Research Article

Redox-Reaction Based Spectrophotometric Assay of Isoniazid in Pharmaceuticals

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Received 21 January 2014; Accepted 25 February 2014; Published 22 April 2014

Academic Editors: B. N. Barman, G. Drochioiu, J. V. Garcia Mateo, B. Rittich, and A. Szemik-Hojniak

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Two spectrophotometric methods are described for the determination of isoniazid (INH) in pharmaceuticals. In the first method (FCR method), INH is reacted with Folin-Ciocalteu reagent in Na_2CO_3 medium and the resulting blue colored chromogen measured at 760 nm. Iron(II), formed as a result of reaction between INH and iron(III), is made to react with ferricyanide, and the resulting Prussian blue is measured at 760 nm, basing the second method (FFC method). The conditions for better performance are optimized. Beer's law is obeyed in the concentration ranges $0.5\text{--}10$ and $0.2\text{--}3.0\ \mu\text{g mL}^{-1}$ for FCR method and FFC methods, respectively, with corresponding molar absorptivity values of 1.12×10^4 and $4.55 \times 10^4\ \text{L mol}^{-1}\text{ cm}^{-1}$. The methods are validated for accuracy, precision, LOD, LOQ, robustness, and ruggedness as per the current ICH guidelines. The validated methods were successfully applied to quantify INH in its commercial formulation with satisfactory results; hence the methods are suitable for isoniazid determination in bulk drugs and pharmaceuticals.

1. Introduction

Isoniazid (INH) (Figure 1), chemically known as pyridine-4-carboxylic acid hydrazide, is an antitubercular drug now widely used together with rifampicin and streptomycin for the chemotherapy of tuberculosis. This has prompted many investigators to devise methods for its determination in its pure form as well as in its tablet form.

The drug is official in Indian Pharmacopoeia (IP) [1], British Pharmacopoeia (BP) [2], and United State Pharmacopoeia (USP) [3]. IP and BP describe titration of the drug with potassium bromate in presence of potassium bromide using methyl red indicator. USP describes HPLC method using L_1 column ($4.6\text{ mm} \times 25\text{ cm}$) and a mobile phase consisting of methanol:water (40:60) (pH adjusted to 2.5 with H_2SO_4) with a flow rate of 1.5 mL min^{-1} and UV-detection at 254 nm. Apart from the above official methods, a number of methods based on several techniques are found in the literature for INH and include titrimetry [4–7], voltammetry [8–13], ion selective electrode-potentiometry [14–19], amperometry [20, 21], spectrofluorimetry [22, 23], chemiluminescence spectrometry [24–33], high performance

liquid chromatography (HPLC) [34–37], gas chromatography (GC) [38–40], LC/LC-MS [41], and capillary electrophoresis [42–44].

Visible spectrophotometry is by far the most widely used technique for the assay of INH. Methods were based on a variety of reaction schemes such as nucleophilic substitution [45], condensation [46–49], charge-transfer and ion-pair [50], derivatization [51–54], diazo-coupling [55, 56], oxidative coupling [57, 58], complex formation [59–61], and redox followed by complexation [62–65]. These methods suffer from one or more of the disadvantages such as drastic experimental conditions, use of organic solvent, longer standing time, poor sensitivity, and narrow linear range. Folin-Ciocalteu (F-C) reagent or iron(III) and ferricyanide have been widely used for the sensitive determination of a wide-ranging phenolic and amine organic compounds of pharmaceutical importance [66–74].

The aim of this work was to investigate the utility of F-C reagent and iron(III) and ferricyanide systems in the assay of INH. The methods had sufficiently good accuracy and precision and presented a simple and time-saving assay of INH.

FIGURE 1: Structure of INH.

2. Experimental

2.1. Apparatus. A Systronics model 166 digital spectrophotometer (Ahmedabad, India) with matched 1-cm quartz cells was used for absorbance measurements.

3. Materials

Chemicals used were of analytical reagent grade. Distilled water was used throughout the investigation. Pharmaceutical grade INH certified to be 99.85% pure was kindly provided by Cipla India Ltd., Bangalore, India, and was used as received. Isokin-300 (Pfizer Ltd., Gandhinagar, Hyderabad, India) tablets containing isoniazid 300 mg with vitamin B₆ 10 mg were purchased from local commercial store.

3.1. Reagents

3.1.1. Folin-Ciocalteu Reagent. Aqueous solution of Folin-Ciocalteu reagent (1:1 v/v) was prepared by mixing 50 mL of reagent (Merck, Mumbai, India) with 50 mL water.

3.1.2. Sodium Carbonate. A 20% solution of sodium carbonate was prepared by dissolving 20 g compound (S.D. Fine Chem. Ltd., Mumbai, India) in 100 mL water.

3.1.3. Potassium Ferricyanide. A 500 $\mu\text{g mL}^{-1}$ potassium ferricyanide solution was prepared by dissolving 50 mg reagent (Glaxo Laboratory, Mumbai, India) in 100 mL water.

3.1.4. Ferric Chloride (FeCl_3). A solution of 0.5 M $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ was prepared by dissolving 14 g of the chemical (S.D. Fine Chem. Ltd., Mumbai, India) in 100 mL of 0.1 M HCl (Merck, Mumbai, India).

3.1.5. Standard Drug Solution. A stock standard solution of INH (100 $\mu\text{g mL}^{-1}$) was prepared by dissolving 10 mg of pure INH in 100 mL water, appropriately diluted to 20 $\mu\text{g mL}^{-1}$ INH for method A and 10 $\mu\text{g mL}^{-1}$ INH for method B with water, and used in the assay.

4. Assay Procedure

4.1. Method A (FCR Method). Into a series of 10 mL volumetric flasks, different aliquots of working standard INH solution (20 $\mu\text{g mL}^{-1}$) ranging from 0.25 to 5.0 mL equivalent to 0.5–10.0 $\mu\text{g mL}^{-1}$ were transferred and the total volume was brought to 5 mL with water. To each flask, 2 mL of 1:1 F-C reagent and 2 mL of 20% Na_2CO_3 solution were successively added by means of a microburette. The flasks were stoppered and the contents were mixed well and kept at room temperature for 10 min. The volume was made up to the mark with water and the absorbance of each solution was measured at 760 nm against a reagent blank similarly prepared in the absence of INH.

4.2. Method B (FFC Method). Different aliquots of standard INH solution (10 $\mu\text{g mL}^{-1}$) ranging from 0.2 to 3.0 mL were transferred into a series of 10 mL of calibrated flasks and the total volume was brought to 3 mL with water. Then, 1.5 mL of 500 $\mu\text{g mL}^{-1}$ potassium ferricyanide and 1.0 mL FeCl_3 solution were accurately added. The volume was made up to the mark with water, content mixed, and the flasks were kept at room temperature. After 10 min, the absorbance was measured at 760 nm against reagent blank prepared simultaneously without adding INH.

Standard graph was prepared by plotting the absorbance versus INH concentration, and the concentration of the unknown was computed from the respective regression equation derived using the absorbance-concentration data.

4.3. Procedure for Pharmaceutical Tablets. Twenty tablets were weighed and ground into a fine powder. An accurately weighed quantity containing 10 mg of INH was transferred to a 100 mL volumetric flask, 60 mL water added, shaken well for 20 minutes and made up to mark with water, and then filtered. This solution was diluted to a 20 and 10 $\mu\text{g mL}^{-1}$ INH with water and analyzed by the recommended procedures.

4.4. Procedure for Placebo Blank and Synthetic Mixture Analyses. A placebo blank containing starch (10 mg), acacia (15 mg), hydroxyl cellulose (10 mg), sodium citrate (10 mg), talc (20 mg), magnesium stearate (15 mg), and sodium alginate (10 mg) was prepared by mixing all components into a homogeneous mixture. 10 mg of the placebo blank was accurately weighed and its solution was prepared as described under Procedure for Pharmaceutical Tablets and then subjected to analysis by following the general procedure.

An accurately weighed 10 mg of INH was added to 10 mg of placebo blank and homogenized. Synthetic mixture was quantitatively transferred into a 100 mL volumetric flask and the extract was prepared as described under the Procedure for Pharmaceutical Tablets. The resulting extract was diluted to get 20 and 10 $\mu\text{g mL}^{-1}$ INH solutions, respectively. Suitable aliquot of the solution was analyzed at three levels by following the general assay procedure.

FIGURE 2: (a) Absorption spectra of A: reaction product of INH ($6 \mu\text{g mL}^{-1}$) with F-C reagent in Na_2CO_3 solution and B: blank. (b) Absorption spectra for method B, reaction product of INH ($1.25 \mu\text{g mL}^{-1}$) with ferric chloride and ferricyanide solution ($\blacktriangle$) and blank ($\circ$).

5. Validation of Method

The method was validated according to the procedures described in ICH guidelines [75] for the validation of analytical methods.

Limits of Detection (LOD) and Quantification (LOQ). The limits of detection (LOD) and quantification (LOQ) were calculated according to the ICH guidelines using the formulae:

$$\text{LOD} = 3.3 \frac{S}{b}, \quad \text{LOQ} = 10 \frac{S}{b}, \quad (1)$$

where S is the standard deviation of blank absorbance values and b is the slope of the calibration plot.

5.1. Precision and Accuracy. The intraday precision was evaluated by analyzing INH solution at three different levels. Similarly the interday precision was evaluated on five consecutive days ($n = 5$). In each case and for each concentration, mean value of the INH found and the relative standard deviations (RSD) were calculated. The accuracy of the methods was determined by the percent mean deviation between the obtained and known concentration of INH.

5.2. Robustness and Ruggedness. Method robustness was studied by making small changes in the optimized experimental variables and their effect on the absorbance was evaluated by calculating the percentage RSD values. In order to determine the method ruggedness, analyses were performed using three instruments and also by three analysts with the same instrument.

6. Results and Discussion

The proposed FCR method is based on the formation of a blue colored chromogen, following the reduction of phosphomolybdotungstic mixed acid of the F-C reagent [73] by INH in the presence of sodium carbonate, which could be measured at 760 nm. The mixed acids in the F-C reagent

TABLE 1: Sensitivity and regression parameters.

Parameter	Method A	Method B
λ_{max} , nm	760	760
Color stability, min	60	90
Linear range, $\mu\text{g mL}^{-1}$	0.5–10.0	0.2–3.0
Molar absorptivity (ϵ), $\text{L mol}^{-1} \text{cm}^{-1}$	1.12×10^4	4.55×10^4
Sandell sensitivity*, $\mu\text{g cm}^{-2}$	0.0123	0.003
Limit of detection (LOD), $\mu\text{g mL}^{-1}$	0.03	0.14
Limit of quantification (LOQ), $\mu\text{g mL}^{-1}$	0.09	0.42
Regression equation, Y^{**}		
Intercept (a)	0.0131	0.0198
Slope (b)	0.0905	0.3176
Standard deviation of a (S_a)	9.98×10^{-2}	9.98×10^{-2}
Standard deviation of b (S_b)	1.24×10^{-2}	3.98×10^{-2}
Regression coefficient (r)	0.9971	0.9979

*Limit of determination as the weight in $\mu\text{g mL}^{-1}$ of solution, which corresponds to an absorbance of $A = 0.001$ measured in a cuvette of cross-sectional area 1 cm^2 and $l = 1 \text{ cm}$.

** $Y = a + bX$, where Y is the absorbance, X is concentration in $\mu\text{g mL}^{-1}$, a is intercept, and b is slope.

are the final chromogen and involve the following chemical species:

INH probably affects reduction of oxygen atoms from tungstate and/or molybdate in the F-C reagent by producing one or more possible reduced species which have characteristic intense blue color.

The FFC method involves the redox reaction of INH with ferric chloride, in the presence of potassium ferricyanide, under mild acidic conditions, to produce a blue colour with maximum absorption at 760 nm. The first step in the colour development is the reduction of iron(III) of ferric chloride to iron(II) which subsequently reacts with ferricyanide to form Prussian blue.

FIGURE 3: (a) Effect of volumes of F-C reagent (1:1) on the reaction product with INH ($6 \mu\text{g mL}^{-1}$) in Na₂CO₃ solution for method A. (b) Effect of 20% Na₂CO₃ on color formation ($6 \mu\text{g mL}^{-1}$ INH) for method A. (c) Reaction time and stability of colored species for method A ($6 \mu\text{g mL}^{-1}$ INH).

7. Method Development

7.1. Spectral Characteristics. The intensely blue colored product (molybdenum-tungsten mixed acid blue in FCR method and Prussian blue in FFC method) formed in both methods exhibited maximum absorption at 760 nm. The absorption spectra of the blue colored products and of the reagent blanks are shown in Figures 2(a) and 2(b).

FIGURE 4: (a) Effect of volume of FeCl₃ for method B ($1.5 \mu\text{g mL}^{-1}$ INH). (b) Effect of volume of ferricyanide for method B ($1.5 \mu\text{g mL}^{-1}$ INH). (c) Reaction time and stability of colored species for method B ($1.5 \mu\text{g mL}^{-1}$ INH).

7.2. Optimization of Experimental Variables. A series of preliminary experiments necessary for rapid and quantitative formation of colored products to achieve the maximum stability and sensitivity were performed. Optimum condition was achieved by varying one parameter at a time while keeping other parameters constant and observing its effect on the absorbance at 760 nm.

TABLE 2: Evaluation of intraday and interday accuracy and precision.

Method	INH Taken ($\mu\text{g mL}^{-1}$)	Intraday accuracy and precision ($n = 7$)			Interday accuracy and precision ($n = 5$)		
		INH Found ^a ($\mu\text{g mL}^{-1}$)	RSD ^b %	RE ^c %	INH found ($\mu\text{g mL}^{-1}$)	RSD ^b %	RE ^c %
A	3.0	2.96	1.24	0.89	3.07	2.34	2.12
	6.0	6.11	1.84	1.03	6.12	2.00	2.53
	9.0	9.14	1.56	1.52	9.15	1.67	2.00
B	1.0	1.01	1.02	1.92	1.03	3.00	1.85
	1.5	1.47	2.01	1.15	1.54	2.67	1.27
	2.0	2.04	1.92	1.47	2.05	2.50	1.62

^aMean value of 7 determinations; ^brelative standard deviation (%); ^crelative error (%).

TABLE 3: Method robustness and ruggedness expressed as intermediate precision.

Method	Nominal concentration	Robustness			Ruggedness	
		Reaction times* ($n = 3$)	Volumes of FC reagent [‡]	Volumes of ferricyanide [‡]	Interanalysts ($n = 3$)	Interinstruments ($n = 3$)
A	3.0	1.45	1.69	—	2.20	2.32
	6.0	1.72	1.35	—	1.74	1.92
	9.0	2.10	1.71	—	2.44	2.57
B	1.0	1.72	—	1.59	1.78	2.12
	1.5	1.84	—	1.61	2.11	1.62
	2.0	2.12	—	1.72	1.07	2.54

*Reaction times were 10 ± 2 min for both methods. [‡]Volumes of FC reagent added were 2 ± 0.2 mL for method A; volumes of ferricyanide added were 1.5 ± 0.1 mL for method B.

7.3. Optimization for FCR Method

7.3.1. Effect of Concentration of F-C Reagent. Several experiments were carried out to study the influence of F-C reagent concentration on the color development and the obtained results are shown in Figure 3(a). It is apparent that 1.0 to 5.0 mL of reagent gave the maximum color intensity; thus 3.0 mL of reagent was used throughout the investigation.

7.3.2. Selection of Reaction Medium and Optimization of the Base. To select a suitable medium for the reaction, different aqueous bases such as sodium hydroxide, sodium carbonate or bicarbonate, sodium acetate, and sodium hydrogen phosphate were investigated. Better results were obtained with sodium carbonate. In order to determine the optimum concentration of Na_2CO_3 , different volumes of 20% Na_2CO_3 solution (0–5 mL) were attempted at a constant concentration of INH ($6 \mu\text{g mL}^{-1}$) and the results of the observation are shown in Figure 3. It was found that different volumes ranging from 1.0 to 3.0 mL of 20% Na_2CO_3 were optimum; thus 2.0 mL was used throughout the work (given in Figure 3(b)).

Maximum color development was obtained in 10 min after mixing the reactants, and the color was stable for at least 60 min thereafter (Figure 3(c)). The sequence of order of addition of the reactants had significant effect on the absorbance value. So, the order used in the general procedure should be followed for maximum absorbance.

7.4. Optimization for FFC Method. To optimize the concentrations of ferricyanide and ferric chloride reagents, different volumes of these reagents were used with a fixed concentration of INH. Constant absorbance was found with 1.0 mL of 0.5 M FeCl_3 and 1.5 mL of $500 \mu\text{g mL}^{-1}$ ferricyanide solutions, as shown in Figures 4(a) and 4(b).

Colour development was complete in 10 min and stable for the next 90 min (Figure 4(c)). Different results were obtained when different order of addition of reactants was followed. The order of addition of reactants followed in the recommended procedure resulted in rapid color formation with maximum sensitivity and stability.

8. Method Validation

8.1. Linearity, Sensitivity, Limits of Detection, and Quantification. A linear correlation was found between absorbance at λ_{max} and concentration of INH in the ranges given in Table 1. Regression analysis of the Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a), and correlation coefficient (r) for each system and the analytical results obtained from these investigations are presented in Table 1. The optical characteristics such as Beer's law limits, molar absorptivity, and Sandell sensitivity values are also given in Table 1. The high values of ϵ and low values of Sandell sensitivity and LOD indicate the high sensitivity of the proposed methods.

TABLE 4: Results of analysis of tablets by the proposed methods.

Tablets analyzed	Label claim (mg/tablet)	Found* (percent of label claim $\pm$ SD)		
		Official method	Proposed methods Method A	Method B
Iskin-300	300	99.86 $\pm$ 1.36	101.5 $\pm$ 1.16	101.2 $\pm$ 0.92
			$t = 2.06$	$t = 1.83$
			$F = 1.37$	$F = 2.19$

* Mean value of five determinations.

Tabulated t -value at the 95% confidence level is 2.77.

Tabulated F -value at the 95% confidence level is 6.39.

TABLE 5: Results of recovery study via standard addition method with tablet.

Method	Tablets studied	INH in tablet $\mu\text{g mL}^{-1}$	Pure INH Added $\mu\text{g mL}^{-1}$	Total Found $\mu\text{g mL}^{-1}$	Pure INH recovered* percent $\pm$ SD
A	Isokin-300	2.96	1.5	4.41	99.28 $\pm$ 2.03
		2.96	3.0	6.01	100.9 $\pm$ 3.12
		2.96	4.5	7.56	101.3 $\pm$ 2.99
B	Isokin-300	1.01	0.5	1.51	99.85 $\pm$ 2.25
		1.01	1.0	2.04	101.3 $\pm$ 2.32
		1.01	1.5	2.55	101.7 $\pm$ 3.14

* Mean value of three determinations.

8.2. Intraday and Interday Precision and Accuracy. The precision and accuracy of the proposed method were studied by repeating the experiment seven times within the day to determine the repeatability (intraday precision) and five times on different days to determine the intermediate precision (interday precision). The assay was performed for three levels of analyte in this method. The results of this study are summarized in Table 2. The percentage relative standard deviation (%RSD) values were $\leq 2.01\%$ (intraday) and $\leq 3.00\%$ (interday) indicating good precision of the method. Accuracy was evaluated as percentage relative error (%RE) between the measured mean concentrations and taken concentrations of INH, and it was $\leq 2.53\%$ demonstrating the high accuracy of the proposed method.

8.3. Selectivity. A selective study was performed to determine the effect of matrix on the absorbance by analyzing the placebo blank. In the analysis of placebo blank solution the absorbance in each case was equal to the absorbance of blank which revealed no interference. To assess the role of the inactive ingredients on the assay of INH, the general procedure was applied on the synthetic mixture extract by taking three different concentrations of INH within the range. The percentage recovery values were in the range 96.3–102.3% and 95.4–101.8% with RSD $< 4\%$ indicating clearly the noninterference from the inactive ingredients in the assay of INH.

8.4. Robustness and Ruggedness. The robustness of the method was evaluated by making small incremental changes in the volume of reagent and reaction time, and the effects of the changes were studied by measuring the absorbance of the colored product. The changes had negligible influence

on the results as revealed by small intermediate precision values expressed as %RSD ($\leq 2.54\%$). Method ruggedness was demonstrated having the analysis done by three analysts and also by a single analyst performing analysis on three different instruments in the same laboratory. Intermediate precision values (%RSD) in both instances indicated acceptable ruggedness. These results are presented in Table 3.

8.5. Application to Tablets. The proposed methods were applied to the quantification of INH in commercial tablets. The tablets were assayed by the official BP method [2], which describes titration of the drug with potassium bromate in presence of potassium bromide using methyl red indicator. The results obtained by the proposed methods agree well with the claim and also are in agreement with those of the official method. Statistical analysis of the results did not detect any significant difference between the performance of the proposed method and reference method with respect to accuracy and precision as revealed by the Student's t -value and variance ratio F -value. The results of assay are given in Table 4.

8.6. Recovery Study. To further assess the accuracy of the method, recovery experiment was performed by applying the standard-addition technique. The recovery was assessed by determining the agreement between the measured standard concentration and added known concentration to the sample. The test was done by spiking the preanalyzed tablet INH with pure INH at three different levels (50, 100, and 150%) of the content present in the preparation and the total was found by the proposed method. Each test was repeated three times. The recovery percentage values ranged between 99.28 and 101.7% with standard deviation in the range 0.99–1.32%. Closeness

TABLE 6: Comparison of performance characteristics of the proposed methods with the existing methods.

Sl. No.	Reagent/s used	Methodology	$\lambda_{\max}$ (nm)	Linear range ($\mu\text{g mL}^{-1}$) ($\epsilon = \text{L/mol/cm}$)	Remarks	References
1	Epichlorohydrin hydroxyphenacylchloride	Measurement of purple colored coupled complex	405 402	2–22 (0.51×10^4) 20–120 (0.10×10^4)	Longer reaction time and heating	[45]
2	Ethyl vanillin	Measurement of yellow colored hydrazone complex	410	2–16 (71×10^3)	—	[46]
3	Vanillin	Measurement of yellow colored hydrazone complex	405	1–12	—	[47]
4	Isatin	Measurement of yellow colored hydrazone complex	340	0–32 (1.2×10^4)	Requires close pH control and 40 min standing time, measurement at a shorter wavelength	[48]
5	Sodium 1,2-naphthoquinone-4- sulfonate and cetyltrimethyl ammonium bromide	Absorbance of condensation product measured	500	2.0–5.6	Employs a costly reagent	[49]
6	Chloranilic acid Tetracyanoethylene 2,3-dichloro-5,6-dicyano- 1,4-benzoquinone Thymol blue Bromophenol blue Bromocresol green	Measurement of charge-transfer complex Measurement of ion-associate complex	500 480 580 390 410 320	1.37–8.2 6.85–34.27 10.96–21.93 6.85–41.13 1.37–6.85 1.37–8.22	Use of organic solvent, requires heating step Require close pH control, liquid-liquid extraction step	[50]
7	2-Hydroxy-1,4- naphthoquinone	Measurement of derivatized product	365	5–25	Requires close pH control, use of nonaqueous medium, measurement at a shorter wavelength	[51]
8	1, 2-Naphthoquinone-4- sulfonate	Measurement of pink colored condensed product	495	0.5–3.0 (1.18×10^4)	Requires close pH control	[52]
9	6-Methyl-2- pyridinecarboxaldehyde	Measurement of hydrazone derivative	328	2–16	Measurement at lower analytical wavelength	[53]
10	9-Chloroacridine	Measurement of absorbance of derivatized complex	500	—	Requires heating step, time consuming	[54]
11	4,4'-Methylene-bis- <i>m</i> - nitroaniline	Measurement of purple colored diazo-coupled complex	495	0.1–15 (5.63×10^4)	Requires low temperature	[55]
12	4,4'-Sulphonyldianiline	Measurement of purple colored diazo-coupled complex	440 335	0.5–20 (5.72×10^4)	Requires low temperature	[56]
13	Tiron- NaIO_4	Measurement of red-colored oxidative-coupled product	507	1.0–15 (1.84×10^4)	Use of multireaction system step	[57]
14	Tiron- KIO_4	Measurement of red-colored oxidative-coupled product	505	1.5–18 (1.77×10^4)	Use of multistep reaction system	[58]
15	Uranyl acetate	Measurement of yellow-colored uranyl isonicotinoyl dithiocarbamate complex	410	—	Multistep reaction, time consuming	[59]

TABLE 6: Continued.

Sl. No.	Reagent/s used	Methodology	$\lambda_{\max}$ (nm)	Linear range ($\mu\text{g mL}^{-1}$) ($\epsilon = \text{L/mol/cm}$)	Remarks	References
16	Neocuproine	Measurement of redox complex	454	0.3–3.5	Requires close pH control, a multistep reaction	[60]
17	Rose bengal	Measurement of acetone-chloroform extractable ion-pair complex	555	2.8–5.6	Requires time consuming and tedious extraction step, use of organic solvent	[61]
18	* NBS	Measurement of starch-iodine Complex	572	0.1–3.4	Multistep reaction, employs an unstable oxidant	[62]
19	* NBS	Absorbance of starch-iodine complex measured	572	0.1–3.4	Multistep reaction, employs an unstable oxidant	[63]
20	Potassium ferricyanide	Measurement of Prussian blue	735	0.04–8 (3.92×10^4)	—	[64]
21	3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine	Measurement of redox complex	558	0.62–6.15	Multistep reaction, requires an expensive reagent	[65]
22	(a)* FC reagent	Measurement of molybdenum-tungsten mixed acid blue absorbance in sodium carbonate medium	760	0.5–10 (1.12×10^4)	Simple, rapid, sensitive, uses aqueous medium and extraction free, no use of organic solvent, based on a single step reaction	Present work
	(b) Ferric chloride and potassium ferricyanide	Measurement of absorbance of Prussian blue in acid medium	760	0.2–3.0 (4.55×10^4)		

* NBS-N-bromosuccinimide, KMnO₄-potassium permanganate, FC reagent-Folin-Ciocalteu reagent.

of the results to 100% showed the fairly good accuracy of the method. The results are shown in Table 5.

9. Conclusion

Two simple, rapid, selective, and sensitive methods have been proposed for the assay of INH in bulk drugs and in tablets. The methods are based on the well-characterized and established redox and complexation reactions and use very common and inexpensive chemicals and easily accessible instrument. The procedures described here are easily carried out and much simpler than the reported methods for INH (Table 6) but have been demonstrated to be more sensitive in terms of linear dynamic range and sensitivity parameters. The methods were applied successfully to the assay of INH in tablets without interferences from the common excipients. The proposed methods are suitable for isoniazid determination in bulk drug and pharmaceuticals; hence these methods can be used in quality control laboratories.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors wish to thank the quality control manager, Cipla Ltd., Bangalore, India, for gifting pure isoniazid and the authorities of the University of Mysore, Mysore, for permission and facilities.

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