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Application Note 355

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Rapid Extraction and Determination of Arsenicals in Fish Tissue and Plant Material Using Accelerated Solvent Extraction (ASE®)

INTRODUCTION

The toxicity of arsenic is species dependent. Inorganic arsenic species such as arsenite (As[III]) and arsenate (As[V]) have been classified as carcinogens. Methylated forms such as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) have recently been labeled as cancer promoters. Arsenobetaine (AsB), arsenocholine (AsC), and arseno sugars have been found to be relatively nontoxic.

Two major pathways for toxic arsenic exposure include drinking water and diet. Seafood (including fish and seaweed) accounts for the majority of ingested arsenic, most of which is nontoxic, however, fruits and vegetables grown in contaminated soils and sediments contribute another significant source.

Due to the variable levels of toxicity associated with arsenic species in foods, total arsenic determination is not sufficient to assess potential harmful contamination. Determination of individual arsenic species is necessary. This has increased the need to improve separation and detection methods for organometallic speciation. Unfortunately, the majority of organometallic sample preparation methods are still laborious and time consuming. The time discrepancies between the improved analytical methods and outdated sample preparation methods create bottlenecks which slow results of vital toxicological monitoring of food products used for human consumption.

To eliminate sample preparation bottlenecks, Accelerated Solvent Extraction (ASE) methods have been developed and proven to be an excellent alternative to the outdated sample preparation methods such as Soxhlet and sonication. ASE dramatically decreases the extraction time while providing good recoveries of arsenic species. ASE uses solvents at elevated temperature and pressure to increase the kinetics of the extraction process, resulting in faster, more efficient extractions.

This application note describes ASE methods for the extraction of various arsenic species from different food matrices, specifically fish tissue, ribbon kelp, and vegetables grown in contaminated soil. This note also includes references for analysis methods such as ICP-MS.

EQUIPMENT

Dionex ASE 200 Extractor with 11-mL stainless steel extraction cells (P/N 048765)

Dionex Cellulose Glass-fiber Filters (P/N 049458 or 047017)

Dionex Collection Vials (40 or 60 mL) (P/N 048783 or 048784)

Analytical Balance (to read to the nearest 0.0001 g or better)

Dionex SE 400 or SE 500 Solvent Evaporator (P/N 063221 or 063222)

REAGENTS

Methanol (HPLC grade)

HPLC water

Ottawa sand (Fisher Scientific)

EXTRACTION CONDITIONS

Fish Tissue[1]

Solvent:	Methanol 100%
Temperature:	100 °C
Pressure:	1500 psi
Cell Heat-up Time:	5 min
Static Time:	2 min
Flush Volume:	60%
Purge Time:	60 s
Cycles:	5
Total Time:	17 min
Total Solvent:	<30 mL

Ribbon Kelp[2]

Solvent:	30/70 (w/w) Methanol/ H_2O
Temperature:	Ambient
Pressure:	1500 psi
Cell Heat-up Time:	N/A
Static time:	1 min
Flush Volume:	90%
Purge Time:	120 s
Cycles:	3
Total Time:	7 min
Total Solvent:	<30 mL

Carrots[3]

Solvent:	Water
Temperature:	100 °C
Pressure:	1500 psi
Cell Heat-up Time:	5 min
Static Time:	1 min
Flush Volume:	100%
Purge Time:	90 s
Cycles:	3
Total Time:	18 min
Total Solvent:	<30 mL

SAMPLE PREPARATION

Freeze-dried samples are used for all the methods described in this document.

Fish Tissue[1]

Place a cellulose filter into an 11-mL extraction cell before loading the sample. Weigh approximately 0.1–0.3 g of freeze-dried fish tissue directly into the cell. Add Ottawa sand to the cell and mix with fish tissue using a stainless steel spatula. Cap the cell and prepare other samples in the same manner.

Ribbon Kelp[2]

Place a cellulose filter into an 11-mL extraction cell before loading the sample. Weigh approximately 0.25–0.5 g of freeze-dried kelp directly into the cell. Add Ottawa sand to the cell and mix with the dried kelp using a stainless steel spatula. Cap the cell and prepare other samples in the same manner. Note: Because the seaweed samples tend to swell when exposed to the solvent, the amount of sample used may need to be varied depending upon the type of seaweed being extracted.

Carrots[3]

Place a glass-fiber filter into an 11-mL extraction cell before loading the sample. Weigh approximately 0.25–1.0 g of freeze-dried carrot directly into the cell. Add Ottawa sand to the cell and mix with the dried carrot using a stainless steel spatula. Cap the cell and prepare other samples in the same manner. Note: Because the carrot samples tend to swell when exposed to the solvent, the amount of sample used may need to be varied depending upon the type of plant material being extracted.

EXTRACTION PROCEDURE

Place the loaded cells onto the ASE 200 instrument. Label the appropriate number of collection vials and place them onto the vial carousel. Set up the method parameters that are suggested for the sample being extracted and push the START button to begin the extraction. When the extraction is complete the extracts should be treated as follows:

Fish Tissue Extracts[1]

Remove an aliquot of the extract from the collection vial and dilute 1:10 with water. Filter each diluted extract using a 0.45 µm nylon/glass syringe filter into an HPLC autosampler vial.

Ribbon Kelp Extracts[2]

Place the extracts onto the SE Evaporator and evaporate to dryness at 50 °C with a nitrogen stream. Redisolve each extract with 20 g of water. Filter an aliquot of each extract using a 0.45 µm nylon/glass syringe filter into an HPLC autosampler vial.

Carrot Extracts[3]

Filter each extract using a 0.45 µm nylon/glass syringe filter. Place an aliquot of the filtered extract into an HPLC autosampler vial.

Analytical Procedures

To determine the individual arsenic species in each extract, the authors used LC-ICP-MS. The LC-ICP-MS Methods for each sample can be found in literature references [1] for fish, [2] for kelp, and [3] for carrots.

RESULTS AND DISCUSSION

Fish Tissue[1]

The following certified reference materials (CRMs) were extracted for method validation: DORM-2 dogfish muscle (National Research Council, Ottawa Canada), BCR 627 tuna fish, and BCR 710 oyster tissue (Brussels, Belgium).

Subsamples of the different CRMs (n = 4–6) were extracted and diluted with water and analyzed via HPLC-ICP-MS.[1] Table 1 shows the results of AsB extracted by ASE as compared to the known CRM value. The results showed a 99.4% recovery for the DORM-2 samples, a 94.6% recovery for the BCR 627 samples and a 97.3% recovery for the BCR 710 samples.

Table 1. Results of ASE Extraction of Fish Tissue CRMs (n=6)

Data Obtained for AsB in Two Certified Reference Materials and a Candidate Reference Material* Extracted with ASE

	Measured Value	Certified Value
DORM-2 (dogfish muscle)	16.3 ± 0.9 (±1s)	16.4 ± 1.1 (±95% C.I.)
BCR 627 (tuna fish)	3.69 ± 0.21 (±1s)	3.90 ± 0.22 (±95% C.I.)
BCR 710** (oyster tissue)	31.8 ± 1.1 (±1s)	32.7 ± 5.1 (±1s)

* Expressed as mg/kg As, unless otherwise stated.
** Concentration as species. The data shown for this material is based on the consensus mean of the final certification round after the removal of statistical outliers.

Ribbon Kelp[2]

Ribbon kelp containing 3 arsenosugars (As 328, As 428, and As 392) was received from Puget Sound, WA. Portions of each kelp sample were digested with HNO_3 and H_2O_2 to determine the total As for comparison to the ASE method. The procedure to determine the As Total Digest concentration was a modification of US EPA method 200.3. Each digested sample was analyzed in triplicate.

In an effort to optimize the ASE method, several different ASE parameters were evaluated. It was determined that temperature and solvent mixture had the most dramatic effect on As recoveries. The tests conducted by the authors show a 19.7% increase in recoveries when increasing the temperature from ambient to 60 °C. Increasing the temp to 120 °C caused the extracts to become discolored. It was determined that this temperature was too high and the discoloration was due to the unknown thermal stability of the species. Further tests showed that extracting with 100% water gave the best recoveries, but caused the sample to swell excessively in the extraction cell. Using a mixture of water and methanol (30/70, v/v) gave similar results and eliminated sample swelling.

Carrots[3]

Freeze-dried carrot samples that contained an undetectable total arsenic concentration were spiked with arsenic as a single species and with a mixed standard containing the following arsenic species: As(III), As(V), MMA, DMA and AsB. These were spiked at two different concentrations. Table 2 summarizes the average ASE recoveries.

Table 2. Summary of ASE Average Recoveries for Arsenic Spiked on Carrots and Calculated by Direct Calibration (n=3)

Standard Type	Standard Concentration/ ng mL^{-1}	As (III)	As (v)	MMA	DMA	AsB
Single	1	107 ± 10	106 ± 5	101 ± 2	91 ± 4	102 ± 4
Mixture of five	1	111 ± 8	121 ± 3	104 ± 5	109 ± 6	112 ± 3
Single	5	102 ± 8	102 ± 3	104 ± 3	94 ± 1	98 ± 1
Mixture of five	5	104 ± 9	108 ± 4	103 ± 7	102 ± 3	101 ± 1

CONCLUSIONS

The data presented in this application note demonstrate that ASE is an excellent technique for extracting arsenicals from food samples such as fish tissue and vegetables. Using ASE, we were able to rapidly extract arsenicals using a minimal amount of solvent while achieving excellent recoveries.

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