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Determination of Elemental Nutrients and Micronutrients in Functional Foods by ICP-OES

Accurate EAM 4.4 compliant results using the Agilent 5900 SVDV ICP-OES with smart tools

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Introduction

Functional foods are foods that supplement the diet to provide health benefits that extend beyond basic nutrition (*1*). The foods can come in many different forms, from unprocessed fruits and vegetables to foods and beverages that contain mineral additives. Sales of functional foods have experienced rapid growth over the last decade, while products that claim to boost immune functionality have experienced heightened consumer demand more recently (*2, 3*). With the rise in popularity of these foods, it is essential that they are produced in accordance with nutritional labeling requirements, as outlined by regulatory bodies such as the US Food and Drug Administration (FDA).

Proper labeling is especially important if the claimed benefits of the product relate to its elemental composition. Consumers also need to be confident that the supplements are free from adulteration, chemical residues, and contaminants. Therefore, most regulations relate to testing foods for nutritional content and elemental impurities that may be introduced into the products during the manufacturing process. The FDA Elemental Analysis Manual (EAM) 4.4 is an example of a method that outlines how to accurately analyze elements in food by inductively coupled plasma-optical emission spectroscopy (ICP-OES) for regulatory purposes (4). The EAM 4.4 method has been adapted in this study for the analysis of elemental nutrients and micronutrients in functional food samples.

Traditionally, flame atomic absorption spectroscopy (FAAS) has been used for the verification of nutritional elements in foods. However, the need for lower detection limits and higher sample throughput has led many food labs towards using a faster, simultaneous analytical technique, such as ICP-OES.

Food quality and testing labs often process large numbers of samples, so require highly productive and robust ICP-OES methods that deliver accurate results over long analytical runs. To meet these requirements, the Agilent 5900 Synchronous Vertical Dual View (SVDV) ICP-OES uses several smart-tools that aid with method development, speed of analysis, and data accuracy. Examples include:

– SVDV technology that uses a dichroic spectral combiner (DSC) to capture both axial and radial views in a single reading (5). This dual view capability allows for the analysis of macro and micronutrient elements at the same time, halving the analysis time compared to non-SVDV instruments, while delivering accurate results.

– IntelliQuant Screening software that allows the user to perform a full semiquantitative spectrum scan of up to 78 elements in just a few seconds (6). The data provides sample insight that greatly simplifies method development and removes the need for guesswork.

– Fitted Background Correction (FBC), a software feature that automatically corrects simple and complex background structures (7). FBC makes it easier than ever to correct background peaks without requiring extensive knowledge or prior investigation into the sample matrix.

In this study, the 5900 SVDV ICP-OES was used to determine Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn in elderberry-based supplements. The method was adapted from the FDA EAM 4.4 ICP-OES method for food and related products.

Experimental

Instrumentation

The 5900 SVDV ICP-OES was fitted with an Agilent SPS 4 autosampler. The sample introduction system consisted of a SeaSpray nebulizer, double-pass cyclonic spray chamber, and a 1.8 mm inner diameter (I.D.) one-piece injector torch. Most of the instrument settings used in this application were the default parameters (Table 1), greatly saving time spent developing the method.

Table 1. Agilent 5900 SVDV ICP-OES instrument and method parameters.

Parameter	Setting
Read time (s)	5
Replicates	3
Sample uptake delay (s)	20
Stabilization time (s)	15
Rinse time (s)	60
Pump speed (rpm)	12
Fast pump	Enabled
RF power (kW)	1.2
Auxiliary flow (L/min)	1.00
Plasma flow (L/min)	12
Nebulizer flow (L/min)	0.7
Viewing height (mm)	8
Sample pump tubing	White-white
Internal standard tubing	Orange-white
Internal standard	Y (5 ppm)
Waste pump tubing	Blue-blue
Background correction	Fitted (FBC)

Sample preparation

Three different commercially available elderberry-based immune support supplement functional foods were prepared for analysis. The samples included a "natural" elderberry syrup (with no additives), an effervescent tablet, and a "blended" syrup that contained additives. The NIST 2385 Slurried Spinach standard reference material (SRM, National Institute of Standards and Technology, Gaithersburg, US) was used for method evaluation. The samples and SRM were prepared by microwave digestion (CEM MARS 6 Microwave Digestion System, Buckingham, UK).
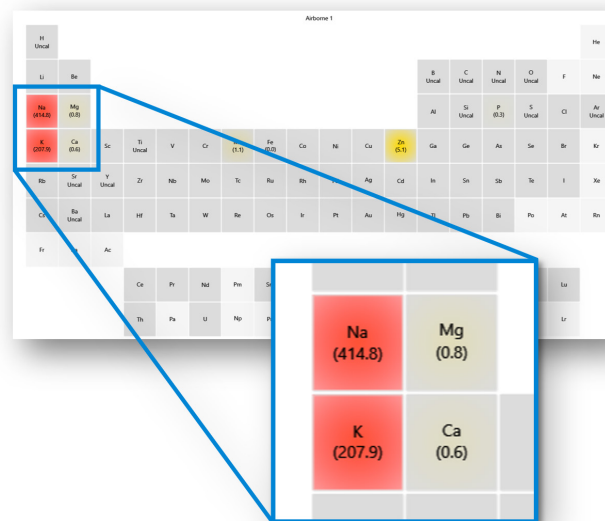
An aliquot of 0.5 g of sample was accurately weighed into a 75 mL PFA MARS Xpress vessel. 8 mL concentrated nitric acid (HNO_3) and 1 mL 30% hydrogen peroxide (H_2O_2) were added to each vessel. Duplicates of each sample type were digested in the same batch using the program outlined in Table 2. The digested solutions were then diluted with de-ionized water to a volume of 50 mL.

Figure 1. IntelliQuant Screening elemental 'heat map', showing which elements are present in the effervescent tablet and the semiquantitative concentration (ppm).

Table 2. Microwave digestion parameters.

Parameter	Setting
Maximum power (W)	1200
Temperature (°C)	200
Ramp time (min)	25
Hold time (min)	15

Method development

The IntelliQuant Screening software function was used to select wavelengths and estimate the concentrations of elements in the samples. The concentration data can be presented as a periodic table 'heat map' view, as shown in Figure 1 for the effervescent tablet sample. The heat map showed that sodium (Na) and potassium (K) were present at a significantly higher concentration than the other elements, requiring a calibration up to 500 ppm. IntelliQuant Screening can quickly and easily inform the user of an appropriate calibration range before starting the quantitative analysis, helping with method development. Yttrium (Y) was chosen as an internal standard to compensate for any matrix effects.

A 5900 SVDV ICP-OES with DSC was used in this study so trace elements could be measured in axial view for the greatest sensitivity, while higher abundant elements were measured in radial view. Using the DSC to capture signals from both views of the plasma in a single reading saves measurement time and reduces instrument running costs.

Generally, the more abundant elements are easily ionizable elements (EIEs), meaning they have low ionization energies and are easily ionized in the plasma. If EIEs are present in a sample at a high enough concentration, the plasma electron density and the atomization-ionization equilibria are affected. These effects cause either an enhancement or suppression of the emission signals, leading to the reporting of either false high or false low element concentrations. Using radial view and optimizing the viewing height can largely alleviate these issues. Typically, dealing with EIEs usually requires trace and macro elements to be separated into axial and radial view analyses to maintain good sensitivity for the trace elements. However, with SVDV technology, the analysis is performed in one measurement, which not only saves time but also argon consumption, crucial for high-throughput laboratories that are looking to control costs.
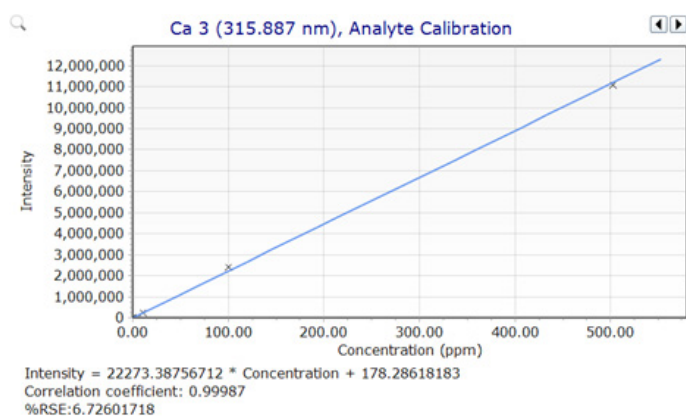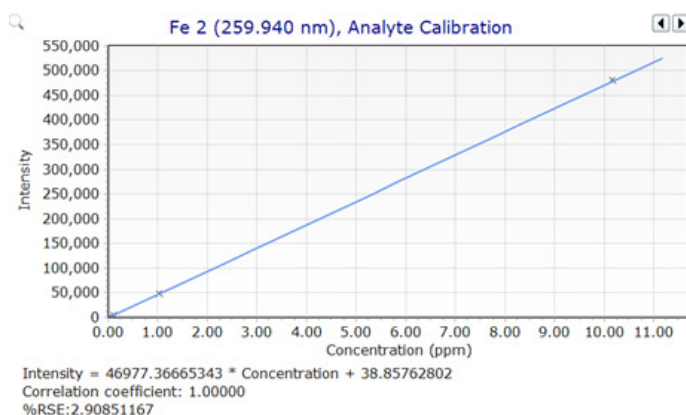
Calibration

All calibration standards were prepared using Agilent single element calibration standard solutions. The solutions were matrix-matched using HNO_3 (Emsure, Merck) and diluted with 18.2 MΩ de-ionized water (Merck Millipore) to form a final matrix of 10% HNO_3. The Y 371.029 nm internal standard solution was prepared at 5 ppm from an Agilent 10,000 ppm stock solution in a 10% HNO_3 matrix.

Elements were calibrated between 0.01 and 500 ppm and linear calibration curves were obtained for all elements, with correlation coefficients >0.9996 (Table 3). The correlation coefficient of the calibration was well below the limit of 0.998 that is stipulated in EAM 4.4. Representative calibration curves are shown in Figure 2.

Table 3. Background correction, internal standard, and calibration information.

Element and Wavelength (nm)	Background Correction	Calibration Range (ppm)	Correlation Coefficient	Internal Standard
Ca 315.887	Fitted	0.01 − 500	0.99987	Y 371.029
Cu 324.754	Fitted	0.01 − 10	1.00000	None
Fe 259.940	Fitted	0.01 − 10	1.00000	None
K 766.491	Fitted	0.1 − 500	0.99964	None
Mg 285.213	Fitted	0.01 − 100	0.99981	Y 371.029
Mn 257.610	Fitted	0.01 − 10	1.00000	None
Na 589.592	Fitted	0.1 − 500	0.99967	None
P 178.222	Fitted	0.1 − 100	1.00000	None
Zn 213.857	Fitted	0.01 − 10	1.00000	None

Intensity = 46977.36665343 * Concentration + 38.85762802
Correlation coefficient: 1.00000
%RSE:2.90851167

Intensity = 22273.38756712 * Concentration + 178.28618183
Correlation coefficient: 0.99987
%RSE:6.72601718

Figure 2. Calibration curve for Fe 259.940 nm (top) and Ca 315.887 nm (bottom).

Automatic background correction

FBC was selected in the Agilent ICP Expert software to correct for background structures on all analyte wavelengths (Table 3). Variable background structures are common in food samples, making FBC highly suitable for this application. Figure 3 shows the accurate correction of an OH emission line, permitting low-level detection of Ca at 315.887 nm in a real functional food sample.
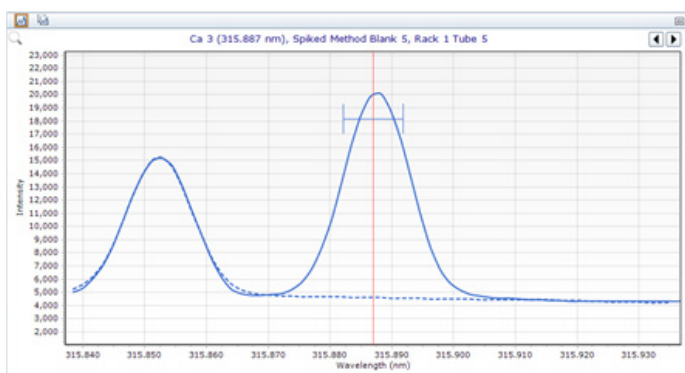
Figure 3. Automatic background correction using FBC for Ca 315.887.

Results and discussion

The EAM method detection limits (LODs) and quantification limits (LOQs) are given in Table 4. The LODs and LOQs were calculated by analyzing six sets of 10 method blank solutions on two separate instruments over three non-consecutive days. The LODs are based on three sigma of the 10 replicate measurements, and the LOQs are based on 10 sigma. The 5900 SVDV ICP-OES analytical limits are lower than the EAM 4.4 nominal analytical limits for all elements.

Table 4. LODs and LOQs for all elements, calculated to account for the 100x dilution factor used in sample preparation.

Element and Wavelength	Quantitative Results (mg/kg)		EAM 4.4 Nominal Analytical Limits (mg/kg)	
	LOD	LOQ	LOD	LOQ
Ca 315.887	0.107	0.357	8	30
Cu 324.754	0.0490	0.163	0.1	0.3
Fe 259.940	0.0295	0.0984	0.2	0.3
K 766.491	7.34	24.5	20	40
Mg 285.213	0.0164	0.0546	2	6
Mn 257.610	0.00684	0.0228	0.2	0.4
Na 589.592	0.893	2.98	2	5
P 178.222	1.70	5.67	2	6
Zn 213.857	0.0216	0.0721	0.3	0.8

SRM analysis

To confirm the accuracy of the quantitative method, the digest of the Slurried Spinach SRM was analyzed in triplicate using the 5900 SVDV ICP-OES. The averaged recoveries for all analytes were within ±10% of the certified value, as shown in Table 5. These recoveries are well within the ±20% acceptance criteria specified in the EAM 4.4 method.

Table 5. Slurried Spinach 2385 SRM data, n=3.

Element & Wavelength	NIST 2385 Slurried Spinach			
	Certified Conc (mg/kg)	Measured Conc (mg/kg)	Recovery (%)	QC Criteria
Ca 315.887	624	638	102	Pass
Cu 324.754	0.9	0.856	95	Pass
Fe 259.940	17.1	18.7	109	Pass
K 766.491	3650	3643	100	Pass
Mg 285.213	368	360	98	Pass
Mn 257.610	3.81	3.70	97	Pass
Na 589.592	47	49.0	104	Pass
P 178.222	323.7	320	99	Pass
Zn 213.857	8.37	8.35	100	Pass

Quantitative data

The quantitative results from the analysis of the three elderberry-based immune support supplement functional food samples are reported in Table 6. The Na and K content of the effervescent tablet was much higher than the other samples, likely due to the composition of the dissolvable matrix. Also, a higher concentration of all elements was determined in the syrup that contained additives compared to the more natural syrup product.

Table 6. Quantitative results of all samples (mg/kg). The data is corrected for dilution.

Element and Wavelength	Sample 1 (Syrup)	Sample 2 (Effervescent Tablet)	Sample 3 (Syrup with Additives)
Ca 315.887	11.9	44.4	83.9
Cu 324.754	0.166	0.0802	68.7
Fe 259.940	0.909	1.68	2.21
K 766.491	403	20857	1125
Mg 285.213	11.3	96.0	143
Mn 257.610	0.135	120	0.635
Na 589.592	42.3	48558	1195
P 178.222	130	29.3	544
Zn 213.857	0.194	576	219

Spike recoveries

Spike recovery tests were performed to test for matrix effects. The Fortified Method Blank (FMB) was prepared by spiking the method blank with trace elements at 5 ppm and the major elements Ca, K, and Na at 50 ppm. A Fortified Analytical Portion (predigest spike) was spiked with elements at the same concentration as present in the sample (sample 3). All results reported in Table 7, which have the 100x dilution factor applied, were within the EAM method acceptance recovery range of 90–110% for the FMB and 80–120% for the FAP.

Table 7. Spike recovery results.

Element and Wavelength	Fortified Method Blank		Fortified Analytical Portion				QC Criteria
	Spiked Concentration (mg/kg)	Recovery (%)	Unfortified (mg/kg)	Fortified (mg/kg)	Expected Spike (mg/kg)	Recovery (%)	
Ca 315.887	0.542	*108	0.829	3.39	2.61	98	Pass
Cu 324.754	0.0512	102	0.681	2.96	2.53	90	Pass
Fe 259.940	0.0542	108	0.0224	0.0459	0.0255	92	Pass
K 766.491	0.512	*102	11.1	59.6	50.5	96	Pass
Mg 285.213	0.0507	101	1.42	6.39	5.26	94	Pass
Mn 257.610	0.0518	104	0.00624	0.0179	0.0127	92	Pass
Na 589.592	0.515	*103	11.9	60.3	51.1	95	Pass
P 178.222	0.0545	109	5.41	14.5	9.99	91	Pass
Zn 213.857	0.0500	100	2.17	7.00	5.11	95	Pass

*Higher spike concentration used.

Long-term stability

To assess the stability of the 5900 SVDV ICP-OES, 360 solutions were measured over 10 hours without recalibration. A QC solution was measured immediately after calibration and then after every 10 samples. Figure 4 shows the stability of all elements over 10 hours to be within ±3%. The relative standard deviation (%RSD) was below 1.5% for all elements, as shown in Table 8.
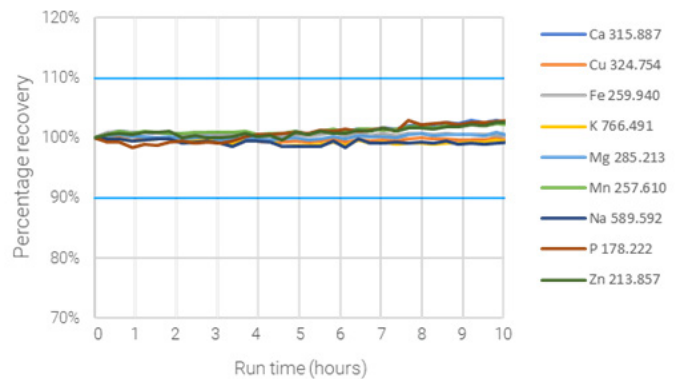
Figure 4. Recovery of elements in a QC solution measured for 10 hours.

Table 8. Concentration of elements in the QC solution and %RSD of measurements taken over 10 hours.

Element and Wavelength	Concentration (ppm)	%RSD
Ca 315.887	5	0.8
Cu 324.754	5	0.9
Fe 259.940	1	0.8
K 766.491	50	0.9
Mg 285.213	5	0.8
Mn 257.610	5	1.0
Na 589.592	50	0.8
P 178.222	5	1.2
Zn 213.857	5	1.5

Conclusion

The Agilent 5900 SVDV ICP-OES was used for the accurate, robust, and fast analysis of multiple elements in functional foods in accordance with US FDA EAM method 4.4 for food and related products.

Method development was streamlined using a range of smart tools that are included in the Agilent ICP Expert software.

- IntelliQuant Screening provided semiquantitative concentrations for all elements in each sample. It also signaled the presence on unexpected elements, aiding wavelength selection. By providing an estimation of sample contents, a calibration range was quickly generated, reducing method development time.

- Synchronous Vertical Dual View (SVDV) removed the need to analyze macro and micronutrient elements separately using radial and axial views. The combination of the two views by the DSC allowed fast measurements while maintaining the best detection limits and reducing argon consumption.

- Fitted Background Correction (FBC) automatically corrected complex background structures that are commonly found in food samples. No time was wasted by manually correcting the background.

The accuracy of the method was evaluated by analysis of an SRM and by conducting multiple spike recovery tests. The tests were carried out on blanks and on samples before digestion. All recoveries were within ±10% in all cases. The instrument also displayed excellent stability over a 10-hour run without failing a single QC.

This study demonstrated the suitability of the 5900 SVDV ICP-OES for the routine measurement of functional foods, irrespective of the sample matrix. The method is ideal for nutritional labeling of elements in foods.

References

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5. Synchronous Vertical Dual View (SVDV) for High Productivity and Low Cost of Ownership, Agilent publication 5994-1513EN

6. Agilent IntelliQuant Screening, Agilent publication 5994-1518EN

7. Fitted Background Correction (FBC) - Fast, Accurate and Fully Automated Background Correction, Agilent publication 5991-4836EN

Agilent part numbers

Description	Part number
Easy-fit 1.8 mm semi-demountable torch for 5000 series VDV/SVDV ICP-OES	G8010-60236
Double-pass spray chamber, glass cyclonic design with ball joint socket and UniFit drain outlet, for Agilent 5000 series ICP-OES	G8010-60256
SeaSpray concentric glass nebulizer for 5000 series ICP-OES	G8010-60255
Peristaltic pump tubing, white/white, 12/pk	3710034400
Peristaltic pump tubing, blue/blue, 12/pk.	3710034600
Peristaltic pump tubing for internal standard, orange/white, 12/pk	3710046900
Y-piece connector for online addition of internal standard/ionization buffer	1610132400
Agilent 10,000 ppm single element stock solution for Ca, 500 mL	5190-8369
Agilent 10,000 ppm single element stock solution for K, 500 mL	5190-8433
Agilent 10,000 ppm single element stock solution for Na, 500 mL	5190-8206
Agilent 1000 ppm single element stock solution for Cu, 500 mL	5190-8349
Agilent 1000 ppm single element stock solution for Fe, 500 mL	5190-8472
Agilent 1000 ppm single element stock solution for Mg, 500 mL	5190-8482
Agilent 1000 ppm single element stock solution for Mn, 500 mL	5190-8484
Agilent 1000 ppm single element stock solution for P, 500 mL	5190-8500
Agilent 1000 ppm single element stock solution for Zn, 500 mL	5190-8558

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