Rapid screening of arsenic species in urine from exposed human by inductively coupled plasma mass spectrometry with germanium as internal standard

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Abstract

In the present work, internal standardization based on species-unspecific isotope dilution analysis technique is proposed in order to overcome the matrix effects and signal drift originated in the speciation of As in urine by HPLC-ICP-MS. To this end, ^{72}Ge has been selected as a pseudo-isotope of As. The resulting mass flow chromatogram of the element allows the calculation of the corrected overall species concentrations without requiring any methodological calibration, providing high-throughput sample processing. The validation was carried out by analyzing a blank human urine fortified at three concentration levels and an unspiked human urine sample containing different species of arsenic. In all cases, recoveries ranging from 90 to 115% and RSD below 10% were attained with this approach. Furthermore, the proposed method provided results in excellent agreement with those obtained using standard additions and internal standard calibration, allowing a fast way to assess human exposure to arsenic species.

1. Introduction

It is widely known that potential health risk to arsenic exposition depends on the chemical form entering the human body, due to the different degree of toxicity of these compounds.[1-4] Urine is regarded as an important biomarker of arsenic intake. In this regard, a speciation analysis of urine is usually performed by HPLC-ICP-MS.[5-10] However, signal drift and matrix effects are observed due to urine matrix, hampering the quantification of such species.[11]

The use of species-unspecific isotope dilution analysis allows the correction for those errors, providing accurate and precise determinations of the sought element. In the case of arsenic, there is only one isotope available to be measured (m/z 75); therefore, an internal standard of an element close to the analyte mass has to be selected to follow the isotope dilution procedure.[12] This approach, which we have called "pseudo-unspecific isotope dilution analysis", could make possible to obtain the accurate concentrations of the above mentioned species in a single run.

The developed method was applied for the analysis of human urine samples fortified at different concentration levels and compared with internal calibration and the standard additions method.

2. Experimental

2.1. Instrumentation

The HPLC system consisted of an Agilent 1100 Series (Agilent, Waldbronn, Germany) binary pump and auto injector with a programmable sample loop (100 μL maximum). The separations were performed on a PRP-X100 (Hamilton, Reno, NV, USA) anion-exchange column (250 x 4.1 mm, 10 μm). An additional Agilent 1100 Series binary pump was used to continuously add the internal standard solution.

The outlet of the chromatographic column was connected through a T piece to a Meinhard concentric nebulizer. An Agilent 7500cx inductively coupled plasma mass spectrometer (Agilent Technologies, Tokyo, Japan) equipped with an octopole reaction cell using helium as a reaction gas to reduce polyatomic interferences on arsenic was used in this work. For HPLC-ICP-MS data acquisition, the "time resolved analysis" mode was used with 1 second of integration time per mass.

For tuning of ICP-MS, a solution containing 10 μg L^{-1} of As made up in double deionized water filtered through 0.45 μm was monitored at m/z 75 and 72; the ion intensity, resolution and mass axis were optimized.

2.2. Standards and reagents

Arsenite (As^{III}), arsenate (As^{V}), dimethylarsinic acid (DMA) and arsenobetaine form (AsB) were delivered by Fluka (Buchs, Switzerland), while monomethylarsonic acid (MMA) was from Carlo Erba (Milano, Italy). The stock solutions of arsenic species containing about 1000 mg L^{-1} of As were prepared in water and maintained at 4 ºC after standardization against an atomic absorption arsenic standard solution (J.T. Baker, Phillipsburg, USA).[13] Appropriate dilutions of the stock solution were prepared daily, by weight, using double deionized water to obtain the required concentration. Germanium internal standard and nitric acid was purchased from Fluka.

A previously developed method[5] based on HPLC-ICP-MS coupling system was optimized in order to separate the five arsenic species in human urine. The mobile phase consisted of 4 mM ammonium phosphate (Merk, Darmstad, Germany), 4 mM ammonium hydrogen carbonate (Fluka) and 4 mM ammonium sulfate (Sigma, St. Louis, MO, USA). The pH value was adjusted to 8.9 by the addition of ammonium hydroxide (Trace Select, Fluka). These solutions were filtered through a 0.45 μm membrane before use.

All the aqueous solutions were prepared with Milli Q Gradient A10 (Millipore, Molsheim, France) water (18.2 MΩ cm).

2.3. Analytical procedure

Urine samples were diluted 5 fold with 0.1 % nitric acid before injection. The flow coming from the column (0.95 mL min^{-1}) was mixed with the internal standard solution containing around 15 ng g^{-1} of Ge and 4 ng g^{-1} of As (0.15 mL min^{-1}). The signals for m/z 75 and 72 were monitored over time. After smoothing of the data using moving average (n = 5) in order to reduce noise level the isotope ratio $^{75}As/^{72}Ge$ was calculated. Then, the on-line pseudo-isotope dilution equation was applied to each point of the chromatogram to obtain the mass flow chromatogram. The amount of arsenic in each fraction was determined by integration of the chromatographic peaks using the Origin 5.0 software (Microcal Software Inc., Northampton, MA, USA). Finally, the concentration of arsenic was computed by dividing the As amount found by the injection volume.

3. Results and discussion

3.1. Selection of internal standard and development of the equation for on-line pseudo-isotope dilution analysis

In order to appropriately correct for matrix-induced signal enhancement or suppression as well as for drift instability of the instrument, the analyte and the internal standard should undergo an equal relative signal intensity drift. To this end, mass-to-charge ratio and ionization potential of both elements should be as close as possible, being especially critical the first factor. In this regard, selenium seems the best candidate to use as internal standard for arsenic speciation.[12,14] However, this element is often present in urine samples, which can produce errors in the normalization. By contrast, germanium is rarely present in urine samples and has been satisfactory used for matrix effects correction.[15] As a consequence, the isotope ^{72}Ge was selected as internal standard.

The proposed procedure is based on post-column isotope dilution analysis.[16] Briefly, this technique consists in the on-line addition of an isotopically enriched solution of the sought element after the chromatography separation to modify the original isotope abundances in the sample. The resulting isotope ratio (in the mixture) of the most abundance isotope in the sample and the spike permits to calculate the endogenous concentration contained in each chromatographic peak. In the case of arsenic, a germanium internal standard is used instead of a spike, owing to its monoisotopic character. Since both elements have different ionization efficiencies, the experimental isotope ratio in the mixture R_m (^{75}As/^{72}Ge) will not provide the As/Ge molar ratio. The instrumental response of Ge present in the mixture must be previously normalized to As in order to correlate R_m with the analyte concentration. For this purpose, a known amount of As was added within the internal standard solution and the corresponding isotope ratio R_{IS} (^{75}As/^{72}Ge) was measured. Consequently, R_m and R_{IS} can be expressed as follows:

105 $$R_m = \frac{N_s^{As} \cdot d_s \cdot f_s + N_{IS}^{As} \cdot d_{IS} \cdot f_{IS}}{N_{IS}^{Ge'} \cdot d_{IS} \cdot f_{IS}} \quad (1)$$

106 $$R_{IS} = \frac{N_{IS}^{As}}{N_{IS}^{Ge'}} \quad (2)$$

107 were N_s^{As} (mol g^{-1}) shows the amount of As in the sample with density d_s (g mL^{-1}) pumped at
108 a flow rate f_s (mL min^{-1}), which is mixed with N_{IS}^{As} (mol g^{-1}) of As arising from the internal
109 standard solution pumped at a flow rate f_{IS} (mL min^{-1}) and density d_{IS} (g mL^{-1}). The term
110 $N_{IS}^{Ge'}$ (mol g^{-1}) is the concentration of Ge normalized to As, which must not be confused with
111 the true amount of Ge. Indeed, $N_{IS}^{Ge'}$ would represent the mol g^{-1} of ^{72}Ge contained in the
112 internal standard solution if the ionization efficiency were the same as As. When we combine
113 eqns. (1) and (2) the following expression is obtained:

114 $$R_m = \frac{N_s^{As} \cdot d_s \cdot f_s + N_{IS}^{As} \cdot d_{IS} \cdot f_{IS}}{(N_{IS}^{As} / R_{IS}) \cdot d_{IS} \cdot f_{IS}} \quad (3)$$

115 Please note that the true amount of Ge is not needed in the calculation. Rearranging eqn.
116 (3) for N_s^{As}, we obtain:

117 $$N_s^{As} \cdot d_s \cdot f_s = N_{IS}^{As} \cdot d_{IS} \cdot f_{IS} \cdot \left(\frac{R_m}{R_{IS}} - 1 \right) \quad (4)$$

118 Concentrations in mol g^{-1} can be expressed as concentrations in weight by taking into
119 account the atomic weight of the element. Since the atomic weight of As in the sample
120 (AW_s^{As}) and in the internal standard solution (AW_{IS}^{As}) are the same, eqn. (4) becomes:

121 $$C_s^{As} \cdot d_s \cdot f_s = C_{IS}^{As} \cdot d_{IS} \cdot f_{IS} \cdot \left(\frac{R_m}{R_{IS}} - 1 \right) \quad (5)$$

122 were C_s^{As} and C_{IS}^{As} are the mass concentrations (ng g^{-1}) of As in the sample and internal
123 standard solution, respectively. $C_s^{As} \cdot d_s \cdot f_s$ has the units of ng min^{-1} and it is the mass flow of

the sample eluting from the column, MF_s. Then, the final pseudo-isotope dilution equation has the form:

$$MF_s = C_{IS}^{As} \cdot d_{IS} \cdot f_{IS} \cdot \left(\frac{R_m}{R_{IS}} - 1 \right) \quad (6)$$

If the analyte concentration changes with time, e.g., during the chromatographic peak, MF_s will also change with time. The integration of the chromatographic peak in the mass flow chromatogram will give the amount of As in that fraction. The concentration is then easily calculated knowing the sample volume injected. Eqn. (6) was thus used for calculations in the present work.

3.2. Analytical results

In a preliminary study, the concentration of the As primary standard in the internal standard solution was optimized. On the one hand, it has to be taken into account that a high enough amount of exogenous As is required to minimize the m/z 75 background influence. On the other hand, the higher amount of ^{75}As coming from the post-column solution the higher baseline noise, leading to poorer detection limits of the endogenous species. Thereby, a concentration of ca. 4 ng g^{-1} was selected as a compromise. The case of Ge internal standard concentration is much less critical because it does not contribute to the signal of the analyte eluting from the column and does not influence the final results. In addition, the chromatographic and post-column flow rates were tested. The final values used for the mobile phase and the internal standard solution (0.95 and 0.15 mL min^{-1}, respectively) allowed the elution of all the species in a proper time without sacrificing the accuracy of the post-column flow rate.

To a better understanding of the procedure, Fig. 1 illustrates the conversion from original ICP-MS intensities to mass flow. The chromatograms corresponding to m/z 75 and 72 (a) are first transformed into the isotope ratio chromatogram (b). It is worth stressing that ^{75}As and ^{72}Ge background signal from mobile phase was negligible (data not shown), thus the baseline

149 of the isotope ratio chromatogram provides R_{IS} (see Fig. 1b). Next, equation (6) is applied to
150 the whole chromatogram. Finally, the mass flow peaks of Fig. 1c are integrated and divided
151 by the injection volume (50 μL). It should be remarked that the present strategy permits to
152 correct for errors derived from instrumental instabilities and matrix effects in the whole
153 chromatogram, since the Ge internal standard is continuously added to the effluent from the
154 column.

155 The proposed procedure was applied to the analysis of a blank urine sample spiked at 1, 5
156 and 10 μg As L^{-1} (concentrations referred to the diluted urine injected). It is worth noting that
157 the blank urine used corresponds to an unexposed human and no As species were found when
158 it was analyzed by the conventional calibration method. Ten replicates for each of the three
159 fortification levels were carried out. Additionally, in order to check the suitability of ^{72}Ge as
160 internal standard the results were compared with those obtained using internal standard
161 calibration. To this end, calibration standards containing 0-20 μg L^{-1} of As for each compound
162 were injected by triplicate within the post-column solution. Then, the isotope ratio
163 chromatogram was plotted (as exemplify in Fig. 1b). Satisfactory recoveries, between 90 to
164 105%, were obtained both for the medium and highest fortification levels when pseudo-
165 unspecific IDA was used. At the lowest fortification level, which was closed to the detection
166 limit, recoveries were in the range of 96-115% and coefficients of variation were below 10%
167 (Table 1). No significant differences were noticed between both methods, thus it seems that
168 calibration-free measurements based on eqn. (6) can be performed for the quantification of As
169 species in urine.

170 Intermediate precision (n = 9) was also estimated by analyzing replicates of the medium
171 fortification level on 3 different days. The coefficient of variation was found to be <8% in all
172 cases. Detection limits, defined as three times the signal-to-noise ratio in the mass flow
173 chromatogram were determined for the blank urine sample spiked at 1 μg L^{-1} of each As

174 species. As can be seen in Table 1, LODs were <0.7 μg L^{-1} in the diluted urine. In fact, the
175 continuous addition of arsenic post-column to normalize de germanium response increase
176 notably the detection limits. However, these values are satisfactory to evaluate the potential
177 risk of people exposed to inorganic arsenic. Actually, the American Conference of
178 Governmental Industrial Hygienists (ACGIH) and Deutsche Forschungsgemeinschaft (DFG)
179 set the BEI and BAT values for occupational arsenic exposure as 35 μg As L^{-1} and 50 μg L^{-1},
180 using the sum of inorganic arsenic, MMA and DMA.[17]

181 The validity of the proposed method for correcting instrument signal drift was tested with
182 the injection of a 15-h batch run. Fig. 2a compares the intensity chromatograms
183 corresponding to the first and last injections of the experiment. Appreciable signal drift was
184 observed between them, resulting in lower peak areas for the second injection. The
185 application of eqn. (6) lead to the mass flow chromatograms presented in Fig. 2b. As
186 expected, no significant differences were noticed between injections, which confirm that
187 appropriate correction of signal drift is achieved. It is worth mentioning the severe signal
188 suppression at the dead volume caused by the high salt content of urine sample (Fig. 2a). In
189 this case, instrumental instability was not totally overcame (Fig. 2b), most probably because
190 the Ge internal standard suffers more signal depression than As in the presence of high
191 concentration of Na. Anyway, such anomalous behavior does not affect any chromatographic
192 peak

193 Finally, the quantification of a human urine sample containing different species of arsenic
194 was performed by the present methodology, internal standard calibration and standard
195 additions (Table 2). The concentrations calculated by pseudo-unspecific IDA were in very
196 good agreement with those obtained using the other quantification strategies. These data
197 confirm the suitability of the developed procedure for arsenic speciation studies in human
198 urine.

Conclusions

A new procedure for the simultaneous determination of AsB, As^{III}, As^{V}, DMA and MMA in human urine which does not require any methodological calibration graph and allows correcting for instrumental instabilities has been developed. For this purpose, species unspecific isotope dilution analysis has been adapted to As using ^{72}Ge as an additional isotope of the sought element.

The proposed method has been successfully validated in spiked and unspiked human urinesamples. In addition, the results were in excellent agreement with internal standard calibration and standard additions.

The need for addition a known amount of As to normalized the Ge concentration increases appreciably the LODs. This fact however, do not hampers the correct quantification of toxic inorganic species of arsenic in urine of exposed humans

Therefore, the possibility to carry out the quantification of As species in a single run provided by the developed procedure could be very useful to assess workplace, drinking water or food exposure to inorganic arsenic.

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Table 1. Analytical characteristics of the pseudo-unspecific IDA procedure obtained for a blank urine sample fortified at three different levels.

Species	1 µg As L^{-1}		5 µg As L^{-1}		10 µg As L^{-1}		Intermediate precision. 5 µg As L^{-1} C.V. (%) (n=9)	LOD[c] (µg L^{-1})
	Pseudo-IDA	IC[b]	Pseudo-IDA	IC	Pseudo-IDA	IC		
AsB	96.8 (8.7)[a]	104.6 (10.0)	90.8 (1.8)	99.4 (1.5)	92.1 (2.2)	101.3 (0.9)	6.2	0.3
As^{III}	99.5 (5.9)	111.0 (4.0)	100.7 (7.4)	106.5 (5.1)	101.1 (4.9)	104.8 (0.9)	7.4	0.6
DMA	102.0 (6.3)	106.5 (3.5)	97.5 (9.5)	94.3 (6.9)	100.0 (2.3)	94.4 (1.6)	3.5	0.3
MMA	114.6 (1.7)	115.6 (2.4)	101.1 (9.2)	101.8 (6.8)	102.6 (3.0)	101.6 (3.3)	5.5	0.4
As^{V}	104.0 (3.6)	104.2 (5.4)	95.7 (6.6)	103.1 (6.8)	96.5 (1.1)	103.1 (0.4)	3.8	0.7

[a] The uncertainty in the values corresponds to 1 s standard deviation of 10 independent HPLC-ICP-MS injections.
[b] Recoveries calculated using internal standard calibration, for comparison.
[c] Detection limits referred to diluted urine sample.

Table 2. Comparison of different methodologies to correct for matrix effects in the analysis of a human urine sample.

Method	AsB, µg L^{-1}	As^{III}, µg L^{-1}	DMA, µg L^{-1}	MMA, µg L^{-1}	As^{V}, µg L^{-1}	Sum of the species
Pseudo-unspecific IDA	15.5 ± 0.3	ND	3.2 ± 0.2	2.5 ± 0.5	3.8 ± 0.3	25.0 ± 1.3
Internal standard calibration	16.4 ± 0.3	ND	3.4 ± 0.2	2.1 ± 0.5	4.0 ± 0.5	25.9 ± 1.5
Standard additions	14.6 ± 0.1	ND	3.0 ± 0.1	2.4 ± 0.1	3.5 ± 0.1	23.5 ± 0.4

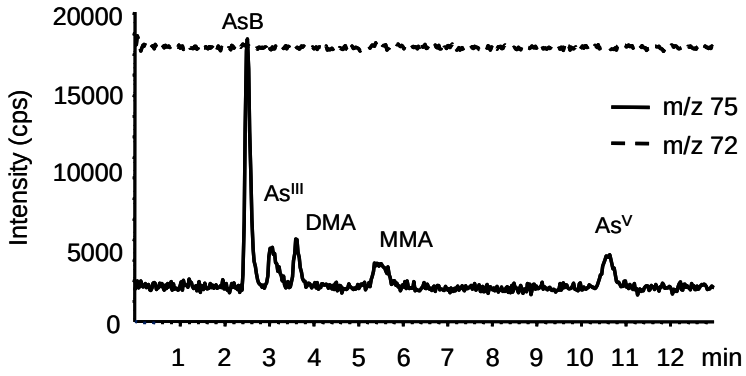
Figure captions

Figure 1.- Conversion process from intensities to mass flow using the pseudo-isotope dilution equation.

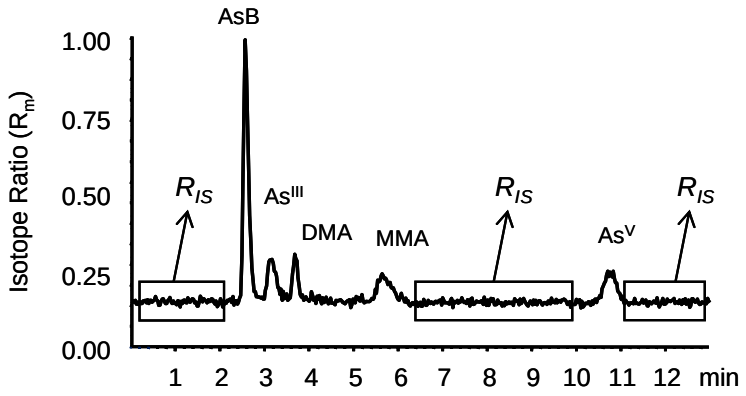
Figure 2.- Use of Ge as a pseudo-isotope of As to correct for instrumental signal drift observed during a 15-h batch run.

Figure 1

a) Intensity chromatogram



b) Isotope ratio chromatogram ($^{75}As/^{72}Ge$)



c) Mass flow chromatogram

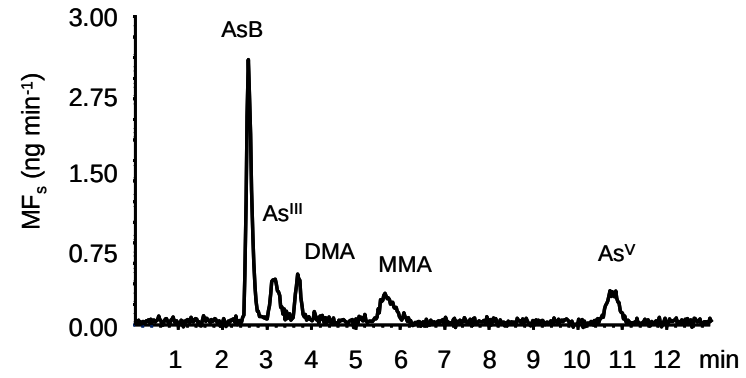
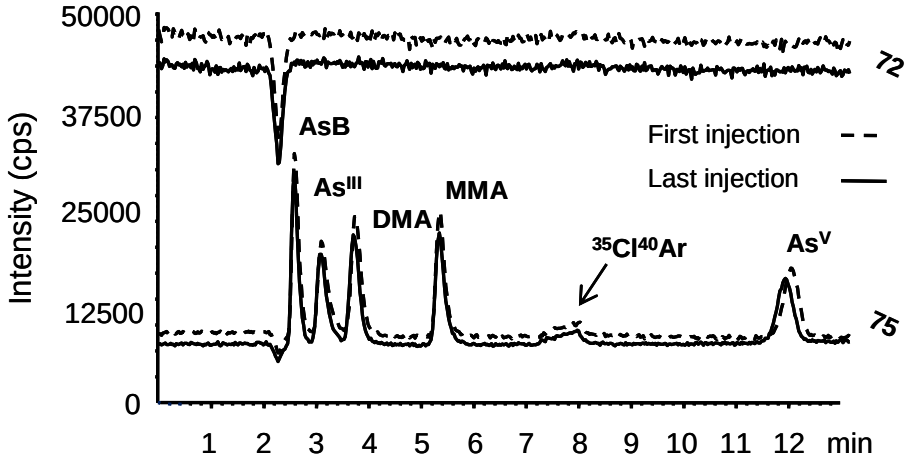


Figure 2

a) Intensity chromatograms



b) Mass flow chromatograms