Validation of method for the separation and quantification of Arsenic species in human urine samples by HPLC/ICP-MS

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Abstract
The importance of arsenic speciation is being increasingly recognized by the scientific community because intrinsic arsenic species are known to exhibit differences in mobility, bioavailability and toxicity. A variety of organic and inorganic species have been identified in environmental and biological samples. The aim of this study was to determine the optimal operating parameters for chemical speciation of arsenic in human urine samples. HPLC/ICP-MS technique was employed to determine arsenobetaine (AsB), dimethyl arsenic acid (DMA), monomethyl arsenic acid (MMA), arsenite [As (III)] and arsenate [As (V)] in human urine samples. The separation was carried out on the Hamilton PRP X100 column, using two mobile phases: phase A containing 10 mM ammonium carbonate and phase B containing 20 mM ammonium carbonate at pH 8.83-9.35. The performance of the method was evaluated in terms of linearity, precision, accuracy and detection and quantification limits. The results demonstrate that the HPLC/ICP-MS methodology proposed is a sensitive, reproducible and accurate technique for the determination of arsenic species at trace levels in human urine.

Introduction
Arsenic is a ubiquitous element which enters the environment through both natural and anthropogenic sources. Its toxicity, environmental mobility and accumulation in living organisms usually depend on the chemical form. It is important for understanding the role of the element present as well as revealing its environmental cycle. These requeriments stimulate the need of information on the arsenic speciation and the development of suitable analytical methodology (McSheehy et al., 2003).
The human intake of arsenic mainly occurs via the diet and drinking water, whilst some individuals may be additionally exposed to arsenic through the workplace environment. Consumers highly exposed to arsenic are those with a high consumption of seafood (mainly as AsB) or people from areas of the world with a naturally high level of arsenic in their drinking water, mainly as inorganic arsenic (Davis et al., 2010). Inorganic forms of arsenic are most toxic, whereas the methylated forms (MMA, DMA) are low in acute toxicity and AsB is considered non-toxic (Xie et al., 2006; Milstein et al., 2003). Consequently, a toxicological evaluation of dietary arsenic intake and environmental exposure should be based on arsenic speciation data (Sloth et al., 2004).
The coupling of high-performance liquid chromatography (HPLC) to inductively coupled plasma mass spectrometry (ICP-MS) is a powerful technique for trace elemental speciation analysis in various sample matrices. HPLC/ICP-MS combines the high separation efficiency of HPLC with the superior selectivity and sensitivity of ICP-MS (Chen et al., 2004).
The aim of this study was to determine the optimal operating parameters for chemical speciation of arsenic in human urine samples.

Materials and Methods
Instrumentation: The chromatographic mobile phase was degassed and pumped through the analytical column to the ICP-MS (Thermo X-Serie II) using a Finnigan Surveyor Quaternary Gradient Pump (20 µL sample injection volume). The ICP-MS technique was employed as detector to quantify the elemental species constituents. The separation was performed on the Hamilton PRP X100 column, using two mobile phases: phase A containing 10 mM ammonium carbonate (J.T. Baker) and phase B containing 20 mM ammonium carbonate at pH 8.83-9.35. The time of analysis was 30 minutes.
Standard substances and sample preparation: Laboratory pure 18 MΩ deionized water was used for standards and samples preparation. Standard solutions of the following chemicals were prepared in water: arsenobetaine (AsB, Sigma Aldrich), cacodylic acid sod. salt (DMA, J.T. Baker), disodium methylarsenate (MMA; Chem Service), standards solutions of 1000 µg ml^{-1} of As_2O_3 in 2% HCl (As III, High Purity Standards) and metallic arsenic in 2% NaOH + $TrBr_2$ (As V, High Purity Standards).
Prior the speciation analysis, samples were thawed at room temperature and filtered with Millipore 0.20 µm filter.

Results and Discussion
To validate the method were analyzed a standard reference material (NIST 2669, arsenic species in frozen human urine) and a internal control samples of

human urine. Six blank samples were analyzed in duplicate to determine the detection (DL) and quantification (QL) limits of the method. The results showed high values of DL and QL for MMA species. It is due to the interference with $^{40}Ar^{35}Cl$ (Table 1).

Table 1 Detection and quantification limits and their correlation coefficients.

As species	DL ($\mu g\ L^{-1}$)	QL ($\mu g\ L^{-1}$)	Correlation coefficients
AsB	0.086	0.225	0.9991
As(III)	0.320	0.738	0.9992
DMA	0.117	0.307	0.9999
MMA	1.900	5.026	0.9999
As(V)	0.104	0.348	0.9994

A dilution of 1:4 with deionized water in urine samples was mandatory in order to minimize matrix effects.
The accuracy of the method was determined by analyzing a standard reference material (SRM 2669, arsenic species in frozen human urine). All values are within the reported uncertainty for each species. The MMA could not be analyzed because its concentration is below to the limit of quantification (Table 2).

Table 2 Quantitative results for standard reference material 2669.

As species	Concentration in urine ($\mu g\ L^{-1}$)	Certified values SRM 2669 ($\mu g\ L^{-1}$)
AsB	11.228	12.4 ± 1.9
As (III)	4.752	5.03 ± 0.31
DMA	3.352	3.47 ± 0.41
As (V)	5.313	6.16 ± 0.95

A 1:4 aliquot of internal control samples of human urine was also spiked with 10 $\mu g\ L^{-1}$ of each species. Table 3 shows the obtained recoveries. As can be seen, excellent recoveries for AsB, As (III), DMA, MMA and As (V) in urine samples have been obtained, demonstrating that the proposed methodology is suitable for arsenic speciation analysis. Retention times for the quantified arsenic species were stable throughout the analysis and were not affected by the sample matrix.

Table 3 Quantitative results for internal control samples of human urine

As species	Concentration in urine ($\mu g\ L^{-1}$)	Recovery % spike 10 $\mu g\ L^{-1}$
AsB	0.240	99.96
As (III)	2.292	110.51
DMA	3.796	99.04
MMA	3.452	103.69
As (V)	0.476	94.32

Conclusions

The results showed that the proposed HPLC/ICP-MS methodology is a sensitive, reproducible and accurate technique for determination of trace levels of arsenic species in human urine. Nevertheless, a minor shortcoming is the time of analysis, greater than 30 min per sample.

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