Measurement of arsenic (As) in biological samples such as urine has important clinical applications and is being undertaken more frequently in epidemiologic studies because of concern about the carcinogenicity of low to moderate levels of As exposure. The objective of this study was to evaluate and improve the accuracy of As determination in urine by inductively coupled plasma mass spectrometry (ICP-MS). Determination of As in biological samples by ICP-MS is difficult for two reasons: the formation of the molecular ion $^{40}$Ar$^{35}$Cl, which overlaps with monoisotopic As at a mass-to-charge ratio ($m/z$) of 75 (causing spectral interference), and signal enhancement due to organic matrix (nonspectral interference).

Available procedures were examined, including the application of different correction procedures using $^{40}$Ar$^{37}$Cl and $^{14}$O$^{35}$Cl molecular-ion formation; the addition of N$_2$ into plasma or nebulizer gas flows; and the addition of organic molecules to the sample and to calibration standards to eliminate or correct for interference due to molecular-ion formation.

The accuracy and precision of determination of As [$m/z$ 75, ionization potential (IP) 9.81 eV] with use of an internal standard was also investigated. Three elements were studied as candidate internal standards: germanium (Ge: $m/z$ 74, IP 7.90 eV), indium (In: $m/z$ 115, IP 5.79 eV), and tellurium (Te: $m/z$ 128, IP 9.01 eV). It was found that these three elements performed more or less equally well with Ar–N$_2$ plasma; it was also found that accuracy was significantly improved when Te was used as the internal standard instead of Ge or In for ethanol-added samples.

Our results indicate that accurate and precise measurement of As in urine by ICP-MS can be obtained by either of two methods ($<5\%$ error, $\sim 2\%$ RSD, limit of detection 0.1 ng ml$^{-1}$): (1) the addition of 1% N$_2$ to plasma gas flow or 3% N$_2$ to nebulizer gas flow, along with use of any of the internal standards tested, or (2) the addition of ethanol to the sample and to calibration standards, with use of Te as the internal standard. The most accurate results ($<1\%$ error) for National Institute of Standard and Technology Standard Reference Material (NIST SRM) 2670 (toxic elements in urine) were obtained with Ar–N$_2$ plasma with either Te or In as the internal standard.

**Keywords:** Arsenic; urine; inductively coupled plasma mass spectrometry; argon–nitrogen; ethanol; tellurium; germanium; indium; internal standard
Matrix-induced signal variation is dependent on the analyte mass number. Several workers have reported that, for optimal precision and accuracy, the internal standard should be as close as possible in terms of mass number to the analyte element(s). However, the calibration of As and Se may be difficult, especially in biological and clinical matrices, and correction for matrix-induced signal variation by use of an improper internal standard can result in systematic error.

Determination of As in urine by ICP-MS is complicated by formation of the argon chloride (40Ar 35Cl) molecular ion, which overlaps at m/z 75 with monoisotopic As. Several workers have tried to eliminate or correct for 40Ar 35Cl formation. The methods they have used fall into three categories: (1) mathematical correction using another molecular ion formed under the same analytical conditions; (2) addition of N2 to plasma gas; and (3) addition of organic solvents to the calibration standards and samples.

In an attempt to find the most accurate method for measuring As in urine samples by ICP-MS, a comparative study of three procedures developed by other workers was conducted: (1) the use of two different correction procedures (i.e., the formation of 40Ar 35Cl and the formation of 16O 35Cl molecular ion to estimate the contribution of 40Ar 35Cl at m/z 75);(8-11) (2) the addition of Ar–N2 to plasma and nebulizer gas flow; and (3) the addition of ethanol to the plasma along with the sample and the calibration standards. In addition, to improve the accuracy and precision of As determination, an internal standard to correct for instrument instability, sample introduction efficiency, and signal enhancement was sought. Three elements were studied as candidate internal standards for As (m/z 75, IP 9.81 eV): germanium (Ge: m/z 74, IP 7.90 eV), indium (In: m/z 115, IP 5.79 eV), and tellurium (Te: m/z 128, IP 9.01 eV). This article reports the results obtained with the three correction procedures and the three internal standards and describes the effect of major matrix components in urine on As determination.

Experimental

Apparatus

The ICP mass spectrometer used in this study was an Elan 5000, (Perkin-Elmer, Norwalk, CT, USA). Nitrogen was introduced into the plasma gas flow through an added mass flow controller and tee connector. A peristaltic pump (Rainin Instrument Co., Woburn, MA, USA) was used for sample introduction. A tee connector and mixing coil were used for online mixing of calibration standards and samples with the internal standard. The parameters for operation of the instrument are summarized in Table 1.

Reagents

All sample handling was performed in a Class 100 clean hood. All glassware was cleaned by soaking in 10% HNO3 for 24 h and rinsing several times with deionized water. The reagents used were As (1000 µg ml−1, High Purity Standards), Ge (1000 µg ml−1, SPEX Plasma Standard, SPEX Industry, Edison, NJ, USA), Te (1000 µg ml−1, Baker Instra-Analyzed Reagent, J. T. Baker, Phillipsburg, NJ, USA), and ethanol (AAPER Alcohol and Chemical, Shelbyville, KY, USA). Two mixed-standard solutions of Ge (20 ng ml−1), In (20 ng ml−1), and Te (250 ng ml−1) were prepared, one in 5% HNO3 and the other in 8% ethanol and 5% HNO3 (after online mixing with the sample and calibration standards to give 4% ethanol).

Samples

Samples used were NIST SRM 2670 toxic elements in urine and interlaboratory comparison study samples from the Quebec Interlaboratory Comparison Program (Le Center de Toxicologie du Québec), whose target values were determined by ETAAS. A 1 ml volume of sample was diluted with deionized water to 10 ml after addition of 1 ml of concentrated HNO3.

Procedures

Effect of major urine matrix components on As determination

Intensities at m/z 75, 77, 82, and 51 were measured and compared with a 5 ng ml−1 As standard and with different aliquots of a 5 ng ml−1 As standard spiked with urea (8–26 mg ml−1), Na+ (1.3–2.7 mg ml−1), PO4 3− (1.4–3.2 mg ml−1), and SO4 2− (1.2–3.6 mg ml−1). The concentrations of the matrix components used were those expected in normal human urine.

Analysis of As in urine by different methods

I. Application of correction equations

(a) Ar–Ar plasma and 40Ar 35Cl correction. Samples were analyzed by external calibration using Ar–Ar plasma and the mixed-element internal standard solution in 5% HNO3. The nebulizer flow rate was optimized to give the maximum signal for m/z 75. The corrected intensity at m/z 75 was then used to estimate the As concentration. Eqn. (1) was used to correct for 40Ar 35Cl interference:

\[ I_{corr} = I_{75} - \left( \frac{A_{37} Cl}{A_{75} As} \right) \times I_{77} \times \left( \frac{A_{77} Se}{A_{82} Te} \right) \times I_{82} \]  

where \( A_i \) is the isotopic abundance and \( I_i \) is the ion intensity at m/z \( X \) (e.g., 175, I25).

(b) Ar–Ar plasma and 16O 35Cl correction. Before analyzing samples by the above-described method to determine the proportionality constant for ArCl: OCl formation, HCl solutions of four strengths (0.0, 0.5, 1.0, 1.5%) were analyzed under different conditions in four strengths (0.0, 0.5, 1.0, 1.5%) were analyzed under different conditions.
the same instrument conditions with the internal standard. The resulting proportionality constant was used to estimate the contribution from $^{40}$Ar$^{35}$Cl on m/z 75, as given in eqn. (2):

$$I_{75_x} = I_{75} - \frac{2 \times \text{molecular ion}}{I_{16,35,Cl}} \times I_{51}$$

(2)

where $I_x$ is the ion intensity at m/z X (e.g., $I_{75}$, $I_{51}$). The proportionality constant for $^{40}$Ar$^{35}$Cl and $^{16}$O$^{35}$Cl was obtained by linear regression of normalized $I_{75}$ vs $I_{51}$ data using the above HCl solutions.10

2. Addition of N$_2$ to nebulizer or plasma gas flow. Nitrogen was introduced with a tee connector into each gas line through a mass flow controller. The amount of N$_2$ introduced was 1% for plasma gas flow or 3% for nebulizer gas flow. The nebulizer flow rate was selected to optimize the As-to-$^{40}$Ar$^{35}$Cl signal ratio for each setting, and the torch position remained constant. Samples were analyzed for As by external calibration with mixed-element internal standard solutions in 5% HNO$_3$.

3. Addition of 4% ethanol to sample and calibration standards. The calibration standards and sample were mixed (1 + 1) online with the internal standard solution containing 8% ethanol. The nebulizer flow rate was set to optimize the As-to-$^{40}$Ar$^{35}$Cl signal ratio for each setting, and the torch position remained constant. As content in the samples was determined by the external calibration method.

Signal enhancement

The mixed-element internal standard solution in 5% HNO$_3$ was mixed (1:1) online with the 50 ng ml$^{-1}$ solution of As before analysis. Intensities at m/z 74, 75, 115, and 128 were measured at nebulizer gas flow rates ranging from 0.6 to 1.5 l min$^{-1}$ with variation by 0.11 min$^{-1}$ increments. This analysis was repeated with the mixed-element internal standard solution prepared in 8% ethanol and 5% HNO$_3$.

Results and discussion

The effect of urea, sodium, and phosphate on As determination by ICP-MS was not significant. The addition of SO$_3$$_2$ to the concentrations expected in normal urine (1.2–3.6 mg ml$^{-1}$) did not notably affect counts at m/z 75, but, did increase counts at m/z 82 and m/z 51. These increases correlated well with the increases in SO$_3$$_2$ concentration (correlation coefficients: 0.9998 and 0.9950, respectively; correlation coefficients for the normalized signal with any one of the three internal standard tested: > 0.99) and may have been due to the formation of the molecular ion $^{34}$Si$^{16}$O$^{16}$O$^{16}$O at m/z 82 and the molecular ion $^{34}$Si$^{16}$O$^{16}$O at m/z 51.

Determination of As content in urine by an Ar–Ar plasma with external calibration procedure, using aqueous calibration standards with any of the internal standards, resulted in estimates about 37% higher than expected. This result was attributable to the formation of the molecular ion $^{40}$Ar$^{35}$Cl and to signal enhancement due to the sample matrix.

The efficacy of the isobaric fractionation (IBF) method in eliminating isobaric interference ($^{40}$Ar$^{35}$Cl) was examined.9 This method is based on the observation that the ion intensity produced by a given species can be estimated from the ion intensity of a different isotope of the same species. For example, of a given amount of $^{40}$ArCl ion species formed, 75% is $^{40}$Ar$^{35}$Cl and 25% is $^{40}$Ar$^{32}$Cl; this distribution reflects the natural isotopic abundance of chlorine. Counts at m/z 75 that are accounted for by $^{40}$Ar$^{35}$Cl can be estimated from counts at m/z 77 due to $^{40}$Ar$^{37}$Cl. The corrected intensity at m/z 75 was calculated with eqn. (1). Then the concentration of As was determined by the external calibration procedure. However, most biological samples contain significant and variable levels of Se, which has a stable isotope at m/z 77 and directly interferes with this correction procedure. This correction method is subjected to additional error since sulfur present in biological samples forms the molecular ion $^{32}$S$^{16}$O$^{16}$O$^{16}$O, which has an m/z value of 82. As mentioned earlier the increase in counts at m/z 82 occurred because of increasing sulfate concentration. The percentage error calculated for As determination in urine samples with the IBF correction procedure (Table 2) ranged from −15.8 to 18.3%. More than 60% of the samples analyzed had > 5% error in estimated As concentration, and about 33% of the samples had > 10% error.

The accuracy of the correction method proposed by Kershishnik et al. was investigated.10 These authors used the $^{16}$O$^{35}$Cl molecular ion species to correct for $^{40}$Ar$^{35}$Cl interference at m/z 75. The proportionality constant for $^{40}$Ar$^{35}$Cl and $^{16}$O$^{35}$Cl was obtained by linear regression of normalized intensity at m/z 51 ($I_{51}$) vs m/z 75 ($I_{75}$), with the analysis of aqueous solutions spiked with different concentrations of chloride under the same instrument conditions. The corrected intensity at m/z 75 was calculated with eqn. (2), and the As concentration was determined by the external calibration procedure. A major drawback of this procedure is that the presence of vanadium (V) in the sample can interfere with the correction procedure, causing erroneous results. Another interference was observed stemming from the SO$_3$$_2$$^{2−}$ present in the sample. Counts at m/z 51 increased with the SO$_3$$_2$$^{2−}$ concentration as a result of the formation of the molecular ion $^{34}$Si$^{16}$O$^{16}$H. The percentage error

<table>
<thead>
<tr>
<th>Sample</th>
<th>ArCl</th>
<th>OCl</th>
<th>Ar–N$_2$</th>
<th>EtOH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ge</td>
<td>In</td>
<td>Te</td>
<td>Ge</td>
</tr>
<tr>
<td>S1</td>
<td>−2.4</td>
<td>−10.8</td>
<td>1.5</td>
<td>−10.6</td>
</tr>
<tr>
<td>S2</td>
<td>−8.6</td>
<td>−14.4</td>
<td>−2.1</td>
<td>−3.6</td>
</tr>
<tr>
<td>S3</td>
<td>−15.8</td>
<td>−5.3</td>
<td>−9.3</td>
<td>−3.7</td>
</tr>
<tr>
<td>S4</td>
<td>−4.4</td>
<td>−10.7</td>
<td>−0.7</td>
<td>6.6</td>
</tr>
<tr>
<td>S5</td>
<td>0.2</td>
<td>−6.5</td>
<td>7.1</td>
<td>3.6</td>
</tr>
<tr>
<td>S6</td>
<td>9.8</td>
<td>3.6</td>
<td>18.3</td>
<td>12.2</td>
</tr>
<tr>
<td>S7</td>
<td>7.1</td>
<td>−0.2</td>
<td>11.3</td>
<td>5.0</td>
</tr>
<tr>
<td>S8</td>
<td>1.5</td>
<td>−4.4</td>
<td>6.3</td>
<td>3.0</td>
</tr>
<tr>
<td>S9</td>
<td>12.8</td>
<td>4.8</td>
<td>17.9</td>
<td>13.7</td>
</tr>
</tbody>
</table>

| NIST SRM 2670 | 16.6 | 5.7 | 15.1 | 3.2 | −6.4 | 1.9 | 7.2 | 0.7 | 0.5 | 10.5 | 8.2 | 2.1 |

* Percentage error = (measure concentration-target concentration) × 100/target concentration.
calculated for As determination in urine samples ranged from −18.5 to 20.9% (Table 2). Overall, only ≈ 50% of the samples tested had < 5% error in estimated As concentration. About 12.5 average percentage error estimate in As concentration in NIST SRM 2670 may be due to the Se present in the sample and to overcorrection for the Se present in the sample, where both the analyte and the interfering element are present at almost the same concentration (As, 0.48; Se, 0.49 μg ml⁻¹). Accurate isobaric correction can be performed only when the interfering element present in significantly lower concentration than the sample. The accuracy of the refined 1⁰⁸⁰Cl correction procedure proposed by Nixon et al. was not tested in this study. They have reported that some bias still remains, but it has been reduced from 28% to about 13%. Addition of N2 to the plasma or nebulizer gas flow dramatically improved the accuracy of As determination. These observations agree with previously published data. Addition of N2 to plasma gas was presented. As shown in Table 2, 80% of the samples analyzed with Te or In as the internal standard yielded As concentration estimates with an error of < 5%; for 40% of these samples, the error was < 2%. Sixty per cent. of the samples analyzed with Ge as the internal standard yielded As concentration estimates with an error of < 5%, and 30% of these samples had an error of < 2%. The precision (RSD) of the As determination with any one of the three internal standards was ≈ 2%, and the limit of detection for As in urine samples was 0.1 μg ml⁻¹.

Addition of a small amount of organic solvent reduced interference on As and Se of polyatomic ions (ArCl, ArAr) at m/z 75, 77, and 78 due to the shift in peak maximum to lower carrier gas flowrates and increased signal intensity in ICP-MS. In this study, 4% ethanol was added to the sample and calibration standards and the As content was determined by external calibration, with Ge, In, or Te as the internal standard. The most accurate results were obtained with Te as the internal standard (Table 2). The precision (RSD) of the As determination with any one of the three internal standards was ≈ 2%; the limit of detection for As in urine samples with Te as the internal standard was 0.1 μg ml⁻¹.

In ICP-MS, internal standardization is used to correct for instrument instability, signal drift, differences in nebulization efficiency, and nonspectral interference (signal suppression or signal enhancement caused by the matrix). Use of an ideal internal standard can improve both the precision and the accuracy of the analysis. The best internal standard for ICP-MS is an element whose mass and ionization potential are close to those of the analyte element. However, it is difficult to find an element that satisfies both of these criteria and is free from interference below m/z 80.

To correct for nonspectral interference, three elements were tested as the internal standard. The first, Ge, closely matches As in terms of mass. The second, Te, closely resembles As in terms of ionization potential. The third, In, is not close to As in terms of either mass or ionization potential but has been used by other workers as a internal standard for many elements, including As.

Relative signal enhancement was calculated by comparison of the signal for the 4% ethanol matrix with that for the 5% HNO₃ matrix. On the basis of the results (Fig. 1), it was concluded that all four elements exhibit signal enhancement when introduced with an organic matrix; however, the degree of enhancement is very small (< 2) for Ge and In and very large (≈ 8) for As and Te. For analysis of As in organic matrices, it is important to use a proper internal standard that gives an enhancement similar to that of As. Therefore, because Te and As exhibit the same degree of enhancement, use of Te as an internal standard for urinary As determination may be prefera-
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