Part II
Stability Methodologies and Best Practices

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Chapter 6
Understanding and Predicting Pharmaceutical Product Shelf-Life

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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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K. Huynh-Ba (ed.), *Handbook of Stability Testing in Pharmaceutical Development*,
DOI 10.1007/978-0-387-85627-8_6, © Springer Science+Business Media, LLC 2009

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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

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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

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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

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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

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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.

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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,

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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.

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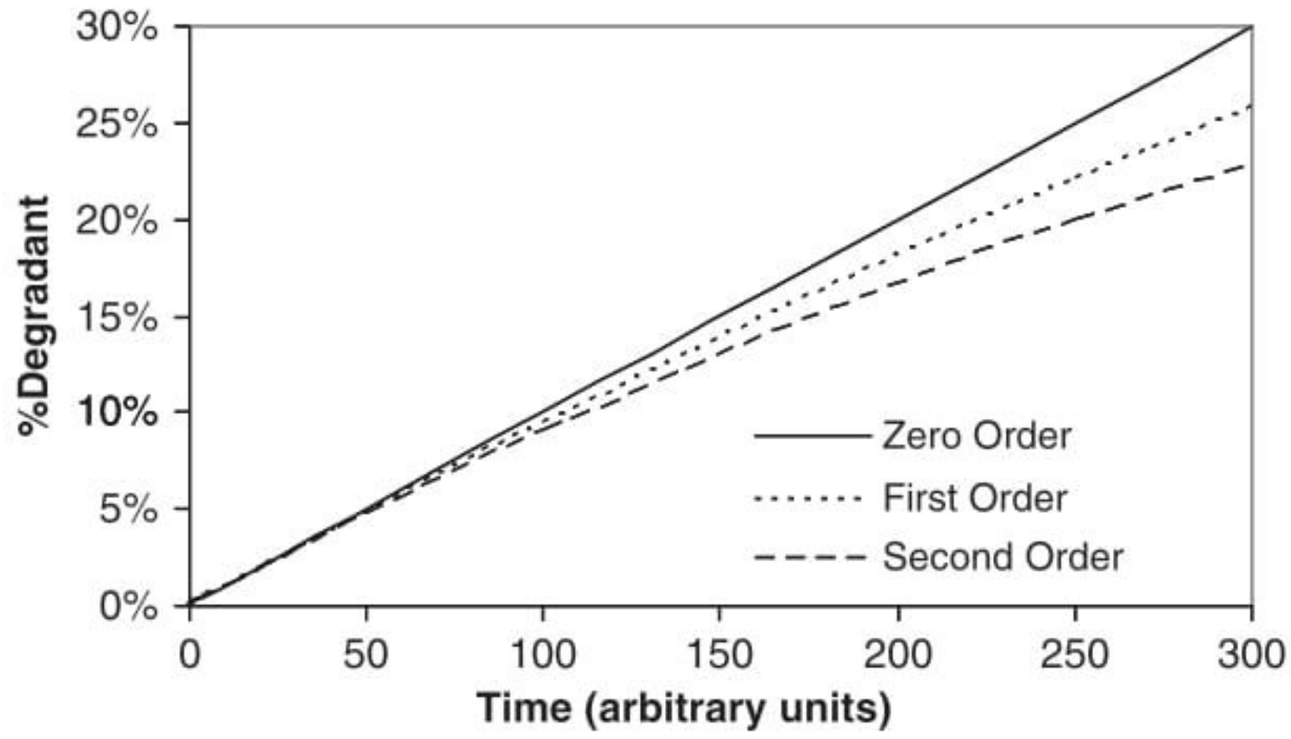

Fig. 6.1 Difference between zero, first, and second order processes. As can be seen, at low conversions (low amounts of degradant formed), the functional differences between the different reaction orders are indistinguishable

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

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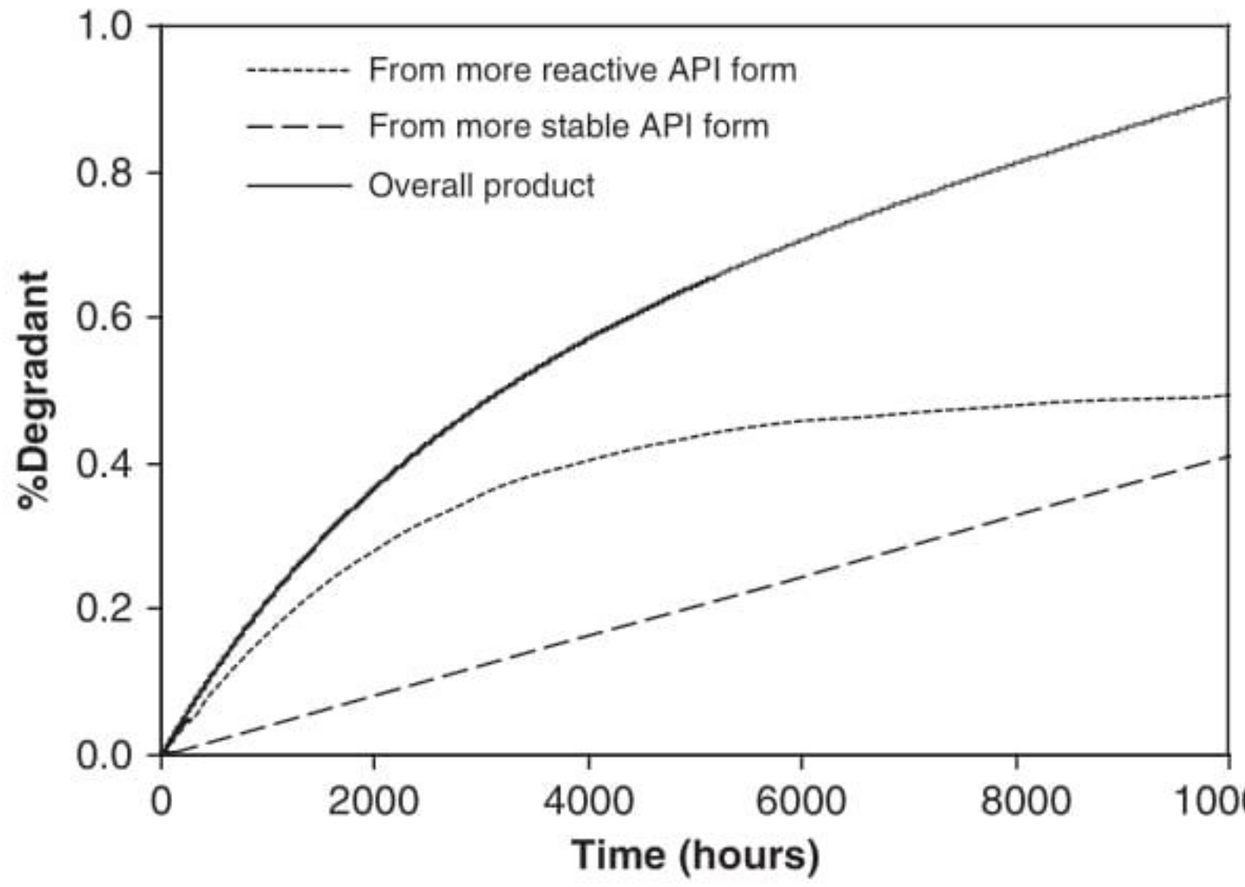

Fig. 6.2 The overall product profile is affected by the slow degradation of the crystalline API as well as the more rapid degradation from API in a more reactive form (e.g., amorphous). In this example, the degradant is formed based on the assumption that there is 0.5% of reactive API form, which reacts 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

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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

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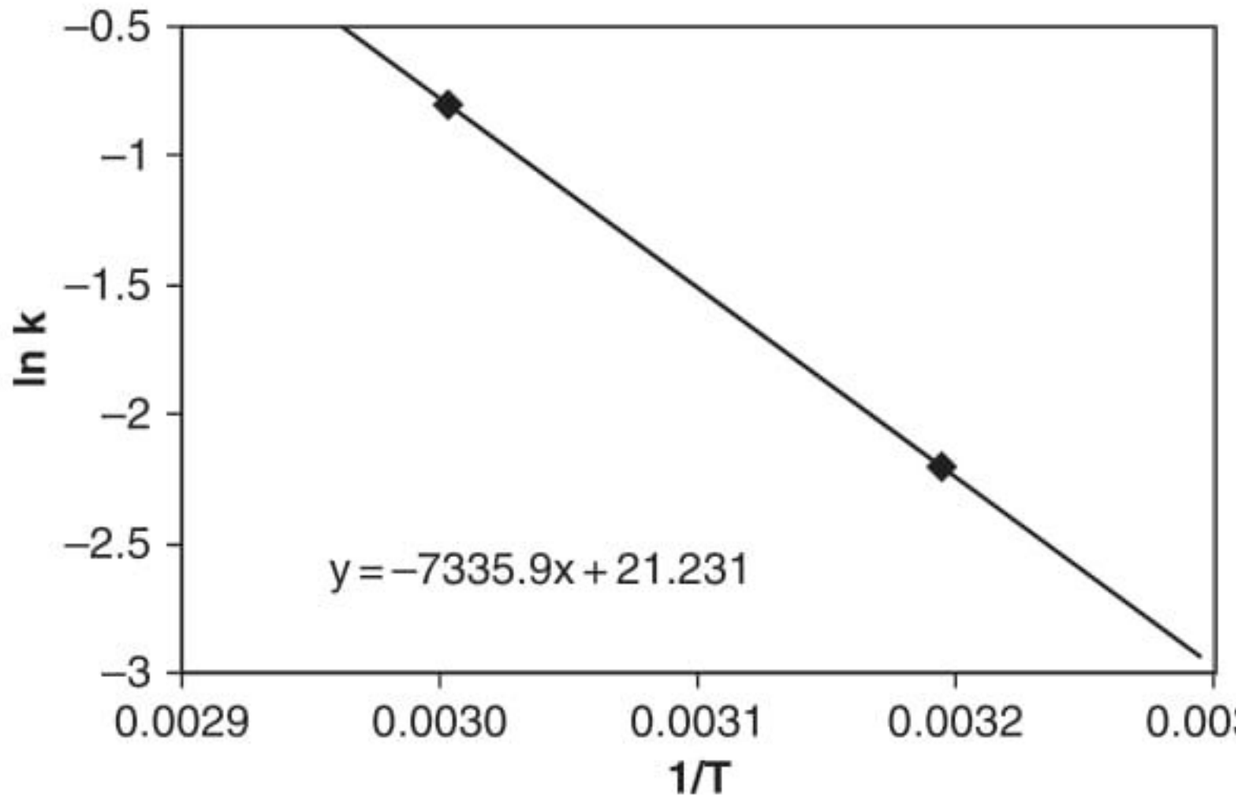

Fig. 6.3 Arrhenius plot used for the example calculation. The intercept is ln A and the slope is $-E_a/R$

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

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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

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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.

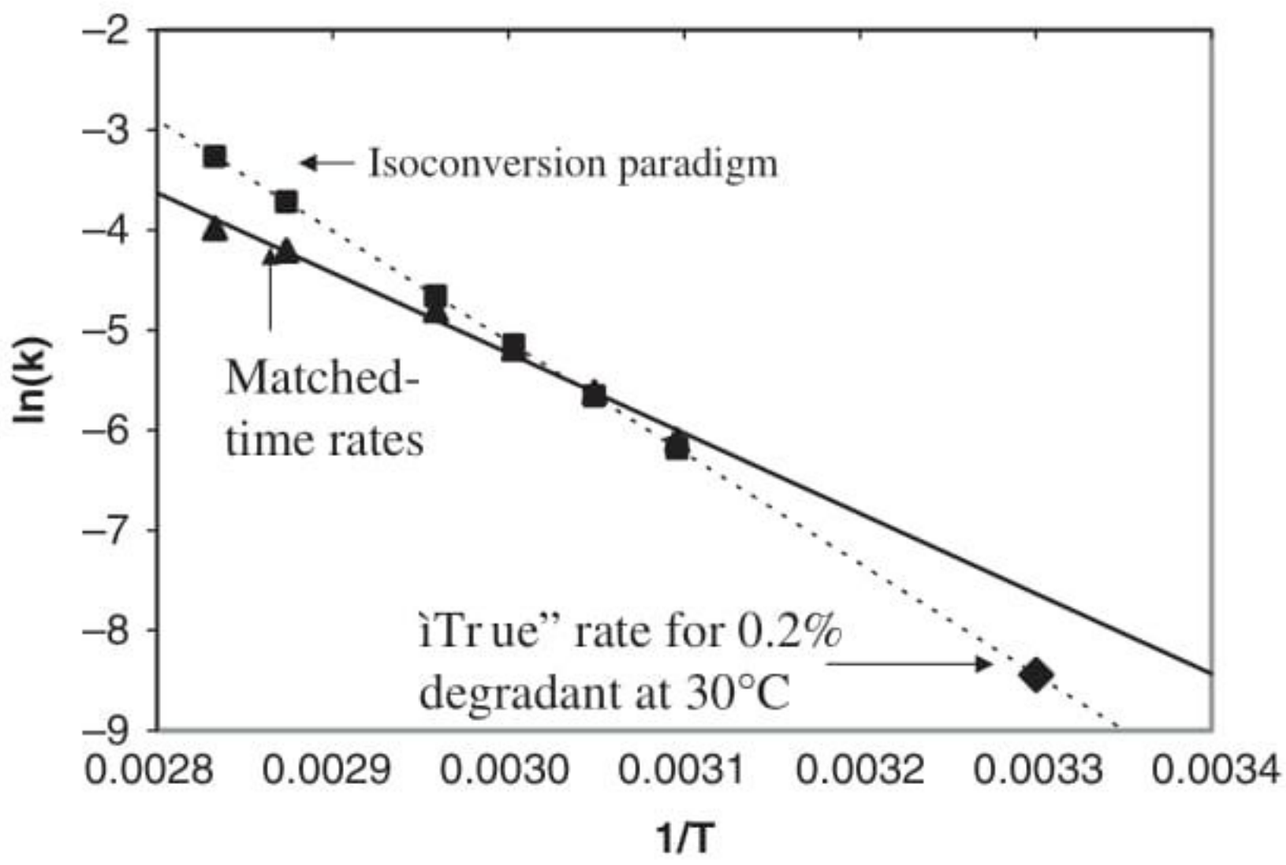

Fig. 6.4 Calculated Arrhenius plots illustrating the contrast between the isoconversion paradigm and constant time-based accelerated stability measurements. The isoconversion (to 0.20%) model (*squares*) and matched exposure time model (*triangles*) extrapolate to shelf-lives (at 30°C) of 2.54 and 1.14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

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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

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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:

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

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

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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

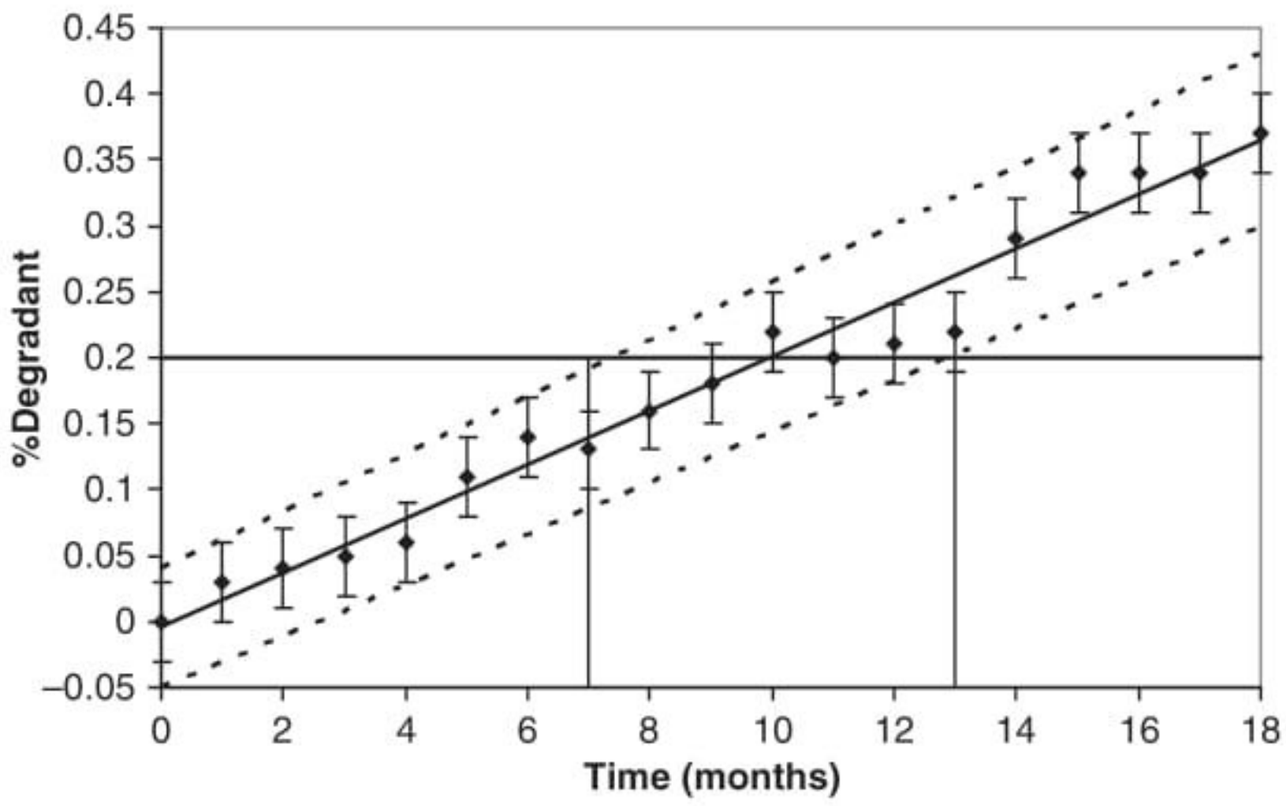

Fig. 6.5 Example of how error bars in real-time stability measurements translate to error bars in shelf-life values. In this example, the 0.2% degradant level used for the limitation of shelf-life is passed at 10 months, but with the error bars, this is really a range of 7–13 months

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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.

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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.

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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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K. Huynh-Ba (ed.), *Handbook of Stability Testing in Pharmaceutical Development*,
DOI 10.1007/978-0-387-85627-8_7, © Springer Science+Business Media, LLC 2009

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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-

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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.

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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)

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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*

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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

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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

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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

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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.

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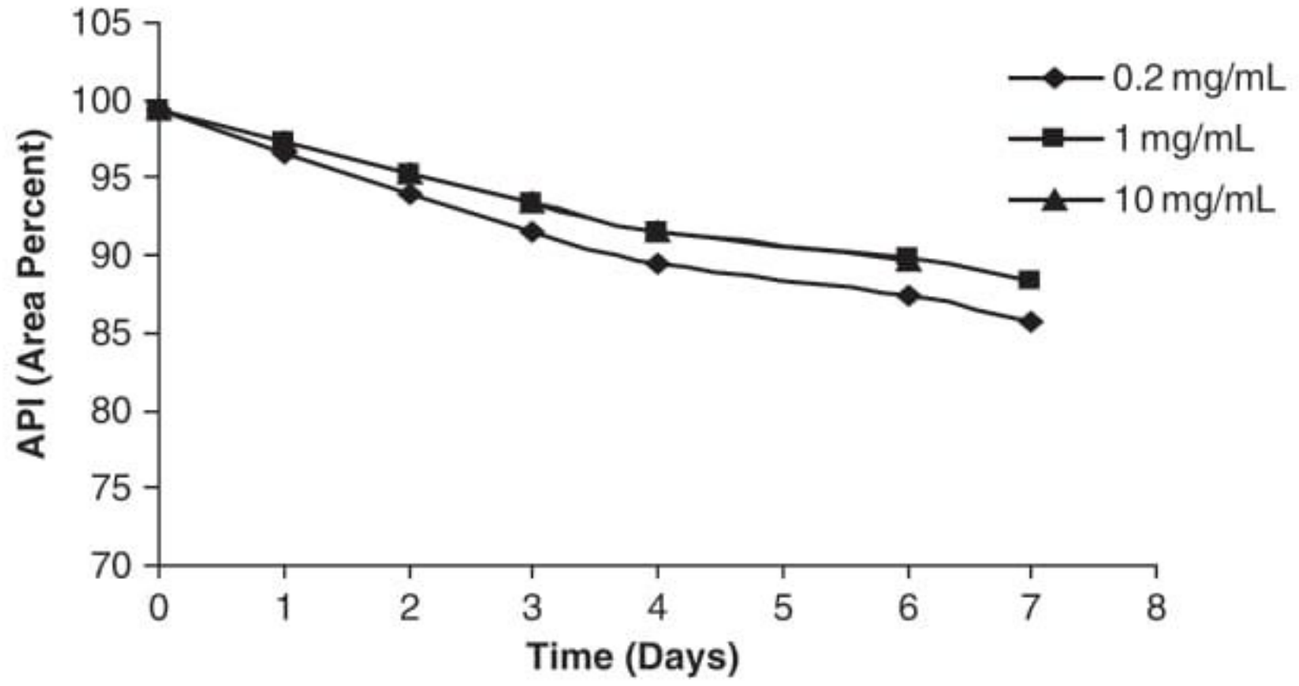

Fig. 7.1 Thermal hydrolysis profile of an API (structure not shown) at 70°C: degradation vs. time at three sample concentrations

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

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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 (radical, light, metal, peroxide mediated)	N-oxide, hydroxylamine
Thiols		Disulfide
Thioethers		Sulfoxide, sulfone
Alkenes		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.

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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, $ArNH_2$

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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

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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

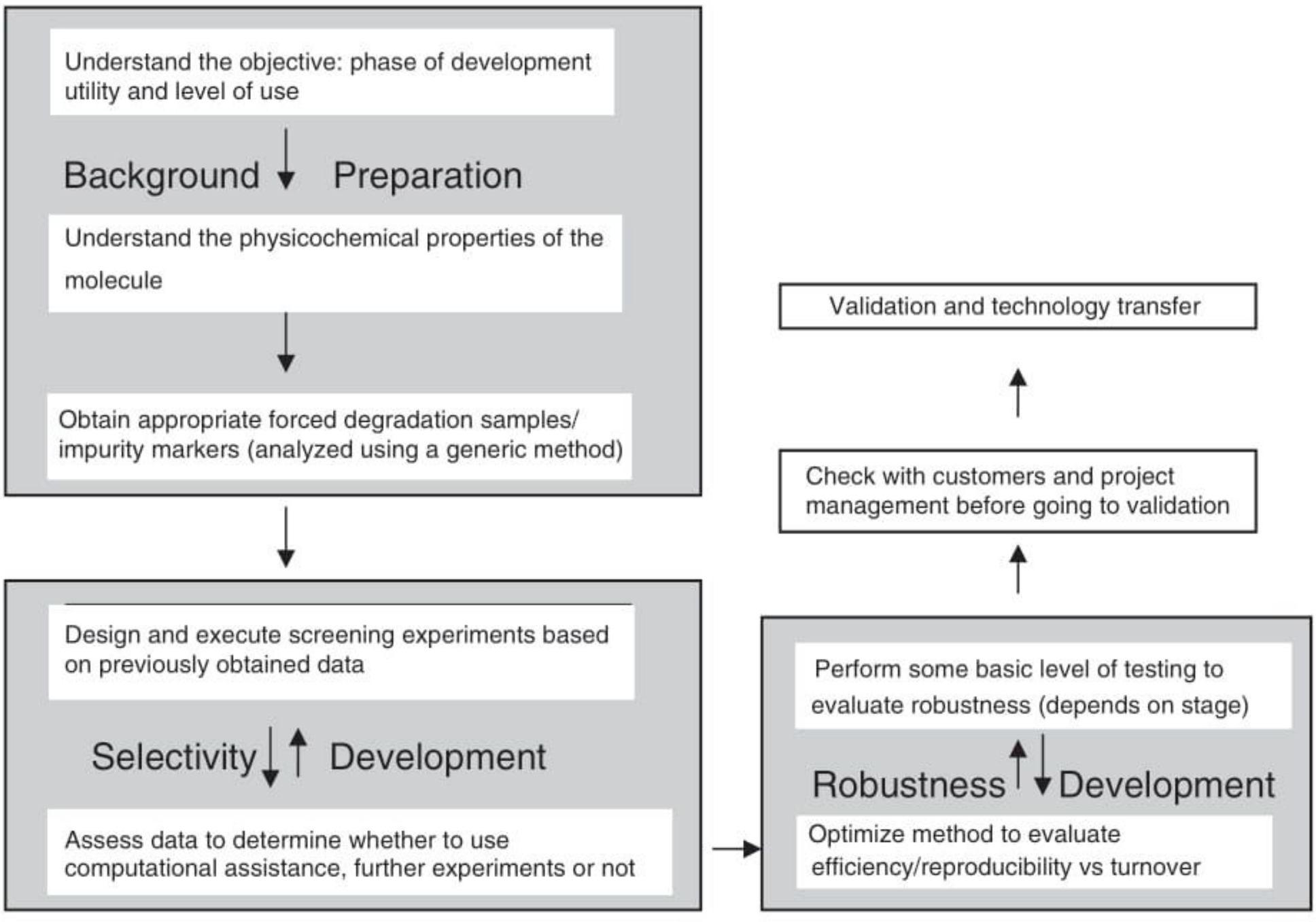

Fig. 7.2 Stability-indicating method development process

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(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

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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

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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.

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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)

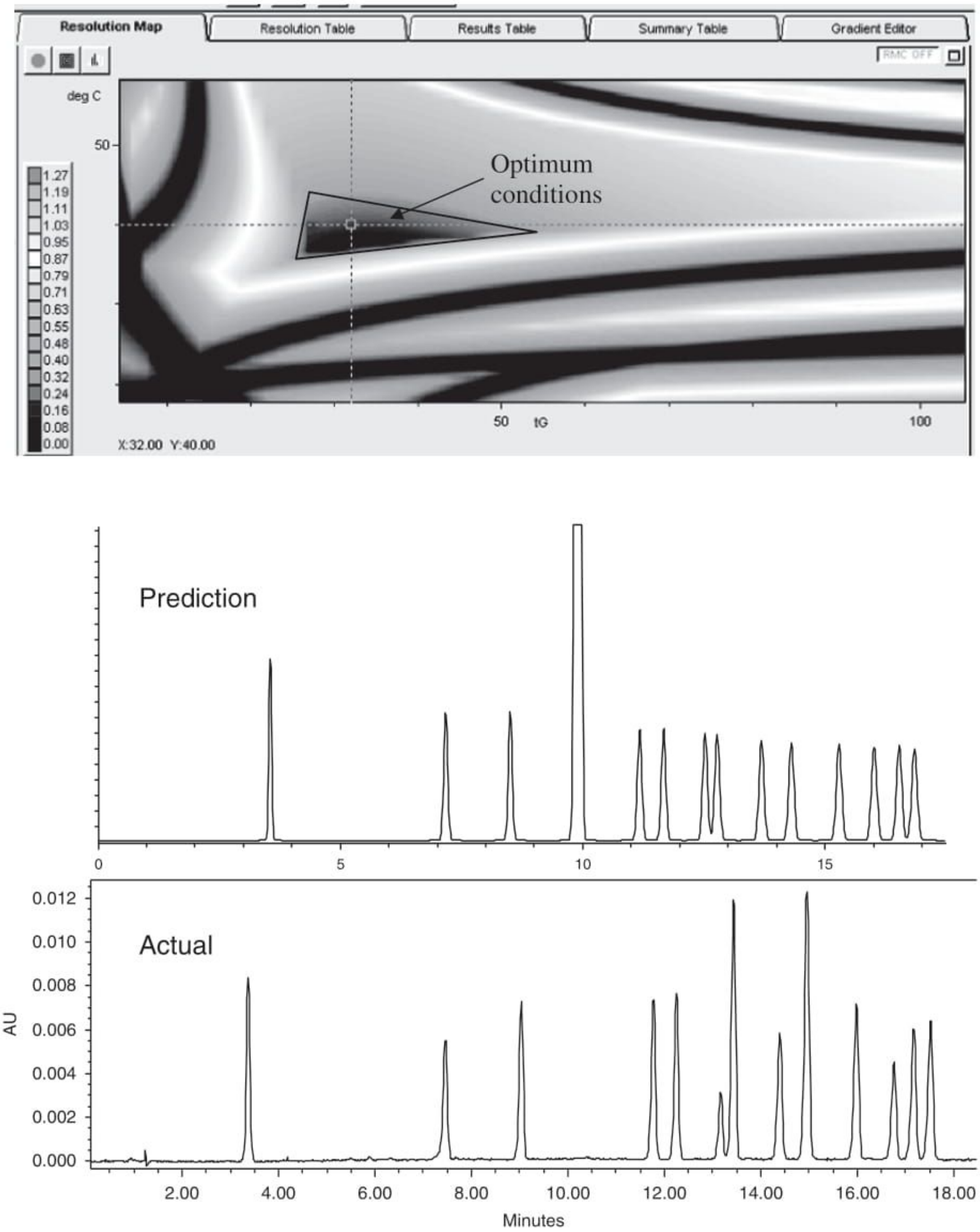

Fig. 7.3 Resolution map as a function of temperature and gradient for a critical pair of impurities in a chromatographic separation. Predicted and actual chromatographic separations of an API and 13 impurities/degradants (API peak absent in bottom chromatogram)

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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

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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

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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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K. Huynh-Ba (ed.), *Handbook of Stability Testing in Pharmaceutical Development*,
DOI 10.1007/978-0-387-85627-8_8, © Springer Science+Business Media, LLC 2009

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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

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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

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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

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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

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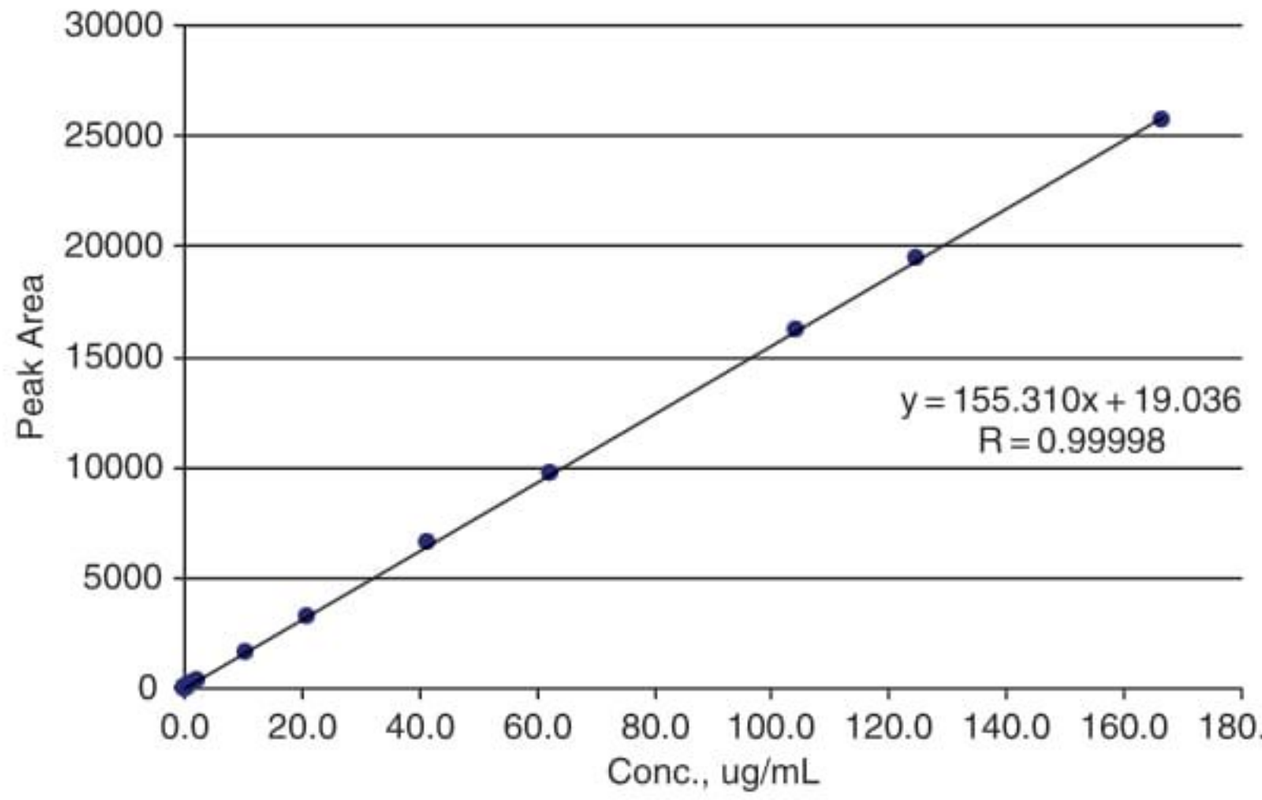

Fig. 8.1 Example of a calibration curve indicating linearity of the analytical method

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.

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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

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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

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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

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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

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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.

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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

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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)

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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

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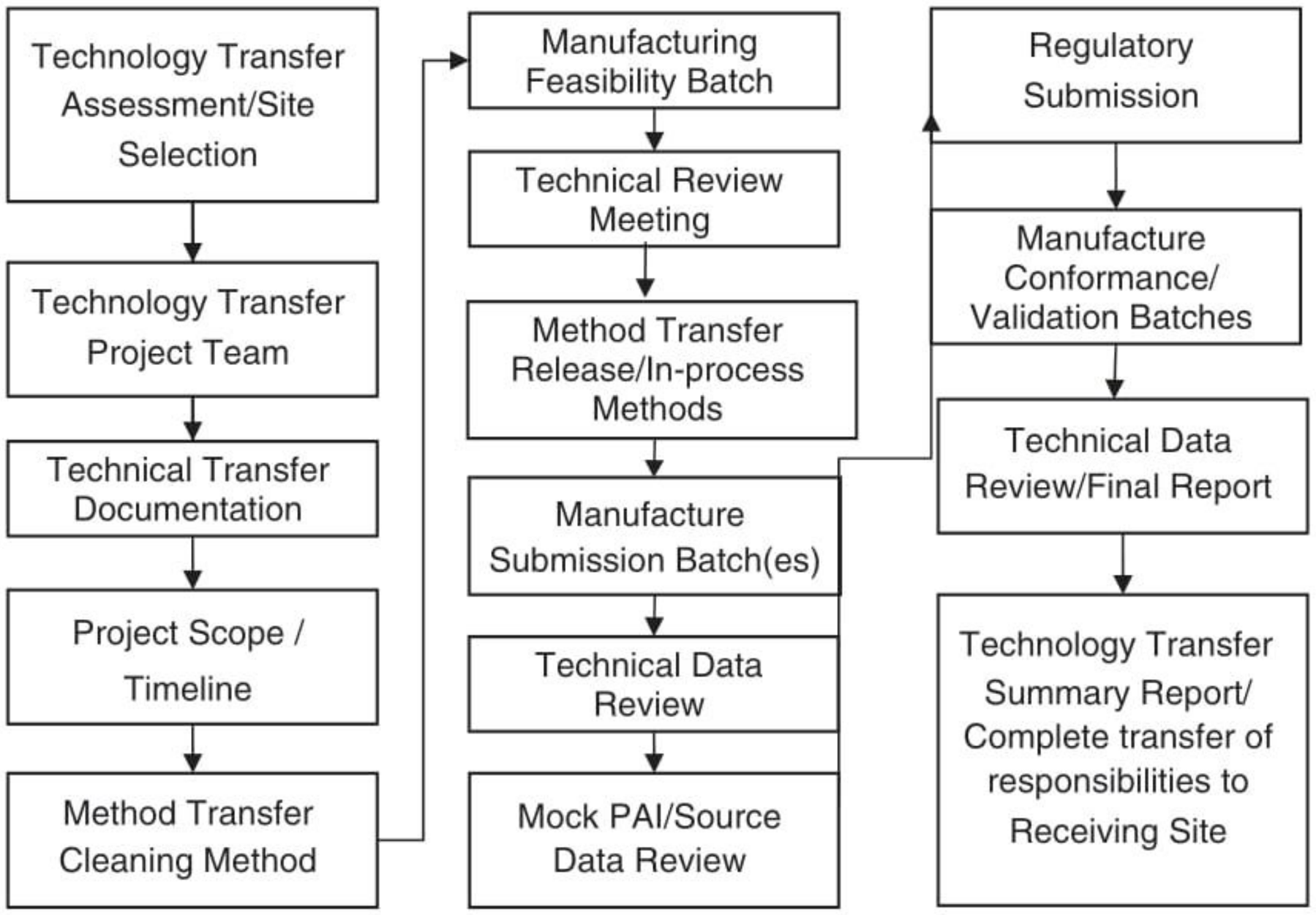

Fig. 8.2 Technology transfer flow chart

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

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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.

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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

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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

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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

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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.

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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

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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

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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

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- 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.

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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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K. Huynh-Ba (ed.), *Handbook of Stability Testing in Pharmaceutical Development*,
DOI 10.1007/978-0-387-85627-8_9, © Springer Science+Business Media, LLC 2009

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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.).

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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

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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

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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.

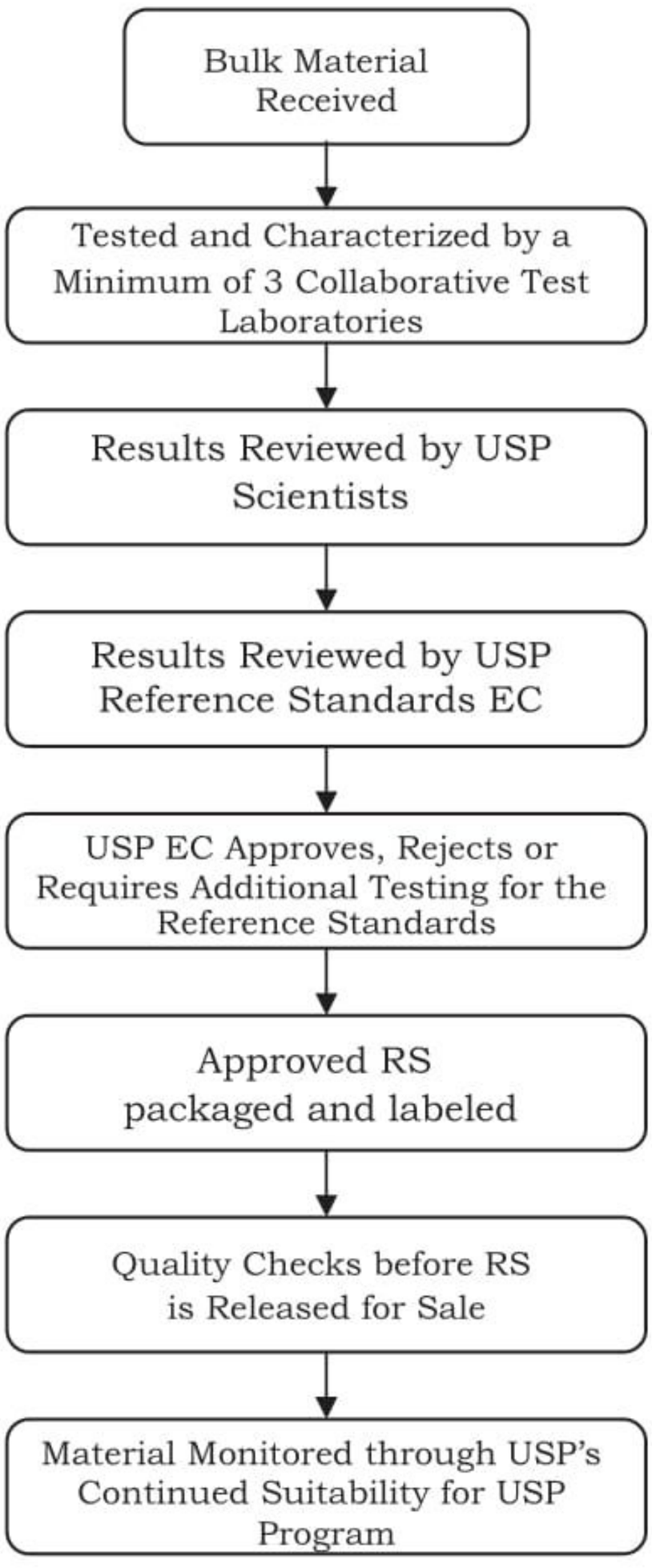

Fig. 9.1 USP reference standard qualification process flow

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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

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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

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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.

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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

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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

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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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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.

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Chapter 10
Non-chromatographic Methods to Support Stability Program

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Contents

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K. Huynh-Ba (ed.), *Handbook of Stability Testing in Pharmaceutical Development*,
DOI 10.1007/978-0-387-85627-8_10, © Springer Science+Business Media, LLC 2009

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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.

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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.

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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

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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

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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

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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.

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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

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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.

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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.

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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

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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.

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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

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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.

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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

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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,

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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>.

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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.

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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 $37.0 \pm 0.5°C$ 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.

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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-

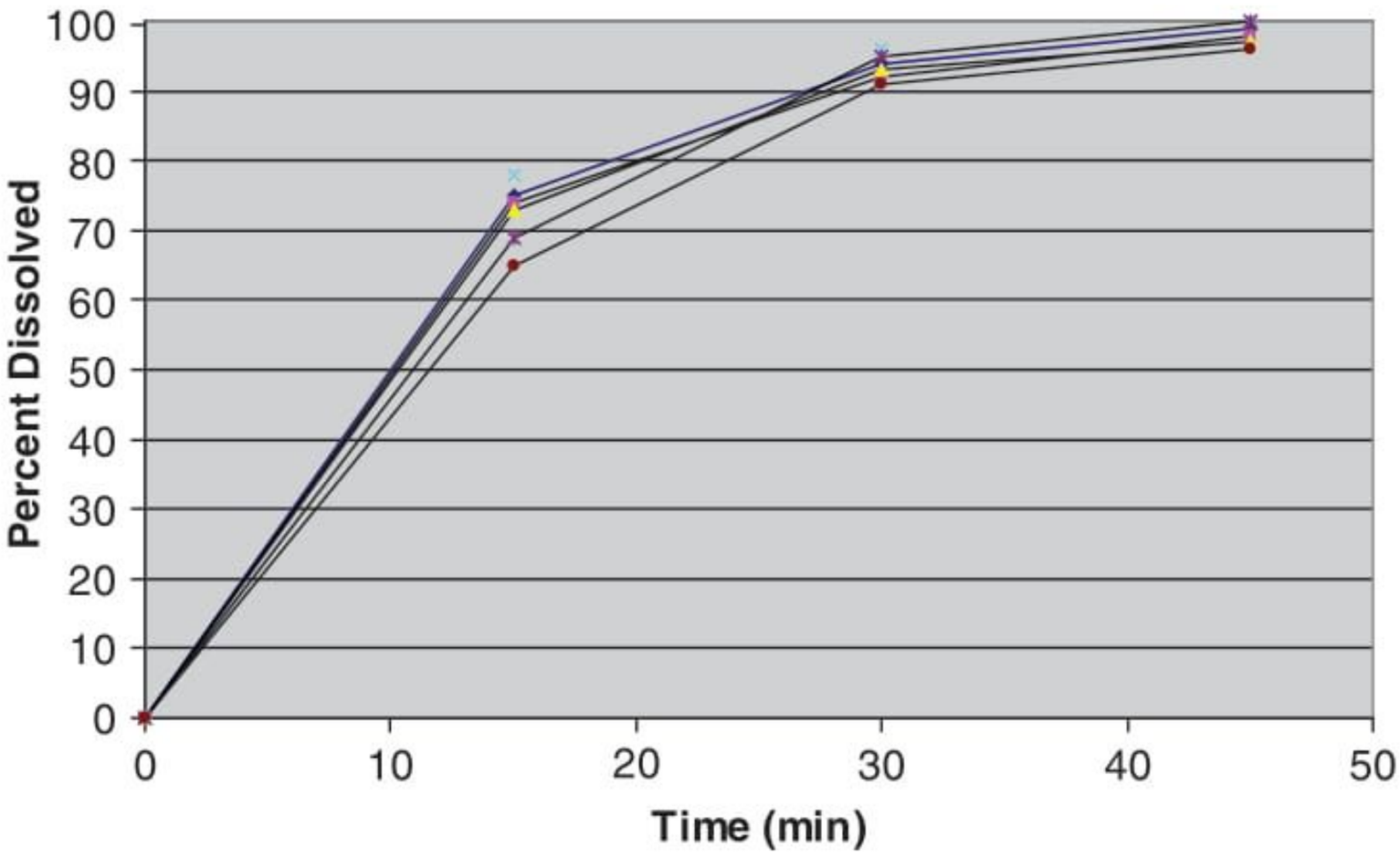

Fig. 10.5 Typical dissolution profile

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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, non-destructive, 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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K. Huynh-Ba (ed.), *Handbook of Stability Testing in Pharmaceutical Development*,
DOI 10.1007/978-0-387-85627-8_11, © Springer Science+Business Media, LLC 2009

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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

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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

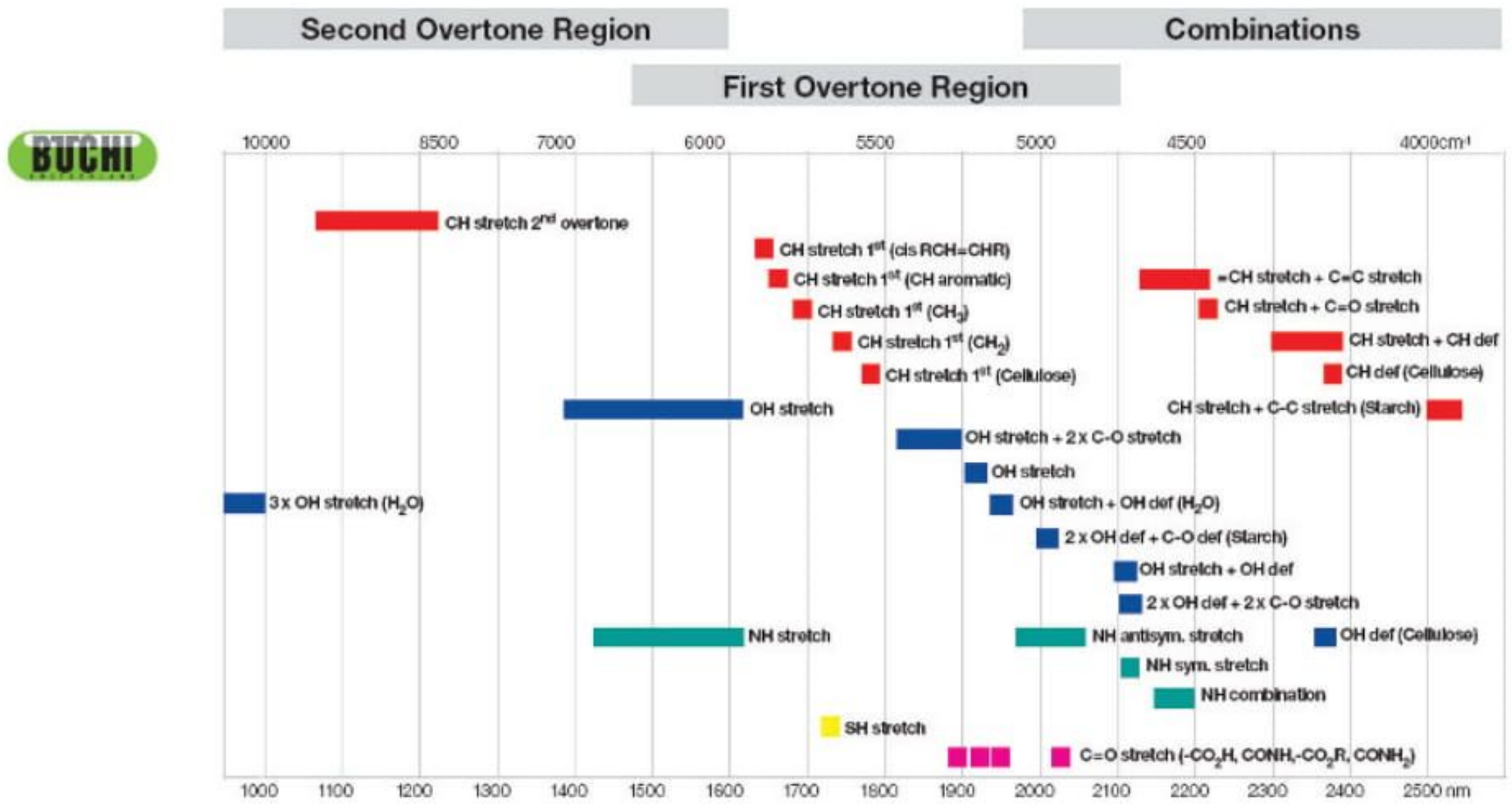

Fig. 11.1 Chart showing the NIR absorbance of different important functional groups, courtesy of Buchi Corporation

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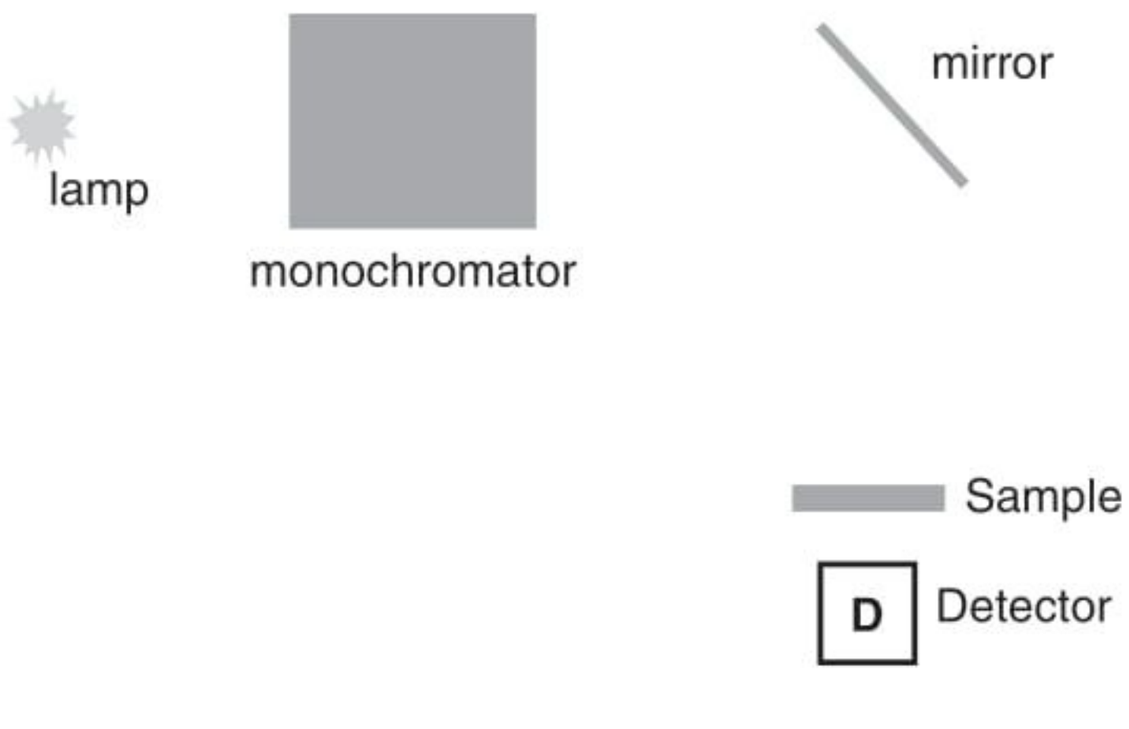

Fig. 11.2 Simple diagram of a NIR spectrometer

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

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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

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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

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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.

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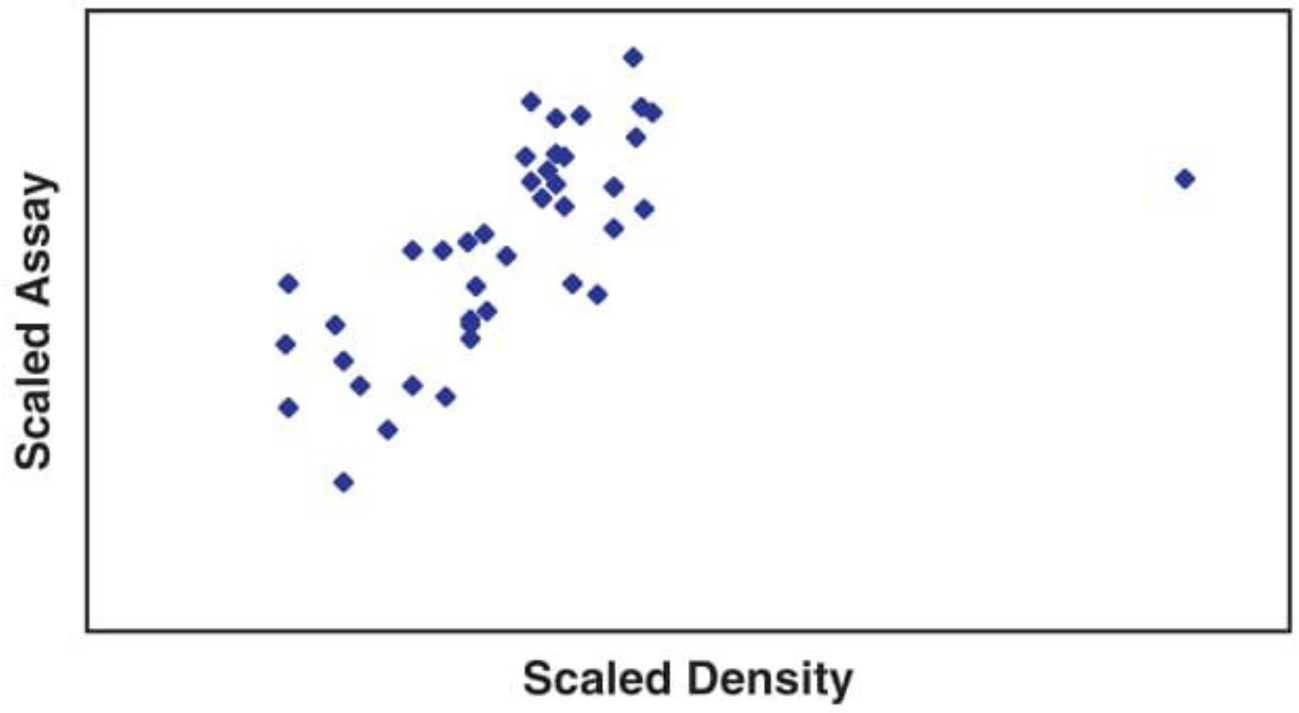

Fig. 11.4 Scaled pharmaceutical quality data. Both the density and assay are scaled by the standard deviation of the data for each variable

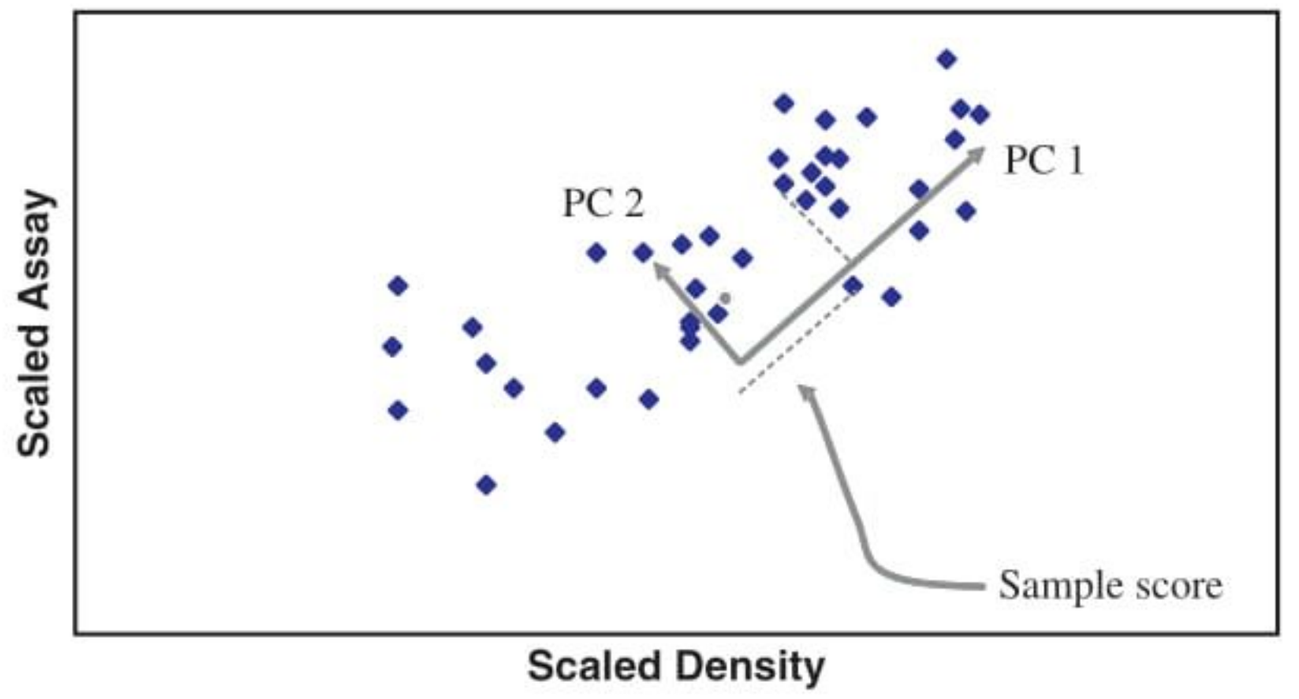

Fig. 11.5 Scaled pharmaceutical quality data showing both the first and second principal components for the data set. The first principal component is the direction of the maximum variation with in the data set. The second principal component is perpendicular to the first PC. The scores for each sample 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

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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

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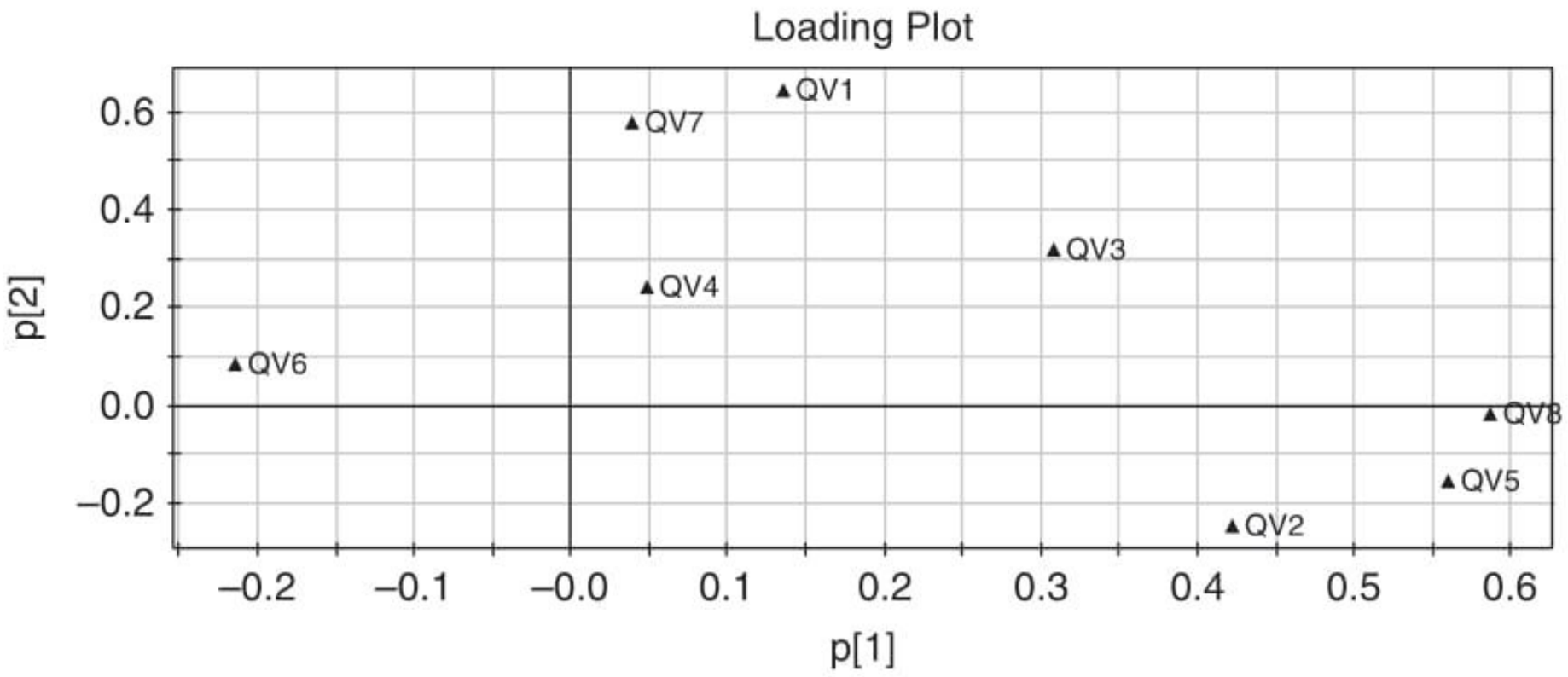

Fig. 11.6 Loading plot for the data set. The first principal component is plotted on the x-axis and the second principal component is on the y-axis. Variables that are close to each other are highly correlated

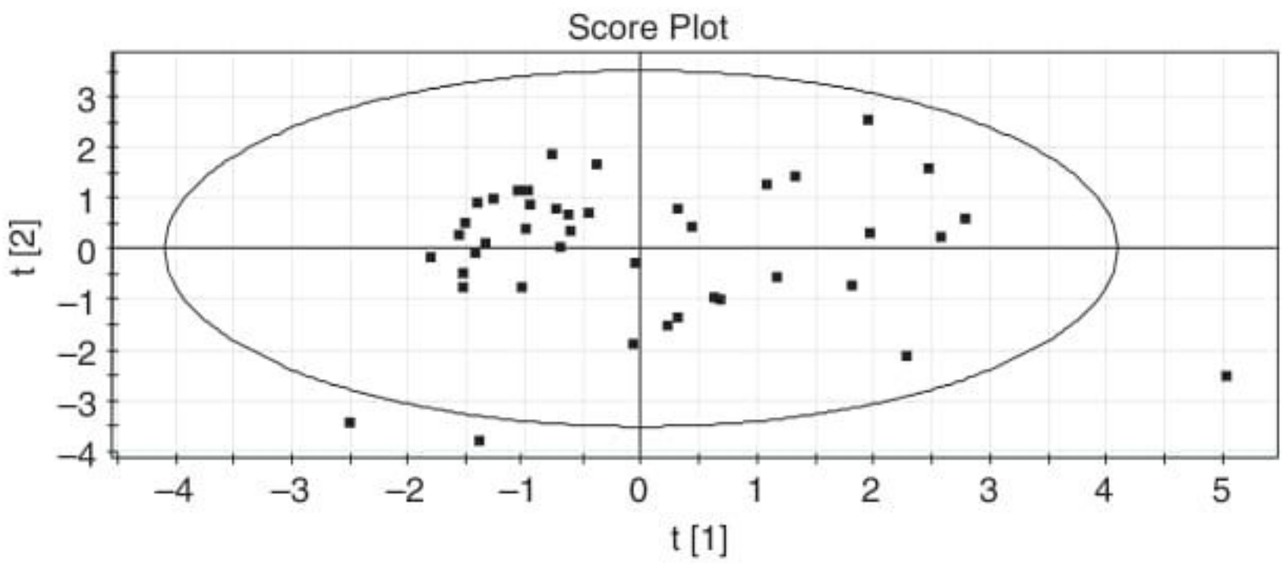

Fig. 11.7 Score plot of the data set

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.

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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].

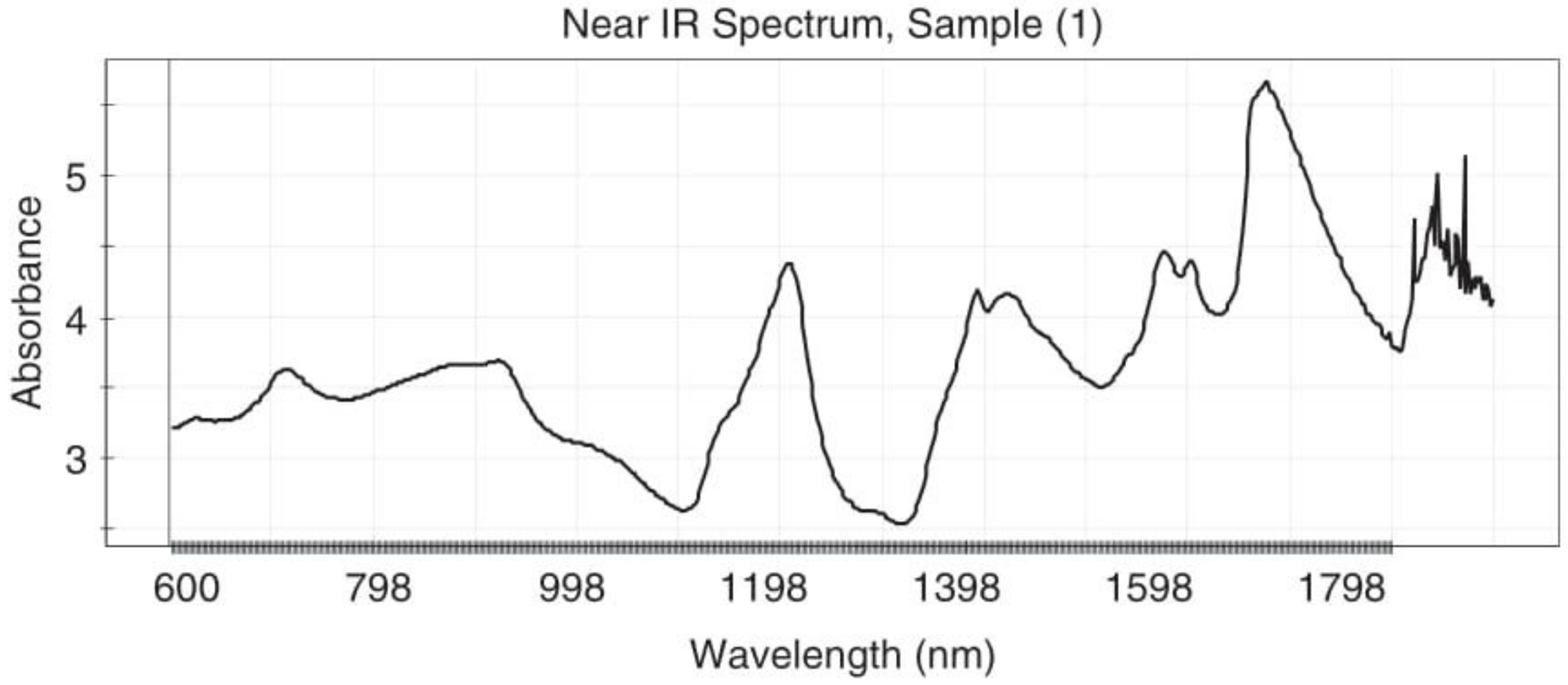

Fig. 11.8 NIR transmission spectrum of a pharmaceutical tablet

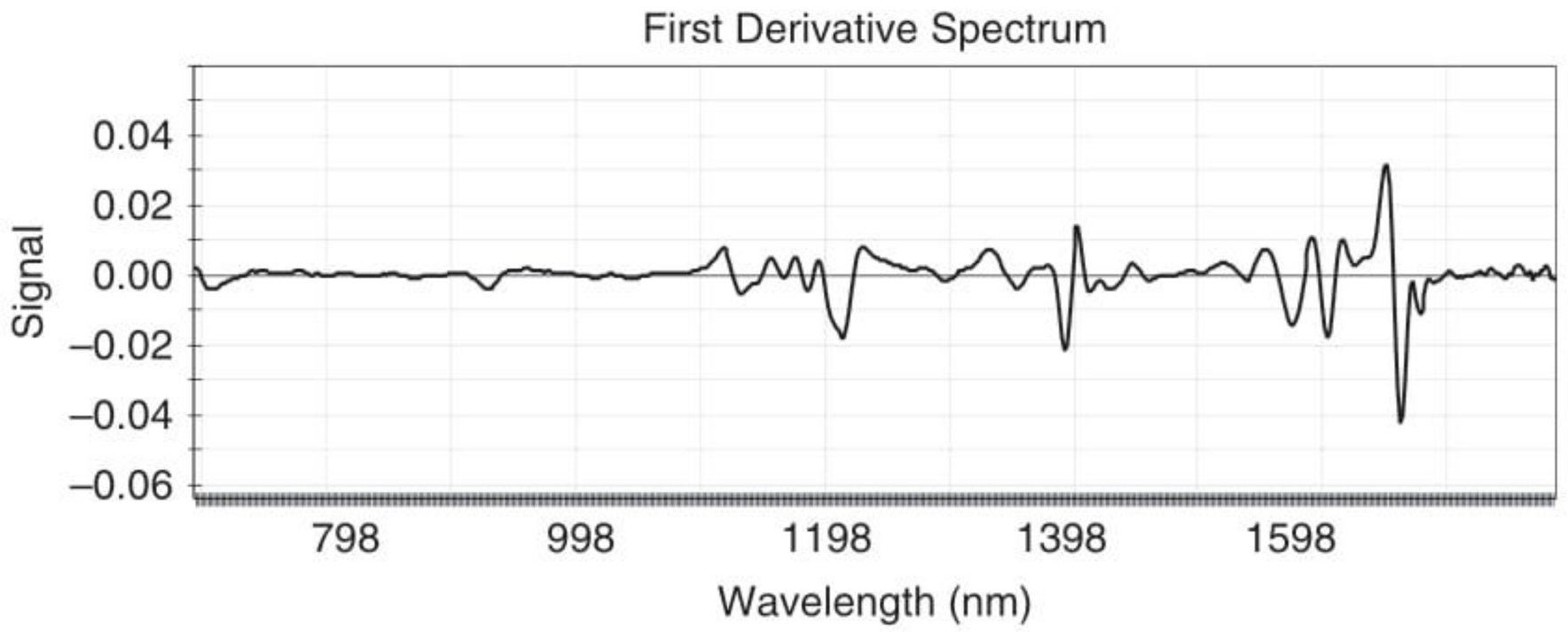

Fig. 11.9 Spectrum from Fig. 11.8 after first-derivative pre-processing

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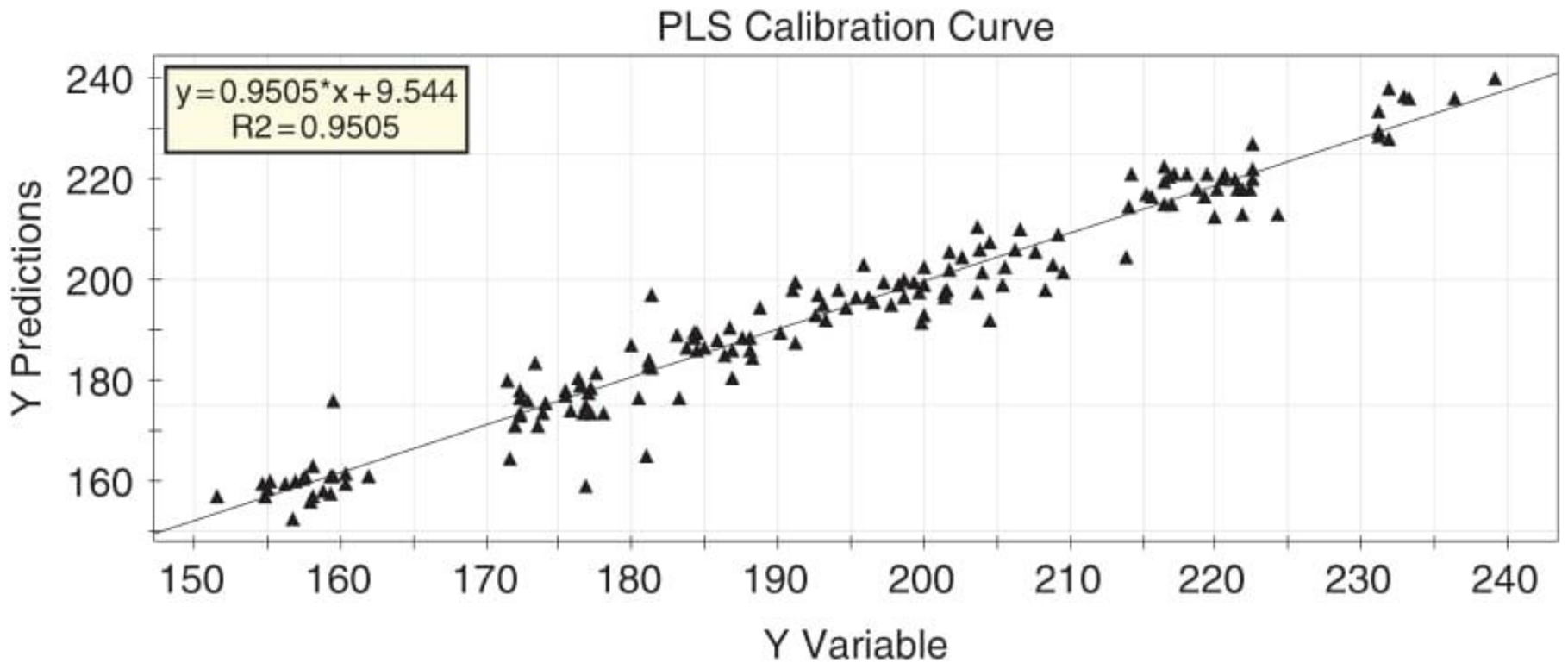

Fig. 11.10 Calibration curve for PLS method for tablet assay value

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.

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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.

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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),

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$$\text{SEP} = \sqrt{\sum \frac{(\text{NIR} - \text{LAB})^2}{n}} \tag{11.2}$$

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

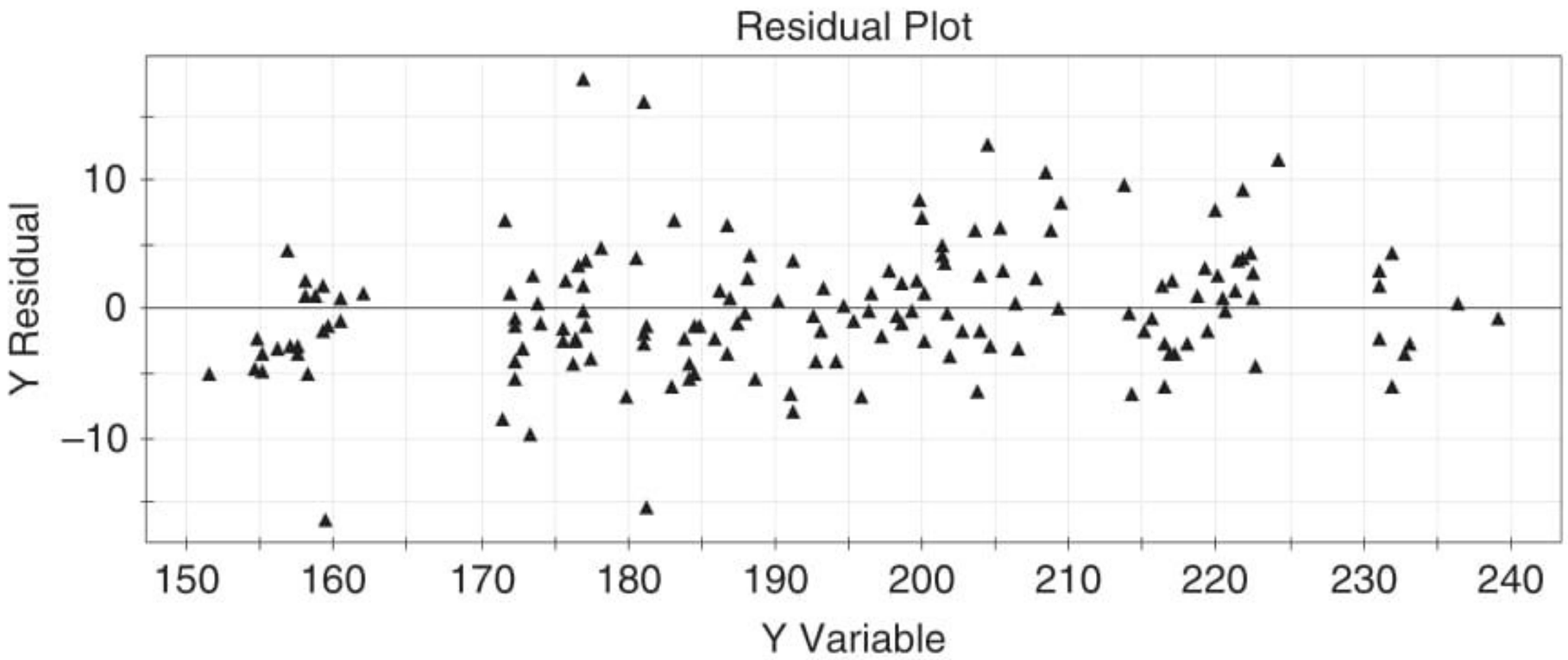

Fig. 11.11 Plot of y residuals versus y values

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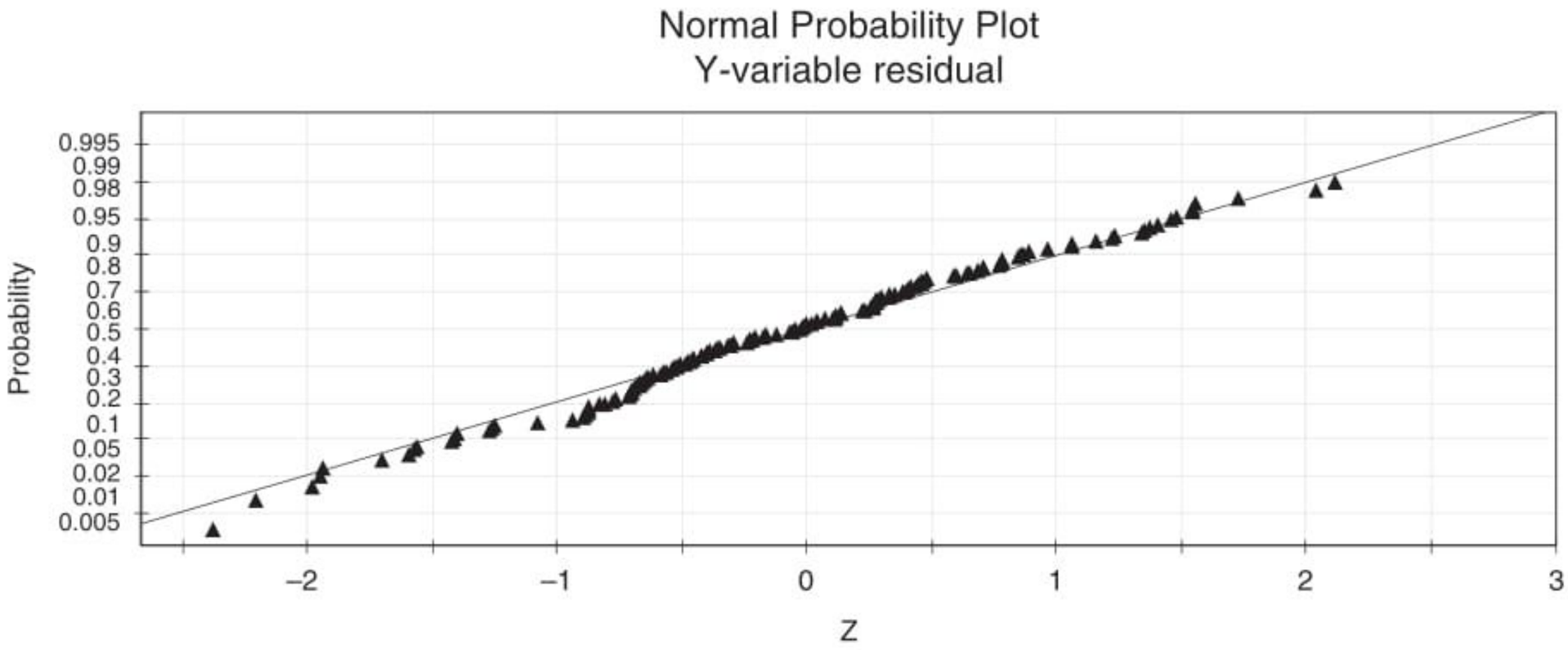

Fig. 11.12 Normal probability plot for residuals. When the residuals fall on straight line, the calibration under consideration is linear

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.

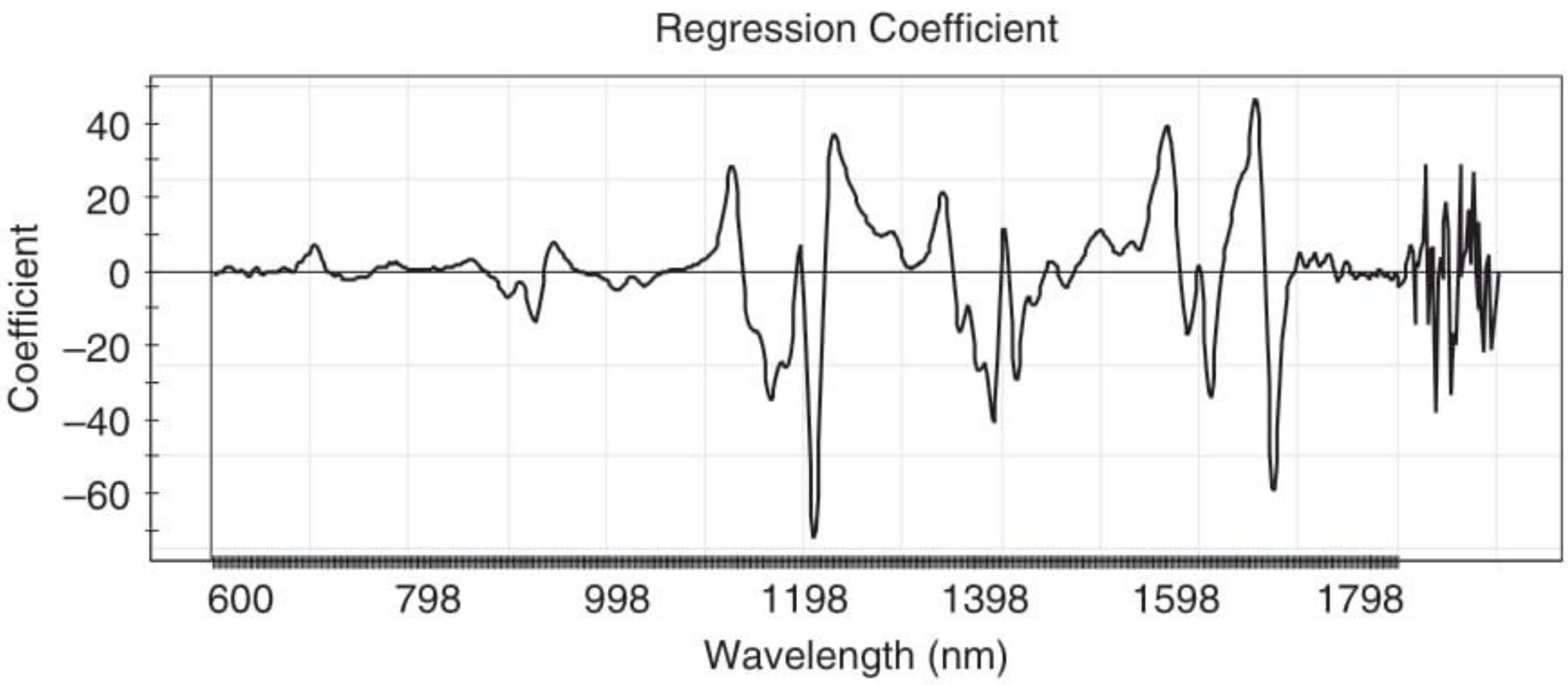

Fig. 11.13 Regression coefficient from the PLS model for tablet assay described earlier. The regression coefficient is a method for documenting and examination of which wavelengths are most important for the PLS calibration

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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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K. Huynh-Ba (ed.), *Handbook of Stability Testing in Pharmaceutical Development*,
DOI 10.1007/978-0-387-85627-8_12, © Springer Science+Business Media, LLC 2009

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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

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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.

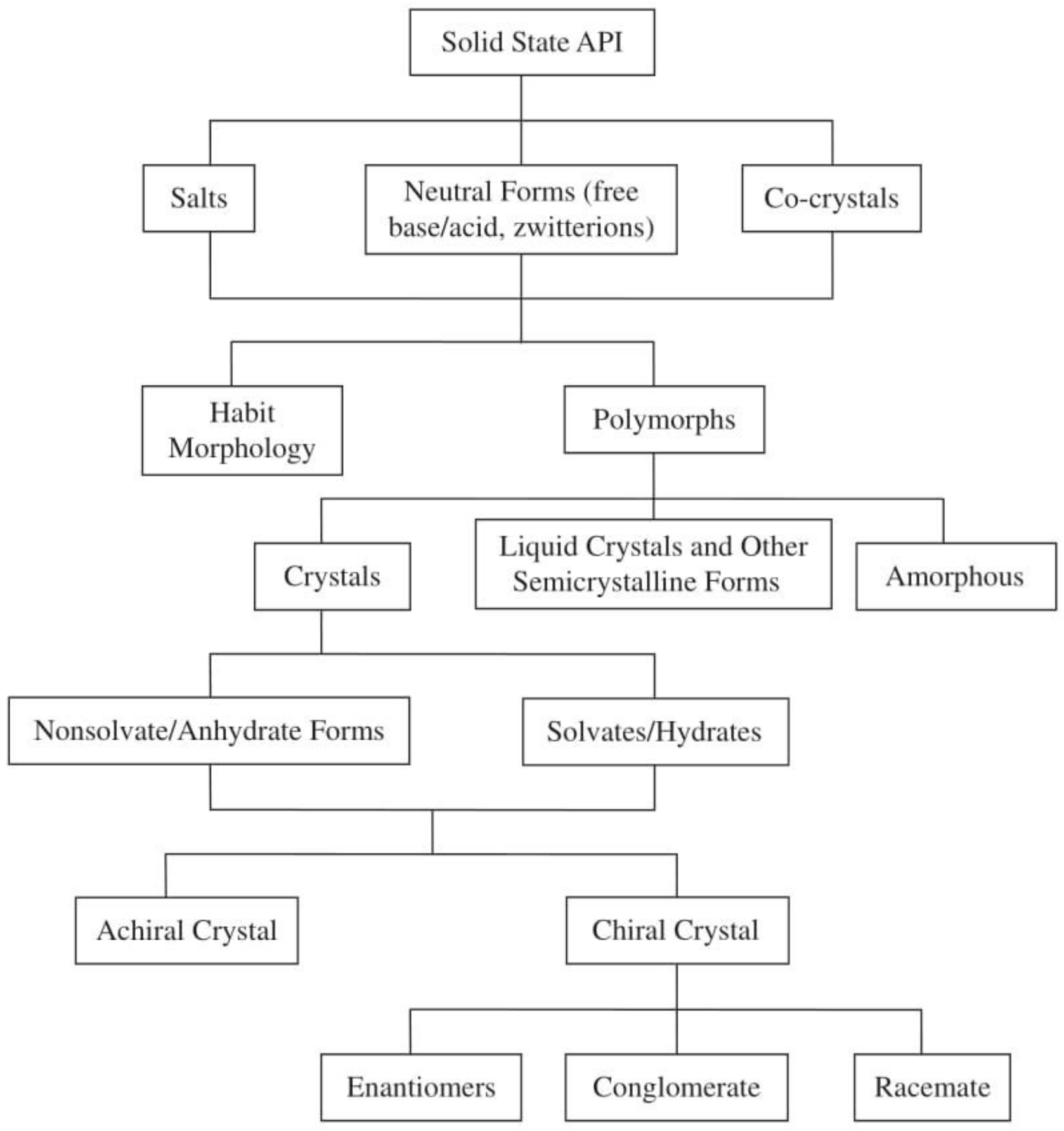

Fig. 12.1 Chemical and solid-state forms of active pharmaceutical ingredients

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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,

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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

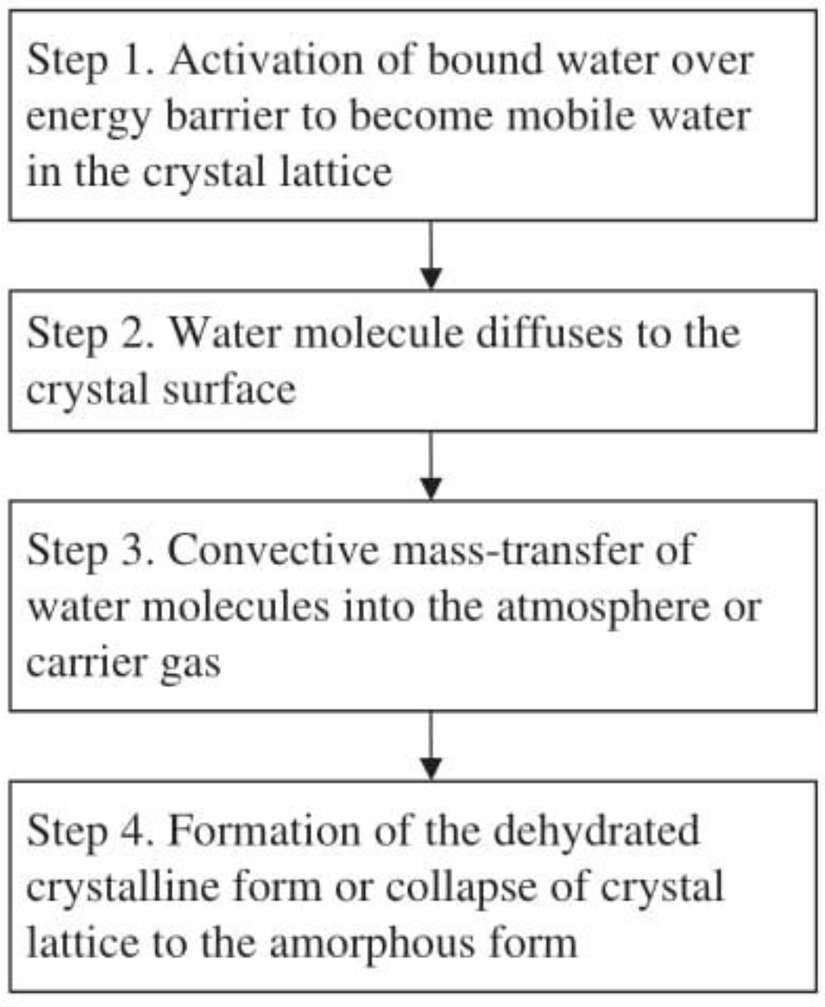

Fig. 12.2 Mechanism of the thermal dehydration process

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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

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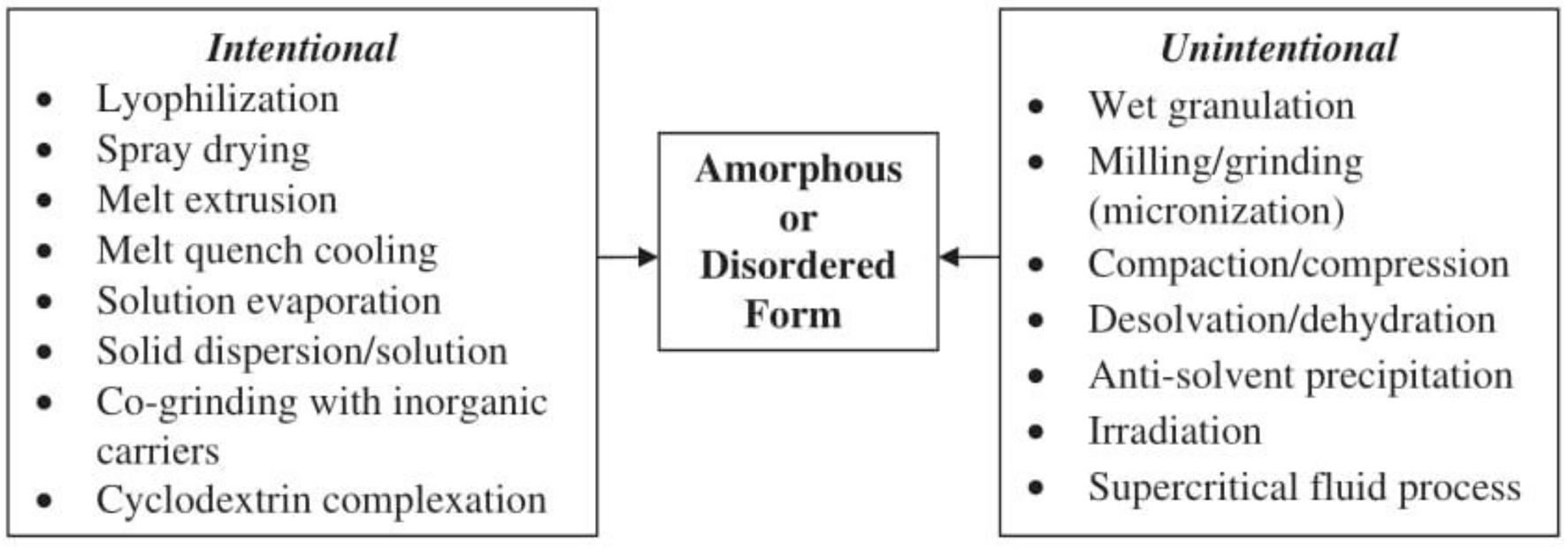

Fig. 12.3 Formation of amorphous or disordered forms during drug manufacturing processes

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

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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].

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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

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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)

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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

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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.

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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

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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

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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

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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)

$$v = \frac{2r^2(\rho_s - \rho_l)g}{9\eta} \tag{12.2}$$

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where

ν = 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,

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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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K. Huynh-Ba (ed.), *Handbook of Stability Testing in Pharmaceutical Development*,
DOI 10.1007/978-0-387-85627-8_13, © Springer Science+Business Media, LLC 2009

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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.

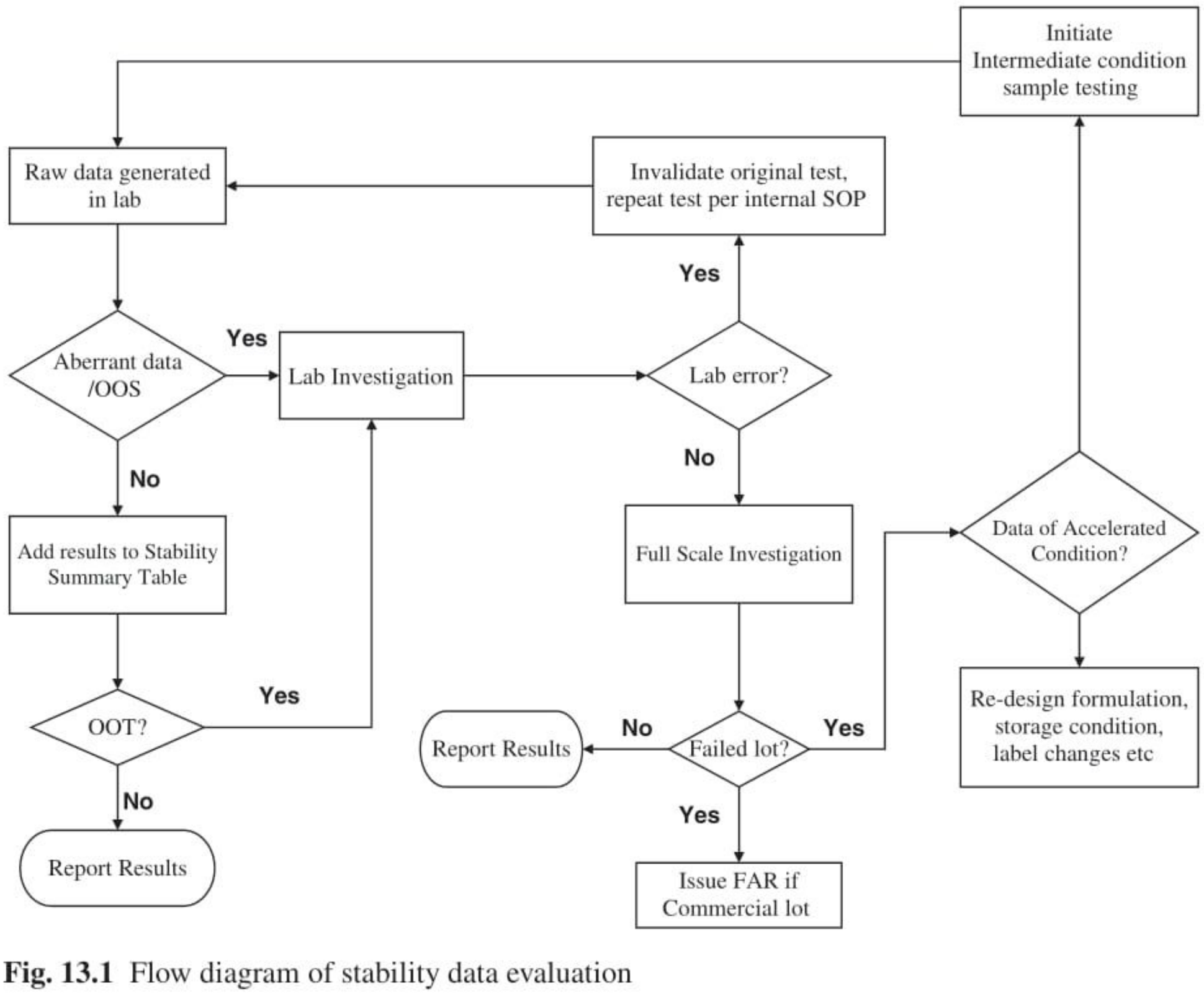

Fig. 13.1 Flow diagram of stability data evaluation

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

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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

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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.

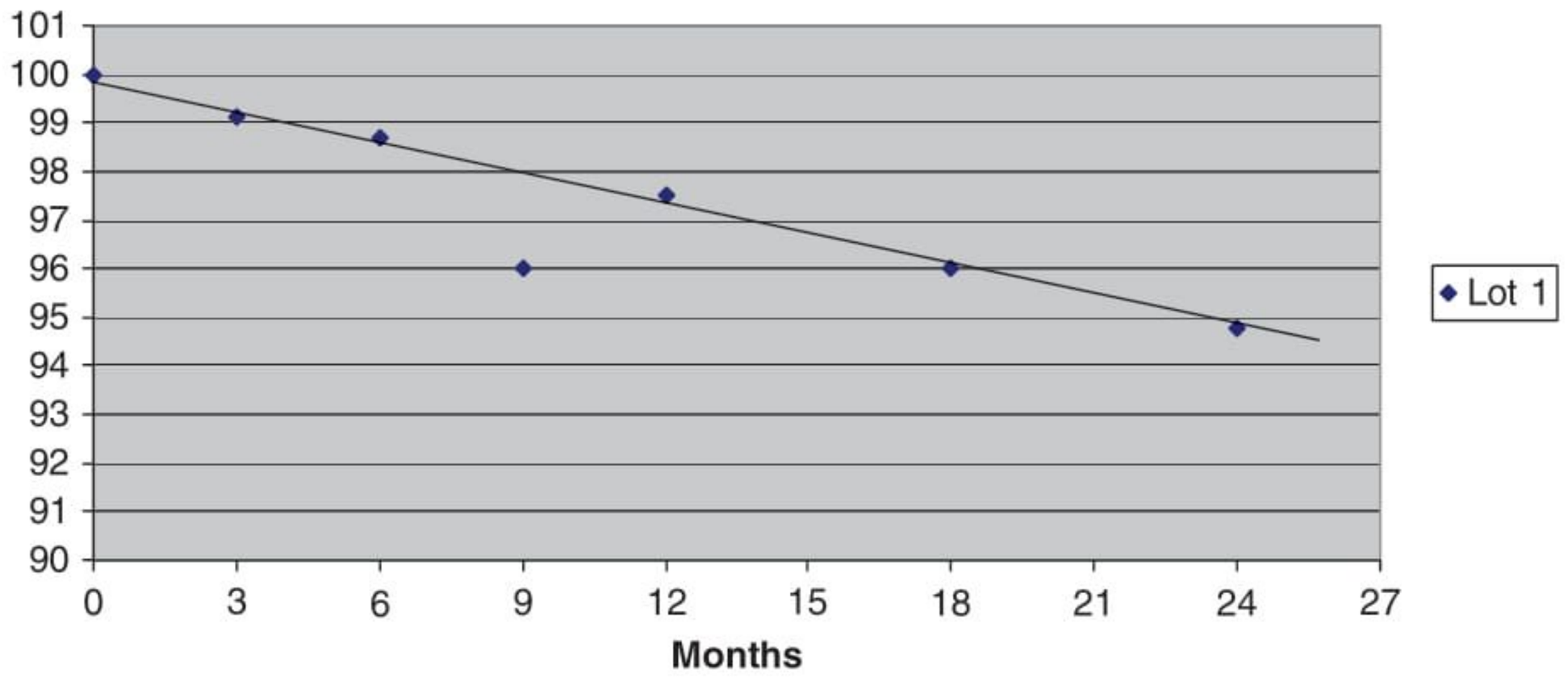

Fig. 13.2 Stability results for a single lot over time. The 9 months result can be considered as OOT

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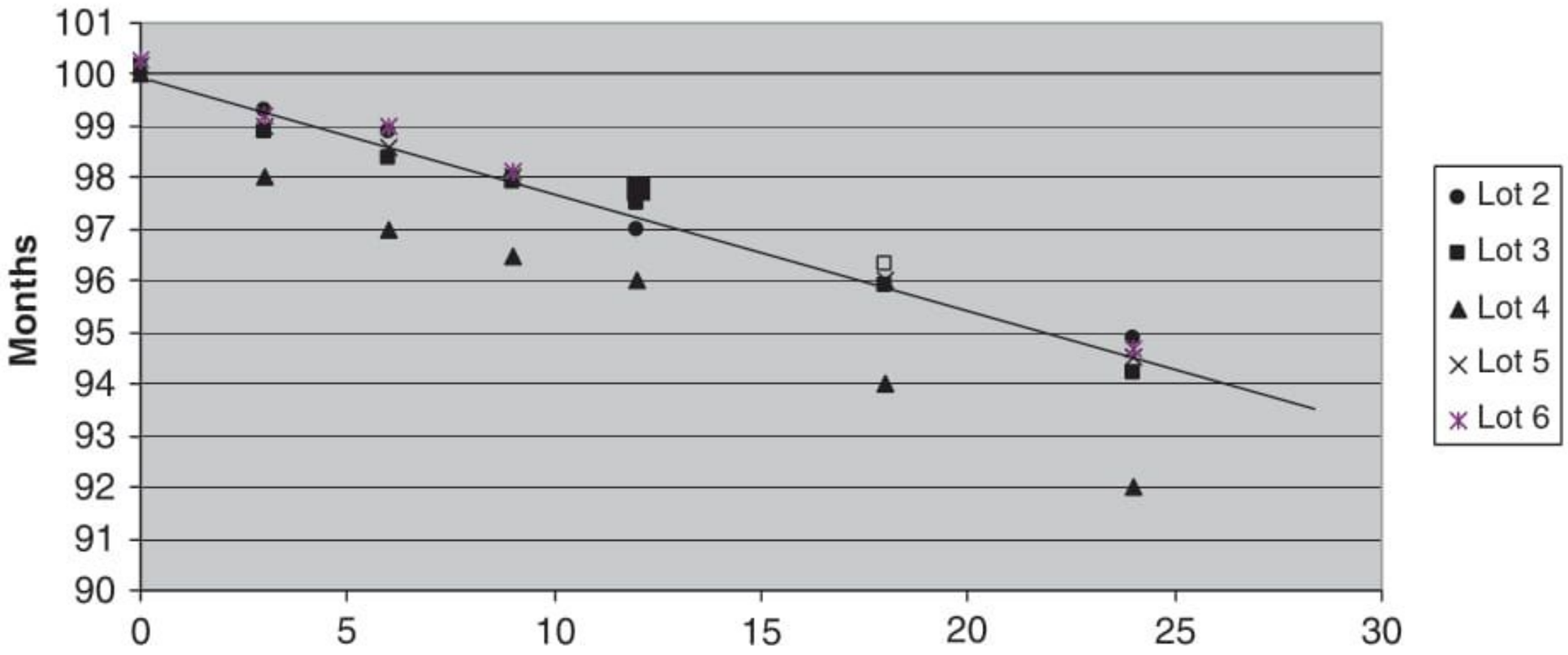

Fig. 13.3 Stability results for 5 lots over time. Here, the rate of change of lot 4 is being compared to that of the other lots and appears to be OOT

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.

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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

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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

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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.

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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

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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

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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

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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.

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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.

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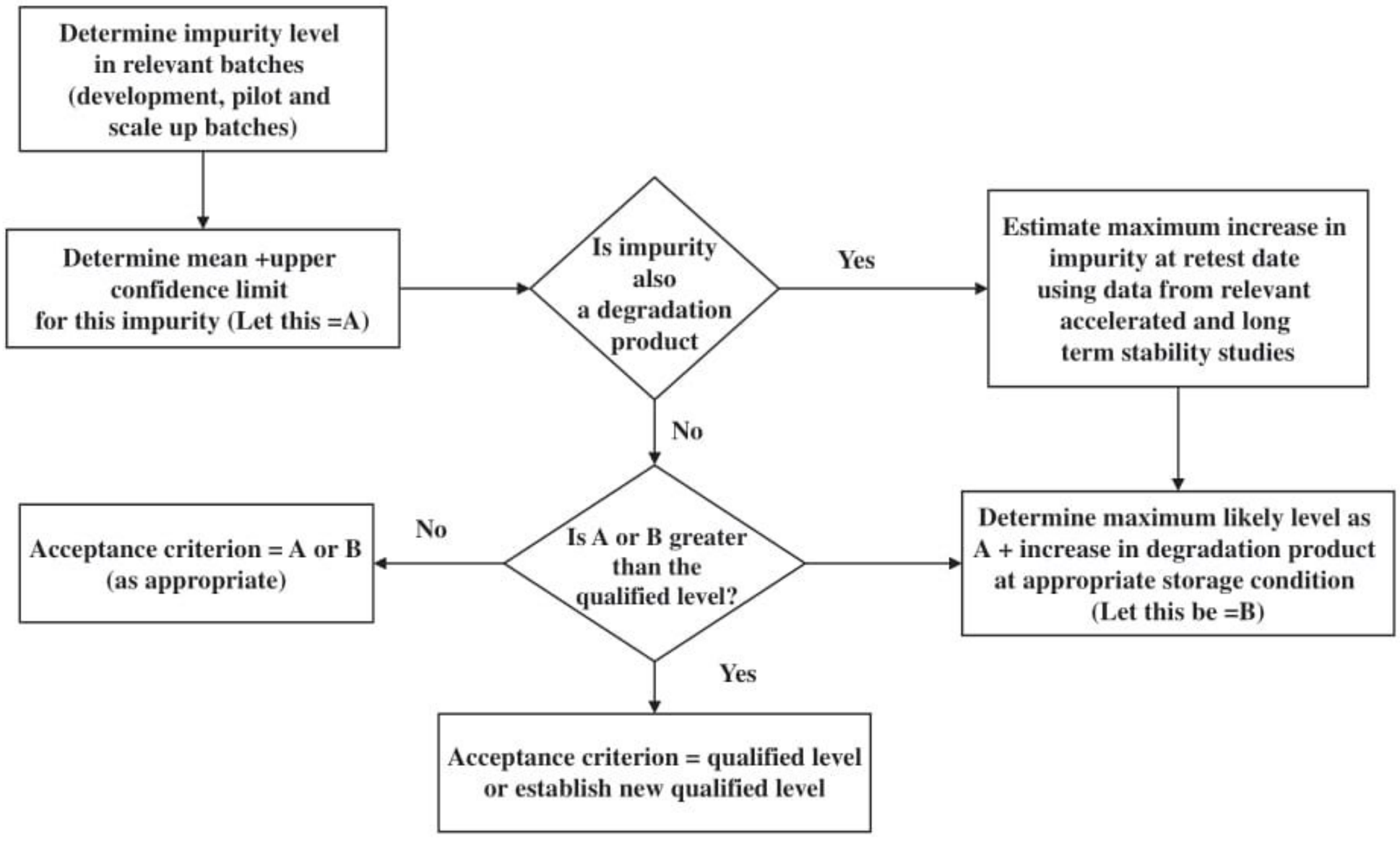

Fig. 13.6 Establishing acceptance criterion for a specified impurity in a new drug substance

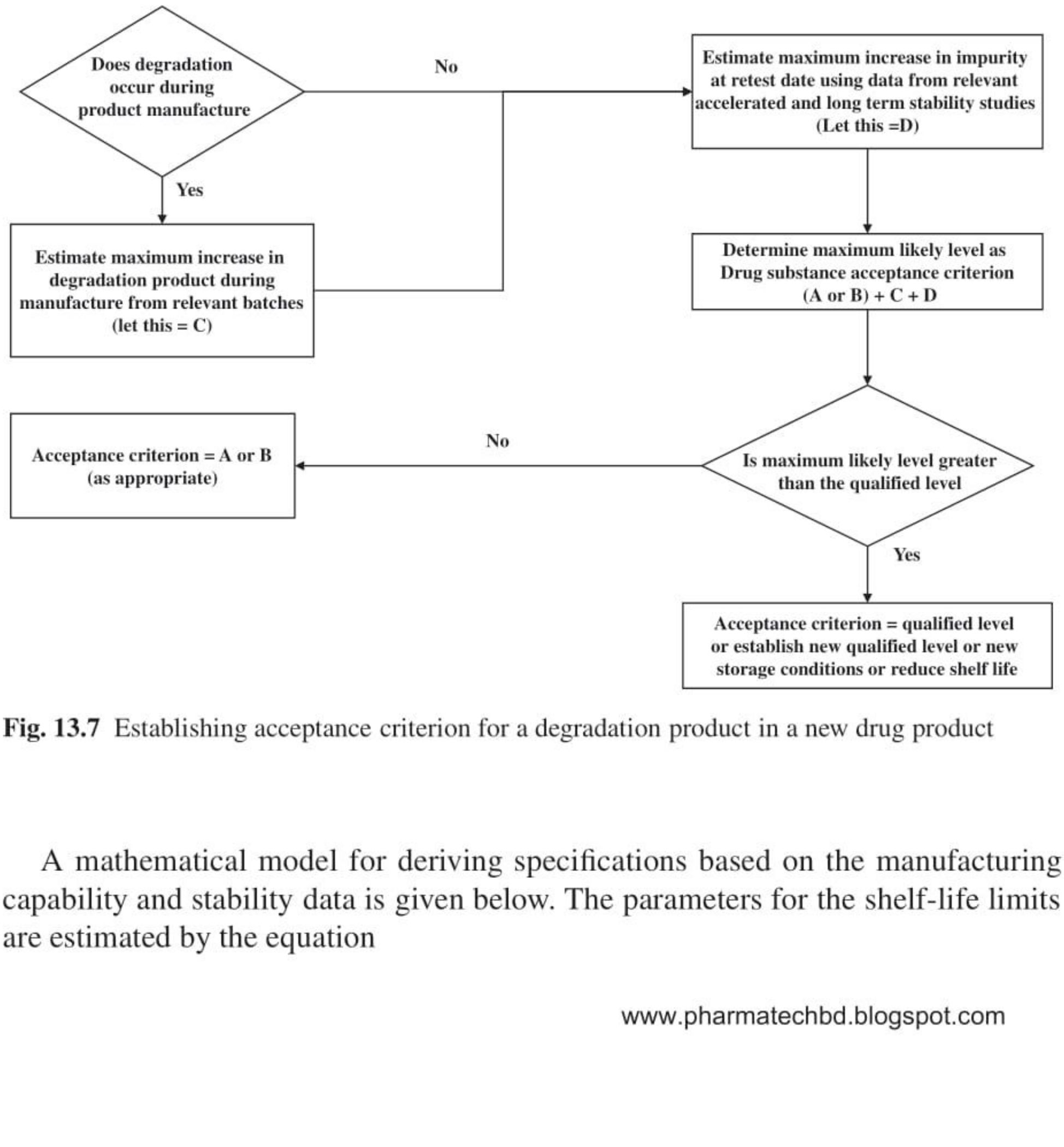

Fig. 13.7 Establishing acceptance criterion for a degradation product in a new drug product

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

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$$\mathrm{LRL} = \mathrm{LR} - \mathrm{EAC} + \ t0.95; \mathrm{DF}\sqrt{S_{\mathrm{T}}^2 + \frac{S_{\mathrm{TOT}}^2}{n}} \tag{13.4}$$

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

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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

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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.

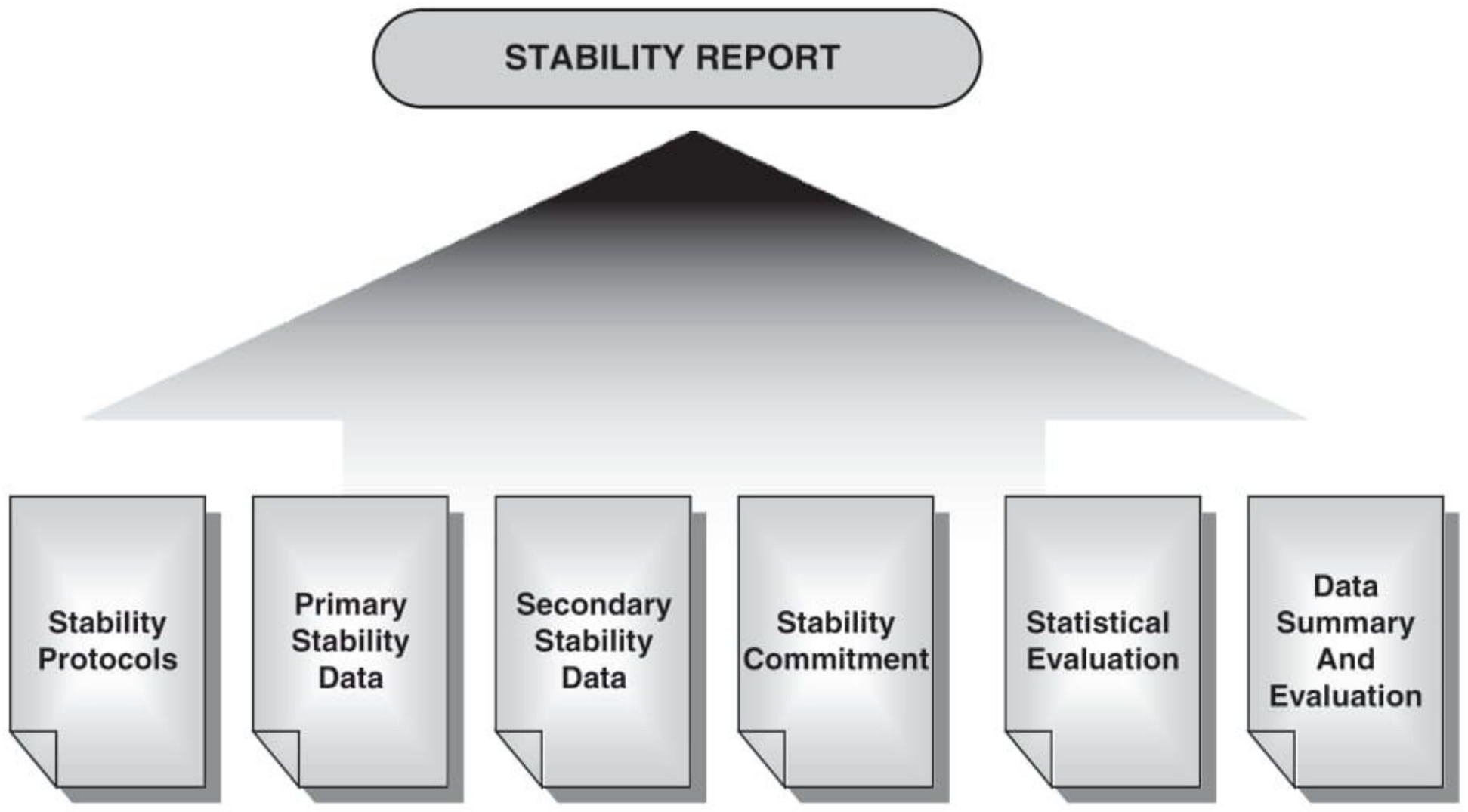

Fig. 13.9 Content of a stability report

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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.

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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:

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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.

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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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K. Huynh-Ba (ed.), *Handbook of Stability Testing in Pharmaceutical Development*,
DOI 10.1007/978-0-387-85627-8_14, © Springer Science+Business Media, LLC 2009

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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.

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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.)*

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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.

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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

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- 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

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- 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.

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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.

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- 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).

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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.

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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

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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).

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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)*

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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 ±2°C and ±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-

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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.

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- 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.

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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

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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.

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Chapter 15
Stability Operation Practices

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Contents

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K. Huynh-Ba (ed.), *Handbook of Stability Testing in Pharmaceutical Development*,
DOI 10.1007/978-0-387-85627-8_15, © Springer Science+Business Media, LLC 2009

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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

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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.

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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

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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.

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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

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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.

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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

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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.

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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

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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

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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.

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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

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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-

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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

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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

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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.

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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.

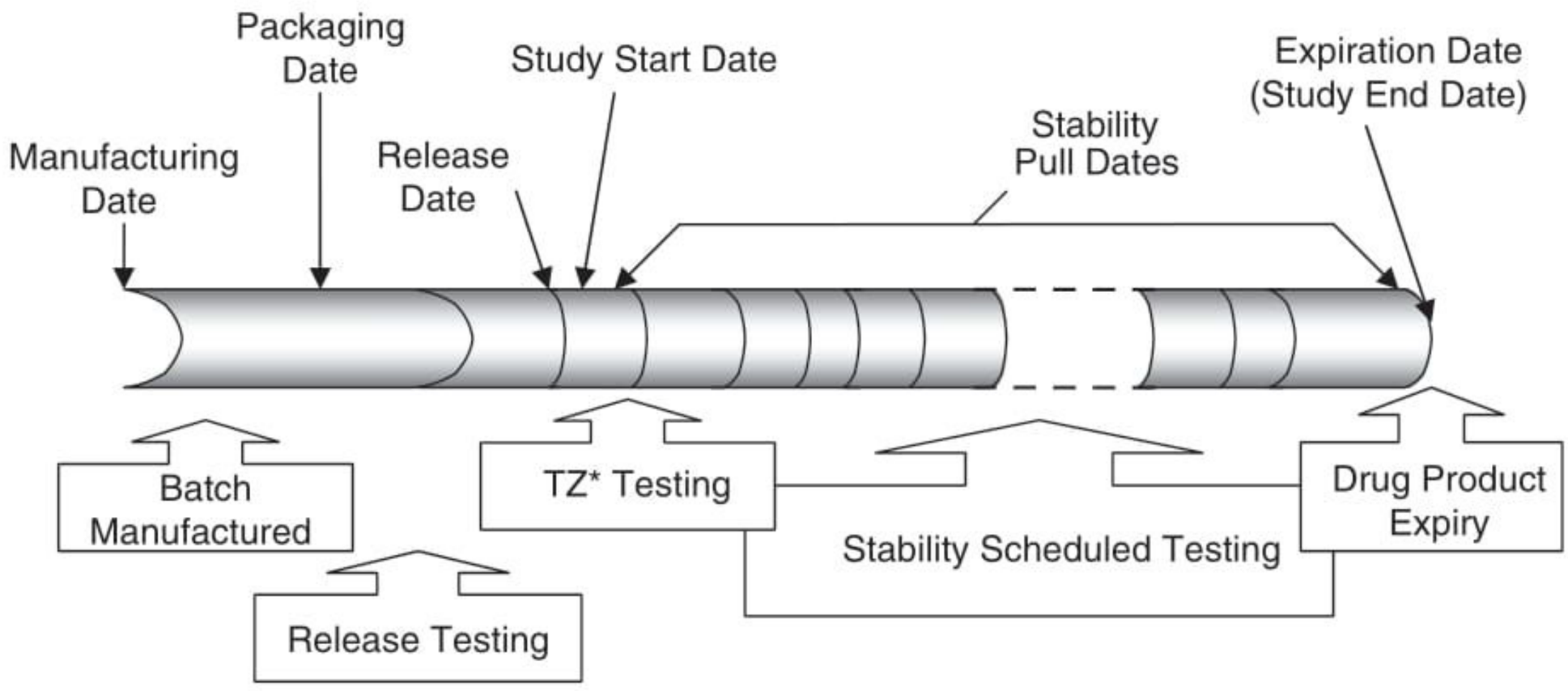

Fig. 15.6 Chronological order of stability activities to support a drug product study

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