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Simultaneous spectrophotometric estimation of Atazanavir sulfate and Ritonavir in tablets

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ABSTRACT

Two simple, sensitive, rapid spectrophotometric methods have been developed for simultaneous estimation of Atazanavir Sulfate (ATV) and Ritonavir (RTV) in tablets. First method involves solving simultaneous equations based on measurement of absorbance at two wavelengths 249.5 nm and 238.5 nm λ max of ATV and RTV, respectively. Second method is based on area under curve(AUC) and the wavelength ranges selected for analysis were 254.5-244.5 nm for Atazanavir Sulfate and 243.5-233.5 nm for Ritonavir. Beer's law was obeyed in the concentration range of 10-50 μg/ml and 10-50 μg/ml for ATV and RTV, respectively. The methods were validated as per ICH guidelines. Statistical analysis proved that the methods were accurate, precise, and reproducible for analysis of ATV and RTV in tablets. The wide linearity range, sensitivity, accuracy and simple procedure imply that the proposed technique demonstrated to be appropriate for routine analysis and quality control assay of tablets.

Keywords: Atazanavir Sulfate, Ritonavir, simultaneous equation method, area under curve method.

INTRODUCTION

Atazanavir Sulfate, chemically is (3S,8S,9S,12S)-3,12-Bis(1,1-dimethyl ethyl)-8-hydroxy-4,11-dioxo-9-(phenylmethyl)-6-[[4-(2-pyridinyl)phenyl]methyl]-2,5,6,10,13-pentaazatetrade anedioic acid dimethyl ester;1-[4-(pyridine-2-yl)phenyl]-5S,2,5-bis[[N-(methoxy carbonyl)-L-tert-leucinyl]amino]-4S hydroxyl-6-phenyl-2-azahexane.[1] It is an oral antiretroviral Protease inhibitors used in the treatment of HIV/AIDS.[2] Ritonavir chemically is (5S,8S,10S,11S)-10-hydroxy-2-methyl-5-(1-methyl ethyl)-1-[2-(1-methyl ethyl)-4-thiazolyl]- 3,6-dioxo-8,11-bis (phenylmethyl)-2,4,7,12-tetraazatridecan-13-oic acid 5-thiazolyl methyl ester.[3] RTV are antiretroviral drugs specifically belongs to protease inhibitors class.[4] Literature survey revealed spectrophotometric and chromatographic methods reported for estimation of ATV[5] and RTV[6-10] individually or in combination with other drugs. However, there is no analytical method reported for the simultaneous determination of these drugs in a pharmaceutical formulation. Present work describes simple, rapid, accurate and precise method for simultaneous

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determination of ATV and RTV in tablets. The proposed methods were validated as per ICH guidelines.[11]

MATERIALS AND METHODS

Standard gift samples of Atazanavir Sulfate and Ritonavir were provided by Emcure Pharmaceuticals Limited, Pune-411026 respectively. Combined dose tablet formulation containing Atazanavir Sulfate (300 mg) and Ritonavir (100 mg), manufactured by Emcure Pharmaceuticals Limited, were purchased from local market. Methanol- AR was used as solvent.

Instruments
A double-beam Shimadzu UV- Visible spectrophotometer, 1700 Pharmaspec, with spectral bandwidth of 2 nm, wavelength accuracy $\pm$ 0.5 nm and a pair of 1-cm matched quartz cells was used to measure absorbance of solution.

Preparation of Standard Stock Solution:
Stock Solution A-Accurately weighed quantity of ATV (5 mg) was transferred to 50.0 ml volumetric flask.Then 15.0 ml of methanol was added and ultrasonicated for 5 min and diluted up to the mark with methanol.(Concentration: 100μg/ml).

Stock Solution B- Accurately weighed quantity of ATV (5 mg) was transferred to 50.0 ml volumetric flask.Then 15.0 ml of methanol was added and ultrasonicated for 5 min and diluted up to the mark with methanol. (Concentration: 100μg/ml).

Method I: Simultaneous Equation Method
For the selection of analytical wavelength, standard solution of ATV (10 μg/ml) and RTV (10 μg/ml) were prepared separately by appropriate dilution of standard stock solution with methanol and scanned in the entire UV range to determine λ max of both the drugs. The λ max of ATV and RTV were found to be 249.5 nm and 238.5 nm, respectively. A series of standard solutions were prepared having concentration range of 10-50 μg/ml for ATV and 10-50 μg/ml for RTV. The absorbance of resulting solutions was measured at 249.5 nm and 238.5 nm and calibration curves were plotted. Both the drugs obeyed linearity in the concentration range under study. Absorptivity values were then determined for both the drugs at selected wavelengths. Two simultaneous equations (in two variables C1 and C2) were formed using absorptivity coefficient values obtained and are as follows:

$$A_1 = 19.59C_1 + 7.81C_2 \tag{1}$$
$$A_2 = 14.28C_1 + 11.30C_2 \tag{2}$$

Where, A_1 and A_2 are the absorbance of sample solution at 249.5 nm and 238.5 nm respectively. C1 and C2 are the concentrations of ATV and RTV (in mg/ml), in sample solutions. Absorptivity values 19.59 & 14.28 are of ATV at 249.5 nm and 238.5 nm, respectively. Similarly, 7.81 & 11.30 are absorptivity values of RTV at 249.5 nm and 238.5 nm, respectively. By applying the Cramer's rule to equation 1 and 2, the concentration C_{ATV} and C_{RTV}, can be obtained as follows,

$$C_{ATV} = \frac{A_2(7.81) - A_1(11.30)}{0.109} \tag{3}$$

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$$C_{RTV} = \frac{A_1(14.28) - A_2(19.59)}{0.109} \qquad (4)$$

Method II: Area Under Curve Method

From the overlain spectra of drugs (Fig.1),AUC in the range of 254.5-244.5 nm(for ATV) and 243.5-233.5 nm (for RTV) were selected for the analysis. The calibration curves for ATV and RTV were plotted in the concentration range of 10-50 μg/ml and 10-50 μg/ml ,respectively. The 'X' values for both the drugs were determined at the selected AUC range. The 'X' value is the ratio of AUC at selected wavelength ranges with the concentration of component in g/lit. A set of two simultaneous equations obtained by using mean'X' values are as follows:

A1 = 206.8C1 + 68.00C2 (at λ254.5-244.5nm) (5)
A2 = 156.3C1 + 113.1C2 (at λ243.5-233.5nm) (6)

Where A1 and A2 are AUC of sample solution at the wavelength ranges 254.5-244.5 nm and 243.5-233.5 nm, respectively. The 'X' values 206.8 and 156.3 are of ATV at wavelength range 254.5-244.5 nm and 243.5-233.5 nm, respectively. Similarly, 68.00 and 113.1 are 'X 'values of RTV at wavelength range 254.5-244.5 nm and 243.5-233.5 nm, respectively. The concentration of ATV and RTV in sample solution was determined by using the equations (5) and (6).

Table No – 1: Result of Analysis of Tablets

Method	Drug	Label Claim (mg/tablet)	Amount Found* (mg/tab)	Label Claim* (%)	S. D. (±) , R.S.D.
I	ATV	300	299.46	99.80	0.3436, 0.1403
	RTV	100	98.54	98.74	1.3950, 0.5697
II	ATV	300	299.4	99.80	0.7483, 0.3055
	RTV	100	101.2	101.2	1.960, 0.800

Mean of six determinations, ATV – Atazanavir, RTV - Ritonavir, S.D – Standard Deviation, R.S.D – Relative standard deviation

Analysis of Tablet Formulation by Proposed Method

For the estimation of drugs in the commercial formulation, twenty tablets were weighed accurately. The average weight was calculated and then crushed to obtain fine powder. A quantity of tablet powder equivalent to about 5 mg of ATV and 5 mg of RTV was transferred to 50.0 ml volumetric flask; 25 ml methanol was added and sonicated for 20 min, volume was then made up to the mark with methanol. The resulting solution was mixed and filtered through Whatmann filter paper and filtrate was appropriately diluted to get approximate concentration of 30 μg/ml of ATV and 10μg/ml of RTV. In method I, the concentration of both ATV and RTV were determined by measuring absorbance of sample solution at 249.5 nm & 238.5 nm and using equations (3) and (4).In method II, concentration of both ATV and RTV was determined by measuring AUC in the range of 254.5-244.5 nm (for Atazanavir Sulfate) and 243.5-233.5nm (for Ritonavir) and values were substituted in equations (5) and (6) to obtain concentration of both the drugs. Results of tablet analysis are shown in Table No. 1.

Validation

The proposed methods were validated as per ICH guidelines.

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Accuracy

To ascertain the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100% & 120%). The results of recovery studies, expressed as percent recovery, were satisfactory and are presented in Table No.2

Table No – 2: Result of Recovery Studies

Level of recovery	Method-I				Method-II			
	Amt of drug recovered		Percent Recovery		Amt of drug recovered		Percent Recovery	
	ATV	RTV	ATV	RTV	ATV	RTV	ATV	RTV
80%	536.7	172.99	99.38	98.10	541.8	182.4	100.3	101.3
	536.8	179.79	99.40	99.88	538.2	182.4	99.66	101.3
	539.1	176.49	99.83	98.05	540.6	181.2	101.1	100.6
100%	596.5	198.6	99.43	99.34	603.6	198.6	100.6	99.3
	594.0	202.5	99.00	101.00	600.6	200.4	100.1	100.2
	596.2	198.9	99.38	99.45	601.2	202.8	100.2	101.4
120%	657.1	217.9	99.57	99.09	658.2	219.0	99.72	99.54
	656.1	219.9	99.44	99.95	659.4	218.4	99.90	99.27
	655.9	221.2	99.39	100.58	659.4	220.2	99.90	100.0
Mean % recovery			99.42	99.49			100.16	100.32
S. D.			0.2156	1.001			0.4583	0.8684
C.V.			0.2168	1.0061			0.4575	0.8656

ATV – Atazanavir, RTV –Ritonavir , S.D. – Standard deviation,
C.V. -– Coefficient of variance

Precision

The reproducibility of the proposed methods was determined by analyzing tablets at different time intervals on same day in triplicates (Intra-day assay precision) and on three different days (Inter-day assay precision). Coefficient of variance for intra-day assay precision was found to be 0.1496 (for ATV) & 0.6149 (for RTV) in simultaneous equation method and 0.3692 (for ATV) & 1.1817 (for RTV) in area under curve method. Inter-day assay precision coefficient of variance was found to be 0.4367 (for ATV) & 0.9804 (for RTV) in simultaneous equation method and 0.3768 (for ATV) & 1.3125 (for RTV) in area under curve method.

RESULTS AND DISCUSSION

The methods discussed in the present work provide a convenient and reliable way for quantitative determination of ATV and RTV in combined dose tablet formulation. Wavelength of maximum absorbance for ATV (249.5 nm) and RTV (238.5 nm) were selected for analysis by simultaneous equation method (method I). In AUC method (Method II) quantitative determination was carried out at wavelength range 249.5 nm (for ATV) and 238.5 nm (for RTV) Linearity for ATV and RTV was observed in the concentration range of 10-50 μg/ml and 10-50 μg/ml, respectively for both the methods. Percent label claim for ATV and RTV in tablet analysis, by both the methods, was found in the range of 99.00 to 101.1 %. Percent recovery for ATV and RTV, by both methods, was found in the range of 99.05 % to 101.4 % with standard deviation well below 2 indicating accuracy of the methods. Intra-day and Inter-day precision studies were carried out by analyzing tablet formulation, by both methods, three times on the same day and on three different days, respectively. Standard deviation and coefficient of variance for intra-day and inter-day precision studies was satisfactorily low indicating high degree of precision and reproducibility of proposed methods.

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CONCLUSION

Based on the results obtained, it can be concluded that the proposed UV- Spectrophotometric method (simultaneous equation method and AUC) for simultaneous determination of ATV and RTV is rapid, economical, accurate, precise and reproducible. The utility of the developed methods have been demonstrated by analysis of combined dose tablet formulation. Hence, the proposed method can be used for quantitative determination of these ingredients in combined dose tablet formulation.

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