KIM HUYNH-BA
Editor

Handbook of Stability Testing in Pharmaceutical Development

Regulations, Methodologies, and Best Practices

Springer

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A dream you dream alone is only a dream
A dream you dream together is reality
John Lennon

Preface

In my professional career as a pharmaceutical scientist, I have been involved with several aspects of the drug development process from pre-IND to commercialization and, somehow, I usually found myself coming back to a stability-related issue. The stability area seemed to draw my utmost interest because in my day-to-day work, my opportunities involved more than one product, and none of the issues were the same. Each situation posed challenges that usually required an exercise of judgment, an understanding of regulations, a knowledge of science, a grasp of compliance, and an appreciation of common practices.

Since early 2000, I have also been involved with several training opportunities and I struggled to find good, concise, practical resources, one of which I could just hand to a new scientist who wishes to gain a greater understanding of stability sciences. In addition, I encountered the same questions posted over and over on different stability best practices discussion forums.

As a book lover, I also have a good collection of technical books. Unfortunately, most of the stability related volumes are outdated. Many of these materials are theoretical and do not contain much practical information. I understand that the pharmaceutical industry during this period is quite volatile, and guidelines are changing rapidly while regulatory agencies are working closely with the pharmaceutical industry to accommodate these changes; however, the fundamental information continues to remain quite the same, just as current Good Manufacturing Practices (cGMP) continue to be the standard industry practice. Therefore, I hoped to assemble a practical handbook to fill this void.

Handbook of Stability Testing in Pharmaceutical Development is a product of several dedicated stability scientists. Collectively, we have over 300 years of experience working in all aspects of the pharmaceutical industry. This volume is intended to bring together a comprehensive overview of a stability program coupled with practical best practices. It can be used to serve the stability community as a handbook to train new scientists who find themselves involved with stability sciences in multidisciplinary functions. It can also be used in an academic setting so students can gain more practical understanding of the pharmaceutical industry.It contains

essential information to guide best practices for development and management of a compliant stability program.

July 2008 Kim Huynh-Ba

Editorial Notes

Contributing authors are responsible for the content and ideas included in their chapters. Although much information is presented and recommendations are drawn based on scientific knowledge of the experts, review perspectives may vary depending on technical background, personal experiences, and discussion preference. In addition, many references are cited from web links that appear to be valid at time of press. Great efforts were made to assure the book is as accurate as possible; however, the editor wishes to hold no responsibility for, nor can she endorse, the material published in this publication.

Acknowledgments

This project would not be possible without support from the following individuals. To them, I am in debt with appreciation.

I would like to thank all contributors for their work. They exemplify the expertise of their field and I am humble to have the privilege of editing and coordinating their work. Many of them have been my mentors, colleagues, and friends who provide enormous support throughout my professional career. I honor their trust in me leading this important project.

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I would like to thank my graduate school mentor and a special friend, the late Dr. Robert Lee Grob, who was an exceptional role model and who had encouraged me to initiate this project.

Last but not least, I would like to thank my family – my husband Thai, my sons John and James – for their encouragement and for putting up with me while getting this important work done. My appreciation also goes to my parents, Mr. Hong Nguon Cao and Mrs. Kimhoa Ngoc Bach, for their unconditional support.

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Part I
Stability Regulations

Chapter 2
Critical Regulatory Requirements for a Stability Program

Alvin J. Melveger and Kim Huynh-Ba

Contents

Abstract This chapter addresses the principles of stability studies in the drug development process. It gives an overview of different types of stability studies that support the entire drug development phases. It also discusses the purpose that one wants to achieve with the data set that these studies generate.

This chapter also discusses stability issues within the framework of the FDA cGMP guidelines as expressed in 21CFR Part 211. This review of cGMP regulations that tie to the stability program as well as to the testing laboratory is essential for pharmaceutical analysts to understand the process. This applies to all phases of stability studies including set up, testing, data review, and follow up on out-of-specification results.

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Details of FDA and ICH regulations are found in Chapter 3 – Understanding ICH Guidelines Applicable to Stability Testing. Other details on how to manage a stability program are addressed in subsequent chapters of this book.

2.1 Stability Role in the Drug Development Process

Stability plays an important role in the drug development process. It explains several factors that affect the expiration dating of drug products, including the chemical and physical stability during the pre-clinical formulation stages, process development, packaging development, and post-marketing life. The evaluation of the physicochemical stability of a given product requires an understanding of the physical and chemical properties of the drug substance [1]. Lack of drug substance or drug product stability may affect the purity, potency, and safety of the drug product.

Pharmaceutical stability may be applied in several ways; therefore, the performance of a drug will be evaluated depending on whether it assesses a drug substance, a formulation, a drug product, or a packaged product [2]. The safety and efficacy of a drug product are established during the development process via preclinical animal and human clinical studies. The quality attributes such as identity, concentration, and purity are defined, and testing is developed. Should drug properties change beyond the accepted criteria during a stability study, then the established safety and efficacy data may no longer be applicable. Changes in drug stability could risk patient safety, since the dosage amount to patient may be lower than expected. Instability may also lead to formation of toxic degradants.

If instability of a drug product leads to these unwelcome effects on patients, it could also lead to expensive costs to manufacturers as they attempt to discover the reasons for instability and methods of minimizing them. An unstable product would highlight an uncontrolled process, and could require a substantial product and process investigation with possible product recalls. FDA has authority to issue cGMP violations with follow-up warning letters and possible consent decrees and criminal prosecutions.

Stability testing therefore allows the establishment of recommended storage conditions, retest periods, and ultimately product shelf-life and expiry dating. Stability considerations will dictate the environment for drug substance preparation and storage, choice of packaging, and allowable shelf-life of the final drug product. Should a drug substance be sensitive to environmental factors such as temperature, humidity, pH, light and oxygen exposure, these must be considered and controlled when designing processing, storage, and final packaging of the drug product.

For example, a light-sensitive drug will require the minimization of exposure to certain light wavelengths during handling and the choice of final dispensing containers. Oxygen-sensitive materials will require handling under an inert atmosphere, such as nitrogen, and the addition of oxygen scavengers in the drug product container. In considering drug stability, attention must be paid to processes which may lead to instability of the product. The reactivity of the drug substance and the environment must be considered as well as potential interaction of all constituents in the drug product, excipients, and packaging. For liquid preparations, the possibility

of contamination by extractables from the container materials may occur during long-term storage. Container materials must be chosen to eliminate or minimize extractables.

2.2 Types of Stability Studies

Stability studies are used to provide data to support clinical trials, registration submission, or commercialization. There are different types of stability studies during the drug development process, which are diagrammed in Fig. 2.1.

Each phase of drug development requires addressing the time period that the drug product continues to maintain its specifications. This period is called *expiration dating* period of a drug product. Current GMP indicates that the purpose of stability testing of the final packaged drug product is to assure that a drug product meets applicable standards of identity, strength, quality, and purity at the time of use.

The use of stability testing is an integral part of the outlined development process and will be further described.

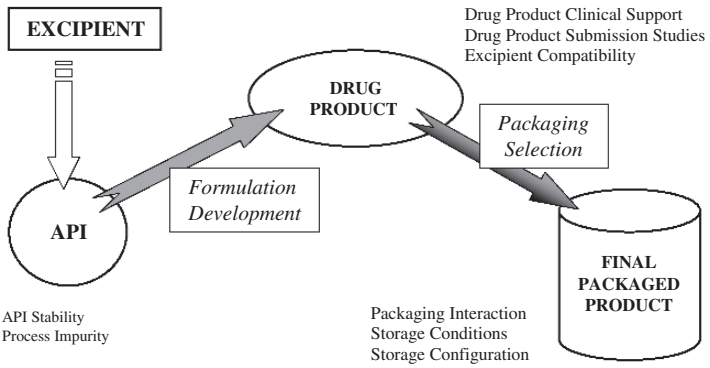

Fig. 2.1 Stability studies to support development of new drug product

2.2.1 Stability of Active Pharmaceutical Ingredient (API)

Before any formulation work is developed, it is necessary to determine the nature of the API. Its purity profile must be established and specifications set for the allowed levels of impurities. The change of impurities with storage time must be established by subjecting the API to various accelerated and stress storage conditions to establish conditions which minimize the formation of degradants. These early stability studies may determine that the API should be stored under non-ambient conditions such as low temperature, low humidity, and non-oxidizing and low-light environments. These stability studies should be continued to determine the optimum storage conditions for holding the bulk API before actual processing. Stability studies of

the API will provide data to establish a retest time for the raw materials used in the process. Stability indicating methods must be developed to monitor the purity of the API as well as identification and quantitation of impurities. If impurities are shown to be process related, then they may be monitored at release but do not need to be monitored during long-term stability. However, if any of these impurities are shown to increase during storage, or if new impurities are developed, these are referred to as "degradants" or "degradation products", and analytical methods must be developed to monitor these degradants during stability studies. Quality specifications and limits must also be set for the degradants as required by ICH.

2.2.2 Stability Studies to Support Formulation Development

Excipients or non-active constituents may be added to an API to develop a formulation which meets the intended performance criteria of the drug product. These excipients may be necessary for purposes of adding color, or controlling pH, moisture, or oxygen content. Interaction of the excipients with one another or with the API will be determined, as well as the rates of these reactions, through stability studies. Data of these studies, so-called *excipient compatibility*, will be used to determine the appropriate formulation for the drug product. If interactions occur, then the products of these interactions (degradants) must be evaluated for safety, and analytical procedures for ID and quantitation must be developed. Krummen gave an overview of some issues which can arise in stability testing during preparation development. He indicated that stability testing is a continuous process as information on the drug substance and the first provisional dosage forms is synergistic and builds the basis for the development of the dosage form which will be marketed [3].

Many companies also manufacture small batches at the extreme of the manufacturing process capabilities. These batches are then placed on stability stations to determine the stability profiles of the drug product, to better understand the process capabilities.

2.2.3 Stability Studies to Support Production and Use of Pre-clinical and Clinical Supplies

During the formulation development studies, batches are made to support clinical studies. Pre-clinical stage formulations are usually used for testing in animals. Stability studies are performed to show that pre-clinical samples maintain their specifications over the entire time span of the animal study. The formulation being tested must be stable to assure that all animals receive the nominal dose and purity from start to finish of the study.

As the drug product enters subsequent clinical phases, materials are needed to support these clinical evaluations. Stability studies are necessary to support these materials. In most cases such studies would only require long-term storage; however, most companies conduct additional accelerated or stress studies on the clinical

materials to gain more understanding of the drug product. This data set is also used to set expiry of clinical supplies.

A stability survey was done in 2007 by AAPS Stability Focus Group, benchmarking industry standards and practices of their stability operations within the pharmaceutical and biopharmaceutical industry. It noted that the majority of the industry has used ambient room temperature as the long-term storage condition to conduct stability studies to support clinical trial application.

2.2.4 Stability Studies to Support Drug Registration

Final packaged product must be shown to be stable up to at least the expiry date. These stability data are obtained by actual testing through the expiry date and beyond. Early term stability data may be submitted to FDA or other regulatory bodies to support preliminary expiry dating. These data as well as data obtained under accelerated storage conditions may be utilized to predict ultimate stability and to establish rates and kinetics of degradation.

ICH requires at least 12 month long-term stability data of three batches of drug products as necessary for drug registration. In addition, accelerated and stress studies are also conducted to establish a tentative expiration date. More detailed information on ICH guidelines are covered in Chapter 3. Global regulations are also discussed in Chapter 4.

2.2.5 Stability Studies to Support Marketed Products

Expiry dating of a drug product must be determined on the actual packaged drug product over the period of time indicated by the expiry date. Although extrapolated stability data may be used to support product registration, real time data must be established to support actual product dating. In addition, sampling of newly manufactured production lots of product must be monitored on a continuing basis, at least to the projected expiration date or beyond, and data submitted to FDA.

After approval is received for the drug product, stability studies are continued to support commercialization of the drug product. Representative lots are put on stability station for annual product monitoring.

In addition, post-approval studies would also be necessary if there is any change to the processing or packaging of the drug product. More details of stability requirements and regulations are discussed in Chapter 5.

2.3 Scientific Principles of Stability Testing

Based on ICH Q1A(R2), *"the purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and*

light" [4]. Therefore, stability studies provide data to justify the storage condition and shelf-life of the drug product. For drug substance, such studies establish the retest date in addition to the storage condition of raw material.

Stability of a drug substance or drug product during drug synthesis, formulation, and storage must be ascertained. Instability could lead to chemical degradation and loss of drug potency and the possible formation of new chemical species with potential toxic side effects. Therefore, early evaluation of a drug substance should include elucidation of stability under a number of environmental conditions. To aid in the prediction of drug stability, forced or accelerated degradation is performed to elucidate potential degradation products, determine their safety, and develop analytical procedures to quantitate these new chemical species. These forced degradation studies may be predictive of the degradation pathways of the drug under normal conditions. In fact, information learned from studying the kinetics of degradation may be used to extrapolate rates of degradation which might apply during normal storage conditions and could be utilized to predict long-term stability under these normal storage conditions [5].

The development of appropriate analytical methods will then aid in the development of purification schemes to remove degradants and to allow the development of drug impurity profiles which will be used for setting purity specifications and for defining the drug which is to be utilized in pre-clinical animal and later human studies.

The analytical procedures to assess stability must encompass the elements common to validating analytical assays. The methods must be validated according to the parameters of accuracy, precision, robustness and specificity, limits of detection and quantitation, linearity of active ingredient assays, degradants, and other reaction products. More information on how to develop stability indicating methods is discussed in Chapter 7. Validation of these methods is discussed in Chapter 8.

These stability studies will expose the drug to potentially degrading conditions including moisture, oxygen, pH, temperature, and light. Discovery that a drug has a very restricted stability range will affect process and packaging development, and labeling for long-term shelf-life. Sensitivity to such environmental factors may also dictate the necessity for inclusion of stabilizers in the formulation and will dictate the choice of dosage form and packaging. It may turn out that such restricted stability and associated developmental costs to remedy the situation will be sufficient to eliminate a potentially viable drug product. For products which are expected to be sold and used worldwide, attention must be given to differing climate zones when considering expiry dating and long-term stability.

Drug stability must be assured during the critical pre-clinical animal testing and subsequent human testing. This requires that the drug that is used from beginning to end of a study be characterized for concentration and impurity levels throughout the study to assure that the drug has not changed. This characterization will then define the drug profile that is to be the specifications as to safety and efficacy.

For solid dosage forms, the solubility, efficacy, and stability of a drug may depend on the particular crystalline state of the drug. Many crystalline drugs can exist in different crystalline states called polymorphs. It is expected that characterization of the solid dosage forms include not only the chemical identity but the polymorphic

distribution as well. The polymorphic content may be characterized by techniques such as x-ray powder diffraction, Raman and infrared spectroscopy. The sensitivity to environmental conditions of different polymorphs of the same drug entity may differ and therefore polymorphic composition may play an important role in determining a drug's stability.

Once the drug sensitivities are determined and the product development process addresses these issues and defines the product, then the long-term official stability studies may begin. The conditions and protocols for these studies are well defined by FDA and ICH guidelines which are discussed in detail in subsequent chapters of this book.

2.4 Review of cGMP Stability Requirements

The development of a new medicine relies heavily on compliance with 21 CFR Part 211. The scope of these regulations indicates that the requirements listed in this section contain only the *minimum current* GMP practice for preparation of drug products for administration to humans or animals. Therefore, companies must adhere to cGMP regulations to avoid regulatory scrutiny. Violations of these regulations could lead to warning letters or even criminal penalties. Thus current GMP plays an important role in guiding development of new drug products. A few selected sections of CFR 211 are discussed in this chapter to clarify the requirements that impact the stability program and testing. It is not meant to be a comprehensive discussion of all applicable cGMP requirements.

2.4.1 Part 211.166 – Stability Testing

The cGMP requirements of a stability program reside in 21CFR Part 211.166. Table 2.1 lists a summary of components needed to support a stability testing program for pharmaceutical products.

Table 2.1 Requirements of stability program

211.166(a) Written program must include: • Sample size and test intervals, • Storage conditions for samples, • Reliable, meaningful, and specific test methods, • Testing of drug product in marketed container, • Testing of drug product for reconstitution at dispensing time and reconstituted time.

Every company must have a written stability program documented in a standard operating procedure (SOP). This program will define the requirements for stability studies to be put up to assess the stability profile and the expiry of the drug product. It is required to have the sample sizes and testing intervals defined along with storage

conditions. Chapter 3 will present in more detail the frequency of stability testing and the conditions under which samples will be stored.

Analytical methods must be developed to allow monitoring the critical characteristics of a drug product. These methods must be stability-indicating and validated. Subsequent chapters will discuss these issues in more detail. Importantly, methods to monitor impurities or degradation products must also be developed and utilized to establish the shelf-life of the drug product. Mass balance is also critical while developing stability indicating methods. This is quite a challenge for Research and Development, where analytical methods continue to evolve as the formulations are being developed.

Current cGMP requires that the drug product must be tested during stability storage in the same container and closure as proposed in the registration. Therefore, stability studies must be set up on stability station, which is the time point in each specific storage condition, in their actual storage container. This may be an issue if there is not enough material available to be placed on stability station. For drug substance, a functionally similar container may be used to mimic the cardboard or plastic drum that is usually used to store raw material.

Part 211.166 (b) stipulates that an *adequate number of batches must be tested to determine an appropriate expiration date*. However, the regulations do not specify what the number of batches is and the size of these batches. This information is further clarified with the issuance of ICH stability guidelines. Similar sets of samples are also placed at higher temperature and higher humidity conditions to speed up degradation. These accelerated conditions generate data that are used to establish *tentative* expiration dates. Most studies use 40°C/75% Relative Humidity (RH) as the accelerated condition. This condition is also the ICH-accelerated condition.

FDA suggests accelerated studies to support tentative expiration dates; however, the real time studies are to be ongoing and continue until the actual projected expiration date is achieved. FDA addresses separately those samples which are claimed to be sterile and/or pyrogen-free. Additional information of the storage conditions for accelerated and stress conditions are discussed in Chapters 3 and 4.

2.4.2 Part 211.170 – Reserve Samples

A sample retention program is required for drug substance and drug product. For drug substance, a representative set of samples of each lot in each shipment of each active ingredient is to be retained to support marketed products. The amount must be twice the quantity needed for all tests to determine whether the active ingredient meets established specifications. In general, these samples must be retained for 1 year after the expiration date of the last lot of manufactured drug product containing the active ingredient. Radioactive drug product, pyrogen-free/sterile, and over-the-counter (OTC) drugs have other requirements as listed in Part 211.170.

For drug product, a representative of each lot or batch of drug product shall be retained and stored under conditions consistent with product labeling, in the same container-closure system that is marketed. Again, the amount is at least twice the

quantity needed to perform all required tests, except for sterility and pyrogens. Similar to drug substance, these samples, in most cases, must be retained for 1 year after the expiration date of the drug product.

2.4.3 Part 211.137 – Expiration Dating

This section of cGMP indicates that the expiry of the drug product is established by the stability program described in Part 211.166. The stability program also establishes the conditions that the product must be stored, and this information must be included on the product label. A manufacture must assure that the product meets quality standards of identity, strength, quality, and purity at the time of use.

Section (g) of this section indicates that the drug product used for investigation does not need to follow cGMP providing that the company will meet their specifications set by stability testing of clinical materials. However, many companies choose to follow cGMP for their late-phase clinical studies.

2.5 Review of Part 211.160 – Laboratory Controls

The general requirement for laboratory controls applies to stability testing (Subpart 211.166) as well as the others. These controls apply to testing instruments, analytical instrumentation, storage chambers, documentation including SOPs, data reporting and storage, data analysis, and sample plans utilizing statistical methods.

This section indicates that the quality unit (QA) is responsible to review and approve all specifications, standards, sampling plans, analytical procedures. QA must also have a change control system to manage changes to the above activities.

This section requires that all activities in the laboratory must be documented at the time of performance. These approvals and sign-offs shall be documented at time of performance. Any deviations must be recorded and justified. Therefore, all activities from sample set-up, sample pulls, sample testing, etc., are included. It requires that the controls shall include the establishment of scientifically sound and appropriate specifications, standards, sampling plans, and test procedures.

These are to assure that components, drug product containers, closures, in-process materials, labeling, and drug products conform to appropriate standards of identity, strength, quality, and purity.

2.6 Part 211.165 – Testing and Release for Distribution

Stability studies run on drug product are useful in defining and establishing the product specifications such as concentration, identity, and purity. These specifications form the criteria for the QA unit's product release activities. This section also

indicates that testing must be done, as needed, for each batch of drug product to assure the absence of objectionable micro-organisms.

Product must be tested utilizing sampling and testing plans which are documented and approved in writing. The testing methods must utilize validated procedures according to pre-approved validation protocols.

2.7 Part 211.194 – Laboratory Records

Section 211.194 details how the testing results are to be documented and the testing methods validated. These criteria also apply to the testing procedures used to perform stability testing, as well as release testing. Table 2.2 lists the requirements of laboratory records specified in this cGMP section. Drug products are only to be released if the test results conform to pre-determined acceptance criteria.

The documentation relating to testing and release must include a complete description of the source of the sample, the amount sampled, the lot number, date received, and date tested. The testing procedures must be completely referenced and any method changes documented and approved by QA with reasons for the change.

All reagents, standards, and instrumentation must be referenced and appropriate documentation for standard and instrument calibrations available for examination.

This requirement is covered by Section 211.280 – General Requirements, which indicates that all the records generated must be available for inspection at any time. Companies must consider their extended laboratories, especially those that are a part of their outsourcing paradigm. For marketed products, these data must be reviewed annually.

Table 2.2 Summary of laboratory records requirements

211.194 Laboratory records
• Complete record of data
• Description of sample (location, quantity, lot, date received, etc.)
• Method used, modification, and reason
• Reagents, standards, and instrumentation
• Stability testing

2.8 Conclusion

The need for stability studies is clearly defined in the above cGMP requirements for the pharmaceutical industry. It forms the basis for the ICH guidelines of specific conditions for stability studies. These guidelines will be discussed in depth in this book in the next two chapters for ICH regions and global regions. Stability studies form an integral part of the drug development process. No drug can be introduced into commerce without a stability studies program which is ongoing. The data generated will assure the drug product's stability and consequent safety and efficacy through at least the expiry date on the label.

Additional information may be obtained by referring to the list of references cited below.

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Chapter 3
Understanding ICH Guidelines Applicable to Stability Testing

Kim Huynh-Ba and Manuel Zahn

Contents

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Abstract This chapter discusses International Conference of Harmonization (ICH) guidelines that are related to the Stability Sciences. It gives a brief history of how the Q1A was initiated. A summary of Q1A(R2) discusses thoroughly the current regulations that the industry supports and practices. While this handbook was being prepared, the FDA Stability Guidance was withdrawn; therefore, a brief discussion of the guidance status has been included. A discussion of mean kinetic temperature is included for a basis of understanding stability testing conditions.

3.1 Introduction

This chapter is a collaborative work that discusses the ICH initiatives evolving around stability testing. To gain a better understanding of the International Conference on Harmonisation process, a brief development history of ICH stability guidelines is given, along with an overview of other ICH stability-related guidelines. Details and applications of these stability-related guidelines can be found in other chapters of this handbook.

The chapter discusses FDA's efforts to harmonize requirements in this area and their withdrawal of the 1987 Stability Guideline and the 1998 Stability Draft Guidance in June 2006. At the same time, ICH also withdrew Q1F which documented the storage condition recommended for Zones III and IV, to make way for development of the World Health Organization (WHO) Stability guidelines. Because ICH Q1A(R2) has been identified as the principal guideline to follow as this book goes to press, a summary highlighting Q1A(R2) requirements is included in this chapter. A discussion of global stability recommendations is further discussed in Chapter 4 of this handbook.

A stability program not only covers registration studies, but also includes studies that are set up to provide supporting data to other programs such as bulk storage, in-process testing, in-use testing, or excursions. This chapter offers details of studies to support these purposes.

To fully understand the decision of ICH storage conditions as well to better design the stability program, the stability professional needs to understand mean kinetic temperature; a discussion of mean kinetic temperature is provided in Section 3.6.

3.2 Development of ICH Stability Guidelines

3.2.1 Brief History

Stability is a critical quality attribute; therefore, the stability program plays an important role when developing new pharmaceutical products. This applies in particular to pharmaceutical products that are to be marketed in several strengths and package types. Multiple strengths and package types combined with multiple

batches, various storage conditions, test parameters, and test intervals require a great number of samples to be tested at considerable cost. Additionally, the requirements of the different regulatory agencies must be taken into account. As a consequence, prior to the early 1990s, an enormous amount of stability testing, much of it redundant; was performed by multinational pharmaceutical companies seeking approvals in more than one country. The compilation of a common set of stability requirements for marketing authorizations was, therefore, considered to be a top priority for the pharmaceutical industry when the International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) was formed in 1990 [1].

Regulators and pharmaceutical industry representatives from the European Union (EU), Japan, and the United States, with observers from the Canadian and Swiss Health Authorities, and the WHO, chose stability testing as one of the first issues to be discussed and harmonized, as announced at the first ICH in Brussels in 1991 [2].

An ICH Guideline on Stability Testing (Q1A) was subsequently developed and published in 1993, after which it was adopted throughout the ICH region, namely the European Union, the USA, and Japan. Other countries followed the ICH guidelines in principle, for example Australia, Canada, Switzerland and so forth. In the following sections, some key aspects of the various documents are summarized.

Development of an ICH guideline consists of five main steps (Fig. 3.1) describing its status in the process. Step 1 is when the process of consensus building begins after the Steering Committee adopts a concept paper. Step 2 is when the consensus is agreed by all parties of Expert Working Group (EWG) members. Step 3 indicates that the draft document is being consulted with all ICH regional regulatory agencies. In USA, it is published as a draft guidance in the Federal Register. In the EU, it is published as a draft Committee for Medicinal Products for Human Use (CHMP) guideline. In Japan, it is translated and issued by the Ministry of Health, Labour and Welfare (MHLW). Step 4 is when the Steering Committee agrees and it is recommended for adoption by the regulatory bodies of the three regions. Step 5 is the final step that the guideline is implemented. Much technical discussion goes among different parties to carry each guideline from one step to the next.

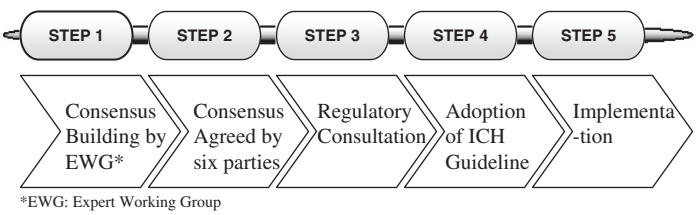

Fig. 3.1 Five steps in ICH Guidelines process

3.2.2 The Parent Guideline (ICH Q1A)

This guideline—released at the ICH meeting in Orlando in 1993—describes the stability testing requirements for a registration application within the ICH region. It was explicitly intended to cover all that is required to get a marketing authorization granted in the ICH region, in other words, *the guideline describes the ceiling, not the floor, of the requirements*. This is of particular importance when the need for site-specific stability data is being discussed. The requirement to submit site-specific stability data is not mentioned anywhere in ICH Q1A, as this has been and is still being regarded as not justified from a scientific point of view. A substance or product manufactured at a different site, following the same procedure, will not change its shelf-life.

The first version of the *parent guideline* was revised twice during the ensuing years, and reached Step 4 of the ICH process on February 6, 2003 [3].

The new version of the guideline takes into account the requirements for stability testing in Climatic Zones III and IV in order to minimize the different storage conditions for submission of a global dossier.

3.2.3 Other ICH Stability Guidelines

3.2.3.1 Photostability Testing (Q1B)

Procedures and tools for testing the light sensitivity of a substance or product were not standardized or used in a common way prior to ICH. It was, therefore, extremely valuable to have some experts, mainly from Japan, to discuss optimal light sources that simulate daylight and the methods to measure light intensity. As a result, the tripartite harmonized ICH guideline Photostability Testing of New Drug Substances and Products (Q1B) was finalized (Step 4) in November 1996, as an annex to the parent stability guideline [4]. Photostability is addressed in more detail in Chapter 14 of this book.

This ICH guideline helped to standardize approaches. In addition, two articles published by Thatcher et al. have provided interpretation of this guideline by defining basic terminology in photochemistry, reviewing photostability testing, characterizing light sources, and measuring output from photolysis sources applied to photostability testing in the pharmaceutical industry [5, 6].

3.2.3.2 Stability Testing for New Dosage Forms (Q1C)

The tripartite harmonized ICH guideline Q1C was finalized (Step 4) in November 1996. It extends the main stability guideline for new formulations of already approved medicines and defines the circumstances under which reduced stability data can be accepted. It is the shortest of all ICH guidelines up to now: just half a page of text. This is because ICH regulators could not agree on the level of

supportive data on similar substances and products or similar dosage forms that could allow the manufacturers to reduce stability testing on the new dosage form [7].

3.2.3.3 Bracketing and Matrixing (Q1D)

Guideline Q1D describes general principles for reduced stability testing and provides examples of bracketing and matrixing designs [8]. The acceptance of this approach by regulators is saving manufacturers a huge amount of unnecessary stability testing. On the other hand, reduced data means an increased risk that the results obtained may not be sufficient to support the expected shelf-life. The tripartite harmonized ICH guideline Q1D was finalized (Step 4) in February 2002, and is addressed in detail in Chapter 15 of this book.

3.2.3.4 Evaluation of Stability Data (Q1E)

The tripartite harmonized ICH guideline Q1E was finalized (Step 4) in February 2003 [9]. This document extends the main guideline by explaining possible situations where extrapolation of retest periods/shelf-lives beyond the real-time data may be appropriate. Furthermore, it provides examples of statistical approaches to stability data analysis. Evaluation of stability data is discussed thoroughly in Chapter 13.

3.2.3.5 Stability Testing of Biotech Products (Q5C)

Because most of the typical proteins and polypeptides are less stable than small molecules, and because test procedures for assay and degradation products are quite unique, very early in the discussion the ICH Steering Committee agreed to let the biotech experts develop a guideline for these types of products separate from Q1A. A tripartite harmonized ICH guideline Stability Testing of Biotechnological/Biological Products (Q5C) was finalized (Step 4) in November 1995 [10]. Stability of biologic products is discussed in greater detail in Chapter 17.

3.3 Status of FDA Draft Guidance (Contributed by Robert Seevers)

FDA issued a document titled Guideline for Submitting Documentation for the Stability of Human Drugs and Biologics in February of 1987. During the next decade there was significant interest within the agency to revise and update that guidance. At the same time, however, FDA joined with European and Japanese regulators, compendia, and industry representatives in the International Conference on Harmonization (ICH). Efforts to revise the 1987 Guideline were placed on hold in order to focus on the ICH negotiations.

Those negotiations bore fruit in terms of the ICH Q1A, Q1B, Q1C, and Q5C publications on stability. Nevertheless, a number of topics were not addressed in the ICH guidances, either because they had not come up or because agreement could not be reached across the three regions. An example of the latter is a primary cause of the brevity of the Q1C guidance (1996). Originally intended to cover stability studies to support post-approval manufacturing changes, it was abbreviated to simply state *"Stability protocols for new dosage forms should follow the guidance in the parent stability guideline in principle."*

Concurrently, FDA was developing new guidances in the area of post-approval changes, beginning with 1995 SUPAC-IR: SUPAC-IR: Immediate-Release Solid Oral Dosage Forms: Scale-Up and Post-Approval Changes: Chemistry, Manufacturing and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation.

FDA's efforts to revise and update the 1987 stability guideline were therefore revived with the goal of touching on areas where ICH could not reach agreement, or on areas that they did not cover. The resulting guidance was published as a draft in June 1998. The goal was to provide, in a single document, access to both the recommendations of ICH and the recent FDA post-approval change guidances. Therefore, it contained the complete text of the ICH Q1A, Q1B, Q1C, and Q5C guidances, and appropriate tables from the SUPAC guidances.

In addition, the 1998 draft FDA stability guidance addressed several key issues not covered by either ICH or the SUPAC guidances. These included the following topics:

- Site-specific stability
- Recommended storage statements
- Certain post-approval changes (e.g., for packaging)
- Generics

The most controversial topic, by far, was site-specific stability. In the 1998 draft guidance, FDA asked for stability data to be provided from the commercial manufacturing site if that was different from the site where the regulatory registration batches of drug product and drug substance were made. This topic was the focus of approximately 3000 public comments received on the guidance, more than any other specific topic. The result was a public meeting, in 1999, of the sub-committee of the FDA's Advisory Committee on Pharmaceutical Sciences. At that meeting a compromise was worked out between industry and the agency wherein it was agreed that submission of Certificates of Analysis for three validation lots at the commercial site would be adequate to demonstrate that the drug product or drug substance technology had been appropriately transferred to the new site. In that case, site-specific stability data would not be necessary for the NDA submission.

The guidance was revised based on the public comments received, but was never released again for public comment. In 2006, with the agency moving in the Quality by Design direction, the detailed 1998 draft stability guidance was withdrawn.

3.4 Summary of Q1A(R2) Guidance

As introduced in Section 3.2.2, Q1A(R2) was adopted by European's Committee for Proprietary Medicinal Products (CPMP) in March 2003, by Japan's Ministry of Health, Labour and Welfare (MHLW) in June 2003, and published in the United States Federal Register in 2003. One of the objectives of this guideline was to define the minimum stability data package for a registration application of a new drug substance or new drug product in the International Conference of Harmonization (ICH) geographic regions. This geographic region encompasses Zone I and Zone II climatic conditions. Some other non-ICH countries adopted this guideline with some modifications specific to the region. This guideline does not cover abbreviated or abridged applications, variations, or clinical trial applications. Much discussion on the global requirements including countries of Zone III and IV will be covered in the next chapter.

It is critical to understand that *"the purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light, and to establish a retest period for the drug substance or a shelf-life for the drug product and recommended storage conditions"* [3].

Based on the above statement, stability data confirm the drug product quality by assuring that the drug product continues to meet its specification throughout its shelf-life in the region that it is registered. The storage conditions impacting the drug product are determined as a combination of light, temperature, and humidity. The ICH process was able to harmonize the expectations and requirements in the three regions: European Union, United States, and Japan.

3.4.1 Stress Testing

Stress testing is necessary to evaluate the drug substance and drug product under various conditions of elevated temperature and humidity. Data from these stress studies could also be useful in understanding the stability profile during manufacturing, storage, shipping, and patient use. These studies provide insight into the potential degradation products and assist in establishing the degradation pathways. These stressed samples could also be used to challenge the stability indicating power of the analytical procedures.

3.4.1.1 Drug Substance

Q1A(R2) requires stress testing to be done on one batch of drug substance. It must be studied under high temperature, high humidity, and across a wide range of pH values when in solution or suspension. Although the guideline does not specify the exact conditions under which these stress studies should be done, many references discuss them thoroughly. It is recommended that these studies should be designed depending on the nature of the drug substance.

Reynolds et al. have summarized the industry collective view on a series of forced degradation studies [11]. It emphasizes that stress conditions must be realistic and not excessive. It depends greatly on the active ingredients and formulation involved. A mass balance assessment is necessary and should be based on the decrease in assay value and the increase in the amount of degradation products. Although the FDA recognizes that mass balance may not be achieved in all cases, it would document the thoroughness and specificity of the analytical method. Klick et al. have developed a generic approach for conducting stress testing on drug substances and drug products to generate relevant and generally predictive results for the development of a stability-indicating method [12]. However, this generic approach should be used as a starting point to set up a stress testing study to develop a stability-indicating method. The study should be designed with common sense and a thorough understanding of the physical and chemical properties of the drug substance.

One of the important stress studies for the drug substance is a light exposure study. This study would help to evaluate the physical and chemical characteristics of the drug substance when exposed to light. According to Q1B, there are two types of studies: confirmatory and forced degradation studies. Q1B describes the required illumination of at least 1.2 million lux hours and an integrated near UV light energy of 200 W h/m^2 for photostability studies. This guideline describes two options that one could take to expose the materials. A flow chart is included in Q1B to demonstrate recommended steps for conducting drug substance photostability stress studies. These stressed samples could also be used to develop stability-indicating analytical procedures.

A study is typically carried out by taking a thin layer of drug substance, typically approximately 1–2 mm thick, which is stored in a quartz Petri dish and protected with a transparent cover. A control sample that is covered with aluminum foil is also prepared. Exposure of the samples is monitored directly by placing a light recorder next to the tested materials.

After the exposure, samples are analyzed according to the method for any physical or chemical change. Impurities, if generated, are recorded and measured.

3.4.1.2 Drug Product

Drug product should be stressed mainly at elevated temperature and humidity to understand the possible degradants that may be developed on long-term storage.

The drug product is also evaluated under light exposure as part of stress studies to determine if the drug product is sensitive to light. Similar to the drug substance, this can be achieved by exposing a layer of drug product in a quartz Petri dish. Care must be taken if the drug product is a liquid as evaporation of the liquid component can result in a more concentrated sample. Results from these studies play a critical role in formulation development as well as packaging development.

This study is performed on one batch of each formulation to see if there is any physical or chemical change. Q1B includes a flow chart of confirmatory

photostability studies. If there is a decrease in potency or an increase in degradation products, then a more protective packaging may be needed and a warning label may be necessary. Reed et al. have evaluated the implications of product photosensitivity and how it influences various aspects of product development [13]. A photosensitivity classification system for pharmaceutical products was introduced to understand and manage the implications of product photosensitivity during manufacturing, packaging, shelf-storage, testing, and administration. Baertschi et al. have discussed the implications of administering transdermal patches containing photosensitive active ingredients [14].

Data from these stress studies also help the manufacturer to select the appropriate packaging for the final drug product. Table 3.1 lists a series of recommended stress conditions for drug substance and drug product. Stress studies should be discontinued when 5–20% of loss of active is obtained. If the sample is allowed to undergo further degradation, there is a high possibility that secondary degradants would result. However, it is important to note that if the nature of the drug substance does not allow for this level of degradation, then the stress studies should be discontinued. There is no need to carry extreme conditions in order to form degradants when the API or drug product is stable.

Table 3.1 Stress testing recommendation for drug substance and drug product

Active Drug Substance	
Temperature	50, 60, 70°C, etc.
Humidity	25°C/75%RH and 25°C/90%RH
Oxidation	Over a wide range of pH
Light	Based on Q1B, exposed and in the drum
Drug Product	
Temperature/humidity	40°C/75%RH, 25°C/80%RH
Temperature	50°C or 60°C for 1 month
Light	Based on Q1B, exposed and in the package

3.4.2 Selection of Batches

Q1A(R2) indicates that three batches per strength are necessary for submission. The recommended batch size is also presented. For drug substance, these batches must be pilot scale batches. For drug products, two out of three batches must be at pilot scale and the third batch can be at lab scale.

The manufacturing process for the drug substance and drug product must be representative of commercial process. One must consider whether there will be any changes that may occur during scale-up. Changes at scale-up have the potential to alter the stability profile of the drug substance or drug product as well as the impurity profile of the materials tested.

A commitment is needed to place three commercial production batches under the same stability testing protocols.

3.4.3 Container Closure System

For the drug substance, stability studies must be conducted using the packaging configuration that is similar to or simulates the packaging proposed for storage and distribution. Normally, the drug substance is stored in the warehouse in polyethylene bags contained in cardboard drums. This set-up is not practical for stability studies due to lack of space and quantity of drug substance needed. Therefore, simulated small telescope drums are typically used for these types of studies. One must be careful that the thickness of the telescoped drum does not provide more or less protection than the warehouse drum.

For the drug product, stability studies will be done in the container closure system proposed for marketing.

3.4.4 Specifications

Specifications need to be established for drug substance and drug product in order to determine the quality of a drug substance or drug product through a set of analytical procedures covering *physical, chemical, biological, and microbiological* attributes. Additional details of establishing specifications for drug substance and drug product may be found in ICH guidelines Q6A and Q6B. The acceptance criteria must be set based on the data obtained for material used in pre-clinical and clinical studies. Different acceptance criteria can be set for release and stability purposes. However, the US regulatory specifications are considered to be the stability specifications.

For impurities, a specification for individual and total impurities must be set. It is recommended that numerical data are reported for individual (known and unknown) and total impurities in place of *conforms* or *complies*. Detailed information is discussed further in Q3A and Q3B. For stability testing of drug products, impurity specifications are set only for degradation products.

3.4.5 Testing Frequency

The guideline recommends that testing will be done every 3 months over the first year, every 6 months over the second year, and annually thereafter. It does indicate that a minimum of three time points (including the initial and final time points) is necessary for accelerated and four time points for intermediate conditions.

Based on the guidelines, the time points listed in Table 3.2 are recommended to be used as pull points for stability studies. Stability-indicating methods are also required. Much more discussion of the development and validation of stability-indicating methods is provided in subsequent chapters.

Table 3.2 Time points for stability studies

	Initial	3 m	6 m	9 m	12 m	18 m	24 m	36 m
Intended storage condition	X	X	X	X	X	X	X	X
Immediate condition	(X)	X	X	X	X			
Accelerated condition	(X)	X	X					

3.4.6 Storage Conditions

Much effort in the ICH process is to harmonize a set of storage conditions that are acceptable in Zone I and Zone II. A combination of temperature and humidity is necessary to evaluate the stability of a drug substance or drug product. Accelerated and intermediate conditions, where available, are also used to evaluate impact of short-term excursions. In addition, the guideline also defines the range of temperature and humidity conditions for control of the storage chamber: The chamber temperature must be controlled within ±2°C, and the humidity controlled within ±5% relative humidity.

For drug product stored at room temperature, the guideline defines an intermediate condition of 30°C/65%RH. Testing at this intermediate condition is needed only when a significant change occurs for samples stored during 6 months under the accelerated condition of 40°C/75%RH. Tables 3.3 and 3.4 list these ICH storage conditions and significant change requirements.

Table 3.3 ICH storage conditions of stability studies

Intended label storage condition	Stability studies	Storage condition	Submission requirements
Room temperature	Long term	25°C/60%RH	12 months
	Intermediate*	30°C/65%RH	6 months
	Accelerated	40°C/75%RH	6 months
Refrigerator	Long term	5°C/Ambient	12 months
	Accelerated	25°C/60%RH	6 months
Freezer	Long term	–20°C/Ambient	12 months

* Test only if there is significant change at 40°C/75%RH

Table 3.4 Definitions of significant changes of data stored at accelerated conditions

API	Significant change is defined as failure to meet the specification
Drug product	1. A 5% potency change from the initial assay value; 2. Any specified degradant exceeding its acceptance criteria 3. Failure to meet acceptance criteria for appearance and physical properties (e.g., color, phase separation, resuspendability, delivery per actuation, caking, hardness); and as appropriate to the product type; 4. The pH exceeding its acceptance criteria; and 5. Dissolution exceeding the acceptance criteria for 12 dosage units.

For drug substance, significant change is noted when any of the analyses of accelerated samples does not meet room temperature specifications. This observation should be done in a timely manner, so that samples stored at the intermediate condition can be pulled from their chamber and tested promptly.

For drug product, significant changes occur if one of the five conditions in Table 3.4 occurs. For the first condition, a 5% change from initial assay value is apparent; however, one must be careful if the assay specification is not ±10% (i.e., 90–100% of label claim). This change may need to be adjusted. In addition, this value needs to be adjusted if the initial assay value is not 100% of label claim.

Secondly, a significant change is when any specified degradant exceeds its specification. This is straightforward if there is a specified degradant included in the specifications. For a new drug application, however, this may be difficult because most likely the degradants have not yet been identified.

Next on the list is a significant change in the physical property of the drug product. Physical testing is subjective and may be difficult to determine. It is recommended that a set of standards are available for comparison purposes. A Pantone color chart may be used to compare the color of the tested materials. Training is critical for analysts to perform testing as consistently as possible. Next would be a pH test, applicable mainly for solutions and, finally, dissolution tests for solid dosage forms or suspension dosage forms. More information about these types of testing can be found in Chapter 10.

If 6-month data at accelerated condition do not meet room temperature specifications, samples at intermediate condition stored to 12 months will be tested. These data will be submitted in the NDA. It is recommended that up to 4 time points will be tested for samples stored at this condition.

For liquids stored in semi-permeable containers, ICH conditions are listed in Table 3.5. However, the guidelines give the option that a room temperature study can be done at 25°C/60% RH, and the weight loss equivalent to 25°C/40% RH can be calculated. Samples stored at 30°C/65% RH could be used in place of 30°C/35% RH as the intermediate condition.

Table 3.5 Conditions for semi-permeable container

Long term	25°C/40% RH (ICH)
Intermediate	30°C/35%RH
Accelerated	40°C/NMT 25%RH

3.4.7 Stability Commitment

ICH Q1A(R2) recommends that a stability commitment be submitted in the registration application. It commits the applicant to perform stability testing on three commercial production batches according to the current protocol through the proposed shelf-life.

For the drug substance, if fewer than three submission batches are submitted then additional batches will be tested with the same stability protocol used for submission batches.

Q1A(R2) also indicates that the commitment batches must be placed on stability with the same protocol as submission batches. Therefore, if there is significant change on the accelerated conditions of the primary batches and samples of intermediate conditions must be tested, then samples of intermediate condition of three production batches must also be tested.

3.4.8 Data Evaluation

Q1A(R2) indicates that data evaluation must be done for submission batches. ICH guideline Q1E provides more details on this topic and is discussed further in Chapter 13. The guidelines also emphasize that no formal statistical analysis is needed if data show little degradation or little variability. A justification of omission is needed to show that the data set remain within method variability and show no particular trend through time.

3.5 Special Stability Studies

3.5.1 Bulk Stability

Stability studies must be conducted to support storage of product between production and packaging. This type of study should be completed before commercialization. Typically, these studies are less than 1 year and conducted at controlled room temperature. Products are stored in a simulated package such as double polyethylene bag in small fiber drum, or in plastic containers mimicking the packaging of the bulk product. Critical testing should be done every 3 months.

In addition, the warehouse conditions where bulk samples are stored must be monitored and mean kinetic temperature calculated.

3.5.2 In-Process Testing

Studies must be conducted to provide data to support bulk holding times for in-process or intermediate materials. For a stable drug product, it is generally acceptable that no formal study is needed if in-process materials are held less than 30 days. For unstable products or materials that need to be held longer than 30 days, stability studies are necessary to verify the holding times do not affect the quality of the in-process materials.

3.5.3 In-Use Testing

In-use studies are necessary to provide support for products that can be used after the container is opened, such as in the multi-dose type of product or product that needs to be reconstituted before use. This requirement is listed in cGMP as well as in European Guidance [15]. This type of study should simulate the use of the product in practice with regard to both usage and storage conditions. The length of the study depends upon how long the product is to be used. Testing includes physical, chemical, and microbiological tests, in order to focus on changes that could happen after the container is opened.

3.5.4 Studies to Support Excursions

Stability studies are necessary to support the storage and shipment of the drug product. These studies are done on packaged drug products as well as on unpackaged drug products. Typically, two types of studies are conducted:

Thermal studies are done for all products by exposing the drug products to a few temperature cycles (i.e., conducting three cycles where each cycle includes drug products stored at 40°C for 4 days and at 25°C for 3 days).

Freeze-Thaw studies are done especially for liquid products by exposing the drug products to a few temperature cycles (i.e., conducting 3 cycles where each cycle includes drug products stored at –10 to –20°C for 4 days and at 25°C/ambient RH for 3 days).

Testing is to be done at the end of the cycles to evaluate any physical and chemical change that may occur with the drug product. These data are also important even after commercialization, to support questions from the sales force or product complaint office.

In some cases where the drug product may be sensitive, these studies could be put on long-term stability storage for the expiration period to evaluate the stability profile of the drug product after the temperature/humidity excursions.

3.6 Mean Kinetic Temperature

A major step forward toward the definition of adequate stability testing conditions based on good science was made by introducing the Mean Kinetic Temperature (MKT) concept by Wolfgang Grimm in the 1990s [16]. In those days, some regulatory authorities required stability testing studies to be conducted at the upper limit of the labeled storage recommendation, for example, a product labeled *Store below* 30°*C* had to be tested at 30°C. When the ICH EWG began to discuss common standards for stability testing, it took the experts several meetings before the regulators accepted the fact that a substance or product that is stable at 25°C (long-term) and 40°C (accelerated) could be labeled *Store below* 30°C. In the following paragraphs, the MKT concept is explained in detail.

3.6.1 Definition

The MKT includes the reaction rate constants in the evaluation of the impact of heat on pharmaceutical products. A suitable definition of the MKT is the following:

MKT is the temperature corresponding to the effects of a given temperature–time distribution on chemical reaction kinetics.

The MKT allows calculating the impact of temperature fluctuations on the chemical degradation of a substance in a given product [17].

3.6.2 Calculations

The MKT can be calculated by using the formula developed by Haynes based on the Arrhenius equation [18].

$$\mathrm{MKT} = \frac{E_a/R}{-\ln\frac{e^{-E_a/R\cdot T_1} + e^{-E_a/R\cdot T_2} + \cdots}{n}} \tag{3.1}$$

MKT = Mean Kinetic Temperature [°K]
E_a = Activation energy [kJ/mol]
R = Universal gas constant = 8.314 [J/°K mol]
T = Temperature [°K]
n = Number of time points

The activation energy E_a is assumed to be 83.144 kJ/mol. This value, which is recommended in the US Pharmacopeia [19], has been derived from evaluating published data for more than 100 chemical substances, namely small molecules that are commonly used as active ingredients in pharmaceutical products, and calculating the mean. If feasible, and definitely in case of biological/biotech products, it is advisable to use the actual activation energy found for the particular substance instead of the mean value. The actual activation energy can be derived by calculating the intercept of the Arrhenius plot with the y-axis [17].

The activation energy E_a (assumed to be 83.144 kJ/mol) is divided by the universal gas constant R (0.00831432 kJ/°K mol):

$$E_a/R = 10000.09622\ [^\circ K^{-1}] \tag{3.2}$$

The result is then divided by the temperature T_n (measured in degrees Kelvin) to get a factor f_n for each timepoint n:

$$f_n = e^{-10000.09622/T_n} \tag{3.3}$$

After that, the sum of the individual results for a defined time period is divided by n, the number of timepoints used.

$$F_n = (f1 + f2 + \cdots + fn)/n \tag{3.4}$$

Then the MKT [°K] for a defined time period is achieved by calculating the negative natural logarithm of the above result using the following equation:

$$MKT = 10000.09622/(-\ln F_n) \tag{3.5}$$

The MKT is converted into degrees Celsius by subtracting 273.1 from the value found.

3.6.3 Examples

The following examples discuss the MKT calculated for Climatic Zone II and IV regions. It shows the difference of temperature fluctuations in these regions and demonstrates that the selected ICH temperatures can adequately be used to study stability of pharmaceutical products marketed in these regions.

3.6.3.1 MKT Versus Arithmetic Mean Temperature

The MKT is normally higher than the arithmetic mean temperature, because the degradation rate increases exponentially with increasing temperature. An arithmetic mean temperature would be adequate only if the increase in the degradation rate were linear. The greater the difference between the lower and the higher temperature, the more important it is to calculate the MKT instead of the arithmetic mean.

As examples,

- For 25 and 30°C, arithmetic mean temperature = 27.5°C, MKT = 27.8°C
- For 20 and 40°C, arithmetic mean temperature = 30°C, MKT = 34.4°C

The MKT provides a more accurate way to present the storage conditions.

3.6.3.2 Temperature Fluctuations in Climatic Zone II

Long-term stability testing for countries in Climatic Zones II is recommended to be conducted at 25°C/60% RH, whereas storage temperatures of pharmaceutical products in pharmacies according to the USP may fluctuate between 15 and 30°C.

Table 3.6 lists the MKT that is calculated for these two different temperature–time distributions.

Table 3.6 MKT is calculated for these two different temperature–time distributions

Long-term testing at 25°C ± 2°C:	Storage between 15 and 30°C:
25°C for 8 h	15°C for 4 months
27°C for 8 h	25°C for 4 months
23°C for 8 h	30°C for 4 months
MKT = 25.1°C in 24 h	MKT = 25.2°C in 12 months

As a result, long-term stability testing at 25 ± 2°C is equal to a MKT of 25.1°C. Temperature fluctuations during storage in a warehouse or pharmacy between 15 and 30°C calculated in the above example result in a MKT of 25.2°C. Therefore, the long-term stability testing condition is a good model for the tolerated storage fluctuations in pharmacies. This example illustrates how the MKT approach facilitates the comparison of two different temperature–time distributions.

In reality, however, the daily and yearly fluctuations of the temperature measured in the open air can be even higher. During a period of 12 months in a fictitious region in Climatic Zone II, the following temperature fluctuations are assumed in order to find out whether long-term testing at 25 ±2°C is applicable to test the impact of storage temperatures on the stability of a substance or a product (Table 3.7).

Table 3.7 Temperature fluctuations in a region in Climatic Zone II

Daily temperatures in April, May, June and September	Daily temperatures in July and August
21°C for 6 h	24°C for 6 h
25°C for 6 h	28°C for 6 h
31°C for 6 h	36°C for 6 h
27°C for 6 h	32°C for 6 h
MKT = 26.7°C in 24 h used for 4 months	MKT = 31.0°C in 24 h used for 2 months
Daily temperatures from October to March	Result
12°C for 6 h	Annual MKT = 24.3°C in 12 months
15°C for 6 h	
20°C for 6 h	
18°C for 6 h	
MKT = 16.8°C in 24 h used for 6 months	

This example with a mean annual temperature of 21.8°C meets the criteria for Climatic Zone II, in other words, mean annual temperature measured in the open air is not higher than 22°C. Therefore, this calculation shows that long-term testing at 25 ± 2°C does cover temperature fluctuations above 30°C that occur during hot summer days. These higher temperatures are compensated by lower temperatures during the night and in winter.

3.6.3.3 Temperature Fluctuations in Climatic Zone IV

Long-term stability testing for countries in Climatic Zone IV is recommended to be conducted at 30°C/65% or 75% RH in general cases. In Table 3.8, the MKT is calculated for the tolerated temperature fluctuations of ±2°C.

Table 3.8 MKT calculated for the tolerated temperature fluctuations of ±2°C

Long-term testing at 30°C ± 2°C:
28°C for 8 h
30°C for 8 h
32°C for 8 h
MKT = 30.2°C in 24 h

As a result, long-term stability testing at 30 ± 2°C is equal to a MKT of 30.2°C. In reality, however, the daily and yearly fluctuations of the temperature measured in the open air can be even higher. During a period of 12 months in a fictitious region in Climatic Zone IV, the following temperature fluctuations are assumed in order to find out whether long-term testing at 30 ± 2°C is applicable to test the impact of storage temperatures on the stability of a substance or a product (Table 3.9).

This example with a mean annual temperature of 28.6°C meets the criteria for Climatic Zone IV, in other words, mean annual temperature measured in the open air is higher than 22°C. In this example, long-term testing at 30 ± 2°C does cover temperature fluctuations above 30°C that occur during hot summer days as these higher temperatures are compensated by lower temperatures during the night and in winter.

Table 3.9 Temperature fluctuations in a region in Climatic Zone IV

Daily temperatures in spring and autumn	Daily temperatures in summer
26°C for 6 h 28°C for 6 h 32°C for 6 h 30°C for 6 h	26°C for 6 h 28°C for 6 h 35°C for 6 h 32°C for 6 h
MKT = 29.3°C in 24 h used for 4 months	MKT = 30.9°C in 24 h used for 4 months
Daily temperatures in winter	Result
22°C for 6 h 26°C for 6 h 30°C for 6 h 28°C for 6 h	*Annual MKT = 29.2°C in* 12 months
MKT = 27.0°C in 24 h used for 4 months	

To illustrate, one of the hottest cities in the world can be evaluated, namely Baghdad, Iraq, where the mean temperature in July reaches a maximum of 44.5°C, but the MKT in 12 months is still just 28.7°C. Figure 3.2 shows the mean daily temperatures for each month in Baghdad, Iraq.

Mean temperatures in Baghdad in July:

At UTC 00:00 = 29.3°C
At UTC 06:00 = 36.7°C
At UTC 12:00 = 44.5°C
At UTC 18:00 = 35.6°C

Mean temperature in July = 36.5°C
MKT in July = 38.0°C

Mean temperature in Baghdad January–December = 22.9°C
MKT in 12 months = 28.7°C

As a result it can be seen that the impact of the total kinetic energy on a substance or product in a hot city in Climatic Zone IV over 12 months is less stressful than the

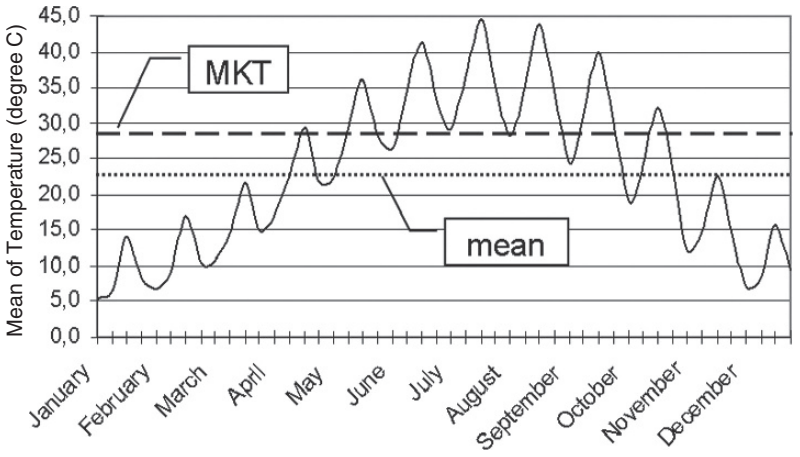

Fig. 3.2 Mean daily temperatures for each month in Baghdad, Iraq

total kinetic energy of 30°C during the same time period. Long-term testing at 30°C is, therefore, adequate to test the stability of a substance or product intended to be marketed in countries located in Climatic Zone III or IV.

3.6.4 Temperature Excursions

As described in ICH Q1A, in addition to long-term stability studies, accelerated studies can be used to assess longer term chemical effects at non-accelerated conditions, and to evaluate the effect of short-term excursions outside the label storage conditions such as might occur during shipping. A substance or product that is stable at accelerated conditions, for example at 40°C/75% RH for 6 months, would not be degraded by temperature fluctuations above 30°C nor by short-term excursions.

The USP recommends monitoring the temperature during storage and shipment, and calculation of the MKT for a defined time period, facilitating the assessment of the impact of temperature excursions on the stability of the substances and products.

3.6.5 Limitations

The MKT approach has some limitations that are to be observed when the impact of temperature on the stability of a substance or product is being evaluated. The most important restriction is the fact that MKT covers only chemical degradation. A drug substance and in particular a pharmaceutical product also has to meet other quality parameters within specified acceptance criteria throughout its shelf-life. Typical examples are a suppository that is not allowed to be transported or stored above 30°C, or a product like cyclophosphamide monohydrate, which melts at 49.5°C

and is freely soluble in water. Short-term storage above 50°C converts the active substance to the anhydrous form that forms a cake with a slow dissolution rate.

Also, at higher temperatures, the mechanism of the chemical degradation may change or may no longer follow zero- or first-order kinetics, which means that the Arrhenius equation would not apply.

3.7 Conclusion

Stability is a critical quality attribute; therefore harmonization of stability requirements is essential to bring new medicines to patients. Established in 1990, ICH has led the industry and regulatory efforts to improve the efficiency of stability requirements from developing to registering new medicinal products. Through this chapter, we have introduced the ICH process, its history and accomplishments. We also reviewed several ICH stability-related guidelines governing the stability program to support the drug product expiry.

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Chapter 4
Global Stability Practices

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Contents

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Abstract This chapter presents the global expectations of a stability program. It includes a thorough discussion of stability requirements of non-ICH regions as well as a discussion on how the climatic requirements are implied in the world. This comprehensive chapter gives an introduction of stability requirements for countries around the world. Discussions of World Health Organization (WHO) stability guidelines and Association of Southeast Asian Nations (ASEAN) stability requirements are also included.

4.1 The Concept of Climatic Zones

4.1.1 Schumacher/Grimm

In order to be able to reduce the amount of stability testing, the number of different testing conditions must be reduced to a sufficient extent. This has been done by Paul Schumacher in 1972 [1] and Wolfgang Grimm in 1986 [2] when they defined four different long-term test conditions, which match with the climatic conditions of the target markets categorised in just four different climatic zones (see Tables 4.1 and 4.2). This concept became an established standard in developing pharmaceutical products.

Table 4.1 Data for Climatic Zones and testing conditions recommended by Wolfgang Grimm in 1986

CZ	Mean annual temperature measured in the open air [°C]	Calculated mean annual temperature [< 19°C = 19°C]	Kinetic average annual temperature [°C]	Average annual relative humidity [%]	Calculated testing conditions [°C / % RH / hPa]
I	10.7	19.7	19.7	43.9	21 / 45 / 11.2
II	18.8	22.3	22.8	52.9	25 / 60 / 19.0
III	24.9	26.9	28.1	31.5	31 / 40 / 18.0
IV	27.0	27.0	27.5	78.0	31 / 70 / 31.5

4.1.2 Amended Climatic Zones

In 2005, the World Health Organization (WHO) proposed as an option to split the Climatic Zone IV into two different zones: IVA and IVB. A new set of criteria have been proposed [3] (see Table 4.3).

Table 4.2 Criteria used to classify Climatic Zones and testing conditions recommended by Wolfgang Grimm in 1998*

CZ	Definition	Criteria Mean annual temperature measured in the open air / Mean annual partial water vapour pressure	Testing conditions [°C / % RH]
I	Temperate climate	≤ 15°C / ≤ 11 hPa	21 / 45
II	Subtropical and Mediterranean climate	> 15 to 22°C / > 11 to 18 hPa	25 / 60
III	Hot and dry climate	> 22°C / ≤ 15 hPa	30 / 35
IV	Hot and humid climate	> 22°C / > 15 hPa	30 / 70

*Grimm W (1998). Extension of the International Conference on Harmonization Tripartite Guideline for stability testing of new drug substances and products to countries of Climatic Zones III and IV. Drug Development and Industrial Pharmacy 24:313–325.

Table 4.3 Amended criteria to classify Climatic Zones and recommended testing conditions

CZ	Definition	Criteria Mean annual temperature measured in the open air / Mean annual partial water vapour pressure	Testing conditions
I	Temperate climate	≤ 15°C / ≤ 11 hPa	21°C / 45% RH
II	Subtropical and Mediterranean climate	> 15 to 22°C / > 11 to 18 hPa	25°C / 60% RH
III	Hot and dry climate	> 22°C / ≤ 15 hPa	30°C / 35% RH
IVA	Hot and humid climate	> 22°C / > 15 to 27 hPa	30°C / 65% RH
IVB	Hot and very humid climate	> 22°C / > 27 hPa	30°C / 75% RH

4.1.3 Köppen–Geiger

A classification of world climates widely used by meteorologists is a system based on the annual and monthly averages of temperature and precipitation. Initially published by Wladimir Köppen in 1918, this scheme has since been modified and refined by Geiger [4]. It is proposed to use the Köppen classification in order to be able to distinguish between different climates in Climatic Zone IV.

4.2 Calculating the Probability of Failure

4.2.1 Risk Factors

Stability testing is a technical experiment conducted at well-defined conditions, for example at fixed temperatures and humidities. These testing conditions provide a model for the climatic conditions in the environment in which the drug substance or medicinal product is stored and shipped during shelf-life. This environment can be sufficiently described by parameters influencing the stability, mainly heat and

moisture, measured as temperatures and dewpoints in warehouses, pharmacies, and in containers used for shipment.

There are, however, several other risk factors, which could have an impact on the stability of a drug substance or a drug product:

- Internal factors like the reactivity of active ingredient(s), excipients, and packaging material, as well as the interactions between these components. The risks are reduced by stress testing as part of drug substance and product development.
- Factors relating to the manufacture, like batch size, equipment, quality of components. The standard approach to reduce these risks is process validation, including cGMP, Installation Qualification (IQ), Operational Qualification (OQ), and applying new technology provided by Process Analytical Technology (PAT).
- External factors like heat and moisture, light, pH, oxygen. The risks are reduced by long-term, accelerated and stress testing, to identify the adequate packaging material, shelf-life and storage recommendations on labels.
- Physical damage during shipment and storage. Using adequate secondary packaging material (drums, cartons and containers) reduces this risk as part of product development.

In the following, only the external risk factors heat and moisture are evaluated.

4.2.2 Built-In Safety Margins

To be on the safe side, the stability tests are normally conducted at more stressful conditions than the climatic conditions in the environment in which the substance or product is expected to remain stable:

- Daily (day/night) and seasonal (summer/winter) fluctuations of temperature and humidity in the environment are replaced by conducting long-term and accelerated tests at constant temperatures (±2%) and relative humidities (±5%).
- The real-time long-term stability testing is conducted at the upper end of the climatic condition of the target market.
- To cover the extremes, short-term stress tests may also be conducted at very high temperatures and extreme humidities.
- Some pharmaceutical forms, for example ointments and emulsions, are also tested by applying freeze/thaw cycles.
- For products to be marketed in more than one specific country, the worst climatic condition of all markets is taken into consideration.

By running stability tests at these artificial conditions, a safety margin is *built in* by default. This fact has to be kept in mind when stability-testing conditions are being established and additional safety margins are regarded as necessary.

4.2.3 Calculating Additional Safety Margins

There is no scientific rationale for setting safety margins. In other disciplines like the construction of buildings, bridges or cars, the safety margins regarded as necessary to cover unexpected extremes is set based on tradition and experience more than on experimental results.

As far as pharmaceutical products are concerned, in exceptional cases where an additional margin of safety may be required, the real climatic conditions in the environment of the target market have to be considered compared to the test condition. Testing temperatures for tropical countries are normally moved up to 30°C, and relative humidities to 65, 70 or 75%.

For temperatures the following equation (4.1) can be applied to calculate a safety margin:

$$Y_T = (T_S - T) \bullet 100/T \tag{4.1}$$

Y_T = Margin of Safety for temperature [%]
T_S = Stability testing storage temperature
T = Temperature measured in the environment or calculated as MKT
$Y_T > 0$ if $T_S > T$
$Y_T = 0$ if $T_S = T$
There is a probability of failure if $Y_T < 0$

The same principle can be applied to other parameters like partial water vapour pressure.

The safety margin required is dependent on

- the impact of the environment on the product during shelf-life (*variability in loading*), and
- on the other hand on the strength of a particular batch of a product to resist heat and moisture (*variability in resistance*).

The distributions of the loading and the resistance result in the *probability of failure* of a particular product in a particular market.

The *variability in resistance* can be neglected, as the batch-to-batch variability of a product's stability is too low to be taken into consideration. As a consequence, the focus is on the *variability in loading*; for example, the fluctuations of heat and moisture in the environment or the frequency of extreme temperatures and humidities during the shelf-life of a particular product.

4.3 Climatic Data

Quantification of the *variability in loading* is started by the calculation of the key parameters temperature and humidity at different times of the day and the year in order to identify the most *loading* part of a country or region. Climatic data for

all parts of the world are available from the European Centre for Medium-Range Weather Forecasts (ECMWF) [5]. Temperatures and dewpoints have been provided, measured four times a day (at 0.00, 6.00, 12.00 and 18.00 UTC [6]) during 23 years (1979–2001), at 2 m above the ground, computerised to the centres of 125 × 125 km^2 [7]. Mean monthly values of daily temperatures and dewpoints have been used to calculate mean daily and monthly fluctuations of temperature and partial water vapour pressure.

As a starting point, the daily temperatures and dewpoints for carefully selected places and major cities in countries of concern (and the area of free trade they may belong to) have been analysed in order to identify

- the daily and monthly fluctuations of temperature and partial vapour pressure;
- their mean maximum values;
- the place representing the most *loading* climatic condition in each country or region.

4.4 Equations

The following equations have been used to calculate climatic parameters.

4.4.1 Temperature (T)

The temperature measured four times per day at each place in degrees Celsius is converted into degrees Kelvin by adding 273.15. The mean temperature per year is calculated by the sum of all 48 temperatures (4 temperatures per day for each month), divided by 48.

4.4.2 Dewpoints

The dewpoints are handled in the same way as described above for the temperatures.

4.4.3 Saturation (P_S) and Partial Water Vapour Pressure (P_D)

The original Wexler's equation published in 1971 [8] has been revised in 1976 [9], and updated in 1998 with coefficients computed for the International Temperature Scale of 1990 (ITS-90) [10]. This updated version of Wexler's equation has been used to calculate the saturation water vapour pressure P_S for each mean daily temperature, and also to calculate the partial water vapour pressure P_D at the same time point and place by taking the corresponding daily dewpoints instead of the temperature.

$$\ln P_S = \sum_{i=0}^{6} g_i \bullet T^{i-2} + g_7 \bullet \ln T \tag{4.2}$$

P_S = Saturation vapour pressure [Pa] over water in the pure phase
T = Temperature [°K]
$g_0 = -2.8365744 \bullet 10^3$
$g_1 = -6.028076559 \bullet 10^3$
$g_2 = 1.954263612 \bullet 10^1$
$g_3 = -2.737830188 \bullet 10^{-2}$
$g_4 = 1.6261698 \bullet 10^{-5}$
$g_5 = 7.0229056 \bullet 10^{-10}$
$g_6 = -1.8680009 \bullet 10^{-13}$
$g_7 = 2.7150305$

To obtain the mean values for P_S and P_D per year, the yearly mean values for temperatures and dewpoints calculated as described above have been used, respectively.

4.4.4 Relative Humidity (RH)

Knowing the saturation and the partial water vapour pressure at each time point and place, the relative humidity RH can be calculated using the following equation:

$$RH = P_D \bullet 100/P_S \tag{4.3}$$

RH = relative humidity [%]
P_S = Saturation vapour pressure
P_D = Partial water vapour pressure

The yearly mean RH values have been obtained by using the yearly mean values for P_D and P_S calculated as described above.

As the saturation vapour pressure increases with increasing temperature, the relative humidity decreases with increasing temperature at constant partial vapour pressure. As an example, the decrease of the relative humidity with increasing temperature is shown in Fig. 4.1 at a constant P_D of 26.0 hPa.

This correlation is of importance when the appropriate relative humidity is calculated for stability testing by keeping the mean partial vapour pressure constant and increasing the temperature to the testing temperature of 30°C.

4.4.5 Mean Kinetic Temperature (MKT)

An additional parameter, which is including the reaction rate constants in the evaluation of the impact of heat on pharmaceutical products, is the Mean Kinetic Temperature (MKT). The MKT was calculated by applying an equation derived by Haynes [11] based on the Arrhenius equation. At first, the activation energy E_a (assumed to be 83.144 kJ/mol) is divided by the universal gas constant R, the result is then divided by the temperature at each time point and place per day measured in degrees

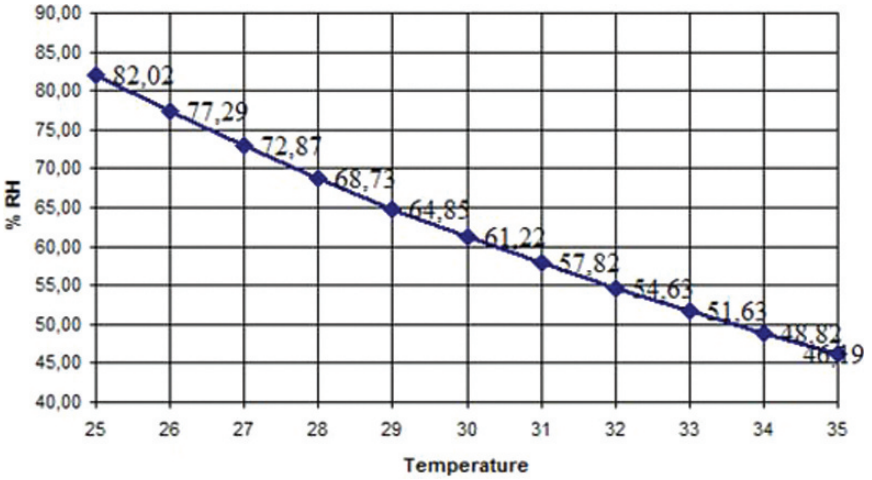

Fig. 4.1 Decreasing relative humidity (RH) with increasing temperature at $P_D = 26.0$ hPa

Celsius after conversion into degrees Kelvin by adding 273.1 to get four different values per day.

$$E_a/R = 10000.09622[^\circ K^{-1}] \tag{4.4}$$

$$f_n = e^{-10000.09622/(273.1+Tn)} \tag{4.5}$$

E_a = activation energy (assumed to be = 83.144) [kJ/mol]
R = universal gas constant = 0.00831432 [kJ/°K • mol]
Tn = temperature measured at each time point per day [°K]

After that, the sum of these four results is divided by 4.

$$F_d = (f_1 + f_2 + f_3 + f_4)/4 \tag{4.6}$$

Then the mean kinetic temperature per day for each month (MKT_d) is achieved by calculating the negative natural logarithm of the above result, converted into degrees Celsius, using the following equation:

$$MKT_d = (E_a/R)/(-\ln F_d) - 273.1 \tag{4.7}$$

The mean value per year is achieved by calculating the sum of all 48 f_n values (= four times per day × 12 months) and applying the equation for MKT_d described above to the 48 values:

$$F_a = (f_1 + f_2 + \ldots f_{48})/48 \tag{4.8}$$

$$MKT_a = (E_a/R)/(-\ln F_a) - 273.1 \tag{4.9}$$

4.5 WHO Stability Guideline

4.5.1 The Development of the WHO Stability Guidelines (Contributed by Sabine Kopp)

Work on stability of pharmaceutical products was initiated by WHO in 1988 and the *WHO Guidelines on stability testing for well-established drug substances in conventional dosage forms* were adopted in 1996 by the WHO Expert Committee on Specifications for Pharmaceutical Preparations following extensive consultation [12].

In 2000, discussions were initiated between the International Conference on Harmonization (ICH) Expert Working Group Q1 (stability) and WHO to harmonize the number of stability tests and conditions undertaken worldwide.

The ICH Expert Working Group, when developing the guidelines ICH Q1F Stability Data Package for Registration Applications in Climatic Zones II and IV, proposed a modification to the WHO guidelines. The proposal concerned the long-term conditions for Climatic Zone IV (hot and humid). The group proposed that WHO change its conditions from 30°C/70% RH to 30°C/60% RH. A detailed paper including the rationale for the change was widely circulated for comments. Non-governmental organisations, international professional organisations and specialists, and members of the WHO Expert Advisory Panel on the International Pharmacopoeia and Pharmaceutical Preparations were among those consulted.

Responses to this proposal were divided. A number of experts agreed that the proposal constituted a sound scientific approach. It was recognised that packaging was very important and common testing conditions should be agreed upon for WHO and ICH guidelines. Other views criticised the approach as being too scientific and impractical while pointing out that actual meteorological and physical storage conditions in these countries would not allow simulation of long-term storage conditions as defined by the new proposal. Arguments were also made against the application of some parameters used in the calculations.

In 2001, in a further round of discussions, it was proposed to change the real-time storage conditions for Zone IV from 30°C and 70% RH to 30°C and 65% RH. This suggestion was again circulated widely for comments and the results discussed in July 2001.

In October 2001 the WHO Expert Committee modified storage conditions and these were subsequently published in the *WHO guidelines for stability testing of pharmaceutical products containing well-established drug substances in conventional dosage forms*, to read 30°C (± 2°C) and 65% (± 5%) RH for real-time stability studies defined for Climatic Zone IV. It was also agreed that where special transportation and storage conditions did not comply with these criteria, additional study data supporting these conditions may be needed [13, 14].

4.5.2 Next Steps in WHO's Harmonization Efforts

In view of the decisions taken by ASEAN described below, WHO responded with the following action plan. First, a WHO document was circulated in early 2004,

in accordance with the WHO consultative procedure, to interested parties for consultation. The document requested comments on whether the WHO guidance on stability testing should be modified for long-term stability testing conditions (hot and humid climatic zone) and sought suggestions on how modifications should be implemented. Thereafter an informal consultation discussed comments received, in preparation of the meeting of the WHO Expert Committee on Specifications for Pharmaceutical Preparations which met in October 2004.

As the ASEAN guidance was confirmed and adopted, WHO organised a meeting including ASEAN, WHO and ICH experts and other interested parties in December 2004 [15]. The following recommendations were agreed during the meeting:

- The existing WHO guideline on stability testing should be reviewed in the light of new information on climatic conditions in Zone IV as raised by the ASEAN countries.
- All concerned parties represented at the meeting should return to their constituencies, consider the options that were discussed, and provide feedback and recommendations to WHO, indicating preferences and giving reasons. Those parties will be invited to be involved in the continuation of the consultative process. The options are as follows:

1. Revert to 30°C/70%RH as the long-term stability testing condition for Zone IV as it is likely that considerable data are already available. This might serve as a potential platform for future harmonization between ICH and WHO.
2. Change to 30°C/75%RH as the long-term stability testing condition for Zone IV in the interest of patient safety worldwide.
3. Add a new climatic Zone IVB to accommodate hot and very humid areas (30°C/75% RH). The present Zone IV (30°C/65%RH) would become Zone IVA.

Feedback was requested by end March 2005. WHO Member States not represented at the meeting were also invited to give their feedback.

Answers were received from some of the WHO Member States and partners. There was, however, no consensus among the various parties. Each option was favoured by at least one party.

4.5.3 Current WHO Status

Based on the above outcome, the experts who met during the 40th WHO Expert Committee on Specifications for Pharmaceutical Preparations in October 2005 [16] had to take a decision about the WHO position for future stability testing. They were faced with a difficult situation. The WHO Secretariat reminded the WHO Expert Committee members that the WHO guideline had been revised in the light of harmonisation efforts in collaboration with ICH. After extensive discussion, the Committee reached consensus that the WHO stability guidelines be amended to reflect conditions for Zone IV as follows:

- Zone IVA (30°C/65% RH), and
- Zone IVB (30°C/75 % RH).

It was agreed that each individual Member State within the former Zone IV would need to indicate which of these conditions (Zones IVA or IVB), would be applicable in its territory. This was intended to accommodate the two conditions currently in use [17].

Meanwhile, the WHO Eastern Mediterranean Region had developed a regional stability guideline, which was released as final in September 2006. In October of the same year at their annual meeting in Geneva, the WHO Expert Committee on Specifications for Pharmaceutical Preparations took this text as a starting point for a new global WHO Stability Guideline [18] that was distributed for comments in 2007. The aim is to use the new text as a replacement for the 1996 stability guideline incorporating a list of all the 193 WHO member countries and their recommended testing conditions.

4.5.4 Future Implementation

It will have to be seen how these new conditions will be implemented in the WHO Member States. The intent is to make this information easily accessible to third parties on an international basis and to see the trend which of the two conditions is most commonly applied.

4.6 Regional Stability Guidelines

4.6.1 ASEAN

The Association of South East Asian Nations (ASEAN) [19] was established in 1967 by five countries, namely Indonesia, Malaysia, Philippines, Singapore and Thailand. Brunei Darussalam joined in 1984, Vietnam in 1995, Laos and Myanmar in 1997, and Cambodia in 1999. The ASEAN region has a population of about 500 million.

ASEAN member countries are establishing a common market in order to facilitate a free movement of goods within the borders of the association [20]. As a consequence, a product which is marketed in a less hot and humid ASEAN member state could easily be distributed to another country which is very hot and humid. Therefore, the hottest and most humid part of the common market determines the stability testing condition.

4.6.1.1 Indonesia

All of the Indonesian islands belong to the Köppen *Group Af* with the exception of some islands in the southeast that belong to *Group Aw*. Kolbano (on the island Timor in the southwest, west of Kupang) is the place with the highest monthly mean temperatures in two consecutive months (October and November), reaching a peak at 31.5°C. The highest monthly mean values for P_D in Indonesia have been found at

the same place: 31.51 hPa in January with values above 31 hPa in four consecutive months (December–March).

4.6.1.2 The Philippines

The Philippines can be characterised as tropical islands with only minor fluctuations of temperatures and partial water vapour pressure during the day or year. Mean maximum temperatures normally do not exceed 31°C, and never drop below 23°C. P_D values can be found in the range of 25.0–33.0 hPa. All of the islands belong to Köppen *Group Am*; just a small part of the northern island Luzon is characterised by *Group Aw*.

The most *loading* place in the Philippines and probably in the whole ASEAN region identified so far is El Nido in the north of the island Palawan with mean temperatures between 25.9 and 31.0°C (average per year: 28.0°C), and P_D values between 27.3 and 33.1 hPa (average per year: 30.3 hPa).

As an example of the graphical presentation of climatic parameters, the daily temperatures and partial vapour pressures per month for El Nido (mean values 1979–2001) are shown in Figs. 4.2 and 4.3.

4.6.1.3 Comparison of Places in ASEAN

A list of the key climatic parameters measured and calculated for all ASEAN member countries (Table 4.4) facilitates the selection of the most *loading* place.

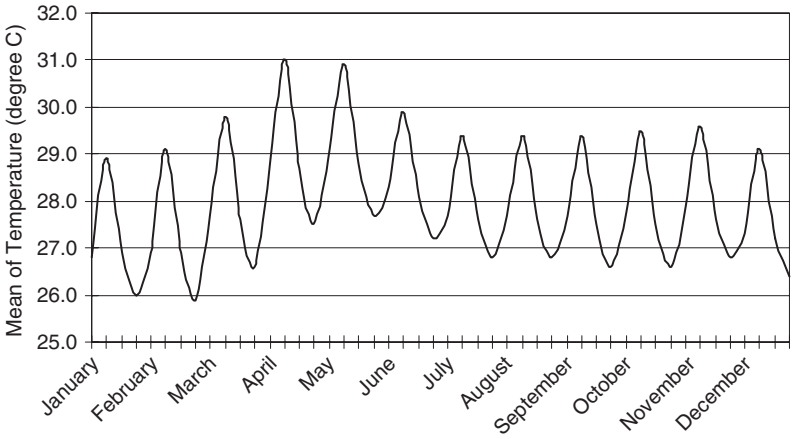

Fig. 4.2 Daily temperature fluctuations – El Nido, The Philippines

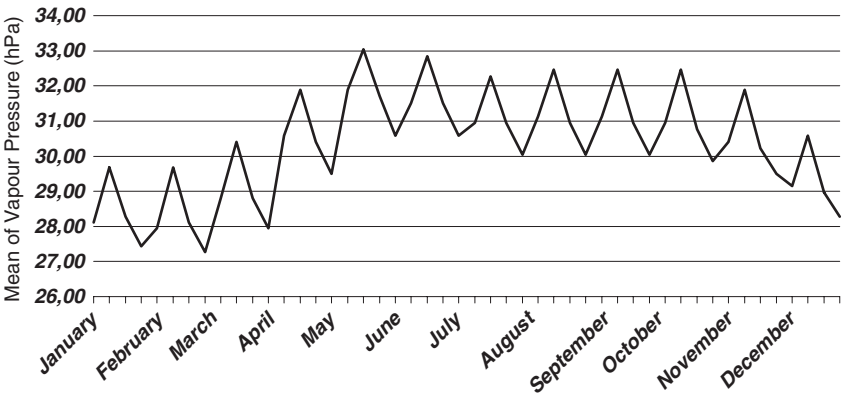

Fig. 4.3 Daily partial vapour pressure fluctuations – El Nido, The Philippines

4.6.1.4 Testing Conditions for ASEAN

With regard to an appropriate testing temperature, a safety margin of 7% is added to the MKT calculated at the place with the highest temperature (El Nido, Philippines: 28.1°C), or a safety margin of 6% to the maximum MKT found (Bangkok, Thailand: 28.4°C), to get a temperature for long-term stability testing of 30°C.

To calculate an adequate relative humidity for stability testing, the highest mean value for P_D (El Nido, Philippines: 30.28 hPa) is kept constant when moving to a testing temperature of 30°C, at which the saturation water vapour pressure is 42.47 hPa, which results in a relative humidity of 71.3% ($RH = P_D \cdot 100 / P_S$). Testing at 30°C/70% RH would represent the climatic conditions at the most *loading* place in the Philippines and the ASEAN region. Relative humidity of 70%, however, would give a negative safety margin of –2% (Table 4.5).

4.6.1.5 The Development of the ASEAN Stability Guideline (Contributed by Sabine Kopp)

In the last couple of years, ASEAN regulators and experts from ASEAN countries have met regularly with WHO and International Federation of Pharmaceutical Manufacturers & Associations (IFPMA) experts to discuss whether the conditions outlined in the WHO and ICH guidelines as described above are appropriate for countries which have vast areas with climatic conditions that are above the mean partial vapour pressure used to characterise Climatic Zone IV.

After consultation and several meetings, the meeting held in Jakarta in January 2004 concluded that the conditions described in WHO and ICH guidelines cited above did not adequately address the climatic conditions prevalent in the majority of ASEAN countries. The conditions shown in Table 4.6 were then adopted for stability studies in ASEAN countries. Arguments supporting this conclusion have been set out. A stability guideline has been released as final in July 2004 [21]. The long-term testing condition required according to this ASEAN guideline is 30°C/75% RH.

Table 4.4 Climatic data for ASEAN member countries

Country	City	T [°C]	MKT [°C]	Y_T [%] T = 30°C	P_D [hPa]	RH [%]	RH [%] at 30°C	Testing condition [°C/% RH]	Y_{PD} [%]
Brunei		25.5	25.8	16	27.35	83.8	64.4	30/65	1
								30/70	9
								30/75	17
Cambodia	Phnom Penh	26.8	27.2	11	27.69	78.5	65.2	30/65	0
								30/70	7
								30/75	15
Indonesia	Jakarta, Java	27.1	27.2	10	28.96	80.6	68.2	30/65	−5
								30/70	3
								30/75	10
	Kolbano, Timor	27.6	27.7	8	27.49	74.5	64.7	30/65	0
								30/70	8
								30/75	16
	SW Sulawesi	27.4	27.5	9	28.82	78.9	67.9	30/65	−4
								30/70	3
								30/75	11
	Palembang, Sumatra	26.7	26.9	11	29.17	83.2	68.7	30/65	−5
								30/70	2
								30/75	9
	Surabaya, Java	27.6	27.7	8	29.07	78.7	68.4	30/65	−5
								30/70	2
								30/75	10
Laos	Vianchan (Vientiane)	24.9	25.6	17	24.22	77.0	57.0	30/65	14
								30/70	23
								30/75	32
Malaysia	Kuala Lumpur	26.1	26.5	13	27.91	82.7	65.7	30/65	−1
								30/70	7
								30/75	14
Myanmar	Yangon	26.6	27.5	9	25.66	73.5	60.4	30/65	8
								30/70	16
								30/75	24
Philippines	Cebu, Cebu	27.3	27.4	9	30.18	83.1	71.1	30/65	−9
								30/70	−2
								30/75	6
	Davao, Mindanao	25.7	25.9	16	28.64	86.7	67.4	30/65	−4
								30/70	4
								30/75	11
	El Nido, Palawan	28.0	28.1	7	30.28	80.2	71.3	30/65	−9
								30/70	−2
								30/75	5
	Manila, Luzon	26.9	27.0	11	29.33	82.9	69.1	30/65	−6
								30/70	1
								30/75	9

Table 4.4 (continued)

Country	City	T [°C]	MKT [°C]	Y_T [%] T = 30°C	P_D [hPa]	RH [%]	RH [%] at 30°C	Testing condition [°C/% RH]	Y_{PD} [%]
	Roxas, Panay	27.4	27.6	9	29.10	79.7	68.5	30/65	−5
								30/70	2
								30/75	10
Singapore		27.2	27.5	9	29.14	80.7	68.6	30/65	−5
								30/70	2
								30/75	9
Thailand	Bangkok	27.9	28.4	6	27.17	72.3	64.0	30/65	2
								30/70	9
								30/75	17
Vietnam	Hanoi	23.8	24.9	21	24.08	81.9	56.7	30/65	15
								30/70	24
								30/75	32
	Ho Chi Minh City (Saigon)	27.3	27.6	9	28.11	77.6	66.2	30/65	−2
								30/70	6
								30/75	13

T = Mean temperature, calculated by using the sum of 48 measured temperatures (4 temperatures per day for each month), divided by 48.
MKT = Mean Kinetic Temperature, calculated as described above.
Y_T = Safety margin for temperature, calculated using the MKT vs. the testing temperature 30°C (for details please refer to chapter *Calculation of safety margins*).
P_D = Mean partial water vapour pressure, calculated by taking the dewpoints.
RH = Mean relative humidity, calculated by using the saturation vapour pressure P_S at the measured temperature, and the value for P_D found in the previous column.
RH at 30°C = Mean relative humidity, calculated by using the saturation vapour pressure P_S at the testing temperature 30°C, and the value for P_D found in the previous column.
Y_{PD} = Safety margin for partial vapour pressure, calculated using the meteorological P_D value vs. the P_D value calculated for the respective testing condition found in the previous column.

ASEAN based its considerations on the principle that testing should be biased towards more stressful rather than less stressful conditions so as to provide a margin of error in favour of the patients and to increase the likelihood of identifying substances or formulations that pose particular stability problems.

Table 4.5 Long-term testing conditions for ASEAN member countries

Country	30°C/65% RH CZ IVA	30°C/70% RH	30°C/75% RH CZ IVB
Brunei		+	
Cambodia		+	
Indonesia			+
Laos	+		
Malaysia		+	
Myanmar	+		
Philippines			+
Singapore			+
Thailand		+	
Vietnam			+

Table 4.6 Conditions for stability testing in ASEAN countries

Type	Conditions
Products in primary containers permeable to water vapour	30°C ± 2°C/75% ± 5% RH
Products in primary containers impermeable to water vapour	30°C ± 2°C/RH not specified
Accelerated studies	40°C ± 2°C/75% ± 5% RH
Stress studies	Unnecessary if accelerated studies at above conditions are available

ASEAN also concluded that stability is obviously affected to a large extent by the permeability of primary packaging materials. Products packed in primary containers demonstrated to be impermeable to water vapour do not require testing at any specific RH, storage at constant temperature of 30°C throughout real-time testing being sufficient. However, guidelines will be needed to specify parameters, such as a thickness and permeability coefficient that indicate demonstrated impermeability of packaging materials.

Implementation of the above decision will be preceded by a transition period during which existing national guidelines will still be applicable. In addition, a science-based approach will be taken to ensure correct evaluation when submitted data is based on conditions that are less stressful than those required (e.g. 30°C /65% RH). Factors to be taken into consideration include:

- complementary data provided to enable proper scientific evaluation;
- detected instability;
- data obtained under accelerated conditions;
- when more protective packaging is provided;
- commitment to generate data under the new guideline conditions (30°C /75% RH, or 40°C /75% RH, or both) within a specified period.

A suitable label recommendation such as *Store below 30°C and protect from moisture* may also be applied.

4.6.2 Brazil

Near the equator, Brazil belongs to Köppen *Group Af and Am*, north and south of the equator to *Group Aw*, in the southeast to *Group Cfa*, and in between to *Group Cw*. The highest temperatures in Brazil have been identified in Pau, Rio Grande do Norte, south of Fortaleza: Four consecutive months (from September to December) show mean maximum temperatures above 34.0°C, and no mean minimum temperature below 23.7°C is found (see Fig. 4.4).

As a consequence, the adequate temperature for long-term stability testing of medicinal products to be marketed in Brazil would be 30°C. That value includes a safety margin of 4% added to the highest MKT calculated for Brazil.

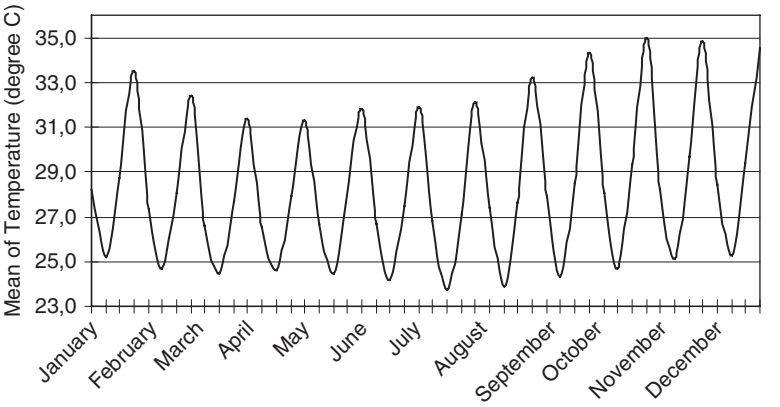

Fig. 4.4 Daily temperature fluctuations – Pau, Brazil

The highest values for P_D are to be found near the Amazonas river, in particular on the Amazonas island Ilha Macuapanim west of Jaú National Park, where the P_D values never decrease below 27.7 hPa and can go up to 32.5 hPa with a mean of 30.2 hPa (see Fig. 4.5).

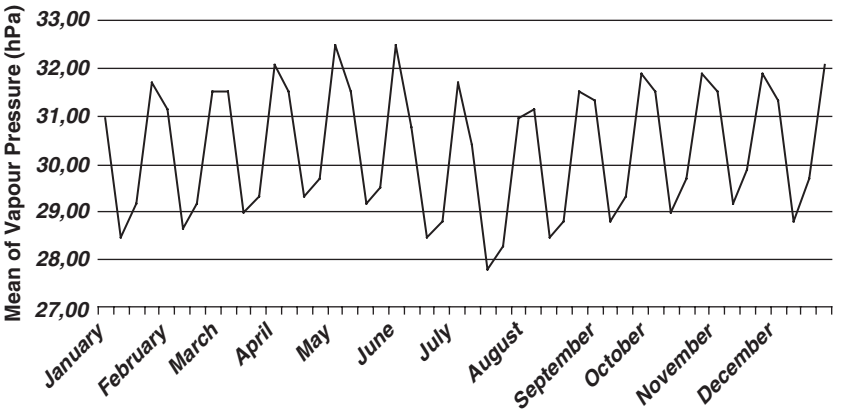

Fig. 4.5 Daily partial vapour pressure fluctuations – Ilha Macuapanim, Brazil

This island, however, is not populated in contrast to the other extreme places identified, such as

- Belém, the capital of Pará,
- Fortaleza, the capital of Ceará,
- Macapá, the capital of Amapá,
- Manaus, the capital of Amazonas,
- Natal, the capital of Rio Grande do Norte, and
- São Luís, the capital of Maranhão.

All of these major cities, however, show a small but positive safety margin for the testing condition 30°C/70% RH.

In the following, the analysis for all major cities in Brazil, all capitals of federal states in tropical climates, as well as the hottest and most humid places are presented. The safety margin for P_D is calculated for testing conditions 30°C/65% RH (first line), 30°C/70% RH (second line), and 30°C/75% RH (third line) for each selected place (see Table 4.7).

4.6.2.1 The Development of the Brazilian Stability Guideline

The Brazilian National Health Authorities (Agência Nacional de Vigilância Sanitária – ANVISA) has revoked the current stability guideline [22] and replaced it just 9 months later with a new document [23], which came into effect on 1 August 2005.

For pharmaceutical products in semi-permeable packaging material, the standard long-term stability testing condition has now been moved to 30°C/75% RH.

The key elements of the new Brazilian guideline are summarised in the following.

At the time of registration, the product may be granted a provisional shelf-life of 24 months based on 12 months stability data at long-term or 6 months accelerated conditions according to Table 4.8 (Item 2.1 of the resolution). At the time of renewal, 24-month stability data have to be presented in order to confirm the shelf-life (Item 2.2 of the resolution).

The Brazilian authorities accept the results of stability studies conducted outside the country for imported products (Item 2.5 of the resolution). The *follow-up studies*, i.e., GMP maintenance studies, however, have to be conducted in Brazil (Item 2.6 of the resolution).

It is not possible to market a product in Brazil with the storage recommendation on the label *Store below 25°C* with the exception of those products that must be stored under 25°C and which are for exclusive use in hospitals and medical clinics. For these products, stability studies conducted at conditions specified for Climatic Zone II (25°C/60% RH) will be accepted. However, the company must assure the recommended storage conditions during transportation and distribution (Item 2.14 of the resolution).

Maintenance studies (GMP) for commercial batches must be conducted at one batch per year if more than 15 batches per year are being produced, or one batch every other year if 15 or fewer batches per year are being produced (Item 3.4 of the resolution).

The frequency of testing is similar to the ICH stability guideline, in other words, at 0, 3, 6, 9, 12, 18 and 24 months for studies at long-term conditions, and at 0, 3 and 6 months for accelerated conditions. For GMP maintenance studies the frequency is every 12 months (Item 4 of the resolution).

It is important for pharmaceutical companies to observe the rules for the transition period:

Table 4.7 Climatic data for Brazil

City, Federal State	T [°C]	MKT [°C]	Y_T [%] T = 30°C	P_D [hPa]	RH [%]	RH [%] at 30°C	Testing condition [°C/% RH]	Y_{PD} [%]
Belém, Pará	26.7	26.8	12	29.29	83.4	69.0	30/65	−6
							30/70	2
							30/75	9
Brasília (Capital)	22.9	23.4	28	18.69	67.1	44.0	30/65	48
							30/70	59
							30/75	70
Fortaleza, Ceará	27.1	27.1	11	28.49	79.5	67.1	30/65	−3
							30/70	4
							30/75	12
Ilha de Marajó, Pará	26.5	26.7	13	29.97	86.6	70.6	30/65	−8
							30/70	−1
							30/75	6
Ilha Macuapanim, Amazonas	26.0	26.3	14	30.24	89.7	71.2	30/65	−9
							30/70	−2
							30/75	5
Macapá, Amapá	26.4	26.7	12	29.24	84.9	68.8	30/65	−6
							30/70	2
							30/75	9
Manaus, Amazonas	27.0	27.4	10	28.33	79.2	66.7	30/65	−3
							30/70	5
							30/75	12
Natal, Rio Grande do Norte	26.7	26.8	12	28.00	79.7	65.9	30/65	−1
							30/70	6
							30/75	14
Pau, Rio Grande do Norte	28.3	28.9	4	22.02	57.1	51.8	30/65	25
							30/70	35
							30/75	45
Pôrto Alegre, Rio Grande do Sul	19.7	20.4	47	18.33	79.8	43.2	30/65	51
							30/70	62
							30/75	74
Recife, Pernambuco	25.9	26.1	15	25.56	76.3	60.2	30/65	8
							30/70	16
							30/75	25
Rio de Janeiro, Rio de Janeiro	22.9	23.3	29	22.41	80.1	52.8	30/65	23
							30/70	33
							30/75	42
Salvador, Bahia	26.0	26.1	15	26.60	79.1	62.6	30/65	4
							30/70	12
							30/75	20
São João, Amapá	26.8	26.8	12	28.82	81.7	67.9	30/65	−4
							30/70	3
							30/75	11
São Luís, Maranhão	27.0	27.0	11	29.06	81.5	68.4	30/65	−5
							30/70	2
							30/75	10

Table 4.7 (continued)

City, Federal State	T [°C]	MKT [°C]	Y_T [%] T = 30°C	P_D [hPa]	RH [%]	RH [%] at 30°C	Testing condition [°C/% RH]	Y_{PD} [%]
São Marcelino, Amazonas	25.3	25.5	18	29.06	90.1	68.4	30/65	−5
							30/70	2
							30/75	10
São Paulo, São Paulo	20.6	21.1	42	18.70	77.2	44.0	30/65	48
							30/70	59
							30/75	70
Teresina, Piauí	27.2	27.6	9	25.77	71.5	60.7	30/65	7
							30/70	15
							30/75	24
Uraricoera, Roreima	27.1	27.5	9	23.72	66.3	55.9	30/65	16
							30/70	25
							30/75	34

Table 4.8 Stability testing requirements

Pharmaceutical product	Labeled storage condition*	Package	Accelerated testing condition**	Long-term testing condition**
Solid	15–30°C	Semi-permeable	40°C ± 2°C / 75% ± 5% RH	30°C ± 2°C / 75% ± 5% RH
Solid	15–30°C	Impermeable	40°C ± 2°C	30°C ± 2°C
Semi-solid***	15–30°C	Semi-permeable	40°C ± 2°C / 75% ± 5% RH	30°C ± 2°C / 75% ± 5% RH
Semi-solid	15–30°C	Impermeable	40°C ± 2°C	30°C ± 2°C
Liquids***	15–30°C	Semi-permeable	40°C ± 2°C / 75% ± 5% RH	30°C ± 2°C / 75% ± 5% RH
Liquids	15–30°C	Impermeable	40°C ± 2°C	30°C ± 2°C
Gases	15–30°C	Impermeable	40°C ± 2°C	30°C ± 2°C
All pharmaceutical forms	2–8°C	Impermeable	25°C ± 2°C	5°C ± 3°C
All pharmaceutical forms	2–8°C	Semi-permeable	25°C ± 2°C / 60% ± 5% RH	5°C ± 3°C
All pharmaceutical forms	–20°C	All	–20°C ± 5°C	–20°C ± 5°C

*Any storage recommendation in temperatures within these ranges must be mentioned in the package inserts and labels. The recommended temperature does not exempt the product from the stability studies within the temperatures established in the two last columns of the table.

**The temperature and humidity values are fixed, and the variations are due to expected oscillations in the climatic chamber and possible openings for removal or entry of material.

***The study for water-based liquids and semi-solid products must be conducted at 25% or 75% RH. In case of 75% RH, the weight loss value must be multiplied by 3.0.

- The submission of stability studies is mandatory at the time of the first renewal of a marketing authorisation after 1 August 2005 – if not submitted earlier – even if the studies have been conducted according to the requirements in force at the beginning of the studies (Item 5.1).
- At the time of registration, post-registration or registration renewal prior to 31 July 2007, ANVISA will accept on-going long-term stability studies with relative humidities below 75% (Item 5.2). After that date, the company must present follow-up stability studies conducted on at least one batch according to the new requirements (Item 5.3).
- If the long-term stability studies have been conducted only with temperature and humidity parameters different from the ones set forth in the new resolution, at the first renewal after 1 August 2007, the company must present 12 month long-term stability studies, or 6 months accelerated study followed by the respective long-term study according to the new requirements; otherwise the registration will not be renewed (Item 5.4).
- If long-term stability studies, conducted according to the new conditions, prove a shelf-life inferior to the one registered, the company must immediately reduce the shelf-life based on the data obtained (Item 5.5).

4.6.3 Other Countries in South America

Similar assessment of meteorological data for other countries in South America concludes in the fact that the climate in those countries indicates long-term stability testing at 30°C/75% RH is appropriate (see Table 4.9), although most of the countries except Ecuador belong to the former Climatic Zone IV and testing at 30°C/70% RH would be sufficient.

4.6.4 China

Mainland China is extremely diverse from a climatic point of view: from tropical parts in the south (*Group Am*) to regions with severe dry winters (*Group Dw*) in the northeast, and humid subtropical regions (*Group Cfa*) in between.

Table 4.9 Long-term testing conditions for South American countries

Country	30°C/65% RH CZ IVA	30°C/70% RH	30°C/75% RH CZ IVB
Bolivia		+	
Brazil			+
Colombia			+
Ecuador	+		
Guyana		+	
Peru		+	
Suriname		+	
Venezuela		+	

Table 4.10 Climatic data for China

City, provinces	T [°C]	MKT [°C]	Y_T[%] T = 25°C T = 30°C	P_D [hPa]	RH [%]	RH [%] at 25°C at 30°C	Testing condition [°C/% RH]	Y_{PD} [%]
Hong Kong (HKG)	23.4	24.4	3 23	22.21	77.4	70.1 52.1	25/60 30/65	−14 25
Macau (MAC)	23.0	24.1	4 24	22.12	78.9	69.8 52.1	25/60 30/65	−14 25
Mengla, Yunnan	21.1	22.1	13 36	19.34	77.3	61.0 45.5	25/60 30/65	−2 43
Nanchang, Jiangxi	18.0	21.5	16 40	15.73	76.0	49.6 37.0	25/60 30/65	21 76
Nanchong, Sichuan	18.1	20.9	19 43	15.29	73.7	48.2 36.0	25/60 30/65	24 81
Sanya, Hainan	26.0	26.4	-5 14	27.16	80.7	85.7 64.0	25/60 30/65	−30 2
Shanghai, Shanghai	15.8	19.0	32 58	13.96	77.8	44.0 32.9	25/60 30/65	36 98

Shanghai at the east coast, Macau and Hong Kong in the southeast, and the city located in the most southern corner of the mainland, Mengla, close to the border to Laos, have been selected. Another city in the south is Sanya on the southern coast of the island Hainan. In addition to these cities, two cities identified as hot and humid *spots* in the centre of the land mass have been analysed: Nanchang in Jiangxi and Nanchong in Sichuan. The results of the calculations are presented in Table 4.10 for both testing at 25°C/60% RH and 30°C/65% RH.

As can be concluded from the table, the most *loading* place in China is Sanya on the island Hainan in the most southern part of the country. Mean maximum

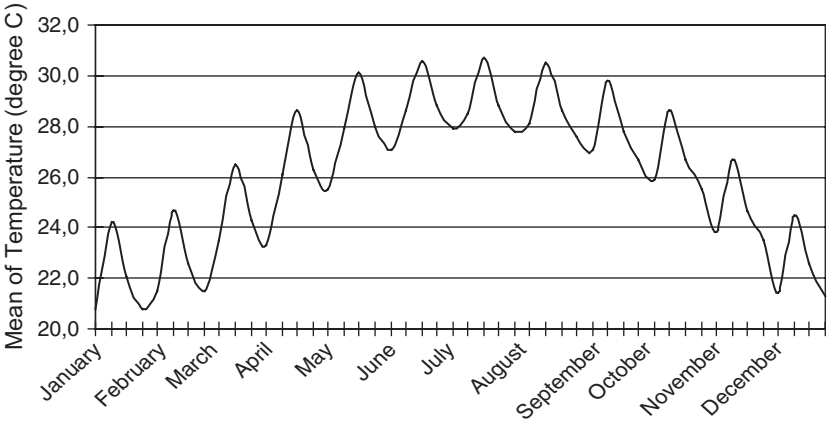

Fig. 4.6 Daily temperature fluctuations – Sanya, Hainan, China

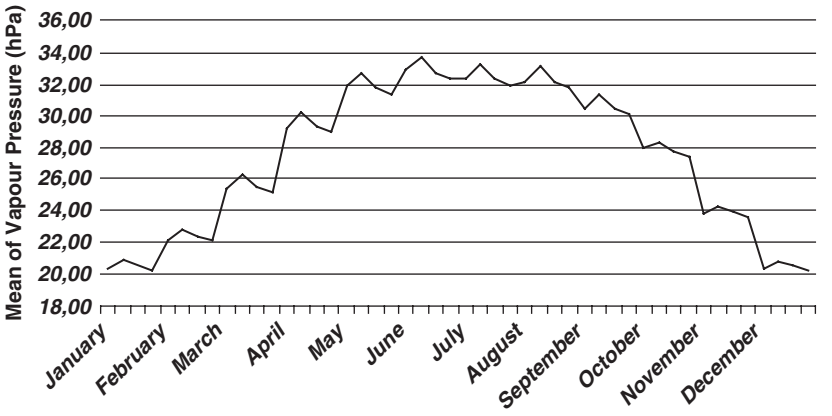

Fig. 4.7 Daily partial vapour pressure fluctuations – Sanya, Hainan, China

temperatures reach more than 30°C in four consecutive months (May–August), and mean minimum temperatures never drop below 20°C (in January, see Fig. 4.6).

The southern part of this island belongs to Köppen *Group Am*, the northern part to *Group Aw*. The maximum P_D values increase to more than 33.0 hPa during 3 months (June–August, see Fig. 4.7).

To calculate the adequate relative humidity for long-term stability testing, the mean partial water vapour pressure calculated for Sanya (27.16 hPa) is used at the standard testing temperature 30°C to get 64.0% RH. Testing at 30°C would include a safety margin of 14% added to the MKT, and testing at 65% RH would include a safety margin of 2% for P_D. Sanya, however, presents an extreme climate compared to the other parts of the country.

4.6.4.1 Stability Testing Requirements

The new *Chinese Pharmacopoeia 2005* (CP 2005) [24], which came into effect on 1 July 2005, provides guidance for stability testing. In principle, these testing conditions are in accordance with the ICH stability guideline Q1A(R2) specified for countries in Climatic Zone II [25]. The key elements of these conditions are summarised as follows and the difference to the ICH guideline is highlighted:

Long-Term Studies

It is confirmed that China in general belongs to Climatic Zone II (subtropical). The long-term storage condition, therefore, is 25°C ± 2°C/60% RH ± *10% RH. Note the difference to the ICH guideline where the fluctuation of the relative humidity (RH) is tighter (± 5%).*

Accelerated Studies

- Three batches of the medicinal product as proposed for marketing should be tested at 40°C ± 2°C/75% RH ± 5% RH for the duration of 6 months in the 1st, 2nd, 3rd and 6th month. This means that there are more testing points required compared to the ICH guideline.
- For products sensitive to temperature, and estimated to be stored in a refrigerator at *4*–8°C (*note the difference to ICH = 2–8°C*), the accelerated storage condition is 25°C ± 2°C/60% RH ± *10% RH* (as compared with ICH: ± 5%) for the duration of 6 months.
- For emulsions, suspensions, aerosol products, effervescent products, etc., the accelerated condition is 30°C ± 2°C/65% RH ± 5% RH for the duration of 6 months.
- For drug products in semi-permeable containers, such as solutions in plastic bag, eye drops or nasal drops in plastic bottle, the accelerated condition is 40°C ± 2°C/*20% RH* ± 2% RH (as compared with *ICH: not more than 25% RH*). This condition can be reached by using a saturated solution of $CH_3COOK \cdot 1.5\ H_2O$.

Intermediate Condition

If the test result cannot meet the specifications at the accelerated condition 40°C ± 2°C/75% RH ± 5% RH, the condition can be modified to the intermediate condition 30°C ± 2°C/65% RH ± 5% RH, for the duration of 6 months.

4.6.5 India (Contributed by Saranjit Singh, Amrit Paudel, Gaurav Bedse, Rhishikesh Thakare, Vijay Kumar)

4.6.5.1 Diverse Physical and Climatic Conditions

According to Köppen, India is truly diverse in its climate, hosting several major climatic subtypes: Alpine tundra and glaciers in the north, arid desert in the west, humid tropical regions supporting rainforests in the southwest and the island territories. Many regions have starkly different microclimates. The nation has four seasons: Winter (January and February), Summer (March–May), Monsoon (rainy) (June–September) and post-Monsoon period (October–December).

4.6.5.2 Calculation of Long-Term Stability Test Conditions

Table 4.11 gives the data for the 18 selected cities (Fig. 4.8). It shows that Srinagar, in the north of India, is the coldest among the selected cities, with a mean temperature of 2.78°C, in line with alpine tundra conditions. Jodhpur in the west is the driest among all, as it falls in a region marked by the Thar Desert. Trivandrum and Chennai in the far south have high temperatures as well as high humidity, but between the two, the former has relatively lower temperatures and higher humidity, whereas the latter has slightly higher temperatures but lower humidity. The data further show

Fig. 4.8 Selection of cities across India

that YP_D values were positive for all the cities at 30°C /65% RH, except Trivandrum where a positive YP_D value was obtained at 30°C /70% RH.

The 18 cities were then distributed according to the WHO's climatic classification system (Table 4.12). The data showed that Srinagar was in Zone I, Jodhpur in Zone III, and all other cities except Trivandrum fell in Zone IVA. Trivandrum alone fell in the WHO's original recommendation 30°C/70% RH [12, 13].

The city of Trivandrum is located in the Indian state of Kerala, the only state with equatorial monsoonal conditions for almost 7 months of the year and is hence the most stringent. It cannot be ignored while determining the stability storage conditions for the country, as it is also one amongst the densely populated. Thus, 30°C/65%RH was ruled out as the condition for the country. Even the WHO's Zone IVB option (30°C/75% RH) was considered improbable, as no city or state in India, including Kerala, matched the equatorial fully humid conditions, as in ASEAN or Brazil. Therefore, the most justifiable and qualifying long-term stability test storage condition for India was determined to be 30°C/70% RH. This storage condition, however, is not listed in the WHO's new classification, although it was originally prescribed by the agency for Zone IV.

Table 4.11 MKT, %RH, %YT and %YPD data for 18 cities across India at testing conditions of 30°C/65% RH, 30°C/70% RH and 30°C/75% RH

Cities	T [°C]	MKT [°C]	YT [%]	PD [hPa]	RH [%]	RH % at 30°C	Testing conditions [°C/% RH]	PD [hPa] at test conditions	YPD [%]
Srinagar	2.78	6.30	376.17	5.52	73.71	13.03	30/65 30/75	27.61 31.85	400.45 477.31
Jodhpur	25.74	28.36	5.76	13.70	41.35	32.35	30/65 30/75	27.61 31.85	101.56 132.51
Ahmedabad	26.87	28.07	6.88	19.38	74.59	45.76	30/65 30/75	27.61 31.85	42.46 64.34
Mumbai	26.24	27.12	10.63	22.03	64.55	52.01	30/65 30/75	27.61 31.85	25.36 44.61
Goa	25.58	26.33	13.94	21.86	66.62	51.62	30/65 30/75	27.61 31.85	26.29 45.68
Trivandrum	27.31	27.48	9.17	29.14	80.20	68.80	30/65 30/70 30/75	27.61 29.73 31.85	−5.24 2.04 9.31
Chennai	28.25	29.09	3.11	25.93	67.56	61.22	30/65 30/75	27.61 31.85	6.49 22.85
Puri	26.69	27.37	9.62	26.16	74.69	61.78	30/65 30/75	27.61 31.85	5.54 21.74

Table 4.11 (continued)

Cities	T [°C]	MKT [°C]	YT [%]	PD [hPa]	RH [%]	RH % at 30°C	Testing conditions [°C/% RH]	PD [hPa] at test conditions	YPD [%]
Kolkata	25.71	26.94	11.36	24.15	73.25	57.04	30/65 30/75	27.61 31.85	14.31 31.86
Mizoram	22.40	23.35	28.51	20.11	74.41	47.49	30/65 30/75	27.61 31.85	37.27 58.35
Cherapunji	22.42	23.50	27.67	21.56	79.71	50.92	30/65 30/75	27.61 31.85	28.05 47.71
Patna	25.16	26.96	11.27	20.33	63.70	48.01	30/65 30/75	27.61 31.85	35.80 35.80
Delhi	24.53	27.40	9.48	17.09	55.62	40.36	30/65 30/75	27.61 31.85	61.54 86.35
Bhopal	25.71	27.78	7.98	15.76	47.80	37.22	30/65 30/75	27.61 31.85	75.16 75.16
Nagpur	26.74	28.49	5.32	19.21	54.81	45.35	30/65 30/75	27.61 31.85	43.75 65.83
Hyderabad	27.27	28.40	5.63	20.88	57.74	49.29	30/65 30/75	27.61 31.85	32.26 52.57
Bangalore	24.99	25.71	16.68	20.99	66.46	49.56	30/65 30/75	27.61 31.85	31.55 51.76
Amritsar	21.49	24.49	22.52	15.64	61.18	36.93	30/65 30/75	27.61 31.85	76.56 103.67

Table 4.12 Assignment of climatic zone for selected cities of India according to current WHO classification criteria

Cities	T [°C]	PD [hPa]	T/PD [°C/hPa]	RH % at 30°C	Storage condition [°C/% RH]	Climatic zones
Srinagar	2.78	5.52	≤15/≤11	13.03	21/45	I
Jodhpur	25.74	13.70	>22/≤15	32.35	30/35	III
Ahmedabad	26.87	19.38	>22/>15–27	45.76	30/65	IVA
Mumbai	26.24	22.03	>22/>15–27	52.01	30/65	IVA
Goa	25.58	21.86	>22/>15–27	51.62	30/65	IVA
Trivandrum	27.31	29.14	>22/>27	68.80	30/70	IV
Chennai	28.25	25.93	>22/>15–27	61.22	30/65	IVA
Puri	26.69	26.16	>22/>15–27	61.78	30/65	IVA
Kolkata	25.71	24.15	>22/>15–27	57.04	30/65	IVA
Mizoram	22.40	20.11	>22/>15–27	47.49	30/65	IVA
Cherapunji	22.42	21.56	>22/>15–27	50.92	30/65	IVA
Patna	25.16	20.33	>22/>15–27	48.01	30/65	IVA
Delhi	24.53	17.09	>22/>15–27	40.36	30/65	IVA
Bhopal	25.71	15.76	>22/>15–27	37.22	30/65	IVA
Nagpur	26.74	19.21	>22/>15–27	45.35	30/65	IVA
Hyderabad	27.27	20.88	>22/>15–27	49.29	30/65	IVA
Bangalore	24.99	20.99	>22/>15–27	49.56	30/65	IVA
Amritsar	21.49	15.64	>22/>15–27	36.93	30/65	IVA

4.6.6 Eastern Mediterranean Region

Stability testing requirements for registration of pharmaceutical products are different in many Arabic countries [26] representing similar climatic conditions. In 1993, the WHO Regional Office for the Eastern Mediterranean (EMRO) initiated a workshop [27] in Amman, Jordan, where experts from ten Arabic countries met to discuss technical standards, and developed a draft regional stability guideline [28] that has been further discussed at a follow-up meeting in Damascus, Syria, in 1994. In November 2003, the Cooperation Council for the Arab States of the Gulf (GCC) released a guideline, which is applicable to the central application procedure valid for the Gulf States. A common stability guideline applicable for products marketed in all of the EMRO countries has recently been developed and recommended for adoption in the region.

In the following, the climates of countries in Northern and Eastern Africa, the Arabian Peninsula, the Middle East and Southern Asia are presented and analysed, and the most appropriate testing conditions for long-term stability studies are proposed.

4.6.6.1 Selection of Countries

In order to support the development of the regional WHO stability guideline for the EMR, the following countries have been selected for detailed evaluation:

- Countries in Northern and Eastern Africa, namely Morocco, Algeria, Tunisia, Libya, Egypt, Sudan, Somalia and Djibouti;
- the Gulf States and Yemen;
- the Middle East countries, namely Lebanon, Jordan, Israel, Palestine, Syria, Iraq and Iran;
- Afghanistan and Pakistan in Southern Asia.

4.6.6.2 Northern and Eastern Africa

This geographical region is dominated by the Sahara desert, presenting a hot and dry climate. The northern part is characterised by a Mediterranean climate, and the south of Sudan by a tropical climate. The results of the calculations are presented in Table 4.13.

4.6.6.3 The Arabian Peninsula

All of the countries, which are members in the GCC, have been selected for climatic evaluation, namely Bahrain, Kuwait, Oman, Qatar, Saudi Arabia and the United Arab Emirates, as well as Yemen. All of these countries are located in a hot and dry climate (desert). The detailed results of the calculations are presented in Table 4.14.

Saudi Arabia

The country is hot and dry. The place with the highest MKT (30.8°C) is Ad-Dammām at the east coast. Mean temperatures never drop below 11.8°C (in January at night), but go up to 44.1°C (in July at noon). The driest place is the capital Ar-Riyāḍ (Riyadh) (see Figs. 4.9 and 4.10). Other cities analysed are Jeddah (Jiddah), Makkah (Mecca) and Al-Madīnah (Medina). The highest mean temperature of 29.1°C has been calculated at 41.8° E, 17.9° N, at the coast to the Red Sea, southeast of Al Birk. Mean daily temperatures fluctuate between 23.7°C (in February at 00:00 UTC) and 34.8°C (in July at 12:00 UTC). This is also the most humid place identified in Saudi Arabia. The lowest mean partial vapour pressure is as high as 22.7 hPa (in January at 12:00 UTC), and the maximum value is 33.8 hPa (in September at 18:00 UTC).

4.6.6.4 The Middle East

The climate is mainly Mediterranean or hot and dry in deserts, and in between arid (steppe). The Mediterranean climate is covered by the standard long-term stability testing condition of 25°C/60% RH, while for the hot and dry places additional conditions should be defined, in particular for populated areas. For details see Table 4.15.

4.6.6.5 Southern Asia

The detailed data for Afghanistan and Pakistan are summarised in Table 4.16.

Table 4.13 Climatic data for Northern Africa

City, region	T [°C]	MKT [°C]	Y_T[%]	P_D [hPa]	RH [%]	RH [%] at 25 or 30°C	Testing condition [°C/% RH]	Y_{PD} [%]
Algeria								
Algiers	18.5	19.8	26 (T = 25°C) 52 (T = 30°C)	15.88	74.4	50.1 (T = 25°C) 37.4 (T = 30°C)	25/60 30/65 30/70 30/75	20 74 87 101
South Corner (Ti-n-Zaouâtene)	26.3	29.5	–15 (T = 25°C) 2 (T = 30°C)	6.04	17.7	19.1 (T = 25°C) 14.2 (T = 30°C)	25/60 30/35 30/65 30/70 30/75	215 146 357 392 427
Djibouti								
Djibouti	29.4	30.9	–3 (T = 30°C)	18.87	46.1	44.4	30/65 30/70 30/75	46 58 69
Egypt								
Al-Iskandarīyah (Alexandria)	20.0	21.6	16 (T = 25°C) 39 (T = 30°C)	15.91	68.0	50.2 (T = 25°C) 37.5 (T = 30°C)	25/60 30/65 30/70 30/75	20 74 87 100
Al-Qāhirah (Cairo)	20.0	22.1	13 (T = 25°C) 36 (T = 30°C)	14.64	62.7	46.2 (T = 25°C) 34.5 (T = 30°C)	25/60 30/65 30/70 30/75	30 89 103 118
As-Suways (Suez)	20.0	22.1	13 (T = 25°C) 36 (T = 30°C)	13.96	59.5	44.0 (T = 25°C) 32.9 (T = 30°C)	25/60 30/65 30/70 30/75	36 98 113 128
Aswān (Aswan)	25.0	28.1	–11 (T = 25°C) 7 (T = 30°C)	9.11	28.8	28.7 (T = 25°C) 21.5 (T = 30°C)	25/60 30/35 30/65 30/70 30/75	109 63 203 226 250
Balât	22.4	26.0	–4 (T = 25°C) 16 (T = 30°C)	9.66	35.7	30.5 (T = 25°C) 22.7 (T = 30°C)	25/60 30/35 30/65 30/70 30/75	97 54 186 208 230
Libya								
Al Jawf	21.7	25.5	–2 (T = 25°C) 18 (T = 30°C)	7.06	27.3	22.3 (T = 25°C) 16.6 (T = 30°C)	25/60 30/35 30/65 30/70 30/75	169 111 291 321 381

Table 4.13 (continued)

City, region	T [°C]	MKT [°C]	Y_T[%]	P_D [hPa]	RH [%]	RH [%] at 25 or 30°C	Testing condition [°C/% RH]	Y_{PD} [%]
Banghāzi (Benghazi)	19.9	21.0	19 (T = 25°C) 43 (T = 30°C)	17.17	73.8	54.2 (T = 25°C) 40.4 (T = 30°C)	25/60 30/65 30/70 30/75	11 61 73 86
Tarābulus (Tripoli)	19.8	22.0	14 (T = 25°C) 37 (T = 30°C)	13.65	58.9	43.1 (T = 25°C) 32.1 (T = 30°C)	25/60 30/65 30/70 30/75	39 102 118 133
Morocco								
Bou Arfa	18.2	22.4	11 (T = 25°C) 34 (T = 30°C)	6.96	33.3	22.0 (T = 25°C) 16.4 (T = 30°C)	25/60 30/35 30/65 30/70 30/75	173 114 297 327 358
Casablanca	18.6	19.1	31 (T = 25°C) 57 (T = 30°C)	17.05	79.5	53.8 (T = 25°C) 40.1 (T = 30°C)	25/60 30/65 30/70 30/75	12 62 74 87
Oujda	17.0	19.5	28 (T = 25°C) 54 (T = 30°C)	11.03	57.0	34.8 (T = 25°C) 26.0 (T = 30°C)	25/60 30/65 30/70 30/75	72 150 170 189
Rabat	17.5	19.4	29 (T = 25°C) 55 (T = 30°C)	13.71	68.5	43.3 (T = 25°C) 32.3 (T = 30°C)	25/60 30/65 30/70 30/75	39 101 117 132
Somalia								
Beled Weyne (Belet Uen)	27.6	8.2	6	21.01	56.9	49.5	30/65 30/70 30/75	31 42 52
Boosaaso (Bender Qaasim)	27.1	28.2	7	18.30	51.1	43.1	30/65 30/70 30/75	51 63 74
Chisimayu (Kismaayo)	27.9	28.3	6	26.26	69.9	61.8	30/65 30/70 30/75	5 13 21
Muqdisho (Mogadishu)	27.5	27.9	7	24.36	66.4	57.4	30/65 30/70 30/75	13 22 31
Sudan								
Al-Khartūm (Khartoum)	29.1	30.8	–19 (T = 25°C) –3 (T = 30°C)	10.38	25.7	32.7 (T = 25°C) 24.4 (T = 30°C)	25/60 30/35 30/65 30/70 30/75	83 43 166 186 207

Table 4.13 (continued)

City, region	T [°C]	MKT [°C]	Y_T[%]	P_D [hPa]	RH [%]	RH [%] at 25 or 30°C	Testing condition [°C/% RH]	Y_{PD} [%]
Būr Sūdān (Port Sudan)	28.8	30.9	–19 (T = 25°C) –3 (T = 30°C)	16.61	42.0	52.4 (T = 25°C) 39.1 (T = 30°C)	25/60 30/65 30/70 30/75	15 66 79 92
Jūbā (Juba)	25.5	26.2	–5 (T = 25°C) 15 (T = 30°C)	20.37	62.2	64.3 (T = 25°C) 48.0 (T = 30°C)	25/60 30/65 30/70 30/75	–7 36 46 56
Kassalā (Kassala)	30.2	31.4	–20 (T = 25°C) –4 (T = 30°C)	14.50	33.8	45.7 (T = 25°C) 34.1 (T = 30°C)	25/60 30/35 30/65 30/70 30/75	31 3 90 105 120
Nyala	24.7	26.2	–5 (T = 25°C) 15 (T = 30°C)	10.79	34.6	34.0 (T = 25°C) 25.4 (T = 30°C)	25/60 30/35 30/65 30/70 30/75	76 38 156 176 195
Tunisia								
Tunis	18.1	20.0	25 (T = 25°C) 50 (T = 30°C)	14.40	69.3	45.4 (T = 25°C) 33.9 (T = 30°C)	25/60 30/65 30/70 30/75	32 92 107 121
South Corner (Fort Saint)	21.1	25.1	0 (T = 25°C) 20 (T = 30°C)	9.31	37.2	29.4 (T = 25°C) 21.9 (T = 30°C)	25/60 30/35 30/65 30/70 30/75	104 60 197 219 242

4.6.6.6 Discussion

Testing Condition: Temperature

Extreme high mean daily temperatures found during the day in summer are 40.1°C in Ti-n-Zaouâtene, Algeria, 41.6°C in Port Sudan, Sudan, 40.1°C in Bahrain, 41.1°C in Kuwait, 44.3°C in Dank, Oman, 44.1°C in Ad-Dammam, Saudi Arabia and 43.0°C in Abu Dhabi, UAE. The hottest place identified is Basra, Iraq (46.2°C at noon in July).

To estimate the impact of heat on pharmaceutical products, however, the MKT has to be taken into consideration rather than single maximum temperatures. While the MKT calculated as described above is below 30°C in most of the countries evaluated, some places have been found that present higher MKT values: Kassala, Sudan (31.4°C), Dank (32.5°C) and Runib, Oman (32.7°C), Ad-Dammam, Saudi

Table 4.14 Climatic data for the Arabian Peninsula

City	T [°C]	MKT [°C]	Y_T[%] T = 30°C	P_D [hPa]	RH [%]	RH [%] at 30°C	Testing condition [°C/% RH]	Y_{PD} [%]
Bahrain								
Al-Manāmah (Manama)	27.2	29.7	1	18.43	51.2	43.4	30/65	50
							30/70	61
							30/75	73
Kuwait								
Al-Kuwayt (Kuwait)	26.3	29.6	1	16.76	48.9	39.5	30/65	65
							30/70	77
							30/75	90
Oman								
Dank, Az-Zāhirah	29.5	32.5	–8	11.97	29.1	28.2	30/35	24
							30/65	131
							30/70	148
							30/75	166
Masqat (Muscat) Matrah, Masqat	28.2	30.4	–1	14.86	38.8	35.0	30/35	0
							30/65	86
							30/70	100
							30/75	114
Runib (oil field), Al-Wusta	30.4	32.7	–8	14.47	33.4	34.1	30/35	3
							30/65	91
							30/70	106
							30/75	120
Salālah, Zufār	25.9	26.1	15	23.50	70.3	55.3	30/65	18
							30/70	27
							30/75	36
Qatar								
Ad-Dawhah (Doha)	27.1	28.7	5	23.44	65.5	55.2	30/65	18
							30/70	27
							30/75	36
Saudi Arabia								
Ad-Dammām, Ash Sharqiyah	26.9	30.8	–3	11.33	31.9	26.7	30/35	31
							30/65	144
							30/70	162
							30/75	181
Al Birk, Acharsīr	29.1	29.6	1	27.72	68.6	65.3	30/35	−46
							30/65	0
							30/70	7
							30/75	15
Ar-Riyād (Riyadh), Ar-Riyā	25.2	29.0	4	6.91	21.5	16.3	30/35	115
							30/65	300
							30/70	330
							30/75	361

Table 4.14 (continued)

City	T [°C]	MKT [°C]	Y_T[%] T = 30°C	P_D [hPa]	RH [%]	RH [%] at 30°C	Testing condition [°C/% RH]	Y_{PD} [%]
Jeddah (Jiddah) Makkah (Mecca), Makkah	28.4	29.9	0	15.72	40.5	37.0	30/35	−5
							30/65	76
							30/70	89
							30/75	103
Al-Madīnah (Medina), Al-Madīnah	25.9	28.9	4	7.86	23.6	18.5	30/35	89
							30/65	251
							30/70	278
							30/75	305
United Arab Emirates								
Abū Zaby (Abu Dhabi),	27.9	30.8	–3	14.18	37.7	33.4	30/35	5
							30/65	95
							30/70	110
							30/75	125
Dubayy (Dubai)	28.4	30.7	–2	16.69	43.2	39.3	30/35	−11
							30/65	65
							30/70	78
							30/75	91
Yemen								
‘Adan (Aden)	24.3	24.8	21 (T = 30°C)	20.10	66.3	47.3 (T = 30°C)	30/65	37
							30/70	48
							30/75	59
‘Adan (Aden) Al-Hudaydah	28.4	28.8	4 (T = 30°C)	28.06	72.5	66.1 (T = 30°C)	30/65	−2
							30/70	6
							30/75	14
‘Adan (Aden) San‘ā’ (Sanaa)	22.0	23.2	8 (T = 25°C)	13.45	51.0	42.4 (T = 25°C)	25/60	41
			30 (T = 30°C)			31.7 (T = 30°C)	30/65	105
							30/70	121
							30/75	137

Arabia (30.8°C), Abu Dhabi, UAE (30.8°C) and Basra, Iraq (31.5°C). Most of these places, however, are deserted areas, and do not have to be considered in this context.

The standard long-term testing temperature, 30°C, for countries in Climatic Zones III, IVA and IVB is regarded as adequate even for MKT values just above 30°C for the following reasons:

- Products proven to be stable at 30°C (long-term testing) are labelled *Store below 30°C*;
- Additional data from 6 months accelerated testing at 40°C/75% RH can be used to evaluate the effect of short-term excursions outside the label storage conditions such as might occur during shipping;

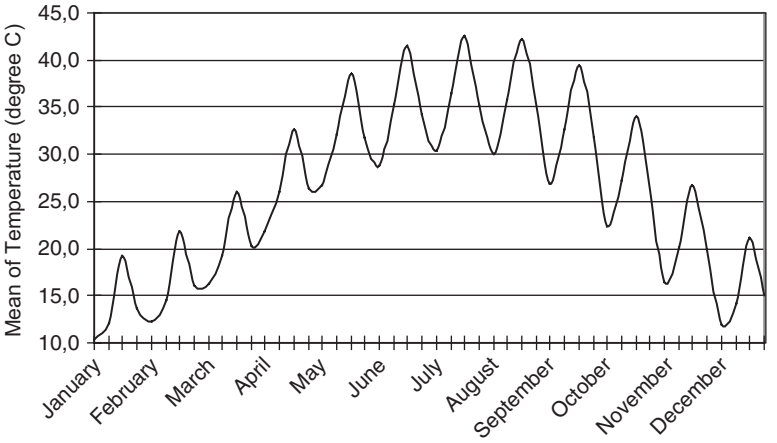

Fig. 4.9 Daily temperature fluctuations – Riyadh, Saudi Arabia

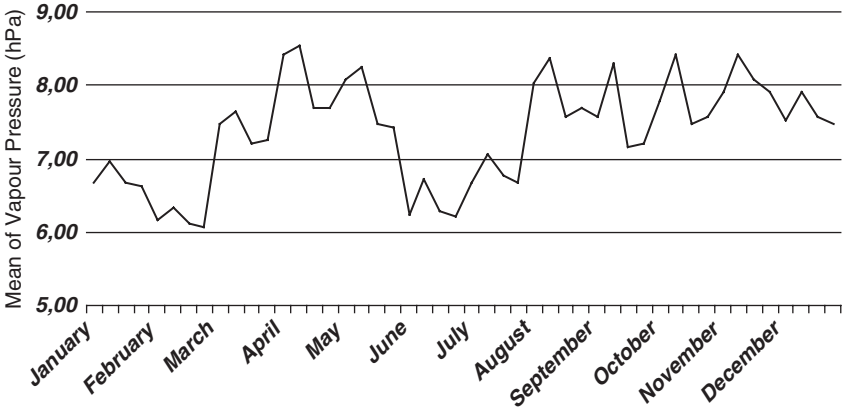

Fig. 4.10 Daily partial vapour pressure fluctuations – Riyadh, Saudi Arabia

- To cover extremely hot and dry conditions, additional stress tests conducted on one batch for up to 3 months at 50°C/ambient humidity may be helpful to select the appropriate packaging material during product development.

Testing Condition: Humidity

Extreme low mean partial water vapour pressure values have been found near Ti-n-Zaouâtene, Sahara desert, Algeria (6.04 hPa), in Khvor in the Salt Desert, Iran (6.24 hPa), in Riyadh, Saudi Arabia (6.91 hPa), and in Bou Arfa, Sahara desert, Morocco (6.96 hPa).

The annual mean RH in Riyadh is 21.5% (annual mean partial water vapour pressure = 6.91 hPa; annual mean temperature = 25.23°C). Moving the temperature

Table 4.15 Climatic data for the Middle East

City	T [°C]	MKT [°C]	Y_T[%]	P_D [hPa]	RH [%]	RH [%] at 25 or 30°C	Testing condition [°C/% RH]	Y_{PD} [%]
Iran								
			–17 (T = 25°C)			29.6 (T = 25°C)	25/60	103
							30/35	59
Ahvāz	24.2	30.0	0 (T = 30°C)	9.38	31.1	22.1 (T = 30°C)	30/65	194
							30/70	217
							30/75	240
			–15 (T = 25°C)			64.5 (T = 25°C)	25/60	−7
							30/65	35
Bandar-e ‘Abbās	27.2	29.4	2 (T = 30°C)	20.46	56.5	48.2 (T = 30°C)	30/70	45
							30/75	56
			–4 (T = 25°C)			19.7 (T = 25°C)	25/60	209
							30/35	138
Khvor	20.5	26.1	15 (T = 30°C)	6.24	25.9	14.7 (T = 30°C)	30/65	342
							30/70	376
							30/75	411
			56 (T = 25°C)			28.5 (T = 25°C)	25/60	110
							30/65	205
Rasht	12.3	16.0	88 (T = 30°C)	9.04	63.4	21.3 (T = 30°C)	30/70	229
							30/75	252
			32 (T = 25°C)			27.0 (T = 25°C)	25/60	122
							30/65	222
Tehran	14.3	18.9	59 (T = 30°C)	8.56	52.7	20.2 (T = 30°C)	30/70	247
							30/75	272
Iraq								
			–21 (T = 25°C)			30.0 (T = 25°C)	25/60	100
							30/35	57
Al-Basrah [Basra]	26.1	31.5	–5 (T = 30°C)	9.50	28.0	22.4 (T = 30°C)	30/65	191
							30/70	213
							30/75	235
			–0 (T = 25°C)			29.6 (T = 25°C)	25/60	103
							30/35	59
Al-Mawsil [Mosul]	19.3	25.1	20 (T = 30°C)	9.37	41.8	22.1 (T = 30°C)	30/65	195
							30/70	217
							30/75	240
			–14 (T = 25°C)			27.1 (T = 25°C)	25/60	121
							30/35	73
An-Najaf	23.8	29.1	3 (T = 30°C)	8.59	29.1	20.2 (T = 30°C)	30/65	221
							30/70	246
							30/75	271
			–13 (T = 25°C)				25/60	109
						28.7 (T = 25°C)	30/35	63
Baghdād [Baghdad]	22.9	28.7	4 (T = 30°C)	9.10	32.6		30/65	203
						21.4 (T = 30°C)	30/70	227
							30/75	250

Table 4.15 (continued)

City	T [°C]	MKT [°C]	Y_T[%]	P_D [hPa]	RH [%]	RH [%] at 25 or 30°C	Testing condition [°C/% RH]	Y_{PD} [%]
Israel								
South of Haifa	19.13	20.8	20 (T = 25°C) 44 (T = 30°C)	15.60	70.4	49.2 (T = 25°C) 36.7 (T = 30°C)	25/60 30/65 30/70 30/75	22 77 91 104
Jordan								
Al ‘Aqabah [Aqaba]	21.9	24.7	1 (T = 25°C) 22 (T = 30°C)	9.47	36.1	29.9 (T = 25°C) 22.3 (T = 30°C)	25/60 30/35 30/65 30/70 30/75	101 57 192 214 236
‘Ammān [Amman]	18.2	21.3	17 (T = 25°C) 41 (T = 30°C)	10.99	52.7	34.7 (T = 25°C) 25.9 (T = 30°C)	25/60 30/35 30/65 30/70 30/75	73 35 151 171 190
Lebanon								
Bayrūt [Beirut]	16.5	19.7	27 (T = 25°C) 52 (T = 30°C)	11.08	58.9	35.0 (T = 25°C) 26.1 (T = 30°C)	25/60 30/65 30/70 30/75	72 149 168 188
Palestine								
West of Hebron [Al Khalīl]	19.17	21.4	17 (T = 25°C) 40 (T = 30°C)	13.82	62.2	43.6 (T = 25°C) 32.5 (T = 30°C)	25/60 30/65 30/70 30/75	38 100 115 131

up to the testing temperature 30°C, by keeping the partial vapour pressure constant, the relative humidity decreases to 16.3%. This RH value is lower compared to the standard long-term testing condition for hot and dry climates, 30°C/35% RH. In other words, testing at 30°C/35% RH represents a higher humidity than the “real” condition measured in the open air.

On the other hand, testing at lower humidities would be less challenging for most of the products, in particular for solid oral dosage forms like tablets, which are generally more stable in a dry environment. It is therefore adequate and justified to test the long-term stability of products for countries in Climatic Zone III at higher humidities, e.g., 30°C/65%.

Concerning aqueous-based products packaged in semi-permeable containers, the recommended long-term and accelerated storage conditions are described in the ICH stability guideline Q1A(2R).

As sensitivity to high humidity or potential for water loss is not a concern for products packaged in impermeable containers, stability studies for these products

Table 4.16 Climatic data for Southern Asia

City	T [°C]	MKT [°C]	Y_T[%]	P_D [hPa]	RH [%]	RH [%] at 25 or 30°C	Testing condition [°C/% RH]	Y_{PD} [%]
Afghanistan								
Chehār Borjak	22.9	27.7	–10 (T = 25°C)	7.01	25.1	22.1 (T = 25°C)	25/60	171
							30/35	112
			8 (T = 30°C)			16.5 (T = 30°C)	30/65	294
							30/70	324
							30/75	354
Herāt	15.2	20.3	23 (T = 25°C)	7.51	43.4	23.7 (T = 25°C)	25/60	153
							30/35	98
			48 (T = 30°C)			17.7 (T = 30°C)	30/65	268
							30/70	296
							30/75	324
Kābol (Kabul)	7.8	13.1	90 (T = 25°C)	5.32	50.2	16.8 (T = 25°C)	25/60	258
							30/35	179
			128 (T = 30°C)			12.5 (T = 30°C)	30/65	419
							30/70	459
							30/75	499
Qandahār (Kandahar)	19.4	25.1	0 (T = 25°C)	6.66	29.6	21.0 (T = 25°C)	25/60	186
							30/35	123
			20 (T = 30°C)			15.7 (T = 30°C)	30/65	314
							30/70	346
							30/75	378
Pakistan								
Islamabad	17.0	20.3	23 (T = 25°C)	12.94	67.0	40.8 (T = 25°C)	25/60	47
							30/65	113
			48 (T = 30°C)			30.5 (T = 30°C)	30/70	130
							30/75	146
Karachi	26.3	27.2	–8 (T = 25°C)	21.24	62.2	67.0 (T = 25°C)	25/60	−11
							30/65	30
			10 (T = 30°C)			50.0 (T = 30°C)	30/70	40
							30/75	50
Lahore	23.8	27.1	–8 (T = 25°C)	17.69	60.0	55.8 (T = 25°C)	25/60	8
							30/65	56
			11 (T = 30°C)			41.7 (T = 30°C)	30/70	68
							30/75	80
Sukkur	26.0	29.5	–15 (T = 25°C)	14.44	42.9	45.6 (T = 25°C)	25/60	32
							30/35	3
			2 (T = 30°C)			34.0 (T = 30°C)	30/65	91
							30/70	106
							30/75	121

can be conducted under any controlled or ambient humidity condition (see ICH Q1A, item 2.2.7.2).

4.6.6.7 Conclusion

As a result of the evaluation of the climatic condition and a risk assessment, an appropriate long-term stability testing condition is proposed for each selected country (see Table 4.17).

The following categorisation can be made:

- Most of the Northern African and Middle East countries at the Mediterranean Sea are in *Climatic Zone II* (Köppen Group *Csa*); some of them exclusively, namely Morocco, Israel and Palestine;

Table 4.17 Climatic zones assigned and recommended long-term stability testing conditions for the countries selected

Country	CZ II	CZ III	CZ IVA	Recommended long- term testing condition*
Algeria (People's Democratic Republic)	+			25°C/60% RH
Afghanistan (Islamic Republic)	+	+		30°C/65% RH
Bahrain (Kingdom)			+	30°C/65% RH
Djibouti (Republic)			+	30°C/65% RH
Egypt (Arab Republic)	+	+		30°C/65% RH**
Iran (Islamic Republic)	+	+	+	30°C/65% RH**
Iraq (Republic)		+		30°C/35% RH
Israel	+			25°C/60% RH
Jordan (Kingdom)	+	(+)		25°C/60% RH
Kuwait (State)			+	30°C/65% RH
Lebanese Republic	+	(+)		25°C/60% RH
Libyan Arab Jamahiriya	+	(+)		25°C/60% RH
Morocco (Kingdom)	+			25°C/60% RH
Oman (Sultanate)		(+)	+	30°C/65% RH
Pakistan (Islamic Republic)	+	+	+	30°C/65% RH
Palestine	+			25°C/60% RH
Qatar (State)			+	30°C/65% RH
Saudi Arabia (Kingdom)		+	+	30°C/65% RH**
Somalia			+	30°C/65% RH
Sudan (Republic)		+	+	30°C/65% RH**
Syrian Arab Republic	+	(+)		25°C/60% RH
Tunisian Republic	+	(+)		25°C/60% RH
United Arab Emirates		+	+	30°C/65% RH
Yemen (Republic)	+		+	30°C/65% RH

+ Climatic zone assigned.

(+) Deserted part of the country.

* The hottest and most humid climatic zone has been selected to establish the adequate stability testing condition for a particular country.

** Aqueous-based solutions in semi-permeable packaging, and dosage forms sensitive to low humidity, e.g., hard-gelatin capsules, may require testing at low humidity according to the procedure described in this guideline.

- Some countries are in Climatic Zone II where the majority of the inhabitants live, and in one additional deserted Climatic Zone III, for example Algeria, Libya, Tunisia, Jordan, Lebanon and Syria;
- There is only one country exclusively presenting a hot and dry climate (*Climatic Zone III*), namely Iraq;
- All of the GCC member states are in *Climatic Zone IVA*, some of them exclusively, namely Bahrain, Kuwait and Qatar;
- Some countries present a mixture of three different climatic zones, for example Iran and Pakistan, where the climate ranges from arctic in the mountains to hot and humid areas;
- None of the selected countries is in the tropical *Climatic Zone IVB*.

Test results conducted at higher temperatures and humidities, for example at 30°C/75% RH, should be acceptable for all countries.

The following testing conditions are proposed:

- For countries exclusively in Climatic Zone II, long-term testing at 25°C/60% RH would be adequate. Testing at higher temperatures and higher humidities, for example 30°C/65% RH or 30°C/75% RH should be acceptable.
- For countries exclusively in Climatic Zone IVA, long-term testing at 30°C/65% RH would be adequate. Testing at higher humidities such as 30°C/75% RH should also be acceptable.
- For Iraq, long-term testing at 30°C/65% RH would also be justified from a scientific point of view for reasons given above.
- For countries in different climatic zones, the long-term testing should be conducted at the condition, which is most challenging for the particular product, for example at 30°C/65% RH. Results generated at 30°C/75% RH should also be acceptable.

4.6.7 South Africa

There is only a small part of South Africa presenting a *Group Cfa* climate, namely the Durban area near the Indian Ocean. The most humid place identified in South Africa is Cape Saint Lucia at the west coast north of Durban. Mean maximum temperatures can go up to 27°C at noon in February, combined with a mean maximum partial vapour pressure of almost 26 hPa. Mean minimum temperatures decrease to just below 18°C at night in July (see Figs. 4.11 and 4.12).

In the centre of South Africa, the *Group BS* is dominating. Pretoria and Johannesburg are located there, while Cape Town is located in a *Group Bsk* climate. At the south coast, South Africa presents a *Group Cfb* climate (see Table 4.18).

4.6.8 Southern Africa

Tropical Climatic *Group Af* is to be found north and south of the equator, surrounded by *Group Aw* regions.

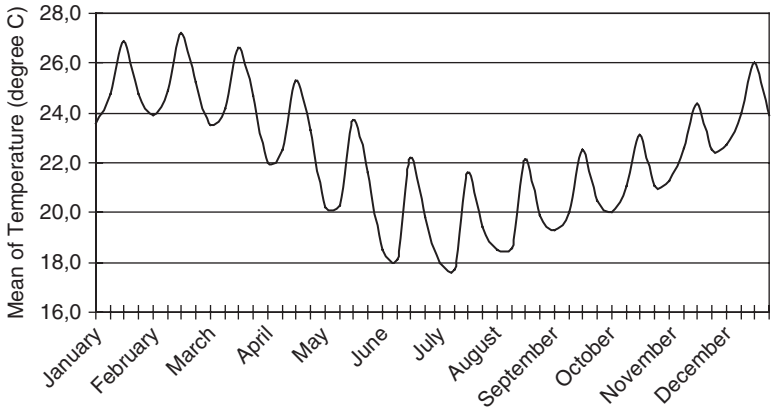

Fig. 4.11 Daily temperature fluctuations – Cape Saint Lucia, Natal, South Africa

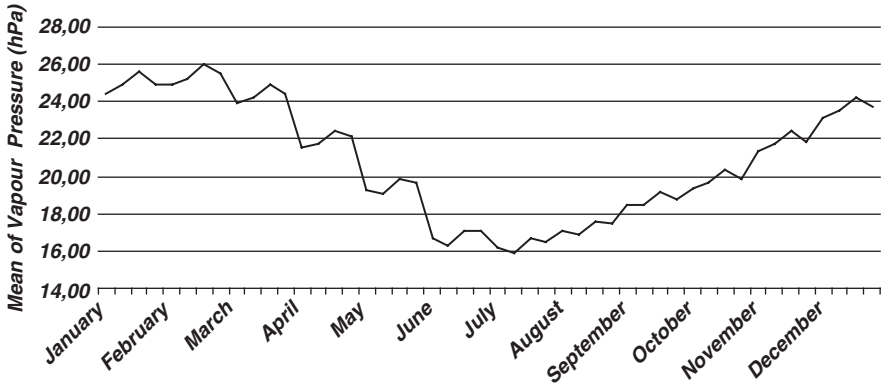

Fig. 4.12 Daily partial vapour pressure fluctuations – Cape Saint Lucia, Natal, South Africa

The hottest place in Southern African Development Community (SADC) identified so far is Dar es Salaam (*Group Aw*) in Tanzania (see Fig. 4.13). Mean maximum temperatures increase to 31.6°C in February, and never decrease below 21°C. The most appropriate temperature for long-term stability testing of medicinal products to be marketed in Tanzania and SADC is 30°C. That value includes a safety margin of 13% added to the highest MKT calculated for Dar es Salaam.

The most humid place is Mbandaka (*Group Af*): values for partial water vapour pressure increase to almost 29.0 hPa in May, and decrease to a minimum of 24.9 hPa in July (see Fig. 4.14).

The mean P_D value of 26.91 hPa calculated for Mbandaka at the testing temperature of 30°C would result in a relative humidity of 63.4% RH. Testing at 30°C/65% RH would include a safety margin of 3% for P_D (see Tables 4.19 and 4.20).

Table 4.18 Climatic data for South Africa

City, provinces	T [°C]	MKT [°C]	Y_T[%] T = 25°C T = 30°C	P_D [hPa]	RH [%]	RH [%] at 25°C at 30°C	Testing condition [°C/% RH]	Y_{PD} [%]
Cape Saint Lucia, KwaZulu-Natal	22.3	22.6	11 33	20.66	76.9	65.2 48.6	25/60 30/65	−8 34
Cape Town, Western Cape	16.7	17.0	47 77	14.96	78.8	47.2 35.2	25/60 30/65	27 85
De Aar, Northern Cape	16.7	19.2	30 57	8.26	43.5	26.1 19.4	25/60 30/65	130 234
Durban, KwaZulu-Natal	20.6	21.0	19 43	19.28	79.4	60.8 45.4	25/60 30/65	−1 43
Kimberley, Northern Cape	17.6	20.3	23 48	9.76	48.4	30.8 23.0	25/60 30/65	95 183
Little Namaland, Northern Cape	21.4	23.6	6 27	9.65	37.8	30.4 22.7	25/60 30/65	97 186
Musina (Messina), Limpopo	21.5	22.8	10 32	15.73	61.3	49.6 37.0	25/60 30/65	21 76
Port Elizabeth, Eastern Cape	18.8	19.1	31 57	16.27	75.1	51.3 38.3	25/60 30/65	17 70
Pretoria, Gauteng	17.5	19.2	30 56	10.70	53.4	33.8 25.2	25/60 30/65	78 158

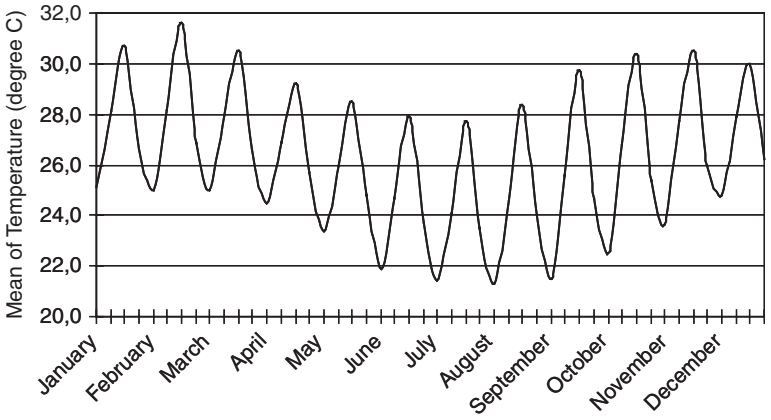

Fig. 4.13 Daily temperature fluctuations – Dar es Salaam, Tanzania

4.6.9 Central America and Panamá

A list of the key climatic parameters measured and calculated for Central America and Panamá (Table 4.21) facilitates the selection of the most *loading* place.

The following interpretation of the data in Table 4.21 can be made:

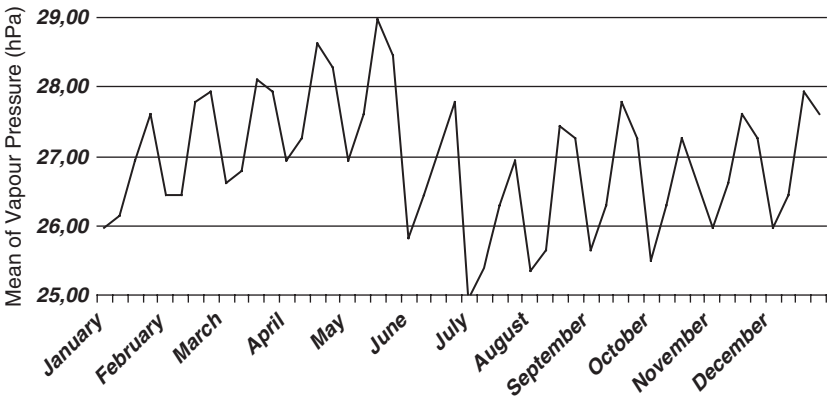

Fig. 4.14 Daily partial vapour pressure fluctuations – Mbandaka, Dem. Rep. Congo

- The mean temperatures measured are all above 22°C and the partial water vapour pressure values are all above 15 hPa, i.e., the countries analysed are either in Climatic Zone IVA or IVB by definition (see Table 4.3).
- All countries present temperatures below 30°C, in other words, the safety margin Y_T is positive in all cases as the MKT calculated is always lower than the testing temperature 30°C.
- All countries except Nicaragua and Panamá show positive safety margins Y_{PD} for the testing condition 30°C/65% RH although some of the partial water vapour pressure values are above 27.0 hPa, the threshold for the Climatic Zone classification according to Table 4.3.
- In Nicaragua, one place shows a P_D value of 27.9 hPa and a negative safety margin at 30°C/65% RH ($Y_{PD} = -1$).
- Panamá is the only country showing partial water vapour pressure values of more than 29 hPa at three places and no place with less than 27 hPa, in other words, testing at 30°C/65% RH would not be sufficient.

4.6.9.1 Recommended Testing Conditions for Central America and Panamá

It is obvious from looking at the above listed data that the adequate long-term testing temperature for the region concerned is 30°C. It is more difficult to select the most appropriate humidity for long-term stability testing, except for Panamá, where 30°C/75% RH is recommended due to its very humid climate, and El Salvador where 30°C/65% RH is adequate as the climate is less hot and humid throughout the year. In the other countries, there is a rainy season of 5–7 months showing partial water vapour pressure values above 27 hPa. These countries are in Climatic Zone IVA for about half of the year but in Climatic Zone IVB in the other period. The yearly mean partial water vapour pressure values, however, classify these countries

Table 4.19 Climatic data for SADC (without South Africa)

Country: City	T [°C]	MKT [°C]	Y_T[%] T=25°C T=30°C	P_D [hPa]	RH [%]	RH [%] at 25°C at 30°C	Testing condition [°C/% RH]	Y_{PD}[%]
Angola: Luanda	25.1	25.7	−3 17	23.48	73.6	74.1 55.3	25/60 30/65	−19 18
Botswana: Gaborone	19.5	21.5	16 40	11.84	52.2	37.4 27.9	25/60 30/65	61 133
Dem Rep								
Congo: Boma	24.6	24.8	1 21	26.25	84.9	82.8 61.8	25/60 30/65	−28 5
Kinshasa	24.1	24.4	3 23	24.14	80.6	76.2 56.8	25/60 30/65	−21 14
Mbandaka	24.5	24.8	1 21	26.91	87.5	84.9 63.4	25/60 30/65	−29 3
Lesotho: Maseru	13.8	15.9	58 89	8.01	50.9	25.3 18.9	25/60 30/65	137 245
Malawi: Linongwe	21.9	22.7	10 32	16.92	64.4	53.4 39.8	25/60 30/65	12 63
Mauritius	24.5	24.7	1 22	23.39	76.2	73.8 55.1	25/60 30/65	−19 18
Mozambique: Maputo	22.8	23.6	6 27	19.22	69.1	60.6 45.3	25/60 30/65	−1 44
Namibia: Windhoek	21.5	23.3	7 29	7.27	28.4	22.9 17.1	25/60 30/65	162 280
Swaziland: Mbabane	18.9	20.1	24 49	16.01	73.1	50.5 37.7	25/60 30/65	19 72
Tanzania: Dar es Salaam	26.2	26.6	-6 13	25.26	74.2	79.7 59.5	25/60 30/65	−25 9
Zambia: Lusaka	21.7	22.7	10 32	15.27	58.9	48.2 36.0	25/60 30/65	25 81
Zimbabwe: Harare	19.2	20.2	24 48	13.63	61.4	43.0 32.1	25/60 30/65	40 103

to be in Climatic Zone IVA, in other words, a testing condition of 30°C/65% RH would be regarded as adequate (see Table 4.22).

4.6.10 Caribbean Islands

All of the northwestern Caribbean Islands are in Köppen *Group Aw*; the southeastern islands belong to *Group Af*. While Cuba belongs to Climatic Zone IVA the other islands would require long-term testing at 30°C/70% RH or higher humidity (see Table 4.23).

Table 4.20 Long-term testing conditions for SADC member countries

Country	25°C/60% RH CZ II	30°C/65% RH CZ IVA
Angola		+
Botswana	+	
Dem. Rep. Congo		+
Lesotho	+	
Malawi	+	
Mauritius		+
Mozambique		+
Namibia	+	
South Africa		+
Swaziland	+	
Tanzania		+
Zambia	+	
Zimbabwe	+	

4.7 Global Stability Testing Protocols

For obvious reasons, global operating pharmaceutical companies are aiming at reducing the amount of stability testing required for different markets. At a first glance, it seems as if testing at the most extreme condition in terms of temperature and humidity covers all countries in the world. There are, however, several aspects to be considered carefully in this context. A single test condition like a combination of high temperature and high humidity, for example, 30 °/75% RH, which is adequate for hot and very humid countries, could force the product for all markets to be packaged in more protective, in other words, more expensive, packaging material, like double aluminium blister for all markets. This would be unnecessary for the majority of the countries that are outside Climatic Zone IVB. To shorten the shelf-life as an alternative would mean that many packs would have to be taken off the market in countries of moderate climates although they would still be within the specified quality. It is, therefore, recommended to conduct stability studies at long-term testing conditions tailored to the climatic conditions of the target region. A minimum of two different testing conditions could cover the world, one for the ICH region, namely Climatic Zones I and II, and another for extreme tropical countries, such as Climatic Zone IVB.

Normally, one accelerated condition has to be part of a global stability testing design. To understand the impact of extreme temperature excursions during shipment, it is recommended to test in addition the product at stress conditions, for example, one batch at 50°C/ambient humidity for 3 months.

A typical testing design for a standard stable oral dosage form intended to be marketed worldwide is presented in the following table (Table 4.24). For aqueous-based products in semi-permeable packaging material testing at low humidity according to ICH Q1A has to be considered. Appropriate testing conditions for less stable products can be defined following a similar pattern.

Table 4.21 Climatic data for Central America and Panamá

Country	City	T [°C]	MKT [°C]	Y_T[%] T = 30°C	P_D [hPa]	RH [%]	RH [%] at 30°C	Testing condition [°C/% RH]	Y_{PD} [%]
								30/65	5
Belize	San Antonio	24.7	25.1	20	26.3	84.3	61.8	30/70	13
								30/75	21
								30/65	2
	San José	25.4	25.8	16	27.1	83.4	63.7	30/70	10
								30/75	18
								30/65	3
Costa Rica	Alajuela	25.0	25.2	19	26.8	84.7	63.2	30/70	11
								30/75	19
	Piedras Blancas	24.0	24.2	24	25.7	85.9	60.5	30/65	8
								30/70	16
								30/75	24
	Perto Limón	24.0	24.2	24	25.9	86.6	61.1	30/65	6
								30/70	15
								30/75	23
								30/65	3
	Amparo	24.9	25.2	19	27.0	85.4	63.1	30/70	11
								30/75	19
								30/65	1
	Carate	25.7	25.8	16	27.3	82.5	64.2	30/70	9
								30/75	17
								30/65	34
El Salvador	Chalatenango	24.4	24.8	21	20.6	67.3	48.4	30/70	45
								30/75	55
								30/65	21
Guatemala	La Gomera	24.5	25.1	20	22.9	74.3	53.9	30/70	30
								30/75	39
								30/65	6
	Carmelita	24.9	25.4	18	26.1	82.9	61.5	30/70	14
								30/75	22
	Los Amates	22.9	23.3	29	22.6	80.9	53.2	30/65	22
								30/70	32
								30/75	41
								30/65	23
Honduras	Santa Rita	23.3	23.6	27	22.5	78.8	53.0	30/70	32
								30/75	42
								30/65	1
	Wampusirpi	25.7	25.9	16	27.5	83.3	64.6	30/70	8
								30/75	16
								30/65	33
	Yuscarán	23.0	23.4	28	20.8	74.0	48.9	30/70	43
								30/75	54
								30/65	28
Nicaragua	Villa Nueva	26.3	26.7	12	21.6	63.1	50.9	30/70	38
								30/75	47

Table 4.21 (continued)

Country	City	T [°C]	MKT [°C]	Y_T[%] T = 30°C	P_D [hPa]	RH [%]	RH [%] at 30°C	Testing condition [°C/% RH]	Y_{PD} [%]
								30/65	3
	Bonanza	25.1	25.3	18	26.9	84.4	63.3	30/70	11
								30/75	19
								30/65	–1
	Colonia Guinea	25.4	25.6	17	27.9	86.0	65.6	30/70	7
								30/75	14
								30/65	–6
Panamá	Chimán	26.9	27.0	11	29.5	82.9	69.3	30/70	1
								30/75	8
								30/65	2
	Chiriquí Grande	25.4	25.6	17	27.2	83.9	64.0	30/70	9
								30/75	17
								30/65	2
	Gualaca	25.4	25.6	17	27.0	83.3	63.5	30/70	10
								30/75	18
								30/65	–6
	San Carlos	26.6	26.7	13	29.3	84.1	69.0	30/70	1
								30/75	9
	Darién							30/65	–5
	National	25.7	25.9	16	29.1	87.8	68.5	30/70	2
	Park							30/75	10

T = Mean temperature, calculated by using the sum of 48 measured temperatures (4 temperatures per day for each month), divided by 48.
MKT = Mean Kinetic Temperature, calculated as described above.
Y_T = Safety margin for temperature, calculated using the MKT vs. the testing temperature 30°C (for details please refer to chapter *Calculation of safety margins*).
P_D = Mean partial water vapour pressure, calculated by taking the dewpoints.
RH = Mean relative humidity, calculated by using the saturation vapour pressure P_S at the measured temperature, and the value for P_D found in the previous column.
RH at 30°C = Mean relative humidity, calculated by using the saturation vapour pressure P_S at the testing temperature 30°C, and the value for P_D found in the previous column.
Y_{PD} = Safety margin for partial vapour pressure, calculated using the meteorological P_D value vs. the P_D value calculated for the respective testing condition found in the previous column.

Table 4.22 Climatic zones assigned and recommended long-term stability testing conditions for Central America and Panamá

Country	CZ IVA	CZ IVB	Recommended long- term testing condition
Belize	+		30°C/65% RH
Costa Rica	+		30°C/65% RH
El Salvador	+		30°C/65% RH
Guatemala	+		30°C/65% RH
Honduras	+		30°C/65% RH
Nicaragua	+		30°C/65% RH
Panamá		+	30°C/75% RH

Table 4.23 Long-term testing conditions for Caribbean Islands

Country	30°C/65% RH CZ IVA	30°C/70% RH
Cuba	+	
Curaçao		+
Puerto Rico		+

Table 4.24 Typical schedule to support global stability testing conditions

	0	3	6	9	12	18	24	36
25°C/60% RH long-term for CZ I & II		+	+	+	+	+	+	+
30°C/65% RH intermediate for CZ I & II		(+)	(+)	(+)	(+)			
30°C/75% RH long-term for CZ III & IV	+	+	+	+	+	+	+	+
40°C/75% RH accelerated		+	+					
50°C/amb. stress test		(+)						

+ = tests required.
(+) = samples required in case tests are to be conducted.

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Chapter 5
Post-approval Changes – Stability Requirements and Regulations

Frank J. Diana

Contents

Abstract There are many reasons for making changes to pharmaceutical products after the original regulatory approval is obtained. Some of these changes may be significant and require a substantial amount of stability data while others are minor and may only require a stability commitment. Company change control procedures should detail how changes are evaluated and implemented as well as how the change impacts stability and what data will be needed to support the change. The regulatory

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group will determine the strategy for submission based on a review of the technical assessment of the change and the appropriate regulatory guidance. The strategy may be more complex if the product is marketed globally. The stability requirements will typically be assessed by a team led by the stability group and including quality assurance (QA), technical and regulatory affairs. Once agreed upon, this information will be captured in a stability protocol and reviewed/approved by the team. Based on the submission strategy, a stability report will be written for inclusion in the supplement, variation (for global changes) and/or the annual report.

In the US, the current regulations around changes are covered in 21CFR314.70 and indicate that "The applicant shall notify the FDA about each change in each condition established in an approved application beyond the variations already provided for in the application". The 1987 stability guideline and the 1998 draft stability guideline (withdrawn in 2006) provide a good background on FDA thinking with regard to stability requirements for post-approval changes. The Scale Up and Post Approval Change Guidances (SUPAC) and the Changes to an Approved NDA or ANDA (issued in April, 2004) offer a significant amount of information to guide the sponsor in filing and data requirements [1–5]. Similarly, for global changes there are several guidances available to provide requirements for various types of changes [6–10]. Web sites and addresses are provided in the references section of this chapter for these guidances from FDA, European Medicines Agency (EMEA), Health Canada, and World Health Organization (WHO). International Conference on Harmonization (ICH) Guidelines can also be found on the FDA web site.

5.1 Evaluating Proposed Changes

Post-approval changes are proposed for various reasons. Once a New Drug Application (NDA) is filed, a limited number of changes to the file are made, typically due to concerns that significant changes may potentially add to FDA review time. Therefore, it is not unusual that right after approval several changes may need to be made to increase the manufacturability or commercial viability of the product. Such changes may involve improvements to the manufacturing process and/or equipment, batch size scale-up, transfer to a new manufacturing site, additional strengths, API synthesis optimization, packaging changes, and testing changes. Any of these changes, if deemed necessary, may have already been evaluated, with appropriate batches manufactured and data generated for a submission shortly after approval to enable implementation as soon as possible after launch. Even several years after approval, changes will still be evaluated and made if warranted due to supplier changes, packaging changes, equipment upgrades, strategic sourcing decisions, and product reformulations due to a stability or manufacturing issue. Improvements to the quality of the product as well as optimization of the supply chain should always be pursued and evaluated against the cost and compliance implications.

The change control process is initiated when a change is recommended proactively for improvement purposes, to resolve a specific product issue, or due to a

supplier change. Typically, a team will get together and evaluate the change to determine the requirements for implementation. Based on the requirements, a timeline will be developed and the cost assessed. For an improvement project, the cost may be prohibitive and the change control project may be rejected. For a supplier change, inventory of the pre-change component may be available for a considerable amount of time or the component could be stock-piled. This may only postpone the inevitable, so the team will review the timeline and decide when work must be started to assure the change is made prior to inventory depletion. For changes which address specific product or compliance issues, the team will still assess the timeline and cost; however, the priority of this type of change may be set by management and implementation will occur even though expensive.

From the stability perspective, each change should be evaluated based on its potential to impact API or product stability. The guidance documents noted above should be consulted to determine if stability requirements are clearly delineated for the proposed change. For example, a manufacturing site change for a solid oral dosage form (immediate release) is covered in the related SUPAC guidance [1] and indicates that for site changes to a different location, 3 months of data at accelerated and room temperature conditions for one to three batches of changed product is required (see discussion of significant body of information below). If there are accelerated data for pre-change product available then the results will typically be compared to determine similarity in stability profiles. If these data do not exist or were generated several years ago, there will be benefit to placing a batch of pre-change product on stability at the same time. If there is more than one strength and/or marketed packages, then multiple put-ups may be needed depending on the type of packages and whether bracketing is appropriate. Not all changes are covered in the guidances. For example, a change in a compendial excipient source for an oral solid dosage form will require an evaluation of the criticality of the component to the formulation, whether the material from the new source is equivalent to material from the original source especially with regard to any special requirements that may impact the product, for example particle size, viscosity, or impurities, and whether there is any potential impact to the stability profile of the product.

Once the stability evaluation is complete, the requirements are added to the change control along with other technical requirements, namely, testing, manufacturing, sampling, packaging, documentation, and quality requirements such as validation, IQ/OQ/PQ, batch disposition. The change control is then sent to the regulatory group for filing requirements, for example annual report, changes-being-effected supplement (CBE), prior approval supplement (PAS), type I variation. Different types of supplements and variations are discussed in Sections 5.2 and 5.3 of this chapter. Alternatively, the initiator can include the appropriate regulatory requirements in the change control based on regulatory input. The change control is then circulated for review/approval to the appropriate individuals including QA and perhaps site management depending on the type of change. Once approval is obtained the activities described in the change control will be initiated.

Based on the stability and filing requirements, the stability group will write a report including the data generated to support the change which will be included

in the NDA supplement. Pilot scale batches can be manufactured and placed on stability to support changes, however it is often advantageous to manufacture at full commercial scale since these batches could be marketed once regulatory approval is obtained and assuming there is enough time left before expiry (at least 12–18 months). The report will include a summary indicating the acceptability of the changed product from a stability perspective and provide a proposal for the expiry period for the changed product based on comparison of the stability profile to the unchanged product. A stability commitment should be included describing what additional studies are needed. For example, if a pilot scale batch was made to support the change, the first 1–3 production scale batches made need to be placed on stability. Post-approval stability requirements should also be included in the change control documentation. For annual reportable changes, a separate stability report may not be necessary, but the data for the changed product should be included in the next annual report and subsequent yearly reports.

5.2 Types of Changes and Filing Requirements – US

The FDA Guidance, Changes to an Approved NDA or ANDA [5], describes many post-approval changes and the filing requirements. Changes are separated into those that are considered minor, moderate, and major and are grouped into reporting categories. Many specific changes are discussed below.

5.2.1 Minor Changes

These are changes which are expected to have a minor impact on the product. Such changes include manufacturing location within the same facility, scale-up of batch size using equipment of the same operational principle, secondary packaging site changes, simple process changes, high density polyethylene (HDPE) bottle changes, small changes in excipient composition, deletion of color, additional manufacturing site for compendial excipient, etc. These types of changes can be filed in the annual report and can be implemented whenever internal requirements are met, such as equipment IQ/OQ/PQ, raw material testing to show equivalence, extended release testing of changed batch, etc. Often these changes are supplemented by placing the first production batch on long-term stability (stability at accelerated conditions is normally not necessary); this batch could also serve as the annual production batch.

5.2.2 Moderate Changes

These are changes which are expected to have a moderate impact on the product. Changes in this category include a manufacturing site change to a new location, which uses the same procedures and equivalent equipment, more significant changes to raw material composition, testing site changes, etc. These changes are filed via a

CBE supplement to the FDA. Stability requirements vary but the submission may require inclusion of 3-month accelerated and long-term stability data from 1 to 3 batches of changed product and/or a commitment to place the first 1–3 production batches on stability. Most of these types of changes can be implemented after 30 days (CBE-30). Change control activities may include a technology transfer for manufacturing and/or testing for those types of changes, raw material and API testing, equipment qualification, process validation, etc.

5.2.3 Major Changes

These are changes which potentially have a major impact on the product. Such changes include reformulation, new test methods, new or relaxed specifications, packaging changes to a less protective package, new packages, new strengths outside of the approved range, new API synthesis, critical excipient changes, etc. These changes are filed to FDA in a prior-approval supplement and stability requirements for the filing as well as post-approval are more substantial. Many of these changes require 3 months of accelerated and long-term stability data on three batches of changed product. If all or any of these batches are pilot scale, then the post-approval commitment will include stability on up to the first three production scale batches using the same protocol.

5.3 Types of Changes and Global Filing Requirements

For products to be marketed globally, changes will need to be considered based on global regulations in addition to the ones mentioned for the US. Type I (minor) and II variation (major) guidances [6, 7] should be consulted for product changes in Europe. Similar guidance is provided by the WHO using equivalent definitions for minor and major changes [8, 9]. The WHO member states include many countries categorized in Zone III or IV in which long-term testing conditions are 30°C/35%RH (Zone III), 30°C/65%RH (Zone IVA), or 30°C/75%RH (Zone IVB). Canadian regulations describe similar definitions and requirements [10]. A supplemental filing is required for a major quality change, while a moderate change is considered notifiable, and a minor change can be implemented without prior approval. Supporting data need be submitted to Health Canada only upon request.

5.3.1 Type I Variation

These changes are somewhat similar to a CBE supplement in that there are some changes that require a notification only and others that require 30 days prior to implementation. Changes can be categorized as 1A (notification) or 1B (applicant is notified within 30 days after validation). It is typical to submit changes individually since bundling changes can often lead to delays. Some of the changes that fit into

Table 5.1 Type I variations

Change	Stability data required	Recommended actions/comments
API batch size	None	Release data on 2 batches
Replacement of excipient with comparable excipient	3-month data on 2 product batches of at least pilot scale	For solid dosage forms, comparative dissolution profile data (old v. new) Need to show no impact on analytical methods due to excipient change
Composition of immediate (primary) packaging material	3-month data on 2 product batches of at least pilot scale	Comparative data on new packaging, (e.g. moisture permeability)
Product batch size	None if up to 10x, 3 months on 1 batch if >10x	Process validation protocol/report
Product manufacture (minor change)	3-month data on 1 batch of at least pilot scale	Manufacturing principle unchanged
Coloring or flavoring system used in the product	3-month data (long term and accelerated) on 2 product batches of at least pilot scale	Perform photo-stability study if warranted If new excipient, no impact on analytical methods
Coating weight for tablets or capsule shell weight	3-month data on 2 product batches of at least pilot scale	Comparative dissolution; type IA filing for IR, IB for MR (coating not a critical factor for release)
Shape or size of container/closure	None	Same container composition, IB for sterile dosage forms, IA for all others
Shape or size of container/closure	3-month data on 2 product batches of at least pilot scale	Same as above except there is a change in headspace or surface/volume ratio
Number of units in a package (e.g. bottle)	Commitment to perform stability, if stability parameters could be affected	If outside approved ranges (e.g. decrease # of units below approved range) then need to commit to perform stability

this category are shown in Table 5.1. A filing fee is due in the EU for each Type I submission.

5.3.2 Type II Variation

These changes are somewhat similar to a PAS and typically require 60–90 days for regulatory authority review. A Type II variation is one which cannot be deemed to be a minor variation or an extension of the marketing authorization. Some of the changes that fit into this category are shown in Table 5.2. A filing fee is due in the EU for each Type II submission. Requirements may differ depending on whether the API is considered stable or unstable. The regulations define a stable API as one which meets specifications when stored at 25°C/60%RH or 30°C/65%RH for 2 years and at 40°C/75%RH for 6 months.

Table 5.2 Type II variations

Change	Stability data required	Recommended actions/comments
API manufacturing process	*API* Stable API: 1 batch/3 months long term and accelerated conditions Unstable API: 3 batches/6 months long term and accelerated conditions	If quality characteristics of API are changed so that stability may be compromised, comparative stability data (before and after change) are required
	Product 2 batches/3 months long term and accelerated conditions	If quality characteristics of API are changed so that stability of product may be impacted, stability data listed may be required
Composition of the product	Conventional dosage forms & stable API: 2 batches/6 months long term and accelerated conditions	IR solid dosage forms, solutions Batches can be pilot scale
	Critical dosage forms or unstable API: 3 batches/6 months long term and accelerated conditions	Extended release dosage forms Batches can be pilot scale
Immediate (primary) packaging	Semi-solid and liquid dosage forms: 3 batches/6 months long term and accelerated conditions	Less protective packaging or risk of interaction; batches can be pilot scale

5.4 Stability Requirements for Various Types of Changes – US

When evaluating changes for stability requirements, the available data needs to be factored in to determine the number of batches that will need to be placed on stability. A Significant Body of Information (SBI) is defined as likely to exist after 5 years of commercial experience for a new product (NCE) and 3 years for a new dosage form for immediate release oral solid dosage forms [1]. For a modified release solid dosage form, SBI is defined similarly for the original complex dosage form and subsequent complex dosage form drug product [2].

5.4.1 Manufacturing Changes

Manufacturing changes include changes to equipment, process, scale, and site. As discussed above, each change needs to be evaluated for its potential adverse effect on the quality of the finished product. Requirements (including stability) will increase as the potential increases. Changed batches need to be assessed for their equivalence. Typically, this is assessed through testing to determine if the product's identity, strength, quality, purity, and potency were affected. For many changes, this comparison will involve a stability profile assessment also. The appropriate guidance documents should be consulted for requirements for manufacturing changes. Many changes such as equipment changes within the same class and operating principle, production scale changes within a factor of 10, changes to mixing times or operating speeds within approved ranges do not require stability data before implementation

although the first changed batch is often placed on stability. Table 5.3 lists some of the more significant changes and the recommended stability data to support them.

5.4.2 Formulation Changes

The available SUPAC guidances provide information on excipient changes within certain ranges and also describe requirements for critical versus non-critical excipients. Minor changes that would not likely impact the product stability include small changes in excipient amounts, deletion of a colorant or flavor, changing tablet or capsule markings (e.g., debossing, printing), and changing tablet shape or dimensions without a change in quantitative composition (this type of change is qualified at release through dissolution profiles).

5.4.2.1 Product Reformulation

Reformulation of the drug product could lead to changes in the stability profile and this type of change will typically require a substantial amount of stability data. For example, the current formulation may contain an ingredient (inactive or another active) which is reacting with the API or causing the API to form a degradation product which increases over time. After several investigations and perhaps a Field Alert report or even a recall, it is concluded that the degradation product increase must be dealt with. A packaging change is assessed but the equipment and component costs are prohibitive. It is determined through experimentation that the degradation is caused by one of the ingredients and just changing the amount will not resolve the issue. Therefore, a new formulation with different excipients is developed. This re-formulation will need to be filed via a prior-approval supplement. A reformulated product will need at least 3 months of stability data on three batches for a prior-approval supplement. An acceptable reformulation should have an improved degradation profile versus the original formulation. It may be difficult to assess the performance of the reformulated product after 3 months, and in some cases 6 months of data at the intended storage and accelerated conditions will be needed to determine the improvement in stability.

5.4.2.2 Change in Critical Excipient

A similar approach would likely be taken for a change in the critical excipient (rate-controlling) of an extended release or transdermal dosage form. In this case the potential event triggering the re-formulation may be a decrease in dissolution results on stability as the formulation ages causing out-of-specification (OOS) results and/or a shortening of the expiration date. Often, to deal with these types of issues an internal requirement for tight release limits is set so that the regulatory (shelf-life) limits are not approached on stability. Thus the successful re-formulation may yield several benefits from a compliance perspective as well as a supply standpoint, such as improved dissolution performance on stability, an extension of the

Table 5.3 Post approval manufacturing changes – US

Change	Stability data required	Dosage forms	Comments	Stability commitment
Site transfer Level 3, different site	1–3 batches/3-month long term and accelerated data	Immediate release, Modified release, Semi-solid	Same procedures, equipment, batch records # of batches in supplement depends on significant body of information availability	1st 3 production batches on long-term stability
Equipment – different design/ operating principle	1–3 batches/3- month long term and accelerated data	Immediate release, Modified release, Semi-solid	# of batches in supplement depends on significant body of information availability	1–3 production batches on long-term stability
Type of process, e.g. wet granulation to direct compression	1–3 batches/3-month long term and accelerated data	Immediate Release	# of batches in prior approval supplement depends on availability of significant body of information	1st production batch on long-term stability
	3 batches/3-month long term and accelerated data	Modified release	Prior approval supplement	1st 3 production batches on long-term stability
Equipment operating conditions outside approved ranges (mixing times/rates/speeds, cooling rate)	None	Immediate release	Changes outside approved application ranges, categorized as SUPAC Level 2, file as CBE-30	
	1 batch/3-month long term and accelerated data	Modified release		1st production batch on long term stability
	1–3 batches/3-month long term and accelerated data	Semi-solid	For SS, # of batches depends on significant body of information	1–3 production batches on long term stability
Excipient ranges – changes that do not meet requirements of Level 1 or 2	1–3 batches/3-month long term and accelerated data	Immediate and modified release	# of batches in prior approval supplement depends on availability of significant body of information	1st 1–3 production batches on long-term stability
		Semi-solid		1st 3 production batches on long-term stability
Release Controlling Excipient	3 batches/3-month accelerated and long-term data	Modified release	Addition/deletion of release controlling excipient or greater than 10% w/w change in release controlling excipient File as prior approval supplement	1st 3 production batches on long-term stability

expiration date, and a decrease in rejected batches at release, since the internal requirements for dissolution may be relaxed.

5.4.2.3 Addition of New Strength

The addition of a new strength outside of the approved range will require stability data to demonstrate a comparable stability profile and a prior approval submission. It is fairly common that the new strength(s) will be of identical formulation, e.g., a decrease or increase in tablet weight or capsule content only. This would likely require the submission of 3 months' accelerated and controlled room temperature (CRT) data for one batch of drug product although in special cases up to three batches of data may be necessary. It is also typical for different colors and shapes to be used to differentiate the strengths; however, this will usually not impact product stability although analytical methods may be affected due to the different dyes used. It is also possible that the colors may exhibit different physical behavior from a photo-stability perspective (e.g. fading) and/or cause different interactions with the active ingredient.

If instead, the new strength(s) are formulated using a different quantitative composition then additional data will be needed as described above for a re-formulated product. In many solid oral dosage formulations, the tablet weight or capsule content weight remains the same and the only change is to the amount of API, leading to a change in drug-to-excipient ratio, for example, tablet weight is kept constant at 250 mg with a change of API over the range of 10–50 mg, the drug-to-excipient ratio is therefore increased from 1:24 to 1:4. If product degradation is an issue then typically the lowest strength will be the most challenging due to the lowest drug to excipient ratio or increased ratio of water to drug due to the formulation ingredients or headspace moisture [11].

5.4.3 Packaging Changes

Changes to the container/closure system need to be evaluated for potential for impact on the product stability profile. Typically, only changes to the primary packaging component (product contact materials) have the potential to affect the product stability. Changes to secondary packaging such as cartons or a change in the packaging site do not typically require stability studies as they will not directly impact product stability. However, deletion of a secondary packaging component that provides additional protection (e.g. light, moisture, or oxygen) will require stability data and rationale for the change (perhaps increased protection from the primary package such as an increase in titanium dioxide content to make a bottle more opaque). Changes to polyethylene bottles for packaging dry oral solid dosage forms are covered in USP <661> [12] and do not require stability data (based on equivalence of the containers), although the first batch packaged in the alternate bottle may be put up and the alternate bottle needs to be added to the annual stability program. Changes to blister card configuration or number of blisters on a card are

acceptable based on stability of the blister itself. Changes such as the addition of a child-resistant (CR) feature to a bottle or blister package or transdermal pouch, in which contact materials do not change, a change to the cap liner in which there is no change to the inner liner material, or a change in cap color should be evaluated but will not typically affect product stability.

Changes to primary packaging components that could affect stability need to be evaluated for their protective properties. A change to a less protective package such as changing from aluminum foil blisters to a PVC film for blister material, although cost effective, will need to be evaluated for stability impact. This type of change would require submission of 3 months' data on one changed batch compared to data on the unchanged product (i.e., previous package). If the dosage form is not affected by moisture, this type of change should be acceptable. Removal of a desiccant from a bottle with tablets will need the same type of evaluation and data, as will a decrease in the tablet count (outside the approved range) in the same size bottle or an increase in bottle size (due to increased headspace volume). Liquid and semi-solid dosage forms would require the same type of evaluation and data for package component changes, for example, bottle resin, cap liner, bottle size, and in addition label components for semi-permeable containers (adhesive, ink) may affect stability and thus need to be assessed. Parenteral (sterile) product container changes that need to be evaluated include type of glass, type of stopper, type of container, and component supplier. Major changes to a sterile product that require stability studies include adding a vial package with an elastomeric closure to an ampoule product line, adding a pre-filled syringe dosage form, changing to a flexible bag (large volume parenteral-LVP) from another container system and change in size or shape of a container.

The addition of a new package, such as a blister, to the already approved bottle for oral solid dosage forms or a pre-filled syringe to the already approved ampoule/vial for parenteral products will require significant stability data and prior-approval from regulatory authorities. Three to six months' data (accelerated and controlled room temperature) will be needed on 1–3 batches depending on the availability of a significant body of information. When selecting blister components, the moisture sensitivity of the product needs to be taken into account to determine the appropriate material based on moisture vapor transmission rates (MVTR). The more sensitive the product, the more important the MVTR is in the selection process due to the moisture protection. As an example, a foil/foil blister will provide better moisture protection than vinyl ACLAR®/foil which will provide better moisture protection than PVC/foil.

5.4.4 Changes to Active Pharmaceutical Ingredient (API)

Often changes to the API are proposed and implemented after product approval. A manufacturing site change (alternate site or company) for the API using similar equipment and synthesis will not typically effect the stability of the drug substance or the drug product; equivalence of impurity profile, chemical and physical

properties is shown by testing three batches according to the approved specifications and utilizing the appropriate testing (e.g., X-ray powder diffraction, solid state NMR) to establish that the polymorph and crystal habit are unchanged. On the other hand, many changes do involve synthetic and/or process equipment changes. Changes early in the synthesis may have less impact on the final drug substance as compared to changes later in the synthesis. A change in the synthesis after the final intermediate step is typically considered a major change. Any change that may impact the physical properties of the API or the impurity profile needs to be evaluated from a stability perspective as well as the potential effect to the finished product. One major change that FDA specifies is a change from filtration to centrifugation or vice versa. In evaluating a critical change to an API, the potential impact on the established retest date needs to be assessed [13].

Often when qualifying a new API manufacturing site (new supplier), the synthesis will be different from the approved synthesis (classified as a major change). This change would necessitate a complete evaluation of the API from a release and stability testing perspective. Frequently, three batches of the new API will be placed on stability according to the approved stability protocol (controlled room temperature and accelerated conditions) and several batches of drug product will be manufactured, packaged, and placed on stability. There is also a good chance that the storage container for the API will be different and this, of course, needs to be taken into account for the stability program along with the establishment of an appropriate retest period for the API. In addition, an appropriate commitment with regard to API production batches would be required (depending upon the scale of primary stability batches for qualification of the new site).

5.4.5 Stability Protocol Changes

The approval of an NDA also establishes the approved stability protocols for future batches that will be placed on stability, whether they represent the first three production batches or the annual stability batches. It is typical that the initial expiration date approved for the drug product is based on extrapolation from real time data and until real time data are available through the expiration dating period, no changes to the stability protocols, except testing changes, should be made. Once enough data are available (e.g., three production scale batches with real time data through the expiration date), then it may be appropriate to update the approved stability protocols. For the annual product monitoring protocol, which includes long-term conditions only (e.g., accelerated conditions are not necessary), testing at fewer time points may be appropriate, for example, 6, 12, 24, and 36 months instead of the ICH-dictated time points of 3, 6, 9, 12, 18, 24, and 36 months. Including time zero, this change reduces the timepoints to be tested from eight to just five for each annual batch, which translates into significant resource savings. This change is most appropriate for products that have a consistent stability profile with acceptable variability, and will need to be filed in a prior-approval supplement. Often, applicants will combine reduction of timepoints with a deletion of one or more tests from the stability protocol. For

instance, at NDA approval there may not have been enough data to support removal of hardness or moisture testing for an immediate release tablet. However, after generation of additional data, it may now be clear that moisture is consistent over time and does not affect other parameters such as degradation products or dissolution, thus, justification can be made to remove the extra testing.

In contrast, adding or removing time points after the expiration date can be filed in the annual report as these changes do not reduce the data generated within the product expiration date.

5.4.5.1 Bracketing and Matrixing Approaches

Bracketing and matrixing approaches can be applied to supplemental change batches and annual stability batches if the original stability protocols in the NDA included either or both of these concepts. Approval of the NDA would therefore indicate approval of the post-approval stability protocols and any bracketing/matrixing approaches included. Generally, these reduced stability testing approaches are not included in the NDA due to the limited amount of available data. However, at post-approval, a significant amount of stability data may have been accumulated, and based on the variability of the data and product stability, a reduced testing protocol may be justified. As described in more detail in Chapter 15, bracketing and matrixing approaches can be applied to a number of factors (e.g., container/fill sizes, strengths) and to many dosage forms and can include various designs and even a combination of bracketing and matrixing. The reduced stability testing protocol for post-approval batches and supportive data justifying the change will need to be submitted via a prior-approval supplement.

Frequently, additional strengths are added post-approval to those already approved in the original application. There are several reasons for doing this including time available to develop new strengths after the filing without delaying approval, competitive products/strengths, patient needs for a wider variety of strengths, pediatric dosing, etc. Bracketing is particularly advantageous for products that are manufactured using a common granulation across several strengths. For example, an immediate release product available in 25-, 50-, 100-, and 200-mg strengths prepared from the same blend (tablet weight is proportionately increased) is an excellent candidate for bracketing. Using the concepts described in Chapter 15, only the extremes would need to be placed on stability, in this case 25 and 200 mg. The intermediate strengths would be covered by the stability data generated for the 25- and 200-mg dosage forms for annual stability. Also any change to the product line such as a package, manufacturing, or API change could be supported by stability data for the two extreme strengths assuming the same change is made to each strength. A similar approach can be used for container sizes, for example 40-, 75-, 150-, and 325-cc HDPE bottles, in which the components are the same. In this case the tablet to volume ratio would need to be taken into account to determine the extremes. In our example, let us conclude that the 40- and 325-cc bottles are the extremes. Taking these factors together, we support 16 combinations (four strengths $\times$ four package sizes) with four stability studies (annual stability

batches or assuming one batch needed for supplemental change); 25 mg/40 cc, 25 mg/325 cc, 200 mg/40 cc, and 200 mg/325 cc. One can see from this example that a good bracketing design can save a substantial amount of stability resources and assure that only value-added testing is performed.

As discussed in Chapter 15, matrixing is another approach that can be used to reduce stability testing requirements. As with bracketing, post-approval changes to the stability protocol to add matrixing needs to be justified with the appropriate data and filed via a prior approval supplement (PAS.) Matrixing involves testing only a fraction of the samples that would be tested in a full stability protocol design. Continuing the example from above, let us say that our product stability profile is excellent and data variability is moderate thus indicating matrixing is applicable. Using matrixing, we propose testing only one-half of the time points for each batch. Let us take the case for annual stability testing of our product in which the full protocol includes time points at 0, 6, 12, 18, 24, and 36 months. Thus, for the full protocol using the bracketing design we would test four batches $\times$ six time points $=$ 24 samples. Using matrixing we can eliminate two of the time points between 0 and 36 months for each batch, yielding 4 samples per batch or 16 samples tested, keeping in mind that for matrixing we need to test the first (time zero) and last (36 months) time point for each batch.

In some cases, matrixing alone will be more appropriate. As an example, let us take the case in which an extended release product is available in two strengths and packaged in two HDPE bottle sizes. In this case, bracketing would not be appropriate since there are not enough combinations to establish extreme/intermediate samples. However, with the appropriate data as described above, matrixing can be justified. For example, applying matrixing (again testing half the samples) to this product for a post-approval change that requires one batch per combination (four batches in this case), we can establish the following:

Full protocol – 0, 3, 6, 9, 12, 18, 24, 30, and 36 months.

Matrixing – all batches 0, 3, and 36 months and then half of the rest of the time points tested = six samples per batch.

Using this approach, we have reduced the testing from four batches $\times$ nine time points $=$ 36 samples reduced to 24 samples. Testing at the 3-month time point is completed for all batches assuming that this data will be submitted to support the post-approval change. As we can see from these examples, matrixing alone or combined with bracketing can save a significant amount of stability testing resources.

5.4.6 Expiration Date Changes

Extension of a product expiration date can be done in two ways. The first method allows an update via the annual report based on three production scale batches completing stability through the desired expiration date. These can be either the original primary stability batches (if made at production scale) or the post-approval commitment production scale batches.

For example, if the original expiration date granted at time of NDA approval is 18 months and the approved protocol filed in the NDA included testing through 36 months, once the three production scale batches reach 24 months with acceptable results, the product expiration date can be updated to 24 months. Similarly, once these batches reach 36 months with acceptable results, the expiration date can be extended to 36 months in the annual report.

The second approach is to use the original registration batches, which are often manufactured at pilot scale. In this case, once the pilot batches are tested at the desired expiration date under an approved protocol with acceptable results, a prior approval supplement can be submitted proposing extension of the expiration date. Using the same example as above, the only difference would be submitting the 24-month data on pilot-scale batches in a PAS submission instead of making the change via the annual report. The applicant would then wait for FDA approval of the shelf-life extension (the FDA goal is to review 90% of prior approval sNDAs within 4 months according to PDUFA goals) before changing the expiration date for subsequent manufactured/packaged product. In this approach, extension of the expiration date can occur more quickly than in the case in which three production scale batches are made post-approval (or right before approval to be ready for product launch) and then data generated through the desired expiration date. Depending on timing and the stability data itself, the second approach can provide an expiration date extension 12 or more months before the other route.

If stability problems occur with a product, the expiration date can be shortened via a CBE-30 supplement. Subsequent product data justifying extending the expiration date can also be submitted in a CBE-30 supplement. The supporting data would be similar to that described above in that three new production scale batches tested through the extended expiration date would be required in the supplement.

For post-approval changes, the previously approved expiration date can be used unless the change will alter the stability of the product. Since the goal of most changes is to show that the changed product and pre-change product are equivalent, the approved expiration is typically proposed based on the required stability data for the change.

For products approved globally, API retest period and finished product shelf-life can be extended through a type IB (major) variation. One of the conditions for shelf-life changes is that the change not be due to stability concerns; therefore, shortening and then re-extending product shelf-life would need to be filed via a Type II variation.

5.4.7 Specifications and Analytical Method Changes

Analytical methods need to be monitored during stability testing for assay, dissolution, degradation products, and other critical tests. Adjustments to the methods need to be made as necessary. As analytical methodology changes that impact new and ongoing stability studies are made, decisions concerning validation, implementation, and reporting need to be made as well. Advances in analytical technology

continue to lead to increased selectivity and sensitivity and thus decreased detection/quantitation levels for impurities and degradation products. Advances in column technology and analytical equipment (e.g., CE, UPLC, UHPLC, LC-MS-MS) lead to method improvements/efficiencies as well as separation/identification of new impurity/degradation product peaks which may need to be specified/quantified.

There are many reasons to propose a change to a product or API specification (limit) and/or an analytical method. With regard to shelf-life limit changes, examples include the addition of a limit for a newly discovered degradation product, an increase in the limit for a specified degradation product, a change in one or more of the dissolution limits (ranges) for an extended release product, and an increase in the pH range for a liquid product.

With regard to analytical methods, changes may be needed due to mass balance or sample preparation issues, enhanced knowledge of API or product (e.g., appearance of new impurity or degradation product), information obtained during a method transfer or from other stability studies such as accelerated or stress studies. Method changes may also be desired to shorten analysis time, take into account experience gained from running the method over an extended period of time or due to automation of the method.

In the case of an analytical method change, the impact on stability studies will need to be assessed. Crossover results will likely be required and a decision as to which method to use for ongoing studies reached. This decision will depend on the nature of the change and the implementation strategy. If the change is a significant quality improvement, then implementation should be immediate once appropriate validation and cross-correlation of the methods is complete. However, the change will need to be filed to regulatory authorities and depending on the type of filing both the old and new method may need to be performed until approval of the new method is received.

In the case where a method change is deemed annual reportable or even as a CBE supplement, implementation can be immediate with perhaps a crossover at the next time point for ongoing studies. In this instance, new studies could be initiated using the new method. Table 5.4 lists some of the typical specification/analytical method changes and potential impact on stability studies.

Table 5.4 Specification/analytical method changes

Change	Regulatory filing	Impact on stability studies
New method, no limit change	Prior approval supplement	Implement upon approval or run both methods
Addition of new specified degradation product (within approved limits)	CBE-0	Implement with filing (no change in method)
Revised method and new specified degradation product	CBE-30 (assumes not a new method)	Crossover testing
Change in limits (e.g. wider range or shift for extended release product dissolution)	Prior approval supplement	Implement new limits upon approval

5.5 Multiple Changes and Changes that Affect Multiple Products

Frequently, more than one change is involved that could impact the drug product and its stability. For example, a move to a new manufacturing site may involve process changes outside the approved ranges or the use of equipment of a different operating principle. As with the reporting category, stability requirements should support the most significant change. In this case, the process or equipment changes may potentially impact product stability and necessitate additional feasibility work and developmental stability studies. Another example would be the development and manufacture of a new dosage form, for example capsule to tablet or vice-versa, to expand the product line. The additional dosage form may be made at another site; however, the stability (and filing) requirements would be dictated by the new dosage form and not considered just a site change for product manufacturing.

Changes can also impact an entire product line or multiple products. For example, the addition of a new API supplier using a different synthetic route, due to cost or quality reasons, could affect all products made with this API. Let us assume that a company markets tablets, capsules, fast dissolving tablets, an oral liquid, and a transdermal dosage form all using the same API. If we factor in the number of strengths and packages per dosage form, the number of stability studies that may be needed could be substantial. However, if there is a significant volume of stability data generated across the product line and substantial product knowledge, then a well-thought-out reduced plan in which all dosage forms, strengths, and packages are covered may be acceptable to the regulatory authorities. One approach would be to evaluate the product line to determine the most challenging dosage form(s), strength(s), and package(s) from a stability perspective (degradation products, dissolution) and ensure that these configurations are placed on stability. In this way, showing that the changed product exhibits stability similar to the unchanged product (product manufactured using API from original supplier) for the most challenging configurations could qualify the entire product line for the API supplier change.

A change that can affect many products of the same dosage form is when a change is made to the primary packaging components, for example rubber stopper in vial products, flexible container closure system for large volume parenterals (LVPs), plastic bottles for oral solutions, or to a polymeric component of a tube for semi-solids. Each of these can lead to a change to several products that are filed in different reviewing divisions of the FDA. As discussed above, a solid stability history as well as product knowledge can facilitate design of the appropriate stability studies.

For example, take the case of LVPs to be packaged in a new flexible container closure system that has been qualified and previously approved for other products on the market; the original flexible package is used in many products. Several factors, such as API and its concentration, formulation pH, ionic strength, and container size

can be evaluated and similarities determined. It may be possible to group several similar products together whether they all contain the same API, or utilize the same size container, or have similar pH ranges. From this evaluation, the appropriate stability design would be developed covering all products and configurations but placing representative combinations on stability instead of testing every possible combination.

Depending on the complexity of the design, it may be important to meet with the regulatory authorities and obtain their recommendations prior to implementation of the product qualification and stability plan. A bundled submission to FDA could be utilized or a strategy used in which the most critical products are submitted first, followed by lower level submissions (e.g., CBE) for similar products to the same division.

Similar to packaging component changes, raw material changes can also affect many products. A wide variety of changes are possible and the potential to impact your products needs to be assessed; changes include site changes, specification changes, manufacturing process changes, starting material or ingredient changes, and change to a different manufacturer due to discontinuation of the material by the current supplier or for cost reasons. If the change is made to a critical excipient or to a release-controlling excipient, the change will require stability data in the change supplement. If the change is made to any other type of excipient, then the change needs to be assessed for potential impact to the product and its stability. For a compendial excipient, a change to the specification can be reported in the annual report and will not likely have any stability impact or requirements. For a site change where the excipient meets the same compendial requirements, a report indicating the equivalence of the raw material produced at both sites should be completed either by the vendor, if the site change is a vendor change, or by the manufacturer, if the site change is to add an alternate vendor. The annual stability program for the variety of products affected by this type of change should be sufficient.

A manufacturing process change to a raw material or a change in starting material will require a similar review with the most important factor being equivalence of the raw material after the change to the pre-change material. If there is a chance that this type of change will modify the physical characteristics of the material or if the excipient is critical to product or manufacturing performance (e.g., dissolution, uniformity) then impact to the product(s) and their stability may need to be evaluated. A qualification protocol should be written and the first batch of product placed on stability; in the case of multiple products, all products could be qualified based on studies of the product or products most likely to be impacted by the change.

Finally, ingredient changes to a raw material made of multiple ingredients (e.g., color coatings, combination excipients) can be the most difficult to assess since often testing of the final material may not be indicative of the individual ingredients. Evaluation by the vendor to assess this change would be required and the manufacturer's assessment may include a qualification protocol and placement of the first lot of representative products on stability.

5.6 Comparability Protocols

Comparability protocols are described in the draft FDA guidance [14]. The inclusion of a comparability protocol in the original NDA or ANDA can be very useful for certain post-approval changes. The protocol prospectively specifies tests and studies including stability testing to be performed based on the type of change to be made. The corresponding acceptance criteria are also included in the protocol. In the NDA, a sponsor may include a comparability protocol for an anticipated change, such as a package change or a manufacturing site change or an API synthesis change, and describe the testing/studies that will be completed to qualify the change prior to implementation. Typically, product and API specifications would remain unchanged in this type of protocol. The advantage to the applicant in filing a comparability protocol is to request that the FDA allow the specified change to be reported at one category lower than normal. If the FDA agrees then, for example, a change that is typically filed as a CBE supplement may instead be filed in the annual report. As discussed in the next section, more effort put into designing quality into the product during pharmaceutical development will facilitate this approach.

A good example is transferring manufacturing for a semi-solid dosage form. The relevant SUPAC guidance indicates that this type of change requires submission of a CBE-30 supplement containing 3-month stability data on one product batch. The comparability protocol would thus describe this type of change and indicate that the new site would meet all of the SUPAC requirements, for example satisfactory GMP inspection for type of product/operation, no changes to manufacturing instructions or test methods, and equivalent manufacturing equipment (same operating principle). The protocol would then go on to describe the testing that would be done, for example one batch of product will be manufactured and fully tested including homogeneity and microbial testing and the requirements for qualification/equivalence between the two sites. The batch would be packaged and placed on stability at long-term storage and accelerated condition (e.g., 25°C/60%RH and 40°C/75%RH) and tested through 3 months. The data obtained at 3 months would be compared to stability data from the previous site to show equivalence. All of this information would be summarized and the effect of the manufacturing site change evaluated with respect to the product's identity, quality, purity, potency, strength, and stability. Once this is satisfactorily and successfully completed, the new manufacturing site would be able to commercialize product from this site. The change and the data (based on the approved comparability protocol) could be filed in the annual report.

5.7 Pharmaceutical Development Considerations

During development, it is important to take into account potential or anticipated changes that may be necessary after product approval. This includes stability testing on product batches manufactured specifically to qualify wider ranges of excipient levels and process parameters. The FDA initiative, GMPs for the 21st Century [15]

was followed by several ICH guidances including Q8 Pharmaceutical Development [16] and Q9 Quality Risk Management [17] as well as the FDA guidance on Process Analytical Technology or PAT [18]. These guidances describe an approach to development in which quality is designed into a product as opposed to testing quality into the product. Product development using this approach leads to a better understanding of the parameters that may affect quality or stability of a product formulation or an API.

Quality by Design (QbD) is defined as a systematic scientific approach to product and process design and development. Studying and understanding the interaction of input variables and process parameters leads to a Design Space, which establishes the ranges for production of a quality product. The design space is proposed by the applicant in an NDA (or supplement) and reviewed and potentially approved by FDA. Working within design space is not considered a change and, therefore, regulatory relief can be obtained (e.g., a supplement would not need to be filed if the change is within the design space). Outside of the design space would necessitate a regulatory post-approval change process as described in this chapter.

The FDA guidance defines PAT as a system for designing, analyzing, and controlling manufacturing through timely measurements of critical quality and performance attributes (CQAs) of raw and in-process materials and processes with the goal of ensuring final product quality. The goal is to enhance control of the manufacturing process and product knowledge by understanding the chemical, physical, and biopharmaceutical characteristics of the drug substance and selecting product components and packaging based on drug attributes.

Taken together, QbD and PAT can facilitate the design of manufacturing processes using engineering, material science, and QA principles to ensure reproducible product quality and performance throughout shelf-life. The objective of pharmaceutical development then is to develop a robust formulation and process as well as rugged and reproducible test methods, which enable process control of critical quality attributes. This objective is not new to pharmaceutical development; however, the tools, as well as regulatory and quality input will lead to improved outcomes and with them we hope a decrease in post-approval changes and corresponding stability requirements.

5.8 Conclusion

A pharmaceutical product can spend many years on the market starting at NDA approval, moving through the peak selling years and into the generic competition period. Throughout this marketing period, changes will be suggested for quality, compliance, technical, and cost reasons. Equipment, suppliers, components, raw materials, and processes will change and impact the drug product. Evaluation of these changes, including assessing the stability requirements, is important to assure changes made are in line with business and quality objectives and are implemented in an effective and efficient manner. All too often one change is evaluated, appropriate batches made, data generated, a submission completed, and the change

implemented only to discover that another change impacting the same product is proposed and the same process needs to be repeated. Or a change is evaluated from a single country's perspective only and global requirements are not taken into account, leading to additional work that would have been better planned up front. Obviously, grouping changes and including all markets when possible is the most efficient use of limited resources; although for global products, this can be particularly challenging.

With quality by design initiatives and comparability protocol use, post-approval changes can be streamlined and/or eliminated completely; however, the ground work needs to be prepared early in the product life cycle. Developmental studies during process and formulation development and the appropriate corresponding stability studies can save a significant amount of resources down the road. Unfortunately, in the desire to get a product approved and on the market as soon as possible, often these studies are not considered and it is left to the post-approval group to manage change.

Product knowledge is gained throughout a product's life cycle. Communicating this knowledge between the development group and the post-approval group, understanding the objectives of both groups, and then working to a common outcome can provide advantages throughout the product life cycle.

Changes are inevitable if just to keep up with technology or to improve processes and costs. Evaluation of changes should be done through a team that includes technical, quality, and regulatory personnel. It must take into account implications to the supply chain. There are many regulatory and technical guidelines available to facilitate this evaluation; however, the ability to implement changes effectively and efficiently is dependent on the plan and its execution.

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16. International Conference on Harmonization (ICH) (May 2006) Guideline Q8, pharmaceutical development.
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Web Addresses

1. Health Canada Drug Products Web site: http://www.hc-sc.gc.ca/dhp-mps/prodpharma/index_e.html
2. Web address: http://www.hc-sc.gc.ca/dhp-mps/prodpharma/activit/proj/post-noc-apres-ac/noc_pn_quality_ac_sa_qualite_e.html
3. WHO Medicines Web site: http://www.who.int/medicines/en/
4. http://www.who.int/medicines/services/expertcommittees/pharmprep/QAS06_179Rev07.pdf
5. http://healthtech.who.int/pq/info_applicants/Guidelines/Variation_Guide.pdf]
6. European Medicines Agency EMEA Web site: http://www.emea.europa.eu/pdfs/human/qwp/057696en.pdf
7. Web address: http://ec.europa.eu/enterprise/pharmaceuticals/eudralex/vol-2/c/var_type_1a1b_guideline_06-2006.pdf

Part II
Stability Methodologies and Best Practices

Chapter 6
Understanding and Predicting Pharmaceutical Product Shelf-Life

Kenneth C. Waterman

Contents

Abstract Pharmaceutical products are assigned a shelf-life which determines the time when a product is considered to be safe and effective under a relevant storage condition. A number of factors are used to assign that shelf-life. Among these factors are the chemical stability of the active pharmaceutical ingredient (API) in its dosage form, and specifically whether any degradation products are potentially hazardous to a patient. In addition, any factors which affect an API's bioavailability can also limit shelf-life. These factors not only include loss of API potency due to degradation,

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but also loss of activity due to precipitation (for liquid dosage forms) or slowing of API release in the gastrointestinal tract. Accelerating the aging process allows the development and production of pharmaceutical products without waiting for the entire shelf-life to elapse before assigning a value. Such factors as temperature and relative humidity can be used to accelerate these processes effectively allowing for accurate and precise predictions.

6.1 Introduction

In the development and ultimate commercialization of a pharmaceutical product, a shelf-life must be assigned. This assignment uses various factors to determine how long a product will be safe and effective for the patient under reasonable storage conditions. A shelf-life is assigned to materials used for clinical trials as well as to products distributed commercially. In some cases, two shelf-life values are assigned: one for a dosage form in its original, bulk packaging, and one corresponding to the safe storage time once the dosage form is removed from the initial packaging. This is particularly important for pharmaceutical products that are prepared (constituted) before dispensing to a patient.

In this chapter, the factors that determine the shelf-life and how these change with time in the majority of pharmaceutical products are explored. After this, the role of accelerating aging in the assignment of shelf-life is explained. In each of these areas, more extensive reading is found in articles referenced in the bibliography section.

6.2 Factors Determining Pharmaceutical Product Shelf-Life

6.2.1 Chemical Stability

Pharmaceutically active ingredients (APIs), be they biological (i.e., protein or nucleic acid) or small molecule, are susceptible to organic chemical degradation processes. To maintain the safety and efficacy of pharmaceutical products, regulatory agencies require that degradation products be assessed to assign a shelf-life. The shelf-life of a pharmaceutical product is set based on the time it takes, at prescribed, likely storage conditions, for any degradation product to reach a level that it becomes a safety concern, or, for the potency (activity) of the active ingredient to drop below a critical level. With limitations based on degradant formation, the amount of such degradants permitted are based on the total daily intake of the API and are described in terms of *reporting*, *identification*, or *qualification* thresholds. A reporting threshold is defined as the level that must be reported to regulatory agencies to alert them of the presence of a degradant, often observed as a peak in a high performance liquid chromatography (HPLC) run. An identification threshold is defined as the level that requires specific chemical identification of the substance. Finally, the qualification threshold is the level that requires a toxicological assessment study to ensure the safety of the degradant. These thresholds are defined as a percent of the API total

Table 6.1 Regulatory guidelines (ICH) for total amount of a degradant allowed for new drug products

Classification	Maximum daily dose (mg)	Maximum daily degradant dose allowable
Reporting	≤ 1000	0.1%
	>1000	0.05%
Identification	<1	1.0% or 5 μg (lower of two)
	1–10	0.5% or 20μg (lower of two)
	10–2000	0.2% or 2 mg (lower of two)
	>2000	0.10%
Qualification	<10	1.0% or 50 μg (lower of two)
	10–100	0.5% or 200 μg (lower of two)
	100–2000	0.2% or 3 mg (lower of two)
	>2000	0.15%

daily intake or an absolute mass amount, whichever is lower. Table 6.1 describes the specific levels of degradants generally allowed for each threshold, consistent with the International Conference on Harmonization (ICH) recommendations.

The factors that are used to determine the level of a specific degradation product that is allowable depend on the following:

1. If a degradant is also a metabolite, a justification exists for allowing greater levels of that degradation product since the degradant is effectively tested for safety as part of the clinical program for the API.
2. When the API is at a low dose, slightly higher percentages of a degradation product may be permitted based on the total patient exposure.
3. If the degradant is a suspected carcinogen, teratogen, or mutagen, lower threshold levels may be applicable.
4. If the API is to be taken chronically, the allowable degradant level may be lower than with a single dose treatment.
5. Degradants with little information about safety will generally be limited to between 0.2 and 0.5% of the API at the end of the shelf-life.

It should be noted that in some cases, a degradant is also a process impurity (i.e., present in the initial un-aged API sample). The stability limitations reflect the total amount of the material including both the amount that is there initially and the amount that forms under storage conditions.

In some cases, the degradants are known to be innocuous (e.g., for prodrug degradation). In these cases, the shelf-life limiting factor will be the loss of potency for the API. While this represents no direct safety risk to the patient, it still can be harmful if the patient is not receiving the expected dose of the API. Generally speaking, the potency needs to remain at or above 95% of the label claim at the end of the shelf-life based on the International Conference on Harmonization [1]. This can be even more significant if the variability in the initial potency is taken into account.

With such large-molecule pharmaceuticals as nucleic acids, proteins, and oligonucleotides, chemical degradation of parts of the molecules not associated with

the active site may not have a significant effect on the biological activity. For this reason, the amount of chemical degradation that may occur can be quite significant without limiting the shelf-life. The activity is often determined using a biological assay to indicate the actual potency. When chemical degradation does occur near the active site of the biological molecule, the potency loss can be as significant as with small molecules; however, even side-chain degradation of proteins on the order of 40% may not impact the overall potency if the degradation does not affect the active site either directly or via secondary and tertiary structural changes.

When determining the shelf-life of a drug product, the most restrictive factor will determine the limitations. For example, if a single degradant has a very restrictive limit based on toxicity concerns, it may be the limiting factor in setting shelf-life even if a different degradation product is more rapidly formed. In general, the percentage conversion of an API to degradants in a drug product remains relatively low even at the end of the shelf-life storage conditions. One important implication of this principle is that it is unnecessary to determine the full course of a degradation process in drug products. Effectively, what happens after the shelf-life limiting factor is reached becomes irrelevant. As will be seen in Section 6.4.1, this can be used to advantage in accelerated aging processes.

For liquid formulations that are constituted by the pharmacist or patient, a separate shelf-life will be indicated before and after constitution. Physical stability before constitution means that the constituted formulation will still perform acceptably (i.e., dissolve or disperse) within the pre-constitution shelf-life. After constitution, the stability concerns will be similar to other liquid formulations. Even for solid-dosage forms, there can be a different shelf-life for the product as shipped (potentially with more protective packaging), and as received by the patient.

6.2.2 Physical Stability

In some cases, shelf-life can be limited by the physical stability of a drug product rather than by its chemical stability. Physical stability is most important when it induces a change in the performance of a dosage form after storage. Of particular concern is any factor that could be anticipated to alter the bioavailability of the API. For solid dosage forms, this can mean a change in dissolution performance (disintegration and subsequent solubilization). Dissolution changes on storage of tablets and capsules can occur due to a number of factors. With tablets, the majority of issues are associated with picking up moisture from the environment. This can result in a change in the effectiveness of a disintegrant. Disintegrants are able to expand rapidly with water, breaking apart a tablet in the stomach. When moisture is adsorbed slowly during storage, some slow expansion occurs, which can prevent the explosive expansion by the disintegrant necessary for fast disintegration in the stomach. When the tablet is exposed to water in the stomach (or dissolution vessel), the disintegrant does not absorb as much additional water and therefore does not break apart as effectively as when the tablet had been kept dry. Especially problematic for such systems is when the disintegrant is exposed to conditions where

water condensation occurs. This happens when an equilibrated tablet at an elevated temperature and humidity is cooled below the dew point, for example by shipping through colder climate zones than where initially packaged.

With gelatin capsules (for both normal capsules and soft-gel capsules), the capsules themselves are subject to physical changes upon long-term storage. In some cases, gelatin will undergo a cross-linking reaction due to low levels of impurities in the formulation or packaging. Such reactions are generally caused by small aldehydes such as formaldehyde and glyoxal. This cross-linking can make the gelatin slow to dissolve in standard dissolution media. It should be noted, however, that in many cases the in vivo release will be unaffected due to enzymatic processes. This effect can be monitored with special dissolution media containing the appropriate digestive enzymes.

Another potential physical change in solid dosage forms involves a change in the form of the API itself. Most pharmaceutical dosage forms employ a crystalline form of the API with a particular packing morphology. In most cases, the way the molecules pack is not unique; that is, the API is capable of assuming polymorphic forms having different energetics. If the polymorph used in a drug product turns out to be a high energy form, the potential exists for the polymorphic form to change during storage [2]. In rare cases, such a change in morphology could result in a change in bioavailability. This can be because of a change in solubility (the more stable polymorph will often have a lower solubility) and corresponding change in dissolution rate. Even if the polymorph that is used is the thermodynamically most stable form, solvation or desolvation can still occur over time. Desolvation results when a solvent molecule (including waters of hydration) is lost from the crystal lattice. Solvation generally involves the addition of water to the crystal lattice. An extreme case of change in morphology results when such a desolvation causes the complete loss of the crystal lattice to give an amorphous form of the API. The result of such loss of crystallinity is generally an increase in drug solubility (dissolution rate), but a decrease in drug stability.

With some APIs, the dosage form takes advantage of the increased transient solubility of amorphous (or other high energy) drug forms to increase bioavailability. APIs in such dosage forms have the potential to spontaneously crystallize since the process is exothermic and often autocatalytic (i.e., once crystal nuclei are generated, they can increase the rate of further crystallization). Nonetheless, with stabilizers, such systems can limit the crystal growth to provide confident stability over extended time periods. Since this change with time can result in decreased API efficacy, shelf-life can be limited by this factor.

For liquid dosage forms, altered bioavailability upon storage is generally manifest in precipitation of API or other formulation components. Precipitation can result from a number of factors. With small molecules, precipitation can be caused by shifts in the pH of the solution (suspension). Such shifts can be due to absorption of carbon dioxide, chemical degradation of a component that generates an acid or base or loss of a buffer component due, for example, to oxidation. Another factor with small molecules is precipitation due to an increase in the API particle size. This effect, called Ostwald ripening, is caused by the gradual dissolution of smaller

crystals with concurrent growth of larger ones due to the lower energetics of the latter (larger crystals have a lower surface-to-volume ratio such that there are fewer molecules on the surface of the crystal where the molecules intrinsically have a higher energy). Chapter 10 provides additional discussion on physical stability of drug substance and drug product.

For biological molecules, a suspension's properties will depend on secondary and tertiary structural features, which in turn involve ionic, van der Waals, and hydrogen bonding forces. In general, there are multiple configurations of large molecules which often have similar energetics, yet can have very different tendencies to aggregate, thus resulting in different biological activities. While an initially formed configuration of the biological API may be monomeric and suspend well, over time parts of the large molecule may denature leading to aggregation and precipitation. Since the aggregated state may not easily re-equilibrate with the suspended material, this process can drive toward greater precipitation based on LeChatlier's principle. Additional information can be found in Chapter 17.

6.2.3 Appearance

At times, a formulated API can change in appearance without any obvious impact on physical stability or chemical degradation. This can manifest itself in a change in color, generally a chemical change for which the most sensitive assay is the human eye. While it is very unlikely that such subtle chemical changes are a risk to the patient, the appearance change can be disturbing to the patient, and as such, can limit the shelf-life of a pharmaceutical product.

The gelatin used with capsules can become brittle enough to crack when stored under dry conditions (such as with desiccant). While the brittleness may not result in a change in biological activity, it nonetheless can give an unacceptable appearance.

For tablets coated with cosmetic film coats, swelling of the core tablet with moisture can in some cases cause cracking of the coating. While this again should have no impact of performance, it still can be disturbing to patients and as such will limit shelf-life (or necessitate special packaging).

6.2.4 Microbial Growth

With parenteral formulations, limitations on shelf-life can be based on the time needed for formation of microbial impurities. For many such formulations, biostats are added to prevent or at least slow microbial growth. Once these are consumed, growth can occur. For other formulations, packaging integrity over time may determine propensity for microbial growth.

6.2.5 Photochemical Degradation

Light sensitivity of a pharmaceutical product can limit shelf-life, or in many cases, determine the packaging requirements for the product. In some cases, light exposure can induce chemical degradation in an API when the light is absorbed and then initiates a chemical reaction. Photochemical reactions commonly include oxidations and free radical rearrangements. Indirect photochemical processes are also possible. In these cases, light is absorbed by a species in the formulation other than the API, which then leads to a reaction with the API. The most problematic ambient light wavelengths are long ultraviolet and short visible (blue) lights, partly because of the energetics (higher energy wavelengths) and because of the overlap with absorption spectra. Most photoprocesses that affect API stability respond to light intensity in a reciprocal fashion such that short duration, high intensity exposures have the same impact as long duration, low intensity exposures, if the total flux of light is the same. Testing can therefore be accomplished with pharmaceutical products using, for example, a light box fitted with a high wattage UV emitting lamp with a filter to remove wavelengths below 320 nm.

6.3 Drug Instability with Time

6.3.1 Extrapolation

For any of the shelf-life determining parameters discussed above, there will generally be a change in that parameter as a function of time. In principle, the shelf-life can be determined by performing the appropriate assay in real time until the threshold is reached. In practice, scientists involved in the pharmaceutical development process want an indication of the stability of a dosage form without waiting an inordinate amount of time (i.e., typically many products have shelf-lives of greater than 2 years). The shelf-life of a product may even be the determining factor for selecting which formulation or process is developed. When the dosage form is progressed to product launch, a shelf-life is often assigned based on data that involve some amount of extrapolation rather than extending for the full shelf-life.

To make a stability extrapolation with time, the functional form of the instability becomes important. For chemical processes, the formation of degradation product or loss of starting API involves a kinetic process. Many chemical reactions follow a first-order process; that is, the reaction rate is proportional to the concentration of API remaining. This can be described in the following kinetic equation:

$$d[\text{API}]/dt = -k[\text{API}] \tag{6.1}$$

where [API] is the concentration of API in molarity, t is time, and k is the first-order rate constant for the reaction. The integral form of Equation (6.1) is the following,

where $[API]_0$ is the initial API concentration and $[API]_t$ is the API concentration at time t:

$$\ln([API]_0/[API]_t) = kt \tag{6.2}$$

In addition to unimolecular processes, reactions where a reactant is at a high enough concentration to remain effectively constant follow the same first order kinetics. This often applies, for example, to hydrolytic reactions.

For one API molecule going to one degradant, the kinetics can be derived as from Equation (6.2) as follows (where [P] is the concentration of the degradation product):

$$[P]_t = [API]_0 - [API]_t \tag{6.3}$$

$$kt = \ln\{[API]_0/([API]_0 - [P]_t)\} = -\ln(1 - [P]_t/[API]_0) \tag{6.4}$$

Some degradation reactions follow zero-order kinetics, i.e., loss of API or formation of degradation product does not depend on the API concentration. This is shown in derivative and integral form below:

$$d[API]/dt = -k \tag{6.5}$$

$$[API]_0 - [API]_t = kt \text{ or } [P]_t = kt \tag{6.6}$$

More rarely, other order reactions (e.g., second order) are involved in degradation processes, especially in solution where collisions between molecules are more likely.

For first order reactions, the loss of API or formation of degradation product follows a logarithmic function, which is different from the linear degradation one expects for zero-order reactions. Similarly, other order reactions will have different functional forms. As it turns out, for the situation of relatively low conversions (as will be the case for shelf-life limitations), the differences between these functions are less significant than they appear at first. This can be seen in Fig. 6.1 for formation of a degradation product (up to 30% of the API).

As can be seen in the graph, the curvatures associated with first or second order processes do not have a significant impact on the functional form of the degradant formation with time curve compared to a zero-order function, up to at least 10% conversion. It is therefore reasonable to assume that all degradation processes follow a zero-order curve shape with respect to the shelf-life. This allows the rate constant for a degradation process to be determined using the initial slope of the degradant versus time plot.

The rate equations discussed above were derived for solutions. In solid-state, molarity is generally replaced by weight percent. This does not change the fundamental equations, based on the assumption that volumes do not change with low API conversions.

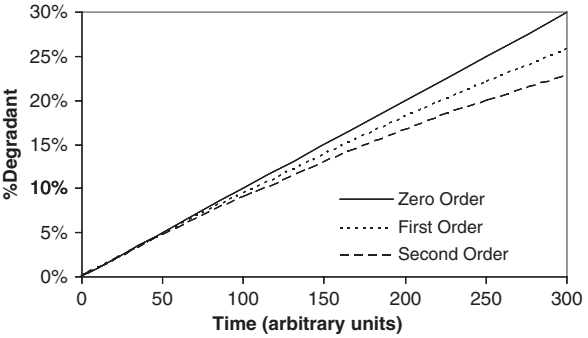

Fig. 6.1 Difference between zero, first, and second order processes. As can be seen, at low conversions (low amounts of degradant formed), the functional differences between the different reaction orders are indistinguishable

6.3.2 Heterogeneous Systems

For solid-state dosage forms, the API can potentially exist in more than one form. While for the majority of drug products, the API is crystalline, some of the API will often be either in a high energy crystalline form (e.g., at crystal defect sites) or in an amorphous form. Amorphous API can either be in a high energy state or be in a solid-solution with excipients, which can be thermodynamically stable. When an API undergoes a degradation reaction from the crystalline state, it typically requires energy to overcome the crystal lattice energy in addition to any energetics involved in bond making or bond breaking. In addition, the mobility of chemical species is usually significantly lower in the crystalline state than in the amorphous state. Even diffusion of small molecules such as water or oxygen is generally reduced in the crystalline phase. The result is that reaction rates are often one to three orders of magnitude higher with non-crystalline API than with crystalline API. This means that pharmaceutical formulations having a small amount of a more reactive API form will show different reaction rates for the first part of the reaction than for later stages. This can be seen in Fig. 6.2, where a small level of reactive drug form is largely depleted before the bulk crystalline form dominates the kinetics.

This heterogeneity in the kinetics can make a rate constant deceptive since it will change with the extent of reaction. This is just one challenge in making extrapolations of degradation with time: a rapid rate due to a reactive API form may not continue once the reactive API form is consumed. Fortunately, the matter is somewhat simplified in pharmaceutical stability testing since only a small amount of degradation determines the shelf-life.

6.3.3 Lag Time Behavior

In some pharmaceutical systems, there is little to no degradation for a period of time, and then degradation proceeds at a rapid pace. This lag time behavior is often caused

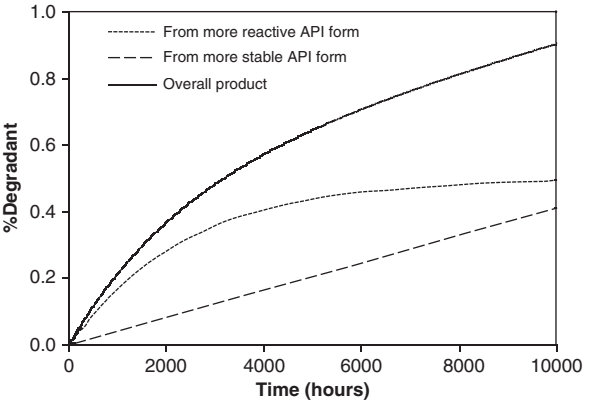

Fig. 6.2 The overall product profile is affected by the slow degradation of the crystalline API as well as the more rapid degradation from API in a more reactive form (e.g., amorphous). In this example, the degradant is formed based on the assumption that there is 0.5% of reactive API form, which reacts with a rate constant of 4.2×10^{-4}%/h, while the 95.5% of the API in its crystalline form reacts with a rate constant of 4.2×10^{-7}%/h

by the presence of a reaction inhibitor which effectively prevents API degradation until it is consumed. One example of such inhibitors is an antioxidant. In some cases, the antioxidant will effectively prevent oxidation of the API until it is consumed, and then API oxidation can proceed. Some oxidizable excipients contain antioxidants as provided by the manufacturer (e.g., polyethylene oxide). Since the decomposition products of some of these materials can then react with an API, this will also result in a lag time behavior in the kinetics. When projecting a shelf-life, pharmaceutical systems showing a lag-time are especially problematic since short-time behavior can dramatically overestimate the stability.

Another process that can lead to a lag time is when the product of a degradation reaction itself affects the degradation rate. This autocatalysis most commonly involves the formation of acidic (or more rarely basic) degradation products. For example, when an ester hydrolyzes to an alcohol and a carboxylic acid, it can drop the pH of its environment, which in turn can speed acid-catalyzed ester hydrolysis.

6.4 Accelerating Aging

6.4.1 Temperature Effects

6.4.1.1 Simple Chemical Degradation

Determining the shelf-life of a pharmaceutical product can sometimes be the slowest step in the effort to bring a new drug product to the market. This is especially important if stability issues arise requiring a change of formulation or process. Consequently, there is a desire in pharmaceutical corporations to determine the shelf-life

of drug products under accelerated conditions, both to meet regulatory requirements and to build confidence that a given formulation and process will not generate stability issues at a later stage of development.

Whether in solid state or in solution, in order for an API to go to a degradation product it must undergo some combination of collisions and molecular reorganizations. Even for chemical degradation processes that are exothermic, the initial API form is usually fairly stable. This means that it is at a local energetic minimum with respect to collisions and rearrangements. The result is that for the majority of chemical degradation processes, energy is needed to overcome the activation barrier. This energy is referred to as the activation energy, E_a. A combination of molecules will intrinsically possess a distribution of energy: some with more, some with less. This energy distribution is dependent on the temperature: with a higher temperature, more molecules will possess greater energy. This leads to a relationship known as the Arrhenius equation relating the rate of a reaction k and the temperature T (in Kelvin). This exponential dependence is related to the distribution of molecules having different energy levels and the height of the energy barrier:

$$k = \mathrm{A}\exp[-\mathrm{E_a}/\mathrm{RT}] \tag{6.7}$$

The other terms in this equation are A, a proportionality term which is sometimes referred to as an "A-factor," and R, which is the gas constant (1.987 cal $K^{-1}mol^{-1}$ or 8.314 J $K^{-1}mol^{-1}$). The Arrhenius equation can be rearranged to the following:

$$\ln \mathrm{k} = \ln A - \mathrm{E_a}/(\mathrm{RT}) \tag{6.8}$$

From this equation, it can be seen that a plot of the natural logarithm of the rate constant versus the reciprocal of the absolute temperature will yield a straight line with a slope equal to the activation energy divided by the gas constant and an intercept equal to the natural logarithm of the A-factor. Typical chemical reactions show activation energies of 10–30 kcal/mol. It is interesting to look at the magnitude of increase in drug reactivity this corresponds to at temperatures around ambient. For example, in going from 20 to 30°C (293–303K), a reaction will increase by a factor of 1.8–5.5, at 10 and 30 kcal/mol activation energies, respectively. This leads to a general rule of thumb that reaction rates will double about every 10°C (corresponds to 12 kcal/mol, at ambient conditions).

Once a set of stability studies are conducted at elevated temperatures, an Arrhenius plot (i.e., ln *k* versus 1/T) can be made. This in turn can be used to predict the rate of formation of a degradation product (or loss of starting API) at the storage condition. The shelf-life will then correspond to the time needed to hit the shelf-life limiting level of degradant (loss of starting API) using Equation (6.9):

$$\text{shelf-life} = ([\mathrm{D}] - [\mathrm{D_0}])/\mathrm{A}\exp(\mathrm{E_a}/\mathrm{RT}) \tag{6.9}$$

where T is the absolute temperature for the storage conditions, [D] is the shelf-life limiting degradant concentration and $[D_0]$ is the concentration of that degradant

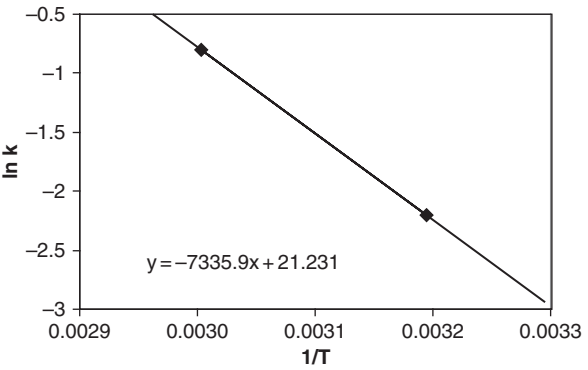

Fig. 6.3 Arrhenius plot used for the example calculation. The intercept is ln A and the slope is $-E_a/R$

present initially. As an example, suppose a formulation initially has a degradant at a concentration of 0.05% and has a regulatory limit for that degradant of 0.5%. Suppose also that the measured level of that degradant is 0.73% at 40°C after 6 months, and 0.50% at 60°C after 1 month. We can then determine the shelf-life for storage at 25°C as follows:

1. Determine the rate constants at each temperature:
   40°C (0.73%–0.05%)/6 month = 0.11%/month
   60°C (0.50%–0.05%)/1 month = 0.45%/month
2. Determine the Arrhenius parameters based on a plot of ln *k* versus 1/T as shown in Fig. 6.3:
   40°C ln (0.11%/month) = ln A − E_a/(1.987 cal $mol^{-1}K^{-1}$ 313 K)
   60°C ln (0.45%/month) = ln A − E_a/(1.987 cal $mol^{-1}K^{-1}$ 333 K)
   Solving for ln A and E_a gives ln A = 21.2 A = 1.61×10^9 %/month and E_a = 14.6 kcal/mol
3. Use the Arrhenius parameters and the limiting degradation threshold to determine the rate at 25°C from Equation (6.9):

$$\begin{aligned} \text{shelf} - \text{life} &= ([D] - [D_0])/A \exp(E_a/RT) \\ &= (0.50 - 0.05\%)/(1.61 \times 10^9\%/\text{month}) \exp\{14600\ \text{cal/mol}/ \\ &\quad (1.987\text{cal mol}^{-1}\text{K}^{-1}298\ K)\} \\ &= 14\text{months} \end{aligned}$$

In this example, the rates were expressed in weight percent of the degradant formed per month; consequently, the final shelf-life is based on the same time unit (i.e., months).

API degradation in some pharmaceutical systems does not show Arrhenius behavior over a wide temperature range due to some combination of the following:

1. Physical changes can occur over the temperature range used. Such transitions typically involve melting (glass transition), vaporization, and changes in

solubility. With many physical changes, there can be an abrupt discontinuity in the Arrhenius curves through the transition. In some cases, the abruptness of the change can make predictions of behavior based on high temperatures (i.e., above the transition) non-predictive of behavior at lower temperatures.

2. Buffers can change pH with temperature, which in turn can impact degradation kinetics in solution. While this is usually a gradual effect, the impact of pH on reaction kinetics can sometimes be significant.
3. Multiple chemical pathways can produce the same reaction products, but with each pathway having different activation parameters. Similarly, the rate-determining step in a degradation process can switch with temperature. The result can be that at higher temperatures a different set of Arrhenius parameters dominate compared to the situation at lower temperatures. This transition between mechanisms is usually gradual, but can lead to poor fitting of Arrhenius data, especially over wide temperature ranges.
4. Humidity can have a profound effect on the degradation kinetics for solid dosage forms. This factor will be discussed in detail in Section 6.4.1.1. A special case of humidity sensitivity involves deliquescence. Deliquescence is a process where liquid water is picked up by a sample when it is stored above its critical relative humidity (CRH). When deliquescence occurs, a dosage form will often display unacceptable changes in appearance and performance. In addition, chemical stability will often change dramatically above the CRH. While the shelf-life generally relates to time at a particular storage condition, one must also take into account the possibility of catastrophic failure when a dosage form is exposed to high relative humidity. If a dosage form has such an issue, it can be necessary to include protective packaging (potentially including desiccants) to enable marketing of a commercial dosage form. A number of excipients have relatively low CRH values. When these excipients are used, they can detrimentally affect the chemical and physical stability of a solid dosage form, even if the API's CRH is not exceeded. When deliquescence occurs, the liquid water can dissolve API, making stability much worse. Table 6.2 shows some low CRH excipients. As shown in the table, for some excipients (as well as APIs), the CRH can change with temperature. When mixtures of excipients and the API are used in a tablet, the possibility exists of having a CRH value below that for any specific compound. This *eutonic* mixture can lead to deliquescence for some pharmaceutical solids, at least at API surfaces, that is considerably lower than one might otherwise anticipate.

6.4.1.2 Heterogeneous Systems

As discussed in Section 6.2.2, solid dosage forms can have API in both crystalline and more reactive API states. If the extent of API conversion changes as the temperature is increased, the possibility exists that the relative contribution from each form may change. Determining the complete Arrhenius expression for such heterogeneous systems can be quite complex. To avoid this complexity, a stability study can be carried out using an isoconversion paradigm; that is, the amount of each

Table 6.2 Some excipients that have critical relative humidities (CRHs) that may affect API stability. When the CRH is exceeded, samples will deliquesce

Excipient	CRH (%) at 20°C	CRH (%) at 40°C
Dextrose	100	88
Fructose	72	64
Sorbitol	80	69
Tartaric acid	84.5	78
Calcium chloride	29	21
Potassium chloride	84	82
Sodium chloride	75	75
Sodium citrate	60.5	78
Polyethylene glycol (3350)	94	85
Sodium carboxymethylcellulose	84	83.5

degradant at each temperature should be kept low and relatively constant. To use the isoconversion paradigm, set the time at each temperature to provide the shelf-life determining degradation product level (or loss of API) at that limiting (specification) level itself. This contrasts with setting fixed times at each accelerated stability condition; where degradant formed at one condition may come predominantly from the reactive API form, while that formed at another condition may be dominated by the crystalline API form. The contrast between the two methods can be seen in Fig. 6.4. The result of using fixed times when the kinetics are indeed heterogeneous can be errors in the Arrhenius extrapolation to ambient conditions. Since the Arrhenius plot involves a logarithm, even small errors in the extrapolation can lead to significant errors in the projected shelf-life.

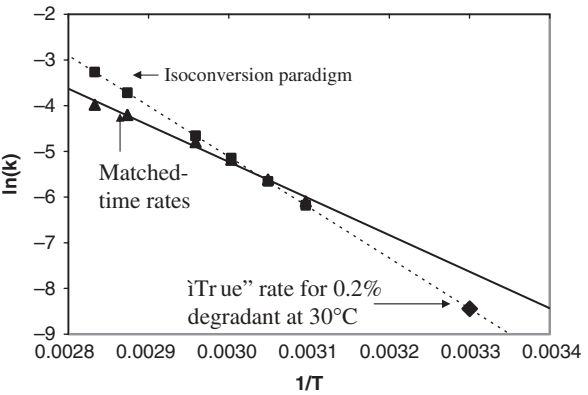

Fig. 6.4 Calculated Arrhenius plots illustrating the contrast between the isoconversion paradigm and constant time-based accelerated stability measurements. The isoconversion (to 0.20%) model (*squares*) and matched exposure time model (*triangles*) extrapolate to shelf-lives (at 30°C) of 2.54 and 1.14 years, respectively, compared to the actual 30°C value (*diamond*) of the model of 2.55 years

To carry out such an isoconversion study, one needs to know the amount of degradant formation as a function of temperature. Such studies will fall into two situations: when one has no knowledge of the actual stability of the sample, and when one has preliminary information. When no information is available, one can use average behavior. This approach is discussed further in conjunction with the combination of relative humidity effects (Section 6.4.3). If preliminary data are available (e.g., for repeats of formulations), the times can be adjusted at any accelerated conditions to meet the isoconversion paradigm. For example, if a degradant has a specification limit of 0.50, and 0.25% was found at one week at 60°C (with 0.00% initially), then it would be appropriate to allow the sample to stay at 60°C for 2 weeks in the follow-up study.

6.4.1.3 Physical Stability

Physical stability may or may not follow the general temperature trends characterized by the Arrhenius equation, since the source of change may not involve an activated process. Even when a process follows the Arrhenius equation, additional factors may make interpretation complex. For example, while increased temperature may increase the rate of rearrangements leading to precipitation of parenteral formulations, the solubility itself may increase with increased temperature. This increased solubility can actually prevent the precipitation being studied. A general situation occurs with proteins in solution. It has been shown that for many proteins, a monomeric protein form can equilibrate with another soluble protein form (e.g., a dimer), which then irreversibly precipitates. This process is shown schematically in Equation (6.10):

$$\text{Native Protein} \rightleftharpoons \text{Dimer} \rightarrow \text{Precipitated Protein} \tag{6.10}$$

The first, fast equilibrium will depend on temperature, which couples with the temperature effects on the second kinetic process since the amount of the dimer present affects the rate of precipitation. The overall result is a bit more complex temperature behavior if there is no easy way to detect the intermediate dimer.

Physical stability related to API polymorphic changes and API crystallization are generally limited by mobility. Since mobility effects generally follow an Arrhenius relation, such processes are often accelerated by elevated temperatures in a predictive manner; however, there will often be a discontinuity at any phase change (such as a melt or glass transition).

6.4.2 Humidity

6.4.2.1 Chemical Stability

Humidity can have a significant effect on solid API stability even for degradation reactions which themselves do not involve water. The ability of water to effect

physical and chemical changes is dependent on the sample's water activity, which in turn is equal to the equilibrium relative humidity (ERH) over a sample (though the former is expressed as an absolute number while the latter is expressed as a percent). ERH represents the moisture content relative to the saturated moisture content at that temperature, which is defined as ERH = 100% (water activity = 1). As one increases temperature, the amount of water in the air for a given ERH increases; however, the water activity depends only on this relative humidity, not the absolute humidity (or absolute water content).

The most obvious effect of changes in a solid API's water activity can be formation or loss of waters of hydration from the API crystal lattice. In some cases, loss of waters of hydration can lead to the complete conversion of the API to an amorphous form, which in turn can lead to greater chemical instability. Fortunately, such effects are generally well-known with a given API based on moisture sorption isotherms (i.e., water uptake and loss measured using sensitive balances as a function of relative humidity).

The more common effect of water activity (ERH) for shelf-life considerations is its effect on the API chemical stability. This effect is mostly due to the impact of moisture on the mobility of species in the solid state. At a given temperature, the effect of the ERH on the rate constant for either degradant formation or API loss (k) is shown in Equation (6.11) (where B and C are constants):

$$\ln\ k = B(ERH) + C \tag{6.11}$$

From Equation (6.11), it can be seen that reaction rates increase exponentially with the ERH. The values for B typically range from 0 to 0.09. This means that in going from dry conditions (10% RH) to moist conditions (75% RH) at a fixed temperature, the degradation rate will range from equal (B = 0) to 347 times faster (B = 0.09) at the damp conditions compared to the dry conditions. To put another perspective on this, in the latter case, a pharmaceutical product with a shelf-life of only 1 week at 75% RH would increase to 6.7 years with effective desiccant.

6.4.2.2 Physical Stability

In general, physical changes associated with moisture uptake, such as any changes in disintegration behavior of tablets, are relatively fast. The time for such moisture uptake to occur can be monitored by weight gain at different relative humidities, and it is generally complete within a couple of days.

6.4.3 Combining Relative Humidity and Temperature

By combining the Arrhenius equation (Equation (6.8)) with the moisture sensitivity equation (Equation (6.11)), one can generate a general equation for the effect of both temperature and ERH on API stability:

$$\ln\ k = \ln\ A - E_a/(RT) + B(ERH) \tag{6.12}$$

This equation states that the degradation of an API depends on the temperature and the ERH, with these dependencies being independent of each other. To be able to use accelerated aging effectively to predict storage at different temperature and humidity conditions, it becomes important that any stability program decouple the two factors. Since Equation (6.12) has three fitted parameters (ln A, E_a/R, and B) based on two independent variables (T and ERH), the minimum number of experiments needed to solve the equation is three (assuming temperature and ERH are both varied). While this would allow the data to be fit, it is generally a good idea to use more points to improve the precision. In practice, a four to six point protocol can be used effectively for fitting the parameters provided that the temperature and ERH are varied independently. As will be discussed below, the precision of any extrapolated stability predictions made from accelerated aging will depend not only on the precision of the data, but also on the distance of the extrapolation. For this reason, longer stability studies can be used with lower temperatures to provide greater precision. In most cases, however, even 2–3 week stability studies can adequately determine the shelf-life of a product at ambient conditions. When designing a protocol, it is important to remember the isoconversion paradigm discussed in Section 6.4.1.2. From this paradigm, it becomes apparent that the time a dosage form is maintained in a stability chamber will not be the same at each condition. Once the fitted parameters are determined, it is possible to establish a dosage form-specific protocol that maintains the isoconversion paradigm (i.e., each sample's conditions provide the specification amount of a degradant that limits the shelf-life). Of course, for an initial stability study, one does not know the parameters ahead of the results. Under these conditions, a generic protocol can be designed based on average parameters. Table 6.3 shows two reasonable protocols that provide shelf-life estimates. The 2-week, 4-point protocol is most appropriate for formulation screening or rough estimations of shelf-life, while the more extensive 3-week, 6-point protocol provides greater precision.

Table 6.3 Two protocols for accelerated stability studies of solid pharmaceutical products. In each case, samples are exposed to the environment of the stability chamber (open conditions) except for those at 5%RH where a desiccant is used with a closed container

Protocol	T (°C)	%RH	Days (1st sample)	Days (2nd sample)
Four-point	50	75	14	
	60	40	14	
	70	75	2	
	80	5	14	
Six-point	50	75	4	14
	60	5	14	21
	60	40	4	21
	70	5	4	21
	70	75	1/3	2
	80	40	1/3	4

6.5 Precision

6.5.1 Extrapolation with Time

The shelf-life assigned to a drug product will intrinsically involve some degree of uncertainty. To understand the role of imprecision in the assignment of shelf-life, it is useful to first look at the case of assigning a shelf-life based on data for the actual storage condition, that is, with no extrapolations involved. Nominally, the shelf-life is the time it takes for the drug product to reach the critical limiting factor, as discussed above. Some form of measurement is used to determine the time when a critical threshold is passed. With any measurement, there will be some variability in the measured value with repetitive tries, representing the precision of the measurement. The range of values can be represented in terms of a confidence interval (CI), that is, the probability that the next measurement is within that range. Typically, one can use a standard deviation (i.e., a 68% confidence interval) or with a greater interval such as 90 or 95%.

In the determination of the shelf-life, one generally measures a value that changes with time. From a plot of the changing value vs. time, it is possible to estimate (with interpolation) the time when the critical parameter value is attained. The change in the value with time may not be linear, yet there are generally insufficient time points in a stability study (due to resource limitations) to explicitly determine the functional form of the changes with respect to time. Even if the linear approximation is valid, the uncertainty needs to reflect the confidence intervals for the measured values. As an example, in Fig. 6.5, a set of measurements are shown as a function of time. As can be seen in the example, the final shelf-life (i.e., the time it takes for the degradant in this example to reach a level of 0.2% of the API) will have a confidence interval (error bar) that reflects the confidence intervals from the measurements. To improve the precision of the shelf-life determination in this case, one needs to

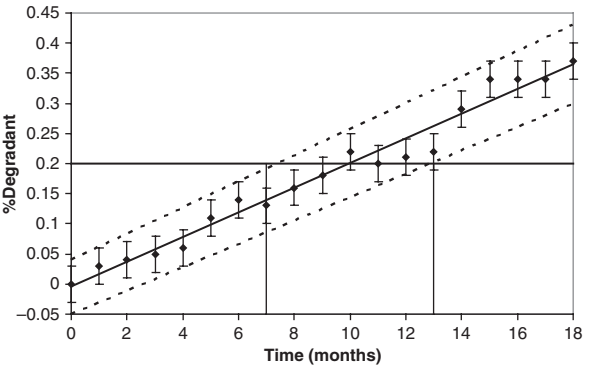

Fig. 6.5 Example of how error bars in real-time stability measurements translate to error bars in shelf-life values. In this example, the 0.2% degradant level used for the limitation of shelf-life is passed at 10 months, but with the error bars, this is really a range of 7–13 months

improve the precision of the actual measurements. This can be accomplished either by intrinsically improving the measurement technique (including sample preparation) or by increasing the sample size. A cautionary note, however: While precision of measurements has improved over the years, regulatory limits have been tightened correspondingly.

The precision of stability measurements is also affected by whether one uses degradation product formation or API loss to set the shelf-life. With the former, a small absolute change can result in a significant relative change. For example, when a degradant level rises from 0.05 to 0.50%, the absolute change is 0.45%, while the relative change is 1000%. With loss of API used to set shelf-life, the same absolute change in API level (i.e., 100.0–99.5%) results in a much smaller relative change of only 0.5%. The consequence is that the precision is generally higher for shelf-lives set by degradation product formation rather than loss of API.

6.5.2 Precision with Accelerated Aging

The imprecision for the rate constant at each temperature and relative humidity affects the precision for the fit of these parameters to the humidity-corrected Arrhenius equation (Equation (6.12)). In addition, since the shelf-life is generally extrapolated on the temperature axis (though often interpolated on the humidity axis), there will be greater imprecision in the predictions depending on how far one must extrapolate. In other words, there are intrinsically larger error bars associated with taking higher temperatures to predict low temperature effects than if the extrapolated temperature is low. It is important to understand that this does not reflect an issue with accuracy (i.e., the model can be exactly correct), yet statistically as one extrapolates to a greater distance, there will be a greater divergence of the lower and upper limits of the confidence intervals. Propagating errors through the logarithmic function of Equation (6.12) is difficult mathematically; consequently, a simulation approach can be used. This approach, known as the Monte-Carlo method, takes the distribution of possible rate constants at each temperature and relative humidity and does a multi-variable least squares fit with all combinations of possible points. This ultimately provides a distribution of fits to the data, which in turn leads to a distribution of extrapolated shelf-life values at any storage condition.

6.6 Prediction of Stability in Packaged Product

Drug product stability ultimately must take into account the packaging since this affects the shelf-life of the product as used. Packaging plays several roles in improving or in some cases worsening shelf-life. These effects include (1) altering the movement of volatile/gaseous materials between the inside and outside of the packaging and (2) providing leachable and extractable impurities into a dosage form.

Foremost, packaging slows equilibration of the external humidity with the API product inside the packaging. In the extreme case, with water-impermeable packaging (e.g., glass bottles, foil-foil blisters), the packaging prevents any transfer of moisture. In that case, the ERH inside the packaging will depend on the moisture content of the API product as packaged and the adsorption tendency for that product at a given temperature (the moisture sorption isotherm). With water-permeable packaging (e.g., plastic bottles or blisters), moisture will enter or leave the package with a rate ($k_{moisture}$) dependent on the MVTR (moisture vapor transmission rate) which is a function of the packaging material, the thickness of the package (d), the surface area of the package (SA), and the difference between the relative humidity inside (RH_{int}) and the relative humidity outside (RH_{out}) the packaging as shown in Equation (6.13):

$$k_{moisture} = (MVTR\ SA|RH_{int} - RH_{ext}|)/d \tag{6.13}$$

As the moisture difference between the internal and external environments becomes closer, the moisture transfer rates slow down. As moisture transfers into or out of packaging, the relative humidity inside the packaging will adjust based on the moisture sorption isotherm for the API product and its total mass. As the moisture level inside a package changes, the rate of chemical degradation will change for the API based on Equation (6.12). Because of this, one can sometimes see different chemical stabilities in packaging as a function of the amount of dosage forms (e.g., tablets or capsules) inside the packaging. One way to control the relative humidity inside bottle packaging is to add desiccants (generally silica gel). Desiccants are materials that have high moisture isotherm values such that they maintain a relatively low relative humidity inside bottles.

Oxygen permeability follows similar trends in packaging as for moisture. In this case, most systems are packaged with ambient oxygen levels such that there is no permeation of oxygen unless there is significant oxygen depletion inside the packaging. Similarly to desiccants, oxygen absorbers (typically iron powder) can maintain a low oxygen level in packaging.

Leachable and extractable chemicals in packaging are generally of greater concern for liquid dosage forms than for solids, due to the ability of direct liquid contact to gradually cause chemicals to migrate into the solution. Commonly, chemicals that can transfer to solutions include residual monomers, plasticizers, antioxidants, colorants, rheology modifiers, rubber vulcanizing agents, accelerants, and other additives. These extractables can either themselves be harmful (i.e., have toxic effects), or destabilize an API chemically or physically. These impurities transfer into API solutions with a rate that is generally temperature dependent. Leaching will often follow an exponential dependence with reciprocal temperature (i.e., an Arrhenius relationship), with a discontinuity at any packaging phase transitions (e.g., melt, glass transition). Because of this, accelerated aging studies must use temperatures below such transitions to predict the ambient behavior.

6.7 Concluding Comments

The shelf-life of a pharmaceutical product is set based on the time that there is little likelihood that harm will come to a patient, whether due to toxic degradation products or due to loss of API potency. To set this time, consideration is given to both the chemical degradation processes and any physical changes in the dosage form with time. Using stability studies with accelerating conditions, it is possible to determine the shelf-life of a pharmaceutical product without having to wait for the entire real-time degradation to occur. It is sometimes prudent to monitor in real-time as confirmation of the accelerated stability process; however, with conservative use of statistics, the real time data should allow extension of shelf-life, rather than require shortening this time period after product introduction.

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Chapter 7
Development of Stability Indicating Methods

Anne-Françoise Aubry, Peter Tattersall, and Joan Ruan

Contents

Abstract The evaluation of the chemical stability studies of small molecule pharmaceuticals rely primarily on the availability of a chromatographic or other separation assay capable of separating and quantifying major impurities and degradation products. A staged approach to the development of stability-indicating HPLC methods, consistent with current regulatory guidelines, is outlined. Practical recommendations are provided for developing forced degradation protocols at every stage of drug development and avoiding common pitfalls that may confuse data interpretation. Consideration is given to special cases such as stereoisomeric drugs, polymorphs, and combination drug products.

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7.1 Introduction

The quality of analytical data generated on stability samples is essential to the successful completion of stability studies and to the ability to draw appropriate conclusions regarding the stability of the product under test [1]. Since the purpose of stability studies is to monitor possible changes to a product or material over time and at different storage conditions, it is expected that all analytical methods applied in the study should be stability-indicating and that only those methods that are truly stability-indicating should be used. Using this broad definition, any method from a X-ray powder diffraction (XRPD) method used to monitor changes of crystalline form to a dissolution method used to evaluate changes in the release rate may be considered stability-indicating if it is demonstrated that it can reliably detect a specific physico-chemical change of the product/material in question. However, for traditional pharmaceutical products, it has become commonplace to reserve the term *stability-indicating* to describe the method (generally a chromatographic method) used to detect chemical degradation of a drug substance or drug product. This is also the viewpoint taken in writing this chapter. It must be noted here that this is not the case for biologics. International Conference on Harmonisation (ICH) guideline Q5C [2] clearly states that not just one method is stability-indicating but that stability can only be inferred by a combination of analytical methods looking at the identity, purity, and potency (or biological activity) of the drug.

A major challenge in developing a stability-indicating method (SIM) is the access to suitable *degraded* samples to aid in method development. In an ideal world, these degraded samples would be real-time stability samples that contain all relevant degradants and only those degradants which form under normal storage conditions. Obviously, this is unrealistic for several reasons: development timeline, and how stability is affected by batch characteristics such as process parameters, quality of excipients, and environmental factors such as humidity or temperature. This is why pharmaceutical chemists have to rely on *forced degradation* samples to develop SIMs. The ability of forced degradation studies (also called stress studies) to forecast real-time degradation has been the object of several studies and is discussed in this chapter.

Formal stability assessment of pharmaceuticals is typically done at three distinct times during development and commercialization: during development, to support the safety and efficacy claims of investigational new drugs; at registration, to ascertain the quality and shelf-life of the marketed product and its ingredients; and finally during the commercialization phase, to ensure the quality of the production and to support site or other changes to the product. Stability information on both drug substance and drug products is required as part of the registration dossier and serves to assign/confirm the shelf-life, determine appropriate storage conditions, define supply chain management, and assure that the quality of the product is unchanged from the time of manufacture to the time of administration to the patient. The approach to SIM development described in this chapter is most suitable for reg-

istrational and marketed product stability studies. Like other development activities, analytical development is an ongoing process and it is generally accepted that early chromatographic methods may not be evaluated for their stability-indicating ability. A staged approach to method development, in which subsequent versions of the method build on knowledge developed previously, is recommended. The notion of SIM is also very much linked to method validation and demonstration of the stability-indicating aspect of the impurity method used to support long-term stability studies (registrational stability studies) is a critical part of its validation protocol.

7.2 ICH Guidelines and Other Worldwide Regulatory Guidance/Pharmacopeias for Method Development and Validation

A number of guidelines have been published that address directly or indirectly the need for SIMs and forced degradation studies. Several of these guidelines are discussed here. Table 7.1 summarizes references made in ICH guidelines [2–9], to the notions of SIM or forced degradation. Table 7.2 summarizes references made to SIM and forced degradation in other guidelines or pharmacopeia.

7.3 Forced Degradation Studies

Forced degradation studies typically involve the exposure of representative samples of drug substance or drug product to the relevant stress conditions of light, heat, humidity, acid/base hydrolysis, and oxidation. These experiments play an important role in the drug development process. The results of forced degradation studies can facilitate SIM development, drug formulation design, selection of storage conditions and packaging, better understanding of potential liabilities of the drug molecule chemistry, and solving of stability-related problems [10–13].

Although the FDA guidance [14] and ICH guidelines [2–9], provide useful definitions and general comments about forced degradation studies, their direction concerning the scope, timing, and best practices is very general and lacking in details. A benchmarking study was conducted to survey forced degradation practices at several pharmaceutical companies [15]. The study revealed that most companies perform some type of forced degradation studies, but company practices vary widely in terms of how and when the studies are performed.

This section serves to illustrate the important role of forced degradation studies by describing the general practices used by the industry. The details include a general study protocol, a description of experiments needed for drug substance and drug products, specific test conditions, and a suggested timeline for conducting the studies relative to the stage of drug development.

Table 7.1 Summary of ICH guidelines referencing SIM or forced degradation

Guideline reference	Title	Ref. to SIM or forced degradation
Q1A (R2)	Stability Testing of New Drug Substances and Product	Page 2: *Stress testing* of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and validate the *stability-indicating* power of the analytical procedures used. The nature of the stress testing will depend on the individual drug substance and the type of drug product involved Page 2: Validated *stability-indicating* analytical procedures should be applied Page 7: Analytical procedures should be fully validated and *stability-indicating*
Q1B	Stability Testing: Photostability Testing of New Drug Substances and Products	The *forced degradation studies* should be designed to provide suitable information to develop and validate test methods for the confirmatory studies
Q1E	Evaluation of Stability Data	Page 2: Factors that can cause an apparent lack of mass balance should be considered, including, for example, the mechanisms of degradation and the *stability-indicating* capability and inherent variability of the analytical procedures
Q2 (R1)	Validation of Analytical Procedures: Methodology	Page 7: [specificity] As appropriate, this should include samples stored under relevant *stress conditions*: light, heat, humidity, acid/base hydrolysis and oxidation
Q3A (R2)	Impurities in New Drug Substances	Page 3: The registration application should include documented evidence that the analytical procedures are validated and suitable for the detection and quantification of impurities (see ICH Q2A and Q2B Guidelines for Analytical Validation)

Table 7.1 (continued)

Guideline reference	Title	Ref. to SIM or forced degradation
Q3B (R2)	Impurities in New Drug Products	Page 2: The registration application should include documented evidence that the analytical procedures have been validated and are suitable for the detection and quantitation of degradation products (see ICH Q2A and Q2B guidelines on analytical validation)
Q5C	Stability Testing of Biotechnological/Biological Products	Page 4: On the whole, there is no single *stability-indicating* assay or parameter that profiles the stability characteristics of a biotechnological/biological product. Consequently, the manufacturer should propose a *stability-indicating* profile that provides assurance that changes in the identity, purity, and potency of the product will be detected Page 5: For the purpose of stability testing, tests for purity should focus on methods for determination of degradation products. Page 6: Studies under accelerated conditions may … assist in validation of analytical methods for the stability program, or generate information which may help elucidate the degradation profile of the drug substance or drug product
Q6A	Specifications: New Chemical Drug Substances and Products	Page 7: Assay: a specific, *stability-indicating* procedure should be included to determine the content of the new drug substance/drug product
Q7	Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients	Page 24: The test procedures used in stability testing should be validated and be *stability-indicating*

Table 7.2 Selection of FDA, EMEA guidelines and pharmacopeia chapters referencing SIM or forced degradation

Guideline reference	Title	Ref. to SIM or forced degradation
USP <1086>	Impurities in Official Articles	Where degradation of a preparation over time is an issue, the same analytical methods that are *stability-indicating* are also purity-indicating
USP <11>	USP Reference Standards	To ensure that the Reference Standards maintain the properties determined at the initial evaluation, USP maintains a Continued Suitability for Use Program. Abbreviated protocols use the *stability-indicating methodology* employed in the initial characterization of the material to confirm the consistency of attributes such as appearance, chromatographic purity, or volatiles content
USP <1150>	Pharmaceutical Stability	Stability of manufactured dosage forms must be demonstrated by the manufacturer, using methods adequate for the purpose. Monograph assays may be used for stability testing if they are *stability-indicating* (i.e., if they accurately differentiate between the intact drug molecules and their degradation products)
USP <1191>	Stability Considerations in Dispensing Practice	At appropriate time intervals, samples of the product are assayed for potency by use of a *stability-indicating method*, observed for physical changes, and, where applicable, tested for sterility and or for resistance to microbial growth and for toxicity and bioavailability

Table 7.2 (continued)

Guideline reference	Title	Ref. to SIM or forced degradation
USP <797>	Pharmaceutical Compounding-Sterile Preparations – Storage and Beyond-Use Dating	. . . quantitative *stability-indicating assays,* such as high performance liquid chromatographic (HPLC) assays, would be more appropriate for certain Compounded Sterile Preparations
EMEA – March 2001	Note for Guidance on In-Use Stability Testing of Human Medicinal Products	The analytical procedures used in the [stability] study should be described and fully validated. *Stability-indicating assays* should be employed
EMEA – December 2004	Guideline on the Chemistry of New Active Substances	The [stability] summary should include results, for example, from *forced degradation* studies and stress condition (light stress, higher temperatures, etc)
FDA – Guidance for Industry (draft)	Analytical Procedures and Method Validation	Page 4: *Stability-indicating assay* is a validated quantitative analytical procedure that can detect the changes with time in the pertinent properties of the drug substance and drug product Page 4: Assay analytical procedures for stability studies should be *stability-indicating*, unless scientifically justified Page 11: Degradation information obtained from *stress studies* (e.g., products of acid and base hydrolysis, thermal degradation, photolysis, oxidation) for the drug substance and for the active ingredient in the drug product should be provided to demonstrate the specificity of the assay and analytical procedures for impurities

7.3.1 Experimental Approach to Forced Degradation Studies

7.3.1.1 Timeline for Conducting Studies

Although ICH guidelines make no mention of any regulatory requirement for forced degradation studies at Phase I or Phase II of development, starting forced degradation experiments at early stage is highly encouraged. There are good reasons for initiating forced degradation studies on drug substances at Phase I. The most important reason is to support the development of a preliminary method that would be highly discriminating due to its ability to detect most if not all of the potential degradation products. Such a method would have stability-indicating power and would require only minimal validation at this stage. Another reason is to further understand degradation pathways and mechanisms occurring in the drug substance and drug product. A good understanding of degradation early in development avoids having to change the method in later development stages, should stability issues arise, a change which would likely require extensive bridging studies. This results in a smoother transition between development phases. During the transition to Phase IIB, new or additional forced degradation studies may be necessary, depending on subsequent changes to process or formulation. Finally, when the synthetic process and formulation are locked, just prior to the start of registrational studies of drug substance and drug product, the forced degradation work is repeated as part of registrational analytical method validation. Even though there are good reasons for initiating forced degradation studies early, doing so requires material and time that may not be available in early development, and it is perfectly acceptable from a regulatory point of view to delay these experiments until after the initial clinical assessment. Forced degradation studies on drug substance and drug product should be completed prior to registrational stability studies and it would be useful to have identified major degradants by that time [16, 17]. In summary, the decision to start forced degradation early or late in development is one that should be driven by quality risk assessment and depends, among other factors, on the chemistry of the molecule (presence of labile moieties), the formulation approach (liquid vs. solid), material availability, and portfolio prioritization.

7.3.1.2 Study Protocol

A general protocol for conducting forced degradation studies, shown in Table 7.3, is arranged according to the type of test material (drug substance, solid or liquid drug product) and type of degradation (hydrolysis, oxidation, etc.) It is essentially based upon the protocol described in Available Guidance and Best Practices for Conducting Forced Degradation Studies [11], with a few additions based on other publications [18–20] and the authors' experience.

7.3.1.3 Conditions for Stress Testing

Specific parameters for stress testing of drug substance and drug product are shown in Tables 7.4 and 7.5, respectively, describing the different stress conditions and

Table 7.3 General protocol for stress testing of drug substances and drug products

Stress condition	Drug substance		Drug product	
	As neat solid	As solution or suspension	Solid dosage form[a]	Liquid[b]
Hydrolysis (Acid, Base, and Thermal)		√		√[c]
Oxidative		√		√
Photo-degradation	√	√	√	√
Thermal	√		√	√
Thermal/Humidity	√		√	

[a]For tablets, capsules or powder blend. Stress intact dosage form; do not grind or put into solution.
[b]For oral solutions, oral suspensions, or parenterals.
[c]Not required for buffered formulations.

Table 7.4 Recommended stress conditions for drug substance

Stress type	Conditions	Time
Acid hydrolysis	1 mg/mL in 0.1 N (up to 1.0 N) HCl; RT or higher	1–7 days
Base hydrolysis	1 mg/mL in 0.1 N (up to 1.0 N) NaOH; RT or higher	1–7 days
Thermal hydrolysis (control)	Aqueous Solution; 70°C	1–7 days
Oxidative/solution	O_2 + Initiator (AIBN) in acetonitrile/H_2O, 80/20; 40°C	1–7 days
Oxidative/solution	0.3% (up to 3%) H_2O_2; RT; protected from light	Few hours to 7 days
Thermal[a]	70°C	Up to 2 weeks
Thermal/Humidity[a]	70°C/75% RH	Up to 2 weeks

[a]If the solid drug substance is unstable to thermal stress at high temperature due to melting, decomposition, etc., use a lower temperature with longer stress time.
[b]ICH guideline for appropriate light exposure: Fluorescent=1.2 million lx hours, UV=200 W h/m^2, timing depends on chamber setting.
[c]AIBN has poor solubility in water, typically a 1 mg/mL API solution is prepared in acetonitrile: water (80/20) premixed with 5 mmol of AIBN. However, ACVA is water soluble.

Table 7.5 Recommended stress conditions for drug product

Stress type	Conditions	Time
Thermal	70°C May vary headspace if oxidation is expected	Up to 3 weeks
Thermal/humidity	70°C/75%RH	Up to 3 weeks
Photo-degradation	Fluorescent and UV light (Option 1 or Option 2)	> 2× ICH

Note: As a control, also perform stress testing on placebo to distinguish drug-related degradants from potential non-drug-related degradation products from the excipients or solvents.

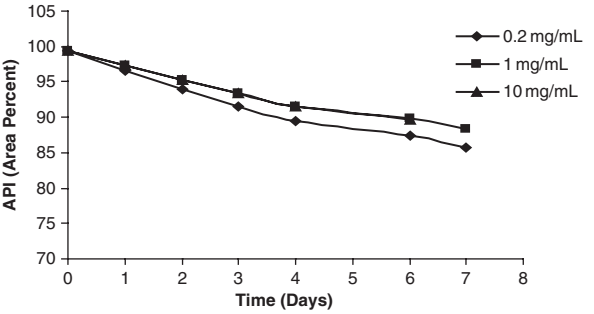

Fig. 7.1 Thermal hydrolysis profile of an API (structure not shown) at 70°C: degradation vs. time at three sample concentrations

range of exposure times. The desired target extent of degradation is approximately 5–20%. This is achieved by varying the stress conditions, for example, exposure time, temperature, or concentration of stressing agent (acid, base, oxidizer, etc.). Overstressing may destroy the compound or may lead to further degradation of the relevant primary degradants. Under-stressing may fail to generate important degradation products. The degradation studies should be terminated after the maximum recommended time/stress conditions, even if sufficient degradation has not been achieved. It is unnecessary and even unwise to try to degrade the drug at all cost as it would only increase the complexity of the method development with little or no benefit in the quality of the data generated by the method.

The concentration of drug in the stressed sample solution may affect the target level of degradation that is ultimately achieved. A more dilute sample concentration generally yields more extensive degradation than does a more concentrated solution, as exemplified in Fig. 7.1 Therefore, lowering the drug concentration may help to increase degradation when necessary. Additional recommendations for preparation of the stressed samples follow.

Acid and Base Hydrolysis of Drug Substance in Solution

Generally, hydrolysis degradation is performed using HCl and NaOH solution as shown in Table 7.4. If the compound is poorly water-soluble, organic co-solvents may be used in combination with acid or base. Organic solvents that have been commonly used for stress-testing studies are discussed in Section 7.3.2.3. Stress is typically first initiated at room temperature; if no degradation occurs, an elevated temperature is applied (50–70°C). A thermal control (i.e., drug in neutral solution at the same stress temperature) should also be run to identify any degradation due to temperature alone. Maximum stress time should not exceed 7 days. The degraded test samples are often neutralized using acid/base/buffer to avoid further decomposition. However, if the degradation is a pH-based equilibrium, this may remove the desired degradants. As always, when conducting stress testing, the analyst should

be wary of possible side reactions that may affect the drug, for example, methanol should be avoided for compounds containing $–CO_2H$, $–CO_2R$, amide groups.

Oxidation

Oxidation may be performed using several conditions [21]. Hydrogen peroxide is the most commonly used oxidant, as shown in Table 7.4. The peroxide concentration may be adjusted as necessary to obtain 5–20% degradation. One disadvantage of using H_2O_2 is that it is non-selective and relatively unpredictable in its results. Stress with hydrogen peroxide often leads to secondary degradation of the primary degradants making results interpretation more difficult. Radical initiators such as AIBN (2,2-azobis isobutyronitrile), ACVA (azobis-cyan valeric acid), and AMPD (azobis methyl propionamidine dihydrochloride) are a better choices for oxidation studies, but are less commonly used. They are generally more selective than peroxides and can be used to confirm or invalidate the peroxide results. An appropriate temperature for the reaction is 40°C. The test may be stopped after 5–20% degradation or after 7 days if no degradation is observed.

Reaction Mechanism/Degradation Pathway

The common reaction mechanisms of chemical degradation of pharmaceutical compounds include hydrolysis, oxidation, isomerization/epimerization, decarboxylation, rearrangement, dimerization/polymerization, photolysis, and reactions with excipients and salt forms. Examples are shown in Table 7.6 Interested readers should consult reference books on drug stability for more information on degradation pathways [13, 22].

Table 7.6 Common degradation routes for functional group

Functional group	Degradation route	Degradants
Acetals	Hydrolysis	Ketones/aldehydes/alcohols
Esters/lactones		Acids/alcohols
Amides/lactams		Amines/acids
Alkenes		Alcohols
2° and 3° Amines	Oxidation	N-oxide, hydroxylamine
Thiols	(radical, light,	Disulfide
Thioethers	metal, peroxide	Sulfoxide, sulfone
Alkenes	mediated)	Epoxide
Allylic Alcohols		α, β, unsaturated ketones
Aldehyde		Acids
Alcohol		Ketones, acids
Oxazoles/imidazoles		Various products
Dienes (able to aromatized)		Aromatic rings
Benzyl/Allylic groups		Benzylic/allylic alcohols

Note: Additional reactions: Rearrangement via hydrolysis, photolysis or intra/inter molecular reaction.

7.3.2 Special Considerations in Conducting Stress Testing

7.3.2.1 Stereochemical Stability

Chemical degradation may affect chiral centers. The impurity/degradant method may or may not be sufficient in assessing stereochemical stability depending on the number of chiral centers [23]. Drugs with one chiral center should be analyzed with a chiral method to assess stereoisomer content. Drugs with two or more chiral centers will most likely convert to diastereomers so achiral analysis should suffice, providing stereoisomers are well separated by achiral HPLC. Complete racemization is very unlikely and can usually be ruled out based on chemistry. Peak purity evaluation using LC-MS and LC-PDA will typically not detect co-eluting stereoisomers, although LC-PDA may detect co-eluting geometric isomers of olefins.

LC-NMR may be used to detect co-eluting diastereomers. Authentic substances of the diastereomers will confirm adequate resolution from the drug peak. Chiral detectors with achiral chromatography can be a useful alternative to chiral separation for monitoring epimerization during stress studies and long-term stability studies. A review article of in-vitro and in-vivo racemization of optically active drugs draws attention to the importance of conducting racemization studies during development of new pharmaceuticals [24].

7.3.2.2 Polymorphism

The physical form of the API can affect both its physical and chemical stability. Physical stability is outside of the scope of this chapter but the potential for chemical stability differences between polymorphs suggests that forced degradation studies (only those that are performed in solid state) should be repeated when a new polymorphic form is advanced at any time during development. Surprisingly, this is not directly addressed in regulations but only touched upon in the FDA guidance for industry on ANDA [25]. The concern is less on the API stability than on the drug product's and the document suggests conducting experiments to understand *the potential effect that a polymorphic form can have on drug product stability.* On a practical point of view, solvates and hydrates present a particular challenge in terms of conducting forced degradation and should be stressed in closed and open containers as different rates of hydrolysis may be observed.

7.3.2.3 Low Solubility Drugs

For drugs that have poor water solubility, stress studies can be conducted either in suspension or in solutions using organic co-solvents.

Co-solvent selection:

- DMSO, acetic acid, and propionic acid are useful for acidic conditions
- DMSO, N-methylpyrrolidone (NMP), and acetonitrile (ACN) work under neutral conditions
- Glyme and 1,4-dioxane facilitate reactions in base
- ACN is the co-solvent of choice for photochemical reaction
- Avoid methanol for $^{-}CO_2H$, amide, ^{-}OH, $ArNH_2$

7.3.2.4 Combination Drugs

Drug products that contain more than one active ingredient should be submitted to stress testing and assessed for degradation products produced by drug–drug and drug–excipient interactions. Degradants of each of the active ingredients are typically well characterized by the time the development of a combination product starts and forced degradation of each API may not need to be repeated. In reality, the compatibility of the two drugs is not always addressed in the published literature on combination products [26, 27]. In one example (atorvastatin and amlodipine combination tablet), the tablet itself, in addition to each drug separately, was submitted to forced degradation [28], hence evaluating additional degradation that may be caused by reactions between the two actives and/or their synthetic impurities.

7.3.2.5 Characterization of Degradants

Primary degradation pathways need to be established as part of the full characterization of new drug substances. In practice, primary degradants obtained in stress conditions are often identified. A decision to isolate and/or characterize a degradation product should be based primarily on results obtained from formal stability studies of the drug substance and drug product whenever possible. Only peaks that occur at or above the ICH identification thresholds from formal stability studies of the drug substance and drug product need to be identified.

7.4 Stability Indicating HPLC Method Development

As discussed in the introduction, the accepted definition of a SIM for a traditional (small molecules) pharmaceutical is a chromatographic (or other separation) method, able to separate the reportable degradants generated upon long-term storage of the product. Traditionally, the stability-indicating quality of the method is demonstrated by using stressed samples or long-term stability samples. If a single method is to be used for quality control and stability of an API, the method should also be able to separate process-related impurities. Stress testing is not the only avenue available for evaluating the validity of the method for stability determination. When available, naturally aged samples or other degraded samples may be more representative of the product's degradation [29].

A literature search for *stability-indicating methods* will bring hundreds of hits, mostly chromatographic methods for the analysis of a specific drug or drug product. An in-depth analysis of these publications, published as a critical review in 2002 [30], demonstrated that the claim of stability-indicating ability was not always well founded and that the approach for method development varied immensely. The authors proposed a five-step approach to developing a SIM that will satisfy the regulations [8, 9]. In their analysis, many of the published methods that claimed to be *stability-indicating* fell short of meeting the current regulations by conducting no stress testing or stress testing at only a few of the recommended conditions. Methods

published in recent years, however, seem to mostly follow the ICH guidelines with a general protocol of stress testing for acid and base hydrolysis, oxidation, and light and heat stress.

7.4.1 Method Scope

As mapped in Fig. 7.2 there are clear steps that are generally accepted [19, 20, 31] in developing a SIM. The first and most important step is to consider what the objective and intended use of the method are. This section deals with the development of SIMs in a systematic manner, by building on knowledge accumulated throughout drug development.

SIMs may be required at different phases of development and the purpose of the method at these different stages is an important consideration. As outlined earlier it is not absolutely necessary, although often it may be beneficial, to conduct an extensive SIM development in the early phase of drug development. As the project develops and the synthesis and degradation pathways become better understood, further method development should be performed. Finally, for formal stability studies a more rugged development should be embarked upon for filing purposes. At early stages, methods need to have a broad gradient because impurities/degradants of the compound may not be known and may alter with changes in synthetic route, form or dosage formulation. IND stability studies serve an important role in development data gathering as well as supporting clinical evaluation. Method improvement

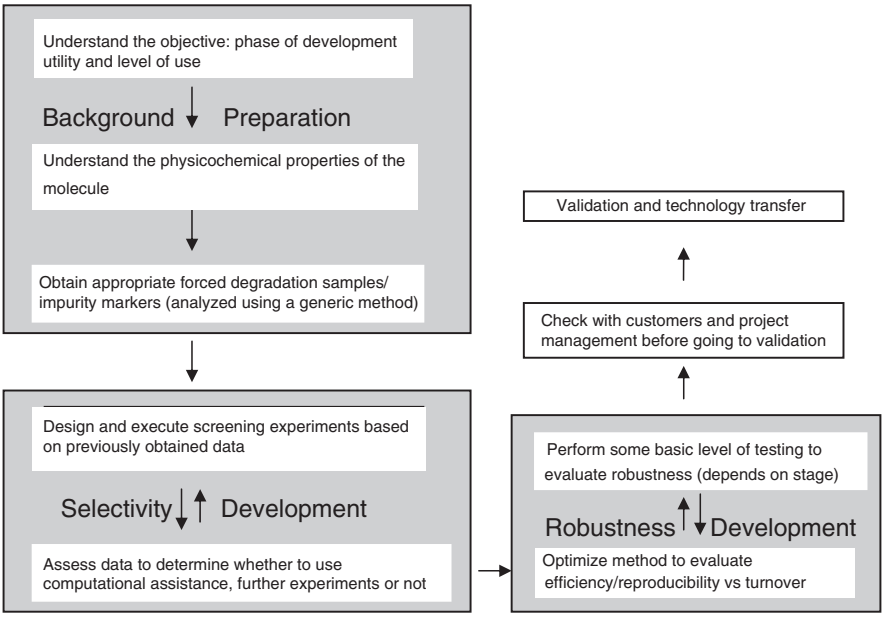

Fig. 7.2 Stability-indicating method development process

(selectivity tuning) is expected as the chemistry evolves and formulation is initiated. At later stages in development the formal long-term stability studies are designed to be confirmatory in nature with the researcher already having a good understanding of the impurities/degradants expected to form at significant amounts. Also, practical experience with the method, along with further method development enables a more subjective insight into the known impurity/degradant profile.

7.4.2 Preliminary Requirements

7.4.2.1 Samples Required for Method Development

Representative samples of the synthetic process with enriched impurities (e.g., mother liquors or reaction mixtures) and individual intermediates, if available, are required to start the development of a selective method. If these impurities can be obtained individually through isolation or suitably characterized from the solution mixture they can serve as markers for positive identification. Other samples from crude batches that have not yet undergone final crystallization, or any other batch containing a large number of process-related impurities, are also useful in testing out the method. A cocktail of impurities and a cocktail of the key degradants will enable the start of method development.

7.4.2.2 Physico-chemical Properties of the Drugs

Information on the compound and the (potential) formulations is essential in helping to frame the development of the method, primarily to determine whether HPLC/UV is appropriate (this chapter assumes that to be the case), to select the diluent and the chromatographic mode. There may be significant background information already available from previous discovery/development scientist reports or experiences. It is highly advisable to consult these people and the literature. In some cases degradation studies have been already undertaken, albeit with different objectives (such as pre-nomination compound screenings or exploratory development work) but they may be useful in selecting the conditions of the stress studies or possibly in proposing degradation mechanisms.

7.4.2.3 Functional Group Effects

Some level of structural understanding of the compound, especially functional groups present that may undergo chemical transformation, is important. Table 7.6 (shown in Section 7.3.1.3) summarizes degradation reaction expected for a series of common groups.

Other valuable information includes the pK_a, pH solubility curve, solubility in common solvents, and log P. They give a valuable insight in solubility and likely structural arrangements in solution. They also guide the selection of chromatographic conditions, including pH of the mobile phase and choice of organic modifiers. Having the pH of a buffered mobile phase >1.5 units away from its pK_a (to

avoid mixed ionization state) is generally accepted, even though the better column performance/selectivity may be at a pH closer to the pK_a. Also due consideration of the pK_a of likely impurities/degradants must be taken.

7.4.2.4 Related Structures

Even for new drug entities, a lot of understanding in this area can be gained from browsing the literature looking at similar compounds which may or may not have been used in drug development [32, 33]. A good example of this is the 3-hydroxy-3-methyl-glutaryl (HMG) compounds that have a common side-group that undergoes similar chemistry with lactonization and oxidation, as illustrated in the paper by Pasha et al. [34]. Small changes in chemical structures, whether backbones or functional groups, can have a profound effect on the reactivity so any information on related compounds should always be used as a guide only and not as extrapolation.

7.4.3 Method Development Approach

7.4.3.1 Stability-Indicating Chromatography Conditions

Principles of chromatography method development, including wavelength, diluent, column and mobile phase selection, have been discussed in a number of chromatography books. An effective solvent and column screen, with relevant samples, cannot be overstated as a valuable foundation for method development [35–37]. In selecting initial chromatographic conditions for a SIM of a new entity, most important is to make sure that degradants are in solution, separated, and detected. To this effect, a diluent of 1:1 water:organic solvent is a good starting point as it will increase the likelihood of solubility of most related materials and ensure proper disintegration of solid dosage forms.

When choosing conditions for method evaluation, broad gradients are appropriate as they maximize the separation of early eluting peaks and increase the opportunity of detecting late eluting peaks. Mass Spectrometry/Evaporative Light Scattering Detector/Charged Aerosol Detector (MS/ELSD/CAD) compatible conditions are beneficial, as they assist in developmental understanding especially in early development.

Most pharmaceuticals have a usable chromophore, allowing for UV detection. UV spectra may be different between the API and the impurities/degradants. Consideration of likely impurities and degradants as to whether they have a chromophore is important for both mass balance reasons as well as experimental setup (choice of detector(s)). At the column scouting phase, the use of a photo-diode array (PDA) detector will increase the likelihood of detecting degradants with different UV spectrum to that of the API. Alternatively, a wavelength in the lower UV range: 210–254 nm may be appropriate. The method sensitivity to impurities compared to the main peak is important to understand when choosing the wavelength. A signal to

noise of 10–1 for limit of quantitation (LOQ) and 3–1 for limit of detection (LOD) are expected with a typical LOQ being 0.05%, although this may vary depending on the known relative response factor (RRF) of the impurities. This can usually be achieved by appropriate adjustment of wavelength, detector settings, sample concentration, and injection volume.

Final selection of a specific UV wavelength is crucial for detection of all relevant degradants. If the λ_{max} of the parent compound is relatively high (e.g., above 280 nm), it should not automatically be selected as the UV detection wavelength, since impurities/degradants may have a significantly different λ_{max} [38]. Alternatively, a dual wavelength detector can be used at both a high λ_{max} and a lower wavelength. At a later stage in development when most or all of the degradants' and impurities' UV spectra are known, any specific wavelength may be justified.

7.4.3.2 Peak Purity

Peak purity (or peak homogeneity) analysis of the main peak, to assess for the presence of impurities under the main peak, is an essential part of the validation of a SIM. Determination of peak purity is more difficult than it seems as one can never be certain that a peak is truly pure. Confidence can be improved by the use of multiple approaches for either direct or indirect evaluation of peak purity.

Direct evaluation can be performed in-line by employing PDA detection [39], LC-MS [40], or LC-NMR. However, PDA only works well for degradants that have a different UV spectrum from that of the drug. LC-MS evaluation will not work if the degradant has the same molecular weight, as is the case for diastereomers, or if the ionization of the degradant is suppressed by the co-eluting API.

Indirect evaluation of peak purity can be accomplished by changing one or more chromatographic parameters (column, mobile phase, gradient composition, etc.) that will significantly impact the separation selectivity. The resulting impurity profile is then compared against that of the original method. If the number of degradant peaks is the same in both separations, and if the area percent of the main component is the same in both separations, then there can be reasonable confidence that all the degradants have been resolved from the main component. Automated versions of this approach have been successfully utilized in a multi-dimensional screening with instrumentation capable of systematically evaluating several different columns and eluents for impurity analysis [19, 23, 41]. Other approaches use alternate separation techniques such as thin-layer chromatography (TLC), normal-phase-HPLC, capillary electrophoresis (CE), or supercritical fluid chromatography (SFC), with similar goals as explained in general terms by Lee Polite in a chapter on liquid chromatography [42].

7.4.4 Method Optimization

Once a method is considered appropriate, the chromatographic conditions and runtime efficiency may be further improved upon by using predictive software.

Figure 7.3 shows an example of the optimization of the resolution of a separation. The resolution map is a graphical representation of how the resolution is affected by temperature and gradient composition. In the example, the optimum is situated on a relative plateau (symbolized by the triangular shaded area in the center), indicating that the separation will be unaffected by small changes in chromatographic conditions. This approach has the advantage of predicting the *robustness zone* for the chromatographic parameters and is consistent with the Quality by Design (QbD)

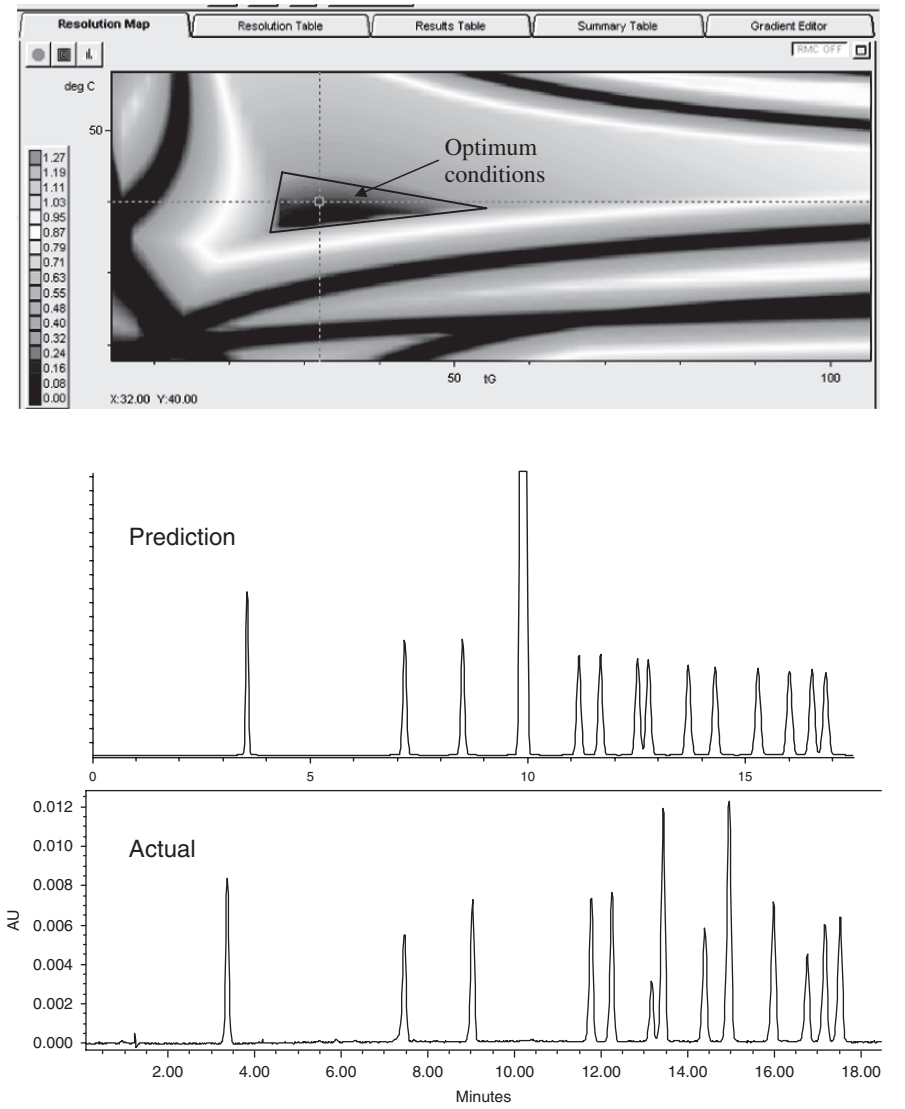

Fig. 7.3 Resolution map as a function of temperature and gradient for a critical pair of impurities in a chromatographic separation. Predicted and actual chromatographic separations of an API and 13 impurities/degradants (API peak absent in bottom chromatogram)

approach to pharmaceutical development. At that stage, if all degradants that need to be monitored have similar polarities, it may be advantageous to evaluate whether an isocratic method would be suitable, instead of a longer gradient method. A successful implementation of a stability-indicating isocratic separation was described for medroxyprogesterone API and injectable formulations [43]. The development of a single gradient method for dexomethazone, its impurities and degradants, and several preservatives in API and multiple formulations is another good example of method optimization for a multi-purpose assay [44]. Revisiting aspects of a method such as solvent selection, extraction, and overall preparation at that point may be beneficial to ensure that nothing was missed and to demonstrate the method robustness. At all stages of development, significant formulation or synthesis changes should trigger further stress studies and peak purity assessments.

7.4.5 Other Considerations

The interpretation of stability results must always been done carefully and with a critical eye to avoid misidentifying an artifact of the analytical method for a drug instability.

7.4.5.1 Sample Stability

Chemical stability in solution is of primary importance, not just the stability of the drug itself but also that of impurities and degradants in the prepared sample. The apparent *disappearance* of peaks in an impurity profile should be investigated and can often be traced back to a chemical change, shift in equilibria, or to a precipitation of the compound in question. It is also worth bringing up again the issue of reactions with co-solvents already discussed in Sections 7.3.1.3 and 7.3.2.3.

7.4.5.2 On-Column Degradation/Rearrangement

There have been many published examples of on-column degradation or reversible reactions between two compounds in the mobile phase leading to misrepresentation of the true levels of degradants or impurities [45–47]. Careful investigations must be planned if this either is or may be occurring. In addition to varying chromatographic conditions (diluent and mobile phase pH and composition, temperature), fast separation techniques and 2D chromatography may be employed to investigate these occurrences.

7.4.5.3 Mass Balance

A question often brought up in discussions of forced degradation and SIMs is whether mass balance should be achieved in all cases. Evidence that the total material detected in the stressed samples is equal to the starting input is certainly helpful for demonstrating the stability-indicating ability of the method. Mass balance results of 96–102% were reported for dipyridamole even with significant degradation of up

to 16% [48]. This level of mass balance may not be achievable in all cases, especially for degradation pathways that give multiple primary and secondary degradants. It may be valuable to at least attempt to reconcile the input and output. Mass balance deficits can be investigated in a number of ways.

- Extending the polarity range of the HPLC gradient. Investigating presence of highly retained compounds by using a stronger mobile phase or by using TLC; looking for poorly retained degradants in the void-volume.
- Comparing UV profiles of detected components because imbalance may occur from different UV responses: systematic use of PDA in early method development allows for a check on significant λ_{max} shifts. RRF must be used for accurate quantitative analysis
- Looking for potential undetected peaks (non-chromophoric degradant) by alternative separations and/or detection such as MS [39], infrared spectroscopy [49], refractive index, chemiluminescence nitrogen detector, TLC (with I_2 or acid/charring visualization), CAD, or ELSD
- GC analysis of volatile degradants [50]
- Investigating the presence of oligomers/polymers by size-exclusion chromatography (SEC) [51].

It is however more difficult to define what constitutes good mass balance and what level of mass balance deficit should be of concern. ICH Q1A attempted to define mass balance but this definition was removed in the 2002 revision. For simple degradation pathways with no significant change in UV response, a mass balance of 95% can be expected but for complex degradation profiles, it may be more useful to focus on assay specificity than on reconciling mass balance. A review published in 2005 provides a more detailed discussion of mass balance in forced degradation studies [52]. This topic is also addressed in a review by Bashki et al., from 2002 [30].

7.4.6 Method Development Report

The value of a method development report (in addition to the validation report) cannot be stressed enough. Such a report can allow future users of the method to efficiently review the work that was done and serve as a starting point for future development (for example, life-cycle development) or redevelopment. In addition, if a related compound comes into development, the method development report can provide useful information that may assist in method development for the new project.

7.5 Conclusion

The staged approach described in this chapter for both forced degradation and method development takes advantage of the knowledge built during drug development to continuously improve the analytical assay of impurities and degradants. The

proposed protocol for forced degradation is not intended to be followed blindly. On the contrary, forced degradation needs to be undertaken with full knowledge of the chemistry of the compound and the results critically evaluated at every step so that the resulting SIM is truly fit for the purpose of monitoring shelf-life stability of the product or material. A complete forced degradation study must be conducted at least once on the final API and formulation to satisfy the regulatory requirements. The risk of a new degradant appearing in real time can be mitigated with comprehensive method development using samples from different sources, stressed and unstressed, judicious application of analytical detection modes, and prudent interpretation of degradation reactions and mass balance information.

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Chapter 8
Method Validation and Transfer

Frank J. Diana

Contents

Abstract Analytical methods used in testing pharmaceutical stability samples need to be validated to the current standards. Often the same methods are employed for release and stability testing which facilitates method validation and allows for the use of initial release data for time zero stability as long as samples are packaged and placed on stability in a reasonably short period of time (typically 30 days). Transfer of these methods between laboratories is also facilitated by the development and validation of the same methods for release and stability testing. Stability testing

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will typically include appearance and a test for assay and degradation products for all dosage forms. The assay method is typically proven to be stability indicating and specific, meaning that all degradation products and synthetic impurities, known or unknown, as well as inactive components are separated from the active ingredient thereby allowing for the accurate measurement of the strength/potency of the dosage form. Similarly, to accurately measure a degradation product level in finished product, all other degradation products and synthetic impurities should be resolved from the peak of interest. Additional tests are typically performed depending on the dosage form, for example dissolution or drug release for solid dosage forms, pH, preservatives and anti-oxidant content for liquid, topical or parenteral dosage forms.

Method validation is covered in the current Good Manufacturing Practices (cGMPs) under section 211.165(e) which indicates that "The accuracy, sensitivity, specificity, and reproducibility of tests methods . . .shall be established and documented". Such validation and documentation may be accomplished in accordance with 211.194(a) (2) which includes the need to "indicate the location of data that establish that the methods used in the testing of the sample meet proper standards of accuracy and reliability as applied to the product tested". Methods included in recognized standard references such as the current USP/NF are understood to be validated. "The suitability of all testing methods used shall be verified under actual conditions of use" (211.194). For new products for which methods are developed, analytical method validation as described in this chapter will be necessary; for methods already included in the USP, method verification will suffice.

8.1 Analytical Method Validation

During method development of an analytical procedure, aspects of method validation need to be considered. For example, specificity of the method through forced degradation studies will be formally completed as part of method validation; however, knowledge of the impurity and degradation profiles of the drug is essential to the development of a good analytical method. The development of a method that was later found not to be stability-indicating would necessitate re-development of the method and any validation which was already completed would likely have to be re-performed and previous data evaluated for acceptability.

There are several resources [1 – 6] available to aid the analytical scientist in performing method validation once a method has been developed. The International Conference on Harmonisation (ICH) guideline on Validation of Analytical Procedures: Text and Methodology was recently updated to combine Q2A and Q2B in one document, Q2(R1) [1]. This guideline provides a defined approach to method validation and offers definitions on the validation elements and recommended data that should be included in the final report.

USP <1225> [3] is another useful source for method validation information. This chapter is specific to compendial procedures but its use for all types of methods

is feasible. Definitions and approaches for validating each element are provided in addition to a table which separates methods into four categories based on their use. For example, Category I covers quantitation of active ingredients in dosage forms and indicates that accuracy, precision, specificity, linearity, and range are required for method validation, while limit of detection and quantitation are not necessary.

After the method(s) are developed and prior to method validation, a validation protocol will be written and approved by the appropriate functional management and perhaps by QA. The protocol will describe the objective and the experimental plan and include acceptance criteria. It will include the number of samples to be tested as well as the number of analysts. The protocol may also reference a method validation SOP for some of the information, for example acceptance criteria, number of samples/analysts, and which validation elements need to be performed for a specific method.

The analytical methods and the validation of these methods will be included in the Chemistry, Manufacturing and Controls (CMC) section of the New Drug Application (NDA) or Common Technical Document (CTD) which is submitted to FDA and other regulatory authorities to initiate the review process for a new drug. FDA will utilize its methods validation program as part of the review process and methods submitted will be validated/verified by an FDA laboratory to ensure their ruggedness and reproducibility [7]. The development and validation of robust methods enables laboratories to generate reliable analytical data that assures the purity, identity, quality, and strength of the drug and facilitates the regulatory review process.

8.2 Validation Parameters

For validating analytical methods for testing stability samples, the following elements need to be considered: accuracy, precision, linearity, range, specificity, robustness, and detection and quantitation limits. Each of these terms is defined and discussed below.

8.2.1 Accuracy

A simple definition of accuracy is *a measure of how close the experimental value is to the true value.* If a pharmaceutical product containing 50 mg of API was analyzed, an accurate method would yield results which would average close to 50 mg. Validation of this element is typically combined with precision (see Section 8.2.2) by performing recovery studies. The placebo matrix is fortified with a known quantity of the analyte at levels consistent with the intended range of the method, for example, for assay 80–120%, for impurity analysis, 0.1 (or limit of quantitation) to 120% of the specification limit. For the impurity method, the API can be added at label claim to represent an actual sample; however, if any of the impurity

Table 8.1 Recovery study for XYZ Tablets showing accuracy and precision of the analytical method for assay

	% Recovery of ABC API in XYZ Tablets		
Sample No.	70% of Nominal sample concentration	100% of Nominal sample concentration	130% of Nominal sample concentration
1	100.3	100.6	99.4
2	99.4	100.8	99.5
3	99.4	99.4	99.7
Mean	99.7	100.3	99.5
%RSD	0.5	0.8	0.2
Overall mean		99.8	
Pooled %RSD		0.6	

is present in the API then this amount will need to be taken into account when measuring the recovery (by subtracting the area of the peak found in the unfortified sample). Fortification of the placebo is done in triplicate at 3 levels, such as 80, 100, and 120%, yielding 9 total determinations per analyst. If possible, a second analyst should perform the spiking studies to show the method can be successfully performed by multiple analysts. If the content uniformity method is the same as the assay method, the range can be widened to 70–130% to cover the allowable results for this test (75–125% of label).

Based on the known amount of analyte spiked into the sample, % recovery is calculated and compared to pre-set acceptance criteria. The acceptance criteria will depend on the ruggedness of the method, for a typical small molecule HPLC assay method; it is usually set at a mean of 98–102% or 97–103% of theoretical, with individual values allowed to be a bit wider. An example of results obtained from a recovery study for XYZ Tablets by one analyst is presented in Table 8.1. For a large molecule assay method, the acceptance criteria would be set wider consistent with the difficulty of the method. Acceptance criteria for impurity methods typically widen as the concentration decreases, thus a 0.1% level, ± 20% (80–120% recovery) may be set where as at a 1% level the criteria may be set at ± 5% for the mean.

8.2.2 Precision

Precision is defined as “the measure of how close the data values are to each other for a number of measurements under the same analytical conditions”. Taken together with accuracy, precision indicates how close an analyst will be to 50 mg (see above) on repeated measurements. Precision includes three sub-sections: repeatability, intermediate precision, and reproducibility.

Repeatability of the analysis is typically performed in combination with accuracy studies using one analyst, instrument, day with multiple measurements. The precision of the method is expressed as %RSD and the results at the upper and lower end of the range of the method should be comparable. It is fairly standard to expect a

Table 8.2 Recovery study for XYZ Tablets showing accuracy and precision of the analytical method for degradation product A (spiked recovery)

% Recovery of degradation product A in XYZ Tablets			
Sample No.	0.1%	0.25%	0.5%
1	94.2	93.8	95.0
2	94.8	92.1	100.6
3	102.1	94.3	96.5
Mean	97.0	93.4	97.4
%RSD	4.4	1.2	2.9
Overall mean		95.9	
Pooled %RSD		3.3	

%RSD of 2% or less for a HPLC assay method for a small molecule drug product or API. For impurity measurements, the %RSD will increase as the spiked level decreases. Typical acceptance criteria at 0.1% levels are 10–25% RSDs, whereas at a 1% level, %RSD criteria are set at 3–5%. An example of accuracy and precision results obtained from a recovery study for Degradation Product A from XYZ Tablets by one analyst is presented in Table 8.2. Another method for measuring repeatability is to analyze a homogenous sample multiple times, for example 6 × samples at 100% of test concentration and then determine the %RSD.

Intermediate precision is determined using different analysts on different days using different equipment, and different standard and sample solutions. By doing this, method ruggedness can be established since multiple analysts and instruments are involved. For HPLC methods, different column batch numbers can also be included, as well as different brands of equipment to broaden the method boundaries. Intermediate precision is typically performed by the lab that developed the method (e.g., Analytical R&D group) although it can be particularly illuminating with regard to method ruggedness to include an analyst who has little experience with the specific method. Based on data gathered during this stage of validation, the system suitability criteria for injection repeatability can be set. USP convention is typically applied here: If the %RSD is 2.0% or less, 5 standard injections are used to establish system suitability for a HPLC run whereas if the %RSD is greater than 2%, 6 injections are made.

Reproducibility expresses the precision between labs and can be determined as part of inter-laboratory qualification, method transfer or collaborative studies. This parameter can be included as part of the method validation studies, but it is more typically performed as part of the method transfer studies.

8.2.3 Linearity

This validation parameter can be defined as “the ability of an analytical procedure to yield test results which are directly proportional to the concentration of the analyte in the sample”. Linearity is evaluated across the range of the method. Five different concentrations are recommended by the ICH Guidance. For example, for a typical

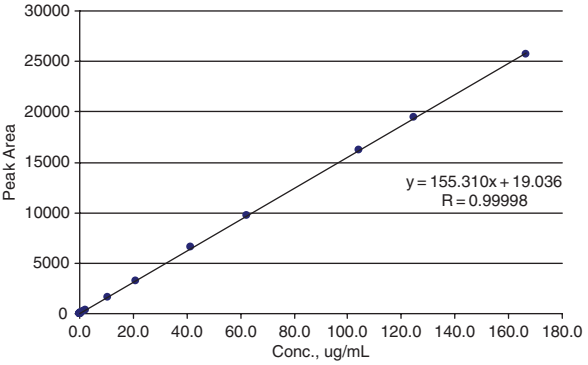

Fig. 8.1 Example of a calibration curve indicating linearity of the analytical method

HPLC assay method, 50, 80, 100, 120, and 150% of target concentration are prepared and analyzed. Typical parameters reported from a linear regression analysis are the correlation coefficient (r) with an acceptance criteria typically greater than 0.99, the y-intercept which should be near zero, and a residual sum of squares. In the case of a method which measures assay and impurities/degradation products simultaneously using a standard at approximately 100% of target, the linearity must be established across the range. In this case, assay is typically quantitated versus the standard, and the related substances are quantitated by area %. In order to correctly measure the related substances at low levels by area %, the linearity must be established within a range covering the quantitation limit (e.g. 0.05%) to 120% of target. In some assay/related substances methods, a second standard (parent compound or related substance standard) is prepared at a concentration consistent with the measurement of low level impurities, for example 0.5%. In this example, linearity is established for the assay (e.g., 50–150% of target) and for determination of related substances (e.g., LOQ to 0.6%). A calibration curve across a wide linearity range is shown in Fig. 8.1.

8.2.4 Range

The range of an analytical procedure takes into account the validation elements described above. It defines the upper and lower concentration bounds for a method for which there is an acceptable level of precision, accuracy, and linearity. For assay methods for a finished dosage form, the range may be described as 80–120% of target, based on recovery studies performed covering this range as well as linearity for an extended range of 50–150% of target, for example. For those finished products which require content uniformity, the range is usually established at a somewhat wider interval, 70–130%. As noted above for combined assay/related substances methods, it is important to establish during validation a range which covers both measurements to assure results are accurate.

Table 8.3 Dissolution requirements, extended release products

Timepoint	Example acceptance criteria	Comments
30 min–2 h	NMT 25%	Immediate release is complete, if applicable
	20–40%	No dose dumping
2–8 h	45–65%	Release profile continues
12–24 h	NLT 80%	Majority of drug dissolved/released
	65–85%	

The range of a dissolution or drug release method needs to be established based on the expected measurements. In the case of an immediate release solid oral dosage form with a Q = 80% acceptance criteria, the range may be fairly narrow, for example 60–100% of target. On the other hand, for a sustained release product, such as an extended release tablet or a transdermal product, the range will need to be evaluated over a wider concentration interval consistent with the acceptance criteria. It is typical for these type products that 3 or 4 time points will be measured and compared to acceptance criteria as listed in Table 8.3. For these products, the range of the method would be established between, for example, 10–110% of target sample concentration.

8.2.5 Specificity

The ICH guidance defines specificity as "The ability to assess unequivocally the analyte in the presence of components which may be expected to be present". For a finished dosage form the components of interest include placebo ingredients such as excipients and preservatives as well as impurities and degradation products. Specificity of a method is evaluated through a series of studies. When the impurity and degradation profile for an API and/or a drug product are well established, spiking each into the sample matrix at appropriate levels and showing adequate separation in representative chromatograms is particularly strong evidence of method specificity. Good recovery results from spiked placebo studies indicating that the matrix is not interfering with extraction or measurement of the active ingredient is important evidence showing method specificity.

Forced degradation studies are performed on new APIs and drug products to provide additional evidence of specificity. The API is typically stressed under heat, light, acid/base, and oxidation conditions. Based on the information obtained in the API studies and the type of drug product, some or all of the forced degradation studies may be repeated on the dosage form. ICH Q1B [8] establishes the requirements for photostability testing for new APIs and drug products. The test article is directly exposed to intense light (1.2 million lux hours) in the first stage of photostability testing. If no adverse effects are observed then the material is unaffected by light. If the material is affected by light then the next stage is to place the material in its immediate container, such as bottle or blister and repeat the test. If there is still an adverse effect then the last stage is to repeat the study in the final package which

would need to protect the material from light, for example aluminum or opaque blisters, opaque bottles, pouches, or cartons. Depending on the results, the product may need to be labeled for light protection. With regard to forced degradation studies, the sample from the first stage of photostability testing will likely be used to establish method specificity.

Heat and humidity are often combined to determine the sensitivity of the API and product, packaged and unpackaged, to these stress conditions. For example studies at 40°C/75% RH and at 50 or 60°C are typically part of a stability program and can also be used to facilitate method specificity studies. Pre-formulation compatibility studies in which the API is combined with various excipients with and without moisture addition and stored at stress conditions may also be helpful in establishing forced degradation conditions for studying method specificity.

The sensitivity of the API to acidic and basic conditions can be studied at room temperature by dissolving the API and allowing the solution to stand for several hours to several days. Depending on the compound, methanol or ethanol may be used to study the stability at either of the pH extremes. If storage at room temperature does not adversely affect the API, then refluxing may be necessary to generate measurable degradation and the establishment of method specificity. Hydrogen peroxide is typically used for oxidation stress studies. For many compounds, this will be a particularly stressful condition and dilution of the hydrogen peroxide and/or short reaction times should be used to control the degradation to reasonable levels.

Each forced degradation solution is appropriately neutralized/diluted and then analyzed by the proposed HPLC method. A loss of the active ingredient measured versus the initial concentration in the range of 3–15% is considered reasonable. A larger decrease, such as 50%, would not be reasonable for drug degradation and may not mimic the real-life degradation profile of the drug as this amount of degradation may include secondary and tertiary pathways that may not realistically occur during drug manufacture/storage. For many drugs, not all stress conditions will cause degradation; however, it is important for establishing method specificity that one or more solutions degrade. Once the analysis is complete, major degradation pathways can be evaluated and major degradation products shown to be resolved from the active ingredient. In some cases, the amount of degradation products formed may approximate the loss in active ingredient from the initial solution. In other cases, the mass balance may not be close to 100%, either because the degradation products formed do not elute at the chromatographic conditions used or do not respond similarly or at all under the detector settings. To determine that a degradation product is not co-eluting with the active ingredient, peak purity tests are run. These typically involve the use of diode array spectroscopy or mass spectrometry to prove that there are no co-eluting compounds; the detection sensitivity should be established. The described studies together establish that the method is specific for the active ingredient.

For stability studies, it is particularly important to understand the potential degradation pathways and degradation products. Product stability is more easily evaluated through an increase in degradation products than through a decrease in assay. For

example, take the case of a tablet which assays at 100, 98, 99, 98, 97, 98, and 96% of label from time zero through the 24-month time point. There appears to be a stability trend – a decrease in assay – but it is difficult to be sure since it could also be normal product variation, for example, content uniformity %RSD was 2.5% at release. On the other hand, if the degradation products have increased from less than 0.5% at time zero to greater than 2% at 24 months than it would establish the stability trend – decreasing assay and increasing degradation products.

8.2.6 Limit of Quantitation and Limit of Detection (LOQ/LOD)

LOQ and LOD are determined for chromatographic methods that measure low concentrations of analyte such as for impurity/degradation product methods, residual solvent methods, and equipment cleaning residue methods. The LOQ is the lowest concentration that can be quantitatively measured with suitable accuracy and precision while the LOD is the lowest concentration that can be detected. At LOD levels the analyte can be determined to be different from the noise level in the baseline but cannot be accurately quantified. The most typical practice for determining the LOQ/LOD is to determine the signal to noise ratio for the peak in question. The noise is measured in an area of the chromatogram that is free of peaks. The analyte concentration that represents a 3 to 1 ratio is the LOD, while a 10 to 1 ratio is the LOQ. These measurements can be affected by several factors including system noise, detector age, and mobile phase components; therefore particularly for method transfers these parameters should be verified for appropriate methods. Another method for determining LOQ/LOD involves analyzing several concentrations of the analyte and determining the minimum concentration that can be quantitated with adequate accuracy and precision (for LOQ) and the concentration which can be consistently observed visually (for LOD). A third acceptable method for determining LOQ/LOD is extrapolation from the calibration curve using the slope of the curve (S) and the standard deviation of the response (σ) based on repeated blank measurements or samples approximating the detection or quantitation limit. The LOD is then calculated from 3.3 σ/S and the LOQ from 10 σ/S.

8.2.7 Robustness

Validation of an analytical method is often done under the best of conditions such as use of a new column on dedicated equipment by an analyst experienced with the method. But what about routine analysis of commercial samples by many analysts in a busy Quality Control laboratory? Robustness establishes the reliability of the method with respect to deliberate variations in the operating parameters, evaluates use of different column lots from the vendor, and also determines the stability of sample and standard solutions. Quality by Design (QbD) principles have begun to impact method development and validation activities to a wider extent and application of QbD concepts should result in higher quality and more robust analytical

Table 8.4 Robustness parameters – HPLC

Mobile phase composition	Vary the amount of organic modifier by +/− 10%
Mobile phase pH and buffer concentration	Vary the pH by +/− 0.2–1 units depending on analyte(s) Vary the buffer (or ion-pairing) concentration by +/− 10%
Column temperature	+/ − 5°C (assuming < 60°C)
Flow rate	+/− 25%
Column	Vendor lot to lot, different suppliers
Injection volume	+/− 25%

Table 8.5 Robustness parameters – GC

Carrier gas	Helium and nitrogen may be used
Carrier gas flow rate	+/− 25%
Oven temperature	+/− 10%
Injection volume	+/− 25%

methods [9]. The design of the method should take into account many factors and robustness studies should assess the effects of variability of chromatographic, sample preparation and method parameters, on the results. Experimental designs can be utilized to study various parameters simultaneously to, for example, establish the factors involved in resolution of a critical pair of peaks.

Some of the HPLC parameters which should be evaluated can be found in Table 8.4 while GC parameters are noted in Table 8.5. Depending on the method being validated, a variety of parameters such as resolution and tailing factor can be measured to determine the suitability of the chromatographic method at the extremes. Other factors can be evaluated during robustness studies including the qualification of an alternate column (e.g., another vendor's C18, different column dimensions or particle size) and use of different vendor equipment (e.g., model or alternate vendor). This evaluation may come in very handy during method transfers (often different equipment vendors are used) and in the situation where a particular brand of column is no longer available and commercial product is awaiting testing.

Sample preparation parameters should also be studied to establish acceptable ranges for the method. Sample solvent and volume, extraction time, filter type, and volume can be studied and modifications made to determine which are critical. For example, sample solvent composition and volume can be modified to assure that the organic solvent level is not on the edge of failure. Similarly the extraction time can be modified over a range (i.e., ± 20%) as can the settings or type of apparatus (e.g., mechanical shakers, sonic baths) used for this sample preparation step. Filter type and the volume discarded before collection of the final sample solution can be evaluated as part of robustness testing and alternate filter types qualified to enhance the method design space.

Solution stability is another important parameter to study as part of robustness. Typically samples and standards (including solutions at the quantitation limit, if appropriate), stock and diluted solutions, are prepared and stored for a period of time and then retested versus freshly prepared standards. One approach is to store the solutions at room temperature and refrigerated conditions and sample them at

Table 8.6 Stability of sample and standard solutions for assay of Tablets XYZ

	Standard solution (% of initial)		Sample solution (% of initial)	
Time (days)	In flask	In vial	In flask	In vial
0	100	100	100	100
1	100.2	99.7	100.1	99.8
3	99.8	99.6	99.9	99.7
5	99.8	99.7	99.6	100.0
7	99.9	99.6	99.7	99.9

24 h (more often within the first day if degradation is anticipated), 3 and 7 days. The % of initial is determined and based on pre-set acceptance criteria the solution stability established (see Table 8.6). Similarly, solution stability for identification and resolution solutions are established based on appropriate acceptance criteria and often storage times for these types of solutions can be extended to weeks or even months (this facilitates efficient use of small amounts of impurity standards). Some solutions need to be stored protected from light or in the refrigerator while others can be allowed to stand on the bench top under laboratory conditions. Sample vials for automated injectors may also need to be evaluated along with the composition of the glass used in the HPLC vials. For some products, solutions will need to be prepared/stored in glassware protected from light. These solutions may then be transferred into amber HPLC vials for analysis. These vials may be treated and contain iron oxide which could react with certain compounds to produce oxidation products; for these compounds an alternate approach to light protection will be necessary [10].

8.2.8 System Suitability Criteria

Once a method is developed and validation complete, the final method can be documented. Based on the experience obtained, critical factors can be defined and system suitability criteria set. Some of the criteria such as injection precision have already been described above. In addition to the precision requirement, acceptance criteria for resolution, peak tailing, and/or theoretical plates will be established. Measurement of these parameters is described in detail in USP <621> [11]. For assay and degradation product methods, resolution for the most difficult to separate pair of peaks should be set. The acceptance criteria should be consistent with the data generated during robustness studies as well as other development and validation studies. Acceptable system suitability results for a chromatographic run establishes that the method is performing adequately and can be used to generate reportable data. Individual methods or general laboratory SOPs should describe how system suitability is applied, for example measure the resolution at the beginning of the run or the beginning and the end, measure the injection precision at the beginning only or throughout the run.

8.3 Re-validation

During product development, analytical methods will be modified frequently with changes to API synthetic routes and product formulations, and due to increased knowledge of the impurity and degradation profiles. Similarly, method transfer to the commercial laboratory may lead to necessary method revisions. Once a product is commercialized and routinely tested in a QC lab, method changes may be brought about in response to troubleshooting, investigations, optimization efforts, newly discovered peaks, column availability/performance issues, etc. or due to changes in API synthesis or product composition. The significance of these method changes must be evaluated to determine if and to what extent re-validation of the method is necessary.

The previous method validation work will be reviewed and those elements which could potentially be affected by the method change re-validated. For example, if the drug substance synthesis is modified (or an alternate supplier with a different synthesis selected for qualification) then elements that would need to be evaluated include specificity, since the impurity profile may have changed along with accuracy and precision. On the other hand, re-validation of the linearity, range, and robustness of the HPLC method may not be necessary. For an analytical procedural change such as a modification of the sample preparation without a change in sample concentration, accuracy and precision testing will be needed; however, linearity/range and robustness would not be needed since there is no change in the chromatographic conditions or sample concentration.

For finished product methods, the addition of a new strength would not require specificity or robustness testing as long as the formulation is the same. If the new strength is one that is bracketed by other strengths and manufactured from a common granulation then additional validation work would not be necessary. In the case in which a new color is used, an evaluation would need to be carried out to assure the dye does not interfere with any of the methods such as by co-elution of peaks, binding with the active ingredient or background absorption for a UV dissolution procedure. If the new strength is outside of the range of strengths validated, then linearity/range, accuracy, and precision may be required. If a product is re-formulated with one or more new excipients, then accuracy, precision, and specificity would be required; but if there was no change in the sample (API) concentration or the chromatographic conditions, then linearity, range, and robustness would be unaffected.

8.4 Method Validation with Stage of Development

An analytical method should be validated according to its intended use. The FDA acknowledges in their Investigational New Drug (IND) guidances [12, 13] “the graded nature of manufacturing and controls information” and that the information needed to assure the proper identification, quality, purity, and strength of the drug substance and product will vary with the stage of development. In early development when little is known about the API and simple dosage forms (e.g., powder in

a bottle) are dosed in the clinic, method validation should be focused on assuring safety and potency of the drug substance/product. The impurity profile should be characterized, although all impurities may not be identified, and stability studies (typically short in duration consistent with the clinical study) should focus on new or increasing degradation products (known or unknown). At this stage, pre-formulation studies will also be in progress and methods to aid selection of formulation ingredients will be important. Limited method validation would be carried out and documented at this stage and internal guidelines instead of formal protocols with set acceptance criteria could be used [14]. FDA's Phase 1 guidance [12] indicates that "Validation data and established specifications ordinarily need not be submitted at the initial stage of drug development".

As a drug proceeds through development, a great deal more information will become available to the analytical chemist. Development/optimization of the API synthesis and the finished product formulation will likely be accompanied by changes in the impurity profile and the analytical methods. At this stage it is important to keep the methods consistent with development to enable appropriate analytical input to facilitate formulation and synthesis design studies. Stability studies will play a key role in determining the final formulation. Once the final formulation and API synthesis are established, the analytical methods can be finalized. Full method validation can then be carried out as described above in preparation for testing of the submission/primary stability batches. Scale-up of the manufacturing process and API synthesis may occur as the organization gears up for validation, regulatory approval, and launch of the product. Method transfers will most likely take place and any significant issues may lead to method revisions and evaluation of the need for re-validation. All of the relevant method information and data generated should be documented in the method validation report/file and provided to the commercial site (see Table 8.7).

Table 8.7 Method development/validation documentation

Analytical methods and specifications	Drug product
	API
	Excipients (if non-compendial) or reference to compendia
	Equipment cleaning samples
	Reference standards
Reports	Validation – API, DP, Cleaning, non-compendial excipients
	Specification justification
	Analytical development/method history
Data	Stability/statistical analysis
	COAs (Ref Stds, Batch release)
	Impurity analysis/identification/synthesis
	Reference standard characterization
Additional documentation	Change control documentation
	Stability protocols
	Critical parameters, design space (may be covered in validation reports)

8.5 Technology Transfer

The transfer of technology from one manufacturing or testing site to another takes place for many different reasons and can occur during drug development or after product launch. In the case of a drug development program within the same company, the product will proceed from discovery through development including clinical studies and eventually to commercialization. In this traditional model, all activities remain within the same company and technology transfer typically occurs between R&D and a manufacturing site at a time in the development process that makes sense, such as before registration or validation batches are manufactured. After commercialization, a transfer may be carried out for many reasons, including positive financial analysis, better utilization of capacity, rationalization of manufacturing sites, mergers, outsourcing initiatives, alternate sites, licensing activities, etc. For those companies that do not have R&D and/or manufacturing facilities, a product may be tested at a contract lab, manufactured at a contract manufacturer and packaged at a contract packager, each of which may involve transfer of technology before product launch or after. This type of transfer involving multiple companies can be particularly challenging owing to the different company cultures and objectives. The technology transfer team needs strong leadership, clear communication lines and agreed upon goals with regard to information sharing and milestones [15]. Global products present additional challenges for technology transfer in that multiple sites are often involved in the manufacture and testing for different regions of the world.

The goals of technology transfer include effective and efficient transfer of knowledge and documentation to the receiving site and ultimately, regulatory approval. To meet these goals, transfer teams are typically formed, timelines generated based on project activities, and responsibilities established. A typical flow chart outlining many of the key steps of technology transfer is provided in Fig. 8.2. This flow chart is an example only; the timing for many of the steps will depend on the phase of product development, for example pre-approval or post-approval, and whether the transfer is intra-company or inter-company. The stability requirements for a product transfer will be one of the key inputs to the project plan and often the last item completed prior to the regulatory submission.

8.6 Analytical Method Transfer

The method transfer process begins with a review of the methods needed for testing at the receiving site. The team will evaluate which methods need to be transferred, the capabilities of and the equipment available at the receiving lab, the samples available for the transfer and several other factors. Typically a technology package will be compiled and provided to the receiving site. This package (see Table 8.7) would include the test methods and specifications, the method validation reports, analytical history and critical parameters, stability protocols and reports, bulk hold reports, Reference standard Certificate of Analysis (COA), and historical data for the finished product and API. Information on the excipients used in the product and

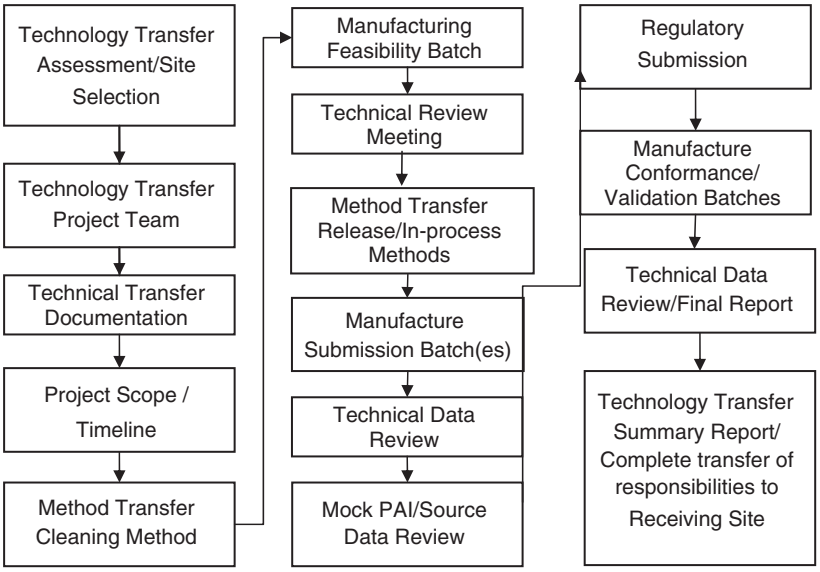

Fig. 8.2 Technology transfer flow chart

the equipment cleaning procedures/methods would also be included. Compendial excipient methods are not typically transferred unless there is something unique (e.g., surface area testing) while similarly API methods that are compendial such as procedures for heavy metals and residue on ignition would not typically be transferred. Even for product testing, a transfer waiver [16] may be granted in some situations where a transfer is not deemed necessary such as the case where the receiving lab is already testing a similar product with the same active ingredient using the same method, or the method differences are judged to be inconsequential.

The receiving site should review the technology package and provide feedback to the originating lab. Such feedback could include questions about the procedures, gaps in the validation versus the receiving lab's SOPs, recommended changes based on routine procedures utilized at the lab, and significant equipment differences. The team will review the receiving site feedback and resolve any significant issues. Once the team reaches agreement on the methods to be transferred, the method transfer protocol is written, reviewed, and approved by both labs. Training at the receiving site may also take place before the method transfer. A face to face meeting at the receiving site is a good opportunity to review any remaining questions and establish contacts in case of issues during the transfer testing.

8.6.1 Method Transfer Protocol

The method transfer protocol is typically written by the originating lab with review and approval by both labs; however, other approaches can be used. In some cases, the receiving lab has their own SOP that they need to follow especially in the

situation where a contract manufacturer/laboratory is involved. In this case since the contractor deals with many customers, they may have their own requirements which are less flexible then the originating lab. In other cases, such as when transferring a method from an API supplier, the manufacturing site laboratory may write a protocol in which the transfer will be based on repeating the method validation or testing several batches and comparing the results generated to the supplier's Certificates of Analysis (COAs).

The method transfer typically has several sections [16 – 18] which are described below.

Objective – indicates what project is involved along with the laboratories that are involved in the method transfer.

Scope – describes what methods will be transferred and those which do not require transfer.

Materials, methods and equipment – lists the batch numbers that will be used, if known, reference standard lot numbers and the method references. Sample age and uniformity is important to the transfer and will be described here. For example, for a product which typically degrades on stability, assure that the protocol takes this into account by having both labs test similarly aged products, for example do not use the release results from the originating lab and compare to 6-month stability samples at the receiving lab. Equipment should be described.

Experimental design – describes the procedure that will be followed including the number of batches, replicates, analysts, instruments, and any additional detail that may not be covered in the method but is critical to the transfer such as sample and standard preparation, number of injections for each as well as how many samples can be injected between standards, dissolution de-aeration procedure, time frame for completing all testing once the samples are received, such as 30 days.

Data and data report forms – the protocol should describe which lab will file the raw data and how the final results will be reported and to whom. Generally, the originating lab receives the results on a data report form which is included with the transfer protocol or the receiving lab agrees to send their Laboratory Information Management System (LIMS) or results report including all needed data.

Data analysis and acceptance criteria – indicates who will perform the analysis and what acceptance criteria have been established. The criteria may be an absolute difference between the labs or statistically derived.

Deviations and investigations – describes how deviations and investigation will be handled, for example, deviations are typically handled according to the lab's SOP (e.g., analyst prepared incorrect mobile phase, equipment malfunction, weighing error, glassware breakage, etc.). Investigations would imply a method problem, such as peaks co-eluting, standard not dissolving or system suitability criteria not met at the receiving lab in which case the transfer team would need to be involved to find a satisfactory resolution.

Review/approval – the protocol should indicate who will review/approve the method transfer report (usually same group that reviews/approves the protocol).

8.6.2 Method Transfer – Experimental Design

Comparative testing and repeating some or all of the method validation parameters are the two practices employed for transferring methods between labs. Comparative testing is frequently used when samples and standards are readily available such as in the traditional model where one lab within a company is transferring the methods to another lab in the same company. Under these circumstances it is typically fairly easy to work out the details with regard to which samples to use, shipment of samples, and availability of reference standards. Also in many cases, both labs share the same document system and perhaps the same LIMS thus facilitating the sharing of information. Comparative testing is also very useful when qualifying methods from suppliers using available COAs for several batches. Typical acceptance criteria for comparative testing are shown in Table 8.8. Validation testing is often used when impurity levels are low in available samples (<0.1%) or when uniform, representative, or stable samples are not available. Recovery studies are performed at the receiving lab using samples that are spiked with impurity standards. Validation testing can also be used in cases where it is difficult to share samples and/or the originating lab or analyst is not available to do the corresponding testing.

8.6.2.1 Assay

Whenever possible it is preferable to use two analysts for method transfer. Each analyst should prepare their own mobile phase, standards, and samples and use a different instrument, if possible. For API and finished product assay, comparative testing is typically done using multiple batches and multiple sample preparations of each batch. All strengths of the product should be covered and bracketing should be used where appropriate. The samples should be chosen carefully to assure they do

Table 8.8 Typical acceptance criteria for method transfers (see text for additional details)

Analytical test	Acceptance criteria
Assay	+/− 2% absolute difference between labs, 2% RSD for each sample/analyst
Impurities/degradation products (low levels, 0.1%)	+/− 40% relative or 0.1% absolute difference between labs
Impurities/degradation products (higher levels, 0.5%)	+/− 25% relative difference between labs, 10% RSD for each sample/analyst
Dissolution (immediate release)	+/− 5% absolute difference between labs at the Q time point
Dissolution/drug release (controlled release, multipoint specification)	5–10% absolute difference between labs at each specified time point

not impact the transfer results, such as samples in which the variability is too great. It is typical to use non-commercial or expired samples if at all possible to avoid complications if Out of Specification (OOS) results are obtained during the method transfer. If the only samples available are marketed product or product being used in the clinic, either release or stability samples, include a statement in the protocol that any OOS results will be investigated with respect to the method transfer; the investigation will be expanded only if the OOS result is determined to be product related. The results for each batch should be calculated for each analyst and compared to the originating lab results or supplier COAs. Acceptance criteria will vary with the method but it is typical for small molecules to set the criteria at 2% absolute difference between the labs. In addition, a requirement for precision can also be set such as 2% RSD for results of a batch per analyst. System suitability requirements also need to be met and should be documented. Another approach to setting acceptance criteria is to perform a statistical analysis of the results between labs. One example is to determine the two one-sided T-test with intersite differences using acceptance criteria of 2% with 95% confidence [17, 18]. Method validation can also be employed especially in cases where appropriate samples are not available; however, this approach is used infrequently since the actual product is not tested and it requires a placebo formulation.

8.6.2.2 Impurities/Degradation Products

For impurities/degradation products either comparative or validation approaches can be used depending on the level of the impurity typically found in actual product or API. For stability testing, the focus should be on degradation products since impurities are controlled in the API at release. If the level of degradation products in typical samples is $>0.1\%$ then comparative testing can be done. The approach is similar to assay described above using multiple samples/measurements, analysts, columns, and instruments. Again, the sample should be chosen carefully so as not to cause problems unrelated to the method transfer. Degradation products that increase with time can be particularly troublesome for a method transfer. For example, the originating lab tests the sample at the 3-month stability time point and the receiving lab, due to other priorities, does not perform the test until 3 months later. To everyone's surprise the receiving lab obtains results that are twice the originating lab's results and fails the transfer protocol. Upon review it is determined that the stability profile for the product indicates that this increase of degradation over time is expected. In some cases the stability profile is well known and this type of problem can be avoided by testing samples within a short timeframe, for example 30 days. Early in development the degradation profile may not be known, so it makes sense to perform the testing at both labs as close to each other as possible. Similarly this type of problem can surface when testing API from a supplier and comparing to their COA. Testing at the supplier may have been done several months before the receiving lab performs the testing. In this case it is important to understand the degradation profile of the API so that problems can be avoided. Acceptance criteria for degradation product testing are based on the level found in the samples. For low degradation

product concentrations, for example 0.1% w/w, a difference of ± 40% between labs or 0.1% absolute between labs is common. At higher levels, such as 0.5% w/w, the acceptance criteria typically narrow to ± 25% or tighter. In addition, precision acceptance criteria are typically set at 25% RSD for the lower level measurements and at 10% RSD as the expected level increases to 0.5–1.0%. A statistical analysis can also be used particularly for higher level results; the two one-sided T-test with inter-site differences as noted above can be used with the appropriate acceptance criteria at the 95% confidence interval.

When samples contain very low levels of degradation products, recovery studies are performed by spiking actual samples with known degradation products at levels consistent with the specification. Acceptance criteria for % recovery and precision should be consistent with the requirements established in the original method validation protocol. Un-spiked samples should also be run to enable correction for any amount present in the actual sample.

Regardless of which procedure is followed the sample chromatograms from the receiving lab should be compared to the chromatograms from the originating lab to assure there are no unexplained or extraneous peaks. In addition the limit of quantitation should be determined by the receiving lab to assure the sensitivity of the method with their equipment. If the method uses response factors for calculation these should also be checked in the new lab to assure there are no significant differences.

8.6.2.3 Dissolution

Method transfer of dissolution or drug release is done by comparison testing of multiple batches that cover the range of product strengths; bracketing should be used as appropriate. For immediate release solid dosage forms, an acceptance criterion of 5% difference between the labs at the Q time point is typical; the dissolution specification should be met in each lab. Including a fast stir after the Q time point to obtain complete release and enable normalization of the results may be important in some cases, especially if tablet to tablet uniformity is fairly large. A couple of items which should not be overlooked during the transfer include de-aeration technique, especially if the product is shown to be sensitive to this parameter during method validation, and type of filter used which should be included in the method based on the validation data. Testing by more than one analyst and using more than one dissolution bath may be appropriate depending on expected method variability.

For modified release dosage forms or transdermal products, a dissolution profile is run during method transfer. Similar to immediate release products, the range of product strengths should be covered. The product specification will include three or more time points and acceptance criteria will need to be incorporated in the protocol for each. The criteria for each time point can be the same as described above (e.g., 5% difference between labs, meet product specifications) although dependent on previous data obtained on release/stability samples the criteria are often widened to 7–10%. It is particularly important for these dosage forms that the uniformity and expected variability in the results are taken into account as well as any special

sampling requirements which may be important for a profile that runs for 12–24 h or even longer. For any dosage form, the protocol should cover how to handle results that do not meet stage/level 1 USP criteria.

Automated methods for dissolution can also be included in method transfer activities although these can be dealt with by qualifying automated equipment/methods afterward using manual methods during the transfer.

Other analytical methods that are typically transferred in the pharmaceutical industry include methods for identification, particle size distribution, and residual solvents and cleaning samples; however, none of these methods are routinely used as part of stability testing, and therefore the reader should consult other references [17, 18].

8.6.3 Method Transfer Report

Once the method transfer testing is complete, the results should be evaluated by the protocol leader and any discrepancies resolved. The report should include tables with the compiled results, specific details with regard to batches tested, equipment used, and any deviations from the protocol. If atypical results were obtained or if any part of the method transfer failed, an investigation should be performed and documented. The investigation should be summarized in the report, any corrective actions described and disposition of the data specified. The report conclusion should summarize the results and indicate whether the transfer was successful. If all results meet acceptance criteria and there are no outstanding deviations/investigations, the receiving site is qualified to perform the referenced testing. The report should be circulated for review, comments resolved, and the report approved by the labs involved in the transfer and quality assurance. Raw data should be available as part of the method transfer files either with the lab that generated the data or in a central file.

Individual protocols and reports can be written for each test to facilitate transfer of specific methods. This approach allows a discrepancy to be investigated while not holding up unaffected method transfer activities. To facilitate internal and regulatory inspections, the method transfer protocols and reports should be compiled for easy review at the receiving site. The raw data should be readily accessible. Transfer of any relevant documentation such as methods, specifications, validation reports, reference standard information should be completed. From this point on, the change control process should assure that methods and specifications remain the same at the qualified testing sites except perhaps for document format.

Method transfer is an important part of technology transfer and should not be treated as a “check the box” activity. An SOP on method transfer should be written and followed for each project transfer. A transfer team should be set up for each project and a process established to assure effective and efficient transfers. Too often, there are problems during the method transfer or shortly thereafter. Table 8.9 lists some problems that can occur, along with comments on how to avoid and/or resolve them.

Table 8.9 Typical method transfer issues

Method not robust	Typically seen when transferring old methods	Originating lab usually knows there are problems; this could be a good time to re-develop and re-validate the method
Method not optimized for routine testing	Can occur with R & D transfer to QC	Include QC in method review and modify based on comments; include QC in method validation (Reproducibility)
Method does not include critical parameters	Originating lab understands what needs to be done based on years of experience	Prior to transfer, review methods with experienced analysts and update the method to include critical information
Samples not uniform or change over time	Can occur more frequently with early development stage transfers where product is not well characterized	Use more uniform product, if possible, or set acceptance criteria based on available data; perform testing within a short period of time or store product to limit change, e.g. refrigerate before testing if will be > 30 days
Different chromatography for gradient methods due to differences in HPLC equipment	Typically caused by low pressure vs. high pressure mixing	Mix organic/aqueous in each MP instead of using organic only and aqueous only; re-set gradient profile based on different equipment and document equivalence; specify equipment that can be used
Different impurity/degradation product results	Investigate product stability issue, if not that then check for potential differences in sample preparation, handling, lab environment, or response factors	Environmental differences which potentially impact sample stability, e.g. refrigerated autosampler used at one lab but not in another; higher temperature or humidity in one lab; differences in light exposure between labs; check for equipment differences yielding different response factors or non-uniform standards
Different dissolution results	Check for differences in de-aeration, filtration, sampling techniques; evaluate product uniformity	Standardize de-aeration technique especially for sensitive products, assure correct type of filter is used and review any differences in time before filtration, automated vs manual sampling can lead to differences as well as location in bath and time needed to take samples; for product with fairly high RSD's may need to normalize results to eliminate variability in amount of active in each tablet
Method validation gaps	Can occur for older methods which were validated according to practices available at the time	Validate method to meet current requirements prior to method transfer or depending on the gaps as part of the method transfer, e.g. impurity recovery/linearity/range since authentic standard now available

8.7 Regulatory Requirements

If only a laboratory transfer is required, the regulations are clear. If the transfer occurs prior to submission of an NDA, such as R&D to QC or R&D to contract lab, include the receiving lab in the filing as one of the commercial labs. The receiving lab would need to be prepared for a potential pre-approval inspection (PAI). If the transfer occurs post-approval, such as QC at one manufacturing site to another or QC to a contract lab, then follow the FDA guidance, Post-Approval Changes – Analytical Testing Lab Site (PAC – ATLS) [19]. This would involve a Changes Being Effected (CBE) filing for the new lab once method transfer is complete. The requirements include use of the same methods as approved in the NDA, satisfactory recent GMP inspection for the new lab, and an indication that the lab is qualified (generally indicated by successful method transfer).

Technology transfers which include manufacturing and testing are covered by several FDA guidances. There are stability requirements for each type of change. If the transfer occurs before NDA filing and the primary stability batches were manufactured at a different facility from the commercial facility, then site-specific requirements will need to be satisfied. These requirements would depend on the amount of primary stability data available, the complexity of the dosage form, and the potential for a site transfer to impact the stability of the finished product. After much discussion, FDA and industry agreed upon two approaches that could be followed to meet site specific requirements. In one approach depending on the factors noted above, no stability data (simple dosage forms) to significant stability data (complex dosage forms) would be required prior to NDA approval. In the second approach, the company would validate the process at the commercial facility and provide certification (prior to approval) to FDA that it was completed along with a commitment to place the batches on stability.

For post-approval changes, the various Scale Up and Post-Approval Changes (SUPAC) guidances [20–22] and the more recent guidance on Changes to an approved NDA or ANDA [23] describe the requirements and should be consulted. Similarly for global products, the type I/II variation requirements should be evaluated. For example, for an immediate release solid dosage form, a level 3 manufacturing site change (new site) would involve the production and packaging of 1–3 batches with 3-month accelerated stability data for each submitted in a CBE-30 supplement. For an extended release oral solid dosage form, a level 3 change would require similar batches and data but a prior approval supplement would be filed.

8.8 Method Transfer Example

To put all the above discussion together, let's work through an example. We will take an immediate release tablet, xyz tablets, which are available in four strengths, 25, 50, 100, and 200 mg per tablet. The analytical methods have been fully validated at the originating lab (Lab A) and now we are going to transfer the methods to another lab (Lab B). A method transfer team is formed and a scope defining the transfer

is written. The timeline is agreed upon. A protocol for each method is drafted and circulated to the team for review. The Assay protocol covers the following points:

- Three lots of expired product will be used, one lot of 25 mg, and two lots of 200 mg tablets (bracketing utilized)
- Samples are pulled from the 25°C/60% chamber and 3 bottles of each lot are provided to both labs
- The stability profile for each batch is reviewed and the results do not indicate any major trends or variability
- The content uniformity method is similar and therefore can be transferred based on the assay data
- Two analysts will perform the transfer testing at each lab in triplicate
- Reference standard source and lot # are provided
- Specific points are defined, for example # of injections per sample/standard vial, # of sample preparations, frequency of standard injections
- Acceptance criteria are defined as the Lab B mean results are within ± 2% of Lab A mean results and %RSD is less than 2.0%; all system suitability must be met
- Forms for reporting results are included
- A face-to-face meeting is held at Lab B and training on the method is provided by Lab A with a focus on sample preparation steps, tablet grinding, filtering, shaking, sonicating, HPLC equipment/columns
- The receiving lab is toured by the transfer team and the HPLC equipment to be used for the transfer reviewed along with any questions from the analysts who will perform the method transfer testing

Once the protocol testing is complete, the result forms are provided to the protocol leader, typically from Lab A. The results from each lab are evaluated and if the acceptance criteria are met the work is summarized in a method transfer report. If there are results which do not meet the acceptance criteria, the transfer team meets to review the situation and determine the next steps. Other tests would be similarly documented; a protocol for a degradation product transfer would include many of the same points as described above for the assay with the following additional points:

- For two known specified degradation products, A and B, the levels are expected to be between 0.1 and 0.3%; therefore, the acceptance criteria are set at ± 0.1% absolute difference between labs with a 25% RSD precision criteria for each analyst per sample (triplicate sample preparations per lot)
- The limit of quantitation will be verified by the receiving lab using a signal to noise (S/N) criteria of 10 (the receiving lab will be instructed to prepare a solution at the LOQ and measure the S/N)
- Chromatograms from each lab will be compared and representative chromatograms included in the transfer report
- Training (and the protocol) will cover specifics around how to identify peaks, for example use of identification solution or authentic standards

- Training will provide an additional opportunity to reinforce critical points, in other words, do not allow the solution to heat up during sonication as this may cause an increase in a specific degradation product; filter the solution immediately after preparation as undissolved dye particles can cause increased degradation
- The protocol clarifies how to report individual peaks less than the reporting limit and how to determine total degradation products, for example, do not include the main API synthesis impurity in the total; sum all peaks greater than the reporting limit

There is no requirement as to whether separate protocols are written for each test or all tests to be transferred are included in the same protocol. One protocol can be effectively used and will likely be more efficient in that many of the general points (e.g., samples to be used, OOS procedures, etc) will not need to be repeated in several protocols and review and approval will be streamlined. However, a difficult issue with one test that needs substantial investigation and problem resolution could hold up approval of the entire method transfer and delay testing by the receiving lab which could negatively impact the overall transfer team's timeline.

8.9 Conclusion

Stability testing is critical to establishment of a finished dosage form's safety and bioavailability. Within the Chemistry, Manufacturing and Controls (CMC) section of an NDA or CTD, the analytical data play a key role in establishing the identity, strength, quality, potency, and safety of the API and the finished dosage form. Analytical methods used in testing for release and stability of an API and finished dosage form need to be validated to assure the data are accurate and reliable since this data will be used to make judgments as to the acceptability of a formulation or a synthetic pathway. These data support the clinical supplies which are used in clinical studies which determine the benefits and risks of a particular drug and ultimately whether or not it will be cleared for approval and launched to the market. Analytical method validation is the foundation on which many decisions are made throughout the development process. Imagine the problems that would be caused if methods were not validated properly, potentially leading to a product not actually containing the expected potency or the purity of an API not being what it was purported to be but instead containing several un-observed impurities.

Transferring validated methods can occur several times in a product lifecycle. Each time there is a chance of losing valuable information if the transfer does not occur according to a sound process. A poor transfer process could mean that the new lab must discover over again what the originating lab knew about the product characteristics and the analytical methods whether it be a particular sensitivity the product has to analytical technique or storage conditions or the identity of a small unknown peak in the related substance's chromatogram.

Both method validation and transfer are important pieces in the drug development puzzle. Without reliable analytical data it is not possible to make informed decisions during product development, and it complicates batch disposition decisions whether for the clinic or the market. Ultimately it is clear that the effort spent on developing and validating robust methods will be time well spent, especially as a product moves through the pipeline toward commercialization. Similarly, the method transfer process should not be seen as a check-the-box activity but rather the transfer of knowledge from a laboratory perspective and an extension of the method development/validation process since the better a method is designed the easier it will likely be for new analysts to perform it well.

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Chapter 9
Overview of USP-NF Requirements for Stability Purposes

Susan Schniepp

Contents

Abstract This chapter discusses how companies can apply compendial procedures to monitor the stability of their product through product expiry. When companies are able to employ compendial procedures for stability monitoring purposes they may save time and money because they will not need to validate new procedures. In addition, the drug approval process may be quickened by referencing methods already approved and familiar to the regulatory authorities.

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9.1 General Introduction to USP

9.1.1 History

The Pharmacopeia of the United States of America – National Formulary (*USP-NF*)[1] is the oldest, continuously revised pharmacopoeia in the world [1]. This government-independent standard setting organization was established in 1820 by physicians whose mission was to produce a manual to be used by pharmacists to help assure the consistency of the product and its name to the patients. While independent of the US government, the USP gained legal recognition in the late 1800s via state laws and permanent national recognition in 1906 with the passage of the Pure Food and Drug Act. The Act states, "the term 'drug,' as used in this Act, shall include all medicines and preparations recognized in the United States Pharmacopoeia. . ." [2].

The original USP monographs were recipes intended to assist pharmacists in formulating medicines. With the emergence of the pharmaceutical industry, monographs morphed into being a collection of specifications and test methods to which products must comply in order to be marketed in the United States. These monographs are intended to ensure the identity, strength, quality, and purity of products through expiration. *The General Notices and Requirements* section states "Every compendial article in commerce shall be so constituted that when examined in accordance with these tests and procedures, it meets all the requirements in the monograph defining it" [3]. The USP also states "Assay and test procedures are provided for determining compliance with the Pharmacopeial standards of identity, strength, quality and purity" [3]. These statements are interpreted as indicating that the requirements set forth in the USP apply throughout a product's shelf-life.

9.1.2 Glossary of Terms

9.1.2.1 General Test Chapter

General Test Chapters provide instructions for performing certain test methodologies that are repeatedly referenced in monographs [4]. Typical types of tests included in the General Test Chapters section of the *USP-NF* include *Residue on Ignition, Loss on Drying,* and *Spectrophotometric Identifications Tests.* General Test Chapters are assigned numbers from 1 to 999.

9.1.2.2 General Information Chapter

General Information Chapters are theoretical and interpretive in nature. They discuss methodology and concepts not referenced by an individual monograph. General Information Chapters are assigned numbers 1000 and higher in the *USP-NF*.

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[1] Other acceptable titles are *United States Pharmacopeia, x Revision*, or *USP x*, x being the current official volume (e.g., 30, 31, etc.).

9.1.2.3 Monograph

Monographs provide specific tests, analytical procedures, and acceptance criteria for determining the strength, quality, purity, and potency for a given compendial article. Additional information such as storage conditions, nomenclature, chemical formulae, and the applicable USP Reference Standards are also included in the monograph.

9.1.3 Standard Development Process for Monographs

The International Conference on Harmonisation (ICH) defines a specification as "A list of tests, references to analytical procedures, and appropriate acceptance criteria which are numerical limits, ranges, or other criteria for the tests described. It establishes the set of criteria to which a drug substance or drug product should conform to be considered acceptable for its intended use. 'Conformance to specifications' means that the drug substance and/or drug product, when tested according to the listed analytical procedures, will meet the listed acceptance criteria" [5, 6].

The USP establishes the specifications and test methods for products marketed in the United States through their monograph adoption process. The process starts when FDA approves a new or generic drug application. After FDA approval, USP sends an official request asking the pharmaceutical company with the approval to assist in setting the public standard for the product by submitting a monograph. The requirements for submitting a monograph are referenced in the letter and set forth the submission criteria by indicating the specific tests, analytical procedures, and acceptance criteria for determining the strength, quality, purity, and potency for the approved active pharmaceutical ingredient and associated dosage forms [7]. The monograph submission includes supporting validation information for the analytical procedures, proper storage conditions for the API and dosage form, nomenclature, chemical formulae, and the appropriate reference standards. The validation information should follow the guidelines set forth in USP General Information Chapters <1225> *Validation of Compendial Procedures* and <1227> *Validation of Microbial Recovery.*

Once the company has compiled the necessary information, they submit the proposed monograph to USP so it may be adopted as an official standard. The appropriate USP expert committee reviews the information, to assess its completeness and scientific merit, and subsequently approves it for publication in Pharmacopeial Forum (PF), USP's bimonthly journal of scientific review and compendial revision. Once a monograph has been published in PF, any interested person, company, or regulatory authority, including USP Expert Committee members, may comment on the contents of the proposal. The USP may chose to accept the comments and revise the original proposal, or reject the comments and proceed to adopt the original submission as initially published.

After the proposal has successfully made it through the public review process, it is adopted as an official standard in USP-NF, in its Supplements, or in an Interim Revision Announcement (IRA). The most common adoption mechanism is

by means of USP-NF and its Supplements. In the rare event that an item needs to be adopted quickly, the IRA may be the chosen adoption vehicle. An example of an item in which adoption in an IRA would be preferred instead of USP-NF or a Supplement would be a situation in which patient safety is at issue; quick adoption of the standard would eliminate any risk to the patient. By keeping pace with the proposals in PF and actively participating in the monograph standards adoption process, industry can be assured that the public standards reflect the current science used to ensure patient health.

9.1.4 Validation Requirements for Monograph Submission

The USP methods contained in the monographs are considered validated. The USP states "...users of analytical methods described in USP-NF are not required to validate the accuracy and reliability of these methods but merely verify their suitability for use. Recognizing the legal status of the USP and NF standards, it is essential, therefore, that proposals of new or revised compendial analytical procedures are supported by sufficient laboratory data to document their validity." The Code of Federal Regulations also recognizes that USP methods are validated. The section on laboratory records [8] states "Laboratory records shall include completed data derived from all tests necessary to assure compliance with established specifications and standards, . . ." and further states "if the method employed is in the current revision of the United States Pharmacopeia [or] National Formulary. . .and the referenced method is not modified, a statement indicating the method and reference will suffice" [8].

The validation requirements for USP monographs are addressed in General Chapters <1225> *Validation of Compendial Procedures* and <1227> *Validation of Microbial Recovery from Pharmacopeial Articles.* Chapter <1225> provides guidance for validating chemical and physical methods intended for submission as an official standard. The test is aligned with ICH documents Q2(R1), Q3A, Q3B, Q3C, Q6A, and Q6B [5–12]. The verification of the compendial procedure should be performed based on General Chapter <1226> *Verification of Compendial Procedures* and FDA guidances on analytical procedures, method validation, and CMC documentation.

9.1.5 Use of Reference Standards in Stability Testing

Many USP methods require the use of USP Reference Standards (RS) to determine the identity, strength, purity, and potency of official articles. USP defines the terms *official substance, official preparation,* and *article* in USP *General Notices and Requirements. Official substances* are the active drug entity, *official preparations* are the drug products, and *article* is an item for which a monograph exists in the USP-NF. Since the USP monographs are applicable through the shelf-life of an article, so is the use of USP Reference Standards. The USP RSs are authentic, highly purified, and characterized substances. These standards are typically employed in

the monograph tests for identification, potency, and impurities regardless of whether the item being tested is the active ingredient or the final product. Not all tests and assay procedures require the use of official RSs. When the use of an RS is required by the monograph procedure, the RS will be designated by *USP xxx RS,* where *xxx* is the name of the particular RS (e.g., *USP Aspirin RS, USP Bisacodyl RS*). The qualification process for USP reference standards is very stringent. The flow diagram below indicates the steps required in order for candidate materials to be certified as official USP RSs (Fig. 9.1).

USP publishes the list of official reference standards in a number of venues. The most accurate information regarding the availability of USP RS can be confirmed on line at USP's website www.usp.org and is updated every 24 hours. Users of USP RSs should note that the storage and handling instructions printed on the RS label take precedence over the storage and handling instructions that might be listed elsewhere on USP's website or in the official USP publications.

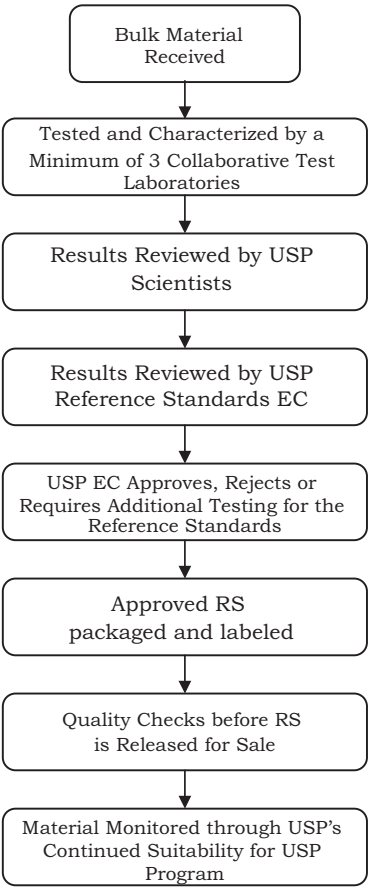

Fig. 9.1 USP reference standard qualification process flow

9.2 General Discussion of Requirements for Stability

9.2.1 Information in General Notices and Requirements

General requirements for stability are discussed in ICH guidelines ICH Q1A(R2), Q1C, Q1E [13–15]. These guidelines define ". . .the stability data package for a new drug substance or drug product that is sufficient for a registration application within the three regions of the EC, Japan, and the United States." Once these pharmaceutical products are approved for market they have the potential to become the subject of a USP monograph.

The issue of the stability of pharmacopeial articles is addressed in various sections of the USP. *The General Notices and Requirements* address the basic concept that the monographs requirements are applicable through the expiration period of an item in commerce. In addition, this section also includes some default requirements for some USP parameters. It is important for users of the pharmacopeia to understand the hierarchy of the information. As a general rule, the information in the monographs takes precedence over the information in the general test chapters which takes precedence over the information in the *General Notices and Requirements.* The General Notices include some general information regarding stability requirements applicable to monographed items. One of these general stability requirements defines what is meant by *Added Substances*. The USP allows for the addition of suitable substances to enhance "stability, usefulness, or elegance. . ." These substances include antimicrobial agents, pharmaceutical bases, carriers, preservatives, and stabilizers to name a few. USP cautions, however, that these substances are ". . .regarded as unsuitable and prohibited unless (a) they are harmless in the amounts used, (b) they do not exceed the minimum quantity required for providing their intended effect, (c) their presence does not impair the bioavailability or the therapeutic efficacy or safety of the official preparation, and (d) they do not interfere with the assays and tests prescribed for determining compliance with the Pharmacopeial standards."

Another general stability requirement addressed in the General Notices is the presence of unlabeled impurities. USP monographs are applicable to all articles regardless of the route of synthesis. Since alternate syntheses yield different impurities the USP needed to have some default conditions for unknown impurities. Since the innovator company typically submits monographs, the impurity tests reflect the impurities identified for their specific synthesis process. Generic versions of these monographed items will generate a different impurity profile because the route of synthesis is presumed to be different from that used by the innovator. To address this potential issue, the USP included default unknown impurity levels in the General Notices. The USP states "The presence of any unlabeled impurity in an official substance is a variance from the standard if the content is 0.1% or greater." and "The sum of all Other Impurities combined with the monograph-detected impurities does not exceed 2.0% (see Ordinary Impurities <466>), unless otherwise stated in the monograph." USP has also published flexible monographs to address the concerns with different impurity profiles arising from different modes of synthesis. Individual

monographs manage the issue of different impurity profiles from different manufacturers through these *flexible monographs*, which incorporate identified impurities and their associated limits from each supplier as permitted by the FDA via their approval of the manufacturer's drug application.

9.2.2 General Information Chapter <1150> Pharmaceutical Stability

USP General Information Chapter <1150> *Pharmaceutical Stability* offers the most definitive stability guidance regarding the use of compendial procedures. It states, "The monograph specifications of identity, strength, quality, and purity apply throughout the shelf life of the product." and "Monograph assays may be used for stability testing if they are stability-indicating (i.e., if they accurately differentiate between the intact drug molecule and their degradation products)." Compendial assay analytical procedures may not be stability indicating, and this should be considered when using the compendial procedures for drug products. The chapter contents include discussion of stability protocols, controlled room temperature, mean kinetic temperature, and world climatic zones. Other General Information Chapters to be considered for stability guidance are <1079> *Good Storage and Shipping Practices,* <1086> *Impurities in Official Articles,* <1118> *Monitoring Devices – Time, Temperature, and Humidity,* <1177> *Good Packaging Practices,* and <1178> *Good Repackaging Practices.* Chapter <1086> *Impurities in Official Articles* is an in-depth look at impurities and degradation products as they apply to drug substances and products including an outline of information required for IND, NDA, and ANDA filings. Pharmacopeial users should review these chapters in their entirety and determine the applicability of the information with respect to their company policies and procedures.

9.2.3 Use of Compendial Procedures for Stability

In addition to General Test Chapters, General Information Chapters, and the monographs, USP also has additional information that might be useful for stability purposes. The *Description and Solubility* information contained in the Reference Tables section of the USP offers information regarding the general characteristics of color, solubility, odor, and compendial use for items used throughout the USP-NF. The introduction to the Description and Solubility Reference Table states "The properties are not in themselves standards or tests for purity even though they may indirectly assist in the preliminary evaluation of the integrity of an article." While not considered official requirements, this section contains valuable information for determining the suitability of many chemicals, reagents, and ingredients used in testing monographed items as well as evaluating the item itself.

Current monographs are expected to contain methods that are stability indicating and that allow for the quantification of impurities and degradation products. It

should be noted that a number of monographs included in the *USP-NF* are in need of revision because they are not up to today's standards. Each user needs to assess the assay procedure in the monograph of interest to assure it is stability indicating. If it is not, a stability-indicating procedure needs to be developed. In addition, some monographs may not include information that would be beneficial to monitor on stability. In order to determine what compendial tests are appropriate for stability, users should review current stability requirements against the appropriate current USP monograph. This review will reveal gaps between regulatory and compendial expectations. Once this is completed, the gaps may be filled, possibly with the use of information from one of the general test chapters in the USP. It is important to realize that stability of a drug substance or drug product goes beyond the production of degradation products and encompasses any functionality that is critical to performance (e.g., dissolution profile, particle size, or other functionally related characteristics important for performance). The attributes to be tested should be those most likely to be affected during the material's expected life and those that have impact on the strength, identity, quality, and purity of the product. The manufacturer needs to demonstrate that its product has maintained the appropriate level of quality through the approved shelf-life in the original container. The selected stability tests to be performed should be based on knowledge of the product and should be capable of determining the physical, chemical, biological, and microbial characteristics of the product, as well as evaluating preservative content, if applicable.

A review of the General Chapter Table of Contents resulted in the development of the following table to assist the reader in determining what tests might be appropriate for stability (Table 9.1). The review focused on parenteral and solid oral products since they are the most prevalent dosage forms addressed in the USP-NF. These General Test Chapters can be verified as suitable for monitoring the strength, identity, quality, and purity of official articles. General Information Chapter <1226> *Verification of Compendial Procedures* should be consulted for information on verification requirements. The table should not be considered all-inclusive. There may be other applicable tests listed in the General Chapters. General Chapter <1> *Injections* should be reviewed for potential requirements. This chapter contains testing information specific to injectable products. There may be test requirements appropriate for stability that are not called out in the specific monograph. An example of this can be found in the monograph for Dextrose Injection, which does not directly reference General Chapter <71> *Sterility.* The sterility test is indirectly referenced through *Other requirements* which refers the user to General Chapter <1> *Injections.* The reference to Chapter <71> is located in Chapter <1>, under the section *Sterility*, where USP states "Preparations for injection meet the requirements under Sterility Test <71>."

Using the table one can determine what compendial tests are suitable for determining the strength, quality, purity, and identity of products on stability. For example, it is important to determine that sterility was maintained throughout the product shelf-life for parenteral products. This can be accomplished by using the General Chapter <71> *Sterility.* Chapter <791> *pH* can be used to determine if there has been chemical degradation of the solution that would cause patient safety concerns.

Table 9.1 Potential compendial tests for stability

Test name	Parenterals	Solid Oral
<1> Injections	X	–
<11> USP reference standards	X	X
<61> Microbiological examination of nonsterile products – microbial enumeration tests	–	X
<62> Microbial examination of nonsterile products – tests for specified microorganisms	–	X
<71> Sterility	X	–
<181> Identification – organic nitrogenous bases	X	X
<191> Identification tests – general	X	X
<197> Spectrophotometric identification tests	X	X
<201> Thin-layer chromatographic identification	X	X
<281> Residue on ignition	–	X
<466> Ordinary impurities	X	X
<621> Chromatography	X	X
<641> Completeness of solution	X	–
<671> Containers – performance testing	X	X
<698> Deliverable volume	X	–
<701> Disintegration	–	X
<711> Dissolution	–	X
<724> Drug release	–	X
<791> pH	X	–

Using Chapter <61> *Microbiological Examination of Nonsterile Products – Microbial Enumeration Tests,* the microbial integrity for solid oral dosage forms can be confirmed. If specific microorganisms are of concern, Chapter <62> *Microbial Examination of Nonsterile Products – Tests for Specified Microorganisms* can also be employed. Chapters <701> *Disintegration,* <711> *Dissolution,* or <724> *Drug Release* can be used to measure that the functionality did not change during storage for a solid oral dosage form product. For both dosage forms, Chapter <671> *Containers – Performance Testing* can be utilized to ensure the integrity of the original container. Additionally, both dosage forms can employ Chapter <466> *Ordinary Impurities* in addition to specific monograph requirements to determine there has been no chemical degradation during storage.

The information and examples presented above demonstrate how compendial procedures and general chapters may be applied for determining the strength, identity, quality, and purity of products on stability. It is up to the users to understand which product attributes are critical for stability and then couple the appropriate compendial test to measure those attributes.

It is important to note that General Information Chapter <1010> *Analytical Data – Interpretation and Treatment* may also be applicable for stability purposes. This chapter provides "...information regarding acceptable practices for the analysis and consistent interpretation of data obtained from chemical and other analyses" and "direction for scientifically acceptable treatment and interpretation of data." This chapter's section on Outlying Results might be helpful in the investigation of unanticipated results obtained during product stability monitoring. When

unexpected or unanticipated results are obtained on stability, it is important to quickly determine if the result is an anomaly or if it reflects the actual condition of the product.

9.2.4 General Case Study: Solid Oral Dosage Form

Solid oral dosage forms must maintain their ability to deliver the therapeutic dose through out its shelf-life. The patient should have confidence that the product delivers the needed dose in the specified time through expiration. The determination of bioavailability is done through performance of the dissolution or disintegration methodologies. Since the bioavailability of the product can be compromised by exposure to heat, humidity, light, moisture, etc., it is important to establish that the container maintained its integrity during storage under approved storage conditions. This can be measured by employing USP's General Chapter <671> *Containers – Performance Testing*. The monograph assay and chromatographic purity tests should also be performed during and at the end of stability to guarantee the potency of the product and the potential impurities and/or degradation products are still within acceptable ranges. When there is no specific test in the monograph for chromatographic purity and a manufacturer has a concern about these attributes, Chapter <466>could be used as a reference, as stated above. Other tests not listed in the table may be used on stability depending on need. For example, if there is knowledge that a dosage form is particularly susceptible to degradation via exposure to moisture it may be advisable to also use one of USP's tests for determining moisture content.

9.2.5 General Case Study: Parenteral

As with solid oral dosage forms, it is important to ensure injectable products maintain their integrity through out their shelf-life. One of the most important performance criteria for an injectable product is sterility. The USP sterility test, coupled with container integrity testing, is used at release and at expiration to confirm the product remained sterile through out the approved shelf-life. As with tablets and capsules, some injectable products can degrade upon exposure to extreme environmental conditions. The pH test can be used during routine stability monitoring to alert the analyst that the product may be degrading. A questionable pH result indicating possible product degradation can be confirmed by performing the monograph tests for assay and chromatographic purity. If the monograph does not have tests for specific impurities, the user should consider referring to Chapter <466> for guidance. Other tests in the USP may be suitable for use on stability depending on the nature of the product. For example, if the product is prone to crystallization upon exposure to heat then the stability testing matrix might include reference to USP's General Chapter <788> *Particulate Matter in Injections.* The purpose of this test is to determine the level of particles that might be present in the injectable product. USP defines particulate matter in injections as ". . .mobile undissolved particles. . .unintentionally present in the solutions." Undissolved, crystallized drug

substance can be interpreted to fit this definition and therefore Chapter <788> could be used on stability if the product has a tendency to crystallize during storage.

9.3 Conclusion

Understanding the product's profile and sensitivities will help users identify the most appropriate USP tests for stability purposes. USP is an invaluable source of test methodology and information applicable for monitoring and confirming product conformance to standards through out the approved shelf-life. These validated procedures can be easily verified and determined to be suitable for measuring the overall quality of pharmaceutical products through their approved expiration period. Companies can save considerable time and effort by recognizing that in many cases USP monographs are appropriate for stability monitoring and can be used to assure the "identity, strength, quality, and purity" attributes of the product remain intact through out the product's life as required by regulatory authorities.

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10. ICH Q3A, Impurities in new drug substances.
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12. ICH Q3C, Impurities: guidelines for residual solvents.
13. ICH Q1A(R2), Stability testing of new drug substances and products.
14. ICH Q1C, Stability testing for new dosage forms.
15. ICH Q1E, Evaluation for stability data.

Chapter 10
Non-chromatographic Methods to Support Stability Program

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Contents

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Abstract Testing of pharmaceutical products is critical in assessing the stability and expected performance of the drug product and API. This chapter will discuss several physical tests as well as those chemical tests that focus on the performance of the drug products. It does not delve into the background theory of the testing but rather gives an overview of the tests and practical information for the analyst performing the tests. Most of these tests are described in detail in the USP. Chapter 9 of this book contains an overview of the USP and its USP-NF requirements for stability purposes.

Physical testing encompasses a wide range of techniques, from visual examination to spectroscopy. It is often the physical attributes which the patient or practitioner can evaluate prior to administration. For example, a particle found in a parenteral formulation can foretell the presence of a new chemical degradant found during stability studies. Many of the procedures in this chapter are performed routinely as part of release or stability testing of API or pharmaceutical products.

Chemical tests such as Karl Fischer testing and pH are also important and conducted routinely.

Analysts in the QC or stability laboratories must be vigilant and attentive to the testing to ensure the products delivered to the public for sale or for a clinical trial meet the design specifications throughout their expiry. All atypical observations must be recorded and elevated to the next level of management for appropriate action. A discussion of investigation is also provided in Chapter 13.

Dissolution testing attempts to characterize a combination of both the physical and chemical nature of the product. The physical aspect of the product may either detract or enhance the dissolution rate; however, so can the chemical nature of the active ingredient. For example, a change in the crystal structure of the API can adversely affect its solubility and hence the dissolution rate.

Due to the rapid advancement of new pharmaceutical delivery systems, this chapter covers only a limited number of techniques. Several of the techniques listed below may be necessary only at time zero or at release rather than being monitored at each stability time point. In addition, this chapter does not cover microbiological tests such as microbial limit, pyrogen, and sterility testing.

10.1 Appearance Testing

Physical appearance is often a required release and stability test. It may also be assessed by the patient, medical professional, or pharmacist. Appearance can be indicative of instability or an error in manufacturing. In this section, appearance testing of various dosage forms will be discussed.

10.1.1 Powders – API, Excipients, and Finished Product

The examination of powders should be performed under white light or fluorescent light with a consistent background (typically white). Powders should be evaluated for color, evidence of clumping or aggregation, flowability, evidence of containments and crystallinity. Appearance testing can be evaluated in the container or by dispensing onto a smooth surface for examination. Each powder must include a specification on appearance. Because appearance testing is subjective, comparative standards are sometimes used to help ensure consistent observations.

10.1.1.1 Color

Color is typically the first characteristic observed; however, a color description can be subjective depending on the chemist's ability to detect color. Color matching is one of the most difficult tests to reproduce. Comparison of the color of the powder to standard color chips (Pantone color chart) is advisable to reduce the variation of the observation. Analysts must have an eye test prior to performing color evaluations. To evaluate color of a sample, the analyst must place the material against the background and record the observation against the appearance specification. Observations will be performed under white light versus yellow light; a colored light will alter the color observed in the sample. To minimize variability, small commercially available bench-top light booths are available with standardized light elements. Color matching is best done in a light booth to minimize the interference of stray ambient light, such as from fluorescent laboratory lighting. If the color does not agree with the appearance specification, a second opinion may be necessary. Care should be taken when determining any appearance specifications, but especially in setting color attributes.

10.1.1.2 Flowability

Other physical parameters of powder, such as flowability, must be observed and recorded. Does the powder flow freely? Does the powder cling to the sides of the container, is the powder fluffy, or does it have the sparkle of a crystal? Refer to the specification document for the physical characteristics of the sample and either confirm if the sample has those characteristics or not. It may be helpful to obtain a retain sample from a previous lot of acceptable material for comparison.

10.1.1.3 Contaminants

Powders must also be examined for physical contaminants. In veterinary pharmaceuticals, especially medicated feeds, the feed matrix can easily be contaminated with insects or other pests. In human pharmaceuticals, samples of powder must be examined for any type of foreign substance, such as black specks, metallic particles, or glass chips. The analyst must be vigilant for the presence of contaminates in powders.

10.1.2 Finished Product Forms

10.1.2.1 Tablets

Appearance assessment of tablets should follow an appearance method in conjunction with a specification document. Tablets should be examined under white light for color, mottling, chips, cracks, sticking and picking for coated tablets, completeness of coating, and the presence and readability of the imprinted or debossed dosage strength or logo. The analyst should record color, tablet shape, and evidence of chips, and cracks. Tablets may be clear or color-coated. The coated tablet will have a sheen appearance to the surface whereas a non-coated tablet typically has a flat finish. Impurities which could be present on the surface of tablets could be metal particles, machine lubricants, or other materials that may come in contact with the tablets during manufacturing or packaging. Figure 10.1 illustrates a few of the tablet deformities that can be encountered in stability or release testing.

10.1.2.2 Capsules (Brittleness)

Appearance testing for capsules should include the examination of the capsule shell and the contents. The contents are typically either a powder or a liquid. If the contents are a powder, the contents should be examined as a powder described above. If the contents are a liquid, the liquid should be examined under white light to determine if it meets specifications. In either case the contents should be examined for any inconsistency with the product specifications.

The capsule shell should be of the desired color, size, and brittleness. Traditional gelatin capsule shells should remain flexible. Under certain storage conditions, gelatin capsule shells can become hard and brittle. When the shells are squeezed between two fingers, a brittle shell will snap and in extreme cases shatter. Brittleness occurs due to cross-linking of the gelatin and this cross-linking adversely affects the dissolution of the capsule. Capsule color has also been known to fade over time.

Fig. 10.1 Examples of tablet deformities

Observations of shell color, logos/lettering printed on the shell, and flexibility of the shell must be made. Observation of brittleness is an important observation to aid in dissolution testing.

A liquid-filled capsule shell should also remain flexible. Furthermore, after removing the contents of the capsule (by expressing the liquid), the analyst should open up the capsule to look for evidence of particulate matter (crystallization of active ingredient) and for unusual discoloration of the interior of the capsule shell.

10.1.2.3 Parenteral and Non-sterile Solutions

Appearance of solution can be performed as the solution is in the clear glass container or after dispensing a portion into a clean container. Observations are made for color, clarity, and absence of particulates. Solutions by their very nature should be clear with no particulate matter and no precipitation on the surface of the bottle or cap. A colorimeter may be used to assess the color of a solution. The use of a colorimeter results in more quantitative assessment of color. However, it is recommended to use the same brand/model of colorimeter for stability or release testing. Figure 10.2 shows an example of a colorimeter.

The appearance testing of a parenteral solution has additional focus on the presence of particulates, fibers, or flecks in the solution. Parenteral appearance should be assessed while the solution is in its original container against a white and/or black background. Vials should be held up in front of the background with indirect white fluorescent lighting. The vials should be examined for about 5–15 s. Typical observations are *"The solution in the vial was clear, colorless, and free of particulates, fibers and any other foreign material"*. When the analyst is presented with a

Fig. 10.2 Klett Summerson colorimeter

product in an amber vial, additional care must be taken to observe the solution. The reduced light passing through the amber vial will make the detection of foreign or precipitated material more difficult. Additional background lighting may be needed to make a full observation. The appearance testing of parenterals should be augmented by particulate matter testing in order to examine and quantitate the number of particles in the solution visible to the naked eye.

10.1.2.4 Lyophilized Products

Lyophilized products are the result of a solution being removed of its water by freeze-drying. The resulting solid mass is called a cake. Typically, the cake sits in the bottom of the vial. Color typically is white or off-white. The consistency of the cake may range from solid and wafer-like to a light crystalline cake. The appearance of the cake would include color, a description of the texture (e.g., solid cake with crystalline nature), and the examination for any foreign particles.

Reconstitution Time

Reconstitution time is the time required to dissolve the cake in the prescribed volume of water for injection (WFI). The procedure is simple; the analyst quickly adds water to the vial by a needle and syringe and records the time it takes for the solids to dissolve. To aid addition of the WFI, a second needle can be placed through the septum so pressure does not build up inside the vial. The reconstitution time is typically recorded, using a stopwatch, from the time the water is introduced to the vial until the solids are completely dissolved. The vial can be vigorously shaken and checked every 5–10 s to see if the cake has dissolved. During a stability study, a gradual lengthening of reconstitution time may occur. During the reconstitution time test, the analyst must be sure not to mistake bubbles in the solution for small particulates.

10.1.2.5 Lotions and Creams

Appearance testing for lotions and creams are similar. Both are typically opaque but can be produced in a number of colors. Color of the products should be uniform and texture must be consistent. Consistency should be smooth and typically not gritty. Using a gloved hand, the analyst can assess consistency of a lotion or cream by rubbing a small portion between the fingers and thumb. A usual inspection should include examination for evidence of cracking, or separation of the aqueous and oil phases. In stability studies, separation could be observed, especially at elevated temperatures, or during freeze-thaw cycling studies. Due to changes in the excipient base for creams and lotions, these products can discolor and generate unusual odors.

10.1.2.6 Pressurized Delivery Systems

Pressurized delivery systems include inhalers (meter dose and dry powder inhalers), foams, and sprays. The appearance test consists of two parts; the examination of the

container and of the content. Initially, the containers should be examined for any sign of leakage around the valve and seal area. Evidence of leakage or no leakage should be recorded by the analyst. The content is then examined in a two-step process. The first assessment is performed by dispensing a portion of the content and catching the material in a clear container for examination. Sprays should be a liquid material. Foams are typically white, and will disperse into a liquid; therefore the appearance should be performed immediately after the material is dispensed. Foams can discolor as they age; discoloring can be due to changes in either the excipients or the API. Appearance testing of inhaled products must include observation for aggregation of particles and for the color of the expelled material.

The container interior of the pressurized products must also be evaluated for any discoloration or crystallization of the interior surface material. Cans are frozen in either liquid nitrogen or in a –70°C freezer and then quickly pierced. As the cans warm up, the propellant is allowed to boil off – preferably in a fume hood. When the cans have warmed to room temperature they are cut open so the entire inside of the can is visible.

10.2 FTIR Spectroscopic Testing

Fourier transform infrared spectroscopy (FTIR) is a common identification test. Chapter 11 also discusses FTIR applications supporting stability. The Fourier transform enhances sensitivity and greatly reduces the time of the spectroscopic measurement. FTIR is commonly used as an identification test, but has been used qualitatively (e.g., dimethicone). Spectra are compared with a reference spectrum for identification purposes. As an identification test, FTIR is used as a release test rather than a stability test. Additional testing information can be found in USP/NF, General Chapter <851>.

10.2.1 Solids

The FTIR spectrum of solids/powders is traditionally obtained using a potassium bromide pellet. The sample is dried, ground to a fine powder, mixed with dried, ground KBr, and then pressed into a pellet. While this process appears straightforward, the control of water in the sample and KBr is of the utmost importance. Even small amounts of water will adversely affect the quality of the pellet and the resulting spectrum. A suitable pellet should be clear. Coloring the pellet is acceptable, but it should be clear.

For a solid sample, a pellet is prepared from the powder sample in a glove box or low humidity lab. It is recommended the pellet be no more than 5 mm in diameter and contain about 0.1–2% active mixed with KBr powder. The pellet is thin and transparent. Otherwise, an opaque pellet or one with white spots may result in poor spectra as little infrared beam passes through.

10.2.2 Liquids

The FTIR of liquids can be measured by either a thin film between two salt plates or a liquid sample cell. The liquid sample cell consists of two KBr plates with a spacer, typically a 0.5-mm thick Teflon ring.

10.2.2.1 Working with a Liquid Sample Cell

Solvents must be dried in molecular sieves or other appropriate drying agents. Cells must be assembled and used throughout the analysis. Reassembling a cell during the analysis may change the pathlength and cause errors. A blank spectrum is obtained using neat solvent when switching between samples and standards.

10.2.2.2 Working with Salt Plates

Salt plates can be used for FTIR testing. A drop of the liquid is placed on the face of a highly polished salt plate, typically KBr or NaCl, and covered with a second plate on top of the first plate to spread the liquid. The plates are then clamped and mounted onto the sample holder for analyses. This option is not feasible for volatile liquid or aqueous solutions. These salt plates must be cleaned with isopropyl alcohol and stored in a desiccator.

10.3 Moisture Testing

Moisture testing is performed during release and stability on pharmaceutical solids, including but not limited to APIs, tablets, and lyophilized cakes. Changes in moisture level can be indicative of the effectiveness of the container-closure system. Moisture increases can adversely affect the stability of the active ingredient; therefore, it is a key parameter in stability studies of solids. Traditionally, moisture testing is performed by two methods, *Loss on Drying* and *Karl Fischer Titration*. Water determination is also discussed in USP/NF, General Chapter <951>.

10.3.1 Loss on Drying

Loss on Drying (LOD) is a non-specific test used when sample material is abundant and will not decompose/melt at 110°C. The expected level of water is greater than 1%. Loss on Drying can be conducted at lower temperatures if a lower atmospheric pressure is used through the use of a vacuum oven or vacuum desiccator. LOD testing is common on tablets, excipients, and very stable APIs. The USP also discusses this testing in USP/NF, General Chapter <731>. This testing is typically conducted with a qualified oven, a calibrated thermometer, and calibrated balance. LOD requires a large quantity of sample, for example in excess of 0.5–1 g. The

amount of sample needed is directly related to the sensitivity of the balance used and the expected water loss. The container must be allowed to cool completely in a desiccator before weighing, since weighing while it is cooling down is inaccurate, due to thermal air flow around the container. Observation of color or texture must be made and recorded. One of the shortcomings of this testing is that LOD is not a specific method, because volatiles, which have a flashpoint greater than 110°C, will also be evaporated and observed as water loss.

10.3.2 Karl Fischer Titration

A volumetric titration used to perform moisture determination is commonly known as a Karl Fischer titration. Karl Fischer developed this technique in the 1930s [1]. This reaction may be considered a form of iodometric titration. The titrimetric determination of water is based upon the quantitative reaction of water with an anhydrous solution of sulfur dioxide and iodine in the presence of a buffer that reacts with hydrogen ions. In the original titrimetric solution, known as Karl Fischer Reagent, the sulfur dioxide and iodine are dissolved in pyridine and methanol. The test specimen may be carried out by a residual titration procedure. The stoichiometry of the reaction is not exact, and the reproducibility of a determination depends upon such factors as the relative concentrations of the Reagent ingredients, the nature of the inert solvent used to dissolve the test specimen, and the technique used in the particular determination. Therefore, an empirically standardized technique is used in order to achieve the desired accuracy. Precision in the method is governed largely by the extent to which atmospheric moisture is excluded from the system. The titration of water is usually carried out with the use of anhydrous methanol as the solvent for the test specimen; however, other suitable solvents may be used for special or unusual test specimens.

In today's lab, pyridine-free Karl Fischer reagents are used. The titration has been adapted to specialized automated titrators made by a variety of instrument manufacturers. The volumetric titration is monitored by two platinum electrodes held at a constant potential. The endpoint is reached when excess reagent results in a constant current. This test method has gained in popularity over LOD testing due to its accuracy, specificity, and smaller sample sizes.

10.3.2.1 Blanking of the Vessel

Because method precision is greatly affected by the extent of atmospheric moisture, all residual water and environmental contributions must be eliminated from the titration vessel prior to titration initiation. Titrant is automatically added to the vessel until equilibrium is reached. The desiccant on the instrument must be charged and fresh, as environmental moisture can bias results drastically.

Table 10.1 Guide to reagent and standard selection

Sample type	Composite	Standard material
USP Standard material	Composite 2	30 μL purified water
0–1% water	Composite 1 or 2	15 or 30 μL purified water or 100 mg sodium tartrate dihydrate
1–5% water	Composite 2	30 μL purified water or 100 mg sodium tartrate dihydrate
≥5% water	Composite 2 or 5	30 μL purified water

10.3.2.2 Standardization

As with any volumetric titration, standardization of the titrant with a primary standard is required. The most common primary standard used will be water. Other primary standards may include sodium tartrate or commercially prepared water standards. Table 10.1 provides a guide to reagent and standard selection.

For sample water content is less than 1%, coulometric moisture analysis is used, if feasible.

10.3.2.3 Percent Recovery Standard

The percent recovery standard must be analyzed at the following times: prior to sample/reference standard analysis, after no more than 10 samples/reference standard titrations, after the final sample/reference standard analysis, and whenever the solvent in the vessel is changed. The solvent in the titration vessel may need to be changed periodically, depending on methods, sample matrix, or volume in the vessel.

10.3.2.4 Sample Analysis

Care must be taken to ensure that the sample is not exposed to environmental moisture prior to analysis. Karl Fischer analysis must be one of the first tests to be performed on stability samples when the container is first opened. If the sample is not solution in solvent, additives may be used to assist with sample solubility.

10.3.2.5 Handling Cautions

Accurate weighing is important. If the sample is hygroscopic, additional care must be taken for weighing samples. Solvent must be changed if excessive solid excipients are present in the vessel.

Karl Fischer reagents are hazardous; thus appropriate safety caution should be taken. These reagents are halogenated and need to be kept with the halogenated waste.

10.4 Residual Solvents Analysis in Pharmaceutical API and Excipients

The USP definition of *residual solvents* is*organic volatile chemicals that are used or produced in the manufacture of drug substance or excipients, or in the preparation of drug product.* According to ICH Q3C, residual solvents have been segregated into three classes based on their toxicity and risk to human health. *Class 1* solvents are considered the highest risk and their use should be avoided whenever possible. *Class 2* solvents should be limited in their use in API/excipients due to their inherent toxicities. *Class 3* solvents are regarded to have little risk to human health at levels normally found in pharmaceuticals. Each class of solvents has different limits for how much is considered acceptable in API or excipients. Like other impurities, residual solvent levels should be minimized as much as possible. When found in API/excipients above their accepted concentration limits, they must be identified and quantitated. Complete lists of the three solvent classes can be found in USP General Chapter <467> or ICH Q3C.

Residual solvent testing is typically performed at release; however, in some cases, where solvents must be monitored closely, companies may choose to conduct this test routinely for stability samples.

10.4.1 Instrumentation

Residual solvents are typically analyzed using a gas chromatograph (GC) outfitted with a flame ionization detector (FID). The sample is introduced either by direct injection or by headspace injection. Headspace injection has grown in popularity in recent years, since it eliminates many of the interferences originating from non-volatile components of the API/excipient. The typical GC column used for residual solvents is a capillary column with a 6% cyanophenyl, 94% dimethylpolysiloxane-phase film, which is referred to as a G43 column by USP, with a unique suffix given by column manufacturers. Certain methods will also use the G16 or Carbowax® 20M columns depending on what solvents are being analyzed.

Many companies have developed and validated their own generic methods to test their commonly used set of residual solvents.

10.4.2 Key Factors for Residual Solvent Testing

10.4.2.1 Standard Preparation

Standard preparation is critical for this analysis due to the volatile nature of the solvents. Standards should be prepared as quickly as possible and glassware should be capped whenever possible. Typically, standards are prepared by adding measured volumes of the solvents, and the standard weight is determined by calculation using

the volume and density. Once standards are made, they should be sealed in air-tight containers and used as soon as possible.

10.4.2.2 Miscibility of Solvents

Miscibility of solvents with the diluent can be an area of concern. It is common to prepare stock standards at higher concentrations, then dilute down to the working standard. Certain solvents may not go completely into solution at these higher concentrations, depending upon the diluent. For example, hexane will not dissolve completely in DMSO at high concentrations. Thus, the stock standard used for hexane will need to be prepared at a lower concentration than that of other solvents.

10.4.2.3 Other Considerations

For headspace gas chromatography, care must be taken for other physical conditions in addition to the injection volume if an autosampler is used. Sample in vapor phase is taken from the headspace of the vial. Headspace sampling is accomplished by heating and agitating a sample in a sealed vial, then opening up a sample loop, which is filled by the vapor phase of the sample and subsequently injected onto the column. The amount of sample that enters the sample loop is dependent upon the pressure inside the vial; therefore, if the pressure varies, the amount injected will be affected and the result will be in error. It is imperative that all vials contain the exact same amount of liquid sample, that all samples are prepared in the same diluent, and that the vials are capped and sealed tightly. Also, each vial can be injected from one time only. After an aliquot is taken from the vial headspace, the analyte will establish equilibrium between the new vapor and liquid phases; resulting in a slightly different concentration than the original sample.

10.5 pH

pH measurement is a very common analytical test for liquid dosage forms. It is a release test and also monitored routinely on stability. However, if not performed correctly, pH measurements can easily produce erroneous results. pH is the *negative log* of molar concentration of hydrogen ions. It is the activity of the hydronium ions, formed by the protonation of water, that creates the change in potential that is then read out by the pH meter.

$$H^+ + H_20 \rightarrow H_3O^+$$

pH electrodes used in today's lab are a combination glass electrode with an Ag/AgCl reference electrode. Electrodes are produced in a variety of shapes and sizes, some for very specialized uses. However, all glass combination pH electrodes work in the same manner and need similar care according to manufacturers' recommendations. USP <791> also provides additional information on these procedures.

10.5.1 Calibration

It is critical that pH electrodes are calibrated often as the slope of their response curve will change with temperature, as defined by the Nernst equation [2]. Calibration should be performed with at least two certified pH calibration buffers that bracket the expected pH of the sample. Calibration is performed as described in the manufacturer's instructions provided with the pH meter. Calibration should be checked by measuring the pH of the first calibration buffer. The result should be no more than 0.02 pH units different from the certified pH of the buffer after adjusted for temperature. pH measurements are best performed when calibration buffers and samples are held at a constant temperature.

10.5.2 Measurement

The pH of solid dosage form and API is measured directly in administered solutions or after material is dissolved. The pH of an API is measured after the compound is dissolved completely in a portion of water, and the sample is allowed to come to the same temperature as that of the calibration buffers. If API does not dissolve in water, the compound cannot react with the water molecules creating a new equilibrium of water and hydronium ions. Even small amounts of polar organic solvents alter the true pH measurement.

$$\mathrm{API} \rightarrow \mathrm{API}^- + \mathrm{H}^+$$
$$\mathrm{H}^+ + \mathrm{H_2O} \rightarrow \mathrm{H_3O}^+$$

pH measurements of solution products are measured in a similar fashion regardless of the route of administration. The samples are equilibrated to the same temperature as the calibration buffers and the pH is measured. Standard buffer measurements should bracket a series of samples to ensure the calibration of the pH meter is maintained. The number of samples to be bracketed depends on the type of sample and the stability of the electrode.

For suspension products, sample must be uniform prior to measurement. The cleaning of the electrode after each sample is important, especially if the suspension contains a high level of taste-masking agents, such as sugars. The pH electrode can easily become coated with particles or viscous liquids that hinder performance. When working with suspensions, as compared with solution products, the number of samples tested within a bracket should be reduced.

10.6 Weight Variation and Fill Volume/Delivery Volume

10.6.1 Weight Variation

Weight variation is commonly tested for tablets. To perform the test, ten tablets are individually weighed on an appropriate analytical balance. The ten weights are

averaged and the relative standard deviation is calculated. This test is used for a batch release, but rarely used in stability testing. Care must be taken for uncoated tablets as moisture can cause the weight measurement to drift.

The analyst should examine the tablets used in this test. All chips, cracks, or abnormalities should be recorded. The tablets should be free of any surface dust. Most tablets can be weighed on a balance with a 0.1-mg resolution; however, it is recommended that a balance accurate to at least three significant figures be used.

10.6.2 Deliverable Volume

Small-volume parenterals are filled to a volume that should allow the practitioner or clinician to withdraw the prescribed amount of solution. Under-filled vials will prevent patients from receiving the full dose, while overfilled vials will result in excess (waste) drug product. A low deliverable volume may indicate an improper seal of the container. A syringe is used to pull all of the solution from the vial. The weight of the syringe containing the solution is determined and the net weight is calculated. Volume of the solution can be calculated with the solution density and the net weight obtained.

Deliverable volume is used for batch release as well as for stability testing. The analyst conducting this testing needs a pre-weighed syringe equipped with a needle (16 or 18 gauge is appropriate). A second needle may also be required as a vent (see below). Either of two approaches can be used: the two-needle technique or the single-needle technique.

10.6.2.1 Two-Needle Technique

The needle without a syringe is inserted through the septum to create an air vent. This vent needle must be inserted far enough in the vial to prevent solution drainage through this vent. The solution is then drawn up into the syringe with another needle.

10.6.2.2 Single-Needle Technique

This technique does not use the vent needle. Prior to inserting the needle with the syringe into the vial, the syringe is filled with air, about three-quarters of the expected volume of solution. The needle is then inserted into the vial and the air is pushed in. If too much air is pushed into the vial, the syringe plunger will be forced back or leakage around the septum may occur. The solution is drawn up into the syringe. The first half of the solution will come into the syringe very easily, but the last part of the solution will become more difficult to remove. As with the two needle system the vial is inverted and manipulated to bring as much solution in to the syringe as possible. Caution must be taken when removing the syringe from the vial as pressure on the syringe plunger is relieved.

10.7 Tablet and Capsule Physical Tests

10.7.1 Friability

Friability is typically tested for tablets as they are constantly subjected to abrasion and mechanical shocks during packaging and transportation as well as during the manufacturing process. Such stresses can lead to chipping, abrasion, and even breakage of the tablets. Therefore, a tablet formulation must be able to withstand such stress without damage to its appearance. In order to predict such damage in tablet appearance, tablets are routinely subjected to a friability test.

Friability testing is conducted by subjecting at least 6 g of drug product (approximately 20 tablets depending on weight) to repeat revolutions using a friability tester, which consists of a drive unit that rotates one or two transparent plastic drums. A shaped radial fixed blade carries the tablets along with it up to the central height and lets them slide off while the drum is in rotation. The tablets rub against each other without any hard impact. Tablets are weighed before and after the testing and results are expressed as percent of weight loss on the original tablet weight. Normally, less than 1% of loss is acceptable. Friability is normally a release test; however, it can also be included in the stability program for uncoated tablets, especially if the tablets are known for their hygroscopicity. Figure 10.3 shows a typical friability tester.

10.7.2 Tablet Hardness

Hardness test is conducted to measure tablet strength. Tablets should be hard enough to withstand manufacturing, packaging, and transportation processes. However, they

Fig. 10.3 Varian friability tester

Fig. 10.4 Varian VK 200 tablet hardness tester

cannot be too hard since that may alter the disintegration or release of the drug product.

Hardness is determined using a hardness tester, where the tablet is placed between two jaws that crush the tablet. The instrument measures the force applied to the tablet and detects when the tablet fractures. Usually 10–20 tablets are tested and the mean value is calculated. Test results can be affected by speed of the testing, the geometry of the tablet contact points, and debris in the testing area, as well as by variation in temperature, humidity, and the age of the tablets. Therefore, the tablets must be oriented consistently in the hardness tester.

This method is used for stability, research & development, and for production quality control. Hardness testers are available from many vendors and today, many labs are using bench top models that can directly be interfaced to a laboratory information management system (LIMS). Figure 10.4 shows a typical hardness tester.

10.8 Content Uniformity

Content uniformity is a measurement of the variation in the active ingredient from one unit to the next. As drug products are manufactured, excipients and fillers are added. Factors such as densities, particle sizes, and particle shapes may contribute to the differences in uniformity. Therefore, uniformity is necessary to assure the individual unit conforms to compendial acceptance criteria of content uniformity. More information on the requirements of this test can be found in USP/NF General Chapter <905>. These are similar to the requirements of the European and Japanese Pharmacopeia. Typically, 10 tablets are analyzed and the average and %RSD are reported. The procedure usually is an HPLC test; however, UV and other methods,

including weight variation, have been used. The manufacturing process is validated to demonstrate that the formulation produces uniform dosage units with respect to the content of the active ingredient.

10.9 Disintegration

Disintegration testing is a procedure to measure the ability of the tablets to disintegrate. Tablets are dropped into an open-ended basket containing six slots; a disk may be put on top of the tablets and the whole assembly is placed in a beaker containing disintegration media. The basket and beaker are placed in a water bath to maintain a constant temperature. In most cases, the disintegration media is water. The basket oscillates up and down until the product is completely disintegrated. The time it takes for the tablets to disintegrate is recorded. Detailed information on this test is illustrated in USP/NF, General Chapter <701>. This test is often included as a release and also a stability test. Many countries require disintegration specifications for new drug products.

10.10 UV/Vis Spectroscopy

According to the USP, absorption spectrophotometry is the measurement of an interaction between electromagnetic radiation and the molecules, or atoms, of a chemical substance. Materials can absorb radiation in the ultraviolet and visible ranges, depending on the arrangement of atoms and the type of bonds between them. In the ultraviolet and visible range of the electromagnetic spectrum (200–780 nm), a quantitative linear relationship exists between the absorption of energy and the concentration of the absorbing species in a given solution, over a concentration range limited by the molar absorptivity of the sample. This relationship is described by the Beer-Lambert law, frequently referred to as *Beer's Law*:

$$A = \mathrm{Log}_{10}(1/T) \tag{10.1}$$

$$A = \varepsilon bc \tag{10.2}$$

where
A is the absorbance,
ε is the molar absorptivity coefficient,
b is the path length of the measurement cell, and
c is the concentration of the absorbing species.

The steps involved in spectrophotometric measurement in the ultraviolet and visible range include irradiation of a sample at a specific wavelength, detection (commonly using a photomultiplier tube or a photodiode array), and transduction into an electronic signal. Specifications and sample analysis procedures are listed in the USP General Chapter <851>.

10.11 Density/Specific Gravity

Density and specific gravity are usually performed for release testing or testing of intermediates. These procedures are typically performed during production as process control steps. Density is the ratio of mass to volume. Specific gravity is the ratio of the density of the product to the density of water. Substances with a specific gravity greater than 1.0 are denser than water and will sink; while those with specific gravity less than 1.0 are less dense than water and thus float in it. More information can be found in USP/NF General Chapter <699>, Density of Solids, and USP/NF General Chapter <841>, Specific Gravity.

10.12 Melting Point

Melting point or more accurately, melting range, may be used for compound identification and to gauge compound purity. The narrower the melting range, the purer the compound. Pure substances can be expected to exhibit a melting range of 1–2°C. Typically, crystalline materials exhibit sharp melting points, while amorphous materials do not. Also, some substances sublime rather than melt, and others may decompose before melting is achieved. Melting point is generally viewed as a physical parameter that can be tested by operators with little specialized training. By comparison, differential scanning calorimetry (DSC), an instrumental thermal analysis technique, may also be used to assess melting point. However, thermal analysis instrumentation is far more complicated, requiring specialized training. USP lists melting range or temperature testing in USP/NF General Chapter <741>.

10.13 Particulate Matter in Parenterals and Intravenous (IV) Solutions

Particulate matter in parenterals and IV solutions is described as undissolved particles, other than gas bubbles, unintentionally present in the solutions. Such particles can have unwanted effects if present at elevated levels. The purpose of the test for particulate matter is to ensure that these particles are below established safety limits. USP General Chapter <788> provides the directive for determining particulate count and size. This test is often conducted at release as well as monitored on stability.

The USP lists two recognized techniques for testing for particulate matter in pharmaceutical solutions. Method 1 is based on the principle of light obscuration, in which light from a laser impinging on a photodiode detector is obscured by any particles present in the test medium. Method 2 employs a light microscope, with which particles are visibly counted per unit volume by the analyst.

10.14 Dissolution

This procedure measures the dissolution rate of the drug from the dosage form in vitro. It is usually expressed as the extent of dissolution (percent of drug content) occurring after a given time under specified conditions. This test is necessary to help in the prediction of the behavior of the drug product/dosage form after ingestion. It is designed to mimic in vivo drug actions and availability.

The USP describes the procedures for dissolution testing. The two most common methods are Apparatus 1 (rotating basket) and Apparatus 2 (rotating paddles). Testing is performed in a dissolution bath containing six vessels. The rotating baskets or paddles are lowered into the vessels and spun at a predetermined rotation in a dissolution media. Media can be dispensed manually or by a media delivery system. Vessels should be covered to minimize the evaporation of the media. Temperature of the media is controlled at $37.0 \pm 0.5^{\circ}C$ and measured either manually or by automatic temperature probes. At specific timepoints, an aliquot is drawn from each vessel, filtered and analyzed either by UV spectroscopy or HPLC. Typically, a dissolution profile is generated for a drug product that is in development, to gather sufficient data to set a dissolution specification. After approval, normally a single-point-pull dissolution would be required to monitor the performance of the drug product on stability.

Use of automated autosamplers is increasing in the industry. At specific timepoints, a tube is dipped in the vessel solution; an aliquot is pulled through a filter, and may be measured directly. Using an autosampler requires fewer manual manipulations and the aliquot collection can be done at more precise intervals.

Often, a paper tape printout is used to record the RPM, temperature, and speed at every sample pull time point. Temperature of the vessels is recorded before the test and after the test to ensure the proper temperature is maintained. The dissolution apparatus is set up and maintained based on manufacturers' recommendations in a space free of vibration, and calibrated according to USP procedures.

10.14.1 Sample Preparation

Sample preparation is critical for dissolution testing. Tablets or capsules may be placed in a sinker when using USP Apparatus 2 (paddle method). If USP Apparatus 1 (basket method) is used, then the baskets should be dry when samples are placed in them.

10.14.2 Sample Introduction – Apparatus 1

When using baskets, the lift assembly is raised and the base of each shaft is dried. Sample is placed in each basket and attached to the corresponding shaft. It is recommended that a wobble test is done for Apparatus 1 to demonstrate the basket has not been misshaped during its attachment to the shaft. The lift assembly is lowered and the baskets are placed into the media and the testing commences.

10.14.3 Sample Introduction – Apparatus 2

Samples (tablets or capsules) are dropped individually into each vessel as close to the same time as possible. Testing starts when all samples reach the bottom of the vessel. If sinkers are used, care must be taken to ensure the tablets or capsules are secured prior to being dropped. If a tablet or capsule gets stuck on a paddle or on the side of the vessel, test results will be inaccurate, the test should be voided, samples discarded, and the test restarted.

For a suspension, the drug being tested must be properly suspended and an accurate weight taken. It is recommended that the sample be introduced using a syringe. The syringe weight must be accurately measured before and after dispensing.

10.14.4 Sampling and Observations

Sampling can be done automatically or manually. Manual sampling is done at the appropriate times using specified disposable or glass syringes fitted with stainless cannulae. Sampling must be performed within ±2% of the specified sampling time. For example, if there is a sample time of 30 min, all vessels must be pulled within the window of 30 min ±36 s.

When sampling six vessels at various time points, it is crucial to organize your syringes and cannulae. Each sample will be identified with the pull point and vessel number.

Observations are very important in dissolution testing. They should be made at the time the drug product is introduced and while sampling at every time point. Observations can vary from the normal; from tablets starting to break up with excipients floating around the vessel, to samples completely broken up, to out of the ordinary observations like sample in vessel 5 not breaking up at all. These obser-

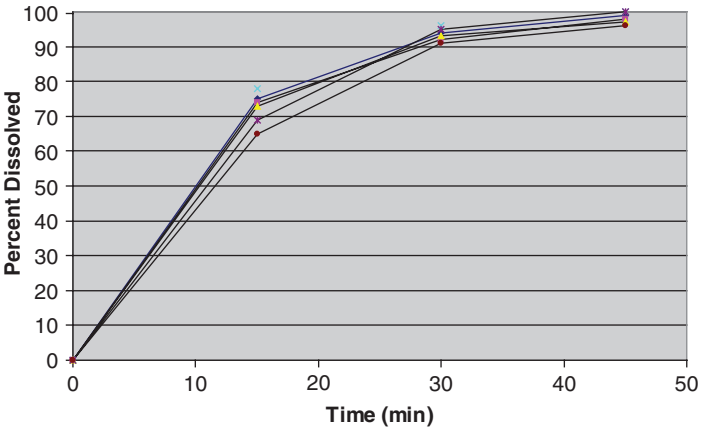

Fig. 10.5 Typical dissolution profile

vations are helpful to establish appearance of a typical dissolution profile of the tested product and also for reconstruction of data when results are atypical. These observations also aid in the training of a new analyst working with the same product. Figure 10.5 shows a typical dissolution profile of a tablet dosage form.

Care must be taken for cleaning and storing dissolution testing units and vessels. Each vessel is calibrated at a certain position of the dissolution unit. All vessels must be cleaned and appropriately stored.

10.15 Conclusion

These analytical tests are critical to establish the stability profile of APIs and drug products. These tests require a level of expertise and attention to detail that an experienced analyst needs. A training program is critical to ensure that the analyst understands the tests as well as recognizes the atypical or out-of-specification results. Proper reporting of results is also crucial for these procedures as some of them are subjective.

Due to the size limitations of this chapter, only the most common techniques are covered. More detailed information can be found in the USP, EP, JP, and other references. Analysts should also consult with their departmental SOPs and training guides.

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Chapter 11
Vibrational Spectroscopic Methods for Quantitative Analysis

Frederick H. Long

Contents

Abstract Spectroscopic methods such as NIR, Fourier transform infrared, and Raman are becoming increasing important in pharmaceutical research and manufacturing. This chapter reviews both quantitative and qualitative applications of spectroscopic analysis for pharmaceutical products. Several applications of these technologies to stability testing are discussed.

11.1 Introduction

Spectroscopic methods such as Fourier transform infrared (FT-IR), Near IR, and Raman are becoming increasingly important in pharmaceutical research and manufacturing [1, 2]. These spectroscopic methods can be used to do rapid, nondestructive, qualitative and quantitative analysis. In addition to analysis of traditional pharmaceutical products, spectroscopic methods can be applied to biological products and drug delivery systems such as drug coated stents. The US FDA has placed great emphasis on this area with recent guidance on process analytical technology (PAT) [3]. PAT has been defined by the FDA as a system for designing, analyzing, and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process

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materials and processes with the goal of ensuring final product quality. Spectroscopic methods such as Near IR and Raman are key PAT tools.

Closely related to PAT is quality by design (QbD) as described in the recent ICH Q8 chapter and the associated Q8 annex [4]. Quality by design is an effort to improve the scientific basis of pharmaceutical development. It is based on the recognition that quality cannot be tested into a product but is created by sound product and process design. A critical concept from QbD is the idea of a design space. A design space is a multidimensional combination and interaction of input variables and process parameters that have been demonstrated to provide assurance of quality [4, 5]. The implementation of QbD is done by a combination of design of experiments, statistical quality control, and PAT tools such as spectroscopic process monitoring.

Spectroscopic methods can provide fast, non-destructive analytical measurements that can replace conventional analytical methods in many cases. The non-destructive nature of optical measurements makes them very attractive for stability testing. In the future, spectroscopic methods will be increasingly used for pharmaceutical stability analysis. This chapter will focus on quantitative analysis of pharmaceutical products. The second section of the chapter will provide an overview of basic vibrational spectroscopy and modern spectroscopic technology. The third section of this chapter is an introduction to multivariate analysis (MVA) and chemometrics. MVA is essential for the quantitative analysis of NIR and in many cases Raman spectral data. Growth in MVA has been aided by the availability of high quality software and powerful personal computers. Section 11.4 is a review of the qualification of NIR and Raman spectrometers. The criteria for NIR and Raman equipment qualification are described in USP chapters <1119> and <1120>. The relevant highlights of the new USP chapter on analytical instrument qualification <1058> are also covered. Section 11.5 is a discussion of method validation for quantitative analytical methods based on multivariate statistics. Based on the USP chapter for NIR <1119>, the discussion of method validation for chemometric-based methods is also appropriate for Raman spectroscopy. The criteria for these MVA-based methods are the same as traditional analytical methods: accuracy, precision, linearity, specificity, and robustness; however, the ways they are described and evaluated can be different.

11.2 Overview of Vibrational Spectroscopy and Equipment

A fundamental property of chemical bonds is that they exhibit vibrations at distinct frequencies. The vibrational frequency of a given chemical bond is intrinsic to the chemical bond of interest [6]. The characteristic frequencies of a given molecule are called a vibrational spectrum. There are many methods for the investigation of vibrational spectra. The most basic measurement technique for molecular vibrations is *IR absorbance spectroscopy*. In practice IR absorbance spectra are measured by FT methods, which are described later in this chapter. The vibrations measured by an FT-IR are often enough to uniquely chemically identify small amounts of

a substance. However, FT-IR is not always ideal for many typical quantification problems of the pharmaceutical and biopharmaceutical industry. The absorbance is so strong in the mid IR, that the FT-IR spectrum is only measuring the top surface of the material of interest, typically 10 μm [1]. This is not adequate for tablet stability monitoring, as well as for other important quantitative measurements common in the industry, because the tablet assay concentration at the surface may not reflect the bulk concentration.

The related technique of *Near IR (NIR)* turns out to be superior for many quantitative problems. NIR is based on the overtones and combination bands of chemical vibrations. Overtones are analogous to octaves in a musical scale. Going up one octave in music will nearly double the frequency of the sound made. Overtones are the harmonics of the fundamental vibrational frequencies. Combination bands are the sum of two different vibrations corresponding to different chemical bonds. Combination and overtone bands are much weaker in absorbance than fundamental transitions; however, in bulk materials this is an advantage and not a liability because there is sufficient material to obtain a strong absorbance. It can be shown that CH, NH, and OH bonds are the most important chemical bonds for NIR spectra. Since most pharmaceutical materials are organic compounds, this is ideal. Although NIR spectra are broad and relatively featureless when compared to mid-IR spectra, with proper MVA, NIR spectroscopy provides chemically specific information about the material being studied. A chart showing the NIR absorbance peaks of common functional groups is shown in Fig. 11.1. The quantitative analysis of NIR spectra using chemometrics is described later in this chapter.

Raman spectroscopy is a vibrational spectroscopy technique that is complementary to IR absorbance. Raman spectroscopy is based on changes in the polarizability of the electron cloud around the atomic nuclei as the molecule vibrates. Using the

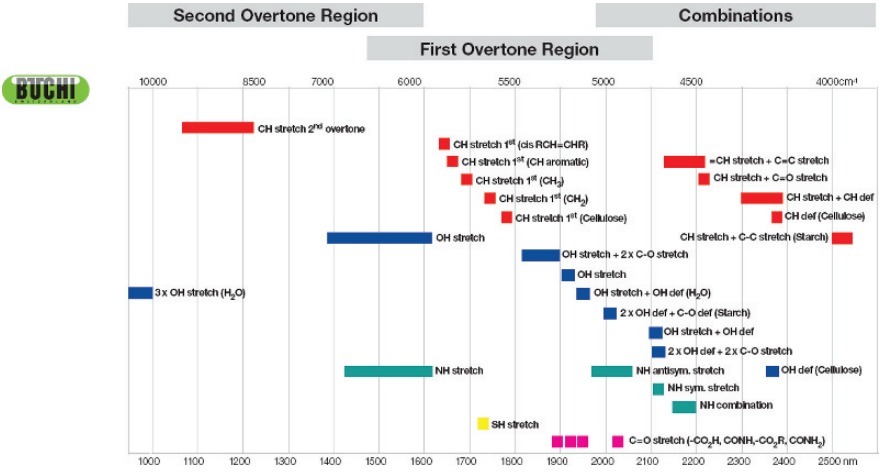

Fig. 11.1 Chart showing the NIR absorbance of different important functional groups, courtesy of Buchi Corporation

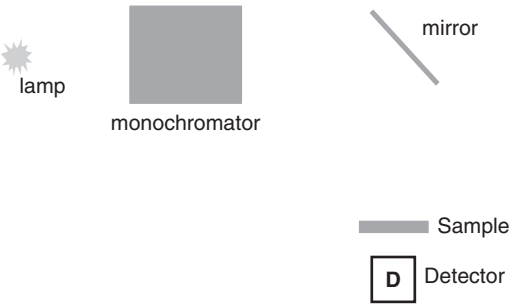

Fig. 11.2 Simple diagram of a NIR spectrometer

intense optical radiation available from a laser, a small fraction of the light scattered will be shifted in frequency. The spectrum of the scattered light is measured and contains peaks that correspond to molecular vibrational frequencies. Often the vibrations seen in a Raman spectrum are complementary to the vibrations seen in an IR spectrum, the reasons for this can be found in the references [2]. In practice, Raman is often complementary to NIR. For example, NIR is a very good method for moisture quantification, while the Raman scattering from water is weak. Therefore, Raman scattering can be used in some cases to quantify low concentrations of analytes in aqueous solution. An important application for Raman scattering in the pharmaceutical industry is polymorph detection [7, 2]. Polymorphs are different physical forms of the same molecule, such as degree or type of crystallinity. The Raman spectra of different polymorphs are often quite distinct. Raman spectroscopy can be used to map out the polymorph phase diagram under different conditions. Raman can also be used in the stability testing of actives and finished products for polymorph stability [7–9].

Figure 11.2 is a simple description of a NIR spectrometer. The spectrometer consists of a light source, a monochromator that separates different wavelengths of light, mirrors that direct the light to the sample, and a detector that monitors the intensity of light. Near IR measurements can be done in reflection mode, transmission mode or both. Modern instrumental designs have made NIR instruments very rugged and suitable for manufacturing environments.

A common variation on the basic design of a NIR spectrometer is a Fourier transform (FT) instrument. The FT-*NIR* instrument is similar to the more common FT-IR instrument. For the FT instrument there is no monochromator or wavelength separation device. The light is split into two beams with a mirror, called a beamsplitter, that both transmits and reflects the light. The two light paths form an interferometer. One arm of the interferometer has a fixed mirror, the other arm has a moving mirror. The light from the two arms is recombined inside the sample of interest. There is constructive and destructive interference of the light in the sample depending upon the difference between the two optical pathlengths. It can be shown that the intensity of the light at different frequencies is related to the signal at different optical pathlengths by a Fourier transform [6]. FT measurements are fundamentally different from dispersion measurements because all wavelengths are measured at the same time. In actual practice both dispersion and FT instruments can provide high quality

Fig. 11.3 Photographs of small, low-cost NIR spectrometer, courtesy of BaySpec Incorporated

NIR spectra with sufficient signal averaging. The typical data acquisition times are around 30 s. A detailed discussion of FT-IR spectroscopy can be found in a recently published book [1].

In recent years new NIR spectrometer technology has appeared out of the telecom industry. Based on different technological innovations, these spectrometers are compact, rugged, low cost instruments. Commercially available instruments include hand-held spectrometers optimized for raw material identification and process instruments that are small enough to be easily mounted on process equipment. They are clearly a disruptive technology that will greatly advance the use of NIR technology in the pharmaceutical and other industries. A photograph of a typical instrument is shown in Fig. 11.3. A list of major NIR vendors can be found in a recent paper [10].

There are several common configurations for Raman spectrometers. Unlike NIR, Raman can be readily done under a confocal microscope. Confocal Raman spectroscopy allows for the chemical composition of materials to be determined with micron spatial resolution including some depth profiling. Confocal Raman measurements have been shown to be useful quantitative analytical tools for the investigation of drug eluting stents [11]. Raman microscopy has been used to quantify thin

Table 11.1 Comparison of NIR and Raman

	Advantages	Disadvantages
Raman	• Sensitive to polytype • Sharp peaks • No sample preparation required • Can examine microscopic samples	• Insensitive to moisture • Fluorescence background for some materials
Near IR	• Excellent method for moisture • No sample preparation needed • Can examine inhomogeneous samples	• Broad, featureless spectra require chemometrics for analysis • Cannot do very small samples

coatings on tablets or other pharmaceutical products [12]. Raman spectrometers are also commercially available for process and laboratory testing. A summary of the advantages and disadvantages of NIR and Raman is given in Table 11.1.

11.3 Chemometrics and Multivariate Analysis

Multivariate analysis (MVA) is the statistical analysis of many variables at once. Many problems in the pharmaceutical industry are multivariate in nature. The importance of MVA has been recognized by the US FDA in the recent guidance on process analytical technology [3]. MVA has been made much easier with the development of inexpensive, fast computers, and powerful analytical software. Chemometrics is the statistical analysis of chemical data. Spectral data from modern instruments is fundamentally multivariate in character. Furthermore, the powerful statistical methods of chemometrics are essential for the analysis and application of spectral data including NIR and Raman. In this section, we will briefly review the subject of chemometrics and MVA.

With spectral data, it is not uncommon to measure several thousand variables at one time. However, it is often hard for beginners to conceptualize so many variables; therefore, we will begin our discussion of MVA with a few simple examples that illustrate important statistical concepts that are essential in chemometrics. The first problem is a set of pharmaceutical quality data. Measurements of density and assay have been collected for 43 lots of material. The data is shown in Table 11.2. Inspection of the data reveals that the density values are near 1.0, while the assay values are closer to 100. A goal of the data analysis is to understand the variation within the data set. It will be advantageous to have the two variables in the data set with similar magnitudes; therefore, we will scale each of the two variables by its own standard deviation.

A simple plot of the scaled data is shown in Fig. 11.4 (Describes x and y axis). Each point represents one of the lots of material. From the plot in Fig. 11.4, one data point is far away from all of the others. Statisticians call data points that do not belong to the data set outliers. Outliers are important to identify and remove from the analysis of the data set, because a single outlier can greatly influence the statistical analysis and obscure underlying trends in the data. We note that while outliers are often removed in a research and development environment during method development, great caution must be used in removing outliers during validation or use in actual production.

The scaled data are replotted in Fig. 11.5, with the outlier point removed. The reader will also note that the origin of the graph has been moved to the center point of the data set. This operation is called *mean centering*, when the average of the overall data set is subtracted from the data. As mentioned earlier, in MVA we are concerned with investigation of the variation within the data set. The average values of the data set are not of primary importance. Two arrows in the figure illustrate the two directions of variation within the data set. P1 is the largest direction of variation and P2 is the second direction of variation. It is important to note that P1 and P2 are

Table 11.2 Pharmaceutical quality data example

Density	Assay
0.801	121.410
0.824	127.700
0.841	129.200
0.816	131.800
0.840	135.100
0.842	131.500
0.820	126.700
0.802	115.100
0.828	130.800
0.819	124.600
0.826	118.310
0.802	114.200
0.810	120.300
0.802	115.700
0.832	117.510
0.796	109.810
0.759	109.100
0.770	115.100
0.759	118.310
0.772	112.600
0.806	116.200
0.803	118.000
0.845	131.000
0.822	125.700
0.971	126.100
0.816	125.800
0.836	125.500
0.815	127.800
0.822	130.500
0.822	127.900
0.843	123.900
0.824	124.100
0.788	120.800
0.782	107.400
0.795	120.700
0.805	121.910
0.836	122.310
0.788	110.600
0.772	103.510
0.776	110.710
0.758	113.800

perpendicular to each other. In MVA, P1 and P2 are the first and second principal components of the data set respectively.

For each one of the data points, the projection of the data point onto the P1 or P2 vector is called a score value. Plots of score values for different principal components, typically P1 versus P2 are called score plots. Score plots provide important information about how different samples related to each other. Principal component plots provide information about different variable relate to each other.

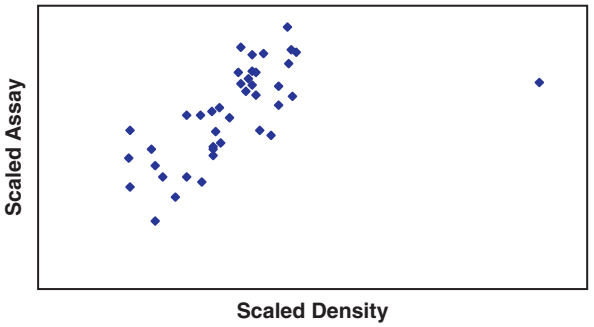

Fig. 11.4 Scaled pharmaceutical quality data. Both the density and assay are scaled by the standard deviation of the data for each variable

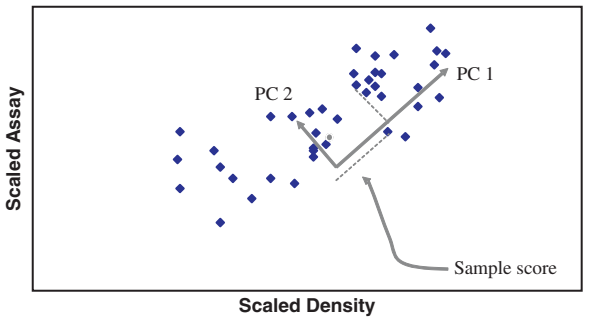

Fig. 11.5 Scaled pharmaceutical quality data showing both the first and second principal components for the data set. The first principal component is the direction of the maximum variation with in the data set. The second principal component is perpendicular to the first PC. The scores for each sample point are given by the projection of the data point onto the principal component vector

The above example is somewhat trivial because only two variables were involved. Let us now consider another example with more variables. In Table 11.3, a set of data describing the properties of 43 raw materials is shown. The variables that describe the raw materials are labeled QV1 to QV8.

Using commercial software, we can do a principal components analysis (PCA) of the data set using the same approach that was used for the first data set, i.e., scaling by standard deviation and mean centering. A few of the critical results are shown in the figures below. The loading (principal component) plot shows some results that are clearly interpretable, Fig. 11.6. The principal component plot shows how different variables relate to each other. In the plot the reader can observe that QV5 and QV8 are close to each other and therefore are well correlated to each other. QV1 and QV7 are also correlated.

A plot of the score values for each one of the 43 raw materials is shown in Fig. 11.7. The origin of the score plot corresponds to the average of the entire data set. The samples that are farther away from the origin are more likely to be possible

Table 11.3 Multivariable quality data set

Primary ID	QV1	QV2	QV3	QV4	QV5	QV6	QV7	QV8
1	110	2	2	180	1.5	10.5	10	70
2	110	6	2	290	2	17	1	105
3	110	1	1	180	0	12	13	55
4	110	1	1	180	0	12	13	65
5	110	1	1	280	0	15	9	45
6	110	3	1	250	1.5	11.5	10	90
7	110	2	1	260	0	21	3	40
8	110	2	1	180	0	12	12	55
9	100	2	1	220	2	15	6	90
10	130	3	2	170	1.5	13.5	10	120
11	100	3	2	140	2.5	8	140	*m*
12	110	2	1	200	0	21	3	35
13	140	3	1	190	4	15	14	230
14	100	3	1	200	3	16	3	110
15	110	1	1	140	0	13	12	25
16	100	3	1	200	3	17	3	110
17	110	2	1	200	1	16	8	60
18	70	4	1	260	9	7	5	320
19	110	2	0	125	1	11	14	30
20	100	2	0	290	1	21	2	35
21	110	1	0	90	1	13	12	20
22	110	3	3	140	4	10	7	160
23	110	2	0	220	1	21	3	30
24	110	2	1	125	1	11	13	30
25	110	1	0	200	1	14	11	25
26	100	3	0	0	3	14	7	100
27	120	3	0	240	5	14	12	190
28	110	2	1	170	1	17	6	60
29	160	3	2	150	3	17	13	160
30	120	2	1	190	0	15	9	40
31	140	3	2	220	3	21	7	130
32	90	3	0	170	3	18	2	90
33	100	3	0	320	1	20	3	45
34	120	3	1	210	5	14	12	240
35	110	2	0	290	0	22	3	35
36	110	2	1	70	1	9	15	40
37	110	6	0	230	1	16	3	55
38	120	1	2	220	0	12	12	35
39	120	1	2	220	1	12	11	45
40	100	4	2	150	2	12	6	95
41	50	1	0	0	0	13	0	15
42	50	2	0	0	1	10	0	50
43	100	5	2	0	2.7	1	1	110

outliers. The ellipse in Fig. 11.7 is called the Hotelling T^2 ellipse and is showing the 95% probability level for outliers.

PCA can be viewed as a method for approximating the original data set. The approximation is based on a linear combination of the principal components where the amplitude coefficients are the previously described scores. The approximation

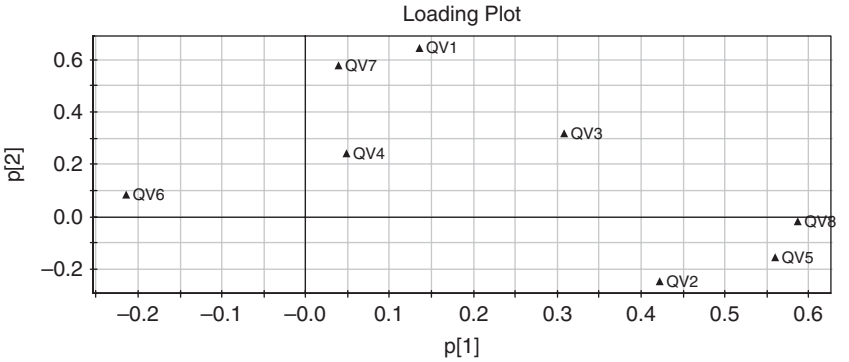

Fig. 11.6 Loading plot for the data set. The first principal component is plotted on the x-axis and the second principal component is on the y-axis. Variables that are close to each other are highly correlated

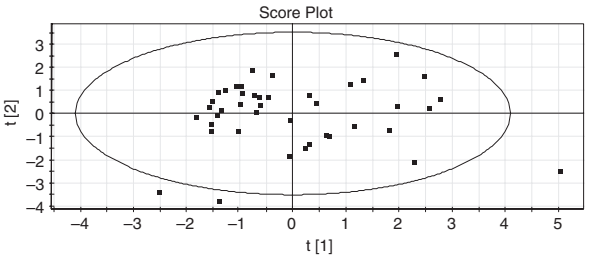

Fig. 11.7 Score plot of the data set

is exact when the number of principal components equals the number of variables in the data set. For most spectral data sets, a small number of principal components (also called factors) can be used to approximate the spectral data set very well. The determination of the correct number of factors can be done by a variety of numerical methods. Too many factors in the PCA model will over-fit the data and the model will not predict reliably. Most multivariate analysis software packages will suggest a suitable number of principal components. The suggested number is usually a good starting point; however, it is a best practice to verify the optimum number of principal components with additional independent test data.

Partial Least Squares (PLS) is an extension of PCA where both the x and y data are considered. In PCA only the x data is considered. The goal of the PLS analysis is to build an equation that predicts y values (laboratory data) based on x (spectral) data. The PLS equation or calibration is based on decomposing both the x and y data into a set of scores and loadings, similar to PCA. However, the scores for both the x and y data are not selected based on the direction of maximum variation but are selected in order to maximize the correlation between the scores for both the x and y variables. As with PCA, in the PLS regression development the number of components or factors is an important practical consideration. A more detailed discussion of the PLS algorithm can be found elsewhere [13, 14]. Commercial software can be used to construct and optimize both PCA and PLS calibration models.

We will now consider an example of a PLS calibration using NIR data. NIR transmission spectra from 155 tablets have been measured [15]. The tablet calibration set included samples with a range of assay values and several lots of production samples in order to capture the typical variations seen in the tablets. After scanning with the NIR instrument, the amount of active ingredient in each tablet was measured by HPLC. The weight of the tablet was about 800 mg and the target value for the drug content was 200 mg. We will use chemometrics to develop a model for the amount of active. This model could be used to monitor the stability of tablets over time in a non-destructive manner. For brevity, we will only outline the analysis procedure. Typical NIR transmission spectra for the pharmaceutical tablet are shown in Fig. 11.8. The broad, overlapping spectra with a considerable background is typical of NIR spectra. Derivative pre-processing can be used to remove the unnecessary background and elucidate the underlying peaks in the spectra. A first derivative spectrum is shown in Fig. 11.9.

A calibration curve showing the predictions of the PLS model versus the laboratory data is shown in Fig. 11.10. The clear quality of the calibration curve is evident. The calibration curve can be evaluated by several methods including outlier detection and removal and optimization of the spectral range used for PLS calibration. A detailed discussion of these issues can be found in the references [16, 14].

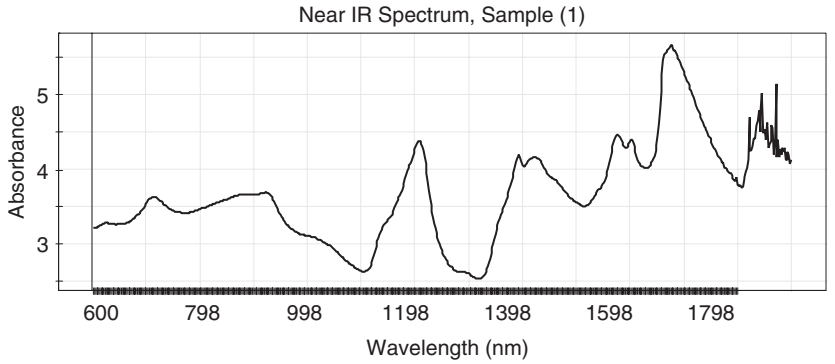

Fig. 11.8 NIR transmission spectrum of a pharmaceutical tablet

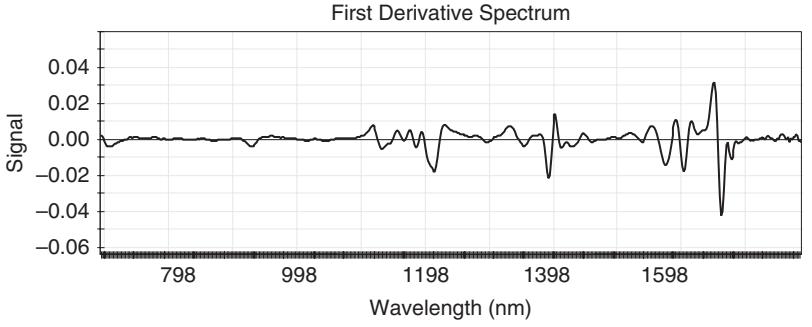

Fig. 11.9 Spectrum from Fig. 11.8 after first-derivative pre-processing

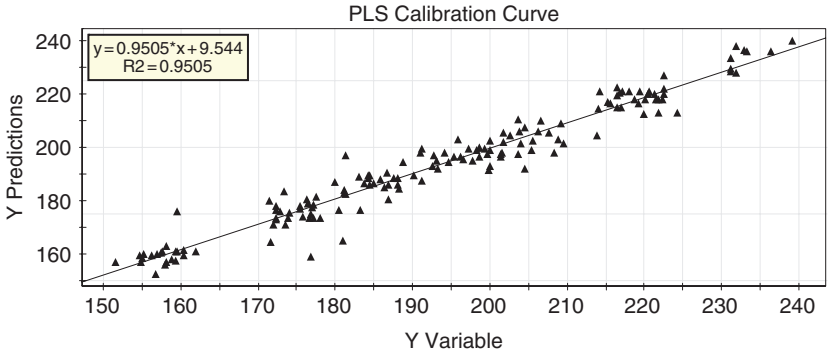

Fig. 11.10 Calibration curve for PLS method for tablet assay value

Common examples of quantitative methods done with NIR data and PLS regression are moisture, particle size, and assay [17].

11.4 Equipment Qualification

The qualification of an analytical instrument is described in a recent USP chapter [18]. The qualification of an NIR spectrophotometer or any piece of equipment can be divided into four parts: a design qualification (DQ), an installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ). The design qualification document describes the functional and/or operational specifications of the instrument and its specified purpose. The design qualification can be done by either the vendor or the end user. The IQ documents the correct installation of the equipment and specific components to assure that the system is qualified. The IQ for an NIR or Raman spectrometer would be similar to other instrumental IQ documents. The IQ for an NIR or Raman instrument should also include documentation of the instrument software and firmware versions. It is not uncommon to combine the hardware and software qualification documents for an NIR instrument. Often the IQ can be performed by the instrument vendor using a pre-approved protocol. The OQ for an NIR instrument will include a series of tests that verify the correct operation of the instrument. The tests specified in the USP chapter for NIR spectrophotometers <1119> are wavelength uncertainty, photometric linearity, and spectrophotometer noise. The wavelength uncertainty and photometric linearity tests are done with external standards. It is essential that the standards used are stable. The commonly used polystyrene internal reference may be subject to aging and degradation effects. A variety of NIST traceable standards are now available for reflection and transmission NIR instruments. The standards are available from instrument vendors or third party companies. A list of the available standards is given in Table 11.4.

Table 11.4 List of NIST traceable standard reference materials (SRM) for NIR

NIST SRM	Description
1920a	Original diffuse reflectance standard
2035	Transmission standard
2036	Improved diffuse reflectance standard

The wavelength uncertainty test verifies the accuracy and precision of the spectrophotometer x-axis. Typically, the x-axis will be in nanometers for a dispersion instrument and cm^{-1} for a FT instrument. The use of cm^{-1} for the spectral axis of an FT instrument is due to the mathematics of the interference term (Atkins 1996). The wavelength standards have stable isolated peaks usually based on a mixture of rare-earth oxides. The *center of mass of the peaks* is compared to standard values established on master instruments at National Institute of Standards and Technology (NIST). The typical tolerance values for the peak accuracy are $\pm$ 1 nm [19]. The observed precision values are usually much smaller than 1 nm due to the high reproducibility of modern spectrophotometers. The photometric linearity verifies that the y-axis of the spectrophotometer is linear over a typical reflectance range. The linearity is verified by scanning a series of standards of known reflectance (absorbance) values. The measured absorbance is plotted versus the standard values. The USP chapter specifies that the slope of this curve is equal to 1.0 ± 0.05 with an intercept of 0.0 ± 0.05. Photometric standards are available from instrument vendors and third party suppliers.

The operational qualification of an NIR spectrometer should also include tests measuring the instrumental noise. The USP chapter on NIR specifies that two kinds of noise tests be performed: a high-flux noise test and a low-flux noise test. For the high-flux test, the instrument noise is measured with a highly reflective (99% = R) reference standard. The test is referred to as a high-flux test because the high reflectance sample will yield a large amount of light on the instrument photodetector. The root-mean-square (RMS) noise is specified to be no greater than 0.8×10^{-3} for the high-flux measurement. The RMS noise is calculated by the Equation (11.1) given below

$$\sqrt{\sum \frac{(A_i - \bar{A})^2}{n}} \tag{11.1}$$

where A is the average absorbance and A_i is the absorbance for a given measure at a specified wavelength. In contrast the low-flux, noise measurement is done with a low reflectivity reference standard. The RMS noise for the low-flux test is specified in the USP chapter to be no greater than 2.0×10^{-3}.

The performance qualification (PQ) protocol should document that the entire system: spectrometer and software can perform as required. Typically, it is good practice to have a mock method included in the PQ protocol. It is also important to verify that the system will perform as expected under a variety of circumstances.

The qualification of a Raman spectrometer is described in USP chapter <1120>. In particular, the tests for the operational and performance qualification of a Raman spectrometer are described: x-axis precision, photometric precision, laser power precision and accuracy. The x-axis of the Raman spectrometer is the Raman shift measured in wavenumbers. Before the Raman shift can be determined, both the laser wavelength and spectrophotometer calibration must be determined. The precision of the Raman shift can then be measured using an American Society for Testing and Materials (ASTM) Raman standard material [20]. A commonly used Raman standard material is acetaminophen. The peak position of the known reference peaks can be determined visually, but is better done with a peak location algorithm. The USP chapter on Raman specifies that the peak location should not vary more than 0.3 cm^{-1} from the previous peak measurement. However, the chapter specifically states that this number can be increased according to the required accuracy of the measurement.

In contrast to NIR spectroscopy, the absolute values of the y-axis of a Raman measurement are difficult to quantify. Possible specific methods are described in USP chapter <1120> [21]. However, it is most common for quantitative Raman measurements to be done using the ratio of two peaks or other approaches which eliminate the need for absolute calibration of the y-axis. The USP chapter on Raman specifies that the photometric consistency or reproducibility specific integrated Raman band intensity should be on the order of 10%.

The USP chapter on Raman specifies that if possible the laser output should be monitored with a power meter from a reputable supplier. The variation of the laser power should be less than 25% and the laser power should be specified during the calibration development process. High laser power values can damage sensitive samples and low laser power values can yield Raman that are very noisy and are of low quality.

11.5 Method Validation

Method validation for NIR or Raman spectroscopic methods using chemometrics is outlined in USP chapter <1119>. The criteria for method validation are the same as other quantitative analytical methods: accuracy, precision, intermediate precision, linearity, specificity, robustness. However, because these methods are statistical in nature and are based on a previously validated analytical method, the validation of MVA methods is somewhat different than traditional analytical methods.

Accuracy of the MVA method refers to how closely the MVA method and the original laboratory method compare. The accuracy of a chemometric method is evaluated by comparing the predictions of the MVA model with the actual lab data for a set of validation samples. The validation samples should be from lots of material not used in the original calibration set. There are several mathematical ways to express the accuracy. The most commonly used approach is the standard error of prediction (SEP). The SEP is defined in Equation (11.2),

$$\text{SEP} = \sqrt{\sum \frac{(\text{NIR} - \text{LAB})^2}{n}} \tag{11.2}$$

where n is the number of validation samples. The SEP value should be close to the actual error of the original laboratory method. The actual error of the laboratory method should include normal sources of variation such as different analysts, different instruments, different lots of material analyzed on different days.

Precision of a chemometric method refers to the reproducibility of the method. For quantitative chemometric methods, it is important to test both the instrument and method precision. Instrument precision is done by repeating measurements on the same sample; method precision is the closeness of replicate sample measurements; while intermediate precision can be evaluated by running the same samples with different analysts on different days.

The *linearity* of a multivariate method is an important topic. Typically the linearity of a chromatographic method is evaluated by the R^2 (coefficient of determination) value of a recovery measurement. The validation of chromatographic methods is described in Chapter 8. R^2 is the fraction of variation in the y-variable explained by the linear fit [22]. In contrast, R^2 is not a good statistical parameter for multivariate methods. The linearity of a multivariate method is evaluated by the inspection of the residual values, in other words, the difference between the predictions of the multivariate model and the actual laboratory data. The residuals for the PLS assay described earlier in this chapter are shown in Fig. 11.11.

A linear model will have residuals that are random, or normally distributed. A non-linear model will have residuals that are not normally distributed. The USP chapter <1119> states that the linearity should be evaluated by examination of the residuals; however, no specific threshold or criteria are given. In the opinion of this author, visual inspection of the residuals using a normality plot is recommended. In Fig. 11.12, a normality plot of residuals is shown. The data points in Fig. 11.12 do follow a straight line, indicating a normal distribution of residuals, consistent with a linear model or calibration [14]. In some cases, the linearity of the model can be

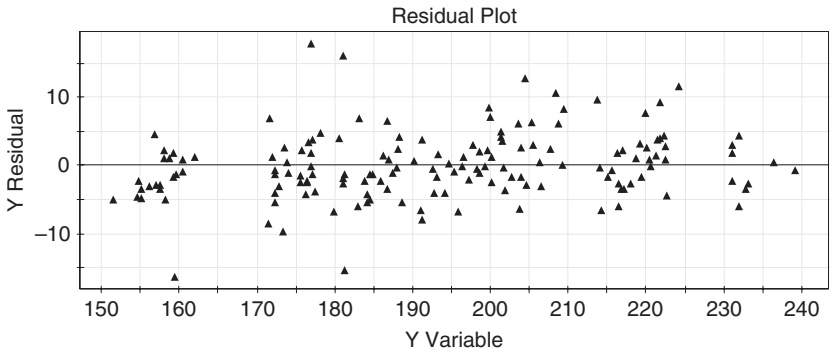

Fig. 11.11 Plot of y residuals versus y values

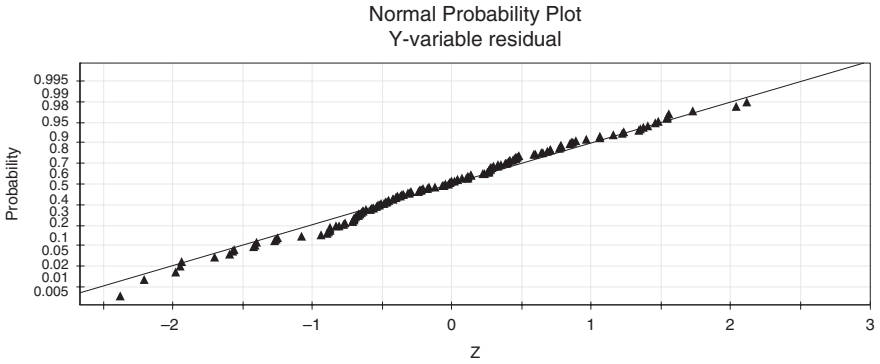

Fig. 11.12 Normal probability plot for residuals. When the residuals fall on straight line, the calibration under consideration is linear

improved by removing some of points in the normality graph which are probable outliers.

Method *specificity* is the extent the multivariate calibration is specific to the analyte of interest. With a PLS calibration, the specificity is documented by the regression coefficient of the calibration. The regression coefficient shows which wavelengths are most important for the PLS calibration. Important wavelengths may have either positive or negative regression coefficient values. The most important wavelengths should correspond to the absorption peaks of the analyte of interest. For example, the regression coefficient for a moisture model will have peaks at the known water absorbance band locations. In practice, the regression coefficient is often documented in the method development report. A regression coefficient from the PLS calibration for tablet assay described earlier in this chapter is shown in Fig. 11.13.

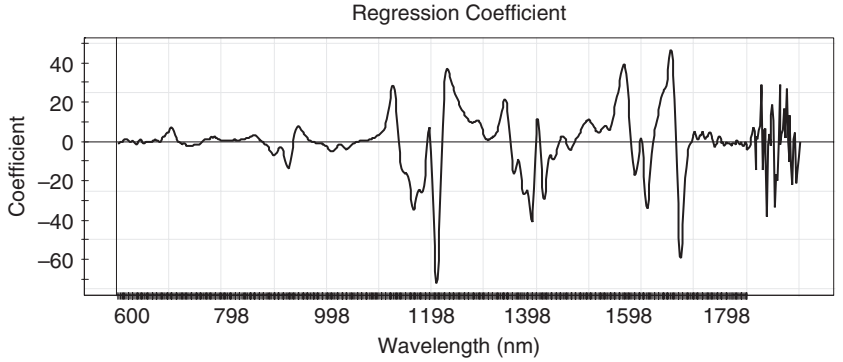

Fig. 11.13 Regression coefficient from the PLS model for tablet assay described earlier. The regression coefficient is a method for documenting and examination of which wavelengths are most important for the PLS calibration

The *range* of a multivariate calibration method is determined by the range of laboratory values in the calibration and validation data sets. A method is validated over the range of lab values of the samples used in the independent validation set. The range of the validation samples can also depend upon the application of the method. For example, in-process testing or testing where a limited number of samples are available may require a fairly small range of values because samples outside of a small range are not available or do not exist.

Multivariate calibrations must also be documented for *robustness.* There are several ways that this can be done. For example, minor changes in sample positioning can be used for robustness testing, for example the effect of rotation of an oval tablet on the predictions of PLS method. Another important issue relevant to method robustness is number of factors in the PLS model. If the PLS model has too many factors, the method will not be robust because the PLS method is fitting noise in the data [14].

11.6 Conclusion

This chapter reviews the use of spectroscopic methods for the quantitative analysis of pharmaceutical products. In recent years, there has been great progress made in the use of techniques such as NIR and Raman for real world pharmaceutical problems. USP chapters for NIR and Raman spectroscopy outline the requirements for equipment qualification and method validation. Because spectroscopic methods for quantitative analysis often involve the use of MVA and chemometrics, the approaches for method validation are somewhat different than that for traditional chromatographic methods.

Spectroscopic methods are mature enough that they can be used for stability testing in favorable cases. Spectroscopic methods have the advantages of being rapid and non-destructive as compared to other methods such as HPLC and Karl Fischer. In particular, NIR is an excellent replacement for Karl Fischer testing [23]. Raman spectroscopy has been shown to be an excellent tool for the investigation of polymorphs [7, 24]. These studies demonstrate that Raman can be applied to the testing for polymorph stability. As spectroscopic methods become more common in the pharmaceutical industry it is clear that they will be increasingly important for many types of stability testing.

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Chapter 12
Impact of Solid-State Characteristics to the Physical Stability of Drug Substance and Drug Product

Yushen Guo

Contents

Abstract In the drug development and commercialization process, the acceptable stability of the drug substance and drug product is one of the basic requirements for clinical studies, regulatory approval, and marketing. The stability of a drug product is related not only to the intrinsic chemical stability of the drug molecule, but also to the physical forms, manufacturing processes, interactions among formulation components, container closure systems, and storage conditions. In the past two decades, there has been a significant increase in the mechanistic understanding of the solid-state characteristics of pharmaceutical systems, along with the advancement of analytical techniques. Physical stability, as one of the quality attributes of drug products, should be designed into the formulation components and critical manufacturing steps using the Quality by Design (QbD) strategy to achieve intended shelf-life and

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product performance. In this Chapter, the major physical stability attributes of drug dosage forms are discussed with focus on the effects of solid-state properties of drug substance and manufacturing processes. The solid-state physical changes involving polymorphs, hydrates, amorphous forms, and the effect of water are highlighted.

12.1 Introduction

Stability, as one of the fundamental pharmaceutical quality attributes, needs to be evaluated during drug discovery and development process, and controlled and maintained for clinical trial materials and commercial drug products. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors, such as heat, humidity, and light. This will help to establish a retest period for the drug substance or a shelf-life for the drug product and determine their storage conditions.

Physical stability, along with chemical stability and microbiological stability of pharmaceuticals, has been of pharmaceutical interest for a long time [1, 2]. Although they likely respond differently to storage conditions, the three stability phenomena often interplay with each other to some extent. For example, a pH shift of a liquid dosage form during storage may alter the drug chemical stability and also the effectiveness of the preservatives. Another well-known example is the chemical degradation of aspirin tablets, which is often accompanied by the apparent odor of acetic acid. Current regulatory stability guidelines, although mostly focusing on chemical stability and related degradation products, have provided useful guidance on the scope and basic techniques for studying physical attributes of drug substances and drug products [3, 4]. Fundamental understanding of physical and chemical mechanisms behind any physical changes is essential to successful drug formulation development. This can only be achieved through the systematic evaluation of drug substances, excipients, and manufacturing processes.

In this chapter, the current understanding of the solid-state properties and characterization techniques of small molecule drugs are reviewed. Their relevance to the physical changes of drug substance and stability of some representative solid and liquid dosage forms are discussed. The special physical stability of large biological molecules (e.g., aggregation, denaturation, and adsorption) is beyond the scope of this article and readers are referred to references in the literature [5, 6].

12.2 Solid-State Characteristics and Physical Stability of Drug Substance

At the drug discovery stage, lead compounds are often prepared as DMSO solutions. The intrinsic chemical stability of the drug molecule in solution under different stress conditions is the main focus of the stability profiling of drug candidates. Candidates with poor chemical stability can be easily identified while their physical

stability is essentially ignored. More comprehensive stability evaluation on the drug substance and formulation is required as the drug development progresses.

Due to its intrinsic physicochemical properties and easy handling, solid-state is still the dominant physical state of drug substances (also known as active pharmaceutical ingredients or API) which can exist in different chemical and physical forms (Fig. 12.1). The usefulness of each form is related to its physicochemical properties (e.g., stability, solubility, and processability) and intellectual property (IP) protection potentials. This scheme is also undergoing dynamic changes with our increasing understanding of solid-state pharmaceutical systems. For example, it has been found recently that a co-crystal can also be formed between a salt and a neutral molecule [7]. Solid-state polymorph transformation is one of the major concerns for physical instability of pharmaceuticals, which can lead to drug product failure in the aspects of esthetic appeal, quality, and safety.

In the following sections, the solid-state physicochemical characteristics of drug substances and their physical changes related to polymorphism, hydrates, amorphous form, and the effect of water will be discussed.

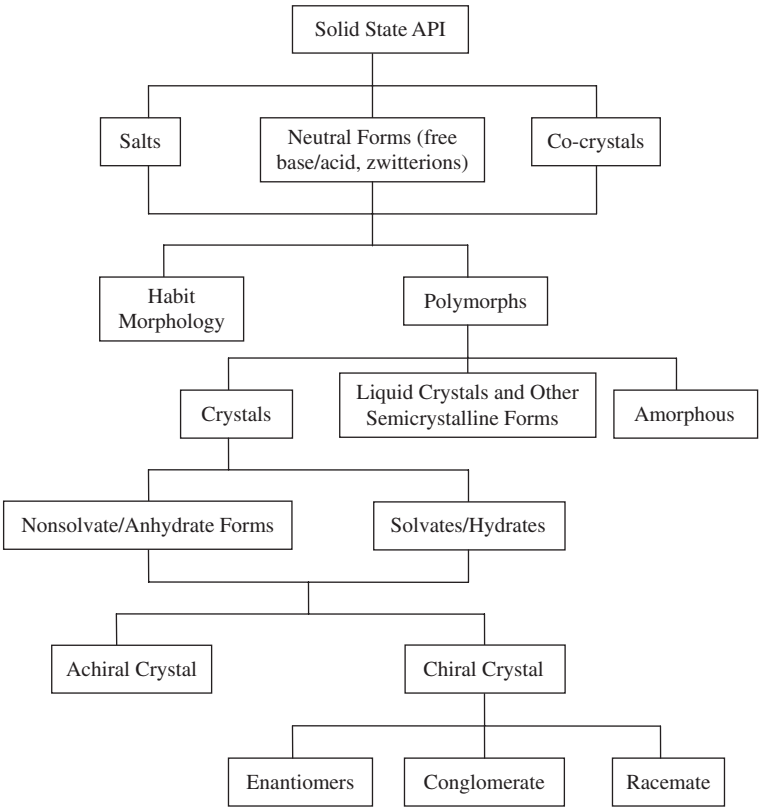

Fig. 12.1 Chemical and solid-state forms of active pharmaceutical ingredients

12.2.1 Polymorphism

Polymorphs are solid phases of a given compound with at least two different molecular arrangements in the crystal lattice or solid structure. In this paper, although the term *polymorph* is used in its broader definition [3], we discuss hydrates and amorphous form in different sections due to their special characteristics. Different polymorphs (including solvates and hydrates, also known as pseudopolymorphs) of the same drug substance display distinct physical and chemical properties [8]. The effect of pharmaceutical processing on drug polymorph transformation and potential impacts on the quality and performance of drug products is of increasing interest to both the pharmaceutical industry and regulatory authorities [9–11]. According to FDA guideline, the applicant needs to establish whether (or not) the drug substance exists in multiple solid-state forms, whether these affect the dissolution and bioavailability of the drug product [12].

Polymorphism screening is often conducted during the drug development process to evaluate the solid-state physical and chemical properties of a drug candidate. In addition to traditional analytical techniques, such as X-ray powder diffraction (XRPD) and differential scanning calorimetry (DSC), other analytical techniques have also been used for the qualitative and quantitative analysis of polymorphs [13, 14]. Most of the characterization techniques of pharmaceutical solids are complementary with each other [15]. They are useful for the exploratory physical stability evaluation when the solid-state complexity of a new drug candidate is unknown. After defining the solid-state space of potential changes and understanding the mechanisms of the changes, only the most suitable and sensitive analytical method will be validated and used in the formal stability studies and manufacturing process control. For a drug substance with several polymorph forms, it is critical to understand their relative stability (e.g., monotropic or enantiotropic relationship, energy–temperature diagram) and any interconversion conditions among them [16]. Decision trees for solid-state pharmaceuticals have provided a useful tool for this purpose [3, 17]. Theoretically, a thermodynamically metastable form will eventually convert to the most stable form. But the kinetics of the transformation is material and environmental condition specific, which adds to the uncertainty on the prediction of the real time physical instability of the metastable forms. The thermodynamically most stable crystal form is generally the preferred form for development to mitigate the risk of undesired phase transformations. The ability to control and produce a stable polymorph with a robust manufacturing process is critical for regulatory approval and marketing [3]. In 1998, Abbott Laboratories had to halt sales of its HIV protease inhibitor (ritonavir) in solution-filled softgel capsules, because a more stable, previously unknown polymorph (Form II) suddenly appeared, causing slowed dissolution and compromising the oral bioavailability of the marketed dosage form. Later it was found that ritonavir has at least five different crystalline forms [18].

Pharmaceutical co-crystals, as an emerging class of pharmaceutical material, provide an alternative to the salt form for drug molecules without ionizable groups [19, 20]. The major goal is to achieve potential improvement of physical properties (e.g., enhanced dissolution rates, mechanical properties, avoidance of moisture sensitivity,

and enhancement of bioavailability). Their developability and stability need to be evaluated on a case-by-case basis.

12.2.2 Hydrates

Hydrate is a special class of solvate form where the solvent molecule in the crystal structure is water. Water can have a significant effect on the physical stability of drug substances due to the wide presence of moisture and the use of water in many manufacturing processes. Many drug substances can form hydrates when crystallized from aqueous solvents or when exposed to higher relative humidity (RH). The water molecules can be incorporated in the crystal lattice in either a stoichiometric or non-stoichiometric way. Thermodynamic stability of hydrate forms may depend on the intrinsic molecular property, crystal lattice structure, and the environmental factors (e.g., relative humidity). The nature of water–solid interactions is less predictable and requires understanding at the molecular level [21]. This often involves the use of several complementary analytical techniques [22]. Humidity-controlled thermogravimetric analysis (TGA) and XRPD have been used to assess the physical stability of pharmaceutical hydrates [23]. The relative stability of the anhydrate and hydrate forms as a function of relative humidity can be evaluated by solution-mediated transformation in aqueous–organic slurries [24]. Diffuse reflectance infrared Fourier-transform spectroscopy (DRIFTS) can be used to monitor the formation of hydrogen bonds between the molecules of the anhydrous drug substance and moisture uptake from the atmosphere [25]. Other nondestructive identification and potential process analytical technologies (PAT) for drug hydrate forms include Near-infrared (NIR) Spectroscopy and Terahertz Pulsed Spectroscopy [26, 27].

The dehydration process of the hydrate form of a drug substance usually follows a multi-step mechanism (Fig. 12.2). The process is related to the physical

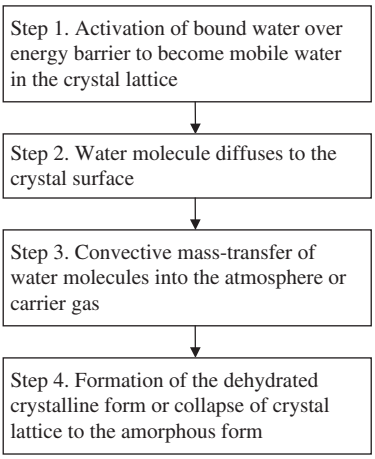

Fig. 12.2 Mechanism of the thermal dehydration process

Table 12.1 Methods to control hydrate form during drying process

Method	Notes
Sampling and off-line analysis	• Water content test with Karl Fischer titration or Loss on Drying (LOD) at various time • XRPD and/or DSC confirmation of crystalline form • Slow
Near-infrared (NIR) spectroscopy	• Fast, online real time and PAT possible • Differentiate free and bound water • Require calibration models
Dew-point hygrometer	• Fast, online real time and PAT possible • Base on minimum RH for stable hydrate form at different temperature (from water vapor sorption isotherms) • Avoid interference of organic solvents

characteristics of the drug substance, such as the crystal structure, the particle size, and interaction forces (e.g., hydrogen bonds) between water and drug molecules. The reversible hydration–dehydration process is often observed when water molecules are located in channels of the crystal lattice. When a hydrate form is selected for development, the endpoint control of the drying process is critical for the physical stability and quality of the drug substance. There are several approaches to control the drying process (Table 12.1), whose objective is to remove free residual water and solvent but preserve crystal-bound water to avoid polymorph transformation or amorphorization. NIR spectroscopy has been used to quantify the water content, either with the probe in direct contact with the wet cake or to measure the water vapor composition in the dryer effluent. A low-cost alternative such as dew-point hygrometer can also be used by measuring the online water content of the dryer vapor effluent [28].

12.2.3 Amorphous Form

The amorphous form, in contrast to the crystalline state, is a thermodynamically metastable solid state which lacks long-range order at the molecular level [29]. Many drug candidates during the discovery phase are first prepared as partially or totally amorphous forms unintentionally due to isolation methods (e.g., lyophilization) and/or a higher impurity level. As a solid state of increasing importance, the amorphous phase can be formed in many ways either intentionally or unintentionally during the manufacturing processes (Fig. 12.3). From the physical stability standpoint, the intentionally formed amorphous drug substance should be preserved and stabilized, while the unintentionally formed one should be prevented. A totally or partially amorphous drug substance often goes through spontaneous transformations toward the thermodynamically lower energy crystalline states. These changes may

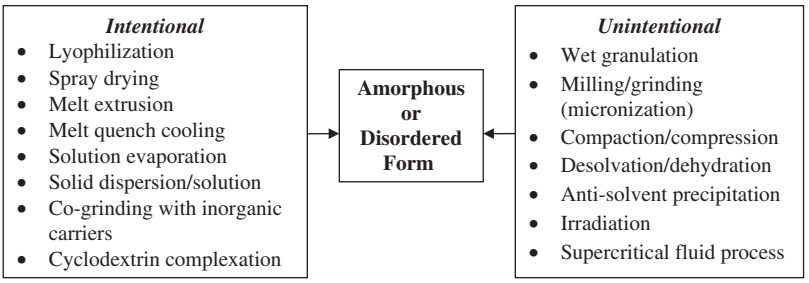

Fig. 12.3 Formation of amorphous or disordered forms during drug manufacturing processes

occur during manufacture or storage of pharmaceutical dosage forms containing the amorphous drug substance, thus influencing their stability and bioavailability.

There have been many attempts to evaluate physical stability of amorphous drug substances and excipients using both theoretical models and experimental techniques, with the focus mainly on the crystallization of amorphous model compounds [30]. For amorphous materials, both chemical and physical instability are related to molecular mobility which increases with increasing temperature. As an empirical rule, amorphous pharmaceutical materials should be stored 50° below its glass transition temperature (T_g) to minimize potential chemical and physical instability [29]. The crystallization process often follows the mechanism of three-dimensional growth of nuclei after an induction period. Amorphous forms of the same compound made by different methods can have different physical stabilities due to kinetic differences of the crystal nucleation and growth processes [31]. When evaluating the physical stability of amorphous systems, the properties of the crystalline counterpart should also be considered regarding the enthalpic driving force for crystallization and activation energy for nucleation [32].

In some cases, amorphous forms are prepared intentionally, to increase the dissolution rate and bioavailability of poorly water soluble compounds. One frequently used strategy is the formation of stable amorphous dispersions (or solid-solutions) with hydrophilic excipients (e.g., carbohydrates and polymers) [33]. Excipients with higher glass transition temperature can act as anti-plasticizers, while those with hydrogen bond donor and/or acceptor groups can interact with the counterpart functional groups of the drug molecules. Both will stabilize the amorphous form by lowering the molecular mobility of the drug in the solid dispersions. Spectroscopic and thermal analyses are routinely used to evaluate the interactions between the drug and the excipient molecules [34]. To evaluate binary amorphous systems, including the plasticizing effect of water, Gordon-Taylor equation (12.1) and related approaches have been widely used.

$$T_g = \frac{(W_1 \cdot T_{g1}) + (K \cdot W_2 \cdot T_{g2})}{W_1 + K \cdot W_2} \tag{12.1}$$

where T_g, T_{g1}, and T_{g2} are the glass transition temperature of the mixture and the individual component, respectively. W_1 and W_2 are the weight fraction of the

components. K is a model specific parameter. The stabilization effect of anti-plasticizers often requires stronger interactions (e.g., hydrogen bonding) between drug and excipient molecules, which often reflected in a higher T_g than that predicted from the equation [35].

Besides hydrophilic excipients, some inorganic materials with high surface area, such as kaolin, hydrotalcite, silica, and their mixtures can also be used to promote and stabilize the amorphous drug form when co-milled with a crystalline drug substance [36]. Hydrogen bonding, and sometimes, a solid-state acid–base reaction between the drug molecules and the inorganic materials are believed to be responsible for the stabilization effect. There is a potential risk that a small number of the residual microcrystals, usually not detectable by conventional analytical techniques, may act as seeds to accelerate the crystallization of the amorphous drug during storage. The physical stability of the above two amorphous drug-carrier systems is often related to both the carrier type and the drug-to-carrier ratio. When the drug load is over a certain limit, the physical stability will decrease dramatically. The presence of other components (e.g., water) should also be taken into consideration with all amorphous systems described above.

Hygroscopicity of amorphous drug substances is an important physical property with impact on both drug stability and handling characteristics. One of the major drawbacks of the amorphous form is its higher moisture sorption tendency compared to its crystalline counterpart. Absorbed water, as a very effective plasticizer ($T_g \approx -136$ K), can significantly lower the glass transition temperature of an amorphous drug substance [37]. With increasing molecular mobility, both chemical and physical stability can be affected [38, 39]. Dynamic water vapor sorption instruments are widely used for the evaluation of hygroscopicity of drug substances and excipients. In recent years, we have seen the increased use of environment (temperature and relative humidity) controlled analytical techniques, such as variable temperature/relative humidity XRPD and moisture-induced thermal activity traces microcalorimetry [40]. The commonly used techniques to evaluate the solid-state physical properties of amorphous drug substances and excipients are summarized in Table 12.2. Of course, almost all of the techniques are also applicable to the characterization of other solid-state forms.

The surface defects and amorphous spots that are common for mechanically processed materials (e.g., milling) can have a significant effect on their downstream performance (e.g., powder flow, static charge). They can also be the centers for chemical degradation or physical transformation, leading to product instability, because the molecular mobility on the surface is likely to be higher than that in the bulk. To make things worse, the absorbed water is mainly localized in the amorphous region (i.e., the surface) which will result in much higher instability, even though the measured total water content may not be significantly high. The phase imaging atomic force microscope (AFM) provides a valuable tool in visualizing the metastable nature of partially disordered material [41]. Inverse gas chromatography (IGC) has been used to measure surface area and surface energy. It can detect small changes in surface characteristics caused by processing and batch-to-batch product variations that could not be detected with most traditional techniques [42, 43].

Table 12.2 Analytical techniques for physical properties and stability studies of amorphous materials

Molecular level/spectroscopy
FT-IR, ATR, DRIFT
FT-Raman
Solid-state NMR
Near infrared spectroscopy (NIR)
Terahertz pulsed spectroscopy
Surface/local
Polarized light microscopy (PLM)
Scan electron microscopy (SEM)
Microscopic image analysis
Atomic force microscopy (AFM)
Inverse gas chromatography (IGC)
Bulk
Thermal microscopy
Differential scanning calorimetry (DSC)
Modulated differential scanning calorimetry (MDSC)
X-ray powder diffractometer (XRPD)
Small-angle X-ray scattering (SAXS)
Water vapor sorption
Isothermal microcalorimetry
Intrinsic dissolution
Triboelectric property

12.2.4 Solid-State Physical Change Mechanism and Stress Stability

Solid-state physical change of drug substances is one of the major concerns of pharmaceutical stability due to its potential effects on the drug dissolution rate and bioavailability. Closely related to the mechanism of solid-state chemical reactions [44, 45], the physical changes of crystalline drug substances may include four steps as described in Fig. 12.4.

Stress stability study is widely used for the chemical stability evaluation of drug substances. This helps to identify the likely degradation pathways and degradation products. For physical stability, a similar approach can be used for both crystalline and amorphous drug substances. The purpose of physical stress stability study is to provide evidence of how the physical quality of a drug substance is influenced by a variety of environmental factors. Drug substances can be stored under different stress conditions to check for physical changes (Table 12.3). This can help to identify the likely polymorph and pseudopolymorph forms, and to establish the interconversion relationships among the forms. Other objectives include finding the most stable polymorph and validating the analytical techniques that are sensitive enough to detect the changes. Results from these studies will form an integral part of the information provided to regulatory authorities and the rationale for the

1 *Loosening of intermolecular interactions*
- Non-covalent bonds, such as hydrogen bonding, van der Waals force
- Under the effect of external energy of environmental factors (e.g., heat, light, mechanic forces, solvent, and moisture)
- Often start on the crystal surface or points with defects

2 *Rearrangement of molecule orientations or intermolecular confirmations*
- Partial or total loss of solvent or water molecules from solvate or hydrate forms may happen depending on storage conditions
- Amorphous phase may be formed as the intermediate phase
- Possible chemical changes depending on intrinsic molecule reactivity and environmental factors (e.g., temperature, presence of oxygen and moisture)

3 *Nucleation of new phase*
- Homogeneous or heterogeneous

4 *Crystal growth and formation of new form*

Fig. 12.4 Solid-state physical change mechanism of crystalline material

Table 12.3 Examples of physical stability stress testing of drug substances

Stress Testing Condition		Objective and Methods
Heat	40–80°C up to 4 weeks with or without control of relative humidity (RH)	– To evaluate polymorph changes and can be combined with stress chemical stability studies – Techniques for fast screening with small amount of sample (DSC, TGA, combination of hot stage with XRPD and spectroscopic instruments)
Moisture	60–95%RH at 25–40°C	– To evaluate polymorph change or hydrate formation, the following methods can be used – Saturated salt solutions (i.e., NaCl 75%RH) – Slurry in water-organic solvent mixtures – Water vapor sorption instrument – XRPD and spectroscopic instruments with temperature and RH% control
Mechanic	Milling or grinding compaction	– To evaluate potential physical changes under mechanic forces during processing (e.g., micronization, tableting) – Mortar and pestle or small mechanical ball mill – Carver press
Vacuum	Ambient to 45°C under vacuum for up to 24 h	– To evaluate the formation of desolvated or dehydrated forms from solvate/hydrate forms under typical drying condition – Vacuum oven or desiccator
Photo	ICH conditions (Q1B)	– To evaluate potential physical changes related to chemical degradations (e.g., coloration and polymorph change)

selection of tests, specifications, and analytical techniques during accelerated and long-term stability studies. It should be noted that the listed stress stability tests are neither comprehensive nor mandatory. They should be customized based on the characteristics of the drug substance, the intended dosage form of the drug product, and the manufacturing processes involved. Effect of milling and grinding are tested for drug substances that need micronization. Gamma radiation, as a sterilization method, can affect the solid-state physicochemical properties of some drug substances [46]. It is essential that potential chemical degradation also be checked for these physical stability samples. Most of the time, the physical stability of drug substance can be incorporated into the stability programs with the physical attributes (e.g., color/appearance, polymorph, water content, and loss on drying) as tests and specifications in the study protocol.

12.3 Physical Stability of Drug Products

Physical stability of drug products, generally dosage form specific, can be affected by both environmental factors (e.g., moisture, heat, light, and oxygen) and product-related factors (e.g., drug substance, formulation composition, manufacturing processes, and packaging). Physical stability of drug products is concerned with not only their esthetic appeal, but also their quality integrity and safety. Consequences of physical instability of drug products are often manifesting as failures of product attributes (e.g., rheological and mechanical properties, dissolution, and efficacy). Mechanisms governing their physical changes are generally more complicated due to presence of various functional excipients and the use of different manufacturing processes. Stability studies should focus on those changes during manufacturing processes and storage that are likely to influence the quality, efficacy, or safety of the drug products. The physical quality of dosage forms cannot be tested into final drug products. It should be achieved by thoroughly understanding the physicochemical properties of the drug substance, excipients, manufacturing processes, and container-closure systems. In the following sections, general considerations of physical stability of drug products and several representative solid and liquid dosage forms are discussed.

12.3.1 General Considerations

The physical tests which should be included in the stability program depend on the nature of the drug product. Table 12.4 lists the general physical stability attributes of major pharmaceutical dosage forms. It is not intended to be exhaustive, nor is it expected that every listed test be included in the design of a stability protocol for a particular drug product. In general, organoleptic properties (e.g., color and appearance) should be evaluated for all dosage forms. Disintegration and dissolution profile are measured for solid dosage forms. Water content can affect various physical and chemical transformations that may occur during manufacturing processes or

Table 12.4 Examples of physical attributes in pharmaceutical dosage forms

Dosage Form	Physical Properties and Stability Attributes
Solid	
Tablets	Disintegration, dissolution profile, hardness, friability, water content, coating integrity if applicable
Hard gelatin capsules	Brittleness, disintegration, dissolution profile
Soft gelatin capsules	Disintegration, dissolution, pH, leakage, pellicle formation
Powder/granules for oral solution/suspension	Water content, hygroscopicity, reconstitution time Reconstituted products in use stability are evaluated as described in the solution and suspension section below
Dry powder inhaler (DPI)	Particle size distribution and physical properties (e.g., shape, crystal habit, morphology, surface texture)
Suppositories	Hardness, softening range, crystallization, content uniformity, dissolution (37°C)
Liquid	
Oral solution	Formation of precipitate, clarity, coloration, pH, viscosity
Large volume parenterals (LVPs)	Coloration, clarity, particulate matter, pH, volume, osmolarity
Small volume parenterals (SVPs)	Color, clarity, particulate matter, pH, formation of precipitate, osmolarity, Powder for Injection Solution: color, water content, reconstitution time, and in use stability of solution
Oral suspension	Polymorphic conversion, pH, viscosity and other rheological properties, particle size distribution/morphology/habits, settling/caking/redispersibility, content uniformity, dissolution profile
Solutions/suspensions for inhalation and nasal sprays	General: interaction of drug with internal container closure system components, internal pressure, weight loss, delivery rate, unit dose reproducibility, net weight dispensed, water content, discharge rates, weight loss/leak rate during storage Solution: foreign particulate matter, pH, osmolality, viscosity, droplet size distribution Suspension-type aerosol: see Oral suspension above
Emulsion	Phase separation (creaming or cracking), pH, viscosity, particle size distribution of dispersed globules
Others	
Semisolids	Particle size change, crystalline form change, viscosity, loss of consistency and flow characteristics (caking, coalescence, bleeding), drug release rate change
Transdermal patches	In-vitro release rate; leakage; peel and adhesive forces

storage of solid dosage forms. As physical stability characteristics of drug dosage forms are the combination of many attributes, in many cases, in vitro release rate or dissolution profile may be used to assess comprehensive product physical stability and product sameness of scale-up or post-approval changes. Stress and accelerated stability testing, although commonly used to predict chemical degradation, may not always be reliable for physical stability prediction of drug products [47]. Some changes in physical attributes (e.g., melting of creams, softening of suppositories) under accelerated conditions may not be realized under normal storage environment.

12.3.2 Solid Dosage Forms

Physical stability of solid formulations is closely related to the solid-state properties of the drug substance as described in the previous sections (12.2). Solid-state transformation of the API is one of the major concerns during development and manufacture of solid oral dosage forms [48, 49]. Unintentional polymorph conversion or amorphorization can result from different manufacturing processes (see Fig. 12.3 in Section 12.2.3). Micronized drug substances are often used in drug products in order to achieve acceptable content uniformity and desired performance (e.g., dissolution rate). The micronization process should be fully validated and the equipment, operation condition, and process controls (e.g., rate of feed, air pressure, air flow rate, fed particle size, cycles) be described in detail in regulatory submissions. Specific physical attributes (e.g., particle size distribution, crystal forms, and amorphous content) should be optimized and controlled to ensure performances in drug products.

12.3.2.1 Powder Mixtures

Powder mixtures and blends usually contain an API and at least one excipient (e.g., diluent/carrier). It is not only a dosage form itself, but also widely used to manufacture other dosage forms (e.g., powder for oral solution/suspension, powder for injection, powder for direct compression, and powder for dry powder inhaler). The effect of the shape and size of the components on the physical stability of the drug-carrier binary mixtures has been extensively studied [50]. Inhomogeneity resulting from a change in the shape of the carriers seems to be smaller than that resulting from size differences. The dry powder mixture for injection, as an alternative to lyophilization, can be filled directly into presterilized vials using suitable filling equipment under aseptic conditions. In this case, the compatibility among ingredients and content uniformity need to be evaluated. The content uniformity is also a critical physical parameter of the blend for the manufacture of other oral dosage forms (e.g., capsule, tablet). Online NIR and Raman spectroscopy have been used for the determination of blend endpoint and confirmation of blend uniformity [51, 52].

Dry powder formulations for inhalation usually comprise a mixture of the micronized drug substance and a coarse carrier (i.e., lactose). The physical stability is affected by both the drug substance and the excipient/carrier. High energy milling may induce defects and amorphous regions in the crystalline material, especially on the surface. The polymorph change of the micronized crystalline drug particles can influence both its chemical stability and its affinity to the large carrier particles during interactive mixing process [53]. Attenuated total reflectance FTIR spectroscopy (ATR) has been used for surface analysis of the powder mixtures. A small proportion of finer particles can drastically influence the surface of powder mixtures, due to their large contribution to the specific surface area [54]. This may affect the deaggregation and dispersion efficiency of a formulation, and cause potential long-term stability issues. When dry powder inhaled drugs are prepared by spray drying from aqueous or aqueous–organic solvent mixtures, the solvent system and processing

conditions may have significant effect on the physical properties and aerosolization behavior of spray-dried drug particles [55].

12.3.2.2 Tablets

Chemical stability, disintegration rate, dissolution profile, friability, and hardness are the major stability attributes for the tablet dosage form. An unoptimized tablet formulation may become soft or very hard after storage, with altered dissolution profiles, and as a result, its dissolution profile and bioavailability may not be appropriate. If effervescent products are not properly formulated, manufactured, and packaged, the premature acid–base reaction will cause the product's self-destruction.

The interaction of moisture with the drug substance and excipient can significantly affect the physical stability of the final drug product [56]. These changes may alter the bioavailability and therapeutic efficacy of a drug product, even though the assayed drug potency and purity may not be significantly affected. Carbamazepine tablets may lose one-third or more of their oral bioavailability when exposed to excessive moisture. Reversible contraction of the crystal lattice due to anhydrate–hydrate interconversion of an API can cause the loss of the tablet integrity during storage. Protective packages are required to ensure drug stability during its shelf-life. NIR and Raman spectroscopy, implementable as PAT tools, allow direct drug hydration state monitoring during wet granulation and drying processes [57, 58]. The effects of moisture content and storage conditions on the physical stability of tablets have been reviewed [59]. Sometimes, physical instability of solid dosage forms may be due to excipients which can lose their functionality under accelerated stability storage condition [47, 60]. This is especially important for special drug delivery systems such as controlled release formulations since changes in functional excipients can have a critical effect on the intended product performance.

For high potency and low drug loading solid formulations, physical change (e.g., polymorph transformation) may not be easily monitored due to interferences from excipients and the detection limit of most analytical techniques. In these cases, surrogate drug product performance testing (e.g., dissolution testing) can generally provide adequate control of polymorph changes for poorly water soluble drugs, which may influence bioavailability and bioequivalence (BA/BE) of drug products. Only in rare cases would polymorphic form characterization in the drug product be recommended [61].

Many tablets are sugar coated or film coated, which includes enteric coated and delayed release products. The volume of coating solution, rate, and temperature are critical process parameters and need to be validated to ensure the long-term physical stability of the final product. It has been well known that the shellac undercoat used for sugar-coated tablets has presented disintegration/dissolution problems. Improper temperature control during the drying process has also been found to cause dissolution failures in aged tablets.

Particle size profiles are important for tablets made by a wet granulation process. The size and the type of granule can affect the pore size in a tablet and can have

an effect on drug dissolution. For example, the dissolution failure of a coated tablet formulation was attributed to a change in the milling screen size, yielding a granulation with larger granules. The slower dissolution profile was caused by increased penetration of the coating solution into the tablet due to larger pores.

12.3.3 Liquid Dosage Forms

Liquid formulations are frequently used in oral, parenteral, inhaled, and topical routes. They may face some common physical stability challenges such as inhomogeneity due to phase segregation, drainability issues due to viscosity changes, and coloration due to oxidation or other degradation reactions. Storage of liquid formulations in a refrigerator or freezer, with the objective to minimize potential chemical degradation and microbiological contamination, can decrease the solubility and potentially cause product haziness/cloudiness due to precipitation of either active drug or functional excipients. Even for room temperature stable formulations, the effect of short-term temperature excursions outside the proposed label storage condition should be evaluated. Specific stability requirements for nasal/inhalation sprays and inhalation solutions/suspensions can be found in FDA guidelines [62].

The liquid formulation for parenteral administration requires additional physical and microbiological functionalities, such as syringeability, sterility, osmolarity, and pyrogen freedom. The particle size change can influence the syringeability of injection of a suspension formulation as well as the level of irritation at the site. Terminal sterilization such as autoclave or gamma irradiation may affect the physical stability of the dosage form. Both formulation and container systems should be evaluated [63].

12.3.3.1 Solutions

Solution formulations are molecularly dispersed homogenous systems, and include oral (e.g., syrups, elixirs), topical, otic, nasal, ophthalmic, irrigation, and parenteral drug products. Solid-state characteristics of raw materials can have an effect on this dosage form in at least two ways. First, particle size and physical characteristics of raw materials can affect their dissolution rate in the manufacturing process. Drug substances of a finer particle size usually dissolve faster than those of a larger particle size. The metastable form (e.g., amorphous) also has a higher dissolution rate and apparent solubility than the stable crystalline form. Second, the solid-state properties of the drug substance may influence the equilibrium between the liquid phase and potential solid phases with respect to supersaturation and precipitation. Heating or sonication may be necessary to increase the dissolution rate of some drug substance or excipients. However, the maximum upper concentration limit should be based on the thermodynamic equilibrium solubility of the most stable crystalline form at its intended storage temperature, with consideration of potential temperature fluctuation and effect of excipients, to avoid supersaturation. If refrigerator storage is required due to chemical stability concern, potential precipitation should be evaluated and easy redissolution of any precipitation upon warming should be

confirmed. The precipitate may be intentionally isolated to examine the solid form. When the precipitate cannot be easily redissolved, there is a great chance that the original solution was supersaturated, a more stable crystalline form was produced, or there was an interaction or incompatibility issue between the drug substance and an excipient.

Injectable liquid formulations are frequently lyophilized to minimize chemical degradation, especially for biological drugs. Excess residual moisture may result in greater product instability. The molecular level and mechanistic understanding of water–solid relationships are important to the manufacture and storage of freeze-dried pharmaceuticals [64]. The control of the drying process and residual moisture level has a great effect on the physical and chemical stability of the drug product. Physical inspection would include the presence of correct volume in the vial and the appearance of the final cake, which may collapse if the sublimation is incomplete. Change in the physical form of the drug substance can also increase the reconstitution time. If the drug is not completely dissolved, partial loss of potency may occur.

Parenteral formulations are often reconstituted or diluted in the clinic and hospital with standard solutions (e.g., 0.9% sodium chloride, 5% dextrose, and Ringer's solution). Compatibility with these diluents and administration sets, as well as in-use stability, should be evaluated. Co-administration of multiple drugs via a Y-site connection is common in hospitals. Precipitation, color change, decomposition or adsorption of the active drugs can occur. Turbidimetric and particulate measurements are often used for the evaluation of solution physical stability in addition to visual inspection [65].

12.3.3.2 Suspensions

Suspension dosage forms contain uniform-sized fine particles with acceptable sedimentation rates. Major stability factors include attributes such as particle size distribution, content uniformity, viscosity, drainability, re-suspendability, dissolution rate, pH, and zeta potential. Small particles have a high degree of surface free energy and an increased tendency to aggregate, and eventually fuse together into a non-dispersable cake. Suspending agents (e.g., surfactants and polymers) are often used to increase physical stability and to make easily redispersed suspensions. Viscosity is important from both processing and dosing aspects. Proper viscosity is required to minimize segregation, as well as to maintain proper drainability. To avoid segregation, many suspensions require continuous or periodic agitation during the filling process. pH shifts of suspension dosage forms during storage may affect the chemical stability and solubility of the active drug in solution.

Stokes' Law provides useful information in determining the main parameters which control the sedimentation rate in a suspension (Equation 12.2)

$$\nu = \frac{2r^2(\rho_s - \rho_l)g}{9\eta} \tag{12.2}$$

where

v = velocity of sedimentation (m/s)
g = gravitational acceleration (~ 9.8 m/s^2)
ρ_s = density of solid (kg/m^3)
ρ_l = density of liquid (kg/m^3)
r = radius of the particles (m)
η = dynamic viscosity of the liquid (Pa·s or kg $\cdot$ m^{-1} $\cdot$ s^{-1})

The physical stability of pharmaceutical suspensions has long been a formulation problem. The effects of particle size, crystal growth due to fluctuating temperature, and solvent-mediated solid-state transformation between different polymorphs of some drug substances are well known [66]. Particle size and crystalline form changes in suspension dosage forms are regarded as level 2 and level 3 changes, respectively, which could have a significant impact on the product quality and performance [67]. Particle size and habits may affect not only the segregation rate but also the dissolution rate of suspension products. The polydisperse system is more likely to form compact aggregation than the monodisperse system. The suspension should be within the particle size specification during its shelf-life. Microscopic image analysis and laser diffraction are often used to measure the particle size distribution. For laser diffraction technique, it is crucial that test procedure and instrumental parameters (e.g., apparatus and accessories, calculation theory, correction principles, software version, sample placement, laser trigger condition, measurement range, beam width, average of runs) be defined thoroughly to ensure consistency and repeatability. Modification of surface morphology (habits) can also affect the physical stability of the suspension formulation in term of sedimentation volume and redispersibility even the polymorphic state and pharmacokinetic profile are not altered [68].

For drug substances with solvates, hydrates, and anhydrate forms, the potential interconversion among them in a suspension formulation need to be evaluated [69]. The most stable polymorph of the drug substance in the vehicle system is generally selected for development. If the drug substance is partially soluble in the dispersion media, Ostwald ripening may occur with the growth of larger crystals from those of smaller size which have a higher solubility than the larger ones. This is likely more serious when formulations experience repeated freeze and thaw cycles. As with other liquid or semisolid formulations, specific instructions should be provided for those that cannot tolerate low temperature storage due to physical instability. An amorphous drug substance may be used when no stable crystalline form is available or when dissolution enhancement is required, although this creates additional uncertainty due to its inherent thermodynamic instability. Stabilizing agents should be evaluated and used to prevent potential crystal nucleation and growth during storage.

In recent years, there has been increased interest in the application of nanoparticles for poorly water soluble compounds due to the enhancement of dissolution rates and bioavailability [70, 71]. Nano-particles can be used for oral, injectable,

pulmonary, and topical formulations. Stabilizers (i.e., surfactants, co-surfactants, polymers) are required to ensure the formulation with acceptable physical stability. Often, nano-suspensions are lyophilized or spray-dried with excipients as the carrier and used for the manufacture of other dosage forms. The whole process should be investigated and optimized to ensure the physical and chemical stability of the final formulations.

12.4 Conclusion

The physical stability of drug substances and drug products should be an integral part of the systematic approach to the stability evaluation of pharmaceuticals due to its potential impacts on drug chemical stability, performance, and safety. Physical stability, as one of the pharmaceutical quality attributes, must be built into the drug substances and products based on high level mechanistic understanding of the pharmaceutical materials and critical manufacturing processes. Identification of different solid forms of a drug substance, determination of their physicochemical properties, thermodynamic stability, and interconversion conditions are essential to minimize unexpected physical instability of pharmaceuticals. With increased knowledge of solid-state chemistry of pharmaceutical systems and advancement of analytical techniques, especially the application of PAT tools, it is possible to control and maintain drug physical stability during manufacture and throughout its shelf-life.

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Chapter 13
Evaluation of Stability Data

Nanda Subbarao and Kim Huynh-Ba

Contents

Abstract This chapter discusses the evaluation of stability data. It follows the stability study information from the point that raw data is generated in the lab, calculations are performed to give test results, and test results are entered in the stability summary sheets, until data is finally entered into a stability report for submission purposes. This chapter also includes a summary of data evaluation addressed in ICH Q1E and a discussion of Out-of-Specification (OOS) and Out-of-Trend

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(OOT) investigations. Specification setting and shelf-life extrapolation, which are performed after evaluating stability data, are also described in this chapter.

13.1 Data Evaluation and Trending

ICH Q1A(R2), Stability Testing of New Drug Substances and Products [1], for drug substances and drug products intended for marketing in the ICH Tripartite region includes sections on the evaluation of stability data. ICH Q1E, Evaluation of Stability Data [2], provides further details for data evaluation and includes recommended procedures for statistical analysis. These ICH guidelines are applicable to New Chemical Entities (NCEs) and associated drug products but do not apply to generics, manufacturing variations, clinical trial batches or devices.

This chapter describes the data evaluation that is to be performed from the time that data are generated until they are reported in a regulatory submission. Figure 13.1 provides a flow diagram for stability data evaluation.

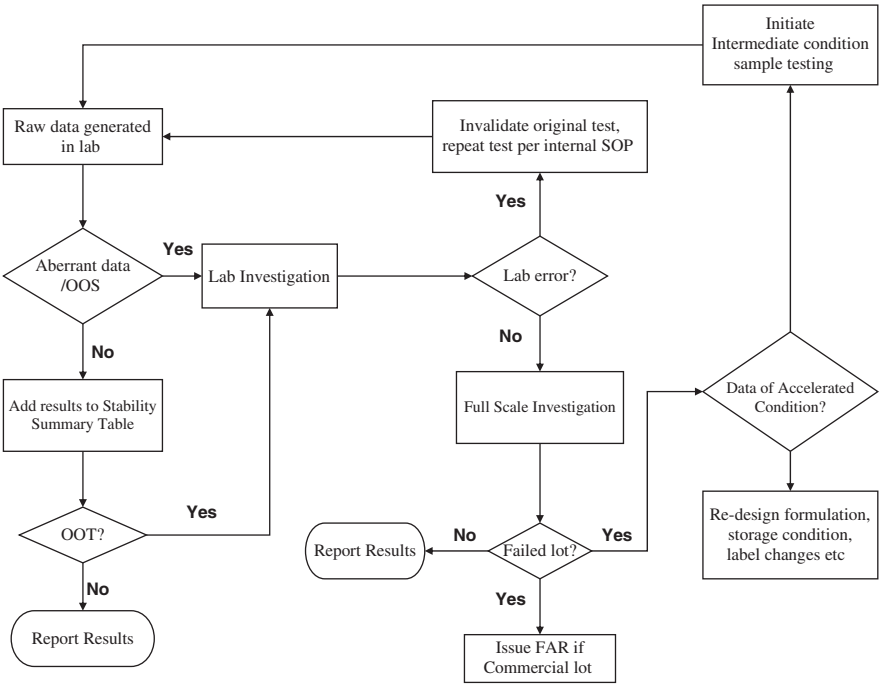

Fig. 13.1 Flow diagram of stability data evaluation

13.1.1 Evaluation of Raw Data

Stability data evaluation must begin when raw data is generated in the laboratory. cGMPs require that drug products and drug substances must meet their

specifications for identity, strength, quality, and purity. Results for tests such as appearance and package integrity are evaluated directly against the specification. ICH Q1A(R2) defines significant changes for stability samples and which can be found in Chapter 3, Table 3.4. Additional information regarding physical testing is discussed in Chapter 10. For other tests such as purity by chromatography, the raw data must be examined for changes such as new or growing impurity peaks. It is important that any *significant changes* or aberrant observations be noted immediately, and investigated promptly, at a time when the original unexpired sample solutions and reagents are still available.

The evaluation of the raw data can be effectively performed only if the analyst has access to the stability specifications as well as to the results and chromatograms from the previous time points of the stability study. Chromatograms of excipient lots and of the drug substance lot used for manufacture of the drug product lot are also useful. Designation of an appropriate person in the laboratory to evaluate data and act promptly if an OOS is found is invaluable for prompt and meaningful laboratory investigations of aberrant raw data. Any OOS results found must be investigated promptly and the procedures for Laboratory Investigations and OOS Investigations are described in Section 13.2.

13.1.2 Evaluation of Stability Results

The results obtained, after calculations, are compared to the Specifications and must be evaluated for OOS and OOT results. The procedures for identification of OOS or OOT incidences are described in this section and the procedures to follow if an OOS or OOT incident occurs are described in Section 13.2.

13.1.2.1 Identification of OOS Results

Although specifications are applicable to products at the intended storage temperature, any stability test result that does not meet specifications is said to be OOS. The individual result, calculated according to the analytical method, rather than the average or mean, must be compared to the specification limit. The result is also considered to have failed the specification limit if it has to be rounded in order to meet the limit. For example, a result of 9.99 units does not pass the specification limit of $\geq$10.0 units.

13.1.2.2 Identification of OOT results

Identification of OOT results is often more complicated than a simple comparison of the results to the specification limits. Guidance documents up to now have provided little guidance on the subject. Yet, trending is a critical part of an effective stability program.

In principle, any data which deviates significantly from the norm for that product, packaging configuration or lot is considered to be OOT. The OOT identification

procedures therefore depend on the availability of data to define the norm. During early development stability studies, where little information about the product or formulation is known, the test results from earlier time points are set as the norm for later time points. Any significant deviation from this earlier result is identified as an OOT incident and action is taken as appropriate. Where a significant amount of stability data is available, a lot or packaging configuration is identified as behaving OOT if its rate of degradation is different from the normal degradation rate for that formulation or package type.

The trend identification can be qualitative and performed by graphing the stability data or could be performed by statistical analysis of the collated data. In both cases, the site OOT Standard Operating Procedure (SOP) defines criteria for designating a data deviation from the norm as an OOT incident. The OOT criteria must be set in such a way that all significant OOT incidences are identified, ideally without false positives.

Graphical OOT Evaluation

Graphing can be used to identify stability data which are OOT within lot or within product/packaging type. Figure 13.2 refers to stability data at different time points for a single lot, with the result at the 9-month time point that can be considered as OOT when compared the other time points in the study. Such an identification of OOT data is of value during development studies where formulation information is limited, and the graph provides information about product degradation or analytical method variability. The presence of an OOT may then be evaluated further by calculating the change from original, change from original per unit time, change from last test or by evaluating the observed value directly, without further calculations.

In Fig. 13.3, lot 4 data could be considered as OOT with respect to the other four lots (lots 2, 3, 5, and 6). This type of plot allows evaluation of multiple lots of different strengths or different pack sizes and can identify OOT of degradation rates in a specific strength or package size and/or configuration.

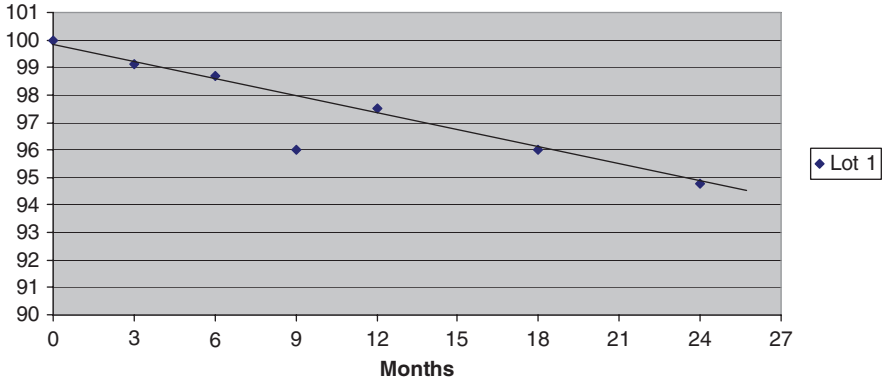

Fig. 13.2 Stability results for a single lot over time. The 9 months result can be considered as OOT

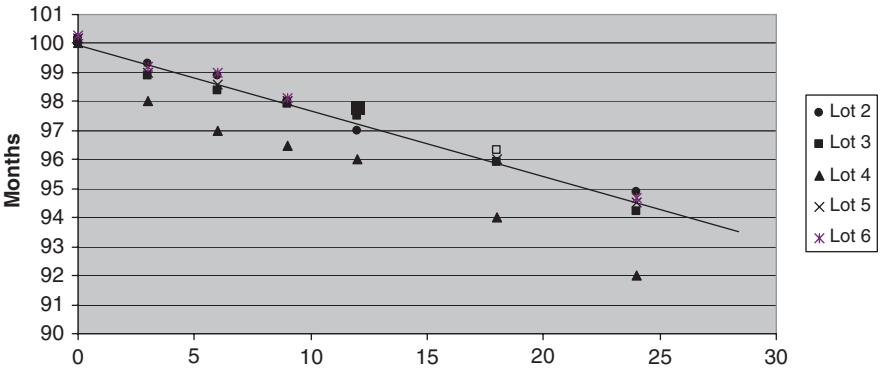

Fig. 13.3 Stability results for 5 lots over time. Here, the rate of change of lot 4 is being compared to that of the other lots and appears to be OOT

Statistical Evaluation of OOT

The data comparison described above in Figs. 13.2 and 13.3 may be performed statistically in several ways. The statistical approach takes data variability into account when setting limits. Therefore, a single acceptance criterion for OOT identification can be set for different types of assays. Three such procedures for normally distributed data are described in a review by the PhRMA CMC Statistics and Stability Expert Teams [3]. Each of these approaches has its own advantages and disadvantages, a summary of which is provided in the paragraphs below.

Regression Control Chart Method

In this approach, a least squares regression line is fit to stability data either from a single batch or from several batches. The expected result for any time point is given by the expression:

$$\text{Expected Result} = \text{intercept} + (\text{Slope} \times \text{Time}) \tag{13.1}$$

The control limits at a given time point is given by

$$\text{Expected Result} \pm (k \times s) \tag{13.2}$$

Where k is a multiplier chosen from a table of normal quantiles to give the desired protection level and s is the square root of the mean square error from the regression. The choice of k value allows the control of the confidence level and thus the rate of false alarms. This approach depends on the data being normally distributed and independent and is applicable only to data with a common linear slope for all batches.

By-Time-Point Method

In this approach, historical data is used to compute a tolerance interval for each stability time point. The tolerance interval can be calculated for the stability results themselves or for the difference between the result and the initial stability result.

The interval at a certain time point can be calculated as:

$$(\text{Mean of result at time point}) \pm k \times s \tag{13.3}$$

where k is the multiplier chosen from a table of normal quantiles to give the desired protection and s is the standard deviation at the time point.

Any result outside the tolerance interval is considered OOT. This approach depends on the data being normally distributed and independent and does not require any assumptions about the shape of the degradation curve.

Slope Control Chart Method

In this approach, a control chart for the slope at each time point is constructed. At each time point, a least squares regression is fit that includes all the earlier time points. The slope estimate for each batch is used to obtain the overall slope estimate for several lots. The OOT limit for the slopes at each time point are obtained from the tolerance interval, in which k is chosen to obtain the desired protection from false negative or false positive values.

13.1.2.3 Special Case – Reviewing Impurity Assay Results

The specific case of impurity assay results requires a more detailed discussion because of its impact on the proposed drug substance and drug product specifications during development studies.

Often, the impurity acceptance criteria in the product specification are rounded as required by the ICH Q3A(R2) guidance document. The impurity data is therefore reported by the laboratory to match the rounding in the product specification, often to one digit past the decimal, while the significant figures for a typical HPLC impurity test are given to two digits past the decimal place. This practice of reporting to match the number of decimals in the ICH guidance limits the power of OOT tools by decreasing the information in the data set. It is therefore advisable to report stability data per the significant figures appropriate to the analytical method, although it may be reported to match the specification for lot release purposes.

The Limit of Quantitation (LOQ) and the reportable limit for impurities also impact impurity test OOT procedures. It is common practice to report impurity peaks below the Limit of Quantitation as $< LOQ$, which is set to the reporting limit for that impurity per ICH Q3A(R2). Therefore, the purity result for peaks just below the LOQ cannot be used for trending although the analytical method variance may be satisfactory at that level. This reporting practice again decreases the information presented in the stability data tables, limits the OOT tools for impurities close to the

LOQ levels, and could be a concern for those impurities with specification limits close to the LOQ.

Some common procedures for evaluating OOT close to the LOQ are given below:

- If all test result values are above the LOQ, the distribution is normal, and the variance is constant, the regression control chart method or the slope control chart method may be applied to the data.
- If all test results are below LOQ, any test result which appears above the LOQ may be considered as OOT. However, this OOT identification procedure will result in false positives if the impurity peak is normally just below the LOQ and its appearance above the LOQ is due to test method variability.
- If some test values are below LOQ, one strategy would be to consider all results which are <LOQ as either the LOQ level or 1/2 (LOQ) for purposes of the statistical calculation leading to the identification of the OOT. This OOT Identification approach is impacted by the distortion of information due to the approximation for peaks <LOQ.

OOT identification for Impurity test results may also point to the need for identification of unknowns which may be increasing in levels. A growing unknown impurity should trigger identification of the impurity and validation of the impurity analytical method, before the peak reaches levels where the guidance documents require its identification.

13.1.2.4 Importance of Prompt Investigations

OOT result identification during development stability studies provides early warning about possible changes required to the formulation or packaging. OOT observations during annual commitment lot stability studies can provide early signals of possible future lot failure.

13.2 Investigation of Out-of-Specification (OOS) Results

21 CFR 211.192 requires that all OOS occurrences be investigated. The FDA issued a guide to inspection of Quality Control laboratories in 1993 and a draft OOS guidance in 1998 [4], following the Barr case. The final OOS guidance document, issued in 2006 [5], provides guidance on the procedures to be followed when OOS or OOT results are observed during stability studies. Where the 1998 guidelines were only applicable to the finished product, the 2006 guidelines apply to APIs, excipients, other components, in-process materials, and finished products. The document describes the laboratory phase of the investigation as well as the full-scale investigation and lists the responsibilities of analysts, supervisors, and the Quality Assurance unit. Performing inadequate investigations for OOS results continues to be a leading cause for Warning Letters in the past 5 years.

It is important that the investigation is timely, unbiased, well documented, and scientifically sound. Typically, an investigation should be closed within 30 days of

OOS discovery. For stability testing handled by a contract research organization (CRO), the sponsor is ultimately responsible for any investigations. Therefore, the responsibilities of the sponsor and CRO must be clearly defined, and the sponsor must be familiar with the CRO's OOS procedure.

An OOS result could be due to errors in the measurement process or in the manufacturing process. Therefore, an investigation must be performed to determine the root cause of the batch failure even if a batch is rejected.

Every pharmaceutical company must have a written procedure for Laboratory Investigation of OOS or OOT events for GMP stability studies. The procedures to be followed and the responsibilities of various personnel as described in the 2006 guidelines are outlined below.

13.2.1 Phase I – Laboratory Investigation

The first phase of the investigation occurs in the lab and is focused on the possible identification of assignable laboratory errors. The responsibilities of the supervisor and the analyst during this phase are listed below.

Analysts are responsible for:

- Ensuring that the equipment used is calibrated and meets the required acceptance criteria.
- Reporting data only if the required system suitability tests pass acceptance criteria.
- Checking the data for compliance to specifications before discarding any test solutions.
- Informing the supervisor if any unexpected results are obtained.
- Stopping testing if an obvious error occurs; they should not knowingly continue testing when they expect to invalidate the data at a later time for an assignable cause, except when the sole purpose is to see what results are obtained when obvious errors are known.

The supervisor is responsible for:

- Performing an objective and timely assessment.
- Confirming the analyst's knowledge and performance of correct procedures.
- Examining the raw data and identifying anomalous or suspect information.
- Confirming the performance of the instruments.
- Examining the solutions, reagents, and standards to confirm that they were appropriate for use during testing.
- Evaluating the performance of the test method.
- Documenting and preserving evidence of the assessment.

Prompt initiation of the investigation is essential for several reasons. Test solutions, reagents, and standard solutions will still be available and may be re-analyzed if necessary. The analyst's memory of all stages of the testing will be clearest on the day of the test, and equipment is more likely to be in the configuration used for testing and can therefore be checked for errors.

Check list for Laboratory Investigations

LIR Number _____ Issued by/Date _____

Product Name: _____ Product Lot Number: _____ Sample ID Number: _____

Stability Study Number: _____ Stability Storage Condition: _____ Stability Time Point: _____

Test type Test Date Analytical Method Number: _____

Observation leading to Investigation: _____

_____

Equipment ID (s) Analyst (s):

System Suitability Passed?

Sample		
	Sample ID and condition satisfactory?	y/n
	Packaging satisfactory?	y/n
Reagent		
	Correct reagent used?	y/n
	Within expiry Date?	y/n
Glassware/supplies		
	Correct glassware type used?	y/n
	Clean glassware used?	y/n
	Solvent washed/dried Glassware used?	y/n
	Correct Volume (volumetric) glassware used?	y/n
Equipment		
	Equipment qualified for intended purpose?	y/n
	Equipment within calibration period?	y/n
	Equipment setting appropriate?	y/n
Chromatography Column:		
	Correct column used as per analytical method?	y/n
	Column wash steps completed prior to injection?	y/n
Analyst Training		
	Trained on use of equipment?	y/n
	Trained on Analytical Method?	y/n
SOP steps		
	Weights in correct range?	y/n
	Dilutions performed per analytical method?	y/n
	All steps performed as per Analytical method	y/n
Calculations		
	Software qualified?	y/n
	All calculations checked and found correct?	y/n
Other		y/n

Investigation by/Date: _____

Laboratory Error Identified? Y/N: _____

Fig. 13.4 Example of a laboratory investigation report checklist

The investigation must be documented and a checklist (see Fig. 13.4 for an example) is often used to aid in reviewing all the relevant facts and serves to speed up the review process.

If the review does not reveal the root cause of the anomalous results, there may be a need to test the final prepared solution, retained samples from earlier steps of the sample preparation or tablet grinds to identify the root cause. The procedures for such testing must be defined in an SOP and the testing must be supervised and

approved by a supervisor, with a review of the results at each stage before proceeding to the next.

If the anomalous result can be unequivocally assigned to laboratory error, the result may be invalidated. Marking the notebook entry as invalid and retaining all related instrument outputs will be invaluable during future audits, to account for the raw data and results which are retained in the instrument electronic database.

The OOS guidance document indicates that laboratory or analyst errors should be relatively rare, and frequent occurrence can be an indication of inadequate training of analysts, poorly calibrated/maintained equipment, or careless work. It should not be assumed that the failing result is attributable to analytical error without performing and documenting an investigation. When a laboratory error is confirmed, the company must determine the source of error, take appropriate corrective actions, and prevent reoccurrence of the incident.

13.2.2 Phase II – Full-Scale OOS Investigation

When the laboratory phase of the investigation does not identify an assignable cause, a full-scale investigation must be initiated. The functional groups involved, in addition to the quality unit, should be included in the investigation team. The investigation should be initiated and completed promptly.

A standard form which will aid in documentation of investigations is provided in Fig. 13.5.

A critical part of the investigation is a review of other related documents to identify the root cause of the OOS result. Some of the documents to be checked include stability data of other time points of the same lot, other lots of the same product, other pack sizes/pack configurations of the same lot or the same product, and the batch production record for other investigations on the same lot/same product. The data can reveal if the anomalous data was developing at earlier time points or whether the root cause discovered as a result of this investigation could impact other lots, other pack sizes, and other time point data.

The investigation may also include experimental work to determine the root cause. Such experimental work must either be described in the OOS SOP or be pre-approved and supervised by a responsible person.

13.2.2.1 Retesting

Retesting is performed using the same homogenous material as the original sample. The concept of retesting as described in the OOS guidance does not apply to some tests such as content uniformity and dissolution.

Companies must have a written procedure that specifies the maximum number of retests. The SOP must define how retesting will be performed. It is understood that the investigation procedure cannot be fully pre-defined and depends on the problem and product. Instead, each testing step must be approved and supervised by a responsible person in the Quality unit. It is important that the retesting be performed

OOS Investigation Form

OOS Number **Issued by/Date** **Close out date**

Product Name: Product Lot Number: Sample ID Number:

Stability Study Number: Stability Storage Condition: Stability Time Point:

Test type Test Date Analytical Method Number:

Observation leading to Investigation:

Equipment ID (s)

Analyst (s):

Repeated Injection authorized by:
Repeated Grinds testing authorized by:
Repeated testing of sample authorized by:

Lab Error identified? Y/N

(Attach Lab Investigation report including Checklist and summary of all repeated testing listed above)

Re-sampling authorization (specify lot number, stability study number, condition, time point:

Other products/lots/packaging configurations that may be afffected

Investigation report including Root Cause and Corrective/Preventive action attached? Y/N

Comments/Recommended follow-up activities:

Investigation Close-out Approval

QA Management Signature/date Laboratory Management Signature/date

Fig. 13.5 Example of an OOS investigation form

by a second analyst if available. Repeating testing until a passing result is obtained and then discarding the originally obtained data is commonly referred to as *testing into compliance* and is objectionable under the cGMPs.

Where retesting of the original sample does not lead to the discovery of the root cause, there may be a need to re-sample the lot. For stability studies, where the original time point often cannot be resampled, due to the passage of time, a later

time point sample is pulled and the results are designated as such. For example, if in a study the 6-month sample test results are under investigation, and additional containers at the 7-month time point are tested as part of the investigation, the results are reported as belonging to the 7-month time point. The investigation may conclude that either the original test result or the original sample tested was not representative of the lot and may therefore be invalidated.

When faced with insufficient samples for testing of stability OOS investigations, some companies may consider taking samples from other programs such as retention programs. However, such practices are not advisable as the storage conditions of the stability and retention programs may differ significantly.

13.2.3 Outlier Test

Outlier testing is a statistical procedure to determine if a value obtained is different than others in a series. Discarding outliers may be appropriate for biological assays that exhibit a high variability. In such cases, the outlier test must be described in advance in a written procedure. For validated chemical tests, the guideline does not recommend the use of outlier tests to invalidate suspect results. Furthermore, the outlier test cannot be applied to data when the variability in the product is being assessed, such as dissolution or content uniformity testing.

13.2.4 When the OOS Result Is Confirmed

If the investigation described above does not identify a laboratory error as a root cause, then the OOS result is considered representative of the lot being tested.

13.2.4.1 Commercial Lots

For those products which are the subject of regulatory applications, regulations require submittal within 3 working days of a Field Alert Report (FAR) concerning the failure of a distributed batch to meet any of the specifications established in the application.

13.2.4.2 Development Lots

OOS at Accelerated Conditions

For registration stability studies, for products intended for long-term storage at room temperature, when stability study result shows *significant change* as defined in ICH Q1A(R2), testing on the intermediate condition samples must be initiated immediately. Failure at accelerated conditions for registration lots may also trigger changes to the labeling of the product. If necessary, the proposed shelf-life can be shortened until data of long-term storage conditions is available.

OOS at Long-Term Storage Conditions

OOS in registration stability results at the long-term storage condition may trigger changes to the product packaging, formulation, storage condition, or proposed shelf-life of the product. The need to remove the lot from ongoing clinical studies should be evaluated.

13.2.5 Trending OOS Results

Trending initial laboratory investigations is a convenient way to identify opportunities for process improvements in the lab. After completion of the lab investigation, the key elements of the investigation, such as product, storage condition, equipment, analytical method, and root cause are entered in a database. The database is periodically queried for occurrence rate per time period. Pareto charts are prepared for the root cause categories. Each root cause, starting with the leading cause is addressed as part of the lab's continuous improvement program.

Trending of stability OOS investigations is considered best practice and is usually included as part of the site OOS monitoring procedure.

13.3 Setting Specifications and Stability Data

The guidance for preparing specifications for drug substances and drug products is provided in ICH Q6A [6] with additional guidance in ICH Q6B [7] for biologics. The discussion below for considering stability data in specifications is applicable only to drug products. The upper and lower acceptance criteria limits in the regulatory specification (shelf-life specification in the EU) are usually set based on the potency and/or impurity levels of the clinical lots and safety and efficacy considerations. The extent of degradation or change in the attributes during the shelf-life of the product is factored in to determine the in-house release acceptance criteria (lot release specification in the EU) to ensure that the product meets the regulatory specification at the end of shelf-life.

The acceptance criteria for some attributes such as package integrity or sterility must not differ between lot release limits and regulatory acceptance criteria, and test results for these attributes must not change over the shelf-life of the product. However, results for other attributes such as potency and impurity profile could change significantly over the shelf-life of the product. Stability data are used in deriving the regulatory specification limits for these attributes.

13.3.1 Refinement of Specifications Using Data from Stability Studies

ICH Q6A provides decision trees given in Figs. 13.6 and 13.7, which address the extrapolation of meaningful limits on degradation products for drug substances and drug products.

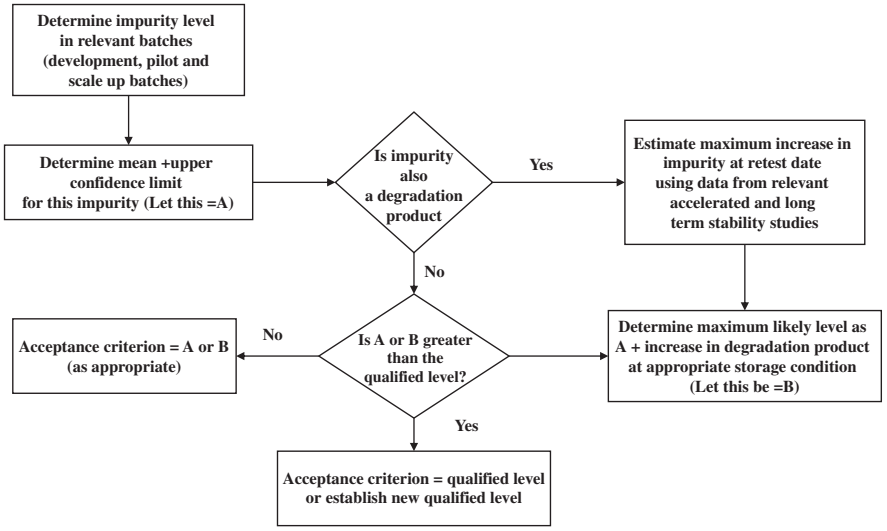

Fig. 13.6 Establishing acceptance criterion for a specified impurity in a new drug substance

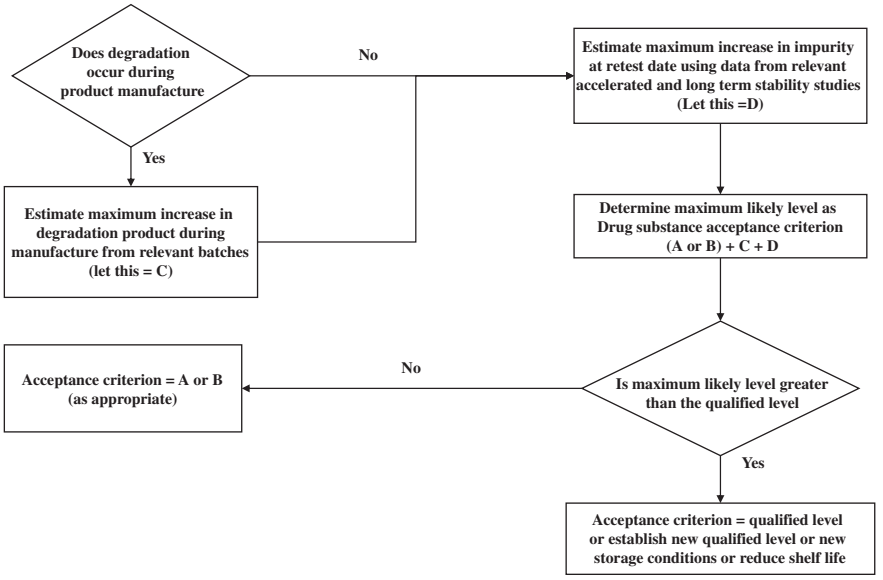

Fig. 13.7 Establishing acceptance criterion for a degradation product in a new drug product

A mathematical model for deriving specifications based on the manufacturing capability and stability data is given below. The parameters for the shelf-life limits are estimated by the equation

$$\mathrm{LRL} = \mathrm{LR} - \mathrm{EAC} + t0.95; \mathrm{DF}\sqrt{S_{\mathrm{T}}^2 + \frac{S_{\mathrm{TOT}}^2}{n}} \tag{13.4}$$

where,

LRL = Lower release limit
LR = Shelf-life limit or Lower registration limit
S_{TOT} = Standard deviation (total) for the analytical method
DF = Degrees of freedom for S
t = Percentile in the t-distribution
n = Number of repeated and independent assay determinations for release
EAC = Average slope for degradation* shelf-life
S_{T} = Standard error of slope* shelf-life

The mathematical model thus provides the procedure for including the degradation slope in the calculation of the specification acceptance criteria.

13.3.2 Expiry Dating of Clinical Materials

Expiry dating for clinical lots is required for clinical trials conducted in Europe. Acceptable procedures for extrapolating expiry dates are described in ICH Q1E.

13.3.3 Commercial Specifications and Extension of Expiration Dating

The drug product and drug substance shelf-life and expiry periods may be extended after product approval when satisfactory data from three stability lots has been obtained. It may also be possible to propose excluding or replacing certain specification tests originally included in the new drug application from the commercial drug product specification. For example, degradation product testing may be reduced or eliminated if it has been conclusively proven that a certain impurity is not formed in the specific formulation and under the specific storage condition proposed in the new drug application. Any testing reduction must be approved if the product has been filed with regulatory authorities.

13.4 Preparation of Stability Reports

13.4.1 GMP Requirements for Records and Reports

21 CFR Part 211 Subpart J indicates that records and reports must reviewed at least annually and be available for inspections at any time. Laboratory records include

a complete record of data and description of samples such as storage, location, quantity, lot, date received, etc. A complete record of instrumentation, reagents, and standards must also be available. Stability data must be well documented in a timely manner. Data must be traceable and defendable during inspection.

Raw data is defined as any record that is the result of original observations and activities of a laboratory study and is necessary for the reconstruction and evaluation of the report of that study. Raw data could be in laboratory notebooks, lab data recording sheets, a laboratory information management system (LIMS), or a combination of these means. Documentation is critical in day-to-day operations; improper documentation continues to be a leading cause of warning letters. Under-documentation leads to the risk of insufficient information and can contribute to filing delays and 483's. However, over-documentation with non-value-added information can be confusing, time consuming and wastes valuable resources.

13.4.2 Elements of a Stability Data Sheet

Stability results from all the time points are collated into tables called stability data sheets. Figure 13.8 presents a typical example of a stability data sheet. The data sheet usually comprises three main sections.

13.4.2.1 Lot Information

This section contains information for identification of the study, such as product name, strength, lot number, batch size, package, formulation identification, storage condition, and sample orientation. This section should also include all relevant dates

STABILITY ANALYTICAL REPORT

Sample Name: Lot#: Study #: Protocol #: Study Start Date: Study Purpose:	Manufacturing Date: Manufacturing Site: Expiration Date: Testing Site: Packaging Site:	Storage condition: Sample Orientation (if applicable): Packaging Information: Packaging Date:

Test Name	Method	Acceptance Criteria	Time Zero Test Date	1 Mo	2 Mo	3 Mo	6 Mo	9 Mo	12 Mo
Pull Date									
Test Date									
LIMS ID									
Appearance									
Assay									
Impurities Individual Total									
Dissolution Average % RSD Range									
Moisture									

Completed By: _____ Date: _____
Reviewed By: _____ Date: _____
Approved By: _____ Date: _____

Fig. 13.8 Example of a stability data sheet

such as manufacturing date, packaging date, and expiration date, and site information such as manufacturing site, packaging site, and testing site.

13.4.2.2 Study Information

This section includes study number, study start date, and time points. Protocol information and purpose of the study should also be included.

13.4.2.3 Testing Information

Testing information should include the current validated stability-indicating methods and corresponding specifications. Any modification of methods must be recorded and justified. This section records any additional information on the study. Most companies have an SOP describing recording of results. Consistent representation of data is required, and data rounding practices usually align the significant figures reported with the specifications for the test. Analysis test dates must also be included in this section.

13.4.3 Anatomy of a Stability Report

The stability data generated from the stability study, the data analysis, interpretations, and conclusions are reported at the end of the study. The stability report contents are an important component in any regulatory submission and the report is one of the documents reviewed in most audits and/or inspections. Figure 13.9 shows sections that contribute to a stability report.

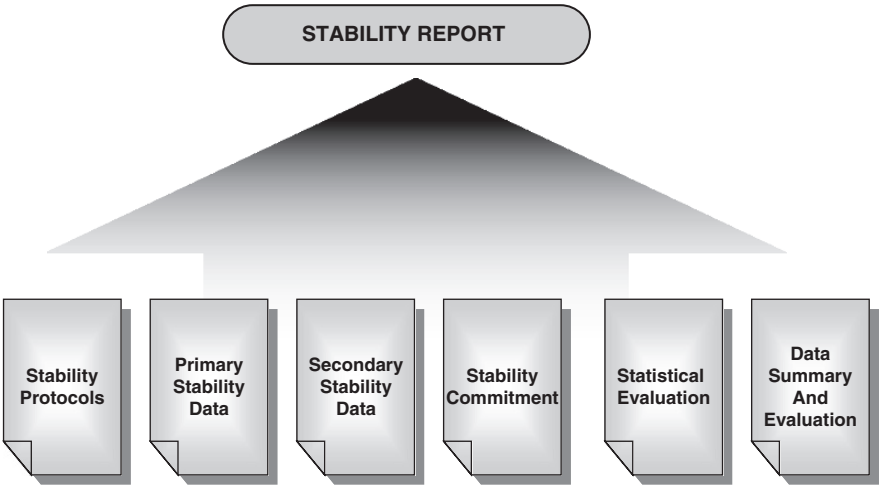

Fig. 13.9 Content of a stability report

13.4.3.1 Stability Commitment

The stability commitment section could include the protocol to be followed for future stability studies. These studies can be for either the first three production batches or for representative batches for annual product monitoring. Commitment should be clearly made and followed through. For a new submission, a commitment is made for the first three production batches, which follows a protocol similar to the submission batches, and also for the annual product monitoring, which generally contains only stability conditions at long-term storage.

13.4.3.2 Stability Summary

The stability summary is a brief section describing the stability profile of the drug substance or drug product. This section should indicate whether all the results meet specifications and support the proposed expiry period. Any differences among the packages, storage conditions, etc. are also discussed.

13.4.3.3 Statistical Analysis

Statistical analysis could be performed via LIMS or by a stability statistician. More information on the prediction of shelf-life can be found in Chapter 6.

13.4.3.4 Stability Protocols

Stability protocols attached to the stability report allow the reviewer to understand the procedures followed for the study. Additional information on this topic is found in Chapter 15.

13.4.3.5 Primary and Secondary Stability Data

Primary stability studies are those used to directly support the expiry dating or shelf-life of the drug substance or drug product, while secondary stability studies are those that provide supporting information. These could be from lab-scale batches, development batches, or experimental batches. The contents of the stability data tables are described above in Section 13.4.2.

13.4.4 Requirements for Stability Section in the CMC

The stability portion of the Chemistry and Manufacturing Controls (CMC) dossier contains the sections from the stability report described above. The requirements for the CMC sections can be found in 21 CFR Part 312 for IND application and Part 314 for NDA and Abbreviated New Drug Application (ANDA). Tables 13.1 and 13.2 provide the requirements for the CMC, and the location of the stability related documents within the CMC are highlighted in Table 13.2.

Table 13.1 Requirements of IND content based on 21 CFR Part 312

Drug substance	• Acceptance limits and analytical methods. • Sufficient information to support the stability of the drug substance during the toxicological studies and the planned clinical studies.
Drug product	• Brief description of the manufacturing and packaging procedure • Acceptance limits and analytical methods • Information sufficient to assure the product's stability during the planned clinical studies

Table 13.2 Structure of CTD

Drug substance	3.2.S.4	Control of Drug Substance
	3.2.S.4.1	Specification
	3.2.S.4.2	Analytical Procedures
	3.2.S.4.3	Validation of Analytical Procedures
	3.2.S.4.4	Batch Analysis
	3.2.S.4.5	Justification of Specification
	3.2.S.5	Reference Standards or Materials
	3.2.S.6	Container Closure System
	3.2.S.7	**Stability**
	3.2.S.7.1	**Stability Summary and Conclusions**
	3.2.S.7.2	**Post-approval Stability Protocol and Stability Commitment**
	3.2.S.7.3	**Stability Data**
Drug product	3.2.P.4	Control of Excipients
	3.2.P.4.1	Specifications
	3.2.P.4.2	Analytical Procedures
	3.2.P.4.3	Validation of Analytical Procedures
	3.2.P.4.4	Justification of Specification
	3.2.P.4.5	Excipients of Human or Animal Origin
	3.2.P.4.6	Novel Excipients
	3.2.P.5	Control of Drug Product
	3.2.P.5.1	Specifications
	3.2.P.5.2	Analytical Procedures
	3.2.P.5.3	Validation of Analytical Procedures
	3.2.P.5.4	Batch Analyses
	3.2.P.5.5	Characterization of Impurities
	3.2.P.5.6	Justification of Specifications
	3.2.P.6	Reference Standards
	3.2.P.7	Container Closure systems
	3.2.P.8	**Stability**
	3.2.P.8.1	**Stability Summary and Conclusions**
	3.2.P.8.2	**Post Approval Stability Protocol and Stability commitment**
	3.2.P.8.3	**Stability Data**

In September 2002, the ICH issued guideline M4, Organization of the Common Technical Document (CTD) for the Registration of Pharmaceuticals for Human Use. Each CTD contains 5 modules:

Module 1 – Region Specific Information
Module 2 – Summaries
Module 3 – Quality (CMC)
Module 4 – Non-clinical Study Reports
Module 5 – Clinical Study Reports

Stability data is included in Module 3. Subsections of Module 3, which include portions of the stability report described in 13.4.3 of this chapter, are highlighted in bold in Table 13.2 above. Stress studies to support the capability of the analytical method are also described in CMC Module 3.2 of the CTD. However, information such as stability data of intermediates designed to support holding times are usually included in the Manufacturing sections.

13.4.4.1 Drug Substance Stability Sections in CMC

Stability data for primary submission studies and supporting studies for drug substance are included in CMC section 3.2.S.7. The amount of stability data required at submission depends on the stability storage condition and the proposed shelf-life of the drug substance. A minimum of 12-months' long-term condition storage data and 6 months' accelerated or intermediate condition data will usually be needed on three primary batches for products intended for storage at room temperature. Cross-over data must be provided for any analytical method changes.

13.4.4.2 Drug Product Stability Sections in CMC

CMC section 3.2.P.8 includes stability data for drug products. The amount of stability data required at submission depends on the intended storage condition and the proposed shelf-life of the drug product. A minimum of 12 months' long-term storage condition data and 6 months of accelerated study data is usually required for products intended to be stored at room temperature.

13.5 Conclusions

Stability raw data and results must be reviewed and evaluated promptly after the analysis. The analyst must also review the stability profile of the batch, as well as stability data of the product after each data point generated. Many companies have implemented LIMS to help making reporting and evaluating stability data more efficient. The stability report is an important segment in the CMC document package. Every company must have an OOS and OOT SOP. If a laboratory error cannot be shown to be the root cause of an OOS or OOT incident, then a cross-functional investigation must be initiated. OOS and OOT investigations are important, as they continue to be one of the leading causes of warning letters.

References

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Chapter 14
Qualification, Calibration, and Maintenance of Stability Chambers

Jack B. Davis

Contents

Abstract An important aspect of all stability studies is the stability chambers themselves. This chapter is intended to provide a description of the different sizes and types of chambers that are available, the chamber tolerances required, and to provide some practical information for qualification, calibration, maintenance and monitoring of the chambers. Temperature, humidity and photo-stability chambers are included. Also included are guidelines on how to handle chamber excursions.

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14.1 Introduction

An important aspect of all stability studies is the stability chambers themselves. This chapter is intended to provide a description of the different sizes and types of chambers that are available, the chamber tolerances required, and to provide some practical information for qualification, calibration, maintenance and monitoring of the chambers. Also included are guidelines on how to handle chamber excursions. You can take the suggestions provided here into consideration as you write and execute your own procedures.

14.2 Chamber Size

Stability chambers can be obtained in a wide variety of sizes from commercially available small bench top reach-in chambers to large custom-designed walk-in rooms. Today the chamber manufacturer can provide high quality chambers at almost any size, for use at just about any condition.

14.2.1 Reach-In Chambers

Most reach-in chambers are floor models that range in size up to 33 cubic feet and larger. These typically have up to 10 adjustable shelves. The system mechanics (compressor, coils, control circuitry, etc.) are built into the chamber cabinet. Reach-in chambers are less expensive initially and are used when the number of samples is relatively small or when the conditions are likely to be changed. There is less potential risk with the failure of a reach-in chamber since the number of samples is likely to be fewer than in a walk-in and it would be easier to transfer the samples to an alternate chamber.

14.2.2 Walk-In Chambers

Walk-in chambers can be built to be any size depending upon the amount of space needed. The system mechanics are typically located above or next to the chamber. Walk-in chambers are more expensive than reach-ins and are used when a larger space is required for sample storage and/or the chamber conditions are likely to remain constant for an extended time. It is generally more efficient to install one large walk-in chamber at a particular condition than several reach-in chambers at that same condition (although having additional, redundant storage capacity among chambers is a good investment and should be considered when planning for chamber excursion or failure). Regardless of the size, every chamber will require qualification, calibration, preventative maintenance, and monitoring. The investment into these activities is not significantly more for a walk-in chamber compared to a reach-in chamber (and is likely to be less on a cubic foot basis). Rolling shelves can be installed to more efficiently utilize space in a walk-in chamber.

Many companies use a mix of chambers – walk-in chambers for standard conditions that are unlikely to change, and reach-in chambers for other conditions.

Refer to Figs. 14.1 and 14.2 for photographs of a reach-in and walk-in chamber.

Fig. 14.1 ES 2000 74 cu ft Reach-in stability chamber (*Photo Courtesy of Environmental Specialties*)

Fig. 14.2 ESI Walk-in stability chamber with CCS Touchscreen control *(Photo Courtesy of Metrics Inc.)*

14.3 Chamber Specifications

When a chamber is set to a specified set-point, it must be demonstrated that the entire chamber interior is maintained at that set-point within a certain tolerance. The chamber specifications described here refer to these tolerances and all stability chambers should be tested to assure that these tolerances are met. The specification will vary depending on the set-point(s) of the chamber as shown in Table 14.1.

Table 14.1 Chamber specifications

Set-point	Tolerance
Any temperature above refrigerated	± 2°C
Refrigerated	± 3°C
Freezer	± 5°C
Ultra-low freezer	± 10°C
Relative humidity	± 5%RH

- For any chamber with both a temperature and a relative humidity component, the specification for the temperature is the set-point ± 2°C and the specification for the relative humidity is the set-point ± 5%. For example, a 25°C/60%RH chamber would have a temperature tolerance of 23–27°C and a relative humidity tolerance of 55–65% RH.
- Chambers with a temperature-only component may have a slightly different specification that depends upon the temperature set-point.
  - Refrigerated chambers with a set-point of 5°C have a tolerance of ± 3°C (2–8°C).
  - Freezers have a tolerance of ± 5°C or, in some cases ± 10°C. For example, a –20°C chamber would have a tolerance of –15°C to –25°C. A –80°C chamber might have a tolerance of –70°C to –90°C.

In practice, many refrigerators and freezers will cycle outside of the temperature ranges given above. The extent of the cycle will be determined at the chamber qualification during the distribution study. Some companies address this by placing the control and monitoring probes in glycol to dampen the fluctuation. Another option is make sure that the alarm delay includes the cycle time. The approach taken should be documented in the appropriate SOP.

- Each firm should make a determination as to the number of significant figures in the tolerance. If the tolerance is 23–27°C, does this mean that 22.5°C (which rounds to 23°C) is within specification? If you take the specification at face value, the answer is yes. If you make the decision that 22.5°C should not be within specification, then the specification should be written as 23.0–27.0°C. I have seen the specification interpreted both ways. Regardless of your interpretation, you should be consistent in your application and to assure consistency, this should be defined in the firm's SOP.

14.4 Chamber Qualification

As with most pieces of equipment used in a GMP operation, stability chambers must go through a qualification process prior to use. This process traditionally includes three stages identified as the Installation Qualification (IQ), Operation Qualification (OQ), and Performance Qualification (PQ). At each stage a qualification protocol is written, approved, and executed. After successful execution a qualification report is written and approved. Upon approval, the next qualification stage is performed. Many components of equipment qualification are common to all pieces of equipment. A brief definition of each stage of qualification will be provided along with what might be included that is unique to stability chambers.

Some firms combine activities into IQ/OQ or OQ/PQ or even a single document without intervening approval steps. This is acceptable so long as all of the elements of the individual protocols are addressed in some manner and no product is placed into the chamber until final approval is obtained. This approach may streamline the process by eliminating time-consuming intermediate signatures.

We are seeing, in some cases, an additional stage of qualification called the Design Qualification (DQ), which precedes the IQ. The DQ assures that the chamber is suitable for its intended purpose and that the equipment manufacturer has utilized appropriate systems for design, manufacturer, and testing. In some cases, especially with a customized chamber, it might be appropriate to have a separate DQ.

For the purpose of this chapter, a separate IQ, OQ, and PQ will be described and the design integrity is incorporated into the IQ.

14.4.1 Installation Qualification

The properly written Installation Qualification (IQ) is a useful tool in that it will help you to consider and prepare for all aspects of the installation in advance. The purpose of the IQ is to demonstrate that the chamber was designed and installed according to manufacturer's specifications and user's expectations as outlined in the IQ protocol. The protocol-defined specifications and expectations would include those listed in Table 14.2.

Table 14.2 Attributes of a stability chamber IQ

Parameter	Specification
Chamber identification	Documented
Size of the chamber	Documented
Chamber location	Documented
Chamber description/design	Documented
Spare parts	Documented
Preventative maintenance	Documented
Environmental conditions	Meets criteria
Electrical requirements	Meets criteria
Water (if applicable)	Meets criteria
Monitoring, back-up, and alarm systems in place	Documented
Test equipment	Documented
SOPs are in place	Documented

- Description of chamber construction is provided, including drawings, which should be provided by the manufacturer, as well as facilities Piping and Instrumentation Diagram (P&ID) including water lines and wiring, floor plans, etc.
- A list of spare parts that will be maintained on site should be included (the manufacturer can help provide this list).
- Ongoing preventative maintenance for the chamber should be included (the manufacturer can help provide this list).
- Literature from the chamber manufacturer will include the requirements for environmental conditions that could affect chamber operation such as the surrounding temperature and humidity, height above sea level, electrical requirements, etc., and this should be included in the IQ.
- The quality of the water is an important component of ongoing humidity chamber maintenance and should be addressed in the IQ. There will be a specification for the water pH and conductivity and this information will be included in the literature from the chamber manufacturer.
- Are the chamber monitoring, alarm, and backup systems in place? The IQ report should include a brief description of these systems.
- Do the chamber controls function as intended, in other words, when you push the ON button does the chamber turn on?
- Test equipment used in the IQ is documented.
- Applicable SOPs should be listed. The chamber-related SOPs would include calibration, maintenance, monitoring, water (for humidity chambers), and chamber excursions.
- Any deviation from the protocol must be explained in the IQ report.
- Upon approval of the IQ report the OQ would be executed.

14.4.2 Operation Qualification

The purpose of the Operation Qualification (OQ) is to demonstrate that the chamber is operating according to manufacturer's specifications and user's expectations as outlined in the OQ protocol. The OQ is performed on an empty chamber. The protocol-defined specifications and expectations would include those listed in Table 14.3.

Table 14.3 Attributes of a stability chamber OQ

Parameter	Specification
IQ has been approved	Documented
Chamber turns on	Documented
Chamber set-point	Programmed and documented
Control variables	Programmed and documented
Calibration	Meets criteria
Distribution study	Meets criteria
Test equipment	Documented

- The chamber should be turned on and allowed to stabilize at the intended conditions.
- There will be programmed controller variables (or other chamber settings) that are preset by the chamber manufacturer such as °C versus °F, alarm delay, calibration offset, etc. Some of these variables will be changed by the user relative to the chamber set-point(s) and function. All variables should be identified, set appropriately, recorded, and then included in the OQ report. Any future change in these variables would warrant a consideration of change control.
- The chamber should be calibrated per SOP. Some firms may elect to perform the calibration separately from the qualification.
- A distribution (or mapping) study will then be performed to demonstrate that the set-point is maintained, within the allowed tolerances, throughout the chamber. In this study, probes are placed on the empty shelves to collect temperature and/or relative humidity data over a period of time. For the chamber to meet specification, all of the data points must be within the tolerance(s) provided in Table 14.1. While there is not a specific requirement for the number of probes to use, the duration of the study, or the data collection rate, the following can be taken as a recommendation:

    - Temperature. 15 probes: 5 top, 5 middle, 5 bottom; at each level there might be one probe in the geographic center and one probe at each corner or side. This layout would apply to reach-in and walk-in chambers.
    - Humidity. The relative humidity (RH) probes should be placed in the same positions as the temperature probes if possible.
    - One of the temperature and/or humidity probes should be placed in close proximity to the chamber controller probe.
    - Timeframe. Minimum of 24 h.
    - Data collection rate. 5 min, this would give 288 data points for each probe for a 24-h period.

- All probes should have been calibrated prior to use and documentation to that effect should be included in the OQ report.
- Any deviation from the protocol must be resolved in the OQ report prior to approval.
- Upon approval of the OQ report the PQ can be executed.

14.4.3 Performance Qualification

The purpose of the Performance Qualification (PQ) is to demonstrate that the chamber is performing according to manufacturer's specifications and user's expectations as outlined in the PQ protocol. The protocol-defined specifications and expectations would include those listed in Table 14.4.

Table 14.4 Attributes of a stability chamber PQ

Parameter	Specification
OQ has been approved	Documented
Program variables	Set and documented
Distribution study	Meets criteria
Open door study	Results documented
Failure study	Results documented
Recovery study	Results documented
Test equipment	Documented

- The OQ has been approved.
- The chamber variables set in the OQ should be documented and confirmed as unchanged in the PQ (or the change justified).
- A second distribution study is performed with the chamber *full*. Empty boxes, trays, shippers, etc. can be used to simulate samples in the chamber. A description of how the chamber was filled should be included in the PQ. Additionally, a photograph can be included. All other variables will be the same as those used in the OQ. The acceptance criteria for the PQ will be the same as that for the OQ. For the chamber to meet specification, all of the data points must be within the tolerance(s) provided in Table 14.1.
- After the distribution study is complete an open door study should be performed. In this study the door to the chamber will be opened for a certain amount of time to simulate access to the chamber for sample retrieval. This time period may vary depending on the size of the chamber and whether it is a reach-in or walk-in chamber. For example, you may anticipate that you would never need to open the door to a reach-in for longer than 3 min at a time. You would perform the open door study for 3 min and evaluate the affect on the chamber (including initiation of the alarm). For a walk-in chamber, 30 s should be an adequate test time. You might consider including these times in an SOP as a guideline for how long the doors can remain open at one time.

    - Upon completion of the distribution and open door study, while the probes are still in the chamber, a failure study should be performed. The purpose of the failure study is to demonstrate the affect of a chamber failure on the chamber conditions and confirm the operation of the alarm. With the chamber equilibrated, turn the power off to the chamber and then observe and document the following:

        - The time required for the chamber set-point(s) to go out of tolerance. If the chamber does not go out of specification after a predetermined period of time (4–6 h) the study could be discontinued.
        - When the chamber set-points go out of tolerance the chamber alarm is automatically initiated (after the programmed delay). If the chamber did not go out of specification, the chamber can be forced out of specification in order to test the alarm.

- Upon completion of the failure study, while the probes are still in the chamber, a recovery study should be initiated. The purpose of the recovery study is to determine the time required for the chamber to return to within tolerance once the power is restored (assuming the chamber went out of tolerance during the failure study). Restore power to the chamber and continue monitoring until the chamber is within specification.
- All probes should have been calibrated prior to use and documentation to that effect should be included in the PQ report.
- Any deviation from the protocol must be resolved in the PQ report prior to approval.

14.4.4 Requalification

Some firms establish a schedule for the periodic remapping of their chambers to assure ongoing compliance with chamber tolerances but there may be other reasons that you would want to re-qualify your chamber as follows:

- The chamber set point is changed. In this case, a new OQ and PQ would likely be performed with corresponding protocols and reports. Some firms have made the argument that, if the initial distribution studies are performed at multiple set-points, it may be unnecessary to repeat them when the set-point is changed.
- The chamber has malfunctioned and has been repaired. The Corrective Action/Preventative Action (CAPA) might result in a limited requalification.
- Some firms re-qualify their chambers periodically to assure continued compliance and this procedure and frequency should be SOP driven. Frequencies of between 1 and 3 years have been observed. Other firms maintain multiple probes in the chamber on an ongoing basis making periodic remapping unnecessary.

Periodic requalification, when performed, generally consists of repeating the distribution study performed in the PQ with the following exceptions:

- The chamber will be mapped with the contents of the chamber *as is*. No simulated samples need be entered into the chamber nor any samples removed.
- The open door, failure and recovery studies are not repeated. It is never a good idea to intentionally take the chamber out of specification when it contains samples.
- As with the original qualification, it must be demonstrated that the temperature and/or humidity specification (Refer to Table 14.1) is met throughout the chamber.

14.5 Chamber Calibration

Ongoing calibration will help to assure that the chamber is working properly over time. Calibration can be performed by placing a temperature and/or relative humidity standard inside the chamber (near the probe used by the chamber controller).

After the chamber has re-equilibrated, record the standard readings and the controller readings. The temperature and relative humidity readings should agree to within a certain range. This range should be set by the firm in the calibration SOP and it should be based on the accuracy of the test equipment being used. I have seen a temperature specification set at ± 1°C and a relative humidity specification set at ± 3%RH when calibration is performed using a Vaisala hand-held monitor, Model HMI41 (with HMP45 probe). This probe is accurate to within ± 0.6°C and ± 2%RH. Other wired or wireless monitors with comparable accuracy may be acceptable.

Calibration is typically scheduled on 6-month intervals but a different interval might be acceptable as long as it is directed by the SOP. An unscheduled calibration may be desired in the event of a chamber failure and this will be directed in the chamber excursion report (or comparable document).

14.6 Preventative Maintenance and Chamber Back-Up

Preventative maintenance (including cleaning) is an important function with any piece of equipment but it is critical with stability chambers since they are in constant operation for long periods of time. Preventative maintenance should be established at the IQ stage of qualification. Usually these procedures, as well as the frequency of execution, can be taken from the chamber literature and would include activities such as cleaning the coils, cleaning the humidity reservoir, checking and cleaning seals, etc., depending on the type of chamber. The procedure and frequency for preventative maintenance should be written into an SOP.

Many firms incorporate various redundant systems into their stability program. Back-up systems are not strictly required but can save some headaches in the long term. The following is a description of some of these systems:

- Back-up power; many companies have installed back-up generators that will automatically start if the primary power is interrupted. These may be powered by diesel fuel, natural gas, or some other energy source.
- Another type of back-up power would include redundant compressors and/or steam generators for each individual chamber. This back-up system could be set up to automatically start if the primary system failed.
- Water; an alternate water system could be available if the primary water supply was interrupted.
- Some companies maintain adequate chamber capacity in alternate chambers in case the primary chamber fails.

Any of the systems used should be described in an SOP and should be qualified where appropriate. For example, if a company incorporates a back-up generator for power, will the generator handle the additional load when a new chamber is brought into operation? This type of information should be included in the qualification of the chamber.

14.7 Monitoring and Alarm System

Monitoring and alarm systems should be an integral part of any stability program. The following is a brief discussion of some types of systems in use today.

The simplest systems are relatively inexpensive in regards to set-up and maintenance. This would include a chart recorder for monitoring, and use of the integral chamber audible alarm if the set point is exceeded. This is satisfactory as long as there is always someone nearby to hear the alarm.

At some point a company may decide that a more automated system is desired and this is usually a call-out type system. This system typically consists of a computer which is wired either into each chamber's integral alarm system or equipped with its own independent probes. A list of phone numbers is programmed into the computer and when a chamber exceeds its tolerance for a given period of time, the computer automatically starts calling these numbers. The phone numbers might be those of a third party contractor or internal employees. This type of system promotes the quickest response to a chamber excursion. Some of these systems will allow you to call in to check on the chamber conditions. These systems should undergo their own qualification when installed.

Ongoing monitoring may be performed with a single probe or with multiple probes throughout the chamber. The use of multiple probes for monitoring may discount the need for periodic remapping of the chamber but this should be planned in advance.

Regardless of the type of monitoring and alarm system used, whenever a new chamber is added to the system, the performance of the system should be tested with the new chamber and documented in the chamber qualification.

14.8 Photo-stability

It doesn't seem long ago that we were placing samples on the roof of the building to expose them to light. Photo-stability has changed quite a bit since then and is continuing to change. There has been more confusion regarding photo-stability compared to temperature and relative humidity studies. Radiation doesn't seem to be as straightforward as temperature or humidity and some of us don't have a clear understanding of its properties. The wording in the ICH guideline is somewhat vague in places and it also gives us choices, and in matters of compliance, it is sometimes easier to not have choices.

14.8.1 Option I or Option II

One of the first choices in regard to photo-stability studies is whether to use Option I or Option II as the light source. The goal of both options is to expose the sample to a range of radiation (approximately 320–800 nm) that simulates filtered sunlight, until a total cumulative exposure is achieved. The difference is that Option I provides for a single radiation source to achieve this exposure and Option II provides for two

Table 14.5 Option I versus option II

Option I	Option II
Chamber is typically benchtop	Chamber can range from benchtop to walk-in
A single radiation source, often Xenon	Two types of bulbs, for example, cool white fluorescence and black light blue.
Xenon more closely matches solar radiation	Bulbs usually last longer
More difficult to control temperature and humidity	Easier to control temperature and humidity
Lowest temperature is 25–30°C	Can perform studies at refrigerated temperatures
There is less lamp-to-lamp variability with Xenon.	Lamps can be purchased at the local hardware store.
Overexposes the sample in the UV range by a factor of 2.5	Can meet ICH requirements over the entire wavelength range
Typically takes hours to perform a study	Typically takes days to perform a study

sources, one for the visible radiation and a second for the UV. Table 14.5 provides a comparison of these two choices.

Regardless of the radiation source, the specification for total exposure is the same. For visible radiation the specification is *not less than* 1.2 million lx h. For the ultraviolet radiation the specification is *not less than* 200 W h/m^2. I want to emphasize the words *not less than*. The total exposure can be more than the required value but not less. The exposure is the product of two variables, intensity and time, and there is not a specification for either of these variables. Since intensity is usually more difficult to control, you would typically measure the intensity and then calculate the time. For example, if the intensity of the visible radiation at the sample shelf is measured at 10,000 lx, the samples would be exposed for 120 h (10,000 lx times 120 h $= 1.2 \times 10^6$ lx h). Some chambers can do this automatically by shutting off the radiation source when then total exposure is reached.

Option II chambers provide choices for completing the exposure as follows.

- The sample can be exposed to UV and visible radiation in sequence. This might be performed in two separate chambers. One chamber could have a visible radiation source and the second a UV radiation source.
- The sample can be exposed to UV and visible radiation simultaneously. Based on the chamber design, it may be possible to balance the UV and visible radiation such that both exposures are completed at about the same time. Otherwise, when one of the exposures is reached, that radiation source can be turned off while the second exposure continues to completion.

For Option I chambers, with a single radiation source, you don't have this choice. And as a result, particularly with a xenon lamp, the sample will be overexposed to UV. For example, due to the relative intensity of the visible radiation to the UV radiation, by the time the visible exposure reaches 1.2×10^6 lx h, the UV will be over 500 W/m^2 (Note that this does meet the specification of not less than 200 W/m^2).

It is also possible to use a combination of Option I and II. Samples could be exposed to xenon until the UV exposure is reached. At that point the radiation source could be changed to cool white fluorescence to complete the visible radiation exposure.

Refer to Figs. 14.3 and 14.4 for photographs of a photo-stability chamber using a xenon radiation source (Option I) and a photo-stability chamber using two separate radiation sources (Option II).

Fig. 14.3 Atlas® SUNTEST® CPS/CPS^{+} photo-stability chamber, ICH option I *(Courtesy of Atlas Material Testing Technology LLC)*

Fig. 14.4 ES 2000 Photo-stablity chamber featuring three independenlhy controlled light banks, ICH option 11 *(Photo Courtesy of Environmental Specialties)*

While there is not currently a temperature or relative humidity requirement for photo-stability studies, it is recommended that a specification (tolerance) be established. This requirement may not necessarily be as tight as that for a temperature/humidity chamber. It should take into consideration the following:

- The affect of temperature and humidity on the drug. This is the primary consideration although using a dark control will compensate for a degree of temperature/humidity degradation. You can deduce that the difference in degradation between the dark control and the exposed sample is due to photo-degradation.
- The type of actinometer being used. A chemical and even a physical actinometer will require a degree of temperature control in order to be accurate. You should know what this temperature requirement is and maintain it.
- The capability of the photo-stability chamber. Many photo-stability chambers will be unable to achieve $\pm 2^{\circ}$C and $\pm 5\%$RH.

14.8.2 Qualification

The temperature and humidity tolerances within the chamber should be challenged as part of the photo-stability chamber qualification just as they are for a temperature/humidity chamber.

As with temperature and humidity chambers, the DQ/IQ/OQ/PQ stages may be handled separately or combined to some degree. There may be very little difference between the qualification of a temperature/humidity chamber and a photo-stability chamber. The following describes what might be added to the qualification guidelines, previously provided in Tables 14.1 (IQ), 14.2 (OQ), and 14.3 (PQ), that make photo-stability unique.

- IQ; the chamber description will state if this chamber is an Option I or Option II chamber and will identify the radiation source(s) being used.
- OQ; if the chamber controls temperature and/or humidity, then distribution (mapping) studies should be performed to test these variables. Even if temperature and humidity are not controlled it would be important to know their profile across the sample shelf. Five temperature and five humidity probes should be adequate for a horizontal sample shelf of up to about 10 feet^2.

The radiation intensity should be mapped in order to determine the range of intensity over the surface of the sample shelf. Radiation intensity drops quickly as the distance from the source increases and so depending on the design of the chamber, the radiation can be much lower at the edge of the shelf area. The shelf should be mapped for both the visible and UV radiation and the number of mapping points would depend on the size of the sample shelf area.

  - For a horizontal shelf area of up to about 10 feet^2, 9 measurement points should be adequate (3 rows of 3).
  - Eight hours would be a reasonable duration for the temperature, humidity, and radiation mapping studies. Fewer measurements need to be taken dur-

ing mapping, especially if hand-held meters are being used. Temperature and humidity should be mapped with the lights on. The chamber should be *empty*.
  - Physical or chemical actinometers would be used to measure the radiation. Physical actinometers (radiometers, lux meters, spectro radiometers) should be appropriately calibrated and chemical actinometers should be validated.
  - If the chamber uses built-in sensors to automatically calculate the total exposure, then this calculation (time vs. intensity) should be verified during qualification.

- PQ; the PQ would repeat the distribution study with the chamber *full* of simulated samples. Based on the distribution study, you should know the areas of lowest and highest radiation intensity. The required time until study completion (total exposure achieved) would be based upon the area of lowest intensity to assure that all samples meet the minimum requirement.

Door opening, chamber failure, and chamber recovery studies are usually not necessary since photo-stability studies are relatively short in duration, require a small amount of sample, and can more easily be repeated if necessary.

- Requalification; Requalification of the chamber should be considered under the following conditions:
  - Some firms will perform periodic requalification of the chamber to assure ongoing performance. This would typically involve repeating the PQ mapping study for temperature, humidity, and/or radiation.

As with temperature/humidity chambers, if multiple temperature, humidity, and/or radiation probes are monitored on an ongoing basis or the chamber was originally qualified at multiple set-points, periodic remapping may not be necessary.

  - Changing the lamps should trigger requalification. Lamps change over time and there will be some degree of lamp-to-lamp variability, especially with Option II.
  - Any changes to the chamber as a result of repair may justify requalification. This would be addressed in the change control process.

Requalification of the chamber, under any of the above scenarios would likely include repeating the mapping study performed during the PQ.

14.8.3 Calibration

The principles for calibration will be very similar to that of a temperature/humidity calibration.

- If the chamber is designed to control temperature and humidity during the photo-stability study then these probes must be calibrated regularly against a qualified standard.

- If the chamber has built-in radiation sensors to measure radiation intensity then these sensors must also be calibrated against a qualified standard. As with the temperature and humidity probes, the radiation sensors should agree within a specified tolerance with instrument error taken into consideration.
- Hand-held meters must be recalibrated periodically. This often means sending them to a third party for calibration.

Six months (unless otherwise specified by the instrument manufacturer) would be an acceptable frequency for calibration. The frequency, process, and specifications should be described in the firm's internal SOP.

14.8.4 Preventative Maintenance and Back-Up

Preventative maintenance (and cleaning) for photo-stability chambers will depend on the type of chamber and variables such as (1) does the chamber control temperature or humidity, (2) does it have built-in radiation monitors and (3) is it Option I or Option II. The information provided for preventative maintenance of temperature/humidity chambers (refer to Section 14.6) applies here. The chamber manufacturer will provide specific procedures and frequencies for preventative maintenance and these should be transferred to an internal SOP.

Photo-stability chambers may be incorporated into the back-up system used for the temperature and humidity chambers although back-up systems are not necessarily as critical for photo-stability as these types of studies can be fairly easily repeated if necessary. Any back-up system in place should be described and tested during qualification.

14.8.5 Monitoring and Alarms

Monitoring and alarm systems also will depend on the type of chamber and what variables are being controlled. The following should be considered when planning for monitoring and alarm systems for photo-stability:

- Many photo-stability chambers have built-in monitoring systems with output to a computer system for temperature, humidity, and even radiation. These chambers may incorporate a call-out type alarm system.
- If an automated monitoring and call-out system is not in place then manual monitoring will be necessary. This may include periodic review of a chart recorder and/or taking periodic chamber readings with a handheld meter(s).
- The location of the monitoring detector should take into consideration the range of radiation observed during qualification. The point of lowest radiation intensity will be the basis for calculating total exposure. Some firms perform a brief mapping study prior to a photo-stability study to confirm the point of lowest intensity.
- The monitoring system should be described during qualification and the alarm system tested during qualification.

14.9 Excursions

Despite all efforts, sooner or later there will be a chamber failure. Chamber excursions are almost right up there with death and taxes, and it is better to give this plenty of forethought in order to be prepared, especially since failures often occur in the middle of the night or on the weekend. The ICH addresses excursions by making the following statements:

1. Short-term spikes due to opening of doors of the storage facility are accepted as unavoidable.
2. The effect of excursions due to equipment failure should be addressed by the applicant and reported if judged to impact results.
3. Excursions that exceed these ranges (i.e., ± 2°C and/or ± 5%RH) for more than 24 h should be described in the study report and their impact assessed.

The first one is simple. Every time the door on the chamber is opened, the conditions inside the chamber will change to some extent (the extent should have been determined during qualification). Documenting that the chamber was accessed is the only action that is required here.

Equipment failure is a different story. The corrective action from an equipment failure is going to depend on several factors including the following:

- Is the chamber out of specification?

Sometimes mechanical failures can occur and be repaired without the chamber ever going out of tolerance. In this case there is likely to be no effect on the samples. Preventative action should be addressed.

- How long is the chamber out of specification?

The longer that the chamber is out of tolerance, the more likely it is that the samples will be impacted. While the ICH states that excursions longer than 24 h should be evaluated and reported, any unplanned excursion should be evaluated and documented internally through some type of formal process.

- How far from the set-point are the chamber conditions?

The extent to which the chamber conditions exceed its tolerances will have an impact on the samples. A 40°C chamber that goes to 43°C is going to have less of an impact than if went to 50°C.

- Are the chamber conditions at a more severe condition than the set point?

A 40°C/75%RH chamber that fails and goes to room temperature is going to have less of an impact than a –20°C chamber going to room temperature.

The procedures for handling the above types of situations should be written into an excursion SOP and anyone with responsibility for chamber monitoring should be trained on these procedures. The SOP should address the circumstances in which samples would be moved to an alternate chamber. For example, if a refrigerated chamber fails, how much time can pass before moving the samples to an alternate

refrigerator? This direction can be based on the chamber failure study that was performed during the qualification. The SOP should also give guidance on completing the formal excursion report, including the impact on the samples themselves (and how to determine this impact), agency notification, chamber repair, and the need for chamber requalification.

Determining the impact of a chamber excursion on the samples themselves can be a challenge. Often, upon failure, the chamber will go to a less severe condition. In this case, the result might be to add time to the study (corresponding to the time the chamber was at the less severe condition). If the chamber goes to a more severe condition, then it needs to be determined if any changes have occurred in the sample as a result of the excursion. This may require the unscheduled removal of a sample from the chamber for physical and/or chemical evaluation.

One other aspect of the excursion SOP that should be considered is a disaster plan. This might include pre-arrangement with an alternate facility for sample storage or some other contingency plan. While a widespread facility failure is less likely to occur, it is always better to be prepared (and to be able to show that you're prepared).

14.10 Conclusion

In conclusion, alternate approaches to chamber qualification and maintenance are acceptable as long as they are scientifically justified and documented. While the worldwide standardization of our approach to drug stability is a positive step for industry, it is recognized that a degree of flexibility is necessary to accommodate different situations.

Along with the flexibility we are given comes the responsibility for adequate planning, execution, and documentation. We must state what we are going to do in our SOPs and protocols, we must follow these procedures, and then we must document the results.

Chapter 15
Stability Operation Practices

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Contents

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Abstract This chapter covers critical activities necessary to maintain an effective stability program. Best practices on day-to-day operational activities such as sample pulling, testing window, and chamber inventory are included in this section to provide guidance on current industrial practices. Development of a stability protocol is also integrated together with a discussion of ICH Q1D-Bracketing and Matrixing concepts.

15.1 Introduction

This chapter introduces day-to-day activities necessary to a successful stability program. It explains critical activities as well as most common practices to manage stability studies from the time that samples are delivered to the stability laboratories to the time that study ends. These activities are usually written into Standard Operating Procedures (SOPs). Many companies have a dedicated group of analysts to manage these operations.

Stability protocols are also discussed in this chapter. Stability requirements based on development phases are also introduced. Bracketing and matrixing concepts are also discussed in this chapter as options to reduce the cost of stability programs.

15.2 Development of SOPs

Stability Operation Practices are guided by SOPs. Each company has its own set of procedures, which describe all activities that are deemed critical. SOPs will help to establish consistency, and thus quality of company stability operations. Therefore, they should be written precisely, based on FDA regulations. Unfortunately, most common deficiencies result from inadequate SOPs.

An SOP should be detailed enough to adequately define the task it describes, but also general enough not to limit the user into a situation where efficiency and effectiveness are minimized. A flowchart is useful to structure an SOP and particularly to clarify responsibility of cross-functional departments.

It is recommended that SOPs be structured numerically in sequential order. Table 15.1 indicates sections that should be included in every SOP.

Table 15.1 Structure of an SOP

1. Title
2. Summary
3. Purpose/objectives
4. Scope
5. Responsibilities
6. References
7. Key words and phrases
8. Safety
9. Procedure
10. History/change control

The procedure should be described in linear fashion and chronological order, minimizing branch points. An SOP says exactly what needs to be done in an unambiguous fashion. Vague statement such as, *As appropriate as needed* should be avoided. Flow charts are recommended to illustrate the order of activities and indicate responsible parties involved.

It is important that roles and responsibilities of operators be clearly defined. However, use of proper nouns (names) must be avoided. Timelines listed in the SOP should be realistic.

All SOPs will include sections to describe when and how exemptions could be justified, how to document these exemptions, and how to secure approvals.

History or a Change Control section is also imperative. It defines the reason for issuing an SOP and why it is being revised. This is very important while a department is fine-tuning its operation or as a response to an observation. It is very helpful for auditing purposes as well.

Table 15.2 shows a suggested list of SOPs for the stability program.

Table 15.2 Typical stability SOPs

• Study initiation
• Study activation
• Sample pulling metrics
• Sample testing turn-around metrics
• Study completion
• Study cancellation
• Sample destruction

15.2.1 Study Set-Up

Study Set-up is typically triggered by a sample request, either from the formulation group or clinical packaging group. The Stability administrator must determine if a new study is necessary and if a standard protocol can be used.

Stability protocols must be approved by a Quality group. Each study must carry a unique, identifying (tracking) number that will contain information necessary to enter the study into a LIMS or a specific tracking system. Lot-specific information is gathered by contacting the appropriate personnel. Alert and test schedule information is determined with input as necessary from appropriate analytical groups. The purpose of the study must also be clearly stated and must be understood by the stability studies team, who will need to determine the impact of the study data.

Table 15.3 lists information needed to initiate a stability study. These items are the minimum requirements in order to identify the drug product as well as the package used. A checklist could be created to ensure all necessary information is collected.

Table 15.3 Information needed to initiate a study

1	Study number
2	Protocol to be followed
3	Formulation description
4	Lot number
5	Dosage form
6	Strength
7	Packaging description
8	# of Pulls (samples per test period)
9	Units to be pulled
10	Etc.

15.2.2 Study Activation

Study activation is an activity showing that the samples are physically placed in the stability chambers. Several tasks must be performed before the study can be activated. The study must be entered into a tracking system, such as LIMS. Once the samples are received, they must be examined for obvious faults, counted, and labeled according to the site SOP.

The analyst must ensure that there are enough samples to conduct all required testing under all conditions. An additional quantity of samples, typically 50–100% of that required for the study, should also be placed on stability for contingency testing; however, this quantity depends greatly on the study purpose and also upon the materials available. Sample inventory must be initiated at this point to track the number of samples that reside in each chamber.

In most cases, initial release data could be used as Time Zero (TZ) if the samples are placed in the chambers within 30 days of testing. Otherwise, Time Zero testing will be performed at the initiation of the study. It is also recommended that Time Zero testing be done if the packaging process could compromise the stability or quality of the drug product.

15.2.3 Sample Pulling

Samples are scheduled to be pulled based on the time points listed in the stability protocol. Many companies allow a brief time window at the scheduled time point for this activity. This is to allow for weekend pull dates or other unanticipated situations. Pulling schedules should depend on the age of the sample as well as the conditions at which it is stored.

Table 15.4 lists the recommended practices for pulling windows.

Table 15.4 Pull windows for stability studies

Room temperature 25°C/60%RH	
0–12 month	Time +3 days
To 24 month	Time +1 week
Accelerated (40°C/75%RH)	
0–6 month	Pull on scheduled date

Samples pulled outside of the allowable windows will be audited. Justification must be documented. Once the samples are pulled, location of the samples must be recorded. If the sample is a Pull and Hold sample, the Hold condition is entered as the new storage condition. The inventory form for each study/condition is updated to reflect the pull and the amount of samples pulled. An example of an inventory form is given in Fig. 15.1.

SAMPLE INVENTORY FORM

Study Number: _____ Product Name: _____
Strength: _____ Lot Number: _____
Activation Date: _____ Manufacturing Date: _____
Last Pull Date: _____ Storage Condition: _____
Package Description: Amount/Package:
Excess Amount/Package:

Unit (Circle): Bottle Blister Vials Open dish Poly Bag in Fiber Drum

Date	Timepoint	Amount Removed	Amount Available	Comments	Analyst Initial

Sample Pending Disposition

Transfer Information

Amount Moved	Current Status*	Current Condition	Initials Date	Comments Sent to/Destroyed	Initials Date

∗Pull/Hold or Overage

Fig. 15.1 Example of a sample inventory form

15.2.4 Sample Testing Turnaround

Sample testing turnaround is the time needed to complete testing of a stability sample. This is the time from the point at which a sample is removed from the storage chamber until the time that all the tests are completed and results are approved for submission. This time should be defined based on available resources as well as the analyst's sample workload. The industry standard for completion of testing is 30 days; however, it depends on the nature of the samples as well as the testing to be done. For samples stored at accelerated or stressed conditions, testing should be started as soon as the samples leave the chambers in order to stop the degradation process.

15.2.5 Study or Protocol Amendment

Once the study is started, any change to the stability protocol needs to be made with appropriate approvals. Justification must be recorded. The Stability administrator must also check to assure that there are enough samples to test the changes. An example of a study amendment could be an addition of testing time points to more completely monitor out-of-trend stability data.

15.2.6 Study or Protocol Deviation

Deviation from a stability protocol can occur throughout the study. There are two forms of deviations: planned and unplanned. Once a deviation occurs, an investigation must be conducted. Corrective actions and preventive actions (CAPA) may also be necessary to avoid recurrence. The impact of the deviation on the study must also be assessed and documented.

15.2.7 Study Completion

The study completion date is the point when the last sample was pulled, tested, and all results are reported. This time marks the end of the study. A study is not considered complete if there is an open investigation on any result.

For a stability study that also supports clinical trials, end of study will encompass the date that the last patient uses clinical materials or the expiry of the clinical materials. If the length of a clinical study is extended, the stability study needs to be amended accordingly to cover the new expiry.

15.2.8 Study Cancellation

If stability information is no longer needed, the study could be cancelled. Appropriate approval must be secured in order to cancel a study. An SOP must define the authority needed to stop the testing. Depending on the nature of the study, departments such as clinical, manufacturing, QA, or RA would need to be part of the team that cancels a stability study.

15.2.9 Sample Destruction/Disposition

Sample destruction is necessary when excess stability samples are removed from the storage. This task is usually part of chamber maintenance activities, and should be done when the end of the study is reached. The lab usually schedules a set time, such as end of the month, to remove all leftover samples of studies completed or

canceled in that month. It is recommended that the samples not be destroyed until all data are approved and the study is complete. As part of good laboratory practices, samples of cancelled or completed studies should not be retained in the chambers.

15.2.10 Sample Inventory Maintenance

Sample inventory is a critical activity in the stability program. All samples must be accounted for at any time. Location and identification of samples is important information, and must be included on the stability label. When a study is completed or canceled, samples must be moved out of the chambers. The number of samples removed must be recorded, and reconciled annually with the inventory system, if electronic tracking is used. Discrepancies must be promptly investigated and documented.

15.3 Training Program

15.3.1 Requirements of a Training Program

Personnel are a critical factor to a successful stability program. To obtain and maintain GMP compliance, every manager and supervisor should provide frequent, meaningful GMP reminders, train and develop all employees, and fully participate in formal, ongoing training programs [1].

Therefore, training is very important. Any critical activity needs an SOP to describe the task. SOPs must be written precisely. Short words should be used where possible, and concrete terms are better than abstract terms. The SOP must be detailed enough to tell a trained analyst how to do the work, but also general enough allow some flexibility. Medical jargon, redundancies, and clichés should be avoided.

Section CFR 211.35 requires that qualified individuals will be trained on a continuing basis. All training must be documented.

15.3.2 Types of Training

15.3.2.1 In-House Training

There are two main focuses to conduct training in a stability laboratory: technique specific or method specific. Most laboratories that deal with early NDA phases prefer technique-specific types of training. Analysts are trained on analytical technologies, such as HPLC, wet chemistry, et cetera, by in-house experienced analysts. At a later phase or after the NDA submission we recommend that training be method specific. At this stage, the analyst is usually qualified to perform the basic technique specific methods.

15.3.2.2 Outside Training

Training can also be accommodated by sending analysts to a variety of courses or conferences available throughout the year. This helps analysts to keep current with industry practices.

15.3.2.3 New Employee Training

New employee training curriculum can include technique-related as well as method-related training. A new employee also needs to be trained on fundamentals of cGMP, as well as relevant SOPs applicable to his or her responsibilities.

All training activities must be documented. Many companies also use short quizzes to test the comprehension of the trainees. Qualified trainers should be used to train new employees on critical activities.

A training guide is also useful for the activities that may not be critical to be written into the SOP. SOPs should be readily available for analysts to consult as needed.

15.3.3 Establishing Laboratory Controls

An effective metrology program will be necessary to ensure the quality of the results generated. Chapter 14 will further discuss stability facilities and key factors of environmental chambers.

Having a good metrology program is not only part of complying with cGMP requirements, but it is also a good business practice. It is beneficial during technology transfer, minimizes instrument downtime, and therefore increases overall product quality.

15.4 Stability Protocols (ICH and Global)

15.4.1 Establishing Stability Protocols

Stability studies are initiated based on approved stability protocols. Much discussion is provided in Chapters 3 and 4 regarding ICH and global requirements for stability protocols.

A summary of stability conditions according to ICH requirements is listed in Table 15.5. These conditions would apply to each package of each lot of drug product and/or drug substance manufactured.

15.4.2 Contents of a Stability Protocol

The typical stability protocol contains a number of significant elements.

Study information comprises all information pertaining to a specific lot of API or product. Detailed information is necessary in order to identify the samples. Purpose

Table 15.5 ICH stability storage conditions

Intended Storage Condition	Study Condition		Data Required at Submission
Room Temperature			
	Long term	25°C/60%RH	12 months
	Intermediate*	30°C/65%RH	6 months
	Accelerated	40°C/75%RH	6 months
Refrigerated			
	Long term	5°C/ ambient	12 months
	Accelerated	25°C/60%RH	6 months
Freezer			
	Long term	–20°C/ ambient	12 months

*Testing if significant change is observed for 40°C/75%RH.

of the study is necessary, as well as packaging information. It is also necessary to list manufacturing site, packaging and testing sites.

Protocol information includes storage conditions and all time points when samples will be pulled. In addition, the protocol should also indicate the configuration (orientation) of the stored samples. For example, liquid products could be stored upright, inverted, on the side, or all three.

Testing information will include all tests to be performed on pulled stability samples. Different tests may be done at different time points and conditions. As an example, sterility may be conducted annually. Chirality or polymorph testing may be done only on room temperature samples. Analytical tests to be performed on pulled stability samples must be fully validated and stability-indicating according to regulatory requirements.

Procedures describing reporting of stability data should be available. Discussion of data reporting and data evaluation can be found in Chapter 13 – Evaluation of Stability Data.

15.4.3 Standard Stability Protocols

To ensure consistency and quality of the stability program, many companies institute standard stability protocols. These are pre-approved protocols based on the phase of development for certain dosage forms.

A study protocol will be drafted by the appropriate organizational unit and must be reviewed and approved by the quality unit. Therefore, it would be time-saving if a company could establish a set of standard stability protocols. These standard protocols should include any specifications, standards, sampling plans, test procedures, and the time points needed for stability studies at different stages of development.

Standard protocols will help to maintain the consistency of the stability program. Companies can design standard protocols for each dosage form at each phase of development, as applicable. Figure 15.2 shows an example of a standard protocol for a stability study of a tablet dosage form at Phase II/III Clinical. In this example, special testing such as chiral assay and x-ray powder diffraction is being done for the first lot, to collect data for these types of testing on stability. Photostability is also performed in an open-dish condition on the first lot. The product is placed in immediate package and exposed to light during the ICH duration. This sample will be tested only if the open-dish sample meets one of the significant change criteria. Samples stored at intermediate condition of 30°C/65%RH will be tested only if there is a significant change at 25°C /60%RH. Also, if a clinical study is done at Climatic Zone III or Zone IV, then testing should be performed for samples stored at 30°C/65%RH to end of study (36 months or until the last patient completes the clinical trial).

Figure 15.3 shows an example of a standard protocol for a study of primary submission batches supporting registration. This is a global protocol as this study not only supports ICH condition but also supports condition for Climatic Zone IVB. Many companies also put 25°C /60%RH samples on hold and use data from 30°C /75%RH condition to support 25°C /60%RH condition. It is also recommended that enough samples be stored at 5°C to conduct critical testing of four time points. In the event that samples stored at 25°C /60%RH do not meet the desired shelf-life, then a more restricted storage condition could be filed, while additional time may be necessary to develop a more protective packaging system. At this time, microbial bioburden testing may be considered for one lot at 25°C /60%RH, especially if moisture is increasing to twice moisture at Time Zero and water activity (a_w) is not less than 0.6.

Similarly, Figs. 15.4 and 15.5 show examples of a liquid product such as an aqueous suspension. Figure 15.4 shows a typical standard protocol of an oral

Conditions	Months on Stability								
Time point (months)	0	1	3	6	9	12	18	24	36
25°C/60%RH	T,X,C	T	T	T	T	T,X,C	T,X,C	T,X,C	T,X,C
30°C/65%RH	HOLD (test if significant change occurs at 40°C/75%RH)								
40°C/75%RH	–	T	T	T,X					

T: Assay, Potency, Degradion Products, Moisture, Dissolution, Appearance, Pysical Tests (as appropriate)
X: X-ray powder diffraction. Test the first lot
C: Chiral assay. Test first lot

Fig. 15.2 Example of standard protocol for a tablet/capsule during phase II and III

Conditions	Months on Stability								
Time point (months)	0	1	3	6	9	12	18	24	36
25°C/60%RH	T,X,C	T	T	T	T	T,X,C	T,X,C	T,X,C	T,X,C
30°C/65%RH	HOLD (test if significant change occurs at 40°C/75%RH)								
30°C/75%RH	–	T	T	T	T	T,X,C	T,X,C	T,X,C	T,X,C
40°C/75%RH	–	T	T	T,X					
50°C/Ambient	–	–	T,X						
5°C/Ambient	HOLD (test if 25°C/60%RH does not meet intended specifications)								

T: Assay, Potency, Degradion Products, Moisture, Dissolution, Appearance, Physical Tests (as appropriate)
X: X-ray powder diffraction. Test first lot
C: Chiral assay. Test first lot

Fig. 15.3 Example of standard protocol for a tablet/capsule to support global registration

suspension at Phase II or III. Since this is an aqueous suspension, a high temperature and low humidity (40°C/25%RH) should be considered to assure that the liquid does not evaporate and caused the product super-potent. Figure 15.5 shows a standard protocol to support global registration for an oral suspension.

Conditions	Months on Stability								
Time point (months)	0	1	3	6	9	12	18	24	36
25°C/60%RH	T,X,C L,P,W	T	T	T	T	T,X,C P,W	T,X,C P,W	T,X,C P,W	T,X,C P,W
30°C/65%RH	HOLD (test if significant change occurs at 40°C/75%RH)								
40°C/25%RH	–	T W	T W	T,X W					
40°C/75%RH	–	T	T	T,X W					

T: Assay/Inspection, Potency, Degradation Products, Preservative Assay, pH, Dissolution, Redispersibility (suspension only), Mean size and Distribution of Particles (as appropriate)
X: X-ray powder diffraction. Test first lot
C: Chiral assay. Test first lot
L: Extractables at Time Zero must be available (for plastic containers)
P: Antimicrobial Preservative Effectiveness testing
W: Weight Change (for aqueous solutions only or solutions containing volatile solvents stored in permeable or semi-permeable plastic containers.)

Fig. 15.4 Example of standard protocol for an oral suspension during phase II and III

Conditions	Months on Stability								
Time point (months)	0	1	3	6	9	12	18	24	36
25°C/60%RH	T,X,C L,P,W	T	T	T	T	T,X,C P,W	T,X,C P,W	T,X,C P,W	T,X,C P,W
30°C/65%RH	HOLD (test if significant change occurs at 40°C/75%RH)								
30°C/75%RH	–	T	T	T	T	T,X,C P,W	T,X,C	T,X,C P,W	T,X,C P,W
40°C/25%RH	–	T W	T W	T,X W					
40°C75%RH	–	T	T	T,X W					
50°C/Ambient	–	–	T,X						
5°C/Ambient	HOLD (test if 25°C/60%RH does not meet intended specifications)								

T: Assay/Inspection, Potency, Degradion Products, Preservative Assay, pH, Dissolution, Weight change, Redispersibility (suspension only), Mean size and Distribution of Particles (as appropriate)
X: X-ray powder diffraction. Test first lot
C: Chiral assay. Test first lot
L: Extractables at Time Zero must be available (for plastic containers)
P: Antimicrobial Preservative Effectiveness testing
W: Weight Change (for aqueous solutions only or solutions containing volatile solvents stored in pereable or semi-permeable plastic containers.)

Fig. 15.5 Example of standard protocol for an oral suspension to support global registration

15.5 Bracketing and Matrixing

Conducting and managing a stability program is very expensive. To reduce the cost of the stability program, many companies employ options such as bracketing or matrixing.

At the inception of ICH Q1A, bracketing and matrixing were merely included in the glossary of the 1993 ICH draft guideline. Therefore, one could interpret that all matrixing or bracketing designs would require prior approval from regulatory agencies. Q1D was subsequently developed by the ICH Expert Working Group and has been examined by the ICH regulatory parties. In November 2000, Q1D was approved by the Steering Committee under Step 2 and released for public consultation. In February 2002, Q1D was published by ICH (Step 4) with recommendation for adoption [2]. It includes specific principles provided for situations in which bracketing and matrixing can be applied without minimal regulatory consultation. Regulatory agencies also encourage the use of these matrixes to reduce testing and minimize redundant testing.

Q1D discusses the use of bracketing and matrixing. A *full design* is a configuration in which samples of all combinations are tested at every time point. A *reduced design* is one in which samples for every factor combination are not all tested at all time points. Assumptions play a critical role in determining whether bracketing or matrixing is appropriate. These assumptions must be assessed and justified prior to the application of any reduced testing.

Q1D indicates that during the course of a reduced design, a change to full testing or to a less-reduced design can be considered if samples are available to accommodate the change; however, the new design must then be carried out though the remaining time points. The following sections discuss bracketing and matrixing designs. Examples are taken directly from Q1D to illustrate the options allowed.

15.5.1 Bracketing

Bracketing is the design of a stability schedule such that at any time point only the samples on the extremes of certain design factors, for example, strength, container size and/or fill, are tested at all time points as in a full design. The design assumes that the stability of the intermediate levels is represented by the extreme levels tested; therefore, there is no need to generate another similar data set. Thus the use of a bracketing design would not be appropriate if the samples selected for testing are indeed not the extreme configuration.

For example, when the stability profile of a range of tablet strengths must be established, only the low and high strengths are put up on stability and tested. Bracketing designs are applicable if the strengths are identical or very closely related in composition (e.g., for a tablet range made with different compression weights of a similar basic granulation, or a capsule range made by filling different plug fill weights of the same basic composition into different sizes of capsule shells). In the case that the extremes are not obvious, justification may be necessary to assure that the stability profiles of the selected sample lots are indeed extremes.

Bracketing can be applied when a range is identified. It could be used to reduce testing of samples in different container sizes or of different fills in the same container/closure system. In cases where different excipients are used among different strengths, then bracketing is not applicable.

Bracketing is a popular choice because the interpolation between the extremes is easy to interpret. If the extremes represent all the configurations in between, then there is no need to generate the same stability profile, which will indeed save resources. However, bracketing also possesses some undeniable challenges.

For instance, extreme presentations may no longer be of interest, or future additions may be outside the tested bracket. Bracketing presents a risky strategy when not all batches have been put up on stability, leaving the firm unable to revert to full testing, or when one of the batches does not meet expected acceptance criteria.

Table 15.6 provides an example of bracketing. Instead of 36 studies to be put up, only 12 studies are required to cover the extremes of strength and container sizes for this set of studies. Stability profiles of the intermediate configurations are

expected to behave similarly to the extremes; therefore, testing of the intermediate configurations is not necessary.

Table 15.6 Example of a simple bracketing design

Strength		50 mg			100 mg			250 mg			500 mg		
Batch		A	B	C	A	B	C	A	B	C	A	B	C
Container size	50 mL	T	T	T	–	–	–	–	–	–	T	T	T
	100 mL	–	–	–	–	–	–	–	–	–	–	–	–
	250 mL	T	T	T	–	–	–	–	–	–	T	T	T

Note: Three batches: A,B,C. Three container sizes: 50mL, 100mL and 250mL

It is important to keep in mind that if a stability profile of one of the extreme configurations does not follow the expected trend, then the intermediate conditions that it represents do not have support data. For more information, one should refer to the Q1D guideline.

15.5.2 Matrixing

Matrixing is a more conservative approach than bracketing. Indeed, it is favored by the regulatory agency, although regulatory experience continues to be limited. It is encouraged that a stability statistician be involved, as interpretation of data may be more complicated. As defined in Q1D, matrixing is a statistical design of a stability schedule. At a specified time point, a selected subset of the total number of possible samples is tested for all factor combinations. At a subsequent time point, another subset of samples for all factor combinations is tested. The design assumes that the stability of each subset of samples tested represents the stability of all samples at a given time point. Long-term trends are approximately linear across the studied presentation, thus the comparative stability of each presentation can be evaluated. Unlike bracketing, where the extremes are evaluated, matrixing is applicable where differences are identified. The differences in the samples for the same drug product must be identified as, for example, covering different batches, different strengths, different sizes of the same container and closure, and, possibly, in some cases, different closure systems. Matrixing can be performed across the packaging systems when a secondary packaging system is used to add to the drug product stability.

In a matrixing design, all factor combinations should be tested at initial and final time points. At intermediate time points, a fraction of these combinations should be tested. If full long-term data for the proposed shelf-life are not available for submission, then all selected combinations should also be tested at 12 months or at the last time point prior to submission.

The most critical advantage of matrixing is the flexibility it offers for design of a stability protocol. Each storage condition can be treated separately under its own matrixing design since the degradation rate may be different for each storage condition. Therefore, realistically only the long-term storage should be matrixed. Regulations require that testing at accelerated or stressed conditions should consist, at a minimum, of three time points for each combination; therefore, data of acceler-

ated or stressed conditions may not have enough data points to support matrixing at these storage conditions.

Matrixing is designed based on the knowledge of the expected stability of drug substances or drug products. Supporting data could help to justify different factorial matrixing designs. Matrixing cannot be performed across test attributes. However, each test could have its own matrixing design, depending on the test variability. Justification may be necessary if different matrixing designs are to be used.

Q1D has introduced the following scenarios of basic matrixing designs that could be exercised. (Tables 15.7, 15.8, 15.9 and 15.10).

Table 15.7 lists a *simple design of a one-half factorial* design, or one-half reduction design. This selection applies to two strengths, of which three batches are made per strength. It is recommended that all testing points of Time Zero and end-of-study are tested to give more confidence to these time points as data would come close to the true value. In addition, all testing is recommended to be performed at 12 months, which is the ICH submission time. These data are helpful to set specifications for new product registration. At all other time points, one-half of the configurations are to be tested, thus this is also called one-half factorial design.

Table 15.7 An example of a one-half factorial matrixing design

Time point		Months on stability							
	Batch	0	3	6	9	12	18	24	36
Strength #1	A	T	T	–	T	T	–	T	T
	B	T	T	–	T	T	T	–	T
	C	T	–	T	–	T	T	–	T
Strength #2	A	T	–	T	–	T	–	T	T
	B	T	T	–	T	T	T	–	T
	C	T	–	T	–	T	–	T	T

Note: Three batches: A,B,C. Two strengths: #1 and #2

Table 15.8 lists a *simple design of a two-third factorial* design or one-third reduction. This is a more conservative choice than one-half factorial design. Similar to the above option, all time points are tested at Time Zero, end of study, and at submission time (12 months). All other time points are reduced to two-thirds of the configurations to be tested.

Table 15.8 An example of a two-third factorial matrixing design

Time point		Months on stability							
	Batch	0	3	6	9	12	18	24	36
Strength #1	A	T	T	–	T	T	–	T	T
	B	T	T	T	–	T	T	–	T
	C	T	–	T	T	T	T	T	T
Strength #2	A	T	–	T	T	T	T	T	T
	B	T	T	–	T	T	–	T	T
	C	T	T	T	–	T	T	–	T

Note: Three batches: A, B, C. Two strengths: #1 and #2

The above examples only apply to cases of two factors, strength and batch. If there is another factor involved, (e.g. container size) the matrix is obviously more complex. Table 15.9 introduces a *complex design* with three batches of three strengths, made and packaged in three different packaging configurations. This design is known as complete complex design, where each configuration is tested under a certain schedule at each time point.

Table 15.9 An example of a complete complex design listed in Q1D

Strength	50 mg			150 mg			250 mg		
Container size	X	Y	Z	X	Y	Z	X	Y	Z
Batch A	T1	T2	T3	T2	T3	T1	T3	T1	T2
Batch B	T2	T3	T1	T3	T1	T2	T1	T2	T3
Batch C	T3	T1	T2	T1	T2	T3	T2	T3	T1

Key for testing	Months on stability							
Time point	0	3	6	9	12	18	24	36
T1	T	–	T	T	T	T	T	T
T2	T	T	–	T	T	–	T	T
T3	T	T	T	–	T	T	–	T

Note: Extracted from Q1D: Three batches: A,B,C; three container sizes: X,Y,Z for each strength.

Table 15.10 shows an *incomplete complex design*, of which only two-thirds of each of the configurations of the complete design are to be tested. As noted earlier, all testing is done at Time Zero, end-of-study, and at 12 months, which is ICH submission time. In this design, only two-thirds of the combination subsets will be tested in place of testing every combination. The design also shows a key that indicates only two-thirds of the testing is being done.

Table 15.10 An example of an incomplete complex design listed in Q1D

Strength	50 mg			150 mg			250 mg		
Container size	X	Y	Z	X	Y	Z	X	Y	Z
Batch A	T1	T2	–	T2	–	T1	–	T1	T2
Batch B	–	T3	T1	T3	T1	–	T1	–	T3
Batch C	T3	–	T2	–	T2	T3	T2	T3	–

Key for testing	Months on stability							
Time point	0	3	6	9	12	18	24	36
T1	T	–	T	T	T	T	T	T
T2	T	T	–	T	T	–	T	T
T3	T	T	T	–	T	T	–	T

Note: Extracted from Q1D: Three batches: A,B,C; three container sizes: X,Y,Z for each strength.

Justification and prior approval would be necessary depending on the differences of the configuration studies in the stability protocol. Q1D establishes a series of possible scenarios where matrixing could be applied without prior approval. Options

include different strengths with identical or closely related formulations, different batches made using the same process and equipment, or different container size and fill in the same container closure system. Justification would be necessary if different strengths are made and the relative amounts of API and excipients change, or if different excipients are used, or different container closure systems are employed. Supporting data would be necessary to show that these differences do not affect the stability profile of the drug product. The matrix could be designed so that the effect of each factor can be determined.

The advantage of matrixing is that it can revert to full testing, if necessary, because all samples are placed on stability. If, at certain time points, any result does not meet specifications, full testing could be started and additional data can be collected.

Matrixing presents a significant saving of resources. However, it also poses serious limitations, for example, all presentations must be set up on storage, data evaluation can be more complex, confidence intervals may be wider, and the design may not be as sensitive to differences as when full testing is done. It is strongly recommended that a stability statistician should be consulted for these applications.

Other matrixing options could be considered, such as complete removal of some presentations from testing, followed by performance of reduced testing of those remaining samples. This is a major reduction and needs justification. It is advisable that firms should work with regulators to apply this option. Factorial designs are extremely useful in a wide variety of experimental situations [3].

Although matrixing is encouraged by FDA, experience is very limited globally. Therefore, discussion with regional regulatory agencies is advisable if a matrixing application is to be submitted globally.

15.6 Annual Product Review

Section 15.21 CFR 211.180 requires that an annual product review must be done annually as part of cGMP requirements. Additional resources provide a thorough discussion of this process, including review of stability data and assessment of the stability profile of the drug products [4]. Performing this assessment will help the firm to determine if changes are needed in product specifications, formulation, process, or analytical procedures. An update of stability data for representative lots that have been placed on the annual product monitoring stability program is submitted to the regulatory agency. It should list any stability trends, deviations, or changes observed since the last review. A discussion of any out-of-specification or out-of-trend of stability data must also be included. It is helpful to present this data update graphically. Statistical analysis is also helpful to demonstrate if the stability program continues to support the approved product expiry. Review of stability data for the annual product review is also a good quality tool to ensure that the drug product continues to demonstrate its safety and effectiveness through its shelf-life.

15.7 Conclusions

Stability operations are critical to any GMP organization. Figure 15.6 describes the chronological order of important stability activities. Stability systems must be designed depending on organization infrastructure, available resources, and the number of studies and products the system must support. There are many ways to run an effective and compliant stability operation; these factors have been well-described [5]. At a minimum, 21 CFR 211.166 requires that a stability program must be written and followed. Regulations such as 21 CFR 211.194, which requires that laboratory records must include a description of the samples received for testing, also applies to stability testing. Other systems such as a training program, a metrology program, and LIMS are also vital to supporting and maintaining high levels of quality and compliance for the stability system in continuous operation.

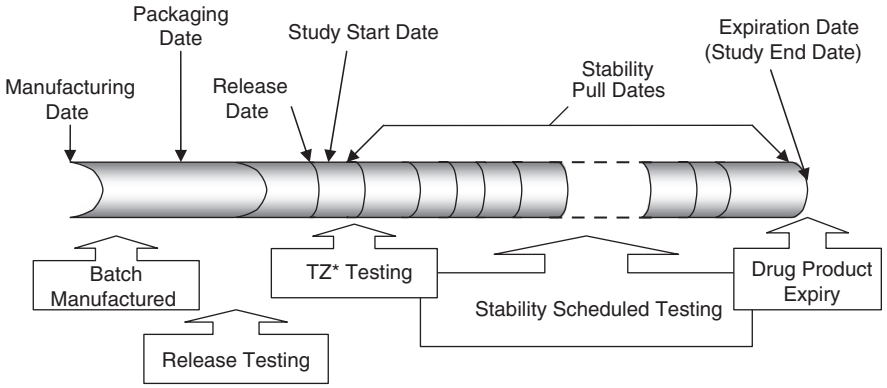

Fig. 15.6 Chronological order of stability activities to support a drug product study

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Part III
Other Stability Programs

Chapter 16
Combination Products/Drugs in Devices

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Contents

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Abstract The purpose of this chapter is to cover additional considerations, guidelines and requirements to help the reader design stability strategies for drug-device combination products. Tests and challenges to be included in stability studies are considered from a regulatory and scientific point of view and these are also related to the stage of development of the product. A number of drug-device combination types including inhaled/nasal products, pen injectors, drug-eluting stents and transdermal products are discussed specifically.

16.1 Introduction

The classification *Combination Product* encompasses a wide variety of different product types. In the United States, the Code of Federal Regulations, 21 CFR 3.2(e), defines what should be considered as a combination product. Essentially any combination of a drug and a device, a biological product and a device, a drug and a biological product, or all three together are considered as combination products. Products as diverse as a monoclonal antibody combined with a therapeutic drug, a condom with spermicide, an inhaler system, and a pre-filled syringe cartridge for use with an auto-injector are all considered to be combination products.

Combination products are a growing area in the field of pharmaceutical development. The purpose of this chapter is to complement the other sections in this book and to cover additional considerations only, including guidelines and requirements that should be taken into account when designing stability studies for drug-device combination products. A number of specific combination types including inhaled/nasal products, pen injectors, drug-eluting stents, and transdermal products are also discussed specifically. Where stability requirements and strategies are the same as for products that are not classified as combination products, readers should refer to other chapters in this book as appropriate.

Medical devices will not be covered in this chapter; the technical requirements of these products are laid out within the European Commission directives, in particular Directive 93/42/EEC and amendments, relevant Food and Drug Administration (FDA) Center for Devices and Radiological Health (CDRH) guidance and other regional/local guidance as appropriate, and will not be discussed here unless they apply to combination products [1–4]. Additional information on medical devices, particularly with respect to efforts to harmonize requirements globally, can be found on the Global Harmonization Task Force (GHTF) website [5].

16.2 Available Guidance and Regulatory Framework

The medical device and pharmaceutical industries have traditionally been separate businesses. The medical device industry generally develops products in line with the EC Medical Device Directive and the guidances issued by the CDRH office of the FDA, whilst the pharmaceutical industry looks to the relevant regulatory guidance available, including that from the FDA Center for Drug Evaluation and Research

(CDER), the FDA Center for Biologics Evaluation and Research (CBER), the European Agency for the Evaluation of Medicines (EMEA) Committee for Medicinal Products for Human Use (CHMP), and the International Conference on Harmonisation (ICH) when developing medicinal products [6–9]. In the emerging field of combination products, there is little specific guidance (akin to ICH) for companies to refer to and there is currently no overarching harmonized framework for developing drug-device combination products for the global market. As a result, the strategies employed during the pharmaceutical development of drug-device combination products are developed on a case-by-case basis. However, understanding the regulatory environment and the available guidance that does exist are important when developing stability strategies for new products.

In the US the FDA Office of Combination Products (OCP), created in 2002, has broad responsibilities covering the regulatory life cycle of combination products [10]. A key role of the OCP is to assign an FDA Center to have primary jurisdiction for regulatory review of a combination product. Early in the development of a combination product, it is wise to submit a Request for Designation (RFD) to the OCP in order to engage with the appropriate FDA center as outcomes from these discussions may have a significant effect on the development strategy. The OCP determine the Primary Mode of Action (PMOA) of the combination product and use this to assign the lead review center. In cases where the PMOA is not obvious or there is insufficient information to assign the PMOA (which may be the case during early development), the OCP will look in the first instance to assign the new product in line with other similar or previously approved products; or failing this to the center with the most expertise in the safety and efficacy of that type of product.

It is also the responsibility of the OCP to work with FDA Centers (CDER, CBER, and/or CDRH as appropriate) to develop guidance or regulations to support the agency regulation of combination products. However, as stated in the FDA guideline Early Development Considerations for Innovative Combination Products "... few guidance documents currently address the scientific and technical issues to consider when combining drug, device and/or biological product constituent parts as a combination product" [11]. The FDA guidance also states "because of the breadth, innovation and complexity of combination products, there is no single developmental paradigm appropriate for all combination products". This could lead to different standards and strategies being applied to individual products even after consultation with regulatory agencies. In summary, the innovator should work with the OCP at an early stage to determine the primary FDA review center to ensure all chemistry, manufacturing and controls (CMC) development aspects of the product are aligned with both the relevant guidance and requirements of that center as well as ensuring that the appropriate submission mechanism (e.g., CTD, 510(k), Device Master File etc.) is followed.

In the European regions covered by the European Medicines Evaluation Agency (EMEA), the key directives for combination products are 93/42/EEC for Medical Devices and 65/65/EEC for Medicinal Products and associated amendments [1, 12]. As with the FDA, it is important to understand early in the development pro-

gram whether the drug/device combination product will be regulated as a device, a medical product, or both. To determine this, the intended purpose of the product (taking into account the way the product is presented) and the method by which the principal intended action is achieved need to be considered. The latter criterion, based on the *principal intended action* is critical. The principal intended action of a product may be deduced from the manufacturer's labeling and claims, but more importantly, from scientific data regarding its mechanism of action. Typically the medical device function is fulfilled by physical means (including mechanical action, physical barrier, replacement of (or support to) organs or body functions). The action of a medicinal product is typically achieved by pharmacological, immunological, or metabolic means.

Medical devices may contain medicinal substances which act on the body in a manner ancillary to the device. However, where such substances act in a manner that is more than ancillary, the product is regulated as a medicinal product rather than a medical device. In addition, in cases where there is doubt as to the classification of the product as a device or medicinal product, the provisions of 2004/27/EC state that the product shall be regulated as a medicinal product [13].

An example of how the Medical Device Directive (MDD) and Medicinal Product Directive (MPD) are applied is outlined below for injection products [1, 14]:

- An empty syringe is classified as a medical device (MDD applied).
- A disposable pen-injector where the drug-containing injector is a single integral unit and only to be used in that given combination, is covered by the MPD. However in addition to this, the relevant essential requirements in Annex 1 of the MDD apply with respect to safety and performance related features of the device (e.g. a syringe forming part of such a product).
- For a drug-containing pen injector that is developed for a specific drug, but whereby the device and drug are available separately, the device and the drug will be considered individually as a medical device and a medicinal product (MDD and MPD applied, respectively).

The EC guideline MEDDEV 2.1/3 rev 2 (July 2001) on demarcation between the directives relating to Active Implantable Medical Devices and Medical Devices, and Medicinal Products is particularly helpful when considering the assignment of products as devices or medicinal products [15]. A device which is intended to deliver a medicinal product is itself regulated as a medical device. The medicinal product which the device is intended to administer must, of course, be approved according to the normal procedures for medicinal products. However, if the device and the medicinal product form a single integral product which is intended exclusively for use in the given combination and which is not reusable, that single product is regulated as a medicinal product. In such cases the essential requirements of the MDD apply as far as the device-related features of the product are concerned (for example as regards the mechanical safety features of a pre-filled pen-injector).

As can be seen from the examples given above, consideration needs to be given to whether the drug-device combination is performed by the patient or manufacturer.

There are two main ways in which the combination of the drug and the device can be achieved:

1. The device is available as a marketed product and the drug that is to be inserted into the device is purchased by the patient separately. Here the combination is undertaken by the patient, not by the manufacturer.
2. The drug and device are combined during the manufacturing/assembly process and the patient receives the product as an integrated drug-containing device.

In summary, if the patient is supplied with the drug and device separately and inserts the drug-containing package into the device for use, the drug is classified as a *standard* drug product and the device as a medical device (in both US and EMEA regions). If the manufacturer combines the drug and device such that the patient receives a drug-device combination (e.g., one that is disposed/not re-used after dose(s) have been delivered), then this combination is classified as a combination product in the US and as a medicinal product in the EMEA region, respectively (with consideration given to appropriate sections of the MDD for the device part for the EMEA).

Within the countries covered by the EMEA, there may be a need to submit a dossier covering both the drug and its combination, and a separate dossier for the device component of the product, whereas in the United States the review of a single dossier for the entire drug-device combination would be primarily handled by one review center. An understanding of the classification rules for medical devices in Europe will help to ensure that both the technical requirements and documentation requirements are met for the device component [1]. In Europe a Class I medical device (a device classed as having the lowest risk) would require a conformity assessment to be undertaken to allow the device to be Conformité Européenne (CE) marked, a necessity for products to be marketed within the EMEA regions. Often, items that are considered accessories to the main device part of the product fall into this Class I category. For example, a needle shield or guard that is used to hide a needle from a patient's view during injection is a non-invasive medical device and would be classified as Class 1 by the EMEA but often as an accessory to the pen-injection device by the FDA. There are often no specific regulatory requirements for these accessories in the US, but this would need to be discussed with the primary review center on a case-by-case basis. However, in Europe, if there is a device component of the product that is a classified as a Class 3 medical device under the MDD, the regulatory review process will include assessment of the conformity assessment and review of the acceptability of the device by both a Notified Body and subsequently by the EMEA Competent Authority responsible for assessment of the product.

The Japanese regulatory process was revised in 2004, creating the Pharmaceutical and Medical Devices Agency (PMDA), and provisions relating to medical devices came into effect on April 1, 2005 [16]. In Japan two submissions may be required: a KIT Drug J-NDA submission and a separate Medical Device Certification; the latter of these has to be filed with PMDA. It should be noted that the review timelines for KIT Drug and Medical Device Certification submissions

can differ significantly (i.e., 12–18 months versus 4–6 months, respectively). As with both the EMEA and FDA, it is important to establish early in development the stability requirements for the drug, device, and the combination and to understand in which submission such data would be submitted. In some cases the Medical Device Certification cannot be submitted for review until the KIT Drug submission is approved, therefore early communication with the PMDA, or engagement with a local medical device consultant, is important in order to understand the stability requirements for each of these submissions. Classification of the device according to the Japanese Pharmaceutical Affairs Law (PAL) may also impact the stability strategy; a summary of the PAL can be found on the Japan Pharmaceutical Manufacturers Association (JPMA) website [17].

16.3 Stability Strategies for Drug in Device Combination Products

The assignment of the innovator's product to an appropriate review agency and an outline agreement on the technical requirements and regulatory submission mechanism/format for the product being developed is important for all development aspects of the product including stability. Stability studies take a finite amount of time to execute and therefore it is vital to know what, if any, stability data may be required by the regulatory agencies as early as possible in the product development cycle. For drug-device combination products in particular, additional considerations include:

- whether additional specific stability studies are required (e.g., transportation studies)
- consideration of the minimum time period to be covered by data at time of submission (depending upon the combination product characteristics) which may in turn affect the testing strategy and bracketing/matrixing design
- the number of units required for testing on any study may be higher for combination products; this may affect manufacturing batch size
- chamber storage capacity (depending on size of device, the number of units needed for testing, and the length of the stability program)
- costs related to stability studies and resources required; these can be significantly higher for these types of products (orientations, numbers of prototypes, specialized testing), and the analytical skills and analyst capabilities required may be more difficult and time consuming to locate or develop

As discussed in the previous section, stability requirements for drug-device combination products are not well defined. For the drug component, ICH guidelines define data points, general testing, test conditions, and other considerations at time of submission. For the device component, stability is related to confirming appropriate and safe functioning of the device over its intended use period. Many of the testing requirements for device components are laid out in the International Organization for Standardization (ISO) standards (e.g., ISO 11608 for standards for

pen-injector devices) [18, 19]. However the bringing together of the drug and device entities, forming the combination product, provides a degree of uncertainty. Innovators need to consider whether the stability of their combination product could be different from that of the individual entities. Questions to consider when developing a stability strategy include:

- Does the drug product come in contact with any part of the device during long-term storage?
- Does the drug product come in contact with any part of the device during patient use?
- Does the device provide protection to the drug product or is its function purely as a delivery system?
- Is there any potential for leakage of the drug product into any of the device components?
- Is the combination product required to be sterile?
- Will all device components function as required over time such that the device will deliver the required dose?
- If the drug product is in direct contact with the device (no impermeable protection included), is there potential for leachables to migrate into the drug product over time?

In addition, the FDA Early Development Considerations for Innovative Combination Products guideline states it may be appropriate to conduct studies to evaluate the potential for the following [11]:

- Changes in stability of the drug constituent when delivered by the device or when used as a coating on the device
- Changes in the stability or activity of a drug constituent when used together with an energy emitting device
- Leaching of the device materials into the drug product

Similarly, consideration must be given to the effects a drug or biological product may have on the device constituent.

Stability scientists, who are usually more familiar with standard dosage forms (e.g., tablets, capsules, and injectables), must understand the stability requirements for the device versus those for the drug component for the device. Figures 16.1, 16.2, and 16.3 outline a series of considerations related to developing a stability strategy for combination products. As can be seen in these flow charts, the type of combination product being developed affects the stability study requirements.

It should be noted that the stability requirements for drug device combinations are still evolving. Some recent interactions with regulatory agencies have led to companies undertaking registration stability programs for combination products consisting of existing formulations in a new device, in which there is no drug-device contact, in order to demonstrate functionality over time (Fig. 16.2). Moreover in some of these cases, companies have been requested to provide additional chemical stability data on the existing formulation in the new combination even though this may be challenging to rationalize scientifically as the new device is not in contact with the

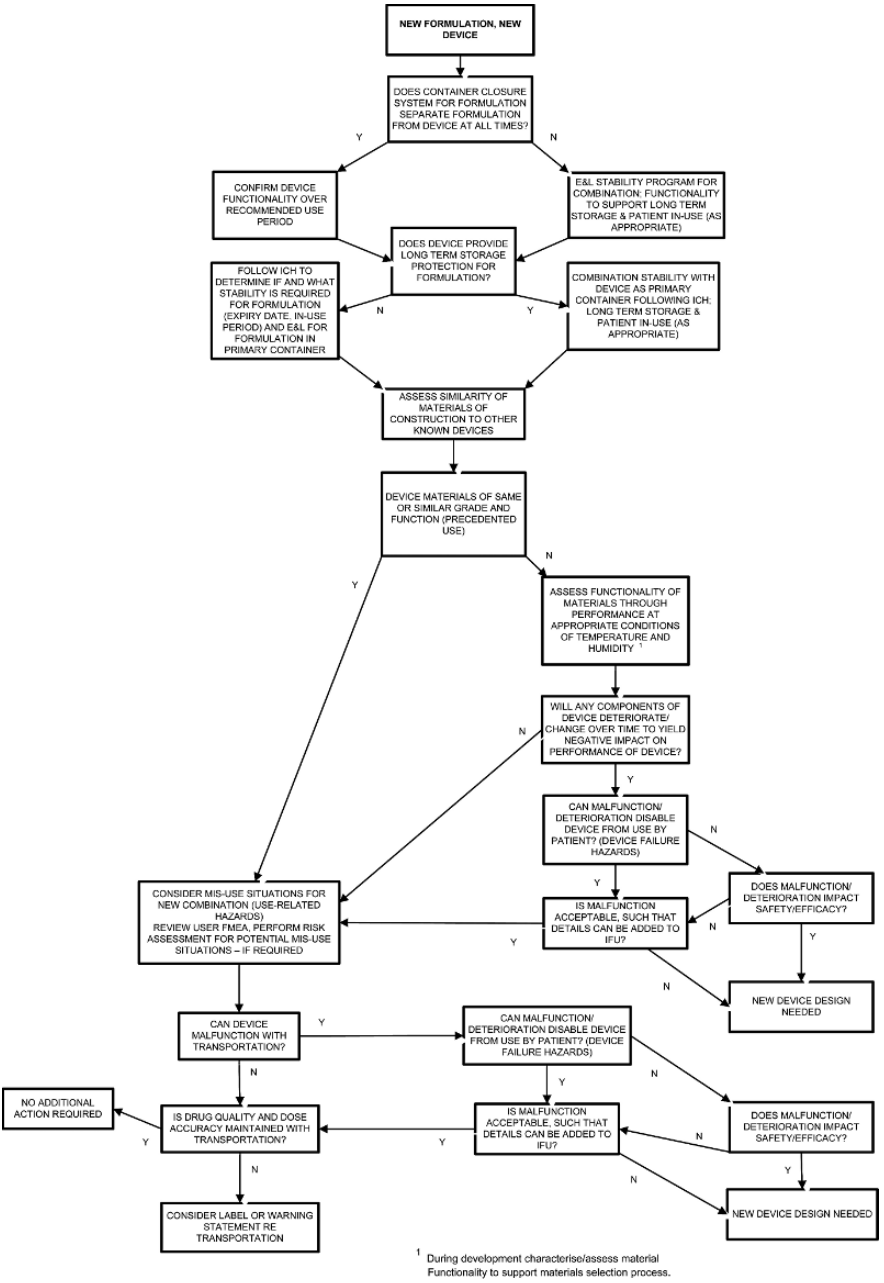

Fig. 16.1 Decision tree for determining stability studies for the registration of combination products with new formulation/new device

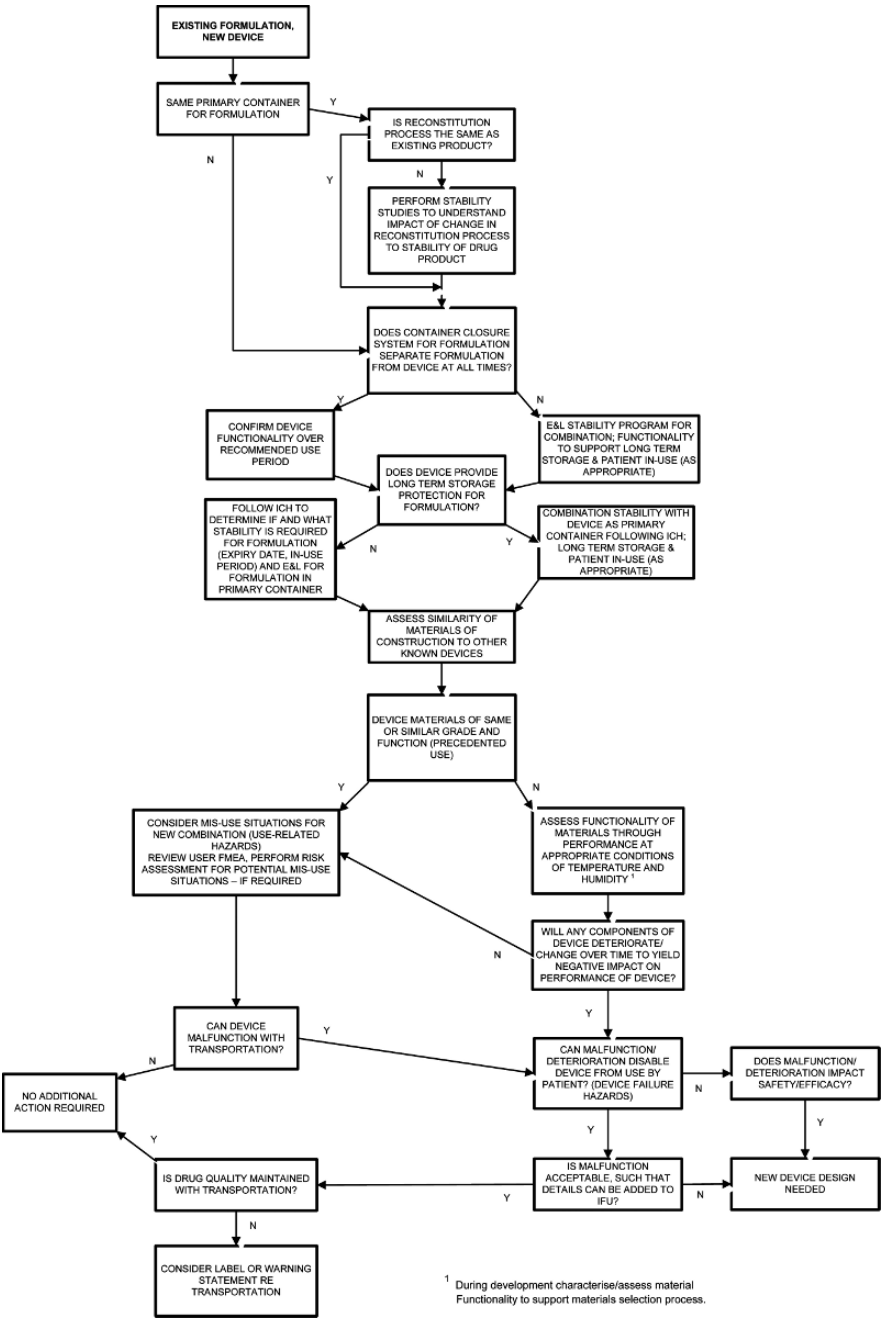

Fig. 16.2 Decision tree for determining stability studies for the registration of combination products with existing formulation/new device

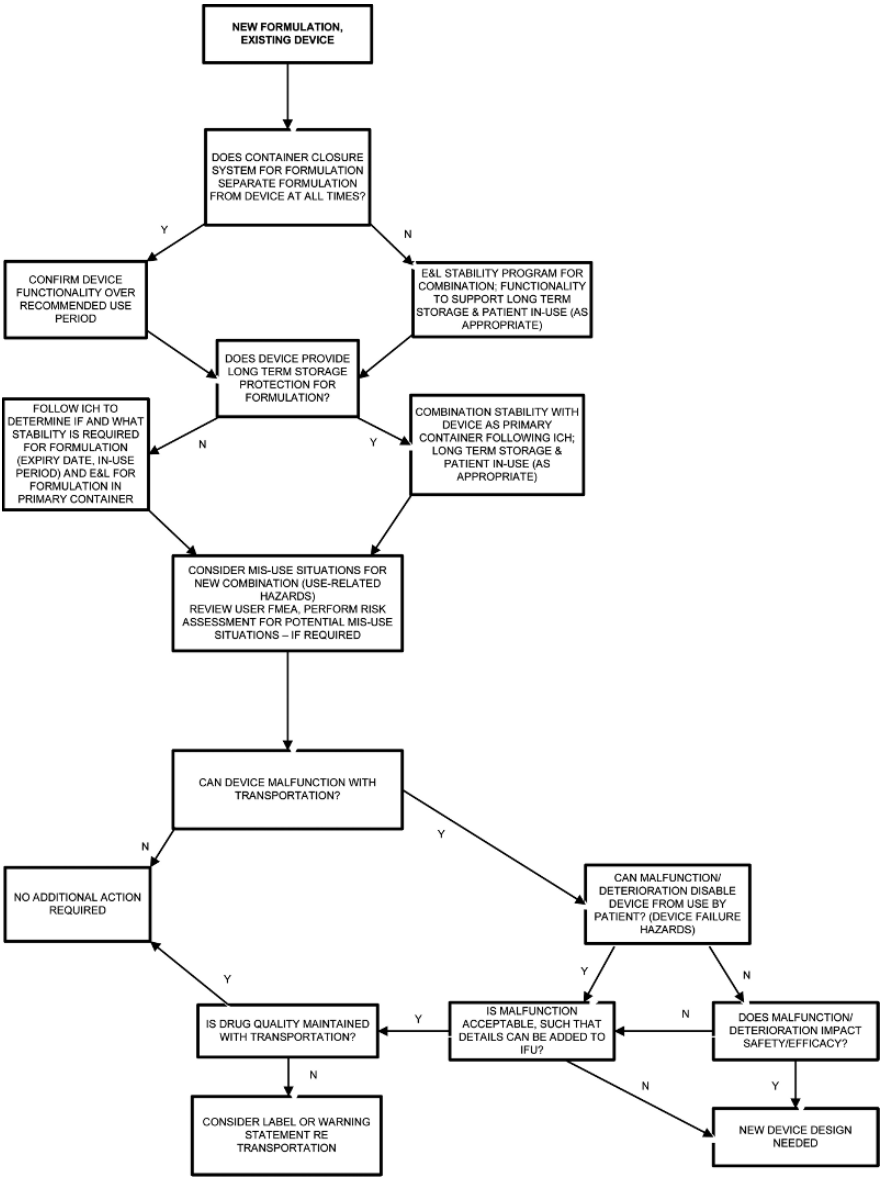

Fig. 16.3 Decision tree for determining stability studies for the registration of combination products with new formulation/existing device

drug nor is it providing any environmental protection. A well thought out and scientifically sound stability strategy needs to be presented to regulatory agencies early in development as requirements could differ significantly depending on mechanism of (primary) submission (e.g., 510(k), CTD, DMF, etc).

16.4 Nasal Spray and Inhaled Products

16.4.1 Introduction

For inhaled products there are a number of guidelines and papers to refer to when developing stability strategies. The European Medicines Agency's Quality Working Party, and Health Canada's Therapeutic Products Directorate have developed a joint guidance document on the Pharmaceutical Quality of Inhalation and Nasal products [20]. In addition, further clarification may be found in the overview of comments received on the guideline as it was being drafted and the responses to the comments [21]. This guidance applies to human medicinal products intended for delivery into the lungs or nasal mucosa.

In the US, two separate guidelines have been developed, one covering both metered dose inhalers (MDIs) and dry powder inhalers (DPIs) and one covering nasal sprays, inhalation solutions, and suspensions and inhalation sprays [22, 23]. The guidance on MDIs and DPIs is however still in draft form nearly 10 years after being published for comment; one of the unresolved issues is the dose content uniformity requirements which are discussed later in this chapter (Section 16.5.4). There are also other sections which would benefit from further discussion, in particular the number and nature of some of the tests that may be expected on stability.

Slightly different terminology is used in the two regions when classifying various types of inhaled products. These are compared in Table 16.1.

There are unique features pertaining to nasal sprays and inhalation products which make stability studies more complex and challenging. Examples include metering and spray production, energy required for spray production, the container closure system, and small doses. Critical attributes include the reproducibility (throughout the shelf-life) of the dose, the spray plume, and the particle/droplet size

Table 16.1 Classification of product types

FDA terminology	EMEA terminology
Nasal spray	Non-pressurized metered dose nasal spray
	Nasal single use sprays
Inhalation spray	Non-pressurized metered dose inhaler
Inhalation solutions and suspensions	Product for nebulization (single and multiple use)
Metered dose inhaler MDI	Pressurized metered dose nasal sprays
	Pressurized metered dose inhaler
Dry powder inhaler DPI, device metered	Dry Powder Inhaler, device metered
	Nasal powders, device metered
Dry powder inhaler DPI, pre-metered	Dry Powder Inhaler, pre-metered
Not included	Nasal drops (single and multiple use)

distribution, the maintenance of sterility or microbial load as well as functionality of the device (spray mechanism, sensors). Additionally, changes to components, manufacturer, or manufacturing process, which might affect any of the key attributes, will require adequate data to demonstrate that significant changes to stability characteristics do not occur.

16.4.2 Overview of Stability Tests

Both the FDA and EMEA guidelines describe tests to be considered on the specification and for stability testing [20, 22, 24]. However the guidelines recommend different attributes and provide limited detail on testing to be performed at the various stages of product development (e.g., early development, registration, post-approval commitment stability studies). The following sections include a comparison of the regulatory requirements and outline stability strategies to be considered during development.

For all products, appearance/description, assay and degradation products would be performed during stability studies at all stages of development. Additional tests to be considered are listed in Tables 16.2 and 16.3. During the development cycle the testing pattern may change as product understanding develops. For example, during early development, the tests considered for a DPI product may include only appearance, assay, degradation products, uniformity of delivered dose, and fine particle dose, whereas in later studies as product knowledge develops, further tests may be added with meaningful specification limits. These in turn may be subsequently eliminated when it has been demonstrated that they are not stability indicating attributes for the product.

16.4.3 Assay

For multi-dose products, the EMEA guideline states that the amount of drug substance should be determined per weight unit or per volume unit as applicable [20]. For single dose products, assay results should be expressed as mass per dosage unit, in other words, concentration. According to the FDA draft guideline, assay for MDIs may be performed indirectly by determining concentration and actual net content i.e. fill weight/volume, whereas for DPIs the amount of drug substance in each individual dosage unit should be determined for pre-metered devices and in the reservoir for device-metered inhalers [22]. Therefore, for MDIs and device metered DPIs, CDER are describing assay as total content rather than concentration. In all cases, for stability testing where degradation trends are important, monitoring assay as concentration over time is also important. If it is possible for fill volume to change on stability (e.g., when semi-permeable containers are used or where gases could be lost through valve elastomers), care should be taken when analyzing the data to ensure that loss of volume (concentration increase) is not offset by degradation (concentration decrease) thereby masking stability trends. For drug substances in salt form, assay of the counterion is unnecessary unless the salt form is known to degrade, for example, as determined via forced degradation studies.

Table 16.2 Tests to be considered on stability for nasal sprays, inhalation sprays, and inhalation solutions and suspensions

	Nasal spray		Inhalation spray	Inhalation solutions
Stability test	Non-pressurized metered dose nasal spray	Nasal single use sprays	Non-pressurized metered dose inhaler	Product for nebulization (single and multiple use)
Mean delivered dose	Yes	Yes	Yes	No
Delivered dose uniformity (+ through container life)[1]	Yes	No	Yes	No
No. of actuations	Yes (EU only)	No	Yes (EU only)	No
Plume geometry[2]	Yes	Yes	Yes	No
Particle/droplet size[3]	Yes	Yes	Yes	Yes (for suspensions)
Microbial count[4]	Yes, unless sterile	Yes, unless sterile	Yes, unless sterile	Yes, unless sterile
Sterility	If sterile	If sterile	If sterile	If sterile
Preservative/stabilizer content[5]	If present	If present	If present	If present
Antimicrobial preservative effectiveness[6]	If present; 1 batch	If present; 1 batch	If present; 1 batch	If present; 1 batch
Particulate matter[7]	Yes	Yes	Yes	Yes
Weight loss	If semi-permeable	If semi-permeable	If semi-permeable	If semi-permeable
pH[2]	Yes	Yes	Yes	Yes
Viscosity	Yes	Yes	No	No
Leachables	Yes	Yes	Yes	Yes

[1]Refer to Section 16.5.4.
[2]May be tested during development only to demonstrate no stability issues; may justify to omit from the specification if appropriate.
[3]Refer to Section 16.5.5.
[4]May consider testing at last checkpoint before submission and annually only.
[5]If present.
[6]May be tested as part of registration stability studies and at selected checkpoints only.
[7]May be tested during development or registration stability studies only to demonstrate no issue; thereafter on release only.

16.4.4 Dose Content Uniformity/Delivered-Dose Uniformity

Various terms are used for this requirement including: dose content uniformity, delivered-dose uniformity, and emitted dose uniformity as well as spray content uniformity for nasal sprays. The EMEA guideline refers to the relevant pharmacopoeia for guidance on requirements [20]. The delivered dose uniformity requirements contained in the draft FDA guideline for MDIs and DPIs are challenging, and during stability testing, where increased numbers of samples are being tested, an

Table 16.3 Tests to be considered on stability for MDIs and DPIs

	Metered dose inhaler MDI		Dry powder inhaler DPI,	Dry powder Inhaler DPI,
Stability test	Pressurized metered dose nasal spray	Pressurized metered dose inhaler	Dry powder inhaler and nasal powders, device metered	Dry powder inhaler pre-metered
Mean delivered dose	Yes	Yes	Yes	Yes
Delivered dose uniformity (+ through container life)[1]	Yes	Yes	Yes	Yes[2]
No. of actuations	Yes (EU only)	No	Yes (EU only)	Yes[2]
Particle/droplet size distribution[3]	Yes	Yes	Yes	Yes
Fine particle mass	No	Yes	DPI only	Yes
Plume geometry[4]	Yes	Yes	No	No
Microscopic evaluation[5]	Maybe	Maybe	Maybe	Maybe
Particulate matter[6]	Yes	Yes	Yes	Yes
Microbial count[7]	Yes	Yes	Yes	Yes
Solid form/polymorph	Suspensions	Suspensions	Yes	Yes
Weight loss[8]	Yes	Yes	No	No
pH[4]	Yes	Yes	No	No
Leak rate	Yes	Yes	No	No
Moisture	Yes	Yes	Yes	Yes
Leachables[9]	Yes	Yes	No[10]	No[10]

[1]Refer to Section 16.5.4.
[2]Not required for capsules.
[3]Refer to Section 16.5.5.
[4]May be tested during development only to demonstrate no stability issues; may justify to omit from the specification if appropriate.
[5]May be tested on stability e.g., if issues noticed with particle size distribution, an increase in the number of foreign particulates, appearance changing, or form changes expected.
[6]Test during development or registration stability studies to monitor trends; if no issues on stability observed, test at release only.
[7]May consider testing during primary/registration stability studies at last checkpoint before submission and annually only.
[8]Generally required as part of in-use testing. Would be required for products in semi-permeable containers on stability.
[9]May be tested as part of registration stability studies only; also refer to Section 16.9.
[10]Assess on a case by case basis.

increase in the number of out of specification results may be observed even when the product is stable [22].

In order to address these challenges, the International Pharmaceutical Aerosol Consortium on Regulation and Science (IPAC-RS) presented a proposal to the FDA to replace the test requirements outlined in the FDA draft guidance with a parametric tolerance interval (PTI) test for dose content uniformity for MDIs and DPIs [25]. As a result of this proposal, a working group consisting of FDA and IPAC-RS members was set up in 2004. In principle FDA have agreed to the use of the PTI test but there is no agreement on the statistical parameters (such as coverage) that might be built into a *universal* PTI test. However in a risk-based approach to product development the test parameters must reflect the therapeutic index and dose–response of the drug. In practice, therefore, a zero-tolerance test approach should be taken (e.g., 10 out of 10 tested must fall within ± 35% of the label claim) until appropriate clinical information is available. To date, the latest editions of the national pharmacopoeias have not adopted the PTI requirements and the current harmonized USP and Ph. Eur. pharmacopeial limits are wider than those outlined in the draft FDA guideline [26, 27]. Although the USP currently requires uniformity of delivered dose over the entire contents for MDIs and DPIs, given the current expectation that MDIs contain a dose counter, information on delivered dose uniformity after actuation of the labelled number of doses is of limited use. In Europe the number of deliveries per inhaler is required.

For metered dose nasal sprays the pharmacopoeias are however different. The Ph. Eur. contains the same requirements as those for MDIs and DPIs, whereas the USP requirements are more challenging, as per those in the original FDA guideline for nasal sprays et al.

An additional requirement detailed in the draft FDA guideline for MDIs and DPIs is valve delivery/shot weight [22]. Although for stability studies the measurement of dose during the determination of dose content uniformity may be more appropriate than valve delivery/shot weight, some regulatory agencies consider that valuable information regarding potential causes of dose variability may still be gained from generation of the data. It is therefore wise to discuss the stability testing strategy with regulatory agencies prior to registration stability studies.

16.4.5 Particle/Droplet Size and Fine Particle Mass

Particle size distribution is a multivariate parameter. In early development it is often described by a single point control known as fine particle mass, typically being the mass of particles *less than or equal to* 5 μm. As development proceeds, more complex specifications are developed whereby the particle size distribution is represented by a number of particle size fractions between 1 and 10 μm, with requirements linked to batches used clinically.

Maintaining particle/droplet size distribution on stability is a key challenge in the development of nasal spray and orally inhaled products. Suspensions have the potential to agglomerate or to undergo particle size changes [28]. For solution products,

moisture ingress may change the evaporative nature of the solvent system and consequently lead to changes in droplet size [29]. In dry powder inhaler products humidity during storage can affect powder properties and the fine particle mass [30, 31].

Microscopy may be used on stability to help determine causes for any changes noted, for example to determine whether agglomeration or particle size growth is occurring in a suspension. Microscopy may also be useful for investigations into appearance observations, for example if foreign particles are noticed. However it is difficult to set acceptance criteria for microscopy as a test, and it is therefore of more use as an investigative tool to understand sources of/causes for particulate formation.

16.4.6 Moisture

As mentioned in the preceding section, moisture can affect the performance of the drug product and therefore a test for water may be required if the product demonstrates sensitivity to moisture.

However moisture *in itself* is not an issue and even if linked to a critical attribute such as particle size, degradation, or microbial growth, this will not often be known during the early stages of development. Thus it is often not feasible to set appropriate acceptance criteria during early development stages, although moisture should still be measured at key stability checkpoints to look for links to key performance attributes. If a correlation is found between moisture and a critical parameter such that moisture has a negative impact on product quality or performance, then moisture itself must be controlled, for example through raw material controls or through appropriate packaging/storage. If moisture is not an issue or is less indicative of an issue than measurement of a critical attribute itself then a justification could be submitted to omit moisture control from the specification and thus from future post-approval stability studies.

16.4.7 Particulate Matter/Foreign Particles in MDIs and DPIs

The draft FDA guideline describes the requirement to monitor foreign particle levels during stability studies [22]. Since this guideline was drafted the IPAC-RS has published two articles/guidelines on particulates testing [32, 33]. These articles include testing and specification development for particulates. Regarding stability studies, the latter article states that particle characterization (e.g., microscopy) should be performed at the initial stability time-point; however, for stability purposes the article indicates that it is necessary at any time-point to characterize only if the number of foreign particles was observed to be increasing (either through appearance testing, via membrane testing e.g., on DPIs, or via a validated method depending on the stage of development). To develop an understanding of performance it may be appropriate to characterize certain batches, but separate from stability activities. If changes are observed on stability it would be prudent to characterize the nature of

the particles and compare for example to *control* samples stored at refrigerated conditions. The first IPAC-RS article states that for commercial batches, release testing only is required in situations where no stability trends were noted in development, and that in time, release testing could also be phased out [32].

16.4.8 Storage Conditions

Storage conditions are described in Chapters 3 and 4 in this book, and depend on the region the product is to be registered in. The one difference in requirements for MDIs and DPIs is described in the draft FDA guideline and is for products needing to be packaged in moisture protective packaging [22]. In this case, storage at the condition of $25°C/75\%RH$ for one-third of the shelf-life is described to check that the packaging is adequate to protect the product. However depending on the regions the product is intended to be registered in, the long-term Zone IVB condition of 30°C/75%RH would essentially be a worst case scenario and should cover registration in all zones [34]. A company could therefore opt not to test product at 25°C/75%RH, assuming adequate stability at the more severe condition.

16.4.9 In-Use Testing

In-use testing is performed without the protective over-pack in which the stability of the product/primary package is being tested. The most recent FDA guideline, for Nasal Spray and Inhalation Solution, Suspension, and Spray Drug Products, states that if additional packaging (e.g., foil over-wrap) is used to protect the drug product from evaporative effects, then adequate stability data conducted at a minimum of 25°C and a maximum of 40% RH should be generated for pertinent parameters for these units without the protective packaging [23]. For MDIs and DPIs, the FDA draft guideline states that data generated at a minimum of 25°C and 75%RH is required if additional packaging (e.g., foil over-wrap) is deemed necessary [22]. It could be scientifically justified that if moisture loss is deemed a potential issue then 25°C/40%RH would be appropriate, if moisture ingress is deemed to be a potential issue then 25°C/75%RH would be appropriate, depending on the properties of the formulation and the packaging. These conditions are appropriate for Zone I/II regions. For Zone III/IV regions, 30°C /35%RH is recommended if moisture loss is a concern, 30°C/75%RH if moisture ingress is a concern [34]. If a global filing is the goal then the Zone III/IV conditions are the most challenging and therefore could be justified as sufficient to cover registration in all regions.

In-use testing should be performed on two batches, at least one of which should be near end of shelf-life or at the final time-point of the submitted stability studies [34, 35]. Thus if a product is to be used within 3 months after removal of the protective packaging (according to the Instructions For Use (IFU)), the product should be removed from the protective packaging 3 months before the end of the shelf-life, and

Table 16.4 Example Registration Stability Protocol for a DPI

		Months							
Stability condition		T0		3	6	9	12	18	24	36
Initial		A[1]		–	–	–	–	–	–	–
Accelerated	40°C/75%RH			A	A[1]	–	–	–	–	–
Long Term	30°C/75%RH			A	A	A	A[1]	A	A[1]	A[1]
Long Term	25°C/60%RH[2] 25°C/75%RH[3]			A	A	A	A[1]	A	A[1]	A[1]
Controls	5°C			C	C	C	C	C	C	C
Photostability	Option 2		A	–	–	–	–	–	–	–
Thermal Cycling			A	–	–	–	–	–	–	–

[1]Samples to be removed for in-use testing, if appropriate as determined by when the data are to be filed and the shelf life being requested.
[2] Depending on stability knowledge of the product, this condition may not be actively tested if the product was found to be stable at 30°C/75%RH during developmental stability studies.
[3]This storage condition is required when moisture protective packaging is deemed necessary for the product; store spares only in case of unexpected results at 30°C/75%RH up to 12 month checkpoint.

then tested at the end of the shelf-life [20]. An example registration stability protocol for a DPI, including in-use testing considerations, is included in Table 16.4.

16.4.10 Other Specific Stability Considerations/Requirements

The FDA guidelines also describe additional stability related studies including device robustness and effects of resting time particularly on priming/re-priming [22, 23]. Additional stability studies also need to be considered when changing the manufacturing facility, manufacturing procedure, source, synthetic route or micronization of the API, source or type (design or composition) of container and closure components, grade of excipient or even source of excipients if they may affect the stability. This may be done via comparability studies. Discussions on leachables and temperature cycling are included in Sections 16.9 and 16.12 of this chapter.

The Association of South East Asian Nations (ASEAN) Guideline on Stability Testing of Drug Product contains a guide on tests to be included in a stability study for this region [36]. In addition to tests already discussed, for MDIs and nasal aerosols both taste and assay for co-solvent are described. For nasal sprays, clarity of solution is also described. Innovators should consider whether these tests add to the information that is being gathered via other tests and, in the case of taste testing, what the safety implications are for analysts.

16.5 Pen Injectors

There are a significant number and variety of pen-injector devices on the market and in development today, including products for treatment of diabetes, rheumatoid arthritis, and growth hormone deficiency. These pen-injector devices are generally

considered as combination products; however, the difference in how the combination is achieved can have a bearing on the requirements for the product. The stability requirements relating to pen-injectors are constantly evolving, in many ways due to the submission mechanism for these types of drug-device combinations. In the past many of these devices have been submitted to FDA as Premarket Notification 510(k)s through CDRH; these have specific requirements in terms of content for submission [37]. More recently a number of pen-injector combination products have been required to be submitted in CTD format through CDER or CBER, therefore the expectations for stability programs have been more akin to ICH requirements [9].

There are two cases in which the combination of the drug and the device can be achieved:

1. The device is available as a marketed product and the drug that is to be inserted into the pen-injector device is purchased by the patient separately. Here the combination is undertaken by the patient, not by the manufacturer.
2. The drug and device are combined during the manufacturing or assembly process and the patient receives the product as an integrated drug-containing pen-injector device.

Case 1 above is relatively straightforward, for example: the pen-injector is registered and approved as a medical device and the drug registered as a medicinal product. The pen-injector device and medicinal product are purchased separately; an individual drug-containing cartridge is inserted into the pen-injector by the patient upon first use. This drug-containing pen-injector combination is then used for a finite period of time until either the shelf-life of the drug-containing cartridge is reached (e.g., the cartridge may have a 14 or 28 day use-by date) or the contents of the cartridge are depleted. After this point the patient takes a new cartridge for insertion into the same pen-injector device for continued medication. This process of using the same pen-injector but inserting new individual cartridges of drug product into the pen-injector device on an ongoing basis would continue until the end of the lifetime of that individual pen-injector. As both the drug and device are packaged (and potentially registered) separately in this case, they each require individual stability programs to meet registration requirements and enable use-by dates to be assigned. Stability for the medicinal product would follow ICH guidance whereas for the device the focus would be to demonstrate functionality over its intended use period. The device needs to meet the requirements of ISO 11608 to confirm its ability to function and meet the requirements of dose accuracy (first, each, and last dose) over its lifetime [19]. This functionality would be performed by calculating the number of times the pen-injector would be used by the patient, for example a once-weekly injection with a pen-injector that could be used over a 2-year period would equate to 104 times that each device could be used by the patient. In this instance dose accuracy would be confirmed over a minimum of 104 uses of an individual pen-injector device. For the medicinal product that is registered, packaged, and sold as a separate entity, the stability program would be executed in line with ICH guidelines.

For Case 2, the requirements will depend to a large degree on the regulatory submission mechanism for the product. The flow charts in Figs. 16.1, 16.2, and 16.3 (see Section 16.3) outline considerations for developing a stability strategy for registration of the product.

16.6 Drug Eluting Stents

The FDA defines the PMOA for a drug eluting stent (DES) is as a device [38, 39]. During the regulatory process CDER would be involved with the review of the pharmacologic agent and CDRH would review the stent platform, the delivery system and the carrier (polymer), if present. In Europe a DES would also be viewed as a medical device [15, 40].

The FDA has issued a draft guidance containing a number of sections relating to the stability recommendations for a coronary DES [41]. Tests described for stability studies are appearance, assay, degradation products, in-vitro drug release, and particulate matter. In addition, sterility and package integrity are included as being required annually and at end of shelf-life. In the example testing protocol for long-term conditions, a test for endotoxins is described; however, for sterile products (tested for endotoxins at release) the need for this test on stability is questionable. Johnson & Johnson (Cypher™ with active drug sirolimus) and Boston Scientific (Taxus™stent with active drug paclitaxel) also included identity, drug content uniformity, residual solvents, and endotoxins in their site-specific registration stability programs [42, 43]. However, these tests are not usually considered necessary for stability studies.

During the development phase, compatibility between the drug, stent, and carrier matrix, if present, must be explored. The stent has to withstand significant expansion during deployment, as well as constant pulsation in the artery after deployment, without cracking or flaking, and thus initiating clotting or liberating potentially harmful particulate matter into the coronary blood stream [44, 45].

For new stent systems, additional stability challenges include engineering, stress, and durability tests. These include stability to magnetic resonance imaging (MRI) scans [46]; chemical stability of any polymer component(s) [47]; the stability of polymer coated stents to degradation during sterilization [48, 49]; predicting degradation rates and determining products for bioresorbable stents [50]; and in-vivo stability [51]. The FDA draft guidance for a coronary DES describes mechanical performance and integrity challenges that should be performed during development, including tests for coating integrity for product aged to the requested shelf-life and under accelerated simulated in vivo conditions, corrosion potential with coating defects, particulate matter after ageing and, if appropriate, durability of degradable coatings [41].

During pre-clinical testing, the FDA has noted many deficiencies related to inadequate stent platform testing (e.g., fatigue and corrosion testing), inadequate analysis of surface modifications (coating integrity/durability, drug content/uniformity)

and inadequate stability and shelf-life information [52, 53]. Developers are urged to work with the FDA early in the development process.

16.7 Implantable Systems

Implantable delivery systems offer a number of advantages over more traditional delivery routes, particularly for biological macromolecules (including peptides, proteins, and oligonucleotides). Some specific delivery mechanisms to date include polymer depots (e.g., Gliadel^{TM}Wafer, prolifeprosan 20 with carmustine implant) and osmotic pumps (e.g., Viadur^{TM} leuprolide acetate implant).

Additional stability challenges for biological molecules in implantable systems include: drug-device interactions; physical stability of drug, especially proteins, during use; and stability of the drug in the device in vivo [54]. Proteins in particular may adsorb onto surfaces, followed by denaturing and subsequently aggregation, or may aggregate as a result of pump operation [54, 55]. Formulations may be developed as non-aqueous solutions or suspensions to ensure in vivo stability. The device must also protect the formulation from ingress of body fluids that may cause degradation of the drug and/or affect the mechanism of the device. Stability studies which include the measurement of the release profile, must therefore be demonstrated at *greater than or equal to* 37°C for the equivalent length of time the implant is to be in the body, which may be for up to a year or more. Similar in-vivo stability concerns are experienced for polymer depot systems in which molecules are stabilized by suspension in polymer solution.

16.8 Transdermal Products

Transdermal systems, frequently combined with enhancement technologies, offer advantages over traditional methods of delivery. Enhancement technologies include chemical enhancement, iontophoresis, sonophoresis, and microneedles (and combinations of these) as well as other innovative approaches in various stages of development [56].

Some specific tests are required for transdermal products on stability studies. It must be demonstrated that the patch maintains adhesive properties over time. This is essential to ensure efficacy of the product particularly if dose is proportional to surface area. Backing degradation or diffusion of drug components through the backing, stiffness caused by moisture vapor and air, drug or excipients undergoing phase changes, and effects on the adhesive by other components may all affect adhesive properties [57]. In-vitro methods for measurement include peel adhesion, tack, and shear adhesion; however, these are essentially quality control tests and are difficult to link to in-vivo performance [58]. During development the various tests available to measure these properties must be evaluated to determine those most appropriate to include in registration and commercial stability programs.

Flatness (which may affect the ability to apply the patch) may be measured during developmental stability studies [59]. Exposure to high or low humidity may affect moisture content and can cause either increased formulation bulkiness or brittleness, respectively [60]. These studies inform packaging decisions prior to commercialization.

Release rate is also included in stability studies. Tests for transdermal patches are described in the pharmacopoeias, with specifications usually containing three time points as with other sustained release dosage forms.

16.9 Leachables Studies

Device components, as part of a drug-device combination product, may contain polymers, elastomers, and other components from which minute quantities of material may migrate (leach) into the medicinal product over time and thus may affect the quality and safety of the product. A number of guidelines outline approaches to be considered for extractables and leachables studies [61, 62]. This chapter outlines additional points to consider specific to the development of drug-device combination products.

For combination products, in addition to the usual consideration for the potential for leachables to migrate during long-term stability studies, an innovator needs to determine whether the formulation will come into contact with the device components during the patient in-use period. For example, during the development of a new pen-injector one consideration is to understand what happens when a drug-containing cartridge is inserted into the pen-injector device during patient use. This consideration equally applies to any combination where the drug is contained within a primary container prior to insertion into the device, namely, the device is not the primary container for the medicinal product, or, when other parts of the device come into contact with the formulation during use.

If the drug is in contact with any part of the device during its storage or use, the developer will need to understand the potential for extractables from the device components to leach into the medicinal product over time. A study should be designed to understand the potential for leachables, taking into account many considerations including the following:

- The specific parts of the device that could come in contact with the medicinal product
- Whether the contact is transient (e.g., only during injection) or sustained during the patient use period (e.g., over the entire period the cartridge remains within the pen-injector)
- The composition of the device components (plastics, springs, elastomers, etc.)
- The composition of the device in terms of moulded and/or assembled component parts, i.e., the moulding process may effect the properties of the components

In 2001 the CMC Leachables and Extractables Technical Team of the Inhalation Technology Focus Group of the American Association of Pharmaceutical Scientists (ITFG)/IPAC-RS Collaboration and its Toxicology Working Group published a paper of points to consider for leachables and extractables testing for MDIs, DPIs, Nasal Sprays and Inhalation Solution, Suspension, and Spray Drug Products [63]. A key point from this document in relation to leachable studies is that the leachables program should be conducted on the drug product packaging configuration employed for long-term stability studies (e.g., capsule with blister, low density polyethylene vial with over-wrap). An in-use study should also be conducted in order to determine the leachables derived from components which are in contact with either the formulation or the patient's mouth or nasal mucosa only during administration (such as mouthpieces and actuators).

Leachables studies can be conducted as part of the stability studies to support registration. A useful document to refer to when designing controlled extraction and leachable studies is Safety Thresholds and Best Practices for Extractables and Leachables in Orally Inhaled and Nasal Drug Products published by Product Quality Research Institute (PQRI), which states: "Since these large drug product stability studies involve analysis of samples at multiple time-points, it is possible to discern trends in drug product leachables profiles over time and storage condition." [64, 65]. Once the potential leachables are identified, stability-indicating methods for each leachable can be developed. Appropriate thresholds for each potential leachable are determined through assessment of toxicological and safety data [64, 66].

16.10 Bracketing/Matrixing

Bracketing and matrixing is described in general stability guidances as well as those guidances for combination products including stents and inhaled products [20, 22, 23, 41, 67]. The FDA however appears reluctant to accept bracketing or matrixing for inhaled products and state that the use of bracketing and matrixing protocols may not be appropriate for MDIs and DPIs, although other agencies have accepted them [22, 68]. When using a bracketing or matrixing approach for designing stability programs for drugs in devices, additional justification will therefore be required and it is recommended that any such strategies are discussed with regulatory agencies prior to commencement of any registration stability activities.

16.11 Storage Orientation

During development, stability studies should include storage of products using different orientations (e.g., upright and inverted) if there is the possibility that orientation could affect stability performance [20, 22, 34, 65, 69]. Storage orientation can affect the stability of the product, and although there is limited information available in the literature demonstrating this, combination products in

solution or suspension may be affected [28]. If no differences are observed in stability performance, subsequent stability studies can then be reduced to one orientation. It may be prudent to store spare samples in alternative configuration(s) during registration stability studies in case of any unexpected regulatory challenges.

16.12 Temperature Cycling

In addition to stability studies at accelerated conditions, a study to determine the effect of extreme temperature variation should be considered to support the product exposed to storage excursions. Drug products susceptible to phase separation, loss of viscosity, precipitation, and aggregation should be evaluated under thermal cycling conditions. As part of the stress testing, the packaged product should be cycled through temperature conditions that simulate the changes likely to be encountered during product distribution. Example temperature cycling protocols are included in Tables 16.5 and 16.6.

Table 16.5 Thermal cycling for product labeled "Protect from Freezing"

	5°C	40°C
Length of storage	2 days	2 days

Table 16.6 Thermal cycling Freezing

	−20°C	25°C
Length of storage	2 days	2 days

The protocols represent one cycle, with the product being subjected to three cycles. Samples should be tested at the end of the third cycle, based on the appropriate test pattern. Guidance on temperature cycling for MDIs and nasal sprays is described in the EMEA and FDA guidelines on inhaled and nasal products [20, 22, 23]. Some of the variations described in the latter may be considered severe, although it is stated that alternative conditions and durations can be used with appropriate justification.

16.13 Transportation Studies

An additional consideration for drug-device combination products is the potential need to undertake transportation studies (also referred to as agitation or rotational studies). This may be particularly relevant where the drug in such a combination product is a biological entity. Biological molecules can be more sensitive to transportation conditions than traditional small molecule medicinal products. In addition, medicinal products of a biological nature that are used in a combination product may raise specific concerns regarding transportation. In a situation where the

combination is achieved by the patient (e.g., through insertion of a drug-containing cartridge in a pen-injector or a drug-containing blister-foil pack in an inhaler) it is possible (even if not prescribed in the Instructions For Use/Patient Information Leaflet) that a patient may carry a "spare" cartridge or blister pack with them to use when the one they currently have in their medical device is depleted.

The manufacturer is responsible for providing information on storage requirements but should also consider providing adequate warnings and precautionary statements covering potential misuse situations (see Figures 16.1, 16.2 and 16.3 in Section 16.3). An assessment of the types of studies that need to be undertaken can be determined through an assessment of the use-related hazards (the potential for patient misuse situations can be assessed through performing for example a User Failure Mode and Effects Analysis (FMEA)).

Development packaging and device pre-verification studies may provide supportive information when developing a protocol/plan for transportation studies. For example, drop testing undertaken to measure the durability/robustness of the device or combination product may highlight potential weak areas of the device that may help in understanding potential issues that can occur during transportation in specific orientations/positions. Examples of areas to consider are as follows:

- If it is a biological product, could it denature during transportation?
- Does the biological product become cloudy or lose solution clarity during agitation?
- Does the device continue to function (e.g., the injection button) after agitation?
- Is there any concern of over-dosing after the drug-containing device is subjected to agitation?

Answers to some of these questions may lead to additional warnings being placed on the label to restrict the conditions of transport where it is shown that transportation or agitation has a negative impact on the quality of the medicinal product. At the other extreme, the potential for over-dosing due to device malfunctions that could occur during transport raise concerns about patient safety and product efficacy, and could therefore impact the viability of the product.

Consider a patient responsible for self-medication for diabetes using a pen-injector that requires refrigerated storage. Questions that should be considered here are what the impact would be on the medicinal-drug and the combination product when the pen-injector is being agitated during normal daily activities if carried by the patient during the in-use period. The developer may provide additional safeguards to avoid misuse situations, for example, a well-designed storage case (e.g., with ice packs). However the developer will still need to consider the consequences of an excursion for short periods; this could be incorporated into the temperature cycling stability study.

Due to the length of time it can take to undertake these types of stability studies and the potential impact of the outcome, it is wise to prioritize these studies appropriately in the development program.

16.14 Commercial Stability Commitment

Similar to other pharmaceutical products, for combination products the FDA requires a stability commitment at time of filing. This commitment must include stability studies on the first three commercial batches and one annual product monitoring batch. It is usually in the form of a protocol detailing checkpoints and test methods and a commitment to communicate the results to the FDA. However, recently there appears to be a trend in expectations for inhaled products whereby 10% of commercial batches fall within the remit of such a stability commitment.

For new devices where the drug product in its primary container remains the same as the current marketed product, and as-such the device provides in fact a secondary packaging for the drug product, it may be appropriate to consider a sunsetting approach to the stability commitment. For example, in the case of pen-injectors that contain a cartridge that is already on the market, it is worth discussing with FDA the possibility of sunsetting the stability commitment such that if the first X number of batches meet the specification criteria and show no change on stability, the stability commitment could be phased out over time.

16.15 Conclusion

In this chapter we have highlighted some of the important considerations for developing stability strategies for drug-device combination products. It is vital to understand the latest regulatory requirements and expectations whilst also adopting a scientific and risk-based approach based on product understanding.

The guidelines for combination products are not as mature or harmonized as for more conventional products, therefore it is recommended to consult with regulatory agencies early in the development program. It is also important to maintain an awareness of emerging and evolving regulatory expectations and industry practice on an ongoing basis. One of the challenges in the future will be that as more products are developed and an increased number of innovators enter the market, airtime with the agencies may become harder to negotiate.

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Chapter 17
Stability Studies for Biologics

Anthony Mazzeo and Patrick Carpenter

Contents

Abstract Biological products represent a growing segment of the pharmaceutical industry. Stability studies of these complex biologics present challenges beyond those found for the typical small-molecule pharmaceutical. Biologic products are typically only marginally stable, not entirely understood, may demonstrate non-Arrhenius behavior, degrade by multiple pathways and possibly different pathways during different stages of shelf life. Further, subtle changes brought on by stresses can have large effects on the therapeutic properties of the product. There are analytical methodology challenges pertaining to monitoring stability as well, in particular the higher variance and complexity of the product and methodology. The issues and strategies involved in studying the stability of biologic protein products, particularly for the purposes of product registration purposes are discussed as well as an overview of ICH Q5C Quality of Biotechnological Products: Stability

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Testing of Biotechnological/Biological Products. Stability protocols should be designed keeping in mind the idiosyncrasies of biologics as well as formulation, analytical, manufacturing, and regulatory knowledge gained during development.

17.1 What Are Biologics?

This chapter discusses the issues and strategies involved in studying the stability of biologic products, particularly for the purposes of product registration, but also for product development purposes. Much of what needs to be performed toward this end is similar to what would be performed for small molecule products; however, the nature of the active biological substances and the resulting more limited knowledge of them requires some careful thinking and different approaches.

Biologics include such products as proteins, monoclonal antibodies, conjugated protein systems, and some polypeptides (some polypeptides can be treated as small-molecule drugs). The drug substances are macromolecules, which are more difficult to formulate and develop as a product than small molecules, but offer the promise of being target-specific and very potent in their medicinal functionality. Generally, the active substance has been produced or at least originated from a biological process, either by fermentation or by a specific cell-culture expression system, by a biotech process such as recombinant DNA (rDNA) technology, or by harvesting from a living organism. A related class of products, usually referred to as biologicals, is pharmaceutical products obtained directly from living organisms. Examples of biologicals are blood plasma products, vaccines, antivenoms, immunoglobulins, and allergenic extracts. Other potential products can be considered biologic or biotechnological products as well. This chapter will emphasize protein-based products, which are the most common biologic pharmaceuticals.

The key aspect of biological drug substances and products is that they are more labile compared to most traditional small molecule pharmaceuticals. Generally, they require low-temperature storage conditions such as refrigeration (2°−8°C), freezing (−10° to −20°C), or even ultra-low (−40° to −80°C) storage temperatures. This necessitates qualifying low-temperature stability chambers. On the other hand, for cold or frozen products, there is no difference whether the registration is targeted for climatic Zone I, II, III, or IV. Another aspect to keep in mind is that biologics are generally very costly and often time consuming to produce and some may be produced only in small batches due to the nature of the technology involved.

Due to the molecule's fragility, cost, and low-temperature requirements, once the biologic material is produced, preserving it in inventory and throughout distribution is of paramount importance. Cold-chain issues become very important, especially when shipping biologics across international borders where delays can be encountered. The stability scientist should be aware of the shipping methods and needs to design stability studies that will support excursions that are likely to be encountered. Likewise, the stability limitations due to stress testing discovered during product development need to be communicated to shipping and packaging engineers so that adequate shipping methods can be planned and qualified prior to product launch.

Because of this additional testing and the need to understand the product's storage and shipping limitations, it is easy to see that there needs to be a balance between the high cost of testing and the need to cover as wide of a *design space* as possible prior to the launch of the product. For this reason, it is important for the stability scientist to leverage knowledge gained during development on the formulation, manufacturing process and packaging, and gain a thorough understanding of the analytical methodology and regulatory aspects of the biologic product to be studied. That knowledge can be used to keep stability designs to a practical level yet cover the important quality parameters.

17.2 Biologics Versus Small Molecules

As mentioned earlier, biologics need to be treated with some extra consideration when addressing their pharmaceutical stability. The biological activity of a protein, for instance, comes not only from its covalently bonded primary structure, but also from the folded conformation that makes up the secondary and tertiary structure. The conformation can be easily altered without breaking any covalent bonds, and once in this denatured state, some or all of the biological activity that makes the protein a useful therapeutic medicine may be lost.

There is also the issue of heterogeneity of the protein forms. For example, a glycoprotein may be produced by a biological process that results in creation of several similar glycoforms. One or more of the forms may possess the desired therapeutic properties. It may be difficult to tell in vitro if there is any activity difference between the forms or whether some forms affect patients more than others.

Purity for a small molecule is a relatively simple concept. Normally, an HPLC method is sufficient to measure the content and impurity levels of a small molecule drug. A macromolecule, such as a protein, has a much more complex behavior. Determining protein concentration by UV absorption spectroscopy can give a measure of the total protein in the product, but it will not necessarily differentiate between active protein and inactive protein (i.e., denatured or otherwise degraded). A validated method or methods to determine the biological activity of the molecule is needed. So, whereas protein concentration is usually tested as part of the specifications, it is also normally accompanied by one or more methods that measure or correlate to biological activity. This is the bioassay. These methods can be animal-based or cell-based, protein interaction assays, binding methods such as surface plasmon resonance or ELISA (enzyme-linked immunosorbent assay) and immunoblot methods.

Size-exclusion HPLC (SE-HPLC), peptide digest mapping, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), isoelectric focusing (IEF), and other electrophoretic gel methods together give a good measure of the distribution of proteins, fragments, and side-chain modifications. Each of these assay methods give different types of information on the impurities present, and together give a purity profile for the biologic. An overview of a variety of these bioanalytical methods, although not exhaustive, can be found in the references [1].

Table 17.1 Comparison of biologic products vs. small molecule products

Topic	"Typical"small molecule products	"Typical" biologic products
Manufacturing Process	Synthetic chemical process	Fermentation or rDNA technology. Generally expensive to produce, often only produced in small batches, production site transfers are difficult
Formulations	Solid oral	Parenteral
Knowledge	Stability and potency generally determined by covalent structure	Very complex molecule relying on both covalent and conformational 3-D structure with a multitude of reaction sites for degradation
Storage	"Room Temperature" products	Refrigerated, frozen, or deep freeze
Specifications	5–10 methods, ICH Q6A	Many methods (10 to 20+) required to profile and characterize the protein, ICH Q6B
Assay	Generally HPLC is sufficient for assay, identification, and impurities	Generally overall protein concentration, plus at least one specific bioassay and one or more assays showing binding correlated with clinical experience during development
Analytical Methods	Typically, HPLC based and relatively sensitive, precise, and accurate. Relatively fast methods.	Biosassay and electrophoretic techniques which are generally less precise, and may lack sensitivity. Methods are slower and generally more costly.
ICH stability guidelines	ICH Q1A through Q1E	ICH Q1A through Q1E and Q5C
US filing	New drug application (NDA)	Biologics license application (BLA)

For stability studies, it is likely several of these types of assays will be used, each providing information on different characteristics of the molecule or information on the different types of degradation pathways. It is common that many tests (relative to what is performed for small molecules) are performed to characterize a biologic substance or product to give assurance of potency, purity, and quality. Table 17.1 (see section 17.3) gives a quick comparison of the differences between a biologic and small molecule drug product.

17.3 Common Degradation Pathways for Proteins

It would be difficult to give a complete picture of the endless possibilities for protein degradation in a handbook on pharmaceutical stability and there are already numerous references in the literature covering the many different degradation pathways, a tiny fraction of which are given in the discussions below. However, it is useful to note that there are several common degradation routes for protein products that are typically studied to determine the stability of biologic products. A key point to remember is that both covalent and noncovalent forces can lead to subtle changes

in the protein conformation and therefore drastically alter its biological activity or physical stability. Whereas, degradation by breaking a covalent bond requires a fair amount of energy (200–400 kJ/mol), the weaker forces, such as hydrophobic interactions and hydrogen bonding, require only about 4–30 kJ/mol to disrupt [2]. Small perturbations that disrupt those weak forces can have a big affect on the protein conformation, and therefore its bioactivity, as well as expose the protein to further chemical degradation. Since macromolecules are such complex structures, it is common to see non-Arrhenius behavior [3–5]. However, many degradation processes of proteins can demonstrate Arrhenius behavior [6, 7]. Protein formulations tend to have poor photostability as well, since many of the amino acid residues themselves are prone to photolytic degradation [8]. It can be summed up simply that biologics are only marginally stable, and relatively minor changes, even to one amino acid residue in the macromolecule, can change their activity, pharmacokinetics, or their ability to fit the receptor. A summary of common problems with the stability of proteins is given in Table 17.2.

The major degradation pathways can be categorized as aggregation, denaturation, oxidation, and deamidation. Although other pathways can be important; these include adsorption onto container components [9], fragmentation [10, 11], deglycosylation, or in formulations containing saccharides, glycosylation [12], and of course destruction of disulfide bonds that may hold the tertiary structure together. Because of the nature of biotech products, other complications can find their way into the spotlight to the consternation of developers. For example, proteolytic enzymes making their way through the purification steps and into the final product (and stability samples) causing proteolytic fragmentation. Although it is the job of the process development scientists to prevent such enzymes from getting into the final product, the stability scientist should be aware that such impurities are possible and the effect of their presence may only show up in longer-duration studies.

Denaturation is described as a disruption or unfolding of the protein's natural secondary or tertiary structure. This is often an irreversible process. It can be initiated by any number of influences, but heat is probably the most common. Unfolding of the protein as a result of a denaturation process can expose otherwise protected amino acid side-chains to chemical degradation [13].

Table 17.2 Common problems with stability of proteins

Usually sensitive to light, heat, air, and trace metal impurities
Small or large stress factors can disrupt protein folding
Numerous chemical degradation routes possible
Numerous physical degradation routes, including agitation, freezing, interaction with surfaces and phase boundaries
Non-Arrhenius behavior
Possibility of different degradation mechanisms appearing depending on the age of the product
Possibility to find proteases left from biotech processes
One type of degradation can facilitate other types of degradation leading to a cascading effect
Limited formulation options

Aggregation is the formation of complexes between macromolecules. They can be dimers, trimers, and heavier multimers. The complexes may be covalently bonded or just associated through hydrophobic interactions. The formation of aggregates can cause changes in protein binding and activity (potency), and have been implicated in immunogenic reactions in the patient. Control of aggregate formation during process and formulation development is, therefore, very important as well as development of methods for the determination of aggregation. Any number of factors can bring about aggregation, most notably heat and pH [14] although it can occur without much stress at all [15]. In the extreme, aggregation can lead to precipitation of the protein [16].

Oxidation occurs generally on the amino acid side chains due to exposure to air, residual peroxide from excipients, or exposure to visible or ultraviolet light. In particular, methionine, cysteine, tryptophan, and tyrosine are prone to oxidation. Metal ions such as iron, zinc, copper, or tungsten from metals that are used in the manufacturing process, leached from contact materials, or present in trace amounts in excipients can catalyze oxidation as well as other degradation processes [2, 17].

Protein deamidation occurs with asparagine and glutamine. It has been shown that protein conformation can affect the rate of deamidation and vice versa [13, 18].

Excipients in the formulation can present additional opportunities for degradation of the protein. As already mentioned, residual peroxides can oxidize side chains [19] and the use of saccharides and polyols, while adding some stability [20, 21], can lead to glycosylation and other reactions that affect the product quality [22, 23].

Degradation processes as discussed above, even those that seem small compared to the relatively large size of the macromolecule, can bring about very large changes in the secondary and tertiary structure of a therapeutic protein. In order to monitor all of these possibilities, multiple types of analytical methods are necessary in the stability studies for biologics, and it is not always clear which method or methods gives the most relevant information on the state of the protein therapeutic agent.

17.4 Other Stability Considerations

As previously mentioned, the nature of biologic products brings along some interesting challenges. The formulations are usually parenteral, with only rare exceptions. While many aspects of stability studies for parenterals hold true whether for small molecule or large molecule, it is important to reiterate some of these aspects for biologics since it is practically a given that if you are developing a biologics product, then you are developing a parenteral. Lyophilized products offer some added stability, but liquid formulations and ready-to-use products are also desired by clinicians since these are easier to use and can, in some cases, be self-administered by patients. In many cases, however, the product may require a constitution step and/or dilution before administration. The compatibility of the diluents as well as all contact materials, for example stainless steel needles, polyvinyl chloride (PVC) and

non-PVC IV bags, filters and associated tubing, should all be considered in an in-use study. Other factors need to be considered as well. Constitution may be performed in the vial with the use of a syringe to add the diluent. Care needs to be taken not to agitate the protein during this process as this can lead to degradation [24]. Syringe plungers and barrels, needles, and other components may be coated with silicone oil that may lead to undesirable interactions with the formulation such as clouding of the solution or aggregation [25]. Add the additional *global complication* that any in-use materials that are tested in the developer's laboratory may not necessarily be considered the same kind or quality as those that will be available in Europe or Asia or South America, etc. Pharmacopeial harmonization efforts may bring some relief to this situation in the future, but for now, care should be taken that constitution stability studies are relevant for the countries targeted for product registration. These compatibility/in-use stability studies may be covered during formulation development, however, as a requirement in ICH Q1A(R2), data must be collected on the stability of the constituted products for inclusion in the filing. Depending upon how many different diluents, administration set-ups, and concentration ranges of the constituted solutions are necessary; these in-use stability studies can become large and laborious. Bracketing and matrixing strategies would be put to good use in designing these compatibility/constitution studies.

Biologics not only tend to be parenteral drugs, but it is also commonly necessarily for them to be stored cold, either refrigerated or frozen. While this does not present a big problem for stability studies as long as the proper qualified storage chambers are available, cold-chain shipping presents a major challenge for these typically labile products. Manufacturers of biologics can go to great lengths and expense to ensure their cold-storage products can be shipped reliably and with minimal temperature excursions. There has been a lot of recent activity in the industry to come to some reasonable solutions for shipping cold and very valuable products around the country and globally [26, 27]. For biologics, special consideration is required to balance the need to keep the products cold enough during shipping, yet keep the cost of doing so to practical levels.

In the previous sections, it has been discussed that biologic products contain complicated molecules, which are only marginally stable and not well understood, the behavior of which is not necessarily Arrhenius, and where subtle changes brought on by large or small stresses can have far-reaching effects on the therapeutic properties of the product. For these reasons, the typical freeze–thaw or short-term heat–stress studies normally performed to support storage and shipping excursions may not necessarily be enough to ensure product quality over the shelf life of the product. Even if a liquid product is shown to survive freeze–thaw testing after several freeze–thaw cycles when compared to the specifications, the data collected from such a study may not cover the worst-case scenario shipping stresses. A protein formulation may survive phase changes that occur quickly in a classic freeze–thaw cycle, for example between –20° and 25°C. However, the rate of freezing (or thawing) may be slower in a real shipping scenario. The rate of freezing has been shown to affect protein denaturation in lyophilization cycles [28] and the possibility of a liquid formulation spending significant time in a partially frozen "slush" condition may induce

more protein unfolding and subsequent aggregation than a quick freeze [24]. In the partially frozen state, the protein can be subjected to pH, ionic strength, and concentration gradients caused by the partial freezing or melting. These conditions can potentially affect the protein conformation permanently, or temporarily expose otherwise protected amino acid residues for degradation.

In many cases, it will be found that that the short-term stress will cause some degradation in a biologic formulation. The question is whether that degradation will also affect the target shelf life of the stressed product. There are theoretical calculations that can be performed to help predict the shelf life of a stressed sample and as long as the models used are shown to be relevant for the product in question, they may be of some use. However, for biologics, experimental data are necessary to lessen the risk of unwanted surprises and/or to mitigate the need for very expensive cold-chain shipping containers and systems. It is therefore recommended that a limited study be performed where stability samples are subjected to an excursion-like stress, by heat or freezing or light exposure, etc., and then placed at the intended storage condition with data being collected occasionally out to the intended shelf life. An example of such a protocol is given in Table 17.7 (see Section 17.5.5.2).

17.5 The ICH Q5C Guideline

Guidance for the design of registrational stability studies of biologics can be found in ICH Guideline Q5C, *Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products,* along with all the ICH Q1 stability guidelines. The ICH Q5C guideline gives general guidance on the expectations for the body of stability data needed to register biologics in ICH countries. It does not necessarily apply to such products as vaccines, antibiotics, heparins, vitamins, allergenic extracts, and other products derived from *traditional* biological or fermentation processes. Consultation with your regulatory department and specific country regulations is recommended to confirm the applicability of the guidance to a specific product. This section will review the salient points for biologic stability studies and those in particular that may differ from the general ICH Q1A(R2) guideline, *Stability Testing of New Drug Substances and Products.* Note however, that the ICH Q1 stability guidelines are generally applicable to biologics and are a good starting place for designing stability protocols.

17.5.1 Drug Substance Stability

Similar to the requirements for small molecules, at least three batches of drug substance of pilot or full scale batch size and representative of the process used in preclinical, clinical, and proposed manufacturing scale should be studied. If pilot scale lots are used in the stability study for the Biologics License Application (BLA), a commitment must be made to place the first three commercial batches on stability.

The containers used to store the samples may be of reduced size, but should be constructed of the same material and fitted with the same container/closure system proposed for the manufacturing process. An important point when determining the container for biologics is the likelihood that the drug substance will be stored long-term in cold temperatures, some down to −80°C. Plastic containers and their closure systems should be checked for their durability and brittleness when subjected to such cold temperatures. What is not specifically mentioned in the guidance is that frozen drug substance obviously will be thawed before use in manufacturing of the product. Sometimes the thawing process is performed in a step-wise manner so as not to damage the macromolecules, and the thawed material kept at a holding temperature until it is ultimately used for manufacturing product. Usually a maximum holding time is determined and shorter times can be used at the discretion of the manufacturing planners. This maximum holding time needs to be supported by stability data and the holding time needs to be considered in determining the ultimate use-period of the bulk drug substance. The hold-time study can be based on a reduced testing scheme as long as the critical quality factors are assured. The stability scientist should consult with manufacturing and perhaps even quality assurance personnel to reach agreement on the maximum hold-time before designing the hold-time stability study. An example is given in Table 17.5 (see Section 17.5.5.2).

17.5.2 Drug Product Stability

Stability information for biologic drug products is expected on at least three batches that are representative of the manufacturing scale batches, packaged in the primary containers, and representative of the product used in clinical trials. Pilot-scale batches may be used with a commitment that the first three manufacturing-scale batches are placed on stability after approval. This is the same strategy as can be used for small molecules. For biologics, it cannot be assumed that a minimum amount of data will receive an extrapolated shelf life from regulatory agencies. Generally, dating of the product will be based on the real-time data collected at the intended storage condition. The reasons for disallowing limited extrapolation for shelf life determination are the non-linear degradation pathways that are more prevalent in biologics and also the possibility that the biologics degrade through different mechanisms as the product ages. This is not to say it is impossible to get some extension of the shelf life with limited data. It may be reasonable to request some extrapolation given a good body of relevant supporting data, product history, clinical experience, etc.

17.5.3 Matrixing and Bracketing

Matrixing and bracketing are of potential use as long as care is taken to show that the stability samples tested properly represent the stability of all samples. In fact, given

the cost of product and bioanalytical test methods, matrixing and bracketing should always be considered. Additional information on the matrixing and bracketing concept can be found in Chapter 15. Prior consultation with manufacturing, analytical, and statistical staff is recommended, as biologic lot-to-lot and bioanalytical method variability can be high, thus thwarting reduced design efforts. It is not uncommon for the stability design to be the subject of an End of Phase II meeting with the FDA prior to starting stability studies. However, the stability scientist should also be aware of the entire filing strategy, including which countries are targeted for filing, when the filing is scheduled, and whether those countries will accept a reduced stability design. Consultation with the regulatory department is also recommended.

In some cases, the biologic may be relatively labile, resulting in a shelf life of 6 months or less. These instances should be discussed with the agency on a case-by-case basis as recommended in the ICH guidance.

17.5.4 Stability Tests

Stability tests for biologics will be determined by the nature of the particular product, manufacturing process, and formulation. Common tests are listed in ICH Guideline Q6B, *Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products.* Some of those tests are important for monitoring the stability of the drug substance or product. Other tests may be substituted if shown to serve the same purpose. As the testing technology advances, it would be advantageous to both manufacturer and regulatory agency to pursue those newer methods. As is the case with any Good Manufacturing Practice (GMP) activity, the stability indicating methods must be validated for use in a registrational stability study (e.g., for a BLA, etc.).

Specifications and tests for drug substance and drug product are categorized in the ICH guideline simply as

- Appearance and description
- Impurities
- Potency
- Quantity

In addition, other tests specific to the drug substance and formulation may apply, such as sterility, microbial limits, bacterial endotoxin, and pH. These types of tests would also be called for in order to comply with local pharmacopoeial requirements. Tests for subvisible particles beyond what is required in the pharmacopoeia may also be necessary due to immunogenicity concerns [29].

The trending of potency and impurities over time can be challenging for biologics and several methods may be necessary to profile each of these attributes. First, as discussed earlier, there is not necessarily a direct link between concentration (quantity) and potency, since the concentration test, usually UV spectrophotometry, does not give information on biological activity, it simply gives protein concentrations

and does not discriminate between active and inactive molecules. Bioassays and binding methods are more relevant for measuring potency. Even so, the potency assay may have to be correlated with clinical results to show that the assay is producing relevant potency information. The inherent problems with the specificity and accuracy of bioassays have resulted in the recommendation in the ICH Guideline Q6B that "the purity of the drug substance and drug product is assessed by a combination of analytical methods." This can cause problems with methodology changes later in development or postapproval. Method transfers from lab to lab can also be problematic for bioassay and gel techniques.

Impurity monitoring is also tricky since there are multiple degradation pathways which are not necessarily detected by a single chromatographic or other method. Size-exclusion chromatography and SDS-PAGE may give some information on aggregation, but peptide mapping methods are needed to determine the degree of side-chain oxidation and deamidation, and isoelectric focusing is needed to detect changes in overall charged sites. It is important to keep in mind how the results of gel methods will be trended over time. Gels can be used for visual comparison with a reference standard, as is often done for identity testing purposes. However, if a gel migration pattern is to be used for trending purposes, it is necessary to develop a quantitative or at least semi-quantitative scheme, for example, based on the number of bands and/or band intensities, so that results can be compared from time point to time point. A list of common techniques used in stability studies are listed in Table 17.3.

17.5.5 Stability Protocols

There is virtually no difference in the general requirements for a stability protocol for biologics from that required for a small molecule. Stability testing must be

Table 17.3 Some common bioanalytical techniques for stability testing of biologics

Test	Method
Aggregation	SE-HPLC, Capillary electrophoresis, SDS-PAGE
Deamidation, Oxidation, Disulfide bond disruption	Peptide mapping
Cleavage, Isomerism	SDS-PAGE
Charge differences, isoforms	IEF
Protein concentration	UV/Vis spectrophotometery
Biological activity (potency)	Animal-based or cell culture-based biological assays, and biochemical assays
Immunochemical properties	Binding assays, ELISA, western-blot (immunoblotting methods)
Appearance, sterility, endotoxin, microbial limits, particulate matter, and other formulation specific tests	Test according to pharmacopeial requirements, as needed

carried out at the long-term storage condition until at least the intended shelf life. Accelerated and photostability tests would be performed as well. The stability indicating tests, of course, will be different for a biologic and it is very likely that there will be more tests than required of small molecules. Since the biologics are typically unstable at room temperature, the stability storage conditions will be aligned for cold storage products. Additionally, there may be more frequent testing for biologics due to this relative instability. Long-term stability test points at 1 month and sometimes days or weeks for the accelerated condition would not be out of place. However, the stability scientist needs to consider the available knowledge of the product gained during development as well as the manufacturing, regulatory, and analytical aspects of the specific drug substance or product before designing the protocol. For instance, a biological drug substance that has demonstrated stability at –70°C during research and development may not need to be tested at the 1-month interval; in other words, testing at three, six, nine, and twelve months, etc., may be just fine.

17.5.5.1 Example Drug Substance Protocol

Table 17.4 gives an example protocol of a biologic drug substance that is stored at –70°C, then thawed before use and held at refrigerated storage for up to several weeks before being used in product manufacturing. The 5°C condition is serving as the accelerated condition. In this full test design, the bioanalytical tests are performed at each time point designated with an *a.* Bacterial endotoxin and microbial limit tests are performed at each time point designated with a *b.* At time points designated with a *c,* a portion of the drug substance is removed from the deep freeze condition and thawed according to thawing instructions specific to the drug substance and container size. The thawed drug substance is stored at the holding temperature, in this example 5°C, and these samples are subjected to further stability tests out to the designated maximum hold-time, as shown in Table 17.5. This removal of samples from the long-term condition at designated time points for study at a different condition can be likened to what is done for constitution stability studies, in other words, some samples are removed, constituted, and studied in the constituted state for a set period of time. However, in this case, the drug substance is thawed and held at the holding temperature of 5°C and tested periodically to demonstrate stability throughout the hold time. Data is also collected at a higher temperature, for example 25°C, to support temperature excursions that may be encountered in commercial manufacturing.

Table 17.4 Example time/temperature schedule for drug substance

	Times in months							
Storage condition	0	1	3	6	9	12	18	24
−70°C	abc	a	a	ac	a	abc	a	abc
5°C	a	a	a	a	a			

Table 17.5 Time/temperature schedule for drug substance after thawing according to thawing instructions

Storage condition	Time in weeks				
	0	1	4	9	13
5°C	a	a	a	a	
25°C/40%RH		a	a		

17.5.5.2 Example Drug Product Protocol

Table 17.6 gives an example protocol for a lyophilized parenteral biologic drug product. Here the long-term condition is 5°C, and 25°C/60%RH is the accelerated condition. The bioanalytical tests occur at each time point designated with an *a*, and sterility and endotoxin tests are performed at each time point designated with a *b*. In addition, 5°C samples are constituted for use-time initially and annually until the end of the study. In the table, the scheduled use-time study is designated with a *u*. The –20°C condition helps support low temperature excursions. Photostability studies would be conducted according to ICH Q1B as well.

Now suppose, for the example given, that the product is a liquid biologic product and sensitive to light. We would also want some assurance that the biologic will survive typical excursions. The excursions may occur inadvertently during shipping and storage, or they may be experienced during handling, for example during a labeling process in a room temperature labeling area. As mentioned before, labile biologics may not immediately show problems from stress right away. In the example in Table 17.7, several stresses are combined, namely freeze–thaw, room temperature, and light exposure, to reduce the amount of testing which would be necessary if each stress was tested individually. This, of course, is a viable time-saving option only if development studies or other product knowledge indicate that the product is likely to survive such stress. Freeze–thaw for a liquid product would put the product through several phase changes in order to show the affect of the stress on the product. For a biologic, it would also be advantageous to know what happens if the product is put in a partially frozen condition as might occur during shipping. This might be done by passing through many freeze–thaw cycles or attempting to hold the samples at the partially frozen condition.

In the example, the stressed samples are tested after 2 days' and 2 weeks' worth of cycling, then the samples are placed in the intended 5°C condition and tested

Table 17.6 Time/Temperature schedule for drug product

Storage condition	Time in months										
	Initial	2w	1	3	6	9	12	18	24	30	36
−20°C		a	a								
5°C	abu		a	a	a	a	abu	a	abu	a	abu
25°C/60%RH			a	a	a						

Table 17.7 Schedule for samples exposed to several stress factors followed by storage at long-term condition 5°C

Storage temperatures/test groups[1]		
Time point	**Freeze–thaw –10°C/25°C and room light**	**Stressed long-term samples Stored at 5°C**
Initial	a	
2 days	a	
14 days	a	
1 m		a
6 m		a
12 m		a
24 m		a
36 m		a

occasionally to show that the samples will pass specifications out to the shelf life or that they are trending similar to the unstressed long-term samples. The examples given are one case of many possibilities and it cannot be stressed enough that before a registrational stability study is designed, the stability scientist needs to understand the product knowledge gained during development, the manufacturing issues, the regulatory issues, and the analytical issues.

17.6 Specification Setting

Setting specifications for biologics has been the subject of debate between industry and regulatory agencies for many years. Much of the debate centers on what the analytical tests really tell about the quality of the product, to what degree the bioassays can predict clinical potency, and what the impurity tests are really telling about the overall quality. The number of specifications that are necessary in terms of assays and *for information only* testing is debated as well. Since we have imperfect knowledge of the complex macromolecule, more specifications are required. Not all of these tests will be stability-indicating, however. In any case, as with small-molecule drugs, good stability data on several batches of product are required, along with clinical experience, knowledge of process consistency (a measure of lot-to-lot variability) and analytical variability, to help set specifications. The lot-to-lot variability and analytical variability can be relatively high for biologics. It is reasonable (and common) for a specification of a bioassay, with a target of

response of 100%, to have specification limits of 50% to 150%. These types of assay limits, of course, are virtually unheard of in the small molecule world. Specification setting for biologics is discussed in the ICH Q6B guideline and in the literature [30, 31].

17.7 Stability for Process Changes

Process changes for a biotech product may have far-reaching effects on a product or substance. Fortunately, many of the process parameters can lend themselves to Quality by Design approaches to reduce the regulatory burden of such changes. Even small changes in biotechnology processes require careful evaluation as to what, if any, stability studies are needed to ascertain if product quality will be affected. Again, subtle changes may have a great affect on the quality of a biologic, and some of the effects may not be detectible immediately after manufacture of the product. Biologics production processes are more sensitive to changes in starting materials and changes in production sites. Even changes in production suites in the same facility may be enough to warrant extra stability studies. Long-term and accelerated stability studies may be needed to demonstrate that the product will retain its quality attributes after the process change. Process changes are discussed in ICH Guideline Q5E, *Comparability of Biotechnological/Biological Products Subject to Changes in Their Manufacturing Process.*

17.8 Summary and Conclusion

Biological products represent a growing segment of the pharmaceutical industry. The interest in biologics stem from their specificity in interacting with complex biological processes in the body. Stability studies of these complex biologics present challenges beyond the typical small-molecule pharmaceutical. Biologic products are typically only marginally stable, not entirely understood, may demonstrate non-Arrhenius behavior, degrade by multiple pathways and possibly different pathways during different stages of shelf life, and subtle changes brought on by large or small stresses can have large effects on the therapeutic properties of the product. There are analytical methodology challenges pertaining to monitoring stability as well, in particular the higher variance and complexity of the product and methodology. Stability protocols should be designed keeping in mind these idiosyncrasies as well as formulation, analytical, manufacturing, and regulatory knowledge gained during development.

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Chapter 1
Introduction

Kim Huynh-Ba

Contents

Abstract The purpose of this chapter is introducing the goal of stability testing and its role in the Drug Development Process. It gives a brief overview of how stability studies are designed to support the development and commercialization of a new medicine. This chapter also acquaints the reader to the content of this book.

1.1 Stability

Stability is a critical quality attribute of pharmaceutical products; therefore, stability testing plays a crucial role in the drug development process. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environment factors, such as temperature, humidity, and light, and to establish a retest period for the drug substance or a shelf-life for the drug product and recommended storage conditions [1]. Therefore, it encompasses all the phases of the drug development process. A testing program for stability samples requires a tremendous amount of

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resources and expertise; however, many stability analysts are not aware of the purposes of these studies and how these studies support the decision-making activities during the drug development process. This chapter will discuss the purposes of the development phases of pharmaceutical products and how they affect the stability program.

1.2 Drug Development Process

The drug development process is a time-consuming process. It would take over 10 years to bring a new chemical entity (NCE) to the market. The drug development process generally consists of three periods: discovery/toxicology, clinical development, and commercialization.

1.2.1 Toxicological Phase

An Investigational New Drug (IND) application is the first regulatory step in the drug development process. The discovery/toxicology (pre-IND) period is where studies are conducted on animals with the purpose to understand the safety and biological activity of the NCE. This phase mainly consists of appropriate animal studies. Characterization of the Active Pharmaceutical Ingredient (API) and drug product must also be well studied to support the IND submission.

1.2.2 Clinical Phases

After the IND submission, the clinical development period starts with four main phases. Phase I concentrates on evaluating the safety and tolerability of the drug product on healthy volunteers. Phase II, focusing on patients, studies efficacy, and extended safety assessment. End of Phase II marks an important go/no-go decision. If promising, Phase III will be initiated on a larger scale with patients to link safety, efficacy, and effectiveness. A New Drug Application (NDA) will be submitted at the end of Phase III to the FDA. Phase IV may start after approval to study long-term side effects, side effects that occur after approval, or to support post-approval changes.

Table 1.1 introduces the development of a pharmaceutical product in several phases. The toxicological phases contain numerous laboratory and animal studies. The purpose of this phase is to study the safety, biological activity, and formulation of the drug substance. Due to recent developments in technology such as high throughput evaluation, genomics development, etc., many compounds have been nominated to enter this phase. After successful review of toxicological data, an IND application is filed to initiate clinical study phases.

Table 1.1 Purpose of drug development phases

Phase	Purpose	Test population
Toxicological (pre-Clinical) phase	Safety, biological activity and formulation	Laboratory and Animal Studies
	IND SUBMISSION	
Phase I	Determine safety and dosage	20–100 Healthy volunteers
Phase II	Evaluate effectiveness and look for side effects	100–500 Patient volunteers
	End-of-Phase II meeting	
Phase III	Confirm effectiveness, monitor adverse reactions from long-term use	1000–5000 Patient volunteers
	NDA/MAA SUBMISSION	
Phase IV	Additional post-marketing testing	
Commercial support	Annual Product Monitoring Post-Approval Changes	

The clinical phases are phases when API is being tested in humans. There are usually three clinical phases: Phase I, Phase II, and Phase III. These phases serve different purposes which are illustrated in Table 1.1.

Phase I studies are usually small studies, thus a stability study supporting this phase is relatively small in number of patients and short study duration. The subjects in this clinical phase are healthy volunteers and the population could range from 20 to 100 subjects. The main purpose of this phase is to determine the safety of the API and dosage form.

If successful, the API will proceed to Phase II. Phase II studies are larger and involving patient volunteers. The size of these studies is approximately 100–500 patients. The purpose of this study is to evaluate effectiveness and look for side effects. At the end of Phase II, companies are usually have an End-of-Phase II meeting with the regulatory agency to discuss the filing strategy. This is advisable before going into Phase III as Phase III usually takes up more resources and investments. Many compounds are dropped at this phase.

Phase III is an expansion of Phase II to a larger population with regards to age, gender, culture, etc... It involves patient volunteers at a range of 1000–5000 subjects. The purpose is to confirm effectiveness and monitor adverse reactions from long-term usage.

1.2.3 Registration Phase

Once Phase III is completed successfully, an NDA or Marketing Authorization Application (MAA) is filed with the regulatory agency. It normally takes from 6 months to a year for the review process to be completed. In general, one out of five

applications may get approved. Once approved, additional post-marketing testing may still be needed. This testing could be required by the regulatory agencies or by the company. Companies may want to expand the packaging configuration or to a different dosage strength.

Stability testing plays an important role in the drug development process. The safety and efficacy of drug products are established during development via clinical studies. If the drug product stability profile changes beyond established acceptance criteria, established safety and efficacy are no longer applicable, and thus, the safety and efficacy of the drug product may need to be re-established. This leads to additional stability studies. During the life of a drug product, there are inevitable changes, which may affect the drug product stability, thus additional studies will be necessary and further data will be needed to support these changes.

The cost of taking an NCE through the drug development process ranges from $800 million to $1.2 billion. Therefore, optimizing the drug development process, fully understanding key factors affecting the stability profile of a drug product, and executing an effective stability program are very important for product commercialization.

1.3 Introduction of this Handbook

This handbook discusses many technical issues that impact a stability program to provide a reference to develop an effective stability program. It comprises several chapters covering topics from regulations to sciences. This book is divided into three main sections: Stability Regulations, Stability Methodologies and Best Practices, and other Stability Programs.

1.3.1 Stability Regulations

Chapter 2 introduces the critical current Good Manufacturing Practices (cGMP) regulations that are applicable to a stability program. It describes different types of stability studies to support the drug development process and discusses the GMP requirements surrounding the stability sciences.

Chapter 3 discusses International Conference of Harmonization (ICH) guidelines that are related to the stability sciences. It gives a brief history of how the Q1A was initiated. A summary of Q1A(R2) discusses thoroughly the current regulations that the industry supports and practices. While this handbook was being prepared, the FDA Stability Guidance was withdrawn; therefore, a brief discussion of the guidance status has been included. A discussion of mean kinetic temperature is included to have a basis of understanding stability testing conditions.

Chapter 4 discusses global expectations of a stability program. It includes a thorough discussion of stability requirements of non-ICH regions as well as a discussion on how the climatic requirements are implied in the world. This comprehensive chapter gives an introduction of stability requirements for countries around the

world. Discussions of World Health Organization (WHO) stability guidelines and Association of Southeast Asian Nations (ASEAN) stability requirements are also included.

Chapter 5 introduces the stability studies needed to support post-approval changes. This chapter also covers change control requirements as well as documentation needed for these changes.

Chapter 6 provides a thorough discussion of several factors that may impact the chemical stability of the API in its dosage form. Understanding these factors would help one to predict shelf-life of pharmaceutical products.

1.3.2 Stability Methodologies and Best Practices

Chapter 7 focuses on how to develop stability indicating methods for API as well as drug products. It also discusses forced degradation studies that challenge the stability indicating power of analytical methods.

Chapter 8 discusses requirements of method validation and transfer. It reviews critical validation characteristics as well as summarizes ICH Q2 Validation guidelines. It also includes strategies that one may take when performing method transfer.

Chapter 9 gives an overview of the Pharmacopeia of the United States of America (USP) and its USP-NF requirements for stability purposes. This chapter also discusses the development process for monographs, the goals for the general chapters, and relevant testing used for stability studies.

Chapter 10 covers non-chromatographic test methods used to monitor stability studies. This chapter also recommends practical practices for appropriate physical testing methods. An overview of dissolution testing is also included.

Chapter 11 introduces an overview of spectroscopic tests used to support stability studies. These types of testing have gained more attention in recent years to provide additional understanding of drug substance and drug product stability.

Chapter 12 provides a review of solid state characteristics. It discusses the major physical attributes and their impact on the stability of drug substances and drug products.

Chapter 13 discusses the collection and presentation of stability data. Evaluation of data (ICH Q1E) is also discussed as well as Out-of-Specification (OOS) and Out-of-Trend (OOT) investigations. In addition, it also introduces the stability report and data trending.

Chapter 14 introduces stability chambers. It also discusses factors to be considered for chamber validation, calibration, and maintenance. This chapter also elaborates on ICH Q1B guideline, which established the requirements for photostability condition.

Chapter 15 covers critical activities necessary to maintain an effective stability program. Best practices on day-to-day operational activities such as sample pulling, testing window, and chamber inventory are included in this section to provide guidance on current industrial practices. Development of a stability protocol is also integrated together with a discussion of ICH Q1D-Bracketing and Matrixing concepts.

1.3.3 Other Stability Programs

Chapter 16 provides a general discussion of stability program for combination products or drug in devices. It covers differences in working with this type of materials as well as applicable regulations in this area.

Chapter 17 gives a general discussion of the stability program for biologics and large molecules.

1.4 Conclusion

As you can see, these 17 chapters cover several different aspects surrounding the stability programs of pharmaceutical products from pre-IND stages to post-approval. It gives a generous overview of stability regulations in the United States and ICH regions as well as in all other climatic conditions around the world. It discusses methodologies to monitor physical as well as chemical stability of drug substance and drug products. It also gives practical information to build effective systems to support stability operations.

We hope that this book will help your journey to discovering the magnitude of Stability Sciences and its significant impact in the Drug Development Process of pharmaceutical products.

Reference

1. ICH Harmonized tripartite guidelines for stability testing of new drug substances and products – Q1A(R2)

List of Abbreviations

Acronym	Definition
ACN	Acetonitrile
ACVA	azobis-cyan valeric acid
AFM	atomic force microscope
AIBN	2,2-azobisisobutyronitrile
AMPD	azobis methyl propionamidine dihydrochloride
ANDA	Abbreviated New Drug Application
ANVISA	Agência Nacional de Vigilância Sanitária
API	Active Pharmaceutical Ingredient
AR & D	Analytical Research & Development
ASEAN	Association of South East Asian Nations
ASTM	American Society for Testing and Materials
ATR	Attenuated total reflectance
BA	Bioavailability
BE	Bioequivalence
BLA	Biologics License Application
CAPA	Corrective and Preventive Actions
CBE	Changes Being Effected
CBE-30	Changes Being Effected – 30 Days
CBER	Center for Biologics Evaluation and Research
CDER	Center for Drug Evaluation & Research
CDRH	Center for Devices and Radiological Health
CE	Capillary Electrophoresis
CE	Conformité Européenne
CFR	Code of Federal Regulations
cGMPs	current Good Manufacturing Practices
CHMP	Committee for Medicinal Products for Human Use
CI	confidence interval
CMC	Chemistry, Manufacturing & Controls
COA	Certificate of Analysis
CQA	Critical Quality Attribute
CR	Child Resistant
CRH	Critical relative Humidity

CRO	Contract Research Organization
CRT	Controlled Room Temperature
CTD	Common Technical Document
DES	Drug eluting stent
DMF	Device Master File
DMSO	Dimethylsulfoxide
DPI	Dry powder inhaler
DRIFTS	Diffuse reflectance infrared Fourier-transform spectroscopy
DSC	Differential scanning calorimetry
ECMWF	European Centre for Medium-Range Weather Forecasts
EEC	European Economic Community
ELISA	Enzyme-linked immunosorbent assay
EMEA	European Medicines Agency
EMRO	World Health Organization (WHO) Regional Office for the Eastern Mediterranean
EP	European Pharmacopoeia
ERH	Equilibrium relative humidity
EU	European Union
FAR	Field Alert Report
FDA	Food and Drug Administration
FID	Flame Ionization Detector
FMEA	Failure Mode and Effects Analysis
FT-IR	Fourier Transform Infrared spectroscopy
GC	Gas Chromatography
GCC	Cooperation Council for the Arab States of the Gulf
GHTF	Global Harmonization Task Force
HDPE	High density polyethylene
HMG	3-hydroxy-3-methyl-glutaryl
HPLC	High performance liquid chromatography
ICH	International Conference on Harmonization
IEF	Isoelectric focusing
IFPMA	International Federation of Pharmaceutical Manufacturers & Associations
IFU	Instructions for use
IGC	Inverse gas chromatography
IND	Investigational New Drug
IP	Intellectual property
IPAC-RS	International Pharmaceutical Aerosol Consortium on Regulation and Science
IQ	Installation qualification
IRA	Interim Revision Announcement
ISO	International Organization for Standardization
ITFG	Inhalation Technology Focus Group
J-NDA	(Japan) New Drug Application
JPMA	Japan Pharmaceutical Manufacturers Association

KBr	Potassium bromide
LC/MS-MS	Liquid chromatography/Mass spectroscopy-Mass spectroscopy
LC-NMR	Liquid chromatography/Nuclear magnetic resonance
LC-PDA	Liquid chromatography/photo-diode array
LIMS	Laboratory information management system
LOD	Limit of Detection
LOD	Loss on Drying
LOQ	Limit of Quantitation
LVP	Large Volume Parenteral
MDD	Medical Devices Directive
MDI	Metered Dose Inhaler
MHLW	(Japan) Ministry of Health, Labour and Welfare
MKT	Mean Kinetic Temperature
MPD	Medicinal Products Directive
MRI	Magnetic Resonance Imaging
MS/ELSD/CAD	Mass Spectrometry/Evaporative Light Scattering Detector/Charged Aerosol Detector
MVA	Multi-variate analysis
MVTR	Moisture vapor transmission rate
NCEs	New Chemical Entities
NDA	New Drug Application
NIR	Near-infrared (IR)
NIST	National Institute of Standards and Technology
NMP	*N*-methylpyrrolidone
NMR	Nuclear Magnetic Resonance
OCP	Office of Combination Products
OINDP	Orally Inhaled and Nasal Drug Products
OOS	Out of Specification
OOT	Out-of-Trend
OQ	Operational qualification
OTC	Over-the-counter
P & ID	
PAC-ATLS	Post-Approval Changes – Analytical Testing Lab Site
PAL	(Japan) Pharmaceutical Affairs Law
PAS	Prior Approval Supplement
PAT	Process analytical technology
PCA	Principal component analysis
PDA	Photo-diode array
Ph. Eur.	European Pharmacopoeia
PLS	Partial least squares
PMDA	(Japan) Pharmaceutical and Medical Devices Agency
PMOA	Primary Mode of Action
PQ	Performance qualification
PQRI	Product Quality Research Institute
PTI	Parametric tolerance interval

PVC	Polyvinyl chloride
QA	Quality Assurance
QA/QC	Quality Assurance/Quality Control
QbD	Quality by Design
QL	Quantitation Limit
RA	Regulatory Affairs
rDNA	Recombinant DNA
RFD	Request for Designation
RH	Relative humidity
RMS	Root-mean-square
RS	Reference Standards
RSD	Relative standard deviation
SADC	Southern African Development Community
SBI	Significant Body of Information
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEC	Size-exclusion chromatography
SE-HPLC	Size-exclusion HPLC
SEP	Standard error of prediction
SFC	Supercritical fluid chromatography
SIM	Stability-indicating method
SOP	Standard Operating Procedure
SUPAC	Scale Up and Post-Approval Changes
TGA	Thermogravimetric analysis
TLC	Thin layer chromatography
UHPLC	Ultra High Performance Liquid Chromatography
UPLC	Ultra Performance Liquid Chromatography
USP	United States Pharmacopeia
USP/NF	United States Pharmacopeia/National Formulary
UTC	Coordinated Universal Time
UV	Ultraviolet
WFI	Water for Injection
WHO	World Health Organization
XRPD	X-ray powder diffraction
YP_D	Yearly mean partial water vapor pressure

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