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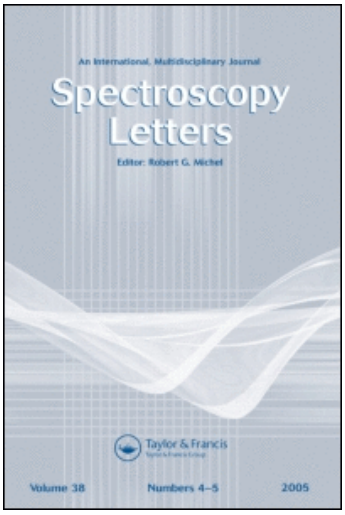

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Optimization of Arsenic Speciation Conditions in Plant Material by HPLC-ICPOES

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Optimization of Arsenic Speciation Conditions in Plant Material by HPLC-ICPOES

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Abstract: Instrument and extraction methods have been investigated and optimized for arsenic speciation in Chinese Brake ferns (*Pteris vittata*) by high-performance liquid chromatography–inductively coupled plasma optical emission spectrometry system components and parameters. The optimum chromatographic conditions were determined to be 30 mM $NH_4H_2PO_4$ (pH = 6) buffer, 1 mL/min flow rate, and 10% methanol. The limits of detection for this method were approximately 80 ng As(III) and 60 ng As(V) per gram of dried fern material. The optimum solvent for extracting arsenic from lyophilized fern material was 50% methanol.

Keywords: Arsenic, high-performance liquid chromatography–inductively coupled plasma optical emission spectrometry, plant

INTRODUCTION

Over the past few decades, arsenic has emerged as one of the most serious, worldwide environmental pollutants.[1,2] One can find numerous resources

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describing the severity of arsenic contamination, especially through agencies like the World Health Organization[3] and the U.S. Environmental Protection Agency.[4] The toxicity of arsenic depends on its chemical environment or oxidation state. For example, inorganic arsenic is more toxic than most organic arsenic compounds, and As(III) is generally more toxic than As(V). Because of these toxicity variations, analytical techniques should distinguish between different arsenic chemicals in addition to quantifying total arsenic. For aqueous samples or extracts, chromatography is typically used to separate arsenic species, and spectroscopy is used as the element detector.

Several arsenic remediation strategies can be used to clean up contaminated areas: excavation, capping, solidification and stabilization, vitrification, soil washing/acid extraction, and soil flushing.[5] A more recent strategy is phytoremediation, which uses green plants to remove arsenic from soil and water. The plants grow in contaminated soil (phytoextraction) or water (phytofiltration, rhizofiltration) and accumulate arsenic. After a period ranging from one to several months, the plants are harvested, which removes arsenic but leaves the original matrix intact. The Chinese Brake fern (*Pteris vittata*) has shown remarkable arsenic-accumulating ability, absorbing 100 times the concentration of soil arsenic in its fronds.[5,6] Other studies using Chinese Brake ferns demonstrate comparable results,[7–9] with differences attributed to soil or climate variations.

Several published articles have characterized arsenic species in Chinese Brake ferns after aqueous extraction.[9–11] Many of the published articles use an aqueous methanol extraction solvent, but mobile phase conditions vary. Based on these articles, the goal of this paper is to optimize instrument and extraction protocols for characterizing arsenic species in Chinese Brake ferns by high-performance liquid chromatography–inductively coupled plasma optical emission spectrometry (HPLC-ICPOES).

MATERIALS AND METHODS

Mature ferns were planted in soil containing up to 200 mg/kg arsenic, mainly as lead arsenate. After 1 month, fern fronds were harvested by cutting 2 cm above the soil with clean, plastic scissors. Fronds were rinsed with 18 MΩ distilled/deionized water, cut into 10-cm lengths, and placed in clean, plastic, sealable bags. The cuttings were frozen in a commercial freezer, placed into 600-mL lyophilizer vessels, and lyophilized for 24 hr (Labconco, Benchtop FreeZone Freeze-Dry Systems, Kansas City, MO, USA). The freeze-dried samples were removed from their vessels, ground in a cleaned, commercially available electric coffee grinder, and stored in clean 100-mL polypropylene vials (Corning Snap-Seal, Acton, MA, USA). Metal contamination from the coffee grinder was checked by grinding dried leaves of a nonaccumulating common blackberry plant (*Rubus fruticosus*). Ground and nonground blackberry leaves were digested with HNO_3 and

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analyzed by ICPOES. No measurable arsenic emission signals were apparent in either type of blackberry leaf sample.

Dried, ground fern samples were extracted with mixtures of water, ethanol, and methanol. Approximately 0.2 g of dried fern material was vortexed for 1 min with 5.0 mL of an extracting solvent. Mixtures were centrifuged, and the supernatant was reserved for analysis. This procedure was repeated several times. The collected supernatant was injected through a 0.45-μm syringe filter (Acrodisc, Pall Corporation, East Hills, NY, USA) into a chromatography injector containing a 100-μL sample loop.

The instrument components and solvents are listed in Table 1. A brief survey of published articles describing arsenic speciation in plant samples[10–14] showed that mobile phase conditions vary widely. In order to determine the optimum mobile phase solvent, varying pHs of ammonium phosphate, ammonium carbonate, and tartaric acid solutions were tested. Tailing of arsenate peaks was noticed with tartaric acid and ammonium carbonate solvents. Ammonium phosphate (pH = 6) was determined to be the optimal mobile phase solvent with regard to peak shapes and resolution. The majority of published articles employed the PRPX100 strong anion exchange column for separation; it was used in this study as well.

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RESULTS

Signal-to-noise studies (data not shown) indicated that the As 228.8-nm emission line should be monitored even though this was not the instrument manufacturer's recommended line. It was determined that interferences from metals would be very rare in our system because they would be

Table 1. High-performance liquid chromatography–inductively coupled plasma optical emission spectrometry system components and parameters

- Acuflow Series I Isocratic Pump, Fisher Scientific (Pittsburgh, PA, USA)
  - 100-μL injection volume
  - 1 mL/min flow rate
- $NH_4H_2PO_4$ mobile phase pH 6 buffer (adjusted with NH_4OH)
- Hamilton PRP-X100 anion exchange column, Hamilton Company (Reno, NV, USA)
- Perkin-Elmer, ICPOES Optima 4100-DV (Waltham, MA, USA)
  - 15 L/min plasma Ar gas
  - 0.5 L/min auxiliary Ar gas
  - 0.5 L/min nebulizer Ar gas
  - 1500 W Radiofrequency power
  - Seven points collected per As emission peak
  - As 228.8-nm line monitored
  - 2.5 s integration per collected spectra
  - Ten minutes per chromatogram (data acquisition after 2 min)

unretained by the strong anion-exchange column and unlikely to co-elute with anionic arsenic species. Also, all solvents were high purity and metal free. No arsenic emission signals were evident from repeated HPLC-ICPOES analyses of blank extracting solutions.

A standard solution containing 2.50 mg/L of As(III) (arsenite) and 5.00 mg/L As(V) (arsenate) was introduced into the HPLC-ICPOES system under various conditions. Figure 1 shows the relationship between mobile phase buffer concentrations and retention times. As expected, higher buffer concentrations expedited arsenate elution. Arsenite is relatively unretained by the column. In Fig. 2, a comparison of flow rates is shown. A rate of 1.0 mL/min was chosen as an adequate flow rate in terms of analysis times and pump pressure. Lastly, the percent methanol concentration in the mobile phase was adjusted to investigate its effect on peak shapes (Fig. 3). Ten percent methanol yielded the largest signal. Most likely, methanol reduces solution viscosity and surface tension, which enhances nebulization and increases sensitivity. Ten percent methanol is the maximum concentration that the plasma tolerates; higher concentrations extinguish the plasma.

In conclusion, the final instrument parameters were determined by considering retention times, peak shape, sensitivity, pump pressure, and plasma stability. Ten percent methanol, 1.0 mL/min flow rate, and 30 mM buffer were the best compromise conditions. Using these parameters, the limits of detection were determined to be 0.032 and 0.025 mg/L for As(III) and As(V), respectively. This corresponds with detection limits of approximately 80 ng As(III) and 60 ng As(V) per gram of dried fern material. These figures are approximately 200 times and 50 times the reported detection limits of Chen et al.[10] who used ICP-MS for element detection. Because ICP-MS is a more sensitive instrument, the results reported here are expected.

Next, the sample preparation and extraction methods were addressed. In general, a method that is simple, fast, and chemically inert is desired. Three sets of samples (10 each) of dried fern material were extracted with water,

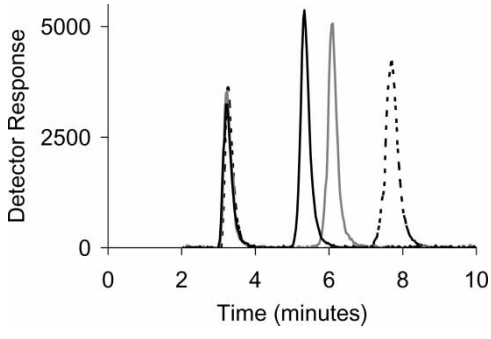

Figure 1. Mobile phase buffer concentration comparison. pH = 6.0 $NH_4H_2PO_4$, 1.0 mL/min, 7.5% MeOH. ——, 60 mM; ——, 45 mM; -----, 30 mM.

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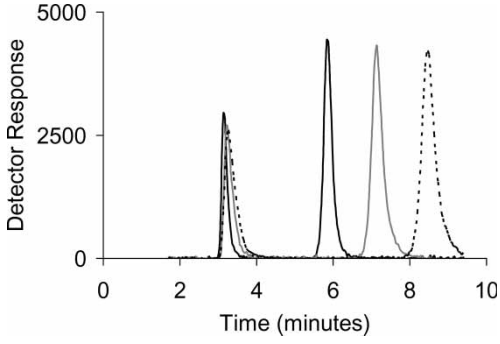

Figure 2. Flow rate comparison (mL/min). 30 mM pH = 6.0 $NH_4H_2PO_4$, 7.5% MeOH. ——, 1.5; ——, 1.0; -----, 0.75.

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50% methanol, and 50% ethanol. Figure 4 shows a representative chromatogram from one fern sample. Because methanol or ethanol concentrations exceeding 10% extinguish the plasma, all sample extracts (including water) were diluted 1:5 with water. Pure methanol and ethanol solvents were avoided because they would require 10-fold dilutions, which would considerably reduce emission signals. The 50% methanol solvent extracted the most arsenic (III) and (V), averaging a 35% total arsenic extraction increase over water. The 50% ethanol solvent averaged a 16% extraction increase over water. Extracted fern samples were re-extracted and analyzed. The presence of arsenic was still evident. It was determined that three extractions were necessary for complete arsenic extraction from fern material.

To compare extraction efficiency, dried fern samples were digested with concentrated nitric acid and analyzed by ICPOES. The extraction-HPLC-ICPOES (three extractions) method recovered 95–105% of total arsenic when compared with the digestion-ICPOES method. These recoveries

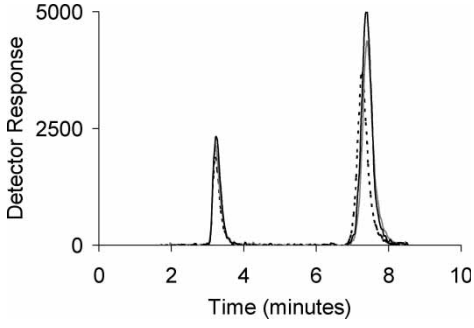

Figure 3. Mobile phase (percent methanol) concentration comparison. 30 mM pH = 6.0 $NH_4H_2PO_4$, 1.0 mL/min. ——, 10%; ——, 7.5%; -----, 5.0%.

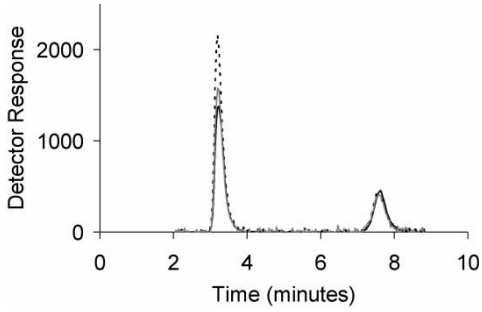

Figure 4. Fern material extracting solvent comparison. Mobile phase: 10% methanol, 30 mM pH = 6.0 $NH_4H_2PO_4$, 1.0 mL/min. ——, water; ——, 50% ethanol; -----, 50% methanol.

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compare favorably with literature results reporting extraction efficiencies ranging from 85 to 100%. Extraction efficiencies were only 50–75% after one extraction. It should be noted that extraction efficiencies were significantly higher when the ferns were lyophilized rather than air-dried (data not shown). It is surmised that freeze-drying more efficiently destroys cell walls, liberating arsenic compounds more readily than air-dying.

CONCLUSIONS

In conclusion, the instrument and fern extraction protocols described in this article are sufficient for HPLC-ICPOES arsenic speciation. However, there is probably considerable room for alternative protocols depending on the instrumental method that is available. Aside from the results discussed in this article, recent results (data not shown) suggest that solvents can be pH-adjusted to attain extraction efficiencies near 100% after one extraction. This may significantly simplify our current extraction protocol. In addition, solid phase extraction (SPE) techniques may be employed to further streamline the extraction protocol. SPE might reduce contamination, increase extraction efficiencies, and allow arsenic to be preconcentrated from plant samples with low levels of arsenic species. In our experience so far, few commercially available SPE stationary phases retain both As(III) and As(V). An effective SPE method may involve chemically modifying the SPE stationary phase, using chelating agents, and/or adjusting the pH of the extracts.

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