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Screening and Quantitation of Trace Metals in Milk by using ICP-MS

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ABSTRACT

Multi-elemental screening and quantification method of trace elements in milk using microwave assisted closed vessel acid digestion followed by ICP-MS technique using Thermo Fisher Scientific iCAP-RQ ICPMS with Qtegra ISDS software. This method covered 18 elements (Pb, Cd, Hg, As, Cu, Sn, Cr, Ni, Co, Se, Sb, B, Sr, Be, Ba, Mn, V, Al). The milk sample was digested with the tri-acid combination (Nitric acid, Hydrogen peroxide, and Hydrochloric acid). An internal standard mixture (Sc, Ge, Y, In, Tb, Rh, Tl, Ir, Bi) with concentration 20µg/L was used, and gold solution (200 ug/L) is used in sample preparation as a stabilizer for mercury. Isopropanol (2%, v/v) is added as an organic modifier to improve the ionization efficiency of elements. The prepared sample is aspirated and quantified against the calibration curve which is plotted with 5 points (calibration ranges from varies with elements). The linearity offered excellent R2 values (> 0.995). LOQs (0.5 to 80 ug/L) are verified through spiking and offered >80% recovery with <10% RSD. The method is assessed for its performance in terms of sensitivity, selectivity, recovery, and precision as per the AOAC 2015.01 guideline. An average recoveries were in the range of 80-115% in compliance with AOAC 2015.01 guideline. This method provides a solution to food testing laboratories

INTRODUCTION

Milk is considered as an important and almost complete dietary food for children and adults. Milk provides all essential macronutrients like carbohydrates, high quality proteins and fats as well as micronutrients like vitamins such as riboflavin, B5 and B12, minerals like calcium, magnesium and selenium. It provides essential amino acids like casein. A 250mL of milk can provide approximately 48% of protein requirement and 9% calorie to 5-6 year children. India is the largest milk producer in world with 20% contribution to total world milk production. In food safety perspective milk is more vulnerable to toxic elemental contamination by the processing activities and adulteration. Food Safety and Standards Authority of India (FSSAI) and European Commission(EC) has set the maximum residual limit (MRLs) in milk for heavy metals. The list of elements and their MRLs are given in table 1. For sample preparation to ICPMS analysis of trace metals, Microwave digestion method was widely accepted technique and the method is implemented in the most of commercial food testing labs of India. AOAC 2015.01 has reported the Microwave digestion method for food samples. The aim of this application note is multi-elemental screening and quantification at trace levels in milk using Thermo Scientific™ iCAP RQ ICP-MS equipped with ASX 560 auto-sampler assisted by the microwave digestion method. The method was validated in accordance with AOAC 2015.01.

Table 1. List of elements and their MRLs

Name of Element	FSSAI MRL (mg/kg)	EC MRL (mg/kg)
Mercury (Hg)	1.0	NA
Arsenic (As)	0.1	NA
Lead (Pb)	0.02	0.02
Cadmium (Cd)	0.1	NA
Copper (Cu)	30	NA
Tin (Sn)	250	50

MATERIALS AND METHODS

Extraction: Microwave digestion protocol

- Weigh 2.5±0.25 g of sample in Microwave digestion vessel
- Add 4 mL Nitric acid (HNO3)
- Add 2.5ml Hydrogen Peroxide (H2O2)
- Add 0.5ml Hydrochloric acid (HCl)
- Keep it for 10min to pre-digestion in a clean fume hood.
- Close the microwave digestion vessels properly and start the Microwave digestion process with set temperature program. Temperature program set for milk matrix is given in table 2.

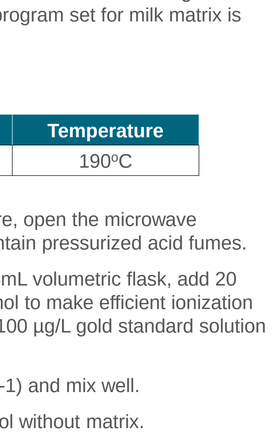
Table 2. Microwave digestion program

Ramp Time (min)	Hold time (min)	Temperature
30	15	190°C

- After completion of digestion, cool to room temperature, open the microwave digestion vessel carefully in a fume hood, as it will contain pressurized acid fumes.
- Quantitatively transfer the content in to precleaned 25mL volumetric flask, add 20 µg/L internal standard solution mix, 0.5mL of 2-propanol to make efficient ionization of high ionization potential elements like Arsenic and 100 µg/L gold standard solution to stabilize Mercury.
- Make up to 25 mL with deionized water (18.20MΩ.cm-1) and mix well.
- Prepare a procedural blank by following above protocol without matrix.

Table 3. ICP-MS Conditions

Parameter	Value
Forward power (RF)	1550W
Nebulizer gas	1.06 L/min
Auxiliary gas	0.8 L/min
Cool gas flow	14.0 L/min
Sample uptake/wash time	45 sec
Dwell time	0.03 sec
Number of readings per single aspiration	3
Total acquisition time	16 sec
Sampling depth	5 mm



RESULTS

ICP-MS kinetic energy discrimination (KED) mode

- ✓ Universal mode for interference removal.
- ✓ This mode utilizes helium as the cell gas because it is inert and does not react with the analyte.
- ✓ Helium has low mass, so it does not degrade signal sensitivity very much.
- ✓ KED creates a potential energy barrier at the collision reaction cell (CRC) exit using ion lenses. Because the exit of the CRC is more positive than the entrance, this slightly repels ions back into the cell. As the sample ions pass through the CRC, they encounter neutral helium atoms and collide. These collisions slow the ions down, so their kinetic energy is reduced. As the ions progress through the cell and collide with additional upstream helium atoms, their kinetic energy is reduced to the point where the potential energy barrier prevents them from leaving the cell as part of the ion beam, resulting in their ejection from the cell. Polyatomic ions are larger than sample ions because they are made up of multiple atoms by definition. Because of this, they encounter more collisions than the analyte ions, and so lose energy faster. By altering the amount of helium in the cell, it is possible to reduce the kinetic energy of the polyatomic interferences to the point where they are ejected by the potential energy barrier set up in the cell, but not the analyte ion.

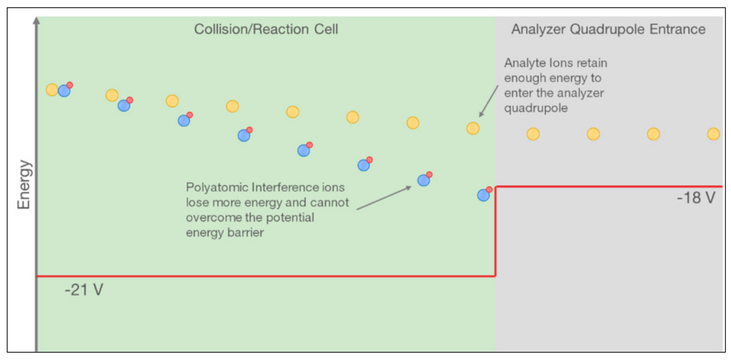


Figure 1: Kinetic energy discrimination (KED) mode

Method performance

Linearity

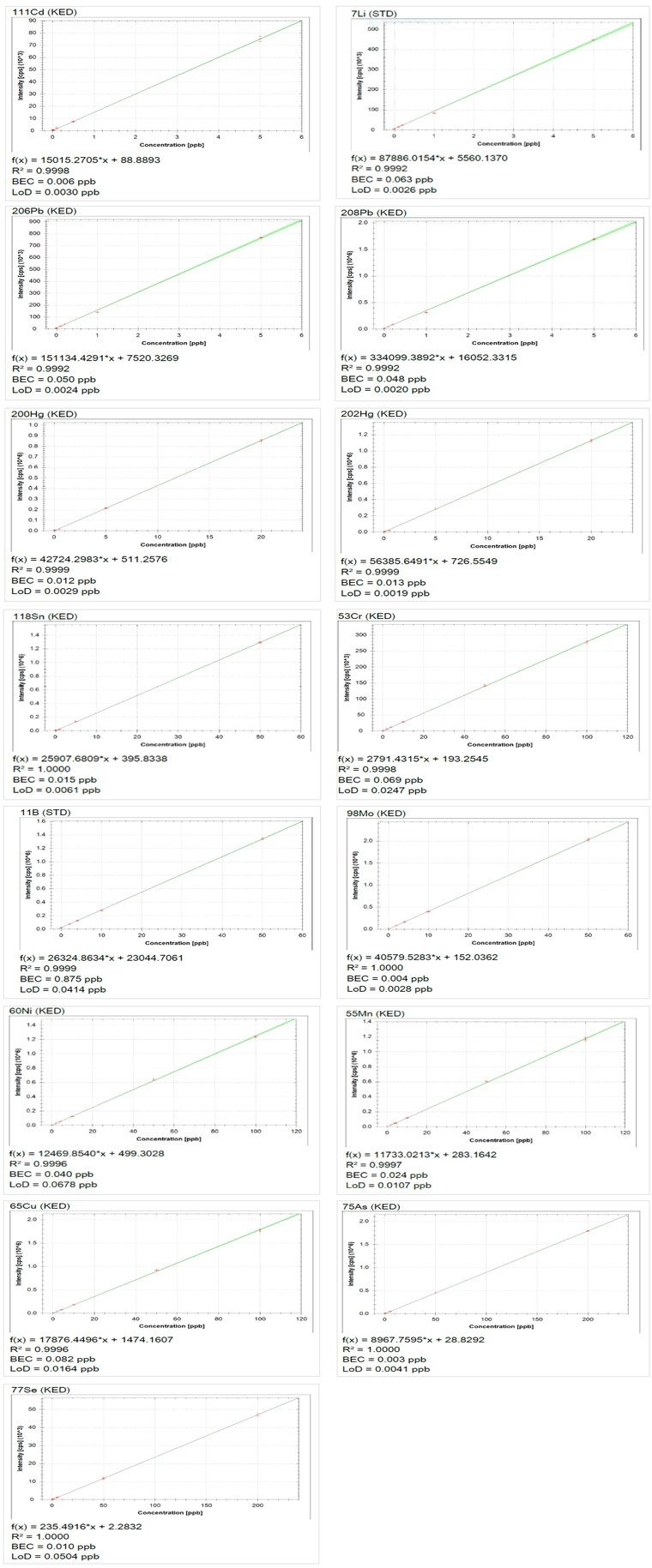


Figure 2. Linearity for the target elements with internal standards

Table 4. List of element with their mode of analysis and internal standards

Name of Element	Molecular weight	Mode of Analysis	Internal standard elements
Cadmium, Cd	111	KED	Rhodium,103
Antimony, Sb	121	STD	Rhodium,103
Cobalt, Co	59	KED	Yttrium,89
Mercury, Hg	200	KED	Bismuth,109
Mercury, Hg	202	KED	Bismuth,109
Arsenic, As	75	KED	Germanium, 72
Tin, Sn	118	KED	Indium,115
Lead, Pb	206	KED	Thalium,105
Lead, Pb	208	KED	Thalium,105
Vanadium, V	51	KED	Scandium, 45
Chromium, Cr	52	KED	Scandium, 45
Chromium, Cr	53	KED	Scandium, 45
Manganese, Mn	55	KED	Scandium, 45
Beryllium, Be	9	KED	Scandium, 45
Nickel, Ni	60	KED	Germanium, 72
Boron, B	11	STD	Scandium, 45
Copper, Cu	65	KED	Germanium, 72
Molybdenum, Mo	98	KED	Yttrium,89
Barium, Ba	137	STD	Terbium, 159
Lithium, Li	7	STD	Scandium, 45

Table 5. Method validation (linearity, recovery and precision (%RSD)

Elements with Mass	R2	LOQ (µg/L)	% Recovery	% RSD	LOQ*4 (µg/L)	% Recovery	% RSD
111Cd (KED)	0.999	0.50	89.4	7.2	2.00	76.6	4.6
121Sb (STD)	0.999	0.50	72.4	8.2	2.00	73.6	2.9
59Co (KED)	0.999	0.50	88.6	5.0	2.00	97.7	1.1
200Hg (KED)	0.999	1.00	92.1	4.6	4.00	104.4	1.2
202Hg (KED)	0.999	1.00	86.2	5.0	4.00	103.8	1.5
75As (KED)	0.999	1.00	79.8	8.1	2.00	111.1	5.7
118Sn (KED)	0.999	2.00	85.3	5.0	8.00	90.3	3.0
206Pb (KED)	0.999	2.00	100.9	4.5	8.00	61.7	6.5
208Pb (KED)	0.999	2.00	99.6	3.7	8.00	60.5	6.1
51V (KED)	0.999	20.00	72.4	2.0	80.00	96.5	1.3
52Cr (KED)	0.999	20.00	76.7	2.40	80.00	95.0	1.5
53Cr (KED)	0.999	20.00	78.6	2.0	80.00	102.4	2.3
55Mn (KED)	0.999	20.00	73.9	3.4	80.00	114.5	3.2
9Be (KED)	0.999	20.00	89.5	7.3	80.00	88.3	3.0
60Ni (KED)	0.999	20.00	82.8	1.6	80.00	99.9	1.4
11B (STD)	0.999	80.00	91.5	4.5	200.00	98.0	4.8
65Cu (KED)	0.999	40.00	83.2	5.8	200.00	89.0	3.1
98Mo (KED)	0.999	40.00	84.5	5.1	200.00	87.6	1.1
137Ba (STD)	0.999	40.00	106.9	4.0	200.00	111.4	1.5
7Li (STD)	0.999	8.00	79.2	2.0	10.00	73.2	3.3
111Cd (KED)	0.999	0.50	89.4	7.2	2.00	76.6	4.6
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118Sn (KED)	0.999	2.00	85.3	5.0	8.00	90.3	3.0

CONCLUSIONS

- Microwave acid digestion method was utilized
- Method offered LOQ in the range of 0.5-40 ppb
- Excellent linearity (r2>0.99)
- Average recoveries and precision data were within acceptance criteria of AOAC 2015.01 guideline.
- Optimized method complied the EU and FSSAI MRLs.

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TRADEMARKS/LICENSING

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