Asian Journal of Chemistry; Vol. 25, No. 5 (2013), 2522-2526

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.13444

# Spectrophotometric Determination of Isoniazid from Pharmaceutical Preparations Using Natural Aldehyde

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(*Received*: 14 January 2012; *Accepted*: 12 November 2012) AJC-12393

A spectrophotometric method has been developed for the micro determination of isoniazid from various pharmaceutical dosage forms. Method is based on a condensation reaction between isoniazid and ethanolic solution of *cis*-cinnamaldehyde to generate an instant yellow coloured schiff's base derivative of isoniazid. The coloured product formed absorbed in visible region and optimal detector response was obtained at a wavelength of 364 nm with a molar absorptivity of $3.1 \times 10^4$ L/mol/cm. The analytical parameters and their effects on the reported systems are investigated. A linear relationship was obtained between the absorbance and the concentration of the derivative which obeyed the Beer's law within 0.5-2.5 µg/mL. The low % relative standard deviation (0.2-1.5) values indicate good precision, high recovery values and accuracy of proposed method.

**Key Words: Isoniazid, *cis*-Cinnamaldehyde, Spectrophotometry.**

## INTRODUCTION

Isoniazid known as isoncotinyl hydrazide (INH), is first line antitubercular agent widely used together with rifampicin and streptomycin[1,2]. Different analytical methods have been reported for the analysis of isoniazid, based on spectrophotometric[3-8], voltametric[9,10], mass spectrometric[11-14], flourimetric[15], capillary electrophoretic[16-20], infra-red spectrophotometric[21], thin layer chromatographic[22] and high performance liquid chromatographic[23-25] techniques. For spectrophotometric analysis, the determination is carried out using either the natural absorbance of isoniazid at 262 nm or it is derivatized with suitable reagents such as vanillin1, copper(II)[4], ethyl 8-quinolinoxy acetate[5], 6,7-dichloroquinoline-5,8-dione[6], 2,3-dichloro-1,4-naphthoquinone[7], *trans*-cinnamaldehyde[8], cerium (IV)[15] and 5-methylfuran-2-carboxaldehyde[24]. The derivatization increases the molar absorptivity of the drug with bathochromic shift.

Eidus and Harnanansingh[8] used *trans*-cinnamaldehyde as derivatizing reagent and measured maximum absorbance at 340 nm but in present method *cis*-cinnamaldehyde is used as a reagent with measurement of absorbance at 364 nm at pH 9.5. The developed method is applied for the determination of isoniazid from pharmaceutical preparations. The method does not require solvent extraction.

Proposed method is based on the condensation reaction between isoniazid and ethanolic solution of *cis*-cinnamaldehyde in boric acid medium pH 9.5 to form a yellow coloured Schiff's base having absorption maximum at 364 nm. The proposed method is free from the interference of the excipients normally found along with isoniazid in various pharmaceutical dosage forms and excess of derivatizing reagent. Further, the method is found to possess adequate accuracy and precision.

## EXPERIMENTAL

All the reagents and chemicals of analytical or pharmaceutical grades were used. The double distilled water used throughout the study was obtained from distillation plant all made of glass. Pure isoniazid, *cis*-cinnamaldehyde, (CIN) and acetic acid from E. Merck, (Germany), sodium acetate and ethanol from Fluka, (Switzerland) were used. Buffer solutions between pH 1-10 at unit interval were prepared from 0.1 M of hydrochloric acid, potassium chloride, acetic acid, sodium acetate, sodium bicarbonate, sodium carbonate, boric acid, sodium hydroxide, ammonium chloride and ammonia solution. The solution of *cis*-cinnamaldehyde (2 % v/v) was prepared in ethanol. The spectrophotometric studies were carried out with a double beam spectrophotometer (UV/visible spectrometer Lambda 25, Perkin-Elmer, USA) with dual silica 1 cm cuvettes. The spectrophotometer was controlled by the

computer with UV WinLab Lambda 25 software. pH meter (Schot Instrument Lab 850, Germany) with glass electrode and internal reference was used.

**Analytical procedure with derivatization:** The aqueous solution (0.5-2.5 mL) containing isoniazid (2.5-12.5 µg) was transferred to a series of 5 mL calibrated well stoppered volumetric flasks and were added 0.6 mL *cis*-cinnamaldehyde (ethanolic 2 %), followed by borate-sodium hydroxide buffer pH 9.5 (0.5 mL). The contents were heated on water bath at 95 ± 5 ºC for 10 min. The solutions were cooled at room temperature and the volumes were adjusted to mark with ethanol. The absorbance was measured at 364 nm against reagent blank which was prepared in a similar way only omitting the addition of isoniazid.

**Procedure without derivatization:** The aqueous isoniazid solution (0.2-1.0 mL) containing isoniazid (20-100 µg) was transferred to 5 mL calibrated well stoppered volumetric flasks and the volumes were adjusted to mark with water. The absorbance was measured at 262 nm against water and the molar absorptivity was calculated $3.9 \times 10^3$ L/mol/cm.

**Analysis of isoniazid from pharmaceutical preparations:** Twenty samples of different pharmaceutical companies were collected and were subjected for the analysis of isoniazid. The powdered sample (0.1 g) from each of the following preparation Rifazol Junior, Rifa-4 (Schazoo Zaka Lahore, Pakistan), Isozide (Nabiqasim Industries (Pvt.) Ltd., Karachi, Pakistan), Polyzide (Polyfine Chem Pharma, (Pvt.) Ltd, Peshawar, Pakistan), Isoniazid, Rifa-plus (Unexo Labs (Pvt.) Ltd., Lahore, Pakistan), Isoniazide (Jawa Pharmaceuticals (PVT) Ltd. Lahore, Pakistan), Rifinah (Pacific Pharmaceuticals Ltd. Lahore, Pakistan), Myrin-P, Myambutol (Wyeth Pakistan Ltd., Karachi.), Rimatol (Dosaco Laboratories Lahore, Pakistan), Acoret (Efroze Chemical Industries (Pvt.) Ltd. Karachi, Pakistan), Afrazid (Consolidated Chemical Laboratories (Pvt) Ltd. Lahore, Pakistan), Cyrex (Rex Pharmaceuticals Pakistan), Fairzide (Ferro Pharmaceutical Laboratories, Pakistan), isoniazid, Rifazid Forte (Geofman Pharmaceuticals, Karachi, Pakistan), I.N.H. (P.D.H. Pharmaceuticals (Pvt) Ltd. Lahore, Pakistan), Isoniazid (Lahore Chemical & Pharmaceutical Works (Pvt.) Ltd., Lahore, Pakistan), Isoniazid (Genera Pharmaceuticals, Islamabad, Pakistan), Rifampicin (Zafa Pharmaceutical Laboratories (Pvt.) Ltd. Karachi, Pakistan) preparation was dissolved/diluted separately in distilled water and filtered through Whatman No. 1 filter paper, volumes were adjusted to 100 mL with water. The solution (0.5 mL) from each of the 100 mL solutions of all brands was further transferred to separate 100 mL calibrated well stoppered flasks and the volumes were made up to the mark with distilled water (0.0005 %). Finally 02 mL from each of the solution of each preparation was taken in separate 5 mL volumetric flask and the procedure was repeated as described in analytical procedure. The amount of isoniazid from each of the sample was calculated using the external calibration curve (Tables 1-4).

TABLE-1
ANALYSIS OF INH FROM SACHET
(AMOUNT LABELED 30 mg/SACHET)

| Drug | Amount found (mg)/Sachet (RSD %) | ± Relative deviation (%) from labeled values | Recovery (%) by standard addition technique |
|---|---|---|---|
| Rifazol Junior | 29.7 (0.7) | 01 | 99.6 |

TABLE-2
ANALYSIS OF INH FROM SYRUPS
(AMOUNT LABELED 50 mg/5 mL)

| Drug | Amount found (mg/5 mL) (RSD %) | ± Relative deviation (%) from labeled values | Recovery (%) by standard addition technique |
|---|---|---|---|
| Isozide | 50.3 (0.9) | 0.5 | 98.9 |
| Polyzide | 50.0 (0.4) | 0.0 | 99.0 |

TABLE-3
ANALYSIS OF INH FROM TABLETS

| Drug | Labeled amount (mg)/tablet | Amount found (mg)/tablet (RSD %) | ± Relative deviation (%) from labeled values | Recovery (%) by standard addition technique |
|---|---|---|---|---|
| Isoniazid | 50 | 49.3(1.5) | 1.4 | 99.8 |
| Isoniazide | 50 | 49.6(1.1) | 0.8 | 98.3 |
| Rifinah | 50 | 48.8(1.4) | 2.4 | 97.9 |
| Myrin-P | 60 | 58.9(0.4) | 1.8 | 98.2 |
| Rifa 4 | 60 | 59.8(0.2) | 0.3 | 99.0 |
| Rimatol | 60 | 59.2(0.9) | 1.4 | 99.5 |
| Acoret | 75 | 74.0(1.0) | 1.3 | 100 |
| Afrazid | 75 | 74.9(0.3) | 0.13 | 99.7 |
| Cyrex | 75 | 75.0(0.5) | 00 | 98.6 |
| Fairzide | 100 | 98.9(0.6) | 1.8 | 97.9 |
| Isoniazid | 100 | 100(1.1) | 00 | 97.4 |
| INH | 100 | 99.9(0.6) | 0.01 | 99.0 |
| Isoniazid | 100 | 99.8(0.3) | 0.2 | 99.8 |
| Isoniazid | 100 | 99.1(0.7) | 0.4 | 95.9 |
| Myambutol | 100 | 98.0(1.6) | 2 | 99.8 |
| Rifa+Plus | 100 | 99.5(1.4) | 0.5 | 99.6 |

## RESULTS AND DISCUSSION

Isoniazid (INH) or isonicotinyl hydrazide reacts with *cis*-cinnamaldehyde (CIN) to form an imine INH-CIN derivative (Fig. 1). The derivative absorbs at 364 nm maximally with bathochromic shift having molar absorptivity of $3.1 \times 10^4$ L/mol/cm. The free CIN was then examined as a derivatizing reagent for the spectrophotometric determination of isoniazid. The amount of CIN added, effects of pH, heating time and temperature and the stability of the (INH-CIN) derivative were studied carefully.

### Optimization of parameters

**Analytical wavelength:** For the quantitative analysis, the wavelength of maximum absorbance plays an important role. It is compulsory to ensure that pure analyte and derivatizing

TABLE-4
ANALYSIS OF INH FROM CAPSULES

| Drug | Labeled amount (mg)/capsule | Amount found (mg)/capsule (RSD %) | ± Relative deviation (%) from labeled values | Recovery (%) by standard addition technique |
|---|---|---|---|---|
| Rifampicin + INH | 150 | 149(1.0) | 0.6 | 99.7 |
| Rifazid forte | 150 | 149.8(0.2) | 0.13 | 98.6 |

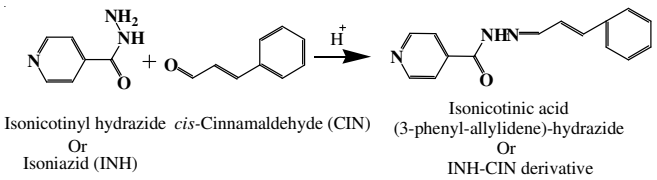


Fig. 1. Formation of isoniazid derivative with *cis*-cinnamaldehyde

reagent should not absorb near to the region where the analyte derivative absorbs. This may cause inaccuracy in absorption of the drug because of the derivatization. The excess volume of derivatizing reagent is added to complete the reaction quantitatively. To stay away from this hinder, it is compulsory to select the wavelength where the analyte derivative shows maximum absorbance and the derivatizing reagent indicates minimum absorbance. The maximum absorbance value of 20-100 µg/mL of pure isoniazid without derivatization and 0.5-2.5 µg/mL of INH-CIN derivative was recorded at different wavelengths between 550-200 nm after heating for 10 min at 95 ºC using borate-sodium hydroxide buffer pH 9.5. It was noted that the maximum absorbance occurs at 364 nm against reagent blank. Therefore, the wavelength of 364 nm was selected as optimum wavelength ($\lambda_{max}$) for INH-CIN derivative while 262 nm was observed for pure isoniazid (Fig. 2).

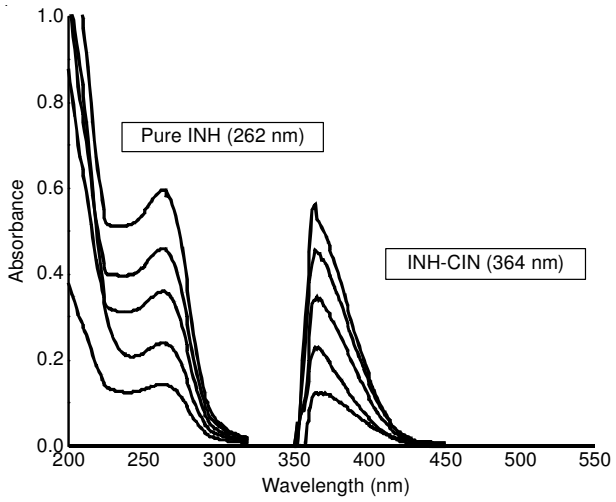


Fig. 2. Optimization of analytical wavelength

**Effect of reagent concentration:** The effects of adding various amounts of CIN solution on absorbance of 02 µg/mL INH-CIN derivative is given in Fig. 3. The volume of 2 % ethanolic CIN was varied between 0.2-1.2 mL with an interval of 0.2 mL and the absorbance was measured at $\lambda_{max}$ 364 nm. Primarily the absorbance was improved slowly and then the similar absorbance was observed with addition of 0.6 mL and above, it was therefore the addition of 0.6 mL (2 % v/v in ethanol) CIN solution was selected.

**Effect of order of mixing the reagents:** The order of adding the reagent during derivatization process has important part in precision of results and enhancement of absorbance. In this study, it was observed that the addition of 2.5 mL isoniazid solution to buffer pH 9.5 (0.5 mL) followed by 0.6 mL reagent (CIN) resulted in a decrease in absorbance value. Taking the buffer pH 9.5 first and then adding the reagent CIN, followed by isoniazid solution also gave lower absorbance

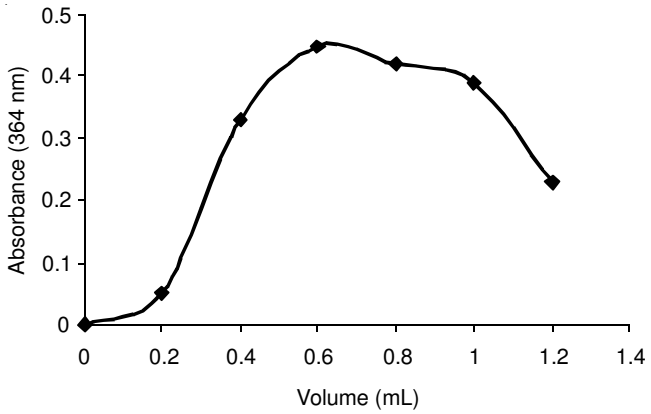


Fig. 3. Effect of volume of reagent CIN 2 % ethanolic on absorbance of isoniazid derivative

value. It was observed that the maximum absorbance value accomplished when 0.6 mL of reagent CIN was added to the standard solution of isoniazid followed by buffer (0.5 mL) pH 9.5. The contents were then heated on water bath and the volume was adjusted to the mark with ethanol.

**Optimization of heating time and temperature for the formation of derivative:** To obtain the maximum absorbance value for derivative, the selection of optimum time and temperature for the formation of unwavering derivative are essential factors. The effect of time and temperature on absorbance of 02 µg/mL isoniazid solution in the presence of 2 % CIN solution was checked at 364 nm from 0-30 min with an interval of 5 min at 95 ± 5 ºC. A similar absorbance was observed after heating for 10 min, so, heating for 10 min at 95 ± 5 ºC was considered as optimal heating time and temperature for derivatization.

**Effect of solvents:** The effect of various solvents such as methanol, 1-propanol, 1-butanol, 2-propanol, amyl alcohol, isoamyl alcohol, acetonitrile and ethyl acetate on the absorbance of 02 µg/mL INH-CIN derivative was examined. Each of the solvent 0.5 and 01 mL was added after the addition of 2 % ethanolic solution of CIN and 0.5 mL borate buffer pH 9.5 followed by heating for 10 min. The ethanol proved to be the best choice in solvents.

**Effect of pH:** The effect of adding 0.5 mL of 0.1 M buffers of pH range 1-10 on the absorbance of 2.5 µg/mL INH-CIN derivative solution at already maximized conditions was studied. It was observed that, with; buffer HCl-KCl pH 1-3 the absorbance was poor, acetate buffer pH 4-7 has very low absorbance, NaOH-NaCl buffer pH 8-10 decreased absorbance and $NaCO_3$-$NaHCO_3$ pH 8-10 shown low absorbance and pH 11 produced turbidity. The absorbance increased gradually with borate buffer pH 7 and it was maximum at pH 9.5 (Fig. 4). Addition of buffer above pH 9.5 decreased absorbance. Therefore, the borate buffer of pH 9.5 was selected as optimal.

**Interference study:** The effect of possible presence of water soluble associated materials such as acacia, ethambutol, fructose, glucose, galactose, mannitol, lactose, pyrizinamide, rifampicin, sorbitol, sucrose, starch, sodium chloride and talc was investigated at double and 10 times the concentration of isoniazid and it was observed that none of these substances interfered by showing any change in absorbance more than ± 2 % (Table-5).

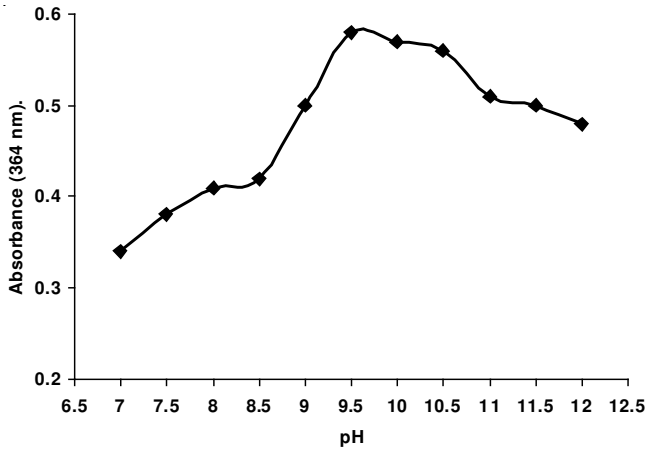


Fig. 4. Effect of pH on derivatization of isoniazid

TABLE-5
EFFECT OF DIFFERENT POSSIBLE EXCIPIENTS ON THE ABSORBANCE OF 02 µg/mL INH-CIN DERIVATIVE

| Excipient added (25 µg/mL) | Absorbance at (460 nm) | Relative error (%) | Recovery of drug (%) ± RSD % (n = 3) |
|---|---|---|---|
| – | 0.451 | – | – |
| Acacia | 0.441 | -2.2 | 98.9 ± 0.27 |
| Ethambutol | 0.451 | 00 | 99.9 ± 0.34 |
| Fructose | 0.449 | -4.4 | 99.3 ± 0.32 |
| Glucose | 0.440 | -2.4 | 98.3 ± 0.22 |
| Galactose | 0.448 | -0.6 | 99.0 ± 0.35 |
| Mannitol | 0.441 | -2.2 | 99.4 ± 0.28 |
| Lactose | 0.450 | -0.2 | 99.7 ± 0.35 |
| Pyrizinamide | 0.451 | 00 | 100.2 ± 0.30 |
| Rifampicin | 0.451 | 00 | 100.4 ± 0.25 |
| Sorbitol | 0.450 | -0.20 | 99.6 ± 0.34 |
| Sucrose | 0.447 | -0.88 | 99.3 ± 0.28 |
| Starch | 0.452 | 0.22 | 99.5 ± 0.35 |
| Sodium chloride | 0.456 | 1.10 | 99.6 ± 0.30 |
| Talc | 0.443 | -1.70 | 99.7 ± 0.35 |

**Calibration plot:** The effect of variation in the concentration of isoniazid on its absorbance as derivative INH-CIN was studied. A linear calibration curve was obtained which obeyed the Beer's law within the concentration range 0.5-2.5 µg/mL of isoniazid with coefficient of determination $r^2$ 0.9992 (Fig. 5).

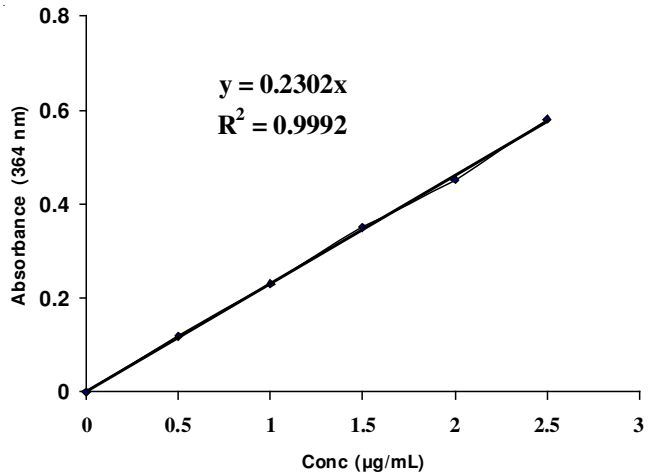


Fig. 5. Calibration curve of isoniazid using *cis*-cinnamaldehyde as derivatizing reagent

**Analytical data:** The optical characteristics such as Beer's law limits, Sandell's sensitivity, relative standard deviation, range of error at 95 % confidence limit, molar absorptivity, limit of detection (LOD) and limit of quantification (LOQ) were calculated and the results are summarized in Table-6. LOD and LOQ were calculated by equations LOD = δ3.3/s and LOQ = δ10/s, respectively, where δ is the standard deviation of blank and s is slope of calibration. Regression analysis of the Beer's law plot at their $\lambda_{max}$ 364 nm exposed a good correlation. The mean values mg/ sample at range of error % at 90 % confidence limit of 30 mg/sachet, 50 mg/5 mL syrup, 60, 75 and 100 mg tablets and 150 mg isoniazid capsules were 29.9 ± 0.2107, 50.15 ± 2.9, 49.2 ± 2.1, 59.3 ± 0.85, 74.6 ± 1.0, 99.3 ± 0.75 and 149 ± 2.7, respectively.

**Stability of the derivative:** The stability of INH-CIN derivative was examined in terms of absorbance at the concentration of 02 µg/mL INH-CIN derivative, but no change in absorbance of more than 3 % was observed within 48 h.

**Recovery (%) of isoniazid from samples by standard addition technique:** The reliability and validity of the proposed method was evaluated by standard addition technique. Isoniazid standard (0.0005 g) was dissolved in 100 mL double distilled water. Two portions, each consisting 2 mL were taken in two different volumetric flasks (5 mL). One was added with 0.5 mL of sample solution containing 5 µg/mL of isoniazid and the derivatization procedure was repeated for both solutions as described in analytical procedure. The % recoveries were calculated using Recovery (%) = $[(C_t - C_s)/C_a] \times 100$ from the increase in the absorbance with added standard (Tables 2-5). Here $C_t$ is the total drug concentration measured after standard addition; $C_s$, drug concentration in the formulation sample; $C_a$, drug concentration added to formulation.

**Day to day reproducibility/repeatability:** For the determination of intra and interday reproducibility of the method, aqueous standard solution (0.5 mL) of 5 µg/mL isoniazid was taken in three different calibrated volumetric flasks (5 mL) and the procedure was followed for each solution as described in analytical procedure. The absorbance was measured against reagent blank at 364 nm. The above procedure was repeated for three days (n = 3). The mean absorbances of intraday and interday reproducibilities were observed as 0.13 and 0.12 with (RSD %) values 0.43 and 0.21, respectively.

**Application of the proposed method:** The proposed method was applied to the determination of isoniazid in commercially available single and multiple ingredient capsules, sachet, syrups and tablets. The same samples were analyzed, simultaneously by thee method without derivatization.

## Conclusion

Simple, economical, selective and sensitive spectrophotometric method was developed and validated. The proposed method does not require expensive and hazardous reagents and sophisticated instruments. The statistical parameters and recovery study data indicate the reproducibility and accuracy of the method. Analysis of the pharmaceutical preparations containing isoniazid as single drug as well as multiple ingredients showed no interference from the common excipients. So, this method could be considered for the determination of isoniazid in pharmaceutical quality control laboratories.

TABLE-6
RESULTS OF OPTIMIZATION, PRECISION AND ACCURACY

| Parameter (s) | Selected values |
|---|---|
| Wave length $\lambda_{max}$ of INH-CIN derivative | 364 nm |
| Beer's law limits (µg/mL) of INH-CIN derivative | 0.5-2.5 |
| Wave length $\lambda_{max}$ of INH | 262 nm |
| Beer's law limits (µg/mL) of INH | 4-20 |
| Molar absorptivity (L/mol/cm) of INH-CIN derivative | $3.1 \times 10^4$ |
| Molar absorptivity (L/mol/cm) of INH | $3.9 \times 10^3$ |
| Sandell's sensitivity of INH-CIN (Conc. µg/mL at 0.005 absorbance unit) | 0.08 |
| Regression equation (y) a | |
| Slope (b) | 0.2302 |
| Intercept (a) | 0 |
| Coefficient of determination ($r^2$) | 0.9992 |
| Relative Standard deviation (%) | 1.6-0.2 |
| Relative deviation (± %) | 2.4-0 |
| LOD (µg/mL) | 0.1 |
| LOQ(µg/mL) | 0.4 |
| Mean value mg/5 mL of syrup ± range of error % at (95 % confidence limit) of two syrups (50 mg/5 mL) of various pharmaceutical companies | 50.15 ± 1.1 |
| Mean value mg/tablet±range of error % at (95 % confidence limit) of three tablets (50 mg) of various pharmaceutical companies | 49.2 ± 1.8 |
| Mean value mg/tablet±range of error % at (95 % confidence limit) of three tablets (60 mg) of various pharmaceutical companies | 59.3 ± 0.85 |
| Mean value mg/tablet±range of error % at (95 % confidence limit) of three tablets (75 mg) of various pharmaceutical companies | 74.6 ± 1.0 |
| Mean value mg/tablet ± range of error % at (95 % confidence limit) of seven tablets (100 mg) of various pharmaceutical companies | 99.3 ± 0.75 |
| Mean value mg/tablet ± range of error % at (95 % confidence limit) of two capsules (150 mg) of various pharmaceutical companies | 149.3 ± 1.2 |

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