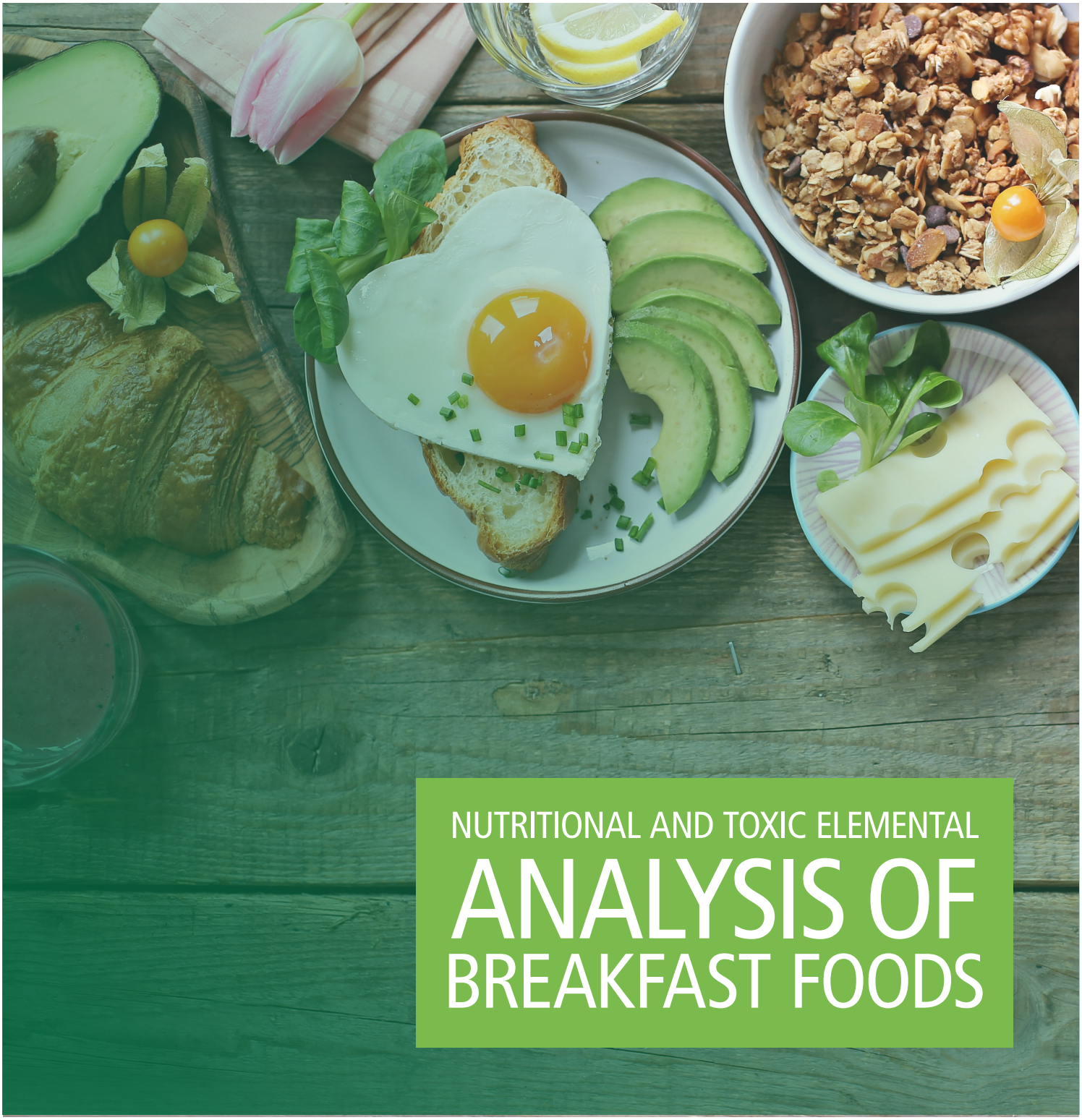

NUTRITIONAL AND TOXIC ELEMENTAL ANALYSIS OF BREAKFAST FOODS

Nutritional and Toxic Elemental Analysis of Breakfast Foods Compendium

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INTRODUCTION

Breakfast is an important meal in providing essential nutrients to keep your energy levels up throughout the day. How do we ensure the food and beverages we consume are healthy, nutritious and safe?

Among the standard techniques utilized for determination of nutritional elements in food and beverages are flame atomic absorption (AA) and inductively coupled plasma (ICP-OES/AES). While graphite furnace AA and ICP-MS can also be used for determination of toxic elements, coupling ICP-MS with HPLC provides the added capability of speciation to differentiate between toxic and non-toxic forms of certain elements to ensure safety.

Following is a collection of application notes highlighting solutions that will help you identify micronutrients in milk, cereal, juice and fresh and dried fruits as well as toxic metals in tea, dairy products and apple juice to ensure safety of your breakfast foods.

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APPLICATION NOTE

Atomic Absorption

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Analysis of Micronutrients in Fresh and Dried Fruits by Flame Atomic Absorption Using Microwave Digestion and FAST Flame Sample Automation

Introduction

As an addition to a morning breakfast, a snack throughout the day or even as a meal itself, fruit is a delicious and healthy food choice. With an increased focus on healthy living and the consumption of healthy foods, interest in the nutritional quality of the fruit has become more important. When fresh fruit is not available, dried fruit is often substituted, and manufacturers and customers would like to know that the dried fruit has not lost some nutritional value during processing when compared to fresh fruit.

One way of monitoring the quality of fresh or dried fruit is by measuring the micronutrient concentration contained within. Micronutrients are represented by trace elements considered to be nutritionally valuable, and it is these elements that can be analyzed via various inorganic analytical methods.

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While inductively coupled plasma optical emission spectroscopy (ICP-OES) is generally favored as a multi-element analytical method, 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 a sample to be analyzed once for each element of interest, with each re-analysis impacting the throughput 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 by reducing technique problems 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 fresh and dried fruit.

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 are outlined in Table 1. The sample introduction system consisted of a high-sensitivity nebulizer, the standard spray chamber and a 10 cm burner head. External calibrations were performed using a single intermediate standard made in 10% 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 associated with 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 lets the FAST Flame 2 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 fruit samples, the elements of interest must be extracted from the fruit 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

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°	0°	0°
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	5	1	1	5	200	10	10
Auto-Diluted Calibration Standards (mg/L)	0.05 0.1 0.2 0.5 1	0.25 0.5 1 2.5 5	0.05 0.1 0.2 0.5 1	0.05 0.1 0.2 0.5 1	0.25 0.5 1 2.5 5	10 20 40 100 200	0.25 0.5 1 8 10	0.5 1 2.5 5 10
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

further filtration or centrifugation prior to introduction into the instrument and can result in reduced recovery with corresponding poor accuracy. Closed-vessel 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.

Fresh fruit and dried fruit samples 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 system ensures accurate digestion method control and zero sample contamination regardless of the sample type. Details of the microwave digestion method are listed in Table 2; each vessel contained 0.5 g dried fruit or 1 g of fresh fruit and 10 mL concentrated nitric acid. All spiking was performed prior to sample digestion with spike concentrations selected based on expected sample concentrations.

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.

Figure 1 shows the results obtained for the analyzed fruit samples, with the dried fruits being in blue and the fresh fruits in orange. From this plot, it is obvious that all of the dried fruits contain significantly higher concentrations of nutrients than the fresh fruits. The elemental concentrations also vary greatly among fruits, but in all cases, the potassium levels are the highest among the elements measured. The FAST Flame 2 accessory automatically diluted the samples by the factors shown in Table 4 so that the results were within the calibration range.

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	2	60
2	195	35	3	25	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	0.99985	0.500	0.494	98.8
Fe	0.99999	2.00	1.98	99.0
Mg	0.99999	0.500	0.517	103
Mn	0.99995	0.500	0.495	99.0
Zn	0.99991	2.00	1.95	97.5
K	0.99860	100	96.7	96.7
Na	0.99865	5.0	4.55	91.0
Ca	0.99975	5.0	5.02	100

Table 4. In-Line Dilution Factors

Fruit	Cu	Fe	Mg	Mn	Zn	K	Na	Ca
Dried Blueberry	1	1	20	1	2	2	1	5
Dried Strawberry	1	1	20	1	2	2	1	5
Dried Raspberry	1	1	20	1	2	2	1	5
Fresh Raspberry	1	1	20	1	2	2	1	5
Fresh Blueberry	1	1	20	1	2	2	1	5
Fresh Strawberry	1	1	20	1	2	2	1	5
Fresh Kiwi	1	1	20	1	2	2	1	5

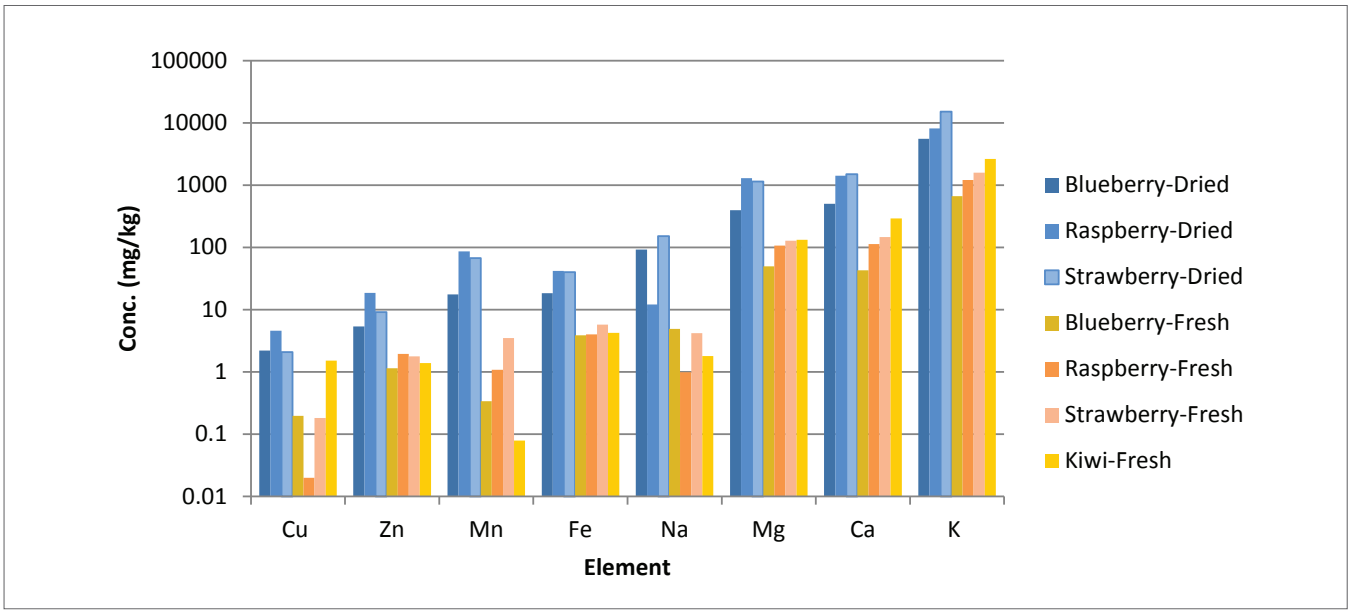

Figure 1. Results for dried (blue) and fresh (orange) fruit samples.

Table 5. Spike Levels (all units in mg/kg)

Fruit	Cu	Fe	Mg	Mn	Zn	K	Na	Ca
Dried Blueberry	49.3	197	493	98.6	197	4880	195	488
Dried Strawberry	46.6	186	466	93.1	186	4930	197	493
Dried Raspberry	50.1	201	501	100	201	5236	209	524
Fresh Raspberry	19.6	78.6	196	39.3	78.6	2078	83.1	208
Fresh Blueberry	18.9	75.7	189	37.9	75.7	1850	74.0	185
Fresh Strawberry	21.0	83.9	210	42.0	83.9	1744	69.8	174
Fresh Kiwi	19.7	78.7	197	39.4	78.7	1991	79.6	199

To assess accuracy, all samples were spiked (pre-digestion) at the levels indicated in Table 5. The recoveries of all sample method spikes are within 10% of the calculated values for all elements, as shown in Figure 2. The spike recovery studies 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 fresh and dried fruit 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 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 validate the accuracy and value of fresh and dried fruit 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 PerkinElmer's PinAAcle 900 AA spectrometer to reliably and effectively analyze fresh and dried fruit samples for Cu, Fe, Mg, Mn, Zn, K, Na, and Ca over a wide range of concentrations. Using the FAST Flame 2 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.

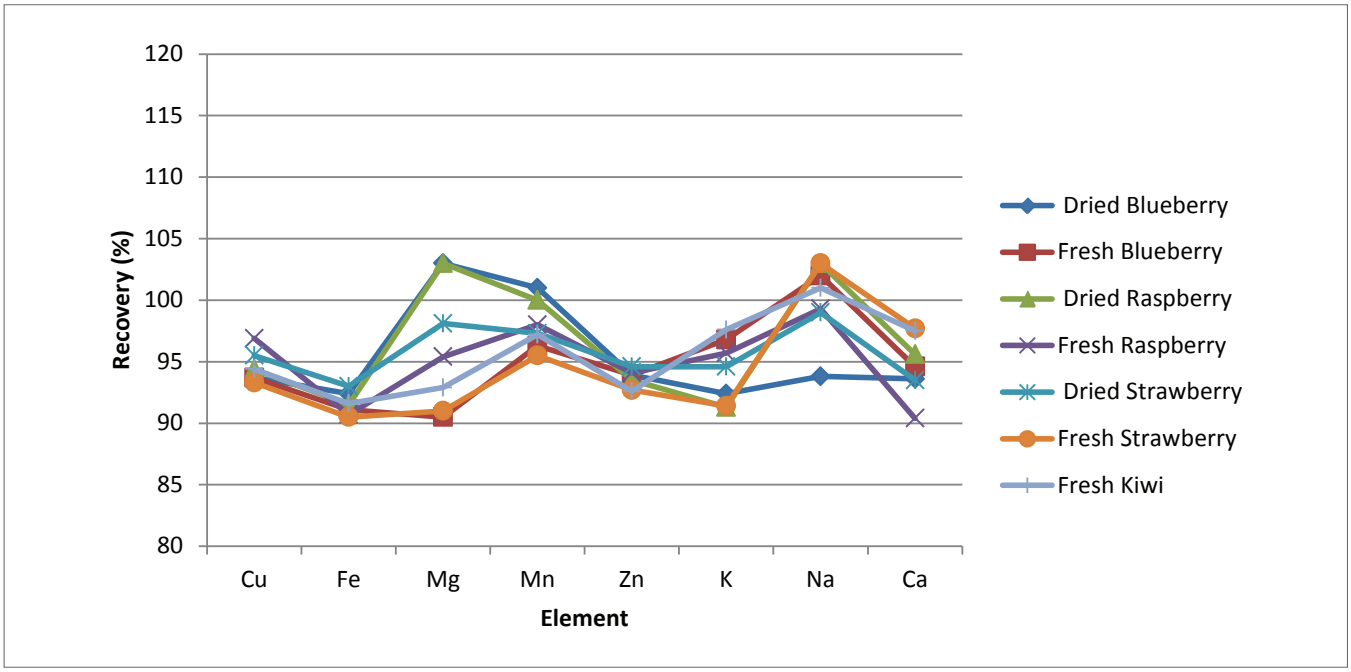

Figure 2. Recovery of pre-digestion spikes for fresh and dried fruit samples.

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 (1000 mg/L)	N9303763 (125 mL) N9300108 (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 (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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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

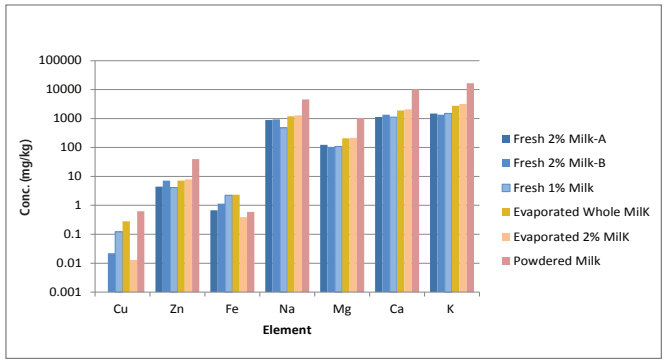

Figure 1. Results from analyses of milk samples.

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.

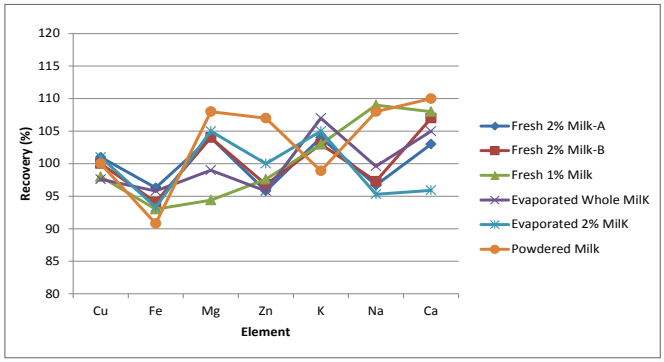

Figure 2. Spike recoveries in the milk samples.

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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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. 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 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 microwave digestion delivers complete sample digestion,

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

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

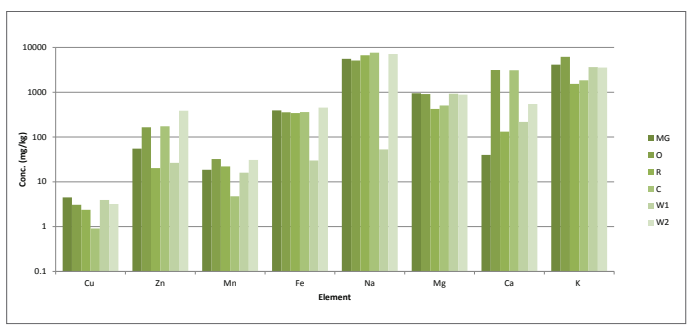

Figure 1. Results from analysis of seven cereals.

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.

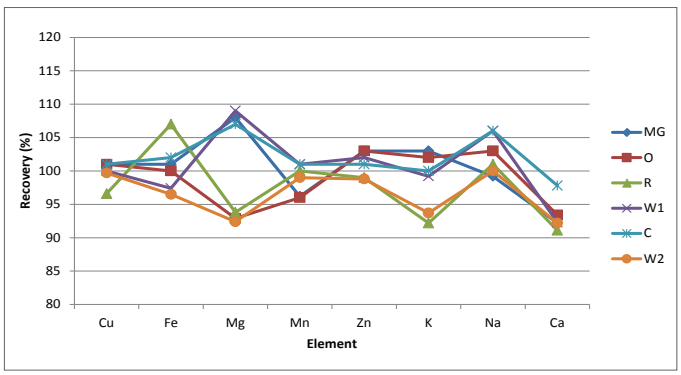

Figure 2. Spike recoveries for all elements for all samples.

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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APPLICATION NOTE

Atomic Absorption

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Analysis of Micronutrients in Fruit Juice Using FAST Flame Sample Automation for Increased Sample Throughput

Introduction

Consumers select fruit juice because it is a tasty, convenient beverage and generally understood to be a more nutritious alternative to carbonated beverages. For 100% juice products, the nutrition content of the original fruit itself is well known, which translates to the expected nutritional value of the final juice product. Detailed labeling is required on food products; for consumers, any comparative variance can be a strong incentive to choose one product over another. In an effort to appeal to consumers and address market needs, many juice products may also be fortified with micronutrients to boost or add to what is already present naturally.

For food manufacturers and processors, it is imperative that there is a means to quantify the content of food products, including micronutrients, for both safety and quality reasons, along with regulatory label-claim requirements. Screening raw materials for elemental contaminants prior to use and then confirming the micronutrient content of the final product are two basic examples of the benefits of analytical testing. Accurate and precise analysis can also help improve the production process by utilizing the analytical data generated and employing statistical analysis to maximize nutrient yield or production volume where appropriate.

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While ICP-OES is generally favored in a multi-element analytical environment, the cost savings, simplicity and speed of a flame atomic absorption (AA) system provides an attractive alternative. However, 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.

This work will focus on the analysis of micronutrients in a variety of commercial juice products using flame AA coupled with a high-throughput sample automation system.

Experimental

Samples and Sample Preparation

With the tremendous variety of juice and juice blends available on the market, samples were selected to be representative of commonly available and purchased juices. Only samples that were made from 100% juice (as accepted under current labeling guidelines) were selected, though this still meant that in many cases the juice was reconstituted from concentrate. The samples analyzed represent two different brands of apple juice and orange juice, two different varieties of grape juice, a pomegranate juice, and a vegetable-fruit juice blend. The analytical elements selected are representative of micronutrients that commonly appear on product labels.

Juice samples were subjected to minimum sample preparation with only nitric acid added to bring the acidity to 2%. Samples were split, and the elements of interest were spiked into one set of the split samples.

Instrumental Conditions

All analyses were performed on a PerkinElmer 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 juice samples are outlined in Table 1. A high-sensitivity nebulizer was used with the standard spray chamber, along with 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. 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 scheme 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 lets the FAST Flame 2 accessory automatically generate all calibration standards in-line as required. In addition, the instrument can identify QC over-range samples and then utilize the in-line dilution capability to automatically re-run a sample that

Table 1. PinAAcle 900 Instrument and Analytical Conditions

Element	Cu	Fe	Mg	Zn	Mn	K	Na	Ca
Mode	Absorption	Absorption	Absorption	Absorption	Absorption	Emission	Emission	Absorption
Wavelength (nm)	324.75	248.33	285.21	213.86	279.48	766.49	589.00	422.67
Slit (nm)	0.7	0.2	0.7	0.7	0.2	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°	45°	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	5	20	2	1	200	200	100
Auto-Diluted Calibration Standards (mg/L)	0.05 0.1 0.2 0.5 1	0.25 0.5 1 2.5 5	0.5 1 2 5 10	0.1 0.2 0.5 1 2	0.05 0.1 0.2 0.5 1	5 10 50 100 200	5 10 25 50 100	5 10 25 50 100
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

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.

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 2. 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.

The analytical results of the juice samples are shown in Figure 1. The juice samples displayed a fairly consistent concentration of elements with a few exceptions. The largest deviation was the Ca concentration in Orange Juice B, which was labelled as "Calcium Fortified" where the amount of Ca present was an order of magnitude greater than the other juices, verifying the label claim. Levels of K and Mg are consistent across all the juice samples, while Na was moderately variable with the vegetable-fruit juice blend having significantly higher levels than the other samples. It is also worth noting that the two grape juice varieties and the vegetable-fruit juice blend had higher concentrations of Mn than the other juices. This elemental distribution highlights how the different balance of nutrients in the raw fruit can translate to the nutrients present in the final product and how monitoring and measuring these can be critical for product quality and labeling accuracy.

Table 2. Calibration Results

Element	Correlation Coefficient	ICV Concentration (mg/L)	Measured ICV (mg/L)	ICV (% Recovery)
Cu	0.99999	0.500	0.508	102
Fe	0.99997	2.50	2.56	102
Mg	0.99998	10.0	10.3	103
Mn	0.99961	0.500	0.503	101
Zn	0.99954	1.00	1.00	100
K	0.99900	100	91.8	91.8
Na	0.99979	20.0	20.8	104
Ca	0.99998	50.0	47.4	94.8

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 accessory.

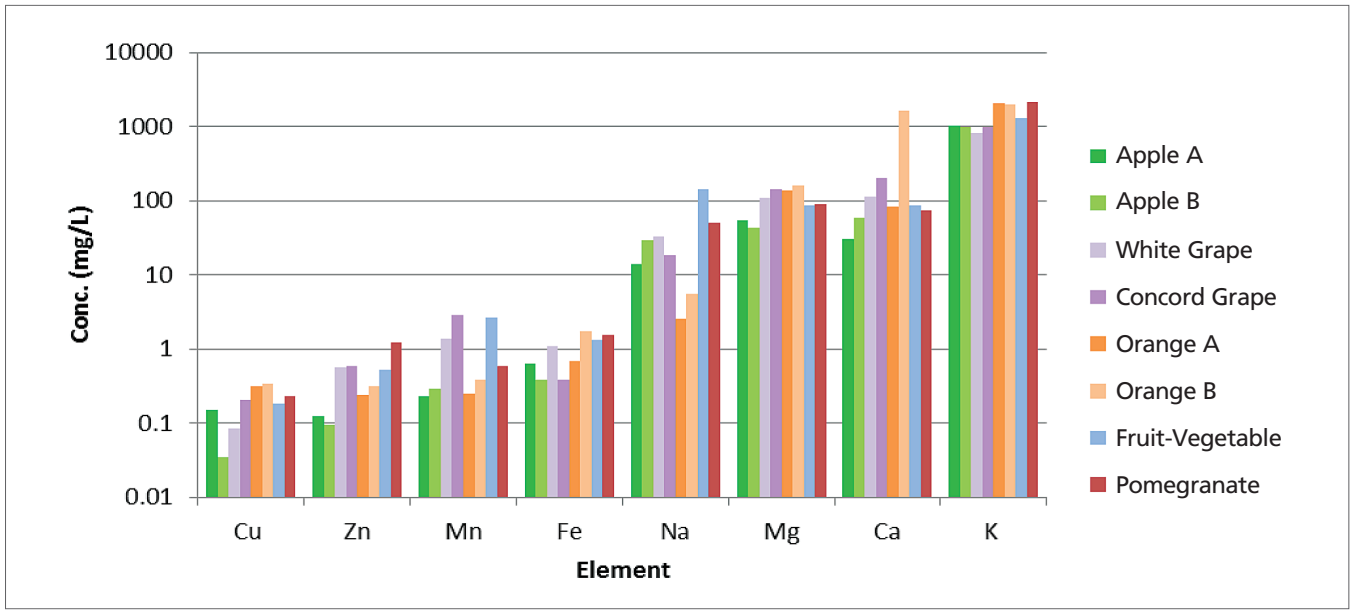

Figure 1. Results from analyses of juice samples.

Table 3. In-Line Dilution Factors

Sample	Cu	Fe	Mg	Mn	Zn	K	Na	Ca
Apple A	2	2	5	2	2	30	2	3
Apple B	2	2	5	2	2	30	2	3
White Grape	2	2	5	3	2	30	2	5
Concord Grape	2	2	5	5	2	30	2	5
Orange A	2	2	10	2	2	30	2	3
Orange B	2	2	10	2	2	30	2	20
Fruit-Vegetable	2	2	8	3	2	30	4	3
Pomegranate	2	2	8	2	2	30	2	3

To assess any possible matrix effects from the various juices, all samples were spiked with all elements at the levels shown in Table 4; the resulting spike recoveries appear in Figure 2. The recoveries of nearly all the sample spikes are within 10% of the calculated values for all elements and did not require per-sample matrix matching. However, there were two recovery values that exceeded 110% for K (Concorde Grape and Orange B), a result of the spike levels (91.9, 95.1 mg/kg, respectively) being significantly lower than the actual K concentrations in the samples (about one-tenth the amount). In all cases, the spike concentrations were established prior to analysis, and therefore, were not ideal. Nevertheless, excellent recoveries were observed. The Ca spike recovery for Orange B is not reported because of the excessively high Ca concentration in this juice. For all remaining elements and samples, the simple spiking and rapid sampling resulted in accurate analysis with good spike recovery, an absolute minimum of labor, and almost no sample preparation.

The addition of the FAST Flame 2 sample automation accessory reduced the number of standards the analyst needed to make from six (one intermediate and five final standards) to a single intermediate standard, with a commensurate reduction in human error during standard preparation. The measured concentrations of K, Mg, Mn, Na, and Ca in the samples varied enough to fall outside the calibration curve, but the in-line dilution capability of the FAST Flame 2 accessory allowed real-time dilution of these samples so that the absorbance fell within the calibration curve, producing accurate analyses. 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 saves time and eliminates additional sample handling and lengthy re-prep.

Comparing typical autosampler performance, the total analytical time for each sample is dramatically reduced, while sample throughput is increased by nearly 4X with the use of the FAST Flame 2 accessory. The sample turnaround was reduced by 45 seconds while still maintaining the advantages of fully automated sample analysis, sample dilution, and calibration standard preparation. FAST Flame 2 retained the full automation benefits and still maintained a speed advantage even when compared with manual operation of the AA.

These results validate the accuracy and value of fruit juice analysis via flame AA along with the speed and increased productivity available from the PinAAcle and the FAST Flame 2 sample automation accessory.

Table 4. Pre-Digestion Spike Levels (all units in mg/kg)

Sample	Cu	Fe	Mg	Mn	Zn	K	Na	Ca
Apple A	0.494	0.494	4.94	0.494	0.494	94.1	94.1	37.7
Apple B	0.508	0.508	5.08	0.508	0.508	92.0	92.0	36.8
White Grape	0.500	0.500	5.00	0.500	0.500	90.4	90.4	36.2
Concord Grape	0.475	0.475	4.75	0.475	0.475	91.9	91.9	36.8
Orange A	0.502	0.502	5.02	0.502	0.502	93.2	93.2	37.3
Orange B	0.484	0.484	4.84	0.484	0.484	95.1	95.1	38.0
Fruit-Vegetable	0.486	0.486	4.86	0.486	0.486	89.1	89.1	35.6
Pomegranate	0.479	0.479	4.79	0.479	0.479	95.8	95.8	38.3

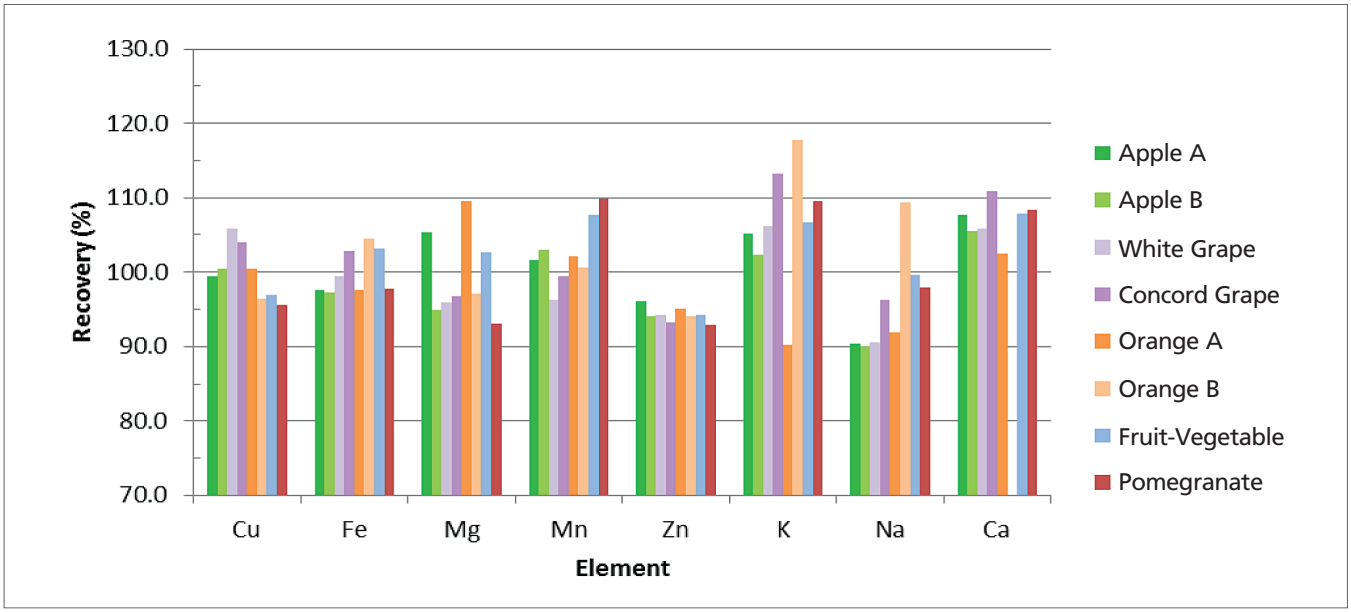

Figure 2. Spike recoveries in the juice samples.

Conclusion

This work has demonstrated the ability of the PinAAcle 900 AA spectrometer to reliably and effectively analyze a variety of fruit juice 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 errors when performing dilutions and making calibration standards while increasing throughput and productivity for the laboratory. (Equivalent results would also be obtained with the PinAAcle 500 AA spectrometer). The same analyses can also be done without the use of a FAST Flame accessory when analyzing smaller sample batches or the auto-dilution needs are not required.

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)
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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APPLICATION NOTE

ICP-Optical Emission Spectroscopy

Author:

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Analysis of Micronutrients in Fruit Juice Using the Avio 200 ICP-OES

Introduction

Fruit juice continues to be a popular and refreshing beverage and can be a better nutritional alternative than typical carbonated beverages. The nutritional content of 100% fruit juices is derived from the fruit itself, and these valuable nutrients are displayed on the detailed bottle label. Customers use these labels to inform themselves on the nutritional content of the juice and for comparative shopping. While these labels provide a valuable reference for customers, they are also legally required in North America, and the veracity of these labels is the responsibility of the manufacturer.

For food manufacturers and processors, it is imperative that there is a means to quantify the content of food products, including micronutrients, for both safety and quality reasons along with regulatory label-claim requirements. Screening raw materials for elemental contaminants prior to use and then confirming the micronutrient content of the final product are two basic examples of the benefits of analytical testing. Accurate and precise analysis can also help improve the production process by providing rapid results and allowing optimization of the production process to maximize nutrient yield or production volume where appropriate.

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Inductively coupled plasma optical emission spectroscopy (ICP-OES) is generally favored in a multi-element analytical environment with detection capabilities appropriate for nutritional analysis as demonstrated in this application. Flame atomic absorption (AA) systems, which provide cost savings, simplicity, and single-element analytical speed, can be attractive alternatives[1]. However, measuring a large number of elements by Flame AA requires each sample to be re-analyzed individually for each element, which can eliminate the speed advantage of Flame AA.

This work will focus on the analysis of micronutrients in a variety of commercial juice products using a PerkinElmer Avio™ 200 ICP-OES with sample preparation performed using a PerkinElmer Titan MPS™ Microwave Sample Preparation System.

Experimental

Samples and Sample Preparation

With the tremendous variety of fruit juice and fruit juice blends available on the market, samples were selected to be representative of commonly available and purchased juices. During selection, a preference for samples that were made from 100% juice (as accepted under current labeling guidelines) was used, though this still meant that, in many cases, the juice was reconstituted from concentrate. The samples analyzed represent two different brands of orange juice, apple juice, and grape juice, as well as a cranberry juice and a cranberry juice cocktail. The analytical elements selected are representative of micronutrients that commonly appear on product labels for these juices.

Juice samples were prepared for analysis by closed-vessel microwave-assisted digestion using a PerkinElmer Titan MPS microwave digestion system. The digestion method, sample parameters, and reagents used are listed in Tables 1 and 2. Samples were delivered by volume into the digestion vessels, and then the digestion reagents and any sample spikes were added. The samples and reagents then sat open in the vessels for 10 minutes, which allowed any early reactions to occur safely. After this time, the vessels were closed and placed into the Titan MPS for heating and digestion. When the digestion had completed, the samples were transferred out of the digestion vessels by triple-rinsing with deionized (DI) water into sample vials and then brought up to the final solution volume with DI water (18 MΩ-cm).

Table 1. Titan MPS Digestion Method.

Step	Target Temp (°C)	Pressure Limit (bar)	Ramp Time (min)	Hold Time (min)	Power Limit (%)
1	150	30	8	5	90
2	200	30	2	20	100
3	50	30	1	20	0

Table 2. Digestion Information.

Parameter	Volume
Reagents Used	8 mL of HNO_3 (70%), 2 mL H_2O_2 (30%)
Initial Sample Volume	5 mL
Final Solution Volume (after dilution)	50 mL

Instrumental Conditions

All analyses were performed on an Avio 200 ICP Optical Emission Spectrometer equipped with a PerkinElmer S10 Autosampler. The elements of interest and instrument conditions for the analysis of the juice samples are outlined in Tables 3 and 4. A Meinhard® glass nebulizer was used with the standard cyclonic spray chamber. External calibration standards were created from a custom PerkinElmer multi-element standard and were diluted with DI water and trace metal grade nitric acid to the final elemental concentrations listed in Table 5. The final nitric acid concentration of the standards was approximately 10% to match the relatively high concentration of acid in the digested and diluted samples.

Table 3. Avio 200 ICP-OES Instrumental Parameters.

Parameter	Value
Nebulizer	Meinhard® Glass Type K1 (Part No. N0777707)
Spray Chamber	Glass Cyclonic Baffled (Part No. N0791352)
Sample Uptake Rate (mL/min)	0.8
RF Power (W)	1500
Nebulizer Gas (L/min)	0.68
Auxiliary Gas (L/min)	0.2
Plasma Gas (L/min)	8

Table 4. Method Parameters.

Element	Wavelength (nm)	Plasma View	Integration Range (sec)
Ca	317.933	Radial	0.1 - 5
Cu	327.393	Axial	0.1 - 5
Fe	238.204	Axial	0.1 - 5
K	766.490	Radial	0.1 - 5
Mg	285.213	Radial	0.1 - 5
Mn	257.610	Axial	0.1 - 5
Na	589.592	Radial	0.1 - 5
P	178.221	Axial	0.1 - 5
S	181.975	Axial	0.1 - 5
Zn	206.200	Axial	0.1 - 5
Y (int std)	371.029	Radial	0.1 - 5
Y (int std)	371.029	Axial	0.1 - 5

Table 5. Calibration Standards.

Element	Std 1 (mg/L)	Std 2 (mg/L)	Std 3 (mg/L)	Std 4 (mg/L)
Ca	-	-	10	50
Cu	0.1	1	-	-
Fe	0.1	1	-	-
K	-	-	10	50
Mg	-	-	10	50
Mn	0.1	1	-	-
Na	-	-	10	50
P	-	-	10	50
S	-	-	10	50
Zn	0.1	1	-	-

Analysis was performed with standard 2-point background correction and no other spectral correction formulas. Yttrium was used as an internal standard for all elements analyzed using axial and radial plasma view.

Along with PerkinElmer's proven track record of ICP performance, the Avio 200 spectrometer benefits from a number of unique capabilities as well. The patented Flat Plate™ plasma technology delivers a robust plasma with zero maintenance and requires no cooling while using nearly half the argon plasma gas of helical load-coil systems. The entire sample introduction system and torch assembly are packaged into a single cassette that is simple to use and maintain. Plus, Avio's Dual View capability allows automated axial or radial viewing and teamed with its outstanding optical resolution, delivers a large linear dynamic range and exceptional stability and detection limits. All instrument control and analyses were done through PerkinElmer's Syngistix™ software.

Results and Discussion

Calibration results and calibration verification check (ICV) results are shown in Table 6. The excellent correlation for the calibration standards demonstrates the accuracy and precision of the Avio 200 ICP-OES. The independent calibration verification recoveries ensure that the calibration is valid, and that the creation of the standards was accurate.

The analytical results of the juice samples are shown in Figure 1. Using the Titan MPS digestion system, the samples were simply and quickly prepared for analysis. This delivered major time savings over the typical process of open vessel digestion, and, as demonstrated by the sample data and spike recoveries, microwave digestion delivers very consistent sample preparation performance. The analytical data for the samples themselves match the amounts provided on the juice labels and demonstrate the capability of the Avio 200 ICP-OES to accurately analyze samples with a large variation of elemental concentrations in a single analysis. It is interesting to note that one of the orange juices selected was fortified with Ca and that the analysis does indeed show a significantly higher amount of Ca for this sample. The results also show the difference between a juice cocktail (Cranberry A), which is a sweetened and blended fruit juice drink, and a pure fruit juice (Cranberry B), where the pure fruit juice had consistently higher concentrations of elements when compared to the juice cocktail. The elemental distributions among the various samples highlights how the different balance of nutrients in the raw fruit can translate to the nutrients present in the final product and how monitoring and measuring these can be critical for product quality and labeling accuracy.

Table 6. Calibration Results.

Element	Correlation Coefficient	ICV Concentration (mg/L)	Measured ICV	ICV (% Recovery)
Ca	0.99998	10.0	10.8	108
Cu	0.99995	0.100	0.106	106
Fe	0.99999	0.100	0.099	99
K	0.99999	10.0	10.6	106
Mg	0.99989	10.0	10.9	109
Mn	0.99999	0.100	0.098	98
Na	0.99999	10.0	10.6	106
P	0.99969	10.0	10.6	106
S	0.99991	10.0	10.5	105
Zn	0.99995	0.100	0.098	98

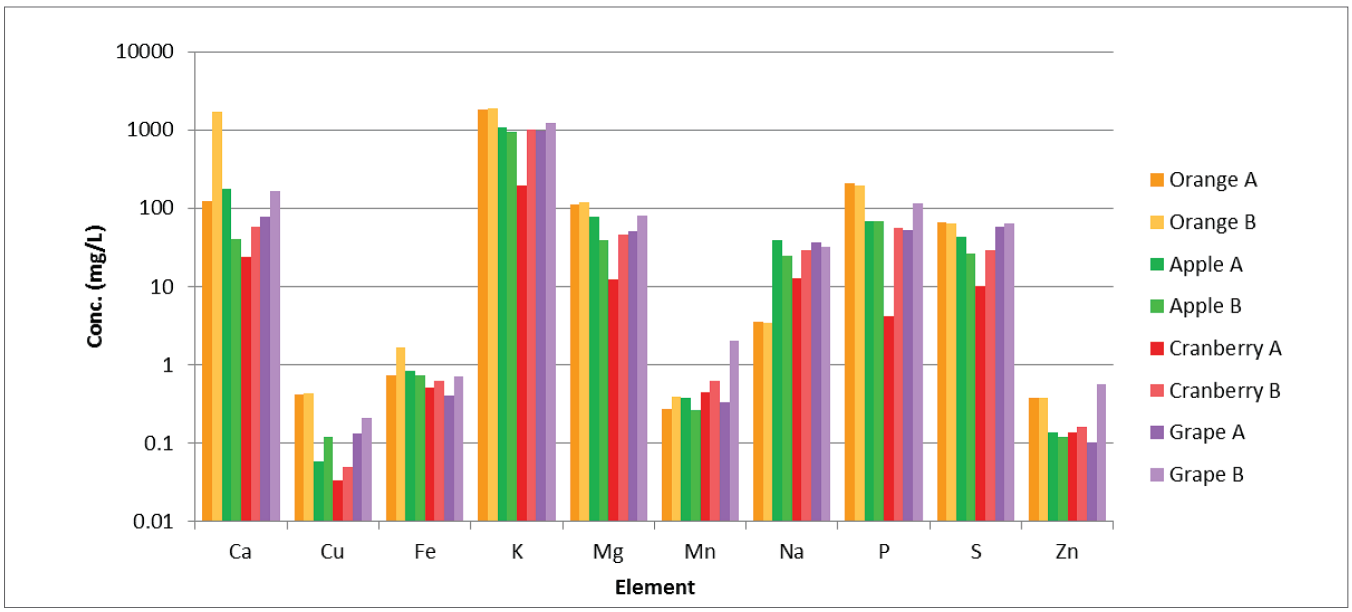

Figure 1. Results from analyses of juice samples.

With the large dynamic range available and the Dual View capability of the Avio 200 system, it was not necessary to make additional sample dilutions to lower any high-element concentrations. This meant that all elements were measured in a single analysis with no need to prepare samples using multiple dilutions or measure the elements over multiple analytical acquisitions. This results in increased productivity and sample throughput. With the incredible linearity of the Avio 200 ICP-OES over a large concentration range, a simple calibration covering the appropriate concentration range for each element ensured that each sample could be analyzed in a single analytical pass.

To assess any remaining matrix effects from the various juices and to verify the entire sample prep method, all juice samples were spiked prior to digestion with all elements at the levels shown in Table 7; the resulting spike recoveries appear in Figure 2. The potassium levels in the juice samples were very high in relation to the spike concentration level, so recovery values for potassium are not included. All other spike recoveries are within 10% of the calculated values, verifying the capabilities and quality of digestion of the Titan MPS and eliminating the need for per-sample matrix-matching or use of a method of standard addition for accurate and precise results.

Table 7. Pre-Digestion Spike Levels.

Element	Spike Concentration (mg/L)
Cu, Fe, Mn, Zn	2
Ca, K, Mg, Na, P, S	50

Conclusion

This work has demonstrated the ability of the Avio 200 ICP-OES to reliably and effectively analyze a variety of fruit juice samples for an array of elements over a wide range of concentrations. With its extended capabilities, the Avio 200 system provides greater multi-element sample throughput when compared to Flame AA while allowing simple analysis of elements which are typically challenging for Flame AA (such as phosphorus and sulfur).

Using the Titan MPS microwave digestion system simplified sample preparation while increasing throughput and productivity for the laboratory, compared to hot plate or hot block digestions. The ability to completely digest the samples eliminated the need to matrix-match calibration standards or use a method of standard addition, thus simplifying the analysis.

The use of the Titan MPS for sample preparation and the Avio 200 ICP-OES for analysis is an ideal combination for fast, simple, and accurate analyses of nutritional elements in fruit juice.

References

1. Spivey, Nick, "Analysis of Micronutrients in Fruit Juice Using FAST Flame Sample Automation for Increased Sample Throughput", Application Note, PerkinElmer, 2015.

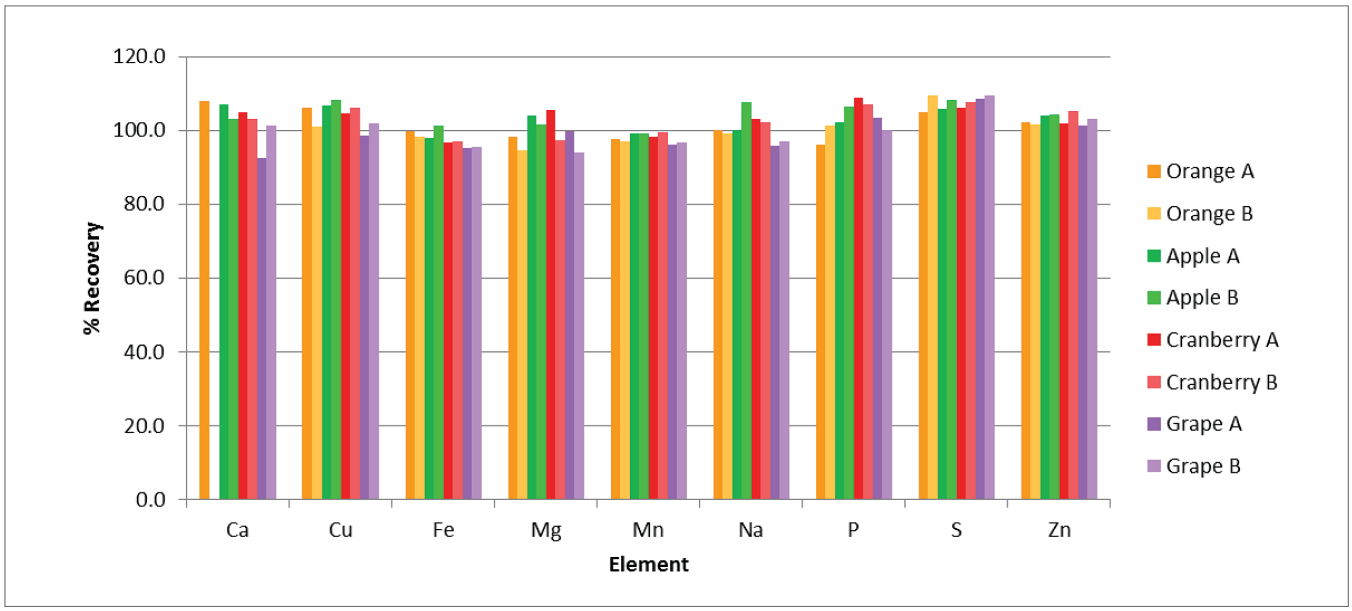

Figure 2. Spike recoveries in the juice samples.

Consumables Used

Avio 200 ICP-OES	
Component	**Part Number**
Red/Red PVC Pump Tubing	09908585
Black/Black PVC Pump Tubing	09908587
Orange/Green PVC Pump Tubing	N0777110
Internal Standard Kit	N0774068
Autosampler Tubes	B0193233 (15 mL) B0193234 (50 mL)
Instrument Calibration Standard 2 (100 mg/L)	N9301721
Pure-Grade Phosphorus Standard (1000 mg/L)	N9303788 (125 mL) N9300139 (500 mL)
Pure-Grade Sulfur Standard (1000 mg/L)	N9303796 (125 mL) N9300154 (500 mL)

Titan MPS Digestion System	
Component	**Part Number**
Consumables Kit for Standard 75 mL Digestion Vessels	N3132000
Rupture Disks for Standard 75 mL Digestion Vessels (25 pieces)	N3132001
Pressure Seal for Standard 75 mL Digestion Vessels (10 pieces)	N3132002
End Cap Plug for Gas Containment Manifold	N3134004
Single Lip Seal Forming Tool for Standard 75 mL Digestion Vessels	N3132015
8-Position Lip Seal Forming Tool for Standard 75 mL Digestion Vessels	N3132014

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APPLICATION NOTE

ICP-Optical Emission Spectroscopy

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Analysis of Micronutrients in Milk Using the Avio 200 ICP-OES

Introduction

Milk is an important source of nutrients not only for children, but for adults as well. With its great importance, milk is available in several different forms: fresh, boxed (ultra-heat treated), powdered, and evaporated. The most commonly consumed form varies globally, being dependent on factors such as geography, culture, and climate.

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

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

Inductively coupled plasma optical emission spectroscopy (ICP-OES) is generally favored in a multi-element analytical environment with detection capabilities appropriate for nutritional analysis and offering a large dynamic range, rapid multi-element throughput, and robust operating conditions. Flame atomic absorption (AA) systems, which provide cost savings, simplicity, and single-element analytical speed, can be attractive alternatives[1]. However, measuring multiple elements by Flame AA requires each sample to be analyzed individually for each element, which eliminates the speed advantage of Flame AA for multi-element analysis.

This work focuses on the analysis of micronutrients in a variety of commercial milk products using a PerkinElmer Avio™ 200 ICP-OES with sample preparation using a PerkinElmer Titan MPS™ Microwave Sample Preparation System.

Experimental

Samples and Sample Preparation

Samples were purchased from local markets and were selected to be representative of commonly available milk varieties, in both fresh and non-perishable forms. The samples analyzed represent whole, reduced-fat, and non-fat versions of fresh, evaporated, boxed, and powdered milk, along with NIST™ SRM 1549a Whole Milk Powder. The analytical elements selected are nutrients commonly found in dairy and dairy-based products.

The milk samples were prepared for analysis by closed-vessel microwave-assisted digestion using a PerkinElmer Titan MPS microwave digestion system. The digestion method, sample parameters, and reagents used are listed in Tables 1 and 2. Samples were weighed and placed into the digestion vessels, and then the reagents and any sample spikes were added. The samples and reagents then sat open in the vessels for 10 minutes to allow any early reactions to occur safely before being sealed and placed into the Titan MPS for digestion. After digestion was complete, the samples were transferred out of the digestion vessels by triple-rinsing with deionized (DI) water into sample vials for analysis.

Table 1. Titan MPS Digestion Method.

Step	Target Temp (°C)	Pressure Limit (bar)	Ramp Time (min)	Hold Time (min)	Power Limit (%)
1	140	35	10	2	80
2	195	35	3	20	100
3	50	35	1	20	0

Table 2. Digestion Information.

Parameter	Volume
Reagents Used	2.5 mL of HNO_3 (70%) + 7.5 mL deionized water
Initial Sample Weight	1 g
Final Solution Volume (after dilution)	50 mL

Instrumental Conditions

All analyses were performed on an Avio 200 ICP Optical Emission Spectrometer. The elements of interest and instrument conditions for the analysis of the milk samples are outlined in Tables 3 and 4. The standard sample introduction system was used, consisting of a Meinhard® glass nebulizer and baffled glass cyclonic spray chamber. For analysis, an auto integration range of 0.1 – 5 seconds was used for each element. This wide range allowed the Avio 200 spectrometer to automatically determine the most appropriate integration time for each element: higher concentration analytes were read with a shorter time, while lower concentration elements used a longer integration time. This capability minimizes sample analysis time and, combined with the low argon consumption (8 L/min), results in a significant savings when considering the cost of argon.

External calibration standards were prepared in 5% nitric acid (v/v) from a multi-element stock and two single-element standards at the concentrations listed in Table 5. The calibration standards were prepared in two ranges to allow maximum accuracy for both low- and high-level elements. The final nitric acid concentration of the standards (5%) was chosen to match the acid concentration of the digested and diluted samples. Yttrium (Y) was added to all solutions at 0.5 ppm as an internal standard.

Table 3. Avio 200 ICP-OES Instrumental Parameters.

Parameter	Value
Nebulizer	Meinhard Glass, Type K1 (Part No. N0777707)
Spray Chamber	Baffled Glass Cyclonic (Part No. N0791352)
Sample Uptake Rate (mL/min)	1.0
RF Power (W)	1500
Nebulizer Gas (L/min)	0.70
Auxiliary Gas (L/min)	0.2
Plasma Gas (L/min)	8

Table 4. Method Parameters.

Element	Wavelength (nm)	Plasma View	Points per Peak	Auto Integration Range (sec)
Ba	455.403	Radial	3	0.1 – 5
Ca	317.933	Radial	3	0.1 – 5
Fe	238.204	Axial	3	0.1 – 5
K	766.490	Radial	3	0.1 – 5
Mg	285.213	Radial	3	0.1 – 5
Na	589.592	Radial	3	0.1 – 5
P	178.221	Axial	3	0.1 – 5
S	181.975	Axial	3	0.1 – 5
Sr	407.771	Radial	3	0.1 – 5
Zn	206.200	Axial	3	0.1 – 5
Y (int Std)	371.029	Axial & Radial	3	0.1 – 5

Table 5. Calibration Standards.

Element	Std 1 (µg/L)	Std 2 (µg/L)	Std 3 (mg/L)	Std 4 (mg/L)
Ba, Fe, Sr, Zn	50	100	----	----
Ca, K, Mg, Na, P, S	----	----	1	10

Results and Discussion

Despite the differences in the milk samples (fat content, density, form), the Titan MPS digestion system was able to simply and quickly prepare them for analysis using a minimal quantity of reagents, providing major time savings over typical open-vessel digestions. All digests yielded clear solutions, which indicates a complete digestion.

To establish the accuracy of the method, NIST™ 1549a Whole Milk Powder was analyzed, with the results appearing in Table 6. This was the most challenging sample due to its high fat content and concentrated (powder) form. Nevertheless, all recoveries are within 10% of the certified values, demonstrating the accuracy of the methodology and highlighting the ability of the Avio 200 ICP-OES to measure large variations of elemental concentrations in a single analysis.

With the accuracy established, the other milk samples were analyzed, with the results appearing in Figure 1. As expected, the concentration of elements in the powdered milks was the highest, followed by the evaporated milks as well as boxed and fresh milks, which had the lowest elemental concentrations.

Table 6. Analysis of NIST 1549a Whole Milk Powder.

Element	Experimental (mg/kg)	Certified (mg/kg)	% Recovery
Ba	0.530	0.566	94
Ca	9203	8810	104
Fe*	1.72	1.8	95
K	11920	11920	100
Mg	845	892	95
Na	3185	3176	100
P	7128	7600	94
S	2239	---	---
Sr	2.04	2.14	95
Zn	33.7	33.8	100

** Reference value*

It is interesting to note that the elemental concentrations are consistent within a sample type (i.e. boxed, fresh, evaporated, or powdered), regardless of the fat content. In addition, the boxed milks contain the same nutrient levels as the fresh milks, indicating that the ultra-heat treatment of the boxed milks (required to keep them stable without refrigeration) does not degrade the nutritional quality. This analysis also shows why milk is such a valued food source: nutrients (such as calcium, potassium, magnesium, and sodium) are present at elevated levels, along with phosphorus and sulfur.

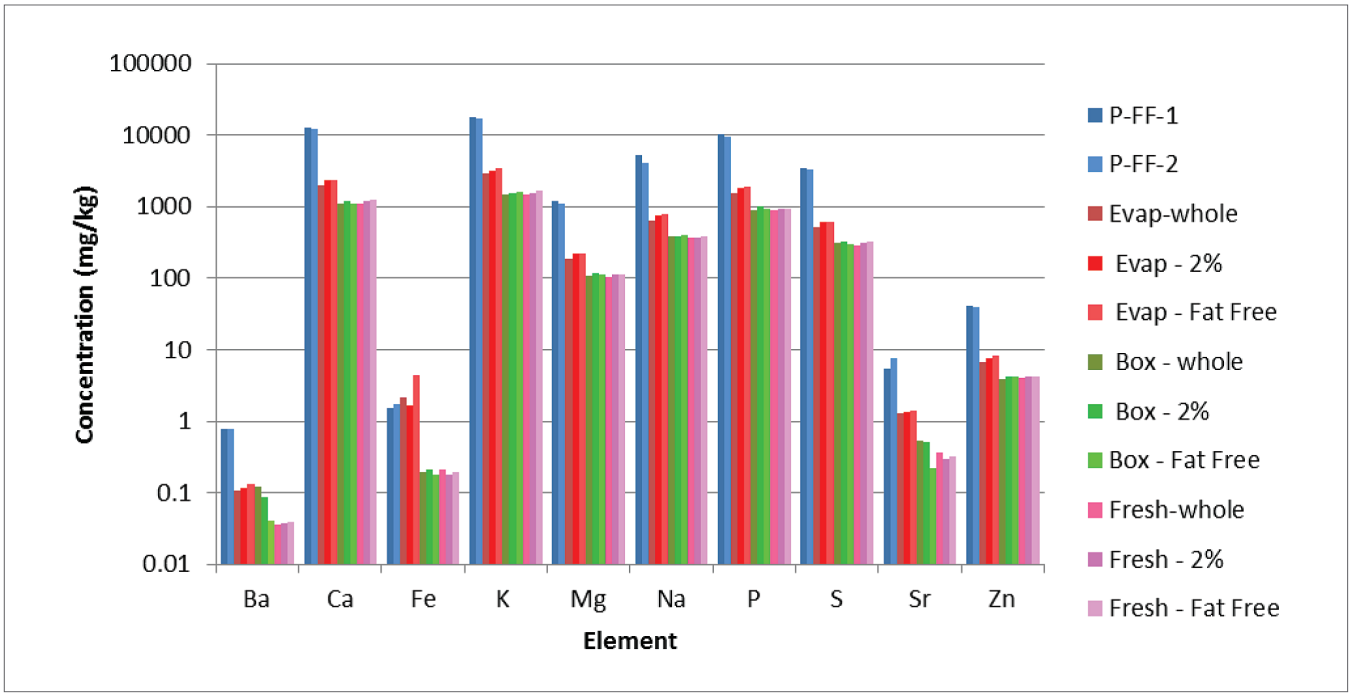

Figure 1. Result s from analyses of milk samples (powdered milks in shades of blue; evaporated milks in shades of red; boxed milks in shades of green; fresh milks in shades of pink).

With the large dynamic range available and the Dual View capability of the Avio 200 ICP-OES, it was not necessary to make custom dilutions per element. With two levels of calibration standards (as shown in Table 5), all elements could be analyzed in a single analytical pass per sample.

To assess any remaining matrix effects from the various samples, all of the evaporated, boxed, and fresh milks were spiked prior to digestion with all elements at the levels shown in Table 7. These spike levels represent the concentrations in solution after sample preparation and are slightly higher than the concentrations of the unspiked milks. This criterion ensures that the spike level is meaningful in relation to the sample signal for the purposes of analytical evaluation. The powdered milk samples were not spiked since analysis of the NIST™ milk powder demonstrated the absence of matrix effects. The resulting spike recoveries appear in Figure 2: all fall within 10% of the spiked value. With the effective digestion provided by the Titan MPS system, per-sample matrix-matching was not required to achieve excellent spike recovery.

Conclusion

This work has demonstrated the ability of the Avio 200 ICP-OES to reliably and effectively analyze a variety of milk samples for an array of elements over a wide range of concentrations. With its extended capabilities, the Avio 200 spectrometer provides greater multi-element sample throughput when compared to Flame AA, while allowing simple analysis of elements, which are typically challenging for Flame AA, such as phosphorus and sulfur.

Table 7. Pre-Digestion Spike Levels .

Element	Spike Level	Units
Ba	10	µg/L
Fe, Sr, Zn	100	µg/L
Mg	5	mg/L
Na, S	15	mg/L
Ca, K, P	50	mg/L

Using the Titan MPS microwave digestion system simplified sample preparation while increasing throughput and productivity for the laboratory compared to hot plate or hot block digestions. The ability to completely digest the samples eliminates the need to matrix-match calibration standards, thus simplifying the analysis.

The use of the Titan MPS digestion system for sample preparation and the Avio 200 ICP-OES for analysis is an ideal combination for fast, simple, and accurate analyses of nutritional elements in milk.

References

1. Spivey, Nick, "Analysis of Micronutrients in Milk by Flame Atomic Absorption Using FAST Flame Sample Automation for Increased Sample Throughput", Application Note, PerkinElmer, 2015.

Figure 2. Spike recoveries in milk samples (evaporated milks in shades of red; boxed milks in shades of green; fresh milks in shades of pink).

Consumables Used

Avio 200 ICP-OES	
Component	**Part Number**
Red/Red PVC Pump Tubing	09908585
Black/Black PVC Pump Tubing	09908587
Autosampler Tubes	B0193233 (15 mL) B0193234 (50 mL)
Instrument Calibration Standard 2 (100 mg/L)	N9301721 (125 mL)
Pure-Grade Phosphorus Standard (1000 mg/L)	N9303788 (125 mL) N9300139 (500 mL)
Pure-Grade Sulfur Standard (1000 mg/L)	N9303796 (125 mL) N9300154 (500 mL)

Titan MPS Digestion System	
Component	**Part Number**
Consumables Kit for Standard 75 mL Digestion Vessels	N3132000
Rupture Disks for Standard 75 mL Digestion Vessels (25 pieces)	N3132001
Pressure Seal for Standard 75 mL Digestion Vessels (10 pieces)	N3132002
End Cap Plug for Gas Containment Manifold	N3134004
Single Lip Seal Forming Tool for Standard 75 mL Digestion Vessels	N3132015
8-Position Lip Seal Forming Tool for Standard 75 mL Digestion Vessels	N3132014

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APPLICATION NOTE

ICP - Mass Spectrometry

Author:

Inorganic Applications Team

PerkinElmer, Inc.

Analysis of Milk for Major and Trace Elements by ICP-MS

Introduction

Milk is a widely consumed food product for both adults and children, while formula milk may constitute a major nutrient source for infants. Furthermore, milk and milk powder are used widely in the food industry for the production of other foods. Because of its nutritional importance and widespread consumption, regulations in many countries necessitate that the quality of the milk is routinely monitored. Both dairies and food manufacturers need to carry out the analysis of major, trace, and contaminant elements in milk and milk powders to fulfill requirements for labelling purposes, monitor nutritional quality, and safeguard against contamination by toxic elements. In Europe, regulations such as Commission Regulation (EC) No 1881/2006 set out maximum levels for some contaminants in foods. Similarly, in India, Food Safety and Standards Regulations (FSSRI) address maximum permitted levels by element over a variety of food groups. In the USA, Proposition 65 regulates contaminants based on maximum daily exposure limits. With elemental concentrations ranging from ng/L to percent levels, samples can present a challenge for ICP-MS instruments in testing laboratories where sample throughput and efficiency is sought. To fulfill this need for the analysis of milk and milk products, reliable testing methods are required.

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Milk contains high concentrations of total dissolved solids (TDS), both from organic and inorganic components. While microwave sample preparation breaks down the organic constituents, the inorganic salts remain in solution at high concentrations. Milk typically contains high levels of phosphorus (P), potassium (K), and calcium (Ca), and moderately high levels of sodium (Na) and magnesium (Mg). Amongst the variety of analytical techniques available for elemental measurements, ICP-MS is unique in its capability to rapidly measure both trace and elevated concentrations of different elements in the same sample.

PerkinElmer's NexION® 2000 ICP-MS includes a variety of design characteristics which enhance its ability to perform these measurements in high-TDS samples. One of the features offered by the NexION 2000 to facilitate analysis of high-TDS samples is the All Matrix Solution (AMS)[1], an innovative argon dilution system designed to precisely dilute the incoming aerosol by 1 to 200x prior to reaching the plasma. This feature allows the introduction of high-TDS samples without the need for off-line liquid dilution, thereby eliminating the possibility of contamination and dilution errors which may be associated with such a step.

This work showcases the NexION 2000 ICP-MS for the analysis of milk samples for major and trace elements, demonstrating the benefits of AMS. Collision mode is applied to target polyatomic interferences, while AMS is used to reduce the level of TDS entering the plasma, ensuring minimal matrix effects. The developed method is simple, fast, and was validated with both certified reference materials and spike recovery studies on a variety of milk samples.

Experimental

Samples and Sample/Standard Preparation

In order to demonstrate the accuracy of the methodology, the following certified reference materials (CRMs) were analyzed:

- ERM-BD 150 skimmed milk powder
- ERM-BD 151 skimmed milk powder
- NMIJ 7512-a milk powder (infant formula milk powder for six-month-old children)

The European Reference Materials (ERM) were obtained from the Joint Research Centre of the European Commission, while the sample from National Measurement Institute of Japan (NMIJ) was obtained from GL Sciences B. V. (Eindhoven, The Netherlands).

The analysis of several materials is advantageous since not all CRMs are certified for the same elements. Thus, a larger selection of elements is covered. Furthermore, the selected CRMs are aiming at different population groups, including infants. Since the materials originate from different continents, the validation reach expands beyond a single region.

However, it is important to provide a method which is not only validated for milk powders, but also for ultra high temperature (UHT) and evaporated milk. For this reason, the following samples were purchased locally and analyzed:

- Skimmed milk powder (non-fat)
- Light evaporated milk (4% fat)
- Evaporated milk (9% fat)
- Sweetened condensed milk
- Skimmed milk (UHT, < 0.5% fat)
- Semi-skimmed milk (UHT, < 2% fat)

To further validate the methodology for various forms of milk with varying fat content, spike recovery studies were performed on these samples.

Samples were digested with a combination of concentrated nitric acid (Fluka™, TraceSELECT® Ultra) and 30% hydrogen peroxide (Sigma-Aldrich™, H_2O_2 ≥ 30%, for Ultratrace analysis). The sample amounts and added volume of water were adjusted, as shown in Table 1. This procedure effectively takes into account the pre-concentration of elements in powdered and evaporated milk, eliminating variation of digestion conditions with milk type due to water content of the sample.

Table 1. Preparation Steps for Various Milk Types.

Milk Type	Weight (g)	Nitric Acid (mL)	Hydrogen Peroxide (mL)	Water (mL)
UHT Milk	5	2.5	2.5	0
Evaporated Milk	2	2.5	2.5	3
Condensed Milk	1	2.5	2.5	4
Milk Powder	0.5	2.5	2.5	5

A Titan MPS™ Microwave Sample Preparation System with standard 75-mL vessels was used for the digestion. The temperature program is given in Table 2. A similar digestion protocol has been successfully used for digestion of milk samples and measurement by ICP-OES[2]. Digests were quantitatively transferred to 50-mL autosampler tubes, spiked with 10 µL of 1000 mg/L gold solution, and made up to the 50 mL volume with deionized water.

Table 2. Microwave Digestion Program for the Titan MPS Microwave Preparation System.

Step	Target Temp (°C)	Pressure Limit (bar)	Ramp Time (min)	Hold Time (min)	Power Limit (%)
1	140	35	10	2	80
2	195	35	3	20	100
3	50	35	1	20	0

Note: The power limit in step 1 is adjusted depending on the number of vessels used in the digestion batch[3].

The moisture content of CRMs was determined following the instructions given on the certificates of analysis. CRM analysis results were corrected for moisture content and are reported on dry-mass basis.

The calibration solutions and internal standard mix were prepared from solutions listed in the Consumables Used table found at the end of this document. Details of the standard concentrations, spike levels, and internal standards are given in Table 3. In addition, glacial acetic acid (Sigma-Aldrich™) was added to the internal standard solution at 1.5% (v/v). The purpose of this organic modifier is to level out carbon content between solutions since digestion of organic samples may leave residual carbon compounds in solution. Because some elements with high ionization potentials are subject to carbon-induced signal enhancement, minimizing the difference in carbon content between standards and samples ensures accurate quantitation. All measurements were made against external calibration curves, with all calibrations being linear and exhibiting $0.99992 \leq r \leq 1.000000$ for all elements. Examples of the high range calibrations are shown in Figure 1 for potassium and sodium. All calibration standards contained 5% nitric acid + 200 µg/L gold, while the autosampler rinse solution consisted of 5% nitric acid. The measured isotopes are listed in Table 7.

Instrumentation

Analysis was carried out on a NexION 2000 P ICP-MS using the conditions and parameters shown in Table 4. No modifications were made to the default sample introduction system: PFA-ST nebulizer, baffled glass cyclonic spray chamber with AMS port, and demountable torch. The Peltier-cooled spray chamber was set to 2 °C, and the AMS flow was set for a 10x dilution. The prepared digests were analyzed straight without any further dilution. No elemental correction equations were used, although the lead (Pb) isotopes were summed (Pb206+Pb207+Pb208) to account for potential geographic differences in Pb isotopic patterns.

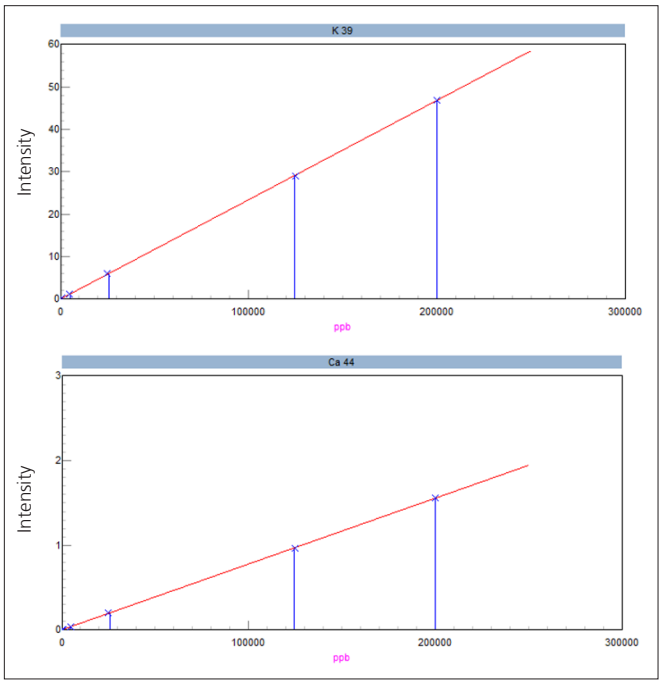

Figure 1. Calibrations for K and Ca.

Table 4. NexION 2000 ICP-MS Parameters.

Component/Parameter	Type/Value
Nebulizer	PFA-ST
Spray Chamber	Glass Cyclonic at 2 °C
Injector	2.0 mm id quartz
Sample Uptake Rate	260 µL/min
Mixing Tee	On-line addition of internal standards
RF Power	1600 W
Collision Flow 1	3.8 mL/min (for As, Se, Ge)
Collision Flow 2	4.7 mL/min (for all remaining elements)
AMS Dilution	10x

Results and Discussion

Certified Reference Materials and Limits of Quantitation

CRMs were measured to validate the accuracy of the method. Table 5 shows the recoveries in NMIJ 7512-a Milk Powder, which are in the range of 94-99% of the certified values.

Table 5. Analysis of NMIJ 7512-a Milk Powder.

	NMIJ 7512a		
Element	Measured (mg/kg)	Certified (mg/kg)	Recovery
Na	1847	1870	99%
Mg	804	819	98%
P	5499	5620	98%
K	8231	8410	98%
Ca	8204	8650	95%
Mn	0.879	0.931	94%
Cu	4.59	4.66	99%
Zn	40.5	41.3	98%
Rb	8.67	8.93	97%
Sr	5.68	5.88	97%
Mo	0.213	0.223	95%
Ba	0.436	0.449	97%

Table 3. Elements, Calibration Levels, and Spike Levels (all units in µg/L).

Element	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6*	Spike Level
Al, As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Rb, Se, Sn, Sr, Ti, V, Zn	1	4	20	100	500	800	200
Hg	0.02	0.08	0.4	2	10	16	4
Na, Mg, K, Ca, P	250	1000	5000	25000	125000	200000	50000
Internal Standards	Sc45, Ga71, Ge72, Rh103, Ir193						

* calibrated for K, Ca, P, and Zn only

Results obtained for ERM-BD 150 and ERM-BD 151 skimmed milk powders are given in Table 6. These CRMs have the same major element composition but differ in levels for some trace elements. There is generally good agreement between the experimental results and certified values, with recoveries ranging between 89-107%. To assess the method repeatability, the ERM-BD 150 reference material was measured seven times, with the average and precision of the seven measurements being shown in Table 6.

Following the recommendations of the Commission Regulation (EC) No 333/2007[4], limits of quantitation (LOQs) were calculated on the basis of 10 times the standard deviation of 10 consecutive blank measurements and have been multiplied by a factor of 100 in order to represent LOQs for milk powders (Table 7). Comparing the LOQs with the CRM values, it can be seen that LOQs are in most cases substantially below certified values. Note, that for milk samples, applicable LOQ values are obtained by dividing the values in Table 6 by 10, due to the larger sample weight for milk (5 g) as compared to powders (0.5 g).

Sensitivity to Measure Legally Stipulated Maximum Levels (ML)

Within the context of milk, Commission Regulation (EC) No 1881/2006 sets MLs for inorganic tin and lead[5]. With the LOQ for lead in milk at 0.00017 mg/kg, the LOQ is two orders of magnitude lower than the regulated level of lead in milk at 0.020 mg/kg. The ML for tin in canned infant formulae and follow-on formulae (including infant milk and follow-on milk) is set to 50 mg/kg, which is four orders of magnitude above the LOQ for tin (Sn) in milk. The comparison is visualized in Figure 2. Digest blanks were below the LOQ for all elements and not of concern.

Table 7. Limits of Quantitation (LOQs) in Different Forms of Milk.

Element	LOQs in Milk Powder (mg/kg)	LOQs in Evaporated Milk (mg/kg)	LOQs in UHT Milk (mg/kg)
Na 23	2.1	0.52	0.21
Mg 24	0.29	0.072	0.029
Al 27	0.40	0.10	0.040
P 31	4.3	1.1	0.43
K 39	3.4	0.85	0.34
Ca 44	6.4	1.6	0.64
Ti 49	0.089	0.022	0.0089
V 51	0.0026	0.00065	0.00026
Cr 52	0.014	0.0035	0.0014
Mn 55	0.021	0.0052	0.0021
Fe 57	0.30	0.075	0.030
Co 59	0.0027	0.00067	0.00027
Ni 60	0.017	0.0043	0.0017
Cu 63	0.0064	0.0016	0.00064
Zn 66	0.099	0.025	0.0099
As 75	0.0099	0.0025	0.00099
Se 78	0.25	0.064	0.025
Rb 85	0.015	0.0037	0.0015
Sr 88	0.010	0.0026	0.0010
Mo 95	0.010	0.0024	0.0010
Cd 111	0.013	0.0033	0.0013
Sn 118	0.012	0.0030	0.0012
Ba 138	0.0034	0.00084	0.00034
Hg 202	0.0083	0.0021	0.00083
Pb 208	0.0017	0.00043	0.00017

Table 6. Analysis of ERM-BD 150 and 151 Skimmed Milk Powders.

	ERM-BD 150				ERM-BD 151		
Element	Measured* (mg/kg)	% RSD*	Certified (mg/kg)	Recovery	Measured (mg/kg)	Certified (mg/kg)	Recovery
Na	4074	1.5	4180	97%	4127	4190	98%
Mg	1225	1.9	1260	97%	1242	1260	99%
P	10368	2.4	11000	94%	10829	11000	98%
K	16343	1.6	17000	96%	16766	17000	99%
Ca	12499	1.4	13900	90%	12927	13900	93%
Mn	0.274	3.9	0.289	95%	0.286	0.29	99%
Fe	4.72	4.3	4.6	103%	49.7	53	94%
Cu	1.04	1.2	1.08	96%	5.05	5.00	101%
Zn	45.3	1.7	44.8	101%	45.5	44.9	101%
Se	< LOQ	---	0.188	---	< LOQ	0.19	n/a
Cd	< LOQ	---	0.0114	---	0.100	0.106	94%
Hg	0.0640	8.5	0.060	107%	0.545	0.52	105%
Pb	0.0170	4.3	0.019	89%	0.200	0.207	97%

* Result of 7 individual measurements

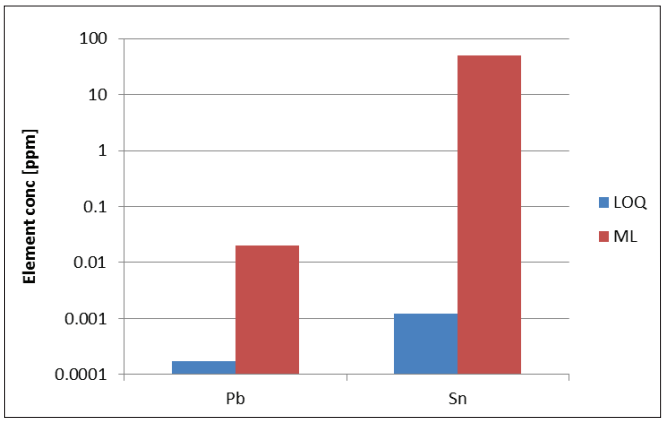

Figure 2. Limit of quantitation (LOQ, 10x SD) vs. maximum level (ML) for lead and inorganic tin, demonstrating sufficient sensitivity of the method.

Analysis of Store-Bought Samples

With the accuracy of the method established for certified elements, the method was applied to the analysis of samples purchased in local grocery stores. UHT milk, milk powder, and evaporated milk samples were analyzed, with the results displayed in Figure 3. The major-element distribution exhibits a clear pattern of highest concentrations in the milk powder sample, intermediate concentrations for the evaporated and condensed milk samples, and lowest concentrations for the UHT milk samples, reflecting the degree of pre-concentration of those elements. The same pattern is also observed for several trace elements (Mn, Cu, Zn, Rb, Sr, Mo, and to a lesser extent Ba and Pb). The evaporated and condensed milk samples show elevated levels of Sn, which may result from the fact that these samples came in tins/cans, which are typically made of tin-coated steel, although further coatings may be involved to protect the food contents. A similar pattern is displayed by iron (Fe), which is also elevated in the canned samples, suggesting packaging could be a possible source there, too. Chromium (Cr) is elevated in the milk powder sample, which may speculatively be caused by processing of the milk sample into powder form. The toxic elements Cd, As, Hg, and other elements not included in Figure 3 were below the quantitation limit throughout.

All samples were spiked at the levels shown in Table 3, which represent 40% of the calibration range for most analytes. The spike levels were chosen so that spike-element concentrations were sufficiently high relative to sample concentrations, to allow accurate quantitation. The spike-to-sample ratio was at minimum between 37-48% for P, K, Ca, and Zn, around 100% for Na, Fe, and Rb, and larger than 400% for the remaining elements. All sample spikes fell within the calibration range, except K, which exceeded the range by 12%. The spike recoveries are plotted in Figure 4, showing recoveries in the range of 86-110%. These results suggest the level of organic modifier (acetic acid) has been set sufficiently high to level out any differences in carbon content between samples and standards. Spike recoveries for the samples and digestion blanks are mostly equivalent, indicating that the sample matrix does not affect recoveries.

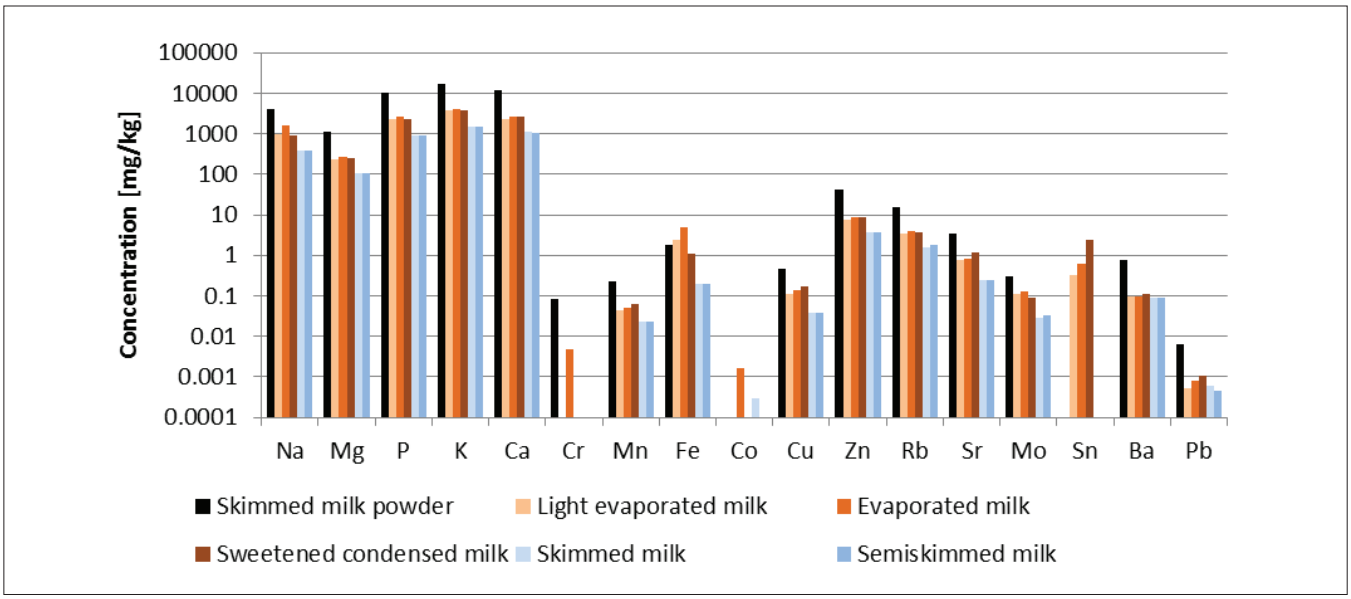

Figure 3. Results of analysis for store-bought samples (milk powder in black; evaporated milks in shades of orange; UHT milks in shades of blue). Values < LOQ have not been plotted.

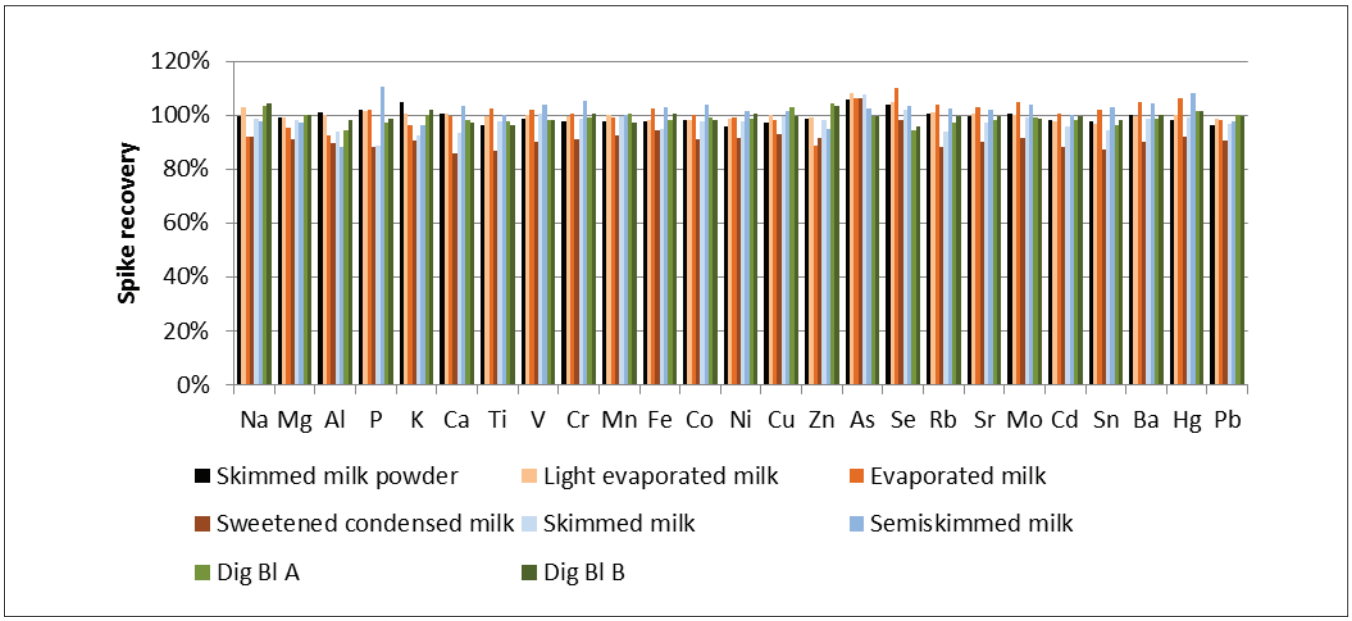

Figure 4. Spike recoveries in store-bought samples (milk powder in black; evaporated milks in shades of orange; UHT milks in shades of blue; digestion blanks in shade of green).

Internal standards stayed within the range of 80-115% and did not show drift over 6.5 hours (Figure 5). Stability over long run times is an important criterion to qualify a method for routine use.

A check standard (Std 4) was measured routinely over the 6.5-hour run. The check standard recoveries are displayed in Figure 6 and show that recoveries are within 92-108% for all elements and are stable over time, showing no drift.

Conclusion

The analysis of major and trace elements in milk, evaporated milk, and milk powder was successfully achieved on the NexION 2000 P ICP-MS using Collision mode with the application of AMS. The analysis of three certified reference materials verified the accuracy of the methodology. Spike recoveries on a variety of store-bought milk samples demonstrated method accuracy for all measured elements, including those where CRMs had no certified values. The method was stable over a 6.5-hour run, showing both no instrument drift and accurate measurement of check standard concentrations. With the combination of unique design characteristics, the NexION 2000 ICP-MS is proven to be an ideal solution for the analysis of both trace and major elements in milk.

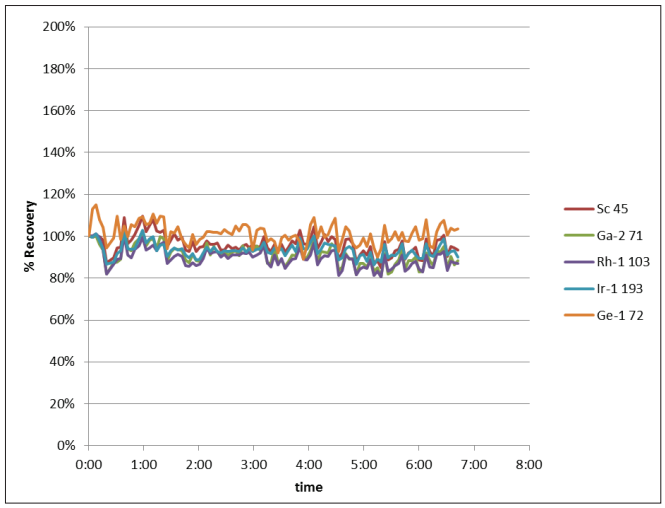

Figure 5. Internal standards behavior during 6.5-hour run.

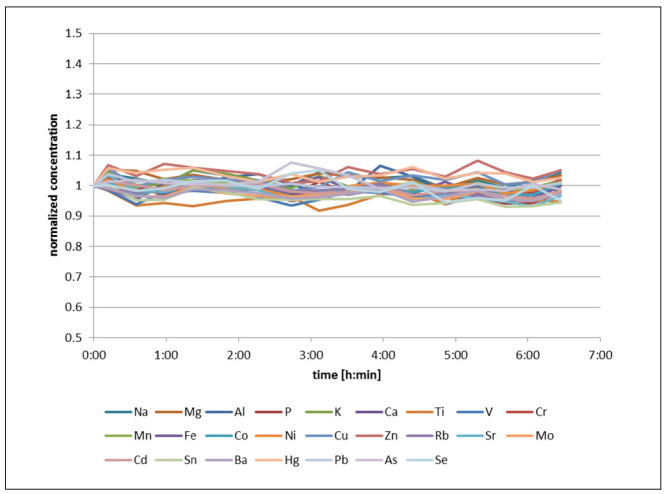

Figure 6. Check standard recoveries over a 6.5-hour run of milk samples.

1. All Matrix Solution System for NexION ICP-MS Platforms, PerkinElmer Technical Note, 2017.
2. Neubauer, K., Spivey, N., Analysis of Micronutrients in Milk Using the Avio 200 ICP-OES, PerkinElmer application note, 2016.
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4. Commission Regulation (EC) No 333/2007: Laying Down the Methods of Sampling and Analysis for the Official Control of the Levels of Lead, Cadmium, Mercury, Inorganic Tin, 3-MCPD, and Benzo(a)pyrene in Foodstuffs, Official Journal of the European Union, European Commission, L88, 2007, pp. 29-38.
5. Commission Regulation (EC) No 1881/2006: Setting Maximum Levels for Certain Contaminants in Foodstuffs, Official Journal of the European Union, European Commission, L364, 2006, pp. 5-24.

Consumables Used

Component	Description	Part Number
Sample Uptake Tubing	Green/yellow (0.44 mm id), flared, PVC, package of 12	N8145198 (MP2 peri pump)
Internal Standard Uptake Tubing	Orange/red (0.19 mm id), flared, PVC, package of 12	N8145195 (MP2 peri pump)
Spray Chamber Drain Tubing	Gray/gray Santoprene (1.30 mm id), package of 12	N8145160 (MP2 peri pump)
Instrument Calibration Standard 2	100 mg/L Ag, Al, As, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sn, Sr, Ti, Tl, V, Zn	N9301721 (125 mL)
Mercury Standard	10 mg/L Hg	N9300253 (125 mL)
Environmental Standard Mix 2	1000 mg/L Na, Mg, K, Ca	N9307805 (125 mL)
Internal Standard Mix	Sc = 200 mg/L; Ga = 20 mg/L; Rh, In, Ir, Tm = 10 mg/L	N9307738 (125 mL)
Germanium Standard	1000 mg/L	N9303774 (125 mL) N9300120 (500 mL)
Gold Standard	1000 mg/L	N9303759 (125 mL)
Phosphorus Standard	1000 mg/L	N9303788 (125 mL) N9300139 (500 mL)
Rubidium Standard	1000 mg/L	N9303792 (125 mL) N9300145 (500 mL)
Autosampler Tubes	50 mL, free-standing 15 mL	B0193234 B0193233

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APPLICATION NOTE

Atomic Absorption

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Analysis of Pb, Cd and As in Tea Leaves Using Graphite Furnace Atomic Absorption Spectrophotometry

Introduction

Tea is drunk by about half of the world's population. It is widely cultivated and consumed in Southeast Asia. Tea is rich in many trace inorganic elements.[1,2] In addition to many essential elements required for human health, some toxic elements may also be present in tea leaves. This could be due to polluted soil, application of pesticides, fertilizers or industrial activities. There is often little information available about the safety of tea leaves and finished tea products with respect to heavy metal contamination. Due to the significant amount of tea consumed, it is important to know the toxic metal contents.

The toxicity and effect of trace heavy metals on human health and the environment has attracted considerable attention and concern in recent years. Among the heavy metals, lead (Pb), cadmium (Cd) and arsenic (As) are especially toxic and are harmful to humans even at low concentrations. They have an inherent toxicity with a tendency to accumulate in the food chain and a particularly low removal rate through excretion.[3] Exposure to heavy metals above the permissible level can cause high blood pressure, fatigue, as well as kidney and neurological disorders. Heavy metals are also known to cause harmful reproductive effects.[4]

A major challenge in the analysis of tea leaves is the extremely low analyte levels and the very high matrix levels. For many years, graphite furnace atomic absorption spectrophotometry (GFAAS) has been a reliable technique and the preferred method for this analysis. The use of longitudinal Zeeman background correction and matrix modifiers help to achieve extremely low detection limits in high matrix samples such as tea leaves, making GFAAS an indispensible tool for carrying out such analyses.

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Experimental Conditions

Instrumentation

The measurements were performed using a PerkinElmer® PinAAcle™ 900T atomic absorption (AA) spectrophotometer (Shelton, CT, USA) equipped with the intuitive WinLab32™ for AA software running under Microsoft® Windows™ 7, which features all the tools to analyze samples, report and archive data and ensure regulatory compliance. The high-efficiency optical system and solid-state detector used in the PinAAcle 900T spectrometer provide outstanding signal-to-noise ratios. The longitudinal Zeeman-effect background correction for graphite furnace analysis provides accurate background correction without the loss of light in other Zeeman systems. The use of a transversely heated graphite atomizer (THGA) provides uniform temperature distribution across the entire length of the graphite tube, eliminating memory effects and potential interferences that may occur with high-matrix sample analyses. Pyrolytically coated THGA tubes with end caps (Part No. B3000655) were used for all measurements. The instrumental conditions are given in Table 1, and the graphite furnace temperature programs are listed in Appendix I (Page 5). Heated injection was used for lead; it can also be used for cadmium and arsenic. A high-performance microwave sample preparation system was used for the microwave-assisted digestion (Table 2). The samples were digested using ten 100 mL high-pressure vessels made of PTFE.

Figure 1. PerkinElmer PinAAcle 900T atomic absorption spectrophotometer.

Table 1. **Optimized parameters for the analysis of tea leaves using the PinAAcle 900T in GFAAS mode.**

Analyte	Pb	Cd	As
Wavelength (nm)	283.3	228.8	193.7
Slit (nm)	0.7	0.7	0.7
Mode	AA-BG	AA-BG	AA-BG
Calibration	Linear through zero	Linear through zero	Linear through zero
Lamp	EDL	HCL	EDL
Current (mA)	440	3	380
Standards (µg/L)	5, 10, 15, 20	0.5, 0.75, 1, 2	10, 20, 30, 40
Correlation Coefficient	0.9991	0.9996	0.9989
Read Time (sec)	3	5	3
Measurement	Peak Area	Peak Area	Peak Area
Injection Temp (˚C)	90	20	20
Sample Volume (µL)	20	20	20
Matrix Modifier	0.05 mg $NH_4H_2PO_4$ and 0.003 mg $Mg(NO_3)_2$	0.05 mg $NH_4H_2PO_4$ and 0.003 mg $Mg(NO_3)_2$	0.005 mg Pd and 0.003 mg $Mg(NO_3)_2$
Modifier Volume (µL)	5	5	5

Table 2. **Microwave digestion program.**

Sequence	1	2
Power (watts)	1000	0
Ramp Time (min)	10	0
Hold Time (min)	10	20
Weight Taken (mg)	~500	
H_2O_2 (mL)	1.0	
HNO_3 (mL)	7.0	
Temp (˚C)	180	

Standards, Chemicals and Certified Reference Materials

PerkinElmer Pure single-element calibration standards for Pb, Cd, and As were used as the stock standards for preparing the working standards (Part Nos. Pb: N9300128; Cd: N9300107; As: N9300102). Working standards were prepared by serial volume/volume dilution in polypropylene vials (Part Nos. B0193233 15 mL Conical; B0193234 50 mL Conical Freestanding) ASTM® Type I deionized water (Millipore® Corporation, Billerica, Massachusetts, U.S.) acidified with 0.2% nitric acid (HNO_3) (Tamapure®, TAMA Chemicals, Japan) was used as the calibration blank and for all dilutions. Thirty percent hydrogen peroxide (H_2O_2) (Kanto Chemicals, Tokyo, Japan) was used for digestion along with nitric acid.

Matrix modifiers were prepared from 10% $NH_4H_2PO_4$ (Part No. N9303445), 1% Mg as $Mg(NO_3)_2$ (Part No. B0190634) and 1% Pd (Part No. B0190635) stock solutions, by diluting with the 0.2% HNO_3 made above. Matrix modifiers were added automatically to each standard, blank and sample by the AS 900 autosampler, an integral part of the PinAAcle 900T spectrometer.

Sample and Certified Reference Material Preparation

Plastic bottles were cleaned by soaking with 10% volume/volume HNO_3 for at least 24 hours and rinsed abundantly in deionized water before use. The polypropylene autosampler cups (Part No. B3001566) were soaked in 20% nitric acid overnight to minimize sample contamination, and thoroughly rinsed with 0.5% HNO_3 acid before use. Five-point calibration curves (four standards and one blank) were constructed for each analyte. The calibration curve correlation coefficient was examined to ensure an $r^2 \geq 0.998$ before the start of the sample analysis.

NIST® 1568a Certified Reference Material (CRM) for Trace Metals in Rice Flour was used to validate the method. Three branded tea leaf samples available in Singapore markets (Tieguanyin tea leaves, Japanese green tea leaves and Loong Jin green tea leaves) were analyzed. Approximately 0.5 g of each sample or CRM, accurately weighed in duplicate, was transferred to the vessel of the microwave digestion system and the sample digestion method (Table 2) was performed in accordance with U.S. Environmental Protection Agency (EPA) Method 3052. The digested samples were diluted with 0.2% HNO_3 and brought up to 25 mL in polypropylene vials.

Results and Discussions

In GFAAS experiments, obtaining reproducible results is a challenging task, as one has to deal with analytes present at low levels in high matrix samples. The role of the sample introduction system is of paramount importance in optimizing the short-term stability of signals. The PinAAcle 900T spectrometer uses a unique built-in camera to monitor sample introduction into the graphite tube. With the furnace camera, it is easier and simpler to position the tip of the injector to the correct depth inside the tube so as to achieve highly reproducible pipetting. The capability to use full Stabilized Temperature Platform Furnace (STPF) conditions along with longitudinal Zeeman background correction and automatic matrix modification made the analysis of low-level analytes in tea leaves an almost effortless task with little to no influence by the concomitant elements in the sample matrix.

The developed method has been validated by incorporating Certified Reference Materials (CRMs) (Table 3). Method detection limits (MDLs) obtained under routine operating conditions were calculated based on the standard deviation of seven replicates of the reagent blank and took into account the 50x dilution factor for the samples (Student's t-value = 3.14, $\rho = 0.02$) (Table 4). The detection limits obtained show the capability of the PinAAcle 900T spectrometer in analyzing difficult matrices at the measured concentrations.

Table 3. **Analysis of certified reference material by GFAAS.**

Analyte	NIST® 1568a Rice Flour	
	Certified Value (µg/g)	Measured Value (µg/g)
Pb	<0.010	0.0093
Cd	0.022 ± 0.002	0.020 ± 0.004
As	0.29 ± 0.03	0.24 ± 0.02

Table 4. **Estimated method detection limits (MDLs).**

Analyte	MDL (µg/L)
Pb	9.5
Cd	2.35
As	9.5

Tea leaves contain a number of organic substances of different stability and impurities of sparingly soluble mineral components. Incomplete mineralization of samples during the microwave-digestion process may cause difficulty in transferring analytes into solution, which can disturb spectrochemical measurements.[5] Application of concentrated HNO_3 along with H_2O_2 for mineralization of tea leaves leads to the complete digestion of samples, which is proven by determination of the values of the analytes in the CRM (Table 3). A post-digestion recovery study was done and the results are summarized in Table 5. The recoveries obtained for the post-digestion spikes indicate there was no interference from the matrix towards the analyte signals.

The results in Table 6 show that the level of lead, cadmium and arsenic in all the samples analyzed were well within the permissible limits of 10, 0.3 and 10 mg/kg respectively, as specified by the U.S. FDA for edible plant parts. The results confirmed that the determination of arsenic, cadmium and lead in tea leaves, after acid solubilization by microwave digestion, can be performed by GFAAS without any interference.

Table 5. **Post-digestion spike recoveries (%).**

Analyte	Pb	Cd	As
Tieguanyin tea leaves	102	96	98
Japanese green tea leaves	92	100	99

Conclusions

Toxicity of food materials is of much greater concern today than ever before. In recent years, greater emphasis has been given to toxic-element contents. A method for the accurate determination of arsenic, cadmium and lead in tea leaves using the PinAAcle 900T atomic absorption spectrophotometer in the GFAAS mode after utilizing microwave-assisted sample digestion was developed. Spike recoveries and the analysis of a CRM showed the method to be accurate, while the MDLs proved the method to be robust and precise. The PinAAcle 900Z (Longitudinal Zeeman Furnace only) spectrometer can also be used for this application.

References

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Table 6. **Results for the detection of toxic metals in tea leaf mixtures (mg/kg) – two replicates (n=2) were performed for each sample or sample duplicate.**

Analyte	Pb		Cd		As	
	Sample	Duplicate	Sample	Duplicate	Sample	Duplicate
Tieguanyin tea leaves	0.68	0.88	0.032	0.026	0.038	0.047
Japanese green tea leaves	0.23	0.27	0.021	0.025	<MDL	<MDL
Loong Jin green tea leaves	0.88	0.95	0.058	0.064	<MDL	<MDL
U.S. FDA limit	10		0.3		10	

Appendix I – Graphite Furnace Temperature Program

Table 7. **Furnace program for lead (Pb).**

Analyte	Step	Temp °C	Ramp Time (sec)	Hold Time (sec)	Internal Gas Flow (mL/min)	Gas Type
Pb	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	850	10	20	250	Argon
	4	1600	0	5	0	–
	5	2450	1	3	250	Argon

Table 8. **Furnace program for cadmium (Cd).**

Analyte	Step	Temp °C	Ramp Time (sec)	Hold Time (sec)	Internal Gas Flow (mL/min)	Gas Type
Cd	1	110	10	30	250	Argon
	2	130	15	30	250	Argon
	3	500	15	35	250	Argon
	4	1500	0	3	0	–
	5	2450	1	3	250	Argon

Table 9. **Furnace program for arsenic (As).**

Analyte	Step	Temp °C	Ramp Time (sec)	Hold Time (sec)	Internal Gas Flow (mL/min)	Gas Type
As	1	110	5	30	250	Argon
	2	130	20	30	250	Argon
	3	800	15	40	250	Argon
	4	1200	15	30	250	Argon
	5	2200	0	5	0	–
	6	2450	1	3	250	Argon

Appendix II – Calibration Graphs for Different Analytes

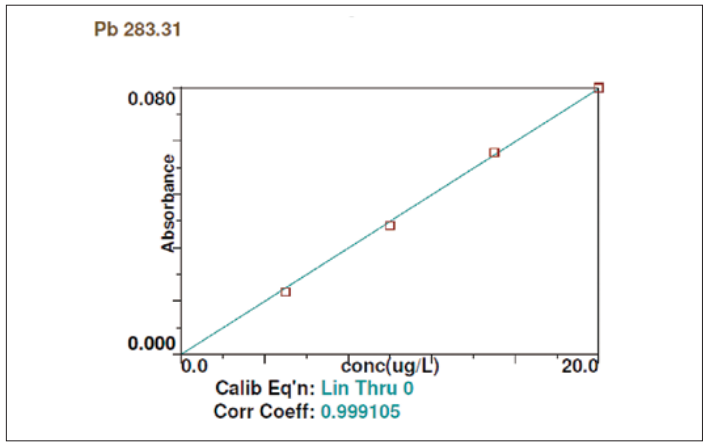

Figure 2. Calibration curve for lead (Pb).

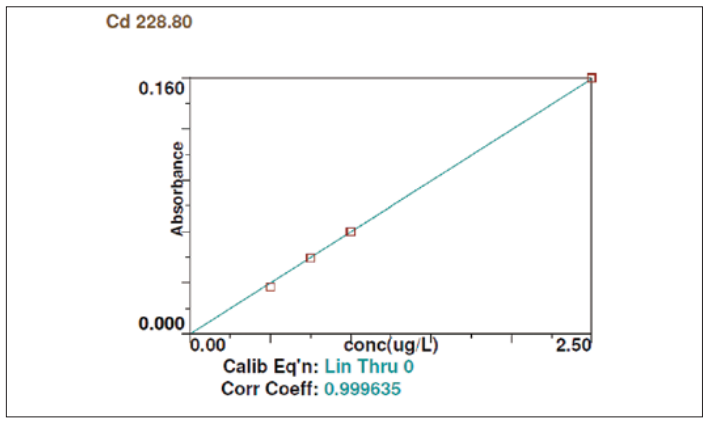

Figure 3. Calibration curve for cadmium (Cd).

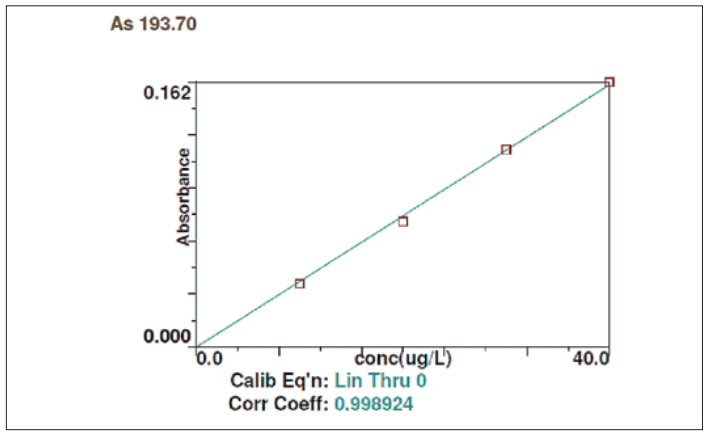

Figure 4. Calibration curve for arsenic (As).

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APPLICATION NOTE

Atomic Absorption

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Accurate Determination of Lead in Different Dairy Products by Graphite Furnace Atomic Absorption Spectrometry

Introduction

Milk is one of the basic food groups in the human diet, both in its original form and as various dairy products. The Chinese contaminated baby formula scandal in 2008 has increased public awareness of contamination possibilities, and has lead to tighter supervision of dairy products as China is faced with demands – both from home and abroad – to improve its food safety record. It is well-known that lead (Pb) is toxic and causes damage to the nervous system; it has a particularly detrimental effect on young children[1] and it has become a cause of major concern since the 1970s. As per World Health Organization (WHO) standards, the permissible limit of lead in drinking water is 10 µg/kg (parts per billion, ppb). Following an in-depth review of the toxicological literature, the Chinese guideline for maximum levels of lead content is set at 20 µg/kg (ppb wet weight) in infant formula (use of milk as a raw material measured by fluid milk diluted from powder, referring to the product ready-to-use) and at 50 µg/kg (ppb) in fresh milk, respectively.[2]

Lead analysis has traditionally been one of the major applications of graphite furnace atomic absorption spectrometry (GFAAS) worldwide. Currently, the Chinese regulatory framework approved standard methods for lead analysis has set GFAAS as the technique for the compulsory arbitration in food testing.[3] In order to ensure protection of consumers, analysis should be sensitive, efficient, and cost-effective so that more effective monitoring can be accomplished. Because GFAAS is a mature technique, it is well-understood and routinely used by technicians and suitable for this determination. Sample preparation is an important part of an analysis and yet can be time consuming.

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Generally, milk is an emulsion or colloid of butterfat globules within a water-based fluid. The exact components of raw milk vary by different animal species, but it contains significant amounts of lactose, fat, protein and minerals as well as vitamins. Due to the relative interference resulting from such a complex matrix, complete decomposition of milk samples prior to instrumental measurement by microwave or heating block acid digestion is generally recommended. This approach, however, is more time-consuming and poses a more rigorous requirement on quality assurance than simple dilution when concentrations of lead are to be determined at µg/kg level in the final solution which is extremely sensitive to reagent blank contribution and environmental contamination.

To overcome these issues, this work describes a simple and direct dilution method for sample preparation, followed by automated analysis using GFAAS. This method minimizes sample preparation, and also reduces potential contamination while still maintaining the speed of analysis.

Experimental Conditions

Instrumentation

A PerkinElmer® PinAAcle™ 900T flame and longitudinal Zeeman atomic absorption spectrometer (Figure 1) was used for the GFAAS measurements of lead (Pb) in different milk samples. The PinAAcle 900T spectrometer's transversely heated graphite atomizer (THGA) with Longitudinal AC Zeeman background correction provide a constant uniform temperature distribution across the entire length of the graphite tube. This allows a full implementation of the Stabilized Temperature Platform Furnace™ (STPF) technique in graphite furnace analysis where we can analyze complex sample matrices using aqueous standard solutions as calibration for suspended sample solutions to get accurate and precise results. Maximum atomic signals can be obtained with minimum memory effect and potential interference.

Figure 1. PinAAcle 900T atomic absorption spectrometer with AS 900 furnace autosampler.

The spectrometer was equipped with an AS 900 autosampler and a PerkinElmer Lumina™ single-element Pb hollow cathode lamp (Part No. N3050157) was used as the light source. A standard THGA tube (Part No. B0504033) and 1.2 mL polypropylene autosampler cups (Part No. B0510397) were used throughout for all measurement. The instrument was controlled by WinLab32™ for AA software running under Microsoft® Windows® 7 operating system. A summary of the PinAAcle 900T instrument settings is listed in Table 1.

***Table 1.* Instrument settings for the PinAAcle 900T spectrometer.**

Parameter	Value
Wavelength:	283.3 nm
Slit Width:	0.7 nm
Lamp Current:	10 mA
Signal Measurement:	Peak Area
Measurement Type:	AA-BG
Integration Time:	5 s
Replicates:	3
Calibration Standard:	4, 10, 15, 20 µg/L
Sample Volume:	16 µL

Sampling

A total of 15 samples of six different dairy products were investigated in this study, representing all the main types of milk commercially available in China, including milk powder, skimmed milk powder, whole milk, low-fat milk, children's milk and yogurt. All the samples collected from the original packaging in a sealed clean polyethylene bag, were labeled and taken to the laboratory then kept refrigerated until analysis.

Sample Preparation

For the preparation of all solutions, ultrapure deionized (DI) water from a MiliQ-Element system (Millipore®, Milford, MA, USA) was used throughout. Concentrated nitric acid (69-70%), HNO_3, and hydrogen peroxide (30%), H_2O_2, were trace-metal grade or better (Jingrui Chemical Co., Ltd., Jiangsu, China). Metal-free polypropylene vials and pipette tips were pre-cleaned with diluted nitric acid (~5% HNO_3) and rinsed thoroughly with DI water before use.

For the subsequent GFAAS analysis, a solution containing 0.5% HNO_3 with 0.1% Triton X-100 (Part No. N9300260), a non-ionic detergent, was prepared daily both as a diluent and as a blank.

A 1-g sample of liquid milk or solid milk powder was accurately weighed and transferred into a 15-mL conical polypropylene tube (Part No. B0193233) which was subsequently diluted to make up the volume of 10 mL, and shaken vigorously for a few minutes to ensure homogeneity. The obtained suspension solution was immediately ready for GFAAS measurement using the autosampler. These suspensions were stable for more than 2 days. Even the more challenging total fat milk powder prepared by this rapid dilute-and-shoot procedure can be stable for this duration, which is sufficient for the inter-day variability check. The same procedure was used to prepare the blanks, and all the samples were prepared in duplicate on a routine analysis basis, unless stated otherwise.

For skimmed milk powders and fortified infant formulas whose protein content characteristics are modified by the manufacturing process, or for any milk powders with a higher protein content, any nitric acid addition will coagulate the dissolution resulting in a non-homogeneous suspension. In these cases, the milk-powder samples can be dispersed in 0.2 to 0.5% Triton X-100 solution, and a short 10-minute sonication will help disperse the milk powder into a more homogeneous solution that is stable for several hours, satisfactory for graphite furnace analysis.

For the validation by ICP-MS determination, a Multiwave™ 3000 high-pressure microwave digestion system (PerkinElmer, Inc., Shelton, CT) was employed to completely decompose the milk sample matrix using an acid mixture of HNO_3 and H_2O_2.

Calibration

As the concentration of Pb in milk samples is generally very low, all the reagents used must be of ultra-pure grade. Thus, Single-Element PerkinElmer Pure Plus Grade Standards (Part No. N9303748, lead in 2% HNO_3) and Matrix Modifiers (Part No. B0190635, 10% Pd as nitrate and Part No. B0190634, 1% Mg as nitrate) were recommended to be used. Calibration curves were constructed using online auto-dilution of a working stock lead standard solution of 20 µg/kg (ppb) by the AS 900 autosampler.

Method Validation

The performance of the procedure using GFAAS measurement was assessed by spike recovery and the evaluation of the Standard Reference Materials (SRMs) from National Institute of Standards and Technology (NIST®), NIST® 1549 Non-Fat Milk Powder, and China National Institute of Metrology (NIM), GBW08509a Skimmed Milk Powder. These two commercial lyophilized SRMs were treated as any dairy product sample.

In addition, these results were also compared to that obtained by the conventional mineralization-based procedures, followed by analysis using the NexION® 300X ICP-MS (PerkinElmer, Inc., Shelton, CT). The complete mineralization was carried out with the Multiwave 3000 microwave digestion system. Instrumental operating parameters for the ICP-MS measurements followed the routinely established protocols.

Results and Discussion

The temperature program for the analysis of lead is optimized to provide maximum matrix decomposition without loss of analyte. The furnace temperature program is given in Table 2.

Due to the challenging characteristics of the sample matrix, an additional drying step, using a special gas of dry compressed air, is recommended to eliminate the carbonaceous residues left after analyzing more than 50 samples in one single batch. The PinAAcle 900T spectrometer's TubeView™ color furnace camera is of great advantage in checking the position of the tip in the furnace, relative to the platform, which brings benefits in optimizing the drying and pyrolysis steps for the complex undigested milk matrix to ensure that no sample boiling or splattering occurred (Figure 2 – Page 4).

Table 2. **Furnace temperature program for the direct measurement of lead in milk samples using the PinAAcle 900T spectrometer with THGA tubes.**

Step		Temp. (°C)	Ramp Time (sec)	Hold Time (sec)	Internal Flow	Read Step	Gas Type
1	Drying	130	5	30	250		Normal
2	Drying	150	15	30	250		Normal
3	Drying	450	15	15	50		Dried Air
4	Pyrolysis	600	10	20	250		Normal
5	Atomization	1600	0	3	0	X	Normal
6	Clean-out	2500	1	5	250		Normal

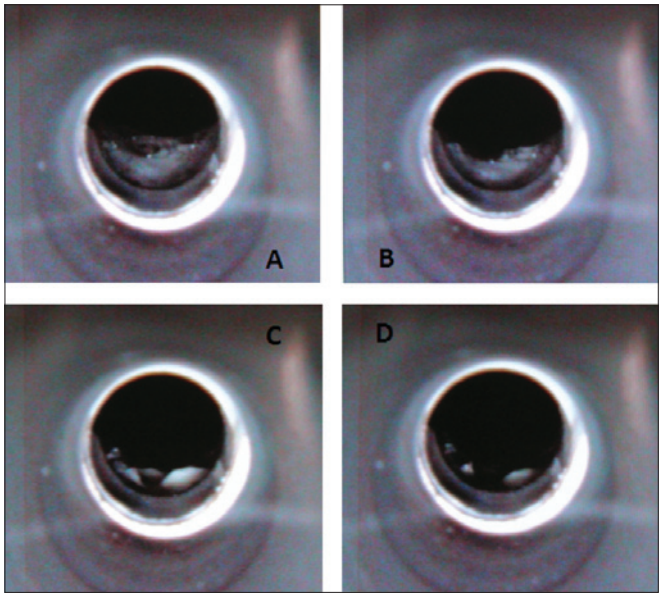

Figure 2. The drying steps of a complex undigested milk sample in the graphite tube, as seen using the TubeView color furnace camera.

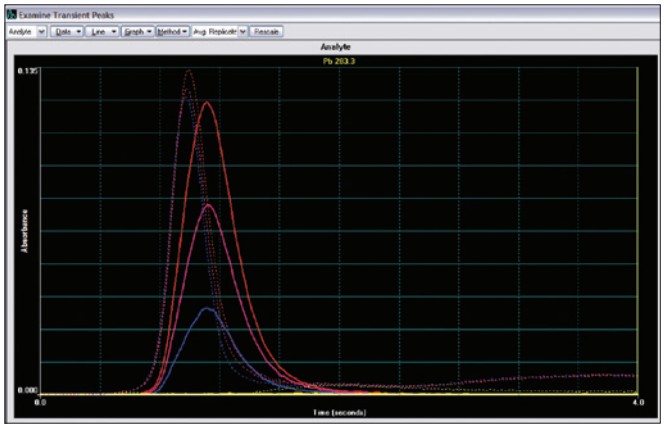

Figure 3. Overlay of typical lead atomic and background signal for the control material of skimmed milk powder. The solid blue line is from the control material of skimmed milk powder, the solid purple line is from the spiked control material, and the solid red line is from the standard at a concentration of 25 µg/kg, while the solid yellow line at the bottom is the reagent blank signal. Dashed lines represent the background absorption profiles.

Therefore, it helped in simpler and faster furnace (temperature) method development.

For Pb determination, complete mineralization of the milk components is not necessary when using the proven and established STPF technique with the patented THGA design which ensures uniform and consistent heating and high atomization efficiency, significantly reducing matrix interferences. All data were calculated from 3 replicate readings for each solution using peak-area (integrated absorbance) integration. Figure 3 depicts the overlay of typical peak profiles of the various solutions. One of the unique benefits of the STPF technique is clearly demonstrated here: even though the peaks may not appear at exactly the same time, the peak-area calculation still provides consistently accurate results.

To test the accuracy of the method, Pb was analyzed in the control material of non-fat milk powder from NIST® 1549 and skimmed milk powder from NIM GBW08509a. The high level of accuracy of the direct method is demonstrated by the good agreement of the results obtained in the analysis of the two SRMs with the certified values, as shown in Table 3. An estimation of analyte recovery was also obtained by spiking one of the SRM samples (GBW08509a) at the 50, 100, and 200% levels with the Pb single-element standard working stock solution, and the data, also collated in Table 3, demonstrates quantitative recovery.

***Table 3.* Results for the direct measurement of NIST® 1549 and GBW08509a by GFAAS (all in µg/kg).**

Sample	Certified Value	Spike Level	Expected Mean	Found Mean	Recovery (%)
NIST® 1549	19 ±3	0	19	19	101
GBW08509a	24 ±6	0	24	23	95
GBW08509a	24 ±6	12	36	35	96
GBW08509a	24 ±6	24	48	48	99
GBW08509a	24 ±6	48	72	71	98

Method detection limits (MDLs), defined as the analyte concentration in micrograms per kilogram (ppb) of dairy products which provides an absorbance reading statistically different from that of the blank, are calculated by dividing 3 times the standard deviation (SD) of the absorbance readings of the reagent blanks by the sensitivity. An impressive characteristic of this method, which uses a sample volume at 16 µL with 10-fold dilution factor, provides the MDL of 0.25 µg/kg (ppb). Thus, the MDL measured in the original dairy products is about two orders of magnitude below the expected level in the typical control materials (around 20 µg/kg). It indicates that this method could prove highly suitable for determining Pb in dairy products.

For additional independent comparative data against GFAAS analysis using this simple method, all collected dairy products were mineralized by conventional microwave total acid digestion, then analyzed for lead by ICP-MS. Table 4 (Page 5) shows the concentrations of Pb found in each dairy product sample.

Table 4. **Lead levels in commercially available dairy products determined by direct GFAAS analysis and conventional ICP-MS measurement (values are means ± SD, all in µg/kg).**

No.	SRMs/Samples	Certified Value	Measured Results GFAAS	ICP-MS
1	GBW08509a (Skimmed milk powder)	24 ±6	23.3 ±0.7	23.9 ±1.7
2	GBW10017 (Milk powder)	70 ±20	23.9 ±2.7	25.7 ±8.7
3	NIST® 1549 (Non-fat milk powder)	19 ±3	19.1 ±1.3	19.3 ±6.5
4	Milk powder	–	40.2 ±1.8	42.1 ±1.9
5	Skimmed milk powder	–	25.7 ±1.3	23.3 ±6.1
6	Whole milk (Brand 1)	–	4.46 ±0.32	4.57 ±0.60
7	Whole milk (Brand 2)	–	2.75 ±0.07	2.73 ±0.09
8	Whole milk (Brand 3)	–	6.13 ±0.07	6.78 ±0.49
9	Whole milk (Brand 4)	–	5.65 ±0.11	5.85 ±0.37
10	Low-fat milk (Brand 1)	–	2.34 ±0.09	2.39 ±0.38
11	Low-fat milk (Brand 2)	–	0.53 ±0.02	0.58 ±0.21
12	Drinkable children's milk (Brand 1)	–	1.70 ±0.09	1.73 ±0.22
13	Drinkable children's milk (Brand 2)	–	0.22 ±0.01	0.54 ±0.15
14	Drinkable yogurt (Brand 1)	–	1.89 ±0.16	2.02 ±0.18
15	Drinkable yogurt (Brand 2)	–	1.36 ±0.02	1.61 ±0.33

It is important to emphasize that there are no significant differences between the two independent testing methods, which further demonstrates the accuracy of the overall methods. However, the relative standard deviation (RSD) was generally higher for data obtained by ICP-MS analysis after conventional mineralization. This is most likely due to the dilution introduced during the digestion step used in the ICP-MS sample preparation. Even though the ICP-MS technique is more sensitive than GFAAS, the dilution of the extremely low levels of Pb present in the samples introduces additional uncertainty. Based on the results, it clearly appears that total digestion of matrix components is unnecessary with all these types of dairy-product samples, and it is more rapid and economical to run the samples with minimal preparation.

As is also shown in Table 4, the Pb concentration in one of the tested SRMs issued by State General Administration of the People's Republic of China for Quality Supervision and Inspection and Quarantine (AQSIQ), GBW10017 milk powder found in this study, is 23.9 ±2.7 µg/kg by direct GFAAS method and 25.7 ±8.7 µg/kg by total digested ICP-MS method, which are both significantly lower than the certified value (70 ±20 µg/kg). This difference has also been observed by other laboratories purchasing this reference material. Based on the higher value of standard deviation (20 µg/kg, 29% of error), the actual certified Pb result in this GBW10017 SRM issued by AQSIQ has yet to be ascertained and needs further investigation.

For an intuitive and illustrative comparison, the differences in Pb concentration and analytical precision are also presented in Figure 4 as a plot with error bar. Our results clearly affirm the great advantage of easy handling and precise analysis using direct determination of Pb concentration by GFAAS, since the need to measure Pb at such a low level (in µg/kg range) in the original dairy product samples requires extremely strict control of reagents, environment and process. This is very challenging, even for experienced professionals, due to the large dilution factor if undergoing the time-consuming and labor-burdened total digestion procedure, taking the poor match of experimental value with the certified value in the SRMs of GBW10017 as an additional proof.

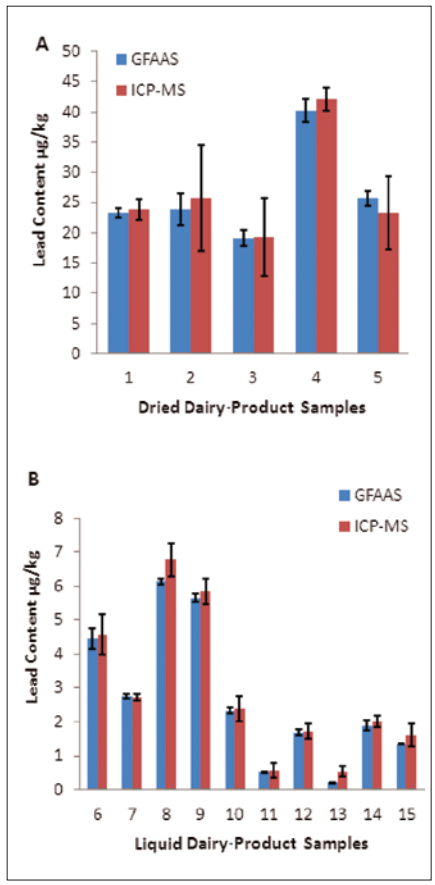

Figure 4. Comparison of lead levels in different dairy-product samples obtained by two independent test methods: A) dried milk powder samples; B) liquid milk samples.

Conclusions

In conclusion, a method involving simple sample dilution and automated PinAAcle 900T GFAAS detection can be successfully applied to the accurate measurement of Pb in different dairy products. Reduced sample handling minimizes the potential for losses or contamination. The advanced THGA technique keeps the risk of chemical interferences to a minimum, which provides a method detection limit well below the normal range of Pb that might be encountered. This method should also be applicable for analysis of samples with equivalent content of fat and complex matrices.

ICP-MS provides multi-element analysis and very high sensitivity. However, the high initial investment and more costly cost of ownership when compared with GFAAS may not offer the best choice for a simple single-element analysis. GFAAS offers not only high selectivity, sensitivity, and ease of operation, but also high tolerance to complex matrices. When coupled with simple sample preparation, it is consequently more appropriate for the trace level determination of a few toxic elements in dairy products as a routine monitoring technique in protecting human health.

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APPLICATION NOTE

ICP - Mass Spectrometry

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Analysis of Milk for Major and Trace Elements by ICP-MS

Introduction

Milk is a widely consumed food product for both adults and children, while formula milk may constitute a major nutrient source for infants. Furthermore, milk and milk powder are used widely in the food industry for the production of other foods. Because of its nutritional importance and widespread consumption, regulations in many countries necessitate that the quality of the milk is routinely monitored. Both dairies and food manufacturers need to carry out the analysis of major, trace, and contaminant elements in milk and milk powders to fulfill requirements for labelling purposes, monitor nutritional quality, and safeguard against contamination by toxic elements. In Europe, regulations such as Commission Regulation (EC) No 1881/2006 set out maximum levels for some contaminants in foods. Similarly, in India, Food Safety and Standards Regulations (FSSRI) address maximum permitted levels by element over a variety of food groups. In the USA, Proposition 65 regulates contaminants based on maximum daily exposure limits. With elemental concentrations ranging from ng/L to percent levels, samples can present a challenge for ICP-MS instruments in testing laboratories where sample throughput and efficiency is sought. To fulfill this need for the analysis of milk and milk products, reliable testing methods are required.

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Milk contains high concentrations of total dissolved solids (TDS), both from organic and inorganic components. While microwave sample preparation breaks down the organic constituents, the inorganic salts remain in solution at high concentrations. Milk typically contains high levels of phosphorus (P), potassium (K), and calcium (Ca), and moderately high levels of sodium (Na) and magnesium (Mg). Amongst the variety of analytical techniques available for elemental measurements, ICP-MS is unique in its capability to rapidly measure both trace and elevated concentrations of different elements in the same sample.

PerkinElmer's NexION® 2000 ICP-MS includes a variety of design characteristics which enhance its ability to perform these measurements in high-TDS samples. One of the features offered by the NexION 2000 to facilitate analysis of high-TDS samples is the All Matrix Solution (AMS)[1], an innovative argon dilution system designed to precisely dilute the incoming aerosol by 1 to 200x prior to reaching the plasma. This feature allows the introduction of high-TDS samples without the need for off-line liquid dilution, thereby eliminating the possibility of contamination and dilution errors which may be associated with such a step.

This work showcases the NexION 2000 ICP-MS for the analysis of milk samples for major and trace elements, demonstrating the benefits of AMS. Collision mode is applied to target polyatomic interferences, while AMS is used to reduce the level of TDS entering the plasma, ensuring minimal matrix effects. The developed method is simple, fast, and was validated with both certified reference materials and spike recovery studies on a variety of milk samples.

Experimental

Samples and Sample/Standard Preparation

In order to demonstrate the accuracy of the methodology, the following certified reference materials (CRMs) were analyzed:

- ERM-BD 150 skimmed milk powder
- ERM-BD 151 skimmed milk powder
- NMIJ 7512-a milk powder (infant formula milk powder for six-month-old children)

The European Reference Materials (ERM) were obtained from the Joint Research Centre of the European Commission, while the sample from National Measurement Institute of Japan (NMIJ) was obtained from GL Sciences B. V. (Eindhoven, The Netherlands).

The analysis of several materials is advantageous since not all CRMs are certified for the same elements. Thus, a larger selection of elements is covered. Furthermore, the selected CRMs are aiming at different population groups, including infants. Since the materials originate from different continents, the validation reach expands beyond a single region.

However, it is important to provide a method which is not only validated for milk powders, but also for ultra high temperature (UHT) and evaporated milk. For this reason, the following samples were purchased locally and analyzed:

- Skimmed milk powder (non-fat)
- Light evaporated milk (4% fat)
- Evaporated milk (9% fat)
- Sweetened condensed milk
- Skimmed milk (UHT, < 0.5% fat)
- Semi-skimmed milk (UHT, < 2% fat)

To further validate the methodology for various forms of milk with varying fat content, spike recovery studies were performed on these samples.

Samples were digested with a combination of concentrated nitric acid (Fluka™, TraceSELECT® Ultra) and 30% hydrogen peroxide (Sigma-Aldrich™, H_2O_2 ≥ 30%, for Ultratrace analysis). The sample amounts and added volume of water were adjusted, as shown in Table 1. This procedure effectively takes into account the pre-concentration of elements in powdered and evaporated milk, eliminating variation of digestion conditions with milk type due to water content of the sample.

Table 1. Preparation Steps for Various Milk Types.

Milk Type	Weight (g)	Nitric Acid (mL)	Hydrogen Peroxide (mL)	Water (mL)
UHT Milk	5	2.5	2.5	0
Evaporated Milk	2	2.5	2.5	3
Condensed Milk	1	2.5	2.5	4
Milk Powder	0.5	2.5	2.5	5

A Titan MPS™ Microwave Sample Preparation System with standard 75-mL vessels was used for the digestion. The temperature program is given in Table 2. A similar digestion protocol has been successfully used for digestion of milk samples and measurement by ICP-OES[2]. Digests were quantitatively transferred to 50-mL autosampler tubes, spiked with 10 µL of 1000 mg/L gold solution, and made up to the 50 mL volume with deionized water.

Table 2. Microwave Digestion Program for the Titan MPS Microwave Preparation System.

Step	Target Temp (°C)	Pressure Limit (bar)	Ramp Time (min)	Hold Time (min)	Power Limit (%)
1	140	35	10	2	80
2	195	35	3	20	100
3	50	35	1	20	0

Note: The power limit in step 1 is adjusted depending on the number of vessels used in the digestion batch[3].

The moisture content of CRMs was determined following the instructions given on the certificates of analysis. CRM analysis results were corrected for moisture content and are reported on dry-mass basis.

The calibration solutions and internal standard mix were prepared from solutions listed in the Consumables Used table found at the end of this document. Details of the standard concentrations, spike levels, and internal standards are given in Table 3. In addition, glacial acetic acid (Sigma-Aldrich™) was added to the internal standard solution at 1.5% (v/v). The purpose of this organic modifier is to level out carbon content between solutions since digestion of organic samples may leave residual carbon compounds in solution. Because some elements with high ionization potentials are subject to carbon-induced signal enhancement, minimizing the difference in carbon content between standards and samples ensures accurate quantitation. All measurements were made against external calibration curves, with all calibrations being linear and exhibiting $0.99992 \leq r \leq 1.000000$ for all elements. Examples of the high range calibrations are shown in Figure 1 for potassium and sodium. All calibration standards contained 5% nitric acid + 200 µg/L gold, while the autosampler rinse solution consisted of 5% nitric acid. The measured isotopes are listed in Table 7.

Instrumentation

Analysis was carried out on a NexION 2000 P ICP-MS using the conditions and parameters shown in Table 4. No modifications were made to the default sample introduction system: PFA-ST nebulizer, baffled glass cyclonic spray chamber with AMS port, and demountable torch. The Peltier-cooled spray chamber was set to 2 °C, and the AMS flow was set for a 10x dilution. The prepared digests were analyzed straight without any further dilution. No elemental correction equations were used, although the lead (Pb) isotopes were summed (Pb206+Pb207+Pb208) to account for potential geographic differences in Pb isotopic patterns.

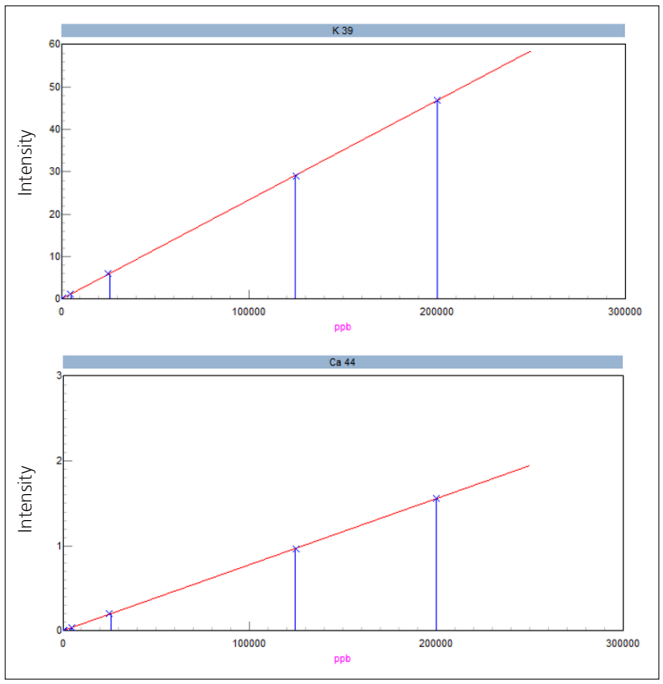

Figure 1. Calibrations for K and Ca.

Table 4. NexION 2000 ICP-MS Parameters.

Component/Parameter	Type/Value
Nebulizer	PFA-ST
Spray Chamber	Glass Cyclonic at 2 °C
Injector	2.0 mm id quartz
Sample Uptake Rate	260 µL/min
Mixing Tee	On-line addition of internal standards
RF Power	1600 W
Collision Flow 1	3.8 mL/min (for As, Se, Ge)
Collision Flow 2	4.7 mL/min (for all remaining elements)
AMS Dilution	10x

Results and Discussion

Certified Reference Materials and Limits of Quantitation

CRMs were measured to validate the accuracy of the method. Table 5 shows the recoveries in NMIJ 7512-a Milk Powder, which are in the range of 94-99% of the certified values.

Table 5. Analysis of NMIJ 7512-a Milk Powder.

	NMIJ 7512a		
Element	Measured (mg/kg)	Certified (mg/kg)	Recovery
Na	1847	1870	99%
Mg	804	819	98%
P	5499	5620	98%
K	8231	8410	98%
Ca	8204	8650	95%
Mn	0.879	0.931	94%
Cu	4.59	4.66	99%
Zn	40.5	41.3	98%
Rb	8.67	8.93	97%
Sr	5.68	5.88	97%
Mo	0.213	0.223	95%
Ba	0.436	0.449	97%

Table 3. Elements, Calibration Levels, and Spike Levels (all units in µg/L).

Element	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6*	Spike Level
Al, As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Rb, Se, Sn, Sr, Ti, V, Zn	1	4	20	100	500	800	200
Hg	0.02	0.08	0.4	2	10	16	4
Na, Mg, K, Ca, P	250	1000	5000	25000	125000	200000	50000
Internal Standards	Sc45, Ga71, Ge72, Rh103, Ir193						

* calibrated for K, Ca, P, and Zn only

Results obtained for ERM-BD 150 and ERM-BD 151 skimmed milk powders are given in Table 6. These CRMs have the same major element composition but differ in levels for some trace elements. There is generally good agreement between the experimental results and certified values, with recoveries ranging between 89-107%. To assess the method repeatability, the ERM-BD 150 reference material was measured seven times, with the average and precision of the seven measurements being shown in Table 6.

Following the recommendations of the Commission Regulation (EC) No 333/2007[4], limits of quantitation (LOQs) were calculated on the basis of 10 times the standard deviation of 10 consecutive blank measurements and have been multiplied by a factor of 100 in order to represent LOQs for milk powders (Table 7). Comparing the LOQs with the CRM values, it can be seen that LOQs are in most cases substantially below certified values. Note, that for milk samples, applicable LOQ values are obtained by dividing the values in Table 6 by 10, due to the larger sample weight for milk (5 g) as compared to powders (0.5 g).

Sensitivity to Measure Legally Stipulated Maximum Levels (ML)

Within the context of milk, Commission Regulation (EC) No 1881/2006 sets MLs for inorganic tin and lead[5]. With the LOQ for lead in milk at 0.00017 mg/kg, the LOQ is two orders of magnitude lower than the regulated level of lead in milk at 0.020 mg/kg. The ML for tin in canned infant formulae and follow-on formulae (including infant milk and follow-on milk) is set to 50 mg/kg, which is four orders of magnitude above the LOQ for tin (Sn) in milk. The comparison is visualized in Figure 2. Digest blanks were below the LOQ for all elements and not of concern.

Table 7. Limits of Quantitation (LOQs) in Different Forms of Milk.

Element	LOQs in Milk Powder (mg/kg)	LOQs in Evaporated Milk (mg/kg)	LOQs in UHT Milk (mg/kg)
Na 23	2.1	0.52	0.21
Mg 24	0.29	0.072	0.029
Al 27	0.40	0.10	0.040
P 31	4.3	1.1	0.43
K 39	3.4	0.85	0.34
Ca 44	6.4	1.6	0.64
Ti 49	0.089	0.022	0.0089
V 51	0.0026	0.00065	0.00026
Cr 52	0.014	0.0035	0.0014
Mn 55	0.021	0.0052	0.0021
Fe 57	0.30	0.075	0.030
Co 59	0.0027	0.00067	0.00027
Ni 60	0.017	0.0043	0.0017
Cu 63	0.0064	0.0016	0.00064
Zn 66	0.099	0.025	0.0099
As 75	0.0099	0.0025	0.00099
Se 78	0.25	0.064	0.025
Rb 85	0.015	0.0037	0.0015
Sr 88	0.010	0.0026	0.0010
Mo 95	0.010	0.0024	0.0010
Cd 111	0.013	0.0033	0.0013
Sn 118	0.012	0.0030	0.0012
Ba 138	0.0034	0.00084	0.00034
Hg 202	0.0083	0.0021	0.00083
Pb 208	0.0017	0.00043	0.00017

Table 6. Analysis of ERM-BD 150 and 151 Skimmed Milk Powders.

	ERM-BD 150				ERM-BD 151		
Element	Measured* (mg/kg)	% RSD*	Certified (mg/kg)	Recovery	Measured (mg/kg)	Certified (mg/kg)	Recovery
Na	4074	1.5	4180	97%	4127	4190	98%
Mg	1225	1.9	1260	97%	1242	1260	99%
P	10368	2.4	11000	94%	10829	11000	98%
K	16343	1.6	17000	96%	16766	17000	99%
Ca	12499	1.4	13900	90%	12927	13900	93%
Mn	0.274	3.9	0.289	95%	0.286	0.29	99%
Fe	4.72	4.3	4.6	103%	49.7	53	94%
Cu	1.04	1.2	1.08	96%	5.05	5.00	101%
Zn	45.3	1.7	44.8	101%	45.5	44.9	101%
Se	< LOQ	---	0.188	---	< LOQ	0.19	n/a
Cd	< LOQ	---	0.0114	---	0.100	0.106	94%
Hg	0.0640	8.5	0.060	107%	0.545	0.52	105%
Pb	0.0170	4.3	0.019	89%	0.200	0.207	97%

* Result of 7 individual measurements

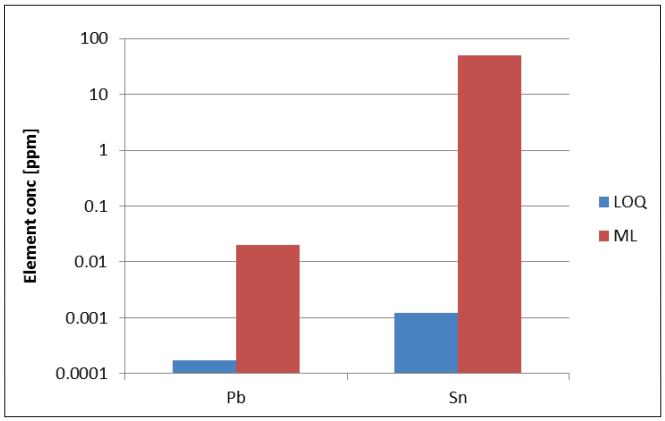

Figure 2. Limit of quantitation (LOQ, 10x SD) vs. maximum level (ML) for lead and inorganic tin, demonstrating sufficient sensitivity of the method.

Analysis of Store-Bought Samples

With the accuracy of the method established for certified elements, the method was applied to the analysis of samples purchased in local grocery stores. UHT milk, milk powder, and evaporated milk samples were analyzed, with the results displayed in Figure 3. The major-element distribution exhibits a clear pattern of highest concentrations in the milk powder sample, intermediate concentrations for the evaporated and condensed milk samples, and lowest concentrations for the UHT milk samples, reflecting the degree of pre-concentration of those elements. The same pattern is also observed for several trace elements (Mn, Cu, Zn, Rb, Sr, Mo, and to a lesser extent Ba and Pb). The evaporated and condensed milk samples show elevated levels of Sn, which may result from the fact that these samples came in tins/cans, which are typically made of tin-coated steel, although further coatings may be involved to protect the food contents. A similar pattern is displayed by iron (Fe), which is also elevated in the canned samples, suggesting packaging could be a possible source there, too. Chromium (Cr) is elevated in the milk powder sample, which may speculatively be caused by processing of the milk sample into powder form. The toxic elements Cd, As, Hg, and other elements not included in Figure 3 were below the quantitation limit throughout.

All samples were spiked at the levels shown in Table 3, which represent 40% of the calibration range for most analytes. The spike levels were chosen so that spike-element concentrations were sufficiently high relative to sample concentrations, to allow accurate quantitation. The spike-to-sample ratio was at minimum between 37-48% for P, K, Ca, and Zn, around 100% for Na, Fe, and Rb, and larger than 400% for the remaining elements. All sample spikes fell within the calibration range, except K, which exceeded the range by 12%. The spike recoveries are plotted in Figure 4, showing recoveries in the range of 86-110%. These results suggest the level of organic modifier (acetic acid) has been set sufficiently high to level out any differences in carbon content between samples and standards. Spike recoveries for the samples and digestion blanks are mostly equivalent, indicating that the sample matrix does not affect recoveries.

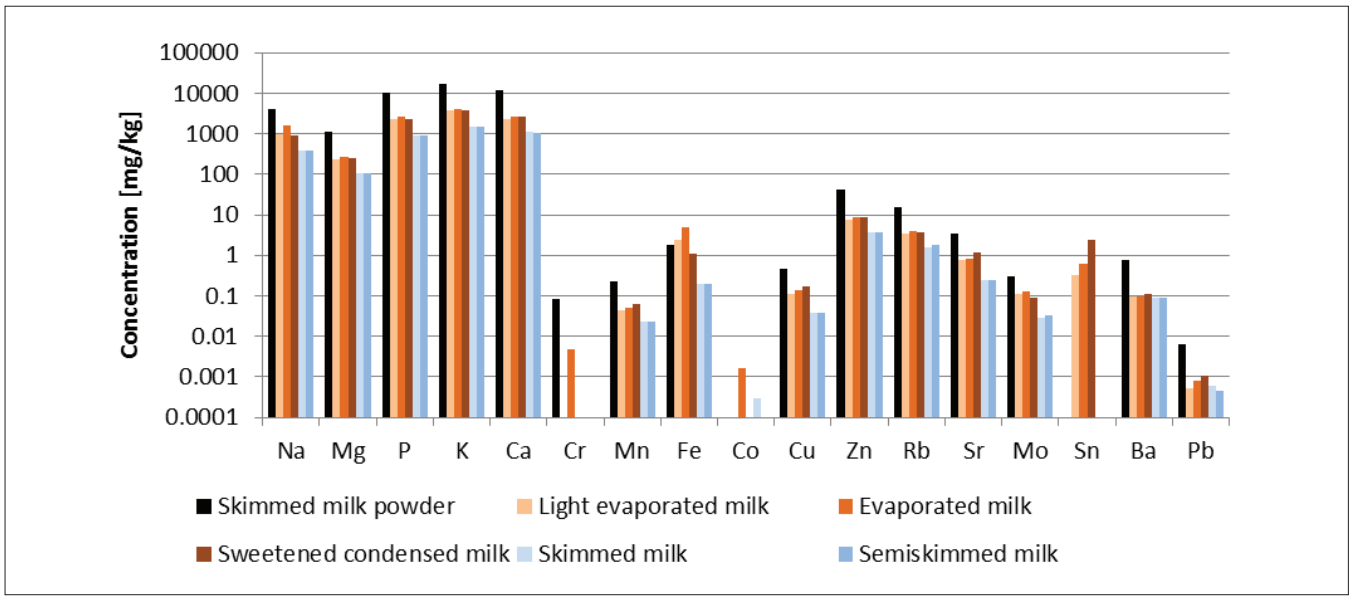

Figure 3. Results of analysis for store-bought samples (milk powder in black; evaporated milks in shades of orange; UHT milks in shades of blue). Values < LOQ have not been plotted.

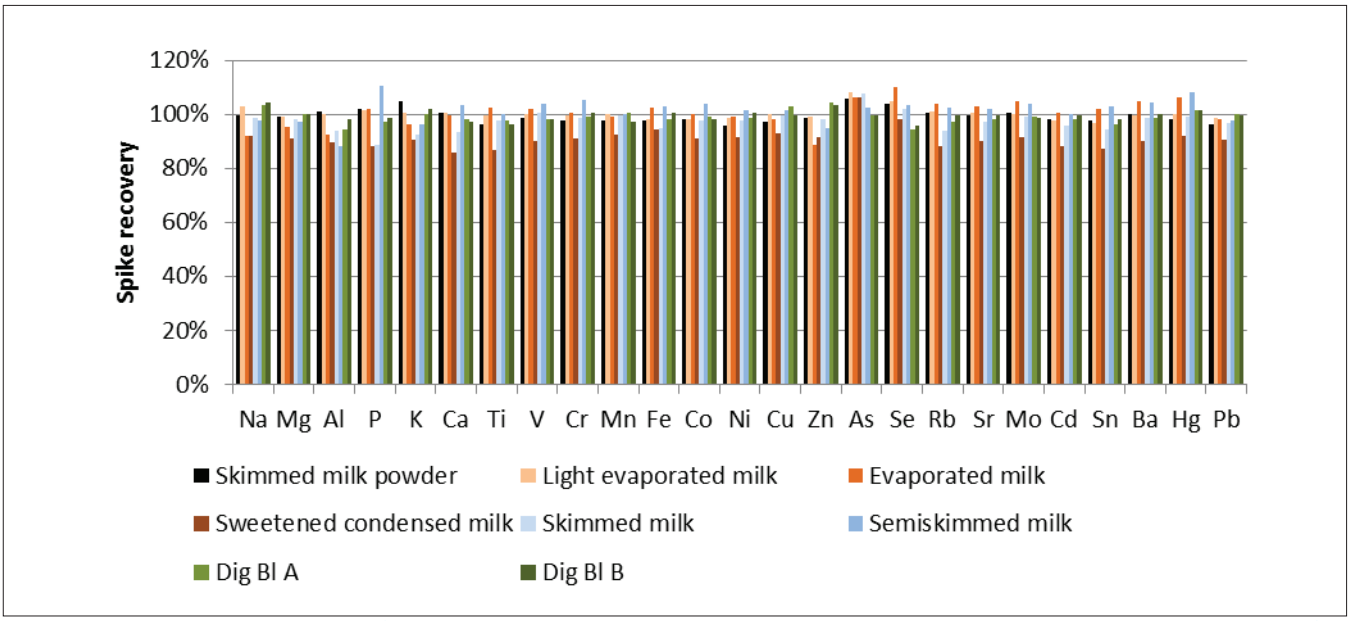

Figure 4. Spike recoveries in store-bought samples (milk powder in black; evaporated milks in shades of orange; UHT milks in shades of blue; digestion blanks in shade of green).

Internal standards stayed within the range of 80-115% and did not show drift over 6.5 hours (Figure 5). Stability over long run times is an important criterion to qualify a method for routine use.

A check standard (Std 4) was measured routinely over the 6.5-hour run. The check standard recoveries are displayed in Figure 6 and show that recoveries are within 92-108% for all elements and are stable over time, showing no drift.

Conclusion

The analysis of major and trace elements in milk, evaporated milk, and milk powder was successfully achieved on the NexION 2000 P ICP-MS using Collision mode with the application of AMS. The analysis of three certified reference materials verified the accuracy of the methodology. Spike recoveries on a variety of store-bought milk samples demonstrated method accuracy for all measured elements, including those where CRMs had no certified values. The method was stable over a 6.5-hour run, showing both no instrument drift and accurate measurement of check standard concentrations. With the combination of unique design characteristics, the NexION 2000 ICP-MS is proven to be an ideal solution for the analysis of both trace and major elements in milk.

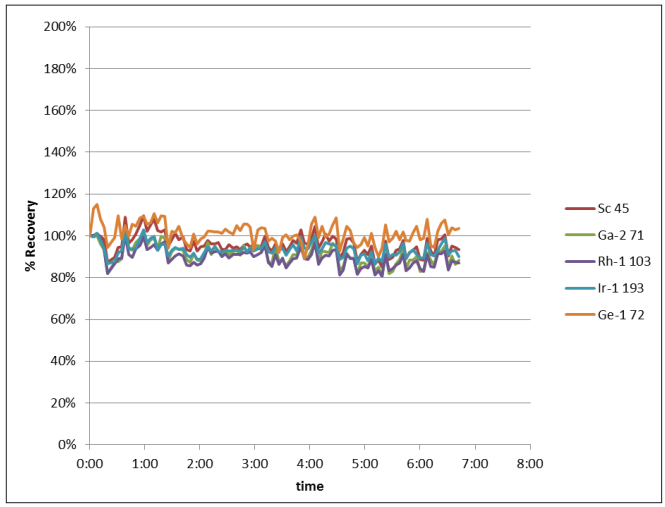

Figure 5. Internal standards behavior during 6.5-hour run.

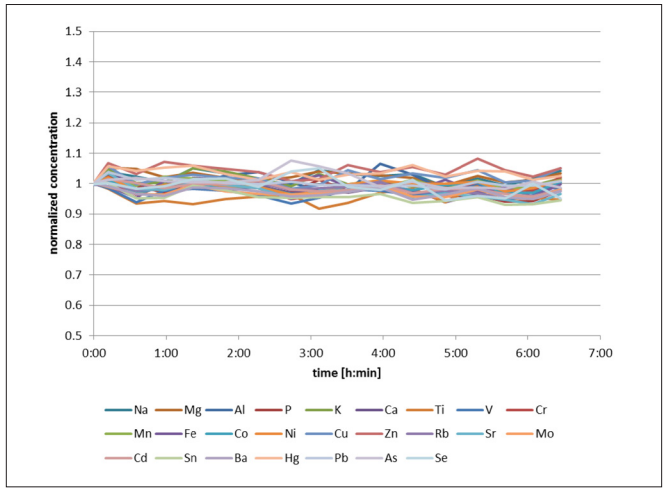

Figure 6. Check standard recoveries over a 6.5-hour run of milk samples.

References

1. All Matrix Solution System for NexION ICP-MS Platforms, PerkinElmer Technical Note, 2017.
2. Neubauer, K., Spivey, N., Analysis of Micronutrients in Milk Using the Avio 200 ICP-OES, PerkinElmer application note, 2016.
3. Titan MPS ™ Microwave Sample Preparation System - A Reference Notebook of Microwave Applications, PerkinElmer, 2016.
4. Commission Regulation (EC) No 333/2007: Laying Down the Methods of Sampling and Analysis for the Official Control of the Levels of Lead, Cadmium, Mercury, Inorganic Tin, 3-MCPD, and Benzo(a)pyrene in Foodstuffs, Official Journal of the European Union, European Commission, L88, 2007, pp. 29-38.
5. Commission Regulation (EC) No 1881/2006: Setting Maximum Levels for Certain Contaminants in Foodstuffs, Official Journal of the European Union, European Commission, L364, 2006, pp. 5-24.

Consumables Used

Component	Description	Part Number
Sample Uptake Tubing	Green/yellow (0.44 mm id), flared, PVC, package of 12	N8145198 (MP2 peri pump)
Internal Standard Uptake Tubing	Orange/red (0.19 mm id), flared, PVC, package of 12	N8145195 (MP2 peri pump)
Spray Chamber Drain Tubing	Gray/gray Santoprene (1.30 mm id), package of 12	N8145160 (MP2 peri pump)
Instrument Calibration Standard 2	100 mg/L Ag, Al, As, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sn, Sr, Ti, Tl, V, Zn	N9301721 (125 mL)
Mercury Standard	10 mg/L Hg	N9300253 (125 mL)
Environmental Standard Mix 2	1000 mg/L Na, Mg, K, Ca	N9307805 (125 mL)
Internal Standard Mix	Sc = 200 mg/L; Ga = 20 mg/L; Rh, In, Ir, Tm = 10 mg/L	N9307738 (125 mL)
Germanium Standard	1000 mg/L	N9303774 (125 mL) N9300120 (500 mL)
Gold Standard	1000 mg/L	N9303759 (125 mL)
Phosphorus Standard	1000 mg/L	N9303788 (125 mL) N9300139 (500 mL)
Rubidium Standard	1000 mg/L	N9303792 (125 mL) N9300145 (500 mL)
Autosampler Tubes	50 mL, free-standing 15 mL	B0193234 B0193233

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APPLICATION NOTE

HPLC-ICP-MS

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Characterization of Arsenic Species in Apple Juice using a NexSAR HPLC-ICP-MS Speciation Analysis Ready Solution

Introduction

Apple juice is a healthy, and often preferred, alternative to artificially flavored and carbonated drinks for many parents to give to children, posing as a valuable source of vitamins, minerals and fiber.[1] However, concern has been raised over the presence of arsenic in apple juices[2] due to high natural abundances and the historical use of arsenic in pesticides, where arsenic from such applications can persist in soil for decades after its use. This is of special concern in exposures involving children, since children elicit a more severe dose-response than adults.[3]

Although the information gained from the total analysis of arsenic can be useful, it may also provide a biased view about toxicity since different chemical forms of arsenic have differing levels of toxicities. Speciation studies, in contrast, are able to provide a wealth of information about toxicity as this relates to chemical species present, and the relative abundance of each species. For arsenic, the inorganic forms (arsenite [As III] and arsenate [As V]) are most toxic, whereas methylated forms of arsenic (dimethylarsinic acid [DMA], monomethylarsonic acid [MMA], etc.) are less toxic. Consequently, the action limit for inorganic As in apple juice set by the FDA is 10 µg/L,[4] where concentrations above this have been found to invoke a toxicological response in children.[1,2,3,5]

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To separate the different chemical species of As, chromatographic techniques, such as HPLC, are often employed, where ICP-MS is the detector of choice for many analytical laboratories. This is due to its wide linear dynamic range, low detection limits, and ability to resolve complex interferences. For analytes other than As, the additional capability of ICP-MS to resolve different isotopes of elements empowers users to employ isotope dilution methods, which can greatly improve analytical accuracy.

A challenge faced by HPLC-ICP-MS users is that the majority of applications utilize salt buffers in strongly acidic [6,7] or strongly basic [9] mobile phases so as to achieve baseline resolution of the different chemical species of the element of interest. In steel-based systems, such extreme conditions may cause corrosion of pump and autosampler hardware over long-term use, where this effect will be more pronounced in components which make contact with the mobile phase. Although these systems can be somewhat passivated by flushing with 20% HNO_3, corrosion is still possible.[10] Any rust formed may become dislodged, blocking and sometimes irreversibly damaging expensive columns. This can be effectively prevented when using an HPLC system which has an inert, metal-free fluid path which would eliminate the issue of rust formation. Moreover, the use of this design would ensure that backgrounds are low for other analytes which are typically of analytical interest in trace speciation applications, such as Cr and Fe.

An additional challenge for users is that despite good pump practice involving flushing the pump with a weak organic solution at the end of each day, salt crystals may still form behind the pump seals. This can cause damage to the seal, affecting analytical results, and increasing maintenance costs and instrument downtime. This issue can be effectively mitigated by using an HPLC pump which has post-seal wash capabilities, thereby actively washing behind the pump seal, and reducing seal wear and tear.

In this study, four arsenic species in commercial apple juices were characterized using an HPLC method which was developed to achieve the fast and reproducible separation and analysis of low concentrations of As species. The reputable and verified method developed by Ernstberger *et al.*[7,8] was used to quantify the various arsenic species commonly found in apple juice (As III, As V, DMA, MMA) and thereby evaluate their potential toxicity. This analysis was performed using a PerkinElmer NexSAR™ HPLC-ICP-MS Solution, which comprises an inert NexSAR Speciation Analysis Ready HPLC system coupled to a NexION® ICP-MS, where the elution was thermostatically controlled using a NexSAR Column Oven.

Experimental

Sample Preparation

Calibration standards with concentrations of 0.1, 0.5, 1, 5, 10, and 20 µg/L were prepared in the mobile phase from the following reagents: As (III) from 999 ± 5 mg/L arsenite (Inorganic Ventures Ltd., Christiansburg, Virginia, USA), As (V) from 1003 ± 6 mg/L arsenate (Inorganic Ventures), DMA from cacodylic acid (≥ 99.0%, Sigma Aldrich, St. Louis, Missouri, USA), and MMA from 1000 mg/L MMA (Chemservice, West Chester, Pennsylvania, USA). These species were selected for evaluation due to their presence in past studies involving the analysis of apple juices.[6,7] Concentrations were chosen to reflect the concentration range around the current action level for inorganic arsenic as set out by the FDA.[4] The analysis took place using an acidic mobile phase (pH 4.0) which has been previously proven to be the most suitable method which accurately reflects the pH of apple juices, while also ensuring the integrity of the various arsenic species.[7]

Four popular, commercially produced apple juices were purchased at a local grocery store. No consideration was made for the length of time these juices were on the shelf. This is because the various chemical species of arsenic are known to be in equilibrium, and stable in commercial-grade apple juices over extensive periods of storage time.[6,7] Juices were well-shaken prior to sampling to ensure homogeneity, and 50 mL of each apple juice was filtered through 0.45 µm PTFE filters (hydrophilic, Millex, Sigma Aldrich) to remove unwanted particulate matter. Analyses were performed on undiluted samples.

During this work, the calibration standards were run, and the four apple juice samples, which had been decanted into a number of different plastic HPLC vials, were repeatedly analyzed. A blank sample was analyzed after every apple juice sample to check for carryover between samples. Due to the absence of a certified reference material, accuracy was ensured through spiking of the samples with 2 µg/L and 10 µg/L of each species (As III, As V, DMA, MMA).

Instrumentation

All analyses were performed using a NexSAR Speciation Analysis Ready HPLC system (PerkinElmer Inc., Shelton, Connecticut, USA) comprised of the NexSAR 200 Inert HPLC Pump, NexSAR Cooled Inert Autosampler, NexSAR Solvent Tray with Degasser, and NexSAR Column Oven (PerkinElmer Inc.). The system was coupled to a NexION ICP-MS (PerkinElmer Inc.). Details pertaining to the HPLC and ICP-MS conditions are shown in Tables 1 and 2, respectively, and were based on previous work.[7] During method development, m/z 75 and 77 were monitored to check for the presence of $^{75}ArCl^+$ interference on $^{75}As^+$. Since no $ArCl^+$ could be detected, the samples were analyzed in Standard mode for the rest of the study. All analyses and the collection of data were performed using Clarity™ software version 8.1.

Table 1. NexSAR Inert HPLC System Conditions.

Parameter	Value
Chromatography	Reversed-phase Ion-pairing Chromatography
Mobile Phase	Ion-pairing Reagent
pH	4.0
Separation Scheme	Isocratic
Injection Volume	20 µL
LC Vials	HPLC Tested PP Vials, 1.5 mL

Table 2. NexION ICP-MS Instrument Conditions.

Parameter	Value
Nebulizer	MEINHARD® plus Glass Type C
Spray Chamber	Glass Cyclonic
RF Power	1600 W
Injector	2.0 mm ID Quartz
Nebulizer Flow	Optimized for <2% oxides
Mode	Standard
Dwell Time	100 ms
Sampling Rate	10 points/sec

Results and Discussion

The correlation coefficients for the standards (0.1 – 20 µg/L) of As V, MMA, As III, and DMA were 0.99990, 0.99999, 0.99999, and 0.99998 respectively (Figure 1), where Figure 2 shows the overlap of the calibration standards. The latter (Figure 2) demonstrates the reliable and consistent flow rate delivered by the pump and shows that there is excellent reproducibility of the retention times, regardless of the concentration.

Owing to the inert fluid path of the NexSAR HPLC system, the chromatogram baseline is negligible for As, where the signal-to-noise ratio (S/N) for the 0.1 ppb standard ranged between 12 and 7 for the various chemical species of As. This allows for the detection of As at levels far below the FDA action limits and also ensures that chemical species, which were present in low abundances – such as MMA – can be easily quantified. This is important since the detection of chemical species, which are present in low concentrations, provides a more holistic view of toxicity. Moreover, in studies where mass-balance equations are used, being able to quantify trace-levels of analytes would greatly improve analyte recoveries, thereby improving the overall quality of such results.

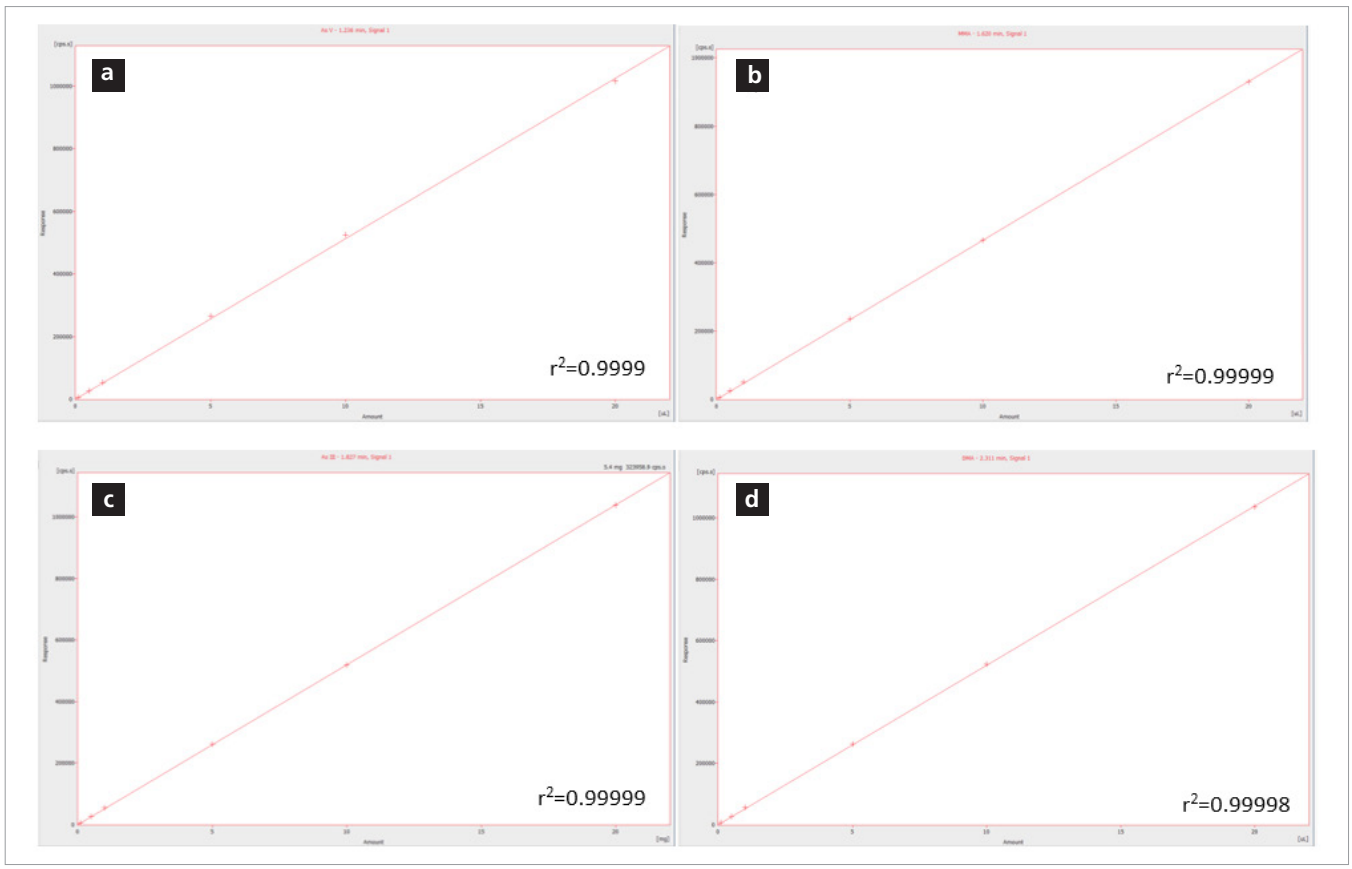

Figure 1. Linear regression of calibration standards ranging in concentration from 0.1-20 µg/L for (a) As V, (b) MMA, (c) As III, and (d) DMA in the mobile phase (pH 4.0) and the respective correlation coefficients.

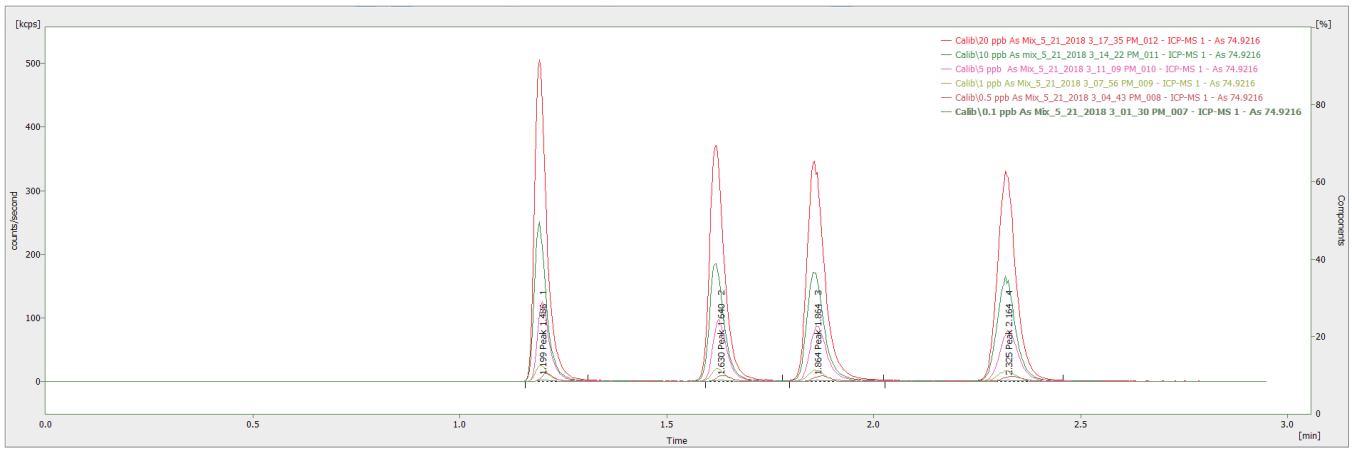

Figure 2. Chromatogram showing overlay of calibration standards (0.1 – 20 µg/L) in the mobile phase at (pH 4.0).

Apple juice is a complex matrix, so achieving reproducible results in undiluted samples can sometimes be challenging. In this study, injections of the same sample from different sample vials were found to exhibit excellent reproducibility (Figure 3) with a percentage relative standard deviation (% RSD) below 2% for all analytes. Moreover, low chromatographic baselines with negligible pulsation for both the samples and calibration standards demonstrates the robustness of the technique in different matrices, and the appropriateness of the HPLC-tested plastic vials for this application. The overlay also shows that injection volumes were highly repeatable, having a near-exact response at each injection. It should be noted that higher quality solvents and chemicals could lower the chromatographic baseline (S/N). Nevertheless, Figure 3 demonstrates that low-ppt analytes can be accurately quantified where the concentrations of As V, MMA, As III, and DMA are 0.94, 0.09, 0.77, and 0.20 µg/L respectively in this particular sample, and the S/N for the lowest concentration analyte (MMA) was 6.

Figure 3 also shows that a complete separation and accurate quantification of the four most common and relevant arsenic chemical species found in apple juices can take place in under three minutes per sample. This elution time is five times faster than traditional techniques, such as anion exchange chromatography, used in arsenic speciation studies. This work was performed without an internal standard and the reproducibility shown demonstrates that there is no need for one; however, if an internal standard is required, previous work has shown that arsenobetaine (AsB) can be used as a suitable internal standard eluting just after three minutes.[7] The analysis of a blank after each apple juice sample indicated that there was no carryover between injections. The absence of carryover is extremely useful, especially in commercial laboratories, where reduced rinsing between samples means less solvent use, lower overheads, and higher productivity.

In order to assess the impact of the apple juice matrix upon analytical accuracy, a low-end (2 µg/L) and high-end (10 µg/L) spike of each arsenic species was added to an undiluted sample. Figure 4 shows the chromatogram for the 2 µg/L and 10 µg/L spikes in comparison to the sample. Spiked samples were found to have similar retention times to the pure sample and exhibited good spike recoveries, ranging between 99-111% for the different arsenic species (2 µg/L spike: 103, 111, 100, 99% recovery and 10 µg/L spike: 106, 103, 111 and 101% recovery for As V, MMA, As III, and DMA respectively). This proves the accuracy of the method across a larger linear dynamic range and shows that samples can be prepared in a relatively simple manner.

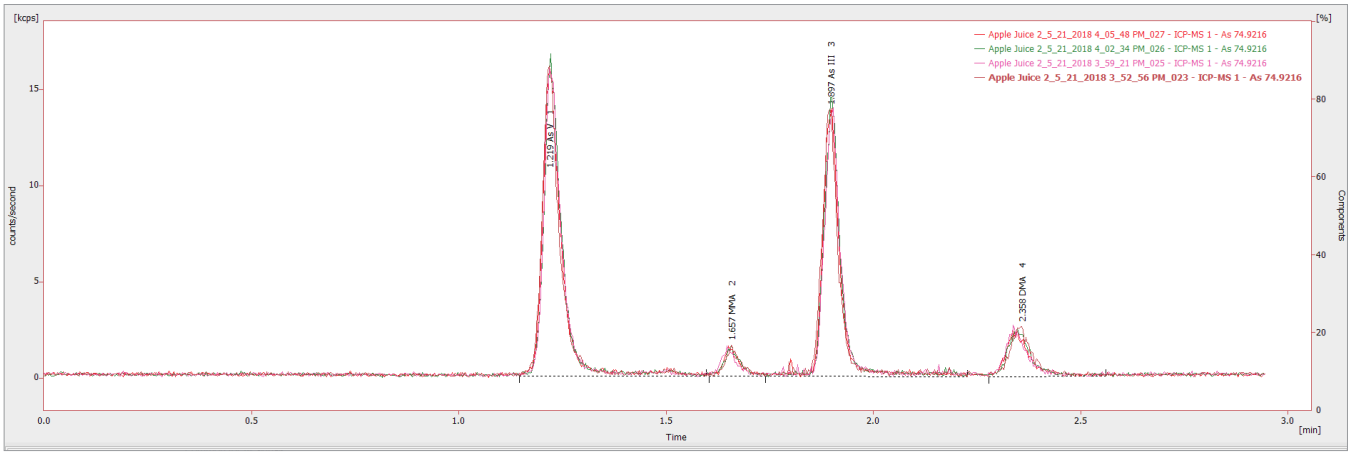

Figure 3. Chromatogram showing four injections (20 µL) of an undiluted apple juice sample from different sample vials.

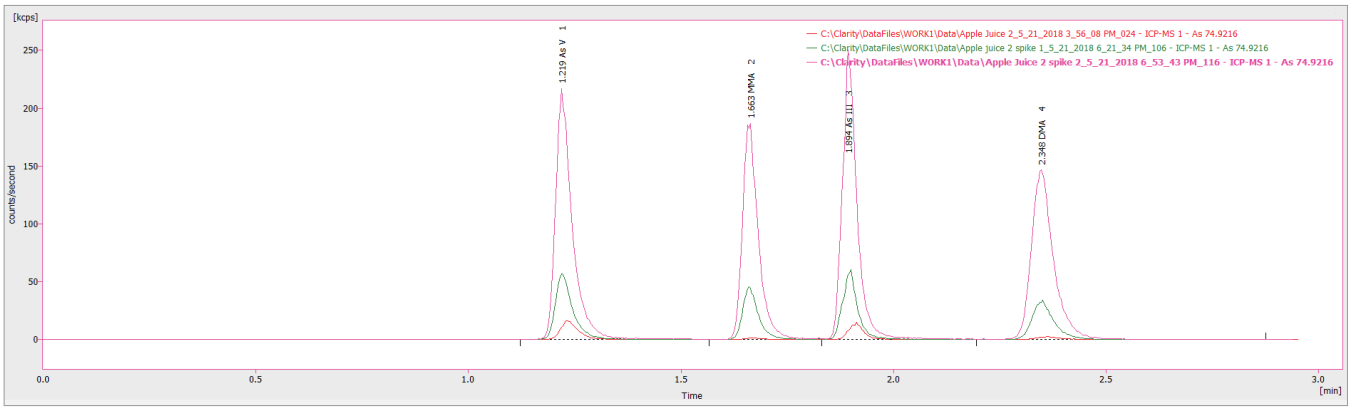

Figure 4. Chromatogram showing an undiluted apple juice sample as well as low-end (2 µg/L) and high-end (10 µg/L) spikes of this sample.

Figure 5 shows the results from the samples collected. As found in previous studies, the dominant forms of arsenic are the inorganic forms As III and As V, where DMA was the third most abundant and MMA was only present in a few samples.[6,7] It is clear that each of the apple juices have different concentrations of the various chemical species of arsenic, however all samples ranged from 16-34% of the action level of inorganic arsenic[5] and therefore is below the toxicological concern threshold as set by the FDA.[4]

Conclusion

This study has shown that the use of the reversed-phase ion-pairing method allows for the complete separation and accurate quantification of the main arsenic species in commercially available apple juices, including the primary toxic forms (As III and As V), in under three minutes. This work was performed using a NexSAR HPLC-ICP-MS Speciation Analysis Ready Solution, where concentrations were in the µg/L and ng/L range. The concentrations of inorganic As in all the apple juice samples analyzed in this study were under the FDA recommended action limit of 10 µg/L.[4]

The strength of the proposed methodology and reliability and robustness of the hardware were demonstrated through repeatability, carryover, and spike recovery studies. Moreover, the inert fluid path of the NexSAR Pump and Autosampler, and the post-seal wash of the pump, ensure peace of mind that samples can be run routinely at a low pH (pH 4.0 in this study), and with salted buffers, without being at risk for corrosion and significant seal damage.

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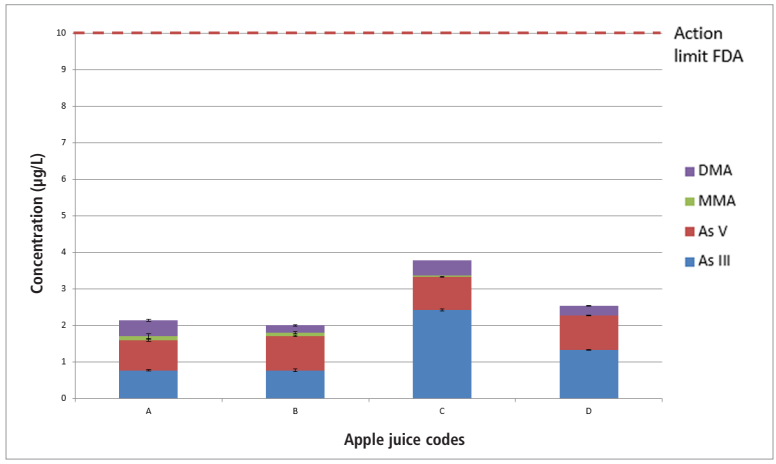

Figure 5. Averaged concentrations of As III, As V, MMA, and DMA in four commercially produced apple juices compared to the action limit of 10 µg/L, where the error bar shows the standard deviation (SD) across replicate analyses.

Consumables Used

Component	Description	Part Number
Reversed Phase Column	4.6 mm I.D x 250 mm, 5 µm	N8145326
HPLC Vials	HPLC Tested Plastic Vials, 1.5 mL PP	N9301736
PEEK Tubing	Yellow, 0.007" ID, 1/16" OD (5 feet)	N9302678
PEEK Fittings	Fingertight for 1/16" OD PEEK Tubing	09920513
Nebulizer Connector	Column-to-Glass Concentric Nebulizer Connector	N8152484

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