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Dual Wavelength Spectrophotometric Method for the Simultaneous Estimation of Rifampicin and Piperine in Their Combined Capsule Dosage Form

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ABSTRACT

The present manuscript describe simple, sensitive, rapid, accurate, precise and cost effective dual wavelength spectrophotometric method for the simultaneous determination of Rifampicin and Piperine in combined capsule dosage form. The utility of dual wavelength data processing program is its ability to calculate unknown concentration of components of interest in a mixture containing an interfering component. The principle for dual wavelength method is “the absorbance difference between two points on the mixture spectra is directly proportional to the concentration of the component of interest”. The method was based on determination of Rifampicin at the absorbance difference between 286 nm and 357 nm and Piperine at the absorbance difference between 356 nm and 479 nm. The linearity was obtained in the concentration range of 10-60 µg/ml for Rifampicin and 1-10 µg/ml for Piperine. The mean recovery was 98.40 ± 0.48 and 98.59 ± 0.46 for Rifampicin and Piperine, respectively. The method was successfully applied to pharmaceutical dosage form because no interference from the capsule excipients was found. The suitability of these methods for the quantitative determination of Rifampicin and Piperine was proved by validation. The proposed methods were found to be simple and sensitive for the routine quality control application of Rifampicin and Piperine in pharmaceutical capsule dosage form. The results of analysis have been validated statistically and by recovery studies.

Keywords: Rifampicin, Dual wavelength, Piperine, Validation, Recovery, Drug analysis.

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INTRODUCTION:

Rifampicin (RIFA) is chemically (12Z, 14E, 24E)- (2S, 16S, 17S, 18R, 19R, 20R, 21S, 22R, 23S) - 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.[1] (Figure 1) is a well known Anti-Tuberculosis drug[2]. It is official in IP[3], BP[4] and USP[5] describe Liquid Chromatography and Visible spectrophotometry method for its estimation. Literature survey reveals HPLC[6], HPTLC[7] and Visible Spectrophotometry[8] methods for determination of RIFA in pharmaceutical dosage forms as well as in biological fluids. Literature survey also reveals spectophotometric, RP-HPLC[9,10], Visible Spectrophotometry[11,12] and HPTLC[13] methods for determination of RIFA with other drugs in combination. Piperine (PIPE) is chemically 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl] piperidine[14] (Figure 2) is a natural alkaloid use as Bio enhancer[15] Piperine is official in IP. IP[14] describe liquid chromatography method for its estimation. Literature survey reveals HPLC[16], UV Spectrophotometry[17] and HPTLC[18, 19] method for the determination of PIPE. Literature survey also reveals HPLC[20, 21] methods for determination of PIPE with other drugs in combination. The combined dosage forms of RIFA and PIPE along with Isoniazid are available in the market and used as anti tuberculosis drugs. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of RIFA and PIPE in their combined dosage forms. Literature survey does not reveal any simple spectrophotometric method for simultaneous estimation of RIFA and PIPE in combined dosage forms. The present communication describes simple, sensitive, rapid, accurate, precise and cost effective spectrophotometric method based on Dual wavelength spectrophotometric method for simultaneous estimation of both drugs in their combined capsule dosage form.

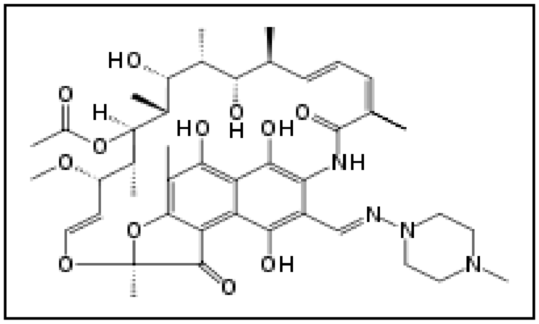

Figure 1: Structure of Rifampicin

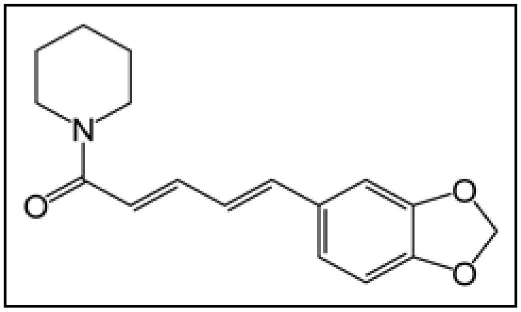

Figure 2: Structure of Piperine

MATERIALS AND METHOD

Materials

A shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study. RIFA and PIPE bulk powder was kindly gifted by Cadila Pharmaceuticals Ltd. Ahmedabad, Gujarat, India. The commercial fixed dose combination product was procured from the local market. Methanol (AR Grade, S. D. Fine Chemicals Ltd., Mumbai, India) were used in the study.

Preparation of Standard Solutions

A 10 mg of standard RIFA and PIPE were weighed and transferred to 100 ml separate volumetric flasks (amber coloured for RIFA) and dissolved in methanol. The flasks were shaken and volumes were made up to mark with methanol to give a solution containing 100 µg/ml each of RIFA and PIPE.

Methodology

The working standard solutions of RIFA and PIPE were prepared separately in methanol having concentration of 10 µg/ml. They were scanned in the wavelength range of 200-800 nm. From the overlain spectra, four wavelengths 286 nm, 356 nm, 357 nm and 479 nm were selected for quantitation of both the drugs by proposed dual wavelength spectrophotometric method. The quantitative determination of RIFA is carried out by measuring the absorbance difference value at between 286 nm and 357 nm where PIPE has same absorbance at both the wavelength. The difference between 286 nm and 357 nm is directly proportional to concentration of RIFA in the mixture. The quantitative determination of PIPE is carried out by measuring the absorbance difference value at 356 nm and 479 nm where RIFA have same absorbance at both the wavelength. The difference between 356 nm and 479 nm is directly proportional to concentration of PIPE in the mixture.

VALIDATION OF THE PROPOSED METHOD

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines [22].

Linearity (Calibration Curve)

Appropriate aliquots from the standard stock solutions of RIFA and PIPE were used to prepare three different sets of dilutions: Series A, B, and C as follows. Series A consisted of different concentration of RIFA (10-60 µg/ml). Aliquot from the stock solution of RIFA (100 µg/ml) was pipette out in to a series of 10 ml volumetric flask and diluted with methanol to get final concentration in range of 10-60 µg/ml. Series B consisted of varying concentrations of PIPE (1-10 µg/ml). Appropriate volume of the stock solution of PIPE (100 µg/ml) was transferred into a series of 10 ml volumetric flask and the volume was adjusted to the mark with methanol. Series C comprised of mixture of RIFA and PIPE having varying concentration of RIFA (10-60 µg/ml) and PIPE (1-10 µg/ml). The solutions of RIFA and PIPE were prepared by transferring 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 ml equivalent to 10, 20, 30, 40, 50 and 60 µg/ml from the stock solution of RIFA and 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 ml equivalent to 1, 2, 4, 6, 8 and 10 µg/ml PIPE (10 µg/ml) into a series of 10 ml volumetric flasks and the volume was adjusted up to the mark with methanol. The absorbance of the solutions of series A, B and C were measured at 286 nm (λ_1), 356 nm (λ_2), 357 nm (λ_3) and 479 nm (λ_4). The difference in absorbance between 286 nm and 357 nm is due to the RIFA and was plotted against RIFA concentration (µg/ml). The difference in absorbance between 356 nm and 479 nm is due to the PIPE and was plotted against PIPE concentration (µg/ml) and two different regression equations were obtained.

Method Precision (Repeatability)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n = 6) for RIFA and PIPE (10 µg/ml for both drugs) without changing the parameter of the proposed Spectrophotometry method.

Intermediate Precision (Reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of RIFA and PIPE (10, 20, 40 µg/ml for RIFA and 2, 4, 6 µg/ml for PIPE). The result was reported in terms of relative standard deviation (% RSD).

Accuracy (Recovery Study)

The accuracy of the method was determined by calculating the recoveries of RIFA and PIPE by the standard addition method. Known amounts of standard solutions of RIFA and PIPE were at added at 50, 100 and 150 % level to pre-quantified sample solutions of RIFA and PIPE (20 µg/ml for RIFA and 2 µg/ml for PIPE). The amounts of RIFA and PIPE were estimated by applying obtained values to the respective regression line equations.

Limit of Detection and Limit Of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by

Calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where,

σ = the standard deviation of the response and

S = slope of the calibration curve.

Analysis of Capsule Sample

Weigh 20 capsules and determine average net content of blend. Remove Isoniazid tablet from blend. Accurately weigh and transfer quantity of capsule contents equivalent to about 200 mg of RIFA and 10 mg of PIPE into 100 ml amber coloured volumetric flask. Add 70 ml of Methanol and sonicate for about 20 minutes. Dilute volume up to mark with Methanol and mix. Take 2 ml aliquot in separate 100 ml amber coloured volumetric flask. Dilute it up to mark with Methanol to get the solution containing 40 µg/ml of RIFA and 2 µg/ml of PIPE. The absorbance of final solution was recorded at selected wavelengths for determination of RIFA and PIPE. The analysis procedure was repeated three times for capsule formulation.

RESULTS AND DISCUSSION

The solution of RIFA and PIPE were prepared separately in methanol and scanned in the UV range of 200 - 800 nm. From the overlain spectra of both drugs, four specific wavelengths are selected. The absorbance at 286 nm (λ_1) and 357 nm (λ_3) wavelengths was found to be with same absorbance for PIPE. These two selected wavelengths were employed to determine the concentration of RIFA from the mixture of RIFA and PIPE. The difference in absorbance at these two wavelengths ($A_{286} - A_{357}$) cancels out the contribution of absorbance of PIPE in mixture. Similarly, the absorbance at 356 nm (λ_2) and 479 nm (λ_4) wavelengths was found to be with same absorbance for RIFA. These two selected wavelengths were employed to determine the concentration of PIPE from the mixture of RIFA and PIPE. The difference in absorbance at these two wavelengths ($A_{356}– A_{479}$) cancels out the contribution of absorbance of RIFA in mixture. The proposed method was found to be simple, sensitive, rapid, accurate, precise and economic for the routine simultaneous estimation of two drugs. Linear correlation was obtained between absorbances and concentrations of RIFA and PIPE in the concentration ranges of 10-60 µg/ml & 1-10 µg/ml, with R^2 value 0.999 for RIFA and 0.998 for PIPE respectively. Figure-3,4,5.

The linearity of the calibration curve was validated by the high values of correlation coefficient of regression. The RSD values of RIFA were found to be 0.21%. The RSD value of PIPE was found to be 0.146%. Relative standard deviation was less than 2 %, which indicates that proposed method is repeatable. The low RSD values of interday (0.61-1.77% for RIFA and 0.19-1.73% for PIPE, respectively) and intraday (0.11-0.84% for RIFA and 0.17-1.59% for PIPE, respectively) variation for RIFA and PIPE, reveal that the proposed method is precise. LOD and LOQ values for RIFA were found to be 1.96 and 5.93 µg/ml, respectively. LOD and LOQ values for PIPE were found to be 0.28 and 0.86 µg/ml, respectively. These data show that method is sensitive for the determination of RIFA and PIPE. The regression analysis data and summary of validation parameters for the proposed method is summarized in Table 1.

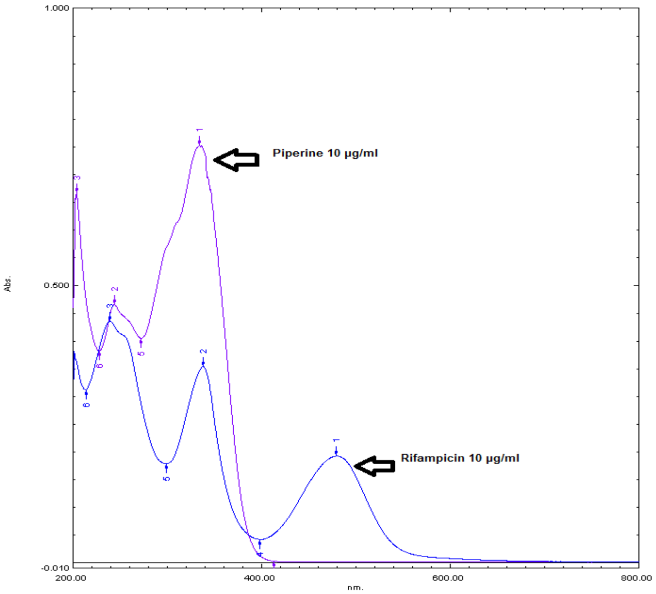

Figure 3: Overlain zero-order absorption spectra of RIFA and PIPE in methanol

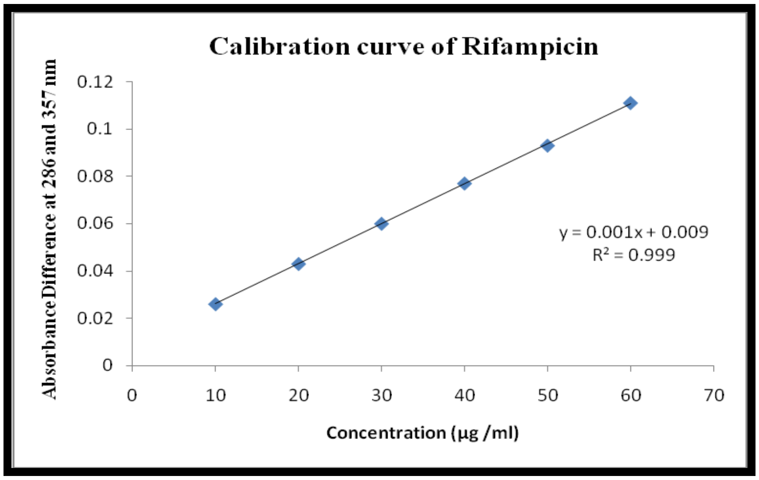

Figure 4: Linearity (Calibration Curve) of Rifampicin at the absorbance difference between 286 nm and 357 nm.

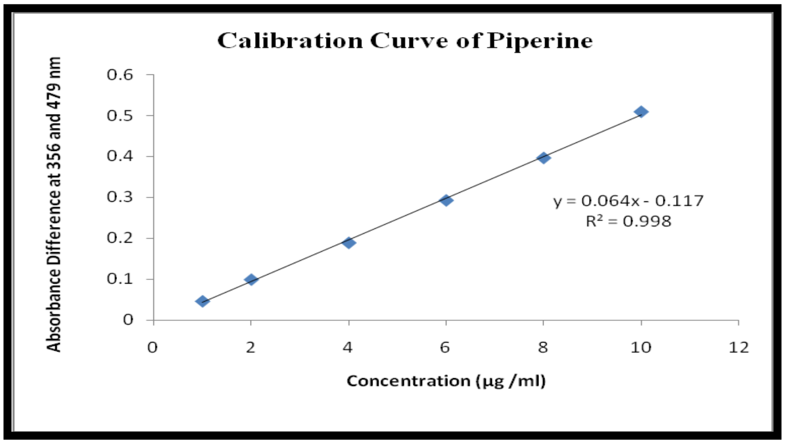

Figure 5: Linearity (Calibration Curve) of Piperine at absorbance difference Between 356 nm and 479 nm

Table 1: Regression Analysis Data and Summary of Validation Parameters for RIFA and PIPE by Dual Spectrophotometric Method

Parameters	**RIFA**	**PIPE**
Wavelength (nm)	286, 357 nm	356, 479 nm
Beer's law limit (µg /ml)	10-60	1-10
Sandell's sensitivity (µg/cm^2/0.001 Absorbance Unit)	0.056	0.018
Regression equation (y = a + bc)	y = 0.001x + 0.009	y = 0.064x – 0.117
Slope (b)	0.0016	0.0643
Intercept (a)	0.009	-0.117
Correlation coefficient (r^2)	0.999	0.998
LOD[a] (µg/ml)	0.628	0.28
LOQ[b] (µg /ml)	1.90	0.86
Repeatability (% RSD[c], n =6)	0.21	0.146
Precision (%RSD, n = 3)		
Interday	0.61-1.77	0.19-1.73
Intraday	0.11-0.84	0.17-1.59
Accuracy ± S.D[d]. (%Recovery, n= 5)	98.40 ± 0.48	98.59 ± 0.46

[a]LOD = Limit of detection, [b]LOQ = Limit of quantification, [c]RSD = Relative standard deviation. [d]S. D. = Standard deviation

Table 2: Recovery Data of RIFA and PIPE by Spectrophotometric Method

Drug	**Amount taken (µg/ml)**	**Amount added (%)**	**%Recovery ± S. D. (n=5)**
RIFA	20	50	98.26 ± 0.27
	20	100	98.42 ± 0.66
	20	150	98.54 ± 0.52
PIPE	2	50	98.11 ± 0.48
	2	100	98.80 ± 0.65
	2	150	98.88 ± 0.26

Table 3: Analysis of RIFA and PIPE by Spectrophotometric Method

Capsule	Label Claim (mg)		Amount Found (mg)		% Label Claim ± S.D. (n=6)	
	RIFA	PIPE	RIFA	PIPE	RIFA	PIPE
I	200	10	201.20	10.13	100.6 ± 0.78	101.30 ± 1.23

The recovery experiment was performed by the standard addition method. The recoveries of RIFA and PIPE were found to 98.40 ± 0.48 and 98.59 ± 0.46 for Rifampicin and Piperine, respectively. The results of recovery studies indicate that the proposed method is highly accurate Table 2. The validation parameters are summarized in Table 1. The proposed validated spectroscopic method was successfully applied to combined dosage form (Capsule). The results obtained for RIFA and PIPE were comparable with the corresponding label claim percentage Table 3. No interference of the method was successfully used to determine the amounts of RIFA and PIPE present in capsule. The results obtained are in good agreement with the corresponding labeled amount. By observing the validation parameters, the method was found to be sensitive, accurate and precise. Hence the method can be employed for the routine analysis of these drugs in combinations.

CONCLUSION

The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of RIFA and PIPE in capsule dosage form. The method utilizes easily available and cheap solvent for analysis of RIFA and PIPE hence the method was also economic for estimation of RIFA and PIPE from capsule dosage form. The common excipients and other additives are usually present in the capsule dosage form do not interfere in the analysis of RIFA and PIPE in method, hence it can be conveniently adopted for routine quality control analysis of the drugs in combined pharmaceutical formulation.

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