Part III
Other Stability Programs

Chapter 16
Combination Products/Drugs in Devices

Jon V. Beaman and Roisin Wallace

Contents

J. V. Beaman (✉)
Pfizer Global Research and Development, Ramsgate Road, Sandwich, Kent, CT13 9NT, UK
e-mail: Jon.V.Beaman@Pfizer.com

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Abstract The purpose of this chapter is to cover additional considerations, guidelines and requirements to help the reader design stability strategies for drug-device combination products. Tests and challenges to be included in stability studies are considered from a regulatory and scientific point of view and these are also related to the stage of development of the product. A number of drug-device combination types including inhaled/nasal products, pen injectors, drug-eluting stents and transdermal products are discussed specifically.

16.1 Introduction

The classification *Combination Product* encompasses a wide variety of different product types. In the United States, the Code of Federal Regulations, 21 CFR 3.2(e), defines what should be considered as a combination product. Essentially any combination of a drug and a device, a biological product and a device, a drug and a biological product, or all three together are considered as combination products. Products as diverse as a monoclonal antibody combined with a therapeutic drug, a condom with spermicide, an inhaler system, and a pre-filled syringe cartridge for use with an auto-injector are all considered to be combination products.

Combination products are a growing area in the field of pharmaceutical development. The purpose of this chapter is to complement the other sections in this book and to cover additional considerations only, including guidelines and requirements that should be taken into account when designing stability studies for drug-device combination products. A number of specific combination types including inhaled/nasal products, pen injectors, drug-eluting stents, and transdermal products are also discussed specifically. Where stability requirements and strategies are the same as for products that are not classified as combination products, readers should refer to other chapters in this book as appropriate.

Medical devices will not be covered in this chapter; the technical requirements of these products are laid out within the European Commission directives, in particular Directive 93/42/EEC and amendments, relevant Food and Drug Administration (FDA) Center for Devices and Radiological Health (CDRH) guidance and other regional/local guidance as appropriate, and will not be discussed here unless they apply to combination products [1–4]. Additional information on medical devices, particularly with respect to efforts to harmonize requirements globally, can be found on the Global Harmonization Task Force (GHTF) website [5].

16.2 Available Guidance and Regulatory Framework

The medical device and pharmaceutical industries have traditionally been separate businesses. The medical device industry generally develops products in line with the EC Medical Device Directive and the guidances issued by the CDRH office of the FDA, whilst the pharmaceutical industry looks to the relevant regulatory guidance available, including that from the FDA Center for Drug Evaluation and Research

(CDER), the FDA Center for Biologics Evaluation and Research (CBER), the European Agency for the Evaluation of Medicines (EMEA) Committee for Medicinal Products for Human Use (CHMP), and the International Conference on Harmonisation (ICH) when developing medicinal products [6–9]. In the emerging field of combination products, there is little specific guidance (akin to ICH) for companies to refer to and there is currently no overarching harmonized framework for developing drug-device combination products for the global market. As a result, the strategies employed during the pharmaceutical development of drug-device combination products are developed on a case-by-case basis. However, understanding the regulatory environment and the available guidance that does exist are important when developing stability strategies for new products.

In the US the FDA Office of Combination Products (OCP), created in 2002, has broad responsibilities covering the regulatory life cycle of combination products [10]. A key role of the OCP is to assign an FDA Center to have primary jurisdiction for regulatory review of a combination product. Early in the development of a combination product, it is wise to submit a Request for Designation (RFD) to the OCP in order to engage with the appropriate FDA center as outcomes from these discussions may have a significant effect on the development strategy. The OCP determine the Primary Mode of Action (PMOA) of the combination product and use this to assign the lead review center. In cases where the PMOA is not obvious or there is insufficient information to assign the PMOA (which may be the case during early development), the OCP will look in the first instance to assign the new product in line with other similar or previously approved products; or failing this to the center with the most expertise in the safety and efficacy of that type of product.

It is also the responsibility of the OCP to work with FDA Centers (CDER, CBER, and/or CDRH as appropriate) to develop guidance or regulations to support the agency regulation of combination products. However, as stated in the FDA guideline Early Development Considerations for Innovative Combination Products "... few guidance documents currently address the scientific and technical issues to consider when combining drug, device and/or biological product constituent parts as a combination product" [11]. The FDA guidance also states "because of the breadth, innovation and complexity of combination products, there is no single developmental paradigm appropriate for all combination products". This could lead to different standards and strategies being applied to individual products even after consultation with regulatory agencies. In summary, the innovator should work with the OCP at an early stage to determine the primary FDA review center to ensure all chemistry, manufacturing and controls (CMC) development aspects of the product are aligned with both the relevant guidance and requirements of that center as well as ensuring that the appropriate submission mechanism (e.g., CTD, 510(k), Device Master File etc.) is followed.

In the European regions covered by the European Medicines Evaluation Agency (EMEA), the key directives for combination products are 93/42/EEC for Medical Devices and 65/65/EEC for Medicinal Products and associated amendments [1, 12]. As with the FDA, it is important to understand early in the development pro-

gram whether the drug/device combination product will be regulated as a device, a medical product, or both. To determine this, the intended purpose of the product (taking into account the way the product is presented) and the method by which the principal intended action is achieved need to be considered. The latter criterion, based on the *principal intended action* is critical. The principal intended action of a product may be deduced from the manufacturer's labeling and claims, but more importantly, from scientific data regarding its mechanism of action. Typically the medical device function is fulfilled by physical means (including mechanical action, physical barrier, replacement of (or support to) organs or body functions). The action of a medicinal product is typically achieved by pharmacological, immunological, or metabolic means.

Medical devices may contain medicinal substances which act on the body in a manner ancillary to the device. However, where such substances act in a manner that is more than ancillary, the product is regulated as a medicinal product rather than a medical device. In addition, in cases where there is doubt as to the classification of the product as a device or medicinal product, the provisions of 2004/27/EC state that the product shall be regulated as a medicinal product [13].

An example of how the Medical Device Directive (MDD) and Medicinal Product Directive (MPD) are applied is outlined below for injection products [1, 14]:

- An empty syringe is classified as a medical device (MDD applied).
- A disposable pen-injector where the drug-containing injector is a single integral unit and only to be used in that given combination, is covered by the MPD. However in addition to this, the relevant essential requirements in Annex 1 of the MDD apply with respect to safety and performance related features of the device (e.g. a syringe forming part of such a product).
- For a drug-containing pen injector that is developed for a specific drug, but whereby the device and drug are available separately, the device and the drug will be considered individually as a medical device and a medicinal product (MDD and MPD applied, respectively).

The EC guideline MEDDEV 2.1/3 rev 2 (July 2001) on demarcation between the directives relating to Active Implantable Medical Devices and Medical Devices, and Medicinal Products is particularly helpful when considering the assignment of products as devices or medicinal products [15]. A device which is intended to deliver a medicinal product is itself regulated as a medical device. The medicinal product which the device is intended to administer must, of course, be approved according to the normal procedures for medicinal products. However, if the device and the medicinal product form a single integral product which is intended exclusively for use in the given combination and which is not reusable, that single product is regulated as a medicinal product. In such cases the essential requirements of the MDD apply as far as the device-related features of the product are concerned (for example as regards the mechanical safety features of a pre-filled pen-injector).

As can be seen from the examples given above, consideration needs to be given to whether the drug-device combination is performed by the patient or manufacturer.

There are two main ways in which the combination of the drug and the device can be achieved:

1. The device is available as a marketed product and the drug that is to be inserted into the device is purchased by the patient separately. Here the combination is undertaken by the patient, not by the manufacturer.
2. The drug and device are combined during the manufacturing/assembly process and the patient receives the product as an integrated drug-containing device.

In summary, if the patient is supplied with the drug and device separately and inserts the drug-containing package into the device for use, the drug is classified as a *standard* drug product and the device as a medical device (in both US and EMEA regions). If the manufacturer combines the drug and device such that the patient receives a drug-device combination (e.g., one that is disposed/not re-used after dose(s) have been delivered), then this combination is classified as a combination product in the US and as a medicinal product in the EMEA region, respectively (with consideration given to appropriate sections of the MDD for the device part for the EMEA).

Within the countries covered by the EMEA, there may be a need to submit a dossier covering both the drug and its combination, and a separate dossier for the device component of the product, whereas in the United States the review of a single dossier for the entire drug-device combination would be primarily handled by one review center. An understanding of the classification rules for medical devices in Europe will help to ensure that both the technical requirements and documentation requirements are met for the device component [1]. In Europe a Class I medical device (a device classed as having the lowest risk) would require a conformity assessment to be undertaken to allow the device to be Conformité Européenne (CE) marked, a necessity for products to be marketed within the EMEA regions. Often, items that are considered accessories to the main device part of the product fall into this Class I category. For example, a needle shield or guard that is used to hide a needle from a patient's view during injection is a non-invasive medical device and would be classified as Class 1 by the EMEA but often as an accessory to the pen-injection device by the FDA. There are often no specific regulatory requirements for these accessories in the US, but this would need to be discussed with the primary review center on a case-by-case basis. However, in Europe, if there is a device component of the product that is a classified as a Class 3 medical device under the MDD, the regulatory review process will include assessment of the conformity assessment and review of the acceptability of the device by both a Notified Body and subsequently by the EMEA Competent Authority responsible for assessment of the product.

The Japanese regulatory process was revised in 2004, creating the Pharmaceutical and Medical Devices Agency (PMDA), and provisions relating to medical devices came into effect on April 1, 2005 [16]. In Japan two submissions may be required: a KIT Drug J-NDA submission and a separate Medical Device Certification; the latter of these has to be filed with PMDA. It should be noted that the review timelines for KIT Drug and Medical Device Certification submissions

can differ significantly (i.e., 12–18 months versus 4–6 months, respectively). As with both the EMEA and FDA, it is important to establish early in development the stability requirements for the drug, device, and the combination and to understand in which submission such data would be submitted. In some cases the Medical Device Certification cannot be submitted for review until the KIT Drug submission is approved, therefore early communication with the PMDA, or engagement with a local medical device consultant, is important in order to understand the stability requirements for each of these submissions. Classification of the device according to the Japanese Pharmaceutical Affairs Law (PAL) may also impact the stability strategy; a summary of the PAL can be found on the Japan Pharmaceutical Manufacturers Association (JPMA) website [17].

16.3 Stability Strategies for Drug in Device Combination Products

The assignment of the innovator's product to an appropriate review agency and an outline agreement on the technical requirements and regulatory submission mechanism/format for the product being developed is important for all development aspects of the product including stability. Stability studies take a finite amount of time to execute and therefore it is vital to know what, if any, stability data may be required by the regulatory agencies as early as possible in the product development cycle. For drug-device combination products in particular, additional considerations include:

- whether additional specific stability studies are required (e.g., transportation studies)
- consideration of the minimum time period to be covered by data at time of submission (depending upon the combination product characteristics) which may in turn affect the testing strategy and bracketing/matrixing design
- the number of units required for testing on any study may be higher for combination products; this may affect manufacturing batch size
- chamber storage capacity (depending on size of device, the number of units needed for testing, and the length of the stability program)
- costs related to stability studies and resources required; these can be significantly higher for these types of products (orientations, numbers of prototypes, specialized testing), and the analytical skills and analyst capabilities required may be more difficult and time consuming to locate or develop

As discussed in the previous section, stability requirements for drug-device combination products are not well defined. For the drug component, ICH guidelines define data points, general testing, test conditions, and other considerations at time of submission. For the device component, stability is related to confirming appropriate and safe functioning of the device over its intended use period. Many of the testing requirements for device components are laid out in the International Organization for Standardization (ISO) standards (e.g., ISO 11608 for standards for

pen-injector devices) [18, 19]. However the bringing together of the drug and device entities, forming the combination product, provides a degree of uncertainty. Innovators need to consider whether the stability of their combination product could be different from that of the individual entities. Questions to consider when developing a stability strategy include:

- Does the drug product come in contact with any part of the device during long-term storage?
- Does the drug product come in contact with any part of the device during patient use?
- Does the device provide protection to the drug product or is its function purely as a delivery system?
- Is there any potential for leakage of the drug product into any of the device components?
- Is the combination product required to be sterile?
- Will all device components function as required over time such that the device will deliver the required dose?
- If the drug product is in direct contact with the device (no impermeable protection included), is there potential for leachables to migrate into the drug product over time?

In addition, the FDA Early Development Considerations for Innovative Combination Products guideline states it may be appropriate to conduct studies to evaluate the potential for the following [11]:

- Changes in stability of the drug constituent when delivered by the device or when used as a coating on the device
- Changes in the stability or activity of a drug constituent when used together with an energy emitting device
- Leaching of the device materials into the drug product

Similarly, consideration must be given to the effects a drug or biological product may have on the device constituent.

Stability scientists, who are usually more familiar with standard dosage forms (e.g., tablets, capsules, and injectables), must understand the stability requirements for the device versus those for the drug component for the device. Figures 16.1, 16.2, and 16.3 outline a series of considerations related to developing a stability strategy for combination products. As can be seen in these flow charts, the type of combination product being developed affects the stability study requirements.

It should be noted that the stability requirements for drug device combinations are still evolving. Some recent interactions with regulatory agencies have led to companies undertaking registration stability programs for combination products consisting of existing formulations in a new device, in which there is no drug-device contact, in order to demonstrate functionality over time (Fig. 16.2). Moreover in some of these cases, companies have been requested to provide additional chemical stability data on the existing formulation in the new combination even though this may be challenging to rationalize scientifically as the new device is not in contact with the

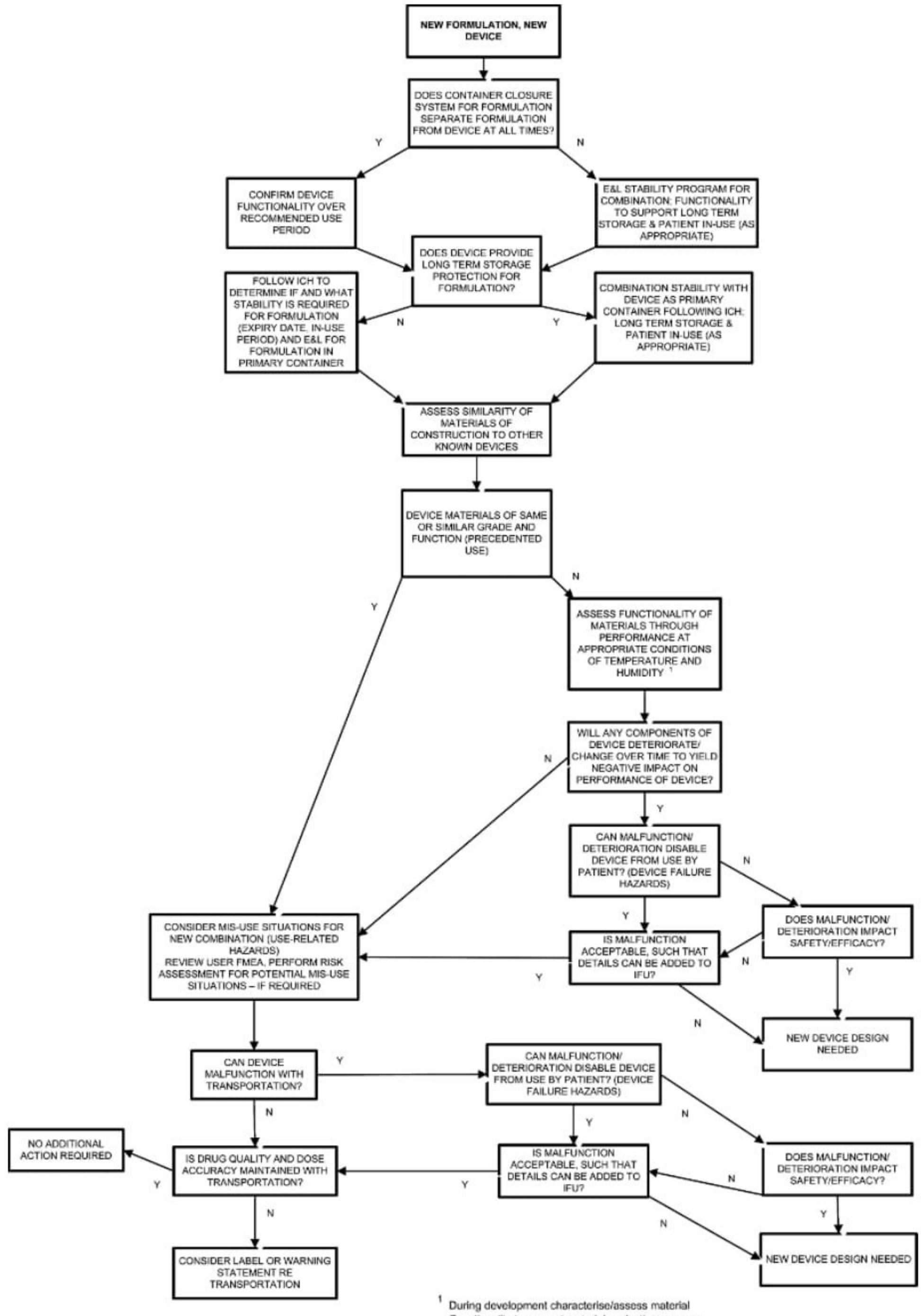

Fig. 16.1 Decision tree for determining stability studies for the registration of combination products with new formulation/new device

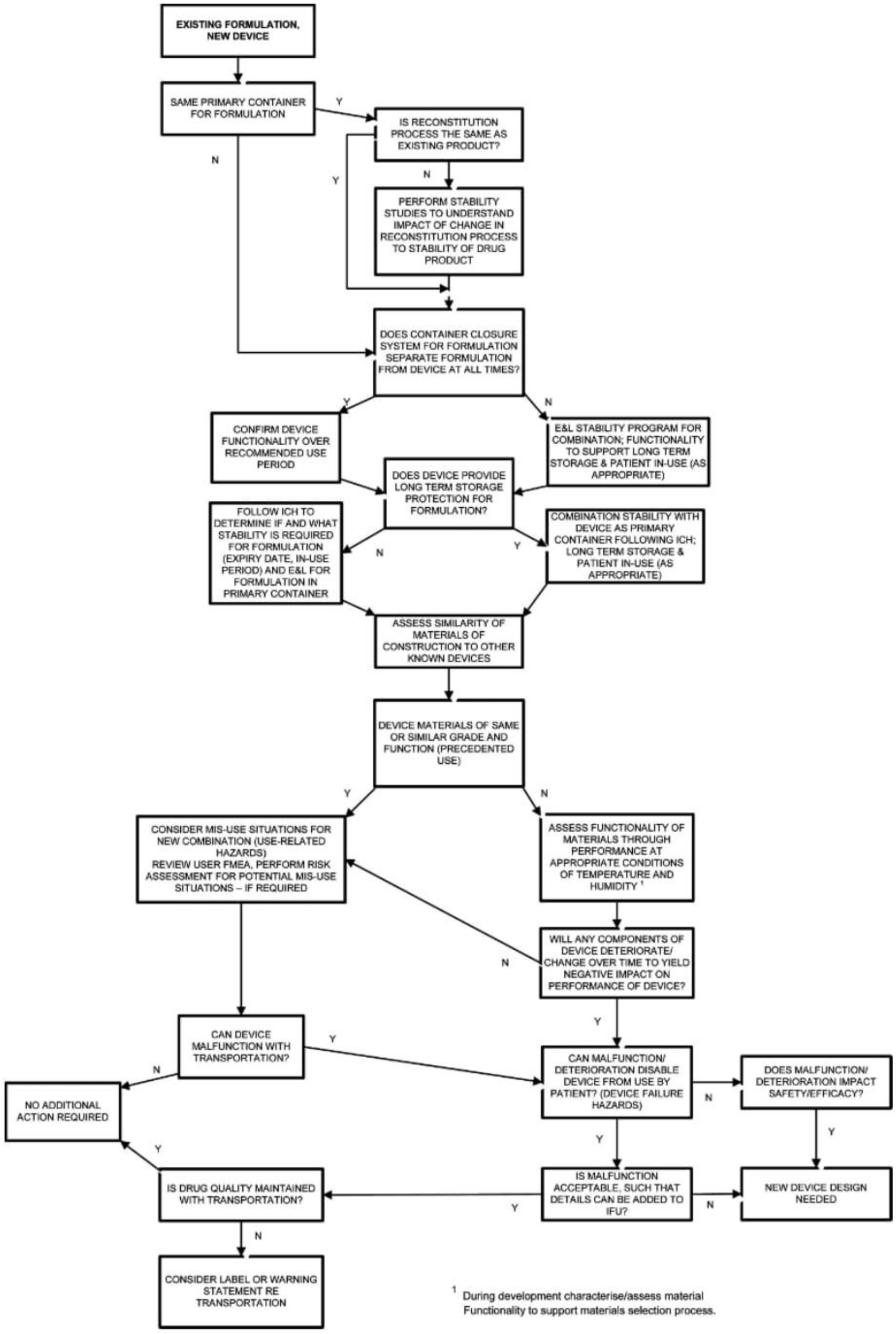

Fig. 16.2 Decision tree for determining stability studies for the registration of combination products with existing formulation/new device

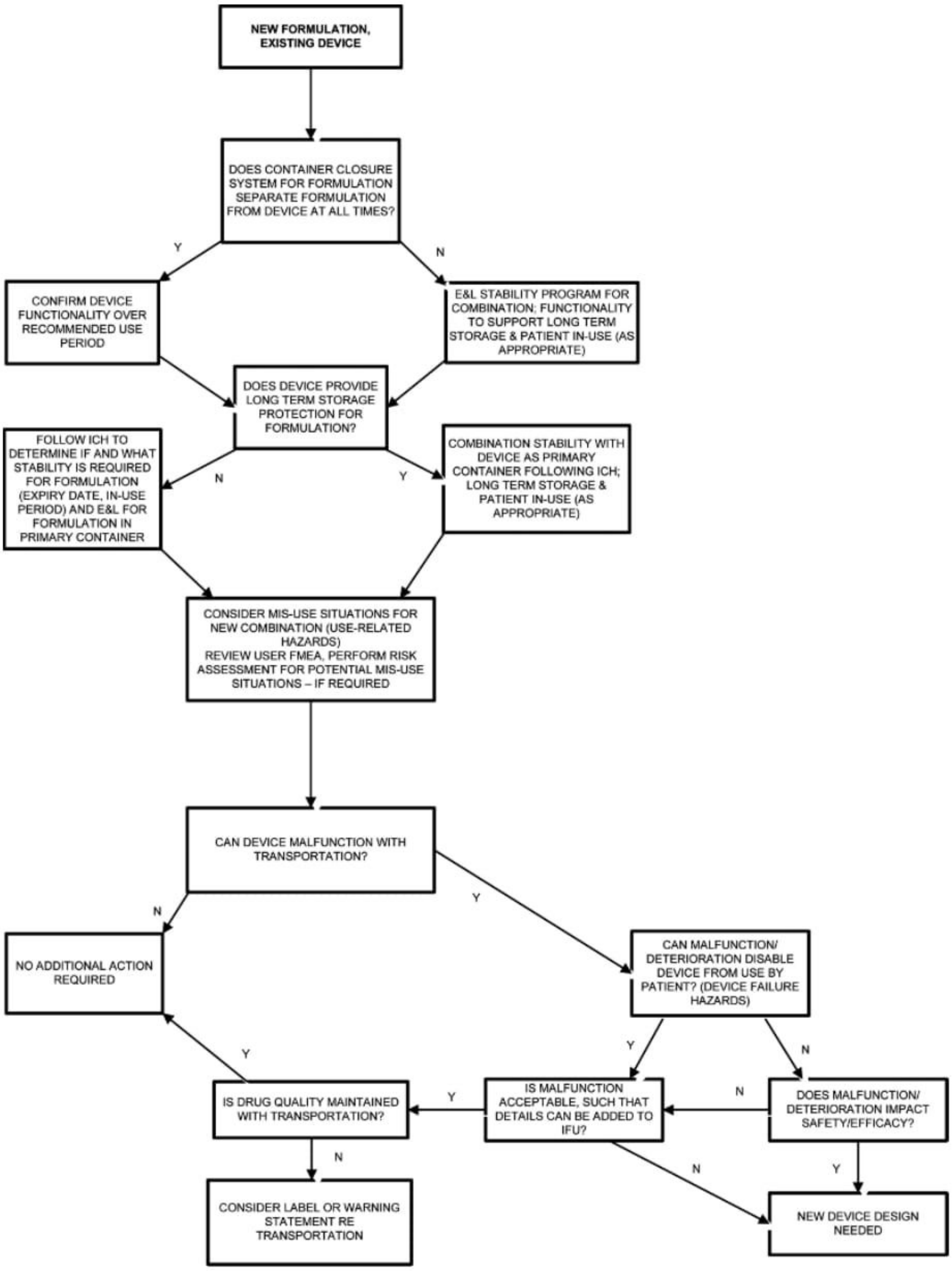

Fig. 16.3 Decision tree for determining stability studies for the registration of combination products with new formulation/existing device

drug nor is it providing any environmental protection. A well thought out and scientifically sound stability strategy needs to be presented to regulatory agencies early in development as requirements could differ significantly depending on mechanism of (primary) submission (e.g., 510(k), CTD, DMF, etc).

16.4 Nasal Spray and Inhaled Products

16.4.1 Introduction

For inhaled products there are a number of guidelines and papers to refer to when developing stability strategies. The European Medicines Agency's Quality Working Party, and Health Canada's Therapeutic Products Directorate have developed a joint guidance document on the Pharmaceutical Quality of Inhalation and Nasal products [20]. In addition, further clarification may be found in the overview of comments received on the guideline as it was being drafted and the responses to the comments [21]. This guidance applies to human medicinal products intended for delivery into the lungs or nasal mucosa.

In the US, two separate guidelines have been developed, one covering both metered dose inhalers (MDIs) and dry powder inhalers (DPIs) and one covering nasal sprays, inhalation solutions, and suspensions and inhalation sprays [22, 23]. The guidance on MDIs and DPIs is however still in draft form nearly 10 years after being published for comment; one of the unresolved issues is the dose content uniformity requirements which are discussed later in this chapter (Section 16.5.4). There are also other sections which would benefit from further discussion, in particular the number and nature of some of the tests that may be expected on stability.

Slightly different terminology is used in the two regions when classifying various types of inhaled products. These are compared in Table 16.1.

There are unique features pertaining to nasal sprays and inhalation products which make stability studies more complex and challenging. Examples include metering and spray production, energy required for spray production, the container closure system, and small doses. Critical attributes include the reproducibility (throughout the shelf-life) of the dose, the spray plume, and the particle/droplet size

Table 16.1 Classification of product types

FDA terminology	EMEA terminology
Nasal spray	Non-pressurized metered dose nasal spray Nasal single use sprays
Inhalation spray	Non-pressurized metered dose inhaler
Inhalation solutions and suspensions	Product for nebulization (single and multiple use)
Metered dose inhaler MDI	Pressurized metered dose nasal sprays Pressurized metered dose inhaler
Dry powder inhaler DPI, device metered	Dry Powder Inhaler, device metered Nasal powders, device metered
Dry powder inhaler DPI, pre-metered	Dry Powder Inhaler, pre-metered
Not included	Nasal drops (single and multiple use)

distribution, the maintenance of sterility or microbial load as well as functionality of the device (spray mechanism, sensors). Additionally, changes to components, manufacturer, or manufacturing process, which might affect any of the key attributes, will require adequate data to demonstrate that significant changes to stability characteristics do not occur.

16.4.2 Overview of Stability Tests

Both the FDA and EMEA guidelines describe tests to be considered on the specification and for stability testing [20, 22, 24]. However the guidelines recommend different attributes and provide limited detail on testing to be performed at the various stages of product development (e.g., early development, registration, post-approval commitment stability studies). The following sections include a comparison of the regulatory requirements and outline stability strategies to be considered during development.

For all products, appearance/description, assay and degradation products would be performed during stability studies at all stages of development. Additional tests to be considered are listed in Tables 16.2 and 16.3. During the development cycle the testing pattern may change as product understanding develops. For example, during early development, the tests considered for a DPI product may include only appearance, assay, degradation products, uniformity of delivered dose, and fine particle dose, whereas in later studies as product knowledge develops, further tests may be added with meaningful specification limits. These in turn may be subsequently eliminated when it has been demonstrated that they are not stability indicating attributes for the product.

16.4.3 Assay

For multi-dose products, the EMEA guideline states that the amount of drug substance should be determined per weight unit or per volume unit as applicable [20]. For single dose products, assay results should be expressed as mass per dosage unit, in other words, concentration. According to the FDA draft guideline, assay for MDIs may be performed indirectly by determining concentration and actual net content i.e. fill weight/volume, whereas for DPIs the amount of drug substance in each individual dosage unit should be determined for pre-metered devices and in the reservoir for device-metered inhalers [22]. Therefore, for MDIs and device metered DPIs, CDER are describing assay as total content rather than concentration. In all cases, for stability testing where degradation trends are important, monitoring assay as concentration over time is also important. If it is possible for fill volume to change on stability (e.g., when semi-permeable containers are used or where gases could be lost through valve elastomers), care should be taken when analyzing the data to ensure that loss of volume (concentration increase) is not offset by degradation (concentration decrease) thereby masking stability trends. For drug substances in salt form, assay of the counterion is unnecessary unless the salt form is known to degrade, for example, as determined via forced degradation studies.

Table 16.2 Tests to be considered on stability for nasal sprays, inhalation sprays, and inhalation solutions and suspensions

	Nasal spray		Inhalation spray	Inhalation solutions
Stability test	Non-pressurized metered dose nasal spray	Nasal single use sprays	Non-pressurized metered dose inhaler	Product for nebulization (single and multiple use)
Mean delivered dose	Yes	Yes	Yes	No
Delivered dose uniformity (+ through container life)[1]	Yes	No	Yes	No
No. of actuations	Yes (EU only)	No	Yes (EU only)	No
Plume geometry[2]	Yes	Yes	Yes	No
Particle/droplet size[3]	Yes	Yes	Yes	Yes (for suspensions)
Microbial count[4]	Yes, unless sterile	Yes, unless sterile	Yes, unless sterile	Yes, unless sterile
Sterility	If sterile	If sterile	If sterile	If sterile
Preservative/stabilizer content[5]	If present	If present	If present	If present
Antimicrobial preservative effectiveness[6]	If present; 1 batch	If present; 1 batch	If present; 1 batch	If present; 1 batch
Particulate matter[7]	Yes	Yes	Yes	Yes
Weight loss	If semi-permeable	If semi-permeable	If semi-permeable	If semi-permeable
pH[2]	Yes	Yes	Yes	Yes
Viscosity	Yes	Yes	No	No
Leachables	Yes	Yes	Yes	Yes

[1]Refer to Section 16.5.4.
[2]May be tested during development only to demonstrate no stability issues; may justify to omit from the specification if appropriate.
[3]Refer to Section 16.5.5.
[4]May consider testing at last checkpoint before submission and annually only.
[5]If present.
[6]May be tested as part of registration stability studies and at selected checkpoints only.
[7]May be tested during development or registration stability studies only to demonstrate no issue; thereafter on release only.

16.4.4 Dose Content Uniformity/Delivered-Dose Uniformity

Various terms are used for this requirement including: dose content uniformity, delivered-dose uniformity, and emitted dose uniformity as well as spray content uniformity for nasal sprays. The EMEA guideline refers to the relevant pharmacopoeia for guidance on requirements [20]. The delivered dose uniformity requirements contained in the draft FDA guideline for MDIs and DPIs are challenging, and during stability testing, where increased numbers of samples are being tested, an

Table 16.3 Tests to be considered on stability for MDIs and DPIs

	Metered dose inhaler MDI		Dry powder inhaler DPI,	Dry powder Inhaler DPI,
Stability test	Pressurized metered dose nasal spray	Pressurized metered dose inhaler	Dry powder inhaler and nasal powders, device metered	Dry powder inhaler pre-metered
Mean delivered dose	Yes	Yes	Yes	Yes
Delivered dose uniformity (+ through container life)[1]	Yes	Yes	Yes	Yes[2]
No. of actuations	Yes (EU only)	No	Yes (EU only)	Yes[2]
Particle/droplet size distribution[3]	Yes	Yes	Yes	Yes
Fine particle mass	No	Yes	DPI only	Yes
Plume geometry[4]	Yes	Yes	No	No
Microscopic evaluation[5]	Maybe	Maybe	Maybe	Maybe
Particulate matter[6]	Yes	Yes	Yes	Yes
Microbial count[7]	Yes	Yes	Yes	Yes
Solid form/polymorph	Suspensions	Suspensions	Yes	Yes
Weight loss[8]	Yes	Yes	No	No
pH[4]	Yes	Yes	No	No
Leak rate	Yes	Yes	No	No
Moisture	Yes	Yes	Yes	Yes
Leachables[9]	Yes	Yes	No[10]	No[10]

[1]Refer to Section 16.5.4.
[2]Not required for capsules.
[3]Refer to Section 16.5.5.
[4]May be tested during development only to demonstrate no stability issues; may justify to omit from the specification if appropriate.
[5]May be tested on stability e.g., if issues noticed with particle size distribution, an increase in the number of foreign particulates, appearance changing, or form changes expected.
[6]Test during development or registration stability studies to monitor trends; if no issues on stability observed, test at release only.
[7]May consider testing during primary/registration stability studies at last checkpoint before submission and annually only.
[8]Generally required as part of in-use testing. Would be required for products in semi-permeable containers on stability.
[9]May be tested as part of registration stability studies only; also refer to Section 16.9.
[10]Assess on a case by case basis.

increase in the number of out of specification results may be observed even when the product is stable [22].

In order to address these challenges, the International Pharmaceutical Aerosol Consortium on Regulation and Science (IPAC-RS) presented a proposal to the FDA to replace the test requirements outlined in the FDA draft guidance with a parametric tolerance interval (PTI) test for dose content uniformity for MDIs and DPIs [25]. As a result of this proposal, a working group consisting of FDA and IPAC-RS members was set up in 2004. In principle FDA have agreed to the use of the PTI test but there is no agreement on the statistical parameters (such as coverage) that might be built into a *universal* PTI test. However in a risk-based approach to product development the test parameters must reflect the therapeutic index and dose–response of the drug. In practice, therefore, a zero-tolerance test approach should be taken (e.g., 10 out of 10 tested must fall within $\pm$ 35% of the label claim) until appropriate clinical information is available. To date, the latest editions of the national pharmacopoeias have not adopted the PTI requirements and the current harmonized USP and Ph. Eur. pharmacopeial limits are wider than those outlined in the draft FDA guideline [26, 27]. Although the USP currently requires uniformity of delivered dose over the entire contents for MDIs and DPIs, given the current expectation that MDIs contain a dose counter, information on delivered dose uniformity after actuation of the labelled number of doses is of limited use. In Europe the number of deliveries per inhaler is required.

For metered dose nasal sprays the pharmacopoeias are however different. The Ph. Eur. contains the same requirements as those for MDIs and DPIs, whereas the USP requirements are more challenging, as per those in the original FDA guideline for nasal sprays et al.

An additional requirement detailed in the draft FDA guideline for MDIs and DPIs is valve delivery/shot weight [22]. Although for stability studies the measurement of dose during the determination of dose content uniformity may be more appropriate than valve delivery/shot weight, some regulatory agencies consider that valuable information regarding potential causes of dose variability may still be gained from generation of the data. It is therefore wise to discuss the stability testing strategy with regulatory agencies prior to registration stability studies.

16.4.5 Particle/Droplet Size and Fine Particle Mass

Particle size distribution is a multivariate parameter. In early development it is often described by a single point control known as fine particle mass, typically being the mass of particles *less than or equal to* 5 μm. As development proceeds, more complex specifications are developed whereby the particle size distribution is represented by a number of particle size fractions between 1 and 10 μm, with requirements linked to batches used clinically.

Maintaining particle/droplet size distribution on stability is a key challenge in the development of nasal spray and orally inhaled products. Suspensions have the potential to agglomerate or to undergo particle size changes [28]. For solution products,

moisture ingress may change the evaporative nature of the solvent system and consequently lead to changes in droplet size [29]. In dry powder inhaler products humidity during storage can affect powder properties and the fine particle mass [30, 31].

Microscopy may be used on stability to help determine causes for any changes noted, for example to determine whether agglomeration or particle size growth is occurring in a suspension. Microscopy may also be useful for investigations into appearance observations, for example if foreign particles are noticed. However it is difficult to set acceptance criteria for microscopy as a test, and it is therefore of more use as an investigative tool to understand sources of/causes for particulate formation.

16.4.6 Moisture

As mentioned in the preceding section, moisture can affect the performance of the drug product and therefore a test for water may be required if the product demonstrates sensitivity to moisture.

However moisture *in itself* is not an issue and even if linked to a critical attribute such as particle size, degradation, or microbial growth, this will not often be known during the early stages of development. Thus it is often not feasible to set appropriate acceptance criteria during early development stages, although moisture should still be measured at key stability checkpoints to look for links to key performance attributes. If a correlation is found between moisture and a critical parameter such that moisture has a negative impact on product quality or performance, then moisture itself must be controlled, for example through raw material controls or through appropriate packaging/storage. If moisture is not an issue or is less indicative of an issue than measurement of a critical attribute itself then a justification could be submitted to omit moisture control from the specification and thus from future post-approval stability studies.

16.4.7 Particulate Matter/Foreign Particles in MDIs and DPIs

The draft FDA guideline describes the requirement to monitor foreign particle levels during stability studies [22]. Since this guideline was drafted the IPAC-RS has published two articles/guidelines on particulates testing [32, 33]. These articles include testing and specification development for particulates. Regarding stability studies, the latter article states that particle characterization (e.g., microscopy) should be performed at the initial stability time-point; however, for stability purposes the article indicates that it is necessary at any time-point to characterize only if the number of foreign particles was observed to be increasing (either through appearance testing, via membrane testing e.g., on DPIs, or via a validated method depending on the stage of development). To develop an understanding of performance it may be appropriate to characterize certain batches, but separate from stability activities. If changes are observed on stability it would be prudent to characterize the nature of

the particles and compare for example to *control* samples stored at refrigerated conditions. The first IPAC-RS article states that for commercial batches, release testing only is required in situations where no stability trends were noted in development, and that in time, release testing could also be phased out [32].

16.4.8 Storage Conditions

Storage conditions are described in Chapters 3 and 4 in this book, and depend on the region the product is to be registered in. The one difference in requirements for MDIs and DPIs is described in the draft FDA guideline and is for products needing to be packaged in moisture protective packaging [22]. In this case, storage at the condition of 25°C/75%RH for one-third of the shelf-life is described to check that the packaging is adequate to protect the product. However depending on the regions the product is intended to be registered in, the long-term Zone IVB condition of 30°C/75%RH would essentially be a worst case scenario and should cover registration in all zones [34]. A company could therefore opt not to test product at 25°C/75%RH, assuming adequate stability at the more severe condition.

16.4.9 In-Use Testing

In-use testing is performed without the protective over-pack in which the stability of the product/primary package is being tested. The most recent FDA guideline, for Nasal Spray and Inhalation Solution, Suspension, and Spray Drug Products, states that if additional packaging (e.g., foil over-wrap) is used to protect the drug product from evaporative effects, then adequate stability data conducted at a minimum of 25°C and a maximum of 40% RH should be generated for pertinent parameters for these units without the protective packaging [23]. For MDIs and DPIs, the FDA draft guideline states that data generated at a minimum of 25°C and 75%RH is required if additional packaging (e.g., foil over-wrap) is deemed necessary [22]. It could be scientifically justified that if moisture loss is deemed a potential issue then 25°C/40%RH would be appropriate, if moisture ingress is deemed to be a potential issue then 25°C/75%RH would be appropriate, depending on the properties of the formulation and the packaging. These conditions are appropriate for Zone I/II regions. For Zone III/IV regions, 30°C /35%RH is recommended if moisture loss is a concern, 30°C/75%RH if moisture ingress is a concern [34]. If a global filing is the goal then the Zone III/IV conditions are the most challenging and therefore could be justified as sufficient to cover registration in all regions.

In-use testing should be performed on two batches, at least one of which should be near end of shelf-life or at the final time-point of the submitted stability studies [34, 35]. Thus if a product is to be used within 3 months after removal of the protective packaging (according to the Instructions For Use (IFU)), the product should be removed from the protective packaging 3 months before the end of the shelf-life, and

Table 16.4 Example Registration Stability Protocol for a DPI

		Months							
Stability condition		T0	3	6	9	12	18	24	36
Initial		A[1]	–	–	–	–	–	–	–
Accelerated	40°C/75%RH		A	A[1]	–	–	–	–	–
Long Term	30°C/75%RH		A	A	A	A[1]	A	A[1]	A[1]
Long Term	25°C/60%RH[2]		A	A	A	A[1]	A	A[1]	A[1]
	25°C/75%RH[3]								
Controls	5°C		C	C	C	C	C	C	C
Photostability	Option 2	A	–	–	–	–	–	–	–
Thermal Cycling		A	–	–	–	–	–	–	–

[1]Samples to be removed for in-use testing, if appropriate as determined by when the data are to be filed and the shelf life being requested.
[2] Depending on stability knowledge of the product, this condition may not be actively tested if the product was found to be stable at 30°C/75%RH during developmental stability studies.
[3]This storage condition is required when moisture protective packaging is deemed necessary for the product; store spares only in case of unexpected results at 30°C/75%RH up to 12 month checkpoint.

then tested at the end of the shelf-life [20]. An example registration stability protocol for a DPI, including in-use testing considerations, is included in Table 16.4.

16.4.10 Other Specific Stability Considerations/Requirements

The FDA guidelines also describe additional stability related studies including device robustness and effects of resting time particularly on priming/re-priming [22, 23]. Additional stability studies also need to be considered when changing the manufacturing facility, manufacturing procedure, source, synthetic route or micronization of the API, source or type (design or composition) of container and closure components, grade of excipient or even source of excipients if they may affect the stability. This may be done via comparability studies. Discussions on leachables and temperature cycling are included in Sections 16.9 and 16.12 of this chapter.

The Association of South East Asian Nations (ASEAN) Guideline on Stability Testing of Drug Product contains a guide on tests to be included in a stability study for this region [36]. In addition to tests already discussed, for MDIs and nasal aerosols both taste and assay for co-solvent are described. For nasal sprays, clarity of solution is also described. Innovators should consider whether these tests add to the information that is being gathered via other tests and, in the case of taste testing, what the safety implications are for analysts.

16.5 Pen Injectors

There are a significant number and variety of pen-injector devices on the market and in development today, including products for treatment of diabetes, rheumatoid arthritis, and growth hormone deficiency. These pen-injector devices are generally

considered as combination products; however, the difference in how the combination is achieved can have a bearing on the requirements for the product. The stability requirements relating to pen-injectors are constantly evolving, in many ways due to the submission mechanism for these types of drug-device combinations. In the past many of these devices have been submitted to FDA as Premarket Notification 510(k)s through CDRH; these have specific requirements in terms of content for submission [37]. More recently a number of pen-injector combination products have been required to be submitted in CTD format through CDER or CBER, therefore the expectations for stability programs have been more akin to ICH requirements [9].

There are two cases in which the combination of the drug and the device can be achieved:

1. The device is available as a marketed product and the drug that is to be inserted into the pen-injector device is purchased by the patient separately. Here the combination is undertaken by the patient, not by the manufacturer.
2. The drug and device are combined during the manufacturing or assembly process and the patient receives the product as an integrated drug-containing pen-injector device.

Case 1 above is relatively straightforward, for example: the pen-injector is registered and approved as a medical device and the drug registered as a medicinal product. The pen-injector device and medicinal product are purchased separately; an individual drug-containing cartridge is inserted into the pen-injector by the patient upon first use. This drug-containing pen-injector combination is then used for a finite period of time until either the shelf-life of the drug-containing cartridge is reached (e.g., the cartridge may have a 14 or 28 day use-by date) or the contents of the cartridge are depleted. After this point the patient takes a new cartridge for insertion into the same pen-injector device for continued medication. This process of using the same pen-injector but inserting new individual cartridges of drug product into the pen-injector device on an ongoing basis would continue until the end of the lifetime of that individual pen-injector. As both the drug and device are packaged (and potentially registered) separately in this case, they each require individual stability programs to meet registration requirements and enable use-by dates to be assigned. Stability for the medicinal product would follow ICH guidance whereas for the device the focus would be to demonstrate functionality over its intended use period. The device needs to meet the requirements of ISO 11608 to confirm its ability to function and meet the requirements of dose accuracy (first, each, and last dose) over its lifetime [19]. This functionality would be performed by calculating the number of times the pen-injector would be used by the patient, for example a once-weekly injection with a pen-injector that could be used over a 2-year period would equate to 104 times that each device could be used by the patient. In this instance dose accuracy would be confirmed over a minimum of 104 uses of an individual pen-injector device. For the medicinal product that is registered, packaged, and sold as a separate entity, the stability program would be executed in line with ICH guidelines.

For Case 2, the requirements will depend to a large degree on the regulatory submission mechanism for the product. The flow charts in Figs. 16.1, 16.2, and 16.3 (see Section 16.3) outline considerations for developing a stability strategy for registration of the product.

16.6 Drug Eluting Stents

The FDA defines the PMOA for a drug eluting stent (DES) is as a device [38, 39]. During the regulatory process CDER would be involved with the review of the pharmacologic agent and CDRH would review the stent platform, the delivery system and the carrier (polymer), if present. In Europe a DES would also be viewed as a medical device [15, 40].

The FDA has issued a draft guidance containing a number of sections relating to the stability recommendations for a coronary DES [41]. Tests described for stability studies are appearance, assay, degradation products, in-vitro drug release, and particulate matter. In addition, sterility and package integrity are included as being required annually and at end of shelf-life. In the example testing protocol for long-term conditions, a test for endotoxins is described; however, for sterile products (tested for endotoxins at release) the need for this test on stability is questionable. Johnson & Johnson (Cypher^{TM} with active drug sirolimus) and Boston Scientific (Taxus^{TM}stent with active drug paclitaxel) also included identity, drug content uniformity, residual solvents, and endotoxins in their site-specific registration stability programs [42, 43]. However, these tests are not usually considered necessary for stability studies.

During the development phase, compatibility between the drug, stent, and carrier matrix, if present, must be explored. The stent has to withstand significant expansion during deployment, as well as constant pulsation in the artery after deployment, without cracking or flaking, and thus initiating clotting or liberating potentially harmful particulate matter into the coronary blood stream [44, 45].

For new stent systems, additional stability challenges include engineering, stress, and durability tests. These include stability to magnetic resonance imaging (MRI) scans [46]; chemical stability of any polymer component(s) [47]; the stability of polymer coated stents to degradation during sterilization [48, 49]; predicting degradation rates and determining products for bioresorbable stents [50]; and in-vivo stability [51]. The FDA draft guidance for a coronary DES describes mechanical performance and integrity challenges that should be performed during development, including tests for coating integrity for product aged to the requested shelf-life and under accelerated simulated in vivo conditions, corrosion potential with coating defects, particulate matter after ageing and, if appropriate, durability of degradable coatings [41].

During pre-clinical testing, the FDA has noted many deficiencies related to inadequate stent platform testing (e.g., fatigue and corrosion testing), inadequate analysis of surface modifications (coating integrity/durability, drug content/uniformity)

and inadequate stability and shelf-life information [52, 53]. Developers are urged to work with the FDA early in the development process.

16.7 Implantable Systems

Implantable delivery systems offer a number of advantages over more traditional delivery routes, particularly for biological macromolecules (including peptides, proteins, and oligonucleotides). Some specific delivery mechanisms to date include polymer depots (e.g., Gliadel™Wafer, prolifeprosan 20 with carmustine implant) and osmotic pumps (e.g., Viadur™ leuprolide acetate implant).

Additional stability challenges for biological molecules in implantable systems include: drug-device interactions; physical stability of drug, especially proteins, during use; and stability of the drug in the device in vivo [54]. Proteins in particular may adsorb onto surfaces, followed by denaturing and subsequently aggregation, or may aggregate as a result of pump operation [54, 55]. Formulations may be developed as non-aqueous solutions or suspensions to ensure in vivo stability. The device must also protect the formulation from ingress of body fluids that may cause degradation of the drug and/or affect the mechanism of the device. Stability studies which include the measurement of the release profile, must therefore be demonstrated at *greater than or equal to* 37°C for the equivalent length of time the implant is to be in the body, which may be for up to a year or more. Similar in-vivo stability concerns are experienced for polymer depot systems in which molecules are stabilized by suspension in polymer solution.

16.8 Transdermal Products

Transdermal systems, frequently combined with enhancement technologies, offer advantages over traditional methods of delivery. Enhancement technologies include chemical enhancement, iontophoresis, sonophoresis, and microneedles (and combinations of these) as well as other innovative approaches in various stages of development [56].

Some specific tests are required for transdermal products on stability studies. It must be demonstrated that the patch maintains adhesive properties over time. This is essential to ensure efficacy of the product particularly if dose is proportional to surface area. Backing degradation or diffusion of drug components through the backing, stiffness caused by moisture vapor and air, drug or excipients undergoing phase changes, and effects on the adhesive by other components may all affect adhesive properties [57]. In-vitro methods for measurement include peel adhesion, tack, and shear adhesion; however, these are essentially quality control tests and are difficult to link to in-vivo performance [58]. During development the various tests available to measure these properties must be evaluated to determine those most appropriate to include in registration and commercial stability programs.

Flatness (which may affect the ability to apply the patch) may be measured during developmental stability studies [59]. Exposure to high or low humidity may affect moisture content and can cause either increased formulation bulkiness or brittleness, respectively [60]. These studies inform packaging decisions prior to commercialization.

Release rate is also included in stability studies. Tests for transdermal patches are described in the pharmacopoeias, with specifications usually containing three time points as with other sustained release dosage forms.

16.9 Leachables Studies

Device components, as part of a drug-device combination product, may contain polymers, elastomers, and other components from which minute quantities of material may migrate (leach) into the medicinal product over time and thus may affect the quality and safety of the product. A number of guidelines outline approaches to be considered for extractables and leachables studies [61, 62]. This chapter outlines additional points to consider specific to the development of drug-device combination products.

For combination products, in addition to the usual consideration for the potential for leachables to migrate during long-term stability studies, an innovator needs to determine whether the formulation will come into contact with the device components during the patient in-use period. For example, during the development of a new pen-injector one consideration is to understand what happens when a drug-containing cartridge is inserted into the pen-injector device during patient use. This consideration equally applies to any combination where the drug is contained within a primary container prior to insertion into the device, namely, the device is not the primary container for the medicinal product, or, when other parts of the device come into contact with the formulation during use.

If the drug is in contact with any part of the device during its storage or use, the developer will need to understand the potential for extractables from the device components to leach into the medicinal product over time. A study should be designed to understand the potential for leachables, taking into account many considerations including the following:

- The specific parts of the device that could come in contact with the medicinal product
- Whether the contact is transient (e.g., only during injection) or sustained during the patient use period (e.g., over the entire period the cartridge remains within the pen-injector)
- The composition of the device components (plastics, springs, elastomers, etc.)
- The composition of the device in terms of moulded and/or assembled component parts, i.e., the moulding process may effect the properties of the components

In 2001 the CMC Leachables and Extractables Technical Team of the Inhalation Technology Focus Group of the American Association of Pharmaceutical Scientists (ITFG)/IPAC-RS Collaboration and its Toxicology Working Group published a paper of points to consider for leachables and extractables testing for MDIs, DPIs, Nasal Sprays and Inhalation Solution, Suspension, and Spray Drug Products [63]. A key point from this document in relation to leachable studies is that the leachables program should be conducted on the drug product packaging configuration employed for long-term stability studies (e.g., capsule with blister, low density polyethylene vial with over-wrap). An in-use study should also be conducted in order to determine the leachables derived from components which are in contact with either the formulation or the patient's mouth or nasal mucosa only during administration (such as mouthpieces and actuators).

Leachables studies can be conducted as part of the stability studies to support registration. A useful document to refer to when designing controlled extraction and leachable studies is Safety Thresholds and Best Practices for Extractables and Leachables in Orally Inhaled and Nasal Drug Products published by Product Quality Research Institute (PQRI), which states: "Since these large drug product stability studies involve analysis of samples at multiple time-points, it is possible to discern trends in drug product leachables profiles over time and storage condition." [64, 65]. Once the potential leachables are identified, stability-indicating methods for each leachable can be developed. Appropriate thresholds for each potential leachable are determined through assessment of toxicological and safety data [64, 66].

16.10 Bracketing/Matrixing

Bracketing and matrixing is described in general stability guidances as well as those guidances for combination products including stents and inhaled products [20, 22, 23, 41, 67]. The FDA however appears reluctant to accept bracketing or matrixing for inhaled products and state that the use of bracketing and matrixing protocols may not be appropriate for MDIs and DPIs, although other agencies have accepted them [22, 68]. When using a bracketing or matrixing approach for designing stability programs for drugs in devices, additional justification will therefore be required and it is recommended that any such strategies are discussed with regulatory agencies prior to commencement of any registration stability activities.

16.11 Storage Orientation

During development, stability studies should include storage of products using different orientations (e.g., upright and inverted) if there is the possibility that orientation could affect stability performance [20, 22, 34, 65, 69]. Storage orientation can affect the stability of the product, and although there is limited information available in the literature demonstrating this, combination products in

solution or suspension may be affected [28]. If no differences are observed in stability performance, subsequent stability studies can then be reduced to one orientation. It may be prudent to store spare samples in alternative configuration(s) during registration stability studies in case of any unexpected regulatory challenges.

16.12 Temperature Cycling

In addition to stability studies at accelerated conditions, a study to determine the effect of extreme temperature variation should be considered to support the product exposed to storage excursions. Drug products susceptible to phase separation, loss of viscosity, precipitation, and aggregation should be evaluated under thermal cycling conditions. As part of the stress testing, the packaged product should be cycled through temperature conditions that simulate the changes likely to be encountered during product distribution. Example temperature cycling protocols are included in Tables 16.5 and 16.6.

Table 16.5 Thermal cycling for product labeled "Protect from Freezing"

	5°C	40°C
Length of storage	2 days	2 days

Table 16.6 Thermal cycling Freezing

	−20°C	25°C
Length of storage	2 days	2 days

The protocols represent one cycle, with the product being subjected to three cycles. Samples should be tested at the end of the third cycle, based on the appropriate test pattern. Guidance on temperature cycling for MDIs and nasal sprays is described in the EMEA and FDA guidelines on inhaled and nasal products [20, 22, 23]. Some of the variations described in the latter may be considered severe, although it is stated that alternative conditions and durations can be used with appropriate justification.

16.13 Transportation Studies

An additional consideration for drug-device combination products is the potential need to undertake transportation studies (also referred to as agitation or rotational studies). This may be particularly relevant where the drug in such a combination product is a biological entity. Biological molecules can be more sensitive to transportation conditions than traditional small molecule medicinal products. In addition, medicinal products of a biological nature that are used in a combination product may raise specific concerns regarding transportation. In a situation where the

combination is achieved by the patient (e.g., through insertion of a drug-containing cartridge in a pen-injector or a drug-containing blister-foil pack in an inhaler) it is possible (even if not prescribed in the Instructions For Use/Patient Information Leaflet) that a patient may carry a "spare" cartridge or blister pack with them to use when the one they currently have in their medical device is depleted.

The manufacturer is responsible for providing information on storage requirements but should also consider providing adequate warnings and precautionary statements covering potential misuse situations (see Figures 16.1, 16.2 and 16.3 in Section 16.3). An assessment of the types of studies that need to be undertaken can be determined through an assessment of the use-related hazards (the potential for patient misuse situations can be assessed through performing for example a User Failure Mode and Effects Analysis (FMEA)).

Development packaging and device pre-verification studies may provide supportive information when developing a protocol/plan for transportation studies. For example, drop testing undertaken to measure the durability/robustness of the device or combination product may highlight potential weak areas of the device that may help in understanding potential issues that can occur during transportation in specific orientations/positions. Examples of areas to consider are as follows:

- If it is a biological product, could it denature during transportation?
- Does the biological product become cloudy or lose solution clarity during agitation?
- Does the device continue to function (e.g., the injection button) after agitation?
- Is there any concern of over-dosing after the drug-containing device is subjected to agitation?

Answers to some of these questions may lead to additional warnings being placed on the label to restrict the conditions of transport where it is shown that transportation or agitation has a negative impact on the quality of the medicinal product. At the other extreme, the potential for over-dosing due to device malfunctions that could occur during transport raise concerns about patient safety and product efficacy, and could therefore impact the viability of the product.

Consider a patient responsible for self-medication for diabetes using a pen-injector that requires refrigerated storage. Questions that should be considered here are what the impact would be on the medicinal-drug and the combination product when the pen-injector is being agitated during normal daily activities if carried by the patient during the in-use period. The developer may provide additional safeguards to avoid misuse situations, for example, a well-designed storage case (e.g., with ice packs). However the developer will still need to consider the consequences of an excursion for short periods; this could be incorporated into the temperature cycling stability study.

Due to the length of time it can take to undertake these types of stability studies and the potential impact of the outcome, it is wise to prioritize these studies appropriately in the development program.

16.14 Commercial Stability Commitment

Similar to other pharmaceutical products, for combination products the FDA requires a stability commitment at time of filing. This commitment must include stability studies on the first three commercial batches and one annual product monitoring batch. It is usually in the form of a protocol detailing checkpoints and test methods and a commitment to communicate the results to the FDA. However, recently there appears to be a trend in expectations for inhaled products whereby 10% of commercial batches fall within the remit of such a stability commitment.

For new devices where the drug product in its primary container remains the same as the current marketed product, and as-such the device provides in fact a secondary packaging for the drug product, it may be appropriate to consider a sunsetting approach to the stability commitment. For example, in the case of pen-injectors that contain a cartridge that is already on the market, it is worth discussing with FDA the possibility of sunsetting the stability commitment such that if the first X number of batches meet the specification criteria and show no change on stability, the stability commitment could be phased out over time.

16.15 Conclusion

In this chapter we have highlighted some of the important considerations for developing stability strategies for drug-device combination products. It is vital to understand the latest regulatory requirements and expectations whilst also adopting a scientific and risk-based approach based on product understanding.

The guidelines for combination products are not as mature or harmonized as for more conventional products, therefore it is recommended to consult with regulatory agencies early in the development program. It is also important to maintain an awareness of emerging and evolving regulatory expectations and industry practice on an ongoing basis. One of the challenges in the future will be that as more products are developed and an increased number of innovators enter the market, airtime with the agencies may become harder to negotiate.

References

1. European Council (1993) Directive 93/42/EEC of 14 June 1993 concerning medical devices.
2. European Council (1990) Directive 90/385/EEC of 20 June 1990 on active implantable medical devices.
3. European Council (1998) Directive 98/79/EC of 27 October 1998 on in vitro diagnostic medical devices.
4. FDA CDRH Overview of device regulations. http://www.fda.gov/cdrh/devadvice/overview.html
5. Global Harmonization Task Force. http://www.ghtf.org/index.html
6. Center for Drug Evaluation and Research (CDER) guidelines, http://www.fda.gov/cder/regulatory/default.htm
7. Center for Biologics Evaluation and Research (CBER) guidelines, http://www.fda.gov/cber/guidelines.htm

8. European Agency for the Evaluation of Medicines (EMEA) Committee for Medicinal Products for Human Use (CHMP) guidelines. http://www.emea.europa.eu/index/indexh1.htm
9. International Conference on Harmonisation technical requirements for registration of pharmaceuticals for human use (ICH) guidelines. http://www.ich.org/cache/compo/276–254–1.html
10. FDA Office of Combination Products. http://www.fda.gov/oc/combination/
11. FDA (2006) Guidance for industry and FDA staff: early development considerations for innovative combination products, Sep. 2006.
12. European Council (1965) Directive 65/65/EEC of 26 January 1965 on the approximation of provisions laid down by law, regulation or administrative action relating to proprietary medicinal products.
13. European Council (2004) Directive 2004/27/EC of 31 March 2004 amending Directive 2001/83/EC on the community code relating to medicinal products for human use.
14. EMEA (2001) Vol. 1, Pharmaceutical Legislation, 2001/83/EC and amendments.
15. EMEA (2001) Guidelines relating to the application of the council directive 90/385/EEC on active implantable medical devices the council directive 93/42/EEC on medical devices, MEDDEV 2.1/3 rev 2 (July 2001).
16. Pharmaceutical and Medical Devices Agency (PMDA) Japan website. http://www.pmda.go.jp/english/index.html
17. Japan Pharmaceutical Manufacturers Association website. http://www.jpma.or.jp/english/index.html
18. International Organization for Standardization (ISO) standards. http://www.iso.org/iso/iso_catalogue.htm
19. ISO 11608 Pen injectors for medical use.
20. EMEA CHMP (Oct 2006) Guideline on the pharmaceutical quality of inhalation and nasal products, EMEA/CHMP/QWP/49313/2005 Final.
21. EMEA CHMP Overview of comments received on draft guideline on the pharmaceutical quality of inhalation and nasal products, EMEA/CHMP/CVMP/QWP/103155/2006.
22. FDA CDER (1998) Guidance for industry: metered dose inhaler (MDI) and dry powder inhaler (DPI) drug products, Oct. 1998, DRAFT.
23. FDA CDER (2002) Guidance for industry nasal spray and inhalation solution, suspension, and spray drug products.
24. Relie H (2007) Implications of the new CHMP Guideline on the Pharmaceutical Quality of Inhalation and Nasal Products, Scientific Thesis for Master of Drug Regulatory Affairs, University of Bonn http://www.dgra.de/studiengang/pdf/master_reile_h.pdf
25. IPAC-RS (2001) A parametric tolerance interval test for improved control of delivered dose uniformity in orally inhaled and nasal drug products.
26. United States Pharmacopoeia (2008) USP 30-NF 25 Aerosols, nasal sprays, metered-dose inhalers, and dry powder inhalers ¡601¿.
27. European Pharmacopoeia (2007) 5th Edition, Preparations for Inhalation, pp 2843–2847.
28. Berry J et al. (2003) Influence of the storage orientation on the aerodynamic particle size of a suspension metered dose inhaler containing propellant HFA-227. Drug Dev Ind Pharm 29 (6):631–639
29. Murata S, Ito H, Izumi T, Chikushi A (2006) Effect of moisture content in aerosol on the spray performance of Stmerin® D HFA Preparations. Chem Pharm Bull 54 (9):1276–1280
30. Borgstrom L et al. (2005) An in vivo and in vitro comparison of two powder inhalers following storage at hot/humid conditions. J Aerosol Med 18 (3):304–310.
31. Chi Lip Kwok P, Chan HK (2008) Effect of relative humidity on the electrostatic charge properties of dry powder inhaler aerosols. Pharm Res 25 (2):277–288
32. IPAC-RS Foreign Particles Working Group (Dec 2004) Foreign particles testing in orally inhaled and nasal drug products. Pharm Res 21 (12):2137–2147
33. IPAC-RS Foreign Particles Working Group (March 2007) Best practices for managing quality and safety of foreign particles in orally inhaled and nasal drug products, and an evaluation of clinical relevance. Pharm Res 24 (3):471–479

34. WHO Stability testing of active pharmaceutical ingredients and pharmaceutical products QAS/06.179/Rev.2 DRAFT
35. EMEA CPMP (2001) Note for guidance on in-use stability testing of human medicinal products CPMP/QWP/2934/99.
36. ASEAN Guideline on stability study of drug product (2005)
37. FDA Device advice, premarket notification 510(k).
38. FDA Jurisdictional Update: Drug-Eluting Cardiovascular Stents.
39. Boam AB (2003) Drug-eluting stents: an FDA case study on regulating combination products. Regul Aff Focus Mag, 18–23
40. EMEA CHMP (2007) Guideline on the development of medicinal substances contained in drug-eluting (medicinal substance-eluting) coronary stents, draft January 2007, EMEA/CHMP/EWP/110540/2007.
41. FDA (2008) Guidance for industry: Coronary drug-eluting stents–nonclinical and clinical studies [DRAFT] March 2008.
42. FDA (2003) Cypher™ Sirolimus-eluting coronary stent, summary of safety and effectiveness data.
43. FDA (2004) Taxus™ Express 2™ coronary stent, summary of safety and effectiveness data.
44. Hunter WL (2006) Drug-eluting stents: Beyond the hyperbole. Adv Drug Delivery Rev 58:347–349
45. Kamath KR, Barry JJ, Miller KM (2006) The Taxus™ drug-eluting stent: A new paradigm in controlled drug delivery. Adv Drug Delivery Rev 58:412–436
46. FDA (2005) Guidance for industry and FDA staff: non-clinical tests and recommended labeling for intravascular stents and associated delivery systems.
47. ANSI Biological evaluation of medical devices – Part 13: Identification and quantification of degradation products from polymeric devices, ANSI/AAMI/ISO 10993-13:1999
48. Dilcher C et al. (2004) Effect of ionizing radiation on the stability and performance of the TAXUS Express2 paclitaxel-eluting stent. Cardiovas Radiat Med 5:136–141
49. Sternberg K et al. (2007) In vitro study of drug-eluting stent coatings based on poly(l-lactide) incorporating cyclosporine A – drug release, polymer degradation and mechanical integrity. J Mater Sci: Mater Med 18 (7):1423–1432
50. Sharkawi T et al. (2007) Intravascular bioresorbable polymeric stents: A potential alternative to current drug eluting metal stents. J Pharm Sci 96 (11):2829–2837
51. Lewis AL et al. (2002) Long-term stability of a coronary stent coating post-implantation. J Biomed Mater Res 63 (6):699–705
52. Boam AB (2003) Innovative systems for delivery of drugs and biologics; drug-eluting stents current approach to review. FDA workshop Innovative systems for delivery of drugs and biologics: scientific, clinical and regulatory challenges.
53. Portnoy S, Koepke S (2005) Obtaining FDA approval of drug/device combination products. Part I: Regulatory strategy considerations for preclinical testing; Part II: Drug testing requirements. http://www.pharmanet-cro.com/pdf/whitepapers/Combo_Products.pdf
54. Van Antwerp B, Gulati P (2003) Protein delivery from mechanical devices; Challenges and opportunities, FDA workshop Innovative Systems for Delivery of Drugs and Biologics: Scientific, Clinical and Regulatory Challenges.
55. Brennan JR, Gebhart SS, Blackard WG (1985) Pump induced insulin aggregation. A problem with the Biostator. Diabetes 34:353–359
56. Jones SA, Brown MB (2005) Evolution of transdermal drug delivery. Pharmaceutical Formulation and Quality, September 2005
57. Wokovich AM et al. (2006) Transdermal drug delivery system (TDDS) adhesion as a critical safety, efficacy and quality attribute, Eur J Pharm Biopharm 64:1–8
58. Minghetti P, Cliurzo F, Casiraghi A (2004) Measuring adhesive performance in transdermal delivery systems, Am J Drug Deliv 2 (3):193–206
59. Mukherjee B et al. (2006) Nefopam containing transdermal-matrix patches based on pressure-sensitive adhesive polymers. Pharm Tech 30(3):146–163

60. Gupta R, Mukherjee B (2003) Development and in vitro evaluation of diltiazem hydrochloride transdermal patches based on povidone–ethylcellulose matrices. Drug Dev Ind Pharm 29 (1):1–7
61. EMEA (2005) Guidance guideline on plastic immediate packaging materials, CPMP/QWP/4359/03.
62. FDA (1999) Guidance for Industry: Container Closure Systems for Packaging Human Drugs and Biologics, May 1999.
63. ITFG/IPAC-RS (2001) Leachables and extractables testing: Points to consider, March 2001.
64. PQRI (2006) Safety thresholds and best practices for extractables and leachables in orally inhaled and nasal drug products. PQRI (September 2006) http://www.pqri.org/pdfs/LE_Recommendations_to_FDA_09-29-06.pdf
65. Norwood DL et al. (2008) Best practices for extractables and leachables in orally inhaled and nasal drug products: An overview of the PQRI recommendations. Pharm Res 25 (4):727–739
66. Ball D et al. (2007) Development of safety qualification thresholds and their use in orally inhaled and nasal drug product evaluation. Toxicol Sci 97 (2):226–236
67. International Conference on Harmonisation Guideline Q1D: Bracketing and matrixing designs for stability testing of drug substances and drug products.
68. Munden R (2005) The role of bracketing and matrixing in efficient design of stability protocols. Pharm Tech Europe 17 (12):48–52
69. International Conference on Harmonisation Guideline Q5C: Quality of biotechnological products: stability testing of biotechnological/biological products.

Chapter 17
Stability Studies for Biologics

Anthony Mazzeo and Patrick Carpenter

Contents

Abstract Biological products represent a growing segment of the pharmaceutical industry. Stability studies of these complex biologics present challenges beyond those found for the typical small-molecule pharmaceutical. Biologic products are typically only marginally stable, not entirely understood, may demonstrate non-Arrhenius behavior, degrade by multiple pathways and possibly different pathways during different stages of shelf life. Further, subtle changes brought on by stresses can have large effects on the therapeutic properties of the product. There are analytical methodology challenges pertaining to monitoring stability as well, in particular the higher variance and complexity of the product and methodology. The issues and strategies involved in studying the stability of biologic protein products, particularly for the purposes of product registration purposes are discussed as well as an overview of ICH Q5C Quality of Biotechnological Products: Stability

A. Mazzeo (✉)
Exploratory Biopharmaceutics and Stability, Pharmaceutical Development Department, Bristol-Myers Squibb Company, 1 Squibb Drive, New Brunswick, NJ 08903 USA
e-mail: Tony.mazzeo@bms.com

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Testing of Biotechnological/Biological Products. Stability protocols should be designed keeping in mind the idiosyncrasies of biologics as well as formulation, analytical, manufacturing, and regulatory knowledge gained during development.

17.1 What Are Biologics?

This chapter discusses the issues and strategies involved in studying the stability of biologic products, particularly for the purposes of product registration, but also for product development purposes. Much of what needs to be performed toward this end is similar to what would be performed for small molecule products; however, the nature of the active biological substances and the resulting more limited knowledge of them requires some careful thinking and different approaches.

Biologics include such products as proteins, monoclonal antibodies, conjugated protein systems, and some polypeptides (some polypeptides can be treated as small-molecule drugs). The drug substances are macromolecules, which are more difficult to formulate and develop as a product than small molecules, but offer the promise of being target-specific and very potent in their medicinal functionality. Generally, the active substance has been produced or at least originated from a biological process, either by fermentation or by a specific cell-culture expression system, by a biotech process such as recombinant DNA (rDNA) technology, or by harvesting from a living organism. A related class of products, usually referred to as biologicals, is pharmaceutical products obtained directly from living organisms. Examples of biologicals are blood plasma products, vaccines, antivenoms, immunoglobulins, and allergenic extracts. Other potential products can be considered biologic or biotechnological products as well. This chapter will emphasize protein-based products, which are the most common biologic pharmaceuticals.

The key aspect of biological drug substances and products is that they are more labile compared to most traditional small molecule pharmaceuticals. Generally, they require low-temperature storage conditions such as refrigeration (2°–8°C), freezing (−10° to −20°C), or even ultra-low (−40° to −80°C) storage temperatures. This necessitates qualifying low-temperature stability chambers. On the other hand, for cold or frozen products, there is no difference whether the registration is targeted for climatic Zone I, II, III, or IV. Another aspect to keep in mind is that biologics are generally very costly and often time consuming to produce and some may be produced only in small batches due to the nature of the technology involved.

Due to the molecule's fragility, cost, and low-temperature requirements, once the biologic material is produced, preserving it in inventory and throughout distribution is of paramount importance. Cold-chain issues become very important, especially when shipping biologics across international borders where delays can be encountered. The stability scientist should be aware of the shipping methods and needs to design stability studies that will support excursions that are likely to be encountered. Likewise, the stability limitations due to stress testing discovered during product development need to be communicated to shipping and packaging engineers so that adequate shipping methods can be planned and qualified prior to product launch.

Because of this additional testing and the need to understand the product's storage and shipping limitations, it is easy to see that there needs to be a balance between the high cost of testing and the need to cover as wide of a *design space* as possible prior to the launch of the product. For this reason, it is important for the stability scientist to leverage knowledge gained during development on the formulation, manufacturing process and packaging, and gain a thorough understanding of the analytical methodology and regulatory aspects of the biologic product to be studied. That knowledge can be used to keep stability designs to a practical level yet cover the important quality parameters.

17.2 Biologics Versus Small Molecules

As mentioned earlier, biologics need to be treated with some extra consideration when addressing their pharmaceutical stability. The biological activity of a protein, for instance, comes not only from its covalently bonded primary structure, but also from the folded conformation that makes up the secondary and tertiary structure. The conformation can be easily altered without breaking any covalent bonds, and once in this denatured state, some or all of the biological activity that makes the protein a useful therapeutic medicine may be lost.

There is also the issue of heterogeneity of the protein forms. For example, a glycoprotein may be produced by a biological process that results in creation of several similar glycoforms. One or more of the forms may possess the desired therapeutic properties. It may be difficult to tell in vitro if there is any activity difference between the forms or whether some forms affect patients more than others.

Purity for a small molecule is a relatively simple concept. Normally, an HPLC method is sufficient to measure the content and impurity levels of a small molecule drug. A macromolecule, such as a protein, has a much more complex behavior. Determining protein concentration by UV absorption spectroscopy can give a measure of the total protein in the product, but it will not necessarily differentiate between active protein and inactive protein (i.e., denatured or otherwise degraded). A validated method or methods to determine the biological activity of the molecule is needed. So, whereas protein concentration is usually tested as part of the specifications, it is also normally accompanied by one or more methods that measure or correlate to biological activity. This is the bioassay. These methods can be animal-based or cell-based, protein interaction assays, binding methods such as surface plasmon resonance or ELISA (enzyme-linked immunosorbent assay) and immunoblot methods.

Size-exclusion HPLC (SE-HPLC), peptide digest mapping, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), isoelectric focusing (IEF), and other electrophoretic gel methods together give a good measure of the distribution of proteins, fragments, and side-chain modifications. Each of these assay methods give different types of information on the impurities present, and together give a purity profile for the biologic. An overview of a variety of these bioanalytical methods, although not exhaustive, can be found in the references [1].

Table 17.1 Comparison of biologic products vs. small molecule products

Topic	"Typical"small molecule products	"Typical" biologic products
Manufacturing Process	Synthetic chemical process	Fermentation or rDNA technology. Generally expensive to produce, often only produced in small batches, production site transfers are difficult
Formulations	Solid oral	Parenteral
Knowledge	Stability and potency generally determined by covalent structure	Very complex molecule relying on both covalent and conformational 3-D structure with a multitude of reaction sites for degradation
Storage	"Room Temperature" products	Refrigerated, frozen, or deep freeze
Specifications	5–10 methods, ICH Q6A	Many methods (10 to 20+) required to profile and characterize the protein, ICH Q6B
Assay	Generally HPLC is sufficient for assay, identification, and impurities	Generally overall protein concentration, plus at least one specific bioassay and one or more assays showing binding correlated with clinical experience during development
Analytical Methods	Typically, HPLC based and relatively sensitive, precise, and accurate. Relatively fast methods.	Biosassay and electrophoretic techniques which are generally less precise, and may lack sensitivity. Methods are slower and generally more costly.
ICH stability guidelines	ICH Q1A through Q1E	ICH Q1A through Q1E and Q5C
US filing	New drug application (NDA)	Biologics license application (BLA)

For stability studies, it is likely several of these types of assays will be used, each providing information on different characteristics of the molecule or information on the different types of degradation pathways. It is common that many tests (relative to what is performed for small molecules) are performed to characterize a biologic substance or product to give assurance of potency, purity, and quality. Table 17.1 (see section 17.3) gives a quick comparison of the differences between a biologic and small molecule drug product.

17.3 Common Degradation Pathways for Proteins

It would be difficult to give a complete picture of the endless possibilities for protein degradation in a handbook on pharmaceutical stability and there are already numerous references in the literature covering the many different degradation pathways, a tiny fraction of which are given in the discussions below. However, it is useful to note that there are several common degradation routes for protein products that are typically studied to determine the stability of biologic products. A key point to remember is that both covalent and noncovalent forces can lead to subtle changes

in the protein conformation and therefore drastically alter its biological activity or physical stability. Whereas, degradation by breaking a covalent bond requires a fair amount of energy (200–400 kJ/mol), the weaker forces, such as hydrophobic interactions and hydrogen bonding, require only about 4–30 kJ/mol to disrupt [2]. Small perturbations that disrupt those weak forces can have a big affect on the protein conformation, and therefore its bioactivity, as well as expose the protein to further chemical degradation. Since macromolecules are such complex structures, it is common to see non-Arrhenius behavior [3–5]. However, many degradation processes of proteins can demonstrate Arrhenius behavior [6, 7]. Protein formulations tend to have poor photostability as well, since many of the amino acid residues themselves are prone to photolytic degradation [8]. It can be summed up simply that biologics are only marginally stable, and relatively minor changes, even to one amino acid residue in the macromolecule, can change their activity, pharmacokinetics, or their ability to fit the receptor. A summary of common problems with the stability of proteins is given in Table 17.2.

The major degradation pathways can be categorized as aggregation, denaturation, oxidation, and deamidation. Although other pathways can be important; these include adsorption onto container components [9], fragmentation [10, 11], deglycosylation, or in formulations containing saccharides, glycosylation [12], and of course destruction of disulfide bonds that may hold the tertiary structure together. Because of the nature of biotech products, other complications can find their way into the spotlight to the consternation of developers. For example, proteolytic enzymes making their way through the purification steps and into the final product (and stability samples) causing proteolytic fragmentation. Although it is the job of the process development scientists to prevent such enzymes from getting into the final product, the stability scientist should be aware that such impurities are possible and the effect of their presence may only show up in longer-duration studies.

Denaturation is described as a disruption or unfolding of the protein's natural secondary or tertiary structure. This is often an irreversible process. It can be initiated by any number of influences, but heat is probably the most common. Unfolding of the protein as a result of a denaturation process can expose otherwise protected amino acid side-chains to chemical degradation [13].

Table 17.2 Common problems with stability of proteins

Usually sensitive to light, heat, air, and trace metal impurities
Small or large stress factors can disrupt protein folding
Numerous chemical degradation routes possible
Numerous physical degradation routes, including agitation, freezing, interaction with surfaces and phase boundaries
Non-Arrhenius behavior
Possibility of different degradation mechanisms appearing depending on the age of the product
Possibility to find proteases left from biotech processes
One type of degradation can facilitate other types of degradation leading to a cascading effect
Limited formulation options

Aggregation is the formation of complexes between macromolecules. They can be dimers, trimers, and heavier multimers. The complexes may be covalently bonded or just associated through hydrophobic interactions. The formation of aggregates can cause changes in protein binding and activity (potency), and have been implicated in immunogenic reactions in the patient. Control of aggregate formation during process and formulation development is, therefore, very important as well as development of methods for the determination of aggregation. Any number of factors can bring about aggregation, most notably heat and pH [14] although it can occur without much stress at all [15]. In the extreme, aggregation can lead to precipitation of the protein [16].

Oxidation occurs generally on the amino acid side chains due to exposure to air, residual peroxide from excipients, or exposure to visible or ultraviolet light. In particular, methionine, cysteine, tryptophan, and tyrosine are prone to oxidation. Metal ions such as iron, zinc, copper, or tungsten from metals that are used in the manufacturing process, leached from contact materials, or present in trace amounts in excipients can catalyze oxidation as well as other degradation processes [2, 17].

Protein deamidation occurs with asparagine and glutamine. It has been shown that protein conformation can affect the rate of deamidation and vice versa [13, 18].

Excipients in the formulation can present additional opportunities for degradation of the protein. As already mentioned, residual peroxides can oxidize side chains [19] and the use of saccharides and polyols, while adding some stability [20, 21], can lead to glycosylation and other reactions that affect the product quality [22, 23].

Degradation processes as discussed above, even those that seem small compared to the relatively large size of the macromolecule, can bring about very large changes in the secondary and tertiary structure of a therapeutic protein. In order to monitor all of these possibilities, multiple types of analytical methods are necessary in the stability studies for biologics, and it is not always clear which method or methods gives the most relevant information on the state of the protein therapeutic agent.

17.4 Other Stability Considerations

As previously mentioned, the nature of biologic products brings along some interesting challenges. The formulations are usually parenteral, with only rare exceptions. While many aspects of stability studies for parenterals hold true whether for small molecule or large molecule, it is important to reiterate some of these aspects for biologics since it is practically a given that if you are developing a biologics product, then you are developing a parenteral. Lyophilized products offer some added stability, but liquid formulations and ready-to-use products are also desired by clinicians since these are easier to use and can, in some cases, be self-administered by patients. In many cases, however, the product may require a constitution step and/or dilution before administration. The compatibility of the diluents as well as all contact materials, for example stainless steel needles, polyvinyl chloride (PVC) and

non-PVC IV bags, filters and associated tubing, should all be considered in an in-use study. Other factors need to be considered as well. Constitution may be performed in the vial with the use of a syringe to add the diluent. Care needs to be taken not to agitate the protein during this process as this can lead to degradation [24]. Syringe plungers and barrels, needles, and other components may be coated with silicone oil that may lead to undesirable interactions with the formulation such as clouding of the solution or aggregation [25]. Add the additional *global complication* that any in-use materials that are tested in the developer's laboratory may not necessarily be considered the same kind or quality as those that will be available in Europe or Asia or South America, etc. Pharmacopeial harmonization efforts may bring some relief to this situation in the future, but for now, care should be taken that constitution stability studies are relevant for the countries targeted for product registration. These compatibility/in-use stability studies may be covered during formulation development, however, as a requirement in ICH Q1A(R2), data must be collected on the stability of the constituted products for inclusion in the filing. Depending upon how many different diluents, administration set-ups, and concentration ranges of the constituted solutions are necessary; these in-use stability studies can become large and laborious. Bracketing and matrixing strategies would be put to good use in designing these compatibility/constitution studies.

Biologics not only tend to be parenteral drugs, but it is also commonly necessarily for them to be stored cold, either refrigerated or frozen. While this does not present a big problem for stability studies as long as the proper qualified storage chambers are available, cold-chain shipping presents a major challenge for these typically labile products. Manufacturers of biologics can go to great lengths and expense to ensure their cold-storage products can be shipped reliably and with minimal temperature excursions. There has been a lot of recent activity in the industry to come to some reasonable solutions for shipping cold and very valuable products around the country and globally [26, 27]. For biologics, special consideration is required to balance the need to keep the products cold enough during shipping, yet keep the cost of doing so to practical levels.

In the previous sections, it has been discussed that biologic products contain complicated molecules, which are only marginally stable and not well understood, the behavior of which is not necessarily Arrhenius, and where subtle changes brought on by large or small stresses can have far-reaching effects on the therapeutic properties of the product. For these reasons, the typical freeze–thaw or short-term heat–stress studies normally performed to support storage and shipping excursions may not necessarily be enough to ensure product quality over the shelf life of the product. Even if a liquid product is shown to survive freeze–thaw testing after several freeze–thaw cycles when compared to the specifications, the data collected from such a study may not cover the worst-case scenario shipping stresses. A protein formulation may survive phase changes that occur quickly in a classic freeze–thaw cycle, for example between –20° and 25°C. However, the rate of freezing (or thawing) may be slower in a real shipping scenario. The rate of freezing has been shown to affect protein denaturation in lyophilization cycles [28] and the possibility of a liquid formulation spending significant time in a partially frozen "slush" condition may induce

more protein unfolding and subsequent aggregation than a quick freeze [24]. In the partially frozen state, the protein can be subjected to pH, ionic strength, and concentration gradients caused by the partial freezing or melting. These conditions can potentially affect the protein conformation permanently, or temporarily expose otherwise protected amino acid residues for degradation.

In many cases, it will be found that that the short-term stress will cause some degradation in a biologic formulation. The question is whether that degradation will also affect the target shelf life of the stressed product. There are theoretical calculations that can be performed to help predict the shelf life of a stressed sample and as long as the models used are shown to be relevant for the product in question, they may be of some use. However, for biologics, experimental data are necessary to lessen the risk of unwanted surprises and/or to mitigate the need for very expensive cold-chain shipping containers and systems. It is therefore recommended that a limited study be performed where stability samples are subjected to an excursion-like stress, by heat or freezing or light exposure, etc., and then placed at the intended storage condition with data being collected occasionally out to the intended shelf life. An example of such a protocol is given in Table 17.7 (see Section 17.5.5.2).

17.5 The ICH Q5C Guideline

Guidance for the design of registrational stability studies of biologics can be found in ICH Guideline Q5C, *Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products,* along with all the ICH Q1 stability guidelines. The ICH Q5C guideline gives general guidance on the expectations for the body of stability data needed to register biologics in ICH countries. It does not necessarily apply to such products as vaccines, antibiotics, heparins, vitamins, allergenic extracts, and other products derived from *traditional* biological or fermentation processes. Consultation with your regulatory department and specific country regulations is recommended to confirm the applicability of the guidance to a specific product. This section will review the salient points for biologic stability studies and those in particular that may differ from the general ICH Q1A(R2) guideline, *Stability Testing of New Drug Substances and Products.* Note however, that the ICH Q1 stability guidelines are generally applicable to biologics and are a good starting place for designing stability protocols.

17.5.1 Drug Substance Stability

Similar to the requirements for small molecules, at least three batches of drug substance of pilot or full scale batch size and representative of the process used in preclinical, clinical, and proposed manufacturing scale should be studied. If pilot scale lots are used in the stability study for the Biologics License Application (BLA), a commitment must be made to place the first three commercial batches on stability.

The containers used to store the samples may be of reduced size, but should be constructed of the same material and fitted with the same container/closure system proposed for the manufacturing process. An important point when determining the container for biologics is the likelihood that the drug substance will be stored long-term in cold temperatures, some down to −80°C. Plastic containers and their closure systems should be checked for their durability and brittleness when subjected to such cold temperatures. What is not specifically mentioned in the guidance is that frozen drug substance obviously will be thawed before use in manufacturing of the product. Sometimes the thawing process is performed in a step-wise manner so as not to damage the macromolecules, and the thawed material kept at a holding temperature until it is ultimately used for manufacturing product. Usually a maximum holding time is determined and shorter times can be used at the discretion of the manufacturing planners. This maximum holding time needs to be supported by stability data and the holding time needs to be considered in determining the ultimate use-period of the bulk drug substance. The hold-time study can be based on a reduced testing scheme as long as the critical quality factors are assured. The stability scientist should consult with manufacturing and perhaps even quality assurance personnel to reach agreement on the maximum hold-time before designing the hold-time stability study. An example is given in Table 17.5 (see Section 17.5.5.2).

17.5.2 Drug Product Stability

Stability information for biologic drug products is expected on at least three batches that are representative of the manufacturing scale batches, packaged in the primary containers, and representative of the product used in clinical trials. Pilot-scale batches may be used with a commitment that the first three manufacturing-scale batches are placed on stability after approval. This is the same strategy as can be used for small molecules. For biologics, it cannot be assumed that a minimum amount of data will receive an extrapolated shelf life from regulatory agencies. Generally, dating of the product will be based on the real-time data collected at the intended storage condition. The reasons for disallowing limited extrapolation for shelf life determination are the non-linear degradation pathways that are more prevalent in biologics and also the possibility that the biologics degrade through different mechanisms as the product ages. This is not to say it is impossible to get some extension of the shelf life with limited data. It may be reasonable to request some extrapolation given a good body of relevant supporting data, product history, clinical experience, etc.

17.5.3 Matrixing and Bracketing

Matrixing and bracketing are of potential use as long as care is taken to show that the stability samples tested properly represent the stability of all samples. In fact, given

the cost of product and bioanalytical test methods, matrixing and bracketing should always be considered. Additional information on the matrixing and bracketing concept can be found in Chapter 15. Prior consultation with manufacturing, analytical, and statistical staff is recommended, as biologic lot-to-lot and bioanalytical method variability can be high, thus thwarting reduced design efforts. It is not uncommon for the stability design to be the subject of an End of Phase II meeting with the FDA prior to starting stability studies. However, the stability scientist should also be aware of the entire filing strategy, including which countries are targeted for filing, when the filing is scheduled, and whether those countries will accept a reduced stability design. Consultation with the regulatory department is also recommended.

In some cases, the biologic may be relatively labile, resulting in a shelf life of 6 months or less. These instances should be discussed with the agency on a case-by-case basis as recommended in the ICH guidance.

17.5.4 Stability Tests

Stability tests for biologics will be determined by the nature of the particular product, manufacturing process, and formulation. Common tests are listed in ICH Guideline Q6B, *Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products.* Some of those tests are important for monitoring the stability of the drug substance or product. Other tests may be substituted if shown to serve the same purpose. As the testing technology advances, it would be advantageous to both manufacturer and regulatory agency to pursue those newer methods. As is the case with any Good Manufacturing Practice (GMP) activity, the stability indicating methods must be validated for use in a registrational stability study (e.g., for a BLA, etc.).

Specifications and tests for drug substance and drug product are categorized in the ICH guideline simply as

- Appearance and description
- Impurities
- Potency
- Quantity

In addition, other tests specific to the drug substance and formulation may apply, such as sterility, microbial limits, bacterial endotoxin, and pH. These types of tests would also be called for in order to comply with local pharmacopoeial requirements. Tests for subvisible particles beyond what is required in the pharmacopoeia may also be necessary due to immunogenicity concerns [29].

The trending of potency and impurities over time can be challenging for biologics and several methods may be necessary to profile each of these attributes. First, as discussed earlier, there is not necessarily a direct link between concentration (quantity) and potency, since the concentration test, usually UV spectrophotometry, does not give information on biological activity, it simply gives protein concentrations

and does not discriminate between active and inactive molecules. Bioassays and binding methods are more relevant for measuring potency. Even so, the potency assay may have to be correlated with clinical results to show that the assay is producing relevant potency information. The inherent problems with the specificity and accuracy of bioassays have resulted in the recommendation in the ICH Guideline Q6B that "the purity of the drug substance and drug product is assessed by a combination of analytical methods." This can cause problems with methodology changes later in development or postapproval. Method transfers from lab to lab can also be problematic for bioassay and gel techniques.

Impurity monitoring is also tricky since there are multiple degradation pathways which are not necessarily detected by a single chromatographic or other method. Size-exclusion chromatography and SDS-PAGE may give some information on aggregation, but peptide mapping methods are needed to determine the degree of side-chain oxidation and deamidation, and isoelectric focusing is needed to detect changes in overall charged sites. It is important to keep in mind how the results of gel methods will be trended over time. Gels can be used for visual comparison with a reference standard, as is often done for identity testing purposes. However, if a gel migration pattern is to be used for trending purposes, it is necessary to develop a quantitative or at least semi-quantitative scheme, for example, based on the number of bands and/or band intensities, so that results can be compared from time point to time point. A list of common techniques used in stability studies are listed in Table 17.3.

17.5.5 Stability Protocols

There is virtually no difference in the general requirements for a stability protocol for biologics from that required for a small molecule. Stability testing must be

Table 17.3 Some common bioanalytical techniques for stability testing of biologics

Test	Method
Aggregation	SE-HPLC, Capillary electrophoresis, SDS-PAGE
Deamidation, Oxidation, Disulfide bond disruption	Peptide mapping
Cleavage, Isomerism	SDS-PAGE
Charge differences, isoforms	IEF
Protein concentration	UV/Vis spectrophotometery
Biological activity (potency)	Animal-based or cell culture-based biological assays, and biochemical assays
Immunochemical properties	Binding assays, ELISA, western-blot (immunoblotting methods)
Appearance, sterility, endotoxin, microbial limits, particulate matter, and other formulation specific tests	Test according to pharmacopeial requirements, as needed

carried out at the long-term storage condition until at least the intended shelf life. Accelerated and photostability tests would be performed as well. The stability indicating tests, of course, will be different for a biologic and it is very likely that there will be more tests than required of small molecules. Since the biologics are typically unstable at room temperature, the stability storage conditions will be aligned for cold storage products. Additionally, there may be more frequent testing for biologics due to this relative instability. Long-term stability test points at 1 month and sometimes days or weeks for the accelerated condition would not be out of place. However, the stability scientist needs to consider the available knowledge of the product gained during development as well as the manufacturing, regulatory, and analytical aspects of the specific drug substance or product before designing the protocol. For instance, a biological drug substance that has demonstrated stability at –70°C during research and development may not need to be tested at the 1-month interval; in other words, testing at three, six, nine, and twelve months, etc., may be just fine.

17.5.5.1 Example Drug Substance Protocol

Table 17.4 gives an example protocol of a biologic drug substance that is stored at –70°C, then thawed before use and held at refrigerated storage for up to several weeks before being used in product manufacturing. The 5°C condition is serving as the accelerated condition. In this full test design, the bioanalytical tests are performed at each time point designated with an *a.* Bacterial endotoxin and microbial limit tests are performed at each time point designated with a *b.* At time points designated with a *c,* a portion of the drug substance is removed from the deep freeze condition and thawed according to thawing instructions specific to the drug substance and container size. The thawed drug substance is stored at the holding temperature, in this example 5°C, and these samples are subjected to further stability tests out to the designated maximum hold-time, as shown in Table 17.5. This removal of samples from the long-term condition at designated time points for study at a different condition can be likened to what is done for constitution stability studies, in other words, some samples are removed, constituted, and studied in the constituted state for a set period of time. However, in this case, the drug substance is thawed and held at the holding temperature of 5°C and tested periodically to demonstrate stability throughout the hold time. Data is also collected at a higher temperature, for example 25°C, to support temperature excursions that may be encountered in commercial manufacturing.

Table 17.4 Example time/temperature schedule for drug substance

	Times in months							
Storage condition	0	1	3	6	9	12	18	24
−70°C	abc	a	a	ac	a	abc	a	abc
5°C	a	a	a	a	a			

Table 17.5 Time/temperature schedule for drug substance after thawing according to thawing instructions

Storage condition	Time in weeks				
	0	1	4	9	13
5°C	a	a	a	a	
25°C/40%RH		a	a		

17.5.5.2 Example Drug Product Protocol

Table 17.6 gives an example protocol for a lyophilized parenteral biologic drug product. Here the long-term condition is 5°C, and 25°C/60%RH is the accelerated condition. The bioanalytical tests occur at each time point designated with an *a*, and sterility and endotoxin tests are performed at each time point designated with a *b*. In addition, 5°C samples are constituted for use-time initially and annually until the end of the study. In the table, the scheduled use-time study is designated with a *u*. The –20°C condition helps support low temperature excursions. Photostability studies would be conducted according to ICH Q1B as well.

Now suppose, for the example given, that the product is a liquid biologic product and sensitive to light. We would also want some assurance that the biologic will survive typical excursions. The excursions may occur inadvertently during shipping and storage, or they may be experienced during handling, for example during a labeling process in a room temperature labeling area. As mentioned before, labile biologics may not immediately show problems from stress right away. In the example in Table 17.7, several stresses are combined, namely freeze–thaw, room temperature, and light exposure, to reduce the amount of testing which would be necessary if each stress was tested individually. This, of course, is a viable time-saving option only if development studies or other product knowledge indicate that the product is likely to survive such stress. Freeze–thaw for a liquid product would put the product through several phase changes in order to show the affect of the stress on the product. For a biologic, it would also be advantageous to know what happens if the product is put in a partially frozen condition as might occur during shipping. This might be done by passing through many freeze–thaw cycles or attempting to hold the samples at the partially frozen condition.

In the example, the stressed samples are tested after 2 days' and 2 weeks' worth of cycling, then the samples are placed in the intended 5°C condition and tested

Table 17.6 Time/Temperature schedule for drug product

Storage condition	Time in months										
	Initial	2w	1	3	6	9	12	18	24	30	36
−20°C		a	a								
5°C	abu		a	a	a	a	abu	a	abu	a	abu
25°C/60%RH			a	a	a						

Table 17.7 Schedule for samples exposed to several stress factors followed by storage at long-term condition 5°C

Storage temperatures/test groups[1]		
Time point	**Freeze–thaw –10°C/25°C and room light**	**Stressed long-term samples Stored at 5°C**
Initial	a	
2 days	a	
14 days	a	
1 m		a
6 m		a
12 m		a
24 m		a
36 m		a

occasionally to show that the samples will pass specifications out to the shelf life or that they are trending similar to the unstressed long-term samples. The examples given are one case of many possibilities and it cannot be stressed enough that before a registrational stability study is designed, the stability scientist needs to understand the product knowledge gained during development, the manufacturing issues, the regulatory issues, and the analytical issues.

17.6 Specification Setting

Setting specifications for biologics has been the subject of debate between industry and regulatory agencies for many years. Much of the debate centers on what the analytical tests really tell about the quality of the product, to what degree the bioassays can predict clinical potency, and what the impurity tests are really telling about the overall quality. The number of specifications that are necessary in terms of assays and *for information only* testing is debated as well. Since we have imperfect knowledge of the complex macromolecule, more specifications are required. Not all of these tests will be stability-indicating, however. In any case, as with small-molecule drugs, good stability data on several batches of product are required, along with clinical experience, knowledge of process consistency (a measure of lot-to-lot variability) and analytical variability, to help set specifications. The lot-to-lot variability and analytical variability can be relatively high for biologics. It is reasonable (and common) for a specification of a bioassay, with a target of

response of 100%, to have specification limits of 50% to 150%. These types of assay limits, of course, are virtually unheard of in the small molecule world. Specification setting for biologics is discussed in the ICH Q6B guideline and in the literature [30, 31].

17.7 Stability for Process Changes

Process changes for a biotech product may have far-reaching effects on a product or substance. Fortunately, many of the process parameters can lend themselves to Quality by Design approaches to reduce the regulatory burden of such changes. Even small changes in biotechnology processes require careful evaluation as to what, if any, stability studies are needed to ascertain if product quality will be affected. Again, subtle changes may have a great affect on the quality of a biologic, and some of the effects may not be detectible immediately after manufacture of the product. Biologics production processes are more sensitive to changes in starting materials and changes in production sites. Even changes in production suites in the same facility may be enough to warrant extra stability studies. Long-term and accelerated stability studies may be needed to demonstrate that the product will retain its quality attributes after the process change. Process changes are discussed in ICH Guideline Q5E, *Comparability of Biotechnological/Biological Products Subject to Changes in Their Manufacturing Process.*

17.8 Summary and Conclusion

Biological products represent a growing segment of the pharmaceutical industry. The interest in biologics stem from their specificity in interacting with complex biological processes in the body. Stability studies of these complex biologics present challenges beyond the typical small-molecule pharmaceutical. Biologic products are typically only marginally stable, not entirely understood, may demonstrate non-Arrhenius behavior, degrade by multiple pathways and possibly different pathways during different stages of shelf life, and subtle changes brought on by large or small stresses can have large effects on the therapeutic properties of the product. There are analytical methodology challenges pertaining to monitoring stability as well, in particular the higher variance and complexity of the product and methodology. Stability protocols should be designed keeping in mind these idiosyncrasies as well as formulation, analytical, manufacturing, and regulatory knowledge gained during development.

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References

1. Mikkelsen SR, Cortón E (2004) Bioanalytical Chemistry. Wiley Interscience, Hoboken.
2. Jacob S, Shirwaikar AA, Srinivasan KK, Alex J, Prabu SL, Mahalaxmi R, Kumar R (2006) Stability of proteins in aqueous solution and solid state. Indian J Pharm Sci 68(2):154–163.
3. Chan H, Dill K (1998) Protein folding in the landscape perspective: chevron plots and non-arrhenius kinetics. Proteins: Struct Funct Genet 30:2–33.
4. Collet O, Chipot C (2003) Non-arrhenius behavior the unfolding of a short hydrophobic α-helix. complementarity of molecular dynamics and lattice model simulations. J Am Chem Soc 125:6573–6580.
5. Roberts CJ, Darrington RT, Whitley MB (2003) Irreversible aggregation of recombinant bovine granulocyte-colony stimulating factor (bG-CSF) and implications for predicting protein shelf life. J Pharm Sci 92(5):1095–1111.
6. Pan B, Abel J, Ricci MS, Brems DN, Wang DIC, Trout BL (2006) Comparative oxidation studies of methionine residues reflect a structural effect of chemical kinetics in rhG-CSF. Biochemistry 45:15430–15443.
7. Yoshioka S, Aso Y, Izutsu K, Terao T (1994) Application of accelerated testing to shelf-life prediction of commercial protein preparations. J Pharm Sci 83(3):454–456.
8. Kerwin BA, Remmele RL (2007) Protect from light: photostability and protein biologics. J Pharm Sci 96(6):1468–1479.
9. Mizutani, T (1981) Estimation of protein and drug adsorption onto silicone-coated glass surfaces. J Pharm Sci 70(5):493–496.
10. Cohen S, Price C, Vlasak J (2007) β-Elimination and peptide bond hydrolysis: two distinct mechanisms of human IgG1 hinge fragmentation upon storage. JACS 129:6976–6977.
11. Cordoba AJ, Shyong B, Breen D, Harris RJ (2005) Non-enzymatic hinge region fragmentation of antibodies in solution. J Chrom B 818:115–121.
12. Werner R, Kopp K, Schlueter, M (2007) Glycosylation of therapeutic proteins in different production systems. Acta Paediatr Suppl 96(455):17–22.
13. Werne SJ, Creighton TE (1989) Effect of protein conformation on rate of deamidation: ribonuclease a. Proteins: Struct Funct Genet 5:8–12.
14. Tsai AM, Van Zanten JH, Betenbaugh MJ (1998) Study of protein aggregation due to heat denaturation: a structural approach using circular dichroism spectroscopy, nuclear magnetic resonance and static light scattering. Biotechnol Bioeng 59:273–280.
15. Kendrick BS, Cleland JL, Lam X, Nguyen T, Randolph TW, Manning MC, Carpenter JF (1998) Aggregation of recombinant human interferon gamma: kinetics and structural transitions. J Pharm Sci 87(9):1069–1076.
16. Chi EY,Krishnam S, Randolph TW, Carpenter JF (2003) Physical stability of proteins in aqueous solution: mechanism and driving forces in nonnative protein aggregation. Pharm Res 20(9):1325–1336.
17. Li S, Shöneich C, Borchardt, RT (1995) Chemical instability of protein pharmaceuticals: mechanisms of oxidation and strategies for stabilization. Biotechnol Bioeng 48:490–500.
18. Xie M, Schowen R, (1999) Secondary structure and protein deamidation. J Pharm Sci 88(1): 8–13.
19. Luo D, Smith SW, Anderson BD (2005) Kinetics and mechanism of the reaction of cysteine and hydrogen peroxide in aqueous solution. J Pham Sci 94(2):304–316.
20. Cleland JL, Lam X, Kendrick B, Yang J, Yang T, Overcashier D, Brooks D, Hsu C, Carpenter J (2001) A specific molar ratio of stabilizer to protein is required for storage stability of a lyophilized monoclonal antibody. J Pharm Sci 90(3):310–321.
21. Constantino HR, Carrasquillo, KG, Cordero RA, Mumenthaler M, Hsu CC, Gribenow K(1998) Effect of excipients on the stability and structure of lyophilized recombinant human growth hormone. J Pharm Sci 87(11):1412–1420.
22. Lai MC, Topp EM (1999) Solid-state chemical stability of proteins and peptides. J Pharm Sci 88(5):489–500.

23. Li S, Patapoff TW, Overcashier, D, Hsu C, Nguyen TH, Borchardt RT (1996) Effects of reducing sugars on the chemical stability of human relaxin in the lyophilized state. J Pharm Sci 85(8):873–877.
24. Kreilgaard L, Jones LS, Randolph TW, Frokjaer, Flink JM, Manning MC, Carpenter JF (1998) Effect of tween 20 on freeze–thawing- and agitation-induced aggregation of recombinant human factor XIII. J Pharm Sci 87(12):1597–1603.
25. Jones LS, Kaufmann A, Middaugh CR (2005) Silicon oil induced aggregation of proteins. J Pharm Sci 94(4):918–927.
26. PDA (Parenteral Drug Association) (2007) Technical report no. 39, Guidance for temperature-controlled medicinal products: maintaining the quality of temperature-sensitive medicinal products through the transportation environment.
27. The Gold Sheet, (September 2006) Global effort to control drug temperature in transit heats up. 40(9):1–19.
28. Martin SWH, Mo J (2007) Stability considerations for lyophilized biologics. Am Pharm Rev 10(4):31–36.
29. Carpenter JF, Randolph TW, Jiskoot W, Crommelin DJA, Middaugh CR, Winter G, Fan YX, Kirshner S, Verthelyi D, Kozlowski S, Clouse KA, Swann PG, Rosenberge A, Cherney B (2008) Overlooking subvisible particles in therapeutic protein products: gaps that may compromise product quality. J pharm sci (early view):1–5.
30. Fairweather WR, Mogg R, Bennett PS, Zhong J, Morrisey C, Schofield TL (2003) Monitoring the stability of human vaccines. J Biopharm Stat 13(3):395–413.
31. Schofield T, Apostol I, Koehler G, Powers S, Stawicki M, Wolfe R (2008) A rational approach to setting and maintaining specifications for biological and biotechnology-derived products. Biopharm International, (July 2008): 1–4.