CHAPTER 3

ANALYTICAL TESTING AND DEVELOPMENT

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METHOD DEVELOPMENT

The need to develop and validate the analytical method is encountered by analytical chemists in the regulated industries on a daily basis. The scope of each development depends on the intended purpose, but the approach remains the same whether the method is for quantitation of a major component or for a trace impurity.

Although there exist different types of chromatographic separations, our focus is on reversed phase high-performance liquid chromatography (HPLC) method development for organic compounds with ultraviolet/visible (UV/Vis) wavelength absorbable chromophores. Most types of pharmaceutical compounds fall within this category.

Prior to starting method development, it is necessary to establish goals of the method. The question to answer might be, Is the method going to be for measurement of the major component (active ingredient) or for minor component(s) (impurity or impurities) or for both? In essence, is it going to be a stability-indicating method that will be validated according to the Food and Drug Administration/International Conference on Harmonisation (FDA/ICH) guidelines? Of equal importance that should not be ignored is the sample information. The information gathering should include the sample matrix, solubility of components in water/organic solvent mixture, chemical structure, polarity of the compound, molecular weight, and dissociation constant (pKa) as well as UV spectra in acidic, basic, and neutral environments.

The number of experiments to be performed could be highly reduced just by gaining full knowledge of the sample composition up-front. The resulting effect is that the choice of separation and type of column selection is made easier. In addition, by defining the purpose of the method before the first experiment is performed, and coupled with the sample information, these could lead to scientifically sound selection of wavelength of detection and the initial composition of the mobile phase.

COLUMN SELECTION

The choice of column for the initial set of experiments is typically based on the number of compounds that require quantitative separation. Generally, separation of component/retention varies inversely with chain length of the bonded phase (Retention: CN < C_4 < Phenyl < C_8 < C_{18}); and C_8 is the column of choice for the

initial set of experiments. The advantage of C_8 as column of first choice is manyfold. It offers the advantage of shorter run time than C_{18} column, is widely available, has stable stationary phase, and is stable over a wide pH range. When the intended use of the method is for separation and quantization of multicomponents such as major components and their respective impurities, C_8 may not provide enough plate number (N) for adequate separation of all the components; then, C_{18} column should be the column of first choice.

Whether the goal of separation is for qualitative identification of compounds or for quantitative determination of the major components with or without trace impurities, the beginning of method development proceeds in the same way for all the cases. Example is use of standard diameter column (150 mm in length, 4.6 mm internal diameter, and 5 mm particle sizes).

MODE OF DETECTION

Before the first sample is injected during HPLC method development, the detector of choice must be capable of detecting all samples of interest with UV scanning capability. Availability of a photo diode-array detector in modern HPLC instruments makes this the detector of choice.

The preferred wavelength for quantitative detection of compound can be obtained directly from the UV scan of the separation. An individual UV spectrum of well-characterized reference material with known purity and injected separately should be obtained if the composition of the sample is known and also available. This approach will help in confirming the elution profile of the sample.

MOBILE PHASE SELECTION

The separation and run time of reversed phase HPLC depends on the organic composition of the mobile phase or its solvent strength. Generally, a strong solvent strength that equates to a high proportion of organic solvent in the mobile phase always results in a decreased retention and shorter run time than with the low-solvent-strength mobile phase. For a stepwise process of method development, 50% water/methanol should be the combination of first choice in an isocratic mode of separation. This organic ratio allows for increase or decrease of the solvent strength depending upon the retention and run time. The separation achieved in the first set of experiments will lead to further optimization of the method.

The following are some practical considerations based on the outcome of the first set of experiments[1]:

1. If there is inadequate separation between components, one should consider one or a combination of the following: (a) changing the length of the column, (b) changing particle size of the stationary phase, and (c) switching the column to a C_{18} column or different bonded phase.
2. If a different elution order is desired to separate unrelated components, a change to a different bonded phase such as a phenyl or cyano column may be considered.
3. For separation of both acidic and basic compounds in the same sample mixture, use of a buffer in the mobile phase to control pH and retention time should be considered.
4. Use of column temperature and change from an isocratic method of elution to gradient should be considered if there is adequate separation of components with long run time. A change in temperature of the column is similar to an increase of organic composition of the mobile phase. As the temperature increases, the relative retention time of the components decreases, without compromising the resolution and with improved peak shape.

METHOD OPTIMIZATION

The separation achieved in the first set of experiments is usually less than adequate. Optimization of the method must be carried out to improve peak resolution, peak shape, and other parameters to meet the set goals. The process amounts to performing another set of experiments in which one parameter is varied at a time while holding others constant.

The variable parameters include some mentioned previously, such as use of ion pairing reagent, buffer, and column temperature; wavelength of detection; mobile phase composition; and change from isocratic mode of separation to gradient. Although the process is slow and time consuming, it helps in understanding how each of the variables affect the method. This approach was used in the separation of sample mixture containing both acidic and basic compounds (acetaminophen, 4-aminophenol, benzoic acid, and phenylephrine). See Figure 3-1.

EFFECT OF BUFFER

Selecting an appropriate buffer salt for the mobile phase is very critical when developing the method for the separation of the sample mixture containing both acidic and basic compounds. The purpose of the buffer is to control the pH of the mobile phase. The selection of buffer salt is based on the pKa of the

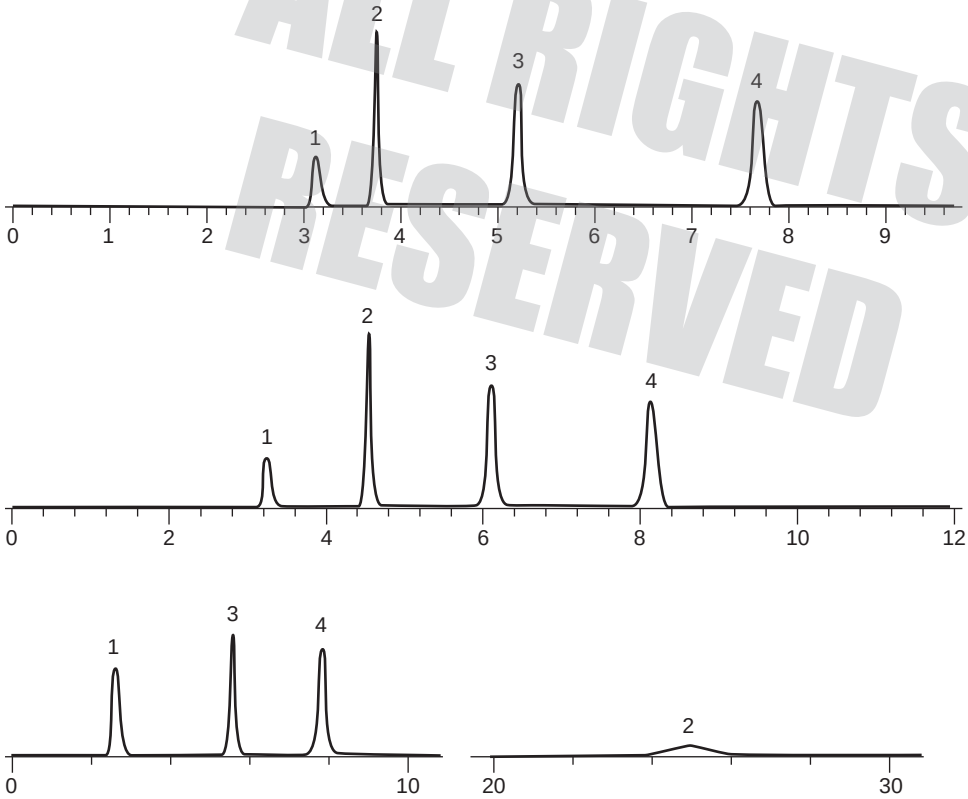

FIGURE 3-1

TABLE 3-1 List of Some Commonly Used Buffers With Their pKa Values

Buffer	pKa	Buffer Range
Acetate	4.8	2.5–5.5
Citrate	3.1	2.0–4.0
	4.7	3.5–4.5
	5.4	4.5–6.5
Phosphate	2.1	1.0–3.0
	7.2	6.0–8.0
	12.3	11.2–13.2
Triethylamine	11.0	10–12.0

separating compound. Maximum pH control of the mobile phase occurs at a pH that is equal to the pKa of the buffer salt. Some of the most commonly used buffers are listed in Table 3-1.

The concentration of buffer in the range of 25 to 50 mM is usually enough to provide buffering capacity. Higher concentration may result in the precipitation of the salt in the presence of organic solvent in the mobile phase.

As shown in Figure 3-1, in the separation of 4-aminophenol, acetaminophen, benzoic acid, and phenylephrine, the retention time of benzoic acid is highly affected by the pH of the mobile phase. As the benzoic acid loses a proton and becomes ionized with increasing pH of the mobile phase, its retention time decreases. The retention time of the other components remains relatively the same.

COLUMN-TO-COLUMN EQUIVALENCY

Method optimization would not be completed without establishing column-to-column equivalency. This is essential for developing a robust, rugged, and reproducible method. Similar columns from the same or different suppliers can cause significant changes in retention time because of variation in plate number, band symmetry, and selectivity, especially when separating polar basic compounds. Unwanted interaction between basic compounds and acidic silanol group on the surface of silica-based stationary phases is the major cause of poor column efficiency and selectivity. Clearly, for the separation of 4-aminophenol, acetaminophen, benzoic acid, and phenylephrine in Figure 3-2, the third column in the experiment showed different selectivity, and thus is not equivalent to the other two columns. A fully reacted and end-capped column from different vendors tends to minimize the effect.

EFFECT OF TEMPERATURE

Changing column temperatures also affects retention time. In Figure 3-3 note the differences in retention time of the compounds at 40°C compared to 25°C.

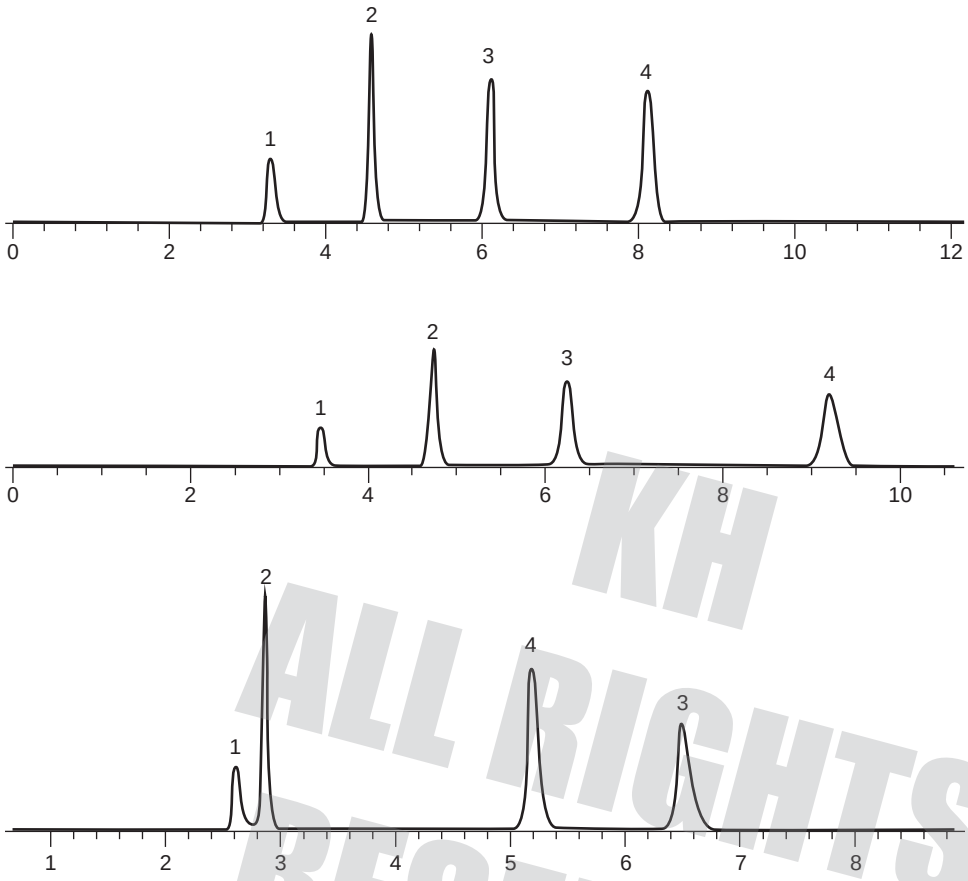

FIGURE 3-2

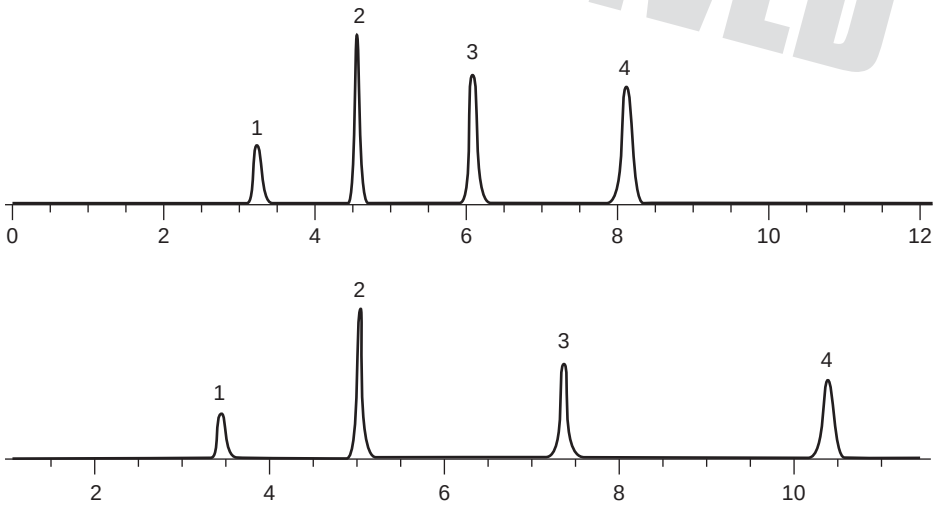

FIGURE 3-3

EFFECT OF ION PAIR REAGENT

The addition of an ion pair reagent to the mobile phase for reverse phase HPLC separation requires more experimental work to be done for optimization of retention. The advantage gained is for better control of retention and selectivity of polar compounds.

The most commonly used ion pair reagents fall into two main classes:

- Tetraalkylammonium salts, such as tetrabutylammonium and cetyltrimethylammonium salts, are for acidic compounds. Interaction with acidic compounds results in an increase of ionized acids and other anions.
- Alkyl sulfonic acid salts (C_4–C_8 sulfonic acid salts) and sodium dodecyl sulfate are for increased retention of protonated bases and other cations.

The alkyl sulfonate and tetrabutylammonium salts are the most frequently used reagents because of their low UV absorption at 210 nm. These reagents work by electrostatic and hydrophobic interactions between the ionic samples and the ion pair reagent. An interchange of any of the ion pair reagents with a different chain length within the same class (e.g., hexanesulfonic acid for butane sulfonic acid salt, as shown in Figure 3-4) does not produce the same retention time effect. To reduce complexity of a method, ion pair reagents should be used in a method only to achieve specific objectives such as to have better control over retention range of polar compounds.

Once the analytical method for analysis has been developed, a regimented series of testing is performed to ensure the method is valid. A method that is valid can be used routinely to test for the stability of excipients, active pharmaceutical ingredients (APIs), and finished dosage forms. The FDA requires that all methods of analysis be validated and that the information be presented when applying for a new drug substance and drug products.

The primary guidances to follow when validating the method are located in the United States Pharmacopeia General Chapter [905], the FDA Guidance to Analytical Procedures and Methods Validation, the FDA "Reviewer Guidance, Validation of Chromatographic Methods," and the ICH guidance Q2B.[2–4]

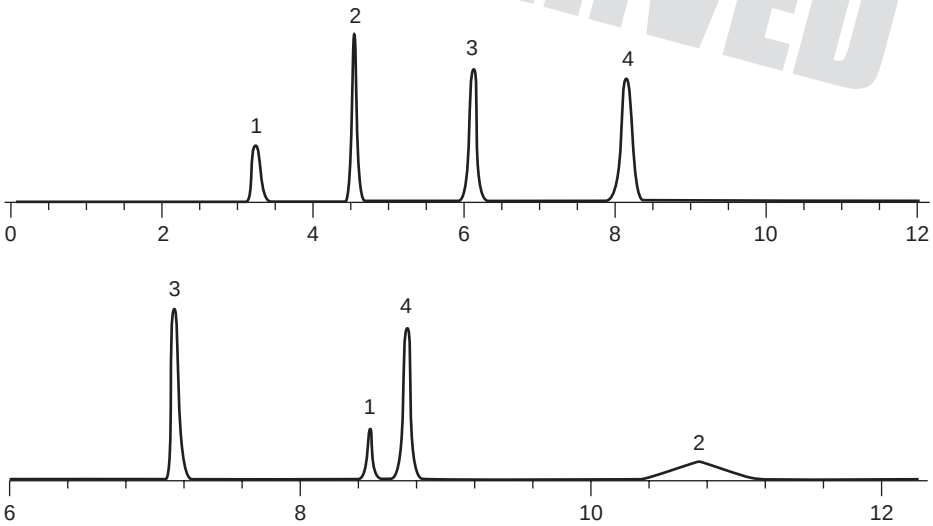

FIGURE 3-4

TABLE 3-2 Recommended Validation Characteristics of Various Types of Tests

Testing for Impurities					
Type of Tests/ Characteristics/ Performance Factors	**Identification**	**Quantitative**	**Limit**	**Assay Dissolution (Measurement Only) Content/Potency**	**Specific Tests**
Specificity	+[2]	+	+	+[5]	+[4]
Linearity	–	+	–	+	–
Range	–	+	–	+	–
Accuracy	–	+	–	+	+[4]
Precision Repeatability	–	+	–	+	+[4]
Precisions Intermediate Precision	–	+[1]	–	+[1]	+[4]
Limit of Detections	–	–[3]	+	–	–
Limit of Quantitations	–	+	–	–	–
Robustness	–	+	–[3]	+	+[4]

Notes:
– Signifies that this characteristic is not normally evaluated.
+ Signifies that this characteristic is normally evaluated.
[1] In cases where reproducibility has been performed, intermediate precision is not needed.
[2] Lack of specificity for an analytical procedure may be compensated for by the addition of a second analytical procedure.
[3] May be needed in some cases.
[4] May not be needed in some cases.
[5] Lack of specificity for an assay for release may be compensated for by impurities testing.

All these references are similar in criteria. Table 3-2 highlights the different validation characteristics of various types of tests.

The FDA and other health authorities require the following general information to be summarized in the final validation report:

1. Data demonstrating suitable accuracy, precision, and linearity over the range of interest (ca. 80% to 120% of the label claim). Data demonstrating specificity of the methods and determination limits for degradation products or impurities should be included. These degradation products or impurities should be adequately identified and characterized.
2. Data demonstrating recovery from the sample matrix where the nature of the product so indicates.

3. Data demonstrating that neither the fresh nor degraded placebo interferes with the proposed method.
4. Legible reproductions of representative chromatographs and instrumental recordings.
5. Data characterizing day-to-day, laboratory-to-laboratory, analyst-to-analyst, and column-to-column variability. These data may be included to provide a further indication of reproducibility and, in a limited sense, ruggedness.
6. A degradation schematic for the active ingredient in the dosage form, where possible (e.g., products of acid/base hydrolysis, temperature degradation, photolysis, and oxidation).
7. System suitability tests (as defined in the current USP–NF [621])[2]:
   a. Number of theoretical plates
   b. Tailing factor
   c. Relative retention
   d. Capacity factor
   e. Resolution
   f. Relative standard deviation

In practice, each method submitted for validation must include an appropriate number of system suitability tests defining the necessary characteristics of that system. Other parameters may be included at the discretion of the applicant or the agency.

The effect of adjustments in mobile phase composition on retention times should be included in the method. Any precolumns or guard columns must be described. The rationale for their use should be logically explained and justified.

Examples of common problems that can delay the acceptance of a successful validation include the following:

1. Failure to include a sample of a critical impurity, degradation product, or internal standard necessary to assess the adequacy of the method.
2. Failure to list complete specifications, or the selection of unsuitable specifications, such as the following:
   a. Unsubstantiated or overly broad ranges (broader than investigational data can support)
   b. Specifications that do not account for assay limitations
3. Failure to provide sufficient detail, or unacceptable choice of procedures, reagents, or equipment, such as the following:
   a. Use of placebo blanks
   b. Use of arbitrary arithmetic corrections
   c. Use of instrumentation not commercially available without a full description of components and their assembly
   d. Use of single-source chromatographic columns, equipment, or reagents without full specifications to permit duplication
   e. Use of specialized tools or equipment that is not commercially available for sample preparation
   f. Use of an internal standard or other reagent that is not commercially available
   g. Failure to provide system suitability tests for chromatographic systems
   h. Differing content uniformity and assay procedures without showing equivalency factors for defining corrections as required by the USP XXI [905]

4. Failure to submit complete or legible data:

a. Failure to label chromatograms and spectra as to sample identity
b. Failure to label *x*-axis and *y*-axis as appropriate

When writing the analytical procedure the following information should be considered. The guideline as issued under 21 CFR 10.90. An applicant (or sponsor) may rely upon the guideline concerning specifications and methodologies as required by 21 CFR 314.50 in the presentation of data, assembly of information, and submission of materials to the FDA, or may follow a different approach. When a different approach is chosen, the applicant is encouraged to discuss the matter in advance with the FDA to prevent the expenditure of money and effort on preparing a submission that may later be determined to be unacceptable.

Some individual drug products may not require submission of all the information described in the guideline. In other cases, additional detail may be needed to provide a rational, scientific foundation for proposed specifications and methodologies. Generally, however, the provisions of 21 CFR 211.194(a)(1) and (2) are descriptive of the kinds of information to be submitted.

This section covers definitions of some of the most common terms used in analytical development from a regulatory perspective.

DEFINITIONS

- Regulatory specifications: Regulatory specifications are the defined limits within which physical, chemical, biological, and microbiological test results for a drug substance or drug product should fall when determined by the regulatory methodology. For compendial articles, the specifications in the current edition of the United States Pharmacopeia–National Formulary (USP–NF) are those legally recognized under Section 501(b) of the Federal Food, Drug, and Cosmetic Act and are used by the agency when determining compliance with the act.
- Regulatory methodology: Regulatory methodology is the procedure or set of procedures used by the FDA to ascertain whether or not the drug substance or drug product is in conformance with the approved regulatory specifications in the new drug application (NDA). Generally, a regulatory assay will be stability indicating. For USP–NF articles, the analytical test methods in the compendial monograph are those legally recognized under Section 501(b) of the act and are used by the agency when determining compliance with the act. However, compendial methods may require validation to establish their suitability for specific drug products.
- Regulatory methods validation: Regulatory methods validation is the process whereby submitted analytical procedures are first reviewed for adequacy and completeness and then are tested as deemed necessary in FDA laboratories. Depending in part on the quality of submitted data, validation may range from step-by-step repetition of an assay procedure to more elaborate studies that include assessment of accuracy, precision, sensitivity, and ruggedness of the method.

STABILITY TESTING

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.[5–8]

Drug Substance

Information on the stability of the drug substance is an integral part of the systematic approach to stability evaluation.

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.

The testing should include the effect of temperatures (in 10°C increments, for example, 50°C or 60°C above that for accelerated testing); humidity (e.g., 75% relative humidity or greater), where appropriate; oxidation; and photolysis on the drug substance. The testing should also evaluate the susceptibility of the drug substance to hydrolysis across a wide range of pH values when in solution or suspension. Photostability testing should be an integral part of stress testing.

Examining degradation products under stress conditions is useful in establishing degradation pathways and developing and validating suitable analytical procedures. However, such examination may not be necessary for certain degradation products if it has been demonstrated that they are not formed under accelerated or long-term storage conditions. Results from these studies will form an integral part of the information provided to regulatory authorities.

The stability studies should be conducted on the drug substance packaged in a container closure system that is the same as or simulates the packaging proposed for storage and distribution. Stability studies should include testing of those attributes of the drug substance that are susceptible to change during storage and are likely to influence quality, safety, and/or efficacy. The testing should cover, as appropriate, the physical, chemical, biological, and microbiological attributes. Validated stability-indicating analytical procedures should be applied.

At the accelerated storage condition, a minimum of three time points, including the initial and final time points (e.g., 0, 3, and 6 months), from a 6-month study is recommended.

Drug Product

The design of the formal stability studies for the drug product should be based on knowledge of the behavior and properties of the drug substance, results from stability studies on the drug substance, and experience gained from preformulation studies. The likely changes on storage and the rationale for the selection of attributes to be tested in the formal stability studies should be stated.

Although normal manufacturing and analytical variations are to be expected, it is important that the drug product be formulated with the intent to provide 100% of the labeled amount of the drug substance at the time of batch release. Variance to this may complicate data handling of the stability study.

Stability testing should be conducted on the dosage form packaged in the container closure system proposed for marketing (including, as appropriate, any secondary packaging and container label).

Stability studies should include testing of those attributes of the drug product that are susceptible to change during storage and are likely to influence quality, safety, and/or efficacy. The testing should cover, as appropriate, the physical, chemical, biological, and microbiological attributes, preservative content (e.g., antioxidant, antimicrobial preservative), and functionality tests (e.g., for a dose delivery system). Analytical procedures should be fully validated and stability indicating. Whether and to what extent replication should be performed will depend on the results of validation studies.

Shelf life acceptance criteria should be derived from consideration of all available stability information. It may be appropriate to have justifiable differences between the shelf life and release acceptance

criteria based on the stability evaluation and the changes observed on storage. Any differences between the release and shelf life acceptance criteria for antimicrobial preservative content should be supported by a validated correlation of chemical content and preservative effectiveness demonstrated during drug development on the product in its final formulation (except for preservative concentration) intended for marketing. A single primary stability batch of the drug product should be tested for antimicrobial preservative effectiveness (in addition to preservative content) at the proposed shelf life for verification purposes, regardless of whether there is a difference between the release and shelf life acceptance criteria for preservative content.[9–12]

For testing frequency at the accelerated storage condition, a minimum of three time points, including the initial and final time points (e.g., 0, 3, and 6 months), from a 6-month study is recommended. Where an expectation (based on development experience) exists that results from accelerated testing are likely to approach significant change criteria, increased testing should be conducted either by adding samples at the final time point or by including a fourth time point in the study design. Photostability testing should be conducted on at least one primary batch of the drug product. The standard conditions for photostability testing are described in ICH Q1B.

When testing at the intermediate storage condition is called for as a result of significant change at the accelerated storage condition, a minimum of four time points, including the initial and final time points (e.g., 0, 6, 9, 12 months), from a 12-month study is recommended.

Reduced designs (i.e., matrixing or bracketing), where the testing frequency is reduced or certain factor combinations are not tested at all, can be applied if justified.

Glossary of Terms for Analytical and Method Development

Listed below are definitions commonly used in analytical and method development in the pharmaceutical industry.

- Accelerated testing: Studies designed to increase the rate of chemical degradation or physical change of a drug substance or drug product by using exaggerated storage conditions as part of the formal stability studies. Data from these studies, in addition to long-term stability studies, can be used to assess longer-term chemical effects at nonaccelerated conditions and to evaluate the effect of short-term excursions outside the label storage conditions such as might occur during shipping. Results from accelerated testing studies are not always predictive of physical changes.
- Bracketing: The design of a stability schedule such that only samples on the extremes of certain design factors (e.g., strength, package size) are tested at all time points as in a full design. The design assumes that the stability of any intermediate levels is represented by the stability of the extremes tested. Where a range of strengths is to be tested, bracketing is 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-sized capsule shells). Bracketing can be applied to different container sizes or different fills in the same container closure system.
- Climatic zones: The four zones in the world that are distinguished by their characteristic, prevalent annual climatic conditions. This is based on the concept described by W. Grimm.[13]
- Container closure system: The sum of packaging components that together contain and protect the dosage form. This includes primary and secondary packaging components if the latter are intended to provide additional protection to the drug product. A packaging system is equivalent to a container closure system.[14]

- Dosage form: A pharmaceutical product type (e.g., tablet, capsule, solution, cream) that contains a drug substance generally, but not necessarily, in association with excipients.
- Drug product: The dosage form in the final immediate packaging intended for marketing.
- Drug substance: The unformulated drug substance that may subsequently be formulated with excipients to produce the dosage form.
- Excipient: Anything other than the drug substance in the dosage form.
- Expiration date: The date placed on the container label of a drug product designating the time prior to which a batch of the product is expected to remain within the approved shelf life specification, if stored under defined conditions, and after which it must not be used.
- Formal stability studies: Long-term and accelerated (and intermediate) studies undertaken on primary and/or commitment batches according to a prescribed stability protocol to establish or confirm the retest period of a drug substance or the shelf life of a drug product.
- Intermediate testing: Studies conducted at 30°C/65% RH and designed to moderately increase the rate of chemical degradation or physical changes for a drug substance or drug product intended to be stored long-term at 25°C.
- Long-term testing: Stability studies under the recommended storage condition for the retest period or shelf life proposed (or approved) for labeling.
- Mass balance: The process of adding together the assay value and levels of degradation products to see how closely these add up to 100% of the initial value, with due consideration of the margin of analytical error.
- Matrixing: The design of a stability schedule such that a selected subset of the total number of possible samples for all factor combinations is tested at a specified time point. 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. The differences in the samples for the same drug product should be identified as, for example, covering different batches, different strengths, different sizes of the same container closure system, and, in some cases, different container closure systems.
- New molecular entity: An active pharmaceutical substance not previously contained in any drug product registered with the national or regional authority concerned. A new salt, ester, or noncovalent bond derivative of an approved drug substance is considered a new molecular entity for the purpose of stability testing under this guidance.
- Retest date: The date after which samples of the drug substance should be examined to ensure that the material is still in compliance with the specification and thus suitable for use in the manufacture of a given drug product.
- Retest period: The period of time during which the drug substance is expected to remain within its specification and, therefore, can be used in the manufacture of a given drug product, provided that the drug substance has been stored under the defined conditions. After this period, a batch of drug substance destined for use in the manufacture of a drug product should be retested for compliance with the specification and then used immediately. A batch of drug substance can be retested multiple times and a different portion of the batch used after each retest, as long as it continues to comply with the specification. For most biotechnological/biological substances known to be labile, it is more appropriate to establish a shelf life than a retest period. The same may be true for certain antibiotics.
- Shelf life (also referred to as expiration dating period): The time period during which a drug product is expected to remain within the approved shelf life specification, provided that it is stored under the conditions defined on the container label.

- Specification, release: The combination of physical, chemical, biological, and microbiological tests and acceptance criteria that determine the suitability of a drug product at the time of its release.
- Specification, shelf life: The combination of physical, chemical, biological, and microbiological tests and acceptance criteria that determine the suitability of a drug substance throughout its retest period, or that a drug product should meet throughout its shelf life.
- Stress testing (drug substance): Studies undertaken to elucidate the intrinsic stability of the drug substance. Such testing is part of the development strategy and is normally carried out under more severe conditions than those used for accelerated testing.
- Stress testing (drug product): Studies undertaken to assess the effect of severe conditions on the drug product. Such studies include photostability testing (see ICH Q1B) and specific testing of certain products (e.g., metered dose inhalers, creams, emulsions, refrigerated aqueous liquid products).

DISSOLUTION TEST FOR SOLID DOSAGE FORMS

The dissolution test is used to determine the dissolution rate of active pharmaceutical ingredients in solid dosage forms (e.g., tablets, capsules, and suppositories). For any active ingredient in solid pharmaceutical dosage form to be available for absorption, it must first be released into the biological medium. The processes which the solid form of dosage undergoes before dissolution or release include wettability of the dosage form, penetrating ability of the dissolution medium, the swelling process, the disintegration of the active into granules, deaggregation of the granules into fine particles, and finally the dissolution and release of the drug into the microenvironment for absorption. To a small extent, dissolution of drug may occur from the intact dosage form after the penetration of the dissolution medium. The rate at which this occurs depends on the composition and method of preparation of the dosage form and may be the rate-limiting step in the bioavailability of the drug for systemic absorption. This can be altered by the formulator.

Dissolution testing of solid dosage forms provides critical means of evaluating formulations from development to end of shelf life. Dissolution testing is routinely used in the following areas and should be performed on individual tablets/capsules to observe intralot variability:

- During the early product development phase, it serves as a means of screening formulations leading to the development of the optimal dosage form.
- The test is commonly used as a quality assurance/quality control (QA/QC) tool to measure batch-to-batch equivalence and surrogate for human clinical testing.
- A well-designed dissolution method can be used to predict physiochemical properties of the drug, thus correlating the in vitro process to in vivo absorption.

Of the types of apparatus that are commercially available, either the basket apparatus (apparatus 1) or the paddle apparatus (apparatus 2) or, in special cases, the flow-through cell apparatus are the most commonly used systems.

Apparatus 1 The apparatus 1 assembly consists of the following: a covered cylindrical vessel made of glass, a metallic drive shaft fabricated with stainless steel, a cylindrical basket made of stainless steel, and a water bath or heating jacket that maintains the dissolution medium at 37°C ± 0.5°C.

Apparatus 2 The apparatus 2 assembly consists of a vessel identical to that described for the basket apparatus (apparatus 1), a vertical metallic shaft with attached blade that serves as stirrer, and a water bath or heating jacket that maintains the dissolution medium at 37°C ± 0.5°C.

Flow-Through Apparatus The flow-through apparatus consists of a reservoir for the dissolution medium, a pump that forces the dissolution medium upward through the flow cell, a flow cell, and a water bath or heating jacket that maintains the dissolution medium at 37°C ± 0.5°C.

Factors That Influence Dissolution Testing The dissolution rate of drug dosage forms can be classified in four main classes:

1. Physiochemical properties of the drug. The physiochemical properties of the drug substance play a critical role in controlling its dissolution rate. The dissolution test medium is in most cases an aqueous base such as buffer and purified water, so dissolution of drug substance in this medium is one of the major factors that govern its dissolution rate. Other factors such as particle size, crystalline nature, amorphic state, state of hydration, free acid, free base or salt form, eutectic, and polymorphic form of the drug substance contribute to the variability of the dissolution rate if they are not well controlled.
2. Product formulation. The dissolution rate of active ingredient in sold dosage form can directly be influenced when mixed with various excipients and by the manufacturing processes. These excipients include diluents, binders, lubricants, granulating agents, surfactants, disintegrants, and coloring agents. Manufacturing processing parameters, such as blending time, sequence of addition of ingredients, method of granulation, water content and density of granules, and size of tablets and their hardness, all contribute to the dissolution rate of solid dosage product.
3. Dissolution testing devices. For reliable and accurate dissolution measurement of drug substance, the dissolution testing devices must be set up precisely. Extreme care should be taken to minimize vibration of the unit and wobbling of the shaft. These affect the flow pattern and the hydrodynamic properties of the dissolution medium. For dissolution rate data to be reproducible and reliable, the flow patterns of the medium must be consistent from test to test.

   With most automated sampling systems and newer fiber optic in-situ analysis techniques currently in operation, a filter-tipped or filter optic probe is immersed in the dissolution medium throughout the duration of the dissolution testing. The size of the probe can also affect the hydrodynamics of the system, resulting in an erroneous dissolution rate.
4. Dissolution test parameters. Several factors associated with dissolution test parameters affect test-to-test reproducibility of dissolution rate. Factors such as temperature, pH, nature and composition, volume, and viscosity of the dissolution medium affect reliability of dissolution measurement. Dissolved air present in the dissolution medium is one of the major sources of error in dissolution measurement processes. With the change in temperature, the dissolved air may be released in the form of bubbles, causing unreliable dissolution measurement.

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