Determination of Mercury in Whole Blood and Urine by Inductively Coupled Plasma Mass Spectrometry

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Abstract

The conventional method for the determination of mercury in clinical samples is cold vapor atomic absorption spectrometry. Sample digestion or pretreatment require large sample volume and long sample preparation time. The inductively coupled plasma mass spectrometry (ICP-MS) method developed in this study requires only 100 μL of sample with practically no preparation, except for dilution with diluent. Significant savings in sample volumes, reagents, technician time, and analysis time are realized. Among different types of diluents, the one containing acid, tert-butanol, and potassium dichromate gave the best results to remove the mercury memory effect. The interassay precisions for whole blood and urine were < 5% and < 8%, respectively, and the intra-assay precisions were < 3% and < 7%, respectively. The lower limits of detection were 0.13, 0.17, and 0.26 pg/L for aqueous standard, urine, and whole blood, respectively. The developed ICP-MS method correlated well with the atomic absorption method and can offer an alternative to the atomic absorption method for mercury analysis with less sample volume requirement as well as shorter analysis time.

Introduction

Mercury is widely distributed in nature, circulating among several media, and is found in different states (gas, liquid, and solid) and different chemical forms (mineral, organic), exhibiting various degrees of toxicity. Mercury may cause kidney injury, central nervous system disorders, intellectual deterioration, and even death (1). Therefore, efficient assessment of suspected mercury intoxication through determination of mercury levels in various biological matrices, particularly blood and urine, is vitally important. Various methods for the determination of total mercury have been reported, including atomic fluorescence spectrometry (2), atomic absorption spectrometry (3), inductively coupled plasma (ICP)-atomic emission spectrometry (4), and ICP-mass spectrometry (MS) (5-10).

The conventional method for the determination of mercury in clinical samples is cold vapor atomic absorption spectrometry (CVAAS) (11-15). Sample digestion or pretreatment is required to decompose organic mercury compounds, and then total mercury can be determined by stannous chloride reduction (15,16). This method requires large sample volume and time consuming sample preparation. Because copious amounts of strong acids and strong oxidants are used to assure the complete decomposition of organic mercury, it is also hazardous. In the last decade, there has been a rapid growth in the use of ICP-MS because of its strong detection power and the possibility of multi-element analysis in a single run. The versatility of the ICP-MS technique makes it a multi-disciplinary analytical tool. It can be used in geological and environmental sciences (17), nuclear and semi-conductor industries (18), materials science, medicine (19), agriculture (20), food, and biological sciences (21). Recently, there has been a growing interest in the use of the ICP-MS for metal analysis in laboratory medicine. This study investigated the application of ICP-MS on clinical analysis utilizing simple dilution techniques. The ICP-MS method developed requires only 100 μL of sample with practically no preparation, except for dilution with diluent containing acid, tert-butanol, and potassium dichromate. Significant savings in sample volumes, reagents, technician time, and analysis time are realized.

The well known “mercury memory effect” is the major obstacle in mercury quantitation by ICP-MS. Long after a sample containing mercury has been nebulized into a conventional spray chamber, a significant residual mercury signal can be detected indicating its failure to return to baseline. The persistence of residual mercury in spray chambers and long acid washout times has been observed (22). Mercury adheres to the spray chamber walls and/or remains as vapor in the spray chamber. Limiting the solution to acid only prevents mercury adsorption onto the spray chamber walls, but mercury volatility persists (23). This problem is overcome through the addition of gold or dichromate, which prevents mercury volatilization and adsorption losses (23). Use of a direct injection nebulizer

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was used, and the nebulizer flow rate was lowered. This adjusted daily to give minimum count for oxide level and doubly as it has been used in mercury analysis for decades. The also as an effective preservative for mercury standard solu-

The analysis was performed on a PerkinElmer Sciex ELAN DRC plus 6100 ICP-MS (PerkinElmer Sciex, Thornhill, ON, Canada) equipped with an autosampler (AS 93 plus, PerkinElmer) and a Meinhard concentric quartz nebulizer. A reduced-size (25-mL) cinnabar spray chamber (Glass Expansion, West Melbourne, Australia) was used to minimize the mercury memory effect. Nickel sampler and skimmer cones were used. Nebulizer gas flow and the lens voltage were adjusted daily to give minimum count for oxide level and doubly charged ions and maximum count for 113mndium. Running conditions for ICP-MS are summarized in Table I.

Table I. Optimized Operating Conditions for the ICP-MS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Setting</th>
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<tr>
<td>Auxiliary Gas Flow</td>
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<td>Plasma Gas Flow</td>
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<tr>
<td>Replicates</td>
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Materials and Methods

Reagents and standards

Stock solutions of mercury (Hg) with a concentration of 1000 mg/L and germanium (Ge) with a concentration of 1000 mg/L were purchased from Chem Service (West Chester, PA). For the preparation of all solutions and reagents, ultra-pure water (18.2 M-ohm) from a MilliQ-Element A10 system (Milpore, Milford, MA) was used. To compensate for instrumental drift, 72Ge was used as internal standard with a final working concentration of 2 µg/L. Hydrochloric acid (HCl, 2%) from TEDIA (trace metal grade, Fairfield, OH), tert-butanol (5%) from BDH (AR grade, BDH Laboratory Supplies, Poole, England), and potassium dichromate (60 mg/L) from Sigma (St. Louis, MO) were used.

Certified controls

Quality control materials were purchased from Seronorm (Trace Elements whole blood control, SERO, Billingstad, Norway) and Bio-Rad (Lyphochek urine metals control, Bio-Rad Laboratories, Diagnostic Group, Hercules, CA). Proficiency testing whole blood and urine samples were obtained by participation in Trace Elements External Quality Assessment Scheme (NEQAS, Centre for Clinical Science and Measurement, University of Surrey, Guildford, Surrey, U.K.).

Sample preparation and calibrators

Working aqueous standards were prepared in 2% HCl at concentrations of 20–1000 µg/L from a stock solution of 1000 mg/L Hg. Calibrators were prepared at Hg concentrations of 0, 2, 5, 10, 30, 60, 80, and 100 µg/L by either dilution with water (aqueous calibrators) or spiking into whole blood or urine (matrix matched calibrators) from a non-exposed subject. The ratio of 209Hg to 72Ge was plotted against the prepared mercury concentrations. The endogenous Hg concentration was derived from the blank specimen and subtracted from the calibration points.

A 100 µL of a standard, whole blood, or urine sample was diluted with 1.7 mL diluent (as later described), and 200 µL internal standard (Ge, 2 µg/L) was added and thoroughly mixed. The final mix was then ready for Hg determination.

Memory effect experiment

The effectiveness of dichromate, gold, HCl, and tert-butanol in reducing the memory effects was determined on 50 µg/L Hg in 2% HCl by taking readings at 2-s intervals with a dwell time of 100 ms and 20 sweeps for the sampling sequences. Ultra-pure water was first measured to ensure that no mercury was present. To test equilibrium and washout, the trial diluent was first measured as a blank. The sample probe was inserted into the test solution containing only the testing diluent. The measurement sequence was initiated as the liquid sample entered
the nebulizer. Measurements at 2-s intervals were accumulated until a steady state signal was observed. The number of counts was recorded. The probe was then inserted into the testing solution containing mercury plus the testing diluent. Once again, measurements were taken at 2-s intervals and the number of counts was recorded. The probe was then reinserted into the diluent and the washout time measured. Data was collected throughout the blank diluent, sample, and washout sequences. Five different kinds of diluents were tested: 2% HCl; 2% HCl with 5% tert-butanol; 2 mg/L gold in 2% HCl and 5% tert-butanol; 60 mg/L dichromate in 2% HCl and 5% tert-butanol; and 60 mg/L dichromate and 2 mg/L gold in 2% HCl and 5% tert-butanol.

HCl was chosen because of its effectiveness in organic mercury solubilization (6,27). The addition of gold or dichromate is well known for preventing mercury volatilization (23,25). The physiochemical properties of a nebulized solution are altered by the introduction of small amounts of some organic solvents, improving desolvation of the aerosol, and thus, an enhanced signal (28).

After the "analysis time" and the "washout time" were known and the suitable diluent selected, the "washout time" study was repeated in both urine and whole blood.

Validation

For validation of the method, the following parameters were determined: range of linearity, intra- and interassay precision, accuracy, recovery, detection limit, and correlation.

Linearity

The Hg aqueous calibration curve ranged from 0 to 100 µg/L and was prepared fresh daily from serial dilution from the Hg stock standard with a concentration of 1000 mg/L with water. Matrix matched calibration curves for urine and whole

Figure 1. Wash-out characteristics of different diluent/rinse solution for 50 µg/L Hg in 2% HCl. The graphs represent 2% HCl (A); 2% HCl with 5% tert-butanol (B); 2 mg/L gold in 2% HCl and 5% tert-butanol (C); 60 mg/L dichromate in 2% HCl and 5% tert-butanol (D); and 60 mg/L dichromate and 2 mg/L gold in 2% HCl and 5% tert-butanol (E).
blood were prepared by spiking non-exposed subject's urine and whole blood with known quantities of mercury.

Precision and accuracy
To study the intra- and interassay precision, urine and whole blood from non-exposed and exposed subjects were used. Intra-assay precision was calculated based on repeat analysis of the same sample within the same batch for 20 times. For inter-assay precision, samples were analyzed once a day for 2 weeks. Accuracy is determined by replicate analysis of urine and whole blood control samples containing known concentrations of mercury. Proficiency testing whole blood and urine samples were also analyzed.

Recovery
Recoveries were evaluated by adding Hg to whole blood and urine to final concentrations of 2 to 100 µg/L and by measuring the Hg concentrations before and after the additions.

Detection limit
The limit of detection is defined as the mercury concentration that produces a signal of 3 times the standard deviation (SD) of the reagent blank readings. In this experiment, 20 blank readings were taken to calculate the SD. The matrix effect on detection limit was studied for water, urine, and whole blood.

Correlation
Unused portions of 21 whole blood specimens and a group of 63 urine specimens routinely analyzed by CVAAS were retested using the new ICP-MS method.

CVAAS facility and method
The AAS used was the Varian AA-400 (Varian Techtron, Mulgrave, Australia) with vapor generation accessory (VGA 76 from Varian Techtron). The sample digestion method (29) is described briefly as follows. Aliquots of 0.5 mL of concentrated sulfuric acid were added to a 0.5-mL standard or sample in a digestion tube and allowed to stand for 30 min at room temperature. A total volume of 4 mL KMnO4 was then added to each tube, 1 mL at a time at 15-min intervals while standing in a dry block heater set at 95°C. The samples were digested at this temperature for 4 h with occasional mixing. A persistent dark color indicates complete digestion (29,30). Two milliliters of 20% hydrazine/HCl was added, followed by 20 µL octanol. The mixture was diluted to a final volume of 8 mL with ultrapure water. Standard CVAAS analysis was performed on these samples (31,32).

Results and Discussion
The traditional CVAAS method for the determination of mercury required sample digestion prior to analysis. Such digestion step at high temperatures may cause losses of mercury, which would result in a falsely low value. In addition, the detection limit of traditional CVAAS method for Hg determination is about 0.5 µg/L, which is not low enough for non-exposed subjects. In order to improve the detection limit, several modifications had been applied, such as on-line microwave sample pretreatment (16) and on-line digestion with flow injection (2). Although a better detection limit can be achieved, the physical set up is rather complicated, which limits its use in routine clinical laboratory. Recent developments on ICP-MS for Hg determination provide better sensitivity and precision, and the detection limit can be achieved.
down to 0.01 μg/L (5). The improved assay performance relied on sophisticated interface technique, such as anodic stripping voltammetry (24), direct-injection high-efficiency nebulizer (24), time-based on-line pre-concentration (5), or expensive Hg isotope (10). The complexity and cost involved practically preclude their application in clinical laboratories in general.

Washout characteristics

Signal recovery time is an important analytical parameter as it provides a reference point for analyzing the next sample in the queue, and thus indicates the rate of analysis. Significant variables determining the signal decay time for potentially volatile analytes, like mercury, in an acidic medium are the volume and the surface area of a spray chamber. The small volume (25 mL) of the cinnabar spray chamber and the constant washing of its surfaces by the sample aerosol resulted in its rather rapid wash-out. The cinnabar spray chamber’s small surface area and appropriate shape also helped reduce memory effects. The cyclonic form prevented the sample from building up around the spray chamber wherein it could possibly be nebulized. Slight tilting of the spray chamber ensured that all droplets thrown against its wall would run away from the tangential nebulizer inlet and run out through the central drain.

Five different kinds of diluent/wash solution were tested (Figure 1). With HCl (Figure 1A) alone, the signal fails to return to baseline level following the analysis of the standard and 30 min of washing. The addition of tert-butanol (Figure 1B) resulted in an increase in Hg signal’s intensity due to improved Hg transport efficiency and its desolvation. For the wash-out time, the addition of tert-butanol did not make any improvement compared with HCl alone. For the diluent containing gold, HCl, and tert-butanol (Figure 1C), the washout time was approximately 118 s. For the diluent containing dichromate instead of gold (Figure 1D), the washout time was significantly improved. It took only 80 s for the signal to return to baseline level. Diluent with both dichromate and gold in HCl and tert-butanol (Figure 1E) had no additional improvement in washout time observed compared with only dichromate in HCl and tert-butanol. Dichromate in HCl and tert-butanol (Figure 1D) was therefore chosen as the diluent for the analysis of mercury by ICP-MS. Matrix effect on washout time was shown in Figure 2, which indicated that there was only 5 s difference in washout time with different matrix. As a result, a washout time of 85 s was used for the study.

Linearity

The calibration curves, which covered the range of Hg concentrations in aqueous standard, whole blood, or urine, were consistently reproducible. We monitored between-day precision of the calibration curves on five days over a period of two weeks. The mean slope, intercept, and coefficient of linear regression ($r^2$) in aqueous standard were 0.0888, 0, and 0.9999, respectively. For whole blood they were 0.0767, 0.125, and 0.9999, respectively. For urine they were 0.0905, 0, and 0.9999, respectively. Calibration curves of the aqueous solution and urine have practically the same slopes and intercepts, which confirms that there is no matrix effect in urine sample. For whole blood sample, different slope and intercept were observed when compared with the aqueous solution. Therefore, a standard addition procedure should be used.

Precision and accuracy

The intra- and interassay precisions are reported in Table II for the whole blood and urine samples. The method was found

<table>
<thead>
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<th>Added Hg (μg/L)</th>
<th>Whole Blood</th>
<th>Urine</th>
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<tr>
<td>2</td>
<td>107.4</td>
<td>95.6</td>
</tr>
<tr>
<td>10</td>
<td>99.4</td>
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<td>100</td>
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</tr>
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</table>

Table IV. Recovery (%) of Hg in ICP-MS (n = 3)
to be highly precise with intra-assay coefficient variations (CVs) < 4% and interassay CVs < 5.0% for whole blood. For urine samples, intra-assay CVs were < 7% and interassay CVs were < 8%.

Results of the quality controls (QCs) studied in Table III indicate the ICP-MS method for mercury has good performance in accuracy. The performance of the ICP-MS method was also tested using proficiency test samples from NEQAS (Centre for Clinical Science and Measurement, University of Surrey, UK) that covered a wide range of mercury levels in whole blood (Figure 3) and urine (Figure 4). The obtained values agree closely with the reported values at the 95% confidence interval.

Recovery
Mean recoveries for five different Hg concentrations (Table IV) ranged from 96.3% to 107.4% and from 95.6% to 105.0% for whole blood and urine, respectively.

Limit of detection
The detection limits obtained were 0.13, 0.17, and 0.26 µg/L for aqueous solution, urine, and whole blood, respectively.

Correlation
The correlations between the ICP-MS method and the CVAAS method were $y = 0.93x + 0.56$ ($n = 22; r = 0.940$) for whole blood and $y = 1.20x - 1.66$ ($n = 8; r = 0.989$) for urine. A Bland–Altman plot indicated equivalence of Hg results over the concentration range found in routine specimen analysis (Figure 5).

Conclusions
It has been shown that acidification of solutions prevents loss of mercury by adsorption, and the addition of gold or dichromate helps prevent mercury volatilization; however, in this study dichromate is much more effective than gold in removing residual mercury and reducing the mercury memory effect. The approach of using a spray chamber of reduced volume and a low-uptake nebulizer demonstrates a significant improvement in the determination of mercury by ICP-MS. The total amount of sample being consumed and processed is reduced to a minimum. Therefore, this approach offers the advantages of simplicity and ease of use as no pre-analytical steps, such as digestion, extraction, and chemical modifications, such as hydride generation, are required.

This study demonstrated that ICP-MS can be effectively used for the determination of mercury in whole blood and urine. Requiring only 100 µL of sample, determination of mercury by ICP-MS yields accurate results compared to the traditional CVAAS method. This fast and simple method is suitable for routine use in a clinical laboratory setting where short turnaround time is needed for proper management of patients suspected of mercury exposure. The small sample size makes it particularly suitable for micro-volume testing in the pediatric population.

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References

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