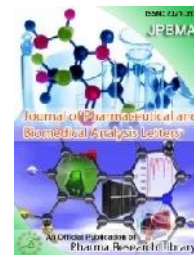




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## RESEARCH ARTICLE

### RP HPLC Method Development and Validation for Simultaneous Estimation of Atazanavir 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 these parathion of Atazanavir and Ritonavir by using Agilent tC185µm (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 LC20 Software. The retention times were found to be 2.443 mins and 2.918 mins. 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, 2114.5 and 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 (r<sup>2</sup>) 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

#### ARTICLE INFO

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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 antiretroviral. It may be used for prevention after a needle stick injury or other potential exposure. It is taken by mouth once a day. Common side effects include headache, nausea, yellowish skin, abdominal pain, trouble sleeping, and fever. Severe side effects include rashes such as erythema multiforme and high blood sugar. Atazanavir appears to be safe to use during pregnancy. It is of the protease inhibitor (PI) class and works by blocking HIV protease.

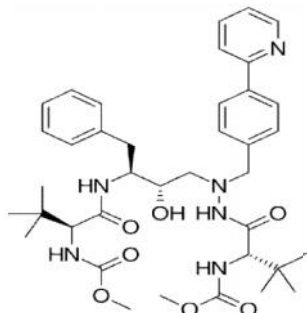


Fig 1: Structure of Atazanavir

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. It may also be used in combination with other medications for hepatitis C. It is taken by mouth. The capsules of the medication do not work the same as the tablets. Common side effects include nausea, vomiting, loss of appetite, diarrhea, and numbness of the hands and feet. Serious side effects include liver problems, pancreatitis, allergic reactions, and arrhythmias. Serious interactions may occur with a number of other medications including amiodarone and simvastatin. At low doses it is considered to be acceptable for use during pregnancy. Ritonavir is of the protease inhibitor class. It is often used to inhibit the enzyme that metabolizes other protease inhibitors. This inhibition leads to higher concentrations of this latter medication.

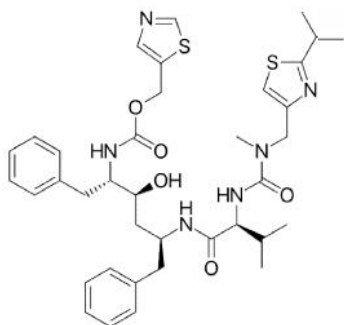


Fig 2: Structure of Ritonavir

## 2. Materials and Methods

**Chemicals:** Ortho phosphoric acid, Acetonitrile, Methanol, Water,  $\text{KH}_2\text{PO}_4$ ,  $\text{K}_2\text{HPO}_4$ .

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## Instrumentation

HPLC Shimadzu Waters 996 LC 20 Software Waters, UV double beam UV 3000 UV Win 5 Lab India, Digital weighing, pH meter, Ultra sonicator, Suction pump.

## Chromatographic conditions

Column	:AgilentC18(4.6*250mm) 5 $\mu\text{m}$
Mobile phase ratio	:Methanol:ACN (70:30%v/v)
Detection wavelength	: 238 nm
Flow rate	: 1 ml/min
Injection volume	:10 $\mu\text{l}$
Temperature	:Ambient

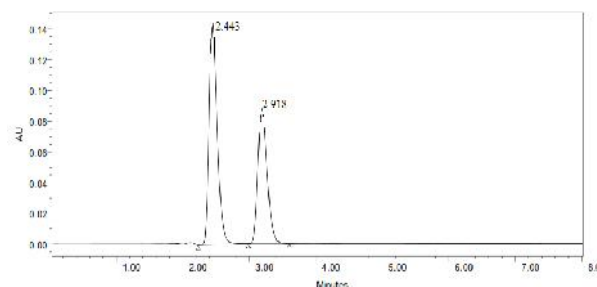


Fig 3: Optimized Chromatogram

## Observation:

The chromatogram is perfect with clear separation of components. The peak symmetry and system suitability parameters are within the limits. Hence this method is chosen as optimized one.

## Preparation of the individual Atazanavir standard preparation:

10mg of Atazanavir working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluent. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluent.

## Preparation of the individual Ritonavir standard preparation:

10mg of Ritonavir working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluent. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluent.

## 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 a 10ml volumetric flask and diluted upto the mark with diluent.

**Method Validation****Accuracy:**

**Preparation of standard solution (Ritonavir and Atazanavir):** Accurately weighed 10 mg of Ritonavir and 10mg of Atazanavir working standard were transferred into a 10mL and 100ml of clean dry volumetric flasks.

**Precision****Repeatability:**

**Preparation of standard stock solution:** Accurately 10 mg of Ritonavir and 10mg of Atazanavir working standard were weighed and transferred into a 10mL and 100ml of clean dry volumetric flasks and about 7mL and 70ml of Diluent was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent.

**Intermediate Precision (Ruggedness):**

To evaluate the intermediate precision (also known as ruggedness) of the method, precision was performed on different days by using different make column of same dimensions.

**Specificity:**

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

**LOD:**

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

**LOQ:**

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y- intercepts of regression lines.

**Linearity:**

**Preparation of stock solution:** Accurately 10 tablets were weighed & crushed in mortar and pestle and weight equivalent to 10 mg of Ritonavir and Atazanavir (marketed formulation) sample were transferred into a 10mL clean dry volumetric flask and about 7mL of Diluent was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution)

**Range:**

Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 1µg-5µg and 100µg- 500µg of Atazanavir and Ritonavir respectively.

**Robustness:**

As part of the robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method.

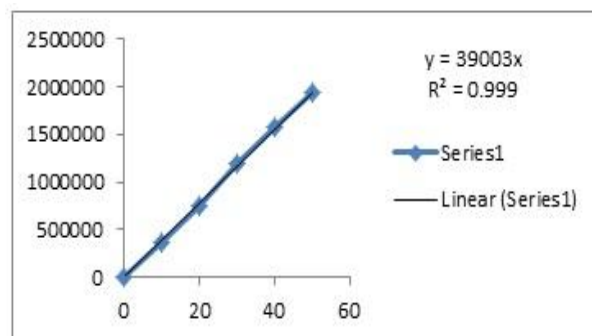
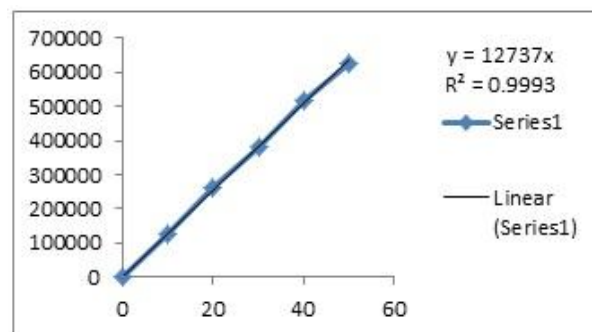
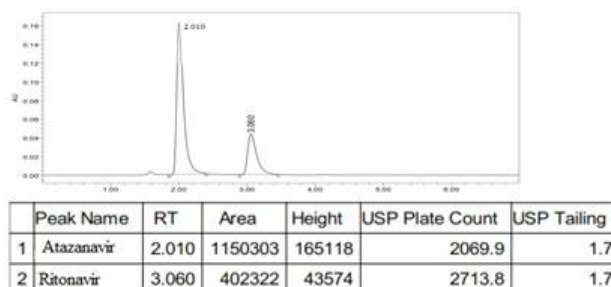
**System suitability:**

5 mg of Atazanavir and 500 mg of Ritonavir working standard was accurately weighed and transferred into a 100ml clean dry volumetric flask and add about 20ml of

diluent and sonicated to dissolve it completely and make volume up to the mark with the same solvent.

**3. Results and Discussion****Table 1:** Linearity Results for Atazanavir and Ritonavir

	PeakName	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	3.458	126156	30269
7	Ritonavir	3.351	261826	39434
8	Ritonavir	3.488	382984	45638
9	Ritonavir	3.712	517383	50538
10	Ritonavir	3.535	627463	65483

**Fig 4:** Calibration curve of Atazanavir**Fig 5:** Calibration curve of Ritonavir**Fig 6:** Chromatogram for Robustness more flow

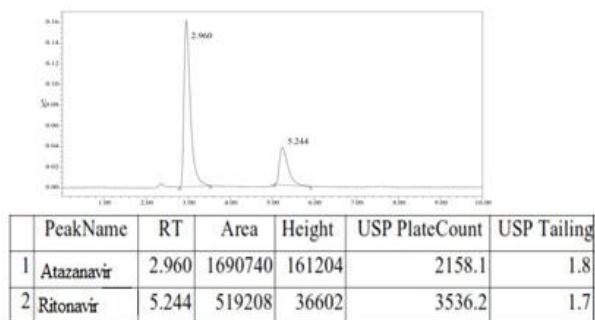


Fig 7: Chromatogram for Robustness less flow

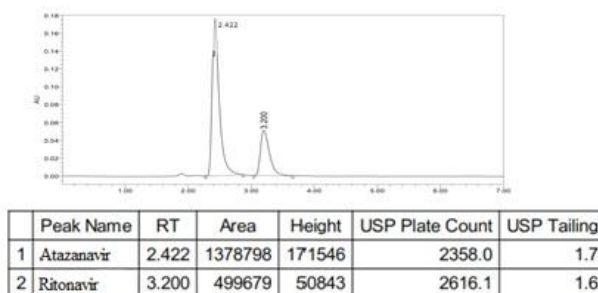


Fig 8: Chromatogram for Robustness more organic

**Mobile Phase:**

The Organic composition in the Mobile phase was varied from 70% to 60%. Standard solution 300 µg/ml of Ritonavir & 3µg/ml of Atazanavir was prepared and analyzed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

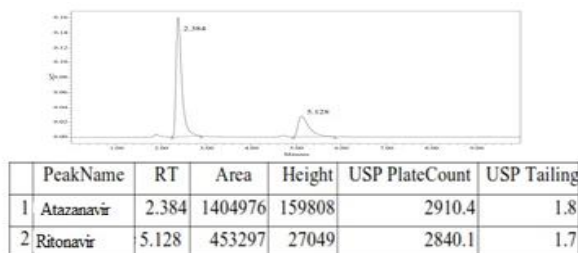


Fig 9: Chromatogram for Robustness less organic

Table 2: System suitability results of Atazanavir and Ritonavir

S.No	Peak name	Rt	Area	Height	USP Plate count	USP Tailing	USP Resolution
1	Atazanavir	2.443	946124	155429	5105	1.3	8.1
2	Ritonavir	2.918	111541	13239	3788	1.4	

Table 6: Accuracy results of Atazanavir

%Concentration (at specification Level)	Area	Amount Added(mg)	Amount found(mg)	% Recovery	Mean Recovery
50%	702873	5	5.10	101.8%	100.5%
100%	139001	10	9.99	99.9%	
150%	220628	15	14.9	99.1%	

Table 7: 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%

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/ Intermediate precision 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	4.607	485203	41640
Mean			933342.8	
Std. Dev.			465781.8	
% RSD			49.9	

**Table 5:** Details of 50%, 100% and 150% Accuracy

**Details of Accuracy 50 %**

	Peak Name	RT	Area	Height
1	Atazanavir	2.346	702873	86026
2	Atazanavir	2.351	704987	85549
3	Atazanavir	2.360	702008	84196
4	Ritonavir	3.639	239401	21744
5	Ritonavir	3.668	239865	21909
6	Ritonavir	3.692	239948	21382
Mean			471513.5	
Std. Dev.			253899.3	
% RSD			53.8	

**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	

**Accuracy 150 %**

	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 8:** Details of Standard Injection

	PeakName	RT	Area	Height	USP Plate Count	USP Tailing
1	Atazanavir	2.318	1333112	164078	2114.9	1.7
2	Atazanavir	2.379	1355521	164511	2127.0	1.7
3	Ritonavir	3.535	462181	44873	2931.4	1.7
4	Ritonavir	3.749	465519	41056	2697.1	1.7

**Table 9:** 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 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 Ritonavir (Mobile phase)

S.No	Change in 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 12:** System suitability results for Atazanavir (Mobile phase)

S.No	Change in 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

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 5 $\mu$ m (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 2.443 mins and 2.918 mins. 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, 2114.5 and 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 1 $\mu$ g-5 $\mu$ g and 100 $\mu$ g-500 $\mu$ g and correlation coefficient (r<sup>2</sup>) 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.

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