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Sample Preparation, Extraction Efficiency, and Determination of Six Arsenic Species Present in Food Composites

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Several sample preparation techniques were investigated to maximize the efficiency of arsenic species extraction from food composites. The optimized method includes lyophilization of food followed by prewashing with acetone and extraction by sonication with 50/50 methanol/water. Six arsenic species were separated and quantitated using an ammonium carbonate buffer system by ion exchange chromatography coupled to inductively coupled plasma mass spectrometry. The performance of the method for speciated arsenic components was evaluated using a matrix containing high fat food composite fortified with arsenic species. A certified reference material, dogfish muscle, was used to evaluate extraction methods for total arsenic content in food composites. More than 200 food composite samples were analyzed during an 18 month period, demonstrating the reliability of the analytical method over a long time period.

KEYWORDS: Food; extraction; ion chromatography; liquid chromatography; plasma mass spectrometry; arsenic speciation; IC-ICP-MS

INTRODUCTION

Because of the varying degrees of toxicity of arsenic species, it has become necessary to develop adequate speciation techniques. Arsenic is ubiquitously distributed throughout the environment (air, water, and soil) as a result of its use in industry, agriculture and wood preservation, and mining operations (*1*−*3*). The species of concern are the inorganic forms: arsenite (AsIII) and arsenate (AsV) and their metabolites monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). The primary source of the toxic and often carcinogenic (*4*−*6*) inorganic forms is drinking water (*7*). The most prevalent arsenic species in dietary origin (mainly marine foods) are the organic forms (about 80−95%), which are nontoxic: arsenocholine (AsC), arsenobetaine (AsB), arsenosugars, and arsenolipids (*8*, *9*, *10*−*12*). It has been shown that a significant consumption of seafoods contributed to an increase in urinary arsenic within 10 h of ingestion (*1*, *13*).

The three stages of speciation analysis include sample extraction, chromatographic separation of species, and quantitation at trace levels (usually parts per billion). Sample preparation techniques must include an extraction method for which the quantitative extraction of arsenic species is achieved while the integrity of the individual species is maintained. Enzymatic digestion of solid tissues using trypsin for extraction of arsenic species has been previously performed on solid food samples, but the enzyme led to degradation of AsB and DMA (*14*, *15*). Combinations of organic solvents (chloroform, methanol, and ethanol) and water have been used with centrifugation (*16*), microwave heating (*17*), or accelerated solvent extraction (*18*). Clearly, from the variety of extraction procedures and solvents used, sample pretreatment must be studied carefully for each particular dietary source. Arsenic compounds have typically been separated using high-performance liquid chromatography (HPLC) in the form of ion pairing chromatography or ion exchange chromatography (IC) (*19*−*21*). These separation methods do not require the species to be derivatized as would be necessary for gas chromatographic analysis. Moreover, the liquid sample flows are compatible with the sample introduction flow rates of sensitive, multielement detection methods such as inductively coupled plasma mass spectrometry (ICP-MS).

In this study, various extraction schemes were evaluated until a final method was developed for a number of composite food samples. The samples were then subjected to IC-ICP-MS using ammonium carbonate buffer, which was previously used by this laboratory to successfully speciate six arsenic compounds: AsB, AsIII, AsV, AsC, MMA, and DMA in human urine samples (*22*).

MATERIALS AND METHODS

Preparation of Composite Foods (LCU, LCS, HCU, and HCT). The goal was to select foods that would contain a variety of food

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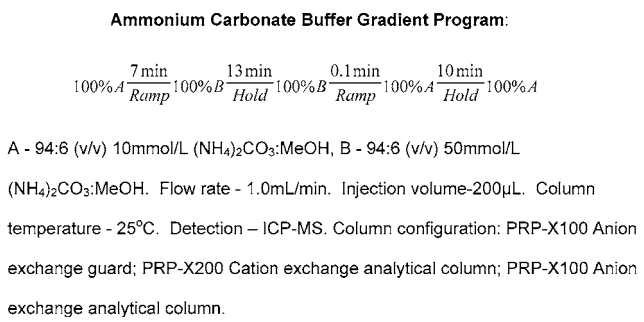

Figure 1. Chromatographic gradient used for speciation of six arsenic compounds.

constituents (fat, protein, fiber, etc.), as well as different species of arsenic to simulate the unknown composite foods for which the method would be applied. It was conjectured that composite foods containing different levels of fat would be the most challenging matrix. A low fat composite (containing 6% calories from fat) was prepared using foods such as wheat bread, vegetables, and fat free cheese. A high fat composite (containing 46% calories from fat) was prepared using foods such as potato chips, all butter pound cake, and pizza. To determine which foods contain the highest arsenic concentrations, the U.S. EPA Dietary Exposure Potential Model (DEPM) database was consulted. Shellfish and tuna are among the foods that contained the highest levels of arsenic. Therefore, the low fat composite was fortified with shrimp and the high fat composite was fortified with tuna, to provide the appropriate matrices for method development and optimization.

The high fat composite food fortified with tuna (HCT) and low fat composite food fortified with shrimp (LCS) and the high and low fat composite food unfortified (HCU and LCU) were prepared using a Robot Coupe RSI 6V food homogenizer (Robot Coupe USA, Ridgeland, MS).

Each of the four composite foods, prepared for method development purposes, was characterized for its As content and homogeneity by measurement of total and speciated arsenic. The total concentration of As in these food composites was determined prior to conducting the extraction optimization experiments. The measured concentrations in HCT, LCS, HCU, and LCU as determined by ICP-MS were 130 ± 2.56, 137 ± 3.88, 13.2 ± 1.97, and 13.8 ± 1.04 ng/g, respectively.

Reagents. The ammonium carbonate buffer (10 and 50 mmol/L) was prepared by dissolving ammonium carbonate (J. T. Baker, Phillipsburg, NJ) in deionized water (Hydro Services, RTP, NC). HPLC grade methanol (Fisher Scientific, Fairlawn, NJ) was also used with the ammonium carbonate mobile phase (6% (v/v) of total mobile phase). All mobile phases were filtered through 0.45 μm filters (Alltech, Deerfield, IL) prior to use. The same chromatographic conditions that were used for urine speciation were employed (**Figure 1**).

The four arsenic standards that were purchased include sodium arsenate (Pfaltz & Bauer Inc, Waterbury, CT) (AsV), arsenious oxide (99.999%, GFS Chemical Inc., Powell, OH) (AsIII), monosodium acid methane arsonate (97% purity, Chem Service, West Chester, PA) (MMA), and cacodylic acid (99%, Pfaltz & Bauer, Inc.) (DMA). Professor William Cullen of the University of British Columbia (Vancouver, Canada) synthesized AsB and AsC. Potassium hexahydroxy antimonate(V) (Aldrich, Milwaukee, WI) and para aminobenzene arsanilic acid (Sigma, St. Louis, MO) were investigated for use as internal standards. The dogfish muscle certified reference material (DORM-2) was obtained from the National Research Council Canada.

Samples. The samples were collected from 300 homes from different individuals as part of the National Human Exposure Assessment Survey (NHEXAS) study and children's study, conducted in EPA region V from July 1995 to May 1997 (*23*−*25*). Region V consists of the Great Lakes area (Minnesota, Wisconsin, Illinois, Indiana, Ohio, and Michigan) in which the demographic characteristics of the population (e.g., races, ethnic groups, and socioeconomic distribution) are similar to the national profile (*25*). The samples were collected in 50 mL polypropylene tubes and stored at −20 °C.

Table 1. ICP-MS Operating Conditions

forward power (W)	1348 ± 2
reflective power (W)	2 ± 2
coolant gas flow (L/min)	16
auxiliary gas flow (L/min)	1.4
nebulizer gas flow (L/min)	0.7
nebulizer	concentric
spray chamber	Scott double pass water-cooled (5 °C)
sampler/skimmer cones	platinum

Instrumentation. A Waters 600S controller (Milford, MA) was used to operate a 626 metal free HPLC gradient pump as well as a 717 plus autosampler for the ion exchange separations. The columns used were Hamilton PRP-X100 (10 μm, 4.1 mm × 250 mm i.d.) and PRP-X200 (10 μm, 4.6 × 150 mm i.d.) (Reno, NV) and were connected in series with PEEK tubing.

Periodic regeneration of the columns individually was necessary (after every 75−100 samples) to ensure reproducible performance of the separation. The cation exchange column was backflushed with 0.1 mol/L nitric acid for approximately 1 h followed by type I deionized water. The anion exchange column was backflushed with 0.1 mol/L sodium hydroxide (Fisher Scientific) for 1 h followed by deionized water.

A VG Elemental PQ-XR ICP-MS instrument (Winsford, U.K.) equipped with a concentric nebulizer and a water-cooled, double-pass spray chamber (CPI, Santa Rosa, CA) was used in this study. Time-resolved data acquisition software was used to simultaneously monitor arsenic species at *m*/*z* 75 and ArCl interference at *m*/*z* 77 as well as any elements used for internal standards (i.e., antimony: *m*/*z* 121). The optimized ICP-MS conditions for the speciation are presented in **Table 1**. The total arsenic content of method performance samples and NHEXAS study samples was measured by ICP-MS.

Analytical Method. After evaluation of the performance (linearity, detection limits, precision, and accuracy) of the IC-ICP-MS method for speciation of six arsenic species in food, the method was applied to the analysis of food composite samples. On each day of sample analysis, the chromatographic system was equilibrated with the mobile phase and 50/50 methanol/water was injected to establish the background. The instrument was then calibrated with four standards prepared in 50/50 methanol/water (0.5, 1.0, 5.0, and 30 ng/mL) prior to any sample analysis. The samples were analyzed in batches, and each batch consisted of 10−15 samples. A duplicate injection of the same aliquot of a sample or a duplicate preparation of the same sample was included with each batch of samples to evaluate the precision of the sample preparation and analysis procedures. At the end of the day, a low calibration standard was reanalyzed to verify the instrument performance.

RESULTS AND DISCUSSION

Optimization of an Extraction Scheme. It was necessary to develop an extraction scheme that results in good extraction efficiency as well as preserves the integrity of the individual arsenic species. Three solvent systems were investigated as follows: (i) methanol−water (MeOH−H_2O), (ii) methanol−chloroform−water (MeOH−$CHCl_3$−H_2O), and (iii) enzymatic (trypsin−ammonium carbonate), for their eventual use in extracting arsenic species from food composites. Each extraction medium was fortified with six arsenic species (served as method controls) and carried through the entire sample preparation process to assess the stability of the species in the extraction media (i.e., to see if the arsenic species are stable or if they interconvert). The arsenic species in methanol−water and methanol−chloroform−water were intact whereas for trypsin−ammonium carbonate, most of AsIII was converted to AsV.

Overall, the 50:50 (v/v) MeOH−H_2O extraction method has shown better extraction efficiency for the six arsenic species

Table 2. Comparison of Extraction Methods: [MeOH–$CHCl_3$–H_2O and MeOH–H_2O] Percent Recovery of Arsenic Species

sample ID[a]	% recovery[b] AsB	AsC	As(III)	DMA	MMA	As(V)	average % recovery[c]
MeOH–$CHCl_3$–H_2O (20:30:20)							
method control-1[d]	79	63	81	94	88	72	79 ± 11
method control-2[d]	74	94	67	71	53	62	70 ± 10
method control-3[d]	109	84	67	107	113	152	105 ± 29
DORM2-1	71			2			73
DORM2-2	120			1			121
spiked HCU[d]	64	27	84	87	71	50	64 ± 23
spiked LCU-1[d]	83	47	53	59	40	41	54 ± 16
spiked LCU-2[d]	80	44	54	59	41	46	54 ± 14
MeOH–H_2O (50:50)							
method control-1[d]	135		152	104	113	97	100 ± 53
method control-2[d]	71	95	72	72	49	80	73 ± 15
method control-3[d]	105	87	95	114	116	136	109 ± 17
DORM2-1	80			2			82
DORM2-2	64			1			65
spiked HCU[d]	159		150	98	107	100	102 ± 57
spiked LCU-1[d]	148	3	76	76	59	71	72 ± 46
spiked LCU-2[d]	80	141	70	70	61	63	81 ± 30

[a] LCU, low fat composite food unfortified; HCU, high fat composite food unfortified. [b] Measured concentration in the aqueous (MeOH–H_2O or MeOH–$CHCl_3$–H_2O) phase as percentage of total arsenic. [c] Average recovery based on the expected total arsenic. [d] Spiked with 250 μg/kg of each arsenic species.

Table 3. Effect of Solvent Composition and pH on Extraction Efficiency of Low Fat Composite Food Fortified with Shrimp (LCS)

solvent composition MeOH:water	% extracted in aqueous phase	% in residue	mass balance (%)
20:80	58 ± 2	21 ± 2	79 ± 1
50:50	64 ± 4	26 ± 2	90 ± 6
80:20	66 ± 2	15 ± 4	82 ± 7
50:50 (buffered)	61 ± 2	21 ± 1	82 ± 3
carbonate buffer	59 ± 2	20 ± 1	79 ± 2

present in food composites than 20:30:20 (v/v) MeOH−$CHCl_3$−H_2O (**Table 2**), particularly for the spiked HCUs and LCUs. The AsC data were not very reproducible because of chromatographic conditions used for these preliminary experiments. The asymmetrical peak shape for AsC made quantification very difficult.

Further studies were performed, however, to evaluate the overall extraction efficiencies by varying the compositions of methanol and water as well as the pH. Three different combinations were investigated as follows: 20:80 (v/v), 50:50 (v/v), and 80:20 (v/v) MeOH/water. Two additional solvent compositions, 50:50 (v/v) methanol/water adjusted to pH 7 and a carbonate buffer (5 mM Na_2CO_3(aq) with 6% (v/v) MeOH, rebuffered to pH 7 after addition to the food sample with 5% NaOH(aq)) were also examined. As shown in **Table 3**, varying the solvent composition and pH did not result in a significant improvement in extraction efficiency; however, 50:50 (v/v) MeOH:H_2O showed a somewhat higher mass balance and was selected as the solvent extraction medium for further studies.

To further increase the extraction efficiency, a lyophilization step was employed prior to extraction to break down the food into fine particles and to increase the surface area during extraction. A prewashing step with an organic solvent prior to chromatography was performed in order to remove unwanted fatty material from the composites. After the investigation of several organic solvents (hexane, methyl *tert*-butyl ether, and acetone), acetone was selected because it resulted in minimum arsenic loss (≤1.3% total arsenic as measured in LCS) during the prewashing of samples. The prewashing of the sample resulted in a cleaner extract that favorably contributed to long column life and less frequent column regeneration.

Table 4. Extraction Efficiency of Food Composite Samples Using the Final Extraction Method

sample	overall % recovery
LCS-1 ($n = 3$)	60 ± 7
LCS-2 ($n = 3$)	69 ± 5
shrimp ($n = 2$)	102 ± 0
HCT-1 ($n = 3$)	84 ± 2
HCT-2 ($n = 3$)	89 ± 3
DORM-2 ($n = 2$)	96 ± 1

Final Extraction Method. The samples were homogenized prior to taking an analytical aliquot (2 g) for analysis. The samples were then lyophilized and prewashed with acetone three times (10 mL each). The food residues were allowed to air-dry in a Class 100 hood until the solvent had evaporated. The samples were then extracted with 10 mL of 50/50 MeOH−H_2O (using an ultrasonic bath) for 30 min. The extraction step was repeated twice with a fresh solvent each time, and the supernatant (~30 mL total) was combined in a clean 50 mL centrifuge tube. The extract was filtered using a 0.2 μm filter (Whatman, Clifton, NJ), and 200 μL was injected onto the column.

Table 4 shows the results of replicate trials of LCS and HCT and shrimp subjected to the final extraction procedure. The low arsenic recovery obtained for LCS in contrast to the high recovery obtained for the shrimp alone is most likely due to the low fat composite matrix. The overall percent recovery for DORM-2 was improved in comparison to the preliminary extraction results presented in **Table 2** (96% vs 82 and 65% overall percent recovery).

Development of a Suitable Speciation Method. The current reported method for determination of six arsenic species in food composites using an ammonium carbonate buffer resulted in acceptable performance and significant improvement over the existing methods reported in the literature (*6*−*14*). The method uses cationic and anionic columns in series for complete baseline separation of six arsenic species in a single chromatographic run, in fewer than 30 min. The method is sufficiently rugged to allow a routine analysis of a large number of samples over a long period.

The ammonium carbonate mobile phase buffer system that was used in this laboratory for the speciation of arsenic in human urine, in which the cationic column and the anionic column were used in series, was applied in this study for determination of arsenic species in food composites. This buffer system had been previously advantageous because urine samples had been successfully analyzed without heavy salt deposits on the sampling cones of the mass spectrometer. The internal standard (potassium hexahydroxy antimonate(V)), previously used for urine speciation, was found to be unstable for the chromatographic analysis of the food composites. The peak was broad with low intensity. Another compound containing arsenic (para aminobenzene arsanilic acid) was evaluated (10 ng/mL as arsenic) for use as an internal standard along with the six arsenic species of interest. The sensitivity of the peak was adequate, but the compound was not well-resolved from MMA. Because the speciation method demonstrated good performance even in the absence of an internal standard, an internal standard was not used.

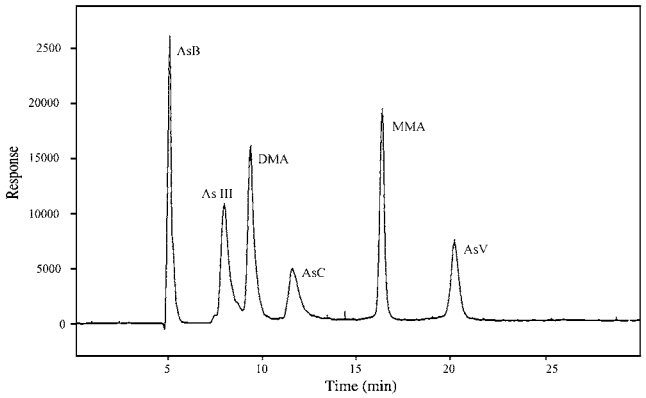

Figure 2. Chromatogram of 5 ppb calibration standard prepared in HCU matrix. Chromatographic condition: mobile phase, ammonium carbonate buffer at pH 9.

Table 5. Detection Limits and Quantitation Limits

arsenic species	MDL (ng/mL)	MQL (ng/mL)	arsenic species	MDL (ng/mL)	MQL (ng/mL)
AsB	0.07	0.2	AsC	0.1	0.4
AsIII	0.08	0.3	MMA	0.04	0.1
DMA	0.04	0.1	AsV	0.2	0.8

Table 6. Analysis Results for Short-Term and Long-Term Precision

arsenic species	short-term (4 h) precision (% RSD) (ng/mL)			long-term (3 day) precision (% RSD) (ng/mL)		
	0.50	2.00	5.00	0.50	2.00	5.00
AsB	4	3	5	2	13	10
AsIII	5	5	4	1	4	3
DMA	3	5	3	0.3	2	2
AsC	7	14	8	0.6	4	3
MMA	3	5	5	0.9	5	4
AsV	14	11	4	1	6	5

Method Performance Evaluation. Determining the linearity, detection limits, precision, and accuracy for each of the six species assessed the analytical performance of the speciation method. The HCU was selected as the "matrix" for the method evaluation and was spiked with all six arsenic species. Good linearity was demonstrated for the six species (AsB, AsIII, DMA, AsC, MMA, and AsV), with the correlation coefficients 0.9990, 0.9985, 0.9990, 0.9985, 0.9976, and 0.9983, respectively. Calibration matrix standards ranging from 0 to 100 ng/mL for all six arsenic species were used to assess linearity. A sample chromatogram of a 5.0 ng As/mL standard is shown in **Figure 2**. Detection and quantitation limits are shown in **Table 5**. The method detection limit (MDL) was calculated as 3 × standard deviation (SD) of three replicate analyses of a food extract spiked at 0.50 ng/mL for each of the six species. The method quantitation limit (MQL) was calculated as 10 × SD of three replicate analyses of a food extract spiked at 0.50 ng/mL for each of the six species. Short-term precision (4 h) was determined for three different matrix standard concentrations (0.5, 2.0, and 5.0 ng/mL), and the percent relative standard deviations (RSDs) were ≤14% (**Table 6**). Long-term (3 day) precision was determined to be ≤13% using 2 ng/mL (**Table 6**). The columns were not regenerated during this 3 day period. The accuracy of the method was demonstrated through the analysis of 0.5, 2.0, and 5.0 ng/mL matrix standards. A percent recovery of ≥89% was calculated for all species except for a percent recovery of 78% for AsB at 5.0 ng/mL (**Table 7**). This exception was attributed to a sample fortification error for that species. The precision and accuracy results clearly indicate acceptable performance of the method even in the absence of an internal standard.

Table 7. Analysis Results for the Method Accuracy

arsenic species	average method accuracy (as % recovery) (ng/mL)		
	0.50 (n=7)	2.0 (n = 8)	5.0 (n = 8)
AsB	96.3	96.4	78.0
AsIII	100	102	91.7
DMA	96.9	94.4	96.6
AsC	102	97.6	101
MMA	103	106	103
AsV	89.0	106	108

Table 8. Comparison of Species Total vs Measured Total for Selected NHEXAS Samples

sample	study	species total (μg/kg)	measured total (μg/kg)	% difference
1	children's	43.5 (AsB)	44.3	1.8
2	children's	299 (AsB)	384	22
3	children's	28.4 (AsB)	66.3	57
4	children's	4.60 (AsB)	32.9	86
5	region V	217 (AsB)	209	–3.8
6	region V	78.4 (AsB)	82.9	5.4
7	region V	75.1 (AsB)	82.9	9.4
8	region V	144 (AsB)	175	18
9	region V	99.7 (AsB)	82.9	–20
10	region V	113 (AsB)	148	24
11	region V	21.9 (AsB)	45.5	52
12	region V	6.80 (AsV)	26.6	75

Sample Analysis Results. A minimum of 80 samples was analyzed in support of NHEXAS. Additional samples (at least 120) were evaluated as part of a cooking experiment study, which is not discussed in this paper. Each sample was aliquotted (a nominal sample mass of 2 g), lyophilized, and kept in the freezer until analysis. After the samples were thawed to room temperature, they were subjected to the final extraction method as previously described and were injected into the chromatographic system. The arsenic species in the samples were separated using an ammonium carbonate buffer as mentioned. For the analyzed NHEXAS children's study samples, 20 samples contained AsB ranging in concentrations from 1.0 to 299 μg/kg and two samples contained AsV in concentrations of 21.4 and 21.2 μg/kg. For the analyzed NHEXAS region V samples, 17 samples contained AsB in concentrations between 6.8 and 217 μg/kg, one sample contained DMA at a concentration of 16.7 μg/kg, and three contained AsV with concentrations between 6.8 and 22 μg/kg. **Table 8** shows a comparison between the summed species total (obtained in this laboratory by ICP-MS) and the measured arsenic total (obtained by Total Diet Study Laboratory, USFDA, Kansas City, MO) as expressed by percent difference. For some samples, the percent difference is low (<25%), indicating good extraction efficiency of arsenic. For the samples in which a larger percent difference was obtained, there may be other arsenic species present (arsenosugars, arsenolipids, etc.) that have not been determined. The separation and quantification of these additional arsenic species may improve the mass balance for some of the NHEXAS samples. Samples prepared in duplicate were analyzed on different days, and results are shown in **Table 9** over the course of 18 months.

Table 9. Duplicate Sample Analysis Results[a]

sample no.	AsB concentration (μg/kg) average ± SD (% RSD)
duplicate sample 1	3.41 ± 0.59 (17)
duplicate sample 2	1.31 ± 0.00 (0.0)
duplicate sample 3	6.55 ± 0.24 (4.5)
duplicate sample 4	5.12 ± 0.15 (3.0)
duplicate sample 5	5.81 ± 1.15 (20)
duplicate sample 6	16.53 ± 0.93 (5.6)
duplicate sample 7	18.40 ± 1.59 (8.6)
duplicate sample 8	1.73 ± 0.62 (36)

[a] Only AsB was detected in these samples.

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