



Spectrophotometric Determination of Cobalt in Medicinal, Biological and Environmental Samples

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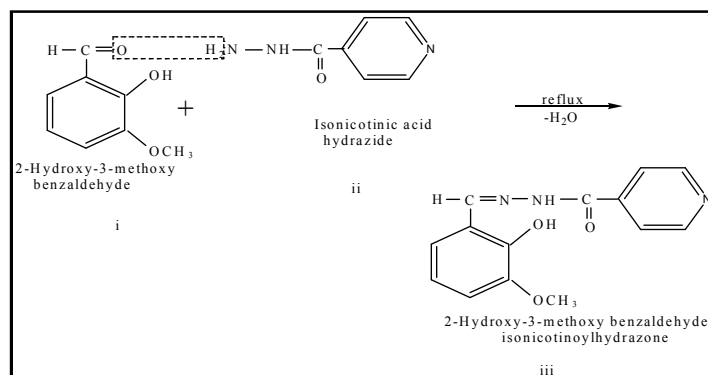
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ABSTRACT

A simple, sensitive and rapid spectrophotometric method was developed for the determination of cobalt(II) using 2-hydroxy-3-methoxybenzaldehydeisonicotinoylhydrazone (HMBAINH) as an analytical reagent. The metal ion forms a yellow colored complex with HMBAINH in aqueous dimethylformamide medium at pH 6.0 in presence of 1% triton x-100. The complex showed absorption maximum at 415 nm in the pH range 5.5-6.5. Beer's law was obeyed in the range of 0.118-3.534 $\mu\text{g mL}^{-1}$ of Co (II). The molar absorptivity and Sandal's sensitivity of the method were $3.5 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ and $0.00703 \mu\text{g cm}^{-2}$ respectively. The interference of various diverse ions was studied. The 2:3 (Metal:Ligand) complex was fairly stable (stability constant 2.72×10^{19}). First and second order derivative spectrophotometric methods were also developed for the determination of cobalt(II) which showed greater sensitivity and selectivity. The proposed methods were applied for the determination of cobalt (II) in environmental, medicinal and biological samples.

Graphical Abstract



Synthesis of HMBAINH and its structure.

Keywords: 2-hydroxy-3-methoxybenzaldehydeisonicotinoylhydrazone (HMBAINH), Determination of Co(II), Medicinal and Environmental studies.

INTRODUCTION

Cobalt is one of the trace elements found in plants. It is one of the important components found in number of enzymes; cobalt increases the drought resistance of seeds. Cobalt is important for nitrogen fixation in legumes. It is also a component of vitamin B12 so is important for human consumption [1]. Some research suggests that cobalt plays a role in the production of ethylene by plants. Interestingly, high levels of cobalt can reduce the amount of cadmium that is taken up by plants. On average, 1 kg of soil contains about 8 mg of cobalt though this amount varies widely around the Earth from 0.1-70 mgkg⁻¹. Cobalt deficiency may result in reduced seed germination in dry conditions and reduced plant growth. In legumes, cobalt deficiency may result in symptoms of nitrogen deficiency. Cobalt toxicity seems to be more common than cobalt deficiency although prevalence does depend on the area. High levels of cobalt can result in iron deficiency in plants so symptoms are often those of iron deficiency. Cobalt can also produce its own toxicity symptoms and these include loss of leaves, from a plant, pale colored leaves and discolored veins [2].

A good number of reagents were reported for the spectrophotometric determination of cobalt (II) 1-Nitroso-2-naphthol, proposed by Ilinsky *et al* was one of the first organic analytical reagents employed for the determination of cobalt. This reagent along with its isomer 2-nitroso-1-naphthal was used for the spectrophotometric determination of cobalt in several real samples [3-5]. A large number of organic compounds were reported as analytical reagents for the determination of cobalt. These include oximes, phenyl hydrazones, azo compounds etc [6-11]. But, many of the proposed spectrophotometric methods either lack sensitivity or suffer due to the less tolerance limits for the other interfering ions. We are now proposing a sensitive, selective and rapid direct and derivative spectrophotometric methods for the determination of Co(II) by using 2-hydroxy-3-methoxy benzaldehyde-isonicotinoylhydrazone (HMBAINH).

MATERIALS AND METHODS

Apparatus: A Perkin Elmer (LAMBDA25) spectrophotometer controlled by a computer and equipped with a 1cm path length quartz cell was used for UV-Vis spectra acquisition. Spectra were acquired between 350- 600nm (1nm resolution). An ELICO model LI-120 pH-meter furnished with a combined glass electrode was used to measure pH of buffer solutions.

Preparation of Co(II) solutions: All chemicals used were of analytical-reagent grade. Doubly distilled water was used for preparing aqueous solutions. 0.01 M solution of Co (II) solution was prepared by dissolving appropriate amounts of cobaltous nitrate (Qualizen) in 100mL distilled water, solutions of lower concentrations were prepared by successive dilution of the stock solution.

Preparation of HMBAINH: Equimolar solutions of 2-hydroxy-3-methoxy benzaldehyde in methanol and is nicotinic acid hydrazide in water were refluxed for two hours on water bath and cooled. The light brownish yellow colored solid obtained was then separated by filtration, washed and dried. The product was recrystallized from aqueous alcohol in the presence of norit. The product showed melting point of 224°C. Figure 1 shows the method of preparation and structure of HMBAINH. The reagent solution 1x10⁻²M of was prepared by dissolving 0.271 g in 100 mL of dimethylformamide (DMF). Working solutions were prepared by diluting the stock solution with DMF [12, 13].

Buffer solutions: Buffer solutions of various pH values were prepared by mixing the appropriate solutions in suitable ratios as shown below. pH Constituents: 1.0–3.0 1 M of sodium acetate + 1 M hydrochloric acid, 3.2–6.0 0.2 M sodium acetate + 0.2 M acetic acid 7.0 1 M sodium acetate + 0.2 M acetic acid, 8.0–11.0 2 M ammonium hydroxide + 2M ammonium chloride. Triton X-100 solution: A 1% solution was prepared by diluting 1.0mL of Triton X-100 (AR Merk) to 100 mL with doubly distilled water.

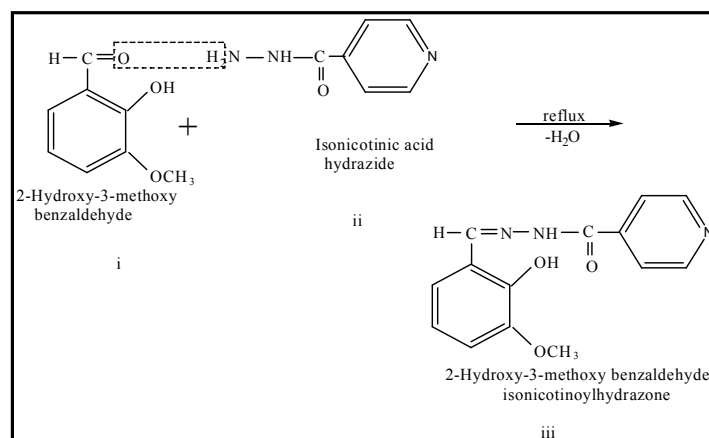


Figure 1. Synthesis of HMBAINH and its structure.

Soil samples: The soil sample (5.0g) was weighed into a 250 mL Teflon high pressure microwave acid digestion bomb and 50 mL aquaregia were added. The bomb was sealed tightly and then positioned in the carousel of a microwave oven. The system was operated at full power for 30 min. The digested material was evaporated to incipient dryness. Then, 50 mL of 5% hydrochloric acid was added and heated close to boiling to leach the residue. After cooling, the residue was filtered and the insoluble residue was washed two times with 5% hydrochloric acid. The filtrates were quantitatively collected in a 250 mL volumetric flask and diluted to the mark with distilled water [14].

Food and biological samples: A wet ash method was employed in the preparation of the sample solution. 0.5 g of the sample was dissolved in a 1:1 mixture of nitric acid and perchloric acid. The solution was evaporated to dryness and the residue was ashed at 300°C. The ash was dissolved in 2 mL of 1M sulphuric acid and made up to the volume in a 25 mL standard flask with distilled water [14].

Blood and Urine Samples: Blood and urine samples of the normal adult and patient (male) were collected from government general hospital, Bangalore. 50mL of sample was taken into 100 mL Kjeldahl flask. 5 mL concentrated HNO₃ were added and gently heated. When the initial brief reaction was over, the solution was removed and cooled. 1mL con. H₂SO₄ and 1 mL of 70% HClO₄ were added. The solution was again heated to dense white fumes, repeating HNO₃ addition. The heating was continued for 30 min and then cooled. The contents were filtered and neutralized with dil. NH₄OH in the presence of 1-2 mL of 0.01% tartarate solution. The solution was transferred into 10mL volumetric flask and diluted to the volume with distilled water [14].

Pharmaceutical samples: A known quantity of the sample was taken in a beaker and dissolved in minimum volume of alcohol. Then added 3 mL of 0.01M nitric acid and evaporated to dryness. The dried mass was again dissolved in alcohol. This was filtered through Whatman filter paper and the filtrate was diluted to 100 mL with distilled water. The lower concentrations were prepared by the appropriate dilution of the stock solution [14].

RESULTS AND DISCUSSION

Direct method of determination of cobalt (II): The absorption spectra fig 2 of [Co (II) - HMBAINH] shows the maximum absorbance of the complex at 415 nm. The preliminary investigations indicate that the absorbance of the complex is maximum and stable in pH range of 5.5 - 6.5 (Fig 3). Hence pH 6.0 was chosen for further studies. A considerable increase in the colour intensity in presence of 0.1% Triton X-100 was observed. Studies on reagent (HMBAINH) concentration effect revealed that maximum 15-fold excess reagent (HMBAINH) is required to get

maximum and stable absorbance for the complex. From the absorption spectra the molar absorptivity coefficient ϵ is calculated as $3.5 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ at 415 nm. Variable amounts of Co (II) were treated with suitable amounts of reagent, surfactant, buffer and validity of Beer's law is tested. The calibration curve [fig 4](#) is linear over the range of $0.118\text{--}3.534 \mu\text{g mL}^{-1}$. The composition of the complex was determined as 2:3 [Co (II): HMBAINH] by Job's method, molar ratio method and slope ratio method [fig 5, 6, 7](#) and [8](#) the stability constant of the reaction was calculated to be 2.72×10^{19} . Other analytical results are presented in [table 7](#).

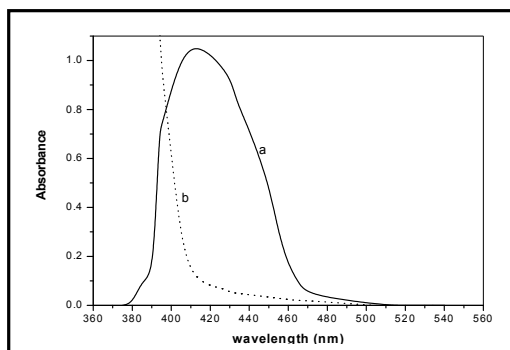


Figure 2. Absorption of (a) (Co(II)-HMBAINH vs Reagent Blend (b) HMBAINH vs Buffer Blank. $[\text{Co(II)}]=3 \times 10^{-5} \text{ M}$, $[\text{HMBAINH}]=4.5 \times 10^{-4} \text{ M}$ pH 6.0

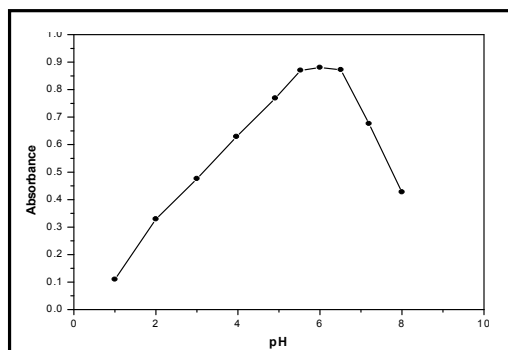


Figure 3. Effect of pH on the absorbance of [(Co(II)-HMBAINH)] system. $[\text{Co(II)}]=3 \times 10^{-5} \text{ M}$, $[\text{HMBAINH}]=1 \times 10^{-4}$ Wavelength =415 nm

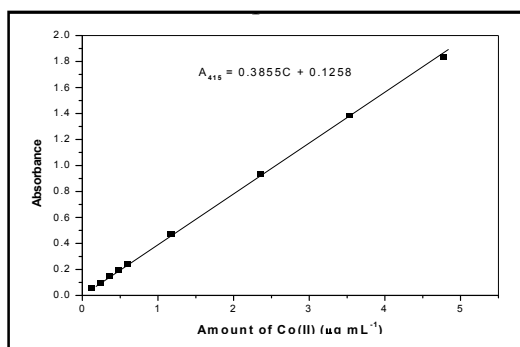


Figure 4. Applicability of Beer's Law. $[\text{HMBAINH}]=4.5 \times 10^{-4} \text{ M}$, Wavelength =415 nm, pH=6.0

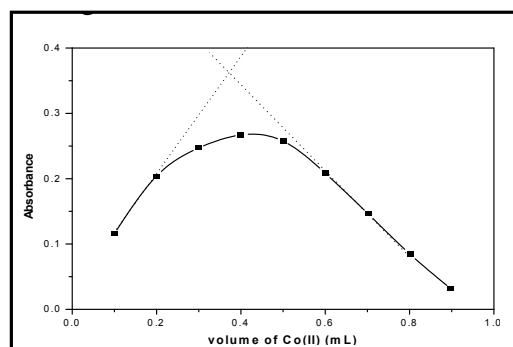


Figure 5. Job's curve. $[\text{Co(II)}]=1 \times 10^{-4} \text{ M}$, $[\text{HMBAINH}]=1 \times 10^{-4} \text{ M}$, Wavelength =415 nm

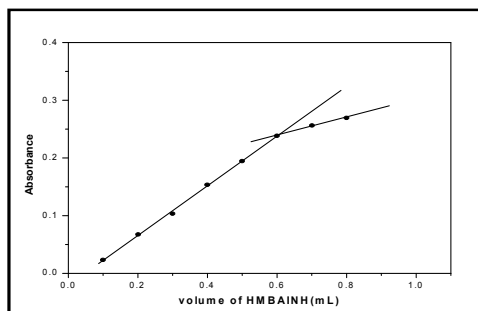


Figure 6. Molar ratio method. $[\text{Co(II)}]=1 \times 10^{-4} \text{ M}$, $[\text{HMBAINH}]=1 \times 10^{-4} \text{ M}$, Wavelength =415 nm, pH=6.0, Volume of Co(II) taken-0.4mL

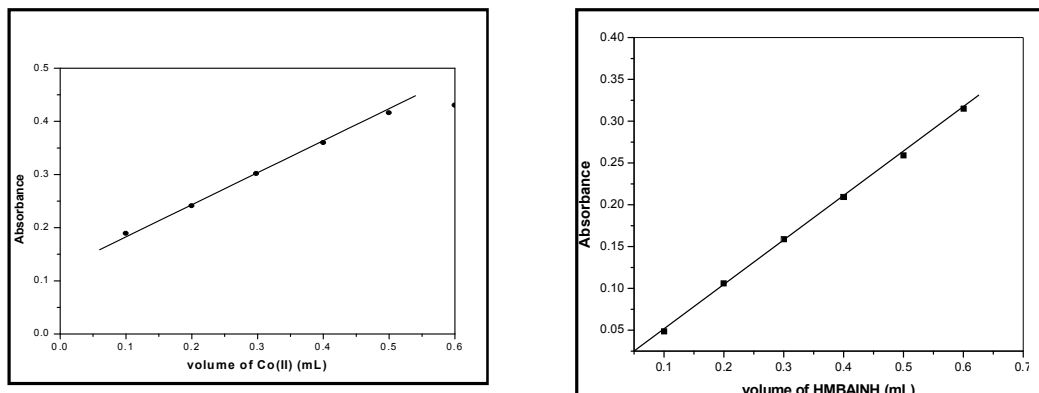


Figure 7 and 8. Slope ratio method.
 $[\text{Co(II)}] = 1 \times 10^{-4} \text{ M}$, $[\text{HMBAINH}] = 1 \times 10^{-4} \text{ M}$, Wavelength = 415 nm

Derivative methods: In order to improve the sensitivity and selectivity of the proposed method, the absorbance data was derivatized once and twice and plotted against the wavelength which gave the resultant first and second order derivative curves respectively. The derivative amplitudes at certain wavelengths were found to be proportional to the amount of cobalt thus obeying Beer's law. Therefore, the method was exploited to develop first and second order derivative spectrophotometric methods for the determination of cobalt.

Derivative curves: The first and second order derivative curves recorded in the wavelength region 350-600 nm, are shown in fig. 9 and 10 respectively. The derivative amplitudes were measured by peak zero method. The first order curves showed maximum amplitudes at 443 nm and second order curves exhibited maximum amplitude at 434 nm and 453 nm.

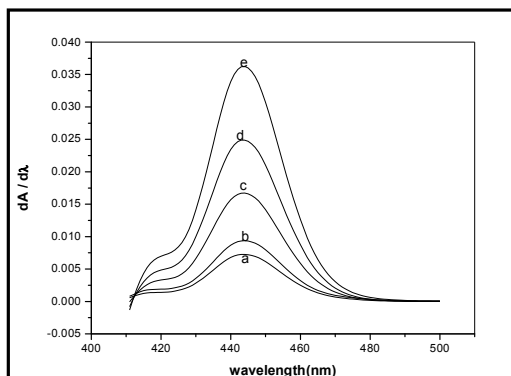


Figure 9. First order derivative spectra of $[\text{Co(II)} - \text{HMBAINH}]$
 Amount of $\text{Co(II)} \mu\text{g mL}^{-1}$: a = 0.059; b = 0.118, c = 0.177,
 d = 0.354, e = 0.472

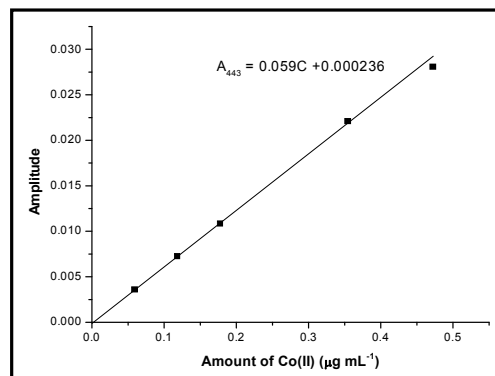


Figure 11. Beer's law plot for first order derivative data
 $[\text{HMBAINH}] = 4.5 \times 10^{-4} \text{ M}$
 pH = 6.0

Effect of diverse ions in the determination of cobalt by direct method: Numerous cations and anions were added individually to a sample solution containing $1.767 \mu\text{g mL}^{-1}$ of cobalt and the influence was examined (Table 1). All the anions and many cations were tolerable in more than 100-fold excess. The tolerance limits of some ions were less than 15 folds. Some of the metal ions, which strongly interfered, could be masked with appropriate masking agents [15, 16].

Determination of cobalt in surface soil, blood and urine samples by direct method: Suitable aliquots of the soil, blood and urine sample solutions were taken and analyzed for cobalt content by

proposed method and the results are presented in table 2 and 3. The soil solutions were further analyzed by a reference method [14] and biological samples were analyzed by flame atomic absorption spectrophotometer and results obtained were compared with the present method, the results indicate the acceptability of present method [13].

Table 1. Tolerance limits of foreign ions
Amount of Co(II) taken = 1.767 $\mu\text{g mL}^{-1}$ pH = 6.0

Foreign ions	Tolerance limit ($\mu\text{g mL}^{-1}$)	Foreign ions	Tolerance limit ($\mu\text{g mL}^{-1}$)	Foreign ions	Tolerance limit ($\mu\text{g mL}^{-1}$)
Iodide	1270	Ba ⁺²	1510	Pb ⁺²	29
Thiosulphate	1120	Na ⁺	1379	Ce ⁺⁴	20
Sulphate	960	K ⁺	1368	Ti ⁺⁴	18
Phosphate	940	Ca ⁺²	1202	Zr ⁺⁴	15
Bromide	800	Sr ⁺²	1051	Au ⁺³	15
Thiourea	760	Mg ⁺²	850	Sn ⁺²	14, 100 ^a
Nitrate	620	La ⁺³	347	Mo ⁺⁶	3, 55 ^c
Thiocyanate	580	Te ⁺⁴	345	Ni ⁺²	3, 80 ^a
Tartrate	512	W ⁺⁶	284	Cr ⁺⁶	3, 100 ^c
Chloride	354	Se ⁺⁴	276	Fe ⁺²	1, 250 ^d
Carbonate	326	Y ⁺³	267	Cu ⁺²	0.5, 150 ^d
Citrate	316	Cd ⁺²	142	Pd ⁺²	0.4, 100 ^e
EDTA	272	Mn ⁺²	121	V ⁺⁵	0.4, 160 ^a
Fluoride	190	Hg ⁺²	65	--	--
Oxalate	96	Ir ⁺³	39	--	--

Increase in tolerance limits in the presence of masking agents
 a = in presence of 600 ppm of Po⁴⁺ c = in presence of 400 ppm of tartrate
 b = in presence of 500 ppm of SCN⁻ d = in presence of 800 ppm of S₂O₃²⁻
 e = in presence of 600 ppm of thiourea

Table 2. Determination of cobalt in surface soil samples

Sample and source	Cobalt ($\mu\text{g mL}^{-1}$)	
	Reference method	Present method
S1. Agricultural land (Redsoil, Bhadravathi.)	16.76 \pm 0.020	16.20 \pm 0.018
S2. Agricultural land (Blacksoil, Gulbarga.)	24.55 \pm 0.022	24.25 \pm 0.020
S3. Riverbed soil (Tungabhadra river, Kurnool)	15.26 \pm 0.018	16.00 \pm 0.022
S4. Industrial soil (electroplating industry, Peenya)	120.12 \pm 0.029	120.12 \pm 0.018

*Average of four determinations

Table 3. Analysis of blood and urine samples for cobalt content

Sample source	Sample	Cobalt ($\mu\text{g mL}^{-1}$)	
		AAS method \pm SD (n=5)	Present method \pm SD (n=5)
Normal adult (male)	Blood	1.78 \pm 0.014	1.66 \pm 0.020
	Urine	0.32 \pm 0.022	0.36 \pm 0.010
Anemia patient (female)	Blood	0.86 \pm 0.020	0.90 \pm 0.020
	Urine	0.28 \pm 0.014	0.24 \pm 0.030
Dengue patient	Blood	4.64 \pm 0.032	5.50 \pm 0.030
	Urine	1.63 \pm 0.025	1.83 \pm 0.020

Determination of cobalt (II): The derivative amplitudes of the first and second order curves measured at 430 nm and 445 nm were plotted against the amount of cobalt figures 11 and 12. The curves were found to be linear obeying Beer's law in the range 0.059–4.712 $\mu\text{g mL}^{-1}$ at 443 nm (first order) and 434 nm and 453 nm (second order). The other analytical and statistical results of the derivative methods are presented in table 7.

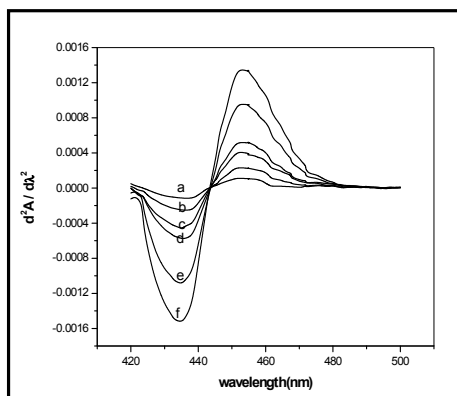


Figure 11. Second order derivative spectra of [Co(II) – HMBAINH]
Amount of Co(II) $\mu\text{g mL}^{-1}$: a= 0.059; b.=0.118, c = 0.177,
d = 0.354, e=0.472

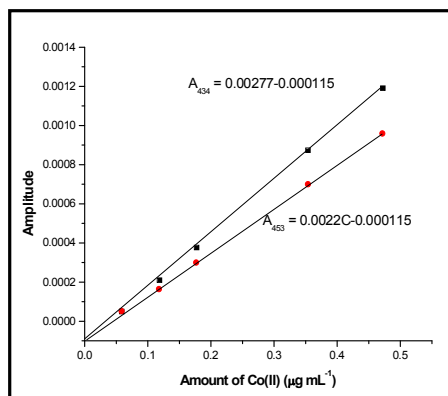


Figure 12. Beer's law plot for second order derivative data.
[HMBAINH] = 4.5×10^{-4} M
pH =6.0

Effect of diverse ions: The tolerance limits of some metal ions which showed serious interference in direct method were evaluated in derivative methods and shown in table 4. It can be seen from the table that the tolerance levels of all the tested ions are much higher in derivative methods than in the zero-order method.

Table 4. Tolerance limit of foreign ions ($\mu\text{g mL}^{-1}$)

Diverse ion	Zero order	First order derivative	Second order derivative
Zr ⁺⁴	15	20	45
Au ⁺³	15	18	25
Sn ⁺²	14	28	15
Mo ⁺⁶	3	35	20
Ni ⁺²	3	20	15
Cr ⁺⁶	3	55	35
Fe ⁺²	1	40	45
Cu ⁺²	0.5	60	20
Pd ⁺²	0.4	80	45
V ⁺⁵	0.4	30	50

APPLICATION

Determination of cobalt in water and pharmaceutical samples by second order derivative method: The second derivative method was applied for the determination of cobalt in different environmental water samples and pharmaceutical samples.

Preparation of water samples: Different water samples were collected from different parts of Anantapur district and filtered and 10 mL of each sample was diluted up to 100 mL. The results obtained in the analysis of water samples by the proposed method are presented in the table 5 and the validity of the results were evaluated by adding known amounts of Co(II) and calculating their recovery percentage.

Preparation of pharmaceutical samples: A known quantity of the sample was taken in a beaker and dissolved in minimum volume of alcohol. Then added 3 mL of 0.01M nitric acid and evaporated to dryness. The dried mass was again dissolved in alcohol. This was filtered through Whatman filter paper and the filtrate was diluted to 100 mL with distilled water. The lower concentrations were prepared by the appropriate dilution of the stock solution. Suitable aliquots were taken and analyzed for cobalt. The results obtained were compared with those obtained by AAS method and presented in table 6. The results of the present direct and derivative methods were compared with those of some of

the already reported methods and presented in table 7. The comparison shows that both the direct and derivative methods proposed are more sensitive than majority of the reported methods. Further, the derivative methods are found to be more selective than large number of the reported methods.

Table 5. Determination of cobalt in environmental samples

Sample	Cobalt ($\mu\text{g mL}^{-1}$)			
	Added	Found	Recovery	RSD
Tap water (Municipality water supply, Bangalore)	0.0	0.32	-	2.5
	1.5	1.80	98.90	1.8
	3.0	3.35	100.90	3
River water (Kaveri, bangalore.)	4.5	4.83	100.20	2.2
	0.0	1.52	-	3.0
	1.5	3.00	99.34	1.6
Drain water (oil Industry, Bangalore)	3.0	4.55	100.66	2.8
	4.5	5.95	98.84	4.0
	0.0	3.60	-	1.7
	1.5	5.31	104.12	3.2
	3.0	6.48	98.18	2.5
	4.5	8.07	99.63	3.6

Table 6. Determination of cobalt in pharmaceutical tablets

Sample (mg tablet^{-1})	Amount of cobalt ($\mu\text{g mL}^{-1}$)		
	Reported	Found	Relative error (%)
Nurobian forte (Cyanocobalamine-15mg)	7.45	7.4	-0.67
Basiton forte (Cyanocobalamine-15mg)	7.42	7.37	-0.67

*Average of four determinations

Table 7. Analytical characteristics of [Co(II)–HMBAINH]

Parameter	Direct method	First derivative	Second derivative	
	415 nm	443 nm	434 nm	453 nm
Beer' law range ($\mu\text{g mL}^{-1}$)	0.118-3.534	0.059-4.712	0.059-4.712	0.059-4.712
Molar absorptivity, ϵ ($\text{L mol}^{-1} \text{cm}^{-1}$)	3.5×10^4	-	-	-
Sandell's sensitivity ($\mu\text{g cm}^{-2}$)	0.00703	-	-	-
Angular coefficient (m)	0.3651	0.0155	0.0007	0.000571
Y-intercept (b)	+0.00132	-0.00011	-0.000093	-0.000098
Correlation coefficient (r)	0.9999	0.9999	0.9998	0.9998
RSD (%)	0.3348	0.48	1.46	0.176
Detection limit ($\mu\text{g mL}^{-1}$)	0.01	0.081	0.014	0.044
Determination limit ($\mu\text{g mL}^{-1}$)	0.03	0.24	0.043	0.132
Composition	2:3	-	-	-
Stability constant	2.72×10^{19}	-	-	-

CONCLUSIONS

The proposed direct and derivative spectrophotometric method of determination of cobalt using HMBAINH is rapid, sensitive and selective. The method was successfully employed in the determination of various biological and environmental samples. Therefore the recommended method can be easily and rapidly used in regular analysis of cobalt.

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