



Multielemental quantification of trace metals in milk and milk products using ICP-MS

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Keywords

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Goal

- To demonstrate the efficiency of Thermo Scientific™ iCAP™ RQ ICP-MS to provide a single method for trace elemental analysis in milk and milk products, such as butter and cheese
- To assess method performance for compliance with AOAC 2015.01 guidelines, the Food Safety and Standards Authority of India (FSSAI), China Food and Drug Administration (CFDA), and European Commission (EC) MRLs

Introduction

Milk is considered an essential and almost complete dietary food for children and adults. It provides necessary macronutrients such as carbohydrates, proteins, and fats as well as micronutrients such as vitamins (e.g., riboflavin, B5, B12) and minerals. India is the largest milk producer in the world, with a 20% contribution to total world milk production.¹ Secondary milk products like cheese and butter are used in a variety of processed foods.

From a food safety perspective, milk and its processed products are vulnerable to potentially toxic elemental contamination by the processing activities and adulteration. The FSSAI,² CFDA,³ and EC⁴ have set maximum residual limits (MRLs) in milk and milk products for heavy metals (Table 1). The composition of milk, cheese, and butter differ in terms of fat, minerals, and water content. Because of these differences, individual methods are used to monitor heavy metals contamination in all three products.

Khan et al. have reported the heating block method for sample preparation in milk and milk products, in which the sample preparation process involves a 10–12 hour digestion period along with an excess amount of acid consumption.⁵ Such long digestion with high acid consumption will reduce the throughput of commercial laboratories. To increase the throughput, reduction in the sample preparation time and the acid amount were considered. Microwave-assisted digestion of food samples was used for ICP-MS analysis. This digestion process reported in AOAC 2015.01,⁶ reduces the amount of acid needed and the preparation time required.

Low-level quantification of trace metals requires high sensitivity with efficient interference removal. Kinetic energy discrimination (KED) uses high purity helium gas to remove polyatomic interferences in the collision reaction

cell (CRC). Yamada et al. have reported the efficiency of KED for interference removal.⁷ The design of the iCAP RQ ICP-MS QCell Collision/Reaction Cell (CRC) efficiently removes the interferences and allows high sensitivity even for low mass elements such as lithium and beryllium. The purpose of this application note is to provide a single method, using the iCAP RQ ICP-MS, for the determination of multiple elements at trace levels in all milk, cheese, and butter using microwave digestion for sample preparation. The method performance was checked by AOAC 2015.01.

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⁻¹, Inorganic™ Ventures (Christiansburg, Virginia, USA)

Table 1. Target analytes with the FSSAI, CFDA, and EU MRL values in mg·kg⁻¹

Element*	FSSAI		EU		CFDA	
	Milk	Secondary milk products	Milk	Milk fat	Milk	Milk fat
Mercury	1.00	1.00	-	-	0.01	-
Arsenic	0.10	1.10	-	-	0.10	-
Lead	0.02	0.02	0.02	0.100	0.05	0.10
Cadmium	1.50	1.50	-	-	-	-
Copper	30.00	30.00	-	-	-	-
Tin	250.00	250.00	-	-	250.00	250.00
Chromium	-	-	-	-	0.30	-

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

Standard preparation and calibration

All the individual standard solutions ($1000 \mu\text{g}\cdot\text{mL}^{-1}$) were procured from Inorganic Ventures (Christiansburg, Virginia USA). All analytes under investigation and the internal standards (IS) are shown in Table 2. For linearity, the individual calibration standards were prepared through serial dilution by diluting the four different groups of mixed working standards in appropriate concentration ranges. The respective concentrations prepared for each element are summarized in Table 3. Gold ($200 \mu\text{g}\cdot\text{L}^{-1}$) was added to all standards and rinse solutions to facilitate the wash out of mercury and to reduce. An internal standard mixture containing Sc, Ge, Y, Rh, Tb, Ir, and Bi (Table 2) was added to all samples at a concentration of $20 \mu\text{g}\cdot\text{L}^{-1}$.

Sample preparation

Milk, paneer (cheese), and butter samples were purchased from the local market. Paneer is a fresh cheese, commonly used in India, made by curdling milk using fruit- or vegetable-derived acids such as lemon juice. Milk was vigorously shaken before an aliquot was taken for analysis. The cheese was cut into small pieces

and homogenized with a mixer and grinder (Maharaja Whiteline, Delhi, India) to get a uniform paste. Butter samples were heated at $50 \text{ }^\circ\text{C}$ on a Thermo Scientific™ Heratherm™ hot air oven and shaken vigorously before taking an aliquot. The samples were weighed to approximately $0.5 \pm 0.05 \text{ g}$ in a pre-cleaned, dry 75 mL capacity microwave digestion vessel. For each recovery experiment, a sample was spiked with analytes before the addition of an extraction solvent. Gold was added to the sample of at $200 \mu\text{g}\cdot\text{L}^{-1}$ as a final concentration in the sample solution to stabilize mercury.

Additionally, 2 mL deionized water, 2 mL nitric acid (HNO_3), 1 mL hydrogen peroxide (H_2O_2), and 0.2 mL hydrochloric acid (HCl) were added to the sample, which was kept for 10 min to pre-digest in a fume hood. The microwave digestion vessels were closed, and the microwave digestion process was started with a set temperature program given in Table 4. A CEM MARS™ 6 one-touch microwave digestion system (CEM Corporation, Matthews, NC, USA) was used to perform the microwave digestion.

Table 2. List of elements with their mass and internal standard elements

Element (symbol)	Mass	Internal standard element	Mass
Lithium (Li)	7	Scandium (Sc)	45
Beryllium (Be)	9	Scandium (Sc)	45
Boron (B)	11	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
Molybdenum (Mo)	98	Rhodium (Rh)	103
Cadmium (Cd)	111	Rhodium (Rh)	103
Tin (Sn)	118	Rhodium (Rh)	103
Antimony (Sb)	121	Rhodium (Rh)	103
Barium (Ba)	137	Terbium (Tb)	159
Mercury (Hg)	202	Bismuth (Bi)	209
Lead (Pb)	208	Bismuth (Bi)	209

Table 3. Calibration level standard preparation

Levels	Linearity concentration	Final volume (mL)	Intermediate standard concentration ($\mu\text{g}\cdot\text{L}^{-1}$)	Required volume (mL)
Set 1: Hg, Cd, Co ($\mu\text{g}\cdot\text{L}^{-1}$)				
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	5.00	25	1000	0.125
STD6	20.00	25	1000	0.50
Set 2: Pb, As, Sn, Sb ($\mu\text{g}\cdot\text{L}^{-1}$)				
STD1	0.10	25	10	0.25
STD2	0.20	25	10	0.50
STD3	0.40	25	100	0.10
STD4	2.00	25	100	0.50
STD5	10.00	25	1000	0.25
STD6	20.00	25	1000	0.50
Set 3: B, Ba, Be, Cu, Cr, Li, Mn, Mo, Ni, V ($\text{mg}\cdot\text{L}^{-1}$)				
STD1	0.002	25	1	0.05
STD2	0.004	25	1	0.10
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	0.50
Set 4: Al, Fe, Zn ($\text{mg}\cdot\text{L}^{-1}$)				
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	100	0.125
STD5	1.00	25	100	0.25
STD6	2.00	25	100	0.50

Table 4. Temperature program for microwave digestion

	Ramp time	Hold time	Temperature	Power
Step 1	40 min	30 min	195 °C	1800 W

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

The microwave digestion vessels were cooled by keeping the rotor at room temperature for 15 min after completion of the digestion process. The vessels were opened slowly and carefully in a fume cupboard, as pressurized acid fumes could evaporate. The digested sample solution was quantitatively transferred to the pre-cleaned 25 mL volumetric flask with multiple rinsing with deionized water. The internal standards were added from a stock solution containing 10 mg·L⁻¹ of each element (final concentration is 20 µg·L⁻¹), and the total volume was adjusted to 25 mL with deionized water. The prepared sample solutions were vortexed well for thorough mixing. A procedural blank was prepared by following the above protocol without sample matrix.

ICP-MS analysis

The multielemental analysis in dairy products was performed using an iCAP RQ ICP-MS. To enable high-throughput analysis, a Teledyne CETAC ASX 560 autosampler (Teledyne CETAC Technologies, Omaha, NE, USA) was used. A summary of all instrument conditions is given in Table 5. All analytes were measured using KED to assure complete removal of polyatomic interferences.

Table 5. ICP-MS instrument conditions

Parameter	Value
Forward power (RF)	1550 W
Nebulizer gas	1.06 L·min ⁻¹
Auxiliary gas	0.8 L·min ⁻¹
Cool gas flow (Argon)	14.0 L·min ⁻¹
CCT gas flow (He gas)	5.1 mL·min ⁻¹
CCT bias	-21 V
Sample uptake/wash time	45 s
Dwell time	0.05 s
Number of readings per sample	3 Main runs with 10 sweeps each
Total acquisition time (3 repetitions including rinse)	150 s

Data acquisition and processing

For data acquisition and processing, the Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ (ISDS) Software was used. Method parameters were set up in a LabBook, the file format used in the Qtegra ISDS Software. The LabBook contains all relevant information associated with the measurement, including acquisition

parameters, sample information, and applicable quality control tests. After data acquisition has finished, the LabBook is populated with calibration data and results for unknown samples and quality control tests. It also contains detailed information about instrumental parameters throughout the acquisition period, assuring full traceability of the data, as well as the operators creating, executing, or modifying the analysis.

Results and discussion

Linearity

The linearity is demonstrated using a six-point calibration curve. The calibration curve for low concentration level lead (Pb) and high concentration level zinc (Zn) in KED mode is shown in Figure 1 with the set calibration points. All the target elements showed excellent correlation coefficient (>0.999).

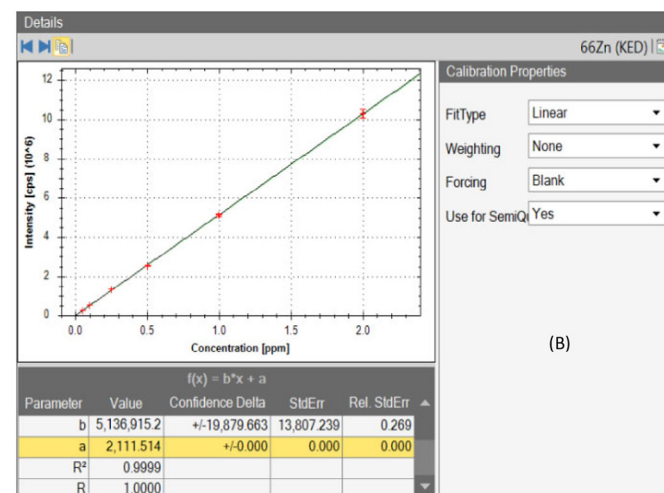
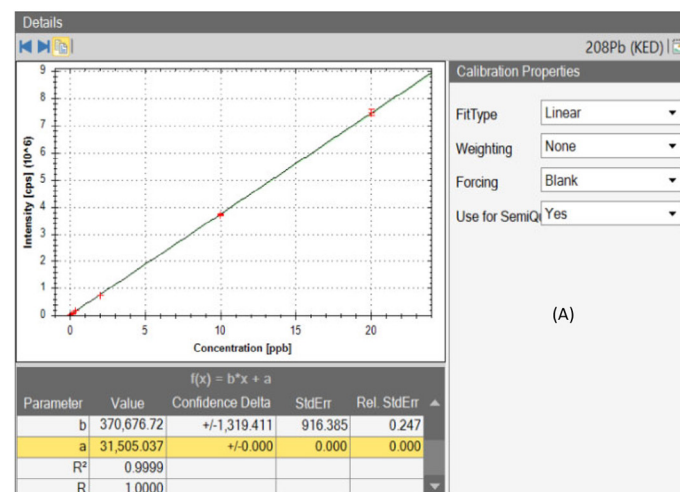


Figure 1. Calibration curves of target analytes lead (A), and zinc (B) obtained from the Qtegra ISDS Software

Method performance

The microwave digested samples were analyzed in KED mode for all elements. The measured value of the analyzed samples is given in Table 6. It can be observed that for most elements, the concentrations in the samples were below the limit of quantification. However, the concentration of lead was higher in the cheese sample than allowed by the regulatory limit ($0.03 \text{ mg}\cdot\text{kg}^{-1}$ as opposed to a limit of $0.02 \text{ mg}\cdot\text{kg}^{-1}$).

Table 6. Incurred values of all analyte elements in milk, butter and cheese samples

Isotope	Milk ($\text{mg}\cdot\text{kg}^{-1}$)	Butter ($\text{mg}\cdot\text{kg}^{-1}$)	Cheese ($\text{mg}\cdot\text{kg}^{-1}$)
^7Li	BLQ	BLQ	BLQ
^9Be	BLQ	BLQ	BLQ
^{11}B	0.32	0.11	0.12
^{27}Al	BLQ	BLQ	BLQ
^{51}V	BLQ	BLQ	BLQ
^{53}Cr	BLQ	BLQ	BLQ
^{55}Mn	BLQ	BLQ	0.18
^{57}Fe	BLQ	BLQ	5.1
^{59}Co	BLQ	BLQ	BLQ
^{60}Ni	BLQ	BLQ	BLQ
^{65}Cu	BLQ	BLQ	BLQ
^{66}Zn	3.3	BLQ	18.7
^{75}As	BLQ	BLQ	BLQ
^{98}Mo	BLQ	BLQ	BLQ
^{111}Cd	BLQ	BLQ	BLQ
^{118}Sn	BLQ	BLQ	BLQ
^{121}Sb	BLQ	BLQ	BLQ
^{137}Ba	BLQ	BLQ	1.2
^{202}Hg	BLQ	BLQ	BLQ
^{208}Pb	BLQ	BLQ	0.03

Note: BLQ = Below limit of quantification (Table 7 (L1) and Table 8)

The limit of quantification (LOQ) of all target elements was determined as 10 times the standard deviation of 20 method blanks. To obtain the practical LOQ, milk and milk product samples were spiked in decreasing order. The verified LOQs, recovery (%), and precision (%RSD) are given in Table 7. As there was no suitable certified reference material available for these target matrices, a spike recovery experiment was conducted to demonstrate the accuracy (recovery) of the method. A graphical representation of percentage recoveries at three different levels in milk for all elements is shown in Figure 2. The observed recoveries were within 81% to 106% with a relative standard deviation (RSD) of <10%. The results are in alignment within the acceptance criteria of AOAC 2015.01 guidelines.

To increase the scope of the method, the optimized method was applied to milk products. Cheese and butter samples were spiked at LOQ (Table 7 (L1)) with six replicates and verified for recovery and precision. It was observed that the copper recovery in cheese was 79% when compared to 108% in butter at the spike level of $0.2 \text{ mg}\cdot\text{kg}^{-1}$. This is due to the incurred value of $0.13 \text{ mg}\cdot\text{kg}^{-1}$ of copper in cheese, which is closer to the spiked value of $0.2 \text{ mg}\cdot\text{kg}^{-1}$ against the incurred value of $0.01 \text{ mg}\cdot\text{kg}^{-1}$ in butter. The results are presented in Table 8 and Figure 3.

The influence of the matrix on analyte element recovery was evaluated without and with internal standard elements. The addition of IS element compensates for matrix suppression and improves accuracy by recovery correction. Without internal standard correction, recoveries of barium and lead in cheese were suppressed to 42% and 60%, respectively. After internal standard correction, the recoveries were 95% and 112% for barium and lead, respectively. In the same way, the addition of rhodium as an internal standard for cadmium in the butter sample corrected the enhanced recovery from 123% to 112%. Bismuth corrected the enhanced recovery of lead in the cheese sample from 124% to 110%. The average % recovery at LOQ level of milk, butter, and cheese without and with the internal standard element is shown in Figure 4.

Table 7. 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)]

Isotope	L1 mg·kg ⁻¹	% Recovery	% RSD	L2 mg·kg ⁻¹	% Recovery	% RSD	L3 mg·kg ⁻¹	% Recovery	% RSD
⁷ Li	0.10	92	2.3	0.50	93	2.4	5.00	91	1.5
⁹ Be	0.10	93	4.7	0.50	94	1.7	5.00	92	1.9
¹¹ B	0.20	87	2.4	0.50	90	2.4	5.00	93	2.1
²⁷ Al	2.50	88	1.2	12.50	99	1.5	50.00	97	1.9
⁵¹ V	0.10	94	2.0	0.50	94	1.7	5.00	96	1.2
⁵³ Cr	0.20	99	6.4	0.50	95	2.6	5.00	93	1.4
⁵⁵ Mn	0.10	88	1.8	0.50	91	2.3	5.00	90	1.7
⁵⁷ Fe	2.50	93	3.6	12.50	93	1.5	50.00	93	1.7
⁵⁹ Co	0.005	91	0.9	0.0125	98	2.6	0.50	90	1.4
⁶⁰ Ni	0.10	83	1.5	0.50	88	1.5	5.00	87	1.5
⁶⁵ Cu	0.10	81	1.5	0.50	87	1.7	5.00	86	1.8
⁶⁶ Zn	2.50	83	1.4	12.50	88	1.4	50.00	88	1.5
⁷⁵ As	0.005	106	2.3	0.02	104	1.8	0.50	102	2.3
⁹⁸ Mo	0.10	94	1.2	0.50	95	2.0	5.00	100	1.5
¹¹¹ Cd	0.003	92	5.1	0.0125	101	1.4	0.50	98	1.0
¹¹⁸ Sn	0.005	83	2.7	0.02	92	1.6	0.50	95	1.9
¹²¹ Sb	0.005	90	6.8	0.02	95	1.8	0.50	97	1.7
¹³⁷ Ba	0.10	96	1.0	0.50	101	1.9	5.00	101	1.7
²⁰² Hg	0.005	75	4.5	0.0125	92	2.0	0.50	106	1.1
²⁰⁸ Pb	0.01	88	4.8	0.02	91	2.0	0.50	93	2.7

*Note: All the concentration values given in the table are back-calculated to sample taken with a dilution factor of 50.

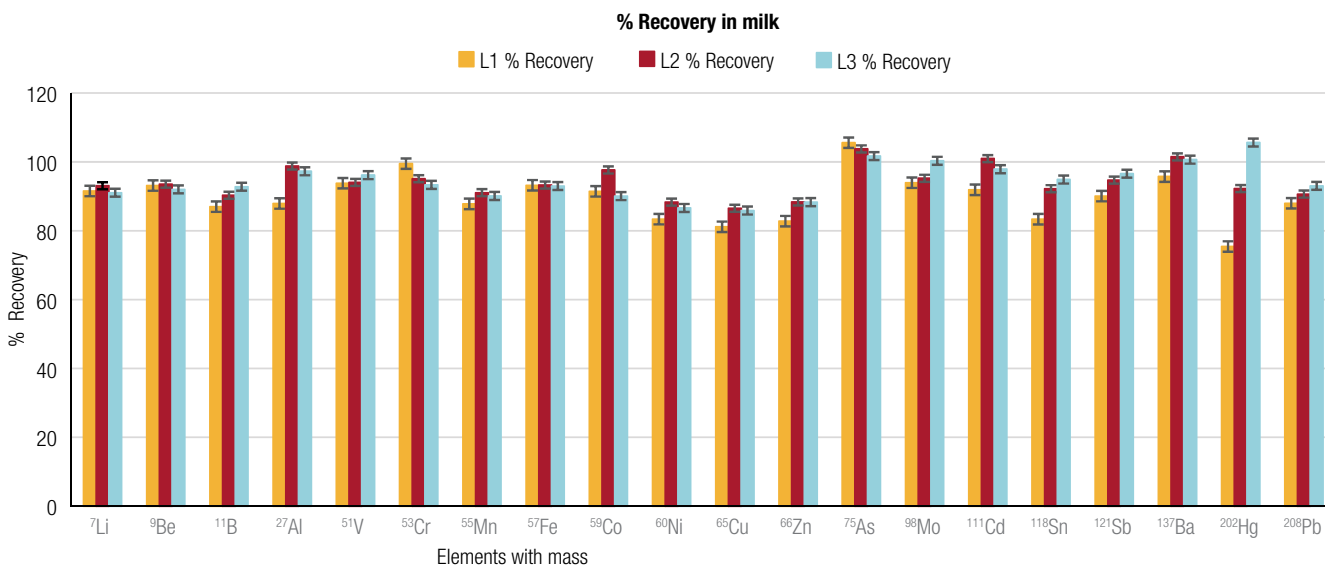


Figure 2. Accuracy in terms of % recovery, precision in terms of % RSD at three different level spikes

Table 8. The recovery (%) and precision (%RSD) observed at LOQ level for spiked butter and cheese samples.

Isotope	Measured spiked sample conc. in Qtegra ISDS Software (mg·kg ⁻¹)	Corrected conc. with dilution factor (mg·kg ⁻¹)	Butter (n=6)		Cheese (n=6)	
			% Recovery	% RSD	% Recovery	% RSD
⁷ Li	0.0020	0.1000	103	0.8	88	4.6
¹¹ B	0.0020	0.1000	104	3.0	80	1.2
²⁷ Al	0.1000	5.0000	111	2.8	109	1.4
⁵¹ V	0.0020	0.1000	103	1.1	97	1.3
⁵³ Cr	0.0040	0.2000	105	3.2	91	1.9
⁵⁵ Mn	0.0040	0.2000	100	2.5	87	1.0
⁵⁷ Fe	0.0500	2.5000	102	1.5	84	1.7
⁵⁹ Co	0.0001	0.0025	107	1.0	87	1.5
⁶⁰ Ni	0.0020	0.1000	112	1.1	81	1.9
⁶⁵ Cu	0.0040	0.2000	108	2.3	79	1.2
⁷⁵ As	0.0003	0.0150	109	2.0	82	3.4
⁹⁸ Mo	0.0020	0.1000	98	1.3	109	1.4
¹¹¹ Cd	0.0001	0.0050	112	2.6	109	2.3
¹¹⁸ Sn	0.0001	0.0050	94	2.2	109	3.0
¹²¹ Sb	0.0002	0.0100	108	1.2	105	1.4
¹³⁷ Ba	0.0020	0.1000	98	1.0	95	1.9
²⁰² Hg	0.0001	0.0050	101	2.4	103	1.3
²⁰⁸ Pb	0.0001	0.0050	95	1.7	110	1.5

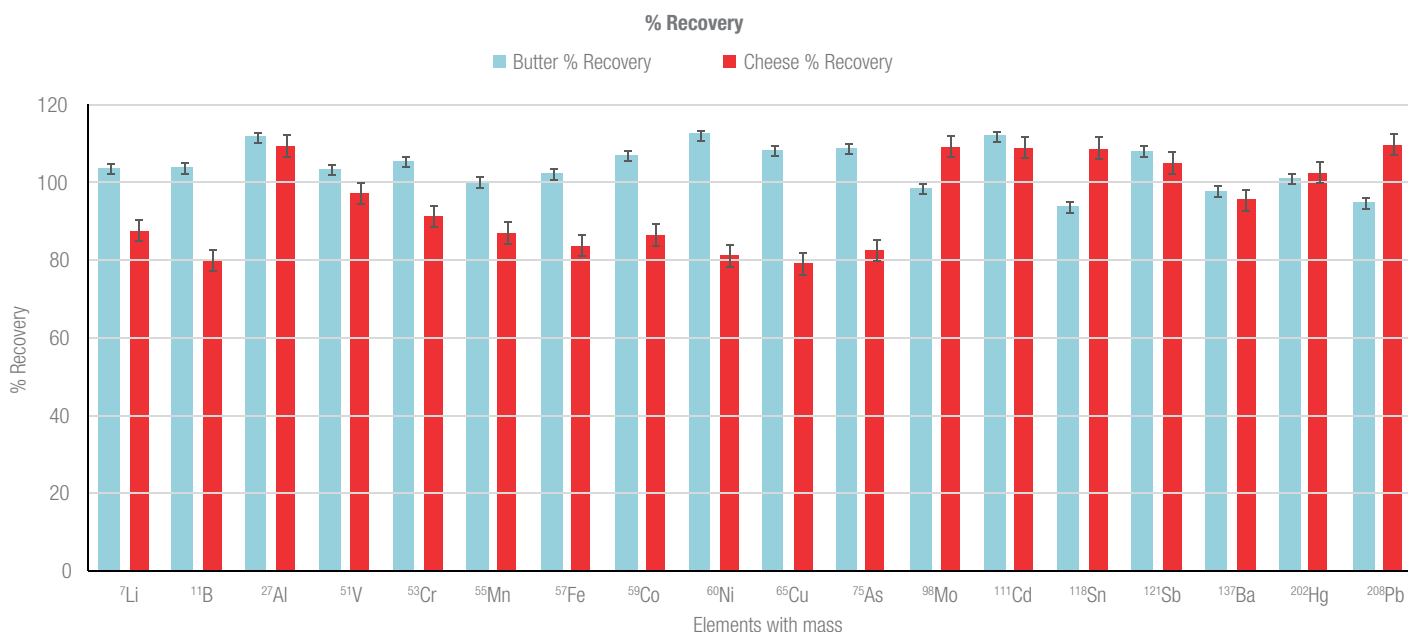


Figure 3. % Recovery observed at LOQ level spike of butter and cheese samples

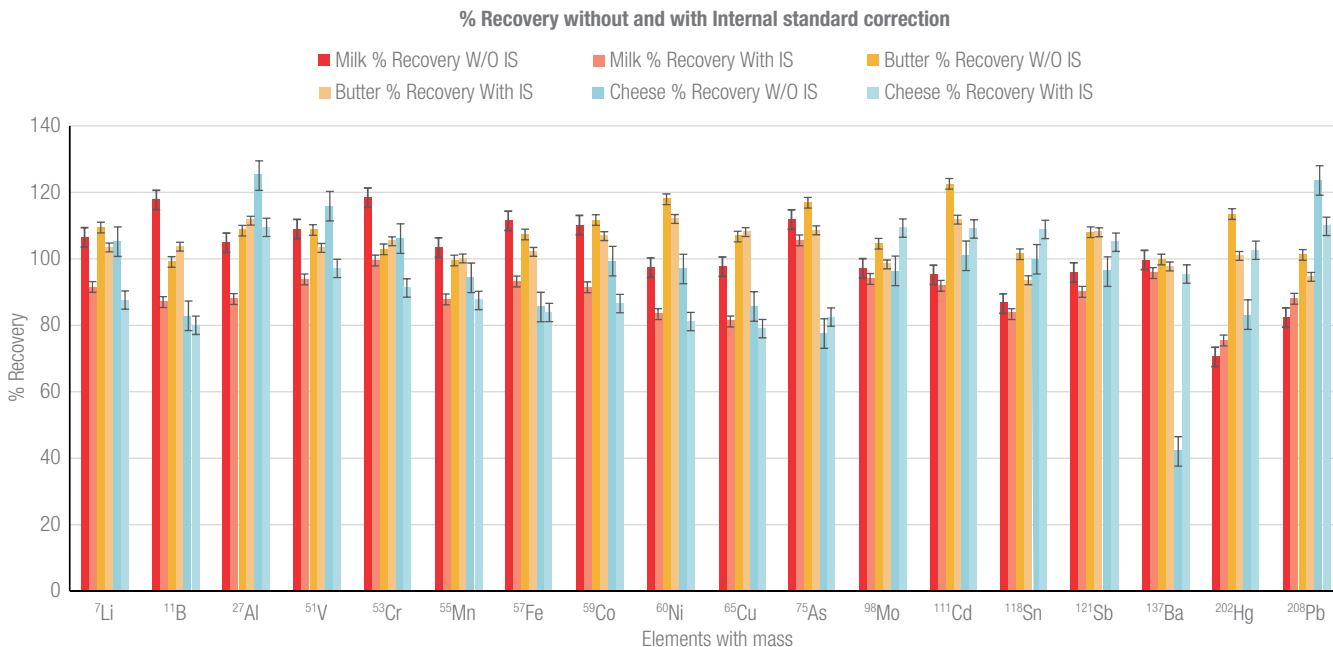


Figure 4. % Recovery without and with internal standards correction in milk, butter, and cheese

Method robustness and drift

The internal standard elements should ideally be close to the analyte elements in terms of mass and the ionization potential, resulting in similar behavior in the plasma and mass analyzer. The effect of the matrix on analyte elements, the robustness of the instrument response to the suppression, and signal drifts can be identified and quantified from the response of internal standard

elements that are added in the same concentration in standards and samples. The response of the internal standard (0.02 mg·L⁻¹) during a sequence of milk samples (n=30) showed excellent robustness. The IS elements showed recoveries in the range of 87% to 120% against the criteria of 60% to 125% (AOAC 2015.01) with the precision of <10% RSD. The Sc, Tb, Ge, In, Ir, Rh, and Bi internal standard recoveries are shown in Figure 5.

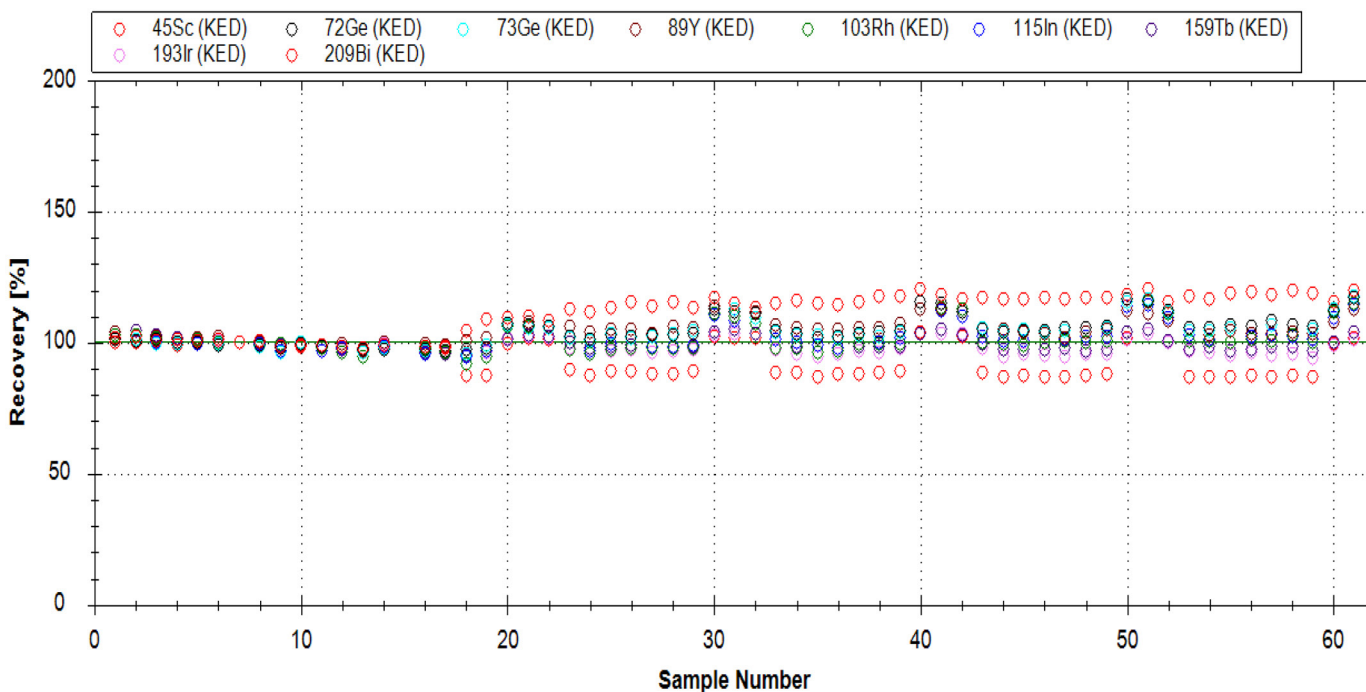


Figure 5. % Recovery of the internal standard in milk samples (n=30)

In addition to internal standards, bracketing standards [continuous calibration verification (CCV)] were aspirated as an intermediate standard check after every six replicates of spike samples. The obtained recoveries of 88–110% were well within the acceptance criteria of 85–115% of AOAC. 2015.01. This showed that there is no signal drift beyond the limit of 85–115% and ensures the quality of data. The observed percentage recoveries of all elements are shown in Figure 6.

Conclusion

This application note provided a complete solution to the quantification of multiple elements at trace levels in milk and milk products using a single methodology. The method allows the lower limits of quantification in milk

(Table 7) and milk products (Table 8), combined with excellent recovery (80–115%) and precision (<10% RSD). Also, the method meets the AOAC 2015.01 standard method performance requirement guidelines as well as the FSSAI, CFDA, and EC MRL requirements. Accuracy and precision are achieved through consistent removal of spectral interferences due to the superior design of the CRC system of the iCAP RQ ICP-MS, combining kinetic energy discrimination with an automatically applied low mass cut off. Excellent robustness is demonstrated by achieving consistent recovery of internal standard response between 87% and 120% with <10% RSD. This optimized method offers a single method solution for commercial food testing laboratories needing to improve sample throughput without sacrificing data quality.

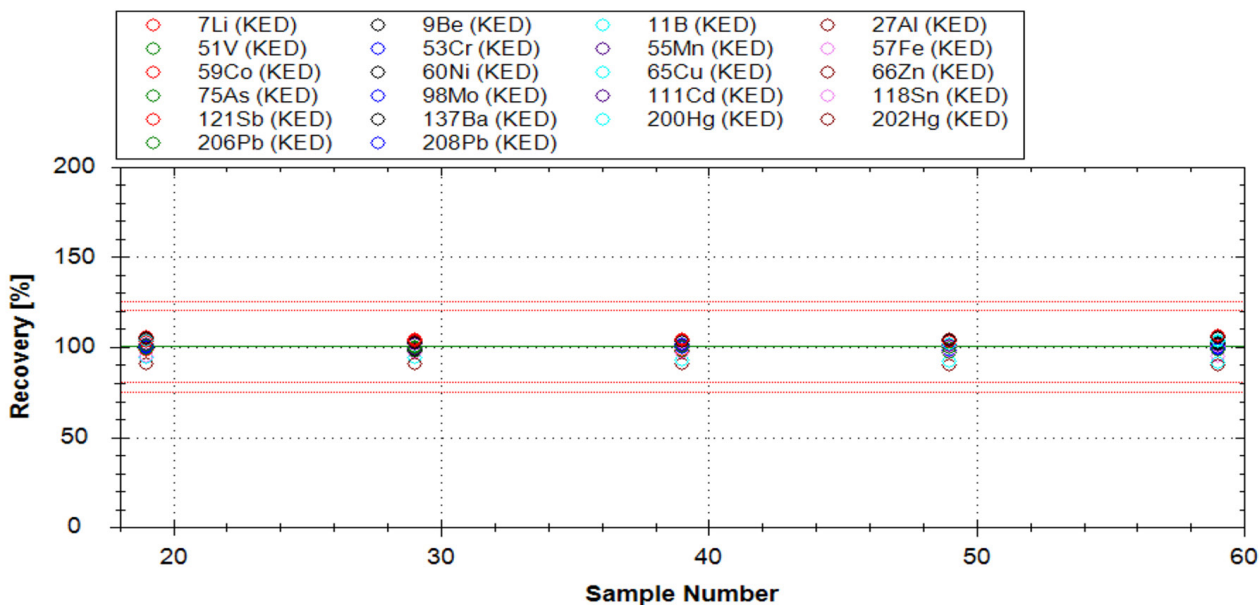


Figure 6. % Recovery of bracketing standards in milk method sequence (continuous calibration verification)

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