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Validation Of Analytical Methodologies: Context And Guidelines For And Safety And Innocuity Of Residues In Brazilian Food

Pedro Prates Valério[1], Margarete Aparecida Pereira[2], Salvador Carlos Grande[3], Anderson Dos Santos De Jesus[4], Esther Sabrina Cordeiro[4], Maria Eduarda Ribeiro Eugênio[4], Tamara Gomes Vitor[4], Wagner Aveino Moreira[4]

1(Professor At Federal Institute Of Education, Science, And Technology Of Southern Minas, Inconfidentes - Brazil)
[2](Chemistry Professor At Trivento Educação. Belo Horizonte - MG, Brazil)
[3](Professor At The Faculty Of Science And Technology, Universidade Zambeze - Beira, Mozambique)
[4](Undergraduate Student At The Faculty Of Science And Technology, Universidade Zambeze – Beira, Mozambique)

Abstract:
Background*: Analytical information is critical for decision-making processes, including product quality control, consumer health protection, and compliance with regulatory standards. Analytical methods for analyzing raw materials and food must undergo rigorous validation to ensure reliability. This study examines the evolution of validation guidelines for analytical methodologies, emphasizing their role in ensuring food safety and consumer protection.*
Materials and Methods*: The research involved a comprehensive literature review on the development of validation guidelines for analytical methods assessing residue safety and innocuity in raw materials and food. A discursive analysis was conducted to contextualize these guidelines within Brazil's role as a significant food exporter and a signatory to international agreements, such as the Codex Alimentarius. The study also examined the standards established by international organizations, including Eurochem and NIST, to validate analytical methods used in reference laboratories.*
Results*: The findings highlight the importance of rigorous validation procedures for analytical methods, ensuring traceability, comparability, and reliability of analyses. The study identifies Brazil's increasing alignment with global food safety demands, addressing the control of chemical substances in production processes to meet international market expectations.*
Conclusion: *Validation guidelines play a vital role in supporting food safety and regulatory compliance, particularly in global markets with high demands for quality and reliability. Brazil's adherence to international standards and agreements reinforces its commitment to safe and sustainable food production, with validated analytical methods serving as a cornerstone for achieving these objectives.*
Keywords*: Analytical Methods; Validation; Food Safety; Guidelines; Residue Analysis*

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I. Introduction

The constant pursuit of effective measures to ensure the safety and innocuity of food tends to favor policies aimed at protecting consumer health. Since the World Declaration on Nutrition in 1992, jointly held by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), access to safe and nutritionally adequate food has been recognized as a fundamental right of every individual. Reinforcing this commitment, governments and multilateral organizations are encouraged to collaborate in ensuring continuous access to safe and affordable food, promoting sustainable development policies integrated with agricultural practices, public health, and social welfare. In this context, aligning such measures with international sanitary requirements and standards, such as those of the Southern Common Market (MERCOSUL), Codex Alimentarius, and the World Trade Organization (WTO), backed by organizations like the World Organisation for Animal Health (WOAH), FAO, and WHO, broadens the scope of quality control and food safety in Brazil, facilitating compliance with the sanitary requirements of international markets importing Brazilian products (BRASIL, 2012b; HIGIENE ALIMENTAR, 2008; WHO, 2021).

It is worth briefly recalling that, in a contextual scope, concern about the indiscriminate use of potentially toxic substances has a referential basis dating back to the 1960s, strengthened by the publication of the book "Silent Spring" by Rachel Carson, which warned about the harmful effects of the extensive use of Dichloro-Diphenyl-Trichloroethane (DDT) in pest control in North American crops. The implementation, perhaps sensitized, of regulatory limits considered safe by various agencies, and the development and validation of analytical methods have been aimed at ensuring more responsible compliance. Similar standards tend to be established by various international organizations, determining methodological designs for tests that also impact the private sector, as a requirement for authorizations that directly affect the nature of businesses, also considering the results of experimental studies that determine human and environmental safety. The validation of analytical methods is an important requirement for any package of information, including those submitted to regulatory agencies, in support of the marketing of new products or in clinical trial applications (FRIEDRICH et al., 2022; SKOOG et al., 2023).

As Brazil is one of the leading countries in global food production, it is essential to consider that the success of its exports is closely related to its ability to meet the requirements of importing countries, particularly regarding quality, safety, control, and the innocuity of raw materials and food products, considering regulated substances. In light of this context, the present study aims to explore primary objectives, including the evaluation of validation guidelines for analytical methodologies, stimulating brief reflections linked to the safety and innocuity of residues in food. Thus, enhancing the integrity of the analytical methods used in the food industry. Throughout the development, the study seeks to identify elements that foster an understanding of the importance of the reliability of analytical results in protecting public health, with implications for the food production chain.

II. Material And Methods

This study is based on an objective bibliographic survey aimed at reflection. Some fundamental concepts related to the validation of analytical methodologies and their importance in food safety, considering regulatory guidelines, are presented, enabling considerations regarding analytical rigor and residues in food within the integrity of the production chain. Thus, to facilitate its development, exploratory research is conducted, from which the generated knowledge is gathered to construct hypotheses and propose an understanding that encourages analytical innovation, while also taking into account the need for continuous adaptations and verifications in commonly used methodologies. The study is complemented by a discursive analysis of ideas in the section on considerations.

III. Development

In the Brazilian context, and in response to the demand for harmonized and scientifically based guidelines for the control of residues and contaminants in food, the Ministry of Agriculture, Livestock, and Supply (MAPA), through the Secretariat of Agricultural Defense (SDA) and the General Coordination of Laboratory Support (CGAL), implemented the Analytical Quality Assurance Manual. This document aims to meet the rigorous quality standards required for analytical results, also focusing on the expectations of international markets. Developed in accordance with the International Vocabulary of Metrology (VIM) and the Codex Alimentarius Guide on Terminology in Analytical Chemistry (CAC-GL72/2009), the manual integrates the requirements of the ABNT NBR ISO/IEC 17025:2005 standard, which establishes criteria for the competence of calibration and testing laboratories. Additionally, it incorporates ISO 11843 standards regarding detection capability and linear calibration, as well as the ISO 5725 series, which emphasizes precision and accuracy in analytical measurements, promoting a solid and globally recognized approach to food safety (BRASIL, 2012a; ABNT, 2005; CODEX, 2009).

It is noteworthy that the aforementioned Manual represents a milestone in the implementation of rigorous standards for method validation and for the assurance of analytical quality in laboratories engaged in the control of residues and contaminants in products of animal and plant origin. By establishing detailed guidelines, the manual provides essential criteria for the validation of analytical methods and for the development of consistent laboratory routines (BRASIL, 2022e; CODEX, 2017; CODEX, 2022).

This framework is fundamental, especially within the scope of the National Residue and Contaminant Control Plan (PNCRC), which aims to ensure that laboratories maintain compliance with international safety standards and have the technical capacity to identify and quantify residues and contaminants. Adhering to these guidelines strengthens the reliability of the data obtained, also ensuring that the results communicate effectively with international markets, increasing the competitiveness of Brazilian products and promoting a culture of safety and analytical quality in the country (BRASIL, 2022e; CODEX, 2017; CODEX, 2022).

Brazilian National Residue and Contaminant Control Program (NRCCP / Brazilian PNCRC)

From a chronological analysis, it is essential to highlight that the National Residue and Contaminant Control Plan (NRCCP / PNCRC) has its roots in the National Program for the Control of Biological Residues in Meat (Brazilian PNCRBC), established on January 26, 1979, by Ministerial Ordinance No. 86 of the Ministry of Agriculture. This initial program aimed to systematize the control of residues in meat products, focusing on collecting information about the occurrence of residues in animals slaughtered in establishments under Federal Inspection, as well as mapping these occurrences by region of origin (PORFÍRIO, 1994). In 1986, the PNCRBC was expanded into the National Residue Control Plan for Products of Animal Origin (PNCR), which became a normative regulatory instrument aimed at controlling residues of substances used in agriculture and environmental pollutants present in meat, milk, honey, fish, and their derivatives (BRASIL, 1986; BRASIL, 1999).

The current National Residue and Contaminant Control Plan (PNCRC) is an evolution of the previous National Residue Control Plan (NRCP / Brazilian PNCR), constituting a federal program for the management, inspection, and supervision of food. This program is based on risk analyses and the proposal to verify the presence of residues of potentially harmful chemical substances to consumer health. Among such residues are veterinary medicines, pesticides, environmental contaminants, and inorganic contaminants. The National Residue and Contaminant Control Plan for animal products (PNCRC/animal) is a risk management program coordinated by the General Coordination of Animal Origin Products, of the Division of Inspection of Animal Origin Products (DIAOP / Brazilian DIPOA). The central objective of the PNCRC is to promote the chemical safety of animal-derived foods produced in establishments under the control of the Federal Inspection Service (FIS) and registered with DIPOA (BRASIL, 2022e; BRASIL, 1999; BRASIL, 2012a).

The PNCRC/animal was established to encompass various sectoral programs, including PNCRC/Fish, PNCRC/Milk, PNCRC/Honey, PNCRC/Eggs, as well as specific programs for cattle, poultry, pigs, horses, and ostriches. The guidelines, programs, and work plans related to these were established by Normative Instruction SDA No. 42, dated December 20, 1999, and evolved through new Normative Instructions, such as SDA No. 24, dated August 9, 2011, which approved the analytical scope for monitoring residue testing (BRASIL, 2012a; BRASIL, 2012b).

In the context of the National Residue and Contaminant Control Plan (PNCRC) for plant products, its implementation was initially established by Normative Instruction SDA/MAPA No. 42, dated December 31, 2008. However, this regulation was revoked in 2022 by Portaria SDA No. 574, dated May 9, 2022. The new ordinance represents a significant evolution from the previous plan, transforming it into a program that aligns with contemporary guidelines from the Secretariat of Agricultural Defense (SAD / Brazilian SDA) and the Department of Plant Origin Products. This change was motivated by the need to update the concepts used in the original plan, which no longer reflected the reality of Inspection of Plant Origin Products. The updates also reflect a shift in focus in monitoring actions, now oriented towards oversight activities based on risk management, aligning with internationally recommended practices (BRASIL, 2008; BRASIL, 2022a).

Thus, in a general sense, it can be stated that both the PNCRC/Animal and PNCRC/Vegetal constitute risk management tools, encompassing a comprehensive set of actions aimed at the official control of residues and contaminants in products of their respective categories, whether imported, exported, or destined for the domestic market. These actions include supervisory, investigative, and evaluative activities, focusing on the analysis, treatment, and dissemination of the results obtained. In addition to annual sampling plans, these programs include monitoring tests to verify maximum limits of chemical residues, which are established by the National Health Surveillance Agency (Anvisa). Specifically, they are defined by Normative Instruction No. 162, dated July 1, 2022, which determines the acceptable daily intake (ADI), the acute reference dose (ARfD), and the maximum residue limits (MRL) for active pharmaceutical ingredients (API) of veterinary medicines in animal-derived foods. Additionally, Normative Instruction No. 160, also dated July 1, 2022, establishes the maximum tolerated limits (MTL) of contaminants in food (BRASIL, 2022c; BRASIL, 2022d; BRASIL, 2022e)

Analytical Consonance: Brazilian Health Regulatory Agency (BHRA - Brazilian ANVISA)

In line with strengthening sanitary control mechanisms in the primary production of food, ANVISA established, through Resolution RDC No. 253, of September 16, 2003 (subsequently revoked), the Program for the Analysis of Residues of Veterinary Medicines in Animal-Derived Foods (PAMVet). This program was designed to monitor the presence of residues of veterinary medicines in animal-derived foods, as part of the commitment to ensure food safety and public health. Initially restricted to the analysis of residues in bovine milk, PAMVet has been expanded over the years in scope, encompassing other food products such as meats and eggs, and implementing improvements in risk assessment techniques (BRASIL, 2003; BRASIL, 2009; PAMVet, 2009; BRASIL, 2010).

Since its inception, PAMVet has established itself as a central structure for the monitoring and control of veterinary medicine residues, playing an essential role in the evolution of the guarantees associated with food

safety and compliance with both national and international reference standards. Resolution RDC No. 166 of 2022, published by Anvisa, reinforces this quality perspective by replacing the former RE No. 899 of 2003 and introducing stricter criteria for the validation of analytical methods. This new resolution, by addressing technical aspects such as selectivity, linearity, accuracy, precision, and robustness, introduces the concept of matrix effect, particularly relevant for methods applied to foods with more complex matrices. Thus, it can be considered that RDC 166 strengthens guidelines for the detection and quantification of residues, ensuring that analytical procedures meet reliability and precision parameters aligned with regulatory requirements (Brasil, 2003; Brasil, 2022b).

Bringing attention to the relevance of more rigorous analytical processes that favor quality assurance in food products, including those subject to Anvisa's sanitary surveillance, it is noteworthy that RDC No. 390 of 2020 (Brasil, 2020) aligns with the Guide for the Provision of Regulatory Data, also published by the agency. The guide provides more direct guidance to the official laboratories of the National Network of Sanitary Surveillance Laboratories (RNLVISA) as well as to accredited laboratories on the procedures for requesting and providing regulatory data, which are subsidiary to the quality assessment of products under sanitary surveillance. The aforementioned laboratories must comply with Good Practices for Quality Control Laboratories, as provided for in RDC No. 512, of May 27, 2021, with adherence to procedures and methods (BRASIL, 2021). Although it is an orientational document without a normative purpose, the guide contributes to a solid approach in the validation of analytical methods.

Thus, continuing the structuring of analytical processes in products under sanitary surveillance, RDC No. 390 of 2020 plays a fundamental contextual role by guiding the necessary regulatory data for quality assessment, in addition to aligning with international requirements for enhancing food product safety. The norm reflects the integration of good practices in the laboratories of the National Network of Sanitary Surveillance Laboratories (RNLVISA), as well as among accredited laboratories. These guidelines also detail the importance of uniformity and precision in laboratory processes and are complemented by RDC No. 512 of 2021, which formalizes Good Practices for Quality Control Laboratories. The resolution applies, as appropriate, to: Holders of products subject to sanitary surveillance; Analytical laboratories providing services located within the national territory that perform analyses on products under sanitary surveillance; Analytical laboratories located within the national territory that belong to importers, distributors, fractionators, and manufacturers of products subject to sanitary surveillance; Companies responsible for ensuring and safeguarding the maintenance of quality, safety, and efficacy of products subject to sanitary surveillance up to the final consumer (manufacturers, importers, distributors, fractionators, transporters, retailers), among others (BRASIL, 2020; BRASIL, 2021).

In brief, the Guide for the Provision of Regulatory Data (Brasil, 2023) serves as a referential base document for providing regulatory data to laboratories of the National Network of Sanitary Surveillance Laboratories and accredited laboratories for the quality assessment of products under sanitary surveillance. Currently in its version 3, although this guide is an orientational document without normative power, it promotes a structured basis for the validation of analytical methods, making the process of data collection and submission more transparent and efficient. This guide strengthens the applicability of the RDCs in the context of control laboratory processes, ensuring greater consistency in results and increasing the reliability of national sanitary inspection. Importantly, it is necessary to emphasize that the promotion of safety, quality, and reliability of analytical results, reflecting safety throughout the entire food product chain, should not be restricted to a specific document but should align with references from regulatory bodies and agencies that maintain the necessary rigor to ensure accountability (ANVISA, 2021).

Analytical Determination: A Brief Context for Residues Detection and Quantification

The application of investigative methods, followed by confirmatory methods applied only to positively characterized samples, is common in the investigation of residues in animal tissues (WHO, 2008).

The most commonly used techniques for quantitatively determining residues in food must be sensitive, accurate, precise, selective, and specific, providing unequivocal information regarding the identity of the analyte. These techniques include: gas chromatography with electron capture detectors (GC-ECD) and mass spectrometry (GC-MS), high-performance liquid chromatography with ultraviolet detection (HPLC-UV), mass spectrometry (HPLC-MS), and, more recently, tandem mass spectrometry (HPLC-MS/MS) (MARTINS JUNIOR et al., 2006; VAN DE REIT et al., 2003; PENNEY et al., 2005).

Different levels of sensitivity of chromatographic techniques depend, among other factors, on the type of substance analyzed and the detector employed. Generally, chromatography is a technique used for separating the components of a mixture, based on the distribution of these components between a stationary phase and a mobile phase. Such separation results from the differing interactions between components carried by the mobile phase and the stationary phase (COLLINS, 1997; CECCHI, 2003; PEREZ et al., 2002; PITTELA, 2009).

Mass spectrometry detectors already have interfaces that facilitate coupling with various chromatographs, reflecting the diffusion of the technique. They provide qualitative and quantitative information,

both atomic and molecular, related to organic and inorganic compounds. When compared to more conventional detectors, they exhibit high levels of reliability and sensitivity, based on classifications that consider ionic transitions, mass-to-charge (m/z) ratios, selection, collision, and the movement of ions in electric and magnetic fields (MARTINS JUNIOR et al., 2006).

The presence of polar functional groups in certain molecules tends to decrease the limit of detection (LOD) for gas chromatography, considering detectors by electron capture (ECD) and mass spectrometry (MS) (WHO, 2008). Usually, the aforementioned derivatizations occur through silylation reactions, for which the reagents used include: a mixture of hexamethyldisilazane (HMDS), trimethylchlorosilane (TMCS), and pyridine (BERRY, 1987; GUDE et al., 1995); N,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) (BORIES et al., 1983); a mixture of BSTFA and TMCS (VAN GINKEL et al., 1990; KEUKENS et al., 1992; GANTVERG et al., 2003); and N-methyl-N(trimethylsilyl)-trifluoroacetamide (MSTFA) (IMPENS et al., 2003).

When GC is coupled with a mass spectrometer, the most frequently applied ionization techniques are chemical ionization (CI) and electron impact (EI) ionization. Negative chemical ionization (NCI) results in a limited number of fragments; however, the molecular ion is always part of a spectrum. Due to the eventual presence of chlorine atoms in some analyzed molecules, the GC-MS technique, in NCI mode, is reported as one of the most reliable and widely used in confirmatory methods. The limit of detection can be less than 0.1 mg/kg in muscle tissues. GC-MS in EI mode is less sensitive; however, it produces reproducible fragment spectra and is therefore suitable for storage in databases (WHO, 2008).

The more recent development of mass detectors where ionization occurs at atmospheric pressure (API), coupled with HPLC (HPLC-MS and HPLC-MS/MS), has become one of the most reliable and widely used techniques for residue analysis, including antibiotics, allowing detection and quantification without the need for preliminary derivatization steps for non-volatile polar analytes (WHO, 2008). IMPENS and collaborators (2003) developed an analytical method for investigating and confirming antibiotic residues in shrimp tissues, using ELISA as an investigative method and GC-MS/MS and HPLC-MS/MS for confirmation; both selective techniques exhibited limit of detection values equal to 0.1 µg/kg.

The highlight of the HPLC-MS/MS technique is related to its high analytical selectivity when in Multiple Reaction Monitoring (MRM) mode. In this mode, the Q1 and Q3 mass analyzers select the precursor and product ions, respectively, defining a specific mass-to-charge (m/z) transition. The second quadrupole (Q2) functions as a collision cell, where the precursor ions selected according to their m/z ratios in Q1 are fragmented by collision-induced dissociation (CID) after collisions with an inert gas (usually N2) under specific energy. Optimizing the detector for such an experiment (MRM), containing more than one transition for the same precursor ion, generates a confirmatory method. Thus, the use of this technique provides information regarding the retention of the compound on the chromatographic column, the monitored transitions, and the signal proportional to the concentration of the analyte, allowing for high levels of reliability and sensitivity in accordance with the established LRM (0.30 µg/kg) (MARTINS JUNIOR et al., 2006; VAN DE REIT et al., 2003; PENNEY et al., 2005).

Validation of Analytical Methods

Numerical and measurable evidence regarding the quality of quantitative, semi-quantitative, and/or qualitative analytical procedures is based on the comparability, traceability, and reliability of results—criteria that are increasingly recognized and required. To ensure that a new analytical method provides safe, accurate, and interpretable information about the sample, it must undergo a thorough statistical evaluation known as validation (RIBANI et al., 2004). The definition of validation by the International Organization for Standardization (ISO) as a process of confirmation through examinations and objective evidence aims to ensure that a method meets specific requirements for its intended use (ABNT, 1994). For analytical methods, the validation process involves characterizing the method's performance, its limitations, and the factors that may influence its accuracy and reliability. Validation is, therefore, essential to ensure that the method is suitable for the intended purpose, considering criteria such as selectivity, accuracy, robustness, among other aspects that directly affect the reliability of results (EURACHEM, 1998).

It is observed that the validation of analytical methods is also justified by technical, legal, and commercial criteria, with guidelines varying between different organizations, which may define specific, sometimes complementary parameters for such validation. Focusing on a classical foundational document within the subject matter, in Brazil, the Ministry of Agriculture, Livestock, and Food Supply (MAPA) specified, through the Validation and Quality Control Guide: Drugs in Food Products and Veterinary Medicines, important performance parameters, in addition to minimum acceptance requirements that must be met for an analytical method to be considered validated. This guide has established itself as a reference for laboratories within the National Network of Agricultural Laboratories, ensuring essential standardization for the reliability of results, as well as compliance with health control regulations. The document defines the necessary parameters for performance determination and acceptance criteria such as linearity, selectivity, detection limit, quantification

limit, recovery (truthfulness), precision (both repeatability and reproducibility), and matrix effect. Furthermore, it recommends the inclusion of studies on robustness and stability (for analytes, samples, and standard solutions) and the evaluation of measurement uncertainty (standard, combined, and expanded) (RIBANI et al., 2004; BRASIL, 2011).

ANVISA published the Guide for Validation of Analytical and Bioanalytical Methods through Resolution - RE No. 899, of May 29, 2003, establishing essential characteristics for validating analytical procedures. As mentioned, this regulation was replaced by Resolution RDC No. 166, of 2022, which introduced stricter validation criteria, encompassing aspects such as selectivity, matrix effect, linearity, accuracy, precision, and robustness, promoting regulatory precision and reliability. In parallel, the European Community, through Directive 2002/657/EC, also defined validation criteria and procedures for analytical methods to ensure the quality and comparability of results, as well as to establish common criteria for the interpretation of results and progressively define minimum performance limits (LMDR) for the detection of substances with no defined maximum limits, which are prohibited for use in the EU. The guide harmonized by the Association of Official Analytical Chemists – AOAC International, International Standards Organization – ISO, and International Union of Pure and Applied Chemistry – IUPAC is also considered an important international reference for validation of testing methods (BRASIL, 2008; PASCHOAL et al., 2008; EC, 2002; EC, 2003; BRASIL, 2017; BRASIL, 2022b; Conrrado 2023).

NBR ISO/IEC 17025:2005 requires that adapted, non-standardized, or laboratory-developed standardized methods undergo the validation process (ABNT, 2005). The EURACHEM Guide for Validation of Methods also suggests that, even for standardized methods, performance parameter checks should be carried out regularly, especially if quality control indicates changes over time, requiring revalidation. In Brazil, ANVISA and the National Institute of Metrology, Quality, and Technology (Brazilian INMETRO) are the main regulatory authorities for the validation of analytical methodologies, and the guidance document DOQ-CGCRE-008, of June 9, 2020, issued by INMETRO, is widely recommended for the validation of methodologies, being part of the National Council of Metrology, Standardization and Industrial Quality (Conmetro) and the National System of Metrology, Standardization, and Industrial Quality (Sinmetro) (INMETRO, 2020; BRASIL, 2022b, Souza & Brito, 2002; Conrrado, 2023).

Table 1: Validation Parameters, referenced by DOQ-CGCRE-008, from Brazilian INMETRO

Evaluated Parameter	DOQ-CGCRE-008 (INMETRO, 2020)	
	Qualitative	Qualitative
Selectivity	Yes	Yes
Linearity / Working Range / Sensitivity		Yes
Detection Limit	Yes	Yes
Quantification Limit		Yes
Bias / Recovery		Yes
Precision		Yes
Robustness	Optional	Optional
Matrix Effect	NA*	NA*

**NA: Not Applicable*

Source: INMETRO, 2020

The presented Table 1 illustrates the main parameters applied to validation procedures and reports according to INMETRO guidelines. Table 2 presents parameters to be considered in analytical validation, while Table 3 presents conditions for evaluating the robustness of the method, based on various analytical techniques, according to RDC No. 166, of 2022, from ANVISA (INMETRO, 2020; BRASIL, 2022b)

Table 2: Parameters to be Considered in Analytical Validation according to RDC 166, 2022 (Brazilian ANVISA)

Evaluated Parameter	**Identification**	**Impurity Testing**		**Dose Determination - Dissolution (quantification) - Content Uniformity - Potência: Potency**
		Quantitative	Limit Test	
Exactness	No	Yes	No	Yes
Precision - Repeatability	No	Yes	No	Yes
Precision - Intermediate	No	Yes	No	Yes
Selectivity	No	Yes	Yes	Yes
Detection Limit	No	No	Yes	Yes
Quantification Limit	No	Yes	No	Yes
Linearity	No	Yes	No	Yes
Range	No	Yes	No	Yes
Matrix Effect	(*)	(*)	(*)	(*)

(*) Applicable to Complex Matrices (including food)
Source: Brasil, 2022b

Table 3: Conditions for the evaluation of the method's robustness, based on various analytical techniques, according to RDC No. 166 of 2022 by Brazilian ANVISA

Sample Preparation	Stability of Analytical Solutions
	Extraction Time
	Compatibility of Filters
Spectrophotometry	Variation of pH of the Solution
	Different Batches or Manufacturers of Solvents
Liquid Chromatography	Variation of pH of the Mobile Phase
	Variation in Composition of the Mobile Phase
	Different Batches or Manufacturers of Columns
	Temperature
	Flow Rate of the Mobile Phase
Gas Chromatography	Different Batches or Manufacturers of Columns
	Temperature
	Gas Flow Rate
Other Analytical Techniques	The variations to be tested should be critically evaluated, and their results should be presented

Source: Brasil, 2022b

From the perspective of exemplification and cross-referencing some sources, JENKE (1996) evaluated the application of performance parameters for the validation of methods in governmental, industrial, and academic spheres. The research revealed that the items accuracy and precision (both repeatability and reproducibility) were the most frequently cited, followed by specificity, linearity, limit of quantification, limit of detection, robustness, and sensitivity. Similarly, SOUZA & BRITO (2002) emphasized the importance of evaluating sensitivity in validation protocols, especially in the comparison of methods. LANÇAS (2004) reported the application of the following validation parameters: linearity, specificity, limit of detection, limit of quantification, application range, accuracy, precision, sensitivity, recovery, robustness, and stability.

In summary, despite existing divergences regarding the performance parameters in the validation processes of analytical methods, it is crucial that studies are based on the intended use of the method, representative, and conducted in a way that accommodates the variation in concentration range and sample types. Thus, the selection of performance parameters must be rigidly aligned with the validation objectives. Therefore, it is up to the laboratory to determine the performance parameters to be characterized (LANÇAS, 2004; Conrrado, 2023; RIBANI et al., 2004; EURACHEM, 1998). The following are definitions of certain performance parameters commonly applied in the validation of analytical methods.

Selectivity

Selectivity refers to the ability of a method to determine the analyte unequivocally in the presence of other substances that may interfere with the determination (LANÇAS, 2004). A specific method should produce a response for a single analyte. In contrast, a selective method produces responses for multiple analytes that can be distinguished from one another. Generally, specificity is considered to be 100% selectivity (EURACHEM, 1998). To ensure the specificity of the method, it must be evaluated whether the signal measured by the equipment is solely due to the analyte or results from the sum of contributions from multiple components (Conrrado, 2023).

Selectivity is the first step in the development and validation of an instrumental separation method and should be continuously re-evaluated during the validation process and subsequent use of the method. If selectivity is not ensured, linearity, accuracy, and precision will be seriously compromised. This parameter can be obtained in various ways. The first way to assess selectivity in separation methods is by comparing the matrix devoid of the substance of interest with the matrix spiked with it (standard), ensuring that no interfering substance is co-eluting at the retention time of that substance. Another procedure to evaluate selectivity is through the collection of the compound of interest and conducting a new analysis using another chromatographic technique or methods and techniques that are specific to the structure of the analyte of interest (RIBANI et al., 2004).

Matrix Effect

The Matrix Effect is a study of selectivity that aims to investigate possible interferences caused by the substances that make up the sample matrix, basically generating phenomena of signal reduction or enhancement of the instrumental response. The study of the matrix effect is essential when working with a calibration curve of the analyte in solvent, that is, with a non-matrix calibration curve. The procedures for determining the Matrix Effect essentially consist of: preparing a calibration curve of the analyte in pure solvent (non-matrix sample), a

curve of the analyte in the extract of the matrix devoid of the analyte (matrix sample), and evaluating the results, including the F-test - Fischer-Snedecor and Student's t-test (BRASIL, 2011).

Linearity and Range of Application

Linearity refers to the method's ability to provide results directly proportional to the concentration of the substance being examined, within a specified range of application (EURACHEM, 1998). Confirmation of linearity and determination of the working range can be achieved by constructing analytical curves of the analyte concentration as a function of the response obtained in the detection system, with replicates around the expected concentration of the analyte. This working range should encompass the expected concentration for the test sample (CONRRADO, 2023; BRASIL, 2011).

The experiments described for evaluating linearity often involve preparing curves with or without matrix, usually with five to six concentration levels, including or excluding the zero point, and with a minimum of two to seven replicates per level. The ordinary least squares (OLS) method is widely accepted as a statistical tool for evaluating linearity. Most references recommend estimating the parameters and the residuals (errors) of the regression, as well as visually inspecting the x-y plot and the residual plot of the regression (SOUZA, 2007; BRASIL, 2022b). Since validation studies are based on statistical hypotheses, a basic check of the assumptions related to statistical tests is fundamental to ensure that the principles of these tests are not affected and that the obtained results are supported (THOMPSON, ELLISON & WOOD, 2002). Thus, before performing any inference, it is necessary to examine the assumptions to determine if the data are suitable for applying the tests (SOUZA, 2007). The method used for data analysis is OLS. Fitting a calibration equation using OLS assumes several premises regarding the regression residuals and the model: i) the residuals are random variables with a mean of zero E (εi) = 0 and variance V (εi) = σ^2 constant and unknown; ii) the residuals are normally distributed $\varepsilon \sim N(0, \sigma^2)$; iii) the residuals are homoscedastic, with a constant distribution across the values of Xi; iv) the residual of one observation εi is not correlated with the residual of another observation εj, that is, cov (εi, εj) = 0, where $i \neq j$. The residuals are not only uncorrelated but also independent; and v) the relationship between Xi and Yi is linear (SOUZA, 2007; CONRRADO, 2023).

The working range is the interval of standards in which the requirements for linearity, as well as those for accuracy and precision, are satisfied. The lower limit of the working range should coincide with the limit of quantification (INMETRO, 2007; INMETRO, 2020).

Accuracy and Precision

Accuracy is the degree of agreement between the mean of a set of experimentally obtained results and the true value or the value recognized as such. Accuracy indicates the difference between the obtained value and the actual value of the analyte in the matrix, generally expressed in terms of bias, that is, the deviation (positive or negative) of the mean of the obtained value relative to the actual value (EURACHEM, 1998). Accuracy is always considered within certain limits, at a given confidence level, and is therefore associated with precision values. These limits can be narrow at high concentration levels and wider at trace levels (RIBANI et al., 2004).

Precision is the degree of dispersion of the results obtained under specified conditions around the mean value. Precision can be evaluated under conditions of repeatability or reproducibility, being expressed in terms of the coefficient of variation or relative standard deviation (SOUZA & BRITO, 2002). Both repeatability and reproducibility are generally dependent on the analyte concentration; thus, they should be determined at a number of concentrations. If relevant, the relationship between precision and analyte concentration should be established (EURACHEM, 1998).

Repeatability is the degree of agreement between results obtained by successive measurements of the same method, conducted under the same measurement conditions, called repeatability conditions, in short time intervals. Repeatability represents the variability obtained by the same analyst applying the same method on the same day over replicates of the same sample (RIBANI et al., 2004). If the same sample is analyzed under varied conditions, the measure of precision is termed reproducibility. This is defined as the degree of agreement between results obtained by applying the same analytical procedure to the same material under predetermined conditions (such as different laboratories, operators, and equipment), which can be partially evaluated by the variation of one or more factors (EURACHEM, 1998). Experimentally, measures of precision can be obtained by conducting a minimum of 10 independent determinations of the analyte at different concentrations (EURACHEM, 1998) or a minimum of nine independent determinations (3 different concentrations/3 replicates each concentration) (ICH, 1996).

Method Sensitivity

Method sensitivity is defined as the ability to distinguish between two close concentrations by altering the measurement response of the instrument at a given level of confidence. The sensitivity of the method should

not be confused with the limit of detection. Sensitivity reflects the capability to discriminate between samples with similar analyte levels (EURACHEM, 1998).

Limit of Detection

The limit of detection is defined as the lowest concentration of an analyte in a matrix that can be identified with a specified level of confidence (EURACHEM, 1998). It corresponds to the smallest amount of an analyte that can be detected, but not necessarily quantified as an exact value (LANÇAS, 2004).Experimentally, the limit of detection can be obtained by various procedures, including: visual method, signal-to-noise ratio method, and method based on parameters of the analytical curve (RIBANI et al., 2004). The visual method involves adding known concentrations of the substance of interest to the matrix, allowing the analytical signal to be distinguished from the noise, by visualizing the smallest detectable concentration. For methods that exhibit baseline noise, the limit of detection can be determined by a signal-to-noise ratio of 3:1 or 2:1, corresponding to the minimum concentration at which the substance can be easily detected (RIBANI et al., 2004). Additionally, the limit of detection can be found from the reading of 20 or more blank samples. In this case, the limit of detection is calculated as the average of the readings, plus three standard deviations of the mean (EURACHEM, 1998; BRASIL, 2022b).

Limit of Quantification

The limit of quantification (LQ) is defined as the lowest concentration of the analyte in the matrix that can be determined at levels where the requirements for accuracy and precision are satisfactory (EURACHEM, 1998). The same criteria used for the limit of detection can be applied to the limit of quantification, using a ratio of 10:1. The LQ can be determined as the concentration of the analyte corresponding to the average value of seven or more injections of the analyte-free sample, plus 5, 6, or, more commonly, 10 standard deviations of these same injections (INMETRO, 2007; BRASIL, 2022b; INMETRO, 2020).

BRASIL (2001) defines the limit of quantification as the lowest concentration level at which it has been demonstrated that the criteria of truthfulness and precision have been met, provided that the signal-to-noise ratio is greater than six ($S/R \geq 6$). The acceptability criterion must be defined by the analyst. For chromatographic analyses, it is common to use the method based on the parameters of the analytical curve, which must include the concentration corresponding to the LQ (RIBANI et al., 2004).

The EC (2002) adopts the parameters decision limit (CCα) and detection capability (CCβ) for assays in specific sectors of the food area, such as contaminants and organic residues in live animals and products of animal origin, within the European Union. For prohibited analytes, which do not have established maximum limits, these parameters are comparable to the limits of detection and quantification, as their concentrations correspond to measurements above the signal obtained for blank samples (matrices free of analyte or for which the analyte was not detected). However, for substances with established maximum limits, these parameters cannot be related to the limits of detection and quantification, as they are expressed in relation to the limits (SOUZA, 2007; BRASIL, 2022b; INMETRO, 2020).

IV. Conclusion

This study emphasizes the critical role of validation guidelines for analytical methodologies in ensuring food safety and public health. The establishment of robust validation protocols by international regulatory bodies is key to maintaining food quality and safety, particularly in global markets. The study underscores Brazil's efforts in advancing food safety standards through national programs like the National Plan for Residue and Contaminant Control (PNCRC), which enhance traceability and monitoring throughout the entire production chain. Additionally, the research highlights the ongoing need for further investigations to support continuous improvement in food safety practices. Suggested areas for future research include: evaluating the effectiveness of public policies in various countries and their impact on food safety and public health; exploring the influence of emerging technologies, such as artificial intelligence and blockchain, on food system traceability and transparency; and conducting comparative analyses of trading practices globally to identify factors affecting food safety. Moreover, the practical development and application of new analytical methods for detecting contaminants in food, in line with established validation parameters, are crucial for fostering innovation. The use of validated methodologies to compare products on the market can further strengthen food safety practices. By addressing these areas, Brazil can continue to demonstrate its commitment to food safety and sustainability, ensuring that the food production chain aligns with global standards and responds proactively to emerging challenges.

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