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Spectrophotometric simultaneous determination of atazanavir and ritonavir in combined tablet dosage form by ratio derivative and area under curve method

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ABSTRACT

Simple, precise, rapid and accurate methods for simultaneous determination of Atazanavir (ATV) and Ritonavir (RTV) in combined tablet dosage form have been developed. Method A is based on Ratio spectra derivative and method B uses integrated area under curve (AUC), methanol is used as solvent for both the methods. The amplitudes at 280.01 nm and 286.12 nm of the first derivative of ratio spectra were selected to determine ATV and RTV, respectively by ratio derivative method and wavelength ranges of 246.97-252.03 nm and 240.78-244.16 nm were selected to determine ATV and RTV by AUC method in combined formulation. Beer's law is obeyed in the concentration range of 15-75 µg/mL and 5-25 µg/mL for atazanavir and ritonavir, respectively by both the methods. The % assay in commercial formulation was found to be in the range 98.81-100.8 % for ATV and 98.91-101.2 % for RTV by the proposed methods. The methods were validated with respect to linearity, precision and accuracy. Recovery was found in the range of 99.20-99.96% for ATV and 99.12-99.97% for RTV by ratio derivative method and 99.08-99.66% for ATV and 99.78-100.6% for RTV by AUC method. The methods developed are simple, economical, precise and accurate and can be used for routine quality control of analytes in combined tablets.

Key words: Atazanavir, Ritonavir, Ratio Spectra Derivative Spectrophotometry, Area Under Curve.

INTRODUCTION

Atazanavir (ATV), is a recently introduced azapeptide inhibitor of HIV-1 Protease. It is formulated as 1:1 sulphate salt. The drug was approved by USFDA on June 20, 2003 [1]. Atazanavir sulphate, chemically[1] is (3S,8S,9S,12S) - 3,12-Bis (1,1-dimethylethyl) - 8-hydroxy - 4,11– dioxo - 9- (phenylmethyl) -6- [[4- (2-pyridinyl) phenyl] methyl] -2,5,6,10,13 - pentaazatetradecanedioic acid dimethyl ester, sulphate (1:1) [1].

Ritonavir (RTV), is chemically known as 2,4,7,12- tetra azatridecan- 13oicacid, 10 hydroxy- 2-methyl- 5- (1- methyl ethyl)- 1- [2- (1-methylethyl)- 4-thiazolyl]- 3,6-dioxo- 8,11-bis(phenyl methyl)- 5-thiazolmethyl ester and its empirical formula is C37H48N6O5S2 with a molecular weight of 720.90. Both the drugs were used as antiretroviral agents. [2, 3]. Few analytical techniques such as spectrophotometry [4-8], HPLC [9-11] have been reported for ATV as single drug formulation of in combination with other drugs. Few spectrophotometry [12-14], HPLC [15-18], HPTLC [19] and bioanalytical methods [20, 21] are available for RTV for its estimation in bulk and formulation. Ratio Spectra Derivative and Area Under Curve these method are suitable for these drug. The method was validated for linearity, accuracy, precision, sensitivity, robustness, etc. in accordance with International Conference on Harmonization (ICH) guidelines [22].

MATERIALS AND METHODS

Instrumentation

An UV-Visible double beam spectrophotometer (Varian Cary 100) with 10 mm matched quartz cells was used. Electronic balance (Model Shimadzu AUW-220D) was used for weighing.

Reagents and Chemicals

Pure drug sample of ATV, % purity 99.86 and RTV, % purity 99.92 were kindly supplied as a gift sample by Cipla Pvt. Ltd. Patalganga, Dist: Raigad. These samples were used without further purification. Spectroscopy grade methanol was used throughout the study. Synthivan tablets (T1) manufactured by Cipla Pvt. Ltd. And laboratory prepared tablets (T2) each containing 300 mg of ATV and 100 mg of RTV were used for analysis.

Preparation of Standard Stock Solutions and Calibration Curve

Standard stock solutions of pure drug containing 1000 µg/mL of ATV and RTV were prepared separately in methanol. Standard stock solutions were further diluted with methanol to get working standard solutions of analytes in the concentration range of 15-75 µg/mL and 5-25 µg/mL of Atazanavir (ATV) and Ritonavir (RTV), respectively and scanned in the range of 200-400nm. For method A first derivative amplitudes (at interval 1.2 and filter size 9) of ratio spectra were measured at 280.01 nm and 286.12 nm for ATV and RTV, respectively. First derivative amplitudes of ratio spectra and concentrations were used to construct calibration curve. For method B integrated area under curve was obtained between wavelength ranges of 246.97-252.03 nm and 240.78-244.16 nm. Integrated area under curve was used to construct two simultaneous equations and these equations were solved and used (equation 3 and 4) to calculate amount of analytes in sample solutions.

Preparation of Sample Solution and Formulation Analysis

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 300 mg of ATV (RTV 100 mg) was weighed and dissolved in the 30 mL of methanol with the aid of ultrasonication for 7 min and solution was filtered through Whatman paper No. 41 into a 100 mL volumetric flask. Filter paper was washed with solvent, adding washings to the volumetric flask, volume was made up to the mark with methanol. The solution was suitably diluted with methanol to get 45 µg/mL ATV and 15 µg/mL of RTV.

Theoretical Aspects

Method A: Ratio Derivative

The method involves dividing the spectrum of mixture by the standardized spectra of each of the analyte to get ratio spectra. First derivative of ratio spectra was obtained which

was independent of concentration of divisor (Fig. 2). Using appropriate dilutions of standard stock solution, the two solutions were scanned separately. The ratio spectra of different ATV standards at increasing concentrations were obtained by dividing spectra of standard mixture with the stored spectrum of RTV (15 µg/mL, Fig 2) solution. Wavelength 280.01 nm was selected for the quantification of ATV in ATV + RTV mixture. The ratio and ratio derivative spectra of the solutions of RTV at different concentrations were obtained by dividing each with the stored standard spectrum of the ATV (45µg/mL, Fig 3). Wavelength 286.12 nm was selected for the quantification of RTV in ATV + RTV mixture. Measured analytical signals at these wavelengths were proportional to the concentrations of the drugs over the selected concentration range. Calibration curves were prepared from the measured signals at the selected wavelength and concentration of the standard solutions. The concentrations of ATV (C_{ATV}) and RTV (C_{RTV}) in sample solution were calculated by using equations (1) and (2), respectively.

At 280.01 nm: C_{ATV} = (ATV Ratio derivative amplitude –0.0008)/0.0712.... (1)
At 286.12 nm: C_{RTV} = (RTV Ratio derivative amplitude – 0.0478)/1.234.… (2)

Method B: Area Under Curve

For the simultaneous determination using the AUC method, suitable dilutions of the standard stock solutions (1000 µg/mL) of ATV and RTV were prepared separately in methanol and further diluted with methanol to make appropriate conc. range. The solutions of drugs were scanned in the range of 200-400 nm, the zero order overlain spectra of the analytes are shown in Fig 1. Integrated area between the selected wavelengths, 246.97-252.03 nm (λ_1-λ_2) and 240.78-244.16nm (λ_3-λ_4) was used to prepare calibration curve. Mixed standards were prepared and their integrated area under the curve was measured at the selected wavelength ranges. Concentration of ATV and RTV in mixed standard and the sample solution were calculated using equation 3 and 4, respectively.

$C_{ATV} = A_2 \times a_{y1} - A_1 \times a_{y2} / a_{X2} \times a_{Y1} - a_{X1} \times a_{Y2}$ …… (3) and
$C_{RTV} = A_2 - a_{X2} \times C_{ATV} / a_{Y2}$ ……... (4)

Where,
a_{X1} (112.1) and a_{X2} (95.9) are the absorptivities of ATVs at (λ1-λ2) and (λ3-λ4), respectively.
a_{Y1} (87.0) and a_{Y2} (72.5) are the absorptivities of RTV at (λ1-λ2) and (λ3-λ4), respectively.

A_1 and A_2 are absorbances of mixed standard at (λ1-λ2) and (λ3-λ4) respectively. C_{ATV} and C_{RTV} are the concentrations in g/100 mL.

Recovery Studies

The accuracy of the proposed methods was checked by recovery study, by addition of standard drug solution to preanalysed sample solution at three different concentration levels (50 %, 100 % and 150 %) within the range of linearity for both the drugs. The basic concentration level of sample solution selected for spiking of the drugs standard solution was 24 and 8 µg/mL of ATV and RTV, respectively.

Solution Stability

Method stability was checked by analyzing solution kept in fridge and at room temperature by both methods. Solution at room temperature was stable for 12 hours and solution in fridge was stable for 30 days (% RSD < 2).

Precision of the Method

Method repeatability was determined by six times repetitions of assay procedure. For intra-day precision method was repeated 5 times in a day and the average % RSD was determined. Similarly the method was repeated on five different days for inter-day precision and average % RSD was determined (Table 1). Precision of analyst was determined by repeating study by another analyst working in the laboratory.

Table1: Optical characteristics of the proposed methods and result of precision and formulation analysis

Parameter		Atazanavir		Ritonavir	
		Method A	Method B	Method A	Method B
wavelength (nm)		280.01	246.97-252.03	286.12	240.78-244.16
Beer's law limit (µg/mL)		15-75	15-75	5-25	5-25
Regression Equation*	Slope (m)	0.2107	-	0.5964	-
	Intercept (c)	0.791	-	0.732	-
Correlation coefficient (r)		0.9992	-	0.9993	-
Precision (%RSD)	Repeatability (n=6)	0.65	0.73	0.54	0.79
	Intra-day (3x5 times)	1.11	0.72	0.52	1.37
	Inter-day(3x5 days)	1.04	0.91	1.18	0.79
	Analyst	0.72	0.91	0.62	0.83
Formulation Analysis (% Assay, % RSD), n=6	T1	98.81, 0.32	100.8, 0.45	98.91, 0.29	101.2, 0.4
	T2	99.01, 0.46	100.9, 0.38	98.91, 0.51	101.72, 0.3

RSD = Relative Standard Deviation, Y = mX + c, where Y is the absorbance and X the concentration in micrograms per milliliter*

Table 2: Result of recovery studies of ATV and RTV by using formulation

Formulation studied	Recovery Level	Recovery of	Amount Spiked (µg/mL)	% Mean Recovery, % RSD by n=6	
				Method A	Method B
Formulation I	50%	RIV	4	99.97, 7.44	100.2, 11.28
		ATV	12	99.96, 7.90	99.08, 33.44
	100%	RIV	8	99.12, 9.60	100.6, 15.10
		ATV	24	99.34, 10.21	99.66, 44.85
	150%	RIV	12	99.15, 11.80	99.78, 18.71
		ATV	36	99.20, 12.55	99.57, 56.01

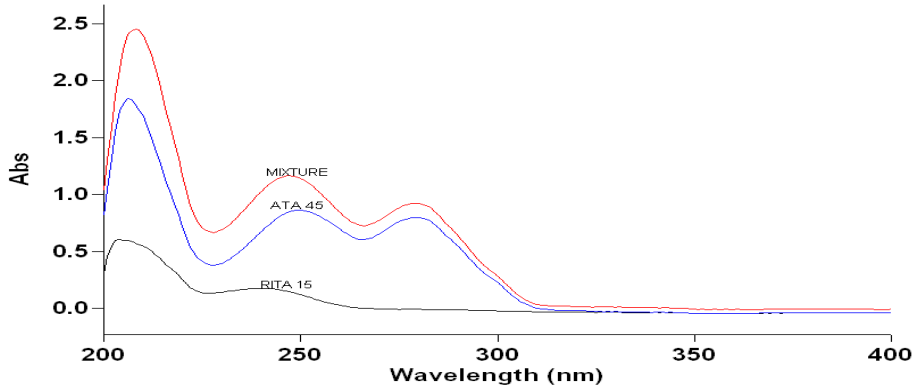

Fig.1: Overlain spectra of Atazanavir (45 µg/mL), Ritonavir (15 µg/mL) and standard mixture in methanol

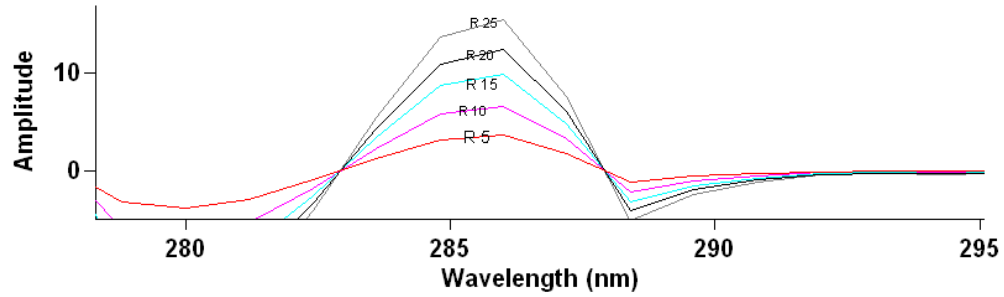

Fig.2: First Derivative of ratio spectra of 5 - 25 µg/ml of RTV when 45 µg/ml of ATV is used as divisor

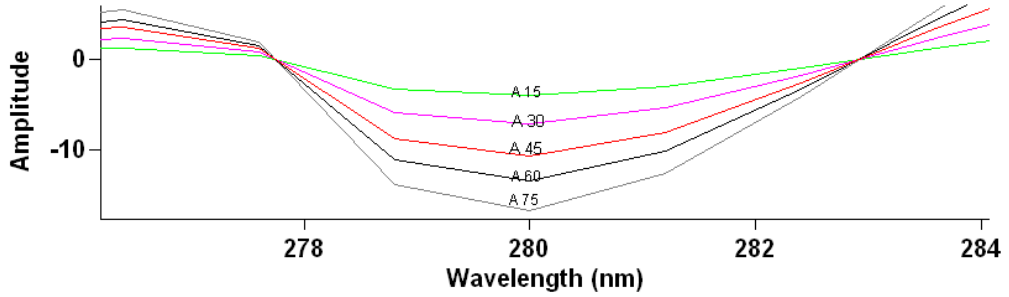

Fig.3: First derivative of ratio spectra of 15 – 75 µg/ml of ATV in mixture, when 15 µg/ml of RTV is used as divisor

RESULTS AND DISCUSSION

Under experimental conditions described, calibration curve, assay of tablets and recovery studies were performed. Using appropriate dilutions of standard stock solution the two solutions were scanned separately. A critical evaluation of proposed method was performed by statistical analysis of data where slope, intercept, correlation coefficient are shown in Table 1. As per the ICH guidelines, the method validation parameters checked were linearity, accuracy and precision. Beer's law is obeyed in the concentration range of 15-75 µg/mL and 5-25 µg/mL for ATV and RTV, respectively. Correlation coefficient was greater than 0.999 for both the drugs. The proposed methods were also evaluated by the assay of commercially available tablets containing ATV and RTV. The results of formulation analysis are presented in Table 1. Recovery was found in the range of 99.20-99.96% for ATV and 99.12-99.97% for RTV by ratio derivative method and 99.08-99.66% for ATV and 99.78-100.6% for RTV by AUC method (Table 2). The accuracy is evident from the data as results are close to 100 % and standard deviation is low. All the statistical data analysis was performed by using MIP Pharmasoft 1.0, software developed and validated in the Institute.

CONCLUSION

The validated spectrophotometric method employed here proved to be simple, economical, precise and accurate. Thus it can be used as IPQC test and for routine simultaneous determination of atazanavir and ritonavir in tablet dosage form.

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