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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

2.5. *Microwave acid digestion procedure*

An acid digestion induced by microwave energy was used in order to determine the total content of the arsenic from human hair samples and also for comparative purposes. Sample (0.50 g) was placed into PTFE reactors and 2 and 8 mL of H_2O_2 and concentrated HNO_3, respectively, were added. The microwave energy program involved three steps; first from 0 to 1000 W (10 min), 5.0 min at 1000 W and finally from 1000 to 0 W (5.0 min) (maximum temperature of 200 °C). A venting stage for 45 min to cool down the reactors was also enclosed. After microwave digestion, the acid liquid phase was filtrated (Whatman n° 40 paper filter). The solutions were made up to 25 mL with ultra-pure water and kept into polyethylene bottles at 4 °C before measurements.

2.6. *ICP-MS measurements*

Total arsenic was measured by ICP-MS under operating conditions listed in Table 2. Determinations were performed by using aqueous standard solutions in 1.0 M nitric acid covering arsenic concentrations from 0 to 10 $\mu g\,L^{-1}$. Total arsenic in pressurized hot water extracts was also measured by ICP-MS using aqueous standard solutions in 150 mM acetic acid. Germanium at a concentration of 5.0 $\mu g\,L^{-1}$ was selected as an internal standard for As determinations. The use of H_2 in the collision cell at a flow rate of 5.85 mL min^{-1} gave the best sensitivity and linear ranges for arsenic determination.

2.7. *HPLC-ICP-MS measurements*

Anion exchange HPLC conditions were used to obtain the separation of four arsenic species (As(III), As(V), MMA and DMA) with a gradient elution consisted of diluted nitric acid solutions as mobile phases. The anion-exchange HPLC conditions as well as ICP-MS settings are summarized in Table 2. Different calibration curves using germanium (5 $\mu g\,L^{-1}$) as an internal standard were obtained by covering As(III), As(V), MMA and DMA concentrations of 0, 0.25, 0.50, 0.75, 1.0, 2.0, 4.0, 8.0 and 10 $\mu g\,L^{-1}$, expressed as As.

3. Results and discussion

3.1. *Variables affecting arsenic leaching by PHWE*

The factors that possibly affect the arsenic leaching from human hair are shown in Table 3. The experiments were performed at two levels for each investigated factors, coded as "−1" (low) and "+1" (high), the so-called boundaries of the experimental domain. These values were chosen according to literature data and instrumental limitations.

Modifiers and additives have been usually applied in PHWE to enhance the solubility of analytes in water or to increase interactions of water with the analytes. The addition of those substances to water alters both the properties of water and the critical temperature and pressure [10]. Several organic modifiers such as ethanol, methanol, acetone and acetonitrile, or carbon dioxide; and inorganic modifiers (mainly nitric acid (1–10% v/v)) have been used for arsenic and other organometallic compounds [10]. The use of high mineral acid concentrations causes clogging of tubes, frits and valves of the extraction system as a consequence of corrosion of stainless steel. Thus, acetic acid (M), at low concentrations (150–450 mM), was selected as modifier. Acetic acid aids to breakdown the ionic interactions and sulphur bonds between arsenic species and the cystine and/or amino acids in the keratin structure and in the follicular melanin of the hair.

The minimum and maximum extraction temperature (*T*) values allowed by the ASE system were room temperature (oven off) and 200 °C, respectively. Therefore, *T* factor was studied from 50 to 100 °C. Static time (*t*) affects the extraction yield. This variable was studied within the 5–10 min range. Analyte leaching could not be completed after only one equilibration step, so the use of several extraction cycles (*S*) is fully recommended. Thus, this variable was studied from 1 to 3. The minimum and maximum pressure values allowed by the ASE system were 500 and 3000 psi, respectively. Therefore, *P* was studied within the 500–1500 psi range. Mean particle size (*s*) could be an important factor. Mean particle size of 180 µm (+) from pulverized human hair (ball mill grinding for 45 min) and 100 µm (−) from pulverized human hair (ball mill grinding for 90 min) were

Table 3
Experimental field definition for the Plackett–Burman designs (PBD)[a].

Variable	Symbol	Low level (−)	High level (+)	Unit
Modifier concentration	M	150	450	mM
Extraction temperature	T	50	100	°C
Static time	t	5	10	min
Extraction step	S	1	3	
Pressure	P	500	1500	psi
Particle size	s	100	180	µm
DE mass/sample mass ratio	R	2	5	
Dummy factor	D	−1	+1	

[a] The human hair mass was 0.50 g for all experiments.

Table 2
ICP-MS and anion exchange HPLC conditions.

ICP-MS	Radiofrequency power/W	1400
	Peristaltic pump speed/rpm	2.5
	Nebulizer type	Beat impact (cooled spray chamber)
Gas flows/L min^{-1}	Plasma	14.0
	Auxiliary	0.8
	Nebulizer	0.85
CCT/mL min^{-1} Mass-to-ratio	H_2/He, 5.85	
	As	75
	Ge[a] (5 $\mu g\,L^{-1}$)	72
HPLC	IonPac AS7 (250 mm × 4 mm i.d.) anion-exchange column	
	Injection volume	100 µL
	Column temperature/°C	25
	Mobile phases flow rate/mL min^{-1}	1.35
Mobile phase A	1.0 mM nitric acid (pH 2.9), 1%(v/v) methanol	
Mobile phase B	80 mM nitric acid (pH 1.3), 1%(v/v) methanol	
	Gradient program	100% A, 3.5 min. 10% A, 5.0 min. 100% A, 1.0 min.

[a] Post-column addition for HPLC-ICP-MS determinations.

fixed. The capacity of the extracting cells (11 mL capacity) allows a maximum of 3.0 g of hair sample/DE mixture. A fixed hair sample mass of 0.50 g implies a maximum DE mass of 2.5 g and a DE mass/sample mass ratio of 5.0. Therefore, DE mass/sample mass ratio (*R*) was studied within the 2–5 range. Finally, a Dummy factor (*D*) (imaginary variable for which the change from one level to another is not supposed to cause any physical change) was also enclosed to evaluate the possible systematic error and/or the existence of an important variable that was not been considered.

3.2. *Statistically significant variables affecting arsenic pressurized hot water extraction*

The statistical significance of the variables commented above was evaluated by applying a $2^8 \times 3/32$ type III resolution PBD, for eight factors and four degrees of freedom. In total 12 non-randomised experiments were performed. The PBD experiments were carried out from 0.50 g of human hair sub-samples. The response variables (Table 4) were the percentages of released analyte (total arsenic, As(III), As(V) and As(III)+As(V)) according to the equation:

$$R(\%) = \frac{C_{\mathrm{PHWE}}}{C_{\mathrm{MAE}}} \times 100$$

where C_{PHWE} is the total As concentration (measured by ICP-MS) or As(III), As(V) and As(III)+As(V) concentrations (measured by HPLC-ICP-MS) obtained after the PHWE procedure and C_{MAE} is the arsenic concentration after microwave acid digestion procedure (70.3 ± 4.4 ng g^{-1}). It must be said that MMA and DMA are not present in the human hair sample used for optimizing PHWE conditions.

Analysis of the results given in Table 4 produces the Pareto chart (*P*=95.0%) shown in Fig. 1. In these charts, the length of each bar is proportional to the standardized effect. The vertical line on the plot judges the effects that are statistically significant. Bars that extend beyond the line correspond to effects that are statistically significant at the 95% confidence level.

Results showed statistical significance for some factors, such as extraction temperature (*T*) and extraction step (*S*). The variable modifier concentration (*M*), static time (*t*), particle size (*s*), extraction pressure (*P*) and DE mass/sample mass ratio (*R*) have not been statistically significant factors. This fact offers several advantages: the use of low acetic acid concentration guarantees the chemical integrity of extracted species for speciation studies; short static times (5 min) can be chosen to perform the extractions; sample pulverization to guarantee a certain particle size is not required. Finally, the dummy factor (*D*) was not significant for

Table 4
PBD for determination of significant parameters involved on PHWE.

Run no.	A	T	t	S	P	s	R	D	Recovery/%[a]			
									Total As	As(III)	As(V)	As(III)+As(V)
1	+	−	+	−	−	−	+	+	−[b]	9.5 ± 0.3	−[b]	9.5 ± 0.3
2	+	+	−	+	−	−	−	+	86.0 ± 2.1	43.8 ± 1.7	44.2 ± 2.3	88.0 ± 2.8
3	−	+	+	−	+	−	−	−	50.5 ± 1.6	24.1 ± 1.8	20.4 ± 0.64	44.5 ± 2.0
4	+	−	+	+	−	+	−	−	22.1 ± 1.2	9.9 ± 0.4	5.6 ± 0.3	15.5 ± 0.5
5	+	+	−	+	+	−	+	−	76.5 ± 1.3	41.5 ± 1.8	38.8 ± 1.0	80.3 ± 2.6
6	+	+	+	−	+	+	−	+	49.5 ± 0.9	36.7 ± 2.3	28.4 ± 2.1	65.1 ± 3.1
7	−	+	+	+	−	+	+	−	66.0 ± 1.4	47.8 ± 3.3	32.1 ± 1.2	79.9 ± 3.6
8	−	−	+	+	+	−	+	+	8.0 ± 0.5	7.1 ± 0.4	7.9 ± 0.2	15.0 ± 0.4
9	−	−	−	+	+	+	−	+	−[b]	4.1 ± 0.1	3.9 ± 0.1	8.0 ± 0.1
10	+	−	−	−	+	+	+	−	10.5 ± 0.7	6.0 ± 0.5	−[b]	6.0 ± 0.5
11	−	+	−	−	−	+	+	+	84.0 ± 3.2	38.3 ± 2.1	38.0 ± 1.9	76.3 ± 2.9
12	−	−	−	−	−	−	−	−	−[b]	4.0 ± 0.1	6.1 ± 0.4	10.1 ± 0.4

[a] N=2.

[b] Concentration are below LOQ.

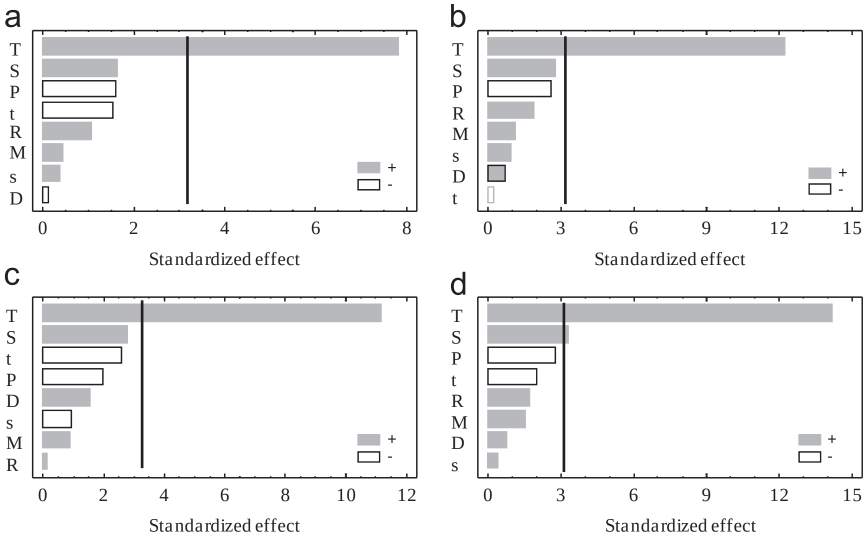

Fig. 1. Standardised (*P*=95%) Main Effects Pareto Charts for the Plackett–Burman Design for (a) Total arsenic, (b) As(III), (c) As(V), and (d) As(III)+As(V).

any case, meaning that there is neither systematic error nor unknown variables affecting the system.

For the next stage in the optimisation process, modifier concentration, static time, pressure and DE mass/sample mass ratio were fixed at 150 mM, 5 min, 1500 psi and 5, respectively. The above commented values were selected according to different aspects such as, reagents saving (low value for modifier concentration) and extraction time saving (low values for static time was chosen).

3.3. Optimisation of significant variables by univariate approach

Screened out the variables that did not have a significant effect on the response, the remaining factor affecting arsenic species leaching (extraction temperature and extraction steps) were optimised by using univariate approaches. 100 °C and four extraction step were selected as optimum values (Figs. 2 and 3). The optimisation of those variables by using experimental design (i.e. central composite design) is limited due to extraction steps only could be fixed at discrete values (1, 2, 3, 4 or 5 steps). Given these findings, we decided to work with the optimum values obtained for the factors studied (Table 1).

Fig. 4 shows a typical chromatogram (HPLC-ICP-MS) for an arsenic species standard (0.5 μg L^{-1} of As(III), MMA, DMA and As(V) (a); and a blank (b) and a hair sample (c) after PHWE by using the optimized conditions commented above, where major species As(III) and As(V) can be seen.

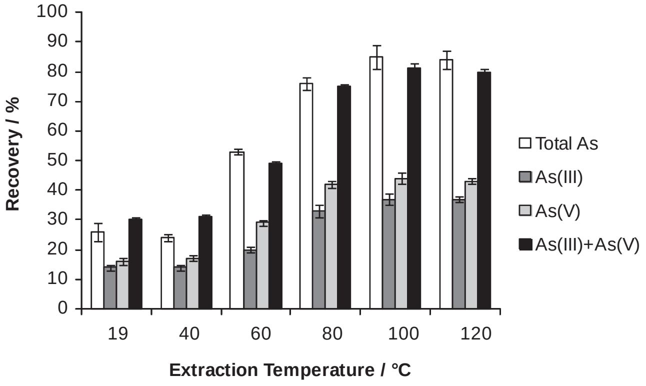

Fig. 2. Effect of extraction temperature for the extraction of total As, As(III), As(V) and As(III)+As(V) from a hair sample (N=3). Other PHWE conditions: 150 mM of modifier concentration, four extraction step of 5 min, 1500 psi and DE mass/sample mass ratio of 5.

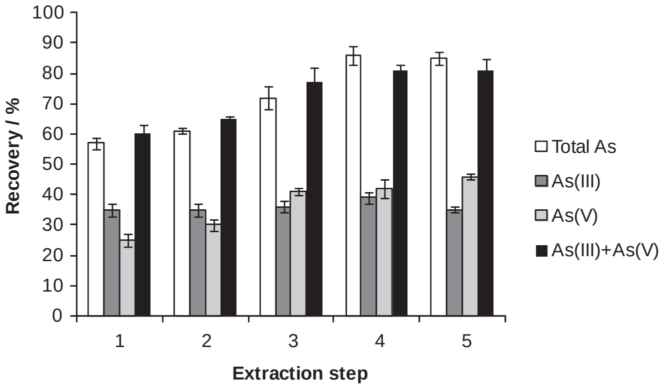

Fig. 3. Effect of extraction step for the extraction of total As, As(III), As(V) and As(III)+As(V) from a hair sample (N=3). Other PHWE conditions: 150 mM of modifier concentration, extraction temperature of 100 °C, extraction time of 5 min, 1500 psi and DE mass/sample mass ratio of 5.

3.4. Figures of merit

Figures of merit of total arsenic (by ICP-MS) and arsenic species (by HPLC-ICP-MS) determination were assessed. Table 5 lists the mean and standard deviation of the slopes of calibration graphs for each analyte. LODs expressed as ng g^{-1} in accordance with sample mass weight and final volumes, are also shown in Table 5. It can be seen that the values are low enough to perform As speciation in human hair. The repeatability of the overall procedure has been obtained by performing a pressurised hot water extract from a CRM (GBW−07601 Human Hair) eleven times. Each leachate was analysed once and the RSD values were 4.4, 7.2 and 2.1 % for total arsenic, As(III) and As(V), respectively. The reproducibility was also assessed for the pressurized hot water method. This study was performed on seven different days by using GBW−07601 Human Hair. Results, expressed as RSD were 15, 18 and 18 % for total arsenic, As(III) and As(V), respectively.

Accuracy of the proposed method was assessed by analysing GBW-07601 (certified total arsenic concentration of 280 ± 5 ng g^{-1}). This CRM was subjected to the optimised PHWE procedure seven times and each extract was analyzed twice by ICP–MS (total As) and HPLC-ICP-MS (mainly As(III) and As(V)). Table 6 lists the analyte concentrations in GBW-07601, expressed as the mean ($\bar{x}$) ± the standard deviation for n=11 measurements (Sd) at a confidence level of 95%.

The extraction efficiency (calculated as: (total As in human hair PHWE extracts/total As certified in human hair) × 100) around 85.2 ± 9% was achieved. The extraction efficiency for total arsenic as sum of As(III) and As(V) is 80.3 ± 1.4%. These extraction efficiencies are higher than previously reported by using PLE [12].

As(III) and As(V) contents are not certified in GBW-07601 but some reported concentrations can be found in the literature. For As(III) and As(V) concentration of 94.2 ± 2.1 and 130.6 ± 3.2 ng g^{-1}, respectively were found, which are similar to most of the As(III) and As(V) concentrations reported in this CRM by other authors (from 72 to 94 and from 81 to 101 ng g^{-1} for As(III) and As(V) respectively) [12–16]. Finally, MMA and DMA concentrations found in GBW-07601 were not quantified due to its concentrations (around 4.2 and 2.1 ng g^{-1} for MMA and DMA, respectively, [14]) are lower than LOQs of the proposed method. These authors used a previous pre-concentration step (dual-column capillary micro-extraction) after electrothermal vaporization inductively coupled plasma mass spectrometry (ETV-ICP-MS) determination [14].

3.5. Evaluation of human hair samples

The proposed method was applied to several human scalp hair samples from healthy people. Each sample was subjected twice to the PHWE process, each extract was measured four times. Concentrations ranged from 60 to 301 ng g^{-1} for total As, 21 to 131 ng g^{-1} for As(III) and 29 to 171 ng g^{-1} for As(V) were obtained. MMA and DMA concentrations in these hair samples were lower than LOQs of the method.

Typical total As background levels are in the range of 80–250 ng g^{-1} [13]. Australian Government Public Service established as indicator for As toxicity level a value of 1.0 μg As g^{-1} [17]. Thus, results found in analysed hair samples showing values within these intervals, indicating absence of critical exposure to arsenic. In addition, results obtained are in agreement with total As levels (ranged from 10 to 285 ng g^{-1}) from As un-exposed people from China, Japan, India, Canada, USA, Poland, Italy, Nigeria and Malaysia [14,18–22]. These As values are very low when comparing with data from population exposed to As contamination. Total As values of 3.43 ± 0.73 μg g^{-1} [23], 2.45 ± 1.11 μg g^{-1} [16], 4.0 ± 2.0 μg g^{-1}

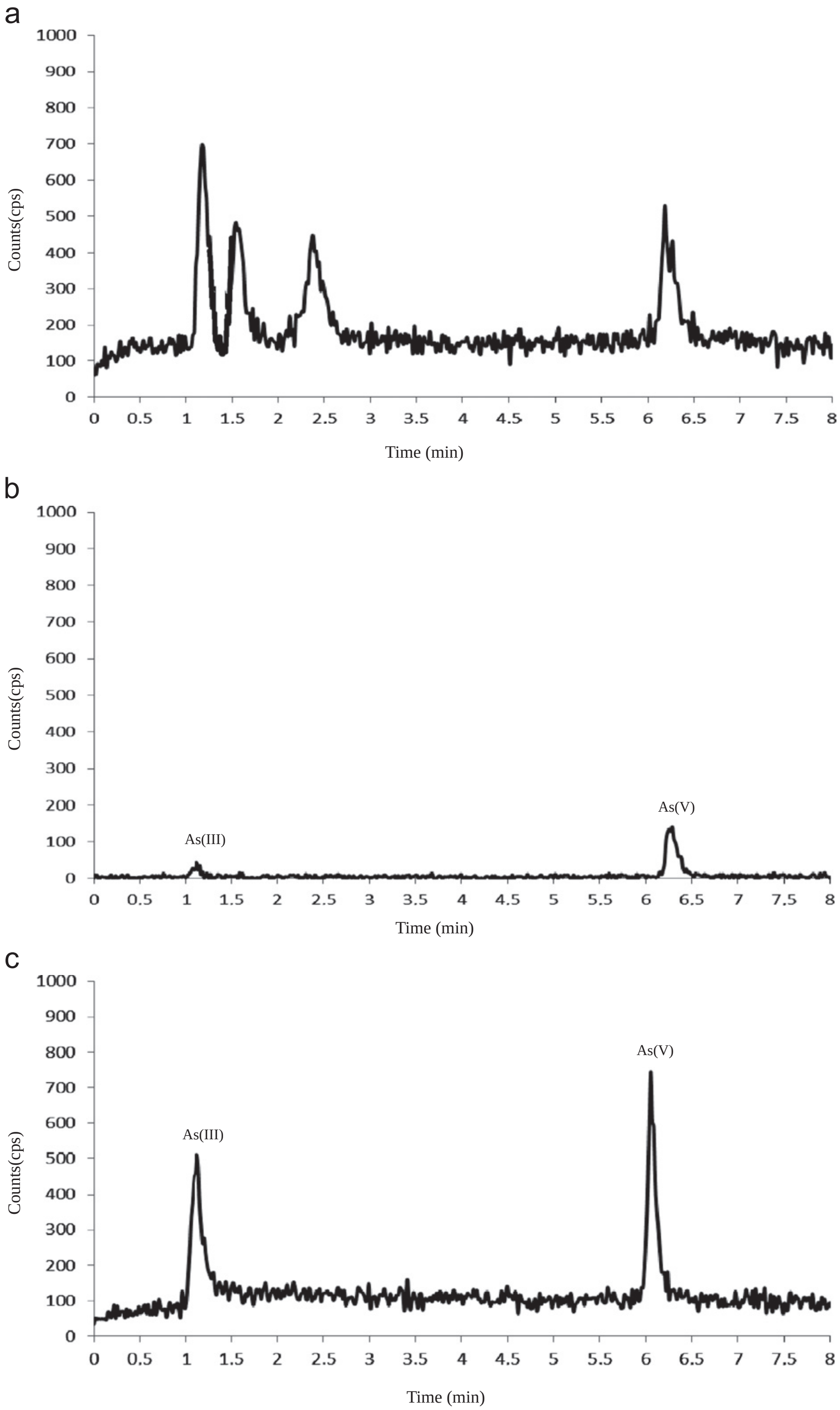

Fig. 4. HPLC-ICP-MS chromatograms of an arsenic species standard 0.5 μg L^{-1} of As(III), MMA, DMA and As(V) (a); and a PHWE extract from a blank (b) and a human hair sample (c) using the optimized conditions: 150 mM of modifier concentration, extraction temperature of 100 °C, four extraction step of 5 min, 1500 psi and DE mass/sample mass ratio of 5.

[15] and 5.96 ± 2.57 μg g^{-1} [13] were found from people of West Bengal (India) [13,15,16,23] and Bangladesh [13] who were drinking As-contaminated water. The arsenic species distribution in hair samples is in good agreement with the few available data [13–16] where the samples contained mainly inorganic arsenic.

4. Conclusions

A simple, green, high efficient and automated leaching procedure for arsenic species extraction by using pressurized hot water from human hair samples has been novelty developed. Automation

Table 5
Mean slopes for calibration, and limits of detection and quantification[a].

	Mean calibratio Slope ± S.D.[b]	LOD[c]/ng g^{-1}	LOQ[c]/ng g^{-1}
Total As	0.025 ± 0.01	2.1	7.0
As(III)	5599 ± 179	1.1	3.7
As(V)	6084 ± 130	1.0	3.3
MMA	5135 ± 233	8.6	28.7
DMA	4827 ± 694	6.3	21.0

[a] Obtained for sample weight of 0.5 g and a final volume of 25 mL.
[b] $n=7$.
[c] $n=11$.

Table 6
Analysis of GBW-07601 after pressurized hot water extraction procedure.

	Certified value/ng g^{-1}	Found value/ng g^{-1}
Total As	280 ± 5	238.6 ± 5.3
As(III)	72–94[a]	94.2 ± 2.1
As(V)	81–10[a]	130.6 ± 3.2
MMA	0.8–4.2[a]	–[b]
DMA	1.6–5.4[a]	–[b]
As(III)+As(V)	–	224.8 ± 3.8

[a] Not certified. Concentration range reported by several authors [12–16].
[b] < LOQ.

(the addition of the extracting solution is totally automated) and the use of non corrosive reagents (pressurised hot water modified with low acetic acid concentration can be considered as an environmental friendly procedure) are the main advantages of the current proposal. Because of centrifugation or filtration steps to separate the extract and the solid residues are omitted (extracts handling is minimised) when using PHWE, the total extraction time is shortened. The application of Plackett–Burman designs, as factor screening, shows that extraction temperature and extraction steps are the most significant variables involved on the extraction of major arsenic species (As(III) and As(V)) in human hair by PHWE. The use of anion exchange chromatography and detection by ICP-MS has offered good sensitivity, repeatability and accuracy. Organic arsenic species (MMA and DMA), although separated by the HPLC method, were not quantified in any studied sample.

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