Solvent Extraction and HPLC Determination of Copper, Iron Nickel and Mercury in Water and Fishes as 2-Pyrrolaldehyde-4-Phenyl-3-Thiosemi-Carbazone as Derivatizing Reagent

M.Y. KHUHAWAR AND S.N. LANJWANI
Institute of Chemistry, University of Sindh,
Jamshoro, Sindh, Pakistan.

(Received 22nd November, 2000, revised 4th August, 2001)

Summary: The reagent 2-pyrroladehyde-4-phenyl-3-thiosemicarbazone(PAPT) was examined as complexing reagent for solvent extraction of copper(II), iron(II) nickel(II) and mercury (II). The study was carried out by spectrophotometry and liquid chromatography. The complexes indicated molar absorptivity in the range of 8.85 to 63.4x10^3 l.mole^{-1} cm^{-1} and eluted from Microsorb C-18, 5um (150x4.6 mm id) with methanol:acetonitrile:water: sodium acetate (1mM) and tetrabutyl ammonium bromide (1mM) (60:28:10:1:1 v/v/v/v/v) using a flow rate of 1 ml/min. Detection UV was at 254 nm. The method was applied for determination of the metal in Fuleli canal water after preconcentration by complexation and solvent extraction and amounts found were within 27-250 ug/L with coefficient of variation (C.V) within 2.7 to 5.1%. The method was also applied for determination of copper(II), iron(II), nickel(II) and mercury(II) in surface water fishes in the range 0.021-1.2 ug/g with C.V 1.1-6.4%. The obtained results indicated good correlation with flame atomic absorption spectrometry.

Introduction

For the determination of metals in natural water systems, a number of preconcentration procedures have been proposed including solvent extraction [1], ion exchange [2], copreciptation [3], evaporation [4] and freeze drying [5]. Solvent extraction procedures are of interest, because they are simple and the required preconcentration could be obtained with low matrix effects. Complexing reagents commonly used for preconcentration are different dithiocarbamates [6,7], 8-hydroxyquinoline [8], dithizone [9] and water soluble polymers [10].

Phenylthiosemicarbazones are interesting chelating reagents for metal ions by binding through sulphur and hydrazino nitrogen atom [11]. The presence of phenyl group at the end of thiosemicarbazone chain increases the sensitivity of the thiazone grouping. A number of phenylthiosemicarbazones have been reported as spectrophotometric reagents for metal ions [11-13] but a few have been investigated for high performance liquid chromatogrpahic (HPLC) determination of metal ion [14-19].

In the present work reagent PAPT has been investigated for preconcentration and HPLC separation and determination of copper, iron and nickel from water and copper(II), iron(II), nickel(II) and mercury(II) from surface water fishes.

Results and Discussion

The reagent is prepared by simple condensation of pyrrolaldehyde and 4-phenylthiosemicarbazide in 1:1 molar ratio. The mass spectrum of PAPT indicates molecular ion peak M$^+$ at m/e 243.8 (55%). A fragment peak at m/e 150.8 is observed due to either loss of $[C_5H_5NH_2]^+$ or [C_4H_4N CH=N] with a base peak at 93(100%) corresponding to $[C_6H_5NH_2]^+$. IR of the reagent in KBr indicates bands at 3260 and 3140 due to NH, five bands within 1615-1508 cm^{-1} are assigned to C=N, C=C, NH and aromatic ring vibration and two bands at 1260 and 1205 cm^{-1} are because of C=S streching vibrations as could be expected from structure (Fig. 1).

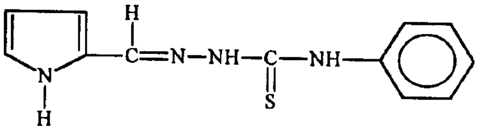

Fig.1 Structural diagram of the reagent.

Spectrophotometric Studies

The reagent reacts with copper(II), nickel(II) and iron(II) to form water-methanol soluble coloured complexes, but mercury(II) and silver(I) under similar conditions develop turbidity. All the complexes are extractable in chloroform. The effect of pH on the maximum colour formation of copper(II), iron(II), nickel(II) cobalt(II), mercury(II) and silver(I) were examined spectrophotometrically in the pH range 1-10. Copper(II), iron(II), cobalt(II) and nickel(II) were examined in water-methanol and mercury(II) and silver(I) after extraction in chloroform. The metal complexes of copper(II), iron(II), cobalt(II) and nickel(II) were stable ranging from pH; 3-10; cobalt(II) even indicated colour reaction at pH 1. Copper(II), cobalt(II) and nickel(II) indicated maximum absorbance at pH 8.5 and iron(II) complex after formation in slightly acidic media gave maximum absorbance at pH 9. Mercury(II) and silver(I) complexes were stable within pH range 3-6.5, but at higher pH 7-10 mercury and silver complexes precipitated out and could not be extracted in chloroform. Mercury(II) and silver(I) complexes showed maximum absorbance at pH 6 (Fig 2). The results of spectrophotometric studies (Table 1) indicate that cobalt(II), copper(II), nickel(II), mercury(II) and silver(I) indicate a band in visible region within 378 to 388 nm with molar absorptivity within 8.85-63.40 x 10^3 l. mole^{-1} cm^{-1}, but iron(II) shows three bands at 450, 560 and 780 nm. The iron(II) bands at 560 nm and 780 nm could be used for selective determination of iron(III) in the presence of cobalt(II), copper(II), nickel(II), mercury(II) and silver(I).

The study of change in molar ratio between metal ions and the reagent was examined at 1:1, 1:2, 1:3 and 1:4 levels. The absorbances at wavelengths of maximum absorbance was recorded. Cobalt(II), copper(II), nickel(II) and iron(II) complexes indicated an increase in absorbance from 1:1 to 1:2, but remained constant from 1:2 to 1:4 metal ligand molar ratio. It leads to suggest that the maximum colour formation of the metal chelates required atleast 1:2 molar metal:ligand ratio. The absorbances of mercury(II) and silver(I) remained constant in metal:ligand molar ratio 1:3 and 1:4. The mercury(II) and silver(I) may be suggested to form metal complexes in 1:3 molar ratio (Fig. 3).

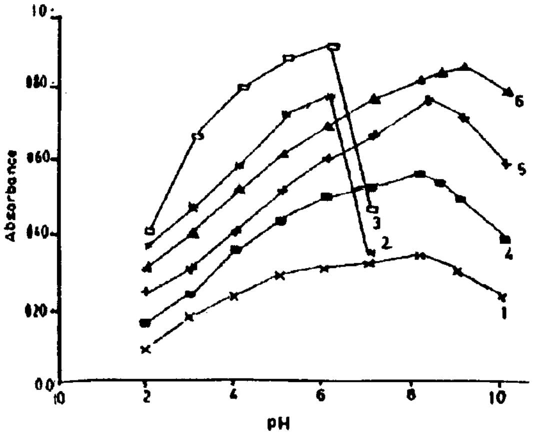

Fig.2 Effect of variation in pH on the absorbance of (1) cobalt(II), (2) silver (3) mercury(II), (4) copper(II), (5) nickel(II), (6) iron(II) as complexes of PAPT against reagent blank at a final concentration 0.5, 10 10.0, 1.0, 0.5 and 2.0 ug/ml at optimal wavelength.

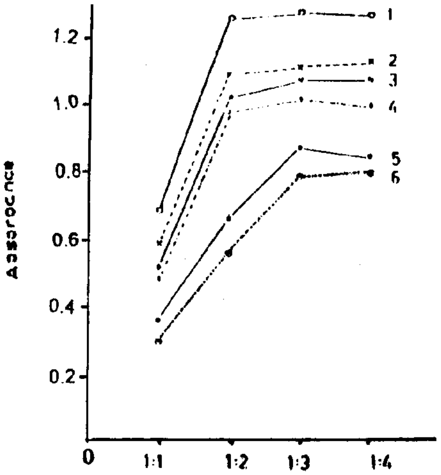

Fig.3 The effect of variation of molar:metal ligand ratio for the formation of maximum metal complexes with (1) nickel(II) (2) cobalt(II), (3) copper(II), (4) iron(II) (5) silver(I) and (6) mercury(II) in methanol and chloroform

Table-1: Quantitative Spectrophotometric data of metal chelates

Reagent	Metal	Solvent	pH of λ-$_{\text{max}}$ Absorbance	λ-$_{\text{max}}$ nm	L.mole^{-1} cm^{-1}x10^3	Calibration Range/ug/mL.
PAPT	Co(II)	Methanol	8.5	385	48.91	0.1-0.5
	Cu(II)	Methanol	8.5	378	38.45	0.2-0.5
	Ni(II)	Methanol	8.5	384	63.40	0.1-0.5
	Fe(II)	Methanol	9	450	22.88	0.2-2.0
	Hg(II)	Chloroform	6	381	22.86	2-10
	Ag(I)	Chloroform	6	388	8.85	2-10

Liquid Chromatographic Studies

The copper(II), nickel(II), iron(II) and mercury(II) as metal complexes of PAPT were simultaneously extracted in chloroform. They were examined for elution from HPLC column Microsorb C-18, 5um. The complexes were long retained and eluted as unsymmetrical peaks, when eluted with mixture of methanol;acetonitrile and water. Quiz and Fritz [16] and from this laboratory [15,19] have been reported that the addition of sodium acetate and tetrabutyl ammonium bromide have a significant effect on the elution capacity factor k' of metal chelates. It was therefore when complexes were eluted with mobile phase containing sodium acetate and tetrabutyl ammonium bromide, symmetrical peaks with retention times within 12 min were obtained (Fig. 4). The resolution factor (Rs) between two adjacent peaks were calculated and were found over 1.78. The capacity factor (k') increased from reagent 0.44 to mercury 6.12. Addition of acetonitrile has also significant effect on the capacity factor (k') of metal chelates. When acetonitrile was decreased by 5% from the mobile phase and the complexes were eluted with metanol:acetonitrile:water:sodium acetate (1m M): tetrabutyl ammonium bromide (1m M) (70:10:18:1:1 v/v/v/v/v); the value of k' increased for reagent to 1.29 and mercury(II) complex 11.78.

The quantitative analysis was carried out by measuring the average peak height (n=3) in the elution chromatogram. The calibration plot was constructed from the solution that contained known concentration of metal and after derivatization 5 ul solution was injected. The linear calibration curves for copper, nickel, iron and mercury as complexes of PAPT were obtained within the range of 10-50 ug/ml, with coefficient of correlation (r) (n=5), 0.994, 0.996, 0.988 and 0.998 respectively. The detection limits were determined by measuring three times the background noice and were found 0.5 ug/ml of each complex, corresponding to 2.5 ng/injection (5 ul).

Aluminium(III), lead(II), cadmium(II), platinum(II), oxovanadium(IV), dioxouranium(VI) and zinc(II) did not react with the reagent and their presence in the same concentration (50 ug) did not affect the determination of copper, iron, nickel and mercury Cobalt also forms coloured complex, but elutes after the reagent and did not affect the determination.

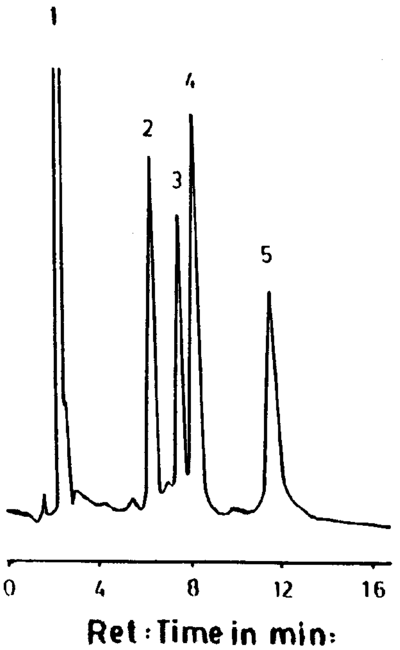

Fig.4 HPLC separation of (1) PAPT, (2) nickel(II), (3) iron(II), (4) copper(II) and (5) mercury(II) chelates on a column (150 x 4.6 mm id) of Microsorb C-18 (5 um) with elution with methanol: acetonitrile: water containing sodium acetate (1 mM) and tetrabutyl ammonium bromide (1 mM) (65:15:18:1:1 v/v/v/v/v) at a flow rate of 1 ml/min. and with UV detection at 254 nm.

The reagent was examined for preconcentration and determination of copper, iron and nickel. Double extraction was carried for qunatitative transfer of the metals from aqueous to organic phase and was processed as assay procedure. The amount of metal found in water samples (Table 2) indicate that copper was present in water samples in the range of 54 to 82 ug/L iron in the range of 182 to 276 ug/L and nickel within 28 to 46 ug/L, with a coefficient of variation (C.V) 3.2-6.7% after preconcentration of 150 times. The results obtained were compared using independent atomic absorption spectrometric method. The preconcentration was obtained by evaporation and determination was carried out after preconcentrations of 12 times. The results in table-2 for copper, iron and nickel with atomic absorption indicate acceptable correlation with C.V. within 1.1-3.2%. The results in table 2 also indicate that the concentration of metals increases as Fuleli canal

Table-2: Analyses of Metal Ion in Fuleli Canal

S.NO.	Amount of Metal Found By HPLC ug/L (C.V%)			Amount of Metal Found by A.A. ug/L (C.V%)		
	Copper	Nickel	Iron	Copper	Nickel	Iron
1.	40 (4.6)	27 (5.1)	250 (2.9)	42 (1.1)	31 (2.2)	260 (2.3)
2.	96 (5.2)	42 (3.8)	360 (4.1)	92 (1.8)	53 (1.6)	372 (1.2)
3.	105 (4.4)	35 (4.2)	340 (3.9)	109 (2.3)	39 (3.2)	356 (1.3)

travels along Hyderabad city due to the addition of sewage water.

The study of fish muscle for metal contents have importance as a direct means for investigation because it may become a source of heavy trace metals entering the human body through food chain enrichments. Five species of fish were caught at locations indicated in table 3 with weight in the range of 390-2200 g.

The reagent PAPT was used for the simultaneous determination of copper, nickel, iron and mercury in surface water fishes. The concentrations of metal ions found are summarized in table 3. The data reveals that the metal contents in fishes varied with species and copper found in muscles of fishes was in the range of 28-300 ng/g in Tenualosa ilisha and Mystus seenghala. The nickel was in the range 78 to 580 ng/g in Tenualosa ilisha and Notopterus notopterus. The contents of iron and mercury in Tenulosa ilisha was the lowest 110 ng/g and 100 ng/g respectively. However iron and mercury were maximum in Mystus Seenghala 1.15 ug/g and 0.92 ug/g respectively. The HPLC results showed C.V 3.8-7.6%. The metal contents of copper, nickel and iron were also analysed using FAAS and indicated close with correlation observed C.V with HPLC of within 1.2-4.5% (Table 3).

Experimental

Preparation of Reagent

4-phenyl-3-thiosemicarbazide (1.83 g) was dissolved in 20 ml methanol water (1:1) to which was added 2-pyrrolaldehyde (0.95 g) in methanol (10 ml). The mixture was added glacial acetic acid (0.5 ml) and was refluxed for 30 min. The mixture was cooled at −10°C for overnight. The dark coloured product obtained was recrystallized three times from methanol. m.p. = 194°C. Calculated for $C_{12}H_{12}N_4S$, expected % C=59.16, H=4.92, N=22.95; found % C=59.47, H=4.90 N=23.15. Mass spectrum indicates M^+ at m/e 243.8 (55%) and main fragment peaks at m/e 150.8 (25%), 136.8(42), 93(100%) and 77 (32%).

A. Spectrophotometric Studies

The metal ion solution (1-2 ml) containing copper(II), nickel(II), cobalt(II) (0-10 ug) or iron(II) (0-20 ug) was transferred to 10 ml volumetric flask, followed by 1 ml reagent PAPT solution, 0.2% in methanol and 2 ml buffer pH 8.5 for cobalt, copper and nickel and 9.0 for iron. The contents were mixed and diluted to volume with methanol.

In case of solution containing mercury(II) or silver(I) 0-100 ug) was transferred to separating funnel and were added 1 ml PAPT solution, 0.2% w/v in methanol, 2 ml sodium acetate-acetic acid buffer pH6 and chloroform 5 ml. The layers were mixed well and allowed to separate. The organic layer was transferred to 10 ml volumetric flask. The extraction was repeated with a fresh aliquot of 5 mL chloroform. The absorption spectra were reocrded against reagent blank.

Table 3 Analysis of Metal Ions in Surcace Water Fishes

	Name of Fish	Weight of Fish	Amount of Metal Found by HPLC ug/g (C.V%)			Amount of Metal Found By FAAS ug/g (C.V.%)			
			Cu	Fe	Ni	Hg	Cu	Fe	Ni
1.	Tenulosa ilish (Palla)	390	0.028 (4.6)	0.11 (5.1)	0.078 (3.9)	0.10 (4.2)	0.030 (2.9)	0.10 (4.5)	0.075 (2.2)
2.	Labeorohita (Kurra)	2000	0.23 (3.2)	0.87 (4.7)	0.29 (3.2)	0.75 (4.5)	0.20 (1.7)	0.80 (2.9)	0.34 (1.1)
3.	Catla Catla (Thaila)	1600	0.12 (6.2)	0.925 (4.2)	0.36 (5.6)	0.55 (3.8)	0.10 (2.9)	0.95 (3.8)	0.40 (3.0)
4.	Notopterus notopterus	2200	0.10 (3.6)	0.97 (4.4)	0.58 (6.7)	1.08 (4.6)	0.10 (1.4)	1.0 (2.4)	0.50 (1.8)
5.	Mystus Seenghala (Singara)	1800	0.30 (4.7)	1.15 (5.3)	0.52 (3.2)	0.96 (6.4)	0.35 (1.6)	1.20 (2.8)	0.61 (1.2)

B. Solvent Extraction Procedure

Solution (5 ml) containing 0-100 ug each of copper(II), nickel(II), iron(II), mercury(II) were transferred to well stoppered test tube (Quickfit) and were added 5 mg ascorbic acid, 2 ml of PAPT, 0.2% in methanol w/v and 2 ml acetate buffer pH 6. The chloroform 2 ml was added and layers were mixed well. The organic layer was separated. The aqueous layer was added 0.5 ml of reagent solution (0.2% in methanol w/v) and 2 ml carbonate buffer pH 9 and extraction was repeated with 2 ml of chloroform. Exactly chloroform (2 ml) from combined extract was transferred to sample vial and solvent was evaporated on water bath and residue was dissolved in methanol (1 ml). The solution 5 ul was injected on column Microsorb C-18 5um (150 x 4.6 mm id) and complexes were eluted with methanol: acetonitrile: water:sodium acetate (1mM): tetrabutyl ammonium bromide (1mM) (65:15:18:1:1 v/v/v/v/v) using flow rate of 1.0 ml. Detection UV was obtained at 254 nm.

C) Preconcentration by Solvent Extraction

An aliquot of solution (300 ml) containing copper(II), iron(II), and nickel(II) 0-100 ug each was transferred to 500 ml separating funnel and were added 10 mg ascorbic acid, 10 ml of PAPT (0.2% in methanol w/v) and 10 ml sodium carbonate sodium bicarbonate buffer pH 8.5. Chloroform 10 ml was added and contents were mixed well. The layers were separated and organic layer was collected. The aqueous phase was added 5 ml chloroform and extraction was repeated. The combined organic layer was warmed on water bath and most of the solvent was evaporated. The residue was dissolved in 2 ml methanol. The solution 5 ul was injected on column Microsorb C-18, 5um (150 x 4.6 mm id) and complex were eluted as B.

D) Analysis of Water Sample

Water samples for metal analyses were collected from Fuleli canal. Fuleli canal takes off from left bank of river Indus and Passes through Hyderabad city where it gets municipal sewerage and industrial effluents. Three samples were collected from Fuleli canal (1) near Akhand village (2) near CIA Centre (3) Sakhi Pir Road, Near Liaquat Colony. Water samples were collected in 1L glass bottle from the surface of water within 3-10 cm. Unfiltered sample was added 1 ml sulphuric acid (98%) per litre sample.

The water samples 300 ml from each was transferred in separating funnel and was added 10 ml reagent PAPT 0.2% in methanol w/v. Further solvent extraction procedure was followed as C. The amounts of metals found in water were evaluated from standard calibration curve constructed from known concentrations of copper, iron and nickel.

E. Analysis of Water Samples by Atomic Absorption Spectrometer

A portion of 300 ml of the water sample from each was transferred in beakers and to it was added 5 ml nitric acid (65%) and 10 ml hydrochloric acid (37%). The mixture heated gently on water bath to dryness and residue was dissolved in water and pH was adjusted to 4. The final volume was adjusted to 25 ml. The blank with double distilled water was also prepared simultaneously.

F. Analysis of Metals in Fishes

Fresh water fishes Tenualosa ilisha (Pala), Labeorohita (Kurra), Calta catla (Thaila), Notopterus notopterus (Gandau) and Mystus Seenghala (Singhara) (weight 390-2200 g) obtained from Kotri Barrage and Kalri lake near Thatta, were weighed and 20-100 g of fish muscle was transferred to 500 ml digestion flask attached with condenser. The flask was added 20 ml sulphuric acid (98%), 60 ml nitric acid (65%) and 5 ml of 2% w/v in water ammonium molybdate. The mixture was heated on water bath at 60-70°C for 1 hr. The cooled mixture was added 80 ml nitric acid (65%) and heated gently till white fumes appeared. The condenser was washed with water and clear solution concentrated to about 10 ml. The volume of the solution was adjusted to 25 ml and 5-10 ml was taken and pH adjusted to 6. the extraction procedure was followed as B.

Atomic absorption determinations were carried out on Varian Spectr AA20 atomic absorption spectrometer using air- acetylene flame with standard burner head under analytical conditions recommended by the manufacturer.The copper iron and nickel were determined at 324.7 nm, 248.3 nm and 232.0 nm respectively. The analyses were carried out in triplicate with delay time 3 sec and integration time 3 sec. The linear calibration curves were obtained with 1.0-5.0 μg/ml, 2.0-10.0 μg/ml and 2.0-10.0 μg/ml for copper, iron and nickel respectrively.

2-Pyrrolaldehyde was procured from Merck, 4-phenylthiosemicarbazide from Fluka, Methanol from Fluka, and acetonitrile from Merck. Freshly prepared double distilled water was used. The elemental micro-analysis and mass spectrum were recorded at HEJ-Research Institute of Chemistry, University of Karachi. The IR spectrum was recorded on Perkin-Elmer 1430 ratio recording infrared spectrophotometer using KBr Pellet in the range of 4000-200 cm^{-1}. Spectrophotometric studies were carried out on Hitachi 220 with 1 cm silica cuvettes. Hitachi 655A liquid chromatograph connected with variable wavelength UV monitor, Rheodyne model 7125 injector and Hitachi D-2500 Chromato-integrator were used. Column Microsorb C-18, 5μm (150 x 4.6 mm id) (Woburn, M.A. USA) was used throughout the study. The stock solutions of copper(II), iron(II), nickel(II), cobalt(II), mercury(II) and silver(I) containing 1mg/ml were prepared. The solutions of lower concentration were prepared after appropriate dilution daily. The buffer solutions in the range 1-10 were prepared from hydrochloric acid (0.1M), potasisum chloride (1M), acetic acid (1M), sodium acetate (1M), sodium bicarboante (1M),sodium carboante (saturated), ammonium acetate (1M), ammonium chloride (1M) and ammonia (1M). pH measurements were made with Orion 420A pH meter.

Conclusion

The work indicates the potentials of reagent PAPT for HPLC determination of copper, iron, nickel and mercury. The complexation with metal ions is rapid with immediate development of coloured solutions. The coloured complexes are extractable in organic solvents, which enabled to separate the metals from matrix and preconcentrate. Metal chelates eluted with simple isocratic system of methanol acetonitrile water containing sodium acetate and tetrabutyl ammonium bromide. HPLC method was developed for determination of copper, iron and nickel in water samples and fresh water fishes.

References

1. B. Kasterka, J. Chojnachi, W. Wojnowski, J.F. Biernat, *Chem. Anal. (Warsaw)* **38**, 287 (1993).
2. S.S. Berman, J.W. Mclaren and S.N. Wallie, *Anal. Chem.* **54**, 488 (1980).
3. N. Toshihiro, H. Oka, M. Ishii, J. Salo, *Analyst*, **119**, 1397 (1994).
4. C. Boutron, S. Martin, *Anal. Chem.* **51**, 140 (1979).
5. A. Hall, M.C. Godinho, *Anal. Chim. Acta*, **113**, 369 (1980).
6. K.W. Bruland, R.P. Franks, G.A. Knaver and J.H. Martin, *Anal. Chim. Acta*, **105**, 233 (1979).
7. D.R. Babu, P.R Naidu, *Talanta*, **38**, 175 (1991).
8. A. Miyazaki, A. Kimura, K. Bansho and Y. Umezaki, *Anal. Chim. Acta*, **144**, 213 (1982).
9. R.G. Smith J.R., H.L. Windom, *Anal. Chim. Acta*, **113**, 39 (1980).
10. V.M. Shiknev, V.N. Gomolitskh, B. Spinvakov, *Talanta*, **36**, 861(1989).
11. R.B. Singh, B.S. Garg, R.P. Singh, *Talanta*, **25**, 619 (1978).
12. E. Cristofol, F. Sanchez Rajas and J.M. Cano Pavon, *Talanta*, **38**, 445 (1991).
13. J.M. Bautinez Aguilar, J.M. Cano Pavon, *Talanta*, **27**, 423 (1980).
14. N. Uehara, K. Morimoto and Y. Shijo, *Analyst*, **117**, 977 (1992).
15. M.Y. Khuhawar, Z.P. Memon and S.N. Lanjwani, *Chromatographia*, **41**, 236 (1996).
16. Y. Qiam, J. Fritz, *J. Chromatogr.* **602**, 103 (1992).
17. S. Hoshi, H. Fujisawa, K. Kakamura, S. Nakata, M. Uto and K. Akatsuka, *Talanta*, **41**, 503 (1994).
18. N. Uehara, M. Hirota and Y. Shijo, *Bunseki Kagaku*, **43**, 195 (1994).
19. M.Y. Khuhawar and S.N. Lanjwani, *Talanta*, **46** 485 (1998).