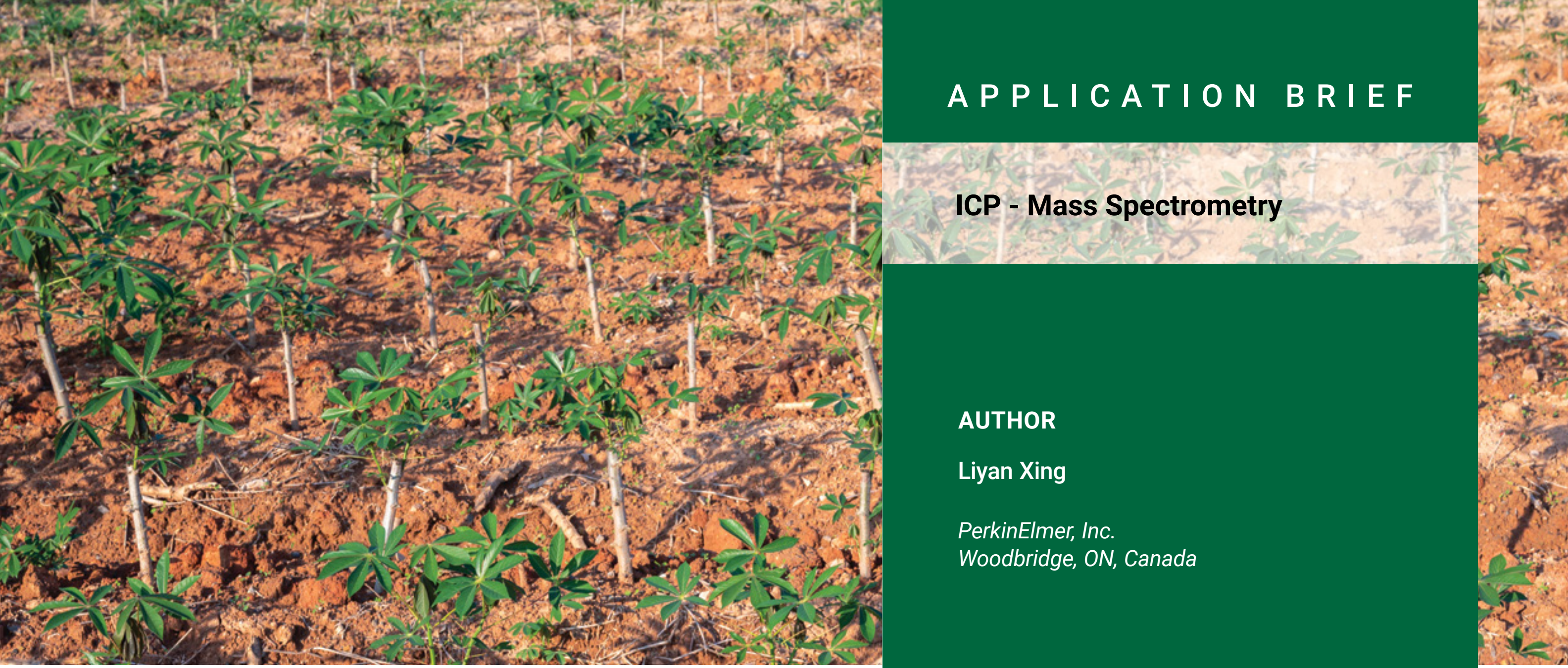

APPLICATION BRIEF

ICP - Mass Spectrometry

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# Determination of Heavy Metals in Cassava Flours Using NexION ICP-MS Following U.S. FDA EAM 4.7 Method

## Introduction

Cassava is a root vegetable grown in tropical and subtropical areas. It is a major source of calories and carbohydrates in many countries around the world.[1] As one of the world's most important staple crops, the levels of harmful chemicals and pathogens in cassava are strictly controlled. The chemicals under control include organic chemicals, like pesticides, and heavy metals. Metals, such as cadmium (Cd), mercury (Hg) and lead (Pb), and metalloids, such as arsenic (As), are harmful to human health, where this is especially notable for children who are undergoing neurological development. These metals and metalloids can be taken up by the cassava plant from air, water, and soil during growth, or introduced as contaminants during processing and storage. The U.S. Food and Drug Administration (FDA) has published an elemental analytical method by ICP-MS: Elemental Analysis Manual (EAM) 4.7, which includes the analysis of the aforementioned elements.[2]

Inductively coupled plasma mass spectrometry (ICP-MS) is a powerful elemental analysis technique with multi-element detection ability, low detection limits, high speed of analysis, wide linear dynamic range, etc. However, it is affected by plasma and matrix-based polyatomic interferences and doubly charged species. PerkinElmer's NexION® ICP-MS systems are equipped with Universal Cell Technology (UCT). One of the modes of operation of this cell is Collision mode with Kinetic Energy Discrimination (KED) which can be used to tackle polyatomic interferences, simply using a single collision gas, such as helium. Doubly charged ions, however, cannot be resolved with Collision mode and require mathematical correction.

One of the interferences which can exist on arsenic (As), is that of doubly charged rare earth elements (REEs). Since REEs have a low second ionization potential and are prone to the formation of doubly charged ions ($M^{2+}$) in the plasma, if they are present in the sample, they need to be addressed. This is best handled using reaction chemistry, which can be used to either mass-shift the analyte or interference depending on the reaction gas being used. This has the added benefit of offering the lowest detection limits on the As analyte without compromising on its sensitivity. In order to somewhat reduce the time of analysis, some labs may prefer to use a single gas, such as a collision gas, in which case a correction equation may need to be applied to the results to ensure that the As result is not over-estimated due to this interference. It should be noted, however, that while REE interferences may occur, carbon enhancement effects from a high carbon content sample in relation to an improperly matrix-matched set of calibration standards may also lead to an over-estimation of As results, an issue which can be easily addressed by adding 1% isopropanol (IPA) to the calibration standards and the samples. Therefore, it is essential to ensure that the REE interference is actually present and not a misdiagnosis of carbon-enhancement effects.

This application brief describes a procedure for the analysis of Cd, Hg, Pb and As in cassava flours following U.S. FDA's EAM 4.7 using the Titan™ MPS microwave digestion system for sample digestion and the NexION ICP-MS for sample analysis.

## Experimental

### Method Development

For this work, following confirmation of the samarium (Sm) and neodymium (Nd) interference in the samples, a collision gas (He) and a half-mass correction[3] approach was applied to resolve the interference of the REEs on As. By taking advantage of the adjustable mass resolution capability of the NexION ICP-MS system, the mass resolution was set to 0.5 amu, and the half-mass ions could be resolved completely, as shown in Figure 1. Since the rate of generation of the doubly charged ions was the same for all isotopes, the ratios of the doubly charged ions should be the same as the natural abundance of the respective isotopes. Half masses at 72.5 ($^{145}Nd^{2+}$) and 73.5 ($^{147}Sm^{2+}$) have no overlap with other ion species, and so could be used to correct for the interference from half mass at 75 ($^{150}Nd^{2+}$). The calculation was as follows, where A represents the isotope natural abundance, and I the signal intensity:

$$I(^{150}Nd^{2+}) = A(^{150}Nd)/A(^{145}Nd) \times I(^{145}Nd^{2+})$$

$$I(^{150}Sm^{2+}) = A(^{150}Sm)/A(^{147}Sm) \times I(^{147}Sm^{2+})$$

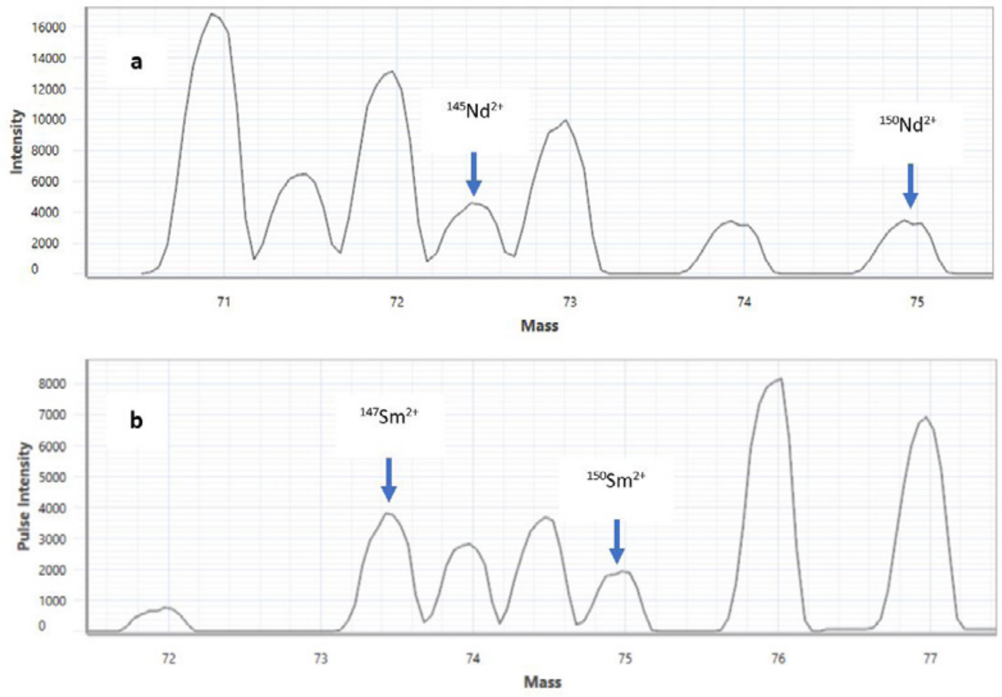

*Figure 1: Mass scan spectrum of single-element standard of Nd (a) and Sm (b) in Collision/KED mode at mass resolution of 0.5 (5% peak height).*

### Reagents and Calibration Standards

Ultrapure water (UPW) (Resistivity ≥18.2 MΩ.cm) and high-purity acids (Tama Chemicals, Kawasaki City, Kanagawa 210-0005, Japan) were used throughout this work. The calibration standards were prepared by the dilution of ICP-MS grade single-element standards (See Consumables Used table) in a diluent made of 3% $HNO_3$ and 0.5% HCl 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 1. Standard 3 was also used as a continuing calibration verification (CCV) standard.

*Table 1: Concentrations of the Calibration Standards.*

| Element | Standard 1 (ppb) | Standard 2 (ppb) | Standard 3 (ppb) | Standard 4 (ppb) | Standard 5 (ppb) |
|---|---|---|---|---|---|
| As, Cd, Pb | 0.1 | 1 | 5 | 10 | 25 |
| Hg | 0.01 | 0.1 | 0.5 | 1 | 2.5 |

The internal standard solution was comprised of 100 ppb In and Ir in the diluent with 5% isopropanol (IPA). Isopropanol was added to address carbon-enhancement effects on As. The internal standard was introduced into the designated port of the High Throughput System (HTS) valve and mixed online with the sample.

The wash solution consisted of 1.5% HCl (v/v) and 0.5% $HNO_3$ (v/v) spiked with 200 µg/L gold and 5% IPA, where IPA was used to facilitate the washout of Hg and organic materials.

### Sample Preparation

A rice flour standard reference material (SRM) 1568b (National Institute of Science and Technology; NIST, Gaithersburg, Maryland, USA) was used to validate the accuracy of the methodology. To evaluate the matrix effect on the analytical performance, EAM 4.7 recommends evaluation recoveries of fortified analytical portions (FAPs), which are prepared by spiking the sample with the standards to proper levels. In this work, two cassava flour samples, namely Garri and Super, were used for FAP test.

Cassava flour samples and the SRM (ca. 0.3 g each) were accurately weighed into 75 mL microwave digestion vessels, and 8 mL $HNO_3$, 2 mL $H_2O_2$, 1.2 mL HCl and 24 µL of gold single-element standard (1000 ppm) were added to each sample. Gold was added as Hg stabilizer. The method blanks, SRM, cassava samples and FAPs were digested on a Titan MPS microwave digestion system (PerkinElmer Inc., Shelton, Connecticut, USA) in the same batch using the built-in method for rice flour. The microwave digestion parameters are shown in Table 2.

At the end of digestion, the digestates were quantitatively transferred into pre-weighed 50 mL centrifuge tubes by decanting the samples into pre-weighed centrifuge tubes, rinsing the vessels twice with 10 mL of UPW and adding these rinsings to their respective centrifuge tubes. UPW was added to the 40 mL mark to get the second sample stock solution and the weight recorded. The second sample stock solution (13.5 mL) was pipetted into a pre-weighed 50 mL centrifuge tube and UPW added to the 40 mL mark, the weight recorded, and the sample analyzed by ICP-MS.

*Table 2: Titan MPS 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 |

### Instrumentation

All samples were digested using a Titan MPS microwave digestion system.

All analyses were performed with a NexION ICP-MS (PerkinElmer Inc., Shelton, Connecticut, USA) equipped with HTS and an S20 series autosampler. Operated under vacuum, HTS can quickly draw and deliver the sample to the plasma and can perform wash procedures more efficiently and effectively than conventional sample introduction systems. The instrument components and operating conditions are shown in Table 3.

*Table 3: NexION ICP-MS Instrument Components and Operating Conditions.*

| Parameter | Value |
|---|---|
| RF Power | 1600 W |
| Cones | Standard Ni |
| Nebulizer | ST-PFA MicroFlow |
| Peripump tubing | Carrier: Yellow/Orange (0.51 mm i.d.)<br>Internal standard: Red/Orange (0.19 mm i.d.) |
| Sample uptake | 0.2 mL/min |
| Nebulizer gas flow | Optimized with oxide <2.5%<br>Doubly charged ions <2.5% |
| Collision gas | Helium |
| Collision gas flow | 4 mL/min |
| Mass resolution | 0.5 (5% peak height) |
| Doubly charged ion correction equation for $^{75}$As | - 0.6747 * mass 72.5 - 0.4923 * mass 73.5 |

## Results and Discussion

### Doubly Charged Ion Correction

To evaluate the efficiency of doubly charged ion correction using the half-mass correction approach, recovery tests were performed on a 5 ppb As single-element standard spiked with Nd and Sm of various concentrations. As shown in Figure 2, without the $M^{2+}$ correction, As was substantially overestimated with Sm and Nd >10 ppb, while with $M^{2+}$ correction, an accurate As result was obtained with Nd and Sm up to 50 ppb, demonstrating the effectiveness of the half-mass approach on the NexION ICP-MS. In the cassava flour samples from this study, however, both Sm and Nd were not observed in significant amounts, i.e., <1 ppb in either sample and consequently were not thought to be a major contributing factor to the results.

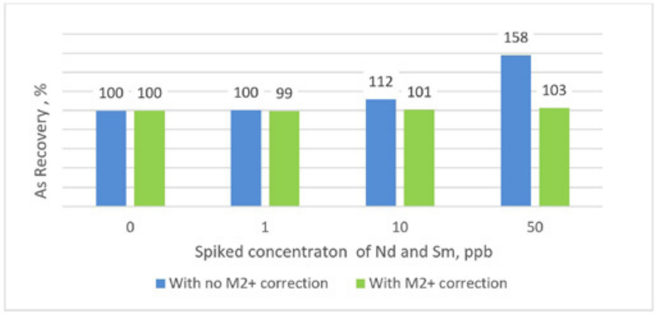

*Figure 2: Recoveries of As in the presence of Nd and Sm (with and without $M^{2+}$ correction).*

### Detection Limits and Limits of Quantifications

Limits of detection (LODs) and limits of quantification (LOQs) were calculated as 3 and 10 times the standard deviation of 10 replicated measurements of the method blank, respectively. The LODs and LOQs of this method were below the nominal limits specified in EAM 4.7 for all target elements, as shown in Figure 3.

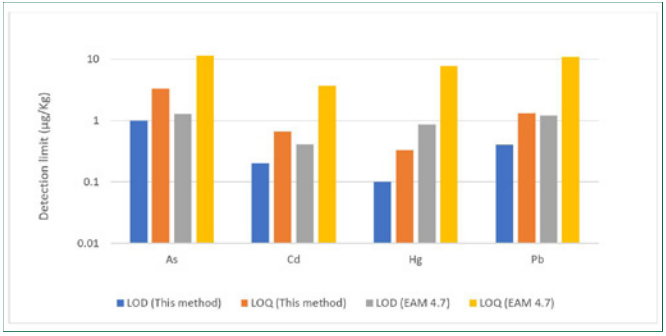

*Figure 3: Comparison of detection limits of this method and EAM 4.7 nominal analytical limits. Three Pb isotopes are measured and summed to address the natural abundance variation.*

### Accuracy

The accuracy of the method was evaluated by the recoveries of SRM 1568b (rice flour) and FAPs.

The SRM was prepared in duplicates and each preparation was measured three times. The mean concentration and the recovery of the duplicates are shown in Figure 4. Recoveries between 90% and 115% were obtained for all target elements, meeting the QC criteria of 80-120% as specified in EAM 4.7.

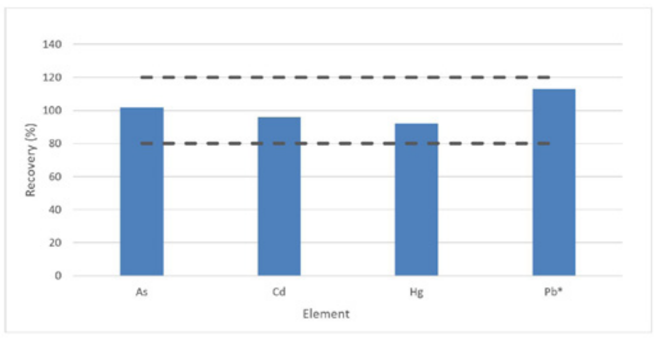

*Figure 4: Recoveries for the certified values in SRM 1568b.* Reference-only quantitative value.*

FAP tests were performed on two cassava samples. The samples were spiked with multi-element standards prior to digestion at a level corresponding to 1 ppb in the analytical solution. Duplicates were prepared for each sample. The mean recoveries are shown in Figure 5. Recoveries between 90% and 110% were obtained for all target elements, meeting the QC criteria of 80-120%, as specified in EAM 4.7.

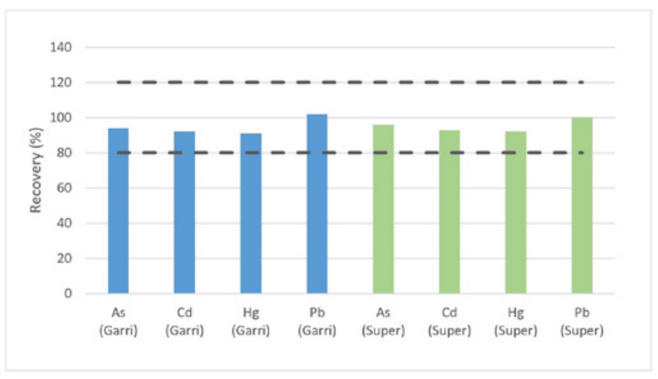

*Figure 5: FAP test results on sample Garri (blue bars) and sample Super (green bars).*

### Precision

Precision was evaluated by the relative percent difference (RPD) of duplicated sample analysis. Relative percent difference tests were performed on two cassava samples and the results are shown in Table 4. For both samples, the RPDs were within the QC criteria of <20%, as specified in EAM 4.7 for all target elements.

*Table 4: RPD Test on Two Samples (Garri and Super).*

| Element | Sample ID | Measured Concentration (mg/Kg) Portion A | Measured Concentration (mg/Kg) Portion B | RPD (%) | QC Criteria (<20%) |
|---|---|---|---|---|---|
| As | Garri | 0.011 | 0.010 | 4.4 | Pass |
| | Super | 0.036 | 0.034 | 2.7 | Pass |
| Cd | Garri | 0.001 | 0.001 | 4.0 | Pass |
| | Super | 0.006 | 0.006 | 1.3 | Pass |
| Hg | Garri | 0.001 | 0.001 | 12.1 | Pass |
| | Super | 0.001 | 0.001 | 9.3 | Pass |
| Pb | Garri | 0.025 | 0.031 | 11.3 | Pass |
| | Super | 0.837 | 0.824 | 0.8 | Pass |

### Stability Check

To validate the stability of the method, cassava samples were measured repeatedly over a period of 8 hours and the CCV recoveries monitored over this period. All the CCV recoveries were normalized to Standard 3 (Table 1) and were well within ±10% of the original reading, as shown in Figure 6, meeting the QC criteria of 100±10% as specified in EAM 4.7.

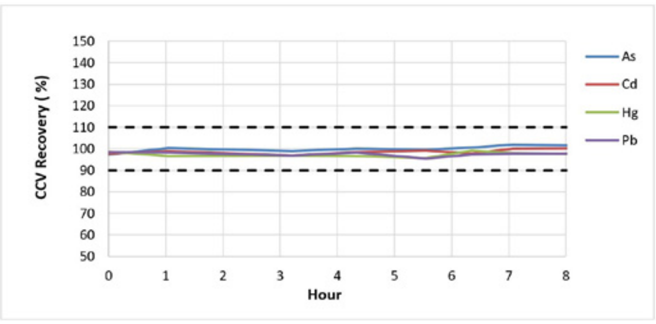

*Figure 6: CCV recoveries over an 8-hour run of cassava samples.*

## Conclusion

The U.S. FDA's EAM 4.7 method was used to prepare and analyze cassava flour samples for the accurate quantification of As, Cd, Hg and Pb using PerkinElmer's NexION ICP-MS.

- Titan MPS microwave system was used to digest the cassava flour samples with the 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.
- Helium Collision/KED mode was used to remove polyatomic interferences. The doubly charged interferences from REEs on $^{75}$As were corrected using the half-mass correction approach.

The method was evaluated in terms of the detection limits, accuracy, precision, and stability in accordance with EAM 4.7, and all met and exceeded the QC criteria in each category, making the method and instrumentation used ideal for the routine analysis of metal(loid)s in cassava and related food samples.

## References

1. Delia M. Pinto-Zevallos, Martín Pareja, Bianca G. Ambrogi; Current knowledge and future research perspectives on cassava (Manihot esculenta Crantz) chemical defenses: An agroecological view; *Phytochemistry*, (2016), 130, 10-21.
2. Patrick J. Gray, William R. Mindak, John Cheng; U.S. FDA Elemental Analysis Manual 4.7 Inductively Coupled Plasma-Mass Spectrometric Determination of Arsenic, Cadmium, Chromium, Lead, Mercury and Other Elements in Food Using Microwave Assisted Digestion, Version 1.2 (February 2020).
3. Skyler W. Smith, Nicole Hanks, Patricia A. Creed, Kasey Kovalcik, Robert A. Wilson, Kevin Kubachka, Judith A. Brisbin, Julio Landero Figueroa, and John T. Creed; Analytical considerations associated with implementing M$^{2+}$ correction factors to address false positives on As and Se within U.S. EPA method 200.8; *Journal of Atomic Analytical Spectrometry*, (2019), 34(10), 2094–2104.

## Consumables Used

| Component | Description | Part Number |
|---|---|---|
| Peripump Tubing | Carrier: Orange/Yellow (0.51 mm i.d.) | N8152405 |
| | ISTD: Orange/Red (0.19 mm i.d.) | N8152401 |
| | Waste: Gray/Gray Santoprene (1.30 mm i.d.) | N8152415 |
| Internal Standard Mix | 10 mg/L of In and Ir | N9307738 |
| Single-element Standard | 1000 µg/mL of As in 2% $HNO_3$ | N9303727 |
| | 1000 µg/mL of Cd in 2% $HNO_3$ | N9303734 |
| | 1000 µg/mL of Pb in 2% $HNO_3$ | N9303748 |
| | 1000 µg/mL of Hg in 10% $HNO_3$ | N9303740 |
| | 1000 µg/mL of Nd in 2% $HNO_3$ | N9303787 |
| | 1000 µg/mL of Sm in 2% $HNO_3$ | N9303800 |
| | 1000 µg/mL of Au in 10% HCl | N9303759 |

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