

ICP - Mass Spectrometry

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Analysis of Milk for Major and Trace Elements by ICP-MS

Introduction

Milk is a widely consumed food product for both adults and children, while formula milk may constitute a major nutrient source for infants. Furthermore, milk and milk powder are used widely in the food industry for the production of other foods. Because of its nutritional importance and widespread consumption, regulations in many countries necessitate that the quality of the milk is routinely monitored. Both dairies and food manufacturers need to carry out the analysis of major, trace, and contaminant elements in milk and milk powders to fulfill requirements for labelling purposes, monitor nutritional quality, and safeguard against contamination by toxic elements. In Europe, regulations such as Commission Regulation (EC) No 1881/2006 set out maximum levels for some contaminants in foods. Similarly, in India, Food Safety and Standards Regulations (FSSRI) address maximum permitted levels by element over a variety of food groups. In the USA, Proposition 65 regulates contaminants based on maximum daily exposure limits. With elemental concentrations ranging from ng/L to percent levels, samples can present a challenge for ICP-MS instruments in testing laboratories where sample throughput and efficiency is sought. To fulfill this need for the analysis of milk and milk products, reliable testing methods are required.

Milk contains high concentrations of total dissolved solids (TDS), both from organic and inorganic components. While microwave sample preparation breaks down the organic constituents, the inorganic salts remain in solution at high concentrations. Milk typically contains high levels of phosphorus (P), potassium (K), and calcium (Ca), and moderately high levels of sodium (Na) and magnesium (Mg). Amongst the variety of analytical techniques available for elemental measurements, ICP-MS is unique in its capability to rapidly measure both trace and elevated concentrations of different elements in the same sample.

PerkinElmer's NexION® 2000 ICP-MS includes a variety of design characteristics which enhance its ability to perform these measurements in high-TDS samples. One of the features offered by the NexION 2000 to facilitate analysis of high-TDS samples is the All Matrix Solution (AMS)¹, an innovative argon dilution system designed to precisely dilute the incoming aerosol by 1 to 200x prior to reaching the plasma. This feature allows the introduction of high-TDS samples without the need for off-line liquid dilution, thereby eliminating the possibility of contamination and dilution errors which may be associated with such a step.

This work showcases the NexION 2000 ICP-MS for the analysis of milk samples for major and trace elements, demonstrating the benefits of AMS. Collision mode is applied to target polyatomic interferences, while AMS is used to reduce the level of TDS entering the plasma, ensuring minimal matrix effects. The developed method is simple, fast, and was validated with both certified reference materials and spike recovery studies on a variety of milk samples.

Experimental

Samples and Sample/Standard Preparation

In order to demonstrate the accuracy of the methodology, the following certified reference materials (CRMs) were analyzed:

- ERM-BD 150 skimmed milk powder
- ERM-BD 151 skimmed milk powder
- NMIJ 7512-a milk powder (infant formula milk powder for six-month-old children)

The European Reference Materials (ERM) were obtained from the Joint Research Centre of the European Commission, while the sample from National Measurement Institute of Japan (NMIJ) was obtained from GL Sciences B. V. (Eindhoven, The Netherlands).

The analysis of several materials is advantageous since not all CRMs are certified for the same elements. Thus, a larger selection of elements is covered. Furthermore, the selected CRMs are aiming at different population groups, including infants. Since the materials originate from different continents, the validation reach expands beyond a single region.

However, it is important to provide a method which is not only validated for milk powders, but also for ultra high temperature (UHT) and evaporated milk. For this reason, the following samples were purchased locally and analyzed:

- Skimmed milk powder (non-fat)
- Light evaporated milk (4% fat)
- Evaporated milk (9% fat)
- Sweetened condensed milk
- Skimmed milk (UHT, < 0.5% fat)
- Semi-skimmed milk (UHT, < 2% fat)

To further validate the methodology for various forms of milk with varying fat content, spike recovery studies were performed on these samples.

Samples were digested with a combination of concentrated nitric acid (Fluka™, TraceSELECT® Ultra) and 30% hydrogen peroxide (Sigma-Aldrich™, H₂O₂ ≥ 30%, for Ultratrace analysis). The sample amounts and added volume of water were adjusted, as shown in Table 1. This procedure effectively takes into account the pre-concentration of elements in powdered and evaporated milk, eliminating variation of digestion conditions with milk type due to water content of the sample.

Table 1. Preparation Steps for Various Milk Types.

Milk Type	Weight (g)	Nitric Acid (mL)	Hydrogen Peroxide (mL)	Water (mL)
UHT Milk	5	2.5	2.5	0
Evaporated Milk	2	2.5	2.5	3
Condensed Milk	1	2.5	2.5	4
Milk Powder	0.5	2.5	2.5	5

A Titan MPS™ Microwave Sample Preparation System with standard 75-mL vessels was used for the digestion. The temperature program is given in Table 2. A similar digestion protocol has been successfully used for digestion of milk samples and measurement by ICP-OES². Digests were quantitatively transferred to 50-mL autosampler tubes, spiked with 10 µL of 1000 mg/L gold solution, and made up to the 50 mL volume with deionized water.

Table 2. Microwave Digestion Program for the Titan MPS Microwave Preparation System.

Step	Target Temp (°C)	Pressure Limit (bar)	Ramp Time (min)	Hold Time (min)	Power Limit (%)
1	140	35	10	2	80
2	195	35	3	20	100
3	50	35	1	20	0

Note: The power limit in step 1 is adjusted depending on the number of vessels used in the digestion batch³.

The moisture content of CRMs was determined following the instructions given on the certificates of analysis. CRM analysis results were corrected for moisture content and are reported on dry-mass basis.

The calibration solutions and internal standard mix were prepared from solutions listed in the Consumables Used table found at the end of this document. Details of the standard concentrations, spike levels, and internal standards are given in Table 3. In addition, glacial acetic acid (Sigma-Aldrich™) was added to the internal standard solution at 1.5% (v/v). The purpose of this organic modifier is to level out carbon content between solutions since digestion of organic samples may leave residual carbon compounds in solution. Because some elements with high ionization potentials are subject to carbon-induced signal enhancement, minimizing the difference in carbon content between standards and samples ensures accurate quantitation. All measurements were made against external calibration curves, with all calibrations being linear and exhibiting $0.99992 \leq r \leq 1.000000$ for all elements. Examples of the high range calibrations are shown in Figure 1 for potassium and sodium. All calibration standards contained 5% nitric acid + 200 µg/L gold, while the autosampler rinse solution consisted of 5% nitric acid. The measured isotopes are listed in Table 7.

Instrumentation

Analysis was carried out on a NexION 2000 P ICP-MS using the conditions and parameters shown in Table 4. No modifications were made to the default sample introduction system: PFA-ST nebulizer, baffled glass cyclonic spray chamber

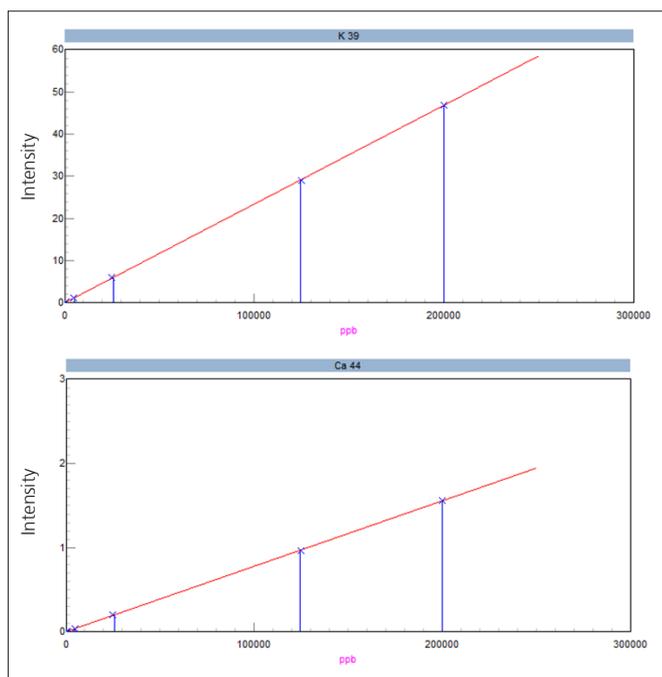


Figure 1. Calibrations for K and Ca.

Table 3. Elements, Calibration Levels, and Spike Levels (all units in µg/L).

Element	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6*	Spike Level
Al, As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Rb, Se, Sn, Sr, Ti, V, Zn	1	4	20	100	500	800	200
Hg	0.02	0.08	0.4	2	10	16	4
Na, Mg, K, Ca, P	250	1000	5000	25000	125000	200000	50000
Internal Standards	Sc45, Ga71, Ge72, Rh103, Ir193						

* calibrated for K, Ca, P, and Zn only

Table 4. NexION 2000 ICP-MS Parameters.

Component/Parameter	Type/Value
Nebulizer	PFA-ST
Spray Chamber	Glass Cyclonic at 2 °C
Injector	2.0 mm id quartz
Sample Uptake Rate	260 µL/min
Mixing Tee	On-line addition of internal standards
RF Power	1600 W
Collision Flow 1	3.8 mL/min (for As, Se, Ge)
Collision Flow 2	4.7 mL/min (for all remaining elements)
AMS Dilution	10x

with AMS port, and demountable torch. The Peltier-cooled spray chamber was set to 2 °C, and the AMS flow was set for a 10x dilution. The prepared digests were analyzed straight without any further dilution. No elemental correction equations were used, although the lead (Pb) isotopes were summed (Pb206+Pb207+Pb208) to account for potential geographic differences in Pb isotopic patterns.

Results and Discussion

Certified Reference Materials and Limits of Quantitation

CRMs were measured to validate the accuracy of the method. Table 5 shows the recoveries in NMIJ 7512-a Milk Powder, which are in the range of 94-99% of the certified values.

Table 5. Analysis of NMIJ 7512-a Milk Powder.

Element	NMIJ 7512a		
	Measured (mg/kg)	Certified (mg/kg)	Recovery
Na	1847	1870	99%
Mg	804	819	98%
P	5499	5620	98%
K	8231	8410	98%
Ca	8204	8650	95%
Mn	0.879	0.931	94%
Cu	4.59	4.66	99%
Zn	40.5	41.3	98%
Rb	8.67	8.93	97%
Sr	5.68	5.88	97%
Mo	0.213	0.223	95%
Ba	0.436	0.449	97%

Results obtained for ERM-BD 150 and ERM-BD 151 skimmed milk powders are given in Table 6. These CRMs have the same major element composition but differ in levels for some trace elements. There is generally good agreement between the experimental results and certified values, with recoveries ranging between 89-107%. To assess the method repeatability, the ERM-BD 150 reference material was measured seven times, with the average and precision of the seven measurements being shown in Table 6.

Following the recommendations of the Commission Regulation (EC) No 333/2007⁴, limits of quantitation (LOQs) were calculated on the basis of 10 times the standard deviation of 10 consecutive blank measurements and have been multiplied by a factor of 100 in order to represent LOQs for milk powders (Table 7). Comparing the LOQs with the CRM values, it can be seen that LOQs are in most cases substantially below certified values. Note, that for milk samples, applicable LOQ values are obtained by dividing the values in Table 6 by 10, due to the larger sample weight for milk (5 g) as compared to powders (0.5 g).

Sensitivity to Measure Legally Stipulated Maximum Levels (ML)

Within the context of milk, Commission Regulation (EC) No 1881/2006 sets MLs for inorganic tin and lead⁵. With the LOQ for lead in milk at 0.00017 mg/kg, the LOQ is two orders of magnitude lower than the regulated level of lead in milk at 0.020 mg/kg. The ML for tin in canned infant formulae and follow-on formulae (including infant milk and follow-on milk) is set to 50 mg/kg, which is four orders of magnitude above the LOQ for tin (Sn) in milk. The comparison is visualized in Figure 2. Digest blanks were below the LOQ for all elements and not of concern.

Table 7. Limits of Quantitation (LOQs) in Different Forms of Milk.

Element	LOQs in Milk Powder (mg/kg)	LOQs in Evaporated Milk (mg/kg)	LOQs in UHT Milk (mg/kg)
Na 23	2.1	0.52	0.21
Mg 24	0.29	0.072	0.029
Al 27	0.40	0.10	0.040
P 31	4.3	1.1	0.43
K 39	3.4	0.85	0.34
Ca 44	6.4	1.6	0.64
Ti 49	0.089	0.022	0.0089
V 51	0.0026	0.00065	0.00026
Cr 52	0.014	0.0035	0.0014
Mn 55	0.021	0.0052	0.0021
Fe 57	0.30	0.075	0.030
Co 59	0.0027	0.00067	0.00027
Ni 60	0.017	0.0043	0.0017
Cu 63	0.0064	0.0016	0.00064
Zn 66	0.099	0.025	0.0099
As 75	0.0099	0.0025	0.00099
Se 78	0.25	0.064	0.025
Rb 85	0.015	0.0037	0.0015
Sr 88	0.010	0.0026	0.0010
Mo 95	0.010	0.0024	0.0010
Cd 111	0.013	0.0033	0.0013
Sn 118	0.012	0.0030	0.0012
Ba 138	0.0034	0.00084	0.00034
Hg 202	0.0083	0.0021	0.00083
Pb 208	0.0017	0.00043	0.00017

Table 6. Analysis of ERM-BD 150 and 151 Skimmed Milk Powders.

Element	ERM-BD 150				ERM-BD 151		
	Measured* (mg/kg)	% RSD*	Certified (mg/kg)	Recovery	Measured (mg/kg)	Certified (mg/kg)	Recovery
Na	4074	1.5	4180	97%	4127	4190	98%
Mg	1225	1.9	1260	97%	1242	1260	99%
P	10368	2.4	11000	94%	10829	11000	98%
K	16343	1.6	17000	96%	16766	17000	99%
Ca	12499	1.4	13900	90%	12927	13900	93%
Mn	0.274	3.9	0.289	95%	0.286	0.29	99%
Fe	4.72	4.3	4.6	103%	49.7	53	94%
Cu	1.04	1.2	1.08	96%	5.05	5.00	101%
Zn	45.3	1.7	44.8	101%	45.5	44.9	101%
Se	< LOQ	---	0.188	---	< LOQ	0.19	n/a
Cd	< LOQ	---	0.0114	---	0.100	0.106	94%
Hg	0.0640	8.5	0.060	107%	0.545	0.52	105%
Pb	0.0170	4.3	0.019	89%	0.200	0.207	97%

* Result of 7 individual measurements

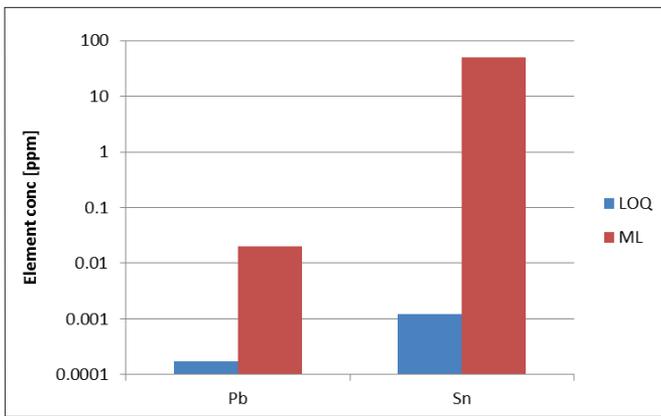


Figure 2. Limit of quantitation (LOQ, 10x SD) vs. maximum level (ML) for lead and inorganic tin, demonstrating sufficient sensitivity of the method.

Analysis of Store-Bought Samples

With the accuracy of the method established for certified elements, the method was applied to the analysis of samples purchased in local grocery stores. UHT milk, milk powder, and evaporated milk samples were analyzed, with the results displayed in Figure 3. The major-element distribution exhibits a clear pattern of highest concentrations in the milk powder sample, intermediate concentrations for the evaporated and condensed milk samples, and lowest concentrations for the UHT milk samples, reflecting the degree of pre-concentration of those elements. The same pattern is also observed for several trace elements (Mn, Cu, Zn, Rb, Sr, Mo, and to a lesser extent

Ba and Pb). The evaporated and condensed milk samples show elevated levels of Sn, which may result from the fact that these samples came in tins/cans, which are typically made of tin-coated steel, although further coatings may be involved to protect the food contents. A similar pattern is displayed by iron (Fe), which is also elevated in the canned samples, suggesting packaging could be a possible source there, too. Chromium (Cr) is elevated in the milk powder sample, which may speculatively be caused by processing of the milk sample into powder form. The toxic elements Cd, As, Hg, and other elements not included in Figure 3 were below the quantitation limit throughout.

All samples were spiked at the levels shown in Table 3, which represent 40% of the calibration range for most analytes. The spike levels were chosen so that spike-element concentrations were sufficiently high relative to sample concentrations, to allow accurate quantitation. The spike-to-sample ratio was at minimum between 37-48% for P, K, Ca, and Zn, around 100% for Na, Fe, and Rb, and larger than 400% for the remaining elements. All sample spikes fell within the calibration range, except K, which exceeded the range by 12%. The spike recoveries are plotted in Figure 4, showing recoveries in the range of 86-110%. These results suggest the level of organic modifier (acetic acid) has been set sufficiently high to level out any differences in carbon content between samples and standards. Spike recoveries for the samples and digestion blanks are mostly equivalent, indicating that the sample matrix does not affect recoveries.

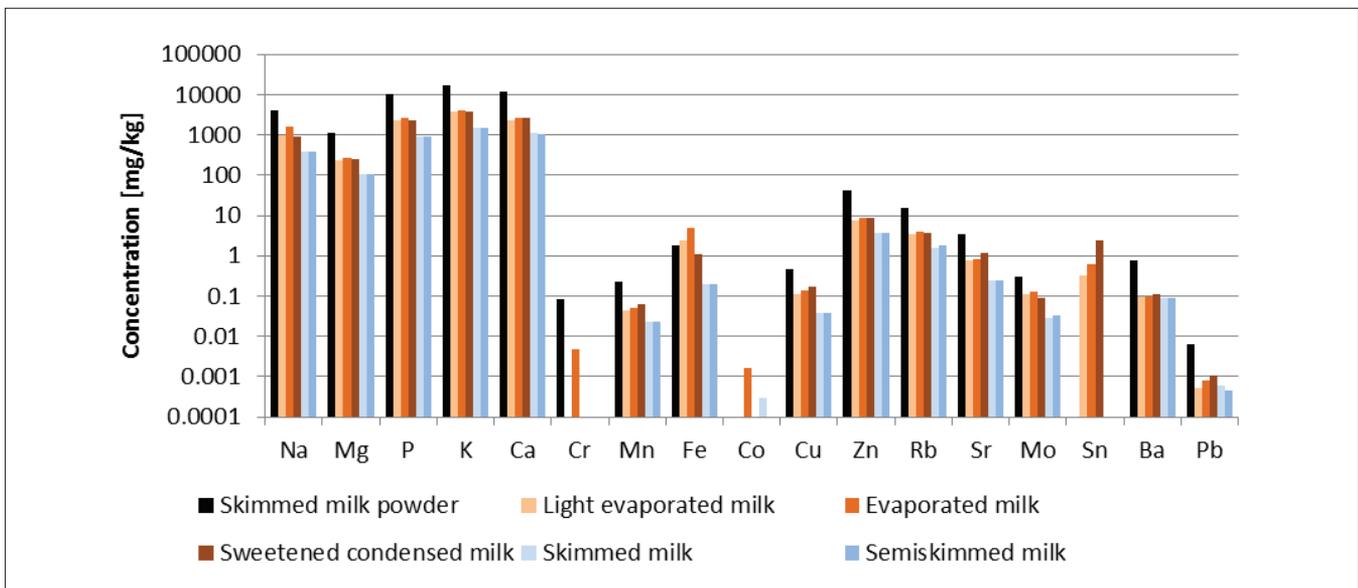


Figure 3. Results of analysis for store-bought samples (milk powder in black; evaporated milks in shades of orange; UHT milks in shades of blue). Values < LOQ have not been plotted.

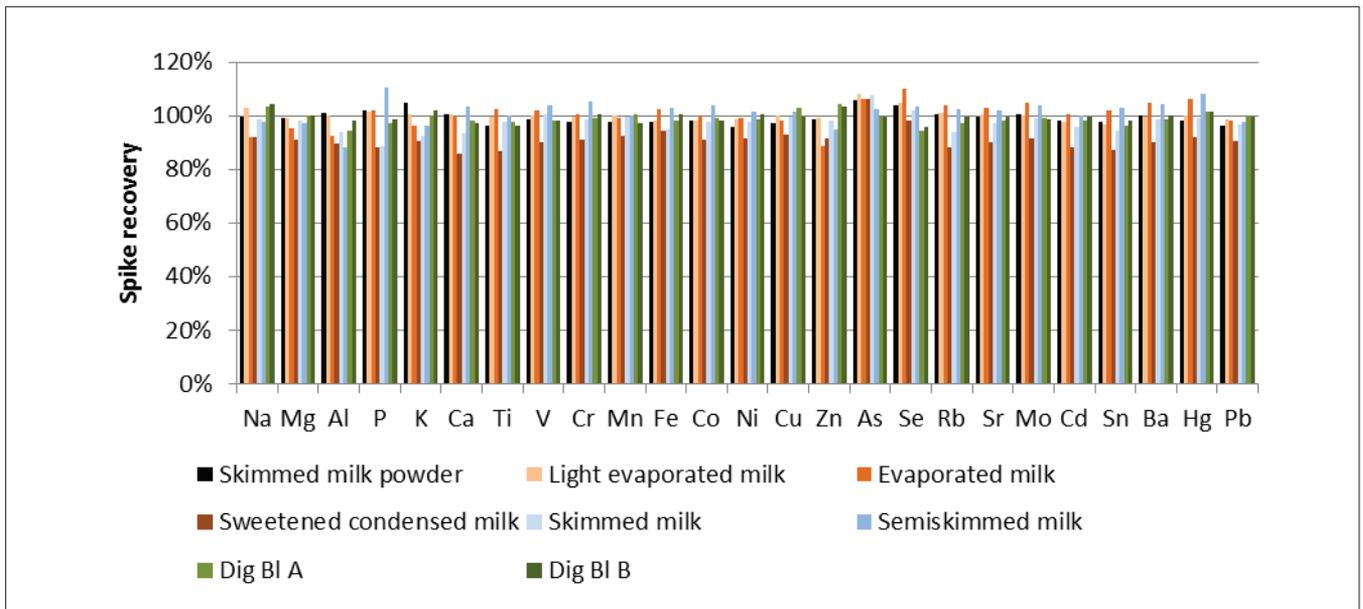


Figure 4. Spike recoveries in store-bought samples (milk powder in black; evaporated milks in shades of orange; UHT milks in shades of blue; digestion blanks in shade of green).

Internal standards stayed within the range of 80-115% and did not show drift over 6.5 hours (Figure 5). Stability over long run times is an important criterion to qualify a method for routine use.

A check standard (Std 4) was measured routinely over the 6.5-hour run. The check standard recoveries are displayed in Figure 6 and show that recoveries are within 92-108% for all elements and are stable over time, showing no drift.

Conclusion

The analysis of major and trace elements in milk, evaporated milk, and milk powder was successfully achieved on the NexION 2000 P ICP-MS using Collision mode with the application of AMS. The analysis of three certified reference materials verified the accuracy of the methodology. Spike recoveries on a variety of store-bought milk samples demonstrated method accuracy for all measured elements, including those where CRMs had no certified values. The method was stable over a 6.5-hour run, showing both no instrument drift and accurate measurement of check standard concentrations. With the combination of unique design characteristics, the NexION 2000 ICP-MS is proven to be an ideal solution for the analysis of both trace and major elements in milk.

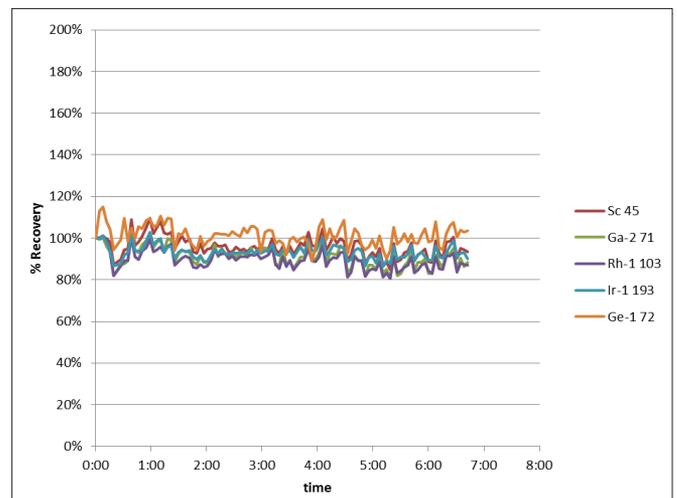


Figure 5. Internal standards behavior during 6.5-hour run.

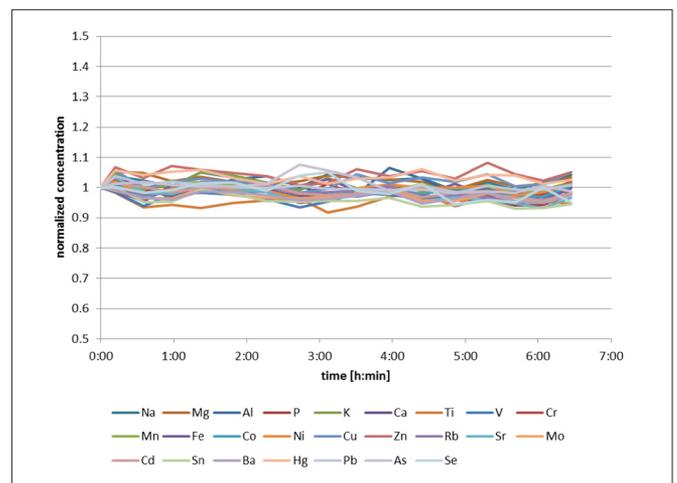


Figure 6. Check standard recoveries over a 6.5-hour run of milk samples.

References

1. All Matrix Solution System for NexION ICP-MS Platforms, PerkinElmer Technical Note, 2017.
2. Neubauer, K., Spivey, N., Analysis of Micronutrients in Milk Using the Avio 200 ICP-OES, PerkinElmer application note, 2016.
3. Titan MPS™ Microwave Sample Preparation System - A Reference Notebook of Microwave Applications, PerkinElmer, 2016.
4. Commission Regulation (EC) No 333/2007: Laying Down the Methods of Sampling and Analysis for the Official Control of the Levels of Lead, Cadmium, Mercury, Inorganic Tin, 3-MCPD, and Benzo(a)pyrene in Foodstuffs, Official Journal of the European Union, European Commission, L88, 2007, pp. 29-38.
5. Commission Regulation (EC) No 1881/2006: Setting Maximum Levels for Certain Contaminants in Foodstuffs, Official Journal of the European Union, European Commission, L364, 2006, pp. 5-24.

Consumables Used

Component	Description	Part Number
Sample Uptake Tubing	Green/yellow (0.44 mm id), flared, PVC, package of 12	N8145198 (MP2 peri pump)
Internal Standard Uptake Tubing	Orange/red (0.19 mm id), flared, PVC, package of 12	N8145195 (MP2 peri pump)
Spray Chamber Drain Tubing	Gray/gray Santoprene (1.30 mm id), package of 12	N8145160 (MP2 peri pump)
Instrument Calibration Standard 2	100 mg/L Ag, Al, As, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sn, Sr, Ti, Tl, V, Zn	N9301721 (125 mL)
Mercury Standard	10 mg/L Hg	N9300253 (125 mL)
Environmental Standard Mix 2	1000 mg/L Na, Mg, K, Ca	N9307805 (125 mL)
Internal Standard Mix	Sc = 200 mg/L; Ga = 20 mg/L; Rh, In, Ir, Tm = 10 mg/L	N9307738 (125 mL)
Germanium Standard	1000 mg/L	N9303774 (125 mL) N9300120 (500 mL)
Gold Standard	1000 mg/L	N9303759 (125 mL)
Phosphorus Standard	1000 mg/L	N9303788 (125 mL) N9300139 (500 mL)
Rubidium Standard	1000 mg/L	N9303792 (125 mL) N9300145 (500 mL)
Autosampler Tubes	50 mL, free-standing 15 mL	B0193234 B0193233