

ARTICLE

Determination and uncertainty analysis of inorganic arsenic in rice by UHPLC-ICPMS

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ABSTRACT The present study arose from the need of to determine inorganic arsenic (iAs) at low levels in rice. Ultra-high performance liquid chromatography coupled with inductively coupled plasma mass spectrometry (UHPLC-ICPMS) using Kinetic Energy Discrimination (KED) mode to eliminate spectral interferences was used for analysis of iAs. Sample preparation involves extraction of inorganic arsenic (sum of As³⁺ and As⁵⁺) with water by heating at 90 °C for 5 min in water bath. Separation is accomplished with a reversed-phase ion pack column using a gradient chromatographic method followed by ICPMS analysis within 5 min. The method was validated in accordance with Commission Regulation (EU) No 836/2011 and performance characteristics were verified. Acceptable values were obtained for specificity, repeatability (HorRat_r < 0.6), within-lab reproducibility (HorRat_r < 0.3) with recovery 80-90%, limit of quantification (0.02 mg/kg), fitness-for-purpose and trueness (using CRM); thus, the method can be considered for official control purposes.

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Introduction

Arsenic (As) is a naturally occurring element in the Earth's crust. It is a metal element that is naturally present in water, air and soil, and is absorbed by some food crops as they grow (Hughes et al. 2011). It is not an additive or ingredient in foods and cannot be eliminated from the food we eat or the water we drink. The main sources of arsenic pollution include certain pesticides and herbicides, wood preservatives, phosphate fertilizers, industrial waste, mining activities, coal burning and smelting (Manjarrez-Domínguez et al. 2019). There are two general types of arsenic compounds. It is present in two general forms: inorganic and organic. These classifications are based on their carbon chemistry and are strictly not classified by the method of farming - as arsenic is in soil and water, both organically- and conventionally grown crops will contain arsenic. The inorganic arsenic is widely considered as detrimental to health (Sanchez et al. 2016). Studies have found alarming levels of arsenic in rice. For many people, rice is a simple and comforting food. In Asia rice is an ancient symbol of wealth, success, fertility, and good health. More than half the world's population, it is a staple food and makes up a large portion of people's diets. Millions of people around the world are exposed to drinking water that contains high amounts of inor-

ganic arsenic mainly south America and Asia (Choi et al. 2010). Fish, shrimp, shellfish, and other seafood may contain significant amounts of organic arsenic, the less toxic form. Rice absorbs more arsenic from water and soil compared to other common food crops. Moreover, studies show that arsenic exposure is more critical in rice than in any other food stuff (Saifullah et al. 2018) and the arsenic level in rice is 10 times higher than in wheat and barley. In addition to direct ingestion, using rice straw for cattle feed increases the risk of arsenic exposure, which is the single biggest food source of inorganic arsenic toxic form. Arsenic may accumulate in the soil of paddy fields, worsening the problem. Paddy rice is particularly susceptible to arsenic contamination, due to the reasons of grown in flooded paddy fields that require high quantities of irrigation water, but in many areas the irrigation water is contaminated with arsenic (Seyfferth et al. 2014).

Brown rice especially might contain high levels of arsenic, particularly in its inorganic forms. Using contaminated water for cooking is another concern because rice grains easily absorb arsenic from cooking water when they are boiled. Young children are also at risk if rice-based products make up a large part of their diet. Studies showed that children who were exposed to arsenic in drinking water scored significantly lower on standardized tests (Wang et al. 2007). Also, pregnant

women who consumed even very low levels of arsenic from food products went on to have children who were much more likely to develop respiratory problems in the first four months of their lives. Arsenic consumption has also been linked to liver, kidney, and prostate damage (Das et al. 2018). The toxicity of arsenic depends not only on the total concentration, but also its chemical forms as these differ in terms of mobility, toxicity, and bioavailability. The inorganic trivalent arsenic (As^{3+}) and pentavalent arsenic (As^{5+}) are the most toxic forms, whereas other common forms including the organic monomethyl arsenic (MMA) and dimethyl arsenic (DMA) have significantly reduced toxicities. It is known that the majority of arsenic in marine organisms is in the form of arsenobetaine, which is non-toxic (Avula et al. 2008).

European Food Safety Authority (EFSA) included rice among the foods that most contribute to iAs exposure and pointed out the need to produce speciation data for different food commodities to estimate the health risk associated with dietary arsenic exposure (Llorente-Mirandes et al. 2012). The European Committee for Standardization (CEN) published in 2008 a standardized method, EN 15517:2008, for the determination of iAs in seaweed. The WHO classifies arsenic as carcinogenic and EFSA's latest risk assessment of arsenic found that exposure to arsenic in Europe is close to the limit that can be considered as not safe (Uysudus et al. 2009). EFSA is therefore encouraging member states to reduce arsenic exposure as much as possible. According to the World Health Organization guidelines, the permissible level for total arsenic in drinking water is 10 ng/ml (WHO 2011). The country that has regulated the level of iAs in rice is China, where the maximum contaminant level permitted is 0.20 mg/kg (Chen et al. 2018). Although no such limit exists for food products, the Food and Agriculture Organization / World Health Organization (FAO/WHO) recommend an intake no greater than 15 $\mu\text{g}/\text{kg}$ body weight per week. The Federal Institute for Risk Assessment (BfR) has assessed the proposed maximum levels in 2014 for rice and rice products from a health point of view and comes to the conclusion that the maximum level of 0.2 mg of inorganic arsenic per kilogram recommended for white rice is only suitable to avoid particularly high levels in rice. Recently, the limit for iAs in rice has been fixed as 0.20 and 0.25 mg/kg by Codex Alimentarius Commission in 2016 and the European Commission as per Regulation No. 2015/1006/EU in 2015. Exposure to inorganic arsenic is primarily of concern because of its cancer-causing properties (Ooki et al. 2018). Arsenic has been classified by the International Agency for Research into Cancer (IARC) as a human carcinogen on the basis of increased incidence of cancers at several sites in people exposed to arsenic at work, in the environment or through

their diet. However, arsenic is also more acutely toxic than other metallic compounds and it was used in earlier times as a rodenticide, while continual low-level exposure to arsenic is associated with skin, vascular and nervous system disorders (Flora et al. 2007). Many methods have been published for the determination of iAs using Hydride Generation-Atomic Absorption Spectrometry. There is a problem in doing the analysis of arsenic speciation due to high pH which leads to additional deprotonation of the arsenate anion (Adrian 2011). In recent years, to measure different forms of arsenic using High-performance liquid chromatography coupled with inductively coupled plasma mass spectrometry (HPLC- ICPMS) is used. In this, UHPLC separates the forms and ICP-MS detects them as they elute from the column. The advantage of ICP-MS is very sensitive and can measure trace levels, as demonstrated by its use to measure impurities in a wide range of environmental samples. Further, this method is applicable to meet the performance criteria considering the maximum levels fixed for iAs in rice as set by the codex and EC. In this article, we describe a fully validated method as per European Union Commission Regulation No. 882/2004/EC.

Materials and methods

Chemicals and consumables

Ammonium carbonate (EMSURE, ACS) were supplied by Merck (Germany) High purity water (18.2 M Ω .cm, 0.22 μm filtered) were from a water purification system (Evoqua Water Technologies, Germany). Standard reference material of arsenite (As^{3+}) and arsenate (As^{5+}), 1000 mg/l solution, traceability to NIST were purchased from Sigma Aldrich (Switzerland). Prepared working standards (10 mg/l) from 1000 mg/l standard by diluting 1 ml to 100 ml with water and used for preparation of linearity standards, 0.5, 1.0, 5.0, 10, 15 and 20 $\mu\text{g}/\text{l}$ mix. Syringe filters (0.22 μm , 25 mm) was purchased from Agilent Technologies (India)

Equipment

Equipment used for sample preparation: analytical balance (Sartorius, Switzerland), refrigerated centrifuge (Sorvall Legend XIR, Thermo Scientific), water bath (Equitron, India), and vortex (Spinax, Tarsons, India).

The chromatographic analysis was performed using ultra-high performance liquid chromatograph (Ultimate 3000, Dionex, Thermo Scientific, Germany) comprising pump, autosampler and column compartment. Mass spectrometric analysis was performed using iCAP Q equipment (Thermo Scientific, Germany). The optimized LC-ICPMS conditions are summarized in Table 1.

Table 1. Optimized UHPLC and ICPMS conditions for inorganic arsenic

HPLC Experimental Conditions	
HPLC column	Dionex IonPac AS7, RFIC, Analytical 4 X 250 mm.
Mobile phase	A) 150 mM ammonium carbonate; B) water
HPLC elution program	0.0 - 2.0 min: 70% A, 30% B 2.2 - 3.0 min: 100% A 4.0 - 6.0 min: 70% A, 30% B
Flow rate (ml/min)	1.0
Column temperature (°C)	40
Injection volume (µl)	20
ICP-MS Experimental Condition	
Plasma power (W)	1550
RF generator supply voltage (V)	38.70
Plasma cooling water flow (l/min.)	0.73
Plasma gas slow (l/min)	14
Auxiliary flow (l/min)	0.80
Collision gas flow, CCT-1 (l/min)	4.83
Nebulizer gas flow (l/min)	1.0550
Spray chamber temperature (°C)	2.7
Operation mode	Kinetic Energy Discrimination (KED)
Peristaltic pump speed (rpm)	40

Sample preparation

Homogenized sample (0.5 g) was made up to 10 ml with water in a 15 ml centrifuge tube, vortexed for at least one minute and kept in a water bath at 90 °C for 5 min. Then vortexed again and centrifuged for 10 min (8000 rpm, 5 °C) and filtered through 0.22 µm syringe filter and transfer to HPLC sample vial. Adopted the same technique for both sample and sample blank. For preparation of spike recovery sample added known volume of As³⁺ and As⁵⁺ standard, adopted the same procedure as sample preparation.

Method validation

The method validation was performed according to Commission Regulation (EU) No 836/2011 amending Regulation of (EC) No 333/2007 and verified performance criteria for applicability, specificity, repeatability (RSD_r), reproducibility (RSD_R), recovery, limit of detection (LOD), limit of quantitation (LOQ) and fitness for purpose.

The validation was performed at four concentration levels with 6 replicates in rice sample. The concentration levels were 0.02, 0.2, 0.5 and 1 mg/kg of inorganic arsenic and a total of 24 samples were spiked and analysed along with blank samples. The experiments were performed on three different occasions to evaluate repeatability and within laboratory reproducibility. As the rice sample con-

tains arsenic naturally and difficult to get blank sample, hence the rice sample was soaked in water overnight and cleaned three times with water then dried in oven at 105 °C for 4 h to reduce the levels of iAs in rice, which was reported in earlier studies carried out by Raab et al. (2009) and EFSA Journal 2014. The dried sample is grinded to fine powder and used as blank material. The same was used for spike recovery study. Specificity was checked by analysing representative blank samples six replicates per day. It was observed that free from matrix or spectral interferences in the region of interest. The spectral interference was eliminated by selecting Kinetic Energy Discrimination (KED) as there is no another isotope for arsenic. Linearity was tested from the calibration curves at 1, 5, 10, 15, 20 µg/l including blank was checked by least-squares linear regression. The calibration curves were best fitted to a linear curve and correlation coefficients (r²) were higher or equal to 0.99.

Results and discussion

Optimization of extraction

Weighted (0.5 g) of homogenized rice is extracted with water using heated water bath technique to determine the best extraction efficiency. Earlier studies show that the methanol method has been widely used for arsenic speciation in plants (Singh and Ma 2006; Mathews et al. 2010). Though As extraction efficiency in the fronds was satisfactory at 80-90%, the efficiency for the roots was low at ~60% (Zhao et al. 2015). This is consistent with Zhang et al. (2002) who reported ~60% for the roots and 85-100% for the fronds. Further, methanol is lethal during extraction process in addition to generating harmful waste. In consequence, it is essential to develop a new method with satisfactory extraction efficiency and less toxic waste. Also, the problem of deprotonation of arsenate anion, due to high pH using acidic extraction method, was eliminated by extracting with water applying heat treatment. However in the present study, the extraction with water typically provided high extraction recoveries. The method was optimized by using less quantity of sample (0.5 ± 0.05 g) to reduce the matrix effect and minimize the ion suppression and ion enhancement.

Specificity

A blank sample (water) was analysed by LC-ICP-MS in each batch, and no signal was observed at the retention times of the As³⁺ and As⁵⁺. Therefore, reagents in the blank did not provoke interferences in the chromatograms. The presence of a high content of chloride (Cl⁻) in the matrices could lead to the misidentification of arsenic with ICP-MS detection (Story et al. 1992; Pretty et al. 1993). A blank

sample (water) spiked at 50 mg/l with Cl standard solution was analysed to check the possible interference with As^{3+} and As^{5+} , and no signal was observed at the retention time of As^{3+} and As^{5+} at 1.5 min (90 sec) and 3.4 min (205 sec), respectively. The ion intensity at m/z 75 (^{75}As) was monitored and additionally, the ion intensities at m/z 77 ($^{40}\text{Ar}^{37}\text{Cl}$ and ^{77}Se) and m/z 35 (^{35}Cl) were monitored to detect possible argon chloride ($^{40}\text{Ar}^{35}\text{Cl}$) interference at m/z 75. However, no possible interferences were occurred, possibly due to the operation of the ICP-MS using KED mode, which was reported to eliminate poly atomic and isobaric interference from co-eluting chloride species (Juskelis et al. 2013; Day et al. 2002; Maher 2015). The selectivity of the method regarding the ($^{40}\text{Ar}^{35}\text{Cl}$) interference for the arsenic species studied was verified.

Establishment of LOD and LOQ

As per Corley (2003), for most modern analytical methods, the detection limit may be divided into two components, instrumental detection limit (IDL) and method detection limit (MDL). In the validation study, IDL and instrumental quantification limit (IQL) were calculated for As^{3+} and As^{5+} on the standard deviation of y -intercepts of regression analysis (σ) and the slope (S) of the standard curves, using the following equation, $\text{IDL} = 3 \sigma/S$. IQLs were calculated from the equation $\text{IQL} = 10 \sigma/S$. Limit of detection (LOD) has been determined analysing a solution fortified with concentration similar to predicted LOD, which is 0.002 mg/kg (0.001 mg/kg As^{3+} and 0.001 mg/kg As^{5+}). Limit of quantitation (LOQ) is set as one tenth of maximum limit (0.2 mg/kg) which is verified by fortifying sample at 0.02 mg/kg (0.01 mg/kg As^{3+} and 0.01 mg/kg As^{5+}). The linearity of As^{3+} , As^{5+} and chromatographic separation of inorganic arsenic is given in Figure 1.

Repeatability and within laboratory reproducibility

Precision was assessed as within-day repeatability and as between-day intermediate precision (Menditto et al. 2007). In both cases, spiking experiments were carried out by adding As^{3+} and As^{5+} standards to rice samples and homogenized. The mixtures were then extracted as stated in sample preparation. Unspiked samples were also analysed in order to calculate the spike recovery. Precision, expressed in terms of relative standard deviation (% RSD) of iAs recovery, was assessed by analyzing spiked rice samples at 0.02 mg/kg (LOQ), 0.2 mg/kg, 0.5 mg/kg, and 1 mg/kg levels. For evaluate the between-day precision, three different analysis days and different analysts for spiking were taken into consideration. For within-day repeatability, six samples for each spiking level were analysed within a day. The precision acceptance criterion (Deventor et al. 2005; Frys et al. 2011) matches the 2/3 Horwitz function (Horwitz 1982). Satisfactory precision

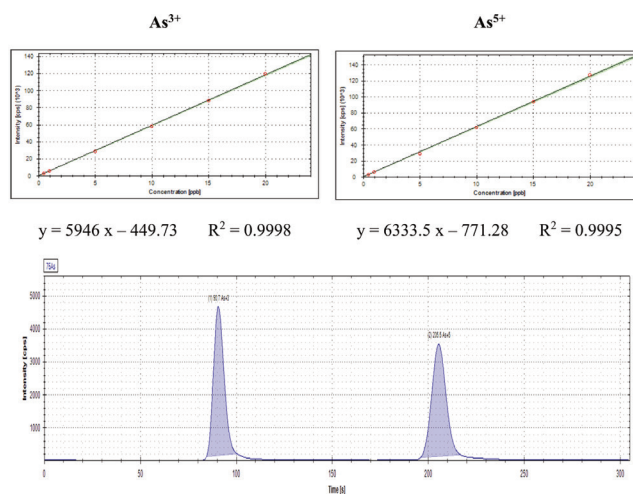


Figure 1: Linearity of As^{3+} and As^{5+} and standard chromatogram (5 $\mu\text{g/l}$)

was obtained in all cases, and the results obtained are consistent with the precision acceptance criteria.

As precision often varies with analyte concentration, precision repeatability was calculated for inorganic arsenic (sum of As^{3+} and As^{5+}) at four spiking levels 0.02, 0.2, 0.5 and 1 mg/kg (individual concentration of As^{3+} and As^{5+} at 0.01, 0.1, 0.25 and 0.5 mg/kg (each concentration 6 times). The repeatability was calculated as relative standard deviation (RSD_r) of measurements for the sample, done by the same analyst, on the same instrument within a short period of time. The % RSD_r and HorRat_r (Horwitz and Albert 2006; Thomson 2000) ranged was tabulated in Table 2.

The reproducibility was determined through the analysis of blank rice samples fortified in six replicates at four spiking levels 0.02, 0.2, 0.5 and 1 mg/kg (each concentration 6 times). Six replicate test portions at each of the four fortification levels ($n = 18$) were analysed on three separate days. Repeatability HorRat_r (Horwitz and Albert 2006; Thomson 2000) and within lab reproducibility data were tabulated in Table 2.

The average recovery for inorganic arsenic was around 89% with HorRat_r ranged from 0.05 to 0.16 and HorRat_R ranged from 0.04 to 0.08 which is less than 2 as per criteria in line with EU 836/2011.

The intra- and interday data were used for the respective determination of the repeatability (r) and within-laboratory reproducibility (R). Precision (intra- and interday) was established through the estimation of HorRat_r and HorRat_R. The Horwitz equation (Horwitz et al. 1980) was used for estimation of precision at 0.20, 0.50 and 1.0 mg/kg, and the modified equation (Thompson 2000) was used for precision at 0.02 mg/kg using the following equations as per EU No. 836/2011:

Table 2. Recovery, repeatability and within lab reproducibility of inorganic arsenic in rice.

Element	Spiked at (mg/kg)	Repeatability				Within laboratory reproducibility			
		Average recovery (%)	RSD _r (%)	HorwitzRSD _r	HorRat _r	Average recovery (%)	RSD _R (%)	Horwitz RSD _R	HorRat _R
As ³⁺	0.01	94.35	2.11	14.52	0.15	90.76	1.53	22.00	0.07
	0.1	95.09	0.47	14.52	0.03	90.80	0.93	22.00	0.04
	0.25	94.27	1.27	12.91	0.10	89.87	1.18	19.56	0.06
	0.5	97.26	0.79	11.63	0.07	92.53	1.57	17.63	0.09
As ⁵⁺	0.01	84.33	8.19	14.52	0.56	80.15	4.51	22.00	0.21
	0.1	83.30	1.18	14.52	0.08	80.12	1.22	22.00	0.06
	0.25	80.32	0.83	12.91	0.06	80.86	1.02	19.56	0.05
	0.5	81.75	0.58	11.63	0.05	80.59	0.86	17.63	0.05
Inorganic As (sum of As ³⁺ + As ⁵⁺)	0.02	89.34	2.35	14.52	0.16	85.46	1.68	22.00	0.08
	0.2	89.20	0.63	13.35	0.05	85.46	0.93	22.00	0.04
	0.5	87.29	0.60	11.63	0.05	83.87	0.78	17.63	0.04
	1.0	89.51	0.58	10.49	0.06	85.06	0.99	15.89	0.06

HorRat_r = observed RSD_r/calculated RSD_R using (modified) Horwitz equation; and

HorRat_R = the observed RSD_R/calculated RSD_R using (modified) Horwitz equation.

RSD_R was calculated by using the Horwitz equation $2C^{(-0.15)}$.

Where, C = the concentration ratio $1.2 \times 10^{-7} \leq C \leq 0.138$; and by using the modified Horwitz equation (22%) for the concentrations less than 1.2×10^{-7} .

Recovery and ruggedness

Since certified reference materials were used for the analytes and matrices of interest, the recovery from spiked blank samples was measured as an alternative to trueness. The recoveries were calculated for iAs by spiking at four levels viz. at 0.02 mg/kg (LOQ), 0.2 mg/kg, 0.5 mg/kg, and 1 mg/kg which are tabulated in Table 2. Also, to check the trueness, certified reference (ERM-BC211) material was used and achieved 125.03 µg/kg (100.8% recovery) against certified value 124 ± 11 µg/kg. The reproducibility (RSD_R) achieved 6.0% and HorRat_R 0.28 which was within acceptance criteria (less than 2) and found satisfactory.

The analytical method is tested with a fortified sample under different experimental conditions and the different analyst to check the ruggedness. The recovery was 80% with RSD_r 3.53% and HorRat_r is 0.24 which is within the acceptance criteria (EU No. 836/2011).

Measurement uncertainty and fitness for purpose approach

The measurement uncertainty in estimation of iAs in rice corresponds to various sources like weighing balance, water bath, volume, centrifuge, temperature, standard purity, dilutions, calibration curve, repeatability. The type A source was the repeatability obtained through the

method, and the type B sources included the calibration graph, standard stock solution preparation, sample weight, make-up volume, and water bath temperature. The standard uncertainty due to type A source was calculated as

$$U_{Rep} = \sigma/\sqrt{p}, \text{ where } p = \text{the number of readings}$$

The standard uncertainty due to the calibration graph was calculated as

$$U(Co) = u(C_0) = \frac{SD_{xy}}{b} \times \sqrt{\frac{1}{p} + \frac{1}{n} + \frac{(C_0 + C_m)^2}{S_{xx}}}$$

where C₀ = mean concentration of readings (µg/kg); SD_{xy} = the residual SD; b = slope; p = number of readings; n = the number of calibration concentrations; C_m = the mean value of calibration standards; S_{xx} = $\sum(C_i - C_m)^2$ where C_i = concentration of calibration standard at level i.

The standard uncertainty due to the standard stock solution was calculated as

where U₁ to U₅ are the relative standard uncertainties

$$U_{STD} = STD_{Conc.} \times \sqrt{(U_1^2 + U_2^2 + U_3^2 + U_4^2 + U_5^2)}$$

due to purity and dilution of standards.

The standard uncertainty due to the weight of the sample was calculated as

where USM = the uncertainty of the balance.

$$U_{Sample\ mass} = \sqrt{2 \times (U_{SM}/2)^2}$$

The standard uncertainty due to volume was calculated as

$$U_{Pipette-vol.} = \sqrt{2 \times (U_{micropipette}/2)^2}$$

Table 3. Measurement uncertainty and fitness-for-purpose for inorganic arsenic in rice.

Element	At level of concentration (mg/kg)	Combined measurement uncertainty (u_c) (mg/kg)	Maximum standard measurement uncertainty (U_f) (mg/kg)	Criteria for fitness-for-purpose ($u_c < U_f$) as per EU No. 836/2011
As ³⁺	0.01	0.001	0.002	Complies
	0.1	0.008	0.018	
	0.25	0.020	0.045	
	0.5	0.040	0.090	
As ⁵⁺	0.01	0.001	0.002	Complies
	0.1	0.007	0.018	
	0.25	0.016	0.045	
	0.5	0.031	0.090	
Inorganic As (sum of As ³⁺ + As ⁵⁺)	0.02	0.002	0.004	Complies
	0.2	0.010	0.036	
	0.5	0.051	0.090	
	1.0	0.101	0.150	

The standard uncertainty due to make-up volume was calculated as

$$U_{\text{Flask-vol.}} = \sqrt{2 \times (U_{\text{Flask-vol.}}/2)^2}$$

The standard uncertainty due to water bath temperature was calculated as

$$U_{\text{Water bath temp}} = \sqrt{2 \times (U_{\text{Water bath temp.}}/2)^2}$$

The standard uncertainty due to centrifuge temperature was calculated as

$$U_{\text{Centrifuge temp}} = \sqrt{2 \times (U_{\text{Centrifuge temp.}}/2)^2}$$

The standard uncertainty due to centrifuge rpm was calculated as

$$U_{\text{Centrifuge rpm}} = \sqrt{2 \times (U_{\text{Centrifuge rpm}}/2)^2}$$

The combined uncertainty was calculated as

$$u_{\text{combined}} = C_0 \times \sqrt{(U_{x1}^2 + U_{x2}^2 + U_{x3}^2 + U_{x4}^2 + U_{x5}^2 + U_{x6}^2)}$$

The expanded uncertainty was calculated at the 95% confidence level using a coverage factor of $k = 2$. Measurement uncertainty was estimated by following the EURACHEM/CITAC Guide CG4. It adopted the approach of grouping the uncertainty components into two categories based on their method of evaluation, i.e. type

Table 4. Summary of method performance characteristics for inorganic arsenic in rice

Parameter	As ³⁺	As ⁵⁺	Inorganic As(sum of As ³⁺ + As ⁵⁺)	Criteria as per EU No. 836/2011
Applicability	Rice and cereal products			Foods specified in Regulation (EC) No. 1881/2006
Specificity	Free from matrix or spectral interferences			Free from matrix or spectral interferences
Repeatability* (RSD _r)	0.15	0.56	0.16	HorRat _r less than 2
Reproducibility* (RSD _R)	0.07	0.21	0.08	HorRat _R less than 2
Recovery* (%)	86-95	75-84	80-90	-
LOD (mg/kg)	0.001	0.001	0.002	0.012 (3/10th of LOQ)
LOQ (mg/kg)	0.01	0.01	0.02	0.040
Fitness-for-purpose*	0.001 $u_c < U_f$ 0.002 Complies	0.001 $u_c < U_f$ 0.002 Complies	0.001 $u_c < U_f$ 0.004 Complies	Combined standard Uncertainty(u_c) less than Maximum standard uncertainty (U_f)
Trueness (using CRM ERM-BC211)* (µg/kg)	-	-	125.03 ± 7.50	124 ± 11 (Certified value)

A and type B. In this case, the type A uncertainty was the repeatability and the type B corresponded to the calibration graph, standard stock solution, sample weight, and make-up volume. The observation was made under the same conditions of measurement at ambient temperature, and the sample temperature was maintained at 25 ± 2 °C. The standard uncertainty due to type A was 0.00044 based on the iAs in rice from ten different trials was measured. In the case of type B, the standard uncertainty due to the calibration graph was estimated as 0.056 and 0.109 for As^{3+} and As^{5+} , respectively; the standard uncertainty due to standard stock solution was 0.013; the standard uncertainty due to sample weight was 0.00014; and the standard uncertainty due to make-up volume was 0.086, the standard uncertainty due to temperature of water bath was 0.64, the standard uncertainty due to temperature and rpm of centrifuge were 0.37 and 19.2, respectively. The combined uncertainty was 0.002 and the expanded uncertainty was 0.004 mg/kg. The final result of iAs was 0.02 ± 0.004 mg/kg. Similarly, uncertainty at 0.2, 0.5 and 1.0 mg/kg was calculated, and the expanded uncertainty for 0.010, 0.051, and 0.101 mg/kg, respectively.

Fitness for purpose

The measurement of uncertainty was calculated at a confidence level of 95% found to be less than the maximum standard measurement uncertainty calculated using formulae below as per EU 836/2011 (C3.3.1 and C 3.3.2) and tabulated in Table 3.

The fitness-for-purpose approach was used to assess the suitability of using the method for official control purposes. Fitness-for-purpose was calculated using the following formula:

where U_f = the maximum standard measurement uncertainty (mg/kg); C = the concentration of interest (mg/kg); and α = the numeric factor to be used depending on the value of C (i.e., 0.2 for concentrations ≤ 0.05 mg/kg, 0.18 for concentrations 0.051-0.500 mg/kg and 0.15 for concentrations 0.500 - 1.0 mg/kg).

This method is applicable for determination of inorganic arsenic (Sum of As^{3+} and As^{5+}) in rice and cereal products by LC-ICPMS at a range of 0.020 - 1.0 mg/kg. The summary of method performance characteristics for inorganic arsenic in rice as per EC 333/2007 amending regulation EC 836/2011 mentioned in Table 4.

Conclusion

One of the main advantages of this method is that it allows quantification of inorganic arsenic in routine analysis in easy and fast sample preparation technique. Using this simple extraction method using water achieves

good repeatability and reproducibility with this method. Further, the trueness of the method is satisfactory with regard to the validation data as well as the results from CRM comparisons. The recoveries between 80-90%, LOD at 0.002 mg/kg, LOQ at 0.02 mg/kg with repeatability of 0.16 (HorRat_r) and reproducibility of 0.08 (HorRat_r) were obtained. The overall analysis time is less because the extraction time is very short and sample preparation is fast and robust. The method takes a full advantage of specificity and no interfering signals to the As^{3+} and As^{5+} compounds used was detected in rice. From the validation study, it can be concluded that trueness (% recovery) and precision (repeatability and within lab reproducibility) of method were satisfactory. The LOQ achieved is low enough and suitable for determining the arsenic species at the low levels found in the samples. The results on CRM shows good agreement with the certified values, as well as with the results on arsenic species reported in the literature. The criteria for acceptance as per validation were met and hence confirming that the method adopted is fit for the intended purpose (quantitative analysis of inorganic arsenic in rice). Based on the above satisfactory validation of methods of inorganic arsenic in rice with respect to method performance criteria as per Commission Regulation (EU) No 836/2011, the method was fit for the purpose and deemed suitable for regulatory analysis of inorganic arsenic in rice by UHPLC-ICPMS at the stated range.

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