

RESEARCH ARTICLE

Synthesis, Identification, and using of New Nitro Hydrazone Ligand in Spectrophotometric Quantification of Co(II) Content in Vitamin B12 Injections

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ABSTRACT

Nitro Schiff base ligand of 2-(biphenyl-4-yl)-3-((2-(2,4-dinitrophenyl)hydrazono) methyl)imidazo[1,2-a]pyridine was synthesized, identified, and used as a new reagent in Co(II) spectrophotometric determination of vitamin B12 injection samples by standard addition technique at 530nm with the recovery of 98-102% and relative error percent of 0-1.3%. The analytical reaction parameters were studied to optimize the analytical conditions of the proposed method. The suggested method performed linearity in the range 25-250 ppm, LoD = 0.193, LoQ = 0.638ppm, and molar absorptivity constant of $238M^{-1}.cm^{-1}$. The formed complex's mole ratio composition was 2:1 of BDNHMIP: Co(II) with a stability constant of 0.519×10^7 .

Keywords: Nitro hydrazone, Schiff base, Hydrazones, Spectrophotometric determination, Chelated reagents.

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INTRODUCTION

Hydrazones are products of a reaction between hydrazine or hydrazine derivatives and carbonyl compounds. Hydrazones are a special class of Schiff base compounds; these categories are specified by a cyclic group that makes hydrazones with other donor atoms high flexible and versatile poly dentate reagents in determining various transition and inner transition metal ions.¹

Hydrazones have a broad range of applications such as analytical reagents^{2,3} for metal ion determination, as biological active compounds,⁴ as enzyme inhibitors⁵ etc.

Nitro group of hydrazone Schiff bases is strong withdrawing group and because of its steric effects nitro group has played a vital role in the reactivity of hydrazone Schiff bases either when it has participated in coordination with metal ions⁶ or not.⁷⁻⁹

A compound of 2-(biphenyl-4-yl)-3-((2-(2,4-dinitrophenyl)hydrazono)methyl)imidazo[1,2-a]pyridine (BDNHMIP) is nitro hydrazone compound with more than one donor atom of heterocyclic nitrogen and nitro group in addition to characterized azomethine group. In our proposed method (BDNHMIP) was suggested as an analytical chelated reagent in Co(II) determination of vitamin B12 injections by standard addition style with high precision and simple economic technique.

EXPERIMENTAL

Instrumentation

1. Double beam spectrophotometer UV-1800 from Shimadzu Corporation Company.
2. Fourier Transform Infrared (FTIR)-8400 spectrophotometer from Shimadzu Company with potassium chloride disk (in region 4000-400) was used to identify the prepared compounds.
3. Single beam spectrophotometer of V-5000(320-1000 nm) from Metash company.
4. Sensitive balance of KERAN ABS.
5. Magnetic Susceptibility Balance (JM) Johnson Matthey.
6. XRD
7. Heater with magnetic stirrer.

Chemicals

The chemicals with high purities from Fluka, BDH, Riedel de-Haen, Alpha, Alpha Aesar, and Merck were used.

Synthetic procedure

Synthesis process of ligand of 2-(biphenyl-4-yl)-3-((2-(2,4-dinitrophenyl)hydrazono)methyl)imidazo[1,2-a]pyridine was included three steps which were.^{10,11}

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1- Synthesis of 2-(4-biphenyl)imidazo[1,2-a]pyridine by cyclization step.

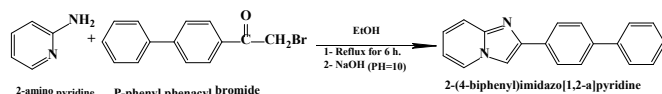
2.75gm (1mmol) of p-phenyl phenacyl bromide (as limiting reactant) was mixed with 0.96gm (1 mmol) of 2-aminopyridine. A 10-12 mL of ethanol was added to the resulted mixture and refluxed with magnetic stirring for 6hours. The resulted solution was cooled and basified to pH = 10 by adding 2-3 mL of 5% sodium hydroxide (5 gm of NaOH in 100ml H₂O). Solid crystals was formed, filtered, washed with water, and dried well in the oven. The product yield was 81.18% and the synthesis process was summarized in Scheme 1.

2- Synthesis of 2-(4-biphenyl)imidazo[1,2-a]pyridine-3-carbaldehyde

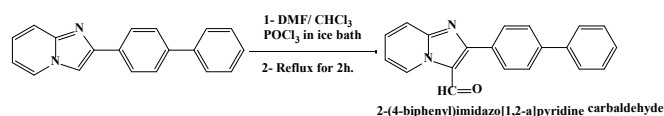
The synthetic process was summarized in Scheme 2 and it included mixing of 5mL of chloroform with 1ml of dimethyl formamide in ice bath. To this mixture, 2ml of POCl₃ was added drop by drop to maintain reaction temperature under 10⁰C because reaction was exothermic, then 2.70 gm (1mmol) of solid 2-(4-biphenyl)imidazo[1,2-a]pyridine with 5–7 mL chloroform were added alternatively to cold mixture with stirring. The resulted mixture was reflexed with magnetic stirring for 2hours. The resulted solution was cooled with ice bath, filtered, washed with ethanol, and dried in oven. The product yield of 2-(4-biphenyl)imidazo[1,2-a]pyridine-3-carbaldehyde yield was 80 %.

3- Synthesis ligand of 2-(biphenyl-4-yl)-3-((2-(2,4-dinitrophenyl)hydrazono)methyl)imidazo[1,2-a]pyridine (BDNHMIP).

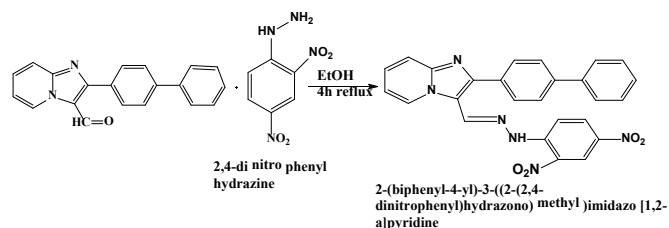
A 10-15 mL of ethanol was added to mixture of 1.491gm (0.5 mmol) of 2-(4-biphenyl)imidazo[1,2-a] pyridine-3-carbaldehyde and 0.99 gm (0.5 mmol) of 2,4-dinitro phenyl hydrazine; then the mixture was refluxed with stirring for 4 hours. The product was cooled, filtered, and washed with water. The product yield was 78.26%. It was recrystallized by ethanol. The synthetic process was summarized in Scheme 3.



Scheme 1: preparation of 2-(4-biphenyl)imidazo[1,2-a]pyridine.



Scheme 2: preparation of 2-(4-biphenyl)imidazo[1,2-a]pyridine-3-carbaldehyde.



Scheme 3: Synthesis of 2-(biphenyl-4-yl)-3-((2-(2,4-dinitrophenyl)hydrazono)methyl)imidazo[1,2-a]pyridine (BDNHMIP).

Synthesis complex of [Co(BDNHMIP)₂]Cl₂.

The complex in this study was synthesized according to the process in reference.12 1mmole (0.478 gm) of 2-(biphenyl-4-yl)-3-((2-(2,4-dinitrophenyl)hydrazono)methyl)imidazo[1,2-a]pyridine (BDNHMIP) was dissolved in 5 mL of ethanol and heated under reflux for few minutes until it dissolved completely. 0.5 mmole (0.119gm) of cobalt chloride hexahydrate CoCl₂.6H₂O was dissolved in 5 mL ethanol and added slowly to ligand solution with refluxing for 4–5 hours, then the precipitate with reddish brown color was filtered and dried.

Preparation of reagents solutions

- Preparation 400 ppm of BDNHMIP by dissolving 40 mg of ligand in 100 mL of DMF.
- Preparation 400 ppm of CoCl₂.6H₂O by dissolving 40 mg of cobalt salt in 100 mL of deionized water, and other concentrations prepared by suitable dilution.
- 0.2M of KCl is prepared by dissolving 1.5gm in 100mL of deionized water.
- 0.2M of HCl is prepared by diluting 8.3mL of stock solution (37% of 1.2 gm/mL) to 1L with deionized water.
- 0.1M of HOAc is prepared by diluting 0.6 gm of stock solution HOAc to 100mL by deionized water.
- 0.1M of NaOAc is prepared by dissolving 0.82 gm of salt in 100 mL of deionized water.
- 0.1M of NaH₂PO₄ is prepared by dissolving 1.2 gm of salt in 100ml of deionized water.
- 0.1M of NaOH is prepared by dissolving 0.4 gm of sodium hydroxide in 100 mL of deionized water.
- Buffers with pH = 1 and 2 are prepared by mixing determined volumes of both of 0.1M hydrochloric acid and 0.1M potassium chloride solutions.
- Buffers with pH = 3, 4, and 5 are prepared by mixing calculated volumes of 0.1M acetic acid and 0.1 M sodium acetate solutions.
- Buffers with pH = 6, 7, and 8 are prepared by mixing determined volumes of 0.1 M sodium hydroxide and 0.1 M sodium dihydrogen phosphate solutions.

Preparation sample of vitamin B12 ampoule

A solution of one ampoule of vitamin B12 was heated in a furnace for 1hour at 700°C. The solution was cooled and 1-2ml of concentrated HNO₃ was added with heating to ensure complete dissolution. The acidity of solution was adjusted by adding sodium hydroxide solution to reach pH = 7 and completing with deionized water to required volume according to cobalt ion content in vitamin B12 ampoules.¹³

Two ampoules of Methylcobal vitamin B12 injection were treated by above procedure and completing volume to 10ml. A 1ml of prepared injection solution was added in each of 5ml volumetric flask and mixed with 0, 0.5, 1, and 1.5 mL of 25ppm standard CoCl₂ respectively, then 2 mL of 400ppm of BDNHMIP ligand was added to each of previous mixtures with completing volume to 5 mL by deionized water.

One ampoule of Panco vitamin B12 injection was treated by above procedure and completed volume to 100mL. 1 mL

of prepared injection solution was added in each of 5 mL volumetric flasks which were contained 0, 0.5, 1, and 1.5 mL of 25 ppm standard CoCl_2 respectively, then 2 mL of 400 ppm of BDNHMIP ligand was added to each of previous mixtures with completing volume to 5 mL by deionized water.

RESULTS AND DISCUSSION

Identification of BDNHMIP (2-(biphenyl-4-yl)-3-((2-(2,4-dinitrophenyl)hydrazono)methyl)imidazo[1,2-a]pyridine and its complex of $[\text{Co}(\text{BDNHMIP})_2]\text{Cl}_2$

According to many analyses, BDNHMIP ligand and its complex $[\text{Co}(\text{BDNHMIP})_2]\text{Cl}_2$ were identified.

Appearance and physical properties

The basic properties of crystals appearance and the melting point of BDNHMIP ligand and its complex of $[\text{Co}(\text{BDNHMIP})_2]\text{Cl}_2$ were listed in Table 1.

Evaluation of Effective Magnetic Moment.

The calculations were done on experimental results of magnetic properties evaluation which were summarized in Table 1.

XRD of BDNHMIP ligand.

The data of XRD analysis of BDNHMIP were treated and summarized in Table 1.

HNMR of BDNHMIP.

As shown in Figure 1 HNMR spectrum of BDNHMIP was included the signals which were depended on and summarized in Table 1 for ligand identification.¹⁴

FTIR Analysis of BDNHMIP and its Complex.

Based on FTIR spectrum of BDNHMIP ligand which was shown in Figure 2 the ligand was identified. The changes in intensities of bonds stretching frequencies of $\text{Co}(\text{BDNHMIP})_2\text{Cl}_2$ complex in comparison with those of BDNHMIP ligand especially of azomethine C=N and N-O

of nitro group in addition to new peaks appearing reflected complex formation.^{7,14} The results were summarized in Table 1.

Shelf Life of Ligand

During storage period, the ligand of BDNHMIP was stable for one month in solvents of ethanol, DMF, and DMSO but only under darkness storage conditions because light exposure changed color of BDNHMIP ligand which was dissolved in DMSO solvent. The spectrum of ligand was changed completely (changing in the position of peaks and valleys) after

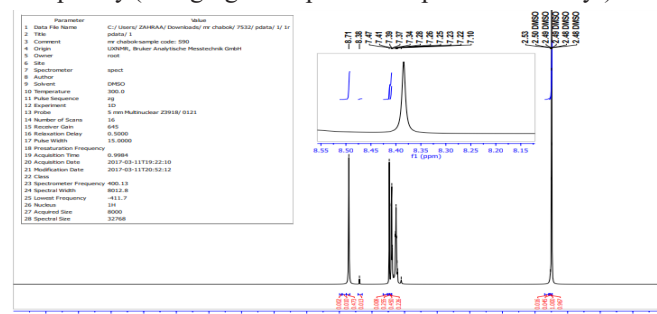


Figure 1: HNMR of 2-(biphenyl-4-yl)-3-((2-(2,4-dinitrophenyl)hydrazono)methyl)imidazo[1,2-]pyridine (BDNHMIP).

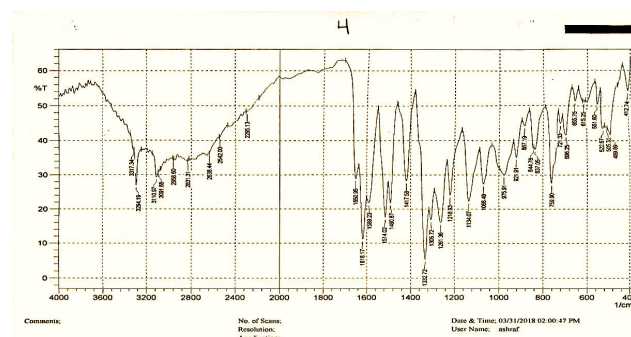


Figure 2: IR spectrum of 2-(biphenyl-4-yl)-3-((2-(2,4-dinitrophenyl)hydrazono)methyl)imidazo[1,2-]pyridine.

Table 1: Physical properties of BDNHMIP ligand and its complex.

	<i>HNMR data in ppm and frequency of bond in cm^{-1} of ligand and complex FT-IR spectra.</i>	<i>Magnetic properties evaluation by Magnetic Balance, emu.mol^{-1}</i>
(BDNHMIP) ligand Light orange crystals 158-160 C°. Unit volume in Å^3 = 511 With Orthorhombic configuration because All three angles of are equal to 90° and each of three lattice dimensions a, b, and c are unequaled	Multiplate (10H) of aromatic=7.10-7.47. Singlet (1H) of HC=N group=8.71 Singlet 1H of NH group=8.38 =CH=3110, aliphatic C-H=2958 aromatic C-H=3091, C=N of ring=1618, C=N of schiff base=1650, NH=3294, C=C aromatic=1514, N-O=symmetric 1332 +asymmetric=1490, Co-N= -	gram magnetic sensitivity is $X_g \times 10^{-6} = 0.909$ negative diamagnetism participation is positive paramagnetism participation is molar magnetic sensitivity, emu. gm^{-1} is effective magnetic moment is .
$[\text{Co}(\text{BDNHMIP})_2]\text{Cl}_2$ Complex. Reddish-brown crystals and its melting point=198-200C°	No distinguish a difference in HNMR data of complex in comparison with ligand. =CH= 3180, aliphatic C-H= 2952 aromatic C-H= 3056, C=N of ring=1618, C=N of schiff base= 1645, NH = 3276, C=C aromatic= 1515 N-O = symmetric 1311+ asymmetric=1456, Co-N = 441+ 582	$X_g \times 10^{-6} = 3.465$ $X_D \times 10^{-6} = - 537$ $X_M \times 10^{-3} = 3.76$ $X_p \times 10^{-3} = 4.3$ = 3.2125

a storage period of one month and as a result, the analytical results of ligand in cobalt ion quantification cannot depend on. The changes in the spectrum of BDNHMIP because of storing were listed in Table 2.

Absorption Spectra of BDNHMIP Ligand and its Complex of $\text{Co}(\text{BDNHMIP})_2\text{Cl}_2$.

The difference between the distinguished wave lengths of complex and its parent ligand (530nm of reddish brown colored complex and 226 nm in addition to 264nm and 318nm of yellow ligand) made the spectrophotometric analysis of complex for Co(II) determination possible and dependable technique. The difference in colors of BDNHMIP and complex in addition to corresponded spectra of both of them were shown Figure 3.

Optimizing the Reaction Parameters for $\text{Co}(\text{BDNHMIP})_2\text{Cl}_2$ Formation.

All reaction parameters were studied by the followed procedure of mixing 2 mL of (400 ppm) BDNHMIP with 2mL of (200 ppm) Co(II) and completing volume to 5 mL with deionized water with or without surfactant or buffer addition.

Influence of Ligand Solvent.

Despite of that the absorbance of complex in DMSO solvent was greater than it's absorbance in other solvents, but it is necessary to keep ligand solution (DMSO solvent) in dark container to avoid ligand color change.

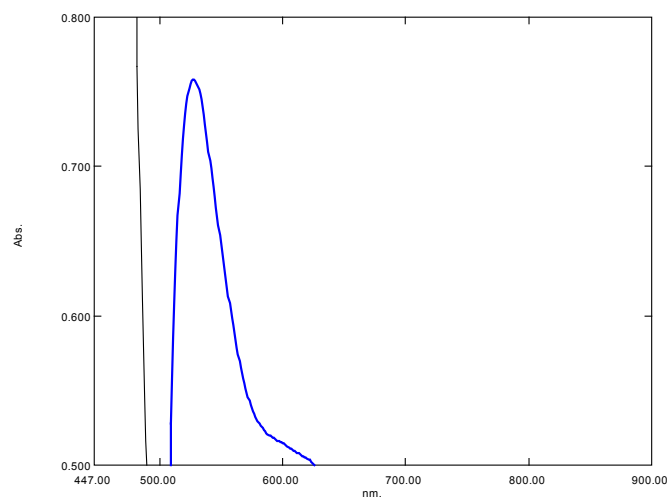


Figure 3: Absorption spectrum of $\text{Co}(\text{BDNHMIP})_2\text{Cl}_2$ complex against ligand as blank (in blue line) and BDNHMIP ligand spectrum against its solvent of DMF (in black line).

Influence of Surfactant Addition.

As summarized in Table 2 the addition of 0.5 mL of 5% nonionic surfactant of TX-114 to reaction solution increased absorbance by turbidity removal, but after few minutes (2 minutes), absorbance value was decreased of precipitation formation.

Influence of Reagents Addition Sequence.

According to the table, an order of reagents addition of L+B+M+ H_2O was the best sequence of addition in which the complex's maximum color intensity has resulted.

Influence of Medium Acidity.

At pH equaled to 7-8, the absorbance of the formed complex was increased, but the color of ligand changed when buffer was added at pH = 7 and turbid solution was formed at pH = 8 because of the possibility of formation of cobalt hydroxide or emulsion solution after mixing ligand with metal ion at this medium and as a result, the absorbance value of resulted complex was unstable and decreasing. The choice was no buffer addition because of ligand color changed in pH = 7-8 before complex formation by Co(II) addition.

Influence of BDNHMIP Concentration.

By mixing 1-2 mL of 200 ppm Co(II) with 1-2 mL of BDNHMIP with concentration range 200-1000ppm and completing volume to 5ml, the effect of ligand concentration on complex color intensity was studied. The best results were performed at 400ppm of ligand concentration because above this concentration the reaction solution became turbid and as a result unstable absorbance was recorded.

Effect of Reaction Time.

Three minutes was the required time for reaction to complete and for absorbance to reach its maximum value. The time which was required to reach maximum complex color intensity was very short and this may be due to the strong electronic withdrawing properties, steric effect,^{15,16} and high possibility of intramolecular bonding of nitro group with other coordinated group, all these factors may be affected on complex stability and decreased reaction time which was required to reach maximum absorbance intensity of formed complex.

Mole Ratio Method.

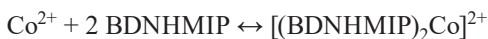
Mole ratio is one of two methods that are used to determine composition of complex (L:M mole ratio). This method was done by mixing different volumes of a ligand with the same volume of metal ion at condition of equaled concentrations

Table 2: Optimum parameters of Co(II) determination reaction.

Reaction parameter	Optimum value	Reaction parameter	Optimum value
Shelf life of BDNHMIP ligand	One month	Reagents addition sequence	BDNHMIP, Buffer, Co(II) and deionized water.
Absorbance wave length, nm	530	Reaction time, min	3
Solvent of BDNHMIP ligand	DMF	Co(II):BDNHMIP mole ratio of complex	1:2
TX-114 detergent	Without addition	Complex stability constant, K	0.519×10^7
Medium acidity, pH	7	ΔG , joule at 300°K	-38566
BDNHMIP concentration, ppm	400		

of both of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ and BDNHMIP ligand (0.00125M). Depending on mole ratio method, the composition of $\text{Co}(\text{BDNHMIP})_2\text{Cl}_2$ was approximately equaled to 2:1 of BDNHMIP: Co(II) as shown in Figure 4.

Estimation Stability Constant of $[(\text{BDNHMIP})_2\text{Co}]^{2+}$ Complex.



According to reaction equation of $\text{Co}(\text{BDNHMIP})_2\text{Cl}_2$, formation Stability constant (K) of complex is expressed as equation below:

$$K = (1 - \alpha)/(4\alpha^3 C^2)$$

where C is an initial concentration of ligand or metal ion in molar (M) and $C = 1.25 \times 10^{-3}$ M in complex formation reaction.

$$\alpha = (A_m - A_s)/A_m$$

where A_m is complex's absorbance after stoichiometric Co(II): BDNHMIP ratio and, A_m of complex = 0.19, A_s is complex's absorbance at stoichiometric Co(II): BDNHMIP ratio and A_s of complex = 0.155

$\Delta G = -RT \ln K$, where ΔG : Gibbs free energy, R: 8.314 joule/mol.k, T: absolute temperature.

The calculated results were summarized in Table 2.

Optimum conditions for Co(II) determination reaction were summarized in Table 2.

Method Validation.

The Co(II) determination method's validity was tested by preparation standard curve and evaluation its linearity. The tested solutions were prepared by addition 3 mL of cobalt chloride CoCl_2 with variable concentrations to 2 mL of 400 ppm and recorded their absorbance. As shown in Figure 5 and listed in Table 3 the results were statistically treated.

Determination of Co(II) in Vitamin B12

Standard addition method (SAM) was used to omit effect of sample matrix¹⁷ in instrumental analysis. SAM was

proposed by Saxberg and Kowalski¹⁸ and performed in spectrophotometric analyses.¹⁹ It is essential to understand that SAM cannot be applied when more than sample matrix interference is achieved such as spectral, instrumental, and methodic interferences because of in exact results which cannot be depended on. As shown in Figures 5 and 6 and listed in

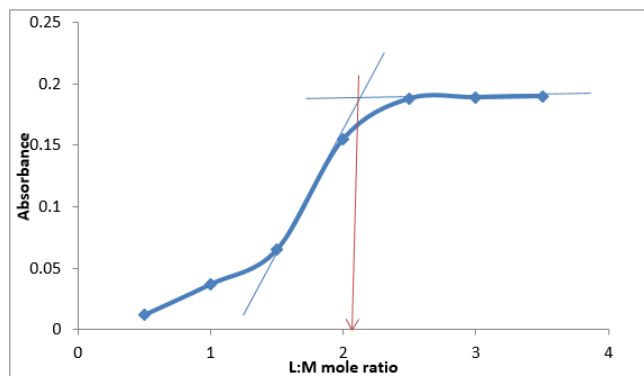


Figure 4: Mole ratio method for determination complex composition of $\text{Co}(\text{BDNHMIP})_2\text{Cl}_2$

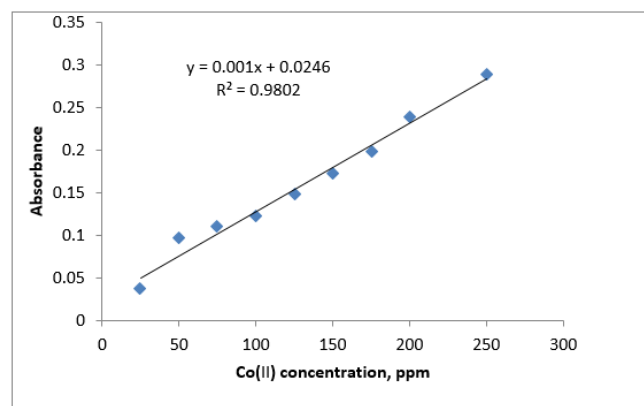


Figure 4: Calibration curve of Co(II) determination.

Table 3: Statistical treatment of standard curve results.

Statistical parameters	equation	Slope, b	0.001
Regression equation	$Y = 0.001X + 0.0246$	Intercept, a	0.0246
Correlation coefficient, r^2	0.9802	Standard deviation of residual, $S_{y/x}$	0.01337
Linearity percentage, $r^2\%$	98.02%	Standard deviation of slope, S_b	6.447×10^{-5}
Linearity range, mg/L (ppm)	25-250	Standard deviation of intercept, S_a	9.367×10^{-3}
Molar absorptivity constant =slope of line $M^{-1}/\text{path length}(\text{cm})$	238	LoD= $3S_b/b$, mg/L	0.193
		LoQ= 3.3 LoD , mg/L	0.638

Table 4: Recovery calculations of standard addition method for Co(II) determination in Vitamin B12 injections

Sample	Description	Co(II) in ppm	Co(II) founded by proposed method in ppm	Recovery %	Relative error R.E%
Methylcobal B12 injection (Japan).	500 in 1ml ampoule	100	100	100	0
Panclo B12 injection (Argentina).	10mg in 3ml ampoule	100	98.87	98.87	1.13

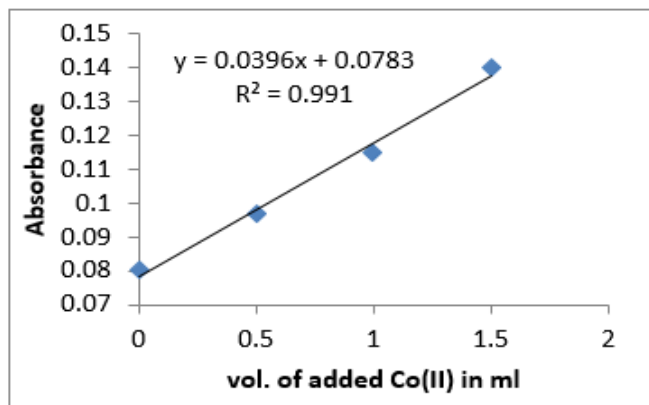


Figure 6: Standard addition method of Co(II) determination in Methylcobal B12 injection.

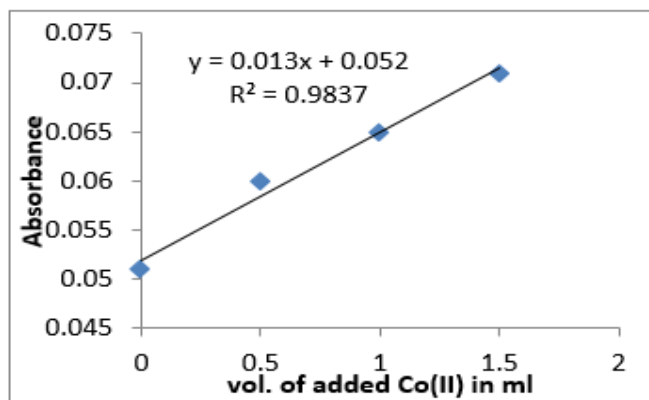


Figure 7: Standard addition method of Co(II) determination in Panclo B12.

Table 4 the Co(II) contents in vitamin B12 injection samples were determined.

CONCLUSION

A new nitro hydrazone compound synthesized, identified, and used as chelated ligand in spectrophotometric determination of Co(II) without need for any pretreatment step such as extraction, pre-concentration, heating or long time of waiting to complete reaction of proposed method, in addition to available inexpensive ligand in suggested quantification method. The proposed method performed Co(II) determination of vitamin B12 injection samples with high precision and minimum error.

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