Challenge
Appropriate sample preparation method providing high extraction efficiency and preventing species interconversion for reliable As speciation

Solution
Mild microwave-assisted extraction (MAE) using 1 M phosphoric acid and 0.1 M ascorbic acid, and arsenic speciation by means of LC-ICP-MS

Intended audience
Industries, organizations, and individuals involved in environmental analysis, regulation, research, and remediation efforts

Arsenic Speciation in Soils and Sediments

Introduction

A wide variety of arsenic (As) compounds are present in the environment and in biological systems. Some inorganic As compounds have been established as human carcinogens.[1-4] Although it is useful to know the total concentration of an element, the determination of the species is important in terms of various analytical aspects due to its significant implications for human health, environmental monitoring, and regulatory compliance.[5] The toxicity and bioavailability of As compounds depend on the chemical form of the As.[6] Currently, mostly hyphenated techniques are used for determining As species which primarily couple ion chromatography (IC) with inductively coupled plasma mass spectrometry (ICP-MS).[7, 8]

For As speciation in soil, the extraction procedure is important regarding the extraction efficiency, since As is differently bonded to various mineral components.[9] Extractants such as water, methanol, ammonium, sodium or potassium nitrate, dilute acetic acid, hydrochloric, phosphoric or nitric acid, sodium carbonate or bicarbonate, ammonium acetate, and ethylenediaminetetraacetic acid are commonly used.[8] However, the extraction efficiency depends on both, the extraction system and the extraction solvent used.[8] For example, mild methods, such as methanol/water, extract only a small percentage (typically 5%) of the As present in the soil and sediment samples.[10] In contrast, various other extractants, such as 0.1 M hydroxylammonium hydrochloride, 0.2 M ammonium oxalate, and 0.3 M orthophosphoric acid, give a much higher efficiency (10–94%) but exhibit large differences between soil, sediment, and sludge.[11] Microwave-assisted extraction (MAE) presents itself as a viable choice for extracting As species from soil due to its automated nature, wherein pressure and temperature are concurrently applied throughout the extraction process. This method exhibits swifter operational pace and reduced labor demand compared to conventional extraction methodologies. It has been found that the use of MAE for extracting As species in solid samples appears to minimize the risk of a redox interconversion of inorganic As forms and that orthophosphoric acid is the best extractant for sediment and sludge, but not for aged soil samples.[11]

This application note reports the development of an MAE extraction procedure for As species in soils and sediments which achieved a good recovery.
For species analysis, HPLC-ICP-MS was applied. The extraction procedure was applied to the National Institute of Standards and Technology standard reference material (NIST SRM) 2711a (Montana soil) and to the International Atomic Energy Agency standard reference material (IAEA) 475 (marine sediment), as well as soil samples collected from an As-affected area of the Pejão mining region in Portugal.

Materials and Methods

Instrumentation

Microwave-assisted extraction

A microwave digestion system (speedwave XPERT, Analytik Jena GmbH & CO. KG) with a rotor for 12 Teflon digestion vessels (DAP60X) was used for sample digestion and extraction.

Inductively coupled plasma mass spectrometry

A PlasmaQuant MS Q ICP-MS was used to determine total As concentration. The Ni sampler and skimmer cone and a Peltier-cooled Scott type spray chamber were used. The ICP-MS system was equipped with a CETAC ASX-560 autosampler (Teledyne CETAC Technologies), and an ESI injection valve (Elemental Scientific Inc.) for automation of the analysis.

HPLC-ICPMS

A PQ LC – HPLC System, non-metal, PEEK (Analytik Jena GmbH+Co. KG) equipped with a Hamilton PRP-X100 (5 µm, 150 mm × 4.6 mm) separation column was used for the chromatographic separation of As species. The samples were injected using an S5300 auto-sampler. The outlet of the separation column was directly connected to a SeaSpray® nebulizer (0.4 mL/min). The PlasmaQuant MS Q ICP-MS was used as an element-specific detector (m/z = 75). The ICP-MS and high-performance liquid chromatography (HPLC) operating conditions are given in Table 1. Data were collected using single-ion monitoring (SIM) to ensure maximum sensitivity. Data treatment was done using the Clarity Chromatography Software.

Samples and reagents

All solutions were prepared with deionized water (Milli Q Plus system; Millipore) with an 18.2 MΩ/cm resistivity. For the speciation studies, standard solutions (1000 mg/l As) of As compounds were prepared as follows: arsenobetaine ($C_5H_{11}AsO_2$, Merck Sigma-Aldrich), sodium arsenite ($NaAsO_2$, Merck Sigma-Aldrich), arsenate ($Na_2HAsO_4.7H_2O$, Merck KGaA), monomethylarsonate (MMA; $CH_3AsNa_2O_3$, Merck Sigma-Aldrich), and dimethylarsinate [DMA; $(CH_3)_2AsO(OH)$, Merck Sigma-Aldrich] were dissolved in water. All the standard solutions were standardized with respect to As and kept at 4 °C in darkness until use. Dilutions of these standard solutions were prepared daily for the analyses.
The mobile phase was prepared from ammonium carbonate ($(NH_4)_2CO_3$, Merck Sigma-Aldrich) and methanol (CH_3OH, purity 99.8% for HPLC, VWR International, LLC). The mobile phase solution was filtered through a 0.22 µm PTFE membrane before use. Concentrated nitric acid (HNO_3, Suprapur 65%; Merck Sigma-Aldrich) and concentrated hydrochloric acid (HCl, Suprapur 30%, Merck Sigma-Aldrich) were used for the aqua regia digestion method. Ortho-phosphoric acid (H_3PO_4, purity 85–90% for HPLC, Fluka, VWR International, LLC), ascorbic acid ($C_6H_8O_6$, purity ≥99%, VWR International, LLC) were assayed for microwave extraction.
NIST SRM 2711a (Montana soil) and IAEA 475 (marine sediment) were used to validate the results. Since soil and sediment standard reference materials with certified concentrations of individual As species are not available, spike experiments were conducted. One contaminated soil sample (EA1) was collected from an As-affected mining area of the Pejão region in Portugal for determination of both total As and species.

Determination of pseudo total As in soil

The concentration of As in soil and sediment samples was carried out by an aqua regia microwave digestion system and further ICP-MS analysis as follows: three extractions of finely powdered soil or sediment sample (0.25 g) were weighed into a dry, clean Teflon digestion vessel to which 10 ml of aqua regia were added. Digestion was done with a ramp up to 180 °C within 15 min and held for 20 min. After cooling for 30 min, the vessels were opened carefully. Each digestion was centrifuged at 5000 rpm for 5 minutes, the supernatant filtered through a Millipore membrane (0.45 µm), transferred to a 50 mL volumetric flask for final dilution to the mark with 1% (v/v) HNO_3, and kept in a plastic container for analysis.

Determination of As species in soil and sediment
About 0.2 g of the soil or sediment sample and 10 mL of phosphoric acid (1.0 M) containing ascorbic acid (0.1 M) were placed in a microwave Teflon vessel, and the mixture was maintained at 150 ºC for 60 minutes with a microwave power of 400 W and 60 bar. Once the solution was at room temperature, the mixture was transferred into a 50 mL volumetric flask, centrifuged at 5000 rpm for 5 minutes, the supernatant filtered through a Millipore membrane (0.45 µm) and then kept in glass container for As speciation analysis. This extraction was repeated twice to check extraction efficiency (Table 3).

Table 1: Optimized HPLC-ICP-MS operating parameters

Parameter (ICP-MS)	Specification	Parameter (HPLC)	Specification
Plasma gas flow	10.5 L/min	Anionic column	PRP-X100 (5µm, 150 mm × 4.6 mm)
Auxiliary gas flow	1.50 L/min	Mobile phase	A: 10 mM $(NH_4)_2CO_3$ + 1% (v/v) MeOH B: 100 mM $(NH_4)_2CO_3$ + 1% (v/v) MeOH
Sheath gas flow	0.00 L/min	Injection volume	100 µL
Nebulizer gas flow	1.05 L/min	Flow rate	1.25 L/min
Sampling depth	5.0 mm	Gradient	0 min (100% A) 5–6 min (90% B) 9 min (100% A)
Plasma RF power	1.35 kW	Elution times	AsB – 1.33 min (IS) As (III) – 2.39 min DMA – 3.90 min MMA – 6.85 min As (V) – 7.94 min
Rump rate	100 rpm – blue/blue PVC pump tubing	Total elution time	10 min
Stabilization delay	0 s		
iCRC gas setting	H_2 40 mL/min		
Dwell time	^{75}As – 100000 µs		
Evaluation	Peak Area of ^{75}As		

Results and Discussion

The results of pseudo total As content and the percentage of recovery of As in the NIST SRM 2711a (Montana soil), IAEA 475 (marine sediment), and the contaminated soil (EA1) are presented in Table 2. These results indicate that the observed value was in good agreement with the certified value (Table 2). The total As content obtained using aqua regia in the SRM sample was also close enough to the certified value. Since aqua regia is a leaching extraction, it was expected that the obtained concentrations would fall below the certified values. However, they remained within the acceptable range (> 80%). As a result, the extraction procedure was extended to environmental soil samples (EA1), which was also consistent with the reported contra lab results.

Table 2: Certified and measured arsenic concentrations in NIST SRM 2711a (Montana soil), IEAE 475 (marine sediment) and EA1 by ICP-MS after microwave digestion.

Sample ID	Certified values (µg/g)	Measured (µg/g)	RSD, % (n=3)	Recovery (%)	Z-score
NIST SRM 2711a	107 ± 5	97.1 ± 3.3	3.4	91	-1.97
IAEA 475	12.6 ± 0.7	10.6 ± 0.5	4.8	84	-2.82
EA1 soil	49.6*	40.5	5.0	82	-

*reported by Bureau Veritas Laboratories, BC, Canada

Table 3 shows extraction efficiency of the two extraction steps performed for both Standard Reference Materials (NIST SRM 2711a and IEAE 475) and the contaminated soil (EA1). It is important to notice that the efficiency of H_3PO_4 was similar to aqua regia (Table 2) which should not be applied for As species determination since it is an oxidizing agent that oxidizes As (III) to As (V). The two consecutive extraction steps provide about 83 to 112% recoveries for the three materials tested, the second extraction step contributes with additional ≈10% of the remaining As and no further extractions steps are needed. Total As species found by phosphoric acid extraction determined by both techniques (HPLC vs. IPC-MS) showed good accuracy with recoveries within 96 to 109%.

Table 3: Extraction efficiency of As species in two extraction steps repeated three times in NIST SRM 2711a, IAEA 475 and soil (EA1) obtained by 1 M H_3PO_4 + 0.1M ascorbic acid. Legend: nd (Not detected).

Sample ID (Total content, mg/kg)	N°. of extractions	Arsenic species (mg/kg), n=3				Total As by HPLC (mg/kg)	Total As in Extract by ICP-MS (mg/kg)	% Recovery (HPLC/ICP-MS)	% Recovery (Total Extr./ Ref.)
		As (III)	DMA	MMA	As (V)				
NIST 2711a (107 ± 5)	1st	0.3 ± 0.1	nd	nd	111 ± 5.2	112 ± 5.3	102 ± 4.6	109	104
	2nd	nd	nd	nd	8.6 ± 0.7	8.6 ± 0.7	7.9 ± 0.1	108	8
	∑extr., n=2	**0.34 ± 0.13**	**nd**	**nd**	**120 ± 5.**2	**120 ± 5.4**	**110 ± 5**	**109**	**112**
IAEA 475 (12.6 ± 0.7)	1st	1.4 ± 0.2	nd	nd	7.9 ± 0.1	9.3 ± 0.3	11.0 ± 0.2	102	74
	2nd	0.09 ± 0.03	nd	0.43 ± 0.05	0.6 ± 0.2	1.1 ± 0.2	1.1 ± 0.1	102	9
	∑extr., n=2	**1.5 ± 0.2**	**nd**	**0.43 ± 0.05**	**8.5 ± 0.2**	**10.4 ± 0.4**	**12.1 ± 0.2**	**102**	**83**
EA1 (49.6)	1st	5.5 ± 0.6	nd	nd	33.2 ± 1.3	38.7 ± 1.9	49.5 ± 3.0	96	78
	2nd	0.9 ± 0.2	nd	nd	**4.1 ± 0.7**	5.0 ± 0.9	5.2 ± 0.8	101	10
	∑extr., n=2	**6.4 ± 0.6**	**nd**	**nd**	37.4 ± 1.5	**43.7 ± 2.1**	**54.7 ± 3.1**	**97**	**88**

Since no soil and sediment SRMs with certified As species contents are available, spike recoveries were conducted (2, 5, 10, and 20 µg/L) in the order of the expected As species concentrations in the original extracts to check the extraction procedure accuracy (Table 4). Spikes were done prior to extraction. The results show good accuracy with recoveries within 72 to 110% for all four of the As species in the three samples matrices tested.

Table 4: Spike recoveries of H_3PO_4 extracts, added prior to extraction. Legend: nd (Not detected).

Sample ID (Total content, mg/kg)	Arsenic species (mg/kg), n=3			
	As (III)	DMA	MMA	As (V)
NIST 2711a_1_2#	nd	nd	nd	9.6
Spike (5, 10 and 20 ppb)	4.9	8.7	10.4	31.7
%REC	**98**	**87**	**104**	**110**
NIST 2711a_3_2#	nd	nd	nnd	8.3
Spike (5, 10 and 20 ppb)	6.1	7.9	10.4	27.6
%REC	**123**	**79**	**104**	**97**
IAEA 475_1_2#	0.2	nd	0.9	0.9
Spike (2 and 5 ppb)	2.2	4.6	4.4	5.5
%REC	**99**	**92**	**72**	**92**
IAEA 475_3_2#	0.2	nd	0.8	1.6
Spike (2 and 5 ppb)	1.8	4.8	4.8	6.5
%REC	**83**	**96**	**81**	**98**
EA1_1_2#	0.7	nd	nd	3.9
Spike (5, 10 and 20 ppb)	5.2	10.2	10.5	18.7
%REC	**90**	**10**2	**105**	**93**
EA1_2_2#	1.1	nd	nd	3.8
Spike (5, 10 and 20 ppb)	6.2	10.0	10.1	19.1
%REC	**101**	**100**	**101**	**96**

Figure 1 illustrates the chromatograms of the three tested samples. In the case of NIST 2711a (Montana soil), the distribution comprises 99.7% of As (V) and only 0.3% of As (III), aligning well with prior studies on this material.[12] The contaminated soil (EA1) shows higher amounts of As (III) reaching 15% and the remaining 85% occur as As (V). The marine sediment tested (IAEA 475) has similar distribution like the contaminated soil (EA1), however, 4% of MMA also exist in the sediment matrix which was extracted only in the second extraction most likely due to strong bonds with potential organic matter in the matrix.

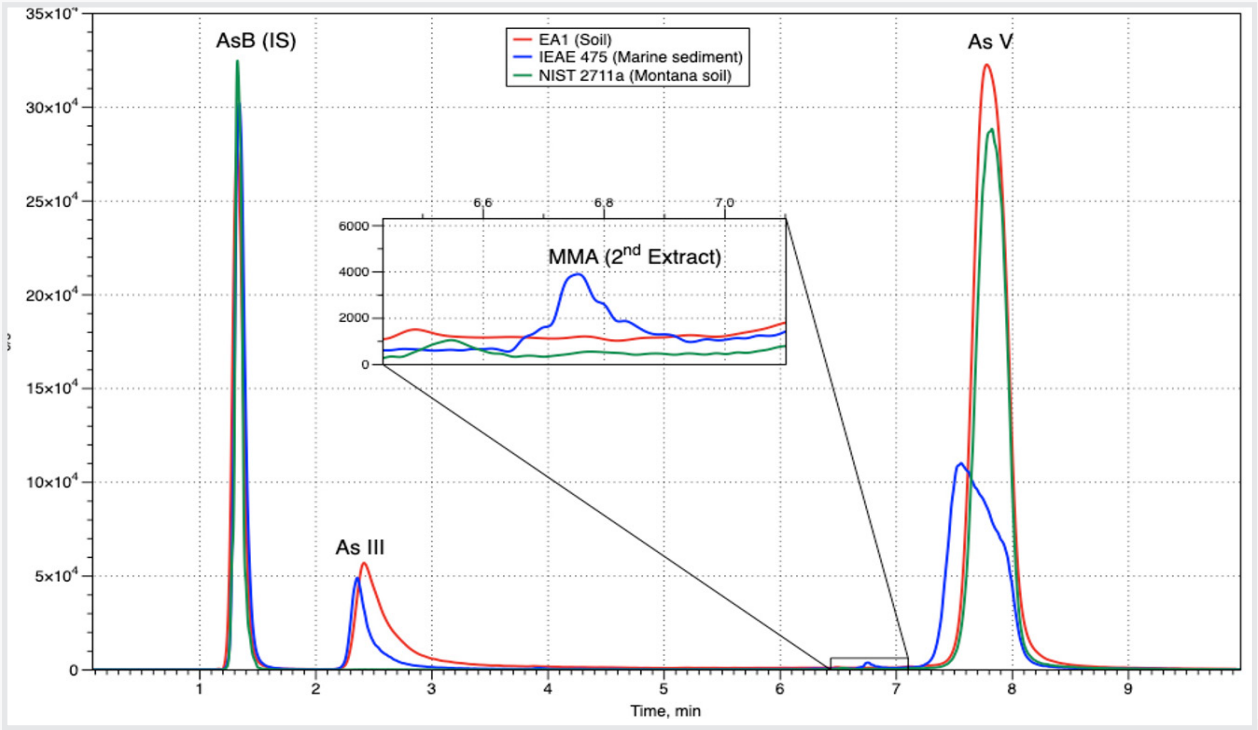

Figure 1: Chromatograms of NIST SRM 2711a (Montana soil), IEAE 475 (marine sediment), and EA1 (Soil), m/z = 75.

Summary

An extraction method utilizing ortho-phosphoric acid was thoroughly investigated, resulting in a remarkable accuracy during the extraction process. Microwave-assisted ortho-phosphoric acid extraction emerges as an efficient technique as it is rapid and effective. Ideally, this study not only yields valuable data but also fuels optimism about potential SRMs candidates for As speciation in soil and sediment, exemplified by NIST 2711a (Montana soil) and IAEA 475 (marine sediment).

Figure 2: PlasmQuant MS Q and PQ LC

Recommended device configuration

Table 5: Overview of devices, accessories, and consumables

Article	Article number	Description
PlasmaQuant MS Q - for sensitive and robust high throughput analysis	818-08011-2	The PlasmaQuant MS Q is a high-performance ICP-MS, which enables efficient and sensitive analysis in quality control and environmental monitoring. The advanced technology and automation of this ICP-MS solution allows to rapidly process many samples, ensuring timely results. This is crucial in environmental monitoring, where a quick and accurate assessment of As species is essential to monitor environmental contamination and thus ensuring public health and safety. The instrument's sensitivity and precision in speciation analysis make it a valuable tool to monitor and mitigate As-related risks effectively.
PQ LC - HPLC system, PEEK	810-88409-0	Separation technique hyphenated to the PlasmaQuant MS
HPLC column PRP X-100 for PQ LC	810-88418-0	Ion exchange column for As species separation

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