

Separation, Preconcentration and Determination of Total Mercury in Aqueous and Biological Samples by cold vapour Atomic Absorption Spectrometry

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Abstract A simple method has been developed for the preconcentration of mercury based on the adsorption of its ammonium pyrrolidine dithiocarbamate (APDTC) complex on amberlite IR-120 column. The influence of complexing agent, pH, amount of resin, eluting agents and flow rate has been investigated in detail. The effects of matrix ions on the retentions of the analyte were also examined. The adsorbed complex could be eluted using 1M HNO₃/ acetone and the concentration of mercury was determined by cold vapour atomic absorption spectrometry (CVAAS). The recoveries of the analyte were generally more than 95% with a low RSD. The proposed method has been successfully applied for the metal content evaluation in samples including water, wastewater, cow milk, dry milk, rice, fish and canned tuna.

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1. Introduction

The toxicity of mercury has been known for a long time. However, the potential hazards of wide spread mercury contamination in the environment to man and animals has become of concentration only recently. The pollution of aquatic environment with heavy metals has become worldwide problem during recent years. Once the mercury enter our biological system, it disturb biological processes, leading in some cases to fatal results, it attack the active sites of enzymes particularly sulfur containing ligands e.g. SCH₃ and -SH in methionine and cysteine amino acids, which are part of the enzyme [1-2] inhibiting their essential enzymes function. Symptology varies according to total dose absorbed and whether the exposure was acute or chronic [3-5].

Cold vapour atomic absorption spectrometry (CVAAS) technique is most widely used in quantitative determination of mercury in biological samples because it highly sensitive and absent of spectral interference [6-8]. However, signal background attenuation could be due to excessive foaming of the sample in the gas- liquid separator, especially if the generated foam or water vapor entered the absorption cell. Cheng- Chieh Yen et al. [9], add an additional gas- liquid separator after the mixing / separator assembly to overcome this problem. The literatures give little information on the validation of procedures for determining total mercury levels in samples with complex matrix composition. The preconcentration techniques such as solid-phase extraction (SPE) [10-12], liquid-liquid extraction [13],

co-precipitation [14], ion-exchange [15-16], electrochemical deposition[17], cloud point extraction [18] and flotation [19] etc. have been widely used for the preconcentration and separation of trace metal ions before analysis .

Solid phase extraction (SPE) offer the opportunity of selectively extracting and preconcentrating only the trace elements of interest, thereby avoiding the presence of major ions. This is crucial in some cases such as determination of heavy metals in biological samples to remove substances present in the sample that may interfere the metal determination by AAS [20-23]. Preconcentration based on column techniques is one of the most effective multi-element preconcentration methods due to its simplicity, rapidly and ability to attain high concentration factor. In addition SPE provides for the removal of potentially interfering matrices. The column can be mostly packed either cross-linked polymeric resins with chelating groups or directly the resin adsorbed the metal complexes formed before the preconcentration step. The basic principle of SPE of trace metal ions is the transfer of analytes from aqueous phase to the active sites of solid phase. For this purpose, various adsorbents including activated carbon [24], silica gel [25], microcrystalline naphthalene [26], C18 cartridges [27], Chelex-100 [28], Alumina [29], and polyurethane foam [30-31] have been used.

Hg²⁺ is a soft acid and has good affinity towards sulfur containing ligand [32-34]. Ammonium pyrrolidine dithiocarbamate (APDTC) is one of these ligands, which has been used in the preconcentration of

many metals [35]. APDTC form much more stable complex in a wide range of pH because of the strong complexation ability of APDTC [36-37].

Among solid-phase adsorbents, Amberlite IR-120 resin which is very useful for preconcentration-separation of metal complexes [38-40] because of their good physical properties such as their porosity, uniform pore size distribution, high surface area as chemical homogeneous non-ionic structure, and good adsorbent.

The aim of this work is the investigation of the optimum conditions for separation of total mercury in different water and biological samples using ammonium pyrrolidine dithiocarbamate as a complexing agent, then adsorbed on amberlite IR-120 as a collector for preconcentration, followed by elution using 1 M HNO₃ in acetone and determination of Hg (II) by CVAAS. This method was successfully applied to tap water, wastewater, cow milk, dry milk, rice, fish, and canned tuna.

2. Experimental

2.1. Instruments

A Perkin-Elmer model 2380 atomic absorption spectrophotometer (USA) equipped with (MHS-10) Mercury/Hydride system was used in this study. Perkin-Elmer hollow-cathode lamp operating at 6 mv, a wavelength of 253.7 nm with a spectral band pass of 0.7 nm, and nitrogen was used as a purge gas and NaBH₄, as a reductant were used. Infrared spectra (IR) were recorded from KBr pellets with Perkin-Elmer model 1430 spectrophotometer (USA) in the range 4000-200 cm⁻¹. pH-meter (Hanna-Instruments, 8519, Italy) with an expanded scale and having an accuracy of ± 0.01 was used for measuring pH values.

2.2. Reagents and solutions materials

Table 1. Properties of Amberlite IR 120

Data	Amberlite IR-120
Matrix	Styrene
Functional group	Sulphonic acid
Divinylbenzene	8%
Mesh size	16-45
Particle size (mm)	0.3-1.2
Ionic form as shipped	H ⁺
Maximum temperature(C ^o)	120
range pH	0-14
Total capacity (equiv./L)	1.9
Swelling	5-7%
exchange capacity	1.9 mequiv./g wet resin
	4.4 mequiv./g dry resin
	4.5 mmol/ml
Moisture content	45%

All the chemicals used were of analytical-grade and purchased from *BDH, UK*. Distilled-deionized

water was used in all determinations. Standard BDH1000ppm Hg solution for AAS was used. The model and standard solutions of the metals were prepared from the stock solutions in appropriate ratios. 0.1 % (W/V) solution of APDC in ethanol was used as the complexing agent. The adsorbent resin amberlite IR-120 is strongly acidic resin and has properties are given in Table 1.

For the pH 2 buffer solution was prepared by mixing of proper volume of 1M sodium sulfate and 1M sodium hydrogen sulfate solutions. Acetate buffers prepared by mixing by mixing different amounts of 1M sodium acetate and 1M acetic acid were used to maintain the pH 3 to 6. 0.1M potassium dihydrogen phosphate and 0.1M Disodium hydrogen phosphate were used for pH7. 0.1M Ammonium chloride buffer solutions were prepared by adding an appropriate amount of ammonia to result in solutions of pH 8-11. pH 12 was prepared by adding amount of 0.1M sodium dihydrogen phosphate to 0.1M sodium hydroxide solutions.

2.3. Preparation of mercury complex

[Hg (PDTC)₂] is prepared by adding (0.271 g, 1 mmol) HgCl₂ in 10 mL ethanol to (0.328 g, 2 mmol) APDTC ligand in 20 mL ethanol. The addition of APDTC in the colorless metal ion solution resulted in formation of off-white precipitate immediately. After stirring for 30 min, the precipitate is filtered off and dried in vacuum desiccators over fused CaCl₂.

2.4. Preparation of the mini-column

The glass mini-column (10cm length and 0.5cm diameter), having a stopcock, used for preconcentration of the metals. The resin was first washed with 1M NaOH, water, 1M HNO₃, water, acetone, and water and kept in a desiccator after drying until filled into the column.

2.5. Sampling and pre-treatments

Mineral, tap, river, and wastewater samples were collected from Dasani (the Coca Cola Co.), Mansoura University, Nile, and effluent from the Talkha Fertilizer Co., respectively. Each sample was acidified to pH~2.0 with HNO₃ after collection and filtered through a filter paper with 0.45µm pore size.

In order to microwave digestion of rice sample, 1.0 g of sample was digested with 6 ml of concentrated HNO₃ and 2 ml of H₂O₂ in microwave system. After digestion the samples, the volume of digested sample was made up to 50 ml with deionized water.

The fish and canned tuna samples were digested as the following. Five gram of fish sample was weighted accurately in a beaker and then 20-25 ml H₂SO₄ was added. Then the microwave digestion of fish sample, the above mixture was digested with 4 ml of concentrated HNO₃ and 2ml of concentrated H₂O₂ in microwave system. After digestion completed, the

volume of digested sample was made up to 50 mL with deionized water.

2.6. Procedures

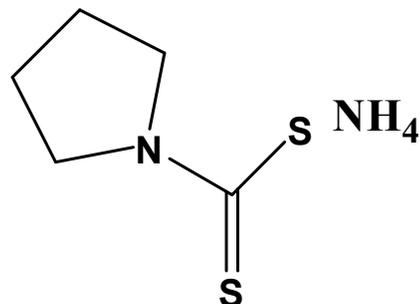
The proposed method was tested first with model solutions prior to the determination of mercury in the real samples. The model solutions (50 mL) containing 50 µg of Hg (II) were prepared. The pH of the model solutions was adjusted to the desired values (in pH range 2-10) by the addition of 2-5mL of respective buffer solution, and 5mL of 0.05% of solution complexing agent was added. The mini-column with the buffer solution at the working pH was used. The solution containing metal complex was passed through the column with a flow rate of 2mL min⁻¹. After the metal complexes retained on the resin in the column were washed with 10mL of distilled-deionized water then were recovered by elution with 1M HNO₃ in acetone into a beaker. The beaker content was evaporated to near dryness on a hot plate at 40-50^o C, and the residue was diluted to 5.0mL with 1M HNO₃. The metals in this solution were determined by CVAAS. These procedures were repeated separately for all optimization parameters.

3. Results and discussion

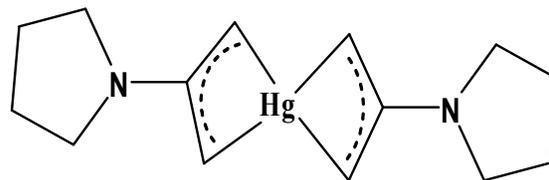
3.1. Characterization of solid complex

The structural formulae of the ligand and its complex are shown in Scheme (1, 2). The prepared compounds are listed in Table 2, 3 and the formulae

given are in agreement with the elemental analyses and IR data.



Scheme 1. Structure of ligand



Scheme 2. Structure of mercury complex

3.2. Elemental analysis and physical data

The complex is stable in prolonged exposure in air. Elemental analyses and other physical data are shown in Table (2).

Table 2. The elemental analysis of the ligand and its complex

Compound	Color	M.p. (°C)	% Found (Calcd.)			
			C	H	N	Hg
C ₅ H ₈ NS ₂ .NH ₄	Off-white	153-155	36.5 (36.5)	7.3 (7.3)	17.0 (17.0)	—
Hg(C ₅ H ₈ NS ₂) ₂	Off-white	270	24.2 (24.4)	3.1 (3.3)	5.4 (5.7)	40.0 40.7

3.3. Infrared spectra (IR)

Extensive use has also been made of infrared spectroscopy for the characterization of mercury-dithiocarbamate complex. C-N and C=S stretching modes, which fall typically in the range 1500±50 and 980±50 cm⁻¹, respectively.

For dithiocarbamate complex, three main regions are of interest the 1580- 1450 cm⁻¹ region, which is primarily associated with the stretching vibration of C-N group of N-CSS⁻ moiety; the 1060- 940 cm⁻¹ region

associated with ν(-CSS) vibration; the 420- 250 cm⁻¹ region which is associated with ν(M-S). Vibrations ν(N-CSS) bands defines a carbon-nitrogen bond (1350 – 1250 cm⁻¹) double bond (1690 – 1640 cm⁻¹) and ν(N-CSS) mode is shifted to higher wave number, showing an increase of C=N in Figure (1,2). Table (3) shows that ν(N-CSS) of PDTC is shifted to higher frequency upon coordination; consist with an increase of carbon-nitrogen double bond character. Methylene C-H bands are observed at 2940 cm⁻¹.

Table 3. IR spectral data (cm⁻¹) for the ligand and its complex

Compound	IR (cm ⁻¹)			
	ν(C=S)	ν(N-CSS)	ν(N-C)	ν(M-S)
C ₅ H ₈ NS ₂ .NH ₄	997	1321	1450	—
Hg(C ₅ H ₈ NS ₂) ₂	1037	1331	1437	415

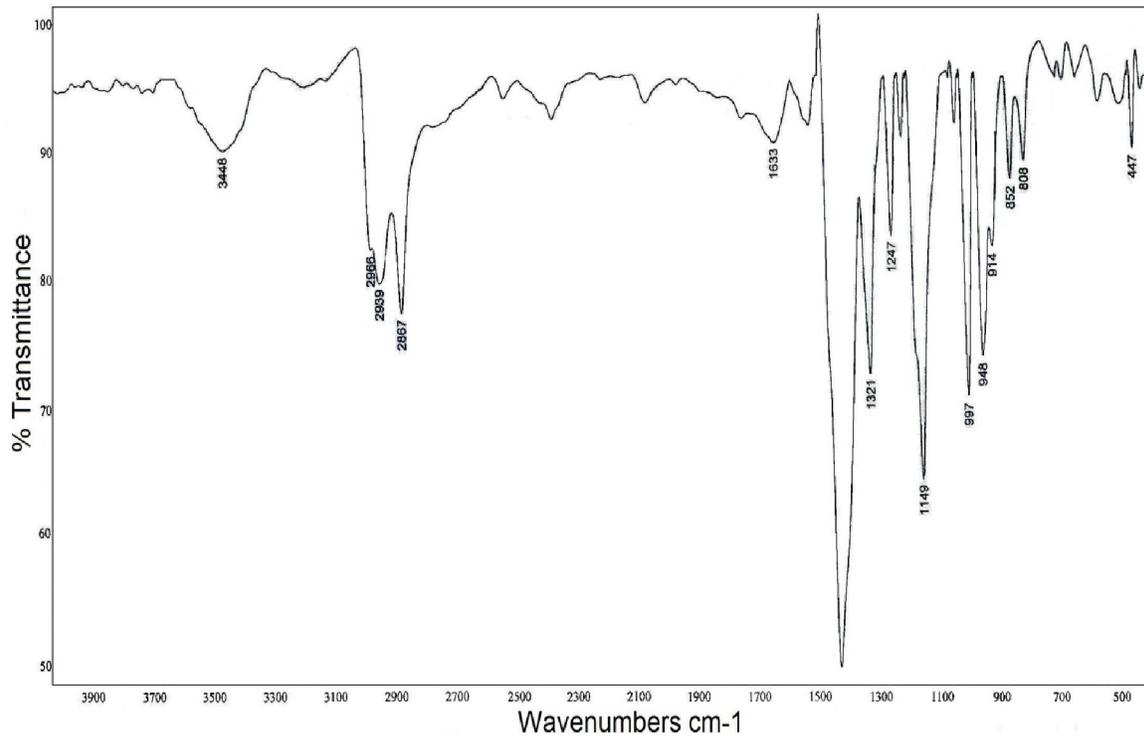


Figure 1. The IR spectrum of the ligand

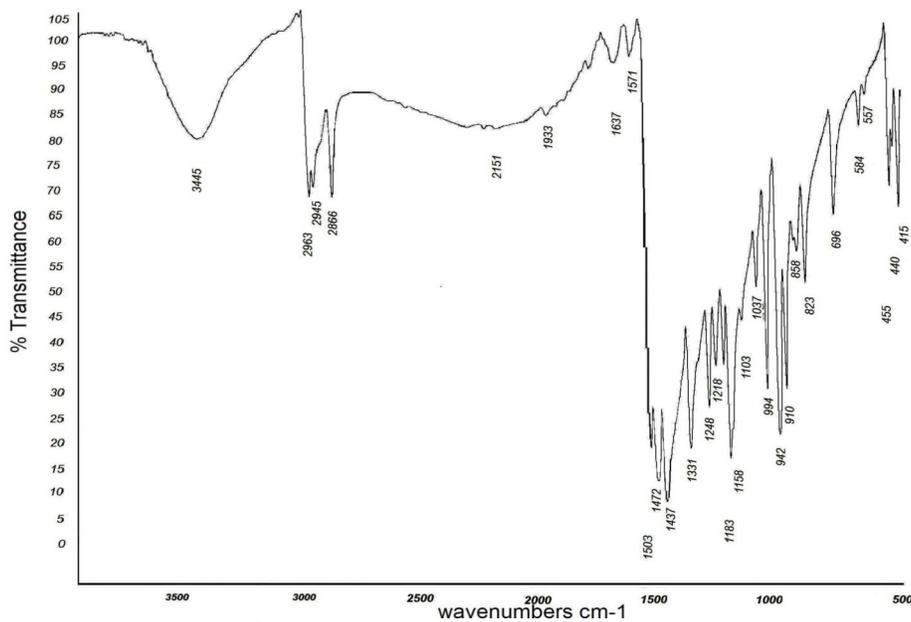


Figure 2. The IR spectrum of the mercury complex

3.4. Optimum pH for sorbent extraction of mercury

The pH value of a sample solution is one of the most important variables controlling the adsorption of the metal ion in the column. Therefore, in order to

determine the optimum pH, the reaction of mercury ions with the complexing agent (APDTC) was investigated in the pH range 2-10, as shown in Figure (3). The recovery was found quantitative in pH 4-8, the

optimized pH of mercury complex sample was established as 5 for the quantitative extraction of mercury-APDTC complex and the metal recovery was about 99%. Therefore, pH 5.0 was selected for all subsequent experiments.

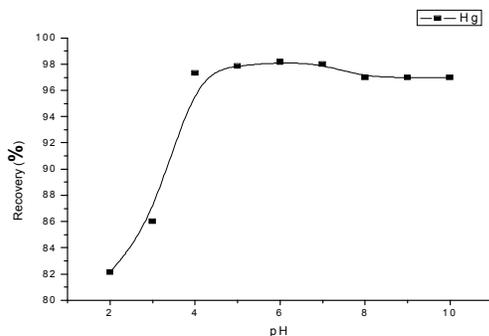


Figure 3. Effect of pH on the recovery of the mercury (N=3)

3.5. Effect of quantity of complexing agent

In order to obtain high recovery, different amount of APDTC were added. So, the effect of APCD quantity on the retention was examined from 1.0 to 10mg. The result in Figure (4) shows that he considerable increase on the recoveries obtained by increasing the complexing agent from 0.5 mg to 2.5mg after that remain constant.

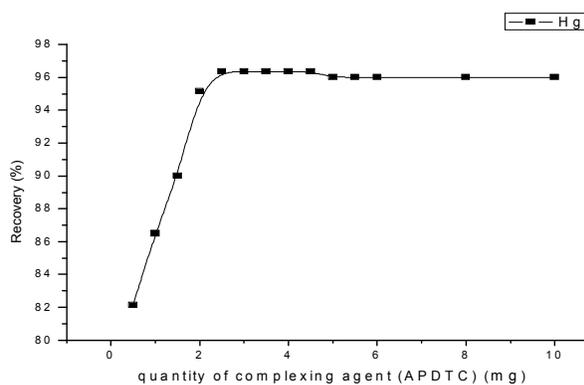


Figure 4. Effect of complexing agent quantity at pH=5 (N=3)

3.6. Effect of resin quantity

In order to estimate the optimum resin quantity, the recoveries of the metals were examined by use of the resin quantities between 50 and 1000 mg, and the best recoveries were achieved between 150 to 400 mg in Figure (5). The optimum resin quantity was 200 mg for the later tests.

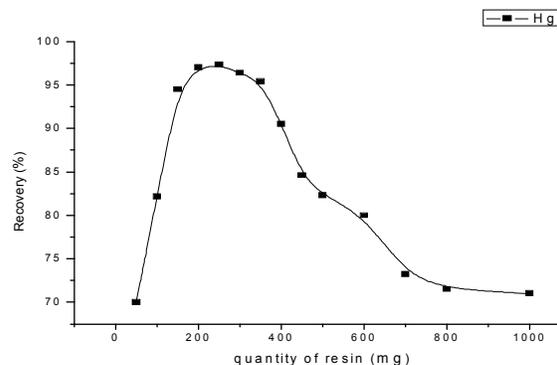


Figure 5. Effect of resin quantity (N=3)

3.7. Effect of eluent type, concentration and volume

A suitable eluent should be used for desorption of metal complexes; (I) the eluent should desorb the metals complexes; (II) the eluent should not destroy the sorbent; (III) the eluent should be suitable for the following determination technique[39].

Various acids and organic solvents were used to choose the best one for the elution of the metal-APDC complexes accumulated on Amberlite ir-120 resin, and the percentage recovery for each eluent type was determined. With using different solvent solutions especially the acids with acetone and acetonitrile provided higher recovery efficiency compared to the acids in the aqueous and alcoholic solutions, and the highest recoveries were obtained with either HNO₃ or HCl in acetone or acetonitrile. At the end, the acetone-HNO₃ or acetonitrile-HNO₃ mixture is specified as the eluent of choice for the later determinations and for and for optimizations of the other parameters Table (4).

Table 4. The effect of different eluents on the recovery of mercury

Eluent solution	Recovery (%) for Hg
HCl(1M) in water	<10
HNO ₃ (1M) in water	<10
Ethanol	17.2±2
HCl(1M) in ethanol	23.5±1
HNO ₃ (1M) in ethanol	29.3±2
Acetone	30.0±2
HCl(1M) in acetone	93.2±2
HNO ₃ (1M) in acetone	98.2±2
Acetonitrile	29.8±2
HCl(1M) in acetonitrile	93.0±1
HNO ₃ (1M) in acetonitrile	98.3±1

The effect of eluent volume on the recovery of mercury was also studied by keeping the eluent concentration constant at 1M HNO₃ in acetone or acetonitrile and varying volumes from 2 to 14 ml as can be seen from Figure (6,7).It was found that quantitative recoveries could be obtained with 1M

HNO₃ in acetone or acetonitrile concentration with 10ml volume. The experimental results under these conditions indicated that quantitative recovery (98%) for the target analyte could be obtained.

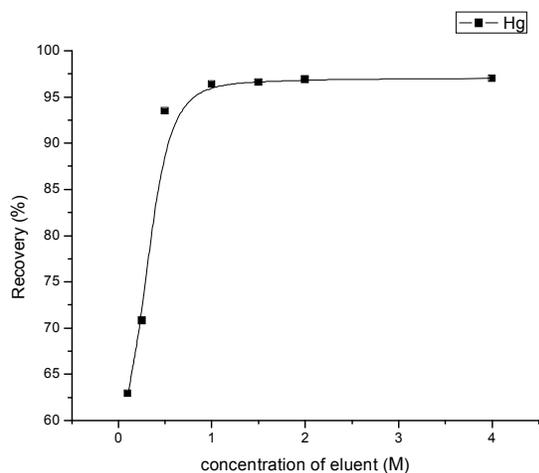


Figure 6. Effect of eluent concentration on the retention of the analyte ions (N=3)

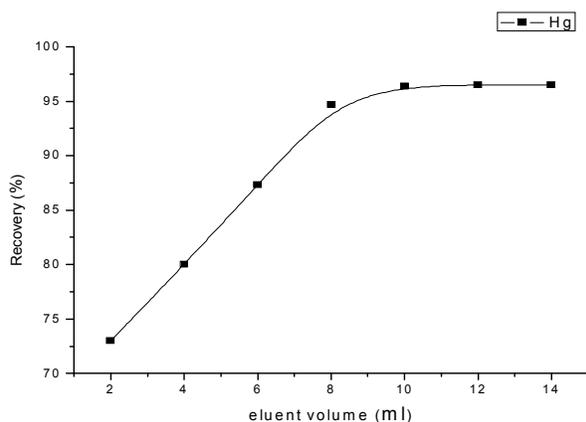


Figure 7. Effect of eluent volume on the retention of the analyte ions (N=3)

3.8. Effect of sample flow rate

The flow rate of the mercury complex solution through the packed column is very important parameter for the time controls of adsorption and analysis. Using the column procedure, the effect of flow rate on sorption of mercury complex was investigated. The results show that the flow rate has more influence on the sorption of mercury complex. The recovery decreases by increasing flow rate sample. The result showed in Figure (8) where the sample flow rate was chosen at 1.0 mL min⁻¹ in further studies.

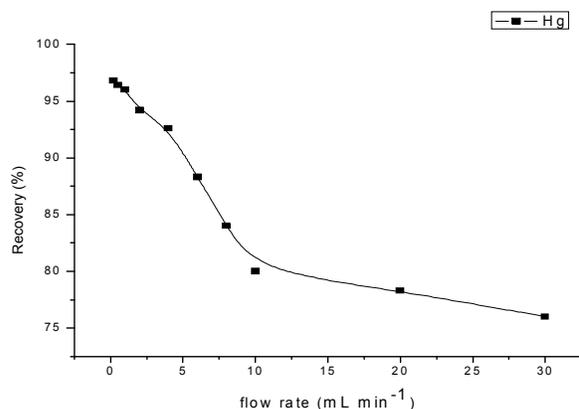


Figure 8. Effect of flow rate on the retention of the analyte ions (N=3)

3.9. Effect of mixed foreign ions

The interfering effect of diverse ions was studied at varying concentrations. The preconcentration studies were carried out as mentioned above using 5 µg Hg (II) maintaining a sample volume of 50 mL. the studies indicated that Na⁺, K⁺, Ca²⁺, Mg²⁺, Cl⁻, NO₃⁻, SO₄²⁻, PO₄³⁻, HCO₃⁻, Fe³⁺, Mn²⁺, Al³⁺ did not cause any significant reduction in the recovery of mercury. The results are presented in Table (5) showing the recovery of Hg (II) with varying concentration of metal ions.

Table 5. Effect of diverse ions on the recovery of the analyte ions by the proposed procedure

Ion	Added as	Conc. (mg L ⁻¹)	Recovery (%)
Na ⁺	NaCl	100	98.2±0.5
		1000	97.5±1
K ⁺	KCl	100	98.1±1
		1000	97.4±1
Ca ²⁺	CaCl ₂	100	98.5±1
		1000	98.0±2
Mg ²⁺	MgCl ₂	100	98.3±0.5
		1000	98.0±1
Cl ⁻	NaCl	100	98.2±0.5
		1000	97.5±1
NO ₃ ⁻	KNO ₃	100	98.3±1
		1000	98.0±1
SO ₄ ²⁻	Na ₂ SO ₄	100	98.4±1
		1000	97.8±2
PO ₄ ³⁻	Na ₃ PO ₄	100	98.1±2
		1000	97.5±2
HCO ₃ ⁻	NaHCO ₃	100	98.1±1
		1000	97.4±1
Fe ³⁺	Fe(NO ₃) ₃	10	98.0±1
		100	97.1±2
Mn ²⁺	Mn(NO ₃) ₂	10	98.3±1
		100	98.0±2
Al ³⁺	Al ₂ (SO ₄) ₃	10	98.1±1
		100	97.6±2
Mixed*			98±2

*A sample containing 1000 mg of Na⁺, K⁺, Ca²⁺ and Mg²⁺ and 1000 mg of SO₄²⁻, Cl⁻ and NO₃⁻

3.10. The accuracy test for spiked recovery

The preconcentration method was applied to standard solution of mercury; the concentration of the tested metal was prepared by appropriate dilutions from 1 mg mL^{-1} (BDH) solutions for AAS. Analytical recoveries for investigated ion were assessed for various concentration levels from $5\ \mu\text{g}$ (0.1 ppm) to $250\ \mu\text{g}$ (5 ppm). The initial sample volumes were 50 mL, pHs of all samples were adjusted to 6 and the final

eluted volumes were 5 mL. The studied metal was analyzed by hydride generation system in FAAS with analytical separation factor 10. The results obtained were given in Table (6). The results indicated that the good recoveries for studied metal from $5\ \mu\text{g}$ to $150\ \mu\text{g}$, while low recoveries were observed at higher concentration due to the saturation of the active sites of the resin under this condition.

Table 6. The accuracy test for spiked mercury recovery (sample volume: 50 mL, N=3)

No of sample		Added (μg)	Found (μg)	Recovery (%)
1	0.1	5	4.9	98.0 \pm 1
2	0.2	10	9.9	99.0 \pm 2
3	0.4	20	19.6	99.0 \pm 2
4	0.8	40	39.6	99.0 \pm 1
5	1.0	50	49.7	99.0 \pm 2
6	2.0	100	98.7	99.0 \pm 1
7	3.0	150	144.3	96.0 \pm 2
8	4.0	200	190.5	95.0 \pm 2
9	5.0	250	229.9	92.0 \pm 2

3.11. Application to real samples

The proposed preconcentration method was applied to the water, wastewater, cow milk, dry milk, rice, fish and canned tuna samples. pHs of all the samples were adjusted to 6. The mercury content of the samples was complexed with APDTC and passed

through the mini-column filled with amberlite ir-120 resin. Mercury complex retained in the column then eluted with 1M HNO_3 in acetone. Final volumes were made 5.0 mL and mercury was analyzed by hydride generation system in AAS. The results for the sample are given in Table (7).

Table 7. Determination of trace metal contents of real samples with the proposed method (n=3)

Sample no.	Sample locations	Concentration of Hg $\mu\text{g mL}^{-1}$	Permissible limit Ppm
1	Tap water (Mansoura city)	0.01 \pm 0.02	0.001 ^a
2	Nile water (Mansoura city)	0.13 \pm 0.03	0.001 ^a
3	Waste water (Talkha Fertilizer plant)	0.42 \pm 0.1	—
4	Milk powder ($\mu\text{g/g}$)	0.02 \pm 0.01	0.02 ppb ^c
5	Fish($\mu\text{g/g}$)	0.09 \pm 0.2	0.5 ^c
6	Tuna sun shine canned	0.12 \pm 0.05	1.0 ^d
7	Canned Tuna golden bell	0.15 \pm 0.02	1.0
8	Tuna Mario canned	0.1 \pm 0.02	1.0
9	Cow milk	0.01 \pm 0.04	—
10	Rice	0.04 \pm 0.01	0.1 ^c
11	Mineral water	0.03 \pm 0.02	0.001 [*]

a: Egyptian standard No. 458(2007), c: Egyptian standard No. 2360 (2007), d: Egyptian standard No. 804 (2005), * Egyptian standard No. 1589 (2007)

4. Conclusion

In the present study, a solid phase extraction technique was developed for determination of mercury in solid/liquid samples, based on adsorption of mercury-APDC complex on Amberlite IRC-120 resin

.The developed procedure is simple sensitive and shows high tolerance to interfering ions, good analytical characteristics such as enrichment separation factor and precision. Accuracy test for spiked mercury contents in range from 5-250 μg yields quantitative

recovery. The proposed separation –enrichment method was applied for the atomic absorption spectrometric determination of the investigated metal ion in tap water, Nile water, cow milk, fish, milk powder, rice and canned tuna from the market of El-Mansoura city, Egypt with satisfactory results (recoveries greater than 95%, relative standard deviation $\leq 2\%$).

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