Low level quantification of trace metals in rice using ICP-MS

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Keywords

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Goal

To demonstrate the capability of an ICP-MS for trace elemental analysis in rice in compliance with the AOAC Official Method 2015.01 validation guidelines and the Food Safety and Standards Authority of India (FSSAI), China Food and Drug Administration (CFDA), and European Union (EU) MRL requirements.

Introduction

Rice is an important staple food with global consumption of 518 million tons in 2016.[1] The main countries in which rice is a major part of the human diet are India and China. Consequently, the FSSAI, CFDA, and the European Commission (EC), have set maximum residue limits (MRLs) for heavy metals in rice as listed in Table 1.[2-4] Heavy metals can occur in rice as a consequence of uptake from agricultural soils contaminated by mining activities and sludge disposal. Processes such as milling and polishing, packaging, and transportation may also be a source of contamination. Contamination with toxic heavy metals in rice is a concern because of the possible adverse effects on human health. Scientists have reported health issues including coughing, anemia, and kidney failure, ultimately leading to death due to heavy metal contamination.[5,6] To verify and quantify the amount of toxic elements in rice, commercial food testing laboratories widely use a procedure based on

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microwave digestion followed by quantification by ICP-MS. For some elements, such as arsenic or selenium, speciation analysis using a hyphenated technique such as IC-ICP-MS may be required to differentiate different chemical forms, which may vary in toxicity or bioavailability.[7]

Table 1. FSSAI, EU, and CFDA MRLs (mg/kg) of rice grain

Element*	FSSAI MRL (mg/kg)	EU MRL (mg/kg)	CFDA (mg/kg)
Mercury	1.0	-	0.02
Arsenic	1.1	0.2	-
Lead	0.2	0.2	0.5
Cadmium	0.1	0.2	0.2
Copper	30.0	-	-
Tin	250.0	-	250
Chromium	-	-	1.0

*For all other elements in the scope of this study, no MRLs are available from the FSSAI, CFDA, or EC.

In this study, a workflow based on microwave digestion followed by quantification using the Thermo Scientific™ iCAP™ RQ inductively coupled plasma mass spectrometer (ICP-MS) was evaluated for the analysis of targeted trace metals in rice. Subsequently, the proposed method was validated in accordance with AOAC 2015.01 guidelines[8] to ensure compliance with FSSAI and EU MRL requirements (see Table 1).

Experimental
Chemicals and reagents
- Nitric acid (65-69%), TraceMetal™ Grade, Fisher Chemical™ (A509-P500)

- Hydrogen peroxide (30-32%), TraceMetal™ Grade, Fisher Chemical™ (H/1820/15)

- Hydrochloric acid (35-37%), TraceMetal™ Grade, Fisher Chemical™ (A508-P500)

- Deionized water (18.20 MΩ·cm), Thermo Scientific™ Barnstead™ MicroPure™ Water Purification System

- Single element standard solutions (for all elements under study, each at 1000 mg/L, Inorganic™ Ventures). The list of all analyte elements including target elements and respective internal standard elements is shown in Table 2.

Table 2. List of elements with their mass, measurement mode, and internal standard (IS) elements

Element (symbol)	Mass	Internal standard element (symbol)	Mass
Lithium (Li)	7	Scandium (Sc)	45
Beryllium (Be)	9	Scandium (Sc)	45
Boron (B)	11	Scandium (Sc)	45
Sodium (Na)	23	Scandium (Sc)	45
Aluminium (Al)	27	Scandium (Sc)	45
Vanadium (V)	51	Scandium (Sc)	45
Chromium (Cr)	53	Scandium (Sc)	45
Manganese (Mn)	55	Scandium (Sc)	45
Iron (Fe)	57	Scandium (Sc)	45
Cobalt (Co)	59	Scandium (Sc)	45
Nickel (Ni)	60	Scandium (Sc)	45
Copper (Cu)	65	Scandium (Sc)	45
Zinc (Zn)	66	Scandium (Sc)	45
Arsenic (As)	75	Germanium (Ge)	72
Selenium (Se)	77	Germanium (Ge)	72
Strontium (Sr)	88	Yttrium (Y)	89
Molybdenum (Mo)	98	Rhodium (Rh)	103
Cadmium (Cd)	111	Rhodium (Rh)	103
Tin (Sn)	118	Rhodium (Rh)	103
Barium (Ba)	137	Terbium (Tb)	159
Mercury (Hg)	202	Bismuth (Bi)	209
Lead (Pb)	208	Bismuth (Bi)	209

Standard preparation and calibration
For calibration of the instrument, four different groups of mixed working standards were prepared in appropriate concentration ranges to match the expected concentrations in the samples. Serial dilution was used to prepare a six-point calibration curve. The respective concentrations for each element are summarized in Table 3. Gold (200 µg/L) was added to all samples as well as all rinse solutions to stabilize and facilitate the washout of mercury and hence reduce memory effects. An internal standard containing Sc, Ge, Y, Rh, Tb, and Bi (Table 2) was added to all samples at a concentration of 20 µg/L.

Table 3. Calibration level standard preparation for elements

Levels	Linearity concentration	Final volume (mL)	Working standard concentration (µg/L)	Required volume (mL)
Set 1: Hg, Cd, Co (µg/L)				
STD1	0.05	25	10	0.125
STD2	0.1	25	10	0.25
STD3	0.25	25	10	0.625
STD4	0.5	25	10	1.25
STD5	5.0	25	1000	0.125
STD6	20.0	25	1000	0.5
Set 2: Pb, As, Sn, Sb (µg/L)				
STD1	0.1	25	10	0.25
STD2	0.5	25	100	0.125
STD3	1.0	25	100	0.25
STD4	5.0	25	1000	0.125
STD5	10.0	25	1000	0.25
STD6	20.0	25	1000	0.5
Set 3: B, Ba, Be, Cu, Cr, Li, Mn, Mo, Ni, Sr, Se, V (mg/L)				
STD1	0.002	25	1	0.05
STD2	0.004	25	1	0.1
STD3	0.01	25	1	0.25
STD4	0.05	25	10	0.125
STD5	0.1	25	10	0.25
STD6	0.2	25	10	0.5
Set 4: Na, Al, Fe, Zn (mg/L)				
STD1	0.05	25	10	0.125
STD2	0.1	25	10	0.25
STD3	0.25	25	10	0.625
STD4	0.5	25	100	0.125
STD5	1.0	25	100	0.25
STD6	2.0	25	100	0.5

Sample preparation

Rice (Basmati) samples labeled as organic were purchased from the local market and assessed for metal contamination. A heavy-duty grinder (Maharaja Whiteline, Delhi, India) was used to grind the rice to a fine powder to facilitate the digestion process. The average particle size after grinding was between 200 and 500 µm. An aliquot of the sample (accurately weighed to 0.25 ± 0.025 g) was placed into a pre-cleaned, dry 75 mL capacity microwave digestion vessel (Note: for the recovery sample, spiking was done before the addition of any solvent). A gold standard (200 µg/L) was added to the sample (to stabilize mercury) followed by addition of 1 mL deionized water, 4 mL nitric acid (HNO_3), 2 mL hydrogen peroxide (H_2O_2) and 0.4 mL hydrochloric acid (HCl). The samples were left to stand in a fume hood for the pre-digest period of 10 min before the microwave digestion process was started using the temperature program shown in Table 4. A CEM MARS 6™ microwave digestion system (CEM Corporation, Matthews, NC, USA) was used to perform the digestions.

Table 4. Temperature program for microwave digestion

	Ramp time (min)	Hold time (min)	Temperature	Power
Step 1	30	20	195 °C	1800 W

Note: Ramp time and microwave power settings may vary depending on a number of vessels.

On completion of the microwave digestion process, the vessels were kept on the working table to cool to ambient temperature. The microwave digestion vessels were opened slowly and carefully in a fume hood as pressurized acid vapors may be released. The digested solution was quantitatively transferred to the pre-cleaned 50 mL volumetric flask. An internal standard (20 µg/L) mix solution (100 µL of 10 mg/L) was added before adjusting to a final volume of 50 mL with deionized water and vortexing for 30 s. A procedural blank was prepared by following the above protocol without the addition of any sample.

ICP-MS analysis

A Thermo Scientific™ iCAP™ RQ ICP-MS was used for all measurements. The sample introduction system consisted of a Peltier-cooled (3 °C), baffled cyclonic spraychamber, MicroMist™ nebulizer and quartz torch with a 2.5 mm i.d. removable quartz injector. The instrument was operated in kinetic energy discrimination (KED) mode using pure He collision gas in the collision/reaction cell (CRC). All analytes are measured using KED for removal of interferences. KED uses helium as a collision gas to remove polyatomic interferences in the CRC. As polyatomic species consist of more than one atom, they have a larger cross section and experience more collisions with helium atoms in the cell compared to the analyte. Therefore, they suffer a higher loss of kinetic energy while transitioning through the CRC and may not enter the mass resolving quadrupole. This allows for selective removal of these interferences and hence a complete elimination of potentially false positive signals. Using KED for all analytes dramatically reduces runtime per sample, since time-consuming gas changes in the cell are avoided. At the same time, the design of the Thermo Scientific™ QCell CRC allows for high sensitivity even for elements with low mass, such as lithium or beryllium.

A Teledyne™ CETAC™ ASX 560 autosampler was connected to the ICP-MS to enable high-throughput analysis. A summary of all instrument-related parameters is given in Table 5.

Table 5. ICP-MS instrument conditions

Parameter	Value
Forward power (RF):	1550 W
Nebulizer gas:	1.06 L/min
Auxiliary gas:	0.8 L/min
Cool gas flow:	14.0 L/min
Sample uptake/wash time:	45 s
Dwell time:	0.05 s
Number of main runs:	3
Time per 10 sweeps:	20 s
Total acquisition time per sample:	150 s

Data acquisition and processing

The data acquisition and processing were performed using the Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ (ISDS) Software.

Method performance

To estimate the level of incurred analytes, rice samples (n=2) were prepared as per the protocol and analyzed. To determine the limit of quantification, rice samples were spiked at decreasing levels and checked for good recovery. To determine the precision and recovery rice samples were spiked (n=6) at three different concentration levels.

Results and discussion

Method performance and validation

Based on Table 3, the calibration standards prepared offered excellent R^2 values (> 0.99) for all target elements. The linearity for sodium and mercury is demonstrated in Figure 1.
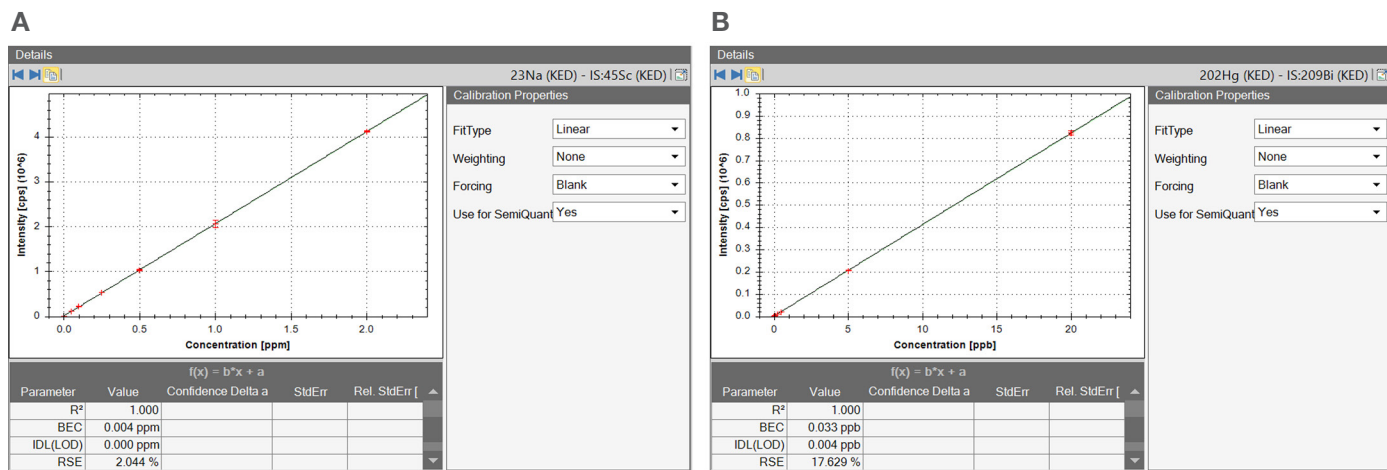
Figure 1. Demonstration of linearity observed for the calibration range in Qtegra software for (A) sodium and (B) mercury

For verification of the analytical method validation parameters such as accuracy and precision, rice samples were spiked with different concentrations of all elements under study. The optimized method provided excellent sensitivity at both low and high concentrations with accuracy within 85–115% against the criteria of AOAC Official Method 2015.01 guidelines[8], i.e, 60–115% at ≤100 µg/kg and within 80–115% at >100 µg/kg. The relative standard deviation between individual replicates was found to be <11% at 0.1–1 mg/kg, and <7.3% above

>1 mg/kg, respectively. The verified limit of quantitation (0.01 mg/kg; Level 1 in Table 6A), recoveries and precision values are presented in Tables 6A and 6B. A graphical illustration of recovery at the limit of quantification for all elements is given in Figure 2. Note that the limit of quantification refers to the amount of contaminant found in the undigested sample. The concentrations in the measured solutions are significantly lower due to the dilution of the sample in the digestion process.

Table 6A. Method validation data [% recovery and precision as %RSD calculated for level 1 (LOQ), level 2, and level 3]

Mass	Element	Level 1 mg/kg	% Rec.	% RSD	Level 2 mg/kg	% Rec.	% RSD	Level 3 mg/kg	% Rec.	% RSD
7	Li	0.4	91	10.9	0.8	98	6.2	2	101	2.4
9	Be	0.4	98	4.1	0.8	99	1.6	2	99	3.4
11	B	0.4	101	5.3	0.8	110	3.7	2	95	2.4
51	V	0.4	93	1.5	0.8	95	2.6	2	93	1.7
53	Cr	0.4	93	2	0.8	103	5.8	2	99	3.2
55	Mn	0.4	105	0.4	0.8	112	1.3	2	98	1.7
59	Co	0.01	96	3.5	0.02	96	1.8	0.05	105	1.1
60	Ni	0.4	110	6.1	0.8	98	2.2	2	94	1.6
65	Cu	0.4	98	5	0.8	113	1.8	2	90	1.2
75	As	0.1	105	2.7	0.2	103	1.7	2	104	1.6
77	Se	0.4	99	8.6	0.8	101	5.8	2	102	3.8
88	Sr	0.4	99	1.3	0.8	105	1.8	2	99	1.2
98	Mo	0.4	86	1.1	0.8	89	2.5	2	90	2.4
111	Cd	0.01	88	3.5	0.02	94	6.1	0.05	98	1.2
118	Sn	0.1	99	2.6	0.2	97	1.2	2	103	2.3
137	Ba	0.4	86	5.2	0.8	99	1.5	2	97	2
202	Hg	0.01	95	8.1	0.02	84	2.9	0.05	86	1.6
208	Pb	0.1	101	3.2	0.2	97	2.3	2	101	1.1

*Note: All the concentration values given in the table are with a dilution factor of 200 fold.

Table 6B. Method validation data (% recovery and precision as %RSD)

Mass	Element	Level 1 mg/kg	% Rec.	% RSD	Level 2 mg/kg	% Rec.	% RSD
23	Na	200	98.1	1.8	400	103.6	1.3
27	Al	200	101.5	2.1	400	102.6	1.1
57	Fe	200	96.5	1.3	400	99.6	1.5
66	Zn	200	97.6	1.8	400	100.4	1.2

*Note: All the concentration values given in the table are with a dilution factor of 200 fold.
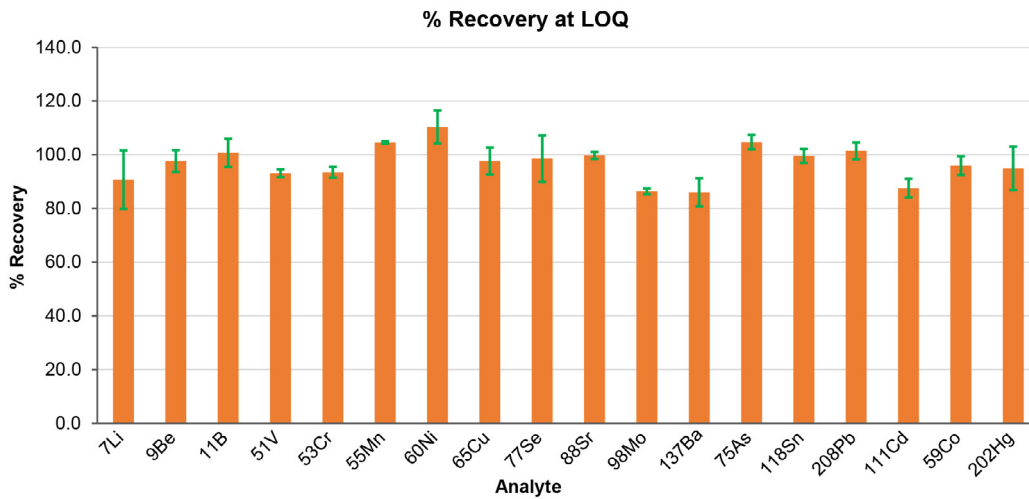
Figure 2. Percentage recovery of all analyte elements at the verified limit of quantification

Method robustness

To verify the robustness of the proposed method, the potential suppression and drift of the signal of the internal standard in the presence of rice matrix was monitored throughout an analytical sequence comprising 30 individual samples in total. The readback of the internal standard recovery demonstrates the robustness and stability of the iCAP RQ ICP-MS for the analysis of food digests. The internal standard showed a recovery between 95% and 120%, which is well within the acceptance criteria (60–125%) set in AOAC Method 2015.01. The recoveries observed for the different internal standards Sc, Y, Tb, Ge, In, Ir, Rh, and Bi are shown in Figure 3.
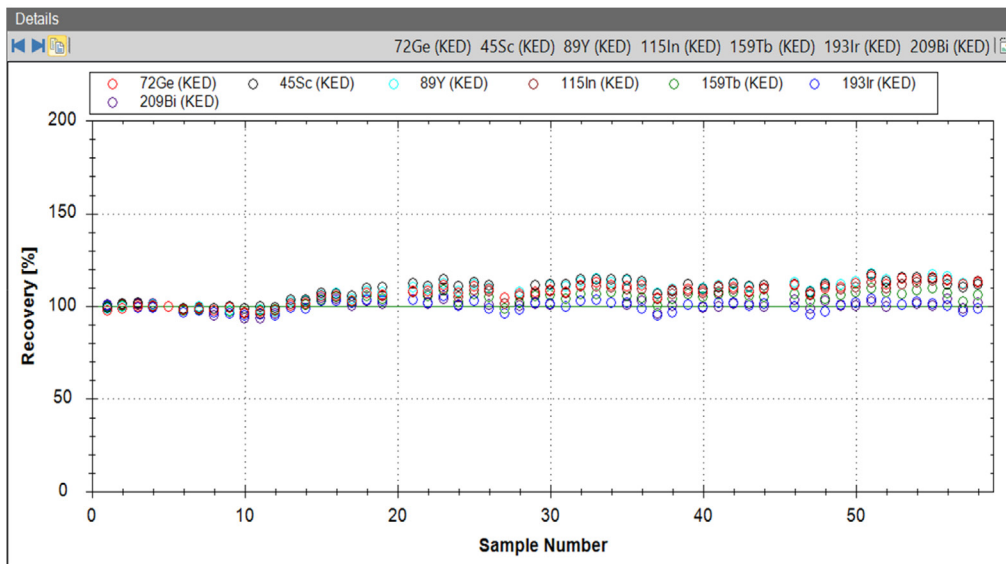

Figure 3. Internal standard recovery in rice matrix for an analytical run with a total of 30 samples

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Conclusion

The workflow described in this application note shows the accurate and precise quantification of multiple elements at trace levels in rice. Samples were acid digested based on a microwave-assisted method followed by quantitative analysis using the iCAP RQ ICP-MS. This methodology allows the lower limits of quantification in rice, combined with excellent recovery (80–115%) and precision (<11% RSD). The proposed method can therefore be used as a turnkey solution for the analysis of metals at trace concentrations in rice. The method meets the requirement of AOAC Official Method 2015.01 standard method performance requirements as well as the FSSAI, CFDA, and EC MRL requirements. Accuracy is achieved through consistent removal of spectral interferences due to the superior design of the CRC system of the iCAP RQ ICP-MS, combining KED with an automatically applied low mass cut off. Excellent robustness is demonstrated by achieving consistent recovery of internal standard response between 95% and 120% with <10% RSD. This optimized method offers an excellent solution for commercial food testing laboratories needing to improve sample throughput without sacrificing data quality.

References

1. Grain Central, March 26, 2018. Global rice consumption continues to grow. https://www.graincentral.com/cropping/global-rice-consumption-continues-to-grow/ (accessed May 27, 2019).

2. FSSAI Manual for food safety, 17th Edition, 2017 (THE FOOD SAFETY AND STANDARDS ACT, 2006).

3. China Food and Drug Administration - National food safety standards https://gain.fas.usda.gov/Recent%20GAIN%20Publications/China%20Releases%20the%20Standard%20for%20Levels%20of%20Contaminants%20in%20Foods%20_Beijing_China%20-%20Peoples%20Republic%20of_5-9-2018.pdf (accessed May 27, 2019).

4. COMMISSION REGULATION (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. 02006R1881 — EN — 28.07.2017 — 021.001 — 1. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02006R1881-20170728&qid=1512388911628&from=EN (accessed May 27, 2019).

5. Horiguchi, H. Current status of cadmium exposure among Japanese, especially regarding the safety standard for cadmium concentration in rice and adverse effects on proximal renal tubular function observed in farmers exposed to cadmium through consumption of self-grown rice, *Nihon Eiseigaku Zasshi* **2012,** *67*(4), 447–454.

6. Aoshima, K. Itai-itai disease: cadmium-induced renal tubular osteomalacia, *Nihon Eiseigaku Zasshi* **2012,** *67*(4), 455–463.

7. Meharg A.A. et al., A review of recent developments in the speciation and location of arsenic and selenium in rice grain, *Analytical and Bioanalytical Chemistry* **2012,** *402,* 3275–3286.

8. Briscoe, M. Determination of Heavy Metals in Food by Inductively Coupled Plasma–Mass Spectrometry: First Action 2015.01, *J. AOAC Int.* **2015,** *98*(4), 1113–1120.

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