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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF SIMULTENIOUS ESTIMATION OF ATAZANAVIR AND RITONAVIR IN BULK AND PHARMACEUTICAL DOSAGE FORMS BY USING RP-HPLC

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ABSTRACT

A simple, rapid reverse phase high-performance liquid chromatographic method was developed and validated for the simultaneous estimation of Atazanavir and Ritonavir in bulk and pharmaceutical dosage forms. Chromatography was carried out by using Agilent XDB, 150 X 4.6 mm, 5µ internal diameter with a mixture of Buffer : acetonitrile in the ratio of 45:55 (v/v) as mobile phase. Determination of the different analytical parameter such as linearity, accuracy, precision and specificity, limit of detection (LOD), limit of quantification (LOQ) was done. The calibration curve was found to be linear for each analyte in the desired concentration range. The % recovery was found to be 99.7 and 99.61 for Atazanavir and Ritonavir respectively. The preposed method is highly sensitive, precise, and accurate, which was evident from the LOD value of 2.4 and 2.2 for Atazanavir and Ritonavir respectively and hence the present method can be applied successfully for the quantification of active pharmaceutical ingredient (API) content in the combined formulation of Atazanavir and Ritonavir.

KEYWORDS

Anti retroviral (HIV), Protease inhibitors (PI), Atazanavir, Ritonavir and RP-HPLC.

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INTRODUCTION

Atazanavir (pronounced *at a za na' veer*) [1], marketed under the trade name Reyataz by Bristol Myers, (formerly known as BMS-232632) is anantiretroviral drug of the protease inhibitor (PI) class. Like other antiretrovirals, it is used to treat infection of human immunodeficiency virus (HIV) [2-8].

Atazanavir is distinguished from other PIs in that it can be given once-daily (rather than requiring multiple doses per day) and has lesser effects on the patient's lipid profile (the amounts of cholesterol and other fatty substances in the blood). Like other protease inhibitors, it is used only in combination with other HIV medications.

The U.S. Food and Drug Administration (FDA) approved atazanavir on June 20, 2003. Atazanavir is the first PI approved for once-daily dosing, and also appears to be less likely to cause lipodystrophy and elevated cholesterol as side effects. It may also not be cross-resistant with other PIs. When boosted with ritonavir it is equivalent in potency to lopinavir for use in *salvage* therapy in patients with a degree of drug resistance, although boosting with ritonavir reduces the metabolic advantages of atazanavir. Atazanavir is a methyl *N*-[(1*S*)-1-{[(2*S*, 3S)-3 hydroxy-4-[(2*S*)-2-[(methoxycarbonyl) amino]-3,3-dimethyl-*N'*-{[4-(pyridin-2yl)phenyl]methyl} butanehydrazido]-1-phenylbutan-2-yl]carbamoyl}-2,2 dimethylpropyl]carbamate (Figure No.1).

Ritonavir, with trade name Norvir (Abbott Laboratories), is an antiretroviral drug from the protease inhibitor class used to treat HIV infection and AIDS[9-15].

Ritonavir is frequently prescribed with HAART, not for its antiviral action, but as it inhibits the same host enzyme that metabolizes other protease inhibitors. This inhibition leads to higher plasma concentrations of these latter drugs, allowing the clinician to lower their dose and frequency and improving their clinical efficacy.

Ritonavir is a peptidomimetic inhibitor of both the HIV-1 and HIV-2 proteases. Inhibition of HIV protease renders the enzyme incapable of processing the gag-pol polyprotein precursor which leads to production of non-infectious immature HIV particles[16-20]. Ritonavir is a 1,3-thiazol-5 yl methyl *N*-[(2*S*,3*S*,5*S*)-3-hydroxy-5-[(2*S*)-3-methyl-2-{[methyl({[2-(propan-2-yl)-1,3-thiazol-4yl] methyl}) carbamoyl] amino} butanamido] - 1,6-diphenylhexan-2-yl]carbamate (Figure No.2).

MATERIALS AND METHODS[21-22]

Chemical reagents

Atazanavir and ritonavir as pure standard reference standards were purchased from the local market, Orthophosphoric acid, acetonitrile, methanol of HPLC grade were purchased from merck specialities private limited, mumbai, india.

Instuments

To develop a high pressure liquid chromatography method for quantitative estimation of atazanavir and ritonavir an isocratic waters 2695 separation model HPLC instrument with Hypersil, 250 x 4.6 mm, 5µ. was used. The instrument with 7 Software Empower V 1.2.2.1 PDA detector 2990. Analytical Balance Afcos, ER-180A, Sartorius- M500P, Meter, AG 104, 2 Microbalance Sartorius-M500P, 3 pH Meter Thermo scientific, 3 pH Meter Thermo scientific.

Mobile phase

Buffer and Acetonitrile taken in isocratic 45:55%v/v.

Buffer

Accurately weighed 0.1% of Ortho Phosphoric acid in 1000ml of volumetric flask added about 900ml of milli-Q water and sonicate to dissolve make up to the final volume pH 3.2.

Preparation of Solutions

Standard Preparation

Accurately Weighed and transferred 15mg of Atazanavir and 5mg of Ritonavir and working Standards into a 100 ml clean dry volumetric flask, add 70ml of diluent , sonicated for 5 minutes and make up to the final volume with diluents.(standard stock) (Table No.1 and Figure No.3).

Sample Preparation

5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 5 tablets was transferred into a 100 mL volumetric flask, 80mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.1ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Label Claim: 300mg of Atazanavir+ 100mg of Ritonavir.

RESULTS AND DISCUSSION

Method and validation

Linearity

The linearity range of atazanavir and ritonavir assay method was determined by preparing and injecting standard solutions of atazanavir and ritonavir (Table No.2, Figure No.4 and Figure No.5).

Precision

The precision of the assay was studied using 6 identical samples with the atzanavir and ritonavir. From the above test repeatability of the combined solution was checked. Peak area and % RSD was calculated from the data obtained in the graphs. Intraday precision Table No.3 and Figure No.6, inter day precision Table No.4 and Figure No.7.

Recovery

The recovery of standard solutions was done taking 50%, 100% and 150% separately. The results were displayed below (Table No.5).

Specificity

Specificity was performed to exclude the possibility of interference with excipients in the region of elution of atazanavir and ritonavir. The specificity and selectivity of the method was tested under normal conditions and the results of the tests proved that the components other than the drug did not produce a detectable signal at the retention place of atazanavir and ritonavir.

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ were determined from standard deviation of y-intercept of regression line and slope method as per ICH guidelines (Table No.6).

Rubustness

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. In this study, the chromatographic parameters monitored were retention time, area, capacity factor, tailing factor and theoretical plates. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above.

Table No.1: Method development

S.No	Peak name	RT	Area	USP plate count	USP tailing
1	Atazanavir	2.291	1303283	3542	1.17
2	Ritonavir	6.494	964618	4214	1.01

Table No.2: Linearity range of Atazanavir and Ritonavir

S.No	Pipetted from stock (mL)	Volume of flask (mL)	Concentration in ppm (Atazana)	Peak areas (Atazana)	Concentration in ppm (Ritonavir)	Areas Ritonavir	% Linearity Level
1	0.25	10	75	629106	25	464841	50
2	0.75	10	112.5	940769	37.5	698709	75
3	1	10	150	1293736	50	958351	100
4	1.5	10	225	1913245	75	1489717	150
5	2.0	10	300	2585527	100	1923185	200

Table No.3: Intraday precision

S.No	Concentration	Atazanavir Peak area	Ritonavir Peak area
1	100ppm	1296864	957958
2	100ppm	1296763	958056
3	100 ppm	1294768	958256
4	100 ppm	1295863	958657
5	100 ppm	1296675	958755
6	100 ppm	1296790	958554
% RSD		0.699	0.6

Table No.4: Interday precision

S.No	Concentration	Atazanavir Peak area	Ritonavir Peak area
1	100ppm	1281925	967725
2	100ppm	1298382	950892
3	100ppm	1305351	961017
4	100ppm	1303039	965595
5	100ppm	1306497	962562
6	100ppm	1298372	965732
% RSD		0.75	0.64

Table No.5: Recovery

S.No	Conc in ppm Atazanavir	% of recovery Atazanavir	Conc in ppm Ritonavir	% of amount recovery Ritonavir
1	50	98.349	50	100.56
2	100	100.259	100	99.321
3	150	100.495	150	98.965
		Avg 99.70%		Avg 99.61%

Table No.6: System suitability and validation parameters

S.No	Parameters	Atazanavir	Ritonavir
1	Theoretical plates	3425	4387
2	Retention time	2.185	6.113
3	Tailing factor	1.19	1.05
4	LOD	2.408486	2.220731
5	LOQ	7.298442	6.729489
6	%RSD	0.7	0.6

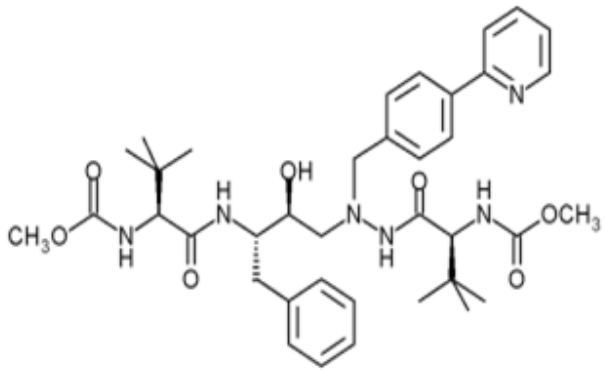

Figure No.1: Structure of Atazanavir

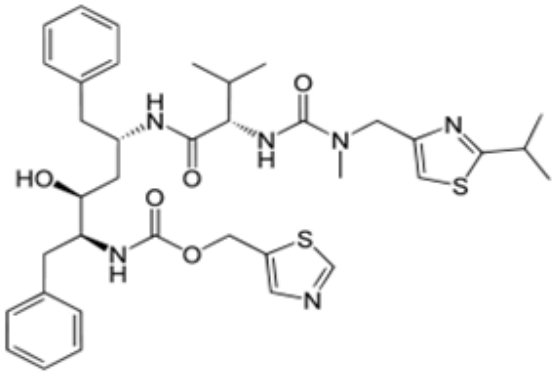

Figure No.2: Structure of Ritonavir

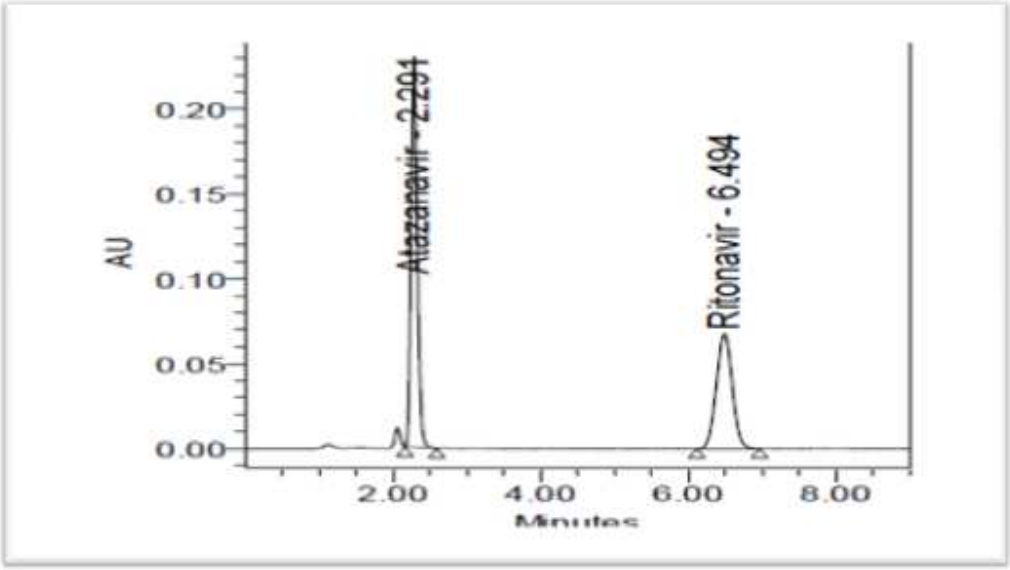

Figure No.3: Method development

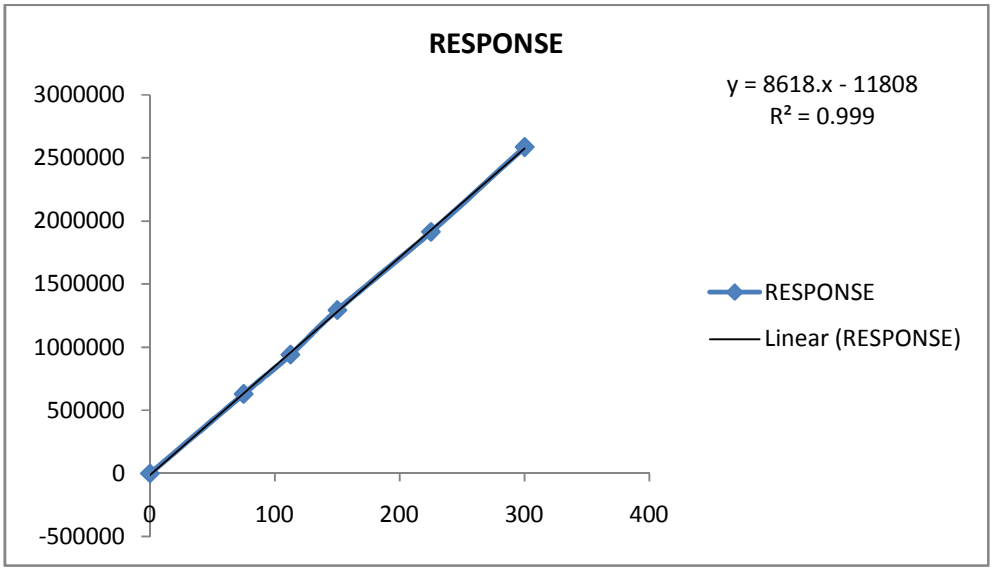

Figure No.4: For Atazanavir

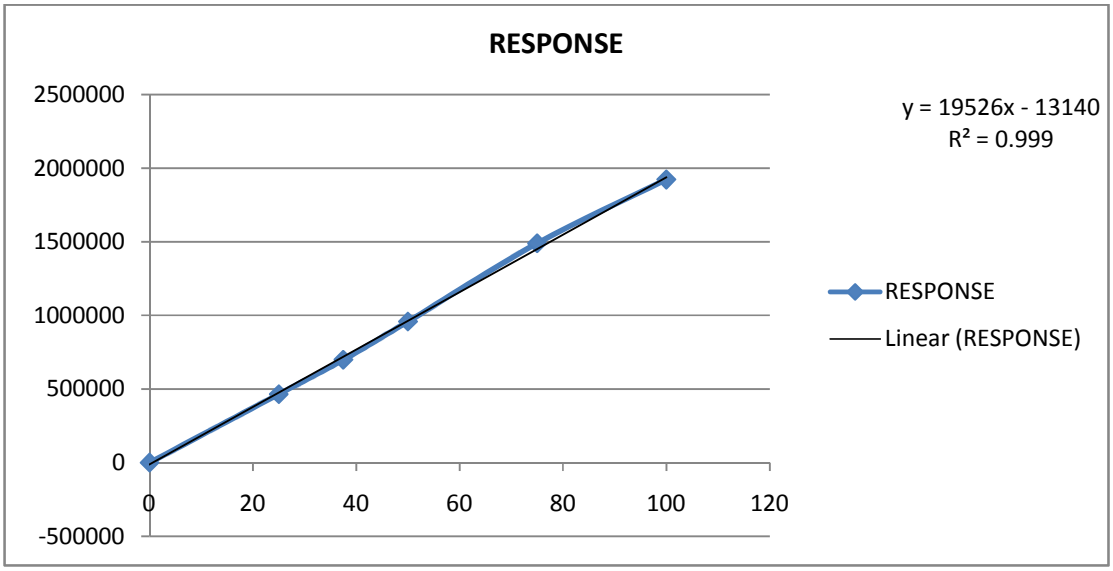

Figure No.5: For Ritonavir

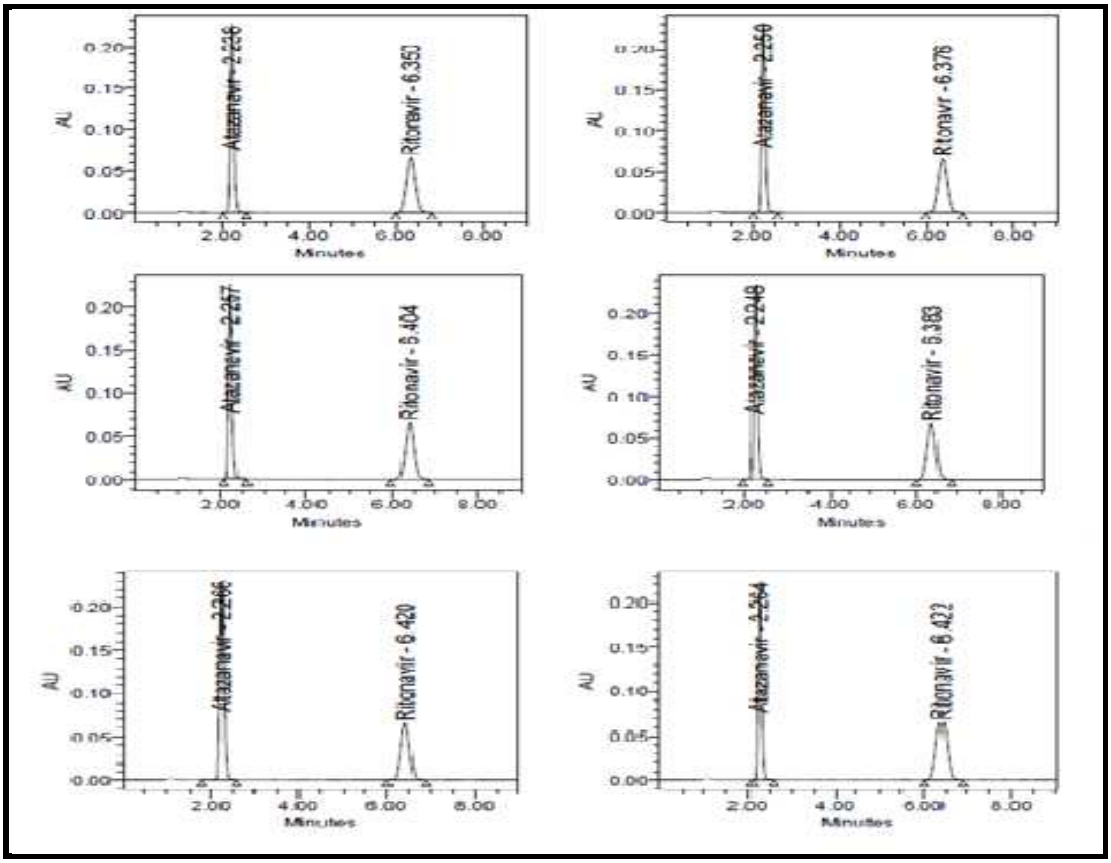

Figure No.6: Intraday precision

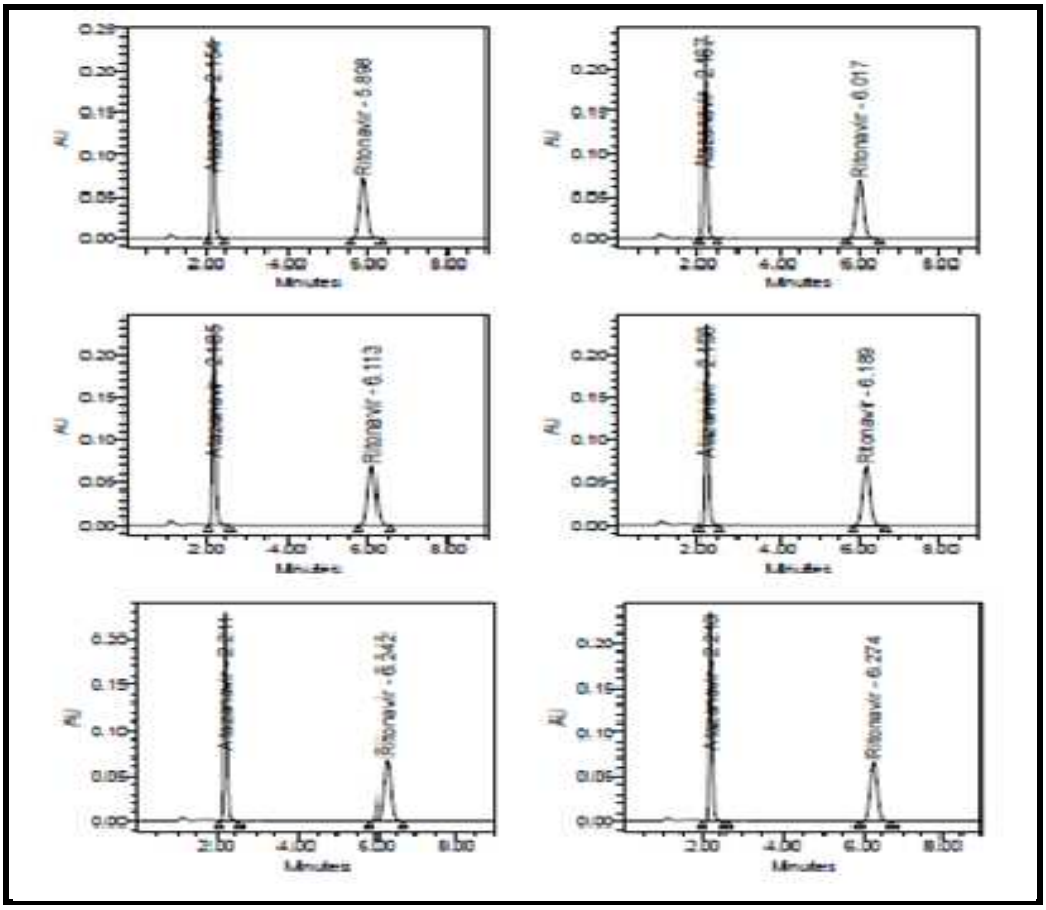

Figure No.7: Interday precision

CONCLUSION

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. In this study, the chromatographic parameters monitored were retention time, area, capacity factor, tailing factor and theoretical plates. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above.

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