Development and comparison of two validated methods to determine cadmium and arsenic in Colombian rice by ICP-MS and ICP-OES*

Desarrollo y comparación de dos métodos validados para determinar cadmio y arsénico en arroz colombiano mediante ICP-MS e ICP-OES

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Cómo citar este artículo

ABSTRACT

Some metals are essential for the diet, such as iron (Fe), copper (Cu), manganese (Mn), and zinc (Zn). However, there are heavy metals that are not biodegradable, so they are not essential for metabolism since they do not fulfill a physiological role in humans or plants. Therefore, they become an adverse factor in terms of health due to their cumulative capacities, such as cadmium (Cd), chromium (Cr), lead (Pb), arsenic (As), and mercury (Hg). This study aimed to develop and compare two methods to determine the content of Cd and As in rice grains (Oryza sativa L.). The samples were subjected to microwave-assisted acid digestion with HNO_3, and H_2O_2 [2:1] as this method is useful to prepare samples with the presence of organic matter since it minimizes the loss of analytes and sample contamination. Subsequently, these were analyzed by quadrupole inductively coupled plasma-mass spectrometry (ICP-MS) equipped with a collision/reaction cell and by inductively coupled plasma-optical emission spectrometry (ICP-OES). The performance of the proposed method was evaluated under selectivity, sensitivity, linear range, limits of detection and quantification, precision, intermediate precision, and reproducibility parameters. The results showed that the digestion method was satisfactory for sample preparation and the ICP-MS technique obtained lower quantification limits compared to ICP-OES. However, due to the quantified concentrations, the results indicated that both methods are suitable to determine Cd and As in rice, considering the maximum limits allowed in different types of rice regulated in the Codex Alimentarius and the European Union.

KEYWORDS:
Heavy metals; Validation; Microwave-assisted acid digestion; Quantification limits; Food.

RESUMEN

Algunos metales son esenciales para la dieta, como el hierro (Fe), el cobre (Cu), el manganeso (Mn) y el zinc (Zn). Sin embargo, hay metales pesados que no son biodegradables, por lo que no son esenciales para el metabolismo ya que no cumplen un papel fisiológico en los seres humanos ni en las plantas. Por ello, se convierten en un factor adverso para la salud por sus capacidades acumulativas, como el cadmio (Cd), el cromo (Cr), el plomo (Pb), el arsénico (As) y el mercurio (Hg). Este estudio tuvo como objetivo desarrollar y comparar dos métodos para determinar el contenido de Cd y As en granos de arroz (Oryza sativa L.). Las muestras se sometieron a digestión ácida asistida por microondas con HNO_3, y H_2O_2 [2:1] ya que este método es útil para preparar muestras con presencia de materia orgánica puesto que minimiza la pérdida de analitos y la contaminación de la muestra. Posteriormente, se analizaron mediante espectrometría de masas con plasma de acoplamiento inductivo cuadrupolar (ICP-MS) equipada con una célula de colisión/reacción y mediante espectrometría de emisión óptica con plasma de acoplamiento inductivo (ICP-OES). El rendimiento del método propuesto se evaluó según los parámetros de selectividad, sensibilidad, rango lineal, límites de detección y cuantificación, precisión, precisión intermedia y reproducibilidad. Los resultados mostraron que el método de digestión fue satisfactorio para la preparación de la muestra y que la técnica ICP-MS obtuvo límites de cuantificación más bajos en comparación con ICP-OES. Sin embargo, debido a las concentraciones cuantificadas, los resultados indicaron que ambos métodos son adecuados para determinar Cd y As en arroz, considerando los límites máximos permitidos en diferentes tipos de arroz regulados en el Codex Alimentarius y la Unión Europea

PALABRAS CLAVE:
Metales pesados; Validación; Digestión ácida asistida por microondas; Límites de cuantificación; Alimentos.

INTRODUCTION

Heavy metals and metalloids are toxic chemical elements that pollute the environment (Tejada Tobar *et al*. 2020). The term "heavy metals" apply to metals and metalloids (Briffa *et al*., 2020) with an atomic density greater than 4 g/cm^3 or those with a density five times higher than the density of water (Tchounwou *et al*., 2012; Fakhar *et al*., 2022). Some metals are essential for the diet, such as iron (Fe), copper (Cu), manganese (Mn), and zinc (Zn). However, there are heavy metals that are not biodegradable, so they are not essential for metabolism (Rai *et al*. 2019; Sandeep *et al*., 2019; Herrero Fernández *et al*., 2021) since they do not fulfill a physiological role in humans or plants. Therefore, they become an adverse factor in terms of health due to their cumulative capacities, such as cadmium (Cd), chromium (Cr), lead (Pb), arsenic (As), and mercury (Hg).

Heavy metals exist naturally in the environment (Briffa *et al*., 2020): however, one way to demonstrate their toxicity at an agronomic level is through the low response in crop productivity (Muhammad *et al*., 2020). The high concentrations of heavy metals can be attributed to natural factors, anthropogenic actions, industrial processes, domestic effluents, application of chemical fertilizers or organic sludge (Sandeep *et al*., 2019; Thomas and Archana, 2023). The dynamics of chemical elements are related to their presence in the soil, water, plants (crops), air, and living beings, and, according to the characteristics of the element and the conditions where it is found, one of the most critical places where these can accumulate is food (Zarei *et al*., 2023)

Rice (*Oryza sativa* L.) is the staple food for more than half of the world's population and one of the most important sources of energy and protein (Roa-Acosta, 2019; Sen *et al*., 2020). Food security is a priority; therefore, there is great interest in knowing the quality of this food in terms of safety because it belongs to the basic family basket. In the case of Colombia, per capita consumption is 42 kg/year, which implies special attention in the evaluation of toxic metals, which is why it is necessary to have validated methodological alternatives that are effective, that allow confidence in the results. However, these must be fast, efficient, with reliable results, have good analytical sensitivity, and can detect low concentrations in food to compare the results with the current regulations that state the maximum permitted limits of contaminants, such as the Codex Alimentarius and the European Union. These are necessary inputs for research or national regulation decision-making.

Among the current analytical methods that allow good analytical sensitivity and detect low concentrations of metalloids such as Cd and As in food, inductively coupled plasma-optical emission spectroscopy (ICP-OES) and inductively coupled plasma-mass spectrometry (ICP-MS) are highlighted. These involve an inductively coupled plasma as an ionization source that, together with a spectrometer, allows analytical quantifications. Unlike the ICP-OES, the ICP-MS has a reaction/collision cell that eliminates polyatomic interference, and later, a quadrupole separates the ions by the mass/charge ratio (Wilschefski and Baxter, 2019; Narukawa *et al*., 2020). Both methods have a wide linear range, low detection limits, and simultaneous analysis (Ondřej *et al*., 2023)accurate methods for their determination in foodstuffs are required. Here, we present a routine-ready method for their co-determination by means of high-resolution continuum source graphite-furnace atomic absorption spectrometry. As the technique allows for monitoring a narrow spectral interval, adjacent secondary lines of Zn (307.588 nm. However, the method selected will be directed by the sensitivity and, therefore, by the concentration found in the food. Accordingly, this research aimed to develop two analytical methods to determine Cd and As in different rice samples with the lowest quantification limits in Colombia, since there is currently no laboratory that offers the primary sector a validated method that allows it to be used for control of marketed product.

The development of the methods will allow providing a reliable analysis tool to the research projects developed by AGROSAVIA and additionally will allow the accompaniment of the Colombian Agricultural Institute - ICA in charge of the annual monitoring of the quality and safety of the primary production of the agricultural sector in Colombia. For this reason, the analysis of different types of rice and different sources was considered in the validation, including products that are marketed by companies duly packaged with sanitary registration and unpackaged products called in bulk that are marketed by weight at the consumer's need.

METHOD

Instrumentation

All rice samples were pre-treated using a drying oven (UL80, Memmert, Schwabach, Germany), a grain mill (Tecator Cyclotec, FOSS, Sweden), and a microwave with a closed digestion system (Speedwave Xpert, Berghof, Eningen, Germany), with pressure and temperature sensors per vessel in a 12-position rack (vessel capacity 100 mL). The quantification of the samples was carried out using two pieces of instrumental equipment. The first is an ICP-MS mass spectrometer (7900, Agilent Technologies, Tokyo, Japan) coupled to an autosampler (SPS4, Agilent Technologies, Tokyo, Japan) and an Integrated Sample Introduction System (ISIS). The second is an ICP-OES atomic emission spectrometer (iCAP 6500 duo, Thermo Scientific, Waltham, USA) coupled to an autosampler (ASX 520, Teledyne Cetac technologies, Omaha, USA). This equipment quantified cadmium in a concentric nebulizer and cyclonic nebulization chamber, and arsenic was registered through a gas exchanger for hydride generation. Instrumental conditions and experimental parameters are described in Table 1.

Table 1. Instrumental conditions for Cd and As analysis

ICP-MS Simple quad Agilent Technologies 7900	
RF power	1,550 W
Plasma gas flow	12 L/min
Auxiliary gas flow	0,10 L/min
Nebulizer gas flow	1,05 L/min
Sample pump tubing	Tygon, 1,02 mm of ID
Internal standard flow rate	0,50 rps
Internal standard pump tubing	Tygon, 0,25 mm of ID
Internal standard solution	100 mg/L of Bi, Ge, In, Li6, Lu, Rh, Sc and Tb
Nebulizer	Concentric MicroMist (Glass)
Spray chamber	Quartz Scott-type double pass spray chamber
Octupole	ORS (Octupole Reaction System)
Helium gas flow	5,0 mL/min
Detector mode	Pulse
Sampler/skimmer cones	Nickel
Scanning mode	Spectrum
Number of sweeps per reading	100
Number of reads per replicate	3
Isotopes monitored	1
ICP-OES Thermo scientific iCAP 6500 duo	
RF power	1150 W
Plasma gas flow	12 L/min
Auxiliary gas flow	0,5 L/min
Nebulizer gas flow	0,5 L/min
Sample pump tubing	Tygon Orange/White
Nebulizer	Concentric type K
Spray chamber	Cyclonic
Scanning mode	Axial
Dwell time	30 s
Number of reads per replicate	3

ICP-MS Simple quad Agilent Technologies 7900	
Berghof Xpert microwave system	
Vessels	DAP-100
Magnetron power	2 x 1000 W
Percent power operation	70–90 %
Ramp time	60 min aprox.
Maximum temperature	180 °C

Reagents a3nd standards

Acid digestion of the rice samples was carried out using 65 % nitric acid (Merck, Germany) distilled through an acid distiller with an infrared lamp (distillacid, Berghof, Achalm, Germany) and 30 % hydrogen peroxide (Merck, Germany). Type I water was obtained from a water deionizer (HX 7040, Milli-Q, Molsheim, France) with ion exchange resin and reverse osmosis.

Quantifications utilized reference solutions of 1,000 mg/L of arsenic (Merck, Germany) and 977 mg/L of cadmium (Instituto Nacional de Metrología de Colombia INM, Bogotá, Colombia). ICP-OES flame alignment was carried out with a solution with 2 mg/L of Zn (Thermo Scientific, China), and ICP-MS tuning utilized a solution with 10 µg/L of Li, Y, Ce, Tl, and Co in 2 % HNO_3 (Agilent technologies, USA). Additionally, in the ICP-MS, the samples were quantified with an internal standard solution of 100 mg/L of Bi, Ge, In, Li6, Lu, Rh, Sc, and Tb (Agilent technologies, USA).

Preparation of solutions and standards

The quantification in ICP-MS was carried out through a calibration curve of 0,25, 0,50, 1,0, 2,0, 3,0, 4,0, 5,0, 6,0, 7,0, 8,0, 9,0, and 10 µg/L of Cd and As, made from a stock solution of 50 µg/L.

The quantification in ICP-OES was carried out through two cadmium calibration curves, low level with concentrations of 1,0, 1,5, 2,0, 2,5, 3,0, 3,5, 4,0, 4,5, and 5,0 µg/L prepared from a stock solution of 50 µg/L and medium level with concentrations of 10, 15, 20, 25, 30, 35, 40, 45 and 50 µg/L elaborated from a stock solution of 500 µg/L. For arsenic, a curve with concentrations of 1,0, 1,5, 2,0, 2,5, 3,0, 3,5, 4,0, 4,5, and 5.0 µg/kg was prepared from a stock solution of 50 µg/kg. All solutions for ICP-MS and ICP-OES were brought to their final volume with 3.25 % w/w nitric acid, a similar percentage of acid present in the samples, to increase the stability of the curves and avoid the precipitation and adsorption of the analytes in the containers (Wiltsche *et al.*, 2015; Raquel-Serrano *et al.*, 2021).

Samples

As internal reference materials (IRM), two rice samples taken in the field with low and medium Cd and As levels were prepared. The certified reference material used was IRMM 804 obtained from the Institute for Reference Materials and Measurements of the Joint Research Centre. Commercial rice samples of different brands and types were purchased in the local market, including 16 white rice samples, three brown rice samples, and one parboiled rice sample. These were analyzed in triplicate.

Method development

Rice sample preparation. Five hundred grams of rice grains without husks were used for the analysis. Pretreatment was carried out through a drying process at a temperature of 70 ± 5 °C for approximately 72 hours; subsequently, grinding and sifting at 0,5 mm were carried out.

Microwave digestion of rice grains. Acid digestion for the quantification of cadmium and arsenic in ICP-MS and cadmium in ICP-OES was developed to obtain adequate recovery percentages for the study matrix. It was carried out by weighing 0,3 g of the sample in dry Teflon beakers and adding 6 mL of 65 % ultrapure nitric acid. These were left in pre-digestion for at least 12 hours, and finally, 3 mL of 30 % hydrogen peroxide was added.

For the quantification of arsenic in ICP-OES by hydride generation, 0,6 g of sample was weighed, 10 mL of 65 % ultrapure nitric acid was added, and left in pre-digestion for at least 12 hours. Finally, 2 mL of hydrogen peroxide 30 % was added.

A pre-digestion process is required to ensure the complete decomposition of the sample. This step is common in food matrices (Lee *et al.*, 2022) since the presence of undecomposed organic matter can obstruct the introduction system of the sample and plasma instability (Wiltsche *et al.*, 2015; Astolfi *et al.*, 2018;"ISSN":"18734324","P-MID":"30327113","abstract":"Breast milk guarantees all the nutrients required by infants during their first few months of life and remains the most important food source for their health and growth. However, the mother may transfer potentially toxic chemicals to the suckling infant through breastfeeding. The aim of this study was to optimize and validate a fast method for the determination of a total content of 34 elements (Al, As, B, Ba, Be, Bi, Ca, Cd, Co, Cr, Cs, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, Sb, Se, Si, Sn, Sr, Te, Ti, Tl, U, V, and Zn Raquel-Serrano *et al.*, 2021).

The digestion program was carried out in three stages: 1) room temperature up to 160 °C in 10 min with 30 bar pressure, 70 % power (potency) for 15 min, 2) temperature increase from 160 °C to 180 °C in 5 min with 35 bar pressure, 90 % power (potency) for 15 min, and 3) in 1 min, a temperature drop to 50 °C with 25 bar pressure, 0 % power (potency) for 10 min. Once the digestion was finished, the rice samples for Cd and As in ICP-MS and Cd in ICP-OES were taken to a final volume of 15 mL; and for As in ICP-OES through hydrides generation to a final volume of 25 mL with MilliQ deionized water.

Development of an analytical methodology

Selectivity. To confirm the selectivity of the method in the ICP-OES due to the presence of spectral interferents and in the ICP-MS due to isobaric and polyatomic interferences, a solution of Mo, Fe, Bi, S, Ni, Cr, Al, Co, K, Hg, Pb, As and Cd at a concentration of 5 µg/L and the analytes of interest were quantified to verify the percentage of cadmium and arsenic recovery in the presence of the elements identified as interferers. Possible interferences in ICP-OES were identified using the iTEVA ICAP Version 2.8.0.97 software that controls the equipment, and in ICP-MS, the isobaric and polyatomic interferences identified in the mass hunter 4.6 software version C.01.06 were studied.

Sensitivity, linear range, and linearity. In the ICP-OES, six calibration curves were elaborated with concentrations of 1 to 500 µg/L Cd and 1 to 5 µg/L As. The value of the slopes and their relative standard deviation were used to evaluate the sensitivity of the method. The average of the CPS (counts per second) obtained at each wavelength was calculated, and the linear range was established by the visual method and the coefficient of determination. The linear range in the ICP-MS was determined considering the maximum levels (ML) established in the European regulation for rice, selecting concentrations of 0,25 to 10 µg/L Cd and As, and quantifying six calibration curves in three days to assess the sensitivity, linear range, and linearity of the method.

The calibration curves were evaluated using the least squares method with the acceptance criterion of a co-rrelation coefficient higher than 0,995. The null hypotheses were evaluated at 95 % confidence using t-student tests: 1. The slope is statistically equal to zero; 2. The intercept is statistically equal to zero; 3. There is no corre-lation between area and concentration. In addition, the residual graphs were reviewed, and the lack-of-fit test was performed.

Limit of Detection (LOD) and Limit of Quantification (LOQ). The instrumental limits of detection (LODi) and quantification (LOQi) were obtained from the analysis of 10 solutions with low concentrations of Cd and As. In ICP-OES, the low concentration used was 0,03 µg/L, while in ICP-MS, a solution of 0,01 µg/L was employed. The limits of detection (LODm) and quantification (LOQm) of the method were obtained from the analysis of 10 method blanks. Equations 1 and 2 were used to calculate the LOD and LOQ, respectively.

$$LOD = X + 3 * SD \qquad \text{(Eq. 1)}$$

$$LOQ = X + 10 * SD \qquad \text{(Eq. 2)}$$

Where X is the average concentration of the method blank or low-concentration solution and SD is the stan-daΩrd deviation.

Repeatability and intermediate precision. The repeatability of the method was determined from the measu-rement of at least nine replicates of the internal reference sample under the same conditions, on the same day, with one analyst and one piece of equipment. Intermediate precision was determined from the measurement of nine replicates over three days. The percentage of relative standard deviation (% RSD) was calculated for each level, and the result obtained was compared with the Horwitz equation according to EURACHEM guidelines (Magnusson and U. Örnemark , 2014).

Reproducibility and veracity. Reproducibility was evaluated by performing an aptitude test acquired from FAPAS® (Accredited under ISO 17043) for the rice matrix, and the veracity of the methods was determined from the recovery percentage obtained from the quantification of six replicates of the Certified Reference Ma-terial IRMM-804 for Cd and As.

Uncertainty. Uncertainty estimation was carried out considering all the sources that contributed dispersion to the measurand. It was estimated based on equation 3.

$$U\,(x) = \sqrt{\begin{array}{l} u^2(CRM) + u^2(stock\ solution) + u^2(calibration\ curve) \\ +u^2(digestion\ of\ the\ sample) + u^2(moisture) \\ u^2(repeatability) + u^2(intermediate\ precision) \end{array}} \qquad \text{(Eq. 3)}$$

RESULTS

Selectivity

The recovery percentages obtained in the evaluation of Cd and As interferences quantified by ICP-OES and ICP-MS (Table 2) were not statistically significant according to the t-test comparison against a reference value for any of the wavelengths and mass-to-charge (m/z) ratios studied, i.e., any wavelength and m/z ratio can be selected for the remainder of the study, if sensitivity is not affected (Drava and Minganti, 2020)in many real cases, the application to routine analysis, e.g. of environmental samples, is still difficult and cumbersome. In this work the use of an internal standard (IS. On the other hand, in the ICP-MS method, m/z 111 is preferred because it is the only isotope that does not show any interference with another metal; the selectivity in the quantification will depend on the selection of the mass (Spanu *et al*., 2020) and, additionally, it was found that m/z 112 and 114 available for Cd analysis reflected higher instrumental responses in the blank analysis than m/z 111. The blank response for the current study is relevant to determine trace elements since it impacts the quantification limit of the method. Thus, the m/z 111 was selected for the rest of the study. In this method, the selectivity is better due to the collision and reaction cell, as it eliminates isobaric interference (Meermann and Nischwitz, 2018; Narukawa *et al*., 2020) through the collision of Helium molecules with interfering molecules in the octupole of the quantification equipment (Astolfi *et al*., 2018;"ISSN":"18734324","PMID":"30327113","abstract":"Breast milk guarantees all the nutrients required by infants during their first few months of life and remains the most important food source for their health and growth. However, the mother may transfer potentially toxic chemicals to the suckling infant through breastfeeding. The aim of this study was to optimize and validate a fast method for the determination of a total content of 34 elements (Al, As, B, Ba, Be, Bi, Ca, Cd, Co, Cr, Cs, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, Sb, Se, Si, Sn, Sr, Te, Ti, Tl, U, V, and Zn Sugiyama, 2021)improving the data quality of trace elements in a variety of matrices. However, spectral interferences caused by doubly charged ions M++.

The selectivity evaluation of each method becomes important since trace elements can be linked to functional groups within the plant material. For example, how Cd is distributed among tissues and how it is co-localized with nutrients in the grain is key because some metals such as Fe, Mn, and Zn share transporters with Cd and S, considering that Cd has a high affinity for thiol groups and usually binds to ligands containing S in plant tissues (Clemens *et al*., 2013; Bari *et al*., 2019)chlorophyll synthesis, soluble protein, cell and membrane stability in Sonarbangla (Cd-tolerant.

Sensitivity, linear range, and linearity

The sensitivity of a method can be defined as the ratio between the instrumental response and the measured concentration (Instituto Colombiano De Normas Técnicas y Certificación, ICONTEC, 2019) This study determined it according to the changes in the slope in each calibration curve. In the ICP-OES, the results indicated relative standard deviations (RSD) lower than 6 % for Cd and RSD<2 % for As, while in the ICP-MS, slopes were obtained with RSD<0,85 % for Cd and As (Table 2), indicating that the methods are sensitive through the repeatability of the slope of the quantified calibration curve on different days since RSDs lower than 8 % are acceptable for specialized techniques (Tanasea *et al*., 2014; Raposo, 2016).

Table 2. Summary of the validation results for selectivity, linear range, and sensitivity for the determination of Cd and As in rice through inductively coupled plasma-optical emission spectroscopy (ICP-OES) (λCd=214,438 nm and λAs=189,042 nm) and inductively coupled plasma-mass spectrometry (ICP-MS) (m/z Cd= 111 m/z As = 75).

Performance parameter	Criterion	Element	ICP-OES	ICP-MS
Selectivity	Recovery in the presence of potential interferents (%)	Cd	99,1 ± 2,1	99,5 ± 1,5
		As	99,5 ± 1,7	100,8 ± 0,4
Lineal range	Linearity intervals (µg/L)	Cd	1-5 10 -50 100-500	0,25-10
		As	1-5	0,25-10
Sensitivity	Slope (CPS L/µg)	Cd	22,068 ± 1,018	5,826,492 ± 49,567
		As	10,019 ± 0,099	1,979,715 ± 15,305

The slope obtained in the calibration curves performed by the ICP-MS was two orders of magnitude higher than those of the ICP-OES for both Cd and As, indicating greater sensitivity in the ICP-MS. Even so, both instrumental techniques showed adequate sensitivity for the determination of Cd and As. On the other hand, the slopes obtained in the curves made by the ICP-OES were higher at the wavelengths (λ) 214,438 nm for Cd and 189,042 in As. For this reason, they were selected for the rest of the study. Further, these same lines were studied by Goncalves *et al.* (2019) in determining trace metals in medicinal plants.

The linear range for Cd and As in the ICP-OES method was determined according to the response of the element to different concentrations and based on the visual method and coefficient of determination. For Cd, three linear ranges were found (from 1 to 5 **µg/L**, from 10 to 50 **µg/L**, and from 100 to 500 **µg/L**) with coefficients of determination higher than 0,995, and the As range was identified from 1 to 5 **µg/L** also with satisfactory results in its coefficient of determination. Da Silva *et al.* (2020) quantified in an ICP-OES Model 5100 equipment (Agilent Technologies) the minerals in rice samples using a linear range for Cd between 1-100 **µg/L**, being this range within the values evaluated in the current study. In the development of the ICP-MS method, a linear range of 0,25 to 10 **µg/L** was identified for Cd and As with satisfactory coefficients of determination ($R^2 > 0,995$) (Table 2).

In the two methods developed, no trends were found in the residual graphs, and it was evidenced that the slope and the intercept were statistically different from zero, with a statistically significant correlation between the concentration and the CPS obtained as an instrumental response. Besides, a lack-of-fit test was carried out on the curves, finding that the regression model of each element and for each method are linear and did not show a lack of fit.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The detection and quantification limits involve the evaluation of the magnitude of the noise level in measuring instruments (Tanasea, 2014; Raposo, 2016) and determine the lowest non-zero concentration that can be quantified with a specified level of confidence (Magnusson and U. Örnemark , 2014). The results of LOD and LOQ for Cd and As of the current study were less than 0,05 mg kg^{-1} in ICP-OES (Table 3) and between 10 and 30 times lower in ICP-MS. These results indicate that the two methods were adequate for determining trace metals/metalloids in rice, considering that regulations such as the Codex Alimentarius and the European Union establish maximum levels (ML) for rice of different types (grain, husked, polished, parboiled, and intended for food production for infants) for Cd between 0,2 mg/kg and 0,4 mg/kg and for As between 0,1 mg/kg and 0,35 mg/kg (Food and Agriculture Organization of the United Nations and World Health Organization, FAO and WHO, 2019).

Similar results were reported by Al-Naimi and Al-Ghouti (2020) on the effect of soaking, acidity, and temperature on the elimination of Cd in rice, where they related LODm values of 0,012 mg/kg and LOQm of 0,040 mg/kg in ICP-OES.

Table 3. Limits of detection of the instrument (LODi) and the method (LODm), and limit of quantification of the instrument (LOQi) and the method (LOQm) identified by the inductively coupled plasma-optical emission spectroscopy (ICP-OES) and inductively coupled plasma-mass spectrometry (ICP-MS) for Cd and As.

Limit	Cd		As	
	ICP-OES	**ICP-MS**	**ICP-OES**	**ICP-MS**
LDi (µg/L)	0,385	0,010	0,298	0,012
LCi (µg/L)	0,483	0,015	0,444	0,019
LDm (mg/kg)	0,034	0,002	0,029	0,001
LCm (mg/kg)	0,043	0,004	0,042	0,002

Repeatability and intermediate precision

The precision measured under repeatability conditions, defined as a set of conditions that include repeated measurements of the same material in a short period (ICONTEC, 2019), was evaluated through the RSD in the analysis of two IRMs of white rice taken in the field and husked, which were analyzed during the same day (n>9) by the same personnel and under the same conditions. The RSD found was less than 15 % (Table 4), indicating that the two methods developed, ICP-OES and ICP-MS, presented adequate repeatability, as established by the Horwitz bell for these concentration levels.

The precision measured under intermediate precision conditions, defined as a set of conditions that include repeated measurements of the same material over a long period (ICONTEC, 2019), was evaluated utilizing the RSD of the IRMs analyzed on three different days (n>27). The RSD obtained was less than 22 % (Table 4), indicating that the two developed methods, ICP-OES and ICP-MS, are accurate at different time intervals, as established by the Horwitz bell for these concentration levels.

Precision at repeatability and intermediate precision levels was higher in ICP-MS than in ICP-OES, but both meet the expected precision as a function of concentration described by the Horwitz equation (Association of Official Agricultural Chemists, AOAC, 2016).

Table 4. Precision obtained at the repeatability and intermediate precision levels for the two reference materials: IRM 1 and IRM 2 in the ICP-OES and ICP-MS, expressed as Relative Standard Deviation (RSD) of the quantified concentration of each material.

Performance parameter	Material	Element	ICP-OES		ICP-MS	
			Concentration (mg/kg)	**RSD (%)**	**Concentration (mg/kg)**	**RSD (%)**
Repeatability	IRM 1	Cd	0,132 ± 0,004	2,9	0,136 ± 0,003	2,3
		As	0,078 ± 0,011	13,6	0,083 ± 0,004	4,6
	IRM 2	Cd	< LOQm	-	0,018 ± 0,001	3,5
		As	< LOQm	-	0,004 ± 0,0004	11,4
Intermediate precision	IRM 1	Cd	0,126 ± 0,010	7,7	0,134 ± 0,004	2,8
		As	0,081 ± 0,017	20,7	0,087 ± 0,004	5,1
	IRM 2	Cd	< LOQm	-	0,018 ± 0,001	3,9
		As	< LOQm	-	0,004 ± 0,0001	11,5

Reproducibility and veracity

Reproducibility is a measurement condition of a material that can include different places (ICONTEC, 2019). For its estimation, an aptitude test was acquired with the provider FAPAS® (accredited under ISO 17043), finding that the validated method has an appropriate reproducibility by obtaining a |z-score| lower than 0,6 in the quantified sample and evaluated under a criterion of |z-score|<2,0 for compliant results (Table 5).

The recovery percentage in the analysis of Cd and As of the Certified Reference Material IRMM 804 showed values between 97 and 106 % (Table 5), indicating that the two methods were accurate. The recovery obtained in this study for the two methods developed demonstrated the efficiency of the digestion process. In this case, it is associated with the application of hydrogen peroxide, as its addition to the digestion vessels has been stated to facilitate organic matter oxidation due to the possibility of producing oxygen in the digestion system and inducing, in situ, the regeneration of nitric acid that was used as the main oxidation reagent, both in the gas phase through the oxygen available in the digestion vessel and the liquid phase through the oxidation of HNO_2 to HNO_3 (Lee *et al.*, 2022).

During the study of the effect of irrigation and genotype on the bioaccumulation of trace elements in rice, veracity was evaluated with the Certified Reference Material (CRM) IRMM 804, providing quantitative recoveries of 95 % for Cd and 99 % for As, as reported by Spanu *et al.* (2020), during the validation of total As in rice samples using ICP-MS.

Table 5. Reproducibility expressed as a function of the Z-Score attributed to each quantified concentration and veracity expressed as a recovery percentage (%R) of the quantified concentration in the IRMM 804 material.

Reproducibility (Proficiency Test FAPAS®)				
	ICP-OES		**ICP-MS**	
Element	**Concentration (mg/kg)**	**Z-Score**	**Concentration (mg/kg)**	**Z-Score**
Cd	0,098	-0,5	0,113	0,1
As	0,215	0,2	0,229	0,5
Veracity (IRMM 804)				
	ICP-OES		**ICP-MS**	
Element	**Concentration (mg/kg)**	**%R**	**Concentration (mg/kg)**	**%R**
Cd	1,596 ± 0,050	99,1	1,568 ± 0,035	97,4
As	0,052 ± 0,002	105,6	0,049 ± 0,003	99,7

Expanded uncertainty

The expanded uncertainty (U (x)) was estimated with a coverage factor Kp of 2 at a confidence interval of 95 % (Table 6), highlighting that the greatest contributions to uncertainty were due to the analyst under intermediate precision conditions, Similar results were reported by Rodriguez *et al.* (2022).

Table 6. Estimated uncertainty in the validation performed

| Parameter	Element	ICP-OES	ICP-MS
Uncertainty (%)	Cd	3,79	3,74
	As	11,65	9,81

Analysis of rice samples

The validated methods were used to analyze Cd and As in 20 samples from 16 brands of rice of three types (Figure 1 and 2). The results showed concentrations below the maximum levels (ML) established by the Codex Alimentarius and the EU for Cd and concentrations below the ML established by the Codex for As in husked rice. Twenty percent of the samples studied showed that the results of As were higher than the ML established by the EU for rice intended for the production of food for infants and young children. The correlation between ICP-MS and ICP-OES was greater than 0,9 (Figure 3), no difference higher than 20% was found between the results of Cd and As quantified by the two validated methods, indicating that they are comparable to each other when analyzed under the intermediate precision criterion.

The concentration of Cd and As in rice obtained by the two analytical methods developed provided valid results that allowed identifying the compliance with current regulations and will allow their implementation as routine analyses for samples with interest for export, agronomic management, or research purposes. These metals are gaining more importance and interest every day as they are mobile and soluble elements and, in terms of absorption, compete for the same transporters that some nutrients use, such as Cd with the metal Zn (Gallego *et al*., 2012;Zn, Cu or Mo Basnet *et al*., 2014; Cao *et al*., 2023).
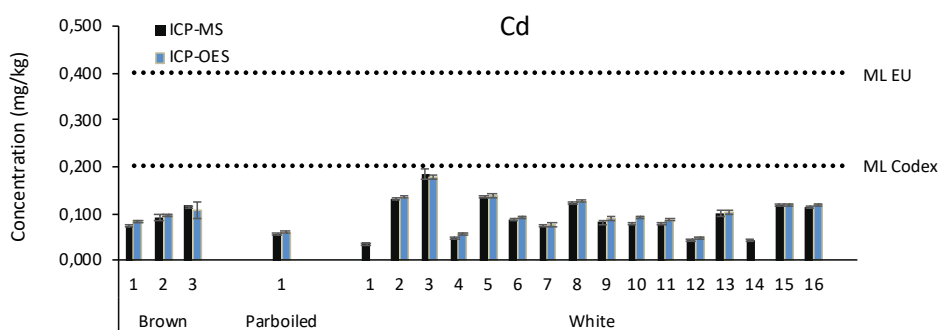
Figure 1. Cd concentration found in the rice samples analyzed by two validated methods (ICP-MS and ICP-OES) and separated by rice type (brown, parboiled and white). Each bar represents the average of three repetitions and its standard deviation, and the results are compared with the maximum levels (ML) of Cd established by the Codex Alimentarius for milled rice and by the European Union (EU) for rice grain.
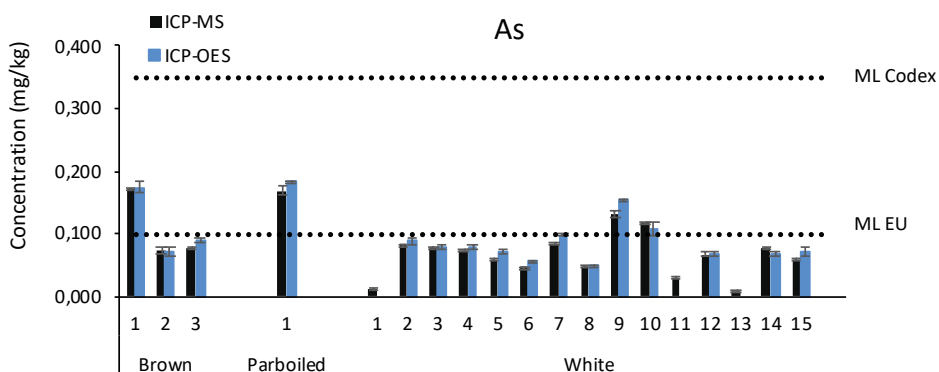
Figure 2. As concentration found in the rice samples analyzed by two validated methods and separated by rice type. Each bar represents the average of three repetitions and its standard deviation, and the results are compared with the maximum levels (ML) of As established by the European Union (EU) for rice intended for production for infant feeding and by the Codex Alimentarius for husked rice.
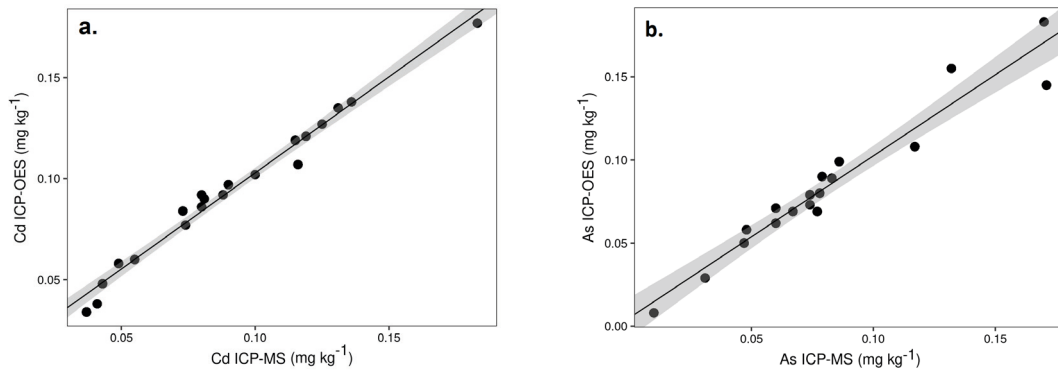
Figure 3. (a.) Comparison graph of cadmium data by ICP-OES (Y axis) and ICP-MS (X axis) with a correlation coefficient of 0,9893. (b.) Comparison graph of arsenic data by ICP-OES (Y axis) and ICP-MS (X axis) with a correlation coefficient of 0,9666.

CONCLUSIONS

Two analytical methods were developed, both by ICP-MS and ICP-OES, allowing the accurate and precise quantification of Cd and As in Colombian rice, with the lowest quantification limits offered by other laboratories, complying with the validation parameters described by Eurachem. The methods were comparable, and the quantification limits found were appropriate for the analysis of rice, considering the maximum limit (ML) established by the Codex Alimentarius and the EU. Still, higher sensitivity and lower quantification limits were evidenced in the ICP-MS method.

The samples collected from the local commerce showed low levels of Cd and As in accordance with the levels established by the Codex and the EU for this food. These results will be useful for the formulation of new national monitoring of contaminants and will give rise to new investigations that allow decisions to be made at the level of research, agronomic management of the crop and commercialization of the product.

ACKNOWLEDGEMENTS

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