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Arsenic species determination in human scalp hair by pressurized hot water extraction and high performance liquid chromatography-inductively coupled plasma-mass spectrometry

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ABSTRACT

Analytical methods for the determination of total arsenic and arsenic species (mainly As(III) and As(V)) in human scalp hair have been developed. Inductively coupled plasma-mass spectrometry (ICP-MS) and high performance liquid chromatography (HPLC) coupled to ICP-MS have been used for total arsenic and arsenic species determination, respectively. The proposed methods include a "green", fast, high efficient and automated species leaching procedure by pressurized hot water extraction (PHWE). The operating parameters for PHWE including modifier concentration, extraction temperature, static time, extraction steps, pressure, mean particle size, diatomaceous earth (DE) mass/sample mass ratio and flush volume were studied using design of experiments (Plackett–Burman design PBD). Optimum condition implies a modifier concentration (acetic acid) of 150 mM and powdered hair samples fully mixed with diatomaceous earth (DE) as a dispersing agent at a DE mass/sample mass ratio of 5. The extraction has been carried out at 100 °C and at an extraction pressure of 1500 psi for 5 min in four extraction step. Under optimised conditions, limits of quantification of 7.0, 6.3 and 50.3 ng g^{-1} for total As, As(III) and As(V), respectively were achieved. Repeatability of the overall procedure (4.4, 7.2 and 2.1% for total As, As(III) and As(V), respectively) was achieved. The analysis of GBW-07601 (human hair) certified reference material was used for validation. The optimised method has been finally applied to several human scalp hair samples.

1. Introduction

Forensic and clinical analysis of trace compounds is usually carried out in blood, urine and hair. Although blood and urine are the most common and preferred matrices used for clinical and toxicological studies, hair is gaining in importance as an alternative specimen because of its advantages. Hair can be easily collected; it shows a high stability which facilitates the storage and a high capacity to accumulate different substances such as metals, organometallic compounds or abuse drug during extended periods [1,2].

The determination of inorganic and organometallic species in biological samples requires sensitive and selective techniques, using sample preparation strategies addressed to shortening and simplifying the stages previous to the analysis. HPLC, mainly in combination with ICP-MS [3,4], is widely used for analysis of inorganic and organic arsenic species in biological samples, offering simplicity and saving time and resources; i.e. the separation of seven arsenic species (arsenite, As(III); arsenate, As(V); monomethylarsonic acid, MMA; dimethylarsinic acid, DMA; arsenobetaine, AsB; arsenocholine, AsC; and trimethylarsine oxide, TMAO) within 11 min in a single chromatographic run and ICP-MS detection [5]. Although the fast separation and detection methods, many of these processes use time-consuming sample pre-treatments, commonly enzymatic hydrolysis [6,7] or extraction procedures by using water, water plus methanol or weak organic acids (orthophosphoric acid and trifluoroacetic acid) aided shaking, heating or sonicating [3,4,7,8]. Such sample pre-treatment for arsenic species isolation may take a relatively long time, because it involves many sample manipulation steps, generate large volumes of waste, and could introduce problems such as sample contamination or analyte losses by volatilisation or during filtration and/or centrifugation steps. Thus the development of automated and rapid extraction methods remains on interest. Modern extraction techniques including pressurized liquid extraction (PLE), pressurized hot water extraction (PHWE) and matrix solid-phase dispersion (MSPD) have demonstrated higher capabilities for organic/inorganic analyte extraction from complex matrices because extraction and clean-up stages

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can be performed simultaneously. Some applications by PLE [7,9], PHWE [10] or MSPD [11] can be found in literature for inorganic and organic arsenic species extraction from biological matrices.

PHWE is based on using deionised water, at high pressure and/or high temperature without reaching the critical point. Pressurized hot water (PHW) is typically used in extractions at temperatures above 100 °C but below the critical temperature (374.1 °C). Pressure is usually high enough (below critical pressure, ~3200 psi) to keep water in liquid state. Under these conditions, the dissolving power of PHW is increased (analyte mass-transfer is enhanced due to viscosity, surface tension and density are reduced due to hydrogen bonding becomes weak). Analyte solubility can be tuned through changes in temperature and pressure (i.e. non-polar compounds are more soluble in water by increasing temperature) [10].

PHWE pre-treatment reduces analysis time, sample manipulation (filtration stage is avoided), and the amount of sample required for analysis; which increases sample throughput and shortens turn-around time. In addition, this procedure enhances safety because no toxic solvents or acid are used (linking to the principles of "Green Chemistry").

Although the commented advantages, only one application of PLE to extracts arsenic species (mainly As(III) and As(V)) from human hair and also nail samples can be found in the literature [12,13]. Extracts were obtained after dispersion with Teflon balls by using sodium dodecyl sulfate (SDS) plus isopropanol. The extraction conditions were two cycles of 5.0 min at 125 °C and 870 psi [12,13].

The aim of this paper is the evaluation of PHW to extract arsenic species (mainly As(III) and As(V) from human scalp hair. The fast and safe extraction procedure combined with an HPLC-ICP-MS can offer a convenient method to assess arsenite and arsenate isolation from scalp hair samples in relatively short times. Because there are many variables that can affect the PHWE process, Plackett–Burman (PBDs) was used to unbiased estimation of all the main effects for all variables requiring few experiments.

2. Materials and methods

2.1. Instrumentation

Arsenic species leaching was carried out using a Dionex ASE-200 system (Sunnyvale, CA, USA) equipped with stainless steel extraction cells of 11 mL and cellulose filters (D28, 1.983 cm diameter, Dionex). An ICP-MS Thermo Finnigan X Series (Thermo Instruments, Austin, TX, USA) was used for analysis. For arsenic species separation a Dionex HPLC UltiMateO 3000LC (Dionex, Sunnyvale, CA, USA), equipped with a GP50 gradient pump (Dionex), an AS50 thermal compartment (Dionex), an AS50 auto-sampler (Dionex) and an IonPacAS7 (250 mm × 4 mm i.d.) anion-exchange column (Dionex) and a guard column Ion PacAG7 (Dionex) was used. The chromatographic system was coupled to an ICP-MS Thermo Finnigan X Series. A Fritsch Planetary Micro Mill Pulverisette 7 ball mill (Fritsch Industriest, Idar-Oberstein, Germany) equipped with zircon cups and zirconium balls was used to hair milling. The particle size distribution was obtained by using a Laser Coulter Series LS200, Fraunhofer optical model particle sizer with a LS-variable speed fluid module plus (Coulter Electronics, Hialeah, FL, USA). An Anton Paar Multiwave, Perkin-Elmer (Graz, Austria) with closed Teflon vessels was used for assisting the acid digestion. Chemometrics package was Statgraphics Plus V 5.0 for Windows, 1994–1999 (Manugistics Inc., Rockville M.D) was used for statistical, graphical, reporting, processing and tabulating procedures.

2.2. Reagents

All chemicals used were of ultra-pure grade, and diluted using ultra-pure water of resistance 18 MΩ cm^{-1} obtained from a Milli-Q purification device (Millipore Co., Bedford, MA, USA). Arsenite and arsenate stock standard solutions, 1000 mg L^{-1}, were from Panreac (Barcelona, Spain). Standard solutions of MMA and DMA, (1000 g L^{-1}) were prepared by dissolving the appropriate amounts of MMA ($CH_3AsO(ONa_2)\cdot 6H_2O$), purchased from Carlo Erba (Milan, Italy) and DMA ($C_2H_6AsNaO_2\cdot 3H_2O$), purchased from Merck (Poole, Dorset, UK). The organic arsenic standard solutions were stored in amber glass bottles and were kept at 4 °C. Diluted standard solutions were prepared daily from stock solution. Acetic acid and diatomaceous earth (DE), 95% SiO_2, were from Aldrich (Milwaukee, WI, USA). GBW-07601 (Human Hair) CRM was supplied from Standard Materials of Soils Components (Harbin, China). Methanol (gradient grade) was from Merck. Nitric acid concentrated, 69–70% (Baker, Phillipsburg, NJ, USA) and hydrogen peroxide, 33% (Panreac, Barcelona, Spain) were used for hair sample digestion. To avoid metal contamination, all glassware and plastic ware were washed and kept for 48 h in 10% (v/v) nitric acid, then rinsed several times with ultra-pure water before use.

2.3. Human hair samples

Human hair samples (length varied between 2 and 3 cm) were taken from healthy people and they were collected from the occipital region of the head, as close as possible to the scalp [1] using a stainless steel scissors. In order to provide an accurate assessment of endogenous metal content, hair samples were first washed with ultra-pure water and then samples were washed three times with acetone. Hair samples were again washed with ultra-pure water (three times) and were finally oven-dried at 80 °C. Then, hair samples were pulverised in a vibrating zirconia ball mill for 45 min using a power of 75% and the particle size distribution was achieved by Laser diffraction spectrometry. A mean particle size around 180 μm was reached after this treatment.

2.4. Pressurized hot water extraction procedure

Eleven milliliters stainless-steel cells with cellulose filters on both the bottom and top were filled with around 0.50 g of sample (human hair) mixed thoroughly with 2.5 g of inert DE (proportion 1:5). DE was used for preventing the aggregation of sample particles and the blockage of the extraction cell outlet. After closing, the cells were situated in the carousel of the ASE 200 system and the samples were extracted by using water modified with acetic acid, under the PHWE conditions shown in Table 1. The cells were purged for 60 s with N_2 gas after the extraction and the extracted solvent was collected in pre-cleaned glass vials. Finally, the acid extracts were transferred to 25 mL volumetric flask which was brought up to its volume with Milli Q water and then placed into polyethylene bottles at 4 °C. At least two different blanks were performed in each set of PHWE conditions.

Table 1
Pressurized hot water extraction conditions.

Modifier concentration (acetic acid)/mM	150
Extraction temperature/°C	100
Static time/min	5
Extraction step	4
Pressure/psi	1500
DE mass/sample mass ratio	5.0
Flush volume solvent/%	60
N_2 purge time/s	60
Cell size/mL	11