

Routine Elemental Analysis of Dietary Supplements using an Agilent 8900 ICP-QQQ

Effective removal of doubly charged and oxide ion interferences ensures accurate measurement of As and Cd



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Introduction

Global consumer demand for dietary supplements is expected to increase steadily over the next few years (1). Dietary supplements contain ingredients such as nutrients, vitamins, and minerals that may be lacking in the consumer's normal diet. Supplements are marketed as supporting a balanced diet as part of a healthy lifestyle, and therefore differ from drugs that are intended to treat or prevent an illness. Supplements may be consumed in forms such as tablets, capsules, softgels, gelcaps, powders, and liquids (2). In the European Union, the term food supplement is usually applied to products that are called dietary supplements in the US (3).

As with any food product, dietary supplements may be subject to contamination. For example, trace metals can be introduced from raw materials, during manufacturing/processing, or from packaging materials. Therefore, the responsibility for a product's quality and safety lies mainly with manufacturers and distributors. In the European Union, manufacturers must adhere to regulations on labeling, contents, claims, and dosage recommendations (3). The U.S. Food and Drug Administration (FDA) also requires accurate labeling according to current Good Manufacturing Practice (cGMP) and labeling regulations (2). Since any unsafe or mislabeled products can be

taken off the market, manufacturers are increasingly testing their products for potential contaminants including trace elements.

To assist manufacturers with quality assuring their products, the United States Pharmacopeia (USP) issued General Chapter <2232>, which deals with the regulation of Elemental Contaminants in Dietary Supplements (4). While <2332> is intended for products labeled as conforming to USP or National Formulary (NF) standards, it contains useful guidance for analysts.

In this study, triple quadrupole ICP-MS (ICP-QQQ) was used for the routine analysis of the four most toxic elements—arsenic, cadmium, lead, and mercury—in dietary supplements.

Improved interference removal with ICP-QQQ

The Agilent 8900 ICP-QQQ features a unique tandem MS configuration, comprising a scanning quadrupole mass analyzer either side of an octopole-based collision/reaction cell (CRC). This configuration enables the 8900 ICP-QQQ to use MS/MS mode to resolve difficult spectral interferences using reactive cell gases in a wide range of sample types (5 to 9). MS/MS mode is also beneficial for the more difficult elements and problematic interferences sometimes encountered in routine applications, such as the analysis of food samples, soils, wastewater, and groundwater (5).

Solving doubly charged and oxide interferences

Due to their toxicity, many countries regulate the permitted concentrations of As and Cd in drinking water, surface water, soils, foodstuffs, and drugs. The only isotope of As (m/z 75) can suffer spectral interferences from polyatomic ions including ArCl^+ and CaCl^+ . These interferences can be reduced using single quadrupole ICP-QMS operating in helium collision mode (He mode), allowing the accurate measurement of As at the concentration levels required to meet typical regulatory demands. While He mode can be considered a universal cell gas for reducing polyatomic interferences using kinetic energy discrimination (KED), KED is not effective against doubly charged (M^{++}) ion overlaps. The lanthanides or rare earth elements (REE) can form doubly charged ions (REE^{++}) which overlap As (and Se) (10, 11). Also, KED does not eliminate oxide interferences well (5). For example, $^{59}\text{Co}^{16}\text{O}^+$ interference on $^{75}\text{As}^+$, $^{95}\text{Mo}^{16}\text{O}^+$ on $^{111}\text{Cd}^+$, and $^{98}\text{Mo}^{16}\text{O}^+$ on $^{114}\text{Cd}^+$. Vitamin B 12 is rich in Co as cyanocobalamin, and multimineral supplements are rich in Mo, included as an essential nutrient (12).

ICP-QQQ has been shown to be effective for the removal of M^{++} and oxide interferences on various analytes using a reactive cell gas (11). Many elemental ions react with O_2 to form an oxide ion, while the M^{++} interference remains unreactive or is much less reactive. This allows the element of interest to be quantified by measuring the oxide ion at + 16 u, free from the spectral interference.

CoO^+ does not react with O_2 , while As^+ reacts with O_2 to form AsO , allowing AsO and Co to be separated. MoO^+ reacts with O_2 to form MoO_2^+ , while Cd^+ does not react with O_2 , allowing MoO and Cd to be separated. To test the method, 1 ppb As was determined in a series of solutions containing 0 to 100 ppm Co, and 1 ppb Cd was determined in solutions containing 0 to 10 ppm Mo. All solutions were analyzed in He mode (to represent conventional ICP-QMS) and MS/MS mode using O_2 cell gas (only available with ICP-QQQ).

As shown in Figure 1, He mode is suitable for the analysis of As and Cd in matrices that contain Co and Mo at low ppm concentrations. However, poor recoveries were achieved for As in the presence of 100 ppm Co and Cd in a 10 ppm Mo matrix. In contrast, ICP-QQQ operating in MS/MS mode with O_2 cell gas avoided the oxide ion interferences on As and Cd, respectively, at all matrix concentrations. The method allows consistent, low-level determination of As, measured as $^{75}\text{AsO}^+$ at m/z 91, and $^{111}\text{Cd}^+$ at m/z 111.

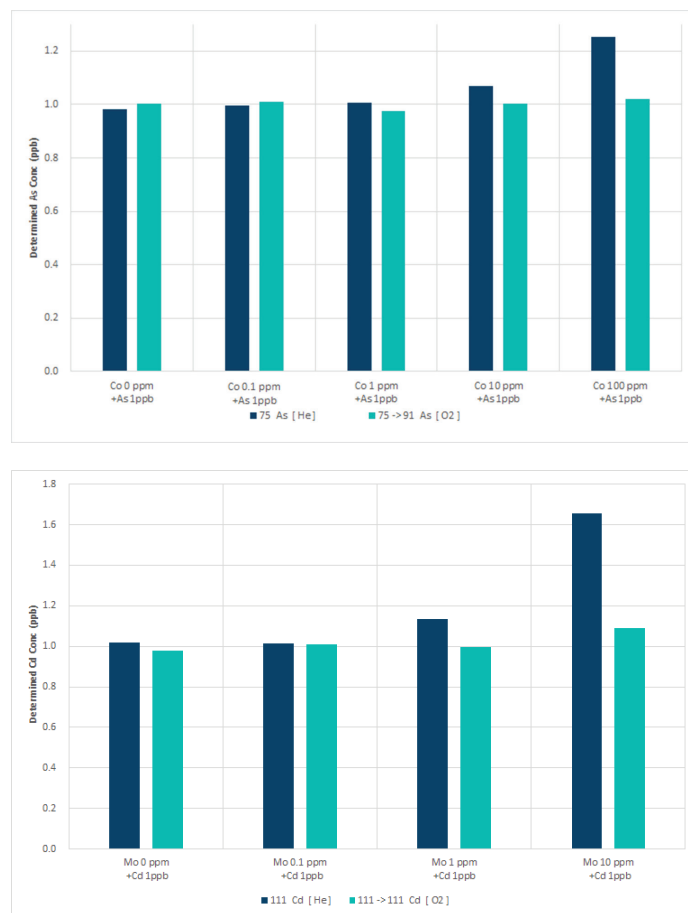


Figure 1. Upper: Recoveries of 1 ppb As spikes in a series of Co matrix solutions. Lower: Recoveries of 1 ppb Cd spikes in a series of Mo matrix solutions. He mode results (blue bars) and MS/MS O_2 mode results (green bars).

Experimental

Standard reference materials

Three National Institute of Standards and Technology (NIST) standard reference materials (SRMs) were analyzed in this study, including 1515 Apple Leaves, 1573a Tomato Leaves, and 3280 Multivitamin/Multielement tablets (Gaithersburg MD, USA). The Apple Leaves SRM contains low $\mu\text{g}/\text{kg}$ levels of As in the presence of mg/kg levels of REEs.

Samples and sample preparation

Three vitamin supplements and two multimineral supplements were bought in a local store in Tokyo, Japan. All SRMs and samples were prepared using a single microwave digestion method. A subsample of 0.20 g was accurately weighed and placed in a PTFE microwave vessel and 6.0 mL of 61% Electronic grade nitric acid and 1.0 mL of 36% Electronic grade hydrochloric acid (Kanto Chemical Co., Inc, Japan) were added. The samples were left for 20 minutes before being placed into a microwave (Mars 6, CEM) and digested using the program given in Table 1. The fully digested samples were then diluted to 50 mL with de-ionized water (DIW). The final digests of the multimineral supplements were further diluted two-fold with acid diluent (total of 12.0 mL HNO_3 , 2 mL HCl, and 86 mL DIW) to match the acid concentration of the other samples.

Although vitamins are water soluble, they were acid-digested because the carbon concentration was expected to be high. Carbon present in the sample solution enhances the ICP-MS signal of some elements, notably As, Se, and P, although the precise mechanism of the enhancement is not clearly understood (13, 14). With the high temperature used in this work (210 °C), the carbon in the samples converts to CO_2 through acid digestion, so the carbon concentration in the sample solution decreases. If any residual carbon did remain, its effect could be mitigated by adding 2% butan-1-ol online with the internal standard (ISTD) solution.

Table 1. Microwave digestion program.

| Temperature (°C) | Ramp Time (min) | Hold Time (min) |
|------------------|-----------------|-----------------|
| 210 | 15 | 15 |
| Ventilation | | 30 |

Calibration standards and internal standards

Calibration standards for As, Cd, Hg, and Pb were prepared from 1000 ppm single element standards (Kanto Chemicals, Tokyo, Japan). The ISTD solution containing Ge, In, Tl, and Bi was also prepared from single element standard (Kanto Chemicals). Calibration standards and ISTDs were prepared to match the acid content of the sample solutions. The ISTD was added to the sample using the standard online ISTD kit.

Instrumentation

An Agilent 8900 Triple Quadrupole ICP-MS (ICP-QQQ, Standard configuration) with UHMI technology was used. The ICP-QQQ was fitted with the standard sample introduction system consisting of a glass concentric nebulizer, quartz spray chamber, and Ni interface cones. The plasma conditions were selected according to the sample type and expected matrix level using the "preset plasma" function of the Agilent ICP-MS MassHunter software. UHMI allows matrices as high as 25% NaCl solution to be analyzed (15).

Acquisition conditions

The method was based on an appropriate preset method for food samples, which was modified to include O_2 cell gas mode. Preset plasma conditions "HMI-4" were selected, where the number 4 represents the approximate aerosol dilution factor. The HMI setting automatically applies the predefined and calibrated parameters for RF power, sampling depth, nebulizer gas flow rate, and dilution gas flow rate. Automating these settings speeds up and simplifies instrument set up as well as ensuring precise and reproducible plasma conditions for the target sample types. The lens voltages were autotuned for maximum sensitivity. Table 2 summarizes the instrument operating parameters.

Table 2. ICP-QQQ operating conditions.

| Parameter | Setting | |
|---------------------------------|-------------|---------------------------|
| | He mode | O_2 mode |
| Cell mode | He mode | O_2 mode |
| Scan type | Single Quad | MS/MS |
| Plasma conditions | HMI-4 | |
| RF power (W) | 1600 | |
| Sampling depth (mm) | 10 | |
| Nebulizer gas flow rate (L/min) | 0.82 | |
| Dilution gas flow rate (L/min) | 0.15 | |
| Extract 1 (V) | 0 | |
| Extract 2 (V) | -250 | |
| Omega bias (V) | -130 | |
| Omega lens (V) | 8.6 | |
| Cell gas flow (mL/min) | 5.5 | 0.45 30% of full scale |
| Energy Discrimination (V) | 5 | -7 |

Shaded parameters are predefined by selecting HMI-4 preset plasma conditions.

Results and discussion

Calibration curves for As and Cd acquired in He mode and MS/MS mode with O₂ cell gas are shown in Figure 2.

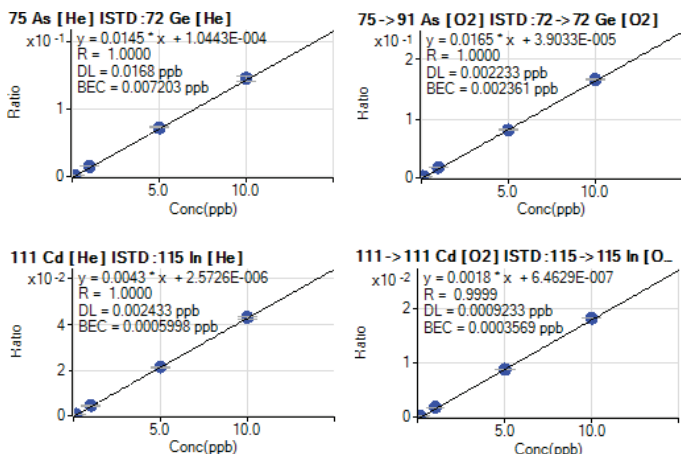


Figure 2. Calibration curves for As (top) and Cd (bottom).

All analytes were measured in He mode, and As and Cd were also analyzed in MS/MS O₂ mode. Three sigma method detection limits (MDLs) and 10 sigma Limit of Quantitation (MLOQs) were calculated from 10 measurements of the preparation blank (Table 3).

Table 3. Method Detection Limits and Method Limits of Quantification.

| Analyte | Q1 | Q2 | Cell Mode | Integration Time (sec) | ISTD | In Solution Prep Blank (n=10) | | In Sample Multimineral | | In Sample Other Samples | |
|---------|-----|-----|----------------|------------------------|-------------------|-------------------------------|-----------|------------------------|-----------|-------------------------|-----------|
| | | | | | | MDL µg/L | MLOQ µg/L | MDL µg/g | MLOQ µg/g | MDL µg/g | MLOQ µg/g |
| As | | 75 | He | 1 | ⁷² Ge | 0.0067 | 0.022 | 0.0033 | 0.011 | 0.0017 | 0.0055 |
| As | 75 | 91 | O ₂ | 1 | ⁷² Ge | 0.0015 | 0.0050 | 0.0008 | 0.0025 | 0.0004 | 0.0013 |
| Cd | | 111 | He | 1 | ¹¹⁵ In | 0.0019 | 0.0062 | 0.0009 | 0.0031 | 0.0005 | 0.0016 |
| Cd | 111 | 111 | O ₂ | 1 | ¹¹⁵ In | 0.0005 | 0.0018 | 0.0003 | 0.0009 | 0.0001 | 0.0004 |
| Hg | | 202 | He | 3 | ²⁰⁵ Tl | 0.0004 | 0.0014 | 0.0002 | 0.0007 | 0.0001 | 0.0004 |
| Pb | | 208 | He | 1 | ²⁰⁹ Bi | 0.0027 | 0.0089 | 0.0013 | 0.0044 | 0.0007 | 0.0022 |

SRM recovery

The accuracy of the method was evaluated by analyzing the three SRMs as unknown samples. Each SRM was measured nine times in the batch. The mean concentrations and relative standard deviation (%RSD) were calculated and compared to the certified value, as shown in Tables 4 to 6. The results for all elements were in good agreement with the certified and reference values. The results for the Apple Leaves SRM and, to a lesser extent Tomato Leaves, show that more accurate recoveries were obtained for As using O₂ mass shift mode compared to He mode. The results illustrate the potential error that can be caused by the relatively high level of REEs in these two reference materials.

Quantitative analysis of bought products and spike recoveries

The 8900 ICP-QQQ was used to analyze three vitamin supplements (Samples 1 to 3) and two multimineral supplements (Samples 4 and 5). The quantitative results and spike recovery results are shown in Tables 7 and 8, respectively. The spikes were added to the microwave vessels before microwave digestion. The spike recoveries for all elements were ±10% of the expected values.

Table 4. Results for NIST 1515 Apple Leaves. The shaded value for As was obtained in single quad mode with He cell gas. The unshaded (accurate) result was obtained using MS/MS mass-shift mode with O₂.

| | Q1 | Q2 | Cell Mode | Measured Solution Concentration (µg/L) | RSD % (n=9) | Calculated Sample Concentration (mg/kg) | Certified Concentration (mg/kg) | Recovery (%) |
|----|-----|-----|----------------|--|-------------|---|---------------------------------|--------------|
| As | | 75 | He | 1.6 | 1.1 | 0.406 ± 0.004 | 0.038 ± 0.007 | 1069 |
| As | 75 | 91 | O ₂ | 0.14 | 2.1 | 0.035 ± 0.001 | | 91 |
| Cd | | 111 | He | 0.057 | 13 | 0.014 ± 0.002 | 0.014 * | 102 |
| Cd | 111 | 111 | O ₂ | 0.059 | 4.3 | 0.015 ± 0.001 | | 105 |
| Hg | | 202 | He | 0.18 | 4.6 | 0.045 ± 0.002 | 0.044 ± 0.004 | 102 |
| Pb | | 208 | He | 2.0 | 2.4 | 0.49 ± 0.012 | 0.47 ± 0.024 | 104 |

*Reference value

Table 5. Results for NIST 1573a Tomato Leaves. The shaded value for As was obtained in single quad mode with He cell gas. The unshaded (accurate) result was obtained using MS/MS mass-shift mode with O₂.

| | Q1 | Q2 | Cell Mode | Measured Solution Concentration (µg/L) | RSD % (n=9) | Calculated Sample Concentration (mg/kg) | Certified Concentration (mg/kg) | Recovery (%) |
|----|-----|-----|----------------|--|-------------|---|---------------------------------|--------------|
| As | | 75 | He | 0.56 | 6.7 | 0.139 ± 0.009 | 0.112 ± 0.004 | 124 |
| As | 75 | 91 | O ₂ | 0.45 | 2.6 | 0.113 ± 0.001 | | 101 |
| Cd | | 111 | He | 6.1 | 7.8 | 1.53 ± 0.12 | 1.52 ± 0.04 | 101 |
| Cd | 111 | 111 | O ₂ | 6.4 | 6.7 | 1.60 ± 0.11 | | 105 |
| Hg | | 202 | He | 0.12 | 8.2 | 0.031 ± 0.003 | 0.034 ± 0.004 | 91 |
| Pb | | 208 | He | 2.6 | 9.1 | 0.64 ± 0.058 | - | - |

Table 6. Results for NIST 3280 Multivitamin/Multielement Tablets.

| | Q1 | Q2 | Cell Mode | Measured Solution Concentration (µg/L) | RSD % (n=9) | Calculated Sample Concentration (mg/kg) | Certified Concentration (mg/kg) | Recovery (%) |
|----|-----|-----|----------------|--|-------------|---|---------------------------------|--------------|
| As | | 75 | He | 0.25 | 2.7 | 0.127 ± 0.003 | 0.132 ± 0.044 | 96 |
| As | 75 | 91 | O ₂ | 0.24 | 2.2 | 0.119 ± 0.003 | | 90 |
| Cd | | 111 | He | 0.17 | 4.4 | 0.0851 ± 0.0037 | 0.08015 ± 0.00086 | 106 |
| Cd | 111 | 111 | O ₂ | 0.16 | 6.5 | 0.0802 ± 0.0052 | | 100 |
| Hg | | 202 | He | < 0.0004 | - | < 0.0002 | - | - |
| Pb | | 208 | He | 0.53 | 4.1 | 0.266 ± 0.011 | 0.2727 ± 0.0024 | 97 |

Table 7. Quantitative results for vitamin supplements.

| | Q1 | Q2 | Cell Mode | Sample 1 | | | | Sample 2 | | | | Sample 3 | | | |
|----|-----|-----|----------------|-----------------|---------------|--------------------|--|-----------------|---------------|--------------------|--|-----------------|---------------|--------------------|--|
| | | | | Unspiked (µg/L) | Spiked (µg/L) | Spike Recovery (%) | Calculated Sample Concentration (µg/g) | Unspiked (µg/L) | Spiked (µg/L) | Spike Recovery (%) | Calculated Sample Concentration (µg/g) | Unspiked (µg/L) | Spiked (µg/L) | Spike Recovery (%) | Calculated Sample Concentration (µg/g) |
| As | | 75 | He | 0.035 | 1.10 | 106 | 0.0086 | <0.0067 | 1.10 | 110 | <0.0017 | 0.024 | 1.08 | 105 | 0.006 |
| As | 75 | 91 | O ₂ | 0.038 | 1.08 | 104 | 0.0093 | <0.0015 | 1.04 | 104 | <0.0004 | 0.021 | 1.07 | 105 | 0.005 |
| Cd | | 111 | He | 0.013 | 1.05 | 103 | 0.0031 | <0.0019 | 1.02 | 102 | <0.0005 | <0.0019 | 1.01 | 101 | <0.0005 |
| Cd | 111 | 111 | O ₂ | 0.014 | 1.10 | 109 | 0.0034 | 0.0008 | 1.07 | 106 | 0.0002 | 0.0009 | 1.05 | 105 | 0.0002 |
| Hg | | 202 | He | <0.0004 | 0.098 | 98 | <0.0001 | <0.0004 | 0.098 | 98 | <0.0001 | <0.0004 | 0.098 | 98 | <0.0001 |
| Pb | | 208 | He | 0.029 | 1.07 | 104 | 0.0069 | 0.011 | 1.04 | 103 | 0.0026 | 0.007 | 1.03 | 102 | 0.0018 |

Table 8. Quantitative results for multiminerals supplements.

| | Q1 | Q2 | Cell Mode | Sample 4 | | | | Sample 5 | | | |
|----|-----|-----|----------------|-----------------|---------------|--------------------|--|-----------------|---------------|--------------------|--|
| | | | | Unspiked (µg/L) | Spiked (µg/L) | Spike Recovery (%) | Calculated Sample Concentration (µg/g) | Unspiked (µg/L) | Spiked (µg/L) | Spike Recovery (%) | Calculated Sample Concentration (µg/g) |
| As | | 75 | He | 0.38 | 1.37 | 100 | 0.18 | 0.47 | 1.46 | 99 | 0.23 |
| As | 75 | 91 | O ₂ | 0.33 | 1.35 | 102 | 0.16 | 0.45 | 1.38 | 92 | 0.23 |
| Cd | | 111 | He | 0.21 | 1.17 | 97 | 0.10 | 0.29 | 1.21 | 92 | 0.14 |
| Cd | 111 | 111 | O ₂ | 0.21 | 1.21 | 101 | 0.10 | 0.29 | 1.26 | 97 | 0.14 |
| Hg | | 202 | He | 0.0019 | 0.098 | 97 | 0.0009 | 0.0015 | 0.094 | 92 | 0.0007 |
| Pb | | 208 | He | 0.29 | 1.29 | 100 | 0.14 | 0.12 | 1.06 | 94 | 0.060 |

Correction techniques using single quadrupole ICP-MS

All five samples could be measured in He mode using a single quadrupole ICP-QMS. However, if the ratio of Co/As and/or Mo/Cd is high, there would be interference from $^{59}\text{Co}^{16}\text{O}^+$ on $^{75}\text{As}^+$ and $^{95}\text{Mo}^{16}\text{O}^+$ on $^{111}\text{Cd}^+$. Because it is difficult for the analyst to be aware of oxide interferences, the Quick Scan function can be used to identify all elements present in the sample. As shown in Figure 3, by checking the Quick Scan data, potential interferences can be identified, such as CoO^+ based on the high concentration of Co in one of the samples.

Once any potential interferences have been identified using Quick Scan, interference correction equations can be applied to compensate for doubly charged and oxide interferences.

Alternatively, it is possible to perform routine analysis without prior knowledge of potential interferences using an 8900 ICP-QQQ operating in MS/MS mode.

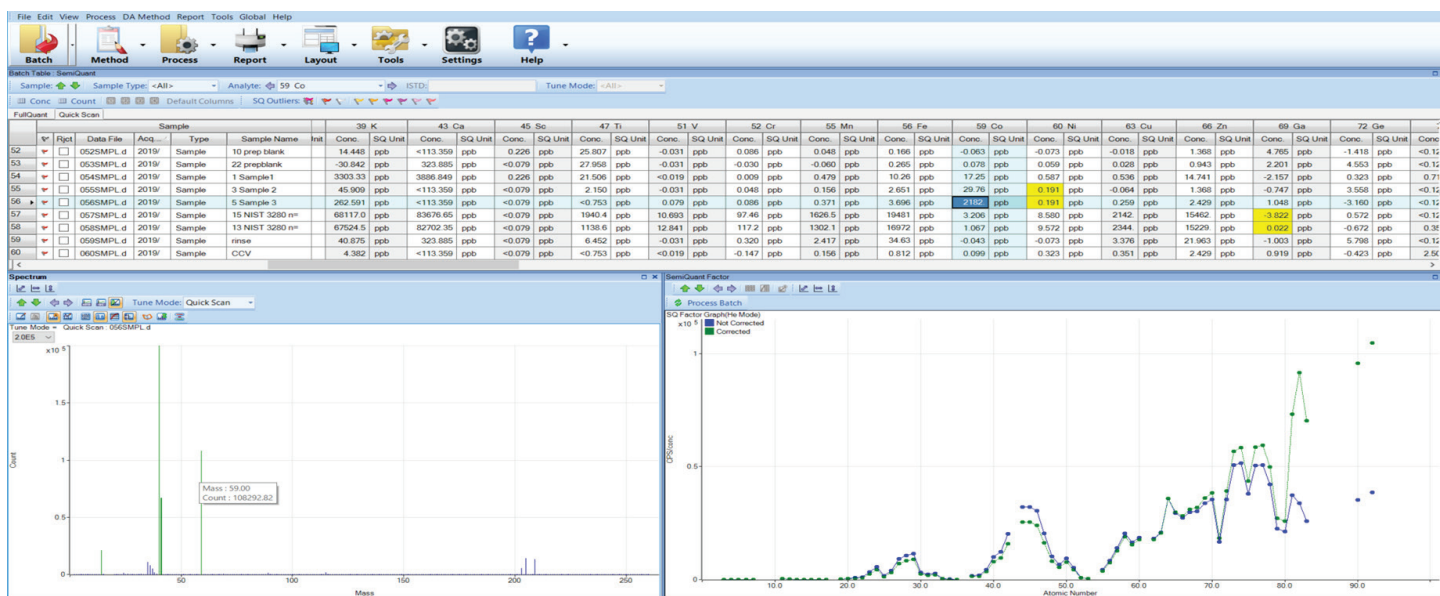


Figure 3. ICP-MS MassHunter Quick Scan qualitative results showing a high concentration of Co in one of the samples.

Conclusion

The Agilent 8900 ICP-QQQ with UHMI effectively removes challenging spectral interferences making it suitable for the routine analysis of the trace elements in high matrix samples such as multiminerals supplements.

Doubly charged REE interferences that can affect As measurement at trace levels were avoided using O_2 cell gas and MS/MS mass-shift mode, as shown by the recoveries for the Apple Leaves SRM. Also, oxide interferences that can affect As and Cd measurement at trace levels were avoided using the same MS/MS method. Excellent recoveries were achieved for Hg and Pb using He mode – a proven technique for the removal of common matrix-based polyatomic interferences in complex and variable matrices. Therefore, it is possible to perform routine analysis of all elements

without prior knowledge of potential interferences using an 8900 ICP-QQQ operating in He mode and MS/MS mode.

For laboratories without access to ICP-QQQ, only ICP-QMS, the Quick Scan function of ICP-MS MassHunter can be used to identify potential interferences from M^{++} and oxide ion interferences. These interferences can then be corrected using interference correction equations.

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