Using $^{16}\text{O}^{35}\text{Cl}$ to Correct for Chloride Interference Improves Accuracy of Urine Arsenic Determinations by Inductively Coupled Plasma Mass Spectrometry

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We have observed inaccurate urine arsenic values with the method of isobaric fractionation, which was designed to correct for the $^{40}\text{Ar}^{35}\text{Cl}$ interference with $^{75}\text{As}$ quantitation by inductively coupled plasma mass spectrometry. Isobaric fractionation, which is based on ion intensities at $m/z$ 77 and 82, consistently underestimates the $^{40}\text{Ar}^{35}\text{Cl}$ interference and overestimates arsenic urine. We present an improved method for identifying the argon-chloride interference. We observed that signal intensities for the species $^{16}\text{O}^{35}\text{Cl}$ and $^{40}\text{Ar}^{35}\text{Cl}$ are proportional ($I_{75} = 0.0295 \times I_x - 14.7, r^2 = 0.998$; where $I_x$ is the normalized ion intensity at $m/z$ $X$) in water and urine, over a broad range of chloride concentrations (0–800 mmol/L). The proportionality constant is remarkably stable within a run (mean and SD, 0.0295 ± 0.0023, based on 10 replicates of five chloride calibrators, 0, 100, 200, 400, and 800 mmol/L). Increased sensitivity (50-fold) for detecting the $^{40}\text{Ar}^{35}\text{Cl}$ interference provides improved accuracy for urine arsenic quantitation as demonstrated by a split-sample comparison with graphite-furnace atomic absorption spectrophotometry.

Additional Keyphrases: intermethod comparison · isobaric fractionation · graphite-furnace atomic absorption spectrophotometry · selenium · vanadium · analytical error

Simultaneous multielement analysis is a primary advantage of inductively coupled plasma mass spectrometry (ICP/MS) over most other analytical methods, including graphite-furnace atomic absorption spectrophotometry (GF/AAS). Sensitive and specific ICP/MS analysis of biological samples have been demonstrated (1–4) despite inherent problems, including isobaric and isotopic interferences in ICP/MS (5–8). When a sample is introduced into the ICP/MS, several ion species unique to plasma are formed, including argon chloride ($\text{ArCl}$) and oxychloride (OCI). Ion-exchange chromatography (9), mixed-gas plasmas ICP/MS (10), and isobaric fractionation (5) have been applied to eliminate isobaric $^{40}\text{Ar}^{35}\text{Cl}$ interference with determinations of $^{75}\text{As}$ (1, 4–6). Because ion-exchange chromatography and mixed-gas plasmas both require special equipment, which can add time and cost to sample analysis, they are perhaps less favorable. The focus of this investigation is on the method of isobaric fractionation (IBF).

The IBF method is based on the premise that the ion intensity produced by a given species can be estimated by observing the ion intensity of a different isotope of that same species. For example, 75% of the $^{40}\text{Ar}^{35}\text{Cl}$ ion species formed is $^{40}\text{Ar}^{37}\text{Cl}$ and 25% is $^{40}\text{Ar}^{37}\text{Cl}$, reflecting the isotopic abundances of chloride. By monitoring $I_{77}$ (normalized ion intensity at $m/z$ 77), which represents the $^{40}\text{Ar}^{37}\text{Cl}$ species, $^{40}\text{Ar}^{35}\text{Cl}$ ion interference can be estimated at $^{75}\text{As}$. However, selenium is also present in most biological samples at significant and variable concentrations. An isotope of selenium, $^{77}\text{Se}$, contributes to $I_{77}$ and therefore interferes with the quantitation of $^{40}\text{Ar}^{35}\text{Cl}$. To circumvent the selenium interference, a proportional amount of the $I_{82}$, based on isotopic abundances of $^{77}\text{Se}$ and $^{82}\text{Se}$, is subtracted from $I_{77}$:

$$\text{Calculated } I'_{75,\text{u}} = I_{75} - \left( \frac{A_{\text{35Cl}}}{A_{\text{77Se}}} \right) \times \left[ I_{77} - \left( \frac{A_{\text{77Se}}}{A_{\text{82Se}}} \right) \times I_{82} \right]$$

$$\text{Calculated } I'_{75,\text{u}} = I_{75} - 3.1271 \times (I_{77} + 2.5831 \times I_{82})$$  (1)

where $A_i$ is isotopic abundance and $I_x$ is normalized ion intensity at $m/z$ $X$.

An assumption made in accepting the IBF correction (ICP/MS method correcting for ArCl interference with arsenic) is that in biological samples the predominance of $I_{77}$ and $I_{82}$ are due primarily to $^{40}\text{Ar}^{37}\text{Cl}$, $^{77}\text{Se}$, and $^{82}\text{Se}$ species. However, the ion species $^{34}\text{S}^{16}\text{O}^{15}\text{O}^{16}$ interfere with $^{82}\text{Se}$ quantitation in biological matrices containing sulfur (11). Also, the signal-to-noise ratio for the ArCl ion species at $^{77}\text{Se}$ is approximately one-third that at $^{75}\text{As}$, and three different ion intensities are used to calculate the final corrected $I_{75}$ for $^{75}\text{As}$. Hence, the error associated with the IBF measurements is propagated through the calculations needed, potentially resulting in a large error associated with the final result.

We present an improved method for correcting the ICP/MS $^{40}\text{Ar}^{35}\text{Cl}$ interference with $^{75}\text{As}$ by using the $^{16}\text{O}^{35}\text{Cl}$ species. A split-sample comparison with the GF/AAS results (12) is also presented.

Materials and Methods

Equipment

A standard Perkin-Elmer (Norwalk, CT) ELAN 500 inductively coupled plasma mass spectrometer equipped with an autosampler was used to measure urine arsenic concentrations. A Radiometer/Copenhagen (Cleveland, OH) chloride titrator was used to determine urine chloride concentrations.
Reagents

Reagent-grade de-ionized water and metal-free polypropylene vials and pipet tips were used. Solutions of beryllium (Be), vanadium (V), arsenic (As), yttrium (Y), rhodium (Rh), and lead (Pb) obtained from PerkinElmer were certified by the National Institute of Standards and Technology (Gaithersburg, MD). Monomethyl arsionic acid [CH₃As(OH)₂] was provided courtesy of J. G. Nicholas (Vineland Chemical Co., Vineland, NJ). Dimethyl arsionic acid [(CH₃)₂As(OH)], arsionic acid [Na₂HAsO₄·2H₂O], o-arsanilic acid (H₂NC₆H₄AsO₃H₂), and arsenic trioxide (As₂O₃) were obtained through Aldrich Chemical Co. (Milwaukee, WI). Arsenobetaine [(CH₃)₂AsCH₂CO₃] was provided courtesy of Bill Cullen and John Nelson (Department of Chemistry, University of British Columbia, Vancouver, BC). Trace-grade nitric acid (HNO₃) was obtained from Mallinckrodt Specialty Chemicals (Paris, KY). Ammonium chloride (NH₄Cl) from Aldrich Chemical Co. was used to prepare stock chloride solutions.

Procedures

**Equipment setup.** The ICP/MS quadrupole filter was tuned daily in continuous-scan, multielement mode, maximizing ion intensities for lead, beryllium, and rhodium. ICP torch alignment and cleaning were performed at least daily, following the Perkin-Elmer users' guide recommendations. Yttrium was used as the internal standard in all experiments presented. ICP/MS sampling variables for the experiments presented were as follows: resolution, low; ion multiplier HV, 4000; rod offset, 0.0 V; plasma RF power, 1.256 kW; auxiliary flow, 2.0 L/min; plasma flow, 14.0 L/min; nebulizer flow, 0.40 L/min; sample flow, 1.0 mL/min; measurements/peak, 1; scanning mode, elemental; measurement mode, sequential; measurement time, 1.0 s; repeats/integration, 3; threshold, 50 ions/s; and counting imprecision, 0.050%. Aqueous stock solutions of all standards and the aforementioned arsenic derivatives were prepared in volumetric polypropylene flasks and were acidified with 50 mL/L HNO₃.

**Sample handling.** This research was approved by the Institutional Review Board at the University of Utah School of Medicine (IRB no. 3702-91). All patients' specimens were stored at 4 °C in polypropylene vials. Samples were handled under a high-volume air-flow hood, and metal-free pipet tips were used only once to minimize contamination. Portions (0.5 mL) of patients' urine samples were diluted 10-fold by adding 4.0 mL of de-ionized water and 0.5 mL of 100 mL/L HNO₃ containing 100 µg/L of yttrium. Preparation of supplemented samples was identical, except that part of the de-ionized water was replaced with the supplement solution. Instrument calibration for arsenic was performed with a blank water sample and 0.5-mL water samples supplemented to contain final arsenic concentrations of 0.0, 20.0, 40.0, 160.0, and 320.0 µg/L. Samples were assayed in sequential analyses of blank, calibrators, and ≤300 samples per assay. Portions (20.0 µL) of patients' urine samples were analyzed directly with the chloride titrator after stable calibration with a 100 mmol/L NH₄Cl standard solution and controls with target chloride values of 83 and 116 mmol/L.

**Data processing.** All measured ion intensities were normalized [Iₓ × (I₁₀₀₈l₄₀₂/I₁₀₀₈l₄₀₂)] by using yttrium (10.0 µg/L) as an internal standard, and equations 1 and 2 were used to calculate corrected ion intensities for each method on each sample (calibrators included). Simple linear regression was performed on calculated calibration ion intensities and calibrator concentrations for each run, which were used to calculate the final results for individual samples. Standard statistical methods were used.

**Results and Discussion**

**Proportionality Constant for ⁴₀Ar_<sup>³⁵</sup>Cl/¹⁸O_<sup>³⁵</sup>Cl**

The equation for the proposed chloride-corrected arsenic ion intensity is as follows:

\[
\text{Calculated } I_{75m} = I_{75} - \frac{I_{40AsCl}}{I_{18OCl}} \times I_{51}
\]

where \( I_5 \) is normalized ion intensity at m/z X and 0.0295 is the proportionality constant for the \( ⁴₀Ar_{³⁵}Cl \) and \( ¹⁸O_{³⁵}Cl \) species.

The proportionality constant for \( ⁴₀Ar_{³⁵}Cl \) and \( ¹⁸O_{³⁵}Cl \) was obtained by linear regression of normalized \( I_{51} \) vs \( I_{75} \) data on chloride-supplemented water samples (Figure 1). Remarkable within-run stability for the \( I_{51} \) vs \( I_{75} \) proportionality constant was observed (mean ± SD,
titrator, Fig. 2. Comparison of urine [Cl\(^-\)] \(\text{mmol/L}\), measured by chloride titrator and both \(^{40}\text{Ar}^{35}\text{Cl}\) (I\(_{75}\)) and calculated \(3.1271 \times (I_{77} - 2.5831 \times I_{82})\) \(^{40}\text{Ar}^{36}\text{Cl}\) (I\(_{77,82}\)) ICP/MS methods; n = 103 patients' samples

Inset: magnitude of difference in urine [Cl\(^-\)] as measured by the chloride titrator and IBF methods in patients' samples

![Chloride by Titrator (mM)](image)

0.0295 ± 0.0023 for 10 replicates of five water-based chloride calibrators at 0, 100, 200, 400, and 800 mmol/L. The proportionality constant should be determined immediately before or within every run, because alterations in quadrupole lens settings significantly affect the proportionality constant. Quadrupole tuning, when required, should be conducted between runs and before determining the proportionality constant. The observed range of the proportionality constant between runs is 0.013-0.035 and is highly dependent on quadrupole tuning. Calculated absolute I\(_{75}\) [3.1271 \(\times (I_{77} - 2.5831 \times I_{82})\)] values for the species \(^{40}\text{Ar}^{35}\text{Cl}\) are represented (Figure 1). Both methods give results that vary linearly with concentration in water. The proportionality constant between chloride and I\(_{61}\) is 50-fold greater than that between chloride and I\(_{75}\) calculated by using I\(_{77}\) and I\(_{82}\). If no chloride correction is used, significant false increases in arsenic values due to \(^{40}\text{Ar}^{35}\text{Cl}\) are observed over the physiological range of urine chloride (Figure 1).

**Chloride Assessment**

To test the accuracy of the correction methods to measure urine chloride and therefore the ArCl interference, we compared the chloride content in 103 urine specimens submitted for arsenic analysis by using a standard chloride titrator and both IBF and the proposed ICP/MS methods. IBF-measured urine chloride is consistently markedly underestimated (Figure 2); thus the IBF correction typically underestimates the \(^{40}\text{Ar}^{35}\text{Cl}\) interference with \(^{75}\text{As}\), resulting in falsely high urine arsenic concentrations (Figure 3). Two additional samples were repeatedly analyzed and had urine chloride values, as determined by the IBF method, that were abnormal (−4349 and −520 mmol/L). Urine chloride for these same two samples, as determined by the proposed method, were within 15 mmol/L of the chloride titrator value (data not shown). These findings suggest the presence of \(^{34}\text{Si}^{16}\text{O}^{14}\text{O}^{16}\) species, selectively increasing the I\(_{82}\), or a human source of selenium having unnatural isotopic abundance.

In only one sample was the chloride concentration determined by the proposed method falsely increased because of vanadium (Figure 2).

**Linearity and Precision**

Both IBF and the proposed ICP/MS methods for measuring urine arsenic demonstrate linearity in the range used (0.0–320.0 \(\mu\text{g/L}\)). Urine samples of patients referred for urine arsenic evaluation (n = 113) were analyzed by both the proposed and IBF ICP/MS methods (Figure 3). The IBF method gave a constant bias of 12.84 \(\mu\text{g/L}\) of arsenic \((P < 0.01)\). This provides additional evidence that the IBF method consistently underestimates the \(^{40}\text{Ar}^{35}\text{Cl}\) interference and overestimates arsenic concentrations.

Within-run precision (n = 10) for the proposed method on urine samples with low and high arsenic concentrations was \((\text{mean} \pm \text{SD}, \text{CV})\) 13.7 ± 1.33 \(\mu\text{g/L}, 9.7\%\), and 81.2 ± 1.52 \(\mu\text{g/L}, 1.8\%\). Between-run precision (n = 10) for the proposed method on the same samples was 10.5 ± 2.56 \(\mu\text{g/L}, 24.3\%\), and 83.9 ± 2.12 \(\mu\text{g/L}, 2.52\%\). The precision of the IBF method was similar to that of the proposed method.

**Chloride Interference**

Chloride interference data for both methods are presented in Table 1. Both methods are able to accurately subtract \(^{40}\text{Ar}^{36}\text{Cl}\) interference at \(^{75}\text{As}\) that is due to added chloride. However, the IBF method consistently underestimates baseline urine chloride and therefore baseline ArCl interference, resulting in an average overestimation of urine arsenic of 12.84 \(\mu\text{g/L}\) (Figures 2 and 3). Two samples had markedly falsely increased
Table 1. Chloride Interference with Arsenic in Three Patients’ Samples

<table>
<thead>
<tr>
<th>Chloride supplemented, mmol/L</th>
<th>As, µg/L*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>l_{77/62} correction</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>27.0</td>
</tr>
<tr>
<td>200</td>
<td>33.1</td>
</tr>
<tr>
<td>400</td>
<td>25.3</td>
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<tr>
<td>600</td>
<td>23.7</td>
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<tr>
<td>l_{9} correction</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10.8</td>
</tr>
<tr>
<td>200</td>
<td>12.4</td>
</tr>
<tr>
<td>400</td>
<td>13.4</td>
</tr>
<tr>
<td>600</td>
<td>11.6</td>
</tr>
</tbody>
</table>

* Average of duplicates.

Table 2. Vanadium Interference at l_{91} in the ICP/MS Method: Suppression of Urine Arsenic

<table>
<thead>
<tr>
<th>Vanadium supplemented, µg/L</th>
<th>Suppression of As, µg/L*</th>
<th>Suppression, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.0</td>
<td>1.45</td>
</tr>
<tr>
<td>2.0</td>
<td>2.6</td>
<td>1.75</td>
</tr>
<tr>
<td>20.0</td>
<td>5.9</td>
<td>2.48</td>
</tr>
<tr>
<td>200.0</td>
<td>40.5</td>
<td>7.80</td>
</tr>
</tbody>
</table>

* Duplicates of 10 assays of a urine specimen with an average arsenic concentration of 49.5 µg/L.

Vanadium Interference

Vanadium interference data is presented for the proposed method (Figure 2, Table 2). Accurate urine chloride determination by the proposed method depends on vanadium concentrations being low. Reported urine vanadium concentrations in exposed workers are <16.0 µg/L (13, 14). If vanadium concentrations are significantly increased, the chloride concentrations as measured by the proposed method will be falsely increased, resulting in falsely depressed results for urine arsenic. Except for one sample (1 of 106), vanadium concentrations were not increased. In that sample, urine chloride determined by the proposed method was falsely greater than the titrator-determined urine chloride (Figure 2), urine arsenic by the proposed method was negative, and urine vanadium was significantly increased (data not shown).

With the proposed method we can identify urine arsenic falsely suppressed because of an increased urinary vanadium. By performing a chloride titration on urine specimens where the l_{91} exceeds the intensity generated by a water sample supplemented to contain 300.0 mmol/L chloride and comparing it with the urine chloride determined by the proposed method, the vanadium interference in a given sample can be closely estimated. For example, 100.0 µg/L of urine vanadium will suppress urine arsenic by ~20.0 µg/L and falsely increase urine chloride by ~250 mmol/L. If the urine chloride values by both the titrator and proposed ICP/MS method agree, then the chloride is increased for another reason (HCl may have been added to urine as a preservative for concomitant biochemical studies; also some urine samples are concentrated because of patient dehydration). Few samples exist where the urine chloride is >300.0 mmol/L. As mentioned, we have identified only one urine specimen in which urine chloride as determined by the proposed and titrator methods did not closely agree; urine vanadium was significantly increased in that sample.

Recovery Studies

Urine specimens may contain five different species of arsenic. Inorganic arsenic compounds are significantly toxic to humans and are metabolized and excreted in the urine as mono- and dimethyl arsenic acids. Seafloor contains a benign type of arsenic to which humans are frequently exposed, arsenobetaine, which is excreted unchanged (12).

To demonstrate recovery of arsenic by the proposed method, we added each of the arsenic compounds listed (Table 3) to water and two different volumes of sample, which represented different chloride concentrations. The proposed method provides good recovery for all forms of arsenic tested and the urine matrix has minimal effect on recovery. With the proposed method, the limit of detection for arsenic by ICP/MS in urine samples diluted 10-fold is 3.0 µg/L, and the aqueous detection limit is 0.3 µg/L.

GF/AAS vs ICP/MS

We compared urine arsenic values determined by the proposed ICP/MS method and by the new GF/AAS method (12) with the palladium–persulfate matrix modifier. Urine specimens submitted for arsenic analysis (n = 113) were analyzed (Figure 4). Both proportional (P < 0.01) and constant (P < 0.01) biases were observed ($S_{f2} = 19.79$ µg/L). A cross-calibration study was performed on two sets of three calibrators (325, 50, and 100 µg/L of arsenic) analyzed by both the proposed ICP/MS method and the improved GF/AAS method (12) showed no significant constant or proportional biases.

To further substantiate improved accuracy of the proposed ICP/MS method over the IBF ICP/MS method,
we used 11 samples from the Quebec Interlaboratory comparison program in the comparison study (above). Analytical data from GF/AAS (12) and both ICP/MS methods are presented in Table 4. Although the target values are determined by GF/AAS methodology, better agreement is observed with the proposed ICP/MS method than with the IBF method.

Values determined by the GF/AAS method (12) agree more closely with target values (Table 4), which is expected because the target values were determined by GF/AAS. For many elements it is difficult to establish which, if any, method (GF/AAS, ICP/MS, or neutron activation analysis) has the greatest true accuracy, because good agreement has not been observed between these methods for other human samples (3, 15). Present data do not allow us to conclude that the proposed ICP/MS method is more or less accurate than the GF/AAS method. However, both GF/AAS and ICP/MS methods provide accuracy sufficient for medical purposes.

The proposed ICP/MS method quantifies urine chloride, $^{40}\text{Ar}^{35}\text{Cl}$ interference, and urine arsenic more accurately and directly than does the IBF ICP/MS method. The IBF method underestimates urine chloride and therefore overestimates urine arsenic. Improved accuracy is due to a high proportionality constant between the $^{18}\text{O}^{35}\text{Cl}$ and $^{40}\text{Ar}^{35}\text{Cl}$ species. Significant interferences were observed at rates of 1% and 2% for the proposed and IBF methods, respectively. Detection and elimination of false-negative arsenic values due to vanadium interference are provided for the proposed method. Because it does not require an in-line ion-exchange column or additional gas-mixing equipment, the proposed method may be preferred.

A split-sample comparison for urine arsenic was performed with the proposed ICP/MS method and the most recently published GF/AAS method, which uses a palladium–persulfate matrix modifier. Both methods adequately identify significant increases in urine arsenic. Both proportional and constant biases are probably due to unidentified matrix effects. Approximately 5% of the proportional bias observed may be due to an ICP/MS signal enhancement that is apparently dependent on urine matrix (Table 3).

We acknowledge the editorial assistance of Thomas P. Moyer (Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN); the technical assistance and expertise of Bill Gordon, Marilyn Cooper, and Richard N. Jones (Associated Regional and University Pathologists, Inc., Salt Lake City, UT); the generosity of Bill Cullen and John Nelson (Department of Chemistry, University of British Columbia, Vancouver, BC); and J. G. Nicholas (Vineland Chemical Company, Inc., Vineland, NJ) for providing arsenobetaine and monomethyl arsenic acid, respectively.

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