



## Trace elemental quantification, including heavy metals in wheat, using ICP-MS

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### Goal

- To develop and validate an analytical solution for routine multielemental analysis in wheat using inductively coupled plasma mass spectrometry (ICP-MS) in accordance with AOAC 2015.01 guidelines
- To assess method performance for compliance with the Food Safety Standards Authority of India (FSSAI), China Food and Drug Administration (CFDA), and European Commission (EC) MRL requirements in wheat

### Introduction

Wheat is an important cereal that is rich in carbohydrates and protein and is consumed worldwide as a staple food, with global wheat production for 2018-2019 at 732 million tons.<sup>1</sup> Wheat flour is processed from wheat grain and is the primary raw material for many bakery products like biscuits and bread. The introduction of toxic elements in wheat may occur through heavy metal deposition in soil and water by industrial and mining processes or agricultural and food processing practices. FSSAI, CFDA, and EC have established maximum residual limits (MRLs) in cereal grains, as listed in Table 1.<sup>2-4</sup> Some trace elements such as zinc or iron are important nutrients and are essential to a healthy and balanced diet; however, other elements like lead, cadmium, mercury, and arsenic pose a serious risk when consumed

even at trace levels. As an example, Itai-itai disease was found to be caused by cadmium poisoning due to contaminated water in Toyama Prefecture.<sup>5,6</sup>

**Table 1. FSSAI, EU, and CFDA MRLs (mg·kg<sup>-1</sup>) of wheat grain**

Element	FSSAI MRL	EU MRL	CFDA MRL
Mercury	1.0	-	0.02
Arsenic	1.1	-	0.5
Lead	0.2	0.2	0.5
Cadmium	0.2	0.2	0.1
Copper	30.0	-	-
Tin	250.0	-	250
Chromium	-	-	1.0

\* For all other elements in the scope of this study, no MRLs are available in the FSSAI, CFDA, and EU regulations.

For food safety regulatory authorities and laboratories, quantification of toxic elements at trace levels is very important to avoid adverse health effects in the population. Sample preparation by microwave digestion is widely accepted and implemented in commercial

laboratories for multielemental analysis. This study demonstrated a workflow for the quantitative assessment of 20 different trace elements in wheat based on microwave digestion followed by quantification using ICP-MS. Subsequently, the method performance was validated in compliance with AOAC 2015.01 guidelines.<sup>7</sup>

## 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
- Individual element standard stock solutions (1000 mg·L<sup>-1</sup>), Inorganic™ Ventures (Christiansburg, VA, USA) are shown in Table 2.

**Table 2. List of elements, masses, and internal standards**

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
Aluminum (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
Molybdenum (Mo)	98	Rhodium (Rh)	103
Cadmium (Cd)	111	Rhodium (Rh)	103
Tin (Sn)	118	Rhodium (Rh)	103
Antimony (Sb)	121	Rhodium (Rh)	103
Mercury (Hg)	202	Bismuth (Bi)	209
Lead (Pb)	208	Bismuth (Bi)	209

## Standard preparation and calibration

For linearity, four different groups of mixed working standards were prepared from the above standards 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 ( $200 \mu\text{g}\cdot\text{L}^{-1}$ ) was added to all samples and rinse solutions to stabilize and facilitate the wash out of mercury and hence to reduce memory effects. An internal standard containing Sc, Ge, Y, Rh, Tb, Bi (Table 2) was added to all samples at a concentration of  $20 \mu\text{g}\cdot\text{L}^{-1}$ .

## Sample preparation

Wheat was purchased from the local market and assessed for metal contamination. A heavy-duty grinder

(Maharaja White line, Delhi, India) was used to grind the wheat to a fine powder to facilitate the digestion process. The average particle size after grinding was between 200 and 500  $\mu\text{m}$ . An aliquot of the sample (accurately weighed to  $0.25 \pm 0.025 \text{ g}$ ) 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 ( $200 \mu\text{g}\cdot\text{L}^{-1}$ ) was added to the sample (to stabilize mercury) followed by addition of 1 mL deionized water, 2 mL nitric acid, 1 mL hydrogen peroxide, and 0.2 mL hydrochloric acid. The samples were left to stand in a fume hood to pre-digest for 10 minutes before starting the microwave digestion process using the temperature program provided in Table 4. A CEM MARS™ 6 microwave digestion system (CEM Corporation, Matthews, NC, USA) was used to perform the digestions.

**Table 3. Calibration level standard preparation for elements**

Levels	Linearity conc.	Final volume (mL)	Working standard conc. ( $\mu\text{g}\cdot\text{L}^{-1}$ )	Required volume (mL)
<b>Set 1: Hg, Cd, Co (<math>\mu\text{g}\cdot\text{L}^{-1}</math>)</b>				
STD1	0.05	25	10	0.125
STD2	0.10	25	10	0.25
STD3	0.25	25	10	0.625
STD4	0.50	25	10	1.25
STD5	5.0	25	1000	0.125
STD6	20.0	25	1000	0.5
<b>Set 2: Pb, As, Sn, Sb (<math>\mu\text{g}\cdot\text{L}^{-1}</math>)</b>				
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
<b>Set 3: B, Ba, Be, Cu, Cr, Li, Mn, Mo, Ni, V (<math>\text{mg}\cdot\text{L}^{-1}</math>)</b>				
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
<b>Set 4: Na, Al, Fe, Zn (<math>\text{mg}\cdot\text{L}^{-1}</math>)</b>				
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

**Table 4. Temperature program for microwave digestion**

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

Note: Ramp time and microwave power settings may vary depending on the 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 vapor could be released. The digested solution was quantitatively transferred to the pre-cleaned 50 mL volumetric flask. An internal standard (20 µg·L<sup>-1</sup>) mix solution (100 µL of 10 mg·L<sup>-1</sup>) was added before adjusting to a final volume of 50 mL with deionized water and vortexing for 30 seconds. 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, micro-mist 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 helium (He) collision gas in the collision/reaction cell (CRC). A summary of all instrument-related parameters is given in Table 5. A Teledyne CETAC ASX 560 autosampler (Teledyne Cetac Technologies, Omaha, NE) was connected to the ICP-MS to enable high-throughput analysis.

**Table 5. ICP-MS instrument conditions**

Parameter	Value
Forward power (RF)	1550 W
Nebulizer gas	1.06 L·min <sup>-1</sup>
Auxiliary gas	0.8 L·min <sup>-1</sup>
Cool gas flow	14.0 L·min <sup>-1</sup>
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

The majority of interferences observed in ICP-MS are caused by so-called polyatomic species. These interferences are formed from all elements present in the sample, including argon (derived from the plasma), water, or acids (e.g. chlorine contained in hydrochloric acid used in the digestion process). This can lead to significant false positive results on almost all elements, but especially on those elements found in the mass range up to *m/z* 100. Key interferences are for example <sup>40</sup>Ar<sup>35</sup>Cl<sup>+</sup> on <sup>75</sup>As<sup>+</sup>, <sup>35</sup>Cl<sup>16</sup>O<sup>+</sup> on <sup>51</sup>V<sup>+</sup>, or <sup>40</sup>Ar<sup>16</sup>O<sup>1</sup>H<sup>+</sup> on <sup>57</sup>Fe<sup>+</sup>. KED can effectively remove these interferences using helium as a collision gas. Although lighter elements such as lithium or beryllium may suffer a reduction in sensitivity in KED mode (due to ion deflection caused by collisions with helium atoms), the design of the QCell CRC system allows the analysis of all elements in a method in one mode while consistently achieving required detection limits throughout the mass range. This eliminates the need for gas exchanges during the measurement and dramatically decreases the runtime per sample.

### Data acquisition and processing

The data acquisition and processing were controlled using the Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ (ISDS) Software. Qtegra ISDS Software is a platform for controlling different instruments for trace elemental analysis as well as state of the art accessories, from autosamplers through dedicated plug-ins. This approach facilitates the operation of multiple instruments without extensive user cross training. Qtegra ISDS Software also contains a comprehensive feature set for automated quality control tests in the analytical sequence. All analysis-related information, such as method parameters, raw data, and analytical results, as well as operator-related information is stored in a single file, the LabBook.

## Results and discussion

### Linearity

The six-point calibration curve was plotted for all the analyte elements with single-run KED mode analysis. The calibration curve for lithium and cadmium in KED mode are shown in Figure 1 with the set calibration points, which offered excellent correlation with R<sup>2</sup> values >0.99 for all elements.

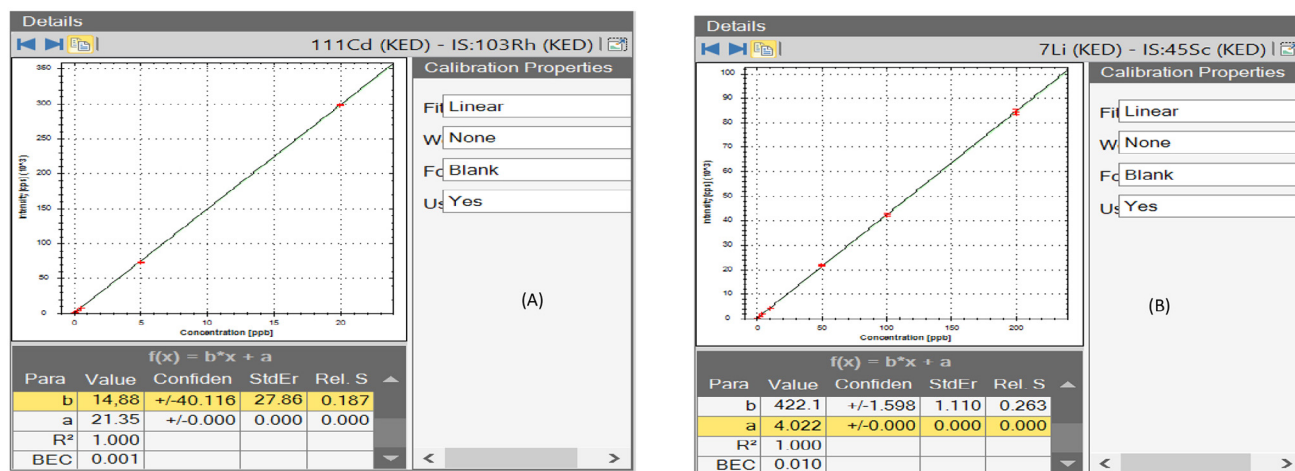


Figure 1. Linearity observed for (A) cadmium and (B) lithium in the target calibration range

Table 6. Method validation data [Accuracy as % recovery and precision as % RSD calculated for three different spike levels: Level 1 (L1) (LOQ), Level 2 (L2), and Level 3 (L3)]

Element symbol	L1 mg·kg <sup>-1</sup>	% Recovery	% RSD	L2 mg·kg <sup>-1</sup>	% Recovery	% RSD	L3 mg·kg <sup>-1</sup>	% Recovery	% RSD
Li	0.4	96.1	3.1	2	93.4	1.7	20	89.9	1.4
Be	0.4	97.8	4.2	2	90.7	1.8	20	89.1	1.1
B	0.4	102.3	3.5	2	89.9	2.5	20	89.7	1.7
Na	10	99.3	0.7	50	93.3	1.6	200	90.5	0.7
Al	10	106.9	0.7	50	103.0	2.3	200	95.2	1.0
V	0.4	94.9	1.1	2	93.5	1.2	20	95.8	0.7
Cr	0.4	100.5	1.0	2	96.4	1.6	20	91.6	0.7
Fe	20	90.4	2.1	50	93.7	0.8	200	86.3	1.0
Co	0.01	99.9	1.1	0.05	97.0	2.0	1	91.7	0.8
Ni	0.4	93.0	1.4	2	90.9	0.9	20	87.7	0.9
Cu	0.4	90.5	3.2	2	82.3	1.8	20	90.3	0.5
Zn	10	93.4	0.4	50	93.8	1.0	200	89.4	0.9
As	0.1	107.9	3.9	0.2	105.7	1.4	2	104.6	1.0
Mo	0.4	93.1	0.6	2	96.1	1.1	20	101.9	0.7
Cd	0.01	104.9	1.8	0.05	101.1	2.2	1	98.8	0.7
Sn	0.1	104.9	0.9	0.2	104.1	1.3	2	103.2	1.0
Sb	0.1	103.5	1.4	0.2	103.3	1.7	2	100.6	1.1
Hg	0.01	88.1	1.6	0.05	105.2	2.3	1	112.7	0.3
Pb	0.1	98.3	2.0	0.2	99.3	5.1	2	102.0	1.0

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

### Method performance

The limit of quantitation (LOQ) was determined based on ten times the standard deviation of method blanks. The practical LOQs were determined by spiking the samples with different concentration levels in decreasing order to check good recoveries. Repeatability was verified by spiking six replicates of samples. The method for wheat provided excellent sensitivity at low concentration with very good accuracy and precision. The verified LOQs, recoveries, and precision values are given in Table 6.

A graphical illustration of spike recovery for all elements at three different levels is given in Figure 2. The observed results of spiked samples were within 82% to 112% in terms of recovery and <10% in terms of % RSD. The results are well within acceptance criteria of AOAC 2015.01 guidelines.

Two different real-life samples were purchased in the local market and analyzed using this method, and the measured values are given in Table 7.

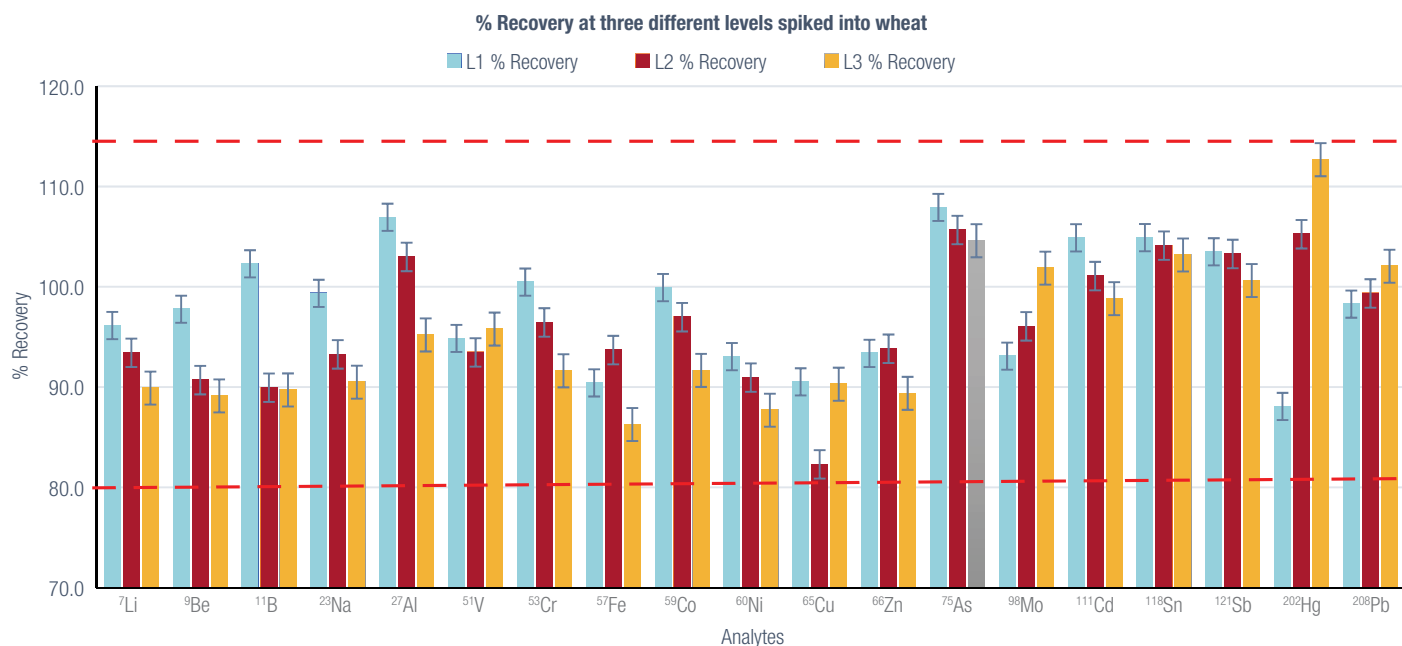
### Method robustness and signal drift

The internal standards (IS) are, wherever possible, similar to the analyte elements in terms of mass and ionization potential such that they have similar behavior in the plasma and mass analyzer. The effect of matrix on analyte elements, robustness of the instrument response to the suppression, and signal drifts can be identified and quantified from the response of IS elements that are added in the same concentration in all standards and samples. The response of internal standard elements (0.02 mg·L<sup>-1</sup>) during the wheat method sequence run containing wheat sample (n=30) by using iCAP RQ ICP-MS showed excellent robustness.

**Table 7. Incurred elemental concentration of two wheat grains**

Element symbol	Wheat A (mg·kg <sup>-1</sup> )	Wheat B (mg·kg <sup>-1</sup> )
Li	BLQ	BLQ
Be	BLQ	BLQ
B	0.8	1.2
Na	17.6	22.5
Al	9.3	13.2
V	BLQ	BLQ
Cr	BLQ	BLQ
Mn	27.9	32.6
Fe	36.3	38.9
Co	0.015	0.019
Ni	BLQ	BLQ
Cu	3.9	4.1
Zn	23.8	21.9
As	BLQ	BLQ
Mo	0.5	BLQ
Cd	0.022	0.016
Sn	BLQ	BLQ
Sb	BLQ	BLQ
Hg	BLQ	0.02
Pb	BLQ	BLQ

Note: BLQ = Below limit of quantification (Table 6 (L1))



**Figure 2. The % recovery, precision in terms of % RSD at three different level spikes**

The internal standard elements showed excellent recovery of 85–115% against the criteria (60–125%) set in AOAC 2015.01 with the precision of <10% RSD. This indicates the instrument ruggedness and stability in the presence of the matrix. The Sc, Tb, Ge, In, Ir, Rh, and Bi internal standard recoveries are shown in Figure 3.

In addition to internal standards, a continuous calibration verification (CCV) was aspirated as an intermediate

standard check using a third linearity point standard, after every six replicates of spike level run. The obtained recoveries were well within the acceptance criteria (85–115%) of AOAC 2015.01. This ensured the high-quality data in each set of different level accuracy and repeatability check in the whole analysis of the wheat method. The observed percentage recoveries of all elements are shown in Figure 4.

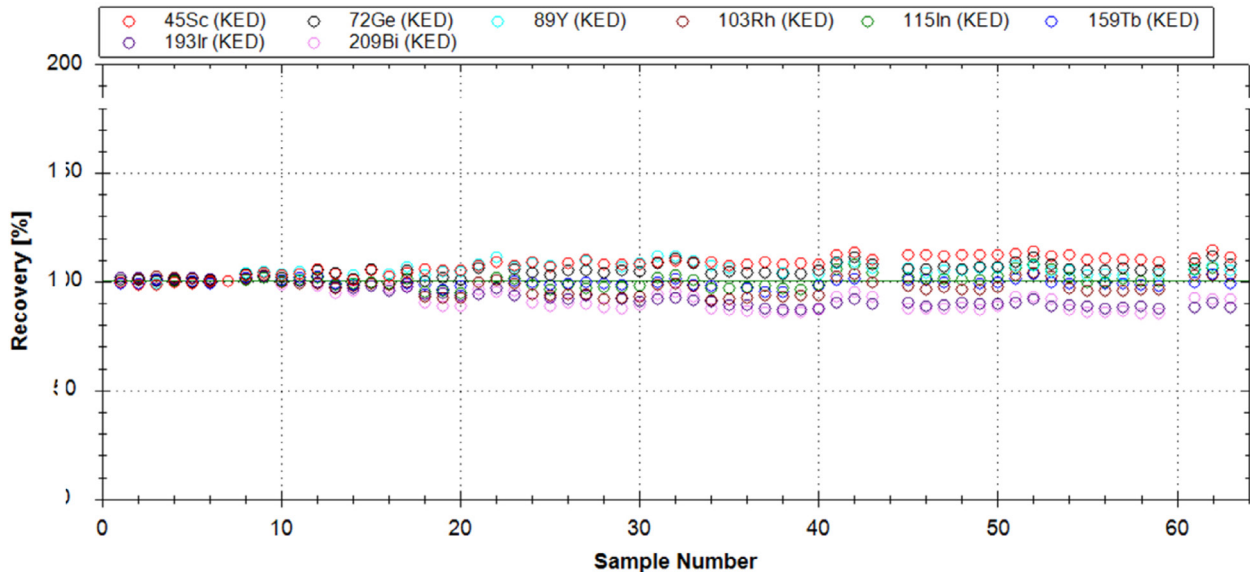


Figure 3. Internal standard recovery in the wheat matrix for an analytical run with 30 wheat samples

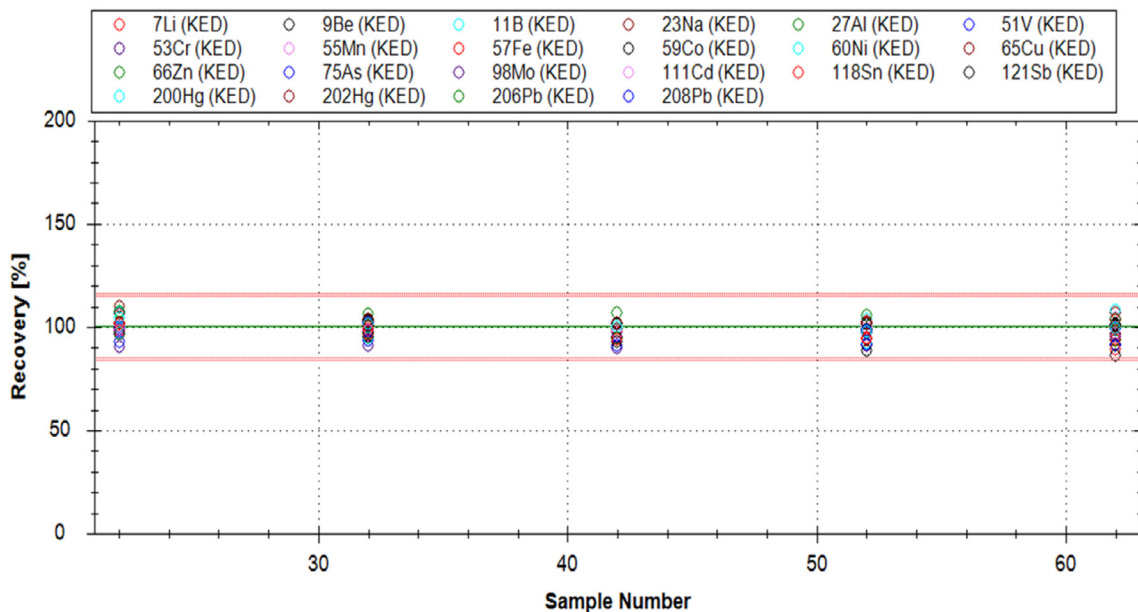


Figure 4. Analyte elements recovery in bracketing standard (n=5)

## Conclusion

This application note provides a complete solution for multielemental quantification at trace levels in wheat based on microwave digestion and quantification using the iCAP RQ ICP-MS. This method meets the the AOAC 2015.01 guidelines and the FSSAI, CFDA, and EU MRL requirements by achieving excellent accuracy (85–112%) and precision (<10%) (Table 6). The verified limit of quantification and performance of method accuracy and precision at three different levels offers a ready-to-use method for the analysis of wheat products. The results of instrumental drift analysis and internal standard recoveries in the presence of wheat matrix show the robustness of iCAP RQ ICP-MS is excellent. This method offers a solution to commercial food testing laboratories to improve both the quality of the data and sample throughput.

## References

1. Grain Central, June 12, 2019. Global wheat production and consumption continues to grow. <https://www.graincentral.com/markets/south-east-asia-becomes-biggest-world-wheat-market-usda/> (accessed June 15, 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](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. 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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