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# Determination of Toxic Elements in Food Following AOAC Method 2015.01 using the NexION ICP-MS

## Introduction

Metal elements are an integral part of foods for humans and animals. Elements can be taken up by crops and animals from the air, water, soil, and food during growth, and/or can be introduced as contaminants during food processing. While some of these elements are essential macronutrients, others, such as As, Cd, Hg, and Pb, are considered toxic. Through the consumption of contaminated food, humans are exposed to toxic elements with the risk increasing proportionately to the amount consumed. These toxic elements can have neurological-, endocrine- and exocrine-disrupting properties as well as, in some cases, may be genotoxic and carcinogenic,[1] therefore the contamination of food and water by toxic elements is of great concern.

Concentrations of toxic elements in foods are present at trace to ultra-trace levels, which requires sensitive and reliable analytical techniques for accurate measurement. ICP-MS is a powerful elemental analysis technique with multi-element detection capabilities, low detection limits, high speed of analysis, and wide linear dynamic range, among many other advantages. However, as with any analytical technique, it is susceptible to interferences, such as those arising in the plasma from the plasma gases, the surrounding atmosphere, and the matrix, leading to the formation of polyatomic and doubly-charged ions.

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Polyatomic interferences are generated by the reaction of matrix ions and plasma-based elements, such as Ar, C, N, O, and H, which can overlap target ions. Examples of such polyatomic interferences relevant to toxic-element analysis include $^{40}Ar^{35}Cl^+$ on $^{75}As^+$, $^{95}Mo^{16}O^+$ on $^{111}Cd^+$, $^{185}W^{16}O^+$ on $^{201}Hg^+$, etc. Doubly-charged ions are formed by the secondary ionization of elements with low second-ionization potentials, such as rare earth elements (REEs). For example, the doubly-charged ions from $^{150}Nd$ and $^{150}Sm$ may interfere with $^{75}As$, which can result in false overestimation. Rare earth elements are not usually present in significant amounts in food samples, but, with increased industrialization and globalization, food products can be sourced from geological regions with high REEs. As such, rare earth elements present in some food products should not be overlooked in elemental analyses.

PerkinElmer's NexION® ICP-MS is equipped with Universal Cell Technology (UCT) that can run in Collision mode with kinetic energy discrimination (KED) as well as Reaction mode with dynamic bandpass tuning (DBT). The Collision mode involves colliding both the analytes and interferences with an inert gas, usually helium. Since polyatomic interferences have a larger cross-sectional diameter than elemental ions at the same mass (e.g., $^{95}Mo^{16}O^+$ is larger than $^{111}Cd^+$), they undergo more collisions and lose more energy, therefore cannot pass the energy barrier at the exit of the collision/reaction cell and are rejected from the ion beam. Interferences from doubly-charged ions and isobars, however, cannot be effectively resolved using Collision mode. A few techniques have been developed to correct for these interferences, which can be either through post-analysis mathematical corrections or via the use of reaction gases. For doubly-charged ions, mathematical corrections using the half-mass approach, i.e., correction equations at higher resolution, are often not preferred as this comes at the expense of analyte sensitivity. A more effective means of removing these interferences is to use reaction gases where, in the case of As, it reacts with oxygen to form $^{75}As^{16}O^+$ at mass 91, so moving away by +16 amu from the respective doubly-charged interferents. An added benefit to this approach is that it offers the lowest detection limit without compromising analyte sensitivity.

The Association of Official Analytical Collaboration (AOAC) International elemental analytical method AOAC 2015.01 describes the analysis of toxic elements in food samples using PerkinElmer's ELAN® DRC II ICP-MS,[2] which is a predecessor to the current NexION ICP-MS. In this work, the analysis of toxic elements As, Cd, Hg, Pb, and Sb in various food matrices following AOAC 2015.01 was validated using the NexION ICP-MS. Though Sb is not a target element in AOAC 2015.01, its analysis was performed due to its regional relevance.

## Experimental

### Instrumentation

Sample digestion was performed using a Titan MPS™ microwave digestion system (PerkinElmer Inc., Shelton, Connecticut, USA). All analyses were performed using a NexION 2000 ICP-MS (PerkinElmer Inc.) equipped with a High Throughput System (HTS) and an S20 series autosampler. The HTS uses flow injection technology to deliver the sample to the plasma during the data acquisition stage and diverts the sample to the waste for the rest of the time, reducing the total sample load on the plasma and resulting in less maintenance and downtime. Since the sample loading and loop wash are enabled by the vacuum pump via Syngistix™ software, HTS can perform the wash procedure more efficiently than conventional sample introduction. The instrument components and operating conditions used for the analysis are shown in Table 1.

*Table 1. NexION ICP-MS Instrument Components and Operating Conditions.*

| Instrument Component | Type/Value |
|---|---|
| Nebulizer | ST-PFA MicroFlow |
| Spray chamber | Quartz cyclonic |
| Torch | One-piece quartz torch, 2 mm i.d. injector |
| Cones | Nickel sampler and skimmer<br>Aluminum hyper-skimmer |
| Peristaltic Pump Tubing | Carrier/internal standard: orange/green (0.38 mm i.d.)<br>Waste: gray/gray Santoprene (1.30 mm i.d.) |
| Sample Uptake Rate | 0.2 mL/min |
| **Operating Conditions** | **Type/Value** |
| RF Power | 1600 W |
| Plasma Gas Flow | 15 L/min |
| Auxiliary Gas Flow | 1.2 L/min |
| Nebulizer Gas Flow | Optimized for CeO⁺/Ce⁺ < 2.5%; Ce⁺⁺/Ce < 2.5% |
| Cell Gas | Oxygen, helium |

## Instrument Optimization and Method Development

The analyzed elements, their respective isotopes, and the mode of analysis used for each element in this method are listed in Table 2. Cobalt, Mo, Zr, and W were monitored for interference evaluation. Among the analytes, As was measured as AsO in Reaction (DRC) mode using oxygen as the cell gas, and the rest of the analytes were measured in Collision (KED) mode using helium as the cell gas.

*Table 2. Elements, Isotopes, and Mode of Analysis.*

| Element | Mass | Mode |
|---|---|---|
| As | 91 | Oxygen DRC |
| Cd | 111 | Helium KED |
| Hg | 201 | Helium KED |
| Sb | 121 | Helium KED |
| Pb* | 206+207+208 | Helium KED |
| Co** | 59 | Helium KED |
| Mo** | 95 | Helium KED |
| Zr** | 91 | Helium KED |
| W** | 182 | Helium KED |
| Lu*** | 175 | Helium KED |

\* Sum of three isotopes to address the natural abundance variation.

\*\* Monitored elements

\*\*\* Element analyzed for digestion recovery evaluation.

## Reagents and Calibration Standards

Ultrapure water (UPW; 18.2 MΩ.cm) and high-purity acids (Tamapure, Tama Chemicals, USA) were used throughout this work. The calibration standards were prepared by the dilution of ICP-MS grade multi-element standards (see Consumables Used table) in a diluent made of 5% $HNO_3$ spiked with 200 ppb of gold to aid the mobility of Hg. This diluent was also used as the calibration blank and carrier solution. The concentrations of the calibration standards are shown in Table 3. Standard 4 was also used as a continuing calibration verification (CCV) standard.

The internal standard solution was made up of 40 ppb of Rh and Ir in a solution of 1% $HNO_3$ and 4% isopropanol (IPA) and spiked with 200 ppb of gold. Isopropanol was added to compensate for the difference in the carbon content in the standards and the samples and among samples to address the carbon-induced signal enhancement effects on As.[3-4] The internal standard was introduced into the designated port of the HTS valve and mixed online with the sample. The wash solution consisted of 1.5% HCl (v/v), 1.5% $HNO_3$ (v/v), and 5% IPA and was spiked with 200 ppb gold, where IPA was used to facilitate the washout of organic materials.

## Interference Removal Checking Solutions

In this work, the interference from $^{59}Co^{16}O_2^+$ and $^{91}Zr^+$ on $^{91}AsO^+$, $^{95}Mo^{16}O^+$ on $^{111}Cd^+$, and $^{185}W^{16}O^+$ on $^{201}Hg^+$ was evaluated using a series of single-element standard solutions of Co, Zr, Mo and W which were prepared in 3% $HNO_3$ with concentrations of 10, 50 and 100 ppb each.

## Sample Preparation

Three food certified reference materials: SRM® 1568b (Rice Flour) and SRM® 2976 (Mussel Tissues) purchased from the National Institute of Science and Technology (NIST) (Gaithersburg, Maryland, USA), and DORM-5 (Fish Protein) purchased from National Research Council Canada (Ottawa, Ontario, Canada) were used to validate the accuracy of the method. They were also used to evaluate the method precision (via method duplicates) and matrix spikes, in which the samples were spiked with multi-element standards to the concentrations of 1 ppb for As, Cd, Sb and Pb, and 0.1 for Hg in the analytical solutions. For all food samples, Lu was added before digestion to assess the potential loss during digestion, as specified in AOAC 2015.01. Three method blanks were included in each digestion batch. The blanks were treated the same as the samples and went through all the preparative steps.

The digestion vessel was pre-wet by adding a few drops of ultrapure water (UPW). Food samples of ca. 0.5 g were accurately weighed and then transferred into 75 mL microwave digestion vessels. Then, 1 mL of UPW was added, followed by 8 mL $HNO_3$, 2 mL $H_2O_2$, and 0.1 mL of 100 mg/L Au and 100 mg/L Lu. The method blanks, samples, and matrix spikes were digested in the same batch using the built-in Rice Flour Method. Microwave digestion parameters are shown in Table 4.

*Table 3. Concentrations of the Calibration Standards.*

| Element | Standard 1 (ppb) | Standard 2 (ppb) | Standard 3 (ppb) | Standard 4 (ppb) | Standard 5 (ppb) | Standard 6 (ppb) |
|---|---|---|---|---|---|---|
| As, Cd, Sb, Pb, Lu | 0.01 | 0.1 | 1 | 10 | 25 | 100 |
| Hg | 0.001 | 0.01 | 0.1 | 1 | 2.5 | 10 |

*Table 4. Microwave Digestion Parameters.*

| Step | Target Temperature (°C) | Pressure (Bar) | Ramp Time (min) | Hold Time (min) | Power (%) |
|---|---|---|---|---|---|
| 1 | 160 | 30 | 5 | 5 | 90 |
| 2 | 190 | 30 | 3 | 20 | 100 |
| 3 | 50 | 30 | 1 | 15 | 0 |

After digestion, the samples were decanted into the pre-weighed centrifuge tubes and brought up to the 40 mL mark with UPW to obtain the intermediate sample solution, and weights were recorded. An aliquot of the intermediate sample solution (10 mL) was pipetted into a pre-weighed 50 mL centrifuge tube, then UPW was added to the 40 mL mark and the weights were recorded. A total dilution factor of *ca.* 320 was achieved and the samples were ready for ICP-MS analysis.

## Results and Discussion

### Evaluation of the Interference Mitigation/Removal

The effectiveness of the interference mitigation/removal was evaluated by the background equivalent concentrations (BECs) of the target elements in the interference removal checking solutions. The results are shown in Table 5. For As, which is analyzed as AsO, the background is at ppt/sub-ppt levels with Zr and Co at concentrations up to 100 ppb; for Hg and Cd, the background is ≤ 10 ppt with W and Mo at concentrations up to 50 ppb. In all cases, the concentrations tested for the interfering elements are much higher than can be expected in normal food materials. Nevertheless, the interfering elements were monitored in this study to look out for extreme situations.

### Lu Digestion Spike

Recoveries higher than 85% were achieved for all digested samples including matrix spikes, blanks, and blank spikes, meeting the acceptance criteria (≥ 75%) as recommended in AOAC Table 2015.01F.

### Detection Limits and Background Equivalent Concentrations

The Limit of Quantification (LOQ) was calculated as 10 times the standard deviation of 10 replicated measurements of the method blank multiplied by the dilution factor.

A comparison of the LOQs achieved in this work and those provided in AOAC 2015.01 is shown in Figure 1. For all the target elements, the LOQs obtained in this work were lower than those of different food types published in AOAC Table 2015.01H.

### Accuracy

The accuracy was verified by evaluating the recoveries of various analytes achieved for the SRMs: NIST 1568b (Rice Flour), NIST 2976 (Mussel Tissues), and CRM DORM-5 (Fish Protein), and the matrix spikes.

The CRMs and matrix spikes were prepared in duplicates and each preparation was measured three times. The mean recoveries of the duplicates are shown in Figure 2. Recoveries between 85% and 114% were obtained for the certified elements, falling well within the QC criteria of 75-125% as provided in AOAC Table 2015.01F.
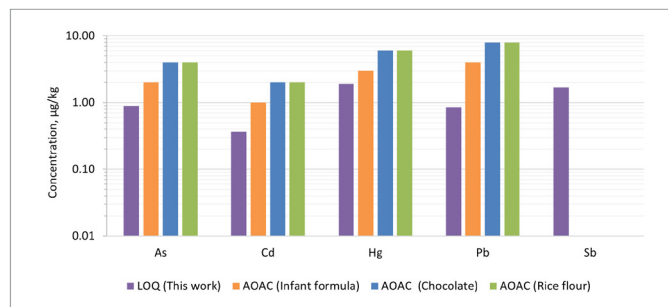


Figure 1. Comparison of limits of quantification (LOQs) achieved in this work with those captured in AOAC Table 2015.01H.
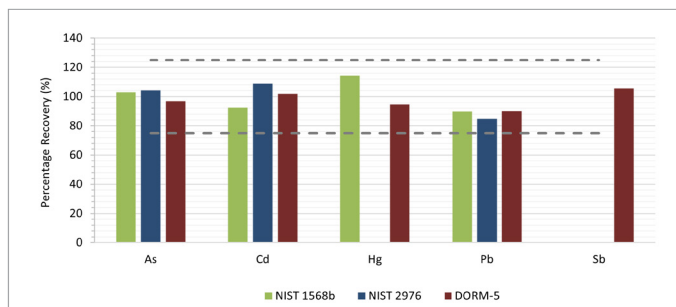
Table 5. Interferences Evaluation for the Target Elements.

| Element | Concentration (ppb) | Interfering Ions | BEC of Interfered Analyte (ppb) | | |
| --- | --- | --- | --- | --- | --- |
| | | | $^{91}AsO^+$ | $^{201}Hg^+$ | $^{111}Cd^+$ |
| Zr | 10 | $^{91}Zr^+$ | <DL | | |
| | 50 | | <DL | | |
| | 100 | | 0.0002 | | |
| Co | 10 | $^{59}Co^{16}O_2^+$ | | 0.001 | |
| | 50 | | | 0.002 | |
| | 100 | | | 0.003 | |
| W | 10 | $^{185}W^{16}O$ | | 0.003 | |
| | 50 | | | 0.010 | |
| | 100 | | | 0.025 | |
| Mo | 10 | $^{95}Mo^{16}O^+$ | | | 0.002 |
| | 50 | | | | 0.006 |
| | 100 | | | | 0.013 |

*Figure 2. Recoveries for the certified elements in the CRMs.*

Matrix spikes were performed on three CRMs. Duplicates were prepared for each spiked sample. The mean recoveries are shown in Figure 3. Recoveries between 75% and 113% were obtained for all target elements, meeting the QC acceptance criteria of 70-130% as specified in AOAC Table 2015.01F.

**Precision**

The precision was evaluated by the relative percent difference (RPD) of duplicated sample analysis. The duplicated samples were separately digested and went through the same sample preparation process as individual samples. These tests were performed on three CRMs. In all cases, the RPDs were within the QC acceptance criteria of < 30% as specified in AOAC Table 2015.01F for all target elements, as shown in Table 6.

**Stability Check**

To validate the stability of the method, various food samples were measured repeatedly over eight hours and the CCV

recoveries were checked over this period. CCV was measured every 10-15 samples. All CCV recoveries were normalized to Standard 4 (Table 3) and were well within ± 10% of the original reading as shown in Figure 4, meeting the QC acceptance criteria of 85-115% as specified in AOAC Table 2015.01F.
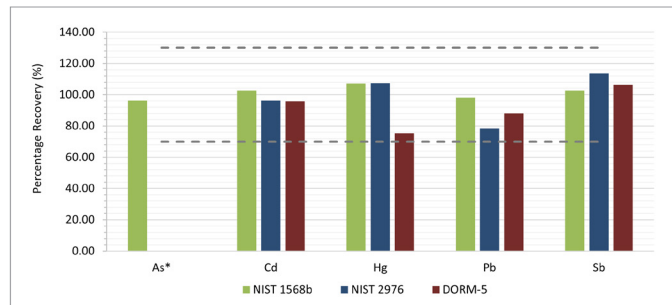


*Figure 3. Spike recoveries for the tested CRMs. *The recoveries of As for NIST 2976 and DORM-5 were not reported. The spike level in this case was too low (<5% of the intrinsic amount), resulting in higher errors.*
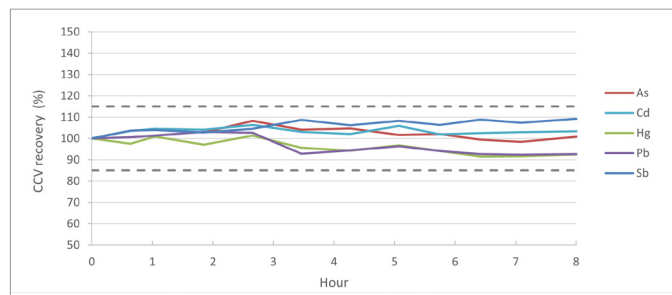


*Figure 4. CCV recoveries over an 8-hour run of food samples.*

*Table 6. RPD Test Results on the CRMs**

| Element | NIST 1568b | | | NIST 2976 | | | DORM-5 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Measured Conc. (µg/kg) Portion A | Measured Conc. (µg/kg) Portion B | RPD (%) | Measured Conc. (µg/kg) Portion A | Measured Conc. (µg/kg) Portion B | RPD (%) | Measured Conc. (µg/kg) Portion A | Measured Conc. (µg/kg) Portion B | RPD (%) |
| As | 296.33 | 290.80 | 2 | 14035.8 | 13680.4 | 3 | 12884.6 | 12879.5 | 0.04 |
| Cd | 19.91 | 21.48 | 8 | 892.99 | 890.26 | 0.3 | 154.01 | 147.66 | 4 |
| Hg | 8.58 | 6.76 | 24 | 64.06 | 57.44 | 11 | 301.34 | 296.21 | 2 |
| Pb | 7.08 | 7.27 | 3 | 1055.0 | 963.99 | 9 | 50.68 | 53.77 | 6 |
| Sb | < LOQ | < LOQ | - | 10.69 | 11.66 | 9 | 6.48 | 6.61 | 2 |
| Se | 383.02 | 375.03 | 2 | 2056.9 | 2005.2 | 3 | 2469.1 | 2464.8 | 0.2 |

* Portion A and Portion B were separately digested

## Conclusions

The method outlined in AOAC 2015.01 was validated with the NexION ICP-MS for the analysis of toxic elements As, Cd, Hg, Pb, and Sb in various food matrices.

- The Titan MPS microwave digestion system was used to digest the food samples using a built-in method.

- Flow injection with HTS was used for sample introduction to increase the sample throughput and reduce the total sample residence time in the plasma.

- The NexION 2000 ICP-MS was used for the quantitation of target elements. Versatile gas modes were used to reduce or remove spectral interferences.

Following AOAC 2015.01, the work was evaluated for the limits of quantitation, accuracy, precision, and stability. All the QC criteria were met and/or improved upon, showing the suitability of the Titan MPS microwave digestion system and the NexION 2000 ICP-MS for this method and routine laboratory analysis.

## References

1. Yiannis Fiamegos, Mitja Vahcic, Håkan Emteborg, James Snell, Georg Raber, Fernando Cordeiro, Piotr Robouch, Beatriz de la Calle; Determination of Toxic Trace Elements in Canned Vegetables. The Importance of Sample Preparation; Trends in Analytical Chemistry, 85, 57-66 (2016).

2. AOAC Official Method 2015.01; Determination of Heavy Metals in Food by Inductively Coupled Plasma-Mass Spectrometry First Action 2015.

3. Erik H. Larsen and Stefan Sturup; Carbon-enhanced Inductively Coupled Plasma Mass Spectrometric Detection of Arsenic and Selenium and Its Application to As Speciation; J. Anal. At. Spectrom., 9, 1099-1105 (1994).

4. Tomoko Ariga, Yanbei Zhu and Kazumi Inagaki; Study on carbon-induced signal enhancement in inductively coupled plasma mass spectrometry: an approach from the spatial distribution of analyte intensities; J. Anal. At. Spectrom., 34, 1865-1874 (2019).

## Consumables Used

| Component | Description | Part Number |
|---|---|---|
| Peripump Tubing | Carrier/Internal Standard: Orange/Yellow (0.38 mm i.d.)<br>Waste: Gray/Gray Santoprene (1.30 mm i.d.) | N8152403<br>N8152415 |
| Internal Standard Mix | 200 ppm Sc, 10 mg/L of Rh and Ir | N9307738 |
| Multi-Element Standard | 100 µg/mL of As, Cd, Pb, and Sb in 5% $HNO_3$/Trace Tartaric Acid/Trace HF | N9301721 |
| Single-Element Standards | 1000 µg/mL of Hg in 10% $HNO_3$ | N9303740 |
| | 1000 µg/mL of Co in 2% $HNO_3$ | N9303735 |
| | 1000 µg/mL of Zr in 2% $HNO_3$ | N9303812 |
| | 1000 µg/mL of Mo in $H_2O$ | N9303745 |
| | 1000 µg/mL of W in $H_2O$ | N9303809 |
| | 1000 µg/mL of Lu in 2% $HNO_3$ | N9303782 |
| | 1000 µg/mL of Au in 10% HCl | N9303759 |

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