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Molybdate Assisted Ninhydrin Based Sensitive Analytical System for the Estimation of Drugs Containing Amine Group

PADMARAJAIAH NAGARAJA*, ASHWINEE KUMAR SHRESTHA,
ANANTHARAMAN SHIVAKUMAR AND NAEF GHALLAB SAEED AL-TAYAR

Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore 570 006, India

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ABSTRACT

A sensitive spectrophotometric method for the analysis of isoniazid, lisinopril dihydrate, amoxicillin trihydrate, ampicillin trihydrate, glucosamine sulfate, phenylpropanolamine hydrochloride and gabapentin is described. The analysis is based on the reaction of drug molecules with ninhydrin and sodium molybdate mixture to give *Ruhemann's* purple product with maximum absorbance (λ_{\max}) at 570 nm. The statistical analysis of intra-day and inter-day estimation of drugs as well as comparison with reported methods demonstrated high precision and accuracy of the proposed method. The method was successfully applied to the analysis of pharmaceutical preparations. The procedure was suitable for quality control application.

Key words: Ninhydrin, sodium molybdate, pharmaceutical analysis, quality control, spectrophotometry

INTRODUCTION

Isoniazid (INH) is one of the most active antituberculosis agents that act by interfering with metabolism of bacterial protein and inhibiting mycolic acid synthesis in bacterial cell wall. Many methods such as chemiluminescence⁽¹⁾, fluorimetry⁽²⁾, voltammetry⁽³⁾, and spectrophotometry⁽⁴⁻⁷⁾ are available for the estimation of INH. Lisinopril (LIS), a lysine analogue of enalaprilate, is an active site-directed ACE inhibitor with zinc ion binding interaction. It has been found effective against hypertension, acute myocardial infarction and has a class effect in patients with chronic heart failure. Several methods have been reported for the determination of LIS, which include radioimmunoassay⁽⁸⁾ and spectrophotometry^(9,10). Amoxicillin (AMX) and ampicillin (AMP) are antibacterial agents that acylate transpeptidase enzyme and inhibit cell wall synthesis in bacteria. AMX can be analyzed by liquid chromatography⁽¹¹⁾, chemiluminescence⁽¹²⁾, capillary electrophoresis⁽¹³⁾ and spectrophotometry⁽¹⁴⁻¹⁶⁾. AMP is determined by fluorimetry⁽¹⁷⁾, and spectrophotometry^(15,18). Glucosamine (GLA), a precursor for glycosaminoglycans, is a major component of joint cartilage, and is considered

to rebuild cartilage and treat arthritis. It is estimated by capillary electrophoresis⁽¹⁹⁾, liquid chromatography⁽²⁰⁾, and spectrophotometry⁽²¹⁾. Phenylpropanolamine (PPL) is a sympathomimetic drug that is a common active component in "over the counter" appetite suppressant and in cough and cold medication. The methods reported for its estimation include liquid chromatography⁽²²⁾, fluorimetry⁽²³⁾ and spectrophotometry^(24,25). Gabapentin (GAB) is an anti-convulsant drug that prevents physiological degradation of γ -aminobutyric acid (GABA) by inhibiting GABA-transaminase. It can be estimated by high-performance liquid chromatography⁽²⁶⁾, spectrofluorimetry⁽²⁷⁾, and spectrophotometry^(28,29).

The reaction of amino acid with ninhydrin is known for a long time⁽³⁰⁾. Several modifications have been proposed to enhance the sensitivity and application of ninhydrin⁽³¹⁾ that include use of cadmium, iron, zinc, lithium and cyanide. In this paper, we suggested a highly sensitive method for the estimation of drugs containing amine group that give positive ninhydrin test, in buffer solution of pH 5.5 at $90 \pm 5^\circ\text{C}$ to give *Ruhemann's* purple with maximum absorbance at 570 nm. This modified method has also been applied to our earlier work for the estimation of glyphosate⁽³²⁾.

* Author for correspondence. Tel: +91-821-2412557;
Fax: +91-821-2421263; E-mail: profpn58@yahoo.com

MATERIALS AND METHODS

I. Materials

A JASCO (Model UVVIDEC-610) UV-VIS spectrophotometer with 1 cm-matched glass cell was used for all absorbance measurements.

All the chemicals used were of analytical grade; double distilled water was used throughout the experiment. LIS, AMX, GLA, and PPL were obtained from Sigma-Aldrich Co., INH was procured from BDH (Poole, England) and AMP from Merck (India).

(I) Preparation of Citrate Buffer of pH 5.5

This was prepared by dissolving 21.0 g of citric acid and 200 mL 1.0 M NaOH in water and making up the volume to 1000 mL with water.

(II) Preparation of Ninhydrin and Sodium Molybdate Mixture (NSM)

An equimolar concentration of 0.206 M ninhydrin and sodium molybdate were dissolved in citrate buffer of pH 5.5 and made up the volume to 25 mL. This solution was used within 8 h after preparation.

(III) Preparation of Standard Solution

Stock solutions of 100 µg/mL of drugs were prepared in water. The solutions were further diluted quantitatively according to their linear calibration range.

(IV) Preparation of Tablet\Capsule Sample Solution

Twenty tablets of each drug were weighed and finely powdered using mortar and pestle. Similarly, ten capsules of AMX, AMP, and GAB were carefully evacuated, and mixed. A quantity equivalent to 10 mg of each drug was transferred to 100-mL volumetric flask. The mixture was shaken mechanically with water for 5 min, sonicated, diluted to the volume with water, mixed and filtered. Appropriate aliquots of the filtrates were further diluted with water to obtain the required concentrations.

II. Method

Into a series of 10-mL volumetric flasks, appropriate volume of 100 µg/mL standard solutions of INH, LIS, AMX, AMP, GLA, PPL and GAB were transferred to get final concentrations range of 0.5 - 5.0, 2.5 - 12.5, 1.5 - 14.0, 3 - 20.0, 1.2 - 7.5, 0.9 - 7.0 and 0.25 - 4.8 µg/mL, respectively. To each flask, 0.5, 1.0, 1.0, 1.0, 1.0, 0.5 and 0.5 mL of NSM solution was added respectively for INH, LIS, AMX, AMP, GLA, PPL and GAB. These solutions were further diluted to 5 mL with citrate buffer (pH 5.5) and heated on a water bath at $90 \pm 5^\circ\text{C}$ for 10 min.

After cooling to room temperature, the solutions were made up to the mark with water. The absorbance of the solutions was measured against reagent blank at 570 nm. The reagent blank had no appreciable absorption at this wavelength. The calibration graph was prepared by plotting absorbance *versus* concentration for each drug. No appreciable change was observed if the order of addition of the reagents was changed.

RESULTS AND DISCUSSION

I. Spectral Characterization

The method is based on the reaction of ninhydrin with drugs in the presence of sodium molybdate in buffer solution of pH 5.5 to give *Ruhemann's* purple colored product with maximum absorbance at 570 nm. The absorption spectrum of the colored product of INH is shown in Figure 1.

II. Optimization of Reaction Variables

The optimal conditions were investigated to achieve maximum color development in the determination of these drugs.

(I) Concentration of NSM

Different concentration combinations of ninhydrin and sodium molybdate in citrate buffer (pH 5.5) were attempted, which showed that the concentration equivalent to 0.206 M of each drug gave the best result. The volume range of 0.3 - 0.7 mL for INH and 0.8 - 1.1 mL for LIS were necessary for maximum color development. Similarly, for AMX and AMP, it was 0.8 - 1.1 and 0.9 - 1.1 mL, respectively and for GLA, PPL, and GAB, 0.8 - 0.1, 0.5 - 0.75 and 0.5 - 0.7 mL, respectively needed for maximum color development. In the analysis, the quantity as mentioned in "General procedure" was taken.

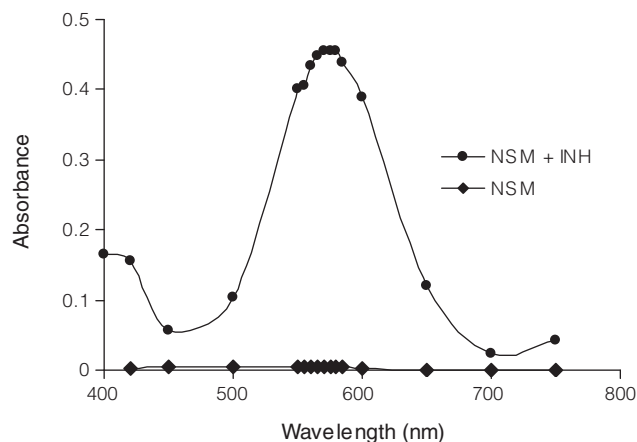


Figure 1. Absorbance spectrum of INH at 3 µg/mL

(II) Effect of pH

The maximum color intensity was observed at pH 5.5 in citrate buffer for all the drugs studied. The effect of different pH for INH is shown in Figure 2.

(III) Effect of Reaction Time and Temperature

The optimum reaction time and temperature were determined by carrying out the reaction at different temperatures (25 - 100°C) and time intervals (0 - 15 min). Satisfactory maximum color intensity and reproducible λ_{max} values were obtained when the reaction mixture was heated at $90 \pm 5^\circ\text{C}$ for 10 min. The effects of reaction time and temperature for INH are shown in Figure 3 and 4, respectively.

(IV) Stability of the Colored Product

The color was developed by heating the solution for about 10 min after the addition of all the reagents, on a boiling water bath followed by cooling to room temperature. The color was stable for at least 24 h at room temperature.

III. Quantification

The Beer's law range, molar absorptivity, sandell sensitivity, regression equation, and correlation coefficient were determined for each drug. A linear relationship was found within the range 0.5 - 5 $\mu\text{g}/\text{mL}$ for INH, 2.5 - 12.5 $\mu\text{g}/\text{mL}$ for LIS, 1.5 - 14 $\mu\text{g}/\text{mL}$ for AMX, 3 - 20 $\mu\text{g}/\text{mL}$ for AMP, 1.2 - 7.5 $\mu\text{g}/\text{mL}$ for GLA, 0.9 - 7 $\mu\text{g}/\text{mL}$ for PPL and 0.25 - 4.8 $\mu\text{g}/\text{mL}$ for GAB. Regression analysis of the Beer's Law plots revealed a good correlation. The graph showed negligible intercept, which was calculated by the least-square method regression equation:

$$A = a + bc,$$

where, A is the absorbance of solution in 1 cm cell, a is the intercept, b is the slope, and c is the concentration of the measured solution in $\mu\text{g}/\text{mL}$. The high molar absorptivities of the resulting colored solutions indicated high sensitivity of the method. The limit of detection (LOD) and the limit of quantification (LOQ) values were determined using the formula

$$\text{LOD or LOQ} = K \text{ SD}/b$$

where, K = 3 for LOD and 10 for LOQ, SD and b represent standard deviations of the intercept and slope, respectively. The results are shown in Table 1.

IV. Interference Study

The effect of common excipients used in the pharmaceutical preparation were studied by analyzing synthetic

sample solutions containing the quantity of drugs as mentioned in Table 2 in presence of 100 fold more concentration of each excipient. For methylcobalamine that accompany GAB in tablet formulation, the tolerance limit

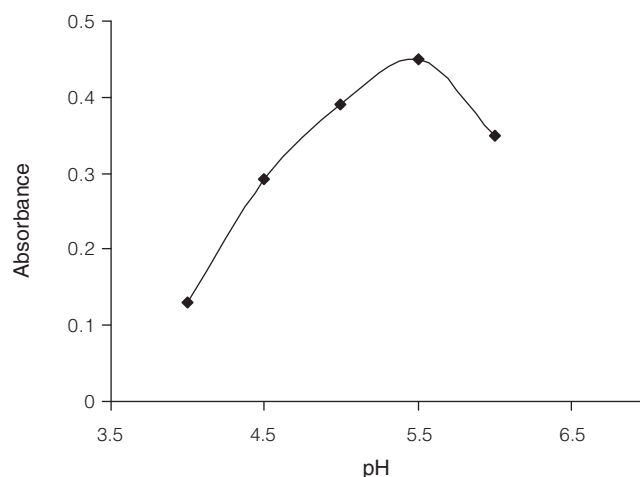


Figure 2. Effect of pH on the absorbance of INH at 3 $\mu\text{g}/\text{mL}$.

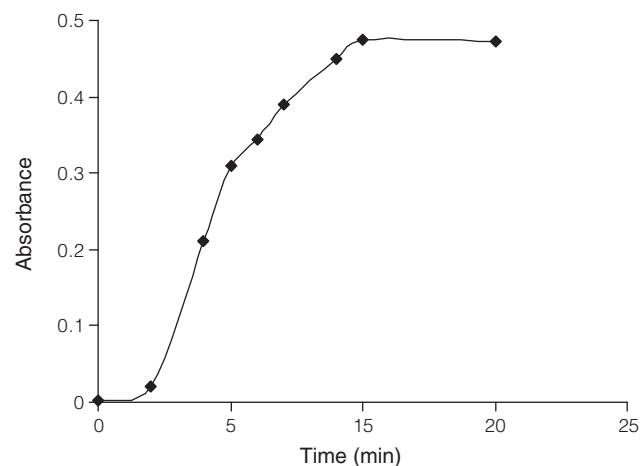


Figure 3. Time course of color development of INH at 3 $\mu\text{g}/\text{mL}$.

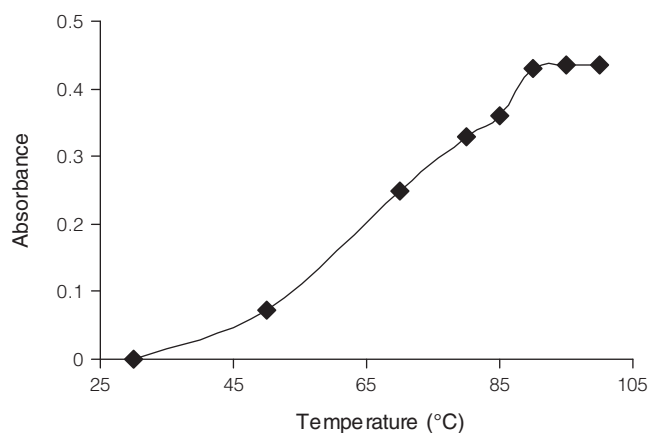


Figure 4. Effect of temperature on the absorbance of INH at 3 $\mu\text{g}/\text{mL}$.

Table 1. Optical characteristics and statistical data of the regression equation for the reaction of the proposed method

Parameters	Characteristic						
	INH	LIS	AMX	AMP	GLA	PPL	GAB
Colour	Purple	Purple	Purple	Purple	Purple	Purple	Purple
λ_{\max} [nm]	570	570	570	570	570	570	570
Beer's law range [$\mu\text{g}/\text{mL}$]	0.5 - 5	2.5 - 12.5	1.5 - 14	3 - 20	1.2 - 7.5	0.9 - 7	0.25 - 4.8
Molar absorptivity [$\text{L}/\text{mol}/\text{cm}$] $\times 10^4$	2.14	2.02	2.14	1.64	4.48	1.85	2.54
Sandell's sensitivity [$\mu\text{g}/\text{cm}$] $\times 10^{-2}$	0.64	2.18	1.9	2.4	1.02	1.01	0.67
Limit of detection [$\mu\text{g}/\text{mL}$]	0.032	0.389	0.491	0.357	0.124	0.116	0.059
Limit of quantification [$\mu\text{g}/\text{mL}$]	0.108	1.296	1.01	1.23	0.414	0.386	0.196
Regression equation ^a							
Slope [b]	0.156	0.0564	0.0491	0.0419	0.0966	0.1035	0.1448
$\pm ts_b \times 10^{-3}$	4.44	4.57	1.92	1.56	2.96	3.73	2.26
Intercept [a]	0.0034	-0.026	0.0051	-0.0063	-0.0151	-0.0144	0.0048
$\pm ts_a$	0.0125	0.038	0.017	0.019	0.014	0.020	0.0063
Correlation coefficient [r]	0.9998	0.9999	0.9996	0.9994	0.9995	0.9999	0.9997
Relative standard deviation ^b	0.666	0.2416	0.138	0.275	0.161	0.232	0.171

^a $A = a + bc$, where c is the concentration of the measured solution in $\mu\text{g}/\text{mL}$.

^b Average of six determinations (concentrations of 2.5, 7.5, 8, 10, 4, 4 and 2.5 $\mu\text{g}/\text{mL}$ of pure drugs of INH, LIS, AMX, AMP, GLA, PPL and GAB respectively).

s_b = Standard deviation of slope, s_a = Standard deviation of intercept.

$\pm ts_b$ = Confidence limit for slope at 95% confidence level for five degree of freedom, $\pm ts_a$ = Confidence limit for intercept at 95% confidence level for five degree of freedom.

Table 2. Recovery of drugs from solution with a 100 fold concentration of various additives used as excipients

Excipients	Recovery \pm R.S.D ^a (%)						
	INH ^b	LIS ^c	AMX ^d	AMP ^e	GLA ^f	PPL ^g	GAB ^h
Dextrose	99.3 \pm 0.4	99.2 \pm 0.8	99.5 \pm 0.8	99.8 \pm 0.4	99.9 \pm 0.2	99.9 \pm 0.5	99.9 \pm 0.5
Lactose	100.1 \pm 0.8	100.9 \pm 0.5	99.8 \pm 0.3	99.9 \pm 0.5	100.2 \pm 0.7	99.9 \pm 0.6	100.9 \pm 0.5
Starch	99.8 \pm 0.2	99.1 \pm 0.7	99.9 \pm 0.5	99.5 \pm 0.2	99.1 \pm 0.3	99.8 \pm 0.3	99.8 \pm 0.1
Sucrose	99.9 \pm 0.2	99.9 \pm 0.8	99.7 \pm 0.3	99.9 \pm 0.3	99.9 \pm 0.3	99.8 \pm 0.3	99.1 \pm 0.7
Carboxymethyl cellulose	99.8 \pm 0.4	100.0 \pm 0.6	99.8 \pm 0.1	99.3 \pm 0.1	99.8 \pm 0.4	99.4 \pm 0.7	99.9 \pm 0.3
Talc	99.8 \pm 0.3	100.0 \pm 0.4	99.6 \pm 0.6	99.7 \pm 0.8	99.2 \pm 0.5	99.4 \pm 0.6	99.6 \pm 0.6
Magnesium stearate	99.6 \pm 0.6	99.4 \pm 0.7	99.5 \pm 0.2	99.2 \pm 0.1	99.4 \pm 0.1	99.6 \pm 0.3	99.4 \pm 0.7
Sodium chloride	100.0 \pm 0.1	100.1 \pm 0.5	100.0 \pm 0.1	100.2 \pm 0.2	100.0 \pm 0.2	100.0 \pm 0.4	100.2 \pm 0.1
Vitamin B ₆	99.4 \pm 0.7						
Chlorpheniramine maleate						99.2 \pm 0.3	

^a Mean of 3 determinations.

^b Concentration of INH used- 2.5 $\mu\text{g}/\text{mL}$.

^c Concentration of LIS used- 7.5 $\mu\text{g}/\text{mL}$.

^d Concentration of AMX used- 8 $\mu\text{g}/\text{mL}$.

^e Concentration of AMP used- 10 $\mu\text{g}/\text{mL}$.

^f Concentration of GLA used- 4 $\mu\text{g}/\text{mL}$.

^g Concentration of PPL used- 4 $\mu\text{g}/\text{mL}$.

^h Concentration of GAB used- 2.5 $\mu\text{g}/\text{mL}$.

Table 3. Intraday and interday precision data

	Amount taken $\mu\text{g/mL}$	Intraday Recovery \pm R.S.D ^a (%)	Interday Recovery \pm R.S.D ^b (%)
INH	1.0	0.99 \pm 0.20	0.99 \pm 0.17
	2.5	2.49 \pm 0.11	2.48 \pm 0.35
	3.0	2.96 \pm 0.10	2.97 \pm 0.34
LIS	5.0	4.99 \pm 0.12	4.99 \pm 0.73
	7.5	7.46 \pm 0.10	7.45 \pm 0.24
	10.0	9.89 \pm 0.72	9.89 \pm 0.11
AMX	5.0	5.03 \pm 1.20	5.13 \pm 1.80
	8.0	8.10 \pm 0.71	8.06 \pm 1.07
	12.0	12.07 \pm 0.62	12.11 \pm 0.80
AMP	5.0	5.03 \pm 1.29	5.06 \pm 1.58
	10.0	9.98 \pm 0.82	10.08 \pm 0.85
	15.0	15.01 \pm 0.76	15.07 \pm 0.65
GLA	2.5	2.49 \pm 0.43	2.49 \pm 0.82
	5.0	5.09 \pm 0.99	5.04 \pm 1.64
	6.5	6.54 \pm 0.12	6.49 \pm 0.39
PPL	3.5	3.52 \pm 0.20	3.52 \pm 0.44
	5.0	5.12 \pm 0.49	5.16 \pm 0.33
	6.5	6.50 \pm 0.13	6.52 \pm 0.71
GAB	1.0	1.04 \pm 0.24	0.98 \pm 0.15
	2.0	1.99 \pm 0.10	2.05 \pm 0.31
	3.0	2.96 \pm 0.12	2.99 \pm 0.23

^a Mean of 5 determinations, ^b Mean of 5 determinations performed over a period of 5 days.

was up to 2 times the quantity of drug taken. Since the ratio of drug and methylcobalamine in pharmaceutical formulation is 600 : 1, no interference was observed in drug analysis. The tolerance limit is the concentration which gives an error of $\pm 3.0\%$ in the determination of drugs. The results indicate that the excipients studied did not interfere the quantitative analysis by the present method.

V. Precision and Accuracy

The short term precision (intraday precision) of the drugs were evaluated by measuring 5 independent samples at 3 different concentration levels (1.0, 2.5, 3.0 $\mu\text{g/mL}$ for INH, 5.0, 7.5, 10.0 $\mu\text{g/mL}$ for LIS, 5.0, 8.0, 12.0 $\mu\text{g/mL}$ for AMX, 5.0, 10.0, 15.0 $\mu\text{g/mL}$ for AMP, 2.5, 5, 6.5 $\mu\text{g/mL}$ for GLA, 3.5, 5, 6.5 $\mu\text{g/mL}$ for PPL and 1, 2, 3 $\mu\text{g/mL}$ for GAB). Similarly, the assay for daily

precision (interday precision) at the same concentration level was repeated for 5 consecutive days. The results are presented in Table 3.

The proposed method was applied to estimate drugs in pharmaceutical formulations. The results were compared statistically with reference methods. In the *t*- and *F*-tests, no significant difference was found between the calculated and theoretical value (95% confidence) of the proposed and reference method. The reliability and accuracy of the proposed method were further ascertained through recovery studies by the standard addition method using extra amount of standard drugs to the pre-analyzed dosage forms such that the cumulative amount after adding the drugs did not exceed their linearity range. The results are presented in Table 4.

Table 4. Analysis of drugs in pharmaceutical formulations

Formulation	Labeled (mg)	Proposed method \pm S.D. (mg) ^a	Reported Method \pm S.D (mg) ^a	%Recovery \pm S.D. ^b	t-Test ^a	F-Test ^a
Solonex DT ^c	100	99.98 \pm 0.07	100.0 \pm 0.06 (6)	99.9 \pm 0.63	0.66	1.36
AKT-3 ^d	300	300.00 \pm 0.12	299.90 \pm 0.17 (6)	100.00 \pm 1.032	1.19	2.0
Odace 5 ^e	5	4.98 \pm 0.02	5.00 \pm 0.02 (9)	99.72 \pm 1.90	1.66	1.32
Listril ^f	5	4.99 \pm 0.03	5.01 \pm 0.03 (9)	99.80 \pm 0.90	1.25	1.07
Mox 250 ^g	250	250.00 \pm 0.10	249.90 \pm 0.12 (14)	100.12 \pm 0.10	1.57	1.44
Roscollin ^h	250	249.90 \pm 0.21	250.20 \pm 0.29 (18)	99.90 \pm 0.25	2.06	1.90
Cartilamine ⁱ	500	500.00 \pm 0.20	500.20 \pm 0.19 (21)	100.34 \pm 0.91	1.2	0.90
Coldact ^j	50	50.10 \pm 0.15	50.20 \pm 0.18 (24)	100.12 \pm 0.35	1.05	1.44
Gabapentin300 ^k	300	300.10 \pm 0.2	300.11 \pm 0.25 (28)	100.61 \pm 0.21	1.5	1.7
Gabapin ME ^l	300	300.30 \pm 0.1	300.10 \pm 0.35 (28)	100.10 \pm 0.32	1.0	1.8

^a Average \pm standard deviation of six determinations; the t- and F- values obtained after comparison to the reference methods which have following theoretical values at 95% confidence limit; t = 2.44 F = 5.05.

^b After adding four different amounts of pure drugs to the fixed concentration of preanalysed pharmaceutical formulations.

^c INH equivalent to 100 mg/tablet (Macleods Pharmaceutical, India).

^d INH equivalent to 300 mg/tablet (Lupin Ltd, India).

^e LIS equivalent to 5 mg/tablet (Indon Health Care, India).

^f LIS equivalent to 5 mg/tablet (Torrent, India).

^g AMX equivalent to 250 mg /capsule (Ranbaxy, India).

^h AMP equivalent to 250 mg/capsule (Ranbaxy, India).

ⁱ GLA equivalent to 500 mg/tablet (Troika, India).

^j PPL equivalent to 50 mg/tablet (Sun Pharma, India).

^k GAB equivalent to 300 mg/capsule (Sun Pharma, India).

^l GAB equivalent to 300 mg/tablet (Intas pharmaceuticals, India).

CONCLUSIONS

The recommended method using ninhydrin and sodium molybdate for the analysis of the drugs is simple, inexpensive and has great sensitivity and accuracy. A comparison of the proposed method with reported methods is shown in Table 5, which also depicts the manifold increase in sensitivity of this system over the method where only ninhydrin was used as seen in LIS, GLA and GAB. The optical parameters and statistical comparisons justify that this method can be applied in routine drug estimation in pure and dosage forms. Also, the procedure does not involve any critical reaction conditions or tedious sample preparation steps. Therefore, this method can be recommended for routine analysis and also for quality control of these drugs.

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Table 5. Comparison of the proposed method with reported method

Drug	Method of analysis	Beer's law range (µg/mL)	λ_{\max}	Molar absorptivity (L/mol/cm)	Remark	Reference
Isoniazid	Condensation of INH with 1, 2-naphthoquinone-4-sulfonic acid	0.5 - 30.0	460 nm	1.18×10^4	samples were extracted in water	4
	Reduction of copper to form Cu (I)-neocuprione complex	0.3 - 3.5	454 nm	-	samples were extracted in water	7
Lisinopril	Ninhydrin and sodium molybdate	0.5 - 5.0	570 nm	2.14×10^4		Proposed method
	Meisenheimer complex formed between Lisinopril and 2,4 dinitrofluorobenzene	4.0 - 20.0	400 nm	-	Use of organic solvents such as methanol and dimethylsulfoxide	9
	Reaction with ninhydrin	10.0 - 150.0	595 nm	4.083×10^3	use of organic solvent such as Dimethylformamide; samples were extracted in methanol	10
Amoxicillin	Ninhydrin and sodium molybdate	2.5 - 12.5	570 nm	2.022×10^4		Proposed method
	Ion pair formed between the drugs and Mo(V)-thiocyanate	7.5 - 8.5	467 nm	5.28×10^3	Low sensitivity, extraction by methanol	15
Ampicillin	Reaction with 1,2-naphthoquinone-4-sulfonate	0.8 - 20.0	468 nm	3.91×10^3	Low sensitivity; samples were extracted in water	16
	Ninhydrin and sodium molybdate	1.5 - 14.0	570 nm	2.14×10^4		Proposed method
	Ion pair formed between the drug and Mo(V)-thiocyanate	1.5 - 77.5	467 nm	6.2×10^3	Low sensitivity, extraction by methanol	15
Glucosamine	Reaction with 1,2-naphthoquinone-4-sulfonate	2.0 - 80.0	463 nm	1.14×10^4	Low sensitivity; samples were extracted in water	18
	Ninhydrin and sodium molybdate	3.0 - 20.0	570 nm	1.64×10^4		Proposed method
Phenylpropanol amine	Reaction with ninhydrin	10.0 - 100.0	570 nm		Low sensitivity; samples were extracted in water	21
	Ninhydrin and sodium molybdate	1.2 - 7.5	570 nm	4.48×10^4		Proposed method
	Reaction of amine group of drug with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole	0.0 - 41.0	455 nm	5.61×10^3	samples were extracted in water followed by chloroform	24
Gabapentin	Ion pair complex with alizarin derivatives	0.5 - 25.0	606 nm	4.6×10^3	Methanol as a solvent; samples were extracted in water	25
	Ninhydrin and sodium molybdate	0.9 - 7.0	570 nm	1.85×10^4		Proposed method
	Reaction with ninhydrin	40.0 - 280.0	569 nm	5.16×10^2	Low sensitivity; samples were extracted in water	28
Proposed method	Reaction with ninhydrin	2 - 30	568 nm	1.25×10^4	Methanol as a solvent for ninhydrin; samples were extracted in water	29
	Ninhydrin and sodium molybdate	0.25 - 4.8	570 nm	2.54×10^4		Proposed method

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