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RP-HPLC Method Development and Validation for Simultaneous Estimation of Atzanavir and Ritonavir in Pharmaceutical Dosage Forms

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ABSTRACT

A new method was established for simultaneous estimation of Atazanavir and Ritonavir by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Atazanavir and Ritonavir by using Agilent C18 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Methanol: ACN (70:30%v/v), detection wave length was 238nm. The instrument used was HPLC Shimadzu Waters 996 LC 20 Software. The retention times were found to be 2.443mins and 2.918mins. The % purity of Atazanavir and Ritonavir was found to be 100.7% and 101.4% respectively. The system suitability parameters for Atazanavir and Ritonavir such as theoretical plates and tailing factor were found to be 1.7, 2114.5and 1.7, 2931.0 the resolution was found to be 8.0.The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for Atazanavir and Ritonavir was found in concentration range of 1µg-5µg and 100µg-500µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 100% and 100.5%, %RSD for repeatability was 2.0 and 2.0, % RSD for intermediate precision was 1.5 and 1.1 respectively. The precision study was precise, robust, and repeatable. LOD value was 2.95 and 3.04, and LOQ value was 9.87 and 10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Atazanavir and Ritonavir in API and Pharmaceutical dosage form.

Keywords: Agilent C18, Atazanavir and Ritonavir, RP-HPLC method

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CONTENTS:

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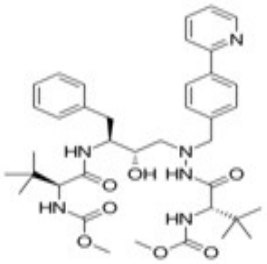
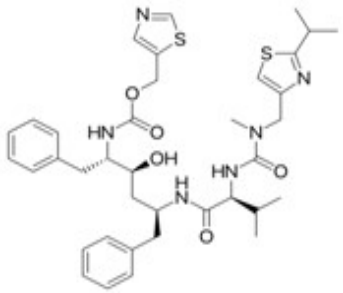
1. Introduction

Atazanavir, sold under the trade name Reyataz among others, is an antiretroviral medication used to treat and prevent HIV/AIDS. It is generally recommended for use with other antiretrovirals. It may be used for prevention after a needlestick injury or other potential exposure.



Atazanavir

Ritonavir, sold under the trade name Norvir, is an antiretroviral medication used along with other medications to treat HIV/AIDS. This combination treatment is known as highly active antiretroviral therapy (HAART). Often a low dose is used with other protease inhibitors.



Ritonavir

2. Materials and Methods

HPLC Auto Sampler:

Shimadzu Model number SPD20A, Software LC Solutions, Detector: Photo diode array detector, Thermosil C18 Column (4.0×1.25mm, 5µ), Sonicator: Model number SE60US Enertech, U.V double beam spectrophotometer: PG Instrument Model number T60 Software UV Win5, pH meter: ADWA Model number AD102U, Digital Weighing machine: a Model number ER200A.

Chemicals

Atazanavir and Ritonavir, KH_2PO_4, Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid, K_2HPO_4.

Chromatographic conditions

Trial-5:

Chromatographic conditions:

Column : Agilent C18 5µm (4.6*250mm)

Mobile phase ratio: Methanol: ACN (70:30%v/v)

Detection wavelength : 238nm

Flow rate : 1ml/min

Injection volume : 10µl Column temperature : Ambient

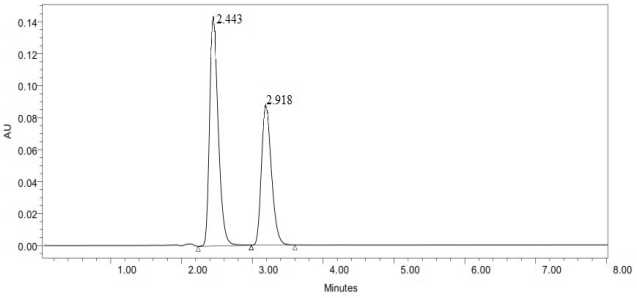


Fig.1 Chromatogram of Trail-5

Preparation of Sample Solution: (Tablet)

Accurately 10 tablets are weighed and crushed in mortar and pestle and weight equivalent to 10 mg of Ritonavir and Atazanavir (marketed formulation) sample into a 10mL clean dry volumetric flask and about 7mL of Diluents is added and sonicated to dissolve it completely and made volume upto the mark with the same solvent. (Stock solution) Further 3 ml of above stock solution was pipetted into a10ml volumetric flask and diluted upto the mark with diluent.

Method Validation

- Linearity
- Accuracy
- Precision
- Intermediate Precision
- Limit of Detection
- Limit of Quantification
- Robustness
- System suitability testing

Table 1 Details of Trail-5

S.No	Peak name	Rt	Area	Height	USP Plate	USP Tailing	USP Resolution
1	Atazanavir	2.443	946124	155429	5105	1.3	8.1
2	Ritonavir	2.9189183	111541	13239	3788	1.4	

3. Results and Discussion

Table.No.2. Linearity Results for Atazanavir and Ritonavir

	Peak Name	Rt	Area	Height
1	Atazanavir	2.297	369216	109198
2	Atazanavir	2.264	748093	145069

3	Atazanavir	2.308	1198858	164962
4	Atazanavir	2.370	1576584	193291
5	Atazanavir	2.322	1936686	238262
6	Ritonavir	2.458	126156	30269
7	Ritonavir	3.251	261826	39434
8	Ritonavir	3.488	382984	45638
9	Ritonavir	3.712	517383	50538
10	Ritonavir	3.535	627463	65483

Table 3 Repeatability results of Ritonavir and Atazanavir

	Peak Name	**Rt**	**Area**	**Height**
1	Atazanavir	2.282	1313235	163051
2	Atazanavir	2.312	1326776	162363
3	Atazanavir	2.344	1347962	163866
4	Atazanavir	2.351	1368872	163893
5	Atazanavir	2.358	1363598	161294
6	Ritonavir	3.433	458218	46160
7	Ritonavir	3.557	452495	45294
8	Ritonavir	3.623	453221	44163
9	Ritonavir	3.639	457145	43079
10	Ritonavir	3.704	458898	43930
Mean			900041.9	
Std.Dev.			468338.8	
% RSD			2.0	

Table 4 Ruggedness results

	Peak Name	**Rt**	**Area**	**Height**
1	Atazanavir	2.381	1366825	164933
2	Atazanavir	2.382	1379095	163608
3	Atazanavir	2.384	1375825	164628
4	Atazanavir	2.395	1364299	164510
5	Atazanavir	2.412	1395271	163964
6	Atazanavir	2.590	1393763	166747
7	Ritonavir	3.784	484545	41393
8	Ritonavir	3.797	484511	40825
9	Ritonavir	3.803	480804	40865
10	Ritonavir	3.845	485023	40309
11	Ritonavir	3.915	504952	39213
12	Ritonavir	4.307	485203	41640
Mean			933342.8	
Std.Dev.			465781.8	
% RSD			49.9	

Table 5 Details of Accuracy 50 %

	Peak Name	**Rt**	**Area**	**Height**
1	Atazanavir	2.346	702873	86023
2	Atazanavir	2.351	704987	85549
3	Atazanavir	2.360	702008	84196
4	Ritonavir	2.639	239401	21744
5	Ritonavir	2.668	239865	21909
6	Ritonavir	2.692	239948	21382
Mean			471513.5	
Std.Dev.			253899.3	
% RSD			53.8	

Table 6 Details of Accuracy 100 %

	Peak Name	**Rt**	**Area**	**Height**
1	Atazanavir	2.372	1390018	163987
2	Atazanavir	2.378	1385589	165904
3	Atazanavir	2.472	1419041	163460
4	Ritonavir	3.728	480779	42641
5	Ritonavir	3.772	480218	41532
6	Ritonavir	4.122	480338	37644
Mean			939330.5	
Std.Dev.			502815.3	
% RSD			53.5	

Table 7 Details of Accuracy 150 %

	Peak Name	**Rt**	**Area**	**Height**
1	Atazanavir	2.462	1390018	251287
2	Atazanavir	2.500	1385589	252406
3	Ritonavir	4.096	1419041	59726
4	Ritonavir	4.252	480779	56682
Mean			1468217.4	
Std.Dev.			848139.6	
% RSD			57.8	

Table 8 Accuracy results of Atazanavir

%Concentration (at specification Level)	Area	Amount added(m)	Amount found(m)	% Recovey	Mean Recovery
50%	702873	5	5.10	101.8%	100.5%
100%	1390018	10	9.99	99.9%	
150%	2206281	15	14.9	99.1%	

Table 9 Accuracy results of Ritonavir

%Concentration(at specification level)	Area	Amount Added(mg)	Amount Found(mg)	% Recovery	Mean Recovery
50%	239401	5	5.0	101.3%	100.0%
100%	480779	10	9.94	99.4%	
150%	733144	15	14.8	99.2%	

Table 10 System suitability results For Ritonavir (Flow rate)

S.No	**Flow Rate(ml/min)**	**System suitability results**	
		USP Plate count	**USP Tailing**
1	0.8	3536	1.7
2	1.0	2931	1.7
3	1.2	2713	1.7

Table 11 System suitability results for Atazanavir (Flow rate)

S.No	**Flow Rate(ml/min)**	**System suitability results**	
		USP Plate count	**USP Tailing**
1	0.8	2158	1.8
2	1.0	2114	1.7
3	1.2	2069	1.7

Table 12 System suitability results for Ritonavir (Mobile phase)

S.No	Changein Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10% Less	2910	1.8
2	Actual	2860	1.7
3	10% More	2358	1.7

Table 13 System suitability results for Atazanavir (Mobile phase)

S.No	Changein Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10% Less	2540	1.7
2	Actual	2458	1.7
3	10% More	2616	1.7

4. Conclusion

The developed RP–HPLC method was found to be suitable for the analysis of Atazanavir and Ritonavir in bulk form, and was found to be simple, reliable, sensitive, economical and precise.

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