

Evaluation of the analytical methodology for the determination of arsenic, cadmium, and lead in coffee samples, according to *Codex Alimentarius* acceptance criteria

Avaliação da metodologia analítica para determinação de arsênio, cádmio e chumbo em amostras de café, segundo os critérios de aceitação do *Codex Alimentarius*

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ABSTRACT

Introduction: The *Codex Alimentarius* establishes international standards in the food segments to protect the health of consumers and ensure legal trade practices between countries. INMETRO, on the other hand, provides guidance documents that help laboratories carry out the validation of analytical methodology. These documents are used as a criteria to validate and verify conformity of the methods applied to determine contamination and residues in different products. **Objective:** To evaluate the validated analytical methodology used to determine arsenic, cadmium and lead in samples of roasted coffee powder according to the acceptance criteria recommended in the *Codex Alimentarius* 27th edition procedure manual. **Method:** This is an observational study of the descriptive exploratory qualitative type using secondary data base. **Results:** With the reading of the *Codex Alimentarius*, the parameters used were established, namely, the minimum application range, the detection limit, the quantification limit, precision and accuracy. Thus, the evaluation of the validated methodology was carried out and it was verified that it is applicable for the analysis of As, Cd and Pb in samples of roasted coffee powder. **Conclusions:** Current Brazilian legislation aimed at controlling the presence of contaminants in food establishes the maximum tolerated limits for As, Cd and Pb, but does not define which methodologies should be used to verify the presence and concentration of these elements. Therefore, the methodology presented in this study is adequate to control the quality of roasted coffee powder regarding the presence of these inorganic contaminants, thus contributing to the protection and safety of the population.

KEYWORDS: *Codex Alimentarius*; Inmetro; Validation Parameters; Roasted Coffee Powder

RESUMO

Introdução: O *Codex Alimentarius* estabelece normas internacionais na área de alimentos para proteger a saúde dos consumidores e garantir práticas legais de comércio entre os países. O Instituto Nacional de Metrologia, Qualidade e Tecnologia (INMETRO) disponibiliza documentos orientativos que auxiliam os laboratórios a realizarem a validação de metodologias analíticas. Estas são utilizadas para estabelecer critérios de validação e verificação de conformidade dos métodos aplicados na determinação dos níveis de contaminantes e resíduos em produtos de interesse sanitário. **Objetivo:** Avaliar a metodologia analítica validada utilizada para determinação de arsênio, cádmio e chumbo em amostras de café torrado em pó segundo os critérios de aceitação preconizados no Manual de Procedimentos do *Codex Alimentarius* 27ª edição. **Método:** Trata-se de um estudo observacional do tipo descritivo exploratório qualitativo utilizando base de dados secundários. **Resultados:** Com a leitura do *Codex Alimentarius*, foram estabelecidos os parâmetros utilizados, sendo eles, a faixa mínima de aplicação, o limite de detecção, o limite de quantificação, a precisão e a exatidão. Assim foi realizada a avaliação da



metodologia validada e verificou-se que esta é aplicável para a análise de As, Cd e Pb em amostras de café torrado em pó. **Conclusões:** A legislação brasileira atual voltada para o controle da presença de contaminantes em alimentos estabelece os limites máximo tolerados para As, Cd e Pb, mas não define quais metodologias devem ser utilizadas para verificar a presença e concentração destes elementos. Sendo assim, a metodologia apresentada nesse estudo é adequada para controlar a qualidade do café torrado em pó quanto a presença destes contaminantes inorgânicos, contribuindo assim para a proteção e a segurança da população.

PALAVRAS-CHAVE: *Codex Alimentarius*; Inmetro; Parâmetros de Validação; Café Torrado em Pó

INTRODUCTION

The main objective of the *Codex Alimentarius* is to establish international standards in the area of food, including standards, guidelines, and guides on good practices and the evaluation of safety and efficacy. In this way, *Codex* has the role of protecting consumer health and guaranteeing legal trade practices between countries¹. Brazil has been a member of this program since 1968 and is one of the Latin American countries with the greatest participation¹.

The *Codex Alimentarius* Commission has become the important international reference point for the development of food parameters² and, through the Manual of Procedures, 27th edition, 2019, has established criteria to ensure that the results provided by official laboratories are generated using appropriate analytical methodologies.

In Brazil, the National Institute of Metrology, Standardization and Industrial Quality (Inmetro) is the regulatory body responsible for accrediting testing and calibration laboratories and assessing compliance with the requirements of ABNT NBR ISO/IEC 17025³. A laboratory that operates with a quality system aims to promote confidence in the operation and guarantee valid results. To do this, laboratories must follow a series of actions including equipment calibration, environmental monitoring, technical validation of methodology, and trained staff³. Inmetro provides a guidance document, DOQ-CGCRE-008, which helps testing laboratories to demonstrate the analytical conditions under which a method is performed⁴.

Once validated, following Inmetro's guidance document, the methodologies for verifying the maximum tolerated limits (MTLs) of contaminants in food must comply with the provisions of the 27th edition of 2019 of the *Codex Alimentarius* Manual of Procedures, according to Article 10 of the Collegiate Board Resolution - RDC N°. 722, of July 1, 2022⁵, of the National Health Surveillance Agency (Anvisa). This manual of analytical procedures presents the acceptance criteria for analytical tests¹ and helps member governments to participate effectively in the work of the Food Standards Programme in conjunction with the Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO)⁶.

Anvisa is the federal authority responsible for establishing MTLs for contaminants in food in Brazil, among other powers⁷. A contaminant is any substance that is unintentionally added to food and is present as a result of production, industrialization, processing, preparation, treatment, packaging, transport or storage, or as a result of environmental contamination⁸.

Therefore, in 2022, RDC N°. 722/2022 was published⁹, which provides for the MTLs of contaminants in food and describes the general principles for establishing MTLs, addressing the methods of analysis for conformity assessment purposes. These limits are defined in Normative Instruction (NI) No. 160 of July 1, 2022⁵.

The aim of this study was to evaluate the validated analytical methodology used to determine arsenic (As), cadmium (Cd), and lead (Pb) in roasted coffee powder samples, according to the acceptance criteria recommended in the *Codex Alimentarius* Manual of Procedures 27th edition (2019).

METHOD

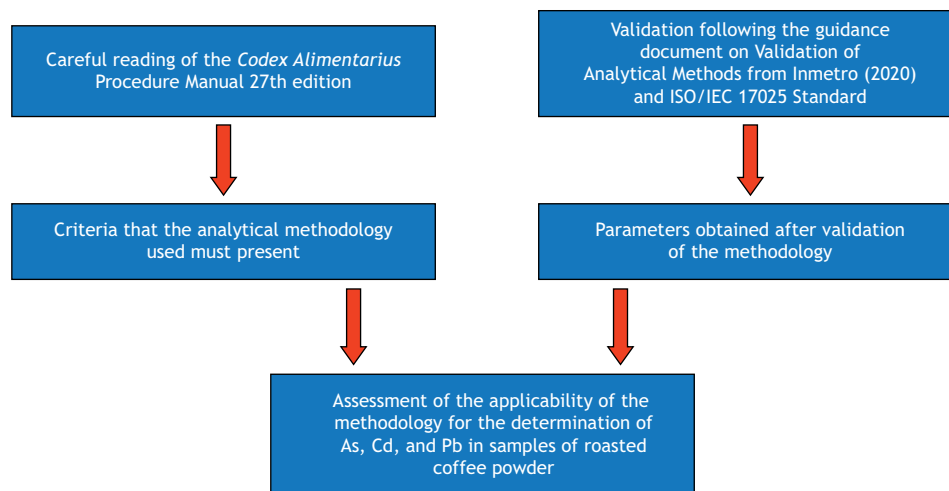
This is an observational descriptive exploratory qualitative study using secondary data. Initially, the Manual of Procedures of the *Codex Alimentarius* 27th edition was carefully read in order to verify which criteria the analytical methodology used should present.

The analytical methodology for the detection and quantification of As, Cd, and Pb, to be evaluated according to the *Codex* analytical criteria, was validated according to document DOQ-CGCRE-008, Revision 9 of INMETRO of 2020^{2,4}, using the following parameters: working range, limit of detection (LOD), limit of quantification (LOQ), precision, and recovery. The validation was carried out in the Inorganic Elements Sector of the Chemistry Department of the National Institute for Quality Control in Health (INCQS/Fiocruz) on an inductively coupled plasma mass spectrometer (ICP-MS), model NeXion 300D from Perkin Elmer, USA, equipped with a concentric nebulizer (Meinhard) and a cyclonic glass nebulizer chamber. The parameters studied in the validation were those described in Inmetro's 2020 guidance document on the Validation of Analytical Methods and the ABNT NBR ISO/IEC 17025 Standard³.

The methodological steps adopted in this study are described in Figure 1.

RESULTS AND DISCUSSION

The calculation of the minimum application range was estimated, considering a concentration greater than 10⁻⁷ (≥ 0.1 mg kg⁻¹) and a confidence level of 99%, since the MTLs for coffee powder for the elements As, Cd, and Pb range from 0.1 mg kg⁻¹ for Cd to 0.5 mg kg⁻¹ for Pb. To calculate the LOD and LOQ, the formulas and



Source: Prepared by the authors, 2023.

Figure 1. Flowchart of the methodological steps used in the present study.

Table 1. Acceptance criteria to evaluate the analytical methodology to determine contaminants in roasted coffee powder with LMT ≥ 0.1 mg kg⁻¹, according to *Codex Alimentarius*².

| | MTL 1 (mg kg ⁻¹) | Working range ² (mg kg ⁻¹) | LOD ³ (mg kg ⁻¹) | LOQ ⁴ (mg kg ⁻¹) | Precision ⁵ (%RSD) | Recovery ⁶ (%) |
|----|---------------------------------|--|--|--|----------------------------------|------------------------------|
| As | 0.2 | 0.07-0.33 | ≤ 0.02 | ≤ 0.04 | ≤ 44 | 80-110 |
| Cd | 0.1 | 0.03-0.17 | ≤ 0.01 | ≤ 0.02 | ≤ 44 | 80-110 |
| Pb | 0.5 | 0.17-0.83 | ≤ 0.05 | ≤ 0.10 | ≤ 44 | 80-110 |

Source: Prepared by the authors, 2023.

¹ Established by RDC No. 722/2022 and defined in NI No. 160, of July 1, 2022.

² For MTL ≥ 0.1 mg kg⁻¹, [MTL - 3 sD, MTL + 3 sD], sD the standard deviation of reproducibility.

³ For MTL ≥ 0.1 mg kg⁻¹, LOD \leq LMT. 1/10.

⁴ For MTL ≥ 0.1 mg kg⁻¹, LOD \leq LMT. 1/5.

⁵ For MTL ≥ 0.1 mg kg⁻¹, HorRat value ≤ 2 , that is, RSD^R/ PRSD^R ≤ 2 . PRSD^R, sendo PRSD^R = 22%.

⁶ For MTL ≥ 0.1 mg kg⁻¹, Recovery 80-110%.

MTL: Maximum tolerated limit; LOD: Limit of detection; LOQ: Limit of quantification.

tables contained in the document were used and the MTL for As, Cd, and Pb for coffee powder⁹ was used as an example.

As for precision, *Codex* determines the relative standard deviation (RSD) for reproducibility and the criterion is calculated on the basis of the maximum tolerated level (MTL), accuracy is assessed when the methodology has a digestion step. If this step is not required, no acceptance range is determined. However, precision was validated for repeatability, which was expressed using the RSD, and accuracy was determined using the recovery study, in which a known concentration of the analyte was added to the samples, with the number of repetitions being equal to seven.

The *Codex Alimentarius* provides a theoretical basis for establishing acceptance criteria to verify that the validated methodology is applicable to the sample to be analyzed. To this end, the MTL for each contaminant in a given sample defines which calculation equations should be used to obtain these values.

Therefore, as the three contaminants of interest have MTL ≥ 0.1 mg kg⁻¹, the values obtained for the acceptance criteria are described in Table 1. In this document, precision is assessed in terms of reproducibility, which is obtained by collaborative

studies and RSD $\leq 44\%$. In this work, reproducibility was not assessed, but rather repeatability.

Table 2 shows the results obtained for validating the methodology for determining As, Cd, and Pb in roasted coffee powder samples by ICP-MS, according to the parameters established by the Inmetro guidance document. The methodology was validated for the simultaneous analysis of the three elements (As, Cd, and Pb) in roasted coffee samples. As a result, the first point of the working range was established as the LOQ for Pb and the values established for the working range include the values recommended by current legislation, IN No. 160/2022⁵.

The precision and accuracy are within the values acceptable to Inmetro, which states that for the method to be considered valid, it must have an RSD $\leq 15\%$ and a recovery between 80%-110%, in the range studied.

After validating the methodology using INMETRO guidance document, we used the *Codex Alimentarius* acceptance criteria to check whether the validated methodology is applicable to the analysis of As, Cd, and Pb in roasted coffee powder samples. To do this, we followed the criteria as shown in Figure 2.

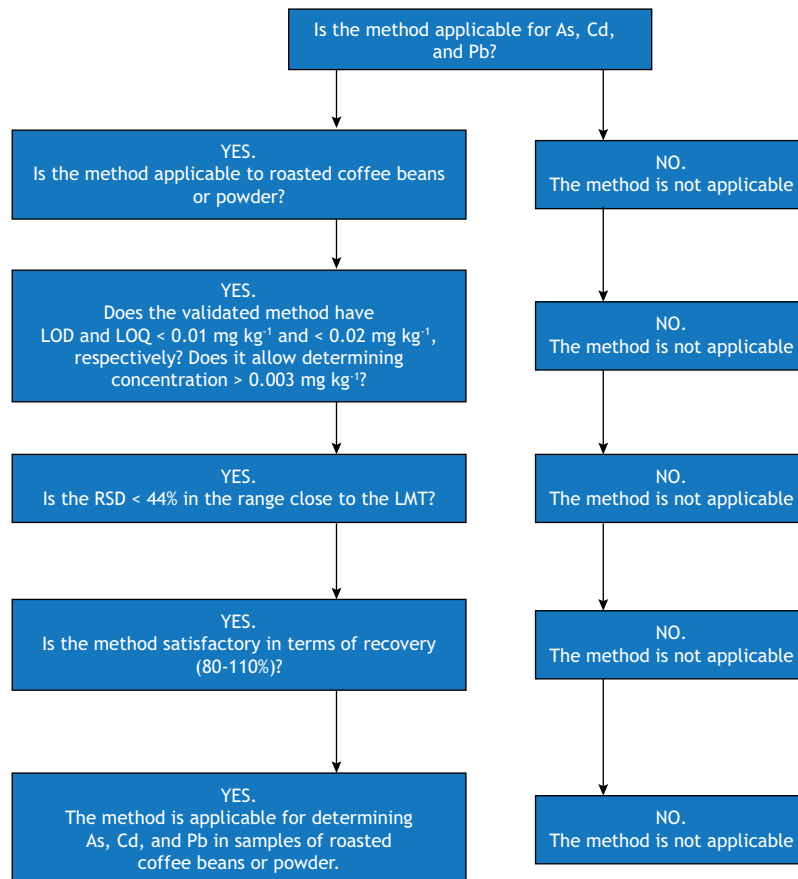


Table 2. Results obtained to validate the methodology for determining arsenic (As), cadmium (Cd), and lead (Pb) in a sample of roasted coffee powder by ICP-MS.

| | Working range (mg kg ⁻¹) | LOD (mg kg ⁻¹) | LOQ (mg kg ⁻¹) | Precision (%RSD) | Recovery (%) |
|----|--------------------------------------|----------------------------|----------------------------|------------------|--------------|
| As | 0.003-0.3 | 0.00003 | 0.0002 | 7 | 98 |
| Cd | 0.003-0.3 | 0.00006 | 0.0003 | 3 | 109 |
| Pb | 0.003-0.3 | 0.001 | 0.003 | 6 | 104 |

Source: Prepared by the authors, 2023.

LOD: Limit of detection; LOQ: Limit of quantification.



Source: Prepared by the authors, 2023.

As: Arsenic; Cd: Cadmium; Pb: Lead; MTL: Maximum tolerated limit; LOD: Limit of detection; LOQ: Limit of quantification.

Figure 2. Flowchart of analysis of the applicability of the validated method for the analysis of roasted coffee powder samples for the presence of arsenic (As), cadmium (Cd) and lead (Pb), according to the Codex Alimentarius.

Given all the positive answers to the questions presented in the previous flowchart, we can see that the methodology validated according to the Inmetro guidance document is applicable for determining As, Cd, and Pb in roasted coffee powder samples and meets the acceptance criteria established by the Codex Alimentarius².

CONCLUSIONS

Current Brazilian legislation aimed at controlling the presence of contaminants in food establishes the MTL for As, Cd, and Pb but does not define which methodologies should be used, but rather the performance criteria aimed at verifying the applicability of

the methods in determining the concentration of contaminants and residues in products intended for health. In this quest to develop appropriate and reliable methodologies, the document produced by the Codex Alimentarius is an excellent tool for guaranteeing reliable results to the national and international bodies responsible for food quality control.

Therefore, the methodology presented in this study has been validated, according to the Inmetro guidance document, has proven to be applicable for the analysis of As, Cd, and Pb in roasted coffee powder and meets the acceptance criteria established by the Codex Alimentarius, thus contributing to the protection and safety of the population.



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Authors' Contribution

Barata-Silva C, Jacob SC, Santos LMG - Conception, planning (study design), analysis, data interpretation, and writing of the work. Magalhães CD, Vicentin-Neto SA - Planning (study design), analysis, data interpretation, and writing of the work. Pereira RA - Analysis and writing of the work. All the authors approved the final version of the work.

Conflict of Interest

The authors inform that there is no potential conflict of interest with peers and institutions, political or financial, in this study.



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