Determination of Nutrients and Micronutrients in Functional Foods

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Introduction

Elderberry-based immune syrups and tablets

Functional foods are foods that supplement the diet to provide health benefits that extend beyond basic nutrition.[1] They can come in a variety of different forms, and the market for them has experienced rapid growth over the last decade. Some products, such as those that claim to boost immune functionality, experienced heightened consumer response in recent years.[2,3]

It is essential that these foods be produced in accordance with the strict nutritional labeling required by regulatory bodies such as the US FDA. Food must also be free from contamination introduced during the manufacturing process.

Experimental

Instrumentation

The Agilent 5900 SVDV ICP-OES (Figure 1) was fitted with an SPS 4 autosampler for the determination of Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn in elderberry-based immune supplements (syrups and tablets).

The analysis was adapted from the FDA EAM 4.4 ICP-OES method for the Determination of Elements in Food Using Microwave Assisted Digestion.[4]

Figure 1. Agilent 5900 SVDV ICP-OES and ICP Expert software.

The instrument and software features beneficial to this application include:

- **Synchronous Vertical Dual View (SVDV)** – both the radial and axial views of the plasma were captured in a single measurement (Fig. 2). Axial view was used for macronutrients (e.g., K) and radial view used for micronutrients (e.g., Cu). Measuring both at the same time halved analysis times.
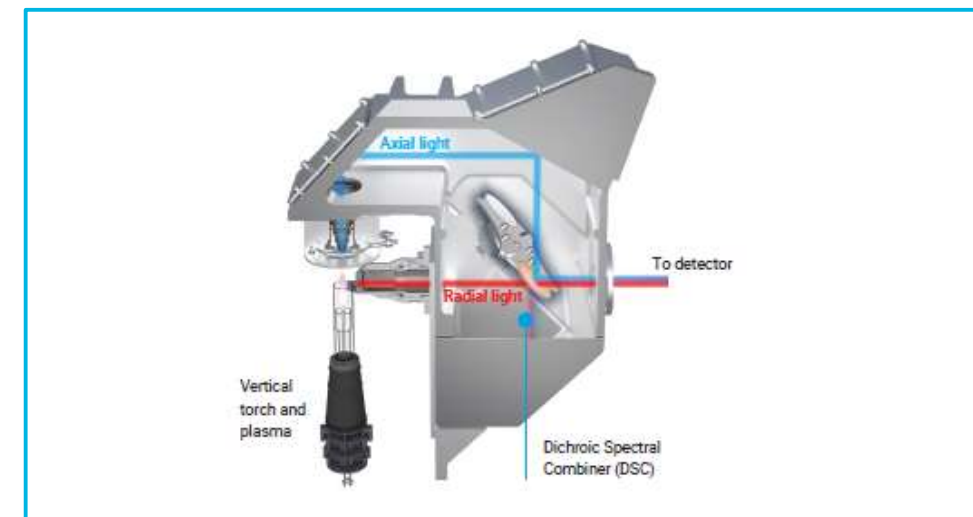

Figure 2. Dichroic Spectral Combiner (DSC) technology, enabling SVDV viewing.

- **IntelliQuant Screening** –fast, semi-quantitative sample scans without having to calibrate the instrument or choose elements and wavelengths. This greatly simplified.
- **Fitted Background Correction** (FBC), – software that automatically corrects simple and complex background structures. FBC makes it easier than ever to correct background peaks without requiring extensive knowledge of the sample matrix.

Assistance with method development

The IntelliQuant Screening function was used to screen samples. The semiquantitative results can be displayed in different formats, including a pie chart (Figure 3) or as periodic heat map (Figure 4). Presenting the results in a visualized format allows users to quickly assess which elements are present in a sample.

In this study, knowing the elemental composition of the samples helped determine the calibration range and wavelengths to be used for the quantitative method.
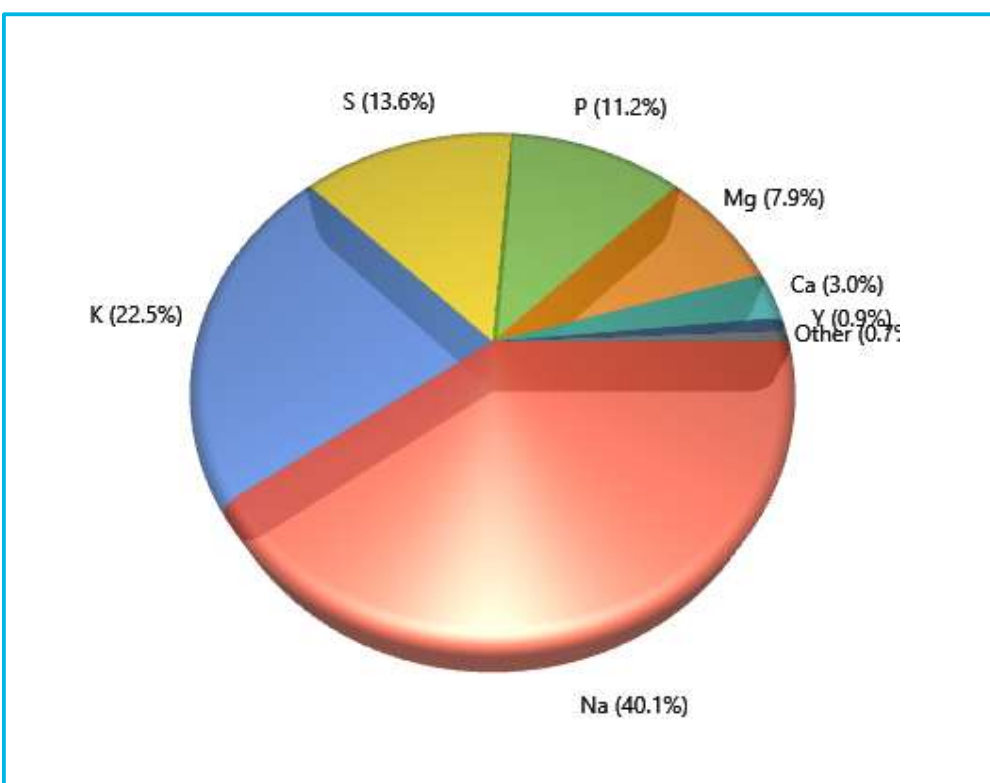

Figure 3. Elemental breakdown of unknown sample, providing percentage composition of a sample.
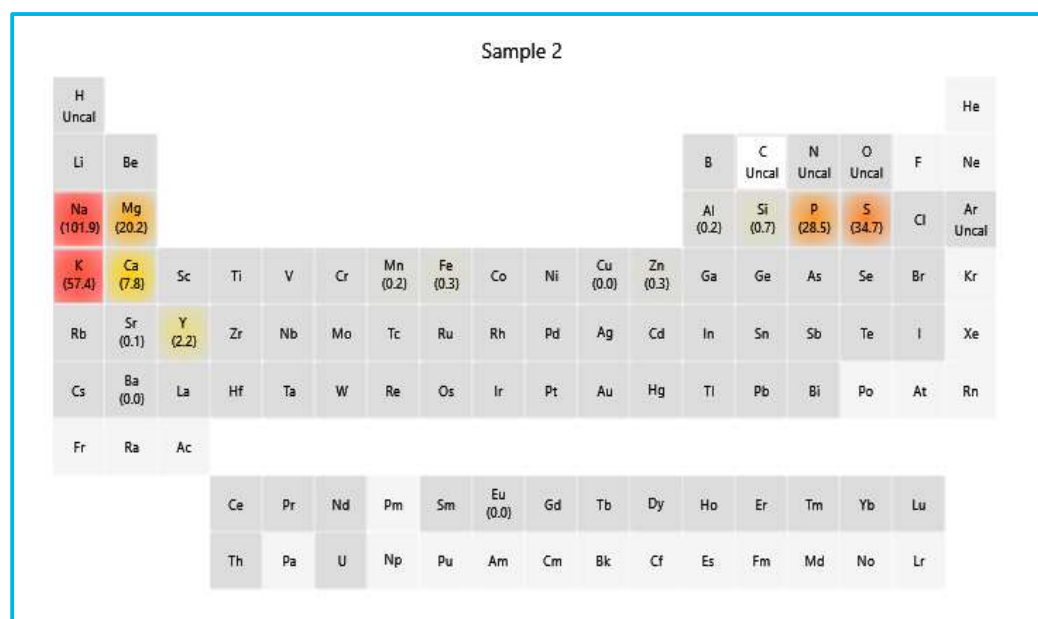

Figure 4. Periodic table elemental 'heatmap'. The semi-quantitative concentrations given can inform the analyst of potential unexpected elements/contaminants in a sample.

Experimental

Calibration

Elements were calibrated between 0.01 and 500 ppm and linear calibration curves were obtained for all elements, with correlation coefficients >0.9996. 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 5.
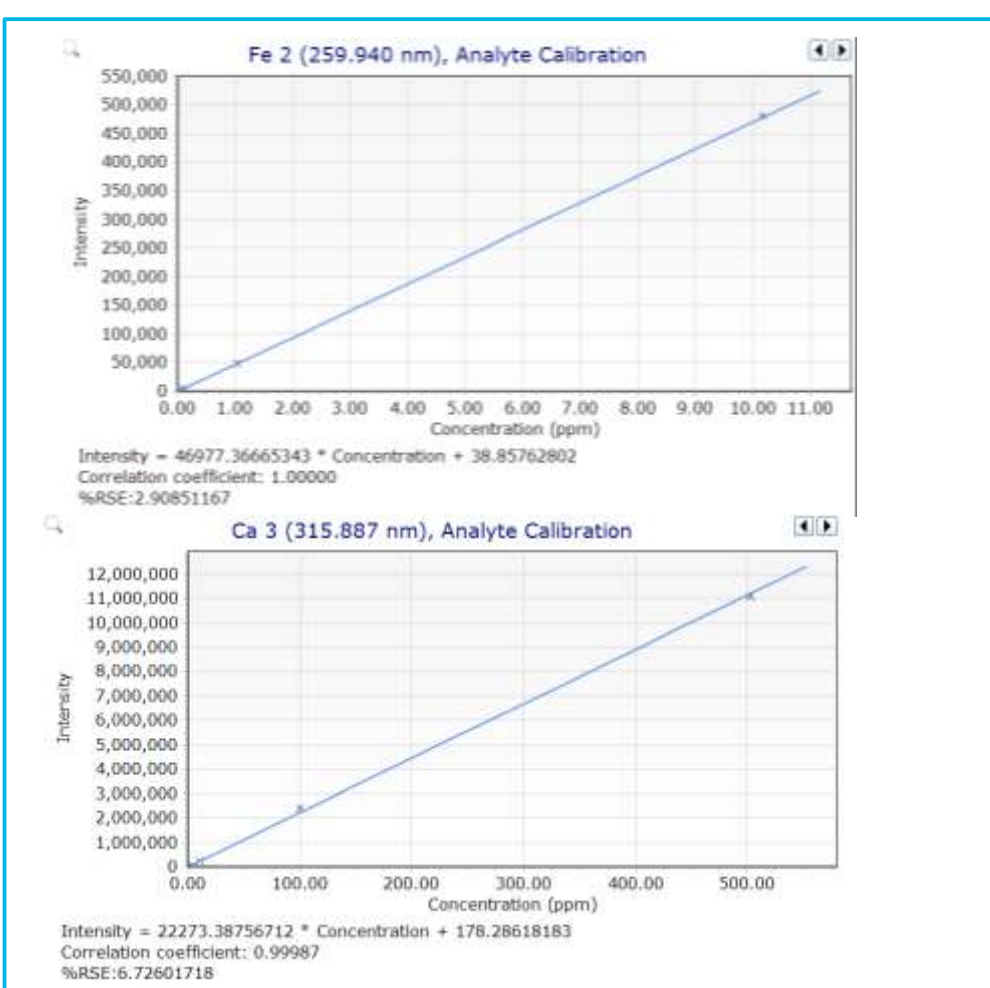

Figure 5. Representative calibration curves.

Sample preparation

Three different commercially available elderberry-based immune support supplement functional foods were prepared for analysis by microwave digestion using the CEM MARS 6 Microwave Digestion System (Table 1). The samples were prepared in triplicate. The following reagents were added to the microwave vessels:

- 0.5 g sample/water (blank)
- 8 mL nitric acid
- 1 mL 30% hydrogen peroxide

Once digested, the contents of the vessels were emptied into 50 mL tubes and filled to the mark with deionized water.

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

Table 1. Microwave digestion program.

The 5900 ICP-OES sample introduction system consisted of a SeaSpray nebulizer, double-pass cyclonic spray chamber and a 1.8mm I.D. injector torch. Instrument operating conditions are given in Table 2.

Parameter	Setting	Parameter	Setting
Read time (s)	5	Plasma flow (L/min)	12.0
Replicates	3	Nebulizer flow (L/min)	0.7
Sample uptake delay (s)	20	Viewing height (mm)	8
Stabilization time (s)	15	Sample pump tubing	White-white
*Rinse time (s)	30	IS tubing	Orange-green
Pump speed (rpm)	12	Internal Standard	Y (5ppm)
Fast pump	Enabled	Waste pump tubing	Blue-blue
RF power (kW)	1.2	Background correction	Fitted
Auxiliary flow (L/min)	1.00		

Table 2. ICP-OES instrument and method parameters.

*Intelligent Rinse can be turned on during the analytical run. This feature optimizes the sample rinse times depending on the sample concentration, saving time and argon without compromising results.

Background correction

FBC was selected to correct for background structures. It was suitable for both the simple and complex matrices that were present. Figure 6 displays the accurate correction of a OH emission line, permitting low-level detection of Ca at 315.887 nm.
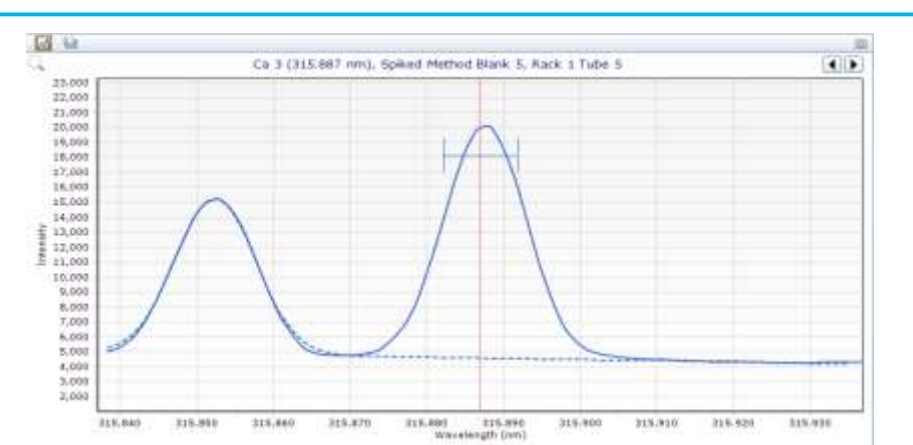

Figure 6. Automatic background correction using FBC for Ca at 315.887 nm.

Results and Discussion

Method validation

A 'Slurried Spinach' Standard Reference Material (SRM 2385) purchased from the National Institute of Standards and Technology (NIST) was used in this study to confirm the efficacy of the sample preparation process and the method.

The SRM, method blank, and spiked samples were subjected to the same sample preparation procedure as the real samples.

The SRM results, shown in Table 3, were all within ±10% of the certified values, proving the accuracy of the method.

Results and Discussion

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

Table 3. Recoveries of elements in NIST 2385 Slurried Spinach SRM.

Spike recoveries

Spike recovery tests were performed to test the accuracy of the method on real samples.

Sample spikes were performed pre-digestion (Fortified Analytical Portion) and post-digestion (Fortified Analytical Solution) to generate the same elemental concentrations as the real sample.

These test the robustness of the method and determine if further dilution is required to account for any matrix interferences.

All spike tests, resulted in recoveries within ±10% (Table 6).

Element & Wavelength	Quantified Conc in Syrup (mg/kg)	Fortified Analytical Solution (post-digest spike)	Fortified Analytical Portion (pre-digest spike)
Ca 315.887	83.9	98	98
Cu 324.754	68.7	90	90
Fe 259.940	2.21	92	93
K 766.491	1125	96	96
Mg 285.213	143	95	94
Mn 257.610	0.635	91	91
Na 589.592	1195	95	94
P 178.222	544	91	90
Zn 213.857	219	95	94

Table 3. Quantitative data for syrup and spike recoveries.

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 every 10 samples. Figure 7 shows the recovery of all elements to be within ±3%, with no QC failures.
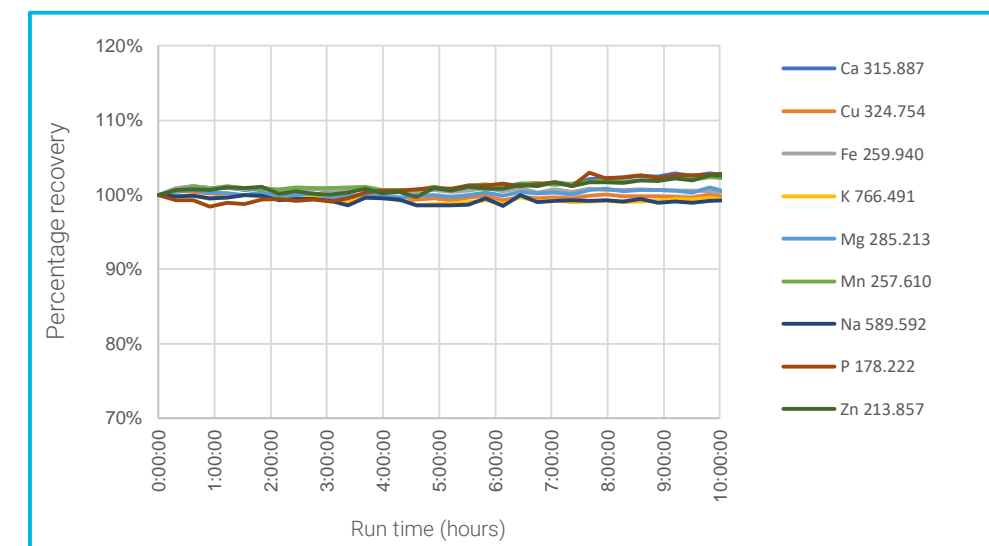

Figure 7. Long term stability data over 11 hours.

Conclusions

Accurate, robust measurements of elements in functional foods can be performed using the Agilent 5900 ICP-OES in accordance with the FDA EAM 4.4 method.

The Agilent 5900 SVDV ICP-OES was used for the quantitative analysis of multiple elements in functional food samples after acid digestion, yielding excellent results.

Method development was greatly simplified and streamlined through the use of smart tools included in the instrument software:

- IntelliQuant Screening
- Synchronous Vertical Dual View (SVDV)
- Fitted Background Correction

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 samples before and after 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.

References

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