Universidade de Lisboa
Faculdade de Farmácia

Analytical methods validation in the regulatory framework. Experimental designs, optimization, and evaluation.

The case of stability testing

Vivian Caroline Dadalt Reichert

Monografia orientada pelo Professor Doutor Luis Filipe Baptista Pleno de Gouveia, Professor Auxiliar

Mestrado Integrado em Ciências Farmacêuticas

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Resumo em português

Procedimentos analíticos são usados em diversos passos do desenvolvimento e fabrico de substâncias ativas e medicamentos. Muitas das decisões importantes são tomadas com base em resultados analíticos, obtidos necessariamente através de metodologias e procedimentos cuja adequabilidade haja sido demonstrada. Esta é uma das definições da validação analítica. A avaliação da estabilidade de uma substância activa ou de um medicamento segue procedimentos bem estabelecidos do ponto de vista regulamentar. O prazo de validade do medicamento, determinado através dos ensaios de estabilidade, corresponde ao tempo durante o qual os seus atributos críticos permanecem dentro dos limites de aceitação pré-definidos. Geralmente, os critérios de aceitação para o doseamento na especificação da libertação de lote são 100,0±5,0% do valor indicado no rótulo, e os critérios de aceitação típicos nas especificações de prazo de validade são de 100,0±10,0% do valor indicado no rótulo. Alguns atributos têm limites de aceitação definidos por documentos oficiais (como por exemplo o doseamento, a friabilidade) enquanto outros são específicos de cada medicamento (como por exemplo a dissolução ou a dureza). Existem vários aspectos a ter em consideração para definir limites apropriados para os atributos específicos do produto, na libertação de lote. Tais considerações devem incluir a incerteza no valor estimado do atributo aquando da libertação e a incerteza associada à estimativa da cinética da degradação. Neste trabalho, são discutidos os principais documentos regulamentares relacionados com a validação analítica e a sua adequabilidade para suportar a aplicabilidade dos procedimentos analíticos aos estudos de estabilidade. Defendemos que os atuais requisitos da ICH, relativamente à validação analítica destinada à avaliação da estabilidade, não garantem robustez adequada a todo o procedimento analítico. A formação adequada dos analistas, a manipulação adequada de materiais e dos equipamentos, a observação das boas práticas como por exemplo a manutenção adequada dos equipamentos, entre outros, são requisitos críticos para garantir que os resultados gerados no laboratório de CQ (controle de qualidade) são exactos e confiáveis. O processo de validação analítica deve ser capaz de detectar antecipadamente com elevada probabilidade a ocorrência de qualquer não conformidade ou de resultados inadequados, devidos a deficiências do procedimento analítico.

Palavras-chave: validação analítica, estudos de estabilidade, requisitos de qualidade ICH

Abstract

Analytical procedures are used throughout the development and manufacturing of drug substances and drug products. Important decisions are based on analytical results, which must be proved suitable for their intended use. This is the definition of analytical validation. Shelf life estimation aims to determine the storage time during which the critical attributes of the drug product stay within the acceptance limits. The stability assessment is a well-established procedure from the regulatory standpoint. Generally, the acceptance criteria for assay (potency) in the release specification are 95.0-105.0% of the label claim, and the typical acceptance criteria in the shelf life specifications are 90.0-110.0% of the label claim. Several considerations need to be given to set appropriate release limits. They include variability in the estimated attribute value at release and uncertainty with degradation rate estimate. In this work, we have reviewed the main regulatory documents related to the validation procedure and its capacity to support stability studies. We defend that the current ICH requirements of the analytical validation, when assessing stability, do not ensure a suitable robustness of the whole analytical procedure. at the QC (quality control) laboratory are accurate and reliable. The analytical validation already described and discussed should be able to detect any nonconformity or unacceptable results. Proper training of analysts, suitable handling of materials and equipment, good practice compliance and proper equipment maintenance are critical requirements to ensure the results generated at the QC (quality control) laboratory are accurate and reliable. The analytical validation should be able to detect any non-conformity or unacceptable results, what means safe medicines for the patients and resources saving for pharmaceutical industry.

Keywords: Analytical Validation, Stability analysis, ICH quality requirements

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Abbreviations

API – active pharmaceutical ingredient

EMA - European Medicines Agency

FDA – Food and Drug Administration, USA

FPP – finished pharmaceutical product

cGMP – current Good Manufacture Practices

ICH - International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

OOS – out-of-specification

RH – relative humidity

WHO - World Health Organization

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1 Introduction

1.1 Goals

The stability assessment of the API as well as the FPP is a well-established procedure from the regulatory standpoint. Several guidance documents were issued by regulatory agencies, for instance the EMA, the FDA, other agencies as the WHO and the ICH.

In fact, the first Quality guideline issued by the ICH addressed several aspects of assessment of the stability of API's and FPP's which demonstrates the relevance of the topic. The Q1A forms the parent guideline to a set of related guidelines (currently Q1B-E) containing additional details on the stability practice.

The next Quality topic addressed by the ICH was the validation of analytical methods. This quality guideline was initially published as two separate documents, "Q2A Text on Validation of Analytical Procedures", in March 1995, and "Q2B Validation of Analytical Procedures: Methodology", in May 1997.

These two general quality guidelines (stability and analytical validation) are obviously related as the qualitative or quantitative assessment of the quality attributes of the API or the FPP requires the previous validation of the analytical methods to be used. The validation of the methods for testing some attributes requires the knowledge of the chemical and/or physical degradation profile (which can be obtained via degradation at mild conditions or at stress conditions).

The validation focuses on generating documented evidence that the analytical method(s) are suitable to its intended purpose. The ICH Q2 provides an indication of the data should be presented in a regulatory context. Different analytical performance parameters are assessed depending on the nature of the tests (identification, quantitative, limit tests for impurities, for instance) and the acceptance limits or ranges are usually not stated and left to the interpretation of the regulatory bodies. This is a desirable approach as this flexibility allows the change or update of such limits as needed without the need to revise the guideline. On the other hand, this may lead to diverse interpretations and heterogeneity which was the reason why the ICH itself was created in the first place. Establishing acceptable criteria for analytical variability is crucial because other acceptance criteria can be derived from such a precision. Acceptable precision ranges can be achieved by validation, but also be extracted from batch release or stability studies. (1)

Almost three decades have passed since Q1A has been first approved (in 1993). In the meantime, the analytical hardware underwent a remarkable development, the computing power (hardware and software) is now millions-fold more powerful (in terms of speed and memory capacity) than before and the ability to compare results and access data and storage is outstandingly faster (2).

Despite these facts, the "science-based" nature of the guidelines issued by the ICH, the validation requirements of Q2(R2) are, in our view, too tolerant and led to real problems in the assessment of the stability of the API and FPP. This affirmation can be easily illustrated through the fact that, while we were writing this work, two batches of drug products were recalled from the Portuguese market because of OOS results during on-going stability studies.[1]

1.2 Ensuring the quality of medicines

The classic definition of a quality product is about exceeding customer's needs and expectations. Unfortunately (history has proved it through tragedies involving patients treated with improperly developed and produced drugs), in the case of pharmaceutical products, the customer (patients and prescribers) cannot assess the quality of the product they take. That is why the FDA statutorily defines acceptable attributes of pharmaceutical products for regulatory purposes. First, a production batch must deliver the same clinical performance as the investigational batches and prove safety, efficacy, and dosing as described by the label claim (3).

Beyond delivering clinical performance per label claims and not introducing additional risks due to contaminants, there is a second and essential part of the FDA definition of drug quality: the enforcement of cGMP regulations, following The Federal Food, Drug, and Cosmetic Act: cGMP regulations are *to assure that such drug meets the requirement of this Act as to the safety*

[1] Lorazepam Labesfal, 1mg, comprimidos, lot 21032, expiry date: 01/2023 (51); Vigantol 0,5mg/mL, oral solution, several lots (52)

and has the identity and strength, and meets the quality and purity characteristics, which it purports or is represented to possess. (4).

Once the concept of the quality drug is understood, one question arises: What aspects should be monitored and controlled to achieve it? The answer is not as simple as it looks. Drug substances and drug products must show conformity to a set of criteria described in the specifications that contain attributes, analytical methods, and acceptance criteria designed to ensure that the product is adequate for its intended use. Still, it is not enough (5). The ICH Q6A and Q6B clearly refers that *specifications are just one part of a comprehensive strategy for the drug substance and drug product designed to ensure product quality and transparency* (6,7) Thus, this strategy should not prescind a holistic view of all developing and manufacturing processes, including preclinical and clinical trials, analytical method development, stability studies, and process validation. Ideally, setting specifications is not compliance-driven anymore but riskbased (5).

Fundamentally, analytical methods provide data that gives information to make decisions. Acknowledging the risk of making the wrong decision is part of the decision-maker process (8). It can drive better decision-making for current product development processes (9).

As defined in ICH Q6A and ratified in WHO (10), a specification is a list of tests, references to analytical procedures, and appropriate acceptance criteria, which makes part of the control strategy, even for the drug substance or drug product. It is a list of analytical methods and the set of acceptance criteria the drug substance or drug product should conform to be acceptable for its intended use. Compliance with specifications proposed by manufacturers is mandatory by regulatory authorities for market approval (6, 11).

The criteria are different between batches for release and stability specification. As stated in the WHO TRS 970, two specifications may be set out: after packaging the drug product (release) and at the end of shelf-life. There is a roll of universal and specific tests and criteria for drug product. It must include tests for appearance, identification, assay, purity, performance tests, like dissolution, physical tests, uniformity of dosage units, and, as applicable, an assay of antimicrobial or chemical preservatives and microbial limit tests (10). Some apply just at release time, while others are used both at batch release and throughout the product's shelf-life

(expiration date). Stability studies are carried out to estimate the shelf-life and to prove the drug product remains safe and efficacious (5,12).

Stability tests are expected to monitor and analyse the drug product attributes susceptible to changes during storage that can influence the quality, safety, and efficacy. The acceptance limit for the drug product's API (active pharmaceutical ingredient) content in the release specifications is usually ± 5% of the label claim (95-105%) (10).

1.3 The assessment of stability and shelf-life estimation

1.3.1 The importance of the assessment of stability

As defined in the WHO TRS 1010, the purpose of assessment stability is to provide evidence of how the quality of an API (active pharmaceutical ingredients) or FFP (finished pharmaceutical product) varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light. It also includes evaluating how characteristics intrinsically related to the product itself can influence the stability parameters, such as the interaction API-excipients and all the different materials of packaging (13).

In the pharmaceutical industry, a drug substance or a drug product is more stable as it can retain the same properties and characteristics that it possessed at the time of its production, regarding its period of storage and use. The stability pre-market supports the clinical trials and is conducted throughout the filing period. Once in the market, the manufacturer must ensure the long-term monitoring of the strength of the drug product, using a stability-indicating method to establish the shelf life (15).

The concept of drug products must be present to understand how stability studies are designed and what shelf-life means. The FDA defines a drug product, or dosage form, as a *finished dosage form, for example, tablet, capsule, solution, etc., that generally contains an active drug ingredient but is not necessarily associated with inactive ingredients. The term also includes a finished dosage form that does not have an active ingredient but is intended to be used as a placebo* (16). The shelf-life period defines the time the drug product remains within approved specifications if stored under specific conditions (12). It indicates to the consumer how often a product is expected to be safe and effective (17).

1.3.2 The Regulatory requirements

The ICH Q1A, *Stability of New Drug Substances and Products*, was the first ICH Quality guideline published, in October 1993, following the foundation of The International Conference for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) in April 1990. The current version of ICH Q1A, the ICH Q1A(R2), results from two previous revisions in 2001 and 2003 (18). Although the FDA requires since 1979 that all prescription drug product have the shelf life printed on the container label, the ICH initiative to harmonize stability guidelines adopted by national regulatory authorities was the milestone to align critical stakeholders with overcoming the log jam in drug product registration and market in several countries and bring in uniformity in testing between manufacturers (17,19).

1.3.3 The WHO climatic zones

The temperature and humidity conditions used in the stability studies depend on the geographical zone where the product is intended to be marketed. As such, the classification of the climatic zones: I (temperate), II (subtropical and mediterranean), III (hot and dry), and IV, this last one split into IVa (hot and humid) and IVb (hot and very humid). The WHO recommends stability tests covering all climatic zones. As we can see in Table 1, the two general storage conditions are long-term (25°C ± 2°C/60% ± 5% RH (relative humidity) and accelerated (40°C ± 2°C/75% ± 5% RH). The intermediate storage condition can be used as an alternative to the long-term storage condition of zone I and II and thus harmonize the same long-term conditions in zone III and IV (20).

Table 1. Difference between the general storage conditions in zone I/II and zone III/IV, adapted from Khan and Akhtar (20)

Study Design	Storage Condition		Minimum time period covered by study for

	Zone I/II	Zone III/IV	submission of data
Long Term	25°C ± 2°C/60% RH ± 5%	30°C ± 2°C/65% RH ± 5%	12 months
Accelerated	40°C ± 2°C/75% RH ± 5%	40°C ± 2°C/75% RH ± 5%	6 months
Intermediate[1]	30°C ± 2°C/65% RH ± 5%	-------	6 months

[1]Alternate stability study condition (i.e. intermediate) in case the drug substance/product fails to meet the specifications at accelerated stability condition, RH=relative humidity

1.3.4 Stability studies regarding drug substance

Considering a systematic approach to stability evaluation, potential attributes and time testing points should be studied in the drug substance. Thus, the retest period, i.e., the period during which the drug substance is expected to remain within its specification and can be used for drug product manufacturing, should be derived from stability testing data. Even after this period, a batch of drug substance can be used for drug product manufacturing if tested for compliance specifications and used immediately. This procedure can be done multiple times as long as the set of drug substance continues to comply with the specification (12).

Overall, appearance, assay, and degradation products should be evaluated for all APIs. Some related substances identified as degradation products from stability studies must be monitored as part of API stability studies. If an API parameter is susceptible to change, like particle size or polymorphism for low-solubility API, its inclusion as a stability study specification is mandatory (12).

1.3.4.1 Stress testing

To retest period assessment, one of the tests the sample of drug substance should be submitted is stress testing to evaluate the effect of temperature, humidity, and, if applicable, oxidation and photolysis of the drug substance, as well as the susceptibility to hydrolysis under some pH ranges when in solution or suspension. A stress test can be carried out on a single batch. It should include the effect of temperature in 10°C increments above the temperature used for accelerated testing, humidity (75% relative humidity or more excellent, for example) manufactured at a minimum of pilot scale by the same route as production batches and packaged in the same container-closure system proposed for storage and distribution (12,13).

1.3.4.2 Related substances

The conditions studied in stress testing should cause degradation to occur to a small extent, not completely degrade the drug substance (the studies are designed to generate a 10-30% degradation in drug substance, compared to non-degraded drug substance), and this is determinant to establishing the conditions and duration of experiments. After ten days of total absence of degradation products, the drug substance is considered stable under those specific conditions. Yet, if a suitable and validated analytical procedure has been demonstrated that certain degradation products are not formed under accelerated or long-term storage conditions, the stress tests for this finality are dispensable (12).

1.3.4.3 Selection of batches

Regarding the data requirements, results from at least three batches of the drug substance should be provided to regulatory authorities. They should be manufactured at a minimum pilot scale by the same synthesis route as production batches. The objective is to obtain results representative of the quality of the material to be made on a production scale. The same concept can be applied to the container-closure system (12).

1.3.4.4 Testing frequency and storage conditions

For long-term studies, the periodicity of testing should be adequate to establish the stability profile of the drug substance. For a retest period of at least 12 months, the frequency for longterm condition testing is supposed to be every three months over the first year, every six months over the second year, and annually throughout the proposed retest period. At accelerated conditions, a minimum of three-time points for a six-month study, including the initial and final periods. Depending on the expected results, additional time points should be added. Any evaluation should cover the assay, degradation products, and other stability-indicating attributes (13). The general conditions for long-term, accelerated, and intermediate storage conditions for drug substance are described in Table 2. If a significant change occurs in long-term studies conducted at 25°C ± 2°C/60% RH ± 5% RH at any time during six months' testing at the accelerated storage condition, additional testing at the intermediate storage condition should be conducted and evaluated against significant change criteria. The initial application should include a minimum of 6 months of data from a 12-month study at the intermediate storage condition (12).

Table 2. General conditions for long-term, accelerated and intermediate storage conditions for drug substance, adapted from ICH Q1A (12).

Study	Storage condition	Minimum time period covered by data at submission
Long term*	25ºC ± 2ºC/60% RH ± 5% RH or 12 months 30ºC ± 2ºC/65% RH ± 5% RH	
Intermediate*	30ºC ± 2ºC/65% RH ± 5% RH	6 months
Accelerated	40ºC ± 2ºC/75% RH ± 5% RH	6 months

*It is up to the applicant to decide whether long term stability studies are performed at 25ºC ± 2ºC/60% RH ± 5% RH or 30ºC ± 2ºC/65% RH ± 5% RH.

** If 30ºC ± 2ºC/65% RH ± 5% RH is the long-term condition, there is no intermediate condition.

When a significant change occurs between 3 month's testing at the accelerated storage condition for refrigerated data, the proposed re-test period should be based on the real-time data available at the long-term storage condition. If a significant change occurs within the first three months of testing at the accelerated storage condition, it would be interesting to evaluate the effect of short-term excursions outside the label storage condition, e.g., during shipping or handling. It can be made, if appropriate, by further testing on a single batch of the drug substance for a period shorter than three months but with more frequent testing than usual. It is considered unnecessary to continue to test a drug substance for six months when a significant change has occurred within the first three months. The same approach is applied for drug substances intended for storage in a freezer, where the re-test period should be based on the real-time data obtained at the long-term storage condition. In the absence of an accelerated storage condition for drug substances intended to be stored in a freezer, testing on a single batch at an elevated temperature for an appropriate period should be conducted to address the effect of short-term excursions outside the proposed label storage condition, e.g., during shipping or handling. The general requirements for drug substances intended for storage in a refrigerator and freezer are shown in table 3. Drug substances intended for storage below -20°C should be treated on a case-by-case basis.

Table 3. Drug substances intended for storage in a refrigerator and freezer. Adapted from ICH Q1A (12)

Study	Storage condition	Minimum time period covered by data at submission
Long term	-20ºC ± 5ºC (freezer)	12 months
Long term	5ºC ± 3ºC (refrigerator)	12 months
Accelerated	25ºC ± 2ºC/60% RH ± 5% RH (refrigerator)	6 months

1.3.5 Stability studies regarding the drug product

1.3.5.1 General aspects

The basis for designing stability studies for drug product comes from results obtained in drug substance studies (12). Some pharmaceutical companies include release data as part of stability study, because it is supposed that release data reflects the condition of the packaged drug product at time 0, what will be discussed forward in this work (14). Stress testing should be conducted on at least one primary batch of drug product. The requirements related to the selection of batches depending on the drug substance. For drug product containing new drug substances, stability data should be provided on at least three primary batches of each proposed strength of the drug product, of which two should be at the pilot scale. In the case of drug product containing existing drug substance, data should be provided on not less than two batches of at least pilot scale, or in the case of an uncomplicated drug product, like immediaterelease solid drug product or non-sterile solutions, at least one batch of at least pilot scale and a second one may be smaller (12,13).

1.3.5.2 Testing frequency and storage conditions

At the time of submission, the long-term testing should cover a minimum of six months for drug product containing already existing drug substances or 12 months for drug products having new drug substances, continuing for a period sufficient to cover the proposed shelf life. The same proceeding is applied to accelerated storage conditions differing in the number of time points (in this case, a minimum of three from a six-month study). If there is an expectation of changing criteria based on development results, is desirable to add samples at the final time point or even including a fourth time point in the study design. An intermediate storage condition test can be called for when a result of significant change is found in accelerated conditions. In this case, a minimum of four time points, including the initial and the final, from a 12-month study is recommended (0, 6, 9, and 12 months). It is interesting to note that the time point t0 is defined as the initial storage date. Each time point must follow the test protocol and be completed as soon as possible; in the same way, that deviation from the protocol should be recorded and justified (12).

Generally, a drug product should be tested under store conditions beyond thermal stability, its sensibility to moisture, or the potential for solvent loss. So, the experimental conditions should cover the storage, shipment, and subsequent use in the climatic conditions the drug product is supposed to be used (12).

1.3.6 Attributes to be monitored during stability studies

The first time a specification is proposed, justification for each procedure and acceptance criteria should be included. All the attributes to be evaluated and information from developing studies must be kept in mind. The ICH Q6A Specifications: *Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances* states tests and acceptance criteria generally and specifically applicable to all new drug substances and products. A list of the less and the most sensibles tests regarding analytical variability is provided in table 4. As stated by ICH Q6A, this list represents a sample of tests and acceptance criteria applicable to specific dosage forms, for instance solid oral drug products, liquid oral drug products, and parenterals. Considering that these examples do not represent the totality of dosage forms available in the market, ICH stimulates the application of the concepts of Q6A to other dosage forms.

Table 4. Test procedures for new drug substances and new drug products, regarding its sensibility to analytical variability, adapted from ICH Q6A(6)

			Attribute
Universal	New drug substance		Description, identification[1]
			Assay, impurities[2]
	New drug product		Description, identification[1]
			Assay, impurities[2]
Specific	New drug substance		Physicochemical properties, particle size, polymorphic forms, tests for chiral new drug substances, inorganic impurities, microbial limits[1]
			Water content[2]
	New drug products	Tablets	Dissolution, disintegration, hardness/friability, uniformity of dosage units, microbial limits[1]
			Water content[2]
		Oral liquids	Uniformity of dosage units, pH, microbial limits, antimicrobial preservative content, antioxidant preservative con tent, extractables, alcohol content, dissolution, particle size distribution, redispersibility, rheological properties, reconstitution time[1].
			Water content[2]
		Parenteral forms	Uniformity of dosage units, pH, sterility, endotoxins/pyrogens, particulate matter, antioxidant preservative content, Extractables, functionality testing of delivery systems, osmolarity, particle size distribution, redispersibility, reconstitution time[1].
			Water content[2]

1- Tests more sensible to analytical variability

2- Tests less sensible to analytical variability

1.3.7 Shelf life estimation

According with the current ICH requirements for shelf life estimation, the stability studies must be carried out according to the guidelines provided by the International Conference of Harmonization, World Health Organization, and other agencies in a scheduled manner (21).

Shelf life estimation aims to determine the storage time during which the critical attributes of the drug product stay within the acceptance limits. It is the length of time the drug product stays within approved specifications, provided it is stored under specified conditions (12). Throughout this expiration period, the consumer is confident that the drug product preserves identity, strength, quality, and purity (17).

As referred by Capen et al., the ICH Q1A links the concept of shelf life to batch analysis. Similarly, ICH Q1E describes the strategy for estimating the shelf life of a drug product as the determination of storage time during which those critical attributes remain acceptable for all future batches, manufactured, packaged, and stored under similar conditions. As we can see in both guidelines, the batch is the principal point of interest (22).

The current ICH methodology for establishing the supported shelf life from stability studies assumes a fixed effect batch analysis, in which differences between batches are interpreted as differences in batch attributes or in the fixed intercept and slope parameters, which characterizes each batch's response over time (usually measured in months). The analytical strategy assumes that a linear regression model is satisfactory for describing the response data from each batch. Next, a series of poolability tests are performed using a regression model selection procedure. The poolability tests determine the best-fitted regression model to identify trends across the storage time in the set stability test, considering these three variations of a simple linear model (22).

Mathematically, shelf life is determined by the maximum time a stability-limiting characteristic remains within the acceptance limit. A graphic representation of the actual shelf life of a pharmaceutical product with linear degradation and lower acceptance limit η is shown in figure 1. Specification acceptance limits are typically no more remarkable than ±10% of the labelled content of the drug product (6, 11).

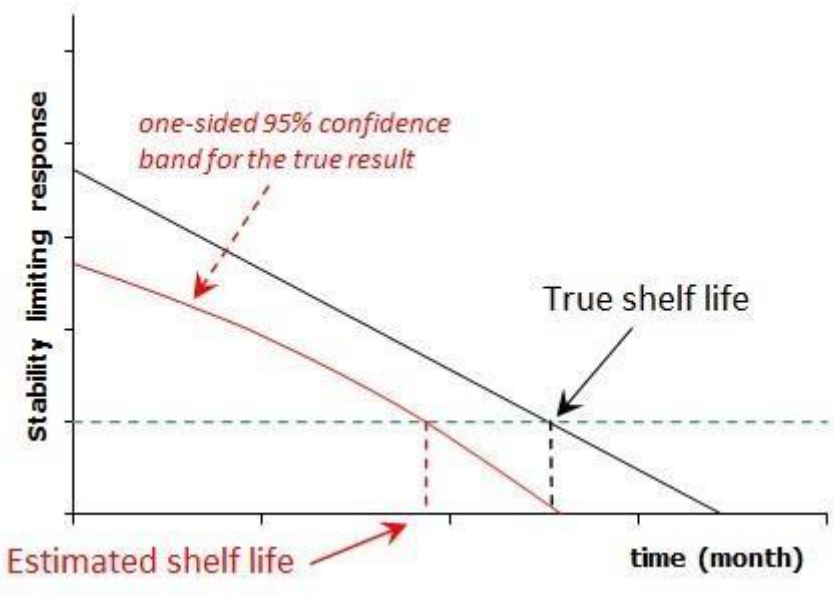


Figure 1. True and Estimated shelf-life. The True shelf-life corresponds to the time at which the product under analysis would reach the acceptance limit in the absence of any uncertainty of any kind or source (the green dashed line = η). The estimated shelf-life is the time at which the probability that product will be within the acceptance limits is, usually, 95%.

Quinlan et al. (17) compared the ICH approach with alternative shelf life estimation procedures to provide a statistically sound and justifiable estimative of shelf life and thus provide a more accurate shelf life applicable to future batches. A thorough statistical analysis and discussion, based on both simulated and on blind real data was presented. It supports the conclusion that, in some situations, the ICH requirements fail to detect issues in the stability data and will wrongly accept longer shelf lives than should be granted. These weaknesses include the number of batches, the poolability tests and other statistical technicalities, outside the scope of this thesis.

Generally, the acceptance criteria for assay (potency) in the release specification are 95.0105.0% of the label claim, and the typical acceptance criteria in the shelf-life specifications are 90.0-110.0% of the label claim. Narrowing the release specification decreases the probability that the product will fail the stability specification during storage but would increase the chances of failure at batch release. Several considerations need to be given to set appropriate release limits. They include variability in the estimated attribute value at release and uncertainty with degradation rate estimate (5).

The use of batch release and stability specifications in ICH Q6A is not harmonized. In the United States, a product must meet a single specification at release and throughout the shelf life (this is also generally true for a pharmacopeial monograph). Contrarily, in the EU, separate specifications are required at batch release and on stability. However, in the absence of a regulatory release specification, tighter "in-house" release specifications can be used to ensure that the product will meet the regulatory specification throughout its shelf life, although reported OOS values require a thorough formal OOS investigation (5).

2 Measuring drug substance and drug product quality attributes

2.1 The analytical validation

2.1.1 The traditional versus new approaches of analytical procedure validation

Analytical procedures are used throughout the development and manufacturing of drug substances and drug products. Important decisions are based on analytical results, which must be proved suitable for their intended use. This is the definition of analytical validation (23). Although the general requirements for test procedures are now well established and understood, it was not always a reality in the pharmaceutical industry.

The turning point of validation in the pharmaceutical industry was in the early 1970s when a failure in the terminal sterilization process in a batch of dextrose IV bottles in the UK had, in consequence, the death from acute endotoxic shock of five patients to whom the microbiologically contaminated bottles were infused (24). The called Devonport incident was a milestone that brought attention to everyone in the pharmaceutical industry about the importance of safety and, more than this, reliability of the drug manufacturing process. The second edition of UK Good Pharmaceutical Manufacturing Practice, the *Orange Guide*, was issued in 1983 and included for the first time the word validation. Currently, the UK orange guide includes EU GMP (25).

In the US, the GMP for drugs, described in FDA's Code of Federal Regulations – CFR21 parts 210 and 211, was firstly issued in 1978 and included the term validation in 1983 (16,26). Although the 1987 FDA guide to process validation did not mention the design of the process, defined validation *as establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined*

specifications and quality attributes. Here we can see a change compared to the first validation activities. The validation activities are not restricted to manufacturing anymore but include the analytical methods used to evaluate the products (27). The United States Pharmacopeia issued an informational general chapter <1225> (Validation of Compendial Methods) (28) in 1989, followed by general chapters <1226> (Verification of Compendial Procedures) (28) and <1224> (Transfer of Analytical Procedures) (29).

In 2004, the FDA published *Pharmaceutical cGMPs for the 21st Century—A Risk-Based Approach*, to encourage the early adoption of new technological advances by the pharmaceutical industry, through the application of modern quality management techniques, including the implementation of quality systems approaches to all aspects of pharmaceutical production and quality assurance. This report also included the stimulus for the implementation of risk-based approaches that focus both industry and agencies' attention on critical areas (30).

In 2011, the FDA published the *Guidance for Industry: Process Validation: General Principles and Practice*. In this guidance, the FDA adopted a life-cycle approach, moving from process qualification to validation in three stages: process design, process qualification, and continued process verification (31). The guidance *Analytical Procedures and Methods Validation for Drugs and Biologics* was issued in 2015 and provide recommendations on how the applicant can submit data regarding analytical procedures and methods validation to support the documentation of the identity, strength, quality, purity, and potency of drug substances and drug products. The FDA refers to this guide as a complement of the International Council on Harmonization (ICH) guidance *Validation of Analytical Procedures: Text and Methodology Q2(R1)* (32,33).

The ICH also identified the need to modernize the quality management approach. Between 2005 and 2009, they published a series of guidelines related to pharmaceutical development, the life cycle, and the framework of quality risk management: ICH Q8 (Pharmaceutical Development) (34), ICH Q9 (Quality Risk Management) (35), and ICH Q10 (Pharmaceutical Quality System) (36). Some quality guidelines are related to drug substances and supply chain assessment: ICH Q6 (Specifications) (6,7), Q7 (Good Manufacturing Practice) (37), Q9 (Quality Risk Management) (35), and Q10 (Pharmaceutical Quality System) (36).

Meanwhile, in 2007, Borman *et al*. (27) suggested that the concepts of Quality by Design initially developed to enhance the robustness of the manufacturing process should be applied

to analytical procedures. As defined by ICH Q8, Quality by Design is *"a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding based on sound science and quality risk management"* (34). Process validation, according to FDA, is *"the collection and evaluation of data, from the process design stage throughout its lifecycle of use, which establishes scientific evidence that a method is capable of consistently delivering quality products"* (31). Thus, Nethercote *et al.* gave a similar definition to method validation lifecycle approach: *"the collection and evaluation of data and knowledge from the method design stage throughout its lifecycle of use, which establishes scientific evidence that a method is capable of consistently delivering quality data."* Here, a method is a synonym for the analytical procedure and includes steps such as sample preparation, analytical technique, calibration, and definition of the reportable result and specification limits (33).

Concerning the ICH, the main guideline that outlines the requirements for method validation is the Q2(38). Firstly published in 1993, its last version was recently issued (24 march 2022), revised by an Expert Working Group formed in 2018 to fix some gaps in Q2 (R1) and elaborate the ICH Q14 (Analytical Procedure Development) (39). In ICH Q2 (R1), there was no description related to procedure development and the focus was mainly on chromatographic analysis. Additionally, the Expert Working Group also had the mission of examining the feasibility of combining both documents, Q2 and Q14, to clarify the understanding of the guidance. Once the work was finished, the merging of both documents did not happen, which was considered by some authors inappropriate, since they consider low the probability that analysts in the pharmaceutical industry read Q2 and Q14 and integrate them (40).

On the other hand, the new USP general chapter 1220 (41) put all together to deal with the whole analytical life cycle in just one document. They adopted the QbD approach, a concept already described by the International Council of Harmonization in the ICH Q8 to Q12. In simple words, the focus was associate analytical validation activities through the entire life cycle of an analytical procedure, and moreover, to understand the impact of total analytical error based on risks. Risk assessment involves identifying and controlling factors that significantly impact the methodology's performance (42,43).

According to ICH Q2(R2), the typical performance characteristics and related validation tests for commonly measured product attributes are specificity, working range, accuracy, and precision (table 5 shows the definitions of some attributes and how it can be demonstrated). Compared to Q2(R1), the ICH Q2(R2) included other important new aspects. Beyond the

possibility to be applied to both chemical and biological/biotechnological products, the use of prior knowledge (for example from development or previous validation) can be used as part of the validation, with appropriate justification. Furthermore, the word linearity has been removed as a primary validation characteristic and replaced with working range, a wider term, which ensures that other types of calibration beyond linear can be used (nonlinear, multivariate) (42).

Although the Expert Working Group has been included in ICH Q2 (R2) validation during the life cycle (section 3.1 of the guidance), they only referred to validation due to changes or transfers and not mentioned ongoing performance verification, which does not characterize properly the life cycle concept (40). It brings to light an inevitable question: How deeply do the available regulatory frameworks related to analytical validation really ensure suitability throughout drug stability studies? We will discuss this topic in the next section.

Table 5. Typical performance characteristics and related validation tests for commonly measured product attributes.

	What is it?	How is it demonstrated?
Specificity/selectivity	Specificity is the capacity of a method to identify unequivocally a specific analyte. Selectivity measure which extent to which particular analytes in mixtures can be measured without interferences from other similar components.	Absence of interference
		Orthogonal procedure comparison
		Technology inherent justification
Working range	The working range is the lowest and the highest concentration in which analytical procedure provides meaningful results.	Response
		Validation of lower range limits
Accuracy	Is the closeness of agreement between the value which is accepted either as a conventional true value or as an accepted reference value and the value measured.	Precision
		Combined approaches for accuracy and precision

3 The critical difference between batch release and stability assessment

As we have seen before, the regulatory guidance for setting specification limits for new drug substances and new drug products ICH Q6A defines specification, concerning quantitative quality attributes, as a list of tests and acceptance criteria that drug substance or drug product should conform to be considered acceptable for its intended use. These specifications limits should be classified into release and shelf life limits. A drug product must meet release limits at the time of manufacture and conformity to shelf life limits during its whole shelf life (6). Regarding drug product, release limits are generally narrower than shelf life limits to enable uncertainty in degradation estimates to be highly assured that the product will remain within the shelf life limits (44). This concept should include assay and impurity levels (6).

Conceptually, the stage-gate between a drug product development and deployment (release) is validation. Frequently, validation proceedings are performed at a single point in time, after which method performance is considered acceptable, all this happening under well-controlled conditions related to laboratory, analysts, and materials. As referred by Verch et al. (8) that is the reason why validation has been viewed as a "well-rehearsed demonstration of method performance." The disconnection between method development and deployment makes findings less predictive of real-world method performance.

The United States Pharmacopeia provides market standards for lot quality that often serve as a basis for lot-release decisions. Each lot is treated in isolation and does not provide statistical power to support decision-making. Additionally, the small sample sizes used for lot release do not allow high decision confidence levels. It is relevant to refer that the literature reports that product specifications based on USP standards do not separate the analytical error component, treating intra-lot manufacturing variability and analytical variability as one source of variation. It does not allow the estimation of true product quality (45).

The stability-indicating analytical methods are validated quantitative analytical procedures that can detect changes in a quality attribute of the drug substance and drug product during storage. According to ICH Q2(R2), to demonstrate specificity/selectivity, challenges must be done using samples spiked with target analytes and all known interferences, samples exposed to stress conditions, and even actual samples stored at high temperature or humidity conditions (38).

Although the same analytical procedure may be suitable to ensure batch release, it does not mean that it is appropriated to assess drug substance and drug product stability. It happens

because the measured data's variability highly influences a drug product's estimated shelf life. This variation in stability data occurs due to the manufacturing process variation (batch-to-batch and within-batch variability) and the uncertainty of the analytical method, i.e., reproducibility and repeatability (46). The degree of variability of individual batches affects the confidence that a future production batch will remain within acceptance criteria throughout its retest period or shelf life (47).

4 Final Discussion / Conclusion

In this work, we have reviewed the main regulatory documents related to the validation procedure and its capacity to support stability studies. We defend that the current ICH requirements of the analytical validation, when assessing stability, do not ensure a suitable robustness of the whole analytical procedure. Even in 2005, Ermer et al. (48) were already worried about the lack of information on the analytical variability in precision determination. They addressed 2915 assay (liquid chromatography) from 156 stability studies of 44 drug substances and drug products, to obtain precision estimates and their typical distribution for typical pharmaceutical applications, calculated and reported as relative standard deviations. The distribution of individual repeatabilities reflected the complexity of the sample and its preparation. The precisions were mainly influenced by the type of drug product and in a minor extent by the concentration. Less complex drug products have small repeatibilities and the importance of the intermediate variability contribution to the overall precision was larger. In the case of assay procedures, precision determines suitability, because analytical variability must be compatible with the acceptance limits of specification (in fact, variability is almost a dominant part of the specification range).

In 2016, a meeting organized by members of the Steering Committees of the Stability Focus Group, the Pharmaceutical Impurities Focus Group and the Chemistry, Manufacturing and Controls Statistic Focus Group of American Association of Pharmaceutical Scientists discussed the approaches to set release limits and managing method variability towards ensuring product quality. To ensure that a drug product consistently meet the registered specification during its shelf life, it is relevant to have an internal control limit for the assay that factors in manufacturing and analytical variability, the typically called release limit, which development is critical for controlling the product quality. Although all the meeting participants perceived

this criticality, they also recognized that the data and procedures used to determine appropriate release limits are not always consistent (9).

The ICH Q1E incorporates the typical variabilities in stability profile in the calculation of shelf life (the regression/covariance approach is one way to characterize the stability change over time and its associated variability). The figure 2 shows how helpful can be use the manufacturing data to assess the capability of meeting the calculated limit based on histogram distribution. Also, the drug substance stability profile is important (maximum allowed level of impurities), as well the acknowledgement of the typic analytical variability established during method validation. After all, appropriate release limits mean consistent quality of drug product through shelf-life (9).

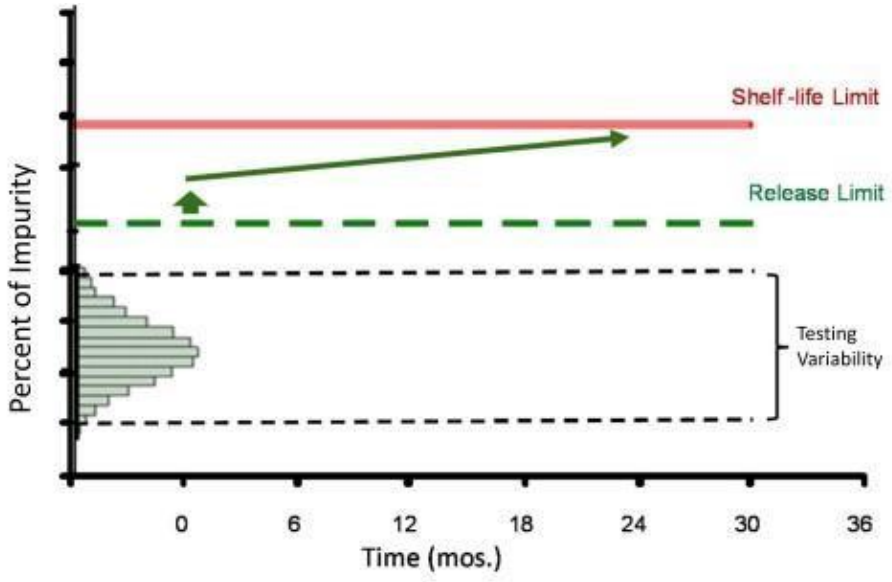


Figure 2. Release Limit as an internal control. Adapted from Huynh-Ba (9).

The stability testing has, in our view, a small but significant conceptual difference when compared with the batch release testing. In the latter case, we have many (sequential) batches tested only once (for as many quality attributes as deemed necessary for that particular FPP) whereas in the former scenario we have the same entity(ies)/batch(es) tested many times (usually at storage time t=0, 3, 6, 9, 12, ... up to 60 month). The figures 3 and 4 show the differences between the batch release and stability studies regarding the OOS investigation. In the batch release procedure we have, as reference, the acceptance criteria of the specification results (basically a pass/fail approach), which makes the OOS scrutiny straightforward, whilst in stability studies the investigation should be based on a holistic overview of the results. In the latter case, the flagpole is the release results, but how true these values are? Have the precision of release results been well set? Was the variability of the initial value considered? In our

opinion, decide if a drug product pass or fail based on individual values during stability studies is inadequate, because in fact we cannot separate what is really loss in the assay and what is assignable to variability itself.

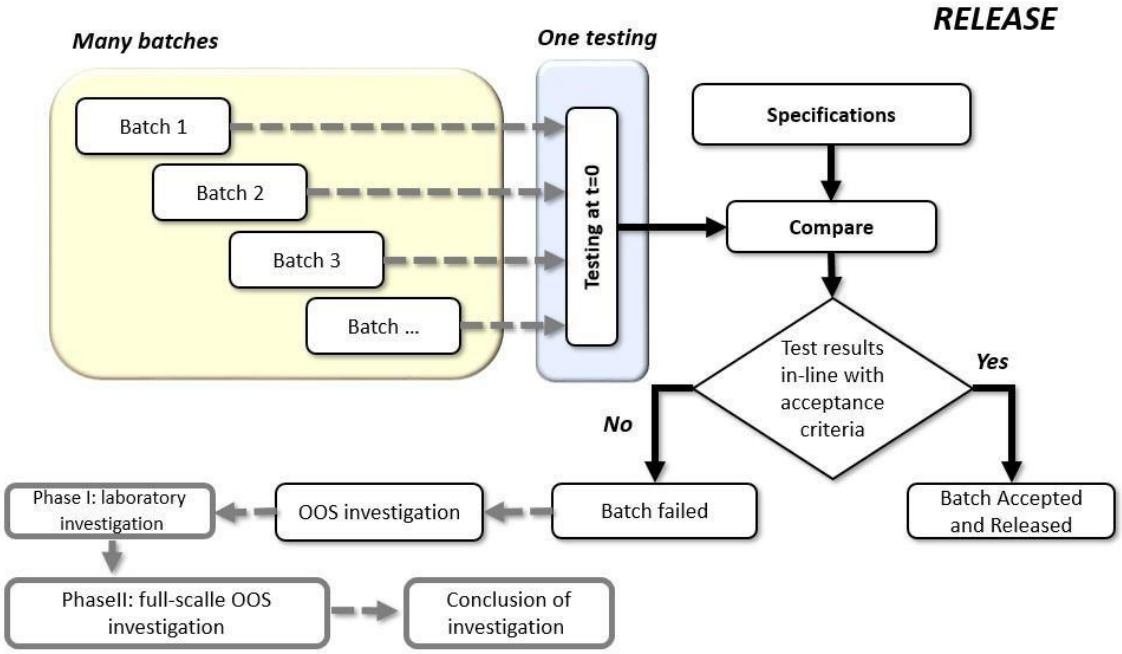


Figure 3. Schematic representation of management of OOS results regarding batch release. Adapted from FDA guidance for industry: Investigating Out-of-Specification (OOS) Test Results for Pharmaceutical Production(49).

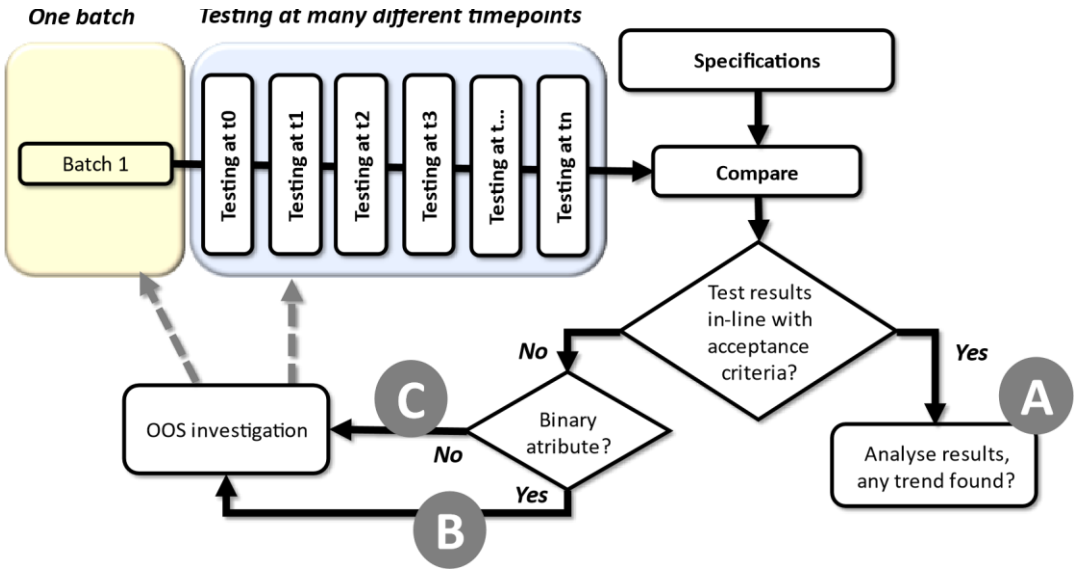


Figure 4. Schematic representation of management of OOS results regarding stability studies.

This makes a huge difference, as the goal of the batch release testing is to decide (based on the test results) weather the batch pass/fails (after comparing with the prespecified approved specifications). Therefore, it is not if the observed inter-batch variability is due to a true batch

variability or the variability of the measuring procedure, as long as the result met the acceptance criteria. In stability, however, the very same entity (the batch) is assessed many times over time and there is no batch-to-batch variability to take into consideration, only intra-batch variability (content uniformity, etc) and analytical variability.

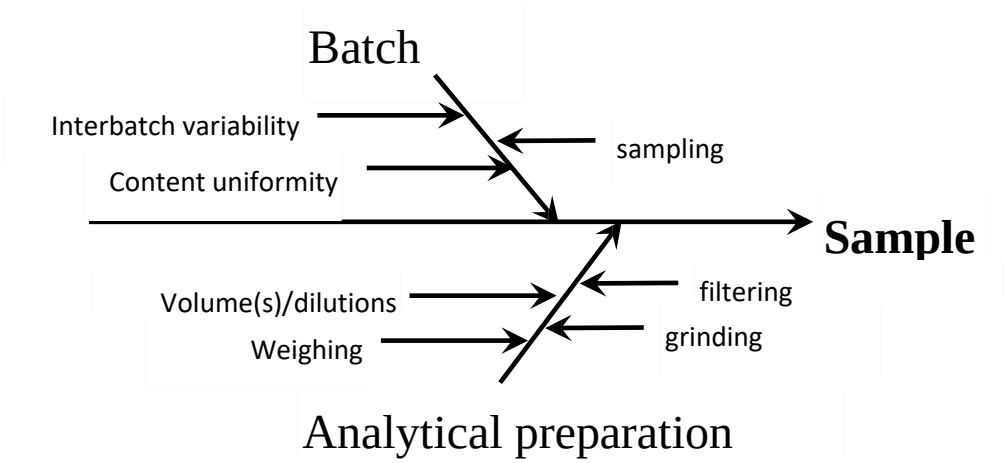


Figure 5. Ishikawa diagram showing some of the sources of the variability observed in the samples.

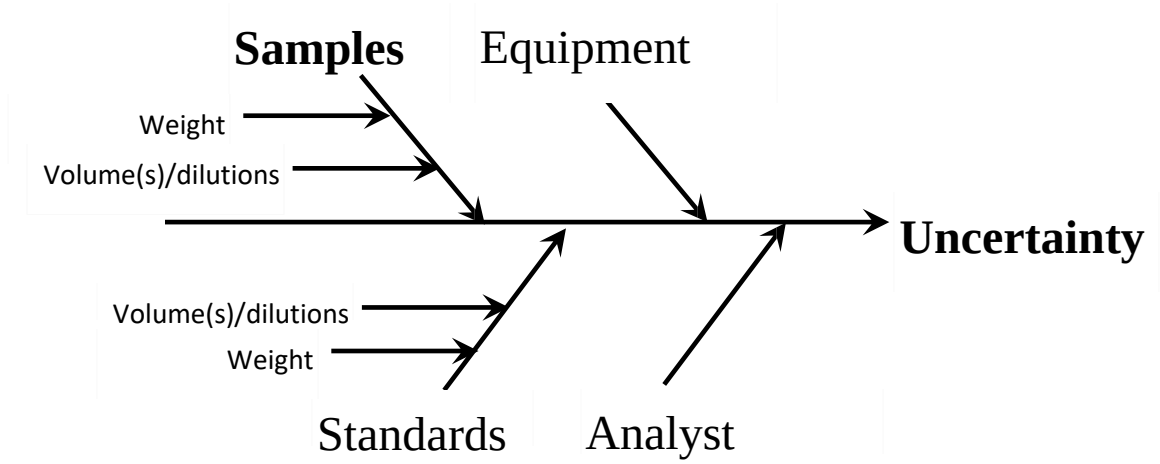


Figure 6. Ishikawa diagram showing some of the sources that contribute to the overall Uncertainty

If an appropriate sample size (for instance the number of tablets) has been chosen then the cause for a high variability in the results can only be due to the measuring process (human, analytical, etc). This variability is frequently large, at least large enough to not allow the detection (visual or even statistical) of any trend in the stability limiting quality attribute over time. Figure 7 shows and example where there is no OOS single result within the requested (or granted) shelf life (ex. 48 month) but the regression prediction confidence band intersect the minimum acceptable value at around 23 months. This is due to the uncertainty arising from the large variability and poor experimental design. If, for instance, have the applicant should performed a regression analysis or the regulatory agency required an integrated "global" analysis of the data instead of a simple comparison of each observed result against the acceptance criteria, this shelf life would have not been accepted.

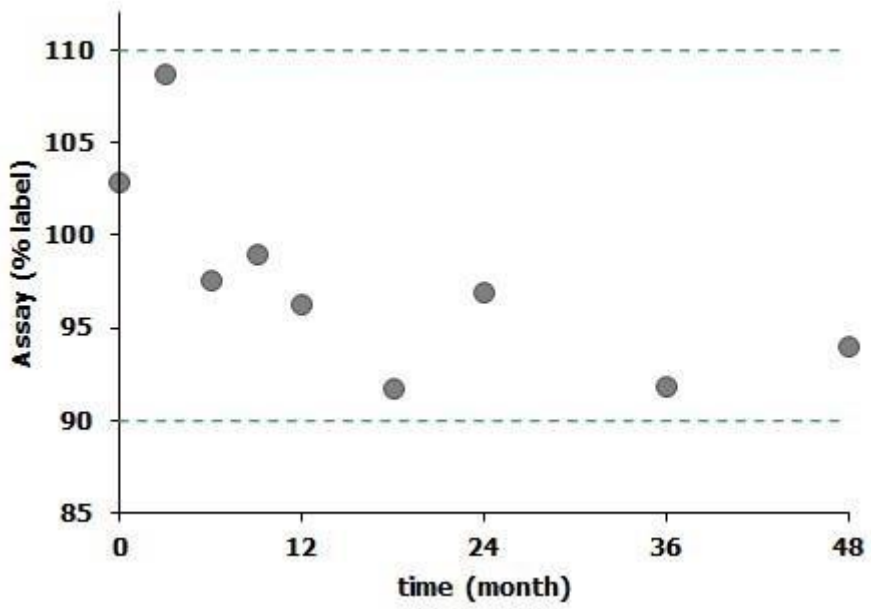


Figure 7. Simulated data[2] with a variability, expressed as standard deviation, of approximately 5%. All the results up to 48 months are within the acceptance limits (100±10%).

[2] Simulated data with an assay decrease of 1% per year and a normally distributed random error with a 5% standard deviation

In the example above, the batch shows acceptable results at the 3, 6, 9, 12, 18, 26, 36, and 48 months just by pure chance or "luck". If the same analysis would have been replicated in the same batch (or any other similar batches) chances that some of these results would turn in to OOSs would be considerable. This is a concern as this uncertainty is unable to ensure that the future batches will comply with the regulatory requirements. In fact, this was already flagged at the ICH Q1 guideline when stating that *"The degree of variability of individual batches affects the confidence that a future production batch will remain within specification until the expiration date."*. It is our opinion that these aspects have not received the due attention from both manufacturers and regulators.

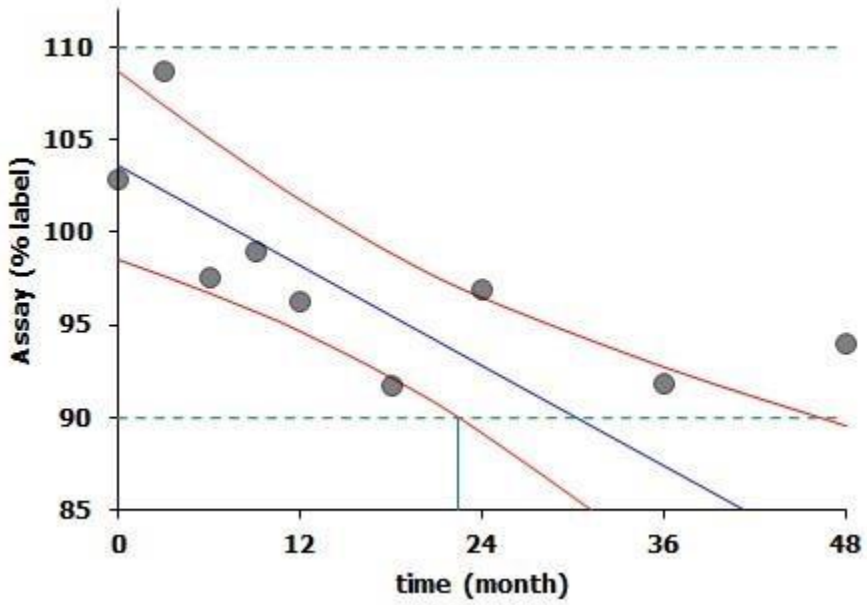
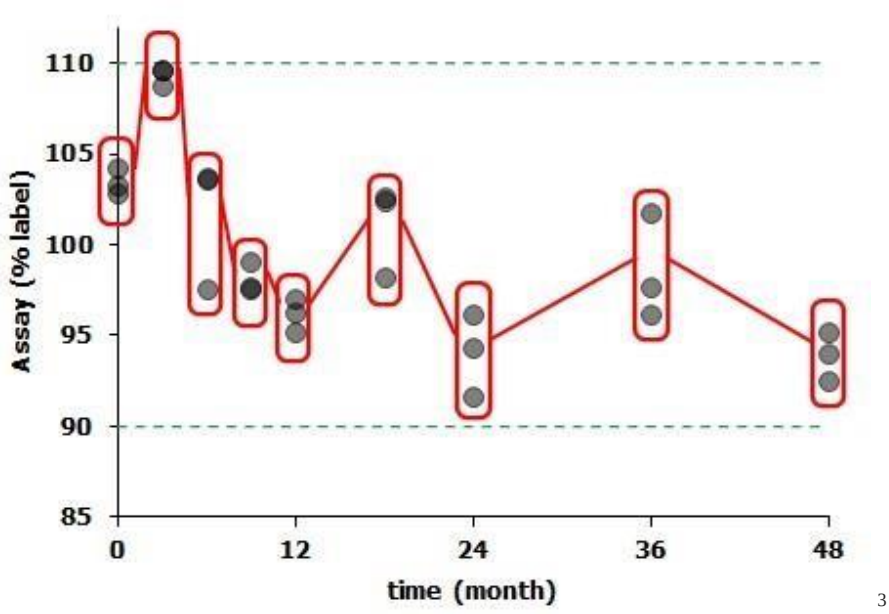


Figure 8. Graphical representation of the regression analysis performed over the same data as in the previous chart. See text for details.

As we can see in figure 8, despite the absence of OOS results, the 95% one-side confidence band crosses the lower acceptance limit before 23 months. As such, based on this example, a shelf life longer than 23 months should not be granted by the regulators. To improve graphical clarity, only one batch is displayed instead of the required three batches. However, the outcome would not be different if the three batches where used.

Another scenario commonly observed is illustrated by the figure 9. In this case, all the observations at the same timepoint seem to go all up or down. This behaviour happens in the

cases where contribution of the intrabath and sample preparation variabilities has been well controlled (to a low level) by using a proper sampling and replication plan, but such care was not taken when preparing and/or analysing the standard solutions. As such, any random and/or systematic errors occurred in the preparation or analysis of the standard solutions will become systematic when estimating the concentration of the samples [1].



[3]

Figure 9. Typical stability assay pattern when the contribution of the variability observed between sampling timepoints is larger than the within and between batch variabilities. The bullets at each time point represent the result for the 3 stability batches.

In addition, it is a usual practice to prepare just one standard concentration corresponding to the expected 100%. As the three batches charged into the stability studies are usually the same batches as the validation ones, they are sequential batches, manufactured within a short period of time (days) and therefore the stability pull out dates or analysis date are usually the same and, as such, they are analysed in the same analytical sessions, sharing the same standard solutions. An error of, for instance, +5.3% in the concentration of the standard solution will turn out in a systematic error of -5.0%[4] in all the samples, without the contribution of any other

[3] This, obviously applies only to the most common cases of the relative quantitative analytical chemistry methods and not to the absolute ones (as, for example, titrimetry or gravimetry)

[4] The bias (b%) in the sample's concentration coming from and error of x% in the standard solution is equal to b%=1-1/(1-x%).

potential causes of variability. This is a "significant change"[5] as defined in the ICH Q1 stability guideline with undesirable consequences that will not be addressed in this dissertation. Therefore, the control of the variability should not focus only on the samples side but also on the standards side.

So, how to control the variability? Proper training of analysts, suitable handling of materials and equipment, good practices compliance, proper equipment maintenance are critical

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requirements to ensure the results generated at the QC (quality control) laboratory are accurate and reliable. The analytical validation already described and discussed should be able to detect any non-conformity or unacceptable results. In the last two decades, defining expectations for medicine quality and establishing conformance to those expectations has been a joint effort involving regulators, the pharmaceutical industry, pharmacopeias, and other stakeholders, to safeguard the quality and consistency of medicines without increasing the barriers to access in less developed countries which may lack the resources or technology to meet or enforce stringent standards (50).

[5] Significant change is, among others, "A 5 percent potency loss from the initial assay value of a batch"

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