



ICP - Mass Spectrometry

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The Determination of Nutritional and Toxic Elements in Plant-Based Foods Using the NexION 2200 ICP-MS

Introduction

With rising concerns about animal cruelty, the environmental impact of farms and a greater

awareness of the health benefits of consuming plant-based foods, the food industry is witnessing significant investments in this sector.

Plant-based foods consumption and demand are on the rise. These foods are made with ingredients derived from fruits, nuts, vegetables, grains, and legumes. From a nutritional standpoint, plant-based foods offer several benefits to prevent diseases, such as obesity, diabetes, and heart disease, as they are lower in cholesterol levels, improve gut microbiome and are a great source of vitamins.¹

While plant-based foods offer many benefits, they carry some challenges associated with their formulation, nutritional content, and safety. Nutritional and toxic elements need to be routinely monitored in incoming raw materials and finished product, ensuring accurate label claims and compliance with adopted quality control criteria per specified regulatory bodies.

Inductively coupled plasma mass spectrometry (ICP-MS) is perfectly poised to achieve such a task, measuring a wide range of elemental concentrations from ppm to ppt levels.

In this work, PerkinElmer's NexION® 2200 ICP-MS was used to analyze a variety of plant-based food samples and several certified materials. This platform is equipped with many features and capabilities that guarantee:

- **Uptime:** This is achieved through the unique design of the NexION 2200 combining the patent-pending OmniRing™ technology with the orthogonal design encompassing the Quadrupole Ion Deflector (QID), requiring a minimal amount of maintenance.
- **Throughput:** This is obtained with the built-in Extended Dynamic Range (EDR),² a selected mass electronic dilution which eliminates the need for manual sample dilutions and duplicate sample preparation; and the optional High Throughput System (HTS) flow injection module.³
- **Ease of use:** In addition to the intuitive Syngistix™ for ICP-MS software, the NexION 2200 is equipped with an LCD touchscreen for a PC-less experience as well as LED lighting to alert users of the instrument's running status.
- **Higher environmental, social, and governance (ESG) score:** Reduced cooling needs with LumiCoil™ RF coil technology⁴ and the GreenCT™ cooling system.⁵

The data quality was evaluated per the QC criteria specified in the U.S. Food and Drug Administration (FDA) EAM 4.7 method.⁶

Experimental

Sample Preparation

Sample digestion was performed using the MPS 320™ microwave digestion system (PerkinElmer, Shelton, Connecticut, USA).

A variety of plant-based food products bought from the local market (vegetable shakes, egg replacers, meatless patties, nuggets, and bologna) and a selection of food certified reference materials (CRMs) including Standard Reference Materials (SRMs) 1568b (Rice Flour), 1549a (Whole Milk Power), and 2976 (Mussel Tissue) bought from the National Institute of Science and Technology (NIST) (Gaithersburg, Maryland, USA), and DORM-5 (Fish Protein) bought from National Research Council (NRC) (Ottawa, Canada) were digested.

The digestion vessels were pre-wet by adding a few drops of ultrapure water (UPW). Food samples and the CRMs of ca. 0.5 g were accurately weighed into 85 mL digestion vessels, then 8 mL HNO₃, 2 mL H₂O₂, and 24 µL of gold single-element standard (1000 ppm) were added. Gold was added as a Hg stabilizer. The method blanks, CRMs, and food samples were digested in the same batch. The digestion parameters are shown in Table 1.

Table 1. Microwave Digestion Parameters.

Step	Target Temperature (°C)	Ramp Time (min)	Hold Time (min)	Power (%)
1	170	20	5	70
2	200	10	20	100
3	50	0	20	0

After the digestion process was completed, the resulting digests were transferred into pre-weighed 50 mL autosampler vials by pouring them carefully. To quantitatively transfer the samples to the autosampler vials, the digestion vessels were then rinsed twice with 10 mL of ultrapure water each time, and the rinsing solutions were combined into their respective autosampler vials. High-purity concentrated hydrochloric acid (0.5 mL) was then added to the autosampler vial, followed by ultrapure water up to the 40 mL mark, creating the digested sample solution. The weight of the solution was recorded. Next, 10 mL of the digested sample solution was pipetted into a pre-weighed 50 mL autosampler vial followed by adding UPW to the 40 mL mark, and the weight was recorded. A total dilution factor of ca. 320 was achieved and the samples were ready for ICP-MS analysis.

Reagents and Calibration Standards

The calibration standards were prepared gravimetrically by diluting ICP-MS grade single-element standards and multi-element standards (see the *Consumables Used* table) in a diluent made of 3% HNO₃ and 0.5% HCl and spiked with 200 ppb of gold to aid the mobility of Hg. This diluent was also used as the calibration blank and the HTS carrier solution. The concentrations of the calibration standards are shown in Tables 2(a) and 2(b). Standards 4 and 9 were used as continuing calibration verification (CCV) standards. A second multi-element standard with certified concentrations of 10 ppm for the target elements (except Hg) was used as the initial calibration verification (ICV) stock source and was diluted to 10 ppb using the same diluent.

Table 2(a). Concentrations of the Calibration Standards (Set 1).

Analytes	Standard 1 (ppb)	Standard 2 (ppb)	Standard 3 (ppb)	Standard 4 (ppb)	Standard 5 (ppb)	Standard 6 (ppb)
Ba, Fe, Sr	1	10	50	100	500	1,000
Mg	10	100	500	1,000	5,000	10,000
Ca, K, Na	50	500	2,500	5,000	25,000	50,000
Ag, Al, As, B, Be, Cd, Co, Cr, Li, Ni, Pb, Rb, Sb, Se, Sn, Te, Th, Ti, Tl, U, V	0.1	1	5	10	50	100
Cu, Mn, Zn	0.3	3	15	30	150	300
Hg	0.01	0.1	0.5	1	5	10

Table 2(b). Concentrations of the Calibration Standards (Set 2).

Analytes	Standard 7 (ppb)	Standard 8 (ppb)	Standard 9 (ppb)	Standard 10 (ppb)
P, S	12.5	250	5,000	50,000
Br	1.25	25	500	5,000

The internal standard solution was made up of 400 ppb Sc, 20 ppb In, Rh, and Ir in a solution of 1% HNO₃ and 0.5% HCl, and 4% isopropanol (IPA). 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 elements with high ionization potential, e.g., As and Se.^{7,8} 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% HCl (v/v) and 3% HNO₃ (v/v) spiked with 200 µg/L gold and 5% IPA, where IPA was used to facilitate the washout of residual organic materials.

Instrumentation

All analyses were performed on the NexION 2200 ICP-MS (PerkinElmer, Shelton, Connecticut, USA) equipped with the High Throughput System (HTS) sample introduction module and an S20 series autosampler.

The instrument components and operating conditions used for the analysis are shown in Table 3.

Method Development

Interferences Mitigation/Removal

To ensure accurate elemental analysis of food products by ICP-MS, possible interferences must be dealt with. These interferences include isobaric, polyatomic, and doubly charged.

Polyatomic interferences are generated by the reaction of matrix ions and the plasma-based ions, such as Ar, C, N, O, and H, which overlap target ions, such as ⁴⁰Ar³⁵Cl⁺ on ⁷⁵As⁺, ⁴⁰Ar¹²C⁺ on ⁵²Cr⁺, ⁴⁰Ar¹⁵N⁺ on ⁵⁵Mn⁺, ⁴⁰Ar³⁸Ar⁺ on ⁷⁸Se⁺, 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, such as ¹⁵⁰Nd⁺⁺ and ¹⁵⁰Sm⁺⁺, may interfere with ⁷⁵As⁺, and doubly charged ions, such as ¹⁵⁶Gd⁺⁺

Table 3. 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 cones Ni Hyper-skimmer cone with OmniRing technology
Peristaltic Pump Tubing	Carrier/Internal standard: orange/green (0.38 mm i.d.) 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 ²⁺ /Ce < 2.5%
Cell Gas	Helium, oxygen

and ¹⁵⁶Dy⁺⁺, can interfere with the ⁷⁸Se⁺. In this section, we will discuss some of the NexION 2200 features that address these challenges.

Equipped with Universal Cell Technology™ (UCT), the NexION 2200 can operate in Collision mode with kinetic energy discrimination (KED) and in Reaction mode with dynamic bandpass tuning (DBT). In Collision mode, an inert gas, such as helium, collides with analytes and interferences. Due to their larger size compared to elemental ions, polyatomic interferences, such as ⁴⁰Ar³⁵Cl⁺ on ⁷⁵As, undergo more collisions and lose more energy, therefore making it difficult for them to overcome the energy barrier at the exit of the Universal Cell.

In Reaction mode, a reaction gas reacts selectively with the analyte or the interferent(s) to form ions/clusters of different masses or neutralize the interferent(s). For example, Reaction mode using oxygen as a reaction gas can be used for the analysis of As and Se, in which As and Se react with oxygen to form $^{75}\text{As}^{16}\text{O}^+$ at mass 91 and $^{78}\text{Se}^{16}\text{O}^+$ at mass 94, respectively, and mass shift by +16 amu from the respective doubly charged interferents. Apart from resolving the doubly charged ion interferents, Reaction mode has the added benefit of offering the lowest detection limits for As and Se without compromising on their sensitivities. However, the accuracy of this oxygen mass-shift for the determination of As and Se can be affected by the presence of Zr, Co, and Mo which either have isotopes at the reaction product masses or can form an additional product (such as CoO_2^+) at the reaction product mass.

The effectiveness of the interference mitigation/removal was evaluated by determining the background equivalent concentration (BEC) of the target element in the interference removal checking solutions, which consists of a series of single-element standard solutions of Co, Zr, and Mo in the calibration blank. The results are shown in Table 4. For As, which is analyzed as AsO, the BEC is at ppt/sub-ppt levels in the presence of Zr and Co concentrations up to 100 ppb, which is well above the amounts normally observed in food samples. Also as shown in Figure 1, the contribution from ^{91}Zr on mass 91 decreased rapidly with the increase of oxygen flow. The profiles of the As solution and the Zr-spiked As solution started overlapping with oxygen gas reaching 1.4 mL/min, indicating the complete removal of the ^{91}Zr interference.

Table 4. Interferences Evaluation for the Target Elements.

Element	Concentration (ppb)	Interfering Ions	BEC of Mass 91 (ppb)
Zr	10	$^{91}\text{Zr}^+$	< DL
	50		< DL
	100		0.0002
Co	10	$^{59}\text{Co}^{16}\text{O}_2^+$	0.001
	50		0.002
	100		0.003

TotalQuant and Survey Scan

In a global supply-chain economy, many raw materials are sourced from various parts of the world. As such, a food laboratory might find the need to completely characterize incoming raw materials. The powerful TotalQuant™ feature in Syngistix for ICP-MS software provides a complete mass spectrum without using multi-point calibration. This feature can be run in Survey Scan mode during routine data acquisition of regulated methods, i.e. EAM 4.7, constantly monitoring all elements across the periodic table or a/multiple customized range(s) for possible interferences.⁹ As some might be aware, rare earth elements (REEs), such as Nd, Sm, Dy, and Er, can possibly interfere on As and/or Se when they form doubly charged ions in the plasma. Enabling such a feature provides laboratories with the confidence of achieving interference-free sample analysis. As shown in Figure 2, the spectrum displays intensity as counts-per-second (cps) on the y-axis and mass numbers on the x-axis. The y-axis uses a logarithmic scale to enhance visualization. No significant amount of Nd, Sm, Dy, and Er was detected by the Survey Scan for all the food samples analyzed in the work.

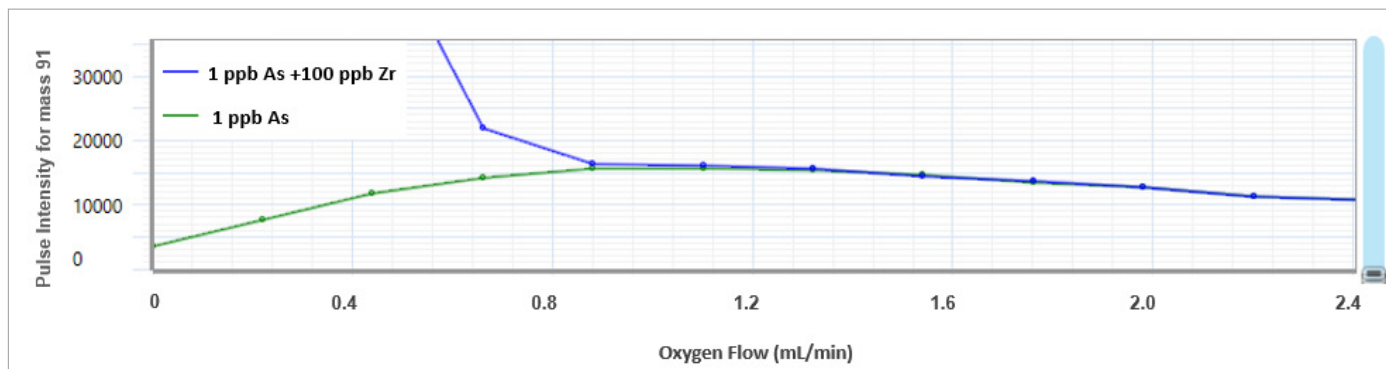


Figure 1. Stacked oxygen gas flow optimization profiles show the removal of interference of $^{91}\text{Zr}^+$ on $^{75}\text{As}^{16}\text{O}^+$. The green line and blue lines represent the signal intensity of mass 91 vs. oxygen flow for a 1 ppb As solution and a 1 ppb As solution spiked with 100 ppb Zr, respectively.

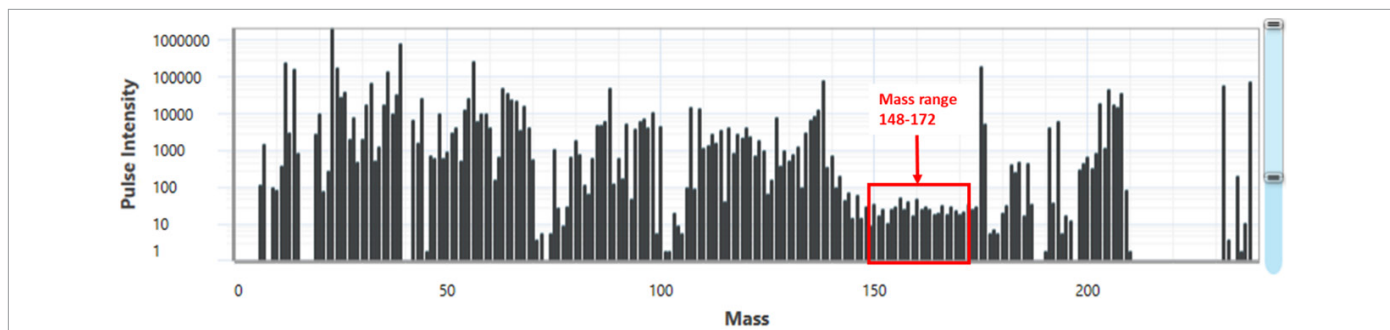


Figure 2. Example TotalQuant spectrum of a food sample spectrum. The mass range for Nd, Sm, Dy, and Er is highlighted in the red square.

Extended Dynamic Range (EDR)

Sodium (Na) and potassium (K) are usually present in food samples at high concentrations. Since they are either mono-isotopic (Na) or greater than 90% of natural abundance at the primary isotope (³⁹K), it could be difficult to measure these elements and the ultra-trace elements in the same sample run at the same sample dilution level. The usual practice of analyzing the major and trace elements in separate analytical runs at different sample dilutions has the drawbacks of reduced productivity, increased consumable usage, and increased risk of sample contamination. The NexION 2200 ICP-MS leverages the proprietary Extended Dynamic Range (EDR) capability to selectively reduce the signal on individual masses or analytes by adjusting the RPa (DC voltage) on the UCT which helps to overcome the challenges mentioned earlier.¹⁰⁻¹² In this study, the analysis of Na and K was conducted by applying different RPa values from the rest of the elements.

Table 5. Elements, Masses, and Modes of Analysis for Different Elements.

Element	Mass	Mode	RPa	Element	Mass	Mode	RPa
Li	7	Helium KED	0	Zn	66	Helium KED	0
Be	9	Helium KED	0	As	91	Oxygen DRC	0
B	11	Helium KED	0	Se	94	Oxygen DRC	0
Na*	23	Helium KED	0.015	Br	79	Helium KED	0
Mg	24	Helium KED	0	Sr	88	Helium KED	0
Al	27	Helium KED	0	Mo	95	Helium KED	0
P	31	Helium KED	0	Ag	107	Helium KED	0
S	34	Helium KED	0	Cd	111	Helium KED	0
K*	39	Helium KED	0.015	Sn	118	Helium KED	0
Ca	44	Helium KED	0	Sb	121	Helium KED	0
Ti	47	Helium KED	0	Te	125	Helium KED	0
V	51	Helium KED	0	Ba	137	Helium KED	0
Cr	52	Helium KED	0	Hg	201	Helium KED	0
Mn	55	Helium KED	0	Tl	205	Helium KED	0
Fe	56	Helium KED	0	Pb**	206+207+208	Helium KED	0
Co	59	Helium KED	0	Th	232	Helium KED	0
Ni	60	Helium KED	0	U	238	Helium KED	0
Cu	63	Helium KED	0	---			

* EDR applied for measurement

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

Method Setup

Arsenic (As) and selenium (Se) were measured in oxygen Reaction (DRC) Mass Shift mode, and the rest of the elements were measured in helium Collision (KED) mode. The elemental isotopes, cell gas modes, and RPa used for analysis are listed in Table 5.

Results and Discussion

Detection Limits

As defined in EAM 4.7 and EAM 3.2,¹³ the limit of quantification (LOQ) was calculated as 30 times the standard deviation of 10 replicated measurements of the method blank multiplied by the dilution factor.

As shown in Figure 3, for all elements specified in EAM 4.7, the LOQs obtained in this work are lower than the EAM 4.7 specifications.

Accuracy

The accuracy of the method was verified by evaluating the recoveries of various analytes in the certified reference materials (CRMs). The CRMs were prepared in duplicates and each preparation was measured three times. The mean recoveries of the duplicates are shown in Figure 4. Recoveries between 85% and 111% were obtained for all the certified elements, falling well within the QC criteria of $\pm 20\%$, as specified in EAM 4.7.

Stability Check

To confirm the stability of the method, plant-based food samples were measured repeatedly over 12 hours and the CCV recoveries were checked over this period. CCV recoveries for all analytes were normalized to Standard 4 (Table 2(a)) or Standard 9 (Table 2(b)) and were found within $\pm 10\%$ of the original reading, as shown in Figure 5, which met the QC criteria of $\pm 10\%$ as recommended in EAM 4.7.

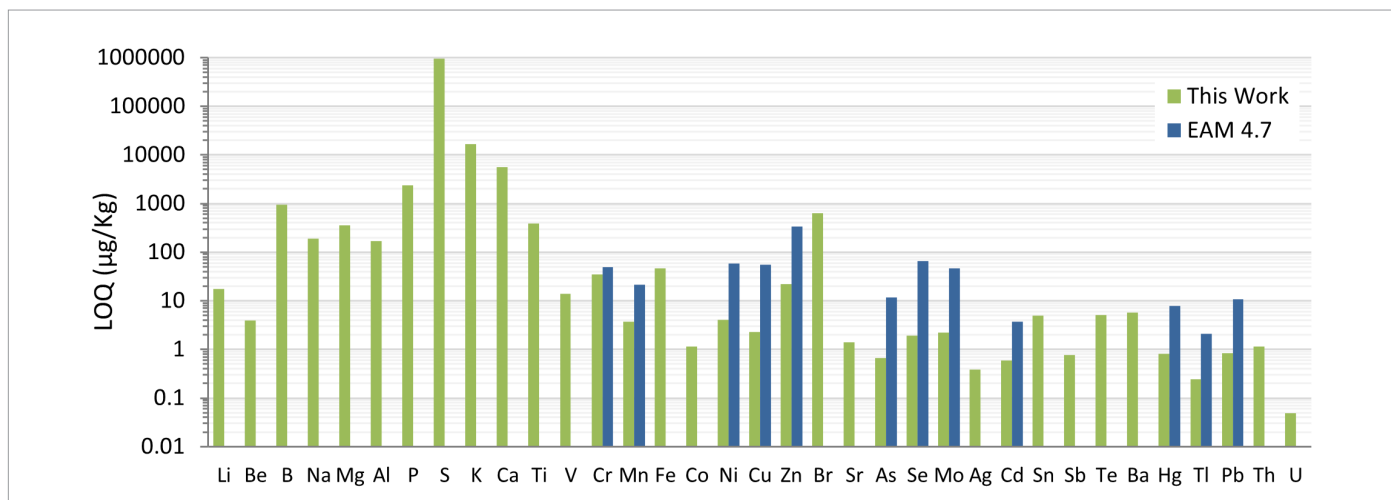


Figure 3. The LOQs obtained in this work in comparison to EAM 4.7 specifications.

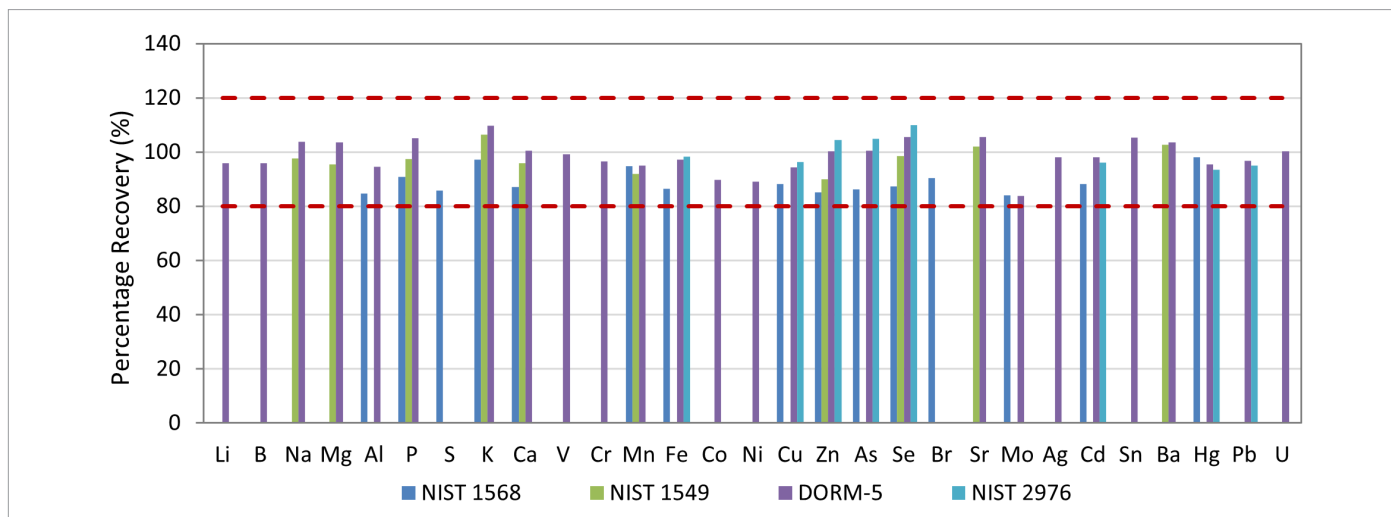


Figure 4. Recoveries for the certified values in NIST 1568b (Rice Flour), NIST 1549a (Whole Milk Power), NIST 2976 (Mussel Tissue), and DORM-5 (Fish Protein).

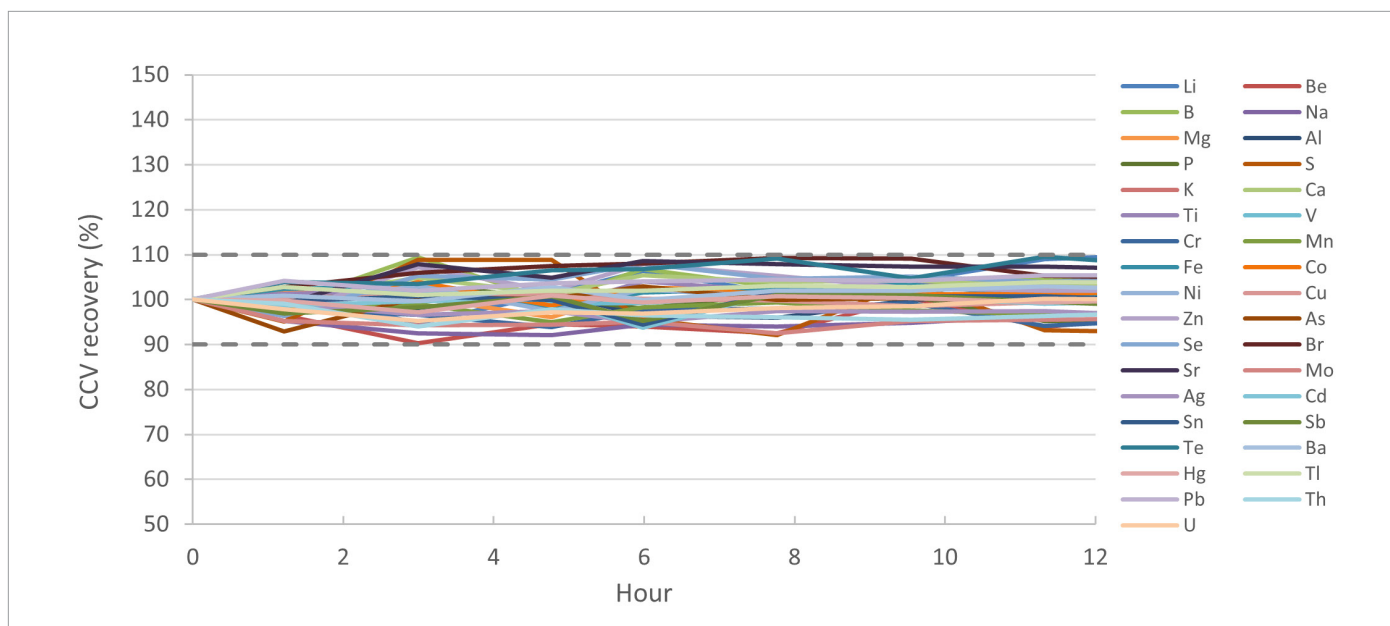


Figure 5. CCV recoveries over a 12-hour run of various plant-based food samples.

Conclusions

In this application note, a method for analyzing major and trace elements in plant-based food samples using the NexION 2200 ICP-MS equipped with a High Throughput System (HTS) was described. For sample preparation, the MPS 320 microwave digestion system with a 32-position rotor and MR-85 auto-venting vessels were used.

With the application of PerkinElmer's unique Extended Dynamic Range (EDR) capability, both major and trace elements could be measured in a single analytical run. Using helium as the collision gas, a single Collision mode was sufficient for analyzing all elements, as shown on an earlier analysis using the prior-generation NexION 2000 ICP-MS. However, with oxygen as the reaction gas, improved sensitivity and detection limits were achieved for the analysis of As and Se, leading to superior performance compared to Collision mode, where method development was more straightforward.

The method was evaluated in terms of its detection limits, accuracy, and stability in accordance with the EAM 4.7 method and was found to meet and exceed the QC criteria in each category, proving that the method and instrumentation used are suitable for the routine analysis of plant-based food products.

This work illustrates that the NexION 2200 ICP-MS can be used to provide trace and major elemental data in a variety of plant-food materials for making informed decisions on product formulation, evaluating composite samples for nutrition labeling purposes, and controlling the contamination of food supplies by heavy metals.

References

1. Craig W. J.; Health Effects of Vegan Diets; *Am J Clin Nutr.* (2009); 89(5):1627S-1633S.
2. <https://www.perkinelmer.com/library/extended-dynamic-range-feature-on-nexion-icp-ms-series-instruments.html>.
3. High Throughput System for ICP-MS/OES; PerkinElmer Product Note; 2020.
4. Advantages of a Novel Plasma Generator for the NexION 1000/2200/5000 ICP-MS Systems; PerkinElmer Technical Note; 2023.
5. GreenCT – Keeping the Instrument and Planet Cool; PerkinElmer Product Note; 2023.
6. 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).
7. 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.* (1994), 9, 1099-1105.
8. Tomoko Ariga, Yanbei Zhu and Kazumi Inagaki; Study on Carbon-induced Signal Enhancement in Inductively Coupled the Plasma Mass Spectrometry: An Approach from the Spatial Distribution of Analyte Intensities; *J. Anal. At. Spectrom.* (2019), 34, 1865-1874.
9. Ewa Pruszkowski and John Birtles; Elemental Analysis of Wines using the NexION 2000 ICP-MS with TotalQuant; PerkinElmer Application Note; 2021.
10. Valentina Lyubomirova, Veronika Mihaylova, and Rumyana Djingova; Determination of Macroelements in Potable Waters with Cell-Based Inductively Coupled Plasma Mass Spectrometry; *Spectroscopy Europe* (2020), 32 (5), 18-21.

11. Cynthia Bosnak, Ewa Pruszkowski, and Kenneth Neubauer; The Analysis of Food Substances by ICP-MS; Spectroscopy, Special Issues-10-01-2008.
12. Liyan Xing, Chady Stephan, Aaron Hineman, and Sherri Naughton; Direct Trace-Element Analysis in Cell Culture Media and Raw Materials with the NexION 5000 ICP-MS; PerkinElmer Application Note; 2021.
13. William C. Cunningham, William R. Mindak, Stephen G. Capar; U.S. FDA Elemental Analysis Manual for Food and Related Products: 3.2. Terminology (September 2014).
14. Liyan Xing; The Analysis of Major and Trace Elements in Plant-based Foods Using the NexION 2000 ICP-MS; PerkinElmer Application Note; 2023.

Consumables Used

Component	Description	Part Number
Nebulizer	PFA-ST3	N8152378
Spray Chamber	Quartz Cyclonic High Sensitivity Spray Chamber with Matrix Gas Port	N8152383
Torch	One Piece Quartz Torch with 2.0 mm injector	N8152472
Sampler Cone	Nickel sampler cone for NexION 1000/2000/2200/5000 ICP-MS	W1033612
Skimmer Cone	Nickel skimmer cone for NexION 2200 ICP-MS	N8171142
Hyper-skimmer Cone/OmniRing	Nickel hyper-skimmer cone with OmniRing	N8160120
Peripump Tubing	Carrier/Internal Standard: Orange/Green (0.38 mm i.d.) Waste: Gray/Gray Santoprene (1.30 mm i.d.)	N8152403 N8152415
Internal Standard Mix	200 ppm Sc, 10 mg/L of In, Rh, and Ir in 5% HNO ₃ /tr.HCl	N9307738
Multi-Element Standard (used for preparing calibration standard)	100 µg/mL of 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, and Zn in 5% HNO ₃ /Trace Tartaric Acid/Trace HF	N9301721
Multi-Element Standard (used for preparing initial calibration verification standard)	10 µg/mL of Ag, Al, As, Ba, Be, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Se, Tl, V, Zn, Th, and U; and 1000 µg/mL of Ca, Fe, K, Mg, Na, and Sr in 5% HNO ₃ /Trace Tartaric Acid	N9303825
Single-Element Standard	1000 µg/mL Mn in 2% HNO ₃	N9303744
	1000 µg/mL Cu in 2% HNO ₃	N9303737
	1000 µg/mL Zn in 2% HNO ₃	N9303758
	1000 µg/mL Li in 2% HNO ₃	N9303781
	1000 µg/mL B in 2% H ₂ O	N9303760
	1000 µg/mL Fe in 2% HNO ₃	N9303738
	1000 µg/mL Ba in 2% HNO ₃	N9303729
	1000 µg/mL Sr in 2% HNO ₃	N9303802
	1000 µg/mL Te in 10% HNO ₃	N9304385
	1000 µg/mL Th in 10% HNO ₃	N9303842
	1000 µg/mL U in 10% HNO ₃	N9303844
	10,000 µg/mL Na in 5% HNO ₃	N9304124
	10,000 µg/mL Mg in 5% HNO ₃	N0691745
	10,000 µg/mL K in 5% HNO ₃	N9304121
	10,000 µg/mL Ca in 5% HNO ₃	N0691581
	10,000 µg/mL P in H ₂ O	N9304119
	10,000 µg/mL S in H ₂ O	N9304126
	1000 µg/mL Hg in 10% HNO ₃	N9303740
	1000 µg/mL Au in 10% HCl	N9303759