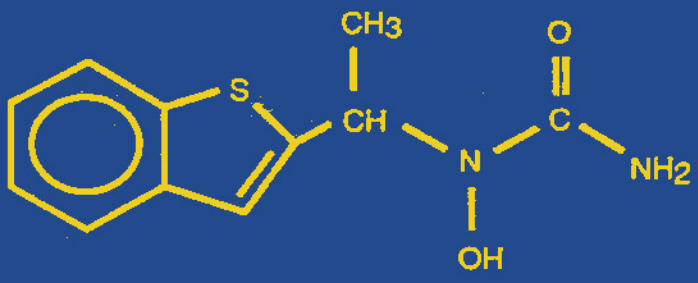
Analytical Profiles of Drug Substances and Excipients

VOLUME 25



Edited by
Harry G. Brittain

Analytical Profiles

of

Drug Substances

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Excipients

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Analytical Profiles of Drug Substances and Excipients

Volume 25

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Harry G. Brittain

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PREFACE

The comprehensive profiles of drug substances and pharmaceutical excipients as to their physical and analytical characteristics are an essential feature of drug development. The compilation of concise summaries of physical and chemical data, analytical methods, routes of compound preparation, degradation pathways, and the like, is a vital function to both academia and industry. It goes without saying that workers in the field require access to current state-of-the-art data, and the *Analytical Profiles* series has always provided information of the highest quality. For this reason, profiles of older compounds are updated whenever a sufficient body of new information becomes available.

This present volume is the 25th member of a long and distinguished series. The first twenty volumes were edited by Klaus Florey as a response to a definite need in the field, and the rationale for producing new volumes is just as strong today as it was for the early members of the series. The content of each analytical profile has expanded in sophistication as the tools available to investigators improves accordingly, and the range of compounds suitable for profiling has increased as well. It is almost ironic to note that the need for analytical profiles has grown to be overwhelmingly important in the current climate of drug development, and yet potential authors seem to have even less time than ever before to develop projects for the series. However, the contributors to the present volume have indeed found the time to write their chapters, and I would like to take this opportunity to salute them for their dedication to this work.

As always, a complete list of available drug and excipient candidates is available from the editor. I continue to explore new and innovative ways to encourage potential authors, and welcome suggestions as to how to get people involved in the writing of analytical profiles. Communication from new and established authors is always welcome, and Email contact (address: greatbrittain@worldnet.att.net) is encouraged. I will continue to look forward to working with the pharmaceutical community on the *Analytical Profiles of Drug Substances and Excipients*, and to providing these information summaries which are of such great importance to the field.

Harry G. Brittain

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ADENOSINE

George S. Mahler

Pharmaceutical Development

Medco Research, Inc.

Research Triangle Park, NC 27709

ANALYTICAL PROFILES OF DRUG SUBSTANCES AND EXCIPIENTS—VOLUME 25
1075-6280/98 $25.00

Contents

1. Description

1.1 Nomenclature

1.1.1 Chemical Name

9-β-D-Ribofuranosyl-9H-purin-6-amine

6-Amino-9-β-D-ribofuranosyl-9H-purine

9-β-D-ribofuranosido adenine

1-(6-amino-9H-purin9-yl)-1-deoxy-β-D-ribofuranose

Adenine 9-β-D-Ribofuranoside

1.1.2 Nonproprietary Names

Adenosine

adenine riboside

1.1.3 Proprietary Names

Adenocard (Fujisawa

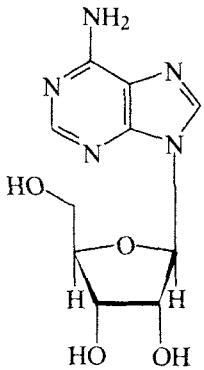
Adenoscan (Medco)

1.2 Formulae

1.2.1 Empirical

$C_{10}H_{13}N_5O_4$

1.2.2 Structural



1.3 Molecular Weight

267.24 Daltons

1.4 CAS Number

[58-61-7]

1.5 Appearance

Adenosine is a white, crystalline powder.

1.6 Uses and Applications

Adenosine is an endogenous nucleoside occurring in all cells of the body, and is currently approved by the U.S. Food and Drug Administration for two uses. The drug is approved for use as an agent to slow conduction time through the atrioventricular (A-V) node, to interrupt the reentry pathways through the A-V node, and to restore normal sinus rhythm in patients with paroxysmal supraventricular tachycardia (PSVT) including PSVT associated with Wolff-Parkinson-White Syndrome. Adenosine is also indicated as an adjunct to thallium-201 myocardial perfusion scintigraphy in patients unable to exercise adequately.

2. Methods of Preparation

Adenosine can be prepared by culturing a mutant of *Bacillus subtilis* in a neutral medium containing glucose, monobasic potassium phosphate, magnesium sulfate, ammonium chloride, guanine, potassium, calcium chloride, and soybean protein hydrolyzate as well as trace amounts of ferrous and manganese sulfate [1].

Adenosine may also be produced by fermentation using *Sarcina ureae*, *Sarcina hansenii*. and their mutants in a broth containing soya hydrolyzate, calcium carbonate, adenine, and glucose [2].

3. Physical Properties

3.1 Particle Morphology

Adenosine can exhibit more than one crystal morphology. Photomicrographs were obtained at a magnification of 200X on a Leica Diastar optical microscopy system, with the samples being suspended in mineral oil for the analysis. Material obtained from Aldrich is shown in Figure 1, where one observes component crystals characterized by an equant rhomb morphology. The particle size of these crystals averaged approximately 25-50 μm in length and approximately 15-30 μm in width. Many aggregate species could be detected which were evidently formed as multiple laminates of thick crystal slabs.

On the other hand, material obtained from Pharma Waldhof was found to exhibit a needle-like morphology. As evident in Figure 2, intact crystals within the examined sample ranged 25-75 μm in length, and 25-75 μm in width. The sample also contained numerous fragment crystals, of a wide variety of particle sizes.

3.2 Crystallographic Properties

3.2.1 Single Crystal Structure

The full single crystal structure of adenosine has been reported [3]. The material crystallizes in the monoclinic space group $P2_1$, with $a = 4.825(1)$, $b = 10.282(2)$, $c = 11.823(1)$, $\beta = 99.30\ (1)^\circ$

An illustration of the bond distances and angles for the heavy atoms obtained as a result of the crystallographic study is given in Figure 3, and Figure 4 illustrates the pattern of hydrogen bonding which links up the adenosine molecules to form the overall crystal lattice. Finally, a stereoscopic view of the molecule is provided in Figure 5.

3.2.2 Polymorphism

Although it is possible to obtain adenosine in more than one crystal morphology, there is presently no definitive information which conclusively

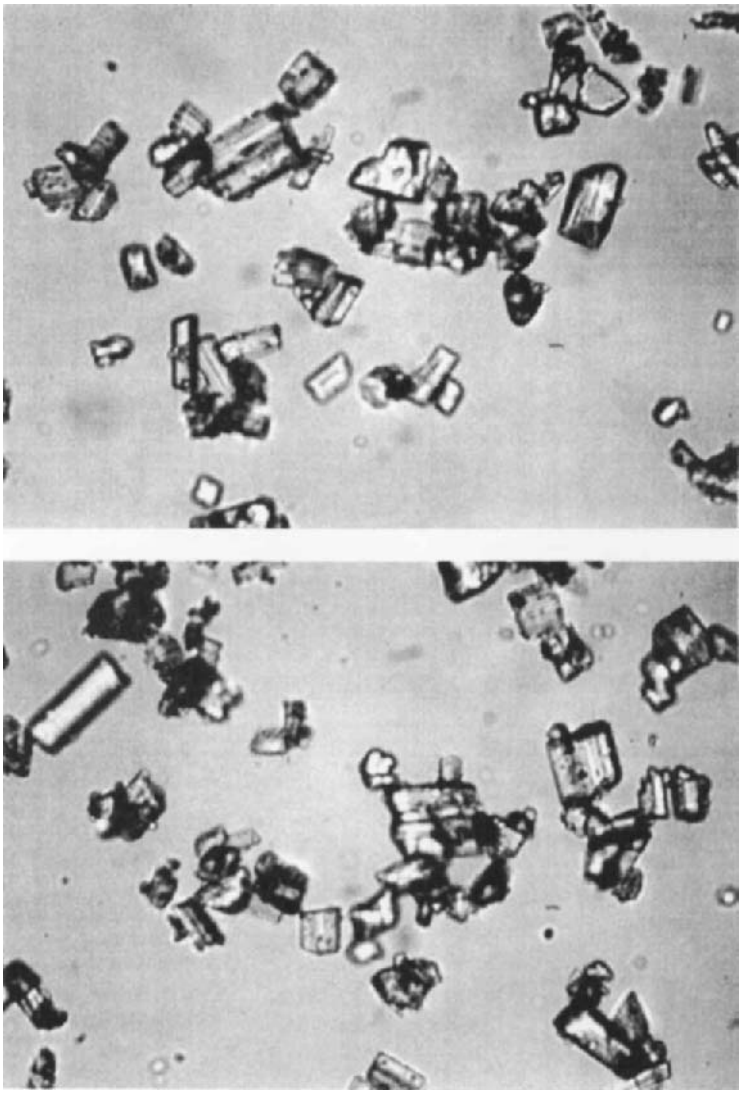


Figure 1. Photomicrographs of adenosine sourced from Aldrich, and obtained at a magnification of 200X.

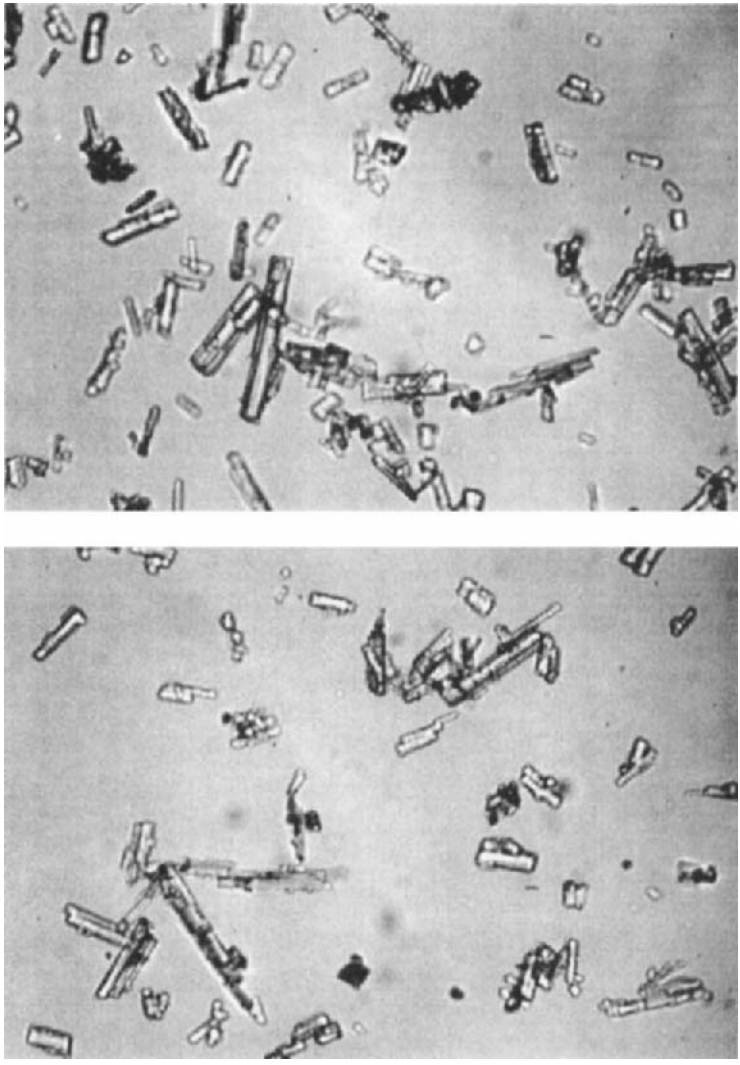


Figure 2. Photomicrographs of adenosine sourced from Pharma Waldhof, and obtained at a magnification of 200X.

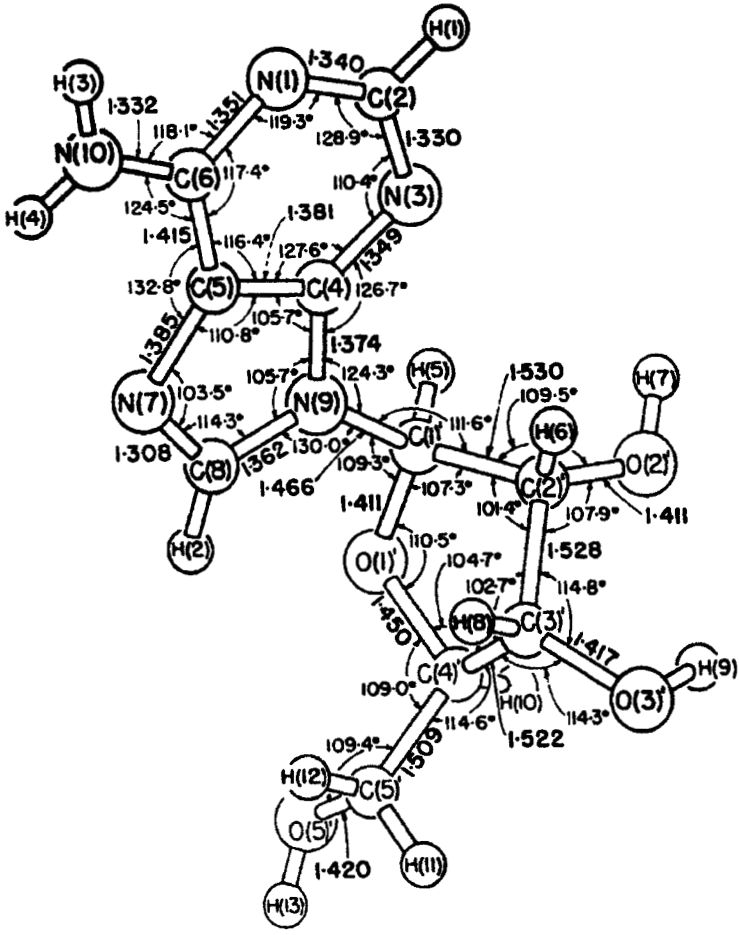


Figure 3. Bond distances and angles involving the heavy atoms of adenosine, obtained from reference [3] and used with permission from the International Union of Crystallography.

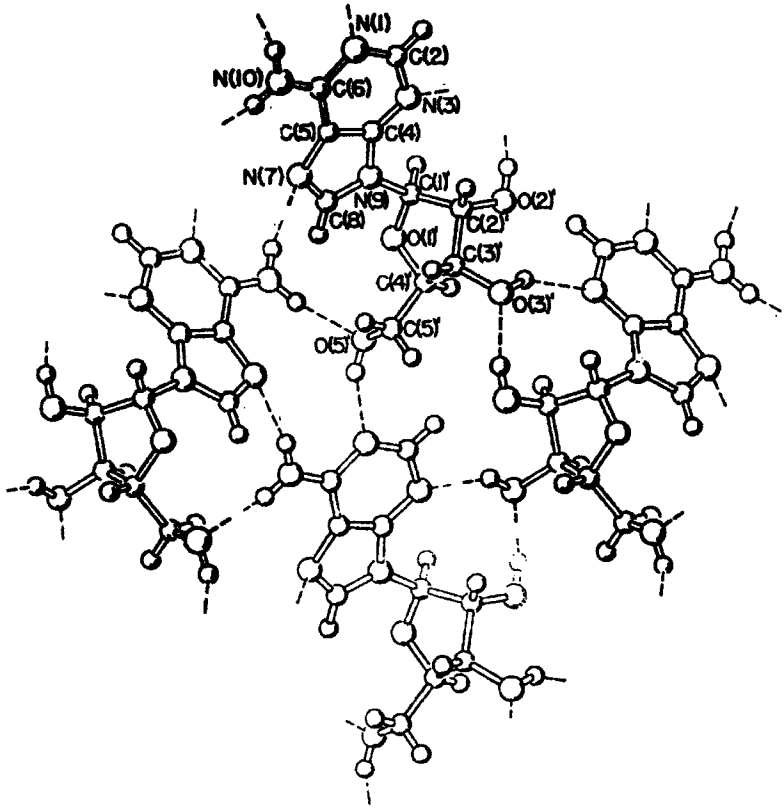


Figure 4. Crystal lattice of adenosine, obtained from reference [3] and used with permission from the International Union of Crystallography.

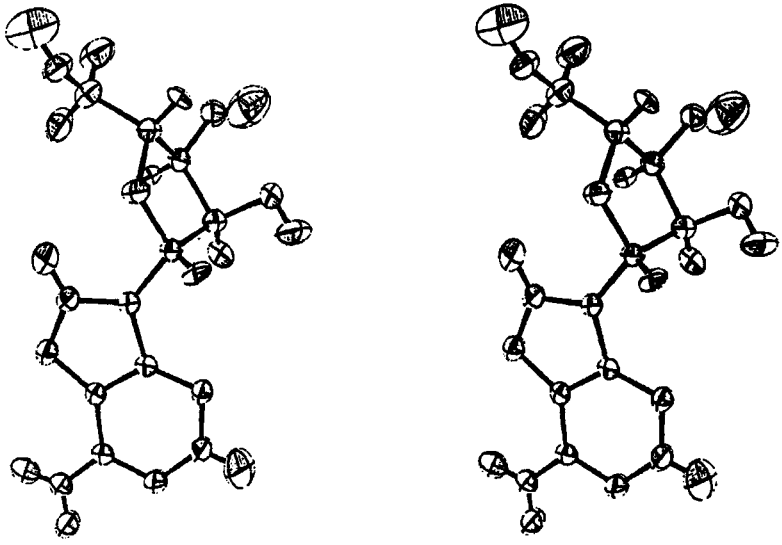


Figure 5. Crystal lattice of adenosine, obtained from reference [3] and used with permission from the International Union of Crystallography.

demonstrates the existence of crystal polymorphs. The compound may, however, be obtained in either the crystalline or amorphous form.

3.2.3 X-Ray Powder Diffraction Pattern

Both samples of adenosine were found to exhibit strong and characteristic x-ray powder diffraction patterns. The XRPD work was performed using a Philips APD 3720 powder diffraction system, equipped with a vertical goniometer in the $\theta/2\text{-}\theta$ geometry. The K-α line of copper at 1.54439 Å was used as the radiation source. Each sample was scanned between 2 and 46 degrees 2-θ, in step sizes of 0.02 degrees (integrated for 0.5 sec at each step).

The adenosine materials obtained from Aldrich and Pharma Waldhof were found to yield identical powder patterns, in spite of the markedly different crystal morphologies. The powder pattern obtained for the Aldrich-sourced material is illustrated in Figure 6, and a summary of scattering angles, d-spacings, and relative intensities is provided in Table 1. The identity of the XRPD data provides further support for the lack of polymorphism in this system.

3.3 Optical Activity

3.3.1 Optical Rotation

The specific rotation (at 589 nm) of adenosine has been determined in aqueous solutions. At a temperature of 11°C (c = 0.706 in water) the specific rotation has been reported as -61.7°, while at 9°C (c = 0.658 in water) the specific rotation has been reported as -58.2°. The optical rotation does not change appreciably if the solution pH is raised, and has been determined to be -69.5° (c = 0.199 in 5% NaOH) at a temperature of 23°C.

3.3.2 Circular Dichroism

Both the optical rotary dispersion [6] and circular dichroism [4] spectra of adenosine have been reported. Most useful are the CD spectra, which were obtained in a variety of solvents. The molar ellipticity of the

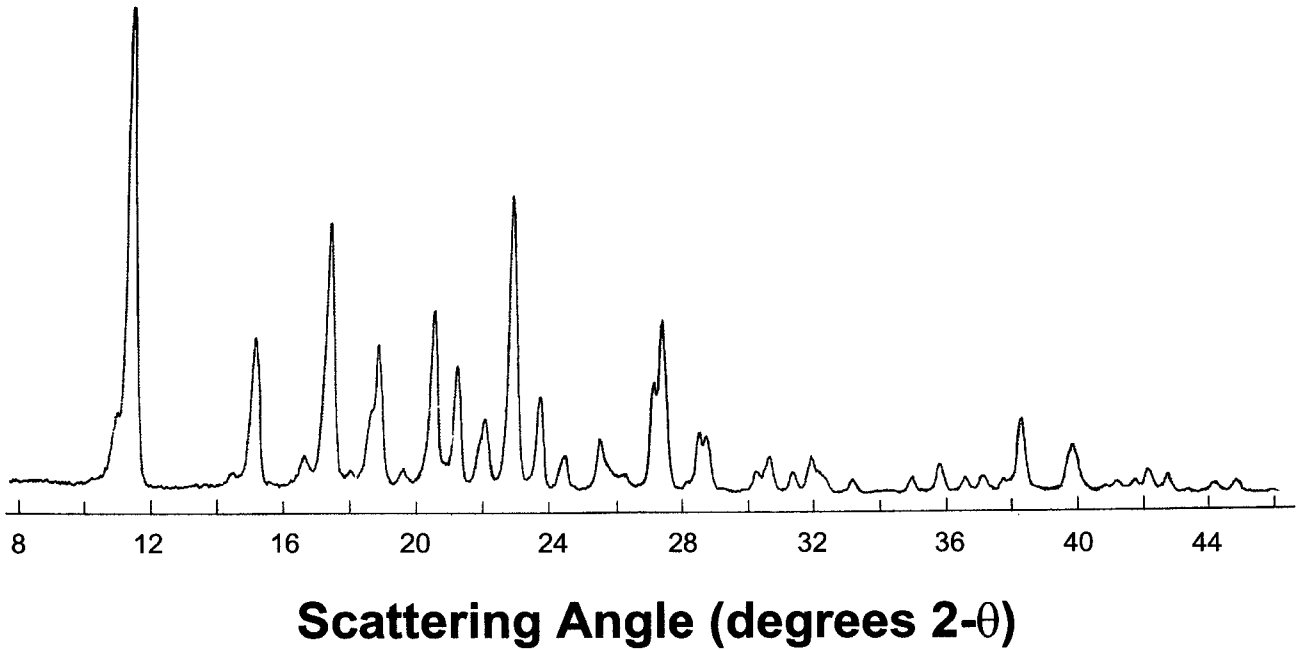


Figure 6. X-ray powder diffraction pattern of adenosine.

Table 1

Data from the X-Ray Powder Diffraction Pattern of Adenosine

Scattering Angle (degrees 2-θ)	**d-spacing (Å)**	**Relative Intensity, I/I_0 (%)**
11.4585	7.7336	100.00
14.4454	6.1405	4.26
15.1921	5.8404	31.38
16.6463	5.3333	7.71
17.4323	5.0946	54.79
18.0218	4.9292	4.79
18.8865	4.7055	29.79
19.5939	4.5372	5.05
20.5371	4.3309	36.70
21.2445	4.1882	25.53
22.0306	4.0405	14.89
22.9345	3.8833	60.11
23.7205	3.7564	19.15
24.4279	3.6492	7.45
25.4498	3.5049	10.37
26.2358	3.4017	3.46
27.1397	3.2904	21.54
27.4148	3.258	34.04
28.5546	3.1305	11.70
28.7511	3.1095	10.90
30.2445	2.9593	3.72

Table 1 (continued)

Data from the X-Ray Powder Diffraction Pattern of Adenosine

Scattering Angle (degrees 2-θ)	**d-spacing (Å)**	**Relative Intensity, I/I_0 (%)**
30.6376	2.9223	6.92
33.179	2.704	2.39
34.9869	2.5683	2.93
35.8122	2.511	5.32
36.559	2.4614	2.66
37.1092	2.4262	3.19
37.738	2.3872	2.39
38.3275	2.3518	14.10
39.8603	2.2648	9.04
40.9607	2.2065	1.06
41.1965	2.1944	1.60
41.7467	2.1668	2.13
42.1397	2.1475	3.99
42.7686	2.1173	3.19
44.1834	2.0528	1.06
44.8515	2.0237	1.60

aromatic band at 262 nm has been reported to be -3000, while the molar ellipticity of one of the better-defined aliphatic bands (198 nm) was determined to be -8800 [5].

The circular dichroism and absorption spectra of adenine and some of its derivatives are collected in Figure 7. Shown are the CD spectra of adenosine-5'-phosphate, adenosine, deoxyadenosine, and deoxyadenosine-5'-phosphate. The results indicate that the chirality experienced by the adenine moiety, and evidenced by the CD spectra of the aromatic group, is not strongly affected by modification on the periphery. However, the various substitutions produce strong effects on the appearance of the aliphatic CD bands.

3.4 Thermal Methods of analysis

3.4.1 Melting Behavior

The melting point of adenosine is 234 - 235°C, and the substance appears to melt without decomposition.

3.4.2 Differential Scanning Calorimetry

The thermal properties of adenosine have been studied through the use of differential scanning calorimetry [7]. In this study, adenosine exhibited a well-defined melting endotherm at 242°C (515 K), a broad exotherm at 294°C (567 K) corresponding to decomposition (glycoside cleavage), and another endotherm at 359°C (632 K) corresponding to a phase transition of the cleaved pyrimidine. DSC thermograms of adenosine and related compounds are shown in Figure 8.

3.4.3 Thermogravimetric Analysis

As an anhydrous material, adenosine exhibits no weight loss until heated beyond the temperature of its thermal decomposition, which is approximately 300°C.

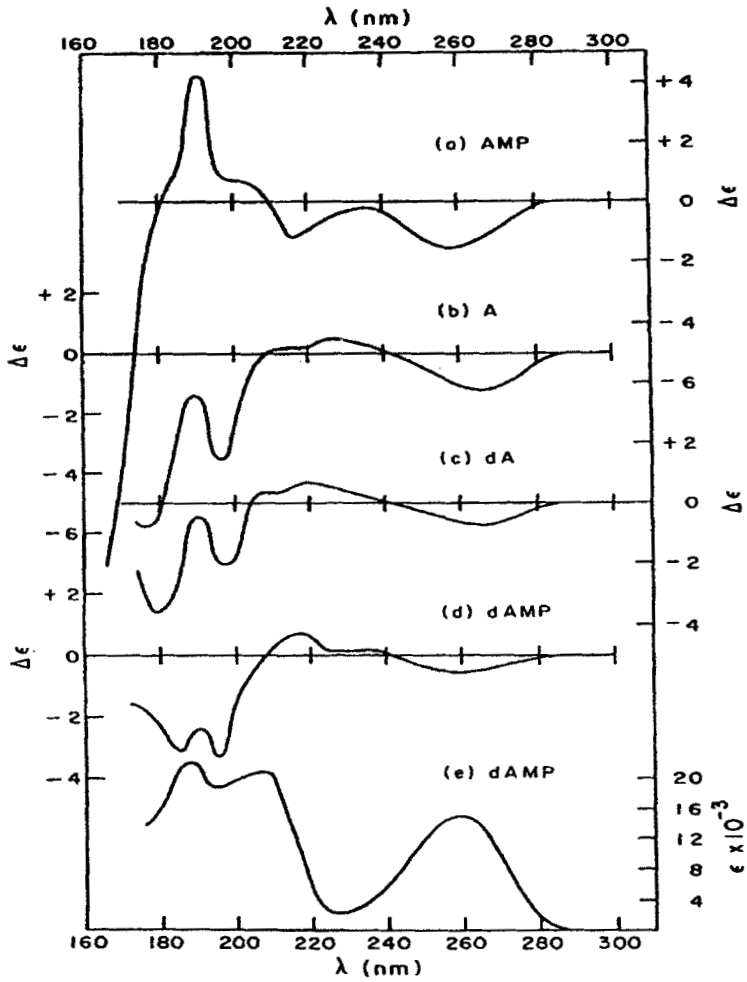


Figure 7. Circular dichroism and absorption spectra of adenine derivatives of in 0.01M sodium phosphate buffer, pH 7.0. CD of (a) adenosine 5'phosphate, (b) adenosine (c) deoxyadenosine (d) deoxyadenosine 5'-phosphate, and absorption of (e) deoxyadenosine 5'phosphate. Reproduced from reference [4b], and used with permission from John Wiley & Sons, Inc., New York.

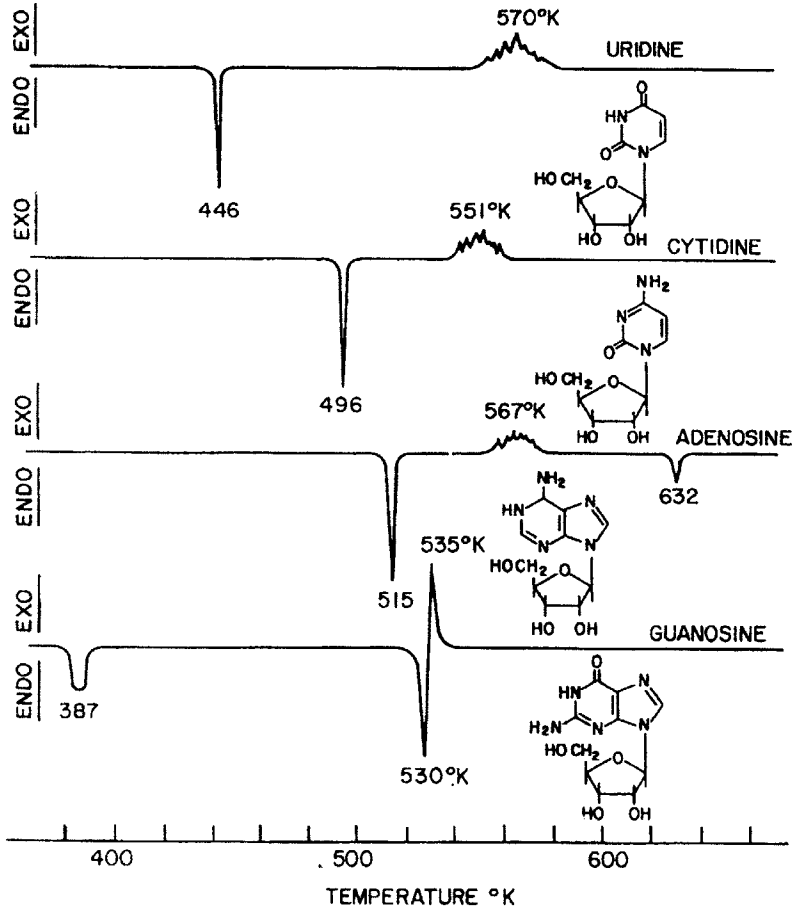


Figure 8. Differential scanning calorimetry thermograms of adenosine and related compounds. Reproduced from reference [7], and used with permission from Elsevier Science, Amsterdam.

3.5 Hygroscopicity

When stored for three months at 40°C and 75% relative humidity, the level of moisture in a sample of adenosine did not increase significantly. This indicates that the compound is essentially non-hygroscopic.

3.6 Solubility Characteristics

The solubility of adenosine in water is approximately 5 mg/mL [8]. The aqueous solubility is appreciably higher in methanol (6 mg/mL), ethanol (13 mg/mL), 1-propanol (20 mg/mL), 2-propanol (12 mg/mL), 1-butanol (9 mg/mL), or ethylene glycol (8 mg/mL).

3.7 Partition Coefficients

The partition coefficient for adenosine has been determined to be 0.105 $\pm$ 0.003, or log P -0.979 [8], demonstrating the hydrophilic nature of the compound. Using the ACD/logP program (Advanced Chemistry Development, Toronto, Canada), a partition coefficient of approximately 0.055 was calculated, further supporting the hydrophilic nature of adenosine.

3.8 Ionization Constants

The pK of adenosine has been reported to be 3.5 [9].

3.9 Spectroscopy

3.9.1 UV/VIS Spectroscopy

The UV spectrum of adenosine in 0.1 N HCl, water, and 0.1 N NaOH were all found to be equivalent, showing neither bathochromic or hypsochromic shifts nor hyperchromic or hypochromic effects. As evident in Figure 9, adenosine exhibits a maximum in its UV spectrum at 259 nm associated with the adenine chromophore, for which the molar absorptivity is 15185.

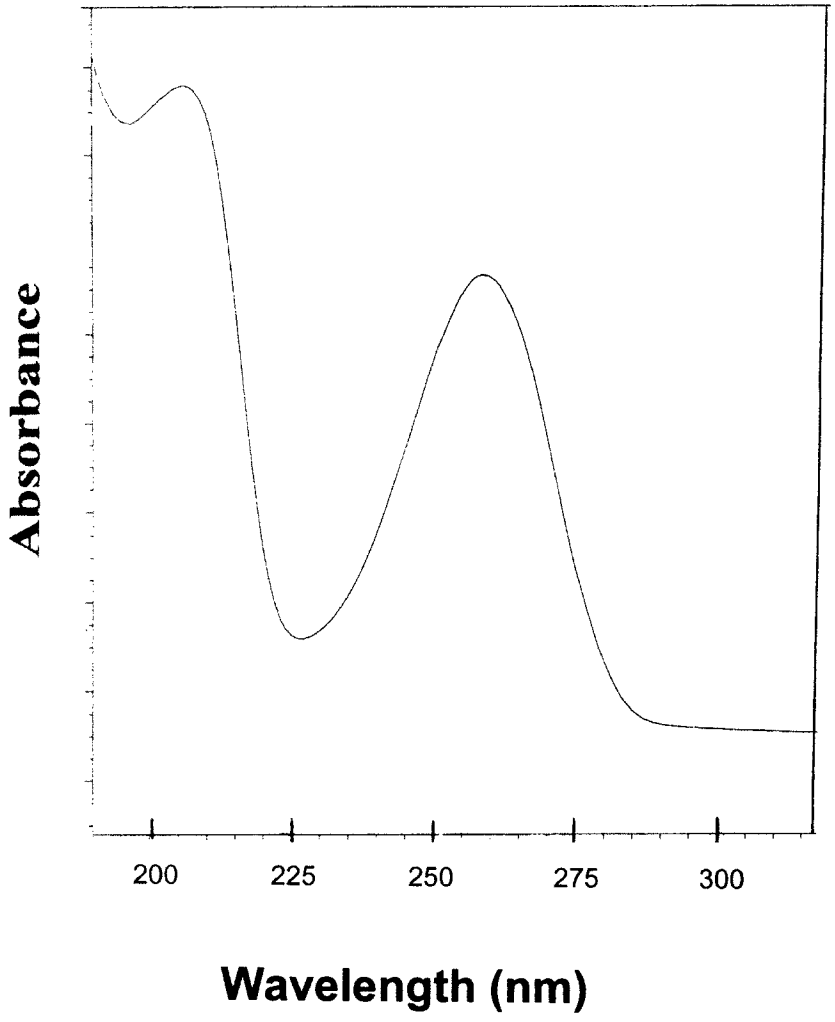


Figure 9. Ultraviolet absorption spectrum of adenosine, obtained in water.

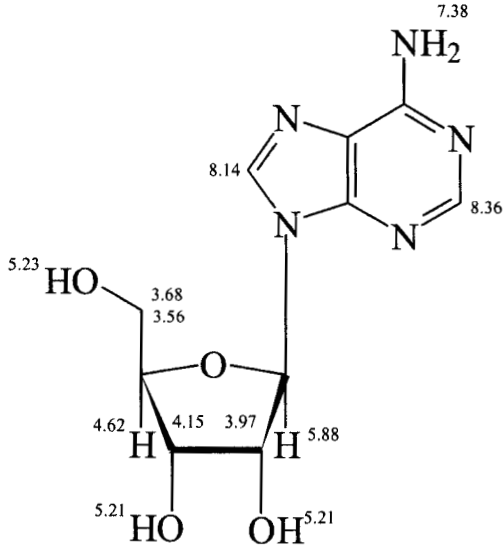
3.9.2 Vibrational Spectroscopy

The infrared absorbance spectrum of adenosine is shown in Figure 10, and the pattern of bands is diagnostic for this particular compound.

3.9.3 Nuclear Magnetic Resonance Spectrometry

3.9.3.1 ^{1}H-NMR Spectrum

The proton NMR spectrum of adenosine is shown in Figure 11, and the peak assignments are summarized as follows:



3.9.3.2 ^{13}C-NMR Spectrum

The ^{13}C NMR spectrum of adenosine is shown in Figure 12, and the peak assignments are summarized as follows:

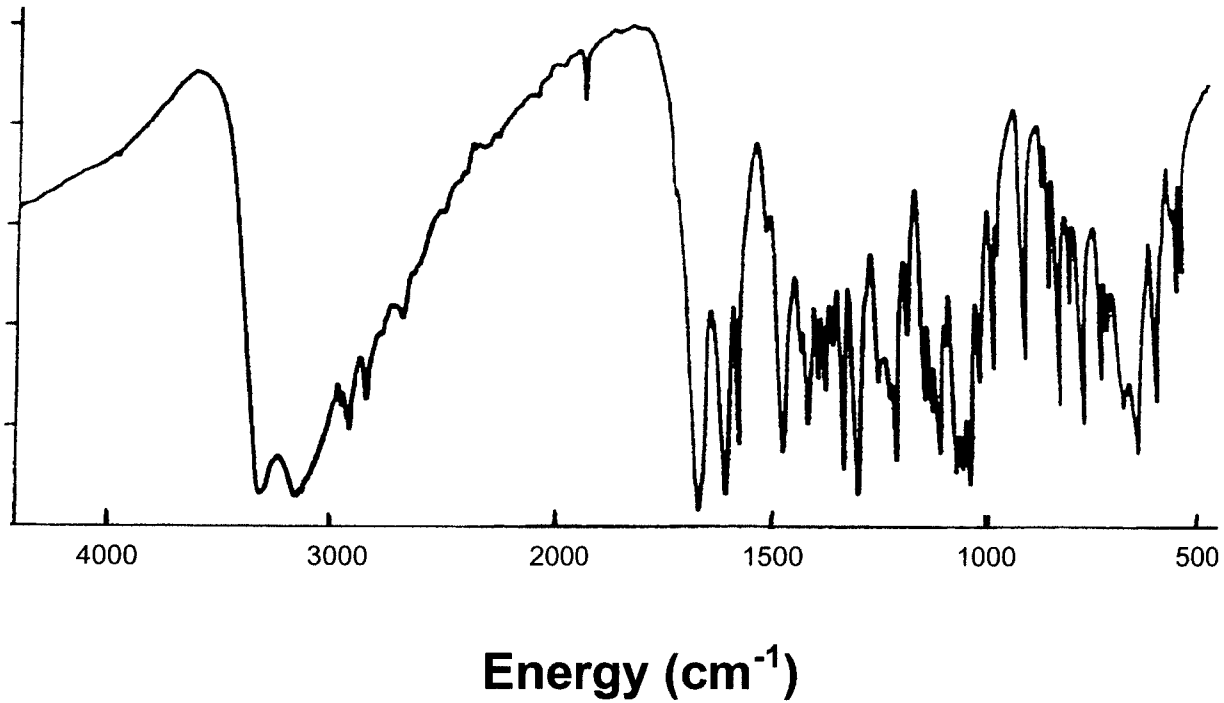


Figure 10. Infrared absorption spectrum of adenosine.

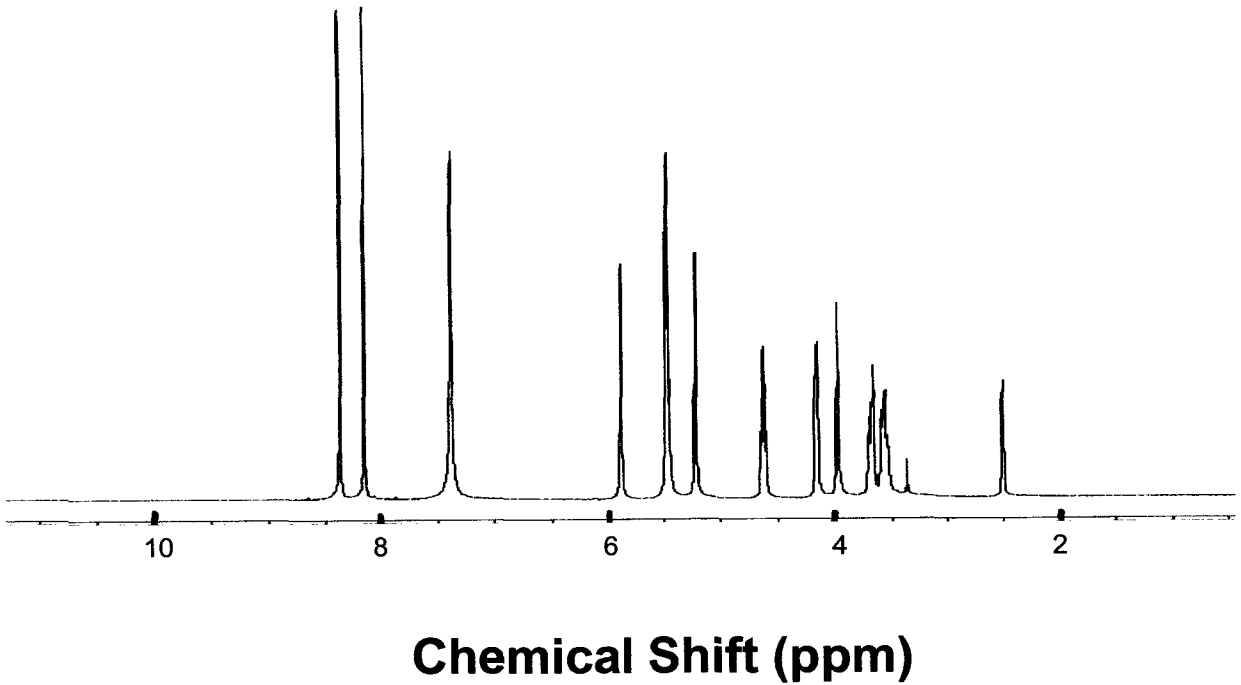


Figure 11. ^{1}H-NMR spectrum of adenosine.

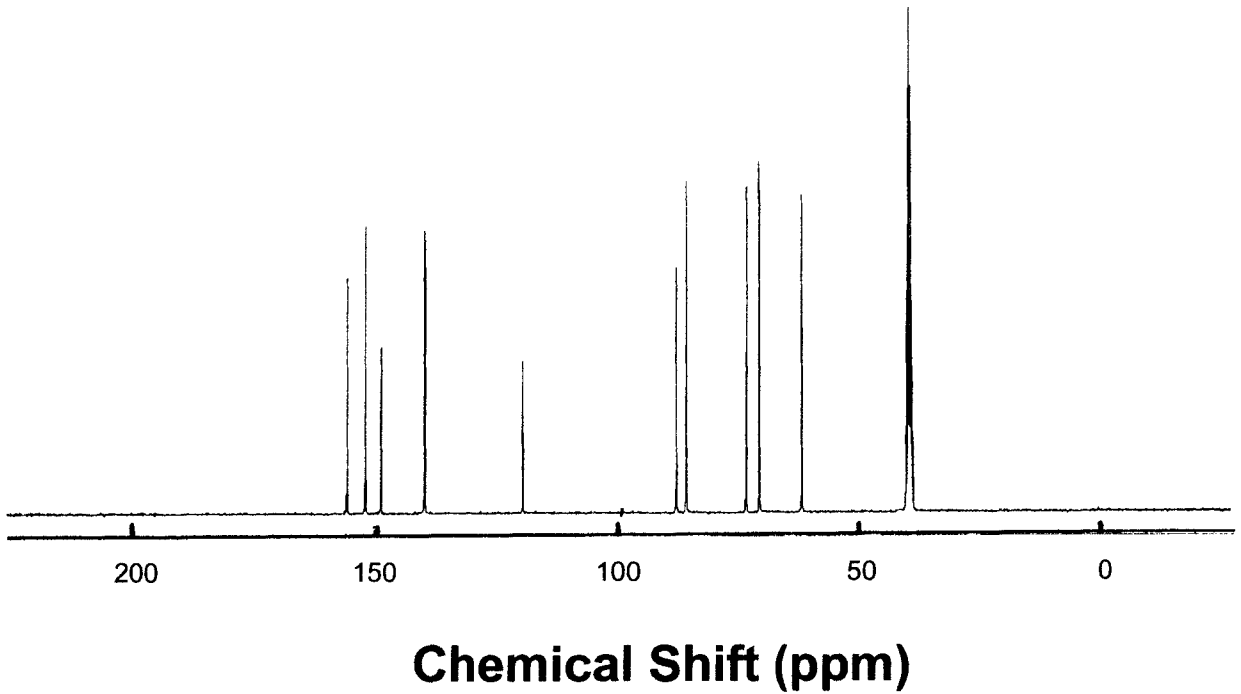
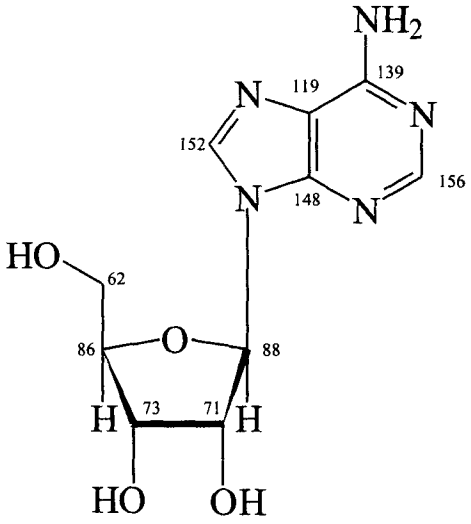


Figure 12. ^{13}C-NMR spectrum of adenosine.



3.9.4 Mass Spectrometry

The electron impact mass spectrum of adenosine is shown in Figure 13. As shown, a weak molecular ion is observed, but the base peak of the spectrum corresponds to cleavage of the glycosidic bond and charge retention on the electrophilic base [1]. Other, less intense peaks, are observed due to fragmentation of the carbocyclic ring.

4. Methods of Analysis

4.1 Identification

The identity of adenosine can be confirmed by comparing the infrared absorption spectrum of the sample to that reported in Figure 9.

4.2 Elemental Analysis

The theoretical elemental composition of adenosine is carbon 44.94%, hydrogen 4.90%, nitrogen 26.21%, and oxygen 23.95%.

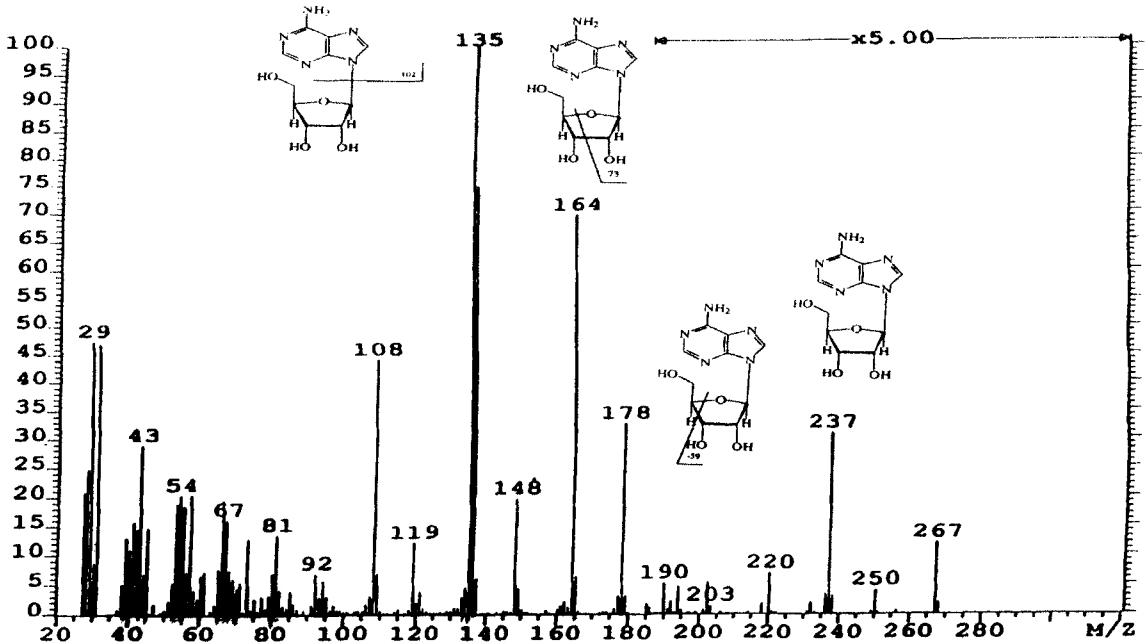


Figure 13. Electron impact mass spectrum of adenosine.

4.3 Titrimetric Analysis

Adenosine can be titrated using perchloric acid in anhydrous acetic acid (glacial acetic acid/acetic anhydride), using a combination glass/calomel electrode to determine the endpoint.

4.4 Chromatographic Methods of Analysis

4.4.1 Thin Layer Chromatography

A variety of thin-layer chromatographic methods for analysis of adenosine and related compounds have been reported. The salient characteristics of these are summarized in Table 2.

The anomers of adenosine may be separated using octadecyl-modified silica gel treated with copper acetate and (2S, 4R, 2'RS)-4-hydroxyl-1-(2-hydroxydodecyl)proline (Chiralplates) [10]. The mobile phase used to effect the separation consists of 50:50:30-400 methanol, water, and acetonitrile.

Sleckman, Touchstone, and Sherma have reported seven systems to separate adenosine from adenosine 5-monophosphate, as well as from other related nucleosides and nucleotides [11].

A fast and efficient separation of adenosine from its polyphosphates has been reported, which used aminopropyl silanized HPTLC plates and 46:60 methanol/water and sodium chloride (0.18mol/L) as the mobile phase [12]. Another reported HPTLC method involves the use of silica gel plates and a mobile phase of 60:30:10 propanol, 25% ammonia and water. Using this system, adenosine is separated from uridine, guanosine, inosine, and adenine.

An interesting reverse-phase thin-layer separation of adenosine from cytidine, guanosine, and uridine has been reported by Armstrong and Terrill [13]. These authors used a mobile phase consisting of 1.5 M sodium dioctylsulfosuccinate and 4.44M H_2O in cyclohexane, and silanized silica gel sheets.

Table 2

Thin-Layer Chromatographic Methods for Adenosine

Author	Mobile Phase	Stationary Phase	R_f
Feldberg, Reppucci [10]	50:50:50 Water:ACN:MeOH	Chiralplates	~0.8
Sleckman, Touchstone, Sherma [11]	7:2:1 IPA: Water:Ammonia	Whatman LK5DF	~0.6
	7:2:1 IPA: Water:Ammonia	Whatman LHPKDF	~0.7
	16:4:1 IPA:Water:DMSO	Whatman $LKC_{18}DF$	~0.6
	16:4:1 IPA:Water:DMSO	Whatman C_2	~0.8
	0.1M $NH_4H_2PO_4$, 1.0 M NaCl	Whatman SCX	~0.7
Jost, Hauck [12]	46:60 MeOH : Water. Sodium Chloride (0.18mol/L)	HPTLC NH_2	~0.8
Armstrong, Terrill [13]	1.5M Sodium Dioctylsulfosuccinate, 4.4M Water in Cyclohexane	Silica	~0.1

4.4.2 Gas Chromatography

Nucleosides are commonly analyzed by gas liquid chromatography after silanation to improve volatility [14-18]. A concise summary of the derivitization reagents and stationary phases is given in Table 3.

In a study published by Gehrke and Patel, various nucleosides (including adenosine, cytidine, guanosine, ionsine, thymidine, uridine, and xanthosine) were separated using 4% OV-11 on 100-120 mesh Supelcoport [16]. These has been previously derivatized with a 225 molar excess of bis(trimethylsilyl)trifluoroacetamide (BSTFA) in acetonitrile. Samples were heated with BSTFA for 15 minutes at 150°C to afford complete reaction. In a later study, the authors reported that BSTFA containing 1% trimethylchlorosilane afforded improved derivatization efficiency. The chromatographic conditions were the same as in the previous report, as was the use of an excess of silylating reagent.

4.4.3 High Performance Liquid Chromatography

Much of the literature on the HPLC analysis of adenosine is presented in the context of measuring levels of this nucleoside in biological matrices, and will be presented in a following section. The present narrative will focus on the purity or stability-indicating analyses developed specifically for the adenosine substance.

Adenosine may be separated from uridine, adenine, inosine, and guanosine by gradient HPLC using an octadecylsilanized silica stationary phase and the following linear gradient conditions:

Mobile Phase A:	0.01M Ammonium Acetate, pH 6.0
Mobile Phase B:	1:1 Acetonitrile : 0.01 M Ammonium Acetate, pH 6.0

Table 3

Gas Chromatographic Methods for Adenosine

Author	**Derivitization Reagent**	**Stationary Phase**
Langridege, McClure, *et al.* [14]	BSTFA	DB-5 (30 m X 0.25mm)
Ballard, Eller, *et al.* [15]	MTBSTFA with 1% TBDMS chloride	DB-5 (30 m X 0.26mm)
Miller, Pavakova [18]	HMDS, BSTFA	3% OV-101 or 3% OV-17 on Chromosorb W HP
Gehrke, Patel [16,17]	BSTFA	4% OV-11 on 100 - 120 mesh Supelcoport

Time (min)	Percent Component A	Percent Component B
0 - 10	96	4
10 - 20		4 to 20
20 - 30	80	20
30 - 30.1		20 to 4

The eluent flow rate used in this method was 1 mL/min, and the analyte detection was made using the UV absorbance at 254nm. The retention times (relative to adenosine) of uridine, adenine, inosine, and guanosine were found to be 0.329, 0.514, 0.6, and 0.667, respectively. A typical chromatogram illustrating the analytical separation possible with this method is shown in Figure 14.

An isocratic version of this method, which used 94% component A and 6% component B (the composition of both just defined above), yields a retention time for adenosine of approximately 9 minutes.

Another isocratic HPLC method for adenosine again uses an octadecyl-silanized silica stationary phase. The mobile phase for this method consists of 0.05M monobasic potassium phosphate, 0.01M tetrabutyl-ammonium hydrogen sulfate, and 0.01% aqueous sodium azide in a ratio of 1:1. The pH of the mobile phase is adjusted to 6.5 with dilute sodium hydroxide. The flow is 1 mL/min, and adenosine is detected at 254nm.

A simple and fast reversed-phase ion-pair HPLC method capable of the simultaneous detection of ATP, ADP, AMP, GTP, GDP, IMP, NADP+, NADPH, NAD+, NADH, ADP-ribose, inosine, adenosine, hypoxanthine, and xanthine has been reported [19], as has a sensitive and specific method using fluorometric detection [20].

4.5 Determination in Body Fluids and Tissues

Sensitive and selective HPLC methods which quantitate adenosine in a variety of biological matrices have been reported. The matrices include rat striatal tissue [21], human blood [22], brain tissue [23], plasma [24-27], urine [28], and red blood cells [29].

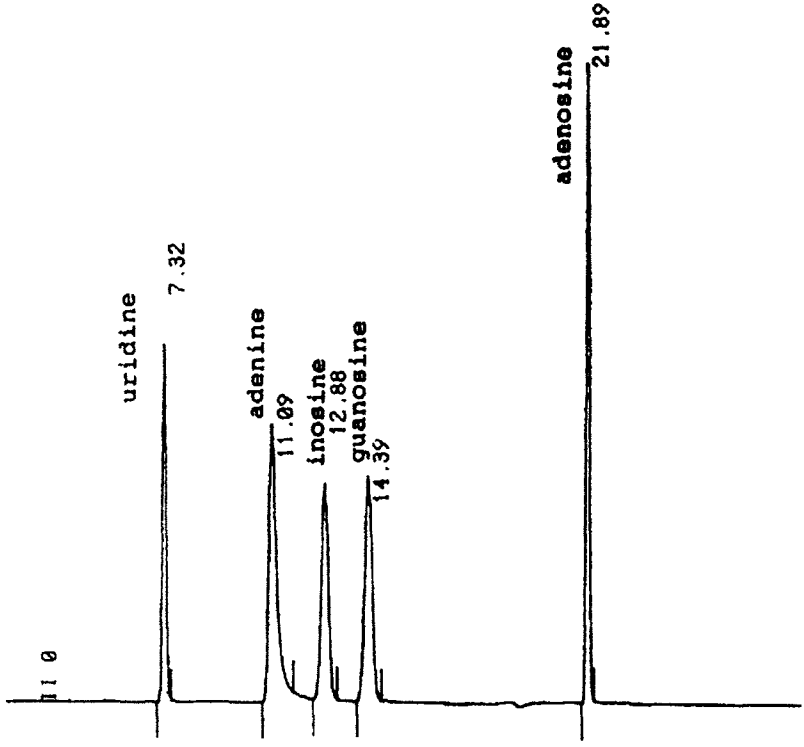


Figure 14. Gradient HPLC analysis of adenosine and related compounds.

A general method for adenosine and related nucleosides in biological materials has been reported [30].

5. Stability

5.1 Solid-State Stability

Adenosine has been stored as a dry solid in polyethylene bags at 20.5 - 23.5°C for five years without any evidence of appreciable decomposition [31].

5.2 Solution-Phase Stability

Solutions of adenosine in a mixture of 0.1 M ammonium acetate (pH 6) and acetonitrile have been stored at room temperature for up to 120 hours, and analyzed against a freshly prepared standard. When analyzed by a stability-indicating gradient HPLC method, no evidence of decomposition was noted [32].

A study on the terminal steam sterilization (120°C, 20 minutes) of 0.5% adenosine solutions in 0.9% saline reported no detectable decomposition [33].

The stability of adenosine formulation for injection has been reported [34], and the substance was again found to be quite stable in solution.

5.3 Stability in Biological Fluids

The stability of adenosine in biological matrices has been addressed in many of the references associated with analysis methods in these fluids (see above). The stability in tissue samples has been described as being dependent on the tissue and species being evaluated [35]. In the case reported, the observed differences in stability were actually due to the varying abilities of different tissue samples to metabolize adenosine.

6. Drug Metabolism and Pharmacokinetics

Adenosine is a naturally occurring nucleoside which is present in various forms in all cells of the body. It is an essential component of the energy production and utilization systems of the body.

Adenosine has properties which make it essentially impossible to perform the classic ADME (absorption, distribution, metabolism, and excretion) studies in man. As a result of an extremely efficient re-uptake system in the body, the half-life after intravenous administration is estimated to be less than 10 seconds. In addition, because adenosine is present in every cell of the body, any administered dose is minute in comparison to the existing body pool.

The metabolic pathways for adenosine have been clearly defined by many investigators [36-39]. Adenosine may be converted to its base, adenine, and then to adenine monophosphate (AMP); adenosine may go directly to AMP, or adenosine may be deaminated to iosine and then converted to AMP. Under normal circumstances, adenosine is added to the system by breakdown of adenosine triphosphate (ATP) and by biosynthesis in the liver [40]. It has been suggested that the erythrocytes serve as the transporting vehicle to move adenosine from the liver to those tissues which do not synthesize it. The basic biochemical pathways appear to be the same in all animals, although the proportions of the various enzymes involved may vary from tissue to tissue.

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CEFIXIME

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ANALYTICAL PROFILES OF DRUG SUBSTANCES
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Contents

1. Description

1.1 Nomenclature

1.1.1 Chemical Names

[6*R*-[6α,7β(Z)]]-5-thia-1-azabicyclo[4.2.0] oct-2-ene-2-carboxylic acid, 7-[[(2-amino-4-thiazolyl)[(carboxymethoxy)imino]acetyl]-amino]-3-ethenyl-8-oxo, trihydrate,

(6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl) glyoxylamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylic acid, 7 ^{2}-(Z)-[O-(carboxymethyl)oxime] trihydrate

[6*R*-[6α,7β(Z)]]-7-[[(2-Amino-4-thiazolyl)[(carboxymethoxy)-imino]acetyl]aminol-3-ethenyl-8-oxo-5-thia-l-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid

7-[2-(2-amino-4-thiazolyl)-2-(carboxymethoxyimino)acetamido]-3-vinyl-3-cephem-4-carboxylic acid

1.1.2 Nonproprietary Names

Cefixime trihydrate

1.1.3 Proprietary Names

Oroken, Cefspan, Suprax

1.2 Formulae

1.2.1 Empirical

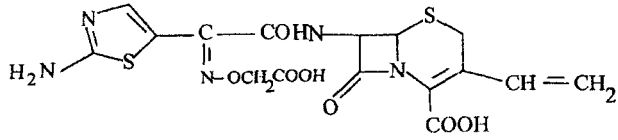
free acid: $C_{16}H_{15}N_5O_7S_2$

trihydrate: $C_{16}H_{15}N_5O_7S_2 \cdot 3\ H_2O$

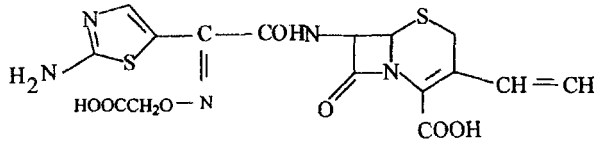
the theoretical water content of the trihydrate phase is 10.65%

1.2.2 Structural

Z-isomer:



E-isomer



1.3 Molecular Weight

free acid: 453.44 $\{C_{16}H_{15}N_5O_7S_2\}$

trihydrate: 507.49 $\{C_{16}H_{15}N_5O_7S_2 \cdot 3\ H_2O\}$

1.4 CAS Number

79350-37-1

1.5 Appearance

The Z-isomer of cefixime, which is the article of commerce used in dosage forms, is a white to light-yellow crystalline powder. It is odorless, or can have a slight characteristic odor.

The E-isomer of cefixime is a pale yellow solid.

1.6 Uses and Applications

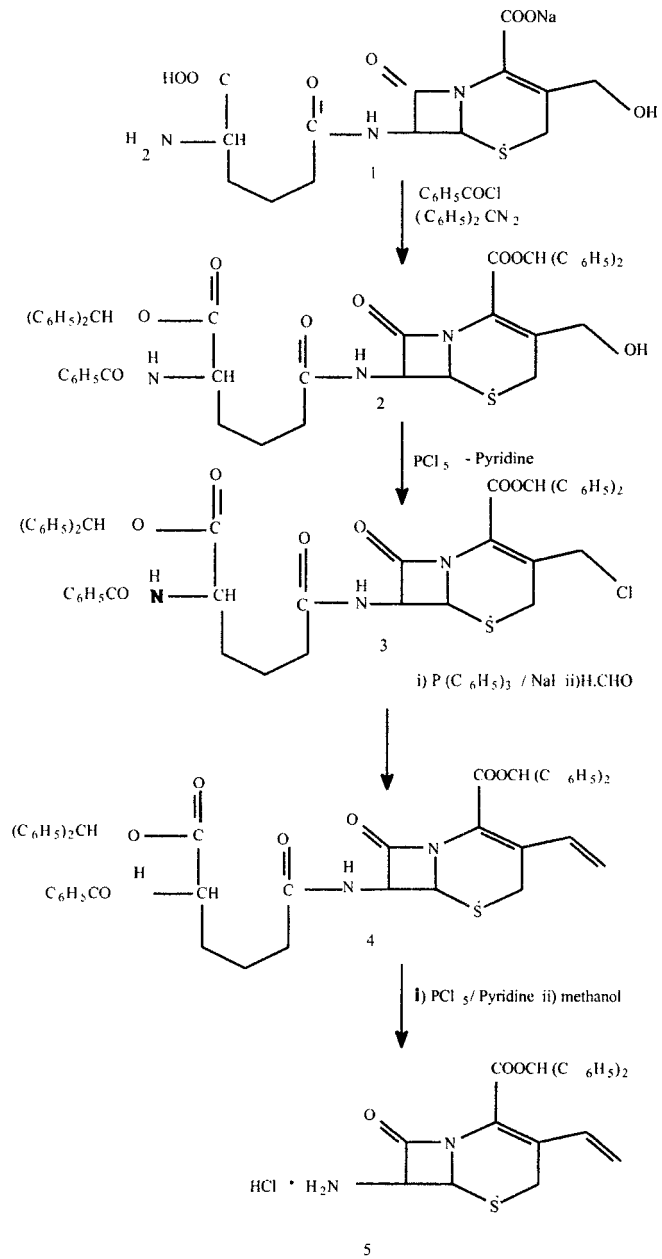
Cefixime is the first member of what is generally termed the third generation orally active cephalosporins. These third generation cephalosporins are distinct from the older β-lactam antibiotics in their intensive antibacterial activity against a wide range of gram-negative bacteria. The exceptional antibacterial activity of the third generation cephalosporins has been shown to be based on both their enhanced affinity

for the target enzymes and their high stability to β-lactamase [1]. That Cefixime shares similiar characteristics with the other third-generation cephalosporins was confirmed by Yasutaka Shigi *et al.* [2]. Although Cefixime is less active against *staphylococci* than are other orally active β-lactam antibiotics, it is far more potent against a wide range of gram-negative bacteria.

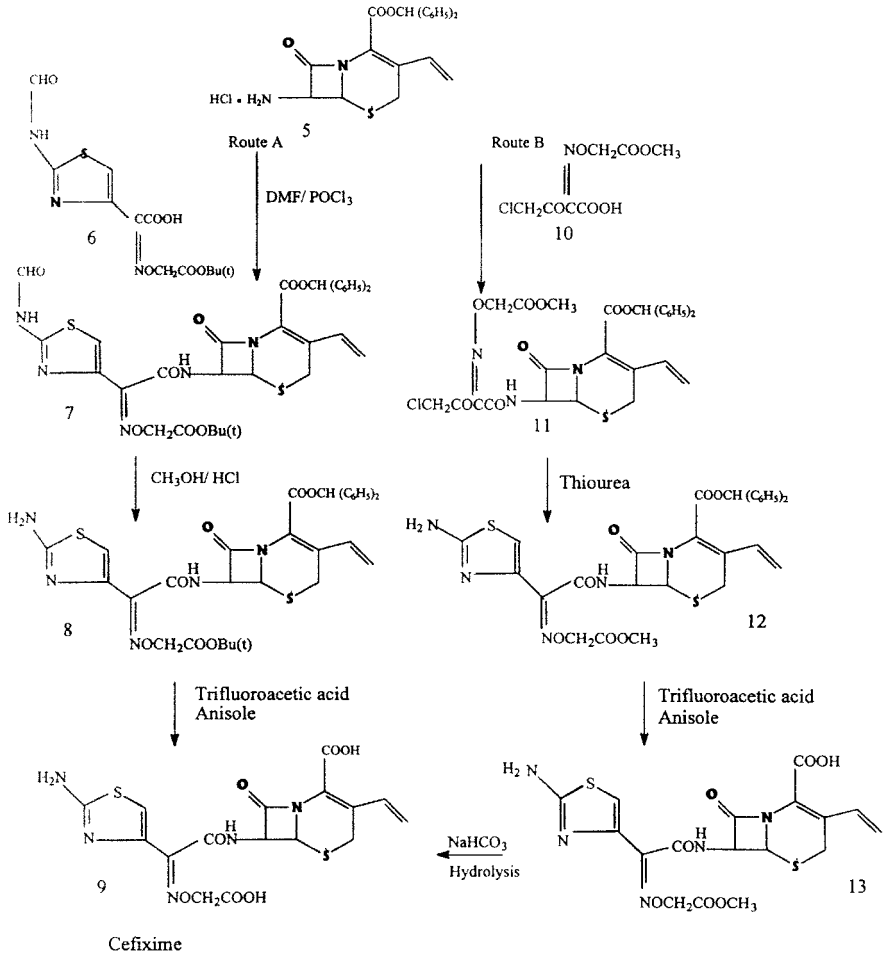
The aminothiazole ring appears to be associated with both excellent activity and oral absorption, and the amino group in the thiazole ring is essential for the potential antibacterial activity [3]. Cefixime exhibits geometrical isomerism with reference to the configuration of the oxime. The Z-isomer is the predominant isomer relative to the E-isomer, and is the article of commerce used in the preparation of dosage forms. The E-isomer is considered as the concomitant component of cefixime, and is therefore not considered as an impurity in the usual pharmacopoeial sense. The antimicrobial activity and oral absorbability of both isomers have been studied is detail. The E-isomer is reported to be 2-32 times less active than the Z-isomer against gram-negative bacteria, although both isomers show appreciable oral absorbability regardless of the configuration of the oxime. In marked contrast to other cephalosporin antibiotics, cefixime has a vinyl group at C-3 and a (Z)-2-(2-amino-4-thiazolyl) -2-(carboxymethoxyimino) acetyl moiety at C-7 which influences its improved activity against gram-negative bacteria and pharmacokinetic properties [4,5].

2. Methods of Preparation

Excellent and efficient routes for the synthesis of the Z-isomer and E-isomer of cefixime have been developed [3,6,7]. The synthetic routes for the Z-isomer are shown in Scheme 1 and Scheme 2. The synthesis involves the preparation of the key intermediate diphenylmethyl 7-amino-3-vinyl-3-cephen-4-carboxylate hydrochloride (**5**), and three different synthetic routes for the preparation of **5** have been developed and described by Kawabata *et al.* [7]. The more efficient of the three routes is described in Scheme 1, which starts with deacetylcephalosporin C sodium salt (**1**). This compound is treated with benzoyl chloride, followed by reaction with diphenyldiazomethane, to obtain the protected deacetylcephalosporin C (**2**). The reaction of **2** with phosphorus pentachloride and pyridine converts the hydoxymethyl group into



Scheme 1. Synthetic route for the preparation of intermediate 5.



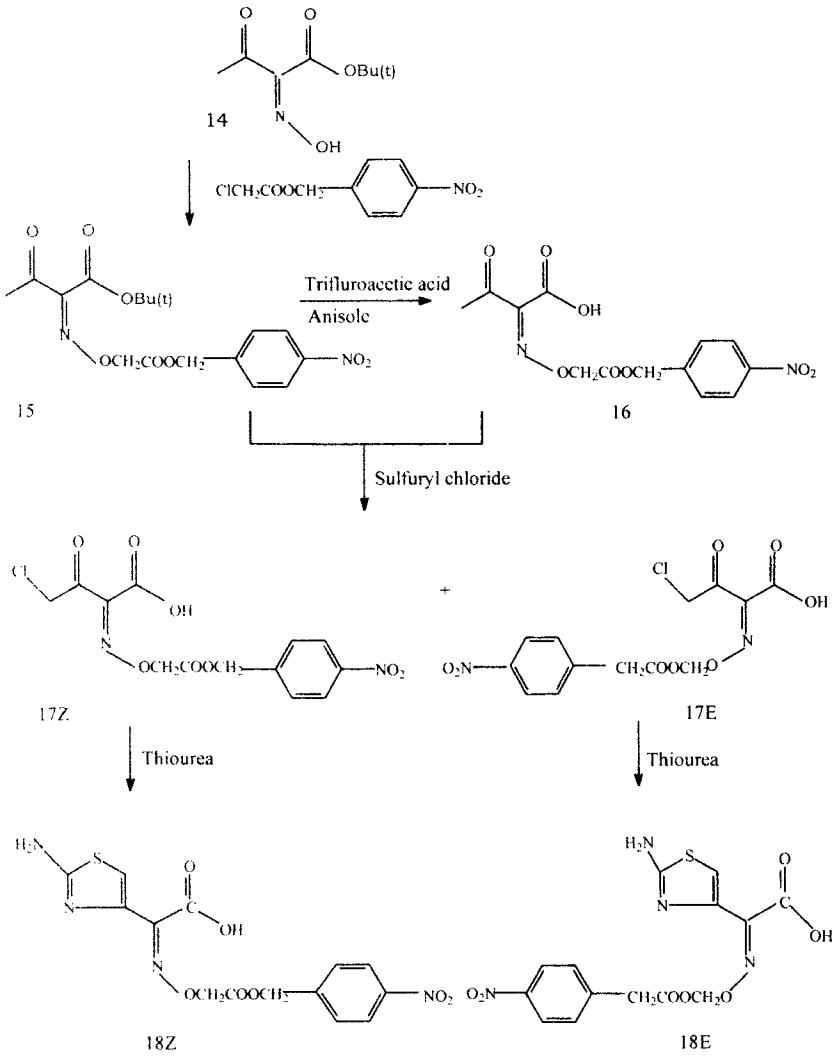
Scheme 2. Synthetic routes from intermediate 5 for the preparation of the Z-isomer of cefixime.

a chloromethyl group, to yield intermediate **3**. The chloromethyl derivative **3** is then treated with triphenylphosphine and sodium iodide in N,N-dimethyl-formamide to yield a phosphonium salt, which then is then treated with formaldehyde in methylene chloride (Wittig reaction) to obtain the 3-vinyl derivative **4**. Cleavage of the acyl side chain in intermediate **4** is achieved by treatment with phosphorus pentachloride and pyridine, followed by methanol, to give the intermediate **5** in good yield.

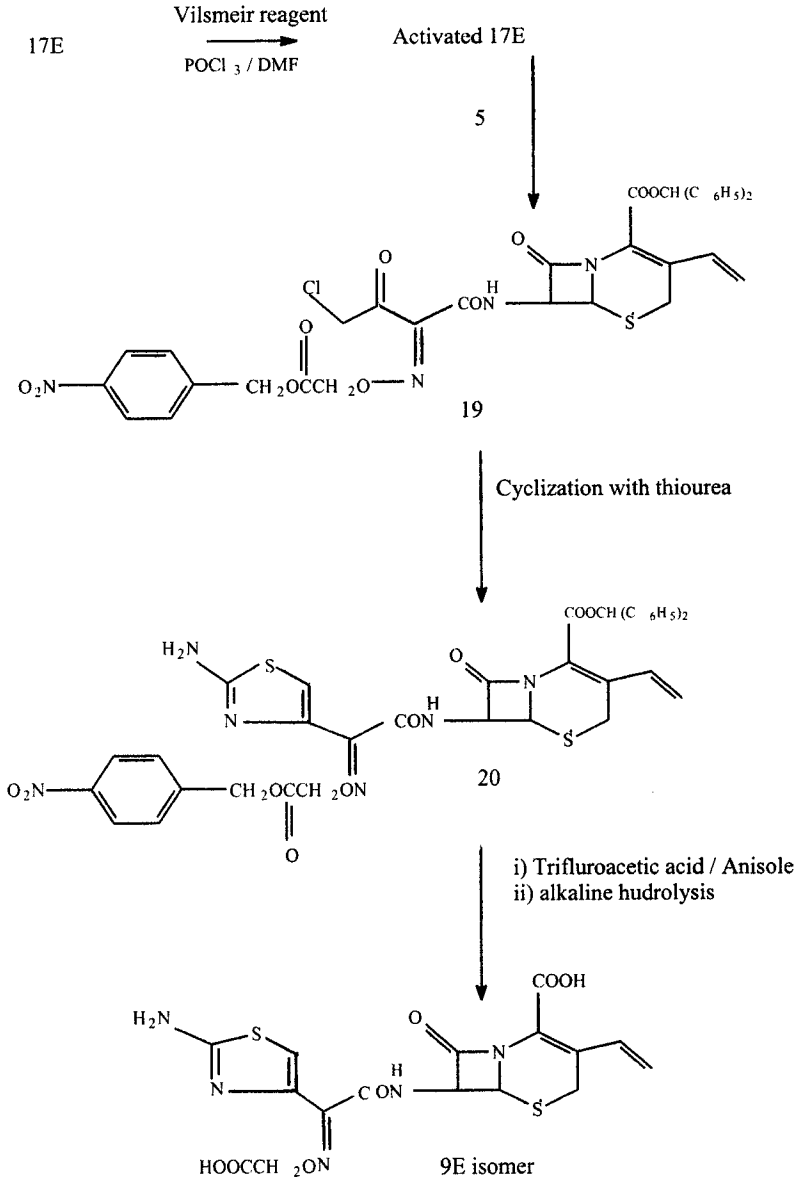
As shown in Scheme 2, the conversion of intermediate **5** into the Z-isomer of cefixime has been achieved by two different routes. In route A, the intermediate **6**, (Z)-2-(2-formamido-4-thiazolyl)-2-(*tert*-butoxycarbonyl-methoxyimino) acetic acid, is initially activated by treating with a mixture of phosphoryl chloride and dimethylformamide (Vilsmeir reagent). This is condensed with intermediate **5** to give the protected intermediate **7**. Deprotection of the N-formyl group in **7** is achieved by treatment with a methanolic solution of concentrated hydrochloric acid, obtaining the deformyl cephem intermediate **8**. The removal of the *tert*-butyl and diphenylmethyl groups in **8** is achieved by treatment with trifluoroacetic acid and anisole to yield cefixime (**9**).

In the alternate synthetic route B, the intermediate **5** is acylated with 4-chloro-2-methoxycarbonylmethoxyimino-3-oxobutyric acid **10** to give the acylated cephem intermediate **11**. This compound is then treated with thiourea to give intermediate **12**, 2-aminothiazol cephem. The cleavage of the diphenylmethyl ester of **12** is achieved by treatment with trifluoro-acetic acid and anisole to give the monoester, **13**, which on hydrolysis with sodium bicarbonate yields cefixime.

During their efforts to investigate the effect of oxime configuration in the 7-acyl side chain on the antibacterial activity and oral absorbability in rats, Kawabata *et al.* synthesized the E-isomer of cefixime by two different methods [6]. In one of their methods, shown in Schemes 3 and 4, the key intermediate, 4-chloro-2-p-nitrobenzyloxycarbonylmethoxyimino-3-oxobutryic acid (**17**) is obtained as a mixture of geometrical isomers starting from *tert*-butyl (Z)-2-hydroxyimino-3-oxybutyrate **14**. The geometrical isomers, **17-Z** and **17-E**, are indistinguishable in their spectral characteristics, and were characterized by separately converting them to the cyclic aminothiazole compounds, **18-Z** and **18-E**. The E-intermediate is activated with the Vilsmeir reagent, and condensed with **5** to give the acylated compound **19**. Intermediate **19** is then cyclized with thiourea to



Scheme 3. **Synthetic route for the preparation of the 17Z and 17E geometric isomers of 4-Chloro-2-p-nitobenzyloxy-carbonylmethoxyimino-3-oxobutyric acid.**



Scheme 4. Synthetic route for preparation of the E-isomer of Cefixime.

yield the aminothiazole cephem, **20**. The diphenylmethyl group in **20** is removed by treatment with trifluoroacetic acid and anisole, and then subjected to alkaline hydrolysis to yield the E-isomer of cefixime.

3. Physical Properties

3.1 Particle Morphology

Scanning electron photomicrographs (obtained using a Hitachi S-800 system) of commercially available cefixime trihydrate are shown in Figure 1. The compound has been processed into spherical aggregates which are approximately 75 μm in diameter to ease its formulation.

As evident in Figure 1c, the individual microcrystals which form the aggregated particles have the flat bladed morphology typical of compounds of this type. The most fully developed crystals are 15-20 μm in length, and approximately 5 μm wide. The existence of rounding at the caps of the microcrystals suggests that the aggregate species were formed by a process which used a considerable amount of tumbling.

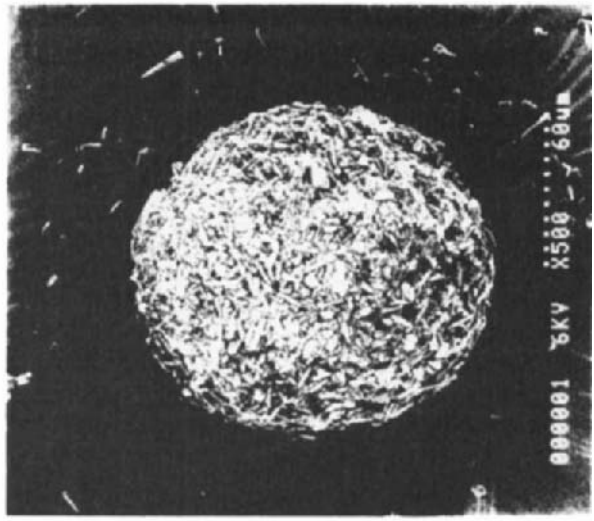
3.2 X-Ray Powder Diffraction Pattern

The x-ray powder diffraction pattern of cefixime trihydrate was obtained using a Philips MPD1880 system, and is shown in Figure 2. The x-ray generator was operated at 40 kV and 30 ma, using the K-α line of copper at 1.544056 Å as the radiation source. Each sample was scanned between 2.5 and 32.5 degrees 2-θ, at a scan rate of 0.10 degrees 2-θ/sec, and in step sizes of 0.02 degrees 2-θ. As evident in the figure, the sample exhibited a number of scattering peaks indicative of the existence of well-formed molecular planes in the crystal. A summary of observed scattering angles, d-spacings, and relative intensities is given in Table 1.

3.3 Optical Rotation

The specific rotation of cefixime at 25°C in a 10mg/mL aqueous solution of sodium bicarbonate is between -75° and -88°.

(a)



(b)

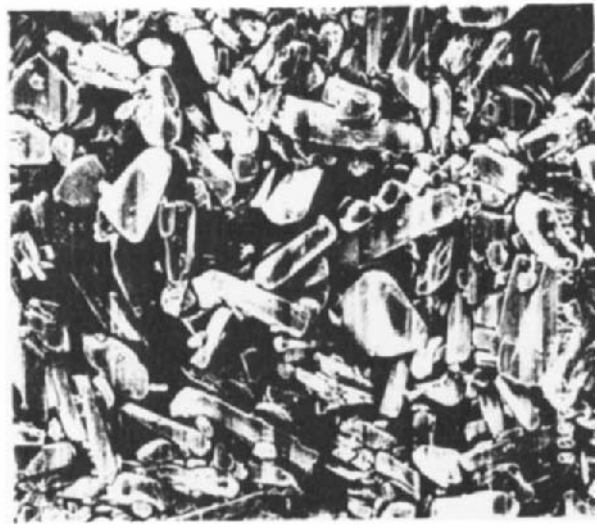


Figure 1. Scanning electron photomicrographs of cefixime trihydrate, obtained at magnifications of (a) 500x and (b) 3000x.

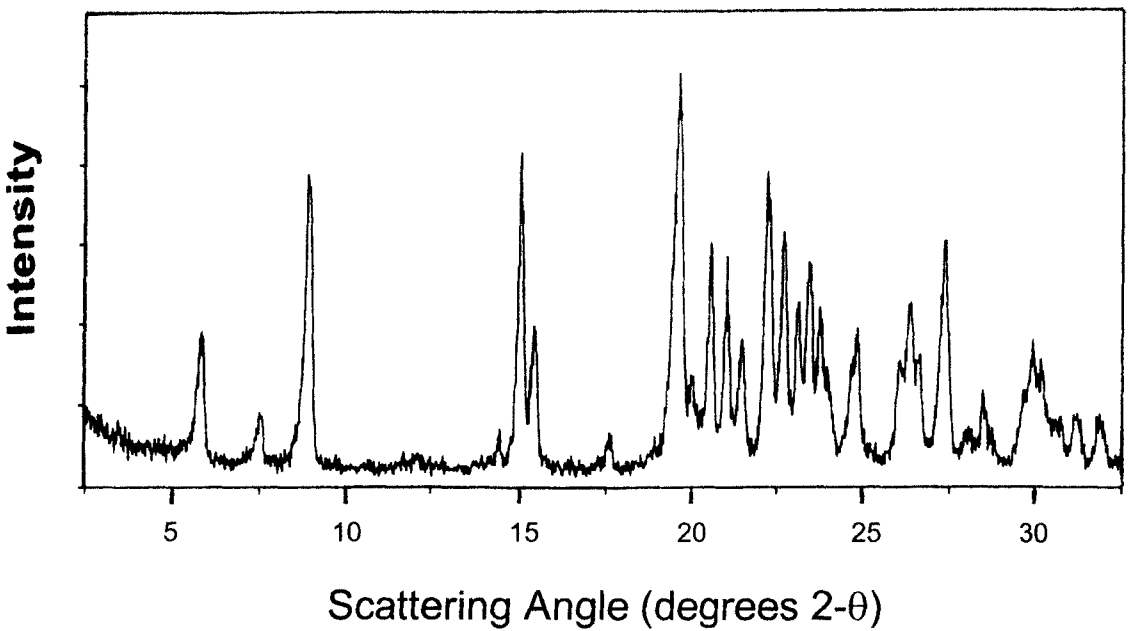


Figure 2. X-ray powder diffraction pattern of cefixime trihydrate.

Table 1

Data from the X-Ray Powder Diffraction Pattern of Cefixime Trihydrate

Scattering Angle (degrees 2-θ)	**d-spacing (Å)**	**Relative Intensity, I/I_0 (%)**
5.888	14.999	28.1
7.573	11.665	11.9
8.968	9.853	74.0
14.455	6.123	8.3
15.070	5.874	81.4
15.455	5.729	36.0
17.615	5.031	8.3
19.673	4.509	100.0
20.0223	4.431	20.8
20.595	4.309	56.8
21.080	4.211	43.6
21.520	4.126	33.3
22.258	3.991	74.8
22.748	3.906	60.3
23.130	3.842	41.8
23.485	3.785	52.7
23.788	3.738	40.0
24.878	3.576	36.0
26.048	3.418	26.2
26.388	3.375	42.4
26.670	3.340	26.7
27.388	3.254	61.1
28.028	3.181	7.8
28.478	3.132	17.1
29.948	2.981	29.6
30.223	2.955	23.9
30.775	2.903	11.9
31.213	2.863	12.5

3.4 Thermal Methods of analysis

3.4.1 Melting Behavior

Neither the Z-isomer nor the E-isomer of cefixime exhibits a sharp melting point. The Z-isomer melts at about 240°C with decomposition [7b]. The melting point of the disodium salt of the Z-isomer is reported to be over 250°C. The trihydrate phase of the E-isomer is found to melt with decomposition over a range of 218-225°C.

3.4.2 Thermogravimetric Analysis

The thermogravimetric analysis (TGA) thermogram of cefixime trihydrate was obtained using a Seiko EXSTAR6000 system 3U-99A, at a heating rate of 10°C/min, and is shown in the upper half of Figure 3. Between ambient and 130°C, the compound exhibits a weight loss of 10.4%, which is in good agreement with the theoretical water content of 10.65% [9]. Beyond 180°C, the desolvated compound continually loses mass, as would be anticipated from its pyrolysis at these elevated temperatures.

3.4.3 Differential Thermal Analysis

The differential thermal analysis thermogram of cefixime trihydrate was obtained using a Seiko EXSTAR6000 system 3U-99A, at a heating rate of 10°C/min, and is shown in the lower half of Figure 3. The dehydration endotherm exhibits its maximum at 107°C, which lies within the low-temperature transition range of the TGA thermogram [9]. The exothermic decomposition of the dehydrated solid exhibits a maximum at 191°C.

3.5 Hygroscopicity

When exposed to relative humidities ranging from 23% to 93% (at an equilibrium temperature of 25°C), cefixime trihydrate did not exhibit any measurable moisture pickup. The trihydrate phase is therefore determined to be non-hygroscopic.

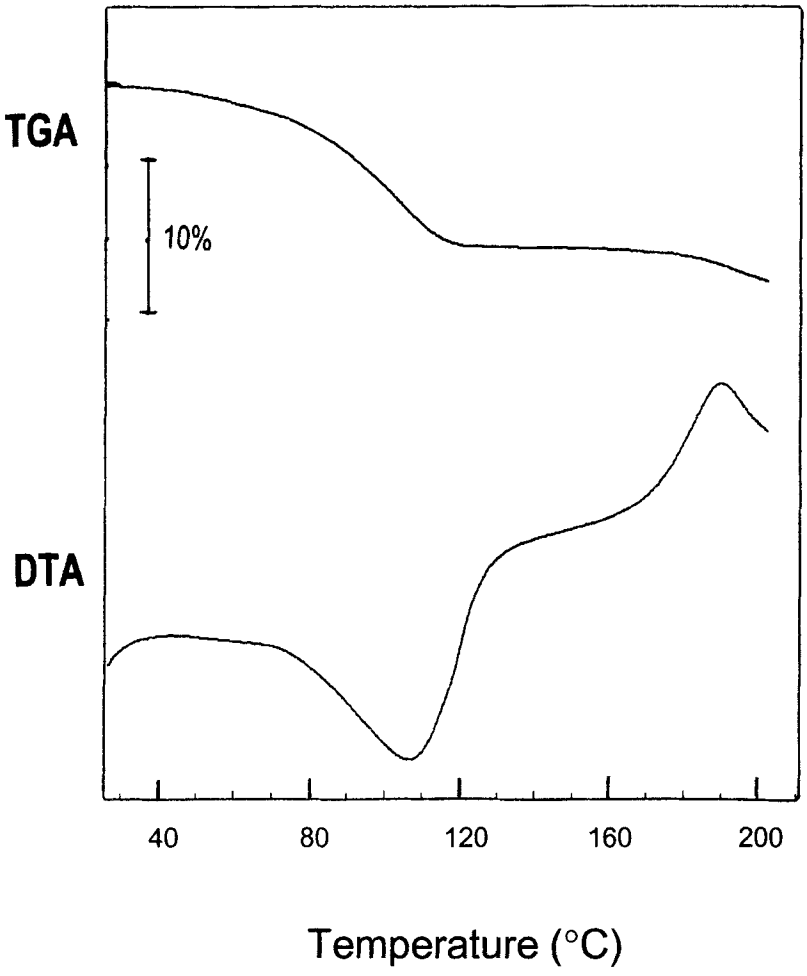


Figure 3. Thermogravimetric analysis (upper trace) and differential thermal analysis (lower trace) thermograms of cefixime trihydrate.

3.6 Solubility Characteristics

Cefixime trihydrate is practically insoluble in diethyl ether, ethyl acetate, hexane, and water. The compound is sparingly soluble in acetone, and is only slightly soluble in alcohol and 70% sorbitol. It is readily soluble, however, in methanol, dimethyl sulfoxide, glycerin, and propylene glycol.

3.7 Partition Coefficients

The octanol/water partition coefficient of cefixime trihydrate is 0.0029.

3.8 Ionization Constants

Cefixime trihydrate exhibits three acidic ionization constants, which are characterized as follows:

pKa1	2.10	-COOH of the cephem group
pKa2	2.69	$-NH_2$ of the amino-thiazol group
pKa3	3.73	-COOH of the 7-sidechain

3.9 Spectroscopy

3.9.1 UV/VIS Spectroscopy

The ultraviolet absorption spectra of the Z- and E-isomers of cefixime were recorded on a Shimadzu UV-2200 spectrophotometer, with the compounds being dissolved in 0.1 M phosphate buffer adjusted to pH 7.0. The spectrum of the Z-isomer is shown in Figure 4, and was obtained at a concentration of 15.42 μg/mL (30.39 μM). The spectrum of the E-isomer is shown in Figure 5, and was obtained at a concentration of 15.77 μg/mL (31.07 μM).

The spectrum of the Z-isomer consists of a strong absorption band at 288 nm, which is characterized by a molar absorptivity of 24,516 L/mol. The spectrum also contains a weaker absorption at 230 nm, whose molar absorptivity equals 17,382 L/mol.

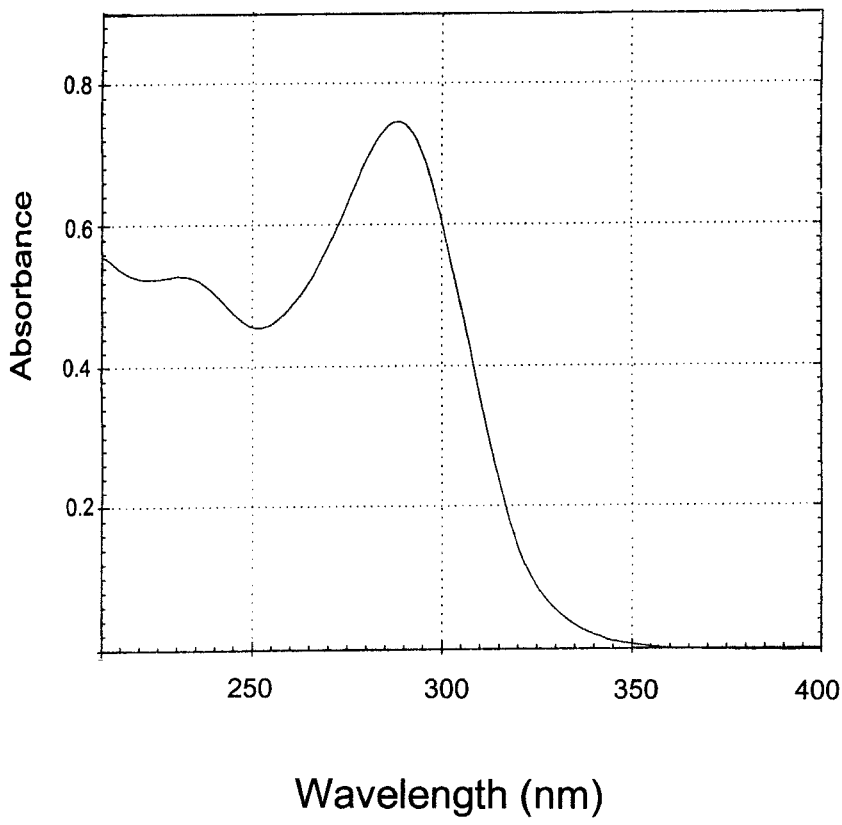


Figure 4. UV absorption spectrum of the Z-isomer of cefixime trihydrate, obtained at a concentration of 15.42 μg/mL (30.39 μM) in aqueous solution.

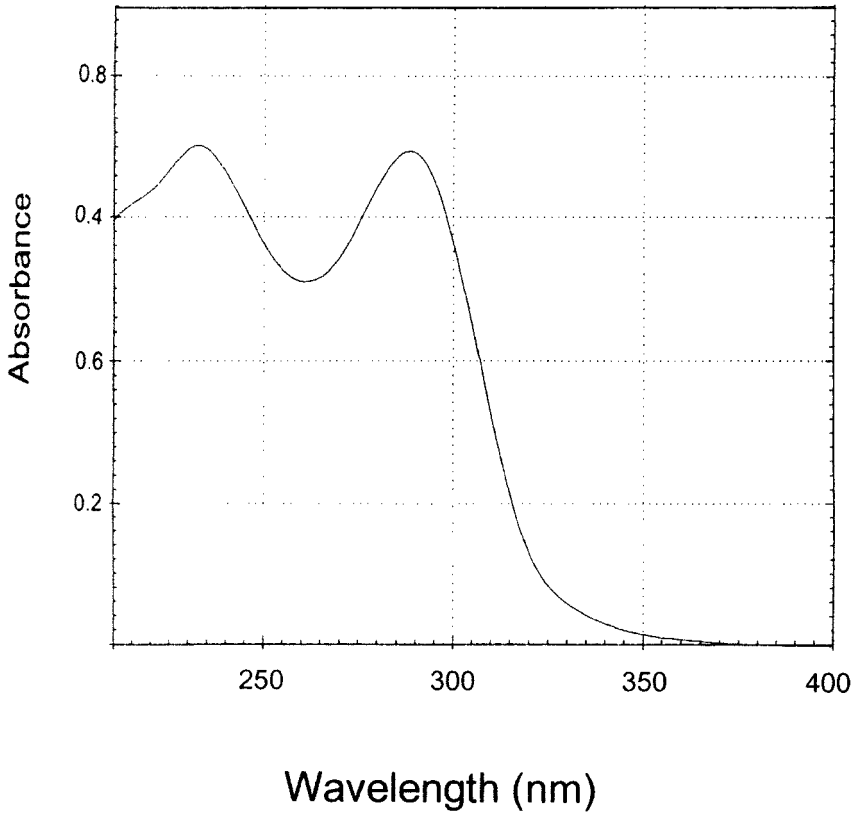


Figure 5. UV absorption spectrum of the E-isomer of cefixime trihydrate, obtained at a concentration of 15.77 μg/mL (31.07 μM) in aqueous solution.

The spectrum of the E-isomer consists of two relatively strong absorption bands. One of these is located at 289 nm (molar absorptivity equal to 22,333 L/mol), and the other is observed at 233 nm (molar absorptivity of 22,581 L/mol).

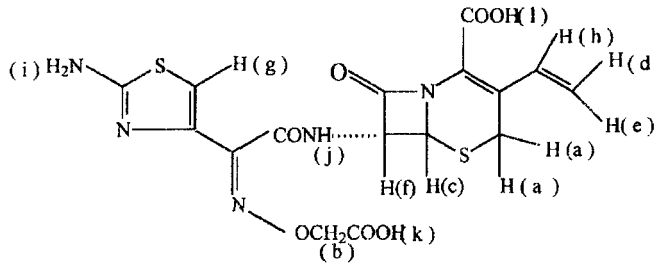
3.9.2 Vibrational Spectroscopy

The infrared absorption spectra of the Z- and E-isomers of cefixime were obtained using a Horiba FT-200 spectrophotometer, where the KBr pellet method of sample preparation was used to prepare the samples. The spectra are illustrated in Figures 6 and 7, and a table of band assignments for the Z-isomer is provided in Table 2. As would be expected, the two isomers may be differentiated on the basis of their absorption bands in the C=N oxime region.

3.9.3 Nuclear Magnetic Resonance Spectrometry

3.9.3.1 ^{1}H-Spectrum

The ^{1}H-NMR spectra of the Z- and E-isomers of cefixime were obtained using a 200 MHz Bruker Instruments model AC 200P NMR spectrometer. The data were obtained at ambient temperature in DMSO-d_6, at a concentration of 100 mg/mL, and were references to tetramethylsilane. The one-dimensional spectra are shown in Figures 8 and 9, and a summary of the band assignments is collected in Table 3. The nomenclature of the assignments is based on the following alphabetical designation:



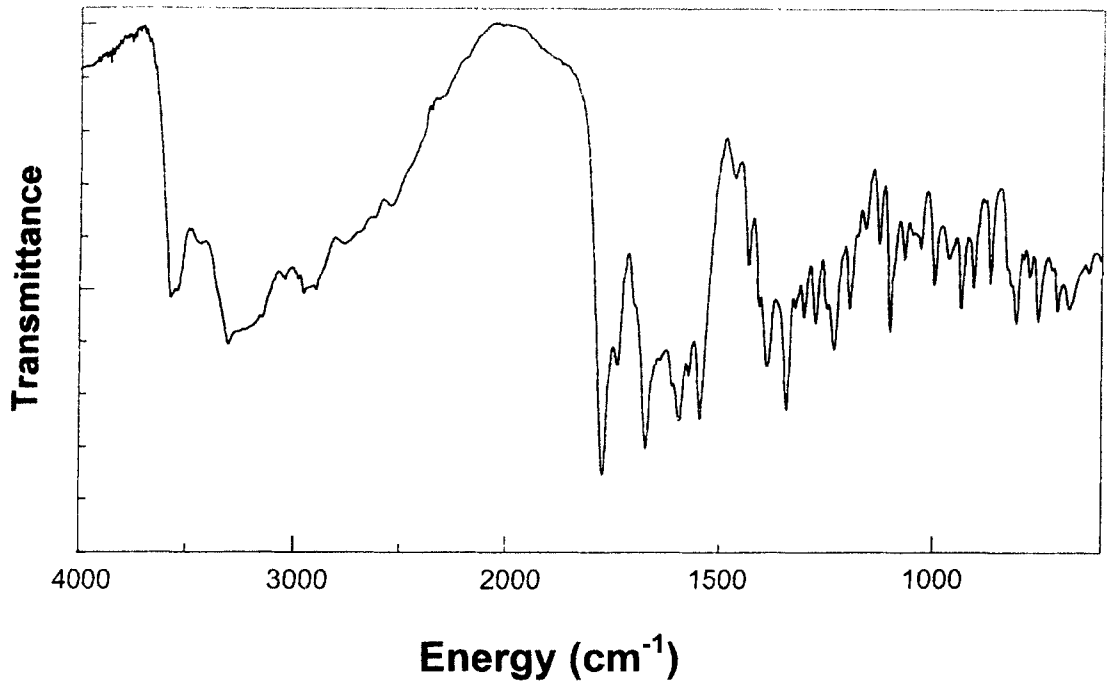


Figure 6. Infrared absorption spectrum of the Z-isomer of cefixime trihydrate.

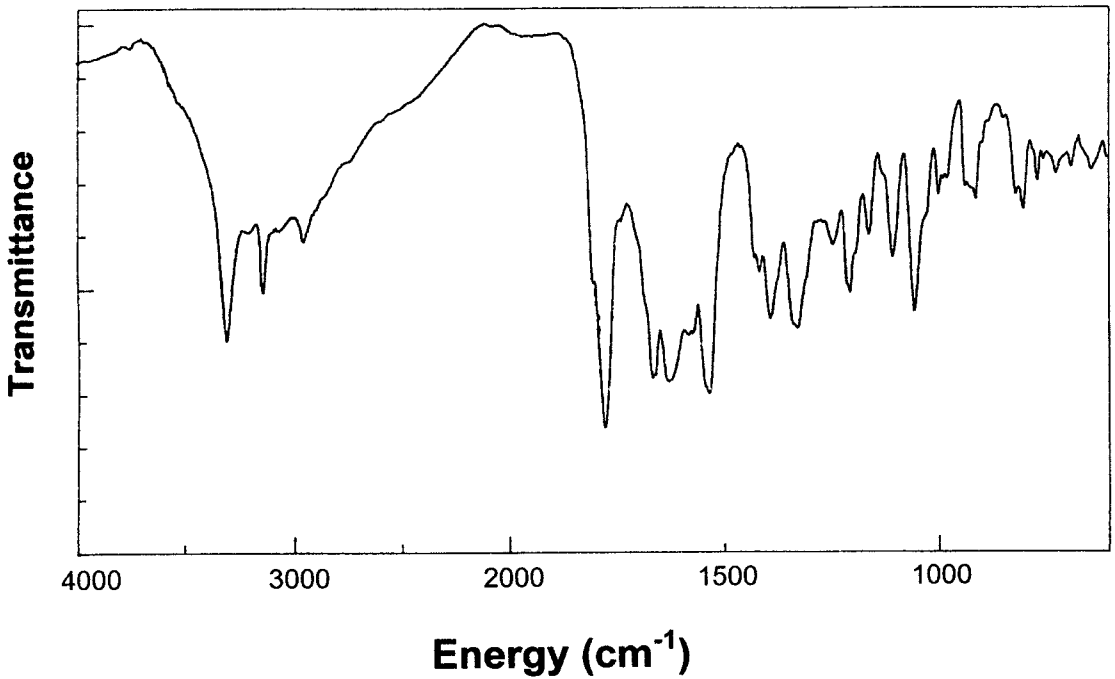


Figure 7. Infrared absorption spectrum of the E-isomer of cefixime trihydrate.

Table 2

Assignments for the Vibrational Transitions of Cefixime Trihydrate

Energy (cm^{-1})	**Band Assignment**
3566, 3531	NH stretch of the hydrogen-bonded amide group
3295	symmetric and antisymmetric NH stretches of the carbamate NH_2 group
2946	6-H, 7-H stretching modes in the β-lactam ring
2946	CH_3 asymmetric stretching mode
1772	β-lactam C=O stretching mode
1735	carbamate C=O stretching mode
1670	amide I C=O stretching mode
1591	oxime C=N stretching mode
1457	CH_3 deformation in the CH_3O group
1336	carbamate NH_2 bending mode
1095	C-O stretch in the CH_3O group
1063, 1025	C-O and N-O stretches of the carbamate and oxime moieties in the CH_2O groups

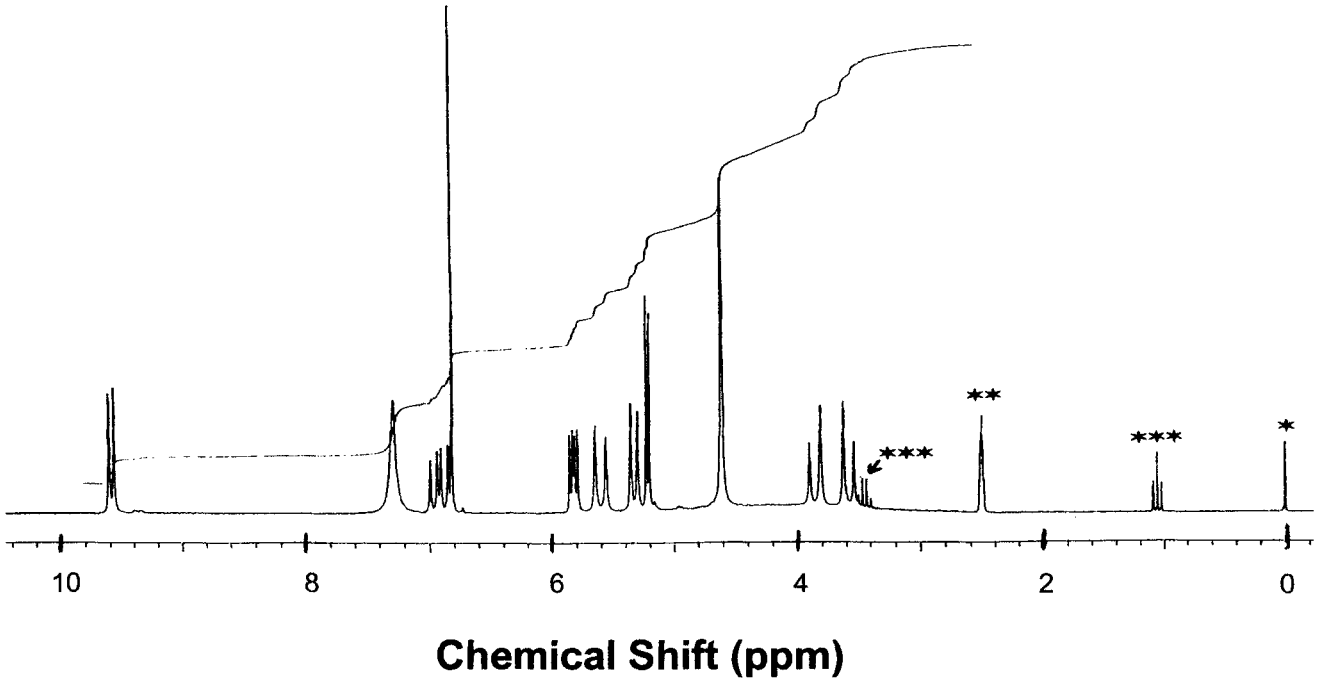


Figure 8. ^{1}H-NMR spectrum of the Z-isomer of cefixime trihydrate. Additional peaks noted are due to tetramethylsilane (*), dimethylsulfoxide (**), and residual ethanol (***).

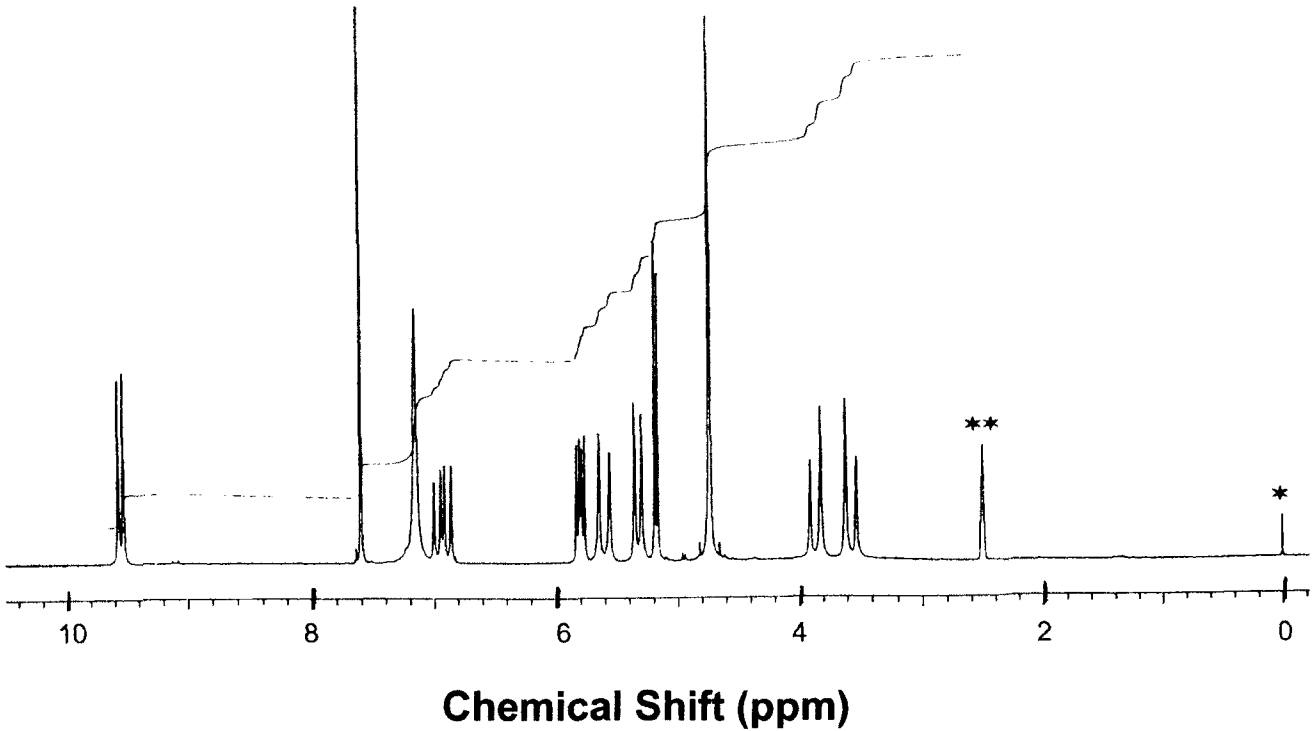


Figure 9. ^{1}H-NMR spectrum of the E-isomer of cefixime trihydrate. Additional peaks noted are due to tetramethylsilane (*) and dimethylsulfoxide (**).

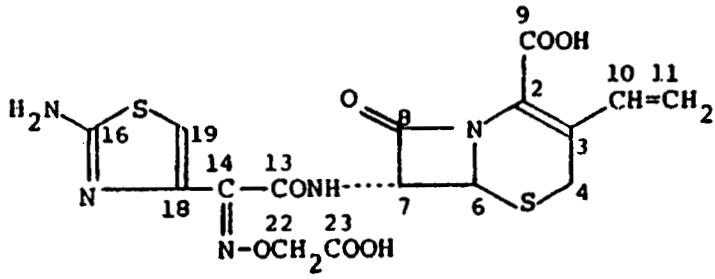
Table 3

Assignments for the Observed ^{1}H-NMR Resonance Bands of Cefixime

Chemical Shift (ppm)	**Multiplicity**	**Relative number of protons**	**Proton Assignment**
9.6	doublet	1	j
7.28	broad	2	i
6.93 - 6.98	multiplet	1	h
6.72	singlet	1	g
5.78 - 5.84	quartet	1	f
5.55	doublet	1	e
5.29	doublet	1	d
5.22	doublet	1	c
4.60	singlet	2	b
3.53	quartet	2	a

3.9.3.2 ^{13}C-Spectrum

The ^{13}C-NMR spectra of the Z- and E-isomers of cefixime were obtained using the spectrometer and data collection conditions as described for the ^{1}H-NMR spectra. The one-dimensional spectra are shown in Figures 10 and 11, and a summary of the band assignments is collected in Table 4. The nomenclature of the assignments is based on the following numerical designation:



As in the ^{1}H-NMR spectra, the spectral differences between the two isomers are all related to the configuration of the oxime group.

3.9.4 Mass Spectrometry

The mass spectra of the Z- and E-isomers of cefixime were obtained using a Perspective Biosystems Voyager Elite instrument, and are shown in Figures 12 and 13. The spectrum of cefixime is characterized by the presence of an intense protonated molecular ion at m/z 454 (MH^+), and other prominent fragment ions at m/z 329, 285, 210, and 126. Less prominent ions were found to occur at m/z 301, 252, 245, 241, 227, 207, 179, and 170.

4. Methods of Analysis

4.1 Compendial Tests

The compendial tests for USP cefixime are given in the drug substance monograph [13].

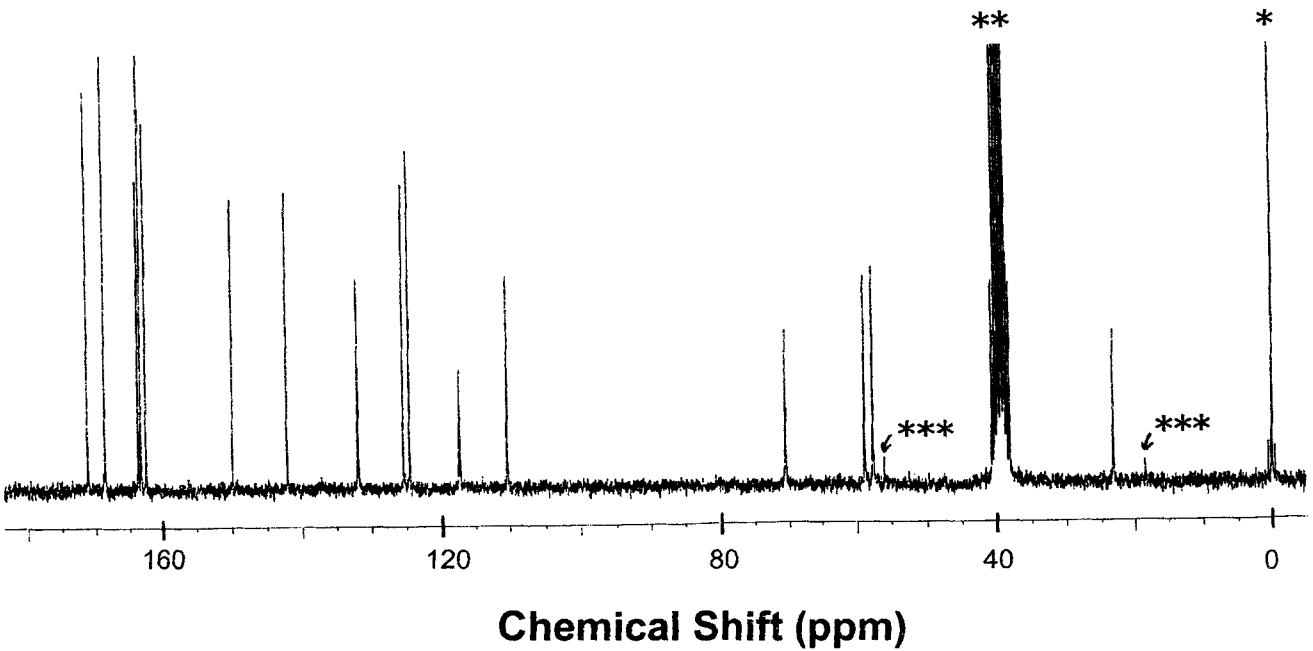


Figure 10. ^{13}C-NMR spectrum of the Z-isomer of cefixime trihydrate. Additional peaks noted are due to tetramethylsilane (*), dimethylsulfoxide (**), and residual ethanol (***).

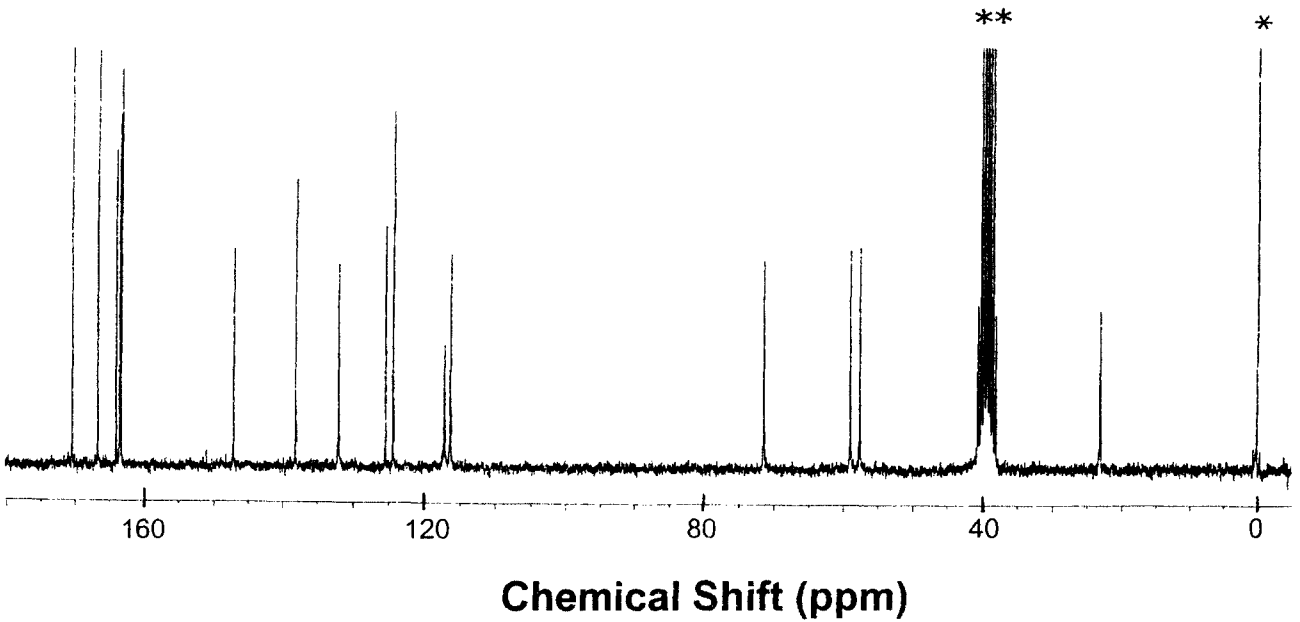


Figure 11. ^{13}C-NMR spectrum of the E-isomer of cefixime trihydrate. Additional peaks noted are due to tetramethylsilane (*) and dimethylsulfoxide (**).

Table 4

Assignments for the Observed ^{13}C-NMR Resonance Bands of Cefixime

Carbon Number	Chemical Shift, Z-Isomer (ppm)	Chemical Shift, E-Isomer (ppm)
4	23.19	23.06
6	57.57	57.51
7	58.79	58.91
22	70.43	71.33
19	110.25	116.33
11	117.30	117.16
3	124.43	124.30
2	125.34	125.37
10	131.84	131.91
18	141.96	138.01
14	149.76	146.98
13	162.35	163.34
9	163.10	163.14
8	163.50	163.81
16	168.42	166.46
23	171.02	170.23

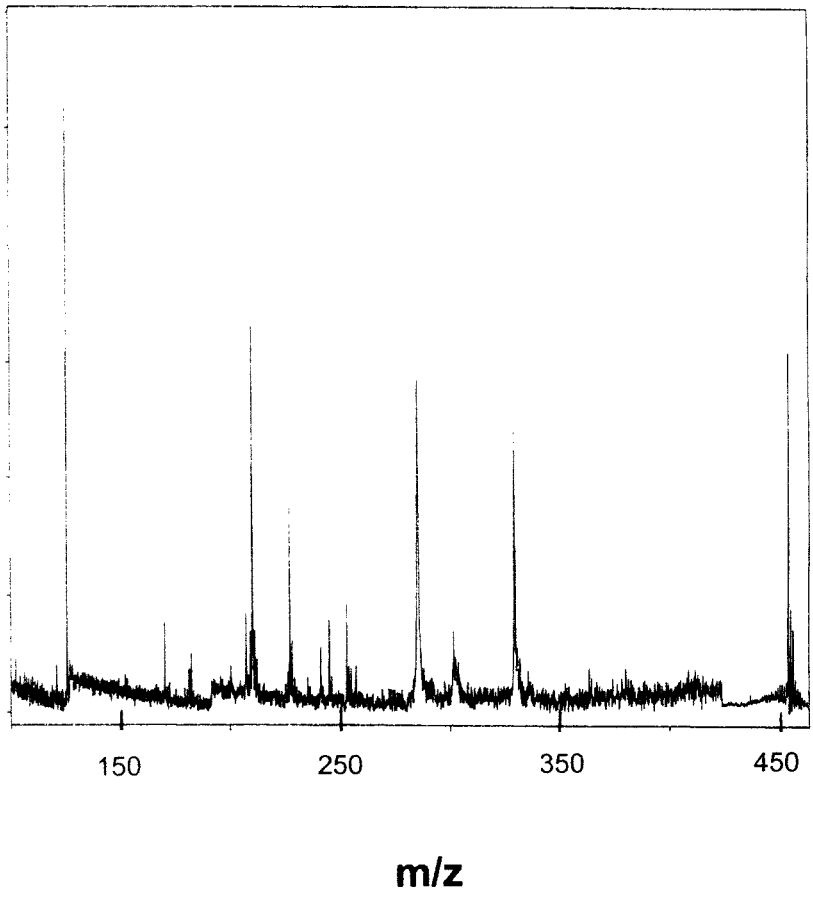


Figure 12. Mass spectrum of the Z-isomer of cefixime trihydrate.

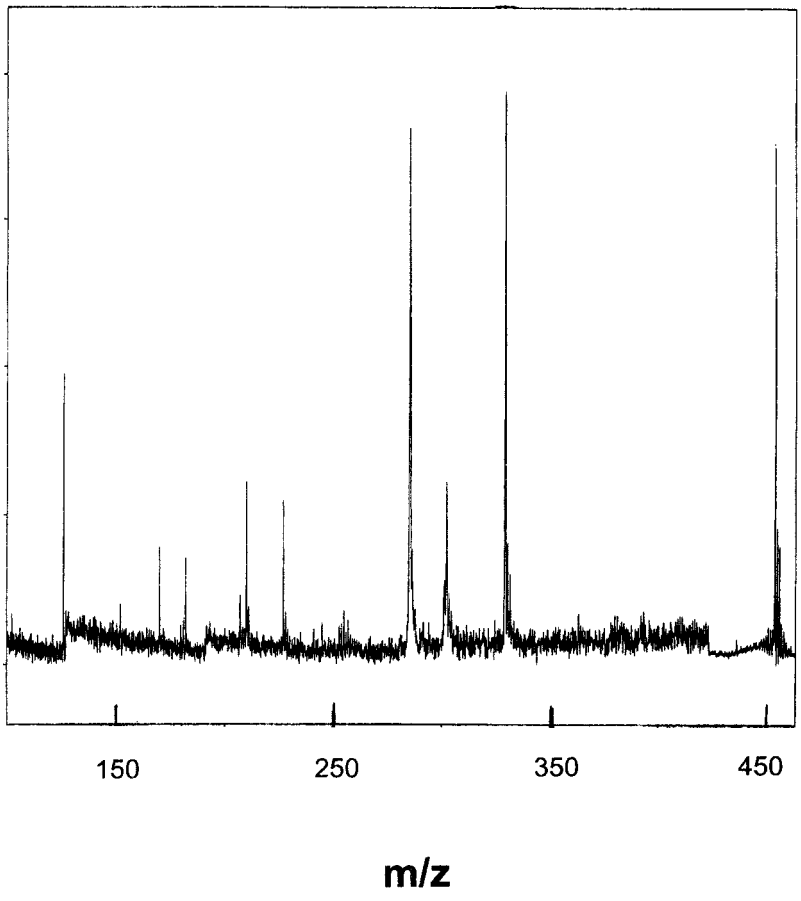


Figure 13. Mass spectrum of the E-isomer of cefixime trihydrate.

4.1.1 Identification

Dissolve about 5 mg of solid by trituration in 2 mL of methanol, and evaporate to dryness with the aid of gentle heat. The infrared absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Cefixime reference standard.

4.1.2 Specific Rotation

The specific rotation is determined according to USP general test <781S>, and should be between -75° and -88°, calculated on an anhydrous basis. The solution concentration is prepared at 10 mg/mL, in sodium bicarbonate solution.

4.1.3 pH

The pH is determined according to USP general test <791>, and should be between 2.6 and 4.1, in a solution containing the equivalent of 0.7 mg/mL of cefixime.

4.1.4 Water Content

When determined according to USP general test <921>, Method I, the water content should be between 9.0% and 12.0%.

4.1.5 HPLC Assay Method

To perform the assay determination, several solutions are required. These are prepared as follows:

Tetrabutylammonium hydroxide solution: Dilute 25 mL of 0.4 M tetrabutylammonium hydroxide solution to 1000 mL with water, and adjust with 1.5 M phosphoric acid to a final pH of 7.0.

Mobile phase: Prepare a suitable filtered and degassed 775:225 v/v mixture of tetrabutylammonium hydroxide solution and acetonitrile.

pH 7.0 buffer: Add 6.8 mL of phosphoric acid to 300 mL of water, adjust to pH 7.0with 10 N sodium hydroxide, dilute to 1000 mL with water, and mix.

Resolution Solution: Dissolve USP cefixime reference standard in water to obtain a solution concentration of approximately 1 mg/mL. Heat this solution at 95°C in an oil bath for 45 minutes, cool, and use promptly.

Standard Solution: Dissolve an accurately weighed quantity of USP cefixime reference standard in pH 7.0 buffer to obtain a solution having a known concentration of about 0.2 mg/mL cefixime. Use this solution promptly.

Sample Solution: Transfer about 110 mg of accurately weighed cefixime to a 50-mL volumetric flask, dilute with pH 7.0 buffer to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with pH 7. 0 buffer to volume, and mix. Use this solution promptly.

Analyte detection is effected on the basis of the UV absorbance at 254 nm. The column separation material octadecyl silane, chemically bonded to porous silica or ceramic microparticles, which are 3 μm in diameter, and is eluted at a flow rate of 1.5 mL/min.

To verify system performance, chromatograph the Resolution Solution, and record the peak responses. The resolution between the cefixime Z-isomer peak and the cefixime E-isomer peak must not be less than 1.1. Then chromatograph the Standard solution, and verify that the column efficiency is not less than 650 theoretical plates, that the capacity factor is not less than 5 and not more than 11, that the tailing factor for the analyte peak is not less than 0.85 and not more than 1.5, and that the relative standard deviation for replicate injections is not more than 2.0%. To assay samples, separately inject equal volumes of the Standard Solution and the Sample Solution onto the HPLC system, record the chromatograms, measure the responses for the major peaks, and calculate the quantity of cefixime in the sample.

4.2 Identification

The USP monograph for cefixime provides a infrared spectroscopic procedure for the identification of this compound, which has been described in section 4.1.1.

4.2.1 Colorimetry

A simple and easy to perform colorimetric identification test has been described in the Requirements for Antibiotic Products of Japan [12]. An aqueous sodium bicarbonate solution of cefixime is added to an alcoholic solution of hydroxylamine hydrochloride, allowed to stand for five minutes, and shaken with 1 mL of acidic ferric ammonium sulfate. The development of a red-brown color is taken as a positive reaction.

In another simple test, 1 mL of dilute hydrochloric acid and 1 mL of a freshly prepared solution of aqueous sodium nitrite is added (cooled in ice) to an aqueous sodium bicarbonate solution of cefixime. After a period of two minutes, 1 mL of ammonium sulfamate solution is added to the cooled solution, which is shaken and allowed to stand for 1 minute. After this, 1 mL of N-(1-naphthyl) ethylenediamine hydrochloride solution is added, whereupon a red-purple color develops.

4.2.2 UV Spectroscopy

A 1 in 62,500 solution of cefixime in 0.1 *M* phosphate buffer (pH 7.0) exhibits absorption maxima at 288 and 230 nm, and a minimum around 253 nm.

4.3 Elemental Analysis

The elemental analysis of cefixime trihydrate has been reported by Takaya *et al.* [7], and is summarized in Table 5.

Table 5

Elemental Analysis of Cefixime Trihydrate

	% Expected	% Found
C	37.87	37.95
H	4.17	4.05
N	13.80	13.73
S	12.63	12.38
O	31.53	31.89 (by difference)

Note: The theoretical water content of the trihydrate phase is 10.65%.

4.4 Microbiological Analysis

A cylinder-plate method using *Bacillus subtilis* ATCC 6633 as the test organism has been described in the Requirements for Antibiotic Products of Japan [12]. 0.1 *M* phosphate buffer (pH 7.0) is specified as the diluting medium for preparing cefixime solutions.

4.5 High Performance Liquid Chromatography

The official pharmacopeial procedure for the assay of cefixime is by HPLC [13], and has been fully described in section 4.1.5. The method is interesting in that the system suitability is monitored by an *in situ* generation of the E-isomer, which is obtained by the heating an aqueous solution of the Z-isomer at 95°C for about 45 minutes.

Another HPLC procedure used in the monitoring of cefixime degradation products uses a mobile phase mixture of acetonitrile and tetrabutylammonium phosphate (pH 7.0; 9:31 and 11: 29), and an

octadecylsilane column. Analyte detection is effected on the basis of the UV absorbance at 254 nm [11].

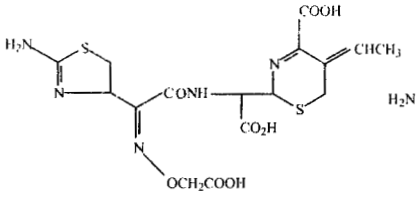
4.6 Determination in Body Fluids and Tissues

Silber *et al.* have developed and validated a simple, accurate, and precise reverse-phase liquid chromatography procedure for the determination of cefixime in human serum and urine [14]. The procedure involves a sample preparation wherein protein is precipitated by the addition of a 6% solution of trichloroacetic acid, and 7-hydroxycoumarin is used as an internal standard. The liquid chromatograph is equipped with a column containing octadecyl silane chemically bonded to porous silica or ceramic particles, and a UV detector that can be set at 280 nm (for the serum assay) or 313 nm (for the urine assay). The mobile phase for the serum assay consists of a 17:83 mixture of acetonitrile and aqueous monobasic sodium phosphate. while the mobile phase for the urine assay consists of a 20:80 mixture of acetonitrile and aqueous monobasic sodium phosphate.

5. Stability

5.1 Drug Substance Stability

The degradation of cefixime under a variety of exposure conditions has been reported by Koda *et al.* [10,11]. Using reverse-phase, preparative chromatography, these workers isolated the degradation products produced at acidic (pH 1.1), neutral (pH 6.05), and alkaline (pH 8.84) solutions. The structures of the six major degradation products are illustrated in Figure 14, and were determined using infrared spectroscopy, ^{1}H-NMR spectrometry, mass spectroscopy, and ultraviolet absorption spectroscopy. Compounds **21** and **22** are the degradation products formed under alkaline conditions, while compounds **23**, **24**, and **25** are the degradation products formed under acidic conditions. The only degradation product that is formed at neutral conditions is compound **26**, which is formed slowly, is rather unstable, and is prone to polymerization. The structures of the degradation products suggest that the β-lactam ring in cefixime is susceptible to ring opening under all conditions.



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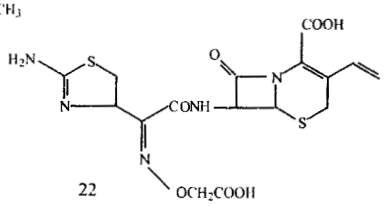

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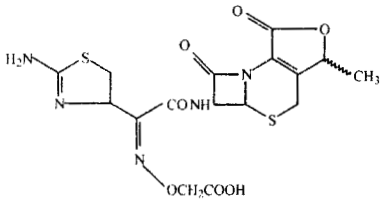

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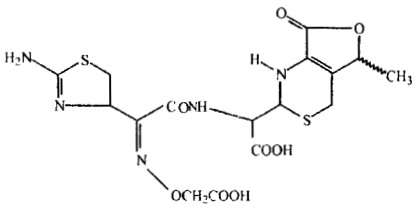

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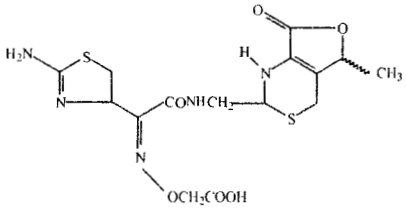

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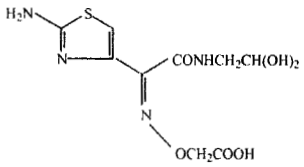

26

Figure 14. Major degradation products of cefixime.

5.2 Effect of Grinding on the Stability

Cefixime crystallizes with three waters of hydration, which is the most stable crystalline form. The water molecules in the crystal lattice are considered to play a significant role in the chemical stability of cefixime, as the dihydrate and anhydrate forms have been found to be less stable than is the trihydrate form [8]. Kitamura *et al.* have studied the effect of grinding on the solid-state stability and physicochemical properties of cefixime trihydrate, using x-ray diffraction, scanning electron microscopy, differential scanning calorimetry, and color measurements [9]. It was found that the crystalline trihydrate phase changes to a non-crystalline solid when the trihydrate is milled for four hours in a ball mill, as evidenced by the decrease in x-ray diffraction peak intensities that accompany prolonged grinding times. The dehydration temperature of ground cefixime trihydrate has been found decrease with increasing grinding times.

5.3 Effect of Humidity on the Stability

Studies conducted on the effects of humidity on the solid-state stability of cefixime trihydrate at 50°C and 70°C have shown that the decomposition of drug components and solid dosage forms depends both on temperature and humidity [10]. It was reported that the loss of drug according to the decomposition pathway which exists at 0% relative humidity is accelerated when the environmental relative humidity is increased. This finding would explains the observation of Mooney *et al.* that cefixime trihydrate stored in fiber-board drums (which allow the diffusion of moisture at higher temperature) decomposed at a more rapid rate than did material stored in glass vials [8]. It appears a relative humidity of approximately 20% at the storage temperature may be critical for the optimal stability of cefixime trihydrate.

6. Drug Metabolism and Pharmacokinetics

6.1 Absorption

Cefixime is well absorbed from the gastrointestinal tract. Following oral administration, the drug reaches a peak plasma concentration between 2 to

6 hours [15-18]. Better bioavailability was obtained with an oral 200 mg suspension, as compared to a 200 mg tablet. The reported peak serum concentrations were 3.4 ± 0.9 mg/L and 3.0 ± 0.9 mg/L for suspension and tablet, respectively.

In another report, a 200 mg oral solution was compared to an oral capsule, and the resultant peak serum concentrations (C_{max}) were found to be 3.22 and 2.92 mg/L, respectively [19]. Plasma peak levels were reached in 3 hours for a 400 mg dose aqueous solution, and in 4 hours for a 400 mg dose tablet [15]. The area under the serum concentration time curve (AUC) was reportedly larger for solution or suspension formulations than for tablets or capsules [18,19]. The absolute bioavailability of cefixime administered in 200 mg and 400 mg oral doses was found to range between 40.2 to 52.3% [19]. The percent of cefixime absorbed after 200 mg and 400 mg doses was determined to be similar and comparable [19,20]. A comparison of 200 mg administered intravenously or orally produced a mean AUC of 47.0 mg•hr/L and 26.0 mg•hr/L respectively [19].

Food has been reported to prolong the time required to reach peak plasma concentrations [16]. Following a 400 mg oral dose, cefixime given with food required approximately one hour longer to reach C_{max} than did cefixime administered to fasted subjects [20,21]. However, Faulkner *et al.* concluded from their work that cefixime may be administered with or without food. It was reported elsewhere that about 40 to 50% of an oral dose is absorbed when administered with or without food [22,23]. However, the time of maximal absorption is found to increase to about 0.8 hours when cefixime is given with food [22-24]. The C_{max} and AUC parameters may be greater in older patients as compared to younger patients, although Faulkner *et al.* reported that there was no need for dosage adjustment in the elderly [25].

6.2 Distribution

Cefixime is readily distributed in most tissues and fluids. The drug concentration in sputum was reportedly very low (0.07 mg/L) after a 400 mg single dose, but reached acceptable levels in bronchial mucosa (2.4 mg/L) after repetitive 400 mg per day dosing [16]. Accumulation was not

evident in the serum or urine of volunteers after 15-days administration of cefixime at 400 mg/day [16,26].

6.3 Elimination

Close to 50% of the absorbed dose has been found to be excreted unchanged in urine [19,22-24]. Between 12 to 20% of a 200 mg dose of Cefixime was recovered unchanged in urine in 24 hours [16]. The total clearance after oral administration of a single dose of 200 mg was reported to be 9.7 L/hr, and 11.4 L/hr after a single dose of 400 mg [19]. The total systemic clearance was found to be approximately 4.4 L/hr after a single intravenous dose of 200 mg, of which renal clearance accounted for 40% [19]. Faulkner *et al.* found the elimination half-life to range between 3-4 hours, and to be dose independent [19-21]. Brittain *et al.* also reported the elimination half-life for the 200 mg and 400 mg doses to be 3 hours [27].

6.4 Effect of Age and Renal Impairment

Faulkner *et al.* compared 12 young and 12 elderly subjects in order to determine the effect of age on cefixime pharmacokinetics. The subjects were administered with a 400 mg tablet daily for 5 days. The mean C_{max} parameters on days one and five were found to be 4.90 and 5.68 mg/L for elderly subjects, and 3.88 and 4.74 mg/L for young subjects. The serum AUC values on days one and five were 41.0 and 49.5 mg•hr/L for the elderly subjects, and 28.6 and 34.9 mg•hr/L for the young subjects. It was also observed that the mean elimination half-life, mean residence time, average concentration, minimal concentration, and renal clearance values were significantly higher (P less than 0.05) in the elderly [25].

Dhib *et al.* reported on the effect of renal function on orally administered cefixime. Their investigation on patients with various degrees of renal insufficiency found the C_{max} and T_{max} (time to reach peak plasma concentration) to be slightly increased. The elimination half-life was increased to 12-14 hours in patients with endogenous creatinine clearance below 20 mL/min, and the apparent volume of distribution was decreased. In addition, the apparent total and renal clearances were lower in proportion to the degree of renal insufficiency. Their overall observation

was that cefixime dosing should be reduced in patients with severe renal failure [17].

7. Acknowledgments

The authors wish to express their sincere thanks to Drs. Masanobu Kohsaka and Mamoru Fujioka of Fujisawa Pharmaceutical Co., Ltd., who have provided significant amounts of data reproduced in this chapter.

8. References

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CLOMIPHENE CITRATE

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ANALYTICAL PROFILES OF DRUG SUBSTANCES AND EXCIPIENTS—VOLUME 25
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Contents

1. Description

1.1 Nomenclature

1.1.1 Chemical Names

Clomiphene citrate:

2-[*p*-(2-chloro-1,2-diphenylvinyl)phenoxy]triethylamine citrate (1:1) [1,2]

2-[2-chloro-1,2-diphenylethenyl)-phenoxy]-N,N-diethylethanamine 2-hydroxy-1,2,3-propanetricarboxylate (1:1) [1]

Ethanamine, 2-[4-(2-chloro-1,2-diphenylethenyl)-phenoxy]-*N,N*-diethyl-, 2-hydroxy-1,2,3-propanetricarboxylate (1:1) [2]

A mixture of the *E* and *Z* isomers of 2-[4-(2-chloro-1,2-diphenylvinyl)-phenoxy]-triethylamine dihydrogen citrate [3,4]

Clomiphene:

2-[4-(2-chloro-1,2-diphenylethenyl)-phenoxy]-*N,N*-diethylethanamine [5]

A mixture of the *E* and *Z* isomers of 2-[*p*-(2-chloro-1,2-diphenylvinyl)-phenoxy]-triethylamine [5,6].

1.1.2 Proprietary Names

Clomiphene Citrate: *Clomid, Clomivid, Clomphid, Clostilbegyt, Dyneric, Ikaclomine, Milophene,Omifin, Pergotime, Prilofen, Serophene* [3,5,6].

Clomiphene: *Chloramiphene, Clomifene* [6].

Clomiphene *E* isomer: *Transclomiphene, Enclomiphene*

Clomiphene *Z* isomer: *Cisclomiphene, Zuclomiphene*

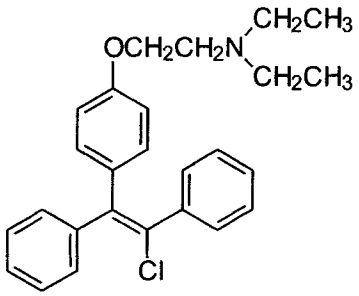
1.2 Formulae

1.2.1 Empirical

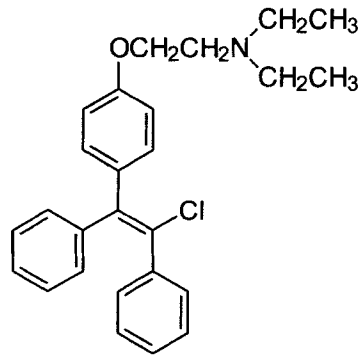
Clomiphene citrate: $C_{26}H_{28}ClNO \bullet C_6H_8O_7$ [3]

Clomiphene: $C_{26}H_{28}ClNO$ [5]

1.2.2 Structural



E-clomiphene
enclomiphene
trans-clomiphene



Z-clomiphene
zuclomiphene
cis-clomiphene

1.2.3 CAS Registry Numbers

911-45-5	(clomiphene)
50-41-9	(clomiphene citrate)
15690-57-0	(*E*-clomiphene)
7599-79-3	(*E*-clomiphene citrate)
15690-55-8	(*Z*-clomiphene)
7619-53-6	(*Z*-clomiphene citrate) [3,5]

1.3 Molecular Weights

Clomiphene citrate: 598.1 [1-5]

Clomiphene: 405.9 [6]

1.4 Elemental Composition

	Clomiphene citrate	Clomiphene
C	64.3%	76.9%
H	6.1%	7.0%
Cl	6.0%	8.7%
N	2.3%	3.5%
O	21.4%	3.9%

1.5 Appearance, Color, and Odor

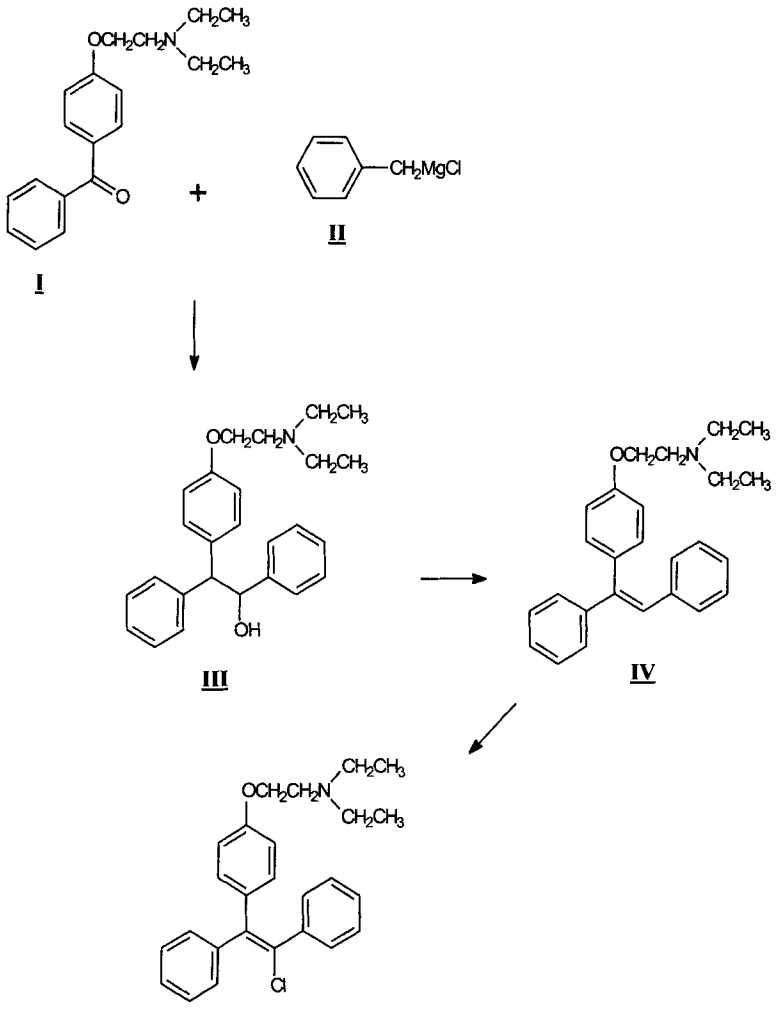
Clomiphene citrate is a yellowish-white, odorless or almost odorless powder [3,4,6,7].

Clomiphene free base is a white to pale yellow solid [6].

2. Method of Preparation

Clomiphene is a triarylethylene compound, prepared according to the procedure outlined in Scheme 1. Reaction of the benzophenone derivative (**I**) with benzylmagnesium halide (**II**), followed by dehydration of the resulting alcohol (**III**), leads to the triarylethylene (**IV**) [8-10]. Treatment of **IV** with *N*-chlorosuccinimide yielded clomiphene in good yield. This process produces a mixture of the geometrical isomers, which could be separated by repeated crystallization of their hydrochloride salts [10]. A stereospecific procedure which yields clomiphene enriched in either isomer is also available [8].

Scheme 1. Synthesis of E-clomiphene.



The preparation of carbon-14 labelled clomiphene, using ^{14}C labelled benzoyl chloride, has been reported [11,12], as has the preparation of tritium labelled clomiphene [13].

3. Physical Properties

3.1 Melting Range

Clomiphene free base melts at 111-115°C , while clomiphene citrate melts with decomposition at about 145°C [6]. However, the actual value measured for the melting point depends on the method used for its determination [7]. For example, if a capillary is inserted in a Berl-Kullmann apparatus at temperature 10°C below the expected melting point, and if the capillary is warmed at a speed of about 0.5°C per minute, the first melting transition occurs between 110-115°C. The material becomes solid again at 120°C, and a second melting transition occurs between 132-140°C.

The Kofler micro-hot stage provides the most distinct results if the powder is spread evenly with a cover glass, and if the temperature is held when melting first occurs (about 112°C). The Kofler hot bench gives an immediate sharp melting point, but when the powder remains on the bench for a longer time, lower melting points are obtained [7].

3.2 Solubility Data

Clomiphene is sparingly soluble in water and alcohol and slightly soluble in chloroform at 25°C [6]. Clomiphene citrate is slightly soluble in water and alcohol, is practically insoluble in diethyl ether [4-7], and is freely soluble in glacial acetic acid [4,6,7].

At room temperature, clomiphene citrate dissolves with great difficulty in most solvents. By gentle heating on a water bath, the compound can be dissolved in a few seconds [7]. After heating in a water bath, cooling, standing for 24 hours at room temperature, filtering, and then evaporating the solvent the following solubilities were obtained [7]:

Solvent	Solubility	Solubility, USP Definition
methanol	1 part in 3 parts	freely soluble
acetone	1 part in 40 parts	sparingly soluble
chloroform	1 part in 50 parts	sparingly soluble

3.3 Spectral Characteristics

3.3.1 Ultraviolet Absorption Spectroscopy

The ultraviolet absorption spectra of clomiphene citrate (15 mg/mL) dissolved in water and in methanol were recorded over a range of 200-350 nm, using a Shimadzu UV-160A UV-VIS spectrophotometer. As shown in Figure 1, the spectra obtained in water exhibit two absorption maxima, located at 230 and 287 nm. On the other hand, three absorption maxima at 205, 230, and 290 nm are observed in methanol. The spectra are in broad agreement with those shown in [6] and [7]. Solutions prepared in either solvent medium are stable for at least 24 hours at room temperature [7].

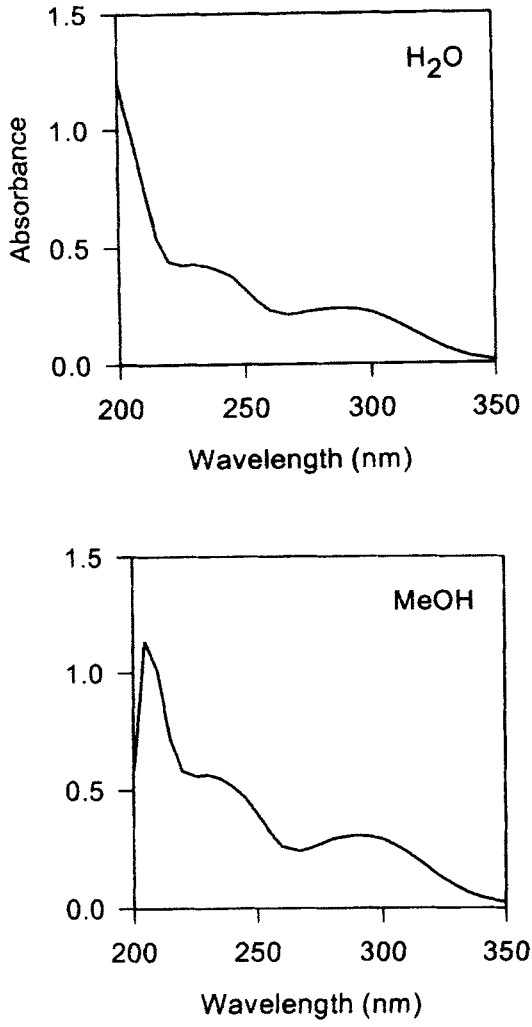
The British Pharmacopoeia reports that the UV spectrum of a 50 mg/mL solution of clomiphene citrate in 0.1 M hydrochloric acid exhibits absorption maxima at 235 nm and 292 nm when scanned between 220 and 350 nm [4]. The absorbance at 235 nm was about 1.58, while the absorbance at 292 nm was about 0.88 [4]. However, for spectrophotometric measurements, the use of hydrochloric acid solutions are less suitable, since clomiphene citrate reacts with HCl to give a precipitate consisting of oily droplets [7]. The oily precipitate becomes solid only after standing for several hours [7].

A molar absorptivity of 10,450 L mol^{-1} cm at 298 nm has been reported [14].

3.3.2 Infrared Spectroscopy

The infrared absorption spectrum of clomiphene citrate was in a KBr disk, was recorded on a Hitachi 270-30 infrared spectrophotometer, and is

Figure 1. Ultraviolet-visible absorption spectra of clomiphene citrate.



shown in Figure 2. The spectrum shows a strong, broad absorption band extending across the region 2500 - 3500 cm^{-1}, indicative of intermolecular hydrogen bonding of the clomiphene salt. The carbonyl group of the citrate counterion is responsible for the strong band as 1740 cm^{-1}. The British Pharmacopoeia provides a reference spectrum for clomiphene citrate over the region from 2000-400 cm^{-1}, which is equivalent with that shown in Figure 2 [4].

3.3.3 Mass Spectrometry

The electron impact (EI) mass spectrum of clomiphene was recorded using a JEOL JMS-DX300 mass spectrometer, using the following spectrometer conditions:

Acceleration voltage	3 kV
Ionization voltage	70 eV
Ionization current	0.1 mA
Ionization chamber temperature	150°C

The spectrum obtained in the present work is shown in Figure 3, which is consistent with those reported in the literature [15-17]. The spectrum contains a molecular ion at m/z=405 (relative intensity 9%), and the expected $(M+2)^+$ peak at m/z=407. Other prominent ions occur at m/z values of 252 (27%), 178 (16%), 100 (21%), and 86 (100%).

The fast atom bombardment (FAB) mass spectrum of clomiphene citrate was also recorded on the same instrument, and is presented in Figure 4. The sample was analyzed in a 3-nitrobenzylalcohol matrix and bombarded with xenon atoms at an acceleration voltage of 6 keV. The spectrum shows a major $(M+H)^+$ peak at m/z=406 and the expected $(M+H+2)^+$ ion at m/z=408.

3.3.4 NMR Spectroscopy

The relative insolubility of clomiphene citrate in water, the presence of two geometric isomers, and the potential interference from the citrate counterion, makes it difficult to obtain NMR data on clomiphene citrate. This situation may explain why no little or no literature data is available. For the present work, it was thought that spectra of the clomiphene free base would be more informative. Accordingly, the free base was prepared

Figure 2. Infrared spectrum of clomiphene citrate.

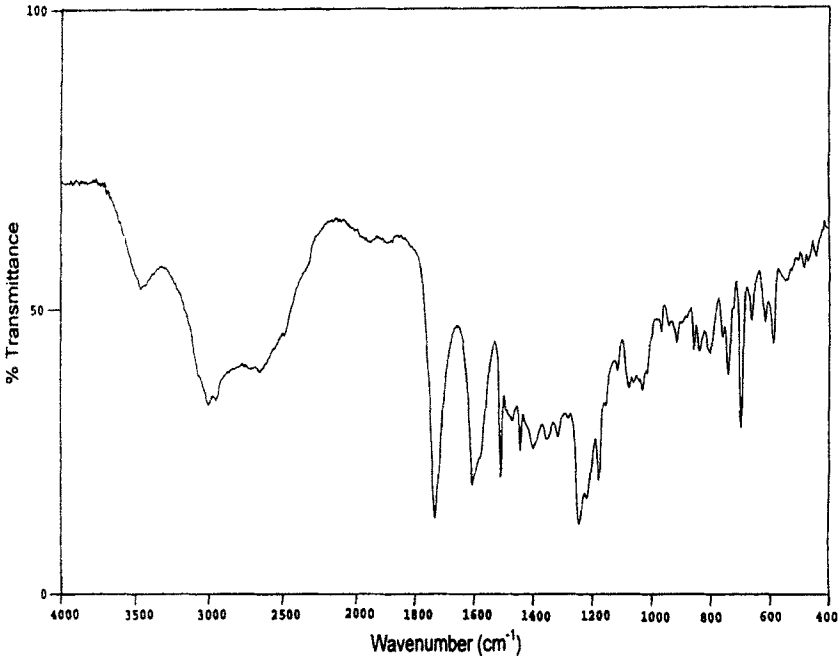


Figure 3. Electron impact mass spectrum of clomiphene citrate.

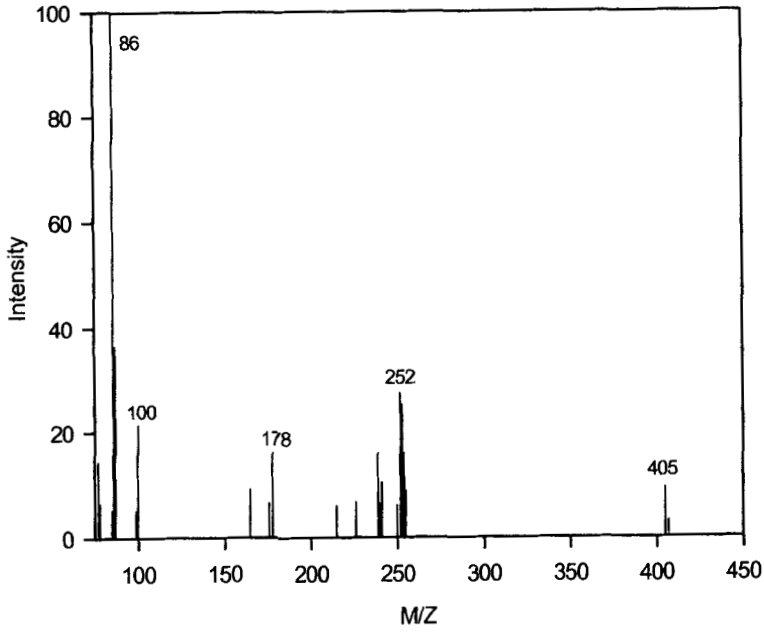
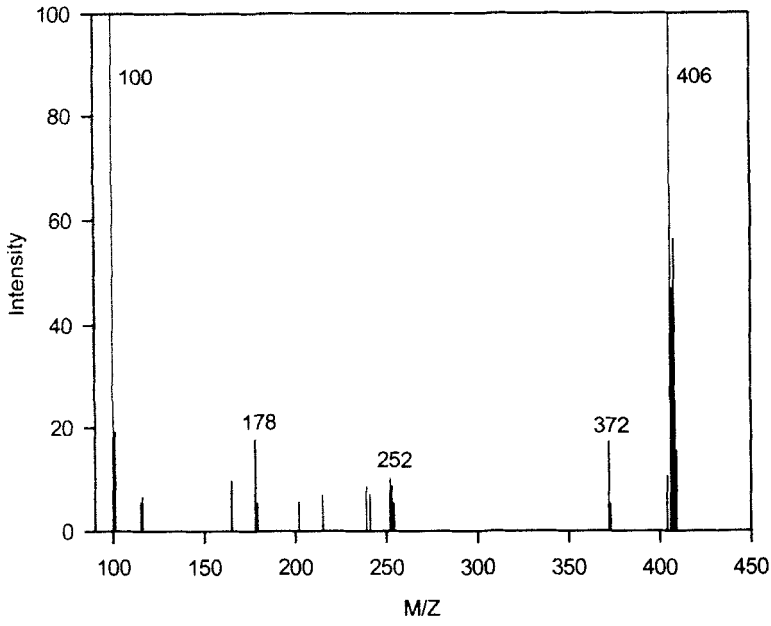


Figure 4. Fast atom bombardment mass spectrum of clomiphene citrate



by dissolving clomiphene citrate (Marion-Merrell-Dow lot S-38016) in 1M NaOH, extracting the liberated base with chloroform, drying the organic phase, and finally removing the solvent.

The ^{1}H-NMR spectrum of clomiphene in $CDCl_3$ was recorded on a Bruker AMX-300 (300 MHz) spectrometer operating at ambient temperature, and is shown in Figure 5. The chemical shift and spectral assignments of the clomiphene protons are presented in Table 1, with all data being reported relative to tetramethylsilane (TMS). As indicated above, the spectrum is complicated by the fact that commercial clomiphene exists as a mixture of the *E*- and *Z*-isomers. The non-equivalent proton environments provided by the two isomers are reflected in the ^{1}H NMR spectrum. In essence, there are two spectra, one for each isomer. This is particularly evident in that there are two well-separated sets of triplets at 4.0 ppm and at 2.9 ppm. The quartets at 2.65 ppm and the triplets at 1.05 ppm are less well separated and overlap in the spectrum. Usually the *E*-isomer is in present in greater quantity, so the downfield triplets in the well-resolved areas of the spectrum are assigned on the basis of integration to that isomer.

The ^{13}C-NMR spectrum of clomiphene in $CDCl_3$ was also recorded on a Bruker AMX-300 (75 MHz) spectrometer, again at ambient temperature and relative to TMS, using the JMOD pulse program. Using this pulse sequence, the primary and tertiary carbons are shown as positive peaks in the spectrum (Figure 6), while secondary and quaternary carbons appear as negative peaks. Again, the presence of two isomers ensures that the spectrum is complex, although some assignments can be made. These are presented in Table 2.

3.4 Crystal Structure

Prior to 1976, the *cis* stereochemistry was assigned to the *E*-isomer of clomiphene, as it exhibited ultraviolet absorption at a higher wavelength than did the *Z*-isomer [18-20]. The results of dipole moment studies appeared to be consistent with this assignment [18], but the NMR data were open to varying interpretations. These inconsistencies and uncertainties were clarified after 1976 through the use of single-crystal x-ray diffraction analysis [18-20].

Figure 5. ^{1}H NMR spectrum of clomiphene in $CDCl_3$.

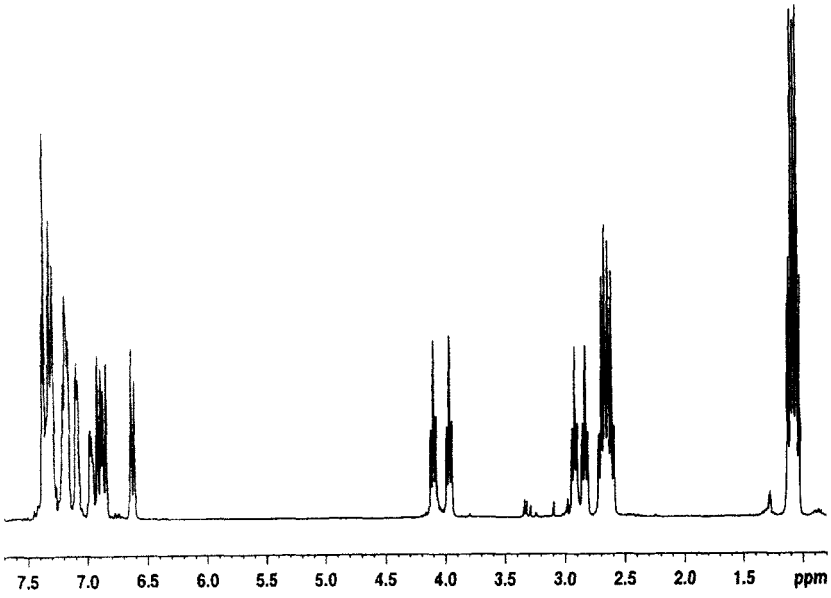
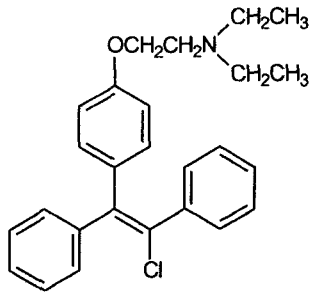


Table 1

^{1}H-NMR Characteristics of Clomiphene



Chemical Shift (ppm)	Multiplicity (number of hydrogens)	Assignment
1.03 - 1.12	2 x t (6H)	N-($CH_2C\underline{H}_3$)$_2$
2.59 - 2.72	2 x q (4H)	N-($CH_2C\underline{H}_3$)$_2$
2.81 - 2.94	2 x t (2H)	O-$CH_2C\underline{H}_2$-N
3.95 - 4.16	2 x t (2H)	O-$C\underline{H}_2CH_2$-N
6.61 - 7.39	m (14H)	Ar$\underline{H}$

Figure 6. ^{13}C NMR spectrum of clomiphene in $CDCl_3$.

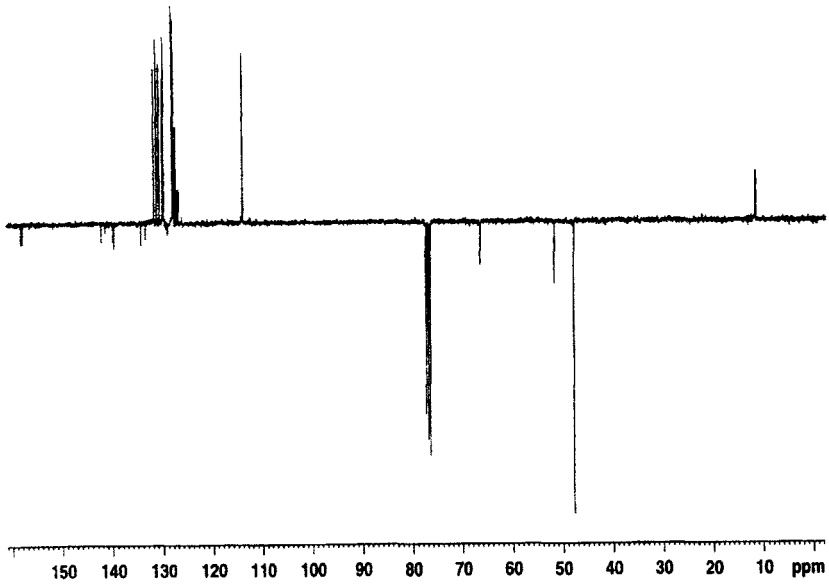
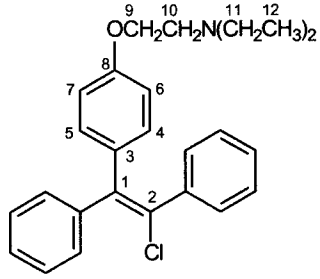


Table 2

^{13}C-NMR Characteristics of Clomiphene



Chemical Shift (ppm)	Assignment
11.8	C12
47.9	C11
51.8	C10
66.5	C9
114.0	C6, C7
126.9 - 142.8	C1, C2, ArC
158.7	C8

Crystals (having dimensions of 0.14 x 0.17 x 0.20 mm) of clomiphene hydrochloride were obtained from pentanone, and the intensity data were collected on a Nonius CAD-IV automatic diffractometer using graphite-monochromated Cu-Kα radiation (λ=1.5418 Å). The *E*-isomer of clomiphene HCl was found to exist in the $P2_1/c$, space group, with the unit cell containing four molecules and being characterized by a =15.564(1) Å, b=9.229(1) Å, c=18.285(2) Å, and β=116.40(1)°. The computed density was deduced to be 1.24 g cm^{-3}, while the measured density was determined as 1.25 g cm^{-3} [10]. From the data, the C(15)-C(1)-C(2)-C(21) torsion angle of 174.4° establishes the *trans* stereochemistry for the *E*-isomer, the ovulation inducing, anti-estrogenic isomer of clomiphene, and the *cis* stereochemistry for the *Z*-isomer. The structure derived from the data in reference [19] is shown in Figure 7.

3.5 Dipole Moment

Dipole moments (μ), measured with a Kahl DM 01 dipolmeter in benzene at 25°C were found to be 1.98 (*Z*-clomiphene) and 2.86 (*E*-clomiphene) [18].

3.6 Storage

Clomiphene citrate should be kept in a well-closed container and protected from light to avoid photo-isomerization. [1,2,7].

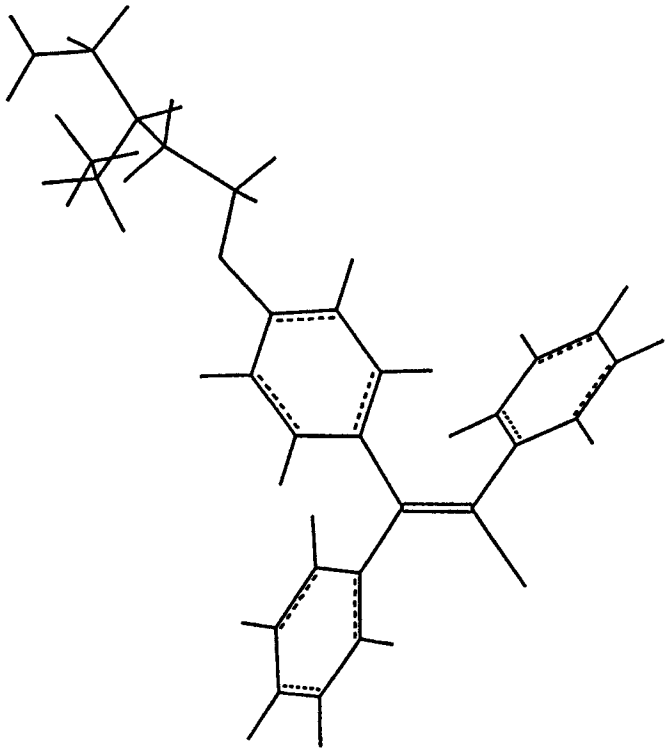
4. Methods of Analysis

4.1 Color Tests

4.1.1 Dissolve about 2 mg of clomiphene citrate in 1 mL of sulfuric acid, and a green color develops. This reaction is diagnostic for the clomiphene moiety[7].

4.1.2 Dissolve 5 mg of clomiphene citrate in 5 mL of a mixture consisting of 1:5 (v/v) acetic acid - pyridine and heat in a water bath. A dark, wine-red color is indicative of the citrate moiety [4,7].

Figure 7 X-ray structure of E-clomiphene.



4.1.3 A solution of 1.0 g clomiphene citrate in 30 mL of methanol is clear and colorless [1].

4.1.4 Reaction with the Liebermann reagent (1g of potassium nitrite dissolved in 10 mL sulfuric acid) yields a brown reaction product [6].

4.1.5 Reaction with the Mandelin reagent (0.5% aqueous solution of ammonium vanadate) test yields a violet to orange-brown reaction product [6].

4.1.5 Reaction with the Marquis reagent (2 drops of formaldehyde solution mixed with 1 mL of sulfuric acid) yields a violet-brown reaction product [6].

4.2 Assay

Two procedures have been described for the assay of clomiphene citrate.

4.2.1. Dissolve 300 mg (accurately weighed) of clomiphene citrate in 25 mL of glacial acetic acid (minimum 95.5% purity). Titrate with 0.1 M perchloric acid, using crystal violet as the indicator. 1.00 mL of 0.100 M perchloric acid is equivalent to 59.81 mg of clomiphene citrate [1,4,7].

4..2.2. Dissolve 100 mg (accurately weighed) of clomiphene citrate in 25 mL of neutralized methanol. Titrate with 0.1M NaOH using phenolphthalein as the indicator. 1.00 mL of 0.100 M NaOH is equivalent to 19.94 mg of clomiphene citrate [7].

4.3 Extraction

Diethyl ether is probably the most commonly employed solvent for the extraction of clomiphene [14,16]. For instance, clomiphene was extracted from 3 mL of plasma in 1 mL borate buffer, pH 9, with an extraction efficiency of 70% at 30 ng/mL [14].

Clomiphene isomers, in addition to metabolites, have been extracted using *tert*-butyl methyl ether (MTB) [21]. In a standard extraction, MTB (12 mL) was added to a 3 mL plasma sample in a screw-top centrifuge tube, and subjected to end-over-end rotation at 50 rpm for 30 minutes. The extraction efficiency was 98 ± 4% for plasma clomiphene concentrations exceeding 1ng/mL, and 85 ± 7% for concentrations less than 1ng/mL [21].

Chloroform also has been used to extract clomiphene and its metabolites from both bile [22] and plasma [15]. An extraction efficiency of 98% was reported for clomiphene at 1 ng/mL [15], and efficiencies of 96% and 85% were reported for 4-hydroxyclomiphene and *N*-desethylclomiphene, respectively, at 100 ng/mL [22].

4.4 Chromatography

4.4.1. Thin Layer Chromatography

Several TLC methods have been developed to detect clomiphene citrate [7,23], as well as clomiphene and its metabolites [16,24]. The salient details of these methods are detailed in Table 3. Analytes can be viewed under UV light, or with Dragendorrf's reagent [7].

4.4.2 Gas Chromatography

Table 4 provides a summary of the methods developed for the GC analysis of clomiphene. In most cases, derivitization was not necessary. Unfortunately, in many cases internal standards were not used and detection limits were not indicated. In one instance, it was stated that GC was much less sensitive than HPLC, and that GC did not allow for baseline separation of clomiphene isomers [25].

4.4.3. High Performance Liquid Chromatography

A number of HPLC methods have been developed, usually as a means of quantitating the geometric isomers of clomiphene. Both normal and reversed phase HPLC methods have been employed, with either UV or fluorescence detection. Details of the reported methods are provided in Table 5.

Table 3

Conditions Employed for the TLC Detection of Clomiphene

Stationary Phase	Eluent	R_f	Reference
Silica gel GF_{254}	methyl ethyl ketone / propionic acid / H_2O (75 : 25 : 30, v/v)	0.8	7
Silica gel 60 F_{250}	ethyl acetate / MeOH/ 30% ammonia (85 : 10 : 15 , v/v)	0.88	23
Silica gel 60 F_{250}	cyclohexane / toluene / diethylamine (65 : 25 : 10 , v/v)	0.64	23
Silica gel 60 F_{250}	acetone (plates pre-dipped in KOH)	0.66	23
Silica gel GF_{254}	chloroform / MeOH / 28% aqueous ammonia (95 : 5 : 0.5 , v/v)	0.73	10, 24

Table 4

Gas Chromatography Methods for the Determination of Clomiphene

Column / support	Detector	Derivitization	Reference
BP1	flame ionization detector	none	25
HP-5	mass-selective detector operating at m/e = 86.1	none	26
3% OV-17 on Gas-Chrom Q	flame ionization detector	N,O-bis(trimethyl-silyl)acetamide	10
3%-OV1 on Chromosorb W-HP	phosphorous nitrogen detector	none	27
3%-OV17 on Chromosorb W-HP	phosphorous nitrogen detector	none	27

Table 5

Conditions Employed for the HPLC Determination of clomiphene

Column	Mobile Phase	Detector	Internal Standard	Sensitivity	Ref.
DuPont Zorbax Sil silica, 6 μm	chloroform / MeOH (80 : 20)	UV, 298 nm	none	5 ng/mL	8
DuPont Zorbax Sil silica, 6 μm	chloroform / MeOH (80 : 20)	fluorescence (257 nm (excitation) (367 nm emission)	none	0.35 ng/mL	8
Spherisorb 10-μm silica OR 10-μm LiChrosorb Si 60	acetonitrile / dichloromethane (20 : 80) with 0.1% (w/v) methenamine	UV, 254 nm	none	not stated	28
μPorasil 10 μm	acetonitrile / dichloromethane(20 : 80) with 0.1% (w/v) methenamine	UV, 298 nm	none	not stated	25

Table 5

Conditions Employed for the HPLC Determination of clomiphene (Continued)

Column	Mobile Phase	Detector	Internal Standard	Sensitivity	Ref.
LC-8 Supelco 5 μm	MeOH / H_2O (80 : 20) with 2.3 mL phosphoric acid and 10 μL triethylamine / L	fluorescence (255 nm excitation) (378 nm (emission)	none	0.06 ng/mL	21
Porasil, 10 μm	n-hexane / alcohol-free chloroform / triethylamine (80 : 20 : 0.1)	UV, 302 nm	none	not stated	2, 4
Spherisorb nitrile S5CN 5 μM	acetonitrile / 0.01 M NaPi (pH 5.5) (40:60)	UV, 195 nm	none	0.35 ng	9

Note: Sensitivity refers to the plasma level of clomiphene.

Generally, fluorescence detection has permitted the attainment of much lower limits of detection than do UV methods. This situation has been attributed to the low molar absorptivity of clomiphene (a = 10,450 L/mol cm), and the superiority of the fluorescence response [20]. Based on studies conducted on tamoxifen, an assay was developed where, after elution from the column, clomiphene was passed through a photolysis coil irradiated by a powerful UV lamp. Clomiphene, a stilbene-based molecule, was converted to highly a fluorescent phenanthrene derivative [14]. The latter species was detected using a fluorescence spectrometer, and allowed the measurement of plasma clomiphene at levels lower than 1 ng/mL. This sensitivity was sufficient for monitoring in the therapeutic range of 50-250 ng/mL [9,14,21], and for pharmacokinetic studies where the levels may be considerably lower [14].

In a later study, the utility of the in-line photochemical reaction was again demonstrated [21]. However, it was shown in this work that pre-column irradiation of clomiphene isomers led to the formation of multiple fluorescent products, which required that that any irradiation be carried out in a post-column fashion. It was also claimed that the reversed phase mode provided greater reliability and reproducibility than did the normal phase mode, and resulted in the elution of clomiphene metabolites prior to the isomers [14]. While this situation would eliminate potential inter-injection wash-out of the highly retained metabolites which could occur in the normal-phase mode, it did result in considerably longer retention times (25 minutes as opposed to 7 minutes) [14,21].

A nitrile column has also been used to separate clomiphene from its 4-hydroxy and *N*-desethyl metabolites, while a second assay (which used a C-18 column) permitted separation of the metabolites of the individual isomers [22]. In the former assay, the clomiphene isomers eluted as a single peak.

5. Metabolism

In rabbit liver microsomes, *trans*-clomiphene (enclomiphene) is metabolized via *N*-dealkylation and 4-hydroxylation [16,22]. However, incubation of clomiphene with rat liver microsomes results in an additional *N*-oxidation pathway [24]. The three pathways, with their

corresponding metabolites, *N*-desmethylclomiphene, clomiphene *N*-oxide and 4-hydroxyclomiphene, are shown in Figure 8.

In both rats and humans, the principal route of elimination appears to be via the feces [22,24,29]. The drug and its metabolites may still be detected in humans, even after six weeks, suggesting enterohepatic recirculation [15,22]. In rats, fecal elimination was in the form of unchanged drug and 4-hydroxyclomiphene [24], while in the perfused rat liver, clomiphene also appeared to be metabolized by quaternary ammonium-linked glucuronidation [22].

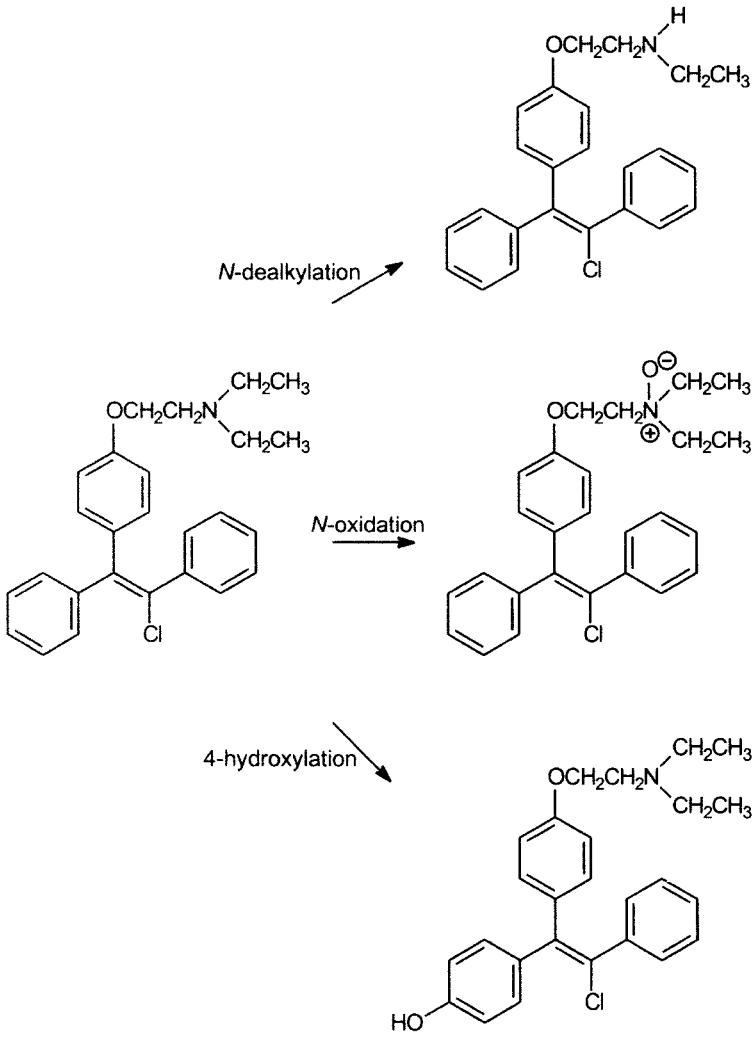
6. Pharmacokinetics

The earliest study reported that the excretion of clomiphene depended on the route of administration [29]. In humans, 5 days after oral administration of ^{14}C-clomiphene, 51% of the total radioactivity was detected in the feces. However, after I.V. administration, only 37% of the administered clomiphene was excreted during the same period. Small amounts could still be recovered up to six weeks after dosing [29].

More recently in a three phase study, a single 50 mg oral dose of clomiphene citrate (containing about 38% of zuclomiphene, the *cis* isomer) was administered to healthy female volunteers [30]. The plasma profiles of the two isomers showed different characteristics, with enclomiphene having the more rapid distribution and elimination. Ratios of the plasma levels of enclomiphene to zuclomiphene varied from about 1:1 at 2 hours to 1:6 at 24 hours, at which point the plasma concentration of enclomiphene was below 0.5 ng/mL.

Finally, in a study conducted by Szutu *et al.* [31], a single 50 mg I.V. dose containing 33% zuclomiphene and 67% enclomiphene was given to two healthy patients. Again the two isomers demonstrated different pharmacokinetic patterns, although the volume of distribution was very high and similar for both isomers. As with the oral dose, enclomiphene had a more rapid distribution and elimination. The elimination rate of enclomiphene was 3-5 fold greater relative to that of zuclomiphene. Plasma levels of enclomiphene exceeded those of zuclomiphene after I.V. administration, while the reverse situation was observed after oral administration.

Figure 8. Metabolism of clomiphene.



In summary, clomiphene is not only a mixture of two drugs with different pharmacodynamics, but the relative plasma concentrations of the two isomers also change with time and with the route of administration. Enclomiphene has a much lower bioavailabilty than does zuclomiphene, since it is eliminated more rapidly. Zuclomiphene also accumulates in the plasma after repeated doses, possibly because of stereoselective enterohepatic recirculation. This accumulation is clinically important since the usual dosage is 50 mg/day for 5 days, and therapy could continue for as long as several months [15,32].

7. Pharmacology

Clomiphene citrate is a non-steroidal synthetic ovulatory stimulant that is used world-wide in the treatment of female infertility of endocrine origin. Prepared as a mixture of *cis* and *trans* isomers, it was first synthesized in 1958 and became available in 1960 for clinical trials [15,33]. Prior to 1976, the *cis* isomer was thought to be the more anti-estrogenic and the *trans*-isomer the more estrogenic. However, following the 1976 x-ray crystallographic analysis of the isomers, the *cis* and *trans* designations were reversed. Today it is accepted that the *cis* isomer (zuclomiphene) is estrogenic, while the *trans* isomer (enclomiphene) is anti-estrogenic [9, 33-35]. The mixture which is administered to induce ovulation in women (among other clinical uses) is generally 38% zuclomiphene and 62% enclomiphene [16,35].

The exact mode by which clomiphene induces ovulation in anovulatory women has not been clearly elucidated. However, it is clear that the compound has mixed estrogen agonist/antagonist activity [9,35,36]. Its antagonistic action is due to a competitive inhibition of estrogen receptors [35,36], particularly at hypothalamic sites [36]. Clomiphene can prevent the entry of estrogen, and speed up its clearance from cell receptors [16,35]. Due to its ability to compete for estrogen receptors, it is capable of interacting with a variety of estrogen dependent tissues, especially the breast, liver, pituitary, hypothalamus, and uterus [35].

Clomiphene has been shown to increase the synthesis and release of the gonadotropins, FSH and LH, in both males and females [9,15,33,35,36]. It is believed that by preventing the entry of estrogen to hypothalamic

receptors, clomiphene counteracts the negative feedback action of estrogen on gonadotropin secretion and stimulates the release of FSH and LH by the pituitary [35]. Conversely, there is also a possibility that ovulation-induction is extrahypothalamic and that the pituitary gland is the major target site. Thus clomiphene may act directly on the pituitary to potentiate the action of GnRH on the synthesis and release gonadotropins, particularly LH [9,15,35].

It is also possible that clomiphene can exert a direct effect on the ovary and testis. Both inhibitory and stimulatory effects have been claimed [9,33,35].

Finally, the available evidence indicates that the sensitivity of different estrogen-sensitive tissues to the various biological actions of clomiphene may vary not only from species to species, but also among the different estrogen-responsive tissues in the same species [35].

8. Clinical Uses and Administration

The prime indication for use of clomiphene is for the induction of ovulation in anovulatory women desiring pregnancy, particularly in cases of ovarian dysfunction [32,35]. The overall rate of ovulation with clomiphene is 70-90%, with conception resulting in 30-40% of these cases (the risk of multiple pregnancies is about 20%) [9,34,35]. Other complications, especially the occurrence of ovarian cysts and hot flashes, have decreased over the years due to careful selection of patients and by decreasing the dose and duration of treatment [9,35].

The recommended starting regimen is 50 mg for 5 days from the fifth day of spontaneous or induced ovulation, and ovulation should occur 5-10 days after the last dose of clomiphene. If ovulation does occur there is no point in increasing the dose in subsequent cycles, but if the results are negative after two cycles with the same dose then the dose may be gradually increased to 100 mg daily [32]. Doses as high as 300 mg/day have been employed, albeit rarely [35]. At these higher doses the possibility of ovarian enlargement is increased, at which point therapy is stopped. The enlarged ovary then readily regresses within one or two months [9,33,35]. Other dose-related side effects of therapy are vasomotor flushes and abdominal or pelvic discomfort [9,32]. If, after 3

cycles the results are still negative, then it is unlikely that conception will occur. However, if ovulation occurs but is not followed by pregnancy, subsequent courses may be administered, to a maximum of six cycles of clomiphene treatment [32].

Clomiphene has also been tried in other conditions with varying degrees of success. In females these include [35]:

- correction of luteal phase defects.
- regulation of menstrual cycles in cases with stress amenorrhoea, post-contraception pill amenorrhoea and dysfunctional uterine bleeding.
- chronic cystic breast disease and breast cancer.
- endometrial hyperplasia and endometriosis.
- *in vitro* fertilization, where clomiphene together with HMG (human menopausal gonadotropin) is commonly used to hyper-stimulate the ovaries of patients undergoing IVF.
- testing hypothalamic-pituitary function; normally, after 50-100 mg of clomiphene daily for 5 days there is an increase in FSH and LH blood levels, while in cases of hypothalamic dysfunction the response is poor or absent.
- prevention of post-partum breast enlargement.
- estrogen replacement therapy in menopause.

In males these include [35]:

- male infertility, particularly oligospermia.
- male hypogonadism.
- testing hypothalamic-pituitary function.
- treatment of painful gynaecomastia.

9. Acknowledgments

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Finally, the help of Stuart Thomson in obtaining mass spectra and in interpreting NMR spectra is greatly appreciated.

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GUAIFENESIN

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ANALYTICAL PROFILES OF DRUG SUBSTANCES
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CONTENTS

1. Description

1.1 Nomenclature

1.1.1 Chemical Name

(*R,S*)-3-(2-methoxyphenoxy)-propane-1,2-diol

1.1.2 Other Chemical Names

(2-methoxyphenoxy)-1,2-propanediol
(o-methoxyphenoxy)-1,2-propanediol
Guaiacyl glyceryl ether
Guaiacol glycerol ether
Glycerylguaiacol
Glyceryl guaiacolate
Guajocolum glycerolatum
Glycerylguayacolum
Glycerol α-(2-methoxyphenyl)ether
o-Methoxyphenyl glyceryl ether

1.1.3 Pharmacopoeia Names

British Pharmacopoeia: Guaiphenesin
United States Pharmacopoeia: Guaifenesin
French Pharmacopoeia: Gaifenesine
German Pharmacopoeia: Guaifenesin
European Pharmacopoeia: Guaifenesinum
Japanese Pharmacopoeia: Guiafenesin

1.1.4 Proprietary Names

Amonidrin, Anti-Tuss, Benylin, Breonesin, Fenesin, Genatuss, Guaituss, Resyl

Component of: Brexin EX, Brondecon, Bronkaid, Brontex, Congestac, Coricidin Syrup, Dimacol, Dristan Cold & cough, Entex, Histalet X, Hycotuss, Isoclor Expectorant C, Kwelcof, Lufyllin-GG, Nalcecon, Nasatab LANeothylline-GG, Organidin

NR, Pneumomist, Pneumotussin HCPolaramine Expectorant, Primatine Dual Action, PV Tussin, Robitussin, Ru-Tuss DE, Slo-Phyllin GG, Sudafed Cough & Cold, Theolair Plus, Triaminic, Tussar, and Tussi-Organidin

1.2 Formulae

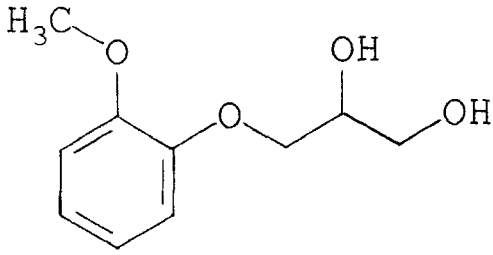
1.2.1 Empirical Formula

$C_{10}H_{14}O_4$

1.2.2 CAS Registry Number

93-14-1

1.2.3 Structure



1.3 Molecular Weight

198.22

1.4 Elemental Composition

Carbon	60.59%
Hydrogen	7.12%
Oxygen	32.29%

1.5 Appearance

Guaifenesin is a white or slightly gray crystalline powder. It is generally odorless or with a slight characteristic odor, and has a slightly bitter aromatic taste

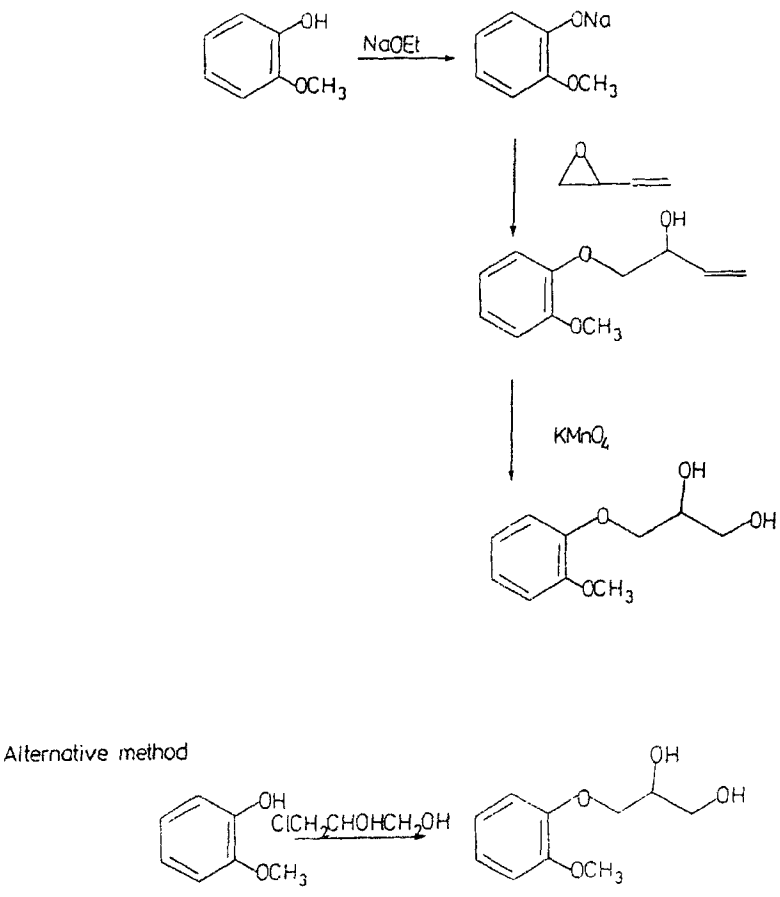
1.6 Uses and Applications

Guaifenesin was first synthesized during the 1940's, and is used as an expectorant in the symptomatic management of coughs associated with the common cold, bronchitis, laryngitis, pharyngitis, pertusis, influenza, measles, and coughs provoked by chronic paranasal sinusitis. While there is clinical evidence that guaifenesin is an effective expectorant (*i.e.*, increasing expectorated sputum volume over the first 4-6 days of a productive cough, decreasing sputum viscosity and difficulty in expectoration and improving associated symptoms), there currently is insufficient evidence to support efficacy of the drug as an antitussive (cough suppressant). Therefore, the principal benefit of guaifenesin is in the symptomatic treatment of coughs, associated with the ability of the drug to loosen and thin sputum and bronchial secretions, and ease expectoration.

Although such facilitation of secretion evacuation may indirectly diminish the tendency to cough, the mechanism of this effect differs from that of antitussives, which inhibit or suppress cough. The effect of guaifenesin on sputum production and viscosity, and on the ease of expectoration, suggests that the drug may prove useful in the management of irritative non-productive cough, and of coughs which only produce scanty amounts of thick, viscous secretions. Guaifenesin is combined with bronchodilators, decongestants, antihistamines, or opiate antitussives in numerous commercial liquid cough preparations [1]. Guaifenesin is also used as an intermediate in the synthesis of the muscle relaxant agent methocarbamol [2], and the muscle relaxant and tranquilizer agent mephenoxalane [3].

2. Synthesis

As shown in Scheme 1, guaiacol (2-methoxyphenol) is treated with sodium ethoxide to give a sodium salt which is quenched with an epoxyalkene. The final step involves the oxidation of the alkene to the diol using potassium permanganate [4].



Scheme 1. Synthesis routes for guaifenesin.

An alternative method of synthesis involves treating guaiacol directly with an equimolar amount of glycerol α-monochlorohydrin in a strongly alkaline solution or suspension (Scheme 2). When the vigorous reaction subsides, the mixture is heated for three hours, concentrated *in vacuo*, and extracted with hot isopropanol to yield (after filtration and cooling) product in greater than 80% yield. The synthesis of guaifenesin has also been reported by others [5,6].

3. Physical Properties

3.1 Microscopy

3.1.1 Particle Morphology

When obtained from ether, guaifenesin is obtained as minute rhombic prisms. A commercial sample was evaluated using optical microscopy, with the data being obtained on a Leica Diastar system. As illustrated in the photomicrograph of Figure 1 (magnification of 200x), the individual crystals were approximately 10-50 μm in length, and 5-20 μm in width. In this particular preparation, the majority of the crystals exhibited a rod-like morphology.

3.1.2 Optical Crystallography

The presence of guaifenesin in an unknown sample can be identified using polarizing light microscopy. The process entails mounting small amounts of the material in successive specific refractive index liquids, and then determining refractive indices, optical sign, type of extinction and estimated optic axial angle [9]. The optical crystallographic data known for guaifenesin are as follows:

Refractive Index (α-axis)	Refractive Index (β-axis)	Refractive Index (γ-axis)	Optic Sign	Extinction Type	Axial Angle
1.584	1.592	1.698	+	parallel/ inclined	Small

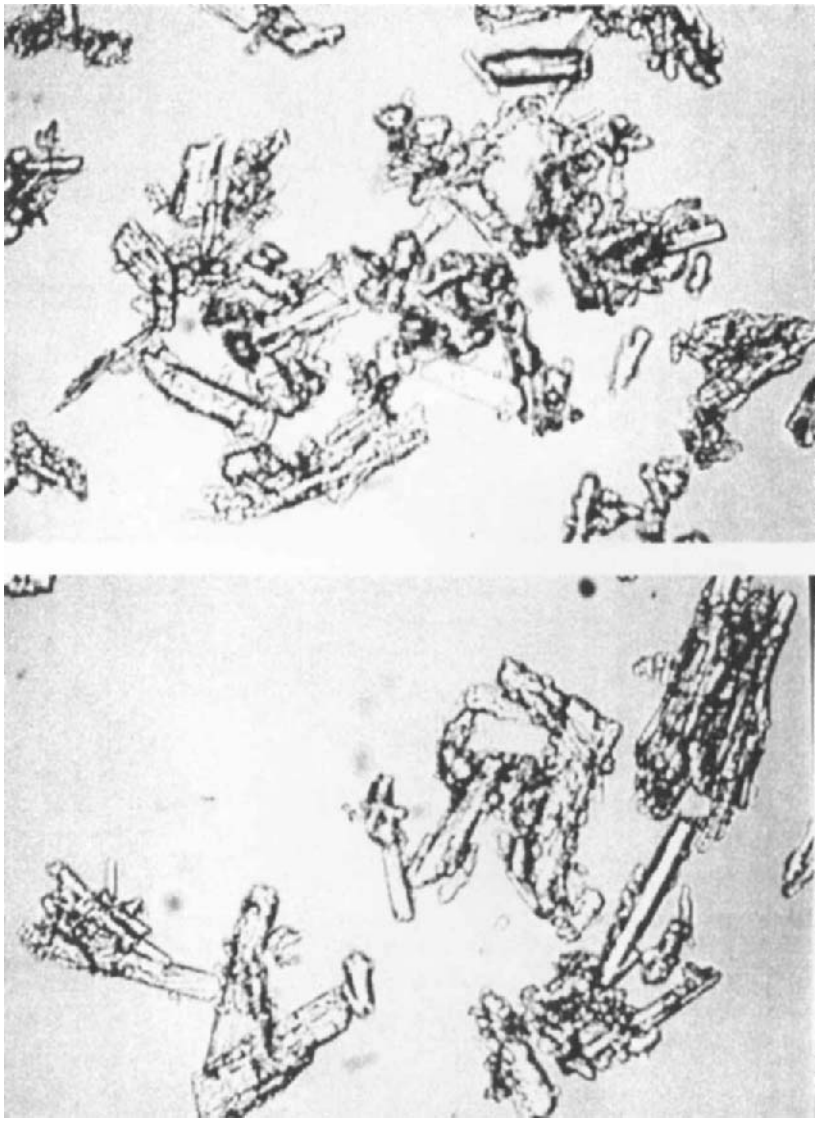


Figure 1. Optical photomicrographs of guaifenesin, obtained at a magnification of 200x.

3.2 X-Ray Powder Diffraction Pattern

Guaifenesin was found to exhibit a strong and characteristic x-ray powder diffraction pattern. The XRPD work was performed using a Philips APD 3720 powder diffraction system, equipped with a vertical goniometer in the θ/2-θ geometry. The K-α line of copper at 1.54439 Å was used as the radiation source. Each sample was scanned between 2 and 40 degrees 2-θ, in step sizes of 0.02 degrees (integrated for 0.5 sec at each step).

The powder pattern obtained for guaifenesin is illustrated in Figure 2, and a summary of scattering angles, d-spacings, and relative intensities is provided in Table 1. Although the powder pattern contains a number of scattering lines, the pattern is dominated by the two intense scattering peaks located at 12.06 and 13.46 degrees 2-θ, and the moderate peak located at 20.83 degrees 2-θ.

3.3 Optical Activity

Although guaifenesin contains one center of dissymmetry, only the racemic form of the compound is commercialized. The racemic nature of the commercial product was confirmed by measuring the specific rotation in 95% ethanol (sodium D line, 2 dm cells) using a Schmidt and Haerisch polarimeter. It was found that at a solute concentration of 4 g/100 mL, the optical rotation was indeed zero.

(*S*)-guaifenesin form has been synthesized in order to compare its pharmacological properties with that of the racemic modification [10]. It was reported that the (*S*)-form exhibited a specific rotation of $[\alpha]_D = -15.4°$ when measured in 95% ethanol at a temperature of 23.5°C.

3.4 Thermal Properties

3.4.1 Melting point

The melting range of racemic guaifenesin can be between 78°C and 82°C, and current USP specifies that the magnitude of the temperature interval spanning the beginning and end of melting cannot exceed 3°C. The latest issue of the Merck Index quotes a melting point of 78.5-79°C.

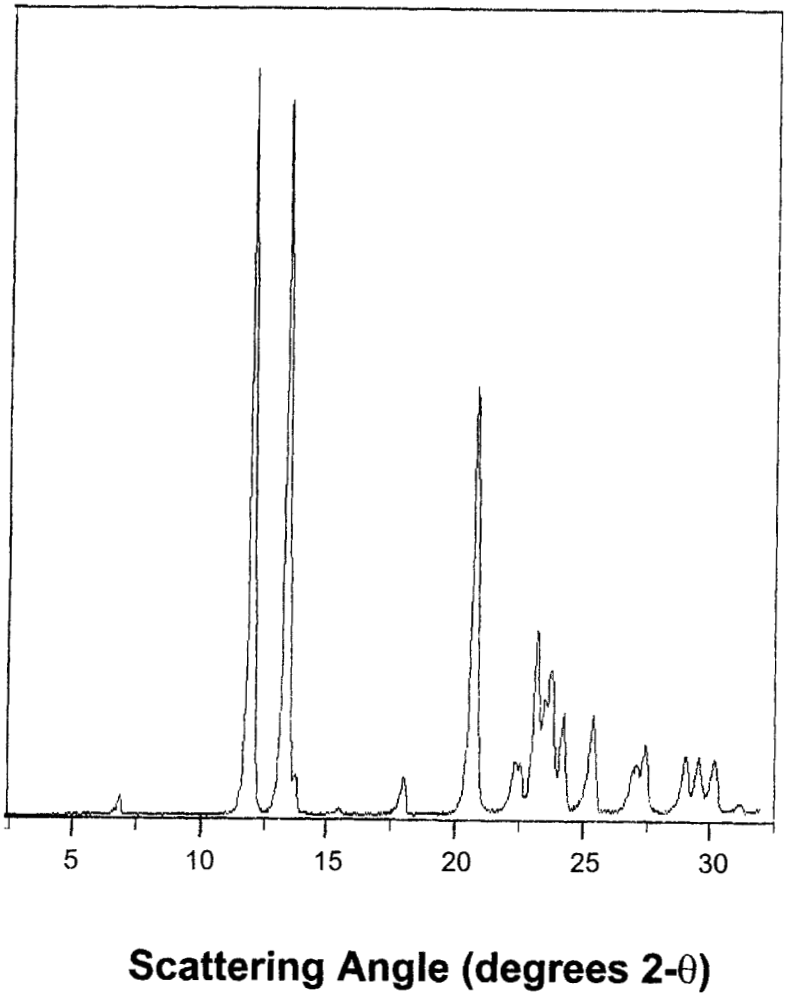


Figure 2. X-ray powder diffraction pattern of guaifenesin.

Table 1

Data from the X-Ray Powder Diffraction Pattern of Guaifenesin

Scattering Angle (degrees 2-θ)	**d-spacing (Å)**	**Relative Intensity, I/I_0 (%)**
6.865	12.897	4.3
12.055	7.354	100.0
13.460	6.589	85.2
13.780	6.437	4.4
15.525	5.717	0.6
18.030	4.928	3.4
20.830	4.272	32.7
22.340	3.986	3.8
22.570	3.946	3.7
23.205	3.840	12.8
23.795	3.746	9.8
24.255	3.676	6.6
25.435	3.508	6.2
27.110	3.295	3.0
27.485	3.251	4.1
29.075	3.076	3.4
29.585	3.024	3.2
30.220	2.962	3.1
31.240	2.868	0.7

It has been reported that the (*S*)-enantiomer of guaifenesin exhibits a melting range of 95-97°C [10].

3.4.2 Differential Scanning Calorimetry

The differential scanning calorimetry thermogram of guaifenesin is shown in Figure 3 and was obtained using a DSC 910S DSC module in conjunction with the DuPont 2000 Thermal Analyst system. The only detected thermal event was the melting endotherm at 78.3°C, which exhibited an onset temperature of 76.3°C. Integration of the melting endotherm yielded an enthalpy of fusion equal to 187 J/g, and the quality of the thermogram indicates that DSC could be used to determine compound purity.

3.2.3 Loss on Drying

Guaifenesin loses not more than 0.5% of its weight after being dried at 105°C for three hours [7]. When dried over P_2O_5 at 60°C at a pressure of 1.5 to 2.5 kPa for three hours, the compound will not lose more than 0.5% of its weight.

3.5 Hygroscopicity

The hygroscopicity of guaifenesin was evaluated by exposing bulk material at a temperature of 40°C and a relative humidity of 75°C for a period of 3 days, and then determining the water content using Karl Fischer titration. The water content only increased from an initial value of 0.57% to 0.66%, thus permitting the deduction that the compound is essentially non-hygroscopic. However, the USP calls for storage of guaifenesin in stored in tight containers to avoid forming lumps.

3.6 Solubility Characteristics

Guaifenesin is soluble to the extent of 1 part in 33 parts of water, and 1 part in 11 parts of alcohol or chloroform. The compound is also slightly soluble in ether. According to the USP, guaifenesin is soluble to the

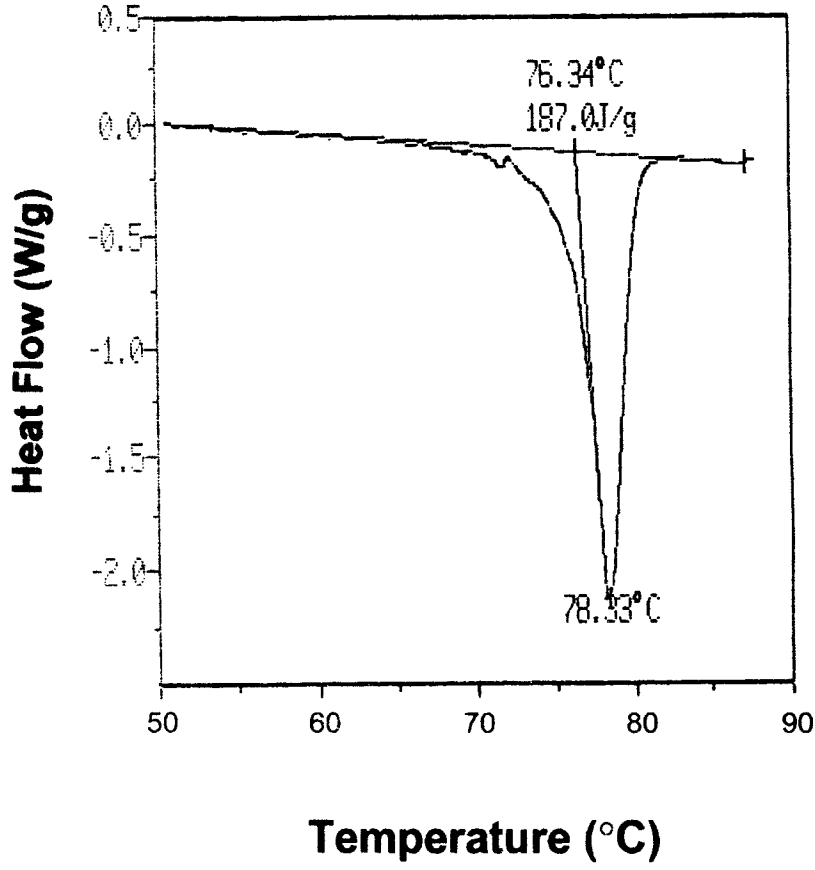


Figure 3. Differential scanning calorimetry thermogram of guaifenesin, obtained at a heating rate of 5°C/min.

degree of 1 part in 60 to 70 parts of water, as well as being soluble in alcohol, chloroform, glycerol, and propylene glycol.

When 1.0 g of guaifenesin is dissolved in 100 mL of water; the resultant pH of the solution is between 5.0 and 7.0.

3.7 Spectroscopy

3.7.1 UV/VIS Spectroscopy

The UV absorption spectrum of guaifenesin was recorded on a Beckman DU-7 spectrophotometer, over the spectral range of 200 - 400 nm. When dissolved in chloroform at a concentration equivalent to 1 part in 25000 parts of solvent, the spectrum shown in Figure 4 was obtained. It was found that the absorption maximum was observed at a wavelength of 275 nm, which is in good agreement with the literature value of a maximum between 272 and 274 nm [7]. The A (1% 1cm) parameter was found to be 118 in chloroform solvent.

3.7.2 Vibrational Spectroscopy

The infrared absorption spectrum of guaifenesin was recorded on a Nicolet FTIR spectrometer, using the potassium bromide pellet method. For the sake of clarity, the spectrum has been shown in two parts, spanning 4000-2000 cm^{-1} and 2000-400 cm^{-1}, respectively. Assignments for the observed bands are provided in Table 2.

3.7.3 Nuclear Magnetic Resonance Spectrometry

3.7.3.1 ^{1}H-NMR

The ^{1}H-NMR spectrum of guaifenesin was obtained on a Bruker 300 MHz spectrometer, using deuterochloroform as the solvent and tetramethyl-silane as the internal standard. The full spectrum is shown in Figure 7, while an expansion of the aliphatic region is found in Figure 8. A summary of the assignments for the observed resonance bands is provided in Table 3.

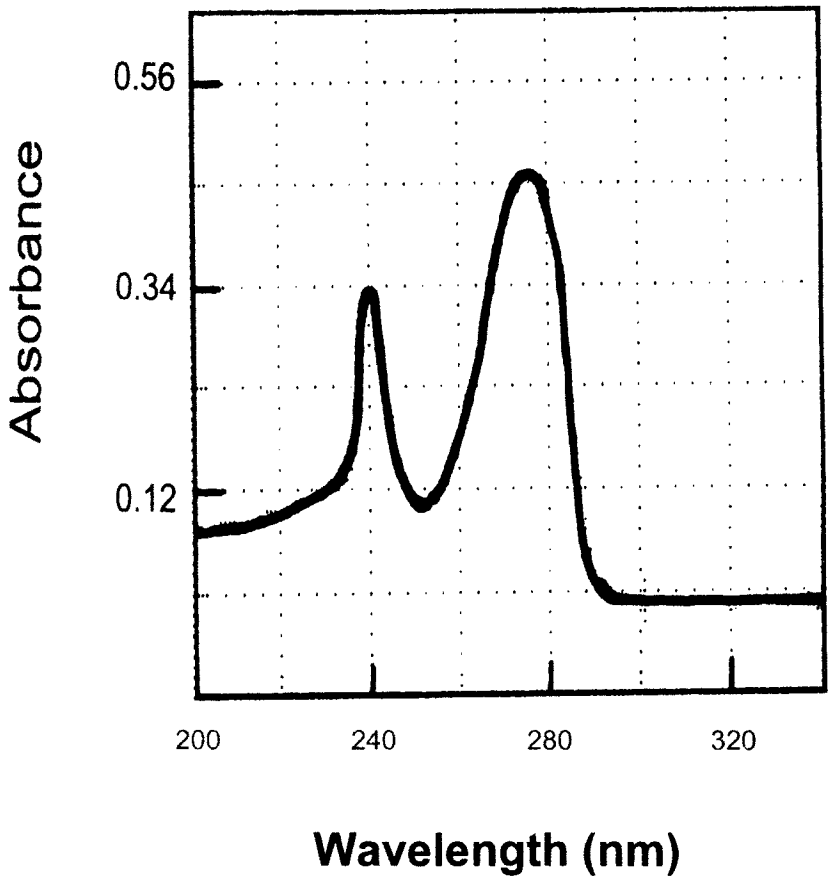


Figure 4. Ultraviolet absorption spectrum of guaifenesin dissolved in chloroform.

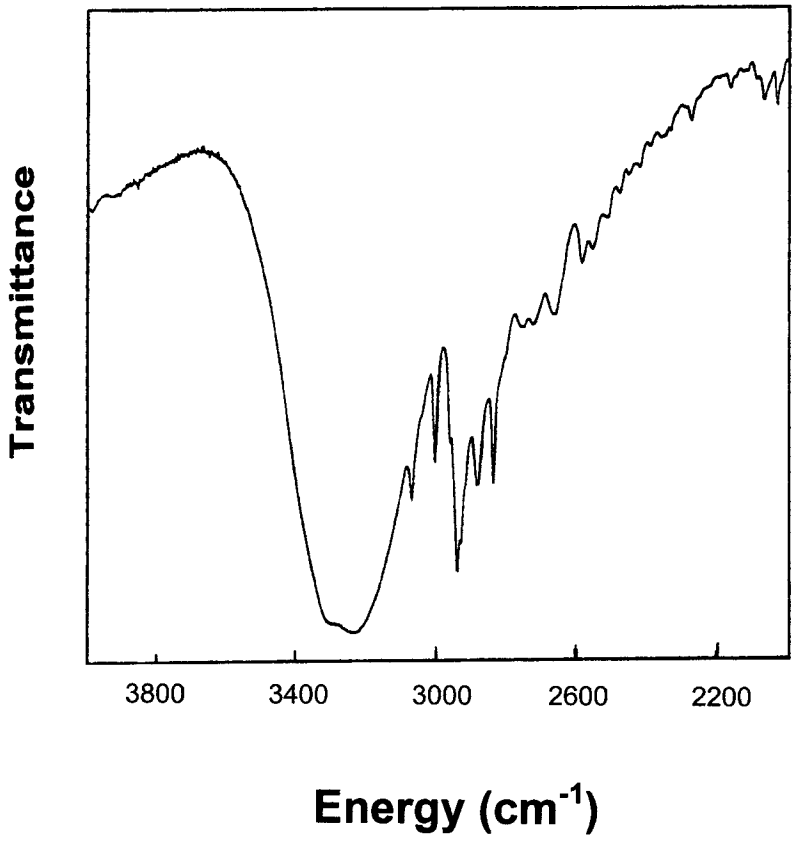


Figure 5. High frequency region infrared absorption spectrum of guaifenesin, obtained in a KBr pellet.

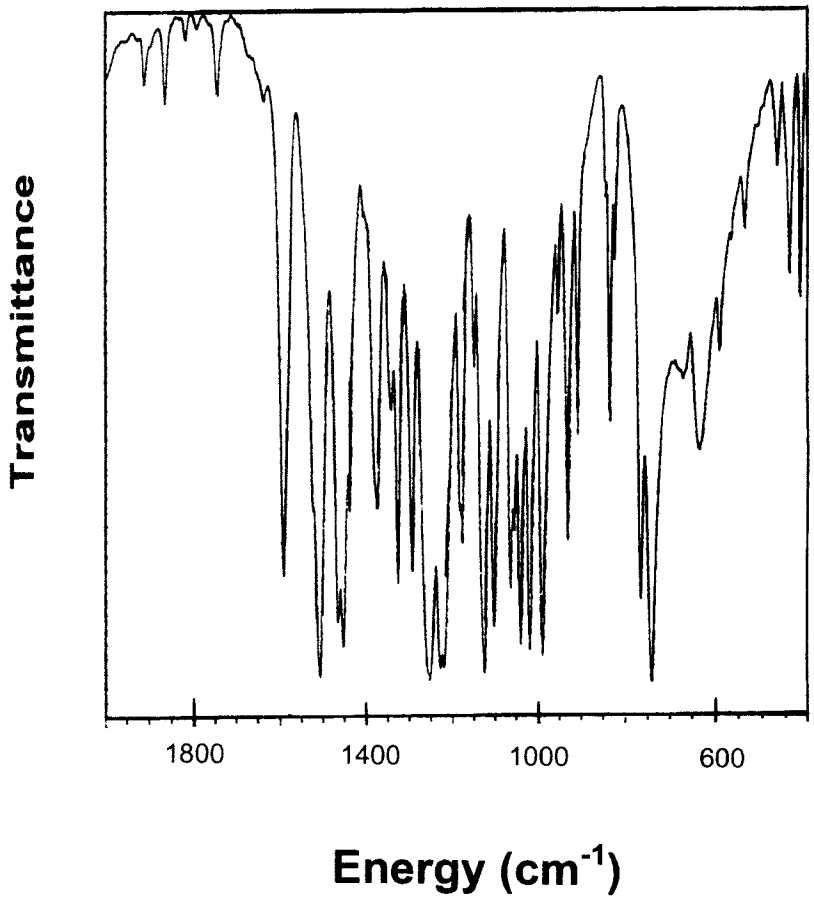


Figure 6. Fingerprint region infrared absorption spectrum of guaifenesin, obtained in a KBr pellet.

Table 2

Assignments for the Vibrational Transitions of Guaifenesin

Energy (cm^{-1})	**Assignment**
3600 - 3200	O-H stretching mode associated with the hydroxyl groups (intermolecular hydrogen bonding)
3200 - 2500	O-H stretching mode (intramolecular hydrogen bonding)
~ 3000	C-H stretch of the aromatic group
1920 - 1600	Ortho-substituted aromatic overtones
1600 - 1510	C=C stretch of the aromatic group
1260 - 1350	O-H deformation of the hydroxyl groups
1300 - 1200	C-O antisymmetric and symmetric stretching mode the of aryl ether
1150 - 1000	C-O stretch of the two hydroxyl groups
748	C-H out-of-plane bending mode of the ortho-substituted aromatic ring
650 - 400	ring deformation of the aromatic group

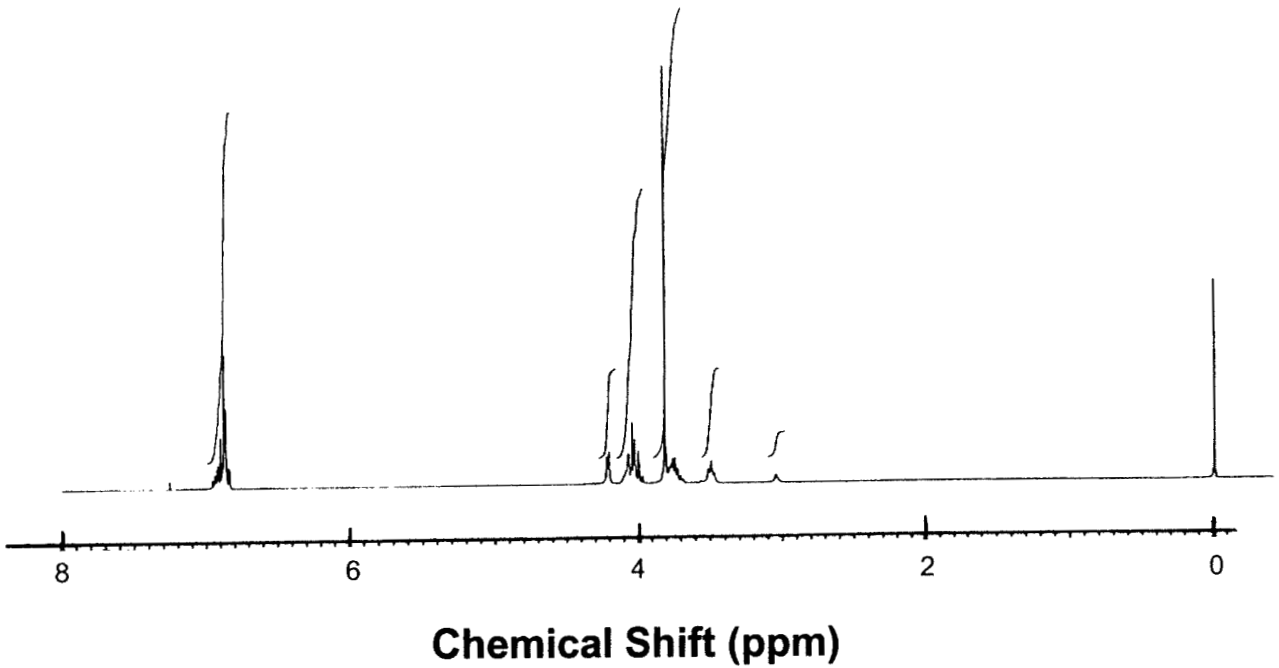


Figure 7. Full ^{1}H-NMR spectrum of guaifenesin.

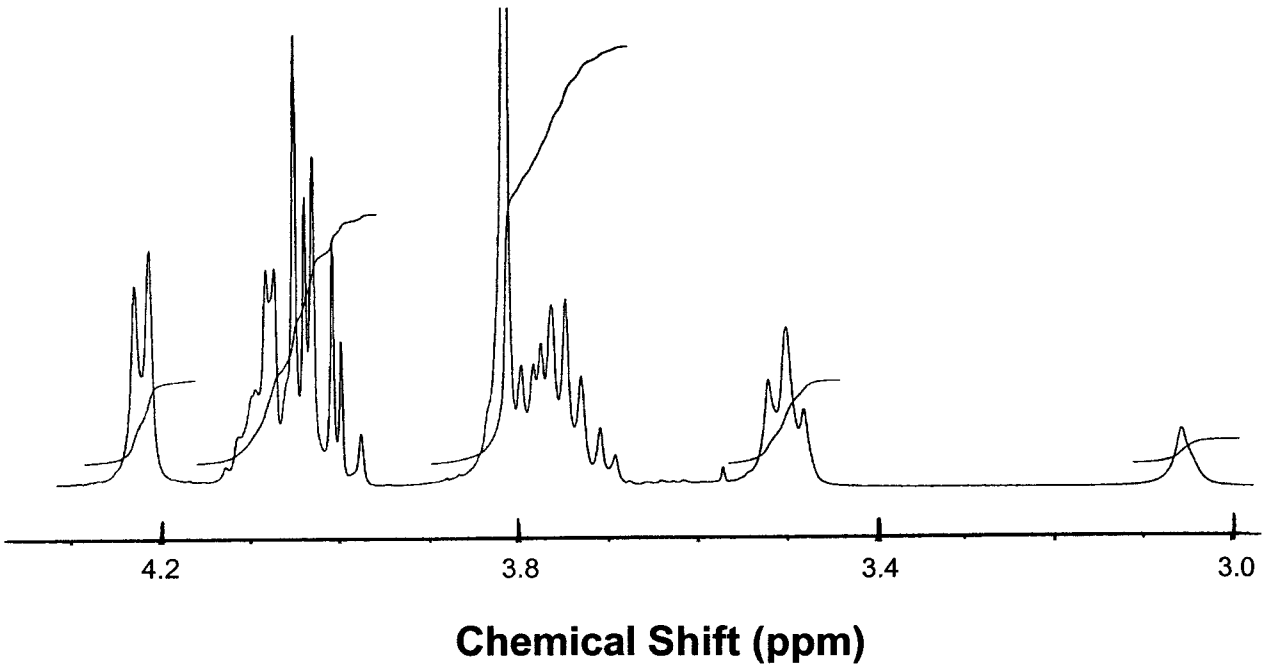


Figure 8. Expanded ^{1}H-NMR spectrum of guaifenesin.

Table 3

Assignments for the Observed ^{1}H-NMR Resonance Bands of Guaifenesin

Chemical Shift (ppm)	**Number of Protons**	**Assignment**
6.96 - 6.83	4	Aromatic ring protons
4.23	1	Hydroxyl proton of the -CHOH group
4.12 - 3.98	3	Methylene protons of the -CHOH group, and of the aromatic -OCH_2 group
3.80	3	Protons of the -OCH_3 group
3.75	2	Methylene protons of the -CH_2OH group
3.48	1	Hydroxyl proton of the -CH_2OH group

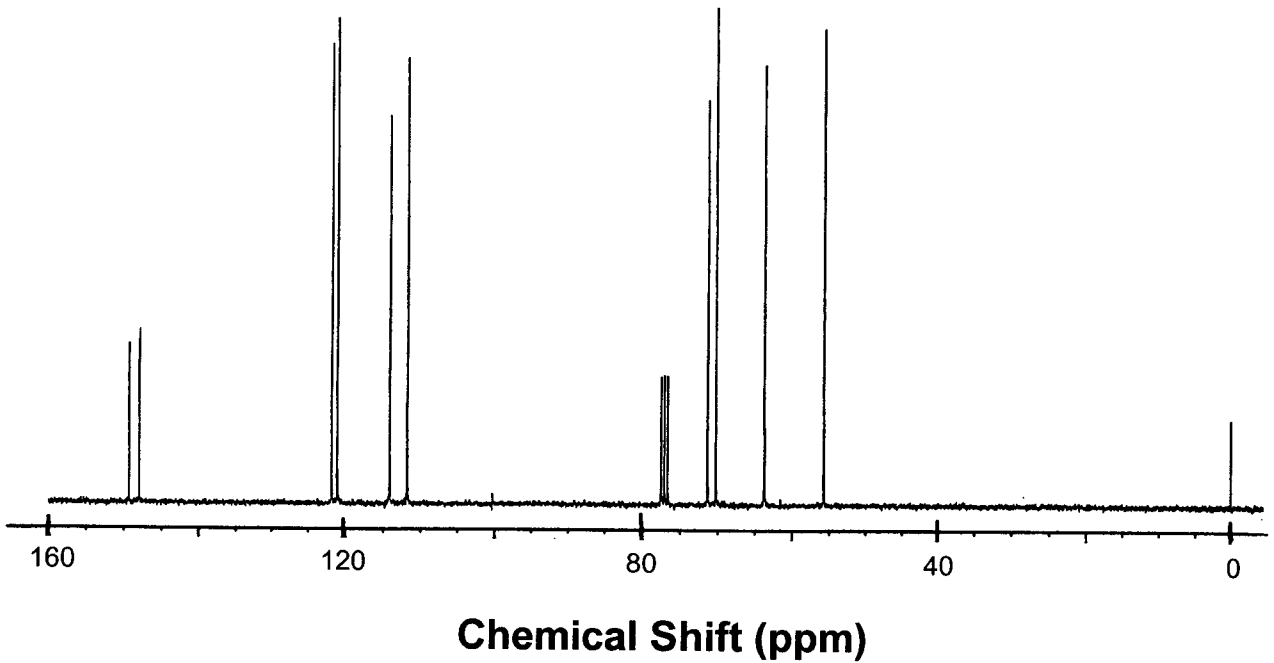


Figure 9. ^{13}C-NMR spectrum of guaifenesin.

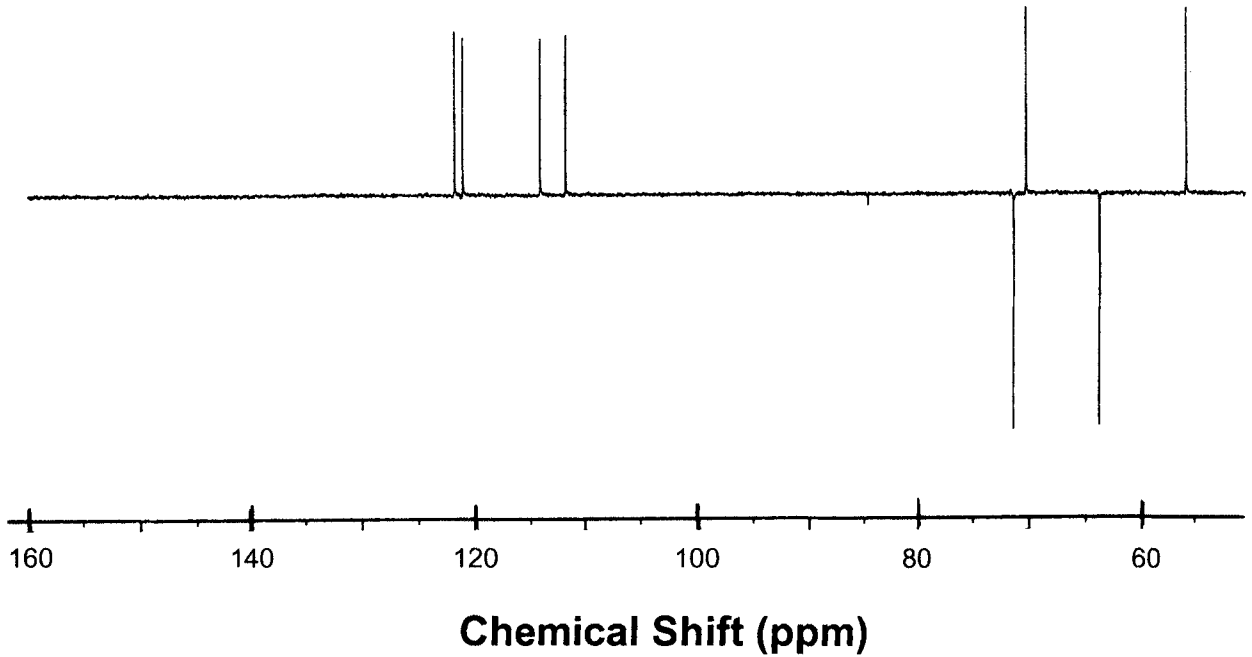


Figure 10. DEPT 135 ^{13}C-NMR spectrum of guaifenesin.

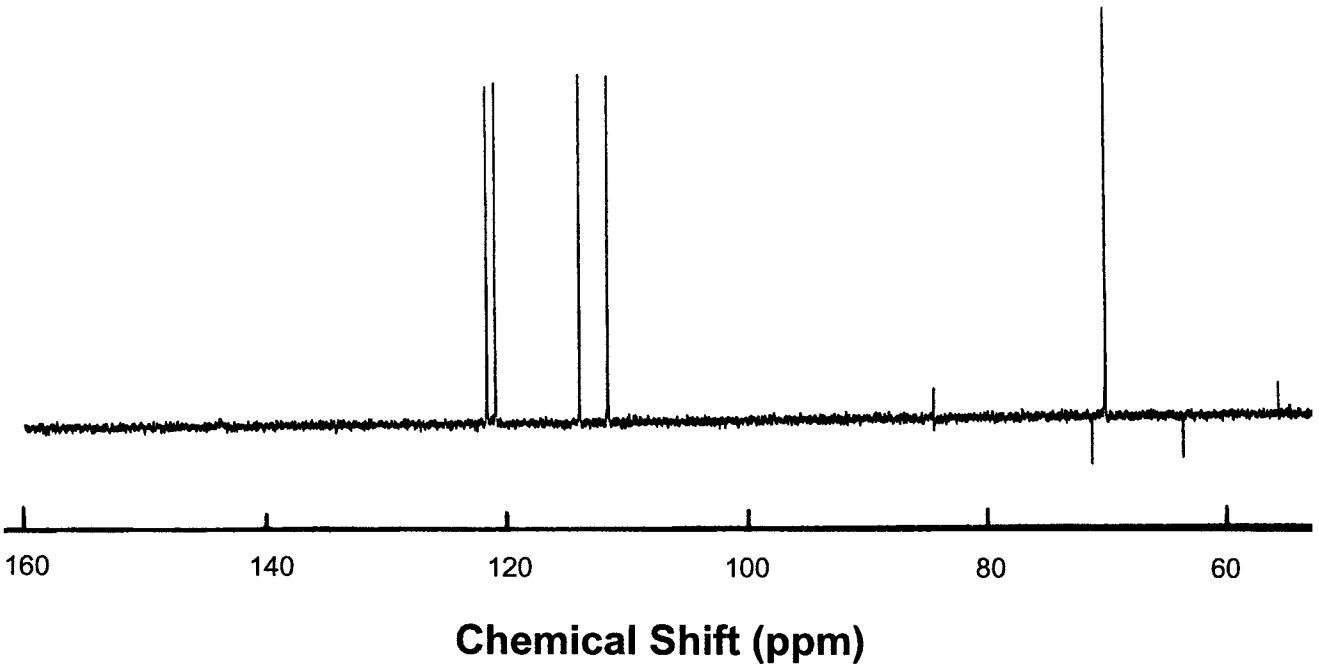


Figure 11. DEPT 90 ^{13}C-NMR spectrum of guaifenesin.

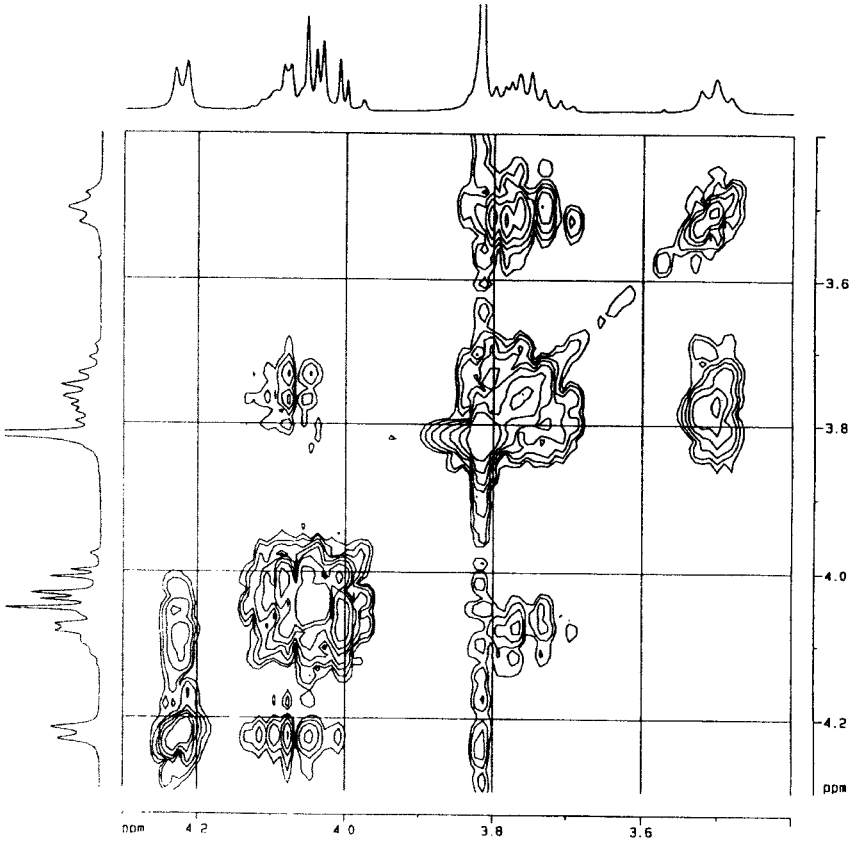


Figure 12. HOMOCOSY NMR spectrum of guaifenesin.

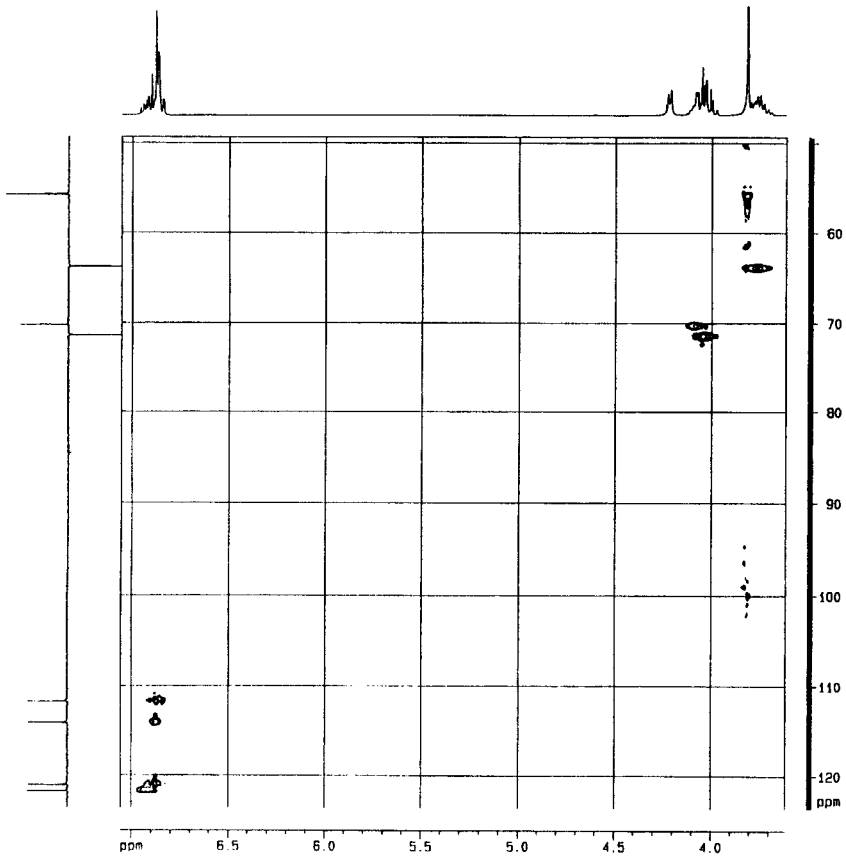


Figure 13. HETEROCOSY NMR spectrum of guaifenesin.

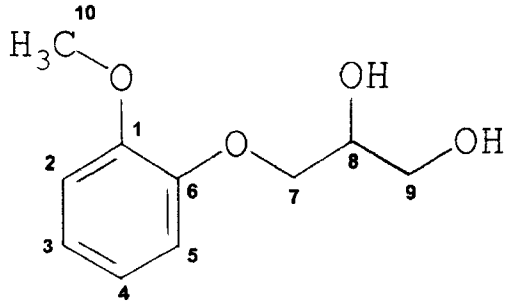
Table 4

Assignments for the Observed ^{13}C-NMR Resonance Bands of Guaifenesin

Chemical Shift (ppm)	Assignment
149.30	1
147.90	6
121.80	3 or 4
121.10	3 or 4
114.10	5
111.80	2
77.10	$CDCl_3$
71.40	7
70.20	8
63.80	9
55.80	10

3.7.3.2 ^{13}C-NMR

The ^{13}C-NMR spectrum of guaifenesin was also obtained in deutero-chloroform at ambient temperature, using tetramethylsilane as the internal standard. The one-dimensional spectrum is shown in Figure 9, while the DEPT 135, DEPT 90, COSY 45 (HOMOCOSY), and INVBTP (HETEROCOSY) are shown in Figures 10 through 13, respectively. All of these spectra were used to develop the correlation between chemical shifts and spectral assignment that are given in Table 4, and which have been identified according to the following numbering system:



3.7.4 Mass Spectrometry

Owing to their similarity in structure, the mass spectrum of guaifenesin should show a similar fragmentation pattern to that of methocarbamol. This compound is a propanediol derivative, whose systematic name is 3(o-methoxyphenoxy)-2-hydroxypropyl 1-carbamate, and which is also known as guaifenesin carbamate [2]. The suggested structures for fragment ions are shown in Figure 14.

3.8 Micromeritic Properties

3.8.1 Particle Size Distribution

The particle size distribution of a commercial sample of guaifenesin was obtained using sieving analysis. Using a Fritsch Laborgeratebau vibrator system, set at amplitude 7 for a period of 10 minutes, the particle size

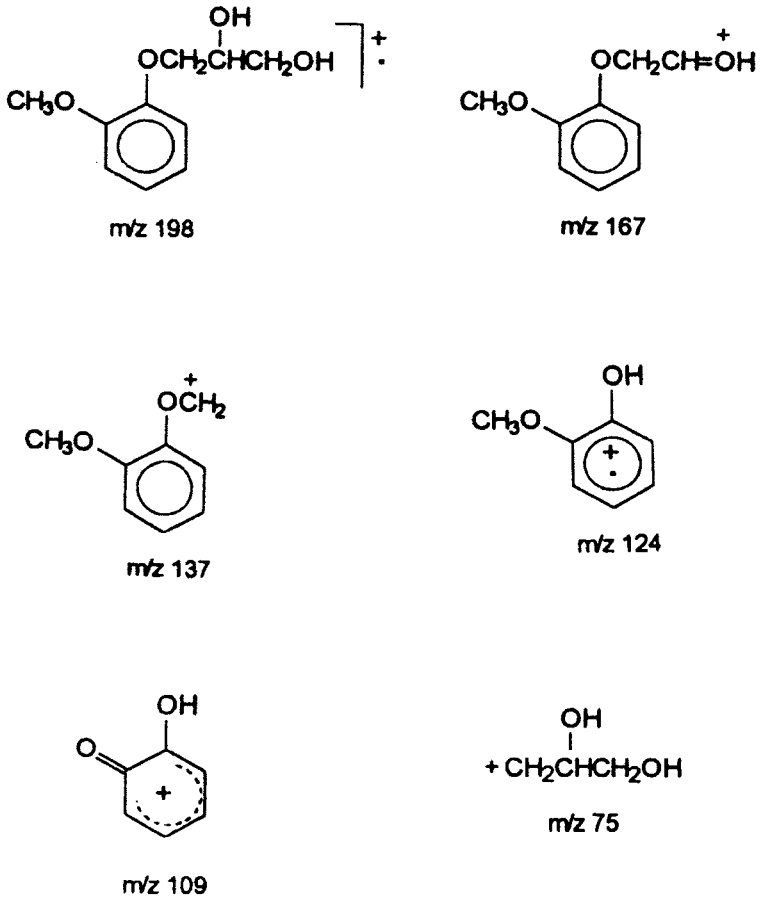


Figure 14. Anticipated molecular ions in the mass spectrum of guaifenesin.

distribution shown in Table 5 was obtained. The data in Table 5 indicate that the average particle size of this particular sample is approximately 35 μm.

Table 5

Particle Size Distribution Obtained for a Commercial Sample of Guaifenesin

Particle Size (μm)	Percent of Particles Found in the Size Band	Cumulative Percent of Particles
6.3	2.16	2.16
9.0	9.52	11.68
25	11.63	23.33
36	29.16	52.49
50	18.58	71.07
71	16.05	87.12
100	7.11	94.23
140	5.79	100.02

3.8.2 Bulk and Tapped Densities

The bulk density was determined by measuring the volume of a known mass of powder that had been passed through a screen into a volume-measuring device, and calculating the bulk density by dividing the mass by the volume. The average bulk density of the guaifenesin sample studied was found to be 0.39 g/mL.

The tapped density was obtained by mechanically tapping a measuring cylinder containing a known amount of sample. After observing the initial volume, the cylinder was mechanically tapped, and volume taken until a constant volume was reached [11]. The tapped density is calculated as the mass divided by the final tapped volume. It was found that the average tapped density of the guaifenesin sample was 0.64 g/mL.

3.8.3 Powder Flowability

The Carr Compressibility Index and Hausner Ratio are two measures which can be used to predict the propensity of a given powder sample to be compressed, and which are understood to reflect the importance of interparticulate interactions. These interactions are generally less significant for a free-flowing powder, for which the bulk and tapped densities will be relative close in magnitude. Poorer flowing materials are characterized by the existence of larger interparticle interactions, so a greater difference between bulk and tapped densities is observed. The two indices are calculated using the following relations [12]:

$$\text{Carr Compressibility Index} = 100\,(V_o - V_f) / V_o$$

$$\text{Hausner Ratio} = V_o / V_f$$

where:

V_o = original bulk volume of powder

V_f = final tapped volume of powder

The Compressibility Index for guaifenesin was found to be approximately 39, indicative that this powder sample would be predicted to exhibit very poor flowability. The Hausner Ratio for guaifenesin was determined to be 1.64, which also is taken to indicate that the powder would exhibit poor flow. These findings are reasonable give the fine particle size distribution of the material, since fine powders generally exhibit poor degrees of flowability.

4. Methods of Analysis

4.1 Compendial Tests

The current monograph in the United States Pharmacopoeia for guaifenesin requires testing to be performed for compound identification, melting range, loss on drying, heavy metals, free guaiacol, impurity profile, and organic volatile impurities [11].

4.2 Identification

The identification of guaifenesin can be made on the basis of its characteristic UV absorbance (see section 3.7.1), or on the basis of its infrared absorption spectrum (see section 3.7.2).

A colorimetric identity test may be performed by mixing about 5 mg of solid with one drop of formaldehyde and a few drops of sulfuric acid. The development of a deep cherry-red to purple color is taken as a positive reaction.

4.3 Titrimetric Analysis

The current British Pharmacopoeia describes an aqueous titration method for guaifenesin. 50 mg of sample is dissolved in 10 mL of water, to which is added 20 mL of sodium periodate solution, and the resulting solution allowed to stand for 10 minutes. After this, 25 mL of sodium arsenate solution and 1 mL of potassium iodide solution is added. This mixture is allowed to stand for 10 minutes, and is then titrated with 0.05 M iodine VS, using 2 mL of starch solution as the indicator. A titration blank is performed by repeating the procedure without the substance being examined. The difference between these two titrations represents the amount of iodine required for the analyte. Each milliliter of 0.05 M iodine VS is equivalent to 9.911 mg of guaifenesin.

4.4 Spectrophotometric Methods of Analysis

The determination of guaifenesin by UV spectrophotometry has been described in the United States Pharmacopoeia [11]. Transfer about 100 mg of accurately weighed guaifenesin to a 100-mL volumetric flask, dilute to volume with chloroform, and mix. Transfer 4.0 mL of this solution to a second 100-mL volumetric flask, dilute with chloroform to volume, and mix. The absorbance of this sample solution is to be determined at the same time as the absorbance of a standard solution of guaifenesin RS dissolved in chloroform, and prepared to have an accurately known concentration in the vicinity of 40 μg/mL. The photometric assay is performed using a suitable spectrophotometer, with the solutions

contained in 1 cm cells and the absorbance determined at a wavelength of 276 nm. Chloroform is to be used as the blank. The quantity (in units of mg) guaifenesin in the sample is obtained using the relation:

$$2.5\ C\ (Au/As)$$

where C is the concentration (in units of μg/mL) of the guaifenesin standard in the standard solution, and Au and As are the absorbencies of the sample solution and the standard solution, respectively.

The United States Pharmacopoeia describes a spectrophotometric test for the presence of guaiacol in samples of guaifenesin [11]. 1.0 g of sample is transferred to a 100-mL volumetric flask, and 25.0 mL of water is added (warming the solution may be required to effect dissolution). To this is added 1.0 mL of potassium ferricyanide TS, the contents swirled to mix, whereupon 5 mL of 4-aminoantipyrine solution (1 in 200) is added. At this point, one begins timing the reaction with a stop watch. The flask is swirled for 5 seconds, and the contents are immediately diluted to volume with sodium bicarbonate solution (1 in 1200) to volume. After an accurately timed fifteen minute time period commencing after the addition of the 4-aminoantipyrine solution, the absorbance of the solution is determined at 500 nm. The instrument blank is sodium bicarbonate solution (1 in 1200). The absorbance of the sample solution is not to be greater than that of a standard solution similarly prepared using 3.0 mL of a 1 in 10,000 solution of USP Guaiacol RS.

The determination of guaifenesin in antitussive pharmaceutical preparations containing dextromethorphan by first and second derivative UV spectrophotometry has been reported [20]. The method was found to be selective, accurate, and precise, but the analysis of guaifenesin by the second-derivative mode deteriorated when the ratio of dextromethorphan to guaifenesin exceeded unity. It was concluded that first-derivative spectrophotometry is the method of choice for the assay of tablets and syrups containing these two drugs.

A UV spectrophotometric method, which used 3-methylbenzothiazolin-2-one hydrazone hydrochloride as a derivitizing reagent, has been reported for the determination of guaifenesin in various formulations [21].

Colorimetric estimation has been used for the determination of guaifenesin in pharmaceutical formulations [25]. This procedure utilized the reaction

with a formaldehyde-sulfuric acid mixture. The assay was found to yield linear results over a concentration range of 10-25 μg/mL, with a maximum error of ±0.5%.

4.5 Chromatographic Methods of Analysis

4.5.1 Thin Layer Chromatography

The British Pharmacopoeia describes a TLC method suitable for the determination of the purity of guaifenesin. The method uses a mixture of 80 volumes of carbon tetrachloride and 20 volumes of ethanol (96%) as the mobile phase. The TLC place is prepared by mixing 0.3 g of carbomer with 240 mL of water, allowing this to stand with moderate stirring for 1 hour, and adjusted to pH 7 by the gradual addition (with stirring) of 2 M NaOH. To this solution is added 30 g of silica gel H, whereupon a uniform layer of suspension (0.75 mm in thickness) is spread on a glass plate. The coated plate is allowed to dry in air for 16 hours, then heated at 100-105°C for 1 hour, allowed to cool, and used immediately. To each plate, 5 μL is separately applied of each of four dichloromethane solutions. These four solutions contain 2.0% w/v of the substance being examined, 0.2% w/v of the substance being examined, 0.01% w/v of the substance being examined, and 0.2% w/v of guaifenesin reference standard. The plate is allowed to air-dry after being removed from the eluting solution. It is then sprayed with a mixture consisting of equal volumes of 1% w/v potassium hexacyanoferrate (III) solution, 20% w/v solution of iron(III) chloride hexahydrate, and 96% ethanol.

The Japanese Pharmacopoeia describes another TLC method suitable for the determination of guaifenesin purity [7]. 1.0 g of guaifenesin is dissolved in 100 mL of ethanol, with this solution being used as the sample solution. 1 mL of the sample solution is pipetted into a 200-mL volumetric flask, diluted to volume with water, and used as the standard solution. 10 μL each of the sample solution and the standard solution are spotted on a silica gel TLC plate. The plate is developed with a 40:10:1 mixture of ether, ethanol, and concentrated ammonium hydroxide to a distance of about 10 cm, and the air-dried. *p*-dimethylaminobenzaldehyde TS is sprayed on the plate, which is then heated at 110°C for 10 minutes.

Tomankova *et al.* reported the densitometric determination of propyphenazone, paracetamol, guaifenesin, caffeine, and aspirin in analgesic-antipyretic preparations after separation by TLC [26]. Methanolic solutions (in aliquots of 2 μL) of the cited drugs were chromatographed on silica gel 60 F_{254}, using 8:1:1 with ethyl acetate / methanol / acetic acid as the mobile phase. The densitometric scanning was performed at a wavelength of 276 nm. The contents in different dosage forms (50-325 mg/tablet) were quantitated through the use of external standards. Analyte recoveries were reported as 102.0-102.6%, 98.4-105.2%, 95.95-102.8%, 101.2-101.8%, and 99.4% for caffeine, guaifenesin, paracetamol, aspirin, and propyphenazone, respectively.

4.5.2 Gas Chromatography

The use of capillary gas chromatography to simultaneously determine guaifenesin, dextromethorphan, and diphenhydramine in a cough-cold syrup was reported [22]. 25 mL of the sample was diluted with 25 mL of water, treated with 1 mL of 1M KOH, and extracted with three 30 mL aliquots of chloroform. The extracts were concentrated at 25 kPa and 33°C, mixed with 15-30 mg of biphenyl (the internal standard), and diluted to 25 mL with chloroform. The solution was dried over anhydrous Na_2SO_4, and a 1 μL portion analyzed by GC with FID detection. The GC system used a fused-silica column (12 m x 0.22 mm) containing bonded methylsilicone, operated with temperature programming from 100 to 250°C at a ramp of 10°C/min, and used helium as the carrier gas. Good separation of the components was achieved, and the method sensitivity was 2.5-4.0 ng.

A capillary GC method for the quantitative determination of guaifenesin, methyl-*p*-hydroxybenzoate and propyl-*p*-hydroxybenzoate in a cough mixture has also been reported [23]. From three different batches of cough mixture, the results were found to yield 98.5-101% of the label claims for each ingredient. Relative standard deviations were in the range of 1-2%. It was shown in this work that some possible degradation products (methanol, formaldehyde, guaiacol, glycerol, and *p*-hydroxybenzoic acid) did not interfere.

4.5.3 High Performance Liquid Chromatography

The current United States Pharmacopoeia monograph for guaifenesin describes a HPLC method which can be used to establish the chromatographic purity [11]. The mobile phase is a 500:500:0.75:0.5 mixture of acetonitrile, water, glacial acetic acid, and triethylamine, where the pH has been adjust to 4.1 through the as-needed addition of glacial acetic acid or triethylamine. The test solution is prepared by dissolving about 100 mg of guaifenesin in 10 mL of water, whereupon 1.0 mL of this solution is further diluted to 200 mL with water. Analyte detection is made on the basis of the UV absorbance at 276 nm, and the analytical separation is effected using a phenyl column eluted at a flow rate of about 1 mL/min. It is important that the analyst chromatograph the dilute test solution first to prevent any carryover from the more concentrated test solution. The test solution is chromatographed for a period of time that is 4 times the retention time of guaifenesin. 10 μL aliquots of the dilute test solution and the test solution are injected into the chromatograph system, the chromatograms recorded, and the peak responses measured. The percentage of each impurity in the guaifenesin sample is obtained using the formula:

$$0.5(r_i/r_s),$$

where r_i is the response of each peak(other than that of the principal guaifenesin peak), in the chromatogram obtained from the test solution. r_s is the response of the main peak in the chromatogram obtained for the diluted test solution.

The isolation and identification of 2-(2-methoxyphenoxy)-1,3-propanediol as an impurity in dosage forms containing guaifenesin was reported [13]. These workers determined the structure of the synthetic impurity in guaifenesin, and modified a HPLC method for phenylephrine hydrochloride, phenylpropanolamine hydrochloride, guaifenesin, and sodium benzoate in dosage forms.

Shervington reported the simultaneous determination of the active ingredients in a multicomponent pharmaceutical product using an isocratic reverse-phase system HPLC method [27]. Pseudoephedrine HCl, guaifenesin, and dextromethorphan HBr were separated and quantitated simultaneously in a cough syrup.

4.5.4 Capillary Electrophoresis

Yururi *et al.* reported the simultaneous determination of guaifenesin and other ingredients in an anti-cold preparation by capillary electrophoresis [24]. Compounds that are either neutral or positively charged in acidic media (such as etenzamide, guaifenesin, paracetamol, or potassium guaiacolsulfonate), were determined in 50 mM-phosphate buffer (adjusted to pH 11.0), whereas those that are charged in alkaline media (such as chlorpheniramine maleate, dextromethorphan hydrobromide, dihydrocodeine phosphate, (±)-methylephedrine hydrochloride, or noscapine) were determined in 50mM-phosphate buffer (adjusted to pH 3.0). Hydrostatic injection for 10 seconds, and on-column detection at 185 nm, were used. The reported recoveries were in the range of 92.7-109%, and the method precision was characterized by a relative standard deviation of 0.5-6.3% (n = 3).

4.6 Determination in Body Fluids and Tissues

Several works have been published which concerned the determination of guaifenesin in plasma. One of these reported a selective reaction suitable for the gas chromatographic determination of guaifenesin in plasma [14]. This procedure involved a selective derivatization and subsequent use of electron capture detection in the GC step.

In another paper, the HPLC determination of guaifenesin in dog plasma was reported [15]. A 1 mL aliquot of plasma was mixed with mephenesin solution (the internal standard), and the proteins precipitated with 1N HCl. Lipids were extracted from the supernatant solution with hexane containing 10% methylene chloride, and then guaifenesin was extracted from the aqueous layer with methylene chloride containing 5% methanol. The organic extract was evaporated to dryness, and the residue dissolved in the HPLC mobile phase. A 5 μL aliquot of this solution was injected onto a μ-Bondapak column, and eluted with 11:11:78 methanol / acetonitrile / 0.05 M KH_2PO_4. Also contained in this mobile phase was 4 mM heptanesulfonic acid (an ion pairing agent) and 1% of glacial acetic acid. Detection was effected on the basis of the UV absorbance at 272 nm. Linearity in analyte response was obtained over the range of 0.25 to 2.0

μg/mL, with a coefficient of variation equal to 1.3 to 6.5% (n = 15). The detection limit was 0.10 μg/mL, and the mean recovery was 80.44 ±1.32%.

The determination of guaifenesin in human plasma by liquid chromatography in the presence of pseudoephedrine has been reported [16]. Plasma was vortexed for 10 seconds together with 100 ng/mL methanolic mephenesin solution (the internal standard), and the mixture adjusted to pH 7 with phosphate buffer solution. This solution was further vortexed for 30 seconds to mix the contents, and then frozen over acetone/dry ice to separate the layers. After centrifugation at 2700 rpm, the bottom organic layer was removed and evaporated to dryness under nitrogen. The residue was dissolved in 100 μL of mobile phase, and a 50 μL portion of this solution was analyzed on Waters μ-Bondapak C18 column. The mobile phase used was 11:11:78 methanol / acetonitrile / 0.05 M KH_2PO_4, containing 4 mM heptanesulfonic acid and 1% anhydrous acetic acid. Detection was made on the basis of the UV absorbance at 272 nm. The method linearity was reported to be 50-1000 ng/mL of guaifenesin, and sample recovery was in the range of 88.6-97.2%. The inter-day relative standard deviation was reported to be 5.0-8.4%, and the intra-day RSD was 4.8-8.7%. The method was applied to the analysis of human plasma samples obtained from clinical studies, and has also been used to confirm the bioequivalence of sustained release tablet formulations.

The simultaneous determination of dextrorphan and guaifenesin in human plasma by liquid chromatography with fluorescence detection has been reported [17]. Dextrorphan and guaifenesin were extracted from plasma by a liquid-liquid extraction procedure which used chloroform containing laudanosine as an internal standard. The analytical separation was effected on a cyano column (Spherisorb 5-CN), using a mobile phase consisting of 10:1:89 acetonitrile / triethylamine / water (pH 6). An excitation wavelength of 280 nm was used, and the fluorescence was monitored at 315 nm. Linearity in the concentration-response relationship for dextrorphan was found to be 23-515 ng/mL, with a lower limit of detection equal to 20 ng/mL. The accuracy of the method was reported to be within 9.53 and 11.07% of the true value. The degree of inter-day and intra-day precision (as reflected in the relative standard deviation values) was reported as 1.88-30.07% (mean 2.28%) and 4.69-7.51% (mean

5.67%), respectively, over the range of the method (33-326 ng/mL). The concentration-response relationship for guaifenesin was found to be linear over a concentration range of 181-8136 ng/mL, with a detection limit of 30 ng/mL. The accuracy for this analyte was within 9.78 and 8.04%, and the inter-day and intra-day precision values were reported to be 2.55-6.07% (mean 3.90%) and 3.12-3.90% (mean 3.52%), respectively, over the range of the method (435-6430 ng/mL). The percent recoveries of dextrorphan, guaifenesin, and laudanosine were found to be 96%, 94%, and 88%, respectively. The benchtop and storage stability of the plasma samples was found to be adequate, and frozen plasma samples could be subjected to three freeze/thaw cycles without undergoing a significant change in the stability of guaifenesin or dextrorphan.

5. Stability

5.1 Solid-State Stability

Bulk guaifenesin drug substance was subjected to a stress temperature of 75°C for a period of 24 hours. The material recovery was found to be 99.06%, and no degradation products were observed. A HPLC method was used to quantitate the assays and any degradation [8].

5.2 Solution-Phase Stability

Guaifenesin has been found to be relatively stable when subjected to acidic (dilute hydrochloric acid), basic)dilute sodium hydroxide), or oxidizing (dilute hydrogen peroxide solution) media for time periods up to 24 hours at 75°C [8]. The material recovery from these stress studies was reported to be 98.61%, 99.74%, and 99.85%, respectively, when using a HPLC method for the analysis.

6. Drug Metabolism and Pharmacokinetics

6.1 Absorption and Bioavailability

The steady-state human pharmacokinetics and bioavailabilty of guaifenesin and pseudoephedrine in a sustained released tablet have been

reported relative to immediate-release liquids [28]. To compare the steady-state *in vivo* performance of sustained released tablets consisting of guaifenesin in combination with pseudoephedrine hydrochloride (Entex) with coadministered immediate-release liquids (Robitussin) and pseudoephedrine (Sudafed), a randomized, crossover study was conducted in 30 healthy men, ages 18-39 years. The subjects received Entex (600 mg guaifenesin and 120 mg pseudoephedrine) twice daily on days 1-4 and once daily on day 5, or they received Robitussin 300 mg and Sudafed 60 mg liquids coadminstered 4 times daily on days 1-4 and twice daily on day 5. A steady-state condition was attained by day 5 of dosing for both the tablet and reference liquids. The sustained drug release from Entex resulted in a significant reduction in the guaifenesin fluctuation index and no difference in the pseudoephedrine fluctuation index relative to the corresponding indices for the reference liquids.

Bhagat *et al.*, reported on the kinetics and mechanism of drug release from calcium alginate coated tablets [29]. Compressed tablets containing guaifenesin and pharmaceutical excipients were prepared and coated with calcium alginate hydrogel using a novel, self-correcting membrane coating technique. The effect of coating time, type of alginate polymer, and pH of the dissolution medium on the rate of drug release were all evaluated.

6.2 Metabolism

The major metabolite of guaifenesin in plasma [18] and urine [19] has been found to be β-(2-methoxy-phenoxy) lactic acid.

6.3 Toxicity

The identification of guaifenesin by thin-layer chromatography and coupled gas chromatography / mass spectrometry during the course of a toxicological investigation was reported [30]. In this work, a new metabolite was found and identified, and the pharmacological and toxicological properties of guaifenesin were summarized. Some cases of intoxication after taking drugs containing guaifenesin were correlated with clinical symptoms.

The relationship between toxicity and dosage of guaifenesin was reported [31], and this work included a general description of expectorants and their efficacy following the FDA classification system for over-the-counter drug review processes.

Gross abuse of a cough syrup was cited [32]. A case was presented in which a 24 year old patient, who abused 200 mL of Tusselix Forte (codeine phosphate, guaifenesin, promethazine hydrochloride, and pseudoephedrine hydrochloride) cough syrup each day as a single dose for 7 months, developed changes in mood, blood pressure and pulse rate.

Acknowledgment

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MEBEVERINE HYDROCHLORIDE

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ANALYTICAL PROFILES OF DRUG SUBSTANCES AND EXCIPIENTS—VOLUME 25
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CONTENTS

1. Description

1.1 Nomenclature

1.1.1 Chemical Name

(*N*-Ethyl-*N*-(4-methoxy-α-methylphenethyl)-*N*-(4-veratroyloxybutyl) ammonium chloride

4-[Ethyl(*p*-methoxy-α-methylphenethyl)-amino]-butyl; 3,4-dimethoxybenzoate hydrochloride

(*RS*)-4-[Ethyl-4-methoxy-α-methylphenethyl amino] butyl veratrate hydrochloride

1.1.2 Proprietary Names

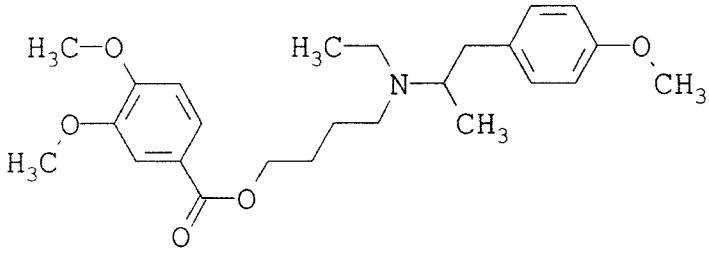
Colofac, Dusphapasmin, Duspatal, Duspatalin(e)

1.2 Formulae

1.2.1 Empirical: $C_{25}H_{36}ClNO_5$

1.2.2 CAS Registry Number: 3625-06-7

1.2.3 Structure



1.3 Molecular Weight: 429.55 (free base)
466.0 (hydrochloride salt)

1.4 Appearance

Mebeverine hydrochloride is a white to off-white crystalline powder [1-3].

1.5 Uses and Applications

Mebeverine hydrochloride has an antispasmodic action on smooth muscle, and is used in the treatment of abdominal pain and spasm associated with gastrointestinal disorders such as mucous colitis [1].

2. Methods of Preparation [4-5]

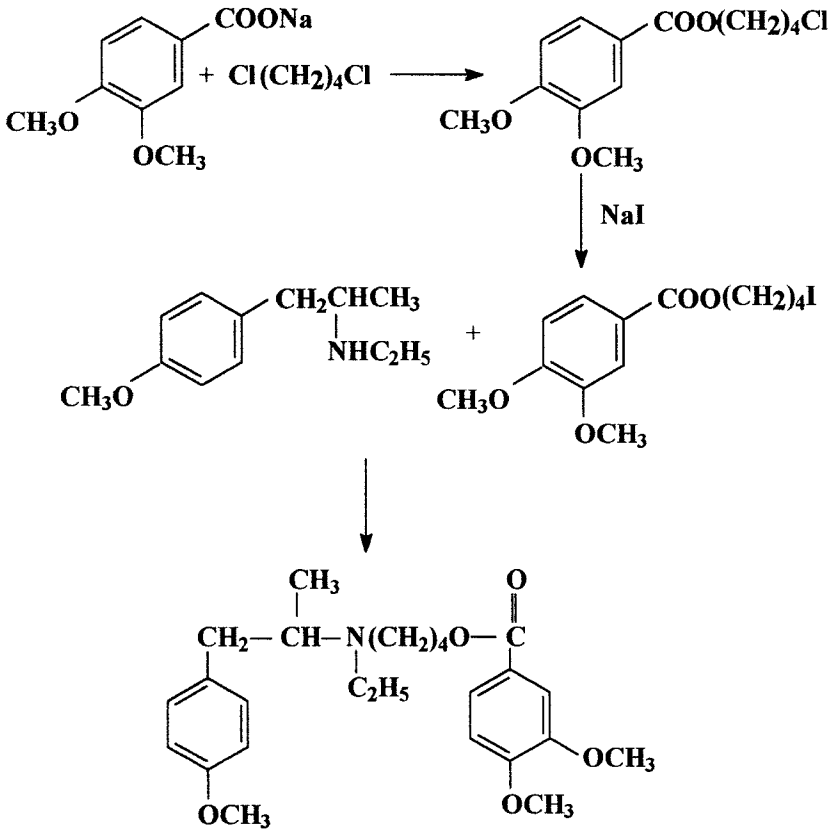
An overview of the synthesis of mebeverine is provided in Scheme 1. The compound is prepared by reacting sodium 3,4-dimethoxybenzoate with 1,4-dichlorobutane to form a chloroester, which is in turn transformed to the corresponding iodide on heating with NaI in methyl ethyl ketone. The alkylation of 2-ethylamino-3-*p*-methoxyphenylpropane with iodoester leads to mebeverine.

3. Physical Properties

3.1 X-ray Powder Diffraction

The X-ray diffraction pattern of mebeverine hydrochloride was determined using a Philips automated X-ray diffraction spectrogoniometer equipped with PW 1730/10 generator. The radiation source was a copper target (Cu anode 2000 W, λ = 1.5418 Å) high intensity X-ray tube operated at 40 kV and 35 mA, and the monochromator was a curved single crystal (PW 1752/00) unit. The divergence and receiving slits were 1^{o} and 0.1^{o}, respectively. The goniometer (PW 1050/81) scanning speed was 0.02 degrees 2θ per second. The goniometer was aligned using a silicon reference sample before use.

As evident in Figure 1, a large number of scattering lines were observed, although the most intense bands were all found below 30 degrees 2θ. A summary of the scattering angles, crystal d-spacings, and relative intensities is provided in Table 1.



Scheme 1 The Synthesis of Mebeverine

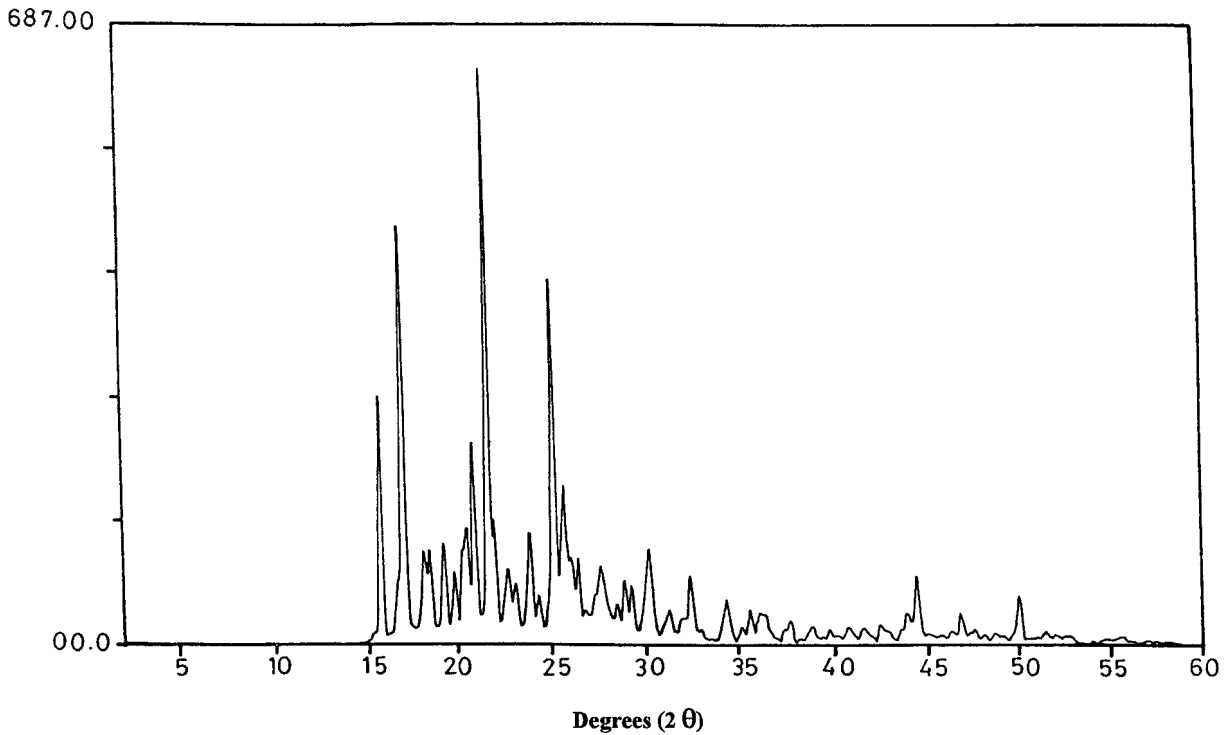


Figure 1 X - Ray Powder Diffraction Pattern of Mebeverine Hydrochloride

Table 1

X-ray Diffraction Data of Mebeverine Hydrochloride

Peak	Scattering Angle (degrees 2 θ)	d-spacing (Å)	Relative Intensity (%)
1	15.28	5.7939	1.92
2	15.734	5.6275	43.03
3	16.660	5.3169	10.98
4	17.020	5.2053	72.45
5	18.143	4.8854	16.34
6	18.448	4.8054	16.15
7	19.198	4.6193	17.39
8	19.773	4.4863	12.79
9	20.342	4.3620	16.36
10	20.517	4.3253	19.86
11	20.930	4.2407	34.83
12.	21.784	4.0764	100.00
13.	22.108	4.0174	20.88
14	22.744	3.9066	12.56
15	23.125	3.8430	10.88
16	23.884	3.7226	19.30
17	24.308	3.6586	8.10
18	25.173	3.5348	63.02
19	25.744	3.4577	27.58
20	26.095	3.4120	14.99
21	26.482	3.3630	15.07
22	27.373	3.2555	8.85
23	27.664	3.2220	13.56
24	28.517	3.1274	6.82
25	28.946	3.0821	11.17
26	29.297	3.0460	9.70
27	30.183	2.9585	16.59
28	31.263	2.8587	5.99
29	32.029	2.7921	4.74
30	32.416	2.7596	12.12

Table 1 (Continued)

X-ray Diffraction Data of Mebeverine Hydrochloride

Peak	Scattering Angle (degrees 2 θ)	d-spacing (Å)	Relative Intensity (%)
31	32.914	2.7190	2.74
32.	34.336	2.6096	7.96
33	35.168	2.5497	2.97
34	35.580	2.5212	6.09
35	36.139	2.4834	5.50
36	36.411	2.4655	5.25
37	37.420	2.4013	2.87
38	37.695	2.3844	4.32
39	38.813	2.3182	3.18
40	39.769	2.2647	2.53
41	40.734	2.2132	3.20
42	41.643	2.1670	2.92
43	42.547	2.1230	3.40
44	42.899	2.1064	2.27
45	43.565	2.0757	2.86
46	43.912	2.0601	5.53
47	44.397	2.0388	12.00
48	46.342	1.9576	2.39
49	46.839	1.9380	5.31
50.	47.532	1.9114	2.45
51	48.133	1.8889	1.72
52	50.073	1.8202	8.04
53	51.546	1.7716	2.30
54	52.776	1.7331	1.58
55	54.765	1.6748	1.09
56	55.681	1.6194	1.35
57	59.859	1.5439	0.44

3.2 Thermal Methods of Analysis

3.2.1 Melting Behavior

The melting range of mebeverine hydrochloride has been reported by a number of workers, and is summarized as follows:

131-136°C	[2]
133°C	[1]
135°C	[3]
129-131°C	[6]

3.2.2 Differential Scanning Calorimetry

The differential scanning calorimetry (DSC) thermogram for mebeverine hydrochloride is shown in Figure 2, and was obtained on a Dupont TA 9900 Thermal Analyzer system at a scan rate of 10°C/min over a temperature interval of 40 to 340°C. The hydrochloride salt was found to melt at 131.72°C, with an associated melting enthalpy equal to 23.17 cal/gm.

3.3 Solubility Characteristics

The free base of mebeverine base is essentially insoluble in water, but its hydrochloride salt is very soluble in water, and freely soluble in alcohol. A 2% solution in water has a pH between 4.5 and 6.5. The hydrochloride salt is practically insoluble in ether [1-3].

3.4 Ionization Constant

Mebeverine is characterized by a single ionization constant, for which the pk_a is approximately 10.

3.5 Spectroscopy

3.5.1 Ultraviolet Absorption

The UV absorption spectrum of mebeverine hydrochloride (0.003%w/v, dissolved in 0.1 N HCl) was obtained over the range of 190-400 nm using

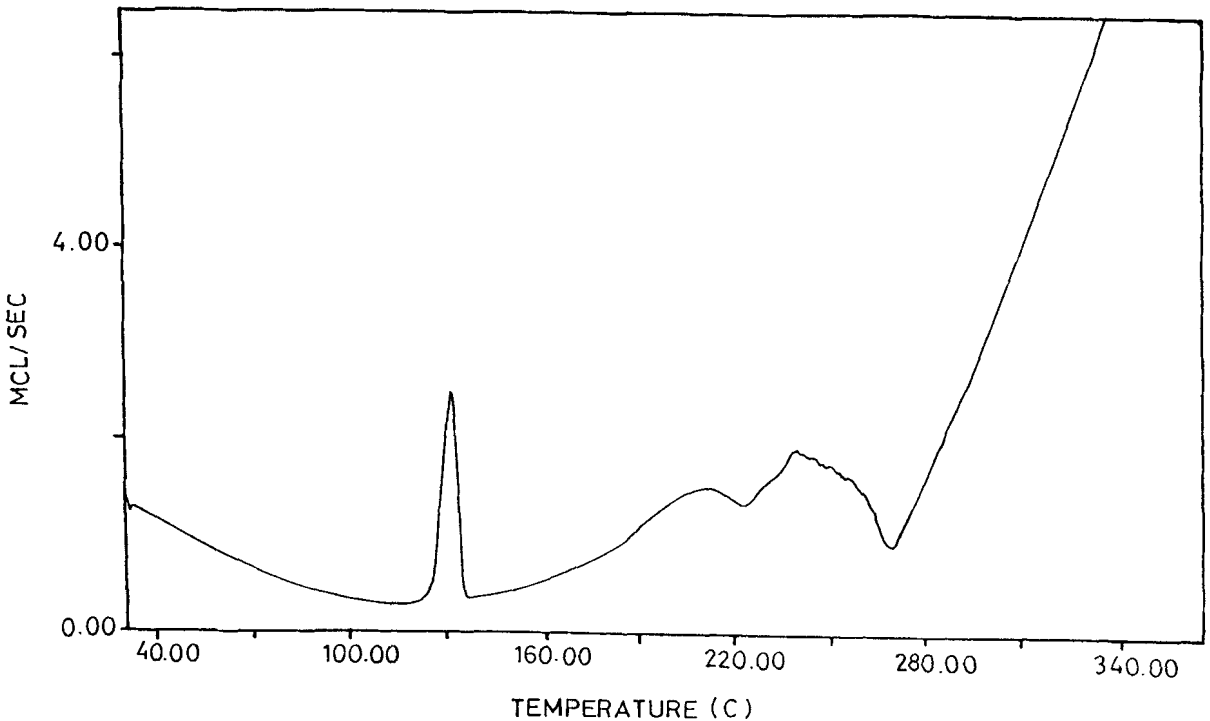


Figure 2 Thermal Curve of Mebeverine Hydrochloride

a Pye Unicam PU 8850 UV/VIS spectrophotometer. As illustrated in Figure 3, the compound exhibits a UV maximum at 263.4 nm, for which $E_{cm}^{1\%}$ equals 301. Mebeverine also exhibits a shoulder at 292 nm, whose intensity is approximately half that of the peak maximum [3].

3.5.2 Vibrational Spectroscopy

The principal absorption peaks found in the infrared absorption spectrum of mebeverine hydrochloride (obtained on a KBr disc using a Perkin-Elmer Model 1760X infrared spectrometer) were found at 1216, 1266, 1132, 1510, 1715, and 1174 cm^{-1} [2]. The IR spectrum is shown in Figure 4, and a summary of functional group assignments for the major observed bands is provided in Table 2.

3.5.3 Nuclear Magnetic Resonance

Both the proton nuclear magnetic resonance (^{1}H-NMR) and carbon nuclear magnetic resonance (^{13}C -NMR) spectra of mebeverine have been obtained in $CDCl_3$, using TMS as the internal standard. All data were obtained at ambient temperature, using was obtained on a Varian XL-300 NMR Spectrometer.

3.5.3.1 ^{1}H-NMR Spectrum

The ambient temperature 300 MHz ^{1}H-NMR spectrum of is shown in Figure 5, and a summary of the chemical shifts and spectral assignments is provided in Table 3.

3.5.3.2 ^{13}C-NMR Spectrum

The ambient temperature 75 MHz ^{13}C-NMR spectrum of mebeverine in $CDCl_3$ is shown in Figure 6, and a summary of the chemical shifts and spectral assignments is provided in Table 4. The ^{13}C signal multiplicities were determined by APT (Figure 7) and DEPT (Figure 8) experiments. The ^{1}H and ^{13}C spectral assignments were confirmed by utilizing the two-dimensional (2D) correlation spectroscopy (COSY) technique. The proton-proton (HH-COSY) and carbon-proton (CH -

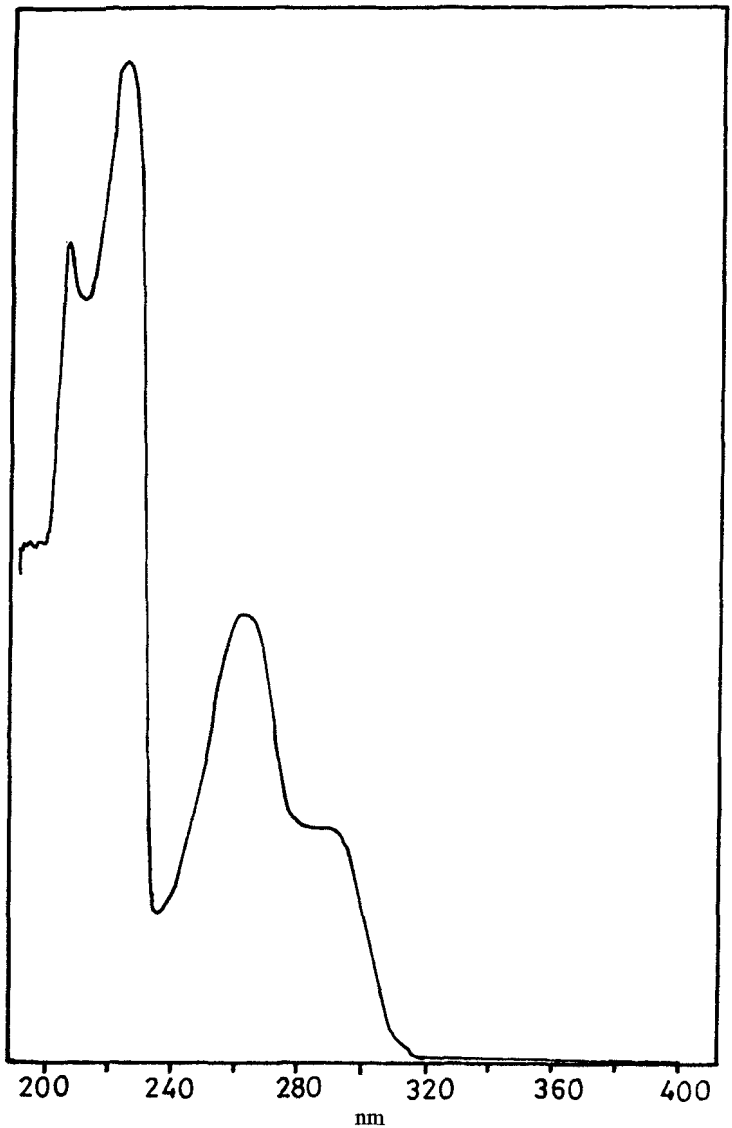


Figure 3 Ultraviolet Spectrum of Mebeverine Hydrochloride in 0.1 N HCL

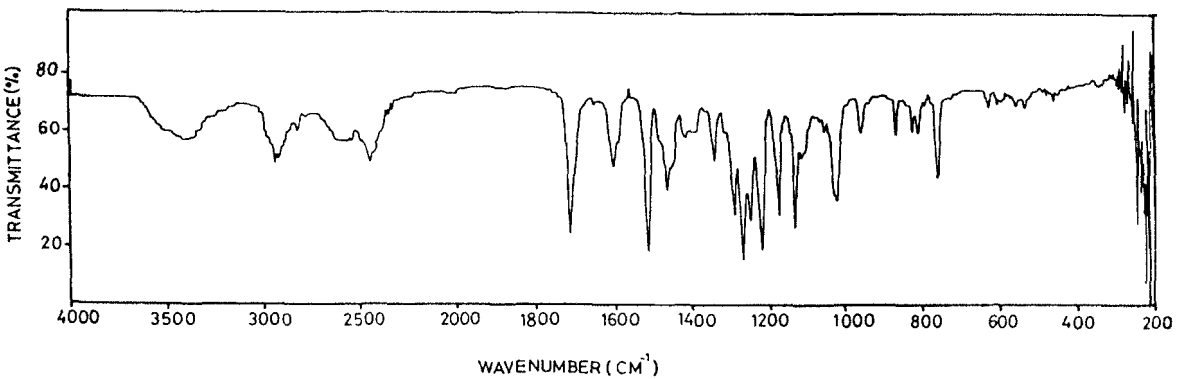


Figure 4 Infrared Spectrum of Mebeverine Hydrochloride, KBr Disc

Table 2

Vibrational Spectral Assignments of Mebeverine Hydrochloride

Frequency (cm^{-1})	**Assignment**
3025-2860	Aromatic and aliphatic C-H Stretchings
2480-2360	N^{+}-H Stretching
1715	C = O Stretching
1600, 1510, 1450	C = C Stretchings
1265-1130	Asymmetrical C-O-C and C-O Stretchings
1020	Symmetrical C-O-C Stretching
950-750	A number of bands due to substituted benzene rings

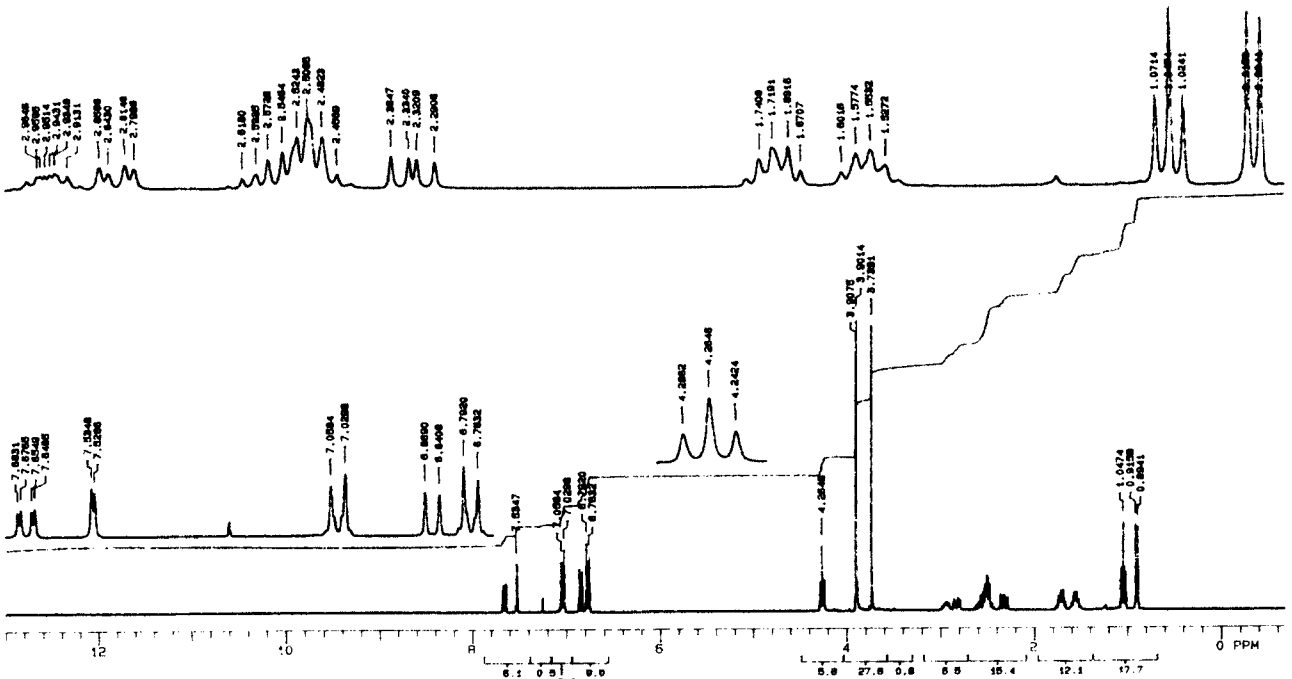


Figure 5 ^{1} **H-NMR Spectrum of Mebeverine in $CDCl_3$ from TMS**

Table 3

Chemical Shifts and Spectral Assignments of the ^{1}H-NMR of Mebeverine

Chemical Shift δ; ppm	Multiplicity	Assignment	Number of Protons
0.90	d	Cα - H	3H
1.05	t	Cγ - H	3H
1.55	dt	C4 - H	2H
1.72	dt	C3 - H	2H
2.29 - 2.36	dd (broad)	C7 - H	1H
2.46 - 2.62	m	C5 - H, Cβ - H	4H
2.80 - 2.86	dd (broad)	C7 - H	1H
2.91 - 2.96	m	C6 - H	2H
3.74	s	C3`- OMe	3H
3.90 - 3.91	s	C4`- OMe, C4``-OMe	6H
4.26	t	C2-H	2H
6.76 - 6.79	d	C3``/ 5``- H	2H
6.84 - 6.87	d	C5`- H	1H
7.03 - 7.06	dd	C2``/ 6``- H	2H
7.53	d	C2`- H	1H
7.65 - 7.68	dd	C6`- H	1H

s: singlet
d: doublet
t: triplet
m: multiplet
dd: doublet of doublets
dt: doublet of triplets

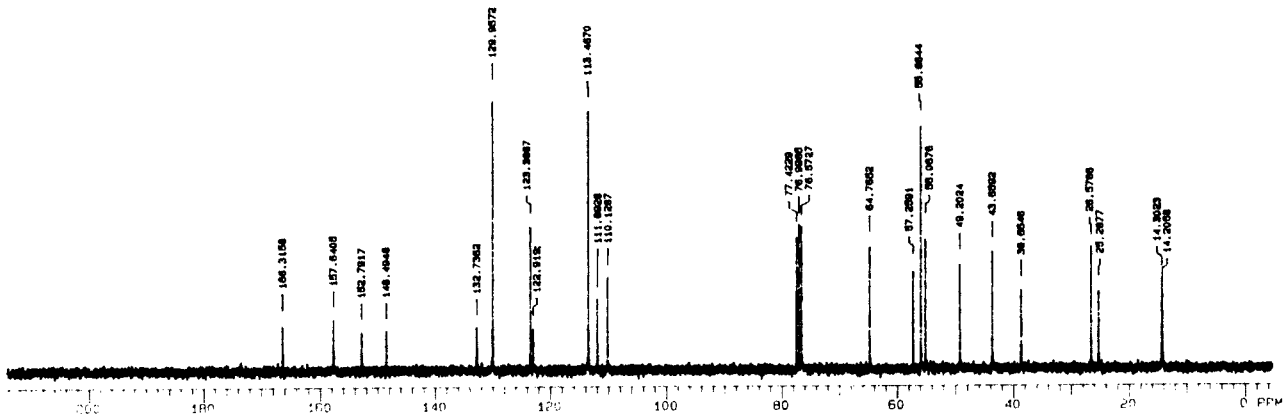


Figure 6 ^{13} **C-NMR Spectrum of Mebeverine in $CDCl_3$ from TMS**

Table 4

Chemical Shifts and Spectral Assignments of the ^{13}C- NMR of Mebeverine

Chemical Shift δ; ppm	Assignment
14.21, 14.31	C - α, C- γ
25.29	C - 4
26.59	C - 3
38.66	C - 7
43.67	C - β
49.21	C - 5
55.07	C3` - OMe
55.87	C4`- OMe, C4``- OMe
57.26	C - 6
64.77	C - 2
110.13	C - 5`
111.90	C - 2`
113.47	C - 3``/5``
122.92	C - 1`
123.39	C - 6`
129.39	C - 2``/6``
132.74	C - 1``
148.50	C - 3`
152.79	C - 4`
157.64	C - 4``
166.31	C - 1

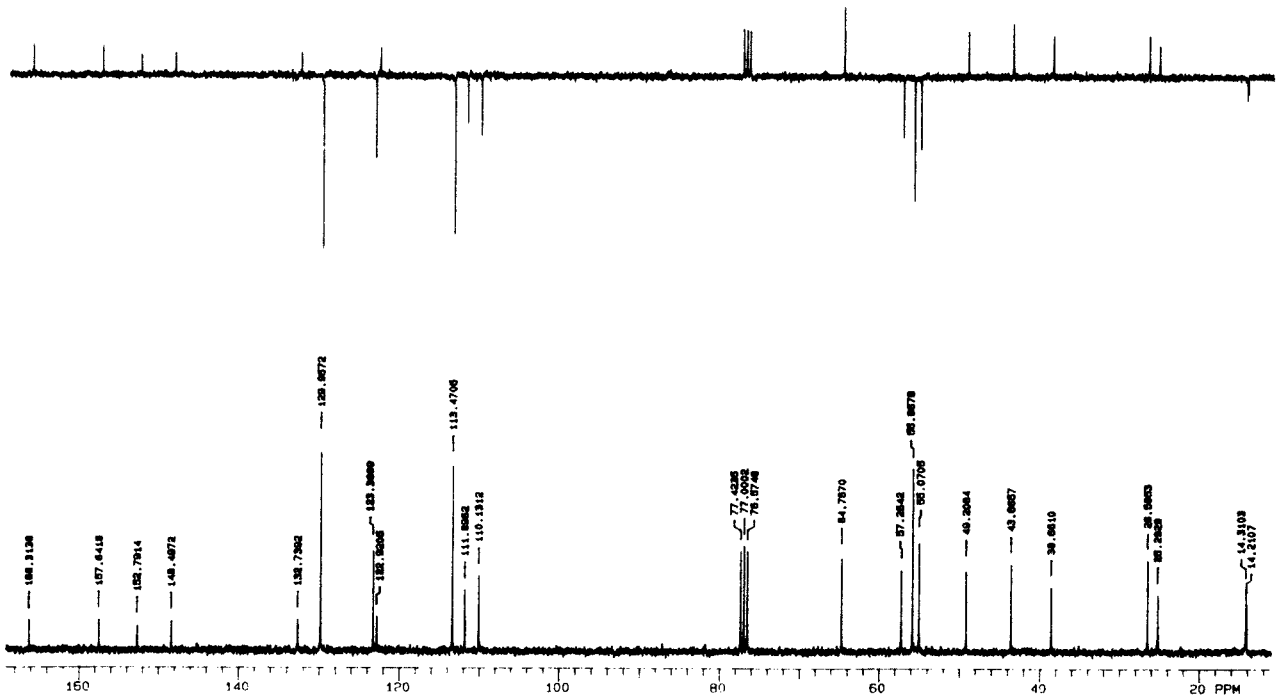


Figure 7 APT Spectrum of Mebeverine

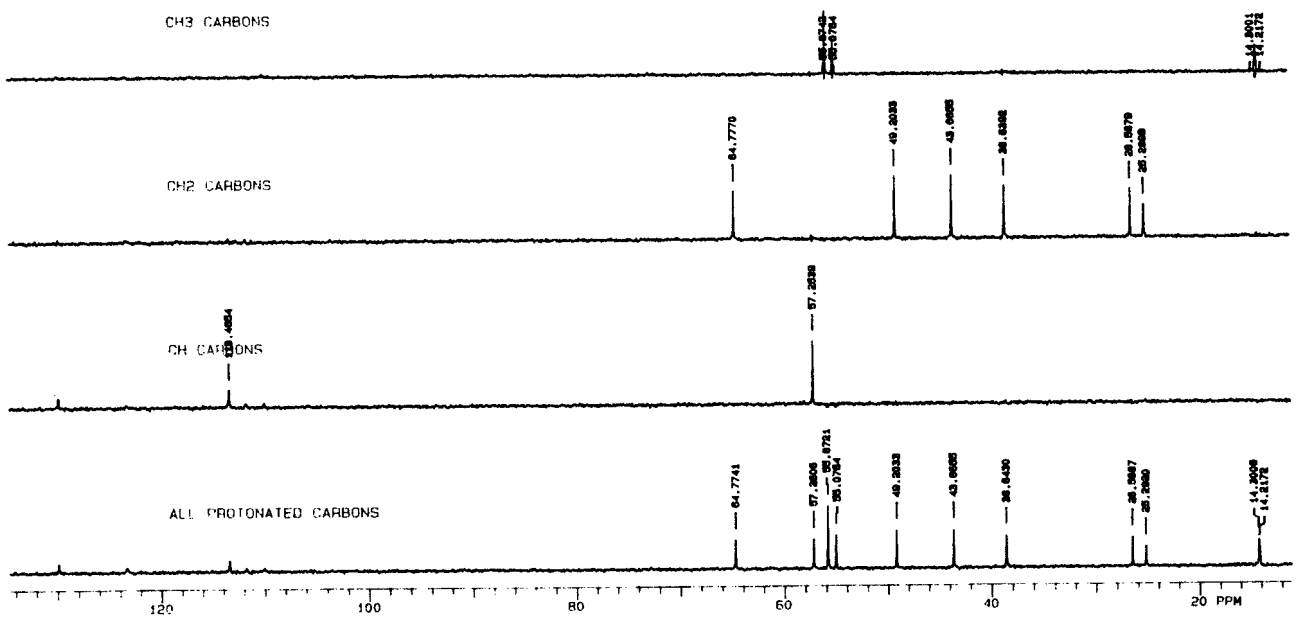


Figure 8 DEPT Spectrum of Mebeverine

COSY or HETCOR) correlations are shown in Figures 9 and 10, respectively.

3.5.4 Mass Spectrometry

The chemical ionization (CI) mass spectrum of mebeverine hydrochloride was obtained on a VG ZAB-HF mass spectrometer, equipped with an ion Tech. Saddle-field FAB gun and a standard VG source. The gun was operated at 8 keV with 2-mA current. As evident in the mass spectrum shown in Figure 11, the molecular ion peak was detectable at m/z = 429 and the base peak $[M + H]^+$ at m/z = 430. Assignments for the observed fragments are given in Table 5. Literature reports [2] have indicated that principal peaks can be observed at m/z values equal to 309, 308, 165, 154, 121, 98, 56, and 55. The mass spectrum of mebeverine is clearly able to provide a sensitive and specific mean its identification in pharmaceutical dosage forms.

4. Methods of Analysis

4.1 Compendial Tests [3]

4.1.1 Method I: Non-Aqueous Titration

4.1.1.1 Drug Substance

Mebeverine hydrochloride (0.4 g) is dissolved in 7 mL of mercury (II) acetate. The solution is titrated with 0.1 M perchloric acid, and the end point is determined potentiometrically. Each mL of 0.1 M perchloric acid is equivalent to 46.6 mg of mebeverine hydrochloride.

4.1.1.2 Drug Product

Twenty tablets of mebeverine hydrochloride are weighed and powdered. The quantity of powdered tablets which contains 0.5 g of mebeverine hydrochloride is heated, with occasional shaking, in 100 mL of 0.1 M hydrochloric acid for 10 minutes on a water bath. After cooling, a sufficient amount of 0.1 M hydrochloric acid is added to produce 250 mL, and the solution is filtered. A sufficient amount of 0.1 M hydrochloric acid is added to 10 mL of the filtrate to produce a total volume of 100 mL,

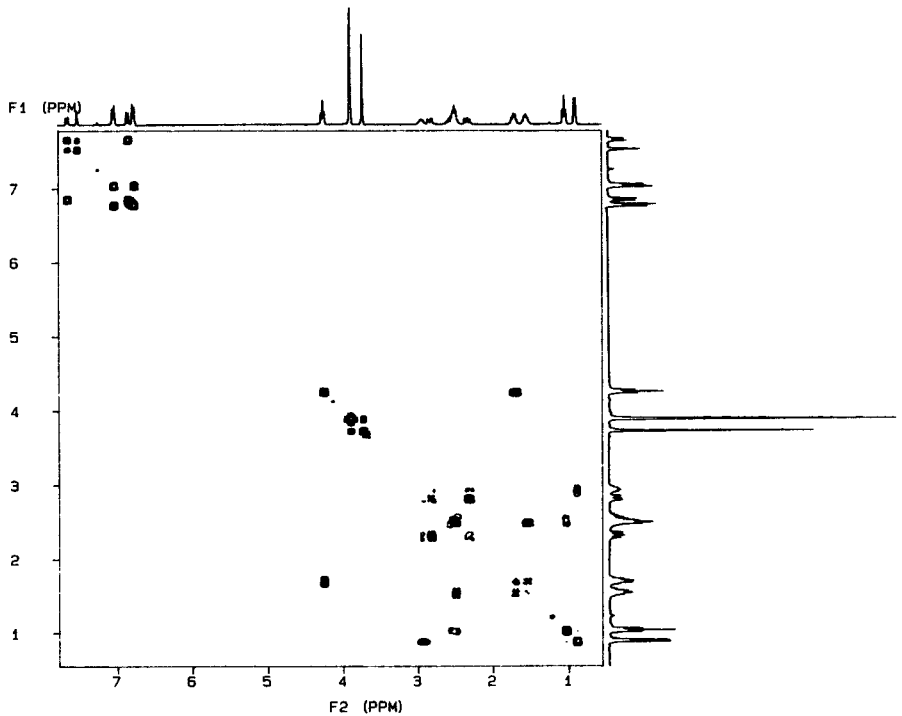


Figure 9 HH - COSY Spectrum of Mebeverine

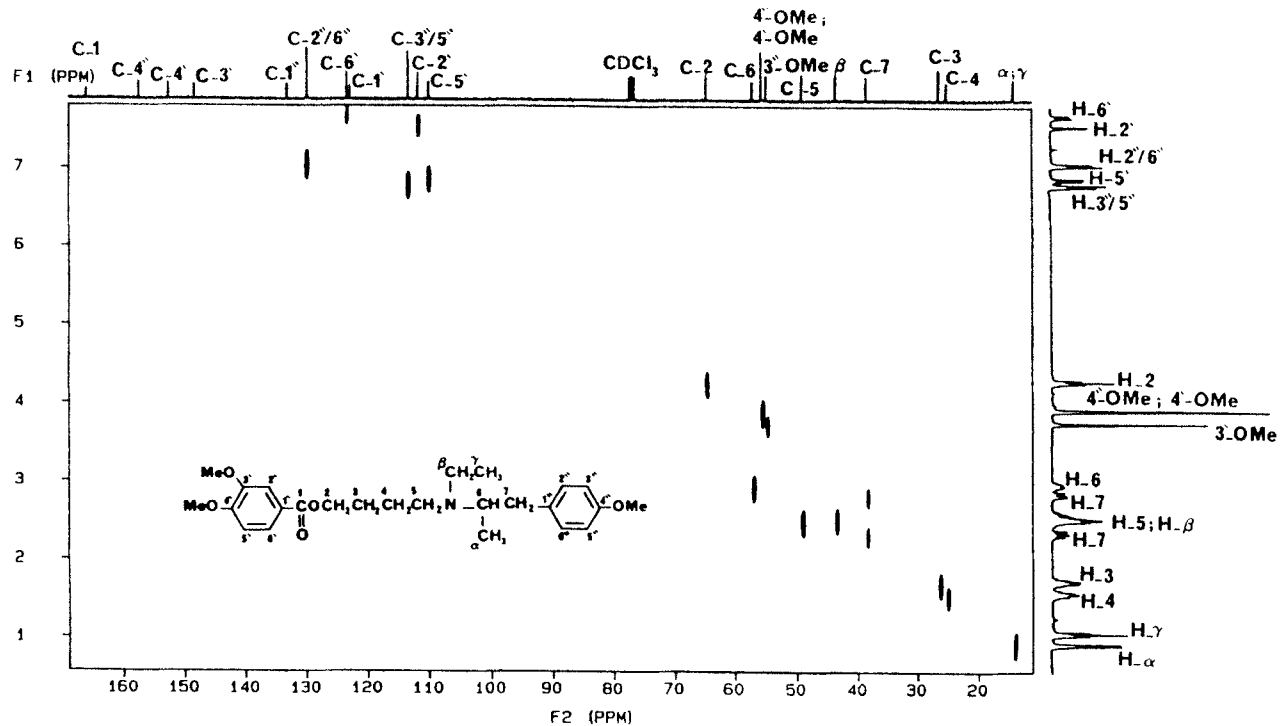


Figure 10 The Assigned CH - COSY (HETCOR) Spectrum of Mebeverine.

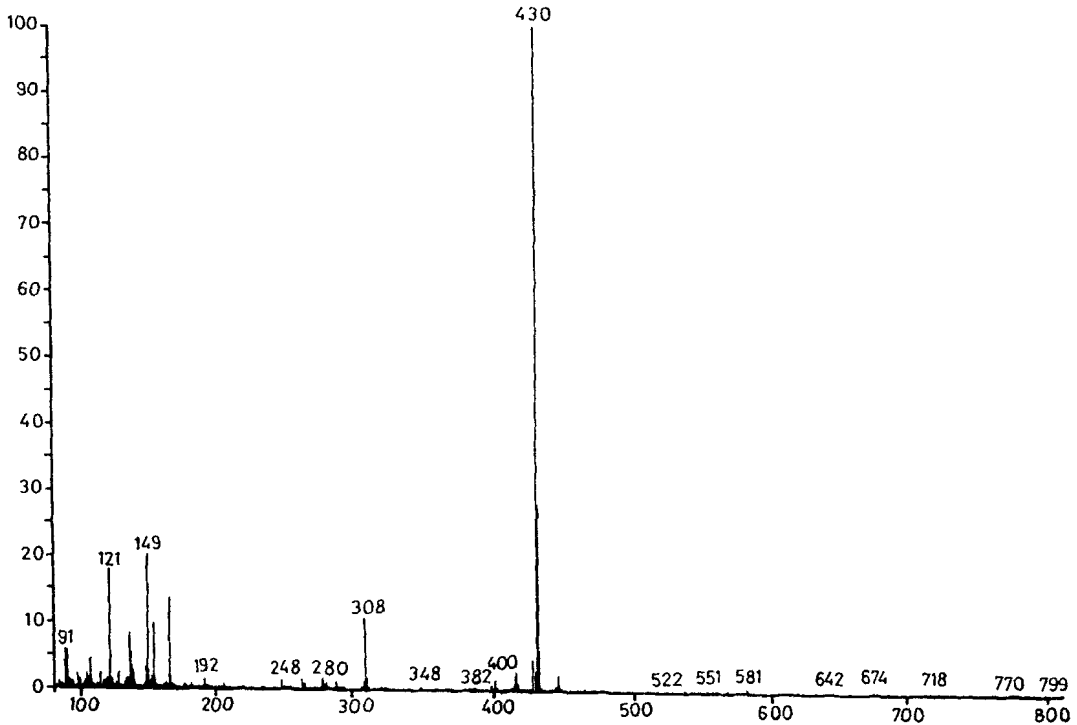


Figure 11 Chemical Ionization Mass Spectrum of Mebeverine Hydrochloride

Table 5

Fragmentation Assignments of the Mass Spectrum

of Mebeverine Hydrochloride

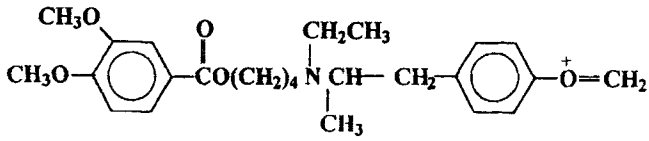
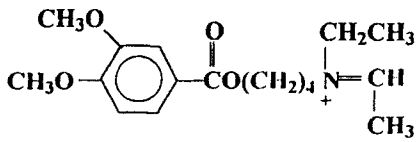
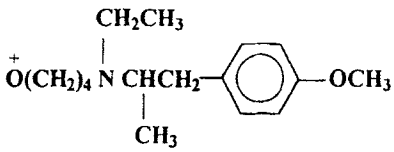
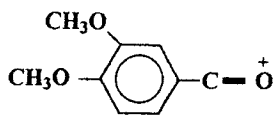
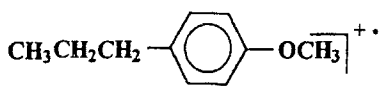
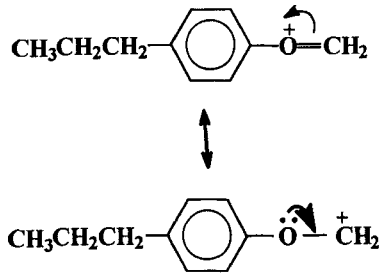
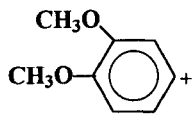
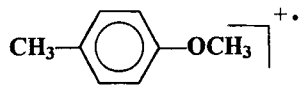
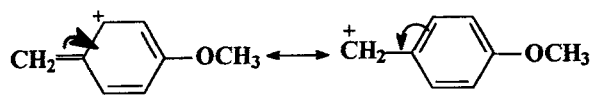
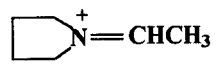
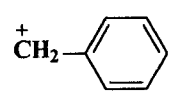
m/z (%)	Fragment Assignment
430 (100)	$[M+H]^+$
429 (3)	$M^{+\cdot}$
428 (5)	
308 (11)	
264 (2)	
165 (14)	
150 (3)	

Table 5 (Continued)

Fragmentation Assignments of the Mass Spectrum of Mebeverine Hydrochloride

m/z (%)	Fragment Assignment
149 (20)	
137 (3)	
122 (3)	
121 (18)	
98 (3)	
91 (5)	

whereupon 15 mL of the that solution is diluted to 100 mL with the same solvent. The absorbance of the solution is measured at 263 nm. The content of mebeverine hydrochloride is then calculated taking 263 as the value of A (1%, 1 cm) at this wavelength.

4.2 Identification [3]

4.2.1 Infrared Absorption Spectrum

Appendix IIA of the British Pharmacopoeia (1993 edition) contains the reference IR spectrum of mebeverine hydrochloride. The five most intense lines of the experimentally determined spectrum should agree with those of the reference.

4.2.2 UV Absorption Spectrum

Appendix IIB of the British Pharmacopoeia (1993 edition) contains the ultraviolet absorption spectrum of 0.003% w/v mebeverine hydrochloride in 0.1 M hydrochloric acid, over the range of 230 to 356 nm. As mentioned in a previous section, the compound exhibits a maximum at 263 nm, and a shoulder at 292 nm. The absorbances at these wavelengths are approximately 0.79 and 0.41, respectively.

4.2.3 Chloride Content

The chloride content is detected according to the method described in Appendix VI of the British Pharmacopoeia (1993 edition). 25 mg of mebeverine hydrochloride are dissolved in 2 mL of water, acidified with 2M nitric acid, and centrifuged. The supernatant liquid is then used in conjunction with the reaction characteristic for the presence of chlorides.

4.2.4 Melting Point

The melting point of mebeverine hydrochloride is approximately 135°C, according to Appendix VA of the British Pharmacopoeia (1993 edition).

4.2.5 Color Test

A Libeimann's color test has been described, which produces a black color with mebeverine hydrochloride. [2].

4.3 Elemental Analysis

The calculated elemental content (%w/w) of both mebeverine [6] and of its hydrochloride salt is shown in Table 6.

Table 6

Elemental Analysis of Mebeverine and Its Hydrochloride Salt

Element	Mebeverine (w/w %)	Mebeverine Hydrochloride (w/w%)
C	69.90	64.377
H	8.21	7.725
N	3.26	3.004
O	18.62	17.167
Cl	-	7.618

4.4 Titrimetric Analysis [3]

Mebeverine hydrochloride is assayed by the non-aqueous titration method described in section 4.1.1.

4.5 Spectrophotometric Methods of Analysis

4.5.1 Mebeverine in Tablets

A spectrophotometric method for the detection of mebeverine hydrochloride in tablets has been described in section 4.1.1.2.

4.5.2 Derivative Spectrophotometric Method

A derivative spectrophotometric determination of mebeverine hydrochloride in drug products, and in the presence of its alkaline-induced degradation product, has been developed [7]. The drug was extracted from powdered tablets with methanolic 0.1 N HCl, and the extract then diluted with methanolic 0.1 M NaOH. The first derivative absorption values were measured at 275 nm and the second derivative values measured at 239 nm. The recovery was found to be 99%, and characterized by a relative standard deviation of 1.4%.

4.6 Chromatographic Methods of Analysis

4.6.1 Paper Chromatography

A paper chromatography method has been described, which uses system PI [8,9]. The particulars of the method are as follows:

Paper: A Whatman No.1 sheet (14 x 6 in) is buffered by dipping in a 5% solution of sodium dihydrogen citrate, blotting, and drying at 25°C for 1 hour. It can then be stored indefinitely.

Sample: 2.5 mL of a 1% solution dissolved in 2 N acetic acid. Other solvents for dissolution of samples could be 2 N hydrochloric acid, 2 N sodium hydroxide, or ethanol.

Solvent: 4.8 g of citric acid is dissolved in a mixture of water and n-butanol, at a ratio of 130:870 v/v.

Development: Ascending in a tank 8 x 11 x 15.5 in, 4 sheets being run at a time. The run time is 5 hours.

Location: Under ultraviolet light, blue fluorescence is observed.

Location Reagent: Iodoplatinate spray strong reaction.

R_f: 0.77

4.6.2 Thin Layer Chromatography

Three different thin layer chromatographic methods for the detection of mebeverine hydrochloride have been reported [10], and are summarized in Table 7.

4.6.3 Gas Chromatography

Liu *et al.* [11] described a combined supercritical fluid extraction/solid-phase extraction method, with octadecylsilane cartridges as the sample preparation technique, for the ultratrace analysis of the mebeverine metabolite, mebeverine alcohol. The absolute recovery, selectivity, precision, and accuracy of the combined supercritical fluid extraction/ solid-phase extraction approach were compared to those of conventional solid-phase extraction combined with GC-MS detection. Although the superficial fluid extraction/solid-phase extraction technique was in an unoptimized stage, its accuracy and precision for the analysis of mebeverine metabolite were comparable to that obtained using the conventional solid-phase extraction method.

Identification of six mebeverine metabolites excreted in urine following oral administration of the drug was carried out using GC-MS detection [12]. In this work, proposed metabolic pathways of mebeverine in man were also provided.

4.6.4 High Performance Liquid Chromatography

Several HPLC systems for the determination of mebeverine hydrochloride have been reported [12-17], and the details of these are given in Table 8.

4.6.5 High Performance Thin Layer Chromatography

Two HPTLC methods have been described. The first of these studied the influence of stabilizers (2,6-di-t-butyl-*p*-cresol and quinol) and impurities in solvents on the reproducibility of the quantitative separation (as stability evaluation) of mebeverine hydrochloride. Si 60 F254 high-performance TLC plates were used [18]. After pre-development with 1:1 methanol-chloroform, the plates were spotted with test solution and equilibrated with a mixed solvent system containing a small amount of aqueous ammonia. One component of the solvent system was 1:1 isopropyl ether-2,2,4-

Table 7

Thin Layer Chromatographic Methods for the Detection of Mebeverine Hydrochloride.

System No.*	Mobile Phase	Stationary Phase	Reference Compound	R_f
TA	Methanol Strong NH_3 solution (100:1.5)	Silica gel G, 250 μm thick, dipped in, or sprayed with, 0.1 M KOH in CH_3OH, and dried.	Diazepam R_f 75, Clorprothixene R_f 56, Codeine R_f 33, Atropine R_f 18.	63
TB	Cyclohexane: Toluene: Diethylamine (75:15:10)	As system TA	Dipipanone R_f 66, Pethidine R_f 37, Desipramine R_f 20, Codeine R_f 06.	40
TC	Chloroform: Methanol (90:10)	As system TA	Meclozine R_f 79, Caffeine R_f 58, Dipipanone R_f 33, Desipramine R_f 11.	53

* According to "Isolation and Identification of Drugs" [2].

Table 8

HPLC Systems for the Determination of Mebeverine Hydrochloride

System No.	Flow rate, ml/min.	Column	Mobile Phase	Detection	Remarks	Ref. No.
1	0.8	Chrom Sep Spherisor CN (10 x 0.3 cm) (5 μm)	Triethylammonium acetate buffer:ACN (55:45)-pH 6.5	UV at 227 nm	Drug was extracted from urine (after alkalization) with diethy ether- Detection limit: 5 ng/ml	12
2	1.5	(10 x 0.8 cm) C_{18} Bondapack (10 μm)	H_20: ACN: acetic acid (59: 40: 1)	Fluorescence: imm.:362 nm, excit. at 270 nm	Detection limit 20 ng/ml plasma.	13
3	2	(25 x 0.4 cm) Lichrosorb CN (10 μm)	ACN: H_20: propylamine (80: 20: 0.01)	UV at 227 nm	For tablets & Biological fluids: extraction using ion-pairing with sod. octyl sulfate. Detection limit: 2 ng/ml	14
4	1	Lichrosorb RP-8 (10 μm)	Aqueous NH_3 & 75% MeOH containing 0.05% hexylamine - pH 5	UV at 263 nm (drug) & at 220 nm (minor components), MS (degradation products)	For the drug, 3 degradation products, 2 intermediate components (from synthesis), & other anti-spasmodic drugs.	15
5	1.1	4 μm C_{18} Nova pack cartridge with guard column C_{18} Corasil Bondapack	H_2O: ACN: Triethylamine (55: 45: 1) - pH 3	UV at 225 nm	*in -vitro & in-vivo* study	16
6		(12.5 x 0.49 cm) Spherisorb S5W Silica (5 μm)	Methanolic Ammonium Perchlorate (10 mM, pH 6.7)	254 nm & electrochem. at +1.2 V	Applied to 462 compounds	17

trimethyl- pentane, and the other was 1:1 methanol-isopropyl ether. The effects of solvent system composition were investigated by varying the ratio of each component. Both the composition of the pre-development mixture, and the purity of the solvents, were found to be critical in obtaining reproducible and adequate separations.

In the second method [19], mebeverine hydrochloride and its degradation products were separated by HPTLC on RP-2 F254S, RP-8 F254S, and RP-18 F254S plates, as well as on Nano SIL C18-50 UV 254, -75 UV 254, and -100 UV 254 plates. The mobile phases consisted of aqueous ammonia with methanol (80%), acetonitrile (77.5%), or acetone (70%) as an organic modifier. For the quantitative determination of mebeverine in tablets, optimal separations were obtained on the RP-8 F 254S plate, using 40:9:1 methanol-water-25% aqueous ammonia as the mobile phase.

4.6.6 Capillary Supercritical Fluid Chromatography Coupled with Mass Spectrometric Detection

Capillary supercritical fluid chromatography system coupled with mass spectrometric detection was used to determine mebeverine in dog plasma and tissues [20]. The assay involved solid phase extraction with ODS cartridges, and linearity was achieved for 5.95 to 59.5 ng/mL. The method precision was approximately 10%.

5. Stability

Mebeverine exhibits the classical property of pH-dependent hydrolysis which is invariably associated with the ester functional group [15]. Thus, neutral solutions of mebeverine are quite stable, but at ambient temperatures the drug degrades in either 0.5 M HCl and 0.1 M NaOH. Half-lives of 90 and 0.2 days, respectively were measured in these systems.

5.1 Solid State Stability

The bulk drug substance should be stored in airtight containers, at temperatures not exceeding 30^{o}C, and should be protected from light [21].

A HPLC study of the stability of the drug substance in tablets has been reported [15], with the degradation products being identified by mass spectrometry. The tablets were found to be stable at 50°C for over a year, after which 96.8% of the drug substance still remained.

5.2 Incompatibilities

The physicochemical compatibilities between mebeverine hydrochloride and a number of common tablet and capsule excipients were investigated by differential scanning calorimetry [22]. Mebeverine was found to be incompatible with stearic acid, lactose, sodium carboxymethyl cellulose, and corn starch.

6. Interaction of Mebeverine Hydrochloride and Carbopol

The interaction of mebeverine hydrochloride and carbopol 934 polymer in an aqueous medium was studied using a dialysis technique [23]. The interaction was found to be inversely proportional to the initial concentration of drug, and directly proportional to the polymer concentration. The drug-polymer interaction was found to be dependent on both the solution pH and on the ionic strength. The solubility and the melting point of the interaction product were significantly different from those of the original compounds. DSC, IR, and X-ray diffraction were used to show the difference between the parent components and the interaction product. The interaction product had a spasmolytic activity towards acetylcholine and barium chloride in guinea-pig isolated colon. The percentage antagonism was 83.8 and 67.7 towards acetylcholine and barium chloride, respectively.

7. Pharmacokinetics and Drug Metabolism

Mebeverine is a spasmolytic agent with a direct nonspecific relaxant effect on smooth muscle [24-29]. Although one clinical trial [30] has failed to prove its efficacy, other trials seem to confirm its clinical significance in the treatment of irritable bowel syndrome [31-33].

Dose for dose, the compound was found to be three times as potent as papaverine for inhibiting the peristaltic reflex of the guinea-pig ileum, and 20 to 40 times more powerful in inhibiting the sphinder of oddi [29].

Mefenamic acid or mebeverine, given at the first sign of dysmenorrhea or at the onset of menstruation and continued for 1 to 4 days, were comparable in reducing the severity of pain in 49 patients with primary dysmenorrhea. Mebeverine produced a lower incidence of nausea and abdominal bloating [34].

Seventy-one patients with functional abdominal pain lasting more than 3 months were randomized to receive either mebeverine or dicyclomine. No differences were detected between the two groups. However, more unwanted side effects were reported in the dicyclomine group [35].

7.1 Absorption

Mebeverine is completely and quickly absorbed after oral administration.

7.2 Metabolism

Although mebeverine has been used clinically since 1965, very little has been published on its biotransformation in man or animals. Since it is an ester of veratric acid and 4-{ethyl-[2-(4-methoxyphenyl)-1-methylethyl] amino}butan-1-ol, these compounds might be expected to be formed upon *in vivo* hydrolysis of the drug. However, only the alcohol moiety was detected in a pig following intravenous administration of mebeverine hydrochloride [14]. Analysis of plasma samples of two fasting male volunteers who ingested 270 mg of mebeverine hydrochloride failed to reveal detectable concentration of the parent drug. However, considerable plasma concentration of veratric acid was detected within one hour after drug administration. These findings suggested that the ester functional group of mebeverine was rapidly and extensively hydrolyzed *in vivo*, and did not reach the systemic circulation in detectable amounts [16].

A Product Monograph (Duphar Company) reported that mebeverine is extensively metabolized as it passes through the gut wall and liver, as

described in Scheme 2A. The first step takes place in the small intestine and liver, and the remaining steps possibly take place in the liver. The metabolites are all rapidly excreted in the urine, with excretion being virtually complete within 24 hours after ingestion of a single oral dose.

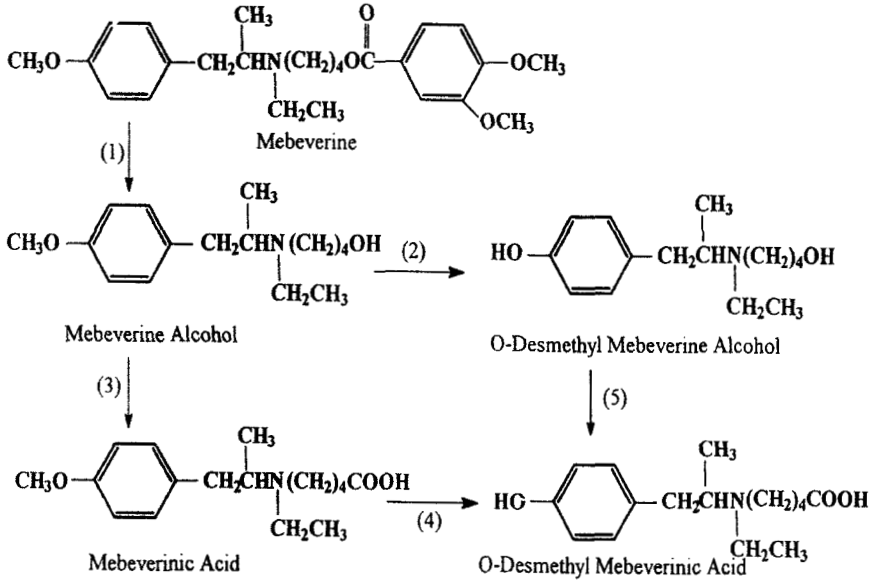
Recently, Kristinsson *et al.* [12] studied the urinary metabolites of orally administered mebeverine hydrochloride (270 mg) in healthy volunteers. No altered mebeverine was detected in the pooled urine samples, which is in agreement with the findings reported earlier by Dickinson *et al.* [16]. Their results indicated that mebeverine is completely metabolized to at least six metabolites following oral administration to man. The proposed pathway of metabolism is depicted in Scheme 2B. The total excretion of the acid moiety (unchanged or in the form of metabolites) was 97.6%, while the alcohol moiety corresponded to 5.5%. All these metabolites were mostly excreted as conjugates. It was also suggested that the effects of the drug are probably mediated through metabolites, rather than through the drug itself. This proposal is supported by the effective use of orally administered mebeverine for relieving the symptoms of primary dysmenorrhea [34], which should be a systemic effect. On the other hand, assuming the gut is the main site of hydrolysis, the effect of mebeverine on irritable bowel syndrome might be explained by the local action of some unabsorbed drug or of its hydrolysis products [12,16].

8. Toxicity

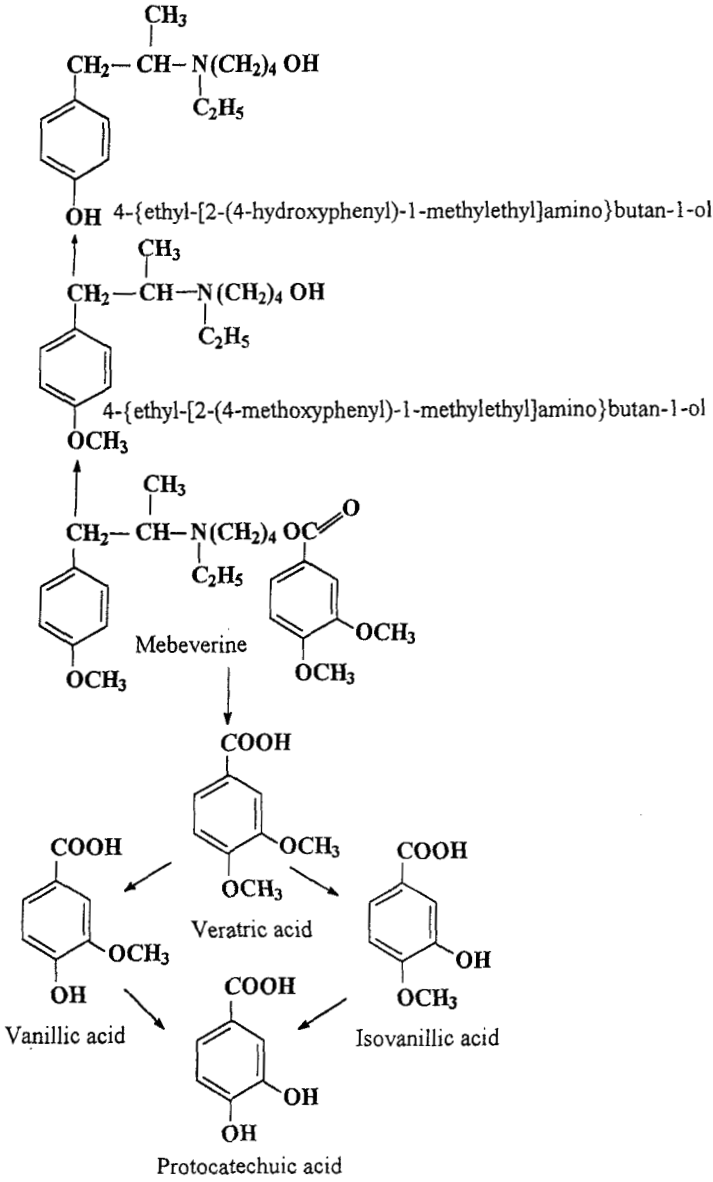
The LD_{50} (oral administration route) of mebeverine in mice is approximately 1 g/kg [2].

9. Adverse Effects

A 24-year old man with cystic fibrosis, who took mebeverine hydrochloride for his lower abdominal pain and constipation, was found to have a perforated stercoral ulcer with generalized peritonitis [36]. It was suggested that mebeverine produced colonic stasis, which predisposed the patient to ulceration. However, the manufacturers considered that concomitant development of constipation and distal intestinal syndrome



Scheme 2A Proposed Metabolic Pathways of Mebeverine



Scheme 2B Proposed Metabolic Pathways of Mebeverine

(meconium ileus equivalent) in the patient precipitated the development of stercoral ulceration [37]. It was recommended that antispasmodics such as mebeverine should not be used for the symptomatic treatment of distal intestinal syndrome in cystic fibrosis [36].

10. Contraindications

It is not safe to give mebeverine to patients with acute porphyria because the compound has been shown to be porphyrinogenic in animals or in other *in vitro* systems [38].

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MESALAMINE

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Contents

1. Description

1.1 Nomenclature

1.1.1 Chemical Names

5-aminosalicylic acid

5-amino-2-hydroxybenzoic acid

5-amino-2-hydroxybenzene-1-carboxylic acid

m-aminosalicylic acid

5-amino-2-salicylic acid

1.1.2 Nonproprietary Names

Mesalamine

5-aminosalicylic acid

1.1.3 Proprietary Names

Asacol; Asacollitin; Claversal; Fisalamine; Lixacol; Mesasal; Mesalazine; Pentasa; Rowasa; Salofalk

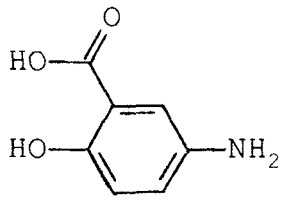
Mesalamine is commercially available as tablets (Asacol, Procter & Gamble Pharmaceuticals, Cincinnati, OH), capsules (Pentasa, Hoechst Marrion Roussel, Kansas City, MO), as well as rectal suppositories, rectal solution, and rectal suspension (Rowasa, Solvay Pharmaceutical, Marietta, GA).

1.2 Formulae

1.2.1 Empirical

$C_7H_7NO_3$

1.2.2 Structural



1.3 Elemental Composition

Based on the empirical formula given above, the theoretical elemental composition of mesalamine is C = 54.90%, H = 4.61%, N = 9.15%, and O = 31.34% [4].

1.4 Molecular Weight

153.14 Daltons

1.5 CAS Number

89-57-6

1.6 Appearance

Mesalamine is obtained as a tan to pink crystalline powder, whose individual crystals exhibit a needle-like morphology. The color of bulk material can darken upon exposure to air. The solid is odorless, or can exhibit a slight characteristics odor.

1.7 Uses and Applications

Mesalamine, or 5-aminosalicylic acid, has been used for several years in the treatment of inflammatory bowl disease [1]. This drug was administered orally as sulfasalzine, which consisted of sulfapyridine linked to 5-aminosalicylic acid through an azo bond. After oral administration, sulfasalazine is hydrolyzed by colonic bacteria to release the active therapeutic moiety [2]. Sulfapyridine was used in the formulation as an inactive carrier to transport mesalamine to the distal part of the intestine. Oral administration of the drug produces a complete systemic absorption from the small intestine [3]. Currently available oral dosage forms are designed to circumvent this proximal absorption problem from the GI tract, and targeted to deliver the drug at the site of infection in the distal part of the intestine. Controlled release mesalamine capsules (Pentasa), and pH sensitive enteric coated tablets (Asacol), are some of the newer generation dosage forms.

Mesalamine is structurally related to the salicylates. It differs both structurally and therapeutically from 4-aminosalicylic acid, also known as *p*-aminosalicylic acid. The mechanism by which mesalamine acts during

the treatment of inflammatory bowel disease remains unknown, but this drug is believed to work through modulation of the chemical mediators of inflammatory response, particularly prostaglandins and leukotrienes. Mesalamine inhibits the cyclooxygenase enzyme in the arachidonic acid cascade, thereby reducing the production of inflammatory prostaglandins.

2. Methods of Preparation

Several methods have been reported in the literature for the synthesis of mesalamine [5-10]. Scheme 1 shows the preparation of the compound from 5-nitrososalicylic acid, using tin and hydrochloric acid as the reducing agent [5]. Gatterman synthesized mesalamine (illustrated in Scheme 2) by electrolysis in concentrated sulfuric acid, using 3-nitrobenzoic acid as the starting material [6].

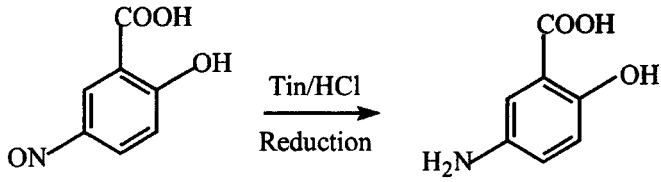
As shown in Scheme 3, mesalamine has also been prepared by the hydrogenation of 5-nitrobenzoic acid [7]. Fierz-David and Blangey used 5-phenylazosalicylic acid as the starting material, and obtained mesalamine using Na_2SO_4 and NaOH solution at a temperature of 80-90°C (Scheme 4) [8].

Scheme 5 shows the route used by Stutts *et al.* to synthesize the compound by the electrochemical reduction of 2-hydroxy-5-nitrobenzoic acid in the presence of 1N NaOH at 25°C [9]. Vasudevan and coworkers have reported the synthesis of mesalamine by the electrochemical reduction of 2-hydroxy-5-nitrobenzoic acid at a Ti/TiO_2 electrode (outlined in Scheme 6) [10].

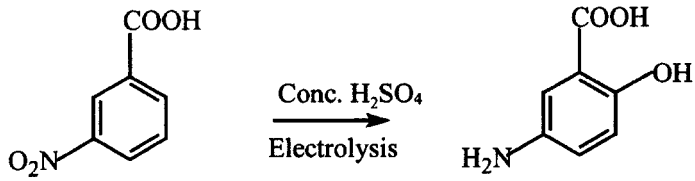
3. Physical Properties

3.1 Particle Morphology

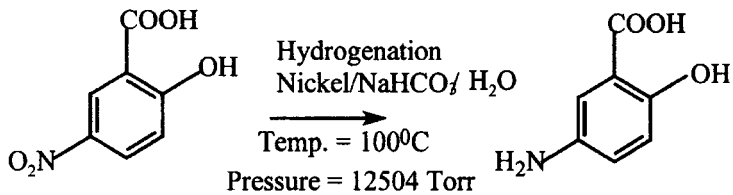
As evident in Figure 1, full unbroken crystals of mesalamine exhibit a needle-like morphology. The photomicrographs were obtained on a Leica Diastar optical microscopy system. Intact crystals within the examined sample ranged 35-40 μm in length, and 5-7 μm in width. However, the



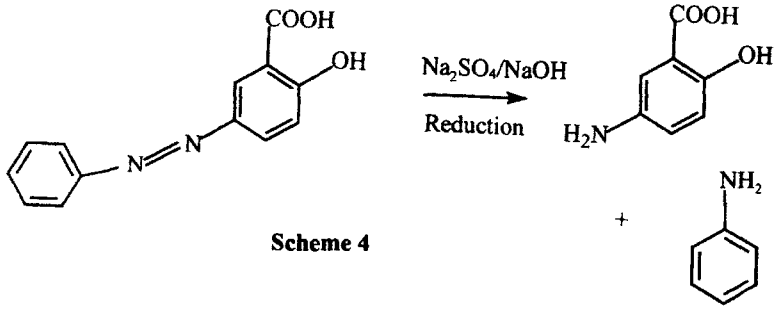
Scheme 1



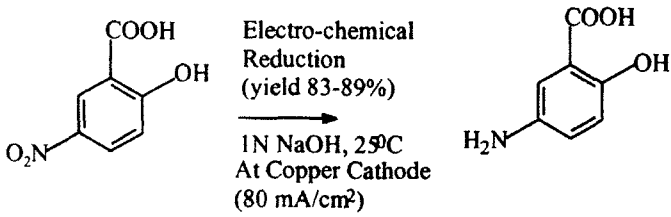
Scheme 2



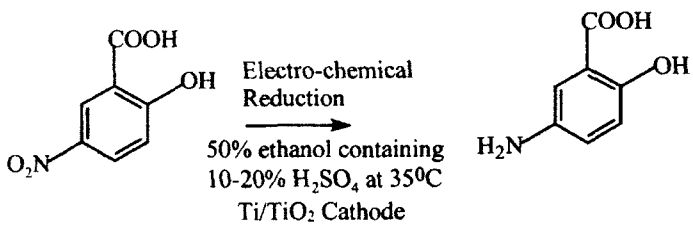
Scheme 3



Scheme 4



Scheme 5



Scheme 6

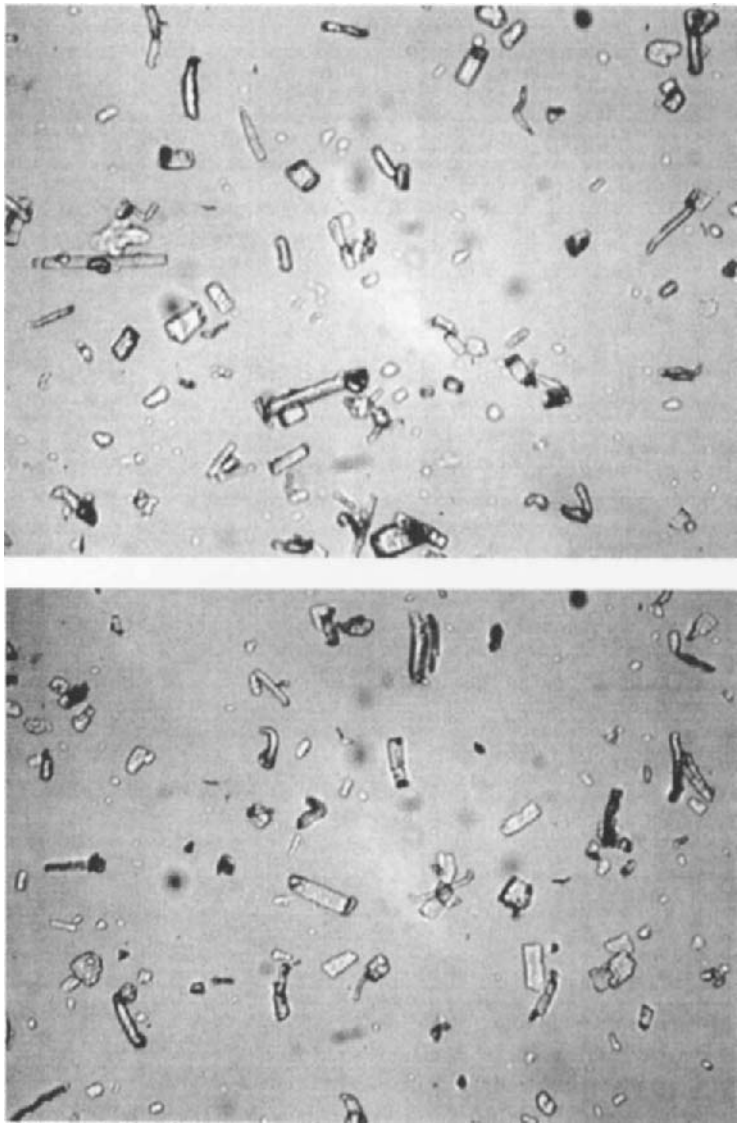


Figure 1. Photomicrographs of mesalamine, obtained at a magnification of 200X.

sample contained numerous fragment crystals, whose sizes fell in a length range of 10-20 μm and a width range of 5-7 μm.

3.2 X-Ray Powder Diffraction Pattern [11]

The x-ray powder diffraction pattern of mesalamine was obtained using a wide angle x-ray diffractometer (model D500, Siemens), and is shown in Figure 2. As evident in the figure, the sample exhibited a number of scattering peaks indicative of the existence of well-formed molecular planes in the crystal. A summary of observed scattering angles, d-spacings, and relative intensities is given in Table 1.

3.3 Thermal Methods of Analysis [11]

3.3.1 Melting Behavior

Mesalamine has been reported to melt with decomposition at about 280°C [4,13].

3.3.2 Differential Scanning Calorimetry

The DSC thermogram of mesalamine is shown in Figure 3a, and was obtained using a Shimadzu model DSC-50 thermoanalytical system. The sample was heated in a non-hermetically crimped aluminum pan at a rate of 10°C/min from 30 to 350°C. An endothermic transition assignable to the melting of the compound was observed at a temperature of 281°C, for which the enthalpy of fusion was determined to be 0.6 kJ/g.

3.3.3 Thermogravimetric Analysis

The TGA thermogram was obtained using a Shimadzu model TGA-50 Thermogravimetric analyzer, programmed to heat the sample as the same rate as used for the DSC analysis, and is shown in Figure 3b. No weight loss was noted between ambient temperature and 200°C, indicating that the sample contained neither adventitious moisture nor any molecules of solvation. Some weight loss was observed to accompany the melting transition, and complete thermal pyrolysis of the compound took place by 320°C.

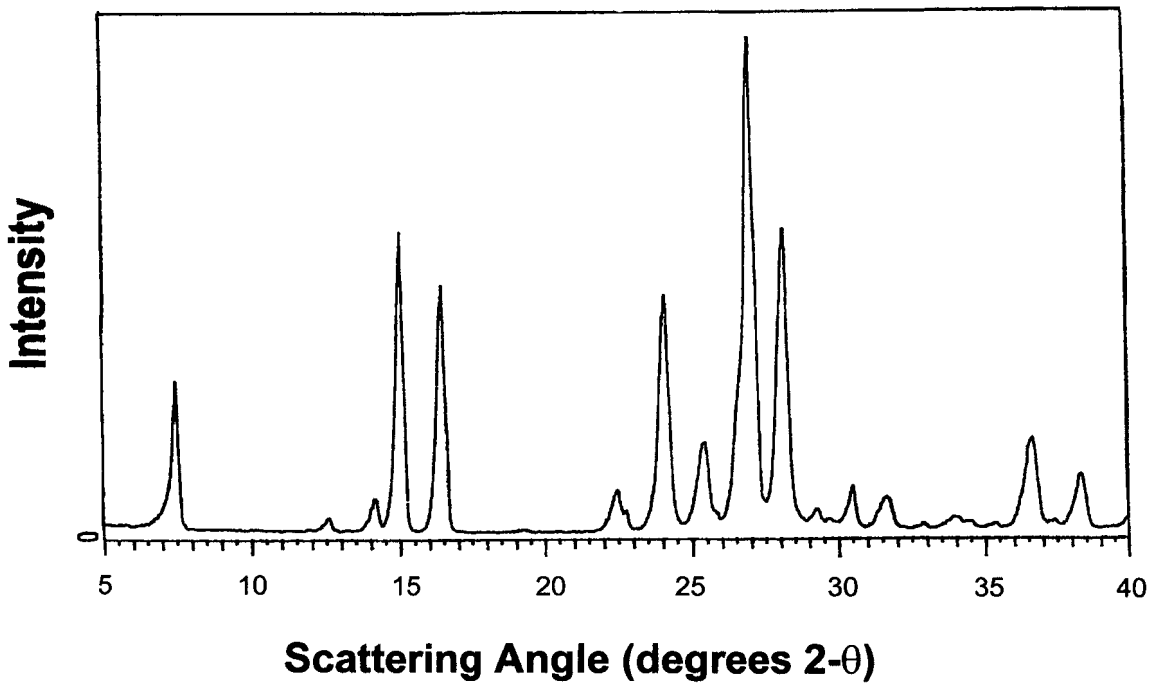


Figure 2. X-ray powder diffraction pattern of mesalamine.

Table 1

Data from the X-Ray Powder Diffraction Pattern of Mesalamine

Scattering Angle (degrees 2-θ)	**d-spacing (Å)**	**Relative Intensity, I/I_0 (%)**
7.444	11.8922	29.95
12.626	7.0209	2.90
14.092	6.2934	6.76
14.972	5.9255	60.87
16.439	5.4000	50.24
22.500	3.9571	7.73
24.064	3.7033	47.34
25.433	3.5071	17.39
27.095	3.2956	100.00
28.268	3.1614	60.87
29.344	3.0480	3.87
30.615	2.9243	8.21
31.690	2.8275	6.28
32.961	2.7213	0.97
33.939	2.6451	2.42
34.525	2.6015	1.93
35.405	2.5388	0.97
36.676	2.4537	17.87
37.458	2.4043	1.45
38.436	2.3454	10.63

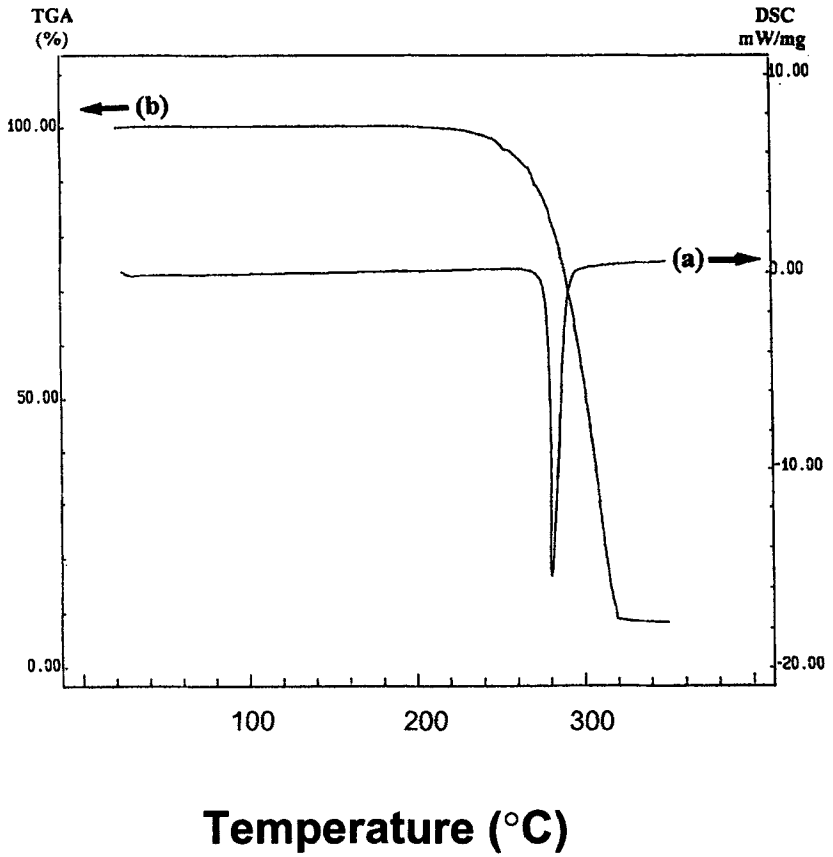


Figure 3. (a) Differential scanning calorimetry, and (b) thermogravimetric analysis thermograms of mesalamine.

3.4 Hygroscopicity

When exposed to a relative humidity of 75% (established through the use of a saturated sodium chloride solution), the substance exhibited a weight gain of only 0.7% [12]. Under ordinary environmental conditions, mesalamine is deduced to be essentially non-hygroscopic.

3.5 Solubility Characteristics

Mesalamine is slightly soluble in water (1 mg/mL at 20°C), but is soluble in dilute hydrochloric acid and in dilute alkali hydroxides. The compound is very slightly soluble in dehydrated alcohol, acetone, and methyl alcohol, and practically insoluble in chloroform, ether, butyl alcohol, ethyl acetate, n-hexane, methylene chloride, and propyl alcohol [4,13,14].

The aqueous solubility of mesalamine has been reported to be 0.844 mg/mL at 25°C, and 1.41 mg/mL at 37°C [15].

3.6 Partition Coefficient [12]

No partition coefficient data have been reported for mesalamine, but an estimation has been obtained using the ACD/logP program (Advanced Chemistry Development, Toronto, Canada). Using this computation, log P was calculated to be 0.46 ± 0.34. The existence of a partition coefficient of this magnitude indicates that the compound would exhibit roughly an equivalent preference for either a hydrophobic or hydrophilic environment.

3.7 Ionization Constants

Following trends in the pH dependence of its ultraviolet absorption spectrum, the dissociation constant of the amino group (pK_{a2}) in 5-aminosalicylic acid was determined to be 5.69. The dissociation constant of the carboxyl group (pK_{a1}) was obtained through studies of the compound solubility at pH values between 1.0 to 2.5, and was reported to be 2.30 [15].

The three dissociation constants of mesalamine have been reported to be pK_{a1} = 3.0 (carboxylate group), pK_{a2} = 6.0 (amino group), and pK_{a3} = 13.9 (hydroxyl group) [13].

3.8 Spectroscopy

3.8.1 UV/VIS Spectroscopy

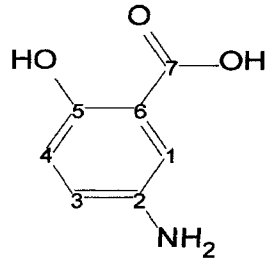
The UV absorption spectrum of mesalamine was recorded on a Perkin-Elmer model Lambda 3B spectrophotometer, over the spectral range of 200 - 400 nm [12]. The absorption spectra obtained in aqueous media of low, neutral, and high pH are shown in Figures 4a through 4c, respectively. Deprotonation of the compound is seen to cause a definite red shift of the absorption maximum, which moves from 300 nm in acidic or neutral media to 335 nm in basic solution.

3.8.2 Vibrational Spectroscopy

The infrared spectrum of mesalamine shown in Figure 5 was obtained in a KBr pellet (0.5% w/w), using a Perkin-Elmer model 1600 FTIR spectrophotometer) [11]. Assignments for the characteristic absorption bands s are listed in Table 2.

3.8.3 Nuclear Magnetic Resonance Spectrometry

Nuclear magnetic resonance studies were conducted on mesalamine using a JEOL GSX-270 spectrometer, operating at 270.05 MHz (^{1}H-NMR) or at 67.8 MHz (^{13}C-NMR). Spectra were obtained in DMSO-d_6 at room temperature, and the resonance assignments make use of the following numbering scheme:



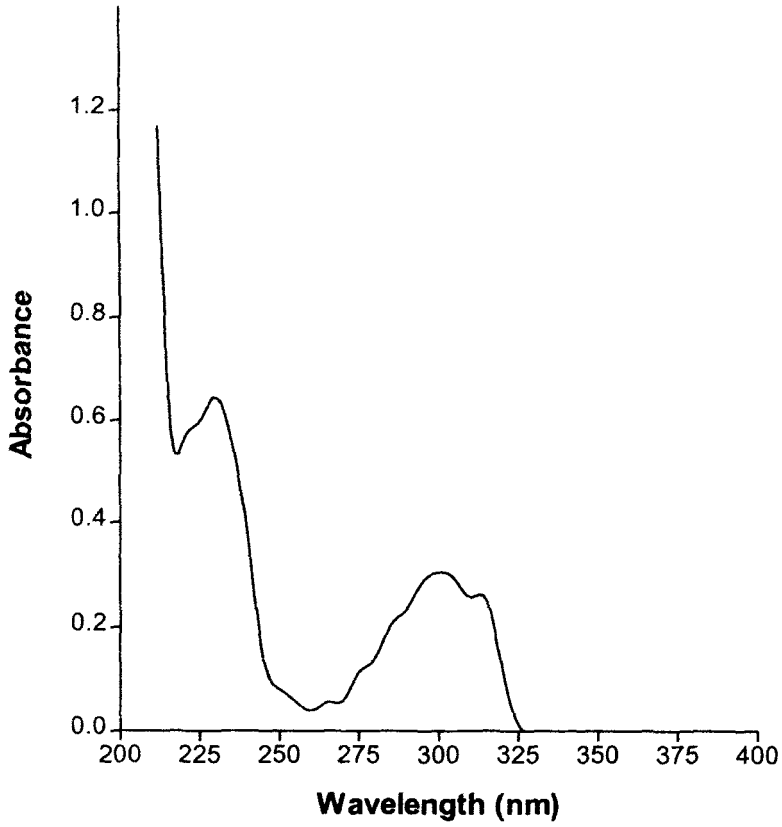


Figure 4a. Ultraviolet absorption spectrum of mesalamine, 0.014 mg/mL in 0.05 N HCl.

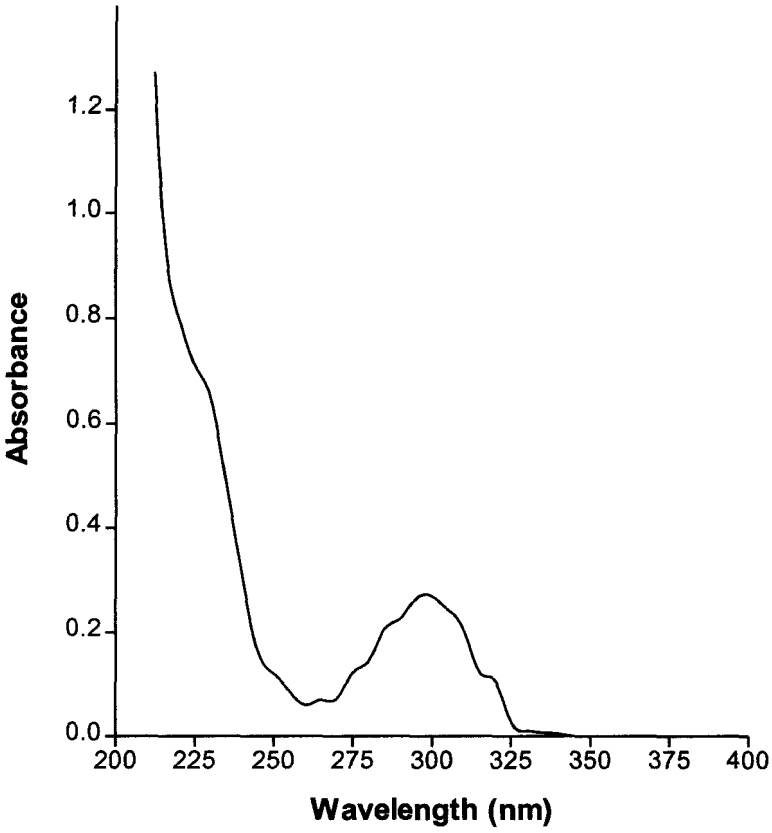


Figure 4b. Ultraviolet absorption spectrum of mesalamine, 0.014 mg/mL in water.

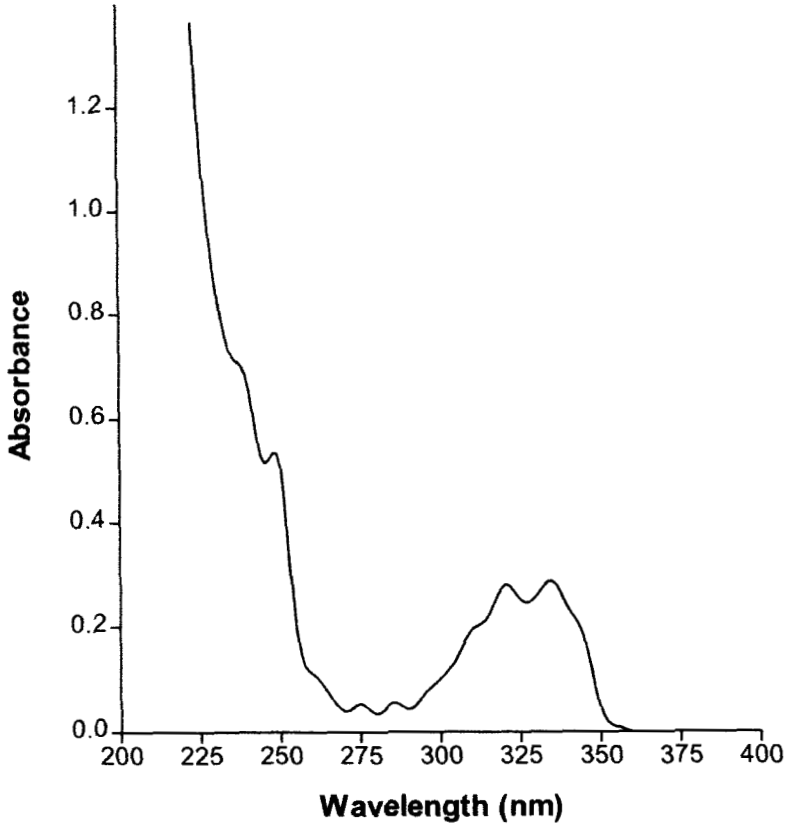


Figure 4c. Ultraviolet absorption spectrum of mesalamine, 0.014 mg/mL in 0.05 N NaOH.

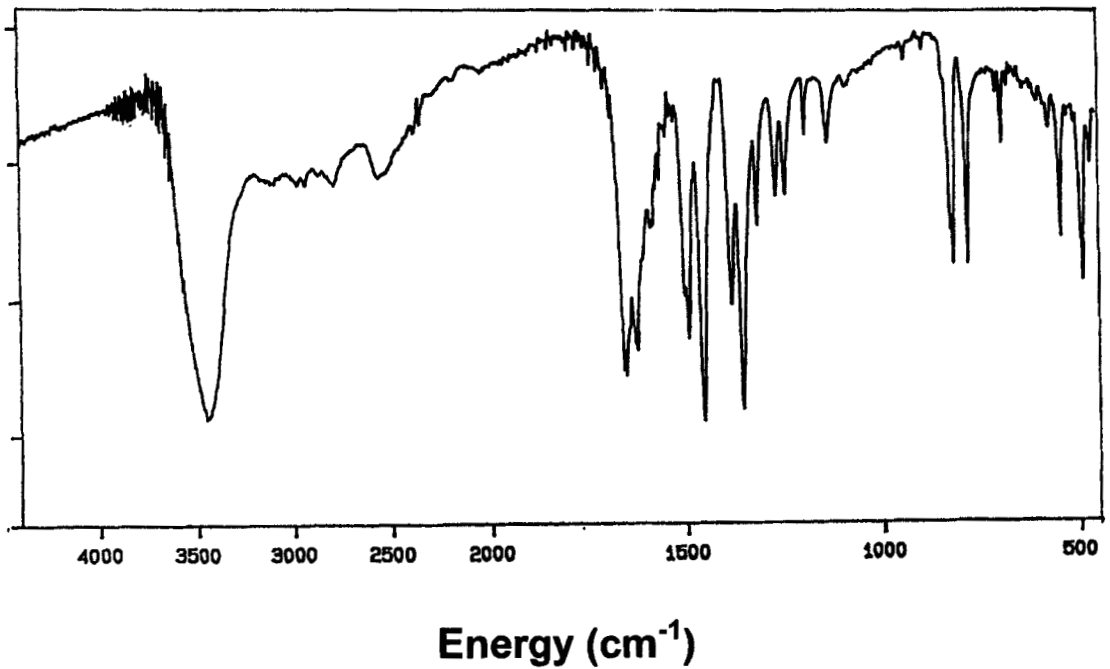


Figure 5. Infrared absorption spectrum of mesalamine.

Table 2

Assignments for the Vibrational Transitions of Mesalamine

Energy (cm^{-1})	**Relative Intensity**	**Assignment**
3600 - 3200	strong	O-H stretching mode associated with the hydroxyl groups
~ 3000	strong	C-H stretch of the aromatic group
1619	medium	C=C stretch of the aromatic group; N-H bond scissoring
1449, 1490	strong	C-C stretching mode
1355, 1378	strong	O-H deformation of the hydroxyl groups
1131	medium	C-O stretching mode
1190 - 1267	medium	In plane bending mode
685 - 808	strong	C-H bond out of plane bending mode; Ring deformation of the aromatic group

3.8.3.1 ^{1}H-NMR Spectrum

The one-dimensional ^{1}H-NMR spectrum of mesalamine is shown in Figure 6, and the assignments for the observed resonance bands are provided in Table 3. Additional justification for the assignments were obtained using two-dimensional proton-carbon correlated spectroscopy.

Owing to their fast exchange, the proton resonance bands associated with the hydroxyl, amino, and carboxylate groups could not be distinguished from each other. These all contribute to the broad band observed at a chemical shift of 7.67 ppm. The remaining bands all originate from protons bound to the aromatic ring, and their assignment is straight-forward.

3.8.3.2 ^{13}C-NMR Spectrum

The one-dimensional ^{13}C -NMR spectrum of mesalamine is shown in Figure 7, and assignments for the observed resonance bands are provided in Table 4. Additional justification for the assignments was obtained using two-dimensional proton-carbon correlated spectroscopy (HETCOR), with the assignments being exactly in accord with prediction.

3.8.4 Mass Spectrometry

The mass spectrum of mesalamine was obtained using a VG-Trio 2 mass spectrometer, operating in the electron impact mode. The spectrum thusly obtained is shown in Figure 8, and the assignments deduced for the major fragment ions are given in Table 5.

4. Methods of Analysis

4.1 Identification

No direct identification tests have been reported for mesalamine. However, two color tests are available for the identification of sulfasalazine, 5-[[*p*-(2-pyridylsulfamoyl)-phenyl]azo]salicylic acid [16]. This structurally related compound gives a orange-brown color when 1% w/v copper sulfate solution is added dropwise to a solution of the

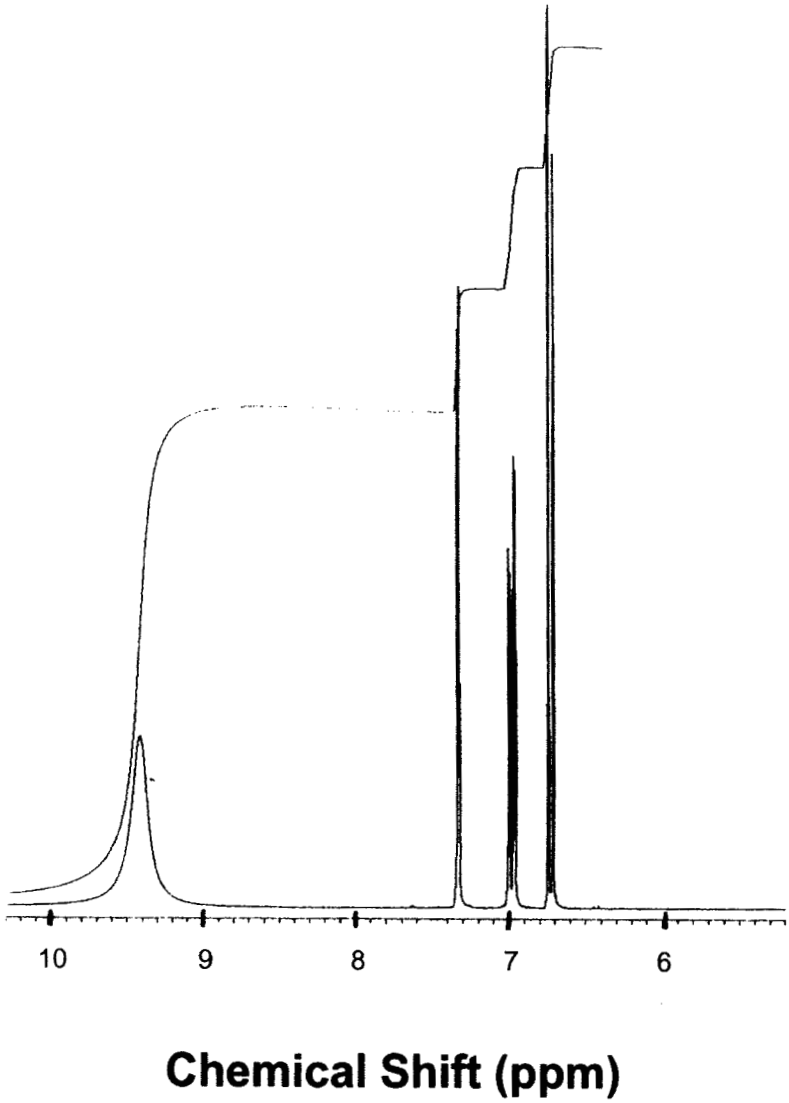


Figure 6. ^{1}H-NMR spectrum of mesalamine.

Table 3

Assignments for the Observed ^{1}H-NMR Resonance Bands of Mesalamine

Chemical Shift (ppm)	**Number of Protons**	**Assignment**
9.42	3	Protons associated with the carboxylate, amino, and hydroxyl groups
7.338, 7.328	1	Proton attached to carbon-1
7.005, 6.989, 6.972, 6.962	1	Proton attached to carbon-3
6.746, 6.714	1	Proton attached to carbon-4

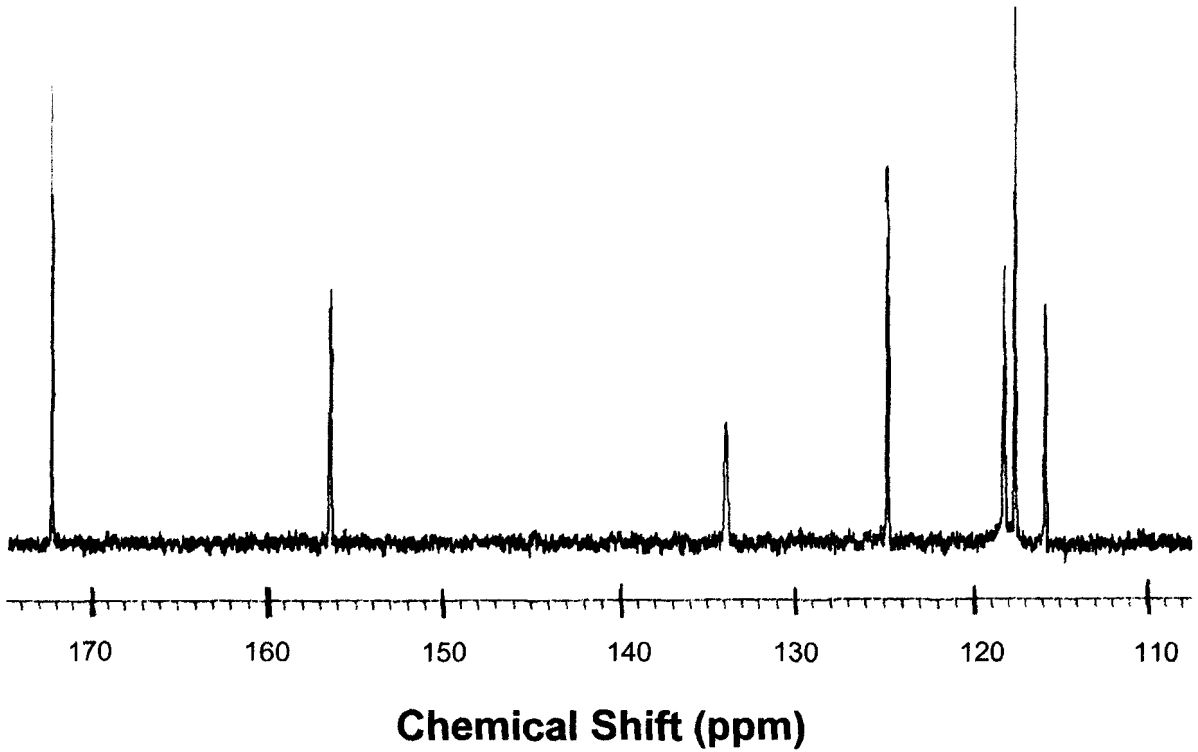


Figure 7. ^{13}C-NMR spectrum of mesalamine.

Table 4

Assignments for the Observed ^{13}C-NMR Resonance Bands of Mesalamine

Chemical Shift (ppm)	**Assignment**
172.377	Carbon-7
156.509	Carbon-5
133.928	Carbon-2
124.865	Carbon-1
118.340	Carbon-4
117.688	Carbon-3
115.893	Carbon-6

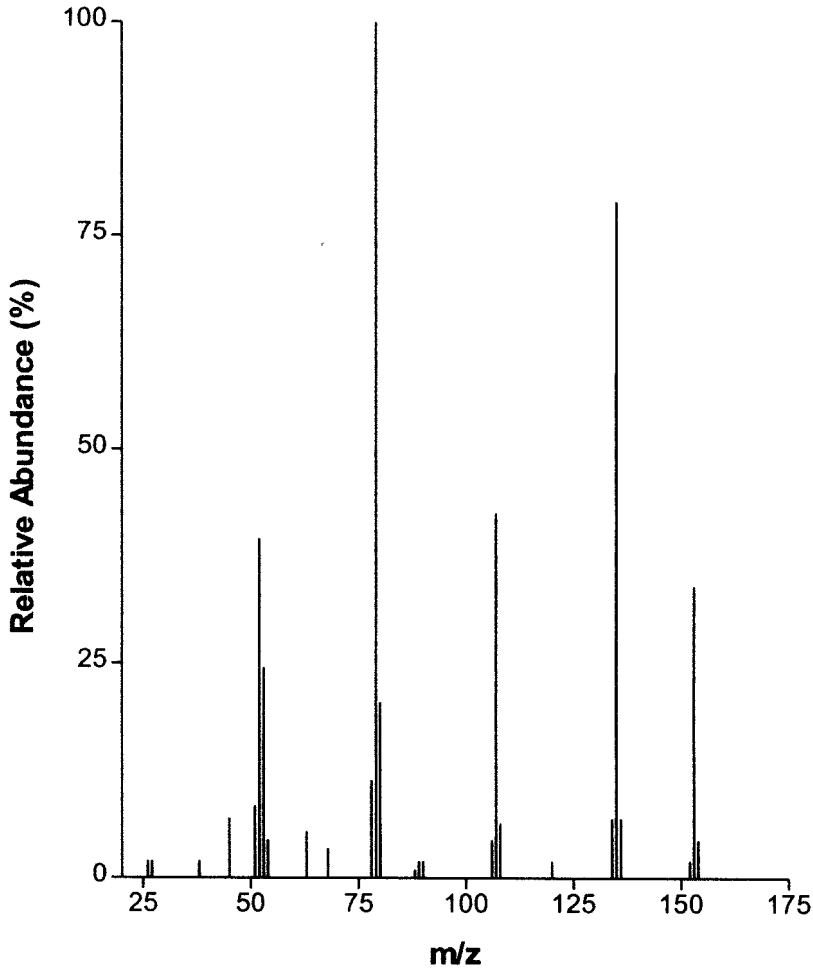


Figure 8. Electron impact mass spectrum of mesalamine.

Table 5

Assignments for the Electron Impact Mass Spectrum of Mesalamine

m/z	**Relative Intensity (%)**	**Fragment**
153	34.7	$[\mathbf{M}]^+$
135	77.8	$[\mathbf{M} - OH]^+$
107	43.1	$[\mathbf{M} - COOH]^+$
79	100	$[\mathbf{M} - COOH, OH, NH_2]^+$
52 , 53	40.3 , 25.5	$[C_3H_2N]^+$, $[C_3H_3N]^+$

Note: **M** denotes the 5-aminosalicylate unit.

compound dissolved in 0.1M sodium hydroxide. Sulfasalazine also gives a black color when subjected to the palladium chloride test.

4.2 Spectrophotometric Methods of Analysis

French and Mauger have reported a spectrophotometric method for the quantitation of mesalamine in aqueous solution [15]. This method uses citrate buffer (pH 3.5) as the medium, and the spectrophotometric analysis is conducted at a wavelength of 298 nm.

4.3 Chromatographic Methods of Analysis

4.3.1 High Performance Liquid Chromatography

Several HPLC methods have been developed for the analysis of mesalamine, and its major metabolite (acetyl-5-aminosalicylic acid), in biological fluids [17-25]. Mesalamine is an amphoteric compound, and therefore cannot be easily extracted into organic solvents without prior derivatization. For this reason, the amino group is ordinarily acylated prior to the performance of extraction of the analyte from plasma [22].

Liquid chromatographic methods with various detection modes have been used to study the stability of mesalamine in aqueous solutions and in pharmaceutical formulations [26-31]. Some of the reported chromatographic conditions are summarized in Table 6.

4.3.2 Gas Chromatography - Mass Spectrometry

A GC-MS method has been reported for the analysis of mesalamine, and a radical-derived oxidation product [30]. The method used the positive-ion electron impact mode at 70 eV, and the analytical separation is effected using a HP1 capillary column (25 m x 0.22 mm ID, 0.33 mm film thickness, and a 1.5 x 0.32 mm retention gap). The gas chromatograph was operated at 80°C for 1 minute; and then heated to 310°C at a rate of 30°C per minute.

5. Stability

5.1 Solution-Phase Stability

According to Palsmeier *et al.*, the solution-phase decomposition of mesalamine occurs rapidly under conditions promoting oxidation, and that the compound is most stable under conditions tending to inhibit oxidation. Decarboxylation was not found to be a significant degradation pathway. They further indicated that this compound undergoes a two-electron, two-proton oxidation, with the formation of 5-aminosalicylic acid quinoneimine, followed by complex formation [28].

Jensen *et al.* have identified four major degradation products of mesalamine after the compound was stressed in buffered solutions at pH 7.0. Study of these products suggests that the degradation of mesalamine leads to the formation of polymeric species by oxidative self-coupling of the 5-aminosalicylate moieties [29].

5.2 Stability in Pharmaceutical Formulations

Montogomery *et al.* have shown that mesalamine suspension retains at least 90% of its original concentration for 90 days. Refrigeration has been found to have no effect on the stability of the suspension [26].

Cendrowska and co-workers have suggested that mesalamine is quite stable in tablet and suppository formulations. Their studies have shown that less than 1% degradation of the drug occurred in these dosage forms after storage at room temperature for one year [27].

5.3 Stability in Biological Fluids

Brendal *et al.* have shown that mesalamine is stable in plasma, if the solutions are stored at -80°C. Much more rapid decomposition was observed for samples stored at -20°C over a period of 4 weeks [25].

6. Drug Metabolism and Pharmacokinetics

6.1 Absorption

Peak serum levels of mesalamine obtained after administration of an oral, delayed-release, formulation were reported as 4-12 hours [33]. The drug was absorbed extensively from the proximal part of the GI tract. Oral administration of a 2.4 g dose of the delayed-release preparation produced an average peak-serum-mesalamine-concentration of 1.27 mg/mL within 6 hours, a concentration of 2.3 mg/mL for N-acetyl-5-aminosalicylic acid (its degradation product).

Absorption of mesalamine via the rectal route after administration of suspensions was found to be very poor. Absorption from the enema depends on the retention time of the fluid, the pH, the volume of the enema, and the disease state of the patient. Rectal administration of a single 4 g dose of mesalamine suspension as an enema produced a peak drug concentration of 3-4 mg/mL within 3-6 hours. Rectal administration of mesalamine suppositories (500 mg) has shown an average of 24% of the dose being absorbed [32].

The rate of drug absorption was reported to be slower when administered orally with food; but the extent of absorption was not significantly different [34].

6.2 Distribution

Following the rectal administration of mesalamine suspension in adults, the drug has been found to distribute from the rectum into the colon, reaching the splenic flexure, and possibly the ascending colon. The apparent volume of distribution of mesalamine was reported to be 0.2 L/kg [35].

When monitored *in vitro*, 44% to 55% of the drug, and 80% of N-acetyl-5-aminosalicylic acid, has been reported to be bound to serum proteins [36].

6.3 Elimination

Absorbed mesalamine is rapidly acetylated in the liver and the colon wall [36-38]. Both hepatic and intestinal acetylation processes are reported to

be saturable and irreversible. The drug is primarily acetylated to N-acetyl-5-aminosalicylic acid, but other possible conjugation products may occur [39].

Following oral administration of mesalamine(500 mg three times daily), the elimination half-life of the drug was found to be 1.4 hours in humans. However, the elimination half-life was found to be 0.6 hour for a lower dose (250 mg three times daily), indicating a dose-dependency [36]. After rectal administration, 13% to 16% of the drug was recovered in the urine almost entirely as an acetylated metabolite [35].

7. Toxicity [14]

The oral LD_{50} value of mesalamine in male rats has been reported as 3.2 g/kg. This value may be contrasted the LD_{50} of 5.5 g/kg obtained for female rats. Mesalamine did not show any mutagenic activity in the Ames mutagen test, nor did it show evidence of reverse mutations or adverse chromosomal effects in mice. No carcinogenic effect associated with long-term use of 5-aminosalicylic acid have yet been reported.

Acknowledgments

Special thanks are due to Dr. Martin Gall for obtaining the nuclear magnetic resonance spectra, and to Dr. Kamalesh Johri for providing the mass spectrometric data.

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METFORMIN HYDROCHLORIDE

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Contents

1. Introduction

1.1 Therapeutic Category

Metformin hydrochloride is a biguanide hypoglycemic agent used in non-insulin-dependent diabetes mellitus. It is sometimes given to patients who no longer responding to the sulfonylureas. The mode of action for this compound is not clear. It does not stimulate insulin release, but does require that some insulin be present for it to exert a hypoglycemic effect [1].

1.2 History

The synthesis of metformin was first reported in the 1920's as one of a series of biguanides [2]. In 1929, Slotta and Tschesche also synthesized metformin as part of a series of biguanides [3] which were examined for hypoglycemic activity [4]. However at that time, metformin was not recognized as being useful as an insulin substitute in humans. Subsequent to this, some biguanides such as metformin and phenformin (1-phenethylbiguanide hydrochloride) were confirmed as having pharmacological hypoglycemic activity [5,6]. Both compounds were marketed as hypoglycemic agents, but phenformin was withdrawn from most markets as it was found to be associated with an unacceptably high incidence of lactic acidosis that often proved fatal [7]. Metformin does not suffer from this toxic effect, and is widely prescribed. Three salt forms of metformin are available (hydrochloride, embonate (pamoate), and chlorophenoxyacetate salt), but the hydrochloride is by far the most commonly used salt form.

This analytical profile describes metformin hydrochloride, however additional information is included in the appendix for the embonate (pamoate) and chlorophenoxyacetate salts.

1.3 Hazard Information

Metformin hydrochloride may be harmful by inhalation, ingestion, or skin absorption. It causes eye and skin irritation, and is irritating to mucous membranes and to the upper respiratory tract. However, it is considered to be of a low order of toxicity, and has a reported LD_{50} in rats of 1g/kg [8].

In case of eye contact, immediately flush with copious amounts of water for at least 15 minutes. In case of skin contact, immediately wash with soap and copious amounts of water. If inhaled, remove to fresh air. If breathing is difficult, give oxygen, and if not breathing give artificial respiration. If ingested, wash out mouth with water and seek medical advice.

2. Description

2.1 Nomenclature

2.1.1 Chemical Names

N,N-dimethylimidodicarbonimidic diamide hydrochloride

1,1-dimethylbiguanide hydrochloride

N,N-dimethyldiguanide hydrochloride

N'-dimethylguanylguanidine hydrochloride

2.1.2 Proprietary Names

Metformin hydrochloride formulations have the principal tradenames of Diabefagos®, Diabetosan®, Diabex®, Glucophage®, Haurymellin®, Meguan®, Metaguanide® , and Metiguanide®.

The p-chlorophenoxyacetate salt formulation has the tradename of Glucinan®, and the embonate salt formulation tradename is Stagid®.

2.2 Formulae

2.2.1 Empirical

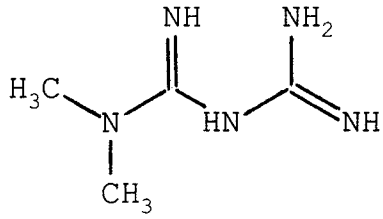
Metformin: $C_4H_{11}N_5$

Metformin hydrochloride: $C_4H_{12}ClN_5$

Metformin p-chlorophenoxyacetate: $C_{12}H_{18}ClN_5O_3$

Metformin embonate (pamoate): $C_{31}H_{48}N_{10}O_6$

2.2.2 Structure of Metformin Free Base



2.2.3 CAS Registry Numbers

The CAS Registry number for metformin free base is 657-24-9, and the CAS Registry number for metformin hydrochloride is 1115-70-4.

2.3 Molecular Weights

Metformin: 129.17

Metformin hydrochloride: 165.63

Metformin *p*-chlorophenoxyacetate: 315.76

Metformin embonate (pamoate): 646.71

2.4 Elemental Composition

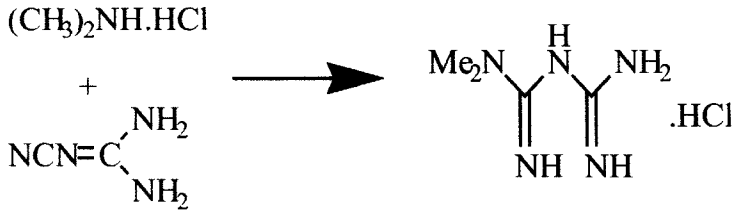
The elemental composition of metformin hydrochloride is C = 29.0%, H = 7.3%, Cl = 21.4%, and N = 42.3%. The elemental composition of metformin free base is C = 37.2%, H = 8.6%, and N = 54.2%.

2.5 Appearance, Color, and Odor

Metformin hydrochloride is a white hygroscopic crystalline powder, which is odorless and has a bitter taste.

3. Synthesis

The synthesis of metformin hydrochloride was reported by Shapiro *et al* [5], and was effected by fusion of equimolar mixtures of dimethylamine and dicyandiamide at 130-150°C for up to 2 hours.



4. Physical Properties

4.1 Particle Morphology

It has been reported that when metformin hydrochloride is crystallized from water, prisms are obtained [2]. Rod-like crystals are obtained when the compound is precipitated from propanol [6].

4.2 Crystallographic Properties

4.2.1 Polymorphism

Metformin hydrochloride has been reported to exist in two polymorphic forms [12]. Form 1 exhibits a fibrous habit, while Form 2 is a flaky aggregate.

Interconversion from Form 1 to Form 2 occurs by heating the crystals at 50-60°C for several hours.

4.2.2 X-Ray Powder Diffraction Pattern

The x-ray powder diffraction pattern of metformin hydrochloride is presented in Figure 1. The pattern was obtained using a water-cooled Siemens Kristalloflex Diffraktometer D500, with the samples being leveled off in a brass sample holder. Scanning was effected over the range 2 to 72 degrees 2-θ, with a step size of 0.05 degrees 2-θ, and a count time of 3 minutes at each step. A complete summary of scattering angles, d-spacings, and relative intensities is provided in Table 1.

The powder pattern was found to contain significant scatter at 12.2 and 22.2 degrees 2-θ, with subsidiary scatter between 22.7 and 23.2 degrees 2-θ (2 peaks), as well as 31.2 and 39.3 degrees 2-θ (5 peaks).

4.3 Thermal Methods of Analysis

4.3.1 Melting Behavior

The prisms obtained when metformin hydrochloride is crystallized from water have been reported to melt at 232°C [2], while the crystals obtained upon precipitation from propanol melt at 218-220°C [6]. Elsewhere, the melting point of metformin hydrochloride has been reported to be approximately 225°C [10,11].

4.3.2 Loss on Drying

Samples of metformin hydrochloride exhibit typical weight losses which are less than 0.5% for an accurately weighed sample which is dried to constant weight at 105°C.

4.3.3 Thermogravimetric Analysis

A thermogravimetric analysis study of metformin hydrochloride was performed using a DuPont Instrument model 951 thermal analysis system. Samples were heated at a rate of 10°C/minute up to a final temperature of 300°C under nitrogen

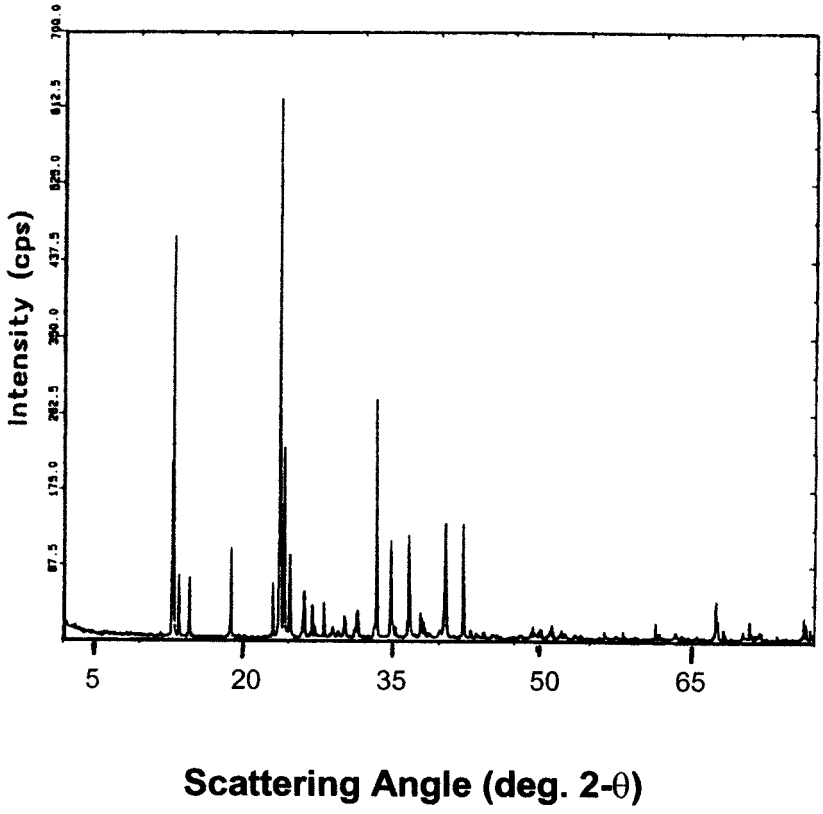


Figure 1. X-ray powder diffraction pattern of metformin hydrochloride.

Table 1
X-ray Diffraction Data for Metformin Hydrochloride

d-spacing (Å)	Scattering angle (degrees 2-θ)	Relative Intensity (%)	d-spacing (Å)	Scattering angle (degrees 2-θ)	Relative Intensity (%)
7.3368	12.053	28.4	37.613	2.3894	22.1
7.2523	12.194	65.9	39.298	2.2907	24.6
6.9754	12.680	8.9	39.989	2.2527	1.8
6.4644	13.687	9.8	41.222	2.1882	1.4
5.0350	17.600	16.9	43.712	2.0691	0.8
4.1121	21.593	10.6	44.563	2.0316	0.9
3.9928	22.246	100.0	45.799	1.9796	2.2
3.9208	22.660	32.8	45.991	1.9717	1.2
3.8321	23.192	14.9	47.532	1.9114	2.3
3.6248	24.538	8.0	48.440	1.8776	1.6
3.5180	25.295	6.6	49.651	1.8346	1.0
3.4901	25.501	2.3	52.415	1.7442	1.6
3.3804	26.343	7.2	54.151	1.6923	1.6
3.2821	27.147	1.7	57.205	1.6090	3.5
3.2235	27.650	1.0	57.581	1.5994	1.2
3.1617	28.202	4.2	59.035	1.5634	1.3
3.0615	29.145	1.6	59.642	1.5489	1.1
3.0356	29.399	4.6	62.836	1.4777	7.9
2.8642	31.202	48.0	63.564	1.4625	2.2
2.7497	32.536	18.1	65.393	1.4259	1.7
2.7345	32.722	2.3	66.009	1.4141	4.0
2.7121	33.000	2.1	66.519	1.4045	1.0
2.6196	34.200	20.9	66.898	1.3975	1.5
2.5401	35.306	5.2	68.555	1.3677	1.1
2.5157	35.660	3.0	71.048	1.3257	4.2
2.4238	37.060	1.3	71.651	1.3160	2.3

(purge rate of 85 mL/minute). As shown in Figure 2a, the TG thermogram exhibits no weight loss attributable to loss of solvent, but does exhibit a significant weight loss at approximately 256°C which is due to the thermal decomposition of the compound. This assignment was confirmed by differential scanning calorimetry and hot stage microscopy observations.

4.3.4 **Differential Scanning Calorimetry**

Differential scanning calorimetry analysis was performed using a DuPont Instruments model 910 thermal analysis system. Approximately 5 mg of sample was hermetically sealed in a coated aluminum pan, with pinholes being punched through the lid. The pan was heated under nitrogen (purged at 85 mL/minute) up to 300°C at a rate of 10°C/minute. The DSC thermogram (shown in Figure 2b) exhibits an endothermic transition which has its maximum at 234.6°C. This transition is attributed to compound melting, and was confirmed by hot-stage microscopy. The exothermic transition observed directly following the melt is due to decomposition of the sample, as supported by the TG analysis and by hot-stage microscopy.

4.4 **Hygroscopicity**

A dynamic vapor sorption analyzer (Surface Measurements Systems, Ltd.) was used to determine the moisture sorption and desorption isotherms of metformin hydrochloride. The procedure followed used a stepwise increase and subsequent decrease in relative humidity from 0 to 95%RH, both as a function of relative humidity (Figure 3a) and time (Figure 3b). It was found that approximately 20% moisture was absorbed when the drug substance was exposed up to a relative humidity of 95%, and that the desorption isotherm was essentially superimposable.

4.5 **Solubility Characteristics**

Metformin hydrochloride is soluble to the degree of 1 part in 2 parts of water, and 1 part in 100 parts of ethanol. It is practically insoluble in chloroform, acetone, methylene chloride and ether [10,11].

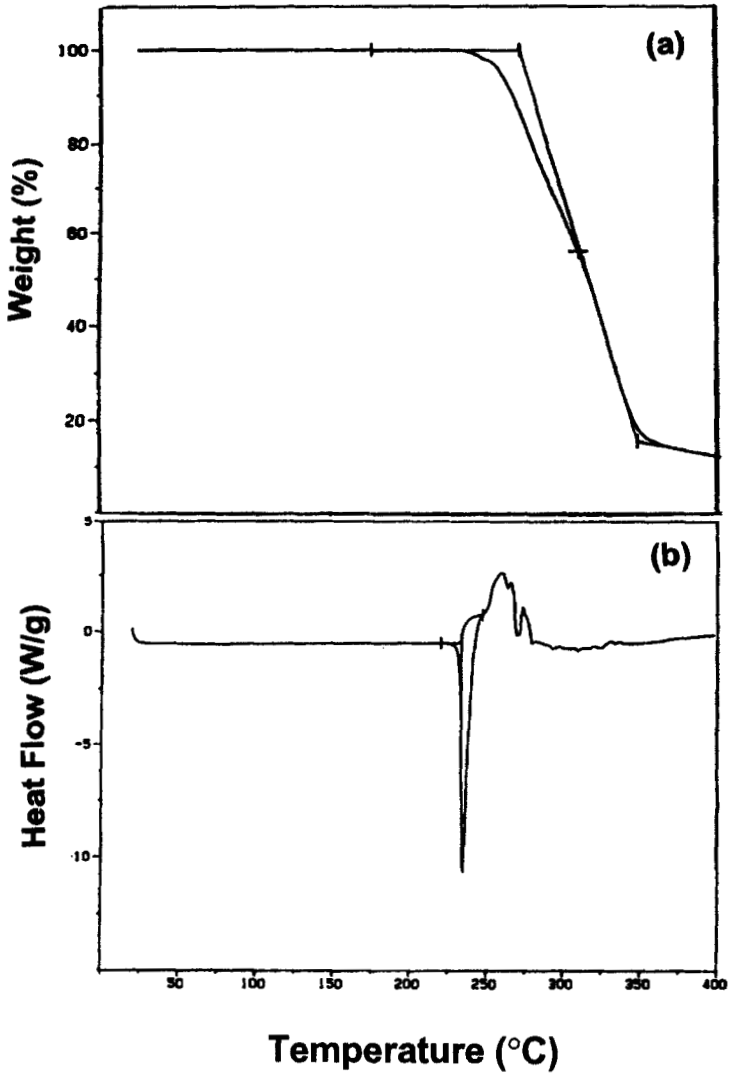


Figure 2. (a) Thermogravimetric analysis and (b) differential scanning calorimetry thermograms of metformin hydrochloride.

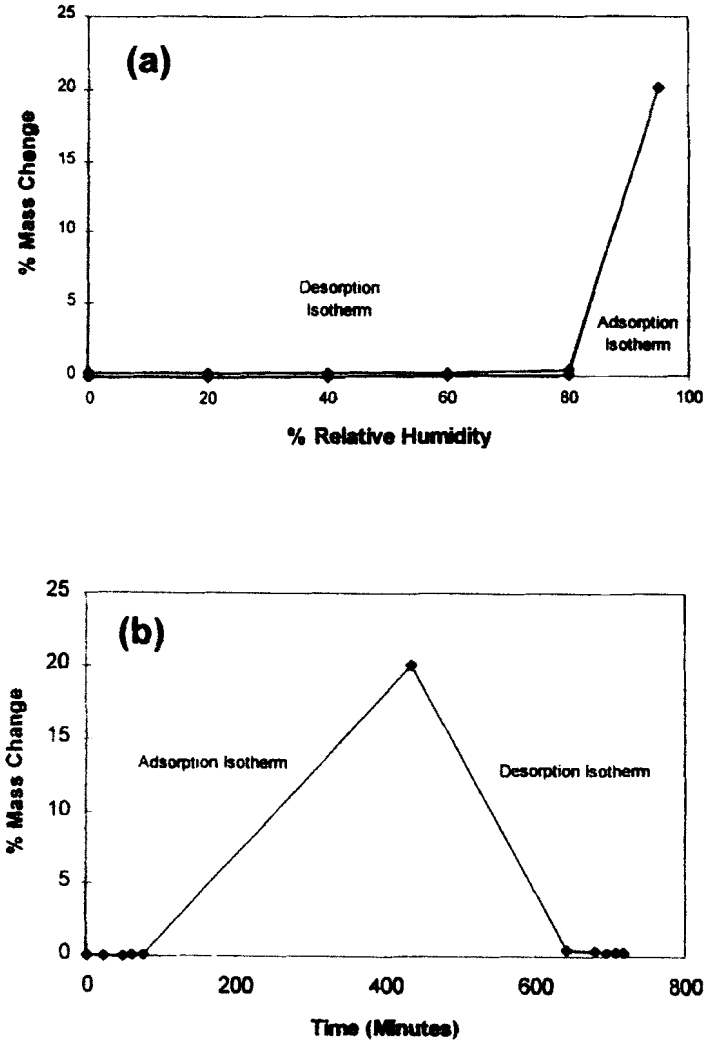


Figure 3. DVS moisture sorption and desorption isotherms of metformin hydrochloride, as a function of (a) relative humidity and (b) time.

A pH profile of the solubility of metformin hydrochloride cannot be drawn, as metformin is a strong base and a saturated solution of metformin hydrochloride becomes self-buffering.

4.6 Ionization Constants

Metformin hydrochloride has two dissociation constants, for which the pK values (at 32°C) have been determined to be 2.8 and 11.5 [13].

4.7 Spectroscopy

4.7.1 UV/VIS Spectroscopy

The ultraviolet absorption spectrum of metformin hydrochloride (at a concentration of 0.01 mg/mL in water) was measured from 210 to 350 nm using a Hewlett Packard 8452A diode array spectrophotometer. As shown in Figure 4, the UV spectrum consists of a single maximum observed at 233nm, which is characterized by a molar absorptivity of 12770 L/mol.

4.7.2 Near-Infrared Spectroscopy

The near-infrared spectrum of metformin hydrochloride was obtained using a Perstorp Analytical/NIR Systems 6500 spectrophotometer equipped with a Direct Contact Analyzer. The sample was scanned in the reflectance mode between 1100 and 2500 nm, and 32 scans were averaged to give the NIR spectrum shown in Figure 5. Data points were recorded at 2 nm intervals, and recorded in absorbance units (log 1/R).

4.7.3 Vibrational Spectroscopy

The infrared spectrum of metformin hydrochloride is provided in Figure 6, and is that given in the British Pharmacopoeia [10]. The spectrum was obtained after

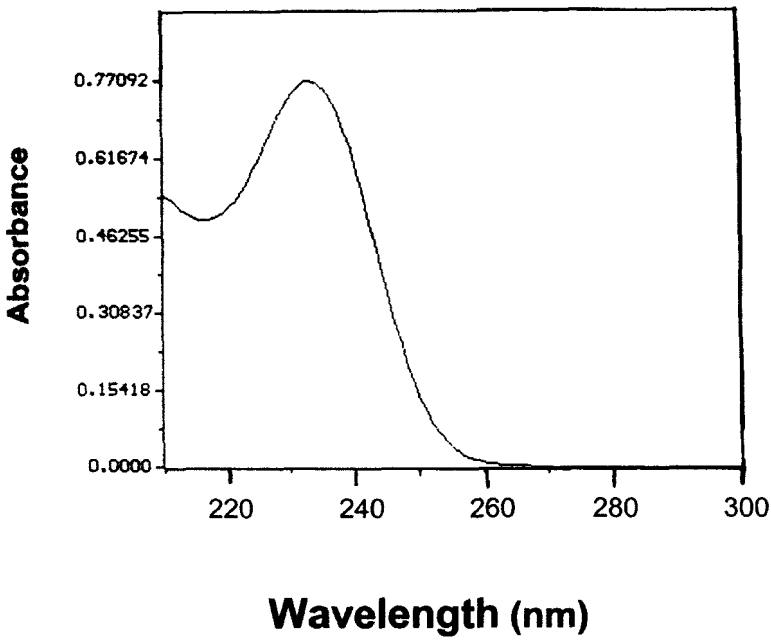


Figure 4. Ultraviolet absorption spectrum of metformin hydrochloride, at a concentration of 0.01 mg/mL in water.

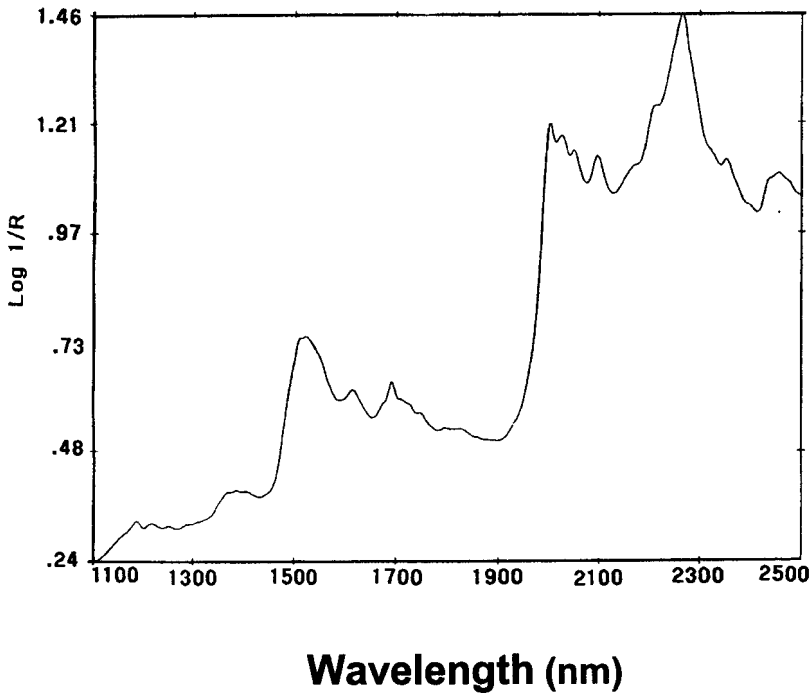


Figure 5. Near-infrared absorption spectrum of metformin hydrochloride.

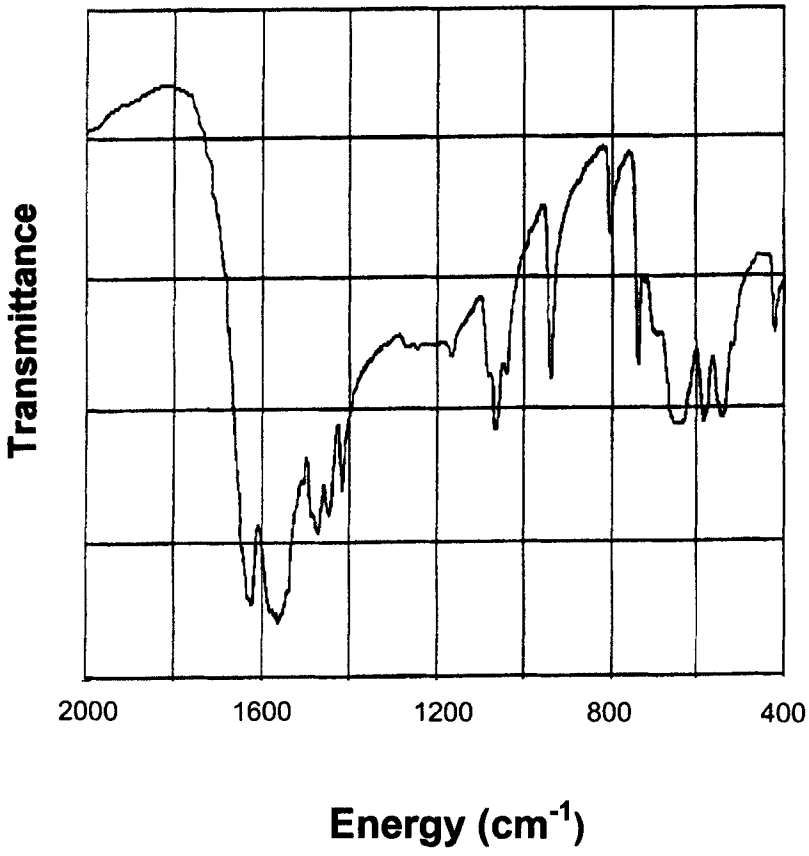


Figure 6. Infrared spectrum of metformin hydrochloride.

dispersion in a potassium chloride disk, and the major bands are assigned in Table 2.

Table 2
Assignment of Major Infrared Absorption Bands

Energy (cm^{-1})	Assignment
1630, 1565	N-H deformation, and asymmetric NCN stretch
1470, 1440, 1410	CH_3 asymmetric and symmetric deformations
1060, 940	C-N stretch and CH_3 rock

4.7.4 Nuclear Magnetic Resonance Spectrometry

The ^{1}H-NMR spectrum of metformin hydrochloride was obtained in both deuterium oxide and in dimethylsulfoxide-d_6, and these spectra are illustrated in Figure 7a and 7b, respectively. In D_2O solution, a sharp singlet resonance was observed at approximately 3.0 ppm, and was assigned as the signal from the magnetically equivalent methyl groups [9]. In dimethylsulfoxide, one also observes two resonance bands, with a ratio of approximately 2 to 4 as compared to the methyl signal. This latter finding implies that the compound exists largely in its indicated tautomeric form under acidic conditions.

The ^{13}C-NMR spectrum analysis in D_2O solution is shown in Figure 8, and consisted of the expected single large aliphatic signal (38.4 ppm) assigned as the resonance for the two rotationally equivalent methyl groups, and two hetero-substituted quaternary resonances (161.0 and 159.4 ppm).

The ^{1}H-NMR and ^{13}C-NMR spectra, together with detailed spectral assignments, of metformin pamoate and metformin *p*-chlorophenoxyacetate are found in the appendices.

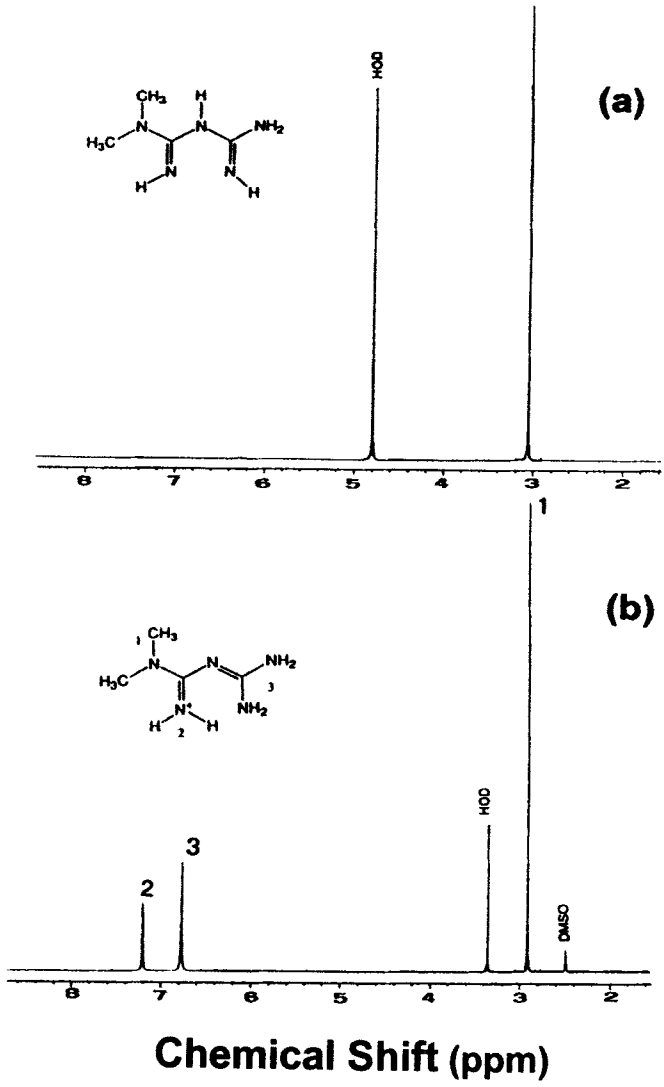


Figure 7. ^{1}H-NMR spectrum of metformin hydrochloride, as obtained in (a) deuterium oxide and (b) dimethylsulfoxide-d_6.

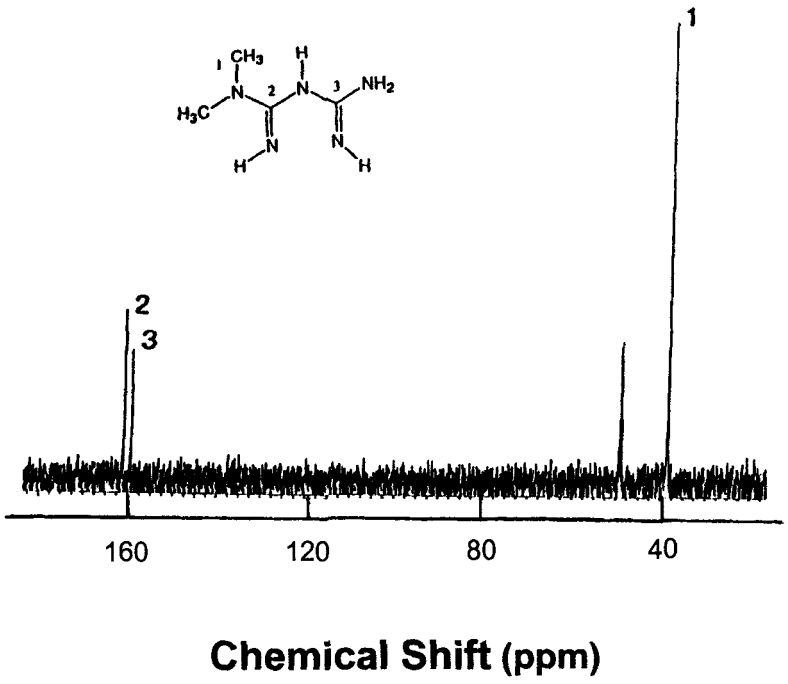


Figure 8. ^{13}C-NMR spectrum of metformin hydrochloride in deuterium oxide.

4.7.5 Mass Spectrometry

The mass spectrum of metformin hydrochloride was obtained using LC/MS on a Sciex API III triple quadrupole mass spectrometer, ionspray ionization, positive ions, and Q1 scan mode [9]. As shown in Figure 9, the positive ion mass spectrum of metformin hydrochloride yielded the expected $[M+H]^+$ ion at m/z = 130.

The mass spectra of the pamoate and *p*-chlorophenoxyacetate salts of metformin are included in the appendices.

5. Stability

5.1. Solid-State Stability

In the solid state, metformin is very stable, and thermally decomposes only at temperatures exceeding 230°C. However on exposure to alkaline solutions, it decomposes to form ammonia and dimethylamine.

5.2. Solution Stability

Solutions of metformin hydrochloride prepared at a concentration of 0.1 mg/mL and within the pH range of 2.5 to 8.2 were found to be stable for 6 days at ambient temperature. At pH 10, however, after 6 days a reduction in potency to 95% was observed.

6. Methods of Analysis

6.1 Identification

The British Pharmacopoeia lists include three procedures for the identification of metformin hydrochloride [10].

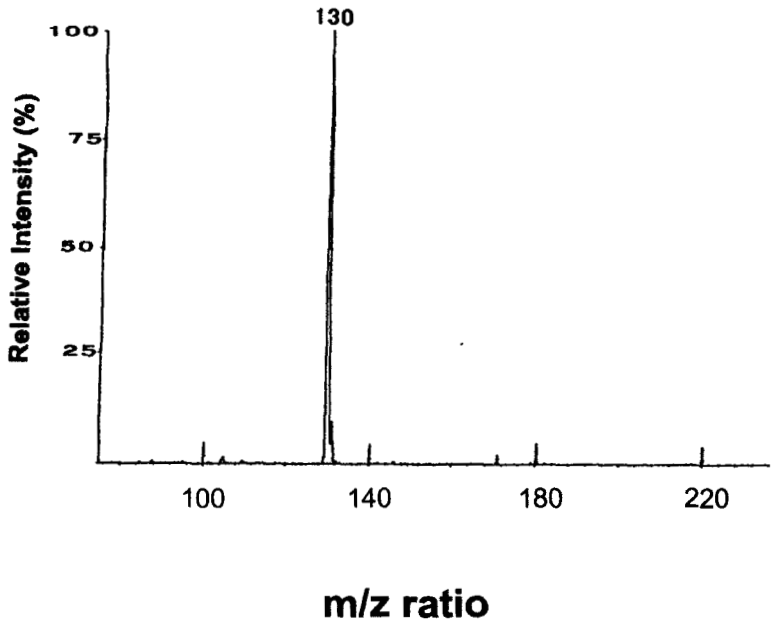


Figure 9. Positive ion mass spectrum of metformin hydrochloride.

6.1.1 Infrared Spectrum

When the infrared absorption spectrum is obtained in a KCl disk, the observed spectrum must be equivalent with the published reference spectrum [10]. This spectrum has been provided earlier in Figure 6.

6.1.2 Color Test (I)

Addition of 5M sodium hydroxide and a concentrated 1-napthol solution to a 0.5% w/v solution of metformin hydrochloride in water, followed by dropwise addition of 0.5 mL of dilute sodium hypochlorite solution, will produce an orange-red color which darkens on standing.

6.1.3 Color Test (II)

Addition of the reagent prepared by mixing equal volumes of 10% w/v sodium nitroprusside, 10% w/v potassium hexacyanoferrate(III), and 10% sodium hydroxide, to a 0.1% solution of metformin hydrochloride in water will produce a red color.

6.2 Titrimetric Analysis

The British Pharmacopoeia describes a non-aqueous titration assay method for metformin hydrochloride bulk drug substance [10]. 0.25 g of sample is dissolved in anhydrous acetic acid, mercury(II) acetate solution added, and the mixture titrated with 0.1 M perchloric acid VS to a potentiometric end-point. Each milliliter of 0.1 M perchloric acid reagent is equivalent to 8.281 mg of metformin hydrochloride.

The European Pharmacopoeia describes another non-aqueous titration method, where the sample is dissolved in formic acid, acetic anhydride is added, and the mixture then titrated with 0.1 N perchloric acid. The end-point is determined potentiometrically [11].

A conductometric titration method has been reported for the determination of metformin in Glucophage tablets [31].

6.3 Spectrophotometric Analysis

The ultraviolet spectophotometric assay of metformin hydrochloride tablets described in the British Pharmacopoeia involves the extraction of metformin into 100 mL of water, filtering to remove any undissolved materials, and determining the absorbance of the resulting solution at 232 nm [14]. The A(1%,1cm) for metformin hydrochloride at 232 nm is 398.

A UV method for the determination of metformin in Glucophage tablets and metformin hydrochloride raw material has been described by El-Bardicy *et al.* [15]. Metformin was extracted into dichloroethane, and allowed to form a 1:1 charge-transfer complex with iodine. After the complex solution was allowed to stand for 20 minutes, the absorbance of the resulting solution was determined at 295 nm against a dichloroethane blank. The method was described as being comparable to the B.P method with respect to precision and accuracy.

6.4 Chromatographic Methods of Analysis

6.4.1 Thin-Layer Chromatography

Several methods have been reported for the identification of metformin using thin-layer chromatography (TLC), and the salient aspects of these are summarized in Table 3. One system identifies metformin by spraying the TLC plate with solutions of different metal ions, which give rise to a variety of color complexes for metformin [17].

Another TLC procedure has been used to identify metformin in herbal mixtures [18]. In this procedure, the metformin is extracted by the addition of 95% v/v ethanol and subsequently analyzed by the conditions described in Table 3. Metformin was visualized under a UV lamp at 254nm, and/or sprayed with Dragendorff reagent. No interference from other components in the complex herbal mixture was reported.

A method to determine metformin using ion-pair extraction with bis(2-ethylhexyl) phosphoric acid, followed by reverse phase (RP-18) or normal phase TLC analysis has been reported, and the conditions are summarized in Table 3 [19].

Table 3

Conditions for Thin-Layer Chromatography Analysis

Support	Solvent System	Detection	Reference
Glass plates coated with talc, activated at 110-170°C for 1-2 hr.	butanol - ethanol - water (40:50:10 v/v)	1% oxine in 95% ethanol and sodium perchlorate.	16
Silica gel F_{254}	butanol-chloroform-methanol-ammonium hydroxide (40:15:15:15 v/v)	(a) cobaltous nitrate: pink spot. (b) cobaltous chloride: greenish-yellow spot. (c) cobalt thiocyanate: green spot. (d) copper acetate: purple spot.	17
Silica gel $G60F_{254}$	(a) ethyl acetate (b) methanol-ammonia (97:3 v/v) (b) Methanol-1% w/v aqueous ammonium chloride (90:10 v/v)	UV at 254 nm, or Dragendorff reagent.	18

Table 3 (continued)

Conditions for Thin-Layer Chromatography Analysis

Support	Solvent System	Detection	Reference
(a) RP - $18F_{254}$	Reverse phase: methanol-water-12 N HCl (50:50:1 v/v)	UV at 254nm, Fast Black K salt reagent, or acidified iodoplatinate reagent..	19
(b) Silica gel $60F_{254}$	Normal phase: toluene-acetone-ethanol-ammonia (45:45:7:3 v/v)		
Silica gel G	water-butanol-glacial acetic acid (50:40:10 v/v)	Equal volumes of 10% (w/v) sodium nitroprusside, 10% (w/v) potassium ferricyanide, and 10% (w/v) sodium hydroxide	11

The system was reported to have no interference from components in biological samples.

6.4.2 **Gas Chromatography**

The metformin content in tablets has been determined using isothermal packed-column GC [21]. The method involves extraction of metformin as the trifluoroacetyl derivative using trifluoroacetic anhydride, and the analytical conditions are given in Table 4.

6.4.3 **High Performance Liquid Chromatography**

A number of authors have reported HPLC methods for metformin, and the details of these are summarized in Table 5.

Metformin and its impurity dicyandiamide have been assayed by HPLC, using a procedure which was designed to be stability indicating. Tablets or raw material were prepared in aqueous solution, and chromatographed on a μ-Bondapak C18 column at 30°C using the conditions given in Table 5. Analyte detection was by UV absorbance at 217 nm for dicyandiamide, and at 233nm for metformin. A typical chromatogram obtained using this method is given in Figure 10.

In conjunction with the HPLC conditions listed in Table 5, solid-phase extraction has been used to detect undeclared potent synthetic substances (such as metformin) in herbal mixtures [18].

The European Pharmacopoeia describes HPLC conditions suitable for the analysis of cyanoguanidine (dicyandiamide), (4,6-diamino-1,3,5-triazin-2-yl)guanidine, *N,N*-dimethyl-1,3,5-triazine-2,4,6-triamine, melamine, and 1-methylbiguanide [11].

Table 4

Gas Chromatography Conditions for Metformin Analysis

Column	Mesh	Oven Temp.	Flow Rate/ Detection	Sample	Internal Standard	Ref.
Glass column (200 cm x 3 mm i.d) packed with 3% OV-17 on silanised Chromosorb W	80 - 100	250°C	40 mL/min N_2 as carrier gas. Nitrogen (KCl salt)	Plasma	Propylbiguanide	20
Glass column (200 cm x 4 mm i.d) packed with 3% OV-210 on Chromosorb W	80 - 100	150°C	40 mL/min N_2 as carrier gas. FID/TCD	Tablets	not specified	21
Glass column (212 cm x 3 mm i.d) packed with 3% OV-17 on Chromosorb W-AW DMCS HP	100 - 200	165°C	30 mL/min 5% CH_4 in Ar as carrier gas. Electron capture	Plasma	Propylbiguanide	22
Glass column (183 cm x 4 mm i.d) packed with 3% OV-17 on Gas Chrom Q	100 - 120	210°C	50 mL/min N^2 as carrier gas. Electron Capture	Various biological fluids	Buformin	23

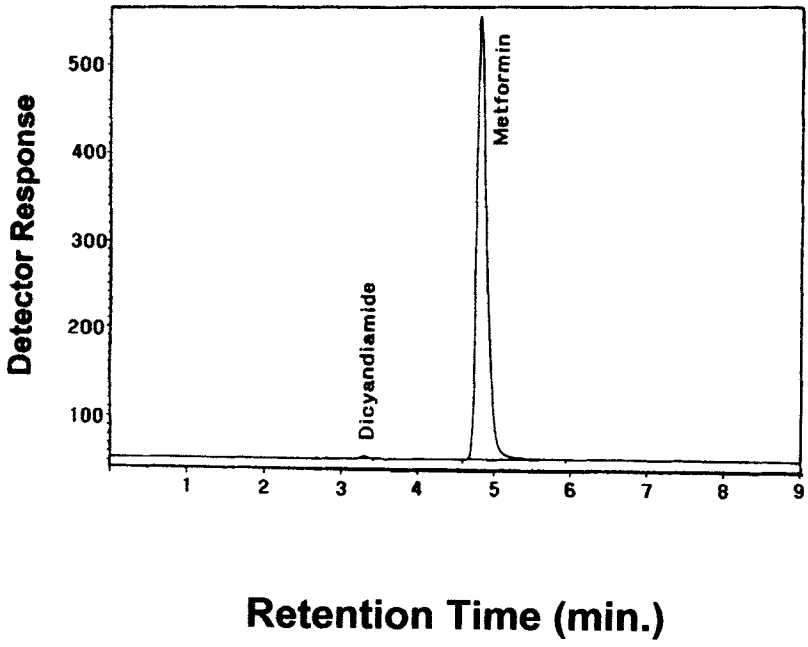


Figure 10. HPLC chromatogram of metformin hydrochloride and its process impurity, dicyandiamide.

Table 5

High Performance Liquid Chromatography Conditions for Metformin Analysis

Column	Mobile Phase	Flow Rate (mL/min)	Detection	Internal Standard	Ref.
μ-Bondapak C_{18} (30 cm x 3.9 mm)	Acetonitrile-0.087% (w/v) pentane-sulfonic acid sodium salt and 0.12% sodium chloride adjusted to pH 3.5, 5:95 (v/v),	1.0	217/233 nm	-	See text
μ-Bondapak Phenyl/Corasil (30 cm x 3.9 mm)	Methanol- Water (40:60 v/v)	1.0	280 nm	-	25
Partisil 10 SC X (25 cm x 4.6 mm)	0.03M ammonium dihydrogen phosphate pH 2.4	3.0	230 nm	1-Propylbiguanide sulphate	26
μ-Bondapak Phenyl (30 cm x 3.9 mm)	0.01M potassium dihydrogen phosphate : acetonitrile (40:60 %v/v) at pH 7	1.35	236 nm	Phenformin	27

Table 5 (continued)

High Performance Liquid Chromatography Conditions for Metformin Analysis

Column	Mobile Phase	Flow Rate (mL/min)	Detection	Internal Standard	Ref.
Spherisorb ODS 2 (15 cm x 4.6 mm)	(0.003M heptanesulfonic acid, 0.05M dipotassium hydrogen orthophosphate) : acetonitrile (92:8 %v/v) at pH 4.	1.0	234 nm	1-Propylbiguanide sulfate	28
μ-Bondapak C_{18} (30 cm x 3.9 mm)	0.01M phosphate buffer : acetonitrile (50:50 %v/v)	not specified	254 nm	-	29
Finepak Sil C_{18} (25 cm x 4.0 mm)	0.1M phosphate buffer pH 2.5:acetonitrile (25:75 % v/v)	not specified	Fluorescence: Ex = 306 nm Em = 450 nm	Phenformin	24
Supercosil LC-PCN (25 cm x 4.6 mm)	0.005M phosphate buffer pH 3 : acetonitrile (60:40 %v/v) and 0.4mM dibutylamine	1.4	not specified	Phenformin	32

Table 5 (continued)

High Performance Liquid Chromatography Conditions for Metformin Analysis

Column	Mobile Phase	Flow Rate (mL/min)	Detection	Internal Standard	Ref.
Lichrosphere 60 ORP Select B (25 cm x 4.0 mm)	1% (v/v) aqueous triethylamine pH 7: acetonitrile (7:3 v/v)	1.0	246 nm	-	18
TSK gel ODS 120T (15 cm x 4.6 mm)	acetonitrile : 0.5M TRIS buffer pH 8: water (6:1:3 v/v)	0.8	Fluorescence: Ex = 315 nm Em = 450 nm	Phenyl guanidine	35
Porous silica (5 or 10 m) bonded with benzene sulfonic acid (25 cm x 4.6 mm)	1.7% (w/v) ammonium dihydrogen phosphate adjusted to pH 3.0 with phosphoric acid	1.0	218 nm	-	31

6.4.4 Capillary Electrophoresis

The authors have used capillary electrophoresis to analyze metformin hydrochloride by means of micellar electrokinetic chromatography. The work was conducted on a Beckman P/ACE 5510 system, using an untreated fused silica capillary having a length of 57 cm (50 cm to the detector) and an internal diameter of 75 μm. The assay was conducted at a temperature of 15°C, and used a background electrolyte consisting of 0.1 M borate buffer (prepared using 4.2 g of boric acid and 3.0 g of disodium tetraborate per liter of water) and which also contained 0.025 M sodium dodecyl sulfate. The applied voltage was 30 kV. Metformin hydrochloride, at a concentration of 0.01 mg/mL, was introduced into the capillary using the pressure mode of injection for 5 seconds, and was detected using UV absorbance at 200 nm. A typical electropherogram is shown in Figure 11.

Metformin has been used as an internal standard in the capillary electrophoresis analysis of phenformin [30]. This method used a capillary which was 120 cm in length (70 cm to the detector) and having an internal diameter of 50 μm. The assay was performed at a temperature of 30°C, and the capillary was conditioned before use with 1 M sodium hydroxide for 20 minutes followed by a 10 minute rinse with water. The run buffer was 200 mM sodium dihydrogen phosphate (pH 4.4), with an applied voltage of 15 kV. Detection was effected by UV absorbance at 236 nm.

6.5 Dissolution Testing

The British Pharmacopoeia describes a dissolution test for metformin hydrochloride tablets [14]. The dissolution rate was determined in 1000 mL of 0.68% w/v potassium dihydrogen orthophosphate (pH 6.8) using a rotating basket at 100 rpm. 10 mL aliquots were removed from the dissolution vessel, filtered, and diluted to 100mL with water. A second dilution was performed, where 10 mL of the previous solution was diluted to 100 mL with water, whereupon the UV absorbance of the samples was obtained at 233 nm. The A(1%,1cm) of metformin hydrochloride was reported to be 806 at 233 nm.

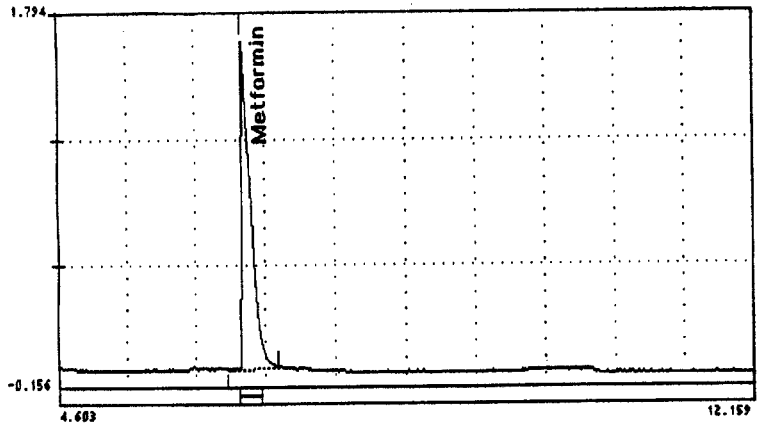


Figure 11. Electropherogram of metformin hydrochloride.

6.6 Determination in Biological Matrices

6.6.1 Spectrophotometric Analysis

A UV method for the determination of metformin in urine samples has been described [33]. Metformin was quantitatively extracted as an ion-pair, based upon the complexation of the compound with bromothymol blue. Once extracted, the metformin was released from its ion-pair by the addition of tetrabutyl-ammonium hydroxide, and subsequently quantitated by its UV absorbance at 232 nm. This method was adapted to determine metformin in whole blood and plasma, by precipitating out the protein present before performing the metformin ion-pair extraction and subsequent quantitation by UV [34].

6.6.2 Gas Chromatography

Gas chromatographic methods to determine metformin in plasma and various other biological fluids have been reported, and the main details of these are summarized in Table 4. The reported methods involve the derivatization of metformin to form the *s*-triazine derivative using *p*-nitrobenzoyl chloride [20], or monochlorodifluroacetic anhydride [22,23] with subsequent GC analysis. These methods yield a linear response for metformin, are precise, and can be used to quantitate metformin at levels of 50 ng/mL in plasma.

6.6.3 High Performance Liquid Chromatography

One of the first HPLC methods reported for the determination of metformin involved derivatization to form the *s*-triazine derivative [25]. The derivative was determined in urine using a μ-Bondapak phenyl/Corasil column, a mobile phase containing 40:60 v/v methanol:water, and UV detection at 280 nm.

Metformin has been reacted with 9,10-phenanthraquinone, and subsequently determined in human plasma by HPLC coupled with fluorescence detection [24]. As indicated in Table 5, this method was found to be specific for both phenformin and buformin, as well as for metformin. Fluorescent labeling of metformin has also been reported using dansyl chloride, where the compound is extracted from

human serum and the derivative analyzed by HPLC with fluorescence detection [35].

A method based on the ion-pair extraction of metformin into methylene chloride with bromothymol blue, and subsequent extraction into water of metformin freed from its ion-pair upon reaction with tetrabutylammonium hydroxide was reported for the extraction of metformin from human plasma [28]. The extracted metformin was analyzed by HPLC using the conditions described in Table 5.

Methods to determine metformin in human plasma and urine using acetonitrile [29] or trichloroacetic acid [26] to deproteinate the biological fluid have been reported. After deproteination, the samples are centrifuged and the supernatant solutions injected directly onto the HPLC using the conditions described in Table 5.

Solid-phase extraction techniques have been reported for the determination of metformin in human plasma with subsequent analysis by HPLC [27,32].

7. Drug Metabolism and Pharmacokinetics

7.1 Absorption and Bioavailability

After single oral doses, maximum plasma concentrations and urinary excretion rates were observed at about 2 hours, indicating that gastrointestinal absorption of metformin is relatively slow. Up to 50% of the drug was recovered as unchanged drug in the urine, with a further 30% remaining unchanged in the feces [36]. Absolute oral bioavailability was 50-60% of the dose.

7.2 Distribution

Metformin is not bound to plasma proteins [36-38].

7.3 Metabolism

Studies conducted in animals have failed to detect any metabolites of metformin [38,39], and when radiolabeled metformin is administered to humans, all of the label in urine samples is obtained in the form of metformin [36]. Simple conjugates of metformin have not been found in urine, and it is concluded that metformin is not metabolized. In this respect, the compound is similar to buformin [40], and is unlike phenformin which is hydroxylated to 4-hydrophenformin and displays genetic polymorphism [41].

7.4 Elimination

After intravenous administration, about 80% of the drug was recovered unchanged in the urine, and none detected in the feces, indicating that the elimination of metformin in healthy subjects occurs entirely by the kidney[36]. Its renal clearance is about five times the creatinine clearance, indicating that tubular secretion is the principal mechanism of metformin elimination.

7.5 Toxicity

Lactic acidosis has been associated as the most severe adverse reaction of biguanides, however this activity is almost exclusively limited to phenformin. It is generally accepted that lactic acidosis associated with biguanide therapy has occurred in patients whose condition is contraindicated for the use of metformin [1,42].

Acknowledgments

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Appendix I: Metformin Pamoate

AI.1 Nuclear Magnetic Resonance Spectra

The ^{1}H-NMR and ^{13}C-NMR spectra were obtained in dimethylsulfoxide-d_6, assigned by inspection, and confirmed with Nuclear Overhauser Effect Spectroscopy (NOSEY), Heteronuclear Multiple Quantum Coherence (HMQC), and Heteronuclear Multiple Bond Coherence (HMBC) experiments. A full summary of the resonance band assignments is provided in Table A1.

As shown in Figure A1a, the ^{1}H-NMR spectrum featured the aromatic patterns consistent with the pamoate 1,2,3-substituted naphthalene system, as well as the upfield singlets expected for the metformin methyl and the pamoate methylene groups [43]. The presence of residual water in the sample precluded the assignment of the exchangeable resonances. The approximate stoichiometry of the sample by proton integration is 2:1 for metformin and pamoic acid, respectively.

The ^{13}C-NMR spectrum is shown in Figure A1b, and yielded a single methylene carbon resonance associated with the pamoate group, as well as a complex system of aromatic resonances. The combination of HMQC and HMBC data analysis allowed complete unambiguous assignments of all the pamoate resonances of Table A1 to be made.

The remainder of the ^{1}H-NMR and ^{13}C-NMR band assignments were consistent with those made for metformin, as described earlier in section 4.7.4.

AI.2 Mass Spectrum

The mass spectrum of metformin pamoate was obtained using LC/MS on a Sciex APIII triple quadrupole mass spectrometer, ionspray ionization, positive ions and Q1 scan mode. The positive ion mass spectrum yielded the expected $[M+H]^+$ ion at m/z = 130 (Figure A2a), while the negative ion spectrum of the pamoate anion showed the $[M-H]^-$ ion at m/z = 387 (Figure A2b).

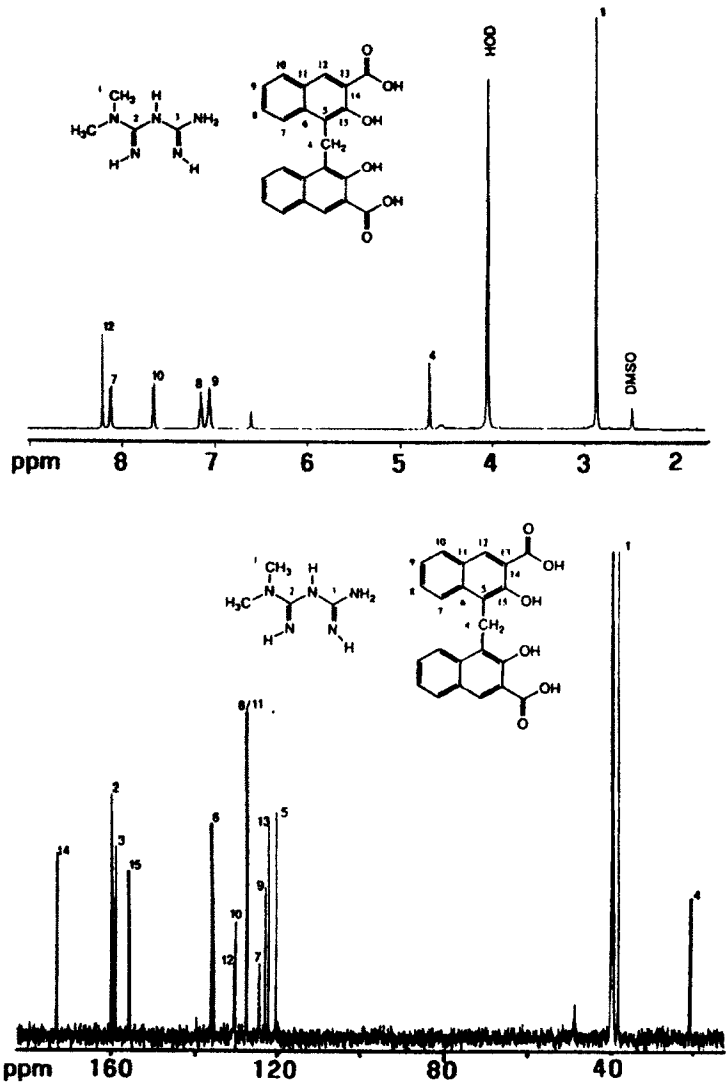

Figure A1. (a) ^{1}H-NMR and (b) ^{13}C-NMR spectra of metformin pamoate in dimethylsulfoxide-d_6.

Table A1

^{1}H and ^{13}C NMR Assignments of Metformin Pamoate in Dimethylsulfoxide-d_6

Assignment (atom #)	Chemical shift (ppm) ^{1}H	Chemical shift (ppm) ^{13}C	Integration (protons)	*JHH* (Hertz)	^{1}H Multiplicity*
1	2.88	38.0	12	-	s
2	-	160.0	-	-	-
3	-	158.9	-	-	-
4	4.68	20.7	2	-	s
5	-	120.2	-	-	-
6	-	135.9	-	-	-
7	8.12	124.2	2	8.3	d
8	7.15	127.42#	2	7.9,8.3	a,t

Table A1 (continued)

^{1}H and ^{13}C NMR Assignments of Metformin Pamoate in Dimethylsulfoxide-d_6

Assignment (atom #)	Chemical shift (ppm) ^{1}H	Chemical shift (ppm) ^{13}C	Integration (protons)	*JHH* (Hertz)	^{1}H Multiplicity*
9	7.06	122.9	2	7.9,8.3	a,t
10	7.66	130.1	2	8.3	d
11	-	127.4#	-	-	-
12	8.22	130.5	2	-	s
13	-	122.0	-	-	-
14	-	173.0	-	-	-
15	-	155.7	-	-	-

resonances are overlapped

*s = singlet
d = doublet
t = triplet
a = apparent

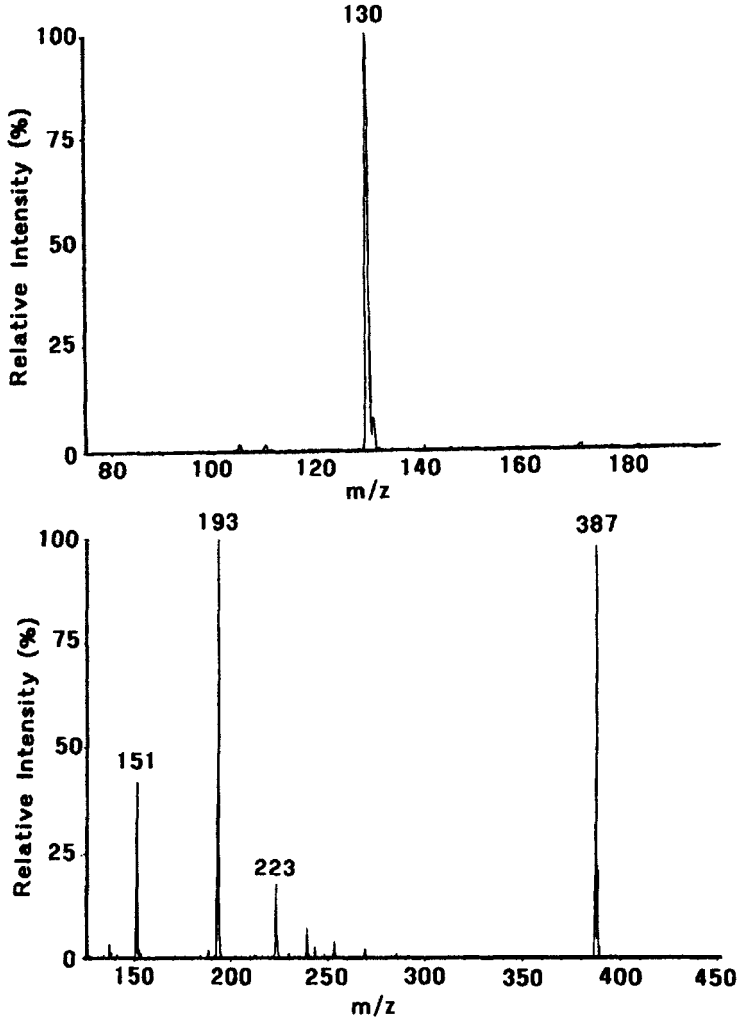

Figure A2. (a) Positive ion and (b) negative ion mass spectra of metformin pamoate.

Appendix II: Metformin *p*-chlorophenoxyacetate

AII.1 Nuclear magnetic Resonance Spectra

The ^{1}H-NMR and ^{13}C-NMR spectra of metformin *p*-chlorophenoxyacetate were obtained in deuterium oxide, and a full summary of the resonance band assignments is given in Table A2.

As shown in Figure A3a, the ^{1}H-NMR spectrum featured the aromatic AB pattern consistent with the 1,4-substituted *p*-chlorophenoxyacetate aromatic ring, as well as the upfield singlets expected for the metformin methyl groups and the *p*-chlorophenoxyacetate methylene group [44].

The ^{13}C-NMR spectrum (shown in Figure A3b) yielded four lines associated with the 1,4-disubstituted aromatic ring, as well as a signal for the acidic carbonyl carbon and the oxygen substituted methylene carbon.

The remainder of the assignments made for the ^{1}H-NMR and ^{13}C-NMR spectra were consistent with the structure of the metformin as described earlier.

AII.2 Mass Spectrum

The mass spectrum of metformin *p*-chlorophenoxyacetate was obtained using LC/MS on a Sciex API III triple quadropole mass spectrometer, ionspray ionization, positive ions and Q1 scan mode. The positive ion spectrum (Figure A4a) of metformin *p*-chlorophenoxyacetate yielded the expected $[M+H]^{+}$ ion at m/z = 130, while the negative ion spectrum (Figure A4b) of *p*-chlorophenoxyacetate showed the $[M-H]^{-}$ ion at m/z = 185/187.

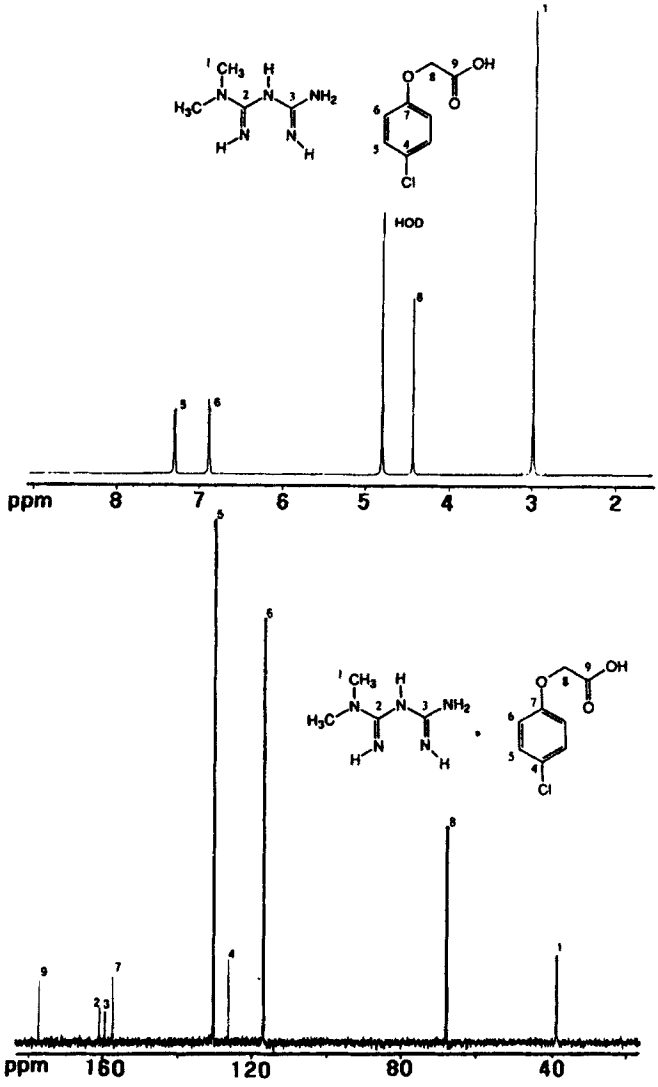

Figure A3. (a) ^{1}H-NMR and (b) ^{13}C-NMR spectra of metformin *p*-chlorophenoxyacetate in deuterium oxide.

Table A2

^{1}H and ^{13}C NMR Assignments of Metformin *p*-Chlorophenoxyacetate in Deuterium Oxide

Assignment (atom #)	Chemical Shift (ppm) ^{1}H	Chemical Shift (ppm) ^{13}C	Integration (protons)	J_{HH} (hertz)	^{1}H Multiplicity*
1	2.98	38.3	6	-	s
2	-	160.9	-	-	-
3	-	159.4	-	-	-
4	-	126.4	-	-	-
5	7.29	130.3	2	9.1	d
6	6.87	116.8	2	9.1	d
7	-	157.4	-	-	-
8	4.43	67.8	2	-	s
9	-	177.3	-	-	-

* s = singlet
d = doublet

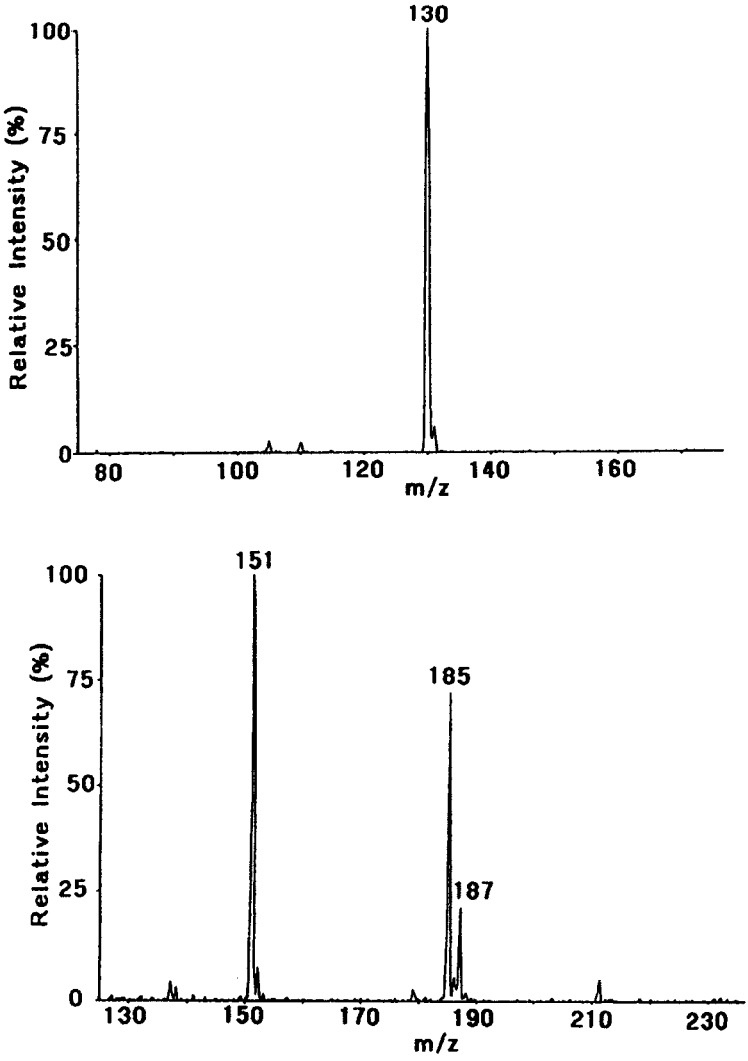

Figure A4. (a) Positive ion and (b) negative ion mass spectra of metformin *p*-chlorophenoxyacetate.

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PENTOXIFYLLINE

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ANALYTICAL PROFILES OF DRUG SUBSTANCES
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Contents

1. Description

1.1 Nomenclature

1.1.1 Chemical Names [1-3]

(oxo-5-hexyl)-1-dimethyl-3,7-dioxo-2,6-tetrahydro-1,2,3,6-purine

1H-Purine-2,6-dione, 3,7-dihydro-3,7-dimethyl-1-(5-oxohexyl)

3,7-dihydro-3,7-dimethyl-1-(5-oxohexyl)- 1H-purine-2,6-dione

1-(5-oxohexyl)-3,7-dimethylxanthine

3,7-dimethyl-1-(5oxohexyl)-1H,3H-purine-2,6-dione

1-(5-oxohexyl) theobromine

1.1.2 Nonproprietary Names [1,2]

Pentoxifylline (USAN)

Oxpentifylline (BAN)

vazofirin

BL 191

1.1.3 Proprietary Names

Trental, Tarontal, Elorgan, Terental, Torental, Erytral, Lentrin, Reotal, Zumavastal

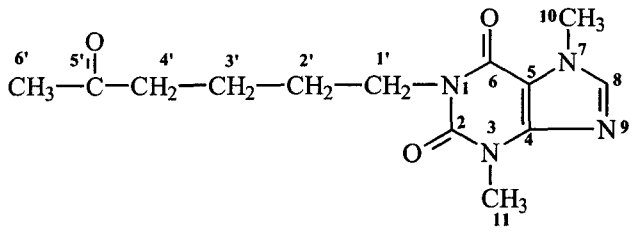
Generally pentoxifylline is available as tablet, film-coated tablet, sugar-coated tablet or caplet, each containing 100, 200 or 400 mg of drug [4].

1.2 Formulae

1.2.1 Empirical

$C_{13}H_{18}N_4O_3$

1.2.2 **Structural**



1.2.3 **CAS Registry Number**

6493-05-6

1.3 **Molecular Weight**

278.31

1.4 **Elemental Composition** [2]

C: 56.10%

H: 6.52%

N: 20.13%

O: 17.25%

1.5 **Appearance, Color, and Odor**

Pentoxifylline is a white, or almost white, crystalline or microcrystalline powder, which has a bitter taste and only a slight characteristic odor [2, 5].

1.6 **Uses and applications**

Pentoxifylline decreases the viscosity of blood, and thereby improves its flow. This increased blood flow helps patients with peripheral arterial disease to obtain better circulation and oxygen delivery to vital tissues [41]. The drug was first introduced in Germany in 1972 (by Albert-Rouseel), in France in 1974 (by Hoechst), in the United Kingdom in 1975

(Hoechst), in Italy in 1976 (by Hoechst), and in Japan in 1977 (by Hoechst) [42]. Pentoxifylline was the first drug approved for the treatment of intermittent claudication [25].

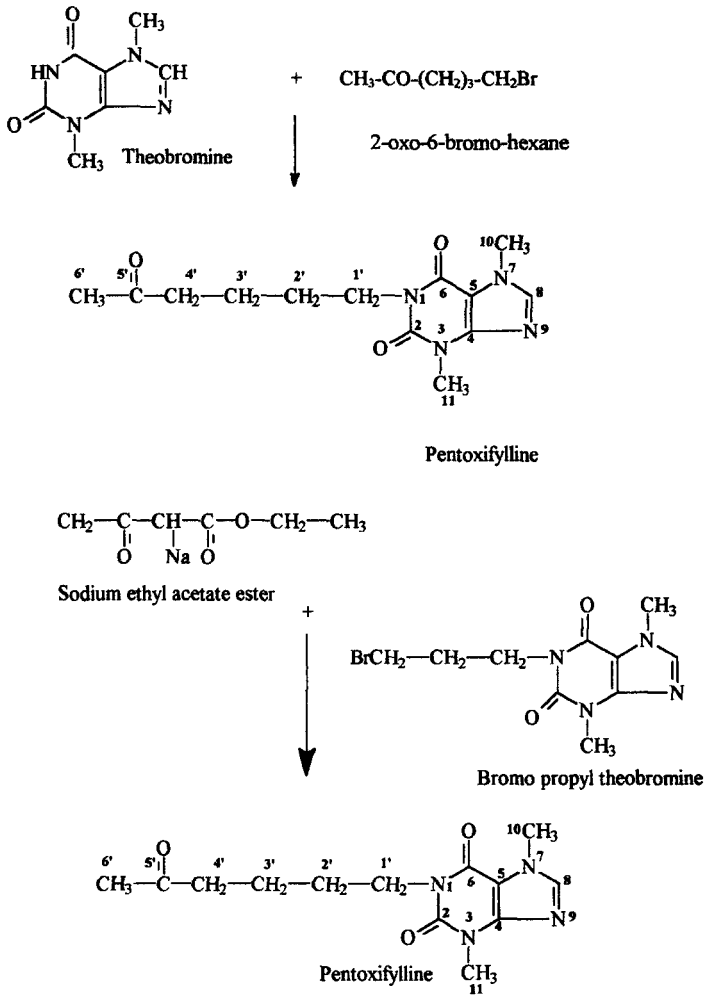
The therapeutic indication for pentoxifylline were for intermittent claudication secondary to chronic occlusive vascular disease, Reynould's syndromes, and transient ischaemic attacks [25]. Pentoxifylline can also be used also to lower blood level of the cytokine called tumor necrosis factor [43]. The usual dose is 400 mg three times daily per oral, reducing to 400 mg twice daily if adverse effects become troublesome. Beneficial effects may not be evident until after 2 to 6 weeks of treatment [34].

Pentoxifylline is contraindicated for patients who have had a recent brain hemorrhage. The compound is structurally related to caffeine and theophylline, and should therefore be used with caution in patients sensitive to these substances [41]. The drug is not used in neonatals and lactating women owing to its secretion in breast milk [25].

The concomitant use of pentoxifylline and aspirin has been reported to cause increase bleeding. Pentoxifylline may occasionally enhance the effect of hypotensive drugs, and its use may require reduction of their dose. Parentally administered pentoxifylline has been reported to enhance the hypoglycaemic effects of insulin and oral hypoglycaemic drugs [25]. Cimetidine concomitantly administrated with pentoxifylline caused a reduction in the total apparent clearance of pentoxifylline [44].

2. Synthesis

Two synthetic methods have been reported for the preparation of pentoxifylline, which are both represented in Scheme 1. The compound can be prepared by the reaction of 2-oxo-6-bromohexane with theobromine in hydro-organic solutions containing sodium hydroxide, following by extraction and crystallization of the product. The other procedure entails the reaction of 3-bromopropyl-theobromine with sodium ethyl acetate ester [3, 11].



Scheme 1. The chemical synthesis of pentoxifylline [11].

3. Physical Properties

3.1 Particle Morphology

When obtained from organic solvents, pentoxifylline is obtained as colorless needles [2]. A commercial sample was evaluated using optical microscopy, with the data being obtained on a Leica Diastar system. As evident in the photomicrographs of Figure 1 (magnification of 200x) and Figure 2 (magnification of 400x), the individual crystals were approximately 30-50 μm in length, and 5-10 μm in width. In this particular preparation, the absence of well-defined crystal faces suggested rapid crystallization of the solid from a solvent above the roughening temperature of the material.

3.2 X-Ray Powder Diffraction Pattern

The powder x-ray diffraction pattern of pentoxifylline was obtained on a Jeol JDX-3530 diffractometer system. The x-ray radiation was provided by copper target, operated at 40.0 kV and 30.0 mA. The powder pattern was obtained using a step angle of 0.040 degrees 2-θ, and each step was averaged for 1.0 sec. The crystallographic data are listed in Table 1, and the powder pattern itself is shown in Figure 3.

3.3 Thermal Methods of Analysis

3.3.1 Melting Behavior

The melting range for pentoxifylline has been reported as 105-106°C [2,6].

3.3.2 Thermal Analysis

The thermal properties of pentoxifylline were evaluated using simultaneous differential thermal analysis (DTA) and thermogravimetric analysis (TGA). The work was performed on a Shimadzu DT-30 Thermal Analyzer system, which was calibrated using indium as the standard. Using a heating rate of 10°C/min, the thermograms presented in Figure 4 were obtained.

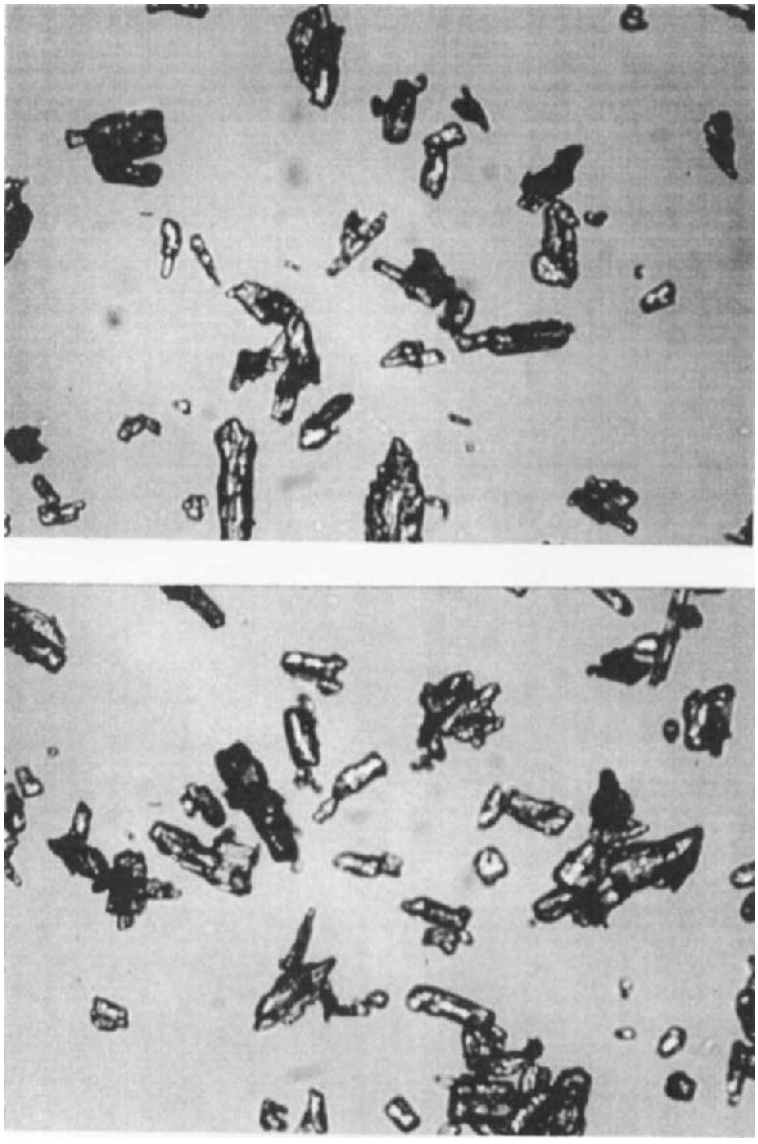


Figure 1. Optical photomicrographs of pentoxifylline, obtained at a magnification of 200x.

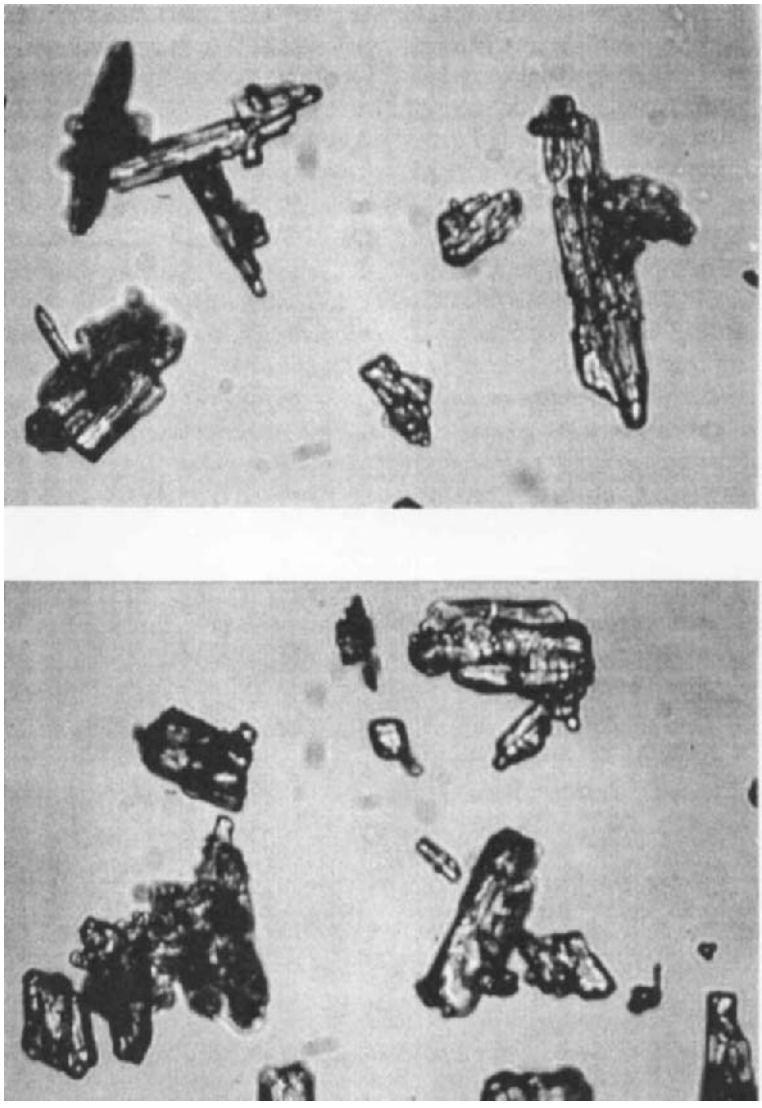


Figure 2. Optical photomicrographs of pentoxifylline, obtained at a magnification of 400x.

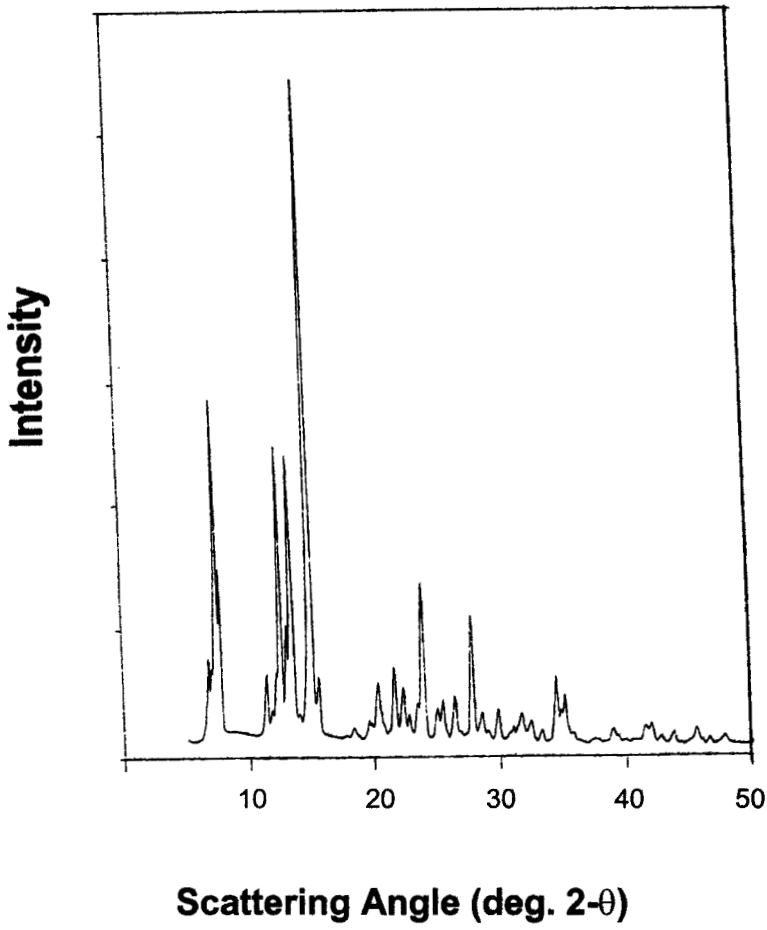


Figure 3. X-ray powder diffraction pattern of pentoxifylline.

Table 1

Crystallographic Data from the X-Ray Powder Diffraction Pattern of Pentoxifylline

Scattering Angle (degrees 2-θ)	**d-spacing (Å)**	**Relative Intensity, I/I_0 (%)**
6.77	13.0452	14.99
7.57	11.6683	50.56
11.45	7.7216	12.70
12.65	6.9916	44.28
13.57	6.5196	47.56
15.13	5.8507	100.00
15.81	5.6006	9.14
20.41	4.3475	13.00
21.69	4.0938	14.91
22.41	3.9638	11.69
24.05	3.6971	27.61
24.93	3.5686	6.76
25.69	3.4647	8.12
26.53	3.3569	9.83
27.85	3.2007	22.23
28.61	3.1174	7.81
29.85	2.9906	8.01
31.65	2.8246	7.74
32.41	2.7600	6.43
34.45	2.6011	12.64
35.13	2.5523	10.16
42.05	2.1469	5.60
45.61	1.9873	5.00

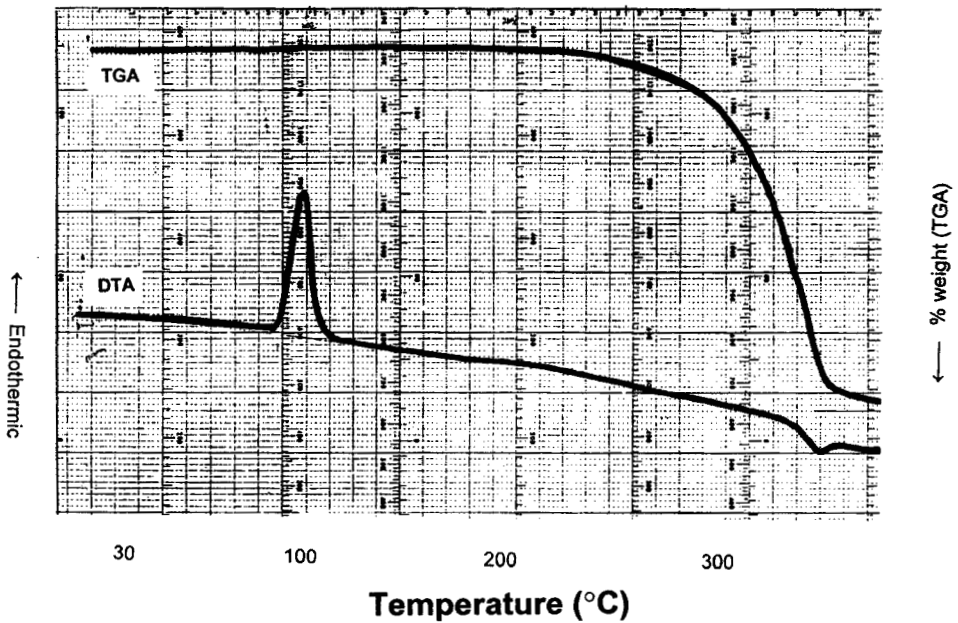


Figure 4. Thermogravimetric analysis (upper trace) and differential thermal analysis (lower trace) thermograms of pentoxifylline.

The anhydrous nature of pentoxifylline is evident in the lack of discernible weight loss when the compound was heated as high as 200°C. Beyond 250°C, an extensive weight loss was observed, which would correspond to the exothermic decomposition of the compound.

In the DTA thermogram, a sharp endothermic peak was observed at 103.6°C. Given the reported melting point range [2,6], and the lack of any associated weight loss in the TGA thermogram, identifies this thermal feature as the melting transition.

3.4 **Hygroscopicity**

Through the performance of moisture uptake experiments, pentoxifylline was found to be able to reversibly sorb and desorb approximately 1% of adventitious water. The compound did not appear to be capable of forming genuine hydrate species.

3.5 **Solubility Characteristics** [2,7-9]

Pentoxifylline has been reported to be very soluble in acetic acid, freely soluble in chloroform, methanol, and acetone, sparingly soluble in ethanol, slightly soluble in ether, and practically insoluble in hexane. In benzene, the reported solubility is 0.11 g/mL.

The water solubility of pentoxifylline has been reported as 77 mg/mL at 25°C, and 191 mg/mL at 37°C. In addition, the pH of a 1% aqueous solution is 5.0-5.7.

3.6 **Ionization Constant**

The pKa of pentoxifylline has been reported to be 0.28 [25].

3.7 **Partition Coefficient**

In *n*-octanol/water, the partition coefficient was found to be 1.96 when measured at 20°C, and 2.82 when measured at 37°C [11].

3.8 Spectroscopy

3.8.1 UV/VIS Spectroscopy

The ultraviolet absorption spectrum of pentoxifylline was recorded on a Shimadzu UV 265 spectrophotometer, and the spectra recorded in methanol and water are shown in Figure 5. The wavelengths observed for the absorption maxima in these two solvents were essentially identical, with the peak being found at 272 nm in methanolic solution and 273 nm in aqueous solution. The absorptivity of pentoxifylline was also not found to be greatly affected by the solvent used. The molar absorptivity in methanol solution was calculated to be 10,350 L/mole, while the molar absorptivity was found to be 10,400 L/mole in water.

The ultraviolet absorbance spectrum of pentoxifylline was also obtained in the reflectance mode using a Camag TLC-Scanner II equipped with CATS 3.17 software. A 1.2 μg sample was spotted on a Silica gel F254 pre coated plate (E. Merck), and then eluted with 15:85 methanol/ethyl acetate. After transformation of reflectance into absorbance, the spectrum shown in Figure 6 was obtained. The main spectral feature was a peak having a maximum at 277 nm, which is barely changed from the maxima observed in methanolic or aqueous solutions.

3.8.2 Fluorescence Spectroscopy

Pentoxifylline was found to be only weakly fluorescent when dissolved either in aqueous or methanolic solutions. Using a Spex Fluorolog II system, the spectrum shown in Figure 7 was obtained upon excitation at 275 nm, and was found to be typical for pentoxifylline solutions. In methanol, the emission maximum was observed at 323 nm, while slight red shifts were noted in 0.1 N HCl (333 nm) and in 0.1 N NaOH (332 nm).

3.8.3 Vibrational Spectroscopy

The infrared absorption spectrum of pentoxifylline was obtained in a KBr disc (using approximately 2 mg dispersed in 200 mg KBr), and recorded on a Jasco 5300 FTIR spectrophotometer. The infrared spectrum thusly

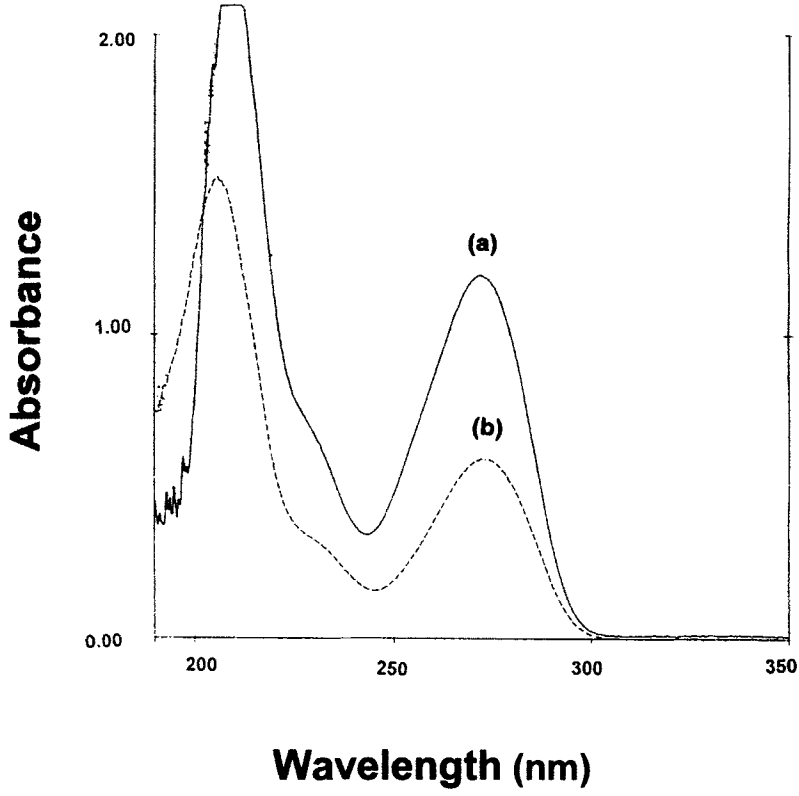


Figure 5. Ultraviolet absorption spectrum of pentoxifylline dissolved in (a) methanol (concentration of 32 ppm) and in (b) water (concentration of 16 ppm).

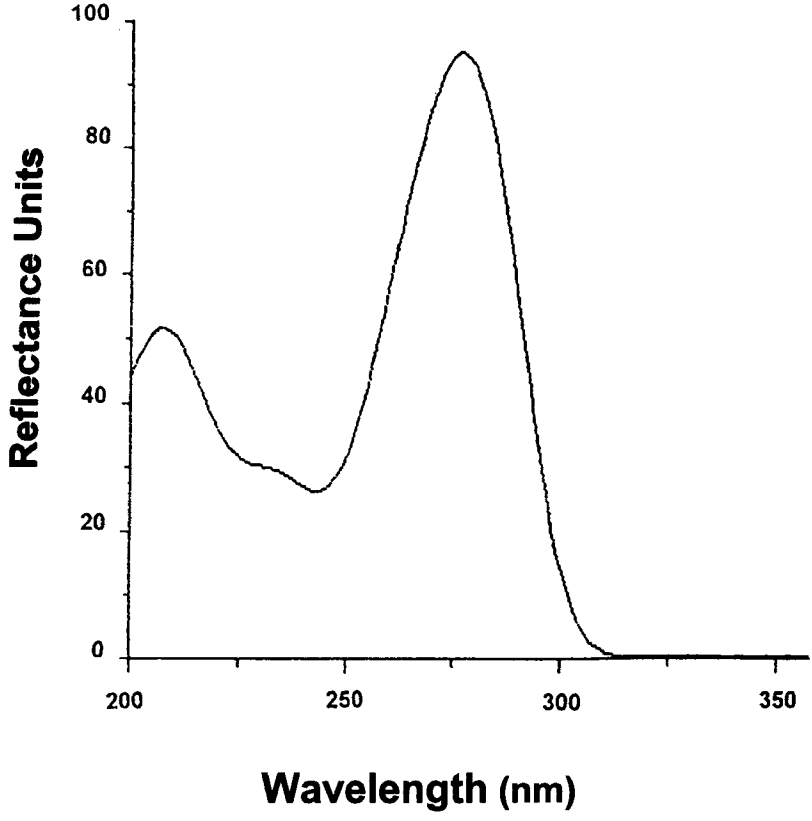


Figure 6. Reflectance ultraviolet absorption spectrum of pentoxifylline, spotted on a Silica gel F254 pre coated plate.

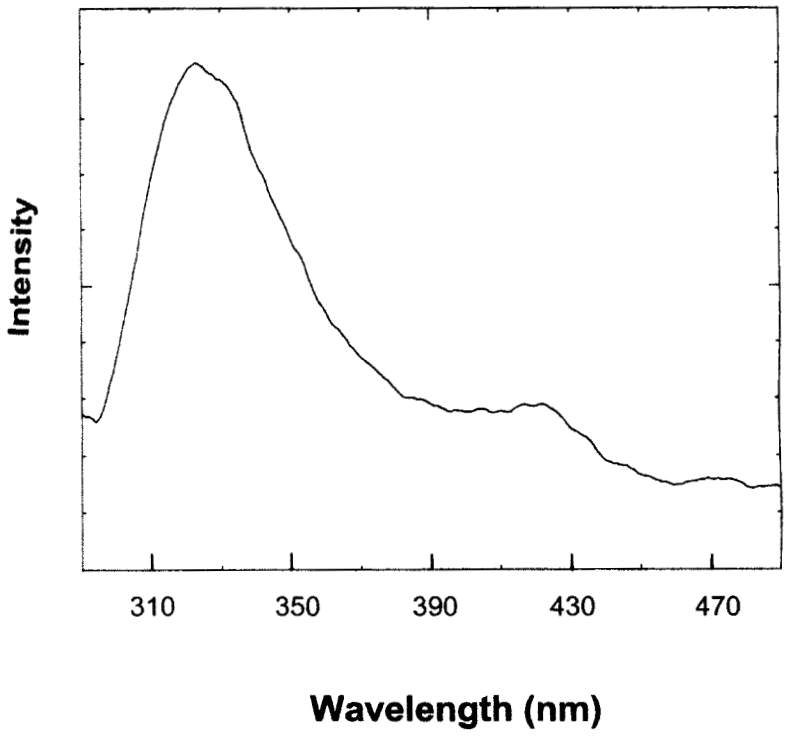


Figure 7. Fluorescence spectrum of pentoxifylline, obtained in methanol (concentration of 40 ppm) at an excitation wavelength of 275 nm.

obtained is shown in Figure 8, and the assignments of the characteristic bands is given in Table 2.

Table 2
Assignments for the Characteristic Infrared Absorption Bands of Pentoxifylline

Energy (cm^{-1})	Band Assignment
2959, 2945	-CH stretching mode
1720, 1701	-CO stretching mode
1658	amide -CO stretching mode
1656, 1433	$-CH_3$ deformation mode
761, 752	$-(CH_2)_n-$ skeletal vibration

3.8.4 Nuclear Magnetic Resonance Spectrometry

3.8.4.1 ^{1}H-NMR

The ^{1}H-NMR spectrum of pentoxifylline was obtained in deuterated chloroform at two different field strengths. The 90 MHz ^{11}H-NMR spectrum was recorded by a Hitachi R-1900 FT-NMR spectrometer, while the 300 MHz spectrum on a Bruker spectrometer. These ^{1}H-NMR spectra are shown in Figures 9 and 10, respectively. Chemical shifts were measured relative to tetramethylsilane, and assignments for the observed bands are found in Table 3.

3.8.4.2 ^{13}C-NMR

The broad band decoupled ^{13}C-NMR spectrum of pentoxifylline is shown in Figure 11, while its J-modulation spin echo ^{13}C-NMR spectrum (with delay $\tau = 1/J = 7$ msec) is shown in Figure 12. Both spectra were recorded in chloroform-d on a Hitachi R-1900 FT-NMR spectrometer (operating at 22.6 MHz) at room temperature. Chemical shifts were measured relative

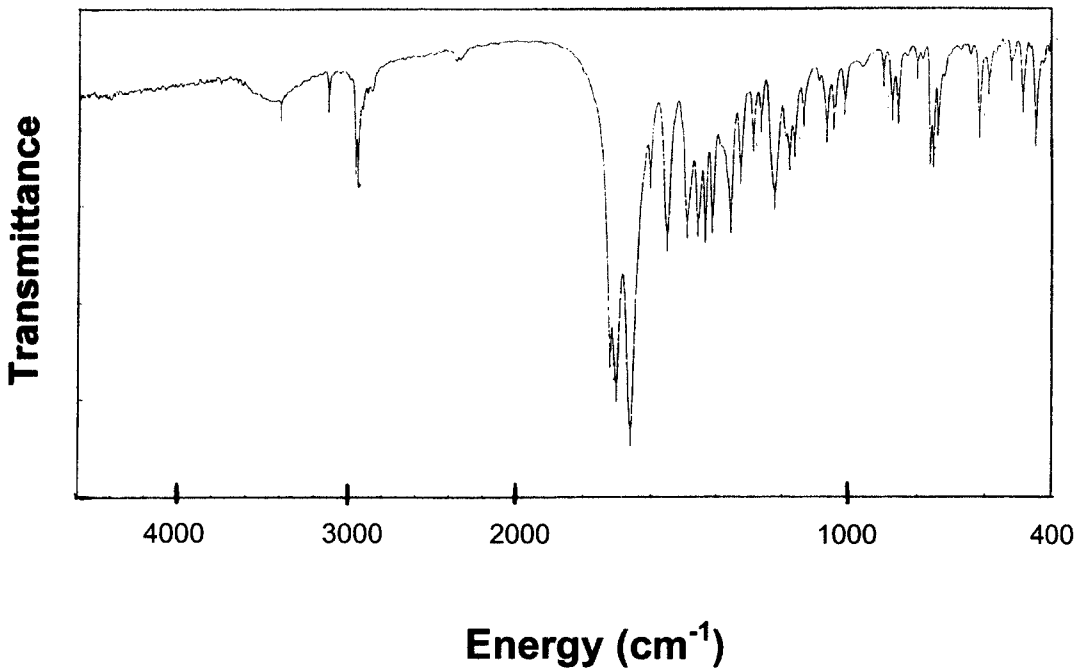


Figure 8. Infrared absorption spectrum of pentoxifylline, obtained in a KBr disk.

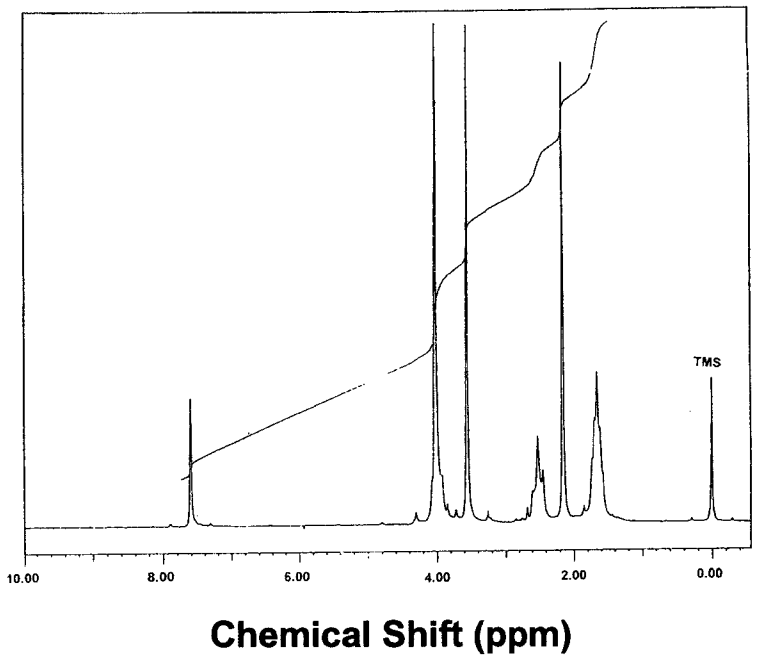


Figure 9. ^{1}H-NMR spectrum (90 MHz) of pentoxifylline in chloroform-d.

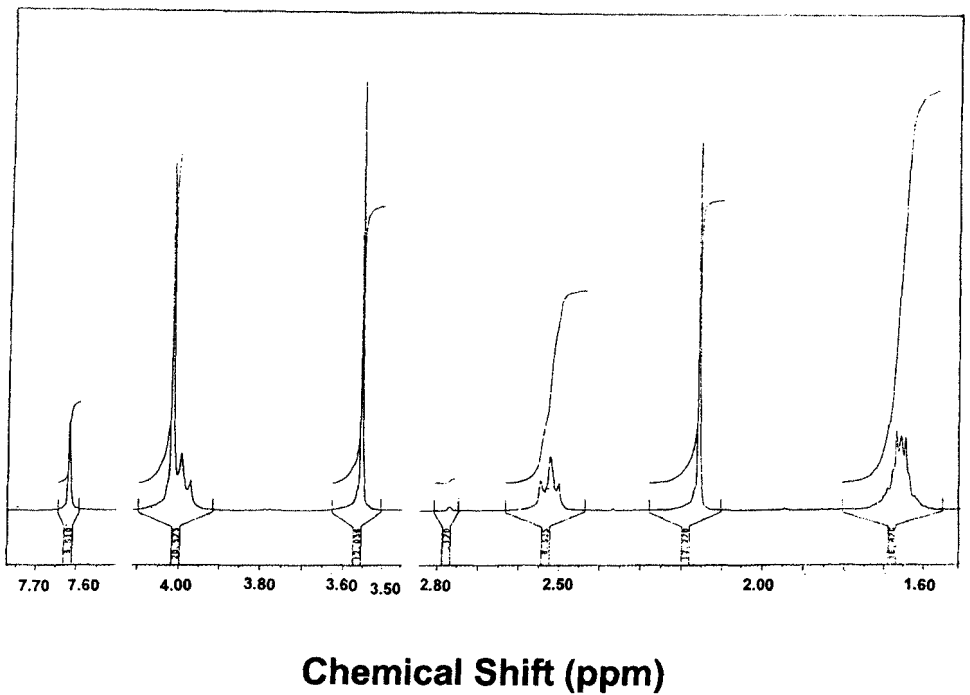


Figure 10. ^{1}H-NMR spectrum (300 MHz) spectrum of pentoxifylline in chloroform-d.

Table 3

^{1}H-NMR Spectral assignments For Pentoxifylline

Chemical Shift (ppm)	**Multiplicity**	**Number of Protons**	**Assignment**
1.40 - 1.70	multiplet	4	H-2', H-3'
2.15	singlet	3	Me-6'
2.52	triplet	2	H-4'
3.55	singlet	3	Me-10
4.01	multiplet	5	Me-11, H-1'
7.61	singlet	1	H-8

Note: The numbering system follows that given in section 1.2.2.

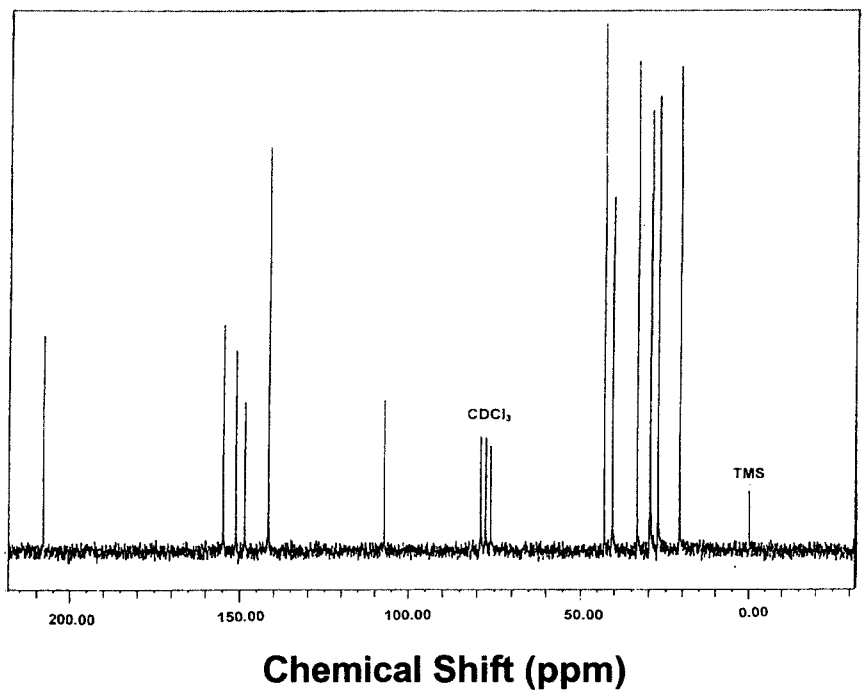


Figure 11. Broad band decoupled ^{13}C-NMR spectrum of pentoxifylline in chloroform-d.

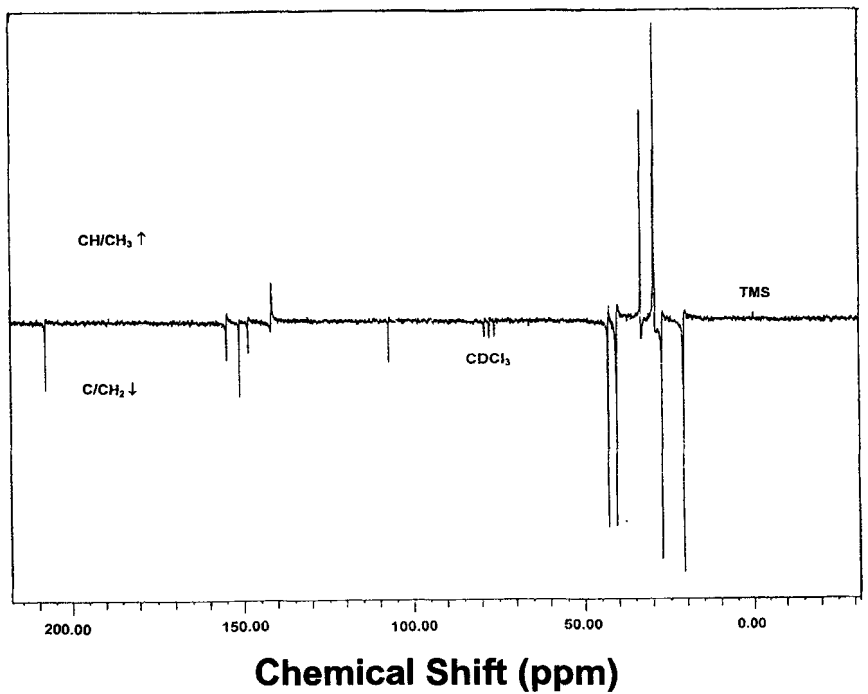


Figure 12. J-modulation spin echo (τ =1/J = 7 msec) ^{13}C-NMR spectrum of pentoxifylline in chloroform-d.

Table 4

^{13}C-NMR Spectral assignments For Pentoxifylline

Chemical Shift (ppm)	Carbon Number
151.1	C_2
148.5	C_4
107.3	C_5
154.9	C_6
141.6	C_8
33.5	C_{10}
29.5	C_{11}
40.6	$C_{1'}$
27.4	$C_{2'}$
21.0	$C_{3'}$
43.0	$C_{4'}$
208.1	$C_{5'}$
29.8	$C_{6'}$

Note: The numbering system follows that given in section 1.2.2.

to tetramethylsilane, and assignments for the observed bands are found in Table 4.

3.8.5 Mass Spectrometry

The electron impact mass spectrum of pentoxifylline is presented in Figure 13, and assignments for the main observed fragments [3] are collected in Table 5. The ion noted at m/z 180 was formed by loss of the 1-position of the side chain, accompanied by the transfer of one H to the xanthine-ring [10].

4. Methods of Analysis

4.1 Identification

The official method for the qualitative identification of pentoxifylline has been given in the British Pharmacopoeia 1993, Addendum 1994 [5], and in the Deutscher Arzneimittelcodex, 1986 [8]. The cited methods are as follows.

4.1.1 Infrared Absorption Spectrum

The absorption maxima in the IR spectrum obtained in a KBr pellet must correspond in energy and in relative intensity when compared to the spectrum of an authentic standard. The spectrum provided in Figure 8 may suffice for this purpose.

4.1.2. Melting Point

When viewed in a capillary melting tube, or through hot-stage microscopy, the melting point of authentic pentoxifylline must be in the range of 102-107°C.

4.1.3 Thin-Layer Chromatography

The principal spot of the sample solution (prepared at 2 mg/mL in methanol) must elute at the same relative retention time as a 2 mg/mL

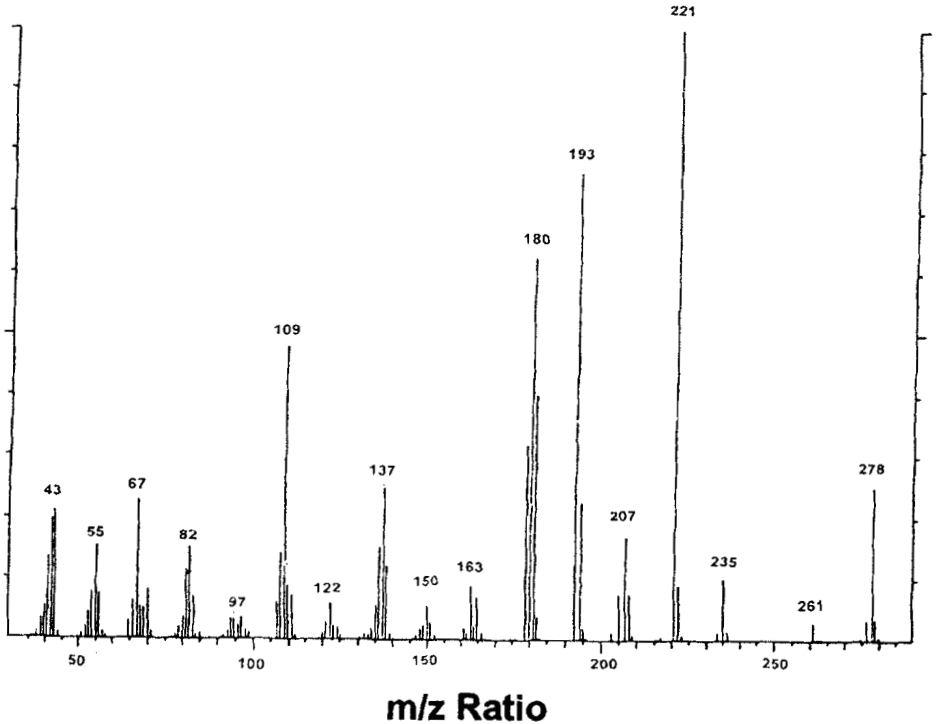
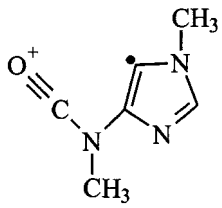
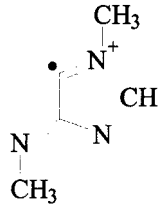


Figure 13. Electron-impact mass spectrum of pentoxifylline.

Table 5

Electron Impact Mass Spectral Data of Pentoxifylline

m/z Ratio (relative intensity)	**Fragment assignment**
278 (25)	$[M^+]$
235 (10)	$[M - 28 (CO) - 15 (CH_3)]^+$
221 (100)	$[M - 14 (CH_2) - 28 (CO) - 15 (CH_3)]^+$
207 (17)	$[M - 28 (2 \times CH_2) - 28 (CO) - 15 (CH_3)]^+$
193 (76)	$[M - 42 (3 \times CH_2) - 28 (CO) - 15 (CH_3)]^+$
180 (62)	$[M -56 (4 \times CH_2) -28 (CO) -15 (CH_3) +1 (1 H)]^+$
137 (25)	
109 (48)	

Note: M = Molecule ion

methanolic solution of an authentic standard. In addition, the relative sizes of the sample and standard spots must be equivalent. Full details of the TLC method are provided in section 4.4.1 of this profile.

4.2 Titrimetric Analysis

Pentoxifylline may be determined by non-aqueous titration, following the method given as described in the British Pharmacopoeia 1993, Addendum 1994 [5], and in the Deutscher Arzneimittelcodex, 1986 [8]. The sample is dissolved in acetic anhydride and titrated with 0.1 M perchloric acid, with the endpoint being determined potentiometrically. Each milliliter of 0.1 M perchloric acid is equivalent to 27.83 mg pentoxifylline.

4.3 Spectrophotometric Analysis

The drug substance content of pentoxifylline tablets can be readily determined using UV spectroscopy. A total of 20 tablets are weighed, and the mean weight of the tablet determined. The tablets were then finely powdered, and a weight equivalent to 25.0 mg of pentoxifylline was dissolved in 25.0 mL of distilled water with ultrasonication. The resulting solution is filtered and diluted to 10.0 mL, whereupon 1.0 mL of this solution was diluted to 100.0 mL with water. The absorbance of the solution is determined at 274 nm.

This method was found to be linear over the range of 2-20 ppm, and the recovery was found to be 101.13 ± 2.1 %. The precision of the method was also found to be acceptable, since relative standard deviations of six replicates fell within the 0.2% to 0.3% range.

4.4 Chromatographic Methods of Analysis

4.4.1 Thin-Layer Chromatography

Smith *et al.* [23] determined pentoxifylline and its metabolite using silica gel as the stationary phase, and 9:1 chloroform/methanol, 7:3 acetone/cyclohexane, or 45:4:1 ethyl acetate/petroleum ether/diethyl ether

as various mobile phases. Detection was effected by the UV absorbance at 254 nm, through the use of Dragendorf's reagent, or through the application of equal parts of 10 % $FeCl_3$ in 20 % tartaric acid and 4 % iodine in acetone.

Bauerova *et al.* [24] reported the determination of pentoxifylline in dog-serum using high performance thin layer chromatography (HPTLC). The serum and standard internal was applied to a Sepacrol SI C 18 mini-column (Institute of Polymers, Slovak Academy of Science), and eluted with acetonitrile. The HPTLC plates (catalog number 13728, E. Merck) were used as the stationary phase, and the mobile phase consisted of 95:5 chloroform/methanol. The method was shown to be linear over the range of 0.02 to 1.5 μg/mL serum, and the recovery was reported to be 95±5 %.

The British Pharmacopoeia 1993, Addendum 1994 [5], and the Deutscher Arzneimittelcodex, 1986 [8], report a method for determination of related substances in samples of pentoxifylline. This TLC method uses silica gel GF_{254} as the stationary phase, and 15:85 methanol/ethyl acetate as the mobile phase. Detection is effected on the basis of the UV absorbance at 254 nm. For the same type of analysis, Kunze Indopharm, Den Haag, [3] used silica gel 60 F_{254} as the stationary phase, and 90:10:1 chloroform/ ethanol/25 % ammonium hydroxide as the mobile phase.

The authors have evaluated the characteristics of the official TLC method [5, 8], and have developed a TLC method for the analysis of pentoxifylline in dosage forms. The method was found to be linear over the range of 0.8 to 2.5 μg/spot, and the accuracy was found to be 100.66 ± 1.4 %. The precision of the method was evaluated by repetitive analysis, and was found to be characterized by a relative standard deviation of 1.3 %. The detection limit was determined to be 0.26 μg/spot, and the quantitation limit was found to be 0.78 μg/spot.

For the assay of ^{14}C-labelled pentoxifylline, Bryce *et al.* [10] used a TLC system to separate the analyte before measuring the total radioactivity by scintillation. These authors effected a separation using a standard silica gel layer, and 80:10:2 chloroform/ethanol/formic acid as the mobile phase.

4.4.2 **Gas Chromatography**

Wills *et al.* [13] developed a method to determine pentoxifylline and its metabolite in plasma samples. In their work, 1-[6-hydroxyhexyl]-3-methyl-7-propylxanthine was used as the internal standard. Before being injected into the GC system, dried plasma extracts were reacted with 5 % trifluoroacetic acid in hexane. The analyses were performed on a GC equipped with nitrogen specific detector, and used a silanized column (2 m x 1.75 mm id.) packed with 3 % OV-25 on Chromosorb W-HP. The oven was programmed to ramp from ambient temperature to 250°C in 6 minutes, then raised at a rate of 80°C/min to a final temperature 270°C. The detector temperature was 300°C, and the helium carrier gas was run at a flow rate of 30 mL/min. Linearity in pentoxifylline response was observed from 20 to 100 ng/mL plasma, and the recovery ranged from 97.8 to 104 %. The detection limit was found to be 1 ng/mL, and the quantitation limit was found to be 15 ng/mL.

Bauza *et al.* [14] developed a GC method for the determination of pentoxifylline and its metabolites in human breast milk. The method required pre-extraction with hexane, followed extraction with 4:1 dichloromethane/isopropanol or 4:1 dichloromethane/isopropanol. Pentoxifylline and its alcoholic metabolites (as the trifluoroacetate salts) and the carboxylic acid metabolites (as their ethyl esters) were measured in a separate GC system which used a nitrogen-phosphorus detector. A glass column (2m x 2 mm id.) was packed with 3 % OV-17 on Chromosorb W-HP was used. The oven, injector, and detector temperatures were 245°C, 300°C, and 300°C, respectively. A flow rate of 25 mL/min was used for the helium carrier gas. By this method, the detection limit was found to be 10 ng/mL, while the recovery of pentoxifylline and its metabolites was in the range of 96-99%.

Marko and Bauerovà [15] developed a GC method for the determination of pentoxifylline and its secondary alcohol metabolites in serum. Before being reacted with trifluoroacetic acid, pentoxifylline and the internal standard {1-(5-oxohexyl)-3-methyl-7-propylxanthine} were extracted from serum by solid phase extraction (SPE). From the various SPE columns that were evaluated, Supelclean LC-18 (Supelco) showed the best analyte recovery of 97 ± 1%. The method used a GC equipped with a nitrogen selective detector, a direct injection port, and a wide-bore fused

silica capillary column (HP-1, 30 m x 0.53 mm id., film thickness 2.65 μm). The separation required an oven temperature programmed from its initial temperature to 200°C for 0.01 minutes, followed by a temperature ramp of 10°C/min to 250°C, and finally a temperature hold for 7 minutes. The temperature of the injection port and the detector were both 300°C. Nitrogen was used as the carrier gas, and run at a flow rate of 25 mL/min). The detection limits of pentoxifylline and the metabolites were found to be 2 and 10 ng/mL, respectively.

In order to determine the pharmacokinetic parameters of pentoxifylline, Bryce *et al.* [10] developed several chromatographic procedures. The method for the quantitative analysis of pentoxifylline and its metabolites was similar to the method of Bauza *et al.*[14]. For characterization of the metabolites, Bryce *et al.* used radio gas chromatography and gas chromatography mass spectrometry. In this work, urine aliquots were reacted with trifluoroacetic acid or BSTFA. Samples were injected into a glass column (2m x 4 mm id.) packed with 3 % OV silicon phase coated onto Chromosorb W-HP. An ESI Nuclear Radio Detector 504 (ESI) and an AEI MS 30 mass spectrometer (Kratos) were used as detectors. Separator and connecting lines were held constant at 250°C.

4.4.3 **High Performance Liquid Chromatography**

Hinze *et al.* [16] developed a HPLC method for the determination of pentoxifylline and its metabolites in human serum. The method required extraction of the analytes with a mixture of 5% isopropanol in chloroform, after addition of 0.1 HCl or NaOH. The column was a Lichrosorb Si 60 (E. Merck), and the mobile phase consisted of 96.5:2.5:1 chloroform/ isopropanol/ acetic acid). 1-(3-oxobutyl)-3,7-dimethylxanthine was used as the internal standard. Detection was effected using the UV absorbance at 273 nm, and the detection limit was found to be 5 ng/mL plasma.

Luke and Rocci [17] developed a method to analyze pentoxifylline and its metabolite (1-(5-hydroxyhexyl)-3,7-dimethylxanthine) in rat plasma. In this method, the analyte was extracted out of the plasma with acetonitrile. A HPLC equipped with a UV-detector (detection at 280 nm) and a reversed phase μ-Bondapak C_{18} column (5 μm particle size, Waters Associates) operating at room temperature were used in this work. The

mobile phase consisted of 24 % acetonitrile in water (sparged with helium) at a flow rate of 1 mL/min. Phenacetin was used as the internal standard. The procedure was able to quantitate pentoxifylline and its metabolite over a concentration range of 25 to 1000 ng/mL. The mean recovery for pentoxifylline and its metabolite were 101.5% and 100.0 %, respectively, and the reported sensitivity limit was 25 ng/mL.

Musch *et al.* [18] proposed a HPLC method to determine pentoxifylline and its 5-hydroxy metabolites in human plasma. The plasma was first deproteinized by adding acetonitrile, centrifuging, and then evaporating to dryness. After reconstitution of the residue with water, the solution was introduced into a cyanopropyl SPE cartridge (J. T. Baker). The analytical separation was effected on a cyanopropyl Lichrosorb (particle size 5 μm, 12.5 cm x 0.4 cm id., E. Merck) column operated with a pre column packed with cyanopropyl bonded Lichrosorb. The mobile phase was 1:99 acetonitrile/water, and detection was made on the basis of the UV absorbance at 280 nm. The recoveries for pentoxifylline and its metabolite were 98.0 % and 86.9 %, respectively, while the detection limits were found to be 10 ng/mL for pentoxifylline and 15 ng/mL for the metabolite.

Lockemeyer and Smith [19] described a method for the analysis of pentoxifylline in rabbit plasma, where sample preparation was virtually eliminated through the use of a Hisep™ (15 cm x 4.6 mm id., Supelco) HPLC column. Acetophenone was used as the internal standard. 10:90 acetonitrile/water was used as the mobile phase, and detection entailed measurement of the UV absorbance at 280 nm. In this work, the mean recovery was found to be 100.08 % and the detection limit to be 3.95 nM.

Mancinelli *et al.* [20] first used selective solid phase extraction (with C_{18} 0.1 g., J.T. Baker) to isolate pentoxifylline, its metabolites, and an internal standard {7-(2'-chloroethyl)-theophylline}. The analytical separation was then performed using a 125 mm x 4 mm id., 5μm, Lichrosphere 100 RP-18 column (E. Merck), and 87:6.5:6.5 water/dioxane/acetonitrile (acidified with 0.5 % acetic acid) as the mobile phase (having a final pH of 3). As usual, the analytes were detected using their UV absorbance at 275 nm. The method recovery was found to be 85%, and the detection limit to be 25 ng/mL.

Raz *et al.* [21] developed a HPLC method to evaluate the pharmacokinetic performance of a sustained-release dosage form of pentoxifylline. After adding 1 mL plasma (containing (1 μg/mL chloramphenicol as an internal standard) and 5 mL dichloromethane, the mixture was vortexed and centrifuged. The organic layer was removed and evaporated to dryness. After this, the residue was re-constituted through the addition of 100 μL of mobile phase. The mobile phase itself consisted of 46% methanol in 50 mM H_3PO_4, adjusted to pH 4.0 with NaOH. The HPLC column used was a 6500 RP-118 (Alltech, 25 cm x 0.46 cm id.) operated with a pre-column (COPELL ODS 30-38 μM, Whatman). The UV absorbance at 273 nm was used to detect the analyte. The method was found to be linear from 25 to 500 ng/mL, while the mean recovery and detection limit were reported to be 95.4 ± 2.8 % and 5 ng/mL, respectively.

The authors have developed a gradient HPLC method to determine pentoxifylline in dosage forms, which uses a reverse phase Lichrospere 100 RP-18 column (5μm, E. Merck) [22]. The mobile phase consists of a mixture of acetonitrile and 0.01 M sodium acetate (10 % acetonitrile for 4 minutes, then raised to 35 % acetonitrile in 4 minutes, and then held for 11 minutes), eluted at a flow rate of 0.9 mL/min. The eluent was monitored using a photodiode array detector (Hitachi L-4500) over the range of 224-400 nm. Linearity (at 288 nm) was achieved from 82 to 814 ppm (injection volume 20 μL), the recovery was 101.5±0.63 %, and the detection limit was found to be 35 ppm. A typical chromatogram of pentoxifylline and its related substances is presented in Figure 14. Using a HPLC system equipped with a photodiode array detector, the purity and identity of analyte peaks could be determined easily as previously reported [22].

The metabolites of pentoxifylline have been isolated using preparative HPLC, and a mobile phase consisting of 0.2 M formic acid and methanol [10]. Urinary aliquots (2 ml) were injected onto a Partisil ODS (5 μm, 50 cm x 10 mm. id.) column, and appropriate fractions were collected over a period of 20 minutes. During the elution period, the mobile phase composition was changed from 8:2 formic acid/methanol to 5:5 formic acid/methanol.

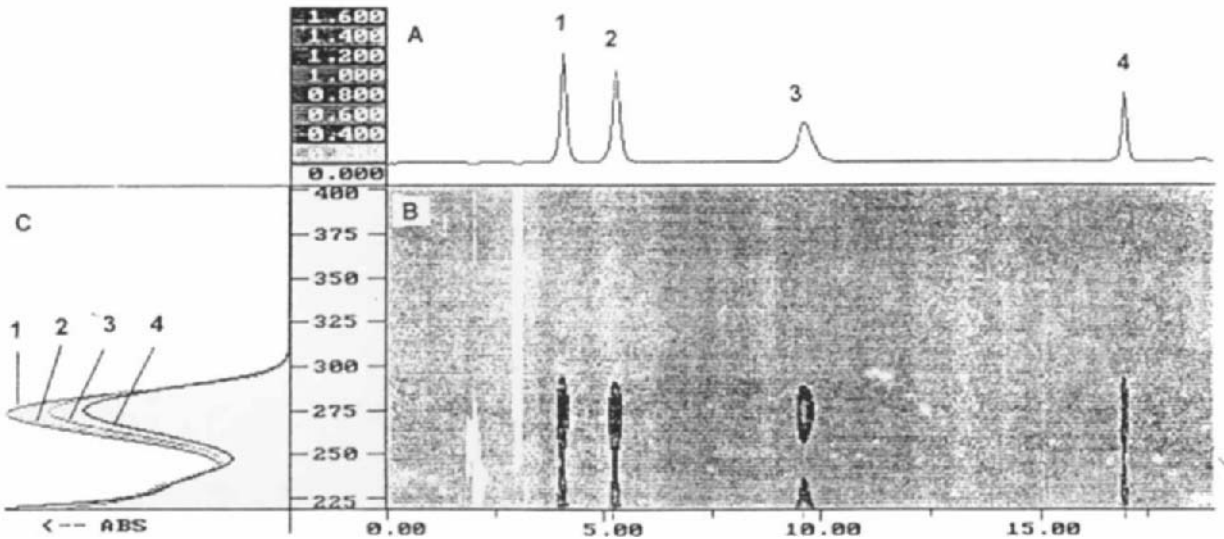


Figure 14. (A) HPLC chromatogram of theobromine (1), theophylline (2), caffeine (3) and pentoxifylline (4).
(B) Contour plot of chromatogram from 224 to 400 nm
(C) Ultraviolet spectrum of peaks 1-4.

5. Stability

Pentoxifylline has been found to be stable when stored at room temperature in well-closed container. The bulk drug substance does not give rise to any degradation product after being stored for 5 years [3].

Tablets should be stored in well-closed and light-resistant containers at room temperature [45], and appear to be stable at room temperature for at least 3 years [3]. Stability data obtained by the aurhors for Tarontal™ (which contains pentoxifylline at levels of 100 and 400 mg/tablet) showed that the products were extremely stable for 5 years at room temperature.

Pentoxifylline intravenous solutions prepared from commercially available ampoules have been reported to be stable at room temperature for at least 24 hours [44].

6. Drug Metabolism and Pharmacokinetics

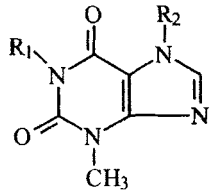
6.1 Metabolism

In humans, pentoxifylline was metabolized both in the red cells and in the liver, and two mayor pathways have been described. One pathway entails demethylation at C7, and the other hydroxylation of the side chain. Of the seven metabolites that were identified (see Scheme 2 for a description of the structures) two of these {1-(5-hydroxyhexyl)-3,7-dimethylxanthine and 1-(3-carboxypropyl)-3,7-dimethylxanthine} were found to be active *in vitro*. 1-(3-carboxypropyl)-3,7-dimethylxanthine was found to constitute almost 80% of all of the metabolic product [10,14,16,25]

6.2 Pharmacology

On isolated organs, such as rabbit ear or rabbit hind limb, pentoxifylline induced vasodilatation, and the coronary flow through isolated guinea pig hearts was also slightly increased. After intravenous and oral application *in vivo*, the drug increased blood flow in the brain, muscle, liver, and kidney of the rat, while blood circulation in the skin remained

Scheme 2. Structure of the Metabolites of Pentoxifylline



Metabolite	R_1	R_2
M_1	$CH_3CHOH(CH_2)_3CH_2$-	CH_3
M_2	$CH_2OHCHOH(CH_2)_3CH_2$	CH_3
M_3	$CH_3CHOHCHOH(CH_2)_2CH_2$-	CH_3
M_4	$HO_2C(CH_2)_3CH_2$-	CH_3
M_5	$HO_2C(CH_2)_2CH_2$-	CH_3
M6	$CH_3CO(CH_2)_3CH_2$-	H
M_7	$CH_3CHOH(CH_2)_3CH_2$-	H

uninfluenced or rose slightly [26]. In general, pentoxifylline and its metabolites are vasodilators, which supports the claim that these compounds improve the flow properties of blood by decreasing its viscosity [25].

Thorough the use of opthalmodynamography, a statistically significant increased in retina blood flow was shown by the administration of pentoxifylline. The effect of pentoxifylline in promoting blood flow to the eye has also been demonstrated by retinal ^{131}I-fluorescence scintigraphy [27]. The effect of pentoxifylline in various stages of arterial obliterations, in arteriovenous perfusion, and in post-thrombotic syndrome became evident by stopping or reducing parasthesia and resting pains, prolongation of walking distance, healing of trophic defects, and through the rise of temperature and reduction of edemas [28]. From the therapeutic and clinical point of view, pentoxifylline produced a significant improvement in the claudication distance in peripheral obstructive arterial disease patients, thus demonstrating both biological activity and clinical efficacy [29]. Schubotz [30] reported a significant improvement in walking distance, reactive hyperaemia, and parathesia in diabetic patients following the administration of the drug. Blood sugar levels showed a slight tendency to improve as well. A clinical trial on patients with cerebrovascular insuffiency demonstrated that after treatment with pentoxifylline, there was a significant improvement in red cell behavior and a significant increased platelet aggregation [31]. Moreover, a fall in plasma fibrinogen and blood viscosity was observed.

Pentoxifylline and its metabolites are inhibitors of superoxide anion production in stimulated leukocytes, so the drug appears to protect cells from the toxic effect of active oxygen production by leukocytes [32]. Recently it was reported that pentoxifylline was found to reverse the vincristine resistance of L1210/VCR mouse leukemic cells [33].

6.3 **Toxicology**

The acute toxicity (as represented as LD_{50} values) of pentoxifylline in various animals is presented in Table 6.

Table 6

LD_{50} Values Found for Pentoxifylline After 7 Days of Observation

Species	By mouth	i.v.	i.p.
Mouse	1385	197	239
Rat	1772	231	305
Rabbit	> 320	100 - 120	
Dog	> 320	> 160	

Note: The data are given in units of mg/kg body weight

No toxic lesions were reported in any organs for studies conducted in dogs or rats after oral administration of 50-200 mg/kg (rats) or 32-100 mg/kg (dogs) body weight over 6.5 months. However, dogs that received higher dose (320-400 mg) exhibited behavioral abnormalities after one year of dosing. Some of the animals died of acute circulatory failure after 4 to 48 weeks [27]. There was no evidence of any teratogenic lesions in female rats that were given daily doses of 0.8, 3.2, or 12.5 mg/kg body weight from the sixth or seventh day of gestation to the sixteenth day [27].

6.4 **Pharmacokinetics**

Pentoxifylline is readily absorbed (at levels exceeding 95 %) from the gastrointestinal tract, but undergoes extensive first-pass hepatic metabolism (60-70 %) [25, 34]. After intravenous introduction, plasma level decline in a biphasic manner, with a terminal $t_{1/2}$ of 1.63±0.8 hours. Plasma clearance was 1333±48 l mL/min, and the volume of distribution was 168±82.3 L [35]. Schaefer *et al.* [36] showed that in patients having a creatinine clearance of less than 10 mL/min, an accumulation of pentoxifylline occurred.

After oral dosing of pentoxifylline (at 100, 200, and 400 mg) in healthy male volunteers, peaks plasma of pentoxifylline occurred between 0.29 to 0.41 hours, with $t_{1/2}$ values being in the range of 0.39 to 0. 84 hours [37]. About 89 % of orally administrated pentoxifylline was excreted in the urine after 6 hours [10].

The excretion of pentoxifylline and its metabolites into human breast milk was reported by Witter et al. [38]. Pentoxifylline and its major metabolites were present in human milk within 2 hours after oral ingestion.

After 100 mg intravenous infusions, the half life of pentoxifylline elimination was prolonged in cirrhotic patients (2.12±1.22 hours) [39]. Wills *et al.* [13] showed that the AUC (0 to 10 hours) values of pentoxifylline and its metabolites were not significantly different after ingestion of food. A significant decrease in peak plasma concentration was observed when the drug was administrated with food.

When pentoxifylline was rectally administrated, more than 80 % of the drug could enter the general circulation, thus bypassing the metabolic effect of the liver. Through this mode, the t_{max} was between 0.10 and 0.90 hour, and the C_{max} ranged from 1.12 to 4.35 μg/mL, depending on the drug formulation [40].

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POVIDONE - IODINE

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Contents

1. Introduction

Iodine was discovered in 1812 by Curtius, and in 1815 Gay-Lussac showed that it was an element. Soon after that, the disinfectant properties of iodine were discovered. The new element, however, had several properties which made its use cumbersome. Its inherent insolubility in water was overcome by dissolving the iodine in alcohol, but the alcoholic iodine solution itself exhibited serious drawbacks. First of all, the concentration of the solution constantly varied due to evaporation of the solvent. Furthermore, at concentrations higher than 5 % the solutions were found to be irritating to the eyes, skin, and mucous membranes. These problems were alleviated to a degree by adding some iodide to the iodine solution to yield the water soluble triiodide, but the irritating effect could not be completely eliminated through this formulation.

Nevertheless, the value of a new disinfectant made from iodine was soon recognized, and the water-alcohol solutions were quickly put in use. The so-called Lugol's Solution (an aqueous solution containing 5% elemental iodine and 10% potassium iodide) was first made in 1829, and "tinctura iodine" was listed in the U.S. Pharmacopoeia by 1830. This latter formulation contains a medium consisting of 45 % aqueous ethanol, formulated with 2 % elemental iodine and 2.4% sodium iodide.

The disinfecting characteristics of iodine originated in its ability to substitute for covalently bound hydrogens in compounds containing -OH, -NH, -SH., or -CH functional groups. These groups can not only be part of the solvent or other constituents of the formula, but also of the material to be disinfected (such as skin, mucous membranes, bacteria, *etc.*) [1].

The exact solution-phase chemistry which yields the germicidal action is not easy to determine owing to the number of reactions which iodine may undergo in solution. The chemistry of iodine in water can be described by a large number of reactions with eight of these being considered important. These reactions, and their respective equilibrium constants, are as follows:

$$I_2 \Leftrightarrow I^+ + I^- \qquad K = 9.9 \times 10^{-9}$$

$$I_2 + H_2O \Leftrightarrow H_2OI^+ + I^- \qquad K = 1.2 \times 10^{-11}$$

$$I_2 + H_2O \Leftrightarrow HOI + H^+ + I^- \qquad K = 3 \times 10^{-18}$$

$$HOI \Leftrightarrow I^+ + OH^- \qquad K = 3 \times 10^{-10}$$

$$HOI \Leftrightarrow H^+ + IO^- \qquad K = 4 \times 10^{-13}$$

$$I_2 + HOI \Leftrightarrow I_2HOI \qquad K = 2.7 \times 10^{-7}$$

$$I_2 + I \Leftrightarrow I_3^- \qquad K = 7.14 \times 10^{2}$$

$$3\,HOI \Leftrightarrow 3\,H^+ + 2\,I^- + IO_3^- \qquad K = 2.5 \times 10^{-11}$$

These equations show that in aqueous solution, iodine can exist in as many as seven different forms. It is also evident that since H^+ participates in many of the reactions, effects of solution pH are always important to the reaction pathways. It has been shown that of the seven forms of the iodine described in the reactions above, only hydrated molecular iodine (I_2), hypoiodous acid (HOI), and iodide ion (I^-) contribute to the antibacterial effect [3]. In pharmaceutical preparations which contain both iodine and iodide, the bactericidal effect can be attributed almost entirely to free molecular iodine [2].

In addition to the equilibrium reactions shown above, a decomposition reaction is also possible:

$$3\,HOI \Leftrightarrow HIO_3 + 2\,H^+ + 2\,I^-$$

This reaction may serve to decrease the bactericidal action, since both iodates and iodides are inactive in this respect [4].

In the presence of polymers having the ability to bind iodine (denoted as an *iodophor* property), the chemistry of iodine becomes even more complex. Based on reactions between small compounds of similar composition [5,6], it may be presumed that polymeric iodophors having oxygen-containing functional groups (*e.g.*, carbonyl groups) will react with iodine to form donor-acceptor complexes in which the iodine is the acceptor. In povidone-iodine, the iodophor consists of poly(*N*-vinyl-2-pyrrolidinone), and its ability to complex with iodine was discovered by Shelanski and Shelanski in 1956 [5,6]. The stability of povidone-iodine solutions was found to be vastly superior to either that of iodine tincture or of Lugol's solution [7].

In the particular instance of povidone-iodine, at least two further reactions must be considered:

$$PVP{\bullet}HI_3 \quad \Leftrightarrow \quad PVP + H^+ + I_3^-$$

$$PVP{\bullet}I_2 \quad \Leftrightarrow \quad PVP + I_2$$

The addition of a iodophor to the system produced great advantages. The complex exhibited excellent water solubility, which eliminated the unpleasant staining properties of common iodine solutions. These solutions have essentially no iodine vapor pressure, which greatly improves the odor of the formulation. In addition, the polymeric iodophor complexes were virtually non-irritating and of very low toxicity [8]. Of greatest importance was the fact that the polymeric iodophors showed considerably higher bactericidal activity relative to other iodine containing preparations [9].

As in the case with all iodophors, the antibacterial activity of povidone-iodine is associated with the elemental iodine in the solution. The difference between a conventional iodine solution and a iodophor is that the latter carries practically all the iodine in a complexed form, so that the concentration of free iodine in the solution is always very low. This property has the effect of reducing the drawbacks associated with the presence of iodine (high toxicity, high level of irritation, and staining power). The bulk of the iodine exists in the triiodide form, which is in equilibrium with iodide and the active iodine. This species dissociates according to the environmental conditions, yielding elemental iodine and iodide ion. This equilibrium is dependent upon solution pH, temperature, the nature of the solvent, and the solution concentration. When these parameters reach a state of equilibrium, the properties and composition of the solution become invariant [10].

In the povidone-iodine complex, the iodine does not exist as a single species, and in fact several forms of iodine have been characterized. The so-called "available iodine" contains all the iodine species which can be titrated with sodium thiosulfate. Also discussed is the negatively charged "iodide" which is necessary for the complexation of iodine. The third type is the so-called "total iodine", which is given by the sum of available iodine and iodide. The so-called "free iodine" is the type of iodine which can be extracted from aqueous povidone-iodine solution with heptane [11],

which is the residual uncomplexed iodine and which can also be determined using dialysis [12].

Dialysis studies were used to ultimately prove that all the iodine species present actually moved through the membrane, showing that all the equilibria described before actually exist. It had been found that all of the available iodine could be determined by thiosulfate titration, which was taken as evidence of the very high speed by which these equilibria are reestablished. Using stopped-flow kinetic methodology, Fuoss showed that the reaction equilibria were established on the millisecond time frame [13].

Based on these studies, one can best describe the solvated povidone-iodine complex as a swollen polymer, which carries a definite number of HI_3 units in complexed form. In addition, a cloud of various iodine species is attached to the polymer. Within this cloud, the system is in a thermodynamically metastable state, and there is a constant and extremely fast change of one species to another according to the intimate details of the solution environment. The free iodine content of a povidone-iodine solution depends upon the concentration of available iodine in the system, and follows a bell-shaped curve having a clear maximum at 100 ppm available iodine [9]. This correlation is illustrated in Figure 1.

1.1 Behavior of the Povidone-Iodine Complex

Elemental analyses, iodine determinations, and the results obtained using various physical methods, have shown that povidone-iodine can be defined as a system that for which every two amide groups complexed with HI, there are an average of seventeen uncomplexed vinylpyrrolidinone units in the molecule. Therefore, approximately 80 mole% of the product is actually unaltered poly(vinylpyrrolidone), and should therefore behave as such. This prediction is in accord with most of the physical properties recorded for povidone-iodine. The strength of the complex has been found to be dependent upon the molecular weight of the polymer, where higher molecular weights of povidone lead to higher degrees of absorption in the ultraviolet region.

Povidone is known to undergo complex bonding to a variety of materials, with the strongest interactions being with compounds having acidic

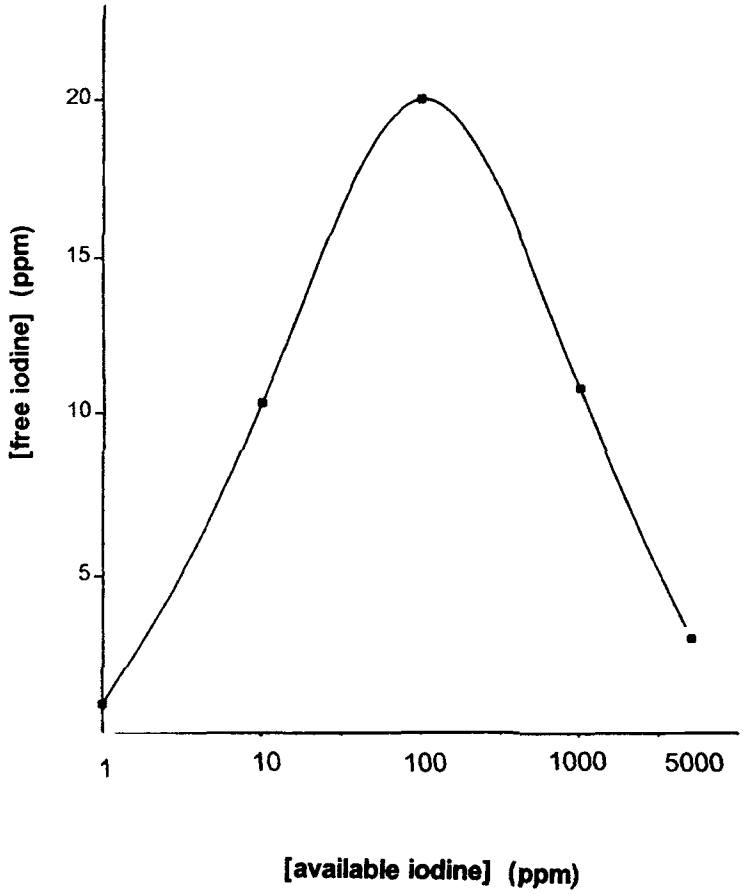


Figure 1. Free iodine levels in povidone-iodine aqueous solutions, as a function of the total available iodine [9].

hydrogens. Examples of this reactivity are phenols [26,27] and poly-acids [28,29]. Barabas has shown that povidone-iodide is capable of forming complexes and multi-complexes as does the unmodified polymer [30].

When studying the cryoprotective properties of ^{14}C-labeled povidone, Digenis determined that the carbonyl group was bound to red blood cells [31]. It is still not clear whether this binding takes place with the involvement of the phenolic tyrosine group or with the carboxylic acid groups of the cell wall protein. Regardless, if povidone can bind to cell walls, then it can be expected that povidone-iodine will bind to cell walls as well.

Rutherford proposed a very persuasive mechanism based on the similarity of povidone and povidone-iodine, and on the poly-ion character of the latter [32].

During the process of dissociation, povidone-HI_3, is transformed into a polymer containing PVP-H^+ poly-cations and I_3^- poly-anions. The PVP-iodine ratio indicates that povidone-iodine contains 17 uncomplexed pyrrolidone units for each iodine complexed unit. With the polymer structure that contains one charged unit for every 19 pyrrolidone units along the polymer chain, povidone-iodine can be considered as a true polyelectrolyte. In fact, the viscosity of PVP-iodine solutions shows typical "electroviscous" behavior, and the plot of reduced specific viscosity versus concentration curve showed a strong increase at diminishing concentrations. The data have been found to follow the empirical behavior developed by Fuoss for polyelectrolytes [13]. Povidone-iodine is therefore characterized as a highly reactive polymer combined with a strong complexing ability, which also acts as a polyelectrolyte carrying charges along the polymer chain.

Digenis and co-workers have shown that when povidone-iodine solution and Lugol's solution are formulated to the same level of "available iodine" (*i.e.*, the thiosulfate titratable iodine), the bactericidal effect of povidone-iodine is considerably higher [31]. Conventional thought attributes the bactericide effect to the amount of "free" iodine in the system, since it is presumed that the effect is due to either iodination of the tyrosine segments of the protein or to the formation of disulfide bridges through the oxidation of sulfhydryl groups [34]. Nevertheless, it remains that at the

same "available iodine" level, the amount of "free" iodine is higher in Lugol's solution, so Lugol's solution should be bacteriologically more effective than povidone-iodine. This is counter to empirical observation, and according to Rutherford this behavior is attributable to the polyelectrolyte nature of povidone-iodine [32]. In his model, the expanded polymer molecule resides in contact with protein molecules in the cell walls, being bound by a combination of PVP complexing power of PVP and ionic binding power of the polyelectrolyte. Povidone-iodine brings along a cloud of various iodine species, capable of producing the bactericidal effect.

The determining factor for bactericidal activity is not the concentration of the "free iodine" in the solution, but instead is the concentration of "free iodine" at the wall of the target bacterium. Poly(vinylpyrrolidinone) itself has no bactericidal effect, but owing to its affinity for the cell membranes it delivers the active ingredient to the target. The delivered iodine species affect the cytoplasm and cytoplasmic membranes through very fast reactions [33]. The lower activity of Lugol's solution can be explained by its lack of an affinity for the cell walls of bacteria.

Rackur reported an interesting and paradoxical behavior of the commercially available 10% povidone-iodine solution [2]. Berkelman and co-workers [36] and Gruen [37] had also independently observed that the microbial action of such solutions increased on dilution, and a gradual decrease in activity only began when the dilution reached 1:100. Furthermore, this behavior seems to be independent of the duration of the interaction between povidone-iodine and the microbes. The correlation is shown in Figure 2.

This unexpected finding was further supported by the results of other studies conducted parallel with the bacteriological studies. Gottardi [38] used potentiometric measurements, and Horn and Ditter [39] used equilibrium dialysis and photometric investigations, to find that the content of uncomplexed iodine initially increased with dilution. This reached a maximum at a solution strength of 0.1%, and then decreased with further dilution. On the other hand, the other iodine species present in a povidone-iodine solution exhibit normal behavior in that their concentration decreases on dilution [2]. This behavior is illustrated in Figure 3.

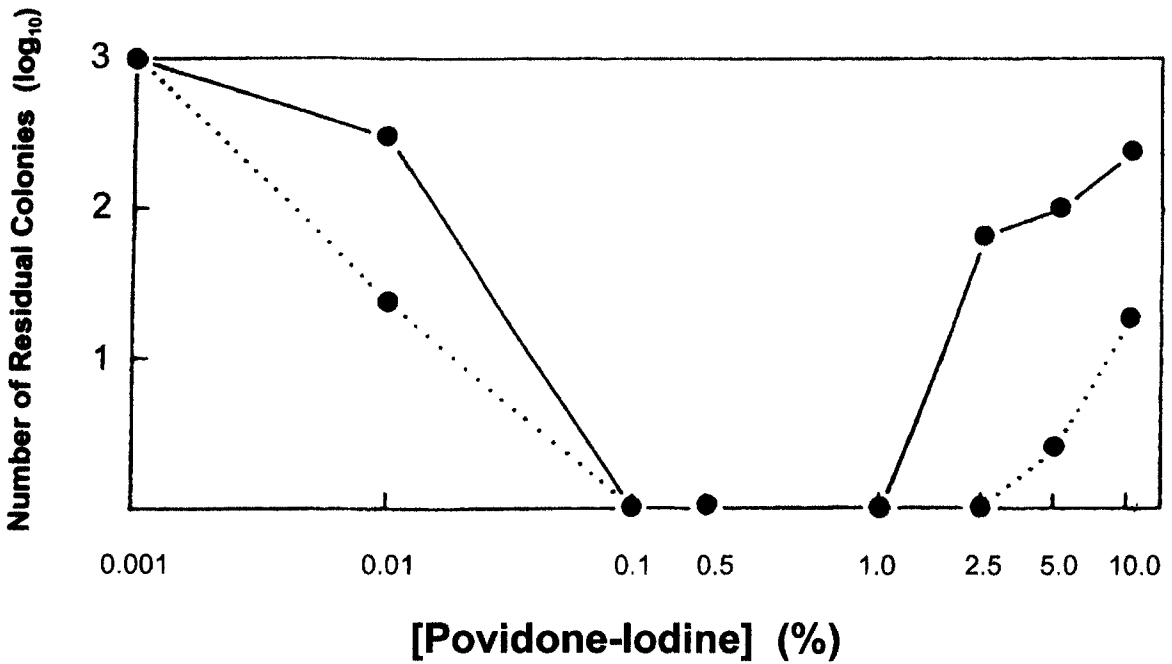


Figure 2. Correlation between survival of *Staphylococcus Aureus* and the povidone-iodine concentration [2].

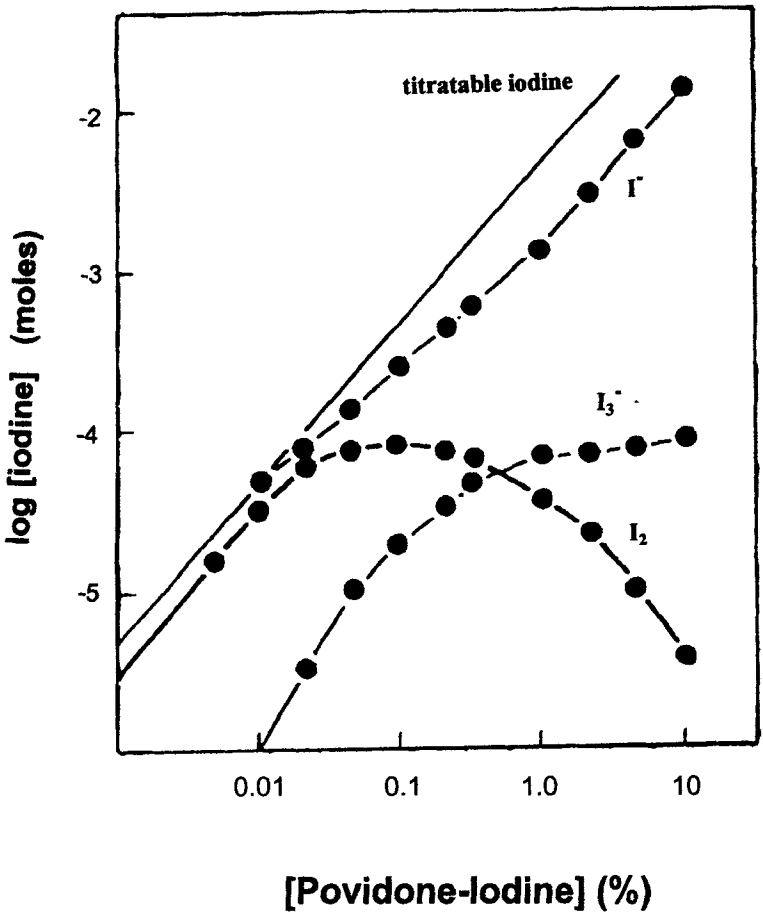


Figure 3. Concentration of iodometrically titratable iodine, and equilibrium of iodine species in aqueous solutions of povidone-iodine [2].

By combining the results of the microbiological studies (illustrated in Figure 2) with the iodine equilibrium concentration curve (given in Figure 3), it becomes evident that the maximum iodine concentration and maximum microbial effect coincide. This provides a strong confirmation of the fact that the microbial effectiveness of povidone-iodine is a function of the concentration of uncomplexed iodine. This relation is is illustrated in Figure 4.

Rackur explained the concentration effectiveness phenomenon by the formation of polymeric aggregates, which contain entrapped uncomplexed iodine [35]. Increasing the amount of solvent causes these aggregates to dissociate, releasing the entrapped iodine and increases the bactericidal effectiveness of the solution.

A very interesting (and potentially very useful) property of povidone-iodine is that this material is impervious to high energy radiation [40]. Povidone-iodine does not crosslink upon irradiation, its molecular weight does not increase as a result of the process, and covalent addition of halogen to polymer does not take place during the irradiation [41,42]. The only observable change associated with high energy irradiation is some loss of iodine potency through the formation of iodide. This can be compensated during formulation by the addition of a slight excess of povidone-iodine. Poly(vinylpyrrolidinone), on the other hand, is not resistant to gamma-radiation, and undergoes both intermolecular and intramolecular crosslinking when irradiated by ^{60}Co source.

This finding is important since it may offer a practical new method for the sterilization of povidone-iodine solutions, since the currently used sterilization methods have serious drawbacks. Heat treatment usually results in unacceptably high loss of iodine, while ethylene oxide is unsuitable owing to its reactivity with many of the substrates which the formulation is intended to be used on. On the other hand sterilization by gamma-irradiation has none of these disadvantages.

1.2 Reactions of Povidone-Iodine with Materials to be Disinfected

As previously discussed, only elemental iodine and hypoiodous acid are capable of bactericidal action. In this process, both povidone and iodine

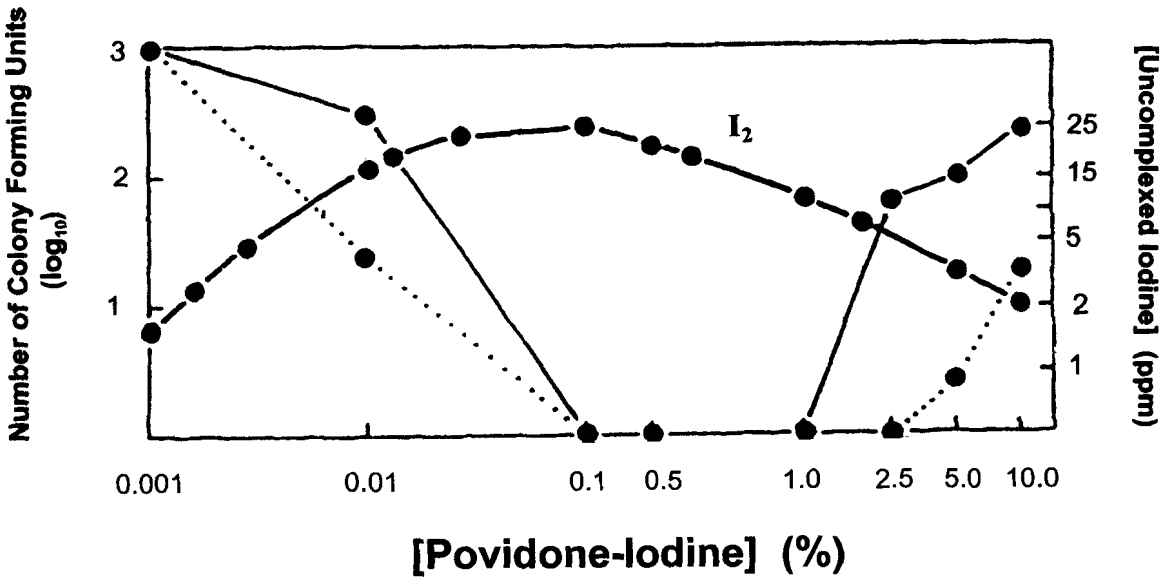


Figure 4. Correlation of the concentration of uncomplexed iodine with the microbial destruction kinetics of various concentrations of aqueous povidone-iodine solutions [2].

are effective, but participate differently. After being targeted to the protein to be attached, the povidone part may react by complexing either the phenolic group of the tyrosines in the protein, or by forming complexes with the carboxylic acid groups of the protein of the bacterium cell walls. The disinfection itself is carried out by the two bactericidal iodine species (I_2 and HIO), keeping in mind that these are indistinguishable with respect to the iodination mechanism.

$$\text{R-H} + \text{I} \quad \Leftrightarrow \quad \text{R-I} + \text{H}^+ + \text{I}^-$$

$$\text{R-H} + \text{HOI} \quad \Leftrightarrow \quad \text{R-I} + \text{H}_2\text{O}$$

According to Gottardi, the nature of the reaction between iodine (or hypoiodous acid) and the functional groups of the proteins present in the bacterial cell walls depends upon the chemical nature of the functional groups in question [38]. First, N-iodide compounds are formed with the N-H function of the basic amino acids (lysine, histidine, and arginine), as well as with such groups in nucleotides (adenine, cytosine, guanine):

$$\text{R-NH}_2 + \text{I}_2 \quad \rightarrow \quad \text{R-NI} + \text{HI}$$

This reaction blocks an important position for hydrogen bonding, effecting noticeable changes in the protein structure. Secondly, the sulfhydryl group in the cysteine can be oxidized, eliminating the formation of disulfide bonds, and thus inhibiting an important part of protein synthesis.

$$\text{R-SH} + \text{I}_2 \quad \rightarrow \quad \text{[R-SI]}$$

$$\text{[R-SI]} + \text{RSH} \quad \rightarrow \quad \text{R-SS-R} + \text{HI}$$

Finally, iodine can substitute for the hydrogen atoms which are ortho to the phenolic hydroxyl group of tyrosine. The large size of a covalent bound iodine atom at the aromatic ring can provide a steric hindrance to the hydrogen bonding between the carbonyl group and the acidic proton of the phenolic hydroxyl group.

2. Description

2.1 Nomenclature

2.1.1 Chemical Name

2-pyrrolidinone-1-ethenyl-homopolymer complex with iodine

2.1.2 Nonproprietary Names

1-vinyl-2-pyrrolidinone polymer, iodine complex

Povidone-iodine

Povidon-Jod

Iodopovidonum

Mundidon

PVP-iodine

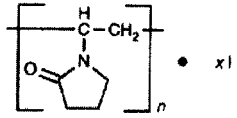
2.1.3 Proprietary Names

ACU-dyne	Acme: Bridgeport, CT, USA
Amyderm S	Schulke & Mayr: Hamburg, Germany
Batticon	Trommsdorff: Alsdorf, Germany
Betadine	Faulding: Salisbury, Australia Multipharma: Basel Napp: Watford Herts: UK Purdue Frederick: Norwalk, CT, USA
Betaisodona	Mundipharma: Limburg, Germany
Betaseptic	Mundipharma: Basel, Switzerland
Braunoderm	Braun: Melsungen, Germany
Braunol	Braun: Melsungen, Germany
Braunosan	Braun: Melsungen, Germany
Braunovidon	Braun: Melsungen, Germany
Bridine	Allen & Hanbury: Greenford, Middlesex UK
Chem-O-Dine	Remedia: Johannesburg, South Africa
Destrobac	Zyma: Nyon, Switzerland

Diofexon	Bago: Buenos Aires, Argentina
Disadine DP	Stuart: Cheadle, Cheshire, UK
Disphex	ICI
Efodine	Fougera: Mellville, N.Y., USA
Eutadine (veterinary)	Gräub: Bern, Switzerland
Femidin	AVP: Clarence, N.Y., USA
Freka-cid	Fresenius: Bad Homburg, Germany
Iodine Tri-Test	Nelson: Ermington, N.S.W., Australia
Iso-Betadine	Belgana: Bruxelles, Belgium
	Leiras: Turku, Finland
Isodine	Purdue Frederick: Norwalk, CT, USA Dagra: Niemen, the Netherlands
Jodobac	Bode Bacillolfabrik: Hamburg, Germany
Jodocur	Farmacologici Milanese: Caronno Petrusella
Nutradine	Restan: Wadeville, Transvaal, South Africa
Orodine	Orapharm: South Yarra, Australia
Peractum	Thornton & Ross: Linthwaite, Huddersfield UK
Pevidine	Berk: Eastbourne, Sussex, UK
Polydine	Fischer: Tel-Aviv, Israel
Povadyne	Chaston: East Farmingdale, N.Y., USA
Povi-Derm	Kinder: Sao Paolo, Brazil
Povidermol	Organon: Os, the Netherlands
Poviodine	Rougier: Montreal, Quebec, Canada
Sabofen	Geyer: Porto Alegre, Brazil
Savion Dry	ICI
SP Betaisodona	Mundipharma: Limburg, Germany
Stoxine	Smith, Kline & French
Topionic	R. Rius: Barcelona, Spain
Traumasept	Smith, Kline & French
Vetedine (veterinary)	Gräub: Bern, Switzerland
Videne	C-Vet: Bury St. Edmunds, UK

2.2 Formula

Povidone-iodine is a stable chemical complex of poly(vinyl-2-pyrrolidinone) and elemental iodine.



Both BASF and GAF (now ISP Corporation) produce a grade of povidone-iodine whose poly(vinyl-2-pyrrolidinone) component has a nominal K-value of 30. BASF also supplies a product whose poly(vinyl-2-pyrrolidinone) component has a nominal K-value of 17.

2.3 Chemical Abstracts Registry Number

25655-41-8

2.4 Appearance

Povidone-iodine is a reddish-brown, free-flowing powder. Micronization of this product alters the color to a brownish-orange hue, and also reduces the flowability.

3. Methods of Preparation

Povidone-iodine can be made by a heterogeneous process, involving a diffusion controlled reaction in which povidone powder is mixed with crystalline iodine at elevated temperatures [45]. Alternatively, povidone-iodine can be produced by a homogeneous method, where the reactants are dissolved in a common organic solvent, and the complex ultimately brought into aqueous solution by solvent exchange [46].

3.1 Heterogeneous Reaction Process

Four hundred parts of povidone, having a K-value of approximately 30 and a water content of 5.0% (adjusted if necessary), are placed in a tumbler-mixer together with 72.3 parts of ground iodine. The mixture is

tumbled at room temperature for 5 hours, whereupon the temperature of the reactants is raised to 90°C. After that, the tumbling is continued, and the mixture is tumbled at 90°C for an additional 10 hours [44].

3.2 Hcmogeneous Reaction Process

A solution consisting of 789.4 g of iodine in 644 g of ethanol is prepared by heating these materials at 70±5°C for 2 hours in a glass-lined kettle equipped with a dropping funnel. An aqueous solution containing 68.6 g of hydrogen iodide is then added to the contents of the kettle through the dropping funnel. The resulting solution is then charged for 2 minutes to a glass reaction maintained at about 85°C. This glass reactor, which is equipped with an agitator and a reflux condenser, contains an 35% aqueous solution of 22.14 g of poly(vinylpyrrolidone) K30. The solutions which are introduced into the reactor provide the desired PVP:I+HL mole ratio, where the HI represents 25-30% of the total iodine content.

The resulting solution is refluxed then at the azeotrope temperature of 85-86°C for 1.25 hours to complete the complexation. The ethanol-water azeotrope is then stripped off, while the removed portion is continuously replaced with deionized water. The solvent stripping is continued until the bath temperature reaches 98°C. After that, the system is held at the reflux temperature for a combined distillation and reflux time of 90 minutes. At this point, cool deionized water is added to bring the final concentration to 24% [45].

3.3 Miscellaneous Methods and Modifications

BASF Aktiengesellschaft received a British patent in which poly(vinylpyrrolidone) is prepared in an anhydrous organic medium, using an organic per-compound initiator and a complex heavy metal salt as a co-catalyst [45a]. It was claimed that the povidone-iodine made from this polymer by the method described in Section 3.1 loses substantially smaller amounts of iodine (as inactive hydrogen iodide), and that the product exhibits excellent stability.

Iodine was treated with hydrogen peroxide in an acidified poly(vinylpyrrolidone) solution. The reaction was performed at 90-95°C

for 1 hour, and yielded a solution of polyvinylpyrrolidone-iodine complex [45b].

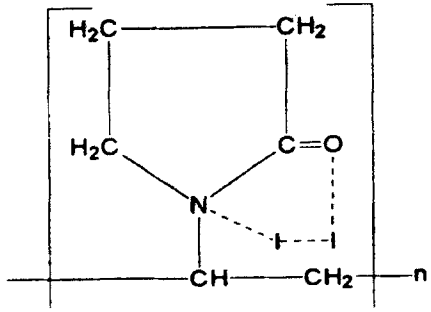
A patent was granted for the preparation of a polyvinylpyrrolidone-iodine complex which involved treating powdered polyvinylpyrrolidone with iodine at 70-100°C in the presence of formic acid, or with an ammonia salt or amide of carbonic, formic, or oxalic acid. By heating the ingredients for 1 hour at 50°C and 8 hours at 100°C, a product was obtained which lost only 5.3 % iodine after 15 hours at 80°C [45c].

4. Physical Properties

4.1 Structure of the Povidone-Iodine Complex

Because of its cyclic imide structure, poly{vinylpyrrolidinone} (PVP) may considered to exist in an equilibrium resonance structure with charges distributed between the cationic carbonyl group and the anionic nitrogen segment. Due to these resonance properties, PVP can form complexes with numerous types of compounds [14].

The first attempt to describe the structure of the povidone-iodine complex was actually based on the resonance structure of PVP, and the complex was envisioned as a system existing of two five-membered condensed rings [10]. One of these consisted entirely of covalently bound atoms, and the other was thought to contain complex bonds. The rings were supposed to share the nitrogen group and the carbonyl group of the pyrrolidonyl unit, as shown below:



In another model, the iodine molecule was proposed to be attached to the nitrogen atom of the adjacent pyrrolidinone ring [15]. A PVP-$(I_2)_x(IO^-,I^-)_y$ structure was also proposed, with the x and y stoichiometry being factors predetermined by the reaction conditions [16]. However, this could only happen if the hydrolysis of iodine in the presence of polyvinylpyrrolidone were to take place to such an extent that the formed complex would contain iodide and hypoiodide in addition to iodine.

Daniels provided the first data useful for determining the correct structure of the povidone-iodine complex [17]. He prepared and characterized a number of insoluble complexes of amides and HBr, using two or three moles of amide for each mole of HBr. Schenck and co-workers prepared insoluble complexes of tertiary amides, whose structures were similar to that of PVP and HI_3, using these ingredients in 2:1 molar ratio [18]. X-ray diffraction studies carried out on the complex formed by *N*-methyl-2-pyrrolidinone and HI_3 proved unambiguously that the proton of the HI_3 molecule was shared by two carbonyl groups in genuine complex formation. From this it seems likely that formation of the povidone-HI_3 complex takes place by a similar mechanism, and that the proton of the HI_3 moiety actually binds two pyrrolidonyl carbonyl groups with split bonds [19]. Based on this evidence, and on infrared spectroscopic investigations, the structure shown in Figure 5 was proposed as best describing the povidone-iodine complex.

The only problem with the proposed structure of Figure 5 is that it requires the formation of a 10-membered ring which is normally considered as being energetically unfavorable. This theoretical objection, however, could be set aside if one presumes that the sharing of the proton does not necessarily take place along the polymer chain in a regular fashion but rather comes about randomly with the participation of any pyrrolidinone ring which is closest to the HI_3 proton.

By carrying out a single crystal x-ray structural analysis on the model compound bis(1-methyl-2-pyrrolidono-O-O')-potassium triiodide), Schenck and co-workers proposed a structure for the iodine complexes of pyrrolidonyl compounds which is shown in Figure 6. These studies suggested that the unit cell of the crystal consists of four independent *N*-methylpyrrolidone units and two I_3 segments. Figure 6 shows the mean

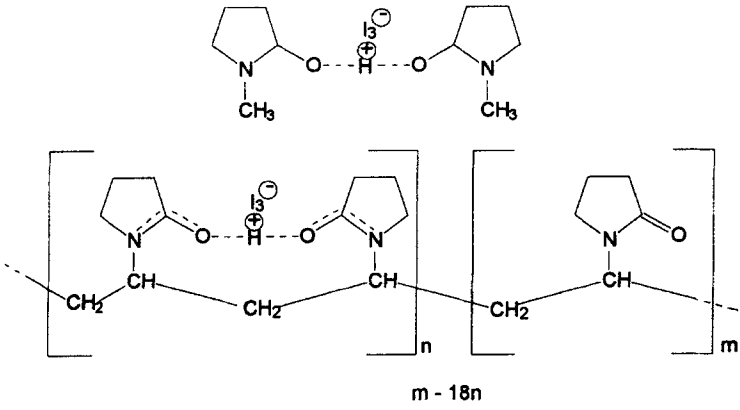


Figure 5. Proposed structure for the povidone-iodine complex [19].

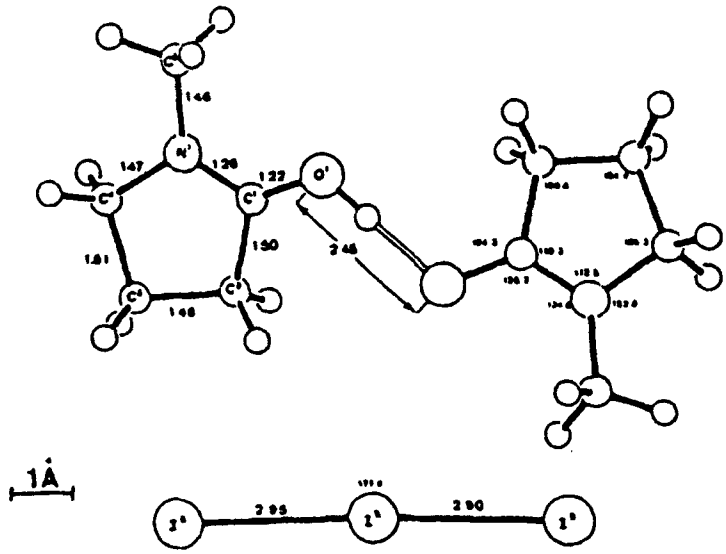


Figure 6. Average bond lengths and angles from the x-ray structural analysis [18].

bond distances and angles. As proposed by Schenck, two 1-methyl-2-pyrrolidinone units are connected through a 2.45Å hydrogen bond. The pyrrolidolyl pair represents the cation of the system, while the I_3 unit is the counter-anion. The atoms of each ring system are coplanar though the hydrogen atoms are located outside of that plane, the iodine atoms also lie in a plane, and the two IO units are oriented at an angle of 135°. Two of the *N*-methylpyrrolidonyl rings are situated in a plane which is parallel to the plane of the iodine atoms, while the other two ring systems are twisted 40° away [18].

However, Vratsanos has proposed a different structure for povidone-iodine [20]. He found that the correlation between the absorbance and concentration of aqueous povidone-iodine solutions is not linear, but shows almost twice the expected absorption at 290 nm. This seems to indicate that a considerable structural change of the complex must take place as the povidone-iodine concentration increases due to the decreasing possibility of solvation of iodine by water. This in turn enhances the formation of triiodide. At the same time, he found a noticeable shift in the absorption maxima of the triiodide. Similar effects had been reported when the solvent was gradually changed from pure water to pure butanol, which demonstrates the effect that more hydrophobic sites can exert on the development of the iodine complex [21]. This finding underlines the importance of the length of the polymer segment involved.

Potentiometric titration results had shown the existence of a distinct inflection during the addition of titrant equivalent to approximately 14 monomer units for each iodine molecule. These results, however, raise the question as to why only one polymer unit out if the 14 satisfies the requirements for successful complexation.

While taking part in complexation, the polymer units have to direct their polar groups toward the bulk solvent water in order to maintain solubility. Since spectrophotometric studies indicated that the complexation sites are mostly hydrophobic, it must be presumed that these reactions take place with the participation of the polymer backbone. These findings seem to support a coiled arrangement for the povidone-iodine complex. Another interesting finding is that the viscosity of povidone is lower in the presence of triiodide ion [22]. Such a reduction of the hydrodynamic

volume would be consistent with the transformation of the expanded polymer chain to a coiled form.

The concept where the complex would consist of iodine surrounded by a helix had been proposed by Rundle and co-workers for amylose [23,24]. However, more recent studies using resonance Raman and and Mössbauer spectroscopies suggested that the pentaiodide ion ($I_2I_3^-$) actually is the species located in the amylose helix [25]. The helical concept proposed by Vrantsos does not exclude an involvement of the carbonyl groups of the polymer with the active protons of the carboxyl and hydroxyl groups of the protein. Neither does it prevent the involvement of the carbonyl bond of the pyrrolidonyl units in hydrogen ion bonding with the triiodide counterion. This donor acceptor interaction actually contributes to the stability of the complex.

4.2 Solubility Characteristics

With agitation, povidone-iodine is completely soluble in cold water at concentrations exceeding 10%. By contrast, the aqueous solubility of elemental iodine is only 0.034%. Povidone-iodine is soluble in 5% sodium hydroxide solution, where it exhibits a reaction and subsequent loss of color.

Povidone-iodine has been found to be soluble in ethyl alcohol, propyl alcohol, and acetone. The complex is insoluble in tetrahydrofuran, ethyl acetate, chloroform, carbon tetrachloride, dichloroethane, and hexane. Swelling of the polymer complex has been observed in dioxane.

4.3 Viscosity of Povidone-Iodine Solutions

As would be anticipated, the viscosity of povidone-iodine solutions is a function of both the molecular weight of the polymer and the concentration of the solution. Typical data determined at 25°C for polymer complexes having K=30 are shown in Table 1.

Table 1

Viscosity of Povidone-Iodine in Aqueous or Ethanolic Solutions

Solution Concentration (%)	Viscosity, Water Solution (mPa•sec)	Viscosity, Ethanol Solution (mPa•sec)
5	2.0	2.0
10	7.0	5.0
20	23.0	20.0

4.4 Spectroscopic Properties

The spectroscopic properties of povidone-iodine strongly resemble those of povidone itself [29]. However, when the bound iodine interacts sufficiently with the polymer, perturbations in vibrational frequencies and nuclear magnetic resonance chemical shifts can be observed. These trends can be used to deduce additional information as to the nature of the bonding existing in the povidone-iodine complex.

4.4.1 Vibrational Spectroscopy

As shown in Figure 7, the infrared spectrum of povidone-iodine exhibits numerous characteristic peaks. The spectrum strongly resembles that of povidone itself [29], which indicates that the observed bands are associated with vibrational modes of the polymer. Particularly diagnostic is the triplet pattern which is observed in the methylene-bending region. A summary of the significant absorption bands, together with assignments for the vibrational transitions, is provided in Table 2.

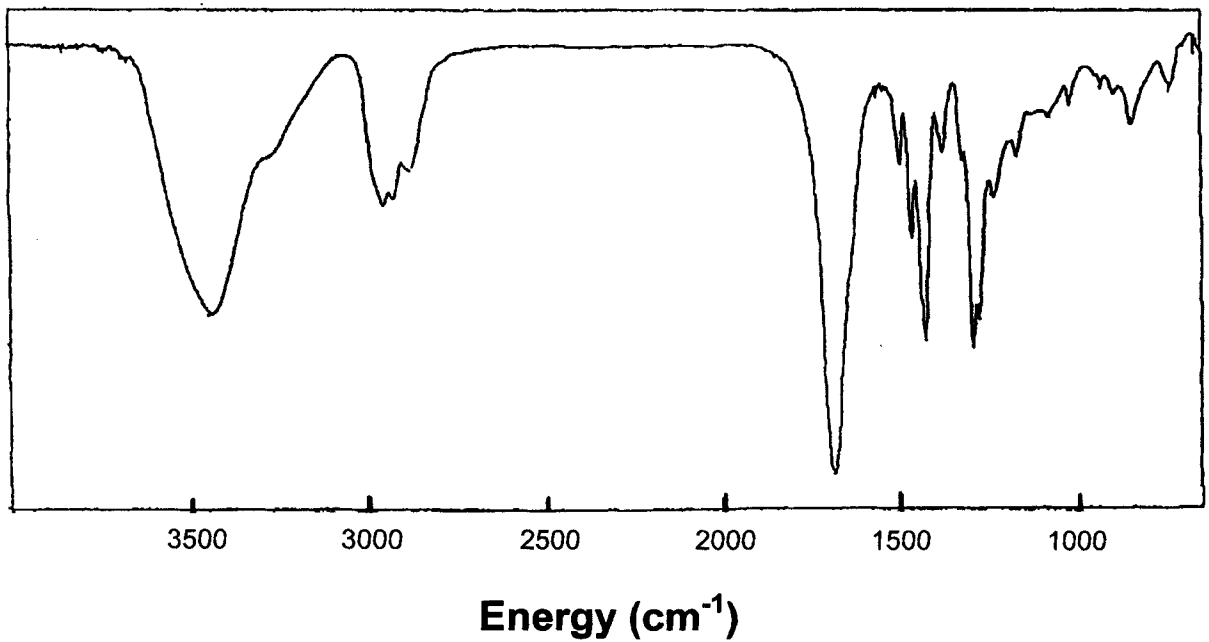


Figure 7. Infrared absorption spectrum of povidone-iodine (Courtesy ISP Corporation).

Table 2

Band Assignments for the Vibrational Transitions of Povidone-Iodine

Energy (cm^{-1})	Assignment
2949	aliphatic -CH-stretching mode
2884	aliphatic -CH-stretching mode
1678	carbonyl stretching mode
1453	heterocyclic ring mode
1420	$-CH_2$ scissors deformation mode
1337	$-CH_2$ and -CH wagging mode
1283	-CH bending mode

The energy maxima for the various peaks are found to be independent of the molecular weight of the polymer. Most significant is the shift observed for the amide stretching mode, which decreases from 1706 cm^{-1} in povidone [29] to 1678 cm^{-1} in povidone-iodine. This observation provides additional evidence for the existence of a strong interaction between the bound iodine and the amide functional group.

4.4.2 ^{1}H-NMR Spectrometry

The proton NMR spectrum of povidone-iodine is given in Figure 8, and strongly resembles that of povidone itself [29]. Owing to the range of molecular weights associated with the polymer, broad resonance bands are obtained rather than the usual sharp bands normally encountered. The peak assignments are given in the spectrum, and are designated according to the following numbering system:

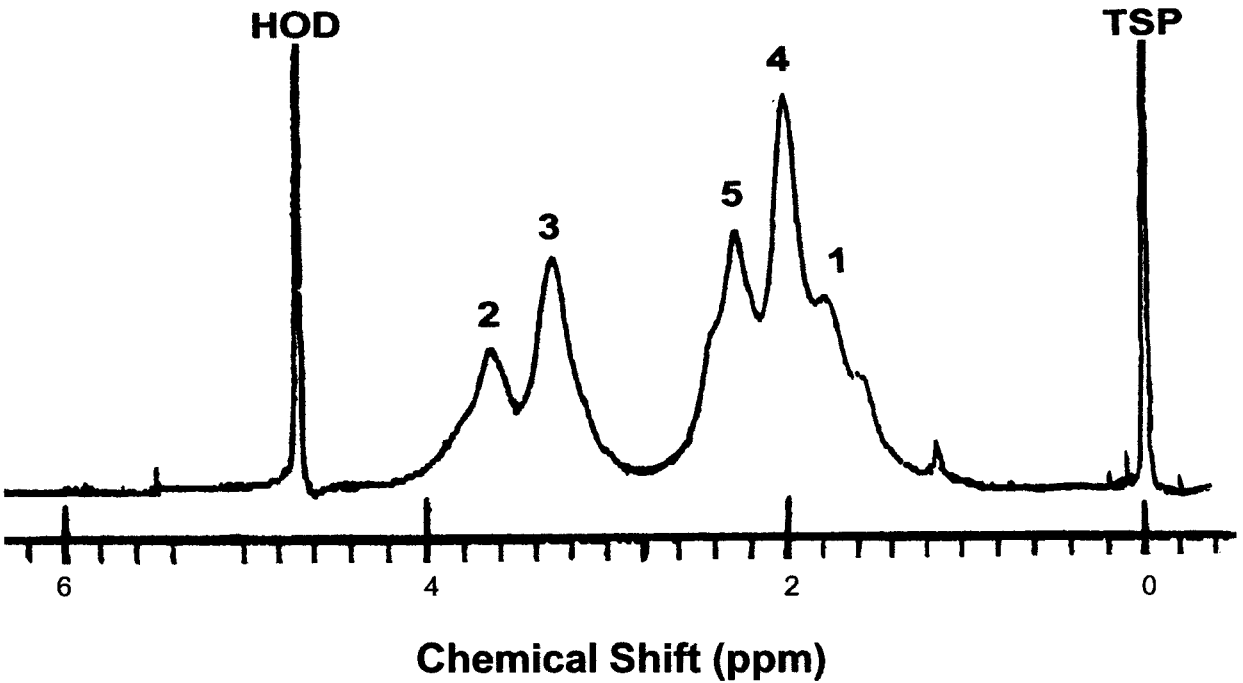
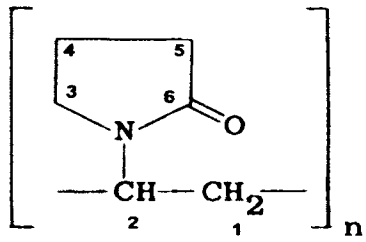


Figure 8. ^{1}H-NMR spectrum of povidone-iodine, obtained using TSP as the internal standard. (Courtesy ISP Corporation).



4.4.3 ^{13}C-NMR Spectrometry

The carbon NMR spectrum of povidone-iodine is shown in Figure 9, and also bears a strong resemblance to that of povidone itself [29]. Once again, the existence of a range of polymer molecular weights causes broadening of the resonance bands. The numbering system used to identify the various bands is the same as given in the previous section.

4.5 Stability of the Complex

Povidone-iodine can be stored as a solid-state powder without significant loss of iodine. Samples stored at 65°C in glass-stoppered bottles for as long as three years showed only 0.5% loss of available iodine. Because povidone-iodine exhibits only negligible vapor pressure (0.01 torr as compared to 3.3 torr for elemental iodine at 56°C), the iodine does not sublime even at elevated temperatures. However, the material is hygroscopic and should be protected from moisture [8].

The stability of povidone-iodine solutions is much higher than that of iodine tincture or of Lugol's solution. Aqueous solutions of the complex containing 2% available iodine were found to remain unchanged when stored for one year at room temperature. Experimental data relating the relative loss of available iodine from povidone-iodine solutions and other iodine preparations are given in Table 3.

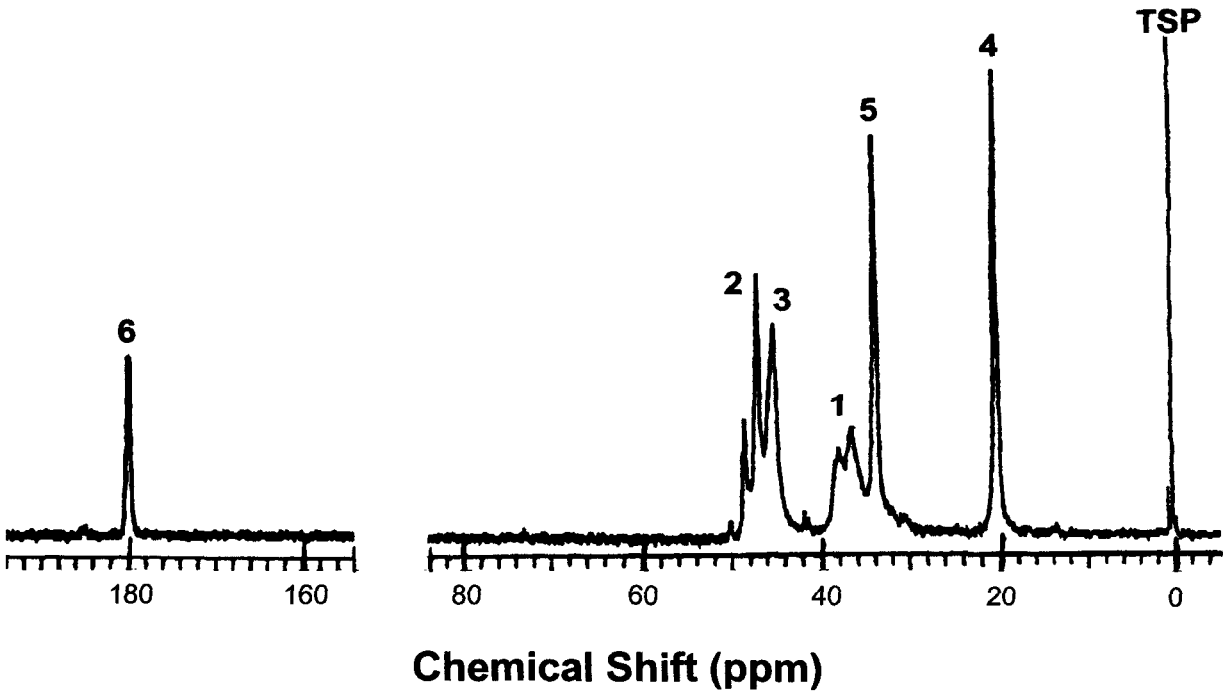


Figure 9. ^{13}C-NMR spectrum of povidone-iodine, obtained using TSP as the internal standard. (Courtesy ISP Corporation).

Table 3

Percent Iodine Remaining After Storage of Various Iodine Preparations

Conditions	Aqueous Povidone-Iodine Solution	Lugol's Solution	Iodine Tincture Solution
Starting concentration 0.03%: room temperature storage for 20 hours in open beaker	90	34	90
Starting concentration 1.0%: room temperature storage for 20 hours in open beaker	87	82	87
Starting concentration 1.0%: 42°C storage for 32 days in sealed bottles [8]	95	92	53

Note: In the open beaker studies, the solution volume was maintained at a constant level through the addition of solvent.

4.6 Compatibility of Povidone-Iodine

Povidone-iodine has been found to be compatible with a number of medicinal vehicles, and has been successfully compounded with a variety of liquids, ointments, gels, soluble tablets, and sprays [8]. However, any formulation must ensure that the effective pH of the system remains on the acidic side in order to prevent complex dissociation. In addition, the system has to be impervious to halogens.

Reducing agents and alkaline materials are known to affect the germicidal action. If such materials are present, the level of available iodine in the formulation must be adjusted so that the germicidal activity is established at the expected level.

Glass, plastic, wood, or stainless steel are suitable materials for the storage of povidone-iodide.

5. Methods of Analysis and Pharmacopoeial Compliance

Povidone-iodine is used in numerous applications, and is packaged either in its neat form, or with the addition of different vehicles and additives corresponding to its intended application. The Pharmacopoeias describe and regulate the following articles:

Povidone-iodine, neat

Povidone-iodine topical aerosol solution

Povidone-iodine ointment

Povidone-iodine cleansing solution

Discussions and work are in progress to harmonize the directives of the various pharmacopoeial agencies for the analysis of povidone [29]. It is anticipated that similar efforts will be made to regulate the analysis and specifications of povidone-iodine.

5.1 Povidone-Iodine, Neat

Neat povidone-iodine is a complex of iodine with povidone. It is a dry hygroscopic powder, which contains not less than 9.0 percent and not more than 12.0 percent of available iodine, calculated on a dry basis.

Additional specifications for commercial povidone-iodine are as follows:

Available Iodine: Dry Basis: As Is Basis:	 12.0% 10.5%
Iodide (Anhydrous Basis):	6.6%
Moisture:	8.0%
Nitrogen (Dry Basis):	10.0-11.5 %

Ash:	0.025 % max.
Heavy Metals:	0.002 % (20 ppm)
Arsenic:	0.00015 (1.5 ppm)

Povidone-iodine should be kept in a well sealed container.

5.1.1 Identification

5.1.1.1 Blue Color Test

Dissolve 1 g of povidone-iodine in 10 mL of distilled water. Add one drop of this solution to a mixture of 1 mL starch TS and 9 mL of distilled water. A deep blue color is developed in the presence of povidone-iodine.

5.1.1.2 Film Test

Spread a solution consisting of 1 g of the test material dissolved in 9 mL of distilled water over a 20 cm x 20 cm area on a glass plate. Allow the film to air dry overnight at room temperature in an atmosphere of low humidity. A brown film is formed, which is readily soluble in water.

5.1.1.3 Precipitation Test

Dissolve 0.1 g of the test material in 5 mL distilled water, and add dropwise 1% (w/v) of an aqueous sodium sulfite solution until the color of the test solution disappears. On the addition of 2 mL potassium dichromate solution TS, a light brown participate is formed.

5.1.1.4 Infrared Absorption Spectrum Test

The infrared absorption spectrum of povidone-iodine, shown in section 4.4.4, is sufficiently distinct from that of povidone so as to permit a differentiation between the two materials. In addition, the spectrum can be used as a general method of identification.

5.1.2 Determination of the Various Forms of Iodine

Povidone-iodine contains the so called "free" iodine, which is responsible for the antimicrobial activity of the product. It also contains "available iodine", which is present in a complexed form and serves as a reservoir for the "free" iodine and which can be titrated against sodium thiosulfate. The " total" iodine value also includes the "iodide" ions which are necessary for formation of the complex.

5.1.2.1 Determination of Available Iodine

This method provides an assay value for a sample of povidone-iodine. About 5 g of accurately weighed povidone-iodine is placed in a 400-mL beaker, and 200 mL of distilled water is added. The beaker is covered and its contents are stirred at room temperature to dissolve these as completely as possible. However, the stirring should not be continued longer than one hour. The solution is titrated immediately with 0.1 N sodium thiosulfate VS until the sample solution becomes light-yellow in color. At that point, 3 mL of starch TS is added, and the sample solution should become blue-black in color. The titration is completed then to a clear endpoint.

The percent available iodine is calculated by the following equation:

$$\% \text{ available iodine} = \frac{(V)(C)(126.92)}{(W)(1000)} \times 100$$

where V is the volume (mL) of thiosulfate solution used, C is the molar concentration of the thiosulfate solution, and W is the mass (g) of sample taken. Computed in this manner, each milliliter of sodium thiosulfate solution is equivalent to 12.69 mg of available iodine.

5.1.2.2 Determination of Total Iodine

About 500 mg of accurately weighed povidone-iodine is placed in a 250-mL glass-stoppered iodine flask. After adding 100 mL of distilled water, the stopper is sealed with electricians tape. The flask is then placed on a shaker, and shaken for 30 minutes. Sodium bisulfite TS is added dropwise until the color of the solution disappears. Using a burette, exactly 25.0 mL of 0.1 N silver nitrate VS and 10 mL of nitric acid are added with mixing.

The excess silver nitrate is titrated with 0.1 N ammonium thiocyanate VS, using ferric ammonium sulfate TS as the indicator. After that a blank titration is carried out exactly the same way but in the absence of sample.

The amount of total iodine is calculated using the following:

$$\% \text{ total iodine} = \frac{([V_b - V_s])\ (N)\ (12.692)}{(W)\ (S)} \times 100$$

where V_b is the volume of ammonium thiocyanate TS titrant used for the blank, V_s is the volume of titrant used for the sample, N is the normality of the ammonium thiocyanate TS, W is the mass of sample taken, and S is the solid content of the sample. Computed in this manner, each milliliter of 0.1 N silver nitrate is equivalent to 12.69 mg of total iodine.

5.1.2.3 Determination of Iodide Ion

The amount of iodide ion present in the povidone-iodine complex can be calculated by subtracting the percentage of available iodine (as determined according to the procedure in section 5.1.2.1) from the percentage of total iodine (as determined in section 5.1.2.2). The amount should not be more than 6.6%, calculated on an anhydrous basis.

5.1.2.4 Determination of "Free" Iodine in Povidone-Iodine

The amount of uncomplexed, or "free", iodine in a sample of povidone-iodine is determined using heptane as the solvent in a solid-liquid extraction procedure. One milliliter of povidone-iodine solution, which contains exactly 1% of available iodine, is placed in a 50-mL volumetric flask, and 25 mL of heptane is added. The flask is stoppered and then shaken for one minute in a 25±1°C water bath. The shaking is discontinued, and the liquid allowed to separate into two layers. The upper layer (heptane) is separated, and its absorbance determined at a wavelength of 520 nm. A reference solution may be prepared by dissolving 4 mg of iodine in 100 mL of heptane. In a 1 cm cell, the absorbance at 520 nm will be 0.142, so therefore the molar absorptivity of iodine in heptane is 901 liter/mole·cm.

The distribution coefficient of iodine between heptane and a given povidone-iodine complex is calculated using the following equation:

$$K = \frac{(\{A_i - I_h\})(25)}{I_h}$$

where K is the distribution coefficient, A_i is the available iodine in 1 mL of povidone-iodine solution, and I_h is the number of milligrams of iodine in heptane [43].

5.1.2.5 Stability Determination of Povidone-Iodine

The stability of a given povidone-iodine sample is determined through the conduct of stress testing. The baseline value for the study requires performance of the available iodine assay described in section 5.1.2.1.

About 250-300 mL of povidone-iodine solution is prepared, which is to contain approximately 1% available iodine. This solution is filtered through fluted filter paper, and subdivided into two 50-mL aliquots. These are placed into glass-stoppered 100-mL weighing bottles, both of which are sealed with electrical tape. Bottle #1 is placed in a 75±0.1°C constant temperature bath in such a way that the level of the bath oil is at the solution level of the sample bottle. The time is recorded, and the available iodine content in bottle #2 (the blank) is determined immediately. Bottle #1 is incubated for 360±5 minutes. At the end of this period, the bottle seal is removed, and the bottle contents chilled to room temperature by submerging the bottle briefly in an ice bath. After that, the available iodine content of the Bottle #1 is determined as per section 5.1.2.1.

The following calculation steps are performed to deduce the 6-hour stability of the povidone-iodine sample. The available iodine value from bottle #2 is used to deduce the amount of iodine blank:

$$\text{Iodine Blank} = \frac{(V_b)(N)(12.692)}{50} = I_b$$

where V_b is the volume (in mL) of thiosulfate TS (having a normality of N) used in this titration. The available iodine value from bottle #1 is used to deduce the incubated amount of iodine:

$$\text{Incubated Sample Iodine} = \frac{(V_b)\ (N)\ (12.692)}{50} = I_s$$

where V_s is the volume (in mL) of thiosulfate TS used in this particular titration. The percent iodine loss incurred over the 6-hour stress test is calculated using:

$$\text{Iodine Loss} = \frac{(\{I_b - I_s\})\ (100)}{I_b}$$

5.1.3 Loss on Drying

In its solid state, povidone-iodine is quite hygroscopic, so depending upon the conditions of its preparation and storage it may contain sizable amounts of water. It may contain other volatile contaminants. The method suitable for the determination of the loss on drying (LOD) is identical with that previously described for povidone itself [29]. The LOD value should not be more than 8.0%.

5.1.4 Residue on Ignition

The residue on ignition (ROI) test is used to determine the total nonvolatile content in a given sample of povidone-iodine. Any contribution to the ROI usually originates from occluded salts, but contributions from other contaminants may add to the ROI value. The test is performed on a 2 gram sample, and the ROI resulting after ignition should be negligible. The ROI method suitable for this analysis is described in the povidone monograph [29].

5.1.5 Nitrogen Content

Povidone-iodine is a complex of poly(vinylpyrrolidone), which itself contains as much as 12.6% nitrogen content. According to the compendial requirements of the United States Pharmacopoeia, the nitrogen content of a povidone-iodine sample should be not more than 11.5%, and not less than 9.5% on an anhydrous basis. The method to be used for the nitrogen content analysis is the same as described in the povidone monograph [29].

5.1.6 Heavy Metal Content

The heavy metal assay detects the trace amounts of included metals which can be precipitated by sulfide ion. Since povidone-iodine is used mostly in various medical applications, and povidone is a metal-ion chelating species, it is essential to determine the metal ion content. The test method suitable for this determination is the same as previously given for povidone [29]. The amount of heavy metal content of povidone-iodine should not be more than 0.002%, determined as lead.

5.2 Povidone-Iodine Topical Aerosol Solution

Povidone-iodine topical aerosol solution is a dispersion of povidone-iodine under nitrogen in a pressurized container. It contains no less than 85 percent and no more than 120 percent of the labeled amount of iodine. The solution is stored in pressurized containers, and should not be exposed to excessive heat [46].

A number of the assay methods conducted on samples of povidone-iodine topical aerosol solution require a common sample pre-treatment step. This procedure provides an analytical sample by isolating the dispersed aerosol solution from its inert carrier. Spray 50 mL of a povidone-iodine topical aerosol solution into a beaker, and allow the contents to remain open to the air so that the propellant will evaporate. After that, transfer the residual liquid into a glass stoppered bottle for further testing.

5.2.1 Identification

5.2.1.1 Blue Color Test

To a mixture consisting of 1 mL of starch TS and 9 mL of distilled water, add 1 mL of a solution that contains about 0.05% iodine. The test is positive if a deep blue color develops.

5.2.1.2 Iodine Vapor Test

Place 10 mL of the solution prepared as described in section 5.2 into a 50-mL flask, avoiding contact with the mouth of the flask. Cover the mouth of the flask with filter paper, and wet this with 1 drop of starch TS. A blue color should not appear after the assembly has stood for 60 seconds.

5.2.2 Total Iodine Content

The total iodine content in a given sample is determined as the assay value of that material. In a 100-mL beaker, place an accurately measured volume of the solution prepared as per section 5.2 and dilute to a volume not less than 30 mL with distilled water. Titrate the solution immediately with 0.02 N sodium thiosulfate VS, determining the endpoint potentiometrically with a platinum-calomel electrode. Each mL of sodium thiosulfate titrant is equivalent to 2.538 milligrams of iodine.

5.2.3 pH

The pH of a povidone-iodine topical aerosol solution is determined on the solution prepared according to the directives of section 5.2, and should not be more than 6.0.

5.3 Povidone-Iodine Ointment

Povidone-iodine ointment is an emulsion, solution, or suspension of povidone-iodine in a suitable, water-soluble base. It contains not less than 85 percent and not more than 120 percent of the stated amount of iodine [46].

5.3.1 Identification

5.3.1.1 Blue Color Test

Dilute the sample with sufficient alcohol to attain a concentration of 0.05% total iodine. Add 1 mL of this dilution to a mixture consisting of 1 mL starch TS and 9 mL of distilled water. A deep blue color should appear in less than one minute.

5.3.1.2 Iodine Vapor Test

Place 10 g of sample into a 50-mL beaker, ensuring that no sample touches the walls of the container. Cover the mouth of the beaker with filter paper, and wet with one drop of starch TS. The typical color of iodine-starch should not appear within one minute.

5.3.2 Total Iodine Content

The assay value of a sample of povidone-iodine ointment is obtained on the basis of the total iodine content. Place an accurately weighed sample containing an equivalent of 50 mg iodine in a 100-mL beaker. Add to this sufficient distilled water to yield not less than 30 mL of solution. Stir the mixture until a clear solution is obtained, and then titrate with 0.02 N sodium thiosulfate VS. Determine the endpoint potentiometrically using a platinum-calomel electrode. Each mL of sodium thiosulfate titrant is equivalent to 2.538 mg of iodine [46].

5.4 Povidone-Iodine Cleansing Solution

Povidone-iodine cleansing solution is a solution a povidone-iodine containing one or more suitable surfactants, which do not react with halogens. The solution should not contain less than 85 percent or more than 120 percent of the stated amount of iodine. It may contain a small amount of alcohol to maintain solubility.

These solutions must be stored at room temperature, in tightly sealed containers [46].

5.4.1 Identification

5.4.1.1 Blue Color Test

To a mixture consisting of 1 mL of starch TS and 9 mL of distilled water, add 1 mL of a solution that contains about 0.05% iodine. The test is positive if a deep blue color develops.

5.4.1.2 Iodine Vapor Test

Place 10 mL of the solution to be tested into a 50-mL flask, avoiding contact with the mouth of the flask. Cover the mouth of the flask with filter paper, and wet this with 1 drop of starch TS. A blue color should not appear after the assembly has stood for 60 seconds.

5.4.2 Total Iodine Content

The total iodine content in a given sample is determined as the assay value of that material. In a 100-mL beaker, place an accurately measured amount of povidone-iodine cleansing solution equivalent to about 50 mg iodine. Adjust the volume of the sample to not less than 30 mL by the addition of distilled water. Add 1 mL of 1 N hydrochloric acid, and place a Teflon-coated stirring bar in the beaker. Initiate the stirring, and titrate the solution with 0.02 N sodium thiosulfate VS, adding 3 mL of starch TS to the solution when the endpoint is near. Each milliliter of 0.02 N sodium thiosulfate is equivalent to 2.538 mg of iodine.

5.4.3 pH

The pH of the solution can be determined by the methods described in the povidone monograph [29], using either a pH-meter or pH indicator test paper. The solution pH should not be higher than 6.0.

6. Antimicrobial Activity of Povidone-Iodine

It has been shown in section 1.1 that povidone-iodine has all the valuable properties of its base polymer, poly(N-vinyl-2-pyrrolidone), combined with those of a polyelectrolyte. Poly(vinylpyrrolidone) is probably the most widely used specialty polymer [47], and the capability of its complex to release iodine in a controlled fashion is the basis for some of its important and varied applications. Iodine attacks the cytoplasm of bacteria, and by its oxidization and iodination properties it renders biologically inactive compounds containing sulfhydryl groups, vitamin C, lipids, enzymes, peptides, cytosine, *etc.* [48].

Povidone-iodine is used in a wide range of applications, most of which are related to various fields of medicine. Some of these uses are prophylactic in nature, such as treatment of the skin and mucous membranes, or disinfection by surgical of hygienic scrubbing. Other applications are therapeutic in nature, consisting of the treatment of burns, diseases of the skin, vaginitis and diseases of the urinary tract, decubitus and varicose ulcers, eye infections, and wound infections in surgery. Additional uses include disinfection in numerous instances, such as surgical processes, dental and oral practice, general gynecology, podiatry, prenatal and postnatal disinfection (*i.e.*, treatment of the umbilical cord), and disinfection of medical instruments and hospital environment.

Encouraging *in vitro* research results have been obtained indicating the effectiveness of povidone-iodine in eliminating the Human Immune Deficiency Virus and even some forms of cancer. Beside human applications, povidone-iodine has found wide use in veterinary medicine, and in some branches of agriculture.

The correlation between property and the advantages warranted by the properties is shown in Table 4.

6.1 Microbiology of Systems Affected by Povidone-Iodine

In the overwhelming majority of its applications, povidone-iodine kills microorganisms, such as bacteria, viruses, yeasts, molds, fungi, and protozoa. Although many of these are non-hostile to humans, and play an indispensable role in maintaining the ecological balance of the

Table 4

Properties of Povidone-Iodine, and Advantages Realized From These [8]

Property	**Advantages**
Broad spectrum biocide	Nonselective germicidal action: Acts as a bactericide, fungicide, viricide, amoebicide, insecticide, nematocide, and sporicide. Useful in human and veterinary medicine. Lacks tendency to develop resistant organisms. Effective in dilute solution, and unparalleled for surface sterilization and in mixed infections.
Detoxified iodine	Low animal and phytotoxicity, and nonirritating to skin and mucous membranes. Generally nonsensitizing, and does not delay healing or formation of granulation tissue. Nonstinging on application, and is a lessened hazard if accidentally ingested.
No detectable vapor pressure	Stable. Can be bandaged without danger of burns, and is retained where applied.
Water-soluble	Easy to formulate in dosage forms having uniform concentrations. Will not permanently stain.
Film-forming	Prolonged germicidal action is possible, since it adheres to treated surfaces where applied. The color delineates treated areas.
Stable complex	Absence of general odor. Free iodine is not extractable by organic solvents, and no loss of iodine occurs by sublimation or volatilization. Rapid action is noted, even in presence of organic matter (such as blood, pus, oil, grease, or soap).

environment, there are significant threats to man from the microorganisms which cause disease.

Bacteriologists describe more than 100 classes of bacteria. Since under microscope they do not show much detail, it is customary to stain them. By this method the bacteria can be classified as Gram-positive or Gram-negative bacteria, and ones identified by acid fast stains. Another way to classify bacteria is through the environment necessary to their growth. Aerobic bacteria grow only in the presence of oxygen, while anaerobic bacteria thrive in a nitrogen atmosphere but cannot grow in the presence of oxygen [49].

Bacteria are present all the time on the skin, in the mouth, or sexual organs, but are completely harmless. It is when they infect other parts of the body that they can cause diseases. Some of them may produce exotoxins, such as the organism which causes tetanus [50]. Others may build endotoxins into their structure, which are released when the cell disintegrates. These highly poisonous toxins can cause a variety of serious and contagious diseases.

6.1.1 Povidone-Iodine as a Bactericide

Three strains of bacteria are of particular interest in that they require control on a regular basis. *Pseudomonas aeruginosa* has a multifaceted way of invasion, and is one of the most infectious opportunistic pathogens [51]. It targets tissues with reduced resistance, invades chronically infected lung tissues, and affects individuals with diminished immune resistance. It produces two exotoxins and two hemolysins, which enhance the virulence of the bacterium [52]. *Staphylococcus aureus* is normally present in the alimentary canal and nasal passages, but can produce enterotoxins which may cause food poisoning and toxic shock syndrome. Disease is caused by this organism through infections, or by spreading the toxin in the whole body [53,54]. *Escherichia coli* can be present in the gastrointestinal tract, and is the most common cause of urinary tract infections, prostatitis, and pyelonephritis. Its virulence is further enhanced by the production of enzymes, toxins, and hemolysins [55,56].

The bactericidal effect of povidone-iodine on these three important bacteria, as well as on other pathogenic Gram-negative and Gram-positive

bacteria, has been thoroughly studied. German found that aqueous solutions of povidone-iodine were more bactericidal to *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* than were Lugol's solution, some quaternary ammonium derivatives in alcohol, hexachlorophene solution, and various mercurial compounds [59]. Scherr and Dodd treated 19 strains of bacteria of the *Staphylococcus*, *Streptococcus*, and *Proteus* type with povidone-iodine, and found the agent to exert a bactericidal effect on all of these [60].

Pseudomonas aeruginosa can be effectively killed by 0.1% Betadine within 15 minutes [61]. The *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella oxytoca* count of the vagina and intrauterine catheter was reduced significantly by the introduction of povidone-iodine during the birth-process [62]. In another study, strains of *Pseudomonas aeruginosa*, *Eschericia coli*, *Klebsiella aerogenes*, and *Serratia marcescens* were treated with serial doses of povidone-iodine in sub-inhibitory concentrations, and no significant changes were observed in the minimum inhibitory concentrations, minimum bactericidal concentrations, and killing times when comparing the activity of the parent strains and those of the 20^{th} subculture [63]. No *in vitro* resistance to povidone-iodine was encountered for species of *Pseudomonas*, *Proteus*, or *Serratia* bacteria, while resistance to chlorhexidine was found after only 5-8 passes [64]. When studying the antibacterial effect of acetic acid, povidone-iodine, and chlorhexidine gluconate, the iodophor was found to be the most effective antibacterial agent among those tested for *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli* [65].).

Ko and Vanderwyk tested povidone-iodine solutions having an iodophor concentration of 1:15,000 available iodine against *Staphylococcus aeureus*, and produced a 99.91 % kill after one minute of contact [66]. Methicillin-resistant (MRSA) *Staphylococcus aureus* was found to be the cause of widespread and difficult-to-treat pathogenic infections. Povidone-iodine solution and cream proved to be effective anti-bacterial agents against both MRSA and methycillin sensitive (MSSA) strains, killing all within 30 seconds. The study also demonstrated that povidone-iodine is more effective in this respect than is chlorhexidine [67]. Yasuda and co-workers designated *Staphylococcus aureus* which showed 7.56 μg/mL as the minimum inhibitory concentration of cloxacillin as MSSA, and 3.13 μg/mL or higher as MRSA. Chlorohexidine gluconate showed

great variation in the activity, but methydiaminoethylglycine hydrochloride and povidone-iodine killed all strains within 20 seconds [68]. In the course of studies on the behavior and activity of MRSA and MSSA, Myoshi and co-workers also found povidone-iodine to be an effective and good disinfectant [69]. In a similar study extended to include ten antibiotics, Sakagami and coworkers found povidone-iodine was effective against MRSA, together with minocycline, doxycyerine, and variomycine [70]. Povidone-iodine was tested against MRSA at a dilution of 1:100, and it killed all bacteria within 15 seconds. Other dilutions were found to be less effective, although each killed all the bacteria in less than 120 seconds. Similar results were obtained with three strains of MSSA. Among the commonly used disinfectants (benzalkonium chloride, chlorhexidine gluconate, and povidone-iodine), the latter was found to yield the most rapid bactericidal effects against both MRSA and MSSA [71].

The *in vitro* and *in vivo* antimicrobial activity of aqueous and alcoholic solutions of povidone-iodine were also compared. *In vitro* alcoholic solutions of povidone-iodine were found to be more active against *staphylococcus* strains, Gram-negative bacteria, and *Candida* strains than were aqueous povidone-iodine solutions, or even alcohol alone. The antimicrobial *in vivo* effect of an alcoholic solution of povidone-iodine was also judged to be superior to an aqueous solution of povidone-iodine or alcohol alone. The presence of serum inhibited the activity of the alcoholic solution less than it did relative to that of the aqueous solution or alcohol [72].

The effects of two nonionic surfactants, Tween 80 (polysorbate) and Emulgin B2 (cetostearyl alcohol), as well as those of two amphoteric detergents, Amphonyl 635 BA (triethanolamine lauryl cycloimidinium-N-I-(ethoxyethionate)-11-ethionate) and Amphosol SP (triethanolamine lauryl aminopropionate), on the antibacterial activities of disinfectants were investigated against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus faecalis*, and *Mycobacterium smegmatis*. Combinations of povidone-iodine and nonionic surfactants showed the greatest reduction of antibacterial activity, while the effect of the amphoteric detergents was less significant [73] Extensive studies were conducted in which 580 Gram-negative bacilli were investigated, and 18.2% of the tested Enterobacteriaceae were found to be resistant to chlorhexidine digluconate, including 92.1% of those belonging to the

Proteus strains. 4% showed resistance towards benzalkonium chloride (with 89.5% of the *Proteus* strains), but povidone-iodine killed all the strains tested [74].

The behavior of 29 bacterial strains, including *Pseudomonas aeruginosa*, *Serratia marcescens*, and *Burkholderia cepacia* was studied against chlorhexidine gluconate, benzalkonium chloride, saponated cresol, and povidone-iodine. As many as 5 strains of *Pseudomonas aeruginosa* were found to be resistant to chlorhexidine gluconate and benzalkonium chloride, 3 strains of *Burkholderia cepacia* were resistant to chlorhexidine gluconate, and 5 of the 8 strains of *Serratia marcescens* tested were resistant to chlorhexidine gluconate and benzalkonium chloride. None of the strains were resistant to saponated cresol or to povidone-iodine. The level of the bacteria tested was at the concentration recommended for disinfection of hands [75].

In order to determine the behavior of povidone-iodine against methicillin sensitive or resistant strains of *Staphylococcus aureus*, 40 clinical isolates were investigated. However, no difference was found in the killing potency of the iodophor, and greater than 99% of the strains were destroyed within 10 seconds regardless of the nature of the bacilli. Since resistance could not be found, it is presumed that the sensitivity to povidone-iodine is dependent upon the degree of local aggregation of the bacteria as well as the penetration degree of the iodophor. It was also found that sulfur containing amino acids are probably the most potent agents to retard the activity of povidone-iodine [76].

The bactericidal effect of povidone-iodine was also studied in 30- and 100-fold diluted preparations, where the complex was found to be bactericidal against all Mycobacterium species [77]. While the antibiotics neomycin (1%), kanamycin (2%), and bacitracin (50 units/mL) were ineffective in human fibroblast cultures, povidone-iodine (and also sodium hypochlorite, acetic acid, and hydrogen peroxide) killed 100% of the cells [78]. Iodophors such as povidone-iodine could be stabilized with acids having pKa values less than 2, while acids characterized by pKa values exceeding 2 caused the loss of iodine. The stabilized preparations were claimed to be suitable for the sterilization of objects with high bioorganic loads, such as urine collection bags, body exudate collectors, *etc.* [79].

Povidone-iodine and tincture of iodine were compared against bacteria commonly encountered in hospital environments. *Streptococcus aureus*, *Pseudomonas aeruginosa*, *Burkholderia cepacia* and *Klebsiella pneumoniae* were killed in 15 seconds by povidone-iodine at various concentrations (1%, 2%, 1:2, and 1:4). Tincture of iodine was also found to be effective against these bacteria. *Streptococcus faecalis*, however, survived in 1:4 and 1: 100 concentrations after 3 seconds of exposure, but was killed when treated for 1 minute. Concentrated povidone-iodine solutions destroyed all bacteria, and none of these survived a 15-second exposure to tincture of iodine [80].

The bacteriostatic and bactericidal performance of povidone-iodine is very susceptible to the presence of organic matter. This was shown in experiments in which the disinfectant was tested against the pure organism *Morganella morganii*, and against the same organism obtained from a wound containing pus. Povidone-iodine was found to be more sensitive than Hibicleans in this study [81]. Studies conducted *in vitro* have shown that some of the organic matter ordinarily present in hospital environments can hinder the bactericidal activity of povidone-iodine. The degree of this effect was inversely proportional to the concentration of the complex, and the pattern was practically identical for all strains tested. A possible explanation is that the contaminants bind the iodine, thus decreasing the amount of the iodine available for bacterial kill [82]. *Escherichia coli*, for instance, releases lipopolysaccharide (LPS) on the addition of disinfectants. While 37% formal and 20% cetaolon inhibit LPS, it persists in the presence of povidone-iodine and chlorhexidine. This demonstrates the necessity for thorough washing of all non-autoclaved equipment exposed to the agents that bring about the release of LPS [83].

Under *in vitro* conditions, povidone-iodine (Betadine) was found to be cytotoxic to human polymorphonuclear cells at concentrations ranging from 0.01% to 1.0% [84]. The use of more dilute *in vitro* concentrations which did not affect the PMN cells were noted not to destroy *Escherichia coli* or *Staphylococcus aureus*. At concentrations of 0.0001% to 0.001%, povidone-iodine did not affect PMN cell phagocytosis, chemotaxis, or superoxide production.

Holoxenic hairless mice were used as experimental models to study the bactericidal performance of antiseptics [85]. The results obtained with povidone-iodine were similar to those found on human skins, the

exception being that the mouse skin flora contains *Staphylococcus xylosus*, *Streptococcus faecalis*, and bacteria of the *Micrococcus* species. The mouse skin is lacking in bacteria of the *Corynebacterium* and *Propionobacterium* species.

Povidone-iodine exhibited strong bactericidal activity against tubercle bacilli BCG and H37Rv [86]. The concentration influenced the degree of bactericidal performance, and at a 1:5000 dilution 6 out of the 7 strains tested were effectively killed by povidone-iodine. This activity against BCG was significantly reduced at temperatures lower than 10°C, and in the presence of proteinaceous substances.

6.1.2 Anti-Viral Activity of Povidone-Iodine

Although povidone-iodine is a wide range microbial disinfectant, it has also been found to effectively destroy a variety of nosocomial viruses. Because of strong public fear about the spreading of Acquired Immune Deficiency Syndrome (AIDS), the currently most widely studied anti-viral property of povidone-iodine is its behavior again the human immuno-deficiency virus (HIV).

Digenis developed a high-energy coprecipitate of nonoxynol-9, povidone, and iodine which acts both as an anti-HIV agent and as a contraceptive when introduced into the vagina [87]. Various preparations of povidone-iodine and chlorohexidine gluconate solution killed HIV virus in cell culture systems. All povidone-iodine products inactivated the virus *in vitro* at concentrations greater than or equal to 0.5%, except for Betadine Lubricating Gel, for which more than 2.5% was necessary for effective elimination of the HIV virus. Betadine douche and medicated douche had to be applied at higher concentrations (0.33% and 0.25%, respectively) to be virucidal against the HIV species [88].

Out of several disinfectants tested as antiseptics for the oral cavity to inactivate human immunodeficiency virus, povidone-iodine, benzathonium chloride, and chlorhexidine digluconate were found to be effective. However, povidone-iodine was the most effective of the three, since it also yielded negative results in the HIV-specific plaque forming assay [89]. Durno and co-workers found that Betadine Surgical Scrub and Betadine solution (both at a concentration of 0.25%) inactivated HIV

within seconds *in vitro*. The study proved that povidone-iodine used in clinically achievable concentrations could serve as a surface disinfectant in hospital settings for which HIV might be present [90].

Extensive studies were conducted on the virucidal action of germicides to the infectious hemotopoietic necrosis virus (IHNV) [91]. Povidone-iodine was found to have effective virucidal activity for the treatment of various forms of herpes. While herpes simplex and herpes zoster have different origins, and differ also in the site and tenacity of their invasion, the respective viruses were both destroyed by povidone-iodine [92-94]. Povidone-iodine was found to be particularly effective in the treatment of herpes genitalis [96]. Daireaux and co-workers reported virucidal activity by povidone-iodine against the poliomyelitis virus (type II) [97]. They showed also that diluted preparations achieved greater reduction in virus titer and faster virucidal action than did the 5% stock solution.

Using Type I (Sabin strain) polio virus as the testing organism, 5% povidone-iodine was found to be rapidly virucidal [98]. In the same study, 2% glutaraldehyde was found to be similarly effective. 0.2% glutaraldehyde and noxythiolin were found to be less effective, while 0.05% chlorhexidine digluconate showed no virucidal activity.

The effect of potential virucides against poliomyelitis virus was investigated by the Pasteur Institute [99]. Agbalika and co-workers studied the virucidal activity of some disinfectants against Mahoney strain poliomyelitis virus type I using a simple dilution method. Povidone-iodine was found to be an active virucide, but was less effective relative to glutaraldehyde. Chlorhexidine and noxyflex showed no virucidal behavior. Similar results were obtained when the virus was separated by centrifugal gel filtration on Sephadex LH20 after contact with disinfectants [100]. The adsorbent retained the disinfectants but did not bind the virus, which could then be quantified by titration. Again, glutaraldehyde was the strongest virucide, followed by Betadine, while chlorhexidine was inactive.

Occurrences of rotaviral gastroenteritis can usually be prevented by suitable disinfection practices, but relatively little is known about the effectiveness of disinfectants in controlling rotavirus. In studies carried out at the University of Ottawa, several commercial disinfectants were investigated [101]. Povidone-iodine was shown to be able to cause more

than three decades reduction (99.9%) in virus plaque titer. This performance was not hindered by the presence of organic matter. Betadine effectively killed salmonid virusus that invaded the bloodcells of fish [95].

6.1.3 Effect of Povidone-Iodine on Other Microbes

Kappensteiner has shown that povidone-iodine can effectively kill a wide range of fungi in a few minutes, and that activity was not inhibited by the presence of organic matter [102].

Iodine complexes were prepared from graft polymers of vinylpyrrolidone on cellulose. The cloth prepared from these copolymers showed fungicidal activity towards *Epidermophyton kaufmanwolff*, *E. rubrum*, and mold-forming fungi [103]. The sensitivity of nine yeast species of the *Candida* genus was tested against various antiseptic agents, and povidone-iodine was found to be effective against all strains at dilutions of 10%, 20%, or 30% [104].

Several disinfectants were tested for fungicide activity against blastospores (*Trychopyton mentagrophytes*, *Asperigillus niger*, and *Candida albicans*), using these at in-use concentrations. Performance was evaluated by the time necessary to achieve a 99.99% kill. An alcoholic solution of chlorhexidine gluconate achieved the desired kill number in 2 minutes, while the 99.99% kill was achieved by Betadine in 10 minutes [105].

Povidone-iodine was found to be a potent fungicide against a number of pathogenic yeasts. It also could neutralize strains of *Candida albicans* that had contaminated animal skins or stainless steel surfaces. The fungicidal activity was lower in the presence of blood serum, although it was still fungicidal at low (3 mg/mL) concentrations even in the presence of organic matter [106].

6.1.4 Apparent Resistance of Some *Pseudomonas*-Type Bacteria Against Povidone-Iodine

In early 1980, several contaminations of povidone-iodine solutions and poloxamer-iodine solutions had been reported [107]. Studies by Bond and co-workers proved that while iodophors are capable of killing bacterial cells, viruses, and even highly resistant spores, under some conditions

members of the *Pseudomonas* genus (particularly *P. cepacia* and *P. aeruginosa*) can survive for prolonged periods. It was reported that they would even grow in iodophors, and subsequently cause infections. There is strong indication that this behavior is due to some type of unidentified interaction that exists between the *Pseudomonas* bacteria and the plastic environment, particularly polyvinyl chloride. The phenomenon may be explained by the ability of the bacteria to form extracellular glycoccalix, a slime-like protective shield which prevents the killing effect of the iodophor. The consequence of this is that close control over the quality of water used in microbiological processes is extremely important, so that the presence of living organisms in the system is to be vigorously excluded [107a].

The matter was further investigated by Anderson and co-workers. Two brands of povidone-iodine (Betadine and Pharmadin) were tested against *Staphylococcus aureus*, *Mycobacterium chelonei*, *Klebsiella pneumoniae*, *Pseudomonas cepacia*, and *Streptococcus mitis*. It was found that all of the microorganisms were promptly killed by both agents. The degree of effectiveness was determined to be directly proportional to the amount of free iodine, and the latter was a function of the degree of dilution. Potassium iodide retarded the bactericide activity.

Pseudomonas aeruginosa was recovered from disinfectant solutions after these had been passed through pipes made of polyvinyl chloride [108]. It was presumed that the origin of the build-up resistance noted in a fouled system was contaminated material on the inside surfaces of the pipes, and the on the threads of pipe fittings.

6.1.5 Other Reactions of Povidone-Iodine in Bacteriology

The fact that free iodine is responsible for the antimicrobial effect of povidone-iodine, and that biological fluid influences this activity, seems to be supported by the results of the experiments of Abdullah and co-workers [109]. When measuring the release of radioactive ^{125}I from povidone iodine, it was found that the amount of iodine released in 24 hours was 9.5% in the presence of saliva, 5.99% in the presence of vaginal smear, and 5.9% from normal saline. The release of iodine was significantly decreased in the presence of blood plasma and blood cells.

The amount of available iodine could also be decreased by irradiation with γ-rays from a cobalt source, and this decrease was accompanied by a diminished degree of antibacterial performance [110]. The effect of irradiation was less pronounced in a nitrogen atmosphere relative to an oxygen-containing atmosphere. The radiation resistance of *Bacillus pumillus* was lower in povidone-iodine solution than in distilled water, but a similar effect could not be found for *Bacillus megaterium*.

Povidone-iodine prepared from polymers whose molecular weights varied between 10,000 and 360,000 Daltons showed antimicrobial activity which was directly proportional to the molecular weight [110a]. This phenomenon maybe attributed to the respective changes in the free and available iodine ratio.

6.2 Disinfection of Hospital Areas and Equipment

Hospital environments can become infested with a variety of nosocomial bacteria and viruses, and eliminating these contaminants is essential to the practice of medical science. According to Spaulding, the inanimate objects of a hospital area can be classified into three groups [111]. Critical items are those which are either introduced inside the body, or are parts of equipment accompanying such work. Instruments used in this fashion must be completely void of any microbial contamination, and must be completely sterile. Semi-critical items are those instruments which may come in contact with various mucous membranes of the body, but which will not be allowed to penetrate body surfaces. When using semi-critical instruments, the complete removal of microorganisms is beneficial, but not required. Finally, non-critical objects would not normally come in contact during use with mucous membranes, and their degree of sterility is less demanding than the other categories with respect of disinfection.

Infections of central venous lines are still encountered in surgery. In order to avoid the removal of the infected catheter, a method was developed by which iodine could be released from the catheter. The antimicrobial performance of the catheter, tested against *Staphylococcus epidermis*, was very good, and the bacterium was completely eliminated around the catheter [112]. The advantage of using povidone-iodine for prolonged

venous catheterization is recommended by several authors to reduce the risk of infection [113,114]. Catheters may become damaged due to chemical exposure used to assure a sterile operative field, but this danger can be eliminated by using povidone-iodine [115].

Povidone-iodine was used with good results as the disinfectant for long-term intravenous hyperalimentation equipment [116]. Prior to hemodialysis treatment, povidone-iodine is to be injected into the blood compartment of the hemodialysis machine, in preparation for the absorbing solution [116a].

The fiber bronchoscope can be disinfected by washing both the inner channel and the outer sheath with hexachlorophene, followed by a second washing with alcoholic povidone-iodine solution [117]. The method was successfully tested on 76 specimens, with all the post-treatment cultures being sterile except for one which contained fewer than 10^2 colonies of *Staphylococcus aureus* per milliliter. The treatment required only five minutes, and completely eliminated the *Pseudomonas aeruginosa*, *Serratia marcescens*, *Klebsia pneumoniae*, *Staphylococcus aureus*, *Candida albicans*, and *Mycobacterium tuberculosis* bacteria, as well as influenza virus and rhinovirus. In another experiment, the instrument was contaminated with five pathogenic microorganisms (*Staphylococcus aureus*, *Staphylococcus pyrogenes*, *Escherichia coli*, *Klebsidia pneumoniae*, and *Pseudomonas aeruginosa*) [118]. The bronchoscope was washed with povidone-iodine, followed by rinses with 70% isopropyl alcohol and saline solution. The treatment effectively reduced the bacterial count on the instrument from 10^9 organisms/mL to 10^4 organisms/mL.

Three outbreaks of *Serratia marcescens* associated with the use of flexible fiber bronchoscopes were reported from two different intensive care units [119]. Attempts to clean the bronchoscope with a combination of 70% alcohol and ethylene oxide gas sterilization proved to be inadequate. Sterilization was shown to be possible using povidone-iodine solution (Betadine) as the disinfectant. Nelson and co-workers, however, presented a dissenting opinion [120]. They isolated a strain of *Mycobacterium tuberculosis* on a surveillance culture taken from a bronchoscope after it had been cleaned and disinfected with an iodophor solution. They found other iodophors which failed to kill *Mycobacterium tuberculosis*, and

documented the transmission of this bacterium from one patient to another by an iodophor disinfected fiber bronchoscope. This group has advised against the use of iodophors to disinfect fiberoptic bronchoscopes.

However, Lindstaedt and co-workers obtained satisfactory results in disinfecting fiberoptic endoscopes [121]. The instruments underwent a short intermediate treatment with povidone-iodine between two investigations, followed by a main disinfection with glutaraldehyde at the end of a series of investigations. These workers emphasized the importance of degerming all channels and accessories.

The time necessary to achieve complete bacterial kill is very important for the disinfection of endoscopes. When povidone-iodine was used without controlling either contact time or concentration, only a disinfection rate of 60% (9 out of 15 instruments) was obtained [122]. However, when the endoscopes were cleaned under pre-set conditions (povidone-iodine concentration of 0.0035%, and a contact time of 4 minutes), all the endoscopes were sterile after the treatment. Wagenwoort and co-workers also reported success in cleaning fiberoptic endoscopes with povidone-iodine [123].

The necessity for careful monitoring and reviewing of sterilization and disinfection processes by hospital infection control communities was evidenced by a care report of *Salmonella gastroenteritis*, which was acquired by 11 patients from contaminated duodenoscopes [124]. *Salmonella typhimurium* organisms were isolated from the gastroscope cytology brush, the colonoscope forceps channel aperture, the lumen of the rubber tube connecting the suction bottle to the endoscope, and the suction collecting bottle. After recognizing the source of infection, the gastroscope was submitted to gas sterilization and soaked in povidone-iodine after each use. Follow-up cultures of the instruments were found to be negative for the presence of intestinal organisms.

When used for the disinfection of whirlpool baths, a minimum concentration of 3 ppm available iodine per each gallon of water (supplied by the addition of povidone-iodine) was sufficient to reduce bacteria to insignificant numbers [125]. Similar results were obtained when the whirlpool tank was washed with povidone-iodine scrub solution.

A fogging germicide preparation has been recommended for the disinfection of hospital areas, such as wards, corridors, waiting rooms, hospital rooms, waiting areas, surgical areas, and other rooms of general use [126]. The active ingredients of the preparation were povidone-iodine and alcohol. Under normal traffic conditions through the areas, the count of airborne bacteria was reduced by as much as 90%.

Povidone-iodine was tried in a 6-day test, with 72 cases, for the disinfection of water in a hydrotherapy machine. Using 30 mL of agent per 20 gallons of water, the bacteria count were decreased from 123 per plate to 57 per plate on the third day of the test [127]. A further decrease to 14 per plate by the end of the cycle was noted.. Aside from some stains on skin and materials, no deleterious effect associated with the use of povidone-iodine was noted. In another study, a whirlpool bath was studied after 50 patients had been treated in it [128]. The water was disinfected by a povidone-iodine solution which contained 4 ppm available iodine, and the walls of the unit were cleaned with surgical scrub containing 0.75% available iodine. The treatment lowered the bacterial count by 90%, and left the protein-bound iodine level and thyroxine level of the patients unaffected.

Hospital equipment in a respiratory intensive care unit was disinfected by washing with 1:100 aqueous solution of povidone-iodine [129]. The germicide was used in an environment infected by selected, highly resistant strains of bacteria. Povidone-iodine gave excellent results against both Gram-positive and Gram-negative species, and was well tolerated by patients and hospital personnel.

Povidone-iodine was declared to be the disinfectant of choice in hospital nurseries for the elimination of pathogenic Gram-negative bacteria in isolettes, infant face masks, suction apparatus, and resuscitation equipment [130]. This disinfectant was impervious to stainless steel or plastic equipment.

6.3 Use of Povidone-Iodine as a Handscrub

One of the most common pathways for the transmission of bacterial pathogenic organisms is associated with hand contact, which normally carries a large number of bacteria (*e.g.*, Staphylococci and enterobacteria)

[131]. The fact that mortal pathogens could be transmitted by the contaminated hands of surgeons and nurses had been recognized over 150 years ago by Lister and then by Semmelweiss. Since then, a variety of bactericides have become indispensable in every branch of medical technology, and one of the most widely recognized of these is povidone-iodine.

One must always keep in mind that not only is the type of disinfectant important, but equally significant is the length of time for treatment. Unlike some disinfectants (such as hexachlorophene) which have no immediate effect but act slowly after the hand been dried, povidone-iodine exerts its sterilizing effect at the time of application. The method by which the disinfectant is applied is also of major importance. The standard procedure for hand washing involves rubbing palm to palm, palm over dorsum, and ending with fingers interlaced is measurably more effective than unregulated ways of rubbing [132]. Similarly, rinsing with a standard number of strokes to cover all surfaces of the hand gives better results then does free-style rinsing.

Water removable, non-staining povidone-iodine is one of the most effective skin sterilizing agents. It rapidly kills fungi, as well as vegetative bacteria and trichomonas. The complex has been shown to kill fungi spores in one hour, and bacteria spores in seventeen hours [133]. In combination with detergents, it was found to be a suitable mixture for degerming patients having *Staphylococcus aureus* infections [134]. Ayliffe found that povidone-iodine hand-scrub showed an immediate reduction in bacterial count of 70% to 80% after application, eventually increasing to 99% [135]. The degerming efficiency of 10% povidone-iodine and 70% isopropanol was compared by applying the disinfectants with cotton swabs on the outer side of the upper arms of 31 patients for over 24 hours. With the exception of one test person, no significant differences were found between the two disinfectants [136].

Aqueous povidone-iodine solutions were found to be very effective bactericidal agents when applied on human or animal skin, and on other living tissues [137]. Bogash reported observations about povidone-iodine when used as topical antiseptic on as many as 5900 patients during a time span of three years, finding that bacterial and mycotic infections responded rapidly [138]. During all this time only two idiosyncrasies

developed which, however, subsequently subsided. No systemic toxicity or iodism was experienced in the course of the observation period.

Davies and co-workers compared three detergent antiseptics (povidone-iodine, chlorhexidine, and hexachlorophene) against soap for daily bath use [139]. In these studies, both the subjects and the bath water were tested for bacterial content. When soap was used as the disinfectant, an increase in total bacterial count was observed. Use of the antiseptics yielded either a decrease or a smaller increase in bacterial count. Although chlorhexidine gave the most consistent reduction, the difference and the bacterial counts produced by the different antiseptics were not statistically different.

231 clinical isolates were tested for susceptibility to povidone-iodine [140]. Of 95 *Staphylococci* isolates tested with a 15 second contact time, only 18 (19%) were killed by exposure to the commercial 10% iodophor concentration, while 77 *staphylococcus* isolates (81%) showed an apparent resistance under the test conditions. In the same study, 39 other Gram-positive isolates and 96 Gram-negative isolates were tested (including 20 containing *Pseudomonas aeruginosa* and 4 containing *Pseudomonas cepacia*), only one strain of *Escherichia coli* remained unkilled. However, 60% of the strains were killed when the contact time was extended to 30 seconds, and none of the isolates survived when the contact time was 120 seconds. The killing potential was found to be concentration dependent, with the maximum killing efficiency being exhibited at a 0.1% povidone-iodine concentration. A new povidone-iodine preparation (BP-Betadine) was 100% effective for all the isolates tested, and needed only a 15 second contact time.

6.4 Use of Povidone-Iodine in Surgery and Its Effect on Wound Healing

Ever since Lord Lister discovered over 100 years ago that fatality following surgery could be dramatically reduced by killing bacteria with the help of carbolic acid, concentrated efforts have been made to achieve optimum hygienic surgical conditions, antiseptic surgical methods and prophylaxis, and developing effective antimicrobial agents. The discovery and utilization of povidone-iodine was a significant step in achieving these

goals. Preparations of povidone-iodine are available as aqueous and ethanolic solutions, as well as in the forms of ointment and surgical scrub.

For the promotion of wound healing, povidone-iodine is used in two phases of the surgical process. The first phase ensures that the site of operation is aseptic. For this phase, the skin flora around the place of incision or puncture is rendered practically germ-free by painting the area with povidone-iodine solution. The second phase entails treatment of the wound with the iodophor. Because of the antibacterial action of povidone iodine, the bacterial contamination of the wound is decreased and regeneration of the infected tissues is accelerated.

In a randomized study, 737 patients were prepared in the pre-op by either a 0.5% chlorhexidine gluconate spray or by a povidone-iodine scrub [141]. The wound infection rate in the two comparable groups was not statistically different, although the results of the spray technique were slightly favorable. As part of a systematic effort to eliminate the potentially nosocomial bacteria from the skin of nine bone marrow transplant patients, they were washed down daily with povidone-iodine soap and water containing 0.5% chlorhexidine [142].

After evaluating the results of 565 operations on the abdominal aorta and lower extremity vasculature, Kaiser and co-workers recommended a brief preoperative course of cefazoline followed by povidone-iodine skin antisepsis for vascular reconstructive surgery [143]. The skin of patients can be effectively sterilized by povidone-iodine under hyperbaric conditions [144]. Such manipulation may become necessary when the need arises to prepare for anesthetic or surgical procedure while the system is under pressure.

During disinfection of the surgical site, chemical burns sometimes occur due to a pooling of the disinfectant [145]. This happens mostly in gynecological operations, or during orthopedic surgery. In former case the irritation or sores appear usually on the buttocks, and in the latter sores are found on the extremities or under a tourniquet. Such occurrences can be avoided, or at least minimized, by judicious selection of the disinfectant to prevent its pooling and seeping under the tourniquet. Povidone-iodine was found to be the most effective and least irritating to the skin.

When undergoing a surgical operation, or suffering a wound through accident, the outcome depends greatly upon the nature of the wound and upon the concentration of pathogenic microorganisms around and in it. Görtz and Häring classified wounds into three categories [146]. The first group comprises all wounds which heal without complication after primary surgical attention, which do not usually need the application of topical disinfectants. To the second group belong wounds having a high risk of infection, such as bites, lacerations, abrasions, punctures, cauterizations, burns, or scalds. Though not originally infected, they are usually subjected to topical treatment so as to prevent infection. The third group consists of wounds which are either already infected or need special surgical therapy, such as wounds, abscesses, gangrenes, or infected cuts. These require topical antiseptic treatment, as well as surgery, to eliminate the cause of infection.

Povidone-iodine has been shown to be an effective, fast acting, and safe wound healing disinfectant. [147,148] which can be used on mucous membranes without danger of burns. The agent is self-indicative, with renewal of the application being indicated by a lessening of the characteristic brown color. Topically applied, povidone-iodine is not only antiseptic, but appears to augment wound healing [149].

In one study, 500 patients undergoing various general surgical procedures were divided randomly into two groups, where the incisions of one group were irrigated with povidone-iodine, and those of the other group were treated with saline solution [150]. The povidone-iodine group exhibited a significantly lower incidence of infections (7 of 242, or 2.9%) than did the saline control group (39 of 258, or 15.1%). In all age groups, the use of povidone-iodine spray reduced the incidence of wound infection in general surgical practice through its bactericidal action [151].

In 451 consecutive appendectomy patients, the infection rate was reduced by using (before and after wound closure) either a dry powder povidone-iodine spray (Disadine), or a combination antibiotic spray consisting of neomycin-bacitracin-polymyxin (Dispray) [152]. The reduction of infection was statistically significant for povidone-iodine, but significant enhancement was not attained when using the antibiotic spray.

Dilute povidone-iodine solution was used in a continuous system for the treatment of mediastinal infection following cardiac surgery [153]. The

treatment eradicated the infection in 14 patients evenly divided between adults and children. Scully and co-workers used povidone-iodine irrigation to treat patients who developed deep sternal infections with mediastinitis [154].

In gastrointestinal surgery, wound sepsis has to be minimized through the avoidance of contamination at the incision. This can be achieved by the use of systemic prophylactic agents, and with topical antimicrobial treatment with disinfectants [155]. Before 1981, the mortality of acute necrotizing pancreatitis was about 70%, but the use of intra- and postoperative rinsing helped reduce the rate that to 50% [156]. When the peritoneal cavity was left open for easier manipulation, only one patient out of seven died. In a wide variety of abdominal procedures, povidone-iodine was found to reduce wound infection in treated patients as compared with untreated randomized controls [151]. The infection rate was 9% (6 out of 70) in the povidone-iodine group, and 24% (18 out of 74) in the control group.

When applied in intestinal resection and peritonitis, interparietal instillation of povidone-iodine substantially reduced wound infection compared to untreated controls [157]. An 88 year old woman with an aortic graft infection occurring after abdominal aortic aneurismectomy was treated successfully with continuous irrigation of the graft bed with povidone-iodine solution [158]. A 6 year old boy developed purulent mediastinitis at the site of the graft after repair of the truncus arteriosus, but the graft and surrounding area became sterilized after a 6 week irrigation with full strength Betadine at the rate of 200 mL/hour combined with intravenous addition of gentamicin [159].

The technique of painting and dressing with povidone-iodine solution, ointment, or dry powder is used today in a variety of disinfecting processes, since it offers a safe, fast and effective method for surgical sterilization in laparoscopy performed with abdominal operations [160]. Teruel and co-workers reported that by the instillation of povidone-iodine into the lymphatic cavity, a safe and simple method was found for lymphocele treatment in renal transplantation [161].

Povidone-iodine has also been proven to be a useful and effective degerming irrigation agent in vascular surgery [162], and has been used successfully in the field of neurosurgery. *In vitro* studies conducted by

Tucker and co-workers showed povidone-iodine to be efficacious in preventing and eradicating infection in cerebrospinal fluid shunts [163]. After flushing the system with the iodophor, the valves could not be colonized. To eradicate infection from a previously colonized valve, three iodophor injections were made at 24 hour intervals.

To evaluate povidone-iodine for its use in effectively degerming the epidermal space, local iodophor lavages were carried out on 20 rabbits upon which lumbar laminectomy had been performed [164]. After one month, the meningeal covering of the operated spinal cords showed no signs of fibrosis or arachanoid adhesions when studied with the naked eye or with the scanning electron microscope.

Sixteen pediatric bone marrow transplant patients were treated by brushing all mucous membrane surfaces within the oropharyngeal cavity with povidone-iodine four times a day. None of the patients developed candida esophagitis or sepsis [165]. This method is an easy and effective way to prevent oropharyngeal candidiasis in pediatric bone marrow transplant patients.

Povidone-iodine is used in three phases of total hip surgery to prevent acute fulminating infection (hematoma) and low grade creeping infection, as well as the need for reimplantation after such infection [166].

6.5 Use of Povidone-Iodine in the Treatment of Burns

When part of the body is burnt, the loss of skin removes the protective barrier against an attack by nosocomic bacteria. Early in the injury the bacterial count is relatively small, but quickly a strong proliferation of bacteria takes place. Initially these are mostly of the Gram positive type, but later Gram-negative bacteria also appear in and around the burn wound. Since these open wounds extend to a large area, it is practically impossible to maintain sterile conditions. Treatment with povidone-iodine helps to maintain a low bacterial count, thus preventing the bacterial count from reaching a life-threatening concentration.

When used in the treatment of burns, povidone-iodine effectively controls bacterial growth and protects the developing epithelium. Unlike antibiotic agents, it has the added advantage in that it does not produce any

resistance in the bacterial flora [167]. In the case of facial trauma resulting from second degree burns, povidone-iodine aerosol spray facilitates sterile crust formation [168,169]. Daily numerous sprayings of the affected areas assures normal healing by keeping the bacterial count at the lowest level. The use of povidone-iodine spray was proven to be the most suitable treatment of injuries of soft tissues, and of the management of infected donor areas.

Betadine ointment was found to be effective in preventing infections over a 2-year period, and was shown to be beneficial in 146 burn cases [170]. Robson and co-workers reported that when tested in rats, 10% povidone-iodine ointment was an effective prophylactic when applied every six hours [171]. While the effectiveness of prophylaxis is generally only limited where the infection spread over the eschars, Zellner and Metzger found that povidone-iodine provided good bactericidal and tanning action on the burn eschar [172]. The necrotic skin remained dry and firm, and epithelization was not interrupted by infection, with the exception of two second degree burn cases. The affected surface area of the patient's skin were found to be sterile or showed low bacterial count.

These findings are supported by the report of Stefanides and co-workers, who demonstrated using the agar diffusion technique, that povidone-iodine could penetrate eschar and would remain effective against Gram-positive bacteria (such as *Staphylococcus aureus*, *Streptococcus nitis*, and *Candida albicans*), and Gram-negative bacteria (including *Pseudomonas aeruginosa*) [173]. There was no significant difference in the healing process with open or closed burn treatment. However, by increasing the number of coatings over the area with povidone-iodine ointment, improved bacterium control was achieved [174]. When comparing povidone-iodine with silver sulfadiazine and gentamycin during the first two week period of a burn treatment, the iodophor was found to be the best performing bactericide [175]. With the iodine complex, the bacterial count never reached the danger threshold (10^5 microorganisms/cm^2) for generalized infections.

Topical treatment of 160 burn patients was described by Höhlfeld and Butenandt [176]. In the treatment of 80 of these patients, povidone-iodine (Betaisodine) replaced Mercurochrome in Grob's three-stage tanning. Betaisodona and Mercurochrome produced similarly firm and fast drying

scabs, the complete separation of which took 16 days with Betaisodone and 14 days with Mercurochrome. With respect to wound infection, both disinfectants performed equally well. However, the serious danger of mercury poisoning was eliminated by the use of the iodophor complex. A remarkable improvement in the rate of burn healing was achieved by adding Aserbine (a combination of malic, benzoic, and salicylic acids) to povidone-iodine ointment [177]. Beside the shorter healing time, a lower bacterial count was obtained as a consequence of the formulation modification, and the findings were judged to be valid for both superficial and deep burn wounds. Interestingly, addition of the carboxylic acids to povidone-iodine cream produced much less improvement.

A patient who has received second degree burns over 3% of his body surface was treated with a sterile gauze dressing which was impregnated with a novel ointment [178]. This consisted of 16 parts of white beeswax, 80 parts of paraffin oil, 3 parts of camphor, and 1 part of micronized povidone-iodine. Though the wound contained *Staphylococcus aureus* contamination, it did not require antibiotic treatment, and complete healing was achieved in 7 days.

Burn injuries, may also be caused by contact with corrosive chemicals. Sàgi and co-workers described skin damages suffered from bromine exposure and some of its compounds [179]. Administration of a povidone-iodine antibacterial agent was recommended to reduce the extent of the injury and assure safe healing conditions, after the exposed area had first been washed with large volumes of water.

By the uncontrolled ingestion of the photoactive drug methoxysporalen (Meladinine), a 20-year old man suffered a large chemical burn injury over 70% of his body surface [180]. In the intensive care unit, he received thorough topical treatment with povidone-iodine in addition to the standard of care. The administration of the iodophor served to prevent infection, and the treatment was decisive for his recovery.

6.6 Povidone-Iodine in the Treatment of Ulcers

Povidone-iodine was found to be eminently suitable for the treatment of external ulcers. These areas of destroyed tissue may be caused by disruption of the blood supply of the skin, or by infection or other injury

of the skin or mucous membranes. Such injuries are termed stasis ulcers, and most often appear on the legs and feet. Vascular ulcers are caused by disturbances in the blood circulation, with the most common of these being the arteriosclerotic ulcer. This is a consequence of the narrowing of the arteries of the legs, which causes a diminished blood supply and an eventual necrotization of skin tissues. Another stasis ulcer is diabetic neuropathy, which appears in the form of deep ulcer wounds, usually on the sole of the foot. Certain types of anemia may cause chronic leg ulcers. Decubitus ulcers (or bedsores) develop on the buttocks, hips, heels, and other pressure points on the bodies of patients who are bedridden for long periods of time. The treatment of such ulcers varies according to the stage of advancement, but invariably the maintenance of low bacterial count is of the utmost importance. In this respect, povidone-iodine can be of great utility.

Twenty-four patients who could not be successfully treated with antibiotics for chronic leg ulcers, received treatment with povidone-iodine ointment and compression bandages [181]. Of this group, 15 exhibited measurable improvement, 2 were completely healed, 3 remained unchanged, and 4 regressed. With the exception of one hypersensitive subject, none of the patients experienced any skin irritation from povidone-iodine. In another study, 30 patients suffering from leg ulcers were treated with povidone-iodine ointment [182]. Within 4 weeks to 6 months, 18 patients within this group achieved complete recovery. Seven patients with chronic history were classed as "no response", while 4 patients showed deterioration. The authors felt that povidone-iodine not only produced an aseptic environment, but actually helped the healing process by stimulating tissue proliferation.

Anania and co-workers reported the healing of diabetic skin ulceration by a treatment which used the combination of povidone-iodine and sugar [183]. Imperial Chemical Industries, Ltd. (ICI) was issued a patent for an aerosol composition useful for the treatment of varicose ulcers [184]. The composition consisted of 1-5% povidone-iodine, 20-65% high boiling propellant, and 35-79% of a low boiling ingredient. The composition could be prepared by milling the povidone-iodine with the liquids in an inert atmosphere.

The infected stasis and decubitus ulcers of 20 elderly patients were treated three times daily with povidone-iodine solutions and ointments [185]. In

spite of the feeble physical condition of the patients, two ulcers healed completely and 18 showed obvious improvement. During the course of this six-week study, all the symptoms (induration, erythema, odor, pain, and itch) diminished, as did the average diameters and depths of the ulcers. The number of infected lesions decreased from 20 to 7, while the number of lesion-yielding microbial growths fell to 5 from 20. At the end of the six week therapy, all patients showed good to excellent symptomatic relief, which can be attributed mostly to the beneficial effect of povidone-iodine. One hundred and ten patients (aged 10-60 years) were submitted to a 10-12 week topical povidone-iodine treatment in order to heal their decubitus ulcers [186]. All patients were hospitalized with spinal cord injuries, and treatment with Betadine was successful.

Povidone-iodine (both in the solution and ointment forms) was evaluated on 18 male outpatients (age range 33-68 years) for controlling infection brought about by decubitus and stasis ulcers [187,188]. During the 42-day test period, the dressings were changed twice daily. The signs and symptoms of ulcer (edema, pain, erythema, ulcer size, and ulcer depth) showed statistically significant improvement, and all patients experienced relief within two weeks after starting therapy. At the end of the study, 67% of the ulcers were clinically healed and 33% showed improvement. None of the patients experienced side effect or sensitization reactions. The compatibility of topical disinfectants with the new anti-ulcer agent for skin, Bucladesine ointment (DT-5621), was investigated for kneading uniformity, change of properties, color change, and mixture potency [189]. The mixtures were sampled and tested after 0 hour, 24 hours, and 14 days after blending. Isodine (povidone-iodine gel) was completely miscible with Bucladesine, and showed no loss of potency in the admixture.

6.7 The Use of Povidone-Iodine in the Treatment of Peritonitis

Although povidone-iodine is universally recognized as a very efficient bactericide, its use with patients suffering peritonitis is a matter of some dispute. Before the discovery of antibiotics, peritonitis was a deadly disease, but even today is still a serious sickness with an unacceptably high rate of mortality. Usually it is a multi-bacterial infection of the abdominal cavity membranes, caused by events which empty bacteria into the abdominal cavity (such as a perforated ulcer or burst appendix, perforated stomach, or damaged colon). The majority of involved bacteria

are *Escherichia coli*, although the microorganisms of the *Bacteroides*, *Enterococci*, *Proteus*, and *Klebsiella* types, as well as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, are often present.

In experimentally induced monomicrobial peritonitis, intraperitoneally added Betadine solution was found to be as effective as intraperitoneal cephalotin sodium or kanamycin sulfate, and also produced increased survival over the untreated controls [190]. The intraperitoneal injection of Betadine was safe to rats at doses less than 2.5 mg/kg available iodine with respect to both acute and chronic toxicity. In studies of experimental peritonitis in mice and rats, Gilmore found that the therapeutically effective dose of povidone-iodine was 6.0 to 7.5 mg available iodine per kg body weight, which is about one tenth of the LD_{50} value [191]. Intraperitoneally introduced povidone-iodine at this dose significantly reduced the mortality of the experimental animals with *Escherichia coli* peritonitis. Similar results were obtained in an extended study, where 220 Theiller Original mice and one hundred Wistar rats were injected intraperitoneally [192]. The injection of povidone-iodine significantly reduced mortality in treated animals as compared with matched controls given the same volume of Ringer's solution.

In a randomized investigation, 168 consecutive patients undergoing laboratory studies for peritonitis received irrigation either with povidone-iodine solution or with saline solution (the control group) [193]. In the povidone-iodine treatment group, only 1 of 80 patients (1.3%) had an abscess, while in the control group 9 of 88 patients (10.2%) had abscesses. Although the serum iodine levels were elevated after the povidone-iodine irrigation, these returned to normal after 72 hours. After induction of peritoneal adhesions in 250 female Wistar rats, the experimental animals were allocated to one of five treatment groups: a) control with no instillate; b) control with Ringer's solution; c) noxythiolin 0.5% solution; d) noxythioline 1.0% solution; and e) povidone-iodine solution [194]. A blinded assessment of the adhesion was made 1 week after closure, and it was found that the povidone-iodine solution significantly reduced the number of adhesions relative to the other four groups.

In a study carried out to determine (besides other questions of bacterial contamination at operation) the prophylactic effect of interparietal povidone-iodine, it was concluded that the iodophor was of significant

value in cases of intestinal resection and peritonitis, particularly with obese patients and in those with paramedian incisions [195]. The authors of this work suggested that povidone-iodine could provide a preferable alternative in related infections.

Unfortunately, the communications of other researchers were sufficient to dampen the enthusiasm about the usefulness of povidone-iodine for the control of peritonitis. While it was reported that noxythiolin (Noxyflex) injected intraperitoneally as a 2-5% solution protected mice from the lethal effect of an intraperitoneal injection of *Escherichia coli* bacteria [196], in a parallel study in which povidone-iodine was used as the antibacterial agent, no protection against the microorganism was observed [196].

Studies were carried out by Lagarde and coworkers in which the efficacy of intraperitoneal povidone-iodine was investigated in established peritonitis. It was found that while dosages exceeding 4.0 mg/kg were fatal to healthy animals, a non-toxic 2.0 ml/kg dose was fatal in animals with peritonitis [197]. In the studies carried out on animals having previously developed peritonitis, the mortality rate was 100%. The authors suggest that the intraperitoneal administration of povidone-iodine can be fatal when the animals are compromised with peritonitis. The favorable results of some laboratory studies were attributed to the animals being treated before peritonitis was allowed to develop. The good results of some clinical studies achieved with povidone-iodine lavage were suggested to be due to mechanical continuous irrigation by the dilute solution. On the basis of their studies, the authors advised against the intraperitoneal administration of povidone-iodine for the treatment of peritonitis.

In dogs with peritonitis caused by appendicitis, intraperitoneal administered povidone-iodine caused death more rapidly than did the induction of a saline solution [198]. This was not associated with differences in peritoneal microflora, but with peritoneal absorption of excessive amounts of iodine. Povidone-iodine diminished mortality when injected immediately, but not when given 1 to 3 hours later. Experiments were carried out with mice challenged with intraperitoneally injected lethal doses of *Escherichia coli*. Povidone-iodine lowered the mortality rate when given immediately, but was ineffective when injected later. Immediate injection lowered the *Escherichia coli* count by 3 orders of

magnitude, but the number of *Escherichia coli* bacteria was lowered only by 1/3 log unit when the povidone-iodine was introduced 3 hours later.

An attempt to control the peritonitis induced by the infusion of *Escherichia coli* through the peritoneal injection of povidone-iodine resulted in 100% mortality [199]. Injection of noxytiolin and taurolin, however, gave protection against the lethal effect of peritonitis. Autopsies of the experimental mice showed staining of the bowels and peritoneum, as well as signs of inflammation and necrosis. The use of povidone-iodine in treatment of the peritoneal cavity was not recommended. Intraperitoneal addition of a 1% solution of either low or high molecular weight (6,000 and 30,000 Daltons, respectively) povidone-iodine was made in rats [200]. Doses of 200 and 300 mg per kg of either preparation in experimental animals often caused mesenteric fatty tissue necrosis with subsequent fibroplastic peritonitis. These effects were presumed to be due to cytotoxicity of the iodine, since iodine-free povidone failed to show similar behavior. These authors concluded that they could not recommend the intraperitoneal use of povidone-iodine.

6.8 Use of Povidone-Iodine in Gynecology and Urology

Povidone-iodine has been found to be extremely useful in various gynecological applications as both a topical and therapeutic agent. For treatment of birth-canal infections, and for various forms of vaginitis, the iodophor is probably the most widely used anti-microbial agent.

Various forms of povidone-iodine were tested in a study conducted using 87 patients, who were suffering from monilial and trichomonal vaginitis or a combination of both [201]. The treatment regimen consisted of swabbing with povidone-iodine solution, a povidone-iodine gel application for the night, and a povidone-iodine douche in the morning. The monilial vaginitis symptoms were cleared, and negative laboratory results were obtained in 1-3 weeks for all the test cases. In cases with combined infections, 13 out of 14 symptoms cleared in 3 weeks and the pathogens disappeared in 4 weeks. Forty-one women were treated for up to 14 days with Betadine pessaries night and morning, as a treatment for vaginitis [202]. When followed up after 3 weeks, 39 out of 41 (95%) patients were clinically cured. Reexamination of 18 patients after 3 months revealed no relapses.

Abdominal hysterectomy patients were treated in 4 groups [203]. The first group received a povidone-iodine gel insertion into the vagina 48 hours before operation, followed by two povidone-iodine douches immediately before operation. The second group received douching on two separate occasions during the 24 hours before operation. The third group received treatment similar to Group 2, but with a chlorhexidine solution. The fourth group was the control, and received no vaginal preparation. The lowest morbidity rate was associated with the first group, which also showed also the greatest decrease in colony counts from the upper vagina before and after operation, as well as the least increase in the number of colonies on the postoperative swab.

In a controlled clinical trial, irrigation of the bladder with povidone-iodine solution prevented urinary infection after prostatectomy, as well as in catheterized non-surgical patients [204]. The use of povidone-iodine in bladder irrigation with for the prevention of urinary tract infection after intermittent catheterization was studied [205]. In the control group the specimen was taken after removal of the urethral catheter, while in the test group 50 mL of 2% povidone-iodine solution was instilled and drained immediately before the removal of the catheter. The incidence of bacteria was 28% in the control group, but only 4% in the povidone-iodine treated test group.

A randomized group of 64 patients with a history of recurrent urinary tract infection was treated with 3 long term (1 year) prophylactic regimens [206]. Twenty patients were given 100 mg trimetoprim at night, 25 subjects took 1000 mg methenamine hyppurate every 12 hours, and 19 patients cleaned the perineum twice daily with povidone-iodine solution. All treatments were equally effective in reducing the symptomatic attacks relative to the 12 months prior to beginning the treatment.

The effectiveness of Betadine gel was tested as a catheter lubricant for the prevention of microbial contamination of bladder urine [207]. Before catheterization, mean colony counts were 0.3 in 5 mL urine for the povidone-iodine treated group, and 0.4 in 5 mL urine for the control group. After catheterization, the respective bacterial counts for these groups were 3.6 and 69.3 per 5 mL urine. Clearly, catheters coated with Betadine gel carried much less bacteria than those coated with the control

"K-Y" jelly. In addition, lubricity, adhesiveness, and cohesiveness were found to be more satisfactory in the test group than in the control samples.

The investigations of Chang and co-workers, however, revealed some problems when using povidone-iodine solution for controlling bacterial infections by intravesical instillation [208]. Experiments carried out on female rats found the urothelium to be damaged by the instillation (as evidenced by the increased weight of the bladder due to edema), as well as by crystal adhesion and bacterial adhesion upon the bladder mucosa after the injury. It was further found that the urothelium recovered after 6 or 7 days after the povidone-iodine injury, and that at least partial protection from bacterial and crystal adhesion could be achieved by immediate treatment of the bladder with heparin.

Ten cases of vulvo-vaginal and cervical herpes virus infections were treated externally and vaginally with povidone-iodine [209]. The healing time and duration of the symptoms was shortened in 9 patients, and the effect of the virucide on the cervical lesions was remarkable. One hundred patients with *Trichomonas*, *Candida*, and other non-specific infections were divided into two groups and treated with vaginal pessaries [210]. The pessaries of one group contained povidone-iodine, while the pessaries of the other group were made with lactic acid. Healing in group the iodophor-treated group was 94% (with a 3% relapse rate), while in the other group the healing rate was only 34% (with a 72% relapse rate). Similar results were reported by Henderson and Tait, who investigated the use of Betadine-pessaries in the treatment of 135 women with candidal and trichomonal vaginitis [211]. Sucessful treatment of post-gonorrheal urethoitis with Betaisodona solution was reported by Zinnage [212].

In order to determine the effect of povidone-iodine prophylaxis on the subpartal change of microflora in the birth canal, 58 women in labor were assigned randomly to povidone-iodine prophylaxis or to a control group [213]. The evaluation was performed on cervical smears at the beginning and after birth, as well as on bacteriological specimens from the intrauterine catheter and the oral cavity of the neonatus. The most frequently found bacteria (*Escherichia coli* in the post partum cervical smears, and *Pseudomonas aeruginosa* and *Klebsiella oxytoca* on the catheter) were all reduced in the povidone-iodine group. The reduction could be seen also on the oral cavity smear of the neonatus, which showed

a bacterial flora almost identical with that of the post-partum cervical smear.

A study was conducted to find a barrier contraceptive agent capable of preventing the sexual transmission of *Ureaplasma urealyticum* from the female genital tract, and to help to reduce nongonococcal urethritis caused by such infection [214]. The study included the use of six intravaginal contraceptives, and povidone-iodine against eight microbial serotypes. Povidone-iodine exhibited a significant ureaplasmacidal effect for dilutions up to 1:64 which could not be attributed to a pH change caused by the bactericide. Thus povidone-iodine may be useful in controlling ureaplasma urealyticum.

6.9 Use of Povidone Iodine in Colorectal Healing

A frequent ailment of the colon is ulceration, which can be either dysenteric (caused by the amoebae *hystolitica* or by bacilli of the *Shiga* type), or non-dysenteric (caused by a variety of microbes). The colon stasis (stagnation of the large bowel) may result in auto-intoxication with severe consequences. In all these cases, the application of suitable antimicrobial agent is of primary importance.

Arango and co-workers reported the successful use of povidone-iodine for the intraoperative segmental preparation of the large bowel [215]. The bowel content and segments of the colon were treated with povidone-iodine, and with saline solution, respectively. 45 of the 50 segments treated with povidone-iodine showed no bacterial growth, while the segments treated with saline maintained 3.5×10^8 colony forming units per milliliter. The segmental preparation of the colon with povidone-iodine may be useful in reducing the endogenous bacteria at the time of the transaction of the colon.

Forty-five patients were randomly assigned to two study groups undergoing major resection for large bowel carcinoma [216]. Among the patients who received 10% povidone-iodine preoperative irrigation, only one (4.6%) developed an abdominal wound infection. All patients had mechanical bowel preparation, as well as pre- and postoperative antibiotic treatment. Among the patients of the control group who were irrigated

only with water, nine (39.1 %) did develop an infection. Swabs from selected patients taken during the course of the operation showed no bacterial growth.

In 20-minute *in vitro* exposure studies to dog feces suspensions, povidone-iodine eliminated aerobic growth and significantly reduced anaerobes (with the exception of *C. perfringens*) [217]. The results of *in vivo* experiments where povidone-iodine was intraluminally injected into 20 dogs immediately before resection, gave similarly satisfactory results. All the test dogs survived 3 weeks without anastomotic leaks. The 20 minute exposure decreased the bacterial count sufficiently in the unprepared sigmoid colon, and no adverse effects were found. Rotstein and co-workers studied this so called "instant" colon preparation in rats [218]. It was found that luminal bacteria were significantly decreased, but that intestinal irrigation with 10% povidone-iodine did not effectively sterilize the cecum. It was presumed that some of the bacteria present in the area of the colonic mucosal surface were protected from the effect of the microbiocide.

To prevent and control bacterial infections during transrectal biopsy of the prostate, the rectum was treated topically with a povidone-iodine saturated gauge preparation [219]. Four patients with partial obstruction of the colon were prepared for tumor resection using povidone-iodine solution for emptying and sterilizing the lumen of the bowel [220]. The biocide was introduced through a colonic tube inserted through the rectum, and the combined method/ agent effect permitted the performance of anastomosis without prior colostomy. Povidone-iodine was reported to be among the intraluminal cytotoxic agents used routinely most often by the surgeons of South West England, following partial resection of the large bowel for cancer [221].

6.10 Use of Povidone-Iodine in Ophthalmology

The eye is one of the most sensitive parts of the human body, and its constituent parts are liable to microbial attack. Such attacks may result in very serious consequences, which range from vision impairment to total blindness. Keratitis is probably the most common disease of the eye, and can be the consequence of primary or secondary microbial infection. Some of the equally serious types of iritis may also be caused by

microbes. Catarrhal conjunctivitis is usually caused by microorganisms, such as the common Morax-Axenfeld bacillus or the equally frequent Koch-Weeks bacillus. This condition can turn to its purulent form in which the vision may be severely damaged before the inflammation would subside. Diphteric conjunctivitis is propagated through the infection with *Bacillus diphteriae*, while trachoma is caused by a virus.

In the last decades, the healing of impaired or diseased eyes through surgery made great advancement and has become practically routine. This requires the use of effective, but gentle, prophylactic agents, and povidone-iodine has the necessary characteristics to be the antiseptic of choice in ophthalmologic surgery.

In a corneal ulcer study, rabbit eyes infected with *Pseudomonas aeruginosa* showed complete sterility after a 2 minute povidone-iodine irrigation [222].. Eyes in the control group which had been irrigated with antibiotics recorded distinct bacterial growth. The treatment was also used in four patients with corneal ulcers. Topical application of 1% povidone-iodine significantly reduced experimental *Aspergillus niger* infections in conjunctival fornices of rabbits without any irritation to the eye [223].

The elimination of (or at least reduction in) bacteria on the conjunctiva before intraocular surgery was hindered due to the lack of suitable antimicrobial agent. Now, with povidone-iodine there is one such agent which is well tolerated by both the cornea and the conjunctiva. This was shown in a successful study on the treatment of bacterial and viral kerato conjunctivitis, in which 41 eyes were treated with povidone-iodine prior to cataract extraction [224]. In another study, 105 cataract surgery patients were treated with povidone-iodine and cultures were obtained before and after operation [225]. The control cultures showed growth of *Staphylococcus albus*, *Staphylococcus aureus*, and some diphteroid *Corynebacteroid* species. In the test group, the *Staphylococcus albus* content was reduced to one third, and the other species were completely eliminated.

York and co-workers studied the effect of frequent dosing on ocular toxicity in rabbit eyes [226]. It was found that 0.5% povidone-iodine was practically non-irritating when instilled six times a day, and that concentrations of 0.33% were sufficient to heal corneal epithelial abrasions. In experimental bacterial conjunctivitis studies in rabbits,

povidone-iodine was compared to the antiseptics Noviform and Biseptol, as well as to the antibiotic Polyspectran [227]. The iodophor was ranked first in the overall ranking, and particularly good in the elimination of *Staphylococcus aureus*. In another investigation, half-strength povidone-iodine solution was used for preparation of the eyes of 30 patients before surgery [228]. The topical treatment reduced the number of colonies in the treated eyes by 91%, while the number of species decreased by 50%. No change was observed in the eyes that served as controls.

When applied topically as preoperative preparations for ocular surgery, Neosporin antibiotic solution and 5% povidone-iodine solution caused similar and significant decrease in the numbers of both colonies and species [229]. When the two agents were used in combination, the results were even better, and 83% of the conjunctivae became sterile. Povidone-iodine has been successfully used against four strains of *Neisseria gonorrhea*, a clinical isolate of *Chlamidia trachomatis*, and a strain of herpes simplex virus, all of which affect the eye through contamination [230].

6.11 Dental and Oral Use of Povidone-Iodine

As early as 1961, Zimmer and co-workers determined that povidone-iodine was a very effective bactericide which destroyed the organisms commonly found in the mouth within 15 seconds [231]. They reported that when dilute povidone-iodine was used to prepare the oral mucosa prior to dental surgery, the danger of bacterial infection was almost totally eliminated. In their study of 99 patients who received as many as 115 injections, in only 5 cases did they find bacteria on the needles used.

The effectiveness of povidone-iodine gargle as a preventive agent for bacteremia when applied before oral surgery was demonstrated by Brenman and Randall [232]. They found that both gingival surface bacteria and post-gingivectomy blood cultures showed reduced counts after irrigation and rinse with the iodophor. In similar studies, it was found that the incidence of bacteremia was reduced to 28%, as compared to the 56% for a group treated with a placebo [233]. Povidone-iodine (along with other disinfectants) effectively inhibited the germination and postgermination growth of *Bacillus subtilis* [234].

Rosling and co-workers studied the effect of hydrogen peroxide formulated with either sodium chloride or sodium bicarbonate (combined with subgingival irrigation with povidone-iodine) in the treatment of periodontal disease [235]. The study showed that subgingival debridement, together with mechanical plaque control, decreased the number of microorganisms (including spirochetes and motile rods) and stopped the breakdown of periodontal tissues. Topical use of the antimicrobial agent reduced the subgingival microflora, and sped healing. This included gain in attachment level and increase in radiographic alveolar bone mass. Another study proved the effectiveness of a combined treatment consisting of subgingival debridement (topical povidone-iodine treatment and a systemic tetracycline therapy) in suppressing *Acetinobacillus actinomycetemcomitans* which had been implicated in the Aetiology of juvenile periodontitis [236].

In a detailed *in vitro* study, Kamoi and co-workers investigated the bactericidal effect of povidone-iodine against six oral pathogenic bacteria (*Bacteroides gingivitis*, *Bacillus melaninogenicus*, *Fusobacterium nucleatum*, *A. actinomycetemcomitans*, *Capnocytophaga sp.*, and *Eicenella corrodens*), and two control species (*Streptococcus intermedius*, *Pseudomonas aeruginosa*) [237]. The best killing efficiency was recorded at a dilution of 400x, and at a contact time of 15 seconds. These authors recommended the use of 0.25% povidone-iodine solution for the treatment of oral mucosa, and for periodontal pocket irrigation. Povidone-iodine has also been used to disinfect dental impressions made from silicon rubber and alginate [238]. The bacteriological and physical testing of samples immersed for 30 minutes in the disinfectant solution showed that effective antimicrobial activity had taken place, and that the treated object exhibited perfect dimensional stability and unimpaired surface hardness [238].

Studies carried out on the *in vitro* reaction between the stimulated parotid salivae of 13 individuals (aged from 7 to 80 years) and povidone-iodine showed that age had no effect on the binding of iodine [239]. On the other hand, the solution pH exerted a strong influence, with maximum binding being noted between 7.0-7.5. Practically no iodine binding occurred below pH 4.0, and Cu(II) and Zn(II) ions also inhibited the binding of iodine. These authors interpreted the results by the existence of an unidentified iodine binding factor which reacted with the disinfectant (when applied orally at low pH) and prevented the loss of iodine.

While povidone-iodine had generally worked very well in various oral and dental application studies, some researchers found shortcomings in the performance of this disinfectant. For instance, Tanzer and co-workers found that when tested against a number of strains of plaque producing bacteria, inorganic iodine was considerably more effective as an anti-plaque agent than was the organic iodophor [240]. Addy and co-workers compared povidone-iodine to chlorhexidine gluconate as antiseptic mouthwashes [241]. When rinsing with povidone-iodine, the salivary bacterium level exhibited an immediate mean fall in total count, but returned to the previous bacterium level in one hour. Chlorhexidine gluconate, on the other hand, not only produced a lower level of bacterial count, but helped to maintain this up to a 7 hour post-rinsing period [241]. On the other hand, Fine found no significant difference in the performance of these two antiseptic mouthwashes, except that povidone-iodine caused less staining of the teeth than did chlorhexidine gluconate [242]. Tao and co-workers have published a review article in which they listed 19 references related to the antibacterial activity of povidone-iodine solutions against oral bacteria [243].

6.12 Use of Povidone-Iodine in Dermatology

Povidone-iodine antiseptic preparations have found a wide variety of applications in the treatment of various diseases of the skin. This iodophor is easy to handle and gentle to the skin, but kills infectious bacteria very efficiently. Povidone-iodine has been used successfully in several thousand cases as a topical antiseptic to heal bacterial and mycotic infections.

In one study, two groups of patients suffering from acne were treated with povidone-iodine [244]. In the group consisting of mild acne patients, 9 out of 10 were either improved or much improved. The other group consisted of more severe cases, but the penetration of the antiseptic was not sufficient to produce lasting improvement. Brown used povidone-iodine aerosol foam twice daily against acne vulgaris, and reported that 19 out of 36 patients (52.8%) benefited from the treatment [245].

Povidone-iodine has been found to be effective in treating the irritation occurring as a consequence of maceration or pressure sores during gynecological or orthopedic procedures [246]. In a group of 50 patients

being treated for eczematoid ringworm with povidone-iodine with, 20 were clinically cured and 17 showed substantial improvement [247]. In addition, itching and burning were greatly ameliorated. A shampoo consisting of combined detergent and povidone-iodine improved the seborrheic dermatitis condition of 102 of 114 (89%) patients undergoing to the treatment, while the detergent alone was found to be only 30% active [248]. The iodophor also showed effectiveness in a formula for healing severe pyodermas of the skin.

Povidone-iodine ointment was used in combination with mycostatin and neosporin as an antiseptic on the skin flora underlying a subclavian catheter dressing during intravenous hyperalimentation [249]. The iodophor prevented overgrowth of skin bacteria, and far outperformed the saline solution used as a control. Povidone-iodine was compared to a silicon cream in a clinical study for protection of the skin of patients encased in plaster of paris casts [250]. The iodophor group showed a 95% benefit of the aerosol treatment, while the dimethicone cream group showed only a 10% beneficial result (the untreated control group exhibited a 3% improvement).

6.13 Use of Povidone-Iodine in Cancer Therapy

It has already been mentioned that the properties of povidone-iodine are very similar with respect to the unsubstituted, nonionic povidone from which it is prepared. Povidone was one of the well studied polymers with potential use in tumor therapy, so it follows that povidone-iodine would be investigated for similar applications.

Bodkin reported the results of studies in which the injection of povidone (either by itself or in combination with iron or bismuth salts) permitted the visualization of carcinomatous tissues of the rectum in x-ray films [251,252]. Later, Young and co-workers modified Bodkin's method by using bismuth sodium iodide with povidone, taking advantage of the heavy-atom effect exerted by iodine for enhancement of radiographic images [253]. The presence of poly(vinylpyrrolidone) in the complex was beneficial in eliminating the toxic effects of the bismuth compounds.

Povidone complexes of appropriate organoplatinum compounds were considered as potential time release, broad spectrum, antitumor agents, in

which the polymer contributed water solubility and reduction in the unpleasant side effects associated with the Cisplatin part of the complex [254]. Povidone has served as a macromolecular probe in the use of 5-fluorouracil therapy for metastatic colon carcinoma [255]. The polymer provided a reproducible measure of the integrity of the mucosal barrier after the 5-fluorouracil therapy, thus preventing the risk of developing infection and fever.

Hodnett developed a copolymer exhibiting antitumor properties, which consisted of 2-methylene1,3-propanediyl-bis(methyl carbamate) and vinylpyrrolidone in a 3:7 ratio (w/w) [256]. The polymer was reported to be of low toxicity, and exhibited a great range of effective doses.

Several cationic polyelectrolytes have shown *in vitro* and *in vivo* antitumor activity. In a study of Ehrlich carcinoma, the survival of mice was increased even when the cationic polymer was introduced 5 days after the tumors were transplanted in the experimental animals [257]. Since povidone-iodine is a cationic polyelectrolyte, some type of antitumor activity for povidone-iodine would be anticipated. Polymers do not only attack tumors directly, but depending upon their functionalities can ward off contamination by harmful microorganisms [258].

A neutral, buffered, povidone-iodine solution containing 250 mg iodine was found to be an effective anticancer agent when used for the treatment of wounds seeded with Walker 256 rat carcinoma [259]. Tumor incidence was reduced to 25% in povidone-iodine treated animals, as compared to 85.5% for the saline treated control animals. When assayed for cytotoxicity against tumor cells which were freshly prepared from human colorectal carcinomas, povidone-iodine (and also chlorhexidine-cetrimide) were found to exhibit a fast killing action at a wide range of concentrations (5-100%) [260]. Both compounds have been identified as the agents of choice to destroy malignant cells that exfoliate into the colorectal lumen.

6.14 Use of Povidone-Iodine in Treatment of the Umbilical Cord

After the umbilical cord of newborns is cut, there is always a possibility of umbilical infection. Almost as soon as it was discovered, povidone-iodine was found to be eminently suitable to prevent such problems. Sarracino and co-workers reported successful results in 1800 hospital cases where

the shortened umbilical stumps were painted with povidone-iodine every 12 hours [261]. As a consequence of this treatment, the stumps looked clean and healed rapidly. Problems related to infection were considerably reduced, and the use of povidone-iodine was found to be very beneficial.

A fast separation of the umbilical stump was achieved through the combination of mechanical and antiseptic means [262]. The umbilical stump was painted daily with povidone-iodine, and a tiny, close fitting, rubber ring was used for closure of the cut umbilical cord. The incidence of umbilical infection was reduced by the daily iodophor treatment, no complications due to the Betadine treatment occurred, and no skin reactions were reported.

6.15 Application of Povidone-Iodine in Minor Injury Cases

Since povidone-iodine is mild but powerful broad spectrum antiseptic, it is eminently suitable for treatment of small accidents. These cuts, bruises, and lacerations usually demand immediate attention in order to avoid serious infections, so summer camps, factories, tourist centers, hospital emergency rooms, and medical emergency facilities are prime examples where such application takes place.

At a children's summer camp, Betadine was used with great success in treating skin traumas with or without accompanying infection [263]. Infections were prevented or quickly eliminated, and prompt healing was achieved in 41 of the 43 cases (95%). Wringer injuries are surprisingly frequent among children. Franz and Root reviewed 92 cases, and reported that the antiseptic of choice for surgical preparation was povidone-iodine (Betadine) [264]. Two thousand out-patients with topical infections were treated with povidone-iodine (Betadine) solution, scrub, and ointment [265]. In 94% of the cases, good to very good results were obtained, and even the remaining 65 cases showed fair recovery. No complaint was received about any side effects, and the germicide preparations were well tolerated.

Three hundred accident patients were randomized into two groups, one of which was topically treated with povidone-iodine before suturing, and the other not treated so as to serve as the control [266]. The rate of wound infection was found to be only 6% in the povidone-iodine treated group,

which may be compared to 14% in the control group. Roberts and co-workers carried out similar studies for the particular instance of hand lacerations [267]. The results obtained showed a significant healing effect associated with povidone-iodine treatment and observed no adverse reactions attributable to the antiseptic. These authors recommended that all hand lacerations should be treated with povidone-iodine.

6.16 Miscellaneous Medical Applications

In the field of otolaryngology, the chronic secretory otitis media is frequent, and the treatment is surgical drainage of the tympanum [268]. Otorrhea is a very common and unpleasant complication, but preparation of the ear canal with povidone-iodine combined with postoperative use of an antimicrobial corticosteroid preparation provides optimal control of infection.

The phenomenon of capsular contracture was investigated in a double-blind study on 124 augmentation mammaplasty patients [269]. The use of 5% povidone-iodine (50% Betadine) in and around inflatable retromamary prosthetic implants reduced the earlier postoperative findings by 85%, and the number of incidents by more than 50%. These results seem to prove that the cause of capsular conjecture was bacterial contamination.

Disinfection of the colon and the rectal area can be best achieved by the insertion of a suitable suppository. Desjardins obtained a patent for the preparation of a polyethylene glycol and sorbital base product, the active ingredient of which is povidone-iodine [270].

Treatment with povidone-iodine is used to make the human bones suitable for transplantation [271]. The bone is first disinfected by 5% povidone-iodine, then after subsequent cleaning with Triton X200 and hydrogen peroxide, it is incubated again in a 1% solution of the iodophor.

Plank and Grande were granted a patent for the preparation of fibers suitable for surgical goods [272]. Their invention is to extrude a pre-polymer solution containing a pharmaceutical additive (such as povidone-iodine) into a suitable precipitant.

A sterilized antimicrobial ointment of povidone-iodine was obtained by room temperature x-ray irradiation of a solution consisting of water, povidone-iodine, a nonionic low sudsing surfactant, and a nonionic gelling agent [273].

An interesting concept is the intrauterine device having a tail thread which releases povidone-iodine in a controlled fashion [274]. The fiber inhibits the growth of a wide range of bacteria for the control of venereal diseases, while the device performs as a contraceptive device.

It has been reported that of various antibacterial shampoos, the greatest reduction in the bacterial count was achieved by shampoos containing povidone-iodine [275]. Povidone-iodine serves as a bacteriostat in an antibacterial soap, in which it is mixed with a compatible soap base [278]. The antibacterial soap is useful for wound cleansing, preoperative cleaning of surgical sites, hand scrubbing, and other antiseptic manipulations.

The use of iodophor-impregnated plastic adhesive drapes was proven to reduce wound contamination in implant surgery [276]. Bacterial sampling at the end of hip replacement surgery showed decreases of wound contamination from 15% to 1.6%.

After washing and sterile rinse of the feet of 25 patients, povidone-iodine solution was applied only to the left foot [277]. Both feet were then bandaged, and after 72 hours the bacterial flora of the treated feet increased by 288% on the dorsal surface and by 239% on the planar surface. This may be compared with the 600% average regrowth increase of the right control feet.

Jungerman and Scott were issued a patent for an antibacterial soap which contains povidone-iodine as an active ingredient [278].

Shanbrom received a patent for the infusing into patients a of platelet-containing liquid that had been in contact with povidone iodine (or a povidone-hydrogen peroxide complex) for a time sufficient to inactivate or destroy pathogenic microorganisms [279].

6.17 Use of Povidone-Iodine in Veterinary Medicine

Owing to its favorable spectrum of antiseptic properties, povidone-iodine has found extensive use in various areas of veterinary medicine. In general, animals respond favorably to treatment with povidone-iodine, and the observations described in the previous sections are usually valid with respect to analogous applications in veterinary practice. Out of the numerous possible uses, a few choice examples will serve to illustrate the broad areas where povidone-iodine is the antiseptic of choice.

The infection in the reproductive tract of mares by *Pseudomonas* bacteria was treated successfully with povidone-iodine [280]. After the treatment, foaling was accomplished without complications. Infected abdominal incisions of 7 cattle and 3 horses were resutured with monofilamentous stainless steel retention sutures, and any superficial wound infection was successfully treated with povidone-iodine irrigation [281].

Witkin obtained a patent for a two-component antiseptic composition consisting of 0.5 - 2.0% povidone-iodine and 0.5 - 5% hydrogen peroxide in water, and used the solution for the disinfection of cow udders and for the treatment of pet ear infections [282]. Cows with breeding problems were given intrauterine povidone-iodine disinfection in their luteal phase [283]. About 70% of the cows exhibited estrus 6-11 days later, and 50% of the animals which were inseminated were able to conceive.

Studies were carried out to identify the causes for loss of 17-β-estradiol from silicon rubber implants in the ears of cattle [284]. It was found that washing the ears of the animals with povidone-iodine solution before implantation, and then treating the sites of the implant with antibiotic after this, was sufficient to reduce the loss to an acceptable level. In abdominal surgery performed on cattle (including Cesarean sections), povidone-iodine was found to be a suitable disinfectant to prevent post-operative complications [285].

The relative effectiveness of povidone-iodine and silver sulfadiazine in fighting *Staphylococcus aureus* infections in the wounds of dogs who had undergone polytetrafluoroethylene carotid artery bypass graft surgery was studied [286]. The group of dogs treated with the silver compound

showed a marginally better rate of survival, but povidone-iodine was superior with respect to destroying the bacteria.

One hundred and forty-two cows were randomly divided 0 to 8 days postpartum into a treatment group and a control group [287]. The treatment group received 100 mL of 2% povidone-iodine infused into the uterus, and showed diminished amounts of pus in the vagina, a diminished rate of bacteria growth, and a smaller number of bacteria cultures. In addition, the interval from weaning to the first estrus was shortened by 25%. These findings indicate that the infusion of povidone-iodine in early postparturient cows stimulates the involution of the uterus and eliminates delays in postweaning estrus.

Seventeen rabbits infected with Trichophyton mentagrophytes were treated with a single topical application of povidone-iodine about 3 months after their lesions first appeared [288]. New hair growth was observed within 3-4 days, and re-growth was complete in about 3 weeks. After that no further lesions appeared, and all the animals remained healthy.

The lethal effect of *Chlamidia trachomatis* in fertile chicken eggs could be stopped rapidly in vitro by treating these with povidone-iodine at 37°C [289]. The germicide, however, did not provide protection when the intracellular replication of the microorganism already has begun. Since high concentrations of the iodophor did not damage the chick embryos, it showed great potentials as an anti-chlamidial antiseptic.

Povidone-iodine has been very useful in sustaining the ecology of places where fish breed by protecting the fish eggs. Through its broad-spectrum microbiocidal activity, it is beneficial also by killing fungal, viral and bacterial pathogens which can be responsible for extensive depletion of fish ponds and rivers.

It was found that povidone-iodine (25 ppm) significantly reduced the colony count for 9 species of bacteria and 2 fungi in 15 seconds. Three strains of Pseudomonas counts gave only I to 7 cells per milliliter, but Aeromonas salmonicida and Saprotegnia parasitica remained unaffected [290].

In vitro studies showed that povidone-iodine could destroy the viruses of hematopoietic necrosis, infectious pancreatic necrosis and hemorrhagic septicemia viruses. The virucide effect was not affected by water hardness, but was reduced above pH 8 and in the presence of organic materials [291]. It was also found, however, that povidone-iodine was safe on fish eggs at pH 6 or higher when used after water hardening [292].

An extensive study by Sako and co-workers with Aeromonas salmonicida, Vibrio anquillarum, and Vibrio ordalii found that 10-30 ppm povidone-iodine was necessary to reduce the viable cell counts at 20°C from 106 cells/mL to less then 10 cells per mL. The use of sea water as solvent, however, reduced the bactericide activity of the iodopho. [293).

Povidone-iodine did not show any significant toxic effect on rainbow trout eggs, and the rate of hatching was not different from the untreated controls [294]. Povidone-iodine registered as a fish egg disinfectant, as most of the requirements in this respect have already been met.

6.18 Miscellaneous Disinfectant Applications of Povidone-Iodine

Crustaceans and fish can be treated with povidone-iodine without the disadvantages of taste and odor of chlorine. The treatment can be used on living animals as well, and its advantage over antibiotics is that it will not produce resistant bacteria [295].

Brine used for meat curing was sterilized by treatment with povidone-iodine [296]. Plastic packaging films and containers are coated or laminated with povidone-iodine to achieve improved bacteriostatic properties [297].

Povidone-iodine is the active material of the antiseptic polish used to disinfect doorknobs, toilet seats, *etc.* The nonwax liquid polish contains about 10% povidone-iodine, and remains antiseptic as a thin film over 30 days [298].

The iodophor complex is used in deodorants for humans, animals, and the environment [299]. It provides germicidal properties to certain semisolid cleaning compositions, which contain (beside povidone-iodine) C14-C26 sulfated alcohols or sulfosuccinates, and about equal amounts of C14-C26

saturated fatty alcohols or C14-C20 fatty acids [300]. Toilet bars suitable for cleaning and disinfecting human skin and inanimate objects were made by combining povidone-iodine, a polyethylene glycol extender, a fatty alcohol, and a detergent. The detergent bars were non-irritating, and for rougher cleaning jobs could be mixed with powdered pumice [301].

Povidone-iodine has already found some promising applications in agriculture. At a concentration of 1000 ppm, the iodophor killed nematodes and meal worm larvae after two to four hours contact time, an effect equal to that of chloropicrin. It was found to be effective also against soil symphilids, red spider mites, and aphids [302]. Povidone-iodine was proven to be effective against Sphaerotheca pannosa on rose plants [303].

Betadine antiseptic solution was selected by the National Aeronautics and Space Administration to decontaminate Apollo spacecraft after splashdown [304].

6.19 Water-Insoluble Poly(Vinylpyrrolidone)-Iodine Complex

Water insoluble poly(vinylpyrrolidone)-iodine complexes may be synthesized by contacting poly(vinylpyrrolidone) with molecular iodine [305]. Recently, the complex was made by mixing poly(vinylpyrrolidone), powdered iodine, and a small amount of isopropanol, and then heating the mixture at 50°C for about 3 hours, and then at 85°C for about 15 hours [306].

Insoluble complexes of this nature are used to sterilize fluids coming in contact with mucous membranes or other living tissues, as well as water (or gases) used in human or Veterinary medicine. Such materials are also used also in certain cosmetic products, such as soaps, pomades, toothpastes, and chewing gums.

7. Toxicology

In contrast to other iodine-based antiseptics, povidone-iodine is of very low toxicity. It does not cause cutaneous reactions in humans or animals, and its effect on mucous membranes is mild and of a passing nature. Still, povidone-iodine, regardless of the route it is applied, can cause systemic absorption of iodine [307].

7.1 Studies on the Skin

Studies on the effects of iodophors on intact skin were carried out on 200 patients, exposing them to povidone-iodine solution and tincture of iodine (each containing 2% available iodine) [5]. It was found that while the tincture of iodine soaked patches had to be removed after 24 hours owing to the presence of severe cutaneous reactions, the povidone-iodine patches produced no reactions even after 96 hours. Tests on human and rabbit skins abraded with course sandpaper gave results similar to those of the intact skin tests [5,8]. Moreover, the wounds did not become infected, but were in the process of healing when the povidone-iodine patches were removed. Bogash administered as many as 5000 topical applications on intact skin and mucosa, and noted only 2 allergic reactions [308]. There were, however, reports about erythema induration and vesicular eruption in special cases, such as patients with diminished immunity or underlying malignancy [309,310].

Iodine may be absorbed also through burn wounds for which povidone-iodine has been used [311]. The iodine enters the bloodstream attached to serum albumin, and is then eliminated by the kidneys. Depending upon the concentration of the antiseptic solution, the area of the burn, and the frequency of the application, the level of the serum protein-bound iodine and the concentration of the iodine in the urine could increase to several times higher than the normal level. Nevertheless, since the excretion of the iodine is fast, the normal iodine level is usually reached within one week after discontinuation of the treatment [312].

Continuous exposure to povidone-iodine could lead to a decrease in iodine binding, and the spontaneous synthesis of free triiodothyroxine and thyroxine, which is the so called Wolff-Chaikoff-block [313]. This

situation is also transitory by nature, and more likely to occur in individuals with goiter [314].

Lautier performed extended studies on guinea pigs, and showed that the cutaneous irritation caused by povidone-iodine was within acceptable levels [315]. He also found that chronic application caused a transcutaneous absorption, which led to the saturation of the thyroid gland. This in turn resulted in transient hypersecretion and increased levels of triiodothyroxine and thyroxine. That, however, triggered the activity of the pituitary gland, which lowered the production of TSH by one-half, which in turn achieved the recurrence of euthyroidism after 90 days of treatment.

7.2 Effect of Povidone-Iodine on the Eyes

Experiments performed with rabbits showed great differences in side effects between Lugol's solution and the povidone-iodine complex [8]. Instillation of 0.5 mL (1% available iodine) of the former caused severe erythema, edema, and progressive corneal damage. Similar eye drops containing the povidone-iodine complex caused only slight conjunctival reddening that disappeared within 2-3 days. Eye drops of Lugol's solution (0.1% potency) also caused such strong reactions that the tests had to be discontinued after 3 days. On the other hand, povidone-iodine drops could be administered daily for the entire 15 day test period. The only effects associated with administration of the complex was only a transitory erythema which disappeared within a few hours.

In order to determine the suitability of povidone-iodine for treating corneal ulcers and conjunctivitis, the effect of frequent dosing on ocular toxicity was determined using rabbits [226]. Concentrations of 0.5% or lower were found to be practically non-irritating, even when applied six times a day, and the epithelial healing was delayed only by one day by this treatment. Because of its broad antibacterial spectrum and low toxicity, povidone-iodine was determined to be a useful drug for treatment of the eyes.

7.3 Effect of Povidone-Iodine on the Mucous Membranes

The mucous membranes in the throats of 25 patients were painted with povidone-iodine solution, including the tonsilar fossae, palate, uvula, and posterior pharingeal wall [8]. About 60% of the patients showed only a minor reaction (slight reddening of the mucous membranes) which disappeared in about 2 hours, and 40% showed no reaction at all. The test was repeated fifteen times daily with approximately the same results.

Allergy tests, which yielded very severe sensitization with Lugol's solution, failed to show any reaction with povidone-iodine [8]. In a three-year study on 5900 patients, only two allergy cases were observed. The manifested dermatitis healed in 7 days, and no systemic toxicity or iodism was found.

7.4 Acute Toxicity of Povidone-Iodine

Povidone-iodine has been found to exhibit significantly lower oral toxicity then do most organic iodine compounds [44]. The intraperitoneal toxicity, as might be expected, is higher than the oral effect.

LD_{50} rat (oral):	5990 mg/kg
LD_{50} mouse (i.p.):	360 mg/kg

7.5 Chronic Oral Toxicity of Povidone-Iodine

Two experimental dogs received daily doses of 1.84 g povidone-iodine in enteric coated tablets over a 5-month period [8]. The daily dose corresponded to 370 mg total or 280 mg available iodine. At the end of the study, the sacrificed test animals showed no gross pathology, and their histology was normal.

7.6 Cell Toxicity of Povidone-Iodine

One hour incubation of rat hearts in povidone-iodine (containing 0.5 to 5.0% available iodine) produced a cell toxicity that was evidenced by

lower growth potential of the subsequently planted cells [316]. The healing of skin wounds, on the other hand, was not affected by painting with these solutions as long as the level of available iodine was less than 2%. Concentrations of the latter as high as 5% slowed the healing of the wound without having a permanent effect.

7.7 Mutagenicity and Teratogenicity of Povidone-Iodine

The potential mutagenicity of povidone-iodine was thoroughly investigated by Peh and Zeller using the intraperitoneally administered dominant lethal test, the micronucleus test, the bone marrow test, and animal experiments [317]. In all these tests, even when using extremely high doses of povidone-iodine, no mutagenic effect could be detected.

Various concentrations (16, 35, and 75 mg/kg/day) of a 15% aqueous povidone-iodine solution were injected intramuscularly to pregnant rabbits for 12 days during the 6-18 days of gestation [318]. The only differences found between the test animals and the controls were some lower weight increases, and lower average weights of fetus and placenta. No toxic or teratogenic effect of povidone-iodine were noticed.

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PRAZIQUANTEL

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ANALYTICAL PROFILES OF DRUG SUBSTANCES AND EXCIPIENTS—VOLUME 25
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CONTENTS

1. Introduction

Praziquantel is a broad spectrum anthelmintic drug which is considered the most successful agent for the treatment of shistososomiasis and cysticercus cellulosa. This compound has proven to be very effective, and is excellently tolerated during long periods of treatment. The drug is normally administered in its racemic form, however, recent theoretical studies have established evidence that the *R*-(-)-*syn*-enantiomer is responsible for interaction with the parasite and therefore the pharmacological effect [1-4]. A single dose of 20-40 mg/kg for trematodes,and 5-10 mg/kg for cestodes,is normally sufficient for the treatment of human infections [5,6]. Praziquantel is mainly used to treat shistososomiasis, a parasitic disease infecting more than 275 million people worldwide [7].

2. Description

2.1 Nomenclature

2.1.1 Generic Name

Praziquantel

2.1.2 Chemical Name

2-(cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4H-pyrazino(2,I-a) isoquinolin-4-one [8]

2.1.3 Proprietary Names

Biltricide, Cesol.

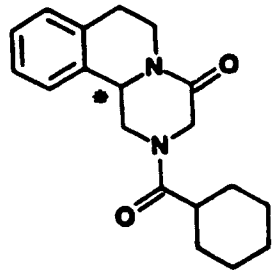
2.1.4 Synonyms [9]

EMBAY-8440, EMD-29,810

2.1.5 CAS Registry Number

55268-74-1

2.2 Structure, Molecular Formula, and Molecular Weight [8,9]



Empirical Formula: $C_{19}H_{24}N_2O_2$

Molecular Weight: 312.41

2.3 Appearance, Color, and Taste

Praziquantel is a colorless, almost odorless, crystalline compound having a bitter taste. It is stable under normal conditions [10].

2.4 Molecular Conformation

The anthelmintic activity of praziquantel is mainly associated with the *R*-(-)-enantiomer. Figure 1 shows its probable conformation, as computed by force field minimization [1].

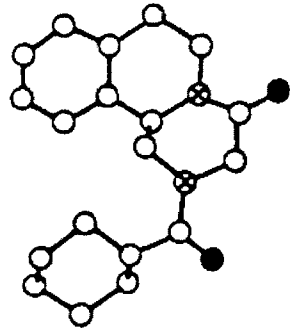


Figure 1. Conformation of *R*-(-)-praziquantel, where O = carbon, ⊗ = nitrogen, and ● = oxygen.

3. Synthesis

A short review on the synthesis of praziquantel was reported in 1978 [14]. Most approaches of practical importance to pyrazino(2,1-a)isoquinolin-4-ones start from the readily available isoquinoline, **1** (Scheme 1). The preparation of intermediate **3** *via* the Reissert reaction and subsequent high-pressure hydrogenation has been reported [15]. Conversion to the final product, **5**, can be accomplished by acylation with chloroacetyl chloride, **4**,followed by base catalyzed cyclization [10,16-19].

As illustrated in Scheme 2, the racemic product, **5**, can be resolved into its optically active components *R*-(-) **(5a)** and S-(+) **(5b)** [17,20,21]. A reaction sequence to recycle the unwanted enantiomer, **5b**, and its conversion into its mirror image, **5a**, is also shown in Scheme 2. This transformation can be accomplished by the dehydrogenation of **5b** to afford **6**, followed by its catalytic hydrogenation to **5** [22].

4. Physical Properties

4.1 Elemental Composition [8,9]

C	73.05%
H	7.74%
N	8.97%
O	10.24%

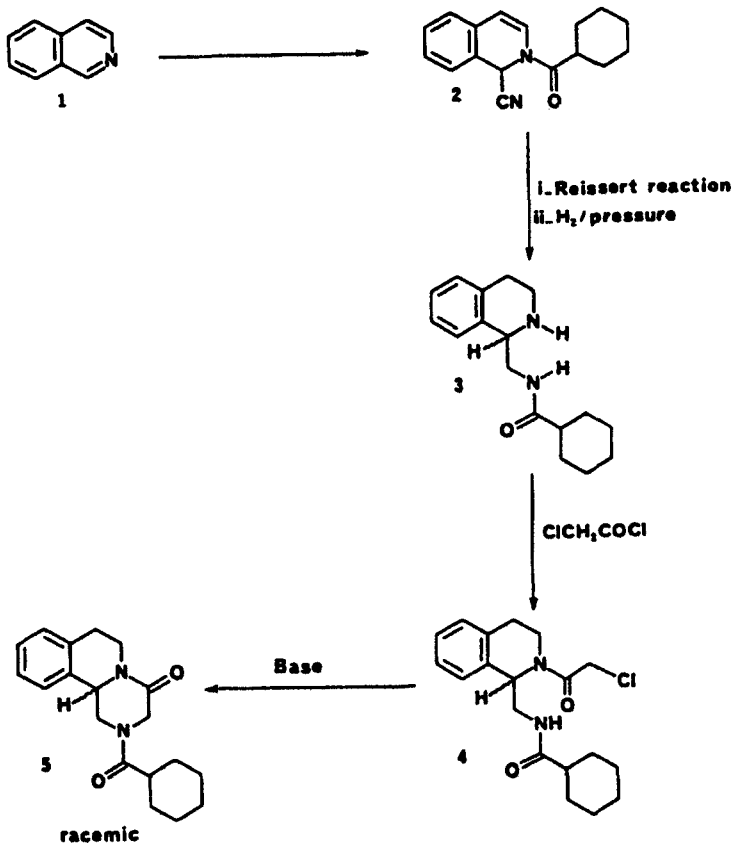
4.2 Melting Range

136-142°C [8]

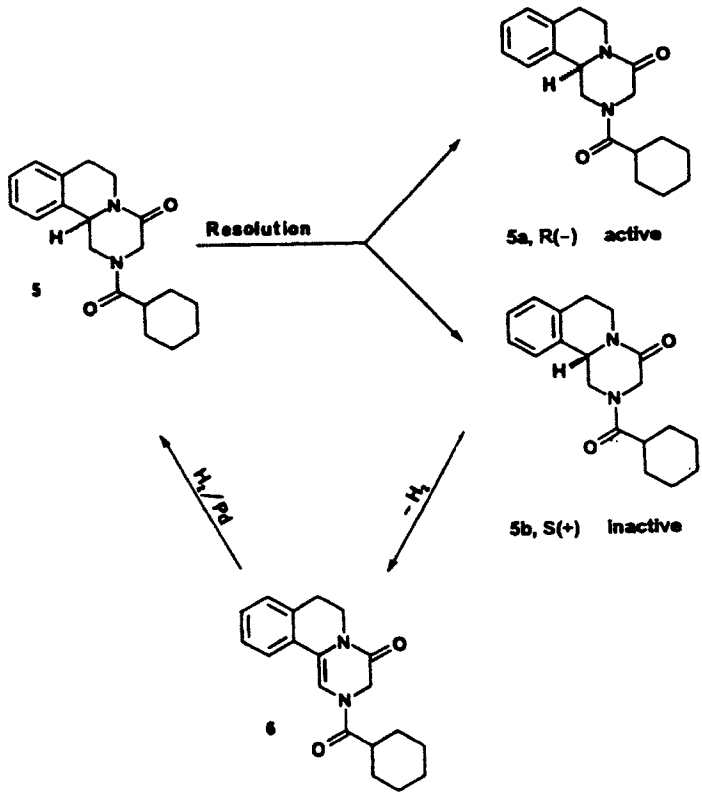
4.3 Solubility

Praziquantel is soluble in ethanol (97 mg/mL) and chloroform (567 mg/mL), but only sparingly soluble in water (0.4 mg/mL) [8].

Scheme 1


1
2
CN
O
i.-Reissert reaction
ii.-H_2/pressure
3
$ClCH_2COCl$
Cl
Base
4
5
racemic

Scheme 2



4.4 X-Ray Powder Diffraction Pattern

The x-ray powder diffraction pattern of praziquantel has been obtained on a Philips PW-1710 diffractometer, equipped with a single crystal monochromator and using copper Kα radiation [11]. The crystallographic data (scattering angles, d-spacings, and relative intensities) were automatically provided by the system, and are found in Table 1. The powder pattern itself is illustrated in Figure 2, and shows the characteristic scattering peaks of pure praziquantel.

4.5 Thermal Behavior

The differential scanning calorimetry (DSC) thermogram for praziquantel is shown in Figure 3, and was obtained using a DuPont TA-9900 Thermal analyzer system interfaced to the DuPont data unit[12]. The thermogram was obtained at from 90-260°C, at a temperature ramp of 10°C/min. The compound was found to melt at 140.5°C, with an enthalpy of fusion of 28.3 kJ/mol being found. The sharpness of the melting endotherm, and the lack of thermal decomposition which accompanies the melting process, indicates that DSC may be successfully used to obtain compound purities.

4.6 Spectroscopic Properties

4.6.1 Ultraviolet Absorption Spectroscopy

The ultraviolet (UV) absorption spectrum of praziquantel solutions has been obtained using a Varian DMS 90 system and matched 1-cm quartz cells [12]. When measured in 95% ethanol against the corresponding blank, absorption maxima were found at 257, 263, and 271 nm. An example of a typical UV absorption spectrum is found in Figure 4.

4.6.2 Infrared Spectroscopy

The IR spectrum of praziquantel was obtained in a KBr disk, using a Perkin-Elmer infrared spectrometer system [12]. The IR absorption

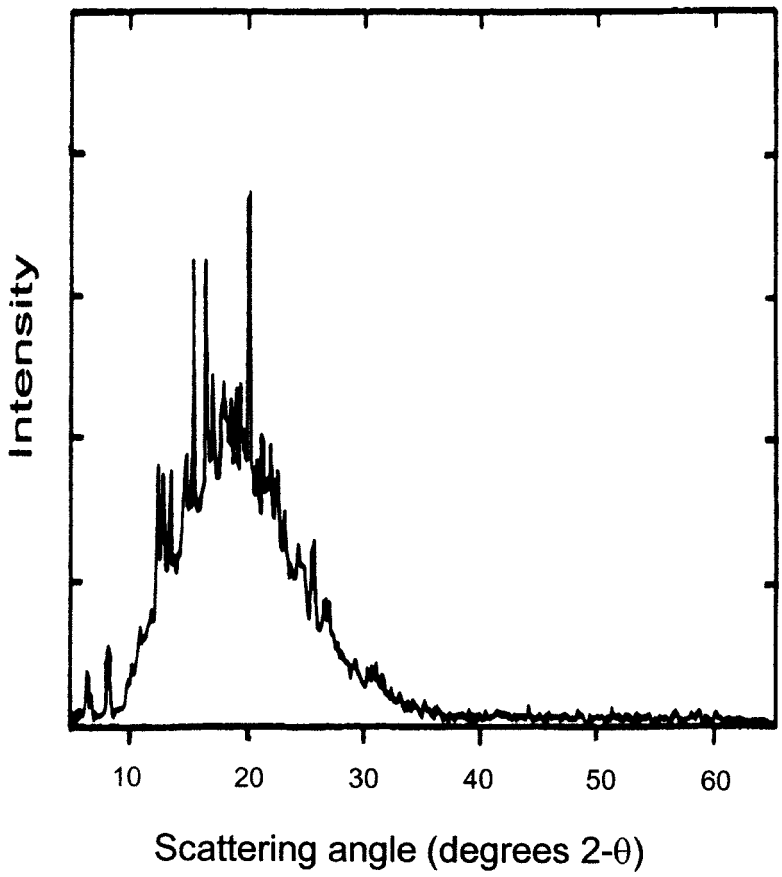


Figure 2. X-ray powder diffraction pattern of praziquantel.

Table 1

Scattering Angles, d-Spacings, and Relative Intensities Observed in the X-Ray Powder Diffraction Pattern of Praziquantel

Scattering angle (degrees 2-θ)	d-spacing (Å)	Relative Intensity (%)	Scattering angle (degrees 2-θ)	d-spacing (Å)	Relative Intensity (%)
6.416	13.7648	9.71	25.706	3.4627	34.41
6.774	13.0388	5.25	26.703	3.3356	24.12
8.137	10.8568	14.63	17.026	3.2965	23.29
9.849	8.9730	8.32	30.335	2.9440	10.90
10.261	8.6137	11.54	30.759	2.9044	10.82
10.845	8.1509	18.05	31.105	2.8729	10.98
12.367	7.1511	47.87	31.722	2.8184	8.77
12.851	6.8830	46.41	33.230	2.6939	5.56
13.472	6.5667	47.35	35.310	2.5398	3.96
14.821	5.9721	49.84	36.089	2.4867	3.19
15.472	5.7225	87.36	36.407	2.4658	3.61
16.530	5.3583	87.45	37.020	2.4263	2.51
17.121	5.1747	65.73	38.413	2.3415	2.16
17.819	4.9735	60.11	39.150	2.2991	2.87
18.612	4.7635	61.30	39.908	2.2572	2.19
19.972	4.6495	63.44	41.431	2.1776	2.85
19.445	4.5613	63.56	44.327	2.0418	3.02
20.189	4.3947	100.00	44.969	2.0142	2.46
20.852	4.2564	48.98	45.993	1.9717	2.51
21.307	4.1667	54.16	48.471	1.8765	2.38
22.056	4.0268	51.86	50.950	1.7909	1.85
22.704	3.9133	47.30	51.398	1.7763	2.65
23.212	3.8288	39.64	54.367	1.6861	1.91
24.491	3.6317	32.92	56.589	1.6251	2.37
24.790	3.5885	30.12	60.091	1.5384	1.81

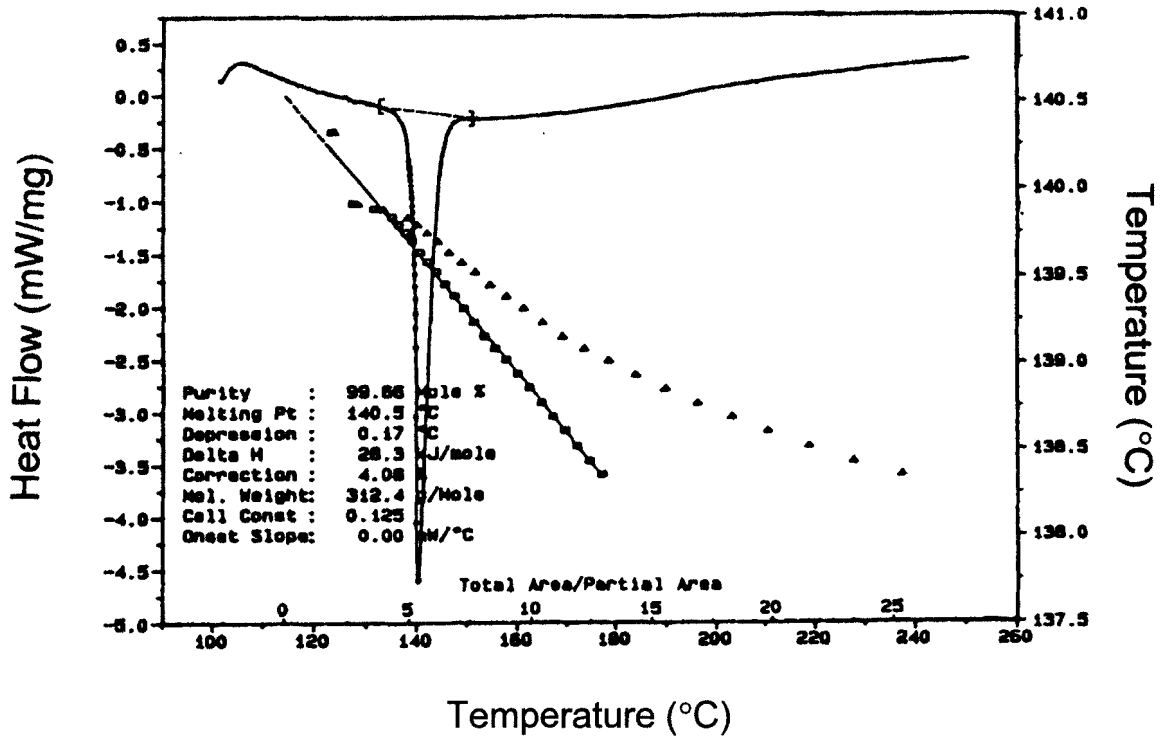


Figure 3. Differential scanning calorimetry thermogram of praziquantel.

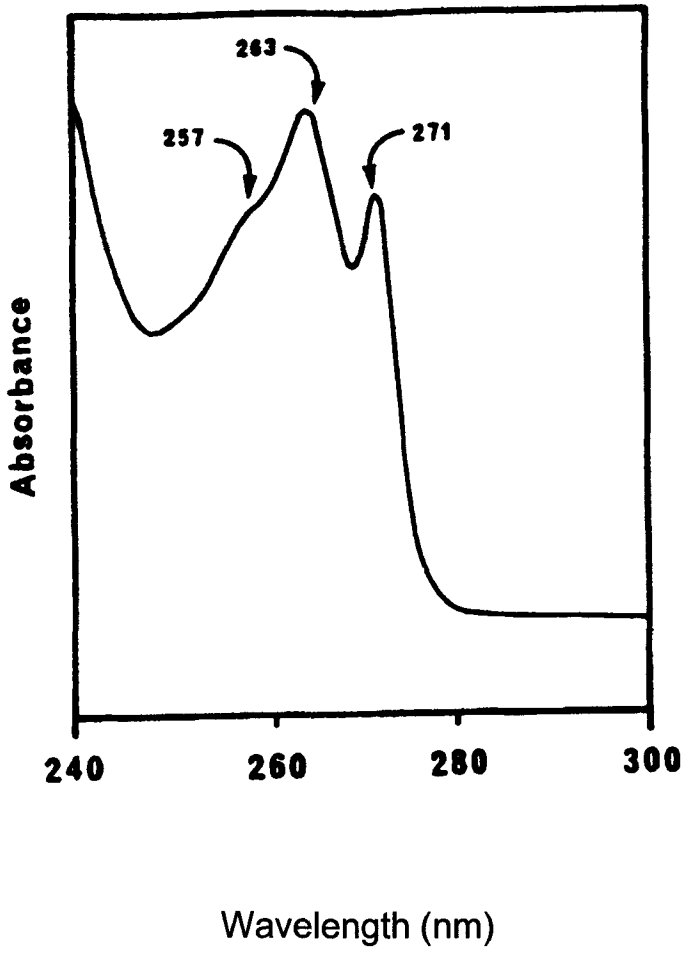


Figure 4. Ultraviolet absorption spectrum of praziquantel.

spectrum thusly obtained is shown in Figure 5, featuring the following prominent peaks:

2952 - 2880 cm^{-1}	$-CH_2$ and -CH stretching modes
1720 - 1655 cm^{-1}	carbonyl stretching mode
1460 cm^{-1}	$-CH_2$ and -CH scissors and asymmetric deformation modes
760 cm^{-1}	Aromatic -CH wagging mode

4.6.3 **Mass Spectrometry**

The mass spectrum of praziquantel was obtained on a Finnigan T3Q45 spectrometer, interfaced with the SuperIncos data system [13]. The parent ion was allowed to collide with argon at a collision energy of 19.8 eV. Figure 6 shows the detailed mass fragmentation pattern, where the base peak appears at m/z=203, and the molecular ion peak at m/z=313.

4.6.4 **Nuclear Magnetic Resonance**

Both the ^1H and ^{13}C-NMR spectra of praziquantel were obtained in $CDCl_3$, using tetramethylsilane as an internal standard [12]. Summaries of the observed ^1H and ^{13}C resonance bands, together with the peak assignments, are listed in Tables 2 and 3, respectively. The detailed ^1H and ^{13}C spectra are shown in Figures 7a and 7b, while the results of DEPT, COSY, and HETCOR experiments are shown in Figures 8a, 8b, and 8c, respectively.

4.4.6.1 **^1H-NMR spectrum**

The ^1H spectrum of praziquantel (Figure 7a) can be conveniently divided into the aliphatic and aromatic regions. In the aromatic portion, one multiplet is observed at 7.10-7.40 ppm corresponding to protons 8, 9, 10, and 11. The aliphatic protons were initially assigned on the basis of

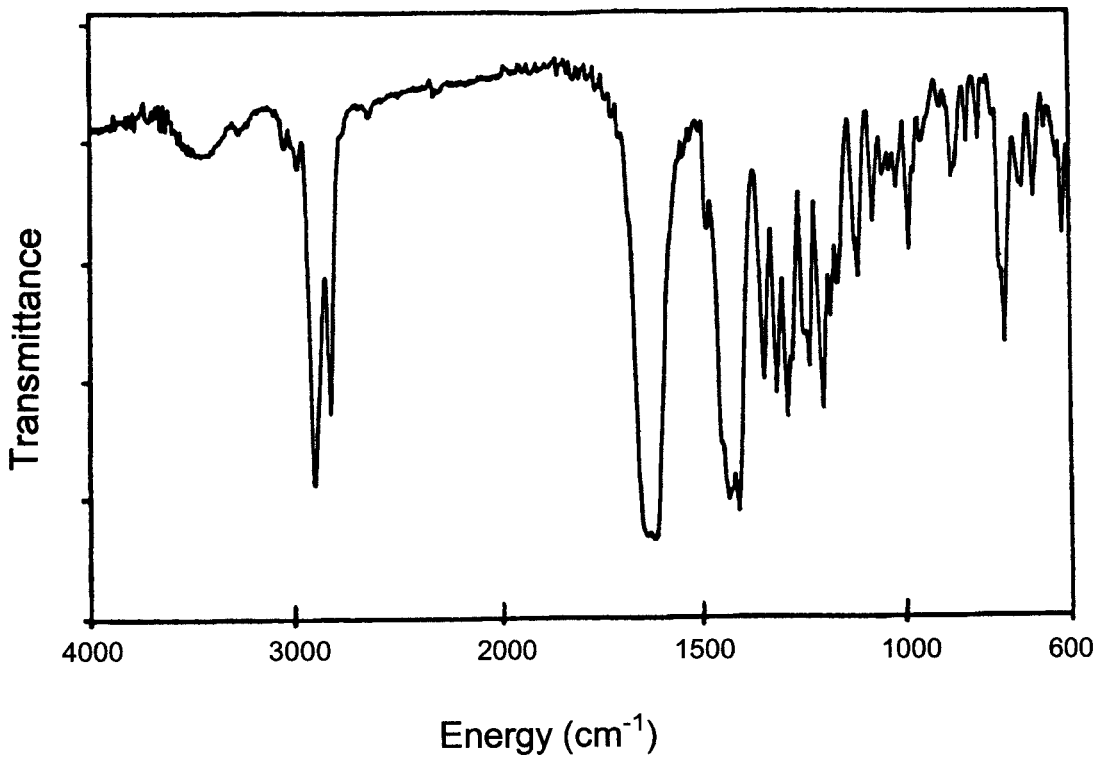


Figure 5. Infrared absorption spectrum of praziquantel.

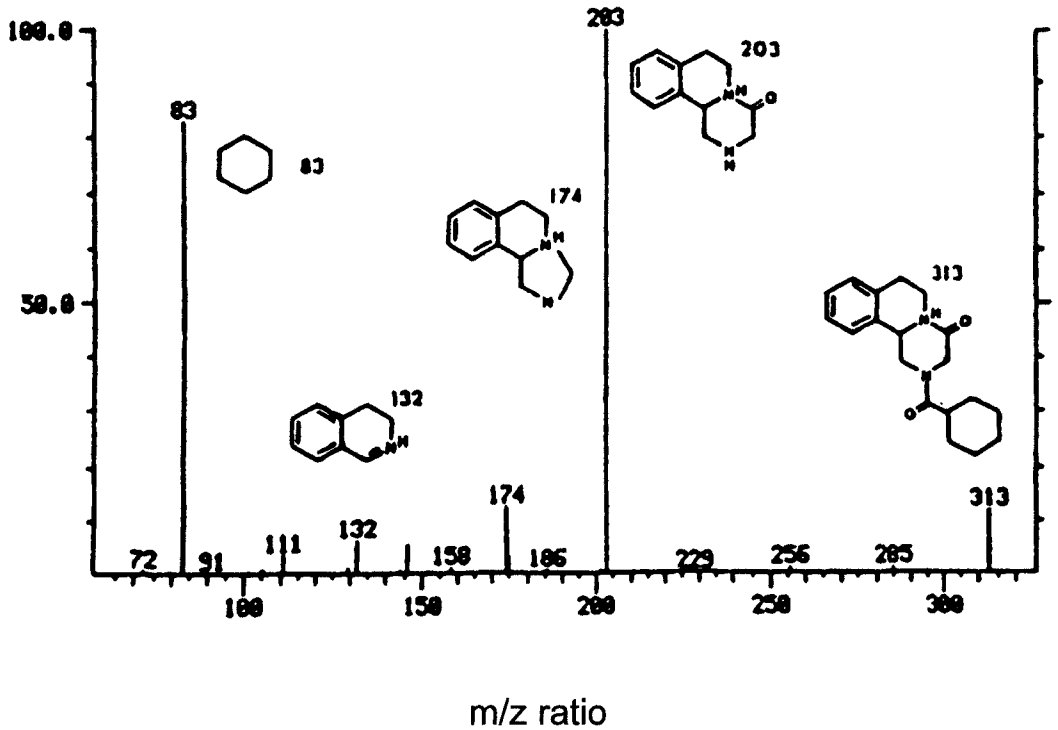


Figure 6. Mass spectrum of praziquantel.

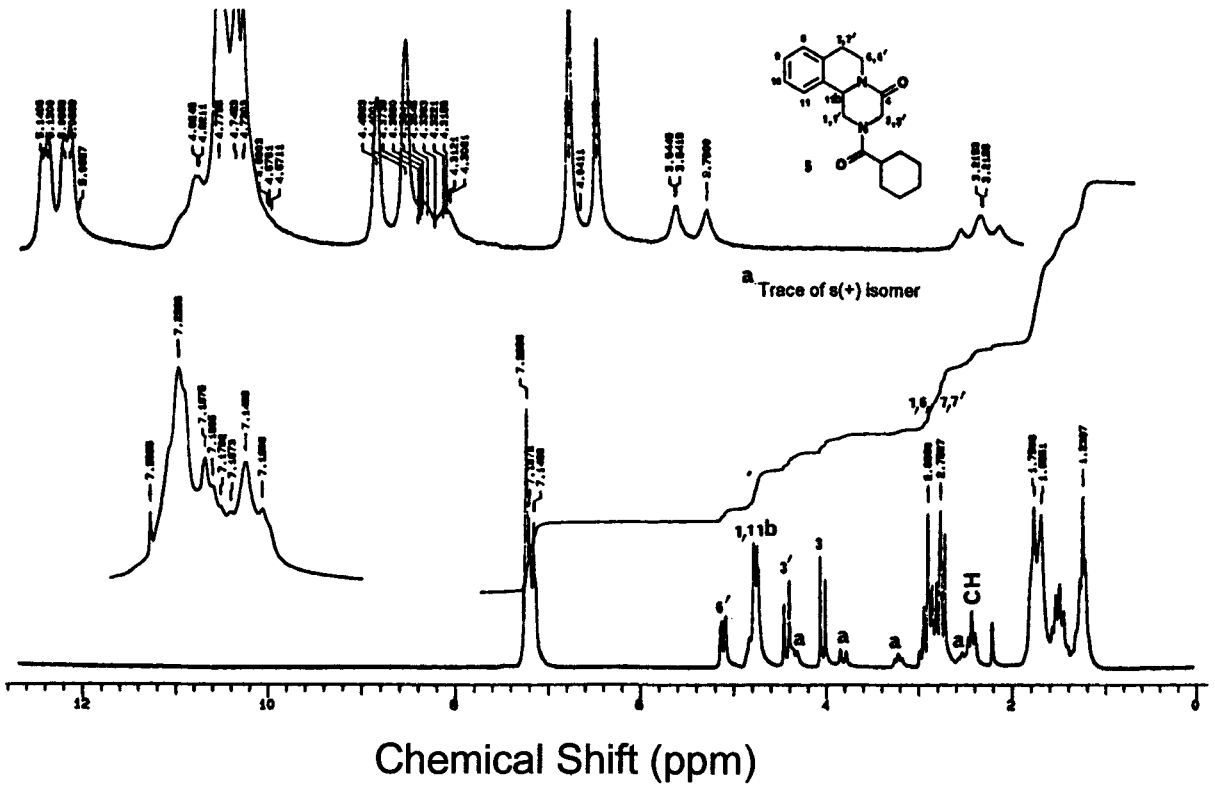


Figure 7a. ^{1}H-NMR spectrum of praziquantel.

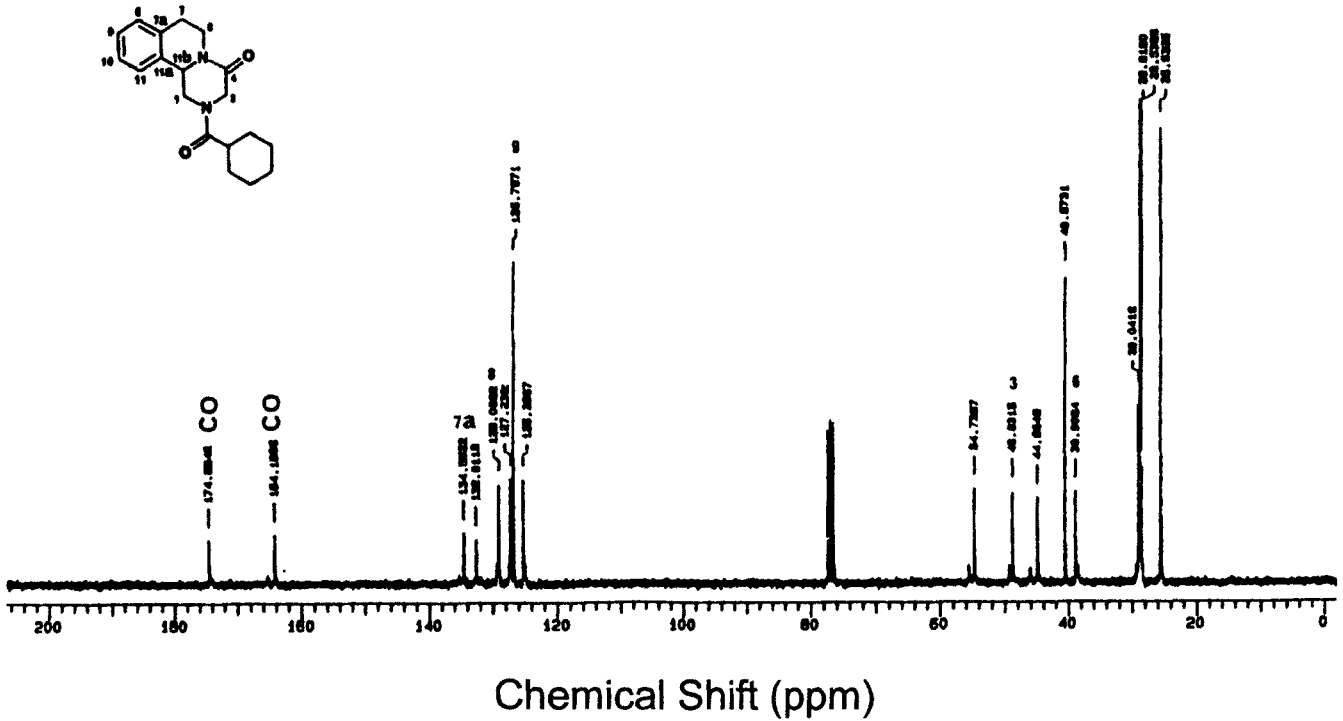


Figure 7b. ^{13}C-NMR spectrum of praziquantel.

Table 2

^{1}H-NMR Resonance Band Assignments for Praziquantel

Chemical shifts (ppm, TMS)	No. of protons	Multiplicity*	Assignment
1.1-1.90	10	m	Cyclohexyl
2.35-2.60	1	m	CH (cyclohexyl)
2.65-3.00	4	m	1,6,7 and 7`
4.10	1	d (17 Hz)	3
4.50	1	d (17 Hz)	3`
4.70-4.90	2	m	1` and 11b
5.10	1	d (14 Hz)	6`
7.10-7.40	4	m	Aromatic H

*d = doublet, m = multiplet.

Table 3

^{13}C-NMR Resonance Band Assignments for Praziquantel

Chemical shift (ppm, TMS)	Assignemnt	Chemical shift (ppm, TMS)	Assignment
174.53	C-4, CO	48.83	C-3
164.20	Cyclohexyl CO	44.96	C-1
134.55	C-7a	40.58	Cyclohexyl
132.51	C-11a	39.90	C-6
129.07	C-8	29.04	Cyclohexyl
127.24	C-11	28.81	C-7
126.77	C-9	28.54	Cyclohexyl
125.26	C-10	25.53	Cyclohexyl
54.75	C-11b		

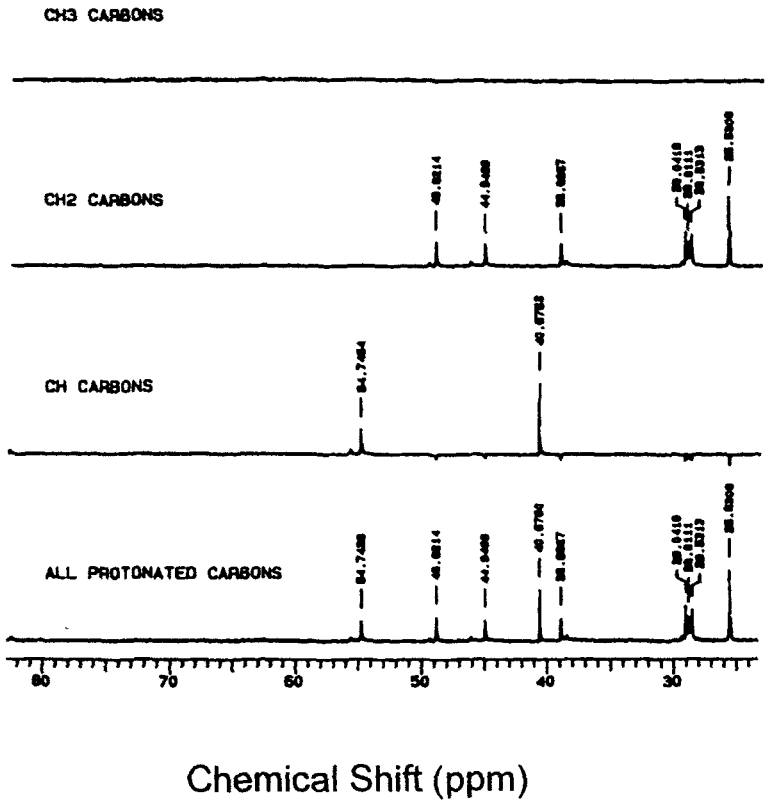


Figure 8a. DEPT spectra of praziquantel.

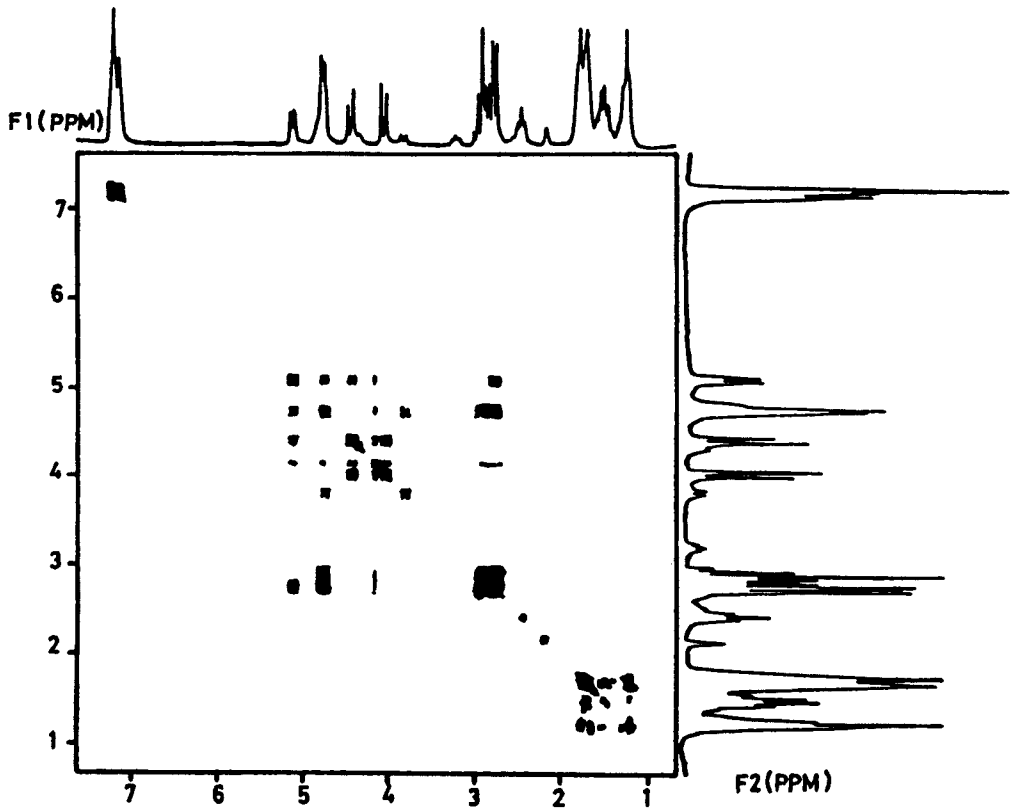


Figure 8b. COSY profile of praziquantel.

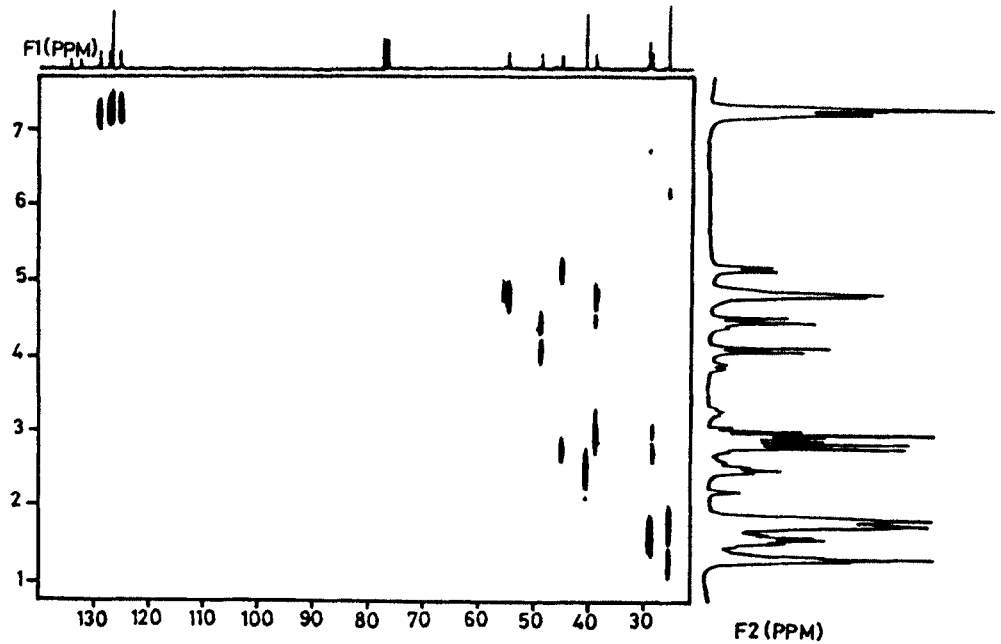


Figure 8c. HETCOR profile of praziquantel.

chemical shift considerations. The multiplet at 1.1-1.90 ppm is assigned to the protons of the cyclohexyl -CH_2 group, while the multiplet at 2.35-2.60 ppm is assigned to the protons of the cyclohexyl -CH group. The multiplet at 2.65-3.00 ppm is assigned to protons 1, 6, 7, and 7'. Protons 3 and 3' form an AB quartet at 4.10-4.50 ppm, with a coupling constant of 17 Hz. Protons 1' and 11b form a multiplet which resonates at 4.70-4.90 ppm, while proton 6' yields a doublet at 5.10 ppm (coupling constant of 14 Hz). All ^{1}H-NMR assignments were confirmed by means of the two dimensional COSY experiment (Figure 8b).

4.4.6.2 ^{13}C-NMR spectrum

A noise-modulated, broad-band decoupled ^{13}C-NMR spectrum (Figure 7b) showed 17 resonance bands, which is in accordance with what would be anticipated for the 19 carbons of praziquantel. Carbon resonance bands at 25.53, 28.54, 29.04, and 40.57 ppm were assigned to the cyclohexyl carbons, while bands at 164.20 and 174.53 ppm were assigned to the two carbonyls of praziquantel. A DEPT experiment (Figure 8a) within the aliphatic region permitted the identification of the methine and methylene carbons. Seven -CH_2 absorptions at 25.53, 28.54, 28.81, 29.04 38.90, 44.96, and 48.83 ppm were assigned to carbons 1, 3, 6, 7, and those of the cyclohexyl group. In addition, two -CH resonance bands at 40.58 and 54.75 ppm were assigned to carbon 11b and the cyclohexyl -CH. These assignments were all confirmed through the performance of a heteronuclear correlation (HETCOR) experiment (Figure 8c).

5. Methods of Analysis

5.1 Spectrometric Determination

5.1.1 Ultraviolet Spectrophotometry

Dissolved in ethanol at a concentration of 0.2 to 1 mg/mL, praziquantel can be determined either at 271-273 nm (first derivative lineshape), or at 268-272 nm (second derivative lineshape) without interference from matrix elements or from turbidity. The recovery was reported to be 99.4 ± 0.5% (first derivative method) or 100.8 ± 0.5% (second derivative method) [23-25].

5.1.2 **Infrared Spectrophotometry**

When dissolved in CCl_4 at a concentration of 22 mg/mL, praziquantel can be determined by measuring its absorbance at 1658.4 and 1720 cm^{-1}. A linear concentration-absorbance response was obtained over the range of 18 to 26 mg/mL, characterized by a variation coefficient (n = 4) of 1.0% [26, 27].

5.1.3 **Colorimetry**

Colorimetric methods for praziquantel have been developed using two different reagents:

5.1.3.1 Reaction with tetrazolium blue:
The developed color was measured at 500 nm. It was reported that recoveries were quantitative, and no interference was observed [28].

5.1.3.2 Reaction with 3-chloro-7-nitro-2,1,3-benzoxadiazole:
The developed color was measured using two different preparations at 478 and 496 nm. It was found that Beer's law was obeyed from 2 to 18 μg/mL, and that determination limits of 0.124 and 0. 15 μg/mL could be obtained with coefficients of variation equal to 0.38 and 0.47%, respectively[29].

5.1.4 **Fluorimetry**

Praziquantel was fluorimetrically determined in biological fluids using dansyl chloride as a derivitizing reagent. In this work, it was reported that the limit of determination was 3 μg/L in blood plasma and 30 μg/dL in urine. In both media, the degree of imprecision was approximately 7.5% [30, 31].

5.2 Chromatographic Techniques

5.2.1 Thin-Layer Chromatography

A low-cost portable TLC method was described for the simultaneous detection and semi-quantitative determination of praziquantel. The plates used for this purpose were glass or plastic (10 x 5 cm) coated with Merck silica gel 60-F254. Praziquantel and standard solutions were spotted alternately across the plate. An appropriate mobile phase was used to develop the solvent front to 1 cm from the top of the plate. Spots were examined with a Shimadzu CS-930 dual-wavelength TLC scanner at 254 nm before dipping the plate into an aqueous I_2/KI solution. The plate was dried and the spots measured by densitometry at 420 nm. It was reported that the detection limits ranged from 0.5 to 10 mg/mL [32].

5.2.2 Gas Chromatography

Praziquantel was determined in biological fluids and in pharmaceutical preparations using a GC method [33,34]. Papaverine was used as a reference substance, and the separation effected using a coiled glass column (60 cm x 2 mm) of chromosorb W (AWDMCS) packed with 3% Dexsil 300 (80-100 mesh), operated at 270°C. The recovery was found to be quantitative, with the coefficient of variation for two samples (containing 0.105 and 1.37 mg/mL) being 0.54 and 0.16% respectively. The detection limit was reported to be 0.5 μg/μL [35].

5.2.3 High Performance Liquid Chromatography

HPLC has been used as a technique for the quantitative analysis of praziquantel, and methods have been reported using different columns and elution systems [36-39].

A HPLC method was developed for the determination of praziquantel in plasma, urine and rat liver homogenates [36]. The method requires 2 mL of biological fluid, an extraction using Sep-Pak cartridges, 0.05M phosphate buffer solution (pH 5) for equilibrating and washing, and ethyl acetate/diisopropyl ether for drug elution. The analysis was performed

using an Ultrasphere ODS C18 column, a mobile phase consisting of acetonitrile/water, and UV detection at 217 nm. It was found that the assay is sensitive (31.2 ng/mL), linear between 0.125 and 4 μg/mL, precise (coefficient of variation = 10%), and selective with respect to other drugs currently administered with praziquantel.

Determination of praziquantel and several of its monohydroxylated metabolites in serum was achieved using a reversed-phase method, based on a LiChrospher 100 RP-18 column (5 μm particle size, 250 x 4 mm I.D., Merck) with a 25 x 4 mm I.D. guard column (LiChrospher 100 RP-18, 5 μm) [37]. The chromatography was performed at 20°C and a flow rate of 1.5 mL/min. The initial mobile phase consisted of 20:80 acetonitrile/ water, which was changed to 29:71 (v/v) after 13 min.and then maintained for 17 min. The retention times for trans-4-hydroxypraziquantel and praziquantel were 6.4 and 20.1 min., respectively.

The enantiomeric ratio analysis of praziquantel and trans-4-hydroxy-praziquantel was performed using a chiral HPLC system [37]. The separation is based on a column packed with cellulose tris-3,5-dimethylphenylcarbamate coated on silica gel (10 μm, 250 x 4.6 mm I.D., Chiralcel OD, Daicel), and a 25 x 4.6 mm guard column (Chiralcel OD, 10 μm). The resolution of *R*(-) and *S*(+)-praziquantel, and of *R*(-) and *S*(+)-*trans*-4-hydroxypraziquantel, was accomplished using a mobile phase of 72:28 (v/v) *n*-hexane/isopropanol. The flow rate was 0.8 mL/min., and the column temperature was 20°C. The K¢ values for the praziquantel enantiomers were 2.4 and 3.2, respectively, and the stereochemical resolution factor (R) was 1.57. The corresponding values for *trans*-4-hydroxypraziquantel were $K_1¢ = 4.1$, $K_2¢ = 5.0$, and R = 1.59.

Praziquantel was also determined after its extraction from fish tissue by dissolving the extract in 50% (v/v) acetonitrile in water [38]. A 25 μL aliquot was analyzed on 150 x 4.6 mm I.D. supelcosil LC-18 column, equipped with a guard column. The method used a mobile phase flow rate of 0.9 mL/min., and UV detection at 210 nm. A linear concentration-absorbance relation was found for the range of 5-500 ng/g of praziquantel in the sample. Recoveries ranged from 78-93%, and the method precision (as estimated by the coefficient of variation) was less than 7%.

Xiao *et al.* have developed a simple, sensitive and quantitative HPLC procedure to measure praziquantel levels in human or mouse serum [39]. The method is clinically applicable, readily validated, and eliminates the need for involved sample preparation or derivatization. Analyses were carried out using a Model M-6000A high-performance liquid chromatograph, equipped with a Model SF-770M-A2 variable-wavelength UV detector. An octadecyl silica gel RP-18, 5 μm (Spheri-5) column a, 10 cm x 4.6 mm HPLC analytical cartridge, and a RP-18, 5 μm (Spheri-5), 3 cm x 4.6 mm precolumn, were used to effect the analytical separation. The mobile phase was 38% aqueous acetonitrile, eluted at a flow-rate of 1.5 mL/min, and detection was effected by the UV absorbance at 210 nm. After 15-20 analyses, the column was cleared with 100% acetonitrile for 20 minutes, using a Waters Associates Model 660 solvent programmer (flow program No. 6).

6. Separation of Enantiomers

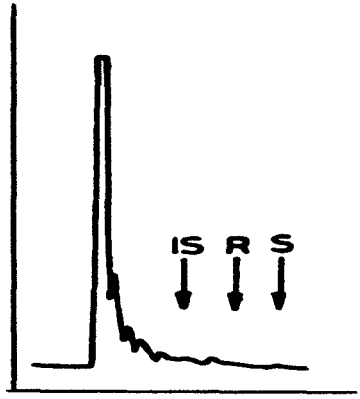
Racemic Praziquantel has been resolved using three different techniques.

6.1 Cellulose-based Chiral Stationary Phase [40]

1 mL of serum was mixed with 20 μL of methanolic 5-chloro-2-methylaminobenzophenone solution (the internal standard, at 12.8 μg/mL) and applied to a cyanopropyl solid-phase extraction column. The column was washed with 10% aqueous acetonitrile, and dried using suction. Elution was effected with ten 0.1 mL portions of ethyl acetate. The eluent was evaporated to dryness under N_2 at 50°C. The residue was dissolved in 0.25 mL of 7:3 hexane/ *i*-propanol, and 0.1 mL of this solution was analyzed on a chiralcel OD column (25 cm x 4.6 mm), fitted with a chiralcel OD guard column (5 cm x 4.6 mm) The chromatography was effected at 1 mL/min, and detected at 212 nm.

As evident in Figure 9, the *R*-(-) and *S*-(+)-enantiomers were well-resolved, and exhibited retention times of 12 and 14.4 minutes, respectively. The internal standard eluted at 8.3 minutes. Calibration curves for each enantiomer were found to be linear from 50-1000 ng/mL in serum, and the detection limit was found to be 5 ng/mL.

(A)



(B)

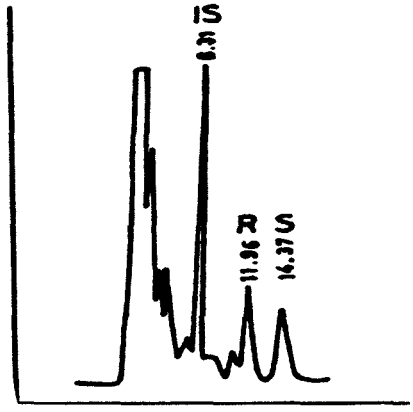


Figure 9. Typical chromatograms of (A) blank serum and (B) serum spiked with 250 ng/mL each of *R*-(-) and *S*-(+)- praziquantel, as well as an internal standard (IS) at 256 ng/mL. UV detection at 212 nm was used, and the retention times are given in minutes [40].

6.2 **Simulated Counter-Current Chromatography** [41]

Counter-current chromatography was conducted using four identical stainless-steel columns (44.5 cm x 12.5 mm), connected in series through solenoid valves and packed with microcrystalline cellulose triacetate. 9:2 hexane/*i*-propanol was used as the mobile phase (flow rate of 4.1 mL/min), and fluorimetric detection at 300 nm (excitation at 270 nm) was used for quantitation purposes. The system was used at the preparative scale, yielding 429 mg/hour of the *S*-(+)-enantiomer and 404 mg/hour of the *R*-(-)-enantiomer from extract and raffinate streams, respectively. The enantiomeric purity of the products exceeded 90%.

6.3 **Resolution on Polyamides and Cellulose Triacetate** [42]

Polyacrylamide and microcrystalline cellulose triacetate were used in liquid-chromatographic separations of the enantiomers of praziquantel, both on analytical and preparative scales.

7. Pharmacokinetics

7.1 **Absorption, Distribution, and Excretion**

Praziquantel is rapidly absorbed after oral administration, even when taken with a meal, and more than 80% of a dose is reported to be absorbed. Peak plasma concentration are achieved within 1 to 3 hours after dosing. The drug undergoes rapid and extensive metabolism in the liver, and is distributed to the cerebrospinal fluid (CSF). The plasma elimination half-life of praziquantel is 1 to 1.5 hours, with the half-lives of the metabolites being about 4 hours. The metabolite products are excreted in the urine, with about 80% of the dose being eliminated within 4 days and more than 90% the dose within the first 24 hours. Praziquantel is also excreted in breast milk [43-46].

7.2 **Biotransformation**

Praziquantel is extensively metabolized on the first pass through the liver, with the mechanism being felt to be dose-dependent [47]. As illustrated in Figure 10, it has been reported that praziquantel is completely metabolized

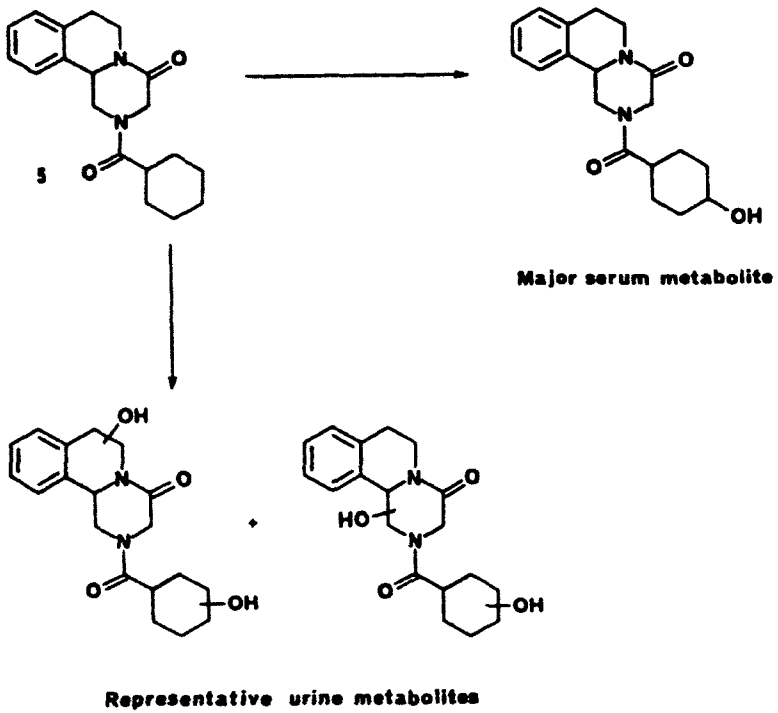


Figure 10. Metabolic biotransformations of praziquantel.

in humans, resulting in monohydroxylated product as a major metabolite and polyhydroxylated product as a minor metabolite [48]. The 4-Hydroxycyclohexylcarbonyl analogue of praziquantel has been identified to be the major metabolite-in serum, which is 400-fold less active than the parent drug and usually excreted as a mixture of glucuronides and/or sulfates [1, 49, 50].

8. Pharmacology

8.1 Mode of Action

Praziquantel is the single major treatment for schistosomiasis and several other trematode and cestode infections, although its target and mechanism of activity are not known [51]. Recent reports of schistosome strains resistant to praziquantel and other antihelminths [52-54] indicate that new therapies must be identified to fight these debilitating diseases. The detoxification enzyme, glutathione S-transferase (GST), which catalyzes the nucleophilic addition of the tripeptide glutathione (GSH) to endogenous and xenobiotic electrophilic toxins was recently shown to be inhibited by praziquantel [7]. As helminths contain very low levels of other detoxification enzymes (such as catalase, superoxide dismutase, and cytochrome P450), GST may provide the worm's primary defense against electrophilic and oxidative damage [55]. Helminths contain two distinct GST classes, having molecular weights of 26,000 and 28,000 and which differ in substrate specificity. These GSTs are believed to prevent toxin accumulation in the worm by catalyzing the conjugation of glutathione to lipid peroxidation products and other toxins [56], and by facilitating the export of toxic host hydrophobic metabolites such as heme [57]. These helminth GSTs show promising vaccine potential, one example being GST injection by Schistosoma japonica and Shistosoma mansorn inducing partial (52-67%) protection against host infection [57, 58]. Recent reports stated that praziquantel selectively binds with parasitic GSTs (and not mammalian GSTs), acting as a non-substrate GST inhibitor. Praziquantel appears to inhibit the catalytic ability of GSTs to detoxify large electrophilic substrate [7].

Table 4

Praziquantel Sensitive Parasites and Their Corresponding Effective Doses

Parazite	Dose	Ref.
1. Clonorchiasis	75 mg/kg given in 3 divided doses for 1 day.	(59,60)
2. Cysticercosis	50 mg/kg per day for 14 to 15 days.	(61)
3. Fasciolopsis Buski	75 mg/kg per day in 3 divided doses for 1 day.	(62)
4. Metagonimus yokogawai	75 mg/kg per day in 3 divided doses for 1 day.	(62)
5. Nanophyetiasis	60 mg/kg for 1 day in 3 divided doses.	(63)
6. Opisthorchiasis	75 mg/kg in 3 divided doses for 1 day	(64)
7. Paragonimiasis	75 mg/kg in 3 divided doses for 2 days.	(64)
8. Schistosomiasis:		(65)
- S. mansoni or haematobium	40 mg/kg per day divided in two doses for one day.	
- S. japonicum or mekongi	60 mg/kg per day in 3 divided doses for 1 day.	(65)

8.2 Therapeutic Uses

The therapeutic use of praziquantel as broad spectrum anthelmintic is summarized in Table 4, which shows the type of parazite, recommended dose, and literature citation.

Acknowledgment

The authors would like to express their gratitude and thanks to Dr. S.R. Michael at Bayer Scientific and Technical Office, Jeddah, Saudi Arabia for the generous gift of praziquantel. Also, our deep thanks are due to the NMR lab personnel in both King Abdulaziz City for Science and Technology (KACST) and College of Pharmacy, University of Mississippi, for carrying out the NMR studies. The authors also would like to thank Mr. Tanvir A. Butt for typing the manuscript.

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TRANYLCYPROMINE SULFATE

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CONTENTS

1. Description

1.1 Nomenclature

1.1.1 Chemical Name

trans-(+)-2-phenylcyclopropanamine sulfate

1.1.2 Synonyms

Tranilcipromina, transamine

1.1.3 Trade Names [5]

Parnate, Tylciprine, Transepin

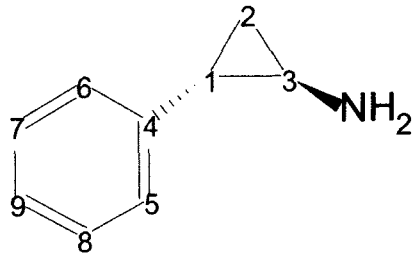
1.2 Formulae

1.2.1 Empirical: $(C_9H_{11}N)_2 \cdot H_2SO_4$

1.2.2 CAS Registry Number: 155-09-9 (free base)
13492-01-8 (sulfate salt)

1.2.3 Structure

Tranylcypromine free base exhibits the following structure:



The sulfate salt is the 2:1 neutralization product of tranylcypromine with sulfuric acid.

1.3 Molecular Weight: 133.19 (free base)
364.47 (sulfate salt)

1.4 Appearance

Tranylcypromine sulfate is a white or off-white crystalline powder, having an acidic taste. It is odorless, or can exhibit a very faint odor of cinnamaldehyde. The free base is a liquid.

1.5 Historical Overview

Tranylcypromine was synthesized and reported in 1946 by Burger and Yost in an attempt to find clinically useful congeners of amphetamine[1]. The compound was tested as a replacement for amphetamine in a proprietary nasal inhaler , but this system proved unrewarding and was never marketed. Later, Meass and Nimmo demonstrated that tranylcypromine could act as an inhibitor of monoamine oxidase (MAO) [2], and this led to clinical trials in which the drug was tested as an antidepressant agent in man. The fact that tranylcypromine was not a hydrazine derivative , as were all other marketed MAO inhibitors at that time, added to enthusiasm for it as a compound that might show far less toxicity than did the other antidepressants. Marketed in February 1961 as Parnate 10 mg tablets, tranylcypromine was accepted in New and Nonofficial drugs in 1962. On February 24,1964, Parnate was withdrawn under formal protest, from the US market by the manufacturer at the request of the United States Food and Drug Administration because of complications resulting from hypertensive crises attributed to its use [3]. In a subsequent FDA study , the conclusion was reached that although such complications occurred frequently, they could be minimized by more stringent controls during the use of the drug. It was also concluded that tranylcypromine is sufficiently effective as an antidepressant agent that it should be made available again for clinical use. Revised, but more restrictive, prescribing information was distributed by Smith-Kline and French, and tranylcypromine was re-marketed in mid-August 1964 [4].

2. Method of Preparation

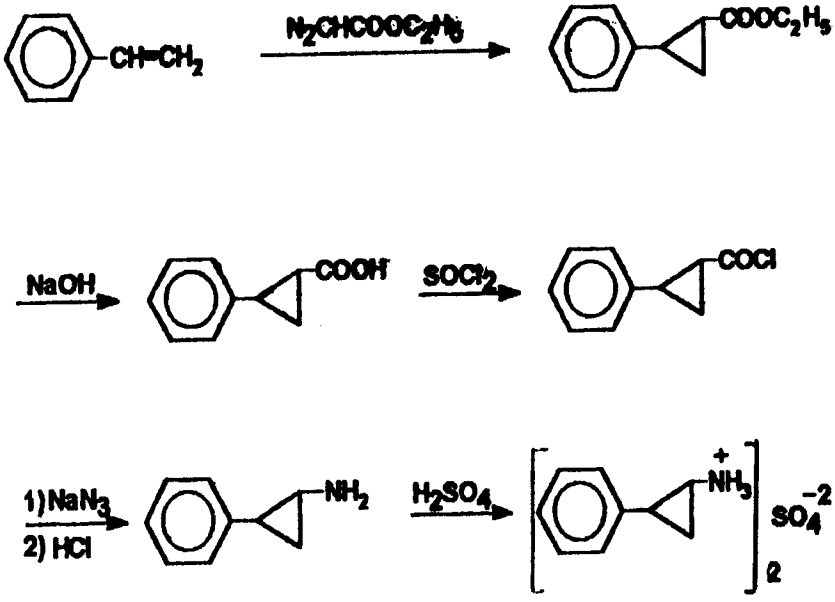
Tranylcypromine base was synthesized in 1984 by Burger and Yost for Smith Kline and French Laboratories [1]. The starting material was ethyl-2-phenyl propan-carboxylate, which had been obtained from the condensation of styrene and ethyldiazo acetate. Selective hydrolysis of the ester with NaOH yielded a mixture of two isomeric carboxylic acids that could be separated by fractional crystallization. The appropriate ratio of acid and *trans*-isomer (having a melting point of 93°C) was refluxed with sulfuryl chloride/dry benzene to obtain the acid chloride. A Curtius degradation, reacting the acid chloride with sodium azide in boiling toluene, and subsequent hydrolysis of the formed isocyanate, yielded *trans*-2-phenylcyclopropane as an oily product having a boiling point of 79-80°C). The sulfate salt was prepared in the usual way. A diagram illustrating the synthesis of tranylcypromine is provided as Scheme 1.

3. Physical Properties

3.1 Particle Morphology

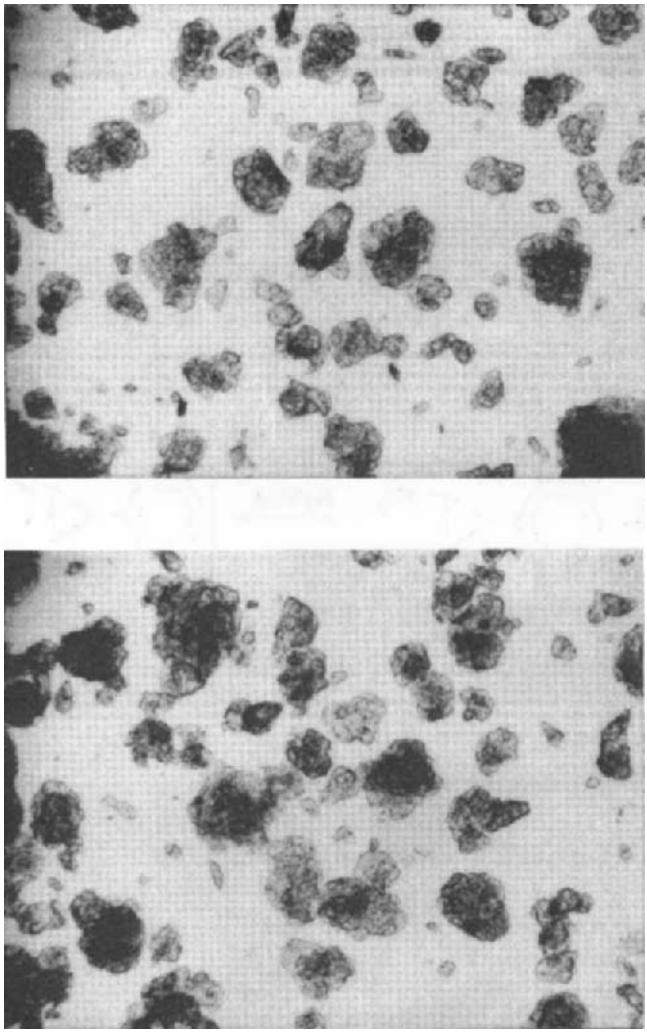
The particle morphology of tranylcypromine sulfate was studied using polarizing optical microscopy. These investigations were performed using a Nikon Labophot-2 system, and the crystals were viewed a magnifications of 40x and 100x. Samples were immersed in mineral oil, and held on the slide under a cover glass. The drug substance was found to exist as essentially spherical aggregates, which typically ranged in size between 25 and 50 μm. As evident in the optical microscopic photographs shown in Figure 1, the aggregates are built up from smaller individual crystals, which were in the range of 1 to 5 μm. No reportable morphology could be described for the component crystals, as these were irregular in form and exhibited no well-formed crystal faces.

When viewed between crossed polarizers, individual crystals, and the aggregates formed from these, are found to exhibit a high degree of birefringence. Multiple colors associated with first-order birefringence were noted, features consistent with strong crystalline character.



Scheme 1. Synthesis of tranylcypromine.

Figure 1. Optical Photomicrographs of Tranylcypromine Sulfate, Obtained at 100x Magnification



3.2 Crystallographic Properties

As would be predicted from the results of the polarizing optical microscope studies, tranylcypromine sulfate was found to exhibit a strong and characteristic x-ray powder diffraction pattern. The XRPD work was performed using a Philips APD 3720 powder diffraction system, equipped with a vertical goniometer in the θ/2-θ geometry. The K-α line of copper at 1.54439 Å was used as the radiation source. Each sample was scanned between 2 and 40 degrees 2-θ, in step sizes of 0.02 degrees (integrated for 0.5 sec at each step).

The powder pattern obtained for tranylcypromine sulfate is illustrated in Figure 2, and the table of scattering angles, d-spacings, and relative intensities is provided in Table 1. Although the powder pattern contains a number of weak scattering lines, the pattern is dominated by the two intense scattering peaks located at 4.81 and 14.32 degrees 2-θ.

3.3 Thermal Methods of Analysis

3.3.1 Melting Behavior

When characterized by conventional means, tranylcypromine sulfate exhibits an apparent melting point at 206-208°C.

3.3.2 Differential Scanning Calorimetry

Measurements of differential scanning calorimetry were obtained on a Perkin-Elmer DSC-7 thermal analysis system. Sample sizes of approximately 2 mg were used, and the samples were heated at a rate of 5°C/min. up to a final temperature of 300°C. As shown in Figure 3, the DSC thermogram of tranylcypromine sulfate featured a continually sloping baseline, indicating that the sample underwent a constant change in heat capacity during the experiment.

A very weak endothermic transition was noted at 210°C, which may be assigned to the melting of tranylcypromine sulfate. This feature is almost immediately followed by a very strong exothermic transition, which

Figure 2. X-Ray Powder Diffraction Pattern of Tranylcypromine Sulfate.

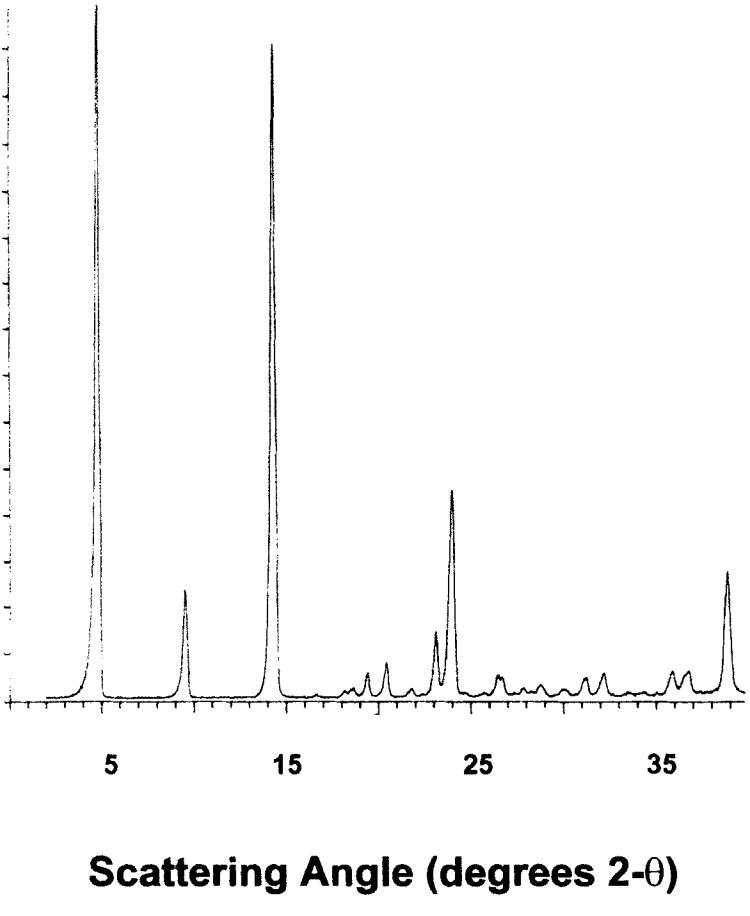
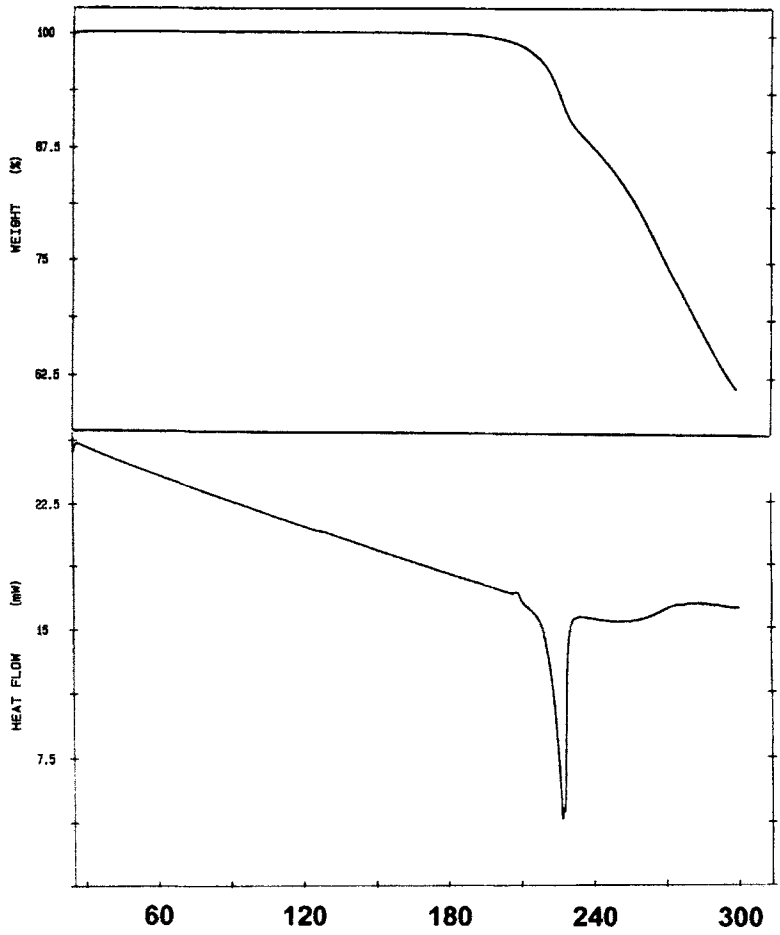


Table 1

Scattering Angles, d-Spacings, and Relative Intensities Associated with the X-Ray Powder Diffraction Pattern of Tranylcypromine Sulfate

Scattering Angle (degrees 2-θ)	d-Spacing (Å)	Relative Intensity (%)
4.810	18.4019	100.0
9.535	9.2910	7.3
11.760	7.5376	0.1
14.320	6.1954	30.3
16.625	5.3412	0.1
18.145	4.8971	0.3
18.640	4.7682	0.3
19.435	4.5749	0.7
20.390	4.3627	1.1
21.805	4.0827	0.3
23.130	3.8517	1.8
24.015	3.7118	5.5
24.700	3.6104	0.1
25.760	3.4642	0.1
26.435	3.3772	0.5
26.710	3.3431	0.5
27.810	3.2133	0.2
28.705	3.1151	0.3
29.895	2.9938	0.2
30.205	2.9637	0.1
31.240	2.8979	0.4
32.180	2.7862	0.5
33.550	2.6755	0.1
34.355	2.6147	0.1
34.985	2.5690	0.1
35.760	2.5151	0.4
36.520	2.4645	0.4
36.775	2.4480	0.5
37.395	2.4088	0.1
38.730	2.3288	1.6
38.850	2.3219	2.2

Figure 3. Thermogravimetry (upper trace) and Differential Scanning Calorimetry (lower trace) Thermograms of Tranylcypromine Sulfate.



exhibited a maximal heat flow around 225°C. This latter feature is characteristic of thermal decomposition.

3.3.3 Thermogravimetry

Thermogravimetric analysis studies were performed using a Perkin-Elmer TGA-7 thermal analysis system. Sample sizes of approximately 4 mg were used, and the samples were heated at a rate of 5°C/min. up to a final temperature of 300°C. The TG thermogram is shown in Figure 3 along with the DSC thermogram. The anhydrous nature of tranylcypromine sulfate is evident in that no weight loss was detected below 180°C. Above 200°C, the compound undergoes an extensive thermal degradation.

3.4 Solubility

The solubility of tranylcypromine sulfate at ambient temperature (26°C) was determined in a number of solvents, with the following results being obtained:

Solvent	Solubility
Water	40 mg/mL
Methanol	4 mg/mL
Ethanol	very slightly soluble
Diethyl Ether	0.5 mg/mL
Chloroform	practically insoluble

3.5 Partition Coefficient

The octanol/water partition coefficient of tranylcypromine sulfate has been reported as log P = 1.58 [6].

3.6 Ionization Constant

The pKa associated with the amine functionality is 8.2.

3.7 Spectroscopy

3.7.1 Ultraviolet/Visible Spectroscopy

Tranylcypromine sulfate absorbs only weakly in the ultraviolet region, with transitions being localized within the phenyl moiety [7]. As shown in Figure 4, the ionization state of the amine group causes spectral differences to exist between acidic and alkaline media. In 0.2 N sulfuric acid, absorption maxima are observed 258 nm (E1% = 14.4), 264 nm (E1% = 16.2), and 271 nm (E1% = 11.6). All bands undergo a small red shift when the compound is dissolved in an aqueous medium of high pH, with maxima 260 nm, 266 nm, and 273 nm being observed.

3.7.2 Vibrational Spectroscopy

The infrared spectrum of tranylcypromine sulfate has been obtained using in a potassium bromide disk [15], and a copy of the absorption spectrum is given in Figure 5. Assignments for the most intense peaks are provided in Table 2.

3.7.3 Nuclear Magnetic Resonance Spectrometry

Nuclear magnetic resonance studies were conducted on tranylcypromine sulfate using a JEOL GSX-270 spectrometer, operating at 270.05 MHz (^{1}H-NMR) or at 67.8 MHz (^{13}C-NMR). Spectra were obtained in D_2O solution at room temperature, and the resonance assignments make use of the numbering scheme given in section 1.2.3 of this profile.

3.7.3.1 ^{1}H-NMR Spectrum

The one-dimensional ^{1}H-NMR spectrum of tranylcypromine sulfate is shown in Figure 6. The assignments for the observed resonance bands are provided in Table 3, with additional justification being obtained using two-dimensional proton-proton correlated spectroscopy and two-dimensional proton-carbon correlated spectroscopy. The complex nature ^{1}H-NMR spectrum is clearly evident in the expanded spectra shown in Figure 7.

Figure 4. Ultraviolet Absorption Spectra of Aqueous Solutions of Tranylcypromine Sulfate, Obtained in Acidic (solid trace) and Basic (dotted trace) Media.

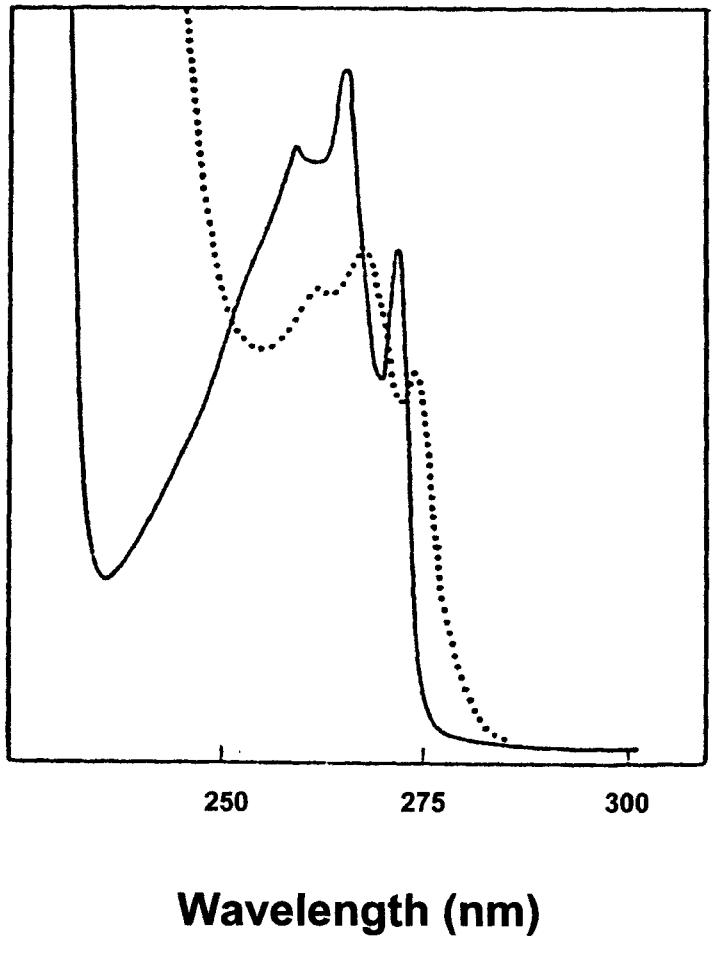


Figure 5. Infrared Absorption Spectrum of Tranylcypromine Sulfate, Obtained in a KBr pellet.

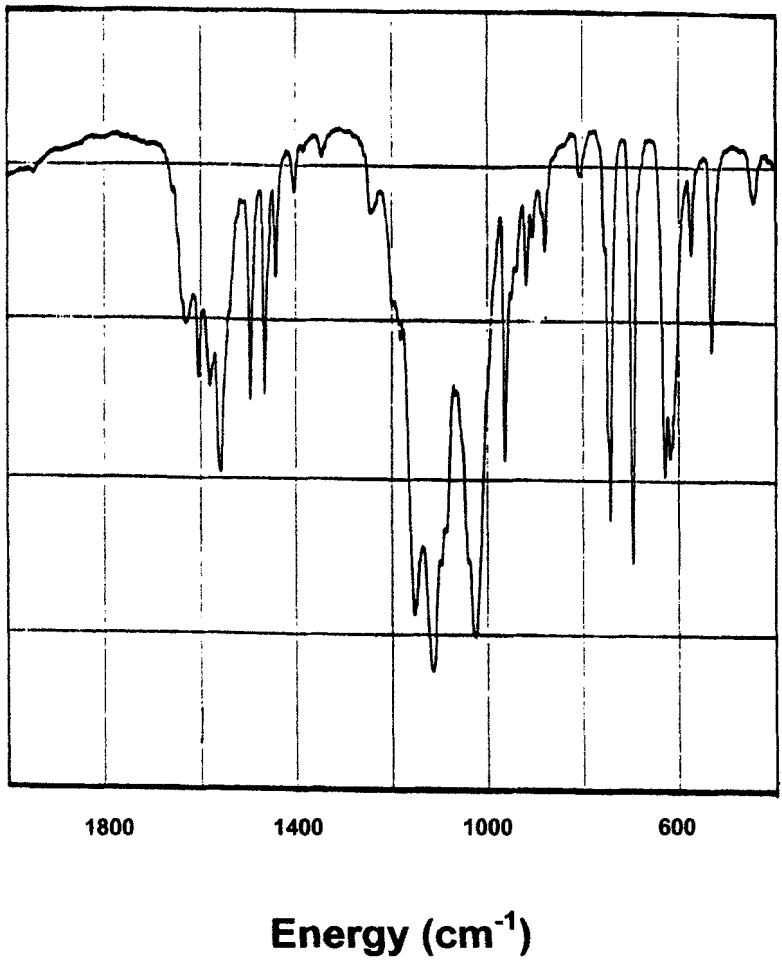


Table 2

Assignments for the Bands Observed in the Infrared Absorption Spectrum of Tranylcypromine Sulfate

Band Energy (cm^{-1})	Assignment
443	out-of-plane phenyl quadrant mode
616,626	sulfate ν_4 mode
697,740	out-of-plane phenyl sextant mode
958	$-CH_2$ rocking mode
1024	in-plane phenyl bending mode
1110	sulfate ν_3 mode
1149	-C-N asymmetric stretching mode
1442	$-CH_2$ scissor deformation mode
1465	-CH bending mode
1493,1559	semicircle phenyl stretching mode
1579,1602	quadrant phenyl stretching mode
1625	$-NH_3^+$ deformation mode

Figure 6. ^{1}H-NMR Spectrum of Tranylcypromine Sulfate, Obtained in D_2O Solution.

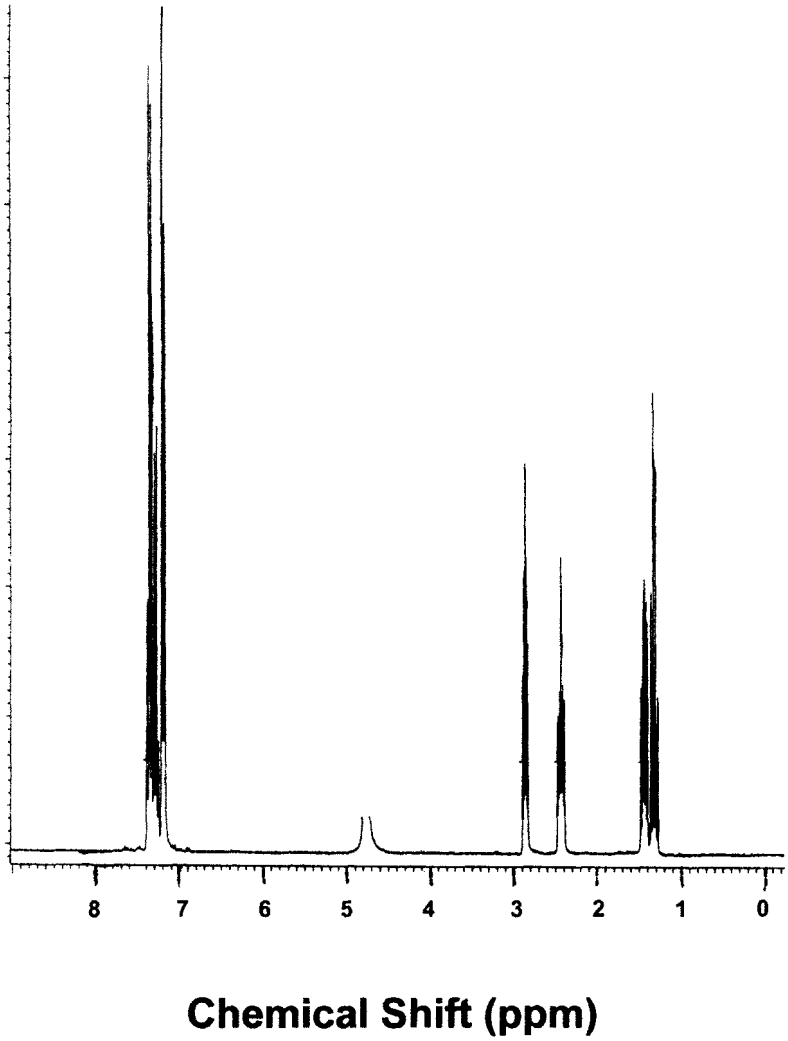
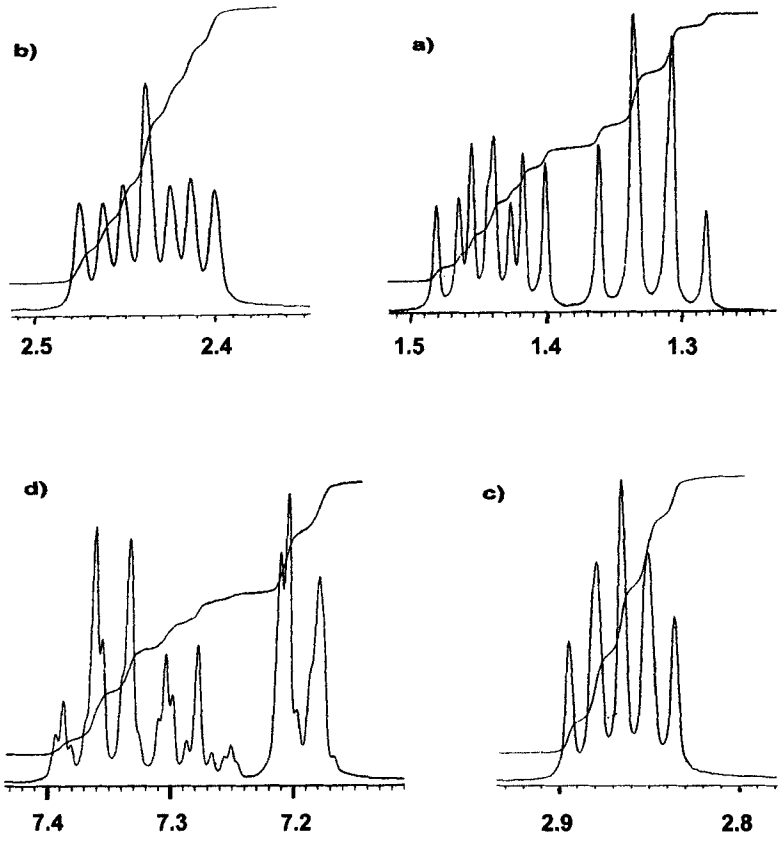


Figure 7. Expanded Views of the ^{1}H-NMR Spectrum of Tranylcypromine Sulfate. Shown are the spectra associated with the protons attached to carbon-2 (trace a), the proton attached to carbon-3 (trace b), the proton attached to carbon-1 (trace c), and the protons attached to the phenyl group (trace d).



As there is complicated coupling existing in all resonance bands, assignment of the ^{1}H-NMR spectrum will be made for a range of chemical shifts. The band system observed at 1.2-1.5 ppm is assigned to the protons attached to carbon-2, the band system observed at 2.4-2.5 ppm is assigned to the proton attached to carbon-3, and the band system observed at 2.8-2.9 ppm is assigned to the proton attached to carbon-1. The very complex pattern noted at 7.1-7.4 ppm is assigned to the protons attached to the phenyl group.

3.7.3.2 ^{13}C-NMR Spectrum

The one-dimensional ^{13}C-NMR spectrum of tranylcypromine sulfate is shown in Figure 8, and the assignments for the observed resonance bands are given in Table 3. Unlike the situation observed for the ^{1}H-NMR spectrum, assignment of the observed resonance bands in the ^{13}C-NMR spectrum is relatively straightforward.

3.7.4 Mass spectrometry

The mass spectrum of tranylcypromine sulfate was obtained using gas chromatography as the means to introduce the sample into the analysis system. The compound was derivatized with (*S*)-*N*-(trifluoroacetyl)propyl chloride to enable an enantiomeric gas chromatographic analysis of the resulting stereoisomers [9]. A Hewlett-Packard (HP) model 5890A gas chromatograph was used to effect the analytical separation, which was linked to a HP 3392A integrator, a mass spectrometer, and a HP 7920 data system. Operating conditions for the mass spectrometer included an ion source temperature of 200°C, an interface temperature of 275°C, an accelerating voltage of 22 kV, an ionization voltage of 70 eV, and a scan speed 100 AMU/sec. The major fragments identified in the mass spectrum of the tranylcypromine derivative identified in Table 4.

4. Methods of Analysis

4.1 Identity Tests

4.1.1 Mandalein Test

Add few drops of the Mandalein reagent (prepared by dissolving 0.5 g of ammonium vanadate in 1.5 mL water, and diluting to 100 mL with H_2SO_4)

Figure 8. ^{13}C-NMR Spectrum of Tranylcypromine Sulfate, Obtained in D_2O Solution. Part of the Phenyl Region is Expanded in the Inset.

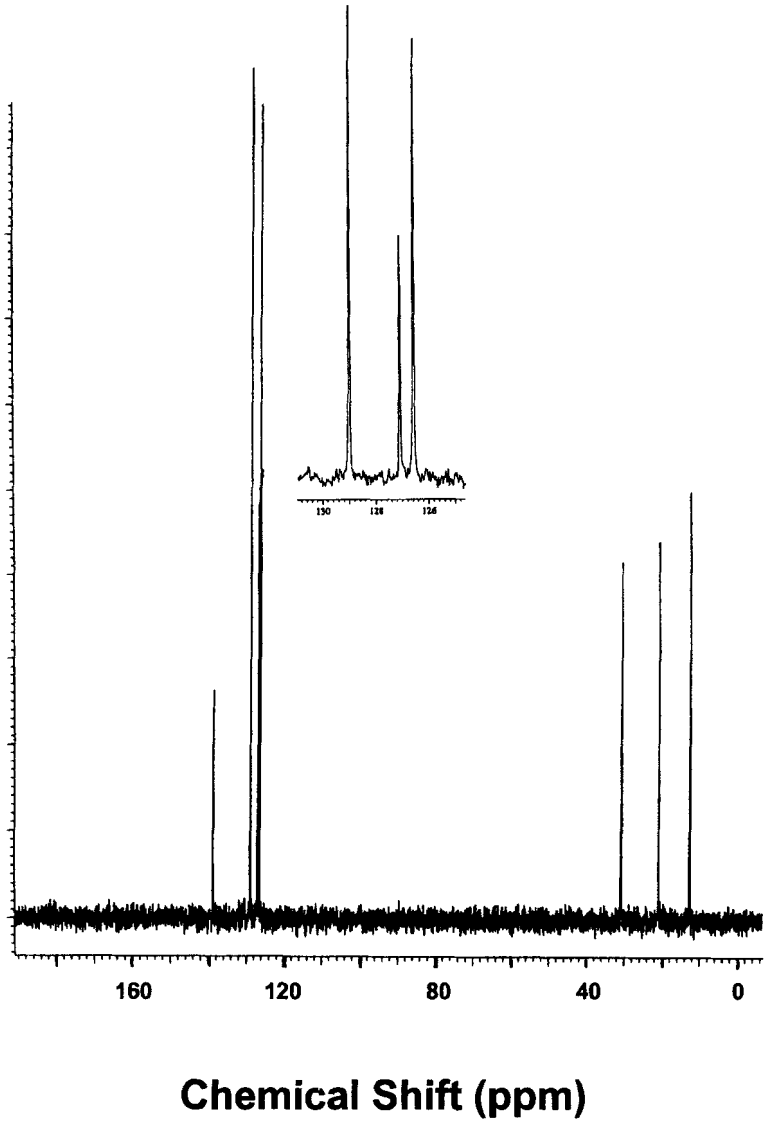


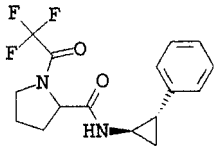
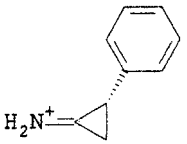
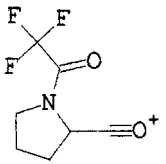
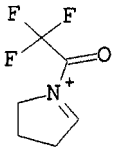
Table 3

Assignments for the Resonance Bands Observed in the ^{13}C-NMR Spectrum of Tranylcypromine Sulfate

Carbon	Chemical Shift (ppm)
1	21.044
2	12.726
3	30.840
4	138.931
5,6	126.603
7,8	129.020
9	127.107

Table 4

Assignments for the Fragments Observed in the Mass Spectrum of Derivitized Tranylcypromine Sulfate

m/z	Structure of Fragment
326	
132	
194	
166	
69	$^{+}CF_3$

to few drops of tranylcypromine solution on a white porcelain spot-plate. A positive reaction is indicated by the production of a green to violet color [7].

4.1.2 Liebermann Test

Add few drops of the Liebermann reagent (which consists of a 10% solution of sodium nitrite dissolved in cooled sulfuric acid) to few drops of the tranylcypromine solution. Heat the mixture in a water bath, and a positive outcome in signified by the development of a red to orange color [7].

4.1.3 Marquis Test

Mix few drops of the Marquis reagent (which consists of 10% formaldehyde in sulfuric acid) with few drops of the tranylcypromine solution. A positive reaction is indicated by the production of a red to brown color [7].

4.1.4 Ninhydrin Test

To about 10 mg of powdered tranylcypromine, add 2 mL of alcohol and 1 mL of the ninhydrin reagent (which consists of 0.5 g ninhydrin dissolved in 100 mL of acetone). After the solution is thoroughly mixed, a violet to purple color is obtained [16].

4.2 Compendial Tests

4.2.1 Drug Substance Assay

Both the United States Pharmacopoeia [16] and the British Pharmacopoeia [17] specify a nonaqueous titration method for the determination of tranylcypromine sulfate. Approximately 1 g of tranylcypromine sulfate (accurately weighed) is dissolved in 75 mL of glacial acetic acid, and titrated with 0.1 N perchloric acid. The endpoint is determined using potentiometric detection and suitable electrodes. Each mL of 0.1 N $HClO_4$ is equivalent to 36.45 mg of tranylcypromine.

4.2.2 Drug Product Assay

Spectrophotometric methods have been adopted for determination of the potency of tranylcypromine tablets. The USP method uses 0.7 N H_2SO_4 to extract the drug substance from powdered tablets, followed by a determination of the absorbance at 285 nm and 271 nm. On the other hand, the BP recommends an extraction using 0.1 N H_2SO_4), and the subsequent measurement of absorbance at 282 nm and 271 nm.

4.3 Chromatographic Methods of Analysis

4.3.1 Thin Layer Chromatography

A number of TLC systems have been reported which are suitable for either the identification or determination of tranylcypromine, and the salient points of these are summarized in Table 5.

4.3.2 High Performance Liquid Chromatography

Several HPLC methods have been developed for tranylcypromine. Davies and Heal had described a HPLC method, which was coupled with electrochemical detection [21]. The mobile phase consisted of 85:15 nitric acid / methanol, adjusted to pH 2.3, and the analytical separation was effected using an ODS column. Detection of analytes was made using a glassy carbon electrode, operating at a potential of +0.85 V. A similar method was reported for tranylcypromine in plasma, which was also based on electrochemical detection after the drug substance had been concentrated using liquid-liquid extraction [22].

An enantiospecific HPLC method with fluorimetric detection was applied to the pharmacokinetic study of tranylcypromine [23]. This method involved the precolumn derivitization of the analyte with *o*-phthaldehyde and mercaptan-*N*-acetylcysteine. A HPLC method for the enantiomeric analysis of racemic tranylcypromine has also been described [24].

4.3.3 Gas chromatography

A combined TLC-GC method was described for the identification of tranylcypromine using a nitrogen-selective flame ionization detector [25].

Table 5

Systems for the Thin Layer Chromatographic Analysis of Tranylcypromine Sulfate

System	R_f	Developing spray	Reference
methanol / strong ammonia (100 : 1.5)	0.54	Dragendorff reagent	18
cyclohexane / toluene / diethylamine (75:15:10)	0.33	Dragendorff reagent	18
chloroform / methanol (90:10)	0.33	Dragendorff reagent	18
cyclohexane / benzene / diethylamine (75:15:10)	0.55	Dragendorff reagent	19
methanol	0.55	Dragendorff reagent	19
acetone	0.58	Dragendorff reagent	19
95% ethanol	0.56	Dragendorff reagent	19
methanol / 12 N NH_4OH (100 :1.5)	0.58	Folin-Ciocalteu reagent Mandelin reagent Furfural reagent	20
cyclohexane / diethylamine / benzene (75:20:15)	0.52	Folin-Ciocalteu reagent Mandelin reagent Furfural reagent	20
acetone	0.66	Folin-Ciocalteu reagent Mandelin reagent Furfural reagent	20
chloroform / methanol (90:10)	0.54	Folin-Ciocalteu reagent Mandelin reagent Furfural reagent	20
benzene / ethanol / 12 N NH_4OH (95:15:5)	0.51	Folin-Ciocalteu reagent Mandelin reagent Furfural reagent	20

A similar GC method was described for the determination of tranylcypromine in blood and urine, and entailed the use of TLC for further confirmation [26].

Morrazi *et al.* (27) reported the I_R and δI_R obtained under programmed and isothermal temperature conditions, which were used as a new approach to GC investigation in the forensic toxicology of tranylcypromine. Volatile derivatives of tranylcypromine were required for quantitation purposes, so the pentaflurobenzoyl [28], trimethylsilyl [29], pentaflurobenzenesulfonyl [30], and isothiocyanate [31] derivatives were prepared and characterized.

4.4 Electrochemical Methods

Belal *et al.* developed a polarographic method for tranylcypromine in its formulations [32]. The method is based on treatment with nitrous acid, where an electrochemically active reducible derivative was produced. A well-defined cathodic wave, with an $E_{1/2}$ value of -0.73 V *vs.* Ag/AgCl, was obtained in Britton-Robinson buffer at pH 5. A linear current-concentration relationship was obtained over a range of 0.011 mM to 0.165 mM.

4.5 Enzymatic Methods

A sensitive method for either enantiomer of tranylcypromine in brain tissue has been described [33]. The method is based on the enzymatic transfer of the ^{14}C-methyl group of *S*-adenosyl-*L*-methionine-^{14}C to tranylcypromine by rabbit lung *N*-methyltransferase. The method was applied to determine levels of tranylcypromine in the brain.

4.6 Spectrophotometric Methods

A rapid differential spectrophotometric method for the determination of tranylcypromine in biological fluids has been described [34]. The method is based on the oxidation of tranylcypromine to benzaldehyde by periodate in alkaline media. The reaction product is either directly measured

spectrophotometrically, or its semicarbazone derivative is formed and that is measured spectrophotometrically.

A spectrophotometric method, based on charge-transfer complexation, has been reported for the determination of tranylcypromine in formulations [35]. In the reaction, tranylcypromine acts as the electron-pair donor, and 7,7,8,8,-tetracyanoquinodimethane acts as the electron-pair acceptor. It was found that Beer's law was obeyed over the range of 0.2 to 4 μg/mL. Iodine monochloride can also be used as the acceptor; but this method is less sensitive than is the former. For the ICl reagent, the calibration curve was linear over the range of 2-20 μg/mL.

Belal *et al.* proposed a simultaneous spectrophotometric method for the analysis of dosage form mixtures consisting of tranylcypromine combined with trifluroperazine in tablets [36]. The treatment of tranylcypromine with nitrous acid resulted in an enhancement in its absorptivity, which permitted the development a method based on this observation [32]. The nitroso derivative was formed at pH 2 and a reaction temperature of 80°C. Beer's law was obeyed over the range of 1-20 μg/mL.

4.7 Fluorimetric Methods

Tranylcypromine sulfate was determined in plasma after derivitization with 1-dimethylaminonaphthalein-5-sulphonyl chloride [37]. The reaction to generate a fluorescent product was carried out after an analytical separation had been performed on TLC plates.

Activated (*R,S*)-benzoxaprofen was reported as a new derivitization reagent for tranylcypromine, where the procedure could be coupled with TLC and HPLC [38].

4.8 Nuclear Magnetic Resonance Methods

The ^{1}H-NMR spectrum of racemic tranylcypromine has been clarified using both chiral and achiral lanthanide shift reagents [39]. The purity of enantiomerically resolved tranylcypromine was evaluated using NMR spectroscopy and the same reagents [8].

5. Pharmacokinetics

5.1 Absorption and Distribution

Tranylcypromine is readily absorbed after oral administration, and is extensively metabolized. Less than 2% of the administered dose can be extracted from urine in an unchanged form after 24 hours. This amount may increase to about 8% if the urine is maintained at an acidic pH [7].

A difference in the pharmacokinetics of the enantiomers of tranylcypromine has been noticed, where (-)-tranylcypromine entered the blood circulation more rapidly, reached significantly higher concentrations, and metabolized more slowly than did (+)-tranylcypromine [10].

5.2 Metabolism

A metabolic study of tranylcypromine in rats led to the detection of *N*-acetyltranylcypromine and the glucouronoid conjugate of *N*-hydroxy-*N*-acetyltranylcypromine as the principal metabolites which could be detected in urine [11].

p-hydroxytranylcypromine has been unequivocally identified as a metabolite of (+)-tranylcypromine. Levels of this metabolite in brain and heart tissue of rats after intraperitoneal administration of TCP were determined. Higher levels of *p*-hydroxytranylcypromine were found to be present in the brain relative to the heart after tranylcypromine treatment [12]. Hippuric acid was also identified as a metabolite of tranylcypromine, being due to cyclopropyl ring cleavage in the body [13].

5.3 Enzyme Inhibition

The nature of MAO blockage by tranylcypromine was extensively studied by Zeller , who demonstrated that this compound competed with amine substrates for the same active sites on MAO [14]. A rapid onset of MAO inhibition after drug exposure and quick recovery of the enzyme activity were noticed.

The effect of both enantiomers of tranylcypromine on the disposition and metabolism of norepinephrine in rat brain were studied [15]. Both isomers inhibited the deactivation of norepinephrine, but the (+)-enantiomer was found to be considerably more potent than was the (-)-isomer. Tranylcypromine is a very potent inhibitor of MAO both *in vivo* and *in vitro*, and is thus effective in the treatment of human depression.

6. Acknowledgments

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ZILEUTON

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ANALYTICAL PROFILES OF DRUG SUBSTANCES AND EXCIPIENTS—VOLUME 25
1075-6280/98 $25.00

Contents

1. Description

1.1 Nomenclature

1.1.1 Chemical Name

N-(l-Benzo[b]thien-2-ylethyl)-N-hydroxyurea

1.1.2 Nonproprietary Names

Zileuton (USAN)

(±)-(l-Benzo[b]thien-2-ylethyl)-N-hydroxyurea

Urea, N-(l-benzo[b]thien-2-ylethyl)-N-hydroxy-, (±)

(±)-(l-Benzo[b]thien-2-ylethyl)-l-hydroxyurea

1.1.3 Proprietary Name

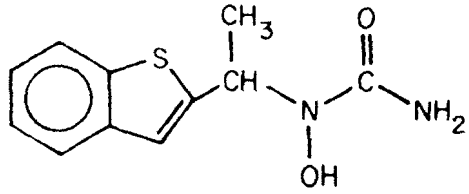
ZYFLO™

1.2 Formulae

1.2.1 Empirical

$C_{11}H_{12}N_2O_2S$

1.2.2 Structural



1.3 Molecular Weight

236.29

1.4 CAS Number

111406-87-2

1.5 Appearance

Zileuton is a white crystalline material with no discernible odor.

1.6 Uses and Applications

Zileuton is a benzothiophene N-hydroxyurea with potent and selective *in vitro* and *in vivo* 5-lipoxygenase inhibitory activity. 5-Lipoxy-genase is an enzyme that converts archidonic acid to 5-hydroperoxy-eicosa-6,8,11,14-tetraenoic acid in the pathway leading to the production of leukotrienes. Inhibition of leukotriene synthesis has many potential therapeutic benefits for conditions in which such synthesis is elevated, such as asthma, rheumatoid arthritis, ulcerative colitis, and psoriasis.

2. Methods of Preparation

A variety of approaches have been reported in the scientific literature for the synthesis of zileuton [1]. Numerous United States patents also exist which describe different synthetic routes, notably US patents 4,873,259, 4,992,464, and 5,250,565. A typical synthetic pathway used for the large-scale manufacture of the drug substance is shown in Figure 1.

3. Physical Properties

3.1 Crystallographic Properties

3.1.1 *Single Crystal Structure*

A crystal suitable for single crystal x-ray diffraction structural analysis was crystallized from ethyl acetate by vapor diffusion against hexane. The experimental parameters for XRAY 391 are listed in Table 1, and the crystal structure itself is presented in Figure 2. The crystal structure is that of a racemate.

3.1.2 Polymorphism

Zileuton can exist as two crystal forms, one of which is an anhydrate phase (denoted as Form 1) and the other of which is a monohydrate phase

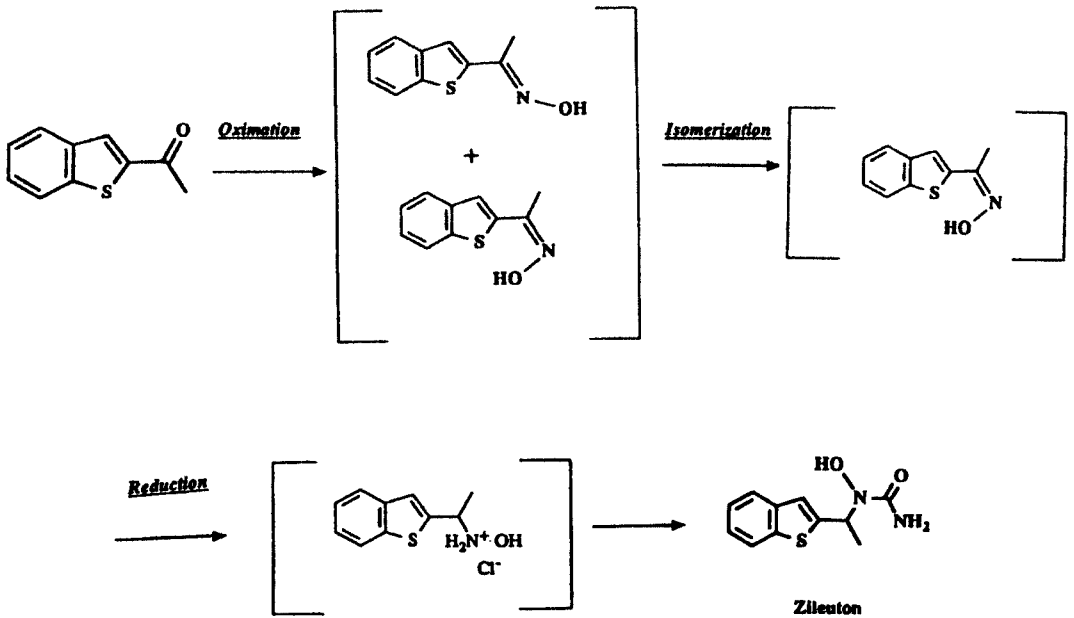


Figure 1. Synthesis of zileuton.

Table 1

Single Crystal X-Ray Parameters from the XRAY 391 Analysis

Crystal Data

Crystal system:	triclinic
	$a = 6.017(2)$ Å
	$b = 36.894(3)$ Å
	$c = 5.215(2)$ Å
	$\alpha = 92.28(2)°$
	$\beta = 97.64(3)°$
	$\gamma = 87.11(2)°$
Space Group:	P1
Z-Value:	4

Intensity Measurements

Diffractometer:	Rigaku AFC5R
Radiation:	Cu Kα (1.54178 Å)
Temperature:	ambient
$2\theta_{max}$:	120.1°
Correction:	Lorentz-polarization Absorption (trans. Factor: 0.93-1.16)

Structure Solution and Refinement

Number of Observations ($I>3.0\sigma$):	2237
Number of Variables:	289
Reflections / Parameters Ratio:	7.74
Residuals: R:R_w:	0.070; 0.091

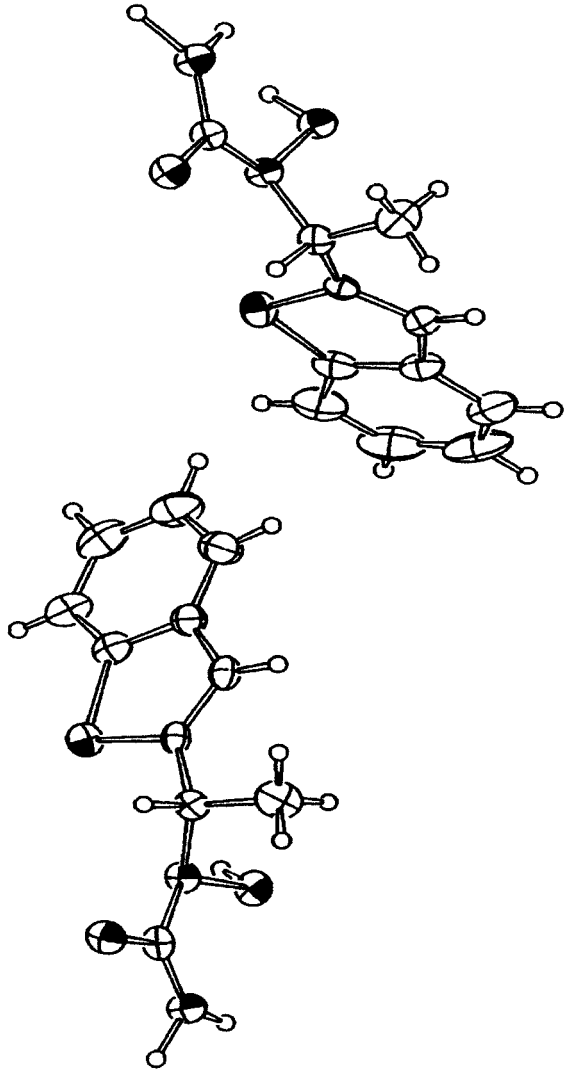


Figure 2. Single crystal structure of zileuton.

(denoted as Form 2). It has been determined that Forms 1 and 2, or any combination of these, has the same solubility in water. In addition, all materials have been found to exhibit the same intrinsic dissolution rate.

The anhydrate phase can be converted to the monohydrate phase through the use of an ethanol slurry, followed by the addition of water. The mixture is heated, allowed the solution to cool, and the monohydrate phase crystallizes from the solution. The monohydrate phase can be converted into the anhydrate phase by drying the sample.

3.1.3 X-Ray Powder Diffraction Patterns

The X-ray powder diffraction patterns of the two polymorphs of zileuton were determined using a Nicolet 12 X-ray diffraction unit, making use of a copper source tube operated at 50 kV and 30 ma.

Typical zileuton samples consist of the partially dehydrated monohydrate phase, and therefore represent a mixture of the anhydrous Form 1 and the monohydrate Form 2. As a result, the x-ray powder pattern of such samples shows lines attributable to both Form 1 and Form 2. Figure 3 contains the powder patterns of the anhydrous, monohydrate, and partial hydrate materials, and the d-spacings and relative intensities of these are collected in Table 2.

Any possible polymorphism in the zileuton system was evaluated using hot stage x-ray powder diffraction (Nicolet 12 x-ray diffraction system and Paar TTK-HC hot stage). A sample was placed in the sample holder, and the powder pattern obtained at 302 K (29°C). The system was programmed to heat the sample to the next desired temperature, held there for a specific time, and to then record the x-ray powder pattern. Temperatures evaluated during this work were 352 K (79°C), 366 K (93°C), and 378 K (105°C). Diffraction lines at 21.1, 10.4, 6.97, and 5.23 A lost intensity during the analysis (Figure 4), but these lines are characteristic of the monohydrate phase and their loss represents mere dehydration of this phase. No other changes in the powder patterns were observed, which was interpreted to rule out conversion to another polymorph of the anhydrous phase.

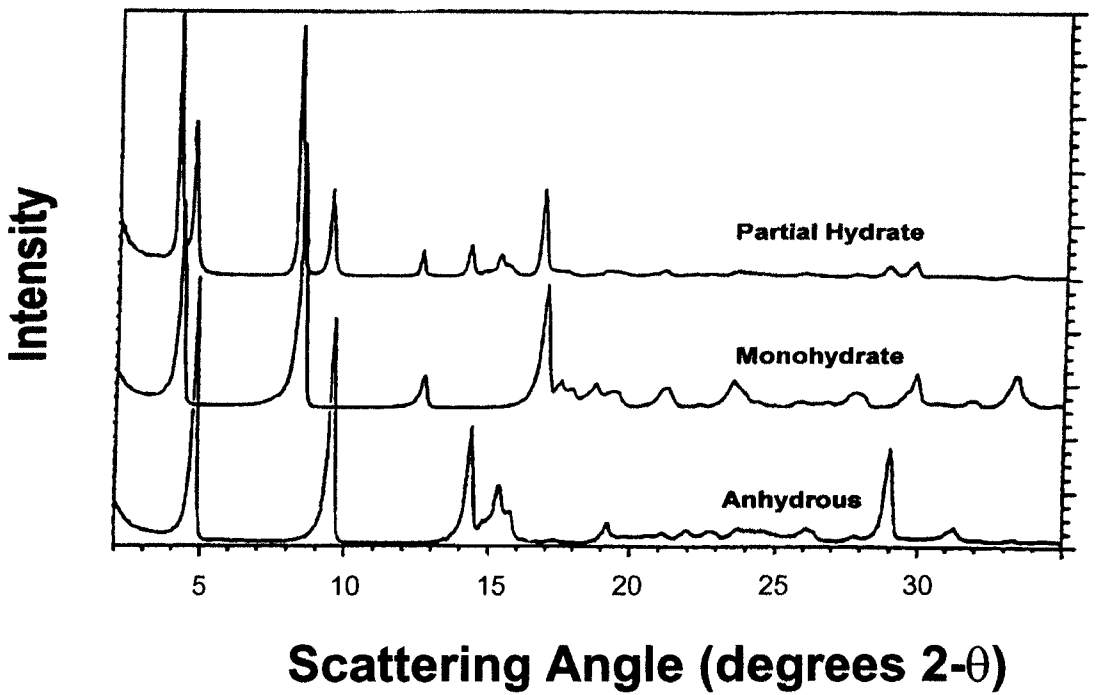


Figure 3. X-ray powder diffraction patterns for the various forms of zileuton.

Table 2

D-Spacings and Relative Intensities Obtained from the X-Ray Powder Diffraction Analysis of the Anhydrate and Monohydrate Phases of Zileuton

Anhydrate Phase (Form 1)		**Monohydrate Phase (Form 2)**	
d-Spacing (Å)	**Relative Intensity (%)**	**d-Spacing (Å)**	**Relative Intensity (%)**
18.48	100	20.84	73
9.25	93	10.46	100
6.16	47	6.97	12
5.95	9	5.23	49
5.77	24	5.07	10
4.52	8	4.96	7
4.21	4	4.73	9
4.05	5	4.56	6
3.90	4	4.19	8
3.75	6	3.78	10
3.42	6	3.32	2
3.20	2	3.21	6
3.08	40	2.99	13
2.86	6	2.81	2
2.69	2	2.69	13

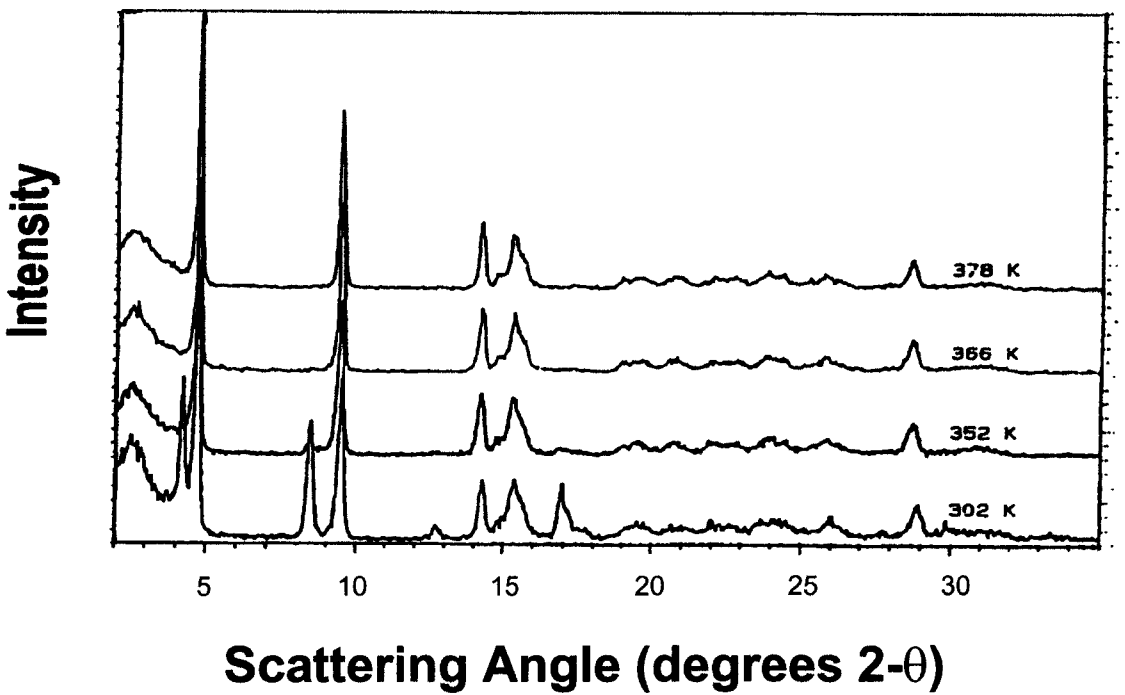


Figure 4. Variable-temperature x-ray powder diffraction study of zileuton.

3.2 Optical Activity

Although zileuton contains one center of dissymmetry, it is synthesized as a racemic mixture. A 2% methanolic solution exhibited no detectable optical activity when its optical rotation was measured on a polarimeter at 589 nm.

3.3 Thermal Methods of Analysis

3.3.1 Differential Scanning Calorimetry

The differential scanning calorimetry (DSC) profile of zileuton (anhydrous phase) was recorded on a DuPont Model 912 Differential Scanning Calorimeter and DuPont Thermal Analyst 2100 Data System. The thermogram shown in Figure 5 was obtained at a scan rate of 1°C/minute, and yielded an apparent melt and decomposition at 145.7°C.

3.3.2 Thermogravimetric Analysis

The thermogravimetric analysis curve of the anhydrate phase of zileuton (Figure 6) was obtained on a DuPont Model 9900 Thermal Gravimetric Analyzer, at a scan rate of 5°C /minute. The thermogram revealed the existence of a weight loss at the same temperature range corresponding to the DSC endotherm, which is probably due to the sublimation and decomposition of the compound.

3.3.3 Sublimation Behavior

Zileuton has been found to exhibit some sublimation within the temperature range of 104-107°C under vacuum.

3.4 Hygroscopicity

The anhydrous form and a partially hydrated form of zileuton displayed some hygroscopicity when exposed to 75% relative humidity, but the monohydrate phase of zileuton was found not to be hygroscopic even at 75% RH. Both the anhydrate and monohydrate forms showed no hygroscopicity when exposed to 50% relative humidity.

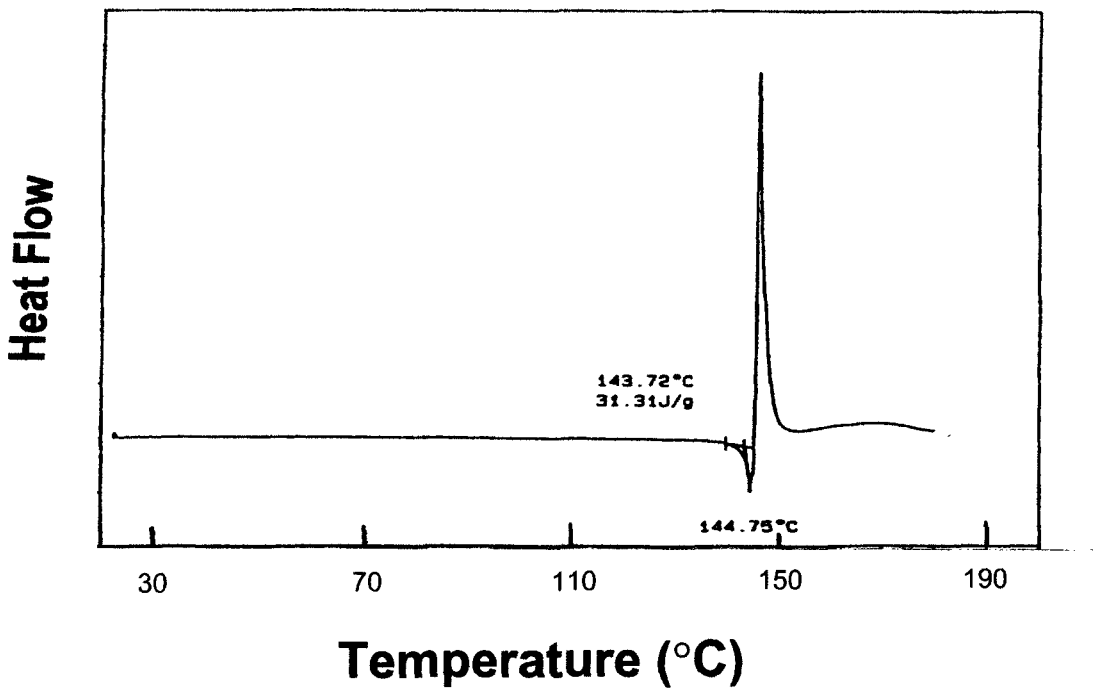


Figure 5. Differential scanning calorimetry thermogram of zileuton.

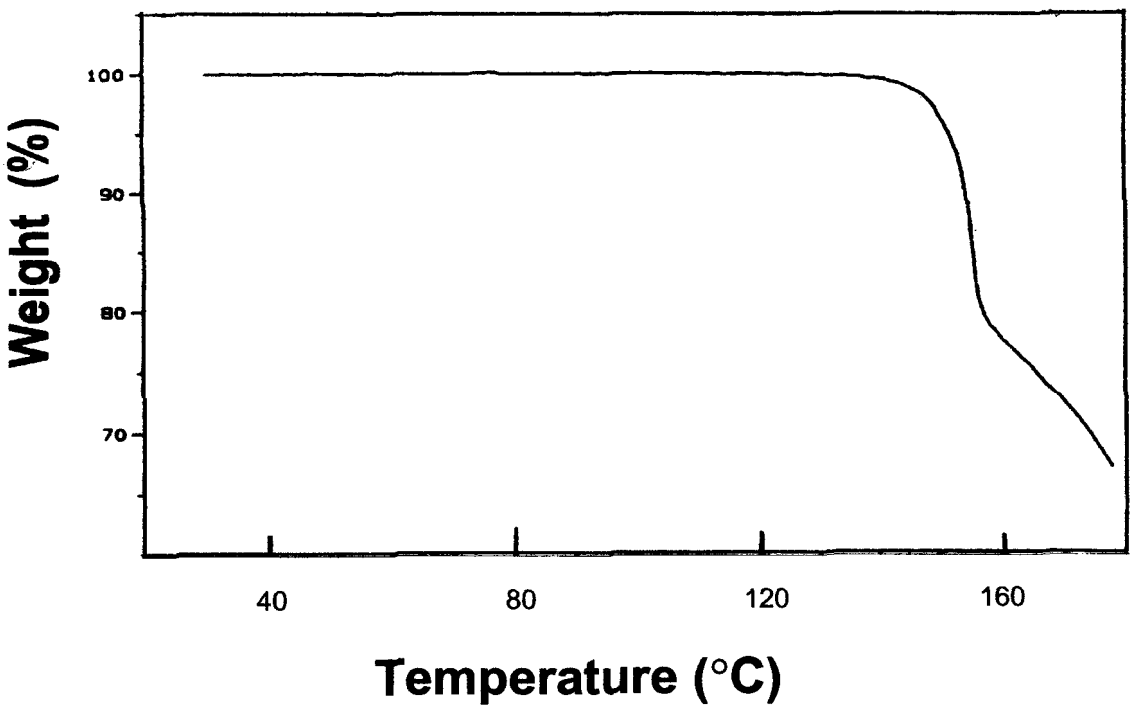


Figure 6. Thermogravimetric analysis of zileuton.

3.5 Solubility Characteristics

It has been determined that zileuton Forms 1 and 2, or any combination of these, have the same solubility in water. The intrinsic rate of dissolution was studied on a partial hydrate sample and on an anhydrous sample, but both samples were found to have the same intrinsic dissolution rate.

The drug substance is practically insoluble in water and hexane, slightly soluble in chloroform, and sparingly soluble in acetonitrile. It is soluble in methanol and ethanol, and freely soluble in N,N-dimethylformamide. A summary of the equilibrium solubility data determined for zileuton at 25°C is found in Table 3.

Zileuton is practically insoluble in acidic and neutral aqueous solutions. However, in basic aqueous solutions, the solubility is significantly increased to about 70 mg/mL at pH 12, as the data in Table 3 show.

3.6 Partition Coefficient

The n-octanol / water partition coefficient of zileuton was determined at 25°C by a shake-flask method, and the average value of four determinations was found to be 127.1 ± 0.6 ($\log P = 2.104$).

3.7 Equilibrium Constants

3.7.1 Acid Ionization Constants

The pKa of zileuton was determined using the Calvin-Bjerrum titration technique [2]. pH measurements were made during the acid titration of aqueous methanolic solutions of sodium hydroxide, both in the presence of and in the absence of zileuton. Extrapolation to pure water gave a pKa value of 10.3.

The pH dependence of zileuton solubility (Table 3) is consistent with a pKa value of approximately 10.5.

Table 3

Solubility of Zileuton in Various Media

Solvent system	Solubility (mg/mL)
Acetonitrile	12.7
Chloroform	4.3
N,N-Dimethylformamide	159.3
Ethanol (200 Proof)	49.8
Ethanol (190 Proof)	53.1
Hexane	0.04
Methanol	89.5
n-Octanol	14.9
2-Propanol	28.0
Water (pH 1.87)	0.18
Water (pH 3.80)	0.10
Water (pH 7.50)	0.12
Water (pH 10.1)	0.28
Water (pH 10.5)	1.50
Water (pH 11.6)	73.7

3.7.2 Metal-Ion Binding Constants

The metal-ion binding constants of zileuton with Ca(II), Mg(II), Cu(II), and Fe(II) were determined using a spectrophotometric technique [3]. The medium for this work was a phosphate buffer, maintained at pH 9.44. The mean pK values for Cu(II) and Fe(II) were found to be 6. 0 and 5.5, respectively, and no evidence for significant binding between zileuton and Ca(II) or Mg(II) could be detected.

3.8 Spectroscopy

3.8.1 UV/VIS Spectroscopy

When a 0. 0 1 % solution of zileuton in acetonitrile was scanned from 400 to 190 nm, the spectrum shown in Figure 7 was obtained [10]. Absorption bands were observed at the following five wavelengths:

Absorption Maximum (nm)	Molar Absorptivity (liter/mole•cm)
201	101.5
230	122.3
260	37.4
289	94
299	9.6

3.8.2 Fluorescence

A 0.002% solution of zileuton in methanol exhibits fluorescence when excited with ultraviolet light. The emission spectra consists of a single band having a maximum at 315-320 nm, with an excitation wavelength of 230 nm being appropriate. An example of a typical emission spectrum is shown in Figure 8.

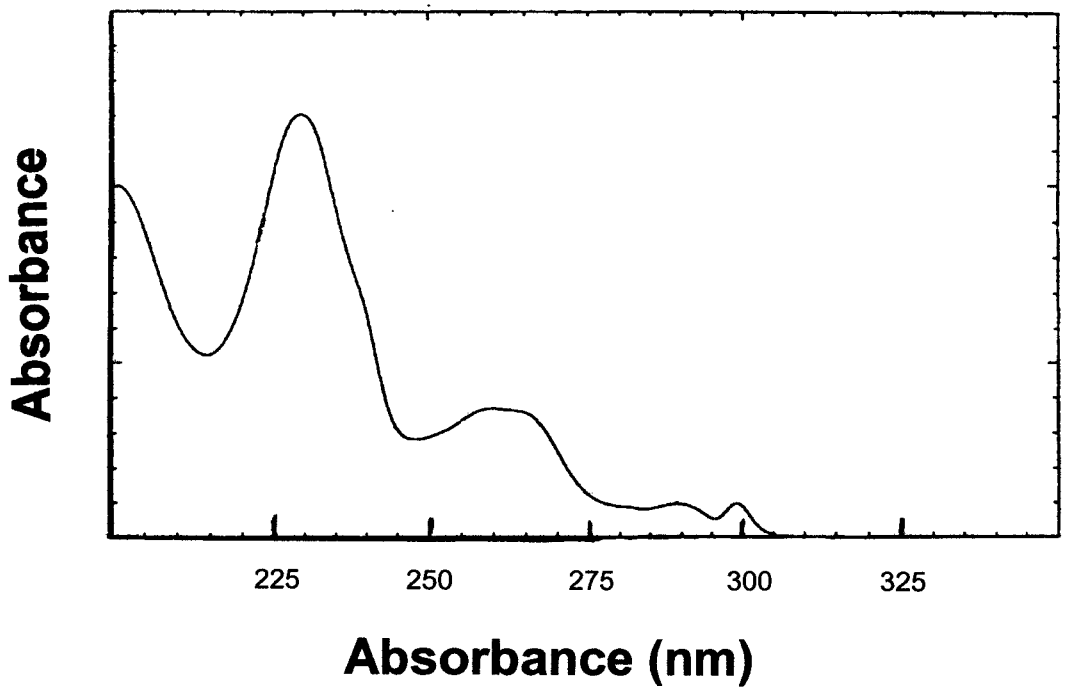


Figure 7. Ultraviolet absorption spectrum of zileuton.

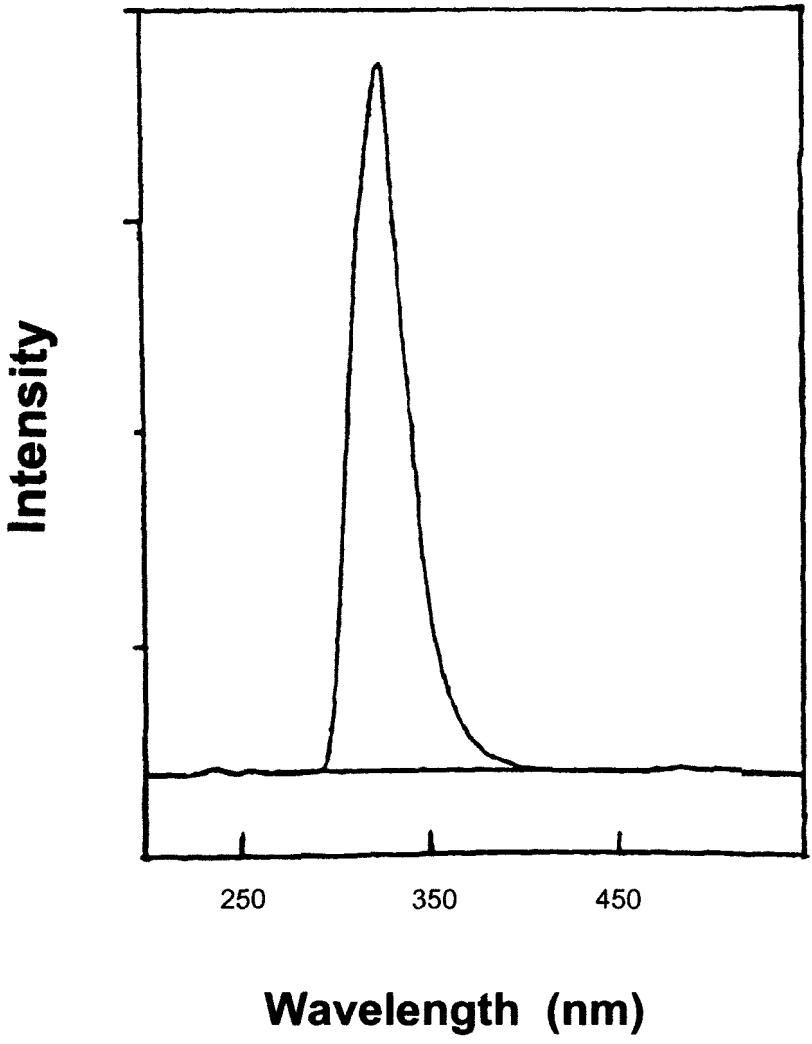


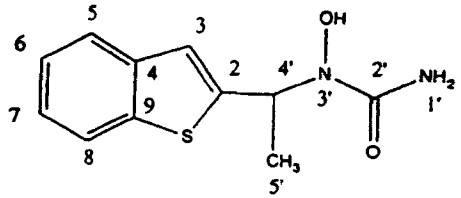
Figure 8. Fluorescence spectrum of zileuton, obtained using an excitation wavelength of 230 nm.

3.8.3 Vibrational Spectroscopy

The infrared spectrum of zileuton was measured as a potassium bromide dispersion, and is presented in Figure 9. The assignments of the prominent bands are collected in Table 4.

3.8.4 Nuclear Magnetic Resonance Spectrometry

The nuclear magnetic resonance spectra of zileuton were obtained on a General Electric GN 300 spectrometer, as a 20% w/v solution in deuterated dimethylsulfoxide. The numbering system used for the band assignments is as follows:



3.8.4.1 ^{1}H-NMR Spectrum

The proton magnetic resonance spectrum of zileuton is shown in Figure 10, and assignments for the observed bands are collected in Table 5. The proton coupling assignments are as follows:

Coupling Pair	J_{H-H} (Hz)
5 - 6	7.3
5 - 7	1.7
6 - 7	7.3
6 - 8	1.6
7 - 8	7.1
4' - 5'	7.0

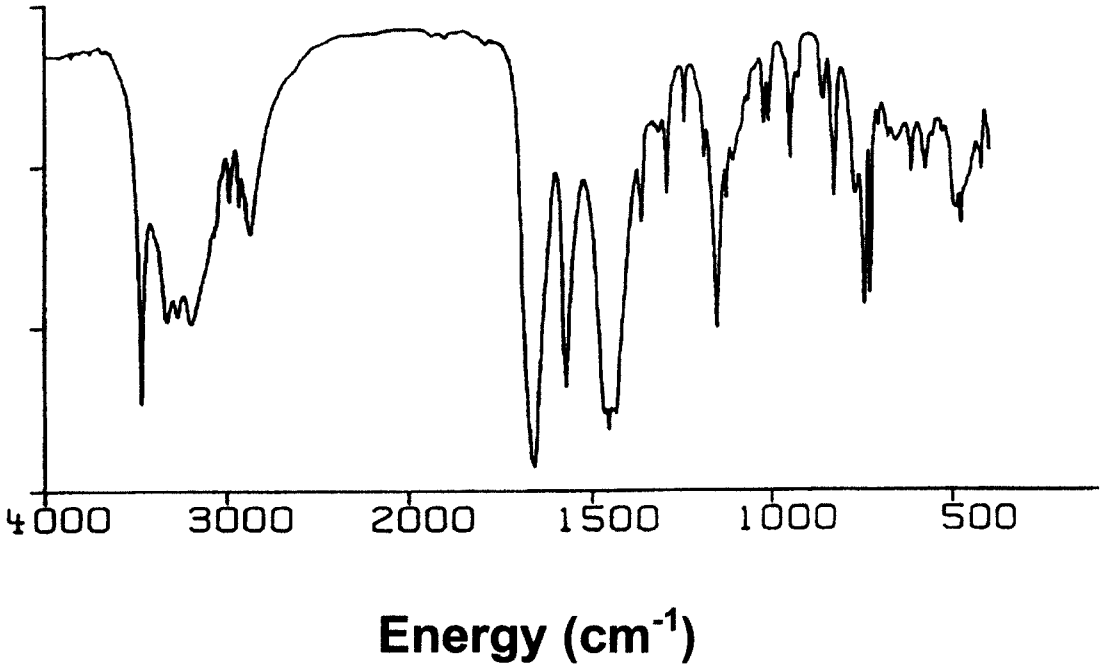


Figure 9. Infrared absorption spectrum of zileuton.

Table 4

Assignments for the Infrared Absorption Bands of Zileuton

Frequency Range (cm^{-1})	Band Assignment
3465	A sharp band is due to the N-H stretching vibrations of the NH_2 group
3420 - 2400	Bands due to the N-H stretching of the NH_2 group, the 0-H stretching of the OH group, the C-H of the aromatic rings, and the C-H of the alkyl CH and CH_3 groups
1750 - 1525	Bands are due to the stretching vibration of the C=O group, the aromatic rings, and the deformation vibrations of the NH_2 group
1525 - 1340	A composite band resulting from the superposition of several bands (plus a sharp band at 1369 cm^{-1}) are due in part to the stretching vibrations of the aromatic rings, the C-N of the $CONH_2$ group, and the deformation vibrations of the CH_3 group
1230 - 1040	A series of bands is due in part to the C-N stretching vibrations of the C-N-C moiety, and to the NH_2 rocking vibrations
744 - 728	Two bands are due to the wagging vibrations of the aromatic ring CH groups

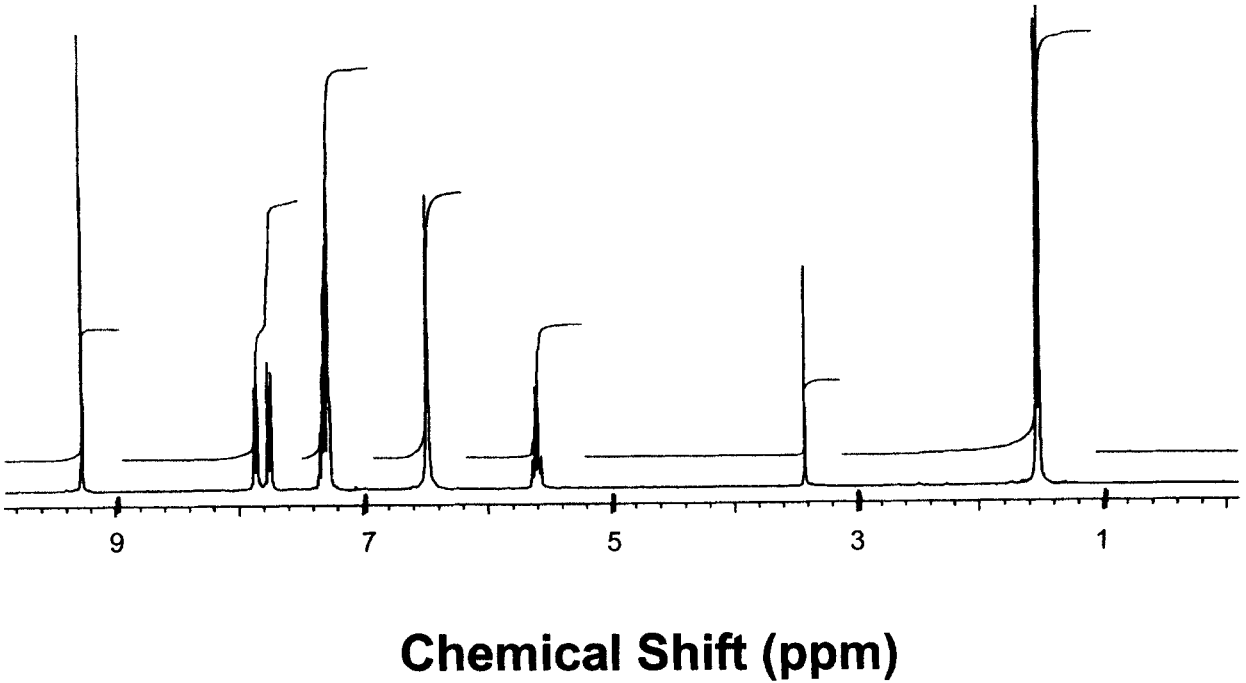


Figure 10. ^{1}H-NMR spectrum of zileuton.

Table 5

Assignments for the Nuclear Magnetic Resonance Spectral Bands of Zileuton

Position	Group	^{1}H-NMR Resonance (ppm)	^{13}C-NMR Resonance (ppm)
2	C		146.18
3	CH	7.28	121.37
4	C		139.14 *
5	CH	7.77	123.28
6	CH	7.33	124.13
7	CH	7.29	123.99
8	CH	7.88	122.19
9	C		139.02 *
1'	NH_2	6.5	
2'	C		161.50
3'	OH	9.29	
4'	CH	5.62	52.44
5'	CH_3	1.54	17.97

* The chemical shifts of C-4 and C-9 could be interchanged.

Notes:

1. The ^{13}C and the ^{1}H chemical shifts are referenced from the solvent DMSO-d^{6} resonances, which are assigned as 39.5 ppm and 2.50 ppm, respectively.
2. The singlet resonance at 3.44 ppm in the ^{1}H-NMR spectrum is due to residual water present in the solvent.
3. If a resonance has more than one peak due to coupling, an average is reported for the chemical shift.
4. The ^{13}C-NMR spectra are proton decoupled.

3.8.4.2 ^{13}C-NMR Spectrum

The carbon-13 magnetic resonance spectrum of zileuton is shown in Figure 11, and assignments for the observed bands are collected in Table 5.

3.8.5 Mass Spectrometry

The electron impact mass spectrum of zileuton was obtained using a Kratos MS-50 Mass Spectrometer, and is shown in Figure 12. The assignments for the prominent ions and subsequent fragments are shown in Table 6, and a scheme for the fragmentation patters in presented in Figure 13.

4. Methods of Analysis

4.1 Identification

The identity of zileuton can be confirmed by comparing the infrared absorption spectrum of the sample to that reported in Figure 9.

4.2 Elemental Analysis

A typical elemental analysis of a sample of zileuton is as follows:

Element	Theoretical (%)	Found (%)
carbon	55.91	55.76
hydrogen	5.12	5.15
nitrogen	11.86	11.86
sulfur	13.57	13.56
oxygen	13.54	13.58

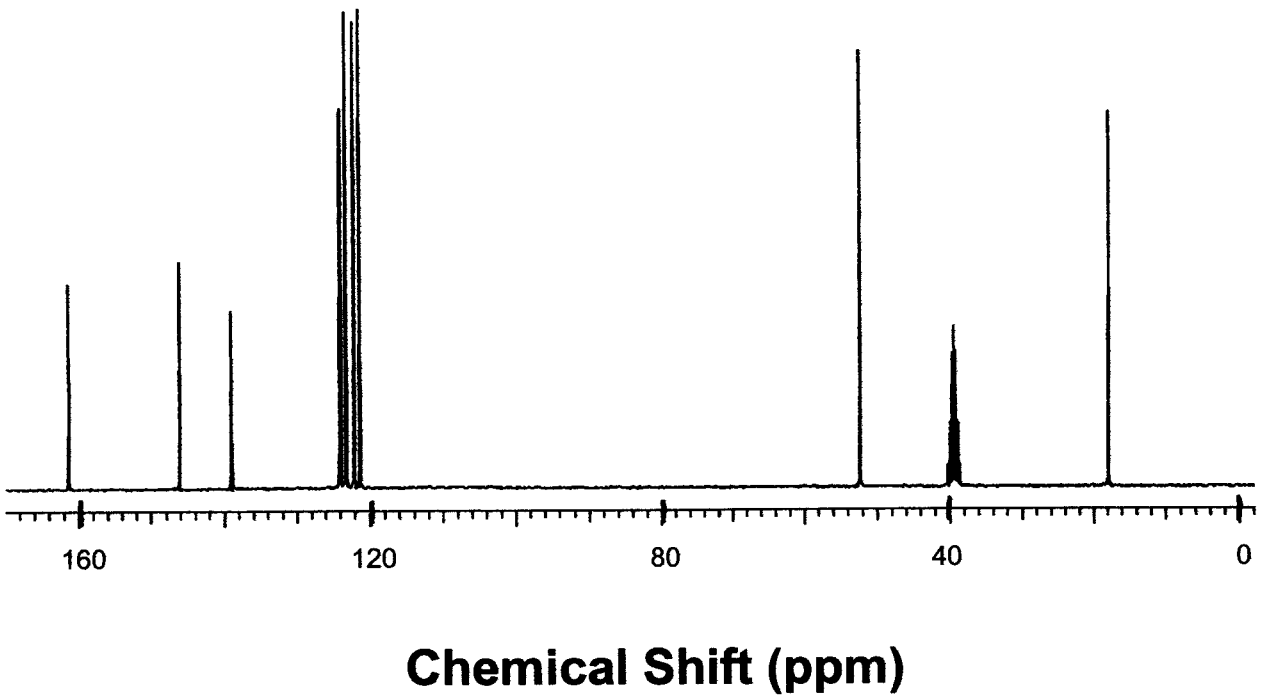


Figure 11. ^{13}C-NMR spectrum of zileuton.

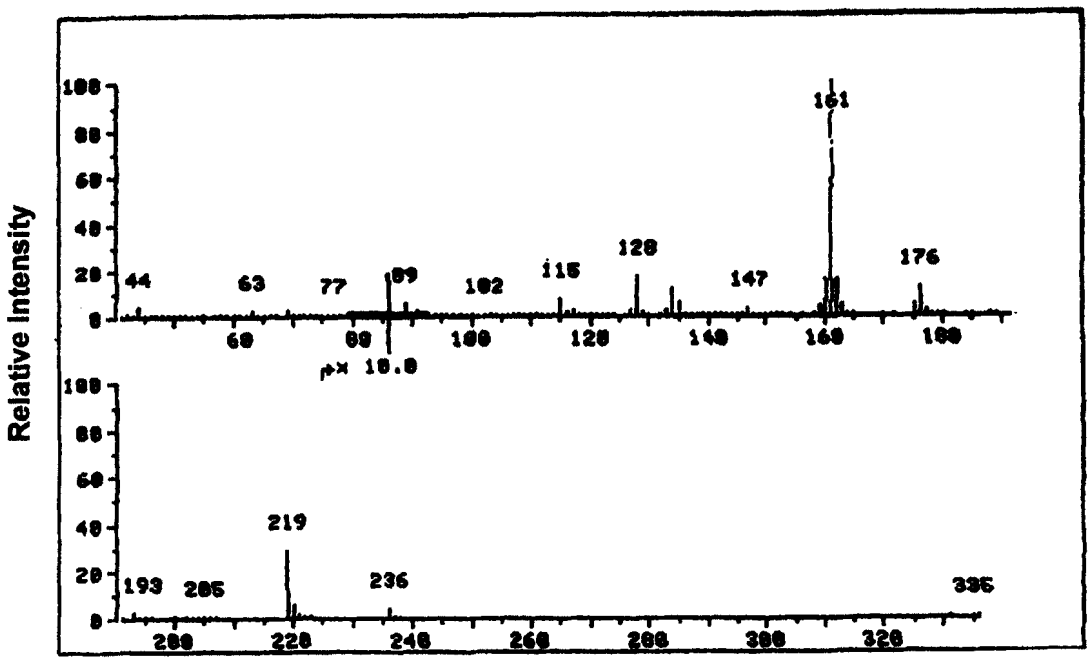


Figure 12. Electron impact mass spectrum of zileuton.

Table 6

Assignments and Exact Mass Measurements for the Electron Impact Mass Spectrum of Zileuton

Formula	Calculated Mass	Measured Mass
$C_{11}H_{12}N_2O_2S$	236.0619	236.0622
$C_{11}H_{12}N_2OS$	220.0670	220.0644
$C_{11}H_{11}N_2OS$	219.0592	219.0594
$C_{10}H_{10}NS$	176.0534	176.0532
$C_{10}H_9S$	161.0425	161.0422
$C_{10}H_8$	128.0626	128.0626

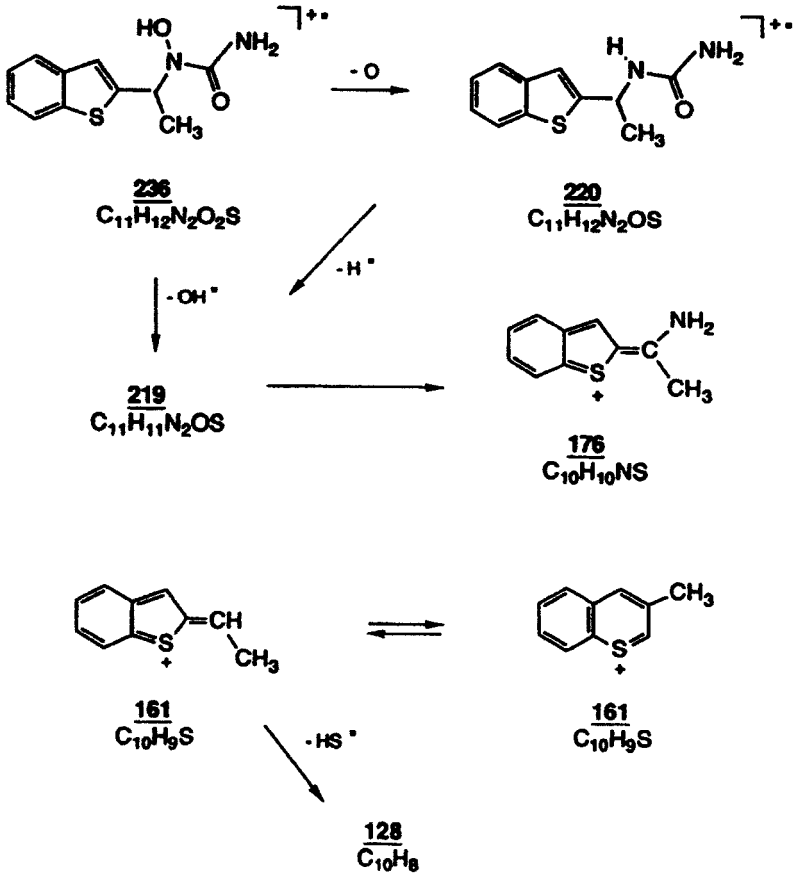


Figure 13. Mass spectral fragmentation of zileuton.

4.3 Chromatographic Methods of Analysis

4.3.1 Thin Layer Chromatography

Several thin-layer chromatographic systems have been investigated to evaluate the purity of zileuton. Two of these have been found to give the best separation of zileuton, its impurities, and degradation products.

System I uses ethyl acetate to effect the analytical separation on silica gel 60 F_{254}, with the detection being made with short-wave UV irradiation. N-(l-benzo[b]thien-2-ylethyl)-urea (R_f, 0.12), (Z)-l-benzo[b]thien-2-ylethanone oxime (R_f, 0.59), (E)-l-benzo[b]thien-2ylethanone oxime (R_f, 0.65), and N-1benzo[b]thien-2-ylethyl) hydroxylamine can be separated from zileuton (R_f, 0.21).

System II uses a mobile phase consisting of 50:50:1 chloroform / methylene chloride / ammonium hydroxide, the separation being performed on silica gel 60 F_{254}, and the detection is made with short-wave UV irradiation. This system can be used to separate (Z)-1-benzo[b]thien-2-ylethanone oxime (R_f, 0.06) (E)-l-benzo[b]thien-2-ylethanone oxime (R_f, 0.17), 1-benzo[b]thien-2-ylethanone (R_f, 0.56), 1-benzo[b]thien-2-ylethyamine (R_f, 0.07), and O-(l-benzo[b]thien-2-ylethyl)-l-benzo[b]thien-2-ylethanone oxime (R_f, 0.33) from zileuton (R_f, origin).

4.3.2 High Performance Liquid Chromatography

Several HPLC methods have been developed to evaluate the quality of zileuton drug substance. System I is used to quantitate the bulk drug substance potency, while systems II and III are used to quantitate the impurities and degradation products in bulk substance. The characteristics of Systems II and III are such that they cover the range of polarities associated with the various impurities in zileuton. System II is used to monitor the degradation products.

System I

Mobile Phase:	0.1 M ammonium acetate solution containing 0.025 % acetohydroxamic acid (adjust the solution with perchloric acid to pH 2.0) / acetonitrile (72:28)
Column:	30 cm x 1/4 " (o.d.) x 4.6 mm (i.d.) packed with Spherisorb S10 ODS
Flow Rate:	approximately 1.5 mL/minute
Detector:	260 nm, 0.1 AUFS

System II

Mobile Phase:	0.1 M ammonium acetate solution containing 0.025 % acetohydroxamic acid (adjust the solution with perchloric acid to pH 2.0) / acetonitrile (82:18)
Column:	30 cm x 1/4 " (o.d.) x 4.6 mm (i.d.) packed with Spherisorb S10 ODS
Flow Rate:	2.2 mL/minute
Detector:	260 nm, 0.01 AUFS

System III

Mobile Phase:	1:1 acetonitrile / 0.5% perchloric acid
Column:	30 cm x 1/4 " (o.d.) x 4.6 mm (i.d.) packed with Spherisorb S10 ODS
Flow Rate:	approximately 1.5 mL/minute
Detector:	260 nm, 0.01 AUFS

In addition, a direct chiral chromatography method for the separation of zileuton enantiomers has been developed, which uses the following conditions:

Mobile Phase:	92:8:0.1 hexane / 2-propanol / trifluoroacetic acid
Column:	Daicel Chiralpak AD, 250 x 4.6 mm (i.d.) (Regis) - operated at 25°C
Injection volume:	10 μL (0.1 mg/mL)
Flow Rate:	1.0 mL/minute
Detector:	260 nm, 0.02 AUFS

4.4 Determination in Pharmaceutical Dosage Forms

The potency and primary degradation products in zileuton tablet formulations can be analyzed by a high performance liquid chromatography procedure using methyl 4-hydroxybenzoate (methylparaben) as the internal standard. The method uses a Spherisorb S10 ODS, 10 μm column, a mobile phase consisting of 72 parts of 0.1 M ammonium acetate solution containing 0.025 % acetohydroxamic acid (adjusted with perchloric acid to pH 2.0) and 28n parts acetonitrile.

4.5 Determination in Body Fluids

Simultaneous determination of zileuton and its N-dehydroxylated metabolite in untreated rat urine by micellar liquid chromatography was developed by Thomas and Albazi [23]. Separation of these compounds is achieved using sodium dodecyl sulfate (SDS) as the mobile phase, a CN-bonded silica column, and UV detection at 262 nm. Because of the solubilizing power of the micellar mobile phase, urine samples were injected into the system without any time-consuming protein precipitation and/or drug extraction steps.

A HPLC method was also developed for the determination of zileuton and its inactive N-dehydroxylated metabolite in plasma [24].

5. Stability

The identified degradation pathways of zileuton are collected in Figure 14.

5.1 Solid-State Degradation

Zileuton in the solid state was determined to be stable after a one-month exposure to sunlight and fluorescent light, a temperature of 60°C, or elevated humidity. Upon exposure to sunlight and fluorescent light at intensities of 600 and 1500 foot-candles for one month, zileuton samples were observed to yellow, with degradation amounting to <0.1%, <0.1%, and about 0.2% respectively. Although solid zileuton was noted to yellow considerably after 6 hours of exposure to high intensity UV radiation, only about 0.7% degradation took place. Zileuton was found to be less stable at upon exposure to higher temperatures (*i.e.*, 90°C for one month), where 14% degradation was found.

The primary degradation products observed in the solid-state degradation studies were (Z)-1-benzo[b]thien-2-ylethanone oxime, (E)-1-benzo[b] thien-2ylethanone oxime, and N-(l-benzo[b]thien-2-ylethyl) urea.

5.2 Hydrolytic Degradation in Solution

Aqueous solutions of zileuton were subjected to unstressed and stressed exposure under various conditions. Aqueous solutions standing for one hour showed essentially no degradation in acid, less than 0.1% in base, and about 0.1 % in neutral solution. After 15 minutes at reflux temperature, a neutral aqueous solution showed approximately 23% degradation, while an acidic solution showed about 60% degradation. Five minutes of refluxing in base produced about 67% degradation.

The primary degradation product noted in the neutral and acidic aqueous solutions was N-(l-benzo[b]thien-2ylethyl) hydroxylamine. The primary degradation products observed in the basic aqueous solution were N-(1-benzo[b]thien-2-ylethy) hydroxylamine, (Z)-1-benzo[b]thien-2-ylethanone oxime, and (E)-1-benzo[b]thien2-ylethanone oxime.

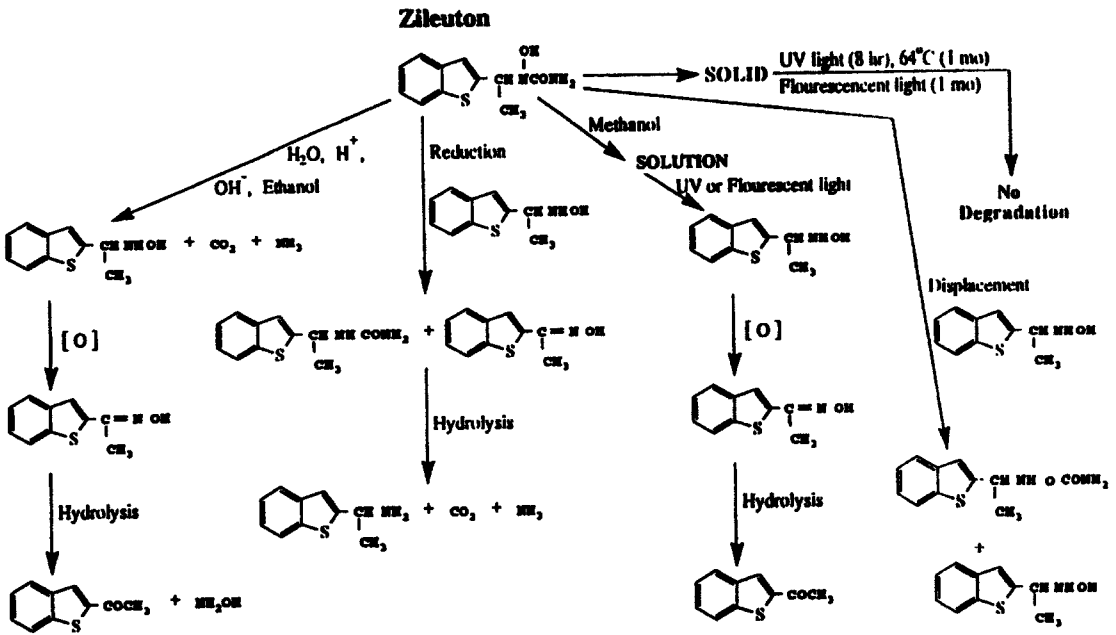


Figure 14. Degradation pathways of zileuton.

5.3 Photolytic Degradation in Solution

Extensive degradation of the compound occurred when acetonitrile solutions of zileuton were subjected to two weeks exposure to 600 and 1500 foot-candles of fluorescent light. The degrees of degradation in these studies were approximately 35% and 55%, respectively. The primary degradation products observed were (Z)-1-benzo[b]thien-2-ylethanone oxime and (E)-1-benzo[blthien-2-ylethaone oxime.

Total degradation of zileuton occurred when an acetonitrile solution was exposed to high intensity UV light for 4 hours, but less degradation took place in methanol solutions. In methanol, 9% degradation was noted at exposure to 600 foot-candles, 14% degradation after exposture to 1500 foot-candles, and 47% after exposure to high-intensity UV light. The primary degradation products observed in this work were (Z)-1-benzo[b]thien-2-ylethanone oxime and (E)-1-benzo[b]thien-2-ylethanone oxime.

When zileuton was dissolved in a solution of 1:1 acetonitrile / water, about 0.6% degradation occurred after 24 hours at ambient conditions. The primary degradation product observed was N-(l-benzo[b]thien-2-ylethyl) hydroxylamine.

5.4 Oxidative Degradation in Solution

Oxidative degradation was examined in aqueous solutions of 5% (v/v) hydrogen peroxide, with about 1% degradation of zileuton occurring after one week exposure to the solution. The primary degradation products observed in the hydrogen peroxide solution were (Z)-1-benzo[b]thien-2-ylethanone oxime and (E)-1-benzo[b]thien-2-ylethanone oxime.

Exposure of zileuton to 0.4% (w/v) sodium hypochlorite solution produced extensive degradation of the compound (about 50%) immediately upon exposure. The primary degradation products observed in this study were 1-benzo[b]thien-2-ylethanone, (Z)-1-benzo[b]thien-2-ylethanone oxime, and (E)-1-benzo[b]thien-2-ylethanone oxime.

6. Drug Metabolism and Pharmacokinetics

Metabolism of arachidonic acid by the 5-lipoxygenase enzyme leads to the formation of a group of biologically active lipids known as leukotrienes. Leukotrienes have been proposed as important mediators in allergic and inflammatory disorders. Inhibitors of 5-lipoxygenase, by blocking leukotriene synthesis, have therapeutic potential in a range of diseases including arthritis and asthma. A review summarizing the biology of leukotrienes and the current knowledge of the mechanism of 5-lipoxygenase has been published by McMillan and Walker [4].

Machinist *et al.* (5) evaluated the metabolism of orally administered ^{14}C-zileuton in Cynomolgus monkeys [5]. After oral administration of a single 5 mg/kg dose ^{14}C -zileuton to female Cynomolgus monkeys, an average of 92% of the administered radioactivity was found in the 0-24 hour urine, 86% of which was recovered after 7 hours. Only 0.3% of the dose was found in the feces. Mean concentrations of total plasma radioactivity peaked at 6.5 μg equivalents/mL by 0.5 hours and then declined to 2.5, 0.6, and 0.3 μg equivalents/mL at 2, 4, and 6 hours, respectively.

Zileuton was subjected to rapid and complete first pass metabolism, predominantly by Phase II reactions. The major metabolites in urine and plasma were two glucuronic acid conjugates of parent drug. Based on their reactivity with β-glucuronidase and identical mass spectra, they may represent isomers of zileuton-N-O-glucuronide. Both of the glucuronides accounted for 87 % of the dose in the 0-24 hour urine. Free zileuton was not detected in any of the urine samples. Plasma levels of both glucuronides peaked at 0.5 hours (approximately 6 μg equivalents/mL) and declined to *ca.* 2.4 μg equivalents/mL at 2 hours. Maximum levels of zileuton also occurred at 0.5 hours (*ca.* 0.32 μg /mL) and diminished to 0.01 μg/mL after 2 hours. From 0.25 to 2 hours after dosing, the plasma concentrations of the glucuronides exceeded that of zileuton by at least 19-fold. The rapid absorption, excretion, and extensive metabolism of zileuton provide an explanation for the very short half-life and low plasma levels of the drug.

Preliminary studies in humans by Braeckman *et al.* demonstrated that zileuton is eliminated primarily as a glucuronide conjugate [6].

The glucuronidation of the (*R*)-isomer and (*S*)-isomer of zileuton was examined by Sweeny and Nellans using human hepatic microsomes [7]. The glucuronidation of both isomers followed Michaelis-Menten kinetics, but glucuronidation rates were between 3.6- and 4.3-fold greater for the (*S*)-isomer. The results of this study indicate that glucuronidation of the zileuton isomers in human hepatic microsomes is stereoselective. The stereoselective glucuronidation may be the basis for the more rapid clearance of the (*S*)-isomer observed in humans receiving zileuton.

In vitro plasma protein binding of zileuton and its N-dehydroxylated metabolite was studied by Machinist and Bopp [8].

The pharmacokinetics of single oral doses of zileuton (200 to 800 mg), its enantiomers, and its metabolites, in normal healthy volunteers were reported by Wong *et al.* [9].

Awni *et al.* reported the effect of mild or moderate impairment (cirrhosis) on the pharmacokinetics of zileuton [10], the effect of food on the pharmacokinetics of zileuton [11], pharmacokinetics and pharmacodynamics of zileuton after oral administration of single and multiple dose regiment of zileuton (600 mg) in healthy volunteers [12], diurnal variation in the pharmacokinetics of zileuton in humans [13], and population pharmacokinetics of zileuton in patients with rheumatoid arthritis [14].

Braeckman *et al.* reported the pharmacokinetics of zileuton in healthy young and elderly volunteers [15], and pharmacokinetics of zileuton in subjects with various degrees of hepatic impairment [16].

The metabolic interaction between zileuton and phenytoin, as determined by Michaelis-Menten kinetics, was reported by Samara *et al.* [17].

The effects of a 5-lipoxygenase inhibitor on asthma induced by cold dry air were studied by Israel *et al.* [18]. The 5-lipoxygenase enzyme catalyzes the metabolism of arachidonic acid to form products that have been implicated in the airway obstruction of asthma. The study indicated that a selective inhibition of 5-lipoxygenase by zileuton is associated with a significant amelioration of the asthmatic response to cold, dry air, suggesting that 5-lipoxygenase products are involved in this response.

Israel *et al.* reported the effect of inhibition of 5-lipoxygenase by zileuton in mild-to-moderate asthma [19]. The results enabled the conclusion that inhibition of 5-lipoxygenase can improve airway function and decrease symptoms and medication use in patients with asthma.

Israel et al. also reported the effect of three months of treatment with zileuton in patients with mild to moderate asthma [20]. They showed that three months of 5-lipoxygenase inhibition produced a significant improvement in asthma control.

The effects of zileuton on antipyrine and indocyanine green disposition were studied in 16 healthy, non-smoking adult men by Peter *et al.* [21]. It was concluded that zileuton therapy has no detectable effect on indocyanine green disposition, but exerts marked effects on antipyrine plasma and urine metabolic disposition.

Pharmacokinetics, safety, and ability to diminish leukotriene synthesis by zileuton were evaluated by Rubin *et al.* [22]. Zileuton was given to normal human volunteers in single doses of 200 mg to 800 mg. These studies indicated that orally administered zileuton was well-absorbed, with an elimination half-life of approximately 2.5 hours. Uukotriene B4 production by *ex vivo* calcium ionophone stimulated whole blood was inhibited by up to 80% of baseline, and correlated with plasma concentrations. Zileuton did not significantly inhibit cyclooxygenase as demonstrated by thromboxane B2 levels.

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