Research Article,

Solid Phase Extraction Using Polymer-Based C18 Cartridge Modified with Two Phenyl Azo Compounds for Spectrophotometric Determination of Mercury in Water and Environmental Samples

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Abstract:

A new highly selective, sensitive, and rapid procedure to determine ng ml^{-1} level of Hg(II) depended on the speed reaction of Hg^{2+} with 5-amino-2-(phenyldiazenyl)phenol (I) and/or 5-amino-2-(p-tolyldiazenyl)phenol (II) and the solid phase extraction (SPE) of the colored complex with a reversed phase polymer-based C18 cartridge have been developed. The I and/or II reacted with Hg^{2+} to form a violet complex of a molar ratio 2: 1 [I and/or II to Hg^{2+}] in the presence of 3.0 M of nitric acid solution and Triton X-100 medium. This complex was enriched by SPE with a polymer-based C18 cartridge. The enrichment factor of 200 was achieved. The molar absorptivity of the complex are 8.81 x 10^8 and 9.57 x 10^8 L mol^{-1} cm^{-1} at 626 and 649 nm, using I and/or II, respectively Beer's law is obeyed in the range of 2.5 – 200 ng ml^{-1}, whereas the optimum concentration ranges obtained from Ringbom plot was 10 – 175 ng ml^{-1}. The detection and quantification limit are given as 0.75 and 2.37 ng ml^{-1} for I, whereas their values for II are 0.80 and 2.48, indicating that a nanogram fraction of Hg^{2+} can be determined. The relative standard deviation was 1.45 and 1.34 % using I and II, respectively, obtained from a series of 10 standards each containing 100 ng ml^{-1} of Hg^{2+}. This procedure was applied successfully to

Keywords: Solid phase extraction, Spectrophotometry, Mercury determination, Water and environmental analysis, Azo dyes.

1. Introduction:

Mercury is one of the most toxic heavy metals. It enters the environment as metallic, inorganic and organic mercury compounds through various industries like pulp and paper industry, gold and silver mining, electrical industry, paints fungicides and pharmaceuticals. The toxicity of mercury depends on its chemical species and it is found that organo-mercurials are more toxic than inorganic mercury compounds [1,2]. Various determination methods of trace mercury have been continuously developed not only for monitoring the component but also for controlling its presence in the environment [3,4]. Different methods including cold vapour generation-atomic spectrometry (CV-AAS) [5], cold vapour generation-atomic fluorescence spectrometry (CV-AFS) [6], inductively coupled plasma optical emission spectrometry (ICPOES) [7], inductively coupled plasma mass spectrometry (ICP-MS) [8] and electrothermal atomization atomic absorption spectrometry (ETAAS) [9] have been reported to determine mercury.

A serious problem in the determination of mercury is related to low concentrations of target species. The main species of mercury in natural waters are inorganic mercury (Hg$_2^{2+}$, Hg^{2+}) and methyl mercury (CH$_3$Hg$^+$). Recent reports estimate that total mercury concentration is in the range of 0.2–100 ng L^{-1} and methyl mercury concentrations are lower (ca. 0.05 ng L^{-1}) in natural waters [10]. The basic principle of SPE is the transfer of the analyte from the aqueous phase to bind to active sites of the adjacent solid phase [11]. A variety of solid phase have been applied in SPE, such as active carbon [12], silica gel [13], microcrystalline naphthalene [14], Amberlite XAD resin [15], polyurethane foam [16] and alumina [17]. Among these solid

phases, silica gel modified with organic compounds as metal chelating agent exhibits some definite advantages such as porosity, large specific surface area, good mechanics, the thermal and chemical stability [18,19].

The use of CPE [20] offers an alternative to conventional extraction systems. Aqueous solutions are used in the CPE method instead of toxic and flammable organic solvents. In addition, CPE offers higher recovery efficiency and a large pre-concentration factor. The CPE method has been used to pre-concentrate mercury ions after the formation of sparingly water-soluble complexes, as a prior step to their determination [21–31].

Recently, solid phase extraction (SPE) cartridges and disks have widely and successfully been used for preconcentrating and separating of trace mercury ions from different matrixes [32, 33–37]. The SPE has several important advantages over solvent extraction: faster operation, easier manipulation, not requiring large amounts of an organic solvent, less stringent requirements for separation, higher preconcentration factor and easier linkage to analytical instruments such as ICP-MS, ICP-AES, HPLC and HP-TLC.

In the present work polymer-based C18 cartridge as a sorbent and 5-amino-2-(phenyldiazenyl)phenol (I) and/or 5-amino-2-(p-tolyldiazenyl)phenol (II) as a ligands are a simple, rapid and reliable method to separate and concentrate trace amounts of Hg^{2+} ions. This proposed procedure was developed to separate Hg^{2+} ions from aqueous environmental samples.

2. Materials and Methods:

2.1. Apparatus:

The extraction was performed on a Waters solid phase extraction (SPE) device (that can prepare 20 samples simultaneously), and a reversed phase polymer C18 TM cartridge (methacrylate polymer functionalized with C18 ligands, 10 mm i.d., 15 mm, 30 μm particle) was obtained from Beijing Genosys Technologies, P. R. China. A Perkin Elmer atomic absorption spectrometry model *AAnalyst* 300 was used for all GFAAS measurements. A Perkin Elmer Lambda 12 UV-Visible spectrophotometer with a 5.0 mm quartz cell was used for all spectral measurements. A Hamilton syringe (10 μL) was used to deliver small volumes of Hg^{2+} into the cell. The reference cell was contained a membrane prepared in the same way without Hg^{2+}.

2.2. Reagent:

Unless otherwise stated, all reagents used were of analytical grade and all solutions were prepared with doubly distilled deionized water. Standard labware and glassware used were repeatedly cleaned with HNO_3 and rinsed with double distilled water, according to a published procedure [38].

Doubly distilled water and analytical reagent grade chemicals were used throughout. ACDP and/or ATDP used in the present investigation was prepared according to the procedure described previously [39]. A stock 3×10^{-3} M solution of I and/or II wa prepared by dissolving an appropriate weight of the reagent in a minimum amount of absolute ethanol [Sigma product] and brought to 100 ml in a calibrated flask with ethanol. The solution was stable for more than one month.

A stock solution of 100 μg mL^{-1} Hg^{2+} ion was prepared by dissolving 0.0068 g of $HgCl_2$ (Merck) in double distilled water and diluted to the mark in a 50 mL volumetric flask. The required volumes of this solution were used to prepare the working solutions. Working standard solutions were made by suitable dilution of this stock solution as required. A 3.0 M solution of nitric acid was used. Triton X-100 [Sigma Company] solution (5.0 %, v/v) was prepared by dissolving Triton X-100 with water.

2.3. General procedure:

A 10 ml of 3.0 M of nitric acid solution (0.3 M nitric acid in the final total 100 ml sample), 5.0 ml of 3 x □10^{-3} M of I and/or II solution and 5.0 ml of 5.0 % Triton X-100 solution were added to a standard or sample solutions containing no more than 20 μg of Hg^{2+} in a 100 ml of measuring flask. The mixture was diluted to volume of 100 ml and mixed well. After 5.0 min, the solution was passed through the polymer based C18 cartridge at a flow rate of 20 ml min^{-1} for enrichment. After the enrichment was accurse on the cartridge and finished, the retained complex is eluted from the cartridge at a flow rate of 5.0 ml min^{-1} with 0.5 ml of acetonitrile in the reverse direction. So only six seconds was required to elute the column. The absorbance of this solution was measured at 626 and 649 nm in a 5.0 mm cell using I and II, Respectively, against a reagent blank prepared similarly without Hg^{2+}.

2.4. Application to real samples:

2.4. Samples preparation:

2.4.1. Determination of mercury in soil and water samples:

Soil sample (from Port Said University Compose, Port Said, Egypt) was homogenized and dried at 105 ºC. After that, 10 g of soil was taken and 3.0 mL of $HNO_3:H_2O_2$ mixture (2:1) was added to the soil. This mixture was slowly shacked and dried on a hot plate. After cooling, 2.0 ml of 0.75 M nitric acid were added to the remainder and centrifuged. The mercury content of the sample solution was determined by applying the general procedure described above.

The analyzed water samples were acidified with 1.0 % HNO_3 after collection from the sources to prevent any losses from the analytes. River and sea water samples (from different locations of river, Benha, and Port Said, Egypt) did not need previous treatment and was analyzed for mercury ion concentration using the proposed method.

2.4.2. Determination of mercury in fish and vegetable samples:

2.0 g of the fish was dried and ashed at 700 ºC. After that the ash was dissolved with 10 ml of concentrated HNO_3 and diluted to 50 ml with distilled water to reach for the analysis conditions. A 0.5 ml of the solution was transferred into a 25 ml calibrated flask.

For the analysis of vegetables, all samples were washed and then dried at 100 ºC. Approximately, 0.5 g of each dried sample put into 250 ml of Pyrex beaker about 0.5 ml of concentrated H_2SO_4 was added to it. Beaker containing sample was placed into ashing furnace at 480 ºC for 4.0-5.0 hour. Then, 3.0 ml of concentrated $HNO_3: H_2O_2$ mixture (2: 1) was added to the ashed sample and dried on a hot plate. After residue was dissolved by using 2.0 ml of 1.0 M HNO_3 and, if necessary diluted to suitable volume.

2.5 Determination of mercury in amalgam and omega 3 tablet samples:

The commercial form of the amalgam takes form of little balls, each weighing 1.0 g. One of these exactly weighing balls was dissolved in 1:1 HNO_3 and the mixture was carefully boiled until complete dissolution of the amalgam was achieved. The pH was adjusted to 8.5 and diluted by appropriate dilution of the mother solution to 100 ml. For preparation of the omega 3 tablet sample, 20 tablets (from PHARCO Manufactory) were accurately weighed and powdered in a mortar. A mass corresponding to a tablet was dissolved in 0.1 M HCl in 100 ml measuring flask. After 30 min of mechanically shaking, the solution was filtrated in a 100 ml measuring flask through Whatman no: 40 filter papers. The mercury content of the each samples solution was determined by the proposed general procedure.

3. Results and Discussion:

3.1. Absorption spectra:

In the present studies, reagents I and II were applied as a complexing agent for Hg^{2+} through SPE using the polymer based C18 cartridge at variable conditions. The absorption bands of I and II and its complex in acetonitrile medium after SPE are located at 525 and 538 nm, respectively, whereas for their Hg^{2+} complexes with that ligands in acetonitrile after SPE are located at 626 and 649 nm, respectively [Fig. 1].

Fig. 1. Absorption spectra for 100 ng ml^{-1} Hg^{2+} complexed with I and II
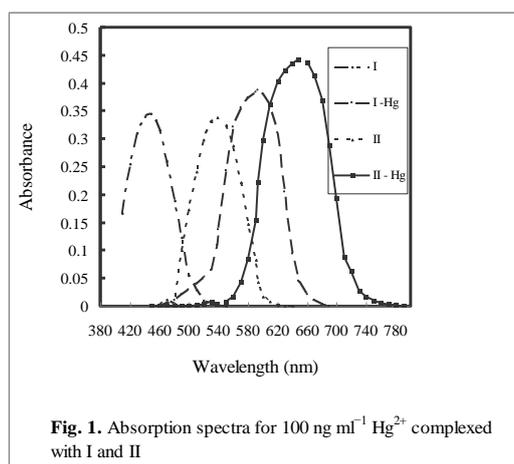
3.2. Effect of acidity:

Results showed that the optimal conditions for the reaction of Hg^{2+} with I and II are in acid medium. Therefore, the effects of hydrochloric, nitric, perchloric, phosphoric, and sulphuric acids, on the color reaction of Hg^{2+} with I and II were studied. Experiments showed that 3.0 M nitric acid has the best effect, and the amount of 9.0–11 ml was found to give a maximum and constant absorbance (Fig. 2), so 0.3 M nitric acid was recommended in the final total 100 ml samples.


Fig. 2. Effect of pH on the complex formation of 100 ng mL^{-1} Hg^{2+} with I and II
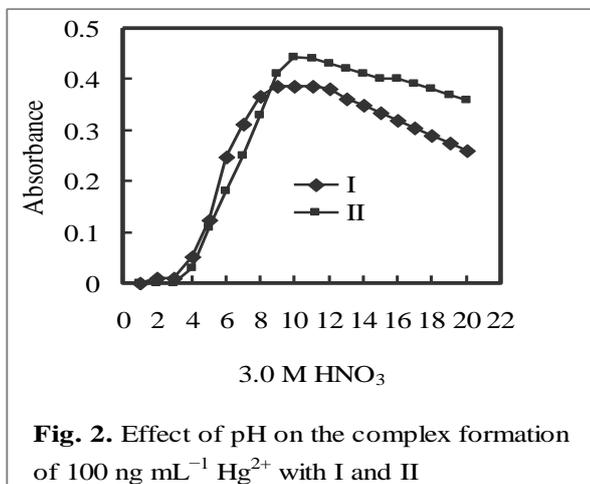
3.3. Effect of surfactants:

The effects of surfactants on the Hg^{2+}-I and/or II system were investigated. The results showed that, in the presence of anionic or cationic surfactants, the Hg^{2+} -I and/or II chromogenic system gives a low absorption, whereas in the presence of nonionic surfactants, the absorption of the chromogenic system increases markedly. Various nonionic surfactants enhance the absorbance in the following sequence: Triton X-100 > Triton X-114 > emulsifier-OP > Tween-80 > Tween-60 > Tween-20. Accordingly, the Triton X-100 was the best additive, and the use of 4.5 – 5.5 ml of 5.0 % Triton X-100 solution gave a constant and maximum absorbance. Consequently, the use of 5.0 ml of 5.0 % Triton X-100 solution was recommended.

3.4. Effect of ligand concentration:

For up to 60 ng ml^{-1} of Hg^{2+}, the use of 5.0 ml of □3 x 10^{-3} M of I and/or II solution was found to be sufficient for a complete reaction (Fig. 3). Accordingly, 5.0 ml of I and/or II solution were added in all further measurements.

Fig. 3. Effect of I and II on the complexation of 100 ng mL^{-1} of Hg^{2+} at the optimum conditions
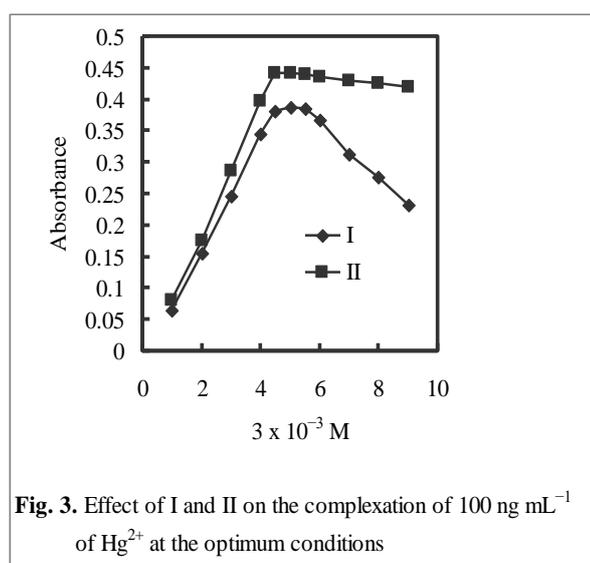
3.5. Stability of the chromogenic system:

After the components are mixed, the absorbance reaches its maximum within 5.0 min at room temperature and remains stable for at least 6.0 hrs. After having been extracted into the acetonitrile medium, the complex was stable for at least 24 hrs.

3.6. Solid phase colorimetric measurements:

Both the enrichment and the elution were carried out on a Waters SPE device. Different flow rates for enrichment and elution were tested and optimized. The results indicated that the flow rate was set to 20 ml min^{-1} for enrichment and 5.0 ml min^{-1} for elution as optimum flow rates.

Some experiments were carried out in order to investigate the retention of I and/or II and their Hg^{2+} complexes on the cartridge. It was found that both I and/or II and their Hg^{2+} complexes are retained on the cartridge quantitatively when they pass the cartridge as nitric acid medium. The blank solution containing I and/or II without Hg^{2+} was treated similarly to avoid the I and/or II absorbance. The capacity of the cartridge was determined as 31 and 27 μg for Hg^{2+} - I and/or II complexes, respectively, in 100 ml of solution. In this experiment, the maximum amount of mercury is only 20 μg. Therefore, the cartridge has adequate capacity to enrich the Hg^{2+}- I and/or II complexes.

In order to choose a proper eluent for the retained I and/or II and its Hg^{2+} complexes. Various organic solvents were used and studied. Out of various solvents (0.5 ml acetonitrile or 1.3 ml DMF or 1.5 ml acetone, 1.8 ml isopentyl alcohol or 2.5 ml for ethanol or 3.0 ml methanol) used for the elution of Hg^{2+} - I and/or II complexes from the cartridge. A 0.5 ml of acetonitrile was found to be sufficient to elute I and/or II and their Hg^{2+} complexes from cartridge for both sample and blank at a flow rate of 5.0 ml min^{-1}, the volume of 0.5 ml was selected. The experiment shows that it was easier to elute the retained I and/or II and their Hg^{2+} complexes in reverse direction than in forward direction, so it is necessary to reverse the cartridge during elution. In the forward direction, the elution is not quantitative, in addition to the elution flow rate is 25 ml min^{-1}

3.7. Stoichiometric ratio:

The nature of the complex was established at the optimum conditions described above using the molar ratio and continuous variation methods. The plot of absorbance versus the molar ratio of I and/or II to Hg^{2+}, obtained by varying the I and/or II concentration, showed inflection at molar ratio 2.0, indicating presence of three I and/or II molecules in the formed complex. Moreover, the Job method showed a ratio of I and/or II to Hg^{2+} = 2.0. Consequently, the results indicated that the stoichiometric ratio was (2 : 1) [I and/or II : Hg^{2+}]. The conditional formation constant (log K), calculated using Harvey and Manning equation applying the data obtained from the above two methods, was found to be 3.24 and 3.47 using I and II, respectively, whereas the true constant was 3.10 and 3.50, respectively.

For the ternary complex with Triton X-100, the obtained results implied that a 1: 1 complex is formed between the [(I and/or II)$_2$Hg] complex and Triton X-100. Consequently, the results indicated that the stoichiometric ratio was 2 : 1 : 1 [(I and/or II)$_2$Hg][Triton X-100], as shown in the following equations.

$$2 \text{ I and/or II} + Hg^{2+} \rightleftharpoons [(\text{I and/or II})_2Hg]$$

$$[(\text{ I and/or II})_2Hg] + [\text{Triton X- 100}] \rightleftharpoons [(\text{I and/or II})_2Hg][\text{Triton X-100}]$$
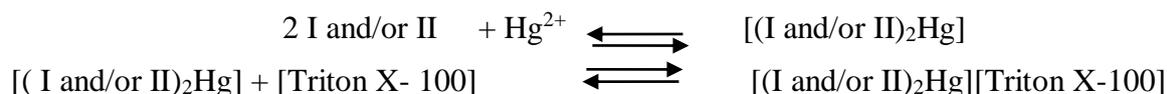
3.8. Selectivity:

To determine of selectivity of the proposed method, it was tested for the determination of 100 ng ml^{-1} of Hg^{2+} ions in the presence of some metal ions including, Tl^+, Ba^{2+}, Cu^{2+}, Co^{2+}, Cd^{2+}, Ni^{2+}, Mn^{2+}, Pb^{2+}, Bi^{3+}, Mg^{2+}, Al^{3+}, Sn^{2+}, Ag^+, Fe^{2+}, Zn^{2+} and Cr^{3+}. The tolerance limit was taken as the concentration causing an error of ± 5.0 % in the absorbance for determination of Hg^{2+} [40]. At the applied pH value, no interference was observed from even 6000-fold excess of the above metal ions except Ni^{2+}. The results showed that the Ni^{2+} ions interfered at concentrations higher than 1000 ng ml^{-1} which can be masked by addition of muroxide (0.01 M) as the optimum masking agent in this studies.

3.9. Calibration curve and sensitivity:

The calibration curve showed that the system obeys Beer's law in the concentration range of 2.5 – 200 ng Hg^{2+} per ml in the original solution. For more Accurate results, Ringbom optimum concentration ranges was found to be 10 – 175 ng Hg^{2+} per ml in the original solution. The linear regression equations obtained was A = 7.05 C (μg ml^{-1}) + 0.011 (r = 0.9996) and A = 7.72 C (μg ml^{-1}) + 0.009 (r = 0.9992) for I and II complexes, respectively. The molar absorptivity was calculated to be 8.81 x □10^8 and 9.57 x □10^8 L mol^{-1}

cm^{-1} at 626 and 649 nm, respectively. Sandell sensitivity was found as 0.0014 and 0.0017 ng cm^{-2}, respectively.

The standard deviations of the absorbance measurements were calculated from a series of 13 blank solution. The limits of detection and of quantification using the proposed method were established and calculated [41] according to the IUPAC definitions ($C_1 = KS_o/s$ where C_1 is the limit of detection, S_o is the standard error of blank, s is the slope of the standard curve and K is the constant related to the confidence interval [K=3 and K=10, for the limits of detection and of quantification, respectively]). . The detection and quantification limit are given as 0.75 and 2.37 ng ml^{-1} for I, whereas their values for II are 0.80 and 2.48, indicating that a nanogram fraction of Hg^{2+} can be determined. The relative standard deviation was 1.45 and 1.34 % using I and II, respectively, obtained from a series of 10 standards each containing 100 ng ml^{-1} of Hg^{2+}.

Table1: The comparison of the proposed method with other extraction methods.

Method	DL (ng ml^{-1})	QL (ng ml^{-1})	RSD (%)	Ads capacity (mg g^{-1})	Ref.
Silica modified-SPE	4.75 x 10^{-3}	0.02–1	1	200	[42]
Chelating resin-SPE	6.00 x 10^{-3}	0.1–30	3.5	–	[43]
Functionalized silica gel-SPE	–	–	–	3.652	[44]
Microextraction	0.01	–	6.1	–	[45]
Functionalized silica gel-SPE	0.90	1–1500	3	8	[46]
Gold-wire microextraction	0.006	–	4.6	–	[47]
Silica modified-SPE	–	–	–	340–700	[48]
Cloud point extraction	4.00 x 10^{-3}		3.4	–	[49]
Cloud point extraction	4.00 x 10^{-3}	–	3–5	–	[50]
Ionic liquid based-preconcentration	2.3	–	2.8	–	[51]
Membrane	4	–	0.1–2	–	[52]
Solid phase spectrophotometry	0.024	0.062–250	2.4	–	[53]
C18 modified-membrane	3.8 x 10^{-3}	–	3.1	–	[54]
C18-SPE	10.92	32.9–263.9	3.2	0.52	[33]
C18 modified-SPE	2.5 x 10^{-3}	–	–	–	[55]
Silica cartridge as a sorbent and 4-BPDB-SPE	1.87 x 10^{-3}	0.0062–0.31	2.98–4.45	0.49	[56]
IIP	2.875			25	[57]
IIP	0.006	0.02–1.0	5–9	6.4	[58]
The proposed method using I	0.75	2.37	1.45	---	----
The proposed method using II	0.80	2.48	1.34	----	----

As recorded in Table 1 the present procedures were compared with other reported methods for determination of mercury via; pH for complexation; λ_{max}; nm; linear dynamic range; µg ml^{-1}; detection limits; µg ml^{-1}; RSD %; and remarks interferences. The proposed method is more selective than all other compared methods. Also, it has a lower detection limits compared to listed methods.

3.10. Accuracy and analytical applications:

The proposed procedure was found to work well under laboratory conditions. In order to confirm the applicability of the present sensor, it has been applied to the determination of nanogram amounts of mercury in the omega 3 tablet, vegetable and fish digest samples spiked with different amounts of mercury ions were measured by the proposed procedure (Table 2). The mercury content of water and soil digest were analyzed by standard addition method and then determined by the proposed procedure (Table 3). From the data given in Table 2, and 3, of this paper is readily seen that the present procedure is useful for the determination of mercury in real samples. The accuracy was further validated by comparison of the results obtained by the proposed procedure with those obtained by the GFAAS as a reference method.

The performance of the proposed procedure was assessed by calculation of the t-value (for accuracy) and F-test (for precision) compared with GFAAS method. The mean values were obtained in a Student's t- and F-tests at 95% confidence limits for five degrees of freedom [60]. The results showed that the calculated values (Table 2 and 3) did not exceed the theoretical values. A wider range of determination, higher accuracy, more stability and less time consuming, shows the advantage of the proposed method over other method.

Table2: Results of mercury ion determination in spiked samples (N=6)

Sample [a]	Hg^{2+} Added	Hg^{2+} found [b] I	II	GF-AAS	RSD %	t-value[c]	F-test[c]
Omega 3(1)[d]	0.00	N.D[d]	N.D[d]	N.D	---		
	30.0	29.6	30.2	29.3	1.8	1.23	2.54
Omega 3 (2)	0.00	N.D	N.D	N.D	---		
	60.0	59.7	59.5	60.7	1.9	1.47	2.92
Omega 3 (3)	0.00	N.D	N.D	N.D	---		
	20.0	19.6	19.8	20.5	1.9	1.36	2.76
Vegetable 1	0.00	N.D.	N.D	N.D	---		
	70.0	71.85	69.2	72.5	1.8	1.17	2.43
Vegetable 2	0.00	N.D.	N.D	N.D	---		
	40.0	41.80	39.5	41.9	1.3	0.84	2.06
Vegetable 3	0.00	N.D.			---		
	50.0	51.5	48.9	51.9	1.9	1.11	2.38
Fish 1	0.00	76.0	75.2	74.5	2.0		
	20.0	95.0	96.8	95.5	1.9	1.57	3.21
Fish 2	0.00	100	99.3	98.5	2.0		
	30.0	127	131.2	133	1.8	1.44	2.78
Fish 3	0.00	140	138.5	136	2.0		
	40.0	181	178.2	179	1.9	1.25	2.61

a) All values are ng g^{-1}

b) Average of six determinations found by the proposed method

c) Theoretical values for t and F at 95% confidence limit (n = 5) were 2.57 and 5.05, respectively.

d) Number of samples

Table3: Determination of Hg^{2+} in soil and water samples

Sample	Hg^{2+} added	Hg^{2+} found[a] I	II	GF-AAS	RSD %	t-value[c]	F-test[c]
Soil[b]	0	2.12	2.15	2.20	1.3		
	5	7.20	7.12	7.10	1.5	1.26	2.45
	10	12.03	12.20	12.30	1.8	1.57	2.91
	15	16.98	17.10	17.40	1.6	1.44	2.67
	20	21.96	22.40	21.80	1.5	1.32	2.53
River water[c]	0	50.00	49.4	48.50	1.7		
	10	59.50	48.6	60.50	1.8	1.60	3.37
	20	71.20	70.5	71.50	1.7	1.49	2.77
	25	75.10	74.3	76.40	1.4	1.22	2.59
	30	79.66	80.8	81.80	1.8	1.03	2.33
Ground water[c]		100.0	101	102.5	1.6		
	10	109.2	110.5	111.4	1.3	1.54	2.89
	20	120.7	119.7	120.9	1.8	1.37	2.60
	30	129.0	130.5	131/5	1.7	1.23	2.39
	40	141.3	140.3	139.6	1.6	1.52	2.91
	50	148.7	150.8	152.8	1.6	1.41	2.74

a) Average of six determinations.

b) All values are $\mu g\ g^{-1}$

c) All values are $ng\ ml^{-1}$

4. Conclusion:

In this work, a new, simple, inexpensive, sensitive, and selective procedure with the Hg^{2+}–I and/or II complex was developed for the determination of mercury in environmental samples, for continuous monitoring to establish the trace levels of mercury in difficult sample matrices. It offers also a very efficient method for speciation analysis. Although many sophisticated techniques such as HPLC, AAS, FAAS, GF-AAS, ICP-AES, and ICP-MS, are available for the determination of mercury at trace levels in numerous complex materials, factors such as the low cost of the instrument, easy handling, lack of requirement for consumables, and almost no maintenance have caused spectrophotometry to remain a popular technique, particularly in laboratories of developing countries with limited budgets. The sensitivity in terms of molar absorptivity and precision in terms of relative standard deviation of the present method are very reliable for the determination of mercury in real samples down to $ng\ ml^{-1}$ levels in aqueous medium at room temperature (25 ± 5 °C).

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