Pak J Sci Ind Res 2001 44 (5) 253-256

Determination of Mercury by Liquid Chromatography in Fresh Water Fishes Using 2-Thiophenaldehyde-4-Phenyl-3-Thiosemicarbazone

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(Received 25 January 1999; accepted 19 October 2000)

Co (II), Ag (I) and Hg (II) or Co (II), Ni (II), Fe (II), Cu (II) and Hg (II) are simultaneously extracted as metal chelates compounds of 2-thiophenaldehyde-4-phenyl-3-thiosemicarbazone (TAPT) in chloroform. The complexes were separated from microsorb C-18, 5 μm column when eluted with methanol/acetonitrile/water/aqueous sodium acetate 1m mol or methanol/acetonitrile/water/sodium acetate (1mmol) tetrabutyl ammonium bromide (1mmol) with a flow rate of 1ml^{-1} and detection UV at 254 nm. Linear calibrations were made with 10-50 μg ml^{-1} and detection limit was 0.4 μg ml^{-1}, corresponding to 2 ng/injection in Co and Hg. The method was used for the determination of mercury in surface water fishes. It was found within 0.125 to 1.18 μg g^{-1} of fish muscles with coefficient of variation (C.V) 3.4-5.8%.

Key words: Liquid chromatographic technique, Fresh water fishes, Mercury.

Introduction

The determination of mercury in fishes by HPLC have been reported by many researches (Bond and Wallace 1983; Ichinoki *et al* 1983; Munder and Ballschmiter *et al* 1986; Parkin 1989; Steen kamp and Coetzee 1993). It was based on the use of different substituted dithiocarbamates. The complexation reagent like dithiozone, 2- mercaptobenzothioazole (Wag and Whang 1993) unithiol and trans-1,2-diaminecyclohexane N-N,N-H tetracetic acid (Valle *et al* 1992) have also been applied for estimation of mercury by HPLC. Thiosemicarbazone (TAPT) and phenylthiosemicarbazones reagents have been used for spectrophotometric determinations (Asuero and Gonzakez 1980; Cristofol *et al* 1991), spectrofluorometric and HPLC determination of a number of metals (Heizmann and Ballsmiter 1977; Hoshi *et al* 1986; Cristofol *et al* 1991; Qian and Fritz 1992; Valle *et al* 1992; Khuhawar *et al* 1995). Thiophenyaldehyde 4-phenyl-3-thiosemicarbazone (TAPT) has been reported for the separation of Fe and Ni (Ueharo *et al* 1994) and for the determination of Cu, Fe and Co in pharmaceutical preparations (Khuhawar and Lanjwani 1998). The present work examines the reagent TAPT for the quantitative separation and HPLC determination of mercury in fresh water fishes.

Experimental

The aqueous solution containing 1 mg ml^{-1} of Co (II), Hg (II) and Ag (I) were prepared from Co $(CH_3COO)_2$, Ag NO_3, Hg $(SO_4)_2$ (E.Merck). Chloroform, methanol and acetonitrile (E. Merck) were used as solvents. Freshly prepared double distilled water was also used for HPLC studies.

Hitachi 220 was used for spectrophotometric studies and Hitachi 655A liquid chromatograph connected with variable wavelength UV monitor, Rheodyne injector 7125 and chromatointegrator Hitachi D-2500 was used.

a) Solvent extraction procedure. Solution (1-5 ml) containing Hg (II), Ag (I) and Co (II) (0-100 μg each) was transferred to well stoppered test tube (Quickfit) and added reagent TAPT solution (2 ml, 0.2% in methanol wv^{-1}), sodium acetate acetic acid buffer (1 M) pH 6 (2 ml), chloroform (4 ml) and contents were mixed well. The organic layer was allowed to separate. Exactly (2 ml) of extract was transferred to a sample vial and solvent was evaporated. The residue was dissolved in 1 ml methanol. The solution (5 μl) was injected on to a column of microsorb C-18, 5 μm (150 x 4.6 mm id) and complexes were eluted with methanol: acetonitrile: water:aqueous sodium acetate (1mM) (60:28:10:2) v^{-1} using flow rate of 1 ml min^{-1} and UV detection at 254 nm.

b) Analyses of mercury in fishes. Fresh water fishes *Tenualosa ilisha* (Palla), *Labeo rohita* (Kurra), *Calta calta* (Thaila), *Notopterus notopterus* (Gandan) and *Mystus seenghala* (Singhara) weighing 390-2200 g are obtained from River Indus at Kotri barrage. Fish muscles weighing 20-100 g was transferred to digestion flask (500 ml) attached with condenser. Sulphuric acid (20 ml, 98%), nitric acid (60 ml, 65%) and ammonium molybdate (5 ml, 2% wv^{-1} in water) were added and the mixture was heated on water bath at 60-70°C for 1 h. To this mixture nitric acid and (80 ml, 65%) was added and heated gently till white fumes appeared. The condenser was washed with water and the clear solution was concentrated to about

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Table 1
Analysis of mercury in surface water fishes from Indus river at Kotri Barrage

Name of the fish	Weight of fish	Amount of mercury found μg g^{-1} (C-V%)
1. *Tenualosa ilisha* (Palla)	390 g	0.14 (4.6)
2 *Tenualosa ilisha* (Palla)	400 g	0.125 (4.6)
3 *Labeo rohita* (Kurra)	1600 g	0.77 (3.4)
4 *Catla catla* (Thaila)	1600 g	0.51 (5.1)
5 *Notopterus notopterus* (Gandan)	2200 g	1.18 (5.8)
6 *Mystus seenghala*	1800 g	1.10 (3.8)

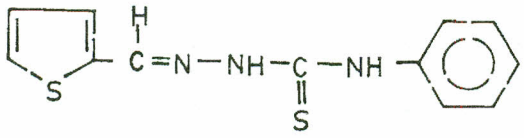

Fig 1. Structural diagram of reagent

10 ml. The volume of solution was adjusted to 25 ml and 5-10 ml was taken and pH was adjusted to 6. Reagent TAPT solution (2 m1, 0.2% wv^{-1} ethanol) was added and solvent extraction procedure 'a' was followed. The complexes were eluted with methanol:acetonitrile:water:tetrabutyl ammonium bromide (TBA) (1mM) sodium acetate (1mM): (78:10:10:1:1) v^{-1} with a flow rate of 1 ml min^{-1} and UV detection at 254 nm. The amount of mercury in fishes were calculated from external calibration curve prepared from standard Hg (II) solution.

C) Analysis of mercury in fish using standard addition technique. The fish *Catla* (Thaila) was analysed and fish muscle (20 g) was added 20, 40, 60, 80 and 100 ug of mercury and procedure b was followed. The amount of mercury in fish was calculated by graphical method.

Results and Discussion

The reagent TAPT reacts with Co (II) to develop orange colour in pH range 2-10 Hg (II) and Ag (I) develop yellow turbid solution in aqueous-methanol. The complexes are extractable in chloroform. The effect of pH shows that the absorbance of Co (II) is fairly constant within pH 6-9 with maximum at 8.5Hg (II) and Ag (I) are extractable in chloroform within pH 2-7 with maximum absorbance at pH 6. The Al (III), Pb (II), Cd (II), Sn

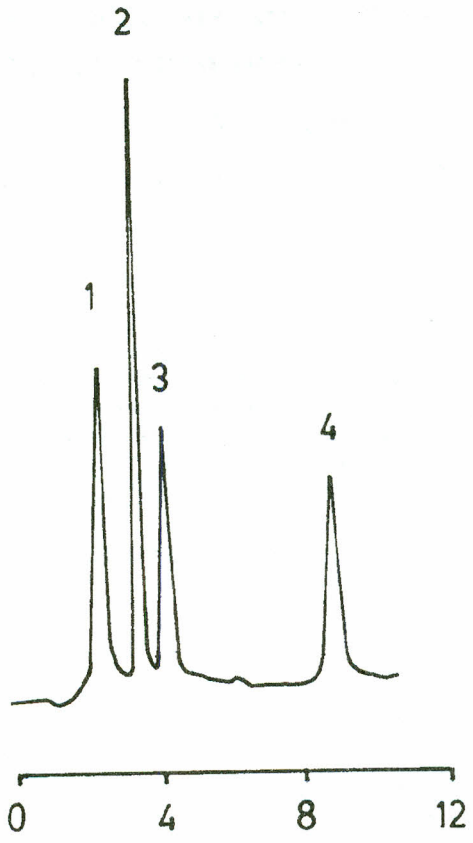

Fig 2. HPLC separation of (1) TAPT and its (2) Co (III) (3) Ag (I) and (4) Hg (II) chelates on column 150 x 4.6 mm i.d. of microsob C-18, 5μm with elution with methanol: acetonitrile: water: sodium acetate (1mM) (60:28:10:2) v^{-1} at a flow of 1.0 ml^{-1} min and UV detection at 254 nm.

(II), OV (IV), O_2U (VI), Pt (II), Pd (II) and Zn (II) did not react with reagent TAPT to form coloured complexes and when present at the same concentration as Co(II), Ag((I) and Hg(II) did not affect the analytical separation. Cu(II), Fe(II) and Ni also form complexes with TAPT and are extracted together with Co(II) and Hg (II) but require the addition of tetrabutyl ammonium bromide (TBA) for the adequate elution and separation of metal chelates Fig 3. However Hg (II) TAPT complexes elutes at the end of chromatogram and there is a large separation of mercury (II) from TAPT metal chelates as observed in Fig 2. Therefore, mercury was determined in fresh water fishes by isocratic elution with a mixture consisting of methanol: acetonitrile: water: aqueous sodium acetate (1mM) and TBA (1mM) (78:10:10:1:1) v^{-1} at a flow rate of 1 ml min^{-1} and UV injection at 254 nm. The calibration curve for mercury here was also obtained with 10-50 μg ml^{-1}. Fish muscles were analysed because it may be a source of heavy trace metals entering the human body. Five species of fishes were caught from river Indus at Kotri barrage (Table I). The results (Table I)

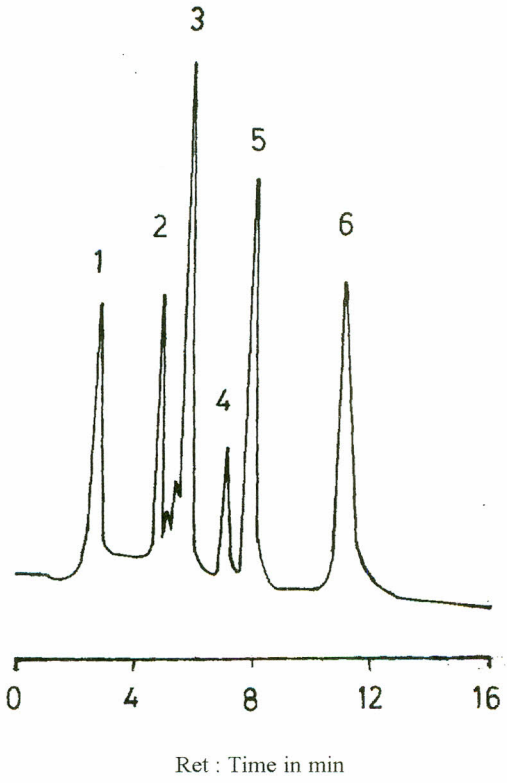

Fig 3. HPLC separation of (1) TAPT and its (2) Co (II), (3) Ni (II), (4) Fe (II), (5) Cu (II) and (6) Hg (II), chelates on a column (150 x 4.6 mm id) of microsorb C-18 (5 μ m) with elution with methanol: acetonitrile: water: contianing aqueous sodium acetate (1mM) and tetrabutyl ammonium bromide (1mM) (78:10:10:1:1) v^{-1} at a flow rate of 1.0 ml min^{-1} and with UV detection at 254 nm.

showed a large variability in the mercury concentration in surface water fishes. The mercury contents in muscle tissues of fishes were found in the range of 0.125 $\mu g\ g^{-1}$ for *Tenualosa ilisha* (Palla) and *Notopterus notopterus* (Gandan) respectively with coefficient of variation (C.V) within 3. 4-6.8%. The mercury contents of *Tenualosa ilisha* (palla) and *Notoptrus notoperous* (Gandan) were compared and T-test was applied. A significant difference was indicated at 95% confidence level.

The fish *Catla catla* (Thaila) was analysed for the contents of mercury using standard addition technique. The amount of mercury was found 0.72 $\mu g\ g^{-1}$ with C.V 4.8%. The amount of mercury found by direct calibration was 0.68 $\mu g\ g^{-1}$ and indicate relative deviation of 5.5% from the result observed using standard addition technique. The amount of mercury in common fishes in Indus water contains within the permissible limits and normal consumption of fishes particularly *Tenusalsa ilisa, Catla catla* and *Labeo rehita* would not increase the permissible limit laid by World Health Organisation ((WHO) for the safe consumption of 0.3 mg $week^{-1}$ per adult person for mercury

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