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FIRST DERIVATIVE 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 First derivative spectrophotometric zero crossing method for the simultaneous determination of Rifampicin and Piperine in combined capsule dosage form. The utility of first derivative data processing program is its ability to calculate unknown concentration of components of interest in a mixture containing an interfering component. The first order derivative absorption at 272 nm (zero cross point for Piperine) was used for Rifampicin and 238 nm (zero cross point for Rifampicin) was used for Piperine. The linearity was obtained in the concentration range of 10-80 µg/ml for Rifampicin and 2-24 µg/ml for Piperine. 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, Derivative spectrophotometric method, Piperine, Drug analysis, Validation, Recovery.

INTRODUCTION

Rifampicin (RIFA) 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.[1] (Figure 1) is a well known Anti-Tuberculosis drug[2]. It is official in IP, BP and USP. 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] and UV Spectrophotometry[22] 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 First derivative spectrophotometric method for simultaneous estimation of both drugs in their combined capsule dosage form.

MATERIALS & METHODS

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.

Methods

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 against solvent methanol as blank. The absorption spectra thus obtained were derivatised from first to fourth order. First order derivative spectrum was selected for the analysis of both the drugs. From the overlain spectra of both the drugs (figure 3) wavelengths selected for quantitation were 272 nm (zero cross point for PIPE) for RIFA and 238 nm (zero cross point for RIFA) for PIPE.

VALIDATION OF THE PROPOSED METHOD

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[23].

LINEARITY (CALIBRATION CURVE)

Appropriate aliquots from the standard stock solutions of RIFA and PIPE were used to prepare two different sets of dilutions: Series A, and B 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 (1, 2, 3, 4, 5 and 6 ml). Series B consisted of varying concentrations of PIPE (2-20 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 to get final concentration in range of 2-24 µg/ml (0.2, 0.4, 0.8, 1.2, 1.6 and 2.0 ml). The calibration curve were constructed by plotting drug concentration versus the absorbance values of first derivative spectrum 272 nm fir RIFA and 238 nm for PIPE. Statistical data for calibration curves is depicated in Table 1. The concentration of individual drugs present in the mixture was determined from the calibration curves in quantitation mode.

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, 8, 16 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 prequantified sample solutions of RIFA and PIPE (20 µg/ml for RIFA and 8 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 were recorded at selected wavelengths for determination of RIFA and PIPE. The analysis procedure was repeated three times capsule formulaton.

RESULTS AND DISCUSSION

The standard solutions of RIFA and PIPE were scanned separately in the UV range and First-order spectra for RIFA and PIPE were recorded. The first order derivative absorption at 272 nm (zero cross point for PIPE) was used for Rifampicin and 238 nm (zero cross point for RIFA) was used for Piperine. These two wavelengths can be employed for the determination of RIFA and PIPE without any interference from the other drug in their combined formulations.

Linear correlation was obtained between absorbances and concentrations of RIFA and PIPE in the concentration ranges of 10-60 µg/ml and 2-20 µg/ml, with R^2 value 0.999 at both the wavelength respectively. 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 0.416% at 272 nm. The RSD value of PIPE was found to be 0.483% at 238 nm. Relative standard deviation was less than 2 %, which indicates that proposed method is repeatable. The low RSD values of interday (0.48-1.64% for RIFA at 272 nm and 0.16-1.73% for PIPE at 238 nm, respectively) and intraday (0.13-0.71% for RIFA at 272 nm and 0.19-1.26% for PIPE at 238 nm, respectively) variation for RIFA and PIPE, reveal that the proposed method is precise. LOD and LOQ values for RIFA were found to be 2.04 and 6.20 µg/ml at 272 nm, respectively. LOD and LOQ values for PIPE were found to be 0.42 and 1.28 µg/ml at 238 nm, 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.

The recovery experiment was performed by the standard addition method. The recoveries of RIFA and PIPE were found to 99.84 ± 0.36 and 100.26 ± 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 excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of RIFA and PIPE in pharmaceutical dosage forms.

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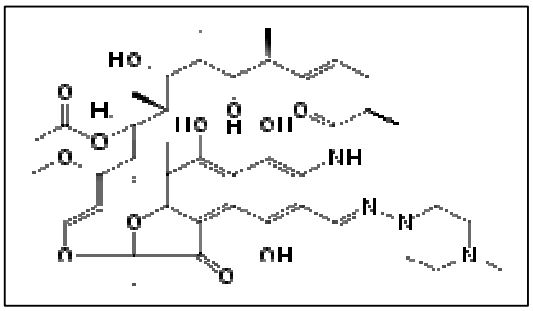

Figure 1: Structure of Rifampicin

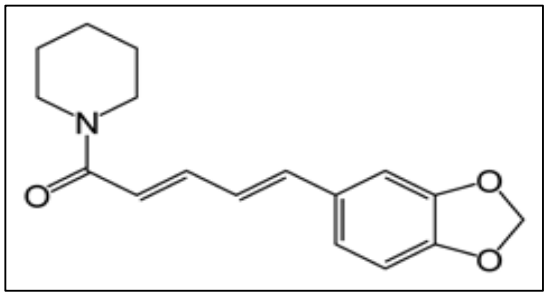

Figure 2: Structure of Piperine

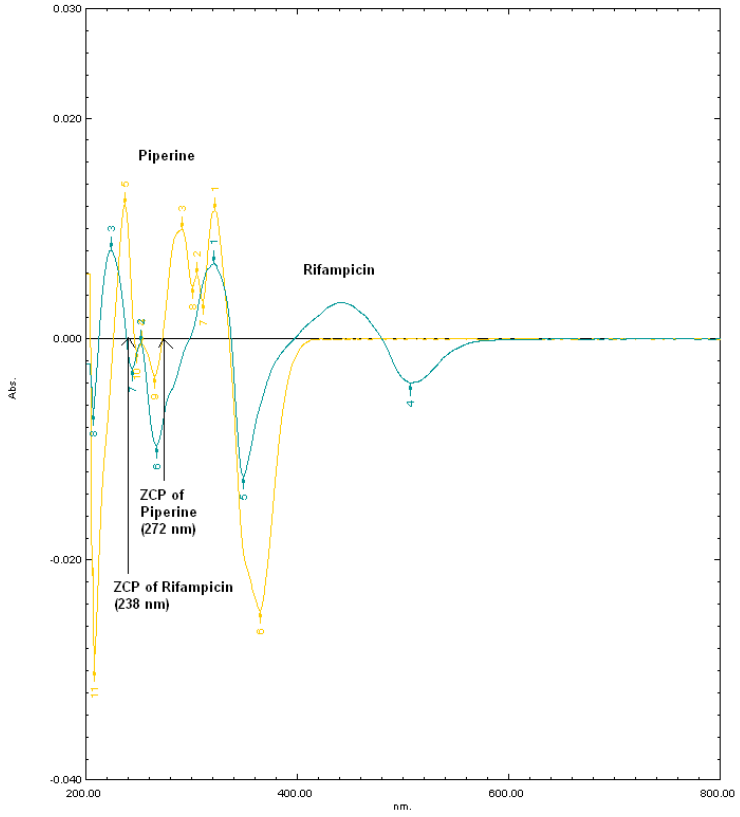

Figure 3: Overlain First order Derivative absorption spectra of RIFA and PIPE in methanol

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.

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

Parameters	RIFA	PIPE
Wavelength (nm)	272	238
Beer's law limit (µg /ml)	10-60	2-20
Regression equation (y = a + bc)	y = 0.0009x – 0.0011	y = 0.0009x – 0.0013
Slope (b)	0.000933	0.000888
Intercept (a)	– 0.0011	– 0.0013
Correlation coefficient (r^2)	0.999	0.999
LOD[a] (µg/ml)	2.04	0.42
LOQ[b] (µg /ml)	6.20	1.28
Repeatability (% RSD[c], n =6)	0.416	0.483
Precision (%RSD, n = 3)		
Interday	0.48-1.64	0.16-1.73
Intraday	0.13-0.71	0.19-1.26
Accuracy ± S.D[d]. (%Recovery, n= 5)	99.84 ± 0.36	100.26 ± 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	99.78 ± 0.17
	20	100	99.91 ± 0.54
	20	150	99.83 ± 0.38
PIPE	8	50	100.11 ± 0.44
	8	100	100.80 ± 0.69
	8	150	99.88 ± 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	198.30	9.93	99.15 ± 0.42	99.30 ± 0.48

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