HUMAN HEALTH

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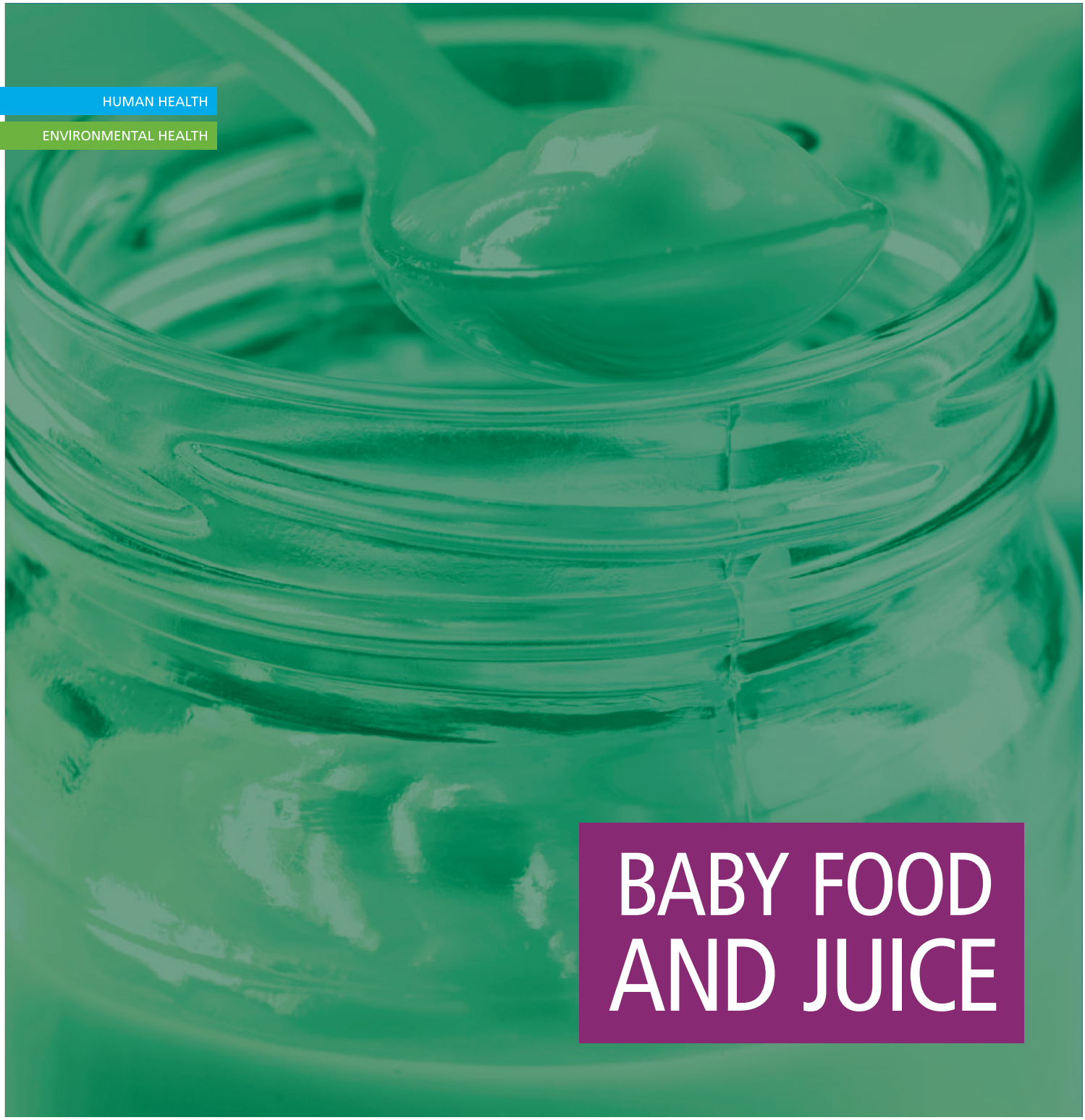

BABY FOOD AND JUICE

Baby Food and Juice Compendium

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BABY FOOD AND JUICE

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APPLICATION NOTE

Food Safety

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The Analysis of Baby Foods and Juices for Metals to Protect a Sensitive Population

Introduction

Babies and small children are an especially sensitive population to exposure to environmental contaminants. Their small mass and developing systems, including brain development may show adverse health effects from even low levels of contamination on a chronic or single dose case. Foods, infant formula, milk, and water provide significant exposure routes for metal contaminants. The effect of lead exposure at low levels has been well established and levels below toxic have been shown to contribute to behavioral and learning issues[1]. Other elements, such as arsenic and cadmium can contribute to cancer, neurological effects and diseases.

Although baby food and juice may contain metals from fruit grown in contaminated soil or introduced during the manufacturing process, there is very little guidance on levels considered to be safe. In the US, the Food and Drug Administration (FDA) has issued a guidance memo on lead in children's candy lollipops for import, suggesting that lead content above 0.1 ppm (mg/kg) would provide an unacceptable risk to children who regularly consume sweets.[2] This is extrapolated to guidance for many food items. Canada has a specific tolerance level of 0.1 mg/kg (ppm) for arsenic and of 0.2 ppm for lead in ready-to-serve fruit juices, nectars, and beverages[3]. Europe has examined the problem more carefully and several elements are regulated in a variety of foodstuffs through Commission Regulation (EC) No 1881/2006.[4] Lead and cadmium are regulated at low levels in materials such as cereals, fruit, and vegetables, although not specifically baby food. Tin is regulated at 50 ppm specifically in canned baby food and infant formula.

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This work will describe measurements of a variety of toxic metals at low concentrations in fruit juices and fruit purees. Sample preparation and the effect on detection limits will be described. Graphite furnace atomic absorption (GFAA) and inductively coupled plasma mass spectrometry (ICP-MS) will be compared and an overall approach to analysis described.

Experimental

Samples of a variety of fruit juices and fruit purees of different brands were purchased from a local supermarket. Two milliliters of juice or one gram of puree were taken in duplicate for digestion. Microwave digestion was used (Multiwave™ 3000, PerkinElmer®, Inc. Shelton, CT USA) to obtain clear solutions. Six mL of nitric and 0.5 mL of hydrochloric acid (GFS Chemical™, Columbus, OH USA) were added to PTFE vessels and the digestion program shown in Table 1 applied. The digestate solutions were then transferred and diluted to 25 mL with ASTM Type I water. The samples were fairly homogeneous and in a form that allowed a representative sample to be easily taken. If the samples were solids, grinding, blending or other procedures might be necessary to ensure a more homogeneous sample to be measured. Preparing replicate samples will allow us to evaluate if our homogeneity assumption is accurate.

Minimal dilution of the sample was chosen to provide a more concentrated digestate solution so that a lower concentration can be measured in the sample. In this case dilution factors of 12.5 and 25 were used for juice and puree samples respectively. Dilution factors can be increased when a lower acid concentration in the extract is desired or a higher concentration is expected in the sample.

Table 1. Microwave Digestion Program.

Step	Power	Ramp	Hold	Fan
1	750	10:00	10:00	1
2	1200	10:00	10:00	1
3	0		15:00	3

The AAnalyst™ 800 atomic absorption system (PerkinElmer, Inc., Shelton, CT USA) was used for the graphite furnace measurements. The AAnalyst 800 uses a transversely heated stabilized temperature platform system and Zeeman background correction to ensure the minimum influence of matrix interferences possible. The instrumental parameters and graphite furnace heating program are shown in Tables 2 and 3.

Table 2. Atomic Absorption Instrumental Conditions for Arsenic Determinations.

Element	Arsenic (As)
Wavelength	193.7 nm
Slit width	0.7 nm
Signal mode	AA-BG Peak Area, 3 replicates
Read time	5 sec
Delay time	0 sec
BOC	2 sec
Lamp and current	Electrodeless Discharge Lamp, 380 mA
Autosampler Sample volume Matrix modifier volume	AS800 24 µL 6 µL
Matrix modifier	0.018 mg Pd + 0.012 mg $Mg(NO_3)_2$
Calibration	2.5, 5.0, and 10.0 µg/L

Table 3. Graphite Furnace Temperature Program.

Step	Temperature (°C)	Ramp Time (sec)	Hold Time (sec)	Argon Gas (mL/min)
1*	120	1	30	250
2	140	5	15	250
3	1100	10	15	250
4**	1900	0	5	0
5	2450	1	3	250

** = Injection Temperature = 100 °C*
***= Atomization Step*

Additional information on the GFAA methodology can be found in reference 5.

The ELAN® DRC-e ICP-MS (PerkinElmer, Shelton, CT USA) was also used for measurement. The instrumental conditions are shown in Table 4.

Table 4. ICP-MS Instrumental Conditions.

Nebulizer	Quartz Concentric
Spray chamber	Quartz Cyclonic
RF power	1500 W
Integration time	1000 ms (per analyte)
Replicates	3
Reaction gas for arsenic	O_2 = 0.6 mL/min
RPq for arsenic as ^{91}AsO	0.5
Internal standard	^{103}Rh
Calibration	1.0 and 2.0 µg/L

The arsenic analysis employed dynamic reaction cell (DRC) technology to move the arsenic ions using oxygen to mass 91, away from chloride interference at mass 75. Chloride may be in the sample arising from natural chlorine content, salt added to a food material or hydrochloric acid used in sample preparation, and can degrade the detection limit for arsenic. The DRC is an active additional quadrupole in the ion path with a rejection parameter q (RPq) of 0.5 for this work. Detection limits are improved compared to other approaches and the precision in varying matrices is more consistent. An instrument detection limit for arsenic measured in 1000 mg/L NaCl was shown to be 2.3 ng/L, comparing favorably to detection limits of 0.6-1.8 ng/L, measured in 1% nitric acid solution, using this technique.[6]

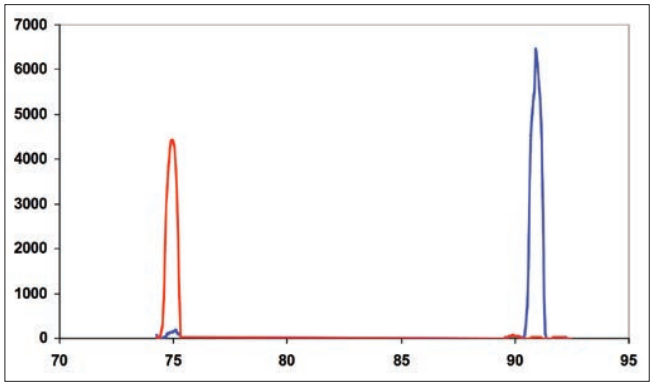

Figure 1. The Dynamic Reaction Cell (DRC™) operates in the PerkinElmer ELAN ICP-MS to react and avoid interferences without allowing competing reactions to add other interferences. Red = As^+ signal at mass 75 with no oxygen in reaction cell. Blue =As^+ signal with oxygen in reaction cell showing formation of AsO^+ at mass 91.

Results and Discussion

A variety of samples from two manufacturers were prepared in duplicate. Table 5 shows the results obtained using graphite furnace atomic absorption (GFAA) analysis for a single element, arsenic. RPD is relative percent difference.

The results show very low levels of arsenic measured in the baby food and juice matrices. The standard deviations are very low showing good agreement between the three replicates measured on each sample. The relative percent differences (RPD) between the duplicate sample preparations show the material is homogeneous. Good agreement is generally considered to be less than 20% RPD, so the measurement in pear puree is likely due to the increase in variation generally seen when measuring close to the detection limit.

The results using ICP-MS for measurement of the same samples is shown in Table 6.

Table 5. GFAA Arsenic Results.

Mean Sample ID	SD (mg/kg)	(mg/kg)	%RSD	% RPD	Recovery
B Pear Juice	0.010	0.001	12	9.9	93.9
G Pear Juice	0.015	0.0006	4.3	3.3	90.0
B Grape Juice	0.027	0.002	8.2	0.7	85.0
B Apple Juice	0.012	0.001	7.8	3.4	92.6
G Apple Juice	0.018	0.0003	1.6	4.7	
B Apple/Cherry Juice	0.010	0.0008	7.5	23	
B Pear Puree	0.005	0.002	35	55	95.7
G Pear Puree	< 0.003				
B Apple Sauce	< 0.003				
HP QC TM-A* (µg/L)	9.995 (µg/L)	0.051 0.51		99.9	

High Purity Water Quality Control Standard = 10 ug/L As. Spike 2.5 ppb

Table 6. ICP-MS Results (mg/kg)*.

	AsO91	Cd111	Sn118	Sb121	Pb Tl205	(sum)
B Pear Juice	0.009	0.002	0.064	0.001	0.001	0.014
G Pear Juice	0.014	0.001	< DL	0.001	0.0009	0.009
B Grape Juice	0.031	0.001	0.005	0.099	0.0004	0.016
B Apple Juice	0.010	0.0003	0.069	0.001	0.0005	0.016
G Apple Juice	0.018	0.0004	< DL	< DL	0.0006	0.018
B Apple/ Cherry Juice	0.008	< DL	0.058	0.0004	0.0006	0.006
B Pear Puree	0.002	0.001	0.33	0.0001	0.001	0.003
G Pear Puree	0.001	0.003	< DL	< DL	0.001	0.003
B Apple Sauce	< DL	< DL	0.067	< DL	0.0004	0.003

** < DL means the result was less than the detection limit, listed in Table 8.*

A variety of elements are measured in the ICP-MS analysis, including toxic elements as well as tin, which might be introduced through packaging. Comparison with the graphite furnace determination for arsenic is compared in the next table.

Table 7. Comparison of Arsenic Measurement by ICP-MS and GFAA (mg/kg).

	ICP-MS	GFAA
B Pear Juice	0.009	0.010
G Pear Juice	0.014	0.015
B Grape Juice	0.031	0.027
B Apple Juice	0.010	0.012
G Apple Juice	0.018	0.018
B Apple/Cherry Juice	0.008	0.010
B Pear Puree	0.002	0.005
G Pear Puree	0.001	< 0.003
B Apple Sauce	< 0.001	< 0.003

Table 8. Detection Limits for ICP-MS and GFAA for this Method (mg/kg).

	ICP-MS	GFAA
As	0.001	0.003
Cd111	0.0002	
Sn118	0.006	
Sb121	0.0008	
Tl205	0.0001	
Pb(sum)	0.003	

Agreement between measurements obtained on the two techniques is excellent at these low concentrations.

Detection limits were estimated for the two methods using the calculation 3 times the standard deviation of the blank, which was carried through the sample preparation procedure.

The detection limits measured are well below the Canadian limits set for As (0.1 mg/kg) and Pb (0.2 mg/kg), ensuring confidence of measurements made near the compliance concentration.

A variety of quality control checks, including spiking, analysis of a reference material and comparison of duplicate sample preparations were done to ensure that the developed ICP-MS method was adequate and under control during measurement of the samples. Table 9 shows agreement between duplicate samples for two juice types and a puree.

The results are shown in mg/kg and show good agreement in most cases. As the results approach the detection limit it is more difficult to show good agreement.

Table 9. Duplicate Agreement ICP-MS (mg/kg).

	Pear-1	Pear-2	RPD	Grape-1	Grape-2	RPD	Pear Puree-1	Pear Puree-2	RPD
AsO91	0.011	0.0085	21%	0.031	0.031	2%	0.0017	0.0015	14%
Cd111	0.0016	0.0016	1%	0.00068	0.00069	1%	0.0014	0.0013	2%
Sn118	0.067	0.061	10%	0.051	0.049	4%	0.35	0.31	10%
Sb121	0.0014	0.00095	41%	99.9	97.7	2%	< DL	< DL	–
Tl205	0.0010	0.0010	1%	0.0004	0.0004	6%	0.0014	0.0013	6%
Pb(sum)	0.016	0.012	27%	0.016	0.015	6%	0.004	< DL	–

Table 10 shows post-digestion spike recoveries at 2 ppb in solution. This represents a spike of 50 ppb in the original juices.

Table 10. ICP-MS % Spike Recoveries.

	AsO91	Cd111	Sn118	Sb121	Tl205	Pb(sum)
B Pear Juice	104	90.3	97.7	83.4	103	103
B Grape Juice	104	84.4	99.6	104	119	108
B Apple Juice	110	83.0	120	119	120	115
G Apple Juice	110	86.4	97.5	81.1	115	110
B Apple/ Cherry Juice	100	83.4	101	80.1	118	113
B Pear Puree	95.8	82.1	106	79.2	102	106
G Pear Puree	95.3	87.1	99.8	81.1	100	101
B Apple Sauce	90.1	83.5	98.7	80.5	101	102

The spikes were recovered within 20% of the spiked value, showing excellent recovery.

A suitable mixed diet food reference material (CRM, High Purity, USA) was measured using the same ICP-MS method. The results are shown in Table 11. Unfortunately the suite of certified metals only included two of the metals of interest here.

Table 11. Mixed Diet Reference Material (mg/kg).

	Concentration	SD	Certified Value	%Recovery
AsO91	0.019	0.0002	0.020	96.7
Cd111	0.0084	0.0001	0.008	105
Sn118	–	–	–	–
Sb121	–	–	–	–
Tl205	–	–	–	–
Pb(sum)	0.0001	0.0000	NV	–

The certified values for arsenic and cadmium showed excellent recovery, further validating the developed methodology was operating properly for this matrix and representative concentrations.

Conclusions

Metals, including arsenic can be hazardous to health, especially that of a sensitive population, such as children. Therefore materials that provide significant exposure, such as food, should be closely monitored to ensure that concentrations of elements that might be hazardous should be very low.

The results measured in this set of samples were very low and did not violate any of the current standards. Additional method quality checks were done to ensure both the GFAA and ICP-MS methods were capable at the concentration levels of interest and under control during sample measurement.

Sample preparation should be matched to the analytical requirements and laboratory workload needs. A variety of techniques can be used, but the measurement of lower concentrations requires that a clean digestion be used so that additional contamination is not introduced during this step in the analysis process. The amount taken for digestion and the dilution can be varied to accommodate sample homogeneity and concentration requirements.

GFAA has detection limit capability well below the level of concern and provides an economical choice for smaller laboratories or those with a smaller workload. ICP-MS provides excellent detection limits and offers efficient multielement capability for the detection of other hazardous elements in the same run, such as lead. As many countries move toward the regulation of additional elements in food materials the list of elements to be measured may increase.

Based on the requirements of the laboratory, an appropriate technique can be chosen for regulations of today and to meet lower limits or additional elements that may be required in the future

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APPLICATION NOTE

Atomic Absorption

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Determination of Arsenic in Baby Foods and Fruit Juices by GFAAS

Introduction

The United States does not have specific regulations specifying the allowable levels of toxic elements in foods, but many other countries do. For example, Canada has a specific tolerance level for arsenic of 0.1 ppm in ready to serve fruit juices, nectars, and beverages[1]. The toxic nature of arsenic is such that chronic exposure to the element can lead to internal cancers of the bladder and kidney, skin cancer, neurological effects, and cardiovascular disease.

Arsenic can find its way into food through a variety of paths. In the recent past, various organic arsenicals were used as herbicides and antimicrobial agents in growth fields as well as applied directly on fruits and fruit trees. Prior to 2003, arsenic was commonly used as a wood preservative. Sawing and/or sanding of this wood would yield arsenic contaminated sawdust. In some areas, arsenic is naturally found in rock formations and can enter soil and water which is used in the growth of food products. Foods can also be contaminated during manufacturing, processing, packaging and transport processes.

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There are a few specific analytical challenges that an analyst must consider in the determination of arsenic in foods by GFAAS. Toxic elements, such as arsenic, which may be present in foods are biologically important at very low concentrations. The U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry (ATSDR) defines a minimal risk level for chronic inorganic arsenic exposure to be 0.0003 mg As/kg/day. For a 45 lb. child drinking a liter of fruit juice a day, the minimal risk level for that juice would then be about 6 µg/L. Therefore, the analytical technique employed for this application must have the capability to accurately measure arsenic in sample digestates at the sub-ppb concentration level.

A complete method has been developed for the determination of arsenic (As) in baby foods and baby fruit juices by Graphite Furnace Atomic Absorption Spectroscopy (GFAAS). This method includes sample preparation steps using microwave assisted closed vessel digestion. Foods come in a wide variety of complex sample types and matrices, but their fundamental major components are water and various carbohydrates. In this work, the samples were totally digested in a microwave oven so that the samples' various carbohydrate matrices were completely destroyed prior to instrumental analysis. Microwave digestion has several analytical advantages for this type of analysis. Because the sample is placed in a sealed PTFE polymer digestion vessel, contamination is minimized and there is no loss of volatile elements during the digestion procedure. In a sealed vessel, higher temperatures of digestion are reached thereby quickly yielding complete matrix decomposition. With the microwave system used here, each sample's digestion process is thoroughly documented as to time, pressure and temperature. This gives an analytically repeatable and transferable digestion process.

Figure 1. Examples of samples used in this work.

Experimental

A Multiwave™ 3000 Microwave Oven (PerkinElmer®, Shelton, CT USA) was used for the microwave-assisted digestion. This is an industrial-type oven which can be equipped with various accessories to optimize the sample digestion. In this case, the foods were digested in the Rotor 8XF100 which is a rotor with 8 high pressure vessels made of PTFE-TFM and surrounded by a ceramic jacket. TFM is chemically modified PTFE that has enhanced mechanical properties at high temperatures compared to conventional PTFE. This vessel has a "working" pressure of 60 bar (580 psi) and can operate at temperatures up to 260 ˚C with an internal volume of 100 mL. All vessels' temperatures were monitored with the IR Temperature Sensor Accessory. This device gives thermal protection to the reactions in all of the vessels by measuring the temperature remotely on the bottom surface of each vessel liner during the digestion process. Pressure is continuously monitored in all vessels using load-cell technology in the upper rotor plate.

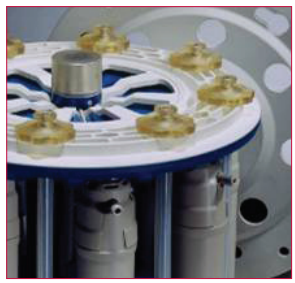

Figure 2. The Multiwave Rotor.

Samples of fruit juices and solid fruit purees were weighed directly into the PTFE-TFM digestion vessel liners (Figure 2). Sample weights were approximately 2 grams for the liquid juices and 1 gram for the fruit purees. To each sample, 6 mL of concentrated nitric acid and 0.5 mL of concentrated hydrochloric acid were added. A pre-digestion spike of arsenic was added to some of the samples to measure analyte recovery through the digestion process. Some vessels contained only the acids with no sample to act as analytical reagent blanks. The vessels were sealed and placed into the rotor for the microwave digestion. The acids used were high purity GFS Chemical™ (Columbus, OH, USA) which are packaged in PTFE containers. After the digestion process, the digestates were transferred to polypropylene 50-mL autosampler vials (PerkinElmer part number B0193234) and laboratory ASTM type I water was added to a final total weight of 25 grams.

Table 1. Microwave Digestion Program.

Step	Power (Watts)	Ramp (min)	Hold (min)	Fan Speed
1	750	10	10	1
2	1200	10	10	1
3	0 (cool-down)	0	15	3

Table 1 shows the power/time program used for the sample digestions. To ensure a safe digestion, the Multiwave 3000's IR sensor measures the temperature of each vessel. If a vessel nears its maximum operating temperature of 260 °C, then the Multiwave oven will automatically decrease the applied power. Also, the pressure sensor sends data to the Multiwave oven controller during the digestion. The Multiwave oven will automatically reduce power if the maximum pressure of 60 bar is approached.

An AAnalyst™ 800 Atomic Absorption Spectrometer (PerkinElmer) was used for the GFAAS measurements of arsenic in the digested samples. The AAnalyst 800 features longitudinal Zeeman-effect background correction[2] and a solid-state detector which is highly efficient at low wavelengths (arsenics's primary AA wavelength is 193.7 nm). The AAnalyst 800 uses a transversely heated graphite atomizer (THGA) which provides uniform temperature distribution across the entire length of the graphite tube. The THGA features an integrated L'vov platform[3] which is useful in overcoming potential chemical interference effects common to the GFAAS technique.

For instrument calibration, a 10 µg/L As standard was prepared from serial dilutions of a 1000 mg/L stock standard (PE Pure, PerkinElmer Part Number N9300102). The AAnalyst 800 autosampler then prepared a calibration curve of 2.5, 5.0 and 10.0 µg/L from that 10 µg/L arsenic standard. A QC standard was also measured by this method, High Purity Standards TM-A, (Charleston, SC 29423) and is certified to be 10 µg/L arsenic. A mixed matrix modifier of palladium and magnesium nitrate was prepared by diluting and combining individual stock matrix modifier solutions. The mixed modifier solution is prepared by combining 5 mL of the stock palladium modifier (1% solution, PerkinElmer Part Number B0190635) and 0.5 mL of the magnesium nitrate stock modifier (PerkinElmer Part Number B0190634) and diluting to 50 mL with ASTM Type I water. Other instrumental parameters are given in Tables 2 and 3.

A typical calibration curve is shown in Figure 3 and calibration standard profiles are shown in Figure 4. The curve has good linearity and the sensitivity is good at low concentrations.

Table 2. AAnalyst 800 Instrumental Parameters.

Wavelength (nm)	193.7
Source Lamp (mA)	EDL 380
Slit Width (nm)	0.7
Background Correction	Zeeman-effect
Measurement Mode	Peak Area, 3 replicates
Calibration Algorithm	Linear thru Zero
Integration Time	5.0
Sample Volume	24
Matrix Modifier Volume	6

Table 3. THGA Heating Program.

Step (°C)	Temperature (sec)	Ramp Time (sec)	Hold Time (mL/min)	Argon Gas
1*	120	1	30	250
2	140	5	15	250
3	1100	10	15	250
4**	1900	0	5	0
5	2450	1	3	250

* = Injection Temperature = 100 °C.
**= Atomization Step

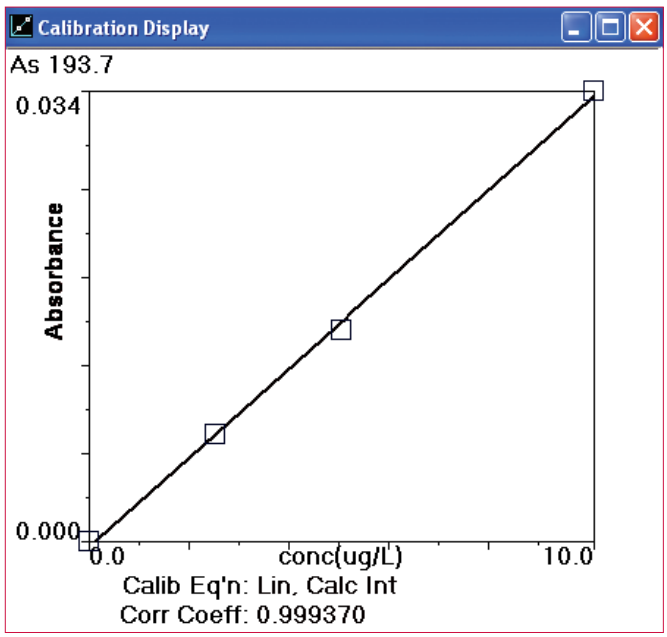

Figure 3. Arsenic calibration curve.

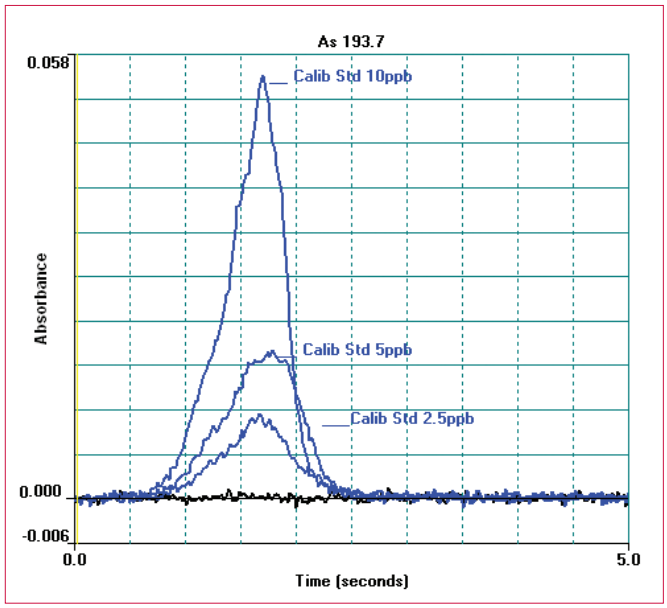

Figure 4. Arsenic atomic profile signals for calibration standards and blank.

Results

The Multiwave 3000 Digestion System with the rotor-8 produced clear, fully digested, sample solutions. No filtration was necessary. The AAnalyst 800 gave a characteristic mass (M_o) of 36 pg for arsenic with these conditions which is in good agreement with the manufacturers recommended Mo value of 40 pg. Nine different samples of baby juice and puree foods were analyzed by this method. The fruit juices and one of the puree samples were prepared in duplicate to check the entire method's reproducibility. These samples were also "spiked" prior to digestion with the equivalent in the undiluted sample of approximately 240 ng/g arsenic. The percent recovery of this spike will be used to check for any losses of arsenic during the digestion and to check for the presence of any matrix interferences. All of those data are given in Table 4.

Table 4. Results for the Analyses of Baby Foods by GFAAS.

Sample ID (ng/g)	Mean (ng/g)	SD	%RSD	% DIFF of Spike*	%Recovery
B_Pear Juice	10.2	1.2	12	9.9	93.9
G_Pear Juice	15.1	0.65	4.3	3.3	90.0
B_Grape Juice	27.4	2.2	8.2	0.70	85.0
B_Apple Juice	12.4	0.96	7.8	3.4	92.6
G_Apple Juice	18.2	0.29	1.6	4.7	
B_Cherry Juice	10.3	0.77	7.5	23	
B_Pear Puree	5.00	2.0	35	55	95.7
G_Pear Puree	<3				
B_Apple Sauce	<3				
HP QC TM-A (µg/L)	10.0 (µg/L)	0.051	0.51		99.9

*Predigestion spike of 5 µg/L in the final solution or analysis

Table 4 shows the mean of the three replicate measurements for the food sample corrected for weight used and final volume, the standard deviation of those measurements (SD), and the relative standard deviation of the three replicates (%RSD). Also, the first seven samples shown were digested in duplicate. The difference between the two are shown in the column labeled % Diff. The relatively high percent differences in the pear puree samples is due to the fact that the concentration of arsenic is very low in this sample, near the method detection limit for the puree of 3 ng/g.

For samples that were split and spiked with arsenic prior to digestion, that measure of the spike recovery is shown as the percentage of the recovery in the last column of Table 4. A recovery value of near 100% shows that there is little or no loss of analyte during the digestion process and that there are no unresolved matrix interferences with the analytical method.

Conclusion

It has been shown that this method can be successfully applied to the determination of arsenic in these types of foods. The Multiwave 3000 Digestion System gave completely digested, clear samples with no loss of arsenic during the high temperature, high pressure process. The AAnalyst 800 with longitudinal Zeeman-effect background correction and THGA tube containing the L'vov platform, gave good spike recoveries with no matrix interference. The detection limit estimated to be 3 ng/g was well below the Canadian limit of 100 ng/g in the original juice or puree and offers room for lower regulatory limits that may be established in the future to also be satisfactorily measured.

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