



*JOURNAL OF CHROMATOGRAPHY
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*chromatography
of antibiotics*

second, completely revised edition

Gerald H. Wagman and Marvin J. Weinstein

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JOURNAL OF CHROMATOGRAPHY LIBRARY

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Preface to revised edition

When the first edition of this volume appeared ten years ago, it was anticipated that progress in antibiotic research and discovery would continue at a productive rate. It would give a great sense of satisfaction if it would be possible to ascertain that the information presented in the initial edition was in some way responsible for an acceleration of the discovery process. That was its objective.

Now it is necessary to keep up with the times. Along with the discovery of new antibiotics came the development of new chromatographic analytical techniques. One of these that has been refined for rapid and precise detection and identification is high-performance liquid chromatography, customarily abbreviated HPLC. This revised edition contains a significant amount of methodology and data based upon HPLC. Columns are packed with particles having an average diameter of less than 50 μm , the velocity of the mobile phase is increased by means of a high inlet pressure, and sensitive sample detectors are applied to the column effluent. This valuable tool is included in this latest volume for numerous antibiotics for which it has been reported. Likewise, there is a significant increase in the text of data obtained by thin-layer chromatography, whenever it has been reported. Modifications of methods to increase the speed of paper or thin-layer separations have also resulted in new data being presented in this volume.

Of major importance is the incorporation of the many new antibiotics which have appeared over the past ten years, particularly the novel β -lactams. There is a significant amount of data on this family of antibiotics in this edition, a group that did not receive a great deal of attention in the earlier volume. New chromatographic methods that have been developed are reported for the numerous penicillins and cephalosporins that have appeared.

If the progress during the past ten years can be projected twenty years hence, the next century should have such a plethora of novel substances that perhaps the bacteria will finally give up the good fight. This is our latest objective... .

December 1983

GERALD H. WAGMAN
MARVIN J. WEINSTEIN

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Preface to first edition

THE AUTHORS REMEMBER...

A little over twenty years ago, in the late 1940's and early 1950's the authors were engaged in antibiotic screening and other microbiological research projects; we remember well the introduction and development of the new analytical tool, paper chromatography. It was finding its place in the analysis of various biologically interesting substances such as alkaloids, steroids, vitamins, amino acids and finally antibiotics.

Researchers struggled with this new technique in antibiotic screening which moved the arena of discovery from the hallowed halls of the organic chemist closer to the corner laboratory of the microbiologist. With this new weapon, properly employed, it became possible to demonstrate differences among substances but not necessarily similarities. This is the goal of any screening program: one searches for novelty, not for sameness. The labors of the chemist could now be conserved for final chemical identification and physical characterization. The microbiologist was now able to declare with a good deal more confidence what the observed activities were not.

The use of paper, and later, thin-layer chromatography, permitted antibiotic screening to make genuine advances. As increasing numbers of new antibiotics tumbled from the fermentation broths, identification of authentic discoveries became increasingly more difficult. The search, however, is far from being over, and for the imaginative and persevering researcher, there are still useful agents to be found.

It is for the diligent screener that this book has been prepared. It is an antibioticist's *vade mecum*. We have found this data compilation on the chromatography of antibiotics most useful for our purposes and hope it will be found applicable and time saving for others.

This book is primarily for use as a consolidated reference for the specific chromatographic identification of antibiotics. As an operating manual, one might ask if this is a propitious time to introduce such a book. Hasn't the antibiotic discovery era come and gone? We think not. In spite of the frequent funereal pronouncements that the discovery of new antibiotics is in a moribund state, new entities are regularly brought to life. From our laboratory, during the past 10 years, the discovery of gentamicin, halomicin, everninomicin, megalomicin, rosamicin and sisomicin have been reported up to the time of this writing.

There was a hiatus in the discovery of clinically useful antibiotics from the middle 1950's, when kanamycin was discovered, until the *Third Interscience Conference on Antimicrobial Agents and Chemotherapy*, in 1963, when both lincomycin and gentamicin were announced. The utility of the new aminoglycoside, gentamicin, was unique with its wide spectrum of gram-negative activity, including *Pseudomonas* organisms, as well as activity against gram-positive bacteria, and most important, limited but definable and minimal toxicity. This initiated a re-evaluation of basic, resin extractable antibiotics

which had been shelved in various screening program refrigerators. In the last few years a plethora of aminoglycoside antibiotics have been reported; tobramycin, kanandomycin, dideoxy-kanamycin B, ambutyrosine, negamycin, sisomicin and BBK-8, to name several.

This renaissance was by no means limited to aminoglycoside antibiotics. Indeed new polypeptides, ansamycins, macrolides and other novel chemical types have been discovered in recent years.

We believe that antibiotics are alive and well and will continue to be found. We offer this book as a guide to the investigator who takes up this hunt.

February 1973

GERALD H. WAGMAN
MARVIN J. WEINSTEIN

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Abbreviations

Most of the abbreviations follow those used in the *Journal of Chromatography*.

A	amperes
abs.	absolute; e.g., abs. alcohol
A f.s.	amperes full scale
APA	aminopenicillanic acid
approx.	approximately
aq.	aqueous
atm	atmosphere
a.u.f.s.	absorbance units full scale
av.	average
b.p.	boiling point
C	Coulombs
°C	degree Celsius, centigrade
ca.	circa, about
calc.	calculated
CCD	counter-current distribution
cm	centimeter(s)
conc.	concentrated
concn.	concentration (<i>c</i> or <i>C</i> in formulae)
const.	constant
corr.	corrected
cpm	counts per minute
cS	centi Stokes
dil.	diluted
dpm	disintegrations per minute
$E_{1\text{cm}}^{1\%}$	extinction coefficient (1% solution, 1-cm light path)
ECD	electron-capture detector
EDTA	ethylenediaminetetraacetate
ELPHO	electrophoresis
equiv.	equivalent
eV	electron volt(s)
°F	degree(s) Fahrenheit
FID	flame ionization detector
Fig., Figs.	figure, figures
FLC	fast liquid chromatography
f.s.d.	full scale deflection
ft.	foot, feet
g	gram(s)

GC	gas chromatography
GLC	gas-liquid chromatography
GSC	gas-solid chromatography
h	hour(s)
HPLC	high-performance liquid chromatography; also, high-pressure liquid chromatography
HSLC	high-speed liquid chromatography
I (or μ)	ionic strength
I.D.	inner diameter
in.	inch(es)
insol.	insoluble
IR	infrared
I.U.	international unit
k	kilo(s)
K	distribution coefficient (CCD)

$$K = \frac{N_{\max.}}{N_t - N_{\max.}}$$

kg	kilogram(s)
l	liter(s)
lb., lbs.	pound, pounds
m	milli ($= \times 10^{-3}$); also, meter(s)
m	molal (mol/kg)
m -	<i>meta</i> -
M	mega ($= \times 10^6$)
M	molar (mol/l)
max.	maximum
mequiv.	milliequivalent
MeV	mega electron volt(s)
mg	milligram(s)
min	minute(s)
ml	milliliter(s)
mol	mole(s)
mol.wt.	molecular weight
m.p.	melting point
mV	millivolt(s)
μ	micro ($= \times 10^{-6}$); also, ionic strength
μm	micrometer ($10^{-6}\text{ m} = 10^{-4}\text{ cm}$)
n	nano ($= \times 10^{-9}$)
n -	normal in organic names, e.g., <i>n</i> -butylamine
N	normal (solution)
$N_{\max.}$	in CCD, number of tube containing maximum concentration
N_t	in CCD, total number of transfers

ng	nanogram = 10^{-9} g
nm	nanometer = 10^{-9} m
No.	number
<i>o</i> -	<i>ortho</i> -
O.D.	outer diameter; also, optical density
OPA	<i>o</i> -phthalaldehyde
<i>p</i> -	<i>para</i> -
PC	paper chromatography
pH	hydrogen ion exponent
<i>pK</i>	<i>K</i> = equilibrium constant, cf., pH
ppm	parts per million
ppt.	precipitate
p.s.i.	pounds per square inch
R_e	value expresses the ratio of the distance traversed by the antibiotic with respect to the end of the tape rather than the solvent front
R_F	distance travelled by the zone divided by the distance travelled by the liquid front
R_M	$\log (1/R_F - 1)$
R_t	distance of the zone from the origin divided by the distance from the origin to the end of the paper, at time <i>t</i>
R_X	distance travelled by the zone divided by the distance travelled by reference substance X
radioactivity	indicated by a superior number preceding the element symbol, e.g., ^{32}P , [^{14}C]amino acids, ^{14}C -labelled
ref., refs.	reference, references
rpm	revolutions per minute
S	Stokes
satd.	saturated
satn.	saturation
S.D.	standard deviation
S.E.	standard error (deviation) of mean of series
sec	second(s)
<i>sec.</i> -	secondary, e.g., <i>sec.</i> -butylamine
soln.	solution
sp.gr.	specific gravity
<i>sym.</i> -	symmetrical, e.g., <i>sym.</i> -tetrachloroethane
<i>t</i>	time
t_R	retention time
temp.	temperature
<i>tert.</i> -	tertiary, e.g., <i>tert.</i> -butanol
TLC	thin-layer chromatography
UV	ultraviolet
V	volt(s)
vol., vols.	volume, volumes

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vs.	versus, against
v/v	volume per volume
wt.	weight
w/v	weight per volume
w/w	weight per weight
>	greater than; faster than
<	less than; slower than
°	degree(s) Celsius, centigrade

CHROMATOGRAPHY OF ANTIBIOTICS

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Chromatographic classification of antibiotics

Over the years many investigators have devised numerous procedures for classification and identification of antibiotics by use of chromatographic techniques. In earlier years, these various chromatographic systems were quite useable because of the relatively small number of antibiotics compared to the present. With the thousands of antibiotics currently known, systematic chromatographic classification of this large number of compounds is extremely difficult. It is possible to group many of these substances, but in order to identify very closely related antibiotics in a particular group the use of many additional chromatographic systems may be required. This book is not written as a means of systematic chromatography but hopefully as an aid in identifying very similar compounds by use of specific chromatographic techniques. A number of methods proposed for classification of antibiotics into groups and within groups will be reviewed for use in the preliminary or presumptive identification of some of these compounds. More definitive identification should be possible by selective use of the Data section comprising the body of the book.

In 1953 Miyazaki et al.¹ described a method of grouping antibiotics according to their salting out chromatograms. The antibiotics were examined by means of ascending paper chromatography. As solvents, distilled water and increasing concentrations of ammonium chloride (0.5 to 20% and saturated solution) were used. Location of the antibiotic zones were determined by bioautographic methods. Using this technique the antibiotics were divided into four major groups. In group A the R_F values were not correlated with the concentration of the ammonium chloride solution, identical values being found at all concentrations and in distilled water. Group B consisted of the antibiotics which had an R_F value of 0 in distilled water and increasing R_F values with increasing concentrations of ammonium chloride. Group C displayed the highest R_F values in distilled water and lowered values with increasing concentration of the ammonium chloride solution. Group D consisted of antibiotics which did not display any movement whatever in the solvent from the starting point. This systematic method was extended by Uri² who added two additional groups. Group E consisted of antibiotics which had an R_F value of 0 in distilled water and an initial increase with rising concentrations of ammonium chloride with the maximum (R_F approximately 1) in 5% salt solution. Beyond this a decrease occurred. This type of paper chromatogram was typical for the macrolides such as oleandomycin, erythromycin, and carbomycin. Within the macrolide group further differentiation was possible. An additional grouping, F, was made, which at that time consisted of only one antibiotic, desertomycin. By connecting the R_F values using a variety of concentrations of ammonium chloride solution, paraboloid curves were obtained which were different for different antibiotics.

Paris and Theallet³ were able to separate 23 antibiotics which were described in the French Pharmacopie into seven groups utilizing paper chromatography (PC) and thin-layer chromatography (TLC), as well as electrophoresis. The groups were as

follows: (1) penicillin and derivatives, (2) heterocyclic compounds containing amino groups such as the aminoglycosides, (3) macrolides, (4) tetracyclines, (5) chloramphenicol and viomycin, (6) polypeptides, and (7) polyenes.

Blinov et al.⁴ in 1969 were able to separate over 300 antibacterial preparations into five groups according to a chromatographic scheme.

Probably the greatest single influence on the systematic analysis of antibiotics was that of Betina⁵ who, in 1964, attempted to establish a systematic chromatographic separation of 62 known antibiotics. These were distributed into five classes and further into 14 subclasses according to their R_F values in four principle solvent systems. Betina felt that antibiotics were a very heterogeneous group of biologically active compounds which causes great difficulty in working out systematic chromatographic analysis. Therefore he developed the analysis along the lines previously noted. By using what are referred to as "summarized chromatograms" it was possible to graph the R_F values of each antibiotic in a number of solvent systems. This resulted in a kind of curve or plot for an unknown which could be compared to plots of known antibiotics belonging to appropriate classes and subclasses.

Betina further analyzed antibiotics by means of "pH-chromatography". By the use of this chromatographic method the ionic character of unknown antibiotics and also the general possibilities of their isolation can be determined. A series of strips of chromatographic papers impregnated with buffers in the range of pH 2–10 is used for each antibiotic. For the development of the chromatograms an appropriate water-saturated organic solvent is used as the mobile phase. Salting out chromatography as described previously was also utilized to further characterize the antibiotics.

Using techniques previously described, Barath et al.⁶ classified the antibiotics from crude concentrates of fermentation media and from the mycelia of 50 strains of soil fungi. Utilizing the systematic PC methods with bioautographic techniques against *Bacillus subtilis*, *Escherichia coli*, *Saccharomyces cerevisiae* and *Candida pseudotropicalis*, the antibiotics were grouped into a series of classes which permitted the authors to choose strains that produced antibiotics with specific activities.

Utilization of hydrolysis–gas chromatography (GC) for differentiation and characterization of antibiotics was described by Brodasky⁷. In most cases, this information, together with conventional chromatography and infrared and ultraviolet spectra, was sufficient to establish identity or dissimilarity. Both high and low temperature pyrolysis were described. The general lability of antibiotics makes them suitable compounds for pyrolysis studies. A number of examples of differentiation of antibiotics in both temperature ranges were given.

For screening of new antibiotics, Maeda et al.⁸ have applied the use of high voltage paper electrophoresis for separation and identification of these substances. The relative mobilities of 92 antibiotics in two groups are listed according to their extraction properties. Antibiotics in Group 1 are those which are able to be extracted by solvent techniques. Most antibiotics assigned to Group 2 are adsorbed on weak cation-exchange resins such as Amberlite[®] IRC-50 or strong cation exchange resins such as Amberlite[®] IR-120.

The behavior of 16 antibiotics was examined by Dobrecky et al.⁹ by means of TLC using silica gel G, aluminium oxide G, and cellulose MN300. A number of solvents

were used. Antibiotic zones were detected by spraying with ninhydrin or sulfuric acid, or by UV.

An extension of the classification of antibiotics by TLC was proposed by Aszalos et al.¹⁰ The authors present what is called "instant thin-layer chromatography (ITLC)" which attempts to rapidly assess the probability that the antibiotic in question is one already known. The authors used 84 antibiotics in this study. They state that the method will not identify an individual antibiotic in a crude mixture, but it will narrow the choice of identities to a small number. Thereafter, additional chemical, physical and microbiological testing are required to distinguish the individual antibiotics. The prime criterion employed by the authors in their method of classification was the occurrence of movement of an antibiotic in a specific solvent system. Analysis of the antibiotics tested, utilizing 3 primary solvent systems, produced a scheme containing four primary groups. Application of 11 additional solvent systems to the members of the four primary groups yielded 15 sub-groups. The authors state that differentiation of antibiotics based on the characteristics of the ITLC system is more reliable than differentiation based on potentially misleading differences in R_F values. The only use made of R_F values in this scheme is to demonstrate the movement of the antibiotic.

In an extension of these methods, Issaq et al.¹¹ classified 151 antibiotics exhibiting antitumor properties using TLC and bioautographic detection. Aszalos and Issaq¹² similarly described TLC systems for classification and identification of various antibiotics.

Sephadex has been used in TLC for the identification of antibiotics by Zuidweg et al.¹³ With this medium a buffer solution was used instead of organic solvents. A combination of Sephadex TLC and bioautography was applied to qualitative analysis of mixtures of particular antibiotics. The authors stated that the advantage of the combination of Sephadex TLC and bioautography is the elimination of false inhibition zones due to the incomplete removal of organic solvents sometimes occurring when conventional chromatographic methods are used. Descending chromatography was used and the layers were prepared by mixing Sephadex G-15 (40–120 μ g) with 0.025 *M* phosphate buffer at pH 6.0, containing 0.5 *M* sodium chloride. The suspension was spread in a layer of 0.5-mm thickness, and the plates were allowed to dry at room temperature for about 1 h, then transferred to a moist chamber where they were kept in a horizontal position for at least 24 h. Plates were run in sandwich arrangements using descending chromatography for about 60–90 min. After development, the Sephadex chromatographic plates were pressed on seeded agar layers covered with a sheet of lens tissue paper and allowed to remain in contact for 30 min, the lens paper removed, and the agar plate incubated at the optimal growth temperature of the test organism. The organisms used were *Bacillus subtilis*, *Staphylococcus aureus* and *Saccharomyces cerevisiae*. Utilizing this technique 17 antibiotics were successfully separated.

TLC using Kieselgel G (Merck) was utilized by Schmitt and Mathis¹⁴ for separation of 42 antibiotics. Utilizing three selective solvent systems, the authors were able to distribute these antibiotics into four groups. The antibiotics of closely related chemical composition generally fell in the same chromatographic group; for example, macrolides in group two, tetracyclines in group three and aminoglycosides in group four. A number of miscellaneous antibiotics fell into the first group. These included cepha-

losporins, penicillins, chloramphenicol, rifamycin, etc. The antibiotics were detected after development by two reagents containing dimethylamino benzaldehyde.

A number of systems have been proposed for classification of specifically related antibiotics as opposed to the previously discussed schemes for classification of a variety of antibiotic types. Yajima¹⁵ was one of several investigators to propose a system for the classification of antifungal antibiotics. The author used summarized papergrams with six different solvent systems and compared the studies of this classification with results obtained by electrophoretograms, diffusion curves, antifungal spectra and identification by ultraviolet absorption spectra. Twenty-four antifungal antibiotics were tested and were classified into 11 groups. It was difficult to differentiate closely related substances such as actidione and formicidin. However, it was concluded that the patterns obtained would be useful not only for the identification of antifungal agents produced in agar or broth but also for the selection of solvents employed in purification procedures. Using electrophoresis, diffusion curves and antifungal spectra, correlations such as those of solvent extractabilities or solvent solubilities were unable to be differentiated. Utilizing the summarized papergram system, the author could classify 17 antifungal substances produced by streptomycetes into seven groups, four substances produced by fungi into two groups, and three substances produced by bacteria into two groups.

At approximately the same time, Ammann and Gottlieb¹⁶ worked out a PC technique for the separation of antifungal antibiotics on the basis of their R_F values in five solvent systems. Rather than utilizing the summarized papergram method, the authors described R_F values of 15 antifungal antibiotics, six of which also showed antibacterial activity, by utilizing a flow chart for the separation of the agents on the basis of the chromatographic data.

In 1956, PC followed by bioautography was shown to be useful for differentiating antibiotics produced by the *Bacillus* species in a screen program by Snell et al.¹⁷ Most of the antibiotics active against gram positive bacteria and several polypeptide antibiotics produced by other microorganisms have been separated. A solvent mixture of *tert.*-butanol, acetic acid and water proved to be most satisfactory for giving a good initial spread of the entire group of antibiotics tested. After determination of the rate of movement in the first solvent, a series of additional solvent systems was used for specific areas of mobility in order to eventually separate the antibiotics. One of the major difficulties in this system is the fact that most of the organisms produced a multiplicity of antibiotics and the production of only one antibiotic appeared to be the exception rather than the rule. Thus, if a major spot was readily observed, it was more easy to key the particular antibiotic.

Blinov et al.¹⁸ worked out a scheme for classification of antibiotics having indicator properties by means of paper chromatography. They applied a PC method to a study of over 20 preparations of indicator antibiotics (blue in an alkaline medium and red in an acid medium). These authors showed that the antibiotics under study contained not less than 15 different compounds which were divided into five groups. The largest group, the mycetin-violarin group was partitioned into approximately 10 different components. A classification scheme was suggested for these antibiotics which enables rapid comparison of the isolated antibiotic with at least 15 different compounds of this group.

TLC was utilized by Ikekawa et al.¹⁹ for resolving approximately 50 antibiotics utilizing seven different solvent systems. The method used silica gel G TLC plates and detection of the spots with 10% potassium permanganate and 0.2% bromophenyl blue solution or by color reactions characteristic to the particular antibiotic under test. Of particular interest are several solvent systems for separation of certain groups of antibiotics. Chromatography using ethanol–conc. ammonium hydroxide–water (8:1:1) was useful for the macrolide antibiotics as a group. Propanol–pyridine–acetic acid–water (15:10:3:12) was useful for differentiating the water soluble basic antibiotics. Differentiation of peptide antibiotics was accomplished with butanol–acetic acid–water (3:1:1), and ethanol–conc. ammonium hydroxide–water (8:1:1) was useful for identification of the polyene antibiotics. The solvent system selected by TLC can be applied to column chromatography for preparative separations.

Using the solvent system propanol–pyridine–acetic acid–water (15:10:3:12) on cellulose powder, TLC plates gave excellent separations of the water-soluble antibiotics according to Ito et al.²⁰ A ninhydrin reagent or an oxidized nitroprusside reagent was used as the identification system. Based on R_F and color, 20 basic antibiotics were separated. For six closely related compounds, in terms of R_F , the solvent system consisting of water-saturated butanol plus 2% *p*-toluenesulfonic acid was successful in differentiation.

A number of schemes for identification of antibiotics of the macrolide group have been composed. Sokolski et al.²¹ were able to chromatograph some of the macrolide antibiotics by using Whatman No. 1 paper and 11 different solvent systems. Iglóy et al.²² felt that the solvent systems proposed were not completely satisfactory for separation of closely related macrolides. In their experiments they endeavored to formulate solvent systems which allowed for maximum separation of the macrolide antibiotics from other antibiotics, together with maximum separation of individual members within this group, and, furthermore, allowed for the reproducible estimation of samples of low biological activity. The principle of the separation method is based on the structural specificity of macrolide antibiotics and utilizes Schleicher–Schüll 2043/b paper strips with four solvent systems. Chromatograms were bioautographed against *B. subtilis*. The solvent systems in each case contained a polar and a non-polar solvent; the water content of the mixtures was standardized by shaking with 1/10 volume of a 30% aqueous sodium chloride solution. The adjustment of the pH of the paper was carried out by impregnating it with 1 *M* phosphate buffers of pH 4.7, 6.0, 7.0 and 8.0, respectively. The most effective solvent mixtures were composed of an alcohol and a non-polar solvent saturated with a concentrated solution of sodium chloride, and buffered to various pH values with mixtures of organic acids and bases. The solvents tested were shown to be suitable for the identification of these antibiotics in fermentation broths of the producing organisms. More recently, silica gel G thin-layer plates were utilized by Ochab and Borowiecka²³ for separation of the macrolide group, which these authors state was difficult to identify on paper. Out of 17 solvent systems investigated, four were found to be the most useful. After development, plates were dried at room temperature for 1 h at 100–110° for about 20 min and sprayed with a 0.05% solution of xanthidrol in glacial acetic acid with 2 ml of concentrated HCl, and finally with a 10% solution of phosphomolybdic acid in ethanol. After heating, various

colors dependent on the macrolide tested were formed. With the four solvent systems used, by noting the R_F and the color it was possible to separate five of the more common macrolide antibiotics.

One of the latter authors, Borowiecka²⁴, devised a thin-layer technique for chromatography of glycosidic antibiotics. Group separation was obtained with all solvent systems tested with a thin layer composed of Kieselgel G plus Kieselguhr G (1:2). Similarly, Roets and Vanderhaeghe²⁵ utilized different color reactions and chromatographic systems on paper or silica gel thin-layer plates for the identification of 14 aminoglycoside and peptide antibiotics.

Cephalosporin C and its semi-synthetic derivatives have been separated and identified, by TLC using silica gel G layers, by Buri²⁶. Using heat activated plates and a solvent consisting of isopropanol-methanol-pH 5 buffer (30:105:15) and spraying the developed, dried plates with an iodine-starch detection system it was possible to separate the cephalosporins and their derivatives.

TLC of compounds with chelating ability, particularly a variety of tetracyclines and their derivatives, was carried out by Nishimoto et al.²⁷. Silica gel thin-layer plates pretreated with disodium-EDTA were utilized with excellent results. Changes in the behavior of tetracycline and oxytetracycline with pH and temperature were studied, and the effects of citric acid, boric acid and Ca^{2+} on these compounds were determined.

A sensitive silica gel TLC method was described by Pauncz and Harsányi²⁸ for the identification of aminoglycoside antibiotics and can be used for detection of these compounds during the course of fermentation, isolation and purification, and for following chemical conversion. Two developing solvents were used: methyl ethyl ketone-96% ethanol-25% ammonia (1:1:1) and chloroform-methanol-25% ammonia (1:7:4). Detection was by use of tetrazolium blue, tetrazolium violet, and bioautography with *Bacillus subtilis* ATCC 6633. Using this method, 0.05-0.2 μg of antibiotic could be detected.

Separation and identification of 14 different antibiotic residues in milk (which are used for mastitis control) by means of TLC were described by Bossuyt et al.²⁹. The limits of detection ranged from 0.1 to 3 $\mu\text{g}/\text{ml}$ except for neomycin which had a minimum detectable level of 15 $\mu\text{g}/\text{ml}$.

A new solvent system consisting of methanol-water (adjusted to pH 2 with phosphoric acid) has been developed for high-performance liquid chromatography (HPLC) of the daunorubicin complex³⁰. This method in conjunction with TLC is useful for identification of the anthracycline group of antibiotics.

A method has been devised³¹ for the separation and detection of 11 antibiotics by the use of silica gel G plates for thin-layer electrophoresis. Various chromogenic reagents were used in the detection of the zones.

Some useful references for chromatography of antibiotics are: *Antibiotics: Isolation, Separation and Purification*³², *Handbook of Chromatography*, Vol. I³³, and a comprehensive review of instrumentation for TLC by Lott et al.³⁴. Two additional references on gel chromatography³⁵ and electrophoretic methods³⁶ will be found very useful.

It is not possible to go into the details of all of the aforementioned chromatographic methods for differentiating various antibiotics because of the enormous amount of data

in some of the publications reviewed here. It is suggested that direct examination of the publications mentioned should be made in order to better evaluate the system or systems most useful for particular separations. It is hoped that this book will be useful in aiding in the positive identification of these antibiotics or antibiotic complexes separated by preliminary systematic chromatography.

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Detection of antibiotics on chromatograms

Numerous methods are used for the detection of antimicrobial agents on chromatograms and these are divided into several categories: chemical detection by use of suitable reagents, bioautographic detection of biologically active components, the use of ultraviolet light for the detection of fluorescent or absorbing spots, and the use of radioisotopic scanning for radioactive antibiotics produced by the addition of tracers to the various media in which the antibiotics were produced, or by other means. Methods for visualization by chemical means or by ultraviolet light will not be discussed in any great detail because these techniques have been known and used for many years in the detection of numerous substances on chromatograms. Several of the more general and useful techniques for the detection of antibiotics will be discussed. Specific chemical methods are given for particular antibiotics listed in the Data section. Likewise, radioisotopic detection is described under the designated antibiotic to which it applies. General methodology will be mentioned. Because of their extreme importance to the detection of antibiotics on both paper and thin-layer plates, various bioautographic techniques will be explored in some detail.

Bioautography of developed chromatograms is carried out by similar methods in most laboratories. The authors use Pyrex baking dishes approximately 8.5 in. wide by 13.5 in. long by 1.75 in. deep ($21.6 \times 34.3 \times 4.4$ cm) with stainless-steel covers. Similar types of flat dishes constructed of plate glass or plastic have also been used by numerous investigators.

A large variety of microorganisms including bacteria, fungi, and viruses have been used to detect various antibiotics under test. Typically, a 200-ml portion of a base layer agar is poured into a baking dish resting on a level surface and allowed to harden. To 100 ml of agar is added 1.0 ml of the working inoculum, mixed well, and poured on top of the base layer.

Occasionally, air bubbles form on the surface of the agar; if so, a bunsen burner flame can be passed rapidly over the agar to break them down. The plates are then allowed to harden. Or, a detergent can be incorporated in the medium.

A number of useful agar media can be found in two excellent books: *Analytical Microbiology* by Kavanaugh¹, or in *Assay Methods of Antibiotics* by Grove and Randall².

Paper chromatograms are placed in contact with the agar and allowed to remain so for a period of several minutes to several hours depending on the organism that is used and the diffusibility of the antibiotic that has been chromatographed. In analytical techniques where paper strips are used to determine unknown quantities of antibiotics in samples for assay, the strips are placed evenly spaced on the seed layer of the agar, carefully laying them on the surface beginning at the origin. Generally, alternate standards and unknowns are plated if possible with no two like standards on a plate, such as described by Wagman et al.³. In a number of techniques, in order to enhance

zone sizes, the strips are allowed to diffuse at 20°C for 1 h before being placed in a 37°C incubator. In all methods that are generally utilized for bioautography of the chromatograms, the plates are incubated for approximately 18 h (overnight) and the zones of inhibition on the grown plates are ready for observation or measurement.

Bioautographic methodology can also be used for characterization of antiviral agents. Herrmann and Rosselet⁴ have described an adaptation of the Dulbecco virus-plaque technique⁵ for this purpose. Paper chromatograms were sterilized by means of ethylene oxide after drying and were placed for 5 min on agar overlays of virus infected cultures. After removal of papers, baking dishes were sealed with Saran Wrap (Dow Chemical Co.), then incubated for 4 days at 36°C. The cell sheet was then stained with a second agar overlay containing indonitrotetrazolium chloride, and within a few hours plaques were readily observed. It was found that zone sizes varied, dependent on the time after virus infection that the paper was applied to the agar. The longer the time period after infection, the smaller the zones that were formed. When very sensitive tests are needed, the authors recommend applying the paper very soon after virus infection of the overlay cultures. When sensitivity is not important, but a more accurate determination of the area of antiviral activity is required, then application of the paper can be delayed.

Bioautography of thin-layer chromatograms is used routinely for the detection of antimicrobial substances but is somewhat more difficult to handle because of the inflexibility of the glass-backed plate which does not always permit the layer to conform to the agar. This lack of contact between the entire surface and the agar can result in poorly defined or missing spots.

To avoid the adherence of the adsorbent to the agar surface, a number of methods have been used. Probably the most common technique is that described by Meyers and Smith⁶ who inserted a sheet of filter paper between the plate and the agar surface. Initially, the Meyers and Smith technique consisted of incubating the developed chromatographic plates for 16 h at 4°C to allow diffusion of the antibiotic into the agar. The chromatographic plate and filter paper were removed and the seeded agar examined after an additional overnight incubation at 37°C. In order to avoid two overnight incubation periods these authors substituted *Streptococcus lactis*, facultative in respect to oxygen, as a replacement for *Staphylococcus aureus*. Using this culture it proved possible to obtain results after overnight incubation at 37°C with the chromatographic plates and filter paper laying on the agar surface. Growth of this organism occurred only under the area covered by the glass plate.

In our laboratories, however, we have modified this procedure to incubate the glass plate on the agar with a strip of Whatman No. 1 filter paper between the plate and the agar for periods ranging from several minutes to 1 h. The paper and glass plate are both removed and the seeded agar incubated as is normal for paper chromatograms. We have had satisfactory results with a variety of microorganisms seeded in the agar using this technique; however, the zone sizes and diffusion rates are dependent upon the amount of material spotted on the chromatographic plate and the thickness and type of layer.

As a modification of the Meyers and Smith method, Meyers and Erickson⁷ have altered the technique by incorporating 0.1% potassium nitrate into both the basal and

seed agar layers described in the previous paper and have found that good growth of the organism occurred under the glass plate. The theory of this technique is that an organism will grow under these conditions if given a compound capable of replacing oxygen as an oxidant in terminal respiration. Under the conditions described, the test organism was as sensitive to a variety of antibiotics as it was when paper chromatograms of the same antibiotics were tested.

Another technique for increasing visualization of the zones of inhibition is to incorporate tetrazolium dye in the agar overlay, or to add a solution of tetrazolium to the grown organism after incubation and let the agar stand for a length of time. Either method results in a reddish area of growth against a clear zone of inhibition. In general, addition of 1.0 ml of a 2% (w/v) aqueous solution of 2,3,5-triphenyl-2H-tetrazolium chloride per 100 ml of seed layer is satisfactory.

In order to enhance the visualization of the zones of inhibition, Begue and Kline⁸ have tested a large variety of tetrazolium salts which are commercially available in order to determine the optimum conditions for color formation with these various compounds. Their conclusion was that not all tetrazolium salts will produce desired results and each investigator may have to find the best agent experimentally for his own system. Of the salts which were tested, the one which was generally most satisfactory was *p*-iodonitrotetrazolium violet. This was sprayed as an aqueous solution at a concentration of 2 mg per ml after incubation and bioautograms allowed to react for approximately 30 min for *Bacillus subtilis* or *Sarcina lutea* and up to 2 h as in the case of *Pseudomonas syringae*. One recent technique for enhancement of bioautography of *Trichomonas* utilizes phenolphthalein monophosphate and was described by Meyers and Chang⁹.

An interesting method used to plate thin-layer chromatograms is described by Narasimhachari and Ramachandran¹⁰ who used the method of taking a micro-thin layer of the developed, dry thin-layer chromatogram by pressing a transparent cellulose adhesive tape such as "Scotch" tape of suitable width on the thin-layer plate. The Scotch tape is then carefully removed from the plate and gently tapped on the non-adhesive side to remove any loose adsorbent material. It is then stretched on a nutrient agar plate freshly seeded with a suitable test organism. It is important that the tape is fully stretched without any folds. These authors recommend testing to determine whether the tape or the solvent-free adsorbent has any effect on the test organism. This is done by pressing a piece of tape of similar length on the thin-layer medium at a place where no compound was applied and then contacting it to the seeded plate as a control strip. They found that neither the tape nor any of the adsorbent material had an inhibitory action on the test organisms used in their studies.

Several methods have been devised by Hamilton and Cook¹¹ while using the phytopathogenic organism *Xanthomonas pruni*, which does not reduce tetrazolium dyes, is an obligate aerobe and whose normal growth is very slow. *X. pruni* hydrolyzes gelatin and starch and produces acid from several sugars. These characteristics can be made indirect indicators of growth. Gelatin hydrolysis as an indicator of microbial growth was determined by incorporating 0.4% gelatin into the nutrient agar. After the incubation period, the agar was flooded with a solution of 10% mercuric sulfate in 2.5 M hydrochloric acid which causes the unhydrolyzed gelatin to form a white

precipitate. The zones of inhibition are white and the growth areas have the normal slight turbidity of nutrient agar. These authors determined starch hydrolysis as an indicator of microbial growth by incorporating 0.2% soluble starch into the nutrient agar. After incubation, the agar was flooded with a mixture of 1% iodine in 2% aqueous potassium iodide. The unhydrolyzed starch forms a blue complex with iodine resulting in blue zones of inhibition and faint yellow areas of growth. Acid production was detected by incorporating bromcresol purple (1 ml of a 1.6% solution in ethanol per liter of medium) into nutrient agar containing 1% glucose. The microorganism produced slight amounts of diffusible acids; zones of inhibition were purple and growth areas yellow.

Another interesting technique was devised by Homans and Fuchs¹² who found that it was possible to spray a thin-layer chromatogram directly with a spore suspension of a fungus contained in a glucose-minimal medium and which gave most reliable results. After locating UV-absorbing spots, the chromatograms are sprayed with a conidial suspension of a fungus in a medium prepared as follows. The stock solution contains (per liter of tap water), 7 g KH_2PO_4 , 3 g $\text{Na}_2\text{HPO}_4 \cdot \text{H}_2\text{O}$, 4 g potassium nitrate, 1 g $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ and 1 g sodium chloride. This solution is autoclaved at 120°C for 20 min. Just before making the conidial suspension, 10 ml of a 30% aqueous solution of glucose are added per 60 ml of this solution. During spraying, care should be taken to avoid the plates becoming too wet. After spraying, the thin-layer plates are incubated in a moist atmosphere for two to three days at 25°C. Inhibition zones indicate the presence of the original fungitoxic product plus, if present, the conversion or decomposition products that are fungitoxic. The authors have applied this technique to a number of compounds using many fungi, e.g., *Aspergillus niger*, *Ascochyta pisi*, *Botrytis cinerea*, *Colletotrichum lindemuthianum*, *Fusarium colmorum* and *Penicillium expansum*.

Thin-layer chromatograms have the disadvantage of adherence of the adsorbent to the agar surface when carrying out bioautographic methods. This problem can be avoided as shown by Wagman and Bailey¹³ by the use of a silicic acid-glass fiber sheet (ChromAR sheet 500, code 2182; Mallinckrodt Chemical Works, St. Louis, MO, U.S.A.). This sheet is composed of approximately 70% silicic acid and 30% micro fiber glass and can be cut to the desired size with a pair of scissors or a paper cutter. Although the sheet does not have high tensile strength, if one is careful it is easily handled. This medium has been used in our laboratories for some length of time with excellent results.

The ChromAR sheet has several advantages over TLC plates: (1) it conforms entirely to the agar surface, making contact complete; (2) the adsorbent does not adhere to the agar, therefore no paper need be used to separate the sheet from the agar; (3) much lower levels of antibiotic often need to be spotted compared to TLC plates, apparently due to a more efficient transfer of material from sheet to agar; and (4) in a number of chromatographic solvent systems development was up to twice as rapid as with TLC plates.

Visualization of antibiotics on chromatograms by chemical means are essentially the same as for numerous other compounds which are routinely separated by chromatography. Probably the most common reagent is ninhydrin which is used primarily for the detection of amino acids. This reagent is extremely satisfactory for detecting amino-

glycoside and polypeptide antibiotics, and a variety of other amino-containing compounds. Because various investigators have their own preferences in making up ninhydrin solutions, the methodology for preparing each of these sprays is discussed under the individual antibiotics in the Data section. Another reagent not often used in most chromatographic methods but which is useful in antibiotic detection, particularly for those antibiotics containing a guanidine group such as streptomycin and viomycin, is the Sakaguchi reagent. An excellent method for detection of Sakaguchi-positive antibiotics by use of *n*-bromosuccinimide is described by Szilagyi and Szabo¹⁴. The chromatographic paper strips were dipped into a 0.01% solution of 1-naphthol in 5% methanolic sodium hydroxide, dried in air, and the spots developed by a cooled 0.5% solution of *n*-bromosuccinimide and stabilized by a 40% solution of urea. A red color develops in the presence of Sakaguchi-positive compounds.

One very useful compound for location of spots on thin-layer chromatograms is iodine vapor. This is most easily accomplished by placing some crystals of iodine in the bottom of a small glass chromatographic chamber, covering with a glass top, and simply standing the dried thin-layer chromatogram in the jar. Upon exposure to the iodine vapor, brownish yellow spots corresponding to those antibiotics which are positive to the iodine can be recognized. An alternative technique is to make a saturated solution of iodine in light petroleum and either dip or spray the plate with this solution. Results similar to those obtained by exposure to iodine vapor are found. Studies by Brown and Turner¹⁵ have indicated that neither calcium sulfate (present as a binder in the silica gel) nor ultraviolet sensitizer affects the rate of iodination. Furthermore, results are unchanged when plates are dried at 80°C prior to iodination to insure that no traces of organic solvent remain adsorbed on the silica gel as a reaction medium. The only disadvantage of this technique is that the color fades rapidly as the plates are exposed to air. The degree of fading can be reduced somewhat by covering the chromatogram with glass.

Radioisotopic scanning for labelled antibiotics will not be discussed here. Several methods are noted under specific antibiotics in the Data section. However, a technique for autoradiography of thin-layer radiochromatograms utilizing Polaroid film has been developed by Tio and Sisenwine¹⁶ which bears noting for permanent records of these TLC plates. The authors have constructed a light-tight cassette for 4×5 in. Polaroid type 57 or 58 film packets. The film holder with the front side of the packet (i.e., the side normally towards the lens) facing the radiochromatogram is inserted into the holder and the protective envelope is then withdrawn. Exposure times depend upon the activity of the material on the TLC plates. For details on construction it is suggested that the original paper be consulted. A typical radiochromatogram containing 10,000 dpm of [¹⁴C]glycine and 1 million dpm of [³H]valine was exposed to Polaroid type 57 film (black and white) for 96 h. The same radiochromatogram, when exposed to Polaroid type 58 film, gave a positive print after 240 h which exhibited a very faint blue area due to the tritiated material and a greenish white area due to ¹⁴C. Greater activities of tritium or a longer exposure time produced brighter areas. The whiteness of the ¹⁴C also increased on longer exposure times. For type 57 film, exposures were similar to those for autoradiographs on X-ray film using a wet process development. In particular, the ability to differentiate ¹⁴C and ³H makes this method useful in studies

where doubly labelled antibiotics must be chromatographed and recorded.

One additional technique for removing chromatographic layers bears note. This method, described by Kuranz¹⁷, is useful for removal of the active silica gel layer from Eastman Chromagram Sheets and for its subsequent replication. The author has found that by firmly pressing number 810 Magic Mending Tape (Minnesota Mining and Manufacturing Co., St. Paul, MN, U.S.A.) over the chromatogram and subsequently removing it, the silica gel layer can be transferred almost intact from the supporting plastic adhesive layer of the tape. Since the thickness of the tape is in the order of 0.0027 in., the thickness of the chromatogram has been greatly reduced. This method is very useful for producing replicates of single chromatograms, and it is very satisfactory for inclusion in notebooks or for other permanent record storage. The author further states that this process can be repeated several times to produce multiple, though obviously less distinct, copies.

Some additional techniques have been found useful in our laboratories for the chromatography of antibiotics and will be described briefly.

A useful method for increasing the detection sensitivity of paper strips during bioautography is to leave them in contact with the agar surface throughout the incubation period, thus increasing the diffusion of even trace quantities of antibiotics present and making such zones readily apparent. Another interesting method, particularly for aminoglycoside antibiotics, is to bioautograph paper chromatograms after first spraying with ninhydrin and developing the color; colored areas and zones of activity can easily be correlated, since in most instances enough active substances remain to give zones of inhibition on the seeded agar plates.

Many chemical methods used for detection of a variety of substances are also useful for location of antibiotics. For both PC and TLC numerous reagents described in *Spot Tests in Organic Analysis* by Feigl¹⁸ can be utilized to detect antibiotics on chromatograms. One method useful for TLC spot detection that we have found particularly helpful is charring with sulfuric acid, i.e., concentrated sulfuric acid in methanol is sprayed onto a developed, preheated (110°C) thin-layer plate. This results in dark zones against a white background and is a very sensitive detection agent for many antibiotics. Finally, chromatography of antibiotic hydrolysates followed by a ninhydrin spray is a useful "fingerprint" technique for comparison of similar substances, most particularly for identification of aminoglycosides.

For measurement of R_F values a very simple, reliable tool is a piece of elastic tape marked with ten equal calibrations and subdivisions (0 to 1.0). This can be mounted with the zero end held fast to a strip of metal and a movable clamp attached to the opposite end of the tape. The "0" is positioned at the origin and the "1.0" placed at the front. The R_F can then be read directly from the scale opposite the antibiotic zone.

A rapid, simple means of recording chromatographic data is the use of Polaroid equipment. Even an elementary camera on an inexpensive stand can be used for photographing chromatograms or bioautographic plates. These can be copied in color or black and white and used as permanent laboratory records and are available immediately after photography.

Other specific techniques for detection of particular antibiotics will be discussed under the chromatography of the antibiotic in the Data section.

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CHROMATOGRAPHIC DATA SECTION

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Comments on the use of the Data section

The Data section is divided into two major parts; the first part lists antibiotics in alphabetical order. The second part lists numbered or letter/number combinations of antibiotics so designated in the literature. Antibiotics which have not been assigned names or numbers are listed under the organism from which they were derived. The organism names will be found in the generalized alphabetical part.

The chromatographic methods for those antibiotics listed are presented in the following order: paper chromatography (PC), thin-layer chromatography (TLC), electrophoresis (ELPHO), counter-current distribution (CCD), gas chromatography (GC), gas-liquid chromatography (GLC), gas-solid chromatography (GSC), high-performance liquid chromatography (HPLC), and high-speed liquid chromatography (HSLC).

In the first edition there were numerous instances where no information was shown under a particular heading. The reason was that either no data regarding the heading were presented in the literature or the data were not defined clearly. For example, in paper chromatography, the type of paper used may not have been specified. In most instances, it can usually be assumed that a paper such as Whatman No. 1 will suffice and that other substitutes will also be satisfactory. R_F values on other papers may not be identical to those presented in the literature. However, it was felt that in most cases enough information could be gleaned to make the system (or systems) described of some use in the laboratory. In those techniques where detection methodology was not given, it could generally be inferred that a bioautographic method was used or can be used against a sensitive organism.

However, in this edition, where data were unavailable or poorly defined, the relevant headings have been omitted entirely.

In many systems the R_F values are denoted as estimated values. These were derived as accurately as possible from photographs, drawings, or graphic reproductions of mobilities, as closely as could be determined. In order to allow comparisons to be made with other chromatographic systems described in the Data section, it was felt that such estimated values would be more useful than a text punctuated with numerous and varied types of illustrations.

Solvent proportions shown in the text are presumed in all instances to be ratios by volume unless otherwise noted.

A list of the abbreviations used can be found on pages XV–XVIII.

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Named antibiotics

AABOMYCIN A

PC

Solvents

- A. Benzene
- B. Chloroform
- C. Ethyl acetate
- D. Ethyl acetate-benzene (1:1)
- E. Chloroform-benzene (1:1)
- F. Benzene-ethyl acetate (1:2)

Detection

Bioautography against *Piricularia oryzae*

R_F

Solvent	R_F	Solvent	R_F
A	0.00	D	0.10
B	0.00	E	0.00
C	0.80	F	0.23

Ref.

German Patent 1,961,746, 1970

TLC

Media

- A. Silica gel G
- B. Alumina

Solvents

- A. Ethyl acetate
- B. Benzene
- C. Ethyl acetate-benzene (1:1)
- D. Ethyl acetate-benzene (2:1)
- E. Ethyl acetate-benzene (1:2)
- F. Chloroform
- G. Diethyl ether
- H. Methanol
- I. Acetone

Detection

- A. Bioautography against *Piricularia oryzae*
- B. Concentrated sulfuric acid followed by heating

R_F

R_F same for both detection methods

Solvent	Medium		Solvent	Medium	
	A	B		A	B
A	0.82	0.70	F	0.00	0.00
B	0.00	0.00	G	0.80	0.79
C	0.35	0.10	H	0.96	0.93
D	0.47	0.23	I	1.00	1.00
E	0.10	0.00			

Ref.

S. Aizawa, Y. Nakamura, S. Shirato, R. Taguchi, I. Yamaguchi and T. Misato, *J. Antibiot.*, 22 (1969) 457-462

ELPHO

Medium

Sephadex sheet

Buffers

- A. Phosphate, pH 7.0
- B. Phosphate, pH 10.5

Conditions

15 mA, 30 min

Detection

Bioautography against *Piricularia oryzae*

Mobility

Buffer (A), no movement; (B) moved slightly to anode

Ref.

As TLC

ABBOTT 29119

(See Numbered antibiotics)

ABLASTMYCIN

PC

Solvent

n-Propanol-pyridine-acetic acid-water (15:10:3:12), descending, 18 h

R_F

Spot moved 23 cm from origin

Ref.

T. Hashimoto, M. Kito, T. Takeuchi, M. Hamada, K. Maeda, Y. Okami and H. Umezawa, *J. Antibiot.*, 21 (1967) 37

ELPHO

Medium

Whatman No. 3MM paper

Buffer

Formic acid–acetic acid–water (25:75:900)

Conditions

3000 V, 100–150 mA for 30 min

Detection

Ninhydrin, UV absorption; biological activity

Ref.

As PC

ACIDOPHILIN**TLC**

Media

A. Silica gel

B. Alumina

Solvent

Chloroform–methanol (90:10)

Detection

A. UV

B. H_2SO_4 charring

C. Iodination

 R_F

Medium A 0.49

Medium B 0.38

Ref.

K.M. Shahani, J.R. Vakil and R.C. Chandan, *U.S. Patent* 3,689,640, Sept. 5, 1972

ACID S**(octahydro and decahydro derivatives)****TLC**

Medium

Silica gel

Solvent

Ethyl acetate–2-propanol–water (85:10:5)

Detection

Iodine

 R_F

One spot

Ref.

D.T. Connor, S.M. Ringel, S. Roemer and M. von Strandtmann, *U.S. Patent* 4,001,398, Jan. 4, 1977

ACLACINOMYCIN**TLC 1**

Medium

Silica gel 60 F₂₅₄ (E. Merck)

Solvents

A. Chloroform–methanol (20:1)

B. Acetone–*n*-hexane (1:1) R_F

Component	Solvent	
	A	B
A	0.36	0.71
B	0.15	0.49

Ref.

T. Oki, Y. Matsuzawa, A. Yoshimoto, K. Numata, I. Kitamura, S. Hori,^{*} A. Takamatsu, H. Umezawa, M. Ishizuka, H. Naganawa, H. Suda, M. Hamada and T. Takeuchi, *J. Antibiot.*, 28 (1975) 830–834

TLC 2

Medium

Silica gel

Solvents

A. Chloroform–methanol (20:1)

B. Chloroform–methanol (5:1)

C. Acetone

D. Acetone–hexane (1:1)

E. Benzene–methanol (1:1)

 R_F

Solvent	Component	
	A	B
A	0.36	0.71
B	0.88	0.90
C	0.43	0.72
D	0.15	0.49
E	0.86	0.93

Ref.

Belgian Patent 831,726, Nov. 17, 1975

ACLACINOMYCIN A**TLC**

Medium

Silica gel

Solvents

- A. Chloroform
- B. Chloroform-methanol (65:1)
- C. Chloroform-methanol (20:1)
- D. Chloroform-methanol (10:1)
- E. Chloroform-methanol (5:1)

Detection

See footnote to table

R_F

Compound	Solvent				
	A	B	C	D	E
Bisanhydro- aklavinone	0.64 •	0.77	0.89	0.93	0.87
7-Deoxy- aklavinone	0.21	0.54 •	0.78	0.86	0.83
Aklavinone	0.13	0.45 •	0.77	0.86	0.83
MA144-EI	0.03	0.31 •	0.74	0.86	0.83
Aclacino- mycin A	0	0.03	0.49 •	0.83	0.83
MA144-MI	0	0	0.15	0.54 *	0.72
MA144-NI	0	0	0.11	0.47 •	0.66
MA144-SI	0	0	0.02	0.17	0.42 *
1-Deoxypyrr- romycin	0	0	0.01	0.11	0.22 •

* These values indicate the suitable solvent system for TLC-fluorescence scanning of each compound

Ref.

I. Kitamura, T. Oki and T. Inui, *J. Antibiot.*, 31 (1978) 919-922

ACRYLAMIDINE**PC****Paper**

Toyo filter paper No. 51

Solvents

- A. Wet butanol
- B. 20% Ammonium chloride
- C. 75% Phenol
- D. 50% Acetone
- E. Butanol-methanol-water (4:1:2) + 1.5% methyl orange
- F. Butanol-methanol-water (4:1:2)
- G. Benzene-methanol (4:1)
- H. Water

Detection

- A. Iodine
- B. Nitroprusside reagent
- C. Potassium permanganate
- D. UV

R_F

Sol-vent	R_F	Sol-vent	R_F
A	0.20 (est)	E	0.68 (est)
B	0.95 (est)	F	0.43
C	0.78 (est)	G	0.00 (est)
D	0.70 (est)	H	0.89 (est)

Ref.

K. Yagishita, R. Utahara, K. Maeda, M. Hamada and H. Umezawa, *J. Antibiot.*, 21 (1968) 444-450

TLC**Medium**

Eastman Chromagram Sheet 6061

Solvent

1-Butanol-glacial acetic acid-water (4:1:5)

Detection

As PC

R_F

0.56

Ref.

As PC

ACTINOBOLIN**PC****Solvent**

n-Butanol-acetic acid-water (10:1:4); ascending

R_F

0.12 to 0.19 (actinobolin acetate)

Ref.

T.H. Haskell, J. Ehrlich, R.F. Pittillo and L.E. Anderson, *U.S. Patent* 3,043,830, July 10, 1962

ACTINOCARCIN**ELPHO****Medium**

Cellulose acetate film

Buffer

pH 8.9

Mobility

Moves toward cathode as a single entity

Ref.

T. Kihara, S. Takeuchi and H. Yonehara, *J. Antibiot.*, 27 (1974) 994-995

ACTINOLEUKIN**TLC****Medium**

Silica gel G

Solvent

Ethyl acetate-acetone (9:1)

R_F

0.11, 0.57, 0.74

Ref.

S. Omura, Y. Lin, T. Yajima, S. Nakamura, N. Tanaka, H. Umezawa, S. Yokoyama, Y. Homma and M. Hamada, *J. Antibiot.*, 20 (1967) 241

ACTINOMYCINS**PC 1****Solvents**

- A. Ethyl acetate-di-*n*-butyl ether-2% aq. naphthalene-2-sulfonic acid (1:1:2)
 B. Ethyl acetate-di-*n*-butyl ether-10% aq. sodium-*o*-cresotinate (1:3:4)

R_F

Actino- mycin	<i>R_D</i> * solvent A	<i>R_D</i> solvent B
A	1.80, 2.16, 2.46	1.24, 1.71, 4.5
B	1.00, 1.80	(0.55)** , 0.76, 1.00, 1.24 *** (1.71), (2.07)
C	1.57, 2.00	1.59, 2.52
D	1.00	(0.55), (0.76), 1.00, 1.59

* *R_D* value is ratio of distance run by component compared with that of major component of actinomycin D

** () indicate minor component

*** Italics indicate major component

Ref.

R.A. Mancher, F.J. Gregory, L.C. Vining and S.A. Waksman, *Antibiotics Annual 1954-1955*, pp. 853-857

PC 2**Solvent**

Ethyl acetate-di-*n*-butyl ether-2% aq. naphthalene-2-sulfonic acid (1:1:2). Acetone solution of actinomycins placed along entire starting line

Detection**A. Color**

B. Bioautography against *B. cereus* made resistant to naphthalene-2-sulfonic acid by gradient plate technique. Chromatograms plated on seeded agar for 3 min and incubated at 28°C for 24 h. Quantitative estimation done by densitometric scanning and measuring area enclosed by each plate

R_F

Indicates % of component at each *R_D* value, where *R_D* is as in PC 1

Actinomycin A (Sample 1): 1.00 (54.1), 1.80 (12.4), 2.16 (0.7), 2.46 (2.8); Actinomycin A (Sample 2): 0.07 (9.5), 1.00 (28.1), 1.80 (59.3), 2.16 (3.1); Actinomycin B: 0.07 (12.5), 1.00 (27.6), 1.80 (59.9); Actinomycin C: 1.00 (10.9), 1.57 (52.0), 2.00 (37.1); Actinomycin D: 1.00 (100)

Ref.

F.J. Gregory, L.C. Vining and S.A. Waksman, *Antibiot. Chemother.*, 5 (1955) 409-416

PC 3**Solvents**

- A. Di-*n*-butyl ether-*n*-butanol (5:1)/2% aq. soln. of β-naphthalene sulfonic acid sodium salt
 B. Isoamylacetate/5% aq. soln. of β-naphthalene sulfonic acid sodium salt

R_F

Solvent	<i>R_{C₂}</i> *							
	C ₀	C ₁	C _{1a}	C ₂	C _{2a}	C ₃	C _{3a}	C ₄
A	0.25	0.69	0.81	1.0	1.15	1.39	1.56	1.75
B	0.20	0.64	—	1.0	1.21	1.55	1.79	—

* *R_{C₂}* value is the ratio of the distance run by the component compared with C₂

Paper

Schleicher and Schull 2043 bmgl

Ref.

K.H. Zepf, *Experientia*, 14 (1958) 207-208

PC 4

Paper

Whatman No. 1 (ascending or circular)

*Solvent*Di-*n*-butyl ether-ethyl acetate-2% naphthalene- β -sulfonic acid (3:1:4). Paper is dipped in aq. phase and blotted between sheets of filter paper*Detection*Color. Deep red color of naphthalene- β -sulfonic acid salt facilitated detection of zones. Identical separations achieved by either ascending or circular chromatography*Ref.*L.C. Vining and S.A. Waksman, *Science*, 120 (1954) 389-390 R_F

Sample	R_F				
	0.02	0.30	0.47	0.54	0.60
Actinomycin A (produced in 1940)		xxx [•]		xx	
Actinomycin A (produced in 1953)	x	xx		xxx	
Actinomycin B	x	xx		xxx	
Actinomycin C		x	xx		xx
Actinomycin D		xxxx			

* x = relative intensity of zones

PC 5

*Solvent**n*-Butanol-pyridine-water (4:1:5, upper phase) R_F

Actinomycin, 0.9; actinomycin monolactone, 0.75; actinomycin acid, 0.5

*Ref.*D. Perlman, A.B. Mauger and H. Weissbach, *Antimicrob. Agents Chemother.*, 1966 (1967) 581-586

PC 6

Paper

Whatman No. 2, circular, 15-cm diameter

*Solvent*Ethyl acetate-di-*n*-butyl ether-2% aq. naphthalene-2-sulfonic acid (1:3:4). Paper dipped in lower phase and blotted between sheets of clean filter paper. Samples applied to segments of circle near center of paper. Development time about 30 min*Ref.*

As PC 2

PC 7

Paper

Whatman No. 2, circular

 R_F

Solvent A

Actino- mycin complex	$R_{D, IV}$ [•] (% composition)					
	I (0.27)	II (0.40)	III (0.56)	IV (1.00)	V (1.35)	VI ^{**} (1.55-1.97)
X	4.9	Trace	Trace	11.5	84.6	Trace
B	9.5	Trace	Trace	28.1	59.3	3.1
A	6.6	2.9	Trace	66.7	23.8	Trace
D	Trace	Trace	Trace	100	Trace	-
	$R_{D, IV}$ [*]					
C	1.00 (C ₁) 10.3	1.43 (C ₂) 48.3	1.99 (C ₃) 41.4			

*^{***} For footnotes, see table for solvent C

Solvent B

$R_{D, IV}^*$	Actinomycin complex group		
	B	V_{VI}	C
I 0.20	x ***		
II 0.39	x		
III 0.63	x		
IV 1.00	x		x (C_1)
V 1.20	x	x	
VI _a 1.77	Trace	x	x (C_2)
VI _b 2.20	Trace	x	
VI _c 2.66	Trace	x	x (C_3)
VI _d 2.90	Trace	x	
VI _e 3.27	Trace	x	

Solvent C

Actinomycin	$R_{D, IV}^*$
B _{VI_c}	2.13
C ₃	2.90

* Relative to D_{IV}

** Values greater than 1.35 have been referred to collectively as component VI

*** Present

Solvents

- A. Di-*n*-butyl ether-*sym*.-tetrachloroethane-10% aq. sodium-*o*-cresotinate (2:1:3)
 B. Di-*n*-butyl ether-*sym*.-tetrachloroethane-10% aq. sodium-*o*-cresotinate (5:1:6)
 C. Di-*n*-butyl ether-ethyl acetate-10% aq. sodium-*o*-cresotinate (2:1:3)

Detection

UV light of 2570 Å; zones appear as dark absorbing areas against a fluorescent background

Ref.

G.G. Rousos and L.C. Vining, *J. Chem. Soc.*, (1956) 2469-2474

PC 8

Paper

Circular

Solvents

- A. As PC 5
 B. Ethyl acetate-di-*n*-butyl ether-10% aq. sodium-*o*-cresotinate (1:3:4)

 R_F

Actinomycin	R_D^* values	
	Solvent A	Solvent B
B zone I	0.00	0.00, 0.08, 0.25
B zone II	0.07	0.40
B zone III	1.00	0.76
B zone IV	1.00	1.00
B zone V	1.80	1.24
C zone I	0.00	0.06, 0.53, 0.73
C zone II	1.00	1.07
C zone III	1.57	1.59
C zone IV	2.00	2.52
D zone I	0.00	0.00, 0.25
D zone II	1.00	1.00

* Relative to actinomycin D

Ref.

L.C. Vining, F.J. Gregory and S.A. Waksman, *Antibiot. Chemother.*, 5 (1955) 417-422

PC 9

Paper

Circular

Solvents

- A. Isoamyl acetate-5% aq. sodium β -naphthalene sulfonate
 B. Dibutyl ether-1-butanol (5:1)/2% aq. sodium β -naphthalene sulfonate

Detection

Color

 R_F

Photographs of circular chromatograms show comparisons and identity of actinomycin C, oncastatin C and actinomycin L

Ref.

W. Woznicka, H. Niemczyk and A. Paskiewicz, *Med. Dosw. Mikrobiol.*, 13 (1961) 47-52

PC 10

Paper

Toyo Roshi No. 50, circular

Solvents

- A. Isoamyl acetate-5% sodium naphthalene sulfonate (1:1)
 B. As PC 4
 C. Ethyl acetate-2% β -naphthalene sulfonic acid-dibutyl ether (2:1:1)

R_F

Solvent	R_D • actinomycin		
	D	S ₂	S ₃
A	1.00 (std)	1.00	1.59
B	1.00 (std)	1.00	1.83
C	1.00 (std)	1.00	1.23

* Relative to actinomycin D

Ref.

M. Furukama, A. Inoue and K. Asano, *J. Antibiot.*, 21 (1968) 568–570

PC 11

Paper

Circular

Solvent

As PC 2

Detection

As PC 2

 R_F Actinomycins S₁, S₂ and S₃ clearly separated

Ref.

J. Kawamata and H. Fujita, *J. Antibiot.*, 13 (1960) 295–297

PC 12

Paper

Circular

Solvent

Amyl acetate–10% aq. sodium *m*-cresotinate (1:1)

 R_F

Separation of actinomycin U complex

Actinomycin	R_{C_2} *
U ₁	0.32
U ₂	0.45
U ₃	0.73
U ₄	1.18

* Relative to actinomycin C₂

Ref.

G. Schmidt-Kastner, C. Hackman and J. Schmid, *German Patent* 1,126,563, March 29, 1962

PC 13

Paper

Whatman No. 3 mm, circular. Useful for chromatography of tritiated actinomycin D

Solvent

As PC 7, Solvent A

Detection

Radioautogram

 R_F Actinomycin-D-H³ has an R_F of about 0.5

Ref.

Schwarz Bioresercher, 3 (1968) 1, 3

PC 14

Paper

Circular

Solvent

n-Butyl acetate–di-*n*-butyl ether (3:1)/10% aq. sodium-*m*-cresotinate. Filter paper is dipped in the aq. layer, blotted and organic solvent phase used for development

Detection

Color

 R_F

Actinomycin	R_{C_2} *
Z ₀	0.35
Z ₁	0.39
Z ₂	0.78
Z ₃	1.63
Z ₄	2.36
Z ₅	2.55
C ₁	0.63
C ₂	1.00
C ₃	1.52
I ₁	0.65
X ₀	0.20
X ₁	0.65
X ₂	1.05

* Relative to C₂

Ref.

R. Bossi, R. Hütter, W. Keller-Schierlein, L. Neipp and H. Zähler, *Helv. Chim. Acta*, 41 (1958) 1645–1652

PC 15

Paper

Circular

Solvents

A. Butanol–di-*n*-butyl ether (2:3)/10% aq. sodium-*m*-cresotinate

B. As PC 14. Procedure for A and B same as PC 14

Detection

Color

R_F	Actinomycin	$R_{C_2}^*$ Solvent A	$R_{C_2}^*$ Solvent B
	C_0^a	0.13	0.10
	C_0	0.13	0.10
	C_1	0.72	0.56
	C_2	1.00	1.00
	C_3	1.39	1.61
	I_0^a	—	0.12
	I_0	0.49	0.27
	I_1	0.74	0.63
	I_2	1.00	0.97
	I_3	—	1.34
	X_0^a	0.14	0.17
	X_0	0.14	0.39
	X_1	0.48	0.56
	X_1^a	—	0.71
	X_2	0.72	0.98
	X_3	0.93	1.49
	X_4	1.11	1.90

* Relative to C_2

Ref.

H. Brockmann and H. Gröne, *Chem. Ber.*, 87 (1954) 1036–1051

PC 16

Paper

Circular

Solvent

Dibutyl ether–butyl acetate (1:1)

Detection

UV (SVD-120A lamp with a UFS-3 filter); yellowish-green fluorescence

 R_F

Actinomycin D, 0.3

Ref.

S.N. Filippova, V.A. Poltorak and V.D. Kuznetsov, *Mikrobiologiya*, 42 (1973) 251–254

TLC

Media

A. Alumina (Merck, G grade)

B. Silica gel (Merck, G grade)

Solvents

A. Ethyl acetate–*sym.*-tetrachloroethane–water (3:1:3, bottom layer)B. Ethyl acetate–*di-n*-butyl ether–water (3:1:3, top layer)C. Ethyl acetate–*di-n*-butyl ether–water (2:1:2, top layer) R_F

Solvent		R_F						
		C-group	C_1	C_2	C_3	F-group	F_1	F_2
Alumina	A	—	0.44	0.51	0.58	—	0.21	0.35
	B	—	0.40	0.46	0.53	—	0.23	0.29
	C	—	0.28	0.30	0.33	—	0.10	0.13
Silica gel	D	0.24	—	—	—	0.13	—	—
	E	0.43	—	—	—	0.13	—	—
	F	0.63	—	—	—	0.53	—	—
	G	0.70	—	—	—	0.50	—	—
	H	0.95	—	—	—	0.75	—	—

D. Benzene–ethyl acetate–methanol (10:2.5:1)

E. Benzene–ethyl acetate–methanol (6:4:1)

F. Butan-1-ol–methanol–water (6:1:3)

G. Butan-1-ol–acetic acid–water (10:1:3)

H. Ethyl acetate–propan-2-ol–water (5:2:1)

Migration time ranged from 30 to 60 min

Detection

A. Bright orange color ($E_{\max}$ 440–450 nm)B. UV light ($E_{\max}$ 240 nm)

Ref.

G. Cassani, A. Albertini and O. Ciferri, *J. Chromatogr.*, 13 (1964) 238–239

ELPHO

Medium

Paper

Buffer

Pyridine–acetate, pH 6.5

Mobility

Actinomycin, no movement; actinomycinic acid, high mobility towards cathode; actinomycin monolactone, one-half mobility of the di-acid

Ref.

As PC 5

HPLC**Apparatus**

ALC 202/6000 p.s.i., Waters Assoc., Framingham, MA, U.S.A.

Column

6 ft. $\times$ 1/8 in. $\times$ 2.3 mm I.D., stainless steel. Bondapak C₁₈/Corasil and Bondapak phenyl/Corasil (Waters Assoc.) packings. Packing retainers consist of porous PTFE plugs and end fittings of 5- μ porous stainless frits embedded in reduced unions

Mobile phase

Acetonitrile–water (1:1) 22°

Flow-rate

1.0 ml/min

Pressure

1000 p.s.i.

Attenuation

08

Retention time

Compound	Retention time (min) *
Actinomycin C ₂	25.0
Actinomycin C ₃	31.5
Actinomycin D (C ₁)	20.5

* Estimated from drawings

Ref.

W.J. Rzeszotarski and A.B. Mauger, *J. Chromatogr.*, 86 (1973) 246–249.

ACTINOMYCINS (from *Micromonospora floridensis*)
(cf., 70591)

TLC**Medium**

Silica gel GF

Solvent

Chloroform–methanol–water (2:1:1)

Detection

Bioautography vs. *Staphylococcus aureus* ATCC 6538P

R_F

Fraction	R _F
1	0.70
2	0.68
3	0.65
4	0.64

Fraction	R _F
5	0.62
6	0.48
7	0.44
8	0.42
9	0.40
10	0.37
11	0.10

Ref.

G.H. Wagman, J.A. Marquez, P.D. Watkins, F. Gentile, A. Murawski, M. Patel and M.J. Weinstein, *Antimicrob. Agents Chemother.*, 9 (1976) 465–469

ACTINOSPECTACIN**PC 1****Paper**

Eaton-Dikeman 613

Solvents

- A. *n*-Butanol satd. with water
 - B. *n*-Butanol with 2% (w/v) *p*-toluenesulfonic acid
 - C. *n*-Butanol with 2% (v/v) piperidine
 - D. Methanol–water (4:1) with 1.5% (w/v) sodium chloride vs. paper buffered with 1 *M* sodium sulfate-bisulfate, pH 2.0
 - E. Ethanol–water (4:1) with 1.5% (w/v) sodium chloride vs. paper buffered with 1 *M* sodium sulfate-bisulfate, pH 2.0
 - F. Water (steaming)
 - G. Water, 5% ammonium chloride
 - H. Water, 20% ammonium chloride
- All systems developed ascending at 28°

Detection

Bioautography against *Bacillus subtilis*

R_F

Solvent	R _F
A	0.35
B	0.32
C	0.36
D	0.87
E	0.60
F	0.12
G	0.79
H	0.98

Ref.

A.C. Sinclair and A.F. Winfield, *Antimicrob. Agents Chemother.*, 1961, (1962) 503–506

PC 2**Paper**

Whatman No. 1

Solvents

- A. *n*-Butanol–water (84:16, v/v)
 B. *n*-Butanol–water plus 0.25% (w/v) *p*-toluene-sulfonic acid
 C. *n*-Butanol–acetic acid–water (2:1:1, v/v/v)
 D. *n*-butanol–water (84:16) with 2 ml piperidine added to 98 ml of butanol–water mixture
 E. *n*-Butanol–water (4:96, v/v)
 F. *n*-Butanol–water (4:96) plus 0.25% (w/v) *p*-toluenesulfonic acid

Detection

Bioautography against *Klebsiella pneumoniae*,
Bacillus subtilis and *Escherichia coli*

R_F

Solvent	R _F *
A	0.02
B	0.17
C	0.42
D	0.18
E	0.82
F	0.81

* Estimated from drawing

Ref.

D.J. Mason, A. Dietz and R.M. Smith, *Antibiot. Chemother.*, 11 (1961) 118–122

ACTINOTIOCIN**TLC****Medium**

Silica gel

Solvent

Methanol–chloroform (1:9)

Detection

UV

R_F

0.39

Ref.

A. Tamura, R. Furuta, S. Naruto and H. Ishii, *J. Antibiot.*, 26 (1973) 343–350

**ACTINOTIOCIN, DEAMINO-
(See deamino-actinotiocin)****ACTINOXANTHIN****ELPHO****Medium**

Acrylamide gel (disc electrophoresis)

Conditions

Monomer concn. 15%; charge, 400 μg, current, 5 mA; time, 60 min; dye, nigrosin, 1.2 mg in 1000 ml of ethanol–acetic acid–water (5:1:4)

Detection*Bacillus subtilis*; *Staphylococcus aureus* 209P**R_F**

Appears to move slightly toward anode

Ref.

A.S. Khoklov, B.Z. Cherches, P.D. Reshetov, G.M. Smirnova, I.B. Sorokina, T.A. Prokoptzeva, T.A. Koloditskaya and V.V. Smirnov, *J. Antibiot.*, 22 (1969) 541–544

ACULEACIN A**TLC****Medium**

Silica gel (Eastman Kodak No. 6060)

Solvents

- A. Chloroform–methanol (10:3)
 B. Ethyl acetate–methanol–water (20:4:1)
 C. Ethyl acetate–*n*-butanol (3:1) water satd.

Detection

Iodine vapor (brown spot)

R_F

Solvent	R _F
A	0.47
B	0.28
C	0.37

Ref.

K. Mizuno, A. Yagi, S. Sato, M. Takada, M. Hayashi, K. Asano and T. Matsuda, *J. Antibiot.*, 30 (1977) 297–302

ACULEACINS**TLC****R_F**

Component	Solvent			
	A	B	C	D
Aculeacin A		0.47	0.28	0.37
Aculeacin B	0.52	0.91		
Aculeacin C	0.46	0.87		
Aculeacin D	0.35	0.67		
Aculeacin E	0.18	0.58		
Aculeacin F	0.15	0.38		
Aculeacin G	0.13	0.32		

Medium

Eastman Chromagram Sheet No. 6060

Solvents

- A. Ethyl acetate–isopropanol–water (10:2:1)
 B. Chloroform–methanol (10:3)
 C. Ethyl acetate–methanol–water (20:4:1)
 D. Ethyl acetate–*n*-butanol (3:1)

DetectionBioautography vs. *Candida albicans***Ref.**

K. Mizuno, T. Matsuda, K. Asano, A. Yagi, M. Takada, T. Saito, M. Toriya, S. Satoi and K. Matasuura, *U.S. Patent* 3,978,210, August 31, 1976; S. Satoi, A. Yagi, K. Asano, K. Misuno and T. Watanabe, *J. Antibiot.*, 30 (1977) 303–307

**7-N-ACYLAMINOCEPHALOSPOR-
 ANIC ACIDS; O-DESACETYL
 DERIVATIVES
 (cf., cephalosporins)**

TLC**Medium**

Silica gel

R_F**Solvents**

- A. Ethyl acetate–pyridine–glacial acetic acid–water (62:21:6:11)
 B. *n*-Butanol–glacial acetic acid–water (67:10:23)

Ref.

R. Bosshardt, B. Fechtig, J. Mueller, H. Peter and H. Bickel, *U.S. Patent* 3,706,746, Dec. 19, 1972.

Derivative	Solvent A	Solvent B
O-Desacetyl-O-(N- β -chloroethylcarbamyloxy)-7-N-D-(–)- α -phenylglycyl]-aminocephalosporanic acid as inner salt of the formula	0.08	0.24
7-N-[N-Carbo- <i>tert</i> -butoxy-D-(–)- α -phenylglycyl]-aminocephalosporanic acid	0.59	0.47
O-Desacetyl-7-N-[N-carbo- <i>tert</i> -butoxy-D-(–)- α -phenylglycyl]-aminocephalosporanic acid	0.37	0.38
O-Desacetyl-O-(N- β -chloroethylcarbamyloxy)-7-N-[N-carbo- <i>tert</i> -butoxy-D-(–)- α -phenylglycyl]-aminocephalosporanic acid	0.63	0.50
O-Desacetyl-O-(N-methylcarbamoylethyl)-7-N-[D-(–)- α -phenylglycyl]-aminocephalosporanic acid trifluoroacetate	0.05	0.13
O-Desacetyl-O-(N-methylcarbamoylethyl)-7-N-[D-(–)- α -phenylglycyl]-aminocephalosporanic acid	0.05	0.13
O-Desacetyl-O-(N-methylcarbamoylethyl)-7-N-[N-carbo- <i>tert</i> -butyloxy-D-(–)- α -phenylglycyl]-aminocephalosporanic acid	0.50	0.50
O-Desacetyl-O-(N-ethylcarbamoylethyl)-7-N-[D-(–)- α -phenylglycyl]-aminocephalosporanic acid trifluoroacetate	0.07	0.18
O-Desacetyl-O-(N-ethylcarbamoylethyl)-7-N-[D-(–)- α -phenylglycyl]-aminocephalosporanic acid	0.07	0.18
O-Desacetyl-O-(N-ethylcarbamoylethyl)-7-N-[N-carbo- <i>tert</i> -butyloxy-D-(–)- α -phenylglycyl]-aminocephalosporanic acid	0.55	0.52

**ADRIAMYCIN, 4'-EPI-6'-HYDROXY-
 (See 4'-epi-6'-hydroxyadriamycin)**

ADRIAMYCINS**PC****Paper**

Whatman No. 1

Solvents

- A. *n*-Butanol satd. with pH 5.4 1/15 M phosphate buffer
 B. Propanol–ethyl acetate–water (7:1:2, v/v)
 C. Methylene chloride–methanol–water (100:20:2)

R_F

Solvent	R_F
A	0.10
B	0.25
C	0.17

Ref.

F. Arcamone, G. Cassinelli, G. Fantini, A. Grein, P. Orezzi, C. Pol and C. Spalla, *Biotech. Bioeng.*, 11 (1969) 1101–1110; F. Arcamone, G. Cassinelli, A. diMarco and M. Gactani, *U.S. Patent* 3,590,028, June 29, 1971

TLC 1

Medium

Kieselgel G layer buffered with 1% oxalic acid in water

Solvents

- A. *n*-Butanol–acetic acid–water (4:1:5)
 B. Benzene–ethyl acetate–petroleum ether, b.p. 80–120°C (8:5:2)
 C. Benzene–ethyl formate (1:2)

 R_F

Solvent	R_F
A	0.33
B	0.0
C	0.0

Ref.

As PC

TLC 2

Medium

Silica gel buffered at pH 7 (1/15 *M* phosphate)

Solvents

- A. Chloroform–methanol–water (130:60:10)
 B. Chloroform–acetone (10:1)

 R_F

	Solvent	R_F
Adriamycin (α -anomer)	A	0.35
4'-Epiadriamycin (α -anomer)	A	0.55
4'-Epiadriamycin (β -anomer)	A	0.43

	Solvent	R_F
9-Desacetyl-9-(2',2'-dimethyl-4'-methoxy-4'-dioxolanyl)-daunomycinone	B	0.38

Ref.

F. Arcamone, A. DiMarco and S. Penco, *U.S. Patent* 4,058,519, Nov. 15, 1977

HPLC

Apparatus

DuPont Series 848 Amplifier Pump at 4500 p.s.i. with no pulse dampener

Column

Stainless-steel analytical column (250 × 2.1 mm) packed with Zorbax SIL, particle diameter 5 μ m

Temperature

Room temperature. A six-port Rheodyne rotary injection valve fitted with a 200- μ l loop was mounted between the pump and the column

Mobile phase

Methylene chloride–methanol–25% ammonia–water (90:9:0.1:8)

Flow-rate

0.9 ml/min

Detector

DuPont Series 837 Spectrophotometer (200–600 nm) fixed at 490 nm with a sensitivity of 0.02 or 0.04 absorbance units

Retention time

Adriamycin, 5 min

Ref.

R. Hulhoven and J.P. Desager, *J. Chromatogr.*, 125 (1976) 369–374

AKLAVIN

TLC

Medium

Silica gel

Solvents

- A. Chloroform–methanol (4:1)
 B. Ethyl acetate–methanol (3:7)

Ref.

V. Kumar, W.R. Remers and R. Grulich, *J. Antibiot.*, 30 (1977) 881–882

ALAZOPEPTIN (cf., OS-3256)

PC

Solvent

Ascending method. 93.8% *n*-Butanol–44% propionate (1:1)

R_F

0.45–ca. 0.50

Ref.

T. Hata, I. Umezawa, Y. Iwai, M. Katagiri, J. Awaya, K. Komiyama, R. Oiwa and K. Atsumi, *J. Antibiot.*, 26 (1973) 181–183

ALBOCYCLINE

PC

Solvents

- A. Wet butanol
- B. Aq. ammonium chloride 3%
- C. Aq. ammonium chloride 20%
- D. Aq. acetone
- E. Butanol–methanol–water (4:1:2)
- F. Benzene–methanol (4:1)
- G. Water

Detection

Biological activity

R_F

Solvent	R_F
A	1.00
B	0.52
C	0.35
D	1.00
E	1.00
F	1.00
G	0.67

Ref.

N. Bagahama, M. Suzuki, S. Awataguchi and T. Okuda, *J. Antibiot.*, 20 (1957) 261–266

TLC 1

Medium

Silica Gel

Solvents

- A. Benzene
- B. *n*-Hexane–ethyl acetate (7:3)
- C. Benzene–ethyl acetate (4:1)
- D. Benzene–ethyl acetate (1:1)
- E. Diethyl ether–isopropyl ether (1:1)
- F. Isopropyl ether
- G. Chloroform

Detection

Biological activity

R_F

Solvent	R_F
A	0.90
B	0.28
C	0.28
D	0.63
E	0.57
F	0.33
G	0.04

Ref.

As PC

TLC 2

Medium

Silica gel Uniplat, Analtech, Inc.

Solvent

Ethyl acetate–cyclohexane (1:1)

Detection

- A. Bioautography vs. *Penicillium oxalicum*
- B. For radioactive antibiotic, after development, 5-mm wide strips of the silica gel were scraped off the plates directly into the counting vials and the radioactivity was determined by conventional scintillation counting techniques

R_F

0.70

Ref.

L. Slechta, J. Cialdella and H. Hoeksema, *J. Antibiot.*, 31 (1978) 319–323

ELPHO

Medium

Paper

Conditions

1/15 *M* phosphate buffer, 10 V/cm, 2.5 h, pH 5.0 and 8.0

R_F

At pH 5.0 or pH 8.0 albocycline did not move

Ref.

As PC

GLC

Column

1.5 m packed with 5% SE-52

Temperature

180°C

Carrier gas

N₂, 90 ml/min

Retention time

6 min (est. from curve)

Ref.

As PC

ALBOMYCIN**PC****Paper**

Whatman No. 1 strips

Solvents

- A. *n*-Butanol–water–acetic acid (4:2:1)
- B. *n*-Butanol–water–acetic acid (1:2:1)
- C. Methanol–0.1 *N* HCl (3:1)
- D. *n*-Propanol–2.5% sodium chloride–acetic acid (10:8:1)
- E. *n*-Butanol–ethanol–water–acetic acid (25:25:47:3)
- F. Acetone–water–acetic acid (60:37:3)

DetectionBioautography against *Escherichia coli*, W. **R_F**

Solvent	R_F
A	0.14, 0.33, 0.46
B	0.83
C	0.68
D	0.79
E	0.75, 0.92
F	0.71

Ref.E.O. Stapley and R.E. Ormond, *Science*, 125 (1957) 587**TLC****Solvents**

- A. 1-Butanol–acetic acid–water (4:1:5), upper phase
- B. *tert*-Butanol–0.004 *N* hydrochloric acid–satd. aq. sodium chloride soln. (2:1:1), upper phase; plate pre-treated with acetone–water–satd. aq. sodium chloride soln. (16:3:1)
- C. Ethanol–water (2:1) containing 2% sodium chloride
- D. 1-Propanol–pyridine–water (15:1:10)
- E. Pyridine–1-pentanol–water (7:7:6)
- F. 2-Propanol–water (7:2); plate pre-treated with 0.2 *M* ammonium sulfate

Detection

Bioautography

 R_F

Solvent	Albomycin δ_1	Albomycin δ_2
A	0.34	0.26
B	0.11	0.03
C	0.73	0.65
D	0.63	0.52
E	0.50	0.40
F	0.19	0.07

Ref.H. Maehr and R.G. Pitcher, *J. Antibiot.*, 24 (1971) 830–834**ALBORIXIN****TLC****Medium**

Silica gel (Merck)

Solvents

- A. Ethyl acetate–cyclohexane (40:60)
- B. Dioxane–hexane (20:80)

Detection

Red color when treated with 50% sulfuric acid at 110°C

 R_F

Solvent	R_F
A	0.40
B	0.50

Ref.P. Gachon, C. Farges and A. Kergomard, *J. Antibiot.*, 29 (1976) 603–610**ALDGAMYCIN E****PC****Solvents**

- A. *n*-Amyl acetate–dibutyl ether–acetic acid–water (20:6:1:10)
- B. Cyclohexane–*sec*-butanol–0.40% ammonium hydroxide (4:1:4)
- C. *n*-Heptane–tetrahydrofuran–*n*-amyl acetate–0.2 *M* aq. acetic acid (4:1:1:4)

 R_F

Solvent	R_F
A	0.87
B	0.72
C	0.18

Ref.

M.P. Kunstmann, L.A. Mitscher and E.L. Patterson, *Antimicrob. Agents Chemother.*, 1964 (1965) 87-90

ALDGAMYCIN F**TLC***Solvent*

Acetic acid-diethyl ether

Detection

Sulfuric acid and heated to 130°C

R_F

0.45

Ref.

H. Aschenbach and W. Karl, *Chem. Ber.*, 108 (1975) 780-789

ALVEOMYCIN**PC***Solvents*

- A. Phenol-butanol-water (5:4:9)
- B. Butanol-acetic acid-water (4:1:5)
- C. Butanol-aminopropanol-water (25:1:25)
- D. Butanol-phenol-water (3:3:4)

R_F

Solvent	<i>R_F</i>
A	0.50
B	0.13
C	0.50
D	0.85

Ref.

G. Schmidt-Kastner and J. Schmid, *Med. Chem. (Leverkusen, Ger.)*, 7 (1963) 528-539; *C.A.*, 60 (1964) 1542e

AMAROMYCIN**PC***Solvents*

- A. 3% aq. ammonium chloride
- B. Acetone
- C. Methanol
- D. Benzene
- E. Butyl acetate
- F. Butanol
- G. Light petroleum

R_F

Solvent	<i>R_F</i>
A	1.0
B	1.0
C	1.0
D	0.1
E	0.2
F	1.0
G	0.0

Ref.

T. Hata, Y. Sano, H. Tatsuta, R. Sugawara, A. Matsumae and K. Kanamori, *J. Antibiot.*, 8 (1955) 9-14

AMBUTYROSIN

(cf., butirosins)

PC*Solvent*

1-Butanol-pyridine-5% boric acid (6:4:3)

Detection

(Method of Pan and Dutcher). Spray paper with sodium hypochlorite (dil. 1 part 5.25% sodium hydrochlorite to 20 parts water). Dry. Spray with 95% ethanol. Dry. Spray with starch-iodide reagent (1% aq. soluble starch-1% aq. potassium iodide, 1:1). Acetylated spots show up as deep blue zones against a colorless background

R_F

Tetra-N-acetyl-ambutyrosin A 0.30-0.38
Tetra-N-acetyl-ambutyrosin B 0.16-0.20

Ref.

S.C. Pan and J. Dutcher, *Anal. Chem.*, 28 (1956) 836; *Netherlands Patent* 69,04408, Sept. 29, 1969

AMETHOBOTTROMYCIN**PC***Solvents*

- A. *n*-Butyl alcohol satd. with 1% aq. acetic acid
- B. *n*-Butyl alcohol satd. with 2% aq. pyridine
- C. Ethyl acetate satd. with 1% aq. acetic acid
- D. Ethyl acetate satd. with 0.1 M phosphate buffer (pH 7)
- E. Benzene-hexane-methanol-5% aq. acetic acid (7:6:10:6)
- F. Benzene-hexane-methanol-5% aq. pyridine (7:6:10:6)
- G. Benzene satd. with 1% aq. acetic acid
- H. Benzene satd. with 2% aq. pyridine
- I. Capryl alcohol satd. with 0.1 M pH 6 phosphate buffer (reversed phase)

R_F

Solvent	R_F
A	0.85
B	0.84
C	0.37
D	0.83
E	0.0
F	0.5
G	0.0
H	0.5
I	0.30

Ref.

F.J. Wolff and W.J. Miller, *U.S. Patent* 3,860,703, Jan. 14, 1975

TLC

Medium

Silica gel

Solvent

Chloroform-methanol (94:6)

Detection

Iodine vapor, brown stain

 R_F

0.60

Ref.

As PC

AMICETIN**PC 1**

Solvents

A. 90% aq. 1-butanol

B. 1-Butanol satd. with water. Both solvents run descending

Detection

Bioautography vs. *Mycobacterium avium* R_F

Solvent	R_F
A	0.22
B	0.46

Ref.

J.W. Hinman, E.L. Caron and C. De Boer, *J. Amer. Chem. Soc.*, 75 (1953) 5864-5866

PC 2

Solvent

1-Butanol satd. with 0.05 M phosphate buffer, pH 7.0, and paper strips impregnated with the buffer soln.

Detection

Bioautography vs. *E. coli* P-D 04863 R_F

0.63

Ref.

T.H. Haskell, A. Ryder, R.P. Forhardt, S.A. Fusari, Z.L. Jakubowski and Q.R. Baetz, *J. Amer. Chem. Soc.*, 80 (1958) 743-747

TLC

Medium

Alumina

Solvent

80% Methanol

 R_F

0.61

Ref.

M. Konishi, M. Kimeda, H. Tsukiura, H. Yamamoto, T. Hoshiya, T. Miyaki, K. Fujisawa, H. Koshiyama and H. Kawaguchi, *J. Antibiot.*, 26 (1973) 752-756; H. Kawaguchi, M. Konishi and K. Tomita, *U.S. Patent* 3,843,449, Oct. 22, 1974

AMICLENOMYCIN**PC**

Paper

Toyo Roshi No. 53

Solvent

n-Butanol-1 N hydrochloric acid (6:1)

Detection

Bioautography vs. *Saccharomyces cerevisiae* ATCC 7754

 R_F

0.4 (Estimated from drawing)

Ref.

T. Kitahara, K. Hotta, M. Yishida and Y. Okami, *J. Antibiot.*, 28 (1975) 215-221

TLC 1

Medium

Silica gel

Solvents

A. Benzene-methanol-acetone-acetic acid (14:4:1:1)

B. *n*-Butanol-acetic acid-water (3:1:1)

C. 95% Ethanol-water (6:4)

Ref.

As PC

TLC 2

Medium

Cellulose F (Merck)

Solvents

A. *n*-Propanol-pyridine-acetic acid-water
(15:10:3:12)

B. *n*-Butanol-acetic acid-water (2:1:1)

 R_F

Solvent	R_F
A	0.45
B	0.03

Ref.

Y. Okami, T. Kitahara, M. Hamada, H. Naganawa, S. Kondo, K. Maeda, T. Takeuchi and H. Umezawa, *J. Antibiot.*, 27 (1974) 656-664

ELPHO**Buffer**

Formic acid-acetic acid-water (25:75:900)

Conditions

3000 V, 20 min

Mobility

Moves toward cathode ($R_M = 1.33$ in relation to 1.0 of alanine)

Ref.

As TLC 2

AMIDINOMYCIN**PC****Solvent**

Butanol-acetic acid-water (2:1:1)

 R_F

0.25-0.27

Ref.

S. Nakamura, H. Umezawa and N. Ishida, *J. Antibiot.*, 15 (1961) 163-164

AMIDOMYCIN**PC****Solvents**

A. Light petroleum vs. paper impregnated with ethylene glycol

B. *n*-Amyl alcohol satd. with water

C. Ethanol-acetic acid-water (3:1:6)

D. Ethanol-water (2:3)

Detection

Bioautography vs. *Candida albicans*

 R_F

Solvent	R_F
A	0.86
B	0.90
C	0.36
D	0.89

Ref.

W.A. Taber and L.C. Vining, *Can. J. Microbiol.*, 3 (1957) 953-965

AMIKACIN**TLC****Medium**

Silica gel F₂₅₄

Solvent

Chloroform-methanol-28% ammonium hydroxide water (1:4:2:1)

 R_F

0.16

Ref.

T. Naito, S. Nakagawa, Y. Narita, S. Toda, Y. Abe, M. Oka, H. Yamashita, T. Yamasaki, K. Fujisawa and H. Kawaguchi, *J. Antibiot.*, 27 (1974) 851-858

L-trans-2-AMINO-4-(2-AMINO-ETHOXY)-3-BUTENOIC ACID**TLC 1****Medium**

Cellulose

Solvent

Phenol-water (80:20)

Detection

Bioautography

Ref.

J. Berger, D. Pruess and J.P. Scannell, *U.S. Patent* 3,775,255, Nov. 27, 1973; *U.S. Patent* 3,751,459, Aug. 7, 1973

TLC 2**Medium**

Silica gel glass plates

Solvent

Ethanol-water-ammonia (49:49:2)

Detection

Ninhydrin

Ref.

As TLC 1

7-AMINOCEPHALOSPORANIC ACID DERIVATIVES (cf., cephalosporins)

TLC

Medium

Silica gel

R_F

Solvents

A. *n*-Butanol–glacial acetic acid–water (75:7.5:21)

B. *n*-Butanol–pyridine–glacial acetic acid–water (42:24:4:30)

Ref.

H. Bickel, J. Mueller, R. Bosshardt, H. Peter and B. Fechtig, *U.S. Patent* 3,646,023, Feb. 29, 1972

Derivative	Solvent A	Solvent B
7-[Tetrazolyl-(1)-acetylaminol]-cephalosporanic acid, sodium salt	0.17	0.47
3-(Desacetoxymethyl)-3-pyridinomethyl-7-[tetrazolyl-(1)-acetylaminol]-cephalosporanic acid	0.01	0.11
3-(Desacetoxymethyl)-3-benzoylthiomethyl-7-[tetrazolyl-(1)-acetylaminol]-cephalosporanic acid sodium salt	0.36	0.48
3-(Desacetoxymethyl)-3-benzoylthiomethyl-7-bromacetylaminol-cephalosporanic acid	0.55	
3-(Desacetoxymethyl)-3-pyridinomethyl-7-[tetrazolyl-(1)-acetylaminol]-cephalosporanic acid	0.01	0.11
O-Desacetyl-O-(β -chloroethylcarbamoyl)-7-[tetrazolyl-(1)-acetylaminol]-cephalosporanic acid	0.23	0.5
O-Desacetyl-O-methylcarbamoyl-7-[tetrazolyl-(1)-acetylaminol]-cephalosporanic acid, sodium salt	0.17	0.40
O-Desacetyl-O-ethyl-carbamoyl-7-[tetrazolyl-(1)-acetylaminol]-cephalosporanic acid, sodium salt	0.21	0.44

3-AMINO-3-DEOXY-D-GLUCOSE

PC

Solvent

n-Butanol–pyridine–water–acetic acid (6:4:3:1)

Ref.

S. Umezawa, K. Umino, S. Shibahara, M. Hamada and S. Omoto, *J. Antibiot.*, 20 (1967) 355

TLC

Solvents

A. Butanol–pyridine–water–acetic acid (6:4:3:1)

B. 14% Aq. ammonia–methanol–chloroform (1:1:2), upper layer

Detection

A. Ninhydrin

B. Bioautography vs. *Micrococcus pyogenes* var. *aureus* 209P

R_F

One major and one minor zone result; major

zone corresponds to 3-amino-3-deoxy-D-glucose. By ninhydrin method, major spot is brown and minor spot is purple

Ref.

S. Umezawa, *U.S. Patent* 3,634,197, Jan. 11, 1972

3-AMINOFLEOMYCIN DERIVATIVES

TLC

Medium

Silica gel G (Merck)

Solvent

Methanol–10% ammonium acetate–10% ammonia (10:9:1)

R_F

Fleomycin derivative	R_F
2-N,N-Dimethylaminoethylamino	0.35
2-N,N-Diethylaminoethylamino	0.52
3-N-Methylaminopropylamino	0.56

Fleomycin derivative	R_F
3-N,N-Dimethylamino-2,3-propylamino	0.35
3-(N,N,N-Trimethylamino)-propyl-aminofleomycin chloride	0.42
3-N,N-Diethylaminopropylamino	0.43
3-N-Butylaminopropylamino	0.60
3-N- <i>tert.</i> -Butylaminopropylamino	0.62
3-N-Propionylaminopropylamino	0.58
3-N-(3-Hydroxypropyl)-aminopropyl-amino	0.61
3-N-(3,3-Dichloropropyl)-amino-propylamino	0.72
3-S,S-Dimethylmercaptopropylamino	0.48
3-N-Cyclohexylaminopropylamino	0.62
3-N-Benzylaminopropylamino	0.57
3-N-(1-Phenylethyl)-aminopropylamino	0.76
3-Pyrrolidinopropylamino	0.39
3-Piperidinopropylamino	0.48
3-Piperazinopropylamino	0.32
3-Morpholinopropylamino	0.65
2-(Imidazol-4-yl)-ethylamino	0.77
3-Amidinopropylamino	0.66
3-(3-N,N-Dimethylaminopropyl-amino)-propylamino	0.11
3-(3-N-Cyclohexylaminopropyl-amino)-propylamino	0.31
3-(3-N-Benzylaminopropylamino)-propylamino	0.48
3-[3-N-(1-Phenylethyl)-aminopropyl-amino]-propylamino	0.54
3-(3-Pyrrolidinopropylamino)-propyl-amino	0.11
3-(3-Piperidinopropylamino)-propyl-amino	0.15
3-(3-Morpholinopropylamino)-propyl-amino	0.41
3-(3-N- <i>n</i> -Butylaminopropylamino)-propylamino	0.25
3-Guanidinopropylamino	0.67

Ref.

Netherlands Patent 7,303,030, Sept. 6, 1973

7-[D- α -AMINO- α -(*p*-HYDROXY-PHENYL)ACETAMIDO]-3-1,2,3-TRIAZOL-5-YLTHIOMETHYL)-3-CEPH-EM-4-CARBOXYLIC ACID

PC

Paper

Whatman No. 1 $\frac{1}{2}$

Solvent

n-Butanol-pyridine-water-glacial acetic acid (6:4:3:1)

Detection

Bioautography vs. *Bacillus subtilis*

Ref.

D. Willner and L.B. Crast, Jr., U.S. Patent 3,899,394, Aug. 12, 1975

7 β -AMINO-3-METHYLCEPH-3-EM-4-CARBOXYLIC ACID
(Cephalosporin compound)

PC

Paper

Whatman No. 3MM (buffered at pH 6 with 50 mM phosphate (5.9 g potassium dihydrogen phosphate, 1.85 g disodium hydrogen phosphate/liter)

Solvent

Butan-1-ol-ethanol-water (4:1:5)

Detection

A. Ninhydrin

B. UV

R_F

Brown stain with ninhydrin

Ref.

I.D. Fleming, M.K. Turner and E.J. Napier, U.S. Patent 3,880,713, April 29, 1975

6-AMINOPENICILLANIC ACID

TLC 1

Medium

Silica gel

Solvents

A. *n*-Butanol-ethanol-water (4:1:5)

B. *n*-Butanol-acetic acid-water (12:3:5)

C. Isopropanol-water (7:3)

R_F

Solvent	R_F
A	0.38
B	0.39
C	0.77

Ref.

German Patent 2,517,316, Oct. 23, 1975

TLC 2

Medium

Silica gel KSK (treated with a citrate buffer soln. pH 5.0)

Solvent

Dry, freshly distilled acetone–glacial acetic acid–water (95:5:10)

Detection

Iodine vapor

 R_F

	$R_{6\text{-APA}}$
6-Aminopenicillanic acid (6-APA)	1.00 *
6-APA Dimer	0.93
Penicillamine	1.09
Benzylpenicillin	1.37

* Assigned value

Ref.

L.I. Aserova, A.D. Tomaschick and V.B. Korchagin, *Antibiotiki*, 18 (1973) 613–618

5''-AMINO-3',4',5''-TRIDEOXYBUTIROSIDIN A

TLC**Media**

- A. Silica gel
- B. Alumina
- C. Cellulose

Solvents

- A. Chloroform–methanol–17% ammonia (2:1:1) upper phase
- B. Chloroform–methanol–29% ammonia–water (1:6:4:2)

 R_F

Solvent	Medium A	Medium B	Medium C
A	0.44	0.70	
B			0.65

Ref.

P.W.K. Woo, *J. Antibiot.*, 28 (1975) 522–529

AMIPURIMYCIN**HPLC****Apparatus**

Model ALC/GPC 244 (Waters Assoc.)

Column

μ Bondapak C₁₈ (Waters Assoc.) 30 cm $\times$ 14 mm I.D., prepacked

Mobile phase

- A. Methanol–0.01 M phosphate buffer (pH 5.7)
- B. Methanol–0.005 M citrate buffer (pH 6.0)
- C. Methanol–0.005 M citrate buffer (pH 5.8)
- D. 0.005 M citrate buffer (pH 5.8)

Flow-rate

1.0 ml/min

Pressure

1500–2000 p.s.i.

Detection

UV Model 440, 254 nm. Single peak observed under various conditions

Injector

Universal Injector U6K (Waters Assoc.) 2 ml of internal volume

Type

Reversed-phase

Retention time

Mobile phase	Retention time (min)
A	3.4
B	3.2
C	4.9
D	9.0

Ref.

S. Harada and T. Kishi, *J. Antibiot.*, 31 (1978) 519–524

AMPHOMYCIN**CCD****Solvent**

1-Butanol–pyridine–acetic acid–water (4:2:1:7)

Distribution coefficient

60 transfers; $K = 3.0$

Ref.

M. Bodanszky, G.F. Sigler and A. Bodanszky, *J. Amer. Chem. Soc.*, 95 (1973) 2352–2357

AMPHOTERICINS**PC 1****Paper**

Whatman No. 1. Paper is soaked in 0.3 M potassium phosphate buffer, pH 3.0, and dried

Solvent

80% Propanol. Antibiotic spotted (1 μ g of B, 30 μ g of A) and papers equilibrated in tank with water vapor for 1 h, then developed 6–7 h. Longer periods of development resulted in destruction of antibiotics

DetectionBioautography vs. *Candida albicans***R_F**

Amphotericin A, 0.7

Amphotericin B, 0.5

Ref.W. Gold, H.A. Stout, J.F. Pagano and R. Donovick, *Antibiot. Ann.*, (1955–1956) 579–586**PC 2****Paper**

As chromin PC

Solvent

As chromin PC

Detection

As chromin PC

R_F

Amphotericin A, 0.45

Ref.

As chromin PC

TLC 1**Medium**Silica gel 60 F₂₅₄ (Merck)**Solvent**

Butanol–ethanol–acetone–32% ammonium hydroxide (2:5:1:3)

Detection

UV

R_F

Compound	R _F
Amphotericin B	0.41
Amphotericin B methyl ester	0.79
Amphotericin B ethyl ester	0.82
Amphotericin B propyl ester	0.84
Amphotericin B butyl ester	0.87

Ref.T. Bruzzese, M. Cambieri and F. Recusani, *J. Pharm. Sci.*, 64 (1975) 462–463**TLC 2****Medium**

Silica gel G

Solvents

- Ethanol–ammonia–water (8:1:1) buffered with phosphate (pH 8)
- Butan-1-ol–acetic acid–water (3:1:1) buffered with phosphate (pH 8)
- Ethanol–ammonia–dioxane–water (8:1:1:1); plate activated for 1 h at 110°C
- Butan-1-ol–pyridine–water (3:2:1); plate activated for 1 h at 110°C

Detection

A. 10% Potassium permanganate and 0.2% bromophenol blue

B. 5% Potassium permanganate

C. Orthophosphoric acid, heat for 5 min at 100°C

R_F

Solvent	Amphotericin A	
	Detection	R _F
A	A	0.33
B	A	0.33
C	B+C	0.19
D	B+C	0.56

Ref.A.H. Thomas, *Analyst (London)*, 101 (1976) 321–340**TLC 3****Medium**

Silica gel G

Solvents

- Methanol–propan-2-ol–acetic acid (90:10:1); plate activated for 1 h at 110°C
- Methanol–acetone–acetic acid (8:1:1); plate activated for 1 h at 110°C
- Ethanol–ammonia–dioxane–water (8:1:1:1); plate activated for 1 h at 110°C
- Butan-1-ol–pyridine–water (3:2:1); plate activated for 1 h at 110°C
- Butan-1-ol–methanol–ammonia–water (20:2:1:4)
- Chloroform–methanol–borate buffer (7:5:1) (pH 8.3)
- Butan-1-ol–ethanol–acetone–32% ammonia (2:5:1:3)

DetectionA. 0.2% *p*-Dimethylaminobenzaldehyde in sulfuric acid containing ferric chloride

B. 5% Potassium permanganate

C. Orthophosphoric acid, heat for 5 min at 100°C

D. Spectrodensitometric

R_F

Solvent	Detection	R _F
A	A	0.18
B	A	0.45
C	B+C	0.19
D	B+C	0.32
E	A	0.07
F	A	0.60
G	D	0.41

Ref.

As TLC 2

ELPHO

Media

A. Whatman No. 4 paper

B. Whatman No. 31 paper

Buffers

A. 0.1 M Boric acid–85% formic acid–sodium veronal–methanol–polyethylene glycol 400 (200 ml–10 ml–2.06 g–100 ml–50 ml)–water to 1000 ml, pH 2.6

B. 2 M Propionic acid–2 M formic acid–sodium veronal–ethylene glycol (100 ml–100 ml–

2.06 g–100 ml)–water to 1000 ml, pH 2.6

C. 0.1 M Boric acid–85% formic acid–glycerol (200 ml–10 ml–100 ml)–water to 1000 ml, pH 2.3

D. Methanol–glacial acetic acid–water (950:50:50), pH 3.3

Conditions

Samples of antibiotic (ca. 20 µg) in methanol soln. were placed onto Whatman No. 4 or No. 31 sheets (23 × 38 cm). Starting line was placed 12 cm from the immersion line on anode side. Developed for 4 h at 350 V

Ref.

S. Ochab, *Diss. Pharm. Pharmacol.*, 23 (1971) 205–208

Mobility

Medium	Buffer	Potential gradient (V/cm)	Current density (mA/cm)	Mobility (cm ² /V sec)	
				A	B
A	A	9.2	0.22	5.12	0.00
B	B	9.2	0.70	6.71	0.00
B	C	9.2	0.80	7.54	0.37
A	D	9.2	0.02	4.52	3.77

CCD 1

Solvent

Chloroform–methanol (with 3% calcium chloride)–water–*n*-butanol (20:20:10:1), 97 transfers

Distribution

The amphotericin A peak was distributed beyond tube 65. Amphotericin B was distributed in the lower and upper phases of tubes 40–64

Ref.

H.A.B. Linke, W. Mechlinski and C.P. Schaffner, *J. Antibiot.*, 27 (1974) 155–160

CCD 2

Solvent

Chloroform–methanol–borate buffer (pH 8.2–8.4) (2:2:1). 200 transfers

Distribution coefficient

Amphotericin B, *K* = 2.70

Ref.

As TLC 2

HPLC

Apparatus

Liquid chromatograph Model ALC/GPC 200 (Waters Assoc.)

Column

µBondapak C₁₈, 30 cm × 4 mm I.D. (Waters Assoc.)

Mobile phase

Methanol–0.005 M soln. Na salt of EDTA (8:2, v/v)

Flow-rate

2.5 ml/min

Detection

UV: 364, 384 and 407 nm

Attenuation

0.01

Type

Reversed-phase

Retention time

Amphotericin B, 4.16 min

Ref.

I. Nilsson-Ehle, T.T. Yoshikawa, J.E. Edwards, M.C. Schotz and L.B. Guze, *J. Inf. Dis.*, 135 (1977) 414–422

AMPICILLIN

PC 1

Solvents

A. *n*-Butanol–ethanol–water (4:1:5)B. *n*-Butanol–acetic acid–water (4:1:5)

Detection

Bioautography vs. *Alcaligenes viscolactis* and *Bacillus subtilis*

R_F

Solvent	R_F
A	0.43
B	0.69

Ref.

H. Troonen, P. Roelants and B. Boon, *U.S. Patent* 4,002,530, Jan. 11, 1977

PC 2

Paper

Toyo Roshi K.K. No. 50

Solvents

- A. Butanol-ethanol-water (5:1:4), upper layer
 B. Pyridine-isopropanol-water (5:65:30)

Detection

Bioautography vs. *Staphylococcus aureus* F.D.A. 209P

 R_F

Solvent	R_F
A	0.3
B	0.6

Ref.

N. Kuwana, T. Kawanmura, M. Fukumura, K. Hiratsuka-shi, *U.S. Patent* 3,716,454, Feb. 13, 1973

PC 3

Paper

Whatman No. 1, 4 × 30 cm

Solvent

n-Butanol-ethanol-water (4:1:5, v/v) organic phase (ascending) 18 h, 4°C

 R_F

0.42

Ref.

B. Gradnick and L. Fleischmann, *Arzneim.-Forsch.*, 23 (1973) 935-938

PC 4

Paper

Whatman 3MM

Solvent

Butanol-acetic acid-water (12:3:5)

Detection

Paper stained with 0.25% ninhydrin in acetone containing 0.01% isatin and 1% lutidine; heat at 100° for 10 min

 R_F

2.0. Migration distance relative to alanine taken as 1.00

Ref.

J.L. Potter, *J. Pediatr.*, 84 (1974) 250-251

TLC 1

Medium

Kieselgel G (Merck)

Solvent

Acetone-*n*-butanol-isopropanol-ethyl acetate-water (7:7:7:5:5, v/v)

 R_F

0.57

Ref.

As PC 1

TLC 2

Medium

Silica gel (Eastman Chromatogram Sheet No. 6061)

Solvent

Ethyl acetate-acetic acid-water (8:1:1)

Detection

Bioautography vs. *Bacillus subtilis* ATCC 6633

 R_F

0.1

Ref.

T. Murakawa, Y. Kono and M. Nishida, *J. Antibiot.*, 25 (1972) 421-426

TLC 3

Media

- A. Cellulose MN 300 (Macherey, Nagel and Co.)
 B. Kieselgel 60 (Merck)

Solvents

- A. *n*-Butanol-methanol-water-acetic acid (45:30:36:9)
 B. *n*-Butanol-methanol-water-ethanol-acetic acid (45:30:36:8:1) adjusted to pH 5.5 with 30% sodium hydroxide
 C. *n*-Butanol-water (45:36) adjusted to pH 4.0 with glacial acetic acid

Detection

Bioautography vs. *Sarcina lutea* ATCC 9341

 R_F

Solvent	Medium A	Medium B
A	0.90	
B	0.88	0.75
C		0.75

Ref.

F. Baldini, G. Frati, G. Pezzani and G. Ambanelli, *Ind. Conserve*, 48 (1973) 135-139

TLC 4*Medium*

Avicel

Solvent

Methanol-isobutyl alcohol-chloroform-10% hydrochloric acid (10:10:10:1)

Detection

- A. Iodine vapor
 B. 0.25% Stannous chloride soln.
 C. 0.2% *p*-Dimethylaminobenzaldehyde soln.

R_F

0.43

*Ref.*G.S. Chung and R.T. Wang, *Tai-Wan K'o Hsueh*, 27 (1973) 27-31**TLC 5***Medium*

Cellulose

*Solvent*Water satd. *n*-butyl alcohol-*tert*-amyl alcohol-water (6:1:4), pH 4.1 (6 h)*Detection*

Starch-iodine spray

R_F

0.22

*Ref.*S.C. Pan, *J. Chromatogr.*, 79 (1973) 251-255**TLC 6***Solvents*

- A. Chloroform-ethyl acetate (85:15)
 B. Chloroform-ethyl acetate (95:5)
 C. Chloroform-ethyl acetate (80:20)
 D. Acetone-acetic acid (95:5)

Medium

Silica gel

*Ref.*J. Bouchaudon, P. Le Roy and M.N. Messer, *U.S. Patent* 3,978,078, Aug. 31, 1976*R_F*

Compound

Solvent

A B C D

Trichloroethyl 6-(2-benzyl-2-hydroxy-5-oxo-4-phenyl-3-trichloroethoxythiocarbonyl-imidazolidin-1-yl)-penicillinate

0.76

Trichloroethyl-6-[N-(trichloroethoxythiocarbonyl-D- α -phenylglycyl) amino]-penicillinate

0.85

Ampicillin hydrochloride

0.15

Phenyl 6-(2-benzyl-2-hydroxy-5-oxo-4-phenyl-3-trichloroethoxythiocarbonyl-imidazolidin-1-yl)-penicillinate

0.61

Phenacyl 6-[N-trichloroethoxythiocarbonyl-D- α -phenylglycylamino]-penicillinate

0.61

6-[N-(Trichloroethoxythiocarbonyl-D- α -phenylglycyl) amino]-penicillanic acid

0.74, 0.75

Benzyl 6-(2-benzyl-2-hydroxy-5-oxo-4-phenyl-3-trichloroethoxythiocarbonyl-imidazolidin-1-yl)-penicillinate

0.80

Benzyl 6-[N-(trichloroethoxythiocarbonyl-D- α -phenylglycyl) amino]-penicillinate

0.77

ELPHO*Medium*

Paper. Whatman 3MM

Buffer

Formic acid-acetic acid-water (6:24:170)

Conditions

3000 V; potential gradient, 60 V per cm; current, 50 mA. Time, 30 min

Detection

Paper stained with 0.25% ninhydrin in acetone containing 0.01% isatin and 1% lutidine at 100° for 10 min

Mobility

0.68. Migration distance relative to alanine taken as 1.00

Ref.

As PC 4

GLC**Apparatus**

Shimadzu GC-6A gas chromatograph

Column

Glass, 2 m × 3 mm I.D., containing 1.5% (w/w) OV-17 on Chromosorb W AW DMCS (60–80 mesh)

Carrier gas

Helium

Flow-rate

65 ml/min

Temperature

Injector port and detector, 270°C. Column, 190°C for 5 min after injection, then programmed at 5°C/min to 250°C for 5 min

Detector

Flame ionization

Sample treatment

Injection of 20–30 µl of monotrimethylsilylated sample soln. made before each day's run in order to minimize column adsorption

Retention time

Compound	Retention time (min) •
5-Cholestane	
(internal standard)	9.0
Tris-TMS-ampicillin	12.0
Bis-TMS-ampicillin	15.0
Mono-TMS-ampicillin	17.0

• Estimated from drawing

Ref.

H. Wu, M. Masada and T. Uno, *J. Chromatogr.*, 137 (1977) 127–133

HPLC**Column**

1 m × 2.1 mm I.D. × 6.3 mm O.D., 316 stainless steel, C₁₈/Porasil B packing

Mobile phase

30% Methanol, 70% 0.05 M ammonium carbonate

Pressure

500 p.s.i.

Flow-rate

0.5 ml/min

Detector

UV (254 nm)

Sensitivity

0.16 a.u.f.s.

Type

Reversed-phase

Retention time

9.0 min (estimated from drawing)

Ref.

E.R. White, M.A. Carroll, J.E. Zarembo and A.D. Bender, *J. Antibiot.*, 28 (1975) 205–214

AMPICILLINS: 6-[D-(–)-α-AMINO-PHENYLACETAMIDO]-PENICILLANIC ACID *p*-(DIPROPYLSULFAMOYL)BENZOYLOXYMETHYL ESTER HYDROCHLORIDE V

ELPHO**Medium**

Paper

Buffers

A. pH 7

B. pH 2

Conditions

100 V/cm for 20 min

Mobility

Buffer	Mobility
A	Single band at origin
B	– 7 cm

Ref.

B.G. Christensen and W.J. Leanza, *U.S. Patent* 3,931,150, Jan. 6, 1976

AMPICILLINS: 6-[D-(–)-α-AZIDO-PHENYLACETAMIDO]-PENICILLANIC ACID *p*-(DIPROPYLSULFAMOYL)-BENZOYLOXYMETHYL ESTER IV

TLC**Medium**

Silica gel

Solvent

5% Ethyl acetate–methylene chloride

R_F

0.4

Ref.

B.G. Christensen and W.J. Leanza, *U.S. Patent* 3,931,150, Jan. 6, 1976

ANGOLAMYCIN**PC****Solvents**

A. 5% Aq. ammonium chloride

B. Benzene–methanol (4:1)

- C. 1 *M* phosphate buffer, pH 7.0
 D. 0.05 *N* (0.175%) ammonia satd. with methyl isobutyl ketone
 E. 1% Aq. ammonia
 F. 1% Ammonia satd. with methyl isobutyl ketone

All solvents developed ascending for 18–20 h

R_F

Solvent	R_F
A	0.75
B	0.88
C	0.26
D	0.48
E	0.49
F	0.71

Ref.

H. Koshiyama, M. Okanishi, T. Ohmori, T. Miyaki, H. Tsukiura, M. Matsuzaki and H. Kawaguchi, *J. Antibiot.*, 16 (1963) 59–66

TLC 1

Solvents

- A. Benzene–methanol (55:45)
 B. Butanol–acetic acid–water (3:1:1)

R_F

Solvent	R_F
A	0.61
B	0.33

Ref.

N. Nishimura, K. Kumagai, N. Ishida, K. Saito, F. Kato and M. Azumi, *J. Antibiot.*, 18 (1965) 251–258

TLC 2

Medium

Silica gel Merck 60 F₂₅₄ precoated

Solvents

- A. Benzene–acetone (2:3)
 B. Chloroform–methanol (10:1)
 C. *n*-Butanol–acetic acid–water (3:1:1)

Detection

Sulfuric acid — brown color

R_F

Solvent	R_F
A	0.23
B	0.42
C	0.44

Ref.

Y. Shimauchi, K. Kubo, K. Osumi, K. Okamura, Y. Fukagawa, T. Ishikura and J. Lein, *J. Antibiot.*, 31 (1978) 270–275

ANISOMYCIN

HPLC

Apparatus

Model 6000A high-pressure metering pump (Waters Assoc.)

Column

30 cm × 4 mm I.D., Bondapak C₁₈ (Waters Assoc.) (a monomolecular layer of octadecyltrichlorosilane chemically bonded to totally porous μ Porasil particles having an average diameter of 10 μ m)

Mobile phase

124 ml acetonitrile + 875 ml pH 6, 0.05 *M* potassium dihydrogen phosphate buffer soln.

Flow-rate

1.0 ml/min

Temperature

Ambient

Pressure

7000 p.s.i.

Detection

UV, 225 nm (Schoeffel Model SF 770)

Sensitivity

0.2 a.u.f.s.

Recorder

Varian Model A-25 (10 mV)

Chart speed

10 in./h

Type

Reversed-phase

Retention time

13 min (estimated from drawing)

Ref.

R.L. Kirchmeier and R.P. Upton, *Anal. Chem.*, 50 (1978) 349–351

ANTHELVEHCINS

PC

Paper

Whatman No. 1

Solvent

Methanol–0.05 *M* sodium citrate buffer, pH 5.7 (7:3) vs. paper impregnated with same buffer

Detection

Bioautography vs. *Bacillus subtilis*

R_F

System useful for distinguishing anthelvecins from netropsin and distamycin A

Ref.

G.W. Probst, M.M. Hoehn and B.L. Woods,
Antimicrob. Agents Chemother., 1965 (1966) 789–795

TLC**Medium**

Cellulose

Solvent

n-Butanol–pyridine–acetic acid–water (15:10:1:12)

Detection

- A. Color: spray dried plates with 1% soln. of Ehrlich's reagent in acetone then heat 3–5 min at 90°C to develop a blue-violet color
B. Bioautography vs. *Bacillus subtilis*

Ref.

As PC

ANTHRAMYCIN**TLC****Medium**

Silica gel

Solvent

Ethyl acetate–methanol (4:1)

 R_F

0.50

Ref.

H. Aoki, N. Miyairi, M. Ajisaka and H. Sakai, *J. Antibiot.*, 22 (1969) 201–206

ANTIAMOEBIN**TLC****Medium**

Silica gel (Eastman)

Solvent

Chloroform–methanol (6:1)

Detection

Bioautography vs. *Sarcina lutea*

 R_F

0.45

Ref.

German Patent 2,352,693, April 25, 1974

ANTIBIOTIC V**TLC****Medium**

Silica gel, aluminium backed (Merck)

Solvents

- A. Chloroform–methanol–water (65:25:4)
B. *n*-Propanol–glacial acetic acid–water (4:1:1)
C. *n*-Butanol–glacial acetic acid–water (4:1:1)

D. Chloroform–*n*-propanol–methanol–water (18:7:2:1)

E. Chloroform–benzyl alcohol–methanol (2:1:1)

Detection

See R_F

 R_F

Solvent	V	TAF *
A	56	56
B	40	40
C	23	23
D	28	28
E	57	57

* TAF = The iron(III)V complex chromatographed as a single spot in 5 solvent systems. A comparison with TAF (triacetylfulsigen) under the same conditions showed that the R_F values were identical for both compounds. Furthermore a mixture of iron(III)V and TAF was indistinguishable in all 5 solvent systems from TAF alone

Ref.

A.J. Middleton, D.S. Cole and K.D. Macdonald, *J. Antibiot.*, 31 (1978) 1110–1115

ANTICAPSIN**PC****Solvents**

- A. *n*-Butanol satd. with water
B. *n*-Butanol satd. with water + 2% *p*-toluenesulfonic acid
C. Methanol–0.1 *N* HCl (3:1)
D. Propanol–pyridine–acetic acid–water (15:13:3:12)
E. Methanol–0.05 *M* sodium citrate at pH 5.7 (70:30)

Detection

Bioautography vs. *Streptococcus pyogenes* or *Salmonella gallinarum*

 R_F

Solvent	R_F
A	0.12
B	0.58
C	0.65
D	0.60
E	0.66

Ref.

R. Shah, N. Neuss, M. Gorman and L.D. Boeck, *J. Antibiot.*, 23 (1970) 613–617

ANTIMYCINS

PC 1

Paper

Whatman No. 1

Solvent

Water-ethanol-acetone (7:2:1)

Detection

Bioautography vs. *Saccharomyces cerevisiae* Y-30

R_F

Component	R_F
A ₀	0.03
A ₁	0.16
A ₂	0.25
A ₃	0.47
A ₄	0.70
A ₅	0.87
A ₆	0.94

Ref.

D. Kluepfel, S.N. Sehgal and C. Vezina, *J. Antibiot.*, 23 (1970) 75-800

PC 2

Paper

Eaton-Dikeman No. 163

Solvent

Water-ethanol-acetone (7:2:1). Develop ascending 21-24 h at 24-25°C

Detection

Bioautography

R_F

Component	R_F
A ₁	0.30
A ₂	0.45
A ₃	0.60
A ₄	0.64

Ref.

W. Liu and K.M. Strong, *J. Amer. Chem. Soc.*, 81 (1959) 4387-4390

PC 3

Paper

Eaton-Dikeman No. 613

Solvent

Butanol-benzene (1:1)

Detection

Bioautography vs. *Saccharomyces cerevisiae*

R_F

Antimycin B 0.05 (estimated from figure)

Antimycin A 0.9 (estimated from figure)

Ref.

H.G. Schneider, G.M. Tener and F.M. Strong, *Arch. Biochem. Biophys.*, 37 (1952) 147

PC 4

Paper

Whatman No. 1

Solvents

A. Water-satd. butanol

B. Butan-1-ol-acetic acid-water (2:1:1)

C. Butan-1-ol-pyridine-water (1:0.6:1)

D. Acetone-water (1:1)

R_F

Solvent	R_F
A	0.93
B	0.98
C	0.97
D	0.86

Ref.

As Amphotericin TLC 2

TLC

Medium

Silica gel G

Solvent

Ethanol-ammonia-water (8:1:1) buffered with phosphate (pH 8)

Detection

10% Potassium permanganate and 0.2% bromophenol blue

R_F

0.72

Ref.

As Amphotericin TLC 2

ANTIMYCOIN

PC

Paper

As chromin, PC

Solvent

As chromin, PC

Detection

As chromin, PC

R_F

Antimycoin complex 0.45, 0.55

Antimycoin A, 0.55

Ref.

As chromin, PC

ANTIVIRAL SUBSTANCE (from *Penicillium cyaneofulvum* Biourge)

ELPHO

Medium

Paper

Buffer

Borate buffer, pH 8.6

Conditions

300 V, 6.8 mA, 2 h

Detection

Stain with mucicarmine (for acidic and neutral polysaccharides) or bromphenol blue (for S-H bonds)

Mobility

Two bands appeared with each dye, one towards the anode, the other towards the cathode

Ref.

P.M. Cooke and J.W. Stevenson, *Can. J. Microbiol.*, 11 (1965) 913; D. Syeklocha, P.M. Cooke and J.W. Stevenson, *Can. J. Microbiol.*, 13 (1967) 1481

APLASMOMYCIN

TLC 1

Medium

Kieselgel 60 F₂₅₄ (Merck)

Solvents

- A. Benzene-ethyl acetate (1:1)
- B. Chloroform
- C. Chloroform-methanol (9:1)

Detection

Sulfuric acid-vanillin

R_F

Solvent	R _F
A	0.72
B	0.08
C	0.97

Ref.

Y. Okami, T. Okazaki, T. Kitahara and H. Umezawa, *J. Antibiot.*, 29 (1976) 1019-1025

TLC 2

Medium

Kieselgel 60 F₂₅₄ (Merck)

Solvents

- A. Benzene-ethyl acetate (1:1)
- B. Diethyl ether

R_F

Solvent	Aplasmomycins		
	B	C	Deboro-
A	0.65	0.61	0.24
B	0.70	0.62	0.31

Ref.

K. Sato, T. Okazaki, K. Maeda and Y. Okami, *J. Antibiot.*, 31 (1978) 632-635

APRAMYCIN

PC 1

Paper

Ascending

Solvent

Chloroform-methanol-17% aq. ammonia (2:1:1) lower layer, 12 h room temp.

R_F

0.02

Ref.

T. Nara, R. Okachi, M. Yamamoto, Y. Mori, M. Sato, M. Sugimoto and Y. Shimizu, *U.S. Patent* 4,048,015, Sept. 13, 1977

PC 2

Paper

Ascending

Solvents

- A. 20% Aq. soln. of ammonium chloride
- B. Water-satd. *n*-butanol
- C. *n*-Butanol-acetic acid-water (3:1:1)
- D. Water-satd. *n*-butanol + 2% (w/v) *p*-toluene-sulfonic acid + 2% (v/v) piperidine
- E. Chloroform-methanol-17% aq. ammonia (2:1:1), lower layer

R_F

Solvent	R _F
A	0.95
B	0.00
C	0.00
D	0.00
E	0.02

Ref.

T. Nara, S. Takasawa, R. Okachi, I. Kawamoto, M. Yamamoto, S. Sato, T. Sato and A. Morikawa, *U.S. Patent* 3,939,043, Feb. 17, 1976

TLC*Medium*

Silica gel

Solvents

- A. Chloroform-methanol-17% aq. ammonia (2:1:1), upper layer
 B. Methanol-10% aq. ammonium acetate (1:1)
 C. *n*-Butanol-ethanol-chloroform-17% aq. ammonia (4:5:2:5)

R_F

Solvent	<i>R_F</i>
A	0.80
B	0.35
C	0.15

Ref.

As PC 1

AQUAYAMYCIN**TLC 1***Medium*

Silica gel G (Merck)

Solvents

- A. Water-satd. butyl acetate
 B. Ethyl acetate-chloroform (3:2)
 C. Chloroform-methanol (10:1)
 D. Benzene-methanol (10:1)
 E. Water

R_F

Solvent	<i>R_F</i>
A	0.41
B	0.09
C	0.30
D	0.06
E	0.70

Ref.

M. Sezaki, T. Hara, S. Ayukama, T. Takeuchi, Y. Okami, M. Hamada, T. Nagatsu and H. Umezawa, *J. Antibiot.*, 21 (1968) 91

TLC 2*Medium*

MN-cellulose powder 300

Solvent

Water

R_F

0.66

Ref.

As TLC 1

ELPHO*Medium*

Toyo filter paper No. 51

Buffer

Formic acid-acetic acid-water (25:75:900)

Conditions

3300 V, 1.5 mA/cm, 15 min

Ref.

As TLC 1

ARANOFILAVINS**TLC***Medium*

Kieselgel G (Merck)

Solvent

Ethyl acetate

Detection

Bioautography

R_F

Aranoflavin A, 0.70

Aranoflavin B, 0.45

Ref.

K. Mizuno, T. Ando and J. Abe, *J. Antibiot.*, 23 (1970) 493-496

ARISTEROMYCIN**PC***Paper*

Whatman No. 1

Solvents

- A. Water-satd. *n*-butanol
 B. Acetic acid-*n*-butanol-water (1:4:5)
 C. Pyridine-*n*-butanol-water (3:4:7)

R_F

Solvent	<i>R_F</i>
A	0.35
B	0.38
C	0.61

Ref.

T. Kusaka, H. Yamamoto, M. Shibata, M. Muroi, T. Kishi and K. Mizuno, *J. Antibiot.*, 21 (1968) 255

TLC*Medium*

Silica gel G (Merck)

Solvent

Ethyl acetate-methanol (2:1)

R_F

0.25

Ref.

As PC

HPLC

Apparatus

Model ALC/GPC 244 (Waters Assoc.)

Column

μ Bondapak C₁₈ (Waters Assoc.) 30 cm $\times$ 14 mm
I.D., prepacked

Mobile phases

- A. Methanol–0.001 *M* phosphate buffer (pH 5.7) (2:8)
B. Methanol–0.005 *M* citrate buffer (pH 6.0) (2:8)
C. Methanol–0.005 *M* citrate buffer (pH 5.8) (5:95)

Flow-rate

1.0 ml/min

Pressure

1500–2000 p.s.i.

Detection

UV (Model 440) 254 nm. Single peak observed under various conditions

Injector

Universal injector U6K (Waters Assoc.), 2 ml of internal volume

Type

Reversed-type

Retention time

Mobile phase	Retention time (min)
A	6.8
B	6.4
C	N.E. •

• Not eluted

Ref.

As Amipurimycin, HPLC

ARMENTOMYCIN

PC

Solvents

- A. 1-Butanol–water (84:16); 16 h
B. 1-Butanol–water (84:16) + 0.25% *p*-toluene-sulfonic acid; 16 h
C. 1-Butanol–acetic acid–water (2:1:1), 16 h
D. 2% Piperidine (v/v) in *n*-butanol–water (84:16); 16 h
E. 1-Butanol–water (4:96); 5 h
F. 1-Butanol–water (4:96) + 0.25% *p*-toluene-sulfonic acid; 5 h

Detection

Bioautography vs. *Proteus mirabilis*

R_F

Solvent	<i>R_F</i> *
A	0.25
B	0.42
C	0.65
D	0.42
E	0.85
F	0.80

* Estimated from drawing

Ref.

Netherlands Patent 66,04978, Oct. 17, 1966

ASCOCHOLIN α and β

TLC

Medium

Silica gel

Solvent

Benzene–methanol (4:1)

Detection

Bioautography vs. *Candida albicans*

R_F

Ascocholin α , 0.92

Ascocholin β , 0.67

Ref.

G. Tamura, S. Suzuki, A. Takatsuki, K. Ando and K. Arima, *J. Antibiot.*, 21 (1968) 539

ASCOCIN

PC

Solvents

- A. Water satd. butanol •
B. Butan-1-ol–acetic acid–water (2:1:1) •
C. Butan-1-ol–pyridine–water (1:0.6:1) •
D. Acetone–water (1:1) •
E. Butan-1-ol–McIlvaine's buffer (pH 6.5)
F. Methanol–ammonia–water (20:1:4)
G. Butan-1-ol–acetic acid–water (20:1:25)
• Whatman No. 1 paper specified

R_F

Solvent	<i>R_F</i>
A	0.33
B	0.88
C	0.79
D	0.43, 0.73
E	0.00, 0.55
F	0.00, 0.61, 0.92
G	0.00, 0.42, 1.0

Ref.

As Amphotericins, TLC 2

ASCOFRANOL**TLC***Medium*

Silica gel G (Merck)

*Solvent**n*-Hexane-acetone (4:1)*Detection*Spray with sulfuric acid; heat at 110°C for 5 min
— green color*R_F*

0.23

*Ref.*H. Sasaki, T. Hosokawa, M. Sawada and K. Ando, *J. Antibiot.*, 26 (1973) 676-680**ASCOFURANONE****TLC 1***Medium*

Silica gel G (Merck)

*Solvent**n*-Hexane-acetone (4:1)*Detection*Spray with sulfuric acid; heat at 110°C for 5 min
— green color*R_F*

0.42

Ref.

As Ascofranol

TLC 2*Medium*

Silica gel

Solvent

Light petroleum-acetone (3:1)

R_F

0.42

*Ref.*H. Sasaki, T. Okutomi, T. Hosokawa, Y. Nawata and K. Ando, *U.S. Patent* 3,873,529, March 25, 1975H. Sasaki, T. Okutomi, T. Hosokawa, Y. Nawata and K. Ando, *Canadian Patent* 986,865, April 6, 1976**ASPERLIN****PC***Solvents*

A. 1-Butanol-water (84:16), 16 h

B. 1-Butanol-water (84:16) + 0.25% *p*-toluene-sulfonic acid, 16 h

C. 1-Butanol-acetic acid-water (2:1:1), 16 h

D. 2% piperidine (v/v) in *n*-butanol-water (84:16), 16 h

E. 1-Butanol-water (4:96), 5 h

F. 1-Butanol-water (4:96) + 0.25% *p*-toluenesulfonic acid, 5 h*Detection*

Bioautography vs. KB cells in agar. (KB cells are human epidermoid carcinoma cells.)

R_F

Solvent	<i>R_F</i> *
A	0.80
B	0.85
C	0.88
D	0.88
E	0.86
F	0.90

* Estimated from drawing

*Ref.*A.D. Argoudelis and J.H. Coats, *U.S. Patent* 3,366,541, Jan. 30, 1968**ASPICULOMYCIN****PC***Paper*

Toyo No. 51

Solvent

Water-satd. butanol

R_F

0.37

*Ref.**Netherlands Patent* 7210880, Feb. 2, 1973**TLC***Medium*

Silica gel (Eastman Chromogram 6065)

*Solvents*A. *n*-Butanol-acetic acid-water (4:1:1)B. *n*-Propanol-pyridine-acetic acid-water (15:10:3:10)*R_F*

Solvent	<i>R_F</i>
A	0.05
B	0.40

Ref.

As PC

ATROVENTIN

TLC

Medium

Polyamide

Solvent

Acetone-acetic acid (5:1)

Detection

Visible yellow color

R_F

Compound	R_F
Atroventin	0.29
Atroventin monomethyl ether (deoxyherqueinone)	0.32

Ref.

As herqueichrysin TLC 1

AURANTININ

TLC

Medium

Silica gel (Merck)

Solvent

Chloroform-methanol (8:1)

R_F

0.35

Ref.

T. Nishikiori, R. Masuma, R. Oiwa, M. Katagiri,
J. Awaya, Y. Iwai and S. Omura, *J. Antibiot.*, 31
(1978) 525-532

AUREOFUNGIN

PC 1

Paper

Whatman No. 1

Solvents

- A. Butanol-acetic acid-water (20:1:25)
- B. *n*-Propanol-water (4:1)
- C. Pyridine-ethyl acetate-water (2.5:6:7)
- D. Pyridine-ethyl acetate-acetic acid-water
(5:5:1:3)
- E. Butanol-methanol-water (1:1:1.5)
- F. Butanol-methanol-water (4:1:2)

R_F

Solvent	R_F
A	0.64
B	0.57
C	0.19
D	0.78
E	0.94
F	0.46 (B minor), 0.65 (A major)

Ref.

G.R. Deshpande and N. Narasimhachari, *Hind. Antibiot. Bull.*, 9 (1966) 76-83

PC 2

Solvents

- A. Butan-2-ol-ethanol-water (2:2:1)
- B. Amyl alcohol-methanol-water (10:5:4)
- C. Butan-1-ol-acetic acid-water (20:1:25)
- D. Propan-1-ol-water (4:1)
- E. Ethyl acetate-pyridine-water (12:5:14)
- F. Butan-1-ol-methanol-water (2:2:3) •
- G. Butan-1-ol-methanol-water (4:1:2) •
- H. Butan-1-ol-pyridine-water (1:1:1) *
- I. Ethyl acetate-pyridine-25% acetic acid
(5:5:4)

• Whatman No. 1 paper specified

R_F

Solvent	R_F
A	0.69
B	0.66
C	0.63
D	0.59
E	0.18, 0.42
F	0.96
G	0.30, 0.67
H	0.26, 0.64
I	0.71

Ref.

As Amphotericins, TLC 2

CCD

Solvent

Chloroform-methanol-sodium acetate (pH 8.4)
(2:1:1)

Distribution coefficient

200 transfers; $K = 0.72$

Ref.

As Amphotericins, TLC 2

AUREOTHRICIN**PC***Paper*

Whatman No. 1

*Solvent*Carbon tetrachloride-acetic acid-water (8:3:1),
organic phase, 4 h*Detection*

Color: visible yellow spot

R_F

0.65

*Ref.*J.H. Marin, W.C. Groth and W.K. Hausmann,
Antimicrob. Agents Chemother., 1963 (1964) 130-
133**AVILAMYCIN****PC***Solvent*Chloroform-benzene (7:3) vs. paper satd. with
formamide as stationary phase*Detection*Bioautography vs. *Bacillus subtilis**R_F*

0.70

*Ref.*F. Buzzetti, F. Eisenberg, H.N. Grant, W.K.
Schierlen, W. Voser and H. Zahner, *Experientia*,
24 (1968) 320**TLC 1***Medium*

Silica gel G (Kieselgel G, Merck)

Solvent

Chloroform-methanol (92:8)

Detection

Concentrated sulfuric acid spray

R_F

0.62

Ref.

As PC

TLC 2*Medium*Silica gel (Merck No. 7731) (activated at 130°C
for 2 h)*Solvent*

Chloroform-methanol (92:8), 20°C

Detection

- A. Bioautography vs. *Bacillus subtilis*
- B. Spray with conc. sulfuric acid and heat for 10
min at 110°C

R_F

0.54

*Ref.*L. Ninet, F. Benazet, Y. Charpentie, M. Dubost,
J. Florent, J. Lunel, D. Mancy and J.
Preud'Homme, *Experientia*, 30 (1974) 1270-1272**AXENOMYCIN D****TLC***Medium*

Silica gel

Solvent

Ethyl acetate-isopropanol-water (100:35:5)

R_F

0.35

Ref.

Belgian Patent 766,606, Nov. 3, 1971

AXENOMYCINS**TLC***Medium*

Silica gel, buffered at pH 7

*Solvent**n*-Butanol-acetic acid-water (4:0.5:1)*R_F*

Axenomycin A, 0.49

Axenomycin B, 0.61

Ref.

Netherlands Patent 69,08269, Dec. 15, 1969

AYAMYCINS**PC***Solvent*Di-*n*-butyl ether-*sym.*-tetrachlorethane-10% aq.
sodium *o*-cresotinate (2:1:3)*R_F*

Ayamycin	<i>R_F</i> *
A ₁	0.05, 0.15
A ₂	1.0
A ₃	0.20, 0.80, 0.90

* Estimated from diagram

*Ref.*K. Sato, *J. Antibiot.*, 13 (1960) 321**AYFACTIN****TLC***Medium*

Silica gel

Solvent

Butanol-ethanol-acetone-conc. aq. ammonium hydroxide (2:5:1:3)

Detection

UV

R_F

Ayfactin	R_F
Ayfactin	0.48
Ayfactin methyl ester	0.75

Ref.

T. Bruzzese and R. Ferrari, *U.S. Patent* 3,936,526, Feb. 3, 1976

5-AZACYTIDINE**TLC****Medium**

Silica gel HF₂₅₄

Solvents

- A. Methyl ethyl ketone-acetone-water (15:5:2)
- B. Chloroform-methanol (1:1)
- C. Methanol

Detection

UV (254 nm); anisaldehyde spray; potassium permanganate-sodium metaperiodate spray

R_F

Solvent	R_F
A	0.4
B	0.2
C	0.55

Ref.

M.E. Bergy and R.R. Herr, *Antimicrob. Agents Chemother.*, 1966 (1967) 625-630

AZALOMYCIN F**PC****Solvents**

- A. 20% Acetone
- B. Water-satd. *n*-butanol

R_F

A	0.09
B	0.63

Ref.

K. Uchida and H. Zahner, *J. Antibiot.*, 28 (1975) 185-193

AZALOMYCINS**PC 1****Solvent**

Butanol-benzene-5% ammonium chloride (1:9:10)

Detection

Bioautography vs. *Sarcina lutea* or *Mycobacterium phlei*

R_F

Azalomycin B, 0.38

Ref.

M. Arai, *J. Antibiot.*, 13 (1960) 51

PC 2**Solvents**

- A. Water satd. *n*-butanol
- B. 20% Ammonium chloride
- C. 75% Phenol
- D. 50% Acetone
- E. Butanol-methanol-water-methyl orange (40:10:20:15, v/v/v/w)
- F. Butanol-methanol-water (40:10:20)
- G. 80% Benzene
- H. Water

Detection

Bioautography vs. *Candida albicans*

R_F

Solvent	Azalomycin F, R_F •
A	0.55
B	0.0
C	0.0-0.1
D	0.95
E	0.7
F	0.7
G	0.0-0.2
H	0.0

* Estimated from diagram

Ref.

As PC 2

TLC**Medium**

Silica gel G (Merck)

Solvent

Upper phase of *sec*-butanol-0.1 *M* phosphate buffer, pH 6.0 (2:1). Spot 2-5 μ g, develop for 3 h at 20°C

Detection

A. Bioautography vs. *Candida albicans* YU 1200

- B. Spray with 6 *N* sulfuric acid and heat at 120°C. Quantitative determination performed by densitometric scan using slit 0.5 mm wide × 6 mm long

R_F

Separates azalomycins F, F₃, F₄ and F₅

Ref.

M. Arai and K. Hamano, *J. Antibiot.*, 23 (1970) 107–112

ELPHO

Medium

Paper

Buffer

1/150 *M* acetate buffer, pH 4.1

Conditions

500 V/34 cm, 0.2 mA/cm, 10 h

Detection

Bioautography vs. *Candida albicans*

Mobility

Moves slightly toward anode

Ref.

As PC 1

AZDIMYCIN

TLC

Medium

ITLC-SA

Solvents

A. Chloroform–methanol (9:1)

B. Chloroform–methanol–water (1:1:1)

Detection

Bioautography vs. *Escherichia coli* ATCC 10536

R_F

Solvent	R_F
A	0.4
B	0.23

Ref.

M.W. Nimeck, E. Meyers and W.-C. Liu, *U.S. Patent* 3,898,327, Aug. 5, 1975

AZIRINOMYCIN

TLC

Medium

Avicel microcrystalline cellulose (Brinkmann)

Solvent

n-Butanol–acetic acid–water (3:1:1)

Detection

Ninhydrin, brom-thymol blue, iodine vapor or bioautography

R_F

0.82

Ref.

T.W. Miller, E.W. Tristran and F.J. Wolf, *J. Antibiot.*, 24 (1971) 48–50

ELPHO

Medium

Schleicher and Schuell SS-598 paper, 52 cm long

Buffer

0.165 *M* phosphate at pH 7.0

Conditions

600 V, 2.5 h, refrigerated

Detection

Bioautography vs. *Proteus vulgaris*

Mobility

12 cm from origin

Ref.

E.O. Stapley, D. Hendlin, M. Jackson and A.K. Miller, *J. Antibiot.*, 24 (1971) 42–47

AZOMULTIN

PC

Solvents

A. Water–satd. *n*-butanol

B. 20% aq. ammonium chloride

C. 75% aq. phenol

D. 50% aq. acetone

E. Butanol–methanol–water (4:1:2) + methyl orange

F. Butanol–methanol–water (4:1:2)

G. Benzene–methanol (4:1)

H. Water

I. *n*-Propanol–pyridine–acetic acid–water (15:10:3:12)

R_F

Solvent	R_F
A	0.10
B	0.25
C	0.85
D	0.28
E	0.58
F	0.40
G	0.00
H	0.10
I	0.60

Ref.

Japanese Patent 6073/70, Feb. 28, 1970

AZOTOBACTER CHROOCOCCUM ANTIBIOTIC

PC

Solvents

- A. Water-satd. chloroform
- B. Water-satd. *n*-butanol
- C. *n*-Butanol satd. with water + 2% piperidine
- D. *n*-Butanol-pyridine-water (10:6:10)
- E. *n*-Butanol-acetic acid-water (2:1:1)
- F. *n*-Butanol satd. with water + 2% *p*-toluene-sulfonic acid

Detection

Bioautography vs. *Candida albicans*

R_F

Solvent	R_F
A	0.54
B	0.80
C	0.86
D	0.86
E	0.88
F	0.84

Ref.

E.N. Mishustin, A.N. Narimova, Y.M. Khoklilova, S.N. Ovshstoper and F.A. Smirnova, *Microbiologia*, 38 (1969) 87-90

AZOTOMYCIN

PC

Paper

Whatman No. 1

Solvents

- A. 95% Ethanol-1 *M* ammonium acetate, pH 7.5 (75:30)
- B. Ethanol-*tert*-butanol-88% formic acid-water (60:20:5:15)
- C. 93.8% Aq. *n*-butanol-40% aq. propionic acid (1:1)

Detection

Bioautography vs. *Escherichia coli*; ninhydrin; UV

R_F

Solvent A, 0.62 (estimated from diagram)

Ref.

R.W. Brockman, R.F. Pittillo, S. Shaddix and D.L. Hill, *Antimicrob. Agents Chemother.*, 1969 (1970) 56-62

BACIMETHRIN

PC

Solvents

- A. *n*-Butanol-acetic acid-water (2:1:1)
- B. Water-satd. *n*-butanol
- C. Water-satd. ethyl acetate

Detection

Bioautography; fluorescein reaction

R_F

Solvent	R_F
A	0.52
B	0.38
C	0.27

Ref.

F. Tanaka, N. Tanaka, H. Yonehara and H. Umezawa, *J. Antibiot.*, 15 (1962) 191-196

BACITRACIN

PC

Paper

Whatman No. 4

Solvent

Acetic acid-*n*-butanol-water (1:4:5)

Detection

After treatment with 0.1% ninhydrin in *n*-butanol the components were localized by examination in visible and UV light

R_F

ca. 0.6

Ref.

J. Prath, *Acta Chem. Scand.*, 6 (1952) 1237-1248

TLC 1

Medium

Silica gel G

Solvent

n-Butanol-acetic acid-water (4:1:2)

Detection

- A. Heat TLC plate at 110°C, 1 h and observe under UV light
- B. Ninhydrin (0.0359 g in 10 ml *n*-butanol + 0.4 ml acetic acid)

R_F

Bacitracin A, 0.35

Bacitracin B, 0.22

Ref.

P.A. Nussbaumer, *Pharm. Acta Helv.*, 40 (1965) 210-218

TLC 2

Media

- A. Cellulose MN 300 (Macherey, Nagel & Co.)
 B. Kieselgel 60 (Merck)

Solvents

- A. *n*-Butanol-methanol-water-acetic acid (45:30:36:9)
 B. *n*-Butanol-methanol-water-ethanol-acetic acid (45:30:36:8:1) adjusted to pH 5.5 with 30% sodium hydroxide
 C. *n*-Butanol-water (45:36) adjusted to pH 4.0 with glacial acetic acid

Detection

Bioautography vs. *Sarcina lutea* ATCC 9341

 R_F

Solvent	Medium A	Medium B
A	0.92	
B	0.89	
C		0.12

Ref

As Ampicillin, TLC 1

TLC 3

Medium

Silica gel 60 (Merck)

Solvent

Ethanol-water-ammonia (8:8:1)

Detection

Bioautography vs. *Micrococcus flavis* ATCC 10240

 R_F

0.45

Ref.

R. Bossuyt, R. Van Tenterghem and G. Waes, *J. Chromatogr.*, 124 (1975) 37-42

ELPHO 1

Medium

Paper; Munktel No. 20

Buffers

- A. 0.1 M acetate, pH 4.8
 B. 0.1 M veronal, pH 8.6

Conditions

8 V/cm, 5 h

Detection

Ninhydrin; bioautography

Mobility

One spot with both buffers. With buffer A, bacitracin moves toward cathode

Ref.

As PC

ELPHO 2

Medium

Acrylamide gel

Buffer

Acrylamide gel:

Buffer sol., pH 4.6-monomer soln.-water-ammonium persulphate soln. 0.14% (1:2:1:4)

Buffer soln.:

1 N potassium hydroxide-acetic acid-tetramethylethylenediamine (48:21.5:4.6) + water to 100 ml

Monomer soln.:

Acrylamide-methylenebisacrylamide (60 g:1.0 g) + water to 100 ml

Conditions

The gel soln. is pipetted into glass tubes, 65 × 3 mm, sealed at one end by parafilm, leaving sufficient space at the drop to add a few drops of water to ensure a flat even surface on the catalysed gel surface. The tubes are allowed to stand at 28°C for 1 h and after removal of the parafilm are ready for use

Detection

Naphthalene black 0.1% in 7% acetic acid

Mobility

Light blue band

Ref.

R.G. Coombe, *Aust. J. Pharm. Sci.*, NS1 (1972) 6-8

HPLC 1

Apparatus

Laboratory Data Control modular liquid chromatograph equipped with a 254 nm UV monitor (Model 1285) together with two Milton Roy mini pumps equipped with a pulse dampener (LDC, Model 709)

Column

Stainless-steel, 1000 × 2.1 mm (DuPont) first rinsed with tetrahydrofuran followed by vigorous cleaning of the inside of the tubing with a cotton string pre-soaked with THF. Chloroform is drawn through the column and the column dried under a stream of dry nitrogen. Bondapak C₁₈ Corasil packing

Mobile phase

- A. Absolute methanol (50 ml) and water (750 ml) poured into a 1000-ml graduated cylinder, then 200 ml of 0.1 M potassium buffer, pH 4.5, added and mixed
 B. Absolute methanol (400 ml), acetonitrile (200 ml) and water (200 ml), poured into a 1000-ml graduated cylinder, after mixing, 200 ml of 0.1 M phosphate buffer, pH 4.5, added and mixed

Flow-rate

Column, 0.90 ml/min, reference side of UV
Monitor, 0.2 ml/min

Temperature

Ambient

Chart speed

0.25 in./min

Pressure

1000 p.s.i. to 1500 p.s.i.

Detection

UV

Retention time

Peak No.	Retention time (min)	Retention volume (ml)
1	1.7	1.5
2	1.8	1.6
3	2.2	2.0
4	2.7	2.4
5 (F ₁)	4.3	3.8
6 (F ₂)	6.6	5.9
7	10.3	9.3
8	12.5	11.3
9	13.8	12.5
10	14.6	13.1
11	15.7	14.1
12	17.1	15.4
13	18.1	16.3
14	19.1	17.2
15	23.4	21.1
16	25.6	23.0
17	27.5	24.8
18	29.8	26.9
19 (B)	31.3	28.1
20 (A)	33.2	29.9
21	35.2	31.7
22 (C)	36.7	33.1

Ref.

K. Tsuji, J.H. Robertson and J.A. Bach, *J. Chromatogr.*, 99 (1974) 597–608

HPLC 2**Apparatus**

Laboratory Data Control modular liquid chromatograph, equipped with a high-pressure Milton Roy Minipump with a pulse dampener (model 709)

Column

Stainless-steel (30 cm × 4.6 mm I.D.), prepacked with μ Bondapak C₁₈ (Water Assoc.) attached to a septumless injector valve (Micromeritics) and to the UV monitor

Mobile phase

Convex gradient from 5% methanol to 50% methanol and 20% acetonitrile, both with 10 mM phosphate buffer, pH 4.5, in 1 h

Flow-rate

1.0 ml/min

Temperature

Ambient

Chart speed

6.4 mm/min

Pressure

7.0 atm (1000 p.s.i.)

Detection

254 nm UV monitor (Model 1285)

Retention time

Component	Retention time (min) *
A	36
B ₁	34
B ₂	35
C	38.5
D	23
E	30, 31.5
F ₁	48
F ₂	46
G	43
X	13

* Estimated from drawing

Ref.

K. Tsuji and J.H. Robertson, *J. Chromatogr.*, 112 (1975) 663–672

BAMICETIN**PC****Solvent**

As amicetin, PC 2

Detection

As amicetin, PC 2

R_F

0.22

Ref.

As amicetin, PC 2

TLC**Media**

A. Silica gel

B. Alumina

Solvents

A. *n*-Butanol–acetic acid–water (3:1:1)

B. Ethanol–28% ammonium hydroxide–water (8:1:1)

C. 80% Methanol

R_F

Solvent	Medium	R_F
A	A	0.36
B	A	0.67
C	B	0.36

Ref.

M. Konishi, M. Kameda, H. Tsukiura, H. Yamamoto, T. Hoshitani, T. Miyaki, K. Fujisawa, H. Koshiyama and H. Kawaguchi, *188th Meeting, JARA, July 27, 1973*

BANDAMYCIN A

PC

Solvents

- A. Water-satd. *n*-butanol
 - B. 3% Aq. ammonium chloride
 - C. Phenol–water (4:1)
 - D. Acetone–water (1:1)
 - E. *n*-Butanol–methanol–water (4:1:2)
 - F. Benzene–methanol (4:1)
 - G. *n*-Butanol–acetic acid–water (74:3:25)
- All solvents were run ascending

 R_F

Solvent	R_F
A	0.96
B	0.89
C	0.94
D	0.96
E	0.96
F	0.96
G	0.95

Ref.

S. Kondo, J. Marie, J. Sakamoto and H. Yumoto, *J. Antibiot.*, 14 (1961) 365–366

BANDAMYCIN B

PC

Solvents

- A. Water-satd. *n*-butanol
- B. 15% Aq. ammonium chloride
- C. Phenol–water (4:1)
- D. Acetone–water (1:1)
- E. *n*-Butanol–methanol–water (4:1:2)
- F. Benzene–methanol (4:1)
- G. *tert*.-Butanol–acetic acid–water (74:3:25)

H. Diethyl ether-satd. water

I. Water

All solvents were run ascending

 R_F

Solvent	R_F
A	0.97
B	0.76
C	0.97
D	0.95
E	0.97
F	0.94
G	0.95
H	0.95
I	0.77

Ref.

S. Kondo, T. Miyakawa, H. Yumoto, M. Sezaki, M. Shimura, K. Sato and T. Hara, *J. Antibiot.*, 15 (1962) 157–159

BENZATHINE PENICILLIN G

TLC

Medium

Avicel

Solvent

Methanol–isobutanol–chloroform–10% hydrochloric acid (10:10:10:1)

Detection

- A. Iodine vapor
- B. 0.25% Stannous chloride soln.
- C. 0.2% *p*-Dimethylaminobenzaldehyde soln.

 R_F

0.86

Ref.

As Ampicillin, TLC 1

BERNINAMYCIN

PC

Solvents

- A. 1-Butanol–water (84:16), 16 h
- B. 1-Butanol–water (84:16) + 0.25% *p*-toluene-sulfonic acid, 16 h
- C. 1-Butanol–acetic acid–water (2:1:1), 16 h
- D. 2% Piperidine (v/v) in 1-butanol–water (84:16) 16 h
- E. 1-Butanol–water (4:96), 5 h
- F. 1-Butanol–water (4:96) + 0.25% *p*-toluene-sulfonic acid, 5 h
- G. 0.1 *M* Potassium phosphate buffer, pH 7.0
- H. 0.075 *N* NH_4OH satd. with methyl isobutyl ketone

- I. Benzene-methanol-water (1:1:2)
 J. 1-Butanol satd. with water + 2% *p*-toluene-sulfonic acid
 K. Methanol-15% sodium chloride (4:1) (strips impregnated beforehand with 0.1 *M* Na₂SO₄ and dried)

 R_F

Solvent	R_F
A	0, 0.77-0.92
B	0, 0.8-0.95
C	0, 0.9
D	No zone
E	0.05; 0.05-0.2 (trace)
F	0.05; 0.05-0.2 (trace)
G	0.03
H	0.03
I	0.0-0.1
J	0.88
K	0.62-0.86

Ref.

M.E. Bergy, J.H. Coats, F. Reusser, *U.S. Patent* 3,689,639, Sept. 5, 1972

TLC

Medium

Silica gel. The thin-layer plates are prepared from silica gel HF₂₅₄ (60 g, E. Merck AG) suspended in 0.2 *M* potassium dihydrophosphate (50 ml), 0.2 *M* disodium acid phosphate (50 ml) and methanol (20 ml). The plates are air dried then activated at 120°C for 2 h

Solvent

Methylene chloride-methanol (95:5)

Detection

UV absorption with 254 nm light; KMnO₄-NaIO₄ spray reagent

 R_F

0.3-0.4

Ref.

As PC

BERROMYCIN

PC

Paper

Circular. Paper treated with 0.1 *M* soln. of trilon

Solvent

Acetone-benzene-water (6:2:0.5)

 R_F

Component	R_F
A	0.87
B	0.57
C	0.40

Ref.

M.K. Kudinova, V.N. Borisova, N.M. Petukhova, M.G. Brazhnikova, *Antibiotiki*, 17 (1972) 690-693

TLC

Medium

Silica gel

Solvent

Chloroform-methanol-benzene (7:3:2)

 R_F

Separates components A, B, C

Ref.

As PC

CCD

Solvent

Ethyl acetate-butanol-0.005 *M* aq. soln. of trilon (12.5:3:12.5). 100 tubes

Distribution coefficients

A = 7.0; B = 3.7; C = 1.4

Ref.

As PC

BESTATIN

TLC

Medium

Silica gel G

Solvents

A. Butyl acetate-butanol-acetic acid-water (4:4:1:1)

B. Butyl acetate-butanol-acetic acid-water (6:4:1:1)

 R_F

Solvent	R_F
A	0.24
B	0.13

Ref.

H. Umezawa, T. Aoyagi, T. Takeuchi, M. Hamada and Y. Okami, *U.S. Patent* 4,052,449, Oct. 4, 1977

ELPHO**Medium**

High voltage

Buffer

Formic acid–acetic acid–water (25:75:900)

Conditions

3500 V, 15 min

Mobility R_M 0.68**Ref.**

As TLC

BIKAVERIN**TLC****Medium**

Silica gel G

Solvent

Chloroform–methanol–acetic acid (94:1:5)

Detection

Single violet zone

 R_F

0.45

Ref.D. Brewer, G.P. Arsenault, J.L.C. Wright and L.C. Vining, *J. Antibiot.*, 26 (1973) 778–781**BLASTICIDIN S****PC****Paper**

Toyo No. 131, 1 × 16 cm

Solvents

- A. Water–satd. butanol
- B. 3% Aq. ammonium chloride
- C. 80% Aq. phenol
- D. 50% Aq. acetone
- E. Butanol–methanol–water (40:10:20) + 1.5 g methyl orange
- F. Butanol–methanol–water (4:1:2)
- G. Benzene–methanol (4:1)

 R_F

Solvent	R_F
A	0.30
B	0.00
C	1.00
D	0.80
E	0.75
F	0.70
G	0.00, 0.35

Ref.K. Funkunaga, T. Misato, I. Ishii and M. Asakawa, *Bull. Agric. Chem. Soc. Japan*, 19 (1955) 181–188**BLASTICIDIN S****PC****Paper**

Toyo Roshi No. 51

Solvents*n*-Butanol–acetic acid–water (2:1:1), ascending**Detection**

- A. UV light at 253.7 nm
- B. Spray with alkaline ferricyanide–nitroprusside reagent; blasticidin S appears as a reddish-brown spot
- C. Bioautography vs. *Bacillus cereus* or *Piricularia oryzae*

 R_F

0.40

Ref.H. Yonehara and N. Otake, *Antimicrob. Agents Chemother.*, 1965 (1966) 855–857**TLC****Medium**

Silica gel (Kieselgel G)

Solvent

Chloroform–methanol–ammoniacal water (2:1:1), upper phase

Detection

As PC, B

Ref.

As PC

HPLC**Apparatus**

Model ALC/GPC 244 (Waters Assoc.)

Column μ Bondapak C₁₈ (Waters Assoc.) 30 cm × 14 mm I.D., prepacked**Mobile phase**

- A. Methanol–0.01 M phosphate buffer (pH 5.7) (2:8)
- B. Methanol–0.005 M citrate buffer (pH 6.0) (2:8)
- C. Methanol–0.005 M citrate buffer (pH 5.8) (5:95)
- D. 0.005 M citrate buffer (pH 5.8)

Flow-rate

1.0 ml/min

Pressure

1500–2000 p.s.i.

Detection

UV Model 440, 254 nm. Single peak observed under various conditions

Injector

Universal injector U6K (Waters Assoc.), 2 ml of internal volume

Type

Reversed-phase

Retention time

Mobile phase	Retention time (min)
A	8.8
B	B.P. *
C	B.P.
D	B.P.

* B.P. = broad peak

Ref.

As Amipurimycin, HPLC

BLEOMYCIN A₂**PC****Paper**

Toyo No. 51, ascending

Solvent

10% Ammonium chloride

R_F

0.85

Ref.

H. Umezawa, T. Takita, A. Fujii and H. Ito, *U.S. Patent* 3,960,834, June 1, 1976

TLC**Medium**

Silica gel G

Solvents

A. Methanol-10% ammonium acetate (1:1)

B. Methanol-10% ammonium acetate-10% ammonia (10:9:1)

R_F

Solvent	R _F
A	0.41
B	0.44

Ref.

As PC

**BLEOMYCIN A₂, DEMETHYL-
(See demethylbleomycin A₂)****BLEOMYCINIC ACID****PC****Paper**

Toyo No. 51

Solvent

10% Ammonium chloride

R_F

0.86

Ref.

H. Umezawa, Y. Takahashi, T. Shirai and A. Fujii, *U.S. Patent* 3,932,374, Jan. 13, 1976

ELPHO**Medium**

Paper

Buffer

Formic acid-acetic acid-water (25:75:900)

Mobility

R_M 0.65

Ref.

As PC

BLEOMYCINS

(cf., YA-56 complex, PC 2 and TLC)

PC 1**Solvent**

10% aq. ammonium chloride

R_F

Bleomycin-copper chelates	R _F
Cu-At 1	0.92
Cu-Bt 1	0.71
Cu-At 2	0.83
Cu-Bt 2	0.72
Cu-At 3	0.85
Cu-At 4	0.85
Cu-Bt 3	0.71
Cu-At 5	0.86
Cu-Bt 4	0.72
Cu-Bt 5	0.70
Cu-At 6	0.88

Ref.

H. Umezawa, Y. Suhara, T. Takita and K. Maeda, *J. Antibiot.*, 19 (1966) 210-215

PC 2**Solvent**

As PC 1

R_F

Bleomycin A, 0.88-0.94

Ref.

French Patent 3.978M, June 3, 1964

PC 3

Paper

Toyo No. 51

Solvent

As PC 1; ascending

Detection

Bioautography vs. *Mycobacterium phlei*

 R_F

Bleomycin A, 0.88–0.99

Bleomycin B, 0.66–0.70

Ref.

H. Umezawa, K. Maeda, T. Takeuchi and Y. Okami, *J. Antibiot.*, 19 (1966) 200–209

PC 4

Paper

Toyo No. 51

Solvent

10% Ammonium chloride

Detection

Bioautography vs. *Bacillus subtilis*

The chromatographic behavior of B'_1 was similar to B_1 , but B'_1 gave a negative Sakaguchi reaction, while B_1 is positive

 R_F

Component	R_F
Demethyl A_2	0.71
A'_2 -a	0.82
A'_2 -b	0.82
A'_2 -c	0.81
B'_1	0.73
B_3	0.52
- A_3 •	0.85
- B_1 •	0.70
- B_5 *	0.70

* Cited from H. Umezawa, Y. Suhara, T. Takita and K. Maeda, *J. Antibiot., Ser. A*, 19 (1966) 210–215

Ref.

A. Fujii, T. Takita, K. Maeda, H. Umezawa, *J. Antibiot.*, 26 (1973) 396–397

TLC 1

Medium

Silica gel G (Merck)

Solvent

10% Ammonium acetate-methanol (1:1)

 R_F

Bleomycin-copper chelates	R_F
Cu-At 1	0.74
Cu-Bt 1	0.75
Cu-At 2	0.40
Cu-Bt 2	0.68
Cu-At 3	0.13
Cu-At 4	0.49
Cu-Bt 3	0.68
Cu-At 5	0.51
Cu-Bt 4	0.60
Cu-Bt 5	0.52
Cu-At 6	0.30

Ref.

As PC 1

TLC 2

Medium

Silica gel

Solvents

- Chloroform-methanol-17% aq. ammonia (2:1:1) upper layer
- 10% Ammonium acetate-methanol (1:1)
- Methanol-10% ammonium acetate-10% aq. ammonia (10:9:1)
- 0.05 M Citric acid buffer soln. of pH 6.5

 R_F

Solvent	Bleomycin			
	A_2	A_5	B_2	B_4
A	0.28	0.05	0.54	0.14
B	0.46	0.60	0.80	0.75
C	0.55	0.25	0.78	0.68
D	0.08	0.08	0.10	0.10

Ref.

T. Nara, S. Takasawa, R. Okachi, I. Kawamoto, M. Kohagura and I. Takahashi, *U.S. Patent* 3,922,343, Nov. 25, 1975

TLC 3

Media

- Silica gel G
- Avicel SF

Solvents

- Methanol-10% ammonium acetate (1:1)
- Methanol-10% ammonium acetate-10% ammonium hydroxide (10:9:1)
- n*-Propanol-pyridine-acetic acid-water (15:10:3:12)

Detection

Bioautography vs. *Bacillus subtilis*

The chromatographic behavior of B₁' was similar to B₁, but B₁' gave a negative Sakaguchi reaction, while B₁ is positive

R_F

Component	Medium	Solvent		
		A	B	C
Demethyl				
A ₂	A	0.80	0.78	
	B			0.72
A' ₂ -a	A	0.70	0.65	
	B			0.49
A' ₂ -b	A	0.70	0.65	
	B			0.46
A' ₂ -c	A	0.71	0.82	
	B			0.53
B' ₁	A	0.80	0.76	
	B			0.55
B ₃	A	0.47	0.39	
	B			0.51
A ₃ •	A	0.13		
B ₁ ★	A	0.77		
B ₅ •	A	0.52		

• Cited from H. Umezawa, Y. Suhara, T. Takita and K. Maeda, *J. Antibiot., Ser. A*, 19 (1966) 210–215

Ref.

As PC 4

TLC 4**Medium**

Silica gel

Solvents

- Chloroform–methanol–17% ammonia water (2:1:1) (upper layer)
- 10% Ammonium acetate–methanol (1:1)
- Methanol–10% ammonium acetate–10% ammonia water (10:9:1)
- 0.05 M Citrate buffer (pH 6.9)

Detection

Bioautography vs. *Bacillus subtilis* KY 4273

Ref.

S. Takasawa, I. Kawamoto, S. Sato, R. Yahashi, R. Okachi, M. Yamamoto, T. Sato and T. Nara, *J. Antibiot.*, 28 (1975) 662–667

R_F

Solvent	Bleomycin			
	A ₂	A ₅	B ₂	B ₄
A	0.27	0.05	0.45	0.10
B	0.45	0.60	0.80	0.75
C	0.52	0.28	0.88	0.68
D	0.03	0.05	0.03	0.03

TLC 5**Medium**

Silica gel

Solvent

Chloroform–10% ammonium acetate–10% ammonium hydroxide (10:9:1)

R_F

Compound	R _F
Bleomycin B ₂	0.66, 0.61
Bleomycin B ₄	0.50
YA-56X	0.68
YA-56Y	0.71
3-N,N-Dimethylamino-propylamino-bleomycin	0.44

Ref.

H. Umezawa, Y. Yakita, Y. Muraoka and A. Fujii, *U.S. Patent* 3,953,594, April 27, 1976

TLC 6**Medium**

Avicel

Solvent

Propanol–pyridine–acetic acid–water (15:10:3:12)

R_F

Compound	R _F
Bleomycin B ₂	0.56, 0.48
Bleomycin B ₄	0.54
YA-56X	0.56
YA-56Y	0.66
3-N,N-Dimethylamino-propylamino-bleomycin	0.55

Ref.

Same as TLC 5

TLC 7

Medium

Silica gel

Solvents

- A. Chloroform-methanol-17% ammonia water (2:1:1) upper layer
 B. 10% Ammonium acetate-methanol (1:1)
 C. Methanol-10% ammonium acetate-10% ammonia water (10:9:1)
 D. 0.05 M Citrate buffer (pH 6.9)

Detection

Bioautography vs. *Bacillus subtilis* KY 4273 R_F

Solvent	Bleomycin			
	A ₂	A ₃	B ₂	B ₄
A	0.28	0.05	0.54	0.14
B	0.46	0.60	0.80	0.75
C	0.55	0.25	0.78	0.68
D	0.08	0.08	0.10	0.10

Ref.

S. Takasawa, I. Kawamoto, R. Okachi, M. Kohakura, R. Yahashi and T. Nara, *J. Antibiot.*, 28 (1975) 366-371 (cf., TLC 2)

ELPHO 1

Buffer

Formic acid-acetic acid-water (25:75:900), pH 1.8

Conditions

2000 V at 25 mA

Mobility

Bleomycin-copper chelates	Relative mobility
Cu-At 1	0.66
Cu-Bt 1	0.58
Cu-At 2	0.79
Cu-Bt 2	0.74
Cu-At 3	0.91
Cu-At 4	0.92
Cu-Bt 3	0.80
Cu-At 5	0.84
Cu-Bt 4	0.78
Cu-Bt 5	0.86
Cu-At 6	0.84

Ref.

As PC 1

ELPHO 2

Medium

Paper, Toyo No. 51

Buffer

Formic acid-acetic acid-water (25:75:900)

Conditions

3000 V, 40 min, R_M (Alanine: 1.0)

Detection

A. UV

B. Coloration with rubeanic acid

The chromatographic behavior of B₁' was similar to B₁, but B₁' gave a negative Sakaguchi reaction, while B₁ is positive

Mobility

Component	R_M
Demethyl A ₂	0.64
A ₂ '-a	0.88
A ₂ '-b	0.88
A ₂ '-c	0.84
B ₁ '	0.65
B ₃	0.94
A ₃	—
B ₁	—
B ₅	—

Ref.

As PC 4

BLUENSIN

 R_F

Solvent	R_F
A	0.0 -0.15
B	0.0 -0.15
C	0.05-0.25
D	0.0 -0.15
E	0.2 -0.9
F	0.05-0.25
G	0.05-0.25
H	0.4 -0.65

PC

Solvents

- A. 1-Butanol-water (84:16)
 B. 1-Butanol-water (84:16) + 0.25% *p*-toluene-sulfonic acid
 C. 1-Butanol-acetic acid-water (2:1:1)

- D. 1-Butanol–water (84:16) + 2% piperidine
 E. 1-Butanol–water (96:4)
 F. 1-Butanol–water (96:4) + 0.25% *p*-toluenesulfonic acid
 G. 1-Butanol–water (84:16) + 2% *p*-toluenesulfonic acid
 H. Methanol–water containing 3% sodium chloride (80:20)

Detection

Bioautography vs. *Bacillus subtilis*

Ref.

T.E. Eble and L.E. Johnson, *U.S. Patent* 3,880,827, April 29, 1975

BLUENSOMYCIN**PC****Paper**

Ascending

Solvent

Chloroform–methanol–17% aq. ammonia (2:1:1) lower layer (developed at room temp. 12 h)

 R_F

0.01

Ref.

As Apramycin, PC 1

ELPHO**Medium**

Whatman No. 4 filter paper

Buffers

- A. 2 *M* Formic acid–0.1 *M* *p*-toluenesulfonic acid–*n*-propanol (500:50:50) in 1000 ml water, pH 1.8
 B. 2 *M* Formic acid–0.1 *M* *p*-toluenesulfonic acid–*n*-propanol (400:50:50) in 1000 ml water, pH 1.9
 C. 2 *M* Formic acid–0.1 *M* *p*-toluenesulfonic acid (400:50) in 1000 ml water, pH 1.9
 D. 1 *M* Ammonium hydroxide–1 *M* sodium hydroxide–0.1 *M* *p*-toluenesulfonic acid–*n*-propanol (200:5:100:100) in 1000 ml water, pH 10.8
 E. 1 *M* Ammonium hydroxide–1 *M* sodium hydroxide (200:2.5) in 1000 ml water, pH 11.5
 F. 50 ml 1 *M* Sodium hydroxide in 1000 ml water, pH 12.2

Conditions

The electropherograms were developed at 350 V for 1–4 h (depending on the electrolyte)

Mobility

Buffer	Toward cathode (cm ² /V sec)
A	22.63
B	19.30
C	21.76
D	8.59
E	10.10
F	9.34

Ref.

S. Ochab, *Pol. J. Pharmacol. Pharm.*, 23 (1973) 105–108

CCD**Solvent**

1-Butanol–water

Distribution coefficient

0.38 (*p*-toluenesulfonate salt)

Ref.

M.E. Bergy, T.E. Eble, R.R. Herr, C.M. Large and B. Bannister, *Antimicrob. Agents Chemother.*, 1962 (1963) 614

BOSEIMYCIN**PC 1****Solvents**

- A. Propanol–pyridine–acetic acid–water (15:10:3:12)
 B. Pyridine–acetic acid–water (50:35:15)
 C. *n*-Butanol satd. with water + 0.25% *p*-toluenesulfonic acid
 D. *n*-Butanol satd. with water + 2% *p*-toluenesulfonic acid
 E. *n*-Butanol–acetic acid–water (2:1:1)

 R_F

Solvent	R_F
A	0.14
B	0.25
C	0.02
D	0.08
E	0.07

Ref.

R.K. Sinha, *J. Antibiot.*, 23 (1970) 360–364

PC 2**Solvent**

Wet *n*-butanol containing 2% (w/v) *p*-toluenesulfonic acid

R_F
0.08

Ref.

K. Arima, T. Kawamura and T. Beppu, *J. Antibiot.*, 25 (1972) 387–392

TLC 1

Medium

Silica gel G

Solvents

A. As PC 1, A

B. As PC 1, B

C. As PC 1, E

R_F

Solvent	R_F
A	0.75
B	0.68
C	0.09

Ref.

As PC 1

TLC 2

Medium

Silica gel G

Solvent

n-Propanol–pyridine–acetic acid–water (15 : 10 : 3 : 12)

R_F

0.75

Ref.

As PC 2

ELPHO

Medium

Paper

Buffers

A. 0.1 *M* acetate, pH 3.9

B. 0.1 *M* phosphate, pH 7.2

Conditions

2 h

Mobility

(as boseimycin HCl)

Buffer A, 2.4 cm towards cathode

Buffer B, 3.8 cm towards cathode

Ref.

As PC 1

BOTRIC ACID

TLC

Medium

Kieselgel G (Merck)

Solvents

A. Wet ethyl acetate

B. Methanol–water (4 : 1)

C. Chloroform–methanol (10 : 1)

Detection

Spraying of 1% aq. potassium permanganate soln.

R_F

Solvent	R_F
A	0.76
B	0.80
C	0.35

Ref.

K. Mizumo, J. Miyazawa and M. Takeda, *J. Antibiot.*, 27 (1974) 552–554

BOTTROMYCIN, AMETHO- (See amethobottromycin)

BOTTROMYCINS

TLC

Medium

Silica gel G

Solvent

Butanol–acetic acid–water (100 : 12 : 10), upper phase

Detection

Spray with 0.1% bromophenol blue

R_F

Bottromycin A, 0.69

Bottromycin B, 0.64

Ref.

S. Nakamura, T. Chikaike, K. Karasawa, N. Tanaka, H. Yonehara and H. Umezawa, *J. Antibiot.*, 18 (1965) 47–52

BRAMYCIN

PC

Solvents

A. 30% Acetone

B. *n*-Hexane–benzene–ethyl acetate (1 : 1 : 1)

Detection

Bioautography vs. *Piricularia oryzae*

R_F

Solvent	R_F
A	0.32
B	0.78

Ref.

Y. Sakagami, H. Sekine, S. Yamabayashi, Y. Kitaura and A. Ueda, *J. Antibiot.*, 19 (1966) 99-103

TLC*Medium*

Silica gel G

Solvents

A. Methanol-ethyl acetate (100:15)

B. Ethanol-water (4:1)

R_F

Solvent	<i>R_F</i>
A	0.70
B	0.69

Ref.

As PC

BRESEIN**PC***Solvents*

A. *tert.*-Butanol-acetic acid-water (74:3:25)

B. Acetic acid-ethyl acetate-water (6:88:6)

C. Methanol-acetic acid-water (25:3:72)

D. Acetone-acetic acid-water (20:6:74)

E. Acetone-water (70:30)

F. *n*-Butanol-acetic acid-water (79:6:15)

G. *tert.*-Butanol-water (80:20)

H. As D, but (80:3:17)

I. As A, but (65:3:32)

J. *n*-Butanol-ethanol-acetic acid-water (25:25:3:47)

K. As A, but (70:6:24)

L. As F, but (4:1:5)

M. As D, but (60:13:17)

N. As F, but (4:1:1)

O. Phenol-*n*-butanol (1:1)

P. As O, but (1:2)

Q. As O, but (1:3)

R. As O, but (4:1)

S. Phenol-diethyl ether (4:1)

T. Phenol-methanol (4:1)

R_F

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.85	K	0.94
B	0.90	L	0.95
C	0.68	M	0.95
D	0.81	N	0.91

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
E	0.95	O	0.88
F	0.90	P	0.86
G	0.83	Q	0.90
H	0.19	R	0.78
I	0.95	S	0.75
J	0.95	T	0.84

Ref.

R.A. Radzhapov, G.G. Zharikova, A.B. Silaev and G.S. Katrukha, *Vestn. Mosk. Univ., Biol., Pochvoved.*, 1968, 23 (4), 42-7; *C.A.*, 69 (1968) 109767f

BREVICIDS**TLC***Medium*

Kieselgel H (Merck)

Solvent

Ethyl acetate-chloroform-methanol (2:6:2)

Detection

Spray with 5% silver nitrate soln. in ethanol-water (9:1)

R_F

Brevicid X	Brevicid Y ₁	Brevicid Y ₂
0.75 (intense yellow)	0.64 (brownish yellow)	0.58 (dark brown)

Ref.

J. Zarnack, H. Tonjes and H. Potter, *Pharmazie*, 26 (1971) 503-504

BULGERIN**PC***Paper*

Toyo Roshi No. 51

Solvents

A. *n*-Butanol-acetic acid-water (4:1:2)

B. *n*-Propanol-pyridine-acetic acid-water (15:10:3:12)

R_F

Solvent	<i>R_F</i>
A	0.20
B	0.60

Ref.

J. Shoji, R. Sakazaki, M. Mayama, Y. Kawamura and Y. Yasuda, *J. Antibiot.*, 23 (1970) 295–299

TLC*Medium*

Silica gel GF

Solvents

- A. Chloroform–methanol–17% aq. ammonia (2:1:1), upper layer
B. *n*-Butanol–ethanol–0.1 *N* hydrochloric acid (1:1:1)

R_F

Solvent	<i>R_F</i>
A	0.80
B	0.50

Ref.

As PC

BUTENOIC ACID, L-*trans*-2-AMINO-4-(2-AMINOETHOXY)-3-

[See L-*trans*-2-amino-4-(2-aminoethoxy)-3-butenic acid]

BUTIROSIN, PENTA[N-(TRIFLUOROACETYL)]-5''-AMINO-5''-DEOXY-
{ See penta[N-(trifluoroacetyl)]-5''-amino-5''-deoxybutirosin }

BUTIROSIN A, 5''-AMINO-3',4',5''-TRI-DEOXY-

(See 5''-amino-3',4',5''-trideoxybutirosin A)

BUTIROSIN A, PHOSPHORYLATED
(See phosphorylated butirosin A)

BUTIROSIN A (BB-K186), 3'-DEOXY-
[See 3'-deoxybutirosin A (BB-K186)]

BUTIROSIN N,N',N'',N'''-TETRA-ACETYL (N-Ac) DERIVATIVE

PC*Paper*

Whatman No. 1 (descending development, 24 h at 25–30°C)

Solvent

1-Butanol–pyridine–5% boric acid (6:4:3)

Detection

The zones are detected by using a modification of the method of Pan and Dutcher (*Anal. Chem.*,

28 (1956) 836–838), chlorine gas being used instead of sodium hypochlorite

R_F

Compound	<i>R_F</i>
N-Ac Butirosin A	0.34–0.38
N-Ac Butirosin B	0.17–0.20

Ref.

As Butirosins, PC 3

BUTIROSINS

(cf., ambutyrosin and BU-1975)

PC 1*Paper*

Ascending

Solvent

Chloroform–methanol–17% aq. ammonia (2:1:1) lower layer, (developed at room temp. for 12 h)

R_F

Butirosin A, 0.00

Butirosin B, 0.01

Ref.

As Apramycin, PC 1

PC 2*Solvent*

1-Butanol–pyridine–water–acetic acid (6:4:3:1)

R_F

0.73

Ref.

German Patent 2,350,169, April 18, 1974

PC 3*Paper*

Whatman No. 1 (buffered at pH 3)

Solvents

A. Methanol–water–acetic acid–25% aq. sodium chloride (600:212:75:20.8)

B. Methanol–5% aq. sodium chloride (2:1)

C. Ethanol–water–acetic acid–25% aq. sodium chloride (250:500:38:7.5)

R_F

Solvent	<i>R_F</i>
A	0.21
B	0.5
C	0.71

Ref.

H.W. Dion, P.W.K. Woo, N.E. Willmer, D.L. Kern, J. Onaga and S.A. Fusari, *Antimicrob. Agents Chemother.*, 2 (1972) 84–88

TLC 1

Medium

Silica gel Quanta Q1F

Solvents

- A. 15 N aq. Ammonia-methanol (1:1)
- B. 10% Methanol in chloroform

- C. 3% Methanol in chloroform
- D. 2.5% Methanol in chloroform
- E. 8% Methanol in chloroform

Ref.

P.W.K. Woo, U.S. Patent 3,960,837, June 1, 1976

R_F

Components	Solvent				
	A	B	C	D	E
Aminotrideoxybutirosin A	0.54				
5''-Aminopenta-N-carbobenzoxy-5''-deoxybutirosin A		0.50			
5''-Aminopenta-N-carbobenzoxy-3',4''-O-cyclohexylidene-5''-deoxybutirosin A		0.65			
2'',2''',3'',6-Tetra-O-acetyl-5''-aminopenta-N-carbobenzoxy-5''-deoxy-3',4'-di-O-methanesulfonylbutirosin A			0.48		
2'',2''',3'',6-Tetra-O-acetyl-5''-aminopenta-N-carbobenzoxy-3',4',5''-trideoxybutirosin-3',4'-ene A				0.42	
5''-Aminopenta-N-carbobenzoxy-3',4',5''-trideoxybutirosin-3',4'-ene A					0.30

TLC 2

Medium

Silica gel

Solvent

Chloroform-methanol-17% ammonia (1:4:3)

R_F

0.24

Ref.

As PC 1

TLC 3

Media

- A. Silica gel
- B. Alumina

R_F

Solvent	Medium	Butirosin			
		A ₁ •	A ₂ •	C ₁	C ₂
A	A	0.41	0.41	0.51	0.51
B	A	0.20	0.20	0.26	0.26
C	B	3.7 cm	0.2 cm	5.3 cm	0.5 cm

• A₁ = Butirosin A; A₂ = Butirosin B

Solvents

- A. Chloroform-methanol-28% ammonium hydroxide-water (1:4:2:1), develop 3 times
- B. Chloroform-methanol-17% ammonium hydroxide (1:3:2)
- C. Chloroform-methanol-17% ammonium hydroxide (2:1:1), upper phase (develop 16 h, location shown in cm from origin)

Ref.

H. Kawaguchi, K. Tomita, T. Hoshiya, T. Miyaki, K.-I. Fujisawa, M. Kimeda, K.-I. Numata, M. Konishi, H. Tsukiura, M. Hatori and H. Koshiyama, *J. Antibiot.*, 27 (1974) 460-470; Additional references: K. Numata, M. Konishi, K. Shimoda, M.K. Hiroshi and H. Kawaguchi, paper presented at the 192nd JARA, March 22, 1974;

H. Kawaguchi, H. Tsukiura, K. Fujisawa and M. Konishi, *German Patent* 2,346,243, March 21, 1974; *Belgian Patent* 016,374, Dec. 16, 1974

TLC 4

Medium

Silica gel

Solvents

A. Chloroform-methanol-17% ammonium hydroxide (2:1:1) upper phase

B. 10% Ammonium acetate-methanol (1:1)

Detection

Bioautography vs. *Bacillus subtilis*

R_F

Solvent	R_F
A	0.30
B	0.70

Ref.

R. Okachi, I. Kawamoto, S. Takasawa, M. Yamamoto, S. Sato, T. Sato and T. Nara, paper presented at the 194th Sci. Meeting, JARA, July 26, 1974

CALVATIC ACID

TLC

Media

A. Silica gel G (Merck)

B. Silica gel H (Mallinckrodt)

Solvents

A. Butanol-acetic acid-water (4:4:1)

B. Benzene-ethanol (5:1)

R_F

Solvent	Medium	R_F
A	A	0.80
B	B	0.60

Ref.

H. Umezawa, T. Takeuchi, H. Iinuma and O. Tanabe, *U.S. Patent* 4,009,155, Feb. 22, 1977

CANADENSOLIDE

PC

Solvents

A. Butanol-satd. water

B. Propanol-water (1:9)

C. Methanol-propanol-water (6:2:1)

D. Methyl ethyl ketone-benzene-water (1:5:1), upper layer

E. Methanol-0.05 M sodium citrate at pH 5.7 (7:3), (paper buffered with 0.05 M sodium citrate at pH 5.7)

Detection

Bioautography vs. *Klebsiella pneumoniae*

R_F

Solvent	R_F
A	0.45
B	0.73
C	0.81
D	0.91
E	0.87

Ref.

D.H. Berg, R.L. Hamill and M.M. Hoehn, *U.S. Patent* 4,001,086, Jan. 4, 1977

TLC

Medium

Silica gel 60 F₂₅₄ (Merck)

Solvents

A. Benzene-ethyl acetate (1:1)

B. Chloroform-acetone (3:2)

C. Ethyl acetate-chloroform (3:1)

Detection

A. Fluorescence quenching under short-wave ultraviolet light

B. Spray with iodine-azide spray reagent

R_F

Solvent	R_F
A	0.44
B	0.56
C	0.54

Ref.

As PC

CANDICIDIN

PC 1

Paper

Whatman No. 1

Solvents

A. Ethyl acetate-pyridine-water (4:3:2)

B. *n*-Butanol-pyridine-water (6:4:5)

C. Methanol-25% ammonium hydroxide-water (20:1:4)

Detection

Bioautography vs. *Candida albicans*

R_F

Solvent	R_F
A	0.66
B	0.71
C	0.55

Ref.

R. Bosshardt and H. Bickel, *Experientia*, 24 (1968) 422-424

PC 2

Solvents

- A. Water-satd. butanol *
- B. Butan-1-ol-acetic acid-water (2:1:1) •
- C. Butan-1-ol-pyridine-water (1:0.6:1) *
- D. Acetone-water (1:1) •
- E. Butan-1-ol-McIlvaine's buffer (pH 6.5)
- F. Methanol-ammonia-water (20:1:4)
- G. Butan-1-ol-acetic acid-water (20:1:25)
- H. Butan-1-ol-pyridine-water (4:1:5)
- I. Butan-1-ol-pyridine-water (6:1:7)
- J. Butan-1-ol-ethanol-water (4:2:1)
- K. Butan-1-ol-ethanol-water (3:3:2)
- L. Butan-1-ol-pyridine-water (6:4:5) *
- M. Methanol-25% ammonia-water (20:1:4) *
- N. Ethyl acetate-pyridine-water (4:3:2) *
- O. Butan-2-ol-ethanol-water (2:2:1)

* Whatman No. 1 paper specified

 R_F

Solvent	R_F
A	0.44
B	0.86
C	0.80
D	0.38, 0.69
E	0.00, 0.52
F	0.00, 0.44, 0.66
G	0.00, 0.44
H	0.39
I	0.28
J	0.53
K	0.89
L	0.71
M	0.55
N	0.66
O	0.60

Ref.

As Amphotericins, TLC 2

TLC 1

Medium

Silica gel G

Solvents

- A. Butan-1-ol-ammonia-methanol-water (20:1:2:4)
- B. Chloroform-methanol-borate buffer (pH 8.3) (7:5:1)
- C. Butan-1-ol-ethanol-acetone-32% ammonia (2:5:1:3)
- D. Chloroform-95% ethanol-water (50:50:8)

Detection

- A. 0.2% *p*-Dimethylaminobenzaldehyde in sulfuric acid containing ferric chloride
- B. Spectrodensitometric

 R_F

Solvent	Detection	R_F
A	A	0.13
B	A	0.49, 0.67
C	B	0.55
D	B	0.34, 0.74, 0.95

Ref.

As Amphotericins, TLC 2

TLC 2

Medium

Silica gel 60 F₂₅₄ (Merck)

Solvent

Butanol-ethanol-acetone-32% ammonium hydroxide (2:5:1:3)

Detection

UV

 R_F

Compound	R_F
Candididin	0.55
Candididin methyl ester	0.86
Candididin ethyl ester	0.89
Candididin propyl ester	0.92
Candididin butyl ester	0.92

Ref.

As Amphotericins, TLC 1

TLC 3

Medium

Silica gel G

Solvent

Chloroform-methanol-20% ammonium hydroxide (2:2:1) lower phase

Detection**Spectrodensitometry****Ref.**

J.F. Martin and L.E. McDaniel, *Eur. J. Appl. Microbiol.*, 3 (1976) 135–144

CCD 1**Solvents**

A. Chloroform–methanol–borate buffer (pH 8.2–8.4) (2:2:1)

B. Chloroform–methanol–water (2:2:1)

Distribution coefficient

Solvent	N_t	K
A	300	0.78
	200	0.61
	600	1.02
B	300	0.38

Ref.

As Amphotericins, TLC 2

CCD 2**Solvent**

Chloroform–methanol–borate buffer (pH 8.2–8.4)

Distribution coefficient

360 transfers, $K = 2.55$

Ref.

As Amphotericins, TLC 2

HPLC**Apparatus**

DuPont Model 830 liquid chromatograph, equipped with a DuPont Model 837 spectrophotometer. Extinction at 380 nm recorded by means of a Hewlett-Packard Model HP 3380 A integrator

Column

Permaphase ODS, 1 m × 2.1 mm I.D., reversed-phase partition column with octadecylsilane as stationary phase, chemically bonded to a controlled porous surface ("porosity beads", 20–37 μ m) by means of Si–O–Si bonds and contains about 1% stationary phase. Pre-packed Permaphase ODS column has 600 theoretical plates, determined by isocratic elution of anthracene with methanol–water (6:4)

Temperature

55 ± 1°C

Mobile phase

A. 0.05 M phosphate buffer (pH 7.0)–methanol (8:2)

B. Methanol**Gradient**

0 → 100% B at 3%/min, hold at limit, concave gradient $c = kt^5$, where c is the percentage change during the run, t is the time fraction passed, and k is a constant

Pressure

1000 p.s.i.

Flow-rate

1.0 ml/min

Detection

380 nm

Attenuation

0.08 a.u.f.s.

Chart speed

5 mm/min

Ref.

S.H. Hansen and M. Thomsen, *J. Chromatogr.*, 123 (1976) 205–211

CANDIDIN**PC****Solvents**

A. Butan-1-ol–McIlvaine's buffer (pH 6.5)

B. Methanol–ammonia–water (20:1:4)

C. Butan-1-ol–acetic acid–water (20:1:25)

 R_F

Solvent	R_F
A	0.34
B	0.49
C	0.12

Ref.

As Amphotericins, TLC 2

TLC**Medium**

Silica gel G

Solvent

Chloroform–methanol–20% ammonia (2:2:1)

Detection

Spectrodensitometric

 R_F

0.27, 0.30, 0.33, 0.37, 0.40

Ref.

As Amphotericins, TLC 2

CCD**Solvent**

Chloroform–methanol–borate buffer (pH 8.2–8.4) (2:2:1)

Distribution coefficient

N_t	K
200	3.44
360	4.16

Ref.

As Amphotericins, TLC 1

CANDIDOIN**CCD****Solvent**

Chloroform-methanol-borate buffer (pH 8.2-8.4)

Distribution coefficient360 transfers, $K = 1.81$

Ref.

As Amphotericins, TLC 1

CANDIHEXIN**TLC****Medium**

Silica gel G

Solvent

Chloroform-methanol-20% ammonia (2:2:1) lower phase

Detection

Spectrodensitometric

 R_F

0.26, 0.29, 0.36, 0.39, 0.43

Ref.

As Amphotericins, TLC 2

CCD**Solvent**

Chloroform-methanol-citrate + phosphate buffer (pH 6.25) (2:2:1)

Distribution coefficient550 transfers, $K = 1.19, 1.37, 1.85, 2.18, 2.50, 3.37$

Ref.

As Amphotericins, TLC 2

CAPREOMYCIN**PC 1****Paper**

Whatman No. 1 buffered with 0.05 M citrate buffer, pH 6.0

Solvent

Methanol-0.05 M citrate buffer, pH 6.0 (7:3)

DetectionBioautography vs. *Bacillus subtilis* ATCC 6633 at pH 8.0 R_F

Capreomycin II moves more rapidly than I

Ref.

W.M. Stark, C.E. Higgins, R.N. Wolfe, M.M. Hoehn and J.M. McGuire, *Antimicrob. Agents Chemother.*, 1962 (1963) 596-606**PC 2****Paper**

As PC 1

Solvent

As PC 1

DetectionBioautography vs. *Mycobacterium butyricum* at pH 8.0 R_F After 30 h, capreomycin II separates from I; after an additional 10 h, capreomycin II separates further into 2 components, IIA and IIB; with continuous flow for 80 h, capreomycin I separates into 2 components, IA and IB. Relative R_F values are IIB > IIA > IB > IA

Ref.

W.M. Stark and L.D. Boeck, *Antimicrob. Agents Chemother.*, 1964 (1965) 157-164**TLC****Medium**

Silica gel G (Kieselgel-G, Merck)

Solvent

10% Aq. ammonium acetate-acetone-10% ammonium hydroxide (9:10:0.5)

 R_F

0.15

Ref.

A. Negata, T. Ando, R. Izumi, H. Sakakibara, T. Take, K. Hayano and J. Abe, *J. Antibiot.*, 21 (1968) 681-687**CAPSIMYCIN****TLC****Medium**Silica gel GF₂₅₄**Solvents**

- A. Chloroform
- B. Chloroform-methanol (1:1)
- C. Chloroform-methanol (3:1)
- D. Chloroform-methanol (5:1)
- E. Ethyl acetate
- F. Ethyl acetate-methanol (1:1)
- G. Ethyl acetate-methanol (3:1)

Detection

A. UV

B. Charring after spraying with 10% sulfuric acid

 R_F

Solvent	R_F
A	0.00
B	0.75
C	0.39
D	0.30
E	0.02
F	0.45
G	0.12

Ref.

S. Aizawa, H. Akutsu, T. Satomi, T. Nagatsu, R. Taguchi and A. Seino, *J. Antibiot.*, 32 (1970) 193–196

O-CARBAMYL-D-SERINE**PC 1****Solvent***n*-Butanol–acetic acid–water (4:1:5)**Detection**

Ninhydrin or biuret

 R_F

0.20

Ref.

Y. Okami, K. Maeda, H. Kondo, T. Tanaka and H. Umezawa, *J. Antibiot.*, 15 (1962) 147–151

PC 2**Solvents**A. *n*-Butanol satd. with water–acetic acid (3:1)

B. 80% Phenol

C. 77% Ethanol

 R_F

Solvent	R_F
A	0.18
B	0.39
C	0.20

Ref.

N. Tanaka, K. Sashikata, T. Wada, S. Sugawara and H. Umezawa, *J. Antibiot.*, 16 (1963) 217–221

PC 3**Paper**

Whatman No. 1

Solvents

A. Phenol–borate buffer pH 9.3

B. Phenol–phosphate buffer pH 11.2

C. Pyridine–isoamyl alcohol–water (8:4:7)

D. Butanol–formic acid–water (75:15:10)

Detection

Ninhydrin

 R_F

Solvent	R_F
A	0.41
B	0.27
C	0.33
D	0.08

Ref.

G. Hagemann, L. Penasse and J. Teillon, *Biochim. Biophys. Acta*, 17 (1955) 240–243

CARBENICILLIN**PC****Paper**

Whatman No. 1

SolventsA. 70% Aq. *n*-propanolB. 60% Aq. *n*-propanolC. Methanol–*n*-hexane (6:4) (bottom layer)D. Water satd. *n*-butanolE. Methanol–*n*-propanol–0.013 *M* aq. sodium citrate (6:2:1)**Detection**A. Bioautography vs. *Bacillus subtilis*

B. Spray with 0.5% aq. 2,6-dichlorophenol-indophenol (dark blue spot)

 R_F

Solvent	$R_{\text{benzylpenicillin}}$
A	0.41
B	0.68
C	0.12
D	0.10
E	0.38

Ref.

M. Betinova, M. Siricova and V. Betina, *J. Chromatogr.*, 101 (1974) 240–243

TLC**Medium**

Avicel

Solvent

Methanol–isobutanol–chloroform–10% hydrochloric acid (10:10:10:1)

Detection

- A. Iodine vapor
 B. 0.25% Stannous chloride soln.
 C. 0.2% *p*-Dimethylaminobenzaldehyde soln.

 R_F

0.55

Ref.

As Ampicillin, TLC 1

HPLC**Apparatus**

ALC/GPC 204 liquid chromatograph (Waters Assoc.)

ColumnIon-pair system with μ Bondapak C₁₈ (30.4 × 4.0 mm I.D.) Waters Assoc., used as stationary phase**Mobile phase**Methanol–0.01 *M* tetrabutylammonium bromide (4:7)**Flow-rate**

3.0 ml/min

Pressure

3500 p.s.i

Detector

UV (254 nm) Waters Assoc., Model 440

Sample10 μ l**Type**

Reversed-phase

Retention time

Estimated, diastereomers 5.0, 6.0

Ref.K. Yamaoka, S. Narita, T. Nakagawa and T. Uno, *J. Chromatogr.*, 168 (1979) 187–193**CARBOMYCIN
(cf., magnamycin)****PC 1****Solvents**

- A. Benzene vs. citrate buffer, pH 4.6
 B. Butyl acetate vs. citrate buffer, pH 4.6

 R_F

Solvent	R_F
A	0.09
B	0.58

Ref.T. Osato, K. Yagishita and H. Umezawa, *J. Antibiot.*, 8 (1955) 161–163**PC 2****Solvents**

- A. Benzene–cyclohexane (1:1) vs. paper treated with formamide
 B. Benzene vs. paper treated with formamide
 C. Benzene–chloroform (3:1) vs. paper treated with formamide
 D. Benzene–chloroform (1:1) vs. paper treated with formamide

 R_F

Solvent	Carbomycin	Carbomycin B
A	0.25	0.45
B	0.76	0.89
C	0.98	0.98
D	0.98	0.98

Ref.K. Murai, B.A. Sobin, W.D. Celmer and F.W. Tanner, *Antibiot. Chemother.*, 9 (1959) 485–490**PC 3****Solvents**

As angolamycin, PC, solvents A through F

 R_F

Solvent	R_F
A	0.75
B	0.88
C	0.26
D	0.48
E	0.49
F	0.71

Ref.

As Angolamycin PC

PC 4**Solvent**

As celesticetin PC 2, G

Detection

As celesticetin PC 2

 R_F

0–0.28 (estimated from diagram)

Ref.

As celesticetin PC 2

PC 5**Paper**

Toyo No. 51

Solvent

Water-satd. ethyl acetate (ascending, 3 h)

Detection

Bioautography vs. *Bacillus subtilis* No. 10707

R_F

0.00, 0.95

Ref.

I. Kawamoto, R. Okachi, H. Kato, S. Yamamoto, I. Takahashi, S. Takasawa and T. Nara, *J. Antibiot.*, 27 (1974) 493–501

TLC**Medium**

Silica gel

Solvents

- A. Chloroform–methanol–17% ammonia (2:1:1)
- B. Butanol–acetic acid–water (3:1:1)

Detection

- A. Bioautography vs. *Sarcina lutea*
- B. Plate heated at 100°C and sprayed with phosphoric acid in methanol (1:1)

R_F

Solvent	Detection	R _F
A	A	0.96
	B	0.96 purple
B	A	0.58
	B	0.58 purple

Ref.

G.H. Wagman, J.A. Waitz, J. Marquez, A. Murawski, E.M. Oden, R.T. Testa and M.J. Weinstein, *J. Antibiot.*, 25 (1972) 641–646

CCD 1**Solvent**

Benzene–acetate buffer, pH 4.5

Plates

60

Distribution

Carbomycin, *K* = 7

Carbomycin B, *K* = 12

Ref.

F.A. Hochstein and K. Murai, *J. Amer. Chem. Soc.*, 76 (1954) 5080–5083

CCD 2**Solvent**

Benzene–cyclohexane–95% ethanol–water (5:5:8:2)

Distribution

Carbomycin, 0.41

Carbomycin B, 0.67

Ref.

As PC 2

CARBOMYCIN A**TLC****Medium**

Silica gel 60 F₂₅₄ pre-coated (Merck)

Solvents

- A. Benzene–acetone (2:3)
- B. Chloroform–methanol (10:1)
- C. *n*-Butanol–acetic acid–water (3:1:1)

Detection

Sulfuric acid, purplish blue

R_F

Solvent	R _F
A	0.54
B	0.63
C	0.58

Ref.

Y. Shimauchi, K. Kubo, K. Osumi, K. Okamura, Y. Fukagawa, T. Ishikura and J. Lein, *J. Antibiot.*, 31 (1978) 270–275

CARMINOMYCIN**TLC****Medium**

Silica gel G

Solvent

Chloroform–methanol–benzene (7:3:3)

R_F

Carminomycin	R _F *
1	0.06
2	0.24
3	0.40
4	0.50
5	0.56
6	0.70
7	0.85
8	0.95

* Estimated from drawing

Ref.

M.G. Brazhnikova, V.B. Zbarsky, V.I. Ponomarenko and N.P. Potapova, *J. Antibiot.*, 27 (1974) 254–259

CARRIOMYCIN**TLC****Medium**

Silica gel (Kieselgel 60, Merck)

Solvents

- A. Ethyl acetate
- B. Benzene-ethyl acetate (1:1)
- C. Benzene-acetone (1:1)
- D. Benzene-acetone (9:1)
- E. Methanol-chloroform (1:19)
- F. Chloroform-ethyl acetate (2:3)

 R_F

Solvent	R_F
A	0.73
B	0.51
C	0.89
D	0.18
E	0.87
F	0.62

Ref.

- A. Imada, Y. Nozaki, T. Hasegawa, E. Mizuta, S. Igarasi and M. Yoneda, *J. Antibiot.*, 31 (1978) 7-14

CATENULIN**PC***Paper*

Whatman No. 1

Solvents

- A. *n*-Butanol satd. with water + 2% *p*-toluene-sulfonic acid, 40 h
- B. 80% aq. Ethanol + 1.5% sodium chloride for 40 h on paper buffered with 0.95 *M* sulfate-bisulfate
- C. Propanol-pyridine-acetic acid-water (15:10:3:12) for 40 h
- D. Water satd. with methylisobutyl ketone + 1% *p*-toluenesulfonic acid, 6 h
- E. Propanol-water (1:1), 24 h on buffered paper with 0.75 *M* phosphate, pH 4.0
- F. *n*-Butanol satd. with water, for 18 h
- G. *n*-Butanol satd. with water + 2% *p*-toluene-sulfonic acid and 2% piperidine, for 18 h

The migration constant for each of the antibiotics is expressed as an R_e rather than the more conventional R_F value. The R_e value expresses the ratio of the distance traversed by the antibiotic with respect to the end of the tape rather than the solvent front. This method of expressing the constant was chosen in view of the fact that the solvent front had progressed beyond the end of the tape during the time interval employed with most of the solvent systems

 R_F

Solvent	R_e
A	0.44
B	0.25
C	0.34
D	0.65
E	0.35
F	0
G	0

Ref.

- R.Q. Thompson, W.M. Stark, C.E. Higgins, *U.S. Patent* 3,691,279, Sept. 12, 1972

CEFAMANDOLE**TLC***Medium*

Silica gel

*Solvent**n*-Butanol-acetic acid-water (4:1:1)*Detection*

- A. UV
- B. Iodine

Ref.

- J.M. Essery, *U.S. Patent* 3,928,334, Dec. 23, 1975

CEFAZOLIN**PC 1***Paper*

Toyo No. 51A

*Solvent**n*-Butanol-acetic acid-water (5:1:4) upper layer*Detection*

Potassium permanganate

 R_F

0.69-0.71

Ref.

- K. Kariyone, H. Harada, M. Kurita and T. Takano, *J. Antibiot.*, 23 (1970) 131-136

PC 2*Paper*

Toyo No. 51

*Solvent**n*-Butanol-acetic acid-water (4:1:2), ascending, 16 h at room temperature*Detection*

- A. For radioactive antibiotic, developed papers exposed to X-ray films for 7-14 days
- B. Bioautography vs. *Bacillus subtilis* ATCC 6633

R_F

0.75 (estimated from photographs)

Ref.J. Kozatani, M. Okui, T. Matsubara and M. Nishida, *J. Antibiot.*, 25 (1972) 86–93**TLC 1****Medium**

Silica gel

Solvent*n*-Butanol–acetic acid–water (6:3:2)**Detection**

Potassium permanganate

R_F

0.53–0.60

Ref.

As PC 1

TLC 2**Medium**

Eastman Chromagram Sheet No. 6061

SolventsA. *n*-Butanol–acetic acid–water (4:1:5), upper phaseB. *n*-Butanol–ethanol–water (4:1:5)C. Methanol–*n*-propanol–water (6:1:2)**Detection**Bioautography vs. *Bacillus subtilis* ATCC 6633**R_F**

A, 0.35 (estimated from photograph); B and C not given

Ref.M. Nishida, T. Matsubara, T. Murakawa, Y. Mine, Y. Yokota, S. Kuwahara and S. Goto, *Antimicrob. Agents Chemother.*, 1969) 1970) 236–243**TLC 3****Medium**Silica gel F₂₅₄**Solvents**

A. As PC 2

B. Ethyl acetate–acetic acid–water (4:2:1)

Detection

As PC 2

R_F

Useful for detection of urine and bile metabolism

Ref.

As PC 2

HPLC**Column**1 m × 2.1 mm I.D. × 6.3 mm O.D., 316 stainless steel. C₁₈ Porasil B packing**Mobile phase**

Water

Pressure

150 p.s.i.

Flow-rate

2.5 ml/min

Detector

UV (254 nm)

Sensitivity

1.28 a.u.f.s.

Type

Reversed-phase

Retention time

115.0 min (estimated from drawing)

Ref.

As Ampicillin, HPLC

**CELESTICETIN, N-DEMETHYL-
(See N-demethylcelesticetin)****CELESTICETIN, N-DEMETHYL-7-O-
DEMETHYL-
(See N-demethyl-7-O-demethylcelesticetin)****CELESTICETINS****PC 1****Solvents**A. *n*-Butanol–water (81:19)B. *n*-Butanol–water–*p*-toluenesulfonic acid (81:18.7:0.25)C. *n*-Butanol–water–glacial acetic acid (50:25:25)D. *n*-Butanol–water–piperidine (80:18:2)E. Water–*n*-butanol (96:4)F. Water–*n*-butanol–*p*-toluenesulfonic acid (94:4:2)**R_F**

Solvent	R _F *
A	0.55–0.65
B	0.45–0.55
C	0.75
D	0.80
E	0.60
F	0.75

* Estimated from diagram

Ref.H. Hoeksma, G.F. Crum and W.H. De Vries, *Antibiot. Ann.*, (1954–1955) 837–841

PC 2

Solvents

- A. Water-satd. *n*-butanol
- B. *n*-Butanol satd. with water + 0.25% *p*-toluene-sulfonic acid
- C. As PC 1, C
- D. *n*-Butanol satd. with water + 2% piperidine
- E. As PC 1, E
- F. Water-*n*-butanol-*p*-toluenesulfonic acid (96:4:0.25)
- G. 1 M Phosphate buffer, pH 7.0

Detection

Bioautography vs. *Bacillus subtilis*

R_F

Solvent	R_F *
A	0.7 -0.87
B	0.53-0.78
C	0.60-0.80
D	0.85
E	0.38
F	0.42-0.66
G	0.25-0.42

* Estimated from diagrams

Ref.

C. De Boer, A. Dietz, J.R. Wilkins, C.N. Lewis and G.M. Savage, *Antibiot. Ann.* (1954-1955) 831-836

TLC

Medium

Silica gel G

Solvents

- A. Methyl ethyl ketone-acetone-water (186:52:20)
- B. 2-Pentanone-methyl ethyl ketone-methanol-water (2:2:1:1)
- C. Chloroform-methanol (6:1)

Detection

Bioautography vs. *Sarcina lutea*

R_F

	Solvent		
	A	B	C
Desalicytin	0.11	0.22	0.23
Celesticetin B	0.41	0.54	0.52
Celesticetin C	0.43	0.66	0.59
Celesticetin D	0.34	0.39	0.53

Ref.

A.D. Argoudelis and T.F. Brodasky, *J. Antibiot.*, 25 (1972) 194-196

CCD

Solvent

1-Butanol-water-glacial acetic acid (4:5:1)

Distribution coefficient

0.70

Ref.

As PC 1

GC

Apparatus

Hewlett-Packard 402 Biomedical GC unit

Column

Glass, 4 ft. $\times \frac{1}{8}$ in. packed with Hewlett-Packard prepared packing (3.8% UCW 98 on 80-100 mesh Diatoport S)

Detection

Flame ionization detection 280° or 290°. Celesticetin (I) and 7-O-demethylcelesticetin were separated using a 3% PPE column

Retention time

Compound	t_R (UCW 98) (min)		t_R (PPE) (min) 268°C
	290°C	280°C	
Celesticetin	10.2	16.7	1.2
Celesticetin B	2.6	3.8	-
Celesticetin C	11.9	17.9	-
7-O-Demethyl-celesticetin	-	16.7	1.6
N-Demethyl-7-O-demethyl-celesticetin	-	18.2	-
Desalicytin	-	2.6	-

Ref.

T.F. Brodasky and A.D. Argoudelis, *J. Antibiot.*, 26 (1973) 131-134

CENOCOCCUM ANTIBIOTIC

PC

Solvent

Two-dimensional chromatography. Direction 1, methanol-ammonium hydroxide-water (3:3:1); develop 130 min, dry 24 h. Direction 2, benzene satd. with 10% acetic acid, dry 25 h

Detection

- A. Blue-green fluorescence under short wavelength UV light
- B. Bioautography vs. *Bacillus cereus*

R_F

Direction 1, 0.69

Direction 2, 0.00

Ref.L.F. Grand and W.W. Ward, *For. Sci.*, 15 (1969) 286–288**CEPHALEXIN****PC 1****Solvent***n*-Butanol–acetic acid–water (3:1:1)**Detection**Bioautography vs. *Sarcina lutea***Ref.**D.R. Brannon, J.A. Mabe and D.S. Fukuda, *J. Antibiot.*, 29 (1976) 121–124**PC 2****Paper**

Whatman 3MM

Solvent

Butanol–acetic acid–water (12:3:5)

Detection

Paper stained with 0.25% ninhydrin in acetone containing 0.01% isatin and 1% lutidine; heat at 100° for 10 min

R_F

1.90. Migration distance relative to alanine taken as 1.00

Ref.

As Ampicillin, PC 1

ELPHO**Medium**

Paper. Whatman 3MM

Buffer

Formic acid–acetic acid–water (6:24:170)

Conditions

3000 V, potential gradient 60 V/cm, current 50 mA, 30 min

Detection

Paper stained with 0.25% ninhydrin in acetone containing 0.01% isatin and 1% lutidine; heat at 100° for 10 min

Mobility

0.65. Migration distance relative to alanine taken as 1.00

Ref.

As Ampicillin, PC 1

GC**Apparatus**

Shimadzu GC-SA Chromatograph

Column

1 m × 3 mm I.D., glass tubing packed with 1.5% OV-101 coated on Chromosorb W AW DMCS (60–80 mesh)

Carrier gas

Helium

Flow-rate

125 ml/min

Temperature

Column, 175°C; injection port, 280°

Detector

Flame ionization

Retention timePeak height: 5 α -cholestane, 6 min; tri-TMS-cephalexin, 10 min**Ref.**T. Nakagawa, J. Haginaka, M. Masada and T. Uno, *J. Chromatogr.*, 154 (1978) 264–266**HPLC****Apparatus**

Model ALC/GPC (Waters Assoc.) high-speed liquid chromatograph

Column μ Bondapak C₁₈, 30 cm × 4 mm I.D.**Mobile phase**

Methanol–water (1:8) containing 5% acetic acid, urine; methanol–water (1:5) containing 5% acetic acid, plasma

Flow-rate

1.8 ml/min, urine; 0.9 ml/min, plasma

Pressure

1800 p.s.i., urine; 1100 p.s.i., plasma

Detection

UV 254 nm

Type

Reversed-phase

Retention time

Urine, 16.0; plasma, 15.5. Estimated from drawing

Ref.T. Nakagawa, J. Haginaka, K. Yamaoka and T. Uno, *J. Antibiot.*, 31 (1978) 769–775**CEPHALOGLYCIN, DESACETYL-
(See desacetylcephaloglycin)****CEPHALOGLYCINS;
CEPHALOGLYCIN METABOLITES****PC****Paper**

Whatman No. 1

Solvent

n-Butanol–acetic acid–water (3:1:1), descending, 12–16 h

Detection

Air-dry chromatogram, steam lightly to remove acetic acid; bioautography vs. *Sarcina lutea* PCI 1001 using agar at pH 6.5

R_F

Desactyl cephaloglycin > cephaloglycin

Ref.

M.N. Hoehn and C.T. Pugh, *Appl. Microbiol.*, 16 (1968) 1132–1133

TLC**Medium**

Eastman No. 6064 cellulose sheet

Solvent

Acetonitrile–ethyl acetate–water (3:1:1), 1.5 h

Detection

Bioautography vs. *Sarcina lutea* at pH 6.5

R_F

Desacetyl cephaloglycin lactone > desacetyl cephaloglycin > cephaloglycin

Ref.

As PC

HPLC**Apparatus**

High-performance liquid chromatograph (Waters Assoc., ALC/GPC)

Column

Stainless steel, 4 mm × 30 cm. A short column (4 mm × 2 cm) packed with Bondapak C₁₃/Corisal B (Waters Assoc.) used to guard the main column

Mobile phase

Water–methanol (4:1) containing 0.004 *m* sodium *n*-heptylsulfonate and 0.01 *m* sodium phosphate

Flow-rate

1.0 ml/min

Pressure

1400 p.s.i.

Retention time

Deacetylcephaloglycin, 7 min

Cephaloglycin, 19 min

Deacetylcephaloglycin lactone, 33 min

Estimated from drawing

Ref.

J. Haginaka, T. Nakagawa and T. Uno, *J. Antibiot.*, 32 (1979) 462–467

CEPHALORIDINE**HPLC****Apparatus**

Model ALC202 chromatograph equipped with a Model U6K injector (Waters Assoc.)

Column

Stainless steel, 60 cm × 2 mm I.D., packed with phenyl Corasil (37–50 μm) reversed-phase column packing material

Mobile phase

18–20% Methanol in 0.2 *M* aq. ammonium acetate

Temperature

Ambient

Flow-rate

2.0 ml/min

Pressure

1500–1700 p.s.i.

Detection

254 nm

Sensitivity

0.02–0.04 a.u.f.s.

Retention time

2.8 min, estimated from drawing. Peak areas determined by triangulation

Ref.

J.S. Wold and S.A. Turnipseed, *J. Chromatogr.*, 136 (1977) 170–173

CEPHALOSPORANES**TLC****Solvents**

A. *n*-Butanol–glacial acetic acid–water (67:10:23)

B. *n*-Butanol–pyridine–glacial acetic acid–water (38:24:8:30)

C. *n*-Butanol–pyridine–glacial acetic acid–water (34:24:12:30)

D. *n*-Butanol–ethyl acetate–pyridine–glacial acetic acid–water (42:21:21:6:10)

E. Ethyl acetate–glacial acetic acid–water (60:20:20)

Detection

Iodine spray

R_F

(see p. 86)

Ref.

H. Bickel and J. Mueller, *U.S. Patent* 3,929,779, Dec. 30, 1975

R_F

Compound	Solvent				
	A	B	C	D	E
7-[(<i>p</i> -Aminopyridinium)-acetyl-amino]-3-(1-methyl-tetrazol-5-ylthio)-methylceph-3-em-4-carboxylic acid	0.08	0.37	0.48	0.07	—
7-[(<i>p</i> -Aminopyridinium)-acetyl-amino]-cephalo-sporanic acid	0.10	0.25	0.37	—	—
7-[(<i>p</i> -Aminopyridinium)-acetyl-amino]-3-(2-methyl-1,3,4-thiadiazol-5-ylthio)-methylceph-3-em-4-carboxylic acid	0.07	0.35	—	0.05	—
7-[(<i>p</i> -Aminopyridinium)-acetyl-amino]-3-(1,3,4-thiadiazol-2-ylthio)-methylceph-3-em-4-carboxylic acid	0.11	0.31	—	0.06	—
7-[(<i>p</i> -Aminopyridinium)-acetyl-amino]-3-(2-methyl-1,3,4-oxadiazol-5-ylthio)-methyl-ceph-3-em-4-carboxylic acid	0.01	0.36	—	0.35	—
7-[(<i>p</i> -Aminopyridinium)-acetyl-amino]-3-(3-methyl-1,2,4-triazol-5-ylthio)-methyl-ceph-3-em-4-carboxylic acid	0.15	0.36	—	0.06	—
7-[(<i>p</i> -Aminopyridinium)-acetyl-amino]-3-(3-methyl-1,2,4-thiadiazol-5-ylthio)-methyl-ceph-3-em-4-carboxylic acid	0.14	0.41	—	0.10	0.21
7-[(4- <i>tert</i> .-Amyloxycarbonylaminopyridinium)acetyl-amino]-3-(1-methyl-tetrazol-5-ylthio)-methylceph-3-em-4-carboxylic acid	0.26	0.35	—	—	0.39
4- <i>tert</i> .-Amyloxycarbonylaminopyridine	0.48	0.69	—	0.86	—
7-[(4- <i>tert</i> .-Amyloxycarbonylaminopyridinium)acetyl-amino]-3-methyltetrazol-5-ylthio)-methylceph-3-em-4-carboxylic acid	0.26	0.53	—	—	0.39
4- <i>tert</i> .-Amyloxycarbonylaminopyridiniumacetic acid	0.39	0.51	—	0.33	—

CEPHALOSPORANIC ACID, 7-AMINO- (DERIVATIVES)

(See 7-aminocephalosporanic acid derivatives)

CEPHALOSPORANIC ACIDS

TLC

Medium

Silica gel

Solvent

0.1 *N* Aq. ammonium acetate

 R_F

Compound	R_F
7-Aminocephalosporanic acid	0.75
7-(4-Amidinophenylacetyl-amino)-cephalosporanic acid	0.47

Compound	R_F
7-(4-Amidinophenoxyacetyl-amino)-cephalosporanic acid	0.49
7-[4-(5,5-Dimethyl-1,4,5,6-tetrahydro-2-pyrimidyl)-phenoxyacetyl-amino]-cephalosporanic acid	0.22
7-[4(9-Oxa-2,4-diazospiro-5,5-undec-2-en-3-yl)-phenoxyacetyl-amino]-cephalosporanic acid	0.18
7-[4-(1-Methyl-1,4,5,6-tetrahydro-2-pyrimidyl)-phenylacetyl-amino]-cephalosporanic acid	0.18
7-[4-(2-Imidazolyl)-phenoxyacetyl-amino]-cephalosporanic acid	0.41

Ref.

M. Schorr and E. Schrinner, *U.S. Patent* 3,865,820, Feb. 11, 1975

CEPHALOSPORANIC ACIDS, 7-N-ACYLAMINO-; O-DESACETYL DERIVATIVES

(See 7-N-acylaminocephalosporanic acids; O-desacetyl derivatives)

CEPHALOSPORINS

There are numerous cephalosporins including derivatives. Certain of these which may be of particular significance are indexed individually

PC 1

Paper

Whatman No. 1 treated with either 0.05 M sodium citrate buffer, pH 5.5 or 1 M sodium phosphate buffer, pH 6. Paper is dried at 37°C before use. Spot approximately 100 µg

Solvent

Methanol, dried over calcium oxide and distilled

R_F

Cephalosporin C, 0.34
Cephalosporin N, 0.44

Ref.

G.G.F. Newton and E.P. Abraham, *Biochem. J.*, 62 (1956) 651–658

PC 2

Paper

Whatman No. 1. Spot 50–200 µg

Solvents

- A. 1-Butanol–acetic acid–water (4:1:4)
- B. 1-Propanol–water (7:3)

Detection

- A. Bioautography vs. *Staphylococcus aureus* (Oxford strain NCTC 6571) or *Salmonella typhi* (strain "Mrs S"; Felix and Pitt 1935)
- B. Ninhydrin
- C. UV absorption at 230–400 nm (Corning filter No. 9863). Cephalosporin C chromophore appears as dark, light-absorbing spot

R_F

Compound	R_{glycine}	
	Solvent A	Solvent B
Deacetyl		
cephalosporin C	0.57	0.60
Cephalosporin C	0.78	0.77
Cephalosporin C _C	0.85	0.98

Ref.

J.D'A. Jeffrey, E.P. Abraham and G.G.F. Newton, *Biochem. J.*, 81 (1961) 591–596

PC 3

Paper

Whatman No. 1

Solvent

1-Butanol–acetic acid–water (4:1:4)

Detection

- A. Bioautography vs. *Salmonella typhi* or *Staphylococcus aureus*
- B. UV light at 230–400 nm. Cephalosporin C_A derivatives show up as dark spots
- C. Ninhydrin. Derivatives appear as purple spots
- D. Compounds formed from cephalosporin C and sulfapyridine or sulfathiazole can be detected by spraying the paper with 0.2% NaNO₂ in 0.1 N HCl, drying at 60°C and then spraying with 1% (w/v) α-naphthylamine in 75% (v/v) acetic acid
- E. The cephalosporin C-nicotinamide derivative can be detected by suspending the paper for 1 h in vapor of 2-butanone–aq. ammonia soln. (sp. gr. 0.88) (1:1). It appears as a blue-white fluorescent spot when the paper is viewed in UV light at 365 nm

R_F

Cephalosporin C _A derived from:	R_{cephC}^*
Nicotine	0.16
2-Aminopyridine	0.29
2-Amino-6-methylpyridine	0.60
Pyridine	0.31
Nicotinamide	0.30
2,4,6-Trimethylpyridine	0.54
2-Hydroxymethylpyridine	0.36
Quinoline	0.58
Sulphapyridine	0.55
Sulphadiazine	0.62
Sulphathiazole	0.74
3-Hydroxypyridine	0.36
Isonicotinic acid	0.20
Nicotinic acid	0.23
Picolinic acid	0.36
Pyridine-2,3-dicarboxylic acid	0.33

* $R_{\text{cephC}} = R_F$ compared to cephalosporin C

Ref.

C.W. Hale, G.G.F. Newton and E.P. Abraham, *Biochem. J.*, 79 (1961) 403–408

PC 4

Paper

Whatman No. 1

Solvents

- A. 1-Butanol-ethanol-water (4:1:5)
 B. Ethyl acetate satd. with aq. sodium acetate buffer (0.1 M to Na) pH 5.2 vs. paper pretreated with the buffer. Paper is soaked in buffer, blotted, air dried and used immediately. Solvent reaches bottom of paper in 3 h, but develop 18 h

Detection

Cephalosporin C, 7-ACA and related compounds converted to N-phenylacetyl derivatives by spraying dried chromatogram with pyridine in 50% acetone (v/v) until barely damp. Then, lightly spray with 2% (w/v) phenylacetyl chloride in acetone, and again with the pyridine solution until a spot of bromocresol green placed on the paper immediately turns blue (pH 5.0). Dry in air 3–5 min and bioautograph vs. *Staphylococcus aureus* (Oxford strain N.C.T.C. 6571)

 R_F

Compound	Solvent A
Cephalosporin C	0.04
N-Phenylacetyl cephalosporin C	0.13
Cephalosporin C _C	0.09
N-Phenylacetyl cephalosporin C _C	0.23
Cephalosporin C _A (pyridine)	0.00
Cephalosporin C _A (pyridine) nucleus	0.07
Cephalosporin C nucleus (7-ACA)	0.14
N-Phenylacetyl derivative of 7-ACA	0.40

Ref.

B. Loder, G.G.F. Newton and E.P. Abraham, *Biochem. J.*, 79 (1961) 408–416

PC 5

Paper

Whatman No. 1

Solvents

- A. 70% *n*-Propanol, descending
 B. Methanol-*n*-propanol-water (6:2:1), 18–20 h

Detection

Bioautography vs. *Bacillus subtilis* R_F

- A. Cephalosporins C and N move as a single zone but are well separated from penicillins G or V and cephalosporin P type
 B. Cephalosporin P type runs off end of paper; others well separated

Ref.

J.L. Ott, C.W. Godzeski, D. Pavey, J.D. Farran and D.R. Horton, *Appl. Microbiol.*, 10 (1962) 515–523

PC 6

Solvent

Methanol buffered at pH 5.5 with 0.013 M citrate

Detection

Bioautography vs. *Salmonella typhimurium*, *Bacillus subtilis*, *Proteus vulgaris*, *Staphylococcus aureus* or *Sarcina lutea*

 R_F

Cephalosporin N (synnematin B) moves as an elongated spot

Ref.

B.H. Olson, J.C. Jennings and A.J. Junek, *Science*, 117 (1953) 76–78

PC 7

Solvents

- A. 0.02 N Acetic acid in 25% (v/v) ethanol
 B. Diisopropyl ether satd. with 0.1 M potassium phosphate, pH 7.0
 C. Amyl acetate satd. with 0.1 M potassium phosphate, pH 7.0

With solvents B and C the paper is treated with 1 M potassium phosphate, pH 7.0, and dried before use

Detection

Bioautography vs. *Staphylococcus aureus* R_F

Antibiotic	Solvent		
	A	B	C
Cephalosporin P ₁	0.45	0.50	1.0
Cephalosporin P ₂	0.45	–	–
Cephalosporin P ₃	0.00	0.00	<0.05
Cephalosporin P ₄	0.40	0.50	1.00
Cephalosporin P ₅	0.20	–	–

Ref.

H.S. Burton and E.P. Abraham, *Biochem. J.*, 50 (1951–1952) 168–174

PC 8

Paper

Whatman No. 1

Solvents

- A. 2-Butanone satd. with water used when chromatography is followed by bioautography
 B. 1-Butanol satd. with 5 N ammonium hydroxide for separation of biologically inactive radioactive metabolites

Detection

- A. Bioautography vs. *Bacillus subtilis*
- B. Radioactive spots indicated by using a scanner

R_F

Cephalothin, related cephalosporins and their radioactive metabolites can be detected by this method

Ref.

H.R. Sullivan and R.E. McMahon, *Biochem. J.*, 102 (1967) 976–982

PC 9**Paper**

Whatman No. 4 impregnated with 0.1 M acetate buffer (pH 4.6) before use

Solvents

Methyl ethyl ketone–acetonitrile–water (MEK) (84:8:8). A mixture of MEK and water (3:2) is placed in bottom of chamber to provide a solvent satd. atmosphere

Detection

Bacillus subtilis ATCC 6633

R_F

Desacetyl cephalothin > cephalothin

Ref.

M.M. Hoehn, H.W. Murphy, C.T. Pugh and N.E. Davis, *Appl. Microbiol.*, 20 (1970) 734–736

PC 10 (Quantitative procedure)**Paper**

Whatman No. 1. An 8 × 20 in. sheet is slotted to give 17, $\frac{1}{4}$ -in. wide strips spaced $\frac{1}{8}$ in. apart. A $3\frac{1}{2}$ -in. margin is left intact at each end of the sheet along with a $\frac{3}{4}$ -in. strip on each side. Samples are applied $4\frac{1}{4}$ in. from one end not to exceed 0.04 mg/ml of parent antibiotics, using 5- μ l spots. Multiple applications of 5 μ l made for dilute samples

Solvent

Water satd. methyl ethyl ketone. Develop for 3 h without prior equilibration

Detection

Bioautography vs. *Bacillus subtilis* ATCC 6633. A 1% inoculum of a standard spore suspension used in Difco Penassay Base Agar adjusted to pH 6.5. Use 200 ml agar per plate measuring $8\frac{3}{4}$ in. × $17\frac{1}{2}$ in. Incubate overnight at 37°C. Measure max. zone widths and compare against standards at 0.05, 0.10 and 0.20 μ g plotted against zone widths on semi-logarithmic paper

R_F

Cephalosporin derivatives and their desacetyls and lactones moved; however, cephalosporin C itself and its desacetyl and lactone derivatives failed to move in this system

Ref.

R.P. Miller, *Antibiot. Chemother.*, 1962 (1963) 689–693

PC 11 (Quantitative procedure for radioactive cephalosporin)**Paper**

Whatman No. 1

Solvent

Cephalosporin C and deacetylcephalosporin C were placed for 16 h in a solvent system of propanol–pyridine–acetic acid–water (15:10:3:12)

Detection

- A. Two of triplicate chromatograms were scanned for radioactivity with a Vanguard Autoscaner model 880 instrument. The chromatogram containing the third sample and the two standards were plated on an indicator organism, *Pseudomonas solanaceum* Lilly X185, to obtain a bioautogram. From the radioactivity scans and the bioautograms, the locations of [9-¹⁴C]deacetylcephalosporin C and [9-¹⁴C]-cephalosporin C on the chromatograms were determined
- B. The sections of the chromatograms containing [9-¹⁴C]cephalosporin C and [9-¹⁴C]-deacetylcephalosporin C were removed and minced, and the antibiotics were eluted with 3 ml of water. The radioactivity in a 0.5 ml portion of each eluate was determined by scintillation counting

Ref.

D.R. Brannon, D.S. Fukuda, J.A. Mabe, F.M. Huber and J.G. Whitney, *Antimicrob. Agents Chemother.*, 1 (1972) 237–241

PC 12**Solvents**

- A. *n*-Butanol–acetic acid (10:1), satd. with water
- B. Water satd. *n*-Butanol + 1% glacial acetic acid
- C. *n*-Butanol–methanol–water (2:1:2)
- D. Water satd. *n*-butanol–glacial acetic acid (98:2)

Detection

- A. Bioautography vs. *Staphylococcus aureus*
- B. Ninhydrin + collidine
- C. Bioautography vs. *Staphylococcus aureus* after sprinkling with 1 M pyridine in acetone–water (1:1) and 1% phenylacetyl chloride in acetone

R_F (see p. 90)

Ref.

B. Fechtig, E. Vischer, H. Bickel, R. Bosshardt and J. Urech, *U.S. Patent* 3,697,515, Oct. 10, 1972

R_F

Component	R_F	Solvent	Detection
N-Phenylacetylcephalosporin C converted into the corresponding 7-aminocephalosporanic acid esters:			
Methyl ester	0.13	A	B or C
Ethyl ester	0.15	A	B or C
<i>n</i> -Butyl ester	0.16	A	B or C
N-Phenylacetylcephalosporin C diesters:			
Dimethyl ester	0.76	B	A
Diethyl ester	0.85	A	A
Dibutyl ester	0.87	B	A
N-2,4-Dinitrophenylcephalosporin C diesters:			
Dimethyl ester	0.73	A	
	0.76	B	
Diethyl ester	0.78	A	
Dibutyl ester	0.82	A	
	0.85	B	
7-[4-(1-Phenyl-2-thiono-5-oxo-imidazolidine-4-yl)-butyryl]-aminocephalosporanic acid			
	0.68	C	
	0.41	D	

* Detection B, color yellowish brown

PC 13

Solvent

n-Butanol-acetic acid-water (3:1:1)

Detection

Bioautography vs. *Bacillus subtilis* R_F

Separates deacetoxycephalosporin

Ref.

D.R. Brannon, J.A. Mabe and D.S. Fukuda, *J. Antibiot.*, 29 (1976) 121-124

PC 14

Paper

Whatman 3MM

Solvents

A. Isopropanol-water (70:30)

B. Butanol satd. with 0.5 M phosphate buffer (pH 7)

Detection

Bioautography

 R_F

Solvent	R_F
A	0.56
B	0

Ref.

E.O. Stapley, M. Jackson, S. Hernandez, S.B. Zimmerman, S.A. Currie, S. Mochales, J.M. Mata, H.B. Woodruff and D. Hendlin, *Antimicrob. Agents Chemother.*, 2 (1972) 122-131

PC 15

Paper

Whatman No. 1, buffered to pH 6 with 0.1 M sodium dihydrogen phosphate

Solvents

A. *n*-Butanol-ethanol-water (4:1:5), equilibrated at room temperature, upper phase in descending manner, in equilibrium with lower phase at 38°B. Ethyl acetate-*n*-butanol-0.1 M sodium acetate pH 5 (8:1:8) equilibrated at 38°C, the upper phase being used as developer in descending manner, in equilibrium with lower phase at 38°C, (paper buffered to pH 5 with 0.1 M sodium acetate) R_F

Cephalosporin	Solvent	R_F
3-(2-Cyanoallyl)-7 β -(2-thienylacetamido)ceph-3-em-4-carboxylic acid	A	0.63
	B	0.46

*Ref.*N.G. Weir, *U.S. Patent* 3,929,780, Dec. 30, 1975

PC 16

Paper

Whatman No. 1

Solvents

- A. *n*-Butanol-ethanol-water (4:1:5), upper phase, paper buffered to pH 5 with 0.1 *M* sodium acetate
- B. Ethyl acetate-*n*-butanol-0.1 *M* sodium acetate pH 5 (8:1:8) equilibrated at 38°C, upper phase, descending. Paper buffered to pH 5 with 0.1 *M* sodium acetate

R_F

Cephalosporin	Solvent	
	A	B
3-(2-Cyanoethyl)-7β-(2-thienyl-acetamido)ceph-3-em-4-carboxylic acid	0.6	0.28
3-Ethyl-7β-(2-thienylacetamido)-ceph-3-em-4-carboxylic acid		0.55
3-Ethyl-7β-phenoxyacetamido-ceph-3-em-4-carboxylic acid		2.47 *
3-Ethyl-7β-phenylthioacetamido-ceph-3-em-4-carboxylic acid		2.64 *
<i>tert</i> .-Butyl-3-ethyl-7β-phenylglyoxamidoceph-3-em-4-carboxylate		2.87 •
7β-Cyanoacetamido-3-ethyl-ceph-3-em-4-carboxylic acid		0.31 *

* *R_p* values. *R_p* = the *R_F* value divided by that of 3-acetoxymethyl-7β-(phenylacetamido)ceph-3-em-4-carboxylic acid

*Ref.*N.G. Weir, *U.S. Patent* 3,974,152, Aug. 10, 1976

TLC 1

Medium

Silica gel G

Solvents

- A. *n*-Butanol-glacial acetic acid (10:1) satd. with water
- B. *n*-Butanol-pyridine-glacial acetic acid-water (38:24:8:30)
- C. Benzene-acetone (4:1)
- D. Benzene-acetone (3:2)
- E. Toluene-acetone (3:2)
- F. Toluene-acetone (4:1)
- G. Toluene-acetone (7:3)

H. Toluene-ethyl acetate (1:1)

I. Toluene-acetone (9:1)

Detection

Color reaction. Spray with a soln. of 1 g ninhydrin in a mixture of 700 ml alcohol + 28 ml 2,4,6-col-lidine + 210 ml glacial acetic acid and heat at 90°C for 5-10 min

R_F

	Sol-vent	<i>R_F</i>
N-Phthalylcephalosporin		
C-dibenzhydryl ester	C	0.48
N-Phthalylcephalosporin		
C-9-benzhydryl ester	A	0.72
	B	0.68
N-Phthalylcephalosporin		
C-9-benzhydryl ester 1'-methyl ester	E	0.59
N- <i>tert</i> .-Butyloxycarbonyl-cephalosporin C dibenzyl ester	A	0.82
	D	0.72
	F	0.35
Cephalosporin-C dibenzhydryl ester	A	0.65
	B	0.78
	G	0.12
Cephalosporin-C dibenzyl ester	A	0.51
	D	0.18
Iso-7-aminocephalosporanic acid benzyl ester	A	0.72
	D	0.53
Piperidine-(6)-carbonic acid (2)-benzyl ester	A	0.65
	F	0.08
7-Aminocephalosporanic acid benzhydryl ester	H	0.31
Iso-7-aminocephalosporanic acid benzhydryl ester	H	0.23
7-Aminocephalosporanic acid	B	0.48
Piperidine-(6)-carbonic acid (2)-benzhydryl ester	A	0.72
7-Aminocephalosporanic acid	A	0.08
	B	0.40
7-Phenylacetamido-cephalosporanic acid	A	0.42
	B	0.62
7-Aminocephalosporanic acid benzhydryl ester	A	0.65
	B	0.74
	G	0.39
	F	0.33

	Solvent	R_F
N-Phthalyl-D- α -aminoadipic acid benzhydryl ester e-methyl ester	I	0.47
N-Phthalyl-DL- α -aminoadipic acid dimethyl ester	A	0.72
N-Phthalyl-D- α -aminoadipic acid e-methyl ester	A	0.63
	B	0.60

Ref.

B. Fechtig, H. Peter, H. Bickel and E. Vischer, *Helv. Chim. Acta*, 51 (1968) 1108–1119

TLC 2

Medium

Diethylaminoethyl cellulose

Solvent

0.05 M Sodium acetate buffer, pH 5.2

Detection

Color. Spray with ninhydrin reagent (0.3 g ninhydrin, 100 ml of 1-butanol and 3 ml of acetic acid)

 R_F

Cephalosporin C, 0.2

Ref.

C.H. Nash and F.M. Huber, *Appl. Microbiol.*, 22 (1971) 6–10

TLC 3

Medium

Silica gel

Solvents

A. *n*-Butanol–pyridine–acetic acid–water (42:24:4:30)

B. Ethyl acetate–*n*-butanol–pyridine–acetic acid–water (42:21:21:6:10)

C. *n*-Butanol–pyridine–acetic acid–water (34:24:12:30)

 R_F

	Solvent		
	A	B	C
Sodium salt of ethoxycarbonyl-acetamidocephalosporanic acid	0.32	0.35	0.52

	Solvent		
	A	B	C
Methoxycarbonyl-acetyl-amino-cephalosporanic acid	0.24	0.42	0.46

Detection

Iodine vapor

Ref.

H. Bickel, R. Bosshardt, E. Menard, J. Mueller and H. Peter, *U.S. Patent* 3,557,104, Jan. 19, 1971

TLC 4

Medium

Silica gel GF₂₅₄ (buffered with phosphate buffer at pH 5.8)

Solvent

Methanol–isopropanol–pH 5.8 buffer (125:25:6.5)

Detection

Iodine/sodium azide, temperature 110°C (gave a white spot)

 R_F

Cephalosporin	R_F
N-Acetoacetyl-cephalosporin C	0.68
N-Dodecanoyl-cephalosporin C	0.70

Ref.

C. Buitar, G. Mascellani and G. Guerra, *U.S. Patent* 3,931,161, Jan. 6, 1976

TLC 5

Medium

Silica gel

Solvents

A. Chloroform–ethyl acetate (80:20)

B. Chloroform–ethyl acetate (85:15)

C. Chloroform–ethyl acetate (50:50)

D. Acetone–acetic acid (95:5)

E. 0.5 M soln. of sodium chloride

Ref.

J. Bouchaudon, P. LeRoy and M.N. Messer, *U.S. Patent* 3,975,385, Aug. 17, 1976

R_F

Cephalosporin	Solvent				
	A	B	C	D	E
Trichloroethyl-6 β -trichloroacetamidopenicillanate 1 β -oxide	0.59				
Trichloroethyltrichloroacetamidopenicillanate	0.85				
3-Methyl-8-oxo-7-trichloroacetamido-2-trichloroethoxy-carbonyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene	0.63				
2- <i>p</i> -Methoxybenzyloxycarbonyl-3-methyl-8-oxo-7-trichloroacetamido-5-thia-1-azabicyclo[4.2.0]oct-2-ene	0.59				
β -Methoxybenzyl-6 β -trichloroacetamidopenicillanate 1 β -oxide	0.50				
4-(2-Benzothiazolyldithio)-3-trichloroacetamido-1-(1-trichloroethoxycarbonyl-2-methyl-prop-2-en-1-yl)-2-azetidinone		0.60			
4-(2-Benzothiazolyldithio)-3-phenylacetamido-1-(1-trichloroethoxycarbonyl-2-methyl-prop-2-en-1-yl)-2-azetidinone			0.70		
4-(5-Methyl-2-thiadiazolyldithio)-3-phenylacetamido-1-(1-trichloroethoxycarbonyl-2-methyl-prop-2-en-1-yl)-2-azetidinone			0.39		
4-(1-Methyl-5-tetrazolyldithio)-3-phenylacetamido-1-(1-trichloroethoxycarbonyl-2-methyl-prop-2-en-1-yl)-2-azetidinone			0.50		
2-Carboxy-7-chloroacetamido-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene				0.64	
7-Amino-2-carboxy-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene					0.40
2-Carboxy-3-methyl-8-oxo-7-trichloroacetamido-5-thia-1-azabicyclo[4.2.0]oct-2-ene				0.71	
7-Amino-2-carboxy-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene					0.40

TLC 6

Medium

Silica gel

Solvents

- A. *n*-Butanol–glacial acetic acid–water (67 : 10 : 23)
 B. *n*-Butanol–pyridine–glacial acetic acid–water (38 : 24 : 8 : 30)
 C. *n*-Butanol–pyridine–glacial acetic acid–water (37 : 24 : 12 : 30)

 R_F

Cephalosporin	Solvent		
	A	B	C
7-[(<i>p</i> -Aminopyridinium)-acetylamino]-cephalosporanic acid	0.08	0.37	0.44
7-Bromoacetylamino-cephalosporanic acid	0.39		

Cephalosporin

Solvent

A B C

Cephaloridine		0.39	0.44
7-[(<i>m</i> -Aminopyridinium)-acetylamino]-cephalosporanic acid	0.37	0.35	0.43

Ref.

H. Bickel, J. Muller, R. Bosshardt, H. Peter and B. Fechtig, *U.S. Patent* Re. 29,119, reissued Jan. 18, 1977

TLC 7

Medium

Silica gel (Merck F₂₅₄)

Solvents

- A. Ethyl acetate–benzene (2 : 1)
 B. Ethyl acetate–benzene (1 : 2)
 C. Benzene–ethyl acetate (2 : 1)

R_F

Compound	Solvent		
	A	B	C
<i>p</i> -Nitrobenzyl-7 β -phenylacetamido-3-methyl-3-cephem-4-carboxylate	0.34	0.34	
7 β -Phenylacetamido-3-methyl-3-cephem-4-carboxylate			0.74
7 β -Phthalimido-3-methyl-3-cephem-4-carboxylate			0.55

Ref.

L. Dall'Astra, *U.S. Patent* 3,959,266, May 25, 1976

TLC 8

Medium

Silica gel

Solvents

- A. Ethyl acetate-benzene (1:3)
 B. Methanol-chloroform (1:4)
 C. *n*-Butanol-acetic acid-water (4:1:1)

 R_F

Compound	Solvent		
	A	B	C
3-Acetoxyethyl-7 β -(3-isoxazolythioacetamido)-7 α -methoxy-3-cephem-4-carboxylic acid		0.34	
Benzhydryl-3-acetoxy-methyl-7 β -(3-isoxazolythioacetamido)-7 α -methoxy-3-cephem-4-carboxylate	0.37		
7 α -Methoxy-7 β -methylsulfonylaceta-mido-3-(1-methyl-1H-tetrazol-5-yl)thio-methyl-3-cephem-4-carboxylic acid			0.26

Compound	Solvent		
	A	B	C
7 β -(Sydnnon-3-acetamido)-7 α -methoxy-3-(1-methyl-1H-tetrazol-5-yl)-thiomethyl-3-cephem-4-carboxylic acid			0.31

Ref.

H. Nakao, H. Yanagisawa, M. Nagano, B. Shimizu, M. Kanedo and S. Sugawara, *U.S. Patent* 4,007,177, Feb. 8, 1977

TLC 9

Medium

Kieselgel G

Solvents

- A. Benzene-ethyl acetate (1:1)
 B. Benzene-ethyl acetate (2:1)

 R_F

Compound	Solvent	
	A	B
Diphenylmethyl-(6 <i>R</i> ,7 <i>R</i> ,2' <i>R</i>)-7-[2'-hydroxy-(<i>p</i> -nitrophenyl)-acetamido]-3-methylceph-3-em-4-carboxylate		0.40
Diphenylmethyl-(6 <i>R</i> ,7 <i>R</i> ,2' <i>S</i>)-7-[2'-hydroxy-(<i>p</i> -nitrophenyl)-acetamido]-3-methylceph-3-em-4-carboxylate		0.47
Diphenylmethyl-(6 <i>R</i> ,7 <i>R</i> ,2' <i>R</i>)-3-acetoxyethyl-7-[2'-hydroxy-(<i>p</i> -nitrophenyl)-acetamido]-ceph-3-em-4-carboxylate		0.1
Diphenylmethyl-(6 <i>R</i> ,7 <i>R</i> ,2' <i>S</i>)-3-acetoxyethyl-7-[2'-hydroxy-(<i>p</i> -nitrophenyl)-acetamido]-ceph-3-em-4-carboxylate		0.17
Diphenylmethyl-(6 <i>R</i> ,7 <i>R</i> ,2' <i>R</i>)-7-(2'-hydroxyphenylacetamido)-3-(5-methyl-1,3,4-thiadiazol-2-ylthiomethyl)ceph-3-em-4-carboxylate		0.25

Compound	Solvent	
	A	B
Diphenylmethyl-(6 <i>R</i> ,7 <i>R</i> ,2' <i>S</i>)-7-(2'-hydroxyphenylacetamido)-3-(5-methyl-1,3,4-thiadiazol-2-ylthiomethyl)-ceph-3-em-4-carboxylate	0.32	
Diphenylmethyl-(6 <i>R</i> ,7 <i>R</i> ,2' <i>R</i>)-7-[2'-hydroxy-2'-(fur-2-yl)-acetamido]-3-(5-methyl-1,3,4-thiadiazol-2-ylthiomethyl)-ceph-3-em-4-carboxylate	0.24	
Diphenylmethyl-(6 <i>R</i> ,7 <i>R</i> ,2' <i>S</i>)-7-[2'-hydroxy-2'-(fur-2-yl)-acetamido]-3-(5-methyl-1,3,4-thiadiazol-2-ylthiomethyl)-ceph-3-em-4-carboxylate	0.31	
Diphenylmethyl-(6 <i>R</i> ,7 <i>R</i> ,2' <i>R</i>)-7-[2'-hydroxy-2'-(thien-3-yl)acetamido]-3-(5-methyl-1,3,4-thiadiazol-2-ylthiomethyl)-ceph-3-em-4-carboxylate	0.44	
Diphenylmethyl-(6 <i>R</i> ,7 <i>R</i> ,2' <i>S</i>)-7-[2'-hydroxy-2'-(thien-2-yl)-acetamido]-3-(5-methyl-1,3,4-thiadiazol-2-ylthio)-ceph-3-em-4-carboxylate	0.365	

Ref.

M.C. Cook and B. Laundon, *U.S. Patent* 3,974,150, Aug. 10, 1976

TLC 10

Medium

Cellulose

Solvents

- A. Isopropanol-formic acid-water (77:4:19)
 B. *n*-Butanol-acetic acid-pyridine-water (37.5:7.5:25:30)
 C. 66% Aq. acetonitrile
 D. *n*-Butanol-acetic acid-water (11:3:7)

 R_F

Compound	Solvent			
	A	B	C	D
N-Acetyldeacetoxycephalosporin C (from fermentation)	0.79	0.59	0.72	0.77

Compound	Solvent			
	A	B	C	D
Deacetoxycephalosporin C	0.32	0.38	0.45	0.51
Cephalosporin C	0.25	0.42	0.60	0.49
N-Acetylcephalosporin C	0.76	0.63	0.77	0.71
N-Acetyldeacetoxycephalosporin C (authentic sample)	0.79	0.60	0.72	0.76

Ref.

P. Traxler, H.J. Treichler and J. Nuesch, *J. Antibiot.*, 28 (1975) 605-606

TLC 11

Medium

Silica gel

Solvents

- A. Benzene-acetone (6:4)
 B. Benzene-acetone (8:2)
 C. Cyclohexane-ethyl acetate (1:1)
 D. Chloroform-methanol (95:5)
 E. *n*-Butanol-pyridine-glacial acetic acid-water (30:20:6:24)
 F. Toluene-acetone (3:1)

 R_F

Component	R_F	Solvent
N-Phthaloylcephalosporin C dibenzyl ester	0.74	A (UV at 264)
N-Phthaloylcephalosporin C dibenzhydryl ester	0.49 0.28	B C
N-2,4-Dinitrophenylcephalosporin C di- <i>p</i> -nitrophenyl ester	0.70	D
Desacetoxy-7-aminocephalosporanic acid	0.48	E
Desacetoxy-7-phenylacetylaminoccephalosporanic acid benzylhydryl ester	0.61	F

Ref.

As PC 11

TLC 12

Medium

Silica gel. Selecta TLC Plastic Sheets F 1500 LS
254 (Schleicher and Schull)

B. Acetonitrile-methanol-water (6:3:1)

C. Acetonitrile-water (80:20)

Solvents

A. Methyl isobutyl ketone-methanol-formic acid
(60:6:2)

Detection

A. UV

B. Bromocresol green and potassium manganate

R_F

All $R_F \pm 0.10$

Compound	Solvent	Detection	R_F
7-(3-Methylureido)-3-(2-methyl-1,3,4-thiadiazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid	A	B	0.10
7-(3-Methylureido)-3-(2-thiazolin-2-ylthiomethyl)-3-cephem-4-carboxylic acid	A	B	0.09
7-(3-Ethylureido)-3-desacetoxycephalosporanic acid	A	B	0.34
7-(3-Ethylureido)-3-(2-methyl-1,3,4-thiadiazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid	A	B	0.17
7-(3-Ethylureido)-3-(2-thiazolin-2-ylthiomethyl)-3-cephem-4-carboxylic acid	A	B	0.17
7-[3-(2-Chloro-ethylureido)]-3-(2-methyl-1,3,4-thiadiazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid	B	A	0.49
7-(3- <i>n</i> -Propylureido)-3-(2-methyl-1,3,4-thiadiazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid	C	B	0.39
7-(3-Isopropylureido)-3-desacetoxycephalosporanic acid	A	B	0.25
7-(3-Isopropylureido)-3-(2-methyl-1,3,4-thiadiazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid	A	B	0.19
7-(3-Isopropylureido)-3-(2-thiazolin-2-ylthiomethyl)-3-cephem-4-carboxylic acid	A	B	0.24
7-(3-Allylureido)-3-desacetoxycephalosporanic acid	A	B	0.28
7-(3-Allylureido)-3-(2-thiazolin-2-ylthiomethyl)-3-cephem-4-carboxylic acid	A	B	0.14
7-(3-Allylureido)-3-(2-methyl-1,3,4-thiadiazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid	A	B	0.12
7-(3- <i>n</i> -Butylureido)-3-(2-methyl-1,3,4-thiadiazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid	C	B	0.48
7-(3- <i>n</i> -Butylureido)-3-(2-methyl-1,3,4-triazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid	B	A	0.51
7-(3- <i>n</i> -Hexylureido)-3-(2-methyl-1,3,4-thiadiazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid	B	A	0.58
7-(3-Benzylureido)-3-(2-methyl-1,3,4-thiadiazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid	B	B	0.56
7-(3- <i>m</i> -Tolylureido)-3-(2-methyl-1,3,4-thiadiazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid	A	B	0.28
7-(3- <i>p</i> -Fluorophenylureido)-3-(2-methyl-1,3,4-thiadiazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid	A	B	0.29
7-(3-Cyclohexylureido)-3-desacetoxycephalosporanic acid	A	B	0.37
7-(3-Cyclohexylureido)-3-(2-methyl-1,3,4-thiadiazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid	C	B	0.46
7-(3-Ethylthioureido)-3-(2-methyl-1,3,4-thiadiazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid	B	A	0.56
7-(3-Allylureido)-cephalosporanic acid	A	B	0.25

Ref.

P. Crooij and A. Colinet, *U.S. Patent* 3,912,728, Oct. 14, 1975

TLC 13

Media

- A. Silica gel
- B. Cellulose

Solvents

- A. Chloroform-methanol-acetic acid (90:16:5)
- B. 70% aq. *n*-Propanol

 R_F

Compound	Medium	Solvent	
		A	B
(6 <i>R</i> ,7 <i>R</i>)-7-[2-(Fur-2-yl)-2-methoxyimino-acetamido]-3-hydroxy-methylceph-3-em-4-carboxylic acid	A	0.17	—
(6 <i>R</i> ,7 <i>R</i>)-7-(<i>D</i> -Benzoyl-amino-5-diphenyl-methoxycarbonylpent-amido)-3-hydroxy-methylceph-3-em-4-carboxylate	B	—	0.59
(6 <i>R</i> ,7 <i>R</i>)-7-(4-Carboxy-butanamido)-3-hydroxymethylceph-3-em-4-carboxylic acid	B	—	0.47

Ref.

A. Smith and S.A. Goulden, *U.S. Patent* 3,912,598, Oct. 14, 1975

TLC 14

Medium

Silica gel. A 200-mg sample of (I)* in 100 ml of 0.1 N hydrochloric acid is stirred mechanically at 45°C for 2 h. The soln. is cooled to room temperature, 5 ml is diluted to 10 ml with phosphate buffer, pH 7 (2.65 g of potassium dihydrogen phosphate and 5.31 g of dipotassium hydrogen phosphate in 100 ml of water) and 10 μ l of this soln. plus reference loadings of (I), (II)** and (III)*** in phosphate buffer are then spotted onto a Merck Silica gel F₂₅₄ plate
 * I = 7-Aminocephalosporanic acid
 ** II = Deacetyl-7-aminocephalosporanic acid (which it is suggested then forms the corresponding lactone (III)***)

Solvent

This plate is then developed in aq. 0.5 M sodium chloride soln. until the solvent is within 1 cm of the top, development time being 2 h

Detection

- A. After drying the plate, initial visualization is effected by UV light (254 nm)
- B. Alternative visualization of the plate is achieved by spraying the plate with a modified form of a reagent described for the detection of primary amines on TLC plates. This consists of two solutions: 1% 2,5-dimethyloxetrahydrofuran in glacial acetic acid and 1% dimethylamino-benzaldehyde in 15% conc. hydrochloric acid in glacial acetic acid, mixed just before use in the ratio 1:2. After heating the plate at 100°C for 3 min, the compounds show up as red spots on a yellow background. The limit of detection of the named compounds using this reagent is 0.05 μ g

 R_F

Compound	R_F
Deacetyl-7-ACA	0.58
7-ACA	0.44
7-ACA lactone	0.29
Reaction soln. components	0.58, 0.44, 0.29

Ref.

C.J. Budd, *J. Chromatogr.*, 76 (1973) 509-511

TLC 15

Medium

Cellulose

Solvents

- A. *n*-Butanol-acetic acid-water (3:1:1)
- B. *n*-Propanol-water (7:3)
- C. Acetonitrile-water (8:2)
- D. Isopropanol-water-pyridine (65:30:5)
- E. Methanol-*n*-propanol-water (6:2:1)
- F. Ethyl acetate-acetone-water (2:4:2)

 R_F

Deacetylcephalosporin C

Solvent	R_F
A	0.16
B	0.22
C	0.03
D	0.25
E	0.13
F	0.19

Ref.

Y. Fujisawa, M. Kikuchi and T. Kanzaki, *J. Antibiot.*, 30 (1977) 775-777

TLC 16

Medium

Kieselgel (Merck)

Solvents

- A. Benzene-ethyl acetate (5:1)
B. Benzene-ethyl acetate (2:1)
C. Benzene-ethyl acetate (3:1)

 R_F

Cephalosporin	Solvent		
	A	B	C
<i>tert.</i> -Butyl-3-(2-cyano-allyl)-7 β -(2-thienylacetamido)-ceph-3-em-4-carboxylate	0.3		
<i>tert.</i> -Butyl-3-(2-methoxycarbonallyl)-7 β -(2-thienylacetamido)-ceph-3-em-4-carboxylate		0.6	
<i>tert.</i> -Butyl-3-(2-ethoxycarbonallyl)-7 β -(2-thienylacetamido)-ceph-3-em-4-carboxylate		0.75	
<i>tert.</i> -Butyl-3-(2-diphenylmethoxycarbonylallyl)-7 β -(2-thienylacetamido)-ceph-3-em-4-carboxylate	0.5		
<i>tert.</i> -Butyl-7 β -(D-2- <i>tert.</i> -butoxycarbonylamino-2-phenylacetamido)-3-(2-methoxycarbonylallyl)-ceph-3-em-4-carboxylate			0.75
<i>tert.</i> -Butyl-7 β -(D-2- <i>tert.</i> -butoxycarbonylamino-2-phenylacetamido)-3-(2-cyanoallyl)-ceph-3-em-4-carboxylate	0.4		
<i>tert.</i> -Butyl-7 β -(D-2- <i>tert.</i> -butoxycarbonylamino-2-phenylacetamido)-3-(2-diphenylmethoxycarbonylallyl)-ceph-3-em-4-carboxylate	0.5		

Ref.

As PC 13

TLC 17

Medium

Kieselgel (Merck)

Solvents

- A. Benzene-ethyl acetate (3:1)
B. Benzene-ethyl acetate (8:1)
C. Chloroform + chloroform-ethyl acetate (8:1)

 R_F

Cephalosporin	Solvent		
	A	B	C
Diphenylmethyl-7 β -(D-2- <i>tert.</i> -butoxycarbonylamino-2-phenylacetamido)-3-ethyl-ceph-3-em-4-carboxylate	0.8		
Diphenylmethyl-3-(<i>cis</i> -2-cyanovinyl)-7 β -(2-thienylacetamido)-ceph-3-em-4-carboxylate		0.6	
Diphenylmethyl-3-(2-cyanoethyl)-7 β -(2-thienylacetamido)-ceph-3-em-4-carboxylate		0.4	0.5

Ref.

As PC 16

TLC 18

Medium

Silica gel

Solvents

- A. 10% Tartaric acid
B. 10% Tartaric acid satd. with *n*-butanol

Detection

- A. Iodine vapor
B. 0.25% Stannous chloride in 20% hydrochloric acid
C. 2% *p*-Dimethylaminobenzyldehyde in 1 *N* hydrochloric acid

Ref.

R.T. Wang and Y.H. Tsai, *Tai-Wan K'o Hsueh*, 27 (1973) 36-39

R_F

Derivative	Solvent		Detection		
	A	B	A	B	C
Sodium cephalothin	0.65	0.82	—	—	light yellow
Cephaloridine	0.35	0.50	dark brown	—	light yellow

TLC 19

Medium

Reversed-phase TLC. Non-polar stationary phase: silica gel plate (Merck 60) impregnated with silicone oil TSF451, 10 cS (Toshiba Silicone Co.)

Solvent

Polar mobile phase: sodium acetate–Veronal buffer (pH 7.0) containing 2% acetone and satd. with the silicone oil

Detection

Spray with 5% potassium permanganate

Before the coating, the silica gel plate was activated by heating for 15 min at 110°C; β -lactam antibiotics were dissolved in distilled water to give about 3 mg/ml and about 1 μ l of soln. was spotted on the thin-layer plate

 R_F

Compound	R_F
Ceftazole	0.68
Cefazolin	0.52
Cephalexin	0.47
Cephalothin	0.37
Cephaloridine	0.13

Ref.

T. Sawai, K. Matsuba, A. Tamura and S. Yamagishi, *J. Antibiot.*, 32 (1979) 59–65

TLC 20

Medium

Silica gel G. The chromatoplates were coated to a thickness of 500 μ m using a standard Desage spreader. Silica gel G (Merck) was applied according to Stahl's method. The plates were dried at room temperature and activated at 120°C for 30 min

Solvents

A. *n*-Butanol–water–ethanol–acetic acid (5 : 2 : 1.5 : 1.5)

B. *n*-Butanol–water–acetic acid (4 : 1 : 1)

C. Acetone–acetic acid (95 : 5)

D. 85% Aq. acetone

The plates were inserted in previously equilibrated filter paper-lined tanks and each solvent was allowed to rise to a height of 14 cm (approximate times taken: (A) 150 min, (B) 120 min, (C) 20 min, (D) 30 min

Detection

Each TLC plate was spread consecutively with the following reagents: (a) 2 *N* sodium hydroxide; (b) the iodine–azide reagent of Awe et al., *Naturwissenschaften*, 41 (1954) 528; (c) 1% starch soln.

 R_F

Product	Solvent			
	A	B	C	D
Cephalosporin C	28 *	14 *	0 *	10 *
Ceporin	10	7	0	7
7-Aminocephalosporanic acid	40	21	43	40

* $R_F \times 100$

Ref.

E.J. Vandamme and J.P. Voets, *J. Chromatogr.*, 71 (1972) 141–148

TLC 21

Medium

Silica gel F₂₅₄ (Merck). A 200-mg sample of 7-ACA in 100 ml of 0.1 *N* hydrochloric acid is stirred mechanically at 450 for 2 h. The soln. is cooled to room temperature, 5 ml is diluted to 10 ml with phosphate buffer, pH 7 (2.65 g of potas-

sium dihydrogen phosphate and 5.31 g of dipotassium hydrogen phosphate in 100 ml of water), and 10 μ l of this soln. plus reference loadings of 7-ACA, deacetyl-7-ACA, and 7-ACA-lactone in phosphate buffer are then spotted. The addition of a small amount of a simple inorganic salt such as sodium chloride to water, prior to development of a Merck precoated silica gel F₂₅₄ plate, leaves the thin layer intact.

Solvent

Aq. 0.5 M sodium chloride developed to within 1 cm of the top, 2 h

Detection

UV at 254 nm

R_F

Product	R _F
Deacetyl-7-ACA	0.58
7-ACA	0.44
7-ACA lactone	0.29

Ref.

C.J. Budd, *J. Chromatogr.*, 76 (1973) 509-511

TLC 22

Medium

Silica gel

Solvent

n-Butanol-acetic acid-water (2:1:1)

R_F

7-(5-Amino-5-carboxyvaleramido)-7-methoxy-3-hydroxymethyl-3-cephem-4-carboxylic acid, 0.31

Ref.

T. Shomura, H. Watanabe, Y. Ogawa, K. Ohba, Y. Kondo, M. Kohima, S. Inoje and T. Niida, *U.S. Patent* 3,974,035, Aug. 10, 1976

TLC 23

Medium

Silica gel

Solvent

n-Butanol-acetic acid-water (3:1:1)

R_F

7-Cyanomethylthioacetamido-3-methoxy-3-cephen-4-carboxylic acid, 0.47

Ref.

W.H. Koster, W.A. Slusarchyk and J. Bernstein, *U.S. Patent* 3,985,746, Oct. 12, 1976

TLC 24

Medium

Silica gel

Solvents

- A. Chloroform-ethyl acetate (2:1)
- B. Ethyl acetate-chloroform (1:1)
- C. Chloroform-ethyl acetate (3:1)

R_F

Cephalosporin	Solvent		
	A	B	C
2-(Methylthio)-7-(phenyl-acetamido)-3-desacetoxy-cephalosporanic acid <i>tert</i> -butyl ester sulfoxide	Band following the starting material band with the lowest R _F		
2-(Methylthio)-7-(phenyl-acetamido)-3-desacetoxy-cephalosporanic acid tri-chloromethyl ester sulfoxide		Not given	
2-(Methylthio)-7-(phenoxy-acetamido)-3-desacetoxy-cephalosporanic acid <i>p</i> -methoxy-benzyl ester sulfoxide			Band following the starting material band with the lowest R _F

Ref.

W.H. Koster and J.E. Dolfini, *U.S. Patent* 3,968,109, July 6, 1976

TLC 25

Media

- A. Silica gel
- B. Cellulose

Solvents

- A. *n*-Butanol-glacial acetic acid-water (11:3:7)
- B. Isopropanol-formic acid-water (74:4:19)
- C. *n*-Butanol-acetic acid-water (67:10:23)

R_F

Cephalosporin	Medium	Solvent	R_F
7-Amino-3-methoxy-ceph-3-em-4-carboxylic acid	A	A	0.51
	A	B	0.33
7-Amino-3-methoxy-3-ceph-4-em-carboxylic acid	B	C	0.16

Ref.

M. Liersch and J. Nuesch, *U.S. Patent* 3,962,036, June 8, 1976

TLC 26

Medium

Silica gel

Solvents

A. *n*-Butanol-ethanol-water (4:1:5)B. *n*-Propanol-water (7:3) R_F

Compound	R_{pac}^* ; Solvent:	
	A	B
Sodium (6 <i>R</i> ,7 <i>R</i>)-3-carbamoyloxy-methyl-7-[2-(fur-2-yl)-2-phenoxy-iminoacetamido]-ceph-3-em-4-carboxylate (<i>syn</i> isomer)	1.1	
(6 <i>R</i> ,7 <i>R</i>)-3-Carbamoyloxy-methyl-7-(2-methoxyimino-2-phenylacetamido)-ceph-3-em-4-carboxylate acid (<i>syn</i> isomer)		1.0
Sodium (6 <i>R</i> ,7 <i>R</i>)-3-carbamoyloxymethyl-7-(2-phenoxyimino-2-phenylacetamido)-ceph-3-em-4-carboxylate (<i>syn</i> isomer)	1.1	1.2
(6 <i>R</i> ,7 <i>R</i>)-3-Carbamoyloxymethyl-7-[2-cyclopentylloximino-2-(fur-2-yl)-acetamido]-ceph-3-em-4-carboxylic acid (<i>syn</i> isomer)	1.0	
(6 <i>R</i> ,7 <i>R</i>)-3-Carbamoyloxy-methyl-7-(2-ethoxyimino-2-phenylacetamido)-ceph-3-em-4-carboxylic acid (<i>syn</i> isomer)	1.0	

Compound

 R_{pac}^* ; Solvent:

A B

Sodium (6*R*,7*R*)-3-carbamoyloxymethyl-7-[2-*tert*-butoxyimino-2-(thien-2-yl)-acetamido]-ceph-3-em-4-carboxylate (*syn* isomer)

1.05 1.1

* $R_{pac} = R_F$ of compound divided by R_F of (6*R*,7*R*)-3-acetoxymethyl-7-phenylacetamidoceph-3-em-4-carboxylic acid

Ref.

M.C. Cook, G.I. Gregory and J. Bradshaw, *U.S. Patent* 3,974,153, Aug. 10, 1976

TLC 27

Medium

Silica gel

Solvent

Chloroform-acetone-acetic acid (50:50:7) developed 1.5 h

Detection

A. UV

B. 0.1 *N* sodium hydroxide at 100° R_F (estimated from drawing)

Compound	Migration (cm from origin)	
	H *	U *
Cephalothin	1, 2, 10-12	3.5
Cephalexin	0-2, 10-12	1.0
Cefazolin	1.0	1.7
Cephaloglycin	0.7, 2, 12.5	0.5
Cephalexin	2, 4.4, 8, 12.5	0.7
Cefamandole	12.5	2.5

* H = hydrolyzed; U = unhydrolyzed

Ref.

A.B.C. Yu, C.H. Nightingale and D.R. Flanagan, *J. Pharm. Sci.*, 66 (1977) 213-216

TLC 28

Medium

Cellulose

Solvent

n-Butanol-glacial acetic acid-water (3:1:1)

Detection

A. Ninhydrin

B. Iodine

 R_F

Cephalosporin N, 0.3

Ref.

K. Nara, K. Ohta, K. Katamoto, N. Mizokami and H. Fukuda, *U.S. Patent* 3,926,973, Dec. 16, 1975

TLC 29

Medium

Silica gel

Solvents

- A. Ethyl acetate–pyridine–glacial acetic acid–water (62:21:6:11)
B. *n*-Butanol–glacial acetic acid–water (67:10:23)

R_F

Component	Solvent	
	A	B
O-Desacetyl-O-(N-β-chlorethyl-carbamyl)-7-N-[D-(–)-α-phenylglycyl]-aminocephalosporanic acid	0.08	0.24
7-N-[N-Carbo- <i>tert.</i> -butoxy-D-(–)-α-phenylglycyl]-aminocephalosporanic acid	0.59	0.47
O-Desacetyl-7-N-[N-carbo- <i>tert.</i> -butoxy-D-(–)-α-phenylglycyl]-aminocephalosporanic acid	0.37	0.38
O-Desacetyl-O-(N-β-chlorethyl-carbamyl)-7-N-[N-carbo- <i>tert.</i> -butoxy-D-(–)-α-phenylglycyl]-aminocephalosporanic acid	0.63	0.50
O-Desacetyl-O-(N-methylcarbamoyl)-7-N-[D-(–)-α-phenylglycyl]-aminocephalosporanic acid trifluoroacetate	0.05	0.13
O-Desacetyl-O-(N-methylcarbamoyl)-7-N-[D-(–)-α-phenylglycyl]-aminocephalosporanic acid	0.05	0.13
O-Desacetyl-O-(N-methylcarbamoyl)-7-N-[N-carbo- <i>tert.</i> -butyloxy-D-(–)-α-phenylglycyl]-aminocephalosporanic acid	0.50	0.50
O-Desacetyl-O-(N-ethylcarbamoyl)-7-N-[D-(–)-α-phenylglycyl]-aminocephalosporanic acid trifluoroacetate	0.07	0.18

Component	Solvent	
	A	B
O-Desacetyl-O-(N-ethylcarbamoyl)-7-N-[D-(–)-α-phenylglycyl]-aminocephalosporanic acid	0.07	0.18
O-Desacetyl-O-(N-ethylcarbamoyl)-7-N-[N-carbo- <i>tert.</i> -butyloxy-D-(–)-α-phenylglycyl]-aminocephalosporanic acid	0.55	0.52

Ref.

R. Bosshardt, B. Fechtig, J. Mueller, H. Peter and H. Bickel, *U.S. Patent* 3,706,746, Dec. 19, 1972

TLC 30

Medium

Silica gel

Solvents

- A. Chloroform–methanol–formic acid (95:5:1)
B. *n*-Butanol–acetic acid–water (3:1:1)

R_F

	Solvent	Medium	<i>R_F</i>
7-(D-α- <i>tert.</i> -Butoxycarbonylaminophenylacetamido)-3-methyl-3-cephem-4-carboxylic acid	A	B	0.24
7-(D-α- <i>tert.</i> -Butoxycarbonylaminophenylacetamido)-3-acetoxymethyl-3-cephem-4-carboxylic acid	B	A	Single spot
7-(α-Carboxyphenylacetamido)-3-methyl-3-cephem-4-carboxylic acid	B	B	0.45

	Sol-vent	Med-ium	R_F
7-(α -Carboxy-phenylacet-amido)-3-acetoxymethyl-3-cephem-4-carboxylic acid	B	B	0.37 (main) 0.58 (minor)
7-(D- α -Formyl-aminophenyl-acetamido)-3-methyl-cephem-4-carboxylic acid	B	B	0.76

Ref.

S. Seki, S. Sugimoto and S. Nakajima, *U.S. Patent* 3,988,326, Oct. 26, 1976

ELPHO 1**Medium**

Whatman No. 1 paper

Buffer

Aq. collidine-acetate soln. 0.05 *M* to acetate, pH 7.0

Conditions

17 V/cm, 2.5–4 h

Detection

As PC 3

Mobility

Cephalosporin C_A derived from	Electro-phoretic mobility •
Nicotine	– 1.4
2-Aminopyridine	– 0.6
2-Amino-6-methylpyridine	– 0.64
Pyridine	– 0.4
Nicotinamide	– 0.4
2,4,6-Trimethylpyridine	– 0.4
2-Hydroxymethylpyridine	– 0.4
Quinoline	– 0.4
Sulphapyridine	+ 0.25
Sulphadiazine	+ 0.25
Sulphathiazole	+ 0.25
3-Hydroxypyridine	+ 0.75
Isonicotinic acid	+ 0.55
Nicotinic acid	+ 1.0
Picolinic acid	+ 0.75
Pyridine-2,3-dicarboxylic acid	+ 3.2

• + = migration toward anode; – = migration toward cathode

Ref.

As PC 3

ELPHO 2**Medium**

Whatman No. 1 paper

Buffers

A. Collidine-acetate, 0.05 *M* to acetate, pH 7.0

B. Pyridine-acetate, 0.05 *M* to acetate, pH 4.5

C. Acetic acid 10% (v/v), pH 2.2

Conditions

14 V/cm, 2–5 h

Detection

As PC 2

Ref.

As PC 2

Mobility

	Migration (cm) with solvent		
	A	B	C
Deacetylcephalo- sporin C	+4.3	+7.2	–1.2
Cephalosporin C	+4.2	+6.8	–0.6
Cephalosporin C_C	–1.0	–1.7	–4.4
Cephalosporin C_A (pyridine)	–1.0	–1.7	–

ELPHO 3**Medium**

As ELPHO 2

Buffer

A. As ELPHO 2, A

B. As ELPHO 2, B

C. As ELPHO 2, B, but pH 4.0

D. Formic acid, 10% (v/v), pH 1.5

E. Acetic acid, 10% (v/v), pH 2.2

Detection

Bioautography vs. *Salmonella typhi*

Mobility (see p. 104)**Ref.**

As PC 4

Mobility

	Migration (cm) in buffer				
	A	B	C	D	E
Cephalosporin C	+5.2	+7.5	+7.2	-3.2	-0.4
N-Phenylacetylcephalosporin C	+8.2	+12.0	-	-	-
Cephalosporin C _C	-1.5	-2.0	-	-4.0	-5.0
N-Phenylacetylcephalosporin C _C	+3.4	-	-	-	-
Cephalosporin C _A pyridine	-1.5	-	-2.9	-	-4.7
Cephalosporin C _A (pyridine) nucleus	-1.5	-	-9.8	-	-9.9
Cephalosporin C _C nucleus	-1.5	-4.5	-	-	-13.1
Penicillin nucleus (6-APA)	+7.5	+2.1	0.0	-6.5	-10.0
Cephalosporin C nucleus (7-ACA)	+7.5	+3.4	+1.0	-4.5	-4.0
N-Phenylacetyl derivative of 6-APA (benzylpenicillin)	+5.2	+7.5	-	-	-
N-Phenylacetyl derivative of 7-ACA	+5.1	+7.5	-	-	-

ELPHO 4

Medium

Paper, Schleicher and Schuell SS-598

Buffer

0.165 M, pH 7.0 phosphate buffer

Conditions

0 to 5°C for 2.5 h at 600 V

Detection

Bioautography

Mobility

Cephalosporin C, -5.2

Ref.

As PC 14

ELPHO 5

Medium

Whatman No. 1 paper

Buffer

0.1 M ammonium carbonate, pH 9.0

Conditions

45 V/cm

Detection

A. UV light

B. Ninhydrin spray

C. 10% Aq. silver nitrate

D. Ammoniacal silver nitrate (10 ml aq. 10% silver nitrate + 10 ml ammonium hydroxide + 80 ml methanol)

E. Starch-iodine (10 ml of 10 mM iodine in 3 mM potassium iodide + 9 ml aq. 2% soluble starch + 1 ml M phosphate buffer, pH 7)

F. Platinichloride reagent [27 ml M potassium iodide + 5 ml of 5% potassium platinum chloride (K₂PtCl₆) + 100 ml water]

Mobility

Cephalosporin C moved 6 cm towards anode

Ref.

J.M.T. Hamilton-Miller, G.G.F. Newton and E.P. Abraham, *Biochem. J.*, 116 (1970) 371-384

ELPHO 6

Medium

Whatman 3MM paper

Buffer

Formic acid-glacial acetic acid-acetone (5:25:30) diluted to 1 l with water

Conditions

20 V/cm for 2 h

Detection

Spray with iodoplatinate reagent; examine immediately under visible light

Mobility

Cephadrine, Cephaloglycin, Cephalixin, Cephalozin, Cephalothin, and Cephaloridine can be separated by comparison of *R_F* values and colors of the zones

Ref.

J.L. Martin, R.E. Duncombe and W.H.C. Shaw, *Analyst (London)*, 100 (1975) 243-248

CCD

Solvent

Hexane-diisopropyl ether-acetone-0.5 M potassium phosphate, pH 6.0 (25:8:25:25); lower phase mobile

Distribution

Using a 15 tube distribution, most activity was present in the central fractions (peak activity tube No. 8)

Ref.

As PC 4

HPLC 1*Apparatus*

Varian liquid chromatograph 4000

Column

Zipax SAX column, 1.8 × 150 cm

Mobile phase

0.5–0.25 M borate buffer, pH 9.6

Flow-rate

0.45–0.7 ml/min

Retention time (min)

Compound	
N-Acetyldeacetoxycephalosporin C (from fermentation)	35
Deacetoxycephalosporin C	17.5
Cephalosporin C	22
N-Acetylcephalosporin C	48
N-Acetyldeacetoxycephalosporin C (authentic sample)	35

Ref.

As TLC 10

HPLC 2*Apparatus*

Varian LC 4200

Column

Micropak CH, 10 μm, 25 cm × 2.1 mm I.D.

Mobile phase

10% Methanol in water with 0.1 M phosphoric acid

Flow-rate

60 ml/h (70 atm)

Temperatures

50°C

Detector

Variscan 260 nm

Chart speed

0.5 cm/min

Sensitivity

0.1 a.u.f.s.

Sample size

5 μl

Recorder

A-25, 10 mV

Type

Reversed-phase

Retention time (estimated from drawing)

Cephalexin	3.0
7-Aminodesacetoxy- cephalosporanic acid	0.7

Ref.

Varian Aerograph Application Notes, August (1974) No. 6

HPLC 3*Column*

1 m × 2.1 mm I.D. × 6.3 mm O.D., 316 stainless steel, C₁₈/Porasil B packing

Mobile phase

Water

Pressure

500 p.s.i.

Flow-rate

1.1 ml/min

Detector

UV (254 nm)

Sensitivity

0.08 a.u.f.s.

Type

Reversed-phase

Retention time (min) (estimated from drawing)

7-Aminodeoxy- cephalosporanic acid	0.05
Cephalosporin C	4.0
7-Tetrazolylaminocephalo- sporic acid	10.5
7-Aminocephalosporanic acid thiadiazol	21.0

Ref.

As Ampicillin, HPLC

HPLC 4*Column*

1 m × 2.1 mm I.D. × 6.3 mm O.D., 316 stainless steel. Vydac-RP packing

Mobile phase

5% Methanol, 95% 0.05 M ammonium carbonate

Pressure

700 p.s.i.

Flow-rate

0.7 ml/min

Detector

UV (254 nm)

Sensitivity

0.08 a.u.f.s.

Type

Reversed-phase

Retention time

Cephalosporin, 5.5 min (estimated from drawing)

Ref.

As Ampicillin, HPLC

HPLC 5*Column*

1 m × 2.1 mm I.D. × 6.3 mm O.D., 316 stainless steel. ODS-Sil-X-II packing

Mobile phase

5% Methanol, 95% 0.05 M ammonium carbonate

Flow-rate

0.5 ml/min

Pressure

800 p.s.i.

Detector

UV (254 nm)

Sensitivity

0.08 a.u.f.s.

Type

Reversed-phase

Retention time (min) (estimated from drawing)

Cephaloridine 5.0

Cefazolin 8.5

Cephadrine 14.0

Cephaloglycin 23.0

Cephalothin 30.0

Ref.

As Ampicillin, HPLC

HPLC 6**Apparatus**

Varian 4100 liquid chromatograph equipped with a Varian fixed-wavelength UV 254 monitor containing an 8- μ l flow cell

Column

Stainless-steel tube (500 $\times$ 2.1 mm I.D.) fitted at the column outlet with a 2- μ m filter union at low dead volume

Mobile phases and conditions

- I. Mobile phase, water; flow-rate, 1 ml/min; temperature, 25°
- II. Mobile phase, sodium phosphate buffer, 10 mM, pH 7.5: a 10 mM sodium dihydrogen phosphate soln. was adjusted to pH 7.5 with sodium hydroxide (mobile phase IIa). Less conc. buffer soln. (1 mM, mobile phase IIb): 0.5 mM, mobile phase IIc) were prepared by dilution of the 10 mM buffer. Flow-rate, 1 ml/min; temperature, 25°
- III. Mobile phase, aq. soln. containing 1 mM sodium phosphate buffer and 1 mM trioctylmethylammonium hydrogen chloride; flow-rate, 1 ml/min; temperature, 25°
- IV. Mobile phase, aq. soln. containing 1 mM trioctylmethylammonium hydrogen chloride in 10 mM sodium phosphate buffer; flow-rate, 1 ml/min; temperature 25°
- V. Mobile phase, aq. soln. containing 1 mM trioctylmethylammonium hydrogen chloride and 7.5% methanol in 10 mM sodium phosphate buffer; flow-rate, 1 ml/min; temperature, 25°
- VI. Mobile phase, aq. soln. containing 1 mM trioctylmethylammonium hydrogen chloride,

1 mM tetraethylammonium hydroxide and 7.5% methanol in 10 mM sodium phosphate buffer; flow-rate, 1 ml/min; temperature, 20°, 25°, 30°, 55°

- VII. Mobile phase, aq. soln. containing 1 mM trioctylmethylammonium hydrogen chloride, 1 mM tetraethylammonium hydroxide and 5% acetonitrile in 10 mM sodium phosphate buffer; flow-rate, 0.8 ml/min; temperature, 55°

Retention time

Retention times varied, depending on system. Useful for separation of cephapirin, desacetyl-cephapirin, cefazolin and cephalothin

Ref.

E. Crombez, W. Van Den Bossche and P. De Moerloose, *J. Chromatogr.*, 169 (1979) 343–350

HPLC 7**Apparatus**

M600 Chromatography Pump and U6K Injector (Waters Assoc.)

Column

μ Bondapak C₁₈, 30 cm $\times$ 4.6 mm I.D., stainless steel, packed (Waters Assoc.)

Mobile phase

1% Acetic acid–methanol (83:17) (plasma)

0.1% Ammonium carbonate–methanol (75:25) (urine)

Flow-rate

0.75 ml/min (10,400–13,800 kPa)

Detection

CE 212 Variable-wavelength monitor (Cecil Instruments) 236 nm

Type

Reversed-phase

Retention time (min)

Compound	Plasma	Urine
3-Methyl-7-[4-(1,4,5,6-tetrahydro-2-pyrimidyl)-phenyl-acetamido]- Δ^3 -cephalo-sporanic acid	16	17
S 74 7534 (analogue of HR 580) (Hoechst AG)	24	35

Ref.

D. Dell and R.M.J. Ings, *Arzneim.-Forsch/Drug Res.*, 28 (1978) 940–944

HSLC 1**Column**

Stainless steel, 1 m $\times$ 2.1 mm I.D., C₁₈/Porasil B packing

Mobile phase

0.1% Ammonium carbonate

Flow-rate

0.75 ml/min

Pressure

200 p.s.i.

Detection

UV (254 nm)

Sensitivity

0.08 a.u.f.s.

Sample solvent

0.1 M Sodium bicarbonate

*Sample size*10 μ l*Type*

Reversed-phase

Retention time

See HSLC 7

*Ref.*E.R. White, M.A. Carroll and J.E. Zarembo, *J. Antibiot.*, 30 (1977) 811–818**HSLC 2***Column*Stainless steel, 30 cm $\times$ 4.6 mm I.D.,
C₁₈/Lichrosorb 10- μ m packing*Mobile phase*

0.03% Sodium dihydrogen phosphate

Flow-rate

1 ml/min

Pressure

600 p.s.i.

Detection

UV (225 nm)

Sensitivity

0.1 a.u.f.s.

Sample solvent

0.1 M Sodium bicarbonate

*Sample size*5 μ l*Type*

Reversed-phase

Retention time

See HSLC 7

Ref.

As HSLC 1

HSLC 3*Column*Stainless steel, 1 m $\times$ 2.1 mm I.D., C₁₈/Porasil B
packing*Mobile phase*

Water

Flow-rate

1.1 ml/min

Pressure

500 p.s.i.

Detection

UV (254 nm)

Sensitivity

0.08 a.u.f.s.

Sample solvent

0.05 M Ammonium carbonate

*Sample size*5 μ l*Type*

Reversed-phase

Retention time

See HSLC 7

Ref.

As HSLC 1

HSLC 4*Column*Stainless steel, 30 cm $\times$ 4.6 mm I.D.,
C₁₈/LiChrosorb, 10- μ m packing*Mobile phase*

0.03% Sodium dihydrogen phosphate

Flow-rate

1 ml/min

Pressure

600 p.s.i.

Detection

UV (225 nm)

Sensitivity

0.1 a.u.f.s.

Sample solvent

0.1 M Sodium bicarbonate

*Sample size*10 μ l*Type*

Reversed-phase

Retention time

See HSLC 7

Ref.

As HSLC 1

HSLC 5*Column*Stainless steel, 1 m $\times$ 2.1 mm I.D., ODS-Sil-X-II
packing*Mobile phase*

5% Methanol, 95% 0.05 M ammonium carbonate

Flow-rate

0.5 ml/min

Pressure

800 p.s.i.

Detection	Mobile phase
UV (254 nm)	17% Methanol, 83% 0.01 M sodium dihydrogen phosphate
Sensitivity	Flow-rate
0.08 a.u.f.s.	1.3 ml/min
Sample solvent	Pressure
50% Methanol, 50% 0.05 M ammonium carbonate	1000 p.s.i.
Sample size	Detection
5 μ l	UV (254 nm)
Type	Sensitivity
Reversed-phase	0.04 a.u.f.s.
Retention time	Sample solvent
See HSLC 7	Mobile phase
Ref.	Sample size
As HSLC 1	10 μ l
	Type
	Reversed-phase
	Retention time
	See HSLC 7
	Ref.
	As HSLC 1

HSLC 6

Column
Stainless steel, 30 cm $\times$ 4.6 mm I.D.,
C₁₈/LiChrosorb, 10 μ m

HSLC 7

Column
Stainless steel, 30 cm $\times$ 4.6 mm I.D.,
C₁₈/LiChrosorb 10 μ m

Mobile phase

Water

Flow-rate

1 ml/min

Pressure

1000 p.s.i.

Retention time (estimated from drawings)

Detection

Refractive index detector (Model R401, Waters Assoc.)

Sensitivity

$\times 32$

Sample solvent

50% 0.5 M sodium bicarbonate, 50% methanol

Sample size

100 μ l

Compound *

Method

	1	2	3	4	5	6	7
7-ACA **	14.0	7.0	8.0	7.0			
7-TACA ***			27.0	17.5			
7-ADCA †			6.0	5.0			
Cephalosporin C			10.5	4.5			
Desacetyl cephalosporin C				3.0			
Cephalexin					8.0	12.5	
Cephadrine					14.0	15.0	17.0
Cephaloglycin					23.0	18.0	
Cephalothin					29.0	22.5	
Cephalexin						12.0	

* All samples carried out at room temperature

** 7-ACA = 7-Aminocephalosporanic acid

*** 7-TACA = 7-Tetrazolylaminocephalosporanic acid

† 7-ADCA = 7-Aminodesacetylcephalosporanic acid

Type

Reversed-phase

Ref.

As HSLC 1

CEPHALOSPORUM GRAMINIUS AN-TIBIOTIC**PC***Solvent*

Butanol-acetic acid-water (4:1:5)

R_F

0.85

*Ref.*G.W. Bruehl, R.L. Millar and B. Crunfer, *Can. J. Plant. Sci.*, 49 (1969) 235-246**CEPHALOTHIN****HPLC 1***Apparatus*

Varian LCS 1000 liquid chromatograph

Column

Stainless steel, 3 ft.×0.07 in I.D., packed with AS Pellionex-SAX resin (H. Reeve Angel)

Mobile phase

0.01 M Sodium nitrate

Flow-rate

26 ml/h

Temperature

50°C

Pressure

350 p.s.i.g.

Detection

UV, 254 nm

Sensitivity

0.32 A and 0.04 A

Type

Ion-pair

*Ref.*T.F. Rolewicz, B.L. Mirkin, M.J. Cooper and M.W. Anders, *Clin. Pharmacol. Therap.*, 22 (1977) 928-935**HPLC 2***Apparatus*

ALC/GPC 200 liquid chromatograph (Waters Assoc.) equipped with a Model 6000 solvent delivery system, a Model U6K universal injector, and a Model 440 absorbance detector

*Column*MicroBondapak C₁₈ (30 cm × 4 mm I.D.) (Waters Assoc.)*Mobile phase*

Methanol-acetic acid (4:6)

Flow-rate

2.5 ml/min

Detection

254 nm

Attenuation

0.01 a.u.f.s.

Chart speed

0.5 cm/min

Recorder

HP 3380A (Hewlett-Packard)

Retention time

Cephalothin 9.99 min; deacetylcephalothin 4.44 min

*Ref.*I. Nilsson-Ehle, T.T. Yoshikawa, M.C. Schotz and L.B. Guze, *Antimicrob. Agents Chemother.*, 13 (1978) 221-227**HPLC 3***Apparatus*

Constametric 1 pump and Spectromonitor 1 (Laboratory Data Control)

*Column*25 cm μ Bondapak C₁₈ (PN-27324; Waters Assoc.)*Mobile phase*

25% Acetonitrile (Photrex, J.T. Baker) in pH 6.0, 0.1 N acetate buffer

Flow-rate

2.0 ml/min

Temperature

40°C

Detection

UV 270 nm (0.04 a.u.f.s.) time constant, 0.5 sec

Type

Reversed-phase

Retention time

2.4 min

*Ref.*J.R. Koup, B. Brodsky, A. Lau and T.R. Beam, Jr., *Antimicrob. Agents Chemother.*, 14 (1978) 439-443**CEPHAMYCINS****PC 1***Paper*

Whatman No. 1

R_F

Solvent	Component A	Component B
A	0.05	0.30
B	0.30	0.58
C	0.29	0.42

Solvents

- A. *n*-Butanol–acetic acid–water (4:1:5)
 B. *n*-Butanol–acetic acid–water (2:1:1)
 C. 70% Isopropanol

Ref.

H. Fukase, T. Hasegawa, K. Hatano, H. Iwasaki and M. Yoneda, *J. Antibiot.*, 29 (1976) 113–120

PC 2**Paper**

Whatman 3MM

Solvents

- A. Isopropanol–water (70:30)
 B. Butanol satd. with 0.5 *M* phosphate buffer (pH 7)

Detection

Bioautography

R_F

Solvent	Cephamycin		
	A	B	C
A	0.64	0.73	0.44
B	0	0	0

Ref.

As Cephalosporins, PC 13

ELPHO**Medium**

Paper. Schleicher and Schuell SS-598

Buffer

0.165 *M* phosphate buffer, pH 7.0

Conditions

0 to 5°C for 2.5 h at 600 V

Detection

Bioautography

Mobility

- Cephamycin A, –5.9
 Cephamycin B, –2.0
 Cephamycin C, –8.0

Ref.

As Cephalosporins, PC 13

CEPHEMIMYCIN**TLC****Medium**

Cellulose Chromatogram Sheet 6065 (Eastman Kodak) (ascending)

Solvents

- A. *n*-Butanol–acetic acid–water (4:1:2)
 B. 40% *n*-Propanol
 C. 3% Ammonium chloride–methanol (3:7)

D. *n*-Propanol–pyridine–acetic acid–water (15:10:3:10)

E. Methyl ethyl ketone–*n*-butanol–water (30:5:65)

Detection

- A. Bioautography vs. *Proteus vulgaris*
 B. Ninhydrin

R_F

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.33	D	0.29
B	0.83	E	0.87
C	0.50		

Ref.

M. Arai, Y. Itoh, M. Nakahara, H. Kayamori and S. Sugawara, *U.S. Patent* 3,865,693, Feb. 11, 1975; *Belgian Patent* 804,341, Feb. 28, 1974

ELPHO**Medium**

Paper. Toyo Filter Paper No. 5

Buffer

Formic acid–acetic acid–water (1:3:36)

Conditions

3300 V, 40 mA, pH 1.8

Detection

- A. Bioautography vs. *Proteus vulgaris*
 B. Ninhydrin

Mobility

0.3

Ref.

As TLC

**CEPHRADINE
(cf., cephalosporins)****HPLC****Column**

1 m × 2.1 mm I.D. × 6.3 mm O.D., 316 stainless steel. ODS-Sil-X-II packing

Mobile phase

Methanol–0.05 *M* ammonium carbonate (7:93)

Pressure

1000 p.s.i.

Flow-rate

0.6 ml/min

Detector

UV (254 nm)

Sensitivity

0.08 a.u.f.s.

Type

Reversed-phase

Retention time (min) (estimated from drawing)

Cephadrine, 10

Cephaloglycin, 16

Ref.

As Ampicillin, HPLC

CEREXIN A and B

TLC

Medium

Silica gel GF (Merck)

Solvents

A. *n*-Butanol–acetic acid–water (3:1:1)

B. Chloroform–ethanol–water (4:7:2)

C. Chloroform–ethanol–14% ammonia (4:7:3)

R_F

Solvent	Component A	Component B
A	0.51	0.59
B	0.50	0.55
C	0.37	0.41

Ref.

J. Shoji, H. Hinoo, Y. Wakisaka, K. Koizumi, M. Mayama, S. Matsuura and K. Matsumoto, *J. Antibiot.*, 28 (1975) 56–59

CHALCIDIN

PC

Solvents

A. Benzene–hexane–acetone (20:5:6), descending

B. Benzene–hexane–acetone (20:5:3)

R_F

A. 7 biologically active spots

B. 3 biologically active spots in fraction II and 2 in fraction III

Ref.

G.F. Gause, M.G. Brazhnikova, V.A. Shorin, T.S. Masimova, O.L. Okhovatova, M.K. Kidinova, I.A. Vishnyakova, N.S. Pevzner and S.P. Shapovalova, *Antibiotiki*, 15 (1970) 483–486

TLC

Medium

Silicic acid

Solvent

Benzene–acetone (5:1)

R_F

7 biologically active spots

Ref.

As PC

CHALCOMYCIN

TLC

Solvent

Ethyl acetate (100)

Detection

Sulfuric acid and heated to 130°C

R_F

0.33

Ref.

As Aldgamycin F, TLC

CHAMPAMYCINS

PC

Solvents

A. Benzene–acetic acid–water (2:2:1), ascending

B. Pyridine–ethyl acetate–water (2.5:6:7), upper phase, descending

C. Isobutanol–pyridine–water (6:4:3), descending

D. Isobutanol–glacial acetic acid–water (20:1:25), descending

E. Pentanol–methanol–water (1:1:1.5), descending

F. Methanol–water–ammonium hydroxide (20:4:1), descending

G. *n*-Butanol–acetic acid–water (20:1:25)

R_F

Solvent	Champamycin A	Champamycin B
A	0.00	0.33
B		0.87
C		0.96
D		0.91
E		0.39
F		0.94
G		0.70

Ref.

U.K. Rao and P.L. Narasimha Rao, *Indian J. Exp. Biol.*, 5 (1967) 39–43

CHAMPAVITIN

PC

Solvent

As champamycins, A

R_F

0.95

Ref.

As champamycins, PC

CHELOCARDIN

PC

Paper

Eaton-Dikeman No. 613, 5/16 in. wide strips

Solvents

- A. Water satd. *n*-butanol
- B. As (A) + 2% *p*-toluenesulfonic acid (w/v)
- C. As (A) + 2% piperidine (w/v)
- D. 80% Aq. methanol + 1.5% sodium chloride (w/v) vs. paper buffered with 0.05 *M* sodium bisulfate + 0.95 *M* sodium sulfate
- E. As (D), replacing methanol with ethanol
- F. Acetic acid–water–*n*-propanol (1:12.5:12.5). All of above equilibrated with solvent for 3 h, developed 16 h ascending
- G. Water satd. with methyl isobutyl ketone + 2% *p*-toluenesulfonic acid (w/v)
- H. Water satd. with methyl isobutyl ketone
- I. As (H) + 1% *p*-toluenesulfonic acid (w/v)
- J. As (H) + 1% piperidine (v/v)
- K. Water–methanol–acetone (12:3:1) adjusted to pH 10.5 with ammonium hydroxide then to pH 7.5 with 30% phosphoric acid

Systems G–K developed 3 h without prior equilibration, ascending

Detection

Bioautography vs. *Sarcina lutea* ATCC 9341

R_F

Solvent	R_F	Solvent	R_F
A	0.13	G	0.22
B	0.79	H	0.12
C	0.51	I	0.18
D	0.80	J	0.34
E	0.90	K	0.26
F	0.81		

Ref.

T.J. Oliver, J.F. Prokop, R.R. Bower and R.H. Otto, *Antimicrob. Agents Chemother.*, 1962 (1963) 583–591

CHILAPHYLIN

PC

Solvent

Ethyl acetate

Detection

Bioautography vs. *Bacillus subtilis*

R_F

0.02

Ref.

A. Rex and L. Arrieta, *J. Antibiot.*, 26 (1973) 126–130

TLC

Medium

Silica gel

Solvent

Acetone–water (9:1)

Detection

15% phosphoric acid

R_F

0.90

Ref.

As PC

CHLORAMPHENICOL

Chloramphenicol derivatives

Chloramphenicol can be identified after chromatography (1) by bioautography, (2) by observation of a dark zone using ultra-violet light or (3) by reducing the nitro group to an aryl amino group which can be detected as a yellow spot by procedures outlined below

PC 1

Paper

Whatman No. 1

Solvent

Water satd. *n*-butanol containing 2.5% acetic acid

Detection

Spray first with solution composed of 3 ml of 15% stannous chloride, 15 ml conc. HCl and 180 ml water. (Prepare fresh before use.) Air dry; spray again with solution composed of 1 g *p*-dimethylaminobenzaldehyde dissolved in a mixture of 30 ml ethanol, 30 ml conc. HCl and 180 ml *n*-butanol. Upon drying yellow zones appear

R_F

0.89

Ref.

A.J. Glazko, W.A. Dill and M.C. Rebstock, *J. Biol. Chem.*, 183 (1950) 679

PC 2

Paper

Whatman No. 1

Solvent

n-Butanol–water–acetic acid (4:5:1)

Detection

As in 1

R_F
0.92

Ref.

V.H. Reber and E. Lichtenberg, *Helv. Medica Acta*, 20 (1953) 396

PC 3

Paper

Whatman No. 1

Solvent

Stationary phase; *sec.*-octyl alcohol. Mobile phase, McIlvaine buffer pH 6. Development time about 1 h

Detection

Dark zones are observed under UV. Quantitative estimation done by eluting with methanol and direct reading of UV absorption at 278 nm

R_F

Chloramphenicol, 0.30; -palmitate, 0.0; -stearate, 0.0; -succinate, 0.75

Ref.

G. Sferruzza and R. Rangone, *Il Farmaco*, 18 (1943) 322

PC 4

Paper

Arches 302 or Whatman No. 1

Solvents

- A. Same as PC 2. Spot 5–20 ml containing 5–20 μ g, ascending about 16 h at $20^\circ\text{C} \pm 1^\circ\text{C}$
- B. *n*-Butanol–pyridine–water (2:1:2), 8–10 h
- C. *n*-Butanol satd. with water on paper impregnated with 0.2 *M* monopotassium phosphate

Detection

Similar to PC 1 but second spray consists of *p*-dimethylaminobenzaldehyde, 1 g; conc. HCl, 20 ml; and 95% ethanol, to total 100 ml

R_F

Solvent A, 0.96
Solvent B, 0.96
Solvent C, 0.97

Ref.

M.R. Rousselet and R. Paris, *Annales Pharm. Franc.*, 22 (1964) 249

PC 5

Paper

Whatman No. 1

Solvents

- A. 80% Methanol + 3% sodium chloride (1:1) descending. Paper buffered with 0.95 *M* sodium sulfate + 0.05 *M* sodium bisulfate
- B. Propanol–pyridine–acetic acid–water (6:4:1:3), ascending

- C. Butanol–acetic acid–water (4:1:5), ascending
- D. 80% Phenol, ascending

Detection

Bioautography vs. *Staphylococcus aureus*

R_F

Solvent	R_F	Solvent	R_F
A	0.84	C	0.92
B	0.91	D	0.93

Ref.

M.J. Weinstein, G.M. Luedemann, G.H. Wagman and J.A. Marquez, *U.S. Patent* 3,819,611, June 25, 1974

TLC 1

Medium

Silica gel G

Solvents

- A. Ethyl acetate satd. with water. Develop about 1 h
- B. *n*-Butanol saturated with water + 2.5% acetic acid. Develop about 4–5 h

Detection

As in PC 1. Heat plate in an oven at 100°C for 5 min after spraying on solution (1), then spray with (2). Sensitivity is 0.2 μ g chloramphenicol

R_F

Solvent A, 0.90
Solvent B, 0.72

Ref.

M.M.J. Lachorine and G. Netien, *Société de Pharmacie de Lyon*, 9 (1963) 120

TLC 2

Media

- A. Silica gel G: 1, activated; 2, not activated
- B. Silica gel G impregnated with pH 6 phosphate buffer: 1, activated; 2, not activated

Solvents

Chloroform–methanol: A, 90:10; B, 85:15; C, 80:20

R_F

Solvent	Medium			
	A1	A2	B1	B2
A	0.47	0.54	0.53	0.42
B	0.65	0.65	0.66	0.64
C	0.82	0.82	0.80	0.86

Detection

- A. Iodine vapor. Stand plate in a chamber with iodine for a few minutes; gives dark brown spots
- B. Spray plate with a 1% alcoholic solution of Rhodamine B
- C. UV lamp (254 nm) shows zones as dark spots

Ref.

M.A. Kassem, A.A. Kassem and A.E.M. El-Nimr, *Pharm. Zeitung*, 111 (1966) 1792

TLC 3**Medium**

Silica gel G. Mix 30 g Silica gel G (Merck) with 60 ml water vigorously for 1 min and spread a 275- μ g layer on a 20 $\times$ 20 cm glass plate. Air dry for 15 min at room temp., then for 30 min at 110°C in a drying oven and store in a desiccator over "Blaugel" (a drying agent)

Solvent

Butanol-acetic acid-water (4:1:1)

Detection

As PC 4. Useful for separation of chloramphenicol or its phthalate or succinate derivative and their decomposition products

R_F

No numerical data presented

Ref.

V.M. Sahll, H. Zeigler and M. Desch, *Pharm. Zeitung*, 44 (1965) 1542

TLC 4**Medium**

Polyamide, according to Wang. E-poly-caprolactam powder (6 g) dissolved in 30 ml 80% formic acid and spread on an 18 $\times$ 25 cm glass plate. Dry overnight, heat at 100°C for 20 min. Layer = 55 mm thick

R_F

	System A	System B
Chloramphenicol	0.35	0.80
Chloramphenicol palmitate	0.95	0.90
Chloramphenicol succinate	0.25	0.72

Detection

0.25% SnCl₂ in 1 N HCl; dry; follow with 2% *p*-dimethylaminobenzaldehyde in 1.2 N HCl. Compounds appear as bright yellow spots after a few hours. Sensitivity: chloramphenicol 10 μ g; palmitate, 40 μ g; succinate, 30 μ g

Solvents

- A. *n*-Butanol-chloroform-acetic acid (10:90:0.5)
- B. *n*-Butanol-water-acetic acid (82:18:0.5)

Ref.

Y. Linn, K. Wang, T. Yang, *J. Chromatogr.*, 21 (1966) 158; K. Wang, *J. Chinese Chem. Soc.*, 8 (1961) 241

TLC 5**Media**

- A. Cellulose MN 300 (Macherey-Nagel)
- B. Kieselgel 60 (Merck)

Solvents

- A. *n*-Butanol-methanol-water-acetic acid (45:30:36:9)
- B. *n*-Butanol-methanol-water-ethanol-acetic acid (45:30:36:8:1) adjusted to pH 5.5 with 30% sodium hydroxide
- C. *n*-Butanol-water (45:36) adjusted to pH 4.0 with glacial acetic acid

Detection

Bioautography vs. *Sarcina lutea* ATCC 9341

R_F

Solvent	Medium A	Medium B
A	0.95	
B	0.95	
C		0.62

Ref.

As Ampicillin, TLC 1

TLC 6**Medium**

Silanized silica gel HF₂₅₄ (Merck) (reversed-phase)

R_F

Solvent	I *	II **	III ***
A	0.00	0.26	0.15
B	0.21	0.76	0.70
C	0.00	0.29	0.56
D	0.00	0.33	0.55
E	0.17	0.65	0.70
F	0.00	0.13	0.36
G	0.00	0.11	0.40
H	0.00	0.23	0.39

* I = Chloramphenicol palmitate

** II = Chloramphenicol

*** III = Chloramphenicol hemisuccinate

Solvents (mobile phase)

- A. Buffer, pH 3–dioxane (7:3)
- B. Buffer, pH 3–dioxane (3:7)
- C. Buffer, pH 7–dioxane (7:3)
- D. Buffer, pH 7–isopropanol (7:3)
- E. Buffer, pH 7–isopropanol (1:1)
- F. Buffer, pH 7–acetone (8:2)
- G. Buffer, pH 7–tetrahydrofuran (7:3)
- H. Buffer, pH 7–methanol (8:2)

Detection

UV at 250 nm

Ref.

R. Rangone and C. Ambrosio, *Farmaco (Ed. Pr.)*,
26 (1971) 237–250

TLC 7*Medium*

Avicel

Solvent

Methanol–isobutyl alcohol–chloroform–10% hydrochloric acid (10:10:10:1)

Detection

- A. Iodine vapor
- B. 0.25% Stannous chloride soln.
- C. 0.2% *p*-Dimethylaminobenzaldehyde soln.

R_F

Chloramphenicol, 0.9
Chloramphenicol sodium succinate, 0.88

Ref.

As Ampicillin, TLC

TLC 8*Medium*

Silica gel

Solvents

- A. 10% Tartaric acid
- B. 10% Tartaric acid satd. with *n*-butanol

Detection

- A. Iodine vapor
- B. 0.25% Stannous chloride in 20% hydrochloric acid
- C. 2% *p*-Dimethylaminobenzyldehyde in 1 *N* hydrochloric acid

R_F

Derivative	Solvent		Detection		
	A	B	A	B	C
Chloramphenicol	0.86	0.94	–	–	Yellow
Chloramphenicol palmitate	0	0	–	–	Yellow

Ref.

As Cephalosporins, TLC 18

TLC 9*Media*

- A. Kieselguhr F₂₅₄ (Merck 5738). Before use, the Kieselguhr plate was impregnated with citrate/phosphate buffer (pH 3.7)–glycerol (19:1)
- B. Silica gel 60 (Merck)
- C. Cellulose (Merck 5716)

Solvents

- A. Chloroform–acetone–impregnation liquid [citrate/phosphate buffer (pH 3.7)–glycerol (19:1)] (5:5:2)
- B. Ethanol–water–ammonia (8:8:1)
- C. Methanol–chloroform (9:1)
- D. Methanol–acetone (3:2)
- E. Methanol–3% ammonium chloride (7:3)

Detection

- A. Bioautography vs. *Bacillus cereus* ATCC 11778
- B. Bioautography vs. *Bacillus subtilis* ATCC 6633
- C. Bioautography vs. *Micrococcus flavis* ATCC 10240
- D. Bioautography vs. *Sarcina lutea* ATCC 9341

R_F

Medium	Solvent	Detection	Chloramphenicol
A	A	A	0 *
B	B	C	0.70
B	C	D	0.65
B	C	B	0.65
B	D	C	0.80
C	E	A	–

* *R_F* value obtained by running the plate twice*Ref.*

As Bacitracin, TLC 3

TLC 10*Medium*Silica gel 60 F₂₅₄ (Merck)*Solvents*

- A. Ethyl acetate
- B. Ethyl acetate–methanol (99:1)
- C. Ethyl acetate–methanol (98:2)
- D. Ethyl acetate–methanol (95:5)
- E. Ethyl acetate–methanol (90:10)

R_F (see p. 116)*Ref.*

G. Janssen and H. Vanderhaeghe, *J. Chromatogr.*,
114 (1975) 266–269

R_F

Compound	Solvent				
	A	B	C	D	E
Chloramphenicol	0.50	0.54	0.58	0.67	0.74
<i>erythro</i> -Chloramphenicol	0.54	0.57	0.61	0.69	0.75
<i>D-threo</i> -1-(<i>p</i> -Nitrophenyl)-2-mono-chloroacetamido-1,3-propanediol	0.28	0.33	0.39	0.53	0.66

GLC 1 (Quantitative)

Apparatus

Barber-Colman Model 5000 with FID, 5 mV recorder with $\frac{1}{3}$ in./min chart speed

Column

Glass, U-shaped, 6 ft. $\times$ 3 mm I.D. packed with 5% SE-30 on Gas-Chrom Q (80–100 mesh)

Temperatures

Column, 240°C; detector, 250°C; injector, 250°C

Carrier gas

N₂ at 20 p.s.i. (50 ml/min), H₂ at 32 p.s.i. and air at 40 p.s.i.

Current

5×10^{-8} A f.s.d. (sensitivity, 100; attenuation, 5)

Reagent solvent

200 mg *m*-phenylene dibenzoate dissolved in 6 ml acetonitrile. 1 ml of N,O-bis(trimethylsilyl)acetamide (BSA) is added and volume made up to 10 ml with acetonitrile; shake until uniform

Derivative

Add 0.5 ml of reagent and stir vigorously

Chromatography and calculations

One ml of each derivatized solution (= to approx. 20 μ g chloramphenicol) is injected and peaks measured. Chloramphenicol content is determined by direct comparison of peak areas (chloramphenicol/internal standard) with chloramphenicol standard treated in an identical manner

Ref.

M. Margosis, *J. Chromatogr.*, 47 (1970) 341

GLC 2

Apparatus

Shimadzu GC-5AF₃P

Column

Glass, 1 m $\times$ 3 mm I.D., packed with 1.5% OV-17 on Chromosorb W AW DMCS, 60–80 mesh

Detection

Flame ionization

Temperature

180° for the first 6 min, then increased to 260° at the rate of 5°/min. Injection port temperature 280°

Carrier gas

Nitrogen

Flow-rate

65 ml/min

Retention time

7 min (estimated from drawing)

Ref.

T. Nakagawa, M. Masada and T. Uno, *J. Chromatogr.*, 111 (1975) 355–364

GLC 3

Apparatus

Pye Series 104 chromatograph

Column

Coiled glass columns, 5 ft. $\times$ 4 mm I.D. packed with 3% QF-1, 3% OV-1 or 3% OV-9 on Gas-Chrom Q (100–120 mesh) (Applied Science Labs.)

Silylation reagents

BSA, BSTFA and "Tri-Sil", a soln. of hexamethyldisilazane and trimethylchlorosilane in pyridine

Carrier gas

Helium or nitrogen

Flow-rate

60 ml/min

Procedure

Chloramphenicol or *erythro*-chloramphenicol (5–7 mg) was dissolved in either 0.5 ml of a mixture of 1 ml of "Tri-Sil" and the resulting soln. was maintained at room temperature. A volume of 1 μ l or less was injected directly into the gas chromatograph

Ref.

G. Janssen and H. Vanderhaeghe, *J. Chromatogr.*, 82 (1973) 297–306

Retention times (min) of the TMS derivatives of chloramphenicol and its erythro-isomer

Derivative	Column		
	3% QF-1	3% OV-1	3% OV-17
Chloramphenicol			
Bis-TMS	22.6/203° •	19.7/193° • 27.4/181° ***	17.2/196° • 6.3/217° ***
Tris-TMS	11.0/203° **		11.3/196° **
Mono-TMS C-1		35.9/181° ***}	14.5/217° ***
Mono-TMS C-3			16.7/217° ***
erythro-Chloramphenicol			
Bis-TMS	22.5/203° •	20.5/193° • 38.3/181° ***	18.8/196° * 7.7/217° ***
Tris-TMS	10.6/203° **		11.4/197° **
Mono-TMS		43.0/181° ***	17.1/217° ***.†

• Silylation performed with "Tri-Sil"

** Silylation performed with BSA in acetonitrile

*** Silylation performed with BSA in ethyl acetate

† Asymmetrical peak

HPLC 1

Column

1 m × 2.1 mm I.D. × 6.3 mm O.D., 316 stainless steel. ODS-Sil-X-II packing

Mobile phase

100% 0.05 M ammonium carbonate

Pressure

750 p.s.i.

Flow-rate

0.6 ml/min

Detector

UV (254 nm)

Sensitivity

0.08 a.u.f.s.

Type

Reversed-phase

Retention time

19.0 min (estimated from drawing)

Ref.

As Ampicillin, HPLC

HPLC 2

Apparatus

Constametric I pump and Spectromonitor I (Laboratory Data Control)

Column

25 cm μ Bondapak C₁₈ (PN-27324; Waters Assoc.)

Mobile phase

25% Acetonitrile (Photrex; J.T. Baker) 0.1 M acetate buffer, pH 6.0

Flow-rate

2.0 ml/min

Temperature

40°C

Detection

UV 270 nm (0.04 a.u.f.s.) time constant, 0.5 sec

Type

Reversed-phase

Retention time (min)

Chloramphenicol, 3.2; peak height 62 mm

Chloramphenicol succinate, 2.2; peak height 40 mm

Ref.

J.R. Koup, B. Brodsky, A. Lau and T.R. Beam, Jr., *Antimicrob. Agents Chemother.*, 14 (1978) 439-443

HSLC 1

Column

Stainless steel, 1 m × 2.1 mm I.D., ODS-Sil-Z-II

Mobile phase

100% 0.05 M Ammonium carbonate

Flow-rate

0.6 ml/min

Pressure

750 p.s.i.

Temperature

Room temperature

Detection

UV (254 nm)

Sensitivity

0.08 a.u.f.s.

Sample solvent

Water

Sample size5 μ l**Type**

Reversed-phase

Retention time

19 min (estimated from drawing)

Ref.

As Cephalosporins, HSLC 1

HSLC 2**Column**Stainless steel, 30 cm $\times$ 4.6 mm I.D., LiChrosorbC₁₈, 10 μ m**Mobile phase**

25% Methanol, 75% 0.01 M sodium dihydrogen phosphate

Flow-rate

1.6 ml/min

Pressure

1000 p.s.i.

Temperature

Room temperature

Detection

UV (254 nm)

Sensitivity

0.64 a.u.f.s.

Sample solvent

Water

Sample size5 μ l**Type**

Reversed-phase

Retention time

3.7 min (estimated from drawing)

Ref.

As Cephalosporins, HSLC 1

**CHLORAMPHENICOL, DEOXY-
(See deoxychloramphenicol)****7-[α -(3-O-CHLOROBENZOYL-3-METHYL-1-UREIDO)- α -PHENYL-ACETAMIDO]-3-(1-METHYL-1H-TETRAZOLE-5-YLTHIOMETHYL)-3-CEPHEM-4-CARBOXYLIC ACID
(cf., cephalosporins)****TLC****Medium**

Silica gel

Solvent

Chloroform-methanol (7:3)

Detection

A. UV

B. Iodine chamber

R_F

One spot

Ref.R.D.G. Cooper and D.K. Herron, *U.S. Patent* 3,925,368, Dec. 9, 1975**CHLORTETRACYCLINE
(cf., tetracyclines)****CHROMIN****PC****Paper**

Whatman No. 2

SolventEthanol-*n*-butanol-water (1:5:5), ascending
18-24 h**Detection**Bioautography vs. *Saccharomyces cerevisiae***R_F**

0.55

Ref.C.P. Schaffner, I.D. Steinman, R.S. Safferman and H. Lechevalier, *Antibiot. Ann.*, (1957-1958) 869-873**CHROMOCYCLOMYCIN****TLC****Medium**

Silufol

Solvents

A. Chloroform-methanol (6:1)

B. Benzene-methanol (3:1)

Detection

UV at 254 nm. Detection, after exposure to ammonia vapours, in visible and UV light

R_F

Solvent	UV	Visible
A	0.52, yellow-orange	Yellow *
B	0.53	

* Visible immediately, without preceding exposure in ammonia vapour

Ref.K. Stajner, M. Blumaureova, D.A.S. Callieri and Z. Vanek, *Folia Microbiologica*, 19 (1974) 498-506

CHROMOMYCINS (cf., SR 1768 A)

PC 1

Paper

Toyo No. 7. Immerse in glycerol-methanol (1:4), dry, briefly insert in excess of methanol, remove and dry in hot air current

Solvents

- A. Benzene-ethyl acetate (4:1 to 2:1)
- B. Diethyl ether-ethyl acetate (190:10 to 170:30)
- C. Isopentyl acetate-*n*-butanol (9:1)
- D. Isopentyl acetate-acetone (19:1 to 14:1)

Detection

- A. Bioautography vs. *Staphylococcus aureus* or *Bacillus subtilis*

R_F

Solvent	Ratio	A Group				
		A ₁	A ₂	A ₃	A ₄	A ₅
A	4:1			0.33 ± 0.05		
	3:1			0.50 ± 0.05		
	2:1			0.70 ± 0.05		
B	185:15	0.60	0.50	0.35 ± 0.04	0.25	0.10
	170:30	0.70	0.60	0.50 ± 0.04	0.30	0.15
C	9:1			0.95 ± 1.00		
D	19:1			0.90 ± 0.05		
	14:1			0.95 ± 0.05		

Solvent	Ratio	B Group				C Group
		O ₁	O ₂	P	F	
A	4:1		0.00		0.00 ~ 0.02	0.00
	3:1		0.00		0.00 ~ 0.02	0.00
	2:1		0.00		0.00 ~ 0.02	0.00
B	185:15		0.00		0.00	0.00
	170:30		0.00		0.00	0.00
C	9:1		0.75 ± 0.07		0.25 ± 0.04	0.00
D	19:1	0.80	0.70	0.55	0.23 ± 0.04	0.00
	14:1	0.87	0.78	0.65	0.30 ± 0.04	0.00

- B. Freshly prepared soln. of ferric chloride and potassium ferricyanide. Chromomycin A, BO₁, BO₂, B_p appear as bluish green spots against a yellow background. Remove excess reagent under tap water to fix blue spots

- C. Spray with ethanol-2*N* alkali (1:1), dry in air at room temperature and spray with 3% hydrogen peroxide. A₂, A₃, A₄ and A₅ appear as red spots, persisting for a few minutes

- D. UV light (wide range)

Ref.

- K. Mizuno, *J. Antibiot., Ser. B*, 13 (1960) 329-331

R_F

Chromomycin A ₃ derivative	Toyo No. 7	Whatman No. 1	Fluorescence under UV light
A ₃	0.53	0.57	Orange
A ₃ -Formate	0.65	0.67	Orange
A ₃ -Me	0.56	0.59	Emerald green
A ₃ -Me-Ac	0.22	0.19	Emerald green
A ₃ -Ac	0.32	0.26	Emerald green

PC 2

Paper

- A. Acetylated Toyo No. 7
- B. Acetylated Whatman No. 1

Solvent

Butyl acetate-pyridine-water (1:5:10)

Detection

UV light

*Ref.*K. Mizuno, *J. Antibiot.*, 16 (1963) 22-39**TLC***Medium*

Mix 30 g silica gel G (Merck) and 60 ml Theorell's buffer soln. (pH 2.3, 0.07 *M*). Spread on a glass plate to form a 0.2-mm layer; dry at 100°C for 1 h

Solvent

Benzene-chloroform-methanol (1:2:1)

*R_F*Chromomycin A₃, 0.67 ± 0.1Chromomycin A₃ hemisuccinate, 0.40 ± 0.1*Ref.*

K. Mizuno, N. Sugita, M. Asai and A. Miyaki,
U.S. Patent 3,501,570, March 17, 1970

CHROTHIOMYCIN**TLC***Medium*

Silica gel

Solvents

A. Water satd. diethyl ether

B. Water satd. butanol

C. Ethyl acetate

D. Butyl acetate

E. Methanol

F. Butanol

G. Water

H. Benzene

I. Chloroform

Detection

Color. Gives purple spot

R_F

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.22	F	0.45
B	0.77	G	0.72
C	0.09	H	0.00
D	0.03	I	0.00
E	0.50		

Ref.

S. Ayukawa, M. Hamada, K. Kojiri, T. Takeuchi,
T. Hara, T. Nagatsu and H. Umezawa, *J. Anti-*
biot., 22 (1969) 303-308

CINEROMYCIN B**GLC***Conditions*

Same as for albocycline GLC

Retention time

9 min (est. from curve)

Ref.

As albocycline PC

CIRCULIN A and B**HPLC***Apparatus*

Laboratory Data Control modular liquid chromatograph, equipped with a high pressure Milton Roy Minipump with a pulse dampener (model 709)

Column

Stainless steel (30 cm × 4.6 mm I.D.) prepacked with a μ Bondapak C₁₈ (Waters Assoc.) attached to a septumless injector valve (Micromeritics) and to a flame-ionization detector

Mobile phase

Linear gradient from 20% acetonitrile to 50% acetonitrile and 20% methanol, both in pH 2.0 phosphate buffer, in 16 min

Flow-rate

1.0 ml/min

Temperatures

Cleaner, 900°; oxidizer, 690°; evaporator, 380°; reactor, 490°; detector, 175°

Chart speed

6.4 mm/min

Pressure

7.0 atm (1000 p.s.i.)

Detection

Moving-wire flame ionization

Retention time (min)

Circulin A, 9.0

Circulin B, 7.4

Ref.

As Bacitracin, HPLC 1

CIRRAMYCINS**PC 1***Solvents*A. Wet *n*-butanol

B. 3% Aq. ammonium chloride

C. 75% Phenol

D. 50% Acetone

E. Butanol-methanol-water-methyl orange
(40:10:20:1.5, v/v/v/w)

F. Butanol-methanol-water (4:1:2)

G. Benzene-methanol (4:1)

H. Water

*Detection*Bioautography vs. *Bacillus subtilis*

R_F

Solvent	R_F^*	Solvent	R_F^*
A	0.9	E	0.95
B	0.75	F	0.95
C	0.95	G	0.4, 0.9 (trace)
D	0.96	H	0.1

* Estimated from drawing

Ref.

H. Koshiyama, M. Okanishi, T. Ohmori, T. Miyaki, H. Tsukiura, M. Matsuzaki and H. Kawaguchi, *J. Antibiot.*, 16 (1963) 59–66

PC 2

Solvent

Sorensen's buffer, pH 7.0

 R_F

Cirramycin A, 0.48
Cirramycin B, 0.15

Ref.

As PC 1

PC 3

Solvents

Solvents A–F same as for angolamycin PC

Ref.

As PC 1

 R_F

Solvent	Cirramycin A	Cirramycin B
A	0.73	0.70
B	0.66	0.94
C	0.48	0.15
D	0.86	0.60
E	0.74	0.78
F	0.84	0.85

PC 4 (Separation of components of cirramycin A)

Solvent

0.05 *N* Ammonium hydroxide satd. with methyl isobutyl ketone

Detection

Bioautography vs. *Bacillus subtilis* R_F

Cirramycin A₁, 0.84 (major zone)
Cirramycin A₂, 0.89
Cirramycin A₃, 0.75
Cirramycin A₄, 0.67
Cirramycin A₅, 0.86

Ref.

H. Koshiyama, H. Tsukiura, K. Fujisawa, M. Konishi, M. Hatori, K. Tomita and H. Kawaguchi, *J. Antibiot.*, 22 (1969) 61–64

PC 5

Solvents

A. Stationary phase: formamide–methanol (1:1);
mobile phase: benzene–cyclohexane (1:1) satd.
with formamide

B. Paper treated with 2% liquid paraffin. Sta-

tionary phase, butyl acetate. Mobile phase,
 $\frac{1}{15}$ *M* Sorensen's buffer (pH 8) satd. with butyl
acetate

C. Methyl isobutyl ketone–methyl ethyl ketone
(4:1), ascending

D. Methyl isobutyl ketone, descending

 R_F

Type of derivative	Derivative of cirramycin A ₁	Solvent			
		A	B	C	D
Esters of monobasic acids	Acetyl	0.45	–	–	0.87
	Triacetyl	0.93	–	0.98	–
	Propionyl	–	0.15	–	0.93
	Phenylacetyl	0.88	–	0.96	–
	Phenoxyacetyl	0.68	–	0.98	–
	Benzoyl	0.88	–	–	0.92
Esters of dibasic acids	Malcoyl	0	0.49	0.33	0.64
	Disuccinyl (III)	0	0.57	0.80	0.80
	Glutaryl	0	0.47	0.82	0.64
	Phthaloyl	0	0.48	0.33	0.68

Type of derivative	Derivative of cirramycin A ₁	Solvent			
		A	B	C	D
Esters of amino acids	Leucyl	0	0.46	—	—
	Phenylglycyl	0	0.45	—	0.02
Esters of other acids	Methylsuccinyl (IV)	0.18	0.50	—	0.88
	β -Sulfopropionyl	0	0.46	—	0
	Methylsulfonyl	0.80	—	—	0.89
	Methylcarbonyl	0	0.35	—	0.80
	Ethoxycarbonyl	0.61	0.30	—	0.89
Des-epoxy (II)	Depoxy (II)	—	0.35	—	0.78
	Depoxypropionyl	—	—	—	0.95
	Tetrahydro	—	0.88	—	0.90
N-Oxide	N-Oxide	0	0.78	0.76	—
	Succinyl N-oxide	0	0.78	—	0.31
Aldehyde modification	Aldoxime	—	0.64	—	—
	Hydantolnylimino	—	—	—	—
	Cirramycin A ₁ (I)	0	0.51	0.28	0.73

Ref.

H. Tsukiura, M. Konishi, M. Saka, K. Fujisawa, T. Ohmori, T. Hoshiya and H. Kawaguchi, *J. Antibiot.*, 22 (1969) 100–105

B. Chloroform–methanol (10:1)

C. *n*-Butanol–acetic acid–water (3:1:1)

Detection

Sulfuric acid, brownish yellow

Ref.

Y. Shimauchi, K. Kubo, K. Osumi, K. Okamura, Y. Fukagawa, T. Ishikura and J. Lein, *J. Antibiot.*, 31 (1978) 270–275

TLC 1*Solvent*

As angolamycin TLC 1

R_F

Solvent	Cirramycin A	Cirramycin B
A	0.23	0.58
B	0.40	0.47

Ref.

As angolamycin TLC 1

TLC 2*Medium*

Silica gel 60 F₂₅₄ (Merck) precoated

R_F

Solvent	Cirramycin A ₁	Cirramycin B ₁
A	0.04	0.49
B	0.07	0.57
C	0.49	0.68

Solvents

A. Benzene–acetone (2:3)

CCD 1*Solvents*

A. Benzene– $\frac{1}{15}$ M Sorensen's buffer (pH 5.8), 50 transfers

B. Benzene– $\frac{1}{15}$ M Sorensen's buffer (pH 7.0), 50 transfers (used for repeated distribution of A component)

C. Benzene– $\frac{1}{15}$ M Sorensen's buffer (pH 4.5), 50 transfers (used for repeated distribution of B component)

Distribution

Solvent	Component	Peak Tube No.
A	Cirramycin A	5
	Cirramycin B	42
B	Cirramycin A	25
C	Cirramycin B	20

Ref.

As PC 1

CCD 2**Solvent**

Benzene-0.1 M Sorensen's buffer (pH 7.0), 100 transfers

Distribution

Cirramycin A₁ found at tube No. 45. Cirramycin A₂ was distributed before A₁ peak. Cirramycin A₃, A₄ and A₅ appeared after the A₁ component

Ref.

As PC 4

CITRININ**TLC****Medium**

Silica gel G (Merck) activated at 120°C for 2 h

Solvent

Toluene-ethyl acetate-formic acid (5:4:1)

Detection

Greenish yellow fluorescence under UV light. If the silica gel slurry was prepared in chloroform, the spots were more compact with a lower R_F value. If the chamber was satd. with the solvent system, good resolution was possible

 R_F

0.8

Quantitative determination

The fluorescence portion was marked and scraped out into tubes containing 3 ml of 0.1 M carbonate/bicarbonate buffer (pH 10). The suspension was allowed to stand for 1 h with intermittent shaking. The tubes were then centrifuged at 2000 rpm for 20 min. The clear supernatant was wholly or partially (a known volume) taken and tested for the citrinin content using the following standardized estimation procedure

Folin's phenol reagent was prepared as reported by Lowry et al., *J. Biol. Chem.*, 193 (1951) 265. Samples containing 5-100 µg of fresh citrinin (in 0.15% sodium carbonate) were pipetted out into a series of test tubes. The final volume was brought to 3 ml with carbonate/bicarbonate buffer. To this, 1 ml of Folin's reagent was added, followed by 1 ml of 15% sodium carbonate soln. The tubes were then gently shaken once and incubated at 37°C for 15 min. The blue color developed was read at 640 nm

Ref.

C. Damodaren, C.S. Ramadoss and E.R.B. Shanmugasundaram, *Anal. Biochem.*, 52 (1973) 482-488

CITROMYCIN**PC****Solvents**

- A. Wet butanol
- B. 20% Ammonium chloride
- C. 75% Phenol
- D. 50% Acetone
- D. Butanol-methanol-water (4:1:2) + 1.5% methyl orange
- F. Butanol-methanol-water (4:1:2)
- G. Benzene-methanol (4:1)
- H. Water

Detection

Bioautography vs. *Bacillus subtilis*

 R_F

Solvent	R_F	Solvent	R_F
A	0.0	E	0.37-0.46
B	1.0	F	0-0.1
C	0.65	G	0.0
D	0-0.17	H	0-0.1

Ref.

Y. Kusakabe, Y. Yamauchi, C. Nagatsu, H. Abe, K. Akasaki and S. Shirato, *J. Antibiot.*, 22 (1969) 112-118

TLC 1**Medium**

Alumina

Solvent

Ethanol-water (4:6)

Detection

Bioautography vs. *Bacillus subtilis* PCI-219

 R_F

0.5 (estimated from drawing)

Ref.

Y. Kono, S. Makino, S. Takeuchi and H. Yonehara, *J. Antibiot.*, 22 (1969) 583-589

TLC 2**Media**

- A. Silica gel G
- B. Avicel SF

Solvents

- A. *n*-Propanol-pyridine-acetic acid-water (15:10:3:12)
- B. Chloroform-methanol-17% aq. ammonia (2:1:1), upper layer

 R_F (see p. 124)**Ref.**

K. Arima, T. Kawamura and T. Beppu, *J. Antibiot.*, 25 (1972) 387-392

R_F

Solvent	Medium A	Medium B
A	0.52	
B		0.35

CLADOSPORIN**TLC***Medium*

Silica gel (Merck)

Solvent

Toluene-ethyl acetate-90% formic acid (6:3:1)

Detection

UV

 R_F

Compound	R_F	Color
Cladosporin	0.55	Light blue
Monomethyl cladosporin	0.80	Light blue
Dimethyl cladosporin	0.37	Deep blue
Monoacetyl cladosporin	0.79	Light blue
Diacetyl cladosporin	0.53	Deep blue (after spray with conc. sulfuric acid)

*Ref.*P.M. Scott and W. van Walbeek, *U.S. Patent* 3,838,171, Sept. 24, 1974**CLAVULANIC ACID****PC***Paper*

Whatman No. 1

*Solvents*A. *n*-Butanol-isopropanol-water (7:7:6)B. *n*-Butanol-ethanol-water (4:1:5) R_F

Solvent A	0.46
Solvent B	0.37

*Ref.**German Patent* 2,517,316, Oct. 23, 1975**TLC***Medium*

Silica gel

*Solvents*A. *n*-Butanol-ethanol-water (4:1:5)B. *n*-Butanol-acetic acid-water (12:3:5)

C. Isopropanol-water (7:3)

 R_F

Solvent A	0.37
Solvent B	0.44
Solvent C	0.78

Ref.

As PC

CLAZAMYCIN A and B**TLC***Medium*

Silica gel G (Merck, Art. 5715)

Solvent

Butanol-acetic acid-water (6:1:2)

 R_F

Clazamycin A	0.40
Clazamycin B	0.27

*Ref.*Y. Horiuchi, S. Kondo, T. Ikeda, D. Ikeda, K. Miura, M. Hamada, T. Takeuchi and H. Umezawa, *J. Antibiot.*, 32 (1979) 762-764**ELPHO***Medium*

Paper

Buffer

Formic acid-acetic acid-water (1:3:36)

Conditions

3500 V for 15 min

Mobility R_M (relative mobility to alanine); both antibiotics move to the cathode with R_M 1.10*Ref.*

As TLC

**CLINDAMYCIN
(cf., lincomycin)****TLC 1***Medium*

Silica gel

*Solvent*Methyl ethyl ketone-acetone-water (9.3:2.6:1)
developed 20-45 min (15 cm)*Detection*

UV at 366 nm

 R_F

0.78

*Ref.*M. Baudet, *J. Pharm. Belg.*, 31 (1976) 247-254

TLC 2 (Quantitative)

Medium

Eastman 6061 sheets

Solvent

Acetone-methyl ethyl ketone-water (20.1 : 72.1 : 7.8)

Detection

The range of standards for quantitative chromatography was determined by the response observed for the samples to be assayed. Standards and samples were intermixed on the chromatography sheets. Zone diameters were read to the nearest millimeter and these values were used to construct the regression lines for estimation of antibiotic concentrations. Appropriate controls were included in these runs

After drying, the chromatographic sheets were applied, silica gel down, to the surface of trays containing agar seeded with lincomycin-sensitive *Sarcina lutea* and left in contact for 20 min. The sheets were removed and the trays were incubated for 16 h at 32°. The zones of inhibition were then read to the nearest millimeter and these data were used in a least-squares regression program with a Hewlett-Packard 9100A computer to estimate antibiotic concentrations

Ref.

T.F. Brodasky, C. Lewis and T.E. Eble, *J. Chromatogr.*, 123 (1976) 33-44

HPLC

Apparatus

Liquid chromatograph (DuPont, Model 830) equipped with a differential refractometer detector

Column

Bondapak C₁₈ micro silica gel, 30 cm × 3.9 mm (Waters Assoc.)

Mobile phase

1 g dioctyl sodium sulfosuccinate, 1 ml formic acid and 125 ml water. Methanol was added to volume. 500-ml volumetric flask

Column pressure

42.18 kg/cm (1 ml/min) and a 10-μl injection volume was used with a loop injector (Valco)

Detection

Refractive index detector

Chart speed

2.5 cm/5 min

Ref.

L.W. Brown, *J. Pharm. Sci.*, 67 (1978) 1254-1257

Relative retention time

Compound	Relative retention time
Clindamycin hydrochloride	
Lincomycin B hydrochloride	0.49
Epilincmoycin hydrochloride	0.55
Lincomycin hydrochloride	0.55
Clindamycin B hydrochloride	0.79
Clindamycin hydrochloride	1.00
Epilindamycin hydrochloride	1.00
Testosterone propionate (internal standard)	1.25
Clindamycin palmitate	
Clindamycin hydrochloride	0.23
Palmitic acid	0.44
Clindamycin 4-palmitate	0.59
Methyl palmitate (internal standard)	0.70
Clindamycin 3-palmitate	0.75
Clindamycin 2-palmitate	1.00
Clindamycin phosphate	
Lincomycin 2-phosphate	0.45
Lincomycin hydrochloride	0.61
Clindamycin 3-phosphate	0.90
Clindamycin 4-phosphate	0.92
Clindamycin 2-phosphate	1.00
Propylparaben (internal standard)	1.29
Clindamycin hydrochloride	1.94

COFORMYCIN

ELPHO

Medium

Paper

Buffers

A. pH 5.0

B. pH 8.0

Conditions

400 V, 2.5 h

Mobility

A. Moved toward cathode

B. No movement

Ref.

Japanese Patent 12278/70, May 4, 1970

COLICIN E₁**ELPHO****Medium**

Cellulose acetate (Separaphore III, Gelman Instruments)

Buffers

- A. β -Alanine-acetic acid, pH 4.5
B. 0.05 M Sodium barbital, pH 8.6

Conditions

Constant current; 1 mA/strip

Detection

0.5% Ponceau S in 5% trichloroacetic acid. Wash in 5% acetic acid to decolorize; make transparent with 5% acetic acid in methanol, dry on glass plate at 60°C for 15 min. Scan in a Joyce-Loebel recording densitometer

Mobility

Migrates as single band toward cathode

Ref.

S.A. Schwartz and D.R. Helinski, *J. Biol. Chem.*, 246 (1971) 6318-6327

COLISAN**PC****Paper**

Whatman No. 1

Solvent

n-Butanol-acetic acid-water (4:1:5), descending, 24 h

Detection

Paper dipped into a satd. soln. of iodine in petroleum ether (60-80°C)

R_F

Elongated spot, 0.4-0.8

Ref.

S.A. Leon and F. Bergmann, *Biotech. Bioeng.*, 10 (1968) 429-444

TLC**Medium**

Silica gel G

Solvents

- A. *n*-Butanol-acetic acid-water (4:1:5)
B. Chloroform-methanol-water (65:36:8)
C. Benzene-chloroform-methanol-acetic acid-water (15:15:23:7:7)

Detection

- A. Ninhydrin
B. As PC

Ref.

As PC

R_F

	Solvent	R _F
Colisan	A	0.38
Colisan	B	0.27
Colisan	C	0.75
Deaminated colisan	A	0.38 (ninhydrin —, iodine +)
Acetylated colisan		0.10 (ninhydrin —, iodine +)

ELPHO**Medium**

Whatman No. 1 paper

Buffer

1 N Acetic acid

Conditions

- A. 1000 V, 1 h (running tap water as coolant)
B. 3000 V, 1 h

Detection

0.3% ninhydrin in acetone. Heat for 5 min at 80°C

Mobility

- A. 10 cm towards cathode
B. 19 cm towards cathode

Ref.

As PC

**COLISTIN
(cf., Polymyxin E)****PC****Paper**

Whatman 3MM

Solvent

Butanol-acetic acid-water (12:3:5)

Detection

Paper stained with 0.25% ninhydrin in acetone containing 0.01% isatin and 1% lutidine at 100° for 10 min

R_F

0.86 (migration distance relative to alanine taken as 1.00)

Ref.

As Ampicillin, PC 1

ELPHO**Medium**

Whatman 3MM paper

Buffer

Formic acid–acetic acid–water (6:24:170)

Conditions

3000 V, potential gradient 60 V per cm; current 50 mA, 30 min

Detection

Paper stained with 0.25% ninhydrin in acetone containing 0.01% isatin and 1% lutidine at 100° for 10 min

Mobility

0.80 (migration distance relative to alanine taken as 1.00)

Ref.

As Ampicillin, PC 1

HPLC**Apparatus**

Laboratory Data Control modular liquid chromatograph, equipped with a high pressure Milton Roy Minipump with a pulse dampener (Model 709)

Column

Stainless steel (30 cm × 4.6 mm I.D.), preppacked with μ Bondapak C₁₈ (Waters Assoc.) attached to a septumless injector valve (Micromeritics) and to a flame-ionization detection

Mobile phase

Linear gradient from 20% acetonitrile to 50% acetonitrile and 20% methanol, both in phosphate buffer, pH 2.0, in 16 min

Flow-rate

1.0 ml/min

Temperatures

Cleaner, 900°; oxidizer, 690°; evaporator, 380°; reactor, 490°; detector, 175°

Chart speed

6.4 mm/min

Pressure

7.0 atm (1000 p.s.i.)

Detection

Moving-wire flame ionization

Retention time (min) (estimated from drawing)

Colistin A, 10.3

Colistin B, 8.5

Ref.

K. Tsuji and J.H. Robertson, *J. Chromatogr.*, 112 (1975) 663–672

COMIRIN**PC****Solvents**

A. Water satd. amyl alcohol

B. Water satd. *n*-butanol

C. *n*-Butanol–pyridine–water (2:1:4)

D. Aq. ammonia (sp. gr. 0.88)

E. Water satd. phenol

F. 60% Aq. dioxane (v/v)

G. 25% Aq. monoethyl amine (v/v)

H. 60% Pyridine–water (v/v)

I. Water

Detection

Bioautography vs. *Aspergillus flavus*

R_F

Solvent	R _F	Solvent	R _F
A	0.05	F	0.88
B	0.16	G	0.92
C	0.42	H	0.96
D	0.75	I	0.00
E	0.81		

Ref.

W.G.C. Forsythe, *Biochem. J.*, 59 (1955) 500–506

CCD**Solvent**

n-Butanol–water containing 10% pyridine (1:5); ten-tube diagonal distribution

Distribution

Comirin was recovered from tubes 8–10

Ref.

As PC

COPIAMYCIN**PC****Solvents**

A. *n*-Butanol–methanol–water (4:1:2)

B. Pyridine–*n*-butanol–water (4:3:7)

C. 50% Acetone

D. Benzene–methanol (4:1)

E. 75% Phenol

F. 3% Ammonium chloride

G. Water satd. with *n*-butanol

H. *n*-Butanol–satd. with water

I. Ethyl acetate–*n*-butanol (1:1)

J. *tert.*-Butanol–water (4:1)

Detection

Bioautography vs. *Candida albicans*

R_F (see p. 128)**Ref.**

T. Arai, S. Kuroda, H. Ohara, Y. Katoh and H. Kaji, *J. Antibiot.*, 18 (1965) 63–67

R_F

Solvent	R_F^*	Solvent	R_F^*
A	0.75	F	0.05
B	0.95	G	0.02
C	0.7–0.9	H	0.4–0.6
D	0.2–0.4	I	0.2
E	0.95	J	0.65

* Estimated from drawing

TLC 1*Medium*

Silica gel G

*Solvents*A. Acetic acid–*n*-butanol–water (6:25:25)B. *n*-Butanol–methanol–water (4:1:2) R_F

Solvent A, 0.4

Solvent B, 0.5

Ref.

As PC

TLC 2*Medium*

Aluminum oxide

*Solvents*A. Pyridine–*n*-butanol–water (4:6:3)

B. Benzene–methanol (4:1)

 R_F

Solvent A, 0.7

Solvent B, 0.3

Ref.

As PC

TLC 3*Medium*

Silica gel

*Solvent*Water–pyridine–*n*-butanol (7:4:3) R_F

Copiamycin, 0.25

Acetylcopiamycin, 0.66

*Ref.*K. Nakano and N. Nishiyama, *U.S. Patent* 3,852,426, Dec. 3, 1974**CORFORMYCIN****TLC***Solvent*

Benzene–methanol (1:1)

 R_F

0.31

*Ref.*H. Umezawa, K. Maeda and S. Kondo, *U.S. Patent* 3,959,257, May 25, 1976**COUMERMYCINS****PC 1***Paper*

Whatman No. 1. Equilibrate 1–2 h before development

*Solvents*A. Paper dipped in capryl alcohol–methanol (1:5) blotted; developed with 0.1 *M* phosphate buffer, descending, 6.5 h

B. As A, but developed 20–25 h

*Detection*Bioautography vs. *Staphylococcus aureus* R_F

	Solvent	R_F^*
Coumermycin complex	A	0 –0.1
Coumermycin D-1	B	0.1–0.3
Coumermycin D-2	B	0.4–0.6
Coumermycin D-3	B	0.7–0.9

* Estimated from drawing

*Ref.*J. Berger, A.J. Schocher, A.D. Batcho, B. Pecherer, O. Keller, J. Maricq, A.E. Karr, B.P. Vaterlaus, A. Furlenmeier and H. Spiegelberg, *Antimicrob. Agents Chemother.*, 1965 (1966) 778–785**PC 2***Paper*

S and S 589 Blue Ribbon; 1.3-cm wide strips

*Solvent*0.1 *M* Triethanolamine adjusted to pH 7.0 with glacial acetic acid (2:3)*Detection*Bioautography vs. *Staphylococcus aureus* ATCC 6538P R_F Coumermycin A₁, 0.3–0.4*Ref.*J.G. Keil, I.R. Hooper, M.J. Cron, H. Schmitz, D.E. Nettleton and J.C. Godfrey, *Antimicrob. Agents Chemother.*, 1968 (1969) 120–127**TLC***Medium*

Silica gel G

Solvent

Glacial acetic acid–methanol–carbon tetrachloride (6:6:90), ascending, 15 cm

R_F	
Coumermycin D-1a,	0.60
Coumermycin D-1b (c),	0.50
Coumermycin D-1d,	0.40
Coumermycin D-2,	0.35
Coumermycin D-3,	0.30
Coumermycin D-4,	0.25

Ref.

As PC 1

CRANOMYCIN

PC

Paper

Toyo No. 50

Solvents

- Water satd. *n*-butanol
 - 1.5% Aq. ammonium chloride
 - 75% Aq. phenol
 - Acetone–water (1:1)
 - n*-Butanol–methanol–water (4:1:2)
 - tert*-Butanol–acetic acid–water (74:3:25)
 - n*-Butanol–acetic acid–water (4:1:1)
- All of above developed ascending

Detection

Bioautography vs. *Sarcina lutea* R_F

Solvent	R_F	Solvent	R_F
A	0.97	E	1.0
B	0.76	F	0.97
C	0.98	G	0.08–0.11
D	0.96		

Ref.

S.-I. Kondo, M. Shimura, M. Sezaki, K. Sato and T. Hara, *J. Antibiot.*, 17 (1964) 230–233

TLC

Medium

Silica gel G

Solvents

- Water satd. ethyl acetate
- Benzene–methanol (4:1)
- Benzene–methanol–28% ammonium hydroxide (80:20:1)
- Benzene–methanol–acetic acid (80:20:1)
- Water
- Water–28% ammonium hydroxide (100:1)
- Water–acetic acid (100:1)

Detection

Color: potassium permanganate solution

 R_F

Solvent	R_F	Solvent	R_F
A	0.06	E	0.18
B	0.17	F	0.58
C	0.49	G	0.23
D	0.08		

Ref.

As PC

CREMEOMYCIN

PC

Solvents

- 1-Butanol–water (84:16), 16 h
- 1-Butanol–water (84:16) + 0.25% *p*-toluenesulfonic acid, 16 h
- 1-Butanol–acetic acid–water (2:1:1), 16 h
- 2% piperidine (v/v) in 1-butanol–water (84:16), 16 h
- 1-Butanol–water (4:96), 5 h
- 1-Butanol–water (4:96) + 0.25% *p*-toluenesulfonic acid, 5 h

Detection

Bioautography vs. *Proteus vulgaris* R_F

Solvent	R_F^*	Solvent	R_F^*
A	0.1–0.5	D	0.35–0.6
B	0.5	F	0.9
C	0.8	F	0.7–0.9

* Estimated from drawing

Ref.

M.E. Bergy and T.R. Pyke, *U.S. Patent* 3,350,269, Oct. 31, 1967

CRINPELLIN

TLC

Medium

Silica gel, Merck 60 F₂₅₄

Solvents

- Cyclohexane–ethyl acetate–formic acid (120:40:5)
- Benzene–acetone–acetic acid (70:30:5)
- Chloroform

Detection

A. Bioautography vs. *Bacillus subtilis*

B. UV at 254 nm

The antibiotic shows positive color reactions with

dichlorofluorescein (pink), potassium permanganate, and Carr-Price and Kagi-Miescher reagents

R_F

Solvent A, 0.39
Solvent B, 0.92
Solvent C, 0.89

Ref.

J. Kupka, T. Anke, F. Oberwinkler, G. Schramm and W. Steglich, *J. Antibiot.*, 32 (1979) 130–135

CRYOMYCIN

PC 1

Solvents

A. Deionized water
B. Methanol
C. Ethanol
D. 80% Phenol
E. Butanol–acetic acid–water (4:1:1)
F. Benzene–methanol (4:1)
G. Wet diethyl ether
H. Wet petroleum ether
I. Chloroform

Detection

Bioautography vs. *Bacillus subtilis* LFO 30357

R_F

Solvent	R_F	Solvent	R_F
A	0.80	F	0.30
B	0.80	G	0.0
C	0.85	H	0.0
D	0.80	I	0.0
E	0.45		

Ref.

N. Yoshida, Y. Tani and K. Ogata, *J. Antibiot.*, 11 (1972) 653–659

PC 2

Solvent

Butanol–acetic acid–pyridine–water
(15:3:10:12)

Detection

Bioautography vs. *Micrococcus lysodeikticus* IFO 3333

R_F

0.6–0.45 estimated from drawing

Ref.

N. Yoshida, S. Hayashi, Y. Tani and K. Ogata, *J. Antibiot.*, 27 (1974) 128–132

ELPHO

Medium

Paper

Buffers

A. 0.025 M Acetate (ca. pH 3–5)
B. Phosphate (ca. pH 6–8)
C. Glycine (pH 9 and 10)

Conditions

ca. 15–20 mA and 2000 V for 40 min each

Mobility

Towards anode

Ref.

As PC 1

CURAMYCIN

PC 1

Solvent

As avilamycin, PC

Detection

As avilamycin, PC

R_F

0.60

Ref.

As avilamycin, PC

PC 2

Paper

As everninomicin PC 1

Solvent

As everninomicin PC 1

Detection

As everninomicin PC 1

R_F

0.42

Ref.

As everninomicin PC 1

TLC 1

Medium

As avilamycin, TLC 1

Solvent

As avilamycin, TLC 1

Detection

As avilamycin, TLC 1

R_F

0.43

Ref.

As avilamycin, PC

TLC 2

Medium

Silica gel (Merck No. 7731) (activated at 130°C for 2 h)

Solvent

Chloroform-methanol (92:8), 20°C

Detection

- A. Bioautography vs. *Bacillus subtilis*
 B. Spray with conc. sulfuric acid and heat for 10 min at 110°C

R_F
 0.50

Ref.

L. Ninet, F. Benazet, Y. Charpentie, M. Dubost, J. Florent, J. Lunel, D. Mancy and J. Preud'Homme, *Experientia*, 30 (1974) 1270-1272

CYANEIN**PC****Solvents**

- A. Water
 B. *n*-Butanol satd. with water
 C. Ethyl acetate satd. with water
 D. Benzene satd. with water
 E. Isoamyl acetate-methanol-formic acid-water (4:2:1:3), bottom layer
 F. *n*-Butanol-methanol-water (4:1:5), bottom layer
 G. Methanol-hexane (6:4), bottom layer
 H. Benzene-cyclohexane-phosphate buffer of pH 7.4 (5:35:60), bottom layer

Detection

Bioautography vs. *Candida pseudotropicalis*

R_F

Solvent	R_F	Solvent	R_F
A	0.00	E	0.88
B	0.93	F	0.65
C	0.97	G	0.87
D	0.00	H	0.00

Ref.

V. Betina, P. Nemec, J. Dobias and Z. Barath, *Folia Microbiol.*, 8 (1962) 353-357

CYATHINS**TLC****Medium**

Silica gel G, 0.5-mm thickness. Plates heated at 100°C for 30 min before use

Solvent

Benzene-acetone-acetic acid (75:25:1)

Detection

Spray with 30% sulfuric acid

R_F

Cyathin No. 1, 0.67
 Cyathin B, 0.51
 Cyathin B₃, 0.56
 Cyathin C₅, 0.54
 Cyathin A₃, 0.27
 Cyathin A₄, 0.06

Ref.

B.N. Johri, *J. Chromatogr.*, 56 (1971) 324-329

CYCLAMIDOMYCIN**ELPHO****Medium**

Paper

Buffer

Formic acid-acetic acid-water (25:75:900)

Conditions

3300 V, 20 min

Detection

Bioautography vs. *Klebsiella pneumoniae*

Mobility

Cyclamidomycin moved 12.9 cm to cathode with R_M (L-alanine = 1.0) of 1.29; two minor components at R_M 0.5, 1.90

Ref.

S. Takahashi, M. Nakajima, Y. Ikeda, S. Kondo, M. Hamada, K. Maeda and H. Umezawa, *J. Antibiot.*, 24 (1971) 902-903

CYCLOHEXIMIDE**PC****Paper**

Whatman No. 1

Solvents

- A. Water satd. butanol
 B. Butan-1-ol-acetic acid-water (2:1:1)
 C. Butan-1-ol-pyridine-water (1:0.6:1)
 D. Acetone-water (1:1)

R_F

Solvent	R_F	Solvent	R_F
A	0.90	C	0.96
B	0.96	D	0.91

Ref.

As Amphotericins, TLC 2

TLC**Medium**

Silica gel

Solvent

Isopropanol-ethyl acetate (2:98)

Detection

Spray with molybdic acid-ceric sulfate-sulfuric acid-water (2.5:1.0:11.8:88, w/w/v/v)

R_F
0.7

Ref.

L.A. Koninek, *U.S. Patent* 3,915,802, Oct. 28, 1975

CYCLOSERINE**PC 1****Solvent**

Ethanol-water (4:1)

Detection

Ninhydrin (produces a brownish-yellow color)

R_F
0.4

Ref.

D.A. Harris, M. Ruger, M.A. Reagon, F.J. Wolf, R.L. Peck, H. Wallich and H.B. Woodruff, *Antibiot. Chemother.*, 5 (1955) 183-190

PC 2**Solvents**

- A. Buffered phenol (pH 12)
- B. *tert.*-Butanol-acetic acid-water

Detection

- A. Ninhydrin
- B. Ferric chloride (deep red color produced)

R_F
Solvent A, 0.60-0.65
Solvent B, 0.60-0.65

Ref.

G.M. Shull and J.L. Sardinas, *Antibiot. Chemother.*, 5 (1955) 398-399

PC 3**Paper**

Whatman No. 3MM

Solvents

- A. *n*-Butanol-acetic acid-water (4:1:5)
- B. 77% Ethanol
- C. Methyl ethyl ketone-pyridine-water (20:5:8)
- D. Isopropanol-ammonia-water (80:2:18)

R_F

Solvent	R_F
A	0.31
B	0.43
C	0.37 (streaking)
D	0.17

Ref.

F.C. Neuhaus and J.L. Lynch, *Biochem.*, 3 (1964) 472

PC 4**Paper**

Whatman No. 1

Solvents

- A. 80% Ethanol
- B. *n*-Butanol-water-acetic acid (3:1:1)
- C. 0.01 *M* Phosphate buffer, pH 6.0-isopropanol (3:7)
- D. 75% Aq. methanol containing 2% sodium chloride
- E. *n*-Propanol-2 *N* ammonium hydroxide (7:3)
- F. *n*-Butanol-acetic acid-water (8:2:5)

Detection

- A. Ninhydrin
- B. Bioautography

R_F

Solvent	R_F	Solvent	R_F
A	0.36-0.42	D	0.66
B	0.32-0.36	E	0.22
C	0.41	F	0.22

Ref.

E.O. Stapley, T.W. Miller and M. Jackson, *Antimicrob. Agents Chemother.*, 1968 (1969) 268-273

DACTYLARIN**TLC****Medium**

Silica gel Silufol® UV₂₅₄

Solvents

- A. *n*-Butanol-chloroform (1:9)
- B. Methanol-chloroform (1:9)
- C. Acetone-chloroform (7:3)

Detection

UV. Due to the UV absorbing chromophores in the molecule of dactylarin, visualization of the spots could be achieved by the luminescent indicator method using silica gel foils with an inert luminescent indicator (Silufol® UV₂₅₄; Lachema, Brno, Czechoslovakia); spots were then visualized under UV light

R_F

Solvent A, 0.59
Solvent B, 0.73
Solvent C, 0.76

Ref.

M. Kettner, P. Nemec, S. Kovac and J. Balanova, *J. Antibiot.*, 26 (1973) 692-696

DANUBOMYCIN

PC

Solvents

- A. *n*-Butanol satd. with water
- B. *n*-Butanol satd. with water + 2% *p*-toluene-sulfonic acid + 2% piperidine
- C. Methyl isobutyl ketone satd. with water
- D. 80% Ethanol with 1.5% sodium chloride. Paper impregnated with 0.95 *M* sodium bisulfate soln.
- E. Butanol-methanol-water (4:1:2)
- F. Water satd. with methyl isobutyl ketone
- G. Water satd. with methyl isobutyl ketone + 1% *p*-toluenesulfonic acid
- H. 75% Water + 25% of a mixture of methanol-acetone (3:1) adjusted to pH 10.5 with ammonia and neutralized with phosphoric acid to pH 7.5

Detection

Bioautography vs. *Bacterium megatherium* or *Micrococcus pyogenes* var. *aureus*

R_F

Solvent	R_F	Solvent	R_F
A	0.65	E	0.67
B	0.90	F	0.05, 0.70
C	0.00	G	0.80
D	0.5	H	0.05, 0.70

Ref.

E. Gaumann and W. Voser, *U.S. Patent* 3,092,550, June 4, 1963

CCD

Solvents

- A. Stage 1; light petroleum (b.p. 40–70°)–benzene–methanol–water (5:5:8:2), 10 transfers
- B. Stage 2; light petroleum–benzene–absolute ethanol–water (7.5:2.5:7.5:2.5), 375 transfers

Distribution

- A. Danubomycin is found in tubes 4–10 and transferred to stage B
- B. Danubomycin resolved into 4 components identified as B1, B2, B3, B4; maxima are in tubes 40, 64, 124 and 164, respectively

Ref.

As PC

DAUNOMYCIN

PC

Paper

As adriamycins, PC

Solvent

As adriamycins, PC

R_F

Solvent A, 0.20, 0.50
Solvent C, 0.35

Ref.

As adriamycins, PC

TLC

Medium

As adriamycins, TLC 1

Solvent

As adriamycins, TLC 1

R_F

Solvent A, 0.4
Solvent B, 0.0
Solvent C, 0.0

Ref.

As adriamycins, PC

HPLC 1

Apparatus

Varian LC 4100

Column

Micropak Si-10, 15 cm × 2 mm I.D.

Mobile phase

Isopropanol–0.5 *M* acetate buffer (pH 4.5) (90:10)

Flow-rate

25 ml/h

Detector

UV photometer

Sensitivity

0.16 a.u.f.s.

Sample size

5 μ l (conc. 0.5 mg/ml)

Type

Reversed-phase

Retention time (estimated from drawing)

Daunomycin, 2.4
Daunomycinone, 2.5

Ref.

As Cephalosporins, TLC 9

HPLC 2

Apparatus

DuPont Series 848 Amplifier Pump at 4500 p.s.i. with no pulse dampener. A six-port Rheodyne rotary injection valve fitted with a 200- μ l loop was mounted between the pump and the column

Column

Stainless-steel analytical column (250 × 2.1 mm) packed with Zorbax SIL, particle diameter 5 μ m

Temperature

Room temperature

Mobile phase

Methylene chloride-methanol-25% ammonia-water (90:9:0.1:0.8)

Flow-rate

0.9 ml/min

Detector

DuPont Series 837 Spectrophotometer (200-600 nm) fixed at 490 nm with a sensitivity of 0.02 or 0.04 absorbance units

Retention time

2.5 min

Ref.

R. Hulhoven and J.P. Desager, *J. Chromatogr.*, 125 (1976) 369-374

DAUNOMYCINOL**HPLC****Apparatus**

See Daunomycin, HPLC 2

Column

See Daunomycin, HPLC 2

Temperature

See Daunomycin, HPLC 2

Mobile phase

See Daunomycin, HPLC 2

Flow-rate

See Daunomycin, HPLC 2

Detector

See Daunomycin, HPLC 2

Retention time

7 min

Ref.

See Daunomycin, HPLC 2

DAUNOMYCINONE**TLC****Medium**

Silica gel

Solvent

Chloroform-acetone (4:1)

R_F

0.60, 0.35

Ref.

French Patent 2,183,710, Dec. 12, 1973

DAUNORUBICIN (13057 RP)**PC****Paper**

Arches No. 302, impregnated with a phosphate buffer, pH 4.8

Solvent

Water satd. *n*-butanol, descending

Detection

Bioautography vs. *Bacillus subtilis* or *Klebsiella pneumoniae*

R_F

0.3

Ref.

S. Pinnert, L. Ninet and J. Preud'Homme, *U.S. Patent* 3,997,662, Dec. 14, 1976, and *U.S. Patent* 3,997,663, Dec. 14, 1976

TLC**Medium**

Silica gel

Solvents

- A. Benzyl alcohol-ethyl formate-formic acid-water (4:4:1:5), ascending
- B. Chloroform-dioxan (95:5)
- C. Ethyl acetate-diethyl ether- $\frac{1}{3}$ M phosphate buffer of pH 4.8 (50:50:25)

R_F

Solvent A, 0.60

Solvent B, 0.25

Solvent C, 0.5

Ref.

J. Lunel and J. Preud'Homme, *U.S. Patent* 3,987,167, Oct. 19, 1976

DEAMINO-ACTINOTIOCIN**TLC****Medium**

Eastman Chromagram sheet

Solvent

Chloroform-methanol (9:1)

R_F

0.91

Ref.

As actinotiocin, TLC

DEKAMYCIN**PC****Paper**

Whatman No. 1

Solvents

- A. 20% Ammonium chloride soln. (w/v)
- B. 80% Methanol in 3% sodium chloride
- C. Butanol-pyridine-acetic acid-water (6:4:1:3)
- D. Chloroform-methanol-conc. ammonia (1:1:1), lower layer
- E. Chloroform-methanol-conc. ammonia (1:1:1), upper layer

Detection

- A. Ninhydrin
- B. Alanine was applied as reference

R_F

Solvent	R_F	Solvent	R_F
A	0.75	D	0.05
B	0.1	E	0.62
C	0.05		

Ref.

T.C. Quyen, T.C. Loc, N.V. Khanh, T.H. Chau, M. Long, N.K. Lien, H.D. Dao, *Biologia Bratislava*, 32 (1977) 217–222

DELTAMYCIN

TLC

Medium

Silica gel precoated (Merck) 60 F₂₅₄

Solvents

- A. Benzene–acetone (2:3)
- B. Chloroform–methanol (10:1)
- C. *n*-Butanol–acetic acid–water (3:1:1)

Detection

Sulfuric acid, all purplish blue

 R_F

Solvent	Deltamycin			
	A ₁	A ₂	A ₃	A ₄
A	0.41	0.47	0.51	0.54
B	0.55	0.59	0.61	0.62
C	0.46	0.50	0.55	0.58

Ref.

Y. Shimauchi, K. Kubo, K. Osumi, K. Okamura, Y. Fukagawa, T. Ishikura and J. Lein, *J. Antibiot.*, 31 (1978) 270–275

DEMETHYLBLEOMYCIN A₂

PC

Paper

Toyo Filter No. 51, ascending

Solvent

10% Ammonium chloride

 R_F

0.71

Ref.

H. Umezawa, T. Takita, A. Fujii and H. Ito, *U.S. Patent* 3,960,834, June 1, 1976

TLC

Medium

Silica gel G

Solvents

- A. Methanol–10% ammonium acetate (1:1)
- B. Methanol–10% ammonium acetate–10% ammonia (10:9:1)

 R_F

Solvent A, 0.80

Solvent B, 0.78

Ref.

As PC

N-DEMETHYLCELESTICETIN

TLC

Medium

Silica gel G

Solvent

Chloroform–methanol (6:1)

Detection

Bioautography vs. *Sarcina lutea*

 R_F

0.15

Ref.

A.D. Argoudelis, J.H. Coats and O.K. Sebek, *U.S. Patent* 3,812,196, May 21, 1974

N-DEMETHYL-7-O-DEMETHYL-CELESTICETIN

TLC

Medium

Silica gel G

Solvent

Chloroform–methanol (6:1)

Detection

Bioautography vs. *Sarcina lutea*

 R_F

0.05

Ref.

As N-demethylcelesticetin TLC

4-DEMETHYLGRISEOFULVIN

TLC

Medium

Silica gel

Solvent

Chloroform–acetic acid–water (4:1:1)

 R_F

0.55

Ref.

M. Rowland and S. Riegelman, *J. Pharm. Sci.*, 62 (1973) 2030–2032

6-DEMETHYLGRISEOFULVIN**TLC****Medium**

Silica gel

Solvent

Chloroform-acetic acid-water (4:1:1)

R_F

0.34

Ref.

As 4-demethylgriseofulvin, TLC

DENEOSAMINYLLIVIDOMYCIN B**TLC****Medium**

Silica gel 60 F (Merck)

Solvent

Chloroform-methanol-7% aq. ammonia (1:4:3)

Detection

Ninhydrin

R_F $R_{\text{lividomycin B}} = 1.24$ **Ref.**T. Yamaguchi, K. Kamiya, T. Mori and T. Oda, *J. Antibiot.*, 30 (1977) 332-333**3'-DEOXYBUTIROSIDIN A (BB-K186)****TLC****Solvent**

Chloroform-methanol-28% ammonium hydroxide-water (1:4:2:1), developed twice

R_F

0.35

Ref.T. Naito, S. Nakagawa and S. Toda, *U.S. Patent* 3,960,833, June 1, 1976**DEOXYCHLORAMPHENICOL****TLC****Medium**

Silica gel (Kodak No. 6060)

Solvents

- A. Chloroform-methanol (93:7 or 87.5:12.5)
- B. *n*-Butanol-chloroform-acetic acid (10:90:0.5)
- C. Water satd. ethyl acetate

Detection

The chromatogram was sprayed with a soln. of 3 ml of 15% stannous chloride, 15 ml of conc. hydrochloride and 18 ml of water. After drying, it was analyzed by spraying with 1 g *p*-dimethylaminobenzaldehyde dissolved in a mixture of 30 ml ethanol, 30 ml conc. hydrochloride and 180 ml of *n*-butanol

R_F

Solvent	DeoxyCAP *
A	0.58
B	0.48
C	0.50

* DeoxyCAP = 1-deoxychloramphenicol

Ref.H. Akagawa, M. Okanishi and H. Umezawa, *J. Antibiot.*, 32 (1979) 610-620**DEOXYHERQUEINONE
(See atroventin)****3'-DEOXYKANAMYCIN****TLC****Solvent***n*-Butanol-pyridine-water-acetic acid (6:4:3:1)**R_F** $R_{\text{kanamycin}} = 2.2.$ **Ref.**S. Umezawa, H. Umezawa and T. Tsuchiya *U.S. Patent* 3,929,761, Dec. 30, 1975**DEOXYNARASIN****PC****Solvents**

- A. Water-methanol-acetone (12:3:1) adjusted to pH 10.5 with ammonium hydroxide and then lowered to pH 7.5 with phosphoric acid
- B. Water-methanol-acetone (12:3:1) adjusted to pH 10.5 with ammonium hydroxide and then lowered to pH 7.5 with hydrochloride acid
- C. 1% Methyl isobutyl ketone, 0.5% ammonium hydroxide in water
- D. Water satd. benzene
- E. Water-methyl isobutyl ketone-ethyl acetate (98:1:1)

R_F

Sol-vent	Nara-sin	A-28086D	Deoxy-nara-sin	Epi-deoxy-nara-sin
A	0.20	0.11	0.08	0.21
B	0.42	0.25	0.21	0.44
C	0.56	0.38	0.33	0.66
D	0.51	0.51	0.74	0.55
E	0.77	0.61	0.51	0.68

Detection

Bioautography vs. *Bacillus subtilis*

Ref.

W.M. Nakatsukase, G.G. Marconi, R. Neuss and R.L. Hamill, *U.S. Patent* 4,141,907, Feb. 27, 1979

TLC**Medium**

Silica gel

Solvent

Ethyl acetate

Detection

Another substance, chromatographically coincident with A-28086-I which is described in U.S. Patent 4,038,384, is co-produced with the deoxynarasin complex. Although this substance initially coprecipitates with the deoxynarasin antibiotics, it is readily separated from them by silica gel chromatography. On silica gel thin-layer chromatography, this substance is less polar than either 20-deoxynarasin or 20-deoxyepi-17-narasin when ethyl acetate is the developing solvent. Vanillin spray reagent (3% vanillin in methanol + 0.5 ml conc. sulfuric acid per 100 ml of soln.) is convenient for detection. After spraying with vanillin and heating, this substance, like A-28086-I, gives a blue spot while the deoxynarasin antibiotics give bright yellow spots which later darken

R_F

Compound	R_F
Narasin	0.29
A-28086D	0.35
Deoxynarasin	0.42
Epideoxynarasin	0.74

Ref.

As PC

4''-DEOXY-4''-OXOERYTHROMYCIN B**TLC****Medium**

Silica gel HF₂₅₄ (Merck)

Solvent

Methanol-benzene-conc. ammonium hydroxide (33 ml:67 ml:3 drops)

Detection

UV

Ref.

P.H. Jones, J.R. Martin, J.B. McAlpine, J.M. Pavulik and J.S. Tadanier, *U.S. Patent* 3,842,069, Oct. 15, 1974

3'-DEOXYPAROMAMINE**TLC****Medium**

Silica gel G

Solvent

Chloroform-methanol-2% ammonium hydroxide (2:1:1)

R_F

0.42

Ref.

M. Yamaguchi and S. Mitsuhashi, *J. Antibiot.*, 27 (1974) 507-515

DERINAMYCIN**PC****Solvents**

- A. 20% Ammonium chloride
- B. Benzene-methanol (4:1)
- C. Water satd. *n*-butanol
- D. Ethyl acetate-pyridine-water (2:1:2)
- E. *n*-Butanol-acetic acid-water (4:1:2)
- F. *n*-Butanol-methanol-water (4:1:2)
- G. Acetone-water (1:1)

Detection

- A. Ninhydrin
- B. Bioautography vs. *Pseudomonas saccharophila*

R_F

Solvent	R_F *	Solvent	R_F *
A	0.0	E	0.48
B	0.0	F	0.55
C	0.20	G	0.90
D	0.45		

* Estimated from drawing

Ref.

K. Uchida and H. Zahner, *J. Antibiot.*, 28 (1975) 266-273

ELPHO**Medium**

Paper, Whatman No. 1

Buffers

- A. 0.1 M Acetate buffer, pH 4.0
- B. 0.1 M Phosphate buffer, pH 7.0
- C. 0.1 M Veronal buffer, pH 8.6

Conditions

250 V, 19 cm × 5 cm, 3 h

Ref.

As PC

DESACETYLCEPHALOGLYCIN**PC****Paper**Whatman No. 1 $\frac{1}{2}$ in. paper strips, descending**Solvent***n*-Butanol-ethanol-water (8:2:10), upper phase**Detection**Bioautography vs. *Bacillus subtilis* ATCC 6633**Ref.**D. Willner, V.Z. Rossomano and V. Sprancmanis, *J. Antibiot.*, 26 (1973) 179-180**TLC****Media**A. Avicel F (250 μ m) prescored, Uniplat plates used for thin layerB. Avicel F (1000 μ m, 20 × 20) Uniplat plates used for thick layer**Solvent**

Acetonitrile-water (75:25)

Detection

UV. In preliminary experiments zone A was identified as that of desacetylcephaloglycin

Ref.

As PC

DESERTOMYCIN**PC****Paper**Whatman No. 1 impregnated with MacIlvaines phosphate buffer at pH 2.2, 3, 4, 5, 6, 7, 8, 9 and 10. 10 μ g antibiotic spotted**Solvent**

Distilled water

DetectionA. Bioautography vs. *Bacillus subtilis* ATCC 6633

B. Alkaline potassium permanganate soln.

C. Iodine vapor

R_F

Varies according to pH. Most rapid movement at pH 2.2, decreasing to slightly off origin at pH 6 or 7, and increasing slightly to pH 10

Ref.F. Sztariczka and J. Uri, *Microchim. Acta*, (1963) 431-441**DESISOVALERYLNIDDAMYCIN
(Niddamycin derivative)****TLC****Medium**

Merck plates

Solvents

A. Methylene chloride-95% aq. methanol-water (85:15:1)

B. Methylene chloride-95% aq. methanol-ammonium hydroxide (90:10:1)

DetectionA. Bioautography vs. *Bacillus subtilis* and *Staphylococcus aureus*

B. UV at 254 nm

C. After spraying the plates with anisaldehyde reagent [95% ethanol-conc. phosphoric acid-anisaldehyde (9:1:1)] and heating to 100°C for 5-10 min in an oven, the conversion product of niddamycin was characterized by a rather unique purple-red color

R_F

Solvent A, 0.57-0.65

Solvent B, 0.3 -0.37

Ref.R.J. Theriault, *U.S. Patent* 3,784,447, Jan. 8, 1974; *German Patent* 2,319,100, April 16, 1973**DESMYCOSIN****PC****Solvents**

A. Methyl ethyl ketone vs. pH 4 buffered paper

B. Methyl ethyl ketone

C. *n*-Butanol satd. with water vs. pH 4 buffered paperD. *n*-Butanol satd. with water

E. Water with 7% sodium chloride + 2.5% methyl ethyl ketone

F. Ethyl acetate satd. with water vs. pH 4 buffered paper

R_F

Solvent	R _F	Solvent	R _F
A	0.40	D	0.74
B	0.63	E	0.76
C	0.90	F	0.11

Ref.R.L. Hamill and W.M. Stark, *J. Antibiot.*, 17 (1964) 133-139

2-DESOXYSTREPTAMINES

TLC

Media

- A. Silica gel
B. Cellulose

R_F

Solvents

- A. Benzene-ethyl acetate (1:1)
B. Chloroform-acetone (8:2)
C. Chloroform-methanol-ammonia (2:2:1)

Ref.

J.B. Chazan, D. Coussediere and J. Gasc, *U.S. Patent* 3,959,255, May 25, 1976

Derivative	Medium	Solvent		
		A	B	C
4-O-(2',6'-Diamino-2',6'- α -D-glucopyranosyl) 2-desoxystreptamine anomer	A	0.43		
6-O-(3''-Methylamino-3'',4'',6''-tridesoxy- α -D-xylohexopyranosyl) 2-desoxystreptamine anomer	A	0.3		
4-O-[2'-(N-Carbobenzoyl)-amino-2'-desoxy-3'-O-benzyl-6'-O-tosyl- α -D-glucopyranosyl]-6-O-[tosyl-2''-O-benzyl-3''-(N-carbethoxy-N-methyl)-amino-3'',4'',6''-tridesoxy- α -D-xylohexopyranosyl]-1,3-dibenzyl-oxycarbonyl-2-desoxystreptamine	A		0.48	
4-O-[2'-(N-Carbobenzoyl)-amino-6'-azido-3'-O-benzyl-2',6'-didesoxy- α -D-glucopyranosyl]-6-O-[2''-O-benzyl-3''-(N-carbethoxy-N-methyl)-amino-3'',4'',6''-tridesoxy- α -D-xylohexopyranosyl]-1,3-dibenzyl-oxycarbonyl-2-desoxystreptamine	A		0.24	
4-O-(2',6'-Diamino-2',6'-didesoxy- α -D-glucopyranosyl)-6-O-(3'',4'',6''-tridesoxy- α -D-xylohexopyranosyl)-2-desoxystreptamine	B			0.48
4-O-(2',6'-Diamino-2',6'-didesoxy- α -D-glucopyranosyl)-6-O-(3''-amino-3'',4'',6''-tridesoxy- α -D-xylohexopyranosyl)-2-desoxystreptamine *	A			0.30

* German Patent 2,510,838, Aug. 16, 1975

DESTOMYCINS

PC 1

Solvents

- A. Water satd. *n*-butanol
B. 1.5% Aq. ammonium chloride
C. Phenol-water (3:1)
D. Acetone-water (1:1)

E. *n*-Butanol-methanol-water (4:1:2)

F. Benzene-methanol (4:1)

G. *tert*.-Butanol-acetic acid-water (2:1:1)

H. 80% Aq. methanol + 15% sodium chloride

R_F (see p. 140)

Ref.

S. Kondo, M. Sezaki, M. Koike, M. Shimura, E. Akita, K. Satoh, K. Hamamoto, T. Niida, and T. Hara, *U.S. Patent* 3,926,948, Dec. 16, 1975

R_F

Solvent	Destomycin A
A	0
B	0.96
C	0.07
D	0.07
E	0.04
F	0
G	0.20
H	0.55–0.62

PC 2

Paper

Ascending

Solvent

Chloroform–methanol–17% ammonia (2:1:1), (12 h)

 R_F

Destomycin A, 0.03

Ref.

As Apramycin, PC 1

TLC 1

*Solvent*Methanol– $\frac{1}{2}$ N sulfuric acid (1:4)*Detection*

Bioautography

 R_F

Destomycin A, 0.50

Destomycin B, 0.53

Ref.

As PC 1

TLC 2

Medium

Silica gel G

Solvent

Chloroform–methanol–4% ammonium hydroxide (12:1:1), upper layer

 R_F

Destomycin B, 0.24

*Ref.*A. Tamura, R. Furuta and H. Kotani, *J. Antibiot.*, 28 (1975) 260–265

DETOXIN

PC

Paper

Toyo-Roshi No. 51

*Solvents*A. Water satd. *n*-butanol

B. Butanol–acetic acid–water (4:1:2)

C. As B but (2:1:1)

D. As B but (4:1:1)

E. As B but (3:3:1)

F. Butanol–formic acid–water (8:3:2)

G. Butanol–ethanol–water (5:2:3)

H. Butanol–methanol–water (4:1:2)

I. Ethanol–acetic acid–water (33:5:62)

J. Butanol–water–conc. HCl (5:1:1)

K. Ethanol–ammonium hydroxide–water (80:4:16)

L. Propanol–pyridine–acetic acid–water (15:10:3:12)

 R_F

Solvent	R_F	Solvent	R_F
A	0.43	G	0.77
B	0.86	H	0.53
C	0.89	I	0.84
D	0.81	J	1.00
E	0.91	K	0.80
F	0.55	L	0.93

*Ref.*H. Yonehara, S. Aizawa, T. Hidaka, H. Sedo and N. Odake, *17th Meeting, Japan Antibiotics Research Assn.*, March 24, 1967

TLC

Medium

Silica gel G

Solvents

A. Ethanol–ammonium hydroxide–water (33:5:62)

B. Butanol–methanol–water (2:1:1)

C. Water satd. butanol

D. Acetone

 R_F

Solvent	R_F
A	0.67
B	0.60
C	0.085
D	0.00

Ref.

As PC

ELPHO**Buffers**

- A. Potassium chloride-HCl, pH 1.7
- B. Potassium dihydrogen phosphate-disodium phosphate, pH 6.1
- C. As B, but pH 7.5
- D. As B, but pH 7.9
- E. Sodium carbonate-sodium bicarbonate, pH 8.5
- F. As E, but pH 9.0

Conditions

10 mA/6 cm

Mobility (estimated relative mobility toward cathode or anode)

Buffer	Mobility	Buffer	Mobility
A	-2.0	D	0.0
B	-1.5	E	+0.5
C	-0.5	F	+1.0

Ref.

As PC

DEXTOCHRYSIN**TLC****Medium**

Silica gel

Solvent

Ethyl acetate-methanol (4:1)

R_F

0.46

Ref.

H. Aoki, N. Miyairi, M. Ajisaka and H. Sakai, *J. Antibiot.*, 22 (1969) 201-206

DIANEMYCIN**PC****Solvents**

- A. Water-ethanol-acetic acid (70:24:6)
- B. 10% Aq. propanol

Detection

Bioautography vs. *Bacillus subtilis*

R_F

Solvent A, 0.28
Solvent B, 0.77

Ref.

R.L. Hamill, M.M. Hoehn, G.E. Pittenger, J. Chamberlain and M. Gorman, *J. Antibiot.*, 22 (1969) 161-164

TLC**Medium**

Silica gel

Solvent

Ethyl acetate

Detection

Vanillin-sulfuric acid spray

R_F

0.25

Ref.

As PC

DIAZOMYCINS**PC****Solvent**

80% Aq. isopropanol

R_F

Diazomycin A, 0.6
Diazomycin B, 0.2
Diazomycin C, 0.9
(Estimated from drawing)

Ref.

K.V. Rao, S.C. Brooks, Jr., M. Kugelman and A.A. Romano, *Antibiot. Ann.*, (1959-1960) 943-949

DICLOXACILLIN**HPLC****Apparatus**

Constametric I pump and Spectromonitor I (Laboratory Data Control)

Column

25 cm μ Bondapak C₁₈ (PN-27324; Waters Assoc.)

Mobile phase

25% Acetonitrile (Photrex; J.T. Baker) in pH 6.0, 0.1 N acetate buffer

Flow-rate

2.0 ml/min

Temperature

40°C

Detection

UV 270 nm (0.04 a.u.f.s.) time constant, 0.5 sec

Type

Reversed-phase

Retention time

5.6 min; peak height, 16 mm

Ref.

J.R. Koup, B. Brodsky, A. Lau and T.R. Beam, Jr., *Antimicrob. Agents Chemother.*, 14 (1978) 439-443

2,5-DIDEOXYSTREPTAMINE**TLC****Medium**

Silica gel

Solvent

Chloroform-methanol-ammonia-water
(1:4:2:1)

R_F

0.88

Ref.

S. Ogawa and T. Suami, *U.S. Patent* 3,959,375,
May 25, 1976

DIENOMYCIN**TLC****Medium**

Silica gel

Solvent

Ethyl acetate-methanol (5:1)

Detection

- A. Color (Wood reagent). Plate heated to 100°C; sprayed while hot with a 1:1 mixture of 0.4% bromphenol blue in acetone and 2% silver nitrate soln. and allowed to cool. The blue-colored plate is immersed carefully into water without disturbing the surface. Water is changed, if necessary, until clearly-stained blue spots appear on a light background

B. Ehrlich's reagent

C. Sulfuric acid

R_F

Detection method	R _F
A	0.27 0.40 0.48
B	0.27 0.40 0.48 0.87
C	0.10 0.26 0.40 0.48 0.87

Ref.

S. Umezawa, T. Tsuchiya, K. Tasuta, Y. Horiuchi and T. Usui, *J. Antibiot.*, 23 (1970) 20-27

DIHYDROERYTHROMYCIN
(See erythromycins)**DIHYDROSTREPTOMYCIN**
(See streptomycins)**DISTAMYCIN A****PC****Solvent**

Butanol-acetic acid-water (2:1:1)

R_F

0.52

Ref.

As kikumycin PC 1

DIUMYCINS**PC 1****Solvent**

n-Propanol-*n*-butanol-0.5 *N* ammonium hydroxide (2:3:4)

Detection

Bioautography

R_F

Diumycin A, 0.32

Diumycin B, 0.45

Ref.

E. Meyers, D.S. Slusarchyk, J.L. Bouchard and F.L. Weisenborn, *J. Antibiot.*, 22 (1969) 490-493

PC 2**Paper**

Schleicher and Schull 2040b (descending, 15 h)

Solvents

- A. *n*-Butanol-pyridine-water (4:1:4), upper phase, (chamber satd. with lower phase)
B. *n*-Butanol-*n*-propanol-0.5 *N* ammonia (3:1:4), upper phase (chamber satd. with lower phase)

Detection

Bioautography vs. *Staphylococcus aureus* FDA 209 P

R_F

Component	R _a *	
	Solvent A	Solvent B
D1	1.16	1.12
D2	1.34	1.31
D3	1.00	1.00
D4	1.24	1.20
D5	0.87	0.90
D6	0.87	0.96

* R_a = Migration in relation to diumycin A = 1.00

Ref.

A. Sattler and F. Kreuzig, *J. Antibiot.*, 28 (1975) 200-204

PC 3**Solvent**

n-Propanol-*n*-butanol-0.5 *N* ammonia (1:3:4)

Detection

Bioautography vs. *Staphylococcus aureus*

R_F

Component	R_F
A	0.32
A'	0.35
B	0.45
B'	0.52

Ref.

W.A. Slusarchyk, J.L. Bouchard-Ewing and F.L. Weisenborn, *J. Antibiot.*, 26 (1973) 391-393

TLC**Medium**

Silica gel F₂₅₄ (Merck)

Solvents

- A. Isopropanol-2 *N* ammonia (7:3)
 B. Chloroform-ethanol-water (2:3:1)

Detection

- A. UV at 254 nm
 B. Dark blue spots by spraying with a reagent consisting of 1% ceric sulfate (w/v) and 1.5% molybdic acid (w/v) in 10% sulfuric acid (v/v) followed by heating for 15 min at 105°C

 R_F

Component	Solvent A	Solvent B
D1	0.30	0.27
D2	0.27	0.34
D3	0.25	0.24
D4	0.22	0.32
D5	0.20	0.22
D6	0.15	0.18

Ref.

As PC 2

ELPHO**Medium**

Paper. Schleicher and Schull 2040b

Buffers and conditions

- A. 26 ml Pyridine + 20 ml glacial acetic acid, 300 V, 12 mA, pH 5.0
 B. 26 ml Pyridine, 300 V, 4 mA, pH 7.0 *
 C. 26 ml Pyridine + 5 ml 0.1 *N* sodium hydroxide 200 V, 20 mA, pH 9.0
 D. 26 ml Pyridine + 20 ml 0.1 *N* sodium hydroxide 250 V, 20 mA, pH 11.0

* All stock soln. were made up to 100 ml with distilled water. For final electrolyte conc. 20 ml of stock soln. were mixed with 400 ml formamide and made up to 1 liter with distilled water. For

electrolyte pH 7.0, 2 ml of 0.1 *N* sodium hydroxide had to be added

Detection

The separated substances were localized by bio-autography after 2 h drying at 100°C

Mobility

Migration occurred in all four soln. with diumycin A migrating faster to the anode than diumycin B

Ref.

As PC 2

CCD 1**Solvent**

As PC 1; 500 transfers

Distribution

Diumycin A was found in tubes 65-90; diumycin B, in tubes 130-160

Ref.

As PC 1

CCD 2**Solvent**

n-Propanol-*n*-butanol-0.5 *N* ammonia (2:3:4)

Distribution coefficients

Diumycin A, 0.18
 Diumycin A', 0.21
 Diumycin B, 0.30
 Diumycin B', 0.40

1000 transfers:

- A. Tubes 1-249, 950 additional transfers
 Diumycin A, tubes 276-310
 Diumycins A and A', tubes 311-330
 Diumycin A, tubes 331-410

- B. Tubes 250-255
 Diumycins A, A', B, B'

- C. Tubes 256-370
 Repeated silica gel chromatography (*n*-propanol-2 *N* ammonia on acid washed silica gel)
 Diumycins B and B'

Ref.

As PC 3

DORICIN**TLC****Medium**

Silica gel

Solvent

Chloroform-methanol (100:15)

 R_F

0.1

Ref.

M. Bodanszky and J.T. Sheehan, *Antimicrob. Agents Chemother.*, 1963 (1964) 38-40

CCD*Solvent*

Toluene–chloroform–methanol–water (5 : 5 : 8 : 2); 1500 transfers

Distribution

Distribution coefficient = 0.4

Ref.

As TLC

ECHANOMYCIN**CCD***Solvent*

Carbon tetrachloride–chloroform–methanol–water (2.63:0.37:2.4:0.6); 82 transfers

Distribution

Echanomycin found in tube 35

Ref.

E. Gaumann, V. Prelog and A. Wettstein, *Swiss Patent* 346,651, July 15, 1960

ECHINOMYCIN**PC 1***Solvent*

Di-*n*-butyl ether–*sym.*-tetrachloroethane–10% aq. sodium *o*-cresotinate (2 : 1 : 3)

R_F

0.13

Ref.

K. Katagiri, J. Shoji, T. Yoshida, *J. Antibiot.*, 15 (1962) 273

PC 2*Solvents*

A. Light petroleum–benzene–methanol–water (66.7:33.3:80:20)

B. 25% Ethanol

C. Amyl acetate satd. with water

R_F

Solvent A, 0.26

Solvent B, 0.54

Solvent C, 0.61

Ref.

T.S. Maksimova, I.N. Kovsharova, V.V. Proshlyakova, *Antibiotiki*, 10 (1965) 298–304

EDEINE**PC***Paper*

Whatman No. 3MM

Solvents

A. 1-Butanol–acetic acid–pyridine–water (6 : 3 : 2 : 3)

B. Isopropanol–conc. ammonia–water (4 : 1 : 1)

R_F

Solvent	Edeine A	Edeine B
A	0.08	0.11
B	0.19	0.11

Ref.

T.P. Hettinger, Z.K. Borowski and L.C. Craig, *Biochem.*, 7 (1968) 4153–4160

ELPHO 1*Medium*

Paper

Buffer

pH 6.4

Mobility

Cathodic mobility nearly equal to that of α,β -diaminopropionic acid. Edeines A and B not resolved from each other

Ref.

As PC

ELPHO 2 (For resolution of edeine A)*Medium*

Cellulose acetate strips

Buffer

pH 6.4

Conditions

40 V/cm, 65 min, 0°C, 25 μ g of edeine A/cm

Detection

Ninhydrin

Mobility

Edeine A resolved into 2 cationic compounds with the following mobility:

Edeine A₁, 23.2–24 cm

Edeine A₂, 22.0–23.0 cm

Ref.

T.P. Hettinger and L.C. Craig, *Biochem.*, 9 (1970) 1224–1232

CCD*Solvent*

88% Phenol–0.15 *M* ammonium acetate + 0.30 *M* acetic acid (1 : 1). Edeine distributed over first 30 tubes of a 500-tube CCD machine; distribution carried to 600 transfers

Distribution

Edeine A, tubes 240–290

Edeine B, tubes 120–170

Ref.

As PC

EFROTOMYCIN**PC***Solvent*

Isopropanol-0.1 M phosphate pH 6.0 (70:30)

R_F

0.9

*Ref.*R. Wax, W. Maise, R. Weston and J. Birnbaum,
J. Antibiot., 29 (1976) 670-673**TLC***Medium*

Silica gel

*Solvent*Chloroform-methanol-conc. ammonium hy-
droxide (80:20:1)*R_F*

0.34

Ref.

As PC

EKATETRONE**PC***Paper*

Whatman No. 1

*Solvent*Chloroform-1-butanol-McIlvaine's buffer, pH
4.5 (4:1:5)*Detection*

Red fluorescent spot, UV at 254 nm

R_F

0.80

*Ref.*M. Podojil, Z. Vanek, V. Prikrylova and M.
Blumauerova, *J. Antibiot.*, 31 (1978) 850-854**TLC***Media*

A. Kieselgel G (Merck)

B. Polyamide (Woelm)

Solvents

A. Chloroform-formamide-acetic acid (15:15:1)

B. Chloroform-1-butanol-McIlvaine's buffer, pH
4.5 (4:1:5)*Detection*

Red fluorescent spot, UV at 254 nm

R_F

Solvent	Medium	<i>R_F</i>
A	A	0.40
B	A	0.40
B	B	0.55

Ref.

As PC

ELAIOPHYLIN**TLC***Medium*

Silica gel

Solvent

Acetone-water (9:1)

Detection

15% phosphoric acid

R_F

0.88

Ref.

As Chilaphylin, PC

ELASNIN**TLC***Medium*

Silica gel

Solvent

Benzene-ethanol (10:1)

Detection

A. UV at 2537 Å

B. Iodine

R_F

0.5

*Ref.*H. Ohno, T. Saheki, J. Awaya, A. Nakagawa and
S. Omura, *J. Antibiot.*, 31 (1978) 1116-1123**EMERIMICIN II, III and IV****TLC***Medium*

Silica gel G

Solvent

Chloroform-methanol (6:1)

*Detection*Bioautography vs. *Sarcina lutea**R_F*

Fraction	<i>R_F</i> *
II	0.12
III	0.33
IV	0.47

* Estimated from drawing

Emerimicin IV is the main antibiotic component
of the fermentation and can be detected in cul-

ture filtrates as early as 72 h after addition of propyl proline. Emerimicin III, which is produced in smaller quantities, can also be detected early in the fermentation (72–96 h after addition of propyl proline). Emerimicin II is produced late in the fermentation and can be detected 144 h after addition of the amino acid

Ref.

A.D. Argoudelis and L.E. Johnson, *J. Antibiot.*, 27 (1974) 274–282

ENACTIN

PC

Solvents

- A. *n*-Butanol–water (87:13)
- B. *n*-Butanol–ethyl acetate–water
- C. Ethyl acetate–methanol–water (10:2:1)
- D. Ethyl acetate–acetone–water (19:3:1)

R_F

Solvent	R_F	Solvent	R_F
A	0.72	C	0.64
B	0.74	D	0.64

Ref.

H. Kondo, H. Sumomogi, T. Otani and S. Nakamura, *J. Antibiot.*, 32 (1979) 13–17

TLC

Medium

Silica gel G

Solvents

- A. *n*-Butanol–methanol–water (8:4:1)
- B. *n*-Butanol–water (87:13)
- C. *n*-Butanol–ethyl acetate–water (87:10:13)
- D. Acetone–water (4:1)
- E. *n*-Butanol–ethanol–water (8:5:2)
- F. Chloroform–methanol–water (65:35:10) lower phase

Detection

- A. Bioautography
- B. Ninhydrin
- C. Heat at 100°C for 15 min after spraying with 40% sulfuric acid

R_F

Solvent	R_F	Solvent	R_F
A	0.39	D	0.89
B	0.27	E	0.48
C	0.26	F	0.45

Ref.

As PC

ENAMINOMYCINS

TLC 1

Medium

Eastman silica gel sheet No. 6060

Solvent

Chloroform–methanol–acetic acid (9:1:1)

R_F

Component	R_F
A	0.64
B	0.52
C	0.39

Ref.

Y. Itoh, T. Miura, T. Katayama, T. Haneishi and M. Arai, *J. Antibiot.*, 31 (1978) 834–837

TLC 2

Media

- A. Eastman Chromagram sheet No. 6060, silica gel
- B. Eastman Chromagram sheet No. 6065, cellulose

Solvents

- A. Chloroform
- B. Ethyl acetate
- C. Acetone
- D. Methanol
- E. Butanol–acetic acid–water (3:1:1)
- F. Chloroform–methanol–acetic acid (9:1:1)
- G. Propanol–pyridine–acetic acid–water (15:10:3:12)
- H. Butanol–acetic acid–water (3:1:1)
- I. Butanol–methanol–water (4:1:2)

R_F

Solvent	Medium	Component		
		A	B	C
A	A	0.01	0	0
B	A	0–0.3	0–0.3	0–0.3
C	A	0.72	0.72	0.72
D	A	0.79	0.79	0.79
E	A	0.72	0.59	0.67
F	A	0.64	0.52	0.39
G	B	0.94	0.94	0.94
H	B	0.84	0.86	0.85
I	B	0.79	0.76	0.74

Ref.

M. Arai, Y. Itoh, R. Enokita, Y. Takamatsu and T. Manome, *J. Antibiot.*, 31 (1978) 829–833

ENDOMYCINS**PC***Paper*

Whatman No. 1

Solvents

- A. Water satd. *n*-butanol
 B. As A vs. paper buffered with 0.1 *N* sodium carbonate
 C. As A vs. paper buffered with 0.1 *N* sodium phosphate, pH 12.0
 D. *tert*-Butanol–water (4:1)
 E. *n*-Butanol–ethyl acetate (1:1) satd. with water

Detection

Bioautography vs. *Candida albicans*

R_F

Solvent	Endomycin A	Endomycin B
A	0.04	0.37
B	0.03	0.25
C	0.10	0.38
D	0.30	0.70
E	0.03	c0.29

Ref.

L.C. Vining and W.A. Taber, *Can. J. Chem.*, 35 (1957) 1461–1466

ENDURACIDIN**PC 1***Paper*

Whatman No. 1

Solvents

- A. *n*-Butanol–acetic acid–water (4:1:5)
 B. *n*-Butanol–pyridine–water (4:3:7)

R_F

Solvent A, 0.45 ± 0.1
 Solvent B, 0.80 ± 0.1

Ref.

M. Asai, M. Muroi, N. Sugita, H. Kawashima, K. Mizuno and M. Miyake, *J. Antibiot.*, 21 (1968) 138–146

PC 2*Paper*

Whatman No. 1

Solvents

- A. Acetic acid–*n*-butanol–water (1:4:5)

B. *n*-Butanol satd. with water

C. Pyridine–*n*-butanol–water (3:4:7)

All solvents run ascending

R_F

Solvent	<i>R_F</i> *
A	0.45 ± 1
B	0.0
C	0.80 ± 0.1

* The substance is easily adsorbed on filter paper under neutral conditions

Ref.

T. Matsuoka, K. Takeda, M. Goto, A. Miyake, *U.S. Patent* 3,697,647, Oct. 10, 1972. M. Shibata, E. Higashide, *U.S. Patent* 3,786,142, Jan. 15, 1974. A. Miyake, S. Horii, J. Ueyanagi, *U.S. Patent* 3,678,160, July 18, 1972

CCD*Solvent*

As PC 1: A, 150 transfers

Distribution

Analyzed by determinations of biological activity against *Sarcina lutea* and UV absorption at 268 nm. *K* = 0.59

Ref.

As PC 1

ENHYGROFUNGIN**PC***Solvents*

- A. 1-Butanol–water (84:16); 16 h
 B. As A + 0.25% *p*-toluenesulfonic acid; 16 h
 C. 1-Butanol–acetic acid–water (2:1:1); 16 h
 D. As A + 2% piperidine; 16 h
 E. 1-Butanol–water (4:96); 5 h
 F. As E + 25% *p*-toluenesulfonic acid; 64 h

Detection

Bioautography vs. *Saccharomyces cerevisiae*

R_F

Solvent	<i>R_F</i> *	Solvent	<i>R_F</i> *
A	0.18–0.50	D	0.18–0.50
B	0.65	E	0.10–0.35
C	0.90	F	0.10–0.35

* Estimated from drawing

Ref.

Netherlands Patent 7008652, Dec. 15, 1970

ENOMYCIN**ELPHO***Medium*

Paper

Buffer

Acetic acid–pyridine–water (8:40:952), pH 6.4

Mobility

Moved toward cathode as a single entity

Ref.

Y. Suhara, M. Ishizuka, H. Naganawa, M. Hori,
M. Suzuki, Y. Okami, T. Takeuchi and H.
Umezawa, *J. Antibiot.*, 16 (1963) 107–108

ENSHUMYCIN A**TLC***Solvent*

Ethyl acetate–acetone (10:0.1)

R_F

0.2

Ref.

Derwent Abstract 93392X/50, April 19, 1975

EPICILLIN

(cf., Penicillins)

TLC*Medium*

Cellulose

*Solvent*Water satd. *n*-butyl alcohol–*tert*-amyl alcohol–
water (6:1:4), pH 4.1 (6 h)*Detection*

Starch–iodine spray

R_F

0.29

*Ref.*S.C. Pan, *J. Chromatogr.*, 79 (1973) 251–255**EPICORAZINE A and B****TLC***Medium*

Silica gel

Solvent

Chloroform–methanol (94:6)

*Detection*Bioautography vs. *Staphylococcus aureus**R_F*

Epicorazine A, 0.7

Epicorazine B, 0.5

Ref.

M.A. Baute, G. Deffieux, R. Baute and A. Neveu,
J. Antibiot., 31 (1978) 1099–1101

EPIDEOXYNEGAMYCIN**TLC***Medium*

Silica gel

*Solvent*Chloroform–methanol–conc. ammonium hy-
droxide–water (1:4:2:1)*R_F*

0.37

Ref.

H. Maehr, J. Smallheer, M. Chin, N. Palleroni, F.
Weiss and C.-M. Liu, *J. Antibiot.*, 32 (1979)
531–532

**EPIERYTHROMYCYLAMINE and EPI-
ERYTHROMYCYL B AMINE****TLC***Medium*

Silica gel G

Solvent

Ethyl acetate–diethylamine–water (98:2:2)

*R_F*Epi amine has an *R_F* value about $\frac{1}{2}$ of the
“normal” amine*Ref.*

E.H. Massay and B.S. Kitchell, *U.S. Patent*
3,652,537, March 28, 1972

4'-EPI-6'-HYDROXYADRIAMYCIN**TLC***Medium*

Kieselgel HF (Merck)

*Solvent*Chloroform–methanol–water (130:60:10,
v/v/v)*R_F*

0.5

Ref.

F. Arcamone, A. Bargiotti, G. Cassinelli and A.
diMarco, *U.S. Patent* 4,025,623, May 24, 1977

EQUISETIN**TLC***Medium*

Silica gel

Solvents

A. Acetonitrile–water–benzene (90:6:4)

B. Toluene–methanol (7:3)

Detection

Visible as reddish spot after spraying with *p*-anisaldehyde reagent and heating to 110°C for 5–10 min

R_F

Solvent A, 0.53

Solvent B, 0.61

Ref.

H.R. Burmelster, *U.S. Patent* 3,959,468, May 25, 1976

ERICAMYCIN**TLC****Medium**

Silica gel

Solvents

A. Ethyl acetate

B. Methanol–benzene (1:5)

R_F

Solvent A, 0.2–0.3

Solvent B, 0.5–0.6

Ref.

Japanese Patent 30970/69, March 18, 1969

ERIZOMYCIN**PC****Solvents**

A. 1-Butanol–water (84:16); 16 h

B. As A + 0.25% *p*-toluenesulfonic acid; 16 h

C. 1-Butanol–acetic acid–water (2:1:1); 16 h

D. As A + 2% piperidine; 16 h

E. 1-Butanol–water (4:96); 5 h

F. As E + 0.25% *p*-toluenesulfonic acid; 5 h

Detection

Bioautography vs. *Mycobacterium avium*

R_F

Solvent	<i>R_F</i> *
A	0.7–0.9
B	0.65–0.9
C	0.85
D	0.6–0.83
E	0.05, streak to 0.8, 0.83
F	0.45–0.8

* Estimated from drawing

Ref.

R.R. Herr and F. Reusser, *U.S. Patent* 3,367,833, Feb. 6, 1968

**ERYTHROMYCIN, DIHYDRO-
(See erythromycins)****ERYTHROMYCIN B, 4''-DEOXY-4''-OXO-**

(See 4''-deoxy-4''-oxoerythromycin B)

ERYTHROMYCIN B METABOLITES**TLC****Medium**

Silica gel G

Solvent

95% Ethanol–chloroform (1:10, v/v)

Detection

Compounds were visualized by spraying with the arsenomolybdate reagent of Nelson (1944) and heating for a few minutes on a hot plate

R_F

5,6-Dideoxy-5-oxoerythronolide B, 0.70–0.78

Ref.

J.R. Martin and R.S. Egan, *Biochemistry*, 9 (1970) 3439–3445

**ERYTHROMYCIN B OXIME,
9-METHYL-
(See 9-methylerythromycin B oxime)****ERYTHROMYCINS****PC 1****Solvent**

Methanol–acetone–water (19:6:75)

Detection

Bioautography vs. *Micrococcus pyogenes* var. *aureus* or *Bacillus subtilis*

R_F

Erythromycin, 0.7

Erythromycin B, 0.6

Ref.

C.W. Pettinga, W.M. Starke and F.R. Van Abeele, *J. Amer. Chem. Soc.*, 76 (1954) 569–571

PC 2**Solvent**

As celesticetin, PC 2; G

Detection

As celesticetin, PC 2

R_F

0.3–0.42 (estimated from drawing)

Ref.

As celesticetin, PC 2

PC 3*Solvent*

As PC 1

R_F

Erythromycin C > erythromycin > erythromycin B

*Ref.*P.F. Wiley, R. Gale, C.W. Pettinga and K. Gerzon, *J. Amer. Chem. Soc.*, 79 (1957) 6074**PC 4***Solvent*

A, B, C, D as carbomycin, PC 2; A, B, C, D

Detection

As carbomycin PC 2

R_F

Solvent	<i>R_F</i>
A	0.00
B	0.02
C	0.24
D	0.50

Ref.

As carbomycin, PC 2

PC 5 (Useful for chromatography of whole blood, serum, plasma, urine or saliva)*Paper*

Whatman No. 1

Solvent

Develop descending using absolute methanol for 1 h. Cut off upper portion of chromatogram 5 cm below point of application and develop descending, with the following solvent mixture. Dissolve 12.5 g of ammonium chloride and 100 g sodium chloride in 100 ml distilled water. Add 25 ml dioxane and 12.5 ml methyl ethyl ketone, dilute to 1 liter with distilled water and adjust to pH 5.7 with 1 N ammonium hydroxide

*Detection*Bioautography vs. *Sarcina lutea* ATCC 9341*R_F*

Erythromycin > propionyl erythromycin

*Ref.*V.C. Stephens, C.T. Pugh, N.E. Davis, M.M. Hoehn, S. Ralston, M.C. Sparks and L. Thompson, *J. Antibiot.*, 22 (1969) 551-557**PC 6***Paper*

Toyo No. 51

Solvent

Water satd. ethyl acetate (ascending, 3 h)

*Detection*Bioautography vs. *Bacillus subtilis* No. 10707*R_F*

0.20-0.40

Ref.

As carbomycin, PC 5

PC 7*Paper*

Whatman No. 1

Solvents

- A. 80% Methanol + 3% sodium chloride (1:1) descending. Paper buffered with 0.95 M sodium sulfate + 0.05 M sodium bisulfate
- B. Propanol-pyridine-acetic acid-water (6:4:1:3), ascending
- C. Butanol-acetic acid-water (4:1:5), ascending
- D. 80% Phenol, ascending

*Detection*Bioautography vs. *Staphylococcus aureus**R_F*

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.90	C	0.78
B	0.93	D	0.86

Ref.

As Chloramphenicol, PC 5

TLC 1*Medium*

Silica gel

Solvent

Methylene chloride-methanol-benzene-formamide (80:20:20:2.5). Quantity of formamide is varied according to laboratory humidity conditions. At a relative humidity of 20%, 5 vols. of formamide gives clear separation; higher humidity (30-40%) requires 3 or 2 vols. Development time is 30-40 min and is complete when solvent front is 10 cm from origin

Detection

- A. Spray plate with 10% phosphomolybdic acid in ethanol; heat on a hot plate. Blue spots appear on a yellow background
- B. Spray with 50% aq. sulfuric acid; heat on a hot plate. Charred zones result

*Ref.*T.J. Anderson, *J. Chromatogr.*, 14 (1964) 127-129

TLC 2

Medium

Talc

Solvent

Water-ethanol-ethyl acetate-acetic acid-25% ammonia (10:9:1:0.2:1)

 R_F

Erythromycin C > B; anhydroerythromycin same R_F as C

Ref.

Z. Kotula and A. Kaminska, *Med. Dosw. Microbiol.*, 19 (1967) 381-387; *C.A.*, 68 (1968) 72292y

TLC 3

Medium

Kieselgel G; Kieselguhr G (1:1, w/w). Dry at 110°C for 1 h and immerse for depth of 1 cm in 15% formamide in acetone. Dry for several min at room temperature and use directly

Solvents

A. Methylene chloride-*n*-hexane-ethanol (60:35:5)

B. Methylene chloride-ethyl acetate-*n*-hexane-ethanol (40:40:15:5)

Detection

A. Spray with 50% sulfuric acid

B. Spray with 1% cerium sulfate and 2.5% molybdic acid in 10% sulfuric acid and heat at 110°C for several minutes. Spots are blue against a white background

 R_F

Solvent A:

Erythralosamine > erythromycin B = anhydroerythromycin A > erythromycin A = anhydroerythromycin C > erythromycin C

Solvent B:

Erythralosamine > anhydroerythromycin A > erythromycin B > anhydroerythromycin C > erythromycin A > erythromycin C

Ref.

A. Banaszek, K. Krowicki and A. Zamojski, *J. Chromatogr.*, 32 (1968) 581-583

TLC 4

Medium

Silica gel G (Merck), 50 g in 100 ml of 0.02 *N* aq. sodium acetate, 0.25 mm thick. Air-dry overnight

Solvent

Methanol-0.02 *N* aq. sodium acetate (120:30)

Detection

Spray with a soln. of glucose-85% phosphoric acid-water-ethanol-*n*-butanol (2 g:10 ml:40 ml:30 ml:30 ml). Heat 5 min at 150°C. Prepare reagent fresh daily

 R_F

	R_F	Color
Anhydroerythronolide	0.82	Red
Erythromycin estolate	0.65	Blue-gray
Erythromycin ethyl succinate	0.67	Blue-gray
Erythromycin ethyl carbonate	0.67	Blue-gray
Erythromycin	0.28	Blue-gray
Erythromycin stearate	0.28	Blue-gray
Erythromycin lactobionate	0.28	Blue-gray
Erythromycin gluceptate	0.28	Blue-gray
Anhydroerythromycin A	0.33	Blue-gray

Ref.

G. Richard, C. Radecka, D.W. Hughes and W.L. Wilson, *J. Chromatogr.*, 67 (1972) 69-73

TLC 5

Medium

Avicel

Solvent

Methanol-isobutyl alcohol-chloroform-10% hydrochloric acid (10:10:10:1)

Detection

A. Iodine vapor

B. 0.25% Stannous chloride soln.

C. 0.2% *p*-Dimethylaminobenzaldehyde soln.

 R_F

Erythromycin estolate, 0.98

Erythromycin lactobionate, 0.82

Ref.

As Ampicillin, TLC 1

TLC 6

Medium

Silica gel

Solvent

Benzene-methanol (9:1) satd. with ammonium hydroxide

Detection

A. Bioautography vs. *Bacillus subtilis*

B. Bioautography vs. *Staphylococcus aureus*

 R_F

9-Dihydroerythromycin B 6,9-epoxide, 0.40

Ref.

P. Kurath, R.S. Egan and P.H. Jones, *U.S. Patent* 3,681,323, Aug. 1, 1972

TLC 7

Medium

Eastman Chromagram (Cat. No. 6060)

Solvents

- A. Chloroform-methanol-acetone (10:3:1)
 B. Benzene-methanol-toluene (85:10:5)
 C. *n*-Butanol-acetic acid-water (4:1:5)
 D. *n*-Hexane-benzene-methanol-ethyl acetate-acetone (30:25:10:20:10)

Detection

Spray with CeSO_4

 R_F

Solvent	Erythromycin	
	A	B
A	0.35	0.34
B	0.07	0.07
C	0.67	0.66
D	0.11	—

Ref.

M.C. Flickinger and D. Perlman, *J. Antibiot.*, 28 (1975) 307-311

TLC 8**Medium**

Silica gel 60

Solvent

Chloroform-methanol-acetic acid (90:10:1)

Detection

Spray with potassium dichromate (5.0 g) dissolved in 40% sulfuric acid (100 ml) and heat for 1 h at 150°

 R_F

	R_F *
Erythromycin	0.05
Erythromycin stearate **	0.04, 0.68
Stearic acid	0.69
Erythromycin ethyl succinate	0.10
Erythromycin estolate	0.10
Erythromycin gluceptate	0.04
Erythromycin lactobionate	0.04

* Average of five plates

** Two spots, viz. erythromycin free base and stearic acid

Ref.

K.C. Graham, W.L. Wilson and A. Vilim, *J. Chromatogr.*, 125 (1976) 447-450

TLC 9**Medium**

Silica gel 60 F_{254} (Merck) plates activated for 30 min at 130° prior to use

Solvents

- A. Methanol
 B. Chloroform-methanol-acetic acid (90:10:1)
 C. Methanol-chloroform-acetic acid (90:5:5)

Detection

Spray reagent: potassium dichromate (5.0 g) dissolved in 40% (v/v) sulfuric acid (100 ml)

 R_F

Compound	Solvent		
	A	B	C
Erythromycin	0.29	0.05	0.40
Erythromycin stearate	0.29	0.04, 0.68	0.38
Stearic acid	N.D. *	0.69	N.D. *
Erythromycin estolate	0.73, 0.93	0.10, 0.06	0.42, 0.80
Sodium lauryl sulfate	0.93	0.06	0.80
Erythromycin ethyl-succinate	0.74	0.10	0.44

* Not detectable after heating for 20 min at 150°

Ref.

A. Vilim, M.J. LeBelle, W.L. Wilson and K.C. Graham, *J. Chromatogr.*, 133 (1977) 239-244

TLC 10**Media**

- A. Kieselguhr F_{254} (Merck 5738). Before use, the Kieselguhr plate was impregnated with citrate/phosphate buffer (pH 3.7)-glycerol (19:1)
 B. Silica gel 60 (Merck)
 C. Cellulose (Merck 5716)

 R_F

Medium	Solvent	Detection	R_E
A	A	A	0.20 *
B	B	C	0.70
B	C	D	0.35
B	C	B	0.35
B	D	C	0.25
C	E	A	—

* This value is the mean of the two determinations

Solvents

- A. Chloroform-acetone-impregnation liquid [citrate/phosphate buffer (pH 3.7)-glycerol (19:1)] (5:5:2)

- B. Ethanol–water–ammonia (8:8:1)
 C. Methanol–chloroform (9:1)
 D. Methanol–acetone (3:2)
 E. Methanol–ammonium chloride (3%) (7:3)

Detection

- A. Bioautography vs. *Bacillus cereus* ATCC 11778

- B. Bioautography vs. *Bacillus subtilis* ATCC 6633
 C. Bioautography vs. *Micrococcus flavus* ATCC 10240
 D. Bioautography vs. *Sarcina lutea* ATCC 9341

Ref.

As Bacitracin, TLC 2

TLC 11**Medium**

Silica gel G (Merck) in phosphate buffer, pH 8

Solvents

- A. Methylene chloride–chloroform–methanol (80:20:20)
 B. Methylene chloride–methanol–formamide (100:20:2)
 C. Methylene chloride–methanol (100:20)
 D. Chloroform–methanol (20:100)

R_F**Detection**

Phenol–sulphuric acid reagent prepared from 3 g of phenol, 95 ml of absolute ethanol, and 5 ml of concentrated sulfuric acid. Solutions for spotting prepared by dissolving each substance in methanol (10 mg/ml)

Ref.

G. Kobrehel, Z. Tamburasev and S. Djokic, *J. Chromatogr.*, 133 (1977) 415–419

Erythromycins	Solvent			
	A	B	C	D
Erythromycin	0.26 0.18	0.48 0.33	0.25 0.14	0.28 0.22
Erythromycin oxime	0.23 0.16	0.48 0.32	0.28 0.13	0.27 0.21
Erythromycylamine	0.05 0.04	0.07 0.06	0.06 0.03	0.07 0.05
Erythromycin propionate	0.76	0.93	0.81	0.80
Erythromycin oxime monopropionate	0.22	0.51	0.28	0.27
Erythromycylamine monopropionyl derivative	0.11	0.23	0.12	0.25
Erythromycin oxime dipropionate	0.81	0.94	0.81	0.77
Erythromycylamine dipropionyl derivative	0.60	0.85	0.70	0.78
Erythromycin ethyl succinate	0.51	0.81	0.66	0.69
Erythromycin oxime monomethylsuccinate	0.28	0.39	0.17	0.22
Erythromycylamine monomethylsuccinyl derivative	0.07	0.18	0.07	0.19
Erythromycin oxime dimethylsuccinate	0.51	0.90	0.70	0.70
Erythromycylamine dimethylsuccinyl derivative	0.04	0.72	0.55	0.68

TLC 12**Medium**

Silica gel GF

Solvents

- A. Chloroform–methanol–17% ammonia (2:1:1)
 B. Butanol–acetic acid–water (3:1:1)

Detection

Spray with conc. sulfuric acid–methanol (1:1), developed by heating at 105°C for several min

R_F

Solvent A, 0.98 (yellow tan)
 Solvent B, 0.26 (green brown)

Ref.

As Chloramphenicol, PC 5

TLC 13**Medium**

Silica gel

Solvents

- A. Chloroform–methanol–17% ammonia (2:1:1)
 B. Butanol–acetic acid–water (3:1:1)
 C. Chloroform–methanol (3:2)

Detection

- A. Bioautography vs. *Sarcina lutea*
 B. Plate heated at 100°C and sprayed with phosphoric acid in methanol (1:1)

 R_F

Solvent	Detection	R_F
A	A	0.94
	B	0.94 (brown)
B	A	0.40
	B	ca. 0.38–0.45 (brown)
C	A	0.29

Ref.

G.H. Wagman, J.A. Waitz, J. Marquez, A. Murawski, E.M. Oden, R.T. Testa and M.J. Weinstein, *J. Antibiot.*, 25 (1972) 641–646

ELPHO**Medium**

Whatman No. 1 paper

Buffer

Borate, pH > 8

Conditions

400 V, 4–6 h

Mobility

Erythromycin B > A > C

Ref.

As TLC 2

CCD**Solvent**

0.1 M Phosphate buffer (pH 6.5)–methyl isobutyl ketone–acetone (10:10:0.5); 100 transfers

Distribution

Erythromycin, tubes 30–50

Erythromycin B, tubes 60–80

Ref.

As PC 1

FLC**Instrument**

JASCO FLC-150

Detector

JASCO UVIDEK-2 (232.5 nm) spectrophotometer

Separation mode

Partition

Column

JASCOPACK SV-02-500

Column temperature

Ambient

Column pressure

65 kg/cm²

Mobile phase

Methanol– $\frac{1}{15}$ M acetate buffer, pH 4.9–acetonitrile (35:60:5)

Flow-rate

1.0 ml/min

Retention time

Erythromycin A, 3.0

Erythromycin B, 2.4

Ref.

S. Omura, Y. Suzuki, A. Nakagawa and T. Hata, *J. Antibiot.*, 26 (1973) 794–796

HPLC**Apparatus**

LDC Model 19-60066-002 high-pressure mini-pump (Laboratory Data Control)

Column

LiChrosorb RP-18 (250 × 4.6 mm I.D.) (Brownlee Labs) and a stainless-steel column (50 × 2.1 mm I.D.) prepacked with μ Bondapak C₁₈ (Waters Assoc.)

Mobile phase

Acetonitrile–0.2 M ammonium acetate–water (65:60:10:25–30), pH 7.0

Flow-rate

0.9 ml/min

Temperature

Room temperature

Pressure

1250 p.s.i.

Detection

Fluorimetric

Type

Reversed-phase

Retention time

Compound	Retention time (min) *
Erythromycin	6.0
Erythralosamine	7.5
4''-Acetylerythromycin or anhydroerythromycin	8.3
Erythromycin ethylsuccinate	12.1

* Estimated from curve

Ref.

K. Tsuji, *J. Chromatogr.*, 158 (1978) 337–348

HSLC**Column**

Stainless steel, 30 cm × 4.6 mm I.D., C₁₈/LiChrosorb 10 μ m

Mobile phase

Methanol–water–ammonium hydroxide (80 : 19.9:0.1)

Flow-rate

1.4 ml/min

Pressure

1000 p.s.i.

Temperature

Room temperature

Detection

RI (Refractive Index Detector, Model R401, Water Assoc.)

Sensitivity

×4

Sample solvent

Methanol

Sample size

10 µl

Type

Reversed-phase

Retention time

5.5 min (estimated from drawing)

Ref.

As Cephalosporin, HSLC 1

ERYTHROMYCYLAMINES**TLC****Media**

A. Alumina

B. Silica gel

Solvents

A. Methylene chloride–methanol (30:1)

B. Chloroform–methanol (30:1)

C. Chloroform–methanol (10:1)

D. Dimethylformamide–methanol (3:1)

 R_F

Component	Medium	Solvent	R_F
N-[2-(Hydroxyethyl-imino)-ethylidene-1]-erythromyclamine	A	A	0.65
N-[2-(Thiomorpholinyl-imino)-ethylidene]-erythromyclamine	A	B	0.7
N-(2-Aminoethyl)-erythromyclamine	A	C	0.1
N-(Hydroxyethyl)-erythromyclamine	B	D	0.3
N-[2-(Thiomorpholinyl-amino)-ethyl]-erythromyclamine	B	D	0.25

Ref.

B. Wetzel, E. Weitun, R. Maier, W. Reuter, H. Goeth and U. Lechner, *U.S. Patent* 4,016,263, April 5, 1977

ESEIN**PC****Solvents**

A. *n*-Butanol–acetic acid–water (2:1:1)

B. As bresein, PC, N

C. Phenol satd. with 0.3% ammonium hydroxide

D. As bresein, PC, O

E. As bresein, PC, P

F. As bresein, PC, Q

G. As bresein, PC, R

H. As bresein, PC, S

I. As bresein, PC, T

 R_F

Solvent	R_F	Solvent	R_F
A	0.92	F	0.67
B	0.88	G	0.60
C	0.70	H	0.62
D	0.60	I	0.66
E	0.64		

Ref.

As bresein, PC

ETAMYCINS**TLC****Medium**

Silica gel HF₂₅₄; (Merck)

Solvent

Benzene–acetic acid–water (42:24:1)

Detection

UV at 254 and 366 nm

 R_F

Component *	R_F
I	0.78 (yellow)
	0.85 (white)
II	0.63 (blue)
III	0.35 (deep blue)
IV	0.65 (violet)
V	0.62 (violet)
VI	0.65 (violet)
VII	0.56 (blue)

Component *	R_F
VIII	0.26 (blue) 0.68 (white) 0.16 (white)
IX	0.65 (violet)

* Where more than one zone was present they are listed in order of decreasing intensity

Ref.

C. Chopra, D.J. Hook, L.C. Vining, B.C. Das, S. Shimizu, A. Taylor and J.L.C. Wright, *J. Antibiot.*, 32 (1979) 392-401

EUROCIDIN

PC

Solvent

n-Butanol-methanol-water (4:1:2)

R_F

0.64

Ref.

D. Brown, *Naturwissenschaften*, 47 (1960) 474

EVERNINOMICIN D

TLC (Quantitative)

Medium

Silica gel F₂₅₄, TLC plates (Merck), 0.25-mm layer thickness

Solvent

Fresh ethyl acetate-acetone (1:1)

Detection

A Schoeffel SD 300 double-beam densitometer was equipped with an optical density computer, Model SDC 300 (Schoeffel Instrument Corp.). The output was recorded on a Honeywell 10-in. strip recorder (Honeywell) provided with a disc integrator Model 252 A (Disc Instruments, Inc.). Scanning and recording took place in the direction of the chromatography at a speed of 4 in./min. The excitation wavelength in the fluorescence quenching transmittance mode was 265 nm, and the monochromator and exit slits were 1.5 and 1 mm, respectively

Ref.

P. Kabasakalian and S. Kalliney, *J. Chromatogr.*, 86 (1973) 145-149

EVERNINOMICINS

PC 1

Paper

Whatman No. 1

Solvent

Benzene-light petroleum (30-60°C)-acetone (20:5:10); descending, 2 h

Detection

Bioautography vs. *Staphylococcus aureus* ATCC 6538P

R_F

Everninomicin A, 0.0
Everninomicin B, 0.17
Everninomicin C, 0.28
Everninomicin D, 0.62

Ref.

M.J. Weinstein, G.M. Luedemann, E.M. Oden and G.H. Wagman, *Antimicrob. Agents Chemother.*, 1964 (1965) 24-32

PC 2

Paper

Whatman No. 1

Solvents

- As PC 1; descending, 1.5 h
- Toluene-*n*-butanol-water-light petroleum 30-60°C (20:1.5:7:1.5); descending, 4.5 h
- Light petroleum 60-90°C-methanol-ethyl acetate-water (5:8:5:2), 2 h
- Ligroin 90-120°C-butyl acetate (3:7) satd. with 15% aq. 4-chloro-2-methylphenoxyacetic acid sodium salt

Detection

Bioautography vs. *Staphylococcus aureus*

R_F

Sol-vent	Component					
	A	B	C	D	E	F
A	0.00	0.14	0.28	0.64	0.74	0.74
B	0.00	0.19	0.42	0.61	0.71	0.71
C	0.00	0.08	0.26	0.60	0.82	0.82
D	-	0.11 *	-	0.36 *	0.16 *	0.77 *

* Front allowed to run off paper. Calculated as distance of zone from origin divided by distance from origin to end of paper

Ref.

G.M. Luedemann and M.J. Weinstein, *U.S. Patent* 3,499,078, March 3, 1970

PC 3

Paper

Toyo No. 51

Solvents

- 20% Ammonium chloride
- Water satd. *n*-butanol

- C. *n*-Butanol–acetic acid–water (3:1:1)
 D. Water satd. ethyl acetate
 E. Water satd. *n*-butanol + 2% *p*-toluenesulfonic acid + 2% piperidine

Detection

Bioautography vs. *Bacillus subtilis* No. 10707

R_F

Solvent	R _F	Solvent	R _F
A	0.78	D	0.40
B	0.86	E	0.87
C	0.83		

Ref.

As Carbomycin, PC 1

TLC 1**Medium**

Silica gel

Solvent

Benzene–acetone (60:40)

Detection

- A. Spray with sulfuric acid–methanol (1:1), heat at 105°C. Dark zones on white background
 B. Dip plate in satd. soln. of iodine in light petroleum, place in air stream to remove excess iodine. Brown zones appear
 C. Bioautography vs. *Staphylococcus aureus*

R_F

Everninomicin D, 0.5 (estimated from photograph)

Ref.

As PC 1

TLC 2**Medium**

Silica gel

Solvent

As TLC 1

Detection

As TLC 1; A, C

R_F

Not given. Method used to determine purity of everninomicin B column fractions

Ref.

M.J. Weinstein, G.H. Wagman, E.M. Oden, G.M. Luedemann, P. Sloane, A. Murawski and J.A. Marquez, *Antimicrob. Agents Chemother.*, 1965 (1966) 821–827

TLC 3**Medium**

Silica gel G (Merck)

Solvent

As TLC 1

Detection

Spray with sulfuric acid–methanol (85:15); heat to develop color

R_F

Everninomicin B, 0.25

Everninomicin D, 0.45

Ref.

H.L. Herzog, E. Meseck, S. deLorenzo, A. Murawski, W. Charney and J.P. Rosselet, *Appl. Microbiol.*, 13 (1965) 515–520

TLC 4**Medium**

Silica gel fluorescent layer (Mallinckrodt Silicar TLC 7GF), 0.5-mm thick

Solvent

As TLC 1

Detection

UV light

R_F

Everninomicin E4*, 0.18

Everninomicin E3, 0.25

Everninomicin E2, 0.32

Everninomicin E1, 0.40

* Everninomicin A = E4; B = E3; C = E2; and D = E1

Ref.

A. Sattler and C.P. Schaffner, *J. Antibiot.*, 23 (1970) 210–215

TLC 5**Solvent**

Acetone–benzene (60:40)

R_F

Everninomicin D, 0.79

Ref.

H.E. Harris, *U.S. Patent* 3,901,973, Aug. 26, 1975

TLC 6**Medium**

Silica gel

Solvents

A. Acetone–benzene (60:40)

B. 50% Acetone in benzene

C. Acetone–ethyl acetate–benzene (2:2:1)

D. Acetone–benzene (1:1)

Detection

UV

R_F (see p. 158)

Ref.

A.K. Ganguly and O. Sarre, *U.S. Patent* 3,915,956, Oct. 28, 1975; A.K. Ganguly, O. Sarre and S. Szmulewicz, *U.S. Patent* 3,920,629, Nov. 18, 1975

R_F

Component	Solvent			
	A	B	C	D
Evernino- micin D	0.79			
Hydroxyl- aminoever- ninomicin D		0.44		
Hydroxyl- aminoever- ninomicin D benzyl nitron				0.5-0.7
Desevernitrone everninomicin D				0.51
Nitrosoever- ninomicin D			0.75-0.85	

TLC 7*Medium*

Silica gel

Solvents

- A. 50% Acetone in benzene
 B. Acetone-ethyl acetate-benzene (2:2:1)
 C. Acetone-benzene (1:1)

Detection

UV

 R_F

Component	Sol- vent	R_F
Hydroxylaminoevernino- micin D	A	0.45
Nitrosoeverninomicin D	B	0.78
Hydroxylaminoevernino- micin B benzyl nitron	C	0.5-0.7
Hydroxylaminoevernino- micin D furfuryl nitron	C	0.75-0.85
Hydroxylaminoevernino- micin C	A	0.27
Hydroxylaminoevernino- micin B	A	0.18

Ref.

A.K. Ganguly and O. Sarre, *U.S. Patent*
 4,006,225, Feb. 1, 1977

EXFOLIATIN**PC***Solvent*

As avilamycin, PC

Detection

As avilamycin, PC

 R_F

0.58

Ref.

As avilamycin, PC

TLC*Medium*

As avilamycin, TLC 1

Solvent

As avilamycin, TLC 1

Detection

As avilamycin, TLC 1

 R_F

0.40

Ref.

As avilamycin, PC

FELDAMYCIN

(cf., U-48266)

PC*Paper*

Impregnated with 0.1 M sodium sulfate

Solvents

- A. 1-Butanol-water (84:16)
 B. 1-Butanol-water (84:16) + 0.25% *p*-toluene-
 sulfonic acid
 C. 1-Butanol-acetic acid-water (2:1:1)
 D. 2% (v/v) Piperidine in 1-butanol-water
 (84:16)
 E. 1-Butanol-water (4:96)
 F. 1-Butanol-water (4:96) + 0.25% *p*-toluene-
 sulfonic acid
 G. 0.5 M phosphate buffer pH 7.0
 H. 0.075 M ammonium hydroxide satd. with
 methyl isobutyl ketone, lower phase
 I. Benzene-methanol-water (1:1:2)
 J. 1-Butanol-water (84:16) + 2% *p*-toluene-
 sulfonic acid
 K. Methanol-15% aq. sodium chloride (4:1)

Ref.

A.D. Argoudelis, F. Reusser, S.A. Mizesak and L.
 Baczynskyj, *J. Antibiot.*, 29 (1976) 1007-1014

R_F

Solvent	R_F^*	Solvent	R_F^*
A	0.05	G	No zone
B	0.05	H	No zone
C	0.15	I	0.05
D	0.0	J	0.15
E	0.85	K	0.30
F	0.85		

* Estimated from drawing

TLC**Medium**

Silica gel G

Solvent

95% Ethanol–water (75:25)

DetectionBioautography vs. *Staphylococcus aureus*, *Sarcina lutea*, or *Escherichia coli* R_F

0.38 (Estimated from drawing)

Ref.

As PC

FERRAMIDO CHLOROMYCIN (FACM)**PC****Solvents**

- A. Distilled water
 - B. 3% Ammonium chloride soln.
 - C. Chloroform
 - D. Ethyl acetate–water (1:1)
 - E. Acetone
 - F. Methanol
 - G. Ethanol–2% sodium chloride (3:1)
 - H. *n*-Butanol–water (1:1)
 - I. *n*-Butanol–acetic acid–water (4:1:5)
 - J. *n*-Butanol–acetic acid–water (2:1:1)
- All solvents developed descending

 R_F

Solvent	R_F	Solvent	R_F
A	0.05	F	0.89
B	0.00	G	0.48
C	0.00	H	0.85
D	0.00	I	0.23
E	0.76	J	0.45

Ref.I.R. Shimi and S. Shoukry, *J. Antibiot.*, 19 (1966) 110–114**FERRIMYCINS****PC 1****Paper**

Whatman No. 1 paper impregnated with acetone–water–satd. aq. sodium chloride (6:3:1)

Solvent*tert*-Butanol–0.004 *N* hydrochloric acid–satd. aq. sodium chloride (2:1:1) R_F Ferrimycin A₁, 0.59Ferrimycin A₂, 0.47**Ref.**E. Gaeumann, E. Vischer and H. Bickel, *U.S. Patent* 3,093,550, June 11, 1963**PC 2****Solvents**

- A. Butanol–glacial acetic acid–water (4:1:5), 10 h
- B. Butanol–butyl acetate–glacial acetic acid–water (100:30:13:143), 24 h
- C. As B but 60 h

DetectionBioautography vs. *Staphylococcus aureus* R_F^*

Solvent	Component			
	A	A ₁	A ₂	B
A	0.4	0.4	0.4	0.6
B	0.1	0.1	0.1	0.5
C	0.4; 0.5	0.5	0.4	–

* Estimated from drawing

Ref.

As PC 1

PC 3**Solvents**

- A. Water satd. butanol
- B. Butanol–glacial acetic acid–water (4:1:5), upper phase
- C. Water satd. butanol + 2% *p*-toluenesulfonic acid
- D. Water satd. butanol + 2% piperidine
- E. Butanol–pyridine–water (6:4:3)
- F. 80% Ethanol + 1.5% sodium chloride (Whatman No. 4 impregnated with 0.95 *M* sodium sulfate + 0.05 *M* sodium bisulfate)

G. Butanol-ethanol-water (1:1:2)

H. Butanol-butyl acetate-glacial acetic acid-water (10:3:1.3:14.3), upper phase

Detection

Bioautography vs. *Staphylococcus aureus* or *Bacillus subtilis*

R_F

Solvent	Ferrimycin A	Ferrimycin B
A	0	0
B	0.49	0.63
C	0.34	0.58
D	0.05	0.15
E	0.32	0.32
F	0.47	0.47
G	0.74	0.74
H	2.7	7.6

Ref.

E. Gaeumann, T. Gaeumann, V. Prelog, E. Vischer and H. Bickel, *U.S. Patent* 3,852,424, Dec. 3, 1974

ELPHO

Medium

Paper

Buffer

0.1 M acetate buffer, pH 4.6

Conditions

500-4000 V

Mobility

Ferrimycins A and B migrate towards cathode

Ref.

As PC 1

CCD

Solvent

Craig apparatus filled with 100 ml upper phase and 100 ml lower phase of a mixture equilibrated at 19°C of benzyl alcohol-*n*-butanol-1 N hydrochloric acid-water-aq. sodium chloride (saturated at 19°C) (200:100:6:300:60). 30 shakes/distribution at 15 min intervals. First two tubes contain solvent; next three tubes contain 2 g mixture; 115 transfers, 120 tubes

Detection

Bioautography; dilute samples 1:50 and test vs. *Staphylococcus aureus*

Distribution

Ferrimycin A found in fractions 40-70; Ferrimycin B found in fractions 75-100

Ref.

E. Gaeumann, T. Gaeumann, V. Prelog, E. Vischer and H. Bickel, *U.S. Patent* 3,852,424, Dec. 3, 1974

FERVENULIN

PC

Solvents

A. 1-Butanol-water (84:16) + 0.25% *p*-toluenesulfonic acid

B. 1-Butanol-acetic acid-water (2:1:1)

C. 1-Butanol-water (4:96)

Detection

Bioautography vs. *Klebsiella pneumoniae*

R_F

Solvent	R_F *
A	0.50
B	0.75
C	0.75

* Estimated from drawing

Ref.

C. DeBoer, A. Dietz, J.S. Evans and R.M. Michaels, *Antibiot. Ann.*, (1959-1960) 220-226

FICELLOMYCIN

(cf., U-47929)

PC

Paper

Impregnated with 0.1 M sodium sulfate

Solvents

A. 1-Butanol-water (84:16)

B. 1-Butanol-water (84:16) + 0.25% *p*-toluenesulfonic acid

C. 1-Butanol-acetic acid-water (2:1:1)

D. 2% (v/v) Piperidine in 1-butanol-water (84:16)

E. 1-Butanol-water (4:96)

F. 1-Butanol-water (4:96) + 0.25% *p*-toluenesulfonic acid

G. 0.5 M Phosphate buffer, pH 7.0

H. 0.075 M Ammonium hydroxide satd. with methyl isobutyl ketone, lower phase

I. Benzene-methanol-water (1:1:2)

J. 1-Butanol-water (84:16) + 2% *p*-toluenesulfonic acid

K. Methanol-15% aq. sodium chloride (4:1)

Ref.

A.D. Argoudelis, F. Reusser, H.A. Whaley, L. Baczynskyj, S.A. Mizsak and R.J. Wnuk, *J. Antibiot.*, 29 (1976) 1001-1006

R_F

Solvent	R_F^*	Solvent	R_F^*
A	0.05	G	0.95
B	0.15	H	0.95
C	No zone	I	0.00
D	0.05	J	0.50
E	0.95	K	0.50
F	0.95		

* Estimated from drawing

TLC*Medium*

Silica gel G

Solvent

95% Ethanol–water (75:25)

*Detection*Bioautography vs. *Staphylococcus aureus*, *Sarcina lutea* or *Escherichia coli* R_F

Component	R_F^*
I	0.25
II	0.37
III	0.60

* Estimated from drawing

*Ref.*As PC 1; and A.D. Argoudelis, F. Reusser, S.A. Mizesak and L. Baczynskyj, *J. Antibiot.*, 29 (1976) 1007–1014**FIGAROIC ACID COMPLEX****TLC***Medium*Silica gel 60 F₂₅₄ (Brinkmann)*Solvents*

- A. Chloroform–methanol (19:1)
 B. Chloroform–methanol–formic acid (90:10:1)
 C. Chloroform–methanol–formic acid (80:20:1)

 R_F

Solvent	R_F^*
A	0.0 (s), 0.39 (w), 0.214 (m), 0.243 (w), 0.349 (m), 0.470 (w), 0.612 (s), 0.651 (s), 0.694 (m)

Solvent

 R_F^*

B	0.0 (w), 0.043 (s), 0.073 (m), 0.149 (s), 0.169 (s), 0.212 (w), 0.272 (m), 0.308 (m), 0.368 (w), 0.467 (m), 0.523 (m), 0.722 (s), 0.795 (s), 0.825 (s)
C	0.0 (very weak), 0.200 (m), 0.233 (w), 0.279 (m), 0.311 (w), 0.446 (m), 0.472 (s), 0.508 (m), 0.622 (w), 0.741 (m), 0.839 (s), 0.892 (m), 0.928 (s)

* R_F values and (in parentheses) zone intensities, qualitatively estimated as strong (s), moderate (m) and weak (w) are indicated. Zones with an R_F of 0.0 are at the point of application

*Ref.*W.T. Bradner, J.A. Bush and D.E. Nettleton, Jr., *U.S. Patent* 4,112,071, Sept. 5, 1978**FILIPIN COMPLEX****TLC***Medium*

Silica gel HF₂₅₄ (Merck). Prepare 60 g of silica gel HF₂₅₄ suspended in 60 ml of 0.2 M monopotassium phosphate and 60 ml of 0.2 M disodium phosphate. Air dry and activate at 130°C for 2 h

Solvent

Methylene chloride–methanol (85:15)

Detection

Fluorescence under 366 nm light. For quantitation use densitometric measurement

 R_F

- Filipin I, 0.8
 Filipin II, 0.7
 Filipin III, 0.6
 Filipin IV, 0.5

*Ref.*M.E. Bergy and T.E. Eble, *Biochem.*, 7 (1968) 653–659**FLAMBAMYCIN****TLC***Medium*

Silica gel (Merck No. 7731) activated at 130°C for 2 h

Solvent

Chloroform-methanol (92:8), 20°C

DetectionA. Bioautography vs. *Bacillus subtilis*

B. Spray with conc. sulfuric acid and heat for 10 min at 110°C

R_F

0.32

Ref.L. Ninet, F. Benazet, Y. Charpentie, M. Dubost, J. Florent, J. Lunel, D. Mancy and J. Preud'Homme, *Experientia*, 30 (1974) 1270-1272**FLAVOFUNGIN****PC****Solvent**

As flavomycoin, PC 1; A-K

R_F

Solvent	R _F	Solvent	R _F
A	0.79	G	0.12
B	0.85	H	0.01
C	0.95	I	0.02
D	0.72	J	0.45
E	0.89	K	0.07
F	0.81		

Ref.

As flavomycoin, PC 1

FLAVOMYCOIN**PC 1****Solvents**

A. Methanol-water-ammonia (80:16:4)

B. *n*-Butanol-methanol-water (4:1:2)C. *n*-Propanol-acetic acid-water (60:4:4)

D. Dimethyl formamide-water (50:50)

E. Pyridine-butanol-water (4:6:5)

F. Dimethyl formamide-water-glacial acetic acid (50:45:5)

G. Chloroform-tetrahydrofuran-formamide (50:50:5)

H. Chloroform-methyl ethyl ketone-tetrahydrofuran-formamide (60:20:20:4)

I. Chloroform-methyl ethyl ketone-formamide (66:33:4)

J. Benzene-methyl ethyl ketone (50:50, formamide satd.)

K. Benzene-dioxane (50:50, formamide satd.)

R_F

Solvent	R _F	Solvent	R _F
A	0.90	G	0.55
B	0.88	H	0.42
C	0.94	I	0.33
D	0.77	J	0.69
E	0.86	K	0.21
F	0.84		

Ref.R. Schlegel and H. Thrum, *J. Antibiot.*, 24 (1971) 360-367**PC 2****Paper**

Schleicher and Schull 2043b

Solvent

Chloroform-tetrahydrofuran-formamide (50:50:5), ascending 2.5 h, 7°C

DetectionBioautography vs. *Penicillium notatum* P 36**R_F**

0.5

Ref.

As PC 1

FLEOMYCIN, 3-AMINO DERIVATIVES

(See 3-aminofleomycin derivatives)

FOLIMYCIN**PC****Solvents**

A. 40% Methanol in water

B. 55% Methanol in water

C. 20% *n*-Propanol in water**R_F**

Solvent A, 0.32

Solvent B, 1.0

Solvent C, 0.35

Ref.

As Ikutamycin, PC

FORMYCINS**ELPHO****Medium**

Toyo Roshi No. 51 paper

Buffers

A. Formic acid-acetic acid-water (5:75:900)

B. Phosphoric acid-monopotassium phosphate (pH 2.45, μ 0.06)

Conditions

Solvent A, 3000 V/40 cm for 15 min

Solvent B, 500 V/30 cm for 2 h

Mobility

For- mycin	Sol- vent	Mobility
A	A	Moves to anode 5.8 cm
A	B	Moves to anode 7.5 cm
B	S	Moves to anode 1.6 cm
B	B	Moves to anode 0.3 cm

Ref.

Derwent Farmdoc No. 30580; Japanese Patent, JA.759/68, Jan. 11, 1968

FOROMACIDINS**PC****Paper**

Whatman No. 1 treated with a 2% aq. soln. of sodium *m*-cresotinate, blotted and used wet

Solvent

Dibutyl ether-butyl acetate (1:3) satd. with sodium *m*-cresotinate soln. 2-3 h

Detection

Dry at 100°C for 2° min; spray with 15% phosphoric acid, heat at 100°C. Blue-green colors result

R_F

Separates foromacidins A, B, and C

Ref.

R. Corbaz, L. Ettlinger, E. Gäumann, W. Keller-Schierlein, F. Kradolfer, E. Kyburz, L. Neipp, V. Prelog, A. Wettstein and H. Zähler, *Helv. Chim. Acta*, 39 (1956) 304-317

CCD**Solvent**

Chloroform-0.2 *M* citrate buffer, pH 4.9 (1:1), 145 transfers

Distribution

Foromacidin A, tubes 71-115

Foromacidin B, tubes 39-70

Foromacidin C and D, tubes 16-38

Ref.

As PC

FORTIMICIN B**PC 1****Paper**

Ascending

Solvent

Chloroform-methanol-17% ammonia (2:1:1), (12 h)

R_F

0.65

Ref.

As Apramycin, PC 1

PC 2**Paper**

Ascending

Solvents

A. 20% Ammonium chloride (develop 3 h)

B. Water satd. *n*-butanol (develop 15 h)

C. *n*-Butanol-acetic acid-water (3:1:1), (develop 15 h)

D. Water satd. ethyl acetate (develop 4 h)

E. Water satd. *n*-butanol + 2% *p*-toluenesulfonic acid + 2% piperidine (develop 15 h)

R_F

Solvent	R _F	Solvent	R _F
A	0.85	D	0.00
B	0.00	E	0.07
C	0.09		

Ref.

As Apramycin, PC 1

TLC**Medium**

Silica gel

Solvents

A. Chloroform-methanol-17% ammonia (2:1:1)

B. Ammonium acetate-10% methanol (1:1)

R_F

Solvent A, 0.80

Solvent B, 0.62

Ref.

As Apramycin, PC 1

FORTIMICINS; FORTIMICIN A**PC 1****Paper**

Ascending (at 28°C)

Solvents

A. 20% Ammonium chloride (3 h)

B. Water satd. *n*-butanol (15 h)

C. *n*-Butanol-acetic acid-water (3:1:1) (15 h)

D. Water satd. ethyl acetate (4 h)

E. Water satd. *n*-butanol + 2% *p*-toluenesulfonic acid + 2% piperidine (15 h)

R_F

Solvent	Fortimicin A
A	0.96
B	0.00
C	0.06
D	0.00
E	0.04

Ref.

As Apramycin, PC 1

PC 2

Paper

Ascending

Solvent

Chloroform-methanol-17% aq. ammonia (2:1:1) lower layer (developed at room temp. for 12 h)

 R_F

Fortimicin A, 0.37

Fortimicin B, 0.65

Ref.

As Apramycin, PC 2; *Belgium Patent*, 313,772, Oct. 16, 1974**TLC**

Medium

Silica gel

Solvents

A. Chloroform-methanol-17% aq. ammonia (2:1:1) upper layer

B. 10% Ammonium acetate-methanol (1:1) (Developed at room temp. 3 h)

 R_F

Solvent	Fortimicin A
A	0.74
B	0.37

Ref.

As Apramycin, PC 2

FUMIGACHLORIN**TLC**

Medium

Kieselgel G (Merck)

Solvents

A. Chloroform-ethyl acetate (3:1)

B. Carbon tetrachloride-ethyl acetate-acetic acid (100:30:1)

 R_F

Solvent A, 0.65

Solvent B, 0.42

Ref.

K. Atsumi, M. Takada, K. Mizuno and T. Ando, *J. Antibiot.*, 23 (1970) 223-224**FUNGICHRONIN****PC**

Paper

Whatman No. 1

Solvents

A. Methyl isobutyl ketone-1-butanol-water (50:15:3)

B. Methyl isopropyl ketone-water (satd. ca. 1.6%)

C. Methyl isopropyl ketone-1-butanol-water (50:10:3.5)

D. Methyl isopropyl ketone-methanol-water (50:1:0.4)

Detection

The spots were detected by their intense greenish fluorescence in UV light

 R_F

Solvent	R_F
A	0.73
B	0.35
C	0.76
D	0.47

Ref.

A.C. Cope, R.K. Bly, E.P. Burrows, O.J. Ceder, E. Ciganek, B.T. Gillis, R.F. Porter and H.E. Johnson, *J. Amer. Chem. Soc.*, 84 (1962) 2170-2178**FURANOMYCIN****PC**

Paper

Toyo Roshi No. 50

Solvents

A. *n*-Butanol-acetic acid-water (4:1:6, upper phase)B. *n*-Butanol satd. with 2 *N* ammonium hydroxide

C. Pyridine-acetic acid-water (10:7:3)

Detection

Color spot

 R_F

Solvent A, 0.35

Solvent B, 0.12

Solvent C, 0.69

Ref.

K. Katagiri, K. Tori, Y. Kimura, T. Yoshida, T. Nagasaki and H. Minato, *J. Med. Chem.*, 10 (1967) 1149-1154

TLC**Medium**

Silica gel G (Merck)

Solvents

- A. *n*-Propanol-water (7:3)
- B. *n*-Butanol-acetic acid-water (3:1:1)
- C. Chloroform-methanol-17% ammonia (2:2:1, upper phase)

R_F

Solvent A, 0.41
Solvent B, 0.41
Solvent C, 0.64

Ref.

As PC

FUSARIUM ANTIBIOTIC**TLC****Medium**

Silica gel F₂₅₄

Solvent

10% Ethyl acetate in benzene

Detection

UV absorption

R_F

0.45

Ref.

R.H. Evans, Jr., M.P. Kunstmann, C.E. Holmlund and G.A. Ellestad, *U.S. Patent* 3,546,074, Dec. 8, 1970

GALANTINS**PC****Paper**

Toyo Roshi No. 51

Solvent

n-Propanol-pyridine-acetic acid-water (15:10:3:12)

Detection

Bioautography vs. *Bacillus subtilis*

R_F

II > I = III

Ref.

J. Shoji, R. Sakazaki, Y. Wakisaka, K. Koizumi, M. Mayama and S. Matsuura, *J. Antibiot.*, 28 (1975) 122-125

TLC**Media**

- A. Carbon plate
- B. Silica gel

Solvents

- A. 0.1 *N* Hydrochloric acid-methanol (4:1)
- B. 5% of dioxane added to a mixture of chloroform-methanol (4:1)

R_F

Medium	Solvent	R _F *
A	A	I > II > III
B	B	Dinitrophenyl derivative of I gave single spot

* Estimated from drawing

Ref.

As PC

GANGTOKMYCIN**PC****Paper**

Paper partition chromatography measured using ascending method on Whatman filter paper No. 1

Solvents

- A. *n*-Butanol-acetone-water (40:15:50)
- B. *n*-Butanol-methanol-water (25:4:16)
- C. *n*-Butanol-acetone-pyridine-water (40:10:5:45)

R_F

Solvent A, 0.55-0.60
Solvent B, 0.56-0.60
Solvent C, 0.53-0.55

Ref.

M. Shibata, H. Yamamoto, E. Higashide, K. Mizuno, M. Imanishi, and A. Miyake, *U.S. Patent* 3,678,161, July 18, 1972

GARDIMYCIN**PC****Paper**

Whatman No. 1

Solvents

- A. Butanol satd. with $\frac{1}{15}$ *M* phosphate buffer (pH 6.0)
- B. Water satd. butanol + 2% *p*-toluenesulfonic acid
- C. Water satd. butanol + 2% ammonia
- D. $\frac{1}{15}$ *M* Phosphate buffer (pH 6.0), satd. with butanol

- E. 20% Aq. sodium chloride
 F. Butanol-methanol-water (40:10:20) + 0.75% methyl orange
 G. Butanol-methanol-water (40:10:20)
 H. Acetone-water (1:1)
 I. Water satd. ethyl acetate

Detection

Bioautography vs. *Staphylococcus aureus*

R_F

Solvent	Gardimycin		
	A	B *	C
A	0.00	0.15	0.85
B	0.75	0.80	0.95
C	0.15	0.10	0.85
D	0.65	0.80	0.00
E	0.00	0.80	0.00
F	0.70	0.65	0.90
G	0.85	0.60	0.90
H	0.80	0.00	0.65
I	0.00	0.00	0.75

- As the monosodium salt

Ref.

German Patent 2,533,447, Feb. 19, 1976

TLC**Medium**

Kieselgel

Solvent

Ammonia-ethanol-water (1:8:1)

Detection

- A. Bioautography vs. *Staphylococcus aureus*
 B. Sulfuric acid
 C. Vanillin

R_F

Gardimycin A, 0.30
 Gardimycin B *, 0.70
 Gardimycin C, 0.00

- * As the monosodium salt

Ref.

As PC

GATAVALIN**PC****Solvent**

n-Butanol-acetic acid-water (3:1:1)

Detection

Bioautography vs. *Staphylococcus aureus* FDA 209P

R_F

0.9

Ref.

N. Nakajima, S. Chihara and Y. Koyama, *J. Antibiot.*, 25 (1972) 243-247

GELBECIDIN**PC****Paper**

Whatman No. 1

Solvents

- A. Butanol-acetic acid-water (4:1:5)
 B. Methanol-benzene (4:6)

Detection

- A. UV and visible light
 B. Bioautography

R_F

Solvent A, 0.94
 Solvent B, 0.80

Ref.

A. Aszalos, R.S. Robison, F.E. Pansy and B. Berk, *U.S. Patent* 3,551,561, Dec. 29, 1970

TLC**Medium**

Eastman Chromagram sheets

Solvents

- A. Methanol
 B. Methanol-chloroform (1:9)

Detection

- A. As PC, A
 B. As PC, B

R_F

Solvent A, 0.60
 Solvent B, 0.45

Ref.

As PC; and German Patent No. 1942694, March 5, 1970

GELDANAMYCIN**PC****Paper**

Whatman No. 1

Solvents

- A. 1-Butanol-water (84:16) + 2% p-toluene-sulfonic acid, 64 h
 B. 0.075 N Ammonium hydroxide satd. with methyl isobutyl ketone, 5 h
 C. Benzene-methanol-water (1:1:2), upper phase, 5 h
 All systems developed descending

Detection

Bioautography vs. *Tetrahymena pyriformis*

R_F

Solvent A, 0.05; 0.4

Solvent B, 0.05; 0.78

Solvent C, 0.05; 0.6–0.7

Ref.

C. DeBoer, P.A. Meulman, R.J. Wnuk and D.H. Peterson, *J. Antibiot.*, 23 (1970) 442–447

TLC*Medium*

Silica gel H (Merck)

Solvent

Chloroform–methanol (9.5:0.5)

Detection

A. Yellow color

B. UV light

 R_F

0.56

Ref.

As PC

GENIMYCIN**CCD***Solvents*

A. Butanol–methanol–hexane–borate buffer, pH 7.5 (1:1:1:3) 0.1 *M*

B. Chloroform–methanol–borate buffer, pH 5.6

*Distribution coefficient*Solvent A, $K = 1.22$ Solvent B, $K = 1.38$

Ref.

Y.L. Severinets, V.M. Efimova, L.O. Bol'shakova, A.I. Karnaushkina, S.N. Solov' and A.N. Egorenkova, *Antibiotiki*, 15 (1970) 5–9

GENTAMICIN C₁**ELPHO 1***Medium*

Paper

Buffer

Formic acid–acetic acid–water (25:75:900)

Conditions

3000 V for 20 min

Mobility

Gentamicin C₁ moved 23.0 cm toward cathode. Inactivated gentamicin C₁ moved 20.0 cm toward cathode. (Acetylated on 3 amino group of 2-deoxystreptamine.)

Ref.

H. Umezawa, M. Yagisawa, Y. Matsuhashi, H. Naganawa, H. Yamamoto, S. Kondo and T. Takeuchi, *J. Antibiot.*, 26 (1973) 612–614

ELPHO 2*Medium*

Paper, Whatman 3MM

Buffer

Formic acid–acetic acid–water (6:24:170)

Conditions

3000 V potential gradient 60 V per cm, current 50 mA, 30 min

Detection

Paper stained with 0.25% ninhydrin in acetone containing 0.01% isatin and 1% lutidine at 100° for 10 min

Mobility

1.3 (migration distance relative to alanine taken as 1.00)

Ref.

As Ampicillin, ELPHO

GENTAMICIN C₂ DERIVATIVES**TLC***Medium*

Silica gel

Solvent

Chloroform–methanol–28% ammonium hydroxide–water (2:8:2:1)

 R_F

Derivative	R_F
BB-K73	0.58
BB-K74	0.33
BB-K75	0.15
BB-K76	0.08

Ref.

T. Naito, S. Nakagawa and Y. Abe, *U.S. Patent* 3,796,698, March 12, 1974

GENTAMICINS**PC 1***Paper*

Whatman No. 1

Solvents

A. Methanol–water (4:1) + 3% sodium chloride vs. paper buffered with 0.95 *M* sodium sulfate + 0.05 *M* sodium bisulfate

B. Propanol–pyridine–acetic acid–water (15:10:3:12)

C. Propanol–water–acetic acid (50:40:5)

D. 80% Aq. phenol

Detection

Bioautography vs. *Staphylococcus aureus* ATCC 6538P

R_F

Solvent	R_F
A	0.59
B	0.26
C	0.10
D	0.30

Ref.

M.J. Weinstein, G.M. Luedemann, E.M. Oden and G.H. Wagman, *Antimicrob. Agents Chemother.*, 1963 (1964) 1-7

PC 2

Paper

Whatman No. 1

Solvent

Lower phase of chloroform-methanol-17% ammonium hydroxide (2:1:1). Upper phase placed in bottom of chamber several hours prior to use. Papers developed descending, ca. 5 h, 25°C. Solvent allowed to drip off end of paper

Detection

A. As PC 1

B. Spray with 0.25% ninhydrin in pyridine-acetone (1:1). Heat at 105°C several min. Purple to blue spots result

R_F

Gentamicin $C_1 > C_2 > C_{1a}$

Ref.

G.H. Wagman, J.A. Marquez and M.J. Weinstein, *J. Chromatogr.*, 34 (1968) 210-215

PC 3 (Quantitative)

Paper

Whatman No. 1 strips $\frac{1}{4}$ in. wide $\pm \frac{1}{64}$ in.

Solvent

As PC 2

Detection

As PC 1. Zone diameters are measured and compared to zone diameters of series of standards. Standard curves are constructed and conc. of each component determined from appropriate curve. Percentage of each component can be calculated for unknown samples

R_F

As PC 2

Ref.

G.H. Wagman, E.M. Oden and M.J. Weinstein, *Appl. Microbiol.*, 16 (1968) 624-627

PC 4 (Quantitative)

Paper

Whatman No. 4. Two replicate spots per sheet

Solvent

As PC 2 but used mixed phases to equilibrate chamber for 24 h prior to use

Detection

Modified Barrolier reagent. To 1 g ninhydrin add 0.1 g cadmium acetate, 3 ml water and 1.5 ml of glacial acetic acid and shake. Add to 100 ml *n*-propanol and shake until soln. is complete. Store in a brown bottle under refrigeration

R_F

As PC 2. Quantitative procedure: cut each paper in half lengthwise; spray one half with ninhydrin reagent and dry at 100°C for 1 min. Using this as a guide, cut other half of paper in segments representing 3 gentamicin components. Cut each segment into small strips and put into separate 125-ml flasks, add 50 ml of 0.1 *M* potassium phosphate buffer, pH 8.0, and shake 30 min. Decant, allow paper to settle, pipet 4 ml of clear soln. in a 25-ml volumetric flask and bring to volume with same buffer. Assay each soln. by microbiological plate assay vs. *Staphylococcus epidermidis*. The percentage of each fraction is calculated as follows:

$$\% C_1 = \frac{A_1}{504} \times \frac{100}{B}$$

$$\% C_{1a} = \frac{A_{1a}}{626} \times \frac{100}{B}$$

$$\% C_2 = \frac{A_2}{656} \times \frac{100}{B}$$

where

A_1 = concentration of the assayed C_1 soln. in $\mu\text{g/ml}$

A_{1a} = concentration of the assayed C_{1a} soln. in $\mu\text{g/ml}$

A_2 = concentration of the assayed C_2 soln. in $\mu\text{g/ml}$

$$B = \frac{A_1}{504} + \frac{A_{1a}}{626} + \frac{A_2}{656}$$

504, 626 and 656 are the activities of C_1 sulfate, C_{1a} sulfate, and C_2 sulfate, respectively, compared to the gentamicin sulfate standard

Ref.

N. Kantor and G. Selzer, *J. Pharm. Sci.*, 57 (1968) 2170-2171

PC 5 (Quantitative)

Paper

Schleicher and Schuell No. 589 blue ribbon chromatographic paper cut to size 20 $\times$ 58 cm (along grain)

Solvent

As PC 1

Detection

After development, strips containing the antibiotic are treated with ninhydrin reagent, developed, and color intensities read on an integrating scanner from which component proportions can be determined. Results are in excellent agreement with the microbiological method. (Integrating scanner-instrument used for the determinations to be described was the model RB Analytrol, manufactured by Beckman Instruments.) Comparison of ninhydrin reactivity for each of the gentamicin base component standards showed that the intensity of color varied with $C_1a > C_2 > C_1$. This was true also for the sulfates, but the ratios were somewhat different. For the free bases, if C_1a is assigned a ninhydrin peak value of 1.00, then an equal quantity of C_2 results in a less intense color reaction and a value of 0.58 (58% of the intensity of color for an equal weight of C_2 compared to C_1a). For the C_1 component the value is 0.52

Comparison of intensity of ninhydrin reactions on chromatograms for individual components of the gentamicin complex

Derivative	Component	Relative intensity of ninhydrin spot	Factor (reciprocal of intensity)
Base	C_1	0.518	1.93
	C_1a	1.000	1.00
	C_2	0.578	1.73
Sulfate	C_1	0.397	2.52
	C_1a	1.000	1.00
	C_2	0.485	2.06

 R_F $C_1 > C_2 > C_1a$

Ref.

G.H. Wagman, J.V. Bailey and M.M. Miller, *J. Pharm. Sci.*, 57 (1968) 1319-1322

PC 6**Paper**

Whatman No. 1

Solvents

A. Chloroform-methanol-17% ammonium hydroxide (2:1:1), lower phase, descending, 16 h, 25°C. Solvent allowed to drip off paper

B. 2-Butanone-*tert.*-butanol-methanol-conc. ammonium hydroxide (16:3:1:6), descending, 16 h

Detection

Bioautography vs. *Staphylococcus aureus* ATCC 6538P

 R_F

	Solvent A mm from origin)	Solvent B * $R_F B_1$
Gentamicin A ₁	0.0	0.36
Gentamicin A	0.0	0.41
Gentamicin B	9.0	0.52
Gentamicin X	13.5	0.55
Gentamicin B ₁	27.0	1.00

$$R_F B_1 = \frac{(\text{distance component from origin})}{(\text{distance } B_1 \text{ from origin})}$$

Ref.

G.H. Wagman, J.A. Marquez, J.V. Bailey, D. Cooper, J. Weinstein, R. Tkach and P. Daniels, *J. Chromatogr.*, 70 (1972) 171-173

PC 7**Paper**

Whatman No. 1

Solvents

- A. *n*-Butanol satd. with water + 2% *p*-toluenesulfonic acid, for 40 h
 B. 80% Aq. ethanol + 1.5% sodium chloride, for 40 h on paper buffered with 0.95 M sulfate-bisulfate
 C. Propanol-pyridine-acetic acid-water (15:10:3:12) for 40 h
 D. Water satd. with methylisobutylketone + 1% *p*-toluenesulfonic acid, for 6 h
 E. Propanol-water (1:1) for 24 h on paper buffered with 0.75 M phosphate, pH 4.0
 F. *n*-Butanol satd. with water, for 18 h
 G. *n*-Butanol satd. with water + 2% *p*-toluenesulfonic acid and 2% piperidine, for 18 h

 R_F

Solvent	R_e^*	Solvent	R_e^*
A	0.83	E	0.51
B	0.75; 0.92	F	0
C	0.62	G	0.10
D	—		

* The migration constant for each of the antibiotics is expressed as an R_e rather than the more

conventional R_F value. The R_e value expresses the ratio of the distance traversed by the antibiotic with respect to the end of the tape rather than the solvent front. This method of expressing the constant was chosen in view of the fact that the solvent front had progressed beyond the end of the tape during the time interval employed with most of the solvent systems

Ref.

R.Q. Thompson, W.M. Stark, C.E. Higgins, *U.S. Patent* 3,691,279, Sept. 12, 1972

PC 8

Paper

Ascending

Solvents

- A. 20% Aq. soln. of ammonium chloride
- B. Water satd. *n*-butanol
- C. *n*-Butanol-acetic acid-water (3:1:1)
- D. Water satd. *n*-butanol + 2% (w/v) *p*-toluenesulfonic acid + 2% (v/v) piperidine
- E. Chloroform-methanol-17% aq. ammonia (2:1:1) lower layer

R_F

Solvent	Component				
	A	B	C _{1a}	C ₁	C ₂
A	0.98	0.98	0.98	0.98	0.98
B	0.00	0.00	0.02	0.03	0.02
C	0.05	0.08	0.08	0.08	0.03
D	0.00	0.00	0.05	0.08	0.09
E	0.00	0.00	0.18	0.59	0.38

Ref.

As Apramycin, PC 1

PC 9

Paper

Ascending

Solvent

Chloroform-methanol-17% aq. ammonia (2:1:1) lower layer (developed at room temp. for 12 h)

R_F

Compound	R_F
Gentamicin A	0.00
Gentamicin B	0.00
Gentamicin C _{1a}	0.18
Gentamicin C ₁	0.59
Gentamicin C ₂	0.38

Ref.

As Apramycin, PC 1

PC 10

Paper

Toyo No. 51

Solvents

- A. 20% Ammonium chloride
- B. Water satd. *n*-butanol
- C. *n*-Butanol-acetic acid-water (3:1:1)
- D. Water satd. ethyl acetate
- E. Water satd. *n*-butanol + 2% *p*-toluenesulfonic acid + 2% piperidine

Detection

Bioautography vs. *Bacillus subtilis* No. 10707

R_F

Solvent	R_F	Solvent	R_F
A	0.98	D	0.00
B	0.00	E	0.01
C	0.05		

Ref.

As Carbomycin, PC 5

PC 11

Paper

Whatman No. 1

Solvents

- A. 80% Methanol + 3% sodium chloride (1:1) descending. Paper buffered with 0.95 *M* sodium sulfate + 0.05 *M* sodium bisulfate
- B. Propanol-pyridine-acetic acid-water (6:4:1:3), ascending
- C. Butanol-acetic acid-water (4:1:5), ascending
- D. 80% Phenol, ascending

Detection

Bioautography vs. *Staphylococcus aureus*

R_F

Solvent	R_F	Solvent	R_F
A	0.59	C	0.00
B	0.26	D	0.50

Ref.

As Chloramphenicol, PC 5

PC 12

Solvents

- A. 80% Methanol + 3% (w/v) sodium chloride (1:1), descending. Paper buffered with 0.05 *M* sodium sulfate + 0.05 *M* sodium bisulfate

- B. Propanol-pyridine-acetic acid-water (6:4:1:3, v/v) ascending
 C. 80% (v/v) Phenol, ascending
 D. Benzene-methanol (9:1, v/v), descending
 E. *n*-Butanol-water-acetic acid (4:5:1), upper phase, ascending
 F. Chloroform-methanol-17% ammonium hydroxide (2:1:1)
 G. 2-Butanone-*tert*.-butanol-methanol-conc. ammonium hydroxide (16:3:1:6), 16 h

 R_F

Solvent	Gentamicin		
	C ₁	C ₂	C _{1a}
A	0.57	0.56	0.48
B	0.34	0.30	0.22
C	0.45	0.45	0.45
D	0.0	0.0	0.0
E	0.0	0.0	0.0
F	0.67	0.40	0.21
G		0.75 *	0.65 •

• R_i

Ref.

M.J. Weinstein, G.H. Wagman and J.A. Marquez,
U.S. Patent 4,001,209, Jan. 4, 1977

PC 13

Paper

Toyo No. 51

Solvent

Methanol-3% aq. sodium chloride (2:1)

 R_F

0.53

Ref.

K. Munakata, T. Oda, T. Mori and H. Ito, *U.S. Patent* 3,891,506, June 24, 1975

PC 14

Paper

Toyo Filter No. 51A

Solvent

Methanol-3% aq. sodium chloride (2:1)

 R_F

0.53

Ref.

K. Munakata, T. Oda, T. Mori and H. Ito, *U.S. Patent* 3,870,698, March 11, 1975

PC 15

Paper

- A. Chromar 500 sheet, ascending
 B. Whatman No. 1, descending

Solvents

- A. Chloroform-methanol-conc. ammonium hydroxide (1:1:1)
 B. Chloroform-methanol-17% ammonium hydroxide (2:1:1)
 C. 2-Butanone-*tert*.-butanol-methanol-conc. ammonium hydroxide (16:3:1:6)

 R_F

Gentamicin	R_F (Medium A, Solvent A)	R_i (Medium B, Solvent B) ($t = 6$ h)	R_i (Medium B, Solvent C) ($t = 16$ h)
C ₁	0.74	0.67	0.57
C ₂	0.65	0.40	0.47
C _{1a}	0.57	0.21	0.38

Ref.

M.J. Weinstein, G.H. Wagman and J.A. Marquez,
U.S. Patent 3,951,746, April 20, 1976

PC 16

Paper

Whatman No. 1

Solvent

Chloroform-methanol-30% ammonium hydroxide (1:1:1), lower phase

Detection

Bioautography vs. *Bacillus subtilis* R_F

C ₁	Component 1 •
0.70	0.63
	$R_F C_1$ ** 0.90

* Biosynthetically produced; related to Gentamicin C₁

** $R_F C_1$ = Mobility relative to Gentamicin C₁

Ref.

S.J. Daum and R.L. Clarke, *U.S. Patent* 3,982,996, Sept. 28, 1976

PC 17

Paper

Macherey-Nagel 214

Solvent

Methyl ethyl ketone-*tert.*-butanol-methanol-6.5 *N* ammonium hydroxide (16:3:1:6)

Detection

- A. Bioautography vs. *Bacillus subtilis* ATCC 6633
B. Ninhydrin

 R_F

Compound	R_F *
New compound (I-1)	1.60
Gentamicin A ₂	0.25
G-418	0.73
New compound (II-2)	0.52
Gentamicin X ₂	0.56
Gentamicin A	0.36
New compound (III-1)	0.46
Garamine	0.91
Gentamicin A ₁	0.37
Gentamicin B	0.41
Gentamicin B ₁	1.00
JI-20A	0.39
JI-20B	0.94
Sisomicin	0.86
New compound (V-2)	0.75
Gentamicin A ₃	0.29
New compound (VII-1)	1.13
Gentamine C ₁	1.35
New compound (VII-3)	0.92
Gentamine C ₂	1.12
New compound (VII-5)	0.71
Gentamine C _{1a}	0.82

* Relative to B₁

Ref.

J. Berdy, J.K. Pauncz, Zs.M. Vajna, Gy. Horvath, J. Gyimesi and I. Koczka, *J. Antibiot.*, 30 (1977) 945-954

PC 18

Paper

FN3 paper

Solvents

- A. Chloroform-propanol-aq. ammonia (2:2:1)
B. Chloroform-methanol-aq. ammonia (2:2:1)
C. Butanol satd. with water-1% *p*-toluenesulfonic acid

Detection

- A. Bioautography vs. *Bacillus mycoides*
B. Ninhydrin

 R_F

Solvent	Components		
	C ₁	C ₂	C _{1a}
A	0.73	0.42	0.25
B	0.62	0.35	0.18
C *			

* No separation: $R_F = 0.47$

Ref.

K.L. Canchev, *Khim. Fan. Zh.*, 7 (No. 7) (1973) 47-49

PC 19

Paper

Whatman No. 1

Solvent

Chloroform-methanol-9 *N* ammonium hydroxide (2:1:1), lower phase, developed for 4 h

Detection

Bioautography vs. *Staphylococcus aureus*

 R_F

Gentamicin component	R_{C_1} *
C ₁	1.00
C ₂	0.56
C _{2b}	0.73
C _{1a}	0.29

* $R_{C_1} = (\text{distance moved by component from origin}) / (\text{distance moved by gentamicin C}_1 \text{ from origin})$

Ref.

P.J.L. Daniels, C. Luce, T.L. Nagabhushan, R.S. Jaret, D. Schumacher, H. Reimann and J. Ilavsky, *J. Antibiot.*, 28 (1975) 35-41

PC 20

Paper

Whatman No. 1 saturated with 0.95 *M* sulfate bisulfate

Solvent

80% Aq. ethanol + 1.5% sodium chloride (descending)

Detection

Bioautography vs. *Bacillus subtilis*

Ref.

S.J. Daum and R.L. Clarke, *U.S. Patent* 3,972,930, Aug. 3, 1976

R_F

Component	R_{C_1} *
Gentamicin C_1	1
Gentamicin C_2	0.89
Gentamicin C_1a	0.50
O-3-Deoxy-4-C-methyl-3-methyl-amino- β -L-arabinopyranosyl-(1 $\rightarrow$ 6)-O-[2-amino-6-methylamino-6-C-methyl-2,3,4,6-tetradeoxy- α -D-erythroglucopyranosyl-(1 $\rightarrow$ 4)]-D-streptamine	0.96
O-3-Deoxy-4-C-methyl-3-methyl-amino- β -L-arabinopyranosyl-(1 $\rightarrow$ 6)-O-[2,6-diamino-6-C-methyl-2,3,4,6-tetradeoxy- α -D-erythroglucopyranosyl-(1 $\rightarrow$ 4)]-D-streptamine	0.76
O-3-Deoxy-4-C-methyl-3-methyl-amino- β -L-arabinopyranosyl-(1 $\rightarrow$ 6)-O-[2,6-diamino-2,3,4,6-tetradeoxy- α -D-erythroglucopyranosyl-(1 $\rightarrow$ 4)]-D-streptamine	0.50

$$* R_{C_1} = \frac{(\text{distance component from origin})}{(\text{distance gentamicin } C_1 \text{ from origin})}$$

TLC 1

Medium

MN Cellulose 300. Mix 1 part cellulose powder with 6 parts water and prepare plates 250 μ m thick. Dry at 100°C, 20 min

Solvent

n-Propanol-pyridine-acetic acid-water (15 : 10 : 3 : 12)

Detection

Ninhydrin

 R_F

0.35, 0.28, 0.20

Ref.

Y. Ito, M. Namba, N. Nagahama, T. Yamaguchi and T. Okuda, *J. Antibiot.*, 17 (1964) 218-219

TLC 2

Medium

Silica gel

Solvent

Chloroform-methanol-conc. ammonium hydroxide-water (1 : 4 : 2 : 1)

Detection

A. Ninhydrin spray. Developed plates dried at 100°C and sprayed with pyridine; reheat to aid removal of ammonia traces

B. Bioautography vs. *Staphylococcus aureus*. The pyridine and heat treatments of the plates are omitted

 R_F

Component	R_F
Gentamicin A	0.60
Gentamicin C_1a (D)	0.69
Gentamicin C_1	0.71
Gentamicin C_2	0.76

Ref.

H. Maehr and C.P. Schaffner, *J. Chromatogr.*, 30 (1967) 572-578

TLC 3

Medium

Silica gel G

Solvent

Chloroform-methanol-17% ammonium hydroxide (2 : 1 : 1), lower phase

Detection

- 0.25% Ninhydrin in pyridine-acetone and consequent heating at 105°C for several minutes. The zones appear as purple or blue spots against a white background
- Starch-potassium iodine reagent spray with which the zones show up as dark blue spots against a white background
- Bioautography vs. *Staphylococcus aureus* ATCC 6538P

 R_F

$C_1 > C_2 > C_1a$

Ref.

G.H. Wagman, J.A. Marquez and M.J. Weinstein, *J. Chromatogr.*, 34 (1968) 210-215

TLC 4

Medium

DC-Alufolien strips (Merck)

 R_F

Spot No.	R_F	Color	Anti-bacterial action
1	0.26	Gray-blue	+
2	0.38	Bluish-purple	+
3	0.51	Bluish-purple	-
4	0.55	Pale pink	-
5	0.62	(a) Reddish-blue (b) Bluish-purple	(+)
6	0.73	Bluish-purple	-
7	0.76	Bluish-purple	-

Solvent

1-Butanol-methanol-glacial acetic acid-water (1 : 1 : 1 : 2)

Detection

- A. Bioautography
B. Ninhydrin

Ref.

U. Ullmann, *Arzneim. Forsch.*, 21 (1971) 263–267

TLC 5**Medium**

ChromAR Sheet 500

Solvent

Lower phase of chloroform–methanol–conc. ammonium hydroxide (1:1:1), ascending, 50 min

Detection

Bioautography vs. *Staphylococcus aureus* ATCC 6538P

R_F

Gentamicin A₁, 0.10
Gentamicin A, 0.16
Gentamicin B, 0.22
Gentamicin X, 0.28
Gentamicin B₁, 0.40

Ref.

As PC 6

TLC 6**Medium**

Silica gel

Solvents

- A. Chloroform–methanol–17% aq. ammonia (2:1:1) upper layer (developed at room temp. for 3 h)
B. 10% Ammonium acetate–methanol (1:1) (developed at room temp. for 3 h)

R_F

Solvent	Gentamicin C complex	Gentamicin C ₂
A	0.71	0.71
B	0.06–0.16	0.08–0.14

Ref.

As Apramycin, PC 1

TLC 7**Medium**

Silica gel

Solvents

- A. Chloroform–methanol–17% aq. ammonia (2:1:1) upper layer
B. Methanol–10% ammonium acetate (1:1)
C. *n*-Butanol–ethanol–chloroform–17% aq. ammonia (4:5:2:5)

R_F

Solvent	Component				
	A	B	C _{1a}	C ₁	C ₂
A	0.66	0.75	0.86	0.87	0.86
B	0.39	0.38	0.18	0.17	0.18
C	0.38	0.28	0.52	0.59	0.61

Ref.

As Apramycin, PC 1

TLC 8**Medium**

Silica gel F₂₅₄

Solvent

Chloroform–methanol–conc. (28%) ammonium hydroxide (1:1:1) lower phase

Detection

Ninhydrin spray on heating

R_F

Component	R _{C₁} [*]
Gentamicin C ₁	1
Gentamicin C ₂	0.83
Gentamicin C _{1a}	0.67
O-3-Deoxy-4-C-methyl-3-methyl-amino-β-L-arabinopyranosyl-(1→6)-O-[2-amino-6-methylamino-6-C-methyl-2,3,4,6-tetra-deoxy-α-D-erythroglucopyranosyl-(1→4)]-D-streptamine	0.92
O-3-Deoxy-4-C-methyl-3-methyl-amino-β-L-arabinopyranosyl-(1→6)-O-[2,6-diamino-6-C-methyl-2,3,4,6-tetra-deoxy-α-D-erythroglucopyranosyl-(1→4)]-D-streptamine	0.75
O-3-Deoxy-4-C-methyl-3-methyl-amino-β-L-arabinopyranosyl-(1→6)-O-[2,6-diamino-2,3,4,6-tetra-deoxy-α-D-erythroglucopyranosyl-(1→4)]-D-streptamine	0.61

$$^* R_{C_1} = \frac{(\text{distance component from origin})}{(\text{distance gentamicin } C_1 \text{ from origin})}$$

Ref.

As PC 7

TLC 9**Medium**

Alumina

Solvent

Chloroform-methanol-17% aq. ammonia
(2:1:1) upper layer

R_F
0.81

Ref.
As PC 14

TLC 10**Medium**

Cellulose

Solvent

Chloroform-methanol-17% ammonium hydroxide (2:2:1), lower layer

R_F
Component C₁, 0.69
Component C₂, 0.39
Component C_{1a}, 0.23

Ref.
T. Naito, S. Nakagawa and Y. Abe, *U.S. Patent* 3,796,699, March 12, 1974

TLC 11**Media**

- A. Silica gel (Quanta Gram)
- B. Cellulose

Solvents

- A. Propanol-pyridine-acetic acid-water (15:10:3:12)
- B. Pyridine-ethyl acetate-acetic acid-water (5:6:1:3)
- C. Methanol-chloroform-ammonia (1:1:1), lower phase

Detection

Ninhydrin with Medium A

R_F

Component	Medium	Solvent		
		A	B	C
C ₁	A			0.59
	B	0.625	0.208	
C ₂	A			0.51
	B	0.575	0.208	
C _{1a}	A			0.45
	B	0.475	0.146	

Ref.
R.S. Egan, R.L. DeVault, S.L. Mueller, M.I. Levenberg, A.C. Sinclair and R.S. Stanaszek, *J. Antibiot.*, 28 (1975) 29-34

TLC 12**Medium**

Silica gel

Solvent

Chloroform-methanol-22% ammonium hydroxide (1:2:1)

R_F
Component C₁, 0.47
Component C₂, 0.47
Component C_{2b}, 0.35

Ref.

P.J.L. Daniels, *U.S. Patent* 4,000,261, Dec. 28, 1976; and P.J.L. Daniels, *U.S. Patent* 4,000,262, Dec. 28, 1976

TLC 13**Medium**

Avicel

Solvent

Methanol-isobutyl alcohol-chloroform-10% hydrochloric acid (10:10:10:1)

Detection

- A. Iodine vapor
- B. 0.25% Stannous chloride soln.
- C. 0.2% *p*-Dimethylaminobenzaldehyde soln.

R_F
0.1

Ref.
As Ampicillin, TLC 4

TLC 14**Medium**

Alumina

Solvent

Chloroform-methanol-17% aq. ammonia (2:1:1), upper layer

R_F
0.81

Ref.
As PC 14

TLC 15**Media**

- A. Silica gel HF
- B. Ion-exchange: Fixion 50-X8 (Na⁺) plates, precoated at 50°C

Solvents

- A. Chloroform-methanol-25% ammonium hydroxide (12:19:20) lower phase
- B. 0.5 M Sodium phosphate soln. (adjusted to pH 6.5) containing 2.5 M sodium chloride and 5% *tert*-butanol

Detection

- A. Bioautography vs. *Bacillus subtilis* ATCC 6633
- B. Ninhydrin

R_F

Compound	Solvent	Medium	
		A	B
New compound (I-1)	A	0.93	
	B		0.16
Gentamicin A ₁	A	0.08	
	B		0.47
G-418	A	0.27	
	B		0.60
New compound (II-2)	A	0.15	
	B		0.73
Gentamicin X ₂	A	0.16	
	B		0.66
Gentamicin A	A	0.08	
	B		0.84
New compound (III-1)	A	0.18	
	B		0.67
Garamine	A	0.31	
	B		0.74
Gentamicin A ₁	A	0.09	
	B		0.82
Gentamicin B ₁	A	0.23	
	B		0.62
JI-20A	A	0.14	
	B		0.76
JI-20B	A	0.25	
	B		0.46
Sisomicin	A	0.60	
	B		0.24
New compound (V-2)	A	0.56	
	B		0.19
Gentamicin A ₃	A	0.06	
	B		0.84
New compound (VII-1)	A	0.69	
	B		0.13
Gentamine C ₁	A	0.73	
	B		0.19
New compound (VII-3)	A	0.55	
	B		0.17
Gentamine C ₂	A	0.59	
	B		0.27
New compound (VII-5)	A	0.48	
	B		0.20
Gentamine C _{1a}	A	0.42	
	B		0.28

Ref.

As PC 18

TLC 16

Medium

Silica gel 60 F₂₅₄

Solvent

Chloroform-methanol-30% ammonium hydroxide (1:1:1), lower phase

Detection

Ninhydrin

 R_F

C ₁	Component 1 *
0.39	0.30
	$R_F C_1$ ** 0.77

* Biosynthetically produced; related to Gentamicin C₁** $R_F C_1$ = Mobility relative to Gentamicin C₁

Ref.

As PC 16

TLC 17

Medium

Silica gel GF (Analtech). Plates were activated by heating for 1 h at 100°C prior to use

Solvents

A. Chloroform-methanol-15 N ammonium hydroxide (1:1:1)

B. Chloroform-methanol-15 N ammonium hydroxide (1:2:1)

C. Chloroform-methanol-15 N ammonium hydroxide (2:1:1) lower phase

Detection

Sulfuric acid charring

 R_F

Solvent	Gentamicin			
	C ₁	C ₂	C _{2b}	C _{1a}
A	0.53	0.42	0.40	0.33
B	0.64	0.63	0.55	0.52
C	0.28	0.22	0.23	0.15

Ref.

As PC 19

TLC 18

Medium

Silica gel 60 (precoated 20 × 20 cm, 0.25 mm thickness, E.M. Labs.)

Solvent

Methanol-chloroform-conc. ammonium hydroxide (1:1:1)

Detection

Ninhydrin: [10 ml of a freshly prepared soln. of ninhydrin (1.0 g) in a mixture of 95% ethanol (50 ml) and glacial acetic acid (10 ml)] heat for 15 min at 135°. The antibiotics are visible as magenta red spots on a white background

R_F

Gentamicin	R_F *
C ₁	0.37
C ₂	0.32
C _{1a}	0.25

* Estimated from drawing

Ref.

W.L. Wilson, G. Richard and D.W. Hughes, *J. Chromatogr.*, 78 (1973) 442-444

ELPHO

Medium

Whatman No. 4 filter paper

Buffers

- A. 2 M Formic acid-0.1 M *p*-toluenesulfonic acid-*n*-propanol (500:50:50) in 1000 ml water, pH 1.8
- B. 2 M Formic acid-0.1 M *p*-toluenesulfonic acid-*n*-propanol (400:50:50) in 1000 ml water, pH 1.9
- C. 2 M Formic acid-0.1 M *p*-toluenesulfonic acid (400:50) in 1000 ml water, pH 1.9
- D. 1 M Ammonium hydroxide-1 M sodium hydroxide-0.1 M *p*-toluenesulfonic acid-*n*-propanol (200:5:100:100) in 1000 ml water, pH 10.8
- E. 1 M Ammonium hydroxide-1 M sodium hydroxide (200:2.5) in 1000 ml water, pH 11.5
- F. 50 ml 1 M Sodium hydroxide in 1000 ml water, pH 12.2

Conditions

The electrophoregrams were developed at 350 V for 1-4 h (depending on the electrolyte)

mobility

Buffer	Toward cathode (cm ² /V sec)
A	30.16
B	31.97
C	32.77
D	4.97
E	5.05
F	5.73

Ref.

S. Ochab, *Pol. J. Pharmacol. Pharm.*, 23 (1973) 105-108

CCD

Solvent

Separations carried out in a 1020 cell automatic Craig distribution apparatus; 10-ml fixed lower-

phase volume. Chloroform-methanol-17% ammonia (2:1:1); 15-stroke equilibrium phase and 3 min settling period

Detection

To test fractions:

1. PC: bioautography vs. *Staphylococcus aureus* ATCC 6538P
2. TLC: ninhydrin
3. Disc diffusion assay of every 5th tube tested vs. *Staphylococcus aureus* ATCC 6538P

Distribution coefficient

Antibiotic	C *	Peak tube
Gentamicin C ₁	1.74	250
Gentamicin C _{2b}	2.39	390
Gentamicin C _{2a}	3.17	500
Gentamicin C ₂	3.65	550
Gentamicin C _{1a}	4.56	620
Polar components	9.00	780

* C = partition ratio, $\frac{r}{n-r}$, where r is the number of peak tube, and n is the number of transfers

Ref.

K.M. Byrne, A.S. Kershner, H. Maehr, J.A. Marquez and C.P. Schaffner, *J. Chromatogr.*, 131 (1977) 191-203

HPLC 1

Column

μ Bondapak C₁₈ column, 30 cm $\times$ 3.9 mm I.D. (Waters Assoc.), precolumn 4.3 cm $\times$ 4.2 mm I.D. Micropart C₁₈ phase-bonded silica gel (5 μ m) packing

Mobile phase

0.2 M Sodium sulfate, 0.02 M sodium pentane-sulfonate, and 0.1% (v/v) acetic acid in water-methanol (97:3)

Flow-rate

Column: 2 ml/min at 184 atm
o-phthalaldehyde 0.5 ml/min

Detection

Continuous-flow, post-column derivatization with o-phthalaldehyde used to form fluorescent products

Type

Ion pair

Retention time (estimated from drawing)

Gentamicin C_{1a}, 3.0
Gentamicin C₂, 4.5
Gentamicin C₁, 6.5

Ref.

J.P. Anhalt, *Antimicrob. Agents Chemother.*, 11 (1977) 651-655

HPLC 2**Apparatus**

Model M6000A, equipped with a Model U6K injector (Waters Assoc.)

Column

30 cm × 4 mm I.D. prepacked with microparticulate μ Bondapak C₁₈ (Waters Assoc.)

Mobile phase

Acetonitrile–water (95:5)

Flow-rate

1 ml/min

Temperature

Ambient

Detection

220 nm (interference filter KV 470)

Recorder

10 mV, potentiometric recorder

Chart speed

40 cm/h

Type

Reversed-phase

Retention time (min)

C_{1a} and C₂, 5.1

C₁, 5.9

Ref.

G.W. Peng, M.A.F. Gadalla, A. Peng, V. Smith and W.L. Chiou, *Clin. Chem.*, 23 (1977) 1838–1844

HPLC 3**Apparatus**

Constametric I pump and Spectromonitor I (Laboratory Data Control)

Column

25 cm μ Bondapak C₁₈ (PN-27324; Waters Assoc.)

Mobile phase

25% Acetonitrile (Photrex; J.T. Baker) 0.1 N acetate buffer, pH 6.0

Flow-rate

2.0 ml/min

Temperature

40°C

Detection

UV 270 nm (0.04 a.u.f.s.), time constant, 0.5 sec

Type

Reversed-phase

Retention time (min)

3.2; peak height, 10 mm

Ref.

J.R. Koup, B. Brodsky, A. Lau and T.R. Beam, Jr., *Antimicrob. Agents Chemother.*, 14 (1976) 439–443

GEOMYCIN**PC****Paper**

Schleicher and Schuell 2043b circular

Solvents

A. Butanol–formic acid–water (2:1:1), 4.75 h

B. As A, but (3:3:2), 5.75 h

C. Butanol–acetic acid–water (1:2:1), 7.3 h

D. Butanol–pyridine–acetic acid–water (15:10:3:12), 4.3 h

Detection

Ninhydrin

R_F

Solvent	R _F
A	Single band
B	Single band
C	Single band
D	Two bands

Ref.

H. Brockmann and H. Musso, *Chem. Ber.*, 87 (1954) 1779–1799

GLEBOMYCIN**PC****Solvents**

A. 100 ml of 80% aq. methanol + 10.5 ml piperidine (adjusted to pH 9.09–9.5 with acetic acid), ascending

B. Methanol–water–glacial acetic acid (80:15:5), ascending

C. 50% Acetone, ascending

D. Wet *n*-butanol containing 2% *p*-toluenesulfonic acid, descending

E. Wet *n*-butanol containing 2% *p*-toluenesulfonic acid and 2% piperidine, descending

F. Wet *n*-butanol containing 2% *p*-toluenesulfonic acid, 2% piperidine and 2% lauric acid, descending

R_F

Solvent	R _F	cm from origin at 24 h
A	0.74	
B	0.66	
C	0.83	
D		3.0
E		2.6
F		5.6

Ref.

B.B. Clyman, C.W. Carlson and H.W. Taylor, Jr., *U.S. Patent* 3,142,671, July 28, 1964

GLIOTOXIN**PC***Solvent*

Butanol–acetic acid–water (4:2:1), descending

R_F

0.75

Ref.

G. Nanda, A. Pal and P. Nandi, *Curr. Sci.*, 38 (1969) 518–519

GLUCONIMYCIN**PC***Paper*

Whatman No. 1

Solvents

- A. Distilled water
 - B. Ethyl acetate
 - C. Ethyl acetate–ethanol–water (5:2.5:2.5)
 - D. Chloroform
 - E. Chloroform–ethanol–water (5:2.5:2.5)
 - F. Ethyl acetate–light petroleum–water (5:2.5:2.5)
 - G. Chloroform–light petroleum–water (5:2.5:2.5)
 - H. Light petroleum–ethanol–water (5:2.5:2.5)
 - I. Light petroleum
 - J. *n*-Butanol–light petroleum–water (5:2.5:2.5)
 - K. *n*-Butanol–acetic acid–water (4:1:5)
- All solvents developed descending

R_F

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.75	G	0.89
B	0.95	H	0.96
C	0.48	I	0.00
D	0.91	J	0.94
E	0.89	K	0.96
F	0.89		

Ref.

I.R. Shimi and A. Dewedar, *Archiv für Mikrobiologie*, 54 (1966) 246–252

GOUGEROTIN**HPLC***Apparatus*

Model ALC/GPC 244 (Waters Assoc.)

Column

μBondapak C₁₈ (Waters Assoc.) 30 cm × 14 mm I.D., prepacked

Mobile phase

- A. Methanol–0.01 *M* phosphate buffer (pH 5.7) (2:8)
- B. Methanol–0.005 *M* citrate buffer (pH 6.0) (2:8)
- C. Methanol–0.005 *M* citrate buffer (pH 5.8) (5:95)
- D. 0.005 *M* citrate buffer (pH 5.8)

Flow-rate

1.0 ml/min

Pressure

1500–2000 p.s.i.

Detection

UV Model 440, 254 nm. Single peak observed under various conditions

Injector

Universal Injector U6K (Waters Assoc.), 2 ml of internal volume

Type

Reversed-phase

Retention time

Mobile phase	min
A	5.2
B	4.1
C	5.1
D	5.2

Ref.

As Amipurimycin, HPLC

GOUGEROXYMYCIN**PC***Paper*

Toyo No. 51

R_F

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.72	E	0.73
B	0.54	F	0.00
C	0.49	G	Tailing spot
D	0.76		

Solvents

- A. Methanol–water–2.5% acetic acid (3:1:0.5)
- B. Water satd. *n*-butanol
- C. Methanol
- D. 50% Acetone
- E. Phosphate buffer pH 9.0 satd. with *n*-butanol

F. Water

G. Ethanol

Ref.

E.L. Wang, N. Kanda and H. Umezawa, *J. Antibiot.*, 22 (1969) 211–214**TLC****Medium**

Silica gel (Wakogel B-O)

Solvents

A. Methanol–water–2.5% acetic acid (3:1:0.5)

B. Methanol–1 *N* ammonium hydroxide (2:1)C. Water satd. *n*-butanol–methylcellosolve (1:1)

D. Methanol–dioxane (1:1)

E. Methanol–water–2.5% acetic acid (3:1:0.5)

F. Ethanol

G. Chloroform

 R_F

Solvent	R_F	Solvent	R_F
A	0.4	E	0.55
B	0.37	F	0.00
C	0.00	G	0.00
D	0.00		

Ref.

As PC

ELPHO**Medium**

Paper

Buffer

Formic acid–acetic acid–water (25:75:900)

Conditions

3500 V/42 cm; 65 mA/20 cm; 15 min

Mobility

Remains at origin

Ref.

As PC

GRAMICIDINS**TLC (for gramicidin J)****Media**

A. Silica gel G (Merck 7731), 0.45–0.65 mm thick. Dry plates at 100–110°C before use

B. Cellulose MN 300 (Machery, Nagel Co., D-516). Dry plates at room temperature for 12 h

SolventsA. *n*-Butanol–acetic acid–water (3:1:6)B. *n*-Butanol–acetic acid–water: 2 *N* ammonia (30:10:60) and 1 ml ammonia added to upper layer

C. As A, but (20:3:20)

D. As A, but (60:15:20)

E. *n*-Propanol–water–benzene–diethylene glycol–acetic acid (75:25:5:1.5:1, v/v)F. *n*-Butanol satd. with water + 20% acetic acid and 10% pyridine

G. Toluene–acetic acid–water (10:10:1)

H. *n*-Butanol–pyridine–water (15:15:10)I. Isopropanol–1 *N* ammonia–water (20:10:10)J. Isopropanol–formamide–2 *N* ammonia–water (20:10:2:10)K. Acetone–water–acetic acid–2 *N* ammonia (15:5:1:2)**Detection**Ninhydrin. Heat plates at 80–110°C and spray with 0.3–0.5% soln. of ninhydrin in water satd. *n*-butanol R_F

	Solvent	R_F Gramicidin J
Medium A	A	0.39
	B	0.50
	C	0.46
	D	0.57
	E	0.76
	F	0.71
	G	0.32
	H	0.15
	I	0.84
	J	0.21
Medium B	C	0.91
	K	0.95

Ref.

K. Obojska, *Acta Pol. Pharm.*, 27 (1970) 285–288**ELPHO****Buffer**

Formic acid–acetic acid–water (4:15:180), pH 1.9

Conditions

15 cm × 40 cm, 600 V, 2 h

Detection

Ninhydrin

Mobility4-5- δ -Aminovaleric acid gramicidin S moves to cathode 11.8 cm (slightly faster than gramicidin S)

Ref.

I. Muramatsu, S. Sofuku and A. Hagitani, *J. Antibiot.*, 25 (1972) 189–190

CCD**Solvent**

Water-methanol-chloroform-benzene (7 : 23:15:15), 100 transfers

Distribution

Peak in tubes 46 and 47

Ref.

J.D. Gregory, L.C. Craig, *J. Biol. Chem.*, 172 (1948) 839-840

HPLC**Apparatus**

DuPont 830 liquid chromatograph

Column

Stainless steel, 25 cm × 2.1 mm I.D., prepacked with Zorbax ODS (DuPont)

Mobile phase

Methanol-0.005 M aq. ammonium sulfate (74:26)

Pressure

2000 p.s.i.

Detector

DuPont 837 Spectrophotometer UV 220 nm

Temperature

Column, 60°

Sample treatment

Samples dissolved in the mobile phase and introduced into the column by means of a six-port valve with a 10-μl loop corresponding to an injected amount of 1 μg gramicidin

Retention time

Compound	Retention time (min) *
Valine gramicidin A ₁	7.9
Valine gramicidin B ₁	12.0
Valine gramicidin C ₁	6.2
Isoleucine gramicidin A ₁	8.5
Isoleucine gramicidin B ₁	13.3
Isoleucine gramicidin C ₁	6.8

* Estimated from drawing

Ref.

K.S. Axelsen and S.H. Vogelsang, *J. Chromatogr.*, 140 (1977) 174-178

GRISEIN**PC****Paper**

Whatman No. 1

Solvents

A. *n*-Butanol-water-acetic acid (4:2:1)

B. *n*-Butanol-water-acetic acid (1:2:1)

C. Methanol-0.1 N hydrochloric acid (3:1)

D. *n*-Propanol-2.5% sodium chloride-acetic acid (10:8:1)

E. *n*-Butanol-ethanol-water-acetic acid (25 : 25:47:3)

F. Acetone-water-acetic acid (60:37:3)

Detection

Bioautography vs. *Escherichia coli* W

R_F

Solvent	R _F
A	0.14, 0.37, 0.46
B	0.83
C	0.68
D	0.78
E	0.97
F	0.75

Ref.

E.O. Stapley and R.E. Ormond, *Science*, 125 (1957) 587

**GRISEOFULVIN, 4-DEMETHYL-
(See 4-demethylgriseofulvin)****GRISEOFULVIN, 6-DEMETHYL-
(See 6-demethylgriseofulvin)****GRISEOFULVINS****PC 1****Solvents**

A. Water satd. butanol-ammonium hydroxide (20:1), vs. chloroform stationary phase

B. Butanol satd. water, vs. ethyl acetate stationary phase

C. Butanol-ethanol-water (5:1:4), vs. ethyl acetate stationary phase

D. Butanol satd. water, vs. methyl ethyl ketone stationary phase

Detection

UV fluorescence

R_F

Solvent	R _F
A	0.40
B	0.22
C	0.37
D	0.24

Ref.

E.G. McNall, *Arch. Dermatol.*, 81 (1960) 657-661

PC 2*Solvent*

Benzene-cyclohexane-methanol-water (5:5:6:4), organic phase; add 0.5% acetic acid after separation

Detection

UV light gives blue fluorescent spots

R_F

Griseofulvin, 0.90
4-Demethylgriseofulvin, 0.70
Griseofulvin acid, 0.20
6-Demethylgriseofulvin, 0.15

Ref.

B. Boothroyd, E.J. Napier and G.A. Somerfield, *Biochem. J.*, 80 (1961) 34-37

PC 3*Paper*

Whatman No. 1

Solvent

Benzene-cyclohexane-methanol-water (5:5:6:4), organic phase; add 0.5% acetic acid after separation

Detection

UV absorption

R_F

Griseofulvin, 0.9 (bright fluorescence)
6-Demethylgriseofulvin, 0.15 (dark blue fluorescence)

Ref.

M.J. Barnes and B. Boothroyd, *Biochem. J.*, 78 (1961) 41-43

TLC 1*Medium*

Silica gel HF₂₅₄ (Merck)

Solvents

A. Butyl acetate-acetone (4:1)
B. Chloroform-methanol (98:2)

Detection

5% Potassium carbonate, 0.2% potassium permanganate

R_F

	Solvent	<i>R_F</i>
(-) Dehydrogriseofulvin	A	0.47
(+) Griseofulvin		0.49
(-) Dehydrogriseofulvin	B	0.65
(+) Griseofulvin		0.68

Ref.

A. Segal and E.H. Taylor, *J. Pharm. Sci.*, 57 (1968) 874-876

TLC 2*Medium*

Silica gel

Solvent

Chloroform-diethyl ether-acetone-acetic acid (65:20:15:0.5)

Detection

The standards are visible as dark spots on the green background under 253 nm light

Ref.

P.A. Harris and S. Riegelman, *J. Pharm. Sci.*, 58 (1969) 93-96

TLC 3*Solvent*

Chloroform-acetic acid-water (4:1:1)

Detection

As TLC 2

Ref.

W.L. Chiou and S. Riegelman, *J. Pharm. Sci.*, 58 (1969) 1505-1510

TLC 4*Medium*

Cellulose Strips (Eastman MN-Polygram 300/UV)

Solvent

Hexane-ethyl acetate-methanol-water (70:30:15:6), lower phase

Detection

UV light

R_F

Component	<i>R_F</i>
Dehydro-1-thiogriseofulvin	0.78
(+)-1-Thiogriseofulvin	0.92
(+)-5'-Hydroxy-1-thiogriseofulvin	0.65

Ref.

H. Newman, P. Shu and W.W. Anders, *U.S. Patent* 3,532,714, Oct. 6, 1970; *U.S. Patent* 3,616,237, Oct. 26, 1971

TLC 5*Medium*

Kieselgel G-HR

Solvent

Chloroform-acetone (93:7)

Detection

First under long-wave UV light and then in normal light after being sprayed with 50% sulfuric acid and heated at 110°C for 30 min

Ref.

R.J. Cole, J.W. Kirksey and C.E. Holaday, *Appl. Microbiol.*, 19 (1970) 106–108

GLC 1 (Quantitative)**Apparatus**

Shimadzu Model GC-LB gas chromatograph equipped with a differential hydrogen FID system

Column

A 150 cm (75 cm × 2) × 4 mm I.D. U-shaped stainless-steel column is packed in a vertical position by tapping and is preconditioned overnight at 260°C before use. Packings are 1.5% SE-30 (methyl silicone; General Electric Co.) on acid-washed and silanized Chromosorb W, 80–100 mesh (Johns-Manville Co.), and 1.5% QF-1 (fluorinated alkyl silicone; Dow Corning Corp.) on acid-washed and silanized Anakrom, 80–100 mesh (Analabs Inc), both prepared by the soln.-coating technique

Temperature

Operating conditions are as follows: column and injection port temperature, 230°C; detector temperature, 240°C

Carrier gas

Nitrogen as carrier gas at 17.5 ml/min (2 kg/cm²) at inlet

Sample preparation

A quantity of suspension or a finely pulverized sample equivalent to 3–15 mg of giseofulvin

Calculations

A series of synthetic mixtures is prepared for injection by accurately adding 1–8 mg of pure giseofulvin to 1 ml of a soln. containing 2 mg per ml diphenylphthalate (internal standard) in acetone. At the fixed sensitivity and range of the instrument, approximately 1 µl of each mixture is injected into the chromatograph. The peak areas are determined by planimeter and/or by triangulation. By plotting the weight ratios against the peak area ratios of giseofulvin to diphenylphthalate, a straight line passing through origin was obtained for the calibration curve. Approximately 1–2 µl of soln. is injected, the ratio of the peak areas again determined, and the amount of giseofulvin is calculated by comparison with the calibration curve

Relative retention time

	1.5% Se-30	1.5% QF-1
Diphenylphthalate (internal standard)	1.00 *	1.00 **
Griseofulvin	1.84	3.25
Isogriseofulvin	2.40	5.15

* Retention time: 5.5 min

** Retention time: 5.2 min

Ref.

S. Iguchi, M. Yamamoto and T. Goromaru, *J. Chromatogr.*, 24 (1966) 182–185

GLC 2**Apparatus**

Barber Coleman series 5000 gas chromatograph equipped with a hydrogen FID and disc integrator

Column

Glass column

Supports

The liquid phases used were 1% QF-1 and 1–2% SE-30 coated onto Anakrom ABS 80 mesh (Analab Corp.) Hamden, Conn.)

Relative retention time *

	SE-30	QF-1
Griseofulvin	6.31	0.81

* Retention time relative to cholestone

Ref.

As TLC 5

GLC 3**Apparatus**

Shimadzu GC-3AE gas chromatograph equipped with electron-capture detector of pulse type

Column

Glass column (2 m × 4 mm) packed with 1.5% SE-30 on Chromosorb W (60–80 mesh)

Temperature

Column and detector, 193°C

Carrier gas

Nitrogen

Flow-rate

100 ml/min

Sensitivity

100 with a range of 0.8

Retention time

15 min, estimated from drawing

Ref.Y. Suenaga and N. Yasukawa, *Jap. J. Antibiot.*, 28 (1975) 665–668**GLC 4****Apparatus**Griseofulvin conc. in the skin was determined by GLC with use of a ^{63}Ni electron-capture detector, and diazepam as an internal standard on a 6-ft. column packed with 3% OV-17 on Gas Chrom W**Ref.**W.L. Epstein, V.P. Shah and S. Riegelman, *Arch. Dermatol.*, 106 (1972) 344–348**HPLC 1****Column**1 m $\times$ 2.1 mm I.D. $\times$ 6.3 mm O.D., 316 stainless steel, ODS-Sil-X-II packing**Mobile phase**

Methanol–0.05 M ammonium carbonate (3:7)

Pressure

1000 p.s.i.

Flow-rate

0.6 ml/min

Detector

UV (254 nm)

Sensitivity

0.16 a.u.f.s.

Type

Reversed-phase

Retention time

12.0 min, estimated from drawing

Ref.

As Ampicillin, HPLC

HPLC 2**Apparatus**

Waters Assoc. high-performance liquid chromatograph equipped with a Waters 450 variable-wavelength UV detector

Column30 cm $\times$ 4 mm, packed with μ Bondapak C₁₈ (Waters Assoc.). Samples were introduced by means of a variable-loop injector (Waters Model U6K)**Mobile phase**

45% Acetonitrile in 45 mM potassium phosphate adjusted to pH 3.0 with phosphoric acid

Flow-rate

2.5 ml/min

Detection

295 nm

Retention time

4.8 min

Ref.L.P. Hackett and L.J. Dusci, *J. Chromatogr.*, 155 (1978) 206–208**HPLC 3****Apparatus**

Model M-6000A (Waters Assoc.)

Column μ Bondapak (Waters Assoc.) 30 cm microparticulate**Mobile phase**

Distilled water–acetonitrile (1:1)

Flow-rate

2 ml/min

Injector

Model U6K (Waters Assoc.)

Temperature

Ambient

Detection

Fluorescence, 260 nm (Model FS 970, Schoeffel Instruments Corp.)

Recorder

Potentiometric 25.4 cm (Laboratory Data Control)

Chart speed

4 mm/min

Type

Reversed-phase

Retention time

3.8 min

Ref.R.L. Nation, G.W. Peng, V. Smith and W.L. Chiou, *J. Pharm. Sci.*, 67 (1978) 805–808**GRISEOLUTEINS****PC 1****Paper**

Toyo Roshi No. 51

Solvent*n*-Butanol–acetic acid–water (4:1:1)**R_F**

Griseo-lutein	R _F	Detection	
		B	C
A	0.84–0.86	Orange	Orange–pink
B	0.89–0.94	Yellow	No color change

Detection

- A. Bioautography
 B. 15% Hydrochloric acid
 C. 10% Sodium hydroxide

Ref.

K. Yagishita, *J. Antibiotics*, 13 (1960) 83-96

PC 2**Paper**

Whatman No. 1. Impregnate with aq. buffer (usually 0.25 M phosphate, pH 6) satd. with methyl isobutyl ketone

Solvent

Buffer satd. methyl isobutyl ketone, descending

Detection

Bioautography vs. *Bacillus subtilis*

R_F

Component	R _F
Griseolutein A	0.5 -0.55
Griseolutein B	0.07-0.1

Ref.

F. Tausig, F.J. Wolf and A.K. Miller, *Antimicrob. Agents Chemother.*, 1964 (1965) 59-64

GRISORIXIN**TLC****Medium**

Silica gel (Merck)

Solvent

Ethyl acetate-cyclohexane (25:75)

R_F

0.40

Ref.

P. Gachon, A. Kergomard, T. Staron and C. Esteve, *J. Antibiot.*, 28 (1975) 345-350

HALOMICIN**PC 1****Solvents**

- A. Benzene-chloroform (93:7) satd. with formamide. Chromatographic paper, prior to use, is dipped in 25% methanolic formamide, blotted and air dried for 5 min to remove methanol, 2 h
 B. Benzene-methanol (9:1)
 C. Methanol-water (80:20) containing 3% sodium chloride. The paper is buffered with a soln. of

0.95 M sodium sulfate + 0.05 M sodium bisulfate and dried prior to developing, 4-6 h

D. Propanol-acetic acid-water (50:5:40), 18 h

E. Butanol-acetic acid-water (4:1:5), 18 h

F. Propanol-pyridine-acetic acid-water (15:10:3:12), 18 h

G. Phenol-water (80 g:20 ml), 18 h

System A developed descending; B-G, ascending

Detection

Bioautography vs. *Staphylococcus aureus* ATCC 6538P

R_F

Solvent	R _F
A	Halomicin A, 0.0, B, 0.35, C, 0.60, D, 0.75
B	1.0
C	0.78-1.0
D	0.95
E	1.0
F	1.0
G	1.0

Ref.

G.M. Luedemann and M.J. Weinstein, *U.S. Patent* 3,511,909, May 12, 1970; M.J. Weinstein, G.M. Luedemann, E.M. Oden and G.H. Wagman, *Antimicrob. Agents Chemother.*, 1967 (1968) 435-441

PC 2**Paper**

Toyo No. 51

Solvents

- A. 20% Ammonium chloride
 B. Water satd. *n*-butanol
 C. *n*-Butanol-acetic acid-water (3:1:1)
 D. Water satd. ethyl acetate
 E. Water satd. *n*-butanol + 2% *p*-toluenesulfonic acid + 2% piperidine

Detection

Bioautography vs. *Bacillus subtilis* No. 10707

R_F

Solvent	R _F	Solvent	R _F
A	0.45	D	0.30
B	0.85	E	0.56
C	0.75		

Ref.

As Carbomycin, PC 5

HAMYCIN

PC 1

Paper

Whatman No. 1

Solvents

- A. Water satd. *n*-butanol, descending, 16 h
- B. *n*-Butanol-pyridine-water (1:0.6:1), ascending, 16 h
- C. 50% Aq. acetone, ascending, 10 h
- D. Methanol-water-ammonium hydroxide (20:4:1), descending, 5.5 h
- E. 60% Aq. isopropanol, ascending, 16 h

Detection

Bioautography vs. *Saccharomyces cerevisiae*

R_F

Solvent	R_F	Solvent	R_F
A	0.26	D	0.67; 0.44
B	0.75	E	0.99
C	0.77		

Ref.

P.V. Divekar, V.C. Vora and A.W. Khan, *J. Antibiot.*, 19 (1966) 63-64

PC 2

Solvents

- A. Butanol-acetic acid-water (20:1:25)
- B. *n*-Propanol-water (80:20)
- C. Pyridine-ethyl acetate-water (2.5:6:7)
- D. Pyridine-ethyl acetate-acetic acid-water (5:5:1:3)
- E. Butanol-methanol-water (1:1:1.5)
- F. Butanol-methanol-water (4:1:2)

R_F

Solvent	R_F	Solvent	R_F
A	0.59	D	0.69
B	0.56	E	0.95
C	0.17	F	0.31; 0.64

Ref.

G.R. Deshpande and N. Narasimhachari, *Hind. Antibiot. Bull.*, 9 (1969) 76-83

PC 3

Solvents

- A. Water satd. butanol *
- B. Butan-1-ol-pyridine-water (1:0.6:1) *
- C. Acetone-water (1:1) *
- D. Methanol-ammonia-water (20:1:4)
- E. Ethyl acetate-pyridine-water (12:5:14) *
- F. Butan-1-ol-acetic acid-water (20:1:25) *

G. Propan-1-ol-water (4:1) *

H. Propan-2-ol-water (3:1) *

I. Ethyl acetate-pyridine-water (12:5:14)

J. Butan-1-ol-methanol-water (2:2:3) *

K. Butan-1-ol-methanol-water (4:1:2) *

L. Butan-1-ol-pyridine-water (1:1:1) *

M. Ethyl acetate-pyridine-25% acetic acid (5:5:4) *

* Whatman No. 1 paper specified

R_F

Solvent	R_F	Solvent	R_F
A	0.26	H	0.87-0.88 *
B	0.75	I	0.17
C	0.77	J	0.95
D	0.44, 0.67	K	0.31, 0.64
E	0.08-0.22 *	L	0.26, 0.61
F	0.22-0.43 *, 0.59	M	0.69
G	0.38, 0.56		

* R_F varied with purity of sample

Ref.

As Amphotericins, TLC 2

TLC

Medium

Silica gel G

Solvents

- A. Butan-1-ol-ammonia-methanol-water (20:1:2:4)
- B. Chloroform-methanol-borate buffer (pH 8.3) (7:5:1)

Detection

0.2% *p*-Dimethylaminobenzaldehyde in sulfuric acid containing ferric chloride

R_F

Solvent A, 0.13
Solvent B, 0.49, 0.67

Ref.

As Amphotericins, TLC 2

CCD

Solvents

- A. Chloroform-methanol-borate buffer (pH 8.2-8.4)
- B. Chloroform-methanol-sodium acetate (pH 8.4) (2:2:1)

Distribution coefficient

200 transfers
Solvent A, $K = 0.61$
Solvent B, $K = 1.10$

Ref.

As Amphotericins, TLC 2

HELVOLIC ACID**HPLC***Column*

1 m × 2.1 mm I.D. × 6.3 mm O.D., 316 stainless steel. ODS-Sil-X-II packing

Mobile phase

Methanol–0.05 M ammonium carbonate (3:7)

Pressure

1000 p.s.i.

Flow-rate

0.06 ml/min

Detector

UV (254 nm)

Sensitivity

0.16 a.u.f.s.

Type

Reversed-phase

Retention time

16.0 min (estimated from drawing)

Ref.

As Ampicillin, HPLC

HEPTAFUNGIN**TLC***Medium*

Silica gel G

Solvents

- A. Butan-1-ol–ammonia–methanol–water (20:1:2:4)
- B. Chloroform–methanol–borate buffer (pH 8.3) (7:5:1)
- C. Butan-1-ol–ethanol–acetone–32% ammonia (2:5:1:3)

Detection

- A. 0.2% *p*-Dimethylaminobenzaldehyde in sulfuric acid containing ferric chloride
- B. Spectrodensitometric

R_F

Solvent	Detection	<i>R_F</i>
A	A	0.10
B	A	0.55
C	B	0.42

Ref.

As Amphotericins, TLC 2

HEPTAFUNGIN A**CCD***Solvent*

Chloroform–methanol–water (2:2:1)

Distribution coefficient

300 transfers, *K* = 0.77

Ref.

As Amphotericins, TLC 2

HERQUEICHRYSIN**TLC***Medium*

Polyamide

Solvent

Acetone–acetic acid (5:1)

Detection

- A. Visible yellow color
- B. Ethanolic ferric chloride (gives deep brown color with desmethyl herqueichrysin)

R_F

Herqueichrysin, 0.73
Desmethylherqueichrysin, 0.67

Ref.

N. Narasimhachari and L.V. Vining, *J. Antibiot.*, 25 (1972) 155–162

**HERQUEINONE, DEOXY-
(See atroventin)****HETICILLIN****TLC***Medium*

Silica gel G

Solvent

Acetone–distilled water–benzene–acetic acid (650:100:100:25) (milliliters)

Detection

Ninhydrin–ethanol spray (300 mg:100 ml)

R_F

Allow the solvent front to travel about 11.5 cm from the bottom of the plate. The sample and standard should have spots of corresponding *R_F* values

Ref.

Fed. Regist., 37 (Nov. 15, 1972) 24175

**6-[(HEXAHYDRO-1H-AZEPIN-1-YL)-METHYLENEAMINO]-PENICILLANIC ACID
(cf., Penicillins)****PC***Paper*

Whatman No. 1

Solvent

n-Butanol–ethanol–water (4:1:5)

R_F
0.5

Ref.

F.J. Lund, *U.S. Patent* 3,957,764, May 18, 1976

HIKIZIMYCIN

PC

Paper

Toyo Roshi No. 51

Solvents

- A. 3% Ammonium chloride
 - B. 50% Acetone
 - C. 80% Phenol
 - D. Wet butanol
 - E. Butanol-methanol-water (40:20:20) + 1.5 g methyl orange
 - F. Butanol-acetic acid-water (1:1:1)
 - G. Propanol-pyridine-acetic acid-water (15:10:3:12)
- All systems developed ascending

Detection

Bioautography vs. *Pseudomonas fluorescens*

R_F

Solvent	R_F	Solvent	R_F
A	1.0	E	0.25
B	0.10	F	0.40
C	0.08	G	0.35
D	0.00		

Ref.

K. Uchida, T. Ichikawa, Y. Shimauchi, T. Ishikura and A. Ozaki, *J. Antibiot.*, 24 (1971) 259-262

ELPHO

Medium

Toyo Roshi No. 51

Buffer

$\frac{1}{30}$ M Sorensen phosphate buffer, pH 5.0

Conditions

300 V/35 cm, 2.0-2.5 mA/5 cm, 1.5 h, 15°C

Mobility

4.1 cm to the cathode

Ref.

As PC

HISTIDOMYCIN

ELPHO

Medium

Schleicher and Schuell No. 598 paper

Buffer

0.25 M Sodium phosphate buffer, pH 7

Conditions

600 V for 6 h

Mobility

Histidomycin A migrates as anion, 7.5 cm

Histidomycin B does not migrate

Ref.

E.A. Kaczka, T.W. Miller, F. Tausig and F.J. Wolf, *Antimicrob. Agents Chemother.*, 1966 (1967) 603-605

T.C. Demny, *U.S. Patent* 3,657,418, April 18, 1972

HODYDAMYCIN

PC

R_F

Solvent	R_F	Solvent	R_F
A	1.00	K	0.60
B	0.92	L	1.00
C	0.40	M	1.00
D	1.00	N	0.60
E	1.00	O	0.55
F	0.00	P	1.00
G	0.00	Q	1.00
H	0.00	R	1.00
I	0.78	S	0.00
J	0.45	T	0.00

Solvents

- A. Ethyl acetate
- B. Ethyl acetate-water (1:1)
- C. Ethyl acetate-light petroleum-water (5:2.5:5)
- D. Ethyl acetate-ammonia (1:1)
- E. Ethyl acetate-acetic acid (1:1)
- F. 3% Ammonium chloride
- G. Distilled water
- H. 0.5% Disodium phosphate
- I. *n*-Butanol
- J. *n*-Butanol-light petroleum-water (5:2.5:5)
- K. *n*-Butanol-ethanol-water (5:2.5:5)
- L. *n*-Butanol-acetic acid-water (4:1:5)
- M. Chloroform
- N. Chloroform-ethanol-water (5:2.5:5)
- O. Chloroform-light petroleum-water (5:2.5:5)
- P. Chloroform-water (1:1)
- Q. Chloroform-ammonia (1:1)

R. Chloroform-acetic acid (1:1)

S. Light petroleum

T. 0.5% Sodium carbonate

Ref.

I.R. Shimi, A. Dewedar and S. Shoukry, *J. Antibiot.*, 23 (1970) 388-393

HOLOMYCIN

PC

Solvents

A. Stationary phase, formamide; mobile phase, benzene

B. Stationary phase, sodium *m*-cresotinate; mobile phase, a 3:1 mixture of *n*-butyl acetate and di-*n*-butyl ether

R_F

	R_{th}^*	
	Solvent A	Solvent B
Holomycin	0.10	0.78
Propionyl holothin	0.28	1.50
Butyryl holothin	0.52	1.87

* R_{th} = distance relative to thiolutin (= 1.00)

Ref.

E. Gaeumann, V. Prelog and E. Vischer, *U.S. Patent* 3,014,922, Dec. 26, 1961

TLC

Medium

Silica gel 60 F₂₅₄ (Merck)

Solvent

Benzene-acetone (1:1)

R_F

n-Propionylholothin, 0.31 (yellow spot)

Holomycin, 0.25 (yellow spot)

Ref.

K. Okamura, K. Soga, Y. Shimauchi, T. Ishikura and J. Lein, *J. Antibiot.*, 30 (1977) 334-336

HON (δ -HYDROXY- γ -OXO-L-NOR-VALINE)

PC

Solvent

n-Butanol-acetic acid-water (4:2:1), ascending

Detection

Ninhydrin reagent

R_F

0.22

Ref.

S. Tasuoka, A. Miyake, H. Hitomi, J. Ueyanagi, H. Iwasaki, T. Yamaguchi, K. Kanazawa, T. Ataki, K. Tsuchiya, F. Hiraiwa, K. Nakazawa and M. Shibata, *J. Antibiot.*, 14 (1961) 39-43

HONDAMYCIN

PC 1

Solvents

A. Benzene-water-acetic acid (1:1:0.2)

B. Ethanol-*n*-hexane (1:2)

C. Water-acetone (75:25)

D. 16% Aq. soln. *n*-propanol

All systems developed ascending

R_F

Solvent	R_F	Solvent	R_F
A	0.62	C	0.41
B	0.65	D	0.19

Ref.

Y. Sakagami, A. Ueda, S. Yamabayashi, Y. Tsurumaki and S. Kumon, *J. Antibiot.*, 22 (1969) 521-527

PC 2

Solvents

A. 16% Hydrated *n*-propanol

B. 18% Hydrated *n*-propanol

R_F

Solvent A, 0.19

Solvent B, 0.27

Ref.

I. Takeda, M. Mizuno, T. Sugawara, Y. Shimojima and S. Horiguchi, *U.S. Patent* 3,780,172, Dec. 18, 1973

TLC

Medium

Silica gel

Solvents

A. Ethanol-water (4:1)

B. Ethyl acetate-methanol (100:15)

C. Benzene-ethyl acetate (1:1)

R_F

Solvent A, 0.75

Solvent B, 0.82

Solvent C, 0.42

Ref.

As PC 1

CCD**Solvent**

Methanol–water–chloroform–carbon tetrachloride (3:1:1.5:4)

Distribution

$K = 0.35$

Ref.

As PC 1

HORDATINES**TLC****Medium**

Microcrystalline cellulose

Solvent

n-Butanol–acetic acid–water (4:1:5), upper phase

Detection

- A. Diazotized nitraniline
- B. Bromocresol green
- C. Sakaguchi reagent

 R_F

Hordatine A, 0.54
Hordatine B, 0.53

Ref.

A. Stoessl, *U.S. Patent* 3,475,459, Oct. 28, 1969

HORDECIN**PC****Paper**

Whatman No. 3MM. Impregnate paper with 20% formic acid for 24 h; evaporate formic acid and wash paper with doubly distilled water

Solvent

n-Butanol–acetone–water (5:1:2)

Detection

UV light and yellow fluorescence

 R_F

0.93

Ref.

I.S. Ezhov and N.V. Novotel'nov, *Prikladnaya Biokhimiya i Mikrobiologiya*, 3 (1967) 178–183

HYBRIMYCINS**PC****Solvent**

1-Butanol–pyridine–water (6:4:3)

Ref.

W.T. Shier, K.L. Rinehart, Jr. and D. Gottlieb, *U.S. Patent* 3,833,556, Sept. 3, 1974

 R_F

Component	R_F	Component	R_F
A1	0.32	B2	0.19
A2	0.19	C1 *	0.36
B1	0.37	C2 *	0.24

* Penta N-acetylated

1-N[(*S*)- α -HYDROXY- ω -AMINO-ACYL]-3',4'-DIDEOXYNEAMINE**PC****Solvent**

n-Butanol–pyridine–acetic acid–water (6:4:1:3)

 R_F

$R_{3',4'}$ -dideoxyneamine, 0.47

$R_{3',4'}$ -dideoxyneamine designates the ratio of migration of the spot of the 1-N-[(*S*)- α -hydroxy- γ -amino-*n*-butyryl]-3',4'-dideoxyneamine to that of 3',4'-dideoxyneamine

Ref.

H. Umezawa, S. Umezawa, E. Akita, Y. Horiuchi, S. Yasuda and O. Tsuchiya, *U.S. Patent* 3,948,882, April 6, 1976

L-threo- β -HYDROXYASPARTIC ACID**PC****Solvents**

- A. Butanol–acetic acid–water (2:2:1)
- B. Butanol–acetic acid–water (2:1:1)
- C. Butanol–ethanol–water (5:2:3)

 R_F

Solvent A 0.41
Solvent B 0.24
Solvent C 0.24

Ref.

T. Ishiyama, T. Furuta, M. Takai, Y. Okimoto, S. Aizawa, A. Shimazu and H. Yonehara, *J. Antibiot.*, 28 (1975) 821–823

ELPHO**Medium****Paper****Buffer**

Formic acid–acetic acid–water (25:75:900, v/v)

Conditions

3500 V, 20 min

Mobility

Mobility of the *threo*-isomer, 2.9 cm, but different from that of the *erythro*-isomer, 4.1 cm

Ref.

As PC

2-HYDROXY-5-IMINOAZACYCLO-PENT-3-ENE

TLC

Medium

Avicel SF (Funakoshi, lot No. 008390)

Solvent

1-propanol–0.001 *N* hydrochloric acid (8:2)

R_F

0.52

Ref.

A. Okuyama, S. Kondo, T. Ikeda, K. Miura, M. Hamada and H. Umezawa, *J. Antibiot.*, 32 (1979) 768–770

ELPHO

Medium

Paper

Buffer

Formic acid–acetic acid–water (1:3:36)

Conditions

3000 V, 20 min

Mobility

R_M (relative mobility to alanine), 1.60

Ref.

As TLC

HYDROXYMYCIN

PC

Paper

Whatman No. 1

Solvents

- A. *n*-Butanol–acetic acid–water (2:1:2)
- B. *n*-Butanol–water–*p*-toluenesulfonic acid (15:15:0.7)
- C. *n*-Butanol–piperidine–*p*-toluenesulfonic acid (98:2:2)
- D. Methanol–soln. of 2% sodium chloride (2:1), with paper buffered at pH 2.4 with sodium bisulfate

Detection

- A. Ninhydrin
- B. Bioautography vs. *Bacillus subtilis*

R_F

Solvent	R_F	Solvent	R_F
A	0.01	D	0.01
B	0.07	D	0.46

Ref.

G. Hagemann, G. Nominé and L. Pénasse, *Ann. Pharm. Franc.*, 16 (1958) 585–596

ELPHO

Medium

Paper

Buffer

0.1 *M* phosphate buffer, pH 4.6

Conditions

240 V, 120 mA (11.4×10^{-5} cm/sec/V/cm)

Detection

Bioautography vs. *Bacillus subtilis*

Mobility

Homogeneous zone

Ref.

As PC

CCD

Solvent

Acetone–water–chloroform (3:1:1.8), 25 transfers

Distribution

Peak is in tube 22

Ref.

As PC

δ -HYDROXY- γ -OXO-L-NORVALINE [See HON (δ -hydroxy- γ -oxo-L-norvaline)]

HYDROXYPEPSTATIN

TLC

Medium

Silica gel

Solvent

Chloroform–methanol–acetic acid (86:12:2)

Detection

Detected by Rydon-Smith reagent

R_F

Hydroxyepstatin A, 0.20
Pepstatin A, 0.35

Ref.

H. Umezawa, T. Miyano, T. Murakami, T. Takita, T. Aoyagi, T. Takeuchi, H. Naganawa and H. Morishima, *J. Antibiot.*, 26 (1973) 615–617

HYDROXYSTREPTOMYCIN (cf., streptomycins)

PC

Paper

Whatman No. 4

Solvent

Wet butanol containing 2% *p*-toluenesulfonic acid and 2% piperidine

Detection

Bioautography vs. *Staphylococcus aureus*

Ref.

F.H. Stodola, O.L. Shotwell, A.M. Borud, R.G. Benedict and A.C. Riley, Jr., *J. Amer. Chem. Soc.*, 73 (1951) 2290

HYGROMYCIN**ELPHO***Medium*

Whatman No. 4 filter paper

Buffer

- A. 2 *M* Formic acid–0.1 *M* *p*-toluenesulfonic acid–*n*-propanol (500:50:50) in 1000 ml water, pH 1.8
- B. 2 *M* Formic acid–0.1 *M* *p*-toluenesulfonic acid–*n*-propanol (400:50:50) in 1000 ml water, pH 1.9
- C. 2 *M* Formic acid–0.1 *M* *p*-toluenesulfonic acid (400:50) in 1000 ml water, pH 1.9
- D. 1 *M* Ammonium hydroxide–1 *M* sodium hydroxide–0.1 *M* *p*-toluenesulfonic acid–*n*-propanol (200:5:100:100) in 1000 ml water, pH 10.8
- E. 1 *M* Ammonium hydroxide–1 *M* sodium hydroxide (200:2.5) in 1000 ml water, pH 11.5
- F. 50 ml 1 *M* Sodium hydroxide in 1000 ml water, pH 12.2

Conditions

The electrophoretograms were developed at 350 V for 1–4 h (depending on the electrolyte)

Mobility

Buffer	Toward cathode (cm ² /V sec)
A	27.44
B	22.16
C	27.55
D	5.51
E	5.73
F	6.83

Ref.

As Bluensomycin, ELPHO

CCD*Solvents*

- A. Equilibrate *n*-butanol–water–glacial acetic acid (1:1:0.4)
- B. *n*-Amyl alcohol–water–glacial acetic acid (1:1:0.4)

Distribution

Solvent A, *K* = 1.0

Solvent B, *K* = 0.3

Ref.

R.L. Mann, R.M. Gale and F.R. van Abeele, *Antibiot. Ann.*, (1953–1954) 167–170

HYGROMYCIN B**PC***Paper*

Ascending

Solvent

Chloroform–methanol–17% aq. ammonia (2:1:1) lower layer (developed at room temp. for 12 h)

R_F

0.02

Ref.

As Apramycin, PC 1

HYGROSTATIN**PC***Solvent*

Water satd. butanol

Detection

Bioautography vs. *Candida albicans*

R_F

0.41

Ref.

M. Arai, *J. Antibiot.*, 13 (1960) 51

ELPHO*Buffer*

$\frac{1}{150}$ *M* Acetate buffer, pH 4.1

Conditions

500 V/34 cm, 0.2 mA/cm, 10 h

Mobility

Migrates toward cathode

Ref.

As PC

IKUTAMYCIN**PC***Paper*

Toyo No. 51

Solvents

- A. 40% Aq. methanol
- B. 40% Aq. ethanol
- C. 20% Aq. *n*-propanol
- D. Water satd. butanol
- E. 3% Ammonium chloride
- F. Butanol–methanol–water (4:1:2)
- G. 55% Aq. methanol

DetectionBioautography vs. *Piricularia oryzae* R_F

Solvent	R_F	Solvent	R_F
A	0.62	E	0.00
B	1.00	F	0.91
C	0.35	G	1.00
D	0.37		

Ref.

Y. Sakagami, A. Ueda and S. Yamabayashi, *J. Antibiot.*, 20 (1967) 299–307**TLC**

Medium

Silica gel

Solvents

- A. Ethyl acetate
- B. *n*-Hexane–ethyl acetate (1:1)
- C. *n*-Hexane–ethyl acetate (3:7)
- D. Chloroform–methanol (15:1)
- E. Methanol–tetrahydrofuran (10:1)
- F. Benzene–ethanol (1:1)
- G. Benzene–acetone (1:1)
- H. Benzene–methanol (1:1)
- I. Acetone–ethanol (9:1)

Detection

As PC

 R_F

Solvent	R_F	Solvent	R_F
A	0.31	F	0.53
B	0.00	G	0.30
C	0.13	H	0.79
D	0.00	I	0.79
E	0.77		

Ref.

As PC

ILEUMYCIN

PC

Solvents

- A. *n*-Butanol–water (87:13)
- B. *n*-Butanol–water–ethyl acetate (87:13:30)
- C. *n*-Butanol–acetone–water (8:2:1)
- D. *n*-Propanol–ethyl acetate–water (7:1:1)
- E. *sec*-Butanol–water–ethyl acetate (87:13:30)

Detection

Ninhydrin

 R_F

Solvent	R_F	Solvent	R_F
A	0.47	D	0.45
B	0.46	E	0.31
C	0.41		

Ref.

Y. Kawakami, S. Matsuoka, T. Otani, H. Kondo and S. Nakamura, *J. Antibiot.*, 31 (1978) 112–116**TLC**

Medium

Silica gel G

Solvents

- A. *n*-Butanol–water (87:13)
- B. *n*-Butanol–methanol–water (4:1:1)
- C. *n*-Propanol–ethyl acetate–water (7:1:2)
- D. *n*-Butanol–acetone–water (4:1:1)
- E. *n*-Butanol–acetone–water (1:1:1)

Detection

- A. Bioautography
- B. Ninhydrin
- C. Heat at 100°C for 20 min after spraying with 40% sulfuric acid

 R_F

Solvent	R_F	Solvent	R_F
A	0.14	D	0.34
B	0.36	E	0.67
C	0.68		

Ref.

As PC

ILICICOLINS

TLC

Medium

Silica gel

 R_F

	Solvent A	Solvent B
Ilicicolin A	0.60	0.67
Ilicicolin B	0.44	0.50
Ilicicolin C	0.37	0.44
Ilicicolin D	0.31	0.43
Ilicicolin E	0.31	0.39
Ilicicolin F	0.23	0.27
Ilicicolin G	0.35	0.27
Ilicicolin H	0.09	0.055

Solvents

- A. *n*-Hexane-acetone (3:1)
 B. Benzene-ethyl acetate (7:1)

Ref.

S. Hayakawa, H. Minato, K. Katagiri, *J. Antibiot.*,
 24 (1971) 653-654

INGRAMYCIN**PC****Solvents**

- A. 1-Butanol-water (84:16), 16 h
 B. 1-Butanol-water (84:16), +0.25% *p*-toluene-sulfonic acid, 16 h
 C. 1-Butanol-acetic acid-water (2:1:1), 16 h
 D. 2% Piperidine (v/v) in 1-butanol-water (84:16), 16 h
 E. 1-Butanol-water (4:96), 5 h
 F. 1-Butanol-water (4:96) + 0.25% *p*-toluene-sulfonic acid, 5 h
 G. Phosphate buffer 0.1 M $\text{KH}_2\text{PO}_4 \cdot 3 \text{H}_2\text{O}$, pH 7.0
 H. 0.075 N NH_4OH satd. with methylisobutyl ketone
 I. Benzene-methanol-water (1:1:2)
 J. 1-Butanol satd. with water + 2.0% *p*-toluene-sulfonic acid
 K. Methanol-15% NaCl (4:1) (strips impregnated beforehand with 0.1 N Na_2SO_4 and dried)

Detection

Bioautography vs. *P. oxalicum*

 R_F

Solvent	R_F^*	Solvent	R_F^*
A	0.90 (trace)	G	0.60
B	0.90	H	0.70
C	0.92	I	0.68
D	0.90	J	0.90
E	0.80	K	0.95
F	0.85		

* Estimated from drawing

Ref.

M.E. Bergy, H. Hoeksema, L.E. Johnson and D.G. Kinch, *U.S. Patent* 3,651,219, March 21, 1972

IONOMYCIN**TLC****Medium**

Silica gel

Solvent

4% Methanol in chloroform

Detection

- A. Bioautography vs. *Staphylococcus aureus* 209P
 B. UV at 254 nm

 R_F

0.35

Ref.

E. Meyers, D.S. Slusarchyk and W. Liu, *U.S. Patent* 3,873,693, March 25, 1975

ISO-LASALOCID A**CCD****Solvent**

Heptane-ethyl acetate-methanol-water (27:18:18:2) (160 ml)

Distribution coefficient

200 Tube distribution apparatus

Solution placed in the first two tubes. After 380 transfers, like tubes were pooled and the solids recovered after the evaporation contained:

- A. Mixture of lasalocid homologs, B, C, D and E
 B. Lasalocid A
 C. Crude Iso-lasalocid A

Fraction C, which was in crude form and contained sodium and potassium as salts, was dissolved in methylene chloride and washed with 0.1 N hydrochloric acid to convert it to the purified Iso-lasalocid A free acid

Ref.

J. Westley, *U.S. Patent* 3,944,573, March 16, 1976

ITURINE**PC****Solvents**

- A. Pyridine-butanol-water (2:3:1.5)
 B. *n*-Butanol-acetic acid-water (4:5:1)

 R_F

Useful for detection of fraction B and impurities

Ref.

L. Delcambe, *Bull. Soc. Chim. Belges*, 73 (1965) 315-328

ELPHO**Medium**

Paper

Buffers

- A. 0.1 N Collidine-0.1 N acetic acid (3:1.85), pH 7.0
 B. 0.1 N Pyridine-0.1 N acetic acid (1:1), pH 5.2
 C. Acetic acid-water (1:10), pH 2.3

Conditions

500 V, 12 mA, 2.5 h

Detection

A. Pauly reagent

B. Bioautography vs. *Penicillium notatum***Mobility**

Purified fraction B is immobile; impurities move to positive pole

Ref.

As PC

JANIEMYCIN**PC****Paper**

Whatman No. 1

SolventsA. *n*-Butanol-acetic acid-water (4:3:7)B. *n*-Butanol-pyridine-water (4:3:7) **R_F**

Solvent A, 0.13

Solvent B, 0.85

Ref.E. Meyers, F.L. Weisenborn, F.E. Pansy, D.S. Slusarchyk, M.H. von Saltza, M.L. Rathnum and W.L. Parker, *J. Antibiot.*, 23 (1970) 502-507**ELPHO****Buffer**

pH 3.3 in the presence of 40% formamide

Detection

Safranin O, cathodic indicator; apalon, electro-osmotic indicator

Mobilities

+85 (major); +44 (minor); +12 (minor)

Ref.

As PC

JOLIPEPTIN**PC****Solvent***n*-Butanol-acetic acid-water (4:1:2) **R_F**

0.65-0.70

Ref.M. Ito and Y. Koyama, *U.S. Patent* 3,883,649, May 13, 1975**JOSAMYCIN****PC 1****Paper**

Toyo No. 50

Solvents

A. Benzene-chloroform (1:1)

B. Benzene-ethyl acetate satd. with water (9:1)

C. Benzene-acetone (95:5)

D. Carbon tetrachloride-chloroform (1:1)

 R_F

Solvent	R_F
A	0.49
B	0.68
C	0.65
D	0.76

Ref.T. Osono, Y. Oka, S. Watanabe, Y. Numazaki, K. Moriyama, H. Ishida and K. Suzuki, *J. Antibiot.*, 20 (1967) 174-180**PC 2****Solvent**

Benzene-chloroform (1:1)

 R_F

0.49

Ref.H. Umezawa and T. Osono, *U.S. Patent* 3,636,197, Jan. 18, 1972**TLC 1****Media**

A. Alumina (Merck)

B. Silica gel (Merck)

Solvents

A. Ethyl acetate

B. *n*-Butanol-acetic acid-water (3:1:1) **R_F**

Solvent A, 0.67

Solvent B, 0.64

Ref.

As PC 1

TLC 2**Medium**

Kieselguhr G, 0.5 mm

Solvent

Benzene-acetone (2:1)

Detection

Coloration: 20% sulfuric acid

 R_F

0.69

Ref.S. Omura, Y. Hironaka and T. Hata, *J. Antibiot.*, 23 (1970) 511-513

TLC 3**Media**

- A. Silica gel
B. Alumina

Solvents

- A. *n*-Butanol-acetic acid-water (3:1:1)
B. Ethyl acetate

 R_F

- Solvent A, 0.64
Solvent B, 0.66

Ref.

As PC 2

TLC 4**Medium**

Alumina B-10 (Wako Pure Chem. Corp.)

Solvent

Butyl acetate-methyl ethyl ketone-water (80:18:2)

 R_F

- Josamycin, 0.46
Monoacetyljosamycin-1, 0.50
Monoacetyljosamycin-2, 0.58
Diacetyljosamycin, 0.64

Ref.

T. Osono, K. Moriyama, K. Murakami and H. Umezawa, *U.S. Patent* 3,959,252, May 25, 1976

JULYMYCINS**TLC****Medium**

Silica gel (Merck). Metal-free silica gel prepared as follows: the silica gel (Merck; less than 0.08 mm for chromatography) was washed with 6 *N* hydrochloric acid being warmed at 90–100°C to remove contaminating metal ions which interfere with the chromatography in forming metal chelate compounds with B-II. After usual waterwashing to remove the absorbed hydrochloric acid, the gel was washed repeated with boiling water until the chlor ion test became negative. The washed gel was then dried at 110°C before use. Thin-layer plate: 10% of gypsum was mixed with the metal free silica gel and used to prepare plates by the usual method

Solvent

- A. Chloroform-methanol (9:1)
B. Benzene-ethyl formate-formic acid (3:2:1)

Ref.

J. Shoji, Y. Kimura and K. Katagiri, *J. Antibiot.*, 17 (1964) 156–160

 R_F

Solvent	Jullymycin					
	B-0	B-I	B-II	S.V.	B-III	B-IV
A	0.85	0.65	0.45	0.35	0.25	0–0.1
B	–	0.50	0.40	0.40	0.45	–

JUVENIMICINS**PC 1****Paper**

Toyo Roshi No. 51

Solvents

- A. Aq. 0.05 *N* ammonium hydroxide satd. with methyl ethyl ketone
B. Methyl isobutyl ketone-methyl ethyl ketone (4:1)
C. Methyl isobutyl ketone
D. $\frac{1}{15}$ *M* Phosphate buffer, pH 8.0, satd. with *n*-butyl acetate. Paper impregnated with 2% liquid paraffin
E. Benzene-cyclohexane (1:1) satd. with formamide. Paper impregnated with formamide-methanol (1:1)
F. Aq. 0.05 *N* ammonium hydroxide satd. with *n*-butyl ethyl ketone

 R_F

Solvent	R_F	Solvent	R_F
A	0.74	D	0.60
B	0.68	E	0.02
C	0.57	F	0.70

Ref.

German Patent 2,034,245, Feb. 25, 1971

PC 2**Paper**

Toyo No. 51

Solvent

Water satd. ethyl acetate (ascending, 3 h)

Detection

Bioautography vs. *Bacillus subtilis* No. 10707

 R_F

0.00, 0.93

Ref.

As Carbomycin, PC 5

PC 3**Paper**

Toyo No. 51

Solvents

- A. 20% Ammonium chloride
 B. Water satd. *n*-butanol
 C. *n*-Butanol–acetic acid–water (3:1:1)
 D. Water satd. ethyl acetate
 E. Water satd. *n*-butanol + 2% *p*-toluenesulfonic acid + 2% piperidine

Detection

Bioautography vs. *Bacillus subtilis* No. 10707

R_F

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.92	D	0.00, 0.93
B	0.92	E	0.90
C	0.85		

Ref.

As Carbomycin, PC 5

TLC 1**Media**

- A. Spotfilm F
 B. Silica gel

Solvent

Chloroform–methanol–7% aq. ammonia (40:12:20)

R_F

	Medium A	Medium B
Juvenimicin		
A group A ₁	0.85	–
A group A ₂	0.80	–
A group A ₃	0.70	0.70
A group A ₄	0.65	
Juvenimicin		
B group B ₁	0.50	–
B group B ₂	0.40	–
B group B ₃	0.33	–
B group B ₄	0.25	–

Ref.

As PC 1

TLC 2**Medium**

Aluminum oxide

Solvent

Ether

R_F

Juvenimicin A₁, 0.65
 Juvenimicin A₂, 0.80
 Juvenimicin A₃, 0.80
 Juvenimicin A₄, 0.29

Ref.

As PC 1

KALAFUNGIN**PC****Paper**

Whatman No. 1

Solvents

- A. 1-Butanol–water (84:16), 16 h
 B. 1-Butanol–water (84:16) + 0.25% *p*-toluenesulfonic acid, 16 h
 C. 1-Butanol–acetic acid–water (2:1:1), 16 h
 D. 1-Butanol–water (84:16) + 2% piperidine, 16 h
 E. 1-Butanol–water (4:96), 5 h
 F. 1-Butanol–water (4:96) + 0.25% *p*-toluenesulfonic acid, 5 h

Detection

Bioautography vs. *Saccharomyces pastorianus* subsp. *arbignensis* ATCC 2366

R_F

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.80	D	0.70
B	0.80	E	0.60
C	0.85	F	0.60

Ref.

L.E. Johnson and A. Dietz, *Appl. Microbiol.*, 16 (1968) 1815–1821

TLC**Medium**

Silica gel HF₂₅₄ (E. Merck), prepared by suspending 0.2 *M* disodium phosphate–0.2 *M* monopotassium phosphate (1:1), pH 6.7. The plates were activated at 120–130°C for 2 h prior to use

Solvent

Ethyl acetate–cyclohexane (1:1)

Detection

- A. UV light (254 nm)
 B. Potassium permanganate–sodium metaperiodate spray

R_F

0.4

Ref.

M.E. Bergy, *J. Antibiot.*, 21 (1968) 454–457

**KANAMYCIN, 3'-DEOXY-
(See 3'-deoxykanamycin)**

KANAMYCIN A**TLC***Medium*Silica gel F₂₅₄*Solvents*

- A. *n*-Propanol-pyridine-acetic acid-water (15:10:3:12)
 B. Acetone-acetic acid-water (20:6:74)
 C. Chloroform-methanol-conc. ammonium hydroxide-water (1:4:2:1)
 D. Methyl acetate-*n*-propanol-conc. ammonium hydroxide (45:105:60)

Detection

- A. Ninhydrin
 B. Anthrone-sulfuric acid

R_F

Solvent	6'-Cbz-Kanamycin K	Kanamycin A
A	0.42 *, 0.33 ** 0.15 **	0.04
B	0.24	0.14
C	0.76	0.50
D	0.22 (with Detection B)	0.04 (with Detection B)

* Major

** Minor

Ref.

German Patent 2,555,405, June 10, 1976

KANAMYCIN A, 1-N-ACYL DERIVATIVES**TLC***Medium*Silica gel F₂₅₄*Solvent*

Chloroform-methanol-28% ammonium hydroxide-water (1:4:2:1)

R_F

Twenty-three 1-N-acylated derivatives of Kanamycin A [prepared by reaction of 6'-N-benzoyloxycarbonyl Kanamycin A with the N-hydroxysuccinimide ester of an appropriate side-chain acid, followed by reductive removal of the protecting group(s)] were chromatographed. *R_F* values ranged from 0.18 to 0.70.

Ref.

As Amikacin, TLC

KANAMYCIN A (transglycosylated)**TLC***Solvent*

Chloroform-methanol-ammonium hydroxide (1:3:2)

*Ref.*T. Endo and D. Perlman, J. *Antibiot.*, 25 (1972) 751**ELPHO***Medium**Paper**Condition*

Ninhydrin-positive and biologically active components were observed upon paper ionophoresis of the mixture (pH 1.8, 53 V/cm, 20 min)

Detection

- A. Bioautography
 B. Ninhydrin

Ref.

As TLC

KANAMYCIN B, 1,2'-DI-N-SUBSTITUTED**TLC***Medium*

Silica gel (ART 5721)

Solvents

- A. Methanol-chloroform-28% aq. ammonia-water (4:1:2:1)
 B. Butanol-ethanol-chloroform-17% aq. ammonia (4:5:2:5)

R_F

Compound	Solvent	<i>R_F</i>
1,2'-Di-N-[(<i>S</i>)-4-amino-2-hydroxybutyryl]-kanamycin B	A	0.04
1-N-[(<i>S</i>)-4-Amino-2-hydroxybutyryl]-kanamycin B	A	0.15
1,2'-Di-N-[(<i>S</i>)-4-amino-2-hydroxybutyryl]-3',4'-dideoxykanamycin B	B	0.09
1-N-[(<i>S</i>)-4-Amino-2-hydroxybutyryl]-3',4'-dideoxykanamycin B	B	0.17
1,2'-Di-N-DL-isoseryl-3',4'-dideoxykanamycin B	A	0.26
1,2'-Di-N-DL-isoseryl kanamycin B	B	0.06

Ref.

H. Umezawa, K. Maeda, S. Kondo and S. Umezawa, *U.S. Patent* 3,940,382, Feb. 24, 1976

KANAMYCINS**PC 1***Solvent*

Water satd. *n*-butanol + 2% *p*-toluenesulfonic acid

Detection

Bioautography vs. *Bacillus subtilis*

R_F

0.1–0.26 (minor)

0.21–0.37 (minor)

Ref.

K. Maeda, M. Ueda, K. Yagishita, S. Kawaji, S. Kondo, M. Murase, T. Takeuchi, Y. Okami and H. Umezawa, *J. Antibiot.*, 10 (1957) 228–230

PC 2*Solvent*

2% *p*-Toluenesulfonic acid (monohydrate) in water satd. *n*-butanol

R_F

0.21–0.26 (major); 0.00, 0.37 (minor)

Ref.

H. Umezawa, M. Ueda, K. Maeda, K. Yagishita, K. Kondo, Y. Okami, R. Utahara, Y. Osato, K. Nitta and T. Takeuchi, *J. Antibiot.*, 10 (1957) 181–188

PC 3*Paper*

Whatman No. 1

Solvent

n-Butanol–water–2% *p*-toluenesulfonic acid

Detection

The dye used to locate the position of the kanamycins on paper chromatograms, commonly called “chromato red”, is made by coupling diazotized *p*-rosaniline with 1-naphthol-4-sulfonic acid. The developed strips are dried thoroughly at room or higher temperatures before being dipped into a 0.1–0.5% aq. soln. of this dye. The stained strips are hung vertically, and the excess dye is removed by several washes alternately with warm water and methanol

R_F

Kanamycin A, 0.13–0.18

Kanamycin B, 0.26–0.28

Kanamycin C, 0.20–0.24

Ref.

J.W. Rothrock, R.T. Goegelman and F.J. Wolf, *Antibiot. Ann.*, (1958–1959) 796–803

PC 4*Paper*

Whatman No. 1

Solvent

Water satd. butanol containing 2% *p*-toluenesulfonic acid

Detection

As PC 3

R_F

Kanamycin A, 0.15–0.22

Kanamycin B, 0.25–0.40

Kanamycin C, 0.20–0.30

Ref.

J.W. Rothrock and I. Putter, *U.S. Patent* 3,032,547, Sept. 12, 1958

PC 5*Paper*

Toyo Roshi No. 50

Solvent

0.4% Aq. ammonium chloride and 20% methanol–water

Detection

Bioautography vs. *Bacillus subtilis*

R_F

Kanamycin methanesulfonate, 1.0

Ref.

H. Umezawa, M. Murase and S. Yamazaki, *J. Antibiot.*, 12 (1959) 341–342

PC 6*Papers*

A. Schleicher and Schuell 589 Blue Ribbon

B. Toyo No. 50

Solvent

n-Butanol–water–2% *p*-toluenesulfonic acid

Detection

Ninhydrin reaction

R_F

Kanamycin A, 0.35, (Paper A)

Kanamycin B, 0.60, (Paper A)

Kanamycin C, 0.21–0.26, (Paper B)

Ref.

D.A. Johnson and G.H. Hardcastle, Jr., *U.S. Patent* 2,967,177, Jan. 3, 1961

PC 7*Paper*

Toyo Roshi No. 51

Solvents

A. Ethanol–conc. ammonium hydroxide–water (8:1:1)

B. *n*-Butanol–conc. ammonium hydroxide–water (8:1:1)

C. *n*-Butanol–acetic acid–water (4:1:4), upper layer

D. *n*-Butanol– $\frac{1}{15}$ *M* phosphate buffer, pH 7.0 (3:1), upper layer. Filter paper was treated with the buffer

E. *n*-Octanol–water–conc. ammonium hydroxide (5:4:1), upper layer

All solvents developed ascending

Detection

A. UV absorption

B. Bioautography vs. *Bacillus subtilis*

Ref.

A. Fujii, K. Maeda and H. Umezawa, *J. Antibiot.*, 21 (1968) 340–349

R_F

	Solvent				
	A	B	C	D	E
Tetra-N-benzylkanamycin	0.858	0.845	0.760	0.175	0.545
Tetra-N-phenylethylkanamycin	0.889	1.000	0.964	0.286	0.710
Tetra-N-phenylpropylkanamycin	0.910	1.000	0.974	0.252	0.761
Tetra-N-cinnamylkanamycin	0.889	1.000	0.976	0.218	0.671
Tetra-N-2-hydroxybenzylkanamycin Na ₂	0.815	0.695	0.646	0.030	0.355
Tetra-N-3-hydroxybenzylkanamycin	0.737	0.423	0.544	0.038	0.020
Tetra-N-2-methoxybenzylkanamycin	0.868	0.859	0.704	0.107	0.360
Tetra-N-4-methoxybenzylkanamycin	0.843	0.648	0.687	0.068	0.139
Tetra-N-2,4-dimethoxybenzylkanamycin	0.868	0.916	0.832	0.077	0.296
Tetra-N-2-chlorobenzylkanamycin	0.864	1.000	0.769	0.727	0.707
Tetra-N-4-chlorobenzylkanamycin	0.884	1.000	0.966	0.489	0.688
Tetra-N-2,4-dichlorobenzylkanamycin	0.902	1.000	0.962	0.987	0.705
Tetra-N-4-methylbenzylkanamycin	0.900	0.920	0.954	0.316	0.725
Tetra-N-4-isopropylbenzylkanamycin	0.942	1.000	0.974	0.496	0.801
Tetra-N-3-nitrobenzylkanamycin	0.779	0.636	0.704	0.084	0.055
Tetra-N-2-hydroxy-3-methoxybenzylkanamycin Na ₂	0.674	0.316	0.588	0.084	0.105
Kanamycin A	0.079	0.000	0.000	0.000	0.000

PC 8

Paper

Ascending

R_F

Solvent	Component		
	A	B	C
A	0.92	0.98	0.96
B	0.00	0.00	0.00
C	0.00	0.00	0.00
D	0.00	0.00	0.00
E	0.02	0.01	0.02

Solvents

A. 20% Aq. soln. of ammonium chloride

B. Water satd. *n*-butanol

C. *n*-Butanol–acetic acid–water (3:1:1)

D. Water satd. *n*-butanol + 2% (w/v) *p*-toluene-sulfonic acid + 2% piperidine (v/v)

E. Chloroform–methanol–17% aq. ammonia (2:1:1), lower layer

Ref.

As Apramycin, PC 1

PC 9

Solvents

A. 80% Methanol + 3% sodium chloride (1:1, w/v), descending. Paper buffered with 0.95 *M* sodium sulfate + 0.05 *M* sodium bisulfate

B. Propanol–pyridine–acetic acid–water (6:4:1:3, v/v) ascending

C. 80% Phenol, ascending (v/v)

D. Benzene–methanol (9:1, v/v), descending

E. *n*-Butanol–water–acetic acid (4:5:1), upper phase, ascending

R_F

Solvent	R_F	Solvent	R_F
A	0.0, 0.28	D	0.0
B	0.08	E	0.00
C	0.0, 0.17		

Ref.

M.J. Weinstein, G.H. Wagman and J.A. Marquez,
U.S. Patent 4,001,209, Jan. 4, 1977

PC 10

Paper

Toyo No. 51

Solvent

Methanol-3% aq. sodium chloride (2:1)

 R_F

0.36

Ref.

As Gentamicin, PC 14

PC 11

Paper

Whatman No. 1

 R_F

Solvent	R_e^*	
	Kanamycin	Kanamycin B
A	0.35	0.57
B	0.35	0.26, 0.51
C	0.41	0.32, 0.39
D	0.65	0.70
E	0.42	0.29
F	0	0
G	0	0.05

* R_e = The migration constant for each of the antibiotics is expressed as an R_e rather than the more conventional R_F value. The R_e value expresses the ratio of the distance traversed by the antibiotic with respect to the end of the tape rather than the solvent front. This method of expressing the constant was chosen in view of the fact that the solvent front had progressed beyond the end of the tape during the time interval employed with most of the solvent systems

Solvents

A. *n*-Butanol satd. with water + 2% *p*-toluenesulfonic acid, for 40 h

B. 80% Aq. ethanol + 1.5% sodium chloride for 40 h on paper buffered with 0.95 *M* sulfate-bisulfate

C. Propanol-pyridine-acetic acid-water (15:10:3:12) for 40 h

D. Water satd. with methylisobutyl ketone + 1% *p*-toluenesulfonic acid, for 6 h

E. Propanol-water (1:1), for 24 h on paper buffered with 0.75 *M* phosphate, pH 4.0

F. *n*-Butanol satd. with water, for 18 h

G. *n*-Butanol satd. with water + 2% *p*-toluenesulfonic acid and 2% piperidine, for 18 h

Ref.

R.Q. Thompson, W.M. Stark and C.E. Higgins,
U.S. Patent 3,691,279, Sept. 12, 1972

PC 12

Paper

Whatman No. 1

Solvent

Methanol-conc. ammonium hydroxide (4:1)

Detection

Bioautography vs. *Bacillus subtilis* R_F

Kanamycin, 0.54

Kanamycin biosynthesis product, 0.18

Ref.

W.J. Shier, K.L. Rinehart, Jr., and D. Gottlieb,
U.S. Patent 3,833,556, Sept. 3, 1972

PC 13

Paper

Toyo Filter No. 51A

Solvent

Methanol-3% aq. sodium chloride (2:1)

 R_F

0.36

Ref.

As Gentamicin, PC 15

PC 14

Paper

Descending

Solvents

A. Water satd. *n*-butanol + 2% *p*-toluenesulfonic acid, 20 h

B. *n*-Propanol-pyridine-acetic acid-water (15:10:3:12), 20 h

C. *n*-Butanol-pyridine-acetic acid-water (6:4:1:3), 7 days

 R_F (see p. 202)

Ref.

T. Shomura, N. Ezaki, T. Tsuruoka, T. Niwa, E. Akita, and T. Niida, *U.S. Patent* 3,697,648, Oct. 10, 1972

R_F

Sol-vent	Kanamycin		
	A	B	C
A	4.7 *	9.0 *	7.3 •
	1.74 **	2.90 **	2.43 **
B	13.5 •	12.4 *	15.8 *
	1.39 **	1.29 **	1.65 **
C	13.1 •	11.3 *	18.2 *
	2.22 **	1.98 **	3.19 **

• Migration distance from original point in cm

** R_{767-L} Represents the ratio of the value for migration distance of one antibiotic substance to the value for migration distance of the SF-767-L substance. In determination of R_{767-L} , an amount of the SF-767-L substance was added as an internal standard to each of the antibiotic substances, and the mixture was then developed

TLC 1

Medium

Silica gel G

Solvent

Methanol–water–conc. ammonium hydroxide (20:4:1)

Detection

10% Sulfuric acid spray and heat

 R_F

Tetra-N-benzylkanamycin	0.83
Tetra-N-phenylethylkanamycin	0.82
Tetra-N-phenylpropylkanamycin	0.81
Tetra-N-cinnamylkanamycin	0.80
Tetra-N-2-hydroxybenzylkanamycin	
Na ₂	0.85
Tetra-N-3-hydroxybenzylkanamycin	0.85
Tetra-N-2-methoxybenzylkanamycin	0.84
Tetra-N-4-methoxybenzylkanamycin	0.78
Tetra-N-2,4-dimethoxybenzylkanamycin	0.77
Tetra-N-2-chlorobenzylkanamycin	0.86
Tetra-N-4-chlorobenzylkanamycin	0.82
Tetra-N-2,4-dichlorobenzylkanamycin	0.84
Tetra-N-4-methylbenzylkanamycin	0.85
Tetra-N-4-isopropylbenzylkanamycin	0.83
Tetra-N-3-nitrobenzylkanamycin	0.80
Tetra-N-2-hydroxy-3-methoxybenzylkanamycin Na ₂	0.65
Kanamycin A	0.03

Ref.

As PC 7

TLC 2

Medium

Silica gel

Solvents

A. Chloroform–methanol–17% aq. ammonia (2:1:1), upper layer

B. Methanol–10% ammonium acetate (1:1)

C. *n*-Butanol–ethanol–chloroform–17% aq. ammonia (4:5:2:5)

 R_F

Solvent	Component		
	A	B	C
A	0.77	0.80	0.84
B	0.76	0.80	0.84
C	0.15	0.16	0.17

Ref.

As Apramycin, PC 1

TLC 3

Medium

Alumina

Solvent

Chloroform–methanol–17% aq. ammonia (2:1:1) upper layer

 R_F

0.75

Ref.

As Gentamicin, PC 9

TLC 4

Medium

Silica gel F₂₅₄

Solvents

A. *n*-Propanol–pyridine–acetic acid–water (15:10:3:12)

B. Acetone–acetic acid–water (20:6:74)

C. Chloroform–methanol–conc. ammonium hydroxide–water (1:4:2:1)

D. Methyl acetate–*n*-propanol–conc. ammonium hydroxide (45:105:60)

Detection

Ninhydrin

Ref.

T. Naito, S. Nakagawa and Y. Abe, *U.S. Patent*, 3,904,597, Sept. 9, 1975

R_F

Compound	Solvent			
	A	B	C	D
Kanamycin A	0.04	0.14	0.50	0.04 *
6'-Carbobenz-oxykanamycin A	0.42 (main) 0.33-0.4 (minor)	0.24	0.76	0.22 •
6'-Carbobenz-oxykanamycin B	0.03	0.16		

• Detected by anthrone-sulfuric acid

TLC 5*Medium*

Cellulose

Solvent

Methyl ethyl ketone-methanol-isopropanol-7.9 N ammonium hydroxide (10:8.5:3:7)

*Detection*Ninhydrin. Spray with 0.3% soln. of ninhydrin in water satd. *tert.*-butanol, heat at 100-105°C for 3-5 min R_F

Kanamycin A, 0.48

Kanamycin B, 0.43

*Ref.*N.R. Chatterjee, *Indian J. Chem.*, 13 (1975) 1282-1284**TLC 6***Medium*

Silica gel (ART 5721)

 R_F

	Solvent	
	A	B
Kanamycin (ref.)	0.39	0.11
1-N-DL-Isoserylkanamycin	0.27	0.08
Kanamycin B		0.09
1-N-DL-Isoserylkanamycin B		0.16
Dideoxykanamycin		0.26
1-N-DL-Isoseryl-3',4'-dideoxykanamycin B		0.17

Solvents

A. Methanol-chloroform-28% ammonia-water (4:1:2:1)

B. Butanol-ethanol-chloroform-17% ammonia (4:5:2:5)

Detection

Ninhydrin

Ref.

German Patent 2,423,591, Dec. 5, 1974

TLC 7*Medium*

Alumina

Solvent

Chloroform-methanol-17% aq. ammonia (2:1:1), upper layer

 R_F

0.75

Ref.

As Gentamicin, PC 15

TLC 8*Medium*

Silica gel

Solvent

Chloroform-methanol-28% ammonia (1:4:2:1)

Detection

Ninhydrin

 R_F

1-N-(L-4-Amino-2-hydroxybutyryl)-6'-N-methylkanamycin, 0.13

1-N-(L-4-Amino-2-hydroxybutyryl)-6'-N-ethylkanamycin, 0.20

Ref.

German Patent 2,512,587, Sept. 25, 1975

TLC 9 R_F

Component	Solvent	
	A	B
1 N-(DL-Isoseryl)-6'-N-methyl-3',4'-dideoxykanamycin B	0.51	
1 N-(L-Isoseryl)-6'-N-methyl-3',4'-dideoxykanamycin B	0.51	
1 N-(L-4-Amino-3-hydroxybutyryl)-6'-N-methyl-3',4'-dideoxykanamycin B	0.38	0.60
1 N-(L-5-Amino-2-hydroxyvaleryl)-6'-N-methyl-3',4'-dideoxykanamycin B	0.39	

Medium

Silica gel ART 5721 (Merck)

Solvents

- A. *n*-Butanol-ethanol-chloroform-20% aq. ammonia (4:5:2:8)
 B. Butanol-ethanol-chloroform-17% ammonia (4:5:2:3)

Detection

- A. Ninhydrin
 B. Rydon-Smith

Ref.

Belgian Patent 834,236, April 6, 1976

ELPHO 1**Medium**

Whatman No. 4 filter paper

Buffers

- A. 2 *M* Formic acid-0.1 *M* *p*-toluenesulfonic acid-*n*-propanol (500:50:50) in 1000 ml water, pH 1.8
 B. 2 *M* Formic acid-0.1 *M* *p*-toluenesulfonic acid-*n*-propanol (400:50:50) in 1000 ml water, pH 1.9
 C. 2 *M* Formic acid-0.1 *M* *p*-toluenesulfonic acid (400:50) in 1000 ml water, pH 1.9
 D. 1 *M* Ammonium hydroxide-1 *M* sodium hydroxide-0.1 *M* *p*-toluenesulfonic acid-*n*-propanol (200:4:100:100) in 1000 ml water, pH 10.8
 E. 1 *M* Ammonium hydroxide-1 *M* sodium hydroxide (200:2.5) in 1000 ml water, pH 11.5
 F. 50 ml 1 *M* Sodium hydroxide in 1000 ml water, pH 12.2

Conditions

The electrophoretograms were developed at 350 V for 1-4 h (depending on the electrolyte)

Mobility

Buffer	Toward cathode (cm ² /V sec)
A	33.77
B	30.46
C	31.36
D	3.77
E	4.22
F	6.23

Ref.

S. Ochab, *Pol. J. Pharmacol. Pharm.*, 23 (1973) 105-108

ELPHO 2**Medium**

Whatman 3MM paper

Buffer

Formic acid-acetic acid-water (6:24:170)

Conditions

3000 V, potential gradient 60 V per cm, current 50 mA, 30 min

Detection

Paper stained with 0.25% ninhydrin in acetone containing 0.01% isatin and 1% lutidine; heat at 100° for 10 min

Mobility

1.3. Migration distance relative to alanine taken as 1.00

Ref.

As Ampicillin, PC 4

GLC**Apparatus**

F and M Model 400 with FID

Column

Glass column, 3 mm × 1830 mm (6 ft.) packed with 3% OV-1 on Gas Chrom Q, 100-120 mesh (Applied Science Labs., Inc.)

Gases

Hydrogen 40 ml/min, air 600 ml/min, and carrier gas (helium) 70 ml/min. Chart speed 0.25 in./min

Temperatures

Column, conditioned at 330°C for 1 h; oven temperature, 300°C; flow at 200 and 250°C; injection, 300°C

Internal standard-silylation reagent

For kanamycin a soln. containing 8 mg of tri-laurin and 25 μl of *N*-trimethylsilyldiethylamine/ml of Tri-Sil Z is prepared

Reference standard

A water soln. containing 10.0 mg of kanamycin sulfate reference standard/ml

Sample

Samples are prepared in the same way as the reference standard

Silylation procedures

One ml of the internal standard-silylation reagent is added to the sample and the reference is added to the sample and the reference standard vials using a tuberculin syringe. Vials are heated in a 75°C oil bath for 45 min, swirling occasionally

Separation of kanamycins

Order of elution is (1) aminoglucosyl deoxystreptamine, (2) kanamycin B, (3) kanamycin A

Ref.

K. Tsuiji and J.H. Robertson, *Anal. Chem.*, 42 (1970) 1661-1663

HPLC*Apparatus*

Varian LC 4200

Column

Aminex A-28, 1 m × 2 mm I.D.

Mobile phase

Water

Flow-rate

12 ml/h

Temperature

40°C

Detector

Refractive index

Sensitivity

Attenuation × 4

Sample size

2 µl (Kanamycin A, 2.1 mg); (Kanamycin B, 2 mg); (Deoxykanamycin, 1.2 mg in 100 µl)

Type

Reversed-phase, anion exchange

Retention time (estimated from drawing)

Kanamycin A, 15.0

Kanamycin B, 6.1

Deoxykanamycin, 5.0

Ref.

As Cephalosporin, HPLC 2

KANCHANOMYCIN**TLC***Solvents*

- A. Acetone-methanol-acetic acid (1:1:1)
- B. 1-Butanol-methanol-10% citric acid (4:2:1)
- C. 1-Butanol-acetic acid-methanol (3:1:1)
- D. Benzene-ethyl acetate-methanol (10:2.5:1)
- E. Water

R_F

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.73	D	0.10
B	0.65	E	0.00
C	0.46		

Ref.

T.F. Kuimova, K. Fukushima, S. Kuroda and T. Arai, *J. Antibiot.*, 24 (1971) 69-76

KASUGAMYCIN**PC 1***Paper*

Toyo No. 51

Solvents

- A. Butanol-acetic acid-water (2:1:1)
- B. Butanol-ethanol-water-ammonia (4:1:4.9:0.1)
- C. Butanol-acetic acid-water (6.3:1:2.7)
- D. Butanol-acetic acid-water (2:1:1)

R_F

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.28	C	0.06
B	0.07	D	0.29

Ref.

H. Umezawa, Y. Okami, T. Hashimoto, Y. Suhara, M. Hamada and T. Takeuchi, *J. Antibiot.*, 18 (1965) 101-103
H. Umezawa, Y. Okami, T. Takeuchi, M. Hamada, *U.S. Patent* 3,358,001, Dec. 12, 1967

PC 2*Paper*

Ascending

Solvent

Chloroform-methanol-17% aq. ammonia (2:1:1) lower layer (developed at room temp. for 12 h)

R_F

0.01

Ref.

As Apramycin, PC 1

TLC*Medium*

Avicel

Solvents

- A. *n*-Propanol-pyridine-water-acetic acid (15:10:12:3)
- B. Methyl acetate-2-propanol-ammonium hydroxide (45:105:60)

Detection

Ninhydrin spray

R_F

Solvent A, 0.35

Solvent B, 0.20

Ref.

M.J. Cron, R.E. Smith, I.R. Hooper, J.G. Keil, E.A. Ragan, R.H. Schreiber, G. Schwab and J.C. Godfrey, *Antimicrob. Agents Chemother.*, 1969 (1970) 219-224

ELPHO 1*Buffer*

Formic acid-acetic acid-water (25:75:900), pH 1.8

Conditions

3000 V/40 cm at 10°C, 15 min

Mobility

Moves to cathode 3.7 cm

Ref.

H. Umezawa, Y. Okami, T. Hashimoto, Y. Suhara, M. Hamada and T. Takeuchi, *J. Antibiot.*, 18 (1965) 101–103

ELPHO 2**Medium**

Paper

Buffer

Formic acid–acetic acid–water (22:75:900)

Conditions

3500 V, 15 min

Mobility

Kasugamycin guanidino derivative, single spot

Ref.

H. Umezawa and Y. Suhara, *U.S. Patent* 3,968,100, July 6, 1976

KETOMYCIN**PC****Paper**

MN 212

Solvent

Methanol–water (90:10)

Ref.

W. Keller-Schierlein, K. Poralla and H. Zähler, *Arch. Mikrobiol.*, 67 (1969) 339–356

TLC**Medium**

Kieselgel G

Solvents

- A. Butanol–acetic acid–water (80:20:20)
 B. Ethyl acetate–water–formic acid (200:60:5), organic phase

Ref.

As PC

KIDAMYCIN**PC****Paper**

Toyo No. 50

Solvents

- A. Acetonitrile
 B. *n*-Butyl acetate–dibutyl ether (3:1)
 C. *n*-Butanol–methanol–water (4:1:2)
 All solvents developed ascending

 R_F

- Solvent A, 0.14
 Solvent B, 0.71
 Solvent C, 0.68

Ref.

N. Kanda, *J. Antibiot.*, 24 (1971) 599–606

TLC**Media**

- A. Silica gel (Merck Kieselgel G)
 B. Schleicher and Schull No. 288 aluminum oxide paper

Solvents

- A. Ethanol–14% ammonia water (4:1)
 B. Ethanol–pyridine (4:1)
 C. Water satd. ethyl acetate

 R_F

Solvent	Medium A	Medium B
A	0.81	–
B	0.06	–
C	–	0.10

Ref.

As PC

KIKUMYCIN**PC 1****Solvent**

Butanol–acetic acid–water (2:1:1)

 R_F

Component	R_F
Kikumycin A	0.04
Kikumycin B	0.72

Ref.

M. Kikuchi, K. Kumagai, N. Ishida, Y. Ito, T. Yamaguchi, T. Furumai and T. Okuda, *J. Antibiot.*, 18 (1965) 243–250

PC 2**Solvents**

- A. Wet butanol
 B. 3% Ammonium chloride
 C. 80% Phenol
 D. 50% Acetone
 E. Butanol–methanol–water (4:1:2), 1.5% methyl orange
 F. Butanol–methanol–water (4:1:2)
 G. Benzene–methanol (4:1)

- H. Water
 I. Pyridine
 J. Butanol satd. water–propanol–acetic acid–water (10:15:3:12)

 R_F

Solvent	Component	
	A	B
A	0.00	0.00
B	0.12	0.12
C	1.00	1.00
D	0.00	0.10
E	0.50	0.60
F	0.10	0.40
G	0.00	0.00
H	0.02	0.05
I	0.02	0.00
J	0.50	0.50

Ref.

As PC 1

ELPHO

Medium

Paper

Buffers

- A. pH 5.0
 B. pH 8.0

Conditions

250 V/30 cm, 2.5 h

Mobility

- A. Moves toward cathode
 B. Moves toward cathode

Ref.

As PC 1

KINAMYCIN**TLC 1**

Medium

Kieselgel G (Merck)

Solvent

Chloroform–ethyl acetate (3:2)

 R_F

- Kinamycin A, 0.89
 Kinamycin B, 0.82
 Kinamycin C, 0.47
 Kinamycin D, 0.39

Ref.

S. Ito, T. Matsuya, S. Omura, M. Otani, A. Nakagawa, H. Takeshima, Y. Iwai, M. Ohtani and T. Hata, *J. Antibiot.*, 23 (1970) 315–317

TLC 2

Medium

Kieselgel G (Merck) treated with 2% sulfuric acid

Solvents

- A. Benzene–acetone (5:1)
 B. Chloroform–ethyl acetate (3:2)

Detection

- A. UV lamp
 B. Visible color

 R_F

Solvent	Component			
	A	B	C	D
A	0.60	0.60	0.25	0.25
B	0.89	0.82	0.47	0.39

Ref.

T. Hata, S. Omura, Y. Iwai, A. Nakagawa, M. Otani, S. Ito and T. Matsuya, *J. Antibiot.*, 24 (1971) 353–359

KIRROMYCIN**TLC**

Media

- A. Kieselgel
 B. Aluminum oxide

Solvents

- A. Benzyl alcohol
 B. Ethyl acetate–methanol (1:1)
 C. Ethyl acetate–methanol–formic acid (17:2:1)
 D. 1-Propanol–water–conc. ammonium hydroxide (7:2:1)

 R_F

Medium	Solvent	R_F
A	A	0.57
	B	0.80
	C	0.63
	D	0.59
B	C	0.45
	D	0.33

Ref.

H. Wolf and H. Zähler, *Arch. Mikrobiol.*, 83 (1972) 147–154

KOBENOMYCIN

PC

Paper

Toyo No. 51

Solvents

- A. *tert*-Butanol-acetic acid-water (2:1:1)
 B. *n*-Butanol-acetic acid-water (4:1:5), upper phase
 C. Acetone-water (1:1)
 All systems developed ascending

 R_F

- Solvent A, 0.60
 Solvent B, 0.15
 Solvent C, 0.83

Ref.

S. Okamoto, M. Mayama, Y. Tanaka, K. Tawara, N. Shimaoka, H. Kato, H. Nishimura, M. Ebata and H. Ohtsuka, *J. Antibiot.*, 21 (1968) 320-326

TLC**Medium**

Silica gel G

Solvents

- A. *n*-Butanol-acetic acid-water (4:1:2)
 B. *n*-Butanol-ethanol-0.1 *N* hydrochloric acid (1:1)
 C. *n*-Butanol-pyridine-acetic acid-water (15:10:3:10)
 D. Chloroform-methanol-17% ammonium hydroxide (2:1:1), upper phase
 E. Ethanol-water (4:1)

 R_F

Solvent	R_F	Solvent	R_F
A	0.30	D	1.00
B	0.50	E	0.57
C	0.75		

Ref.

As PC

ELPHO**Medium**

Toyo Paper No. 51

Buffer

pH 2.0, 5.0, 6.0, 7.0, 8.0 and 11.4 buffer solns.

Conditions

300 V for 3 h

Mobility

Moves slight to anode at pH 11.4; moves slightly to cathode at other pH values

Ref.

As PC

KOMAMYCINS**TLC****Solvents**

- A. *n*-Butanol-acetic acid-water (3:1:1)
 B. Phenol-water (4:1)
 C. Methanol-water (17:3)
 D. *n*-Propanol-water (7:3)

 R_F

Solvent	Component	
	A	B
A	0.40	0.40
B	0.52	0.40
C	0.52	0.44
D	0.63	0.63

Ref.

Japanese Patent 23381R, March 27, 1970

KUJIMYCIN**TLC****Media**

- A. Silica gel
 B. Alumina

Solvents

- A. Benzene-acetone (1:1)
 B. Ethyl acetate

Detection

Bioautography vs. *Sarcina lutea*

 R_F

Component	Medium	Solvent	R_F
Kujimycin A	A	A	0.46
	B	A	0.12
		B	0.38
Kujimycin B	A	A	0.62
	B	A	0.55
		B	0.65

Ref.

S. Omura, S. Namiki, M. Shibata, T. Muro, H. Nakayoshi and J. Sawada, *J. Antibiot.*, 22 (1969) 500-505

KUNDRYMYCIN**TLC****Medium**

Silica gel

Solvent

Methanol-toluene-formic acid (95:5:0.5)

DetectionBioautography vs. *Bacillus subtilis* ATCC 6633**R_F**

0.78

Ref.J.A. Bush, C.S. Cassidy, K.E. Crook, Jr., and L.B. German, *J. Antibiot.*, 24 (1971) 143-148**KUWAITIMYCIN****TLC****Media**A. Silica gel GF₂₅₄

B. Alumina

C. Kieselguhr

Solvents

A. Ethanol

B. Ethanol-water (75:25)

C. Acidic ethanol (pH 3.5)

D. Alkaline ethanol (pH 8.5)

E. Acetone

F. Acetone-water (70:30)

G. Acetone-benzene (1:1)

H. Acetone-benzene (1:1) pH 3.5

I. Acetone-benzene (1:1) pH 8.5

J. Chloroform-light petroleum (b.p. 60-80°) (1:1)

K. Ethyl acetate-light petroleum (b.p. 60-80°C) (1:1)

Detection

UV

R_F

Solvent	Medium		
	A	B	C
A	0.83	0.86	0.80
B	0.52	0.49	0.42
C	0.83	0.81	0.81
D	0.79	0.77	0.78
E	0.96	0.98	0.94
F	0.48	0.51	0.48
G	0.70	0.73	0.69
H	0.68	0.70	0.65
I	0.64	0.68	0.66
J	0.53	0.46	0.50
K	0.60	0.52	0.57

Ref.I.R. Shimi, A. Dewedar and S. Shoukry, *J. Antibiot.*, 26 (1973) 593-601**LACTENOCIN****PC****Solvents**

A. Methyl ethyl ketone on pH 4 buffered paper

B. Methyl ethyl ketone

C. *n*-Butanol satd. with water on pH 4 buffered paperD. *n*-Butanol satd. with water

E. Water with 7% sodium chloride and 2.5% methyl ethyl ketone

F. Ethyl acetate satd. with water on pH 4 buffered paper

R_F

Solvent	R _F	Solvent	R _F
A	0.20	D	0.60
B	0.38	E	0.86
C	0.75	F	0.05

Ref.R.L. Hamill and W.M. Stark, *J. Antibiot.*, 17 (1964) 133-139**LAGOSIN****PC****Paper**

Whatman No. 1

Solvents

A. Methyl isobutyl ketone-1-butanol-water (50:15:3)

B. Methyl isopropyl ketone-water (satd. ca. 1.6%)

C. Methyl isopropyl ketone-1-butanol-water (50:10:3.5)

D. Methyl isopropyl ketone-methanol-water (50:1:0.4)

Detection

UV light; greenish fluorescence

R_F

Solvent	R _F	Solvent	R _F
A	0.72	C	0.76
B	0.35	D	0.47

Ref.A.C. Cope, R.K. Bly, E.P. Burrows, O.J. Adar, E. Ciganek, B.T. Gillis, R.F. Porter and H.E. Johnson, *J. Amer. Chem. Soc.*, 84 (1962) 2170-2178

LARGOMYCIN

TLC

Media

- A. Silica gel
- B. Aluminum oxide

Solvents

- A. Ethanol-ammonium hydroxide-water (8:1:1)
- B. *n*-Butanol-methanol-water (4:1:2)

R_F

Solvent	Medium A	Medium B
A	0.95	—
B	—	0.74

Ref.

T. Yamaguchi, T. Kashida, K. Nawa, T. Yajma, T. Miyagishima, Y. Ito, Tl. Okuda, N. Ishida and K. Kumagai, *J. Antibiot.*, 23 (1970) 373–381

LASALOCID

(cf., *Iso-Lasalocid A*)

CCD

Solvent

Heptane-ethyl acetate-ethanol-acetic acid-water (10:5:9:1:3)

Distribution coefficient

2800 transfers, 500 tubes

After 2800 redistributions, one separates lasalocids B, C, D, E

Ref.

Belgian Patent 827,415, Oct. 1, 1975

LASPARTOMYCIN

PC 1

Solvents

- A. Benzene-methanol (4:1)
- B. *n*-Butanol satd. with water
- C. *n*-Butanol-pyridine-water (2:1:1)
- All systems developed ascending

R_F

Solvent A, 0.70
Solvent B, 0.45
Solvent C, 0.41

Ref.

H. Naganawa, M. Hamada, K. Maeda, Y. Okami, T. Takeuchi and H. Umezawa, *J. Antibiot.*, 21 (1968) 55–62

PC 2

Paper

Toyo No. 51

Solvents

- A. 1-Butanol satd. with water
- B. 5% Ammonium chloride
- C. 1-Butanol-acetic acid-water (4:1:2)
- D. 1-Butanol-pyridine-water (2:1:1)
- E. 1-Butanol-pyridine-acetic acid-water (20:5:5:10)
- F. Benzene-methanol (4:1)
- All solvents developed ascending

Detection

Bioautography

R_F

Solvent	R_F	Solvent	R_F
A	0.45	D	0.41
B	0.23	E	0.80
C	0.91	F	0.70

Ref.

H. Umezawa, M. Hamada, H. Naganawa, T. Takeuchi, K. Maeda and Y. Okami, *U.S. Patent* 3,639,582, Feb. 1, 1972

TLC 1

Medium

Silica gel

Solvents

- A. 1-Butanol satd. with water
- B. Acetic acid-chloroform (2:1)
- C. *sec*-Butanol-formic acid-water (75:15:10)
- D. *n*-Propanol-acetic acid-chloroform (1:4:2)
- E. 1-Butanol-acetic acid-water (4:1:2)
- F. 1-Butanol-pyridine-water (2:1:1)

Detection

Permanganate

R_F

Solvent	R_F	Solvent	R_F
A	0.14	D	0.16
B	0.13	E	0.58
C	0.45	F	0.78

Ref.

As PC 1

TLC 2

Medium

Silica gel

Solvents

- A. *n*-Butanol-satd. with water
- B. Acetic acid-chloroform (2:1)
- C. *n*-Butanol-acetic acid-water (4:1:2)
- D. *n*-Butanol-pyridine-water (2:1:1)

Detection

- A. Decolorization of permanganate
B. Bioautography

 R_F

Solvent	R_F	Solvent	R_F
A	0.14	C	0.58
B	0.13	D	0.76

Ref.

As PC 1

LATERIOMYCIN F**PC****Paper**

- A. Whatman No. 1 •
B. Arches No. 302 •
C. Toyo Roshi No. 51 •
• Impregnate papers with McIlvaine's buffer, pH 4.8

Solvents

- A. *n*-Butanol satd. with water
B. *n*-Butanol satd. with water, phosphate buffer pH 5.6
C. *n*-Butanol-acetic acid-water (4:1:5)
D. Acetone-benzene-water (12:3:2)

 R_F

Solvent	Paper	R_F
A	A	0.8 -0.9
A	B	0.8 -0.9
A	C	0.8 -0.9
B	A	0.65-0.95
C	A	0.75-0.95
D	A	0.75-0.99

Ref.

French Patent 1,523,522, March 31, 1967; E. Higashide, T. Hasegawa, M. Shibata, T. Kishi, S. Harada and K. Mizuno, *U.S. Patent* 3,660,567, May 2, 1972

TLC**Medium**

Silica gel G (Merck)

Solvent

Ethyl acetate-methanol (10:1), ascending

 R_F

0.2-0.45

Ref.

As PC

LEMACIDINE**PC****Solvent**

Ethanol-water (3:1) + 2% sodium chloride

 R_F

	R_B *
Lemacidine B ₁	0.34
Lemacidine B ₂	0.61
Lemacidine B ₃	1.00

$$* R_B = \frac{\text{distance zone moved}}{\text{distance B}_3 \text{ moved } (=1.00)}$$

Ref.

E. Gaeumann and F. Benz, *U.S. Patent* 3,089,816, May 14, 1963

LEMONOMYCIN**PC****Paper**

Whatman No. 1

Solvent

Pyridine-chloroform-acetic acid-water
(140:35:10:30), upper phase

Detection

Bioautography vs. *Bacillus subtilis* on agar medium, pH 6

 R_F

0.40

Ref.

H.A. Whaley, E.L. Patterson, M. Dann, A.J. Shay and J.N. Porter, *Antimicrob. Agents Chemother.*, 1964 (1965) 83-86

LEUCINAMYCIN**PC****Paper**

Toyo No. 51A

Solvents

- A. Wet *n*-butanol, ascending
B. 1.5% Ammonium chloride, ascending
C. 50% Aq. acetone, ascending
D. *n*-Butanol-methanol-water (4:1:2), ascending
E. *n*-Butanol-acetic acid-water (4:1:5), upper phase, descending

 R_F (see p. 212)

Ref.

K. Mizuno, Y. Ohkubo, S. Yokoyama, M. Hamada, K. Maeda and H. Umezawa, *J. Antibiot.*, 20 (1967) 194-199

R_F

Solvent	R_F	Solvent	R_F
A	0.09	D	0.50
B	0.40	E	0.31
C	0.86		

ELPHO*Medium*

Toyo No. 51

Buffer

Formic acid-acetic acid-water (25:75:900), pH 1.8

Conditions

3500 V/46 cm. 50 mA/20 cm, for 20 min

Mobility

0.35 towards cathode

Ref.

As PC

LEUCOMYCINS**PC 1***Solvents*

- A. Citrate buffer of pH 4.6 and benzene
- B. Citrate buffer of pH 4.0 and butyl acetate

*Detection*Bioautography vs. *Bacillus subtilis* R_F

- Solvent A, 0.40
- Solvent B, 0.60

*Ref.*T. Osato, K. Yagashita and H. Umezawa, *J. Antibiot.*, 8 (1955) 161-163**PC 2***Solvents*

- A. Benzene
- B. *n*-Butanol
- C. Methanol
- D. Acetone
- E. Ethyl acetate
- F. Diethyl ether
- G. Light petroleum
- H. Chloroform
- I. 3% Aq. ammonium chloride

*Detection*Bioautography vs. *Bacillus subtilis* 219*Ref.*Y. Sano, *J. Antibiot.*, 7 (1954) 93-97 R_F

Solvent	R_F	Solvent	R_F
A	0.17	F	0.86
B	0.84	G	0.00
C	0.73	H	0.90
D	1.00	I	0.83
E	0.83		

PC 3*Paper*

Toyo No. 51

Solvent

Water-satd. ethyl acetate (ascending, 3 h)

*Detection*Bioautography vs. *Bacillus subtilis* No. 10707 R_F

0.97

Ref.

As Carbomycin, PC 1

TLC*Medium*

Kieselgel G, 0.5 mm

Solvent

Benzene-acetone (2:1)

Detection

Coloration: 20% sulfuric acid

 R_F

0.69

*Ref.*S. Omura, Y. Hironaka and T. Hata, *J. Antibiot.*, 23 (1970) 511-513**CCD***Solvents*

- A. Benzene- $\frac{1}{15}$ M sodium phosphate buffer, pH 6.5-methanol (5:2:5)
- B. Benzene- $\frac{1}{15}$ M sodium citrate buffer, pH 4.9-chloroform-methanol (10:9:8:20)
- C. Chloroform- $\frac{1}{15}$ M sodium acetate buffer, pH 4.5-acetone (1:2:2)

Distribution

	Solvent		
	A	B	C
Leucomycin A ₁	0.92	0.33	1.86
Leucomycin A ₂	2.33	0.14	3.00

Ref.

T. Watanabe, H. Nishida, J. Abe and K. Satake,
Bull. Chem. Soc. Japan, 33 (1960) 1104–1108

FLC*Instrument*

JASCO FLC-150

Detector

JASCO UVIDEDEC-2 (232.5 nm) spectrophotometer

Separation mode

Partition

Column

JASCOPACK SV-02-500

Column temperature

Ambient

Column pressure

65 kg/cm²

Mobile phase

Methanol– $\frac{1}{15}$ M acetate buffer (pH 4.9)–acetonitrile (35:60:5)

Flow-rate

1.0 ml/min

Retention time (min)

Leucomycin A₁, 17.5
Leucomycin A₃, 25.8
Leucomycin A₅, 8.0
Leucomycin A₄, 11.5
Leucomycin A₇, 4.0
Leucomycin A₆, 6.0
Leucomycin A₉, 2.5
Leucomycin A₈, 3.5
Leucomycin U, 2.0
Leucomycin V, 1.5

Ref.

S. Omura, Y. Suzuki, A. Nakagawa and T. Hata,
J. Antibiot., 26 (1973) 794–796

LEUCOPEPTIN**PC***Paper*

Toyo No. 50

Solvents

A. Water satd. *n*-butanol
B. 1.5% Aq. ammonium chloride
C. 75% Aq. phenol
D. 50% Aq. acetone
E. *n*-Butanol–methanol–water (4:1:2)
F. Water
G. *tert*-Butanol–acetic acid–water (74:3:25)
H. *n*-Butanol–acetic acid–water (4:1:2)
All systems developed ascending

Detection

Bioautography vs. *Bacillus subtilis*

R_F

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.05	E	0.54
B	0.26	F	0.13
C	0.97	G	0.61
D	0.47	H	0.47

Ref.

S. Kondo, M. Sezaki, M. Shimura, K. Sato and
T. Hara, *J. Antibiot.*, 17 (1964) 262–263

LEUCYLNEGAMYCIN**ELPHO***Buffer*

Formic acid–acetic acid–water (25:75:900)

Conditions

3500 V for 20 min

Detection

A. Ninhydrin
B. Red tetrazolium
C. Rydon–Smith reactions (starch–potassium iodide)

Mobility

14.3 cm to cathode (relative mobility against alanine = 1.24)

Ref.

S. Kondo, H. Yamamoto, K. Maeda and H.
Umezawa, *J. Antibiot.* 24 (1971) 732–734

LEVOMYCIN**PC 1***Solvents*

A. *n*-Butanol–water
B. *n*-Butanol–10% acetic acid
C. Methyl isopropyl ketone
D. Methyl isopropyl ketone–2% toluenesulfonic acid
E. Methyl isopropyl ketone–2% piperidine

R_F

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.94	D	0.63
B	0.95	E	0.84
C	0.55		

Ref.

H.E. Carter, C.P. Schaffner and D. Gottlieb,
Arch. Biochem. Biophys., 53 (1954) 287

PC 2*Solvent*

Di-*n*-butyl ether-*sym.*-tetrachloroethane-10% aq.
sodium *o*-cresotinate (2:1:3)

R_F
0.13

Ref.

K. Katagiri, J. Shoi, T. Yoshida, *J. Antibiot.*, 15
(1962) 273

PC 3*Solvents*

- A. Light petroleum-benzene-methanol-water
(66.7:33.3:80:20)
B. 25% Ethanol
C. Amyl acetate satd. with water

R_F
Solvent A, 0.26
Solvent B, 0.32
Solvent C, 0.60

Ref.

T.S. Maksimova, I.N. Kovsharova and V.V. Proshlyakova, *Antibiotiki*, 10 (1965) 298-304

LEVORIN**TLC***Medium*

Silica gel G

Solvents

- A. Butan-1-ol-ammonia-methanol-water
(20:1:2:4)
B. Chloroform-methanol-borate buffer (pH 8.3)
(7:5:1)

Detection

0.2% *p*-Dimethylaminobenzaldehyde in sulfuric
acid containing ferric chloride

R_F
Solvent A, 0.13
Solvent B, 0.49, 0.67

Ref.

As Amphotericins, TLC 2

CCD*Solvents*

- A. Chloroform-methanol-borate buffer (pH 8.2-
8.4) (2:2:1)
B. Pyridine-ethyl acetate-water (3.5:6.5:8.3)

Ref.

As Amphotericins, TLC 2

Distribution coefficient

Compound	Solvent	N_t	K
Levorin	A	200	0.61
Levorin A	A	300	0.74
	A	49	0.90
	B	49	1.00
Levorin A1	A	80	0.60
Levorin A2	A	80	1.07
	A	600	1.07
Levorin A3	A	80	1.37
Levorin B	A	49	5.1
	B	49	0.46

HPLC*Apparatus*

DuPont Model 830 liquid chromatograph,
equipped with a DuPont Model 837 spectropho-
tometer. Extinction at 380 nm recorded by means
of a Hewlett-Packard Model HP 3380 A integra-
tor

Column

Permaphase ODS, 1 × 2.1 mm, I.D. reversed-
phase partition column with octadecylsilane as
stationary phase, chemically bonded to a con-
trolled porous surface ("porosity beads", 20-37
μm) by means of Si-O-Si bonds and contains
about 1% stationary phase. Pre-packed Per-
maphase ODS column has 600 theoretical plates,
determined by isocratic elution of anthracene
with methanol-water (6:4)

Temperature

55° ± 1°

Mobile phase

- A. 0.05 M Phosphate buffer (pH 7.0)-methanol
(8:2)
B. Methanol

Gradient

0 → 100% B at 3%/min, hold at limit, concave
gradient $c = kt^5$

Pressure

100 p.s.i.

Flow-rate

1.0 ml/min

Detection

380 nm

Attenuation

0.08 a.u.f.s.

Chart speed

5 mm/min

Ref.

As Candicidin, HPLC

LEVORIN A**PC 1***Paper*

Whatman No. 1

Solvents

- A. Butan-1-ol-pyridine-water (6:4:5)
- B. Methanol-25% ammonia-water (20:1:4)
- C. Ethyl acetate-pyridine-water (4:3:2)

R_F

Solvent A, 0.71
 Solvent B, 0.55
 Solvent C, 0.66

Ref.

As Amphotericins, TLC 2

PC 2*Paper*

Whatman No. 1

Solvents

- A. Acetic acid-pyridine-water (4:3:2)
- B. *n*-Butanol-pyridine-water (6:4:5)
- C. Methanol-25% ammonia-water (20:1:4)

*Detection*Bioautography vs. *Candida albicans**R_F*

Solvent A, 0.66
 Solvent B, 0.71
 Solvent C, 0.55

*Ref.*R. Bosshard and H. Bickel, *Experientia*, 24 (1968) 422-424**LIBRAMYCIN A****PC***Solvents*

- A. Water satd. *n*-butanol
- B. 3% Ammonium chloride
- C. 75% Phenol
- D. 50% Acetone
- E. *n*-Butanol-methanol-water (4:1:2)
- F. Benzene-methanol (4:1)

R_F

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.80	D	0.96
B	0.97	E	0.83
C	0.90	F	0.66

*Ref.*T. Yahagi, H. Sugawara, M. Uramoto and S. Suzuki, *J. Antibiot.*, 27 (1974) 143-144**TLC***Medium*

Silica gel (Kieselgel G, Merck)

Solvents

- A. Water satd. *n*-butanol
- B. *n*-Butanol-methanol-water (4:1:2)
- C. Propanol-pyridine-acetic acid-water (15:10:3:10)
- D. *n*-Butanol-acetic acid-water (3:1:1)
- E. Methanol-ethyl acetate (15:100)

R_F

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.53	D	0.87
B	0.56	E	0.32
C	0.88		

Ref.

As PC

LICHENIFORMIN**PC***Paper*

Whatman No. 4

*Solvent*Collidine-lutidine-aq. 2 *N* ammonia (1:1:2)*Detection*

- A. Ninhydrin spray
- B. Bioautography vs. *Staphylococcus aureus*
- C. Bioautography vs. *Mycobacterium phlei*

R_F

Licheniformin C > B > A

*Ref.*R.K. Callow and T.S. Work, *Biochem. J.*, 51 (1952) 558-567**LINCOMYCIN****PC 1***Solvents*

- A. 1-Butanol-water (84:16), 16 h
- B. 1-Butanol-water (84:16) + 0.25% *p*-toluene-sulfonic acid, 16 h
- C. 1-Butanol-acetic acid-water (2:1:1), 16 h
- D. 1-Butanol-water (84:16) + 2% piperidine, 16 h
- E. 1-Butanol-water (4:96), 5 h
- F. 1-Butanol-water (4:96) + 0.25% *p*-toluene-sulfonic acid, 5 h

*Detection*Bioautography vs. *Sarcina lutea*

R_F

Sol-vent	R_F^*	Sol-vent	R_F^*
A	0.40–0.50	D	0.60–0.81
B	0.40–0.45	E	0.81–0.95
C	0.70	F	0.90

* Estimated from drawing

Ref.

D.J. Mason, A. Dietz and C. Deboer, *Antimicrob. Agents Chemother.*, 1962 (1963) 554–559

PC 2

Paper

Whatman No. 1

Solvent

n-Butanol–water–isoamyl alcohol–dichloroacetic acid (100:75:50:1) R_F

S-ethyl homolog of lincomycin, 0.50

Ref.

E.L. Patterson, J.H. Hash, M. Links, P.A. Miller and N. Bohonos, *Science*, 146 (1964) 1691–1692

PC 3

Solvents

- A. 1-Butanol–water (84:16), 16 h
 B. 1-Butanol–water (84:16) + 0.25% *p*-toluene-sulfonic acid, 16 h
 C. 1-Butanol–acetic acid–water (2:1:1), 16 h
 D. *n*-Butanol–water (84:16) + 2% piperidine
 E. 1-Butanol–water (4:96), 5 h
 F. 1-Butanol–water (4:96) + 0.25% *p*-toluene-sulfonic acid, 5 h

 R_F

Solvent	R_F^* Lincomycin B
A	0.40–0.70
B	0.20–0.40
C	0.65
D	0.75
E	0.95
F	0.88

* Estimated from drawing

Ref.

A.D. Argoudelis, M.E. Bergy and J.A. Fox, *U.S. Patent* 3,359,164, Dec. 19, 1967

TLC 1

Medium

Silica gel G (Merck)

Solvents

- A. Methyl ethyl ketone–acetone–water (9.3:2.6:1)
 B. Methyl propyl ketone–methyl ethyl ketone–water–methanol (2:2:1:1)

Detection

Iodine vapor

 R_F

Lincomycin > U21,699 > U11,973

Ref.

T.F. Brodasky and W.L. Lummis, *Antimicrob. Agents Chemother.*, 1964 (1965) 18–23

TLC 2

Medium

Silica gel

Solvent

Ethyl acetate–acetone–water (8:5:1)

 R_F

- Clindamycin, 0.70 *
 1'-Demethylclindamycin, 0.17 *
 * Estimated from drawing

Ref.

A.D. Argoudelis, J.H. Coats, D.J. Mason and O.K. Sebek, *J. Antibiotics*, 22 (1969) 309–314

TLC 3

Medium

Silica gel G

Solvent

Ethyl acetate–acetone–water (8:5:1)

Detection

Bioautography vs. *Sarcina lutea* R_F

- Clindamycin, 0.6 *
 N-Demethyl-N-hydroxymethyl-clindamycin (compound A), 0.4 *
 Lincomycin, 0.3 *
 N-Demethylclindamycin, 0.2 *
 * Estimated from drawing

Ref.

A.D. Argoudelis, J.H. Coats and B.J. Magerlein, *J. Antibiot.*, 25 (1972) 191–193

TLC 4

Medium

Avicel

Solvent

Methanol–isobutyl alcohol–chloroform–10% hydrochloric acid (10:10:10:1)

Detection

- A. Iodine vapor
 B. 0.25% Stannous chloride soln.
 C. 0.2% *p*-Dimethylaminobenzaldehyde soln.

R_F

0.57

Ref.

As Ampicillin, TLC 4

TLC 5**Medium**

Silica gel

SolventMethyl ethyl ketone-acetone-water (9.3:2.6:1)
developed 30-45 min (15 cm)**Detection**

UV at 366 nm

R_F

0.54

Ref.M. Baudet, *J. Pharm. Belg.*, 31 (1976) 247-254**GLC****Apparatus**

Barber-Colman Model 500 with FID

ColumnGlass, U-shaped, 6 ft. × 3 mm I.D.; 5% SE-30 on
Gas-Chrom-Q (80-100 mesh)**Temperature**

Column 257°; detector 280°; injector 280°

Carrier gasN₂ at 20 p.s.i., 150 ml/min; H₂ at 32 p.s.i.; air
40 p.s.i.**Current**2 × 10⁻⁸ f.s.d. (sensitivity 100 and attenuation 2)**Silylating reagent**Nine parts of hexamethyldisilane mixed with one
part of trimethylchlorosilane. The mixture is
cleared by filtration**Lincomycin standard**About 4 mg of lincomycin reference standard,
accurately weighed and transferred to a centri-
fuge tube**Internal standard**A saturated soln. of tetraphenylcyclopentadienone prepared in cyclohexane, and cleared
by filtration**Derivatization**A lincomycin standard is treated in the same
manner as the samples. Each dry or dried sample
is dissolved in 1 ml of pyridine, and 0.2 ml of the
silylating reagent is added. The reaction mixtures
are allowed to stand not less than 30 min. 1 ml of
the internal standard soln. and 2 ml of water are
added, and the mixture is shaken vigorously. The
phases are separated by gravity or centrifugation**Chromatography and calculations**

Five μl of the cyclohexane phase are injected into

the gas chromatograph. The areas of each peak
are measured by planimetry or by disc integra-
tion. The lincomycin content is determined by
direct comparison of the ratio of the peak areas
(lincomycin-internal standard) with that of the
lincomycin reference standard treated in an iden-
tical manner. Lincomycin B content is de-
termined as a fraction of the combined linco-
mycin plus lincomycin B**Retention times of lincomycin and other compounds**

	Retention time (min)	Relative retention time
Lincomycin	7.65	1.00
Lincomycin B	6.12	0.78
Lactose	4.11, 6.00	0.54, 0.78
Sucrose	4.35	0.57
Internal standard	11.10	1.45

Ref.M. Margosis, *J. Chromatogr.*, 37 (1968) 46-54**LINCOMYCIN 2-MONOESTERS****TLC****Medium**

Silica gel G

SolventHexane-diethyl ether-2-pentanone-methanol-
conc. ammonium hydroxide (60:20:20:9:1)**Detection**Plates sprayed with ammonium sulfate and heated
to about 180°**Ref.**W. Morozowich, A.A. Sinkula, F.A. MacKellar
and C. Lewis, *J. Pharm. Sci.*, 62 (1973) 1102-105**GC****Procedure**Conversion of lincomycin 2-acyl esters to corre-
sponding 3,4,7-tris-O-trimethylsilyl ethers. A 1%
soln. of the ester in pyridine containing 20%
hexamethyldisilazane and 20% trimethylchloro-
silane used to give the silyl ethers. After 5 min
reaction period, injected 1 ml into chromato-
graph**Apparatus**

Gas chromatograph

ColumnStainless steel (0.6 × 33 cm) containing 3% OV-1
on Gas Chrom Q (100-120 mesh)

Detection

Flame-ionization

Temperature

235–290°C depending on ester chain length

Ref.

As TLC

LIPIARMYCIN**PC****Paper**

Whatman No. 1

Solvents

- A. Water satd. *n*-butanol
- B. Water satd. *n*-butanol + 2% *p*-toluenesulfonic acid
- C. Water satd. *n*-butanol + 2% conc. ammonia
- D. *n*-Butanol satd. water
- E. Ammonium chloride (20% in water)
- F. *n*-Butanol–methanol–water (40:10:20) containing 0.75 g methyl orange
- G. *n*-Butanol–methanol–water (40:10:30)
- H. Water–acetone (1:1)
- I. Water satd. ethyl acetate

DetectionBioautography vs. *Staphylococcus aureus* **R_F**

Solvent	R_F	Solvent	R_F
A	0.95	F	0.95
B	0.95	G	0.95
C	0.95	H	0.80
D	0.00	I	0.65
E	0.00		

Ref.C. Coronelli, R.J. White, G.C. Lancini and F. Parenti, *J. Antibiot.*, 28 (1975) 253–259**TLC****Medium**Silica gel HF/UV₂₅₄**Solvent**

Chloroform–methanol (9:1)

Detection

Spot detected under UV light and by spraying with conc. sulfuric acid containing vanillin; heat at 100°C

 R_F

0.35

Ref.

As PC

LIPOXAMYCIN**PC****Paper**

Whatman No. 1

Solvents

- A. 1-Butanol–water (4:96) + 0.25% *p*-toluenesulfonic acid, 5 h
- B. 1-Butanol–water (84:16), 16 h
- C. 1-Butanol–water (84:16) + 0.25% *p*-toluenesulfonic acid, 16 h
- D. 1-Butanol–acetic acid–water (2:1:1), 16 h
- E. 2% Piperidine in 1-butanol–water (84:16), 16 h
- F. 1-Butanol–water (4:96), 5 h
- G. 1-Butanol–water (4:96) + 0.25% *p*-toluenesulfonic acid, 5 h

DetectionBioautography vs. *Saccharomyces pastorianus* **R_F (estimated from drawing)**

Solvent	R_F	Solvent	R_F
A	0.70	E	0.20, 0.50
B	0.40–0.80	F	0.00, 0.50
C	0.42–0.70	G	0.38–0.65
D	0.38, 0.78		

Ref.O.K. Sebek and H.A. Whaley, *U.S. Patent* 3,629,402, Dec. 21, 1971. H.A. Whaley, O.K. Sebek and C. Lewis, *Antimicrob. Agents Chemother.*, 1970 (1971) 455–461**CCD****Solvent**Ethyl acetate–ethylene glycol monoethyl ether–water (2:1:1); 200 transfers of lipoxamycin–*p*-toluenesulfonic acid salt**Distribution**

Lipoxamycin, free from coproduced antibiotic, was obtained from a pool of the contents of tubes 40–49

Ref.

As PC

**LIVIDOMYCIN B, DENEOSAMINYL-
(See deneosaminyllividomycin B)****LIVIDOMYCINS****PC 1****Paper**

Toyo No. 51

Solvent

n-Butanol satd. with water-*p*-toluenesulfonic acid-*tert*-butanol (88:2:10), 40 h

Detection

As TLC 2

R_F

Lividomycin B moves 11.2 cm

Ref.

T. Mori, Y. Kyotani, I. Watanabe and T. Oda, *J. Antibiot.*, 25 (1972) 149–150

PC 2**Paper**

Ascending

Solvent

Chloroform-methanol-17% aq. ammonia (2:1:1) lower layer (developed at room temp. for 12 h)

R_F

Lividomycin A, 0.00

Lividomycin B, 0.03

Lividomycin D, 0.02

Ref.

As Apramycin, PC 1

PC 3**Solvent**

n-Butanol-pyridine-water-acetic acid (6:4:3:1)

R_F

Lividomycin B, 1.0

6'-Amino-6'-deoxylividomycin B, 0.5

Ref.

S. Umezawa, H. Umezawa, O. Tsuchiya and I. Watanabe, *U.S. Patent* 3,956,274, May 11, 1976

TLC 1**Media**

A. Silica gel D-5 (Camag)

B. Aluminium Oxide G, Type E (Merck)

Solvent

Chloroform-methanol-17% ammonia (2:1:1), upper layer

R_F

	Medium A	Medium B
Lividomycin A	0.64	0.36
Lividomycin B	0.65	0.73

Ref.

T. Mori, T. Ichianagi, H. Kondo, K. Tokunaga and T. Oda, *J. Antibiot.*, 24 (1971) 339–346

TLC 2**Media**

A. Silica gel D-5 (Camag)

B. Aluminium Oxide G, Type E (Merck)

Solvents

A. Chloroform-methanol-17% ammonium hydroxide (2:1:1), upper layer

B. *n*-Butanol-acetic acid-water (1:1)

C. Methanol-10% ammonium acetate (1:1)

Detection

A. Ninhydrin

B. Bioautography

R_F

Solvent	Medium	Lividomycin B
A	A	0.57
B		0.26
C		0.54
A	B	0.79

Ref.

As PC 1

TLC 3**Medium**

Cellulose

Solvent

Methyl ethyl ketone-methanol-isopropanol-7.9 *N* ammonium hydroxide (10:8.5:3:7)

Detection

Ninhydrin. Spray with 0.3% soln. of ninhydrin in water satd. *tert*-butanol, heat at 100–105° for 3–5 min

R_F

Lividomycin A, 0.43

Lividomycin B, 0.70

Ref.

N.R. Chatterjee, *Indian J. Chem.*, 13 (1975) 1282–1284

ELPHO 1**Medium**

Toyo No. 51

Buffer

Formic acid-acetic acid-water (2:75:900), pH 1.8

Conditions

3000 V (20 mA/10 cm)

Detection

Ninhydrin

Mobility (R_M*)

Lividomycin A, 1.78

Lividomycin B, 2.10

* Relative mobility to alanine as 1.0

Ref.

As TLC 1

ELPHO 2**Medium**

Paper, Toyo No. 51

Buffer

Formic acid–acetic acid–water (22:75:900), pH 1.8

Conditions

3000 V (1 mA/1 cm)

Detection

As TLC 2

Mobility $R_M = 2.14$ (Lividomycin B). R_M = relative mobility to alanine as 1.0**Ref.**

As PC 1

GLC**Column**

0.3 × 100 cm tube, packed with 1.0% OV-1 on Gas-Chrom Q (100–120 mesh)

Carrier gas N_2 at a flow-rate of 30 ml/min**Temperature**

Oven temperature 265°C

Retention time

5.5 min

Ref.

As PC 1

LOMOFUNGIN**PC****Solvents**A. *n*-Butanol–water (84:16), 16 hB. *n*-Butanol–water (84:16) + 0.25% *p*-toluene-sulfonic acid, 16 hC. *n*-Butanol–acetic acid–water (2:1:1), 16 hD. *n*-Butanol–water (84:16) + 2% piperidine, 16 hE. *n*-Butanol–water (4:96), 5 hF. *n*-Butanol–water (4:96) + 0.25% *p*-toluene-sulfonic acid, 5 h **R_F**

Solvent	R_F^*	Solvent	R_F^*
A	0.00	D	0.00
B	0.10 and 0.20–0.30	E	0.30
C	0.79	F	0.10

* Estimated from drawing

Ref.L.E. Johnson and A. Dietz, *Appl. Microbiol.*, 17 (1969) 755–759**TLC****Medium**Silica gel H₂₅₄ (Merck). Solution of buffer salts (pH 6.7) composed of 0.2 *M* disodium phosphate and 0.2 *M* monopotassium phosphate. Air dry but not activate**Solvent**

Methyl ethyl ketone–methanol (94:6)

Detection

A. Visible light

B. Potassium permanganate–sodium metaperiodate spray

 R_F

0.4

Ref.M.E. Bergy, *J. Antibiot.*, 22 (1969) 126–128**LUCKNOMYCIN****CCD****Solvent**

Pyridine–ethyl acetate–water (35:65:83)

Distribution coefficient

261 transfers, 100 tubes

The optical densities of the bottom and top phases are measured; only one peak is observed

Ref.V.C. Vora and A.V. Mody, *U.S. Patent* 4,056,615, Nov. 1, 1977**LYBANOMYCIN****PC** **R_F**

Solvent	Component		
	A	B	C
A	0.65	0.78	0.40
B	0.60	0.85	–
C	0.55	0.75	–
D	0.90	0.90	–
E	0.50	0.75	0.30

Solvents

A. Butanol–pyridine–water (6:4:3)

B. Butanol satd. with phosphate buffer $\frac{1}{15}$ *M* at pH 3

C. Same as B with buffer at pH 5.4

D. Same as B with buffer at pH 7.0

E. Same as B with buffer at pH 8.0

Detection

Bioautography vs. *Bacillus subtilis*

Ref.

Belgium Patent 714,243, Oct. 25, 1968

LYDIMYCIN

PC

Solvents

A. 1-Butanol–water (86:16), 16 h

B. 1-Butanol–water (84:16) + 0.25% *p*-toluene-sulfonic acid, 16 h

C. 1-Butanol–acetic acid–water (2:1:1), 16 h

D. 2% Piperidine in 1-butanol–water (84:16), 16 h

E. 1-Butanol–water (4:96), 5 h

F. 1-Butanol–water (4:96) + 0.25% *p*-toluene-sulfonic acid, 5 h

Detection

Bioautography vs. *Saccharomyces pastorianus*

R_F

Solvent	$R_F^{\bullet}$	Solvent	$R_F^{\bullet}$
A	0.18	D	0.19
B	0.45	E	0.90
C	0.80	F	0.88

[•] Estimated from drawing

Ref.

M.E. Bergy, J.H. Coats and L.J. Hanka, *U.S. Patent* 3,395,220, Feb. 23, 1965

LYSOCELLIN

TLC

Medium

Silica gel

Solvents

A. Ethyl acetate

B. Benzene–methanol (9:1)

C. Chloroform–methanol (9:1)

R_F

Solvent A, 0.54

Solvent B, 0.30

Solvent C, 0.63

Ref.

E. Ebata, H. Kasahara, K. Sekine and Y. Inoue, *J. Antibiot.*, 28 (1975) 118–121

LYSOZYME

ELPHO

Medium

Whatman No. 1 paper

Buffers

A. 0.05 *M* Veronal (pH 8.0 and 9.0) and glycine (pH 10.0) buffers were used

B. Trisethylenediamine tetraacetate buffer, pH 8.9

Conditions

A. A current of 4.5 mA and 90 V for 18 h at room temperature, for "A" buffer

B. A current of 6.5 mA for 16 h, for "B" buffer

Detection

After drying, the strips are stained with amide black 10B

Ref.

J. Hawiger, *J. Bacteriol.*, 95 (1968) 376–384

MACARBOMYCINS

PC 1

Solvent

n-Butanol–pyridine–water (4:1:4)

R_F

0.29 (major)

0.47 and 0.56 (minor)

Ref.

H. Umezawa, K. Maeda, K. Nitta, M. Okanishi and S. Takahashi, *U.S. Patent* 3,564,090, Feb. 16, 1971

PC 2

Paper

Toyo Roshi No. 51

Solvent

n-Butanol–pyridine–water (4:1:4)

Detection

Bioautography

R_F

Component	R_F
Macarbomycin	0.34
Macarbomycin I _a	0.55
Macarbomycin I _b	0.26
Macarbomycin II	0.26
Macarbomycin III	0.40

Ref.

S. Takahashi, M. Miyamoto, S. Fukatsu, K. Maeda and H. Umezawa, *J. Antibiot.*, 26 (1973) 542–544

PC 3**Paper**

Schleicher and Schull 2040b (descending, 15 h)

Solvents

- A. *n*-Butanol-pyridine-water (4 : 1 : 4), upper phase, (chamber satd. with lower phase)
 B. *n*-Butanol-*n*-propanol-0.5 *N* ammonia (3 : 1 : 4), upper phase (chamber satd. with lower phase)

DetectionBioautography vs. *Staphylococcus aureus* FDA 209 P **R_F**

Component	R_a^*	
	Solvent A	Solvent B
M1	1.16	1.12
M2	1.34	1.31
M3	1.00	1.00
M4	1.24	1.20
M5	0.87	0.90
M6	0.87	0.96

* R_a = Migration in relation to diumycin A = 1.00**Ref.**

As Diumycin, PC 2

TLC 1**Medium**Silica gel GF₂₅₄ (E. Merck)**Solvent***n*-Propanol-2 *N* aq. ammonia (70 : 30)**Detection**

- A. UV light at 253.6 nm
 B. Spray with chlorosulfonic acid-acetic acid (1 : 2)
 C. Exposure to iodine vapor

 R_F

0.25

Ref.S. Takahashi, A. Okanishi, R. Utahara, K. Nitta, K. Maeda and H. Umezawa, *J. Antibiot.*, 23 (1970) 48-50**TLC 2****Medium**Silica gel (F₂₅₄, E. Merck)**Solvents**

- A. Isopropanol-2 *N* ammonia (7 : 3)
 B. Chloroform-ethanol-water (2 : 3 : 1)

Detection

- A. UV at 254 nm
 B. Dark blue spots by spraying with a reagent

consisting of 1% (w/v) ceric sulfate and 1.5% (w/v) molybdic acid in 10% (v/v) sulfuric acid followed by heating for 15 min at 105°C

 R_F

Component	Solvent A	Solvent B
M1	0.30	0.27
M2	0.27	0.34
M3	0.25	0.24
M4	0.22	0.32
M5	0.20	0.22
M6	0.15	0.18

Ref.

As Diumycin, PC 2

TLC 3**Medium**Silica gel GF₂₅₄ (E. Merck)**Solvents**

- A. *n*-Propanol-2 *N* ammonia (7 : 3)
 B. Isopropanol-water-0.5 *N* borate buffer, pH 9.0 (70 : 25 : 5)

Detection

- A. UV at (2536Å) nm
 B. Iodine vapor

 R_F

Component	Solvent A	Solvent B
Macarbomycin	0.25	0.33
Macarbomycin I _a	0.20	0.40
Macarbomycin I _b	0.20	0.34
Macarbomycin II	0.15	0.25
Macarbomycin III	0.30	0.29

Ref.

As PC 2

MACROMOMYCIN**ELPHO****Medium****Paper****Buffer**Barbital buffer (pH 8.6, μ = 0.05)**Conditions**

450 V, 4.5 h

Mobility

Moved 2.0 cm to cathode

Ref.H. Umezawa, T. Takeuchi, M. Hamada, M. Ishizuki, H. Chimura and K. Maeda, *U.S. Patent* 3,595,954, July 27, 1971

MACROSIN

PC

Solvents

- A. Methyl ethyl ketone on pH 4 buffered paper
- B. Methyl ethyl ketone
- C. *n*-Butanol satd. with water on pH 4 buffered paper
- D. *n*-Butanol satd. with water
- E. Water with 7% sodium chloride and 2.5% methyl ethyl ketone
- F. Ethyl acetate satd. with water on pH 4 buffered paper

R_F

Solvent	R_F	Solvent	R_F
A	0.45	D	0.80
B	0.66	E	0.74
C	0.92	F	0.39

Ref.

R.L. Hamill and W.M. Stark, *J. Antibiot.*, 17 (1964) 133–139

MAGNAMYCIN

(cf., carbomycin)

PC 1

Solvents

- A. Ethanol–hexane–water (90:10:0.15), 4 h, descending
- B. Benzene–water–acetic acid (1:9:0.5), 4 h, ascending
- C. *n*-Butanol–water–acetic acid (4:5:1), 16 h, ascending
- D. Water satd. with *n*-butanol, 4 h, ascending

Detection

Bioautography vs. *M. pyogenes* var. *aureus*

R_F

Solvent	R_F	Solvent	R_F
A	0.70	C	0.90
B	0.80	D	0.95

Ref.

J.F. Pagano, M.J. Weinstein and C.M. McKee, *Antibiot. Chemother.*, 3 (1953) 899–902

PC 2

Paper

Whatman No. 1

Solvents

- A. 80% Methanol + 3% sodium chloride (1:1) descending. Paper buffered with 0.95 *M* sodium sulfate + 0.05 *M* sodium bisulfate
- B. Propanol–pyridine–acetic acid–water (6:4:1:3), ascending
- C. Butanol–acetic acid–water (4:1:5), ascending
- D. 80% Phenol, ascending

Detection

Bioautography vs. *Staphylococcus aureus*

R_F

Solvent	R_F	Solvent	R_F
A	0.90	C	0.78
B	0.93	D	0.86

Ref.

As Chloramphenicol, PC 5

TLC 1

Solvents

- A. Benzene–methanol (55:45)
- B. Butanol–acetic acid–water (3:1:1)

R_F

Solvent A, 0.75
Solvent B, 0.49

Ref.

N. Nishimura, K. Kumagai, M. Ishida, K. Saito, F. Kato and M. Asumi, *J. Antibiot.*, 18 (1965) 251–258

TLC 2

Medium

Silica gel GF

Solvents

- A. Chloroform–methanol–17% ammonia (2:1:1)
- B. Butanol–acetic acid–water (3:1:1)

Detection

Spray with conc. sulfuric acid–methanol (1:1), developed by heating at 105°C for several min

R_F

Solvent A, 0.98 (blue purple)
0.40 (purple)
Solvent B, 0.47 (red purple)

Ref.

As Chloramphenicol, PC 5

MAGNAMYCIN A and B

FLC

Instrument

JASCO FLC-150

Detector

JASCO UVIDEK-2 (232.5 nm) spectrophotometer

Separation mode

Partition

Column

JASCOPACK SV-02-500

Column temperature

Ambient

Column pressure

65 kg/cm²

Mobile phase

Methanol- $\frac{1}{15}$ M acetate buffer (pH 4.9)-acetonitrile (35:60:5)

Flow-rate

1.0 ml/min

Retention time (min)

Magnamycin A, 18.5 min

Magnamycin B, 28.0 min

Ref.

As Tylosin, FLC 1

MAGNESIDIN**TLC****Media**

A. Silica gel F₂₅₄ on aluminum foil

B. Kieselguhr

Solvents

A. Benzene-acetone (1:1)

B. Light petroleum (60-80°)-acetone (3:2)

C. Benzene-ethyl acetate (1:1)

D. Acetic acid-water (4:1)

Detection

A. Spray with 5% iron trichloride in 0.5 M hydrochloric acid; both compounds give a reddish brown color

B. Spray with 2,4-dinitrophenyl hydrazine; both compounds show an orange-yellow color

R_F

Solvent	Medium A	Medium B
A	0.54	
B	0.19	
C	0.15	
D		0.50-0.77) (two compounds)

Ref.

J. Nazareth, N.M. Gandhi, P.V. Divekar, N.J. de Souza and H. Kohl, *U.S. Patent* 3,968,122, July 6, 1976

MALTOTETRAOSE**TLC****Medium**

Silica gel

Solvents

A. *n*-Butanol-ethanol-water (5:3:2)

B. *n*-Propanol-ethyl acetate-water (6:1:3)

C. *n*-Butanol-acetic acid-water (2:3:1)

Detection

Spot detected by heating at 100°C for 10 min after spraying 2 N aniline-2 N phosphate soln.

R_F

Solvent A, 0.21

Solvent B, 0.47

Solvent C, 0.37

Ref.

H. Kondo, T. Honke, R. Hasegawa, T. Shimoda and S. Nakamura, *J. Antibiot.*, 28 (1975) 157-160

MANNOPEPTINS**PC****Solvents**

A. Water satd. butanol

B. 80% Aq. phenol

C. 50% Aq. methanol

D. 50% Aq. acetone

E. *n*-Butanol-pyridine-acetic acid-water (15:10:3:12)

F. Water

G. *n*-Butanol-acetic acid-water (4:1:2)

H. 0.1 N Sulfuric acid-dimethylformamide (1:1)

I. 0.1 N Sulfuric acid-acetone (1:1)

Detection

Bioautography vs. *Staphylococcus aureus* FDA 209P

R_F

Solvent	R _F	Solvent	R _F
A	0.00	G	0.1 ≈ 0.25
B	0.00		(tailing)
C	0.00	H	0.8
D	0.00	I	0.67
E	0.00		(Mannopeptin A)
F	0.1 ≈ 0.25		0.80
	(tailing)		(Mannopeptin B)

Ref.

T. Hayashi, Y. Harada and K. Ando, *J. Antibiot.*, 28 (1975) 503-507

MANNOSIDOSTREPTOMYCIN (See streptomycin)

MARIDOMYCINS (cf., B-5050 and tetrahydro-B-5050)

PC

Paper

Toyo Roshi No. 50 impregnated with a 2% liquid paraffin soln. in ligroin

Solvent

$\frac{1}{15}$ M phosphate buffer soln. of pH 8.0 satd. with *n*-butyl acetate

Detection

Bioautography vs. *Bacillus subtilis*

R_F

Component	R_F	Component	R_F
A	0.32	D	0.72
B	0.38	E	0.74
C	0.66	F	0.32

Ref.

E. Higashide, T. Hasegawa, H. Ono, M. Asai, M. Muroi and T. Kishi, *U.S. Patent* 3,691,280, Sept. 12, 1972

TLC 1

Media

- Spotfilm (Tokyo Kasei)
- Kieselgel G (Merck)

Solvents

- Benzene-acetone (3:2)
- Benzene-methanol (10:1)

Detection

- Conc. sulfuric acid
- 5% Iodine in chloroform
- 5% Phosphomolybdic acid in ethanol
- 5% Ceric sulfate in sodium sulfate

R_F

	Solvent (Medium)		
	A (A)	A (B)	B (B)
Maridomycin I	0.48	0.68	0.71
Maridomycin II	0.42	0.63	0.66
Maridomycin III	0.37	0.57	0.61
Maridomycin IV	0.32	0.53	0.55
Maridomycin V	0.30	0.50	0.52
Maridomycin VI	0.43	0.43	0.48

Ref.

M. Muroi, M. Isawa, M. Asai, T. Kishi and K. Mizuno, *183rd Scientific Mtg. of Japan Antibiotic Res. Assn.*, May 26, 1972

TLC 2

Medium

Silica gel (Spotfilm f) Tokyo Kasei K.K. *

* Same as Kieselgel G

Solvent

Benzene-acetone (3:2)

Detection

Conc. phosphoric acid spray

R_F

Component	R_F
I	0.48
II	0.42
III	0.37
IV	0.32
V	0.30
VI	0.27

Ref.

As PC, plus M. Muroi, M. Izawa, M. Asai, T. Kishi and K. Mizuno, *J. Antibiot.*, 26 (1973) 199-205

TLC 3

Medium

Silica gel (Kieselgel G) (Merck)

Solvents

- Benzene-acetone (3:2)
- Benzene-methanol (10:1), developed three times

R_F

Component	Solvent A	Solvent B
I	0.68	0.71
II	0.63	0.66
III	0.57	0.61
IV	0.53	0.55
V	0.50	0.52
VI	0.43	0.48

Detection

- 5% Phosphomolybdate soln. in ethanol sprayed and dried at 110°C for 5 min
- 5% Cerium sulfate soln. in *N* phosphoric acid sprayed and dried at 110°C for 5 min
- 10% Aq. phosphoric acid soln. sprayed and dried at 110°C for 5 min

- D. Conc. phosphoric acid
E. 5% I₂ soln. in chloroform

Ref.

As TLC 2

TLC 4

Medium

Precoated plate, Spot film (20 × 20 cm) (Tokyo-Kasie Co.)

Solvent

Hexane-isopropyl ether-ethanol-water
(1:4:3:2)

Detection

A Beckman Model DU spectrophotometer was used for the determination

R_F

Component of PMDM III TSC **	R_s *
I'	1.4
II'	1.2
III'	1.0
IV'	0.8
V'	0.7
VI'	0.6

$$* R_s = \frac{R_F \text{ value of each separated zone of sample}}{R_F \text{ value of standard PMDM III TSC}}$$

** 9-Propionylmaridomycin III thiosemicarbazone

Ref.

N. Kunishige, K. Kawamura, M. Muroi and T. Kishi, *Chem. Pharm. Bull.*, 23 (1975) 3075-3080

TLC 5

Medium

Silica gel G type 60 (E. Merck)

Solvent

n-Hexane-diisopropyl ether-ethanol-water
(3:20:5:4) upper layer

R_F

9-Propionylmaridomycin	R_F (estimated from drawing)
I	0.60
II	0.55
III	0.47
IV	0.40
V	0.34
VI	0.29

Detection

Iodine vapor

The plates were dried overnight at room temperature, then activated at 110° for 2 h

Ref.

K. Kondo, *J. Chromatogr.*, 169 (1979) 337-342

MECILLINAM

TLC

Medium

Silica gel GF₂₅₄

Solvent

n-Butyl acetate-isopropanol-acetic acid-0.07 M citrate buffer (pH 7) (90:60:75:30)

Detection

Iodine vapor followed by spraying with 1% aq. starch soln.

Ref.

R.B. Hagel, E.H. Waysek and D. Humphrey, *J. Chromatogr.*, 170 (1979) 391-398

HPLC

Apparatus

All of the following components were used interchangeably. Pumps: Model 396 Mini Pump (Laboratory Data Control); Model 6000 A Chromatographic Pump (Waters Assoc.)

Column

30 cm × 3.9 mm I.D. μ Bondapak C₁₈ (Waters Assoc.)

Mobile phase

Acetonitrile-buffer (15:85)

Flow-rate

1.0 ml/min

Injectors

Model U6K (Waters Assoc.); Model 7120 (Rheodyne)

Detection

Model 970A variable wavelength (Tracor); Model 1202 variable wavelength (Laboratory Data Control), 220 nm

Sensitivity

0.32 a.u.f.s.

Recorder

10 in. strip chart recorder

Sample solutions

0.1 mg/ml

Retention time

9.0, estimated from drawing

Ref.

As TLC

MEGALOMICIN A, 4'-PROPIONYL- (See 4'-propionyl-megalomicin A)

MEGALOMICINS

PC 1

Solvents

- A. 80% Methanol + 3% sodium chloride (1:1), descending. Paper buffered with 0.95 *M* sodium sulfate + 0.05 *M* sodium bisulfate
- B. Propanol-pyridine-acetic acid-water (6:4:1:3), ascending
- C. Butanol-acetic acid-water (4:1:5), ascending
- D. 80% Phenol, ascending

Detection

Bioautography vs. *Staphylococcus aureus*

R_F

Solvent	R_F	Solvent	R_F
A	0.90	C	0.78
B	0.93	D	0.86

Ref.

M.J. Weinstein, G.M. Luedemann, G.H. Wagman and J.A. Marquez, *U.S. Patent* 3,632,750, Jan. 4, 1972

PC 2

Paper

Toyo No. 51

Solvent

Water satd. ethyl acetate (ascending, 3 h)

Detection

Bioautography vs. *Bacillus subtilis* No. 10707

R_F

0.00

Ref.

As Carbomycin, PC 1

PC 3

Paper

Toyo No. 51

Solvents

- A. 20% Ammonium chloride
- B. Water satd. *n*-butanol
- C. *n*-Butanol-acetic acid-water (3:1:1)
- D. Water satd. ethyl acetate
- E. Water satd. *n*-butanol + 2% *p*-toluenesulfonic acid + 2% piperidine

Detection

Bioautography vs. *Bacillus subtilis* No. 10707

R_F

Solvent	R_F	Solvent	R_F
A	0.60	D	0.00
B	0.85	E	0.92
C	0.75		

Ref.

As Carbomycin, PC 5

TLC 1

Medium

Silica gel G

Solvent

Chloroform-methanol (60:40)

Detection

Bioautography vs. *Staphylococcus aureus*

R_F

Megalomicin A,	0.19
Megalomicin B,	0.38
Megalomicin C ₁ ,	0.52
Megalomicin C ₂ ,	0.65

Ref.

M.J. Weinstein, G.H. Wagman, J.A. Marquez, E.M. Oden, R.T. Testa and J.A. Waitz, *Antimicrob. Agents Chemother.*, 1968 (1969) 260-261.
J.A. Marquez, A. Murawski and G.H. Wagman, *J. Antibiot.*, 22 (1969) 259-264

TLC 2

Solvents

- A. Chloroform-methanol-17% ammonia (2:1:1)
- B. Butanol-acetic acid-water (3:1:1)

Detection

- A. Plates are sprayed with a mixture of conc. sulfuric acid-methanol (1:1) and developed by heating at 105°C for several min
- B. Bioautography vs. *Staphylococcus aureus*

R_F

Solvent	Color by sulfuric acid spray	R_F
A	Blue black	0.98
B	Red purple	0.13

Ref.

M.J. Weinstein, G.M. Luedemann, G.H. Wagman and J.A. Marquez, *U.S. Patent* 3,632,750, Jan. 4, 1972. M.J. Weinstein, G.H. Wagman, J.A. Marquez, R.T. Testa, E.M. Oden and J.A. Waitz, *J. Antibiot.*, 22 (1969) 253-258

TLC 3*Medium*

Alumina Type E (Merck)

Solvent

Ethyl acetate-methanol (9:1), 3 h

*Detection*Bioautography vs. *Bacillus subtilis* No. 10707*R_F*Components separated; *R_F* values not given*Ref.*

As Carbomycin, PC 5

TLC 4*Medium*

Silica gel

Solvents

A. Chloroform-methanol-17% ammonia (2:1:1)

B. Butanol-acetic acid-water (3:1:1)

*Detection*A. Bioautography vs. *Sarcina lutea*

B. Plate heated at 100°C and sprayed with phosphoric acid in methanol (1:1)

R_F

Solvent	Detection	<i>R_F</i>
A	A	0.97
	B	0.97 black
B	A	0.21
	B	0.21 purple

Ref.

G.H. Wagman, J.A. Waitz, J. Marquez, A. Murawski, E.M. Oden, R.T. Testa and M.J. Weinstein, *J. Antibiot.*, 25 (1972) 641-646

TLC 5*Medium*

Aluminum Oxide Type E (Merck)

Solvent

Ethyl acetate-methanol (9:1)

R_F

Megalomicin C ₂ ,	0.85
Megalomicin C ₁ ,	0.80
Megalomicin B,	0.45
4'-Propionylmegalomicin A,	0.55
Megalomicin A,	0.20

Ref.

T. Nara, S. Takasawa, I. Kawamoto and S. Yamamoto, *German Patent* 2,301,080, July 19, 1973

MELANOSPORIN**TLC***Medium*

Silica gel

Solvent

Acetone-water (9:1)

Detection

15% Phosphoric acid

R_F

0.10

*Ref.*A. Rex and L. Arrieta, *J. Antibiot.*, 26 (1973) 126**MELINACIDINS****PC 1***Solvents*

A. 1-Butanol-water (84:16)

B. 1-Butanol-water (84:16) + 0.25% *p*-toluene-sulfonic acid

C. 1-Butanol-acetic acid-water (2:1:1)

D. 2% Piperidine in 1-butanol-water (84:16)

E. 1-Butanol-water (4:96)

F. 1-Butanol-water (4:96) + 0.25% *p*-toluene-sulfonic acid

G. 0.5 M Phosphate buffer pH 7.0

H. 0.075 N Ammonium hydroxide satd. methyl isobutyl ketone, lower phase

I. Benzene-methanol-water (1:1:2)

*Detection*Bioautography vs. *Bacillus subtilis**R_F*

Solvent	<i>R_F</i> *	Solvent	<i>R_F</i> *
A	0.65-0.90	F	0.30; 0.50
B	0.95	G	0.05-0.22
C	0.92	H	0.10; 0.30
D	0.95	I	0.25; 0.60;
E	0.20-0.48		0.80

* Estimated from drawing

Ref.

A.D. Argoudelis and F. Reusser, *J. Antibiot.*, 24 (1971) 383-389. *Netherlands Patent* 68,15117, April 29, 1969

PC 2*Solvents*

A. 1-Butanol-water (84:16), 16 h

B. 1-Butanol-water (84:16) + 0.25% *p*-toluene-sulfonic acid, 16 h

- C. 1-Butanol-acetic acid-water (2:1:1), 16 h
 D. 2% Piperidine (v/v) in 1-butanol-water (84:16), 16 h
 E. 1-Butanol-water (4:96), 5 h
 F. 1-Butanol-water (4:96) + 0.25% *p*-toluene-sulfonic acid, 5 h
 G. 0.5 *M* Phosphate buffer, pH 7.0, 5 h
 H. 0.075 *N* Ammonium hydroxide satd. with methyl isobutyl ketone, lower phase, 5 h
 I. Benzene-methanol-water (1:1:2), equilibrated 16 h, developed 5 h

Detection

Bioautography vs. *Bacillus subtilis*

R_F

Sol-vent	R_F^*	Sol-vent	R_F^*
A	0.60-0.80	F	0.30 and 0.50
B	0.80-0.90	G	0.10-0.20
C	0.88	H	0.10 and 0.30
D	0.95	I	0.25; 0.60
E	0.20-0.45		0.80

* Estimated from drawing

Ref.

A.D. Argoudelis, J.H. Coats and F. Reusser, *U.S. Patent* 3,369,581, Feb. 1, 1972

PC 3

Solvent

Benzene-methanol-water (1:1:2)

Detection

Bioautography vs. *Bacillus subtilis*

R_F (estimated from drawing)

Melinacidin II, 0.9
 Melinacidin IV, 0.8
 Melinacidin III, 0.7

Ref.

A.D. Argoudelis, *J. Antibiot.*, 25 (1972) 171-178

TLC

Medium

Silica gel G

Solvent

Toluene-ethyl acetate mixtures (50:50 or 60:40)

Detection

- A. Bioautography vs. *Bacillus subtilis*
 B. Periodate-permanganate spray reagent

R_F

Melinacidin II, 0.40
 Melinacidin III, 0.25
 Melinacidin IV, 0.20

Ref.

As PC 3

CCD

Solvent

Cyclohexane-ethyl acetate-95% ethanol-water (1:1:1:1), 1000 transfers

Distribution

Tubes 260-300 contained melinacidin IV

Ref.

As PC 3

METACYCLINE**PC**

Solvents

- A. Benzene-nitromethane-pyridine (10:20:3); Whatman No. 4 satd. with McIlvaine's buffer, pH 3.5, ascending
 B. *n*-Butanol-acetic acid-water (4:1:5)
 C. *n*-Butanol-ammonium hydroxide-water (4:1:5); Whatman No. 1 satd. with 0.1 *M* disodium ethylene diaminetetraacetic acid, descending

Detection

- A. Bioautography vs. *Staphylococcus aureus* 8357 NCTO 82
 B. For better visibility the spots were treated with triphenyltetrazolium chloride after incubation

R_F

Solvent A, 0.49
 Solvent B, 0.81
 Solvent C, 0.39

Ref.

I.L. Kahan and H. Hammer, *Arzneim. Forsch.*, 25 (1975) 234-237

METHAMPICILLIN**PC**

Paper

Whatman No. 1

Solvent

n-Butanol-ethanol-water (4:1:5, v/v), 4°C organic phase (ascending), 18 h

R_F

0.23

Ref.

B. Gradnick and L. Fleischmann, *Arzneim.-Forsch.*, 23 (1973) 935-938

TLC

Medium

Kieselgel G (Merck)

Solvent

Acetone-*n*-butanol-isopropanol-ethyl acetate-water (7:7:7:5:5, v/v)

R_F

0.31

Ref.

As PC

METHICILLIN**HPLC***Column*

1 m × 2.1 mm I.D. × 6.3 mm O.D., 316 stainless steel. C 18/Porasil B packing

*Mobile phase*30% Methanol–70% 0.05 *M* ammonium carbonate*Pressure*

500 p.s.i.

Flow-rate

0.6 ml/min

Detector

UV (254 nm)

Sensitivity

0.16 a.u.f.s.

Type

Reversed-phase

Retention time

10.0 min (estimated from drawing)

Ref.

As Ampicillin, HPLC

METHOBOTTROMYCIN**PC** R_F

Solvent	R_F	Solvent	R_F
A	0.85	F	0.5
B	0.84	G	0.0
C	0.37	H	0.3
D	0.83	I	0.19
E	0.0		

Solvents

- A. *n*-Butyl alcohol satd. with 1% aqueous acetic acid
- B. *n*-Butyl alcohol satd. with 2% aqueous pyridine
- C. Ethyl acetate satd. with 1% aqueous acetic acid
- D. Ethyl acetate satd. with 0.1 *M* phosphate buffer (pH 7)
- E. Benzene–hexane–methanol–5% aqueous acetic acid (7:6:10:6)
- F. Benzene–hexane–methanol–5% aqueous pyridine (7:6:10:6)
- G. Benzene satd. with 1% aqueous acetic acid

H. Benzene satd. with 2% aqueous pyridine

I. Capryl alcohol satd. with 0.1 *M* pH 6 phosphate buffer (reverse phase)

Ref.

F.J. Wolf, W.J. Miller and L. Chalet, *U.S. Patent* 3,683,073, Aug. 8, 1972**TLC***Medium*

Silica gel

Solvent

Chloroform–methanol (94:6)

Detection

A. Iodine vapor

B. A brown stain indicates the presence of methobottromycin

 R_F

0.64

Ref.

As PC

METHOXYMETHYL 3-METHYL-7-PHENOXYACETAMIDOCEPH-3-EM-4-CARBOXYLATE**TLC***Medium*Silica gel F₂₅₄*Solvent*

Acetone–acetic acid–water (1% potassium permanganate) (50:10:15)

 R_F

0.8

Ref.

C. Sapino, Jr. and M.V. Ruggeri, *U.S. Patent* 3,890,310, June 17, 1975**9-METHYLERYTHROMYCIN B OXIME****TLC***Medium*

Silica gel G

Solvents

A. Chloroform–methanol–ammonia (95:5:1)

B. Benzene–methanol (85:15) (atmosphere satd. ammonia)

 R_F

Solvent A, 0.39

Solvent B, 0.62

Ref.

A.M. Von Esch, *U.S. Patent* 3,681,326, Aug. 1, 1972

METHYLNEOTHARAMYCIN A and B**TLC****Medium**

Silica gel

Solvents

A. Chloroform-methanol (10:1)

B. Anhydrous methanol at room temperature for 16 h

R_F

Solvent	Component A	Component B
A	0.71	
B		0.61

Ref.

T. Takeuchi, M. Miyamoto, M. Ishizuka, H. Naganawa, S. Kondo, M. Hamada, and H. Umezawa, *J. Antibiot.*, 29 (1976) 93-96

9-METHYLSTREPTIMIDONE**TLC****Medium**Silica gel, Kieselgel GF₂₅₄ (Merck)**Solvent**

Ethyl acetate

Detection

Bioautography vs. HeLa S3 cells

R_F

0.55

Ref.

N. Saito, F. Kitame, M. Kijuchi and N. Ishida, *J. Antibiot.*, 27 (1974) 206-214

METHYMYCIN**CCD****Solvent**

10 ml volumes of mutually satd. diethyl ether and $\frac{1}{2}$ M potassium phosphate buffer, pH 6.8, 24 transfers

DistributionPeak tubes No. 15 and 16. $K = 1.85$ **Ref.**

M.N. Donin, J. Pagano, J.D. Dutcher and C.M. McKee, *Antibiot. Ann.*, (1953-1954) 179-185

MIAMYCIN**CCD****Solvent**

Ethyl acetate-0.1 M phosphate buffer, pH 6.9, 120 transfers

Distribution

The peak concentration was found around tube 40

Ref.

H. Schmitz, M. Misiek, B. Heinemann, J. Lein and I.R. Hooper, *Antibiot. Chemother.*, 7 (1957) 37-39

MICROMONOSPORA CHALCEA ANTIBIOTIC**PC****Solvent**

Benzene-hexane-acetone (20:5:6)

R_F

7 biologically active components

Ref.

G.F. Gause, M.G. Brazhnikova, U.A. Shorin, T.S. Moksimova, O.L. Olkhovatova, M.K. Kudinova, I.A. Vishnyakova, N.S. Pezner and S.P. Shapovalova, *Antibiotiki*, 15 (1970) 483-486

TLC**Medium**

Silicic acid

Solvent

Benzene-acetone (5:1)

R_F

As PC

Ref.

As PC

MICROMONOSPORIN**PC 1****Paper**

As everninomicins, PC 1

Solvent

As everninomicins, PC 1

Detection

As everninomicins, PC 1

R_F

1.0

Ref.

As everninomicins, PC 1

PC 2**Paper**

Toyo No. 51

Solvents

A. 20% Ammonium chloride

B. Water satd. *n*-butanolC. *n*-Butanol-acetic acid-water (3:1:1)

D. Water satd. ethyl acetate

E. Water satd. *n*-butanol + 2% *p*-toluenesulfonic acid + 2% piperidine

Detection

Bioautography vs. *Bacillus subtilis* No. 10707

R_F

Solvent	R_F	Solvent	R_F
A	0.18	D	0.25
B	0.75	E	0.70
C	0.78		

Ref.

As Carbomycin, PC 1

MICROPOLYSPORINS

PC

Solvents

- A. Butanol satd. with water
- B. Methanol
- C. Butanol satd. with water and 2% piperidine
- D. Butanol-pyridine-water (1:0.6:1)
- E. Butanol-acetic acid-water (2:1:1)
- F. Butanol satd. with water and 2% *p*-toluenesulfonic acid

Detection

Bioautography vs. *Sarcina lutea*

R_F

Solvent	Component	R_F
A	Micropolysporin A	0.35-0.41
	Micropolysporin B	0.07-0.13
B	Micropolysporin A	0.82
C	Micropolysporin A	0.40
D	Micropolysporin A	0.67; 0.84
E	Micropolysporin A	0.83
F	Micropolysporin A	0.60

Ref.

N.O. Blinov, E.A. Babkova and L.V. Kalakutskii, *Antibiotiki*, 11 (1966) 487-590

MIHARAMYCINS

TLC

Media

- A. Silica gel
- B. Cellulose

Solvents

- A. Chloroform-methanol-17% aq. ammonia (2:1:1), upper layer

B. *n*-Propanol-pyridine-acetic acid-water (15:10:3:10)

Detection

A. Ninhydrin with Medium A

B. Potassium permanganate. The A substance is quickly oxidized but the color of the B substance takes place after 2-3 min with Medium B

R_F

Solvent	Medium A		Medium B	
	Miharamycin			
	A	B	A	B
A	0.84	0.62	0.72	0.55
B	0.70	0.64		

Ref.

T. Niida H. Yumoto, T. Tsuruoka, K. Hamamoto, J. Shomura and T. Ohashi, *U.S. Patent* 3,678,159, July 18, 1972

ELPHO

Medium

Paper

Buffer

pH 5.0

Conditions

350 V, 3 h

Mobility

Miharamycin A: migrates 2.3 cm toward (-) pole

Miharamycin B: migrates 2.5 cm toward (-) pole

Ref.

As TLC

MILDIOMYCIN

PC

Paper

Whatman No. 1 (W & R Balston)

Solvents

- A. Butanol-acetic acid-water (2:2:1)
- B. Propanol-water (7:3)
- C. Isopropanol-5% ammonium hydroxide (6:4)

R_F

Solvent A, 0.13

Solvent B, 0.27

Solvent C, 0.26

Ref.

As Ampipurimycin, HPLC

TLC**Medium**Kieselgel F₂₅₄ (Merck)**Solvents**

- A. Chloroform-methanol-17% ammonium hydroxide (2:1:1), upper layer
 B. Propanol-pyridine-acetic acid-water (15:10:3:10)
 C. Ethyl acetate-acetone-acetic acid-water (45:5:5:45), lower layer

Detection

Single spot in all solvent systems

R_F

- Solvent A, 0.34
 Solvent B, 0.15
 Solvent C, 0.38

Ref.

As Amipurimycin, HPLC

ELPHO**Medium**

Paper, Whatman No. 1, 500 V, 2 h

Buffers

- A. 0.1 M Citrate buffer, pH 3.62
 B. 0.15 M Phosphate buffer, pH 7.05
 C. 0.1 M Glycine/sodium chloride-sodium hydroxide (pH 9.32)

Mobility

- Buffer A, 3.3 cm
 Buffer B, 1.1 cm
 Buffer C, 0.7 cm

Ref.

As Amipurimycin, HPLC

HPLC**Apparatus**

Model ALC/GPC 244 (Waters Assoc.)

Column

μBondapak C₁₈ (Waters Assoc.) 30 cm × 14 mm
 I.D., prepacked

Mobile phase

- A. Methanol-0.01 M phosphate buffer, pH 5.7 (2:8)
 B. Methanol-0.005 M citrate buffer, pH 6.0 (2:8)
 C. Methanol-0.005 M citrate buffer, pH 5.8 (5:95)
 D. 0.005 M Citrate buffer, pH 5.8

Flow-rate

1.0 ml/min

Pressure

1500-2000 p.s.i.

Detection

UV Model 440, 254 nm. Single peak observed under various conditions

Injector

Universal Injector U6K (Waters Assoc.), 2 ml of internal volume

Type

Reversed-phase

Retention time (min)

Compound	Mobile phase			
	A	B	C	D
Mildio-mycin	6.0	4.0	6.2	8.6
Mildio-mycin formate	6.0	4.1	6.4	8.6
Mildio-mycin N-benzoate	14.7	12.9	N.E. *	
Dihydro-N-mildio-mycin	6.6	4.3	5.1	13.5

* Not eluted

Ref.

As Amipurimycin, HPLC

MINIATOMICINS**PC****Solvents**

- A. Water
 B. 0.1% Aq. ammonium chloride
 C. 0.5% Aq. ammonium chloride
 D. 1.0% Aq. ammonium chloride
 E. 2.0% Aq. ammonium chloride
 F. 3.0% Aq. ammonium chloride
 G. 5.0% Aq. ammonium chloride
 H. 10.0% Aq. ammonium chloride
 I. 20.0% Aq. ammonium chloride
 J. Satd. ammonium chloride
 K. Water satd. *n*-butanol
 L. 75% Phenol
 M. *n*-Butanol-methanol-water-methyl orange (40 ml:10 ml:20 ml:1.5 g)
 N. *n*-Butanol-methanol-water (40:10:20)
 O. Benzene-methanol (80:20)
 P. Water
 Q. pH 8 buffer
 R. Satd. soln. picric acid
 S. 96% Methanol
 T. Acetone
 U. Water satd. soln. ethyl acetate
 V. Water satd. *n*-butanol

- W. Water sat. diethyl ether
 X. Chloroform
 Y. Benzene
 Z. Light petroleum

Detection

Bioautography

R_F (estimated from drawing)

Solvent	Miniatomicin	
	M ₂	M ₃
A	0.00–0.2	0.00–0.32
B	0.00–0.3	0.00–0.50
C	0.00–0.4	0.08–0.70
D	0.00–0.45	0.08–0.73
E	0.00–0.47	0.15–0.79
F	0.00–0.55	0.18–0.83
G	0.00–0.60	0.25–0.90
H	0.00–0.55	0.15–0.87
I	0.00–0.43	0.07–0.84
J	0.00–0.32	0.00–0.59
K	0.45–1.00	0.00–0.80
L	0.00–0.35	0.45–0.90
M	0.80–1.00	0.70–1.00
N	0.75–1.00	0.52–0.90
O	0.60–1.00	0.47–0.90
P	0.05, 0.90	0.60–0.97
Q	0.00–0.20	0.00–0.48
R	0.05	0.08
S	—	—
T	0.25–0.50	0.00–0.2 0.35–0.8
U	0.05	0.00–0.60
V	0.05	0.08
	0.10–0.25	
W	0.47–1.00	0.03–0.41 0.85
X	0.05	0.10
Y	0.05	0.10
	0.1 –0.25	
Z	0.05	0.10
AA	0.05	0.10

Ref.

M.F. de Albuquerque, F.D. de Andrade Lyra, O.G. de Lima, L.L. de Oliverira, J.S. de Barros Coelho, G.M. Maciel and M. da Salete Barros Cavalcanti, *Recife*, 6 (1966) 35–51

ELPHO

Medium

Paper

Buffers

- A. Phosphate buffer, pH 5.4
 B. Phosphate buffer, pH 8.0

Conditions

- A. For buffer A, 6.04 V/cm, 6 h
 B. For buffer B, 6.6 V/cm, 6 h

Mobility

Miniatomicin M₂

- A. Moves slightly toward cathode
 B. Moves slightly toward cathode

Miniatomicin M₃

- A. Moves toward anode
 B. Moves slightly toward anode

Ref.

As PC

MINIMYCIN**PC****Solvents**

- A. Water satd. butanol
 B. Butanol–acetic acid–water (4:1:2)
 C. Ethanol–water (4:1)
 D. Propanol–pyridine–acetic acid–water (15:10:3:12)

 R_F

- Solvent A, 0.25
 Solvent B, 0.49
 Solvent C, 0.67
 Solvent D, 0.85

Ref.

S. Shirato, J. Nagatsu, M. Shibuya, Y. Kusakabe, *U.S. Patent* 3,755,293, Aug. 28, 1973

TLC**Medium**

Silica gel

Solvents

- A. Water satd. butanol
 B. Butanol–acetic acid–water (4:1:2)
 C. Ethanol–water (4:1)
 D. Chloroform–methanol–17% ammonia (2:1:1) upper layer of mixture
 E. Propanol–pyridine–acetic acid–water (15:10:3:12)

 R_F

Solvent	R_F	Solvent	R_F
A	0.42	D	0.88
B	0.57	E	0.85
C	0.75		

Ref.

As PC; Y. Kusakabe, J. Nagatsu, M. Shibuya, O.K. Kawaguchi, C. Hirose and S. Shirato, *J. Antibiot.*, 25 (1972) 44-47

MITHRAMYCINS**TLC 1***Medium*

Silufol

Solvents

A. Chloroform-methanol (6:1)

B. Benzene-methanol (3:1)

Detection

UV at 254 nm. Detection, after exposure to ammonia vapours, in visible and UV light

R_F

Solvent	UV	Visible
A	0.37 yellow-orange	Yellow *
B	0.10	

* Visible immediately, without preceding exposure in ammonia vapour

Ref.

As Chromocyclomycins TLC

TLC 2*Medium*

Silica gel G

Solvents

A. Methyl ethyl ketone-isopropanol-methanol (8:1:1)

B. Methanol-isopropanol-methyl ethyl ketone (1:1:8)

R_F

Solvent	Mithramycin A	Mithramycin C
A	0.60	
B	0.6-0.7	0.15-0.25

Ref.

B.A. Sobin, J.B. Routlen, K.V. Rao, W.S. March and A.L. Garretson, *U.S. Patent* 3,906,093, Sept. 16, 1975

TLC 3*Medium*

Silica gel

Solvent

Methanol-isopropanol-methyl ethyl ketone (1:1:8)

R_F

Component	<i>R_F</i>
Mithramycin A	0.6 -0.7
Mithramycin C	0.15-0.25

Ref.

B.A. Sobin, J.B. Routien, K.V. Rao, W.S. Marsh and A.I. Garretson, *U.S. Patent* 3,646,194, Feb. 29, 1972

MITOCHROMINS**PC 1***Solvent*

Benzene-chloroform-acetic acid-water (2:2:1:1), vs. wet paper

Detection

Bioassay

R_F

Mitochromin A, 0.6
Mitochromin B, 0.5
Mitochromin C, 0.3
Mitochromin D, 0.05

Ref.

W. Liu, W.P. Cullen and K.V. Rao, *J. Antibiot.*, 22 (1969) 608-611

PC 2*Paper*

Whatman No. 4 impregnated with acetone-water (7:3)

Solvent

Benzene-chloroform-acetic acid-water (2:2:1:1)

R_F

Mitochromin A, 0.35
Mitochromin B, 0.25

Ref.

French Patent 2,024,316, Oct. 29, 1969

TLC*Medium*

Silica gel

Solvent

Chloroform-methanol (9:1) with 1% formic acid

R_F

0.2-0.3

Ref.

As PC 1

MITOMYCIN C

CCD

Solvent

Chloroform–phosphate buffer, pH 6.0, containing 10% (w/v) sodium chloride

Distribution coefficient

30 transfers; Mitomycin C peak, tubes 20 to 25

Ref.

T. Hata, H. Kamada, S. Wakaki, S. Kudo, K. Tomioka, H. Marumo, E. Kato and M. Shimizu, *U.S. Patent* 3,660,578, May 2, 1972

MITOMYCINS

HPLC

Apparatus

ALC/GPC 242 single detector liquid chromatograph, equipped with a Model 660 solvent-flow programmer and Model U6K universal injector (Waters Assoc.)

Column

Stainless-steel analytical columns packed with Corasil II (37–50 μ m particle size, 61 cm $\times$ 2 mm I.D.), μ Porasil (10 μ m particle size, 30 cm $\times$ 4 mm I.D.), and for preparative work Porasil A (37–75 μ m particle size, 122 cm $\times$ 7 mm I.D.) (Waters Assoc.)

Mobile phase

Chloroform–methanol (9:1), ethyl acetate–methanol (95:5)

Flow-rate

1 ml/min

Pressure

1000 p.s.i.

Detector

Model 202 UV (254 nm)

Chart speed

10 min/in.

Retention time

Mitomycin A, 2.0
Mitomycin B, 10.5
Mitomycin C, 18.3

Ref.

S.C. Srivastava and L. Hornemann, *J. Chromatogr.*, 161 (1978) 393–395

MOENOMYCINS

PC 1

Solvent

n-Butanol–pyridine–water (4:1:4)

R_F

0.29, 0.38, 0.64, 0.47 (minor)

Ref.

H. Umezawa, K. Maeda, K. Nitta, M. Okanishi and S. Takahashi, *U.S. Patent* 3,564,090, Feb. 16, 1971

PC 2

Solvents

- A. *n*-Butanol–triethanolamine–methyl isobutyl ketone–water (14:1:1:5)
- B. Benzene–glacial acetic acid–water (2:2:1)
- C. *n*-Butanol–glacial acetic acid–water (4:1:5)
- D. *tert*-Butanol–glacial acetic acid–water (60:6:34)
- E. Butanol satd. with water
- F. *sec*-Butanol–glacial acetic acid–water (4:1:1)

R_F

Solvent	R_F	Solvent	R_F
A	0.05	D	0.70
B	0	E	0
C	0.88	F	0.05

Ref.

U. Schacht, R. Tschesche and I. Duphorn, *U.S. Patent* 3,660,569, May 2, 1972

TLC 1

Medium

Silica gel GF (Merck)

Solvent

Isopropanol–2 *N* ammonia (70:30)

Detection

Spray with chlorosulfonic acid–acetic acid (1:2) and heat for 10 min at 100°C; appear as red-violet spots

R_F

Moenomycin A, 0.5
Moenomycin B₁, 0.4
Moenomycin B₂, 0.3
Moenomycin C, 0.6

Ref.

G. Huber, U. Schacht, H.L. Weidenmüller, J. Schmidt-Thome, J. Duphorn and R. Tschesche, *Antimicrob. Agents Chemother.*, 1965 (1966) 737–742

TLC 2

Medium

Silica gel GF

Solvents

- A. Chloroform–ethanol–water (40:70:20)
- B. Isopropanol–water–borate buffer, pH 9.0 (70:25:5)

R_F

Sol-vent	Moenomycin				
	D	E	F	G	H
A	0.20	0.25	0.14	0.22	0.14
B	0.48	0.67	0.47	0.65	0.35

Ref.

Derwent Farmdoc, No. 31774 (1966)

TLC 3

Medium

Silica gel GF₂₅₄ (E. Merck)

Solvent

n-Propanol-2 *N* aq. ammonia (70:30)

Detection

A. UV light at 253.6 nm

B. Spray with chlorosulfonic acid-acetic acid (1:2)

C. Exposure to iodine vapor

 R_F

0.25, 0.33, 0.41, 0.47

Ref.

As PC 1

TLC 4

Medium

Silica gel

Solvents

A. Isopropanol-2 *N* ammonia (70:30)

B. Isopropanol-water-borate buffer, pH 9.0 (70:25:5)

C. Ethanol-water (4:1)

 R_F

Solvent	Moenomycin		
	A	B	C
A	0.45	0.36	0.53
B	0.38	0.55	0.38
C	0.70	0.44	0.77

Ref.

As PC 1

ELPHO

Medium

Paper

Buffer

pH 1.9, 7.8, or 9.8

Mobility

Migrates to anode at pH 9.8 only

Ref.

As PC 1

MONAMYCINS**TLC 1**

Medium

Kieselgel G (E. Merck). Activate before use by heating at 120°C for 1 h

Solvent

Chloroform-methanol (20:1)

Detection

A. Spray with soln. of iodine (2 g/100 ml of methanol-water, 50:1)

B. Scrape sections (0.5 by 2 cm) and apply the Kieselgel to small wells (7-mm diameter) in the agar medium of an assay plate seeded with *Staphylococcus aureus* R_F

0.4-0.6

Ref.

M.J. Hall and C.H. Hassall, *Appl. Microbiol.*, 19 (1970) 109-112**TLC 2**

Medium

Kieselgel G (Merck)

Solvent

Benzene-methanol-acetic acid (10:2:1)

Detection

A. 0.1% soln. of ninhydrin in acetone, heat at 100°C for 5 min

B. 5% soln. of *p*-dimethylaminobenzaldehyde in hydrochloric acid-methanol (5:100) (Ehrlich's reagent)

C. 5% Aq. potassium permanganate

D. Exposure to iodine vapor

 R_F

Monamycin A, 0.31

Monamycin B₁, 0.38Monamycin B₂, 0.38Monamycin B₃, 0.38

Monamycin C, 0.35

Monamycin D₁, 0.43Monamycin D₂, 0.43

Monamycin E, 0.38

Monamycin F, 0.45

Monamycin G₁, 0.55Monamycin G₂, 0.55Monamycin G₃, 0.57Monamycin H₁, 0.57Monamycin H₂, 0.60

Monamycin I, 0.60

Ref.

K. Bevan, J.S. Davies, C.H. Hassall, R.B. Morton and D.A.S. Phillips, *J. Chem. Soc., (C)* (1971) 514–522

TLC 3

Solvents

- A. Butanol satd. with water
- B. Butanol–glacial acetic acid–water (4:1:5)
- C. Butanol satd. with water + 2% *p*-toluene-sulfonic acid
- D. Butanol satd. with water + 2% piperidine
- E. Butanol–pyridine–water (6:4:3)
- F. 80% Ethanol + 1.5% sodium chloride; Whatman No. 4 paper impregnated with 0.95 *M* sodium sulfate + 0.05 *M* sodium hydrosulfate
- G. Butanol–ethanol–water (1:1:2)
- H. Butanol–butyl acetate–glacial acetic acid–water (10:3:1.3:14.3), supernatant phase

Detection

Bioautography vs. *Staphylococcus aureus* or *Bacillus subtilis*

 R_F

Solvent	Component	
	A	B
A	0.00	0.00
B	0.49	0.63
C	0.34	0.58
D	0.05	0.15
E	0.32	0.32
F	0.47	0.47
G	0.74	0.74
H	2.7 *	7.6 *

* Distance in cm from origin after 16 h

CCD

Solvent

n-Butanol–benzyl alcohol–0.001 *N* hydrochloric acid–satd. aq. sodium chloride (10:5:15:3)

Distribution

25°C

Component A, $K = 0.372$

Component B, $K = 0.175$

Ref.

As TLC 1

MONAZOMYCIN

PC

Solvents

- A. Water satd. butanol
- B. Butanol–acetic acid–water (4:2:1)
- C. Ethanol–acetic acid–water (33:5:62)
- D. Butanol–acetic acid–water (2:2:4)

 R_F

Solvent A, 0.23

Solvent B, 0.77

Solvent C, 0.93

Solvent D, 0.95

Ref.

K. Akasaki, K. Karasawa, M. Watanabe, H. Yonehara and H. Umezawa, *J. Antibiot.*, 16 (1963) 127–131

MONENSIN

PC

Paper

Whatman No. 1

Solvent

Water–methanol–acetic acid–benzene (72.0:24.5:3.0:0.5)

Detection

Bioautography vs. *Bacillus subtilis* ATCC 6633

 R_F (estimated from drawing)

Monensin A, 0.50

Monensin B, 0.90

Monensin C, 0.35

Ref.

M.E. Haney, Jr. and M.M. Hoehn, *Antimicrob. Agents Chemother.*, 1967 (1968) 349–352

TLC 1

Medium

Silica gel

Solvents

Ethyl acetate

Detection

Spray the dried plates with 3% vanillin in 1.5% ethanolic sulfuric acid and then heat the plates at 100°C for 5 min to develop a bright red color

 R_F (estimated from drawing)

Monensin A, 0.4

Monensin B, 0.5

Monensin C, 0.2

Monensin D, 0.3

Ref.

As PC

TLC 2**Medium**Silica gel F₂₅₃**Solvent**

Benzene-methanol (7:3)

Detection

Vanillin spray reagent (prepared by adding fuming sulfuric acid (2 ml) to a soln. of vanillin (3 g) in absolute ethanol (100 ml))

R_F

0.62

Ref.

D.R. Brannon and D.R. Horton, *U.S. Patent* 3,932,619, Jan. 13, 1976

MONOKETO-ORGANOMYCIN**PC****Paper**

Whatman No. 1

Solvent

Butanol-acetic acid-water (4:1:4)

R_F

0.84 (Estimated from drawing)

Ref.

G.M. Imam and H. Kuntzel, *J. Antibiot.*, 30 (1977) 314-320

ELPHO**Medium**

Whatman No. 3MM paper

Buffer

Formic-acetic acid-water (4:1:45) pH 4.5

Conditions

60 V/cm, 30 min

Mobility

Moves towards cathode

Ref.

As PC

MULTHIOMYCIN**TLC****Medium**

Silica gel buffered with sodium phosphate

Solvents

A. Ethyl acetate-methanol (10:1)

B. Chloroform-methanol (10:1)

R_F

Solvent A, 0.69

Solvent B, 0.42

Ref.

T. Endo and H. Yonehara, *J. Antibiot.*, 31 (1978) 623-625

MUTALOMYCIN**TLC****Medium**

Silica gel

Solvents

A. Toluene-acetone (4:1) + 1% triethylamine

B. Chloroform-ethyl acetate (1:1)

C. Chloroform-methanol (95:5)

R_F

Solvent A, 0.57

Solvent B, 0.80

Solvent C, 0.65

Ref.

T. Fehr, H.D. King and M. Kuhn, *J. Antibiot.*, 30 (1977) 903-907

MUTAMICINS¹**PC****Paper**Whatman No. 1²**Solvent**

Chloroform-methanol-17% ammonium hydroxide (2:1:1), lower phase

DetectionBioautography vs. *Staphylococcus aureus***R_F (estimated from photograph)**

(*R*_{sisomicin}) Mutamicin 1 (Mu 1), 0.7; Mutamicin 2 (Mu 2), 0.2, 0.6 (trace), 1.6

Ref.

R.T. Testa, G.H. Wagman, P.J.L. Daniels and M.J. Weinstein, *J. Antibiot.*, 27 (1974) 917-921

¹ Names changed to Mu 1 and 2² R. Testa, personal communication**MYCOBACILLIN****PC****Paper**

Whatman No. 1

Solvents

A. Water satd. phenol

B. Water satd. butanol

C. *n*-Butanol-acetic acid-water (4:1:1)D. *n*-Butanol-pyridine-water (2:1:4)

E. Pyridine-water (3:2)

F. Ammonium chloride in water (3.0%)

DetectionBioautography vs. *Aspergillus niger***R_F (see p. 240)****Ref.**

S.K. Majumdar and S.K. Bose, *Arch. Biochem. Biophys.*, 90 (1960) 154-158

R_F

Solvent	R_F	Solvent	R_F
A	0.92	D	0.92
B	0.83	E	0.92
C	0.93	F	0.10

ELPHO**Medium**

Whatman No. 3 MM paper previously soaked in a buffer soln.

Buffers

- A. Veronal buffer, pH 8.5, μ 0.05
B. Acetate buffer, pH 4.8, μ 0.1

Conditions

220 V, 9 mA, 5.5 h

Detection

- A. Blue color with 20% sodium carbonate and phenol reagent of Folin and Ciocalteu
B. Bioautography vs. *Aspergillus niger*

Mobility

Migrates to anode 3 cm at pH 8.6 and 0.0–1.0 cm at pH 4.8

Ref.

As PC

MYCOBACTOCIDINS**PC****Paper**

Whatman No. 1

Solvent

0.067 M Phosphate buffer, pH 7

Detection

Strain with Amid Black 10B

 R_F

- Fraction B separated into 2 areas
Fraction D has only 1 area

Ref.

G.B. Fregman and D.W. Smith, *J. Bacteriol.*, 83 (1962) 1069–1076

MYCOHEPTIN**TLC****Medium**

Silica gel G

Solvents

- A. Butan-1-ol–ammonia–methanol–water (20:1:2:4)
B. Chloroform–methanol–borate buffer (pH 8.3) (7:5:1)

Detection

0.2% *p*-Dimethylaminobenzaldehyde in sulfuric acid containing ferric chloride

 R_F

- Solvent A, 0.06
Solvent B, 0.18

Ref.

As Amphotericins, TLC 2

CCD**Solvent**

Chloroform–methanol–borate buffer (pH 8.2–8.4) (2:2:1)

Distribution coefficient

200 Transfers, $K = 4.26$

Ref.

As Amphotericins, TLC 2

MYCOPHENOLIC ACID**PC****Paper**

Whatman No. 1

Solvent

n-butanol satd. with water + 2% *p*-toluenesulfonic acid and 2% piperidine

Detection

Bioautography vs. vaccinia virus-infected BSC-1 monkey kidney cells

 R_F

0.6

Ref.

R.H. Williams, L.D. Boeck, J.C. Cline, D.C. DeLong, K. Gerzon, R.S. Gordee, M. Gorman, R.E. Holmes, S.H. Larsen, D.H. Lively, T.R. Matthews, J.D. Nelson, G.A. Poore, W.M. Stark and M.J. Sweeney, *Antimicrob. Agents Chemother.*, 1968 (1969) 229–233

TLC**Medium**

Silica gel

Solvent

Amyl acetate–*n*-propanol–acetic acid–water (4:3:2:1)

Detection

Spray with a soln. of 1% ferric chloride in methanol

 R_F

0.65

Ref.

As PC

MYCOPLASMA ANTIBIOTICS, I. *ACHOLEPLASMA LAIDLAWII* B

PC

Solvent

n-Butanol-ethanol-water (2:1:1)

Detection

Bioautography vs. *Bacillus subtilis*

R_F

The purified material contains 3 bioactive substances

Ref.

D. Perlman, C.C. Fraterrigo and J.L. Schwartz, *J. Antibiot.*, 25 (1972) 535-536

MYCOPLASMA sp. RP III

PC 1

Solvents

- A. Water satd. *n*-butanol
- B. 3% Ammonium chloride (w/v)
- C. *n*-Butanol-methanol-water (4:1:2)
- D. Water
- E. Benzene-methanol (4:1)

Detection

Bioautography vs. *Pseudomonas convexa*

R_F

Solvent	Factor	
	I	II
A	0.06	n.d. *
B	0.56	n.d.
C	0.44	n.d.
D	0.53	n.d.
E	0.02	0.63

* n.d.=Antibiotic could not be detected and it was concluded it was inactivated by the solvent system

Ref.

T. Sakai and D. Perlman, *J. Antibiot.*, 28 (1975) 743-756

PC 2

Solvents

- A. *n*-Propanol-pyridine-acetic acid-water (15:10:3:12)
- B. *n*-Propanol-acetic acid-water (50:5:40)

Detection

Bioautography

R_F

Solvent A, 0.6
Solvent B, 1.0

Ref.

M. Sylvestre and D. Perlman, *J. Antibiot.*, 28 (1975) 73-74

TLC

Medium

Silica gel

Solvents

- A. Chloroform-methanol (8:1)
- B. Chloroform-methanol (1:1)
- C. Chloroform-acetone (8:2)
- D. Chloroform-acetone (1:1)
- E. Chloroform-*n*-hexane (2:1)
- F. Chloroform-benzene (2:1)
- G. Ethyl acetate-acetone-water (6:2:1)

Detection

Bioautography vs. *Pseudomonas convexa*

R_F

Solvent	Factor	
	I	II
A	0.44	0.88
B	0.49	0.25
C	Inactivated	0.81
D	Inactivated	0.42
E	0	0.31
F	0	0.47
G	0.52	-

Ref.

AS PC 1

ELPHO 1

Medium

Whatman No. 1 paper

Buffer

Acetic acid-formic acid-water (20:2:78), pH 1.9

Conditions

5 V/cm for 20 min

Mobility

Factor I: 3.3 cm towards the cathode

Ref.

As PC 1

ELPHO 2

Buffer

Acetic acid-formic acid-water (20:2:78)

Conditions

pH 1.8, 34 V/cm, 20 min

Mobility

1.5 cm toward cathode

Ref.

As PC 1

MYCORRHODIN**CCD***Solvents*

- A. Methanol–water–chloroform–carbon tetrachloride (3:1:1.5:4), 200 transfers
 B. Methanol–methyl isobutyl ketone–water (1:1:1), 100 transfers

Distribution

Mycorrhodin A, peak in tube 65

Mycorrhodin B, peak in tube 86

Ref.

M. Misiek, A. Gourevitch, B. Heinemann, M.J. Cron, D.F. Whitehead, H. Schmitz, I.R. Hooper and J. Lein, *Antibiot. Chemother.*, 9 (1959) 280–285

MYCOSPOCIDIN**TLC***Media*

- A. Silica gel
 B. Alumina
 C. Cellulose

Solvents

- A. Chloroform–methanol (1:1)
 B. Chloroform–methanol (1:2)
 C. Chloroform–methanol (2:1)
 D. Benzene–methanol (2:3)
 E. Water satd. butanol
 F. Water satd. collidine
 G. Butanol–pyridine–acetone–water (15:10:3:12)

Detection

Bioautography

R_F (estimated from drawing)

Solvent	Medium		
	A	B	C
A	0.65–0.83	0.0	–
B	0.60–0.90	0.05	–
C	0.10	–	–
D	0.58–0.80	0.00	–
E	–	–	0.55
F	–	–	0.70–0.95
G	–	–	0.75

Ref.

A. Takatsuki, K. Arima and G. Tamura, *J. Antibiot.*, 24 (1971) 215–223

MYCOTRIENIN**CCD***Solvent*

Chloroform–ligroin–water–methanol (2:1:1:2)

Distribution

$K = 0.56$

Ref.

C. Coronelli, R.C. Pasqualucci, J.E. Thiemann and G. Tamoni, *J. Antibiot.*, 20 (1967) 329–333

MYOMYCIN**PC***Paper*

Ascending

Solvent

Chloroform–methanol–17% aq. ammonia (2:1:1) lower layer (developed at room temp. for 12 h)

 R_F

0.00

Ref.

As Apramycin, PC 1

TLC*Medium*

Silica gel

Solvent

95% Ethanol–water–acetic acid–2 M ammonia (79:21:5:10)

Detection

- A. Bioautography vs. *Bacillus subtilis* ATCC 6633
 B. 0.2% Ninhydrin in acetone containing 10% pyridine

 R_F

Component A, 0.21
 Component B, 0.41
 Component C, 0.63

Ref.

J.C. French, L.E. Anderson, R.H. Bunge and J.D. Howells, *U.S. Patent* 3,795,668, March 5, 1974

MYRIOICIN**TLC***Medium*

Silica gel G

Solvent

Chloroform–ethanol–glacial acetic acid–water (40:33:10:10)

 R_F

Uniform spot

Ref.

D. Kluepfel, A. Kudelski and J. Bagli, *U.S. Patent* 3,928,572, Dec. 23 1975

MYROTHECIUM ANTIBIOTICS**TLC***Medium*

Kieselgel G

Solvents

A. Chloroform-methanol (98:2)

B. Benzene-methanol (4:1)

R_F (estimated from drawing)

Component	Solvent A	Solvent B
A-1	0.90	0.90
A-2	0.80	0.85
B-1	0.65	0.75
B-2	0.50	0.73
C	0.40	0.45
D	0.35	0.35
E	0.25	0.25

Ref.

M. Okuchi, M. Itoh, Y. Kaneko and S. Doi, *J. Antibiot.*, 26 (1973) 562-568

MYXOCOCCUS CORALLOIDES ANTIBIOTIC**PC***Paper*

Whatman No. 1, descending technique

 R_F

The antibiotic migrated as a single zone in several solvent systems

Ref.

J.M. Arias, C. Rodriguez and E. Montoya, *J. Antibiot.*, 32 (1979) 205-211

TLC*Medium*

Silica gel

Solvents

A. Ethanol-chloroform (5:95)

B. Ethanol-chloroform (10:90)

C. Ethanol-chloroform (20:80)

D. Ethyl acetate-isopropanol-water 5:23:12)

E. Acetic acid-ethanol-chloroform (1:10:89)

F. Ammonia-ethanol-chloroform (1:10:89)

Detection

The antibiotic migrated as a single ultraviolet-ab-

sorbing spot on silica gel TLC. The active spot reacted positively with iodine vapors and negatively with sulphuric acid, ninhydrin, Dragendorff reagent, hydroxylamine hydrochloride, dinitrophenylhydrazine, perchloric-hydrochloric molybdate and bismuth subnitrate and KI

 R_F

Solvent	R_F	Solvent	R_F
A	0.28	D	0.75
B	0.50	E	0.5
C	0.87	F	0.81

Ref.

As PC

ELPHO*Medium*

Paper, Whatman No. 1

Buffers

A. 1% Borate buffer, pH 9, 300 V for 3 h

B. Pyridine-acetic acid-water (25:1:475), pH 6.5, 300 V for 3 h

C. Pyridine-acetic acid-water (3:1:487), pH 4, 350 V for 4 h

D. 0.25 N Acetic acid buffer, pH 2.9, 350 V for 4 h

Conditions

An V-inverted electrophoretic tank, the right size for Whatman No. 1 paper strips 31 × 30 cm was used

Mobility

Buffers A and B, migrated toward anode as a single active zone

Buffers C and D, immobile

Ref.

As PC

NAEMATOLIN**TLC***Medium*

Kieselgel GF

Solvents

A. Cyclohexane-chloroform-ethanol (2:8:1)

B. Benzene-methanol (9:1)

C. *n*-Hexane-acetone (4:1)

D. Benzene-acetone (4:1)

E. Diethyl ether

F. *n*-Hexane-ethyl acetate (4:1)

G. Chloroform-methanol (4:1)

H. *n*-Hexane-diethyl ether (1:4)

I. Diethyl ether-ethyl acetate (1:1)

R_F

Solvent	R_F	Solvent	R_F
A	0.47	F	0.05
B	0.25	G	0.91
C	0.19	H	0.50
D	0.35	I	0.91
E	0.71		

Ref.

Y. Ito, H. Kurita, T. Yamaguchi, T. Okuda and M. Sato, *156th Scientific Meeting of Japan Antibiotics Res. Assn*, July 21, 1967

NAFCILLIN

HPLC

Column

1 m $\times$ 2.1 mm I.D. $\times$ 6.3 mm O.D., 316 stainless steel. C 18/Porasil B packing

Mobile phase

Methanol-0.05 M ammonium carbonate (30:70)

Pressure

500 p.s.i.

Flow-rate

0.6 ml/min

Detector

UV (254 nm)

Sensitivity

0.16 a.u.f.s.

Type

Reversed-phase

Retention time

45 min (estimated from drawing)

Ref.

As Ampicillin, HPLC

NANAOMYCIN A and B

TLC

Medium

Silica gel

Solvent

Chloroform-methanol (5:1)

 R_F

Nanaomycin A, 0.25

Nanaomycin B, 0.15

Ref.

H. Tanaka, Y. Koyama, J. Awaya, H. Marumo, R. Oiwa, M. Katagiri, T. Nagai and S. Omura, *J. Antibiot.*, 28 (1975) 860-867

NANAOMYCIN C

TLC

Medium

Silica gel

Solvent

Chloroform-methanol (10:1)

 R_F

0.35

Ref.

H. Tanaka, H. Marumo, T. Nagai, M. Okada, K. Taniguchi and S. Omura, *J. Antibiot.*, 28 (1975) 925-930

NAPHTHYRIDINOMICIN A and B

TLC

Media

A. Silica gel

B. Alumina

Solvents

A. Acetone-*n*-propanol-ethylene dichloride (6:2:3)

B. Benzene-acetone-methanol (7:1.5:1.5)

 R_F

Solvent	Medium	Naphthyridinomicin	
		A	B
A	A	0.66	0.50
B	B	0.78	0.66

Ref.

D. Kluepfel, S.N. Sehgal and C. Vezina, *U.S. Patent* 4,003,902, Jan. 18, 1977

NARASIN

PC

Solvents

A. Water satd. with methyl isobutyl ketone

B. Water satd. with methyl isobutyl ketone + 2% *p*-toluenesulfonic acid and 1% piperidine

C. Benzene satd. with water

D. 17.4 g Potassium phosphate + 30 ml ethanol in 1 l water

Detection

Bioautography vs. *Bacillus subtilis*, pH 6.8

Ref.

D.H. Berg and R.L. Hamill, *J. Antibiot.*, 31 (1978) 1-6

R_F

Solvent	Narasin	Narasin B
A	0.11	0.09
B	0.41	0.16
C	0.24	0.51
D	0.15	0.33

TLC**Media**

- A. Silica gel
B. Cellulose

Solvents

- A. Water satd. with methyl isobutyl ketone
B. Water-methanol-acetone (12:3:1) adjusted to pH 10.3 with ammonium hydroxide, then pH 7.5 with hydrochloric acid
C. Ethyl acetate
D. Ethyl acetate-diethylamine (95:5)

Detection

- A. Bioautography vs. *Bacillus subtilis*, pH 6.8
B. Narasin methyl ester detected with 3% vanillin-0.5% sulfuric acid spray, 100°C

 R_F

Solvent	Medium	Narasin	Narasin B
A	B	0.18	0.06
B	B	0.25	0.31
C	A	0.19	0.35
		(Narasin methyl ester)	0.54
D	A	0.34	0.20
		(Narasin methyl ester)	0.66

Ref.

As PC

**NARASIN, DEOXY-
(See deoxynarasin)****NATAMYCIN****TLC****Medium**

Silica gel G

Solvents

- A. Ethanol-ammonia-water (8:1:1) buffered with phosphate (pH 8)
B. Butan-1-ol-acetic acid-water (3:1:1) buffered with phosphate (pH 8)

- C. Methanol-propan-2-ol-acetic acid (90:10:1) activated for 1 h at 110°C
D. Methanol-acetone-acetic acid (8:1:1) activated for 1 h at 110°C
E. Ethanol-ammonia-dioxan-water (8:1:1) activated for 1 h at 110°C
F. Butan-1-ol-pyridine-water (3:2:1) activated for 1 h at 110°C

Detection

- A. 10% Potassium permanganate and 0.2% bromophenol blue
B. 0.2% *p*-Dimethylaminobenzaldehyde in sulfuric acid containing ferric chloride
C. 5% Potassium permanganate
D. Orthophosphoric acid, heat for 5 min at 100°C

 R_F

Solvent	Detection	R_F
A	A	0.34
B	A	0.34
C	B	0.40
D	B	0.54
E	C + D	0.18
F	C + D	0.55

Ref.

As Amphotericins, TLC 1

ELPHO**Media**

- A. Whatman No. 4 paper
B. Whatman No. 31 paper

Buffers

- A. 0.1 M Boric acid-85% formic acid-sodium veronal-methanol-polyethylene glycol 400-water to 1000 ml (200 ml:10 ml:2.06 g:100 ml:50 ml), pH 2.6
B. 2 M Propionic acid-2 M formic acid-sodium veronal-ethylene glycol-water to 1000 ml (100 ml:100 ml:2.06 g:100 ml), pH 2.6
C. 0.1 M Boric acid-85% formic acid-glycerol-water to 1000 ml (200:10:100), pH 2.3
D. Methanol-glacial acetic acid-water (950:50:50), pH 3.3

Conditions

Samples of antibiotic (ca. 20 µg) in methanol soln. were placed onto Whatman No. 4 or No. 31 paper sheets (23 × 38 cm). Starting line was placed 12 cm from the immersion line on anode side. Developed for 4 h at 350 V

Mobility (see p. 246)**Ref.**

S. Ochab, *Dissert. Pharm. Pharmacol.*, 23 (1971) 205-208

Mobility

Med- ium	Buf- fer	Potential gradient (V/cm)	Current density (mA/ cm)	Mobil- ity (cm ² /V sec)
A	A	9.2	0.22	6.41
B	B	9.2	0.70	7.99
B	C	9.2	0.80	8.81
A	D	9.2	0.02	4.75

NEAMINE

(See neomycins)

NEAMINE, 1-N[(S)- α -HYDROXY- ω -AMINOACYL]-3',4'-DIDEOXY-
{ See 1-N[(S)- α -hydroxy- ω -aminoacyl]-
3',4'-dideoxyneamine }

NEBRAMYCINS

PC 1

Paper

Whatman No. 1

Solvent

Water satd. *n*-butanol-*p*-toluenesulfonic acid-
tert-butanol (88:2:10), 40 h

Detection

Bioautography

 R_F

Component	R_F *
Factor 2	0.35
Factor 3	0.35
Factor 4	0.50
Factor 5	0.58
Factor 6	0.75

* Estimated from drawing

Ref.

W.M. Stark, M.M. Hoehn and N.G. Knox, *Anti-
microb. Agents Chemother.*, 1967 (1968) 314-323

PC 2

Paper

Whatman No. 1

Solvents

A. Water satd. *n*-butanol containing 2% *p*-tolu-
enesulfonic acid, 40 h

B. 80% Ethanol + 1.5% sodium chloride, paper
buffered with 0.95 *M* sulfate-bisulfate, 40 h

C. Propanol-pyridine-acetic acid-water
(15:10:3:12), 40 h

D. Water satd. with methyl isobutyl ketone + 1%
p-toluenesulfonic acid, 6 h

E. Propanol-water (1:1); paper buffered with
0.75 *M* phosphate, pH 1.0, 24 h

F. *n*-Butanol satd. with water, 18 h

G. Water satd. *n*-butanol containing 2% *p*-tolu-
enesulfonic acid and 2% piperidine, 18 h

 R_F Component R_E * Solvent:

	A	B	C	D	E	F	G
Factor 1	0.20	-	-	-	-	-	-
Factor 1'	0.27	-	-	-	-	-	-
Factor 2	0.40	0.17	0.32	-	-	-	-
Factor 3	0.40	0.34	0.41	-	-	-	-
Factor 4	0.49	0.20	0.32	-	-	-	-
Factor 5	0.55	0.22	0.35	-	-	-	-
Factor 6	0.71	0.31	0.38	-	-	-	-
Nebra- mycin complex	-	-	-	-	-	-	-

* R_E = Ratio of distance traversed by the anti-
biotic with respect to the end of the tape

Ref.

R.Q. Thompson and E.A. Presti, *Antimicrob.
Agents Chemother.*, 1967 (1968) 332-340; *Belgian
Patent* 697,319; Oct. 20, 1967

PC 3

Solvent

Water satd. *n*-butanol-*p*-toluenesulfonic acid-
tert-butanol (88:2:10)

Detection

Bioautography vs. *Bacillus subtilis* R_F

Component	R_F *
Factor 2	0.35
Factor 3	0.35
Factor 4	0.45
Factor 5	0.60
Factor 6	0.80

* Estimated from drawing

Ref.

W.M. Stark, N.G. Knox and R.M. Wilgus, *Folia
Microbiologica*, 16 (1971) 206-217

PC 4*Paper*

Ascending

Solvent

Chloroform-methanol-17% aq. ammonia (2:1:1) lower layer (developed at room temp. for 12 h)

R_F

Factor 4, 0.01; Factor 5, 0.00; complex, 0.01

Ref.

As Apramycin, PC 1

PC 5*Paper*

Ascending

Solvents

- A. 20% Aq. soln. of ammonium chloride
- B. Water satd. *n*-butanol
- C. *n*-Butanol-acetic acid-water (3:1:1)
- D. Water satd. *n*-butanol + 2% (w/v) *p*-toluene-sulfonic acid + 2% (v/v) piperidine
- E. Chloroform-methanol-17% aq. ammonia (2:1:1) lower layer

R_F

Solvent	Factor 4	Factor 5
A	0.95	0.95
B	0.00	0.00
C	0.05	0.05
D	0.00	0.00
E	0.01	0.00

Ref.

As Apramycin, PC 2

TLC 1*Medium*Silica gel F₂₅₄ (E. Merck)*Solvent*

Chloroform-methanol-ammonium hydroxide (1:3:2)

Detection

- A. As PC 3
- B. Spray as follows:
 1. With 5 ml of 5% sodium hypochlorite in 95 ml water
 2. With 3A ethanol
 3. With benzidine (100 mg) + potassium iodide (100 mg) in 100 ml water + 2 ml of glacial acetic acid

Ref.

As PC 3

TLC 2*Medium*

Silica gel

Solvents

- A. Chloroform-methanol-17% aq. ammonia (2:1:1) upper layer
- B. Methanol-10% aq. ammonium acetate (1:1)
- C. *n*-Butanol-ethanol-chloroform-17% aq. ammonia (4:5:2:5)

R_F

Solvent	Factor 4	Factor 5
A	0.78	0.79
B	0.45	0.45
C	0.16	0.14

Ref.

As Apramycin, PC 1

TLC 3*Medium*

Silica gel

Solvents

- A. Chloroform-methanol-17% ammonium hydroxide (2:1:1), upper phase
- B. 10% Ammonium acetate-methanol (1:1)

*Detection*Bioautography vs. *Bacillus subtilis**R_F*

Solvent	Nebramycin factors			
	2	4	5	6
A	0.80	0.80	0.75	0.54
B	0.35	0.50	0.73	0.54

Ref.

R. Okachi, I. Kawamoto, S. Takasawa, M. Yamamoto, S. Sato, T. Sato and T. Nara, paper presented 194th Sci. Meeting, JARA, July 26, 1974

NEGAMYCIN**TLC***Medium*Kieselgel 60 F₂₅₄*Solvents*

- A. Chloroform-ethanol (100:8.2)
- B. Amino acid ethanol-34% ammonia (7:3)

Ref.

W. Steicher and H. Reinshagen, *Chem. Ber.*, 108 (1975) 813-819

ELPHO 1*Medium*

Paper, Whatman No. 3MM

Buffer

Formic acid–acetic acid–water (25:75:900)

Conditions

2800 V, 55 mA

Mobility

Moved toward cathode after 45 min

Ref.

As TLC

ELPHO 2*Buffer*

Formic acid–acetic acid–water (25:75:900)

Conditions

3500 V for 15 min

Mobility

Moved 12 cm to cathode

Ref.

M. Hamada, T. Takeuchi, S. Kondo, Y. Ikeda,
H. Naganawa, K. Maeda, Y. Okami and H.
Umezawa, *J. Antibiot.*, 23 (1970) 170–171

NEGAMYCIN, EPIDEOXY-**(See epideoxynegamycin)****NEGAMYCIN, LEUCYL-****(See leucylnegamycin)****NEOCARZINOSTATIN****ELPHO***Buffer*Barbital buffer (pH 8.6; $\mu = 0.05$)*Conditions*

450 V, 4.5 h

Mobility

Moves to cathode 5.6 cm

Ref.

H. Umezawa, T. Takeuchi, M. Hamada, M.
Ishizuka, H. Chimura and K. Maeda, *U.S. Patent*
3,595,954, July 27, 1971

NEO-ENACTIN**PC***Solvents*

- A. *n*-Butanol–water (87:13)
- B. *n*-Butanol–ethyl acetate–water (9:9:1)
- C. Ethyl acetate–methanol–water (10:2:1)
- D. Ethyl acetate–acetone–water (1:3:1)

R_F

- Solvent A, 0.80
- Solvent B, 0.76
- Solvent C, 0.76
- Solvent D, 0.73

Ref.

H. Kondo, H. Sumomogi, T. Otani and S.
Nakamura, *J. Antibiot.*, 32 (1979) 13–17

TLC*Medium*

Silica gel G

Solvents

- A. *n*-Butanol–methanol–water (8:4:1)
- B. *n*-Butanol–water (87:13)
- C. *n*-Butanol–ethyl acetate–water (87:100:13)
- D. Acetone–water (4:1)
- E. *n*-Butanol–ethanol–water (8:5:2)
- F. Chloroform–methanol–water (65:35:10)
lower phase

Detection

- A. Bioautography
- B. Ninhydrin
- C. Heat at 100°C for 15 min after spraying with
40% sulfuric acid

R_F

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.12	D	0.38
B	0.27	E	0.20
C	0.07	F	0.31

Ref.

As PC

**NEOMYCIN A; NEOMYCIN A
DERIVATIVES
(cf., neamine)****PC 1***Paper*

Ascending

Solvents

- A. 20% Aq. soln. of ammonium chloride
- B. Water satd. *n*-butanol
- C. *n*-Butanol–acetic acid–water (3:1:1)
- D. Water satd. *n*-butanol + 2% (w/v) *p*-toluene-
sulfonic acid + 2% (v/v) piperidine
- E. Chloroform–methanol–17% aq. ammonia
(2:1:1) lower layer

R_F

Solvent	R_F	Solvent	R_F
A	0.97	D	0.02
B	0.00	E	0.00
C	0.02		

Ref.

As Apramycin, PC 1

PC 2

Solvent

n-Butanol–pyridine–acetic acid–water (6:4:1:3) R_F

Compound	R_{neamine}
1-N-[(<i>S</i>)- α -Hydroxy- γ -amino- <i>n</i> -butyryl]-neamine	0.44
1-N-[(<i>S</i>)- α -Hydroxy- δ -amino- <i>n</i> -valeryl]-neamine	0.50
1-N-[(<i>S</i>)- α -Hydroxy- ϵ -amino- <i>n</i> -caproyl]-neamine	0.50
1-N-[(<i>S</i>)- α , γ -Diamino- <i>n</i> -butyryl]-neamine	0.64
1-N-[(<i>S</i>)- α -N-Acetyl- α , γ -diamino- <i>n</i> -butyryl]-neamine	0.59
1-N-[(<i>S</i>)- α , δ , δ -Diamino- <i>n</i> -valeryl]-neamine	0.5
1-N-[(<i>S</i>)- α , ϵ -Diamino- <i>n</i> -caproyl]-neamine	0.44

Ref.

German Patent 2,342,946, March 14, 1974

TLC 1

Medium

Cellulose

Solvent

Methyl ethyl ketone–methanol–isopropanol–7.9 *N* ammonium hydroxide (10:8.5:3:7)

Detection

Ninhydrin. Spray with 0.3% soln. of ninhydrin in water satd. *tert*.-butanol, heat at 100–105° for 3–5 min R_F

0.61

Ref.

As Kanamycins, TLC 5

TLC 2

Medium

Silica gel

Solvents

A. Chloroform–methanol–17% aq. ammonia (2:1:1) upper layer

B. Methanol–10% aq. ammonium acetate (1:1)

C. *n*-Butanol–ethanol–chloroform–17% aq. ammonia (4:5:2:5) R_F

Solvent A, 0.40

Solvent B, 0.71

Solvent C, no zone

Ref.

As Apramycin, PC 1

NEOMYCINS

PC 1

Solvents

n-Butanol–acetic acid–water (50:25:25), 16 h, descending

Detection

A. Ninhydrin

B. Bioautography vs. *Bacillus subtilis* R_F

Neomycin, 0.0

Neamine, 0.05–0.10

Ref.

B.E. Leach and C.M. Teeters, *J. Amer. Chem. Soc.*, 73 (1951) 2794–2797

PC 2

Paper

Whatman No. 1

Solvent

Butanol–pyridine–water (6:4:3)

Detection

Reagents:

A. Sodium hypochlorite. Add 1 part of "Chlorox" (5.25% sodium hypochlorite in water) to 20 parts of water

B. Ethanol, 95%

C. Starch–iodide reagent. Mix equal volumes of a 1% soluble starch soln. and a 1% potassium iodide soln.

The developed paper chromatogram is sprayed with A. When dry, it is sprayed with B; finally after B has evaporated, the chromatogram is sprayed with C. The acetylated neomycins show up as deep blue spots against a colorless background

 R_F (see p. 250)

Ref.

S.C. Pan and J.D. Dutcher, *Anal. Chem.*, 28 (1956) 836–838

R_F

Component	R_F
N-Acetyl neomycin B	0.29
N-Acetyl neomycin C	0.19

PC 3

Paper

Toyo Roshi No. 50

Solvent

0.4% Aq. ammonium chloride and 20% methanol-water

Detection

Bioautography vs. *Bacillus subtilis* R_F

Neomycin methanesulfonate, 1.0

Ref.

H. Umezawa, M. Murase and S. Yamazaki, *J. Antibiot.*, 12 (1959) 341-342

PC 4

Solvent

n-Propanol-glacial acetic acid-water (9:1:10), descending R_F

Neomycin B, 0.26

Neomycin C, 0.30

Neamine, 0.43

Ref.

K.L. Rinehart, Jr., A.D. Argoudelis, W.A. Goss, A. Sohler and C.P. Schaffner, *J. Amer. Chem. Soc.*, 82 (1960) 3938-3946

PC 5 (Quantitative Radioisotopic Method)

Paper

Whatman No. 4

Solvent

1-Butanol-water-piperidine (84:16:2), descending, 48 h

Detection

Carbon ^{14}C N-acetyl derivatives are located by passing the chromatogram through an automatic scanner and quantitated by liquid scintillation counting R_F

N-Acetyl neomycin B, 0.43

N-Acetyl neomycin C, 0.24

Neamine, 0.68

Ref.

D.G. Kaiser, *Anal. Chem.*, 35 (1963) 552-554

PC 6

Solvent

1-Butanol-pyridine-water (3:2:2), descending, 18-20 h

Detection

As PC 2

 R_F

N-Acetate reference	R_F	N-Acetate to be correlated	R_{rel}
Neomycin B	0.44		
		Neomycin C	0.75
		Neamine	0.93

Ref.

H. Maehr and C.P. Schaffner, *Anal. Chem.*, 36 (1964) 104-108

PC 7

Paper

Toyo No. 50

Solvent

n-Butanol-pyridine-water-acetic acid (6:4:3:1), descending

Detection

Ninhydrin; 0.25% in pyridine

 R_F Neamine: $R_{F_{\text{paromomine}}}$ 0.47

Ref.

K. Tatsuta, E. Kitazawa and S. Umezawa, *Bull. Chem. Soc. Japan*, 40 (1967) 2371-2375

PC 8

Paper

A. Whatman No. 1

B. Whatman No. 4

 R_F

		Paper			R_{rel} * values in solvent:		
					A	B	C
N-Acetyl neomycin B	B		0.69	1.11	1.09		
N-Acetyl neomycin C	A		0.35	0.73	0.84		
N-Acetyl neamine	A		1.00	1.00	1.00		

* R_{rel} = Migration of the substance relative to N-acetyl neamine

Solvents

- A. 1-Butanol–water–piperidine (84:16:2), descending, 34°C, 24 h
 B. 1-Butanol–water–piperidine (6:3:4)
 C. Isoamyl alcohol–water–pyridine (1:0.8:1), ascending, 28°C

Detection

As PC 2

Ref.

M.K. Majumdar and S.K. Majumdar, *Anal. Chem.*, 39 (1967) 215–217

PC 9**Solvent**

1-Butanol–pyridine–water (6:4:3)

Detection

As PC 2

 R_F

Modified neomycins	R_F
N-Acetyl hybrimycin A ₁	0.32
N-Acetyl hybrimycin A ₂	0.19
N-Acetyl hybrimycin B ₁	0.37
N-Acetyl hybrimycin B ₂	0.19
N-Acetyl neomycin B	0.41
N-Acetyl neomycin C	0.30

Ref.

W.T. Shier, K.L. Rinehart, Jr. and D. Gottlieb, *Biochemistry*, 63 (1969) 198–204

PC 10**Paper**

Whatman No. 1

Solvent

Methyl ethyl ketone–*tert.*-butanol–methanol–6.5 *N* ammonium hydroxide (16:3:1:6), descending, 24–36 h

Detection

Sprayed with ninhydrin reagent; 0.25 g of ninhydrin, 10 ml of methanol, 47 ml of butanol, 3 ml of water, and 50 ml of pyridine and finally heated at 80 to 90°C for 30 min. For quantitation, spots were cut and extracted for 30 min with 3 ml of 75% methanol, and the absorbances of the soln. were measured spectrophotometrically at 570 nm with a 1-cm cell. The amount of neomycin was determined by reference to standard curves

Ref.

M.K. Majumdar and S.K. Majumdar, *Appl. Microbiol.*, 17 (1969) 763–764

 R_F

	R_{rel}^*
Neamine	1.00
Neomycin B	0.54
Neomycin C	0.30

* Migration of the substance relative to neamine

PC 11**Solvent**

Butanol–water–acetic acid–pyridine–sodium chloride (30:12:7:2:0.1)

Detection

A. Ponceau S

B. Ninhydrin

 R_F

Useful for separation of neomycin from polymyxin B and bacitracin

Ref.

J.P. Carr, R.J. Stretton, J.W. Watson, *Final Year Study Proj. Theses*, 10 (1969) 17–18; (*Eng.*) *Pharm. Anal.*, 73 (1970) 247

PC 12**Solvents**

- A. 80% Methanol + 3% (w/v) sodium chloride, (1:1), descending. Paper buffered with 0.95 *M* sodium sulfate + 0.05 *M* sodium bisulfate
 B. Propanol–pyridine–acetic acid–water (6:4:1:3), ascending, (v/v)
 C. 80% Phenol, ascending, (v/v)
 D. Benzene–methanol (9:1, v/v), descending
 E. *n*-Butanol–water–acetic acid (4:5:1), upper phase, ascending

 R_F

Solvent	R_F	Solvent	R_F
A	0.00, 0.17	D	0.0
B	0.05	E	0.0
C	0.0, 0.12		

Ref.

M.J. Weinstein, G.H. Wagman and J.A. Marquez, *U.S. Patent* 4,001,209, Jan. 4, 1977

PC 13**Paper**

Whatman No. 1

Solvents

- A. *n*-Butanol satd. with water + 2% *p*-toluene-sulfonic acid for 40 h

- B. 80% Aq. ethanol + 1.5% sodium chloride for 40 h on paper buffered with 0.95 M sulfate-bisulfate
- C. Propanol-pyridine-acetic acid-water (15:10:3:12) for 40 h
- D. Water satd. with methylisobutyl ketone + 1% *p*-toluenesulfonic acid for 6 h
- E. Propanol-water (1:1), for 24 h on paper buffered with 0.75 M phosphate, pH 4.0
- F. *n*-Butanol satd. with water for 18 h
- G. *n*-Butanol satd. with water + 2% *p*-toluenesulfonic acid and 2% piperidine for 18 h

 R_F

Solvent	R_e^*	Solvent	R_e^*
A	0.60	E	—
B	0.12	F	0
C	0.19	G	0.05
D	—		

* The migration constant for each of the antibiotics is expressed as an R_e rather than the more conventional R_F value. The R_e value expresses the ratio of the distance traversed by the antibiotic with respect to the end of the tape rather than the solvent front. This method of expressing the constant was chosen in view of the fact that the solvent front had progressed beyond the end of the type during the time interval employed with most of the solvent systems

Ref.

As Catenulin, PC

PC 14

Solvent

1-Butanol-pyridine-water (6:4:3)

 R_F

Component C, 0.30

Component B, 0.41

Ref.

W.T. Shier, K.L. Rinehart, Jr. and D. Gottlieb,
U.S. Patent 3,833,556, Sept. 3, 1974

PC 15

Paper

Ascending

Solvent

Chloroform-methanol-17% aq. ammonia
(2:1:1) lower layer (developed at room temp. for 12 h)

 R_F

Neomycin A, 0.00

Neomycin B, 0.03

Neomycin C, 0.00

Ref.

As Apramycin, PC 1

PC 16

Paper

Toyo No. 51

Solvents

A. 20% Ammonium chloride

B. Water satd. *n*-butanolC. *n*-Butanol-acetic acid-water (3:1:1)

D. Water satd. ethyl acetate

E. Water satd. *n*-butanol + 2% *p*-toluenesulfonic acid + 2% piperidine

Detection

Bioautography vs. *Bacillus subtilis* No. 10707 R_F

Solvent	R_F	Solvent	R_F
A	0.96	D	0.00
B	0.00	E	0.00
C	0.00		

Ref.

As Carbomycin, PC 5

PC 17

Paper

Toyo No. 51

Solvent

Methanol-3% aq. sodium chloride (2:1)

 R_F

0.20

Ref.

As Gentamicin, PC 1

PC 18

Paper

Toyo Filter No. 51A

Solvent

Methanol-3% aq. sodium chloride (2:1)

 R_F

0.20

Ref.

As Gentamicins, PC 15

PC 19

Paper

Whatman No. 1

Solvent

Methyl ethyl ketone-*tert*-butanol-methanol-6.5*N* ammonium hydroxide (16:3:1:6)

Detection

Ninhydrin

R_F (Estimated from drawing)

Neomycin B, 0.34

Neomycin C, 0.21

Neamine, 0.63

Ref.

P.J. Claes, F. Compennolle and H. Vanderhaeghe,
J. Antibiot., 27 (1974) 931–942

PC 20

Paper

Whatman No. 1

Solvents

A. Methyl ethyl ketone-*tert*-butanol-methanol-
6.5 *N* ammonia (16:3:1:6).

B. 2% *p*-Toluenesulfonic acid in water satd. 1-
butanol

R_F

Neomycin	R_{rel} *	
	Solvent A	Solvent B
Neomycin B	1.00	1.00
Neomycin C	0.55	1.00
Neamine	1.65	1.00

* R_{rel} = Relative to Neomycin B

Ref.

H. Baud, A. Bentencourt, M. Peyre and L.
Penasse, *J. Antibiot.*, 30 (1977) 720–723

PC 21

Solvent

1-Propanol-pyridine-acetic acid-water
(15:10:3:12), 21 h

R_F

Neomycin	Migration
5''-Amino-5''-deoxyneomycin	9.2 cm
Neomycin	5.2 cm

Ref.

S. Hanessian, R. Masse and M.-L. Capmeau, *J.*
Antibiot., 30 (1977) 893–896

PC 22

Paper

Descending

Solvents

A. Water satd. *n*-butanol + 2% *p*-toluenesulfonic
acid, 20 h

B. *n*-Propanol-pyridine-acetic acid-water
(15:10:3:12), 20 h

C. *n*-Butanol-pyridine-acetic acid-water
(6:4:1:3), 7 days

R_F

Solvent	R_{767-L} *		
	Neomycin		
	A	B	C
A	(11.1) **	(7.7)	(7.8)
	3.70	3.50	3.50
B	(13.2)	(10.6)	(10.7)
	1.45	1.00	1.00
C	(17.3)	(7.4)	(7.5)
	2.75	1.00	1.00

* This term represented the ratio of the value for migration distance of one antibiotic substance to the value for migration distance of the SF-767-L substance. In determination of R_{767-L} , an amount of the SF-767-L substance was added as an internal standard to each of the antibiotic substances, and the mixture was then developed.

** Numbers in parentheses are the migration distances from original point in cm

Ref.

T. Shomura, N. Ezaki, T. Tsuruoka, T. Niwa, E.
Akita and T. Niida, *U.S. Patent* 3,697,648, Oct.
10, 1972

TLC 1

Medium

Carbon. Thirty grams of Nuchar (C-190-N) vegetable carbon black and 1.5 g of $\text{CaSO}_4 \cdot \frac{1}{2} \text{H}_2\text{O}$ were slurried with 220 ml of distilled water adjusted to pH 2 with sulfuric acid. The carbon prepared with distilled water was applied to the glass plates immediately; however, the acidified carbon must stand a minimum of 16 h prior to preparation of the plates. The carbon was applied with a film thickness of 3×10^{-2} cm and air dried

R_F

Neo- mycin	Acid carbon, Solvent:		Untreated carbon, Solvent:	
	A	B	A	B
A	0.60	0.61	0.00	0.51
B	0.10	0.21	0.00	0.24
C	0.10	0.43	0.00	0.45

Solvents

- A. Water
B. 0.5 N Sulfuric acid

Detection

Bioautography vs. *Bacillus pumilus* after neutralizing by exposure to ammonia 5 min

Ref.

T.F. Brodasky, *Anal. Chem.*, 35 (1963) 343–345

TLC 2**Medium**

Silica gel

Solvent

3% Ammonia–acetone (160:40), 1 h

Detection

Ninhydrin

R_F

Neomycin B, 0.33
Neomycin C, 0.33

Ref.

F. Foppiano and B.B. Brown, *J. Pharm. Sci.*, 54 (1965) 206–208

TLC 3**Medium**

Kieselgel G (Merck); activate at 110°C for 45 min

Solvents

- A. *n*-Butanol–glacial acetic acid–water–pyridine (30:22:38:6)
B. *n*-Butanol–water–pyridine–glacial acetic acid–ethanol (60:10:6:15:5)

Detection

Ninhydrin spray reagent followed by heating at 105°C for 5 min

R_F

Solvent A, 0.14
Solvent B, 0.05

Ref.

R.J. Stretton, J.P. Carr and J. Watson-Walker, *J. Chromatogr.*, 45 (1969) 155–158

TLC 4**Medium**

Silica gel

Solvent

Butanol–water–pyridine–acetic acid–95% ethanol (60:10:6:15:5)

Detection

- A. Ponceau S stain
B. Ninhydrin

R_F

Useful for separation of neomycin from polymyxin B and bacitracin

Ref.

As PC 11

TLC 5**Medium**

Alumina

Solvent

Chloroform–methanol–17% aq. ammonia (2:1:1) upper layer

R_F

0.74

Ref.

As Gentamicins, PC 12

TLC 6**Media**

- A. Cellulose MN 300 (Macherey-Nagel)
B. Kieselgel 60 (Merck)

Solvents

- A. *n*-Butanol–methanol–water–acetic acid (45:30:36:9)
B. *n*-Butanol–methanol–water–ethanol–acetic acid (45:30:36:8:1) adjusted to pH 5.5 with 30% sodium hydroxide
C. *n*-Butanol–water (45:36) adjusted to pH 4.0 with glacial acetic acid

Detection

Bioautography vs. *Sarcina lutea* ATCC 9341

R_F

Solvent	Medium A	Medium B
A	0.00	
B	0.00	0.00
C	0.00	0.00

Ref.

As Ampicillin, TLC 3

TLC 7**Medium**

Alumina

Solvent

Chloroform–methanol–17% aq. ammonia (2:1:1), upper layer

R_F

0.74

Ref.

As Gentamicins, PC 15

TLC 8**Media**

- A. Cellulose MN-300 (Macherey-Nagel)
B. Silica gel G (Merck)
C. Kieselguhr G purified (BP 68)
D. Silica gel G (Merck) + Kieselguhr G (1 + 1)

Solvents

- A. *n*-Propanol-ethyl acetate-water-25% ammonia (100:20:60:20)
 B. *n*-Propanol-pyridine-acetic acid-water (100:66:20:80)
 C. Methanol-3% sodium chloride (2:1)
 D. 3.85% Ammonium acetate
 E. Methyl ethyl ketone-*tert*-butanol-methanol-6.5 *N* ammonia (160:30:10:60)
 F. *n*-Propanol-ethanol-ethyl acetate-water-25% ammonia-pyridine-3.85% ammonium acetate (100:100:20:60:20:10:200)

 R_F *Detection*

The spots were detected with a reagent containing 200 mg of ninhydrin and 500 mg of *p*-dimethylaminobenzaldehyde dissolved in a mixture of cyclohexane (30 ml), ethanol (70 ml) and pyridine (10 ml). After being sprayed with the reagent, the plates were heated at about 100°C for 5–10 min and then sprayed once again

Ref.

B. Borowiecka, *Dissert. Pharm. Pharmacol.*, 24 (1972) 210–215

Medium	Solvent	Time of devel.	Neomycins			M(A + B + C) **		
			A	B	C			
A	B	3 h	0.47	0.25	0.20	0.43	0.31	0.22
A	B	75 min & 100 min *	0.60	0.33	0.25			
B	A	45 min	0.42	0.23	0.25			
B	B	70 min	0.73	0.57	0.66			
B	F	5 h	0.38	0.18	0.15			
B	E	70 min	0.22	0.14	0.11			
C	E	60 min	0.84	0.46	0.68			
D	D	70 min	0.39	0.22	0.22			
D	F	4 h	0.32	0.18	0.11			
E	B	340 min	0.61	0.40	0.32			
E	B	3 h & 2 h, 20 min *	0.73	0.53	0.46			
E	B	17 h & 4 h, 30 min *	0.66	0.43	0.37	0.55	0.40	
E	C	75 min	0.43	0.36	0.17			

* Chromatographed twice

** Mixture of three components as found in pharmaceutical neomycin preparations

TLC 9*Medium*

Mix 6 g silica gel with 15 ml of distilled water, or with 15 ml of a 9:1 mixture of ethanol and water, into a homogeneous mass which is then uniformly applied onto the plates. After preliminary drying at room temperature the plates are held at 100°C for 30 min in a drying cabinet to activate the sorbent if the latter had been made with water, and for 10 min at the same temperature if the alcohol-water mixture is used. The separation chamber is satd. with the solvent vapor and sealed

Solvent

3.85% Ammonium acetate, freshly prepared soln.

Detection

- A. The plates are dried in a current of warm air to remove the ammonia vapor and then in air at room temperature. The chemical visualization is carried out with ninhydrin
 B. Bioautography vs. *Staphylococcus aureus* ATCC 6538P

R_F (see p. 256)

Ref.

L.P. Snezhova and L.N. Astanina, *Antibiotiki*, 17 (1972) 263–266

R_F

Component	R_F
Neamine	0.4
Neomycins B and C (single violet spot)	0.2

TLC 10

Medium

Silica gel 60 (precoated 20 × 20 cm, 0.25 mm thickness (E.M. Labs., Inc.))

Solvent

Methanol-chloroform-conc. ammonium hydroxide (1:1:1)

Detection

Ninhydrin: 10 ml of a freshly prepared soln. of ninhydrin (1.0 g) in a mixture of 95% ethanol (50 ml) and glacial acetic acid (10 ml); heat for 15 min at 135°. The antibiotics are visible as magenta red spots on a white background

 R_F

0.02 (Estimated from drawing)

Ref.

W.L. Wilson, G. Richard and D.W. Hughes, *J. Chromatogr.*, 78 (1973) 442-444

TLC 11

Medium

Silica gel

Solvent

Methyl ethyl ketone-acetone-water (9.3:2.6:1) developed 30-45 min (15 cm)

Detection

UV at 366 nm

 R_F

0

Ref.

M. Baudet, *J. Pharm. Belg.*, 31 (1976) 247-254

TLC 12

Medium

Silica gel

Solvent

Chloroform-methanol-28% ammonium hydroxide (1:3:2)

 R_F

5''-Amino-5''-deoxy-neomycin, 0.16

Neomycin, 0.20

Ref.

As PC 20

TLC 13

Medium

Silica gel

Solvents

A. Chloroform-methanol-17% ammonium hydroxide (2:1:1), upper phase

B. 10% Ammonium acetate-methanol (1:1)

Detection

Bioautography vs. *Bacillus subtilis*

 R_F

Solvent	R_F (neomycin B)
A	0.37
B	0.50

Ref.

R. Okachi, I. Kawamoto, S. Takasawa, M. Yamamoto, S. Sato, T. Sato and T. Nara, *paper presented 194th Sci. Meeting, JARA, July 26, 1974*

ELPHO 1

Media

A. Cellulose acetate (Oxoid) strips of 2.5 × 20 cm

B. Whatman No. 1

Buffers

A. Glacial acetic acid-formic acid-water (60:30:910)

B. Pyridine-glacial acetic acid-water (75:2.5:922.5), pH 6.6

Conditions

Paper, 700 V for 40 min; cellulose acetate strips, 400 V, for 60 min

Detection

A. Ninhydrin

B. Stain with Ponceau S reagent for 10 min and remove excess stain by soaking for 15 min in 5% acetic acid. Quantitative estimations of the antibiotics present in the electrophoresis strips are made by eluting the Ponceau S stained material with 2 ml of 0.1 N sodium hydroxide and estimating the color produced at 510 nm. Alternatively two strips were run in parallel, the presence of the antibiotics located on one and the corresponding section removed from the other and the antibiotic eluted from this with 2 ml of acetate buffer, pH 5.0. To this soln., 1 ml of quantitative ninhydrin reagent was added and the color was developed by heating at 98°C for 10 min. The color produced was estimated at 570 nm and by comparison with calibration curves of the pure antibiotic the amount in each sample could be determined

Mobility

Medium	Neomycin
A	11.2 cm towards the cathode
B	17.7 cm towards the cathode

Ref.

As PC 11, and TLC 3

ELPHO 2**Medium**

Paper, Whatman 3MM

Buffer

Formic acid–acetic acid–water (6:24:170)

Conditions

3000 V, potential gradient 60 V per cm, current 50 mA, 30 min

Detection

Paper stained with 0.25% ninhydrin in acetone containing 0.01% isatin and 1% lutidine at 100° for 10 min

Mobility

1.3, migration distance relative to alanine taken as 1.00

Ref.

As Ampicillin, PC 1

ELPHO 3**Medium**

Acrylamide gel

Buffer

Acrylamide gel:

Buffer soln., pH 4.6–monomer soln.–water–0.14% ammonium persulphate soln. (1:2:1:4)

Buffer soln.:

1 N potassium hydroxide–acetic acid–tetramethylethylenediamine (48:21.5:4.6) + water to 100 ml

Monomer soln.:

Acrylamide–methylenebisacrylamide (60 g:1.0 g) + water to 100 ml

Conditions

The gel soln. is pipetted into glass tubes 3 mm × 65 mm sealed at one end by parafilm, leaving sufficient space at the top to add a few drops of water to ensure a flat even surface on the catalysed gel surface. The tubes are allowed to stand at 28° for 1 h and after removal of the parafilm are ready for use

Detection

0.1% Naphthalene black in 7% acetic acid

Mobility

Blue-black band

Ref.

As Bacitracin, PC

GLC 1**Apparatus**

F and M Model 400 with F.I.D.

Column

Glass column 1830 (6 ft.) × 3 mm and packed with 0.75% OV-1 on Gas-Chrom Q, 100–120 mesh (Applied Science Labs.) was used

Temperature

Column, 330°C; over, 290°C; temperature programming: 10°C/min from 150 to 310°C

Gases

Gas flow-rates: hydrogen, 20 ml/min; air, 550 ml/min; and carrier gas (helium), 40 ml/min

Internal standard

Prepare a pyridine soln. containing approximately 3 mg/ml of trilaurin (Supelco). TRI-SIL Z (Pierce) may be substituted for pyridine for better stability of silylated neomycin

Reference standard

Use USP lot I neomycin Reference Standard at 767 µg neomycin base per mg anhydrous neomycin sulfate. Weigh approximately 6 mg neomycin powder into a one-dram vial and dissolve with 1 ml water. Place this vial in an appropriate apparatus and freeze-dry. The resulting freeze-dried sample dissolves more readily during silylation

Sample

As Reference standard

Derivative

Silylation procedure. Add 1.0 ml of the internal standard soln. and 80 µl of N-trimethylsilyldiethylamine (Pierce) to each vial containing the freeze-dried sample. Heat the vial in a 75°C oil bath for 40 min. Silylated neomycins thus prepared are extremely sensitive to moisture and better stability may be obtained when the sample is prepared in a 1.5-ml serum vial with a 13-mm natural red rubber closure

Calculations

$[R_1/R_2] \times [W_r/W_s] \times F = \mu\text{g neomycin per mg of sample}$

Where:

$$R_1 = \frac{\text{Area of the neomycin sample peak}}{\text{Area of the internal standard peak}}$$

$$R_2 = \frac{\text{Area of the neomycin standard peak}}{\text{Area of the internal standard peak}}$$

W_r = Weight of neomycin reference standard in mg

W_s = Weight of neomycin sample in mg

F = Assigned value of neomycin reference standard expressed in µg of anhydrous neomycin base per mg of reference standard

A mixture of silylated neamine, neobiosamine,

and neomycins B and C were chromatographed using temperature programming. Neamine and neobiosamine were clearly separated from each other and from neomycins B and C

Ref.

K. Tsuji and J.H. Robertson, *Anal. Chem.*, 41 (1969) 1332–1335; cf., K. Tsuji, J.H. Robertson, R. Baas and D.J. McInnis, *Appl. Microbiol.*, 18 (1969) 396–398

GLC 2

Apparatus

An F and M model 402 gas chromatograph with flame-ionization detector was used

Column

Glass, 61 cm (2 ft) × 3 mm I.D. packed with 3% OV-1 on Gas-Chrom Q, 100–120 mesh is used. To precondition the column before packing, fill the empty column with a 50% soln. of dimethyl-dichlorosilane in hexane and allow to stand for 5 min. Empty the column and wash with 50 ml of hexane followed by 50 ml of chloroform. Dry the column with a stream of dry air. Pack the column to within 8–9 mm of each end, and fill the remaining portions of the column with a small piece of silylated glass wool

Temperature

Oven temperature, 290°C; detector temperature, 310°C; and flash heater, 290°C

Gases

Gas flow-rates: hydrogen, 50 ml/min; air, 600 ml/min; and helium, 70 ml/min. Chart speed: 0.64 cm (0.25 in.)/min

Internal standard

Add 50 μ l N-trimethylsilyldiethylamine and 2 mg trilaurin/ml

Reference standard

Prepare a water soln. containing 5.0 mg/ml of neomycin sulfate, USP Lot I Reference Standard. Before using, dry the neomycin sulfate standard at 60° in a vacuum oven (< 5 mmHg) for 3 h

Derivatives

Add 1 ml of internal standard–silylation mixture to each vial, using 1-ml tuberculin syringe. Heat the vials in a 75° oil bath for 35 min, swirling occasionally

Calculations

Measure each peak area. Add one-third of the area of neomycin C to the area of neomycin B, and determine the peak area ratio of neomycin to trilaurin. The biopotency is determined by comparing area ratios between sample and standard soln.

Retention times

Estimated from curve

Neomycin B, 9.5 min

Neomycin C, 11–12 min

Ref.

B. Van Giessen and K. Tsuji, *J. Pharm. Sci.*, 60 (1971) 1068–1070

NEOPLURAMYCIN

TLC

Medium

Silica gel G (E. Merck)

Solvents

- A. Acetone
- B. Acetone–ethanol (4:1)
- C. Acetone–ethanol (1:1)
- D. Acetone–ethanol (1:4)
- E. Acetone–methanol (4:1)
- F. Acetone–methanol (9:1)
- G. Methanol
- H. Chloroform
- I. Chloroform–methanol (9:1)
- J. Ethanol–28% ammonia–water (8:1:1)
- K. *n*-Butanol–acetic acid–water (4:1:2)

R_F

Sol-vent	<i>R_F</i>	Sol-vent	<i>R_F</i>
A	0.05	G	0.05–0.20
B	0.03–0.09	H	0.00
C	0.06–0.18	I	0.00–0.05
D	0.00–0.10	J	0.80–0.90
E	0.05–0.10	K	0.25–0.30
F	0.00–0.10		

Ref.

S. Kondo, T. Wakashiro, M. Hamada, K. Maeda, T. Takeuchi and H. Umezawa, *J. Antibiot.*, 23 (1970) 354–359

NETILMICIN

HPLC 1

Column

μ Bondapak C₁₈ column, 30 cm × 3.9 mm I.D. (Waters Assoc.), precolumn 4.3 cm × 4.2 mm I.D. Micropart C₁₈ bonded-phase silica gel (5 μ m) packing

Mobile phase

0.2 *M* Sodium sulfate, 0.02 *M* sodium pentane-sulfonate, and 0.1% (v/v) acetic acid in water–methanol (97:3)

Flow-rate

Column, 2 ml/min at 184 atm OPA, 0.5 ml/min

Detection

Continuous flow, post-column derivatization with *o*-phthalaldehyde used to form fluorescent products

Type

Ion-pair

Retention time

4.7, estimated from drawing

Ref.

As Gentamicins, HPLC 1

HPLC 2**Apparatus**

Liquid chromatograph (Model 601, Perkin Elmer) equipped with a sample injector (Glenco Scientific)

Column

Reversed-phase, μ Bondapak C₁₈ (Waters Assoc.)

Mobile phase

Acetonitrile–water (95:5)

Flow-rate

1.0 ml/min

Temperature

Ambient

Detection

Fluorescence excitation wavelength, 220 nm; interference filter (KV470) used to select emission wavelength

Retention time

5.85 min

Ref.

G.W. Peng, G.G. Jackson and W.L. Chiou, *Antimicrob. Agents Chemother.*, 12 (1977) 707–709

NETROPSIN**PC****Solvent**

Butanol–acetic acid–water (2:1:1)

R_F

0.39

Ref.

As kikumycin, PC 1

NEUTRAMYCIN**PC****Solvents**

A. Dibutyl ether–diethyl ketone–0.2 M acetic acid (3:2:4)

B. Cyclohexane–*sec.*-butanol–0.4% ammonium hydroxide (4:1:4)

C. *n*-Heptane–diethyl ketone–tetrahydrofuran (8:3:3:8)

Detection

Bioautography vs. *Bacillus cereus*, *Corynebacterium xerosis* or *Staphylococcus aureus*

R_F

Solvent A, 0.50

Solvent B, 0.18

Solvent C, 0.40

Ref.

D.V. Lefemine, F. Barbatschi, M. Dann, S.O. Thomas, M.P. Kunstmann, L.A. Mitscher and N. Bohones, *Antimicrob. Agents Chemother.*, 193 (1964) 41–44

NIDDAMYCIN**PC 1****Paper**

Circular

Solvents

A. *sec.*-Butanol–acetic acid–water (4:1:1)

B. Pyridine–*n*-butanol–water (4:6:3)

C. *sec.*-Butanol–triethanolamine–methyl isobutyl ketone–water (14:1:1:5)

D. Cyclohexane–tetrahydrofuran (1:2), paper impregnated with formamide

E. Toluene, paper impregnated with propylene glycol

F. 3% Aq. ammonium chloride

G. 0.01 N Ammonium hydroxide satd. with methyl isobutyl ketone

Detection

A. Bioautography vs. *Staphylococcus aureus* 209P

B. UV fluorescence

C. Spray with 15% phosphoric acid and heat 5 min at 105°C to give a dark brown color

R_F

Solvent	R _F	Solvent	R _F
A	1.00	E	0.70
B	1.00	F	1.00
C	1.00	G	0.90
D	0.45		

Ref.

G. Huber, K.H. Wallhäuser, L. Fries, A. Steigler and H.-L. Weidenmüller, *Arztl. Forschung*, 12 (1962) 1191–1195

PC 2**Paper**

Toyo No. 51

Solvent

Water satd. ethyl acetate (ascending, 3 h)

Detection

Bioautography vs. *Bacillus subtilis* No. 10707

R_F

0.95

Ref.

As Carbomycin, PC 1

TLC 1**Medium**

Silica gel

Solvent

Diisopropyl ether-methanol (8:2)

Detection

As PC 1, C

R_F

0.8

Ref.

As PC 1

TLC 2**Medium**

Silica gel (Merck)

Solvents

A. Methylene chloride-95% aq. methanol-water (85:15:1)

B. Methylene chloride-95% aq. methanol-ammonium hydroxide (90:10:1)

Detection

A. Bioautography vs. *Bacillus subtilis* and *Staphylococcus aureus*

B. UV at 254 nm

C. Anisaldehyde reagent

R_F

	Solvent A	Solvent B
Niddamycin	0.77-0.82	0.65-0.70
Desisovaleryl-niddamycin	0.57-0.65	0.4 -0.37

Ref.

R.J. Theriault, *U.S. Patent* 3,948,884, April 6, 1976

ELPHO**Medium**

Paper

Buffer

Buffers at pH 1.9 and 7.8

Mobility

Moved toward cathode

Ref.

As PC 1

**NIDDAMYCIN, DESISOVALERYL-
(See desisovaleryl niddamycin)****NIFIMYCIN****PC****Solvents**

A. Water satd. butanol

B. Water satd. butanol + 2% piperidine

Detection

A. UV absorption

B. Ninhydrin

C. Bioautography vs. *Candida albicans*

D. Bioautography vs. *Bacillus subtilis*

R_F

Solvent A: 0.27, 0.65 (vs. *Candida albicans*)

Solvent B: 2 spots active against *Candida albicans*; additional zone ca. *R_F* 0.9 active vs. *Bacillus subtilis* only

Ref.

E.I. Khlebarova and N.O. Blinov, *Farmatsiya (Sofia)*, 19 (1969) 1-6; *C.A.*, 72 (1970) 296

NIGERICIN**TLC****Medium**

Silica gel

Solvents

A. Toluene-acetone (4:1) + 1% triethylamine

B. Chloroform-ethyl acetate (1:1)

C. Chloroform-methanol (95:5)

R_F

Solvent A, 0.11

Solvent B, 0.13

Solvent C, 0.30

Ref.

T. Rehr, H.D. King and M. Kuhn, *J. Antibiot.*, 30 (1977) 903-907

NIKKOMYCIN**ELPHO****Medium**

Paper

Buffers

A. Pyridine-glacial acetic acid, pH 3.9, 1 h

B. Pyridine-glacial acetic acid, pH 6.1, 1 h

C. 5,5-Diethylbarbituric acid, pH 8.9, 1 h

Ref.

U. Dahn, H. Hagenmaier, H. Hohne, W. Konig, H. Schienpflug and H. Zahner, *U.S. Patent* 4,046,881, Sept. 6, 1977

Mobility

Buffer	Migration
A	- 12 mm
B	- 1 mm
C	+ 16 mm

NOBORITOMYCINS**TLC****Medium**

Silica gel, Merck Kieselgel 60, 0.25 mm

Solvents

- A. Hexane-acetone (3:1)
- B. Toluene-acetone (9:1)
- C. Chloroform-ethyl acetate (2:1)
- D. Chloroform-acetone (9:1)

Detection

Detection was performed by spraying with a soln. of Ceric sulfate (0.2%) in 50% sulfuric acid followed by heating at 110-130°C thus producing brown spots

R_F

Solvent	Component	
	A	B
A	0.37	0.38
B	0.32	0.37
C	0.52	0.56
D	0.27	0.31

Ref.

C. Keller-Juslen, H.D. King, M. Kuhn, H.R. Loosli and A. von Wartburg, *J. Antibiot.*, 31 (1978) 820-828

NOCARDIA ANTIBIOTIC 2326**PC****Paper**

Circular

Solvent

n-Butanol-pyridine-water (1:0.6:1)

Detection

Bioautography vs. *Bacillus mycoides*

R_F

2326 complex > 2326K > 2326M

Ref.

N.T. Chormonova, T.P. Preobrazhenskaya, R.S. Ukholina, G.N. Komarova, G.B. Fedorova and V.N. Borisova, *Antibiotiki*, 23 (1978) 195-198

NOCARDIA ARGENTINENSIS ANTI-BIOTIC**TLC****Medium**

Silica gel

Solvent

Chloroform-acetone (3:1)

Detection

- A. Bioautography vs. *Staphylococcus aureus* or *Micrococcus luteus*
- B. UV at 254 nm

Ref.

W.D. Celmer, W.P. Cullen, C.E. Moppett, M.T. Jefferson, L.H. Huang, R. Shibakawa and J. Tone, *U.S. Patent* 4,148,883, April 10, 1979

NOCARDICIN E and F**TLC****Medium**

Cellulose, Eastman Chromagram Sheet No. 6065 (containing fluorescent agent)

Solvents

- A. *n*-Butanol-acetic acid-water (4:1:2)
- B. Water satd. *n*-butanol
- C. *n*-Propanol-water (7:3)

Detection

UV

R_F

Solvent	Nocardicin E	Nocardicin F
A	0.84	0.89
B	0.29	0.41
C	0.67	0.74

Ref.

J. Hosoda, H. Aoki and H. Imanake, *U.S. Patent* 4,146,536, March 27, 1979

NOGALAMYCIN**PC****Solvents**

- A. 1-Butanol-water (84:16), 16 h
- B. 1-Butanol-water (84:16) + 0.25% *p*-toluene-sulfonic acid, 16 h
- C. 1-Butanol-acetic acid-water (2:1:1), 16 h
- D. 2% Piperidine in *n*-butanol-water (84:16), 16 h
- E. 1-Butanol-water (4:96), 5 h
- F. 1-Butanol-water (4:96) + 0.25% *p*-toluene-sulfonic acid, 5 h

R_F (see p. 262)**Ref.**

B.K. Bhuyan and A. Dietz, *Antimicrob. Agents Chemother.*, 1965 (1966) 836-844

R_F

Solvent	R_F	Solvent	R_F
A	0.80	D	0.70
B	0.60	E	0.18
C	0.82	F	0.62

NOGALARENE; NOGALAROL**TLC***Medium*

Silica gel

Solvent

Chloroform-methanol-water (78:20:2)

 R_F

Nogalarol, 0.16

Nogalarene, 0.37

*Ref.*P.F. Wiley and E.L. Caron, Jr., *U.S. Patent* 3,501,569, March 17, 1970**NOJIRIMYCIN****TLC***Medium*

Silica gel G

Solvent

95% Ethanol-water (75:25)

*Detection*Bioautography vs. *Escherichia coli* R_F

0.60 (Estimated from drawing)

Ref.

As Feldamycin, PC

NOVOBIOCIN**PC 1***Paper*

The lower part of the paper was dipped in a mixture of capryl alcohol-methanol (1:5) up to the point of application of the sample and blotted

Solvent

0.1 M Phosphate buffer, pH 8.2, equilibrated with capryl alcohol

 R_F

Novobiocin, 0.25

Dihydronovobiocin, 0.61

*Ref.*E.J. Wolf and R. Nescot, *Antibiot. Ann.*, (1956-1957) 1035-1039**PC 2***Solvent*

Benzene-hexane-methyl ethyl ketone-ethanol (45:39:13:3)

Detection

Spectrophotometric procedure

 R_F

	Descending	Ascending
Novobiocin	0.22	0.08
Isonovobiocin	0.33	0.13
Descarbamyl novobiocin	0.47	0.20

*Ref.*V.B. Korchagin, V.V. Stepushkina and Z.E. Voinova, *Antibiotiki*, 11 (1966) 107-112**PC 3***Paper*

Whatman No. 4 or 20 dipped in ethylene glycol containing 2% of 85% lactic acid as stationary phase

Solvent

Diisopropyl ether satd. with ethylene glycol, descending, 16 h at 28°C and dried

Detection

UV absorbance at 324 nm

 R_F

Novobiocin acid > decarbamylnovobiocin > isonovobiocin > O-demethyldecarbamylnovobiocin > O-demethylnovobiocin (no R_F given)

*Ref.*L.A. Kominek, *Antimicrob. Agents Chemother.*, 1 (1972) 123-134**TLC 1***Media*

- Kieselguhr F₂₅₄ (Merck 5738). Before use, the Kieselguhr plate was impregnated with citrate/phosphate buffer, pH 3.7-glycerol (19:1)
- Silica gel 60 (Merck)
- Cellulose (Merck 5716)

Solvents

- Chloroform-acetone-impregnation liquid [citrate/phosphate buffer, pH 3.7-glycerol (19:1)] (5:5:2)
- Ethanol-water-ammonia (8:8:1)
- Methanol-chloroform (9:1)
- Methanol-acetone (3:2)
- Methanol-3% ammonium chloride (7:3)

Detection

- A. Bioautography vs. *Bacillus cereus* ATCC 11778
 B. Bioautography vs. *Bacillus subtilis* ATCC 6633
 C. Bioautography vs. *Micrococcus flavis* ATCC 10240
 D. Bioautography vs. *Sarcina lutea* ATCC 9341

 R_F

Medium	Solvent	Detection	Novobiocin
A	A	A	— •
B	B	C	0.80
B	C	D	0.80
B	C	B	0.80
B	D	C	0.80
C	E	A	—

* This R_F value was obtained by running the plate twice

Ref.

As Ampicillin, TLC 1

TLC 2**Medium**

Silanized silica gel HF₁₅₄ (Merck), reversed-phase

Solvents**Mobile phase**

- A. Buffer, pH 7–dioxane (7:3)
 B. Buffer, pH 7–isopropanol (7:3)
 C. Buffer, pH 7–acetone (7:3)
 D. Buffer, pH 7–tetrahydrofuran (7:3)
 E. Buffer, pH 7–methanol (6:4)
 F. Buffer, pH 7, satd. with 25% methyl ethyl ketone

Detection

UV light at 250 nm

 R_F

Solvent	Dihydranovobiocin	Novobiocin
A	0.21	0.28
B	0.25	0.32
C	0.23	0.30
D	0.15	0.21
E	0.14	0.20
F	0.17	0.26

Ref.

R. Rangone and C. Amoroso, *Il Farmaco (ed. Pr.)*, 26 (1971) 237–250

TLC 3**Medium**

Silica gel

Solvent

Methyl ethyl ketone–acetone–water (9.3:2.6:1) developed 30–45 min (15 cm)

Detection

UV at 366 nm, yellow-green spot

 R_F

0.91

Ref.

M. Baudet, *J. Pharm. Belg.*, 31 (1976) 247–254

TLC 4**Medium**

Silica gel

Solvent

Ethyl acetate–methanol (4:1)

 R_F

Novobiocin, 0.46

Hydroxynovobiocin, 0.24

Ref.

O.K. Sebek and A. Dolak, *U.S. Patent* 4,148,992, April 10, 1979

HPLC 1**Apparatus**

Laboratory Data Control modular liquid chromatograph with a Milton Roy Minipump (LDC) and a pulse dampener (LDC Model 709)

Column

Stainless steel (1000 × 2.1 mm I.D.) cont. Zipax HCP

Mobile phase

15% Methanol in 0.02 *M* monobasic and dibasic sodium phosphate buffer at pH 7.0

Flow-rate

0.85 ml/min

Temperature

Ambient

Pressure

68 atm (1000 p.s.i.)

Chart speed

0.25 in./min

Retention time (min)

Novobiocic acid, 3.5
 Novenamine, 4.0
 Desmethyldescarbamyl-novobiocin, 5.5
 Descarbamylnovobiocin, 12.5
 Isonovobiocin, 19.0
 Novobiocin, 23.5
 Dihydranovobiocin, 32.0

Ref.

K. Tsuji and J.H. Robertson, *J. Chromatogr.*, 94 (1974) 245–253

HPLC 2**Apparatus**

Varian LC 8500

ColumnMicropak CH, 10 μ m, 25 cm $\times$ 2.1 mm I.D.**Mobile phase**

60% Methanol + 40% of 1% acetic acid in water

Flow-rate

120 ml/h (320 atm)

Temperature

Ambient

Detector

Variscan 307 nm

Chart speed

0.5 cm/min

Sensitivity

0.5 a.u.f.s.

Sample size2 μ l**Recorder**

A-25, 10 mV

Type

Reversed-phase

Retention time

Novobiocin calcium, 2.1; estimated from drawing

Ref.

As Cephalosporin, HPLC 2

NYSTATIN**PC 1****Paper**

As chromin, PC

Solvent

As chromin, PC

Detection

As chromin, PC

 R_F

0.40

Ref.

As chromin, PC

PC 2**Paper**

Whatman No. 1

SolventWater satd. *n*-butanol, descending**Detection**A. Bioautography vs. *Saccharomyces carlsbergensis* K-20

B. 2,3,5-Triphenyltetrazolium chloride in glucose

 R_F

0.22

Ref.J. Burns and D.F. Holtman, *Antibiot. Chemother.*, 9 (1959) 398-405**PC 3****Paper**

Whatman No. 1

Solvents

A. Water satd. butanol

B. Butan-1-ol-acetic acid-water (2:1:1)

C. Butan-1-ol-pyridine-water (1:0.6:1)

D. Acetone-water (1:1)

 R_F

Solvent	R_F	Solvent	R_F
A	0.25	C	0.73
B	0.76	D	0.82

Ref.

As Amphotericins, TLC 2

TLC 1**Medium**Silica gel 60 F₂₅₄ (Merck)**Solvent**

Butanol-ethanol-acetone-32% ammonium hydroxide (2:5:1:3)

Detection

UV

 R_F

Nystatin, 0.42

Nystatin methyl ester, 0.77

Nystatin ethyl ester, 0.79

Nystatin propyl ester, 0.80

Nystatin butyl ester, 0.81

Ref.

As Amphotericins, TLC 1

TLC 2**Medium**

Silica gel G (Merck), activate 30 min at 105°C

SolventsA. *n*-Butanol-acetic acid-water (4:1:2)

B. Butanol-pyridine-water (2:1:2)

Detection0.02 *N* Potassium permanganate **R_F**

Solvent A, 0.45

Solvent B, 0.73-0.75

Ref.P.-A. Nussbaumer, *Pharm. Acta Helv.*, 43 (1968) 462-464**TLC 3****Medium**

Silica gel G

Solvents

- A. Ethanol-ammonia-water (8:1:1) buffered with phosphate, pH 8
 B. Butan-1-ol-acetic acid-water (3:1:1) buffered with phosphate, pH 8
 C. Methanol-propan-2-ol-acetic acid (90:10:1); plate pre-activated for 1 h at 110°C
 D. Methanol-acetone-acetic acid (8:1:1); plate pre-activated for 1 h at 110°C
 E. Ethanol-ammonia-dioxan-water (8:1:1:1); plate pre-activated for 1 h at 110°C
 F. Butan-1-ol-pyridine-water (3:2:1); plate pre-activated for 1 h at 110°C

Detection

- A. 10% Potassium permanganate and 0.2% bromophenol blue
 B. 0.2% *p*-Dimethylaminobenzaldehyde in sulfuric acid containing ferric chloride
 C. 5% Potassium permanganate
 D. Orthophosphoric acid, heat for 5 min at 100°C

 R_F

Solvent	Detection	R_F
A	A	0.18
B	A	0.18
C	B	0.54
D	B	0.66
E	C & D	0.28
F	C & D	0.65

Ref.

As Amphotericins, TLC 2

ELPHO**Media**

- A. Whatman No. 4 paper
 B. Whatman No. 31 paper

Buffers

- A. 0.1 *M* Boric acid-85% formic acid-sodium veronal-methanol-polyethylene glycol 400-water to 1000 ml (200 ml:10 ml:2.06 g:100 ml:50 ml) pH 2.6
 B. 2 *M* Propionic acid-2 *M* formic acid-sodium veronal-ethylene glycol-water to 1000 ml (100 ml:100 ml:2.06 g:100 ml) pH 2.6
 C. 0.1 *M* Boric acid-85% formic acid-glycerol-water to 1000 ml (200:10:100) pH 2.3
 D. Methanol-glacial acetic acid-water (950:50:50) pH 3.3

Conditions

The antibiotic sample (ca. 20 µg) in methanol soln. was placed onto Whatman No. 4 or No. 31 paper sheets (23×38 cm). Starting line was

placed 12 cm from the immersion line on anode side. Developed for 4 h at 350 V

Mobility

Med-ium	Buf-fer	Poten-tial/gra-dient (V/cm)	Cur-rent den-sity (mA/cm)	Mobil-ity (cm ² /V sec)
A	A	9.2	0.22	4.59
B	B	9.2	0.70	6.63
B	C	9.2	0.80	7.16
A	D	9.2	0.02	4.28

Ref.

S. Ochab, *Dissert. Pharm. Pharmacol.*, 23 (1971) 205-208

CCD**Solvent**

n-Amyl alcohol-isoamyl alcohol-citrate + phosphate buffer (pH 5) (12:17:29)

Distribution coefficient

200 transfers

Nystatin A1, *K* = 4.6

Nystatin A2, *K* = 16.9

Ref.

As Amphotericins, TLC 2

OCHRAMYCIN**PC****Paper**

Whatman No. 1

 R_F

Solvent	R_F	Solvent	R_F
A	0.70	F	0.32
B	0.90	G	0.22
C	0.73	H	0.67
D	0.73	I	0.26
E	0.12		

Solvents

- A. Methanol-water-ammonium hydroxide (20:4:1)
 B. Propan-2-ol-water (6:4)
 C. Butanol-pyridine-water (6:4:3)
 D. Butanol-acetic acid-water (4:1:5)
 E. Butanol satd. with $\frac{1}{15}$ *M* phosphate buffer, pH 4.1

F. As E but pH 5.4

G. As E but pH 6.0

H. As E but pH 7.0

I. As E but pH 8.0

Ref.

G. Cassinelli, A. Grein, P. Orezzi, P. Pennella and A. Sanfilippo, *Archiv Mikrobiologie*, 55 (1967) 358-368

TLC*Media*

A. Kieselgel G

B. Alumina G

Solvents

A. Ethanol-butanol-0.2 N hydrochloride acid (1:1:1)

B. Butanol-acetic acid-water (4:1:5)

C. Ethanol-water-ammonia (8:1:1)

D. Butanol-acetic acid-water (4:1:5)

E. Propanol-ethyl acetate-water-25% ammonium hydroxide (6:1:3:1)

R_F

Medium	Solvent	<i>R_F</i>
A	A	0.60
	B	0.30
	C	0.10
B	D	0.80
	E	0.70

Ref.

As PC

OLEANDOMYCINS**PC 1***Solvents*

A. Benzene-cyclohexane (1:1); formamide treated papers

B. Benzene; formamide treated papers

C. Benzene-chloroform (3:1); formamide treated papers

D. Benzene-chloroform (1:1); formamide treated papers

R_F

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.00	C	0.32
B	0.02	D	0.63

Ref.

K. Murai, B.A. Sobin, W.D. Celmer and F.W. Tanner, *Antibiot. Chemother.*, 9 (1959) 485-490

PC 2*Paper*

Whatman No. 4 impregnated with 50% methanolic formamide

Solvents

A. Benzene-cyclohexane (1:1)

B. Benzene-cyclohexane (2:1)

C. Benzene-chloroform (3:1)

D. Benzene-chloroform (1:1)

All systems satd. with formamide

Detection

Bioautography vs. *Bacillus subtilis* ATCC 6633

R_F

	Solvent			
	A	B	C	D
Oleandomycin base	0.02	0.05	0.05	0.30
3-Mono-acetyl-oleandomycin	0.05	0.10	0.15	0.50
2-Mono-acetyl-oleandomycin	0.10	0.25	0.40	0.70
2,3-Diacetyl-oleandomycin	0.20	0.35	0.70	0.95
1-Mono-acetyl-oleandomycin	0.30	0.50	0.95	0.95
1,3-Diacetyl-oleandomycin	0.40	0.65	0.95	0.95
1,2-Diacetyl-oleandomycin	0.80	0.90	0.95	0.95
Triacetyl-oleandomycin	0.95	0.95	0.95	0.95

Ref.

T.M. Lees, P.J. DeMuria and W.H. Boegemann, *J. Chromatogr.*, 5 (1961) 126-130

PC 3*Paper*

Whatman No. 1

Solvents

- A. 80% Methanol + 3% sodium chloride (1:1), descending. Paper buffered with 0.95 *M* sodium sulfate + 0.05 *M* sodium bisulfate
- B. Propanol–pyridine–acetic acid–water (6:4:1:3), ascending
- C. Butanol–acetic acid–water (4:1:5), ascending
- D. 80% Phenol, ascending

Detection

Bioautography vs. *Staphylococcus aureus*

R_F

Solvent	R_F	Solvent	R_F
A	0.90	C	0.78
B	0.93	D	0.86

Ref.

As Chloramphenicol, PC 1

PC 4**Paper**

Toyo No. 51

Solvent

Water satd. ethyl acetate (ascending, 3 h)

Detection

Bioautography vs. *Bacillus subtilis* No. 10707

R_F

0.30–0.60

Ref.

As Carbomycin, PC 1

TLC 1**Medium**

Silica

Solvent

Chloroform–methanol–toluene (80:17:23)

Detection

Dragendorff reagent

R_F

Triacetyloleandomycin, 0.7

Diacetyloleandomycin, 0.5

Monoacetyloleandomycin, 0.2

Ref.

P. Gantes, J.-C. Garinot, J. Barat and J.P. Juhasz, *Annales Pharm. Franc.*, 23 (1965) 137–140

TLC 2**Medium**

Silica gel–plaster of Paris–water (6:0.3:15)

Solvent

Butanol–acetic acid–water (3:1:1)

Detection

- A. Visualized by spraying with sulfuric acid and heat at 100° for 5 min

B. Bioautography vs. *Bacillus subtilis*

R_F

0.53

Ref.

M.V. Kalinina and E.I. Surikova, *Antibiotiki*, 13 (1968) 112–114

TLC 3**Medium**

Silica gel GF

Solvents

A. Chloroform–methanol–17% ammonia (2:1:1)

B. Butanol–acetic acid–water (3:1:1)

Detection

Spray with conc. sulfuric acid–methanol (1:1), developed by heating at 105°C for several min

R_F

Solvent A, 0.98 (green)

Solvent B, 0.19 (green)

Ref.

As Chloramphenicol, PC 1

TLC 4**Medium**

Silica gel

Solvent

Methylene chloride–95% aq. methanol–conc. ammonium hydroxide (90:10:1) (run through twice)

Detection

Bioautography vs. *Bacillus* sp. 1283 B

R_F

0.7

Ref.

R.W. Kierstead, R.A. LeMahieu and D. Pruess, *U.S. Patent* 3,928,387, Dec. 23, 1975

TLC 5**Medium**

Silica gel

Solvents

A. Chloroform–methanol–17% ammonia (2:1:1)

B. Butanol–acetic acid–water (3:1:1)

C. Chloroform–methanol (3:2)

Detection

A. Bioautography vs. *Sarcina lutea*

B. Plate heated at 100°C and sprayed with phosphoric acid in methanol (1:1)

R_F (see p. 268)

Ref.

G.H. Wagman, J.A. Waitz, J. Marquez, A. Murawski, E.M. Oden, R.T. Testa and M.J. Weinstein, *J. Antibiot.*, 25 (1972) 641–646

R_F

Solvent	Detection	R_F
A	A	0.95
	B	0.78, 0.95 black
B	A	0.36
	B	0.14, 0.36 black
C	A	0.29

TLC 6

Media

- A. Kieselguhr F₂₅₄ (Merck 5738). Before use, the Kieselguhr plate was impregnated with citrate/phosphate buffer (pH 3.7)–glycerol (19:1)
 B. Silica gel 60 (Merck)
 C. Cellulose (Merk 5716)

Solvents

- A. Chloroform–acetone–impregnation liquid [citrate/phosphate buffer (pH 3.7)–glycerol (19:1)] (5:5:2)
 B. Ethanol–water–ammonia (8:8:1)
 C. Methanol–chloroform (9:1)
 D. Methanol–acetone (3:2)
 E. Methanol–ammonium chloride (3%) (7:3)

Detection

- A. Bioautography vs. *Bacillus cereus* ATCC 11778
 B. Bioautography vs. *Bacillus subtilis* ATCC 6633
 C. Bioautography vs. *Micrococcus flavis* ATCC 10240
 D. Bioautography vs. *Sarcina lutea* ATCC 9341

 R_F

Medium	Solvent	Detection	Oleandomycin
A.	A	A	0.20 *
B	B	C	0.70
B	C	D	0.35
B	C	B	–
B	D	C	0.25
C	E	A	–

* This R_F value was obtained by running the plate twice

Ref.

As Bacitracin, TLC 1

CCD

Solvent

Benzene–cyclohexane–95% ethanol–water (5:5:8:2)

Distribution coefficient

$K = 0.25$

Ref.

As carbomycin, PC 2

OLEFICIN

PC

Solvent

Propanol–ethyl acetate–0.25 *N* ammonium hydroxide (6:1:4)

Detection

Bioautography vs. *Bacillus subtilis*

 R_F

0.84

Ref.

J. Gyimesi, I. Ott, I. Horvath, I. Koczka and K. Magyar, *J. Antibiot.*, 24 (1971) 277–282

TLC

Medium

Kieselgel G

Solvents

- A. As PC, A
 B. Butanol–acetic acid–water (4:1:5)
 C. Ethanol–water–ammonium hydroxide (8:1:1)
 D. Benzene–ethyl acetate (1:1)

 R_F

Solvent	R_F	Solvent	R_F
A	0.60	C	0.62
B	0.79	D	0.10

Ref.

As PC

CCD

Solvent

Pyridine–ethyl acetate–water (3.5:6.5:8.3), 100 transfers

Distribution

Peak in tube 59

Ref.

As PC

OLIGOMYCINS

PC 1

Paper

Eaton-Dikeman No. 613

Solvent

Water-ethanol-acetic acid (70:24:6), 18–20 h at 30°

Detection

Bioautography vs. *Glomerella cingulata*

 R_F

Component	R_F
A moves 22 cm	0.70
B moves 29 cm	0.85
C moves 13 cm	0.60

Ref.

S. Masamune, J.M. Sehgal, E.E. van Tamelen, F.M. Strong and W.H. Peterson, *J. Amer. Chem. Soc.*, 80 (1958) 6092–6095; M.H. Larson and W.H. Peterson, *Appl. Microbiol.*, 8 (1960) 182–189; E.W. Marty, Jr. and E. McCoy, *Antibiot. Chemother.*, 9 (1959) 286–293

PC 2**Solvents**

- A. 16% Hydrated *n*-propanol
- B. 18% Hydrated *n*-propanol

 R_F

Solvent A, 0.15, 0.23, 0.30
Solvent B, 0.24, 0.35, 0.44

Ref.

I. Takeda, M. Mizuno, T. Sugawara, Y. Shimojiam and S. Horiguchi, *U.S. Patent* 3,780,172, Dec. 18, 1973

OLIVANIC ACIDS (MM 4550, MM 13902, MM 17880)

PC**Paper**

Ion-exchange paper DE-81 (Whatman)

Solvent

0.2 *m* Sodium chloride in 0.05 *m* phosphate buffer, pH 7. MM 4550 is inactivated in this system

Detection

The developed tapes were laid on an agar plate seeded with *Neisseria catarrhalis* NCIC 3622 and incubated at 37°C for 24 h. Quantitative: zones of inhibition due to MM 13902 and MM 17880 were traced, cut out and weighed

 R_F

Component	R_F
MM 13902	0.40
MM 17880	0.60

Ref.

J.D. Hood, S.J. Box and M.S. Verrall, *J. Antibiot.*, 32 (1979) 295–304

TLC 1**Medium**

Cellulose (Eastman Kodak)

Solvents

- A. *n*-Propanol–water (4:1)
- B. *n*-Butanol–isopropanol–water (7:7:6)
- C. Isopropanol–water (7:3)

 R_F

Solvent	MM 4550	MM 13902	MM 17880
A	0.45	0.75	0.70
B	0.55	0.70	0.70
C	0.70	0.85	0.80

Ref.

As PC

TLC 2**Medium**

Cellulose (Eastman Kodak chromatogram sheets)

Solvent

n-Butanol–isopropanol–water (7:7:6, v/v)

The culture filtrate (25 ml) was extracted with 12.5 ml of benzyldimethyl-*n*-hexadecylammonium chloride in dichloromethane. After separation of the phases, 2.5 ml of 0.5% sodium iodide in water was added to the organic phase, the mixture shaken and the aq. phase chromatographed by spotting 5 μ l onto the cellulose chromatogram sheets

Detection

The zones of β -lactamase inhibitory activity were detected by contacting the chromatograms with agar seeded with *Klebsiella aerogenes* A and containing 6 μ g/ml benzylpenicillin

Ref.

D. Butterworth, M. Cole, G. Hanscomb and G.N. Rolinson, *J. Antibiot.*, 32 (1979) 287–294

OLIVOMYCIN

PC**Paper**

Circular

Solvents

- A. Benzene–acetic acid–water (20:25:5)
- B. Benzene–butanol–water (18:2:20)
- C. Chloroform–carbon tetrachloride (satd. with water)–methanol (5:4:1)

D. Diisoamyl ether (satd. with water)–butanol (20:10)

Detection

Bioautography vs. *Staphylococcus aureus* 209P

R_F

Olivomycin (greatest R_F) can be separated from aburamycin, NSCA-649 and LA-7017

Ref.

E.V. Kruglyak, V.N. Borisova and M.G. Brazhnikova, *Antibiotiki*, 8 (1963) 1064–1067

OOSPORA VIRESCENS (Link) Wallr. ANTIBIOTIC GLYCOSIDES

TLC

Medium

Kieselgel H (Fluka), activate at 110°C

Solvents

A. Chloroform–methanol (85:15)

B. Chloroform–methanol–acetic acid (80:15:5)

Detection

50% Sulfuric acid; heat at 110°C approximately 5 min

R_F

Components	Solvent A	Solvent B
A	0.16	
B	0.34	
C	0.52	
D	0.65	
E	0.36	
F		0.32
G		0.18
H	0.42	

Ref.

German Patent 2100918, July 15, 1971

ORYMYCIN

PC

Solvents

A. 16% Hydrated *n*-propanol

B. 18% Hydrated *n*-propanol

R_F

Solvent A, 0.21

Solvent B, 0.34

Ref.

I. Takeda, M. Mizuno, T. Sugawara, Y. Shimojima and S. Horiguchi, *U.S. Patent* 3,780,172, Dec. 18, 1973

ORYZOXYMYCIN

PC

Paper

Toyo No. 51

Solvents

A. 80% ethanol

B. *n*-Butanol–acetic acid–water (4:1:2)

R_F

Solvent A, 0.66

Solvent B, 0.13

Ref.

T. Hashimoto, S. Kondo, T. Takita, M. Hamada, T. Takeuchi, Y. Okami and H. Umezawa, *J. Antibiot.*, 21 (1968) 653–658

TLC

Medium

Kieselgel G (Merck)

Solvents

A. Methanol

B. *n*-Butanol–acetic acid–water (4:2:1)

C. *n*-Propanol–acetic acid–pyridine–water (50:6:20:24)

D. Methanol–ethyl acetate (1:1)

Detection

A. Bioautography vs. *Xanthomonas oryzae*

B. Color with 0.5% potassium permanganate

R_F

Solvent	R_F	Solvent	R_F
A	0.42	C	0.64
B	0.32	D	0.07

Ref.

As PC

ELPHO

Medium

Paper

Buffer

Formic acid–acetic acid–water (25:75:900)

Conditions

3300 V/42 cm, 65 mA/20 cm, 15 min

Mobility

Moves 7 cm to cathode

Ref.

As PC

OSSAMYCIN

TLC

Media

A. Silica gel

B. Cellulose powder

Solvents

- A. Benzene-methanol-water (100:110:10)
 B. Benzene-methanol-Skellysolve B-water
 (25:150:25:15)

R_F

- Solvent A, 0.66
 Solvent B, 0.43

Ref.

- H. Schmitz, S.D. Jibinski, I.R. Hooper, K.E. Crook, Jr., K.E. Price and J. Lein, *J. Antibiot.*, 18 (1965) 82-88

CCD**Solvent**

- Skellysolve-benzene-ethanol-water (2:3:4:1),
 100 transfers

Distribution

- Peak in tube 71

Ref.

- As TLC

OUDEMANSIELLA MUCIDA ANTI-BIOTIC**TLC****Medium**

- Aluminum oxide

Solvent

- Light petroleum-diethyl ether-acetic acid
 (9:10:1)

Detection

- Bioautography vs. *Saccharomyces cerevisiae*

R_F

- 0.5 (estimated from figure)

Ref.

- Belgian patent 704076, March 20, 1968

OXACILLIN**TLC****Medium**

- Avicel

Solvent

- Methanol-isobutyl alcohol-chloroform-10% hydrochloric acid (10:10:10:1)

Detection

- A. Iodine vapor
 B. 0.25% Stannous chloride soln.
 C. 0.2% *p*-Dimethylaminobenzaldehyde soln.

R_F

- 0.83

Ref.

- As Ampicillin, TLC 1

HPLC 1**Column**

- 1 m × 2.1 mm I.D. × 6.3 mm O.D., 316 stainless steel; C₁₈/Porasil B packing

Mobile phase

- Methanol-0.05 M ammonium carbonate (30:70)

Pressure

- 500 p.s.i.

Flow-rate

- 0.5 ml/min

Detector

- UV (254 nm)

Sensitivity

- 0.16 a.u.f.s.

Type

- Reversed-phase

Retention time

- 22.5 min (estimated from drawing)

Ref.

- As Ampicillin, HPLC

HPLC 2**Apparatus**

- Constametric I pump and Spectromonitor I
 (Laboratory Data Control)

Column

- 25 cm μBondapak C₁₈ (PN-27324; Waters Assoc.)

Mobile phase

- 25% Acetonitrile (Photrex; J.T. Baker) in 0.1 N acetate buffer, pH 6.0

Flow-rate

- 2.0 ml/min

Temperature

- 40°C

Detection

- UV 270 nm (0.04 a.u.f.s.) time constant, 0.5 sec

Type

- Reversed-phase

Retention time

- 3.0 min, peak height 15 mm

Ref.

- J.R. Koup, B. Brodsky, A. Lau and T.R. Beam, Jr., *Antimicrob. Agents Chemother.*, 14 (1978) 439-443

OXAMICETIN**TLC****Media**

- A. Silica gel
 B. Alumina

Solvents

- A. *n*-Butanol-acetic acid-water (3:1:1)

B. Ethanol–28% ammonium hydroxide–water (8:1:1)

C. 80% Methanol

Detection

A. Bioautography vs. *Bacillus subtilis* with Medium A

B. Spray with anthrone reagent

R_F

Solvent	Medium	R_f
A	A	0.25
B	A	0.72
C	B	0.54

Ref.

M. Konishi, M. Kameda, H. Tsukiura, H. Yamamoto, T. Hoshiya, T. Miyaki, K. Fujisawa, H. Koshiyama and H. Kawaguchi, *J. Antibiot.*, 26 (1973) 752–756; H. Kawaguchi, M. Konishi and K. Tomita, *U.S. Patent* 3,843,449, Oct. 22, 1974

OXYTETRACYCLINE (cf., tetracyclines)

PC

Paper

Whatman No. 1

- A. Impregnated with McIlvaine's buffer, pH 5.0
- B. Impregnated with McIlvaine's buffer, pH 5.0, containing 10% w/v urea

Solvents

- A. Chloroform–ethyl acetate–pyridine (1:1:1)
- B. Chloroform–ethyl acetate–pyridine (1:1:1), previously satd. with urea

Detection

UV

R_F

Component	Medium	Solvent A	Solvent B
α - or β -Apoterramycin	A	0.95	
	B		0.76
Oxytetracycline	A	0.61	
	B		0.48
6 β -Deoxyoxy-tetracycline	A	0.02	

Ref.

A. Sina, M.K. Youssef, A.A. Kassem and I.A. Attia, *J. Pharm. Sci.*, 60 (1971) 1544–1547

TLC

Medium

Avicel

Solvent

Methanol–isobutyl alcohol–chloroform–10% hydrochloric acid (10:10:10:1)

Detection

- A. Iodine vapor
- B. 0.25% Stannous chloride soln.
- C. 0.2% *p*-Dimethylaminobenzaldehyde soln.

R_F

0.65

Ref.

As Ampicillin, TLC 1

OXYTOCIN

PC

Paper

Whatman No. 1

Solvent

Butanol–acetic acid–water (4:1:5)

R_F

- 4-Glycine-oxytocin, 0.58
- 3-Glycine-oxytocin, 0.36
- 2-Glycine-oxytocin, 0.38

Ref.

S. Drabarek, *J. Amer. Chem. Soc.*, 86 (1964) 4477

PAECILOMYCEROL

TLC

Medium

Silica gel

Solvents

- A. Benzene–acetone (2:1)
- B. Chloroform–methanol (7:1)
- C. Ethyl acetate

R_F (Estimated from drawing)

- Solvent A, 0.50
- Solvent B, 0.25
- Solvent C, 0.50

Ref.

A. Kato, K. Ando, T. Kimura, G. Tamura and K. Arima, *J. Antibiot.*, 22 (1969) 419–422

PAMAMYCIN

TLC

Media

- A. Silica gel
- B. Alumina

Solvents

- A. Chloroform
- B. Benzene–ethyl acetate (70:30)

- C. Ethyl acetate
 D. Chloroform-methanol (90:10)
 E. Benzene-methanol (55:45)
 F. Chloroform-acetic acid-water (3:1:1)
 H. Ethyl acetate-methanol (50:50)

R_F
 R_F values obtained on glass plates

Solvent	Medium A	Medium B
A	0	—
B	—	0.09
C	—	0.11
D	0.12	—
E	0.58	—
F	0.56	—
G	0.76	—
H	—	0.5

Ref.

P.A. McCann and B.M. Pogell, *J. Antibiot.*, 32 (1979) 673-678

PAPULACANDINS

TLC

Medium

Silica gel F₂₅₄

Solvents

- A. Chloroform-methanol (4:1), repeated three times
 B. Ethyl acetate-acetone-water (72:24:4), repeated twice

Detection

- A. Bioautography vs. *Candida albicans*
 B. UV
 C. Iodine

R_F

Solvent	Papulacandin				
	A	B	C	D	E
A	0.48	0.40	0.35	0.58	0.60
B	0.35	0.28	0.25	0.60	0.38

Ref.

P. Traxler, J. Gruner and J.A.L. Auden, *J. Antibiot.*, 30 (1977) 289-296

PAROMAMINE, 3'-DEOXY- (See 3'-deoxyparomamine)

PAROMOMYCIN DERIVATIVES

TLC

Medium

Silica gel

Solvent

Chloroform-methanol-28% ammonium hydroxide-water (1:4:2:1)

R_F

Component	R_F
BB-K47	0.17
BB-K48	0.07
BB-K49	0.27
BB-K50	0.27
BB-K51	0.18

Ref.

T. Naito and S. Nakagawa, *U.S. Patent* 3,897,412, July 29, 1975

PAROMOMYCINS

PC 1

Solvents

- A. 80% Methanol + 3% sodium chloride (1:1, w/v), descending. Paper buffered with 0.95 M sodium sulfate + 0.05 M sodium bisulfate
 B. Propanol-pyridine-acetic acid-water (6:4:1:3, v/v), ascending
 C. 80% (v/v) Phenol, ascending
 D. Benzene-methanol (9:1, v/v), descending
 E. *n*-Butanol-water-acetic acid (4:5:1), upper phase, ascending

R_F

Solvent	R_F	Solvent	R_F
A	0.0, 0.28	D	0.0
B	0.07	E	0.0
C	0.0, 0.2		

Ref.

M.J. Weinstein, G.H. Wagman and J.A. Marquez, *U.S. Patent* 4,001,209, Jan. 4, 1977

PC 2

Paper

Ascending

Solvent

Chloroform-methanol-7% aq. ammonia (2:1:1) lower layer (developed at room temp. for 12 h)

R_F

0.00

Ref.

As Apramycin, PC 1

PC 3

Solvent

1-Butanol-pyridine-water (6:4:3)

 R_F

N-Acetyl derivatives:

Paromomycin I, 0.33

Paromomycin II_c, 0.21

Ref.

W.T. Shier, K.L. Rinehart, Jr. and D. Gottlieb,

U.S. Patent 3,833,056, Sept. 3, 1974

PC 4

Paper

Toyo No. 51

Solvent

Methanol-3% aq. sodium chloride (2:1)

 R_F

0.31

Ref.

As Gentamicins, PC 14

PC 5

Paper

Descending

Solvents

A. Water satd. *n*-butanol + 2% *p*-toluenesulfonic acid, 20 h

B. *n*-Propanol-pyridine-acetic acid-water (15:10:3:12), 20 h

C. *n*-Butanol-pyridine-acetic acid-water (6:4:1:3), 7 days

 R_F

Solvent	R_{767-L}^*	
	Paromomycin I	Paromomycin II
A	2.33 (5.6) **	2.33 (5.7)
B	1.25 (11.4)	1.25 (11.3)
C	1.60 (9.1)	1.60 (9.0)

* This term represented the ratio of the value for migration distance of one antibiotic substance to the value for migration distance of the SF-767-L substance. In determination of R_{767-L} , an amount of the SF-767-L substance was used as an internal standard to each of the antibiotic substances, and the mixture was then developed

** Numbers in parentheses are migration distances from original point in cm

Ref.

T. Shomura, N. Ezaki, T. Tsuruoka, T. Niwa, E. Akita and T. Niida, U.S. Patent 3,697,648, Oct. 10, 1972

PC 6

Paper

Whatman No. 1

Solvent

Methyl ethyl ketone-*tert*.-butanol-methanol-6.5 *N* ammonium hydroxide (16:3:1:6)

Detection

Ninhydrin

 R_F (Estimated from drawing)

Paromomycin I, 0.44

Paromomycin II, 0.26

Paromamine, 0.77

Ref.

P.J. Claes, F. Compennolle and H. Vanderhaeghe, *J. Antibiot.*, 27 (1974) 931-942

TLC 1

Medium

Mixture of Kieselgel G and aluminum oxide

Solvent

n-Propanol-ethyl acetate-water-25% ammonium hydroxide (50:10:30:10)

Detection

Ninhydrin

 R_F

$$R_{\text{glucosamine}} = \frac{(\text{distance spot moved})}{(\text{distance glucosamine moved})} = 0.31$$

Ref.

V.R. Huttenrauch and I. Schulze, *Pharm. Zentralblatt*, 104 (1965) 85-87

TLC 2

Medium

Alumina

Solvent

Chloroform-methanol-17% aq. ammonia (2:1:1) upper layer

 R_F

0.70

Ref.

As Gentamicins, PC 14

TLC 3

Medium

Cellulose

Solvent

Methyl ethyl ketone-methanol-isopropanol-7.9 *N* ammonium hydroxide (10:8.5:3:7)

Detection

Ninhydrin. Spray with 0.3% soln. of ninhydrin in water satd. *tert.*-butanol, heat at 100–105°C for 3–5 min

R_F

Paromomycin I, 0.55
Paromomycin II, 0.23

Ref.

As Kanamycins, TLC 5

TLC 4**Medium**

Silica gel

Solvent

Chloroform–methanol–28% ammonium hydroxide–water (1:4:2:1)

R_F

Compound	R _F
BB-K47 [*]	0.17
BB-K48	0.07
BB-K49	0.27
BB-K50	0.27
BB-K51	0.18

^{*} BB-K47 = 1-[L-(–)-γ-Amino-α-hydroxybutyryl] paromomycin

Ref.

T. Naito and S. Nakagawa, *U.S. Patent* 3,897,412, July 29, 1975

TLC 5**Medium**

Alumina

Solvent

Chloroform–methanol–17% aq. ammonia (2:1:1), upper layer

R_F

0.70

Ref.

As Gentamicins, PC 15

TLC 6**Medium**

Silica gel

Solvent

Methyl ethyl ketone–acetone–water (9.3:2.6:1) developed 30–45 min

Detection

UV at 366 nm

R_F

0

Ref.

M. Baudet, *J. Pharm. Belg.*, 31 (1976) 247–254

TLC 7**Medium**

Silica gel 60, precoated 20 × 20 cm, 0.25 mm thickness (E.M. Labs.)

Solvent

Methanol–chloroform–conc. ammonium hydroxide (1:1:1)

Detection

Ninhydrin: 10 ml of a freshly prepared soln. of ninhydrin (1.0 g) in a mixture of 95% ethanol (50 ml) and glacial acetic acid (10 ml) heat for 15 min at 135°. The antibiotics are visible as magenta red spots on a white background

R_F

0.02 (estimated from drawing)

Ref.

W.L. Wilson, G. Richard and D.W. Hughes, *J. Chromatogr.*, 78 (1973) 442–444

ELPHO**Medium**

Whatman No. 4 filter paper

Solvents

- 2 M Formic acid–0.1 M *p*-toluenesulfonic acid–*n*-propanol (500:50:50) in 1000 ml water, pH 1.8
- 2 M Formic acid–0.1 M *p*-toluenesulfonic acid–*n*-propanol (400:50:50) in 1000 ml water, pH 1.9
- 2 M Formic acid–0.1 M *p*-toluenesulfonic acid (400:50) in 1000 ml water, pH 1.9
- 1 M Ammonium hydroxide–1 M sodium hydroxide–0.1 M *p*-toluenesulfonic acid–*n*-propanol (200:5:100:100) in 1000 ml water, pH 10.8
- 1 M Ammonium hydroxide–1 M sodium hydroxide (200:2.5) in 1000 ml water, pH 11.5
- 1 M Sodium hydroxide–water (50:1000, v/v), pH 12.2

Conditions

The electrophoregrams were developed at 350 V for 1–4 h (depending on the electrolyte)

Mobility

Buffer	Towards cathode (cm ² /V sec)
A	33.17
B	30.91
C	30.96
D	2.71
E	4.14
F	6.33

Ref.

S. Ochab, *Pol. J. Pharmacol. Pharm.*, 23 (1973) 105-108

GLC (Procedure for GLC as kanamycins, GLC)

Separation of Paromomycin I and II

Paromomycin II is retained longer on the OV-1 column than paromomycin I

Ref.

As kanamycins, GLC

PARTRICIN

TLC 1

Medium

Silica gel

Solvent

Butanol-ethanol-acetone-25% ammonium hydroxide (2:5:1:3)

R_F

0.50

Ref.

T. Bruzzese and R. Ferrari, *U.S. Patent* 3,773,925, Nov. 20, 1973

TLC 2

Medium

Silica gel 60 F₂₅₄ (Merck)

Solvent

Butanol-ethanol-acetone-32% ammonium hydroxide (2:5:1:3)

Detection

UV

R_F

Partricin, 0.51

Partricin methyl ester, 0.84

Partricin ethyl ester, 0.88

Partricin propyl ester, 0.90

Partricin butyl ester, 0.92

Ref.

As Amphotericins, TLC 1

TLC 3

Medium

Silica gel F₂₅₄

Solvent

Butanol-ethanol-acetone-conc. aq. ammonium hydroxide (2:5:1:3)

Ref.

T. Bruzzese and G. Ghielmetti, *U.S. Patent* 3,961,047, June 1, 1976

R_F

Derivative	<i>R_F</i>
N,N'-Diacetyl partricin	0.65
N,N'-Dipropionyl partricin	0.67
N,N'-Dibutyl partricin	0.68
N,N'-Disuccinyl partricin	0.45
N-Maleoyl partricin	0.45
Partricin ethyl ester	0.85
Partricin N-propyl ester	0.88
N-Butyl partricin	0.90
N,N'-Diacetyl partricin methyl ester	0.86
N,N'-Disuccinyl trimethyl ester	0.90
N,N'-Disuccinyl partricin methyl ester	0.73

PATHOCIDIN

PC

Solvents

A. Acetone-water (3:7)

B. Acetone-water (1:1)

C. Acetone-water (8:2)

D. Pyridine-water (2:1)

E. Methanol-phosphate buffer, pH 9 (4:1)

F. Butanol satd. with water

G. Butanol-acetic acid-water (4:1:2)

H. Phosphate buffer, pH 9

Detection

A. Fluorescence under UV light

B. Bioautography vs. *Penicillium chrysogenum*

R_F

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.70	E	0.38
B	0.52	F	0.00
C	0.10	G	0.00
D	0.70		

Ref.

K. Anzai, J. Nagatsu and S. Suzuki, *J. Antibiot.*, 14 (1961) 340-342

PATULIN

TLC 1

Solvents

A. Ethanol-water (4:1)

B. Toluene-ethyl acetate-90% formic acid (6:3:1)

- C. Benzene-methanol-acetic acid (24:2:1)
 D. Benzene-propionic acid-water (2:2:1)
 E. Chloroform
 F. Chloroform-methanol (1:1)
 G. Methanol

Detection

Spray with dianisidin in acetic acid

R_F

Solvent	R _F	Solvent	R _F
A	0.71	E	0.04
B	0.37	F	0.71
C	0.13	G	0.66
D	0.64		

Ref.

J. Reiss, *Chromatographia*, 4 (1971) 576-577

TLC 2**Media**

- A. Silica gel, Mallinckrodt AR-7GF
 B. Kieselgel G (Merck)

Solvent

Benzene-methanol-acetic acid (18:1:1)

Detection

- A. UV at 360 nm
 B. Spray with 1% aq. ammonia, then with 4% aq. phenylhydrazine hydrochloride and dry under a gentle stream of warm air from a hand-dryer

R_F

0.25, yellow spot

Ref.

Y. Fujimoto, T. Suzuki and Y. Hoshino, *J. Chromatogr.*, 105 (1975) 99-106

GLC**Apparatus**

Shimadzu GC-4BM gas chromatograph equipped with an electron-capture detector (⁶³Ni; 10 mCi)

Column

Glass column (2.0 m × 3 mm I.D.) packed with 10% of DC-200 plus 15% of QF-1 (1:1, v/v) on Gas-Chrom Q (80-100 mesh)

Temperature

Column, 175°; injector and detector, 190°

Carrier gas

Nitrogen, 60 ml per min

Electrometer range

100 MΩ × 0.16 V

Retention time

7.8 min

Ref.

T. Suzuki, Y. Fujimoto, Y. Hoshino and A. Tanaka, *J. Chromatogr.*, 105 (1975) 95-98; Y. Fujimoto, T. Suzuki and Y. Hoshino, *J. Chromatogr.*, 105 (1975) 99-106

PELIOMYCIN**TLC****Medium**

Silica gel

Solvent

Ligroin-ethyl acetate (1:1)

Detection

- A. 0.3% Aq. potassium permanganate
 B. Exposure to iodine vapor

Ref.

H. Schmitz, S.B. Deak, K.E. Crook, Jr. and I.R. Hooper, *Antimicrob. Agents Chemother.*, 1963 (1964) 89-94

CCD**Solvents**

- A. Chloroform-carbon tetrachloride-methanol-water (2:2:3:1), 100 transfers
 B. Skellysolve B-benzene-80% ethanol (2:3:5), 100 transfers

Distribution

- A. Peak in tube 27
 B. Peak in tube 37

Ref.

As TLC

PENAM-3-CARBOXYLIC ACID DERIVATIVES (cf., penicillins)**TLC****Medium**

Silica gel

Solvents

- A. *n*-Butanol-glacial acetic acid-water (67:10:23)
 B. *n*-Butanol-ethanol-water (40:10:50), upper phase
 C. *n*-Butanol-pyridine-glacial acetic acid-water (38:24:8:30)
 D. *n*-Butanol-pyridine-glacial acetic acid-water (42:24:4:30)

R_F (see p. 278)

Ref.

K. Kocsis, B. Fechtig and H. Bickel, *U.S. Patent* 3,954,802, May 4, 1976

Derivative	Solvent			
	A	B	C	D
6-[D-(-)- α -(3-Acetoxyacetyl-1-ureido)-phenylacetamido]-penicillanic acid	0.62	0.23	0.60	0.55
6-[D-(-)- α -(3-Phenylsulphonylacetyl-1-ureido)-phenylacetamido]-penicillanic acid	0.56	0.31	0.60	0.54
6-{D-(-)- α -[3-L-(+)- α -Acetoxypionyl-1-ureido]-phenylacetamido}-penicillanic acid	0.70	0.45	0.67	0.61
6-[D-(-)- α -(3- α -Acetoxyphenylacetyl-1-ureido)-phenylacetamido]-penicillanic acid	0.63	0.35	0.60	0.57
7-[D-(-)- α -(3-Acetoxyacetyl-1-ureido)-phenylacetamido]cephalosporanic acid	0.33	0.23	0.59	0.55
1,6-[D-(-)- α -(3- <i>p-tert.</i> -Butylphenylsulphonylacetyl-1-ureido)-phenylacetamido]-penicillanic acid	0.72	0.44	0.63	0.68
1,6-{D-(-)- α -[3-L-(+)- α -Butyryloxypropionyl-1-ureido]-phenylacetamido}-penicillanic acid	0.66	0.42	0.61	0.63
1,6-{D-(-)- α -[3-L-(+)- α -(2-Furoyloxy)-propionyl-1-ureido]-phenylacetamido}-penicillanic acid	0.78	0.45	0.70	0.55
1,6-{D-(-)- α -[3-L-(+)- α -(2-Thenoyloxy)-propionyl-1-ureido]-phenylacetamido}-penicillanic acid	0.79	0.43	0.70	0.55
1,6-{D-(-)- α -[3-D.L- α -Acetoxy-(2-furylacetyl)-1-ureido]-phenylacetamido}-penicillanic acid	0.64	0.33	0.57	0.62
1,6-{D-(-)- α -[3-D.L- α -Acetoxy-(2-thienylacetyl)-1-ureido]-phenylacetamido}-penicillanic acid	0.67	0.32	0.61	0.60
1,6-{D-(-)- α -[3-L-(+)- α -(<i>p</i> -Nitrobenzoyloxy)-propionyl-1-ureido]-phenylacetamido}-penicillanic acid	0.72	0.67	0.63	0.64
1,6-{D-(-)- α -[3-L-(+)- α -Chloroacetoxypropionyl-1-ureido]-phenylacetamido}-penicillanic acid	0.68	0.35	0.56	0.54
1,6-{D-(-)- α -[3-L-(+)- α -Phenoxyacetoxypropionyl-1-ureido]-phenylacetamido}-penicillanic acid	0.70	0.42	0.59	0.61
1,6-[D-(-)- α -(3- <i>n</i> -Propylmercaptocarbonyl-1-ureido)-phenylacetamido]-penicillanic acid	0.70	0.45	0.65	0.61
1,6-[D-(-)- α -(3-Benzylmercaptocarbonyl-1-ureido)-phenylacetamido]-penicillanic acid	0.68	0.43	0.60	0.59
1,6-[D-(-)- α -(3- <i>p</i> -Chlorophenylsulphonylacetyl-1-ureido)-phenylacetamido]-penicillanic acid	0.68	0.38	0.59	0.52
1,6-[D-(-)- α -(3- β' -Naphthylsulphonylacetyl-1-ureido)-phenylacetamido]-penicillanic acid	0.73	0.40	0.61	-

PENICILLAMINE**PC***Paper*

Whatman 3MM

Solvent

Butanol-acetic acid-water (12:3:5)

Detection

Paper stained with 0.25% ninhydrin in acetone containing 0.01% isatin and 1% lutidine at 100° for 10 min

 R_F

0.67; migration distance relative to alanine taken as 1.00

Ref.

As Ampicillin, PC 1

ELPHO*Medium*

Whatman 3MM paper

Buffer

Formic acid-acetic acid-water (6:24:170)

Conditions

3000 V, potential gradient 60 V per cm, current 50 mA, 30 min

Detection

Paper stained with 0.25% ninhydrin in acetone containing 0.01% isatin and 1% lutidine at 100° for 10 min

Mobility

1.2, migration distance relative to alanine taken as 1.00

Ref.

As Ampicillin, PC 1

PENICILLANIC ACID, 6-AMINO (See 6-aminopenicillanic acid)

PENICILLANIC ACID, 6-[(HEXA- HYDRO-1H-AZEPIN-1-YL)-METHYL- ENEAMINO]-

{See 6-[(hexahydro-1H-azepin-1-yl)-methyleneamino]-penicillanic acid}

PENICILLANIC ACIDS**TLC****Medium**

Silica gel

Solvents

- A. Acetone-acetic acid (95:5)
B. Chloroform-ethyl acetate (85:15)

R_F

Solvent	A *	B **
A	0.55	
B		0.34

* A = 6-(1,3-Dithiol-2-on-4-yl)-acetamido-penicillanic acid

** B = 3,3-Dimethyl-6-[(1,3-dithiol-2-on-4-yl)-acetamido]-7-oxo-2-pivaloyloxymethoxycarbonyl-4-thia-1-aza-bicyclo[3.2.0]heptane

Ref.

J. Bouchaudon, *U.S. Patent* 3,969,342, July 13, 1976

PENICILLIC ACID**TLC****Media**

- A. Silica gel, Mallinckrodt AR-7GF
B. Kieselgel G (Merck)

Solvent

Benzene-methanol-acetic acid (18:1:1)

Detection

- A. UV at 360 nm
B. Spray with 1% aq. ammonia, then with 4% aq. phenylhydrazine hydrochloride and dry under a gentle stream of warm air from a hand-dryer

R_F

0.25, yellow fluorescence

Ref.

Y. Fujimoto, T. Suzuki and Y. Hoshino, *J. Chromatogr.*, 105 (1975) 99-106

GLC**Apparatus**

Shimadzu GC-4BM gas chromatograph equipped with an electron-capture detector (⁶³Ni; 10 mCi)

Column

Glass column (2.0 m × 3 mm I.D.) packed with 10% of DC-200 plus 15% of QF-1 (1:1, v/v) on Gas-Chrom Q (80-100 mesh)

Temperature

Column, 175°; injector and detector, 190°

Carrier gas

Nitrogen, 60 ml per min

Electrometer range

100 MΩ × 0.16 V

Retention time

3.6 min

Ref.

As Patulin, GLC

PENICILLINATE HYDROCHLORIDE, PHTHALIDYL D- (See PC-183)

PENICILLIN G, BENZATHINE (See benzathine penicillin G)

PENICILLIN N**PC****Paper**

Whatman 3MM

Solvents

- A. Isopropanol-water (70:30)
B. Butanol satd. with 0.5 M phosphate buffer, pH 7

Detection

Bioautography

R_F

Solvent A, 0.54
Solvent B, 0

Ref.

As Cephalosporins, PC 13

ELPHO**Medium**

Paper, Schleicher and Schuell SS-598

Buffer

*0.165 M phosphate buffer, pH 7.0

Conditions

0 to 5°C for 2.5 h at 600 V

Detection

Bioautography

Mobility

—4.0

Ref.

As Cephalosporins, PC 13

PENICILLINS

(cf., penam-3-carboxylic acid derivatives)

PC 1 (Quantitative radioactive method)**Paper**

Whatman No. 1, pH 6.2 buffered strips

Solvent

Diethyl ether

Detection

The developed strips are left in contact with X-ray film in a cassette for several days and the film is then developed

- A. With these radio-autographs as guides, the corresponding paper strips are cut into squares or, where necessary, rectangles, in such a way as to avoid having parts of different penicillin zones on the same square. The squares are then fitted into planchettes and measured radiometrically in a thin end-window Geiger-Müller counter. From the total counts for each penicillin species, the proportions by weight (more strictly, the molar proportions) can readily be calculated
- B. Alternatively, again using the radio-autographs as a guide, a strip is cut into sections each containing the whole of one penicillin species. Each section is then extracted by boiling for a few minutes with very dilute phosphate buffer. An aliquot of each extract is evaporated down on a planchette and the radioactivity measured

Ref.

E.L. Smith and D. Allison, *Analyst*, 77 (1952) 29–33

PC 2**Paper**

Whatman No. 4

Solvent

Water-satd. diethyl ether. The wet ether is kept at the temperature of development for several hours

before use. Layers of water and ether are kept at the bottom of each tank and for the purpose of maintaining the equilibrium, unbuffered filter papers are suspended from the top of the tank, close to the walls and dipping into the water layer

Detection

A. Bioautography vs. *Bacillus subtilis* 288

B. Methylene blue prints. Flood the surface with a 1.0% aq. soln. of methylene blue (containing 1.0% phenol to kill the test organism), wash off the surplus stain after a minute or so, blot with Whatman No. 1 filter paper by quickly smoothing a sheet over the surface, leave the paper in contact with the surface until the dye has been taken up sufficiently to give a clear print

R_F (Estimated from drawing)

Penicillin G, 0.85

Penicillin V, 0.7

Penicillin K, 0.15

Ref.

J. Stephens and A. Grainger, *J. Pharm. Pharmacol.*, 7 (1955) 702–705

PC 3**Paper**

Whatman No. 1

Solvent

Butanol–ethanol–water (40:10:50), upper layer

Detection

The reaction with phenyl acetyl chloride in the presence of sodium bicarbonate is readily adapted to the detection of 6-aminopenicillanic acid on paper chromatograms, which are sprayed with the appropriate reagents before plating on agar seeded with a sensitive bacterium in the usual way. This conversion of 6-aminopenicillanic acid to benzylpenicillin on paper strips also provides a convenient method of assay, being similar to the well known paper disc method

R_F

Penicillin G > 6-aminopenicillanic acid

Ref.

F.R. Batchelor, F.P. Doyle, J.H.C. Nayler and G.N. Rolinson, *Nature*, 183 (1959) 257–258

PC 4**Solvent**

Butanol–ethanol–water (40:10:50), upper layer

Detection

Bioautography vs. *Bacillus subtilis*

R_F

Penicillin K, $FH_2 > F,G >$ penicillin 4 > penicillin 3 > penicillin 2 > penicillin 1

Ref.

A. Ballio, E.B. Chain and F.D. Di Accadia, *Nature*, 183 (1959) 180-181

PC 5*Paper*

Whatman No. 1

Solvents

A. *n*-Butanol-ethanol-water (40:10:50), 5°C

B. 70% Aq. *n*-propanol

R_F

	Solvent	<i>R_F</i>
4-Carboxy- <i>n</i> -butylpenicillin	A	0.00
	B	0.35
Benzylpenicillin	B	0.75

Ref.

A. Ballio, E.B. Chain, F.D. Di Accadia, M.F. Mastropietro-Cancellieri, G. Morpurgo, G. Serlupi-Crescenzi and G. Sermonti, *Nature*, 185 (1960) 97-99

PC 6*Paper*

Whatman No. 1

Solvent

Butanol-acetic acid-water (4:1:5), 4 h

Detection

A. Spray with 0.5 *N* sodium hydroxide then, after an interval of 10-15 min to allow partial drying, it is further sprayed with a reagent composed of a mixture of 1% aq. starch, glacial acetic acid and 0.1 *N* iodine in 4% potassium iodide soln. (50:3:1). Decolorization of the iodine reagent by the hydrolysed penicillins is fairly rapid, yielding a maximum contrast after 5-10 min

B. The reagent contains a mixture of SchenLabs (SchenLabs Pharmaceuticals Inc., New York) purified *Bacillus cereus* penicillinase (10,000 units/ml), 1% starch, 0.1 *N* iodine and *M* sodium phosphate buffer, pH 7, in the ratio 5:50:1:1. The rate of decolorization of the spray by the penicillin substrate is found to be dependent on the penicillinase conc. and, with this mixture, development is complete at room temperature (ca. 22°) in 10-15 min after hydrolysis of the β -lactam ring with alkali or penicillinase. The resulting penicilloic acid rapidly consumes nine equivalents of iodine. Under suitable conditions it is found that both penicillins and the related products cepha-

losporin C and cephalosporin N are readily detected as white zones against a dark blue background, with a sensitivity of 1-2 μ g

R_F

Cephalosporin C > cephalosporin N > 6-amino-penicillanic acid

Ref.

R. Thomas, *Nature*, 191 (1961) 1161-1163

PC 7*Paper*

Toyo No. 50; immerse in 2% liquid paraffin in diethyl ether and dry in air

Solvent

Butyl acetate, 2 h

Detection

Bioautography

R_F

Penicillin F, 0.22

Penicillin G, 0.48

Penicillin K, 0.14

Penicillin V, 0.36

Penicillin X, 0.59

Ref.

T. Watanabe, S. Endo and Y. Iida, *J. Antibiot.*, 15 (1962) 112

PC 8*R_F*

Solvent	Benzyl-penicillin methyl ether	Phenoxy-methyl-penicillin methyl ether
A	0.18	0.80
B	0.76	0.80
C	0.83	0.76
D	0.86	0.86
E	0.77	0.77
F	0.53	0.53
G	0.75	0.63
H	0.90	0.64
I	0.81	0.67

Solvents

A. Light petroleum

B. Carbon tetrachloride

C. Methanol

D. Isooctanol

E. Dipropyl ether

F. Dioctyl ether

G. Water satd. with butanol

H. Water

I. 3% Aq. soln. of ammonium chloride

Solvents which are not miscible with water are preliminary satd. with it

Detection

Chromatograms are first processed in alkali and then in iodine-starch reagent. Penicillin and related substances isomerize into penicilloic acids which are detected through the decoloration of the iodine-starch reagent

Ref.

A.S. Khokhlov and I.N. Blinova, *Antibiotiki*, 15 (1962) 35-39

PC 9

Paper

Whatman No. 1 paper buffered with a soln. of 10% citric acid monohydrate adjusted with aq. satd. sodium hydroxide to pH 5.7 and dried at room temperature

Solvent

Water satd. diethyl ether

Detection

Bioautography vs. *Sarcina lutea*

 R_F

Penicillin derived from:	R_{bp}^*
Methionine	0.54
Ethionine	0.94
S-Methylcysteine	0.39
S-Ethylcysteine	0.88

* R_{bp} = Mobility relative to benzyl penicillin (= 1.00)

Ref.

E. Albu and R. Thomas, *Biochem. J.*, 87 (1963) 648-652

PC 10

Paper

Whatman No. 1

Solvent

Butanol-acetic acid-water (4:1:5), descending, 16 h

Detection

Dry and dip in 0.2% ninhydrin in acetone containing 1% pyridine and heat at 80° for 10 min

 R_F

Penicillin G, 0.80
6-Aminopenicillanic acid, 0.53

Ref.

J.M.T. Hamilton-Miller, *Biochemistry*, 87 (1963) 209-214

PC 11

Paper

Whatman No. 1 buffered with $\frac{1}{15}$ M phosphate, pH 4.5

Solvent

Butanol-diethyl ether-water-acetone (7:2:2.5:2), 4 h

Detection

Air dry, expose to ammonia vapor 30 min; spray with 0.02 N iodine soln.

 R_F

Useful for chromatography of 6-amino-penicillanic acid

Ref.

B. Vassileva, *Comptes rendus de l'Academie bulgare des Sciences*, 16 (1963) 369-372

PC 12

Paper

Whatman No. 1

Solvent

n-Butanol-2% aq. oxalic acid (2:1), upper layer. Spot paper, allow to dry and then expose the strip to ammonia fumes in a closed container for 10 min. Remove and air dry. Place in the developing tank so that about $\frac{1}{4}$ in. of the bottom of the strip is immersed in the mobile phase. Allow to develop to the 15-cm mark, remove, and air dry

Detection

Place the strip in ammonia vapor for 10 min, remove, air dry, and spray once lightly with 0.02 M iodine soln. White spots on an iodine-colored background indicate the presence of phenethicillin

 R_F

Phenethicillin, approximately 0.55

Ref.

W.H. Cox and B.E. Greenwell, *J. Pharm. Sci.*, 54 (1965) 1076-1077

PC 13

Solvents

- A. 2-Butanol-formic acid-water (75:15:10)
- B. 1-Propanol-water (60:40)
- C. 1-Propanol-water (70:30)
- D. 1-Propanol-ethanol-water (30:40:30)
- E. 1-Propanol-ethanol-water (50:20:30)
- F. 1-Propanol-ethanol-water (50:30:20)
- G. 1-Butanol-1-propanol-water (25:40:35)
- H. 1-Butanol-1-propanol-water (20:50:30)
- I. 1-Butanol-1-propanol-water (25:50:25)

Detection

Dip paper in a soln. of silver nitrate in acetone (1 ml satd. soln. silver nitrate added drop wise to 100 ml acetone until a precipitate begins to form). Air dry. Dip in a soln. of 2.5 ml of 50% sodium hydroxide in methanol to maximum color and wash briefly in water. Decolorize the background by dipping in 6 *N* ammonium hydroxide and wash in running water

R_F

Solvent	Penicillin V	6-Aminopenicillanic acid
A	0.92	0.52
B	0.81	0.66
C	0.70	0.42
D	0.80	0.65
E	0.71	0.59
F	0.65	0.43
G	0.60	0.43
H	0.60	0.43
I	0.55	0.38

Ref.

M. Rohr, *Mikrochim. Acta*, 4 (1965) 705–707

PC 14*Paper*

Whatman No. 1

Solvents

- A. *n*-Butanol–pyridine–water (1:1:1)
 B. *n*-Butanol–ethanol–water (4:1:5)

Detection

Bioautography vs. *Bacillus subtilis* after activation by phenylacetylation if required

R_F

	Solvent	<i>R_F</i>
Methylpenicillin	A	0.58
Penicillin X	A	0.3
6-Aminopenicillanic acid	A	0.48
Penicillin	A	0.75
Penicillin	B	0.4–0.5

Ref.

M. Cole, *Appl. Microbiol.*, 14 (1966) 98–104

PC 15*Paper*

Whatman No. 1

Solvent

n-Butanol–ethanol–water (4:1:5), upper phase

Detection

Bioautography vs. *Bacillus subtilis*

R_F

α-Azidobenzylpenicillin > ampicillin

Ref.

E. Hansson, L. Magni and S. Wahlqvist, *Antimicrob. Agents Chemother.*, 1967 (1968) 568–572

PC 16*Paper*

Papers impregnated with phthalate buffers, pH 4 or 5

Solvent

Both phases of diisopropyl ether–isopropanol–water (70:30:100)

Detection

- A. Iodine–sodium azide reagent in combination with starch soln. produces white spots on a blue-gray background, but they are labile and have to be photographed
 B. Positive spots are produced by an alkaline silver reagent (0.1 *M* silver nitrate, 1 *M* ammonia, 1 *M* sodium hydroxide), but the chromatograms have to be treated with sodium thiosulfate and rinsed well with water

R_F

Penicillins	pH 5.0	pH 4.0
Ampicillin	0.00	0.00
Methicillin	0.04	0.12
Benzyl penicillin	0.23	0.65
Phenoxymethylpenicillin	0.33	
Oxacillin	0.50	
Phenoxylethylpenicillin	0.55	
Cloxacillin	0.56	
Phenoxypropylpenicillin	0.80	

Ref.

H. Hellberg, *J.A.O.A.C.*, 51 (1968) 552–557

PC 17*Paper*

Whatman No. 1

Solvents

- A. Butanol–ethanol–water (4:1:5), upper phase
 B. Water satd. diethyl ether, pH 6.2

Detection

- A. Bioautography vs. *Bacillus subtilis* ATCC 663
 B. 6-Aminopenicillanic acid is detected by phenylacetylation of one of a pair of chromatograms before bioautography

R_F

Reaction mixture 6-aminopenicillin acid + carboxylic acid	R_F , A*	Migration (cm), B*
<i>n</i> -Butyric	0.36	3.0
<i>n</i> -Valeric	0.46	8.0
<i>n</i> -Hexanoic	0.49	15.5
3-Hexanoic	0.46	13.0
<i>n</i> -Heptanoic	0.54	12–15.5
<i>n</i> -Octanoic	0.56	11.0
Pimelic	—	5.0
γ -Amino- <i>n</i> -valeric	—	4.8
ϵ -Aminocaproic	—	4.5
α -Hydroxyisocaproic	—	4.2
Butylthioacetic	0.53	10.0
Phenylacetic	0.47	6.5
N-phenylglycine	0.37	3.0
α -Ketophenylacetic	0.37	2.5
DL- α -Hydroxyphenyl- acetic	0.40	3.4
DL- α -Ethylphenylacetic	0.43	—
DL- α -Methoxyphenyl- acetic	0.41	3.5–6.5
<i>p</i> -Hydroxyphenylacetic	0.29	0.5
<i>p</i> -Aminophenylacetic	0.21	0.0
<i>m</i> -Aminophenylacetic	0.22	0.0
<i>p</i> -Methoxyphenylacetic	0.39	4.2
3,4-Dichlorophenyl- acetic	0.57	6.4
3,4-Dihydroxyphenyl- acetic	0.24	0.0
<i>p</i> -Hydroxy- α -hydroxy- phenylacetic	0.32	0.0
α -Methyl- α -hydroxy- phenylacetic	0.46	6.9
Homogentisic acid lactone	0.31	0.0
Phenoxyacetic	—	7.0
<i>p</i> -Chlorophenoxyacetic	—	6.5
2-Thienylacetic	0.40	5.3
1-Naphthylacetic	0.48	6.0
2-Naphthylacetic	0.52	7.4

* A and B refer to solvents

Reaction mixture of 6-aminopenicillin acid + amide	R_F , A*	Migration (cm), B*
Valeramide	0.49	7.5
Hexanamide	0.55	13.5
Heptanamide	0.58	15.5
Phenylacetamide	0.5	7.2
Phenylacetic acid control (pH 5)	0.5	7.5
<i>p</i> -Aminophenylacet- amide	0.23	0.0
DL-Mandelamide	0.48	4.5
DL- α -Aminophenyl- acetamide	0.25	0.0
DL- α -Phenoxypro- pionamide	0.58	16.0

* A and B refer to solvents

Reaction mixture of 6-aminopenicillanic acid + N-acyl derivatives of glycine	R_F , A*	Migration (cm), B*
Valerylglycine	0.51	7.5
Hexanoylglycine	0.55	14.0
Heptanoylglycine	0.58	10.5
Octanoylglycine	0.62	25.0
Benzoylglycine	0.00	0.0
Phenylacetyl-glycine	0.50	8.0
Phenoxyacetyl-glycine	0.50	12.0
DL- α -Phenoxy- propionylglycine	0.54	14.5
DL- α -Hydroxyphenyl- acetyl-glycine	0.45	5.0
DL- α -Aminophenyl- acetyl-glycine	0.03	0.0
	0.24	
Phenylacetic acid control (pH 5.0)	0.50	8.0
Phenylacetamide control (pH 7.0)	0.50	8.5

* A and B refer to solvents

Ref.

M. Cole, *Biochem. J.*, 115 (1969) 747–756

PC 18

Paper

Whatman No. 1

Solvents

A. Butanol-ethanol-water (4:1:5), upper phase

B. Butanol-pyridine-water (1:1:1)

C. Butanol-acetic acid-water (12:3:5)

Detection

As PC 17

 R_F

	Solvent		
	A	B	C
Benzylpenicillin			
amide	0.9	—	—
Benzylpenicillin			
methyl ester	0.95	—	—
Benzylpenicillin			
cyanomethyl ester	—	—	0.92
Benzylpenicillin			
acetoxymethyl ester	0.92	—	—
Benzylpenicillin			
diethylaminoethyl ester HI	0.78	—	—
Benzylpenicillin			
phenacyl ester	0.92	—	—
Benzylpenicillin			
acetonyl ester	—	—	0.94
Benzylpenicillin			
thiomethyl ester (contaminated with some benzylpenicillin)	0.94	—	—
Benzylpenicilloic acid	—	0.51	—
N-Phenylacetyl-cyclic-DL-cysteiny-D-valine	—	0.7	—
2-Furylmethylpenicillin methyl ester	0.89	—	—
n-Propoxymethylpenicillin cyano-methyl ester	—	—	0.85
2-Thienylmethylcephalosporin	0.32	0.71	0.75
2-Thienylmethylcephalosporin pyridine	0.26	0.63	0.45

*Ref.*M. Cole, *Biochem. J.*, 115 (1969) 733-739

PC 19

Solvents

A. Butanol-ethanol-water

B. Butanol-acetic acid-water

 R_F

Compound	Solvent A	Solvent B
D- α -Triphenylphosphiniminobenzylpenicillin triethylamine salt	0.80	0.86
D- α -Triphenylphosphiniminobenzylpenicillin	0.79	0.87
D- α -Dimethylphenylphosphiniminobenzylpenicillin	0.48	0.86
D- α -Triethylphosphiniminobenzylpenicillin	0.46	0.46
D- α -Tri(<i>n</i> -butyl)phosphiniminobenzylpenicillin	0.85	0.93
D- α -Tri(<i>p</i> -tolyl)phosphiniminobenzylpenicillin	0.95	0.86
D- α -Tri(<i>p</i> -methoxyphenyl)phosphiniminobenzylpenicillin	0.95	0.89
D- α -Tri(<i>m</i> -tolyl)phosphiniminobenzylpenicillin	0.88	0.91
Pivaloyloxymethyl-D- α -triphenylphosphiniminobenzylpenicillanate	0.93	—
Acetoxymethyl-D- α -triphenylphosphiniminobenzylpenicillanate	0.92	0.90
Acetoxymethyl-D- α -triphenylphosphiniminobenzylpenicillin	0.93	0.94
Benzyl-D- α -triphenylphosphiniminobenzylpenicillanate	0.88	0.9
Benzyl-D- α -triphenylphosphiniminobenzylpenicillanate ester	0.92	0.93
D- α -Tri(isopropyl)phosphiniminobenzylpenicillin	0.77	0.88
D- α -Tri(2-cyanoethyl)phosphiniminobenzylpenicillin	0.21	0.76
D- α -Tri(<i>n</i> -octyl)phosphiniminobenzylpenicillin		0.86
D- α -Triethoxyphosphiniminobenzylpenicillin	0.56	0.86

Compound	Solvent A	Solvent B
D- α -Tri(dimethylamino)-phosphininobenzylpenicillin		0.95

Ref.

J.P. Clayton and R. Hubbard, *U.S. Patent* 3,923,787, Dec. 2, 1975

PC 20

Paper

Whatman No. 1

Solvents

- A. 80% Methanol + 3% sodium chloride (1:1) descending. Paper buffered with 0.95 *M* sodium sulfate + 0.05 *M* sodium bisulfate
- B. Propanol-pyridine-acetic acid-water (6:4:1:3), ascending
- C. Butanol-acetic acid-water (4:1:5) ascending
- D. 80% Phenol, ascending

Detection

Bioautography vs. *Staphylococcus aureus* R_F

Solvent	R_F	Solvent	R_F
A	0.63	C	0.85
B	0.91	D	0.91

Ref.

As Chloramphenicol, PC 5

PC 21

Solvent

Butanol-ethanol-water (1:1:1)

Detection

Chromatogram was sprayed with phenylacetyl chloride prior to detection

 R_F

Component	R_F
2,2,2-Trichloroethyl-6-aminopenicillanate benzenesulfonic acid salt	0.9
Phenyl-6-aminopenicillanate benzenesulfonic acid salt	0.81
<i>p</i> -Chlorophenylthio-6-aminopenicillanate benzenesulfonic acid salt	0.84
<i>n</i> -(6-Aminopenicillanoyloxy)benzylidene imine benzenesulfonate	0.84

Component	R_F
<i>n</i> -(6-Aminopenicillanoyloxy)benzylidene imine <i>p</i> -toluene sulfonate	0.91
<i>n</i> -(6-Aminopenicillanoyloxy)-2-furfurylidene imine benzenesulfonate	0.82

Ref.

G.R. Fosker and J.H.C. Nayler, *U.S. Patent* 3,663,563, May 16, 1972

PC 22

Paper

Whatman No. 1

Solvents

- A. Water satd. *n*-butyl alcohol-*tert*.-amyl alcohol (6:1), pH 4.1 (16 h)
- B. Water satd. *n*-amyl alcohol, pH 6.7 (16 h)
- C. Water satd. *n*-amyl alcohol-amyl acetate (3:1), pH 6.7 (30 h)
- D. Water satd. *n*-butyl alcohol-*tert*.-amyl alcohol (2:1), pH 6.7 (30 h)

Detection

Bioautography vs. *Staphylococcus aureus* R_F R_F values measured when solvent front was at end of paper in every case

Compound	Solvent				Distance moved (cm)
	A	B	C	D	
Ampicillin	0.22				9.0
N-Acetyl-ampicillin		0.22			10.5
Penicillin G		0.47 *			8.6
<i>p</i> -Hydroxy-benzylpenicillin			0.08		10.6
<i>p</i> -Hydroxy-ampicillin				0.06	8.1
Epicillin	0.29				11.3
<i>n</i> -Acetylepencilin		0.31			14.0
Penicillin V		0.63 *			11.4
<i>m</i> -Hydroxy-benzylpenicillin			0.09		13.2
<i>m</i> -Hydroxy-ampicillin				0.07	11.3

* Development = 4.5 h

Ref.

S.C. Pan, *J. Chromatogr.*, 79 (1973) 251-255

PC 23

Paper

Whatman No. 1

Solvent

n-Butanol–pyridine–water (1:1:1)

Detection

Bioautography vs. *Staphylococcus aureus* R_F

Compound	R_F
4-Benzoyloxycarbonyl-4-carbenzyl-oxyamino- <i>n</i> -butylpenicillin	0.83–0.84
4-Carboxy-4-carboxybenzyloxy-amino- <i>n</i> -butylpenicillin	0.43–0.47
4-Carboxy-4-amino- <i>n</i> -butyl-penicillin	0.15–0.16

Ref.

H. Vanderhaeghe, A. Vlietinck, M. Claesen and G. Parmentier, *J. Antibiot.*, 27 (1974) 169–177

PC 24 (Radioactive penicillins)

Paper

Whatman No. 1

Solvent

n-Butanol–ethanol–water (4:1:5)

Detection

- A. Bioautography vs. *Bacillus subtilis*; 6-APA detected after phenylacetylation and drying
 B. Radioactive scan

 R_F

Penicillin	R_F
6-APA	0.19
Penicillin V	0.63

Ref.

E.Z. Gordee and L.E. Day, *Antimicrob. Agents Chemother.*, 1 (1972) 315–322

PC 25

Solvents

- A. Butanol–ethanol–water
 B. Butanol–acetic acid–water

 R_F

Penicillin	Solvent	
	A	B
6-(DL- α -Aminocyclobutyl-acetamido)-penicillanic acid	0.25	

Penicillin

Solvent

A B

6-(DL- α -Aminocyclopentyl-acetamido)-penicillanic acid	0.28	
6-(D- α -Aminocyclopentyl-acetamido)-penicillanic acid	0.37	
6-(L- β -Aminocyclopentyl-acetamido)-penicillanic acid	0.3	
6-(DL- α -Aminocycloheptyl-acetamido)-penicillanic acid	0.38	
6-(DL- α -Aminocyclopropyl-acetamido)-penicillanic acid	0.17	
6-(D- α -Aminocyclopropyl-acetamido)-penicillanic acid	0.24	0.59
6-(L- α -Aminocyclopropyl-acetamido)-penicillanic acid	0.16	0.48
6-(D- α -Aminocyclobutyl-acetamido)-penicillanic acid	0.21	0.69
6-(L- α -Aminocyclobutyl-acetamido)-penicillanic acid	0.20	0.59

Ref.

G.R. Fosker, J.B. Harbridge and R. Hubbard, *U.S. Patent* 3,987,178, Oct. 19, 1976

TLC 1

Medium

Silica gel; activate 30 min at 110°C

Solvent

Benzene–isopentyl acetate–carbon tetrachloride–acetic acid–water (20:39:35:6:0.5)

Detection

Spray with 40% sulfuric acid

 R_F

Separates phenethicillin and phenoxymethyl penicillin

Ref.

P.J. Weiss, B. Taliaferro, R. Huckins and R. Chastonay, *J. Ass. Offic. Anal. Chem.*, 50 (1967) 1294–1297

TLC 2

Media

- A. Silica gel G
 B. Silica gel G adjusted to pH 6.1 with McIlvaines buffer

Solvent

Nitromethane–toluene–butanol–pyridine–acetic acid (60:30:15:9:6)

 R_F

Useful for separation of semisynthetic penicillins

Ref.

F. Saccani, *Boll. Chim. Farm.*, 106(9) (1967) 625–628; also *C.A.*, 68 (1968) 2375

TLC 3

Medium

Silica gel H

Solvent

Acetic acid–acetone (5:95)

Detection

Bioautography vs. *Bacillus subtilis*

 R_F

Useful for detection of α -azidobenzyl penicillin and a metabolite; also for ampicillin

Ref.

As PC 15

TLC 4

Media

A. Cellulose MN 30

B. Silica gel G

Solvents

A. 0.1 M Sodium chloride soln.

B. 0.3 M Citric acid soln. satd. with *n*-butanol

C. Isoamyl acetate–methanol–formic acid–water (65:20:5:10)

D. Acetone–acetic acid (95:5)

Detection

A. 10% Ferric chloride–5% aq. potassium ferricyanide (20:10 ml) are mixed with 20% sulfuric acid (70 ml) and used on the day of preparation

B. Ninhydrin

C. 50% Aq. sulfuric acid

 R_F

	Medium A		Medium B	
	A	B	C	D
Benzylpenicillin Na	0.90	0.90	0.61	0.58
Ampicillin Na	0.97	0.98	0.12	0.15
Cloxacillin Na	0.65	0.38	0.64	0.77
Dicloxacillin Na	0.47	0.22	0.65	0.77
Nafcillin Na	0.47	0.22	0.64	0.77
Oxacillin	0.74	0.49	0.65	0.63
Phenethicillin K	0.84	0.73	0.66	0.77

	Medium A		Medium B	
	A	B	C	D
Phenoxy-methylpenicillin K	0.82	0.76	0.52	0.75
Methicillin Na	0.93	0.93	0.52	0.59
Hetacillin K	0.96	0.98	0.30	0.64

Ref.

I.J. McGilveray and R.D. Strickland, *J. Pharm. Sci.*, 56 (1967) 78

TLC 5

Solvent

Butanol–diethyl ether–butyl acetate–water (14:4.5:4.5:1)

Detection

Bioautography

 R_F

Useful for chromatography of 6-(α -aminoacylamido)penicillanic acids

Ref.

J. Cieslak, B. Wasilewa, D. Roslik, *Acta Pol. Pharm.*, 25(2) (1968) 515–516; *C.A.*, 69 (1968) 8121

TLC 6

Medium

Kieselguhr G (Merck) buffered to pH 5.3. Prepare slurry with a mixture of 100 ml 0.05 M potassium hydrogen phthalate and 32 ml 0.1 M sodium hydroxide. Dry the plates 1 h at 105°C

Solvent

Carbon tetrachloride–isopropanol–water (6.5:3.5:0.4). Pour the solvent, after clarification, on the bottom of the tank and moisten the paper lining. Use the tank preferably 10–48 h after preparation. Develop 30–40 min

Detection

Iodine–azide soln.: Dissolve 1 g sodium azide in a mixture of 10 ml 0.1 M iodine soln. and 90 ml water. Starch soln.: 0.5% soln. of soluble starch. Dry in air and spray well with starch soln. Finally dry with warm air and spray with iodine–azide reagent; repeat this alternate drying and spraying with iodine–azide until the white spots are distinct (2–3 times)

 R_F

Useful for separation of semi-synthetic penicillins

Ref.

As PC 16

TLC 7

Medium

Eastman Chromagram Sheet No. 6061

Solvent

Ethyl acetate-acetic acid-water (8:1:1)

Detection

Bioautography vs. *Bacillus subtilis* ATCC 6633 R_F

Separation of ampicillin and cloxacillin. The inhibition zone near the origin is due to ampicillin; zone near the front is cloxacillin

Ref.

T. Murakawa, Y. Wakai, M. Nishida, R. Fujii, M. Konno, K. Okada, S. Goto and S. Kuwahara, *J. Antibiot.*, 23 (1970) 250-251

TLC 8

Medium

Silica gel

Solvent

Butyl acetate-butanol-acetic acid-methanol-phosphate buffer, pH 5.8 (80:15:40:5:24)

 R_F

Benzylpenicilloic acid > benzylpenicillanic acid > benzylpenicillin > benzylpenicillic acid

Ref.

E.E. Imozemtzeva, D.M. Trachtenberg and E.N. Navoilneva, *Khim. Farm. Zh.*, (1970) 26-30

TLC 9

Solvents

A. Isopropanol-acetone-water (1:1:1)

B. *n*-Butanol-ethanol-isopropanol-acetone-water (4:1:2:2:2)C. Isopropanol-acetone-*n*-butanol-water (4:2:4:2)D. *n*-Butanol-ethanol-acetone-water (4:1:4:1)E. *n*-Butanol-ethanol-water (4:1:5)F. *n*-Butanol-ethanol-isopropanol-acetone-water (4:1:1:1:1) R_F

Solvent	6-Aminopenicillanic acid	Benzyl penicillin
A	0.76	0.87
B	0.44	0.63
C	0.53	0.71
D	0.37	0.58
E	0.20	0.45
F	0.28	0.50

Ref.

J. Mikolajczyk, J. Kazimierzczak and J. Cieslak, *Chemia Analityczna*, 16 (1971) 877-887

TLC 10

Medium

Silica gel

Solvent

Acetone-acetic acid (95:5), followed by methanol-butanol-formamide-heptane (46:16:11:5)

Detection

The separated compounds are detected by spraying with 0.01 *N* iodine soln. containing 20 mg sodium azide per 100 ml, followed by spraying with 1% aq. starch soln. The spots appear as white zones on a bluish violet background

 R_F

	R_s^*
Phenoxymethylpenicillic acid	0.25
Phenoxymethylpenicilloic acid	0.33
Phenoxymethylpenilloic acid	0.73
Phenoxymethylpenicillin	1.00
Phenoxymethylpenicillanic acid	1.15

* R_s = Mobility relative to phenoxymethylpenicillin (= 1.00)

Ref.

V.B. Korchagin, L.I. Serova, S.P. Dement'eva, I.N. Navol'neva, I.I. Inozemtseva, D.M. Trakhtenberg and N.I. Kotova, *Antibiotiki*, 16 (1971) 8-11; *C.A.*, 74 (1971) 218, 79535n

TLC 11

Medium

Silica gel

Solvents

A. Acetone-acetic acid (95:5)

B. Butan-2-one-acetic acid (95:5)

C. 4-Methylpentan-2-one-acetic acid (95:5)

D. Butan-2-one-chloroform-acetic acid (20:70:5)

Detection

Bioautography vs. *Bacillus subtilis* R_F (see p. 290)

Ref.

P.J. O'Neil, *Proc. Assoc. Anal. Chem.*, (1974) 39-32

R_F

Solvent	R_F	Solvent	R_F
A	0.85	C	0.30
B	0.50	D	0.16

TLC 12

Medium

Alumina

Solvent

Acetone-chloroform-acetic acid-water
(50:45:5:1)

Detection

Ammonia vapor; starch-iodine spray

 R_F

Penicillin G, 0.51-0.53

Ref.

J. Birner, *J. Pharm. Sci.*, 63 (1974) 603-605

TLC 13

Medium

Silica gel

 R_F

Penicillin	Solvent				
	A	B	C	D	E
Penicillin G	0.85	0.62	0.65	0.90	0.65
Benzylpenicilloic acid	0.78	0.45	0.56	0.74	0.56
Benzylpenilloic acid	0.76	0.42	0.55	0.66	0.55

Solvents

A. Acetone-acetic acid (95:5)

B. Organic phase of amyl acetate-methanol-formic acid-water (65:20:5:10)

C. Chloroform-isopropanol-water (60:40:4)

D. Ethyl acetate-acetic acid-water (8:1:1)

E. *n*-Propanol-water (70:30)

Detection

Typically, 5 μ g of each degradation product were spotted from an aq. soln. Chromatograms were developed in each solvent system until the mobile phase had travelled 15 cm. Development occurred in chromatographic chambers (7 $\times$ 28 $\times$ 22

cm) lined with Whatman No. 1 filter paper and presaturated with vapors of the mobile phase. Chromatograms were visualized by first spraying with ferricyanide reagent [*J. Pharm. Sci.*, 56 (1967) 77] followed by exposing the plate to iodine vapor. The latter procedure made visualization more sensitive

Ref.

P.E. Manni, R.A. Lipper, J.M. Blaha and S.L. Hem, *J. Chromatogr.*, 76 (1973) 512-515

TLC 14

Medium

Silica gel

Solvent

Acetone-chloroform-glacial acetic acid
(50:45:5)

Detection

Spray with ferricyanide reagent followed by iodine vapor

 R_F

Penicillin	R_F
Penicillin G	0.33
Benzylpenicilloic acid	0.15
Benzylpenilloic acid	0.25
Benzylpenicillenic acid	0.38
α -Methyl-D-benzylpenicilloate	0.53

Ref.

As TLC 13

TLC 15

Medium

Silica gel plates

 R_F

Solvent	Penicillin G (potassium salt)	Benzylpenicilloic acid	Benzylpenilloic acid	Dev. time (h) **
A	0.85	0.78	0.76	1.5
B	0.62	0.45	0.42	2
C	0.65	0.56	0.55	3.5
D	0.90	0.74	0.66	2.5
E	0.65	0.56	0.55	4
F*	0.33	0.15	0.25	-

* In Solvent F, benzylpenicillenic acid and α -methyl-D-benzylpenicilloate had R_F values of 0.38 and 0.53, respectively

** Time for solvent front to travel 15 cm

Solvents

- A. Acetone-acetic acid (95:5)
- B. Organic phase of amyl acetate-methanol-formic acid-water (65:20:5:10)
- C. Chloroform-isopropanol-water (60:40:4)
- D. Ethyl acetate-acetic acid-water (8:1:1)
- E. *n*-Propanol-water (70:30)
- F. Acetone-chloroform-glacial acetic acid (50:45:5)

Detection

Chromatograms developed in each solvent system until the mobile phase travels 15 cm. First spray with ferricyanide reagent followed by exposing the plate to iodine vapor

Ref.

As TLC 13

TLC 16**Media**

- A. Kieselguhr F₂₅₄ (Merck 5738). Before use, the Kieselguhr plate was impregnated with citrate/phosphate buffer (pH 3.7)-glycerol (19:1)
- B. Silica gel 60 (Merck)
- C. Cellulose (Merck 5716)

Solvents

- A. Chloroform-acetone-impregnation liquid [citrate/phosphate buffer (pH 3.7)-glycerol (19:1)] (5:5:2)
- B. Ethanol-water-ammonia (8:8:1)
- C. Methanol-chloroform (9:1)
- D. Methanol-acetone (3:2)
- E. Methanol-3% ammonium chloride (7:3)

Detection

- A. Bioautography vs. *Bacillus cereus* ATCC 11778
- B. Bioautography vs. *Bacillus subtilis* ATCC 6633
- C. Bioautography vs. *Micrococcus flavis* ATCC 10240
- D. Bioautography vs. *Sarcina lutea* ATCC 9341

R_F

Clo = cloxacillin; Amp = ampicillin; Oxa = oxacillin

Medium	Solvent	Detection	Clo	Amp	Oxa
A	A	A	- *	0.35 *	0 *
B	B	C	-	-	-
B	C	D	0.70	0.50	0.60
B	C	B	0.70	-	0.60
B	D	C	-	-	-
C	E	A	-	-	-

* These R_F values were obtained by running the plate twice

Ref.

As Ampicillin, TLC 3

TLC 17**Type**

Reversed-phase TLC

Phases

- A. Polar mobile phase: sodium acetate-Veronal buffer (pH 7.0) containing 2% acetone and satd. with silicone oil TSF451, 10 cS (Toshiba Silicone Co., Tokyo)
- B. Non-polar stationary phase: silica gel plate (Merck 60) impregnated with the silicone oil

Detection

Spray with 5% potassium permanganate

Before the coating, the silica gel plate was activated by heating for 15 min at 110°C, β -lactam antibiotics were dissolved in distilled water to give about 3 mg/ml, and about 1 μ l of soln. was spotted on the thin-layer plate

R_F

Ampicillin, 0.45
Benzylpenicillin, 0.29

Ref.

As Cephalosporins, TLC 19

TLC 18**Medium**

Silica gel

Solvent

Dry freshly distilled acetone-glacial acetic acid-water (95:5:3.2)

R_F

A = Phenoxymethylpenicillic acid
B = Phenoxymethylpenicilloic acid
C = Phenoxymethylpenilloic acid
D = Phenoxymethylpenicillin
E = Phenoxymethylpenicillanic acid
E > D > C > B > A

Ref.

V.B. Korchagin, L.I. Serova, S.P. Dementieva, I.N. Navolneva, I.I. Inozemtseva, D.M. Trakhtenberg and N.I. Kotova, *Antibiotiki*, 16 (1971) 410-413

TLC 19**Medium**

Reversed-phase. Glass plates, 20 × 20 cm, were coated with a 0.5-mm layer of Camag microcrystalline cellulose (67.5 g to 330 ml water) using a motorized spreader

Solvent

The plates were dried at room temp. overnight, heated in an oven at 110° for 1.5 h cooled on the bench for 10 min and then impregnated with

n-octanol by developing in a 10% soln. of *n*-octanol in acetone for 1 h in a chromatographic chamber previously equilibrated with the octanol soln. The *n*-octanol was purified by successive washings with dilute hydrochloric acid, water, dilute sodium hydroxide and water before distillation. Acetone was allowed to evaporate from the plates on the bench for 15 min after removing them from the chamber. Spots (1 μ l) of the penicillin soln. (2 mg/ml in water) were then applied, and the plates were placed in the developing buffer 30 min after removal of the plates from the octanol soln. The buffer was a 0.5 *M* soln. of 6-aminohexanoic acid adjusted to the chosen pH

R_F

Penicillin	R_M^*			ΔR_M	
	pH 3	pH 4	pH 5	pH 3-4	pH 4-5
Methicillin	-0.60	-1.34	**	0.74	-
Benzyl	0.09	-0.66	-1.21	0.75	0.55
Phenoxymethyl	0.34	-0.37	-0.87	0.71	0.50
Phenethicillin	0.50	-0.23	-0.95	0.73	0.72
Propicillin	0.97	0.21	-0.52	0.76	0.73
Oxacillin	0.57	-0.15	-0.71	0.72	0.56
Cloxacillin	0.71	0.01	-0.64	0.70	0.65
Dicloxacillin	1.18	0.44	-0.23	0.74	0.67

* Each R_M is the mean of between 5 and 10 spots on 3 to 5 plates. Each plate had at least one spot of each penicillin

** At this pH, methicillin ran very close to the solvent front so that an accurate R_M value could not be obtained

with hydrochloric acid and satd. with *n*-octanol. The chromatographic chamber was equilibrated with buffer soln. in the normal way

Detection

Development was continued until the solvent front reached the 10-cm mark (about 2 h). The wet plates were placed in a tank satd. with ammonia vapor for 15 min dried, sprayed with 10% acetic acid in acetone and then with starch iodine soln. The penicillins appear as white spots on a blue background

Ref.

A.E. Bird and A.C. Marshall, *J. Chromatogr.*, 63 (1971) 313-319

TLC 20

Medium

Silica gel HF₂₅₄ (Merck)

R_F

Penicillin	Solvent			
	A	B	C	D
Acetoxy-methyl- α -aminobenzylpenicillinate	0.51	0.52		
Acetoxy-methyl-6-tritylaminopenicillinate	0.71	0.78		

Penicillin	Solvent			
	A	B	C	D
Acetoxy-methyl-6-aminopenicillinate, <i>p</i> -toluenesulphonate	0.51	0.53		
Pivaloyloxymethyl-6-aminopenicillinate, <i>p</i> -toluenesulphonate			0.66	0.12
Pivaloyloxymethyl-6-aminopenicillinate, hydrochloride			0.86	0.45

Solvents

- A. Butanol-ethanol-water (8:2:2)
 B. Butanol-acetic acid-water (8:2:2)
 C. *n*-Butanol-ethanol-water (4:1:1)
 D. Cyclohexane-ethyl acetate (1:1)

Ref.

E.K. Frederiksen and W.O. Godtfredsen, *U.S. Patent* 3,697,507, Oct. 10, 1972

TLC 21**Medium**

Silica gel (Merck). The chromatoplates were coated to a thickness of 500 μ m. Silica gel G (Merck) was applied according to Stahl's method. The plates were dried at room temp. and activated at 120° for 30 min

Solvents

- A. *n*-Butanol-water-ethanol-acetic acid (5:2:1.5:1.5)
 B. *n*-Butanol-water-acetic acid (4:1:1)
 C. Acetone-acetic acid (95:5)
 D. 85% Aq. acetone

The plates are inserted in a previously equilibrated filter paper-lined tank and each solvent allowed to rise to a height of 14 cm. Approximate times taken: (A) 150 min (B) 120 min (C) 20 min (D) 30 min

Detection

- A. Each TLC plate was sprayed consecutively with the following spray reagents: (a) 2*N* sodium hydroxide; (b) the iodine-azide reagent of Awe, et al., *Naturwissenschaften*, 41 (1954) 528, (c) 1% starch soln.
 B. Ninhydrin: adequate for the detection of 6-APA, 7-ACA, penicic acid, Cephalosporin C, penicillamine, Ampicillin and the penicilloic acid of Ampicillin, with this restriction; the mixtures may not contain other ninhydrin-positive interfering products. 6-APA and 7-ACA yield brown and yellow spots, respectively, while the other products mentioned above yield red-purple spots. Other intact penicillins and other degradation products were scarcely visible

$R_F (\times 100)$

Product	Solvent			
	A	B	C	D
Penicillin V	84	78	66	70
Penillic acid	60	50	15	32

Product	Solvent			
	A	B	C	D
Phenoxyethylpenicilloinate	64	55	10	25
Phenoxyethylpenilloinate	78	70	60	50
6-Aminopenicillanic acid	57	43	50	43
6-Aminopenicilloic acid	43	35	0	10
CDAT (D-4-Carboxy-5,5-dimethyl-2-amino-methylthiazolidine)	55	40	7	14
Penicillin G	84	78	66	70
Benzylpenicilloinate	64	57	10	25
Benzylpenilloinate	78	70	60	50
Oxacillin	84	78	66	70
Penicilloic acid of oxacillin	64	57	10	30
Cloxacillin	84	78	66	70
Penicilloic acid of cloxacillin	64	57	10	30
Methicillin	79	70	60	60
Penicilloic acid of methicillin	64	57	10	20
Ampicillin	64	50	20	57
Penicilloic acid of ampicillin	44	28	0	14
DL-Penicillamine	60	43	58	43

Ref.

E.J. Vandamme and J.P. Voets, *J. Chromatogr.*, 71 (1972) 141-148

TLC 22**Medium**

Silica gel G (Merck)

Solvent

n-Butanol-acetic acid-water (60:15:25)

Detection

The developed plates are thoroughly dried, and the spots detected by spraying with a chloroplatinic reagent comprising 1 ml of 0.2% platinum chloride soln., 0.1 ml 20% potassium iodide soln., 0.1 ml of 3-4% hydrochloric acid and 20 ml of acetone. It is convenient to combine the prepared soln. of platinum chloride, hydrochloric acid and acetone and to add the potassium iodide soln. just before spraying; these two soln. stored in a

refrigerator are stable for several weeks if kept separately, but the final combined reagent is stable for only 1 day

R_F

Compound	R_F
6-Aminopenicillanic acid	0.46
Penicilloic acid	0.36
Penilloic acid	0.43
8-Hydroxyphenicillic acid	0.65
Penicillamine	0.18
Benzylpenicillin	0.85
Benzylpenicilloic acid	0.65
Benzylpenicillenic acid	0.82
Benzylpenicillic acid	0.85
Benzylpenicillinic acid	0.55
Ampicillin	0.58
N-Boc-Ampicillin	0.95
Amoxicillin	0.58
Hetacillin	0.58
Epicillin	0.55
Thenoilpenicillin	0.88
Tritilpenicillin	0.90
Meticillin	0.78
Oxacillin	0.83
Cloxacillin	0.85
Dicloxacillin	0.85
Fluocloxacillin	0.95
Phenoxymethylpenicillin	0.82
1-Hydroxy-2-methyl-3-phenyl-2-propenylpenicillin	0.90
2-Methyl-3-phenylpropenylpenicillin	0.95
2-Methyl-2-phenylpropenylpenicillin	0.83
6-Aminopenicillanic acid sulfoxide	0.10
Benzylpenicillin sulfoxide	0.12
Phenoxymethylpenicillin sulfoxide	0.12
Tritilpenicillin sulfoxide	0.08
Benzylpenicillin benzyl ester	0.95
Benzylpenicillin ethyl ester	0.95
Phenoxymethylpenicillin benzyl ester	0.98
Phenoxymethylpenicillin sulfone	n.d. *
Benzylpenicillin sulfone	n.d.
Tritilpenicillin sulfone	n.d.

* n.d. = Not detected over 20 μ g

Ref.

M. Pokorny, N. Vitezic and M. Japelj, *J. Chromatogr.*, 77 (1973) 458-460

TLC 23

Medium

Silica gel G

Solvents

A. Acetone-methanol (50:80)

B. 95% Ethanol

C. Acetone

D. Acetone and 2 drops of 0.1 N sodium hydroxide

Detection

A. Copper acetate in acetone (satd. soln.)

B. Copper acetate in methanol (satd. soln.)

C. 5% Copper sulfate in water

D. 2% Cobaltous nitrate in water

E. 2% Cobaltous nitrate in methanol

F. Ferric chloride in methanol (satd. soln.)

G. 2% Nickel chloride in water

H. 2% Ceric sulfate in water using 4 ml (conc. sulfuric acid)

R_F (see p. 295)

Ref.

M.L. Walash and S.M. Hassan, *J. Drug Res. Egypt*, 5 (1973) 111-115

TLC 24

Medium

Silica gel (Merck)

Solvent

n-Propanol-*n*-butyl acetate-acetone-water (2:2:2:1)

Detection

UV at 254, 366 nm

R_F

Propicillin, Cloxacillin, Phenethicillin, Methicillin, Ampicillin, Amoxycillin, Carbenicillin, Benzylpenicillin, Phenoxymethylpenicillin, Procaine penicillin, Benzathine penicillin, can be distinguished by spot pattern and colors against green background

Ref.

As Cephalosporins, ELPHO 6

TLC 25

Medium

Silica gel G

Solvent

Acetone-methanol-0.1 M sodium chloride (2:1:1)

Detection

0.4% Aq. potassium permanganate, yellow spots on violet background

R_F

Penicillin G, 0.89

Procaine penicillin G, 0.5

TLC 23, R_F

Penicillin	Solvent	R_F	Detection							
			A	B	C	D	E	F	G	H
Phenoxy-methyl Penicillinic acid	A	0.64	Yellowish	Greenish	Blue	Reddish	Pink	Brown	–	Purple
Ampicillin	C	–	Green	Yellow	–	Yellow	–	–	–	–
	D	0.75	Yellow	Light green	Greenish yellow	Yellow	Yellow	Reddish brown	Yellowish green	–
Oxacillin sodium	B	0.82	Green	Green	Green	Yellow	Yellowish	–	–	Purple
Cloxacillin sodium	B	0.81	Green	Green	Green	Faint yellow	Faint yellow	–	–	Purple
Dicloxacillin sodium	B	0.68	Light green	Light green	Bluish green	Light pink	Pink	Reddish brown	–	Purple

Ref.

M. Shahjahan, B.K. Dutta and A. Rashid,
Bangladesh Pharm. J., 1 (1972) 14-17

TLC 26 (Quantitative)

Medium

Silica gel (E.M. Labs.)

Solvent

Acetone-chloroform-glacial acetic acid
(50:45:5)

Standard soln. of potassium penicillin G prepared in doubly distilled water at conc. of 1 and 10 µg/µl. Appropriate volumes of the standard soln. and the potassium penicillin G dosage forms are applied with a 10-µl Hamilton syringe, each application being dried in a nitrogen stream before another is made. Chromatograms developed in 7 × 28 × 22 cm glass-lined tanks lined with Whatman No. 1 filter paper. All plates developed until the solvent rises to within 5 cm of the top (ca. 50 min)

Densitometry

A Schoeffel model SD-3000 doublebeam spectrodensitometer equipped with a high-pressure mercury-zenon lamp is used with a Schoeffel SDR 303 recorder. All measurements made at 230 nm in the reflectance mode at an attenuation output of 0.2 a.u.f.s. Peak areas determined by multiplying peak height by the peak width at half-height. Linearity noted between 1-20 µg between peak area and concentration

Ref.

P.E. Manni, M.F. Bourgeois, R.A. Lipper, J.M. Blaha and S.L. Hem, *J. Chromatogr.*, 85 (1973) 177-180

TLC 27

Medium

Silica gel GF

Solvents

A. Benzene-ethyl acetate (1:1)

B. Ethyl acetate

C. Methylene chloride-methanol-acetic acid
(90:8:2)

R_F

Penicillin	Solvent		
	A	B	C
<i>cis</i> -3-Azido-1-(2,3-dimethoxybenzyl)-4-oxoazetidine-2-carboxylate	0.64		

Penicillin

Solvent

A

B

C

<i>cis</i> -4-Oxo-3-phenoxyacetylaminoozetidiine-2-carboxylate	0.21	0.44	
<i>cis</i> -1-(2,4-Dimethoxybenzyl)-4-oxo-3-phenoxyacetylaminoozetidine-2-carboxylate	0.38		
<i>cis</i> -2-Hydroxymethyl-4-oxo-3-phenoxyacetylaminoozetidine		0.10	
<i>cis</i> -2-Azidomethyl-4-oxo-3-phenoxyacetylaminoozetidine		0.38	
<i>cis</i> -4-Oxo-3-phenoxyacetylaminoo-2- <i>p</i> -toluenesulfonyloxymethylazetidine		0.47	
3-Acetyl-7-oxo-6β-phenoxyacetylaminoo-1,3-diazabicyclo-[3.2.0]-heptane-2-carboxylic acid			0.31
3-Acetyl-7-oxo-6β-phenoxyacetylaminoo-1,3-diazabicyclo-[3.2.0]-heptane-2-carboxylic acid benzyl ester		0.55	

Ref.

J.G. Gleason, K.G. Holden and W.F. Huffman,
U.S. Patent 4,000,154, Dec. 28, 1976

TLC 28

Medium

Polyamide

Solvents

A. Water-acetic acid-isopropanol (50:15:8.5)

B. Water-acetic acid-isopropanol-methanol
(50:16.5:7.1:5)

Detection

The spots were detected by spraying with 0.25% w/v fluorescein sodium salt soln. The minimum amounts detectable with the method were 3.0 µg for phenoxymethyl penicillin K and 1.0 µg for ampicillin, penicillin G, penicillin GK, penicillin G sodium salt, cloxacillin, dicloxacillin and dicloxacillin sodium salt

Ref.

J.T. Wang, H.H. Chou, C.H. Lin and K.S. Chen,
Tai-Wan K'o Hsueh., 24 (1970) 19-20; *C.A.*, 74 (1971) 115942d

TLC 29**Medium**Silica gel F₂₅₄ (Merck), precoated**Solvents**

- A. Benzene–acetone (80:20)
 B. Acetone–acetic acid (95:5)

Detection

- A. UV
 B. Iodine vapor

R_F

Penicillin	Solvent	
	A	B
6- <i>epi</i> -Hetacillin		0.70
6- <i>epi</i> -Hetacillin methyl ester	0.29	
6- <i>epi</i> -Ampicillin		0.15
α -Hydroxybenzylpenicillin		0.68
α -Hydroxybenzylpenicillin methyl ester	0.24	
6- <i>epi</i> - α -Hydroxybenzylpenicillin		0.70
6- <i>epi</i> -N-Nitrosohetacillin potassium salt		0.77
6- <i>epi</i> -N-Nitrosohetacillin methyl ester	0.54	

Ref.

E. Roets, A. Vlietinck and H. Vanderhaeghe, *J. Antibiot.*, 30 (1977) 847–855

TLC 30**Medium**

Silica gel

Solvent*n*-Butanol–*n*-pentane–glacial acetic acid (10:3:1)**R_F**

D-(–)- α -(3-Methoxycarbonyl)-3-methylureido-benzylpenicillin, 0.45

Ref.

H. Disseinkotter and K.G. Metzger, *U.S. Patent* 3,933,795, Jan. 20, 1976

ELPHO 1**Medium**

Paper

Buffer

0.1 M Phosphate buffer, pH 6.8

Conditions

25 V/cm

Mobility

Benzylpenicillin, 10.5 cm/h
 4-Carboxy-*n*-butylpenicillin, 20 cm/h

Ref.

As PC 5

ELPHO 2**Medium**

Agarose plates (1% agarose)

Buffer

Potassium phosphate, pH 7.0; ionic strength = 0.02

Conditions2 V mm⁻¹, 18 mA, 30 min**Detection**

- A. Bioautography vs. *Bacillus subtilis*. 6-Aminopenicillanic acid is converted into benzylpenicillin before the addition of the seeded agar layer. This is achieved by placing a series of filter papers, impregnated with either a 5% aq. soln. of sodium hydrogen carbonate or with a 5% soln. of phenylacetyl chloride in acetone on the gel surface for 5 min each and finally repeating the 5% aq. sodium hydrogen carbonate soln.
- B. The penicilloic acids are detected by placing the gel in iodine vapor from a few crystals of iodine contained in a chromatographic tank. The acids were visible as dark blue spots on a blue background. Penicillins are detected by this method after they have been hydrolyzed *in situ* to the corresponding penicilloic acids. The plate is immersed in 0.5 N hydrochloric acid for 5 min to hydrolyse the penicillins (alkali treatment at this stage interferes with the iodine reaction). The gel surface is thoroughly freed from liquid by wiping with paper tissues and the plate exposed to iodine vapor. The penicilloic acids already present in the gel are unaffected by the hydrolytic procedure and both the penicilloic acids and the hydrolysed penicillins are visible as dark blue spots

Mobility

6-Aminopenicillanic acid, ampicillin and carbenicillin are readily distinguished; separation of the other penicillins tested is only marginal

Ref.

A.H. Thomas and R.A. Broadbridge, *Analyst (London)*, 95 (1970) 459–462

GLC 1**Column**

1.5 m $\times$ 4 mm column of stationary phase on acid-washed silanized Gas-Chrom P 60–80 mesh

Temperature

Column, 230°C; detector, 240°C; flash heater, 300°C

Carrier gasN₂, 75 ml/min**Retention times**

(Est. from graph): Penicillin G, 7.3 min; penicillin V, 8.7 min (as methyl esters)

Ref.S. Kawai and S. Hashiba, *Bunseki Kagaku (Jap. Anal.)*, 13 (1964) 1223–1226**GLC 2****Apparatus**

Argon Chromatograph (Pye Instruments) modified to permit the injection of a sample directly on the top of the column through a silicone rubber septum

Column

130 cm × 4 mm I.D. borosilicate glass tubing, filled with acid-washed, silanized Gas-Chrom P,

Relative retention time

100–120 mesh, coated with stationary phase at the percentage indicated in table

Temperature

Column, 300°C; detector, 250°C

Carrier gasArgon, inlet pressure, 1 kg/cm²**Detector voltage**

800–1000 V

Reagent solvent

Acetone

Derivative

Different penicillanic acids are transformed into their methyl esters by reaction with an ethereal soln. of diazomethane

Ref.E. Evrard, M. Claesen and H. Vanderhaeghe, *Nature*, 201 (1964) 1124–1125**Methyl ester of:****Relative retention time**

	QF-1, 80%	QF-1, 0.75%	SE-30, 0.4%	SE-52, 0.4%	SE-52, 0.4%
Penicillanic acid	0.59	—	—	—	—
6-Chloropenicillanic acid	0.18	—	—	—	—
6-Bromopenicillanic acid	1.04	—	—	—	—
Benzoylglycine	1.00	—	—	—	—
Benzylpenicillin	—	1.00	1.00	1.00	1.00
α-Phenoxyethylpenicillin side-chain:					
D-Isomer	—	0.864	1.07	1.08	—
L-Isomer	—	0.913	1.14	1.20	—
3,4-Dichloro-α-methoxybenzyl side chain:					
L-Isomer	—	—	—	—	2.69
D-Isomer	—	—	—	—	3.14
6-Tritylaminopenicillanic acid	—	2.72	—	—	6.05

GLC 3**Apparatus**

A Varian Aerograph Model 21000 gas chromatograph equipped with F.I.D.

Column

660 × 4 mm I.D. glass U-tube column packed with 2% OV-17 (Applied Science Labs.) on 80–100 mesh Supelcoport (Supelco)

Temperature

Column oven temperatures of 245 and 275°C are used; injector and detector temperatures are maintained at 275°C

Gases

Helium, 165–215 ml/min; hydrogen, 85 ml/min; air, 260 ml/min

Internal standard-silylating reagent

A 50% soln. of HMDS in pyridine containing 0.375 mg/ml of 5-α-cholestane or 5-α-cholestan-3-one

Reference standard

Penicillin reference standards are dissolved in water at a conc. of 20 mg/ml. To 2.0 ml of the standard soln., 8.0 ml of chloroform and 2.0 ml of pH 2.2 buffer are added. The mixture is immediately shaken vigorously for 1 min and centrifuged. A 2.0-ml aliquot of the organic phase is transferred to an 8.2-ml serum vial for silylation

Silylation procedure

To each vial is added 2.0 ml of internal

standard-silylating reagent. The vials are sealed, mixed, and allowed to stand at room temperature with occasional shaking

Relative retention time

5- α -Cholestan-3-one	1.00 (2.0 min) •
Methicillin	1.51
Oxacillin	1.58
Cloxacillin	2.16
Dicloxacillin	2.83
5- α -Cholestane	1.00 (2.3 min) **
Penicillin G	1.65
D-Phenethicillin	1.60
L-Phenethicillin	1.71
Penicillin V	2.05

• 275°C at 215 ml/min

** 245°C at 165 ml/min

Ref.

C. Hishta, D.L. Mays and M. Garofalo, *Anal. Chem.*, 43 (1971) 1530–1535

GLC 4

Apparatus

Pye 104 gas chromatograph

Column

Coiled glass, 150 cm $\times$ 4 mm I.D., packed with (a) 3% OV-1 or (b) 3% OV-17 on Gas-Chrom Q (100–200 mesh) (Applied Science Labs.)

Carrier gas

Nitrogen

Flow-rate

60 ml/min

Temperature

After 10 min: 150° (OV-1 column); 180° (OV-17 column)

Temperature increased at the rate of 4°/min for 10 min, then held constant to the end of the run

Sample size

1–3 μ l

Retention time (min) (Estimated from drawing)

Methylpenicillin	12.0
n-Propylpenicillin	18.0
n-Pentylpenicillin	24.5
n-2-Pentylpenicillin	24.0
n-Heptylpenicillin	32.5
Benzylpenicillin	35.0
n-Nonylpenicillin	37.5
Phenoxymethylpenicillin	39.5

Ref.

B. Meesschaert, P. Adriaens and H. Eyssen, *J. Chromatogr.*, 136 (1977) 162–164

GLC 5

Apparatus

Pye Series 104 gas chromatograph

Column

Glass U-tube (150 $\times$ 4 mm I.D.) packed with 3% OV-17 on Gas-Chrom Q (100–120 mesh) (Applied Science Labs.)

Carrier gas

Nitrogen with air and hydrogen calibrated for optimum sensitivity

Flow-rate

60 ml/min

Silylation reagent

5% soln. of BSA in dry acetone

Temperature

Column, 230°C; injector and detector, 270°C

Detector

Hydrogen-flame ionization

Relative retention time

Phenoxymethyl penicillin	1.00 (16.5 min)
Benzylpenicillin	0.80
Hetacillin	2.25
N-Nitrosohetacillin	1.74
L- α -Hydroxybenzylpenicillin	0.96
D- α -Hydroxybenzylpenicillin	1.09

Ref.

As TLC 29

HPLC 1

Column

1 m $\times$ 2.1 mm I.D. $\times$ 6.3 mm O.D., 316 stainless steel, C₁₈/Porasil B packing

Mobile phase

0.1% Ammonium carbonate

Pressure

200 p.s.i.

Flow-rate

0.75 ml/min

Detector

UV (254 nm)

Sensitivity

0.08 a.u.f.s.

Type

Reversed-phase

Retention time (min) (estimated from drawing)

6-Aminopenicillanic acid	8.5
8-Aminopenicillanic acid	15.0

Ref.

As Ampicillin, HPLC

HPLC 2

Column

1 m $\times$ 2.1 mm I.D. $\times$ 6.3 mm O.D., 316 stainless steel, C₁₈/Porasil B packing

Mobile phase

Methanol–0.05 M ammonium carbonate (30:70)

Pressure

500 p.s.i.

Flow-rate

0.5 ml/min

Detector

UV (254 nm)

Sensitivity

0.04 a.u.f.s.

Type

Reversed-phase

Retention time (min) (estimated from drawing)

Penicillin G 13.5

Penicillin V 24.0

Ref.

As Ampicillin, HPLC

HPLC 3*Apparatus*

Varian LC 8500

*Column*Micropak CH, 10 μ m, 25 cm $\times$ 2.1 mm I.D.*Mobile phase*

Methanol–1% acetic acid (30:70) in water

Flow-rate

120 ml/h (380 atm)

Temperature

Ambient

Detector

Variscan 268 nm

Chart speed

0.5 cm/min

Sensitivity

0.5 a.u.f.s.

*Sample size*10 μ l*Recorder*

A-25, 10 mV

Type

Reversed-phase

Retention time (estimated from drawing)

Penicillin V, 3.5

Penicillin V acid, 4.0

Ref.

As Cephalosporins, HPLC 2

HPLC 4*Apparatus*

Varian LC 8500

*Column*Micropak CH, 10 μ m, 25 cm $\times$ 2.1 mm I.D.*Mobile phase*

20% Methanol in water

Flow-rate

60 ml/h (310 atm)

Temperature

60°C

Detector

Variscan 268 nm

Chart speed

0.5 cm/min

Sensitivity

0.5 a.u.f.s.

*Sample size*5 μ l*Recorder*

A-25, 10 mV

Type

Reversed-phase

Retention time (estimated from drawing)

Penicillin V benzathin salt 2.5

Penicillin V acid 3.0

Ref.

As Cephalosporins, HPLC 2

HPLC 5*Apparatus*

Varian LC 8500

*Column*Micropak CH, 10 μ l, 25 cm $\times$ 2.1 mm I.D.*Mobile phase*

20% Methanol in water with 0.01 M phosphoric acid

Flow-rate

60 ml/h (310 atm)

Temperature

60°C

Detector

Variscan 268 nm

Chart speed

0.5 cm/min

Sensitivity

0.5 a.u.f.s.

*Sample size*10 μ l*Recorder*

A-25, 10 mV

Type

Reversed-phase

Retention time (estimated from drawing)

Penicillin V benzathin salt 9.0

Penicillin V acid 8.5

Penicillin V calcium 7.2

Ref.

As Cephalosporins, HPLC 2

HPLC 6*Apparatus*

Varian LC 8500

ColumnMicropak CH, 10 μ l, 25 cm $\times$ 2.1 mm I.D.**Mobile phase**20% Methanol in water with 0.01 *M* phosphoric acid**Flow-rate**

60 ml/h (380 atm)

Temperature

60°C

Detector

Variscan 268 nm

Chart speed

0.5 cm/min

Sensitivity

0.5 a.u.f.s.

Sample size1 μ l**Recorder**

A-25, 10 mV

Type

Reversed-phase

Retention time (estimated from drawing)

Procaine penicillin G, 2.2

Ref.

As Cephalosporins, HPLC 2

HPLC 7**Apparatus**

Modular liquid chromatograph with a 254 nm UV monitor (Model 1285, Laboratory Data Control), minipump (Milton Roy), and pulse dampener (Model 709, Laboratory Data Control)

ColumnStainless steel (DuPont), 1000 $\times$ 2.1 mm**Column packing**

Anionic exchange resin

Temperature

Ambient

Mobile phase0.02 *M* Sodium nitrate in 0.01 *M* sodium borate, pH 9.15 borate buffer**Flow-rate**

0.45 ml/min, column pressure about 34 atm (500 p.s.i.)

Chart speed

0.6 cm (0.25 in.)/min

Sample size2.0 μ l**Retention time (min) (estimated from drawing)**

Ampicillin	6.5
Penicillin G	9.5
Penicillin V	15.0

Ref.K. Tsuji and J.H. Robertson, *J. Pharm. Sci.*, 64 (1975) 1542–1545**HPLC 8****Apparatus**

Spectra Physics 3500 B high-performance liquid chromatograph

Column15 cm $\times$ 4.6 mm I.D., packed with LiChrosorb RP-8 (particle size 5 μ m) (Chrompack)**Injector**Injection loop, 100 μ l**Mobile phase**A. Potassium dihydrogen phosphate buffer (Sorensen buffer, pH 4.6, 0.067 *M*)

B. Potassium dihydrogen phosphate–methanol (425:75), pH 4.6

Flow-rate

1.2 ml/min

Detection

225 nm

Relative retention time

Penicillin	Mobile phase	
	A	B
Amoxycillin	1.00	0.22
Ampicillin	–	1.00
I in urine *	0.52	–
II in urine *	0.63	–
6-Aminopenicillanic acid	0.35	0.01
Benzylpenicilloic acid	–	0.01

* Unidentified

Ref.T.B. Vree, Y.A. Hekster, A.M. Baars and E. van der Kleijn, *J. Chromatogr.*, 145 (1978) 496–501**HSLC 1****Column**Stainless steel, 1 m $\times$ 2.1 mm I.D., C₁₈/Porasil B packing**Mobile phase**

0.1% Ammonium carbonate

Flow-rate

0.75 ml/min

Pressure

200 p.s.i.

Temperature

Room temp.

Detection

UV (254 nm)

Sensitivity

0.08 a.u.f.s.

Sample solvent0.1 *M* Sodium bicarbonate

*Sample size*10 μ l*Type*

Reversed-phase

Retention time

See HSLC 4

Ref.

See HSLC 4

HSLC 2*Column*Stainless steel, 30 cm $\times$ 4.6 mm I.D.,
C₁₈/Lichrosorb 10 μ m packing*Mobile phase*

0.03% Sodium dihydrogen phosphate

Flow-rate

1 ml/min

Pressure

600 p.s.i.

Temperature

Room temp.

Detection

UV (225 nm)

Sensitivity

0.1 a.u.f.s.

Sample solvent

0.1 M Sodium bicarbonate

*Sample size*5 μ l*Type*

Reversed-phase

Retention time

See HSLC 4

Ref.

See HSLC 4

HSLC 3*Column*Stainless steel, 1 m $\times$ 2.1 mm I.D., C₁₈/Porasil B*Mobile phase*

30% Methanol, 70% 0.05 M ammonium carbonate

Flow-rate

0.5 ml/min

Pressure

500 p.s.i.

Temperature

Room temp.

Detection

UV (254 nm)

Sensitivity

0.04 a.u.f.s.

Sample solvent

0.05 M Ammonium carbonate

*Sample size*5 μ l*Type*

Reversed-phase

Retention time

See HSLC 4

Ref.

See HSLC 4

HSLC 4*Column*Stainless steel, 30 cm $\times$ 4.6 mm I.D., C₁₈
LiChrosorb 10 μ m*Mobile phase*Methanol-0.01 M sodium dihydrogen phosphate
(35:65)*Flow-rate*

1 ml/min

Pressure

1300 p.s.i.

Temperature

Room temp.

Detection

UV (225 nm)

Sensitivity

0.04 a.u.f.s.

Sample solvent

Mobile phase

*Sample size*8 μ l*Type*

Reversed-phase

Retention time (min)

Com- pound	Column			
	(See HSLC 1)	(See HSLC 2)	(See HSLC 3)	(See HSLC 4)
6-APA	7.5	5.5		
Ampi- cillin			9.0	11.0
Peni- cillin G			13.0	13.0
Peni- cillin V			24.0	22.0
Methi- cillin				13.5
Oxa- cillin				25.0

Ref.

As cephalosporins, HSLC 1

PENTA[N-(TRIFLUOROACETYL)]-5''-AMINO-5'-DEOXYBUTIROSIDIN

TLC

Solvent

Chloroform-methanol (7:2)

R_F

0.40

Ref.

T.P. Culbertson, D.R. Watson and T.H. Haskell,
J. Antibiot., 26 (1973) 790-793

PENTENOMYCIN I and II

TLC

Medium

Silica gel Kiesel gel G (Merck)

Solvents

A. Chloroform-methanol (80:20)

B. Benzene-ethanol (7:3)

C. Chloroform-methanol (9:1)

Detection

The antibiotics were visualized as dark brown spots after spraying with a silver nitrate soln. followed by 5% sodium hydroxide soln.

R_F

Solvent	Pentenomycin	
	I	II
A	0.35	0.82
B	0.35	0.51
C	0.10	0.18

Ref.

K. Umino, T. Furumai, N. Matsuzawa, Y. Awataguchi, Y. Ito and T. Okuda, *J. Antibiot.*, 26 (1973) 506-512

PEPSTATIN, HYDROXY-(See hydroxypepstatin)

PEPSTATINS

TLC

Medium

Silica gel

Solvent

n-Butanol-pyridine-acetic acid-water (100:1:1:1)

Detection

Rydon-Smith reagent

R_F

Pepstatin AC 0.40

Pepstatin A 0.65

Pepstatin Bu 0.61

Pepstatin Pr 0.51

Ref.

T. Aoyagi, Y. Yagisawa, M. Kumagai, M. Hamada, H. Morishima, T. Takeuchi and H. Umezawa, *J. Antibiot.*, 26 (1973) 539-541

PERIMYCIN

PC

Solvent

Pyridine-1-butanol-water (4:6:5)

Detection

Bioautography vs. *Candida albicans* 204

R_F

0.80

Ref.

E. Borowski, C.P. Schaffner, H. Lechevalier and B.S. Schwartz, *Antimicrob. Agents Annu.*, (1960) 532-538

CCD

Solvents

A. Pyridine-ethyl acetate-water (3.5:6.5:8.3), 200 transfers

B. Chloroform-methanol-borate buffer (2:2:1)

Distribution

Solvent A: $K = 2.2$

Solvent B: $K = 0.1$

Ref.

As PC

PETRIN

PC

Solvent

Chloroform-*n*-butanol-water (46:4:50)

Detection

Activity vs. *Haemophilus pertussis*

R_F

Separation of petrin into three fractions: (1) antibiotic; (2) haemolytic; (3) fluorescent

Ref.

A.I. Tiffin, *Nature*, 181 (1958) 907-908

PHENAZINES and PHENOXAZINONES

PC

Paper

A. Whatman No. 1, previously washed with 2.8% ammonium hydroxide

B. Schleicher and Schuell No. 2497 (fully acetylated)

Solvents

- A. Acetic acid–chloroform (1:10)
- B. Toluene–ethanol–water (4:17:1)
- C. Ethanol–water (1:1)
- D. Butanol–acetic acid–water (4:1:1)
- E. Butanol–acetic acid–water (4:1:5), upper layer
- F. Toluene vs. Whatman No. 1 paper dipped in acetone–dimethyl sulfoxide (3:1) and dried 15 min in air
- G. 15% Acetone
- H. 50% Methanol
- I. Methanol–10% hydrochloric acid (1:1)

R_F

	Paper	Solvent	R_F
1,6-Phenazinediol-5,10-dioxide (iodinin)	B	B	0.15
		D	0.21
		C	moved (18 h)
1,6-Phenazinediol	B	B	0.61
		D	0.65
		F	0.82
		E	0.78
2-Aminophenoxazin-3-one	A	G	0.59
		H	0.22
		I	0.55
		E	0.76
2-Acetamidophenoxazine-3-one	A	F	0.90
	B	B	0.52
		D	0.64

Ref.

N.N. Gerber and M.P. Lechevalier, *Biochemistry*, 3 (1964) 598–602

TLC

Medium

Silica gel

Solvent

As PC, A

R_F

Compound	R_F
1,6-Phenazinediol-5,10-dioxide (iodinin)	0.80
2-Aminophenoxazin-3-one	0.30
2-Acetamidophenoxazine-3-one	0.60

Ref.

As PC

PHENOMYCIN

ELPHO

Medium

Cellulose acetate film

Buffer

pH 7.0 buffer (0.01 M phosphate and 0.1 M sodium chloride)

Conditions

10 mA/4 cm, 1 h

Detection

Purple-red spot by treatment with Ponceau 3R

Mobility

Moves 2.3 cm to cathode

Ref.

S. Nakamura, T. Yajima, M. Hamada, T. Nishimura, M. Ishizuka, T. Takeuchi, N. Tanaka and H. Umezawa, *J. Antibiot.*, 20 (1967) 210–216

PHENOXAZINONES

(See phenazines and phenoxazinones)

PHLEOMYCIN D

TLC

Media

A. Silica gel

B. Avicel

Solvents

A. Chloroform–10% ammonium acetate–10% ammonium hydroxide (10:9:1)

B. Propanol–pyridine–acetic acid–water (15:10:3:12)

R_F

Solvent	Medium	R_F
A	A	0.74
B	B	0.58

Ref.

As Bleomycins, TLC

PHLEOMYCINS

(See YA-56 complex, PC 2 and TLC)

PC 1

Solvents

A. 0.5% Ammonium chloride

B. 1.0% Ammonium chloride

R_F

	Solvent	
	A	B
Phleomycin C	0.73	0.79
Phleomycin D	—	0.88
Phleomycin D ₁	0.81	0.88
Phleomycin D ₂	0.76	0.88
Phleomycin E	0.84	0.88
Phleomycin F	0.78	0.76
Phleomycin G	0.81	0.86
Phleomycin H	0.73	0.87
Phleomycin I	0.83	0.88
Phleomycin J	—	0.81
Phleomycin K	—	0.88

Ref.

T. Ikekawa, F. Iwami, H. Hiranaka and H. Umezawa, *J. Antibiot.*, 17 (1954) 194–199

PC 2

Solvent

10% Ammonium chloride

Detection

Sakaguchi reaction

 R_F

All phleomycins showed R_F below 0.80 and gave positive Sakaguchi reaction

Ref.

H. Umezawa, Y. Suhara, T. Takita and K. Maeda, *J. Antibiot.*, 19 (1966) 210–215

TLC

Solvent

10% Ammonium acetate–methanol (1:1)

 R_F

Phleomycin C	0.72
Phleomycin D ₁	0.65
Phleomycin D ₂	0.64
Phleomycin E	0.56
Phleomycin F	0.50
Phleomycin G	0.48
Phleomycin H	0.42
Phleomycin I	0.38
Phleomycin J	0.35
Phleomycin K	0.23

Ref.

As PC 1

ELPHO

Medium

Paper

Buffer

Formic acid–acetic acid–water (25:75:900)

Conditions

3300 V and 25 mA for 20 min

Mobility

	R_F^*
Phleomycin C	0.75
Phleomycin D ₁	0.94
Phleomycin D ₂	0.98
Phleomycin E	1.08
Phleomycin F	1.12
Phleomycin G	0.86
Phleomycin H	0.88
Phleomycin I	0.89

* L-Alanine as the standard (= 1.00)

Ref.

As PC 1

PHOLIPOMYCIN

TLC

Media

A. Silica gel (Chromagram Sheet 6060, Eastman Kodak)

B. Cellulose (Chromagram Sheet 6065, Eastman Kodak)

Solvent

n-Propanol–2 *N* ammonium hydroxide (7:3)

Detection

A. Bioautography vs. *Staphylococcus aureus* 209P

B. UV

 R_F

Medium A, 0.25

Medium B, 0.74

Ref.

M. Arai, A. Torikata, H. Fukatsu, N. Kitano and T. Matsuzawa, *U.S. Patent* 3,912,811, Oct. 14, 1975

PHOSPHONOMYCIN

PC 1

Solvents

A. *n*-Propanol–2 *N* methylamine (7:3)

B. *n*-Propanol–2 *N* isopropylamine (7:3)

C. *n*-Butanol–acetic acid–water (3:1:1)

D. *n*-Butanol–acetic acid–water (4:1:1)

E. Isopropanol–conc. ammonia–water (7:1:2)

F. Methanol–water–triethylamine (80:20:5)

Detection

A. Bioautography vs. *Proteus vulgaris* MB-838

B. Reagent containing 3% perchloric acid and 1% ammonium molybdate in 0.01 *N* hydrochloric acid readily reveals an intense blue zone after heating for 5 min at 85°C

C. Spray with 0.1% ferric chloride in 80% aq. ethanol. After drying, the antibiotic is revealed as a white or light buff colored zone on a pinkish background by spraying with a 1% soln. of sulfosalicylic acid in 80% ethanol

R_F

Solvent	R_F	Solvent	R_F
A	0.19	D	0.26
B	0.26	E	— *
C	— •	F	— •

• See TLC 2

Ref.

H. Shafer, W.J.A. Vandenheuvel, R. Ormond, F.A. Kuehl and F.J. Wolf, *J. Chromatogr.*, 52 (1970) 111–117

PC 2

Paper

Whatman No. 3M

Solvents

A. Isopropanol–0.01 *M* phosphate buffer, pH 6.0 (7:3)

B. Methanol–2% sodium chloride (7.5:2.5)

Detection

Bioautography vs. *Proteus vulgaris* or *Erwinia atroseptica*

R_F

Solvent A, 0.25

Solvent B, 0.74

Ref.

E.O. Stapley, D. Hendlin, J.M. Mata, M. Jackson, H. Wallick, S. Hernandez, S. Mochales, S.A. Currie and R.M. Miller, *Antimicrob. Agents Chemother.*, 1969 (1970) 284–290

TLC 1

Medium

Silica gel G

Solvents

A. Methanol–isopropanol–water (7:3)

B. Methanol–water (1:1)

C. Ethanol–water–conc. ammonia (25:3:4)

D. Aq. ammonia and diethylamine

Detection

Bioautography

R_F

Solvent	R_F	Solvent	R_F
A	0.0	C	Slight mobility
B	0.0	D	1.0

Ref.

L. Chalet, T.W. Miller, R.T. Goegelman, A.J. Kempf and W.J. Wolf, *J. Antibiot.*, 23 (1970) 336–347

TLC 2

Media

A. Cellulose

B. Silica gel G

Solvents

A. As PC 1, C

B. As PC 1, D

C. As PC 1, E

D. As PC 1, F

Detection

As PC 1

R_F

Medium	Solvent	R_F
A	B	0.26
	C	0.18
B	A	0.33
	D	0.75

Ref.

As PC 1

ELPHO

Medium

Schleicher and Schuell SS-598

Buffer

Refrigerated unit, 0.165 *M* phosphate buffer, pH 7.0, 2.5 h

Conditions

600 V

Detection

As PC 1

Mobility

Moves to anode 12.7 cm

Ref.

As PC 1

PHOSPHORYLATED BUTIROSIN A

ELPHO

Medium

Paper

Buffer

Formic acid–acetic acid–water (25:75:900 by volume)

Conditions

3500 V for 15 min

Mobility

The inactivated butirosin A moves toward the cathode 14.6 cm, while butirosin A moves 19.0 cm

Ref.

M. Yagisawa, H. Yamamoto, H. Naganawa, S. Kondo, T. Takeuchi and H. Umezawa, *J. Antibiot.*, 25 (1972) 748–750

PHTHALIDYL D-AMINO BENZYL-PENICILLINATE HYDROCHLORIDE (PC-183)

TLC**Medium**

Eastman Chromagram Sheet No. 6061

Solvent

Chloroform–ethanol (9:1) (until the solvent travelled 8 cm from origin)

Detection

Bioautography vs. *Sarcina lutea* ATCC 9341

R_F

0.88

Ref.

Y. Shiobara, A. Tachibana, H. Sasaki, T. Watanabe and T. Sado, *J. Antibiot.*, 27 (1974) 665–673

PHYSARUM GYROSUM ANTIBIOTIC

PC**Solvent**

Ethyl acetate–pyridine–water (2:2:1)

R_F

Fraction X, 0.99

Fraction Y, 0.33

Ref.

H.R. Schroeder and M.F. Mallette, *Antimicrob. Agents Chemother.*, 4 (1973) 160–166

TLC 1**Medium**

Silica gel G

Solvents

A. Methanol–water–ammonia (20:40:1), descending

B. 1-Propanol–water–ammonia (40:2:1)

C. Benzene–methanol (80:20)

Detection

Bioautography vs. *B. cereus*

R_F

Component	Medium	R _F
Z	A	0.87
O	A	0.48
C-1	B	0.82
C-2	B	0.72
C-3	B	0.37
D-1	C	0.46
D-2	C	0.16

Ref.

As PC

TLC 2**Medium**

Silica gel G

Solvents

A. 1-Propanol–water–ammonia (40:2:1)

B. Benzene–methanol (80:20)

C. 2-Butanone–1-butanol–water (30:5:65)

D. 2-Butanone–pyridine (75:4)

E. Acetone–methanol (1:1)

F. Light petroleum–benzene–methanol–isopropanol (20:5:8:5)

Detection

Charred with chromic–sulfuric acid spray

R_F

Component	Solvent	R _F
D-1	A	0.70
	B	0.33
	C	0.83
	D	0.65
	E	0.61
	F	0.85

Ref.

As PC

PICROMYCIN

PC**Paper**

Whatman No. 1. Saturate with 0.3 M phosphate buffer (pH 3.0) and air dry

Solvent

1-Hexanol satd. with water, developed 24 h; solvent runs off strips

Detection

Bioautography vs. *Corynebacterium xerosis*

R_F

Picromycin moved 15 in. (38.1 cm) from origin

Ref.

S.E. DeVoe, H.B. Renfroe and W.K. Hausmann, *Antimicrob. Agents Chemother.*, 1963 (1964) 125-129

PILOSOMYCINS**PC****Paper**

Whatman No. 1, except for Solvent F (below)

Solvents

- A. Butanol satd. with water
- B. Butanol-glacial acetic acid-water (4:1:5), upper phase
- C. Butanol satd. with water + 2% *p*-toluene-sulfonic acid
- D. Butanol satd. with water + 2% piperidine
- E. Butanol-pyridine-water (6:4:3)
- F. 80% Ethanol + 1.5% sodium chloride; Whatman No. 4 paper impregnated with 0.95 *M* sodium sulfate + 0.05 *M* sodium hydrosulfate
- G. Butanol-ethanol-water (1:1:2)
- H. Butanol-butyl acetate-glacial acetic acid-water (10:3:1.3:14.3), upper phase, developed 16 h

Detection

Bioautography vs. *Staphylococcus aureus* or *Bacillus subtilis*

R_F

Solvent	Pilosomycin A	Pilosomycin B
A	0.00	0.00
B	0.49	0.63
C	0.34	0.58
D	0.05	0.15
E	0.32	0.32
F	0.47	0.47
G	0.74	0.74
H	2.7 *	7.6 *

* Distance from origin in cm after 16 h

Ref.

E. Gaeumann, H. Bickel and E. Visher, *U.S. Patent* 3,033,760, May 8, 1962

ELPHO**Medium****Paper****Buffer**

0.1 *M* Acetate buffer, pH 4.6

Mobility

Pilosomycin migrates toward cathode

Ref.

As PC

PIMARICIN**PC****Paper**

Whatman No. 1

Solvents

- A. Butanol-ethanol-water (5:1:4)
- B. Propanol-water (7:3)
- C. As B, but (8:2)

Detection

Bioautography vs. *Saccharomyces cerevisiae*

R_F

Solvent A, 0.25
Solvent B, 0.15

Ref.

A.P. Struyk, I. Hoette, G. Drost, J.M. Waisv T. van Eek and J.C. Hoogerheide, *Antibiot. A* (1957-1958) 878-885

PIOMYCIN**PC****Paper**

Toyo No. 51

Solvents

- A. Butanol-acetic acid-water (4:1:2)
- B. 75% Phenol

Detection

- A. Bioautography vs. *Piricularia oryzae*
- B. Ninhydrin
- C. UV lamp

R_F

Solvent A, 0.17
Solvent B, 0.36

Ref.

M. Matsuoka, N. Hattori and T. Ishiyama, *presented at Japan Antibiotics Research Association March 22, 1968; Netherlands Patent* 67,13 April 16, 1968

TLC 1**Medium**

Silica gel G

Solvents

- A. Butanol-acetic acid-water (4:1:2)
- B. Butanol-ethanol-0.1 *N* hydrochloric acid (1:1:9)
- C. Propanol-pyridine-acetic acid-water (15:10:3:12)
- D. Chloroform-methanol-17% ammonium dioxide (2:1:1)
- E. Ethanol-water (4:1)

Detection

- A. Ninhydrin
- B. Potassium permanganate
- C. UV lamp

R_F

Solvent	R_F	Solvent	R_F
A	0.22	D	0.82
B	0.35	E	0.50
C	0.88		

Ref.

As PC

TLC 2

Medium

As TLC 1

Solvents

As TLC 1, A-E

Detection

As TLC 1, A-C

 R_F

Solvent	R_F	Solvent	R_F
A	0.21	D	0.87
B	0.45	E	0.74
C	0.86		

Ref.

As PC, reference 2

ELPHO

Medium

Paper, Toyo No. 51

Buffer

Formic acid-acetic acid-water (25:75:900), pH 2.0

Conditions

Temperature, 0°C; 3000 V/40 cm; 30 mA; 20 min

Mobility

Piomycin moves 1.8 cm toward cathode

Ref.

As PC, reference 2

PLATOMYCINS**PC**

Solvents

- A. 10% Ammonium chloride
- B. 5% Ammonium chloride
- C. Water satd. *n*-butyl alcohol
- D. *n*-Butyl alcohol-acetic acid-water (3:1:1)
- E. Water satd. *n*-butanol + 2% *p*-toluenesulfonic acid + 2% piperidine
- F. Water satd. ethyl acetate

Detection

Bioautography vs. *Bacillus subtilis* KY 4273 R_F

Solvent	Platomycin	
	A	B
A	0.67	0.63
B	0.65	0.60
C	0.00	0.00
D	0.02	0.01
E	0.02	0.01
F	0.00	0.00

Ref.

As Bleomycins, TLC 3

TLC 1

Medium

Silica gel

Solvents

- A. Chloroform-methanol-17% ammonia water (2:1:1) (upper layer)
- B. 10% Ammonium acetate-methanol (1:1)
- C. Methanol-10% ammonium acetate-10% ammonia water (10:9:1)
- D. 0.05 *m* Citrate buffer, pH 6.9

Detection

Bioautography vs. *Bacillus subtilis* KY 4273 R_F

Solvent	Platomycin	
	A	B
A	0.43	0.10
B	0.73	0.60
C	0.62	0.47
D	0.15	0.05

Ref.

As Bleomycins, TLC 3

TLC 2

Media

- A. Silica gel GF
- B. Silica gel GF sheet (Woelm)
- C. Alumina-Kieselguhr (6:1)

Solvents

- A. Chloroform-methanol-7% ammonia water (40:12:20)
- B. Benzene-acetone (7:3)
- C. Ethyl acetate-acetone (8:2)
- D. Benzene-acetone (1:1)
- E. Benzene-acetone-water (70:29:1)

R_F

Compound	Medium-Solvent							
	A-A	B-A	B-B	B-C	C-B	C-C	C-D	C-E
3-O-Propionyl-5-O-mycarosyl platenolide	0.65	0.90	0.33	0.54	0.32 0.68	0.56 0.47	0.58	0.48
3-O-Propionyl-5-O-mycarosyl platenolide II	0.50	0.71	0.26	0.52	0.20 0.40	0.34	0.40	0.32
9-Dehydrodemycarosyl-platenomycin	0.35 0.20	0.34	0.40	—	0.24 0.12	0.24 0.11	—	—

Ref.

T. Furumai and M. Suzuki, *J. Antibiot.*, 28 (1975) 775-783; *J. Antibiot.*, 28 (1975) 783-788

Detection

As amicitin, PC 2

 R_F

0.86

Ref.

As amicitin, PC 2

PLEUROMUTILINS

TLC

Medium

Silica gel G 60 (E. Merck)

Solvents

A. Benzene-ethyl acetate (1:1)

B. Toluene-ethyl acetate-glacial acetic acid (50:50:1)

C. Ethyl acetate

Detection

Spray with 1% ferric chloride, 1% acetic acid in conc. sulfuric acid. Heat for 3 min at 120°C

 R_F

Compound	Solvent		
	A	B	C
Pleuro-mutilin	0.34	0.30	0.48 (greenish spot)
Compound M1	0.47	0.45	0.54 (greenish spot)
Compound M2	0.56	0.55	— (greenish spot)
Compound M3	0.44	0.40	0.54 (greenish spot)
Compound M4	—	—	0.20 (red spot)

Ref.

F. Knauseder and E. Brandl, *J. Antibiot.*, 29 (1976) 125-131

PLICACETIN

PC

Solvent

As amicitin, PC 2

PLURALLIN

TLC

Medium

Alumina

Solvent

n-Butanol-ethanol-water (4:1:2)

Detection

Bioautography vs. *Corynebacterium xerosis* R_F

0.0 (plurallin); 0.5-0.7

Ref.

H. Umezawa, H. Ogawara, K. Maeda, K. Nitta, Y. Okami and T. Takeuchi, *U.S. Patent* 3,655,877, April 11, 1972

POLYANGIUM CELLULOSUM var. fulvum ANTIBIOTICS

PC

Paper

Whatman No. 1

Solvents

A. Water

B. Water satd. *n*-butanol

C. Water satd. ethyl acetate

Detection

Bioautography vs. *Microsporum canis* R_F

Solvent A, 0.74

Solvent B, 0.93

Solvent C, 0.71

Ref.

S.M. Ringel, S. Roemer and A.L. Gutt, *U.S. Patent* 3,651,216, March 21, 1972

TLC 1**Media**

- A. Cellulose Eastman Sheet 6065
- B. Silica gel Eastman Sheet 6060
- C. Silica gel G plates

Solvents

- A. Butanol
- B. 95% Ethanol
- C. *n*-Propanol-ethyl acetate-water (7:2:1)
- D. Methanol-ethanol (1:1)
- E. Water-methanol-ethanol (2:3:5)
- F. Water-ethanol (5:95)
- G. Methanol-ethyl acetate (1:1)
- H. Ammonium hydroxide-water-isopropanol (5:15:85)
- I. Water-ethanol (1:4)

Detection

As PC

 R_F

Medium	Solvent	Complex	R_F		
			A	B	C
A	A	0.23			
A	B	0.65			
A	C	0.70			
B	B		0.69	0.38	0.00
B	C		0.50	0.28	0.00
C	D		0.05	0.30	0.50
C	E		0.50	0.75	0.82
C	F		0.10	0.85	0.35
C	G		0.02	0.33	0.18
C	H			0.57	0.61
C	I				0.75

Ref.

As PC

TLC 2**Medium**

Silica gel

Solvent

Ethyl acetate-isopropanol-water (85:10:5)

 R_F

- Component F, 0.68
- Component S, 0.56

Ref.

M.V. Strandtmann, S. Ringel and A. Gutt, *German Patent* 2,303,418, Sept. 6, 1973

CCD**Solvent**

Water-satd. *n*-butanol-butanol-satd. water (1:1),
30 transfers

Distribution

Component A in tubes 16-29

Ref.

As PC

POLYETHERIN A and B**PC****Solvent**

Water-satd. diethyl ether; paper impregnated with
buffers of pH 2, 4, 6 and 8

DetectionBioautography vs. *Bacillus subtilis* **R_F**

- Component A 0.4 (est.)
- Component B 0 (est.)

Ref.

S. Shoji, S. Kozuki, S. Matsutani, T. Kubota, H. Nishimura, M. Mayama, K. Motokawa, Y. Tanaka, N. Shimaoka and H. Otsuka, *J. Antibiot.*, 21 (1968) 402-409

TLC**Media**

- A. Silica gel G
- B. Aluminum oxide

Solvents

- A. Chloroform-methanol (9:1)
- B. Benzene-ethyl acetate-methanol (6:4:1)
- C. Ethyl acetate-tetrachloroethane-water (3:1:3)

Detection

Sulfuric acid

 R_F (Component A)

Medium	Solvent	R_F
A	A	0.44-0.60
A	B	0.40-0.46
B	C	0.33-0.48

Ref.

As PC

POLYFUNGINS**PC 1****Solvent**

Water satd. butanol

DetectionBioautography vs. *Saccharomyces cerevisiae* **R_F**

- Component A 0.12-0.38 (est.)
- Component B 0.39-0.61 (est.)

Ref.

German Patent 2,044,004, April 1, 1970

PC 2*Solvent*

Water satd. butanol

*Detection*Bioautography vs. *Saccharomyces cerevisiae* R_F (Estimated from drawing)

Complex, 0.1–0.4

Polyfungin A, 0.4–0.6

Polyfungin B, 0.1–0.3

Ref.

D.M. Kotiuszko, K.M. Wituch, D.J. Siejko, H. Morawska, N. Porowska, M.T. Horodecka, H.W. Wolkowicz, M. Nowacka, Z.E. Makarowska-Plociennik, and L. Halski, *U.S. Patent* 3,891,505, June 24, 1975

TLC 1*Solvent*

Chloroform–methanol–water (2:2:2)

 R_F $B > A_3 > A$ *Ref.*

Z. Plociennik, L. Halski and Z. Kowszyk-Gindifer, *Chemia Anal. (Warsaw)*, 18 (1973) 151–156

TLC 2*Medium*

Silica gel

Solvent

Methanol–chloroform–water (2:2:1)

*Detection*Bioautography vs. *Saccharomyces cerevisiae* R_F (estimated from drawing)

Complex, 0.52, 0.77

Polyfungin A, 0.5

Polyfungin B, 0.7

Ref.

As PC 2

CCD*Solvents*

A. Methanol–chloroform–borate buffer (2:2:1), pH 8.2

B. Amyl alcohol–isoamyl alcohol–citrate buffer (12:17:29)

Distribution coefficient

400 transfers, Solvent A:

Polyfungin A: tube 352; Polyfungin B: tube 305

Polyfungin A, 400 transfers, Solvent B:

Polyfungin A₁ separated from Polyfungin A₂*Ref.*

As PC 2

POLYKETO ACIDOMYCIN (PKAM)**PC***Solvents*

A. Distilled water

B. 3% Ammonium chloride soln.

C. Chloroform

D. Ethyl acetate–water (1:1)

E. Acetone

F. Methanol

G. Ethanol

H. *n*-Butanol–water (1:1)I. *n*-Butanol–acetic acid–water (4:1:5)

J. Light petroleum–water (1:1)

K. Diethyl ether–water (1:1)

*Detection*A. Bioautography vs. *Bacillus subtilis*

B. Potassium permanganate spray

C. Ninhydrin

 R_F (estimated from drawing)

Solvent	R_F	Solvent	R_F
A	0.80	G	0.80
B	0.75	H	0.70
C	0.85	I	0.65
D	0.82	J	0.05
E	0.90	K	0.55
F	0.85		

Ref.

I.R. Shimi, G.M. Inam and Y.M. Shehata, *J. Antibiot.*, 20 (1967) 204–209

POLYMYXIN B₁ and B₂**HPLC***Apparatus*

Laboratory Data Control modular liquid chromatograph, equipped with a high pressure Milton Roy Minipump with a pulse dampener (Model 709)

Column

Stainless steel (30 cm × 4.6 mm I.D.), prepacked with μ Bondapak C₁₈ (Waters Assoc.) attached to a septumless injector valve (Micromeritics) and the UV monitor

Mobile phase

Linear gradient from 20% acetonitrile to 50% acetonitrile and 20% methanol, pH 2.0 phosphate buffer, in 16 min

Flow-rate

1.0 ml/min

Temperature

Ambient

Chart speed

6.4 mm/min

Pressure

1000 p.s.i.

Detection

254 nm UV monitor

*Retention time (min) (estimated from drawing)*Polymyxin B₁ 14Polymyxin B₂ 11*Ref.*K. Tsuji and J.H. Robertson, *J. Chromatogr.*, 112 (1975) 663–672**POLYMYXIN DERIVATIVES****TLC***Medium*

Silica gel

*Solvent**n*-Butanol–pyridine–acetic acid–water
(50:20:6:24)*R_F*

Derivative	<i>R_F</i>
Penta[N-(3-pyridylmethoxycarbonyl N-oxide)]polymyxin B	0.30
Tri[N-γ-(3-pyridylmethoxycarbonyl N-oxide)]colistamine	0.22
Penta[N-(3-pyridylmethoxycarbonyl N-oxide)]colistamine	0.32
N-α-[N(α)-7-Methyloctanoyl N-γ-benzyloxycarbonyl-L-α, γ-diaminobutyryl-L-threonyl-N-γ-benzyloxycarbonyl-L-α, γ-diaminobutyryl]-tris[N-γ-(3-pyridylmethoxycarbonyl N-oxide)]colistamine	0.55
N-α-[N(α)-7-Methyloctanoyl L-α, γ-diaminobutyryl-L-threonyl-N-α-L-α, γ-diaminobutyryl] colistamine pentahydrochloride	0.37
N-α-(N-α- <i>n</i> -Octanoyl N-γ-benzyloxycarbonyl L-α, γ-diaminobutyryl-L-threonyl-N-γ-benzyloxycarbonyl L-α, γ-diaminobutyryl)-tris[N-γ-(3-pyridylmethoxycarbonyl N-oxide)]colistamine	0.63
N-α-(N-α-3,5,5-Trimethylhexanoyl L-α, γ-diaminobutyryl-L-threonyl-N-α-L-α, γ-diaminobutyryl) colistamine pentahydrochloride	0.34

Derivative	<i>R_F</i>
N-α-(N-α-Nonanoyl N-γ-benzyloxycarbonyl L-α, γ-diaminobutyryl-L-threonyl-N-γ-benzyloxycarbonyl L-α, γ-diaminobutyryl)-tris[N-γ-(3-pyridylmethoxycarbonyl N-oxide)]colistamine	0.60
N-α-(N-α-Heptanoyl N-γ-benzyloxycarbonyl L-α, γ-diaminobutyryl-L-threonyl-N-γ-benzyloxycarbonyl L-α, γ-diaminobutyryl)-tris-[N-γ-(3-pyridylmethoxyloxy-carbonyl N-oxide)]colistamine	0.61
N-α-(N-α-Heptanoyl L-α, γ-diaminobutyryl-L-threonyl-N-α-L-α, γ-diaminobutyryl) colistamine pentahydrochloride	0.26
N-α-(N-α-2-Norbornylacetyl N-γ-benzyloxycarbonyl L-α, γ-diaminobutyryl-L-threonyl-N-γ-benzyloxycarbonyl L-α, γ-diaminobutyryl)-tris[N-γ-(3-pyridylmethoxycarbonyl N-oxide)]colistamine	0.64
N-α-(N-α-2-Norbornylacetyl L-α, γ-diaminobutyryl-L-threonyl-N-α-L-α, γ-diaminobutyryl) colistamine	0.30
N-α-(N-α-3-Cyclohexylpropionyl N-γ-benzyloxycarbonyl L-α, γ-diaminobutyryl-L-threonyl-N-γ-benzyloxycarbonyl L-α, γ-diaminobutyryl)-tris[N-γ-(3-pyridylmethoxycarbonyl N-oxide)]colistamine	0.61
N-α-(N-α-3-Cyclohexylpropionyl L-α, γ-diaminobutyryl-L-threonyl-N-α-L-α, γ-diaminobutyryl) colistamine	0.27
N-α-(N-α-2-Hydroxyoctanoyl N-γ-benzyloxycarbonyl L-α, γ-diaminobutyryl-L-threonyl-N-γ-benzyloxycarbonyl L-α, γ-diaminobutyryl)-tris[N-γ-(3-pyridylmethoxycarbonyl N-oxide)]colistamine	0.62
N-α-(N-α-2-Hydroxyoctanoyl L-α, γ-diaminobutyryl-L-threonyl-N-α-L-α, γ-diaminobutyryl) colistamine pentahydrochloride	0.25

Derivative	R_F
N- γ -(3-Pyridylmethoxycarbonyl N-oxide)colistamine; 1.40 g	
N- α -(N- α -2-oxononanoyl N- γ -benzyloxycarbonyl L- α , γ -diaminobutyl-L-threonyl-N- γ -benzyloxycarbonyl L- α , γ -diaminobutyl)-tris[N- γ -(3-pyridylmethoxycarbonyl N-oxide)]colistamine	0.55
N- α -(N- α -2-Hydroxynonanoyl L- α , γ -diaminobutyl-L-threonyl-N- α -L- α , γ -diaminobutyl)-colistamine	0.28
N- α -(N- α -11-Cyanoundecanoyl N- γ -benzyloxycarbonyl L- α , γ -diaminobutyl-L-threonyl-N- γ -benzyloxycarbonyl L- α , γ -diaminobutyl)-tris[N- γ -(3-pyridylmethoxycarbonyl N-oxide)]-colistamine	0.61
N- α -(N- α -11-Cyanoundecanoyl L- α , γ -diaminobutyl-L-threonyl-N- α -L- α , γ -diaminobutyl)colistamine-pentahydrochloride	0.27
N- α -(N- α -4-Methoxycinnamoyl N- γ -benzyloxycarbonyl L- α , γ -diaminobutyl-L-threonyl-N- γ -benzyloxycarbonyl L- α , γ -diaminobutyl)-tris[N- γ -(3-pyridylmethoxycarbonyl N-oxide)]colistamine	0.63
N- α -(N- α -4-Methoxyphenylpropionyl L- α , γ -diaminobutyl-L-threonyl-N- α -L- α , γ -diaminobutyl)colistamine-pentahydrochloride	0.22
N- α -(N- α -4-Phenylbutyl N- γ -benzyloxycarbonyl L- α , γ -diaminobutyl L-threonyl-N- γ -benzyloxycarbonyl L- α , γ -diaminobutyl)-tris[N- γ -(3-pyridylmethoxycarbonyl N-oxide)]-colistamine	0.67
N- α -(N- α -4-Phenylbutyl L- α , γ -diaminobutyl-L-threonyl-N- α -L- α , γ -diaminobutyl)-colistamine-pentahydrochloride	0.27
N- α -(N- α -3-Hydroxynonanoyl N- γ -benzyloxycarbonyl L- α , γ -di-	

Derivative	R_F
aminobutyl-L-threonyl N- γ -benzyloxycarbonyl L- α , γ -diaminobutyl)-tris[N- γ -(pyridyl-3-methoxycarbonyl N-oxide)]colistamine	0.67
N- α -(N- α -3-Hydroxynonanoyl L- α , γ -diaminobutyl-L-threonyl N- α -L- α , γ -diaminobutyl)-colistamine	0.30
N- α -(N- α -Cinnamoyl N- γ -benzyloxycarbonyl L- α , γ -diaminobutyl-L-threonyl-N- γ -benzyloxycarbonyl L- α , γ -diaminobutyl)-tris[N- γ -(3-pyridylmethoxycarbonyl N-oxide)]colistamine	0.61
N- α -(N- α -3-Phenylpropionyl L- α , γ -diaminobutyl-L-threonyl-N- α -L- α , γ -diaminobutyl)-colistamine-pentahydrochloride	0.23
N- α -(N- α -Dioctylsulfonyl N- γ -benzyloxycarbonyl L- α , γ -diaminobutyl L-threonyl-N- γ -benzyloxycarbonyl L- α , γ -diaminobutyl)-tris[N- γ -(3-pyridylmethoxycarbonyl)]colistamine	0.67
N- α -(N- α -Octylsulfonyl L- α , γ -diaminobutyl-L-threonyl-N- α -L- α , γ -diaminobutyl)-colistamine	0.33
N- α -(N- α -L-6-Methyloctanoyl N- γ -benzyloxycarbonyl L- α , γ -diaminobutyl-L-threonyl N- γ -benzyloxycarbonyl L- α , γ -diaminobutyl)-tris[N- γ -(3-pyridylmethoxycarbonyl N-oxide)]colistamine	0.66
N- α -(N- α -L-6-Methyloctanoyl L- α , γ -diaminobutyl-L-threonyl-N- α -L- α , γ -diaminobutyl)-colistamine-pentachlorhydrate or colistamine A pentahydrochloride	0.42

Ref.

Netherlands Patent 7,200,997, Aug. 4, 1972

POLYMYXINS

PC 1

Paper

Whatman No. 1 pretreated with 0.2 M glycine-HCl at pH 2.5

Solvent

Butanol, 6–18 h

DetectionBioautography vs. *Brucella bronchiseptica***R_F**

Polymyxin A 0.18

Polymyxin B 0.56

Polymyxin D 0.38

Polymyxin E 0.54

Ref.H.A. Nash and A.R. Smashey, *Arch. Biochem.**Biophys.*, 30 (1951) 237**PC 2****Paper**

Whatman No. 1

Solvent

Butanol–water–isopropylamine (125:60:4)

R_F

Polymyxin B 0.43

Polymyxin D 0.23

Ref.A.G. Mistulta, *Antibiot. Chemother.*, 6 (1956)

196–198

PC 3**Paper**

A. Whatman No. 1

B. Whatman No. 2

C. Whatman No. 4

D. Whatman No. 20

Solvents

A. 1-Butanol–acetic acid–water (120:30:50)

B. As A, but (4:1:5), upper phase

C. As B, but lower phase

D. 1-Butanol–pyridine–acetic acid–water
(30:20:6:24)E. 1-Butanol–acetic acid–1% aq. sodium chloride
(120:30:50)F. 1-Butanol–acetic acid–5% aq. sodium chloride
(120:30:50)G. 1-Butanol–pyridine–acetic acid–1% aq.
sodium chloride (30:20:6:24)**R_F**

Solvent	Paper	Polymyxin		
		A	B	E
A	A	0.39	0.54	0.51
	B	0.29	0.42	0.40
	C	–	0.52	0.51
	D	0.15	0.31	0.30

Solvent	Paper	Polymyxin		
		A	B	E
B	A	–	0.50	0.46
	C	–	0.46	0.45
	D	–	–	0.20
C	A	0.92	0.86	0.90
D	A	0.58	0.73	0.77
	C	–	0.73	0.72
	D	–	–	0.64
E	A	–	0.42	0.42
F	A	–	0.37	0.37
	C	–	0.46	0.46
G	A	–	0.24	0.24

Ref.S. Wilkinson and L.A. Lowe, *J. Chem. Soc.*,
(1964) 4107**PC 4****Paper**

Whatman No. 1

SolventsA. *n*-Butanol–acetic acid–water (4:1:5)B. *n*-Butanol–pyridine–acetic acid–water
(30:20:6:24)C. *n*-Butanol–acetic acid–water (12:3:5)**R_F**

Component	Solvent		
	A	B	C
Polymyxin A ₁	0.33	0.58	0.39
Polymyxin A ₂	0.33	0.58	0.39
Polymyxin B ₁	0.48	0.73	0.54
Polymyxin B ₂	0.48	0.73	0.54
Polymyxin E ₁	0.46	0.77	0.51
Polymyxin E ₂	0.46	0.77	0.51

Ref.S. Wilkinson, *Antimicrob. Agents Chemother.*,
1966 (1967) 651–654**PC 5****Solvents**A. *n*-Butanol–acetic acid–water (4:1:5)B. *n*-Butanol–pyridine–acetic acid–water
(15:10:3:12)C. *tert*-Butanol–acetic acid–water (74:3:25)D. *tert*-Butanol–methyl ethyl ketone–formic
acid–water (8:6:3:3)

Detection

Ninhydrin

 R_F

Solvent	Polymyxin B
A	0.23
B	0.76
C	0.54
D	0.60

Ref.

M.J. Daniels, *Biochim. Biophys. Acta*, 156 (1968) 119–127

PC 6

Paper

Whatman 3MM

Solvent

Butanol–acetic acid–water (12:3:5)

Detection

Paper stained with 0.25% ninhydrin in acetone containing 0.01% isatin and 1% lutidine; heat at 100° for 10 min

 R_F

Polymyxin, 1.0. Migration distance relative to alanine taken as 1.00

Ref.

As Ampicillin, PC 1

PC 7

Paper

Toyo Roshi No. 51

Solvent

n-Butanol–acetic acid–water (4:1:2)

Detection

A. Bioautography vs. *Escherichia coli*

B. Ninhydrin

 R_F Polymyxin S₁ 0.45Polymyxin T₁ 0.63

Ref.

J. Shoji, H. Hinoo, Y. Wakisaka, K. Koizumi, M. Mayama and S. Matsuura, *J. Antibiot.*, 30 (1977) 1029–1034

PC 8

Paper

Whatman No. 1

Solvents

A. Butanol–pyridine–acetic acid–water (15:10:3:12)

B. Butanol–acetic acid–water (4:1:5), upper phase

DetectionA. Bioautography vs. *Escherichia coli*

B. Reagents that give color reactions with primary amines

 R_F

Solvent	Polymyxin B	Polymyxin F
A	0.30	0.57
B	0.45	0.69

Ref.

W.L. Parker, M.L. Rathnum, L.D. Dean, M.W. Nimeck, W.E. Brown and E. Meyers, *J. Antibiot.*, 30 (1977) 767–768

TLC 1

Medium

Kieselgel G (Merck)

Solvent

Acetone–water–acetic acid–2 *N* ammonium hydroxide (15:5:1:2)

Detection

A. Ninhydrin

B. Bioautography vs. *Bordetella bronchiseptica* R_F

Polymyxin B 0.45

Polymyxin D 0.51

Polymyxin E 0.45

Polymyxin M 0.36

Polymyxin E

methanesulfonate 0.95

Ref.

M. Igloy and A. Mizsei, *J. Chromatogr.*, 28 (1967) 458–461; and *J. Chromatogr.*, 34 (1968) 546–547

TLC 2

Medium

Silica gel

Solvent

n-Butanol–acetic acid–water (4:1:3) and 1/20 vol. of pyridine; upper layer

 R_F

Polymyxin A 0.34

Polymyxin B 0.48

Ref.

Y. Kimura, E. Murai, M. Fujisawa, T. Tatsuki and F. Nobue, *J. Antibiot.*, 22 (1969) 449–450

TLC 3

Medium

As gramicidin, TLC, A and B

Solvent

As gramicidin, TLC, A–K

Detection

As gramicidin, TLC

 R_F

Med- ium	Sol- vent	Polymyxin	
		E	B
A	A	0.21	0.23
	B	0.35	0.36
	C	0.21	0.23
	D	0.07	0.08
	E	0.43	0.44
	F	0.32	0.33
	G	0.00	0.00
	H	0.00	0.00
	I	0.78	0.80
	J	0.00	0.00
B	C	0.38	0.38
	K	0.68	0.69

Ref.

As gramicidin, TLC

TLC 4

Medium

Silica gel G

Solvent

As PC 1, A

 R_F

Polymyxin	R_F
A ₁	0.47
A ₂	0.47
B ₁	0.57
B ₂	0.57
E ₁	0.57
E ₂	0.57

Ref.

As PC 1

TLC 5

Medium

Gelman ITLC, Type SAF (glass microfiber sheets impregnated with silicic acid)

Solvents

A. Propanol–conc. ammonium hydroxide (7:3)

B. Propanol–pyridine–acetic acid–water (15:10:3:12)

C. Butanol–acetic acid–water (3:1:1)

*Detection*A. Bioautography vs. *Escherichia coli*

B. Reagents that give color reactions with primary amines

 R_F

Solvent	Polymyxin B	Polymyxin F
A	0.62	0.69
B	0.85	0.85
C	0.27	0.56

Ref.

As PC 8

TLC 6

*Medium*Silica gel F₂₅₄*Solvent*

Acetone–water–acetic acid–2 N ammonium hydroxide (15:5:1:2)

*Detection*A. Bioautography vs. *Escherichia coli*

B. Ninhydrin

 R_F Polymyxin S₁ 0.35Polymyxin T₁ 0.12*Ref.*

As PC 7

ELPHO 1

Medium

Whatman 3MM paper

Buffer

Formic acid–acetic acid–water (6:24:170)

Conditions

3000 V, potential gradient 60 V per cm, current 50 mA, 30 min

Detection

Paper stained with 0.25% ninhydrin in acetone containing 0.01% isatin and 1% lutidine; heat at 100° for 10 min

Mobility

Polymyxin, 0.90; migration distance relative to alanine taken as 1.00

Ref.

As Ampicillin, PC 1

ELPHO 2

Medium

Acrylamide gel

Buffer

Buffer soln., pH 4.6–monomer soln.–water–0.14% ammonium persulphate soln. (1:2:1:4)

Buffer soln.

1 *N* Potassium hydroxide–acetic acid–tetramethylethylenediamine (48:21.5:4.6) + water to 100 ml

Monomer soln.

Acrylamide–methylenebisacrylamide (60 g:1.0 g) + water to 100 ml

Conditions

The gel soln. is pipetted into glass tubes (65 × 3 mm) sealed at one end by parafilm, leaving sufficient space at the top to add a few drops of water to ensure a flat, even surface on the catalyzed gel surface. The tubes are allowed to stand at 28° for 1 h and after removal of the parafilm are ready for use

Detection

0.1% Naphthalene black in 7% acetic acid

Mobility

Light blue band

Ref.

As Bacitracin, ELPHO 2

CCD (For separation of polymyxin P components)

Solvent

n-Butanol–sec.-butanol–0.1 *N* hydrochloric acid (6:30:40), 2100 transfers

Distribution

Polymyxin P₁ *K* = 0.056

Polymyxin P₂ *K* = 0.041

Ref.

As TLC 2

POLYOXIN L

HPLC**Apparatus**

Model ALC/GPC 244 (Waters Assoc.)

Column

μBondapak C₁₈ (Waters Assoc.) 30 cm × 14 mm I.D., prepacked

Mobile phases

A. Methanol–0.01 *M* phosphate buffer, pH 5.7 (2:8)

B. Methanol–0.005 *M* citrate buffer, pH 6.0 (2:8)

C. Methanol–0.005 *M* citrate buffer, pH 5.8 (5:95)

D. 0.005 *M* Citrate buffer, pH 5.8

Flow-rate

1.0 ml/min

Pressure

1500–2000 p.s.i.

Detection

UV Model 440, 254 nm. Single peak observed under various conditions

Injector

Universal Injector U6K (Water Assoc.), 2 ml of internal volume

Type

Reversed-phase

Retention time (min)

Mobile phase A 2.8

Mobile phase B 2.6

Mobile phase C 3.0

Mobile phase D 3.4

Ref.

As Amipurimycin, HPLC

POLYOXINS

PC 1**Solvent**

n-Butanol–acetic acid–water (4:1:2)

R_F

At least four active components seen, the following *R_F* values are given (main components):

Polyoxin A 0.23

Polyoxin B 0.13

Ref.

K. Isono, J. Nagatsu, Y. Kawashima and S. Suzuki, *Agr. Biol. Chem.*, 29 (1965) 848–854

PC 2**Paper**

Toyo No. 51

Solvents

A. As PC 1

B. 75% Phenol

R_F

Polyoxin	Solvent A	Solvent B
A	0.21	0.53
B	0.10	0.18
D	0.10	0.08
E	0.13	0.12
F	0.21	0.38
G	0.12	0.30
H	0.27	0.66

Ref.

South African *Derwent Farmdoc* 29960, April 10, 1967

PC 3**Paper**

As PC 2, ascending

Solvents

A. As PC 1

B. Butanol–pyridine–water (4:1:2)

C. As PC 2, B

Detection

- A. UV light
B. Ninhydrin
C. Biological activity

 R_F

Polyoxin	Solvent A	Solvent B	Solvent C
A	0.19	0.07	0.53
B	0.07	0.03	0.18
C	0.09	0.03	0.27
D	0.07	0.01	0.06 *
E	0.08	0.01	0.09 •
F	0.18	0.03	0.38
G	0.09	0.03	0.30
H	0.25	0.12	0.66
I	0.23	0.08	0.61

• Tailing was observed

Ref.

K. Isono, J. Nagatsu, K. Kobinata, K. Sasaki and
S. Suzuki, *Agr. Biol. Chem.*, 31 (1967) 190-199

PC 4*Paper*

As PC 2

Solvent

As PC 1

 R_F

Polyoxin J	0.08
Polyoxin K	0.22
Polyoxin L	0.08

Ref.

Netherlands Patent 68,09186; Dec. 31, 1968

PC 5*Paper*

As piomycin PC 1

Solvent

As piomycin PC 1, A and B

Detection

As piomycin PC 1, A-C

 R_F

Solvent	Polyoxin			
	A	B	G	H
A	0.19	0.07	0.09	0.25
B	0.63	0.16	0.30	0.66

Ref.

As piomycin PC 1, ref. 2

PC 6*Paper*

Toyo No. 51

Solvents

- A. Butanol-acetic acid-water (4:1:2)
B. 75% Phenol

 R_F

Polyoxins	Solvent A	Solvent B
A	0.61	0.53
B	0.10	0.18
D	0.10	0.08
E	0.13	0.12
F	0.21	0.38
G	0.12	0.30
H	0.27	0.66

Ref.

S. Suzuki, K. Isono and J. Natatsu, *U.S. Patent*
3,703,506, Nov. 21, 1972

PC 7*Paper*

Toyo Roshi filter paper No. 51

Solvent

Butanol-acetic acid-water (4:1:2, v/v)

 R_F

Polyoxin A	0.28
Polyoxin B	0.15

Ref.

S. Suzuki, K. Isono, J. Nagatsu, T. Akashiba and
S. Sasaki, *U.S. Patent* 3,700,655, Oct. 24, 1972

TLC 1*Medium*

Silica gel G

 R_F

Poly-oxin	Solvent				
	A	B	C	D	E
A	0.15	0.39	0.66	0.85	0.32
B	0.11	0.23	0.56	0.83	0.23
C	0.12	0.33	0.59	0.83	0.23
D	0.08	0.07	0.25	0.03	0.00
E	0.08	0.07	0.29	0.04	0.00
F	0.16	0.16	0.47	0.06	0.00
G	0.12	0.36	0.59	0.84	0.30
H	0.20	0.44	0.67	0.85	0.39
I	0.18	0.41	0.66	0.85	0.35

Solvents

- A. As PC 1
 B. Butanol-ethanol-0.1 *N* hydrochloric acid (1:1:1)
 C. Butanol-pyridine-water (15:10:3)
 D. Chloroform-methanol-17% ammonia (2:1:1)
 E. Ethanol-water (4:1)

Detection

- A. Permanganate spray
 B. As PC 3, A
 C. As PC 3, C

Ref.

As PC 3

TLC 2**Medium**

Silica gel G

Solvent

As piomycin TLC 1, C and E

Detection

As piomycin TLC 1, A-C

 R_F

Solvent	Polyoxin			
	A	B	G	H
C	0.66	0.56	0.59	0.67
E	0.28	0.23	0.28	0.32

Ref.

As piomycin TLC 1

TLC 3**Medium**

Cellulose Avicel

Solvent

75% Phenol

Detection

UV

 R_F (estimated from drawing)

Polyoxin A 0.67
 Polyoxin B 0.20
 Polyoxin C 0.29
 Polyoxin I 0.73

Ref.

M. Hori, K. Kakiki and T. Misato, *J. Antibiot.*, 28 (1975) 237-239

PORFIROMYCIN**PC****Solvents**

- A. 1-Butanol-water (84:16)

- B. As A + 0.25% *p*-toluenesulfonic acid
 C. 1-Butanol-acetic acid-water (2:1:1)
 D. As A + 2% piperidine
 E. Methanol-benzene-water (1:1:2)
 F. Water satd. ethyl acetate

Detection

- A. Bioautography vs. *Sarcina lutea*
 B. UV at 360 nm

 R_F

Solvent	R_F	Solvent	R_F
A	0.60-0.75	D	0.58-0.75
B	0.55-0.72	E	0.10-0.22
C	No zone	F	0.55-0.80

Ref.

German Patent 1,122,671, Jan. 25, 1962

PRIMYCIN**PC****Paper**

Schleicher and Schuell 2043/b

Solvent

Butanol-acetic acid-water (4:1:5)

Detection

- A. Bioautography vs. *Bacillus subtilis*
 B. N-Bromosuccinimide modification of Sakaguchi color test

 R_F

0.56-0.61

Ref.

I. Szilagyi, T. Valyi-Nagy and T. Keresztes, *Nature*, 205 (1965) 1225-1227

PROACTINOMYCINS**CCD****Solvent**

Diethyl ether- $\frac{1}{2}$ *m* potassium phosphate buffer, pH 6.8; 24 transfers

Distribution

Antibiotic	Peak tubes
Proactinomycin A	4
Proactinomycin B	8-9
Proactinomycin C	20

Ref.

R.Q. Marston, *Brit. J. Exptl. Pathol.*, 30 (1949) 398-407

PROCEOMYCIN

PC

Solvents

- A. Wet butanol
- B. Aq. 3% ammonium chloride
- C. 75% Phenol
- D. 50% Acetone
- E. Butanol-methanol-water (4:1:2) + 1.5% methyl orange
- F. Butanol-methanol-water (4:1:2)
- G. Benzene-methanol (4:1)
- H. Water

All solvents developed ascending

R_F

Solvent	R_F	Solvent	R_F
A	0.95	E	0.90
B	0.10	F	0.90
C	0.95	G	0.65
D	0.90	H	0.25

Ref.

H. Tsukiura, M. Okanishi, H. Koshiyama, T. Ohmori, T. Miyaki and H. Kawaguchi, *J. Antibiot.*, 17 (1964) 223-229

TLC

Medium

Silica gel

Solvents

- A. Ethyl acetate-ethanol-water (92:5:3)
- B. Ethanol-methanol-water (10:9:1)

R_F

Solvent A 0.25
Solvent B 0.49

Ref.

L.J. Hanka and P.F. Wiley, *U.S. Patent* 3,988,441, Oct. 26, 1976

PROPIONIN B

PC

Paper

Whatman No. 1

Solvent (two-dimensional)

First direction:

- A. *n*-Butanol-acetic acid-water (4:1:5)

Second direction:

- B. Phenol-water (4:1)

Detection

Ninhydrin

Ref.

S. Ramanathan, E. Furusawa, H. Yee and W.C. Cutting, *Chemotherapy*, 19 (1973) 16-21

4'-PROPIONYL-MEGALOMICIN A

PC

Solvents

- A. 20% (w/v) Ammonium chloride
- B. Water satd. *n*-butanol
- C. *n*-Butanol-acetic acid-water (3:1:1)
- D. Water satd. acetic acid-ethyl acetate
- E. Water satd. *n*-butanol, 2% (w/v) *p*-toluene-sulfonic acid and 2% (w/v) piperidine
- F. 5% (w/v) Ammonium chloride
- G. Water satd. methyl isobutyl ketone
- H. Water satd. methyl isobutyl ketone, 1% (w/v) *p*-toluenesulfonic acid
- I. Acetone-water (1:1)
- J. Methanol-*n*-butanol-water (1:4:2) with 21% (w/v) methyl orange

R_F

Solvent	R_F	Solvent	R_F
A	0.60	F	0.70
B	0.85	G	0.75
C	0.73	H	0.87
D	0.00	I	0.80-0.90
E	0.87	J	0.89

Ref.

As Megalomicins, TLC 1

PROTICIN

TLC

Medium

Silica gel F₂₅₄ (E. Merck)

Solvent

Chloroform-methanol (3:2)

Detection

Staining was done with a mixture of chloro-sulfonic acid and glacial acetic acid by heating to 110°

R_F

0.38

Ref.

L. Vertesy, *J. Antibiot.*, 25 (1972) 4-10

PROTOMYCIN

PC

Solvents

- A. Water satd. diethyl ether
- B. Water satd. benzene
- C. Water satd. chloroform
- D. Water satd. methyl isobutyl ketone
- E. Water satd. light petroleum
- F. Water satd. cyclohexane

G. 3% Aq. ammonium chloride
All solvents developed ascending

R_F

Solvent	R_F	Solvent	R_F
A	0.85	E	0.00
B	0.35	F	0.00
C	0.75	G	0.86
D	0.78		

Ref.

R. Sugawara, *J. Antibiot.*, 16 (1963) 115-120

CCD

Solvent

Benzene-methanol-0.001 *N* hydrochloric acid
(10:2:8), 30 transfers

Detection

UV at 232 nm

Distribution

$K = 0.9$

Ref.

As PC

PRUMYCIN

TLC

Medium

Silicic acid (Kieselgel G, Merck)

Solvents

A. Propanol-pyridine-acetic acid-water
(15:10:3:12)

B. Butanol-acetic acid-water (3:1:2)

R_F

Solvent A 0.68

Solvent B 0.21

Ref.

T. Hata, S. Omura, M. Katagiri, K. Atsumi, J. Awaya, S. Higashikawa, K. Yasui, H. Terada and S. Kuyama, *J. Antibiot.*, 24 (1971) 900-901

PRUNACETIN

ELPHO

Medium

Starch block

Buffer

5 mM Phosphate, pH 8.5, $\mu = 0.045$

Detection

Color (purple band)

Mobility

Migrates toward anode

Ref.

T. Arai, S. Kishikata, K. Takamiya, F. Yanagisawa and T. Koyama, *J. Antibiot.*, 20 (1967) 334-343

PSEUDOMONAS ANTIBIOTIC

TLC

Medium

Cellulose

Solvent

Butanol-acetic acid-water (12:3:5)

Detection

Ninhydrin

R_F

0.35

Ref.

R. Grandpierre and A. Robert, *U.S. Patent* 3,705,237, Dec. 5, 1972

PSEUDOMONAS ANTIFUNGAL SUBSTANCE

PC

Solvents

A. *n*-Butanol-acetic acid-water (4:1:5)

B. Isopropanol-ammonium hydroxide-water
(20:1:2)

C. Water-satd. phenol

D. *n*-Butanol-pyridine-water (1:1:1)

E. *n*-Amyl alcohol satd. with 3% ammonium hydroxide

F. Benzene-acetic acid-water (2:2:1)

G. Hydrous acetone (50%)

H. *n*-Butanol-satd. water

I. Ethyl acetate-pyridine-water (2:1:2)

J. *n*-Propanol-2.5% sodium chloride-acetic acid
(10:8:1)

K. Methanol-0.1 *N* hydrochloric acid (3:1)

L. Methanol-water (3:1)

M. Ammonium chloride (3%)

N. Water

R_F

Solvent	R_F	Solvent	R_F
A	1.00	H	0.00
B	0.99	I	1.00
C	0.96	J	0.99
D	0.98	K	0.83
E	0.97	L	0.80
F	0.95	M	0.02
G	0.98	N	0.00

Detection

- A. Bioautography
B. Visible yellow zone color

Ref.

W.A. Ayers and G.C. Papavizas, *Appl. Microbiol.*, 11 (1963) 533–538

PSEUDOMONAS REPTILIVORA AN-TIBIOTIC

PC**Paper**

Ascending

Solvent

n-Butanol–acetic acid–water (12:3:5)

Detection

- A. Bioautography vs. *Staphylococcus aureus* ATCC 12600
B. UV at 254, 360 nm

R_F

B₁ and B₂ were found near solvent front and separated from UV visible impurities

Ref.

L.A. del Rio, J.L. Gorge, J. Olivares and F. Mayor, *Antimicrob. Agents Chemother.*, 2 (1972) 189–194

ELPHO**Medium**

Whatman No. 3MM paper

Buffer

Pyridine–acetic acid–water (25:1:475)

Conditions

350 V for 3.25 h

Detection

A V-inverted electrophoretic tank able to fit Whatman 3MM paper strips 31 × 30 cm was used. The extracts obtained after gel filtration were assayed, and the active fractions were eluted from the paper and concentrated *in vacuo* to a small volume

Mobility

B₂ > B₁

Ref.

As PC

PSICOFURANINE

PC 1**Paper**

Whatman No. 1

Solvents

- A. *n*-Butanol–water (84:16)
B. As A + 0.25% *p*-toluenesulfonic acid

C. *n*-Butanol–acetic acid–water (2:1:1)

D. As A, but 2 ml piperidine added to 98 ml solvent mixture

E. Water–*n*-butanol (96:4)

F. As E + 0.25% *p*-toluenesulfonic acid
As solvents developed ascending

Detection

UV at 262 nm

R_F (Estimated from drawing)

Solvent	R _F	Solvent	R _F
A	0.15	D	0.15
B	0.15	E	0.65
C	0.45	F	0.55

Ref.

W.T. Sokoloski, M.J. Eilers and T.E. Eble, *Antibiot. Chemother.*, 9 (1959) 435–438

PC 2 (Quantitative procedure)

Paper

Schleicher and Schuell 589 (blue ribbon special). 0.5 × 22.5 in. strips. Satd. soln. containing 1 mg psicofuranine per ml water applied to strips at doses of 1, 2, 4, 5, 6, 8, 10 or 20 μl/strip. Each test soln. applied to 3 strips using 3 different doses estimated to contain 1–20 μg antibiotic

Solvent

As PC 1, D, developed without equilibration, 40 h

Detection

UV scan at 262 nm with recording scanning spectrophotometer. O.D. proportional to quantity of antibiotic

Ref.

As PC 1

CCD**Solvent**

1-Butanol–water

Distribution

K = 0.28–0.35

Ref.

T.E. Eble, H. Hoeksema, G.A. Boyack and G.M. Savage, *Antibiot. Chemother.*, 9 (1959) 418–420

PUROMYCIN

ELPHO**Medium**

Whatman No. 1 paper

Buffer

Formic acid–acetic acid

Conditions

pH 1.8 at 50 V/cm for 80 min at 7°C

Detection

Detection by cpm of labelled compounds

Mobility (estimated from drawing)

Formyl-methonyl-puromycin 4.5

Methionyl-puromycin 9.5

Ref.G. Lucchini, R. Bianchetti and P. Crosti, *Biochem. Biophys. Res. Commun.*, 51 (1973) 144-150**PURPUROMYCIN****PC****Paper**

Whatman No. 1, descending

SolventsA. Water satd. *n*-butanolB. Water satd. *n*-butanol + 2% *p*-toluenesulfonic acidC. Water satd. *n*-butanol + 2% conc. ammonia

D. Ammonium chloride (20% solution in water)

E. *n*-Butanol-methanol-water (40:10:20) containing 0.75 g methyl orangeF. *n*-Butanol-methanol-water (40:10:30)

G. Water-acetone (1:1)

H. Water satd. ethyl acetate

DetectionBioautography vs. *Staphylococcus aureus***R_F**

Solvent	R _F	Solvent	R _F
A	0.15	E	0.75
B	0.68	F	0.70
C	0.0	G	0.48
D	0.0	H	0.98

Ref.C. Coronelli, H. Pagani, M.R. Bardone and G.C. Lancini, *J. Antibiot.*, 27 (1974) 161-168**TLC****Medium**

Silica gel

Solvent

Chloroform-methanol (95:5) (solvent moved 10 cm)

R_F

0.55

Ref.

As PC

PURPUROSAMINE**TLC****Medium**

Cellulose

Solvents

A. Propanol-pyridine-acetic acid-water (15:10:3:12)

B. Pyridine-ethyl acetate-acetic acid-water (5:6:1:3)

R_F

Solvent	Purpurosamine		
	A	B	C
A	0.625	0.575	0.475
B	0.208	0.208	0.146

Ref.

As Gentamicins, TLC 12

PYRACRIMYCIN A**PC****Paper**

Whatman No. 1

SolventsA. Water satd. *n*-butanolB. Water satd. *n*-butanol + 2% *p*-toluenesulfonic acidC. Water satd. *n*-butanol + 2% conc. ammoniaD. *n*-Butanol satd. water

E. Ammonium chloride (20% soln. in water)

F. *n*-Butanol-methanol-water (40:10:20) containing 0.75 g methyl orangeG. *n*-Butanol-methanol-water (40:10:30)

H. Water-acetone (1:1)

I. Water satd. ethyl acetate

DetectionBioautography vs. *Staphylococcus aureus***R_F**

Solvent	R _F	Solvent	R _F
A	0.60	F	0.65
B	0.29	G	0.71
C	0.53	H	0.52
D	0.14	I	0.00
E	0.71		

Ref.C. Coronelli, G. Tamoni, G. Beretta and G.C. Lancini, *J. Antibiot.*, 24 (1971) 491-496

TLC*Medium*Silica gel HF/UV₂₅₄*Solvent*

Chloroform-methanol (9:1)

Detection

UV absorption

R_F

0.22

Ref.

As PC

PYRIDOMYCIN**TLC***Medium*

Eastman chromatogram sheet type K301R2

Solvent

1-Butanol-acetic acid-water (3:1:1)

*Detection*A. Bioautography vs. *Mycobacterium* 607

B. UV light

C. Spray sheet with 3% ferric chloride

R_F

0.25

*Ref.*H. Ogawara, K. Maeda and H. Umezawa, *Biochemistry*, 7 (1968) 3296-3302**PYRROLE ANTIBIOTIC
(from marine bacterium)****TLC***Medium*

Silica gel G

Solvent

Chloroform

Detection

A. Iodine vapor

B. Conc. sulfuric acid

R_F

0.50

*Ref.*P.R. Burkholder, R.M. Pfister and F.H. Leitz, *Appl. Microbiol.*, 14 (1966) 649-653**PYRROLNITRIN****PC***Solvents*

A. 50% Acetone

B. 1% Ammonium chloride

C. *n*-Butanol-methanol-water (4:1:2)D. *n*-Butanol-methanol-water satd. with methyl orange (4:1:2)E. *n*-Butanol-acetic acid-water (2:1:1)

F. Benzene-methanol (8:2)

G. *n*-Butanol satd. with water

H. Water

R_F (estimated from drawing)

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.90	E	0.90
B	0.00	F	0.85
C	0.95	G	0.90
D	0.90	H	0.00

*Ref.*K. Arima, H. Imanaka, M. Jousaka, A. Fukuda and G. Tamura, *J. Antibiot.*, 18 (1965) 201-204**TLC 1***Solvents*

A. Chloroform

B. Benzene

C. Benzene-*n*-hexane (1:1)*R_F* (estimated from drawing)

Solvent A 0.70

Solvent B 0.60

Solvent C 0.25

Ref.

As PC

TLC 2*Solvent*

Benzene

Detection

Purplish green spot with sulfuric acid spray

R_F

0.75

*Ref.*D.H. Lively, M. Gorman, M.E. Haney and J.A. Mabe, *Antimicrob. Agents Chemother.*, 1966 (1967) 462-469**TLC 3***Medium*

Silica gel G

Solvent

Benzene-Skellysolve F (3:7)

R_F

Component A 0.31

Component B 0.37 (fluoropyrrolnitrin)

*Ref.*M. Gorman, M.E. Haney, Jr., D.H. Lively and J.D. Davenport, *U.S. Patent* 3,590,051, June 29, 1971

GLC 1 (Pyrrolnitrin and derivatives)

Apparatus

F and M model 402 gas chromatograph

Column

Glass, U-shaped, 120 × 0.3 cm I.D.

- A. Packed with 3.8 UCC-W98 [silicone gum rubber (methyl vinyl)] coated on 80–100 mesh Diatoport S
- B. 3% OV-1 coated on 100–200 mesh Gas-Chrome Q

Temperatures

- A. 230°C for flash heater, 180°C for column oven, 210°C for flame detector
- B. 240°C for flash heater, 200°C for column oven, 210°C for flame detector
- C. 240°C for flash heater, 180°C for column oven, 200°C for flame detector

Carrier gas

Helium, 70 ml/min

Solvent

Ethyl acetate

Chromatography

Injection, 1 µg

Relative retention time

Compound	Column-Temperature		
	A-A	A-B	B-C
Aminopyrrolnitrin	0.52	0.75	0.76
Isopyrrolnitrin		0.86	
Pyrrolnitrin	1.00	1.00	1.00
2-Chloropyrrolnitrin	1.50	1.38	1.47
Oxypyrrolnitrin		1.68	
3'-Methyl-3'-dechloro-pyrrolnitrin	0.66		
4'-Fluoropyrrolnitrin	0.86		
3'-Fluoro-3'-dechloro-pyrrolnitrin	0.50		
4'-Trifluoromethyl-3'-dechloroamino-pyrrolnitrin	0.23		

Ref.

R.L. Hamill, H.R. Sullivan and M. Gorman, *Appl. Microbiol.*, 18 (1969) 310–312

GLC 2

Apparatus

F and M model 402 gas chromatograph

Column

4 ft. × 0.25 in. glass column of 3.8% UC-W 98 on Diatoport S

Temperatures

Inlet temperature, 240°C; column temperature, 200°C

Detection

The eluate from the column is split (1:1) and passed through a flame ionization detector (220°C) and an electron-capture detector (210°C). The resulting signals are recorded separately

Solvent

Compounds are run at a conc. of 1 mg/ml in ethyl acetate. Samples of 1 µl are injected

Chromatography

Substances are eluted in the following order: amino derivative, isopyrrolnitrin, pyrrolnitrin, 2-chloropyrrolnitrin and oxypyrrolnitrin

Ref.

R. Hamill, R. Elander, J. Mabe and M. Gorman, *Antimicrob. Agents Chemother.*, 1967 (1968) 388–396

QUINOMYCINS

PC

Solvent

Di-n-Butyl ether-sym.-tetrachloroethane–10% o-cresotinate (2:1:3)

Detection

Bioautography vs. *Bacillus subtilis*

R_F

Quinomycin A 0.13
Quinomycin C 0.59

Ref.

T. Yoshida and K. Katagiri, *J. Antibiot.*, 14 (1961) 330–334

TLC 1 (Circular TLC)

Medium

Aluminum oxide GF₂₅₄ (Merck)

Solvent

Ethyl acetate–tetrachloroethane–water (3:1:3), lower phase

R_F

1. Quinomycin C > B₀ > A
2. Quinomycin C > E > B > D > A

Ref.

H. Otsuka and J.-I. Shoji, *J. Antibiot.*, 19 (1966) 128–131

TLC 2

Medium

Silica gel G (Merck)

Solvent

Methyl ethyl ketone

Detection

UV light

R_F

Quinomycin A	0.34 (UV absorbing)
NX Quinomycin A	0.52 (fluorescent)
QN Quinomycin A	0.70 (fluorescent)

Ref.

T. Yoshida, Y. Kimura and K. Katagiri, *J. Anti-biot.*, 21 (1968) 465–467

QUINTOMYCIN A, B and D**PC****Paper**

Toyo No. 51A

Solvent

Methanol–3% aq. sodium chloride (2:1)

R_F

Quintomycin A	0.25
Quintomycin B	0.27
Quintomycin D	Not given

Ref.

As Gentamicins, PC 14

TLC**Medium**

Alumina

Solvent

Chloroform–methanol–17% aq. ammonia
(2:1:1), upper layer

R_F

Quintomycin A	0.23
Quintomycin B	0.50
Quintomycin D	0.75

Ref.

As Gentamicins, PC 14

RABELOMYCIN**TLC****Medium**

Silica gel

Solvents

- A. Chloroform–methanol–piperidine (94:5:1)
B. Benzene–methanol (9:1)

R_F

Solvent A	0.4
Solvent B	0.5

Ref.

W.C. Liu, W.L. Parker, D.S. Slusarchyk, G.L. Greenwood, S.F. Graham and E. Meyers, *J. Antibiot.*, 23 (1970) 437–441

RACEMOMYCIN A SULFATE**PC 1****Paper**

Whatman No. 1, descending, 24°C for 48 h

Solvent

n-Propanol–pyridine–acetic acid–water
(30:20:6:20)

Detection

- A. Bioautography vs. *Sarcina lutea*
B. Ninhydrin

R_F

Detection A: 21–26 cm from origin
Detection B: 24 cm from origin

Ref.

S.N. Sehgal, K. Singh and C. Vezina, *U.S. Patent* 3,911,112, Oct. 7, 1975

PC 2**Paper**

Whatman No. 1, circular

Solvent

n-Propanol–pyridine–acetic acid–water
(30:20:6:24)

Detection

Ninhydrin

R_F

0.44–0.46

Ref.

As PC 1

RACEMOMYCINS**PC 1****Paper**

Toyo-Roshi No. 51, UH-type; circular development

Solvent

n-Butanol–pyridine–acetic acid–water–*tert.*-
butanol (15:10:3:12:4)

Detection

- A. Bioautography vs. *Bacillus subtilis*
B. Ninhydrin

R_F

Racemomycin A	0.33
Racemomycin B	0.18
Racemomycin C	0.24
Racemomycin D	0.11

Ref.

H. Taniyama, Y. Sawada and T. Kitagawa, *J. Chromatogr.*, 56 (1971) 360–362

PC 2**Solvent**

n-Propanol–pyridine–acetic acid–water
(15:10:3:12)

R_F

0.38

Ref.

K. Arima, T. Kawamura and T. Beppu, *J. Antibiot.*, 25 (1972) 387–392

PC 3*Papers*

A. Toyo Roshi No. 51, ascending

B. Toyo Roshi No. 51 UH

Solvents

A. *n*-Butanol–pyridine–acetic acid–water–*tert*-butanol (15:10:3:12:4)

B. *n*-Propanol–pyridine–acetic acid–water (15:10:3:12)

R_F

	Racemomycin	Medium	Solvent	
			A	B
H	A	B	0.23	0.53
			0.44	0.73
I	A	B	–	0.44
			–	–
J	A	B	–	0.35
			–	–
K	A	B	–	0.28
			–	–

Ref.

Y. Sawada and H. Taniyama, *J. Antibiot.*, 27 (1974) 535–543

TLC 1*Medium*

Avicel SF (Funakoshi Co.)

Solvent

n-Propanol–pyridine–acetic acid–water (15:10:3:12)

R_F

Racemomycin H 0.50

Racemomycin I –

Racemomycin J –

Racemomycin K –

Ref.

As PC 3

TLC 2*Media*

A. Silica gel G

B. Cellulose MN 300

C. Avicel SF

Solvents

A. *n*-Propanol–pyridine–acetic acid–water (15:10:3:12)

B. Wet *n*-butanol containing 2.0% (w/v) *p*-toluenesulfonic acid

C. Chloroform–methanol–17% aq. ammonia (2:1:1) (upper layer)

R_F

Solvent	Medium		
	A	B	C
A			0.25
B		0.20	
C	0.36		

Ref.

As PC 2

**RADIOACTIVE PENICILLINS
(cf., Penicillins, PC 24)****RAMIHYPHINS****TLC***Medium*

Silica gel (Silufol)

Solvent

Ethyl acetate, three-fold elution

Detection

Bioautography vs. *Neurospora sitiphila*

R_F

Compound	Relative mobilities (<i>R</i> _{ramihyphin A})
A	1.0
B	0.76
C	0.65
D	0.28
E	0.19

Ref.

Z. Barath, H. Barathova, V. Betina and P. Nemec, *Folia Microbiologica*, 19 (1974) 507–511

RAMNACIN**PC***Solvent*

n-Butanol–acetic acid–water (4:1:5)

Detection

Bioautography

R_F
0.92

Ref.
K. Ahmad and M.F. Islam, *Nature*, 176 (1955)
646-647

RAPAMYCIN

TLC

Medium

Silica gel (E. Merck)

Solvents

- A. Diethyl ether-hexane (40:60)
- B. Isopropyl alcohol-benzene (15:85)
- C. Ethanol-benzene (20:80)

R_F
Solvent A 0.42
Solvent B 0.5
Solvent C 0.43

Ref.
German Patent 2,347,682, Sept. 29, 1972
S.N. Sehgal, T.M. Blazekovic and C. Vezina, *U.S. Patent* 3,929,992, Dec. 30, 1975

RELOMYCIN

PC

Paper

Whatman No. 1; 0.5 in. wide strips

Solvent

Isopropyl ether-methyl isobutyl ketone-2% aq.
ammonium carbonate (2:1:2)

Detection

Bioautography vs. *Bacillus subtilis* at pH 7.9

R_F
0.24

Ref.
H.A. Whaley, E.L. Patterson, A.C. Dornbush,
E.J. Backus and N. Bohonus, *Antimicrob. Agents Chemother.*, 1963 (1964) 45-48

REQUINOMYCIN

TLC

Medium

Silicic acid

Solvents

- A. *n*-Butanol-pyridine-water (3:2:1.3)
- B. *n*-Butanol-pyridine-benzene-water (5:3:1:3)
- C. Benzene-methanol (1:1)

R_F
Solvent A 0.77
Solvent B 0.89
Solvent C 0.59

Ref.

M. Hori, K. Takemoto, I. Honma, T. Takeuchi,
S. Kondo, M. Hamada, T. Okazaki, Y. Okami
and H. Umezawa, *J. Antibiot.*, 24 (1972) 393-399

ELPHO

Medium

Paper

Buffer

Formic acid-acetic acid-water (25:75:900) pH
1.8

Conditions

3300 V for 20 min

Mobility

R_M 0.5 (α -alanine = 1.0); moved toward cathode

Ref.

As TLC

RESISTAPHYLLIN

TLC

Medium

Silica gel

Solvents

- A. Ethyl acetate
- B. Ethyl acetate-benzene (1:1)
- C. Ethyl acetate-methanol (10:1)
- D. Chloroform
- E. Acetone

Detection

Bioautography vs. *Bacillus subtilis*

R_F

Solvent	R_F	Solvent	R_F
A	0.45	D	0.00
B	0.00	E	1.00
C	0.90		

Ref.

S. Aizawa, J. Nagatus, M. Shibuya, H. Sugawara,
C. Hirose and S. Shirato, *172nd Meeting Japan Antibiotics Assn. April 4, 1970*

RHODOMYCINS

PC

Paper

Circular paper chromatography

Solvent

Butanol- $\frac{1}{15}$ M phosphate buffer, pH 5.8

Detection

Color

R_F

Rhodomycin B > iso-rhodomycin B >
rhodomycin A > iso-rhodomycin A

Ref.

H. Brockmann and P. Patt, *Chem. Ber.*, 88 (1955)
1455–1468

RIBOSTAMYCIN

PC 1

Paper

Ascending

Solvents

- A. 20% Aq. soln. of ammonium chloride
- B. Water satd. *n*-butanol
- C. *n*-Butanol–acetic acid–water (3:1:1)
- D. Water satd. *n*-butanol + 2% (w/v) *p*-toluene-sulfonic acid + 2% (v/v) piperidine
- E. Chloroform–methanol–17% aq. ammonia (2:1:1) lower layer

 R_F

Solvent	R_F	Solvent	R_F
A	0.98	D	0.01
B	0.01	E	0.00
C	0.03		

Ref.

As Apramycin, PC 2

PC 2

Paper

Whatman No. 1

Solvents

- A. Methyl ethyl ketone–*tert*.-butanol–methanol–6.5 *N* ammonia (16:3:1:6)
- B. 2% *p*-Toluenesulfonic acid in water satd. 1-butanol

 R_F

Solvent	R_{rel} •
A	1.35
B	0.85

• R_{rel} = Relative to Neomycin B

Ref.

H. Baud, A. Betencourt, M. Peyre and L. Penassa,
J. Antibiot., 30 (1977) 720–723

PC 3

Solvent

n-Butanol–pyridine–acetic acid–water (6:4:1:3)

 R_F

Compound	$R_{ribostamycin}$
1-N-[(<i>SR</i>)- α -Hydroxy- β -amino- <i>n</i> -propionyl]-ribostamycin	0.43
1-N-[(<i>S</i>)- α -Hydroxy- β -amino- <i>n</i> -propionyl]-ribostamycin	0.43
1-N-[(<i>S</i>)- α -Hydroxy- γ -amino- <i>n</i> -butyryl]-ribostamycin	0.47
1-N-[(<i>S</i>)- α -Hydroxy- δ -amino- <i>n</i> -valeryl]-ribostamycin	0.47
1-N-[(<i>S</i>)- α -Hydroxy- ϵ -amino- <i>n</i> -caproyl]-ribostamycin	0.50
1-N-[(<i>S</i>)- α , γ -Diamino- <i>n</i> -butyryl]-ribostamycin	0.61
1-N-[(<i>S</i>)- α -N-Acetyl- α , γ -diamino- <i>n</i> -butyryl]-ribostamycin	0.59

Ref.

As Neomycin A, PC 2

TLC 1

Medium

Cellulose

Solvent

Methyl ethyl ketone–methanol–isopropanol–
7.9 *N* ammonium hydroxide (10:8.5:3:7)

Detection

Ninhydrin. Spray with 0.3% soln. of ninhydrin in
water satd. *tert*.-butanol, heat at 100–105° for
3–5 min

 R_F

0.51

Ref.

As Kanamycins, TLC 5

TLC 2

Medium

Silica gel

Solvents

- A. Chloroform–methanol–17% aq. ammonia (2:1:1), upper layer
- B. Methanol–10% aq. ammonium acetate (1:1)
- C. *n*-Butanol–ethanol–chloroform–17% aq. ammonia (4:5:2:5)

 R_F

Solvent A 0.75

Solvent B 0.72

Solvent C 0.12

Ref.

As Apramycin, PC 2

TLC 3

Medium

Silica gel

Solvents

A. Chloroform-methanol-28% ammonia (1:3:2)

B. Chloroform-methanol-28% ammonia-water (1:4:2:1)

C. Chloroform-methanol-17% ammonia (2:1:1), upper phase

R_F

Solvent A 0.17

Solvent B 0.33

Solvent C 0.59

Ref.

T. Fujiwara, T. Tanimoto, K. Matsumoto and E.

Kondo, *J. Antibiot.*, 31 (1978) 966-969

RIBOSTAMYCIN, 3-N-CARBOXY-METHYL DERIVATIVE

PC 1

Paper

Toyo No. 50

Solvent

n-Propanol-pyridine-acetic acid-water (15:10:3:12)

Detection

Ninhydrin

R_F

0.82 (R_M : purple spot)

Ref.

M. Kojima, S. Inouye and T. Niida, *J. Antibiot.*, 28 (1975) 48-55

PC 2

Paper

Toyo No. 50

Solvent

Butanol-pyridine-acetic acid-water (6:4:1:3), 5 days

Detection

A. Bioautography vs. *Bacillus subtilis*

B. Ninhydrin

R_F

Moved 10 cm from origin; estimated from drawing

Ref.

As PC 1

TLC

Medium

Silica gel

Solvent

Chloroform-methanol-4% ammonium hydroxide (2:1:1)

Detection

Ninhydrin

R_F

0.66

Ref.

As PC 1

RIFAMPICINS (cf., rifamycins)

TLC

Medium

Kieselgel H treated with phosphate buffer pH 6.0, at 105°C for 1 h

Solvent

Chloroform-cyclohexane (95:5)

R_F (estimated from drawing)

Rifampicin 0.65

Desacetyl-rifampicin 0.45

4-N-Desmethyl-rifampicin 0.22

Ref.

K. Winsel, H. Iwainsky, E. Werner and H. Eule, *Pharmazie*, 31 (1976) 95-99

RIFAMYCINS

PC 1

Paper

Whatman No. 1. Paper dipped in aq. buffered phase and dried

Solvents

A. *n*-Amyl alcohol-*n*-butanol (9:1) satd. with phosphate buffer, pH 8.6

B. As A, but 0.1% sodium ascorbate added

Detection

Bioautography vs. *Sarcina lutea*, pH 5.9

R_F

Rifamycin	Solvent A	Solvent B
B	0.25, 0.73	0.40
O	0.25, 0.73	0.40
S	0.73	0.87
SV	0.73	0.87

Ref.

P. Sensi, C. Coronelli and B.J.R. Nicolaus, *J. Chromatogr.*, 5 (1961) 519-525

PC 2

Paper

As PC 1

Solvents

- A. Phosphate buffer, pH 7.3, containing 0.1% sodium ascorbate satd. with *n*-amyl alcohol-*n*-butanol (9:1)
 B. *n*-Butanol satd. with phosphate buffer, pH 7.3, containing 0.1% sodium ascorbate
 C. Phosphate buffer, pH 8.6, containing 0.1% sodium ascorbate satd. with *n*-butanol

Detection

As PC 1

 R_F

Rifamycin	Solvent		
	A	B	C
B	0.67	0.75	0.86
SV	0.51	0.95	0.67

Ref.

As PC 1

PC 3

Paper

Whatman No. 1

Solvent

Water containing 3% ammonium chloride + 1% ascorbic acid

Detection

Bioautography vs. *Sarcina lutea* R_F (estimated from drawing)

Rifamycin	R_F
A	0.05
B	0.25
C	0.45
D	0.60
E	0.75

Ref.

P. Sensi, A.M. Greco and R. Ballotta, *Antibiot. Ann.*, (1959-1960) 262-270

PC 4

Paper

Whatman No. 1

Solvents

- A. Water containing 3% ammonium chloride + 1% ascorbic acid
 B. Butanol satd. with water

C. Water satd. with butanol

D. Butanol-acetic acid-water (4:1:5)

E. Butanol-acetic acid-ethanol-water (25:3:25:47)

F. Acetone-water (1:1)

G. Chloroform-cyclohexane-water (8:1:2)

Detection

As PC 3

 R_F

Rifamycin B

Solvent	R_F	Solvent	R_F
A	0.25	E	0.85
B	0.87	F	0.88
C	0.87	G	0.92
D	0.95		

Ref.

As PC 3

PC 5 (Centrifugal circular chromatography)

Paper

As PC 1

Solvent

As PC 1, A. Solvent flow aimed 2 cm from center of paper rotating at 1500 rpm; about 4 min

Detection

As PC 1

 R_F

Rifamycin B can be separated from rifamycin SV

Ref.

As PC 1

PC 6

Paper

Reversed-phase paper partition chromatography. Paper impregnated with *sec*-octyl alcohol

Solvent

$\frac{1}{15}$ M Phosphate buffer, pH 8.6, with or without addition of sodium ascorbate.

Detection

Color

 R_F

Rifamycin	Color	R_F
S	Violet	0.04
O	Yellow	0.08
SV	Brown	0.50
B	Yellow	0.80

Ref.

S. Sferruzza and R. Rangone, *Il Farmaco, Ed. Pr.*, 19 (1964) 486–490

PC 7*Paper*

Whatman No. 1

Solvent

n-Amyl alcohol–*n*-butanol (9:1), satd. with phosphate buffer, pH 8.6

Detection

Bioautography vs. *Staphylococcus aureus* ATCC 6538P

R_F

Component	<i>R_F</i>
Rifamycin B	0.40
Rifamycin O	0.40
Rifamycin S	0.87
Rifamycin SV	0.87

Ref.

M.J. Weinstein, G.H. Wagman, M.G. Patel and J.A. Marquez, *U.S. Patent* 3,901,764, Aug. 26, 1975

PC 8*Paper*

Phosphate treated paper, pH 8.6

Solvent

Amyl alcohol–*n*-butanol, 9:1 in normal and reducing (0.1% ascorbic acid) systems

R_F

Rifamycin SV, not given

Ref.

J. Birner, P.R. Hodgson, W.R. Lane and E.H. Baxter, *J. Antibiot.*, 25 (1972) 356–359

TLC 1*Medium*

Silica gel G. Plates dried at 105–110°C for 30 min and cooled in a desiccator

Solvent

Acetone

Detection

Color. Rifamycins B, O and SV are yellow; rifamycin S is red–violet

R_F

Rifamycin SV > O = S > B. Mixture of O and S can be detected by color difference

Ref.

As PC 1

TLC 2*Medium*

Silica gel G

Solvent

Chloroform–ethanol (2:1)

Detection

Color (yellow–orange spot)

R_F

Rifampicin [3-(4-methyl-piperazinylimino-methyl)rifamycin SV]

Ref.

N. Maggi, C.R. Pasqualucci, R. Ballotta and P. Sensi, *Chemotherapia*, 11 (1966) 285–292

TLC 3*Medium*

Silica gel

Solvent

Chloroform–acetone (6:4)

R_F

0.51

Ref.

H. Bickel and B. Fechtig, *U.S. Patent* 3,644,337, Feb. 22, 1972

TLC 4*Medium*

Eastman Chromagram Sheet No. 6060

Solvent

Chloroform–ethanol–0.1 *N* hydrochloric acid (84:15.9:0.1)

Detection

The components are identified by their individual colors

R_F

0.55

Ref.

O.T. Kolos and L.L. Eidus, *J. Chromatogr.*, 68 (1972) 294–295

TLC 5*Medium*

Silica gel *.*.*

Solvents

A. Acetone *

B. Acetone–chloroform (4:1) **

* Silica (Merck)

** Silica-Avicel (microcrystalline cellulose, technical grade)

R_F

Rifamycin SV, *R_F* not given

Ref.

As PC 8

TLC 6

Medium

Silica gel 60 F

Solvent

Chloroform-methanol-water (80:20:2.5). The plate was equilibrated in a filter paper-lined chromatographic chamber which had been satd. with solvent vapor for 1 h prior to use. The plate was developed to a height of 15 cm (ca. 45 min), removed from the chamber and allowed to dry at room temperature

Detection

A. The rifamycins appear as colored spots

B. Visualization may also be obtained by viewing the plate under short-wavelength ultraviolet light

 R_F

Compound	R_F *	Spot color
Rifamycin O	0.83	Yellow
Rifamycin S	0.81	Brown
Rifampin quinone	0.75	Purple
25-Desacetyl-rifampin quinone	0.66	Purple
Rifampin	0.60	Orange
25-Desacetyl-rifampin	0.41	Orange
3-Formylrifamycin SV	0.27	Pink
Rifamycin B	0.15	Yellow
Rifampin N-oxide	0.09	Yellow

* Average of 5 plates

Ref.

W.L. Wilson, K.C. Graham and M.J. LeBelle, *J. Chromatogr.*, 144 (1977) 270-274

TLC 7

Solvent

Ethyl acetate-tetrahydrofuran-water (4:1:5)

Detection

Bioautography vs. *Staphylococcus aureus* R_F

Separates rifamycins S and SV

Ref.

German Patent 2,500,898, July 17, 1975

TLC 8

Medium

Silica gel (Merck)

Solvents

A. Benzene-methanol-ethyl acetate (13:2:2)

B. Benzene-methanol-ethyl acetate (13:4:2)

C. Chloroform-methanol (9:1)

 R_F

Compound	Solvent	R_F
3-Azido rifamycin S	A	0.65
3-Amino rifamycin S	A	0.51
3-Amino rifamycin SV	B	0.11
3-Amino-25-desacetyl-rifamycin S	C	0.65

Ref.

L. Marsili, V. Rossetti, and C. Pasqualucci, *U.S. Patent* 4,007,169, Feb. 8, 1977

TLC 9

Medium

Silica gel 60 F₂₅₄ (Merck)

Solvents

A. Chloroform-methyl alcohol (95:5)

B. Chloroform-methanol (1:1)

 R_F

Rifamycin	Solvent A	Solvent B
A	0.59	
U		0.63
P		0.57
Q		0.32

Ref.

Belgian Patent 832,921, Dec. 17, 1975

TLC 10

Medium

Silanized silica gel HF₂₅₄ (Merck), (reversed-phase) R_F

Component	Solvent			
	A	B	C	D
Rifampicin	0.13	0.14	0.40	0.13
Rifamycin SV	0.27	0.20	0.36	0.08
Rifamycin B diethylamide	0.27	0.25	0.45	0.18
Rifamycin B	0.56	0.54	0.61	0.40

Solvents

A. Buffer, pH 7-dioxane (6:4)

B. Buffer, pH 7-acetone (6:4)

C. Buffer, pH 7-tetrahydrofuran (6:4)

D. Buffer, pH 7, satd. with methyl ethyl ketone 25%

Mobile phase

Detection

Visible color

Ref.R. Rangone and C. Ambrosio, *Il Farmaco (Ed. Pr.)*, 26 (1971) 237–250**TLC 11****Medium**

Silica gel, Soviet KSK No. 2, impregnated with acetate buffer, pH 4.6, affixed layer

Solvent

Chloroform–methanol (100:5)

R_F

Compound	<i>R_s</i> *	Color
Rifamycin O	5.3	Yellow
Rifamycin S	3.8	Violet
3- <i>tert.</i> -Butylimino-methylrifamycin S	4.5	Orange
3- <i>tert.</i> -Butylimino-methylrifamycin SV	4.1	Blue
3-Formylrifamycin SV	1.0	Brown

* The *R_s* values are averages from five determinations. The reference substance used was 3-formylrifamycin SV

Ref.N.B. Dzegilenko, R.A. Vladimirskaia and V.B. Korchagin, *Antibiotiki*, 3 (1977) 206–208**CCD****Solvent**Methanol–0.01 *N* hydrochloric acid–benzene–light petroleum (10:5:15:5), 100 transfers**Distribution**

Rifamycin	Tube No.
E	1– 10
D	40– 55
C	60– 75
A	90–100

Ref.

As PC 3

RIMOCIDIN**PC****Paper**

Whatman No. 1

SolventWater satd. *n*-butanol, descending, 18 h**Detection**Bioautography vs. *Saccharomyces carlsbergensis***R_F**

0.38

Ref.J. Burns and D.F. Holtman, *Antibiot. Chemother.*, 9 (1959) 398–405**PC 2****Paper**

As chromin, PC

Solvent

As chromin, PC

Detection

As chromin, PC

R_F

0.55

Ref.

As chromin, PC

TLC**Medium**

Silica gel G

Solvents

A. 1-Butanol–ethanol–water (1:1:1)

B. 1-Butanol–ethanol–water (4:1:5), upper layer

Detection

A. Ninhydrin

B. UV light

C. 2,4-Dinitrophenylhydrazine (DNPH)–2 *N* hydrochloric acid spray

D. Sulfuric acid spray

Detection	Spot color
A	Violet
B	–
C	Orange–yellow
D	Black

R_F

Solvent A 0.70

Solvent B 0.37

Ref.R.C. Pandey and K.L. Rinehart, Jr., *J. Antibiot.*, 30 (1977) 146–157**RINAMYCIN****PC 1****Solvents**

A. 20% Acetone

B. Water satd. *n*-butanol

Detection

Bioautography vs. *Bacillus subtilis*

R_F

Solvent A 0.54

Solvent B 0.46

Ref.

As Azalomycin F, PC

PC 2**Solvents**

A. Water satd. *n*-butanol

B. 20% Ammonium chloride

C. Acetone–water (1:1)

D. Benzene–methanol (4:1)

E. *n*-Butanol–methanol–water (4:1:2)

F. Ethyl acetate–pyridine–water (2:1:2), upper phase

G. *n*-Butanol–acetic acid–water (4:1:2)

Detection

Bioautography vs. *Pseudomonas saccharophila*

R_F

Solvent	R _F	Solvent	R _F
A	0.41	E	0.67
B	0.00	F	0.38
C	0.87	G	0.76
D	0.16		

Ref.

As Azalomycin F, PC

TLCL**Medium**

Silica gel

Solvents

A. *n*-Butanol

B. Ethyl acetate–pyridine–water (2:1:2), upper phase

C. Ethyl acetate–methanol (1:1)

Detection

Bioautography vs. *Pseudomonas saccharophila*

R_F

Solvent A 0.00

Solvent B 0.33

Solvent C 0.48

Ref.

As Azalomycin F, PC

ELPHO**Medium**

Paper

Buffers

A. $\frac{1}{150}$ M Acetate buffer, pH 4.1

B. $\frac{1}{10}$ M Phosphate buffer, pH 7.0

C. $\frac{1}{10}$ M Veronal buffer, pH 8.6

Conditions

250 V/19 cm, 5 cm, 3 h

Detection

Bioautography vs. *Pseudomonas saccharophila*

Mobility

Buffer A 0.0 mm

Buffer B 0.0 mm •

Buffer C (+) 5 mm •

• Run for 3 and 10 h

Ref.

As Azalomycin F, PC

RISTOCETIN**PC 1****Paper**

Eaton-Dikeman No. 613

Solvents

A. *n*-Butanol satd. with water; equilibrate 3 h, descending 16–17 h

B. As A + 2% *p*-toluenesulfonic acid, 16–17 h

C. As B + 2% piperidine, 16–17 h

D. Methyl isobutyl ketone satd. with water; no equilibration, 3 h

E. As D + 2% *p*-toluenesulfonic acid, 3 h

F. As D + 2% piperidine (v/v), 3 h

G. Water satd. with methyl isobutyl ketone; no equilibration, 3 h

H. As G + 1% *p*-toluenesulfonic acid, 3 h

I. As G + 1% piperidine (v/v), 3 h

J. Water–methanol–acetone, 3:1 (3:1)

Adjust to pH 10.5 with ammonium hydroxide then back to pH 7.5 with phosphoric acid; no equilibration, 3 h

K. Methanol–water (80:20) + 1.5% sodium chloride. Paper buffered with soln. containing 0.95 M sodium sulfate + 0.05 M sodium bisulfate; equilibration 3 h; develop 16–17 h

L. Amyl acetate satd. with 0.1 M potassium phosphate buffer, pH 6.15; equilibration 3 h; develop 16–17 h

M. *n*-Butanol–methanol–water (40:10:20)

Add excess methyl orange (ca. 1.5 g) and let stand at 28°C. Separate from insolubles; equilibration 3 h; develop 16–17 h

All solvents run ascending except A

Detection

Bioautography vs. *Bacillus subtilis*

Ref.

J.E. Philip and J.R. Schenck, *U.S. Patent* 2,990,329, June 27, 1961

R_F

Solvent	R_F	Solvent	R_F
A	0.00	H	0.68
B	0.05	I	0.90
C	0.00	J	0.25
D	0.00	K	0.40
E	0.00	L	0.00
F	0.00	M	0.44
G	0.05		

PC 2*Paper*

Whatman No. 1

*Solvents*A. Pyridine-sym.-collidine-sec.-butanol-water
(2:2:1:1)

B. As PC 1, K

Both solvents run descending

*Detection*Bioautography vs. *Bacillus subtilis* or *Corynebacterium xerosis* R_F

Solvent A	0.85
Solvent B Ristocetin A	0.40
Ristocetin B	0.15

*Ref.*M.P. Kunstmann, L.A. Mitscher, J.N. Porter,
A.J. Shay and M.A. Darken, *Antimicrob. Agents
Chemother.*, 1968 (1969) 242-245**ROLITETRACYCLINE****TLC***Medium*

Avicel

Solvent

Methanol-isobutyl alcohol-chloroform-10% hydrochloric acid (10:10:10:1)

Detection

- A. Iodine vapor
B. 0.25% Stannous chloride soln.
C. 0.2% *p*-Dimethylaminobenzaldehyde soln.

 R_F

0.41

Ref.

As Ampicillin, TLC 4

RORIDINES**TLC 1***Medium*

As verrucarines, TLC 1, A, B

Solvent

As verrucarines, TLC 1, A, B

Detection

As verrucarines, TLC 1, A, B

 R_F

Roridine	R_F			Color	
	(Medium-Solvent)			Iodine	UV
	(A-A)	(B-A)	(B-B)		
A	0.70	0.18	0.21	Yellow brown	Dark
B	0.55	0.26	0.49	Dark green	Dark
C	—	—	0.41	Yellow brown	Dark

Ref.

As verrucarines, TLC 1

TLC 2*Medium*

As verrucarines, TLC 2

Solvent

As verrucarines, TLC 2, A, B, C

Detection

As verrucarines, TLC 2

 R_F

Roridine	Solvent		
	A	B	C
E	0.40	0.35	0.24
D	0.35	0.29	0.18
A	0.21	0.20	0.14

Ref.

As verrucarines, TLC 2

ROSARAMICIN**PC 1***Paper*

Toyo No. 51

Solvents

- A. 20% Ammonium chloride
B. Water satd. *n*-butanol
C. *n*-Butanol-acetic acid-water (3:1:1)
D. Water satd. ethyl acetate
E. Water satd. *n*-butanol + 2% *p*-toluenesulfonic acid + 2% piperidine

DetectionBioautography vs. *Bacillus subtilis* No. 10707 R_F

Solvent	R_F	Solvent	R_F
A	0.65	D	0.00, 0.90
B	0.85	E	0.90
C	0.78		

Ref.

As Carbomycin, PC 5

PC 2**Paper**

Whatman No. 1

Solvent*n*-Butanol–pyridine–acetic acid–water
(6:4:1:3), descending**Detection**

Radioactivity scan

 R_F (estimated from drawing)[¹⁴C]Methyl rosaramicin 0.5**Ref.**B.K. Lee, R.G. Condon, M. Patel, J.A. Marquez
and G.H. Wagman, *J. Appl. Bacteriol.*, 40 (1976)
217–221**TLC 1****Medium**

Silica gel

Solvent

Chloroform–methanol (3:2)

DetectionBioautography vs. *Sarcina lutea* R_F

0.48

Ref.G.H. Wagman, J.A. Waitz, J. Marquez, A.
Murawski, E.M. Oden, R.T. Testa and M.J.
Weinstein, *J. Antibiot.*, 25 (1972) 641–646**TLC 2****Medium**

Silica gel

Solvents

A. Chloroform–methanol–17% ammonia (2:1:1)

B. Butanol–acetic acid–water (3:1:1)

DetectionPlate heated at 100°C and sprayed with phos-
phoric acid in methanol (1:1) R_F

Solvent A 0.82, 0.98 tan

Solvent B 0.31, 0.37, 0.44 tan

Ref.

As TLC 1

TLC 3**Medium**

Silica gel

Solvent

Chloroform–methanol (4:1)

 R_F

0.51; two minor active components 0.16, 0.31

Ref.

As TLC 1

TLC 4**Medium**

Silica gel GF

Solvents

A. Chloroform–methanol–17% ammonia (2:1:1)

B. Butanol–acetic acid–water (3:1:1)

C. Chloroform–methanol (4:1)

D. Chloroform–methanol (3:2)

DetectionBioautography vs. *Sarcina lutea* R_F

Solvent A 0.98

Solvent B 0.37

Solvent C 0.75

Solvent D 0.48

Ref.M.J. Weinstein, G.H. Wagman and J.A. Marquez,
U.S. Patent 4,161,523, July 17, 1979**TLC 5****Medium**Silica gel precoated 60 F₂₅₄ (Merck)**Solvents**

A. Benzene–acetone (2:3)

B. Chloroform–methanol (10:1)

C. *n*-Butanol–acetic acid–water (3:1:1)**Detection**

Sulfuric acid; brownish yellow

 R_F

Solvent A 0.04

Solvent B 0.13

Solvent C 0.49

Ref.Y. Shimauchi, K. Kubo, K. Osumi, K. Okamura
and J. Lein, *J. Antibiot.*, 31 (1978) 270–275**TLC 6****Medium**

Silica gel

Solvent

Chloroform–methanol (4:1), ascending

Detection

Bioautography vs. *Bacillus subtilis* ATCC 6633

R_F

[¹⁴C]Methyl rosaramicin, 0.5

(estimated from drawing and photograph of bioautogram)

Ref.

As PC 2

HPLC**Column**

25 cm × 4.6 mm stainless steel; Partisil 10-ODS packing

Mobile phase

Acetonitrile–0.01 M acetate buffer, pH 4.0 (3:1)

Pressure

1500 p.s.i.

Flow-rate

1.3 ml/min

Detector

UV (254 nm); full scale 0.01 O.D.

Recorder

Full scale of 5 mV

Temperature

Ambient

Type

Reversed-phase

Retention time (min)

Rosaramicin N-oxide	8.5
Rosaramicin	16.0
20-Dihydrosaramicin	17.5
Desepoxyrosaramicin	19.0
9-Hydrodesepoxyrosaramicin	20.5
20-Hydroxyrosaramicin	22.5

Comments

Used for quantitative determination in serum; sensitivity of about 0.01 µg/ml

Ref.

C. Lin, H. Kim, D. Schuessler, E. Oden and S. Symchowicz, *Antimicrob. Agents Chemother.*, 18 (1980) 780–783

ROSEOFILAVIN**TLC****Medium**

Cellulose

Solvents

- A. *n*-Butanol–acetic acid–water–diethyl ether (8:4:4:3)
- B. Methyl ethyl ketone–isoamyl alcohol–acetic acid–water (40:40:7:13)
- C. *n*-Butanol–acetic acid–water (4:1:5), upper layer

D. *n*-Butanol–acetic acid–water (15:3:7)

E. *n*-Butanol–formic acid–water (4:1:1)

F. *n*-Butanol–formic acid–water–diethyl ether (77:10:13:15)

G. Benzyl alcohol–acetic acid–water (3:1:3), upper layer

H. *n*-Butanol–pyridine–water (6:4:3)

R_F (estimated from drawing)

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.24	E	0.30
B	0.05	F	0.05
C	0.30	G	0.73
D	0.33	H	0.40

Ref.

M. Takatsu, M. Nakano and S. Otani, 190th Scientific Meeting, JARA, November 22, 1973

ROSOXACIN**HPLC****Apparatus**

LDC Mini-pump, Model 709

Column

Whatman Partisil-PXS 10/25 PAC, 250 × 4.6 mm I.D., with a Corasil pre-column, 60 × 4 mm I.D.

Mobile phase

Redistilled acetonitrile–0.2 M phosphoric acid (92:8)

Flow-rate

2 ml/min

Temperature

20°

Pressure

1500 p.s.i.

Detection

Altex Model 153 ultraviolet detector with a 280-nm filter

Retention time (min)

Component:

Rosoxacin-N-oxide	5.4
Internal standard	7.5
Rosoxacin	12.0

Ref.

M.P. Kullberg, R. Koss, S. O'Neil and J. Edelson, *J. Chromatogr.*, 173 (1979) 155–163

RUBIDIN**PC****Solvents**

- A. Dioxane–water (1:1)

- B. Ethanol–water (1:1)
 C. Water
 D. Dioxane–water–benzene (12:2:3)
 E. *n*-Butanol– $\frac{1}{15}$ *M* phosphate buffer, pH 5.8 (circular chromatography)

 R_F

Solvent	R_F	Solvent	R_F
A	0.80	D	1.00
B	0.78	E	1.00
C	0.63		

Ref.

A.K. Banerjee, G.P. Sen and P. Nandi, *Antibiot. Ann.*, (1955–1956) 640–647

RUBIFLAVIN

PC

Paper

Whatman No. 1

Solvents

- A. 2 *N* Ammonium hydroxide–*tert*.-amyl alcohol (1:1)
 B. Ethyl acetate–ethanol–water (3:1:3)

 R_F

Solvent A 0.66 (tailing)
 Solvent B 0.90 (tailing)

Ref.

A. Aszalos, M. Jelinek and B. Berk, *Antimicrob. Agents Chemother.*, 1964 (1965) 68–74

RUBRADIRIN

PC

Paper

Schleicher and Schuell No. 589 blue ribbon

Solvent

Toluene–Skellysolve-C–methanol–0.1 *M* phosphate buffer, pH 7.0 (5:5:7:3) Equilibrated overnight; developed descending, 6 h

Detection

Bioautography vs. *Sarcina lutea* R_F

0.6–0.8

Ref.

B.K. Bhuyan, S.P. Owen and A. Dietz, *Antimicrob. Agents Chemother.*, 1964 (1965) 91–96

CCD

Solvent

Hexane–acetone–water (5:5:1), 200 transfers

Distribution

Pools made from tubes 84–95 and 96–113

Ref.

C.E. Meyer, *Antimicrob. Agents Chemother.*, 1964 (1965) 97–99

SAFYNOL

TLC

Medium

SilicAR (TLC-4GF, Mallinckrodt) 1 part, mixed with 40 parts Kieselgel (D5, A.H. Thomas)

Solvents

- A. Benzene–ethyl acetate–formic acid (75:24:1)
 B. Chloroform–acetone–formic acid (95:4:1)
 C. Diethyl ether–light petroleum–formic acid (80:19:1)

Detection

UV at 254 nm

 R_F

Solvent A 0.21
 Solvent B 0.17
 Solvent C 0.50

Ref.

C.A. Thomas and E.H. Allen, *Phytopath.*, 60 (1970) 261–263

SAGAMICIN (XK-62-2)

PC 1

Paper

Ascending

Solvent

Chloroform–methanol–17% aq. ammonia (2:1:1) lower layer (developed at room temp. for 12 h)

 R_F

0.49

Ref.

As Apramycin, PC 1

PC 2

Paper

Ascending

 R_F

Solvent	R_F	Solvent	R_F
A	0.98	D	0.00
B	0.00	E	0.03
C	0.06		

Solvents

- A. 20% (w/v) Ammonium chloride (3 h)
 B. Water satd. *n*-butanol (15 h)
 C. *n*-Butanol–acetic acid–water (3:1:1), (15 h)
 D. Water satd. ethyl acetate (4 h)

E. Water satd. *n*-butanol + 2% (w/v) *p*-toluene-sulfonic acid + 2% (v/v) piperidine (15 h)

Detection

Bioautography vs. *Bacillus subtilis* KY 4273

Ref.

T. Nara, I. Kawamoto, R. Okachi, S. Takasawa, M. Yamamoto, S. Sato, T. Sato and A. Morikawa, *J. Antibiot.*, 28 (1975) 21–28

N. Takashi, T. Seigo, O. Ryo, K. Isao, Y. Mitsuyoshi, S. Tomoyasu and M. Atsuko, *German Patent* 2,326,781, Dec. 13, 1973

PC 3

Paper

Whatman No. 1 (developed 4 h)

Solvent

Chloroform–methanol–9 *N* ammonium hydroxide (2:1:1), lower phase

Detection

Bioautography vs. *Staphylococcus aureus*

R_F

$R_{C_1} = 0.73$

$R_{C_1} = \frac{\text{distance moved by component from origin}}{\text{distance moved by gentamicin } C_1 \text{ from origin}}$

Ref.

P.J.L. Daniels, C. Luce, T.L. Nagabhushan, R.S. Jaret, D. Schumacher, H. Reimann and J. Ilavsky, *J. Antibiot.*, 28 (1975) 35–41

TLC 1

Media

- A. Silica gel (Quanta Gram)
- B. Cellulose

Solvents

- A. Propanol–pyridine–acetic acid–water (15:10:3:12)
- B. Pyridine–ethyl acetate–acetic acid–water (5:6:1:3)
- C. Methanol–chloroform–ammonia (1:1:1), lower phase

Detection

Ninhydrin with Medium A

R_F

Solvent	Medium A	Medium B
A		0.550
B		0.167
C	0.54	

Ref.

As Gentamicins, TLC 12

TLC 2

Medium

Kieselgel; chromatograms run at room temperature

Solvents

A. Chloroform–methanol–17% aq. ammonia (2:1:1)

B. 10% Ammonium acetate–methanol

R_F

Solvent A 0.86

Solvent B 0.21

Ref.

N. Takashi, T. Seigo, O. Ryo, K. Isao, Y. Mitsuyoshi, S. Tomoyasu and M. Atsuko, *German Patent* 2,326,781, Dec. 13, 1973

TLC 3

Medium

Silica gel GF (Analtech). Plates were activated by heating for 1 h at 100°C prior to use

Solvents

A. Chloroform–methanol–15 *N* ammonium hydroxide (1:1:1)

B. Chloroform–methanol–15 *N* ammonium hydroxide (1:2:1)

C. Chloroform–methanol–15 *N* ammonium hydroxide (2:1:1), lower phase

Detection

Sulfuric acid charring

R_F

Solvent A 0.40

Solvent B 0.55

Solvent C –

Ref.

As PC 2

TLC 4

Medium

Kieselgel

Solvent

Isopropanol–conc. ammonia–chloroform (4:1:1)

Detection

Ninhydrin

R_F

Compound	R_F
1-N-(α -Hydroxy- β -carboxyaminopropionyl)-XK-62-2	0.63
1-N-(α -Hydroxy- β -amino-propionyl)-XK-62-2	0.42

Ref.

German Patent 2,509,885, Sept. 11, 1975

SAGAMICIN (XK-62-2) [N-(2-hydroxy-3-aminobutyl) derivatives]

TLC

Medium

Silica gel

Solvent

Isopropanol–conc. ammonia–chloroform (2:1:1)

Detection

Ninhydrin

R_F

Derivatives	R_F
XK-62-2 IA	0.75
XK-62-2 IIA	0.81
XK-62-2 IIIA	0.86
XK-62-2 IVA	0.92

Ref.

Netherlands Patent 7,416,211, Dec. 12, 1974

SALINOMYCIN

PC

Solvents

- Water satd. with methyl isobutyl ketone
- Water satd. with methyl isobutyl ketone + 2% *p*-toluenesulfonic acid + 1% piperidine
- Benzene satd. with water
- Potassium phosphate–ethanol–water (17.4:30:1000, w/v/v)

Detection

Bioautography vs. *Bacillus subtilis*, pH 6.8

R_F

Solvent A	0.32
Solvent B	0.57
Solvent C	0.31
Solvent D	0.32

Ref.

As Narasin, PC

TLC

Media

- Silica gel
- Cellulose

Solvents

- Water satd. with methyl isobutyl ketone
- Water–methanol–acetone (12:3:1) adjusted to pH 10.3 with ammonium hydroxide, then to pH 7.5 with hydrochloric acid
- Ethyl acetate
- Ethyl acetate–diethylamine (95:5)

Detection

Bioautography vs. *Bacillus subtilis*, pH 6.8

R_F

Solvent	Medium A	Medium B
A		0.55
B		0.53
C	0.24	
D	0.43	

Ref.

As Narasin, PC

SARGANIN

PC

Paper

Whatman No. 1

Solvents

- 1-Butanol–water (84:16)
 - As A + 0.25% *p*-toluenesulfonic acid
 - 1-Butanol–acetic acid–water (2:1:1)
- All systems developed 22 h

Detection

Bioautography vs. *Escherichia coli*

R_F (estimated from drawing)

Solvent	R_F
A	0.80 (major) 0.90 (minor)
B	0.85 (major) 0.92 (minor)
C	0.92

Ref.

N.G.M. Nadal, L.V. Rodriguez and C. Casillas, *Antimicrob. Agents Chemother.*, 1964 (1965) 131–134

SCLEROTHRICIN

PC

Solvents

- Butanol–methanol–ammonium hydroxide–water (10:4:3:3)
- n*-Propanol–pyridine–acetic acid–water (15:10:3:10)

Detection

Bioautography vs. *Bacillus subtilis* PCI-219

R_F (estimated from drawing)

Solvent A	0.35
Solvent B	0.45

Ref.

Y. Kono, S. Makino, S. Takeuchi and H. Yonehara, *J. Antibiot.*, 22 (1969) 583–589

TLC 1**Medium**

Silica gel G

Solvent

Chloroform-methanol-14% ammonium hydroxide (2:1:1)

Detection

As PC

R_F (estimated from drawing)

0.8

Ref.

As PC

TLC 2**Medium**

Alumina

Solvent

Ethanol-water (4:6)

Detection

As PC

R_F (estimated from drawing)

0.35

Ref.

As PC

TLC 3**Medium**

Silica gel G

Solvent

Chloroform-methanol-17% aqueous ammonia (2:1:1) (upper layer)

R_F

0.78

Ref.K. Arima, T. Kawamura and T. Beppu, *J. Antibiot.*, 25 (1972) 387-392**SCOPAFUNGIN****PC****R_F (estimated from drawing)**

Solvent	R _F	Solvent	R _F
A	0.18-0.50	D	0.18-0.50
B	0.65	E	0.10-0.35
C	0.85	F	0.10-0.35

Solvents

- A. 1-Butanol-water (84:16), 16 h
 B. As A + 0.25% *p*-toluenesulfonic acid, 16 h
 C. 1-Butanol-acetic acid-water (2:1:1), 16 h
 D. As A + 2% piperidine, 16 h
 E. 1-Butanol-water (4:96), 5 h
 F. As E + 0.25% *p*-toluenesulfonic acid, 5 h

DetectionBioautography vs. *Saccharomyces cerevisiae***Ref.**L.E. Johnson and A. Dietz, *Appl. Microbiol.*, 22 (1971) 303-308**TLC****Medium**Silica gel HF₂₅₄ (E. Merck) suspended in a soln. of buffer composed of 0.2 M disodium phosphate-0.2 M potassium dihydrogen phosphate (1:1). Air dry, activate 2 h at 130°C prior to use**Solvent**

Methyl ethyl ketone-acetone-water (150:50:34)

Detection

Spray with freshly prepared mixture of anisaldehyde-95% ethanol-conc. sulfuric acid-glacial acetic acid (0.5:9:0.5:0.1). Heat at 90-100°C for 5-10 min. Scopafungin appears as a dark blue spot

R_F

0.23

Ref.M.E. Bergy and H. Hoeksema, *J. Antibiot.*, 25 (1972) 39-43**SENFOLOMYCINS****PC****Paper**

Whatman No. 1

Solvents

- A. *n*-Heptane-diethyl ketone-tetrahydrofuran-water (8:3:3:8)
 B. Chloroform-carbon tetrachloride-methanol-water (4:4:1:8)

DetectionBioautography vs. *Bacillus subtilis*, pH 6.0**R_F**

Solvent	Senfolomycin	
	A	B
A	0.75	0.54
B	0.47	0.15

Ref.L.A. Mitscher, W. McCrae, S.E. DeVoe, A.J. Shay, W.K. Hausmann and N. Bohonos, *Antimicrob. Agents Chemother.*, 1965 (1966) 828-831

SETOMIMYCIN**TLC****Medium**

Silica gel (Merck)

Solvents

A. Chloroform-methanol (5:1)

B. Benzene-acetone (4:1)

C. Benzene-ethyl acetate (1:3)

D. Benzene-methanol (4:1)

R_F

Solvent A 0.59

Solvent B 0.18

Solvent C 0.37

Solvent D 0.31

Ref.

S. Omura, H. Tanaka, Y. Iwai, K. Nishigaki, J. Awaya, Y. Takahashi and R. Masuma, *J. Antibiot.*, 31 (1978) 1091-1098

SHARTRESIN**PC****Paper**

Leningradskaya M

Solvents

A. Methanol-ammonium hydroxide-water (20:1:4)

B. Benzene-acetic acid-water (2:2:1)

C. Benzene-methanol-water (1:1:2)

D. *n*-Butanol-acetic acid-water (2:1:2)**R_F**

Solvent A 0.72

Solvent B 0.43

Solvent C 0.35

Solvent D 0.91

Ref.

D.Yu. Shenin, E.N. Sokolova and E.Yu. Konev, *Antibiotiki*, 15 (1970) 9-14

SHINCOMYCINS**PC****Solvents**

A. Wet butanol

B. 3% Aq. ammonium chloride

C. 80% Phenol

D. 50% Acetone

E. Butanol-methanol-water (4:1:2)

F. 1.5% Methyl orange

G. Benzene-methanol (4:1)

H. Water

I. Butanol satd. water

J. Pyridine-propanol-acetic acid-water (10:15:3:12)

R_F (estimated from drawing)

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	1.0	F	1.0
B	0.7	G	0.8
C	1.0	H	0.6
D	1.0	I	1.0
E	1.0	J	1.0

Ref.

N. Nishimura, K. Kumagai, N. Ishida, K. Saito, F. Kato and M. Azumi, *J. Antibiot.*, 18 (1965) 251-258

TLC**Medium**

Silica gel containing 5% calcium sulfate

Solvents

A. Methanol-benzene (45:55)

B. Chloroform-methanol (4:1)

C. Acetone

D. Butanol-acetic acid-water (3:1:1)

Detection

Spray with 10% sulfuric acid; heat at 120°C for 5 min. Shincomycins A and B give dark green and yellowish brown colors, respectively

R_F

Solvent	Shincomycin A	Shincomycin B
A	0.65	0.40
B	0.66	0.38
C	0.32	0.18
D	0.39	0.39

Ref.

N. Ishida, K. Kumagi and N. Nishimura, *U.S. Patent* 3,534,139, Oct. 13, 1970

ELPHO**Medium****Paper****Buffers**

A. pH 5.0

B. pH 8.0

Conditions

225 V/30 cm for 2 h

Mobility

Buffer A: Shincomycin A moves toward cathode

Buffer B: Shincomycin A moves slightly toward cathode

Ref.

As PC

SHOWDOMYCIN

PC 1

Solvent

n-Butanol–water–acetic acid (4:5:1, upper phase), descending

Detection

UV absorption

R_F

0.41

Ref.

S. Roy-Burman, P. Roy-Burman and D.W. Visser, *Cancer Res.*, 28 (1968) 1605–1610

PC 2

Paper

Whatman No. 3MM

Solvents

- A. *n*-Butanol–ethanol–water (50:15:35)
- B. *n*-Butanol–formic acid–water (77:10:13)
- C. *n*-Butanol–methanol–water (20:7:8)
- All solvents developed ascending, 15–18 h

Detection

- A. Bioautography vs. *Escherichia coli* K-12
- B. UV
- C. For analysis of [^{14}C]showdomycin, paper-grams are cut into 0.5–1.0 cm pieces and counted using a liquid scintillation spectrometer with a toluene phosphor soln.

R_F (estimated from drawing)

Solvent A 0.47

Solvent B 0.30

Solvent C 0.42

Ref.

Y. Komatsu, *J. Antibiot.*, 24 (1971) 566–571

SIBIROMYCIN

TLC

Medium

Silica gel

R_F

Solvents

A. Chloroform–methanol (5:1)

B. Ethyl acetate–*n*-butanol satd. with water (1:1)

Detection

Diazo reaction

R_F

Solvent A 0.5

Solvent B 0.45

Ref.

M.G. Brazhnikova, N.V. Konstantinova and A.S. Mesentsev, *J. Antibiot.*, 25 (1972) 668–673

SICCANIN

TLC

Medium

Eastman Chromagram Sheet 6061

Solvents

A. Benzene–acetone (10:1)

B. Benzene

C. *n*-Hexane–ethanol (9:1)

All solvents developed to a height of 15 cm (10–30 min)

Detection

Reagents:

- a. 0.5 g Benzidine dissolved in 1.4 ml conc. hydrochloric acid and 10 ml water, then made up to 100 ml
- b. 10% aq. Sodium nitrite
- c. Mix a–b–acetone (2:2:1) immediately before use
- d. 0.5 *N* Hydrochloric acid–acetone (1:1)

Procedure

Dry sheet in warm air stream dipping into reagent c for 20 sec to develop red color of diazo compound. Remove excess reagent by blotting with filter paper immediately followed by dipping into reagent d for 40 sec. Blot excess hydrochloric acid and dry in warm air stream. Colors are as indicated under section R_F . Quantitative determinations can be done by densitometric scan at 500–520 nm

	R_{siccanin} in solvent systems			Color	R_F in solvent systems		
	A	B	C		A	B	C
Siccanin	1.00	1.00	1.00	Dull red	ca. 0.64	ca. 0.21	ca. 0.65
Siccanochromene A	0.99	1.50	0.88	Reddish brown			
Siccanochromene B	0.85	0.50	0.67	Reddish brown			
Prechromene A	0.91	0.91	0.66	Light brown			

Ref.

M. Arai, K. Hamano, K. Nose and K. Nakano, *Annual Rept. Sankyo Res. Lab.*, 20 (1968) 93–98
 M. Arai, K. Ishibashi and H. Okazaki, *Antimicrob. Agents Chemother.*, 1969 (1970) 247–252

SIOMYCINS

PC 1

Solvent

Methanol–acetic acid–water (25:3:72)

 R_F

Siomycin A 0.08–0.10
 Siomycin B 0.30–0.31
 Siomycin C 0.00

Ref.

M. Ebata, K. Miyazaki and H. Otsuka, *J. Antibiot.*, 22 (1969) 364–368

PC 2

Paper

Ascending

Solvents

- A. Chloroform–methanol (9:1)
- B. Chloroform–methanol (19:1)
- C. Chloroform–*n*-butanol (6:1)
- D. Ethyl acetate–*n*-hexane–2 *N*-ammonia (4:1:1)
- E. Methanol–acetic acid–water (25:3:72)

 R_F

Solvent	R_F	Solvent	R_F
A	0.50	D	0.43
B	0.30	E	0.14
C	0.29		

Ref.

N. Miyairi, T. Miyoshi, M. Kohsaka, M. Ikushima and H. Imanaka, *U.S. Patent* 3,761,587, Sept. 25, 1973

TLC

Medium

Silica gel G

Solvents

- A. Chloroform–methanol (95:5)
- B. Chloroform–methanol (90:10)

 R_F

Solvent	Siomycin		
	A	B	C
A	0.14	0.21	0.42
B	0.60	0.67	0.94

Ref.

As PC 1

TLC 2

Medium

Silica gel GF (Merck)

Solvent

Chloroform–methanol (8:2)

 R_F

Siomycin A	R_F
Half ester: I * with butanedioic acid	0
Half ester: II with butanedioic acid	0.3
Half ester: I with pentanedioic acid	0
Half ester: II with pentanedioic acid	0.49
Half ester: I with hexanedioic acid	0.10
Half ester: II with hexanedioic acid	0.56
Sodium salt of half ester: II with hexanedioic acid	0.56
Half ester: I with heptanedioic acid	0.10
Half ester: II with heptanedioic acid	0.47
Half ester: III with heptanedioic acid	0.56
Half ester: I with octanedioic acid	0.10–0.30
Half ester: II with octanedioic acid	0.53
Half ester: III with octanedioic acid	0.49
Half ester: I with dodecanedioic acid	0.35
Half ester: II with dodecanedioic acid	0.58
Half ester: I with <i>cis</i> -butenedioic acid	0
Half ester: I with <i>cis</i> -2-methylbutenedioic acid	0
Half ester: II with <i>cis</i> -2-methylbutenedioic acid	0.37
Half ester: I with cyclohexane-1,2-dicarboxylic acid	0.20–0.44
Half ester: II with cyclohexane-1,2-dicarboxylic acid	0.52
Half ester: I with 1,2-benzene-dicarboxylic acid	0.01

Siomycin A	R_F
Half ester: II with 1,2-benzene-dicarboxylic acid	0.21
Half ester: III with 1,2-benzene-dicarboxylic acid	0.40

* I, II and III refer to the number and the position of the ester group in the siomycin A moiety

Ref.

K. Okabe, M. Mayama and S. Matsuura, *U.S. Patent* 3,983,230, Sept. 29, 1976

SIRODESMINS

TLC

Medium

Silica gel GF

Solvents

- A. Toluene-ethyl acetate (1:2)
- B. Chloroform-methanol-formic acid (95:4:1)
- C. Chloroform-ethyl acetate (1:1)

Detection

Spray with chromic acid soln. and then heat. The spots first appear yellow, then black

R_F

Compound	Solvent		
	A	B	C
Sirodesmin A	0.23	0.35	0.19
Sirodesmin B	0.07	0.24	0.06
Sirodesmin C	0.17	0.29	0.14
Sirodesmin D	0.31	0.29	0.25
Sirodesmin E	0.36	0.33	0.27
Sirodesmin F	0.42	0.35	0.34
Sirodesmin G	0.38	0.39	0.32
Sirodesmin H	0.23	0.28	0.17
Sirodesmin J	0.38	0.35	0.28
Desacetyl-sirodesmin A	0.23	0.24	0.15
Sirodesmin A bisacetate	0.39	0.51	0.36
Desacetyl-sirodesmin G	0.33	0.27	0.23
Sirodesmin G bisacetate	0.52	0.55	0.49
Sirodesmin A monoacetate	0.31	0.45	0.26
Sirodesmin monosulphide	0.24	0.29	0.19

Ref.

D. Broadbent, H.G. Hemming and B. Hesp, *U.S. Patent* 3,932,394, Jan. 13, 1976

SISOMICIN

PC 1

Paper

Whatman No. 1

Solvents

- A. 80% Methanol + 3% sodium chloride (w/v) (1:1), descending; paper buffered with 0.95 *M* sodium sulfate + 0.05 *M* sodium bisulfate dried before use
- B. Propanol-pyridine-acetic acid-water (6:4:1:3), ascending
- C. 80% Phenol, ascending
- D. Benzene-methanol (9:1), descending
- E. *n*-Butanol-water-acetic acid (4:5:1), upper phase, ascending
- F. Water satd. *n*-butanol + 2% *p*-toluenesulfonic acid, descending 26 h
- G. Chloroform-methanol-17% ammonium hydroxide (2:1:1), descending 16 h

Detection

- A. Bioautography vs. *Staphylococcus aureus* ATCC 6538P
- B. Ninhydrin spray (0.25% in pyridine-acetone, 1:1)

R_F

Solvent	R_F	Solvent	R_F
A	0.49	E	0.00
B	0.29	F	0.51 *
C	0.45	G	0.21 *
D	0.00		

* R_i value

Ref.

M.J. Weinstein, J.A. Marquez, R.T. Testa, G.H. Wagman, E.M. Oden and J.A. Waitz, *J. Antibiot.*, 23 (1970) 551-554
G.H. Wagman, R.T. Testa and J.A. Marquez, *J. Antibiot.*, 555-558

PC 2

Paper

Ascending

Solvent

Chloroform-methanol-17% aq. ammonia (2:1:1) lower layer (developed at room temp. for 12 h)

R_F
0.18

Ref.
As Apramycin, PC 1

PC 3

Paper
Toyo No. 51

Solvents

- A. 20% Ammonium chloride
- B. Water satd. *n*-butanol
- C. *n*-Butanol–acetic acid–water (3:1:1)
- D. Water satd. ethyl acetate
- E. Water satd. *n*-butanol + 2% *p*-toluenesulfonic acid + 2% piperidine

Detection

Bioautography vs. *Bacillus subtilis* No. 10707

R_F

Solvent	R_F	Solvent	R_F
A	0.97	D	0.00
B	0.00	E	0.01
C	0.04		

Ref.
As Carbomycin, PC 5

PC 4

Solvent

2-Butanone–*tert*.-butanol–methanol–conc. ammonium hydroxide (16:3:1:6)

R_F
 $R_t = 0.59$

Ref.
M.J. Weinstein, G.H. Wagman and J.A. Marquez,
U.S. Patent 4,001,209, Jan. 4, 1977

PC 5

Papers

- A. Chromar 500 sheet, ascending
- B. Whatman No. 1, descending

R_F

Medium–Solvent

A–A	B–B	B–C
R_F	R_t ($t = 6$ h)	R_t ($t = 16$ h)
0.58	0.21	0.34

Solvents

- A. Chloroform–methanol–conc. ammonium hydroxide (1:1:1)

- B. Chloroform–methanol–17% ammonium hydroxide (2:1:1)

- C. 2-Butanone–*tert*.-butanol–methanol–conc. ammonium hydroxide (16:3:1:6)

Ref.

M.J. Weinstein, G.H. Wagman and J.A. Marquez,
U.S. Patent 3,951,746, April 20, 1976

PC 6

Paper

Ascending

Solvents

- A. 20% Aq. soln. of ammonium chloride
- B. Water satd. *n*-butanol
- C. *n*-Butanol–acetic acid–water (3:1:1)
- D. Water satd. *n*-butanol + 2% (w/v) *p*-toluenesulfonic acid + 2% (v/v) piperidine
- E. Chloroform–methanol–17% aq. ammonia (2:1:1), lower layer

R_F

Solvent	R_F	Solvent	R_F
A	0.98	D	0.10
B	0.00	E	0.18
C	0.06		

Ref.

As Apramycin, PC 2

TLC 1

Medium

Silica gel

Solvents

- A. Chloroform–methanol–17% aq. ammonia (2:1:1) upper layer
- B. Methanol–10% ammonium acetate (1:1)
- C. *n*-Butanol–ethanol–chloroform–17% aq. ammonia (4:5:2:5)

R_F

Solvent A	0.89
Solvent B	0.66
Solvent C	–

Ref.

As Apramycin, PC 2

TLC 2

Medium

Silica gel

Solvents

- A. Chloroform–methanol–17% aq. ammonia (2:1:1), upper layer (3 h)
- B. 10% Ammonium acetate–methanol (1:1), 3 h

R_F

Solvent A 0.71

Solvent B 0.18

Ref.

As Apramycin, PC 1

HPLC*Column*

μ Bondapak C₁₈ column, 30 cm $\times$ 3.9 mm I.D.
(Waters Assoc.) precolumn 4.3 cm $\times$ 4.2 mm I.D.
Micropart C₁₈ bonded-phase silica gel (5 μ m)
packing

Mobile phase

0.2 M Sodium sulfate, 0.02 M sodium pentane-
sulfonate, and 0.1% (v/v) acetic acid in water-
methanol (97:3)

Flow-rate

Column, 2 ml/min at 184 atm; OPA, 0.5 ml/min

Detection

Continuous-flow, post-column derivatization with
o-phthalaldehyde used to form fluorescent products

Type

Ion-pair

Retention time

3.0 (estimated from drawing)

Ref.

As Gentamicins, HPLC 1

SPARSOGENIN**CCD***Solvents*

A. 1-Butanol-ethyl acetate-water (1.2:0.5:1.9),
300 transfers

B. 2-Butanol-water, 200 transfers

*Distribution*A. *K* = 0.55B. *K* = 0.739*Ref.*

A.D. Argoudelis, C. DeBoer, T.E. Eble and R.R.
Herr, *U.S. Patent* 3,629,406, Dec. 21, 1971

SPARSOMYCIN**CCD***Solvent*

2-Butanol-water (1:1)

*Distribution**K* = 0.74*Ref.*

A.D. Argoudelis and R.R. Herr, *Antimicrob.*
Agents Chemother., 1962 (1963) 780-786

SPECTINOMYCIN**PC***Paper*

Ascending

Solvent

Chloroform-methanol-17% aq. ammonia
(2:1:1) lower layer (developed at room temp. for
12 h)

R_F

0.45

Ref.

As Apramycin, PC 1

TLC*Medium*

Silica gel

Solvents

A. Chloroform-methanol-17% ammonium hy-
droxide (2:1:1), upper phase

B. 10% Ammonium acetate-methanol (1:1)

*Detection*Bioautography vs. *Bacillus subtilis**R_F*

Solvent A 0.70

Solvent B 0.63

Ref.

R. Okachi, I. Kawamoto, S. Takasawa, M.
Yamamoto, S. Sato, T. Sato, and T. Nara, *paper*
presented at 194th Sci. Meet., JARA, June 26,
1974

SPHAEROPSIDIN**TLC 1***Medium*Silica gel GF₂₅₄ (E. Merck)*Solvent*

Ethyl acetate

Detection

A. UV absorbance

B. Spray with 10% phosphomolybdic acid and
heat at 100°C

R_F

ca. 0.33

Ref.

J.M. Coats, M.E. Herr and R.R. Herr, *U.S. Patent*
3,585,111, June 15, 1971

TLC 2*Medium*Silica gel GF₂₅₄ (E. Merck) microslide plates*Solvent*

Ethyl acetate

Detection

Could be detected as a UV absorbing spot which moved approximately one third the distance of the solvent front

R_F

ca. 0.3

Ref.

J.H. Coats, M.E. Herr and R.R. Herr, *U.S. Patent* 3,701,790, Oct. 31, 1972

SPINAMYCIN**PC****Paper**

Toyo No. 51

Solvents

- A. Acetone
- B. Acetone-methanol (1:1)
- C. Methanol
- D. Ethyl acetate
- E. Ethyl acetate-chloroform (1:1)
- F. Butanol
- G. Acetone-diethyl ether (1:1)

R_F

Solvent	R _F	Solvent	R _F
A	1.00	E	0.95
B	0.72	F	1.00
C	0.62	G	1.00
D	1.00		

Ref.

E.L. Wang, M. Hamada, Y. Okami and H. Umezawa, *J. Antibiot.*, 19 (1966) 216-221

TLC**Medium**

Silica gel

Solvents

- A. Methanol
- B. Ethyl acetate-chloroform (1:1)

R_F

Solvent A 0.66
Solvent B 0.27

Ref.

As PC

SPIRAMYCINS**PC 1****Paper**

Toyo No. 51

Solvent

Water satd. ethyl acetate (ascending, 3 h)

Detection

Bioautography vs. *Bacillus subtilis* No. 10707

R_F

0.95

Ref.

As Carbomycin, PC 5

PC 2**Paper**

Whatman No. 1

Solvents

- A. 80% Methanol + 3% sodium chloride (1:1) descending. Paper buffered with 0.95 M sodium sulfate + 0.05 M sodium bisulfate
- B. Propanol-pyridine-acetic acid-water (6:4:1:3), ascending
- C. Butanol-acetic acid-water (4:1:5), ascending
- D. 80% Phenol, ascending

Detection

Bioautography vs. *Staphylococcus aureus*

R_F

Solvent A 0.90
Solvent B 0.93
Solvent C 0.78
Solvent D 0.86

Ref.

As Chloramphenicol, PC 5

TLC 1**Medium**

Silica gel GF

Solvent

Butanol-acetic acid-water (3:1:1)

Detection

Spray with concentrated sulfuric acid-methanol (1:1), developed by heating at 105°C for several minutes

R_F

0.6, 0.30, purple, gray

Ref.

As Chloramphenicol, PC 5

TLC 2**Medium**

Silica gel

Solvents

- A. Chloroform-methanol-17% ammonia (2:1:1)
- B. Butanol-acetic acid-water (3:1:1)

Detection

- A. Bioautography vs. *Sarcina lutea*
- B. Plate heated at 100°C and sprayed with phosphoric acid in methanol (1:1)

R_F

Solvent	Detection A	Detection B
A	0.95	0.95 Red-brown
B	0.16	0.16, 0.31, 0.35, 0.45 Red-brown

Ref.

G.H. Wagman, J.A. Waitz, J. Marquez, A. Murawski, E.M. Oden, R.T. Testa and M.J. Weinstein, *J. Antibiot.*, 25 (1972) 641–646

TLC 3**Medium**

Silica gel GF₂₅₄ (Merck)

Solvents

- A. Chloroform-methanol-1.5 *N* aq. ammonia (2:1:1) bottom layer + 0.1% butyrate (72 h)
 B. Chloroform-methanol-1.5 *N* aq. ammonia (2:1:1) bottom layer + 0.15% butyrate

Detection

- A. UV at 232 nm
 B. The UV absorption was measured by the dual-wavelength chromatogram scanner (Aloka, Model JIC 202B)
 C. By color reaction with 20% sulfuric acid heated for a few minutes

 R_F

Spiramycin			
	I	II	III
Solvent A	0.60	0.70	0.90
Neospiramycin			
	I	II	III
Solvent B	0.29	0.48	0.71, 0.90

Ref.

C. Kitao, H. Ikeda, H. Hamada and S. Omura, *J. Antibiot.*, 32 (1979) 593–599

FLC**Instrument**

JASCO FLC-150

Detector

JASCO UVIDEK-2 (232.5 nm) spectrophotometer

Separation mode

Partition

Column

JASCOPACK SV-02-500

Column temperature

Ambient

Column pressure

65 kg/cm²

Mobile phase

Methanol- $\frac{1}{15}$ *m* acetate buffer, pH 4.9-acetonitrile (35:60:5)

Flow-rate

1.0 ml/min

Retention time (min)

Spiramycin I 2.5
 Spiramycin II 5.1

Ref.

S. Omura, Y. Suzuki, A. Nakagawa and T. Hata, *J. Antibiot.*, 26 (1973) 794–796

HPLC**Apparatus**

Varian LC 8500 chromatograph

Column

LiChrosorb (15 cm × 4.7 mm I.D.) (particle size 10 μm)

Mobile phase

Conc. sulfuric acid (1% in water)-acetonitrile (76:24)

Flow-rate

100 ml/h

Pressure

500 p.s.i.

Detector

UV (231 nm)

Type

Reversed-phase

Sensitivity

0.20 a.u.f.s.

Retention time (sec)

Spiramycin I 206
 Spiramycin II 290
 Spiramycin III 395
 Spiramycin IV 470

Ref.

D. Mourrot, B. Delepine, J. Boisseau and G. Gayot, *J. Chromatogr.*, 161 (1978) 386–388

SPORANGIOMYCIN**PC 1****Solvents**

- A. Water satd. butanol
 B. As A + 2% *p*-toluenesulfonic acid

- C. As A + 2% conc. ammonia
 D. Butanol satd. water
 E. 20% Ammonium chloride
 F. Phenol-water (75:25)
 G. *n*-Butanol-methanol-water (40:10:20), containing 0.75 g methyl orange
 H. *n*-Butanol-methanol-water (40:10:30)
 I. Water-acetone (1:1)
 J. Water satd. ethyl acetate

Detection

Bioautography vs. *Bacillus subtilis*

 R_F

Solvent	R_F	Solvent	R_F
A	0.88	F	0.98
B	0.83	G	0.94
C	0.82	H	0.91
D	0.10-0.50	I	0.10
E	0.00	J	0.88

Ref.

J.E. Thiemann, C. Coronelli, H. Pagani, G. Beretta, G. Tamoni and V. Arioli, *J. Antibiot.*, 21 (1968) 525-531

PC 2**Paper**

Ascending

Solvents

- A. Chloroform-methanol (9:1)
 B. Chloroform-methanol (19:1)
 C. Chloroform-*n*-butanol (6:1)
 D. Ethyl acetate-*n*-hexane-2 *N* ammonia (4:1:1)
 E. Methanol-acetic acid-water (25:3:72)

 R_F

Solvent	R_F	Solvent	R_F
A	0.50	D	0.41
B	0.30	E	0.14
C	0.27		

Ref.

As Siomycins, PC 2

SPOROCYTOPHAGE CAULIFORMIS ANTIBIOTIC

PC**Solvent**

Water satd. butanol

Detection

- A. Ninhydrin
 B. UV fluorescence

 R_F

0.028 (ninhydrin +); 0.10 (fluorescent)

Ref.

German Patent 467,923, March 27, 1969

SPOROVIRIDININ

PC**Solvents**

- A. Butanol-pyridine-water-acetic acid (6:4:3:1)
 B. Butanol-methanol-water (4:1:2)

Detection

- B. Bioautography
 B. UV light at 360 nm (yellowish-blue fluorescence; sporoviridin is not fluorescent)

 R_F

Solvent A 0.51
 Solvent B 0.37

Ref.

M. Suzuki, T. Takaishi and I. Takamori, *Derwent Farmdoc* 41472 (JA.30481/69), Sept. 12, 1969

STAPHYLOMYCIN

PC**Solvent**

Propylene glycol-benzene, descending, 72 h

Detection

- A. Bioautography vs. *Bacillus subtilis*
 B. Spray with 0.5% *p*-dimethylaminobenzaldehyde in 1 *N* hydrochloric acid and heat; red colored zones result

 R_F

Factor S > factor M₁

Ref.

H. Vanderhaeghe, P. Van Dijck, G. Parmentier and P. De Somer, *Antibiot. Chemother.*, 7 (1957) 605-614

STEFFIMYCIN; STEFFIMYCIN B

PC**Paper**

Schleicher and Schuell 589 Blue Ribbon

Solvent

Benzene-methanol-water (1:1:2), upper phase

 R_F (estimated from drawing)

Steffimycin 0.3
 Steffimycin B 0.8

Ref.

T.F. Brodasky and F. Reusser, *U.S. Patent* 3,794,721, Feb. 26, 1974

TLC*Medium*

Silica gel

Solvent

Methanol–methylene chloride (5:95)

R_F

0.12

*Ref.*R.C. Kelly, *U.S. Patent* 3,976,667, Aug. 24, 1976**STEFFIMYCINONE**

(U-25055)

TLC*Medium*

Silica gel

Solvent

Methanol–methylene chloride (5:95)

R_F

0.31

Ref.

As Steffimycin, TLC 1

STEFFISBURGENSIMYCIN**PC***Solvents*

A. 1-Butanol–water (84:16), 16 h

B. As A + 0.25% *p*-toluenesulfonic acid, 16 h

C. 1-Butanol–acetic acid–water (2:1:1), 16 h

D. As A + 2% (v/v) piperidine, 16 h

E. 1-Butanol–water (4:96), 5 h

F. As E + 0.25% *p*-toluenesulfonic acid, 5 h*R_F* (estimated from drawing)

Sol- vent	<i>R_F</i>	Sol- vent	<i>R_F</i>
A	0.50–0.85	D	0.20–0.65
B	0.50–0.85	E	0.15–0.62
C	0.70–0.90	F	0.05–0.57

*Ref.*M.E. Bergy, J.H. Coats and F. Reusser, *U.S. Patent* 3,309,273, March 14, 1967**STREPTAMINE****TLC***Medium*

Cellulose

*Solvent*Methyl ethyl ketone–methanol–isopropanol–7.9 *N* ammonium hydroxide (10:8.5:3:7)*Detection*Ninhydrin. Spray with 0.3% soln. of ninhydrin in water satd. *tert*.-butanol, heat at 100–105° for 3–5 min*R_F*

0.64

Ref.

As Kanamycins, TLC 5

**STREPTAMINE, 2,5-DIDEOXY-
(See 2,5-dideoxystreptamine)****STREPTAMINES, 2-DESOXY-
(See 2-desoxystreptamines)****STREPTIMIDONE****CCD***Solvent*1-Butanol–cyclohexane–water (1:4:5), 25 trans-
fers*Distribution**K* = 1.10*Ref.*R.P. Forhardt, H.W. Dion, Z.L. Jakubowski, A.
Ryder, J.C. French and Q.R. Bartz, *J. Amer.
Chem. Soc.*, 81 (1959) 5500**STREPTIMIDONE, 9-METHYL-
(See 9-methylstreptimidone)****STREPTOLIN****PC***Paper*Eaton-Dikeman No. 613, buffered with 0.95 *M*
sodium sulfate and 0.05 *M* sodium acid sulfate
and air dried before use*Solvent*

75% Ethanol, 25°C, descending, 30–50 h

*Detection*Bioautography vs. *Bacillus subtilis**R_F*

(Inches from origin; estimated from drawing)

Streptolin A 4.6

Streptolin B 6.5

*Ref.*L.M. Larson, H. Sternberg and W.H. Peterson, *J.
Amer. Chem. Soc.*, 75 (1953) 2036

STREPTOLYDIGIN**PC****Solvents**

- A. *n*-Butanol–water (81:19)
 B. *n*-Butanol–water–*p*-toluenesulfonic acid (81:18.7:0.25)
 C. *n*-Butanol–water–piperidine (81:17:2)
 All solvents developed 16 h
 D. 1 *M* Phosphate buffer, pH 7.0, descending, 4 h

Detection

Bioautography vs. *Mycobacterium avium* ATCC 7992

***R_F* (estimated from drawing)**

Solvent A	0.85 (tail)
Solvent B	0.90 (tail)
Solvent C	0.90 (tail)
Solvent D	0.15–0.30

Ref.

C. De Boer, A. Dietz, W.S. Silver and G.M. Savage, *Antibiot. Ann.*, (1955–1956) 886–892

STREPTOMYCES H 359 NSY 6 ANTI-BIOTIC**TLC****Medium**

Silica gel

Solvents

- A. *n*-Butanol–ethanol–water (5:3:2)
 B. *n*-Propanol–ethyl acetate–water (6:1:3)
 C. *n*-Butanol–acetic acid–water (2:3:1)

Detection

Spot detected by heating at 100°C for 10 min after spraying 2 *N* aniline–2 *N* phosphate soln.

R_F

Solvent A	0.21
Solvent B	0.47
Solvent C	0.37

Ref.

As Maltotetraose, TLC

STREPTOMYCES ATROOLIVACEUS ANTI-BIOTIC**TLC****Medium**

Silufol

Solvents

- A. Chloroform–methanol (6:1)
 B. Benzene–methanol (3:1)

Detection

UV at 254 nm. Detection, after exposure to ammonia vapours, in visible and UV light

R_F

Sub-stance	<i>R_F</i>		Appearance in light	
	Solvent		UV	Visible
	A	B		
A	0.10	–	Red	Colorless
B	0.15	–	Violet •	Colorless
C	0.21	–	Yellow •	Colorless
D	0.27	–	Black *	Yellow
E	0.44	–	Violet •	Colorless
F	0.48	0.28	Red	Red–violet *
G	0.73	0.60	Violet *	Pink to yellowish
H	0.78	0.55	Red	Colorless
I	0.90	0.89	Yellow–brown **	Yellow
J	0.94	–	Blue–violet	Yellow
K	0.52	0.53	Yellow–orange	Yellow •

* Visible immediately, without preceding exposure in ammonia vapor

** Quenching fluorescence

Ref.

As Chromocyclomycin, TLC

**STREPTOMYCES COLLINUS Linden-bein
DIPEPTIDE ANTI-BIOTIC****PC****Paper**

Whatman No. 1

Solvents

- A. Water–satd. 1-butanol + 2% *p*-toluenesulfonic acid
 B. Propanol–pyridine–acetic acid–water (15:10:3:12)

Detection

Bioautography vs. *Salmonella gallinarum*

Ref.

B.B. Molloy, D.H. Liverly, R.M. Gale, M. Gorman, L.D. Boeck, C.E. Higgins, R.E. Kastner, L.L. Huckstep and N. Neuss, *J. Antibiot.*, 25 (1972) 137–140

STREPTOMYCES RAMOCISSIMUS R_F **ANTIBIOTIC**

TLC

Medium

Silica gel

Solvent

Acetone-ethyl acetate-water (12:8:1)

R_F

Antibiotic separates into 3 components

Ref.

Belgian Patent 771331, Feb. 14, 1972

STREPTOMYCES VIRIDOCHROMOGENES **ANTIBIOTIC**

PC

Paper

Whatman No. 4 impregnated with acetone-water (7:3)

Solvent

Benzene-chloroform-acetic acid-water (2:2:1:1)

R_F

Component A 0.35

Component B 0.25

Ref.

German Patent 1,954,047, June 11, 1970

STREPTOMYCIN, DIHYDRO- **(See streptomycins)**

STREPTOMYCINS

PC 1

Paper

Whatman No. 1 or Eaton-Dikeman No. 613

Solvent

Water satd. *n*-butanol + 2% *p*-toluenesulfonic acid monohydrate; develop 24 h

Detection

Bioautography vs. *Bacillus subtilis*

R_F

Streptomycin > dihydroxystreptomycin > mannosidostreptomycin

Ref.

D.H. Peterson and L.M. Reineke, *J. Amer. Chem. Soc.*, 72 (1950) 3598-3603

PC 2

Solvent

As PC 1

Detection

As PC 1

	$R_{\text{streptomycin}}$
Streptomycin	1.00
Mannosidostreptomycin	0.37
Dihydroxystreptomycin	0.62
Hydroxystreptomycin	0.64

Ref.

J.N. Pereira, *J. Biochem. Microbiol. Tech. Eng.*, 3 (1961) 79-85

PC 3

Paper

Whatman No. 20

Solvent

Equal vols. of amyl alcohol containing 1% (v/v) di-2-ethyl hexyl phosphate and 0.5% sodium chloride in borate buffer (0.62 g boric acid, 0.21 g borax) are mixed. Adjust pH to 8.0 with sodium hydroxide while stirring for 30 min. Separate phases; dip paper in lower phase, blot, apply samples and dry several minutes. While still wet develop with organic phase either ascending or descending

Detection

A. As PC 1

B. Spray or dip in alkaline α -naphtholdiacetyl color reagent [mix equal vols. of 40% (w/v) potassium hydroxide in 50% methanol, 2.5% (w/v) α -naphthol in methanol and 0.1% (w/v) diacetyl in methanol immediately before use in order given]

R_F

	$R_{\text{streptomycin}}$
Streptomycin	1.00 ($R_F = 0.7$)
Dihydrostreptomycin	0.83
Hydroxystreptomycin	0.73
Mannosidostreptomycin	0.43

Ref.

H. Heding, *Acta Chem. Scand.*, 20 (1966) 1743-1746

PC 4

Paper

Ascending

Solvent

Chloroform-methanol-17% aq. ammonia (2:1:1) lower layer (developed at room temp. for 12 h)

R_F

Streptomycin A	0.02
Streptomycin B	0.00

Ref.

As Apramycin, PC 1

PC 5

Paper

Whatman No. 1

Solvents

- A. 80% Methanol + 3% sodium chloride (1:1) descending. Paper buffered with 0.95 M sodium sulfate + 0.05 M sodium bisulfate
- B. Propanol-pyridine-acetic acid-water (6:4:1:3), ascending
- C. Butanol-acetic acid-water (4:1:5), ascending
- D. 80% Phenol, ascending

Detection

Bioautography vs. *Staphylococcus aureus*

 R_F

Solvent A	0.57
Solvent B	0.40
Solvent C	0.00
Solvent D	0.06

Ref.

As Chloramphenicol, PC 5

PC 6

Paper

Whatman No. 20

Solvents

- A. 1-Butanol-pyridine-2.5% sodium chloride in distilled water (4:8:3) + 1% (w/v) *p*-toluenesulfonic acid monohydrate
- B. 1.5% Sodium chloride in 80% methanol
- C. Water satd. 1-butanol + 2% (w/v) *p*-toluenesulfonic acid monohydrate

 R_F

Solvent A	0.72
Solvent B	0.49
Solvent C	0.17-0.19

Ref.

K. Nagagka and A.L. Demain, *J. Antibiot.*, 28 (1975) 627-635

PC 7

Paper

Whatman No. 20

Solvent

Water satd. *n*-butanol + 2% *p*-toluenesulfonic acid

Detection

Location was visualized by placing the chromatogram on the surface of 500 ml of medium (Bacto

Antibiotic Medium 5) that had been autoclaved for 15 min, cooled to 48°C and mixed with a 0.2-ml suspension of spores of *Bacillus subtilis*, then allowed to set in a 9 × 16 in. dish. After 15 min, the paper was removed and the medium incubated at 28°C for 20 h

 R_F

2-Deoxystreptomycin A	0.35
2-Deoxystreptomycin B	0.08

Ref.

A.L. Demain and K. Nagaoka, *U.S. Patent* 3,956,275, May 11, 1976

TLC 1

Medium

Kieselgel G; heat at 110°C, 30 min. Spot compounds as phenyl hydrazones

Solvent

n-Butanol-water-methanol (40:20:10) + 1 g *p*-toluenesulfonic acid

Detection

Dry plate for 5 min at 110°C. Spray with a 1 + 4 dilution of the following mixture: sodium nitroprusside, 10 g; potassium permanganate, 0.15 g; 0.5 N sodium hydroxide, 2 ml; water, to 100 ml

 R_F

Streptomycin	0.60-0.62
Dihydroxystreptomycin	0.90-0.92

Ref.

P.A. Nussbaumer and M. Schorderet, *Pharm. Acta Helv.*, 40 (1965) 205-209 and 477-482

TLC 2

Medium

Silica gel G; heat at 110°C for 30 min and cool prior to use

Solvents

- A. 3% Aq. sodium acetate
- B. As A, but 4% aq. soln.

Detection

1% Alcohol soln. of bromthymolblue (yellowish spots on green background)

 R_F

Compound	Solvent	
	A	B
Streptomycin	0.46	0.58
Dihydrodesoxystreptomycin	0.28	0.38
Dihydrostreptomycin	0.34	0.44

Ref.

T. Sato and H. Ikeda, *Sci. Papers, Inst. Phys. Chem. Res., Tokyo*, 59 (1965) 159–164

TLC 3*Medium*

Silica gel G (Merck)

Solvent

Water satd. *n*-butanol + 2% *p*-toluenesulfonic acid + 2% piperidine. Develop at 16°C, 3–5 h

Detection

Mix equal vols. of 10% sodium hydroxide, 10% potassium ferricyanide and 10% sodium nitroprusside about 30 min prior to application to plate. Dry plate at 110°C, 15 min before spraying. Vermilion spots on a dark yellowish background

R_F

Run	Streptomycin	Dihydrostreptomycin	Dihydrodesoxystreptomycin
1	0.49	0.31	–
2	–	0.35	0.35
3	0.41	–	0.30

Ref.

T. Katayama and H. Ikeda, *Sci. Papers, Inst. Phys. and Chem. Res., Tokyo*, 60 (1966)

TLC 4*Medium*

Cellulose

Solvent

Methyl ethyl ketone–methanol–isopropanol–7.9 *N* ammonium hydroxide (10:8.5:3:7)

Detection

Ninhydrin. Spray with 0.3% soln. of ninhydrin in water satd. *tert*-butanol, heat at 100–105° for 3–5 min

R_F

0.72

Ref.

As Kanamycins, TLC 5

TLC 5*Medium*

Avicel

Solvent

Methanol–isobutyl alcohol–chloroform–10% hydrochloric acid (10:10:10:1)

Detection

- A. Iodine vapor
- B. 0.25% Stannous chloride soln.
- C. 0.2% *p*-Dimethylaminobenzaldehyde soln.

R_F

0.49

Ref.

As Ampicillin, TLC 4

TLC 6*Medium*

Cellulose (Merck 5716)

Solvent

3% Methanol–ammonium chloride (7:3)

Detection

Bioautography vs. *Bacillus cereus* ATCC 11778

R_F

Streptomycin	0.50
Dihydrostreptomycin	0.40

Ref.

As Bacitracin, TLC 3

TLC 7*Medium*

Silica gel

Solvent

Methyl ethyl ketone–acetone–water (9.3:2.6:1) developed 30–45 min (15 cm)

Detection

UV at 366 nm

R_F

0

Ref.

M. Baudet, *J. Pharm. Belg.*, 31 (1976) 247–254

TLC 8*Medium*

Silica gel 60 (precoated 20 × 20 cm, 0.25 mm thickness, E.M. Laboratories)

Solvent

Methanol–chloroform–conc. ammonium hydroxide (1:1:1)

Detection

Ninhydrin; [10 ml of a freshly prepared soln. of ninhydrin (1.0 g) in a mixture of 95% ethanol (50 ml) and glacial acetic acid (10 ml)]; heat for 15 min at 135°. The antibiotics are visible as magenta red spots on a white background

R_F (estimated from drawing)

Streptomycin	0.01
Dihydrostreptomycin	0.01

Ref.

W.L. Wilson, G. Richard and D.W. Hughes, *J. Chromatogr.*, 78 (1973) 442–444

TLC 9**Medium**

Silica gel G

SolventAcetone-methanol-0.1 M sodium chloride
(2:1:1)**Detection**0.4% Aq. potassium permanganate, yellow spot
on violet background**R_F**

0.15

Ref.M. Shahjahan, B.K. Dutta and A. Rashid,
Bangladesh Pharm. J., 1 (1972) 14-17**TLC 10****Medium**

Silica gel

Solvents

- A. Chloroform-methanol-17% ammonium hydroxide (2:1:1), upper phase
B. 10% Ammonium acetate-methanol (1:1)

DetectionBioautography vs. *Bacillus subtilis***R_F**

Solvent A 0.04

Solvent B 0.87

Ref.R. Okachi, I. Kawamoto, S. Takasawa, M. Yamamoto, S. Sato, T. Sato and T. Nara, *Paper presented 194th Sci. Meet., JARA, June 26, 1974***ELPHO****Medium**

Whatman No. 4 filter paper

Buffers

- A. 2 M Formic acid-0.1 M *p*-toluenesulfonic acid-*n*-propanol (500:50:50) in 1000 ml water, pH 1.9
B. 2 M Formic acid-0.1 M *p*-toluenesulfonic acid-*n*-propanol (400:50:50) in 1000 ml water, pH 1.9
C. 2 M Formic acid-0.1 M *p*-toluenesulfonic acid-(400:50) in 1000 ml water, pH 1.9
D. 1 M Ammonium hydroxide-1 M sodium hydroxide-0.1 M *p*-toluenesulfonic acid-*n*-propanol (200:5:100:100) in 1000 ml water, pH 10.8
E. 1 M Ammonium hydroxide-1 M sodium hydroxide (200:2.5) in 1000 ml water, pH 11.5
F. 50 ml 1 M Sodium hydroxide in 100 ml water, pH 12.2

Conditions

The electrophoregrams were developed at 350 V for 1-4 h (depending on the electrolyte)

Mobility

Buffer	Toward cathode (cm ² /V sec)
A	29.55
B	25.33
C	27.14
D	9.50
E	9.57
F	8.74

Ref.

As Bluensomycin, ELPHO

STREPTONIGRIN**CCD****Solvent**

Ethyl acetate-3% phosphate buffer, pH 7.5; 100 transfers

Distribution

Peak at tube 34 (estimated from curve)

Ref.K.V. Rao and W.P. Cullen, *Antibiot. Ann.*, (1959-1960) 950-953**STREPTONIVICIN****PC****Solvents**

- A. *n*-Butanol-water (81:19) + 0.25% *p*-toluenesulfonic acid
B. *n*-Butanol-water-acetic acid (50:25:25)
C. *n*-Butanol-water-piperidine (78.4:18.6:2)
D. Water-*n*-butanol (96:4)
E. As D + 2% *p*-toluenesulfonic acid

R_F (estimated from drawing)

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.40-0.75	D	0.80
B	0.90	E	0.65
C	0.35-0.70		

Ref.H. Hoeksema, M.E. Bergy, W.G. Jackson, J.W. Shell, J.W. Hinman, A.E. Fonken, G.A. Boyack, E.L. Caron, J.H. Ford, W.H. Devries and G.F. Crum, *Antibiot. Chemother.*, 6 (1956) 143-148

STREPTOSPORANGIUM VULGARE ATCC 21906 ANTIBIOTIC

TLC

Medium

Silica gel G/HF₂₅₄ plates

Solvent

Chloroform-methanol (98:2)

Detection

Conc. sulfuric acid at 100°C

R_F

Fraction A 0.39

Fraction B 0.2

Fraction C 0.07

Ref.

C. Coronelli, M.R. Bardone and H. Pagani, *U.S.*

Patent 3,889,396, Aug. 12, 1975

STREPTOTHRICIN GROUP ANTI-BIOTIC

(See S-15-1 and S-15-2)

STREPTOTHRICINS

(streptothricin-like antibiotics)

PC 1 (Circular paper chromatography)

Paper

Filter paper circles. Samples spotted (ca. 4 μl) along an arc drawn 3.1 cm in diameter. Hydrochloride or sulfate derivatives used

Solvent

Propanol-pyridine-acetic acid-water (15:10:3:12), ca. 5.5 h, 23-24°C

Detection

A. Bioautography vs. *Bacillus subtilis* (sections cut out and tested)

B. Ninhydrin spray

R_F

Antibiotic	Minimum No. of com- po- nents	HCl	SO ₄ ²⁻
Strepto- thricin	1	0.50	0.43
Strepto- thricin VI	2	0.35, 0.50	0.32, 0.43
Pleocidin complex	4	0.22, 0.35, 0.42, 0.50	0.20, 0.32, 0.37, 0.43

Antibiotic	Mini- mum No. of com- po- nents	HCl	SO ₄ ²⁻
Viomycin	1	0.42	0.38
Antibiotic 136	3	0.35, 0.40, 0.50	0.31, 0.43
Streptolin A	2	0.24, 0.33	0.20, 0.33
Streptolin B	3	0.30, 0.35, 0.50	0.27, 0.32, 0.43
Antibiotic VIIa	3	0.30, 0.33, 0.44	0.27, 0.31, 0.41
Antibiotic IXa	2	0.30, 0.44	0.27, 0.41
Mycothricin complex	4	0.26, 0.35, 0.42, 0.50	0.23, 0.32, 0.37, 0.43
Geomycin	2	0.30, 0.33 (diffuse bands)	0.35, 0.26 (diffuse bands)
Roseo- thricin A	2	0.30, 0.50	0.32, 0.43
Roseo- thricin B	1	0.31	0.27
Roseo- thricin C	1	0.28	0.24

Ref.

M.I. Horowitz and C.P. Schaffner, *Anal. Chem.*, 30 (1958) 1616-1620

PC 2

Solvent

n-Propanol-pyridine-acetic acid-water (15:10:3:12)

R_F

0.38

Ref.

K. Arima, T. Kawamura and T. Beppu, *J. Anti-biot.*, 25 (1972) 387-392

PC 3 (Circular)

Paper

Circular

Solvent

Pyridine-*n*-butanol-acetic acid-water
(10:15:3:12)

For purposes of comparison, streptothricins F and D were applied to the chromatogram. When grown in the medium used in the experiments, the initial culture produces a mixture of streptothricins F and D

R_F

Streptothricin F > D

Ref.

A.V. Lesnikova, Y.M. Khokhlova, Y.I. Rautenshtein, N.Y. Solov'eva, A.D. Filatova and O.I. Voronina, *Mikrobiologiya*, 41 (1972) 748-749

TLC**Media**

A. Silica gel G

B. Cellulose MN 300

Solvents

A. *n*-Propanol-pyridine-acetic acid-water
(15:10:3:12)

B. Chloroform-methanol-17% aq. ammonia
(2:1:1), upper layer

R_F

Solvent	Medium A	Medium B
A	0.72	0.26
B	0.26	

Ref.

As PC 1

ELPHO**Medium**

Paper

Buffer

0.02 *M* Formic acid soln., pH 2.0

Mobility

Moves toward cathode

Ref.

As PC 1

STREPTOVARICINS**PC 1****Paper**

Schleicher and Schuell No. 589 (Blue Ribbon Special) impregnated with 0.2 *M* phosphate buffer, pH 4.1, and air dried

Solvent

Cyclohexane-chloroform-water (1:8:2). Equilibrate for 2 h in atmosphere of both phases and develop 5 h in organic phase

Detection

Bioautography vs. *Mycobacterium ranae*

R_F

Streptovaricin A	0.13
Streptovaricin B	0.37
Streptovaricin C	0.77
Streptovaricin D and E	0.88

Ref.

P. Siminoff, R.M. Smith, W.T. Sokolski and G.M. Savage, *Amer. Rev. Tuberc.*, 75 (1957) 576-583

PC 2**Paper**

As PC 1. For quantitative determinations 0.25 in.-wide strips used and standards applied as 0.025, 0.05, 0.10, 0.20, 0.30 and 0.50 μ g streptovaricin A or 0.05, 0.10, 0.20, 0.40, 0.60 and 1.0 μ g of B, C, or D. One set of standard used for every nine strips consisting of at least 3 replicates of each sample

Solvents

- A. Methanol-benzene-water (1:1:2); equilibrate 16 h (chamber with both phases); develop 6 h
B. Toluene-Skellysolve C-methanol-water (5:5:7:3). Strips buffered with 0.2 *M* phosphate buffer, pH 4.1, and dried prior to use. Proceed as in A

Detection

Bioautography vs. *Mycobacterium ranae* UC161, *Sarcina lutea* PC11001 or *Bacillus subtilis* UC564. Component potencies estimated from satd. curves plotted as zone width vs. logarithmic dose

R_F (estimated from drawings)

Component	Solvent	
	A	B
A	0.35	0.02
B	0.65	0.07
C	0.72	0.15
D	0.82	0.60
E	0.88	0.75

Ref.

W.T. Sokolski, N.J. Eilers and P. Siminoff, *Antibiot. Ann.*, (1957-1958) 119-125

See also H. Uamazaki, *J. Antibiot.*, 21 (1968) 204-208

TLC**Solvents**

- A. Chloroform-methanol (95:5)
B. Chloroform-methanol (97:3)

Detection

UV

 R_F

Compound	Sol- vent A	Sol- vent B
Streptovaricin C	0.36	
Atropisostreptovaricin C	0.20	0.26
Atropisostreptovaricin F _c	0.11	
Streptovaricin F _c	0.07	

Ref.

K.L. Rinehart, Jr., and W.M.J. Knoll, *U.S. Patent* 3,954,737, May 4, 1976

CCD**Solvent**

Water-95% ethanol-cyclohexane-ethyl acetate (1:1:1:1), 200 transfers

Distribution

3 components separate cleanly and a fourth peak contains at least 2 components

Ref.

G.B. Whitfield, E.C. Olson, R.R. Herr, J.A. Fox, M.E. Bergy and G.A. Boyack, *Amer. Rev. Tuberc.*, 75 (1957) 584-587

STREPTOVIRUDINS**PC 1****Paper**

Ascending

Solvent

Chloroform-water (1.5:8.5)

 R_F

Com- po- nent	R_F	Com- po- nent	R_F
A ₁	0.74-0.79	C ₁	0.25-0.30
A ₂	0.68-0.73	C ₂	0.22-0.27
B ₁	0.55-0.62	D ₁	0.18-0.23
B ₂	0.48-0.53	D ₂	0.13-0.19

Ref.

German Patent 102,162, May 12, 1973

PC 2**Paper**

Schleicher and Schuell No. 2043b Mg1, ascending, developed twice

Solvent

n-Propanol-water (1.5:8.5)

Detection

Bioautography vs. *Bacillus subtilis*

 R_F

Component A > B > C > D > E

Ref.

H. Thrum, K. Eckardt, G. Bradler, R. Fugner, E. Tonew and M. Tonew, *J. Antibiot.*, 28 (1975) 514-521

STREPTOVITACINS**PC 1****Paper**

Schleicher and Schuell 589 (Blue Ribbon special)

Solvents

A. Water satd. ethyl acetate; paper pre-impregnated with 0.1 *M* phosphate buffer, pH 4.0; equilibrate 16 h; develop 6 h

B. Upper phase of benzene-methanol-water (1:1:2). Equilibrate and develop as A

C. Butanol-water (84:16); develop 16 h without equilibration

Detection

Bioautography vs. *Saccharomyces pastorianus* ATCC 2366

 R_F (estimated from drawing)

Component	Solvent		
	A	B	C
A	0.35	0.0-0.1	0.52
B	0.45	0.0-0.1	0.55
C	0.55	0.0-0.1	0.60
D	0.72	0.0-0.1	0.68
E	0.87	0.0-0.1	0.75

Ref.

W.T. Sokolski, N.J. Eilers and G.M. Savage, *Antibiot. Ann.*, (1958-1959) 551-554

PC 2**Paper**

Whatman No. 1, 0.25 in. width

Solvent

As PC 1

Detection

Strips cut into 4-cm sections and exposed to ethylene oxide vapors for 1 h. Each section dropped into a tube containing 10 ml *Trichomonas vaginalis* culture containing 10⁴ cells/ml. After incubation at 37°C for 48 h, cells counted on a hemacytometer. Low counts indicated antitrichomonal activity

Ref.

As PC 1

PC 3 (Chromatography of streptovitacin A)*Solvent*Ethyl acetate-cyclohexane-McIlvaine's buffer,
pH 5.0 (7:1:8)

Ref.

T.E. Eble, M.E. Bergy, C.M. Large, R.R. Herr
and W.G. Jackson, *Antibiot. Ann.*, (1958-1959)
555-559**CCD (Purification of streptovitacin A)***Solvent**n*-Amyl alcohol-isoamyl alcohol-water
(12:17:29)

Ref.

As PC 3

STREPTOZOTOCIN**PC 1***Solvents*

- A. *n*-Butanol-water (84:16)
- B. As A + 0.25% (w/v) *p*-toluenesulfonic acid
- C. *n*-Butanol-acetic acid-water (2:1:1)
- D. Solvent A-piperidine (98:2)
- E. Water-*n*-butanol (96:4)
- F. As E + 0.25% *p*-toluenesulfonic acid

Detection

Bioautography

 R_F (estimated from drawing)

Solvent	R_F	Solvent	R_F
A	0.3	D	No zone
B	0.3	E	0.7
C	0.6	F	0.7

Ref.

J.J. Vavra, C. De Boer, A. Dietz, L.J. Hanka and
W.T. Sokolski, *Antibiot. Ann.*, (1959-1960) 230-
235**PC 2***Paper*

Whatman No. 3, ascending (Useful for radioactive streptozotocin)

Solvent

1-Butanol-acetic acid-water (2:1:1)

*Detection*Spraying a portion of the chromatogram with a
soln. consisting of 20 ml 6*N* hydrochloric acid,
50 ml 1% sulfanilic acid in 30% acetic acid, and50 ml 0.1% *N*-1-naphthylethylenediamine in 30%
acetic acid or by scanning the chromatogram
with a radiochromatogram scanner. The band
containing the streptozotocin was eluted with
water R_F

0.45-0.60

Ref.

S. Singaram, R.S. Lawrence and U. Hornemann,
J. Antibiot., 32 (1979) 379-385**CCD***Solvent*

Methyl ethyl ketone-water, 775 transfers

Distribution

Streptozotocin found in fractions 120-170

Ref.

R.R. Herr, T.E. Eble, M.E. Bergy and H.K.
Johnke, *Antibiot. Ann.*, (1959-1960) 236-240**SUBSPORINS****TLC***Medium*

Silica gel G

Solvent

Chloroform-methanol-70% ethanol (7:3:5)

 R_F

Subsporin C > B > A

Ref.

M. Ebata, K. Miyazaki and Y. Takahashi, *J.*
Antibiot., 22 (1969)**SUBTILIN****CCD***Solvents*

- A. *n*-Butanol-water (1:1)
- B. 20% Acetic acid-*n*-butanol (5:4)
- C. 20% Acetic acid-isobutanol (3:2)
- D. 20% Acetic acid-*n*-butanol (1:1)
- E. 4% Acetic acid-*sec.*-butanol (6:5)
- F. 20% Acetic acid-*n*-butanol (3:2)

Distribution

Sol-vent	K	Sol-vent	K
A	0.50	D	0.45
B	0.56, 0.55	E	0.33
C	0.33, 0.30, 0.28	F	0.40

Ref.

G. Alderton and N. Snell, *J. Amer. Chem. Soc.*,
81 (1959) 701

SUCCINIMYCIN

PC

Solvents

- A. *n*-Butanol–acetic acid–water (4:1:5)
- B. Isopropanol–0.2 *M* acetate buffer, pH 6.0 (70:30)
- C. Ethanol–0.05 *M* acetate buffer, pH 6.0 (80:20)

Detection

Bioautography vs. *Bacillus subtilis*

R_F

Solvent A	0.42
Solvent B	0.40
Solvent C	0.52

Ref.

T.H. Haskell, R.H. Bunge, J.C. French and Q.R. Bartz, *J. Antibiot.*, 16 (1963) 67–75

ELPHO

Medium

Paper

Buffer

0.05 *M* Acetate, pH 4.0

Conditions

360 V, 2.5 h

Mobility

7.9 cm

Ref.

As PC

CCD

Solvent

tert-Butanol–aq. 0.1 *M* sodium acetate buffer, pH 5.6, containing 142 g/l sodium sulfate (2:3)

Distribution

Two peaks; major peak, $K = 0.26$; minor peak, $K > 30$

Ref.

As PC

SULBENICILLIN

HPLC

Apparatus

ALC/GPC 204 liquid chromatograph (Waters Assoc.)

Column

Ion-pair system with μ Bondapak C₁₈ (30.4 cm $\times$ 4.0 mm I.D.) (Waters Assoc.) used as stationary phase

Mobile phase

Methanol–0.01 *M* tetrabutylammonium bromide (4:7)

Flow-rate

2.0 ml/min

Pressure

3000 p.s.i.

Detector

UV (254 nm) (Waters Assoc., Model 440)

Sample

Urine sample, 20 μ l

Type

Reversed-phase

Retention time

Estimated, 24.0 min

Ref.

As Carbenicillin, HPLC

SULFOCIDIN

PC

Solvent

Aq. 3% ammonium chloride, descending

Detection

Bioautography vs. *Sarcina lutea*

R_F

0.0, 0.06, 0.13, 0.30

Ref.

M. Zeif, R. Woodside and G.E. Horn, *Antibiot. Annu.*, (1957–1958) 886–892

SULFOMYCINS

PC 1

Solvents

- A. Wet *n*-butanol
- B. 20% Aq. ammonium chloride
- C. 50% Aq. acetone
- D. *n*-Butanol–methanol–water–methyl orange [40:10:20, (v/v):1.5 w/v]
- E. *n*-Butanol–methanol–water (40:10:20)
- F. Benzene–methanol (4:1)
- G. Water

Detection

Bioautography vs. *Bacillus subtilis*

R_F

Solvent	R_F (complex)	Solvent	R_F (complex)
A	0.79	E	0.87
B	0.00	F	0.63
C	0.63	G	0.00
D	0.92		

Ref.

Y. Egawa, K. Umino, Y. Tamura, M. Shimizu, K. Kaneko, M. Sakurazawa, S. Awataguchi and T.O. Kuda, *J. Antibiot.*, 22 (1969) 12–47

PC 2**Paper**

Toyo Roshi No. 51

Solvents

- A. Ethyl acetate satd. with water
 B. *n*-Butanol satd. with water
 C. Chloroform-methanol (10:1)
 All solvents developed ascending, 5 h

Detection

As PC 1

 R_F

Solvent	Sulfomycin		
	I	II	III
A	0.59	0.80	0.35
B	0.70	0.80	0.63
C	0.38	0.46	0.30

Ref.

As PC 1

TLC**Medium**Kieselgel GF₂₅₄**Solvents**

- A. Ethyl acetate satd. with water
 B. Ethyl acetate-*n*-butanol (1:1) satd. with water
 C. Chloroform-methanol (10:1)

Detection

As PC 1

 R_F

Solvent	Sulfomycin			
	I	II	III	Minor
A	0.13	0.26	0.05	—
B	0.87	0.93	0.84	—
C	0.38	0.46	0.30	0.42, 0.44, 0.49

Ref.

As PC 1

TAIMYCINS**TLC****Media**

- A. MN Kieselgel G₂₅₄
 B. Kieselgel G
 C. Kieselgel HF₂₅₄₋₃₆₆
 D. Alumina

Solvents

- A. Butanol-acetone-water (4:5:1)
 B. Ethyl acetate-pyridine-isopropanol-water (7:2:3:2)
 C. Propanol-ethyl acetate-water (8:1:1)
 D. Butanol-pyridine-water (6:4:3)

 R_F

Medium	Solvent	Taimycin		
		A	B	C
A	A	0.40	0.30	0.70
B	A	0.60	0.30	0.75
C	A	0.35	0.25	0.50
A	B	0.50	0.50	0.80
B	C	0.45	0.25	0.50
D	D	0.50	0.30	0.70

Ref.

G. Cassinelli, E. Cotta and R. Mazzoleni, *U.S. Patent* 3,644,619, Feb. 22, 1972

TALARON**ELPHO****Medium****Paper****Buffer**

Cellulose acetate at 600 V for 50 min (0.1 M phosphate buffer, pH 7.0)

Mobility

Migrated 2.4 cm toward anode

Ref.

K. Mizuno, A. Yagi, M. Takada, K. Matsuura, K. Yamaguchi and K. Asano, *J. Antibiot.*, 27 (1974) 560-563

TALLYSOMYCINS**TLC****Medium**

Silica gel

Solvents

- A. Methanol-10% ammonium acetate (1:1)
 B. Methanol-10% ammonium acetate-10% ammonium hydroxide (10:9:1)

Detection

- A. Ninhydrin
 B. UV

Ref.

H. Kawaguchi, H. Tsukiura, K. Tomita, M. Konishi, K. Saito, S. Kobaru, K. Numata, K. Fujisawa, T. Miyaki, M. Hatori and H. Koshiyama, *J. Antibiot.*, 30 (1977) 779-788

R_F

Tally- somy- cin	Solvent A		Solvent B	
	Cu- chelated	Cu- free	Cu- chelated	Cu- free
A	0.22	0.16	0.05	0.04
B	0.41	0.31	0.11	0.09
C	0.58	—	0.49	—
D	0.10	—	0.02	—

TBILIMYCIN**CCD***Solvents*

- A. Methanol–chloroform–borate buffer, pH 8.2 (2:2:1), 100 transfers
 B. As A, but 220 transfers

Distribution

- A. $K = 1.93$
 B. Tbilimycin peak, tube 145

Ref.

D.Yu. Shenin, E.N. Sokolova and E.Yu. Konev, *Antibiotiki*, 15 (1970) 9–14

TENEMYCIN**PC***Paper*

Descending

Solvents

- A. Water satd. *n*-butanol + 2% *p*-toluenesulfonic acid, (20 h)
 B. *n*-Propanol–pyridine–acetic acid–water (15:10:3:12), (20 h)
 C. *n*-Butanol–pyridine–acetic acid–water (6:4:1:3), (7 days)

 R_F

Solvent	R_F
A	9.0 • (two spots)
	4.29 ** (two spots)
B	10.9 •
	1.20 **
C	8.1 •
	1.45 **, 1.77 **

• Migration distance from original point in cm

** R_{767-L} represents the ratio of the value for migration distance of one antibiotic substance to the value for migration distance of the SF-767-L

substance. In determination of R_{767-L} , an amount of the SF-767-L substance was added as an internal standard to each of the antibiotic substances, and the mixture was then developed

Ref.

T. Shomura, N. Ezaki, T. Tsuruoka, T. Niwa, E. Akita and T. Niida, *U.S. Patent* 3,697,648, Oct. 10, 1972

TENNECETIN**PC***Paper*

Whatman No. 1

Solvent

Water satd. *n*-butanol, descending

Detection

Bioautography vs. *Saccharomyces carlsbergensis* K-20

 R_F

0.33

Ref.

J. Burns and D.F. Holtman, *Antibiot. Chemother.*, 9 (1959) 398–405

TERTIOMYCINS**PC 1***Solvents*

- A. Benzene–citrate buffer, pH 4.6
 B. Butyl acetate–citrate buffer, pH 4.0

 R_F

Tertiomycin B

Solvent A 0.08–0.11

Solvent B 0.69–0.85

Ref.

T. Osato, K. Yagashita and H. Umezawa, *J. Antibiot.*, 8 (1955) 161–163

PC 2*Solvents*

- A. 1% Aq. ammonium hydroxide
 B. 20% Dioxane
 C. Butanol–acetic acid–water (70:5:25)
 D. Butanol–ammonium hydroxide–water (70:5:25)
 E. Ethyl acetate–acetic acid–water (88:6:6)
 F. Ethyl acetate–ammonium hydroxide–water (88:6:6)
 G. Water
 H. Benzene–water (80:20)
 I. Butanol–methanol–water (4:1:2)
 J. 5% Acetone
 K. 20% Ammonium chloride

L. Water satd. diethyl ether

M. 1% Acetic acid

N. Ethyl acetate

R_F

Tertiomycin A

Solvent	R_F	Solvent	R_F
A	0.41	H	1.00
B	0.00	I	1.00
C	1.00	J	1.00
D	1.00	K	0.84
E	1.00	L	1.00
F	0.00	M	0.85
G	0.33	N	1.00

Ref.

A. Miyake, H. Iwasaki and T. Takewaka, *J. Antibiot.*, 12 (1959) 59-64

CCD

Solvent

Citrate buffer, pH 4.6-benzene; 30 transfers

Distribution

Peak found at tube 21

Ref.

T. Osato, M. Ueda, S. Fukuyama, K. Yagishita, Y. Okami and H. Umezawa, *J. Antibiot.*, 8 (1955) 105-109

TETRACYCLINE, OXY- (See oxytetracycline)

TETRACYCLINES

This section includes data on chlor-tetracycline, 6-demethyltetracycline, oxytetracycline, tetracycline and various tetracycline derivatives and degradation products. Because the published data often includes a variety of these antibiotics in a given chromatographic system, the tetracyclines have been grouped by system rather than by compound. It may be necessary to examine each chromatographic category in order to locate a particular member of this family of antibiotics.

Many of the tetracyclines can be detected by exhibition of a bright yellow or orange fluorescence under UV light which is enhanced by gentle treatment with am-

monia vapor. Bioautography against a number of susceptible organisms is useful in detecting trace quantities of these antibiotics

PC 1

Paper

Whatman No. 4

Solvent

n-Butanol-acetic acid-water (4:1:5)

Detection

A. UV fluorescence

B. Bioautography vs. *Bacillus subtilis*

R_F

Oxytetracycline 0.55

Ref.

P.P. Regna and I.A. Solomons, *Ann. N.Y. Acad. Sci.*, 53, Art. 2 (1950) 229-237

PC 2

Solvents

A. Paper treated with McIlvaine's buffer, pH 3.5, as stationary phase; mobile phase: nitromethane-chloroform-pyridine-*n*-butanol (20:10:5:3)

B. Stationary phase as A, but pH 4.2; mobile phase, toluene-pyridine (20:3)

C. Stationary phase as B; mobile phase, ethyl acetate satd. with water

Detection

UV light

R_F

Compound	Solvent system		
	A	B	C
12 α -Desoxy-tetracycline	0.70-0.75	-	-
10,12 α -(O,O-Di-acetyl)-5-oxy-tetracycline	-	0.13-0.21	0

Ref.

German "Auslegeschrift" 114138, Dec. 27, 1962

PC 3

Paper

Eaton-Dikeman No. 613 soaked in 0.3 *M* phosphate buffer, pH 3, and air-dried

Solvents

A. *n*-Butanol satd. with water

B. *n*-Amyl acetate satd. with water, 48-72 h (solvent allowed to drip off paper)

DetectionBioautography vs. *Bacillus cereus* at pH 6.0**R_F**

Solvent system A

Tetracycline 0.40 ± 0.06

Oxytetracycline 0.40 ± 0.06

Chlortetracycline 0.65 ± 0.05

Solvent system B

Oxytetracycline > tetracycline; chlortetracycline moves off paper

Ref.

N. Bohonos, A.C. Dornbush, L.I. Feldman, J.H. Martin, E. Pelcak and J.H. Williams, *Antibiot. Ann.*, (1953-1954) 49-55

PC 4**Paper**

Schleicher and Schuell No. 507

SolventAcetic acid-*n*-butanol-water (1:2:1)**Detection**

Bioautography

R_F

Chlortetracycline, 0.77

Ref.

R.J. Hickey and W.F. Phillips, *Anal. Chem.*, 26 (1954) 1640

PC 5**Paper**

Whatman No. 1

Solvent

Mix *n*-butyl acetate-methyl isobutyl ketone-*n*-butanol-water (5 ml:15 ml:2 ml:22 ml). Add 2 ml formic acid to the separated organic phase. Develop ascending

Detection

Spray with 5% methanolic soln. of ferric chloride

R_F

Chlortetracycline > tetracycline > oxytetracycline

Ref.

H. Fischbach and J. Levine, *Antibiot. Chemother.*, 5 (1955) 610-612

PC 6**Paper**

Whatman No. 1. Buffer paper with McIlvaine's buffer, pH 3.5, blot firmly, spot antibiotics and develop in solvent while still damp

Solvent

Chloroform-nitromethane-pyridine (10:20:3), ascending

Detection

UV fluorescence; greatly enhanced by fuming with ammonia vapor

R_F

Chlortetracycline 0.50

Tetracycline 0.28

Oxytetracycline 0.13

epi-Chlortetracycline 0.08*epi*-Tetracycline 0.05**Ref.**

G.B. Selzer and W.W. Wright, *Antibiot. Chemother.*, 7 (1957) 292-296

PC 7**Solvents**A. 0.3 M Sodium phosphate, pH 3.0-*n*-butanol

B. McIlvaine's buffer, pH 4.7-ethyl acetate

C. 0.3 N Phosphoric acid + 0.1% trichloroacetic acid/chloroform-*n*-butanol (9:1)**R_F**

	Solvent system		
	A	B	C
7-Chloro-5a(11a)-dehydrotetracycline	0.49	0.87	0.39
5- <i>epi</i> -Tetracycline	0.65	—	—

Ref.

J.R.D. McCormick, P.A. Miller, J.A. Growich, N.O. Sjolander and A.P. Doerschuk, *J. Amer. Chem. Soc.*, 80 (1958) 5572

PC 8**Paper**

Whatman No. 1 impregnated by drawing through 0.1 M disodium ethylenediaminetetraacetic acid and drying in air

SolventsA. *n*-Butanol-acetic acid-water (4:1:5), upper phase, descending, 16-20 h

B. *n*-Butanol-ammonium hydroxide-water (4:1:5), upper phase, descending, 16-20 h. Purge chamber with nitrogen (to remove O₂) before use

Detection

Expose chromatogram to ammonia vapor and view under UV light. Most compounds give yellow to orange spot; *iso* compounds, blue fluorescence; chlortetracycline, blue fluorescence in solvent A

R_F

	Solvent System	
	A	B
Tetracycline	0.65	0.39
4- <i>epi</i> -Tetracycline	0.65	0.15
Oxytetracycline	0.59	0.27
Chlortetracycline	0.76	0.47
Isochlortetracycline	0.67	0.47
Anhydrotetracycline	0.87	0.62
4- <i>epi</i> -Anhydrotetracycline	0.87	0.40
Demethylchlortetracycline	0.72	0.35
4- <i>epi</i> -Demethylchlortetracycline	0.72	0.17
Isotetracycline	0.46	0.21
Anhydromethylchlortetracycline	0.89	0.70
4- <i>epi</i> -Anhydromethylchlortetracycline	0.92	0.44

Ref.

R.G. Kelly and D.A. Buyske, *Antibiot. Chemother.*, 10 (1960) 604-607

PC 9

Paper

Whatman No. 1; paper is moistened with a soln. composed as follows: 500 ml 0.1 *N* citric acid, 208 ml 0.2 *N* disodium phosphate containing 10 mg of sodium benzoate (as preservative) and satd. with a solvent mixture of nitromethane-chloroform-pyridine (20:10:3)

Solvent

Organic phase of above mixture, descending

 R_F

Tetracycline	0.45
4- <i>epi</i> -Tetracycline	0.10
6-Demethyl-7-chlortetracycline	0.37
4- <i>epi</i> -6-Demethyl-7-chlortetracycline	0.12

Ref.

M.M. Noseworthy, *U.S. Patent* 3,009,956, Nov. 21, 1961

PC 10

Paper

Whatman No. 4 satd. with aq. citrate-phosphate buffer, pH 4.2

Solvent

Toluene-pyridine (20:3), satd. with water

 R_F

6-Demethyl-6-deoxytetracycline	0.47
C-4 epimer of above	0.30

Ref.

As PC 9

PC 11

Paper

Whatman No. 1; useful for radioactive 5-hydroxytetracycline. Paper buffered with a mixture of 0.3 *M* sodium dihydrogen phosphate adjusted to pH 3.0 with phosphoric acid, and air dried

Solvents

- A. Ethyl acetate/phosphate-citrate buffer, pH 4.5. Buffer composed of equal vols. of 0.4 *M* disodium phosphate and 4.5% citric acid. Develop descending 18 h
- B. Nitromethane-benzene-pyridine-buffer, pH 3.4 (20:10:3:3). Buffer composed of 30 vols. of 0.2 *M* disodium phosphate and 70 vols. of 2.24% citric acid. Develop descending 18 h

Detection

Scan with Geiger-Muller counter

 R_F

5-Hydroxytetracycline

Solvent A 0.58

Solvent B 0.23

Ref.

P.A. Miller and J.R.D. McCormick, *U.S. Patent* 3,023,148, Feb. 22, 1962

PC 12

Paper

Whatman No. 1 satd. with disodium phosphate-citric acid buffer at pH 3.5

Solvents

- A. Nitromethane-chloroform-pyridine (20:10:3)
- B. Nitromethane-toluene-butanol-pyridine (20:10:5:3)

 R_F

0.35 in both solvents

Ref.

R.K. Blackwood, *U.S. Patent* 3,026,354, March 20, 1962

PC 13

Paper

Whatman modified cellulose phosphate cation-exchange paper

Solvent

0.1% (w/v) Aq. ammonium chloride

 R_F

Tetracycline	0.59
<i>epi</i> -Tetracycline	0.36
Chlortetracycline	0.61
Oxytetracycline	0.61
6-Demethylchlortetracycline	0.53
<i>epi</i> -6-Demethylchlortetracycline	0.32

6-Demethyl-6-desoxytetracycline	0.42
<i>epi</i> -6-Demethyl-6-desoxytetracycline	0.21
6-Methylene oxytetracycline	0.46
Anhydrotetracycline	0.14

Ref.

E. Addison and R.G. Clark, *J. Pharm. Pharmacol.*, 15 (1963) 268–272

PC 14 (Circular chromatography)*Paper*

Whatman No. 1, 28-cm diameter

Solvent

McIlvaine's buffer, pH 4.5–chloroform–*n*-butanol (4:1), 90 min

Detection

UV fluorescence

R_F

Chlortetracycline	0.70–0.73
Demethylchlortetracycline	0.56–0.60
Tetracycline	0.47–0.50
Demethyltetracycline	0.38–0.41
Epimers	0.27–0.30

Ref.

M. Urx, J. Vondrackova, L. Kovarik, O. Horsky and M. Herold, *J. Chromatogr.*, 11 (1963) 62–65

PC 15*Paper*

Treated with buffer, as below

Solvents

- Buffer, pH 4.2–benzene–chloroform (1:1) satd. with water
- Buffer, pH 4.2–toluene–pyridine (20:3) satd. with buffer, pH 4.2
- Buffer, pH 3.5–nitromethane–chloroform–pyridine (20:10:3)
- Buffer, pH 3.5–ethyl acetate satd. with water

R_F

	Solvent system			
	A	B	C	D
4a,12a-Anhydro-tetracycline	0.86	0.88	0.90	1.00
12a-(O-Formyl)tetracycline	0.05	0.27	0.63	0.65
12a-(O-Formyl)chlortetracycline	–	0.52	–	–

Ref.

C.R. Stephans, Jr., and R.K. Blackwood, *U.S. Patent* 3,081,346, March 12, 1963

PC 16*Paper*

Leningrad "Quicky" soaked with McIlvaine's buffer, pH 4.5, and dried

Solvent

Ethyl acetate–water (1:1), 3–4 h

Detection

UV fluorescence after exposing to ammonia vapors 5–10 sec

R_F

From starting line; *iso*-tetracycline, *epi*-tetracycline, *epi*-chlortetracycline, *iso*-chlortetracycline, tetracycline, hydroxytetracycline, demethylchlortetracycline, chlortetracycline, anhydrotetracycline, anhydrochlortetracycline

Ref.

T.N. Lasnikova and N.G. Makarevich, *Antibiotiki*, 9 (1964) 579–583

PC 17*Paper*

Leningrad "Quicky" treated with phosphate buffer, pH 2.5 (0.3 *M* soln. phosphoric acid brought to pH 2.5 with strong soln. of potassium hydroxide). Paper is used while still damp

Solvent

n-Butanol–acetic acid–water (4:1:5), 20 h

Detection

UV fluorescence

R_F

From starting line; *iso*-tetracycline, hydroxytetracycline, tetracycline, *iso*-chlortetracycline, demethylchlortetracycline, chlortetracycline, anhydrotetracycline

Ref.

As PC 16

PC 18*Paper*

Paper satd. with 0.3 *M* phosphate buffer, pH 2.5, and air dried

Solvents

- n*-Butanol satd. with water; equilibrate 4 h, develop overnight
- Ethyl acetate satd. with water; equilibrate overnight
- Chloroform–2-chloroethanol–water (2:1:1), organic phase; equilibrate overnight

R_F (see p. 370)*Ref.*

J.H. Martin, L.A. Mitscher, P.A. Miller, P. Shu and N. Bohonos, *Antimicrob. Agents Chemother.*, 1966 (1967) 563–567

R_F

	Solvent system		
	A	B	C
5-Hydroxy-7-chlorotetracycline	0.65	0.42	0.56
7-Chlortetracycline	0.65	0.03; 0.25 •	0.35; 0.65
Tetracycline	0.38	0.01; 0.05	0.16; 0.38
7-Chloro-6-demethyltetracycline	0.52	0.01; 0.15	0.23; 0.25
2-Acetyl-5-hydroxytetracycline	0.63	—	0.65
5-Hydroxytetracycline	0.38	0.08	0.17
Reduction product of oxychlorotetracycline	0.37	0.08	0.17

• The double zones reflect the separation of the 4-epimers. In each case the natural product has the higher R_F value and its 4-epimer the lower

PC 19

Paper

Whatman No. 4 impregnated with 0.15 *M* phosphate buffer, pH 3.0, and dried

Solvent

n-Butanol–chloroform (9:1), 6 h, 22–25°C

 R_F

Tetracycline	0.34–0.42
Chlortetracycline	0.57–0.68
Anhydrotetracycline	0.79–0.88

Ref.

J. Vondráčková and O. Štrauchová, *J. Chromatogr.*, 32 (1968) 780–781

PC 20

Paper

Treated with buffer at pH 3.5 or 4.2 as noted under solvent

Solvents

- Pyridine–toluene (3:20) satd. with water, pH 4.2 paper
- Toluene–1-butanol–nitromethane–pyridine (10:5:20:3), pH 3.5 paper
- Ethyl acetate–nitromethane–chloroform (40:25:7), pH 4.2 paper

D. Ethyl acetate–chloroform–pyridine (40:15:5), pH 4.2 paper

E. Ethyl acetate satd. with water, pH 4.2 paper

R_F (estimated from drawing)

DL-6-demethyl-6-deoxytetracycline

Solvent	R_F	Solvent	R_F
A	0.5	D	0.6
B	0.9	E	0.7
C	0.8		

Ref.

J.J. Korst, J.D. Johnston, K. Butler, E.J. Bianco, L.H. Conover and R.B. Woodward, *J. Amer. Chem. Soc.*, 90 (1968) 439–457

PC 21

Paper

Whatman No. 1 buffered at pH 4.5 with 0.05 *M* potassium citrate and hydrated by dipping into an aq. soln. of 80% (v/v) acetone and air drying to evaporate the acetone

Solvent

Hexane–ethyl acetate (3:1), descending

Detection

- UV light at 254 nm to detect absorbing and fluorescent compounds. Spots are outlined, the chromatogram exposed momentarily to ammonia vapor and re-examined under UV light
- Bioautography vs. *Staphylococcus aureus* 209P

 R_F

Compound	R_F	Inhibitory activity	Fluorescence	
			Before ammonia	After ammonia
7-Chloro-6-demethyl-4-dedimethyl-amino-tetracycline	0.80	+	Dull orange	Green
7-Chloro-6-demethyl-5a,-6-anhydrotetracycline	0.94	+	Red-orange	Orange
7-Chloro-6-demethyl-5a,-6-anhydro-4-dedimethyl-aminotetracycline	0.98	+	Red-orange	Orange

Compound	R_F	Inhibi- tory activ- ity	Fluorescence	
			Before am- monia	After am- monia
9-Hydroxy-7-chloro-5-demethyl-4-dedimethyl-amino-tetracycline	0.15	+	Orange	Orange
9-Hydroxy-7-chloro-6-demethyl-tetracycline	0.00	+	Orange	Orange

Ref.

S.L. Neidleman, R.W. Kinney and F.L. Weisenborn, *U.S. Patent* 3,375,276, March 26, 1968

PC 22*Paper*

Whatman No. 1 satd. with phosphate buffer, pH 3.0, and dried at room temperature

Solvent

n-Butanol satd. with water, 5–10°C, ascending, 48 h

Detection

Air dry chromatograms and hang in ammonia chamber for 15 sec to neutralize acid. Expose to UV for 30 sec. Spray with arsenomolybdate reagent and heat 10–15 min at 90°C. Greenish spots result

 R_F

Chlortetracycline > tetracycline

Ref.

Abou-Zeid A. Abou-Zeid, *Indian J. Pharm.*, 32 (1970) 59–61

PC 23*Paper*

Whatman No. 1 treated with 0.3 *M* phosphate buffer, pH 2.0, as stationary phase

Solvents

- Butanol satd. with stationary phase
- Upper layer of *n*-butyl acetate–0.3 *M* phosphate buffer, pH 2.0–5% trichloroacetic acid (5:4:1)

Ref.

M. Tobkes and R.G. Wilkinson, *U.S. Patent* 3,549,681, Dec. 22, 1970

 R_F

	Sol- vent	R_F
6-Demethyltetracycline-6-sulfuric acid ester	A	0.25
7-Chloro-6-demethyltetracycline-6-sulfuric acid ester	A	0.29
7-Chloro-6-demethyl-9-nitrotetracycline-6-sulfuric acid ester	A	0.29
5a,6-Anhydro-7-chloro-6-demethyltetracycline	B	0.68
5a,6-Anhydro-6-demethyl-tetracycline	A	0.72
	B	0.53
5a,6-Anhydro-7-chloro-6-demethyl-9-nitrotetracycline	A	0.70

PC 24*Solvents*

- 0.3 *M* Sodium phosphate, pH 3.0–*n*-butyl acetate
- 0.3 *M* Sodium phosphate, pH 3.0–*n*-butanol
- McIlvaine's buffer, pH 4.7–ethyl acetate
- 0.3 *N* Phosphoric acid–0.1% trichloroacetic acid/chloroform–*n*-butanol (9:1)

 R_F

Solvent A	0.30
Solvent B	0.30
Solvent C	0.27
Solvent D	0.22

Ref.

J.A. Growich, Jr., *U.S. Patent* 3,616,240, Oct. 26, 1971

PC 25*Paper*

Whatman No. 1

Solvents

- 80% Methanol + 3% sodium chloride (1:1) descending. Paper buffered with 0.95 *M* sodium sulfate + 0.05 *M* sodium bisulfate
- Propanol–pyridine–acetic acid–water (6:4:1:3), ascending
- Butanol–acetic acid–water (4:1:5), ascending
- 80% Phenol, ascending

Detection

Bioautography vs. *Staphylococcus aureus*

 R_F (see p. 372)*Ref.*

As Chloramphenicol, PC 5

R_F

Solvent	Tetra- cycline	Chlortetra- cycline
A	0.72	0.74
B	0.70	0.75
C	0.38	0.46
D	0.83	0.87

TLC 1 (Circular chromatography)

Media

- A. Silica gel G (according to Stahl). Plates activated at 110°C for 1 h and stored in a vacuum desiccator
- B. 9 g of EDTA dissolved in 60 ml water and 30 g silica gel G added. Plates coated to give a 250 μ m thickness. Dry at room temperature before placing in oven and store as in (A)
- Both A and B plates contain $\frac{1}{8}$ in.-diameter hole in center. Compounds spotted around center hole

Solvents

- A. *n*-Butanol-oxalic acid-water (100 ml:5 g:100 ml), organic phase
- B. *n*-Butanol-tartaric acid-water (100 ml:6 g:100 ml), organic phase
- C. *n*-Butanol satd. with water
- Development carried out using 14-cm Petri dishes; Whatman No. 1 paper wick inserted in center hole and dipped into solvent, plate placed face down. Top of hole is covered with a vial. Development allowed to proceed until solvent front reaches 6.5 cm from center

Detection

- A. UV light
- B. Spray with 5% methanolic soln. of ferric chloride. Dark grayish bands result

 R_F

	Medium-Solvent		
	A-A	A-B	B-C
Tetracycline	0.38	0.26	0.23
Oxytetracycline	0.46	0.31	0.27
Chlortetracycline	0.49	0.35	0.30
HCl	0.49	0.35	0.30

Ref.

G.J. Kapadia and G.S. Rao, *J. Pharm. Sci.*, 53 (1964) 223-224

TLC 2

Medium

Kieselguhr G impregnated with glycerol/phosphate-citrate buffer soln. (pH 3.7). Buffer is prepared by mixing 34 ml 0.2 *M* disodium hydrogen phosphate with 66 ml 0.1 *M* citric acid. For impregnation, 95 ml buffer is brought to 100 ml with glycerol. The plate is impregnated by placing the edge in the buffer solution in a chamber. After impregnation, air dry for 45 min at room temp.

Solvent

Chloroform-acetone (1:1) saturated with impregnating soln.

Detection

UV light at 350 nm

 R_F

Chlortetracycline > tetracycline > oxytetracycline

Ref.

D. Sonamini and L. Anker, *Pharm. Acta Helv.*, 39 (1964) 518-523.

TLC 3

Medium

Coat plates with a mixture of silica gel G (after Stahl) and sodium ethylenediaminetetraacetate (sodium EDTA). Mix 30 silica gel in a soln. of 9 g sodium EDTA in 50 ml water and coat to a thickness of 0.25 mm. Dry at 100°C for 30 min

Solvent

n-Butanol satd. with water

Detection

UV light after exposure to ammonia vapor

 R_F

Tetracycline	0.36
4- <i>epi</i> -Tetracycline	0.36
4- <i>epi</i> -Anhydrotetracycline	0.40
Anhydrotetracycline	0.50

Ref.

L. Rustici and M. Ferappi, *Boll. Chim. Farm.*, 104 (1965) 305-308

TLC 4

Medium

Microcrystalline cellulose (50 g) is passed through a 100 mesh sieve and mixed in a mortar and pestle for 2 min with 180 ml of 0.05% ammonium chloride soln. A 0.25-ml layer is applied to glass plates, dried at room temperature for 10 min and heated at 90°C for 30 min. No special storage conditions are necessary

Solvent

0.1% Aq. ammonium chloride, pH 5.6, developed 20 min

Detection (anhydrotetracycline)

- A. Qualitative: visible yellow zones
 B. Quantitative: individual zones scraped off and collected in 3-ml sintered glass funnels. Wash into 10-ml volumetric flasks with hot methanol and determine absorbance at 428 nm against a methanol blank. Average 5 determinations for each concentration and read against standard curve in range 10–60 μ g anhydrotetracycline per spot

R_F

0.35

Ref.

D.L. Simmons, C.K. Koorengel, R. Kubelka and P. Seers, *J. Pharm. Sci.*, 55 (1966) 219–220

TLC 5**Medium**

Diatomaceous earth prepared as follows: Wash diatomaceous earth with hot 6 *N* hydrochloric acid until washings contain no calcium or iron. Wash with water to neutral pH and dry at 105°C. Triturate a slurry of 8 g of acid-washed diatomaceous earth and 16 ml buffer [5 ml of 20% (v/v) PEG 400 in glycerin with 95 ml of 0.1 *M* ethylenediaminetetraacetic acid (EDTA) previously adjusted to pH 7.0 with ammonium hydroxide]. Pour plates, air dry for 35–50 min and use immediately

Solvent

Ethyl acetate–0.1 *M* EDTA previously adjusted to pH 7.0 (6:1); use organic layer. Equilibrate jar for 30 min prior to use

Detection

UV light

R_F

- A. Anhydromethyl chlortetracycline > demethylchlortetracycline > demethyltetracycline > *epi*-demethylchlortetracycline
 B. Chlortetracycline > demethylchlortetracycline > tetracycline
 C. Anhydrotetracycline > chlortetracycline > tetracycline > *epi*-tetracycline
 D. Anhydrochlortetracycline > chlortetracycline > tetracycline > *epi*-chlortetracycline

Ref.

P.P. Ascione, J.B. Zagar and G.P. Chrekian, *J. Pharm. Sci.*, 56 (1967) 1393–1395

TLC 6**Medium**

Kieselguhr; prepared as follows: 500 g Kieselguhr stirred with 3 l hydrochloric acid (1:2) for 2 h, decanted, filtered by suction, and repeated 3 times. For decanting wash with water until chlorides are eliminated, dry at 100°C, and sift through sieve No. 200 ASTM. A slurry of 40 g of Kieselguhr is made with 80 ml of aq. 5% EDTA neutralized to pH 7.5 or 9.0 with 20% sodium hydroxide. A 0.3-mm coating is made, dried at room temperature for 1 h and then at 100°C for 1 h

Solvents

- A. Acetone–water (10:1)
 B. Acetone–ethyl acetate–water (20:10:3)

Detection

UV light at 366 nm

R_F

	pH–Solvent	
	7.5–B	9.0–A
Anhydrotetracycline	0.88	0.84
Tetracycline	0.71	0.69
<i>epi</i> -Anhydrotetracycline	0.46	0.55
<i>epi</i> -Tetracycline	0.38	0.22

Ref.

A.A. Fernandez, V.T. Noceda and E.S. Carrera, *J. Pharm. Sci.*, 58 (1969) 443–446

TLC 7**Medium**

Silica gel G buffered with phosphate buffer, pH 3.0

Solvent

n-Butanol satd. with water

Detection

- A. UV light
 B. Bioautography vs. *Bacillus subtilis* NRRL B-543

R_F

Chlortetracycline > tetracycline

Ref.

As PC 22

TLC 8**Medium**

Microgranular cellulose (Whatman), 30 g/75 ml distilled water. Apply 0.5-mm layer, air dry for 10 min at room temp., then heat at 90°C for 30 min

Solvent

Spray plate uniformly with 10 ml of buffer (0.1 *M* disodium EDTA–0.1% ammonium chloride) and immediately develop with buffer-satd. chloroform for 16 cm (about 45 min). Air dry

Detection

Expose to ammonia for 2 min and view under short-wave UV

R_F

Tetracycline hydrochloride	0.0–0.25
Anhydrotetracycline hydrochloride	0.93
4- <i>epi</i> -Anhydrotetracycline hydrochloride	0.48

Ref.

P.B. Lloyd and C. Cornford, *J. Chromatogr.*, 53 (1970) 403–405

TLC 9**Medium**

Kieselguhr G (Merck) prepared as follows: 50 g Kieselguhr slurried in a mixture of 0.1 *M* aq. EDTA: 20% (v/v) PEG 400 in glycerin (95:5). Coat plates with 0.25-ml layer, dry at room temp. 4 h overnight

Solvents

- Methyl ethyl ketone satd. with McIlvaine's buffer, pH 4.7
- Dichloromethane–ethyl formate–ethanol (9:9:2) satd. with McIlvaine's buffer, pH 4.7

Detection

A. UV light

B. Spray reagents:

- Fast Blue B (Diazo-Reagent). Spray soln. A: 0.5% aq. freshly prepared soln. of Fast Blue B. Spray soln. B: 0.1 *N* aq. sodium hydroxide
- Diazotized *p*-nitroaniline. Spray soln. A: just before spraying, 5% aq. sodium nitrite soln. (1.5 ml) is added to 0.3% *p*-nitroaniline in 8% hydrochloric acid (25 ml). Spray soln. B: 20% aq. sodium carbonate soln. After spraying with soln. A, spray with soln. B taking care not to make the plate transparent with excess of the sprays
- Modified Sakaguchi Reagent. Boric acid (5 g) is dissolved in water (150 ml) and conc. sulfuric acid (350 ml). The reagent is stored in a glass-stoppered bottle in a refrigerator and is used cold
- Diphenylpicrylhydrazyl (DPPH) reagent. Soln. A: methanolic soln. of DPPH (~1 mg/2 ml). Soln. B: 25% aq. sodium hydroxide

R_F

	<i>R_F</i> *		Color **				
	Solvent system		Reagent				
	A	B	1	2	3 ***	4	
Tetracycline	0.53	0.36	Pk	Y	Y		Pk
Chlor-tetracycline	0.76	0.60	Pk	Y	Y		Y–Pk
Demethylchlor-tetracycline	0.73	0.44	Pk	Y	Y		Pk–Y
Oxytetracycline	0.60	0.20	Y	Pk–Y	Y		Br–Y
Methacycline	0.44	0.29	Pk–Br	Y	Y		Y
Doxy-cycline	0.53	0.57	Pk–Br	Y	Y		Pk
4- <i>epi</i> -Tetracycline	0.20	0.12	Y	Y	Y		Pk–Y
Anhydro-tetracycline	0.93	0.83	Pk	Y	Y		Y–Pk
<i>epi</i> -Anhydro-tetracycline	0.47	0.50	Y–Pk	Y	Y		Y–Pk
4- <i>epi</i> -Chlor-tetracycline	0.33	0.21	Y–Pk	Y	Y		Pk–Y
Anhydro-chlor-cycline	0.83	0.57	Pk	Y	Y		Y

* *R_F* values vary considerably with tank temp., especially in the case of stored plates. If very low *R_F* values are obtained, the chromatograms after brief drying may be rechromatographed in the same solvent system

** Pk=pink, Y=yellow, Br=brown

*** Colors change with excess of spray reagent and with time

Ref.

N.D. Gyanchandani, I.J. McGilveray and D.W. Hughes, *J. Pharm. Sci.*, 59 (1970) 224–228

TLC 10*Medium*

Kieselguhr (E. Merck) prepared as follows: Slurry 40 g Kieselguhr with 80 ml of 5% aq. EDTA neutralized to pH 7.5 with either 20% sodium hydroxide or conc. ammonium hydroxide. Coating, 0.3 mm; dry overnight

Solvent

Acetone–ethyl acetate–water (80:40:12), develop to height of 15 cm

Detection

- A. Isolate band, measure absorbance of extracted material at 430 nm
- B. Spray with 0.5% aq. Fast Blue Salt B and heat for 3 min at 110°C. Purple–pink spots result. For quantitation, these can be analyzed by densitometry and compared with known concentrations

Ref.

C. Radecka and W.L. Wilson, *J. Chromatogr.*, 57 (1971) 297–302

TLC 11*Medium*

Diatomaceous earth

Solvent

Ethyl acetate satd. with 0.1 M EDTA

R_F

6-Methylenetetraacycline > tetracycline > 6-deoxy-5-oxytetraacycline > 5-oxytetraacycline > 6-methylene-5-oxytetraacycline

Ref.

W. Sobiczewski and M. Domradzki, *Chemia Analityczna*, 16 (1971) 131–134

TLC 12*Medium*

Talc suspended in citrate–phosphate buffer (pH 3.5–4.5) plates sprayed with 0.1 M EDTA, pH 7.0 before use

Solvent

As TLC 1

R_F

6-Methylenetetraacycline > 5-oxytetraacycline > tetracycline > 6-deoxy-5-oxytetraacycline > 6-methylene-5-oxytetraacycline

Ref.

As TLC 11, pp. 433–437

TLC 13*Medium*

Polyamide 11 (Rislan)

Solvents

- A. *n*-Butanol–chloroform–acetic acid (10:90:0.5)
- B. *n*-Butanol–acetic acid–water (4:1:5)
- C. *n*-Butanol–methanol–10% aq. citric acid (4:3:2)
- D. McIlvaine's buffer soln., pH 4.6–*n*-butanol–chloroform–methanol (5:3:1:1)
- E. McIlvaine's buffer soln., pH 4.6–*n*-butanol–chloroform (5:4:1)

R_F

Component	Solvent				
	A	B	C	D	E
Tetracycline	0.82	0.79	0.80	0.74	0.73
Oxytetracycline	0.48	0.52	0.73	0.62	0.62
Chlortetracycline	0.81	0.79	0.78	0.51	0.49
Rolitetra-cycline	0.80	0.76	0.80	0.75	0.74
Demethyl-chlortetra-cycline	0.73	0.78	0.73	0.54	0.55
Doxycycline hyclate	0.59	0.76	0.73	0.53	0.31
Metacycline	0.38	0.52	0.64	0.28	0.25

Ref.

M.S. Santos, M.I. Mamede Santos, L.C. Goncalves and M.T. Morgadinho, *Bol. Fac. Farm., Univ. Coimbra, Ed. Cient.*, 33 (1973) 127–134

TLC 14*Medium*

Silica gel

Solvents

- A. 10% Tartaric acid
- B. 10% Tartaric acid satd. with *n*-butanol

Detection

- A. Iodine vapor
- B. 0.25% Stannous chloride in 20% hydrochloric acid
- C. 2% *p*-Dimethylaminobenzyldehyde in 1 N hydrochloric acid

R_F (see p. 376)*Ref.*

As Cephalosporins, TLC 17

R_F

L=Light; D=dark; B=brown; G=green; O=orange; P=pink; and Y=yellow

Tetracycline	Solvent		Detection		
	A	B	A	B	C
Oxytetracycline hydrochloric acid	0.65	0.85	LB	GB	GB
Doxycycline hydrochloric acid	0.55	0.73	LB	—	LP
Demeclocycline hydrochloric acid	0.48	0.67	LB	—	LY
Chlortetracycline hydrochloric acid	0.44	0.63	LB	OY	OY
Tetracycline hydrochloric acid	0.40	0.60	LB	YB	YB
Metacycline hydrochloric acid	0.57	0.80	LB	DB	DB

TLC 15

Medium

Kieselgel (with 5% Titriplex soln.)

Solvent

Benzene-acetic acid-formic acid (10:3:1)

 R_F

6-Desmethyl-6-desoxytetracycline, 0.41

Ref.

German Patent 2,349,909, April 17, 1975

TLC 16

Medium

Cellulose pre-coated (Merck)

Solvents

- A. 0.25 M Magnesium chloride
- B. 0.20 M Calcium chloride
- C. 0.25 M Barium chloride
- D. 0.15 M Zinc chloride

Detection

UV

 R_F

Tetracycline	Solvent			
	A	B	C	D
Tetracycline	0.72	0.67	0.63	0.65
4- <i>epi</i> -Tetracycline	0.68	0.63	0.60	0.65
Anhydrotetracycline	0.29	0.25	0.24	0.22
4- <i>epi</i> -Anhydrotetracycline	0.25	0.16	0.17	0.18
Oxytetracycline	0.72	0.68	0.63	0.65
Chlorotetracycline	0.62	0.59	0.58	0.52
Demethylchlorotetracycline	0.64	0.57	0.54	0.55
Methacycline	0.54	0.47	0.44	0.50
Doxycycline	0.53	0.50	0.49	0.48
Minocycline	0.76	0.70	0.60	0.68

Ref.

E. Ragazzi and G. Veronese, *J. Chromatogr.*, 132 (1977) 105-114

TLC 17

Media

A. Cellulose MN 300 (Machery-Nagel)

B. Kieselgel 60 (Merck)

Solvents

- A. *n*-Butanol-methanol-water-acetic acid (45:30:36:9)
- B. *n*-Butanol-methanol-water-ethanol-acetic acid (45:30:36:8:1) adjusted to pH 5.5 with 30% sodium hydroxide
- C. *n*-Butanol-water (45:36) adjusted to pH 4.0 with glacial acetic acid

Detection

Bioautography vs. *Sarcina lutea* ATCC 9341

 R_F

Tetracycline

Solvent	Medium A	Medium B
A	0.74	
B	0.00	0.00
C		0.00

Ref.

As Ampicillin, TLC 3

ELPHO*Media*

- A. Whatman No. 4 paper
B. Whatman No. 31 paper

Buffers

- A. 0.1 *N* Hydrochloric acid–sodium acetate · 3H₂O–sodium veronal–water (120 ml:1.94 g:2.94 g:460 ml) pH 7.3
B. 2 *M* Propionic acid–2 *M* formic acid–formamide–pyridine–sodium veronal–ethylene glycol–water ad 1000 ml (100 ml:100 ml:100 ml:20 ml:2.06 g:100 ml) pH 4.6
C. 0.2 *M* Sodium acetate–0.1 *N* hydrochloric acid–0.1 *M* boric acid–ethylene glycol–sodium veronal–water ad 1000 ml (200 ml:200 ml:200 ml:100 ml:2.06 g) pH 5.5

Mobility

Me- dium	Buf- fer	Potential gradient (V/cm)	Cur- rent den- sity (mA/ cm)	Tetra- cycline	Oxy- tetra- cycline	Chlor- tetra- cycline	De- methyl- chlor- tetra- cycline	Roli- tetra- cycline	Meta- cycline	Vibra- mycin
A	A	9.2	1.16	4.92	4.72	1.90	2.01	2.31	1.50	1.80
B	B	3.9	2.09	3.54	3.45	3.54	3.61	4.59	3.39	3.39
A	C	9.2	0.70	3.99	3.99	3.77	3.77	3.39 & 7.54	4.49	3.84
A	D	9.2	0.21	7.30	6.38	7.23	7.23	7.61	6.25	6.48
B	E	9.2	0.73	9.56	9.04	9.27	9.27	9.88	8.36	8.74
B	F	9.2	0.78	10.45	10.40	10.63	10.45	10.93	9.65	10.17

D. 0.1 *M* Boric acid–85% formic acid–sodium veronal–methanol–polyethylene glycol 400–water ad 1000 ml (200 ml:10 ml:2.06 g:100 ml:50 ml) pH 2.6

E. 2 *M* Propionic acid–2 *M* formic acid–sodium veronal–ethylene glycol–water ad 1000 ml (100 ml:2.06 g:100 ml) pH 2.6

F. 0.1 *M* Boric acid–85% formic acid–glycerol–water ad 1000 ml (200 ml:10 ml:100 ml) pH 2.3

Conditions

Samples of antibiotics (ca. 20 µg) in methanol soln. were placed into Whatman No. 4 or No. 31 paper sheets (23 × 38 cm). Starting line was placed 12 cm from the immersion line on anode side. Developed for 4 h at 350 V

Ref.

S. Ochab, *Dissert. Pharm. Pharmacol.*, 23 (1971) 205–208

CCD 1*Solvent*

0.01 *N* Hydrochloric acid–*n*-butanol, 230 transfers

Distribution

Tetracycline peak at tube 66 (*K* = 0.40); 2-acetyl-2-decarboxy tetracycline peak at tube 109 (*K* = 0.9)

Ref.

G.C. Lancini and P. Sensi, *Experientia*, 20 (1964) 83–84

CCD 2*Solvent*

0.01 *N* Hydrochloric acid–*n*-butanol (1:1), 100 transfers

Distribution

Chlortetracycline peak, tube 52; *K* = 1.11

Oxytetracycline peak, tube 30; *K* = 0.435

Phases analyzed by assaying against *Bacillus megatherium*

Ref.

R.J. Hickey and W.F. Phillips, *Anal. Chem.*, 26 (1954) 1640–1642

HPLC 1*Apparatus*

ALC/GPC Liquid Chromatograph (Waters Assoc.) equipped with a Model 6000 solvent delivery system and Model U6K Universal injector

Column

µBondapak C₁₈ (30 cm × 4 mm I.D.) (Waters Assoc.) reversed-phase system

Mobile phase

Methanol-EDTA (3:7)

Flow-rate

2.8 ml/min

Detection

UV at 355 nm

Retention time

Tetracycline peak 3.75 min

Ref.

I. Nilsson-Ehle, T.T. Yoshikawa, M.C. Schotz and L.B. Guze, *Antimicrob. Agents Chemother.*, 9 (1976) 754-760

HPLC 2**Apparatus**

Varian LC 8500

Column

Pellionex SCX, 30-40 μ m, 1.5 m $\times$ 2.2 mm I.D., stainless steel

Mobile phase

0.1 M Ammonium sodium phosphate, pH 8.2

Flow-rate

40 ml/h (50 atm)

Temperature

45°C

Detector

UV photometer, 280 nm

Chart speed

1 cm/min

Sensitivity

0.32 a.u.f.s.

Sample size1 μ l**Recorder**

A-25, 1 mV

Type

Cation exchange

Retention time (estimated from drawing)

Compound (hydrochlorides)	Time
Oxytetracycline	2.5
Tetracycline	3.0
Doxycycline	4.6
Chlortetracycline	3.0

Ref.

Varian Aerograph Application Note, August (1974) No. 6

HPLC 3**Apparatus**

Varian LC 8500

Column

Pellionex SCX, 30-40 μ m, 1.5 m $\times$ 2.2 mm I.D., stainless steel

Mobile phase

0.1 M Ammonium sodium phosphate, pH 8.2

Flow-rate

40 ml/h (50 atm)

Temperature

45°C

Detector

UV photometer, 280 nm

Chart speed

1 cm/min

Sensitivity

0.04 a.u.f.s.

Sample size1 μ l**Recorder**

A-25, 1 mV

Type

Cation exchange

Retention time (estimated from drawing)

Compound (hydrochlorides)	Time
Oxytetracycline	2.2
Tetracycline	3.0
Doxycycline	5.2

Ref.

As Tetracyclines, HPLC 2

HPLC 4**Apparatus**

Varian LC 8500

ColumnMicropak CN 10 μ m, 25 cm $\times$ 2.1 mm I.D.**Mobile phase**

Ethyl acetate-methylene chloride-methanol (64:30:6)

Flow-rate

120 ml/h (160 atm)

Temperature

Ambient

Detector

Variscan 268 nm

Chart speed

0.5 cm/min

Sensitivity

0.5 a.u.f.s.

Recorder

A-25, 10 mV

Type

Cation exchange

Retention time (estimated from drawing)

Oxytetracycline 2.0

Tetracycline 4.1

Chlortetracycline 4.0

Ref.

As Tetracyclines, HPLC 2

HPLC 5*Apparatus*

Varian LC 8500

*Column*Micropak CN 10 μ m, 25 cm $\times$ 2.1 mm I.D.*Mobile phase*Ethyl acetate-methyl chloride-methanol
(64:30:6)*Flow-rate*

120 ml/h (160 atm)

Temperature

Ambient

Detector

Variscan 268 nm

Chart speed

0.5 cm/min

Sensitivity

0.5 a.u.f.s.

Recorder

A-25, 10 mV

Type

Cation exchange

Retention time (estimated from drawing)

Tetracycline 0.9

Ref.

As Tetracyclines, HPLC 2

HPLC 6*Column*1 m $\times$ 2.1 mm I.D. $\times$ 6.3 mm O.D., 316 stainless
steel, ODS-Sil-X-II packing*Mobile phase*

Methanol-0.05 M ammonium carbonate (8:92)

Pressure

1000 p.s.i.

Flow-rate

0.56 ml/min

Detector

UV (254 nm)

Sensitivity

0.08 a.u.f.s.

Type

Reversed-phase

Retention time (min) (estimated from drawing)

Oxytetracycline 5.0

Doxycycline 10.5

Demeclocycline 16.0

Tetracycline 25.0

Ref.

As Ampicillin, HPLC

HPLC 7*Apparatus*Perkin-Elmer liquid chromatograph 1220, with a
Hewlett-Packard*Column*2.6 $\times$ 0.25 mm I.D., packed with silica gel SI-100
(Merck) 10 μ m, coated with octadecylsilane
chemically bonded organic phase*Mobile phase*A. Water-acetonitrile-70% perchloric acid
(76.2:22:1.8)B. Water-acetonitrile-70% perchloric acid
(61.2:37:1.8)*Flow-rate*

1 ml/min

Pressure

A. 800 p.s.i.

B. 600 p.s.i.

Detection

254 nm

Recorder

Varian Aerograph Model 20, 1 mV

Type

Reversed-phase

Retention time (estimated from drawing)

Compound	Mobile phase- Pressure	
	A-A	B-B
Tetracycline	11.0	3.0
<i>epi</i> -Tetracycline	8.5	
<i>epi</i> -Anhydrotetracycline		8.0
Anhydrotetracycline		10.0

*Ref.*S.L. Ali and T. Strittmatter, *Int. J. Pharmaceut.*,
1 (1978) 185-188**HPLC 8***Column* μ Bondapak phenyl, 30 cm $\times$ 3.9 mm*Mobile phase*A. 240 ml degassed acetonitrile + 27 ml phos-
phoric acid to 1650 ml degassed, distilled de-
ionized water, pH adjusted to 2.2 with 45% aq.
KOH and diluted to 2 liters; degassed for 15
sec prior to use. Condition column for 10 min

B. 440 ml degassed acetonitrile + 27 ml phosphoric acid + 1500 ml degassed, distilled, de-ionized water. pH adjusted to 2.2 with 25% aq. KOH; degassed for 15 sec prior to use. This mixture used to elute column

Flow-rate

Column run for ca. 15 min

Pressure

7000 p.s.i.

Detection

Waters Assoc. Model 440 absorbance detector

Sample size

20 μ l

Comments

Used for detection and quantitation

Retention time (min) (estimated from drawing)

Tetracycline	7.5
Chlortetracycline	14.0
4- <i>epi</i> -Tetracycline	5.5
4- <i>epi</i> -Anhydrotetracycline	17.7
Anhydrotetracycline	20.0

Ref.

N. Muhammad and J.A. Bodnar, *J. Pharm. Sci.*, 69 (1980) 928-930

HSLC

Method A

Column

Stainless steel, 1 m $\times$ 2.1 mm I.D., ODS-Sil-X-II

Mobile phase

Methanol-0.05 M ammonium carbonate (8:92),
0.005 M ethylenediaminetetraacetic acid

Flow-rate

0.56 ml/min

Pressure

1000 p.s.i.

Temperature

Room temp.

Detection

UV (254 nm)

Sensitivity

0.08 a.u.f.s.

Sample solvent

Water

Sample size

5 μ l

Type

Reversed-phase

Retention time

See Method B

Ref.

See Method B

Method B

Column

Stainless steel, 25 cm $\times$ 3.2 mm I.D.,
C₈/LiChrosorb, 10 μ m

Mobile phase

Methanol-0.05 M ammonium carbonate (20:80),
0.02 M ethylenediaminetetraacetic acid

Flow-rate

0.9 ml/min

Pressure

1500 p.s.i.

Temperature

Room temp.

Detection

UV (254 nm)

Sensitivity

0.08 a.u.f.s.

Sample solvent

Water

Sample size

5 μ l

Type

Reversed-phase

Retention time (min) (estimated from drawing)

Compound	Method A	Method B
Oxytetracycline	5.0	5.0
Doxycycline	10.5	16.0
Demeclocycline	16.0	18.0
Tetracycline	25.0	23.0
Methacycline		9.5
Chlortetracycline		34.0

Ref.

As Cephalosporin, HSLC 1

2,3,4,5-TETRAHYDROXYCYCLOHEX-ANEMETHANOL

TLC

Medium

Analtech Avicel plates

Solvent

n-Butanol-water-acetic acid (3:1:1)

Detection

Bioautography vs. *Klebsiella pneumoniae*, MB-1264

R_F

0.38

Ref.

T.W. Miller, B.H. Arison and G. Albers-Schonberg, *Biotechnol. Bioengineer.*, 15 (1973) 1075-1080

TETRAMYCIN

PC

Paper

Schleicher and Schuell No. 2043b mgl.

Solvents

- A. Water satd. *n*-butanol
- B. 20% Ammonium chloride
- C. 3% Ammonium chloride
- D. 75% Aq. phenol
- E. 50% Aq. acetone
- F. *n*-Butanol-methanol-water-methyl orange (40:10:20:1.5 g)
- G. *n*-Butanol-methanol-water (40:10:20)
- H. Benzene-methanol (80:20)
- I. Distilled water
- J. *n*-Butanol-acetic acid-water (40:10:50)
- K. *n*-Butanol-pyridine-water (60:40:30)
- L. Dimethyl formamide-water (10:90)
- M. Dimethyl formamide-water (50:50)
- N. 70% Aq. propanol

Detection

Bioautography vs. *Candida albicans*

R_F

Solvent	R_F	Solvent	R_F
A	0.30	H	0.00
B	0.04	I	0.59
C	0.59	J	0.65
D	0.82	K	0.55
E	0.93	L	0.62
F	0.62	M	0.90
G	0.54	N	0.70

Ref.

K. Dornberg, R. Fugner, G. Bradler and H. Thrum, *J. Antibiotics*, 24 (1971) 172-177

TETRANACTIN

TLC

Medium

Silica gel

Solvents

- A. Benzene-acetone (4:1)
- B. Chloroform-ethyl acetate (1:2)
- C. *n*-Hexane-diethyl ether (1:2)

R_F

Solvent A	0.12
Solvent B	0.31
Solvent C	0.21

Ref.

K. Ando, H. Oishi, S. Hirano, T. Okutomi, K. Suzuki, H. Okazaki, M. Sawada and T. Sagawa, *J. Antibiot.*, 24 (1971) 347-352

TETRANGOMYCIN

PC

Solvents

- A. *n*-Amyl acetate-dibutyl ether-water (20:6:1)
- B. *n*-Heptane-diethyl ketone-tetrahydrofuran-0.2 *M* acetic acid (50:3:3:50)

R_F

Solvent A	0.90
Solvent B	0.80

Ref.

M. Dann, D.V. Lefemine, F. Barbatschi, P. Shu, M.P. Kunstmann, L.A. Mitscher and N. Bohonos, *Antimicrob. Agents Chemother.*, 1965 (1966) 832-835

TLC

Medium

Eastman Chromagram Type K301R

Solvent

Ethyl acetate

R_F

0.47

Ref.

As PC

TETRIN

PC

Solvents

- A. Water satd. *n*-butanol
- B. 3% Ammonium chloride
- C. 50% Acetone
- D. 70% Propanol

Detection

UV fluorescence

R_F

Solvent A	0.15-0.22
Solvent B	0.50-0.69
Solvent C	0.82-0.90
Solvent D	0.65-0.69

Ref.

D. Gottlieb and H.L. Pote, *Phytopath.*, 50 (1960) 817-822

THAIMYCINS

TLC

Media

- A. Silica gel G
- B. Silica gel HF
- C. Aluminum oxide G

Solvents

- A. Butanol-acetone-water (4:5:1)
- B. Propanol-ethyl acetate-water (8:1:1)

C. Ethyl acetate–pyridine–isopropanol–water (7:2:3:2)

D. Butanol–pyridine–water (6:4:3)

R_F

Medium	Solvent	Thaimycins		
		A	B	C
A	A	0.60	0.30	0.75
A	B	0.45	0.25	0.50
B	C	0.50	0.50	0.80
C	D	0.50	0.30	0.70

Ref.

G. Cassinelli, E. Cotta, G. D'Amico, C.D. Bruna, A. Grein, R. Mazzoleni, M.L. Ricciardi and R. Tintinelli, *Arch. Microbiol.*, 70 (1970) 197–210

THIAMPHENICOL

TLC

Medium

Silica gel 60 F₂₅₄ (Merck)

Solvents

- A. Ethyl acetate
- B. Ethyl acetate–methanol (99:1)
- C. Ethyl acetate–methanol (98:2)
- D. Ethyl acetate–methanol (95:5)
- E. Ethyl acetate–methanol (90:10)

R_F

Compound	Solvent				
	A	B	C	D	E
Thiamphenicol	0.35	0.39	0.41	0.55	0.66
erythro-Thiamphenicol	0.36	0.40	0.42	0.56	0.67
D-threo-1-(p-Methylsulphonylmethyl)-2-monochloro-acetamido-1,3-propanediol	0.16	0.19	0.23	0.37	0.54
DL-erythro-1-(p-methyl-sulphimylphenyl)-2-dichloro-acetamido-1,3-propanediol	0.05	0.06	0.09	0.16	0.32

Ref.

As Chloramphenicol, TLC 1

GLC 1

Apparatus

Shimadzu GC-5AF₃P

Column

Glass, 1 m × 3 mm I.D., packed with 1.5% OV-17 on Chromosorb W AW DMCS, 60–80 mesh

Detection

Flame ionization

Temperature

180° for the first 6 min, then increased to 260° at the rate of 5°/min; injection port temp. 280°

Carrier gas

Nitrogen

Flow-rate

65 ml/min

Retention time

15 min (estimated from drawing)

Ref.

As Chloramphenicol, GLC 2

GLC 2

Apparatus

Hewlett-Packard 5750 gas chromatograph equipped with dual-flame ionization detectors

Column

Glass column, 10 ft. × 2 mm I.D., packed with 6.6% OV-3 and 3.3% OV-17 on Chromosorb W-AW DMCS, 70–80 mesh. The column was conditioned at 290° for 3 days with a helium flow-rate of 15 ml/min

Temperatures

The injection port was maintained at 280°, the column oven at 268° and the detector at 310°

Flow-rates

Helium (carrier gas), 45 ml/min; air, 500 ml/min; hydrogen, 30 ml/min. The air and hydrogen flow-rates sometimes required slight adjustment in order to obtained maximal response

Retention time

Thiamphenicol, approx. 10 min

Internal standard, 6 min

Quantitative

After the chromatography, a baseline was drawn and peak heights were recorded for the thiamphenicol (TAP) and internal standard peaks. The ratio of the peak height of TAP to that of the internal standard, corrected for attenuation if necessary, was calculated. Quantitation was carried out by referring to a standard curve constructed from values of known concentrations of TAP added to TAP-free samples

Ref.

A. Gazzaniga, E. Pezzotti and A. Cotta Ramusino, *J. Chromatogr.*, 81 (1973) 71–77

GLC 3

Apparatus

Hewlett-Packard 5750 gas chromatograph equipped with dual-flame ionization detectors

Column

A glass column, 10 ft. $\times$ 2 mm I.D. packed with 6.6% OV-3 and 3.3% OV-17 on Chromosorb W-AW DMCS, 70–80 mesh. The column was conditioned at 290° for 3 days with a helium flow-rate of 15 ml/min

Temperatures

The injection port was maintained at 280°, the column oven at 268° and the detector at 310°

Flow-rates

The flow-rates used were: helium (carrier gas), 45 ml/min; air, 500 ml/min; hydrogen, 30 ml/min

The silylating reagent was Sil-Prep, (Applied Science Labs.). Stock solutions were prepared by dissolving 1 g of thiamphenicol and of internal standard in 100 ml of methanol. The internal standard (I.S.) was D-(+)-threo-(*p*-methylsulphonylphenyl)-2-acetamido-1,3-propane-diol

An ethyl acetate extract phase was dried. The residue was dissolved in about 0.2 ml of methanol, evaporated to dryness, then added to 10–50- μ l vol. of Sil Prep and allowed to react for 15 min after which time a 205- μ l vol. of the reaction mixture was injected in the gas chromatograph

Under the chromatographic conditions described above, thiamphenicol has a retention time of approximately 10 min and the internal standard 6 min

After the chromatography, a baseline was drawn and peak heights were recorded for the TAP and internal standard peaks. The ratio of the peak height of TAP to that of the internal standard, corrected for attenuation if necessary, was calculated. Quantitation was carried out by referring to a standard curve constructed from values of known concentrations of TAP added to TAP-free samples. The ratio of peak heights was plotted against the concentration of TAP (μ g/ml) and shows that there is satisfactory linearity for peak ratios between 0.2 and 6. The internal standard should be added so as to obtain a peak ratio that lies between these limits

Ref.

As GLC 2

GLC 4

Apparatus

Varian Model 2100 gas chromatograph equipped with a ^{63}Ni electron-capture detector (8.5 mCi ^{63}Ni beta source), operating with a constant polarizing voltage (d.c. mode) and a Varian Model A-25 1-mV recorder

Column

U-shaped glass column (15 m $\times$ 2 mm) I.D. packed with 3% OV-17 on Gas-Chrom Q (60–80 mesh), conditioned at 250°C for 2 days with a nitrogen flow-rate of 30 ml/min

Temperatures

Injection port, 250°C; column oven, 200°C; detector, 280°C

Detector

Electron capture

Carrier gas

Pre-purified nitrogen

Flow-rate

15 ml/min

Relative retention time

Internal standard 1.00

Thiamphenicol 1.88

Ref.

T.A. Plomp, R.A.A. Maes and M. Thiery, *J. Chromatogr.*, 121 (1976) 243–250

THIENAMYCIN

PC

Paper

Whatman No. 1 (descending)

Solvent

Ethanol–water (70:30)

Detection

- Bioautography
- UV fluorescence quenching
- Ninhydrin
- Iodoplatinate reagent

R_F

0.63

Ref.

J.S. Kahan, F.M. Kahan, E.O. Stapley, R.T. Goegelman and S. Hernandez, *U.S. Patent* 4,006,060, Feb. 1, 1977

TLC

Media

- Silica gel HF (Analtech) (pre-washed with ethanol)
- Silica gel G
- Cellulose Avicel

Solvents

- A. Ethanol–water (70:30)
 B. Butanol–acetic acid–water (40:10:50)
 C. *n*-Propanol–water (70:30)

Detection

- A. Bioautography vs. *Staphylococcus aureus* ATCC 6538P
 B. UV light
 C. A peach-colored reaction followed by ninhydrin spray and heating
 D. A slow bleaching of the iodoplatinate spray reagent

 R_F

Solvent	Medium		
	A	B	C
A	0.44	0.49	0.48 0.45–0.50
B			0.43
C		0.31	0.36

Ref.

As PC

THIOLUTIN**PC****Paper**

As aureothricin, PC

Solvent

As aureothricin, PC

Detection

As aureothricin, PC

 R_F

0.45

Ref.

As aureothricin, PC

THIOPEPTINS**PC 1****Solvents**

- A. Ethyl acetate–*n*-hexane–2 *N* ammonium hydroxide (4:1:1)
 B. Methanol–acetic acid–water (25:3:72)

Ref.

N. Miyairi, T. Miyoshi, H. Aoki, M. Kohsaka, H. Ikushima, K. Kunugita, H. Sakai and H. Im-anaka, 176th Meeting Japan Antibiotics Res. Assn., November 20, 1970

 R_F

Thiopeptin	Solvent A	Solvent B
A ₁	0.95	0.18
A ₂	0.85	0.25
A ₃	0.57	0.52
A ₄	0.57	0.38
B	0.00	0.23

PC 2**Paper**

Ascending

Solvents

- A. Chloroform–methanol (9:1)
 B. Chloroform–methanol (19:1)
 C. Chloroform–*n*-butanol (6:1)
 D. Ethyl acetate–*n*-hexane–2 *N*-ammonia (4:1:1)
 E. Methanol–acetic acid–water (25:3:72)

 R_F

Solvent	Thiopeptins			
	A ₁	A ₃	A ₄	B
A	0.83	0.60	0.50	0.10
B	0.48	0.37	0.30	0.00
C	0.73	0.48	0.38	0.00
D	0.95	0.57	0.57	0.00
E	0.18	0.52	0.38	0.23

Ref.

As Siomycin, PC 2

TLC 1**Media**

- A. Silica gel G
 B. Spotfilm (silica gel, Tokyo-kasei Co.)

 R_F

Thiopeptin	Medium A			Medium B	
	Solvent				
	A	B	C		
A ₁	0.83	0.48	0.73		
A ₂	0.70	0.42	0.62		
A ₃	0.60	0.37	0.48		
A ₄	0.50	0.30	0.38		
B	0.10	0.00	0.00		

Solvents

- A. Chloroform-methanol (9:1)
 B. Chloroform-methanol (19:1)
 C. Chloroform-*n*-butanol (6:1)

Ref.

N. Miyairi, T. Miyoshi, H. Aoki, M. Kohsaka, H. Ikushima, K. Kunugita, H. Sakai and H. Imanaka, *Antimicrob. Agents Chemother.*, 1 (1972) 192-196

TLC 2**Medium**

Silica gel

Solvent

Chloroform-methanol (9:1)

 R_F

Thiopeptin A ₁	0.78
Thiopeptin A ₂	0.68
Thiopeptin A ₃	0.60
Thiopeptin B	0.19

Ref.

As Siomycin, PC 2

THIOSTREPTON**PC 1****Paper**

Whatman No. 1

Solvents

- A. Acetone-water (70:30), ascending, 5 h
 B. Acetone-propanol-water (40:40:20), ascending, 7 h
 C. 0.2 *N* Acetic acid, ascending, 6 h

Detection

Bioautography vs. *M. pyogenes* var. *aureus*

 R_F

Solvent A	0.50
Solvent B	0.80
Solvent C	0.08

Ref.

J.F. Pagano, M.J. Weinstein, H.A. Stout and R. Donovan, *Antibiot. Ann.*, (1955-1956) 554-559

PC 2**Paper**

Ascending

Solvents

- A. Chloroform-methanol (9:1)
 B. Chloroform-methanol (19:1)
 C. Chloroform-*n*-butanol (6:1)
 D. Ethyl acetate-*n*-hexane-2 *N*-ammonia (4:1:1)
 E. Methanol-acetic acid-water (25:3:72)

 R_F

Solvent	R_F	Solvent	R_F
A	0.50	D	0.53
B	0.30	E	0.38
C	0.38		

Ref.

As Siomycin, PC 2

TILOMYCIN**CCD****Solvent**

Upper phase, *tert*-butanol; lower phase, 4% sodium chloride; 200 transfers

Distribution

Single, symmetrical peak with maximum at tube 148

Ref.

M. Misiek, O.B. Fardig, A. Gourevitch, D.C. Johnson, I.R. Hooper and J. Lein, *Antibiot. Ann.*, (1957-1958) 852-855

TOBRAMYCIN

(cf., Nebramycin, PC 2, R_F , Factor 6)

PC 1**Paper**

Ascending

Solvent

Chloroform-methanol-17% aq. ammonia (2:1:1) lower layer (developed at room temp. for 12 h)

 R_F

0.02

Ref.

As Apramycin, PC 1

PC 2**Paper**

Ascending

Solvents

- A. 20% Aq. soln. of ammonium chloride
 B. Water satd. *n*-butanol
 C. *n*-Butanol-acetic acid-water (3:1:1)
 D. Water satd. *n*-butanol + 2% (w/v) *p*-toluene-sulfonic acid + 2% (v/v) piperidine
 E. Chloroform-methanol-17% aq. ammonia (2:1:1) lower layer

R_F

Solvent	R_F	Solvent	R_F
A	0.97	D	0.02
B	0.00	E	0.02
C	0.02		

Ref.

As Apramycin, PC 2

TLC

Medium

Silica gel

Solvents

- A. Chloroform-methanol-17% aq. ammonia (2:1:1) upper layer
 B. Methanol-10% aq. ammonium acetate (1:1)
 C. *n*-Butanol-ethanol-chloroform-17% aq. ammonia (4:5:2:5)

 R_F

- Solvent A 0.80
 Solvent B 0.54
 Solvent C 0.25

Ref.

As Apramycin, TLC

TOLYPOMYCIN R**PC**

Solvent

n-Hexane-benzene-acetone-water
 (30:10:18:32)

 R_F

0.65

Ref.

T. Kishi, Y. Yamana, M. Muroi and K. Mizuno,
163rd Meeting Japan Antibiotics Res. Assn., September 27, 1968

TLC

Medium

Silica gel, containing 2% oxalic acid

Solvent

Ethyl acetate containing 1% oxalic acid

 R_F

0.2

Ref.

As PC

TOLYPOMYCIN Y**PC**

Paper

Whatman No. 1

Solvents

- A. *n*-Hexane-benzene-ethanol-water (1:3:1:3)
 B. *n*-Hexane-benzene-acetone-water (30:10:18:2)
 C. *n*-Hexane-diethyl ether-acetone-water (15:5:8:12)

All solvents developed ascending

Detection

- A. As visible colored spots
 B. Bioautography vs. *Staphylococcus aureus*

 R_F

- Solvent A 0.78
 Solvent B 0.68
 Solvent C 0.27

Ref.

T. Kishi, H. Yamana, M. Muroi, S. Harada, M. Asai, T. Hasegawa and K. Mizuno, *J. Antibiot.*, 25 (1972) 11-15

TOLYPOMYCINONE DERIVATIVES**TLC**

Medium

Silica gel G (Merck F₂₅₄)

Solvents

- A. Ethyl acetate
 B. Benzene-ethyl acetate-methanol (10:90:1)
 C. Ethyl acetate-chloroform-methanol (60:37:5)
 D. Ethyl acetate-methanol (50:50)
 E. Ethyl acetate-pyridine (70:30)
 F. Ethyl acetate-methanol (96:4)
 G. Ethyl acetate + 1% oxalic acid
 H. Ethyl acetate-methanol (60:40)
 I. Methanol-acetic acid (9:1)
 J. Ethyl acetate-methanol (20:1)
 K. Ethyl acetate-benzene-methanol (70:25:5)
 L. Chloroform-methanol (95:5)
 M. Ethyl acetate-methanol (75:25)
 N. Chloroform-methanol (90:10)
 O. Benzene-methanol-ethyl acetate (10:1:90)
 P. Chloroform-methanol-ethyl acetate (20:2.5:2.5)
 Q. Chloroform-acetone (20:10)
 R. Chloroform-methanol (25:5)
 S. Ethyl acetate-acetone (15:15)
 T. Ethyl acetate-methanol (24:1)
 U. Chloroform-ethyl acetate-methanol (40:5:5)
 V. Ethyl acetate-methanol (23:5:1.5)
 W. Chloroform-methanol (23:2)
 X. Ethyl acetate-methanol (23:3)
 Y. Chloroform-methanol (27:4)
 Z. Chloroform-methanol (32:3)

R_F

Derivative	R_F (one)	Sol- vent
3-Benzylamino-tolpomy- cinone	1.36	A
3,16-Di-(benzylamino)-16,17- dihydro-tolpomycinone	1.73	A
3-Methylamino-tolpomy- cinone	0.86	B
3,16-Di-(methylamino)-16,17- dihydro-tolpomycinone	0.54	B
3-(2-Morpholinoethylamino)- tolpomycinone	0.58	C
3,16-Bis-(2-morpholinoethyl- amino)-16,17-dihydro- tolpomycinone	0.08	D
3-(2-Hydroxyethylamino)- tolpomycinone	0.64	G
3-[3-(N,N-Dimethylamino)- propylamino]-tolpomy- cinone	0.16	E
3-Benzylamino-16,17-di- hydro-tolpomycinone	1.32	F
3-[2-(Dimethylamino)-ethyl- amino]-tolpomycinone	0.58	H
3-[4-(N-Methylpiperidyl)- methylamino]-tolpomy- cinone	0.31	I
3-(3-Hydroxypropylamino)- tolpomycinone	0.33	A
3-Methylamino-16,17-dihydro- tolpomycinone	1.04	J
3-(4-Pyridyl)-methylamino- tolpomycinone	0.77	K
3-(2-Tetrahydrofuryl)-methyl- amino-tolpomycinone	0.87	K
3-(Piperonylamino)-tolpomy- cinone	1.20	K
3-(<i>p</i> -Dimethylaminobenzyl- amino)-tolpomycinone	0.82	L
3-[2-(4-Imidazolyl)ethyl- amino]-tolpomycinone	0.46	M
3-[γ -(4-Methylpiperazine-1- yl)-propylamino]-tolpomy- cinone	0.84	N
3-(2-Thenylamino)-tolpomy- cinone	1.07	A
3-(β -Imidazolidine-2-one-1-yl- ethylamino)-tolpomy- cinone	0.55	N

Derivative	R_F (one)	Sol- vent
3-[β -(<i>p</i> -Hydroxyphenyl)-ethyl- amino]-tolpomycinone	0.9	O
3-(4-Hydroxy-3-methoxy- benzylamino)-tolpomy- cinone	0.75	P
3-[β -(2-Pyridyl)-ethylamino]- tolpomycinone	0.46	Q
3-(3-Pyridylmethylamino)- tolpomycinone	0.79	R
3-(β -Piperidine-ethylamino)- tolpomycinone	0.36	S
3-[β -(5-Nitropyridine-2-yl)- aminoethylamino]- tolpomycinone	1.27	T
3-(N-Methylpyrrol-2-yl- methylamino)- tolpomycinone	1.2	A
3-(N-Methylpyrrolidine-2-yl- methylamino)- tolpomycinone	0.19	U
3-(Pyrazinylmethylamino)- tolpomycinone	0.78	V
3-[β -(1,2,4-Triazol-1-yl)-ethyl- amino]-tolpomycinone	0.58	W
3-[γ -(1,2,4-Triazol-1-yl)-pro- pylamino]-tolpomycinone	0.48	W
3-[γ -(1,2,3,4-Tetrazol-1-yl)- propylamino]-tolpomy- cinone	0.71	X
3-(N-Methylpiperidine-3-yl- methylamino)-tolpomy- cinone	0.44	Y
3-(Pyridine-2-yl-methyl- amino)-tolpomycinone	0.78	Z

Ref.

Belgian Patent 826,615, Sept. 15, 1975

TOMAYMYCIN**TLC***Medium*

Kieselgel (E. Merck)

Solvents

A. Ethyl acetate

B. Ethyl acetate-chloroform (1:1)

C. Chloroform-methanol (50:1)

D. Ethyl acetate-benzene (1:1)

 R_F

Solvent A 0.50

Solvent B	0.21
Solvent C	0.24
Solvent D	0.02

Ref.

K. Arima, M. Kosaka, G. Tamura, H. Imanaka and H. Sakai, *174th Meeting Japan Antibiotics Res. Assn.*, July 7, 1970

TREHALOSAMINE

PC

Paper

Toyo Roshi No. 50

Solvent

Butanol-acetic acid-water (4:1:5), ascending

Detection

Ninhydrin

 R_F

0.14

Ref.

S. Umezawa, K. Tatsuta and R. Muto, *J. Antibiot.*, 20 (1967) 388-389

TRICHOMYCIN

PC 1

Paper

Whatman No. 1

Solvent

As aureofungin, PC 1, A-F

 R_F

Solvent	R_F	Solvent	R_F
A	0.60	D	0.72
B	0.58	E	0.94
C	0.17, 0.14	F	0.32

Ref.

As aureofungin, PC 1

PC 2

Paper

Whatman No. 1

Solvents

- A. Water satd. *n*-butanol, descending, 16 h
- B. *n*-Butanol-pyridine-water (1:0.6:1), ascending, 16 h
- C. 50% Aq. acetone, ascending, 10 h
- D. Methanol-water-ammonium hydroxide (20:4:1), descending, 5.5 h
- E. 60% Aq. isopropanol, ascending, 16 h

Detection

Bioautography vs. *Saccharomyces cerevisiae*

 R_F

Solvent	R_F	Solvent	R_F
A	0.26	D	0.67, 0.42
B	0.75	E	0.99
C	0.78		

Ref.

P.V. Divekar, V.C. Vora and A.W. Khan, *J. Antibiot.*, 19 (1966) 63

PC 3

Solvents

- A. Water satd. butanol *
- B. Butan-1-ol-acetic acid-water (2:1:1) *
- C. Butan-1-ol-pyridine-water (1:0.6:1) *
- D. Acetone-water (1:1) *
- E. Butan-1-ol-McIlvaine's buffer (pH 6.5)
- F. Methanol-ammonia-water (20:1:4)
- G. Butan-1-ol-acetic acid-water (20:1:5)
- H. Butan-1-ol-pyridine-water (4:1:5)
- I. Butan-1-ol-pyridine-water (6:1:7)
- J. Butan-1-ol-ethanol-water (4:2:1)
- K. Butan-1-ol-ethanol-water (3:3:2)
- L. Butan-2-ol-ethanol-water (2:2:1)
- M. Amyl alcohol-methanol-water (10:5:4)
- N. Ethyl acetate-pyridine-water (12:5:14) *
- O. Butan-1-ol-acetic acid-water (20:1:25) *
- P. Propan-1-ol-water (4:1) *
- Q. Propan-2-ol-water (3:1)
- R. Butan-1-ol-methanol-water (2:2:3) *
- S. Butan-1-ol-methanol-water (4:1:2) *
- T. Butan-1-ol-pyridine-water * (1:1:1)
- U. Ethyl acetate-pyridine-25% acetic acid (5:5:4) *

* Whatman No. 1 paper specified

 R_F

Solvent	R_F	Solvent	R_F
A	0.35	J	0.48
	0.26	K	0.84
B	0.85	L	0.59
C	0.76	M	0.49
	0.75	N	0.17
D	0.69		0.14
	0.78	O	0.34
E	0.00		0.60
	0.51	P	0.41
F	0.00, 0.43		0.58
	0.65, 0.42	Q	0.88
	0.67	R	0.94

Solvent	R_F	Solvent	R_F
G	0.00	S	0.32
	0.40	T	0.28
H	0.54	U	0.72
I	0.32		

Ref.

As Amphotericins, TLC 2

TLC

Medium

Silica gel G

Solvents

- A. Ethanol-ammonia-water (8:1:1) buffered with phosphate, pH 8
- B. Butan-1-ol-acetic acid-water (3:1:1) buffered with phosphate, pH 8
- C. Ethanol-ammonia-dioxan-water (8:1:1:1) activated for 1 h at 110°C
- D. Butan-1-ol-pyridine-water (3:2:1) activated for 1 h at 110°C
- E. Butan-1-ol-ammonia-methanol-water (20:1:2:4)
- F. Chloroform-methanol-borate buffer, pH 8.3 (7:5:1)

Detection

- A. 10% Potassium permanganate and 0.2% bromophenol blue
- B. 0.2% *p*-Dimethylaminobenzaldehyde in sulfuric acid containing ferric chloride
- C. 5% Potassium permanganate
- D. Orthophosphoric acid, heat for 5 min at 100°C

 R_F

Solvent	Detection	R_F
A	A	0.45
B	A	0.17
C	C&D	0.30
D	C&D	0.58
E	B	0.13
F	B	0.49, 0.67

Ref.

As Amphotericins, TLC 2

ELPHO

Media

- A. Whatman No. 4 paper
- B. Whatman No. 31 paper

Buffers

- A. 0.1 M Boric acid-85% formic acid-sodium

veronal-methanol-polyethylene glycol 400-water to 1000 ml (200 ml:10 ml:2.06 g:100 ml:50 ml), pH 2.6

- B. 2 M Propionic acid-2 M formic acid-sodium veronal-ethylene glycol-water to 1000 ml (100 ml:100 ml:2.06 g:100 ml), pH 2.6
- C. 0.1 M Boric acid-85% formic acid-glycerol-water to 1000 ml (200:10:100), pH 2.3
- D. Methanol-glacial acetic acid-water (950:50:50), pH 3.3

Conditions

Samples of antibiotic (ca. 20 µg) in methanol soln. was placed into Whatman No. 4 or No. 31 paper sheets (23 × 38 cm). Starting line was placed 12 cm from the immersion line on anode side. Developed for 4 h at 350 V

Mobility

Med-ium	Buf-fer	Potential gradient (V/cm)	Current density (mA/cm)	Mobility (cm ² /V sec)
A	A	9.2	0.22	0.00
B	B	9.2	0.70	0.00
B	C	9.2	0.80	0.00
A	D	9.2	0.02	4.28

Ref.

S. Ochab, *Dissert. Pharm. Pharmacol.*, 23 (1971) 205-208

CCD

Solvents

- A. Chloroform-methanol-borate buffer, pH 8.2-8.4 (2:2:1)
- B. Chloroform-methanol-sodium acetate, pH 8.4 (2:2:1)

Distribution coefficient

Compound	Sol-vent	N_t	K
Trichomycin (major fraction)	A	200	0.61
Trichomycin (minor fraction)	A	200	1.31
Trichomycin A	A	240	0.411
	B	240	0.47
Trichomycin B	A	240	0.739

Ref.

As Amphotericins, TLC 2

HPLC

Apparatus

DuPont Model 830 liquid chromatograph, equipped with a DuPont Model 837 spectrophotometer. Extinction at 380 nm recorded by means of a Hewlett-Packard Model HP 3380A integrator

Column

Permaphase ODS, 1 m × 2.1 mm I.D. reversed-phase partition column with octadecylsilane as stationary phase, chemically bonded to a controlled porous surface ("porosity beads" 20–37 μm) by means of Si–O–Si bonds and contains about 1% stationary phase. Pre-packed Permaphase ODS column has 600 theoretical plates, determined by isocratic elution of anthracene with methanol–water (6:4)

Temperature

55° ± 1°

Mobile phases

A. 0.05 M Phosphate buffer, pH 7.0–methanol (8:2)

B. Methanol

Gradient

0 → 100% B at 3%/min, hold at limit

Pressure

1000 p.s.i.

Flow-rate

1.0 ml/min

Detection

380 nm

Attenuation

0.08 a.u.f.s.

Chart speed

5 mm/min

Ref.

As Candicidin, HPLC

TRICHOSTATIN A DIACETATE

TLC

Medium

Silica gel AR-7G

Solvent

Chloroform–methanol

Detection

UV

Ref.

N. Tsuji, M. Kobayashi, K. Nagashima, Y. Wakisaka and K. Koizumi, *J. Antibiot.*, 29 (1976) 1–6

TRIDECAPTIN A, B and C

PC

Paper

Toyo Roshi No. 51

Solvents

A. Butanol–acetic acid–water (4:1:2)

B. Butanol–pyridine–acetic acid–water (10:6:1:4)

R_F

Solvent	Tridecaptin		
	A	B	C
A	0.65	0.60	0.60
B	0.34	0.20	0.25

Ref.

J. Shoji, H. Hinoo, P. Sakazaki, T. Kato, Y. Wakisaka, M. Mayama, S. Matsuura and H. Miwa, *J. Antibiot.*, 31 (1978) 646–651

TLC

Medium

Silica gel precoated (Merck)

Solvents

A. Chloroform–ethanol–14% ammonium hydroxide (4:7:2)

B. Isopropanol–14% ammonium hydroxide (2:1)

R_F

Solvent	Tridecaptin		
	A	B	C
A	0.10	0.05	0.10
B	0.47	0.34	0.41

Ref.

As PC

TRIENE

PC

Solvents

A. Light petroleum

B. Carbon tetrachloride

C. Chloroform

D. Diethyl ether

E. Diisoamyl ether

F. Benzene

G. Ethyl acetate

H. Acetone

I. Water satd. with *n*-butyl alcohol

Detection

- A. Bioautography vs. *Saccharomyces cerevisiae*
 B. Visually according to the yellow coloration of the antibiotic

 R_F

Solvent	R_F	Solvent	R_F
A	0	F	0.8
B	0	G	1.0
C	1	H	1.0
D	0.95	I	0
E	0.8		

Ref.

V.A. Poltorak, Z.G. Sokolova, K.A. Vinogradova and A.B. Silaev, *Antibiotiki*, 17 (1972) 738–742

TRIENINE**PC****Solvents**

- A. Butanol–acetic acid–water (4:1:5)
 B. 5% Dimethylformamide in methanol

 R_F

Solvent A	0.55
Solvent B	0.70

Ref.

A. Aszalos, R.S. Robison, P. Lemanski and B. Berk, *J. Antibiot.*, 21 (1968) 611–615

TLC**Medium**

Eastman Chromagram ITLC

Solvent

Methanol

 R_F

0.0

Ref.

A. Aszalos, R.S. Robison, F. Pansy and B. Berk, *U.S. Patent* 3,632,749, Jan. 4, 1972

TRIOSTIN**PC 1****Solvents**

- A. Light petroleum–benzene–methanol–water (66.7:33.3:80:20)
 B. 25% Ethanol
 C. Amyl acetate satd. with water

 R_F

Solvent A	0.40
Solvent B	0.26
Solvent C	0.76

Ref.

T.S. Maksimova, I.N. Kovsharova and U.V. Proshlyakova, *Antibiotiki*, 10 (1965) 298–304

PC 2**Solvent**

Dibutyl ether–*sym.*-tetrachloroethane–10% sodium *o*-cresotinate (2:1:3)

Detection

- A. Bioautography vs. *Bacillus subtilis*
 B. Radioactive triostin detected by scanning

 R_F

Triostin C > A (estimated 0.5, 0.3 respectively, from drawing)

Ref.

T. Yoshida and K. Katagiri, *Biochemistry*, 8 (1969) 2645–2651

TLC 1 (Circular TLC)**Medium**

As quinomycins, TLC 1

Solvent

As quinomycins, TLC 1

 R_F

Triostin C > B > A

Ref.

As quinomycins, TLC 1

TLC 2**Media**

- A. Aluminum oxide GF₂₅₄, circular chromatography
 B. Silica gel GF₂₅₄, ascending

Solvent

Lower phase of ethyl acetate–*sym.*-tetrachloroethane–water (3:1:3)

Detection

UV light

 R_F

Both systems separate triostin A from C

Ref.

As PC 2

TRYPANOMYCIN A₂**PC****Paper**

Schleicher and Schuell No. 2043b

Solvents

- A. Water satd. butanol
 B. Methanol
 C. 50% Acetone
 D. Acetone–benzene–water (12:3:2)
 E. Butanol–methanol–water (4:1:2)
 Solvents A–D, ascending; E, circular

DetectionBioautography vs. *Escherichia coli* C600**R_F**

Solvent	R _F	Solvent	R _F
A	0.65	D	0.87
B	0.90	E	0.70
C	0.48		

Ref.

W. Fleck, D. Straus, C. Schönfeld, W. Jungstand,
C. Seiber and H. Prauser, *Antimicrob. Agents
Chemother.*, 1 (1972) 385–391

TLC**Medium**

Silica gel D (Merck)

SolventChloroform–acetone–methanol–water
(200:20:50:50)**Detection**

Color of zone

R_F

0.46

Ref.

As PC

TSUSHIMYCIN**TLC****Medium**

Silica gel G

SolventsA. *n*-Butanol–acetic acid–water (3:1:1)

B. Ethanol–14% aq. ammonia (4:1)

Detection

A. Bioautography

B. Ethanol–14% aq. ammonia (4:1)

R_F

Solvent A 0.37–0.40

Solvent B 0.16–0.19

Ref.

J. Shoji, S. Kozuki, S. Okamoto, R. Sakazaki and
H. Otsuka, *J. Antibiot.*, 21 (1968) 439–443

TUBERACTINOMYCINS**TLC 1****Medium**

Silica gel G

Solvent10% Aq. ammonium acetate–acetone–10% am-
monium hydroxide (9:10:0.5)**R_F**

0.44

Ref.

A. Nagata, T. Ando, R. Izumi, H. Sakakibara, T.
Take, K. Hayano and J. Abe, *J. Antibiot.*, 21
(1968) 681–687

TLC 2**Medium**

As TLC 1

Solvents

A. As TLC 1, but (9:10:1)

B. Phenol–water–conc. ammonium hydroxide
(30:10:0.6)**R_F**

	Solvent A	Solvent B
Tuberactinomycin	0.53	0.13
Tuberactinomycin N	0.53	0.28

Ref.

T. Ando, R. Izumi, K. Matsura, A. Nagata and J.
Abe, *175th Meeting Japan Antibiotics Res. Assn.*,
September 25, 1970

TLC 3**Medium**

Kieselgel G (Merck)

SolventsA. 10% Ammonium acetate–acetone–10% am-
monium hydroxide (9:10:1)

B. As TLC 2, B

R_F (estimated from drawing)

Tuberactinomycin	Solvent	
	A	B
A	0.50	0.13
B *	0.25	0.13
N	0.50	0.30
O	0.25	0.30

* Identical to viomycin

Ref.

R. Izumi, T. Noda, T. Ando, T. Take and A.
Nagata, *J. Antibiot.*, 25 (1972) 201–207

TLC 4**Medium**

Silica gel

SolventsA. Chloroform–methanol–17% aq. ammonia
(2:1:1), upper layer

- B. 10% Ammonium acetate-methanol (1:1)
 C. 10% Ammonium acetate-acetone-10% aq. ammonia (9:10:0.5)

R_F

Compound	Solvent		
	A	B	C
Tuberactinomycin	0.12	0.67	0.58
Tuberactinomycin N	0.12	0.67	0.58

Ref.

T. Nara, S. Takasawa, R. Okachi, I. Kawamoto, M. Kumakawa, M. Yamamoto and S. Sato, *U.S. Patent* 3,901,972, Aug. 26, 1975

TLC 5

Medium

Silica gel

Solvent

10% Aq. ammonium acetate-acetone-ammonia water (9:10:0.5)

Detection

Ninhydrin reagent

R_F

Separates tuberactinomycin from viomycin

Ref.

A. Nagata, T. Ando, R. Izumi and H. Sakakibara, *U.S. Patent* 3,639,580, Feb. 1, 1972

TUMIMYCIN

TLC 1

Medium

Eastman Cellulose ITLC

Solvent

Methanol-benzene (3:97)

R_F

0.9

Ref.

A. Aszalos, R.S. Robison, N. Kraemer, J. Henshaw and M.S. Giannini, *U.S. Patent* 3,674,867, July 3, 1972

TLC 2

Medium

Eastman Si-Gel Chromagram sheets

Solvent

Methanol-chloroform (10:90)

Detection

Visible yellow and red fluorescent spot

Ref.

As TLC 1

TLC 3

Medium

Eastman Si-Gel Chromagram

Solvent

Methanol-chloroform (1:9)

R_F

0.65 (major); 0.55 (minor)

Ref.

A. Aszalos, R.S. Robison, N.V. Kraemer, J. Henshaw and M.S. Giannini, *German Patent* 2139261, 1972

TUNICAMYCIN

TLC

Media

A. Silica gel

B. Alumina

C. Cellulose

Solvents

A. Chloroform-methanol (1:1)

B. Chloroform-methanol (1:2)

C. Chloroform-methanol (2:3)

D. Benzene-methanol (2:3)

E. Water satd. butanol

F. Water satd. collidine

G. Butanol-pyridine-acetone-water (15:10:3:12)

Detection

Bioautography

R_F (estimated from drawing)

Solvent	Medium		
	A	B	C
A	0.70-0.90	0.0	-
B	0.70-0.95	0.05	-
C	0.15	-	-
D	0.70-0.90	0.0	-
E	-	-	0.60-0.80
F	-	-	0.85-1.00
G	-	-	0.85

Ref.

As Mycospocidin, TLC

TYLOSIN

PC 1

Paper

As relomycin, PC

Solvent

As relomycin, PC

Detection

As relomycin, PC

 R_F

0.48

Ref.

As relomycin, PC

PC 2**Solvents**

- A. Methyl ethyl ketone on pH 4 buffered paper
- B. Methyl ethyl ketone
- C. *n*-Butanol satd. with water on pH 4 buffered paper
- D. *n*-Butanol satd. with water
- E. Water containing 7% sodium chloride and 2.5% methyl ethyl ketone
- F. Ethyl acetate satd. with water on pH 4 buffered paper

 R_F

Solvent	R_F	Solvent	R_F
A	0.46	D	0.84
B	0.81	E	0.57
C	0.90	F	0.89

Ref.R.L. Hamill and W.M. Stark, *J. Antibiot.*, 17 (1964) 133-139**PC 3****Paper**

Toyo No. 51

Solvent

Water satd. ethyl acetate (ascending, 3 h)

DetectionBioautography vs. *Bacillus subtilis* No. 10707 R_F

0.95

Ref.

As Carbomycin, PC 5

TLC 1 (Two-dimensional and one-dimensional chromatography)**Medium**Silica gel GF₂₅₄ (Merck), 0.25-mm thick**Solvents**

- A. First direction; chloroform-acetone (60:40)
- B. Second direction; ethyl acetate-methanol (85:15)

Detection

- A. UV absorbance at 254 nm
- B. Consecutive spraying with the following:
 1. Iodoplatinate. 1 g of $\text{PtCl}_4 \cdot 2\text{HCl} \cdot 6\text{H}_2\text{O}$

and 20 g potassium iodide are dissolved in 8 ml conc. hydrochloric acid and diluted to 400 ml with distilled water. Chromatograms are sprayed until uniformly pink with faint brown color for tylosinxxx

2. Draggendorf's reagent modified by Meunier and Macheboeuf. Soln. (a): 0.85 g basic bis-muth nitrate, 10 ml 96% acetic acid and 40 ml distilled water. soln. (b): 20 g potassium iodide dissolved in 50 ml water. Both solutions mixed and kept in a brown bottle. 10 ml acetic acid and 35 ml water added to 5 ml of mixture just before spraying. This reagent makes tylosin spots more pronounced
3. Satd. soln. of silver sulfate in 10% sulfuric acid. After this spray, tylosin spots become orange-brown against a dark background

 R_F

Solvent A	0.12
Solvent B	0.60

Ref.M. Debackere and K. Baeten, *J. Chromatogr.*, 61 (1971) 125-132**TLC 2****Media**

- A. Cellulose MN 300 (Machery-Nagel)
- B. Kieselgel 60 (Merck)

Solvents

- A. *n*-Butanol-methanol-water-acetic acid (45:30:36:9)
- B. *n*-Butanol-methanol-water-ethanol-acetic acid (45:30:36:8:1) adjusted to pH 5.5 with 30% sodium hydroxide
- C. *n*-Butanol-water (45:36) adjusted to pH 4.0 with glacial acetic acid

DetectionBioautography vs. *Sarcina lutea* ATCC 9341 R_F

Solvent	Medium A	Medium B
A	0.97	
B	0.97	
C		0.16

Ref.

As Ampicillin, TLC 3

FLC**Instrument**

JASCO FLC-150

Detector

JASCO UVIDEK-2 (232.5 nm) spectrophotometer

Separation mode

Partition

Column

JASCOPACK SV-02-500

Column temperature

Ambient

Column pressure

65 kg/cm²

Mobile phase

Methanol- $\frac{1}{15}$ M acetate buffer, pH 4.9-acetonitrile (35:60:5)

Flow-rate

1.0 ml/min

Retention time

3.5 min

Ref.

S. Omura, Y. Suzuki, A. Nakagawa and T. Hata, *J. Antibiot.*, 26 (1973) 794-796

TYROCIDIN**ELPHO****Medium**

Toyo No. 51 paper

Buffer

Formamide-formic acid-acetic acid-water (2:1:3:4) pH 1.4

Conditions

1700 V, 56 cm for 450 min

Detection

- A. Radioactivity (paper strip scanner)
- B. After scanning, tyrocidin was stained by dipping the paper in a 1% aq. bromphenol blue solution and then washing out the excess dye with running water

Mobility

Tyrocidin moved 12.0 cm toward the cathode

Ref.

K. Fujikawa, T. Suzuki and K. Kurashashi, *J. Biochem.*, 60 (1966) 216-218

TYROTHRIN**TLC****Medium**

Kieselgel G

Solvent

n-Butanol-acetic acid-water (100:10:30), 3 h

Detection

Dry plates 2-3 min in air followed by 10 min at 45°C. Suspend in a jar containing solution of 100 ml potassium permanganate, 1.5% to which has

been added 100 ml of 10% hydrochloric acid heated about 50°C. The plate is exposed to the chlorine vapor for approximately 20 min and excess chlorine removed by exposing the plate to a stream of air. Spray plate with a mixture prepared by adding 160 mg of *o*-tolidine in 30 ml conc. acetic acid and diluting to 500 ml

R_F

0.34

Ref.

P.A. Nussbaumer, *Pharm. Acta Helv.*, 89 (1964) 647-652

UMBRINOMYCIN**PC****Paper**

Whatman No. 4

Solvent

n-Butanol-water-acetic acid (4:5:1)

Detection

Bioautography vs. *Staphylococcus aureus* 209P

R_F

0.9

Ref.

Belgian Patent 708601, June 27, 1968

USNIC ACID**HPLC****Column**

1 m × 2.1 mm I.D. × 6.3 mm O.D., 316 stainless steel. ODS-Sil-X-II packing

Mobile phase

Methanol-0.05 M ammonium carbonate (30:70)

Pressure

1000 p.s.i.

Flow-rate

0.06 ml/min

Detector

UV (254 nm)

Sensitivity

0.16 a.u.f.s.

Type

Reversed-phase

Retention time

5.0 min (estimated from drawing)

Ref.

As Ampicillin, HPLC

VALIDAMYCINS**TLC 1****Medium**

Silica gel G

SolventsA. *n*-Propanol-acetic acid-water (4:1:1)

B. Benzene-ethyl acetate (1:1)

Detection

1% Aq. potassium permanganate

 R_F (estimated from drawing)

	Solvent A	Solvent B
Validamycin A	0.30	0.7
Validamycin B	0.43	

Ref.T. Iwasa, Y. Kameda, M. Asai, S. Horii and K. Mizuno, *J. Antibiot.*, 24 (1971) 119-123**TLC 2****Medium**

Silica gel G

Solvent*n*-Propanol-acetic acid-water (4:1:1) R_F

Validamycin C 0.15

Validamycin D 0.24

Validamycin E 0.15

Validamycin F 0.15

Ref.

Netherlands Patent 7,205,375, Oct. 24, 1972

GLC**Apparatus**

Hitachi Model 063 with FID

Column

Glass, 2 m × 3 mm I.D. packed with 1% silicone OV-1 on Chromosorb W AW DMCS

Temperature

Column, 250°C; injection, 300°C

Carrier gas

He at 60 ml/min

Silylation procedureApproximately 1 mg sample dissolved in 100 μ l pyridine. Bis(trimethylsilyl)acetamide, 100 μ l and trimethyl chlorosilane, 50 μ l added. Heat at 70-80°C for 30 min**Chromatography**

Peaks, in min, estimated from graph are:

Validamycin A 4

Validamycin B 5

Validamycin C 27

Validamycin D 4.5

Validamycin E 23

Validamycin F 24

Ref.S. Horh, Y. Kameda and K. Kawahara, *J. Antibiot.*, 25 (1972) 48-53**VANCOMYCIN****PC 1****Paper**

Whatman No. 1

Solvents

A. 5% Aq. ammonium chloride, descending

B. 90% Phenol-*m*-cresol-pyridine-acetic acid-water (25:25:1:1:25), descending**Detection**Bioautography vs. *Bacillus subtilis* or *Corynebacterium xerosis* R_F

Solvent A 0.85

Solvent B 0.35, 0.59

Ref.M.P. Kunstmann, L.A. Mitscher, J.N. Porter, A.J. Shay and M.A. Darken, *Antimicrob. Agents Chemother.*, 1968 (1969) 242-245**PC 2****Paper**Whatman 3MM washed extensively with *M* ammonium acetate, then water, and dried before use**Solvents**

A. Ethanol-ammonium acetate (5:2), 18 h

B. Isobutyric acid-0.5 *M* aq. ammonia (5:3), 18 h R_F

	$R_{\text{vancomycin}}$	
	Solvent A	Solvent B
Iodinated vancomycin	1.02	1.01

Ref.H.R. Perkins and M. Nieto, *Biochem. J.*, 116 (1970) 83-92**TLC (Qualitative and quantitative)****Media**

A. Qualitative; silica gel G activated 105°C for 10 min before use

B. Quantitative; Kieselguhr G (Merck), no pre-activation. Range, 2-10 μ g**Solvents**A. Benzene-*n*-butanol-water (20:20:100), aq. phase

B. Methanol-water (1:99)

DetectionA. Qualitative: Spray with 20% sodium carbonate solution followed by Folin-Ciocalteu reagent. Gray-blue zones result. Detection limit <0.1 μ g

B. Quantitative: Developed with solvent A. Plates dried in warm air stream and immediately sprayed with 20% aq. sodium carbonate, dried thoroughly and sprayed with fresh Folin-Ciocalteu reagent diluted 1:3 and dried 15 min in warm air stream. Scan with integrating densitometer (Photovolt model 520 M with TLC stage used; slit 0.1×6 mm; 420-nm filter; search unit, 1 mm; response setting, 10). Read each spot against standard curve

R_F

Solvent	R_F^*	Solvent	R_F^*
A	0.98	B	0.54

* Silica gel G

Ref.

J.R. Fooks, I.J. McGilveray and R.D. Strickland, *J. Pharm. Sci.*, 57 (1968) 314-317

HPLC

Apparatus

Model 6000A high-pressure metering pump (Waters Assoc.)

Column

30 cm $\times$ 4 mm I.D., Bondapak C₁₈ (Waters Assoc.) (a mono-molecular layer of octadecyltrichlorosilane chemically bonded to totally porous μ Porasil particles having an average diameter of 10 μ m)

Mobile phase

124 ml Acetonitrile + 875 ml 0.05 M potassium dihydrogen phosphate buffer soln., pH 6

Flow-rate

1.0 ml/min

Temperature

Ambient

Pressure

7000 p.s.i.

Detection

UV, 225 nm (Schoeffel Model SF 770)

Sensitivity

0.2 a.u.f.s.

Recorder

Varian Model A-25 (10 mV)

Chart speed

10 in./h

Type

Reversed-phase

Retention time

3.5 min (estimated from drawing)

Ref.

As Anisomycin, HPLC

VARIOTIN

CCD

Solvent

70% Methanol-carbon tetrachloride (1:1), 60 transfers

Distribution

Variotin found in tubes 29-38

Ref.

N. Tanaka, K. Sashikata and H. Umezawa, *J. Gen. Appl. Microbiol.*, 8 (1962) 192-200

VENTURICIDINS

TLC

Medium

Silica gel

Solvent

Ethyl acetate

R_F

Ventricidin A 0.49

Ventricidin B 0.41

Ventricidin X 0.80

Ref.

H. Zachner and W. Keller, *U.S. Patent* 3,636,198, Jan. 18, 1972

VERDAMICIN

PC 1

Paper

Whatman No. 1

R_F

Solvent	R_F	Solvent	R_F
A	0.55	E	0.0
B	0.30	F	0.40 *
C	0.45	G	0.44 **
D	0.0		

* R_f at $t = 6$ h

** R_f at $t = 16$ h

Solvents

A. 80% Methanol plus 3% sodium chloride (1:1, w/v), descending; paper buffered with 0.95 M Na₂SO₄ and 0.05 M NaHSO₄

B. Propanol-pyridine-acetic acid-water (6:4:1:3, v/v), ascending

C. 80% (v/v) Phenol, ascending

D. Benzene-methanol (9:1, v/v), descending

E. *n*-Butanol-water-acetic acid (4:5:1), upper phase used ascending

F. Chloroform-methanol-17% ammonium hydroxide (2:1:1), lower phase

G. 2-Butanone-*tert.*-butanol-methanol-conc. ammonium hydroxide (16:3:1:6)

Ref.

M.G. Weinstein, G.H. Wagman, J.A. Marquez, R.T. Testa and J.A. Waits, *Antimicrob. Agents Chemother.*, (1975) 246-249

PC 2

Papers

- A. Chromar 500 sheet, ascending
B. Whatman No. 1, descending

Solvents

- A. Chloroform-methanol-conc. ammonium hydroxide (1:1:1)
B. Chloroform-methanol-17% ammonium hydroxide (2:1:1), 6 h
C. 2-Butanone-*tert.*-butanol-methanol-conc. ammonium hydroxide (16:3:1:6), 16 h

Detection

Bioautography vs. *Staphylococcus aureus* ATCC 6538P

R_F

Solvent A, Medium A 0.65
Solvent B, Medium B 0.40 *
Solvent C, Medium B 0.44 *

* R_f value

Ref.

M.J. Weinstein, G.H. Wagman and J.A. Marquez, *U.S. Patent* 3,988,316, Oct. 26, 1976

PC 3

Paper

Whatman No. 1

Solvent

Chloroform-methanol-17% ammonium (2:1:1), lower phase

Detection

Bioautography vs. *Bacillus subtilis*

R_F

0.38

Ref.

R. Okachi, I. Kawamoto, S. Takasawa, M. Yamamoto, S. Sato, T. Sato and T. Nara, *paper presented 194th Sci. Meeting, JARA, July 26, 1974*

VERMICULINE

TLC

Medium

Silica gel (Silufol)

Solvents

- A. Chloroform-methanol (98:2)
B. Chloroform-acetone (8:2)

C. Benzene-acetone (7:3)

D. Benzene-acetone (8:2)

E. Benzene-methanol (9:1)

F. Benzene-acetic acid (1:1)

G. Ethyl acetate-acetic acid (10:1)

Detection

A. Bioautography vs. *Bacillus subtilis* SDPC 1:220

B. Conc. sulfuric acid

C. Potassium permanganate

R_F

Solvent	R_F	Solvent	R_F
A	0.71	E	0.44
B	0.47	F	0.48
C	0.52	G	0.59
D	0.23		

Ref.

J. Fuska, P. Nemec and I. Kuhr, *J. Antibiot.*, 25 (1972) 208-211

VERNAMYCIN B

TLC

Medium

Silica gel

Solvent

Chloroform-methanol (100:15)

Detection

Compound I, iodine vapor

Compound II, UV fluorescence

R_F

Compound I 0.6

Compound II 0.5

Ref.

G. Beau de Lomenie, A. Armengaud, G. Housard, J.F. Boissel and M. de Haas, *French Patent* 1,505,434, Sept. 15, 1966

CCD

Solvent

Toluene-methanol (1:1)

Distribution coefficient

100 Transfers

Vernamycin B

α $K = 2.2$

γ $K = 1.4$

β $K = 0.8$

δ $K = 0.5$

Ref.

As TLC

VERRUCARINES

TLC 1

Media

- A. Aluminum oxide
- B. Kieselgel G

Solvents

- A. Chloroform-methanol (98:2)
- B. Chloroform-methanol (97:3)

Detection

- A. Iodine vapor
- B. UV light

R_F

Verru- carine	Medium-Solvent		
	A-A	B-A	B-B
A	0.70	0.28	0.59
B	0.83	0.47	0.69
C	0.74	0.28	0.52
D	0.70	0.28	0.55
E	0.00	0.00	0.09
F	—	0.54	—
G	—	0.49	—

Verru- carine	Color	
	Iodine	UV
A	Yellow-brown	Pale
B	Yellow-brown	Pale
C	Yellow-brown	Brightly fluorescent
D	Yellow-brown	Dark
E	Violet	Dark
F	Light yellow-brown	Dark
G	Brown	Not visible

Ref.

E. Härr, W. Loeffler, H.P. Sigg, H. Stähelin, Ch. Stoll, Ch. Tamm and D. Weisinger, *Helv. Chim. Acta*, 45 (1962) 840-853

TLC 2

Medium

Kieselgel G

Solvents

- A. Chloroform-methanol (98:2)
- B. Benzene-tetrahydrofuran (85:15)
- C. Diethyl ether (twice)

Detection

Iodine vapor

R_F

	Solvent		
	A	B	C
Verrucarine B	0.58	0.63	0.37
Verrucarine H	0.59	0.72	0.51
Verrucarine J	0.59	0.64	0.42

Ref.

B. Böhner, E. Fetz, E. Härr, H.P. Sigg, Ch. Stoll and Ch. Tamm, *Helv. Chim. Acta*, 48 (1965) 1079-1087

VERSICOLIN

PC

Paper

Whatman No. 1

Solvents

- A. *n*-Butanol-acetic acid-water (6:1:2)
- B. Benzene-acetic acid-water (6:7:3)
- C. Light petroleum (b.p. 40-60°C)-methanol (1:1)
- D. Diethyl ether satd. with water
- E. Toluene-light petroleum (b.p. 40-60°C)-methanol (5:4:1)

All solvents developed ascending

Detection

Bioautography

R_F

Solvent	R_F	Solvent	R_F
A	1.00	D	0.85
B	0.88	E	0.50
C	0.97		

Ref.

A.K. Dhar and S.K. Bose, *Appl. Microbiol.*, 16 (1968) 749-752

TLC

Medium

Silica gel G (E. Merck)

Solvents

- A. Light petroleum (b.p. 40-60°C)-benzene
- B. Chloroform-benzene (3:1)
- C. Benzene-methanol (4:1)
- D. Benzene-methanol-acetic acid (80:20:1)
- E. Benzene-methanol-ammonia (80:20:1)

Detection

Permanganate-BPB reagent

 R_F

Solvent	R_F	Solvent	R_F
A	0.00	D	0.40
B	0.80	E	0.20
C			0.50

Ref.

As PC

VERTISPORIN**TLC**

Medium

Silica gel

Solvent

Chloroform-methanol (20:1)

 R_F

0.40

Ref.

S. Hayakawa, E. Kondo, Y. Wakisaka, H. Minato and K. Katagiri, *J. Antibiot.*, 28 (1975) 550-551

VICTOMYCIN (XK 49-1-B-2)

(cf., XK-49-1-B-2)

PC

Solvents

- A. 20% Aq. ammonium chloride
- B. 10% Aq. ammonium chloride
- C. 1% Aq. ammonium chloride
- D. 0.5% Aq. ammonium chloride
- E. Water satd. *n*-butyl alcohol
- F. *n*-Butyl alcohol-acetic acid-water (3:1:1)
- G. Water satd. *n*-butyl alcohol + 2% *p*-toluene-sulfonic acid + 2% piperidine

 R_F

Solvent	R_F	Solvent	R_F
A	0.75	E	0.00
B	0.72	F	0.08
C	0.40	G	0.02
D	0.15		

Ref.

As Bleomycins, TLC 5

TLC

Medium

Silica gel

Solvents

- A. Chloroform-methanol-17% ammonia water (2:1:1), upper layer
- B. 10% Ammonium acetate-methanol (1:1)
- C. Methanol-10% ammonium acetate-10% ammonia water (10:9:1)
- D. 0.05 *M* Citrate buffer, pH 6.9

DetectionBioautography vs. *Bacillus subtilis* KY 4273 R_F

Solvent A	0.20
Solvent B	0.75
Solvent C	0.35
Solvent D	0.40

Ref.

As Bleomycins, TLC 5

VIOMYCIN**TLC 1**

Medium

Kieselgel G (Merck)

Solvent

10% Aq. ammonium acetate-acetone-10% ammonium hydroxide (9:10:0.5)

 R_F

0.24

Ref.

A. Nagata, T. Ando, R. Izumi, H. Sakakibara, T. Take, K. Hayano and J. Abe, *J. Antibiot.*, 21 (1968) 681-687

TLC 2

Medium

Silica gel

Solvents

- A. Chloroform-methanol-17% aq. ammonia (2:1:1), upper layer
- B. 10% Ammonium acetate-methanol (1:1)
- C. 10% Ammonium acetate-acetone-10% aq. ammonia (9:10:0.5)

 R_F

Solvent A	0.12
Solvent B	0.67
Solvent C	0.36

Ref.

As Tuberactinomycins, TLC 4

TLC 3

Medium

Eastman Kodak

Solvent

tert.-Butyl alcohol-acetone-glacial acetic acid-5% ammonium hydroxide-water (5:5:5:3:1)

Detection

UV

Ref.

J.H. Carter, II, R.H. DuBus, J.R. Dyer, J.C. Floyd, K.C. Rice and P.D. Shaw, *Biochemistry*, 13 (1974) 1221–1227

ELPHO**Medium**

Whatman No. 1 paper

Buffer

Sodium veronal, 6.23 g + sodium acetate, 4.11 g in 1 liter water adjusted to pH 8.0 with *N* hydrochloric acid

Conditions

300 V (11.5 V/cm), 3 h

Detection

Ninhydrin

Mobility

Separates from co-crystalline polypeptide

Ref.

Z. Kotula, P. Bukowski and Z. Kowszyk-Gindifer, *Med. Dosw. Mikrobiol.*, 22 (1970) 95–100

GLC

A simple method for routine analysis of viomycin preparations has been elaborated. In this method an alkaline soln. of viomycin sulfate is introduced directly to the gas chromatographic column. Viomycin suffers degradation, one of the degradation products being 2-aminopyrimidine. This compound gives a symmetrical peak which can be easily integrated

Apparatus

Carlo Erba gas chromatograph model GP 515

Column

1.80 × 3 mm I.D., stainless steel, packed with Porapak Q 110–120 mesh

Temperatures

Column temperature, 150°C; vaporizer temperature, 200°C

Carrier gas

40 ml/min nitrogen

Detection

Flame ionization detector

Solutions**Viomycin:**

About 0.1 g of viomycin sulfate was weighed accurately into a 1-ml volumetric flask and dissolved in 0.5 ml of water. 0.25 ml of 1 *N* sodium hydroxide was added, the soln. made up to 1 ml with water and stirred

Standard:

About 10 mg of 2-aminopyrimidine was weighed accurately into a volumetric flask, made up to

1 ml with water and stirred. An alkaline soln. of viomycin sulfate may also be used as a standard, provided the yield of 2-aminopyrimidine formed from the preparation is known

Procedure

5 µl of a viomycin or standard soln. were injected into vaporizer of the gas chromatograph. After about 7 min, a symmetrical peak appeared on the chromatogram. When the recorder returned to zero, 5 µl of water were injected. After a further 7 min, a second peak appeared on the chromatogram. Both peaks have the same retention times, but the area of the second peak is 5–10% of the area of the first one. The areas of both peaks were integrated and added

Ref.

L. Lechowski, *Chemia Analityczna (Warsaw)*, 16 (1971) 9–12

VIRGINIAMYCIN S**TLC****Medium**

Silica gel 60, precoated (Merck)

Solvents

A. Chloroform–methanol (95:5)

B. Chloroform–methanol (96:4)

Detection

A. UV at 360 nm

B. UV at 360 nm

R_F

	Detection	Solvent	
		A	B
Virginiamycin S	A	0.64	
	B		0.50
Allodihydro-virginiamycin S	A	0.51	
Norma-dihydro-virginiamycin S	A	0.40	
Deoxyvirginiamycin S	B		0.59

Ref.

G. Janssen, J. Anne and H. Vanderhaeghe, *J. Antibiot.*, 30 (1977) 141–145

VIRIDENOMYCIN**TLC****Medium**

Silica gel (Tokyo Kasei: Spot Film)

Solvents

- A. Chloroform-methanol (9:1)
 B. Benzene-chloroform-methanol (70:28:2),
 TLC plate pre-treated with oxalic acid

R_F

Solvent A	0.2
Solvent B	0.2

Ref.

T. Hasegawa, T. Kamiya, T. Henmi, H. Iwasaki
 and S. Yamatodani, *J. Antibiot.*, 28 (1975) 167-
 175

XANTHOCIDIN**PC****Solvents**

- A. Wet butanol
 B. 3% Ammonium chloride
 C. 75% Phenol
 D. 50% Acetone
 E. Butanol-methanol-water-methyl orange (40
 ml:10 ml:20 ml:1.5 g)
 F. Butanol-methanol-water (40:10:20)
 G. Benzene-methanol (80:20)
 H. Water
 All solvents developed ascending

Detection

Bioautography vs. *Xanthomonas oryzae*

R_F (estimated from drawing)

Solvent	R _F	Solvent	R _F
A	0.75	E	0.70
B	0.82	F	0.75
C	0.75	G	0.50
D	0.80	H	0.75

Ref.

K. Asahi, J. Nagatsu and S. Suzuki, *J. Antibiot.*,
 19 (1966) 195-199

XANTHOCILLIN**TLC****Medium**

Kieselgel G (Merck)

Solvents

- A. Benzene-acetone-methanol-25% ammonium
 chloride (70:10:20:2)
 B. Chloroform-ethyl acetate-methanol-acetic
 acid (80:10:10:1)
 C. Benzene-acetone (70:30)
 D. Chloroform-acetone-methanol (80:10:10)
 E. Chloroform-acetone-methanol (85:10:5)
 F. Benzene-acetone-methanol (90:10:10)

Detection

- A. Kieffer-reagent, blue
 B. 6 g Silver nitrate in water-acetone (10:90),
 yellow

R_F

Solvent	R _F	Solvent	R _F
A	0.42	D	0.65
B	0.62	E	0.65
C	0.48	F	0.65

Ref.

S. Pfeiffer, H. Baer and J. Zarnack, *Pharmazie*,
 27 (1972) 536-542

XANTHOMYCIN A**PC****Paper**

Eaton-Dikeman 613 (0.5 in. wide strips)

Solvent

1-Butanol containing 1% (v/v) 1 N hydrochloric
 acid

Detection

Bioautography vs. *Bacillus subtilis*

R_F

0.0, 1.0

Ref.

K.V. Rao and W.H. Peterson, *J. Amer. Chem.*
Soc., 76 (1954) 1335
 D. Dougall and E.P. Abraham, *Nature*, 176 (1955)
 256

XYLOSTASIN**TLC****Solvent**

Chloroform-methanol-aq. ammonia-water
 (4:3:2:1)-methanol (5:3), upper layer

R_F

Component	R _F
Xylostin	0.23
3'-Chloro-3'-deoxy-xylostin	0.41
3'-Deoxy-xylostin	0.26

Ref.

S. Igarasi and M. Kida, *U.S. Patent* 3,933,790,
 Jan. 20, 1976

ELPHO**Medium**

Whatman No. 1 paper

Buffer

0.025 M Borate buffer, pH 9.5

Conditions

2 kV/36 cm for 90 min

Mobility

5 to 6 cm to cathode

Ref.S. Horii, I. Nogami, T. Hasegawa and M. Yoneda,
U.S. Patent 3,953,293, April 27, 1976**YAZUMYCIN****PC 1****Solvents**

- A. *n*-Propanol-pyridine-acetic acid-water
(10:15:3:10)
B. 80% Methanol-piperidine (10:1) adjusted to
pH 9.3 with acetic acid
C. Water satd. *n*-butanol containing 2% *p*-tol-
uenesulfonic acid
D. *n*-Butanol-acetic acid-water (2:1:1)

R_F

Solvent A	0.42
Solvent B	0.53
Solvent C	0.09
Solvent D	0.16

Ref.K. Akasaki, H. Abe, A. Seino and S. Shirato, *J.*
Antibiot., 21 (1968) 98-105**PC 2****Solvent**Wet *n*-butanol containing 2.0% (w/v) *p*-toluene-
sulfonic acid**R_F**

0.09

Ref.K. Arima, T. Kawamura and T. Beppu, *J. Anti-
biot.*, 25 (1972) 387-392**TLC 1****Medium**

Silica gel

Solvents

- A. Chloroform-methanol-17% ammonium hy-
droxide (2:1:1), upper layer
B. *n*-Propanol-pyridine-acetic acid-water
(15:10:3:10)

R_F

Solvent A	0.3-0.3
Solvent B	0.6

Ref.

As PC 1

TLC 2**Medium**

Silica gel G

Solvents

- A. *n*-Propanol-pyridine-acetic acid-water
(15:10:3:12)
B. Chloroform-methanol-17% aq. (2:1:1), up-
per layer

R_F

Solvent A	0.64
Solvent B	ca. 0.3-0.33

Ref.

As PC 1

YEMINIMYCIN**PC****Solvents**

- A. Light petroleum, b.p. 40-60°C
B. Light petroleum, b.p. 100-120°C
C. Distilled water
D. *n*-Butanol-water (1:1)
E. Methanol
F. *n*-Butanol-acetic acid-water (4:1:5)
G. Ethyl acetate-water (1:1)
H. Chloroform satd. with water
I. Ethyl acetate-light petroleum
J. Ethanol
K. Chloroform
L. Ethyl acetate
M. Acetone

Detection

- A. Bioautography vs. *Bacillus subtilis* NRRL-B-
543 or *Penicillium chrysogenum* Q176
B. Spray with dilute potassium permanganate and
heat

R_F (estimated from drawing)

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.00	H	0.70
B	0.00	I	0.70
C	0.25	J	0.80
D	0.30	K	0.95
E	0.50	L	0.95
F	0.65	M	0.95
G	0.70		

Ref.I.B. Shimi, A. Dewedar and N. Abdallah, *J.*
Antibiot., 24 (1971) 283-289

ZERVACIN I and II**TLC****Medium**

Silica gel (Eastman Chromagram)

Solvent

Chloroform-methanol (6:1)

DetectionBioautography vs. *Sarcina lutea* R_F (estimated from drawing)

Component I 0.05

Component II 0.20

Ref.A.D. Argoudelis and L.E. Johnson, *U.S. Patent* 3,907,990, Sept. 23, 1975**ZORBAMYCIN;
ZORBONOMYCINS
(cf., YA-56 complex, PC 2 and TLC)****TLC****Medium**

MN-Polygram CEL 300 (Brinkmann)

SolventsA. 0.05 *M* Sodium citrate buffer, pH 6.9B. 0.1 *M* Aq. ammonium chloride adjusted to pH 7.5 with aq. ammonium hydroxideC. 0.2 *M* Aq. ammonium chloride adjusted to pH 7.5 with aq. ammonium hydroxide**Detection**Bioautography vs. *Bacillus subtilis*, *Klebsiella pneumoniae* or *Staphylococcus aureus* R_F (estimated from drawing)

	Solvent		
	A	B	C
Zorbamycin	0.40	0.40– 0.55	–
Zorbomycin B	–	0.25	0.50– 0.55
Zorbomycin C	–	0.10	0.40– 0.50

Ref.A.D. Argoudelis, M.E. Bergy and T.R. Pyke, *J. Antibiot.*, 24 (1971) 543–557**ZORBONOMYCIN B****TLC 1****Medium**

Silica gel

Solvents

A. Chloroform-methanol-17% aq. ammonia (2:1:1) upper layer

B. 10% Ammonium acetate-methanol (1:1)

C. Methanol-10% ammonium acetate-10% aq. ammonia (10:9:1)

D. 0.05 *M* Citric acid buffer soln., pH 6.5 R_F

Solvent A 0.05

Solvent B 0.77

Solvent C 0.83

Solvent D 0.25

Ref.

As Bleomycins, TLC 2

TLC 2**Medium**

Silica gel

Solvents

A. Chloroform-methanol-17% ammonia water (2:1:1), upper layer

B. 10% Ammonium acetate-methanol (1:1)

C. Methanol-10% ammonium acetate-10% ammonia water (10:9:1)

D. 0.05 *M* Citrate buffer, pH 6.9**Detection**Bioautography vs. *Bacillus subtilis* KY 4273 R_F

Solvent A 0.35

Solvent B 0.77

Solvent C 0.84

Solvent D 0.29

Ref.

As Bleomycins, TLC 5

ZYGOMYCINS**PC****Paper**

Toyo Roshi No. 131

Solvents

A. 50% Aq. phenol, descending, 44 h

B. *n*-Butanol-acetic acid-water (2:1:2)

C. As B, but (4:1:5)

DetectionA. Bioautography vs. *Bacillus subtilis*

B. Ninhydrin

C. Sakaguchi reaction

Ref.K. Nakazawa, M. Shibata, E. Higashide, T. Kanzaki, H. Yamamoto, A. Miyake, H. Hitomi, S. Horii, T. Yamaguchi, T. Araki, K. Tsuchiya, Y. Oka and A. Imai, *U.S. Patent* 3,089,827, May 14, 1963

R_F

Compound	Solvent		
	A	B	C
Zygomycin A	4 cm from origin •	0.33	0.01
Zygomycin B	14 cm from origin **	—	—

• Sakaguchi (—)

** Sakaguchi (+)

ZYGOSPORINS

TLC

Medium

Silica gel

Solvents

A. Chloroform–methanol (9:1)

B. Toluene–methanol (10:1)

 R_F

	Solvent	
	A	B
Zygosporin A	0.50	—
Zygosporin D	0.40	—
Zygosporin E	0.55	—
Zygosporin F	0.57	0.28
Zygosporin G	0.57	0.35

Ref.

M. Minato, M. Matsumoto and T. Katayama,
167th Meeting Japan Antibiotics Res. Assn., May
28, 1969

Numbered antibiotics

15

PC

Paper

Whatman No. 1

Solvent

Propanol-pyridine-acetic acid-water
(15:10:3:12), descending, 48 h, 25°C; front runs
off end of paper

Detection

Bioautography vs. *Klebsiella pneumoniae*

R_F

Com- ponent	R_F *	Com- ponent	R_F *
15A	0.53	15D	0.24
15B	0.41	15E	0.17
15C	0.30	15F	0.13

* Distance of spot from origin/distance of origin
to end of paper

Ref.

M.J. Weinstein, G.H. Wagman and G. Luedemann, *U.S. Patent* 3,458,626, July 26, 1969

19A

PC

Paper

Whatman No. 1; buffer as stationary phase

Solvents

- Ethyl acetate-0.1 M phosphate buffer, pH 3-9
- Isopropanol-water (70:30)
- Sodium chloride-water-methanol (2.0% : 25:75)
- Butanol-0.1 M phosphate, pH 7.0
- Methyl isobutyl ketone-0.1 M phosphate, pH 5.0

All solvents developed descending

Detection

Bioautography

R_F

Solvent system	R_F	Solvent system	R_F
A (pH 3)	1.00	A (pH 9)	0.00
A (pH 5)	1.00	B	0.54
A (pH 6)	0.69	C	0.79
A (pH 7)	0.70	D	0.08
A (pH 8)	0.08	E	0.60

Ref.

I. Putter and F.J. Wolf, *Antimicrob. Agents Chemother.*, 1961 (1962) 454-461

60-6

TLC

Medium

Silica gel

Solvents

- n*-Butanol-acetic acid-water (3:1:1)
- Chloroform-ethanol-14% aq. ammonia (4:7:2)
- Chloroform-ethanol-water (4:7:2)

Detection

- Bioautography vs. *Staphylococcus aureus* 209P
- Ninhydrin
- Sulfuric acid spray, heated

R_F

Solvent A	0.50
Solvent B	0.37
Solvent C	0.50

Ref.

J. Shoji, M. Mayama, S. Matsuura, K. Matsumoto and Y. Wakisaka, *U.S. Patent* 3,923,979, Dec. 2, 1975

61-26

TLC

Medium

Silica gel GF

*Solvent**n*-Butanol–acetic acid–water (4:1:2)*Detection*

The zone of the antibiotic was visualized by iodine

*Ref.*J. Shoji, R. Sakazaki, Y. Wakisaka, K. Koizumi and M. Mayama, *J. Antibiot.*, 28 (1975) 129–131**67-121****TLC***Solvent*

Chloroform–methanol–water (2:2:1), lower phase

R_F

Component	<i>R_F</i>
A	0.17
B	0.14
C	0.11
D	0.07

*Ref.*M.J. Weinstein, G.H. Wagman, J.A. Marquez and M.G. Patel, *U.S. Patent* 4,027,015, May 31, 1977**68-1147****TLC***Solvent*

Diethyl ether–hexane (3:2)

Detection

- A. Bioautography
B. Iodine vapor

R_F

Component	<i>R_F</i>
68-1147 I	0.51
68-1147 II	0.42
68-1147 III	0.31
68-1147 IV	0.03

*Ref.*M.J. Weinstein, G.H. Wagman, J.A. Marquez and R.T. Testa, *U.S. Patent* 4,078,056, March 7, 1978**106-7****PC***Solvents*

- A. Ethanol–water (4:1)
B. Butanol–water–acetic acid (4:2:1)

Detection

Ninhydrin (0.2% in 93% ethanol). Gives characteristic yellow–blue color

R_F

Solvent A	0.33
Solvent B	0.37

*Ref.**U.K. Patent* 757,089, Sept. 12, 1956**136****PC***Paper*

Whatman No. 1

*Solvent*1-Butanol satd. with water containing 2% *p*-toluenesulfonic acid*Detection*Bioautography vs. *Bacillus subtilis**R_F*

Component 136A > B > C > D > E

*Ref.*W.F. Phillips and H.S. Ragheb, *J. Chromatogr.*, 19 (1965) 147–159**156-B1****PC***Solvents*

- A. *n*-Propanol–pyridine–acetic acid–water (15:10:3:12)
B. *n*-Butanol–pyridine–acetic acid–water (15:10:3:12)
C. Water satd. *n*-butanol
D. 1.5% Ammonium chloride
E. 75% Phenol
F. 50% Acetone
G. *n*-Butanol–methanol–water–methyl orange (40 ml:10 ml:20 ml:1.5 g)
H. *n*-Butanol–methanol–water (40:10:20)
I. Benzol–methanol (4:1)
J. Water
K. Water satd. *n*-butanol + 0.2% *p*-toluenesulfonic acid

R_F (see p. 408)*Ref.*Y. Shimojima, I. Takeda, E. Takeda, M. Mizuno, Y. Mizuno and T. Ohoka, *U.S. Patent* 3,925,546, Dec. 9, 1975

R_F

Solvent	R_F	Solvent	R_F
A	0.35	G	0.51
B	0.29	H	0.03
C	0.05	I	0.00
D	0.91	J	0.05
E	0.44	K	0.05
F	0.04		

235 S**PC***Solvents*

- A. *n*-Butanol satd. with aq. 0.25 *M* disodium hydrogen phosphate, pH 9.3
 B. *n*-Butanol satd. with 0.1 *N* ammonium hydroxide

The paper is first satd. with the aq. buffer or the aq. ammonia soln. before applying the sample to the paper. Development time is 6–17 h

 R_F

Solvent	S_1	S_2	S_3
A	0.14	0.28	0.47
B	0.15	0.33	0.51

Ref.

L. Chalet and J. Mata, *U.S. Patent* 3,851,054, Nov. 26, 1974

289F, 289FO, and ACETYL 289F**PC***Paper*

- A. Toyo No. 50, developed ascending
 B. Circular aluminum oxide paper

Solvents

- A. Acetonitrile
 B. Butyl acetate–dibutyl ether (3:1)
 C. Coloration with nickel acetate

 R_F

	Paper–Solvent	R_F
289F	A–A	0.14
	A–B	0.71
289FO	A–A	0.40–0.45
	A–B	0.80–0.85
	B–C	0.28–0.39
Acetyl 289F	B–C	1.00

Ref.

South Africa Appl. No. 695196, July 16, 1969

TLC*Medium*

Silica gel (Kieselgel G, Merck)

Solvents

- A. Ethanol–14% ammonia (4:1)
 B. Ethanol–pyridine (4:1)

Detection

As PC; A, B

 R_F

Compound	Solvent	R_F
289F	A	0.8
	B	0.06
Acetyl 289F	B	0.55

Ref.

As PC

342-14-I**TLC 1***Medium*

Silica gel GF

Solvents

- A. Chloroform–ethanol–14% aq. ammonia (4:7:2)
 B. Chloroform–ethanol–10% acetic acid (4:7:2)
 C. *n*-Butanol–acetic acid–water (3:1:1)

Detection

- A. Bioautography vs. *Staphylococcus aureus* 209P
 B. Ninhydrin
 C. Heat treatment of the plate sprayed by sulfuric acid. Single spot in each of the solvent systems

 R_F

Solvent A	2.0
Solvent B	0.14
Solvent C	0.40

Ref.

J. Shoji, M. Mayama, S. Matsuura, K. Matsumoto and Y. Wakisaka, *U.S. Patent* 3,969,501, July 13, 1976

TLC 2*Medium*

Silica gel GF

Solvents

- A. Chloroform–ethanol–14% ammonia (4:7:2)
 B. Chloroform–ethanol–10% acetic acid (4:7:2)
 C. *n*-Butanol–acetic acid–water (3:1:1)

Detection

- A. Bioautography vs. *Staphylococcus aureus*
 B. Ninhydrin

R_F

Solvent A	2.0
Solvent B	0.14
Solvent C	0.40

Ref.

Netherlands Patent 7,507,346, June 19, 1975

460**PC 1**

Paper

Whatman No. 1

Solvents

- A. Propanol-pyridine-acetic acid-water
(15:10:3:12)
B. Propanol-acetic acid-water (50:40:5)

Detection

Bioautography

 R_F

Component	Solvent A	Solvent B
1	0.20	0.13
2	0.25	0.20
3	0.95	0.93

Ref.

M.J. Weinstein, G.H. Wagman, J.A. Marquez
and G. Luedemann, *U.S. Patent* 3,454,696, July
8, 1969

PC 2

Paper

Toyo No. 51

Solvents

- A. 20% Ammonium chloride
B. Water satd. *n*-butanol
C. *n*-Butanol-acetic acid-water (3:1:1)
D. Water satd. ethyl acetate
E. Water satd. *n*-butanol + 2% *p*-toluenesulfonic
acid + 2% piperidine

Detection

Bioautography vs. *Bacillus subtilis* No. 10707 R_F

Solvent	R_F	Solvent	R_F
A	0.95	D	0.00
B	0.00	E	0.00
C	0.00		

Ref.

As Carbomycin, PC 1

PC 3

Paper

Ascending

Solvent

Chloroform-methanol-17% aq. ammonia
(2:1:1) lower layer (developed at room temp. for
12 h)

 R_F

0.01

Ref.

As Apramycin, PC 1

484, 484-A, and 484-B**PC 1**

Paper

Whatman No. 1, descending, 24°C for 48 h

Solvents

- A. *n*-Propanol-pyridine-acetic acid-water
(30:20:6:20)
B. *n*-Propanol-pyridine-acetic acid-water
(6:4:1.5:4)

Detection

- A. Bioautography vs. *Sarcina lutea*
B. Ninhydrin

 R_F

Com- pound	Solvent- Detection	cm from origin
484	A-A	25 and 11
484-A	A-A	23-25
	A-B	13-15 and 23-25
484-A sulfate	A-A	18-20
	A-B	10 and 18-20 (R_F = 0.15-0.16 and 0.22-0.24)
484-B	B-A	17 (main zone) 24 and 8 (minor zones)

Ref.

As Racemomycin A sulfate, PC 1

PC 2

Paper

Whatman No. 1, circular

Solvent

n-Propanol-pyridine-acetic acid-water
(30:20:6:24)

Detection

Ninhydrin

R_F

484-A: 0.40–0.42 (one zone); 0.30–0.32 and 0.40–0.42 (two zones)

484-B: 0.16, 0.29, 0.42

Ref.

As Racemomycin A sulfate, PC 1

TLC

Medium

Silica gel

Solvent

Methanol–chloroform–28% ammonia (2:1:2)

 R_F

484-B: 0.18, 0.36

Ref.

As Racemomycin A sulfate, PC 1

833A**PC**

Paper

Whatman No. 3

Solvents

A. Isopropanol–0.01 phosphate buffer, pH 6.0 (70:30)

B. 2% Sodium chloride + 17% aq. methanol

Detection

A. Bioautography vs. *Proteus vulgaris* MB 383B. Bioautography vs. *Erwina atrosepica* MB 1159 R_F

Solvent	Detection A	Detection B
A	0.36	0.73
B	0.28	0.71

Ref.

D. Hendlin, E.O. Stapley, S. Mochales Del Val and J. Martinez Mata, *U.S. Patent* 3,914,231, Oct. 21, 1975**ELPHO**

Medium

Paper

Buffer

0.165 M Phosphate buffer, pH 7.0

Conditions

600 V, 2½ h

Detection

Bioautography vs. *Erwina atrosepica*

Mobility

Movement from point of origin to center of inhibition zone: 11.5 cm

Ref.

As PC

1308**PC**

Solvents

A. 16% Hydrated *n*-propanolB. 18% Hydrated *n*-propanol R_F

Solvent A 0.19

Solvent B 0.27

Ref.

I. Takeda, M. Mizuno, T. Sugawara, Y. Shimojima and S. Horiguchi, *U.S. Patent* 3,780,172, Dec. 18, 1973**1745 A/X****TLC**

Medium

Silica gel

Solvent

Methylene chloride–95% aq. methanol–conc. ammonium hydroxide (90:10:1) (run through twice)

Detection

Bioautography vs. *Bacillus* sp. 1283 B R_F

0.5

Ref.

As Oleandomycins, TLC 4

CCD

Solvent

n-Butanol–acetic acid–water (10:1:10) adjusted to pH 2.95 with 2 N sodium hydroxide (upper and lower phases)

Distribution

100 Tube apparatus (80-ml tube vol.)

Detection

Activity obtained from tubes 78–88

Ref.

As Oleandomycins, TLC 1

1985/11**PC**

Solvent

As 3035/48, PC

 R_F

0.20, 0.26, 0.41, 0.54

Ref.

As 3035/48, PC

1998**PC***Solvents*

- A. *n*-Butanol–acetic acid–water (4:1:5)
- B. *n*-Butanol–pyridine–acetic acid–water (15:10:3:12)
- C. Water satd. *n*-butanol
- D. Water
- E. 50% Acetone
- F. 3% Ammonium chloride

Detection

- A. Bioautography vs. *Staphylococcus aureus*
- B. UV

R_F

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.65	D	0.12
B	0.81	E	0.90
C	0.40	F	0.71

Ref.

Netherlands Patent 7,406,919, Nov. 26, 1974

2814P**PC***Solvent*

As eurocidin, PC

R_F

0.58

Ref.

As eurocidin, PC

3035/48*Solvent*

n-Butanol–acetic acid–water–pyridine (15:3:12:10)

R_F

0.20, 0.24, 0.32, 0.43

Ref.

R.A. Zhukova, R.V. Kirsanova, L.A. Kovaleva, T.V. Kotenko, W.I. Kuznetsova, A.A. Medvedova, B.V. Sokolov, M.D. Paikin, M.A. Frolova and Y.D. Shenin, *Mikrobiologiya*, 35 (1966) 312–318

3950*Solvents*

As echinomycin, PC 2; A, B, C

R_F

Solvent A 0.26, 0.76
Solvent B 0.32, 0.54
Solvent C 0.60, 0.80

Ref.

As echinomycin, PC 2

4205**PC***Paper*

Whatman No. 1

Solvent

1-Butanol–acetic acid–water (4:1:5), organic phase, ascending, 17.5 h

Detection

- A. 0.25% Ninhydrin in acetone
- B. Bioautography vs. *Staphylococcus albus* or *Escherichia coli*

R_F

4205A 0.69
4205B 0.67

Ref.

M. Shaw, R. Brown and A.G. Martin, *Appl. Microbiol.*, 14 (1966) 79–85

TLC*Medium*

Silica gel G (E. Merck)

Solvent

n-Butanol–acetic acid–water (2:1:1); develop to 15 cm from origin

Detection

- A. Heat plates at 120–150°C for 10–15 min; while hot, spray with 0.2% ninhydrin in 95% *n*-butanol + 5% acetic acid
- B. After ninhydrin spray, re-heat again for a short time at 120–150°C and spray with a satd. soln. of potassium dichromate in conc. sulfuric acid

R_F

4205A 0.67
4205B 0.63
4205C 0.66

Ref.

As PC; also. Ehrhardt and F. Cramer, *J. Chromatogr.*, 7 (1962) 402–407

6270**PC***Solvents*

As echinomycin, PC 2; A, B, C

R_F

Solvent A 0.76
Solvent B 0.31
Solvent C 0.80

Ref.

As echinomycin, PC 2

9865 RP**PC***Paper*

Arches No. 310, impregnated with a soln. of acetone containing 20% formamide

Solvent

Chloroform-benzene satd. with formamide (2:1)

R_F

0.86

Ref.

S. Pinnert, L. Ninet and J. Preud'Homme, *U.S. Patent* 3,989,598, Nov. 2, 1976

TLC*Medium*

Silica gel

Solvent

Benzyl alcohol-ethyl formate-formic acid-water (4:4:1:5), ascending

R_F

0.60

Ref.

As Daunorubicin (13057 RP), TLC

13057 RP

[See Daunorubicin (13057 RP)]

13213 RP**PC***Paper*

Arches No. 302, impregnated with a phosphate buffer, pH 4.8

Solvent

Water satd. *n*-butanol, descending

Detection

Bioautography vs. *Bacillus subtilis* or *Klebsiella pneumoniae*

R_F

0.7

Ref.

As Daunorubicin (13057 RP), PC

13330 RP**PC***Paper*

Arches No. 302, impregnated with a phosphate buffer, pH 4.8

Solvent

Water satd. *n*-butanol, descending

Detection

Bioautography vs. *Bacillus subtilis* or *Klebsiella pneumoniae*

R_F

0.9

Ref.

As Daunorubicin (13057 RP), PC

16511 RP**TLC***Medium*

Kieselgel H_{254, 336}; buffered at pH 5 with $\frac{1}{15}$ M phosphate

Solvent

Ethyl acetate

Detection

A. UV light

B. Bioautography vs. *Neisseria catarrhalis* or *Sarcina lutea*

R_F

Component	<i>R_F</i>
16511A	0.1
16511B •	0.2
16511C	0.35
16511D	0.60

• Major zone = 18051 RP

Ref.

South Africa Patent 671147, July 19, 1967

17967 RP**TLC***Media*

A. Silica gel F₂₅₄ (Merck)

B. Silica gel H (Merck), buffered with $\frac{1}{3}$ M phosphate, pH 8.0

Solvents

A. Chloroform-methanol-acetone (78:20:2)

B. Chloroform-methanol (87:13)

C. Acetone-dioxane (50:50)

Detection

A. UV light

B. Bioautography vs. *Bacillus subtilis*

R_F

Medium	Solvent	<i>R_F</i>
A	A	0.6
A	B	0.4
B		0.6 C

Ref.

Netherlands Patent 69,06827, Nov. 11, 1969

18051 RP (See 16511 RP)

18631 RP

PC

Papers

- A. Arches 302
B. As A, but buffered with $\frac{1}{3}$ M phosphate, pH 7

Solvents

- A. Water satd. butanol
B. Benzene-methanol (4:1)
C. Ammonium chloride (30 g/l)
D. Butanol-acetic acid-water (4:1:5), upper phase
E. Ethyl acetate-cyclohexane (1:1) satd. with water
F. Chloroform

Detection

Bioautography vs. *Staphylococcus albus*

R_F

Paper	Solvent	R_F
A	A	0.95
	B	0.95
	C	0.05
	D	1.00
	E	0.50
B	F	0.50

Ref.

Netherlands Patent 69,02381, Aug. 18, 1969

TLC

Media

- A. Aluminium oxide
B. Silica gel G

Solvents

- A. Methanol-water (95:5)
B. As PC, D
C. Carbon tetrachloride-ethanol-acetic acid (90:6:6)

Detection

As PC

R_F

Medium	Solvent	R_F
A	A	0.27
B	B	1.00
B	C	0.50

Ref.

As PC

18887 RP

CCD 1

Solvent

Ethyl acetate-butanol-3 M phosphate buffer of pH 5.3 (80:20:100, v/v) the pH is kept at 5.3 with N sodium hydroxide soln.

Distribution

103 cells

250 Transfers. The contents of the first 16 cells of the apparatus are combined

Ref.

D. Mancy, L. Ninet and J. Preud'Homme, U.S. Patent 3,712,945, Jan. 23, 1973

CCD 2

Solvent

The antibiotic obtained is dissolved in a mixture of 100 ml of each of the phases of the system benzene-chloroform-water-methanol (15:25:11:26, v/v)

Distribution

Automatic counter-current distribution comprising 103 cells of 50 ml unit volume. 100 Transfers of 25 ml of upper phase are effected, followed by an alteration of 50 transfers of 25 ml of each phase. The contents of cells 30 to 58 of the apparatus are combined

Ref.

As CCD 1

19402 RP

PC

Paper

Arches 302, unbuffered

Solvents

- A. n-Butanol-acetic acid (60:40)
B. n-Butanol-acetic acid-water (60:40:10)
C. Ethyl acetate-pyridine-water (50:40:20)

Detection

Bioautography vs. *Staphylococcus aureus* 209P

R_F

Solvent A	0.62
Solvent B	0.48
Solvent C	0.30

Ref.

D. Mancy, L. Ninet and J. Preud'Homme, U.S. Patent 3,966,557, June 29, 1976

TLC

Media

- A. Silica gel G
B. Silica gel G + alumina G (70:30)
C. Silica gel G + alumina G (50:50)
D. Silica gel H

Solvents

- A. Isopropanol–2 *N* ammonia (70:30)
 B. Isopropanol–*n*-butanol–water (50:40:30)
 C. Dioxan–water (80:20)

Detection

Bioautography vs. *Staphylococcus aureus* 209P

 R_F

Solvent	Medium	R_F
A	A	0.37
B	B	0.35
	C	0.30
C	D	0.20

Ref.

As PC

20798 RP**TLC****Medium**

Silica gel

Solvents

- A. Benzyl alcohol–ethyl formate–formic acid–water (4:4:1:5), ascending
 B. Chloroform–dioxan (95:5)
 C. Ethyl acetate–diethyl ether– $\frac{1}{3}$ *M* phosphate buffer of pH 4.8 (50:50:25)

 R_F

Solvent A	0.57
Solvent B	0.1
Solvent C	0.3

Ref.

As Daunorubicin (13057 RP), TLC

21190 RP**TLC****Medium**

Kieselgel H (Merck)

Solvents

- A. Chloroform
 B. Benzene
 C. Benzene–methanol (90:10)
 D. Benzene–methanol (80:20)
 E. Benzene–methanol (50:50)
 F. Acetone
 G. Methanol

Detection

- A. Bioautography vs. *Staphylococcus aureus* 209P
 B. Spray with sulfuric acid and heat at 110°C

 R_F

Solvent	R_F	Solvent	R_F
A	0	E	0.87
B	0	F	0.80
C	0.15	G	0.85
D	0.60		

Ref.

D. Mancy, J. Florent and J. Preud'Homme, *U.S. Patent* 3,892,849, July 1, 1975

21906**TLC****Medium**

Silica gel G/HF₂₅₄

Solvent

Chloroform–methanol (98:2)

Detection

conc. Sulfuric acid at 100°C

 R_F

Fraction A	0.39
Fraction B	0.2
Fraction C	0.07

Ref.

C. Coronelli, M.R. Bardone and H. Pagani, *U.S. Patent* 3,949,078, April 6, 1976

23671 RP**TLC****Medium**

Silica gel F₂₅₄

Solvent

Chloroform–methanol (87:13)

Detection

Spray with conc. sulfuric acid

 R_F

0.55

Ref.

D. Mancy, J. Florent and J. Lunel, *U.S. Patent* 3,857,937, Dec. 31, 1974

23672 RP**TLC****Medium**

Silica gel F₂₅₄

Solvent

Chloroform–methanol (87:13)

Detection

Spray with conc. sulfuric acid

R_F
0.45

Ref.

As 23671 RP, TLC

24010

TLC

Medium

Silica gel

Solvents

A. *n*-Butanol-acetic acid-water (4:1:5, 4:1:2, 2:1:1)

B. *n*-Butanol-ethanol-water (10:3:7)

Detection

A. Anisaldehyde-sulfuric acid; heat

B. Potassium permanganate-sodium carbonate reagent

C. UV light

D. Bioautography vs. *Bacillus subtilis*

R_F

One spot under all conditions

Ref.

M. Mizuno, Y. Shimojima, T. Sugawara and I. Takeda, *J. Antibiot.*, 24 (1971) 896-899

30504 RP

TLC

Medium

Silica gel

Solvents

A. Ethyl acetate-cyclohexane-water-butanol (50:50:25:5)

B. Methylene chloride-methanol (94:6)

R_F

Solvent A 0.45

Solvent B 0.55

Ref.

German Patent 2,508,914, Sept. 4, 1975

70591

[cf., actinomycins (from *Micromonospora floridensis*)]

TLC

Additional ref.

M.J. Weinstein, G.H. Wagman, J.A. Marquez and P.D. Watkins, *U.S. Patent* 3,954,970, May 4, 1976

A-2

PC

Solvents

A. Water satd. *n*-butanol

B. 50% Acetone

C. *n*-Butanol-methanol-water (4:1:2)

D. Benzene-methanol (4:1)

E. Water

F. 0.5% Ammonium chloride

G. 3.0% Ammonium chloride

H. 20% Ammonium chloride

R_F

Solvent	R_F	Solvent	R_F
A	1.0	E	1.0
B	1.0	F	0.9
C	1.0	G	0.85
D	1.0	H	0.7

Ref.

M. Okuchi, M. Itoh, Y. Kaneko and S. Doi, *J. Antibiot.*, 26 (1973) 562-568

TLC

Medium

Kieselgel G

Solvents

A. Benzene-methanol (4:1)

B. Chloroform-methanol (98:2)

C. Benzene-ethyl acetate (10:1)

R_F

Solvent A 0.9

Solvent B 0.8

Solvent C 0.2

Ref.

As PC

A-59

PC

Paper

Ascending

Solvents

A. Chloroform-methanol (9:1)

B. Chloroform-methanol (19:1)

C. Chloroform-*n*-butanol (6:1)

D. Ethyl acetate-*n*-hexane-2 *N* ammonia (4:1:1)

E. Methanol-acetic acid-water (25:3:72)

R_F (see p. 416)

Ref.

As Siomycins, PC 1

R_F

Solvent	R_F	Solvent	R_F
A	0.50	D	0.43
B	0.30	E	0.14
C	0.27		

A-150A**PC***Paper*

Whatman No. 1

Solvents

- A. Water satd. butanol
- B. 2% *p*-Toluenesulfonic acid in water satd. isobutyl ketone
- C. Water satd. methyl isobutyl ketone
- D. 2% *p*-Toluenesulfonic acid in water satd. methyl isobutyl ketone
- E. 2% Piperidine in water-methanol-acetone (12:3:1)
- F. 10% Aq. propanol
- G. Butanol-ethanol-water (13.5:15:150)

*Detection*Bioautography vs. *Sarcina lutea* R_F

Solvent	R_F	Solvent	R_F
A	0.87	E	0.51
B	0.72	F	0.50
C	0.73	G	0.75
D	0.67		

Ref.

R.L. Hamill, M.E. Haney, Jr. and M.M. Hoehn, *U.S. Patent* 3,711,605, Jan. 16, 1973

TLC*Solvent*

Ethyl acetate

Detection

- A. Bioautography vs. *Sarcina lutea*
- B. Vanicillin
- C. Sulfuric acid spray

 R_F

0.61

Ref.

As PC

A-201A**PC***Paper*

Whatman No. 1

Solvents

- A. Water satd. butanol
- B. Water satd. butanol + 2% *p*-toluenesulfonic acid
- C. Water satd. butanol + 2% *p*-toluenesulfonic acid + 2% piperidine
- D. Water satd. methyl isobutyl ketone
- E. Water satd. methyl isobutyl ketone + 2% *p*-toluenesulfonic acid
- F. Water satd. methyl isobutyl ketone + 2% piperidine
- G. Water-methanol-acetone (12:3:1). This soln. is adjusted to pH 10.5 with ammonium hydroxide and then lowered to pH 7.5 with phosphoric acid
- H. 80% Ethanol with 1.5% sodium chloride. Paper is impregnated with 1 *M* sodium sulfate soln.
- I. Methanol-0.1 *N* hydrochloric acid (3:1)
- J. Water satd. benzene
- K. Water-ethanol-acetic acid (70:24:6)

*Detection*Bioautography vs. *Sarcina lutea* R_F

Solvent	R_F	Solvent	R_F
A	0.87	G	0.70
B	0.89	H	0.87
C	0.88	I	0.88
D	0.44	J	0.13
E	0.47	K	0.83
F	0.48		

Ref.

R.L. Hamill and M.M. Hoehn, *U.S. Patent* 3,843,784, Oct. 22, 1974

TLC*Medium*Silica gel (Brinkmann F₂₅₄ plates)*Solvents*

- A. Ethyl acetate-ethanol (4:1)
- B. Ethyl acetate-ethanol (1:1)

Detection

- A. Naphthorescorcinol reagent
- B. Sulfuric acid
- C. UV light
- D. Bioautography

R_F

Solvent A	0.49
Solvent B	0.83

Ref.

As PC

A-201B

PC

Paper

Whatman No. 1

Solvents

- A. Water satd. methyl isobutyl ketone + 2% *p*-toluenesulfonic acid + 1% piperidine
- B. 80% Ethanol + 1.5% sodium chloride. Paper is impregnated with 1 *M* sodium sulfate soln.

R_F

Solvent A	0.65
Solvent B	0.92

Ref.

R.L. Hamill and M.M. Hoehn, *U.S. Patent* 3,843,784, Oct. 22, 1974

A204

PC

Paper

Whatman No. 1

Solvents

- A. Water-methanol-acetone-benzene (72:24.5:4:0.5)
- B. 10% Aq. *n*-propanol
- C. Water-methanol-acetic acid (12:3:1); adjust to pH 10.5 with ammonium hydroxide then to pH 7.3 with phosphoric acid

Detection

Bioautography vs. *Bacillus subtilis*

R_F

Solvent A	0.14
Solvent B	0.87
Solvent C	0.33

Ref.

Belgian Patent 728,382, Aug. 13, 1969

TLC 1

Medium

Silica gel

Solvent

Ethyl acetate

Detection

Sulfuric acid with vanillin

R_F

0.8

Ref.

As PC

TLC 2

Medium

Silica gel

Solvent

Ethyl acetate-methanol (9:1)

Detection

Spray with sulfuric acid and heat

R_F

A204	0.26, 0.33
A204I	0.53

Ref.

J.W. Chamberlin, *U.S. Patent* 3,985,872, Oct. 12, 1976

A 204A

TLC

Medium

Silica gel, Kieselgel 60, 0.25 mm (Merck)

Solvents

- A. Hexane-acetone (3:1)
- B. Toluene-acetone (9:1)
- C. Chloroform-ethyl acetate (2:1)
- D. Chloroform-acetone (9:1)

Detection

Detection was performed by spraying with a soln. of 0.2% ceric-sulfate in 50% sulfuric acid followed by heating at 110–130°C thus producing brown spots

R_F

Solvent A	0.35
Solvent B	0.11
Solvent C	0.16
Solvent D	0.08

Ref.

C. Keller-Juslen, H.D. King, M. Kuhn, H.R. Loosli and A. von Wartburg, *J. Antibiot.*, 31 (1978) 820–828

A204I

PC 1

Solvents

- A. Water-methanol-acetone (12:3:1) adjusted to pH 10.5 with ammonium hydroxide then lowered to pH 7.5 with phosphoric acid
- B. Water-methanol-acetone (12:3:1) adjusted to pH 10.5 with ammonium hydroxide then lowered to pH 7.5 with dil. hydrochloric acid
- C. 1% Methyl isobutyl ketone, 0.5% ammonium hydroxide in water
- D. Water satd. benzene
- E. Water with 1% piperidine and 2% *p*-toluenesulfonic acid
- F. Water-ethanol-acetic acid (70:24:6)

Detection

Bioautography vs. *Bacillus subtilis* ATCC 6633 (pH 6.0)

 R_F

Compound	Solvent		
	A	B	C
A204I	0.41	0.50	0.34
Ethyl ether	0.35	0.74	0.44
Methyl ether	0.33	0.70	0.56
<i>n</i> -Propyl ether	0.21	0.77	0.21

Compound	Solvent		
	D	E	F
A204I	0.49	0.71	0.61
Ethyl ether	0.57	0.57	0.52
Methyl ether	0.68	0.74	0.67
<i>n</i> -Propyl ether	0.53	0.62	0.59

Ref.

R.L. Hamill, *U.S. Patent* 3,907,832, Sept. 23, 1975

PC 2**Paper**

Whatman No. 1

Solvents

- A. Water-methanol-acetic acid-benzene (72:24.5:3:0.5)
- B. 10% Aq. *n*-propanol
- C. Water-methanol-acetone (12:3:1), (soln. was adjusted to pH 10.5 with ammonium hydroxide and then to pH 7.3 with phosphoric acid)

Detection

Bioautography vs. *Bacillus subtilis*

 R_F

Solvent A	0.14
Solvent B	0.87
Solvent C	0.33

Ref.

R.L. Hamill and M.M. Hoehn, *U.S. Patent* 3,705,238, Dec. 5, 1972

TLC 1**Medium**

Silica gel

Solvent

Ethyl acetate

Detection

- A. Vanillin
- B. Sulfuric acid spray

 R_F

0.8

Ref.

As PC 1

TLC 2**Media**

- A. Silica gel
- B. Cellulose

Solvents

- A. Ethyl acetate
- B. Ethyl acetate-benzene (1:1)
- C. Ethyl acetate-chloroform (2:1)
- D. Water-methanol-acetone (12':3:1) adjusted to pH 10.5 with ammonium hydroxide then lowered to pH 7.5 with dil. hydrochloric acid

Detection

Bioautography vs. *Bacillus subtilis* ATCC 6633 (pH 6.0)

 R_F

Compound	Medium-Solvent			
	A-A	A-B	A-C	B-D
A204I	0.66	0.23	0.43	0.27
Ethyl ether	0.66	0.41	0.49	0.53
Methyl ether	0.48	0.30	0.38	0.67
<i>n</i> -Propanol ether	0.69	0.51	0.60	0.22

Ref.

As PC 1

A204II**TLC****Medium**

Thin-layer chromatography of the mixed sodium potassium salt of A204II

Solvent

Ethyl acetate

Detection

Sulfuric acid spray

 R_F

0.75

Ref.

As A204I, PC 1

A-396-I**TLC****Medium**

Silica gel GF (Merck)

Solvent

Chloroform-methanol-4% ammonia (2:1:1),
upper layer

R_F

0.5 (estimated from drawing)

Ref.

S. Shoji, S. Kozuki, M. Mayama, Y. Kawamura
and K. Matsumoto, *J. Antibiot.*, 23 (1970) 291

A 477**PC***Solvents*

- A. Water satd. butanol
- B. Water satd. butanol + 2% *p*-toluenesulfonic acid
- C. Methanol-acetone-water (19:6:75)
- D. Methanol-0.1 *N* hydrochloric acid (3:1)
- E. Water satd. methyl isobutyl ketone + 1% *p*-toluenesulfonic acid

Detection

Bioautography vs. *Bacillus subtilis*

R_F

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.05	D	0.40
B	0.26	E	0.62
C	0.84		

Ref.

R.L. Hamill, M.E. Haney, Jr. and W.M. Stark,
U.S. Patent 3,780,174, Dec. 18, 1973

A/672**PC***Solvents*

- A. Water-satd. *n*-butanol
- B. Water-satd. *n*-butanol containing 2% *p*-toluenesulfonic acid
- C. Water-satd. butanol containing 2% conc. ammonia
- D. *n*-Butanol-satd. water
- E. 20% Aq. ammonium chloride
- F. Phenol-water (75:25)
- G. *n*-Butanol-methanol-water (40:10:20), containing 0.75 g methyl orange
- H. *n*-Butanol-methanol-water (40:20:20)
- I. Water-acetone (1:1)
- J. Water-satd. ethyl acetate

Detection

Bioautography vs. *Bacillus subtilis*

R_F

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.0	F	0.90
B	0.0	G	0.20
C	0.05	H	0.20
D	0.85	I	0.0
E	0.20	J	0.0

Ref.

J.E. Thiemann, G. Beretta, C. Coronelli and H. Pagani, *J. Antibiot.*, 22 (1969) 119-125

A-2315**PC***Solvents*

- A. Methyl ethyl ketone-benzene-water (1:5:1), upper layer
- B. Water satd. butanol
- C. Water satd. butanol + 2% *p*-toluenesulfonic acid
- D. Water satd. methyl isobutyl ketone
- E. Water satd. methyl isobutyl ketone + 2% *p*-toluenesulfonic acid
- F. Water-methanol-acetone (12:3:1), adjusted to pH 10.5 with ammonium hydroxide and then lowered to pH 7.5 with phosphoric acid
- G. 1% Methyl isobutyl ketone-0.5% ammonium hydroxide in water
- H. 17.4 g Potassium phosphate, 30 ml ethanol per liter water
- I. 7% Sodium chloride + 2.5% methyl ethyl ketone
- J. Propanol-water (1:9)
- K. Butanol-ethanol-water (13.5:15:150)
- L. Ammonium chloride (12.5 g):sodium chloride (35 g), dioxane (25 ml) and methyl ethyl ketone (12.5 ml) per liter water, adjusted to pH 5.7 with dilute ammonium hydroxide

Detection

Bioautography vs. *Sarcina lutea*

R_F

Solvent	Factor		
	A	B	C
A	0.19	0.37	0.60
B	0.91	0.91	0.91
C	0.91	0.91	0.91
D	0.56	0.64	0.71

Solvent	Factor		
	A	B	C
E	0.50	0.66	0.74
F	0.83	0.78	0.72
G	0.75	0.64	0.57
H	0.72	0.65	0.53
I	0.65	0.55	0.46
J	0.84	0.81	0.78
K	0.90	0.85	0.80
L	0.69	0.59	0.51

Ref.

R.L. Hamill and W.M. Stark, *U.S. Patent* 3,923,980, Dec. 2, 1975, and *U.S. Patent* 3,968,204, July 6, 1976

TLC**Medium**

Silica gel

Solvent

Chloroform-methanol (9:1)

 R_F

Factor A 0.23
Factor B 0.38
Factor C 0.47

Ref.

As PC

A-4696**PC****Paper**

Whatman No. 1

Solvents

- A. Butanol satd. water + 1% *p*-toluenesulfonic acid
- B. Methyl isobutyl ketone satd. water + 1% *p*-toluenesulfonic acid
- C. Methyl isobutyl ketone satd. water + 1% *p*-toluenesulfonic acid and 1% piperidine
- D. Water-methanol-acetone (12:3:1), adjusted to pH 10.5 with ammonium hydroxide, then lowered to pH 7.5 with phosphoric acid
- E. Methanol-0.1 *N* hydrochloric acid (3:1)
- F. 1% Methyl isobutyl ketone + 0.5% ammonium hydroxide in water
- G. 7% Sodium chloride + 2.5% methyl isobutyl ketone in water
- H. 10% Propanol in water
- I. Butanol-ethanol-water (150:15:13.5)
- J. Propanol-pyridine-acetic acid-water (15:10:3:12)

K. Water-ethanol-acetic acid (70:24:6)

L. *n*-Butanol-pyridine-acetic acid-water (5:10:3:12)

Detection

Bioautography vs. *Bacillus subtilis*

 R_F

Solvent	R_F	Factor
A	0.88	
B	0.72	
C	0.80	
D	0.59	
E	0.35	
F	0.77	
G	0.80	
H	0.74	
I	0.87	
J	0.59	
K	0.77	
L	0.307	A
	0.448	B
	0.516	C
	0.602	D
	0.205	E

Ref.

R.L. Hamill, W.M. Stark and D.C. DeLong, *U.S. Patent* 4,064,233, Dec. 20, 1977

TLC**Media**

A. Silica gel Quantum Q6F

B. Cellulose

 R_F

Factor	Medium	Solvent	
		A	B
A	A	0.275	
	B		0.40
B	A	0.525	
	B		0.51
C	A	0.625	
	B		0.63
D	A	0.725	
	B		0.69
E	A	0.125	
	B		0.20

Solvents

- A. Acetate-water-ammonium hydroxide (160:40:1)
- B. *n*-Butanol-pyridine-acetic acid-water

DetectionBioautography vs. *Bacillus subtilis***Ref.**

As PC

A4993A; A4993B**PC****Paper**

Whatman No. 1

SolventsA. Propanol-pyridine-acetic acid-water
(15:10:3:12)B. *n*-Butanol satd. with 2% *p*-toluenesulfonic acid

C. As B + 2% piperidine

R_F

	Solvent		
	A	B	C
A4993A	0.75	0.45	0.53
A4993B	0.60	0.31	0.32

Ref.R.L. Hamill and M.M. Hoehn, *U.S. Patent*
3,629,405, Dec. 21, 1971**A-9145****PC 1****Paper**

Whatman No. 1

Solvents

A. Water satd. butanol

B. Water satd. butanol + 2% *p*-toluenesulfonic acidC. Water-methanol-acetone (12:3:1) adjusted to
pH 10.5 with ammonium hydroxide and then
to pH 7.5 with phosphoric acidD. Methanol-0.1 *N* hydrochloric acid (3:1)

E. 10% Aq. propanol

F. Methanol-0.05 *M* sodium citrate, pH 5.7
(70:30) with paper buffered with 0.05 *M*
sodium citrate, pH 6.7**Detection**Bioautography vs. *Saccharomyces pastorianus***R_F**

Solvent	R _F	Solvent	R _F
A	0	D	0.47
B	0.08	E	0.09
C	0.55	F	0.34

Ref.R.L. Hamill and M.M. Hoehn, *U.S. Patent*
3,758,681, Sept. 11, 1973**PC 2****Paper**

Whatman No. 4

Solvents

A. Butanol satd. water

B. Butanol satd. + 2% *p*-toluenesulfonic acidC. 80% Ethanol with 1.5% sodium chloride, im-
pregnated with 1 *N* sodium sulfate buffer**Detection**Bioautography vs. *Saccharomyces pastorianus***R_F**

Solvent A 0.19

Solvent B 0.9

Solvent C 0.07

Ref.

As PC 1

PC 3**Solvents**

A. Methanol-0.1 ammonium chloride (3:1)

B. Butanol satd. water

DetectionA. Bioautography vs. *Saccharomyces pastorianus*

B. Ninhydrin

C. UV

R_F

Solvent A 0.47

Solvent B 0.19

Ref.R.L. Hamill and M.M. Hoehn, *J. Antibiot.*, 26
(1973) 463-465**TLC****Medium**

Cellulose

Solvent

Butanol-acetic acid-water (5:2:3)

DetectionA. Bioautography vs. *Saccharomyces pastorianus*

B. Ninhydrin

C. UV

R_F

0.33

Ref.

As PC 3

A-16316-C**TLC****Medium**

Silica gel G

Solvent

Chloroform-methanol-4% ammonium hydroxide (12:1:1), upper layer

R_F

0.24

Ref.

As Destomycins, TLC 2

A16884**PC****Paper**

Whatman No. 1

Solvents

- A. Propanol-acetonitrile-water (1:1:1)
- B. Ethanol-water (80:20) containing 1.5% sodium chloride. Paper impregnated with 1 *N* sodium sulfate
- C. Methanol-propanol-water (6:2:1); paper buffered with 0.75 *M* potassium phosphate, pH 4.0
- D. Propanol-pyridine-acetic acid-acetonitrile-water (45:30:9:40:36)
- E. *tert.*-Amyl alcohol-acetone-water (2:1:2)
- F. Ethyl acetate-acetic acid-water (3:1:1)
- G. Methyl ethyl ketone-water (92:8); paper buffered with 0.1 *N* sodium acetate, pH 4.6
- H. Propanol-water (70:30)
- I. Water satd. butanol
- J. As I + 2% *p*-toluenesulfonic acid

Detection

Bioautography vs. *Salmonella gallinarum*

R_F

Solvent	R _F	Solvent	R _F
A	0.79	F	0.36
B	0.58	G	0.00
C	0.21	H	0.30
D	0.40	I	0.00
E	0.40	J	0.60

Ref.

Belgium Patent 754,424, Aug. 5, 1970

TLC**Media**

- A. Silica gel
- B. Cellulose

Solvent

Acetonitrile-water (70:30)

Detection

Ninhydrin spray

R_F

Medium A 0.47

Medium B 0.45

Ref.

As PC

A16886B**PC****Paper**

Whatman No. 1

Solvent

Propanol-pyridine-acetic acid-acetonitrile-water (45:30:9:40:36), 16 h, 25°C

Detection

- A. Bioautography vs. *Pseudomonas solanacearum* Lilly culture X185
- B. Radioactive A16886B detected by scanning

R_F

Resolves factor A from B

Ref.

J.G. Whitney, D.R. Brannon, J.A. Mabe and K.J. Wicker, *Antimicrob. Agents Chemother.*, 1 (1972) 247-251

A16886-I; A16886-II**PC****Solvents**

- A. As A16884, PC; B
- B. As A16884, PC; I
- C. As A16884, PC; J
- D. Water satd. methyl isobutyl ketone
- E. As D + 2% *p*-toluenesulfonic acid
- F. As D + 2% piperidine
- G. Acetonitrile
- H. Propanol-acetonitrile-methanol-water (4:3:2:1)
- I. Propanol-pyridine-acetic acid-water (15:10:3:12)
- J. As A16884, PC; D
- K. Butanol-acetic acid-water (3:1:1)
- L. As A16884, PC; F
- M. As A16884, PC; H
- N. As A16884, TLC

Detection

- A. Ninhydrin
- B. Bioautography vs. *Salmonella gallinarum*

Ref.

Netherlands Patent 7,011,805, Feb. 16, 1971

R_F

Solvent	A16886-I	A16886-II
A	0.38	0.33
B	0.00	0.00
C	0.39	0.32
D	0.00	0.00
E	0.00	0.00
F	0.00	0.00
G	0.00	0.00
H	0.00	0.00
I	0.32	0.27
J	0.21	0.15
K	0.20	0.17
L	0.29	0.22
M	0.17	0.17
N	0.72	0.65

TLC*Media*

- A. Silica gel
B. Cellulose

Solvents

- A. Acetonitrile–water (70:30)
B. Acetonitrile–isopropanol–water (1:1:1)

Detection

As PC

 R_F

Component	Medium–Solvent	
	A–A	B–B
16886-I	0.51	0.36
16886-II	0.42	0.29

Ref.

As PC

A 21101**TLC***Medium*

Silica gel

Solvent

Toluene–ethyl acetate (2:1)

Detection

Spray with phosphomolybdic acid and heat for about 5 min

Ref.

D.C. DeLong, D.H. Lively and N. Neuss, *U.S. Patent* 3,745,158, July 10, 1973

 R_F

Component	R_F
Factor I	0.24
Factor II	0.31
Factor III	0.36
Factor IV	0.31

A22765**PC***Solvents*

- A. *n*-Butanol–acetic acid–water (4:1:5)
B. As A, but (4:1:2)
C. As A, but (1:1:2)
D. *n*-Propanol–pyridine–water (60:4:40)
E. *n*-Propanol–acetic acid–2.5% aq. sodium chloride (10:1:8)
F. *n*-Propanol–acetic acid–water (25:2:25)
G. *n*-Butanol–ethanol–acetic acid–water (25:25:3:47)
H. Acetone–acetic acid–water (60:3:37)
I. Methanol–0.1 *N* hydrochloric acid (3:1)

Detection

Bioautography vs. *Staphylococcus aureus* or *Bacillus subtilis*

 R_F

Solvent	R_F	Solvent	R_F
A	0.25	F	0.69
B	0.35	G	0.70
C	0.85	H	0.72
D	0.50	I	0.75
E	0.65		

Ref.

E. Gaumann, E. Vischer and H. Bickel, *German Patent* 1129259, May 10, 1962

A 23187; A 23187 METHYL ESTER**PC***Paper*

Whatman No. 1

Solvents

- A. Methanol–0.1 *N* hydrochloric acid (3:1)
B. Propanol–pyridine–acetic acid–water (15:10:3:12)
C. Methanol–0.05 *M* sodium citrate, pH 5.7 (7:3), paper buffered with 0.05 *M* sodium citrate, pH 5.7

D. Methanol–propanol–water (6 : 2 : 1) paper buffered with 0.75 M potassium dihydrogen phosphate soln., pH 4.0

Detection

Bioautography vs. *Bacillus subtilis*

R_F

Solvent A	0.67
Solvent B	0.91
Solvent C	0.69
Solvent D	0.77

Ref.

R.M. Gale, C.E. Higgins and M.H. Hoehn, *U.S. Patent* 3,923,823, Dec. 2, 1975; *U.S. Patent* 3,960,667, June 1, 1976

TLC

Medium

Silica gel

Solvents

- A. Benzene–ethyl acetate (4 : 1)
- B. Benzene–ethyl acetate (2 : 1)
- C. Benzene–ethyl acetate (1 : 1)
- D. Benzene–ethyl acetate (1 : 3)

Detection

UV

R_F

Solvent	A 23187	A 23187 methyl ester
A	0.74	0.22
B	0.89	0.37
C	0.98	0.63
D	0.93	0.74

Ref.

As PC

A-25822

PC

Paper

Whatman No. 1

Solvent

Water satd. methyl isobutyl ketone–*p*-toluene-sulfonic acid (98 : 2)

Detection

Bioautography vs. *Trichophyton mentagrophytes*

Ref.

L.D. Beock, M.M. Hoehn, J.E. Westhead, R.K. Wolter and D.N. Thomas, *J. Antibiot.*, 28 (1975) 95–101

TLC

Medium

Silica gel

Solvent

Diethyl ether–ethanol (3 : 1)

R_F

Factor	R_F	Factor	R_F
A	0.44	L	0.67
B	0.28	M	0.42
C	0.27	N	0.40
D	0.54		

Ref.

K.H. Michel, R.L. Hamill, S.H. Larsen and R.H. Williams, *J. Antibiot.*, 28 (1975) 102–117

GLC

Apparatus

F&M Scientific, 402 High Efficiency Gas Chromatograph (Hewlett-Packard)

Column

Glass, 4 ft. × 4 mm I.D. packed with 3% OV-101 on Chromosorb W HP, 80–100 mesh (Pierce)

Temperature

Column 275°C; flash heater 285°C

Carrier gas

Hydrogen flow, 47 ml/min; helium flow, 75 ml/min; air flow, 350 ml/min

Retention time

Factor	min	Factor	min
A	10.0	L	8.4
B	7.0	M	9.2
D	8.8	N	7.1
H	8.4		

Ref.

As TLC

A25822A

TLC

Medium

Silica gel F₂₅₄, 0.22 mm (Brinkmann)

Solvent

Diethyl ether–ethanol (3 : 1)

Ref.

R.H. Williams, M.H. Hoehn and K.H. Michel, *U.S. Patent* 3,845,203, Oct. 29, 1974

A-26771

TLC 1

Medium

Silica gel F₂₅₄, pre-coated, 0.25 mm thickness, on glass (20 × 20 cm) (E. Merck)

Solvent

Chloroform-methanol (98:2)

Detection

A. UV

B. Silver nitrate spray No. 224

R_F

Factor A 0.48

Factor B 0.05

Factor C 0.37

Ref.

K.H. Michel and M.M. Hoehn, *J.S. Patent* 3,932,219, Jan. 13, 1976; *U.S. Patent* 3,954,972, May 4, 1976

TLC 2**Medium**Silica gel F₂₅₄ (E. Merck)**Solvent**

Chloroform-methanol (98:2)

Detection

A. UV

B. Silver nitrate spray reagent No. 224

R_F

Factor A 0.48

Factor B 0.05

Factor C 0.37

Ref.

K.H. Michel and M.M. Hoehn, *U.S. Patent* 3,883,561, May 13, 1975

A-27106**TLC****Medium**Silica gel F₂₅₄**Solvent**

Benzene-methanol (7:3)

Detection

Vanillin spray reagent [prepared by adding fuming sulfuric acid (2 ml) to a soln. of vanillin (3 g) in absolute ethanol (100 ml)]

R_F

0.49

Ref.

D.R. Brannon and D.R. Horton, *U.S. Patent* 3,932,619, Jan. 13, 1976

A-28086 (Factors A, B and D)**PC****Solvents**

A. Methyl isobutyl ketone satd. water

B. Methyl isobutyl ketone satd. water + 2% *p*-toluenesulfonic acid and 1% piperidine

C. Water-methanol-acetone (12:3:1) adjusted to pH 10.5 with ammonium hydroxide and then lowered to pH 7.5 with phosphoric acid

D. 1% Methyl isobutyl ketone, 0.5% ammonium hydroxide in water

E. 17.4 g Potassium phosphate, 30 ml ethanol per liter of water

F. Water satd. benzene

G. Water

H. Water-methyl isobutyl ketone-ethyl acetate (98:1:1)

DetectionBioautography vs. *Bacillus subtilis* ATCC 6633**R_F**

Solvent	Factor		
	A	B	D
A	0.11	0.09	0.10
B	0.41	0.16	0.26
C	0.54	0.46	0.36
D	0.48	0.36	0.29
E	0.15	0.33	0.25
F	0.24	0.51	0.26
G	0.24	0.11	0.09
H	0.75	0.61	0.64

Ref.

D.H. Berg, R.L. Hamill and M.M. Hoehn, *U.S. Patent* 4,035,481, July 12, 1977

TLC**Medium**Silica gel F₂₅₄, precoated (Merck)**Solvent**

A. Benzene-ethyl acetate (3:2)

B. Ethyl acetate-diethylamine (95:5)

DetectionBioautography vs. *Bacillus subtilis* ATCC 6633**R_F**

Solvent	Factor		
	A	B	D
A	0.24	0.42	0.26
B	0.54	0.34	0.66

Ref.

As PC

A28694 A and B**PC***Paper*

Whatman No. 1

Solvents

- A. Butanol satd. water
- B. Butanol satd. water + 2% *p*-toluenesulfonic acid + 1% piperidine
- C. Methyl isobutyl ketone satd. water + 2% *p*-toluenesulfonic acid + 1% piperidine
- D. Water-methanol-acetone (12:3:1). This soln. is adjusted to pH 10.5 with ammonium hydroxide and then the pH is lowered to pH 7.5 with phosphoric acid
- E. Water satd. benzene

*Detection*Bioautography vs. *Bacillus subtilis**R_F*

Solvent	Compound	
	A	B
A	0.53	0.83
B	0.64	0.76
C	0.58	0.74
D	0.25	0.54
E	0.57	0.48

*Ref.*R.L. Hamill and M.M. Hoehn, *U.S. Patent* 3,839,559, Oct. 1, 1974**TLC***Medium*

Silica gel

Solvents

- A. Benzene-ethyl acetate (1:1)
- B. Chloroform-ethyl acetate (2:3)
- C. Benzene-acetone (9:1)

Detection

Vanillin spray

R_F

Solvent	Compound	
	A	B
A	0.71	0.61
B	0.69	0.61
C	0.29	0.20

Ref.

As PC

A-28829**TLC***Medium*

Silica gel

Solvent

Ethyl acetate

Detection

- A. Bioautography vs. *Spicaria* sp.
- B. Spray with conc. sulfuric acid and heat to 140°C

R_F

0.75

*Ref.*V. Prelog, H. Zaehner and H. Bickel, *U.S. Patent* 3,769,418, Oct. 30, 1973**A-30641****PC***Solvents*

- A. Butanol satd. water
- B. Propanol-water (1:9)
- C. Methanol-propanol-water (6:2:1) (paper buffered with 0.75 *M* potassium phosphate, pH 4.0)
- D. Methyl ethyl ketone-benzene-water (1:5:1) upper layer
- E. Methanol-0.05 *M* sodium citrate, pH 5.7 (7:3) (paper buffered with 0.05 *M* sodium citrate, pH 5.7)

*Detection*Bioautography vs. *Klebsiella pneumoniae**R_F*

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.33	D	0.83
B	0.56	E	0.76
C	0.69		

*Ref.*D.H. Berg, R.L. Hamill and M.M. Hoehn, *U.S. Patent* 3,991,052, Nov. 9, 1976**TLC***Medium*Silica gel 60 F₂₅₄, precoated (E. Merck)*Solvents*

- A. Benzene-ethyl acetate (1:1)
- B. Chloroform-acetone (3:2)
- C. Ethyl acetate-chloroform (3:1)

Detection

- A. UV at short-wave nm
- B. Iodine-azide spray reagent

R_F	
Solvent A	0.31
Solvent B	0.42
Solvent C	0.39

Ref.

As PC

A 32204

PC

Medium

Cellulose

Solvent

n-Butanol–acetic acid–water (4:1:)

Detection

Ninhydrin

R_F

Component I	0.56 (yellow)
Component II	0.28 (violet)
Component III	0.27 (yellow)

Ref.

Swiss Patent 568,386, Oct. 31, 1975

A-32390

TLC

Medium

Silica gel F₂₅₄ (Merck)

Solvent

Chloroform–methanol (9:1)

Detection

Bioautography vs. *Sarcina lutea* ATCC 3241

R_F

Factor A	0.32
Factor B	0.23
Factor C	0.15
Factor D	0.05

Ref.

G.G. Marconi and M.M. Hoehn, *U.S. Patent* 3,986,928, Oct. 19, 1976

A32390A

TLC

Medium

Silica gel G

Solvent

Chloroform–methanol (9:1)

Detection

- Bioautography vs. *Micrococcus luteus*
- UV
- Iodine vapor

R_F

Factor A	0.32
Factor B	0.23

Factor C 0.15

Factor D 0.05

Ref.

L.D. Boeck, M.M. Hoehn, T.H. Sands and R.W. Wetzel, *J. Antibiot.*, 31 (1978) 19–26

G.G. Marconi, B.B. Molloy, R. Nagarajan, J.W. Martin, J.B. Deeter and J.L. Occolowitz, *J. Antibiot.*, 31 (1978) 27–32

AA-57

TLC

Medium

Silica gel GF₂₅₄ (Kieselgel)

Solvents

- Ethyl acetate–methanol (1:1)
- Chloroform–methanol (2:1)

Detection

- Bioautography
- 10% Sulfuric acid

R_F

Solvent A	0.56
Solvent B	0.60

Ref.

S. Aizawa, H. Akutsu, T. Satomi, S. Kawabata and K. Sasaki, *J. Antibiot.*, 31 (1978) 729–731

AB-64

PC

Paper

Ascending paper strip chromatography

Solvents

- Water
- 50% Aq. acetone
- Water satd. *n*-butanol
- n*-Butanol–methanol–water (4:1:2)
- n*-Butanol–pyridine–water (6:4:3)

R_F

Solvent	R_F	Solvent	R_F
A	0	D	0.74
B	0.59	E	0.72
C	0.54		

Ref.

A. Tamura, R. Furuta, H. Kotani and S. Naruto, *J. Antibiot.*, 26 (1973) 492–500

AB-65

TLC

Medium

Cellulose (Merck, 5552/0025)

Solvents

- A. *n*-Butanol-ethanol-water (4:1:1)
 B. *n*-butanol-methanol-benzene-water (2:1:1:1)
 C. *n*-Butanol-acetic acid-water (4:1:5)
 D. *n*-Butanol-acetone-water (1:1:1)

Detection

Iodine

 R_F

Solvent A	0.17
Solvent B	0.37
Solvent C	0.64
Solvent D	0.77

Ref.

A. Tamura and I. Takeda, *J. Antibiot.*, 28 (1975) 395-397

AB-664 α **PC****Paper**

Whatman No. 1

Solvent

m-Cresol-pyridine-acetic acid-water (200:1:1:100), lower phase, descending

Detection

Wash papergrams with diethyl ether to remove cresol; bioautography vs. *Bacillus subtilis* at pH 6.0

 R_F

0.4-0.5

Ref.

W.K. Hausmann and S.O. Thomas, *U.S. Patent* 3,495,003, Feb. 10, 1970

ABBOTT 29119**PC****Solvents**

- A. 0.1 *M* Aq. ammonium hydroxide satd. with methyl isobutyl ketone
 B. 0.5 *M* Aq. ammonium chloride satd. with *p*-dioxane

DetectionBioautography vs. *Bacillus subtilis* **R_F**

Solvent A	0.35
Solvent B	0.60

Ref.

P.P. Hung, C.L. Marks and P.L. Tarchew, *Appl. Microbiol.*, 13 (1965) 216-217

AC 98**PC****Paper**

Whatman No. 1

Solvent

5% Aq. ammonium chloride

Detection

Bioautography vs. *Bacillus subtilis* or *Corynebacterium xerosis*

 R_F

AC 98 mixture:	0.01-0.15, 0.15-0.45, 0.45-0.70

AC 98 complex A: 0.04, 0.23, 0.57

AC 98 complex B: 0.03, 0.18, 0.63

Ref.

S.E. DeVoe and M.P. Kunstmann, *U.S. Patent* 3,495,004, Feb. 10, 1970

AC541A; AC541B**PC****Solvent**

90% Phenol-*m*-cresol-acetic acid-pyridine-water (100:25:4:4:75)

 R_F

AC541A	0.40
AC541B	0.58

Ref.

W.K. Hausmann, V. Zbinovsky and A.J. Shay, *U.S. Patent* 3,522,349, July 28, 1970

AF 283 α **PC****Solvents**

- A. 5% Ammonium chloride
 B. 90% Phenol-water plus 2% dichloroacetic acid (added to bottom phase)
 C. *m*-Cresol-90% phenol-0.2 *M* morpholine-0.2 *M* acetic acid (5:5:7:3)
 D. Water satd. *m*-cresol plus 2% heptafluorobutyric acid (added to lower phase)
 E. *sec*-Butanol-acetic acid-water (1000:375:500)

 R_F

Solvent	R_F	Solvent	R_F
A	0.32	D	0.24
B	0.65	E	0.24
C	0.25		

DetectionBioautography vs. *Corynebacterium xerosis***Ref.***Australian Patent Appl.* 23,886/67, June 29, 1967D. *sec.*-Butanol-pyridine-*sym.*-collidine-water
(3:6:6:3)

E. 90% Phenol-2% dichloroacetic acid (1:1)

DetectionBioautography vs. *Corynebacterium xerosis***R_F**

Solvent	R _F	Solvent	R _F
A	0.20	D	0.01
B	0.58	E	0.80
C	0.01		

Ref.

As AF 283α

AF 283β**PC****Solvents**

- A. 3% Aq. ammonium chloride
 B. Water satd. *m*-cresol with 2% perfluorobutyric acid added
 C. Chloroform-pyridine-acetic acid-water
 (10:4:4:5)

B-41**TLC****Media**

- A. Silica gel (Spotfilm fluorescent)
 B. Alumina F

Detection

- A. Iodine-chloroform
 B. Ninhydrin
 C. Sulfuric acid spraying with heating

The detection was made according to the intensity of fluorescence emitted when each substance was irradiated with ultraviolet rays of 2536 Å

Solvents

- A. Acetone-*n*-hexane (30:70)
 B. Ethyl acetate-benzene (50:50)
 C. Ethyl acetate-chloroform (25:75)
 D. Acetone-benzene (40:60)
 E. Acetone-benzene (15:75)
 F. Ethanol-*n*-hexane (2:98)

Ref.

A. Aoki, R. Fukuda, T. Nakayabu, K. Ishibashi, C. Takeichi and M. Ishida, *U.S. Patent* 3,992,527, Nov. 16, 1976; *U.S. Patent* 3,992,551, Nov. 16, 1976; and *U.S. Patent* 3,992,552, Nov. 16, 1976

Detection	A ₁	A ₂	A ₃	A ₄	B ₁	B ₂	B ₃	C ₁	C ₂
A	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
B	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
C	Brown	Red-dish purple	Brown	Brown	Red-dish purple	Brown	Brown	Brown	Brown

R_F

Medium	Solvent	A ₁	A ₂	A ₃	A ₄	B ₁	B ₂	B ₃	C ₁	C ₂
A	A	0.47	0.32	0.42	0.44	0.48	0.61	0.63	0.22	0.24
A	B	0.61	0.62	0.63	0.65	0.79	0.80	0.82	0.45	0.47
A	C	0.27	0.35	0.39	0.41	0.75	0.79	0.81	0.12	0.13
A	D	0.85	0.75	0.77	0.79	0.92	0.92	0.92	0.60	0.63
A	E	0.39	0.32	0.39	0.39	0.57	0.51	0.53	0.11	0.13
A	F	0	0	0	0	0	0	0	0	0
B	A	0.55	0.16	0.32	0.34	0.67	0.92	0.92	0	0
B	B	0.56	0.10	0.21	0.23	0.73	0.81	0.83	0	0
B	C	0.40	0.10	0.15	0.17	0.63	0.65	0.67	0	0
B	D	—	—	—	—	—	—	—	—	—
B	E	0.27	0.05	0.09	0.11	0.35	0.45	0.47	0	0
B	F	0.17	0.03	0.07	0.09	0.20	0.42	0.44	0	0

B-2847-R; B-2847-RB; B-2847-Y**PC***Paper*

Whatman No. 1

*Solvents*A. *n*-Hexane–benzene–ethanol–water (1:3:1:3)B. *n*-Hexane–benzene–acetone–water
(15:5:8:12)

All solvents developed ascending

*Detection*Bioautography vs. *Staphylococcus aureus**R_F*

Solvent	B-2847-Y	B-2847-R
A	0.78	—
B	0.60	0.65
C	0.27	—

*Ref.**Netherlands Patent* 68,02679, Aug. 26, 1968**TLC 1***Medium*

Silica gel

Solvents

A. 1% Oxalic acid containing ethyl acetate–acetone (1:1)

B. 1% Oxalic acid containing ethyl acetate

C. 1% Oxalic acid containing acetone

R_F

Solvent	B-2847-Y	B-2847-R
A	0.05	0.70
B	0.00	0.20
C	0.20	—

Ref.

As PC

TLC 2*Media*

A. Silica gel

B. Silica gel containing 2% oxalic acid

Solvents

A. Ethyl acetate–acetone (1:1)

B. 1% Oxalic acid containing ethyl acetate–acetone (1:1)

Detection

Visible color: yellow zone

*Ref.*T. Kishi, M. Asai, S. Harada, M. Muroi and K. Mizuno, *U.S. Patent* 3,717,707, Feb. 20, 1973*R_F*

Medium	Solvent	<i>R_F</i> (B-2847-RB)
A	A	0.65
B	B	0.5

B-5050 and TETRAHYDRO-B-5050 (MARIDOMYCIN)**TLC***Solvent*

Chloroform–methanol

R_F

Compound	<i>R_F</i>
B-5050	0.1–0.2
α -Propionate	0.37–0.52
β -Propionate	0.25–0.30
α,β -Propionate	0.67–0.80

*Ref.*T. Kishi, S. Harada, M. Muroi and M. Izawa, *U.S. Patent* 3,853,842, Dec. 10, 1974**B-15645****PC***Solvents*A. *n*-Butanol–water–pyridine (4:7:3), ascendingB. *n*-Butanol–water (1:1), ascending*R_F*

Solvent A	0.72
Solvent B	0.35

*Ref.**Japanese Patent* 20559/1970, July 13, 1970**TLC***Medium*Silica gel HF₂₅₄ (Merck)*Solvent*

Ethyl acetate–ethanol (4:1)

R_F

0.35

Ref.

As PC

B-98891**PC***Paper*

Whatman No. 1

Solvents

- A. Butanol-acetic acid-water (2:2:1)
 B. Propanol-water (7:3)
 C. Isopropanol-5% water satd. ammonia (6:4)

R_F

Solvent A	0.13
Solvent B	0.27
Solvent C	0.26

Ref.

German Patent 2,513,249, Oct. 9, 1975; and T. Kishi, T. Iwasa, T. Kusaka and S. Harada, *U.S. Patent* 4,007,267, Feb. 8, 1977

TLC**Medium**

Kieselgel F₂₅₄

Solvents

- A. Chloroform-methanol-17% water satd. ammonia (2:2:1), upper layer
 B. Propanol-pyridine-acetic acid-water (15:10:3:10)
 C. Ethyl acetate-acetone-acetic acid-water (45:5:5:45) lower layer

R_F

Solvent A	0.34
Solvent B	0.15
Solvent C	0.37

Ref.

As PC

ELPHO**Medium**

Whatman No. 1 paper

Buffers

- A. $\frac{1}{10}$ M glycine-sodium chloride-sodium hydroxide, pH 9.32
 B. $\frac{1}{15}$ M phosphate buffer, pH 8.05
 C. $\frac{1}{10}$ M citrate-hydrochloric acid buffer, pH 3.62

Conditions

500 V, 2 h

Mobility

Buffer	Mobility (cm)
A	-0.7
B	-1.1
C	-3.3

Ref.

As PC

BA-6903**CCD****Solvent**

Chloroform-methanol-water-ligroin (3:4:1:1), 100 transfers

Distribution

Main component found in tubes 20-40 based on O.D. at 275 nm and activity vs. *Bacillus subtilis*

Ref.

K.V. Rao and S.C. Brooks, *Antimicrob. Agents Chemother.*, 1961 (1962) 491-494

BB-K8**TLC****Medium**

Silica gel plate (Kieselgel F₂₅₄)

Solvent

Chloroform-methanol-28% ammonia-water (1:4:2:1)

Detection

Bioautography of the plates overlaid with *Bacillus subtilis*

R_F

BB-K8 0.15-0.18

Ref.

H. Kawaguchi, T. Naito, S. Nakagawa and K. Fujisawa, *J. Antibiot.*, 25 (1972) 695-708

BB-K47 through BB-K51
(See paromomycin, TLC 3)

BB-K186

(See 3'-deoxybutirosin A)

BD-12**PC 1****Solvents**

- A. Wet butanol
 B. 1.5% Aq. ammonium chloride
 C. Phenol-water (3:1)
 D. 50% Aq. acetone
 E. *n*-Butanol-methanol-water-methyl orange (40:10:20 ml:1.5 g)
 F. *n*-Butanol-methanol-water (40:10:20)
 G. Benzene-methanol (4:1)
 H. Water
 I. *n*-Propanol-pyridine-acetic acid-water (60:40:10:30) + 1.2 g sodium *p*-hydroxybenzene sulfonate/140 ml
 J. Wet butanol + 2% *p*-toluenesulfonic acid

K. *n*-Butanol–pyridine–acetic acid–water (15:10:3:12)

L. Butanol–acetic acid–water (4:1:5)

Detection

Bioautography vs. *Bacillus subtilis*

R_F

Solvent	R_F	Solvent	R_F
A	0.00	G	0.00
B	0.93	H	0.05
C	0.87	I	0.36
D	0.08	J	0.29
E	0.60	K	0.54
F	0.09	L	0.21

Ref.

Y. Ito, Y. Ohashi, Y. Sakurai, M. Sakurazawa, H. Yoshida, S. Awataguchi and T. Okuda, *J. Antibiot.*, 21 (1968) 307–312

PC 2

Solvents

A. *n*-Propanol–pyridine–acetic acid–water (15:10:3:12)

B. Wet *n*-butanol containing 2.0% (w/v) *p*-toluenesulfonic acid

R_F

Solvent A	0.54
Solvent B	0.29

Ref.

K. Arima, T. Kawamura and T. Beppu, *J. Antibiot.*, 25 (1972) 387–392

TLC

Medium

Silica gel G

Solvent

Chloroform–methanol–17% aq. ammonia (2:1:1), upper layer

R_F

0.78

Ref.

As PC

BH 890 α and β

CCD

Solvent

Ethyl acetate–*sec*.-butanol–methanol–buffer (900:303:160:600). Buffer prepared by stirring together triethylamine (6.3 ml) and water (2 l) and adjusting the pH to 7.5 with glacial acetic acid and diluting to 2400 ml with water
310 Transfers, 200-tube apparatus

Distribution

BH 890 β : tubes 85–120

BH 890 α : tubes 113–150

Ref.

J.H.E.J. Martin and J.N. Porter, *U.S. Patent* 3,700,769, Oct. 24, 1972

BL 580 α and β

TLC

Medium

Silica gel F₂₅₄ (Merck)

Solvent

Chloroform–ethyl acetate (1:1)

Detection

Spray with sulfuric acid followed by heating on a hot plate. Components appear as black zones

R_F

BL 580 α , 0.44

BL 580 β , 0.27

Ref.

J.H.E.J. Martin and S. Kantor, *U.S. Patent* 3,812,249, May 21, 1974

BM 123

PC

Paper

Whatman No. 1

Solvent

Phenol–*m*-cresol–acetic acid–pyridine–water (100:25:4:4:75)

Detection

Bioautography vs. *klebsiella pneumonia*

R_F

Component	R_F
BM 123 γ	0.88
BM 123 β_1	0.62
BM 123 β_2	0.71
BM 123 α_1	0.20
BM 123 α_2	0.47

Ref.

German Patent 2,351,344, April 25, 1974

TLC

Medium

Cellulose Polygram Cel UV 254

Solvent

Water–1% trisodium citrate

Detection

Bioautography

R_F

Component	R_F
BM 123 α_1	0.90
BM 123 α_2	0.65

Ref.

As PC

BN-109**TLC***Solvents*

- A. *n*-Butanol–acetic acid–water (2:1:1)
 B. *n*-Butanol–acetic acid–water (4:1:5), upper layer
 C. *n*-Butanol–pyridine–acetic acid–water (6:4:1:3)

 R_F

Solvent A	0.86
Solvent B	0.38
Solvent C	0.66

Ref.

T. Shomura, S. Omoto, S. Miyado, H. Watanabe, S. Inouye, Y. Yamada and T. Niida, *U.S. Patent* 3,940,479, Feb. 24, 1976

ELPHO*Medium*

Paper

Buffer

pH 1.9

Conditions

3500 V, 15 min

Mobility

6.5 cm towards cathode

Ref.

German Patent 2,433,932, March 13, 1975**BN-130****TLC***Medium*

Silica gel

Solvents

- A. Ethyl acetate–methanol (20:1)
 B. Chloroform–isopropanol (9:1)

 R_F

Solvent A	0.45
Solvent B	0.23

Ref.

German Patent 2,516,615, Oct. 30, 1975**BU-1709 E₁ and E₂****TLC***Media*

- A. Silica gel
 B. Alumina

Solvents

- A. Chloroform–methanol–28% ammonium hydroxide–water (1:4:2:1)
 B. Chloroform–methanol–17% ammonium hydroxide (2:1:1), upper phase

 R_F

Antibiotic	Medium	Solvent	R_F
BU-1709 E ₁	A	A	0.25
BU-1709 E ₂	B	B	0.05

Ref.

H. Tsukiura, K. Saito, S. Kobary, M. Konishi and H. Kawaguchi, *J. Antibiot.*, 26 (1973) 386–388

BU-1880**PC***Paper*

Ascending

Solvents

- A. Water satd. *n*-butanol
 B. Aq. 3% ammonium chloride
 C. Acetone–water (1:1)
 D. *n*-Butanol–methanol–water (4:1:2)
 E. *n*-Butanol–methanol–water (4:1:2) + 1.5% methyl orange
 F. Benzene–methanol (4:1)
 G. Water

 R_F

Solvent	R_F	Solvent	R_F
A	0.35	E	0.75
B	0.70	F	0.00
C	0.00	G	0.00
D	0.60		

Ref.

H. Kawaguchi, H. Tsukiura, K. Fujisawa and K. Numata, *U.S. Patent* 3,880,994, April 29, 1975

TLC 1*Medium*

Silica gel

Solvents

- A. *n*-Propanol-17% aq. ammonium hydroxide
 B. *n*-Butanol-acetic acid-water (3:1:1)
 C. *n*-Propanol-pyridine-acetic acid-water (15:10:3:2)
 D. Chloroform-pyridine-28% ammonium hydroxide-water (1:4:2:1)

 R_F

Solvent	R_F	Solvent	R_F
A	0.10	C	0.70
B	0.40	D	0.85

Ref.

As PC

TLC 2**Medium**

Cellulose

Solvent*n*-Butanol-ethanol-acetic acid-water (25:25:3:47) R_F

0.85

Ref.

As PC

Bu-1975 (BUTIROSIN)**TLC****Media**

- A. Silica gel
 B. Alumina

Solvents

- A. Chloroform-methanol-28% ammonium hydroxide-water (1:4:2:1) (develop 3 times)
 B. Chloroform-methanol-28% ammonium hydroxide (1:3:2)
 C. Chloroform-methanol-28% ammonium hydroxide (2:1:1) (develop 16 h, location is shown in cm from the origin)

 R_F

Solvent	Medium	Bu-1975			
		A ₁	A ₂	Ib	Ic
A	A	0.41	0.41	0.51	0.51
B	A	0.20	0.20	0.26	0.26
C	B	3.7	0.2	5.3	0.5

Ref.

T. Naito, S. Nakagawa and S. Toda, *U.S. Patent* 3,923,783, Dec. 2, 1975

BY-81**PC****Solvents**

- A. *n*-Propanol-pyridine-acetic acid-water (15:10:3:12)
 B. Wet *n*-butanol containing 2.0% (w/v) *p*-toluenesulfonic acid

 R_F

Solvent A	0.45
Solvent B	0.17

Ref.

K. Arima, T. Kawamura and T. Beppu, *J. Antibiot.*, 25 (1972) 387-392

TLC**Media**

- A. Silica gel G
 B. Avicel SF plate

Solvents

- A. *n*-Propanol-pyridine-acetic acid-water (15:10:3:12)
 B. Chloroform-methanol-17% aq. ammonia (2:1:1), upper layer

 R_F

Solvent	Medium A	Medium B
A		0.36
B	0.52	

Ref.

As PC

C-2449**PC****Paper**

Whatman No. 2, washed with water satd. *n*-amyl alcohol

Solvent

n-Amyl chloride (for feeding the sheets of paper buffer, a mixture of McIlvaine's buffer, pH 2.2 to 8.0 and borate buffers, pH 9.0 to 10.0 were used)

Detection

Bioautography vs. *Staphylococcus* No. 209 (used for determination of dissociation constants)

Ref.

D.E. Dykhovichnaya, B.N. Bondarenko, R.P. Harionova and Yu.V. Rapp, *Khim-Farm. Zh. (Russ.)* 5 (1971) 56-58; *C.A.*, 75 (1971) 9909j

C-2801X**PC***Paper*

Whatman No. 1

*Solvents*A. *n*-Butanol–acetic acid–water (4:1:5)B. *n*-Butanol–acetic acid–water (2:1:1)

C. 70% Isopropanol

R_F

Solvent A 0.12

Solvent B 0.42

Solvent C 0.33

*Ref.*H. Fukase, T. Hasegawa, K. Hatano, H. Iwasaki and M. Yoneda, *J. Antibiot.*, 29 (1976) 113–130**C-9154****TLC***Medium*

Silica gel (Spot Film F, Tokyo (Kasei))

Solvents

A. Chloroform–methanol (5:1)

B. Ethyl acetate–acetone (3:1)

R_F

Solvent A 0.6

Solvent B 0.3

*Ref.*T. Hasagawa, M. Asai, K. Haibara, T. Yamano, H. Iwasaki and M. Yoneda, *J. Antibiot.*, 28 (1975) 713–716**CP 21635****PC***Solvents*

A. 5% Aq. ammonium chloride

B. Methanol–1.5% aq. sodium chloride (4:1); paper buffered with 0.95 *M* sodium sulfate + 0.05 *M* sodium bisulfate

C. Water satd. methyl isobutyl ketone–piperidine (100:1)

D. Water satd. methyl isobutyl ketone–glacial acetic acid (100:1)

E. Benzene satd. with 25% aq. methanol

*Detection*A. Bioautography vs. *Staphylococcus aureus*

B. UV light

*Ref.*F.C. Sciavolino, J.B. Routien, E.J. Tynan and W.D. Celmer, *U.S. Patent* 3,655,876, April 11, 1972*R_F*

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.29	D	0.86
B	0.81	E	0.36
C	0.88		

TLC*Medium*

Silica gel

Solvents

A. Ethyl acetate

B. Chloroform–acetone (1:1)

C. Ethyl acetate–methanol (1:1)

D. Chloroform–methanol (9:1)

E. Butanol

Detection

A. As PC; A

B. As PC; B

C. Sulfuric acid

D. Van Urk's reagent (0.125 g *p*-dimethylamino-benzaldehyde and 0.1 ml of 5% ferric chloride in 100 ml of 65% sulfuric acid)*R_F*

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.15	D	0.62
B	0.32	E	0.70
C	0.37		

Ref.

As PC

E-749-C**PC***Solvents*A. *n*-Butanol–acetic acid–water (4:1:2)B. *n*-Propanol–pyridine–acetic acid–water (15:10:3:12)*R_F*

Solvent A ca. 0.11

Solvent B 0.45–0.55

*Ref.*J. Shoji, S. Kozuki, M. Ebata and H. Otsuka, *J. Antibiot.*, 21 (1968) 509–511**EM-2, EM-3 and EM-4****TLC***Medium*

Silica gel (Eastman)

Solvent

Chloroform-methanol (6:1)

DetectionBioautography vs. *Sarcina lutea***R_F**

EM-2	0.15
EM-3	0.38
EM-4	0.40

Ref.

German Patent 2,352,693, April 25, 1974

EM 49**PC****Paper**

Whatman No. 1, chromatographic grade

Solvents

- A. *n*-Butanol-acetic acid-water (4:1:5)
- B. *n*-Propanol-*n*-butanol-water (2:3:4)
- C. Chloroform-methanol-water (5:4:2)
- D. Chloroform-methanol-1% acetic acid (5:4:2)
- E. Chloroform-methanol-0.5 *N* ammonium hydroxide (5:4:2)

DetectionBioautography vs. *Escherichia coli***R_F**

Solvent	R _F	Solvent	R _F
A	0.71	D	0.35
B	0.57	E	0.91
C	0.38		

Ref.

E. Meyers, W.E. Brown, P.A. Principe, M.L. Rathnum and W.L. Parker, *J. Antibiot.*, 26 (1973) 444-448

TLC**Medium**

Silicic acid (Gelman ITLC, type SAF)

Solvent*n*-Butanol-propionic acid-water (3:1:1, v/v)**R_F**

0.75

Ref.

As PC

EM-49 components (α , β , γ , δ)**PC****Paper**

Whatman No. 1 (real-partition chromatography)

Solvent*n*-Amyl alcohol-ethyl acetate-propionic acid-water (6:9:5:15)**Detection**A. Bioautography vs. *Escherichia coli*

B. Chloramide reaction

The solvent system separates into two phases. The lower phase is mixed with an equal volume of acetone and the paper strip, on which sample spots have been applied, is drawn through the mixture. After evaporation of the acetone (by hanging the paper strip in air for 3 min) the paper strip, while still wet, is developed descending with the upper phase of the solvent. The jar is lined with paper that has been wetted with the lower phase and allowed to develop 16 h at room temperature of 21.5-24°C

R_F

Component	R _F
α	0.13
β	0.16
γ	0.22
δ	0.30

Ref.S.C. Pan, *J. Chromatogr.*, 97 (1974) 112-114**EM-49 derivatives****TLC****Medium**

Silica gel

Solvent

Conc. propanol-ammonium hydroxide (7:3)

R_F

Derivatives	R _F
O-Butyryl-EM-49 tetrahydrochloride	0.57
O-Hexanoyl-EM-49 hydrochloride	0.49

Ref.

W.L. Parker, *U.S. Patent* 3,941,762, March 2, 1976

EM 49; 2,4-dinitrophenyl derivatives of EM 49**TLC****Medium**

Silica gel

Solvent

Methanol-chloroform (1:9)

R_FA yellow band between R_F 0.5 and 0.7**Ref.**W.L. Parker and M.L. Rathnum, *J. Antibiot.*, 26 (1973) 449-456**ELPHO****Medium**

Whatman No. 3MM paper

Buffer

85% Formic acid-acetic acid-formamide-water (6:4:5:5)

Conditions

330 V

DetectionBioautography vs. *Escherichia coli*

Treatment of EM 49 with a 2,4-dinitrofluorobenzene, using 1.5 moles of the reagent per mole of EM 49, gave a mixture that was resolved by electrophoresis into four yellow spots and the unchanged antibiotic

Mobility

Derivative	Moved towards cathode (cm)
Tetra-DNP-EM 49	0.0
Tri-DNP-EM 49	1.6
Di-DNP-EM 49	2.9
Mono-DNP-EM 49	4.6
EM 49	6.4

Ref.

As TLC

EM-49; tetraamidino derivative**PC****Paper**

Whatman No. 1

Solvent*n*-Butanol-acetic acid-water (4:1:5)**Detection**Bioautography vs. *Staphylococcus aureus* FDA 209P; *Escherichia coli* ATCC 10536**R_F**

0.71

Ref.

German Patent 2,357,858, May 22, 1974

EM-98**PC****Paper**

Whatman No. 1

Solvent*n*-Butanol-water-acetic acid (4:5:1)**Detection**Bioautography vs. *Escherichia coli* ATCC 10536**R_F**

0.3

Ref.German Patent 2,316,893, April 4, 1973; E. Meyers, D.S. Slusarchyk and W. Liu, *U.S. Patent* 3,853,992, Dec. 10, 1974**TLC****Medium**

Cellulose

Solvent*n*-Butanol-water-acetic acid (4:5:1)**Detection**

UV at 360 nm

R_F

0.3

Ref.

German Patent 2,316, 893, April 4, 1973

F-1028**TLC****Medium**

Silica gel

SolventsA. *n*-Propanol-pyridine-acetic acid-water (15:10:3:12)

B. Butanol-acetic acid-water (3:1:2)

R_F

Solvent A 0.68

Solvent B 0.21, 0.25

Ref.T. Hata, S. Omura, M. Katagiri, J. Awaya, S. Kuyama, S. Higashikawa, K. Yasui and H. Terada, *U.S. Patent* 3,956,487, May 11, 1976; *U.S. Patent* 3,956,276, May 11, 1976**FR-02A****PC****Solvent**

Isopropanol-phosphate buffer, pH 6.0 (70:30)

R_F

0.9

Ref.

German Patent 2,450,813, April 30, 1975

TLC**Medium**

Kieselgel

Solvent

Chloroform-methyl alcohol-conc. ammonium hydroxide (80:20:1)

R_F

0.344

Ref.

As PC

FR-1923**TLC****Medium**

Cellulose (Merck)

SolventsA. *n*-Butanol-acetic acid-water (4:1:2)B. *n*-Butanol-methanol-10% aq. ammonium hydroxide (10:2.5:5)**Detection**Ninhydrin (*n*-butanol soln.)**R_F**

Solvent A 0.34

Solvent B 0.16

Ref.H. Aoki, J. Hosoda, T. Komori, M. Izeki, Y. Kubochi, T. Kamiya and H. Imanaka, *U.S. Patent* 3,923,977, Dec. 2, 1975**FR-33289****TLC****Medium**

Cellulose, Eastman Chromagram sheet cellulose No. 13254

Solvent

60% Aq. propanol

R_F

0.6

Ref.Y. Kuroda, M. Okuhara, H. Aoki and H. Imanaka, *U.S. Patent* 4,143,135, March 6, 1979**FR-900098****TLC****Medium**

Cellulose, Eastman Chromagram sheet cellulose No. 13254

Solvents

A. 75% Aq. propanol

B. Water satd. *n*-butanol

C. 70% Aq. acetonitrile

R_F

Solvent A 0.5

Solvent B 0

Solvent C 0.4

Ref.

As FR-33289, TLC

G-52**PC****Solvents**A. 80% Methanol + 3% sodium chloride (w/v), (1:1), descending. Paper buffered with 0.95 *M* sodium sulfate + 0.05 *M* sodium bisulfate

B. Propanol-pyridine-acetic acid-water (6:4:1:3, v/v) ascending

C. 80% (v/v) Phenol, ascending

D. Benzene-methanol (9:1, v/v) descending

E. *n*-Butanol-water-acetic acid (4:5:1), upper phase, ascending

F. Chloroform-methanol-17% ammonium hydroxide (2:1:1)

G. 2-Butanone-*tert*.-butanol-methanol-conc. ammonium hydroxide (16:3:1:6)**R_F**

Solvent	R _F	Solvent	R _F
A	0.55	E	0.0
B	0.29	F	0.48
C	0.45	G	0.73 *
D	0.0		

* R_F value at *t* = 18 h**Ref.**M.J. Weinstein, G.H. Wagman and J.A. Marquez, *U.S. Patent* 4,001,209, Jan. 4, 1977**G-253****PC****Solvent**

Water satd. chloroform

R_F

Component	R _F
G-253A	0.40-0.50
G-253B ₁	0.27-0.38
G-253B ₂	0.13-0.17
G-253B	0.06-0.10
G-253C	0.01-0.06
G-253C ₁	0.00

Ref.

S. Nomura, H. Yamamoto, I. Umesawa, A. Matsumae and T. Hata, *J. Antibiot.*, 20 (1967) 55-61

TLC**Medium**

Kieselgel G

Solvents

A. Methanol-ethyl acetate (4:25)

B. Methanol-ethyl acetate-benzene (1:5:5)

R_F

Component	Solvent A	Solvent B
G-253B ₁	0.67	0.25
G-253B ₂	0.65	0.23
G-253C ₁	0.63	0.21

Ref.

As PC

G-418**PC****Paper**

Whatman No. 4 (descending)

Solvent

2-Butanone-*tert.*-butanol-methanol-conc. ammonia (16:3:1:6) (16 h)

Detection

Bioautography vs. *Staphylococcus aureus* ATCC 6538 P

R_F

One major product, five minor products

Ref.

M.J. Weinstein, G.H. Wagman, R.T. Testa and J.A. Marquez, *U.S. Patent* 3,997,403, Dec. 14, 1976

G-1499-2**TLC****Medium**

Silica gel, 20 × 20 cm (E. Merck)

Solvents

A. Methanol

B. Acetone

C. Dioxane

D. Ethyl acetate

E. Chloroform

F. Benzene

G. Toluene-ethyl acetate (2:1)

H. Toluene-ethyl acetate (3:1)

I. Hexane-ethyl acetate (1:1)

J. Hexane-ethyl acetate (2:1)

Detection

A. Bioautography vs. *Flavobacterium* 1980 or *Staphylococcus aureus* Oxford H strain VI

B. UV at 254 nm

R_F

Solvent	R _F	Solvent	R _F
A	0.85	F	0
B	0.70	G	0.11
C	0.75	H	0.08
D	0.60	I	0.15
E	0.12	J	0.06

Ref.

J.R. Evans, E.J. Napier and R.A. Fletton, *J. Antibiot.*, 31 (1978) 952-957

G2201-C**TLC****Media**

A. Silica gel 60 F₂₅₄, Merck

B. Cellulose Eastman Kodak, 13254

Solvents

A. Benzene

B. Chloroform

C. Ethyl acetate

D. Dioxane

E. Acetone

F. Methanol

G. Butanol-acetic acid-water (3:1:1)

H. Ethyl acetate-methanol (3:1)

I. Propanol-pyridine-acetic acid-water (15:10:3:12)

J. Butanol-methanol-water (4:1:2)

K. Acetonitrile-water (7:3)

Detection

A. Bioautography

B. UV at 254 or 356 after exposure to ammonia vapor

C. Blue color after spraying with 0.5% blue tetrazolium in methanol-2 N sodium hydroxide (1:10)

R_F

Solvent	Medium A	Medium B
A	0	
B	0	
C	0.50	

Solvent	Medium A	Medium B
D	0.80	
E	0.78	
F	0.78	
G	0.48	0.67
H	0.76	
I		0.95
J		0.70
K		0.85

Ref.

M. Noble, D. Noble and R.A. Fletton, *J. Antibiot.*, 31 (1978) 15-18

G-2847RB

TLC

Media

- A. Silica gel
- B. Silica gel containing 2% oxalic acid

Solvents

- A. Ethyl acetate-acetone (1:1)
- B. 1% Oxalic acid containing ethyl acetate-acetone (1:1)

R_F

Solvent	Medium	R_F
A	A	0.65
B	B	0.5 (yellow zone)

Ref.

T. Hishi, M. Asai, S. Harada, M. Muroi and K. Mizuno, *U.S. Patent* 3,717,707, Feb. 20, 1973

GP-3

TLC

Medium

Silica gel

Solvents

- A. *n*-Butanol-acetic acid-water (3:1:1)
- B. Chloroform-ethanol-14% aq. ammonia (4:7:2)
- C. Chloroform-ethanol-water (4:7:2)

Detection

- A. Bioautography vs. *Staphylococcus aureus* 209P
- B. Ninhydrin
- C. Heat treatment of plate with sulfuric acid

R_F

Solvent A 0.60

Solvent B 0.40

Solvent C 0.55

Ref.

J.I. Shoji, M. Mayama, S. Matsuura, K. Matsumoto and Y. Wakisaka, *U.S. Patent* 3,890,436, June 17, 1975

HW-64

TLC

Solvents

- A. Water satd. butanol
- B. 3% Ammonium chloride
- C. 80% Phenol
- D. 80% Phenol (in ammonia)
- E. 50% Acetone
- F. *n*-Butanol-methanol-water-methyl orange (40 ml:10 ml:20 ml:1.5 g)
- G. *n*-Butanol-methanol-water (40:10:20)
- H. Benzene-methanol (4:1, v/v)
- I. Distilled water
- J. *n*-Propanol-pyridine-acetic acid-water (15:10:3:2)
- K. *n*-Butanol-pyridine-*p*-toluenesulfonic acid (98 ml:2 ml:2 g)

R_F

Solvent	R_F	Solvent	R_F
A	0.0	G	0.0
B	0.80	H	0.03
C	0.03	I	0.05
D	0.94	J	0.42
E	0.07	K	0.0
F	0.73		

Ref.

German Patent 2,450,619, May 7, 1975

HW-70

TLC

Solvents

As HW-64, TLC; A-K

R_F

Solvent	R_F	Solvent	R_F
A	0.0	G	0.0
B	0.78	H	0.03
C	0.03	I	0.05
D	0.93	J	0.41
E	0.07	K	0.0
F	0.72		

Ref.

As HW-64, TLC

ELPHO**Medium**

Whatman No. 4 filter paper

Buffers

- A. 2 M Formic acid–0.1 M *p*-toluenesulfonic acid–*n*-propanol (500:50:50) in 1000 ml water, pH 1.8
- B. 2 M Formic acid–0.1 M *p*-toluenesulfonic acid–*n*-propanol (400:50:50) in 1000 ml water, pH 1.9
- C. 2 M Formic acid–0.1 M *p*-toluenesulfonic acid (400:50) in 1000 ml water, pH 1.9
- D. 1 M Ammonium hydroxide–1 M sodium hydroxide–0.1 M *p*-toluenesulfonic acid–*n*-propanol (200:5:100:100) in 1000 ml water, pH 10.8
- E. 1 M Ammonium hydroxide–1 M sodium hydroxide (200:2.5) in 1000 ml water, pH 11.5
- F. 50 ml 1 M Sodium hydroxide in 1000 ml water, pH 12.2

Conditions

The electrophoregrams were developed at 350 V for 1–4 h (depending on the electrolyte)

Mobility

Buffer	Toward cathode (cm ² /V sec)
A	31.66
B	31.97
C	32.77
D	2.86
E	3.54
F	5.63

Ref.

As Bluensomycin, ELPHO

K13-1A**TLC****Medium**

Avicell SF

Solvent

Butanol–acetic acid–water (4:1:5), upper phase

R_F

0.40; 0.80

Ref.

K. Yasuo, C. Shiro, H. Katsumi, M. Gunma, I. Wako, T. Shyoichi and K. Yoshio, *German Patent* 2,317,399, Oct. 24, 1974

K-288**PC****Solvents**

- A. Wet butanol
- B. 20% Ammonium chloride
- C. 72% Phenol
- D. 50% Acetone
- E. Butanol–methanol–water–methyl orange (40 ml:10 ml:20 ml:1.5 g)
- F. Butanol–methanol–water (40:10:20)
- G. Benzene–methanol (80:20)
- H. Water

R_F (estimated from drawing)

Solvent	R _F	Solvent	R _F
A	0.05	E	0.45
B	0.95	F	0.35
C	0.90	G	0.05
D	0.95	H	0.05

Ref.K. Matsumoto, *J. Antibiot.*, 14 (1961) 141–146**ELPHO****Medium**

Paper

Buffers

- A. pH 5.0 buffer
- B. pH 8.0 buffer

Mobility (estimated from drawing)

- A. Slight movement towards cathode
- B. Slight movement towards anode

Ref.

As PC

KM-8**TLC 1****Medium**

Silica gel (Merck Kieselgel G)

Solvents

- A. Chloroform–methanol–water (5:4:2)
- B. Chloroform–methanol–1% acetic acid in water (5:4:2)

Detection

- A. Bioautography
- B. Ninhydrin

R_F

Solvent A 0.35

Solvent B 0.70

Ref.

R. Oiwa, M. Katagiri, N. Tanaka, Y. Takahashi, K. Sato, R. Masuma and S. Omura, *J. Antibiot.*, 28 (1975) 819–820

TLC**Medium**

Cellulose (Avicel SF)

Solvents

- A. *n*-Butanol–acetic acid–water (3:1:1)
 B. *n*-Propanol–pyridine–acetic acid–water (15:10:3:12)

Detection

- A. Bioautography
 B. Ninhydrin

 R_F

Solvent A 0.33
 Solvent B 0.71

Ref.

As TLC 1

L.A. 5352**PC****Solvents**

- A. 3% Aq. ammonium chloride
 B. Methanol–0.1 *N* hydrochloric acid (3:1)
 C. Butanol–ethanol–acetic acid–water (25:25:3:47)
 D. Butanol–acetic acid–water (1:1:2)
 E. Water satd. butanol
 F. As D, but (4:1:2)
 G. As D, but (2:1:1)

DetectionBioautography vs. *Micrococcus aureus* **R_F**

Solvent	R_F	Solvent	R_F
A	0.80	E	0.17
B	0.75	F	0.54, 0.72
C	0.87	G	0.35, 0.45
D	0.88		

Ref.

P. Sensi and M.T. Timbal, *Antibiot. Chemother.*, 9 (1959) 160–166

L.A. 5937**PC****Solvent**

As L.A. 5352 (A–G)

Detection

As L.A. 5352

Ref.

As L.A. 5352, PC

 R_F

Solvent	R_F	Solvent	R_F
A	0.80	E	0.20
B	0.75	F	0.14, 0.37, 0.46
C	0.83	G	0.07, 0.17, 0.31
D	0.90		

LA-7017**PC (Circular paper chromatography)****Solvents**

- A. Benzene–acetic acid–water (20:25:5)
 B. Benzene–butanol–water (18:2:20)
 C. Chloroform–water satd. carbon tetrachloride–methanol (5:4:1)
 D. Water satd. diisoamyl ester–butanol (20:10)

DetectionBioautography vs. *Staphylococcus aureus* 209P **R_F**

Useful for comparing LA-7017 with chromomycins and NSCA-649

Ref.

E.V. Kruglyak, V.N. Borisova and M.G. Brazhnikova, *Antibiotiki*, 8 (1963) 1064–1067

LL-21220**TLC****Medium**Silica gel F₂₅₄ (E. Merck)**Solvents**

- A. Methanol
 B. Acetone
 C. Ethyl acetate

Detection

- A. UV quenching
 B. 1% Aq. potassium permanganate spray

 R_F

Solvent A 0.52
 Solvent B 0.47
 Solvent C 0.06

Ref.

D.B. Borders, F. Barbatschi, A.J. Shay and P. Shu, *Antimicrob. Agents Chemother.*, 1969 (1970) 233–235

LL-AB664**PC****Paper**

Whatman No. 1

Solvent

m-Cresol-acetic acid-pyridine-water
(200:1:1:100), lower phase, descending

Detection

Wash air-dried strips with diethyl ether to remove cresol; bioautography vs. *Klebsiella pneumoniae*, pH 7.9, or *Bacillus subtilis*, pH 6.0

R_F

0.45–0.50

Ref.

K.J. Sax, P. Monnikendam, D.B. Borders, P. Shu, L.A. Mitscher, W.K. Hausmann and E.L. Patterson, *Antimicrob. Agents Chemother.*, 1967 (1968) 442–448

LL-AC541**PC 1****Paper**

Whatman No. 1

Solvent

90% Phenol-*m*-cresol-acetic acid-pyridine-water (100:25:4:4:75)

R_F

0.58

Ref.

V. Zbinovsky, W.K. Hausmann, E.R. Wetzel, D.B. Borders and E.L. Patterson, *Appl. Microbiol.*, 16 (1968) 614–616

PC 2**Solvent**

n-Propanol-pyridine-acetic acid-water
(15:10:3:12)

R_F

0.50

Ref.

K. Arima, T. Kawamura and T. Beppu, *J. Antibiot.*, 25 (1972) 387–392

TLC**Media**

- A. Silica gel G
- B. Avicel SF plate

R_F

Medium	Solvent	R _F
A	A	0.52
B	B	0.35

Solvents

- A. *n*-Propanol-pyridine-acetic acid-water (15:10:3:12)

- B. Chloroform-methanol-17% aq. ammonia (2:1:1), upper layer

Ref.

As PC 2

LL-AO341A; LL-AO341B**PC****Paper**

Whatman No. 1

Solvent

Isoamyl alcohol-methyl isobutyl ketone-acetic acid-water (2:3:1:4), upper layer, descending

Detection

Bioautography vs. *Bacillus subtilis*

R_F

LL-AO341A 0.35

LL-AO341B 0.22

Ref.

H.A. Whaley, E.L. Patterson, M. Dann, P. Shu, M.E. Swift, J.N. Porter and G. Redin, *Antimicrob. Agents Chemother.*, 1966 (1967) 587–590

LL-AV290**PC****Paper**

Whatman No. 1

Solvents

- A. 5% Aq. ammonium chloride
- B. Pyridine-*sym*.-collidine-*sec*.-butanol-water (2:2:1:1)
- C. Methanol-1.5% aq. sodium chloride (4:1); paper buffered with 0.95 *M* sodium sulfate + 0.05 *M* sodium bisulfate
- D. 90% Phenol-*m*-cresol-pyridine-acetic acid-water (25:25:1:1:25)

All solvents developed descending

Detection

Bioautography vs. *Bacillus subtilis* or *Corynebacterium xerosis*

R_F

Solvent A 0.75

Solvent B 0.58

Solvent C 0.54

Solvent D 0.20

Ref.

M.P. Kunstmann, L.A. Mitscher, J.N. Porter, A.J. Shay and M.A. Darken, *Antimicrob. Agents Chemother.*, 1968 (1969) 242–245

LL-BL136
(cf., SF-701)**PC***Solvents*

- A. Water satd. 1-butanol containing 2% *p*-toluenesulfonic acid
- B. Pyridine-*sym*.-collidine-tetramethylammonium hydroxide-water (50:25:1:125)
- C. Phenol-*m*-cresol-acetic acid-pyridine-water (100:25:4:4:75)
- D. 1-Butanol-methanol-water-*p*-toluenesulfonic acid (40:10:20:1)

R_F

Solvent A	0.21
Solvent B	0.70
Solvent C	0.55, 0.68
Solvent D	0.63

Ref.

D.B. Borders, J.P. Kirby, E.R. Wetzel, M.C. Davies and W.K. Hausmann, *Antimicrob. Agents Chemother.*, 1 (1972) 403-407

LL-BM408**PC***Paper*

Whatman No. 1

Solvent

Ethyl acetate-pyridine-water (360:110:115)
(20 h at 25°C)

Detection

TOLLENS reagent

R_F

Compound	<i>R</i> _{ribose}
BM408 hydrolysate	1.00
BM408 glucose	0.33
BM408 mannose	0.50
BM408 xylose	0.73

Ref.

J.P. Kirby, D.B. Borders and G.E. van Lear, *J. Antibiot.*, 30 (1977) 175-177

TLC*Medium*

Silica gel

Solvent

Chloroform-methanol-ammonium hydroxide
(1:3:2)

R_F

*R*_{paromamine}: 0.83

Ref.

As PC

M-81**PC***Solvents*

- A. Butanol-acetic acid-pyridine-water (15:3:10:12)
- B. Water satd. *n*-butanol
- C. 3% Ammonium chloride
- D. 80% Phenol
- E. *n*-Butanol-methanol-water (4:1:2)
- F. 50% Acetone
- G. Phenol in ammonia gas

Detection

- A. Bioautography vs. *Micrococcus lysodeikticus* IFO 3333, Solvent A
- B. Bioautography vs. *Serratia polymycticum* IFO 3055, Solvents B-G

R_F (estimated from drawing)

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.15-0.4	E	0.0-0.1
B	0.0	F	0.0-0.2
C	0.75	G	0.95
D	0.0		

Ref.

N. Yoshida, S. Hayashi, Y. Tani and K. Ogata, *J. Antibiot.*, 27 (1974) 128-132

ELPHO*Medium*

Paper

Buffers

- A. $\frac{1}{40}$ M Acetate, pH 3-5
- B. Phosphate, pH 6-8
- C. Glycine, pH 9-10

Conditions

15-20 mA and 2000 V for 40 min each

Mobility

Moved toward cathode

Ref.

As PC

M-141**PC***Solvents*

- A. Deionized-water satd. *n*-butanol (develop 16.5 h)
- B. Water satd. *n*-butanol + 2% *p*-toluenesulfonic acid + 2% piperidine (develop 16.5 h)

C. Water satd. *n*-butanol + 2% *p*-toluenesulfonic acid + 2% piperidine (develop 16.5 h)

Detection

Bioautography vs. *Bacillus subtilis* ATCC 10707

R_F

Solvent	R_F
A	0.03
B	0.25–0.30 (principal zone) 0.40 (minor zone)
C	0.45

Ref.

A.W. Goldstein, *U.S. Patent* 3,859,170, Jan. 7, 1975

M-13902

TLC

Medium

Cellulose

Solvents

- A. Butanol–isopropanol–water (7:7:6)
- B. Isopropanol–water (7:3)
- C. *n*-Butanol–ethanol–water (7:7:6)
- D. *n*-Propanol–water (4:1)

Detection

- A. Bioautography vs. *Streptomyces alivaceus* ATCC 31126
- B. UV at 305 and 225 nm

R_F

Solvent A	0.72
Solvent B	0.85
Solvent C	0.81
Solvent D	0.75

Ref.

German Patent 2,513,854, Oct. 2, 1975

MC696-SY-2 (A and B)

TLC

Media

- A. Silica gel GF₂₅₄
- B. Cellulose DEAE

Solvents

- A. *n*-Propanol–0.1 M phosphate buffer, pH 7 (7:3)
- B. *n*-Butanol–methanol–water (4:1:2)
- C. 0.2 M Sodium chloride in 0.01 M phosphate buffer, pH 7

Detection

Bioautography

R_F

Solvent	Medium	MC696-SY-2	
		A	B
A	A	0.32–0.37	0.41–0.46
B	A	0.20–0.23	0.23–0.25
C	B	0.54–0.60	0.42–0.53

Ref.

H. Umezawa, S. Mitshuhashi, R. Utahara and T. Ishikawa, *U.S. Patent* 3,928,596, Dec. 23, 1975

MC 916

TLC

Medium

Silica gel

Solvent

Chloroform–methanol (10:1)

R_F

Component	R_F
MC 916 A	0.57
MC 916 B	0.50

Ref.

German Patent 2,510,568, Sept. 18, 1975

MM4462 (from *Fusarium lateritium*)

TLC

Medium

Silica gel

Solvents

- A. Chloroform–acetic acid (1950:50)
- B. Chloroform–acetone (3:2)
- C. Toluene–ethyl acetate–acetic acid (2:4:1)
- D. Benzene–methanol (9:1)
- E. Benzene–dioxane–acetic acid (90:25:4)
- F. *n*-Butanol–acetic acid–water (8:2:2)
- G. *n*-Butanol–ethanol–water (4:2:2)

R_F

Solvent	R_F	Solvent	R_F
A	0.51	E	0.61
B	0.73	F	0.85
C	0.68	G	0.85
D	0.49		

Detection

MM4462 complex gave a violet zone when sprayed with concentrated sulfuric acid. Potassium permanganate in acetone produces an orange zone

Ref.

M. Cole and G.N. Rolinson, *U.S. Patent* 3,793,449, Feb. 19, 1974

MM 4550 (cf., olivanic acids)

TLC 1**Media**

- A. Silica gel F₂₅₄ (Merck)
- B. Cellulose

Solvents

- A. Butanol-isopropanol-water (7:7:6)
- B. Isopropanol-water (7:3)
- C. *n*-Butanol-ethanol-water (7:7:6)
- D. *n*-Propanol-water (4:1)
- E. *n*-Propanol-0.1 *M* phosphate buffer, pH 7 (7:3)
- F. *n*-Butanol-methanol-water (4:1:2)

R_F

Solvent	Medium A	Medium B
A	0.6	
B	0.7	
C	0.7	
D	0.6	
E		0.6
F		0.6

Ref.

M. Cole, J.D. Hood and D. Butterworth, *German Patent* 2,513,855, Oct. 2, 1975

TLC 2**Medium**

Cellulose

Solvent

Acetone-pyridine-acetate buffer (ELPHO buffer) (2:1)

Detection

- A. Bioautography
- B. Spray with Ehrlich's reagent (300 mg 4-dimethylaminobenzaldehyde dissolved in 9 ml conc. hydrochloric acid + 9 ml ethanol + 54 ml *n*-butanol) MM 4550 reacts to give a blue color

R_F

0.85

Ref.

D. Butterworth and G.N. Rolinson, *U.S. Patent* 3,919,415, Nov. 11, 1975

ELPHO**Medium**

Whatman 3MM paper, 40 cm length, with cellophane-covered paper wicks; total length = 20 cm

Buffer

0.067 *M* Acetic acid adjusted to pH 5.3 with pyridine

Conditions

3000 V for 25 min (approx. 60 V/cm)

Detection

- A. Bioautography
- B. Spray with Ehrlich's reagent (300 mg 4-dimethylaminobenzaldehyde dissolved in 9 ml conc. hydrochloric acid + 9 ml ethanol + 54 ml *n*-butanol) MM 4550 reacts to give a blue color

Mobility

Moves 18 cm to anode

Ref.

As TLC 2

MM 13902 and MM 17880 (See olivanic acids)

MM 17880**TLC****Media**

- A. Silica gel plates (Merck) F₂₅₄
- B. Cellulose Eastman Kodak 13255

Solvents

- A. *n*-Butanol-methanol-water (4:1:2)
- B. *n*-Propanol-0.1 *M* phosphate buffer, pH 7.0 (7:3)
- C. Butanol-isopropanol-water (7:7:6)
- D. *n*-Propanol-water (4:1)
- E. Isopropanol-water (7:3)

R_F

Solvent	Medium	
	A	B
A	0.4	
B	0.6	
C		0.7
D		0.7
E		0.8

Ref.

S.J. Box and J.D. Hood, *U.S. Patent* 4,163,051, July 31, 1979

MSD-235**PC***Paper*

Whatman 3MM

Solvent

n-Butanol satd. with 0.1 *N* ammonium hydroxide, 17 h, 25°C

Detection

Bioautography vs. *Escherichia coli*

R_F

Component	cm from origin
MSD-235S ₁	3-5
MSD-235S ₂	10
MSD-235S ₃	16

Ref.

L. Chalet, T.W. Miller, F. Tausig and F.J. Wolf, *Antimicrob. Agents Chemother.*, 1963 (1964) 28-32

MYC 8003**TLC***Media*

- A. Silica gel
- B. Kieselgel F₂₅₄

Solvents

- A. Acetone-ethyl acetate-water (12:8:1)
- B. Methyl isobutyl ketone-acetone-water (50:45:5)
- C. Benzene-ethanol-33% ammonia (65:40:9)

Detection

UV

R_F

Medium	Solvent	Component		
		I	II	III
A	A	0.5	0.7	0.3
B	B	0.29	0.44	0.16
B	C	0.10	0.20	0.76

Ref.

C. Vos and J. Den Admirant, *U.S. Patent* 3,927,211, Dec. 16, 1975

NK-1003**PC***Paper*

Ascending

Solvents

- A. 20% Aq. soln. of ammonium chloride
- B. Water satd. *n*-butanol
- C. *n*-Butanol-acetic acid-water (3:1:1)
- D. Water satd. *n*-butanol + 2% (w/v) *p*-toluene-sulfonic acid + 2% (v/v) piperidine
- E. Chloroform-methanol-17% aq. ammonia (2:1:1) lower layer

R_F

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.93	D	0.00
B	0.00	E	—
C	0.05		

Ref.

As Apramycin, PC 1

TLC*Medium*

Silica gel

Solvents

- A. Chloroform-methanol-17% aq. ammonia (2:1:1), upper layer
- B. Methanol-10% aq. ammonium acetate (1:1)
- C. *n*-Butanol-ethanol-chloroform-17% aq. ammonia (4:5:2:5)

R_F

Solvent A	0.66
Solvent B	0.91
Solvent C	—

Ref.

As Apramycin, PC 1

NSCA-649**PC (Circular paper chromatography)***Solvent*

As LA-7017, PC; A-D

Detection

As LA-7017, PC

R_F

Useful for comparing NSCA-649 with LA-7017 and chromomycins

Ref.

As LA-7017, PC

O-2867**PC***Paper*

Toyo Roshi No. 51

*Solvent**n*-Butanol–acetic acid–water (4:1:2)*Detection*Bioautography vs. *Pellicularia oryzae**R_F*O-2867- α 0.66O-2867- β 0.53*Ref.*

T. Sato, K. Yamaguchi, M. Katagiri, J. Awaya, I. Iwai, S. Omura and T. Hata, *J. Antibiot.*, 24 (1971) 774–778

ELPHO*Medium*

Paper

Buffers

A. McIlvaine's buffer, pH 2.0

B. Pyridine–acetic acid–water (1:10:289), pH 3.7

C. $\frac{1}{15}$ M Phosphate, pH 8.0*Conditions*

200 V, 2.5 h

Mobility

Buffer	Mobility	
	O-2867- α	O-2867- β
A	Toward cathode	Toward cathode
B	Origin	Origin
C	Toward anode	Toward anode

Ref.

As PC

OS-3256 (ALAZOPEPTIN)**PC***Paper*

Ascending method

*Solvent*93.8% *n*-Butanol–44% propionic acid (1:1)*R_F*

0.45–0.5

Ref.

T. Hata, I. Umezawa, Y. Iwai, M. Katagiri, J. Awaya, K. Komiyama, R. Oiwa and K. Atsumi, *J. Antibiot.*, 26 (1973) 181–183

OS-3256-B**PC***Solvents*A. 93.8% *n*-Butanol–44% propionic acid (1:1)B. Ethanol–*tert.*-butanol–formic acid–water (60:20:5:15)*Detection*A. Bioautography vs. *Bacillus subtilis*

B. Ninhydrin

R_F

Solvent A 0.34

Solvent B 0.50

Ref.

S. Omura, I. Umezawa, K. Satoh, J. Awaya, K. Komiyama and R. Oiwa, *U.S. Patent* 3,995,028, Nov. 30, 1976

TLC*Media*

A. Silica gel

B. Avicel

*Solvents*A. *n*-Butanol–methanol–water (2:1:3)B. *n*-Butanol–methanol–water (3:1:2)C. *n*-Butanol–acetic acid–water (3:1:2)

D. Ethanol–ammonium hydroxide–water (8:1:1)

E. *n*-Butanol–acetic acid–water (3:1:2)F. *n*-Butanol–ethanol–water (2:2:3)*Detection*A. Bioautography vs. *Bacillus subtilis*

B. Ninhydrin

R_F

Solvent	Medium	<i>R_F</i>
A	A	0.56
B	A	0.22
C	A	0.31
D	B	0.16
E	B	0.46
F	B	0.55

Ref.

As PC

OS-4742 (A₁, A₂, B₁, B₂)**TLC***Media*

A. Silica gel

B. Acid-treated silica gel (prepared with 0.2% sulfuric acid instead of water)

C. Avicel SF

Solvents

- A. Chloroform-methanol (10:1)
 B. Benzene-acetic acid (3:1)
 C. Benzene-methanol (5:1)
 D. Benzene-acetic acid (4:1)
 E. Benzene-methanol (150:1)

 R_F

Solvent	Medium	A ₁	A ₂	B ₁	B ₂
A	A	0.77	0.77	0.56	0.55
B	A	0.56	0.56	0.06	0.03
C	B			0.36	0.36
D	B			0.50	0.12
E	C	0.83	0.81	0.63	0.25

Ref.

S. Omura, H. Tanaka, R. Oiwa, J. Awaya, R. Masuma and K. Tanaka, *J. Antibiot.*, 30 (1977) 908-916

PA-108; PA-133A; PA-133B; PA-148**PC****Paper**

Treated with formamide as stationary phase

Solvents

- A. Benzene-cyclohexane (1:1)
 B. Benzene
 C. Benzene-chloroform (3:1)
 D. Benzene-chloroform (4:1)
 All solvents developed at 24°C

 R_F

	Solvent			
	A	B	C	D
PA-108	0.05	0.58	0.89	0.90
PA-133A	0.75	0.75	0.95	0.95
PA-133B	0.20	0.24	0.76	0.90
PA-148	0.03	0.18	0.58	0.90

Ref.

K. Murai, B.A. Sobin, W.D. Celmer and F.W. Tanner, *Antibiot. Chemother.*, 9 (1959) 485-490

CCD**Solvent**

Benzene-cyclohexane-95% ethanol-water (5:5:8:2)

Distribution

PA-108 $K = 0.43$

PA-133A $K = 1.50$

PA-133B $K = 0.50$

PA-148 $K = 0.57$

Ref.

As PC

PC-183 (PHTHALIDYL D-AMINO-BENZYL PENICILLINATE HYDROCHLORIDE)**TLC****Medium**

Eastman Chromgram Sheet No. 6061

Solvent

Chloroform-ethanol (9:1) (until the solvent traveled 8 cm from origin)

Detection

Bioautography vs. *Sarcina lutea* ATCC 9341

 R_F

0.88

Ref.

Y. Shiobara, A. Tachibana, H. Sasaki, T. Watanabe and T. Sado, *J. Antibiot.*, 27 (1974) 665-673

PS-5**PC****Paper**

Descending

Solvents

- A. *n*-Propanol-water (7:3)
 B. *n*-Propanol-isopropanol-water (7:7:6)
 C. Acetonitrile-water (8:2)
 D. Acetonitrile- $\frac{1}{10}$ M tris buffer, pH 7.5-EDTA (120:30:1)
 E. Ethanol-water (7:3)

Detection

Bioactivity

 R_F

Solvent	R_F	Solvent	R_F
A	0.68	D	0.34
B	0.70	E	0.63
C	0.36		

Ref.

K. Okamura, S. Hirata, A. Koki, K. Hori, N. Shibamoto, Y. Okumura, M. Okabe, R. Okamoto, K. Kouno, Y. Fukagawa, Y. Shimauchi and T. Ishikura, *J. Antibiot.*, 32 (1979) 262-271

TLC**Media**

- A. Silica gel F₂₅₄
B. Avicel cellulose

Solvents

- A. Ethanol-water (7:3)
B. Isopropanol-water (7:3)

Detection

Bioactivity

R_F

Solvent	Medium A	Medium B
A	0.82	
B		0.96

Ref.

As PC

ELPHO**Medium**

Paper

Buffer

Veronal buffer, pH 8.6

Conditions

$I = 0.05$, 42 V/cm, 30 min

Mobility

Migration distance to anode, 28 mm

Ref.

As PC

PSX-1**TLC****Medium**

Silica gel (Silufo®)

Solvent

Chloroform-methanol (10:1)

Detection

2,4 dinitrophenylhydrazine and potassium permanganate. When the thin-layer chromatograms are allowed to stand for 30–60 min in the open air the antibiotic can be detected as a deep red spot

R_F

0.5

Ref.

J. Fuska, I. Kuhr, P. Nemec and A. Fuskova, *J. Antibiot.*, 27 (1974) 123–127

R4H**PC****Papers**

- A. Toyo Roshi No. 51, ascending
B. Toyo Roshi No. 51 UH

Solvents

- A. *n*-Butanol-pyridine-acetic acid-water-*tert*-butanol (15:10:3:12:4)
B. *n*-Propanol-pyridine-acetic acid-water (15:10:3:12)

R_F

Compound	Medium	Solvent	
		A	B
R4H A	A	0.16	0.38
	B	0.32	0.58
R4H B	A	0.06	0.23
	B	0.19	0.44
R4H C	A	0.09	0.31
	B	0.24	0.51
R4H D	A	0.04	0.19
	B	0.11	0.36

Ref.

As Racemomycins, PC 1

TLC**Medium**

Avicel SF (Funakoshi Co.)

Solvent

n-Propanol-pyridine-acetic acid-water (15:10:3:12)

R_F

Compound	R _F
R4H A	0.31
R4H B	0.22
R4H C	0.26
R4H D	0.13

Ref.

As Racemomycins, PC 1

R-468**PC****Solvents**

- A. Water satd. *n*-butanol
B. 3% Ammonium chloride
C. 50% Aq. phenol
D. 50% Aq. acetone
E. Butanol-methanol-water-methyl orange (40 ml:10 ml:20 ml:1.5 g)
F. Butanol-methanol-water (4:1:2)
G. Benzene-methanol (4:1)
H. Water
I. Butanol satd. water

J. *n*-Propanol-pyridine-acetic acid-water (15:10:3:12)

R_F (estimated from drawing)

Solvent	R_F	Solvent	R_F
A	0.05	F	0.45
B	1.00	G	0.00
C	0.35	H	1.00
D	0.75	I	1.00
E	0.50	J	0.60

Ref.

T. Nishikawa and N. Ishida, *J. Antibiot.*, 18 (1965) 132-133

ELPHO

Buffers

A. $\frac{1}{15}$ M phosphate, pH 5.0

B. $\frac{1}{15}$ M phosphate, pH 8.0

Conditions

225 V/20 cm, 3 h

Mobility

A. Toward cathode

B. Slightly toward cathode

Ref.

As PC

RIT D-2214

PC

Paper

Whatman No. 4

Solvents

A. *n*-Butanol-ethanol-water (4:1:5)

B. *n*-Butanol-acetic acid-water (4:1:5)

Detection

Bioautography vs. *Alcaligenes viscolactis* and *Bacillus subtilis*

R_F

Solvent A 0.18

Solvent B 0.43

Ref.

As Ampicillin, PC

TLC

Medium

Silica gel (Schleicher and Schuell; ready plastic sheets F1500 LS 254)

Solvents

A. *n*-Butanol-ethanol-acetic acid-water (10:1.5:1.5:2)

B. *n*-Butanol-acetic acid-water (4:1:1)

C. Acetonitrile-water (75:25)

Detection

Ninhydrin spray

R_F

Solvent A 0.72

Solvent B 0.63

Solvent C 0.22

Ref.

As Ampicillin, PC 1

Ro 5-2667; Ro 7-7730; Ro 7-7731

TLC

Media

A. MN-polygram cel 300 (Brinkmann)

B. As A, but buffered by dipping plate into 0.2 M ammonium sulfate and allowing excess liquid to drip off plate

Solvents

A. 2-Propanol-water (7:3)

B. 1-Butanol-acetic acid-water (4:1:5)

R_F

	Medium-Solvent	
	A-B	B-A
Ro 5-2667	0.07	0.06
Ro 7-7730	0.19	0.13
Ro 7-7731	0.02	0.04

Ref.

H. Maehr and J. Berger, *Biotech. Bioeng.*, 11 (1969) 1111-1123

S-15-1 (STREPTOTHRICIN GROUP ANTIBIOTIC)

PC

Solvents

A. *n*-Propanol-pyridine-acetic acid-water (15:10:3:12)

B. Wet *n*-butanol containing 2.0% (w/v) *p*-toluenesulfonic acid

R_F

Solvent A 0.10

Solvent B 0.00

Ref.

K. Arima, T. Kawamura and T. Beppu, *J. Antibiot.*, 25 (1972) 387-392

TLC

Solvents

A. Water satd. butanol

B. 3% Ammonium chloride

- C. 80% Phenol
 D. 80% Phenol (in ammonia)
 E. 50% Acetone
 F. *n*-Butanol-methanol-water-methyl orange (40 ml:10 ml:20 ml:1.5 g)
 G. *n*-Butanol-methanol-water (40:10:20)
 H. Benzene-methanol (4:1)
 I. Distilled water
 J. *n*-Propanol-pyridine-acetic acid-water (15:10:3:2)
 K. *n*-Butanol-pyridine-*p*-toluenesulfonic acid (98 ml:2 ml:2 g)

R_F

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.0	G	0.0
B	0.80	H	0.03
C	0.03	I	0.05
D	0.94	J	0.40
E	0.07	K	0.0
F	0.72		

Ref.

- A. Mimura, M. Wada, Y. Hashimoto, K. Tsuzuki, T. Iguchi, T. Kawamura and K. Arima, *U.S. Patent* 3,917,514, Nov. 4, 1975

S-15-2 (STREPTOTHRICIN GROUP ANTIBIOTIC)

TLC

Media

- A. Silica gel G
 B. Cellulose MN 300
 C. Avicel SF plate

Solvents

- A. *n*-Propanol-pyridine-acetic acid-water (15:10:3:12)
 B. Wet *n*-butanol containing 2.0% (w/v) *p*-toluenesulfonic acid
 C. Chloroform-methanol-17% aq. ammonia (2:1:1), upper layer

R_F

Solvent	Medium		
	A	B	C
A	0.50	0.05	0.08
B		0.15	
C	0.28		

Ref.

- K. Arima, T. Kawamura and T. Beppu, *J. Antibiot.*, 25 (1972) 387-392

S-520

TLC

Medium

Silica gel GF

Solvents

- A. *n*-Butanol-acetic acid-water (3:1:1)
 B. Chloroform-methanol (4:1)

R_F

	Solvent	
	A	B
S520	0.58 ± 0.05	—
DNP-S-520	—	(I*) 0.80 (II) 0.20
Acetyl-S-520	—	(I) 0.70 (II) 0.10

- * Roman numerals represent component numbers

Ref.

- J. Shoji, S. Kozuki, M. Mayama and N. Shimaoka, *J. Antibiot.*, 23 (1970) 429-431
 J. Shoji and R. Sakazaki, *J. Antibiot.*, 432-436

S-583-A-II; S-583-A-III; S-583-B

TLC

Medium

Metal-free silica gel plate

Solvents

- A. Chloroform-methanol (85:15)
 B. Benzene-ethyl formate-formic acid (3:2:2)

R_F

- Solvent A: S-583-B HCl (streak) > S-583-A-III HCl > S-583-A-II HCl
 Solvent B: S-583-A-II HCl > S-583-B HCl > S-583-A-III HCl

Ref.

- J. Shoji, S. Kozuki, H. Nishimura, M. Mayama, K. Motokawa, Y. Tanaka and H. Otsuka, *J. Antibiot.*, 21 (1968) 643-648

S-666

PC

Solvent

n-Butanol-acetic acid-water (4:1:5)

R_F

Single spot

Ref.

Derwent Farmdoc 55495, July 22, 1970

S 7481/F-1 and F-2**TLC****Media**

- A. Kieselgel glass plate
- B. Kieselgel "Polygram"

Solvents

- A. Chloroform + 4% methanol
- B. Hexane-acetone (1:1)
- C. Chloroform-acetone (2:1)

Detection

Iodine vapor

 R_F

Solvent	Medium	S 7481/F-1	S 7481/F-2
A	A	0.52	0.46
B	B	0.37	0.28
C	B	0.34	0.26

Ref.

German Patent 2,455,859, June 12, 1975

Sch 16656 (POLYENE ANTIFUNGAL)**TLC****Medium**

Silica gel G

Solvent*n*-Butanol-acetic acid-water-dioxane (6:2:2:1)**Detection**Bioautography vs. *Candida albicans* R_F

0.43

Ref.

G.H. Wagman, R.T. Testa, M. Patel, J.A. Marquez, E.M. Oden, J.A. Waitz and M.J. Weinstein, *Antimicrob. Agents Chemother.*, 7 (1975) 457-461

SF-689**ELPHO****Buffer**

pH 1.9

Conditions

3000 V, 20 min

Mobility

Migrates 4.5 cm toward cathode

Ref.

Japanese Patent 6076/1970, Feb. 28, 1970

SF-701**PC (Circular)****Solvent***n*-Butanol-pyridine-acetic acid-water (15:10:3:12) R_F

0.39

Ref.

T. Tsuruoka, T. Shoumura, N. Ezaki, T. Niwa and T. Niida, *J. Antibiot.*, 21 (1968) 237-238

TLC 1**Media**

- A. Silica gel
- B. Cellulose

Solvents

- A. Chloroform-methanol-17% ammonium hydroxide (2:1:1), upper layer
- B. *n*-Butanol-acetic acid-water (2:1:1)
- C. *n*-Propanol-pyridine-acetic acid-water (15:10:3:12)
- D. Wet butanol containing 2% *p*-toluenesulfonic acid
- E. Phenol-water (6:4)

 R_F

Medium	Solvent	R_F
A	A	0.72
	B	0.15
B	C	0.66
	B	0.40
	D	0.23
	E	0.42

Ref.

As PC

TLC 2**Media**

- A. Silica gel G
- B. Cellulose MN 300
- C. Avicel SF plate

Solvents

- A. *n*-Propanol-pyridine-acetic acid-water (15:10:3:12)
- B. Wet *n*-butanol containing 2.0% (w/v) *p*-toluenesulfonic acid
- C. Chloroform-methanol-17% aq. ammonia (2:1:1), upper layer

R_F

Solvent	Medium		
	A	B	C
A		0.66	0.33
B		0.23	
C	0.72		

Ref.

K. Arima, T. Kawamura and T. Beppu, *J. Antibiot.*, 25 (1972) 387-392

SF-733

PC

Solvents

- A. Water satd. *n*-butanol containing 2% *p*-toluenesulfonic acid
 B. *n*-Butanol-pyridine-acetic acid-water (6:4:1:3)

Detection

- A. Ninhydrin
 B. Bioautography vs. *Bacillus subtilis*

 R_F

Solvent	Distance of zone from origin (cm)
A	14.8
B	8.0

Ref.

Netherlands Patent 68,18105, June 20, 1969; T. Shomura, N. Ezaki, T. Tsuruoka, T. Niwa, E. Akita and T. Niida, *J. Antibiot.*, 23 (1970) 155-161

TLC 1

Medium

Silica gel

Solvents

- A. *tert*-Butanol-acetic acid-water (2:1:1)
 B. *n*-Butanol-acetic acid-water (3:1:1)
 C. *n*-Butanol-pyridine-water (6:4:3)

Detection

As PC

 R_F

Solvent A	0.40
Solvent B	0.16
Solvent C	0.50

Ref.

As PC; ref. 1

TLC 2

Medium

Silica gel

Solvent

80% Aq. methanol

Detection

- A. Bioautography vs. *Bacillus subtilis*
 B. Ninhydrin

 R_F

0.70

Ref.

T. Shomura, N. Ezaki, T. Tsuruoka, T. Niwa, E. Akita and T. Niida, *U.S. Patent* 3,661,892, May 9, 1972

ELPHO

Medium

Paper

Buffer

Buffer, pH 1.8

Conditions

3300 V, 15 min

Mobility

Migrates 15 cm toward cathode

Ref.

As PC; ref. 1

SF-767-A; SF-767-L

PC

Solvents

- A. As SF-733; A
 B. As SF-733; B
 C. *n*-Propanol-pyridine-acetic acid-water (15:10:3:12)

Detection

As SF-733; A, B

 R_F

Solvent	Distance of zone from origin (cm)	
	SF-767-A	SF-767-L
A	3.9	2.5
B	6.1	5.7
C	11.4	9.7

Ref.

German Patent 1926458, Feb. 12, 1970

TLC

Medium

Silica gel G (Merck)

Solvents

- A. As SF-733; A
B. As SF-733; C

 R_F

Derivative	Solvent	
	A	B
<i>n</i> -Acetyl SF-767-A	0.89	1.00
<i>n</i> -Acetyl SF-767-L	0.78	1.00

Ref.

As PC

ELPHO**Medium**

Paper

Buffer

pH 1.8

Conditions

3000 V, 20 min

Mobility

Both compounds migrate 11.5 cm to the cathode

Ref.

As PC

SF-837**TLC 1****Media**

- A. Silica gel
B. Alumina

Solvents

- A. Benzene–acetone (2:1)
B. *n*-Butanol–acetic acid–water (3:1:1)
C. Methanol
D. Ethyl acetate–benzene (2:1)
E. Ethyl acetate

 R_F

	Medium–Solvent				
	A–A	A–B	A–C	B–D	B–E
SF-837	0.45	0.67	0.82	0.34	0.78
SF-837-A ₂	0.51	0.68	0.83	0.40	0.84
SF-837-A ₃	0.50	0.68	0.83	0.45	0.87
SF-837-A ₄	0.55	0.69	0.84	0.52	0.91

Ref.

T. Tsuruoka, T. Shomura, N. Ezaki, E. Akita, S. Inoue, S. Fukatsu, S. Amano, H. Watanabe and T. Niida, *Belgian Patent* 745-430, July 16, 1970

T. Niida, T. Tsuruoka, N. Ezaki, T. Shomura, E. Akita and S. Inoue, *J. Antibiot.*, 24 (1971) 319–320

TLC 2**Medium**

Silica gel (Eastman sheet 6061)

Solvent

- A. Benzene–acetone (2:1)
B. Chloroform–methanol (10:1)

Detection

Spray with 10% sulfuric acid and heat at about 80° for 3 min

 R_F

- SF-837 Violet
SF-837M1 Reddish violet
SF-837M2 Bluish violet

Ref.

T. Shomura and K. Unemura, *Chem. Pharm. Bull.*, 22 (1974) 2427–2433

SF-973 B**TLC****Medium**

Silica gel

Solvent

Ethyl acetate–methanol (10:1)

Detection

10% Sulfuric acid followed by heating

 R_F

0.5

Ref.

T. Tsuruoka, T. Shomura, Y. Ogawa, N. Ezaki, H. Watanabe, S. Amano, S. Inoue and T. Niida, *J. Antibiot.*, 26 (1973) 168–174

SF-1130**PC****Solvent***n*-Butanol–acetic acid–water (4:1:5) R_F

0.02

Ref.

Derwent 56459U-BD, Sept. 19, 1973

SF-1293**PC****Solvents**

- A. Water satd. *n*-butanol
B. 3% Aq. ammonium chloride
C. 75% Aq. phenol

- D. 50% Aq. acetone
 E. *n*-Butanol-methanol-water (4:1:2)
 F. Benzene-methanol (3:1)
 G. Distilled water

Detection

Ninhydrin

 R_F

Solvent	R_F	Solvent	R_F
A	0.02	E	0.11
B	0.98	F	0.01
C	0.50	G	0.93
D	0.92		

Ref.

T. Niida, S. Inoue, T. Tsuruoka, T. Shomura, Y. Kondo, Y. Ogawa, H. Watanabe, Y. Sekizawa, T. Watanabe and H. Igarashi, *Netherlands Patent* 7,210,308, Jan. 30, 1973; *U.S. Patent* 3,832,394, Aug. 27, 1974

TLC**Media**

- A. Silica gel
 B. Cellulose

Solvents

- A. Ethanol-ammonia-water (8:1:1)
 B. Ethanol-water (4:1)
 C. *n*-Propanol-pyridine-acetic acid-water (5:10:3:10)
 D. *n*-Butanol-acetic acid-water (2:1:1)
 E. *n*-Butanol-methanol-water (4:1:2)

Detection

Ninhydrin

 R_F

Solvent	Medium A	Medium B
A	0.65	0.43
B	0.25	0.66
C	0.45	0.40
D	0.42	0.65
E	0.21	0.41

Ref.

As PC

ELPHO**Buffers**

- A. pH 1.9
 B. pH 9.5

Conditions

- A. 3500 V
 B. 250 V

Mobility

Buffer	Condi- tions	Mobility
A	A	4 cm toward cathode (20 min)
B	B	5.5 cm toward anode (2 h)

Ref.

As PC

SF-1306**TLC 1****Media**

- A. Silica gel
 B. Cellulose

Solvents

- A. *n*-Butanol-acetic acid-water (2:1:1)
 B. *n*-Butanol-acetic acid-water (4:1:2)
 C. *n*-Butanol-pyridine-acetic acid-water (6:4:1:2)

 R_F

Solvent	Medium A	Medium B
A	0.36	0.22
B	0.28	0.20
C	0.34	0.13

Ref.

Derwent Farmdoc 00944V-BD

TLC 2**Media**

- A. Silica gel
 B. Cellulose

Solvent*n*-Butanol-acetic acid-water (4:1:1) R_F

Component	Medium A	Medium B
SF-1306 I	0.16	0.22
SF-1306 II	0.20	0.28

Ref.

Derwent Abstract 08179V/05 = J7 9012-55

SF-1623**TLC****Media**

- A. Kieselgel F₂₅₄ (E. Merck)
 B. Aluminum sheet-cellulose

Solvents

- A. *n*-Butanol-acetic acid-water (2:1:1)
 B. *n*-Butanol-acetic acid-methanol-10% aq. ammonium acetate-water (8:2:2:1:4)

R_F

Solvent	Medium A	Medium B
A	0.17	0.33
B	0.50	

Ref.

S. Inouye, T. Shomura, M. Kojima, Y. Ogawa, H. Watanabe, Y. Kondo, K. Saito, Y. Yamada and T. Niida, *U.S. Patent* 4,010,155, March 1, 1977

SOB-7**TLC****Media**

- A. Silica gel G
 B. Cellulose

Solvents

- A. *n*-Propanol-pyridine-acetic acid-water (15:10:3:12)
 B. Ethanol-water (1:1)
 C. 20% Ammonium acetate-water (1:1)
 D. *n*-Butanol-acetic acid-water (2:1:1)
 E. *n*-Butanol-pyridine-acetic acid-water (15:10:3:12)
 F. Chloroform-methanol-14% ammonium hydroxide (2:1:1)

R_F

Solvent	Medium A	Medium B
A		0.29
B		0.20
C		0.78
D		0.17
E		0.08
F	0.12	

Ref.

Netherlands Patent 7,202,275, Aug. 28, 1972

SR 1768 A**TLC****Media**

- A. Silica gel (E. Merck)
 B. Silica gel plates treated with oxalic acid

Solvents

- A. Chloroform-ethyl acetate-methanol (2:4:1)
 B. Benzene-ethyl acetate-dimethylformamide (3:2:1)
 C. Ethyl acetate containing 1% oxalic acid

R_F

Component	Medium	Solvent		
		A	B	C
A	A	0.51	0.60	
	B			0.26
B	A	0.47	0.57	
	B			0.23
C	A	0.44	0.43	
	B			0.32
D	A	0.43	0.45	
	B			0.18
E	A	0.28	0.39	
	B			0.16
F	A	0.26	0.28	
	B			0.23
G	A	0.10	0.39	
	B			0.07
Chromomycin A ₃	A	0.28	0.39	
	B			0.16
Chromomycin A ₂	A	0.47	0.57	
	B			0.23

Ref.

T. Oki, Y. Matsuzawa, K. Numata and A. Takamatsu, *J. Antibiot.*, 26 (1973) 701-704

SS-228 Y**TLC****Solvents**

- A. Chloroform-methanol (9:1)
 B. Benzene-methanol (97:3)
 C. Benzene-ethyl acetate (4:1)

Detection

Color, iodine and potassium permanganate

R_F

Solvent A	0.87
Solvent B	0.32
Solvent C	0.21

Ref.

T. Okazaki, T. Kitahara and Y. Okami, *J. Antibiot.*, 28 (1975) 176–184

T-2636**TLC***Medium*

Kieselgel F₂₅₄ (Merck)

Solvents

- A. chloroform–methanol (93:7)
- B. Ethyl acetate–acetone (95:5)
- C. Methyl ethyl ketone–diethyl ether (1:3)
- D. Benzene–acetone (1:1)

Detection

- A. Conc. sulfuric acid
- B. Iodine vapor
- C. Bioautography vs. *Sarcina lutea* PCI 1001

R_F

Component	Solvent			
	A	B	C	D
A	0.87	0.77	0.85	0.82
B	0.85	0.67	0.78	0.81
C	0.51	0.51	0.57	0.69
D	0.41	0.47	0.53	0.56
E	0.33	0.35	0.35	0.49
F	0.22	0.27	0.25	0.40
M	0.00	0.00	0.00	0.00

Ref.

S. Harada, T. Kishi and K. Mizuno, *J. Antibiot.*, 24 (1971) 13–22
 T. Fugono, S. Harada, E. Higashide and T. Kishi, *J. Antibiot.*, 23–28

TA 2407**PC***Paper*

Toyo No. 131

Solvents

- A. Water satd. *n*-butanol
- B. 20% Aq. ammonium chloride
- C. 75% Phenol
- D. 50% Acetone
- E. Butanol–methanol–water (4:1:2)
- F. Butanol–methanol–water–methyl orange (40:10:20:1.5, v/v/v/w)
- G. Water
- H. Ethyl acetate–conc. ammonium hydroxide–water (3:1:1)

All solvents developed ascending

R_F

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	1.00	E	1.00
B	0.33	F	1.00
C	1.00	G	0.66
D	1.00	H	0.90

Ref.

Japanese Patent 956/1970; Jan. 15, 1970

TLC*Medium*

Silica gel GF₂₅₄

Solvents

- A. Benzene
- B. Chloroform
- C. Ethyl acetate
- D. Hexane–ethyl acetate (7:3)
- E. Benzene–ethyl acetate (4:1)
- F. Benzene–ethyl acetate (1:1)
- G. Diethyl ether–diisopropyl ether (1:1)
- H. Diisopropyl ether

R_F

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.00	E	0.28
B	0.04	F	0.63
C	0.90	G	0.57
D	0.28	H	0.33

Ref.

As PC

TL-119**TLC***Medium*

Silica gel GF

Solvent

Chloroform–methanol (50:1)

Detection

UV lamp

Ref.

J. Shoji, H. Hinoo, Y. Wakisaka, K. Koizumi and M. Mayama, *J. Antibiot.*, 28 (1975) 126–128

TS 885**TLC***Solvents*

- A. Chloroform–ethanol (10:3)
- B. Ethanol

- C. Ethyl acetate
D. Benzene-ethyl acetate (1:1)
E. Benzene

R_F

Solvent	R_F	Solvent	R_F
A	0.8	D	0.25
B	0.65	E	0.0
C	0.5		

Ref.

N. Ishida, T. Okada and A. Kamata, *U.S. Patent* 3,880,858, April 29, 1975

U-12898

PC

Solvents

- A. 1-Butanol-water (84:16), 16 h
B. As A + 0.25% *p*-toluenesulfonic acid, 16 h
C. 1-Butanol-acetic acid-water (2:1:1), 16 h
D. As A + 2% piperidine, 16 h
E. 1-Butanol-water (4:96), 5 h
F. As E + 0.25% *p*-toluenesulfonic acid, 5 h
G. As A + 2% *p*-toluenesulfonic acid, 64 h
H. Methanol-15% sodium chloride (4:1), paper impregnated with 0.1 *M* sodium sulfate, 5 h

Detection

Bioautography vs. *Bacillus subtilis*

R_F (estimated from drawing)

Solvent	R_F	Solvent	R_F
A	0.05	E	0.90
B	0.05	F	0.90
C	0.15	G	0.15
D	0.00	H	0.50

Ref.

D.J. Mason, A. Dietz and L.J. Hanka, *Antimicrob. Agents Chemother.*, 1962 (1963) 607-613

CCD

Solvent

1-Butanol-water

Distribution

Distribution coefficient of *p*-toluenesulfonic acid salt was 0.38

Ref.

M.E. Bergy, T.E. Eble, R.R. Herr, C.M. Large and B. Bannister, *Antimicrob. Agents Chemother.*, 1962 (1963) 614-618

U-13714

PC

Paper

Whatman No. 1

Solvents

As U-12898; A, B, C, E, F

Detection

Bioautography vs. vaccinia-chick-embryo kidney monolayer

R_F (estimated from drawing)

Solvent	R_F	Solvent	R_F
A	0.00	E	0.68
B	0.00	F	0.65
C	0.30		

Ref.

J.J. Vavra and A. Dietz, *Antimicrob. Agents Chemother.*, 1964 (1965) 75-79

CCD

Solvent

2-Butanol-water (1:1), 1000 transfers

Distribution

Peak found in tube 365; $K = 0.57$

Ref.

M.E. Bergy and R.R. Herr, *Antimicrob. Agents Chemother.*, 1964 (1965) 80-82

U-13933

PC

Solvent

As U-12898; A-F

Detection

Bioautography vs. KB cells

R_F (estimated from drawing)

Solvent	R_F	Solvent	R_F
A	0.80	D	0.90
B	0.85	E	0.87
C	0.90	F	0.90

Ref.

A.D. Argoudelis, J.H. Coats and R.R. Herr, *Antimicrob. Agents Chemother.*, 1965 (1966) 801-803

U-20661

PC

Solvents

A-F as U-12898, PC

G. 0.1 M Potassium phosphate buffer, pH 7.0; 5 h

H. 0.075 N Ammonium hydroxide satd. with methyl isobutyl ketone; 5 h

I. Benzene-methanol-water (1:1:2). Paper equilibrated with vapor phase at 25°C; developed with 5 h with upper phase

Detection

Bioautography vs. *Sarcina lutea*

R_F (estimated from drawing)

Solvent	R_F	Solvent	R_F
A	0.50–0.85	F	0.10–0.60
B	0.50–0.85	G	0.00–0.20
C	0.70–0.90	H	0.30–0.50
D	0.20–0.70	I	0.05–0.25
E	0.15–0.65		

Ref.

M.E. Bergy and F. Reusser, *Experientia*, 23 (1967) 254–255

U-21963

PC

Solvent

As U-12898, PC; A–F

Detection

Bioautography

R_F (estimated from drawing)

Solvent	R_F	Solvent	R_F
A	0.37–0.50	D	No zone
B	0.50–0.80	E	0.75–0.90
C	0.75–0.93	F	0.75–0.90

Ref.

T.R. Pyke and A. Dietz, *Appl. Microbiol.*, 14 (1966) 506–510

J.H. Coats, C.E. Meyer and T.R. Pyke, *U.S. Patent* 3,627,882, Dec. 14, 1971

U-22324

TLC

Medium

Silica gel G

Solvent

1-Butanol-acetic acid-water (2:1:1)

Detection

Bioautography vs. *Sarcina lutea*

Ref.

F. Reusser, *J. Biol. Chem.*, 242 (1967) 243–247

U-22956

PC

Paper

Whatman No. 1

Solvent

As U-12898, PC; A–F

Detection

Bioautography vs. *Salmonella gallinarum*

R_F (estimated from drawing)

Solvent	R_F	Solvent	R_F
A	0.20–0.43	D	No zone
B	0.45–0.60	E	0.85
C	0.83	F	0.85

Ref.

D.J. Mason, W.L. Lummis and A. Dietz, *Antimicrob. Agents Chemother.*, 1965 (1965) 110–113

U-25055

(See steffimycinone)

U-42126

PC

Solvents

A. 2% *p*-Toluenesulfonic acid in water satd. 1-butanol

B. 1-Butanol-glacial-acetic acid-water (2:1:1)

Detection

Bioautography vs. *Bacillus subtilis*

R_F

Solvent A 0.45

Solvent B 0.6

Ref.

L.J. Hanka and A. Dietz, *Antimicrob. Agents Chemother.*, 3 (1973) 425–431

U-43120

TLC

Medium

Silica gel

Solvents

A. Ethyl acetate-ethanol-water (92:5:3)

B. Ethanol-methanol-water (10:9:1)

C. Methyl ethyl ketone-acetone-water (70:20:11)

R_F

Solvent A 0.31

Solvent B 0.58

Solvent C 0.62

Ref.
As Proceomycin, TLC

U-43795

TLC

Medium

Silica gel

Solvent

Tetrahydrofuran–acetone–water (50:30:20)

Detection

Ninhydrin

R_F

0.55

Ref.

German Patent 2,514,984, October 30, 1975; and D.G. Martin and L.J. Hanka, *U.S. Patent* 3,944,562, March 16, 1976

U-44590

TLC

Medium

Silica gel

Solvent

Methanol–methylene chloride (1:9)

Detection

Potassium permanganate spray, and 50% aq. sulfuric acid followed by heating at 110°C for ca. 10 min

R_F

0.11

Ref.

C. DeBoer and B. Bannister, *U.S. Patent* 3,907,643, Sept. 23, 1975; *U.S. Patent* 3,907,779, Sept. 23, 1975

U-47929 (cf., ficellomycin)

TLC

Medium

Silica gel

Solvent

95% Ethanol–water (75:25)

Detection

Bioautography vs. *Staphylococcus aureus*

R_F

0.17

Ref.

A.D. Argoudelis and F. Reusser, *U.S. Patent* 3,969,515, July 13, 1976; A.D. Argoudelis, F. Reusser, H.A. Whaley, L. Baczynskyj, S.A. Mizesak and R.J. Wnuk, *J. Antibiot.*, 29 (1976) 1001–1006

U-48266 (cf., feldamycin)

TLC

Medium

Silica gel

Solvent

95% Ethanol–water (75:25)

Detection

Bioautography vs. *Sarcina lutea*

R_F

0.33

Ref.

A.D. Argoudelis and F. Reusser, *U.S. Patent* 3,969,515, July 13, 1976; A.D. Argoudelis, F. Reusser, S.A. Mizesak and L. Baczynskyj, *J. Antibiot.*, 29 (1976) 1007–1014

U-51640

TLC

Medium

Silica gel

Solvent

95% Aq. ethanol–water (75:25)

Detection

Bioautography vs. *Escherichia coli*

Ref.

A.D. Argoudelis and F. Reusser, *U.S. Patent* 3,998,698, Dec. 21, 1976

VD 844

TLC

Solvent

Ethyl acetate–water, pH 2

Detection

Bioautography vs. *Neisseria gonorrhoeae*

R_F

0.15–0.20

Ref.

W. von Daehne, W.O. Godtfredsen and L. Tyb-ring, *J. Antibiot.*, 22 (1969) 233–236

WS-3442

TLC

Solvents

A. *n*-Butanol–acetic acid–water (4:1:2)

B. Acetic acid–ethyl acetate (1:2)

C. Acetonitrile–water (5:2)

Detection

Ninhydrin

R_F

Sol-vent	Component		
	A	D	E
A	0.19		0.27–0.32 0.24–0.25
B	0.58	0.49, 0.67	
C		0.55	

Ref.

Netherlands Patent 73,08948, June 27, 1973

WS-4545**TLC****Medium**

Kieselgel (Merck)

Solvents

- A. *n*-Butanol–acetic acid–water (4:1:5), upper layer
 B. *n*-Butanol–pyridine–water (4:1:1)
 C. Chloroform–methanol (5:1)
 D. Ethyl acetate–methanol (1:1)
 E. Benzene–methanol–chloroform (1:1:1)

Detection

1% Potassium permanganate soln. containing 2% sodium carbonate was used as the coloring agent

 R_F

Solvent	R_F	Solvent	R_F
A	0.41	D	0.63
B	0.55	E	0.41
C	0.39		

Ref.

H. Imanaka, T. Kamiya, S. Maeno, T. Miyoshi, N. Miyairi, H. Aoki, M. Kohsaka, T. Komori and H. Sakai, *U.S. Patent* 3,923,790, Dec. 2, 1975

X-206**TLC****Medium**

Silica gel

Solvents

- A. Toluene–acetone (4:1) + 1% triethylamine
 B. Chloroform–ethyl acetate (1:1)
 C. Chloroform–methanol (95:5)

 R_F

Solvent A	0.25
Solvent B	0.44
Solvent C	0.49

Ref.

T. Fehr, H.D. King and M. Kuhn, *J. Antibiot.*, 30 (1977) 903–907

X5108**TLC 1****Medium**

ITLC-SA (Gelman thin-layer sheet)

Solvents

- A. Chloroform–methanol (9:1)
 B. Chloroform–methanol–water (1:1:1)

DetectionBioautography vs. *Escherichia coli* ATCC 10536 R_F

Solvent A	0.6
Solvent B	0.45

Ref.

As Azdimycin, TLC

TLC 2**Media**

- A. Silica gel F₂₅₄
 B. Kieselguhr

Solvent

Chloroform–methanol–aq. ammonium hydroxide

Detection

- A. Bioautography
 B. UV

 R_F

Medium	Detection	R_F
A	B	0.19
B	A	0.29

Ref.

J. Berger, *U.S. Patent* Re. 28,700, Jan. 27, 1976; reissue of *U.S. Patent* 3,657,421, April 19, 1972

CCD**Solvent**Ethyl acetate–isopropanol–aq. 0.1 *M* sec.-sodium phosphate (12:9:20)**Distribution coefficient**200 Transfers; peak tube: 159; *K* = 3.88

Ref.

As TLC 2

XB-94-F2**TLC****Medium**

Silica gel

Solvents

- A. Benzene-ethanol (7:3)
 B. Chloroform-methanol (9:1)

R_F

Solvent A	0.51
Solvent B	0.18

Ref.

Derwent No. 73121U-BD, Aug. 29, 1973

XK-19-2**PC****Solvents**

- A. 20% Aq. ammonium chloride
 B. Water satd. *n*-butanol
 C. *n*-Butanol-acetic acid-water (3:1:1)
 D. Water satd. ethyl acetate
 E. Water satd. *n*-butanol containing 2% *p*-toluenesulfonic acid and 2% piperidine

R_F

Solvent	R _F	Solvent	R _F
A	0.97	D	0.00
B	0.00	E	0.00
C	0.01		

Ref.

S. Takasawa, M. Yamamoto, S. Sato, I. Kawamoto, R. Okachi, M. Kumakawa and T. Nara, *J. Antibiot.*, 26 (1973) 471-474

TLC**Medium**

Silica gel

Solvents

- A. Methanol-10% aq. ammonium acetate-10% ammonia water (10:9:1)
 B. *n*-Butanol-pyridine-acetic acid-water (3:2:1:2)

Detection

Bioautography vs. *Bacillus subtilis*

R_F

Solvent A	0.34
Solvent B	0.15

Ref.

As PC

XK-33-F₂**TLC****Medium**

Silica gel

Solvents

- A. Chloroform-methanol-17% aq. ammonia (2:1:1), upper layer
 B. 10% Ammonium acetate-methanol (1:1)
 C. 10% Ammonium acetate-acetone-10% aq. ammonia (9:10:0.5)

R_F

Solvent A	0.50
Solvent B	0.67
Solvent C	0.71

Ref.

Netherlands Patent Appl. No. 7,118,132, July 4, 1972

XK-41-B₂**PC****Paper**

Ascending

Solvents

- A. 20% Ammonium chloride
 B. Water satd. *n*-butanol
 C. *n*-Butanol-acetic acid-water (3:1:1)
 D. Water satd. ethyl acetate
 E. Water satd. *n*-butanol + 2% *p*-toluenesulfonic acid + 2% piperidine
 F. 5% Ammonium chloride
 G. Water satd. methyl isobutyl ketone
 H. Water satd. methyl isobutyl ketone + 1% *p*-toluenesulfonic acid
 I. Acetone-water (1:1)
 J. Methanol-*n*-butanol-water (1:4:2) + 21% methyl orange

R_F

Solvent	R _F	Development (h)
A	0.60	3
B	0.85	15
C	0.73	15
D	0.00	
E	0.87	15
F	0.70	3
G	0.75	3
H	0.87	3
I	0.80-0.90	3
J	0.89	15

Ref.

R.S. Egan, S.L. Mueller, L.A. Mitscher, I. Kawamoto, R. Okachi, H. Kato, S. Yamamoto, S. Takasawa and T. Nara, *J. Antibiot.*, 27 (1974) 544-551

XK-41 COMPLEX**PC 1***Paper*

Toyo No. 51

Solvent

Water satd. ethyl acetate (ascending, 3 h)

*Detection*Bioautography vs. *Bacillus subtilis* No. 10707*R_F*

0.00

Ref.

As Carbomycin, PC 1

PC 2*Paper*

Toyo No. 51

Solvents

A. 20% Ammonium chloride

B. Water satd. *n*-butanolC. *n*-Butanol-acetic acid-water (3:1:1)

D. Water satd. ethyl acetate

E. Water satd. *n*-butanol + 2% *p*-toluenesulfonic acid + 2% piperidine*Detection*Bioautography vs. *Bacillus subtilis* No. 10707*R_F*

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.60	D	0.00
B	0.84	E	0.91
C	0.75		

Ref.

As Carbomycin, PC 1

TLC 1*Medium*

Alumina Type E (Merck)

Solvent

Ethyl acetate-methanol (9:1), 3 h

*Detection*Bioautography vs. *Bacillus subtilis* No. 10707*R_F* $A_1 > A_2 > B_2 > B_1 > C$ *Ref.*

As Carbomycin, PC 1

TLC 2*Medium*

Alumina, Type E (Merck)

Solvent

Ethyl acetate-methanol (9:1), 3 h

R_F

Component	<i>R_F</i>
A ₁	0.85
A ₂	0.80
B ₁	0.45
B ₂	0.55
C	0.20

Ref.

R.S. Egan, S.L. Mueller, L.A. Mitscher, I. Kawamoto, R. Okachi, H. Kato, S. Yamamoto, S. Takasawa and T. Nara, *J. Antibiot.*, 27 (1974) 544-551

CCD*Solvent*

$\frac{1}{15}$ M Phosphate buffer soln. (the stationary phase, lower layer), and ethyl acetate (the mobile phase, upper layer); 500 transfers

Distribution

Tubes	Fraction
5-30	XK-41-C
40-115	XK-41-B ₁
119-142	XK-41-B ₂
149-249	XK-41-A ₂
258-420	XK-41-A ₁

Ref.

As Carbomycin, PC 1

XK-46**PC***Solvents*

A. 20% Aq. ammonium chloride

B. Water satd. *n*-butanolC. *n*-Butanol-acetic acid-water (3:1:1)

D. Water satd. ethyl acetate

E. Water satd. *n*-butanol + 2% piperidine and 2% *p*-toluenesulfonic acid*R_F*

Solvent	<i>R_F</i>	Solvent	<i>R_F</i>
A	0.38	D	0.29
B	0.04	E	0.00
C	0.75		

Ref.

S. Takasawa, I. Kawamoto, R. Okachi, Y. Machida and T. Nara, *J. Antibiot.*, 27 (1974) 502-506

TLC*Media*

- A. Silica gel
B. Cellulose (Eastman Kodak)

Solvents

- A. Benzene-ethyl acetate-methanol-acetic acid (10:5:5:1)
B. Benzene-ethyl acetate-acetic acid (5:5:1)
C. Benzene-chloroform-ethyl acetate-acetic acid (10:5:5:1)
D. Diethyl ether-chloroform-ethyl acetate-acetic acid (10:5:5:1)
E. Water satd. *n*-butanol

 R_F

Solvent	Medium A	Medium B
A	0.70	
B	0.77	
C	0.68	
D	0.48	
E		0.57

Ref.

As PC

XK-49-1-B₂**PC***Paper*

Ascending

Solvents

- A. 20% Ammonium chloride (develop 3 h)
B. 10% Ammonium chloride (develop 3 h)
C. 1% Ammonium chloride (develop 3 h)
D. 0.5% Ammonium chloride (develop 3 h)
E. Water satd. *n*-butanol (develop 15 h)
F. *n*-Butanol-acetic acid-water (3:1:1) (develop 15 h)
G. Water satd. ethyl acetate (develop 4 h)
H. Water satd. *n*-butanol + 2% *p*-toluenesulfonic acid + 2% piperidine (develop 15 h)

 R_F

Solvent	R_F	Solvent	R_F
A	0.75	E	0.00
B	0.72	F	0.08
C	0.40	G	0.00
D	0.15	H	0.02

Ref.

As Bleomycins, TLC 8

TLC*Medium*

Silica gel

Solvents

- A. Chloroform-methanol-17% aq. ammonia (2:1:1) upper layer
B. 10% Ammonium acetate-methanol (1:1)
C. Methanol-10% ammonium acetate-10% aq. ammonia (10:9:1)
D. 0.05 *M* Citric acid buffer soln., pH 6.5

 R_F

Solvent A	0.20
Solvent B	0.75
Solvent C	0.35
Solvent D	0.40

Ref.

As Bleomycins, TLC 8

XK-62-2

(cf., sagamicin)

PC*Paper*

Whatman No. 1

Solvent

Chloroform-methanol-17% ammonium (2:1:1), lower phase

*Detection*Bioautography vs. *Bacillus subtilis* R_F

Component	R_F
XK-62-2	0.49
XK-62-1	0.19

Ref.

R. Okachi, T. Kawamoto, S. Takasawa, M. Yamamoto, S. Sato, T. Sato and T. Nara, *paper presented 194th Sci. Meeting, JARA, July 26, 1974*

TLC*Medium*

Silica gel

Solvents

- A. Chloroform-methanol-17% ammonium hydroxide (2:1:1), upper phase
B. 10% Ammonium acetate-methanol (1:1)

*Detection*Bioautography vs. *Bacillus subtilis*

R_F

Component	Solvent A	Solvent B
XK-62-1	0.85	0.25
XK-62-2	0.86	0.27

Ref.

As PC

XK-88

PC

Paper

Ascending

Solvents

- A. 20% Aq. soln. of ammonium chloride
- B. Water satd. *n*-butanol
- C. *n*-Butanol–acetic acid–water (3:1:1)
- D. Water satd. *n*-butanol + 2% (w/v) *p*-toluene-sulfonic acid + 2% (v/v) piperidine
- E. Chloroform–methanol–17% aq. ammonia (2:1:1), lower layer

 R_F

Solvent	Component			
	1	2	3	5
A	0.97	0.98	0.97	0.75
B	0.00	0.00	0.00	0.00
C	0.10	0.08	0.00	0.05
D	0.00	0.00	0.00	0.00
E	0.00	0.00	0.00	0.01

Ref.

As Apramycin, PC 1

TLC

Medium

Silica gel

 R_F

Solvent	Component			
	1	2	3	5
A	0.80	0.73	0.75	0.81
B	0.58	0.45	0.39	0.20
C	0.31	0.38	0.27	0.50

Solvents

- A. Chloroform–methanol–17% aq. ammonia (2:1:1), upper layer

B. Methanol–10% aq. ammonium acetate (1:1)

C. *n*-Butanol–ethanol–chloroform–17% aq. ammonia (4:5:2:5)

Ref.

As Apramycin, PC 1

XK-90

PC

Solvents

- A. 20% Aq. ammonium chloride
- B. Water satd. *n*-butanol
- C. *n*-Butanol–acetic acid–water (3:1:1)
- D. Water satd. ethyl acetate

 R_F

Solvent A	0.80
Solvent B	0.71
Solvent C	0.37
Solvent D	0.84

Ref.

S. Takasawa, M. Yamamoto, R. Okachi, I. Kawamoto, S. Sato and T. Nara, *J. Antibiot.*, 29 (1976) 1015–1018

TLC

Medium

Silica gel

Solvents

- A. Chloroform
- B. Chloroform–acetone (6:1)
- C. Ethyl acetate
- D. Ethyl acetate–acetone (6:1)
- E. Benzene–methanol (6:1)

 R_F

Solvent	R_F	Solvent	R_F
A	0.0	D	0.42
B	0.13	E	0.38
C	0.20		

Ref.

As PC

YA-56 COMPLEX

(cf., bleomycins, TLC 5 and 6)

PC 1

Paper

Toyo No. 51A, ascending

Solvents

- A. Water satd. butanol
- B. Acetone–water (1:1)
- C. Phenol–water (1:1)

D. *n*-Butanol-methanol-water (4:1:2)

E. *n*-Butanol-methanol-water-methyl orange (40 ml:10 ml:20 ml:1.5 g)

F. *n*-Butanol-pyridine-acetic acid-water (15:10:3:12)

G. *n*-Butanol-acetic acid-water (4:1:5)

Detection

Bioautography vs. *Bacillus subtilis* PCI 219

R_F

Solvent	YA-55 X Hydrochloride (Cu-complex)	YA-56 X Hydrochloride
A	0.00	0.00
B	0.05	0.05
C	0.93	0.93
D	0.10	0.10
E	0.47	0.47
F	0.30	0.30
G	0.23	0.23

Solvent	YA-56 Y Hydrochloride (Cu-complex)	YA-56 Y Hydrochloride
A	0.00	0.00
B	0.05	0.05
C	0.93	0.93
D	0.10	0.10
E	0.47	0.47
F	0.45	0.45
G	0.31	0.31

Ref.

Y. Ito, Y. Ohashi, S. Kawabe, M. Sakurazawa, T. Ogawa, Y. Egawa and T. Okuda, *J. Antibiot.*, 26 (1973) 77-83

PC 2

Paper

Toyo No. 51A, descending

Solvent

n-Butanol-pyridine-acetic acid-water (15:10:3:12)

Detection

Bioautography vs. *Bacillus subtilis* PCI 219; *Klebsiella pneumoniae* PCI 602

R_F (estimated from drawing)

Component	R_F
YA-56	0.45, 0.75
YA-56 X	0.50
YA-56 Y	0.75
Phleomycin complex	0.30, 0.50, 0.70
Phleomycin D ₁	0.65
Phleomycin D ₂	0.70
Bleomycin complex	0.25, 0.35, 0.70
Zorbamycin	0.45
Zorbamycin B	0.50

Ref.

As PC 1

TLC

Medium

Cellulose (E. Merck)

Solvent

0.1 M Ammonium chloride, pH 7.5

Detection

Bioautography vs. *Bacillus subtilis* PCI 219; *Klebsiella pneumoniae* PCI 602

R_F (estimated from drawing)

Component	R_F
YA-56	0.50, 0.65 (Trace) 0.75
YA-56 X	0.75
YA-56 Y	0.50
Phleomycin complex	0.30, 0.45, 0.65
Phleomycin D ₁	0.65
Phleomycin D ₂	0.45
Bleomycin complex	0.50, 0.70
Zorbamycin	0.75
Zorbamycin B	0.65

Ref.

As PC 1

YC 73

PC

Paper

Toyo Roshi No. 51A

Solvents

- Wet *n*-butanol
 - 20% Aq. ammonium chloride
 - 50% Aq. acetone
 - n*-Butanol-methanol-water (4:1:2)
 - Benzene-methanol (4:1)
 - Water
- All solvents developed ascending

DetectionBioautography vs. *Staphylococcus aureus* **R_F**

Solvent	R_F	Solvent	R_F
A	0.73	D	0.69
B	0.65	E	0.82
C	0.85	F	0.73

Ref.Y. Egawa, K. Umino, S. Awataguchi, Y. Kawano and T. Okuda, *J. Antibiot.*, 23 (1970) 267-270**TLC****Medium**Kieselgel GF₂₅₄**Solvents**

- A. Chloroform
B. Ethyl acetate

Detection

- A. Visible color
B. Bioautography

 R_F

Solvent A	0.19
Solvent B	0.57

Ref.

As PC

ELPHO**Medium**

Paper

Buffer $\frac{1}{15}$ M Phosphate, pH 5.0 and pH 8.0**Conditions**

10 V/cm, 2.5 h

Mobility

YC 73 moves slightly to the cathode with both buffers

Ref.

As PC

Y-G-19Z D3**TLC****Medium**

Cellulose (Avicel SF)

Solvents

- A. Acetonitrile-water (5:2)
B. *n*-Butanol-acetic acid-water (6:1.5:2.5)
C. *n*-Butanol-acetic acid-water (4:1:2)

Detection

0.25% Ninhydrin soln. in pyridine followed by heating

 R_F

Solvent A	0.35
Solvent B	0.15
Solvent C	0.19

Ref.T. Osono, S. Watanabe, T. Saito, H. Gushima, K. Murakami, I. Takahashi, H. Yamaguchi, T. Sasaki and Y. Oka, *U.S. Patent* 4,039,660, Aug. 2, 1977**ELPHO****Medium**

Whatman No. 1 paper

Buffer

10% Acetic acid, pH 2.2

Conditions

42 V/cm, 1 h

Mobility

-5.2 cm

Ref.

As TLC

YL-704**TLC****Media**

- A. Alumina
B. Silica gel

Solvents

- A. Benzene-acetone (4:1)
B. Benzene-acetone (7:3)
C. Benzene-acetone (2:1)

Detection

Antibiotics are visualized as dark violet or purple-colored spots by spraying with 40% sulfuric acid, followed by heating

 R_F

Component	Medium	Solvent		
		A	B	C
W ₂	A	0.56	0.92	
	B			0.72
W ₁	A	0.37	0.88	
	B			0.68
A ₀	A	0.22	0.75	
	B			0.51
A ₁	A	0.20	0.72	
	B			0.50
A ₂	A		0.60	
	B			0.48
A ₃	A		0.53	
	B			0.43

Component	Medium	Solvent		
		A	B	C
B ₁	A		0.58	
	B			0.36
B ₂	A		0.45	
	B			0.34
B ₃	A		0.38	
	B			0.33
C ₁	A		0.30	
	B			0.29
C ₂	A		0.43	
	B			0.30
C ₃	A		0.41	
	B			0.39
C ₄	A		0.29	
	B			0.35

Ref.

A. Kinumaki, I. Takamori, Y. Sugawara, N. Nagahama, M. Suzuki, Y. Egawa, M. Sakurazawa and T. Okuda, *J. Antibiot.*, 27 (1974) 102–106

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