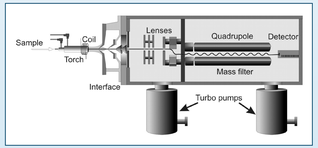
Determination of Heavy Metals in Food: AOAC First Action Method 2015.01

Tamas Ugrai and Michelle L. Briscoe
Brooks Applied Labs, 18804 Northcreek Parkway, Ste 100, Bothell, WA 98011 USA

Abstract

AOAC initiated a call for methods (CFM) for the determination heavy metals in food using Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) for the quantification of arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) in a variety of food items, including rice, chocolate, fruit juice, and infant formula. Brooks Applied Labs (BAL) responded to the CFM, submitting their in-house procedure and supplying method validation data that met all standard method performance requirements (SMPR), which included a required limit of quantitation (LOQ) of 10 µg/kg for most foods. BAL's method was the only method selected for First Action Status by the Expert Review Panel (ERP) and it was assigned method number AOAC 2015.01.

Method detection:
AOAC 2015.01 utilizes ICP-MS, a powerful tool for the simultaneous quantification of multiple elements at sub-part-per-billion (ppb) levels. An aqueous sample is aspirated via a nebulizer attached to a spray chamber. The aerosol then passes through a quartz torch where it is atomized in a stream of argon superheated by an RF coil. The atoms are then directed to the detector, selected by their mass to charge ratios (m/z) by a quadrupole mass filter. The concentration of metals are then quantified by the instrument software.



Challenges to Achieving SMPR

Matrix Effects:
The AOAC SMPR dictated very low LOQs (8 – 10 ppb). LOQs at this level can be challenging to achieve in a food matrix due to the large mass of sample relative to the final dilution of the preparation, as well as high levels of total suspended solids (TSS) and total dissolved solids (TDS) in the digestate. For example according to EPA Method 200.8, it is recommended that TDS concentration in the diluted extract should not exceed 0.2% (w/v), and even then further dilution may be needed (REF 1). High dilution factors result in elevated LOQs and decreased reproducibility in low-level samples.

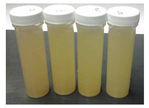


Image of aliquots of 3050B.

To achieve a complete digestion, AOAC 2015.01 employs a microwave digestion technique. The advantage of a microwave system is the rapid heating inside a pressurized vessel allows for efficient throughput and complete dissolution of biota matrices. The reagents are a complementary mix of nitric acid and hydrogen peroxide where the hydrogen peroxide reduces the nitrous vapors which not only recycles the breakdown of the nitric acid, but also raises the temperature resulting in faster and more complete digestions. (REF 2)


Image of microwave clean MW prep.

The use of microwave digestion in AOAC 2015.01 contributed to the ability to achieve LOQs lower than or equal to all SMPR for all element matrix combinations.

The achieved Limit of Detection (LOD) and Limit of Quantification (LOQ) achieved using the standard deviation of method blanks.

Achieved LODs and LOQs (µg/kg)

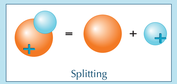
	As	Cd	Pb	Hg
LOD	1.6	0.5	3.5	2.3
LOQ	3.3	1.6	7.1	4.6

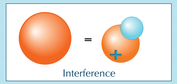
The achieved LODs using the standard deviation of results in specific matrices.

Achieved Matrix-Specific LOQs (µg/kg)

	As	Cd	Pb	Hg
Infant formula	2	1	4	3
Chocolate	4	2	8	6
Rice flour	4	2	8	6
Fruit juice	3	1	2	2

Polyatomic Interferences:
Polyatomic interferences are caused by ions, composed of multiple atoms, which have the same m/z as the analyte of interest, and which cannot be resolved by the mass spectrometer. For example, monoisotopic arsenic has a m/z of 75. When chlorine (m/z 35) reacts with argon (m/z 40) in the plasma, a polyatomic molecule with the same m/z as As is created. The presence salt (sodium chloride) in a food sample can result in an erroneous high bias for the determination of arsenic.





The use of collision or reaction cell (CRC) technology can be critical to removing polyatomic interferences. {Elements not determined with CRC can be corrected by using correction equations in the ICP-MS software.}

When utilizing CRC, a cell (between the ion optics and the mass filter) is filled with an inert or reactive gas, depending on the mode of operation. The gas can interact with the polyatomic molecule in a variety of ways, including:

- Collision-induced dissociation
- Chemical reaction
- Electron transfer (charge transfer)
- Collisional retardation and differential transmission – also known as kinetic energy discrimination (KED)

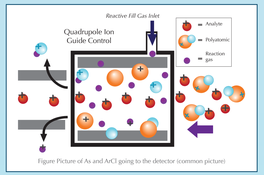


Figure Picture of As and ArCl going to the detector (common picture)

While the SMPR did not specifically require the use of CRC technology with the ICP-MS, it's use is included in Method 2015.01 and it was critical of obtaining the method validation results reported.

Quality Control Protocols Table

QC sample	Measure	Minimum frequency	Acceptance criteria	Corrective action
Calibration standards	Linearity of the calibration curve	Analyzed once per analytical day	Correlation coefficient ≥0.995, 1st standard ≤MRL, standard recoveries = 80-120%	Reanalyze suspect calibration standard. If criteria still not met, then reprepare standards and recalibrate the instrument.
Internal standards	Variation in sample properties between samples and standards	Each standard, blank, and sample is spiked with internal standard	60-125% recovery compared to calibration blank	Rerun the sample at an additional 2x dilution. If not, then samples must be reanalyzed with a new calibration.
Lu Digestion Check Spike	Assessment of potential loss during digestion	Added to every digested samples	Recovery ≥ 75%	Re-prepare the sample.
Initial calibration verification (ICV)	Independent check of system performance	One following instrument calibration	Recovery = 90-110%	Correct problem prior to continuing analysis. Recalibrate if necessary.
Continuing calibration verification (CCV)	Accuracy	At beginning and end of analysis and one per 10 injections	Recovery = 85-115%	Halt analysis, correct problem, recalibrate, and reanalyze affected samples.
Method blanks (MB)	Contamination from reagents, lab ware, etc.	Minimum of three per batch	Mean ≤ MRL; SD ≤ MDL or MBs <1/10th sample result	Determine and eliminate cause of contamination. Affected samples must be reprepared and reanalyzed.
Method duplicates (MD)	Method precision within a given matrix	Minimum of one per 10 samples	RPD ≤ 30% or ±2x LOQ if results ≤5x LOQ	If RPD criteria not met, then sample may be reprepared and reanalyzed, but this is not required. Sample matrix may be inhomogeneous. The sample can be re-analyzed to confirm instrument stability
Matrix spikes/matrix spike duplicates (MS/MSD)	Method accuracy and precision within a given matrix	Minimum of one per 10 samples	Recovery = 70-130% and RPD ≤ 30%	If RPD > 30%, results must be qualified
Laboratory fortified blank (LFB) or blank spike (BS)	Method accuracy	Minimum of one per batch	Recovery = 75-125%	If LFB recovery is outside of the control limit, then batch must be reprepared and reanalyzed
Certified reference material (CRM)	Method accuracy	Must be matrix matched to samples; minimum of one per batch	Recovery = 75-125% unless limits set by CRM manufacturer are greater.	If CRM true value is ≥5x the LOQ and recovery is outside of the control limit, then batch must be reprepared and reanalyzed

In addition to common ICP-MS quality control protocols that can be found in EPA methods, BAL uses a lutetium (Lu) digestion spike similar to surrogate spikes used in organics analyses. The recovery of this spike is used to evaluate sample loss during preparation. Separately, internal standards are introduced into the sample during the sample introduction process to monitor for drift and matrix effects. Therefore, potential issues are monitored for at both sample preparation and sample analysis.

Method Performance

The SMPR included:

- A limit of quantitation of at least 10 ppb (µg/kg) for food matrices (8 ppb for infant formula)
- Repeatability (between analyses of a single sample preparation) requirements for relative standard deviation (RSD) and spike recovery, including criteria at different concentrations
- Reproducibility (between replicate sample preparations) requirements of RSD and spike recovery, including criteria at different concentrations

SMPR requirements	Repeatability	Reproducibility	Recovery
≥ 8 ppb to 100 ppb	15%	32%	60 - 115%
> 100 ppb to 1 ppm	11%	16%	80 - 115%
> 1 ppm to 10 ppm	7.3%	8.0%	80 - 115%

ppb = µg/kg
ppm = mg/kg

Repeatability study for blank spikes:

Repeatability Study for Blank Spike Samples

Element	Spiking Level (µg/kg)	n	RSD	SMPR RSD
As	20	5	5%	15%
	100	5	4%	15%
	1000	5	4%	11%
	10,000	5	2%	7%
Cd	20	5	4%	15%
	100	5	2%	15%
	1000	5	1%	11%
	10,000	5	1%	7%
Hg	20	5	4%	15%
	100	5	3%	15%
	1000	5	0%	11%
	10,000	5	5%	7%
Pb	20	5	2%	15%
	100	5	1%	15%
	999	5	1%	11%
	9,990	5	5%	7%

The repeatability study for blank spike samples analyzed at the SMPR levels analyzed in in five replicate injection of the same prepared sample were all well within the RSD criteria range of the method.

Reproducibility and recovery study for blank spikes:

Reproducibility and Recovery Study for Blank Spike Samples

Element	Spiking Level (µg/kg)	n	RSD	SMPR RSD	Recovery	SMPR Rec
As	20	5	7%	32%	93%	60 - 115%
	100	5	6%	32%	100%	60 - 115%
	1000	5	4%	16%	87%	80 - 115%
	10000	5	4%	8%	88%	80 - 115%
Cd	20	5	5%	32%	69%	60 - 115%
	100	5	2%	32%	68%	60 - 115%
	1000	5	2%	16%	99%	80 - 115%
	10000	5	2%	8%	106%	80 - 115%
Hg	20	5	7%	32%	149%[a]	60 - 115%
	100	5	9%	32%	146%[a]	60 - 115%
	1000	5	2%	16%	85%	80 - 115%
	10000	5	2%	8%	97%	80 - 115%
Pb	20	5	5%	32%	160%[a]	60 - 115%
	100	5	3%	32%	151%[a]	60 - 115%
	999	5	2%	16%	84%	80 - 115%
	9990	5	1%	8%	88%	80 - 115%

[a]Potential error identified with the creation of the spiking solution used for these blank spikes.

The reproducibility of the blank spikes analyzed in five replicates of different preparations spiked at the same level show extremely low RSD levels compared to the SMPR requirements. The low level spikes for Hg, and Pb were found to be erroneously spiked at preparation.

Matrix Specific Repeatability, Reproducibility, and Recovery Studies

To determine the method stability in actual food matrices BAL digested and analyzed actual anonymous food samples purchased from a local grocery store.

Apple Juice		As (µg/kg)	Cd (µg/kg)	Hg (µg/kg)	Pb (µg/kg)
Repeatability Study	Mean (n=3)	11	< 2	< 2	< 2
	RSD	4%	N/A	N/A	N/A
Reproducibility Study	Mean (n=3)	10	< 2	< 2	< 2
	RSD	2%	N/A	N/A	N/A
Mean Spike Recovery (n=4)		95%	89%	85%	112%

Apple Juice:
The repeatability and reproducibility of the arsenic in apple juice was low 4%, 2% respectively. All other analytes were less than 5 times the minimum limit of the SMPR, therefore RSD's were not calculated.

Infant Formula		As (µg/kg)	Cd (µg/kg)	Hg (µg/kg)	Pb (µg/kg)
Repeatability Study	Mean (n=3)	7	< 8	< 8	13
	RSD	16%	N/A	N/A	7%
Reproducibility Study	Rep 1	< 8	< 8	< 8	11
	RSD	15%	N/A	N/A	N/A (33%)
Mean Spike Recovery (n=4)		106%	95%	79%	97%

Infant formula:
The first analysis of infant formula the concentrations were somewhat variable for arsenic since the infant formula was prepared from a dry powder and not reconstituted to a liquid prior to analysis. Detectable results for arsenic are reported from a milk based formula where the repeatability study was analyzed on 5 samples.

Cocoa Powder		As (µg/kg)	Cd (µg/kg)	Hg (µg/kg)	Pb (µg/kg)
Repeatability Study	Mean (n=3)	52	500	72	65
	RSD	11%	0%	2%	2%
Reproducibility Study	Mean (n=3)	60	499	69	68
	RSD	3%	2%	6%	2%
Mean Spike Recovery (n=4)		95%	81%	85%	93%

Cocoa Powder:
Although considered to be a tough matrix due to the high level of fats in cocoa powder, the repeatability and reproducibility studies for the samples produced results that were well below the SMPR required limits. The cocoa powder was the only matrix that resulted detectable results for all analytes. As, Hg and Pb were all within the lowest criteria range between 10 to 100 ppb. The mean reproducibility and repeatability was less than 5% for those analytes and almost 0 for Cd at the 500 ppb level.

Rice Flour		As (µg/kg)	Cd (µg/kg)	Hg (µg/kg)	Pb (µg/kg)
Repeatability Study	Mean (n=3)	405	< 10	1004	11
	RSD	3%	N/A	2%	5%
Reproducibility Study	Mean (n=3)	412	< 10	981	11
	RSD	3%	N/A	5%	3%
Mean Spike Recovery (n=4)		108%	88%	67%[a]	95%

[a]Spiking level was less than sample concentration.

Rice Flour:
Although a rather simple matrix for this digestion method, rice is used extensively in the food industry. Due to the large amount of rice used in food components it can lead to significant levels of heavy metals, especially arsenic, in the final food product. Alarmingly, the mercury content of this specific sample of rice was unusually high, resulting in the analytical spike to be at an improper level, causing the recoveries to be low.

Method Accuracy

The CRM's analyzed were:

- National Institute of Standards and Technology (NIST) 1946 - Lake superior fish tissue certified for mercury (433 µg/kg) and arsenic (277 µg/kg)
- NIST 1568a - Rice Flour certified for arsenic (290 µg/kg) and cadmium (22 µg/kg)
- NIST 1548a - Typical Diet an SRM containing food items that appear on a 4 day typical diet. The CRM is certified for arsenic (200 µg/kg), cadmium (35 µg/kg), and lead (44 µg/kg)
- National Research Council Canada (NRCC) DORM-3 – Fish Protein CRM certified for arsenic (6,800 µg/kg), cadmium (306 µg/kg), mercury (410 µg/kg), and lead (416 µg/kg)
- NRCC TORT-3 - Lobster hepatopancreas CRM certified for arsenic (59,500 µg/kg), cadmium (42,300 µg/kg), mercury (292 µg/kg), and lead (225 µg/kg)

SRM	Element	Certified Value (µg/kg)	Mean	Rec	RSD/ RPD
NIST 1568a - rice flour	As	290 ± 30	303	105%	3%
NIST 1568a - rice flour	Cd	22 ± 2	22	100%	1%
DORM-4 - fish protein	As	6,800 ± 640	7110	105%	2%
DORM-4 - fish protein	Cd	306 ± 15	279	91%	2%
DORM-4 - fish protein	Hg	410 ± 55	332	81%	1%
DORM-4 - fish protein	Pb	416 ± 53	379	91%	4%
TORT-3 - lobster hepatopancreas	As	59,500 ± 3,800	67500	113%	1%
TORT-3 - lobster hepatopancreas	Cd	42, 300 ± 1,800	37800	89%	1%
TORT-3 - lobster hepatopancreas	Hg	292 ± 22	252	86%	2%
TORT-3 - lobster hepatopancreas	Pb	225 ± 18	194	86%	2%
NIST 1548a - typical diet	As	200 ± 10	202	101%	6%
NIST 1548a - typical diet[1]	Cd	35 ± 2	< 20	N/A	N/A
NIST 1548a - typical diet	Pb	44 ± 9	44	101%	6%
NIST 1946 - fish tissue	As	277 ± 10	342	124%	6%
NIST 1946 - fish tissue	Hg	433 ± 9	438	101%	2%

[1]Only half of the recommended mass of the sample was digested in this study, hence increasing the detection limit of cadmium

Conclusion

This robust method for the analysis of four heavy metals (As, Cd, Hg, and Pb) in food provides sufficient detail to allow any trained ICP-MS analyst to easily follow it. The method submitted by Brooks Applied Labs was the only submittal selected for First Action Status for this CFM by the ERP, and the method was published in the Official Methods of Analysis as AOAC First Action Method 2015.01.

REF 1: US EPA Method 200.8, Revision 5.4 (1994)
REF 2: Milestone, Inc. (Shelton, CT), Ethos D User Manual Rev. 0 (2001)