***Review Article***

# A Comprehensive Guide to Applicative Requirements for Analytical Method Validation Across Various Regulatory Guidance: ICH, USP, ChP and ANVISA

## Abstract

This comprehensive guide provides a detailed comparison of analytical method validation requirements across four major regulatory bodies: the International Council for Harmonisation (ICH), United States Pharmacopeia (USP), Chinese Pharmacopoeia (ChP), and Brazilian Health Regulatory Agency (ANVISA). The study examines key aspects of method validation, including scope, validation parameters, acceptance criteria, and regulatory compliance. These guidelines have been chosen due to major differences between regulations requirements and ICH guidelines, leading to a huge challenge for Health Authority and the locally established Pharmaceutical Companies to implement these guidelines. The implementation of the revised or upcoming ICH guidelines will bring many benefits for the industry and health authorities. Recent revision by the guidelines will help to align with other chosen regulatory authority to reduce duplicate work and contribute to the global regulatory harmonization, which will bring a great benefit to the regulatory bodies review process, industrialist approval and important medicines approval will be faster available to the patients.

**Keywords:** ICH guidelines, United States of Pharmacopoeia (USP), Chinese Pharmacopoeia (ChP), Brazil/ANIVISA guidelines, WHO, Analytical method validation, Analytical method verification.

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# 1 Introduction

This comprehensive guide provides a detailed comparison of analytical method validation requirements across four major regulatory bodies: the International Council for harmonization (ICH), United States Pharmacopeia (USP), Chinese Pharmacopoeia (ChP), and Brazilian Health Regulatory Agency (ANVISA). The study examines key aspects of method validation, including scope, validation parameters acceptance criteria, and regulatory compliance. The guide highlights significant differences in approach, such as the ChP's more stringent dissolution testing criteria compared to USP, and ANVISA's efforts to align with ICH guidelines. It also explores the harmonization efforts between these regulatory bodies, noting China's recent inclusion in the ICH and the progressive alignment of the ChP with international standards. Special attention is given to unique requirements of each pharmacopeia, such as the ChP's approach to microbiological testing and ANVISA's implementation timeline for ICH guidelines. The guide also addresses practical considerations for pharmaceutical companies operating in multiple markets, offering strategies for navigating these diverse regulatory landscapes. By providing a thorough analysis of these regulatory requirements, this guide serves as an essential resource for pharmaceutical professionals involved in analytical method development, validation, and regulatory compliance across global markets.

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## 1.1 ICH (International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use)

In the 1960s, European regulatory authorities recognized the importance of multiple regional approvals for medicinal products. In the 1980s, the EU began harmonizing regulatory requirements for Europe, Japan, WHO, and the US. The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) was established in 1990 to harmonize technical guidance between these bodies. In 2015, ICH underwent reforms and became a non-profit association in Switzerland, aiming to transform it into a global initiative with a transparent governance structure [1-3].

The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) is an initiative that aims to promote public health by achieving greater harmonization through the growth of technical guidelines and requirements for pharmaceutical product registration. The ICH comprises the ICH Assembly, ICH Management Committee, MedDRA Management Committee, and ICH Secretariat. The Assembly adopts decisions on ICH Guidelines, admission of new Members and Observers, and the ICH Association's work plans and budget. The ICH topics are divided into four categories: Quality, Safety, Efficacy, and Multidisciplinary Guidelines [4].

## 1.2 United States Pharmacopeia (USP)

The USP is a pharmacopeia published annually by the United States Pharmacopeial Convention, a nonprofit organization that owns the trademark and copyright on the pharmacopeia itself. Established in 1820, it is part of the United States pharmacopeial Convention and is published under the name USP and NF. It contains legally recognized standards for drug substances, dosage forms, and therapeutic products, including nutritionals and dietary supplements. USP-NF standards also have a role in US federal law, and set standards for dietary supplements and food ingredients [6].

### 1.3 Chinese Pharmacopoeia (ChP) or Pharmacopeia of the People's Republic of China (PPRC)

The Chinese Pharmacopoeia (PPRC) is an official compendium of drugs, covering both traditional Chinese and western medicines. It provides information on purity, description, test, dosage, precaution, storage, and strength for each drug. The first edition was published in 1953 and is updated every five years. The 1997 English version consists of two volumes, with a third volume added in 2005. The Chinese version has two volumes, but they are not direct translations. The English edition has 2691 monographs, with 992 for traditional Chinese medicines and 1699 for modern western drugs.

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### 1.4 ANVISA

The Brazilian Health Regulatory Agency (ANVISA) is a regulatory body established in 1999 to regulate pharmaceutical drugs, sanitary standards, and the food industry. It is linked to the Ministry of Health and aims to legitimize the Brazilian Unified Health System through modern, transparent health surveillance and regulation. ANVISA's mission is to promote public health and intervene in risks caused by health surveillance products, coordinating with states, municipalities, and the Federal District. In 2016, ANVISA joined the International Council on Harmonization of Technical Requirements for Pharmaceutical Registration (ICH), with 13 members and 22 observers [5].

## 2 Analytical Method validation requirement comparison

### 2.1 Validation of analytical methods requirements: ANVISA

The use of an analytical method that is not described in the official compendium recognized by ANVISA requires an analytical validation, as per the parameters established in resolution 166, considering the technical and operational conditions [7-10]. Typical parameters to be considered for validation depend on the test to be performed and are presented in table 1.

**Table 1 Validation requirements as per ANVISA**

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| Parameter assessed | Identification | Impurity test | | Assay<br>-dissolution (quantification)<br>-content uniformity<br>-potency |
|---|---|---|---|---|
| | | Quantitative | Limit test | |
| Accuracy | no | yes | no | yes |
| Repeatability Precision | no | yes | no | yes |
| Intermediate precision | no | yes | no | yes |
| Selectivity | yes | yes | yes | yes |
| Limit of Detection | no | no | yes | no |

| | | | | |
|---|---|---|---|---|
| Limit of Quantitation | no | yes | no | no |
| Linearity | no | yes | no | yes |
| Interval | no | yes | no | yes |

Validation ensures reliable results and suitability for purpose, and for Anvisa-not recognized analytical methods, it must be conducted with established parameters and technical operating conditions.

The compendial analytical methods should have demonstrated their suitability for the intended use, the operating conditions in the laboratory, by presenting a partial validation study.

The partial validation should evaluate at least the parameters of precision, accuracy and selectivity.

1. In the case of analytical methods for the quantification of impurities, partial validation shall include the quantification limit.
2. In the case of limit test, replacing the heading of parameters should be evaluated the parameters of selectivity and detection limit.

Transfer methods between laboratories are valid if they involve partial validation premise-receiver laboratories. Revalidation of analytical methods can be considered in cases of changes in synthesis, product composition, analytical method, or other factors that may significantly impact the validated method [13,14].

### 2.2 Validation of analytical methods requirements: ICH

ICH Requirements: Types of Analytical Procedures to be validated.

The discussion of the validation of analytical procedures is directed to the four most typical varieties of analytical procedures:

- Identification tests
- Quantitative tests for impurities' content
- Limit tests for the management of impurities
- Quantitative tests of the active moiety in samples of drug substance or drug product or alternative selected component(s) in the drug product.

The text highlights the importance of validation of various analytical procedures, including identification tests, impurities tests, and assay procedures. These procedures aim to identify an analyte in a sample, accurately reflect its purity characteristics, and measure the analyte present in a given sample. The aim of the analytical procedure should be clearly understood to govern the evaluation of validation characteristics.

The Validation characteristics which need to be considered are listed in table 2 [14].

**Table 2 Validation requirement as per ICH**

| Type of Analytical procedure Characteristics | Identification | Testing for Impurities | | Assay<br>-dissolution (measurement only)<br>-content/potency |
|---|---|---|---|---|
| | | Quantitat. | limit | |
| Accuracy | - | + | - | + |
| Precision<br>Repeatability<br>Intermediate Precision | <br>-<br>- | <br>+<br>+(1) | <br>-<br>- | <br>+<br>+(1) |
| Specificity (2) | + | + | + | + |
| Detection Limit | - | -(3) | + | - |
| Quantitation Limit | - | + | - | - |
| Linearity | - | + | - | + |
| Range | - | + | - | + |

- signifies that this characteristic is not normally evaluated
+ signifies that this characteristic is normally evaluated
(1) In case, where reproducibility has been performed, Intermediate precision is not needed.
(2) Lack of specificity of one analytical procedure could be compensated by other supporting analytical procedure(s).
(3) May be needed in some cases.

## 2.3 Validation of analytical methods requirements: USP Pharmacopoeia General Chapter <1225> VALIDATION OF COMPENDIAL PROCEDURES [17]:

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Compendial test requirements vary from highly exacting analytical determinations to subjective evaluation of attributes. Considering this broad variety, it is only logical that different test procedures require different validation schemes. This chapter covers only the most common categories of tests for which validation data should be required. These categories are as follows:

- **CATEGORY I :** Analytical procedures for quantitation of major components of bulk drug substances or active ingredients (including preservatives) in finished pharmaceutical products.
- **CATEGORY II :** Analytical procedures for determination of impurities in bulk drug substances or degradation compounds in finished pharmaceutical products. These procedures include quantitative assays and limit tests.
- **CATEGORY III:** Analytical procedures for determination of performance characteristics (e.g., dissolution, drug release, and others).
- **CATEGORY IV :** Identification tests.

For each category, different analytical information is needed. Listed in below table 3. are data elements that are normally required for each of these categories. [NOTE—For detailed information regarding the validation of dissolution procedures, see the Dissolution Procedure: Development and Validation ⟨1092⟩.

**Table 3 Data Elements required for Validation as per USP**

| Analytical performance characteristics | Category I | Category II | | Category III | Category IV |
|---|---|---|---|---|---|
| | | Quantitative | Limit test | | |
| Accuracy | Yes | Yes | * | * | No |
| Precision | Yes | Yes | No | Yes | No |
| Specificity | Yes | Yes | Yes | * | Yes |
| Detection Limit | Yes | Yes | Yes | * | No |
| Quantitation Limit | Yes | Yes | No | * | No |
| Linearity | Yes | Yes | No | * | No |
| Range | Yes | Yes | * | * | No |

*May be required, depending on the specific test.

Verification of general procedures is crucial for determining their suitability for new products or raw materials. Physical property methods should be validated based on the same performance characteristics as any analytical procedure, with acceptance criteria consistent with the intended use. Physical methods can be classified into four validation categories: quantitative, qualitative, and quantitative. Documentation of successful laboratory studies is essential for determining the procedure's suitability. Current compendial procedures require demonstration of suitability under actual conditions of use, and appropriate documentation should accompany any proposal for new or revised procedures.

## 2.4 Validation of analytical methods requirements: CPh (9101-Guidance for Validation of Analytical Method)

Validation of analytical methods ensures they meet intended applications, such as drug quality specification, pharmaceutical process changes, or method revisions. For biological product quality control, physico-chemical analytical methods and biological determination methods can be used, with physico-chemical analytical method applicable for both. However, biological determination method has more influencing factors [19].

The analytical items to be validated include identification, impurity test (limit or quantification test), assay procedures (including characteristic parameters and content/ potency, the characteristic parameters such as dissolution test and drug release test of pharmaceuticals) [20-22].

Validation parameters for analytical methods include accuracy, precision, specificity, detection limit, quantitation limit, linearity, range, and robustness. Standard substances may be used, but parameters should be determined based on method and analyte influence.

**Table 4 List of Validation characteristics required to be evaluated in the test of each type**

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| Parameters | Identification | Impurity test | | Assay-Characteristic parameters-content/potency |
|---|---|---|---|---|
| | | Quantitation | Limit of test | |
| Specificity[2] | + | + | + | + |
| Accuracy | - | + | - | + |
| Precision | | | | |
| Repeatability | - | + | - | + |
| Intermediate Precision | - | +[1] | - | + |
| Detection Limit | - | -[3] | + | - |
| Quantitation Limit | - | + | - | - |
| Linearity | - | + | - | + |
| Range | - | + | - | + |
| Robustness | + | + | + | + |

[1] It is not necessary to validate the intermediate precision when the reproducibility has been developed.
[2] Lack of specificity of an individual analytical method maybe compensated by other supporting analytical methods.
[3] It depends on the specific condition.

## 3 Analytical method validation parameters definition

### 3.1 Specificity

**ICH Requirements,**

The pharmaceutical industry should investigate the specificity parameter during identification, purity, impurity determination, and assay quantification. A combination of multiple analytical procedures is recommended for complete discrimination. Identification tests should discriminate between compounds with similar structures, while assay and impurity tests should use representative chromatograms and label components appropriately. Alternate analytical procedures should be used when using non-specific assays, and specificity can be demonstrated when impurities or degradation product standards are unavailable [19].

**United Sates of Pharmacopeia (USP)**

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The ICH defines specificity as the ability to accurately assess an analyte in the presence of expected components like impurities, degradation products, and matrix components, with the term "selectivity" reserved for procedures that are completely selective. *Identification tests:* Ensure the identity of the analyte.

- *Purity tests:* Ensure that all of the analytical procedures performed allow an accurate statement of the content of impurities of an analyte (e.g., related substances test, heavy metals limit, or organic volatile impurities).
- *Assays:* Provide an exact result, which allows an accurate statement on the content or potency of the analyte in a sample [17].

**Chinese Pharmacopoeia (CPh)**

Specificity tests assess an analyte for expected components like impurities, degradants, and matrix. Issues should be investigated for identification, impurity test, and content determination. Identification tests distinguish between compounds and active ingredients. Assays and tests for impurity can be confirmed by graphs. If impurities are not available, methods like accelerating decomposition, assay, impurity tests, and purity tests can be used.

**Assessment of Brazilian Requirements**

Analytical methods demonstrate selectivity by identifying or quantifying analyte in sample The demonstration of identification methods for active pharmaceutical ingredients of vegetal origin and drug products requires demonstrating selectivity by comparing the method to structurally similar substances and a negative test result. To achieve selectivity, a combination of analytical methods may be needed. Quantitative methods and limits tests demonstrate selectivity by demonstrating the analytical response is solely due to the analyte, without interference from diluent, matrix, impurities, or degradation products16,17].

## 3.2 General definition for Forced degradation by different guidelines

Forced degradation studies are crucial in drug substance and product development to select the most effective compounds and excipients for formulations, packaging, and storage conditions. These tests are often repeated when the final drug substance route and market formulation are defined or the compound enters phase 3 clinical trials. Forced degradation studies also demonstrate specificity during the development and validation of stability-indicating methods, often at conditions exceeding accelerated storage conditions. The goal is to produce samples with representative and realistic degradation products, assessing their relationship to the drug substance or excipients or due to drug substance-excipient interactions. The extent of targeted degradation should be between 5% and 10%. Forced degradation studies can identify degradation pathways and mechanisms for the drug substance and product.

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Table 5 represents the forced degradation requirements as per ICH, USP, CPh and ANVISA

**Table 5 Forced degradation conditions as per ICH, USP, CPh and Brazil [23,24,25]**

| Conditions | ICH/USP/CPh only recommendations | Brazil |
|---|---|---|
| pH | Hydrochloric acid or sulfuric acids (0.1–1 M) for acid hydrolysis and sodium hydroxide or potassium hydroxide (0.1–1 M) for base hydrolysis are suggested as suitable reagents for hydrolysis | 10mg in 2mL water<br>10mg in 2 mL of 0.1M HCl<br>10mg in 2 mL of 0.1M NaOH<br>All in amber volumetric flasks and<br>at room temperature<br>Duration : 1 day and 3 days |
| Oxidation (Peroxide) | 0.1–3% hydrogen peroxide at neutral pH and room temperature for seven days or up to a maximum 10-20% degradation could potentially generate relevant degradation products. | 10mg/2mL 3% $H_2O_2$<br>At 5°C and room temperature in amber volumetric flasks. If DS is not soluble, then pH modification may be necessary. |

|  |  |  |
|---|---|---|
|  |  | Duration:1,2,3 days |
| Oxidation (Metal ion Catalyzed) | Not available or no recommendations | 10mg/2mL water containing.<br>100ppm Fe3+, Ni2+, Cu2+ saturated with bubbled oxygen in amber volumetric flasks.<br>Duration:1,2,3 days |
| Oxidation (Saturated with Oxygen) |  | 10mg/2mL saturated with bubbled oxygen in amber volumetric flasks.<br>Duration:1,2,3 days |
| Light | Samples of drug substance and solid/liquid drug product should be exposed to a minimum of 1.2 million lx h and 200 W h/$m^2$ light. The most accepted wavelength of light is in the range of 300–800 nm to cause the photolytic degradation. | 50mg/10mL wáter ambient<br>Duration: Maximum 1.2 million lux hours and 200 watt hours/square meter, 6hr, 1 day and 2 days |
| Heat | Thermal degradation (e.g., dry heat and wet heat) should be carried out at more strenuous conditions than recommended ICH Q1A accelerated testing conditions. Samples of solid-state drug substances and drug products should be exposed to dry and wet heat, while liquid drug products should be exposed to dry heat. Studies may be conducted at higher temperatures for a shorter period or thermal degradation study is carried out at 40–80 °C for 72 hours or 5 days | 10mg in 2 mL water at 50°C<br>Duration: 6 hours, 1 day and 2 days |
| Thermal (Closed Container) | Not available or no recommendations | 50°C and 80°C<br>(Ambient RH)<br>Duration: 1wk and 2wks |
| Thermal/Oxidative (Open Container |  | 50°C and 80°C<br>(Ambient RH)<br>Duration: 1wk and 2wks |
| Thermal/humidity (Open Container) |  | 40°C/75% RH<br>Duration: 1wk and 2wks |
| Light(Closed Container |  | Ambient<br>Duration: Maximum 1.2 million lux hours and 200-watt hours/square meter |
| Light/Oxidative (Open Container) |  | Ambient<br>Duration: Maximum 1.2 million lux hours |

| | | |
|---|---|---|
| | | and 200 watt hours/square meter |

The temperature/humidity used conditions may be more severe than the typical accelerated stability testing conditions in order to generate potential degradation products in a reasonable time. The typical forced degradation conditions include thermolytic, hydrolytic, oxidative, photolytic (more than ICH conditions), high pH (alkaline conditions), and low pH (acidic conditions). Outlined in above table conditions are some solid-state and solution forced degradation studies, respectively, that could be conducted.

### 3.3 Linearity

**ICH requirements**

To evaluate a linear relationship in analytical procedures, it is essential to measure it directly on the drug substance or synthetic mixtures. Visual inspection of signals as a function of analyte concentration or content can also help. Test results should be evaluated using appropriate statistical methods, such as regression line calculation. In some cases, mathematical transformation may be necessary to obtain linearity. For immunoassays, the analytical response should be detailed by the concentration of an analyte in a sample [25].

**United Sates of Pharmacopoeia (USP)**

- Linearity refers to an analytical procedure's ability to produce test results proportional to analyte concentration in samples. To achieve linearity, concentration and measurement may be transformed, such as log, square root, or reciprocal. If linearity isn't achievable, a nonlinear model may be used to describe the concentration-response relationship.
- **Detection Limit:** Limit tests detect the lowest amount of analyte in a sample, usually expressed as the concentration of the analyte, substantiating that the amount is above or below a certain level.
- **Quantification Limit:** Quantitation limit is a quantitative assay characteristic for low levels of compounds in sample matrices, such as impurities in drug substances and degradation products in finished pharmaceuticals, expressed as analyte concentration.

**Chinese Pharmacopoeia (CPh)**

- Linearity in analytical methods is determined by eliciting test results directly proportional to analyte concentration in samples. Samples with varying concentrations are prepared by accurately diluting stock solutions or measuring analyte separately. A regression line is calculated using least squares and mathematical transformation. Nonlinear models for concentration-response relations are acceptable. Data requirements include regression equation, correlation coefficient, residual sum of squares, and linear graph.

The detection limit is the minimum amount of analyte in a sample that can be detected, used for limit tests and qualitative identification, and the quantitation limit is the lowest amount of analyte that can be determined accurately.

**Assessment of Brazilian Requirements**

The calibration curve represents the relationship between the instrument response and the known concentration of the analyte. A calibration curve should be generated for each drug and analytical run, which will be used to calculate the drug concentration in the samples using the same biological matrix proposed for the study.

The calibration curve shall include analysis of the white sample (drug-free and internal standard-free biological matrix), the zero sample (biological matrix plus the internal standard) and at least 6 (six) samples containing drug and internal standard, contemplating the expected range of variation, from LOQ up to 120% of the highest concentration to be analyzed.

To determine the calibration curve, samples extracted from the appropriate matrix should be analyzed for at least 6 (six) different concentrations.

Alternative procedures should be justified, such as in obtaining a nonlinear correlation, where a greater number of standard concentrations will be required.

Results should be analyzed by appropriate statistical methods, such as the least-squares linear regression calculation. The curves obtained (experimental and the resulting from the mathematical treatment), the linear correlation coefficient, the angular coefficient and the line intercept must be presented [26].

Calibration curve acceptance criteria

- less than or equal to 20% (twenty percent) deviation from the nominal concentration for the LOQ;
- deviation less than or equal to fifteen percent (15%) from the nominal concentration for the other concentrations of the calibration curve;
- At least four out of six calibration curve concentrations must meet the above criteria, including the LOQ and the highest calibration curve concentration.
- The linear correlation coefficient must be equal to or greater than 0.98.

## 3.4 Range

### ICH Requirements

The specified range is normally derived from linearity studies which depends on the intended application of the procedure. It is established by confirming that the analytical procedure provides an acceptable degree of accuracy, linearity and precision when applied to samples containing amounts of analyte within or at the limit of the specified range of the analytical procedure. The following minimum specified ranges will be considered:

- For the assay of a drug substance normally from 80 to 120 % of the test concentration.
- For content uniformity, which covers a minimum of 70 to 130 % of the test concentration, unless a wider more appropriate range, based on the nature of the dosage form (e.g., metered dose inhalers), is justified.
- For dissolution testing the range is +/-20 % over the specified range (Example: if the specifications for a controlled released product cover a region from 20%, after 1 hour, up to 90%, after 24 hours, the validated range would be 0-110% of the label claim.)
- For the determination of an impurity: from the reporting level of an impurity from 1 to 120% of the specification.
- For impurities known to be unusually potent or which produce toxic or an unexpected pharmacological effect, the detection/quantitation limit should be commensurate with the level at which the impurities must be controlled;
- For validation of impurity test procedures carried out during development, it may be necessary to consider the range around a suggested limit.

- If purity and assay are performed together as one test and only a 100% standard is used, linearity should cover the range from the reporting level of the impurities from to 120% of the assay specification [2,3].

**United Sates of Pharmacopoeia (USP)**

The range of the procedure is validated by verifying that the analytical procedure provides acceptable precision, accuracy, and linearity when applied to samples containing analyte at the extremes of the range as well as within the range.

It is also recommended that the following minimum specified ranges should be considered:

- *Assay of a drug substance (or a finished product):* From 80% to 120% of the test concentration
- *Determination of an impurity:* From 50% to 120% of the acceptance criterion
- *For content uniformity:* A minimum of 70%–130% of the test concentration, unless a wider or more appropriate range based on the nature of the dosage form (e.g., metered-dose inhalers) is justified.
- *For dissolution testing:* ±20% over the specified range (e.g., if the acceptance criteria for a controlled-release product cover a region from 30% after 1 h, and up to 90% after 24 h, the validated range would be 10%–110% of the label claim).

**Chinese Pharmacopoeia (CPh)**

The range of the analytical method should be determined based on specific application of the method, its linearity,accuracy and precision, and related requirement.

- For content determination of drug substance and preparation, the range should be 80 % to 120 % of the test concentration.
- For content uniformity of preparation, the range should be 70% to 130 % of test concentration and this range may be widened appropriately for special dosage forms, such as aerosols and sprays.
- For dissolution test and drug release test, the range should be ± 30% of the limit. If the range of limit is provided, it should be -20% of lower limit to + 20% of upper limit. For impurity determination, the range should be stipulated from -20% to + 20% of the provided limit on the basis of preliminary actual determination.
- If the content determination and impurities test are performed simultaneously in a test, and only 100% of the reference substance is used, the linear range should cover the reported level of impurities to 120% of the specified content.
- For traditional Chinese medicine, the range of analytical method should be determined based on specific application, linearity, accuracy and precision of the method, and related requirement. For toxic ingredients or those with unique efficacy or pharmacological effect, the range to be validated should be wider than the range of content. For dissolution test and drug release test, the range should be ±30% of the limit.

**Assessment of Brazilian Requirements**

**Interval**

The range specified is the range between the upper quantitation limits and the bottom of an analytical method. It is usually derived from the study of linearity and depends on the intended application of the method. It is established by confirmation that the method has adequate accuracy, precision and linearity when applied samples containing quantities of substances within the specified range.

- Quantitative determination of analyte in raw materials or pharmaceutical forms should be between 80% to 120% of theoretical concentrations.
- Determination of Impurities: from the expected impurity level up to 120% of the specified upper limit . Where they are of toxicological significance or unexpected pharmacological effects, the limits of quantification and detection should be appropriate to the amount of impurities to be controlled.
- Content Uniformity: 70 5 to 130% of theoretical concentrations
- *For dissolution testing:* ±20% over the specified value for the range . If the specification for dissolution involves more than one time , the method ranges should include -20% over than smallest value and +20% over than largest value. (e.g., if the acceptance criteria for a controlled-release product cover a region from 30% after 1 h, and up to 90% after 24 h, the validated range would be 10%–110% of the label claim).

### 3.5 Accuracy

#### ICH Requirements

Accuracy in analytical procedures should be accepted across the specified range. Methods for determining accuracy include applying the procedure to synthetic mixtures of drug product components, adding known quantities of the analyte to the drug product, or comparing results from a second, well-characterized procedure. Accuracy should be concluded once precision, linearity, and specificity have been established. Impurities should be assessed on drug substance/drug product spiked with known amounts, and in cases where samples are impossible, results obtained by an independent procedure or the established response factor can be used. ICH recommends evaluating accuracy using a minimum of 9 determinations over three concentration levels covering the specified range.

#### United Sates of Pharmacopoeia (USP)

Accuracy in analytical procedures refers to the closeness of test results obtained by a procedure to the true value. It should be established across its range and combines concepts of unbiasedness and precision. Accuracy can be determined by applying the analytical procedure to an analyte of known purity or by comparing results with a second, well-characterized procedure. For assaying a drug in a formulated product, accuracy can be determined by applying the procedure to synthetic mixtures of drug product components with known amounts of analyte added within the procedure range. If samples of all drug product components are not available, adding known quantities of analyte to the drug product or comparing results with a second, well-characterized procedure can be acceptable. Accuracy is calculated as the percentage of recovery by the assay of the known added amount of analyte in the sample, or as the difference between the mean and the accepted true value, along with confidence intervals.

#### Chinese Pharmacopoeia (CPh)

Analytical method accuracy is the agreement between the found value and the accepted true or reference value. It is often measured in percent recovery and should be determined within a specified linearity range. Accuracy can be inferred from the method's precision, linearity, and specificity. To evaluate accuracy, at least six samples of the same concentration or nine samples with different concentrations and solutions should be used. The choice of strategy should consider the analysis purpose and sample concentration range.

- **Accuracy method for Assay of Chemical medicine:** The accuracy of drug substances can be determined using a reference or test substance with known purity, or by comparing results with established methods. For drug preparation, accuracy can be determined by spiking the exact amount of blank excipient in prescription dosage, adding known amounts of analyte, or comparing results.

- **Accuracy of quantitative determination of impurity for chemical medicine:** Accuracy of drug substance or preparation can be determined by spiking with a known impurity reference substance or by comparing results with matured methods.
- **Accuracy of determination of ingredients for traditional chinse medicines:** Reference substances with known purity can be used to determine the recovery of added samples. A precise amount of the reference substance is added to a test sample with known analyte content. The recovery ratio is calculated by dividing the determined value by the amount of the substance being examined. Proper amount of reference substance ensures authenticity [27].
- **For traditional Chinese medicine**, the text presents a table detailing the correlation between the test substance content in the sample and the limit of percent recovery, which can be adjusted based on the case.

**Table 6: The content of test substance in sample and the limit of percent recovery**

| **Content of test substance in sample** | | | **Mass fraction of test substance in sample** | **Limit of Percent recovery (%)** |
|---|---|---|---|---|
| **(%)** | **(ppm or ppb)** | **(mg/g or µg/g)** | **(g/g)** | |
| 100 | - | 1000 mg/g | 1.0 | 98-101 |
| 10 | 100 000 ppm | 100 mg/g | 0.1 | 95-102 |
| 1 | 10 000 ppm | 10 mg/g | 0.01 | 92-105 |
| 0.1 | 1000 ppm | 1 mg/g | 0.001 | 90-108 |
| 0.01 | 100 ppm | 100 µg/g | 0.0001 | 85-110 |
| 0.001 | 10 ppm | 10 µg/g | 0.00001 | 80-115 |
| 0.0001 | 1 ppm | 1 µg/g | 0.000001 | 75-120 |
| | 10 ppb | 0.01 µg/g | 0.0000001 | 70-125 |

**This table comes from AOAC "Guidelines for single Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals"**

**Assessment of Brazilian requirements**

The accuracy of an analytical method is the proximity of the results obtained by the method under study to the true value. Several methodologies for determining accuracy are available:

**a) Drug**

1. Applying the proposed analytical methodology in the analysis of a substance of known purity (reference standard)

2. Comparison of the results obtained with those resulting from a well characterized second methodology, the accuracy of which has been established.

**b) Pharmaceutical form**

1. Analyzing a sample in which known amount of drug was added to a mixture of drug components (contaminated placebo);
2. Where samples of all drug components are unavailable, analysis by the standard addition method is accepted, in which known amounts of analyte (reference standard) are added to the drug [28].

**c) Impurities**

1. Analysis by the standard addition method, in which known amounts of impurities and / or degradation products are added to the medicament or drug
2. In the case of unavailability of samples of certain impurities and / or degradation products, comparison of the results obtained with a second well-characterized method (pharmacopoeial methodology or other validated analytical procedure)

Accuracy is calculated as a percentage recovery of the known amount of analyte added to the sample, or as the percentage difference between the means and the accepted true value plus confidence intervals (Table 7).

The accuracy of the method must be determined after establishing the linearity, the linear range and the specificity of the method, being verified from at least 9 (nine) determinations considering the linear range of the procedure, i.e. 3 (three). Concentrations, low, medium and high, with 3 (three) replicates each. Accuracy is expressed by the relationship between the experimentally determined mean concentration and the corresponding theoretical concentration.

**Table 7 Analyte recovery at different concentration**

| **Active Ingredient (%)** | **Analyte ratio** | **Unit** | **Mean recovery (%)** |
|---|---|---|---|
| 100 | 1 | 100% | 98-102 |
| ≥10 | $10^{-1}$ | 10% | 98-102 |
| ≥1 | $10^{-2}$ | 1% | 97-103 |
| ≥0.1 | $10^{-3}$ | 0.1% | 95-105 |
| ≥0.01 | $10^{-4}$ | 100 ppm | 90-107 |
| ≥0.001 | $10^{-5}$ | 10 ppm | 80-110 |
| ≥0.0001 | $10^{-6}$ | 1 ppm | 80-110 |
| ≥0.00001 | $10^{-7}$ | 100 ppb | 80-110 |
| ≥0.000001 | $10^{-8}$ | 10 ppb | 60-115 |
| ≥0.0000001 | $10^{-9}$ | 1 ppb | 40-120 |

## 3.6 Precision

**ICH Requirements**

Validation of tests for quantitative determination of impurities and for assay includes an investigation of precision.

- Repeatability: Repeatability should be assessed using:
  a) a minimal of 9 determinations covering the specified range for the procedure (e.g., 3 concentrations/3 replicates each); or
  b) a minimal of 6 determinations at 100% of the test concentration.
- Intermediate Precision
  The range to which intermediate precision should be proved depends on the circumstances under which the procedure is intended to be used. The applicant should prove the effects

of random events on the precision of the analytical procedure. Typically, the variations which need to be studied include days, analysts, equipment, etc.

- Reproducibility
  Reproducibility is evaluated by means of an inter-laboratory trial. Reproducibility should be considered in the case of the standardization of an analytical procedure, for inclusion, for instance of procedures in pharmacopoeias.
- Recommended Data
  The standard deviation, relative standard deviation that is coefficient of variation and confidence interval should be reported for each type of precision investigated.

**United Sates of Pharmacopoeia (USP)**

The precision of an analytical procedure is determined by using a minimum of nine determinations covering the specified range for the procedure or using a minimum of 6 determination at 100% of the test final concentration.

**Chinese Pharmacopoeia (CPh)**

- Repeatability: In specified range, the repeatability should be evaluated using results from at least 6 samples of test substance at the same concentration (the concentration of the sample determined by the analytical method, equivalent to 100 % concentration level), or at least 9 samples with at least 3 different concentrations of test substance and at least 3 test solutions at each concentration. When the repeatability is evaluated by at least 9 testing results, concentration settings should consider the concentration range of the sample.
- Intermediate Precision: A scheme should be designed to inspect the effect of random variable factors on the precision. The variable factors include different dates, different analysts and different equipment's.
- Reproducibility: Reproducibility should be assessed when an analytical method is adopted as the national drug quality standard, for example, reproducibility should be inspected by different laboratory studies. Both the process of the collaborative study and result of the reproducibility should be recorded in the description of draft file. Where a reproducibility testing is to be conducted, the sample should be uniform properly stored and transported to obtain reliable result.

The Precision of an analytical method can be expressed as the standard deviation, relative standard deviation and confidence interval should be reported. Below table can be referenced (Table 8).

**Table 8 The content of test substance in sample and acceptable range of the RSD for precision**

| **Content of test substance in sample** | | | **Mass fraction of test substance in sample** | **Repeatability (RSD$_r$%)** | **Reproducibility (RSD$_R$%)** |
|---|---|---|---|---|---|
| **(%)** | **(ppm or ppb)** | **(mg/g or µg/g)** | **(g/g)** | | |
| 100 | - | 1000 mg/g | 1.0 | 1 | 2 |
| 10 | 100 000 ppm | 100 mg/g | 0.1 | 1.5 | 3 |

| | | | | | |
|---|---|---|---|---|---|
| 1 | 10 000 ppm | 10 mg/g | 0.01 | 2 | 4 |
| 0.1 | 1000 ppm | 1 mg/g | 0.001 | 3 | 6 |
| 0.01 | 100 ppm | 100 μg/g | 0.0001 | 4 | 8 |
| 0.001 | 10 ppm | 10 μg/g | 0.00001 | 6 | 11 |
| 0.0001 | 1 ppm | 1 μg/g | 0.000001 | 7 | 16 |
| | 10 ppb | 0.01 μg/g | 0.0000001 | 15 | 32 |

**This table comes from AOAC "Guidelines for single Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals"**

**Assessment of Brazilian Requirements**

Precision is the evaluation of the proximity of the results obtained in a series of measurements of a multiple sampling of the same sample. This is considered on three levels.

**Repeatability (intra-run precision**)

Agreement between results within a short time with the same analyst and same instrumentation.

The repeatability of the method is verified by at least 9 (nine) determinations, considering the linear range of the method, that is, 3 (three) concentrations, low, medium and high, with 3 (three) replicates each or minimum of 6. 100% determinations of the test concentration.

**Intermediate precision (inter-race precision)**

Agreement between results from the same laboratory, but obtained on different days, with different analysts and / or different equipment.

For the determination of intermediate accuracy, a minimum of 2 different days with different analysts is recommended.

**Reproducibility (inter-laboratory precision)**

Agreement between results obtained in different laboratories as in collaborative studies, generally applied to standardization of analytical methodology, for example, to include methodology in pharmacopoeias. This data need not be submitted for registration grant.

The Precision of an analytical method can be expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements.

Precision can be expressed as relative standard deviation (DPR) or coefficient of variation (CV%) according to the formula,

$$\mathrm{DPR} = \frac{\mathrm{DP}}{\mathrm{CMD}} \mathrm{x}100$$

where SD is the standard deviation and CMD the mean concentration determined.

The maximum acceptable value should be defined according to the methodology employed, the concentration of the analyte in the sample, the type of matrix and the purpose of the method, with values not exceeding 5%.

## 3.7 Robustness

**ICH, USP, CPh and ANVISA Requirements**

The evaluation of robustness depends on the type of procedure under study and should be considered during the development phase. It should show the accuracy of an analysis with respect to deliberate variations in method parameters [2]. If measurements are similar to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure. One outcome of the evaluation of robustness should be that a sequence of system suitability parameters (e.g., resolution test) is established to ensure that the validity of the analytical procedure is maintained whenever used. Examples of typical variations are:

- stability of analytical solutions;
- extraction time.

For liquid chromatography, examples of typical variations are:

- impact of variations of pH in a mobile phase;
- influence of variations in mobile phase composition;
- different columns (different lots and/or suppliers);
- temperature;
- flow rate.

In the case of gas-chromatography, examples of typical variations are:

- different columns (different lots and/or suppliers);
- temperature
- flow rate.

The robustness of an analytical method is a measure of its ability to withstand small and deliberate variations of analytical parameters. Indicates your confidence during normal use.

During the development of the methodology, the robustness assessment should be considered. Given the susceptibility of the method to variations in analytical conditions, these should be controlled, and precautions should be included in the procedure [5].

## 4 Conclusion

The aim of this work was the critical assessment of the ICH guidelines in USP, CPh and Brazil. Even though all the guidelines are harmonized with ICH except Brazil. The harmonization of the documents will reduce duplication of studies such as, analytical validation and pharmaceutical development which are currently performed in order to comply with different regulations of individual countries. This will speed up the access to medicinal products for the patients in all over the regulated marketplace. The efficient development and validation of analytical methods are critical elements in the development of pharmaceuticals and ensuring regulatory compliance. Analytical method validation is an important tool for ensuring the performance of the method. Various guidelines by different regulatory bodies and organizations disagree on different points. Though ICH guidelines have resolved the differences between Europe, the USA, Chinese and Japan, organizations like IUPAC and AOAC still have differences on some points. There should be an effort to put forward uniform guidelines for validation throughout the world and to create a similar platform for acceptance criteria to reduce duplications and cost reduction to applicants.

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