APPLICATION NOTE

Atomic Absorption

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Analysis of Micronutrients in Fortified Breakfast Cereal by Flame Atomic Absorption Using Microwave Digestion and FAST Flame Sample Automation

Introduction

Enjoyed throughout the world at the start of the day, a breakfast of fortified cereal with the addition of milk[1] and fruit[2] can be a simple and quick solution to a nutritious meal. Fortified breakfast cereals are also an important source of nutrition for children, and consumers have come to expect high quality from a variety of cereals and continue to select fortified products over non-fortified products in the marketplace.

The efficient production of these nutritionally fortified breakfast cereals requires careful formulation and uniformity batch to batch. Ongoing analytical measurement of nutritional additives and the total micronutrient content in the cereal is one way in which food producers can quantify the quality and consistency of their cereal products. The ability to quickly, accurately, and easily analyze their samples is also key to timely data reporting, allowing real-time batch adjustments to be made and enhancing continuous process control. Food producers must also meet nutritional labeling guidelines which require an accurate assessment of micronutrients for regulatory labeling compliance.

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While inductively coupled plasma optical emission spectroscopy (ICP-OES) is generally favored in a multi-element analytical environment, the cost savings, simplicity, and speed of operation of a flame atomic absorption (AA) system provides an attractive alternative. Measuring multiple elements by flame AA requires each sample to be analyzed individually for each element, which impacts the speed advantage of flame AA.

To address the speed issue, a fast high-throughput sample automation system can be used. Although samples still need to be analyzed multiple times, the analysis time per sample is significantly reduced, thus increasing sample throughput compared to manual sample introduction. In addition, an automated sample introduction system increases the precision of the analysis and frees the chemist to perform other tasks.

In this work, we demonstrate the ability of PerkinElmer's PinAAcle™ 900 atomic absorption spectrometer (operating in flame mode) coupled to a FAST Flame sample automation accessory to analyze common nutritional elements in a variety of fortified cereals.

Experimental

All analyses were performed on a PinAAcle 900T atomic absorption spectrometer operating in flame mode using a FAST Flame 2 sample automation accessory. The elements of interest and instrument conditions for the analysis of the cereal samples are outlined in Table 1. A high-sensitivity nebulizer was used with the standard spray chamber and a 10 cm burner head. External calibrations were performed using a single intermediate standard made in 2% HNO_3/deionized water which was then diluted in-line using the capabilities of the FAST Flame 2 accessory.

The FAST Flame 2 accessory is a combination of high-speed autosampler, peristaltic pump, and switching valve which provides quick sample turnaround with fast rinse-out, short signal stabilization times, and no sample-to-sample memory effect. The FAST Flame 2 accessory rapidly fills a sample loop via vacuum and then switches to inject while the autosampler moves to the next sample. This removes the time delay associated with self-aspiration or peristaltic pumping and eliminates the long rinse-in and rinse-out times as a result of autosampler movement and flushing, resulting in complete sample-to-sample analytical times as short as 15 seconds.

The ability of the FAST Flame 2 accessory to mechanically pump the sample during injection allows for ideal optimization of nebulizer and flame conditions, eliminates variability due to changes in sample viscosity, dissolved solids, and tubing length, and also provides long-term sample flow stability. The in-line dilution capability allows the analyst to create a single intermediate standard and then let the FAST Flame 2 accessory automatically generate all calibration standards in-line as required. In addition, the instrument can be set to identify QC over-range samples and then utilize the in-line dilution capability to automatically re-run a sample that falls outside the calibration range at an increased dilution factor, bringing the signal within the calibration range and providing accurate measurement along with a successful QC check.

For accurate analysis of the cereal samples, the elements of interest must be extracted from the cereal into an instrument-ready solution. Open-vessel digestion using nitric acid and a simple heating block can be effective, but may leave undigested matter behind requiring further filtration or centrifugation prior to introduction into the instrument, which can result in reduced recovery with corresponding poor accuracy. Closed-vessel

Table 1. PinAAcle 900 Instrument and Analytical Conditions

Element	Cu	Fe	Mg	Mn	Zn	K	Na	Ca
Mode	Absorption	Absorption	Absorption	Absorption	Absorption	Emission	Emission	Absorption
Wavelength (nm)	324.75	248.33	285.21	279.48	213.86	766.49	589.00	422.67
Slit (nm)	0.7	0.2	0.7	0.2	0.7	0.2	0.2	0.7
Acetylene Flow (L/min)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.7
Air Flow (L/min)	10	10	10	10	10	10	10	10
Burner Head Rotation	0°	0°	0°	0°	0°	45°	45°	45°
Acquisition Time (sec)	1	1	1	1	1	1	1	1
Replicates	3	3	3	3	3	3	3	3
Sample Flow Rate (mL/min)	6	6	6	6	6	6	6	6
Intermediate Standard (mg/L)	1	10	1	1	5	400	100	400
Auto-Diluted Calibration Standards (mg/L)	0.05 0.1 0.2 0.5 1	0.5 1 2.5 5 10	0.05 0.1 0.25 0.5 1	0.05 0.1 0.25 0.5 1	0.25 0.5 1 2.5 5	20 40 100 200 400	10 20 50 100	20 40 100 200 400
Calibration Curve Type	Non-Linear Through Zero	Non-Linear Through Zero	Non-Linear Through Zero	Non-Linear Through Zero	Non-Linear Through Zero	Non-Linear Through Zero	Non-Linear Through Zero	Non-Linear Through Zero

microwave digestion delivers complete sample digestion, eliminating the need for any additional steps and ensuring maximum element recovery while providing higher throughput and increased safety.

A variety of breakfast cereal samples (Table 2) and NIST™ SRM 3233 (Fortified Breakfast Cereal standard reference material) were prepared both spiked and unspiked using a PerkinElmer Titan MPS™ microwave sample preparation system, a sample digestion oven that utilizes unique vessel and system design with an emphasis on safety, throughput, and ease of use. With non-contact temperature control for every vessel and pressure control via a reference vessel, the Titan MPS ensures accurate digestion, method control, and zero sample contamination regardless of the sample type. Details of the microwave digestion method are listed in Table 3. Each vessel contained approximately 1 g of crushed cereal and 10 mL of concentrated nitric acid. All spiking was performed prior to sample digestion with spike concentrations selected based on the reported SRM values.

Results and Discussion

The calibration curves for individual elements were created from a single intermediate standard with the in-line dilution capabilities of the FAST Flame 2 accessory preparing the final standards in real-time. Calibration results are shown in Table 4. The excellent correlation for the calibration standards demonstrates the value of the automatic in-line sample and standard dilution capabilities. The independent calibration verification recoveries ensure that the calibration is valid and that the creation of standards via the dilution system is accurate.

Table 5 shows the results for the analyses of NIST™ SRM 3233 Fortified Breakfast Cereal. All elements read within 10% of the certified values, validating the accuracy of the methodology. It is also important to note that several different dilution factors were used for the various elements, all of which were performed in-line, without the need for user intervention.

With the accuracy of the methodology established, the cereal samples were analyzed. The results are shown in Figure 1 and show a few interesting trends. First, Cu and Mn are present at the lowest concentrations in all samples, while Na and K are present at the highest concentrations. It is interesting to note that Wheat Cereal 1 (W1) contains significantly less Na, Cu, Mn, and Fe than all the other samples, which could indicate that this cereal is less fortified and more natural than the others. In contrast, Oat Cereal (O) is at or near the top for all elements, indicating that it is among the most fortified cereal. Zinc, calcium, and, to a lesser extent, potassium fall into distinct levels equally divided among all the samples, indicating different levels of fortification.

Table 2. Cereal Types Analyzed and Corresponding Data Labels

Cereal Type	Data Label
Multi Grain	G
Oat	O
Rice	R
Corn	C
Wheat	W1, W2

Table 3. Titan MPS System Digestion Method

Method Step	Target Temp (°C)	Pressure Limit (bar)	Ramp Time (min)	Hold Time (min)	Power Limit (%)
1	140	35	10	2	60
2	195	35	3	25	100
3	50	35	1	20	0

Table 4. Calibration Results

Element	Correlation Coefficient	ICV Concentration (mg/L)	Measured ICV (mg/L)	ICV (% Recovery)
Cu	0.99997	0.500	0.494	98.8
Fe	0.99998	5.00	5.06	101
Mg	0.99996	0.500	0.456	91.2
Mn	0.99999	0.500	0.511	102
Zn	0.99990	2.50	2.54	102
K	0.99936	200	208	104
Na	0.99962	50.0	48.6	97.2
Ca	0.99999	200	207	104

Table 5. NIST™ SRM 3233 Fortified Breakfast Cereal Recovery Values

Element	In-line Dilution Factor	Certified SRM Concentration (mg/kg)	Measured SRM Concentration (mg/kg)	% Certified Value Recovery
Cu	1	3.97	4.26	107
Fe	5	766	751	98.0
Mg	40	1093	1142	105
Mn	1	33.1	30.9	93.4
Zn	10	628	587	93.5
K	3	3060	3278	107
Na	5	6830	7249	106
Ca	20	36910	37870	103

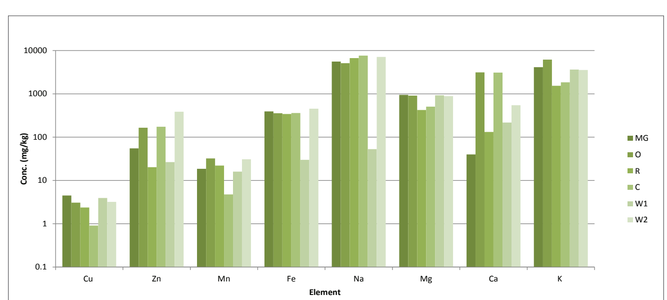

Figure 1. Results from analysis of seven cereals.

Table 6. In-Line Dilution Factors

Sample	Cu	Fe	Mg	Mn	Zn	K	Na	Ca
MG	1	5	40	1	10	3	4	6
O	1	5	40	1	10	3	4	8
R	1	5	40	1	10	3	4	8
C	1	5	40	1	10	3	5	6
W1	1	5	40	1	10	3	4	8
W2	1	5	40	1	10	3	4	10

Table 7. Pre-Digestion Spike Levels (all units in mg/kg)

Sample	Cu	Fe	Mg	Mn	Zn	K	Na	Ca
MG	28.9	578	578	28.9	578	2634	5268	10536
O	32.3	646	646	32.3	646	2995	5989	11979
R	29.6	592	592	29.6	592	2815	5631	11261
C	29.6	593	593	29.6	593	2828	5656	11312
W1	26.5	530	530	26.5	530	2865	5729	11459
W2	28.8	576	576	28.8	576	2847	5693	11387

Because of the wide range of elements among the samples, the same dilution factor was not always applied to all the samples for the same element. Table 6 shows the dilution factors which were automatically determined and performed in-line with the FAST Flame 2 accessory.

To assess any possible matrix effects from the various samples, all samples were spiked (pre-digestion) with all elements at the levels shown in Table 7; the resulting spike recoveries appear in Figure 2. The recoveries of all sample method spikes are within 10% of the calculated values for all elements and did not require per-sample matrix matching, demonstrating the value and labor savings of using the Titan MPS system to digest the samples safely and completely. The variety of cereal types all exhibited spike recoveries within 10%, further demonstrating the robustness of the sample preparation and instrument methods.

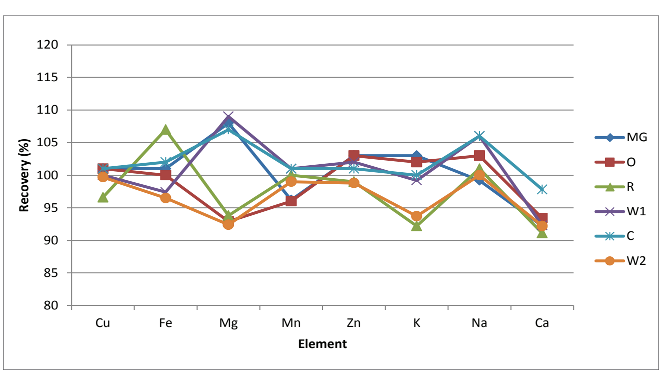

Figure 2. Spike recoveries for all elements for all samples.

The addition of the FAST Flame 2 accessory reduced the creation of standards from one intermediate and five final standards to a single intermediate standard with a commensurate reduction in human error during standard creation. The measured concentrations of many of the elements in the samples varied enough to fall outside the calibration curve. The in-line dilution capability of FAST Flame 2 allowed real-time dilution of these samples so that the absorbance fell within the calibration curve, and the results represented accurate analysis. The ability of FAST Flame 2 to react to the over-range samples and auto-dilute the samples accurately and consistently without interaction from an analyst saved time and eliminated additional sample handling and lengthy re-prep.

These results demonstrate the accuracy and value of breakfast cereal analysis via flame AA along with the speed and increased productivity available from the PinAAcle 900 AA spectrometer coupled with the FAST Flame 2 accessory.

Conclusion

This work has demonstrated the ability of the PinAAcle 900 AA spectrometer to reliably and effectively analyze breakfast cereal samples for Cu, Fe, Mg, Mn, Zn, K, Na, and Ca over a wide range of concentrations. Using the FAST Flame 2 sample automation accessory along with the PinAAcle 900 minimizes user error when performing dilutions and making calibration standards, increases throughput and provides excellent long-term stability, increasing productivity for the laboratory. (Equivalent results would also be obtained with the PinAAcle 500 AA spectrometer). Use of the Titan MPS for sample digestion eliminated sample and matrix problems and permitted the use of external standards without the need for matrix matching or specialized analytical parameters. The same analyses can also be done without the use of a FAST Flame 2 accessory when analyzing smaller sample batches.

References

1. Spivey, N., "Analysis of Micronutrients in Milk by Flame Atomic Absorption Using FAST Flame Sample Automation for Increased Sample Throughput", PerkinElmer Application Note, 2015.
2. Spivey, N., "Analysis of Micronutrients in Fresh and Dried Fruits by Flame Atomic Absorption Using FAST Flame Sample Automation", PerkinElmer Application Note, 2015.

Consumables

Component	Part Number
Red/Red PVC Pump Tubing	09908585
Black/Black PVC Pump Tubing	09908587
Autosampler Tubes	B0193233 (15 mL) B0193234 (50 mL)
Ca Hollow Cathode Lamp	N3050114
Cu Hollow Cathode Lamp	N3050121
Fe Hollow Cathode Lamp	N3050126
Mg Hollow Cathode Lamp	N3050144
Mn Hollow Cathode Lamp	N3050145
Zn Hollow Cathode Lamp	N3050191
Pure-Grade Ca Standard (10,000 mg/L)	N0691581 (125 mL) N9303764 (500 mL)

Component	Part Number
Pure-Grade Cu Standard (1000 mg/L)	N9300183 (125 mL) N9300114 (500 mL)
Pure-Grade Fe Standard (1000 mg/L)	N9303771 (125 mL) N9300126 (500 mL)
Pure-Grade K Standard (10,000 mg/L)	N9304121 (125 mL) N9304120 (500 mL)
Pure-Grade Mg Standard (1000 mg/L)	N9300179 (125 mL) N9300131 (500 mL)
Pure-Grade Mn Standard (1000 mg/L)	N9303783 (125 mL) N9300132 (500 mL)
Pure-Grade Na Standard (10,000 mg/L)	N9304124 (125 mL) N9304123 (500 mL)
Pure-Grade Zn Standard (1000 mg/L)	N9300178 (125 mL) N9300168 (500 mL)

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