Mercury determination in work place air and human biological samples based on dispersive liquid-liquid micro-extraction coupled with cold vapor atomic absorption spectrometry

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A B S T R A C T

Mercury as a toxic heavy metal is important factor must be determined and controlled in work place air and human biological samples. It should be mentioned that, mercury (Hg) get distinguished from other toxic environment pollutants, due to their non-biodegradability which accumulate in living tissues of human body. By NIOSH method, the briefing work place air of worker was measured by flow injection cold vapor atomic absorption spectrometry (FI-CV-AAS). For separation and preconcentration mercury from blood/urine samples, a new procedure based on benzyl 1H-pyrrole-1-carbodithioate (BPDC; $C_{12}H_{11}NS_2$) was used by ultrasonic liquid-liquid micro-extraction (ULLME) coupled with cold vapor atomic absorption spectroscopy (CV-AAS). The influences of various analytical parameters including pH, BPDC concentration, sample volume and ionic liquid volume were investigated. The quantitative recoveries and enrichment factor were obtained more than 95% and 9.8, respectively at pH=7. The detection of limit (LOD) and detection of quantification (LOQ) of mercury were 30 ng L^{-1} and 0.1 μg L^{-1} respectively. In order to calculate the validation and accuracy of proposed method, the certified reference materials (NIST, CRM 3133 Lot 061204) was used and analyzed by ULLME-CVAAS. So, proposed method had good potential for preparation and preconcentration mercury in human blood / urine samples of worker and workplace air before analysis.

1. Introduction

Heavy metal is important factor must be controlled in environmental air and patients. There are some heavy metals with toxic effects such as mercury, cadmium, nickel, vanadium, arsenic, lead and aluminum which have no known beneficial effect on organisms. Mercury has been documented to cause autoimmune and neurological diseases. Mercury simply vaporizes at room temperature and easily enters to the environment and human lungs. High concentration of mercury vapors in work place air can accumulate in human tissues as compared to non- occupationally exposed individuals. Adverse health effects of this exposure including subtle neurological side-effects have also been well documented in most Petrochemical workers even at the lowest levels of exposure; consequently, measurement of mercury and methyl mercury in blood, urine, hair and air briefing seems to be important [1-7]. Chlor-alkali workers are mostly exposed through breathing air of mercury vapors which was released from electrochemical system to human body such as lungs and skin.

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Family members of these workers may also become exposed to mercury through personnel's clothes contaminated with mercury particles. Ingested metallic mercury enters the body through the stomach or intestines but even in large amounts very little enters the body. On the other hand, breathing mercury vapors results in direct absorption of most it (about 80%) from the lungs which rapidly moved to other organs, including the brain and kidneys. Mercury get distinguished from other toxic pollutants due to their non-biodegradability can accumulate in living tissues of human body. Even a very small amount of them can cause severe physiological or neurological damage to the human body [8-14]. The concentration of mercury vapor in air reported by occupational safety and health administration guidelines (OSHA, 0.1 mg m^{-3}). In addition, national institute for occupational safety and health (NIOSH) has established a recommended exposure limit for mercury vapor of 0.05 mg m^{-3} for up to an 8-hour workday and a 40-hour workweek. American conference of governmental industrial hygienists (ACGIH) has assigned mercury vapor a threshold limit value of 0.025 mg m^{-3} for a normal eight-hour workday and a 40-hour work week .Mercury levels in blood can be used to help diagnose recent mercury exposure and to evaluate patient response to chelation therapy. Normal mercury concentration in human blood/urine is less than 10-20 μg L^{-1} [15-19]. Many analytical methods such as atomic fluorescence spectrometry [20-24] high-performance liquid chromatography [25] Gas-chromatographic [26] plasma mass spectrometry [27] high-performance liquid chromatography on-line coupled with cold-vapor atomic fluorescence spectrometry [28, 29] gas chromatography-mass spectrometry [30] ion chromatography using photo-induced chemical vapor generation atomic fluorescence spectrometric detection [31] ion chromatography coupled with ICP-MS [32] liquid chromatography hyphenated to cold vapor atomic fluorescence spectrometry [33] UV–Vis spectrophotometric [34] were used for mercury spices determination. Samples preparation and preconcentration before

analysis is an important factor for determination of pollutants in different matrixes. Recently, the various methods for the preparation and preconcentration of mercury compounds, including solid phase extraction (SPE) [35-43], gold trap [44], ionic liquid-based dispersive liquid-liquid microextraction (IL-DLLME) [45, 46], cloud point extraction (CPE) [47,48], electromembrane extraction [34], dispersive solid phase micro-extraction [49], single-drop microextraction [50], and Liquid–liquid extraction (LLE) [51], were reported. Since 2010, the DLLME method has been used for extraction and/or preconcentration of different analytes from aqueous samples [52, 53]. By DLLME method, extraction solvent such as ionic liquids, liquid phase (sample) and disperser solvent (acetone) was used [54]. The DLLME procedure has many advantages including simple, rapid, low time and cost, and efficient extraction. The green analysis such as, decrease solvent volume and less waste generation due to preparation and analysis samples was achieved [53-54]. In this study, the mercury concentration in human blood and urine samples based on BPDC –IL was determined by FI-CVAAS after ULLME procedure in 50 samples. In addition, 50 briefing air based on Hopcalite was analyzed by NIOSH method (6009).

2. Experimental
2.1. Apparatus
The experiments were performed using the flow injection cold vapor atomic absorption spectrometer (FI-CVAAS, GBC – 932, 3000, Australia). All containers (quartz crucibles, plastic tubes) were cleaned with detergent and treated successively by the hydrochloric acid and rinsed with de-ionized water. Microwave digestions were carried out with a multi-wave 3000 (Anton Paar, 100 mL, 20 bars; Austria). The pure argon gas (99.99%) was used as a carrier gas for CV-AAS analysis and the pH values of the solutions were measured by a digital pH meter (Metrohm 744). Personal sampling pump, Sampler (glass tube, 7 cm long, 6-mm OD, 4 mm ID, flame sealed ends with plastic caps containing one section of 200 mg Hopcalite held in place by

glass wool plugs (SKC, Inc., Cat. Num. 226-17-1A, or equivalent) and BOD bottle were used for collection of air and blood/urine in the industrial factory respectively.

2.2. Reagents and Materials

All chemicals of analytical reagent grade such as nitric acid, hydrochloric acid, benzyl 1H-pyrrole-1-carbodithioate (BPDC; $C_{12}H_{11}NS_2$) (CAS no 60795-38-2), Polyoxyethylene octyl phenyl ether (TX-100), and sodium borohydride (NaBH4) were from Merck Germany. Mercury standard solutions were prepared from a stock solution of 1000 mg L^{-1} in 1% nitric acid from Fluka Switzerland. Reducing agents (aqueous solution of 0.6% sodium borohydride in 0.5% sodium hydroxide) were prepared freshly and filtered before use. Ionic liquid (1-butyl-3-methylimidazolium hexafluorophosphate; [BMIM][PF6]; $C_8H_{15}F_6N_2P$) (1-Ethyl-3-methylimidazolium hexafluorophosphate ;[EMIM][PF6]; $C_6H_{11}F_6N_2P$) (Trimethyl imidazolium hexafluorophosphate ; [DMMIm][PF6]) was purchased from Sigma Aldrich. Buffer solutions were prepared from 2-1 mol L^{-1} sodium acetate and acetic acid for pH= 3-7. Ultrapure water was prepared from Millipore (Germany).

2.3. Sampling

For sampling, all glass tubes (sampling vessel) were washed with a 1 moL L^{-1} HNO_3/HCl solution for at least 24 h and rinsed 10 times with DW before using. Due to low mercury concentrations in whole blood/urine, even minor contamination at any stage of sampling, sample storage and handling, or analysis has the potential to effect on the accuracy of the results. 10 mL blood and 100 mL urine samples were collected from factory workers and healthy matched controls (20-55 years), living in Abadan (IRAN). For analysis of 45 blood samples, 5 microliter of heparin (free metals) was added. The human blood and urine sample was maintained at –20 °C in a cleaned glass tube.

45 air samples were collected in an employee's breathing zone according to 6009 NIOSH analytical method. Each personal sampling pump

was calibrated with a representative sampler and the end of sampler was broken immediately prior to sampling. Samplers were attached to the pumps with flexible tubing and air was collected at a rate of 200 to 300 mL min^{-1}.

2.4. General procedure

In this research, human blood and urine and briefing air samples of factory workers were studied. The determination of mercury in blood/urine and air was carried out using a flow injection cold vapor atomic absorption spectrometry system after sample treatment according to the ULLME procedure. Based on procedure, the BPDC as complexing agent was added to human samples and mercury extracted by ULLME as a new mode of liquid phase extraction with high recovery and extraction efficiency. In this work, 0.5 mL of 2% (w/v) BPDC solution was prepared and added to 10 mL of blood and urine samples and pH was adjusted to 7 with buffer solution in a centrifuge tube. Then, 0.2 g of different IL was added to the mixtures and they were shaken with a vortex apparatus for 5 min. Mercury (Hg^{II}) was complexed and pre-concentrated as Hg-BPDC-IL ([BMIM][PF6]). The phases were separated by centrifuging of turbid solution at 4 min with 3500 rpm. After separation of ionic liquid from liquid phase, the remained solution (Hg-BPDC-IL) was back extracted with nitric acid (0.5 M, 0.5 mL) and the mercury concentration in blood/urine samples was determined by FI-CV-AAS (Fig 1).

Air samplers were capped and pack securely for shipment. Based on NIOSH procedure, the Hopcalite sorbent and the front glass wool plug from each sampler were placed in separate 50-ml volumetric flasks and mixture of 2.5 mL of HNO_3/HCl concentration added to volumetric flasks. Hopcalite sorbent was dissolved in acids and diluted to 50 mL of deionized water (blue color), then the mercury concentration was determined with FI-CV-AAS.

3. Results and Discussion

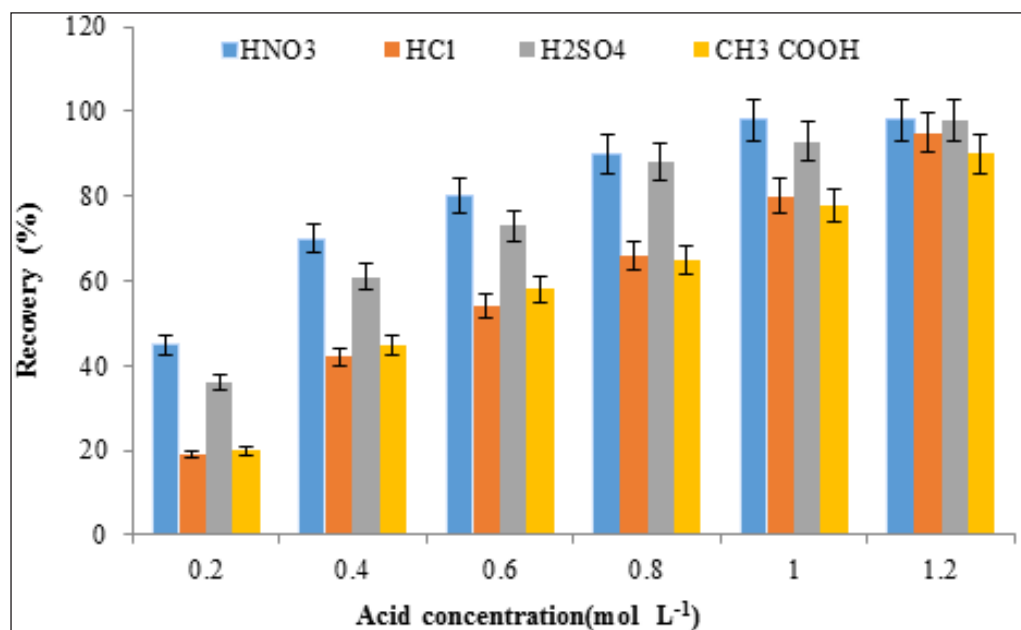Analytical conditions for mercury determination

Fig. 1. Back extraction of Ionic liquid with different acids

were performed in briefing air and human blood and urine samples of chemical factory workers at this work. Absorption (S/N) and repeatability of the results were investigated for the determination mercury by FI-CVAAS. The instrumental and extraction conditions are listed in Table 1. Working range was between 0.05- 7.1 μg L^{-1} for samples at peak area.

The complexation phenomenon is strongly conditioned by the pH. The results showed us the

Table 1. Instrumental and extraction conditions for mercury determination by FI- CVAAS

Instrumental Parameters	Mercury
Wavelength (nm)	253.7
Lamp current (mA)	3-4
Spectral bandwidth (nm)	0.5
LOD (μg L^{-1})	0.2
LOQ (μg L^{-1})	0.6
Working range(μg L^{-1})	0.5-70
ULLME method by BPDC	**Mercury**
LOD (μg L^{-1})	0.015
LOQ (μg L^{-1})	0.05
Working range (μg L^{-1})	0.05-7.1
Enrichment Factor	9.8
Volume sample (mL)	10
Amount of IL (g)	0.2
pH	7

pH from 5.5 to 7.5 was good recovery for mercury extraction by BPDC. So pH=7 selected as favorite pH for further analysis in blood samples (Fig 2). The minimum BPDC concentration necessary to achieve maximum extraction efficiency is 1.4×10^{-6} moL L^{-1}. So the 15×10^{-6} moL L^{-1} was used by ULLME procedure (Fig 3).

Different ionic liquids were used by ULLME method. Based on Figure 4, maximum extraction was occurred by [BMIM][PF6]. The high extraction was observed by volume higher than 0.2 mL for [BMIM][PF6] (Fig. 4). The effect of sample volume was evaluated with different volume of blood and urine samples from 1-25 mL and quantitative extraction was observed in 10 mL of blood/urine sample (Fig. 5).

The concentration of Hg(II) based on BPDC as ligand was determined by ULLME procedure in blood and human samples which was coupled to spectrometer of FI-CVAAS. In optimized conditions, the means of five times determinations, for Hg (II) were obtained by proposed method. The real samples were spiked with standard concentration of Hg(II) in LLOQ and ULOQ of linear range at pH=7 (Table 1). As validation methodology, the good accuracy results was achieved by spiking standard mercury species (0.1-7.0 μg L^{-1}) to human
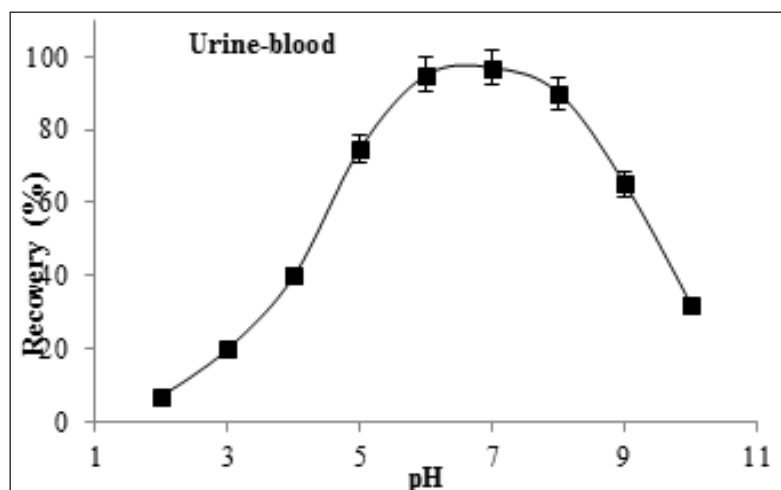
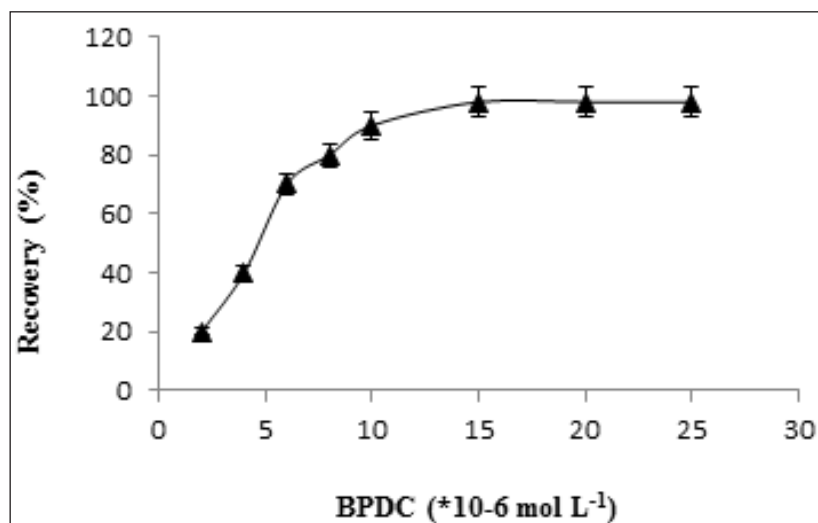**Fig. 2.** Effect of pH on mercury extraction in human blood /urine samples

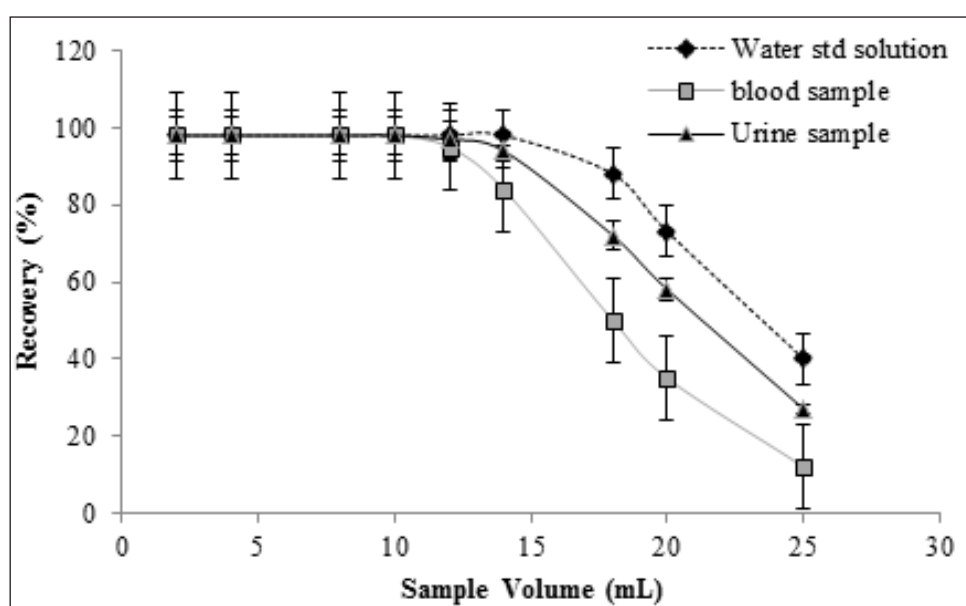**Fig. 3.** Effect of BPDC on mercury extraction in human blood /urine samples

Fig. 4. Effect of sample volume on mercury extraction in human blood /urine samples
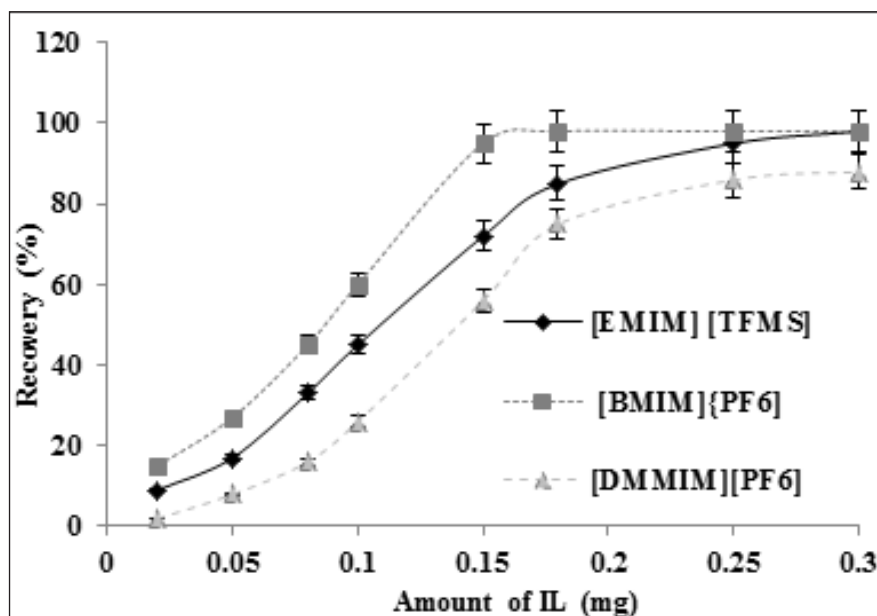
Fig. 5. Effect of ionic liquid on mercury extraction in human blood /urine samples

samples. Mercury concentrations in workers have higher than threshold limit value (TLV) and all of them have almost clinical problem. Validation of

Table 1. Validation of proposed method for determining blood mercury by BPDC ($\mu g\ L^{-1}$)

Sample	Added mercury	Found mercury	Recovery (%)
Blood A	-----	2.33± 0.09	-----
	2	4.26 ± 023	96.5
	4	6.28 ± 035	98.7
Blood B	-----	1.78 ± 0.07	-----
	1	2..80 ± 0.14	102
	2	3..73 ± 0.18	97.5
Urine A	-----	3.12 ± 0.16	-----
	2	5.15 ± 0.28	101.5
	3	6.09 ± 0.32	99.0
*Urine B	-----	7.45 ± 0.33	-----
	5	12.32 ± 0.65	97.4
	10	17.38 ± 0.32	99.3

[a] Mean of five determinations ± confidence interval (P = 0.95)
• Urine diluted with DW(1::5)

proposed method for determining blood and urine mercury was shown by CRM, NIST in Table 2.

Also Statistical parameters for determining mercury in blood and briefing air sample were calculated in Table 3. In addition, the results of mercury concentration in blood samples of worker and control were shown in Figure 6.

4. Conclusions

Mercury has toxic effect in humans. In high exposures, observed mostly in occupational settings, the severity of response correlates with the duration and intensity of the exposure. Increase mercury exposure depended on time of working and volume of air briefing which was determined based on NIOSH 6009. The results showed us, the mercury concentration in human blood/urine and briefing air in workers were higher than control group. Also, the increasing mercury doses in human blood and briefing air may be lead to an important neuropsychological problem in workers. Therefore, the concentration of mercury in human blood and briefing air is very important factor that must be

Table 2. Analytical results of mercury determination in certified reference material (CRM)

Analyte	CRM	Certified Value ($\mu g\ L^{-1}$)	Found ($\mu g\ L^{-1}$)	Recovery%
Mercury	NIST SRM 3133 Lot 061204	6.50 ± 0.29	6.38 ± 0.33	98.2

Mean value ± standard deviation based on three replicate measurements.

Table 3. Statistical parameters for determining mercury in blood and briefing air sample by BPDC

sample	Mean Conc.	Median Conc.	RSD%	Range Conc.
[a] Worker	25.37± 0.93	20.15± 0.57	3.6	4.0-52
[a] control group	1.15± 0.08	0.94± 0.04	4.2	0.7-5.0
[b] Worker	32.72± 2.24	29.81± 1.08	2.7	8.0-64
[b] control group	0.74± 0.03	0.62± 0.02	2.9	0.2-1.1
[c] Worker	0.045± 0.01	0.038± 0.01	4.4	0.01-0.05
[c] control group	ND	ND	---	ND

[a] Blood concentration, BPDC ($\mu g\ L^{-1}$)

[b] Urine concentration, BPDC ($\mu g\ L^{-1}$)

[c] Briefing air concentration ($mg\ m^{-3}$)
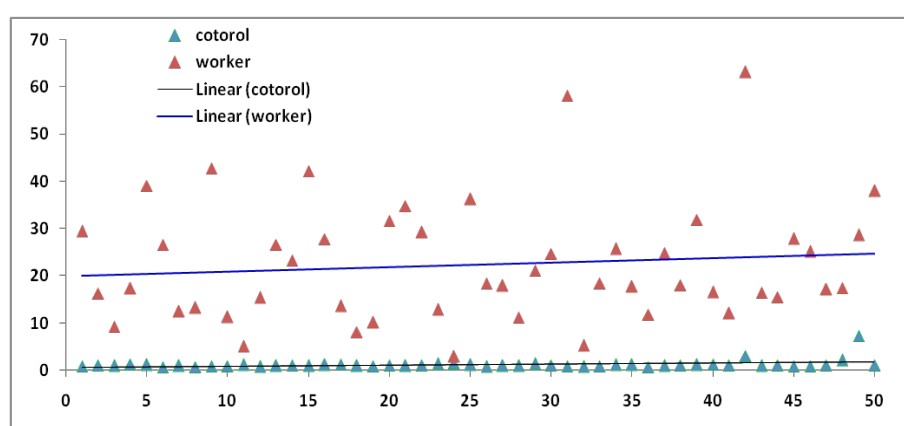
Fig. 6. Dispersive of blood mercury concentration in worker and control group

controlled and determined in industrial workers. In this study the precise and accurate method based on BPDC was used for mercury determination in blood and urine samples by ULLME coupled with FI-CVAAS. The experimental showed, the concentration mercury in worker were higher than OSHA/ACGHI references.

5. Acknowledgements

6. References

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