APPLICATION NOTE

Atomic Absorption

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Analysis of Micronutrients in Milk by Flame Atomic Absorption Using FAST Flame Sample Automation for Increased Sample Throughput

Introduction

Analysis of micronutrients in food continues to be an important facet in the monitoring of food quality. Micronutrients can either be present naturally or added to fortify food, reflecting market demands and, in some cases, regulatory requirements. Regulatory oversight and the mandatory addition of micronutrients continue to grow as organizations seek to prevent systemic malnutrition and improve the food supply in general. Populations are also responding by requesting the addition of micronutrients to improve the quality of the food and by selecting fortified products over non-fortified products in the marketplace.

For food producers, internal quality control and the possibility of external monitoring provide strong incentive for the ability to quickly, accurately and easily monitor micronutrients in their products. In addition, nutritional labeling guidelines also require an accurate assessment of micronutrients for regulatory compliance.

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Milk is an important source of nutrients, mainly for children. Because of its importance, milk is available in several different forms, most commonly as fresh, but it is also available in non-perishable forms (such as powdered and evaporated). Therefore, the requirement exists to analyze several forms of milk for nutritional elements.

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 milks.

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 milk 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. To control ionization during the analysis of potassium (K), sodium (Na), and calcium (Ca), La_2O_3 was added to the solutions, standards, and diluent at a concentration of 0.5% by weight.

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 rapidly fills a sample loop via vacuum and then switches to inject the sample loop 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.

Table 1. PinAAcle 900 Instrument and Analytical Conditions

Element	Cu	Fe	Mg	Zn	K	Na	Ca
Mode	Absorption	Absorption	Absorption	Absorption	Emission	Emission	Absorption
Wavelength (nm)	324.75	248.33	285.21	213.86	766.49	589.00	422.67
Slit (nm)	0.7	0.2	0.7	0.7	0.2	0.2	0.7
Acetylene Flow (L/min)	2.5	2.82	2.5	2.5	2.5	2.5	2.7
Air Flow (L/min)	10	9.56	10	10	10	10	10
Burner Head Rotation	0°	0°	0°	0°	45°	45°	0°
Acquisition Time (sec)	1	1	1	1	1	1	1
Replicates	3	3	3	3	3	3	3
Sample Flow Rate (mL/min)	6	6	6	6	6	6	6
Intermediate Standard (mg/L)	1	2	1	5	400	50	10
Auto-Diluted Calibration Standards (mg/L)	0.05 0.1 0.2 0.5 1	0.1 0.2 0.4 1 2	0.05 0.1 0.25 0.5 1	0.25 0.5 1 2.5 5	20 40 100 200 400	2.5 5 10 25 50	0.5 1.0 2.0 5.0 10.0
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

While it is possible to analyze the milk samples via flame on the PinAAcle using simple dilution, this would require per-sample compensation for the matrix effects and aspiration inefficiencies, which becomes very labor intensive and is very dependent on analyst's skill and technique. A more effective solution is to eliminate the sample matrix via sample digestion. Open-vessel digestion using a simple heating block is an option and can be effective, but closed-vessel microwave digestion delivers higher throughput, superior digestion capabilities, and increased safety while still providing ease of use.

The milk samples and SRM 1549a (Whole Milk Powder 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. To each vessel, one gram of sample and 10 mL of concentrated nitric acid were added. Details of the microwave digestion method are listed in Table 2. 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 3. 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 4 shows the result for the analyses of SRM 1549a Non-Fat Milk Powder. All of the elements recovered within 10% of the certified values, demonstrating the accuracy of the methodology. With the accuracy established, a variety of commercial milk samples were analyzed, which included fresh, evaporated, and powdered milk. The results are shown in Figure 1. All samples contained significantly higher levels of Na, Mg, Ca, and K than the other elements, while Cu was consistently the least abundant element, not even being present in Fresh 2% Milk-A, yet it varied the most among the samples. There are also not many significant differences between the fresh and evaporated milks. However, the nutrient level was consistently highest in the powdered milk (with the exception of Fe). This observation is in line with expectations: since the powdered milk is diluted prior to consumption, the mineral levels should be elevated in the powder.

Table 2. 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	1	60
2	195	35	2	20	100
3	50	35	1	20	0

Table 3. Calibration Results

Element	Correlation Coefficient	ICV Concentration (mg/L)	Measured ICV (mg/L)	ICV (% Recovery)
Cu	0.99998	0.500	0.490	98.0
Fe	0.99996	0.500	0.502	100
Mg	0.99995	0.500	0.527	105
Zn	0.99867	2.50	2.64	106
K	0.99876	100	102	102
Na	0.99925	10.0	10.9	109
Ca	0.99999	5.00	5.39	108

Table 4. SRM Recovery Values

Element	In-line Dilution Factor	Certified SRM Concentration (mg/kg)	Measured SRM Concentration (mg/kg)	% Certified Value Recovery
Cu	1	0.638	0.609	95.5
Fe	1	1.80	1.82	101
Mg	30	892	880	98.7
Zn	1	33.8	31.7	93.8
K	2	11920	12080	101
Na	10	3176	3462	109
Ca	30	8810	8343	94.7

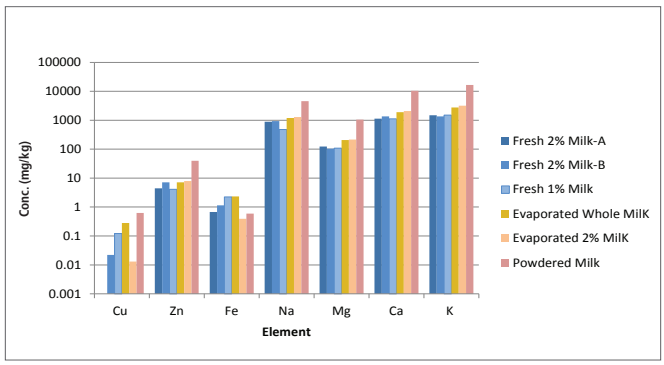

Figure 1. Results from analyses of milk samples.

Table 5. In-Line Dilution Factors

Sample	Cu	Fe	Mg	Zn	K	Na	Ca
Fresh 2% Milk- A	1	1	30	1	2	5	30
Fresh 2% Milk- B	1	1	30	1	2	5	30
Fresh 1% Milk	1	1	30	1	2	5	30
Evaporated Whole Milk	1	1	30	1	2	5	30
Evaporated 2% Milk	1	1	30	1	2	5	30
Powdered Milk	1	1	30	1	2	10	30

Table 6. Pre-Digestion Spike Levels (all units in mg/kg)

Sample	Cu	Fe	Mg	Zn	K	Na	Ca
Fresh 2% Milk- A	24.8	37.8	495	49.5	1986	1986	1986
Fresh 2% Milk- B	25.3	30.3	506	50.6	2002	2002	2002
Fresh 1% Milk	24.8	32.8	497	49.7	1986	1986	1986
Evaporated Whole Milk	24.9	35.5	498	49.8	1994	1994	1994
Evaporated 2% Milk	24.5	37.3	491	49.1	1942	1942	1942
Powdered Milk	33.1	57.5	662	66.2	2608	2608	2608

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 5 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 6; 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 milk types all exhibited spike recoveries within 10%, further demonstrating the robustness of the sample preparation and instrument methods.

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 potassium, magnesium, sodium, and calcium 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 milk 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.

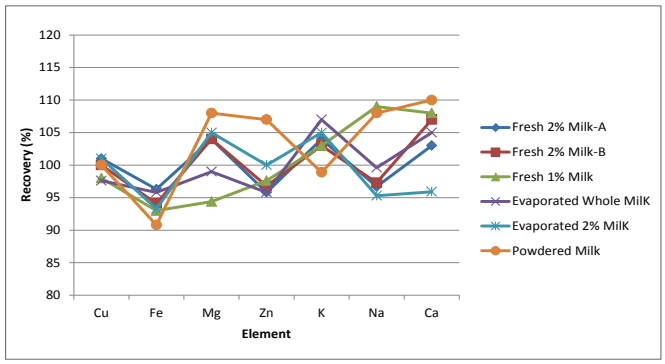

Figure 2. Spike recoveries in the milk samples.

Conclusion

This work has demonstrated the ability of the PinAAcle 900 AA spectrometer to reliably and effectively analyze a variety of milk samples for Cu, Fe, Mg, 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.

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
Zn Hollow Cathode Lamp	N3050191

Component	Part Number
Pure-Grade Ca Standard (1000 mg/L)	N9303763 (125 mL) N9300108 (500 mL)
Pure-Grade Cu Standard (1000 mg/L)	N9300183 (125 mL) N9300114 (500 mL)
Pure-Grade Fe Standard (1000 mg/L	N9303779 (125 mL) N9300141 (500 mL)
Pure-Grade K Standard (1000 mg/L)	N9303779 (125 mL) N9300141 (500 mL)
Pure-Grade Mg Standard (1000 mg/L)	N9300179 (125 mL) N9300131 (500 mL)
Pure-Grade Na Standard (1000 mg/L)	N9303785 (125 mL) N9300152 (500 mL)
Pure-Grade Zn Standard (1000 mg/L)	N9300178 (125 mL) N9300168 (500 mL)

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