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ARTICLE

Assessment and Validation of Ultrasound-Assisted Extraction Coupled with HPLC-ICP-MS for Arsenic Speciation in Husked Rice

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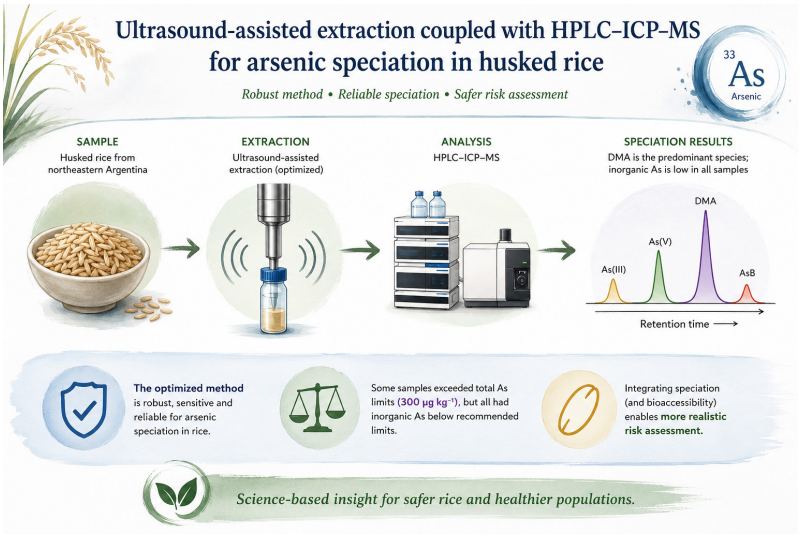

In this study, a high-frequency ultrasound-assisted extraction (HUAE) method coupled with high-performance liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICP-MS) was optimized to evaluate arsenic (As) in husked rice from the northeastern region of Argentina. The optimal conditions were identified as 0.05 mol L^{-1} HNO_3, 5 minutes of sonication, and maximum power (100 W), using a Box-Behnken design. The effective recovery of total As content (t-As) was 98%. The limit of detection (LOD) was 0.39 µg kg^{-1} and the limit of quantification (LOQ) was 1.29 µg kg^{-1}. The optimized method was then used to analyze a total of 64 samples collected from the local farmers. The results showed a similar concentration in terms of t-As content, with certain samples above the maximum permitted limits (300 µg kg^{-1}) established by the Brazilian

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Health Regulatory Agency (ANVISA), Codex Alimentarius, and the World Health Organization (WHO). However, the speciation analysis revealed a predominance of dimethylarsinic acid (DMA), with all samples showing inorganic arsenic values well below the recommended limits. The proposed method offers a robust, sensitive, and reliable analytical tool for quality control in rice.

Keywords: arsenic, chemical speciation, food analysis, Box-Behnken design, rice

INTRODUCTION

Arsenic (As) is a ubiquitous element found in soil and water worldwide, and it is recognized as toxic by many researchers, with dietary intake representing the primary route of human exposure.[1,2] Human exposure to arsenic has become a growing public health concern, as it has been classified as a carcinogenic agent since 1980 by the International Agency for Research on Cancer (IARC).[3] Consequently, as in foodstuffs has become a central focus for regulatory agencies and the scientific community. Arsenic occurs in both organic forms (such as monomethylarsonic acid, dimethylarsinic acid, arsenobetaine, and arsenocholine, among others) and inorganic forms (as arsenite and arsenate, among others), existing in various oxidation states (-3, 0, +3, +5). Literature indicates that arsenic toxicity depends on its chemical form and oxidation state, following the order: AsH_3 > As^{3+} > As^{5+} > monomethylarsenous acid > dimethylarsinic acid > arsenobetaine, arsenocholine, trimethylarsine oxide.[4,5]

In this context, rice is one of the most widely consumed cereals worldwide and is particularly noteworthy due to its high capacity to accumulate As, potentially reaching concentrations exceeding 1000 μg kg^{-1}.[6,7] Consequently, rice has become one of the most widely studied foods in arsenic research. Due to associated health risks, several international regulatory bodies have established limits for inorganic arsenic (i-As) in rice. The European Commission (EC) and China set a maximum limit of 200 μg kg^{-1} for i-As in polished rice. For rice intended for infants and young children, the European Union (EU) has a stricter limit of 100 μg kg^{-1}, aligning with the action level recommended by the U.S. Food and Drug Administration (FDA) for baby food (EC, 2015; U.S. FDA, 2016).[8,9] Additionally, EU regulations define a 250 μg kg^{-1} limit for i-As in husked rice, while Commission Regulation (EU) 2015/1006 reinforces the 100 μg kg^{-1} limit for infant food products. In South America, the Brazilian Health Regulatory Agency (ANVISA) and Southern Common Market (Mercosur) have established a maximum limit of 300 μg kg^{-1} for total arsenic (t-As) in rice[10] (ANVISA, 2013). The Food and Agriculture Organization (FAO)/ World Health Organization (WHO) also recommends the specific determination of inorganic arsenic when total arsenic exceeds 200 μg kg^{-1} in polished rice or 350 μg kg^{-1} in brown rice.[11]

Various analytical methodologies have been developed to address this issue and achieve high throughput, sensitivity and precision for the reliable, routine determination of inorganic and organic arsenic species.[12] Sample preparation is the most critical step in arsenic speciation, as analyte stability and prevention of interconversion are essential.[13] Extraction techniques such as ultrasound-assisted extraction (UAE)[14,15] and microwave-assisted extraction (MAE) are widely employed for food samples.[16] High-frequency ultrasound systems, such as cup horns, deliver up to 50 times more energy than conventional ultrasonic baths, allowing simultaneous processing of multiple vials and smaller sample masses.[17] This method also requires lower volumes of concentrated acids and enables faster extraction than microwave-assisted acid extraction. Analytical throughput can be further improved by applying response surface methodologies, such as Box-Behnken designs, to optimize all variables in the arsenic extraction process.[18,19]

The selection of analytical technique is crucial for the trace-level determination of arsenic species after sample preparation. Hyphenated methods, typically using high-performance liquid chromatography (HPLC) for separation, are widely applied in speciation analysis.[20,21] HPLC can be coupled with element-specific detectors, such as atomic fluorescence spectrometry (AFS)[22] or inductively coupled plasma mass spectrometry (ICP-MS).[23] Among these techniques, high-performance liquid chromatography coupled with inductively coupled plasma mass spectrometry (HPLC-ICP-MS) provides high selectivity, sensitivity, and reproducibility, making it ideal for arsenic speciation in rice. Recently, Kato *et al.* (2024)[24] demonstrated that

the coupling capabilities of HPLC for arsenic speciation can be enhanced by co-eluting the compounds to both electrospray ionization mass spectrometry (ESI-MS) and ICP-MS simultaneously, further expanding these capabilities to enable speciation determinations of both target and non-target analytes. Despite its advantages, ICP-MS-based speciation faces challenges due to mass spectrometric interferences, including polyatomic ions and doubly charged ions.[25] Understanding the sample matrix and potential interferents is therefore critical. Recent developments in collision/reaction cell technology have significantly reduced these interferences, enabling more accurate and reliable results.[26]

In this context, the present study aimed to optimize an extraction method for arsenic species in husked rice samples from the northeastern region of Argentina for analysis by HPLC-ICP-MS. A Box-Behnken design was employed to optimize high-frequency ultrasound-assisted extraction parameters, achieving optimal extraction conditions with shorter processing times and high analytical throughput. This approach is particularly relevant for monitoring arsenic in rice, a globally monitored food product due to its ability to accumulate this element. Finally, the dissemination of these findings is of significant current relevance.

MATERIALS AND METHODS

Instrumentation

An ICP-MS (iCAP TQ®, Thermo Scientific, Bremen, Germany) was used to perform measurements in MS/MS mode, employing oxygen (~0.2 mL min^{-1} O_2) as the reaction gas. The system was also pressurized with helium and operated in kinetic energy discrimination (KED) mode (4.3 mL min^{-1} He). The ICP-MS was tuned daily using a multi-element solution containing Bi, Li, Co, Ce, In, and U (1 µg L^{-1}) to ensure optimal sensitivity under the specified operating conditions. High-purity argon (99.999%), supplied by White Martins-Praxair (São Paulo, Brazil), was used to sustain plasma generation in the ICP-MS. A centrifuge (5804 R, Eppendorf, Hamburg, Germany) was used during sample preparation to separate the supernatant from the solid residue. The five arsenic species were separated using a Hamilton PRP-X100 column (10 µm, 250 mm × 4.1 mm) connected to a Thermo Scientific UltiMate™ 3000 HPLC system coupled to the ICP-MS. The optimized conditions for both the HPLC and ICP-MS are summarized in Table I.

Table I. ICP-MS and HPLC instrumental parameters and conditions.

ICP-MS operational conditions	
RF power	1550 W
Nebulizer	borosilicate glass micromist concentric nebulizer pumped at 30 rpm
Spray chamber	Quartz cyclonic spray chamber (cooled at 2.7 °C)
Plasma gas flow	14 L min^{-1}
Nebulizer gas flow	0.99 L min^{-1}
Auxiliary gas flow	0.8 L min^{-1}
HPLC for As speciation analysis	
Column	Hamilton PRP-X100 column (10 µm, 250 mm × 4.1 mm)
Temperature	30 °C
Flow rate	1.00 mL min^{-1}
Eluent	4 mmol L^{-1} ammonium nitrate 60 mmol L^{-1} ammonium nitrate; pH 8.5
Injection volume	50.0 µL
Elution program	Gradient 100% of 4 mmol L^{-1} ammonium nitrate (0.0 – 6.2 min) 100% of 60 mmol L^{-1} ammonium nitrate (6.2 – 15.0 min)

For total As determination and speciation, Q1 was set to *m/z* 75, while Q2 was operated in mass-shift mode at *m/z* 91 ($^{75}As^{16}O^{+}$). Arsenic extraction from husked rice samples was carried out using a cup-horn sonicator (Qsonica®, Connecticut, USA) operating at 100 W and 20 kHz.

Reagents and standards

All solutions were of analytical grade and prepared with ultrapure water (resistivity 18.2 MΩ·cm) obtained from a Milli-Q Millipore purification system (Bedford, MA, USA). Nitric acid (65% m/m, Synth, Diadema, Brazil) and hydrogen peroxide (30% m/m, EMSURE® Merck, Darmstadt, Germany) were used for sample preparation. Prior to use, nitric acid was purified via sub-boiling distillation. All glassware was cleaned with 10% (v/v) HNO_3 for 24 h and then thoroughly rinsed with deionised water.

Stock solutions of arsenic species (1000 µg L^{-1}) were prepared from $NaAsO_2$ (arsenite, As^{3+}), $Na_2HAsO_4 \cdot 7H_2O$ (arsenate, As^{5+}), $C_5H_{11}AsO_2$ (arsenobetaine, AsB), $(CH_3)_2AsO(OH)$ (dimethylarsinic acid, DMA⁵) and $CH_3AsO(OH)_2$ (monomethylarsonic acid, MMA). As^{3+}, AsB, DMA, and MMA were obtained from Sigma–Aldrich (Steinheim, Germany), while As^{5+} was obtained from Santa Cruz Biotechnology (Dallas, USA). Solutions were stored at 10 °C in the freezer prior to analysis.

The mobile phase used for arsenic species separation by high-performance liquid chromatography (HPLC) was ammonium nitrate (NH_4NO_3, Sigma-Aldrich, St. Louis, MO, USA).

Samples

A total of 64 whole-grain husked rice samples, representing thirteen different varieties (IRGA424, n=31; SCS 121, n=5; INOV, n=3; Taim, n=3; Titán, n=1; Yeruá, n=2; Fortuna, n=1; Puitá, n=4; XP102, n=1; Guri, n=4; IC110, n=2; IC109, n=5; IC107, n=2), were collected from different sites from the northeastern region of Argentina, including: Berón de Astrada (n=31) (27°32′00″S, 57°33′00″W), Saladas (n=4) (28°15′00″S, 58°37′00″W), Paso de Los Libres (n=7) (29°43′00″S, 57°05′00″W), San Martín (n=3) (29°06′00″S, 56°40′00″W), Curuzú Cuatiá (n=2) (29°47′00″S, 58°03′00″W), Esquina (n=1) (30°00′00″S, 59°32′00″W), Itatí (n=4) (27°16′00″S, 58°15′00″W), Empedrado (n=1) (27°57′00″S, 58°48′00″W), General Paz (n=3) (27°46′00″S, 57°37′00″W), Lavalle (n=1) (29°01′00″S, 58°58′00″W), San Roque (n=5) (28°34′00″S, 58°43′00″W), and Mercedes (n=2) (29°11′00″S, 58°05′00″W).

Optimization of the extraction method

The proposed extraction procedure represents an adaptation and optimization of previously reported methods based on diluted acid combined with ultrasound-assisted extraction for arsenic determination in food matrices.[27,28] In those studies, HNO_3 concentration and extraction time were identified as key factors influencing arsenic extraction efficiency in rice and related matrices. Building on this foundation, the present work employed a Box-Behnken experimental design to systematically optimize the extraction conditions including acid concentration, sonication time, and ultrasonic power. The method, based on diluted nitric acid extraction (0.05 mol L^{-1}), combined with ultrasound-assisted extraction (UAE), employed a cup-horn system coupled to a high-frequency ultrasonic transducer. Optimization was performed using multivariate approaches, specifically a Box-Behnken design, to determine the optimal experimental conditions for arsenic extraction. The Box-Behnken design was selected over other response surface methodology (RSM) designs, such as the Central Composite Design (CCD), because the Box-Behnken design requires only 15 experimental runs (including triplicate centre points), compared to 20 for the CCD, resulting in reduced consumption of certified reference material (CRM) and shorter analytical time, and the Box-Behnken avoids combinations in which all factors are simultaneously evaluated at their extreme levels.[29,30] The mass for optimization applied to the real samples was 50.0 mg; all analyses were realized in triplicate. The variables studied were HNO_3 concentration (mol L^{-1}), sonication time (min), and ultrasonic power (W), each evaluated at three levels. Recovery of total arsenic from a certified reference material (CRM), NIST Rice Flour 1568a, was used as the response.

After high-frequency ultrasound-assisted extraction, the samples were centrifuged at 10,000 rpm for 10 min, and the supernatants were collected for analysis by ICP-MS/MS. All experiments were performed in random order and in triplicate. Extractions were conducted inside an acoustically insulated box to minimize noise and vibration. The proposed method is illustrated in the corresponding Figure 1.

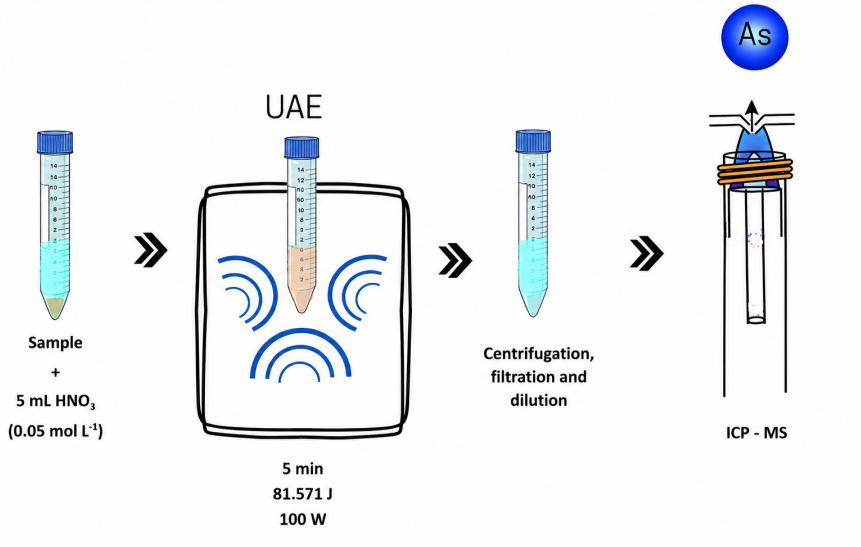

Figure 1. Diagram of the experimental steps in the proposed method using diluted acid as the extractor in cup horn ultrasound-assisted extraction (UAE).

Microwave-assisted acid decomposition

To compare the extraction efficiency, a microwave-assisted acid decomposition was performed. A DTG 100 microwave oven (Provecto Analítica, Jundiaí, Brazil), equipped with a temperature sensor and a 2450 ± 13 MHz magnetron, was employed. Initially, 500 µL of HNO_3 (65% w w^{-1}) and 200 µL of H_2O_2 (30% w w^{-1}) were added to 50.0 mg of NIST Rice Flour 1568a. The microwave-assisted digestion was performed in closed small-volume digestion vessels. The micro decomposition system consisted of a PTFE holder with four positions designed to accommodate PTFE micro-vials dimensionally equivalent to 2 mL Cryovial® polypropylene (PP) mini-vials, the holder was placed inside a microwave digestion vessel containing 10 mL of water, which acted as the heat-transfer medium. It is important to note that all components of the micro decomposition assembly were manufactured from PTFE, minimizing contamination risks and ensuring high chemical resistance. Under these conditions, the use of 500 µL of HNO_3 (65%) and 200 µL of H_2O_2 (30%) was adequate and within the manufacturer's recommended specifications for the sample mass employed (50.0 mg).[31,32] The microwave-assisted acid decomposition oven digestion program was executed in the following steps (time, power): 5 min at 240 W, 5 min at 420 W, 5 min at 600 W, 15 min at 800 W, and 10 min at 0 W. The acid digestion was executed in triplicate.

Arsenic speciation

The separation of arsenic species was initially evaluated by injecting individual standards (As^{3+}, As^{5+}, AsB, DMA, and MMA) to determine their retention times (RT). The chromatographic method was adapted from Richter *et al*. (2019).[33] A mixed standard solution was injected both before and after the rice samples to detect any potential alterations.

For arsenic speciation, the ultrasound-assisted extraction procedure described in the "Optimization of the extraction method" section was followed. The filtrates were then diluted 5–10 times with ultrapure water and analyzed by HPLC coupled to ICP-MS. Blank experiments were performed using the same procedure.

Arsenic species were quantified using an external calibration curve ranging from 0.5 to 50.0 μg L^{-1} for each species. Limits of detection (LOD) and quantification (LOQ) were determined based on signal-to-noise ratios (S/N) of 3 and 10, respectively, according to the IUPAC recommendation.

Statistical analysis

Pairwise comparisons were performed using the Student's *t*-test, Analysis of variance (ANOVA) was used to assess significant differences in the mean arsenic content of rice grains across geographic sites at a 95% confidence level ($p < 0.05$). Tukey's post hoc test was performed at $\alpha = 0.05$ when ANOVA detected significant differences. Statistica software, version 12.5 (StatSoft, Tulsa, USA), was used to optimize arsenic extraction at three levels using the Box–Behnken design.

RESULTS AND DISCUSSION

Strategy for optimizing the sample preparation procedure

The optimization of variables affecting the extraction of total arsenic (t-As) was carried out using a Box–Behnken design. Nitric acid concentration and extraction time were identified as key factors influencing t-As extraction in food matrices such as rice. Since ultrasound energy is involved in the extraction, the ultrasonic power applied in the cup-horn system was also evaluated. The experimental matrix and the results for the recovery of total arsenic from the certified reference material (CRM) Rice Flour 1568a are presented in Table II.

Arsenic recovery was evaluated using two ICP-MS modes: direct monitoring of the ^{75}As isotope and mass-shift monitoring via $^{75}As^{16}O$ (*m/z* = 91). Differences in arsenic recovery were observed between the two modes, with higher values obtained when monitoring *m/z* 75 corresponding to arsenic. This increase is attributed to polyatomic interferences caused by argon (^{40}Ar) and chlorine (^{35}Cl), the latter originating from CRM Rice Flour 1568a, which contains 300 mg kg^{-1} of chlorine, sufficient to generate spectral interferences and lead to inaccurate results. To minimize interfering ions reaching the detector, arsenic determination was performed using the ^{16}O mass-shift on ^{75}As.

The recovery of total arsenic (t-As) was used as the response for optimization via the Box-Behnken design as part of the response surface methodology (RSM). The effect of each factor on t-As extraction from rice samples was evaluated, with trueness ranging from 69% to 101%. Recoveries exceeding 100% when monitoring $^{75}As^{+}$ (*m/z* 75) arise from the overlap between the arsenic signal and the polyatomic interference $^{40}Ar^{35}Cl^{+}$ (*m/z* 75), which is generated by the presence of chlorine in the NIST Rice Flour 1568a CRM matrix (300 mg kg^{-1} Cl). This interference artificially enhances the measured signal, resulting in overestimated recovery values, the mass-shift mode ($^{75}As^{16}O^{+}$, *m/z* 91) effectively eliminates this interference by promoting the reaction of arsenic with oxygen in the collision/reaction cell, shifting the analyte signal to a mass free from polyatomic interferences.

Based on the results presented in Table II, the effects of each variable and their interactions were calculated, as illustrated in Figure 2. The Pareto chart displays the experimental design results, highlighting the effects of each evaluated factor (nitric acid concentration, extraction time, and ultrasonic power) as well as their respective synergistic interactions.

Table II. Experimental matrix from a Box-Behnken design for multivariate optimization of t-As extraction and its determination as As^+ or as AsO^+ using mass-shift strategy.

Exp	Power (W)	Time (min)	HNO_3 (mol L^{-1})	$^{75}As^{16}O^+$		$^{75}As^+$	
				Found (mg kg^{-1})	Recovery (%)	Found (mg kg^{-1})	Recovery (%)
1	20	1	0.08	0.26	87.99	0.27	92.5
2	100	1	0.08	0.20	69.02	0.28	95.0
3	20	5	0.08	0.25	84.71	0.36	122.5
4	100	5	0.08	0.29	100.99	0.40	138.0
5	20	3	0.05	0.25	85.30	0.36	123.8
6	100	3	0.05	0.28	98.22	0.43	150.0
7	20	3	0.10	0.23	79.89	0.36	124.4
8	100	3	0.10	0.24	81.09	0.43	148.1
9	60	1	0.08	0.22	74.61	0.36	122.8
10	60	5	0.08	0.24	82.50	0.41	139.7
11	60	1	0.08	0.20	70.66	0.35	120.3
12	60	5	0.08	0.22	75.10	0.38	129.9
13	60	3	0.08	0.21	73.03	0.37	127.0
14	60	3	0.08	0.22	74.59	0.38	129.4
15	60	3	0.08	0.21	73.81	0.35	122.3

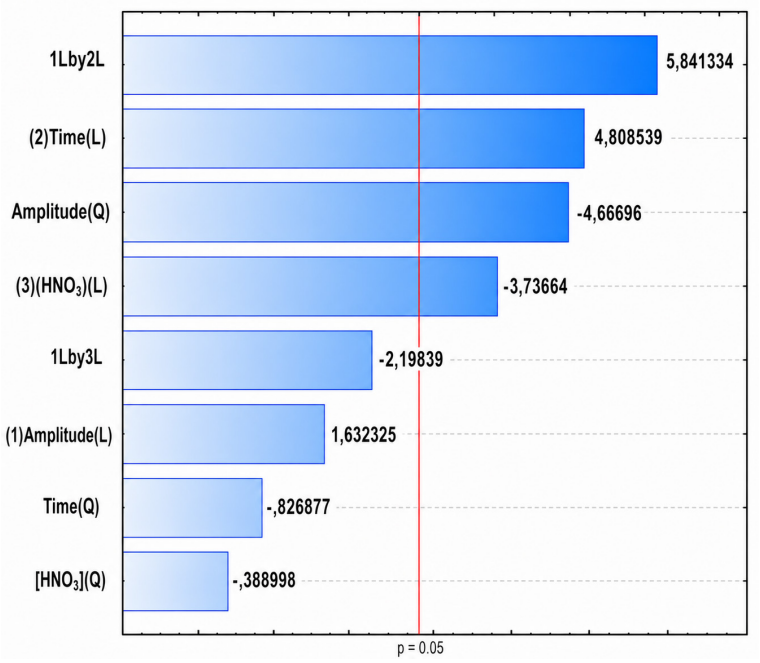

Figure 2. Pareto charts for the effects of the factors on the response of t-As. The red line corresponds to the critical value.

As shown in Figure 2, the most significant factors were variable 2 (extraction time) and variable 3 (HNO_3 concentration), as well as the interaction between variables 1 (ultrasonic power) and 2. This synergistic interaction was identified as the most important contributor to an increased response. However, despite its significance, HNO_3 concentration exhibited an adverse effect on arsenic extraction from rice. In other words, high concentrations of HNO_3 do not allow for effective extraction using high-power ultrasound-assisted extraction. Despite its significance, HNO_3 concentration exerted a negative effect on arsenic extraction from rice at higher levels. This behavior may be attributed to physicochemical factors, in particular, increasing HNO_3 concentration increases the viscosity of the extraction medium, which can alter cavitation dynamics and consequently reduce extraction efficiency during ultrasound-assisted extraction.[34] Higher viscosity dampens the propagation of acoustic waves and raises the cavitation threshold, reducing the intensity of microbubble implosion and, consequently, the efficiency of analyte release from the matrix.[35]

The increase in ionic strength resulting from the dissociation of HNO_3 in the aqueous medium enhances analyte extraction, making the acidic environment important for the method. This suggests that a diluted acid provides better conditions for the efficient propagation of ultrasonic waves in the reaction medium.[36] Nonetheless, the primary interaction driving the increase in response was between extraction time and ultrasonic amplitude, as illustrated in the response surface plot shown in Figure 3.

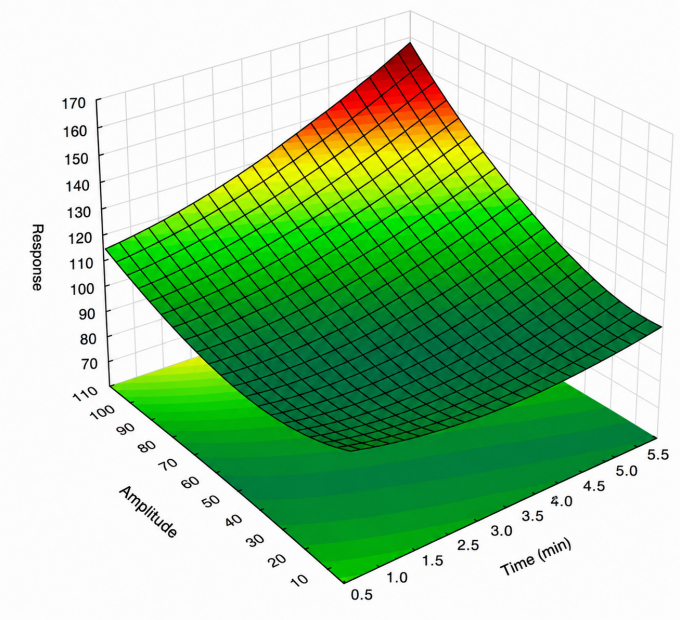

Figure 3. Response surface in function of the Arsenic recuperation in Rice CRM obtained from Box-Behnken design.

In ultrasound-assisted extraction (UAE), acoustic cavitation is the primary physical phenomenon driving the extraction process. The implosion of microbubbles within the extraction solvent disrupts the matrix and facilitates the release of analytes into the solution.[37] As shown in Figure 3, ultrasonic amplitude is directly related to ultrasonic power, and when combined with longer sonication times, it resulted in improved arsenic recoveries (highlighted in red). However, due to instrumental limitations, higher amplitudes could not be applied. The optimal conditions for total arsenic extraction, 0.05 mol L^{-1} HNO_3 and 5 minutes in a cup-horn sonicator at 100% power allow for increased analytical throughput while achieving low LOD and LOQ values of 0.39 µg kg^{-1} and 1.29 µg kg^{-1}, respectively. The method also demonstrated an accuracy of 98 ± 1%, as evidenced by the measured value of 0.28 ± 0.02 mg kg^{-1} compared to the certified value of CRM NIST Rice Flour 1568a, as demonstrated in Table III.

Table III. Comparison between arsenic concentration in NIST Rice Flour 1568a applying the proposed procedure and microwave-assisted acid decomposition (average ± standard deviation, n = 3). The certified value of total arsenic in the NIST Rice Flour 1568a certified reference material (CRM) is 0.290 ± 0.030 mg kg^{-1}.

Analyte	Microwave-assisted digestion (mg kg^{-1})	$t_{calculated}$	Ultrasound-assisted extraction (mg kg^{-1})
As	0.26 ± 0.01	1.73	0.28 ± 0.02

$t_{tabulated}$ = 4.30

A statistical comparison using Student's *t*-test revealed no significant difference between the certified and measured values at the 95% confidence level. Similarly, when comparing the proposed method with conventional microwave-assisted acid decomposition, no significant difference was found. Therefore, the high-frequency ultrasound method demonstrates comparable analytical performance to traditional methods, requiring only 5 minutes for effective arsenic extraction from the husked rice.

Speciation analysis of CRM NIST 1568a rice flour

For arsenic extraction, total arsenic recovery alone is not a sufficient indicator of an efficient method for food matrices. It is essential to perform arsenic speciation, and, consequently, to prevent interconversion between species. The same ultrasound-assisted extraction method was applied for arsenic speciation. Figure 4 shows the chromatograms of the five arsenic species, demonstrating excellent chromatographic resolution, which enabled accurate identification and quantification of the investigated species.

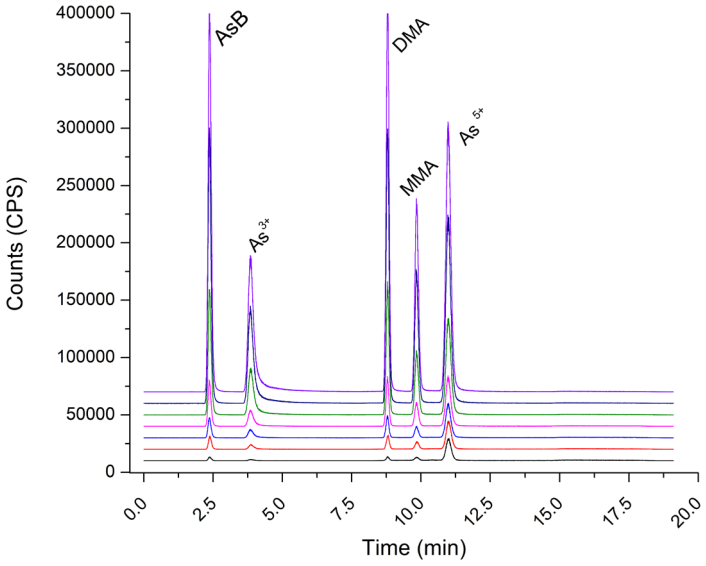

Figure 4. Chromatograms of arsenic calibration standards (0.5; 1.5; 2.5; 5.0; 15.0; 30.0; 50.0 µg L^{-1}) for arsenic compounds.

Once the retention times for the arsenic compounds had been established, 2.20, 3.85, 8.80, 9.90 and 11.0 minutes for AsB, As^{3+}, DMA, MMA and As^{5+} respectively, the extraction efficiency of the arsenic species was evaluated using the NIST CRM 1568a rice flour. Table IV shows the concentrations of the arsenic species found in the CRM. The calibration curves for the standards exhibited coefficient of determination (R^2) > 0.9990 for all arsenic species (Figure S1, Supplementary Material). Limits of detection (LOD) and quantification (LOQ) were calculated using blank samples, as described in the "Arsenic speciation" section of the Materials and Methods.

For AsB, As^{3+}, DMA, MMA, and As^{5+}, the LOD values were 0.002, 0.01, 0.02, 0.05, and 0.35 μg kg^{-1}, respectively, while the corresponding LOQ values were 0.01, 0.04, 0.06, 0.17, and 1.17 μg kg^{-1}, respectively. These values are well below the maximum concentrations established by legislation[8,10] for safe arsenic levels in rice.

Table IV. Mass balance of CRM NIST 1568[a] rice flour, concentration of AsB, As (III), DMA, MMA and As (V). Results expressed in mg kg^{-1}.

Method	AsB	As (III)	DMA	MMA	As (V)	Total As (∑ species)
SRM NIST 1568[a]	–	–	–	–	–	0.290 ± 0.030
This study	–	0.018 ± 0.002	0.210 ± 0.050	0.011 ± 0.005	0.022 ± 0.002	0.260 ± 0.020
(Farías *et al.* 2015)[22]	–	0.066 ± 0.017	0.200 ± 0.030	< LOQ	0.033 ± 0.010	0.299 ± 0.075

Mean values and standard deviations are shown; – : information not available.

The concentration of arsenic species obtained in this study indicated a recovery of approximately 95% of the certified total arsenic content, demonstrating the efficiency of the proposed method not only for total extraction but also for the preservation of species during the process. Although individual species values are not provided in the reference material, the results are consistent with literature data,[22,38] confirming the method's reliability and reproducibility.

In HPLC-ICP-MS analysis, chromatographic separation can resolve the $^{40}Ar^{35}Cl^{+}$ interference temporally, since chloride ions elute at different retention times from the arsenic species. However, the decision to employ the mass-shift mode ($^{75}As^{16}O^{+}$, *m/z* 91) for arsenic speciation in this study was motivated by the use of the same detection mode for both total arsenic determination and arsenic speciation. This approach facilitates direct comparison between the two datasets and minimizes potential systematic errors arising from the use of different analytical modes.[39]

Figure 5 shows the correlation of retention times between the CRM rice flour extract and arsenic standards. These chromatograms indicate not only that the arsenic species remained stable during acid extraction, but also that no significant matrix interferences were observed.

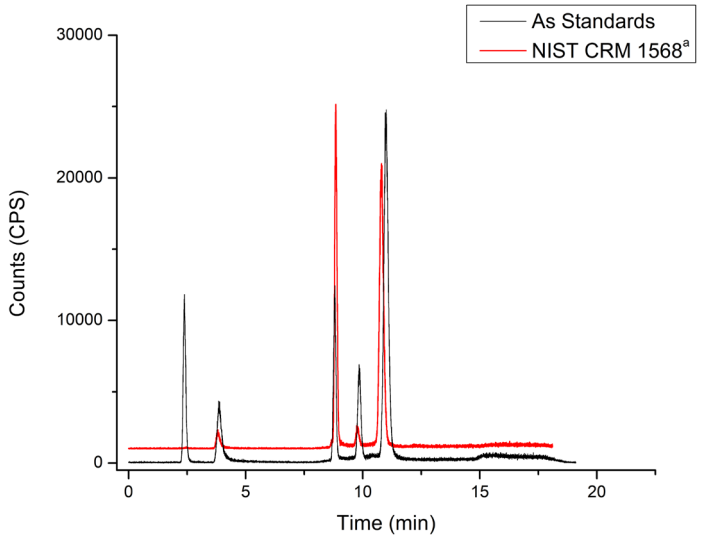

Figure 5. Chromatograms CRM Rice Flour NIST 1568[a] (red line) and As Standards (black line).

The predominance of DMA, followed by lower concentrations of As^{3+} and As^{5+}, is consistent with the speciation profile commonly observed in rice samples.[40] Therefore, the optimized method proved effective for both total quantification and speciation of arsenic in complex food matrices such as rice.

Application of the optimized method to husked rice samples

The developed method was applied to determine t-As in 64 husked rice samples from the different sites in the northeastern region of Argentina, with additional details provided in Table S1 of the Supplementary Material. The total arsenic concentration in husked rice from this region ranged from 16.0 to 1206.8 μg kg^{-1}. Figure 6 shows the mean values of the total arsenic concentration for the different sites of this study and the maximum t-As limit established by ANVISA, MERCOSUR, Codex Alimentarius, and the WHO (300 μg kg^{-1}). Only ten out of 64 husked rice grain samples exceeded this limit, with the highest mean t-As values exhibited by samples from Paso de los Libres, San Martín, and Itatí. Figure 7 shows the distribution of arsenic concentrations in three samples with a higher total arsenic concentration that HPLC-ICP-MS analyzed for speciation. The results revealed higher concentrations of organic arsenic, primarily in the form of DMA, followed by inorganic arsenic in the form As^{5+}.

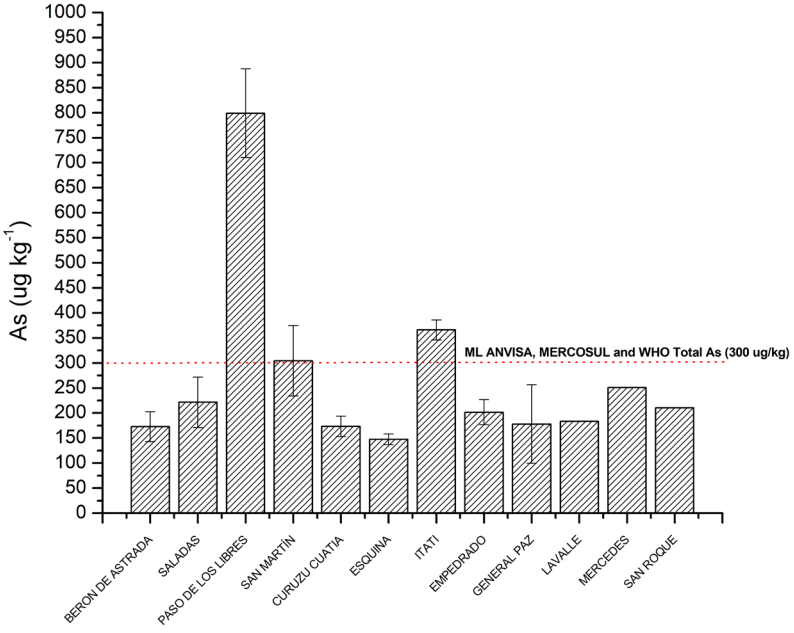

Figure 6. Total As in husked rice from different sites in the northeastern region of Argentina compared to maximum levels (ML) established by legislation.

Considering the toxicity of arsenic species, organic forms are generally less toxic than inorganic arsenic. It should be highlighted that the samples with higher total arsenic concentrations predominantly contained methylated species, such as DMA and MMA. Soil conditions can promote arsenic methylation, producing species such as DMA.[41] Furthermore, studies have demonstrated that DMA is more mobile within plants, leading to increased translocation rates from roots to grain.[42,43] This is consistent with the findings of Kato *et al*. (2019),[44] who reported a similar profile in rice produced in southern Brazil. Future studies should focus on understanding the environmental parameters or conditions that favour As methylation in rice in the region of study.

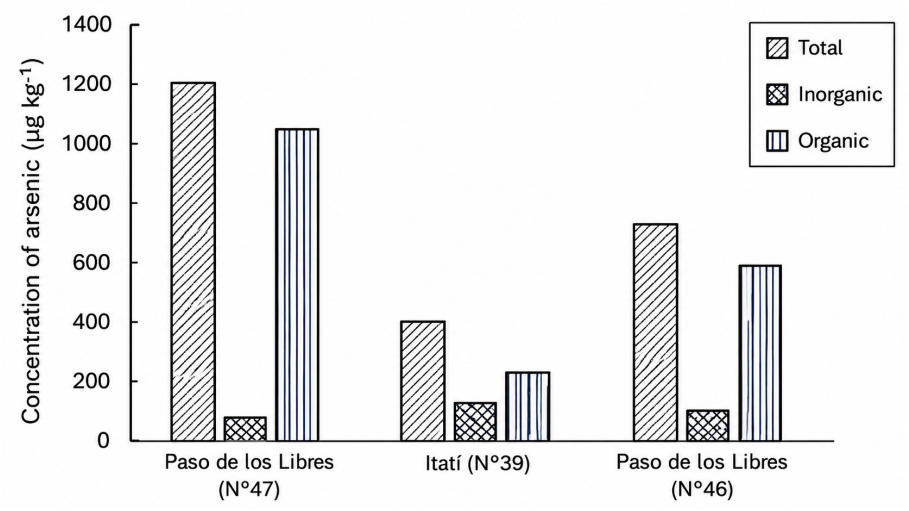

Figure 7. Distribution of arsenic species (µg kg^{-1}) in husked rice samples with t-As greater than 300 µg kg^{-1}.

These results highlight the importance of arsenic speciation data for accurate health assessments. Therefore, chemical speciation requires quantification, as it provides information on the concentration of arsenic species in various food matrices. In this sense, the implementation of a rice traceability system appears to be an excellent alternative as a tool to monitor the rice production process. In terms of risk assessment, understanding the origin and variety of grain, along with information on soil, water, and cultivation characteristics, enables the development and adoption of suitable arsenic mitigation methods, such as crop practices, water management, and rice variety selection. Another important consideration is that exposure estimates based solely on total arsenic concentrations assume complete gastrointestinal availability of the element, potentially leading to conservative risk assessments. Consequently, the integration of arsenic speciation and bioaccessibility data would provide a more realistic estimate of human exposure and a more accurate evaluation of the potential health risks associated with rice consumption.

CONCLUSIONS

A rapid and sensitive method using diluted nitric acid combined with high-frequency ultrasound was developed for arsenic extraction from husked rice, enabling accurate determination of total arsenic and its speciation. The results demonstrate that this methodology, based on ultrasound-assisted extraction optimized via a Box-Behnken experimental design, allows efficient and precise extraction of total arsenic from rice in just five minutes, with detection and quantification limits suitable for regulatory monitoring. Furthermore, applying the method to arsenic speciation enabled the identification and quantification of the main species present in the matrix without promoting interconversion, a critical consideration for toxicological risk assessment. This finding highlights the importance of chemical speciation, as it provides an accurate and comprehensive perspective on food safety. The proposed method represents a robust, sensitive, and reliable analytical tool for quality control, food safety monitoring of arsenic in rice, and potentially in other food matrices.

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Conflicts of interest

The authors declare that there is no conflict of interest.

Use of artificial intelligence (AI) tools

The authors declare that they used ChatGPT and Grammarly for grammar correction. The authors take full responsibility for the accuracy, integrity, and originality of the article's content and confirm that no AI tool was used to generate novel scientific ideas, analyze data independently, or replace the critical role of the authors.

REFERENCES

(1) Ganie, S. Y.; Javaid, D.; Hajam, Y. A.; Reshi, M. S. Arsenic Toxicity: Sources, Pathophysiology and Mechanism. *Toxicol. Res*. **2024**, *13* (1), tfad111. https://doi.org/10.1093/toxres/tfad111

(2) Visciano, P. Arsenic in Water and Food: Toxicity and Human Exposure. *Foods* **2025**, *14* (13), 2229. https://doi.org/10.3390/foods14132229

(3) International Agency for Research on Cancer (IARC). *Arsenic, Metals, Fibres, and Dusts. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*. IARC, Lyon, 2012, Vol. 100.

(4) Cui, D.; Zhang, P.; Li, H.; Zhang, Z.; Luo, W.; Yang, Z. Biotransformation of Dietary Inorganic Arsenic in a Freshwater Fish Carassius auratus and the Unique Association between Arsenic Dimethylation and Oxidative Damage. *J. Hazard. Mater*. **2020**, *391*, 122153. https://doi.org/10.1016/j.jhazmat.2020.122153

(5) El-Ghiaty, M. A.; El-Kadi, A. O. S. The Duality of Arsenic Metabolism: Impact on Human Health. *Annu. Rev. Pharmacol. Toxicol*. **2023**, *63*, 341–358. https://doi.org/10.1146/annurev-pharmtox-051921-020936

(6) Mondal, R.; Majumdar, A.; Sarkar, S.; Goswami, C.; Joardar, M.; Das, A.; Mukhopadhyay, P. K.; Roychowdhury, T. An Extensive Review of Arsenic Dynamics and Its Distribution in Soil-Aqueous-Rice Plant Systems in South and Southeast Asia with Bibliographic and Meta-Data Analysis. *Chemosphere* **2024**, *352*, 141460. https://doi.org/10.1016/j.chemosphere.2024.141460

(7) Islam, S.; Rahman, M. M.; Islam, M. R.; Naidu, R. Arsenic Accumulation in Rice: Consequences of Rice Genotypes and Management Practices to Reduce Human Health Risk. *Environ. Int*. **2016**, *96*, 139–155. https://doi.org/10.1016/j.envint.2016.09.006

(8) U.S. Food and Drug Administration (FDA). *Supporting Document for Action Level for Inorganic Arsenic in Rice Cereals for Infants*. FDA, Silver Spring, MD, 2020.

(9) European Commission. Commission Regulation (EU) 2015/1006 of 25 June 2015 Amending Regulation (EC) No 1881/2006 as Regards Maximum Levels of Inorganic Arsenic in Foodstuffs. Off. J. Eur. Union 2015, 14–16. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32015R1006 (accessed 2026-01-15).

(10) Agência Nacional de Vigilância Sanitária (ANVISA). Resolução RDC Nº 42, de 29 de agosto de 2013. Dispõe sobre o Regulamento Técnico MERCOSUL sobre Limites Máximos de Contaminantes Inorgânicos em Alimentos. Available at: https://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2013/rdc0042_29_08_2013.html (accessed 2026-01-15).

(11) Codex Alimentarius Commission. General Standard for Contaminants and Toxins in Food and Feed. Codex Stan 193-1995. FAO/WHO, Rome, 1995 (revised 2019).

(12) Sadee, B. A.; Galali, Y.; Zebari, S. M. S. Recent Developments in Speciation and Determination of Arsenic in Marine Organisms Using Different Analytical Techniques. A Review. *RSC Adv*. **2024**, *14* (30), 21563–21589. https://doi.org/10.1039/D4RA03000A

(13) Viana, J. L. M.; Menegário, A. A.; Fostier, A. H. Preparation of Environmental Samples for Chemical Speciation of Metal/Metalloids: A Review of Extraction Techniques. *Talanta* **2021**, *230*, 122119. https://doi.org/10.1016/j.talanta.2021.122119

(14) Qu, Y.; Yang, Z. G.; Li, H.; Yao, J.; Qiu, B. Ultrasound-Assisted Enzymatic Extraction Method for Multi-Element Analysis of Rice. *Food Analytical Methods* **2020**, *13* (8), 1549–1555. https://doi.org/10.1007/s12161-020-01779-3

(15) Jones, R. R.; Chaparro, J. M.; Maloley, K.; Evans, J. C.; Boyko, S.; Gruszczynski, M.; Prenni, J. E. Rapid, Semi-Quantitative Arsenic Speciation across Diverse Food Matrices via Reverse Phase HPLC-ICP-MS. *J. Food Compos. Anal*. **2025**, *147*, 108000. https://doi.org/10.1016/j.jfca.2025.108000

(16) Sel, S.; Koyuncu, İ. Microwave-Assisted Sample Preparation for Simultaneous Determination of Trace Elements in Vegan Foods with Inductively Coupled Plasma Mass Spectrometry. *Food Analytical Methods* **2025**, *18* (3), 428–441. https://doi.org/10.1007/s12161-024-02723-5

(17) Santos, H. M.; Capelo, J. L. Trends in Ultrasonic-Based Equipment for Analytical Sample Treatment. *Talanta* **2007**, *73* (5), 795–802. https://doi.org/10.1016/j.talanta.2007.05.039

(18) Gamela, R. R.; Costa, V. C.; Pereira-Filho, E. R. Multivariate Optimization of Ultrasound-Assisted Extraction Procedure for the Determination of Ca, Fe, K, Mg, Mn, P, and Zn in Pepper Samples by ICP OES. *Food Analytical Methods* **2020**, *13* (1), 69–77. https://doi.org/10.1007/s12161-019-01524-5

(19) Silva, A. B. S.; Sá, Í. P.; Higuera, J. M.; Nogueira, A. R. A. Feasibility of Dispersive Liquid–Liquid Microextraction for Molybdenum Determination in Lamb Meat by MIP OES. *Food Analytical Methods* **2021**, *14* (3), 496–502. https://doi.org/10.1007/s12161-020-01898-X

(20) Pedron, T.; Silva, V.; Paniz, F. P.; Batista, B. L. Speciation of Arsenic and Concentration of Cadmium and Arsenic in Rice (*Oryza sativa* L.) Cultivated under Co-Exposure of Cadmium/Arsenic and Different Water Ponding. *J. Trace Elem. Miner*. **2025**, *12*, 100240. https://doi.org/10.1016/j.jtemin.2025.100240

(21) Tiwari, R.; Ranjan, N.; Chaurasia, M.; Flora, S. J. S. Hyphenated Mass Spectroscopic Detection of Heavy Metals in Environmental and Biological Samples: A Review. *J. Trace Elem. Miner*. **2026**, *15*, 100273. https://doi.org/10.1016/j.jtemin.2025.100273

(22) Farías, S. S.; Londonio, A.; Quintero, C.; Befani, R.; Soro, M.; Smichowski, P. On-Line Speciation and Quantification of Four Arsenical Species in Rice Samples Collected in Argentina Using a HPLC–HG–AFS Coupling. *Microchem. J*. **2015**, *120*, 34–39. https://doi.org/10.1016/j.microc.2014.12.010

(23) Barnet, L. S.; Pozebon, D.; Dressler, V. L.; Cioato, D. Method Validation for As Speciation in Rice Using LC-ICP-MS and the Inorganic Arsenic Limit for Brazilian Rice. *J. Food Compos. Anal*. **2021**, *99*, 103849. https://doi.org/10.1016/j.jfca.2021.103849

(24) Kato, L. S.; da Silva, V. H. C.; de Andrade, D. C.; Cruz, G.; Pedrobom, J. H.; Raab, A.; Feldmann, J.; Arruda, M. A. Z. Multimodal Chemical Speciation Techniques Based on Simultaneous High Resolution Molecular/Atomic Mass Spectrometry Applied to Online Target/Non-Target Analysis: A Tutorial Review. *Anal. Chim. Acta* **2024**, *1331*, 343084. https://doi.org/10.1016/j.aca.2024.343084

(25) Tanaka, Y. K.; Matsuhashi, K.; Ogra, Y. Analytical Techniques for Arsenic Speciation. *Anal. Sci*. **2025**, *41* (4), 317–321. https://doi.org/10.1007/s44211-025-00722-y

(26) Balaram, V. Strategies to Overcome Interferences in Elemental and Isotopic Geochemical Analysis by Quadrupole Inductively Coupled Plasma Mass Spectrometry: A Critical Evaluation of the Recent Developments. *Rapid Commun. Mass Spectrom*. **2021**, *35* (10), e9065. https://doi.org/10.1002/rcm.9065

(27) Ribeiro, R. V.; Vieira, J. C.; Lobo, F. A.; Froes-Silva, R. E. S. Comparison between Ultrasound Assisted Extraction and Microwave Digestion in the Determination of Arsenic in Edible Grains. *J. Braz. Chem. Soc*. **2018**, *29* (4), 873–880. https://doi.org/10.21577/0103-5053.20170184

(28) de Avila, L. O.; Torres, D. P.; Oreste, E. Q.; Lisboa, M. T.; Silveira, C. A. P.; Ribeiro, A. S.; Nunes, A. M.; Martinazzo, R. Speciation of Inorganic Arsenic in Retorted Oil Shale Slurry by Hydride Generation Atomic Absorption Spectrometry (HG AAS) after Ultrasound-Assisted Acid Extraction. *Anal. Lett*. **2024**, *57* (7), 1065–1077. https://doi.org/10.1080/00032719.2023.2240453

(29) Cerqueira, U. M. F. M.; Bezerra, M. A.; Ferreira, S. L. C.; de Jesus Araújo, R.; da Silva, B. N.; Novaes, C. G. Doehlert Design in the Optimization of Procedures Aiming Food Analysis – A Review. *Food Chem*. **2021**, *364*, 130429. https://doi.org/10.1016/j.foodchem.2021.130429

(30) Ferreira, S. L. C.; Bruns, R. E.; Ferreira, H. S.; Matos, G. D.; David, J. M.; Brand, G. C.; Silva, E. G. P.; Reis, P. S.; Souza, A. S.; Santos, W. N. L. Box-Behnken Design: An Alternative for the Optimization of Analytical Methods. *Anal. Chim. Acta* **2007**, *597* (2), 179–186. https://doi.org/10.1016/j.aca.2007.07.011

(31) da Silva, A. B. S.; da Silva Leal, K. N.; Arruda, M. A. Z. An Acidless Microwave-Assisted Wet Digestion of Biological Samples as a Greener Alternative: Applications from COVID-19 Monitoring to Plant Nanobiotechnology. *Anal. Bioanal. Chem*. **2025**, *417* (7), 1271–1282. https://doi.org/10.1007/s00216-024-05472-w

(32) da Silva, V. H. C.; de Lima, R. F.; Mayer, J. L. S.; Arruda, M. A. Z. Feasibility of Using Silica (Na_2SiO_3 and SiO_2NPs) to Mitigate Mercury in Transgenic Soybeans Grown in Contaminated Soils and Respective Effects on Nutrient Homeostasis. *Environ. Sci. Pollut. Res*. **2025**, *32*, 1–20. https://doi.org/10.1007/s11356-025-36179-9

(33) Richter, L.; Hernández, A. H.; Pessôa, G. S.; Arruda, M. A. Z.; Rezende-Filho, A. T.; de Almeida, R. B.; Menezes, H. A.; Valles, V.; Barbiero, L.; Fostier, A. H. Dissolved Arsenic in the Upper Paraguay River Basin and Pantanal Wetlands. *Sci. Total Environ*. **2019**, *687*, 917–928. https://doi.org/10.1016/j.scitotenv.2019.06.147

(34) Lučić, M.; Onjia, A. Ultrasound-Assisted Microextraction for Food Chemical Contaminant Analysis: A Review. *Processes* **2025**, *13* (11), 3677. https://doi.org/10.3390/pr13113677

(35) Carreira-Casais, A.; Carpena, M.; Pereira, A. G.; Chamorro, F.; Soria-Lopez, A.; Perez, P. G.; Otero, P.; Cao, H.; Xiao, J.; Simal-Gandara, J.; Prieto, M. A. Critical Variables Influencing the Ultrasound-Assisted Extraction of Bioactive Compounds—A Review. *Chem. Proc*. **2021**, *5* (1), 50. https://doi.org/10.3390/csac2021-10562

(36) Costa, F. S.; Leal, R. V. P.; Pacheco, C. S. V.; Amorim, F. A. C.; de Jesus, R. M.; Santos, L. N.; da Silva, E. G. P. Multivariate Optimization of Ultrasound-Assisted Extraction Procedure for the Determination of Cu, Fe, Mn, and Zn in Plant Samples by Flame Atomic Absorption Spectrometry. *Anal. Methods* **2020**, *12*, 5351–5358. https://doi.org/10.1039/d0ay00554a

(37) Fu, X.; Belwal, T.; Cravotto, G.; Luo, Z. Sono-Physical and Sono-Chemical Effects of Ultrasound: Primary Applications in Extraction and Freezing Operations and Influence on Food Components. *Ultrason. Sonochem*. **2020**, *60*, 104726. https://doi.org/10.1016/j.ultsonch.2019.104726

(38) Huang, J. H.; Fecher, P.; Ilgen, G.; Hu, K. N.; Yang, J. Speciation of Arsenite and Arsenate in Rice Grain – Verification of Nitric Acid Based Extraction Method and Mass Sample Survey. *Food Chem*. **2012**, *130* (2), 453–459. https://doi.org/10.1016/j.foodchem.2011.07.059

(39) Quarles, C. D.; Sullivan, P.; Bohlim, N.; Saetveit, N. Rapid Automated Total Arsenic and Arsenic Speciation by Inductively Coupled Plasma Mass Spectrometry. *J. Anal. At. Spectrom*. **2022**, *37*, 1796–1803. https://doi.org/10.1039/d2ja00055e

(40) Befani, M. R.; Quintero, C. E.; Panozzo, J. A.; Zamero, M. de los Á.; Risso, L. F.; Sansó, M. C.; Hernández, J. P. Agronomic Strategies to Reduce the Arsenic (As) Concentration in Rice in Environments Susceptible to High As Concentration in Grains from Argentina. *Pedosphere* **2025**, *35* (2), 415–423. https://doi.org/10.1016/j.pedsph.2023.12.001

(41) Mishra, S.; Mattusch, J.; Wennrich, R. Accumulation and Transformation of Inorganic and Organic Arsenic in Rice and Role of Thiol-Complexation to Restrict Their Translocation to Shoot. *Sci. Rep*. **2016**, *6*, 40522. https://doi.org/10.1038/srep40522

(42) Abedi, T.; Mojiri, A. Arsenic Uptake and Accumulation Mechanisms in Rice Species. *Plants* **2020**, *9* (2), 129. https://doi.org/10.3390/plants9020129

(43) Song, Y.; Liu, Y.; Li, H.; Fang, Y.; Lu, D.; Yang, Z. The Crucial Elements for Lettuce (*Lactuca sativa* L.) Growth under DMA Stress and the Linkage with DMA Behavior: A New Application of Ionome. *J. Environ. Manage*. **2023**, *347*, 119124. https://doi.org/10.1016/j.jenvman.2023.119124

(44) Kato, L. S.; De Nadai Fernandes, E. A.; Raab, A.; Bacchi, M. A.; Feldmann, J. Arsenic and Cadmium Contents in Brazilian Rice from Different Origins Can Vary More than Two Orders of Magnitude. *Food Chem*. **2019**, *286*, 644–650. https://doi.org/10.1016/j.foodchem.2019.02.043

SUPPLEMENTARY MATERIAL

Table S1. Total arsenic concentration in rice samples from the northeastern region of Argentina.

Sample code	Variety	Sites	As total ($\mu g\ kg^{-1}$ ± SD)
1	IRGA424	Beron de Astrada	152.2 ± 6.4
2	IRGA424	Beron de Astrada	129.1 ± 6.3
3	IRGA424	Beron de Astrada	287.5 ± 4.5
4	IRGA424	Beron de Astrada	152.9 ± 11.7
5	IRGA424	Beron de Astrada	109.5 ± 0.6
6	IRGA424	Beron de Astrada	198.4 ± 9.1
7	SCS 121	Beron de Astrada	226.3 ± 4.3
8	IRGA424	Beron de Astrada	264.6 ± 10.2
9	SCS 121	Beron de Astrada	131.9 ± 1.0
10	SCS 121	Beron de Astrada	193.7 ± 6.2
11	SCS 121	Beron de Astrada	209.1 ± 4.1
12	SCS 121	Beron de Astrada	113.2 ± 1
13	IRGA424	Beron de Astrada	204.4 ± 12.1
14	IRGA424	Beron de Astrada	194.9 ± 5.7
15	IRGA424	Beron de Astrada	97.9 ± 0.6
16	IRGA424	Beron de Astrada	121.6 ± 24.8
17	IRGA424	Beron de Astrada	94.2 ± 18.1
18	IRGA424	Beron de Astrada	126.9 ± 10.9
19	IRGA424	Beron de Astrada	50.6 ± 0.4
20	Taim	Beron de Astrada	96.3 ± 19.2
21	Yeruá	Beron de Astrada	183.1 ± 12.9
22	Puitá	Beron de Astrada	193.8 ± 5.8
23	Guri	Beron de Astrada	200.8 ± 0.6
24	Gurí	Beron de Astrada	82.5 ± 1.3
25	IC110	Beron de Astrada	161.2 ± 5.7
26	IC110	Beron de Astrada	118.9 ± 0.8
27	IC109	Beron de Astrada	293.1 ± 4.6
28	IC109	Beron de Astrada	158.2 ± 0.9
29	IC109	Beron de Astrada	263.2 ± 0.8

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Table S1. Total arsenic concentration in rice samples from the northeastern region of Argentina (continued).

Sample code	Variety	Sites	As total ($\mu g\ kg^{-1}$ ± SD)
30	IC109	Beron de Astrada	288.2 ± 5.9
31	IC109	Beron de Astrada	143.8 ± 0.1
32	IRGA424	Curuzu Cuatia	237.8 ± 12.1
33	Gurí	Curuzu Cuatia	108.9 ± 1.7
34	INOV	Empedrado	201.6 ± 65.8
35	IRGA424	Esquina	147.4 ± 6.5
36	INOV	General Paz	132 ± 10.4
37	Titán	General Paz	129.3 ± 20.7
38	Puitá	General Paz	272.8 ± 13.1
39	IRGA424	Itati	395.8 ± 17.5
40	IRGA424	Itati	82.3 ± 3.3
41	IC107	Itati	146.3 ± 1.5
42	IC107	Itati	839 ± 6.0
43	IRGA424	Lavalle	183.1 ± 4.1
44	Yeruá	Mercedes	16.5 ± 2.1
45	Gurí	Mercedes	484.9 ± 6.1
46	IRGA424	Paso de los Libres	730.5 ± 9.5
47	IRGA424	Paso de los Libres	1206.8 ± 33.9
48	IRGA424	Paso de los Libres	490.8 ± 2.3
49	IRGA424	Paso de los Libres	325.8 ± 62.9
50	IRGA424	Paso de los Libres	96.5 ± 4.4
51	IRGA424	Paso de los Libres	2679.1 ± 184.1
52	IRGA424	Paso de los Libres	67.1 ± 12.5
53	IRGA424	Saladas	155.4 ± 12.9
54	IRGA424	Saladas	229.5 ± 2.2
55	Taim	Saladas	130.5 ± 11.6
56	Taim	Saladas	370.4 ± 19.5
57	IRGA424	San Martín	221.9 ± 0.8
58	IRGA424	San Martín	615.1 ± 3.2
59	IRGA424	San Martín	75.6 ± 0.1

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Table S1. Total arsenic concentration in rice samples from the northeastern region of Argentina (continued).

Sample code	Variety	Sites	As total (μg kg^{-1} ± SD)
60	INOV	San Roque	183.8 ± 11.2
61	Fortuna	San Roque	221.4 ± 3.8
62	Puitá	San Roque	159.5 ± 14.9
63	Puitá	San Roque	420.4 ± 40.4
64	XP102	San Roque	66.5 ± 2.1

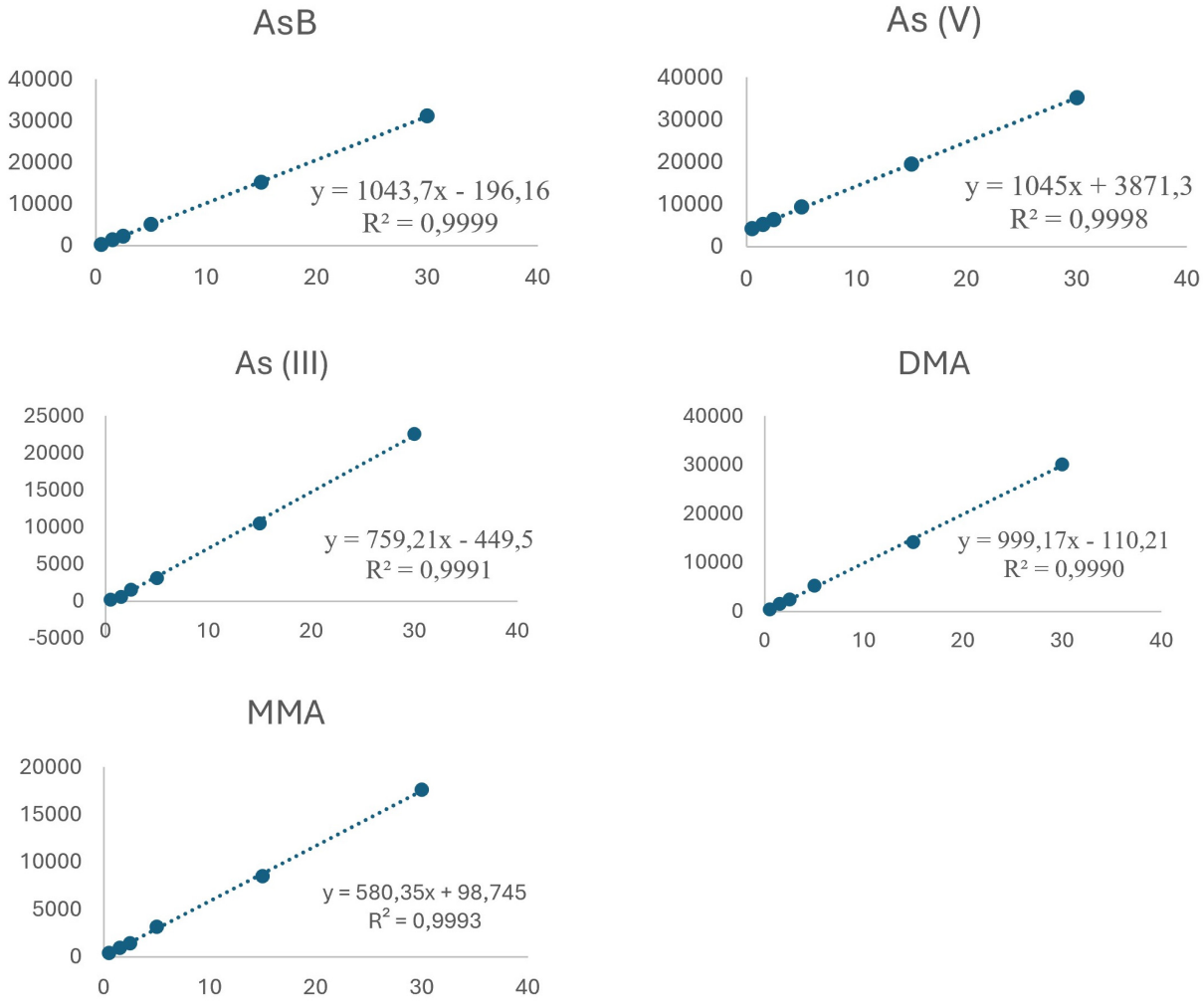

Figure S1. Calibration curves for AsB, As^{5+}, As^{3+}, DMA, and MMA.