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METHOD DEVELOPMENT AND VALIDATION OF ATAZANAVIR AND RITONAVIR IN THE DOSAGE FORM BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

R.Rajesh*, Rakesh K Jat

Institute of Pharmacy, Shri Jagdish Prasad Jhabarmal Tibrewala University, Vidyanagri, Jhunjhunu, Rajasthan-333001

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ABSTRACT

The present work describes a validated reverse phase high performance liquid chromatographic method for the estimation of Atazanavir and Ritonavir in the dosage form.The quantification was carried out using Sunfire C18 (250 mm x 4.6 mm), 5µm and mobile phase comprised of Buffer (pH 2.5) and Acetonitrile in proportion of 50: 50 (v/v).The flow rate was 2.0ml/min and the eluent was monitored at 210nm.The selected chromatographic conditions were found to effectively quantitate Atazanavir and Ritonavir at retention time of about 3.5min and 9min respectively. Linearity were found to be in the range of 29.9792-449.6885 µg/mL for Atazanavir and 9.9939 - 149.9089µg/ml for Ritonavir.The percentage recoveries of all the drugs were found to be 98.0-102.0%.The proposed method were found to be fast,specific,accurate,precise and reproducible and can be used for the estimation of Atazanavir and Ritonavir drug.

Keywords: Atazanavir, Ritonavir, Reversed phase-HPLC.

INTRODUCTION

Atazanavir, which is typically administered with low-dose ritonavir (atazanavir/r), has been an important innovation in the treatment of adult HIV infection owing to its ease of dosing, virologic potency, minimal toxicity, high genetic barrier to resistance, favorable resistance profile and lower effect on lipid and glucose metabolism. Important potential limitations to treatment with Atazanavir/r are interactions with acid-reducing agents and those mediated by low-dose Ritonavir, benign hyperbilirubinemia with jaundice and a rare risk of nephrolithiasis. Atazanavir received US FDA approval for the treatment of adults with HIV-1 infection in 2003 and, since then, it has been widely prescribed throughout the USA

***Corresponding Author:**
R. Rajesh,
Institute of Pharmacy,
SJPJT, University,
Jhunjunu, Rajasthan, India

and Europe. Additionally, Atazanavir/r-based cART has become increasingly available in resource-limited settings. Atazanavir/r is currently being studied in novel HIV treatment strategies, including combinations with a CCR5 inhibitor or an integrase inhibitor, to provide NRTI-sparing treatment options. This article examines the latest reports on the treatment of adults with HIV using atazanavir/r-based cART.

MATERIALS AND METHODS

Reagents and Chemicals:

Atazanavir Sulphate and Ritonavir drug sample, Triethylamine, Orthophosphoric acid, Acetonitrile, Methanol and Water of HPLC grade.

Instruments and Chromatographic Conditions:

HPLC system with UV/PDA detector was used for method development and validation.The separation was achieved on Sunfire C18 (250 mm x 4.6 mm) 5µ.The column was maintained at 30°C and the eluent was monitored at 210nm using UV detector. A mixture of Buffer 2.5 and Acetonitrile 50:50 v/v at flow rate of 2ml/min was used as mobile phase.The injection volume was 20µL.

Preparation of Buffer (pH 2.5)

Pipette out 1 mL of tri-ethylamine into a beaker containing 1000 mL of water and mix well. Adjust the pH to 2.50 ± 0.05 with dilute ortho phosphoric acid. Filter through 0.45µm or finer porosity membrane filter.

Preparation of Mobile phase

Prepare a suitable quantity of degassed mixture of Buffer (pH 2.5) and Acetonitrile in the ratio of 50: 50 (v/v) respectively.

Preparation of Diluent

Use mobile phase as diluent.

Preparation of Standard solution

Accurately weigh and transfer about 114 mg of Atazanavir sulfate working standard (equivalent to 100 mg of Atazanavir) and 34 mg of Ritonavir working standard into a 50 mL volumetric flask. Add about 30 mL of methanol and sonicate to dissolve the content completely. Make up the volume with methanol and mix well. Further pipette out 3 mL of this solution to 20 mL volumetric flask. Make up the volume with diluent and mix well. Filter the solution through 0.45 µm PVDF filter by discarding first 2 mL of filtrate.

Preparation of Sample solution

Determine the average weight not less than 20 tablets.

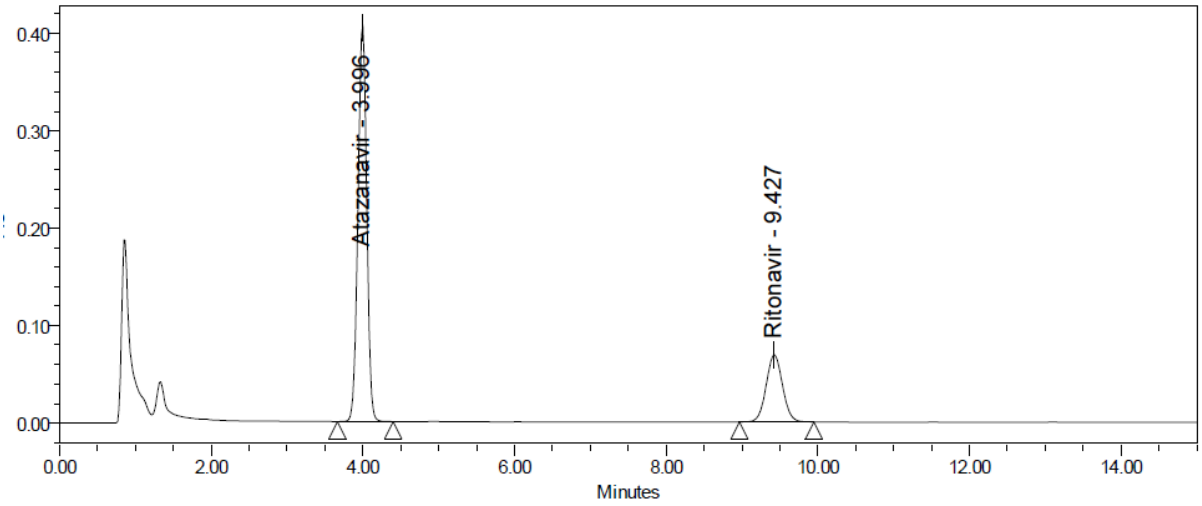
Crush not less than 10 tablets in to fine power with mortar and pestle. Accurately weigh and transfer tablet powder equivalent to 100 mg of Ritonavir to 100 mL volumetric flask. Add about 70 mL of methanol and sonicate for 30 minutes with intermediate shaking. Make up to volume with methanol and mix well. Centrifuge a portion of the above solution for 10 minutes at 5000 RPM. Transfer 5 mL of the supernatant solution in to a 50 mL volumetric flask and dilute with diluent and mix well. Filter the solution through 0.45 µm PVDF filter by discarding first 2 mL of filtrate.

Chromatographic conditions

Column : Sunfire C18 (250 mm x 4.6 mm) 5µ
Column oven temperature : 30°C

Sample temperature : 25°C
Flow Rate : 2 mL / minute
Detection wavelength : 210 nm
Injection volume : 10 µL
Run Time : 15 minutes

Typical Chromatogram of Sample



Validation of RP-HPLC method

1. System Precision

The % RSD for the peak area response of six replicate injection for standard solution found to be 0.3% for Atazanavir and 0.3% for Ritonavir which is within the acceptance criteria of 2.0%

S.No	Area Counts (µV*sec)	
	Atazanavir	Ritonavir
1	3460687	1105098
2	3464314	1106761
3	3469169	1107963
4	3465775	1106466
5	3480168	1111227
6	3489541	1113632
Mean	3471609	1108524
% RSD	0.3	0.3

2. Specificity

There are no any interference or peak observed from blank and placebo chromatogram at the retention time of Atazanavir and Ritonavir peak.

3. Method Precision

The %RSD for %Assay of six replicate sample preparation were found to be 0.5% for Atazanavir and 0.6% for Ritonavir which is within the acceptance criteria of 2.0%.

Sample Number	% Assay	
	Atazanavir	Ritonavir
1	98.7	99.3
2	98.6	98.7
3	97.6	100.2
4	98.3	99.1
5	98.6	99.3
6	97.8	100.3
Mean	98.3	99.5
% RSD	0.5	0.6

4. Accuracy

The accuracy of the method was determined by analyzing solutions of common placebo spiked with Atazanavir and Ritonavir active pharmaceutical ingredient (API) at 10%, 50%, 100% and 150% levels of concentrations in triplicate.

Results obtained for Atazanavir (% Recovery)

Concentration Theoretical (%)	Amount of Atazanavir added (mg)	Amount of Atazanavir Found (mg)	Recovery (%)	Mean % Recovery	%RSD
10% (1)	29.86	30.27	101.4	101.2	0.2
10% (2)	30.23	30.53	101.0		
10% (3)	30.00	30.31	101.1		
50% (1)	150.19	151.97	101.2	101.4	0.3
50% (2)	150.36	152.34	101.3		
50% (3)	150.45	152.94	101.7		
100% (1)	300.90	302.49	100.5	100.5	0.1
100% (2)	300.38	301.58	100.4		
100% (3)	300.47	302.15	100.6		
150% (1)	450.14	448.80	99.7	100.1	0.5
150% (2)	449.97	449.67	99.9		
150% (3)	449.88	453.14	100.7		

Results obtained for Ritonavir (% Recovery)

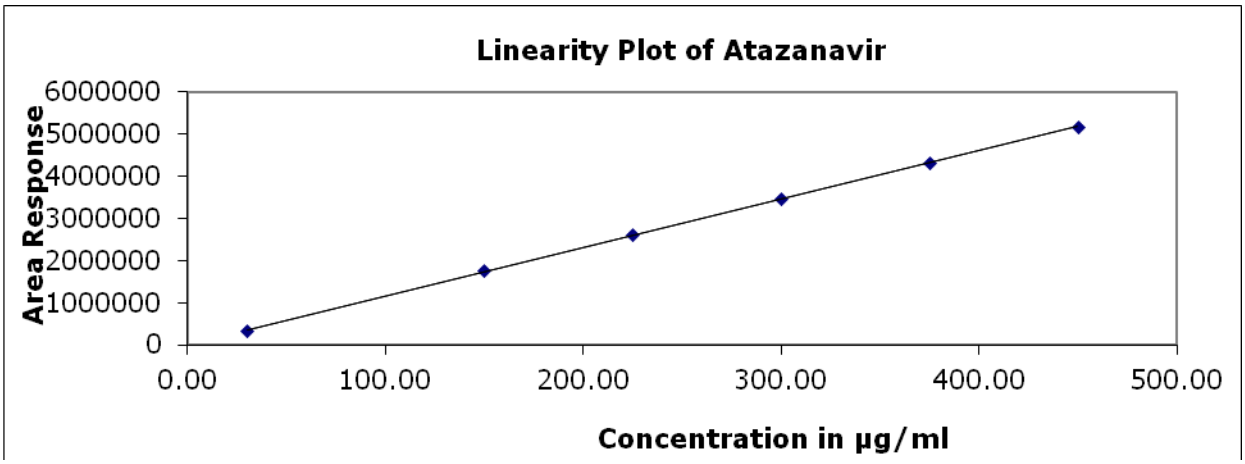
Concentration Theoretical (%)	Amount of Ritonavir added (mg)	Amount of Ritonavir Found (mg)	Recovery (%)	Mean % Recovery	%RSD
10% (1)	9.98	10.02	100.4	100.3	0.1
10% (2)	10.11	10.14	100.3		
10% (3)	10.10	10.13	100.3		
50% (1)	50.19	50.12	99.9	100.1	0.2
50% (2)	50.03	50.02	100.0		
50% (3)	50.05	50.17	100.3		
100% (1)	101.34	101.21	99.9	99.8	0.1
100% (2)	100.04	99.89	99.8		
100% (3)	99.94	99.73	99.8		
150% (1)	149.71	149.09	99.6	99.8	0.5
150% (2)	150.11	149.33	99.5		
150% (3)	150.81	151.38	100.4		

5. Linearity

Linearity was performed on 6 concentration level considering 29.9792 µg/mL to 449.6885 µg/mL of Atazanavir and 9.9939 µg/mL to 149.9089 µg/mL for Ritonavir.The correlation co-efficient found to be 0.99997 for Atazanavir and 0.99997 for Ritonavir respectively.

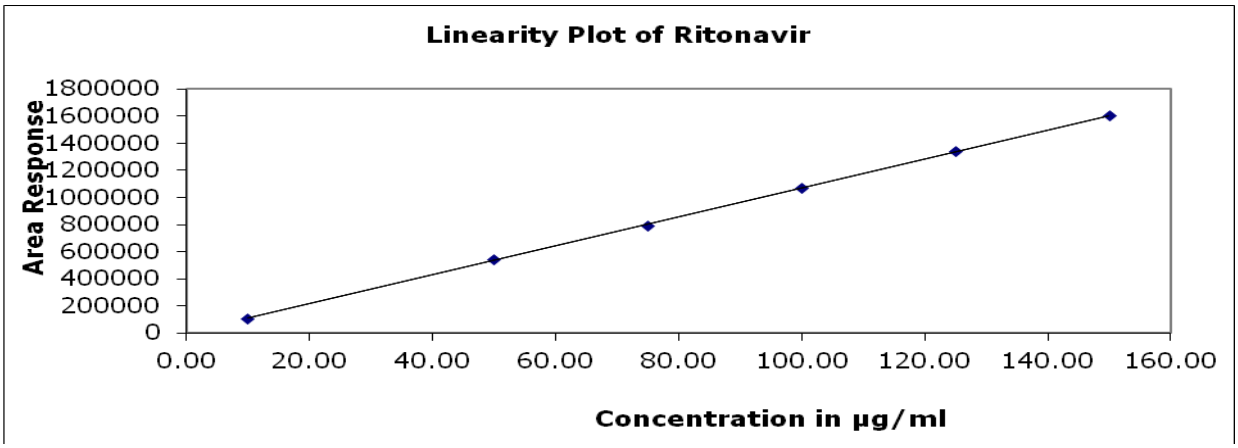
S. No.	Linearity level	Atazanavir Concentration (µg/mL)	Area (µV*sec)
1	Level-1 -20%	29.9792	351262
2	Level-2 -40%	149.8961	1764276
3	Level-3 -80%	224.8442	2614551
4	Level-4 -100%	299.7923	3479450
5	Level-5 -120%	374.7404	4330451
6	Level-6 -150%	449.6885	5175917
		Correlation coefficient (r)	0.99997
		Slope	11484.9893
		Intercept	26006.5668
		Residual sum of squares	1005888523.8933
		% Deviation of the Y-intercept	0.7

Results obtained for Linearity (Atazanavir)



Results obtained for Linearity (Ritonavir)

S. No.	Linearity level	Ritonavir Concentration (µg/mL)	Area(µV*sec)
1	Level-1 -20%	9.9939	106585
2	Level-2 -40%	49.9696	540654
3	Level-3 -80%	74.9544	796333
4	Level-4 -100%	99.9392	1072368
5	Level-5 -120%	124.9241	1339699
6	Level-6 -150%	149.9089	1604790
		Correlation coefficient (r)	0.99997
		Slope	10706.8641
		Intercept	541.0586
		Residual sum of squares	78695416.8172
		% Deviation of the Y-intercept	0.1



6.Robustness

Following parameters were changed one by one and their effect was observed on system suitability for standard preparation.

1.Flow rate of Mobile Phase was changed (±0.2 ml/min) 1.8ml/min and 2.2ml/min.

2. Column oven Temperature was changed (± 5°C) 25°C and 35°C.

3. Detector wave Length was changed (± 2nm) 208nm and 212nm.

4. Mobile phase buffer pH was changed (± 0.2 pH) pH 2.3 and pH 2.7.

5. Mobile phase composition was changed (± 10% Acetonitrile).

The system suitability parameter was found to be within the acceptance criteria in all above conditions.

CONCLUSION

From the above discussion it can be concluded that the proposed method is specific, precise, accurate, linear and robust.Results are in good agreement with label claim which indicates there is no interference of excipients.Therefore the proposed method can be used for routine analysis of Atazanavir and Ritonavir drug product.

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