Biomedical & Pharmacology Journal Vol. **2**(1), 145-148 (2009)

Method development and analysis of total mercury in human urine samples by hydride generator accessory coupled with atomic absorption spectrophotmeter of the population residing near Okhla Industrial area, New Delhi, India

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(Received: April 10, 2009; Accepted: May 22, 2009)

ABSTRACT

The study involves analysis of total mercury in urine of human samples by Hydride generator accessory (Hg-A.A.S.) in the mercury contaminated ground water near Okhla industrial area, New Delhi India. Mercury enters the body mostly through the contaminated drinking water. The technique involves the measuring of the AA signal (Atomic absorption) and involves coupling of HGA with Atomic absorption instrument. Study involves the open digestion of urine samples with strong oxidizing agents. The limit of detection obtained using the technique was 0.1 ppb and urine control-I & II Bio-Rad showed a %CV of 4.6 and 5.5 respectively. Urine sample analysis showed that nearly all the urine samples had detectable and one sample showing borderline values of mercury.

Key words: - HGAAS, FIAS digestion, Mercury

INTRODUCTION

Mercury also known as quicksilver is one of the oldest industrial hazards. Mercury is found in various forms including elemental mercury, inorganic mercury compounds, and organic mercury compounds such as methyl mercury, mercury is a persistent pollutant that exists naturally in the environment [1]. However, levels have risen because of human activity and pollution.

This paper mainly deals in development of analytical technique for analysis of total mercury in urine samples and also analysis of random urine samples of locality near Okhla Industrial area where people generally use ground water for drinking and other domestic use. A recent research in the ground water of Okhla Industrial area New Delhi showed the existence of mercury in ground water [2]. A recent study conducted by the Environmental Science Department of the Guru Gobind Singh Indraprastha University, Delhi, reveals that the concentration of contaminants like mercury and other contaminants in the groundwater of Delhi (a stretch between Palla to Okhla) exceeds the permissible limits [3].

A variety of clinical manifestations of mercury intoxication may be encountered depending mainly upon the mercury involved. Elemental mercury results in pulmonary, central nervous system and kidney damage, inorganic mercury leads to gastrointestinal damage, organic mercury leads to CNS effect and may result to coma and death, urine is the most suitable matrix for analysis of mercury exposure [4].

The cold vapor technique of AAS (HGA-AAS) is the best routine method in the biological/environmental field [5].

To check quality control of the method and analysis universally accepted commercial Bio-Rad urine controls were analyzed in the same way as other urine samples and these were labeled as urine control-I and Urine Control-II. All the random urine samples were labeled as RU-1to RU-12. All the random urine samples were collected in 20 ml polypropylene scintillation vials which were properly washed with 20% nitric acid and subsequently with deminierialized water of milli-Q system (ion exchange and filtration processes). Chemicals, glassware used in the analysis were of grade 'A', acids.

MATERIAL AND METHODS

For this analysis a Perkin Elmer Model AA 600 with FIAS-100 (HGA) were used together with a Mercury Electrodeless Discharge lamp (N305-0634) was used. The FIAS technique involves the reaction of acidified aqueous samples with a reducing agent Sodium borohydride. The sodium borohydride acid reduction generates hydrides shown below [6]:-

$$NaBH_4 + 3H_2O + HCL - H_3BO_3 + NaCl + 8H$$

$$Em + H \text{ (Excesses)} - EHn + H_2 \text{ (excesses)}$$

Where

E= analyte of interest and m may or may not be equal to n

This reaction generates a volatile hydride which combines with mercury element and it is carried to the quartz cell by means of argon gas. It is believed that atomization of the hydride occurs form collisions of free hydride radicals. In the quartz cell, the generated analyte atoms are contained in the path of the source lamp and a signal is generated by the measuring the amount of light absorbed[6].

Work was started by replacing the furnace from AAS instrument and replacing it with quartz cell of FIAS-100 (HGA). First AAS-600 is switched on and the online computer. After this the FIAS-100 is switched on Argon gas flow rate is adjusted between 40-60%. Software AAWinlab is launched and EDL lamp is switched on. Hydride technique is selected from the software and after this the method Hg-NaBH4 is selected.

The carrier is 3% HNO_3 and reductant is 0.02% NaBh4 in one liter of metal free polypropylene bottle[4]. All the reagents and standards are freshly prepared. System is made ready and all the probes are inserted in the desired reagents/acids [7]. A linear zero intercept curves of 2, 5, 10 and 20 PPB is prepared in 3% HNO3 by using the software of the instrument. A reagent blank is also performed.

The procedure for analysis for total mercury in urine was validated by running universally accepted two levels Bio-Rad Controls which was in the same matrix and Standards. The Lot numbers of the controls are 69121 and 69122.

1 ml random urine sample of urine is mixed with 100 µL of 35% HNO_3 and 200 µL of 50% HCl and 500 µL of 5% potassium permanganate. In the same manner the reagent blank and commercial Controls of Bio-Rad is also prepared. The reagent/ sample solution is allowed to stand at room temperature for 15 minutes. If the solution color has changed from purple to brown then a further 500 µL of potassium permanganate solution is added and allowed to stand at room temperature for 15 minutes. This processes of adding successive aliquots of potassium permanganate solution and allowing the reaction mixture to proceed is maintained until the purple color is sustained. After potassium permanganate reaction is complete and 500 µL of butan-1-ol (used to control foaming) and 500 µL of 5% hydroxylamine hydrochloride in 3 % sodium chloride. Total volume is made up to 10 ml using demineralised water. Before analyzing the prepared urine samples universal Bio-Rad urine control levels I & II are analyzed to check the efficacy of instrument/reagents and the work proceeded only after the controls fits into the range. The urine control-I and II are analyzed in 10 replicates and % CV (Coefficient variation) is calculated. The random urine samples were carried in three replicates.

RESULTS AND DISCUSSION

The value obtained by the Bio-Rad urine controls were well within the range and is shown in table 1. The urine control-I obtained the value of 27.6 µg/L with %CV of 4.6 and urine control-II obtained the value of 88.4 µg/L with a %CV of 5.5 The total number of replicates for both the controls were 10. The results of controls show that both the instrument and reagent preparation are O.K.

The analyzed urine samples are presented in table 2. Random urine samples are showing a mixed trend. Sample RU1, RU2, RU3, RU5, RU6, RU7, RU8 RU-09, RU10 are well within the normal reference range while sample number RU-4 was showing borderline trend but was below the normal reference levels[8]. This method of analysis of mercury in urine by hydride generation accessory in combination with AAS is useful in urine analysis in not only in persons affected through ground water but also in persons affected though air inhalation, skin contact of mercury. The results obtained in the random urine samples were expected to be normal since in the previous study done in the ground water in pervious study had shown only normal and borderline values[2]. Many reputed research agency have also reported the findings that mercury has been detected in the ground water in 22 km stretch of Palla to Okhla[3]. The ill effect produced by the inorganic mercury samples ingested through drinking water is not considered very harmful to human health in terms of the levels found in the drinking water[7]. But if the levels in the ground water exceeds in thousands folds then it would lead to very harmful ill effects. For this purpose the ground

Table 1:

	Range of Urine Control-I	Urine Control Level-II	Values Obtained	Values Obtained
1	17.6-38.2 µg/L	45.1-110 µg/L	28	95
2			27	89
3			26	85
4			27	91
5			29	93
6			30	92
7			28	91
8			26	82
9			28	81
10			27	85
Mean			27.6	88.4
SD			1.264911	4.835057
CV			4.6	5.5

Table 2:

	RU-1	RU-2	RU-3	RU-4	RU-5	RU-6	RU-7	RU-8	RU-9	RU-10
Normal reference range in 24 hrs collection of urine Adults non exposed:- <20 µg/L Toxic Concentration:->150µg/L Lethal Concentration:- > 800 µg/L [8]	6.5	4.5	2.9	18.9	7.6	10.5	3.4	7.8	8.6	9.2

water of the city should be monitored on regular frequency. The doctors in the area should be well aware with the problem and if any person reports with signs and symptoms of mercury poisoning the specimen of urine should be send for testing total mercury urine samples

The present study gives technique for analysis of urine because it can be easily collected.

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