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Simultaneous estimation of rifampicin and isoniazid in combined dosage form by a simple UV spectrophotometric method

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ABSTRACT

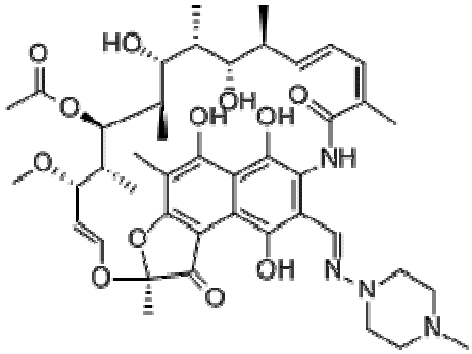
A simple, specific, accurate, precise and reproducible method has been developed and validated for the simultaneous estimation of Rifampicin and Isoniazid in combined dosage form by UV Spectrophotometric method. UV spectrophotometric method includes simultaneous equation method using 337.0 nm and 263.0 nm λmax of both the drugs were selected for estimation of Rifampicin and Isoniazid respectively. Rifampicin and Isoniazid follow Beer's law over the concentration range of 5-35 µg/ml and 5-25 µg/ml. The percentage recoveries of the both the drugs were found to be nearly 100 % representing the accuracy of the proposed methods. Validation of the proposed methods was carried out for its accuracy, precision, and specificity according to ICH guidelines. The proposed method can be successfully applied in routine laboratory analysis for the determination of Rifampicin and Isoniazid in combined dosage form.

Keywords: Rifampicin, Isoniazid, UV spectroscopy, Simultaneous Equation method
Abbreviations: RIF – Rifampicin, INH – Isoniazid

INTRODUCTION

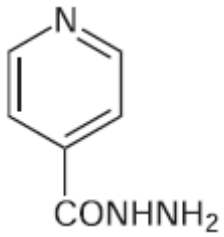
Rifampicin (Fig. 1), a complex semisynthetic macrocyclic antibiotic derived from *Streptomyces mediterranei*, is a member of the rifamycin class of antibiotics used for the treatment of tuberculosis and other infectious diseases [1]. It is categorized as one of the first line antituberculous agents. Tuberculosis remains a major health public problem and is the single most deadly infectious disease. It kills approximately two million people each year [2]. Rifampicin is chemically (12*Z*, 14*E*, 24*E*)- (2*S*, 16*S*, 17*S*, 18*R*, 19*R*, 20*R*, 21*S*, 22*R*, 23*S*) - 1,2 -dihydro- 5, 6, 9, 17, 19 - pentahydroxy, 23 -methoxy- 2, 4, 12, 16, 18, 20, 22 heptamethyl -8- (4-methylpiperazin -1 yliminomethyl) -1, 11 - dioxo 2, 7 (epoxypentadeca -1, 11, 13 trienimino) naphtha [2,1-*b*] furan -21-yl acetate[3]. It is official in IP[4], BP[5] and USP[6]. IP, BP and USP describe Liquid Chromatography and Visible spectrophotometry method for its estimation. Literature survey reveals HPLC[7], HPTLC[8] and Visible Spectrophotometry[9] methods for determination of Rifampicin in pharmaceutical dosage forms as well as in biological fluids. Literature survey also reveals spectrophotometric[10], RP-HPLC[11], Visible Spectrophotometry[12] and HPTLC[13] methods for determination of Rifampicin with other drugs in combination.

Fig.1. Structure of Rifampicin



Isoniazid (Fig. 2), the hydrazide of isonicotinic acid is a synthetic analog of pyridoxine [14]. It is the first line antitubercular medication never used on its own to treat active tuberculosis because resistance quickly develops [15]. It is widely used together with rifampicin, ethambutol and pyrazinamide among others, for the chemotherapy of tuberculosis. Several methods for the determination and quantitation of Isoniazid have been described. These include H-point standard addition method[16], selective adsorption using a piezoelectric sensor[17], voltametric method[18], amperometric method[19], chromatographic methods[20,21,22,23] (HPLC, GC and HPTLC), titrimetric methods[24], chemiluminisence[25].

Fig.2. Structure of Isoniazid



No UV spectrophotometric studies on Rifampicin and Isoniazid in combined dosage form in pharmaceutical preparations have been found in recent literature survey.

The aim of present work is to find out a simple, sensitive, specific, spectrophotometric method and its validation for the simultaneous estimation of Rifampicin and Isoniazid in combined dosage form in pharmaceutical formulations.

MATERIALS AND METHODS

Instruments

Lab India double beam UV visible spectrophotometer (UV 3092) with 1 cm matched quartz cells were used for all absorbance measurements with UV WIN Software. Shimadzu AX 200 balance was used for weighing the samples**.**

Materials

Rifampicin was kindly provided by Lupin Limited, Mandideep, Madhya Pradesh, India. Isoniazid was received as a gift sample from Amar Chemicals, Ahmedabad, Gujarat, India. Ethanol of analytical reagent grade was purchased by Loba Chemie (India). All the solutions were protected from light and were analyzed on the day of preparations. Multicomponent Capsule, R-Cinex (Rifampin 450 mg and Isoniazid 300 mg) manufactured by Lupin Ltd, Aurangabad, Maharashtra, India was obtained from local pharmacy. All the chemicals used were of AR grade.

Selection of common solvent

Ethanol of analytical reagent grade was selected as common solvent for developing spectral characteristics of drug. The selection was made after assessing the solubility of both the drugs in different solvents.

Preparation of Standard Stock Solution

The standard stock solution containing Rifampicin and Isoniazid were prepared by dissolving 100 mg of Rifampicin and 100mg of Isoniazid separately in 20 ml of ethanol. It was then sonicated for 10 minutes and the final volume of both the solutions were made up to 100 ml with ethanol to get stock solutions containing 1000 µg/ ml each of Rifampicin and Isoniazid in two different 100 ml volumetric flasks.

Procedure for Determining the Sampling Wavelength for Simultaneous Analysis

By appropriate dilution of two standard drug solutions with ethanol, solutions containing 10 µg/ ml of Rifampicin and 10 µg/ml of Isoniazid were scanned separately in the range of 400-200 nm to determine the wavelength of maximum absorption for both the drugs. Rifampicin and Isoniazid showed absorbance maxima at 337 nm and 263 nm respectively as shown in Fig. 3 and 4.

Fig.3.Absorption spectrum of Rifampicin in ethanol (10 µg/ml) showing λ max 337 nm

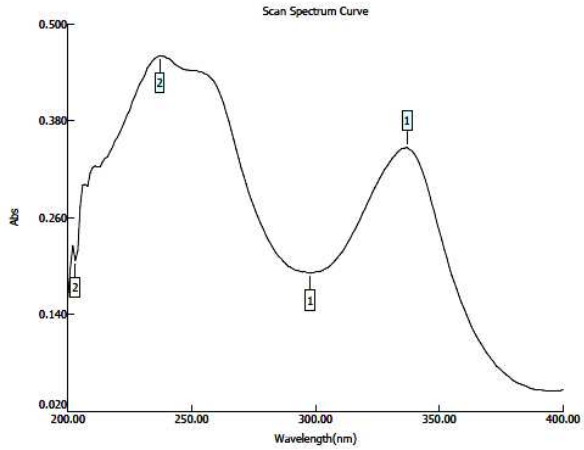


Fig.4. Absorption spectrum of Isoniazid in ethanol (10 µg/ml) showing λ max 263 nm

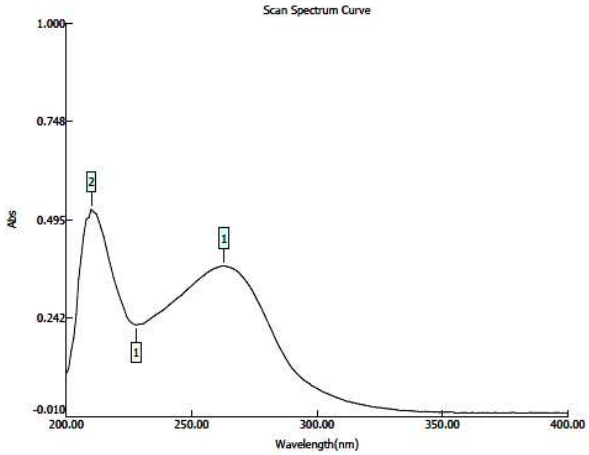
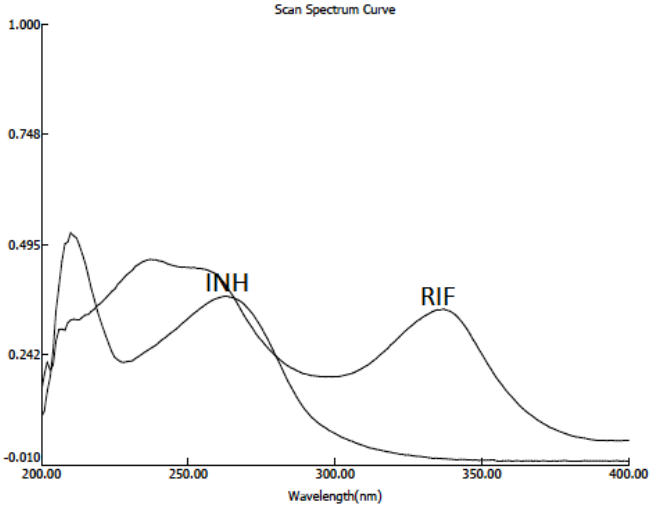


Fig.5. Overlain spectra of Isoniazid and Rifampicin in ethanol (10 µg/ml) showing λ max 263 nm and 337 nm in standard solution



Selection of Method and Wavelength

For estimation of Rifampicin, simultaneous equation method employing 337 nm as analytical wavelength was used. For estimation of Isoniazid, 263 nm was selected as the analytical wavelength. In the simultaneous equation method developed for simultaneous estimation of Rifampicin and Isoniazid, the wavelengths were selected from the overlain spectra as shown in Fig. 5.

Procedure for plotting calibration curve

Rifampicin and Isoniazid showed linearity with absorbance in the range of 5- 35 µg/ml and 5- 25 µg/ml at their respective wavelength maxima, which were validated by least square regression method. Coefficients of correlation were found to be 0.9991 for Rifampicin and 0.9998 for Isoniazid. For simultaneous estimation of Rifampicin and Isoniazid, a series of linearity solutions were prepared by diluting appropriate volume of standard stock solution. The scanning of the solutions of Rifampicin and Isoniazid were carried out in the range of 400- 200 nm against ethanol as blank. Absorbance of series of linearity solutions were recorded at selected wavelengths 337 nm and 263 nm as shown in Fig. 6 and 7.

Fig.6. Overlay spectrum of Rifampicin in ethanol from 5-35 µg/ml

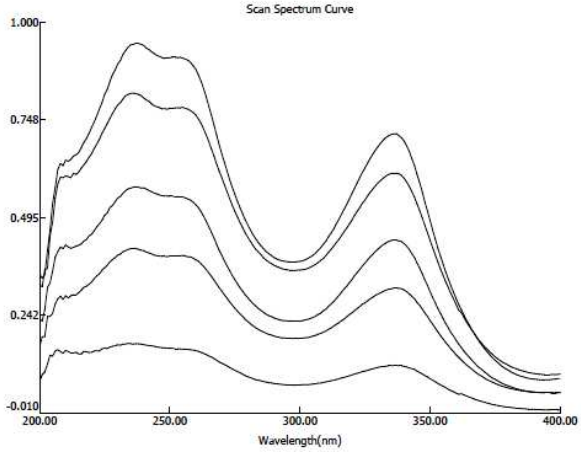
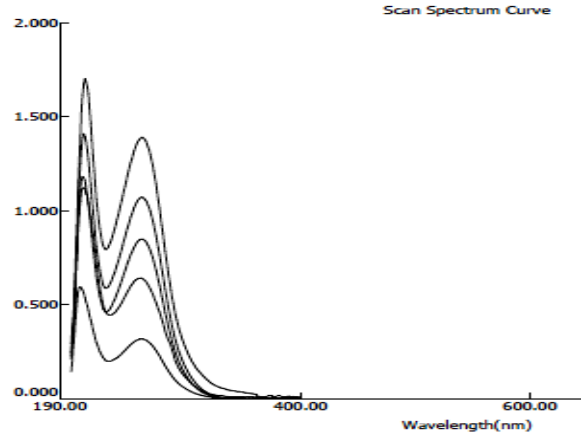


Fig.7. Overlay spectrum of Isoniazid from 5-25 µg/ml



Analysis of Capsule Formulation

The average weights of twenty capsule contents were determined and a quantity equivalent to 100 mg of Rifampicin was transferred to a 100 ml volumetric flask. The contents were dissolved by using 70 ml of ethanol, filtered through whatman filter paper no. 41 and then made up to volume with the same. The solution was further diluted with ethanol to give concentrations of 10 µg/ml of Rifampicin and 10 µg/ml of Isoniazid. The solution was scanned in the range 200-400 nm as shown in Fig. 8. Absorbance of these solutions was measured at 337 nm and 263 nm as A1 and A2 respectively and concentrations of these two drugs in the sample were calculated using Simultaneous equation method. Results of analysis of the capsule formulations were reported in Table 1.

Fig.8. Overlain spectra of Isoniazid and Rifampicin in ethanol (10 µg/ml) showing λ max 263 nm and 337 nm in Formulation

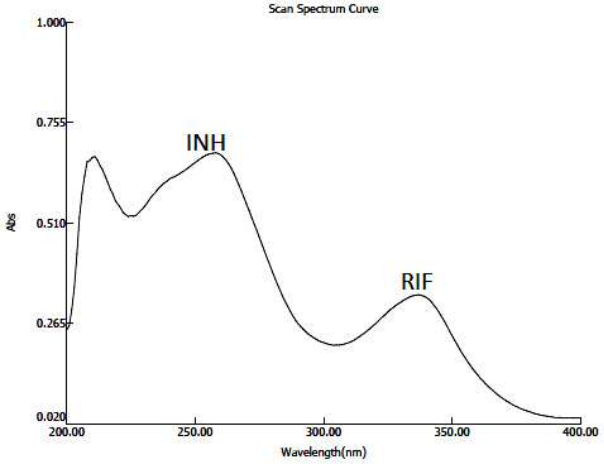


Table 1: Results of Analysis of Capsule Formulation

Drug	Label claim mg/cap	Amount found mg/cap	% Label claim*	% RSD*
RIF	450	452.26	100.50	0.691
INH	300	302.86	100.95	0.597

Mean of six determinations

Determination of absorptivity value

The solutions of each drug in triplicate were read against solvent blank at the selected wavelengths and A (1% 1 cm) value were calculated using below formula:

Absorptivity, A (1% 1 cm) = Absorbance at selected wavelengths/ Concentration in g / 100 mL

Simultaneous equation method by

C x = A2 ay1 – A1 ay2 / ax2 ay1 – ax1 ay2

C y = A1 ax2 – A2 ax1 / ax2 ay1 – ax1 ay2

where:
ax1= The absorptivity of Rifampicin at 337.0 nm,
ax2= The absorptivity of Rifampicin at 263.0 nm
ay1= The absorptivity of Isoniazid at 337.0 nm,
ay2= The absorptivity of Isoniazid at 263.0 nm
C x and C y are the concentrations of Rifampicin and Isoniazid

A1 is the absorbance of mixture at 337 nm and A2 is the absorbance of mixture at 263 nm

Validation Parameters

The method was validated according to ICH Q2B guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for the analyte.

Linearity

The linearity of measurement was evaluated by analyzing different concentrations of the standard solution of Rifampicin and Isoniazid. For simultaneous equation method, the Beer- Lambert's concentration range was found to be for 5-35µg/ml for Rifampicin and 5-25µg/ml for Isoniazid as shown in Fig. 9 and 10.

Fig.9. Calibration curve of Rifampicin in ethanol (5-35 µg/ml)

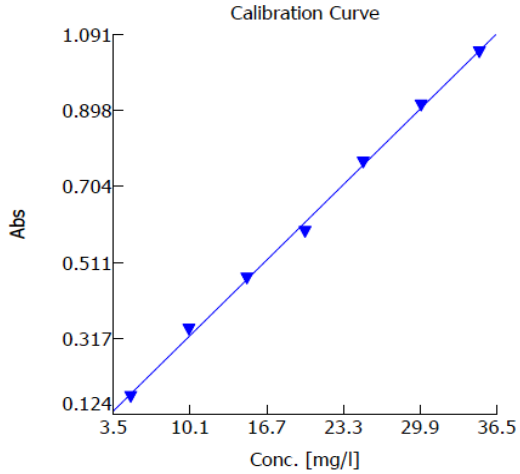
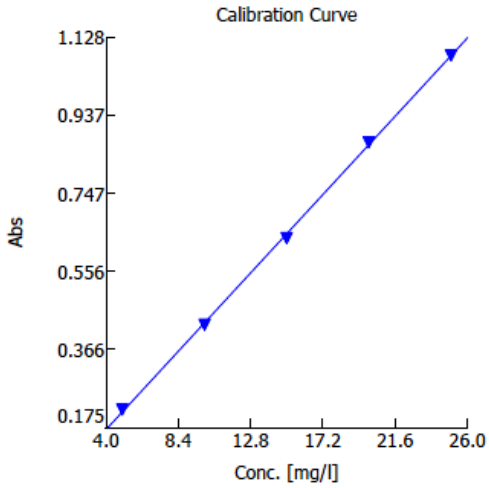


Fig.10. Calibration curve of Isoniazid in ethanol (5-25 µg/ml)



Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the following equation LOD=3.3σ/s and LOQ=10 σ/s, Where σ is standard deviation of y intercept of calibration curve (n=6) and s is slope of regression equation. The results of the same are shown in Table 4.

Precision

The precision of the method was established by carrying out the analysis of the analytes (n=6) using the proposed developed method. The low value of relative standard deviation showed that the method was precise. The results are shown in Table 2.

Inter-day precision

It was done by analyzing the solutions by same analyst on alternate days till 3rd day. The % RSD is shown in Table 2.

Intraday precision

It was done by analyzing the solutions by same analyst three times within a day at intervals of 1 hr. The % RSD is shown in Table 2.

Table 2: Results of Precision Studies

Precision**					
Concentration (µg/ml)		Inter-day		Intraday	
RIF	INH	RIF	INH	RIF	INH
20	20	0.784	1.374	0.578	0.673

***%RSD of six determinations*

Accuracy

To check the accuracy of the developed method and to study the interference of formulation excipients, analytical recovery experiments were carried out by using standard addition method at 50, 100 and 150% levels. From the total amount of drug found, the percentage recovery was calculated. The results revealed no interference of excipients. The results of recovery studies were summarized in Table 3.

Table 3: Results of Accuracy of the method

Drug	% Amount added	Label claim (mg)	Amount Recovered (mg)	% Recovery	Mean % Recovery
RIF	50	450	676.1	99.61	99.23
	100		898.7	99.23	
	150		1125.6	98.86	
INH	50	300	450.9	99.89	99.80
	100		600.4	99.96	
	150		751.3	99.56	

RESULTS AND DISCUSSION

The proposed method for simultaneous estimation of Rifampicin and Isoniazid in combined sample solutions was found to be simple, accurate and reproducible. Beer's law was obeyed in the concentration range of 5-35 µg/ml for Rifampicin and 5-25 µg/ml for Isoniazid respectively. The correlation coefficients were found to be 0.9991 for Rifampicin and 0.9998 for Isoniazid which shows the good linear relationship for both drugs. The capsule assay results obtained by proposed method was very close to labeled claim and low value of relative standard deviation, suggesting that the developed method has high precision. In order to check the accuracy of the developed methods, known quantities of standard drugs of Rifampicin and Isoniazid in three different levels were added to its pre-analyzed capsule sample and analyzed by the developed methods. The mean percentage recoveries were found in the range of 99.0-100.0 and it showed the non interference of the excipients from the capsule formulation. The results of optical characteristics such as Beer's law limits, correlation coefficient, slope, intercept and molar absorptivity values were summarized in Table 4.

Table 4: Optical Characteristics and Summary of Validation Parameters of the proposed method

S.No.	PARAMETER	RESULTS	
		RIF	INH
1	λ max (nm)	337	263
2	Beer's law limit (µg/ml)	5-35	5-25
3	Molar absorptivity (L mole^{-1} cm^{-1})	25349	9341
4	Corrélation coefficient (r^2)	0.9991	0.9998
5	Regression equation (y = a + bC) ** Slope (b) Intercept (a)	y = 0.029x + 0.030 0.029 0.030	y = 0.043x - 0.005 0.043 0.005
6	Inter-day Precision (% RSD) *	0.784	1.374
7	Intraday Precision (% RSD) *	0.578	0.673
8	Limit of detection (LOD) (µg/ml)	1.653	0.585
9	Limit of quantification (LOQ) (µg/ml)	5.007	1.772

**Average of six determinations (n=6)*

***y = a + bC where C is the concentration in µg/ml*

CONCLUSION

The proposed simultaneous UV Spectrophotometric method was developed and validated thoroughly for quantitative determination of Rifampicin and Isoniazid in combined capsule dosage form. The developed method was found to be simple, rapid, accurate, precise and economical and give an acceptable recovery of the analytes, which can be directly and easily applied for routine analysis in quality control analysis of Rifampicin and Isoniazid mixture in pharmaceutical capsule formulations.

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