Determination of Chromium, Selenium, and Molybdenum in Nutritional Products by ICP-MS

Application Note

Food Testing and Agriculture

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Abstract

An ICP-MS validated method for the rapid determination of chromium (Cr), selenium (Se), and molybdenum (Mo) in infant formula and adult nutritional products was successfully transferred from the Agilent 7500cx ICP-MS to the Agilent 7700x ICP-MS, and from lab 1 to lab 2, respectively. The key advantage of the method is that all samples can be analyzed using a single-cell gas mode (helium mode) that provides effective removal of polyatomic interferences on Cr, Mo, and Se. This results in significantly improved productivity. Samples were spiked with internal standards before digestion in a closed-vessel microwave oven, followed by detection using ICP-MS. This method was found to be a suitable candidate for use as a global reference method, and has been granted Final Action/Official status by AOAC for the determination of Cr, Mo, and Se at trace levels in nutritional products.

Introduction

The fortification of foodstuffs with essential elements is widely used as a simple way to improve nutrition, especially for populations and groups at risk of dietary deficiency. Examples include infant formulas, which are fortified with Se, and pediatric and adult medical nutritional products, which are fortified with Se, Cr, and Mo [1,2]. These elements are difficult to analyze at the levels typically present in foodstuffs using traditional techniques, such as inductively coupled plasma-atomic emission spectrometry (ICP-AES). Such analytical techniques can, therefore, require the use of a nonstandard sample introduction technique such as ultrasonic nebulization to provide sufficient sensitivity for the determination of Cr and Mo, or hydride generation for the determination of Se [3,4,5]. If graphite furnace-atomic absorption spectrometry (GF-AAS) is used, it may require a complicated extraction procedure to obtain the required sensitivity for the determination of these elements.

Agilent Technologies

This method is the only official method (AOAC Official Method 2011.19) for the analysis of Cr, Mo, and Se in infant formula. Hydride generation-AAS methods do exist for the determination of Se in pet foods (AOAC 986.15, 1988), feeds (AOAC 996.17, 1997), and foodstuffs (European Norm EN 14627). ICP-AES can be used for the determination of Se, Cr, and Mo in fertilizers using AOAC 2006.03, and GF-AAS can be used for the determination of Cr and Mo in foodstuffs after dry ashing (EN 14082) or pressure digestion (EN 14083; 8). All the EN methods cited here have Type IV status, meaning they are well-regarded methods but not Codex-approved.

ICP-MS is a fast, multielement analysis technique with the necessary sensitivity and selectivity to measure Cr, Se, and Mo (plus many other elements) in nutritional products through external calibration [1,6,7]. ICP-MS offers extremely low quantitation limits at ng/L (parts-per-trillion, ppt) level. The latest generation of instruments is fitted with collision reaction cells (CRCs) that reduce or eliminate spectral interferences caused by polyatomic ions formed from the plasma gas, matrix components, and solvent acids [8,9]. For example, Cr can now be determined accurately at low concentrations using its primary isotope (*m/z* 52) in the presence of matrix-based polyatomic interferences such as $^{40}Ar^{12}C$ and $^{35}Cl^{16}O^{1}H$ that occur at the same mass. Se can be determined using its preferred isotope ^{78}Se, which was previously not suitable for trace level analysis due to the presence of the $^{40}Ar^{38}Ar$ polyatomic overlap [10,11]. Examples of other possible polyatomic interferences on Cr, Mo, and Se in ICP-MS are listed in Table 1.

Table 1. Typical ICP-MS polyatomic interferences for Cr, Mo, and Se.

Element	*m/z*	Abundance (%)	Interferences
Cr	52	83.8	$^{40}Ar^{12}C^+$, $^{37}Cl^{14}N^1H^+$, $^{36}Ar^{16}O^+$, $^{35}Cl^{16}O1H^+$
Cr	53	9.5	$^{40}Ar^{13}C^+$, $^{37}Cl^{16}O^+$, $^{38}Ar^{15}N^+$, $^{38}Ar^{14}N^1H^+$, $^{36}Ar^{17}O^+$, $^{36}Ar^{16}O^1H^+$, $^{35}Cl^{17}O^1H^+$, $^{35}Cl^{18}O^+$, $^{36}S^{17}O^+$
Mo	95	15.9	$^{79}Br^{16}O^+$
Se	78	23.2	$^{38}Ar^{40}Ar^+$
Se	80	49.8	$^{40}Ar_2^+$, $^{32}S^{16}O_3^+$, $^{32}S_2{}^{16}O^+$
Se	82	9.2	$^{40}Ar_2{}^1H_2^+$, $^{12}C^{35}Cl_2^+$, $^{34}S^{16}O_3^+$

Our method for the determination of Cr, Se, and Mo in infant formula and adult nutritional products using an Agilent 7500cx ICP-MS, described in detail in Pacquette *et al.* (2011). The method has since been successfully transferred to an Agilent 7700x ICP-MS, with a CRC method using helium mode only. We present here some details of the method transfer validation procedure.

Experimental

Instrumentation and reagents

An Agilent 7700x ICP-MS with Octopole Reaction System (ORS^3) CRC was used throughout. The instrument was fitted with the standard sample introduction system (MicroMist glass concentric nebulizer, quartz Scott-type spray chamber, quartz torch with 2.5 mm id injector), and interface (Ni sampler and skimmer cones). The ORS^3 was operated in helium collision mode for the determination of Cr, Mo, and Se. Sampling was facilitated through an Agilent ASX-520 autosampler. Instrument settings and parameters are given in Table 2.

Table 2. Agilent 7700x ICP-MS operating parameters.

Parameter	Value
RF power	1,600 W
Sampling depth	9 mm
Carrier gas flow	0.9 l L/min
Extract 1 lens	0 V
Make-up gas flow	0.2 L/min
Spray chamber temp.	2 °C
He cell gas flow rate	4.5 mL/min
Analyte/internal standard	$^{52}Cr/^{60}Ni$, $^{8}Se/^{130}Te$, $^{95}Mo/^{60}Ni$

The official method employs ^{72}Ge as internal standard instead of Ni, given that background Ni is sometimes found in chocolate-flavored products.

Custom Laboratory control sample

Until recently, a homogeneous powder containing all essential nutritional elements was used as the inhouse Custom Laboratory control sample for the validation of this method. Presently, we use National Institute of Standards and Technology (NIST) 1849a Infant/Adult Nutritional Formula Standard Reference Material (Gaithersburg, MD).

Sample preparation

The nutritional laboratory control sample was digested using a MARS 5 (CEM Corp., Matthews, NC) temperature-controlled, closed-vessel microwave oven. Internal standards were added before sample digestion to correct for losses and remove the need for sample dilution using calibrated volumetric labware. Digestion was carried out in a two-step procedure to break down as much organic matter as possible, using the microwave parameters given in Table 3.

Table 3. Microwave operating parameters for HNO_3 and H_2O_2 two-step sample digestion.

Parameters	Step 1 HNO_3	Step 2 H_2O_2
100% power	1,600 W	1,600 W
Ramp-to-temperature time	20 minutes	15 minutes
Hold time	20 minutes	15 minutes
Temperature	200 °C	180 °C
Cool down time	20 minutes	20 minutes

It is widely reported that the presence of carbon in the sample solution enhances the ICP-MS signal of some poorly ionized elements, including Se [10,11,12]. One theory is that the increased population of C^+ in the plasma increases the degree of ionization of Se by promoting the transfer of electrons from Se (ionization energy 9.75 eV) to C^+ (ionization energy 11.26 eV) [10]. Typically, this signal enhancement effect is overcome by ensuring that there is a consistent level of carbon in all samples, for example by adding methanol to both standards and samples. In this work, methanol was added to samples after closed-vessel microwave digestion [13,14,6]. Digestion takes approximately 1.5 hours (including cool down), is automated, and can achieve complete digestion of the sample without the need for perchloric acid [15].

Calibration

Calibration curves were prepared from 0, 0.8, 4, and 20 µg/L (ppb) standard solutions for Cr and Mo, and 0, 0.4, 2, and 10 µg/L standard solutions for Se. The calibration curves of all three analytes produced R values of 0.9995 or better (Figures 1 to 3). It should be noted that the calibration standards (working standards) were prepared from individual 1 ppm stock solutions of each analyte. Calibration standards were prepared on a weight:weight basis. Alternatively, calibration standards were prepared with a multielement stock solution consisting of all analytes and dispensing with Class A volumetric pipets.

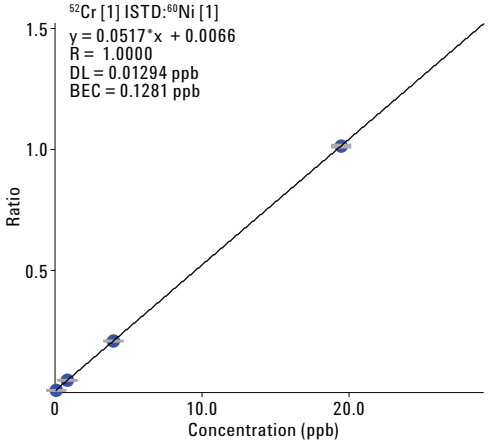

Figure 1. Typical calibration curve for Cr with R ≥ 0.9995.

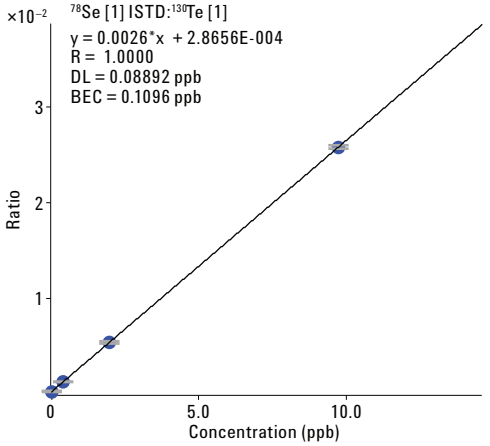

Figure 2. Typical calibration curve for Se with R ≥ 0.9995.

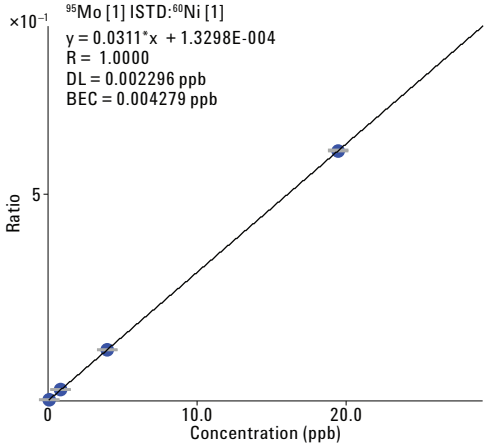

Figure 3. Typical calibration curve for Mo with R ≥ 0.9995.

Method transfer data

In lab 2, four replicate digestions of the laboratory control sample were analyzed on 12 separate days by three different analysts, in line with a typical methodology, to assess method ruggedness. Results of labs 1 and 2 were also compared.

Table 4 shows the 12 independently measured results (12 separate days) produced by all three analysts for Cr in Custom Laboratory control sample 10 CLC10_B. Excellent precision (0.48% to 2.87% RSD) was obtained for the four replicate control samples analyzed on each day. Also, precision for duplicate sample concentrations (samples 1 and 2, or samples 3 and 4) analyzed on each day was less than 2% RSD (protocol requirement is for less than 7% RSD). The overall mean concentration and precision for the 12 independent points obtained by all three analysts were 1,059.36 ng/g Cr and 2.33% RSD, respectively. Table 4 shows that the mean concentrations of Cr obtained on each day (n = 4) and overall (n = 12) were within ± 10% and 3 sigma of the Lab 1 generated control chart mean (1,053.00 ng/g Cr) shown in Figure 4. The Lab 1 results indicate that a precision value of 1.6% RSD was obtained over the 30 days by the three analysts (Table 4). The difference between the Lab 1 control chart mean value (1,053.00 ng/g Cr) and the Lab 2 laboratory mean value (1,059.36 ng/g Cr) was 0.6%.

Table 4. Custom Laboratory control sample 10 analyzed for Cr over 12 separate days by three analysts.

Cr	Custom Laboratory control sample 10 (CLC10_B)						
Analyst	**Day**	**Sample concentration (ng/g)**				**Mean (ng/g)**	**RSD (%)**
1	1	1084.33	1090.81	1096.95	1092.14	1091.06	0.48
	2	1048.87	1062.05	1055.25	1057.55	1055.93	0.52
	3	1063.33	1053.73	1049.73	1054.43	1055.30	0.54
2	1	1082.79	1091.97	1151.06	1090.13	1103.99	2.87
	2	1108.71	1096.43	1062.40	1071.40	1084.73	1.98
	3	1070.82	1055.64	1052.06	1040.06	1054.64	1.20
	4	1074.54	1073.78	1062.18	1069.69	1070.05	0.53
	5	1037.76	1038.63	1048.23	1067.80	1048.11	1.3
	6	1029.96	1025.96	1023.91	1036.40	1029.06	0.5
3	1	1060.53	1056.44	1069.93	1055.33	1060.56	0.63
	2	1031.59	1049.70	1030.33	1032.50	1036.03	0.88
	3	1027.68	1016.48	1022.01	1025.38	1022.89	0.48
Lab 2 mean	12					1059.36	2.33
Lab 1 mean	30					1053.00	1.6

Difference between overall means = 0.6%

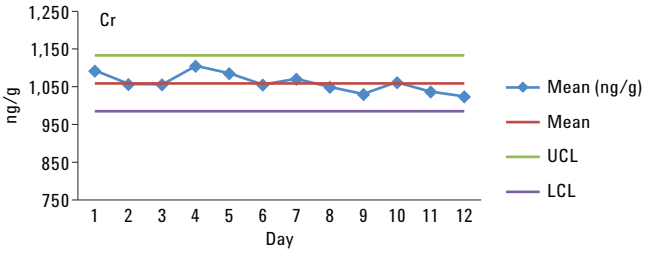

Figure 4. Custom Laboratory control results produced by three analysts for Cr. UCL = upper control limit; LCL = lower control limit.

Table 5 shows the 12 independently measured results (12 separate days) produced by all three analysts for Se in CLC-10. Excellent precision (0.33% to 2.53% RSD) was obtained for the four replicate control samples analyzed on each day. Also, precision for consecutive duplicate sample concentrations (samples 1 and 2, and samples 3 and 4) analyzed on each day was less than 5% RSD (protocol requirement is less than 7% RSD). The overall mean concentration and precision for the 12 independent points obtained by all three analysts were 814.72 ng/g Se and 2.95% RSD, respectively. Table 5 also shows that the mean concentrations of Se obtained on each day (n = 4) and overall (n = 12) were within ± 10% and 3 sigma of the Lab 1 generated control chart mean (1,053.00 ng/g Se) shown in Figure 5. Table 5 shows that a precision value of 1.8% RSD was obtained at Lab 1 over the 30 days by three analysts. The difference between the Lab 1 control chart mean value (813.80 ng/g Se) and the Lab 2 laboratory mean value (814.72 ng/g Se) was 0.11%.

Table 5. Custom Laboratory control sample 10 analyzed for Se over 12 separate days by three analysts.

Se	**Custom Laboratory control sample 10 (CLC10_B)**						
Analyst	**Day**	**Sample concentration (ng/g)**				**Mean (ng/g)**	**RSD (%)**
1	1	828.10	828.22	822.61	824.78	825.93	0.33
	2	845.40	840.98	832.88	804.89	831.04	2.19
	3	798.69	797.53	777.17	797.34	792.68	1.31
2	1	825.87	837.62	862.65	813.01	834.79	2.53
	2	834.92	821.62	836.35	827.63	830.13	0.82
	3	769.73	783.47	791.78	787.63	783.15	1.22
	4	782.21	767.83	759.76	768.17	769.49	1.21
	5	832.14	858.41	806.10	822.45	829.77	2.6
	6	802.30	826.72	835.72	839.35	826.02	2.0
3	1	787.79	791.76	786.04	794.90	790.12	0.50
	2	830.32	856.54	845.63	838.65	842.78	1.32
	3	821.72	811.66	826.68	822.78	820.71	0.78
Lab 2 mean	12					814.72	2.95
Lab 1 mean	30					813.80	1.78

Difference between overall means = 0.11%

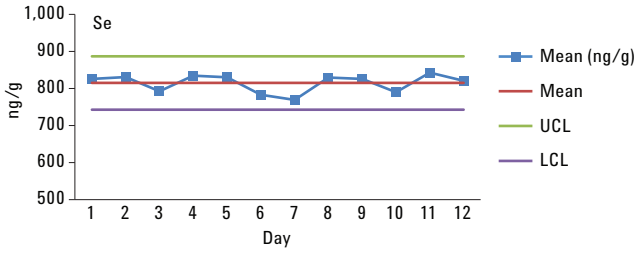

Figure 5. Custom Laboratory control results produced by three analysts for Se. UCL = upper control limit; LCL = lower control limit.

Table 6 shows the 12 independently measured results (12 separate days) produced by all three analysts for Mo in CLC10_B. Excellent precision (0.28% to 5.44% RSD) was obtained for the four replicate control samples analyzed on each day. Also, precision for consecutive duplicate sample concentrations (samples 1 and 2, and samples 3 and 4) analyzed on each day was less than 5% RSD (protocol requirement is less than 7% RSD) for all duplicates. The exception was 8% RSD (1462.98 ng/g Mo and 1641.52 ng/g Mo) obtained by analyst 2 on day 2. This results is highlighted in Table 6. The overall mean concentration and precision for the 12 independent points obtained by all three analysts were 1641.51 ng/g Mo and 1.94% RSD, respectively. Table 6 also shows that the mean concentrations of Mo obtained on each day (n = 4) and overall (n = 12) were within ± 10% and 3 sigma of the Lab 1-generated control chart mean (1,696.00 ng/g Mo). Table 6 shows that a precision value of 1.59% RSD was obtained at Lab 1 over the 30 days by the three analysts. The difference between the Lab 1 control chart mean value (1,696.00 ng/g Mo) and the Lab 2 laboratory mean value (1,641.51 ng/g Mo) was 3.0%.

Table 6. Custom Laboratory control sample 10 analyzed for Mo over 12 separate days by three analysts. %RSD outside protocol is highlighted.

Mo	Custom Laboratory control sample 10 (CLC10_B)						
Analyst	**Day**	**Sample concentration (ng/g)**				**Mean (ng/g)**	**RSD (%)**
1	1	1628.33	1614.66	1620.39	1641.70	1626.27	0.72
	2	1625.22	1621.36	1621.52	1638.34	1626.61	0.49
	3	1624.45	1625.69	1622.69	1633.36	1626.55	0.29
2	1	1639.53	1644.06	1757.45	1655.59	1674.16	3.34
	2	1462.98	1641.52	1631.97	1634.99	1592.86	5.44
	3	1626.86	1635.01	1627.34	1624.31	1628.38	0.28
	4	1706.91	1706.40	1704.52	1725.93	1710.94	0.59
	5	1637.73	1640.49	1652.09	1637.21	1641.88	0.4
	6	1634.95	1631.87	1627.26	1651.66	1636.44	0.6
3	1	1678.21	1679.87	1690.34	1673.59	1680.50	0.42
	2	1626.27	1613.64	1617.34	1619.95	1619.30	0.33
	3	1643.49	1630.91	1631.93	1630.47	1634.20	0.38
Lab 2 mean	12					1641.51	1.94
Lab 1 mean	30					1696.00	1.59

Difference between overall means = 3.0%

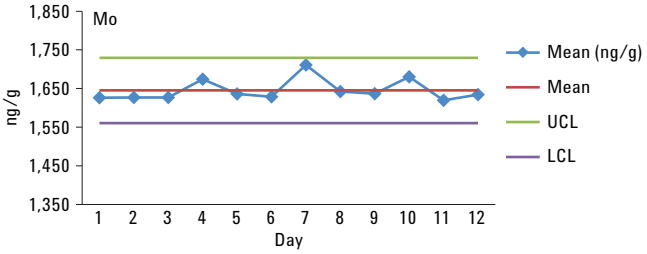

Figure 6. Custom Laboratory control results produced by three analysts for Mo. UCL = upper control limit; LCL = lower control limit.

Practical limit of quantitation

The practical limit of quantitation (PLOQ) values for Cr, Se, and Mo were determined by measuring spike solutions. These solutions were approximately half the concentration of the lowest calibration standards, that is, 0.39 ng/mL for Cr and Mo, and 0.195 ng/mL for Se. Table 7 shows the recoveries obtained by three analysts over six separate days.

The recoveries for Cr (except on one day, 94.1%) and Mo were between 96% and 103%. The overall average recovery for the six days was 98.2% for Cr and 100.5% for Mo. Table 7 also shows the recoveries for Se. On one day, Se recoveries between 95% and 105% were achieved. However, because the overall average recovery for Se was 93.2%, it was decided that the PLOQ for Se should be equivalent to the lowest calibration standard, that is, 0.4 ng/mL. In summary, the PLOQ for Cr, Se, and Mo was 0.4 ng/mL using the 7700x ICP-MS in the He gas mode. It should be noted that PLOQ values of 0.2 ng/mL with Se recoveries between 95% and 105% was achieved in lab 1 using H_2 cell gas mode with our Agilent ICP-MS instruments (7500cx and 7700x).

Table 7. Determination of values for the PLOQ in He gas mode using an Agilent 7700x ICP-MS.

Day	Element	Spike conc. (ng/g)	Analyst 1	Analyst 2	Analyst 3	RSD% (n = 3)	Recovery (%)
1	**Cr**	0.39	0.381	0.379	0.38	0.372	97.44
2			0.373	0.384	0.379	2.055	97.05
3			0.365	0.369	0.367	0.771	94.1
4			0.374	0.379	0.377	0.939	96.54
5			0.4	0.393	0.397	1.248	101.67
6			0.397	0.401	0.399	0.709	102.31
					Overall accuracy (%)		**98.2**
					Overall RSD (%)		**3.2**
1	**Se**	0.195	0.182	0.158	0.17	9.983	87.18
2			0.202	0.168	0.185	12.995	94.87
3			0.179	0.173	0.176	2.411	90.26
4			0.175	0.212	0.194	13.521	99.23
5			0.18	0.188	0.184	3.074	94.36
6			0.173	0.191	0.182	6.993	93.33
					Overall accuracy (%)		**93.2**
					Overall RSD (%)		**4.4**
1	**Mo**	0.39	0.393	0.394	0.394	0.18	100.9
2			0.389	0.392	0.391	0.543	100.13
3			0.384	0.392	0.388	1.458	99.49
4			0.386	0.39	0.388	0.729	99.49
5			0.397	0.393	0.395	0.716	101.28
6			0.396	0.398	0.397	0.356	101.79
					Overall accuracy (%)		**100.5**
					Overall RSD (%)		**1**

Conclusions

An ICP-MS method for the rapid determination of Cr, Se, and Mo in infant formula and adult nutritional products was successfully transferred to the next-generation ICP-MS. The key advantage of the new method is that all samples can be analyzed using a single-cell gas mode (helium mode). This mode provides effective removal of polyatomic interferences on Cr, Mo, and Se, resulting in significantly improved productivity. Samples plus internal standard spikes were prepared using convenient, closed-vessel microwave digestion, followed by sensitive and specific detection by ICP-MS. The study indicates that this method would be suitable as a global reference method (that is, AOAC and International Formula Council) for the determination of Cr, Mo, and Se at trace levels in nutritional products.

For a full account of this methodology, see Pacquette *et al. J. AOAC Int.* **2011**, *94*, 1240-1252.

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