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SIMULTANEOUS SPECTROPHOTOMETRIC ESTIMATION OF ISONIAZID AND PYRIDOXINE

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ABSTRACT

Simple, precise, accurate, rapid and economical UV-Visible spectrophotometric analytical method for simultaneous estimation of Isoniazid and Pyridoxine has been developed. For development of the method distilled water used as solvent. The method shows maximum absorbance at 262 nm and 291 nm for Isoniazid and Pyridoxine respectively. The proposed method was validated as per ICH guideline. The linearity was established over the concentration range of 3 – 18 µg/mL and 0.4 – 2.4 µg/mL for Isoniazid and Pyridoxine with squared correlation coefficients (r^2) 0.999 and 0.999 respectively. The percentage recovery of Isoniazid and Pyridoxine were found to be 99.92 ± 0.053 and 99.93 ± 0.134 respectively. The relative standard deviation for six replicates was found less than 2.0%. The Statistical analysis gives evidences that proposed method is suitable for routine analysis of Isoniazid and Pyridoxine in pharmaceutical formulation without any interference from the excipients.

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INTRODUCTION

Economic, simple, rapid, precise and accurate analytical method has been developed for the simultaneous determination of Isoniazid (INH) and Pyridoxine (PYR) by UV-Visible spectrophotometrically. For the estimation of INH and PYR distilled water is used as solvent because both drugs are freely soluble in water. INH gives maximum absorption at 262 nm while PYR gives at 291 nm in water without any interference.

Chemically INH is pyridine-4-carbohydrazide while PYR is 5-hydroxy-6- methylpyridine-3, 4-dimethanol. INH is a prodrug activated by catalase-peroxidasehemoprotein, KatG. INH inhibits InhA, a nicotinamide adenine dinucleotide (NADH)-specific enoyl-acyl carrier [1-4]. PYR is a water-soluble vitamin used in the prophylaxis and treatment of vitamin B6 deficiency and peripheral neuropathy in patient receiving isoniazid (isonicotinic acid hydrazide, INH) [5, 6]. The structure of INH and PYR are shown in Figure 1.

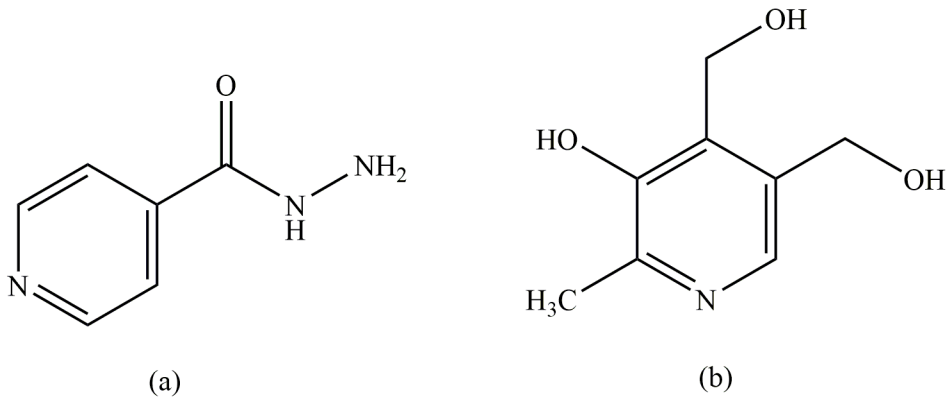

Figure 1: (a) Structure of Isoniazid (b) Structure of Pyridoxine.

This paper is in continuation with our work [8-12] where we studied spectrophotometric method for single or multicomponent drugs. There are methods to estimate the drugs individually for INH and PYR [13-18] or in combination with other drugs, but not a single method is reported for its simultaneous estimation. So here an attempt has been made to develop simple, accurate, sensitive, rapid and economic method for simultaneous estimation of Isoniazid and Pyridoxine in combined dosage form using UV–Visible spectroscopy. The proposed method was validated according to ICH guidelines [19-20].

MATERIALS AND METHODS

Chemicals and Reagents

Standard drug sample of INH and PYR were pursued as a gift sample from Lupin Ltd. Aurangabad. All chemicals and solvents of AR grade and were purchased from Qualigens fine Chemicals, Mumbai, India. Marketed formulation *Isokin-300* tablet containing INH 300 mg and PYR 10 mg was used as sample which is purchased from local market. Distilled water was used as solvent.

Instruments

A Shimadzu model UV-1800 double beam UV-Visible spectrophotometer attachedwith computer operated software UV probe with spectral width of 2 nm, with apair of 1 cm matched quartz cells was used to measure absorbance of the resulting solutions. Analytical weighing balance (AA-2200), digital pH meter (Systronic) and ultrasonic bath (HMG India: CD-4820) were used during the study.

Preparation of standard stock solutions

An accurately weighed quantity of about 10 mg of pure drug of INH and PYR were dissolved in distilled water and diluted to 100 ml in individual flask which gives stock solution of concentration 100 µg/mL. Proper dilution were done to give working standard solution of concentration 10 µg/mL respectively.

Selection of analytical wavelengths

By using above concentration solution absorption spectra were taken for both the drugs. INH shows maximum absorption at 262 nm (Figure 2) while PYR at 291 nm (Figure 3). From the overlain spectra of both the drugs wavelength selected for quantification were 262 nm for INH and 291 nm for PYR. Figure 4 represents the overlay spectra for INH and PYR.

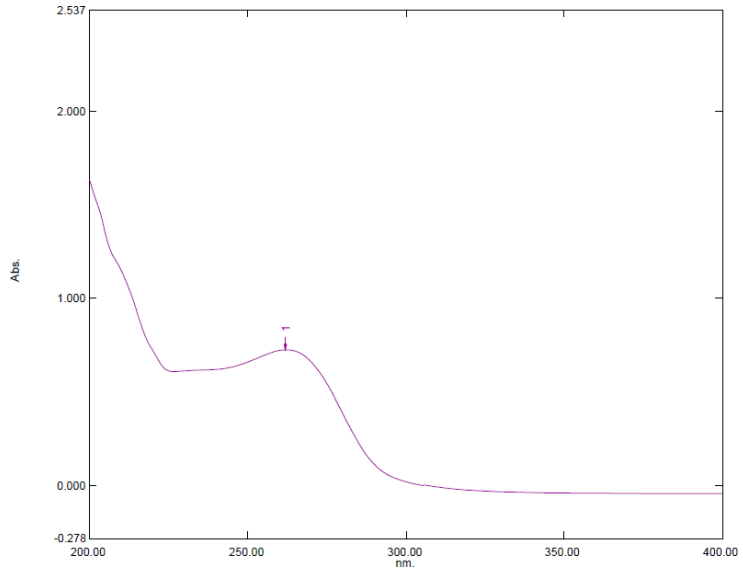

Figure 2: Absorption spectrum of INH.

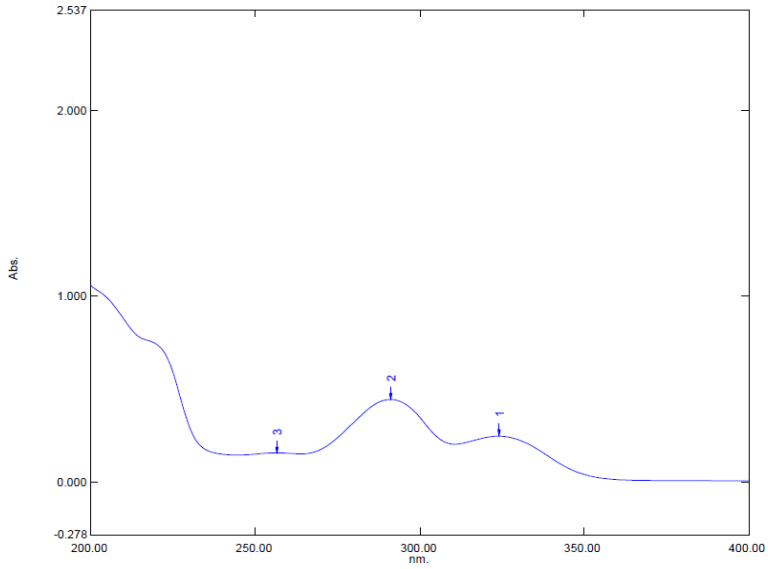

Figure 3: Absorption spectrum of PYR.

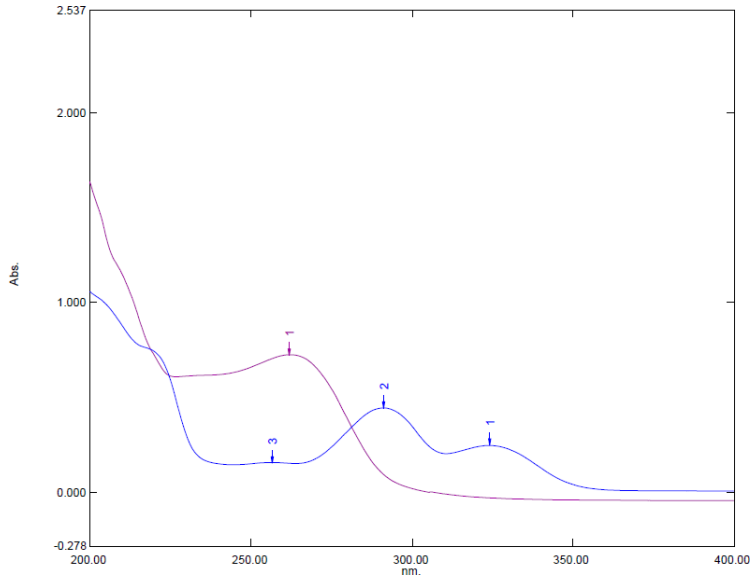

Figure 4: Overlain spectrum of INH and PYR.

Method Validation

The proposed method was validated for accuracy, precision, recovery, linearity and robustness. The method validation was performed as per ICH guideline..

Determination of absorptivity coefficients at analytical wavelengths

The absorptivity coefficients for the two drugs were determined at both the selected wavelengths. The values obtained as the mean of six independent determinations were used for forming the simultaneous equations.

The simultaneous equations formed were:

$A_1 = 63.75 \times C_1 + 45 \times C_2$ ---------- (1) at 262 nm
(For Isoniazid)

$A_2 = 44.68 \times C_1 + 64.29 \times C_2$ ---------- (2) at 291 nm
(For Pyridoxine)

Where A_1 and A_2 are the absorbance of sample solution at 262 nm and 291 nm respectively and C_1 and C_2 are the concentrations of Isoniazid and Pyridoxine respectively (in grams per liter) in the sample solution. By solving the two simultaneous equations, the concentration of Isoniazid (C_1) and Pyridoxine (C_2) in sample solutions can be obtained.

Selection of analytical concentration ranges

From the stock solution of INH and PYR with subsequent dilution by distilled water respectively 3 – 18 µg/mL and 0.4 – 2.4 µg/mL concentration solutions were prepared (Table 1). The calibration curves were plotted for these concentrations against absorbance value obtained at respective λ_{max} (Figure 5 and 6). Optical characteristics and other parameters results were shown in Table 2.

Table 1: Standard calibration table for INH and PYR.

Sr. No.	Isoniazid		Pyridoxine	
	Concentration (µg/mL)	Absorbance at 262 nm	Concentration (µg/mL)	Absorbance at 291 nm
1	3	0.145	0.4	0.018
2	6	0.329	0.8	0.041
3	9	0.524	1.2	0.062
4	12	0.705	1.6	0.083
5	15	0.885	2.0	0.106
6	18	1.054	2.4	0.127

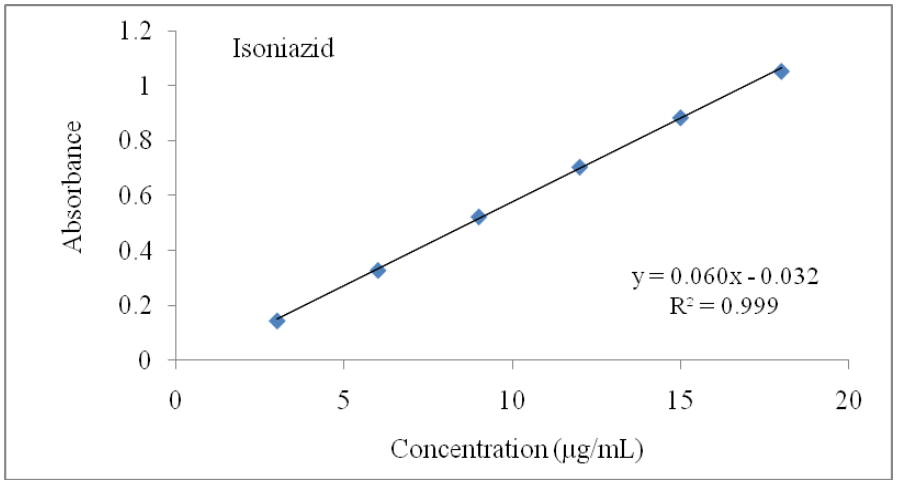

Figure 5: Calibration curve of INH.

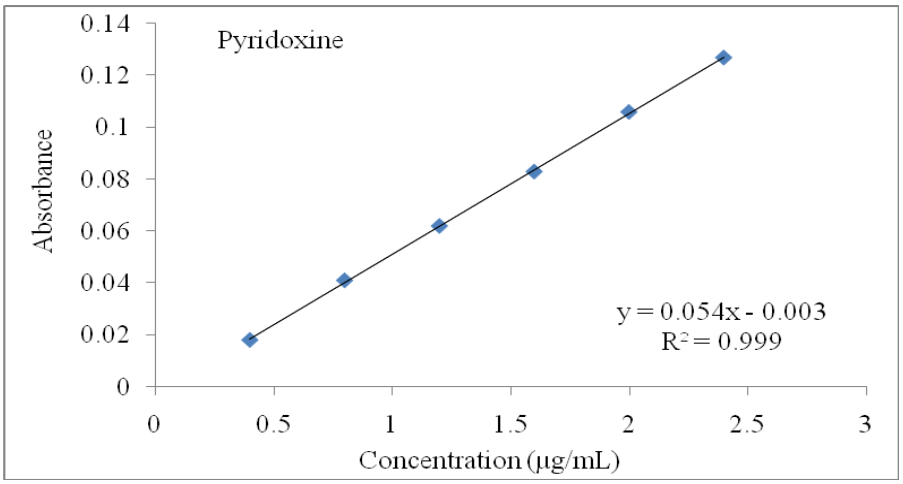

Figure 6: Calibration curve of PYR.

Table 2: Optical characteristics and other parameters.

Parameters	INH	PYR
Working wavelength (nm)	262	291
Linearity range (µg/mL)	3 – 18	0.4 – 2.4
Limit of detection (µg/mL)	0.610	0.147
Limit of quantitation (µg/mL)	2.012	0.485
Molar absorptivity	63.75	45
y = mx + c	y = 0.060x - 0.032	y = 0.054x - 0.003
Slope	0.060	0.054
Intercept	– 0.032	– 0.003
Regression Coefficient	0.999	0.999

Procedure for analysis of tablet formulation

Accurately weighed 20 tablets of marketed formulation *Isokin-300* and average weight were found to be 430 mg. Then these tablets were crushed to fine powder and from this 430 mg of powder weighed containing equivalent to 300 mg of INH and 10 mg of PYR. It has been transferred into 250 mL of volumetric flask and volume is made up by distilled water up to the mark. This mixture is sonicated for about 10 min and filtered through Whatman filter paper. From this sample solution with appropriate dilution, concentrations 12 µg/mL and 0.4 µg/mL of INH and PYR were obtained respectively. The analysis procedure was repeated in six replicates. The results of marketed tablet formulation are given in Table 3.

Table 3: Results of marketed tablet formulation.

Sr. No.	Label claim (mg/tab)		Amount found (mg/tab)		% of Label claim	
	INH	PYR	INH	PYR	INH	PYR
1	300	10	299.91	9.987	99.97	99.87
2	300	10	299.67	9.973	99.89	99.73
3	300	10	299.73	9.983	99.91	99.83
4	300	10	299.58	9.971	99.86	99.71
5	300	10	299.37	9.992	99.79	99.92
6	300	10	299.82	9.996	99.94	99.96
				Mean	99.89	99.84
				SD	0.0635	0.1007
				% RSD	0.0635	0.1009

Recovery studies

To ascertain the accuracy of the proposed methods, recovery studies were carried at three different levels (80 %, 100 % and 120 %) as per ICH guidelines.

To perform recovery study at 80%, tablet powder was weighed about 430 mg containing equivalent to 300 mg of INH and 10 mg of PYR and to this standard 240 mg of INH and 8 mg of PYR were added. The mixture was triturated well and from this 376.96 mg of powder was taken and added into 250 mL of distilled water and by serial dilution technique required concentration solution was prepared containing 12 µg/mL of INH and 0.4 µg/mL of PYR.

To perform recovery study at 100%, tablet powder was weighed about 430 mg containing equivalent to 300 mg of INH and 10 mg of PYR and to this standard 300 mg of INH and 10 mg of PYR were added. The mixture is triturated well and from this 370 mg of powder was taken and added into 250 mL of distilled water and by serial dilution technique required concentration solution was prepared containing 12 µg/mL of INH and 0.4 µg/mL of PYR.

To perform recovery study at 120%, tablet powder is weighed about 430 mg containing equivalent to 300 mg of INH and 10 mg of PYR and to this standard 360 mg of INH and 12 mg of PYR were added. The mixture is triturated well and from this 364.90 mg of powder was taken and added into 250 mL of distilled water and by serial dilution technique required concentration solution was prepared containing 12 µg/mL of INH and 0.4 µg/mL of PYR.

The results of the recovery studies were also validated statistically. The results of recovery studies are given in Table 4.

Table 4: Results of recovery studies.

Level of (%) Recovery	Amountpresent (mg/tab)		Amount of standard added(mg)		Total amount recovered (mg)		% Recovery*	
	INH	PYR	INH	PYR	INH	PYR	INH	PYR
80	300	10	240	8	539.64	17.98	99.93	99.87
100	300	10	300	10	599.50	19.99	99.91	99.95
120	300	10	360	12	659.51	21.99	99.92	99.97
						Mean	99.92	99.93
						SD	0.0528	0.1342
						% RSD	0.0528	0.1343

*Each value is the mean of three observations.

Precision

The precision of the method was evaluated by intra-day and inter-day variation studies. In intra-day studies, working solutions of standard and sample were analyzed triplicate in a day and percentage relative standard deviation (% RSD) was calculated.In the inter-day variation studies, working solution of standard and sample were analyzed on two consecutive days and percentage relative standard deviation (% RSD) was calculated (Table 5).

Table 5: Results of precision study.

Formulation	Parameter	Intra-day precision	Inter-day precision
INH	Mean	99.92	99.84
	SD	0.0458	0.0755
	% RSD	0.0459	0.0756
PYR	Mean	99.72	99.56
	SD	0.3205	0.3195
	% RSD	0.3214	0.3209

CONCLUSION

Novel and economic analytical method is developed for simultaneous estimation of INH and PYR using distilled water as solvent. INH follows the Beers- lamberts law in the range of 3 – 18 µg/mL while PYR in range of 0.4 – 2.4 µg/mL. Formulation containing INH and PYR were analyzed by developed method. Mean assay values in tablet formulation *Isokin-300* were found to be 99.89 ± 0.0635 and 99.84 ± 0.1009 respectively. The accuracy method was determined by recovery studies. The mean recovery was found to be 99.92 ± 0.0528 and 99.93 ± 0.1343 respectively, indicating that the method has required accuracy and there was no interference from the common excipients present in tablets. The RSD value below 2.0 % indicated that developed method has the required precision. LOD and LOQ values at 262 nm for INH were found to be 0.610 and 2.012 µg/mL while at 291 nm for PYR were 0.147 and 0.485 µg/mL respectively. Thus the developed method was simple, economic, accurate and precise and can be used for routine analysis of INH and PYR in Pharmaceutical formulation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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