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Direct Spectrophotometric Determination of Mercury (II) using 2-Acetylpyridine Thiosemicarbazone in Environmental Samples

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ABSTRACT:
2-Acetylpyridine thiosemicarbazone (APT) is used as new analytical reagent for the direct non-extractive spectrophotometric determination of mercury (Hg). It reacts with mercury in acidic medium (pH 6.0, sodium acetate-acetic acid buffer) to form yellow colored (λ_{max}, 351 nm) 1:2(Hg-APT) complex. The colour reaction is instantaneous and the absorbance remains constant for about 24h. The molar absorptivity and sandell's sensitivity are found to be $5.4x10^4$ $L.mol^{-1}$.$cm^{-1}$and 0.0037µgcm^{-2} of Hg(II)respectively. The system obey Beer's law for 0.240-2.407 µg/ml of Hg(II). Large number of cations, anions and complexing agents (e.g. citrate, fluoride and thiocyanate) do not interfere in the determination of mercury. The method is successfully applied for the determination of mercury in a number of water samples (potable and polluted), biological samples, soil samples and ayurvedic medicinal samples. The method has high sensitivity, selectivity precision and accuracy.

Key Words: *Thiosemicarbazones, spectrophotometry, complexing agents, mercury*

1. INTRODUCTION

The analytical monitoring of mercury in environmental, biological, industrial and food samples is extremely important because of the high toxicity of this metal both in its inorganic and organic compounds [1]. One example of acute mercury poisoning is "Minamata diseases" which causes mental disturbance; a loss of balance, speech, sight and hearing difficulty; in swallowing; and finally coma and death [2]. The toxicity of mercury depends on its chemical state[2]. Inorganic mercury has a very high affinity for protein sulfhydryl groups, and accumulated in the kidneys, whereas organic mercury has a greater affinity for the brain. The ability of living organisms to convert inorganic mercury to organic mercury compounds which are more toxic and accumulate to a greater extent in living organisms, additionally increases the danger of mercury exposure , even at trace levels [3] However, people who eat a lot of fish may consume much more; for instance, a level of 0.6 mg Hg Kg^{-1}. Fish could provide 0.15 mg of methyl mercury in one meal. All these findings cause great concern regarding public health, demanding an accurate determination of this metal ion at trace and ultra-trace levels.

In the analysis of environmental and biological samples, there is an increasing need to develop the simple, sensitive and selective analytical techniques that do not use expensive or complicate experimental method test equipment. However, the spectrophotometric method still has the advantages of being simple and not requiring expensive or complicated test equipment. For this reason, a wide variety of spectrophotometric methods for the determination of mercury has been developed. Several authors have reported on the extractive spectrophotometric determination of mercury(II) using complexes formed variety of reagents[4,5]. In most of the methods cited above, mercury forms soluble / insoluble complexes with regents in the aqueous phase, which are subsequently extracted with various solvents for spectrophotometric determination of mercury. Most of these reagents are expensive and non-recoverable.

This paper describes the non-extractive spectrophotometric determination of mercury(II) as its APT complex in aqueous medium. A close literature survey [6-9] reveals that APT has so far not been employed for the spectrophotometric determination of mercury(II). This method does not require a solvent extraction step; hence the use of carbon tetrachloride or chloroform as solvent is avoided which are reported as toxic and environmental pollutants and carcinogens. Compared to even some recently published spectrophotometric methods for the determination of mercury(II) the present method offers several distinct advantages.

2. EXPERIMENTAL

The reagent (APT) was prepared by simple condensation of 1 mol of 2-acetylpyridine and 1 mol of thiosemicarbazide. In a 250-ml Erlenmeyer flask, a hot methanolic solution of 2-acetylpyridine(5ml, 0.0438mol in 5ml of methanol), thiosemicarbazide (4g, 0.0438 mol, dissolved in10 ml of hot water) were taken in 250-ml round bottom flask. Suitable quantity (~ 2ml) of glacial acetic acid was added to the reaction

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mixture and refluxed for 3 hours. On cooling the reaction mixture, light brown coloured product was separated out. It was collected by filtration and washed several times with hot water and 50 percent cold methanol. This compound was recrystallised from ethanol and dried in *vacuo*, 60% yield 4.2 g; m.p. 175 ^{0}C as shown in Scheme 1.

The compound was characterized by IR and ^{1}H-NMR spectral data. Infrared spectrum of APT shows bands at [3459(m) and 3369(m,br)], 3184(m), 1608(m), 1501(s), 1466(s), 1367(w),1150(m), 840(δ) and 664(δ) cm^{-1} respectively corresponding to υ NH (asymmetric and symmetric), υ (C-H) aromatic stretch, υ (C=N) stretching(Schiff base), υ (C-C) aromatic ring, δ (C-H) of pyridine ring, υ (N-H)stretch (primary amide), υ (C=S), δ (C-H)-oop bend (aromatic) and δ(C-C)-oop bend aromatic ring vibrations.^{1}H-NMR spectrum of APT ($CDCl_3$+ DMSO-d_6) showed signals at 2.25 (3H, s), 7.15-7.49(4H, m), 8.15-8.30(2H,s) and 10.08(1H, s) due to CH_3, C_5H_4N (pyridine), -NH_2 and =N-NH (hydrazine) groups of thiosemicarbazone.

The pK_a values were determined by recording the UV-Visible spectra of micro molar (2x10^{-5} M) solutions of the reagent at various pH values and by taking the arithmetic mean of the values obtained from the measurements at different wavelengths determined spectrophotometrically using Phillips and Merrit method [10].The values of deprotonation of APT are 5.46(pK_1) and 8.56 (pK_2) as shown in Scheme 2.

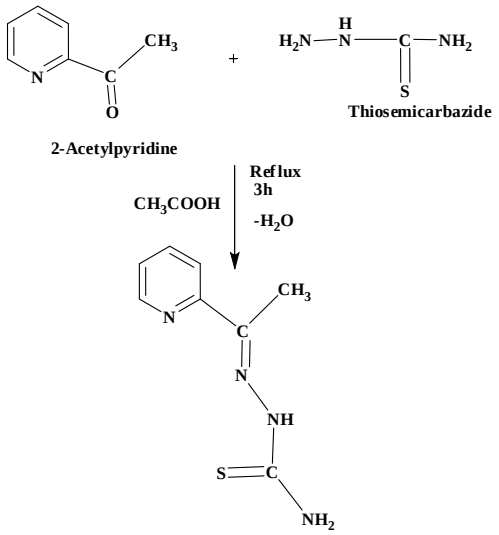
The reagent solution (0.01M) was prepared by dissolving 50 mg of the compound in dimethylformamide (DMF) in 25-ml standard flask. The reagent solution is stable for at least 12h. Hydrochloric acid (1M) sodium acetate (1 M) (pH 0.5-3.5);0.2 M NaOAc-0.2 M AcOH (pH 4-6) and 2 M NH_4Cl-2 M NH_4OH (pH7-10) solutions were used.

Stock solution (1 x 10^{-2} M) of divalent mercury was prepared by dissolving 0.27 g of mercuric chloride (Merck Darmstadt) in deionized water containing few drops of concentrated hydrochloric acid and made up to the mark in a 100-mlvolumetric flask. Aliquots of this solution were standardized [11] with EDTA using xylenol orange as an indicator. Dilute solutions were prepared from this stock solution. Solutions of large number of inorganic ions and complexing agents were prepared form their Analar grade or equivalent grade water soluble salts. In the case of insoluble substance, special dissolution methods were adopted.

2.1 Recommended procedures

(a) *Determination of mercury(II) (Zero order)*
An aliquot of the solution containing 0.240-2.407 µg/ml (or ppm) of mercury(II),10 ml of NaOAc-AcOH buffer solution (pH, 6.0) and 1.5 ml of 0.01 M APT were combined in a 25 ml volumetric flask and resulting solution was diluted to the mark with distilled water. The absorbance of this solution was read at 351nm against reagent blank. The measured absorbance is used to compute the amount of mercury form predetermined calibration plot.

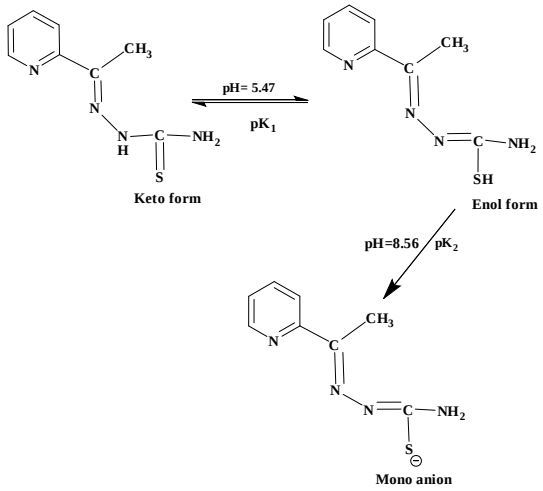
(b)Determination of mercury(II) using second derivative spectrophotometry
A sensitive second order derivative spectrophotometric method was developed for the determination of mercury (II). For the second derivative spectra Fig1, the derivative amplitudes at 410 nm (peak) and at 473 nm (valley) were proportional to the concentration of Hg(II). The derivative amplitudes were measured for different concentration of Hg(II) and plotted against the amount of Hg(II). The plots were linear and obeyed Beer's law in the range 0.664- 0.0286 µg ml^{-1} at 410 nm and 0.08-0.008 µg ml^{-1} at 473 nm. In the second order derivative method, Schimadzu 160A UV-visible spectrophotometer equipped with 1.0 cm quartz cell and an ELICO model LI - 610 pH meter were used in the present study.



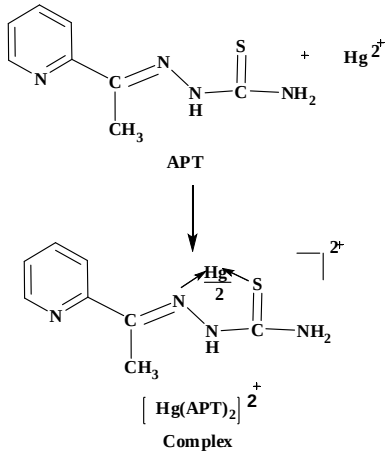
Scheme I: Schematic Chemistry of 2-Acetylpyridine thiosemicarbazone (APT).

3. RESULTS AND DISCUSSION

The reagent, APT (Scheme 2) is easily obtained just as any mono thiosemicarbazone. A 0.01M solution of this reagent is stable for 12h. The bath chromic shift divalent metal ion as mono anion to give neutral complex of absorption band from 290 to 300 nm indicates that in solution on increasing the pH, the acid is neutralized and



Scheme II: Schematic Chemistry Different species of APT in solution at different pH values.



Scheme III: Schematic Chemistry Complex formation reaction.

the>C=S groups is enolized and dissociated [12]. The colour reactions of some important metal ions with APT are summarized in Table 1. In basic medium (above pH 8.56) the ligand presumably exists in enolic form and coordinates the divalent metal ion as mono anion to give neutral complex.

3.1. *Determination of mercury(II)*

Mercury(II) react with APT in acidic pHs to give coloured complexes in Table1. The colour reaction is instantaneous at room temperature. The order of addition of metal ion, reagent, buffer has no effect on the absorbance of complex. Various physico-chemical and analytical characteristics of the complex are summarized in Table 2. The stoichiometry of the complex (M:L=1:2)as shown in Scheme 3, was determined by well-known Job's continuous variation, molar ratio methods. Sodium acetate (0.2M)-acetic acid (0.2M) buffer solution (pH 6.0 μ, 0.2 and T=300K) and equimolar ($4.8x10^{-5}$M) solution of Hg(II) and APT were used in these methods. The data obtained in Job's method were used in the calculation of stability constant of the complex.

Derivative spectrophotometry is a very useful technique in the sense that it decreases the interference i.e., increases the tolerance limit value of foreign ions and it may be adventageously used for the second derivative determination of metal ion having peak-valley method is more sensitive and hence is adopted for the determination of Hg(II).

3.2. Interference

The effect of various cations and anions which are generally associated with the metal ion on the determination of mercury(II) was studied by measuring the absorbance of the mercury complex containing 1.20 μg/ml of mercury(II) in solution. The colour reaction is developed as described in the standard procedure. An error of ± 2% in the absorbance or amplitude reading in the case of derivative methods considered tolerable. The results are given in Table3. The dada obtained in second derivative method are also incorporated. The data suggest that several associated anions and cations do not interfere when they are present in large excess. The tolerance limit values for many anions and cations are more in derivative methods. The interference of associated metal ions such as Fe(III) and Al(III) is decreased with triethanolamine. Larger amount of copper(II) and zinc(II) do not interfere in the presence of citrate and fluoride respectively.

3.3. *Applications*

The present method was successfully applied to the determination of mercury when present alone. The method was also extended to the determination of mercury in a number of environmental, soil, biological and ayurvedic medicinal samples.

3.4. *Determination of mercury in environmental waters*

Each filtered (with what man No. 40) environmental water sample (250 ml) was mixed with 10 ml of concentrated nitric acid in a 500 ml distillation flask. The sample was digested in the presence of an excess potassium permanganate solution according to the recommended procedure [13] The solution was cooled and neutralized with a dilute NH_4OH solution. The digest was transferred into a 25ml calibrated flask and diluted upto the mark with deionized water. The amount of mercury in different water samples was estimated by using recommended procedure. The results are given in Table 4.

3.5. Determination of mercury in soils

A 2 g weight of soil, 5-7 ml of concentrated H_2SO_4 and an excess of $KMnO_4$ are placed into a flask

Table 1. Chromogenic characteristics of APT

Metal ion	λ_{max}	$\varepsilon \times 10^4$ *	pH range	Colour of the complex
Cu(II)	376	0.26	5.0-70	Pale greenish yellow
Ni(II)	375	1.6	5.0-7.0	Yellow greenish
Co(II)	355	1.25	5.0-8.0	Orange yellow
Zn(II)	360	4.06	5.0-7.0	Pale greenish yellow
Fe(II)	370	0.17	4.0-7.0	Deep yellow with red tinge
Fe(III)	375	0.14	4.0-7.0	Deep yellow with red tinge
Hg(II)	351	5.4	5.0-7.0	Yellow colour
V(V)	360	0.37	3.0-7.0	Yellow colour

* $L.mol^{-1}cm^{-1}$

Table 2. Physico-chemical and analytical characteristics of Hg(II)- APT complex

Characteristics	Results
λ_{max} (nm)	351
pH range (optimum)	5.0 – 7.0
Mole of reagent required per mole of metal ion for full colour development	10 fold
Time stability of the complex (in minutes)	90
Beer's law validity range (µg/ml)	0.240 - 2.407
Molar absorptivity (lit $mol^{-1}cm^{-1}$)	5.4×10^4
Specific absorptivity (ml $g^{-1}cm^{-1}$)	0.270
Sandell's sensitivity (µg of Hg (II) cm^{-2})	0.037
Composition of the complex as obtained in Job's and molar ratio methods (M : L)	1 : 2
Stability constant of the complex	7.5×10^{12}
Standard deviation in the determination of 1.20 µg/ml of Hg (II) for ten determinations	0.0042
Relative Standard deviation (RSD), (co-efficient of variation)	0.35%

Table 3. Tolerance limit of foreign ions in the determination of 1.20 ppm of mercury

Ions	Tolerance limits(µg/ml)	
	Zero order	Second derivative
Tartrate, citrate, triethanolamine	5200	7150
Phosphate, ascorbic acid, bromide , thiourea, citrate	450	630
Sulphate, bicarbonate, cynate, thiosulphate, carbonate, nitrate,	175	200
Fluoride, Mn(II),EDTA,	95	120
Iodide, iodate, Ba(II), W(VI), Cd(II), Mo(II)	27	30
TI(III),Pt(IV)	8	10
Al(III)b,Mg(II),Pb(II),Sr(II), Fe(III) b, Ni(II),Co(II),Fe(II),Au(III),Ag(I).	6	22
Pd(II), Cu(II)a , Zn(II)c, Cr(VI)	3	11

aMasked with 700 µg/ml of citrate, bMasked with 1000 µg/ml of triethanolamine, cMasked with 100 µg/ml fluoride.

Table 4. Determination of mercury (II) in water samples.

Sample	Mercury[a] / μg L^{-1}		Recovery (%)
	Added	Found	
Waste water	0	1.42	—
	100	101.11	96.69
	500	501.00	99.91
Sea water[b]	0	2.02	—
	100	103.10	101.05
	500	503.02	100.19
Laboratory[c]	0	123.00	—
	100	224.00	100.40
	500	625.40	100.38

[a]Average of five determinations
[b]Bay of Bengal, Chennai
[c]S.K. University Chemistry Dept. Lab Water

Table 5. Determination of mercury in soil samples

Sample	Mercury [a] / μg L^{-1}		Recovery (%)
	Added	Found	
Urban soil [b]	0	10.9	—
	100	110.52	99.65
	500	512.10	100.23
Roadside soil [c]	0	94.2	—
	100	194.32	100.06
	500	592.10	99.64
Agricultural soil [d]	0	98.13	—
	100	197.64	99.75
	500	599.01	100.14

[a] Average of five determinations
[b] Urban Soil (Anantapur slum area old town)
[c] Traffic Soil (Anantapur bus terminal)
[d] Agricultural Soil (Anantapur S.K. University Campus)

Table 6. Analysis of Fish and Sheep liver samples

Sample	mercury[a] / μg L^{-1}		Recovery (%)
	Added	Found	
Sheep liver	0	0.172	—
	100	101.02	100.80
	500	500.92	100.15
Fish liver	0	1.10	—
	100	101.21	100.10
	500	502.10	101 .20

[a] Average of five determinations

equipped with a reflux condenser [14]. The crystals of $KMnO_4$ are added slowly in small portions, while stirring. It is heated until vapours of SO_3 are evolved. After cooling down, 10 ml of distilled water are added. The excess of $KMnO_4$ and manganese oxides are eliminated by adding H_2O_2. Iron is isolated by precipitation as hydroxide. After filtration the solution is transferred into 25ml standard flask and the volume is brought to the mark with distilled water. Aliquots of this solution are taken for analysis by following recommended procedure. The results are given in Table 5.

3.6. Biological and ayurvedic medicinal samples

A 2-5 g of dried fish, sheep liver samples or ayurvedic medicine samples was taken in a 250ml beaker. A 6 ml of concentrated nitric acid was added and gently heated for half-an-hour. After the disappearance of the forth, 6 ml of 1:1 nitric acid and perchloric acid were added [15]. The contents were digested for one hour and repeatedly treated with 6 ml portions of nitric acid and perchloric acid mixture until the solution becomes colourless. The acid solution was evaporated to dryness and the resulting white residue was dissolved in minimum volume of 1M nitric acid and made upto the volume in a 50-ml volumetric flask. Aliquots of this solution were taken for analysis by following recommended procedure. The results are given in Table 6,7.

Table 7. Determination of mercury in some medicinal samples

Sample	mercury[a] / μg L^{-1}		Recovery (%)
	Added	Found	
Siddama karadhwaja[b]	0	0.19	—
	100	100.22	100. 03
	500	501.20	101. 20
Pancha bhanaras[b]	0	0.26	—
	100	100.31	100. 05
	500	500.11	99. 97
Vasanthaku sumakaram[b]	0	1.2	—
	100	101.15	99. 95
	500	502.03	100.16

[a] Average of five determinations
[b]Purchased from venkateswara ayurvedic nilayam, Chintaluru.

3.7. Human blood serum samples

Human blood samples were collected from four patients with a 10 ml polypropylene syringe

Table 8. Determination of mercury in blood samples

Sample	mercury / μg L^{-1}				Sample source[a]
	Present method n = 5		AAS method n = 5		
	Found	RSD %	Found	RSD %	
Blood	95.786	0.88	99.62	1.5	Kidney damage patient (M)
Blood	228.11	1.3	230.37	1.9	Paralysis Patient (M)
Blood	9.62	1.21	7.72	1.5	Normal adult (M)

[a] Sample were from Government Hospital, Anantapur

equipped with stainless steel needle. These samples were transferred in to glass tube and centrifuged at 3000 rpm for 20 min [16].The supernatants were collected as the blood serum samples. Freeze-dried human serum reference material (GHPA No. 4) issued by the Government Hospital patients Anantapur was also used as a test sample in the preliminary study.

3.8. Digestion procedure for human blood serum

Blood serum samples were digested as follows. Blood serum (4 ml) was placed in a Teflon beaker (100 ml) and after adding 1ml of concentrated HNO_3 the serum sample was heated almost to dryness on a hot plate at 110^0C. Then 1ml of concentrated $HNO_3$was again added to the residue and the solution was heated at 150^0C for two hours. A 1ml of concentrated $HNO_3$and 1ml of 60 percent $HClO_4$ were added and the solution was heated at until white fumes appeared. This procedure was repeated twice. Finally, 0.35 ml of concentrated HNO_3 and 1ml of pure water were added to dissolve the residue with heating at 110^0C for 1 hour, and solution was diluted to 50ml with distilled water. The amount of mercury was estimated using recommended procedure. The results are given in Table 8.

4. CONCLUSION

The present method for the determination of mercury (II) is compared with other recently reported spectrophotomeric methods [17-26].The present method seems to rank among highly sensitive method in Table 9 for the determination of mercury(II).The results obtained in the analysis of different samples using present method are comparable to the data obtained using dithizone method. The present ligand containing heterocyclic ring is found to be potential and cost effective for the determination of mercury(II) without the need for extraction using the toxic solvent. Forther, the reagent is easy to synthesize using commercially available precursors. Moreover, the present method is simple, rapid and very sensitive for non-extractive spectrophotometric determination of mercury(II) in aqueous medium.

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5. REFERENCES

[1]. Agency for Toxic Substance and Disease Registry ATSDR, (1999), ***Toxicology profile for mercury***, Public Health Service, Atlanta.

[2]. C. D. Klaassen, Casarette and Doull's, (2001), ***Toxicology***, McGraw-Hill, New York, 834.

[3]. National Health and Medical Research Council, Drinking water Guidelines, (1996) ***http/www.health.gov.au/nhmrc/publication/pdf/eh***,Pdh, **19**

[4]. T. Perez Ruiz, J.A. Ortuno, M.C.Torrecillos, (1984), Extraction- spectrophotometric determination of mercury with 1,2,4,6-tetraphenylpyridinium perchlorate, ***Analytica Chimica Acta***,**165**, 275.

[5]. B. saad, Salah M. Sultan, (1995), Extraction spectrophotometric determination of mercury(II) using thiacrown ethers and Bromocresol Green,***Talanta***, **42**, 1349-1354.

[6]. R.B. Sing, B.S. Garg, R.P. Sing ,(1979), Oximes as spectrophotometric reagents- a review, ***Talanta*** ,**26**, 425-444.

[7]. K.H. Reddy, D.V. Reddy , (1985), Analytical data and comparison of analytical potentialities of thiosemicarbazones and semicarbazones**,** ***Quarterly Chemistry Reviews***, **1**, 47-98.

Table 9. Comparison of spectrophotometric methods for the determination of mercury (II) .

Name of the reagent	λ_{max}	pH	Determination range µg/ml	ε x 10^4 $Lmol^{-1}$ cm^{-1}	Extraction / aqueous	Ref.
Tetraphenyl Phridinium Perchlorate	310	0.5M H_2So_4	0.04-0.5	2.64	Isopentyl acetate	4
Thiokrown ether and Bromocresol Green	420	3.5	0.5-12.0	0.14µg/ml Detection limit 2.25	Chloroform	5
Glyoxaldithio semicarbazone (GDT)	335	1.0-7.0	-	4.4	Aqueous	17
Bipyridyl glyoxal bis (4-phynyl - 3 - thiosemicarbazone)	370	5.1	-	2.54	Aqueous	18
2-(4,6-Dimethyl-2-pyrimidalazo)-1-Napthol-4-sulphonate, sodium salt	530	6.6-7.6	-	2.25	Aqueous	19
1,5–Diphenylthiocarbazone and sodiumdedecyl sulphate	490	0.8-1.2	0.05-10	5.02	Aqueous	20
2-acetylpyridine4-methyl-3-thiosemicarbazone(2-APMT)2-acetyl furan thiosemicarbazone(AFT)	350	6.0	1.6-16.05	1.08	Aqueous	21
5-methylfuran2-carbaxaldehyde thiosemicarbazone 3-methylthiophene-2-carbaxaldehyde	350	6.0	0.8-8.02	1.92	Aqueous	21
thiosemicarbazone(3-MTAT)	365	8.0	0.4-4.0	2.5	Aqueous	22
Diacetylmonoximeisonicitinoylhydrozone (DMIH)	375	8.0	0.8-8.0	1.8	Aqueous	22
4 - hydroxy 3, 5 - dimethoxy benzaldehyde - 4 - hydroxy henzoylhydrazone	351	5.5	1.0-12.0	2.29	Aqueous	23
Diphenylcarbazone(Dithiozone)	420	4.0	0.60-2.70	4.56	Micellar media	24
6-Hydroxy-3-(2-oxoindolin-3-ylideneamino)	488	0.8M H_2SO_4	0.1-25	2.5	50% Aqueous	25
2-thioxo-2H-1,3-thiazin-4(3H)one	505	4-6	0.2-2.0	4.0	Dioxane	26
2-Acetylpyridine Thio semicarbazone	351	5.0-7.0	0.24-2.40	5.4	Aqueous	PM

[8]. B.S. Garg,V.K. Jain, (1988), Analytical applications of thiosemicarbazones and semicarbazones, Review artical, ***Microchemical Journal***, **38**, 144-169.

[9]. K.H. Reddy, N.B.L. Prasad, T.S. Reddy,(2003) Ananlytical properties of 1-phenyl-1,3-propanedione-2-oxime thiosemicarbazone: Simultaneous spectrophotometric determination of copper(II) and nickel(II) in edible oils and seeds, ***Talanta***, **59**,425-433.

[10]. J.P. Phillips, L.L. Merrit, (1948) Ionization constants of some substituted 8-hydroxyquinolines, ***Journal of American Chemical Society***, **70**, 410-411.

[11]. H. Khan, M.J. Ahmed, M.I. Bhanger, (2005) A simple spectrophotometric determination of trace level mercury(II) using 1,5-diphenyl

thiosemi carbazone solubilized in micelle ***Analytical Science***, **21**, 507-512.

[12]. B.A.Gringas, R.L.Somorjai, C.H. Bayley,(1961) The preparation of some thiosemicarbazones and their copper complexes, ***Canadian Journal of Chememistry***, **39**, 973-985.

[13]. F.W. Fifield, P.Haines, (2000), *Environmental Analytical chemistry*, second edition, Blackwell science, London, UK, **378-380**.

[14]. M. Kamburova (1993), Spectrophotometric determination of mercury in soils with triphenyltetra zolium chloride, ***Talanta,* 40**,719.

[15]. K.Hussain Reddy, D.V. Reddy,(1984), ***Acta ciencia India Vol. XC* 4**, 207-215.

[16]. K. Lnagaki, H. Haraguchi, (2000), Determination of rare earth elements in human blood serum by inductively coupled plasma mass spectrometry after chelating resin preconcentraction, ***The analyst***, **125**, 191-196.

[17]. B.W. Budetinsky, Svee, (1971), Photometric determination of silver and mercury with glyoxal dithiosemicarbazone, ***Journal* of *Anaytical Chimica. Acta***, **55**,115-124.

[18]. Gonazlez Balairon, J.M. Cano pavon, F.Pino, (1979), Ananlytical properties of bipyridyl glyoxal bis(4-phenyl-3-thiosemicarbazone), ***Talanta,* 26**, 73-76.

[19]. Ishwar Singh, Sushma, (2000), Spectro-photomertic determination of Zn(II),Cd(II), Hg(II), Fe(II), Co(II), Ni(II) and Cu(II) with 2-(4,6-dimethyl-2-pyrimidylazo)-1-napthol-4-sulphonate; sodium salt, ***Indian Journal of Chemistry***, **39**, 545-551.

[20]. H. Khan , M.J.Ahmed, Bhanger,(2005), A Simple Spectrophotometric Determination of Trace Level Mercury Using 1,5-Diphenylthiocarbazone Solubilized in Micelle ***Analytical Science,* 21**, 507-512.

[21]. K.Vasudeva Reddy, S.Vidyasagar Babu, K. Hussain Reddy, (2009) Spectrophotometric determination of mercury(II) in water, soil, and biological samples using heterocyclic thiosemicarbazone, ***Journal of Indian Chemical Society,* 86**,162-164.

[22]. D. Nagarjuna Reddy, K. Hussain Reddy, (2010), A simple and rapid spectrophotometric determination of mercury(II) in environmental sample using heterocyclic thiosemicarbazone, ***Ultra Chemistry***, **6**, 287-294.

[23]. G. Chandrasekhar Reddy, N. Devanna, K.B. Chandrasekhar, (2011), Derivative spectrophotometric determination of mercury(II) using diacetylmonoxime isonicotinoyl hydrazone, ***International Journal of Chemistry,* 3,** 227-232**.**

[24]. D. Gopala Krishna, Ch. Kethani Devi, (2011), Direct and derivative spectrophotometric determination of mercury(II) in presence of micellar medium using 4-hydroxy-3,5-dimethoxy benzaldehyde 4-hydroxy benzylhydrazone, ***Indian Journal of Green Chemistry,*1,** 10-12**.**

[25]. M.J. Ahmed, Md. Shah Alam, (2003), A rapid spectrophotometric method for the determination of mercury in environmental, biological, soil and plant samples using diphenyl thiosemicarbazone, ***Spectroscopy***, **17**, 45.

[26]. A. Hazma, A.S. Basammakh, A.A. Al-Sibaai, M.H. Al-Saidi, M.S. El-Shahawi, (2010), spectrophotometric determination of trace mercury(II) in dental-unit wastewater and fertilizer using the novel reagent 6-hydroxy-3-(2-oxoindolin-3-ylideneamino)-2-thioxo-2H-1,3-thiazin-4(3H)-one and the dual-wavelength-correction spectrophotometry, ***Journal of Hazardous Materials***, **178**, 287.