

Research Article

Novel Reagents for the Spectrophotometric Determination of Isoniazid

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Received 11 April 2012; Accepted 10 May 2012

Academic Editors: A. A. Ensafi, D. V. Konarev, and N. Zanatta

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Isoniazid is an antitubercular drug, widely used for tuberculosis. Owing to its importance in therapeutics, the present study aims to develop simple method for the spectrophotometric determination of isoniazid (INH). Two novel reagents, epichlorohydrine (ECH) and 4-hydroxyphenacylchloride (HPC) are used for the spectrophotometric determination of INH. Based on the nucleophilic substitution reactions of INH with EPI & HPC in basic medium, rapid, simple, inexpensive, precise, and accurate visible spectrophotometric method is proposed for the determination of INH in bulk drug and in formulations. Method involves the reaction of INH with EPI and HPC in basic medium to form yellow-colored chromogen, measuring the absorbances at 405 and 402 nm for INH-EPI & INH-HPC, respectively. The optimum experimental conditions have been studied. The absorbance was found to increase linearly with the concentration of the drug and formed the basis for quantification. The calibration graphs were linear from 2.00–22.00 $\mu\text{g mL}^{-1}$ and 20.00–120.00 $\mu\text{g mL}^{-1}$ for INH-EPI & INH-HPC, respectively. The apparent molar absorptivity and Sandell's sensitivity are calculated to be 0.51×10^4 & $0.10 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ and 0.027 & 0.134 $\mu\text{g cm}^{-2}$ for INH-EPI & INH-HPC, respectively. The procedure is used to determine INH in pharmaceutical products. The associated pharmaceutical materials do not interfere in the measurements.

1. Introduction

The enhanced prevalence of infectious diseases threatens world population. Tuberculosis (TB) is characterized as a chronic bacterial infection caused by a germ called *Mycobacterium tuberculosis*. TB is contagious and spreads through the air when a person with TB of the lungs or throat coughs, sneezes, or talks. Worldwide statistics on tuberculosis surprisingly reveals that, one-third of the world's population, over 2 billion people, carry the bacillus that causes TB and 2 million people die of the disease each year. Tuberculosis is on the increase in recent years, largely owing to HIV infection, immigration, increased trade, and globalization [1].

Among the many drugs discovered for the treatment of TB, isoniazid (INH) is one of the powerful drug candidates. The discovery of INH was based on the nicotinamide activity against tubercle bacilli in the animal model observed by Chorine in 1945 [2] and the reshuffling of chemical groups in the thiosemicarbazone [3, 4]. INH represented a major

milestone in the chemotherapy of TB because it is highly active, inexpensive, and without significant side effects [5, 6]. INH keeps on to be the cornerstone of all antituberculosis regimens and remains the only agent recommended for tuberculosis chemoprophylaxis in children [7, 8].

INH is still designated as an essential antituberculosis agent by the World Health Organization (WHO), and is now largely used together with rifampicin and streptomycin for the chemotherapy of TB. This has prompted many investigators to plan methods for the rapid determination of INH in its pure form as well as in pharmaceutical preparations. There are various analytical procedures for the assay of INH, the most important being titrimetry [9, 10], visible spectrophotometry [11–13], polarography [14], coulometry [15], high-performance liquid chromatography [16], and fluorimetry [17] methods. The spectrophotometric methods involve the use of reagents such as chloranil [18], 4-nitrobenzaldehyde, pyridoxal [19], 4-dimethylaminobenzaldehyde [20], and so forth. In the

present work two methods have been developed for the determination of INH using two novel reagents. The methods entail the nucleophilic substitution reactions of INH with epichlorohydrine (EPI) and 4-hydroxyphenacylchloride (HPC).

2. Experimental

2.1. Apparatus. A UV-Visible spectrophotometer (SHIMADZU, Model no.: UV-2550) with 1 cm matched quartz cells was used for the absorbance measurements.

2.2. Reagents and Solutions. All the reagents used were of analytical reagent grade. The solutions of EPI in ethanol (10%), HPC in ethanol (0.2%), and NaOH (1 M) were prepared. A $1000 \mu\text{g mL}^{-1}$ of INH solution was prepared using ethanol.

2.3. Procedures

2.3.1. Using EPI as Reagent. Aliquots containing $2.00\text{--}22.00 \mu\text{g mL}^{-1}$ of INH were transferred into series of 10 mL calibrated flasks. To this, 1 mL of EPI (10%) was added followed by 1 mL of NaOH (0.25 M) and heated for 5 min. The reaction mixture was cooled and made up to 10 mL with distilled water. The absorbance of each was measured at 405 nm.

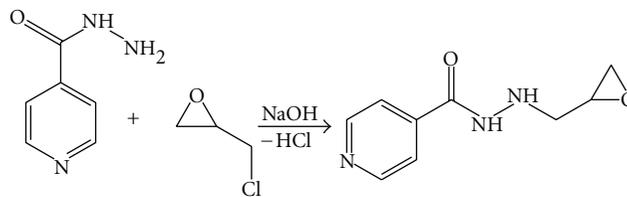
2.3.2. Using HPC as Reagent. Aliquots containing $20.00\text{--}120.00 \mu\text{g mL}^{-1}$ of INH were transferred into series of 10 mL calibrated flasks. To this, 1 mL of HPC (0.2%) was added followed by 0.5 mL of NaOH (1 M) and heated for 5 min. The reaction mixture was cooled and made up to 10 mL with distilled water. The absorbance of each was measured at 402 nm.

2.3.3. Assay of Formulations. The proposed method has been applied successfully for the determination of INH in some pharmaceutical formulations. Commercial INH tablets (Solonex and Isokin) were analyzed using the developed method. To minimize a possible variation in the composition of the tablets, the mixed contents of 20 tablets were weighed and ground, then the powder equivalent to 300 mg INH was dissolved in water by stirring for 10 min and filtered through Whatman No. 41 filter paper. Solutions of working concentration were prepared by proper dilution of this stock solution with water and followed the above procedures for the analysis.

3. Results and Discussion

3.1. Absorption Spectra and Optimization of Reagent Concentrations

3.1.1. Using EPI as Reagent. The proposed method is based on the nucleophilic substitution reaction of EPI in presence of NaOH to form yellow-colored chromogen (Scheme 1), the absorbances of which can be measured at 405 nm (Figure 1).



SCHEME 1: Reaction of INH with ECH.

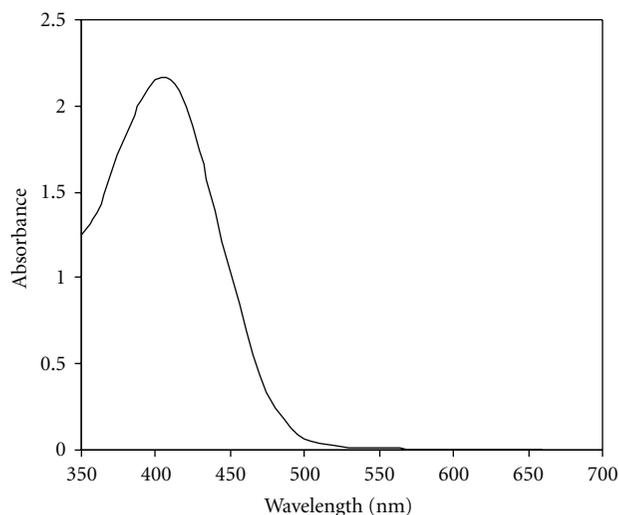


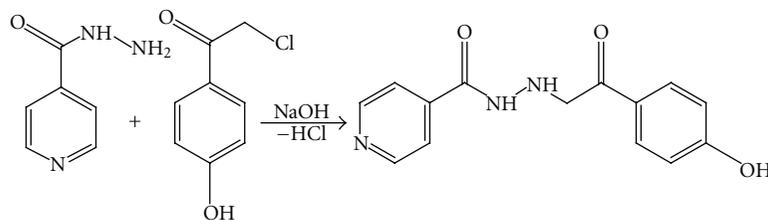
FIGURE 1: Absorption spectrum of INH-EPI system.

Conditions for the assay procedures have been established by studying the reactions as a function of heating time, concentration of reagents, solution stability, and the stability of the colored species.

Preliminary experiments are carried out to fix the initial concentration of the reagents. The influence of the volume of 0.25 M NaOH on the formation of yellow color is studied. This is performed by keeping other parameters constant and taking different volumes of (0.1–5.0 mL) of 0.25 M NaOH. The maximum absorbance is obtained with 1 mL of NaOH. Above this volume absorbance remains constant. Therefore, this volume is used for all the absorbance measurements. To investigate the optimum heating time for color development, the content of the mixture is heated on water bath at 60°C for 5–10 min. The maximum intensity of color is obtained at 5 min of heating at 60°C and remains constant. To study the effect of concentration of EPI, different volumes of 10% ECH are tested. It is found that 1 mL of EPI is sufficient for very good color intensity.

3.1.2. Using HPC as Reagent. The method is based on the nucleophilic substitution reaction of HPC with isoniazid in presence of NaOH (Scheme 2). The absorbance of the product formed is measured at 402 nm (Figure 2).

Preliminary experiments were carried out to fix the initial concentration of the reagents. The influence of the volume of 1 M NaOH on the formation of yellow color is studied. This is performed by keeping other parameters constant



SCHEME 2: Reaction of INH with HPC.

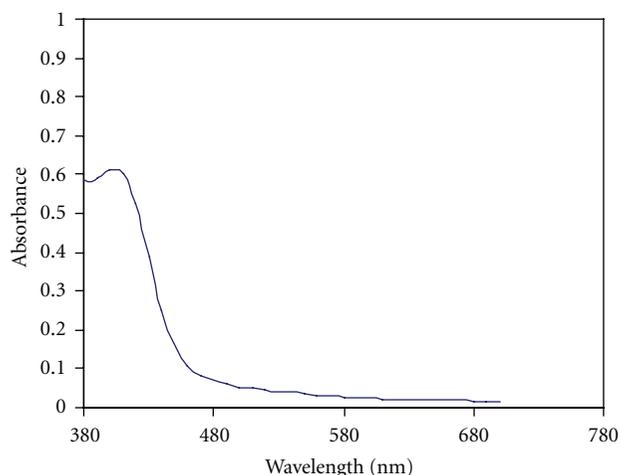


FIGURE 2: Absorption spectrum of INH-HPC system.

and different volumes of (0.1–5.0 mL) of 1 M NaOH. The maximum absorbance is obtained with 1 mL of NaOH. Above this volume absorbance remains constant. Therefore this volume is used for all the absorbance measurements. To investigate the optimum heating time for color development, the content of the mixture is heated on a water bath at 60°C for 5–30 min. The maximum intensity of color is obtained at 15 min of heating and remains constant. To study the effect of concentration of HPC, different volumes of 0.2% HPC are tested. It is found that 1 mL of HPC is sufficient for very good color intensity.

3.2. Analytical Data. The linearity between two parameters is apparent from the correlation coefficient obtained by the method of least squares. The optical characteristics such as absorption λ_{\max} , Beer's law limits, molar absorptivity, Sandell's sensitivity, detection limit and quantification limit, are calculated. The regression analysis of the Beer's law plots at their respective λ_{\max} values revealed a good correlation. Graphs of absorbance v/s concentration show negligible intercept and are described by the regression equation $Y = a + bX$. Where Y is the absorbance, b is the slope, a is the intercept, and X is the concentration of the drug in $\mu\text{g mL}^{-1}$ obtained by the least-squares method (Table 1).

3.3. Method Validation. Validation of an analytical procedure is the process by which it is ascertained, by laboratory studies, that the performance characteristics of the procedure

TABLE 1: Analytical parameters.

	EPI	HPC
λ_{\max} nm	405	402
Beer's law limit ($\mu\text{g mL}^{-1}$)	2.00–22.00	20.00–120.00
Molar absorptivity ($\text{L mol}^{-1} \text{cm}^{-1}$)	0.51×10^4	0.10×10^4
Sandell's sensitivity ($\mu\text{g cm}^{-2}$)	0.027	0.134
Regression equation*	$Y = a + bX$	$Y = a + bX$
Slope (b)	0.0336	0.0064
Intercept (a)	0.022	0.018
Correlation coefficient (r)	0.9910	0.9979
Limit of Detection** ($\mu\text{g mL}^{-1}$)	1.500	5.150
Limit of Quantitation** ($\mu\text{g mL}^{-1}$)	4.545	15.620

* Y is the absorbance and X is the concentration in $\mu\text{g mL}^{-1}$.

**Calculated using ICH guidelines.

meet the conditions for its proposed use. All analytical methods planned to be used for analyzing any experimental samples will need to be authenticated. The accuracy of the method was established by analyzing the pure drugs at diverse levels within working limits and the precision is ascertained by calculating the relative standard deviation of replicate determinations on the same solution containing the drugs at different levels and are presented in Tables 2(a) and 2(b). The relative error and relative standard deviation indicate the high accuracy and precision for the method. In order to check the validity of the proposed method, INH is determined in some commercial formulations. From the results it is clear that there is close agreement between the results obtained by the proposed method and the label claim.

3.4. Interference Study. The specificity of an analytical method may be defined as the ability to clearly determine the analyte in the presence of additional components such as impurities, degradation products, and matrix. The specificity in the current case is evaluated by preparing the analytical placebo and it is confirmed that the change in absorbance with respect to the reagent blank is caused only by the analyte. A solution of the analytical placebo (containing all the tablet excipients except INH) is prepared according to the sample preparation procedure and subjected to analysis using the procedures described earlier. The absorbance measured is nearly the same as that of the reagent blank. To identify the interference by these excipients, a synthetic mixture of inactive ingredients (placebo) including INH with the following composition: INH (10 mg), talc (20 mg), starch

TABLE 2: (a) Evaluation of accuracy and precision (using ECH as reagent). (b) Evaluation of accuracy and precision (using HPC as reagent).

(a)

Amount taken ($\mu\text{g mL}^{-1}$)	Amount found* ($\mu\text{g mL}^{-1}$)	RE (%)	SD ($\mu\text{g mL}^{-1}$)	RSD (%)
2.00	1.98	1.00	0.015	0.79
4.00	3.97	0.75	0.0207	0.52
6.00	5.96	0.66	0.0239	0.4
8.00	7.97	0.37	0.0259	0.32
10.00	9.96	0.4	0.0270	0.27
12.00	11.95	0.41	0.0404	0.33

*Mean of five determinations.

(b)

Amount taken ($\mu\text{g mL}^{-1}$)	Amount found* ($\mu\text{g mL}^{-1}$)	RE (%)	SD ($\mu\text{g mL}^{-1}$)	RSD (%)
20.00	19.99	0.05	0.09	0.45
40.00	40.03	-0.08	0.06	0.14
60.00	59.96	0.06	0.12	0.20
80.00	79.93	0.08	0.08	0.10

*Mean of five determinations.

RE: relative error; SD: standard deviation; RSD: relative standard deviation.

(40 mg), glucose (50 mg), and lactose (40 mg) is prepared. The entire mixture is transferred into a 100 mL calibrated flask, the content shaken for 20 min, volume diluted to the mark with distilled water, mixed well, and filtered. The filtrate after suitable dilution is analyzed by proposed methods. The difference between the measured absorbance of the above extract and that of a standard INH solution of the same concentration is less than 2% indicating the absence of interference by the excipients.

3.5. Applications. The proposed method has been applied to the determination of INH in pure and dosage form. The results are compared statistically with those of the tabulated value at 95% confidence level. The calculated student's *t*-test (Table 3) does not exceed the tabulated value, indicating that there is no significant difference between the proposed method and the tabulated value. The described method has been extensively validated in terms of specificity, linearity, accuracy and precision, and system suitability.

4. Conclusions

The new approach of utilizing epichlorohydrine and 4-hydroxyphenyl chloride as reagents in spectrophotometry is the first of such reports. The method makes use of very easily available and cheaper reagents which demonstrates its cost-effectiveness. Compared to many existing instrumental methods for INH, the proposed spectrophotometric method has two additional advantages of simplicity of operations and low-cost instrument. These advantageous features advocate its use in quality control laboratories for routine use.

TABLE 3: Results of assay of formulations.

Brand name	^a Isokin	^b Solonex
Labeled amount (mg)	300	300
(1) Using ECH		
(i) Amount found* (mg)	299.5	299.0
(ii) % Label claim \pm SD	99.83 \pm 0.010	99.66 \pm 0.018
(iii) <i>t</i> -test	2.23	2.48
(2) Using HPC		
(i) Amount found* (mg)	299.70	299.81
(ii) % Label claim \pm SD	99.90 \pm 0.08	99.93 \pm 0.06
(iii) <i>t</i> -test	1.67	1.62

*Mean value of five determinations.

^aPfizer Ltd., India.^bMacleods Pharmaceuticals Ltd.Calculated Student's *t*-value at 95% confidence level is 2.77.

Acknowledgments

B. Narayana thanks the UGC for financial assistance through SAP and BSR one-time grant for the purchase of chemicals. Divya N. Shetty thanks the UGC-RFSMS scheme (under SAP-Phase1) for providing financial help for the research work.

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