DETERMINATION OF MERCURY IN URINE AND WHOLE BLOOD BY FLOW INJECTION ANALYSIS-COLD VAPOR ATOMIC ABSORPTION SPECTROMETRY

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ABSTRACT

A flow injection-cold vapor atomic absorption spectrometric method has been developed for the determination of mercury in urine and whole blood. The calibration graph is linear up to 25 μg/l (A = 0.001 + 0.015 Hg (μg/l); r = 0.9997), with a detection limit of 0.1 μg/l (0.1 mg of mercury), considering a signal to noise ratio of three. The relative standard deviation varied between 1.4 and 1.7 %. The accuracy of the analysis was evaluated using NBS 2670 freeze-dried urine and Seronorm trace elements in whole blood; the values obtained were in good agreement with the certified values. The mean urine and blood contents of 128 unexposed subjects from Mérida City was found to be 1.0 ± 0.3 and 2.5 ± 1.5 μg/l, respectively.

INTRODUCTION

Increasing occupational exposure of humans to toxic chemicals seems to constitute one of the causal agents leading to the drastically increasing prevalence of either acute of chronic intoxication events in modern society. The most commonly employed methods for determining mercury in blood, urine and other samples have used a cold vapor atomic absorption technique, based upon the reduction of inorganic mercury either by sodium borohydride or by a stannous compound, followed by a process of sweeping the free mercury atoms into a quartz cell mounted in an atomic absorption unit (1). A flow injection analysis (FIA) procedure for the determination of trace amounts of mercury by the cold vapor atomic absorption (CVAA) method has been presented using Teflon for elemental mercury separation (2). Recently, the inorganic mercury can be determined by a procedure without using membrane

separators (3). Nevertheless, none of these procedures have been employed for the determination of mercury in whole blood and urine. Therefore, the present work describes a method for the determination of total mercury in urine and whole blood using a FIA procedure for CVAA.

EXPERIMENTAL

Apparatus

A Varian Techtron AA-1475 atomic-absorption spectrometer, and a mercury standard hollow-cathode lamp was used for all evaluations. A Varian Techtron VGA-76 generation accesory was used with a Tecator V-100 injection valve.

Glassware

Glassware used for mercury determinations was soaked in 20 % (v/v) nitric acid solution and then rinsed sequentially with 10 % (v/v) nitric acid, water, 20 % (w/v) stannous chloride in 6N hydrochloric acid, distilled water again and finally, three times with deionized-distilled water.

Reagents

Deionized-double distilled water was used throughout. All reagents used were of analytical-reagent grade, mercury free. A stock solution (1000 mg/l) of mercury was prepared by dissolving 1.3535 g of mercury(II) chloride in 100 ml of concentrated hydrochloric acid and diluting to 1:1 with water. From this stock solution, working standard solutions were freshly prepared by appropriate dilution with a solution 10 % (v/v) hydrochloric acid.

Sample Digestion

Prior to injection, a total of 10 ml urine samples were added to a 25 ml Erlenmeyer flask. Then 5 ml of concentrated nitric acid and 5 ml (5 % w/v) potassium permanganate were added and allowed to settle for nearly 8 h. The excess of potassium permangante and the manganese dioxide were removed by adding several drops of hydrogen peroxide (30 % w/v). Then the solution was heated at 80°C

during 15 min in order to destroy any excess of hydrogen peroxide. Finally, the volumen was diluted to 25 ml with water in a volumetric flask. 5 ml blood samples were diluted 1:1 with a silicone antifoam agent and water before injection.

Manifold and Procedure

Fig. 1 shows a schematic diagram of the FIA-CVAA configuration used for the determination of total mercury in urine and whole blood samples. A 1.0 ml of digested urine samples or dilute whole blood samples were injected into a continuous flowing stream of water, which was mixed.

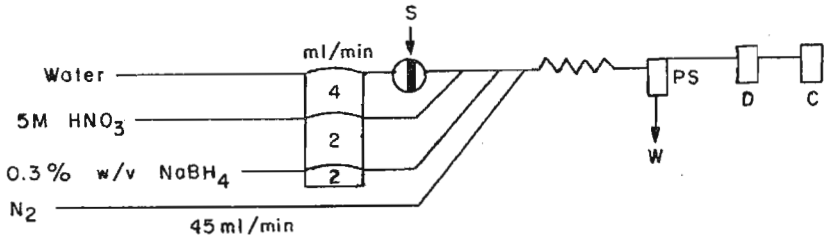

Fig. 1 Flow injection manifold for determination of mercury in urine and whole blood with the cold vapor atomic absorption spectrometric technique. (S) point of injection; (PS) gas-liquid separator; (D) spectrometer; (C) computer; (W) waste

with a stream of nitric acid. After reaction with sodium borohydride in a mixing coil the evolver mercury vapor was swept into the spectrometer quartz cell and the atomic absorption signal evaluated.

RESULTS AND DISCUSSION

Optimization of Analytical Parameters

The quartz cell was aligned vertically and horizontally to allow maximum transmittance. The atomic absorption spectrometer conditions were set at 253.7 nm wavelength, 0.5 nm slitwidth and 5.0 mA lamp current without background correction. The influence of the FIA manifold and chemical parameters were optimized in order to obtain the most reproducible and highest peaks with least tailing and the some of them are indicated in Fig 1. The internal diameter of tubing was of 1.0 mm, the tubing length from the injector to the gas-liquid separator was of 0.5 m, whereas the sample volume was of 1.0 ml.

Fig. 2 indicates that the flow rates of the carrier, nitric acid and reductor appreciably effect the sensitivity. The best sensitivity was observed with 4.2 and 2 ml/min of carrier, nitric acid and reductor flow rates

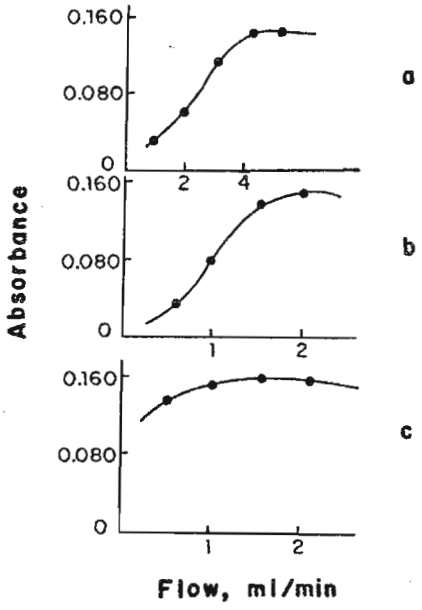

Fig. 2 Effect of (a) carrier solution, (b) nitric acid, and (c) reductor flow rates on the analytical signal. Conditions as specified in the text.

The FIA signal was not affected by nitric acid concentrations. Only if the pH was above 7 an appreciable reduction of the analytical signal was observed. A maximum signal was obtained when the sodium borohydride concentration was between 0.2 to 0.5 % (w/v). Thus, all subsequent work was done using 5 M nitric acid to assure an adequate acidic medium within the flowing system and 0.3 % w/v sodium borohydride.

Tube internal diameters of 0.5, 1.0 and 1.5, were examined. The diameter of tubing of the nitric acid and reductor streams did not affect the analytical signal, whereas by increasing the inner diameter from 0.5 to 1.5 mm of the carrier stream tubing decreased the height of the height of the peaks but only to a small extent. Ocassional clogging of the 0.5 mm inner diameter tubing by blood samples was observed. Therefore, tubing of 0.5 mm inner diameter was used for the nitric acid and reductor streams, whereas tubing of 1.0 mm inner diameter was used for the carrier stream.

A study was undertaken to assess the analytical characteristics by using sodium borohydride and stannous chloride. Under both reductors optimezed concentrations, the maximum peah heights increased linearly with mercury concentration as expressed by the equations: $A = 0.001 + 0.015$ Hg (0.5-25 μg/l); $r = 0.9997$; detection limit ($\pm 3\sigma$) = 0.1 μg/l (0.1 ng of mercury) (using sodium borohydride as reductor), and $A = 0.001 + 0.016$ Hg (0.5-25 μg/l); $r = 0.9996$; detection limit ($\pm 3\sigma$) = 0.075 μg/l (0.075 ng of mercury) (using stannous chloride as reductor). 3σ means the signal to noise ratio. As the sodium borohydride and stannous chloride optimal concentrations were of 0.3 % and 20 % w/v, respectively, and not appreciably effect of sensitivity was observed by using either of these reductos, it was decided to use sodim borohydride throughout.

Interference Studies

The effect of some species on the determination of 2 and 10 μg/l of mercury by the FIA-CVAA procedure described by this work was studied. The following chemical species did not interfere when at a level 1000-fold in excess of mercury: Ca^{2+}, Mg^{2+}, Fe^{3+},

Cu^{2+}, Zn^{2+}, Mn^{2+}, Na^{+}, K^{+}, NH_4^{+}, PO_4^{3-}, S^{2-}, Cl^{-}, I^{-} and Br^{-}. An specie was defined as an interferent if a change of more than two standard deviations in the measurements was observed.

Effect of Sample Pretreatment

Urine and blood samples, and distilled deionized water which had been treated with known addition of mercury prior to evaluation were analyzed and regression lines plotted from the absorbance values obtained. The slope from the undigested urine samples was found to be statistically different with those obtained from digested urine and water samples. As the slopes of the lines obtained from digested urine and water samples were found to be statistically equivalent ($p < 0.05$) when tested using a F-statistic as described by Snedecor and Cochran (4), it was concluded that the organic matter present in urine severly interfere with the method use. Therefore, before each analysis urine samples underwent through the digestion procedure previouly described. The slopes of undigested and digested blood samples and distilled deionized water were found to be statitically equivalent ($p < 0.05$), therefore it was concluded that no matrix effect was found in this case.

Precision and Accuracy

The relative standard deviation for the determination Of 5.0 and 10 µg/l of mercury, obtained from ten replicate analysis were 1.7 and 1.4 %, respectively. Accuracy routine control of the results was established with the conventional CVAA spectrometric technique (5) and the agreement was always within the 96-102 confidence range. The accuracy of the analysis was further evaluated using NBS SRM Freeze-dried urine and Seronorm trace elements in whole blood, Nycomed, batchs Nos. 904 and 905, and the values obtained were in good agreement (within the 95 confidence limit).

Mercury Basic Values in Urine and Blood

With the procedure described, the mercury content was

determined in urine and whole blood of 128 unexposed to the metal subjects from Mérida City. The mean ± SD values found for urine and whole blood were of 1.0 ± 0.3 and 2.5 ± 1.5 μg/l, respectively.

CONCLUSIONS

The FIA-CVAA method described here is quick, with a sampling frequency ranging from 60 to 80 per hour, simpler and at least as accurate as the conventional CVAA. However, with this method less sample is required and reagents consumption is greatly reduced.

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