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Simultaneous Estimation of Atazanavir & Ritonavir in API & Marketed Formulations by Using RP-HPLC

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ABSTRACT: Development and validation of RP-HPLC method for simultaneous estimation of Atazanavir and Ritonavir in their combined tablet dosage form. Atazanavir and Ritonavir are antiviral agents used in treatment of HIV. A simple, precise, rapid, accurate and cost-effective high- performance liquid chromatography (HPLC) method was successfully developed and validated for simultaneous estimation of Atazanavir and Ritonavir in their combined tablet dosage form. The selected mobile phase was Methanol: Phosphate Buffer in proportion 65:35 v/v respectively. The optimized columns used are C18 column, Symmetry and Zodiac column. X bridge C18 (4.6×150 mm, 5 µm) particle size was found to be ideal as it gave good peak shape and resolution at 1ml/min flow for Atazanavir and Ritonavir. In this study, the validation of Atazanavir and Ritonavir in API and marketed formulations were performed keeping in accordance with the parameters like system suitability, specificity, linearity, accuracy, precision (reproducibility & repeatability), robustness. The developed stability indicating method is capable for determination of impurities of Atazanavir and Ritonavir in combined tablet dosage form as well as individual dosage forms also. The method has been successfully validated according to ICH guidelines and the results obtained by using RP - HPLC are rapid, accurate and precise. The proposed methods were successfully applied for the analysis of synthetic mixtures and pharmaceutical formulations of Atazanavir and Ritonavir.

KEYWORDS: Atazanavir, Ritonavir, RP-HPLC, Method Development, Validation

INTRODUCTION:

Atazanavir is chemically Methyl N-[(1S)-1-{[(2S, 3S)-3-hydroxy-4-[(2S)-2- [(methoxycarbonyl) amino]-3,3-dimethyl-N'-{[4-(pyridine 2yl) phenyl] methyl} butane hydrazido]-1-phenylbutan-2- yl] carbamoyl}-2,2-dimethylpropyl] carbamate . The drug profile of Atazanavir is given in Table 1 and its structure is shown in Figure 1. Atazanavir (ATV) is an azapeptide HIV-1 protease inhibitor (PI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1). Ritonavir is chemically 1, 3-thiazol-5-ylmethyl N-[(2S, 3S, 5S)-3-hydroxy- 5-[(2S)-3-methyl-2-{[methyl ({[2-(propan-2-yl)-1, 3-thiazol-4-yl] methyl}) carbamoyl] amino} butan amido]-1, 6- diphenylhexan-2-yl] carbamate. Ritonavir is a protease inhibitor with activity against Human Immunodeficiency Virus Type 1 (HIV-1). Protease inhibitors block the part of HIV called protease. HIV-1 protease is an enzyme required for the proteolytic cleavage of the viral polyprotein precursors into the individual functional proteins found in infectious HIV-1. The drug profile of Ritonavir is given in Table 2 and its structure is shown in Figure 2.

Validation is a fundamental piece of value affirmation; it includes the deliberate study of frameworks, offices and procedures went for figuring out if they perform their planned capacities sufficiently and reliably as determined [1-3]. A composed arrangement depicting the procedure to be approved, including production equipment and how validation is conducted [4]. This method provides information about the relative amount of the components of validation of Atazanavir and Ritonavir [5-7].

DRUG PROFILE

Table 1: Drug Profile of ATAZANAVIR

Drug	Atazanavir
Synonym	Zrivada
Category	Anti- retroviral agent

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IUPAC	Methyl N-[(1S)-1-{[(2S, 3S)-3-hydroxy-4-[(2S)-2-[(methoxycarbonyl) amino]-3,3-dimethyl-N'-{[4-(pyridine 2yl) phenyl] methyl} butane hydrazido]-1-phenylbutan-2-yl] carbamoyl}-2,2-dimethylpropyl] carbamate
Molecular formula	$C_{38}H_{52}N_6O_7$
Melting point	205°C to 209°C
pKa	11.2
Log P	4.08

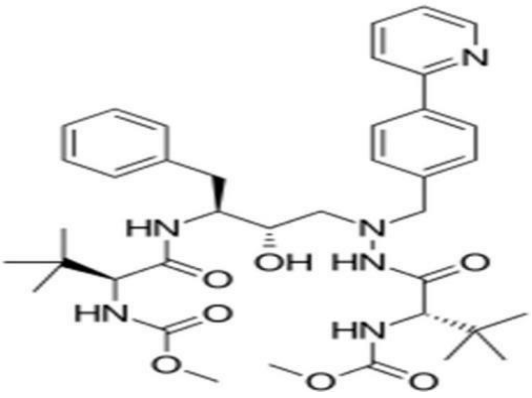

Fig 1: Chemical structure of Atazanavir

Table 2: Drug Profile of RITONAVIR

Drug	Ritonavir
Synonym	Ritonavirum
Category	Antiviral
IUPAC	1, 3-thiazol-5-ylmethyl N-[(2S, 3S, 5S)-3-hydroxy- 5-[(2S)-3-methyl-2-{[methyl ({[2-(propan-2-yl)-1, 3-thiazol-4-yl] methyl}) carbamoyl] amino} butanamido]-1, 6-diphenylhexan-2-yl] carbamate
Molecular formula	$C_{37}H_{48}N_6O_5S_2$
Melting point	126 to 132 ℃
pKa	13.6
Log P	3.9

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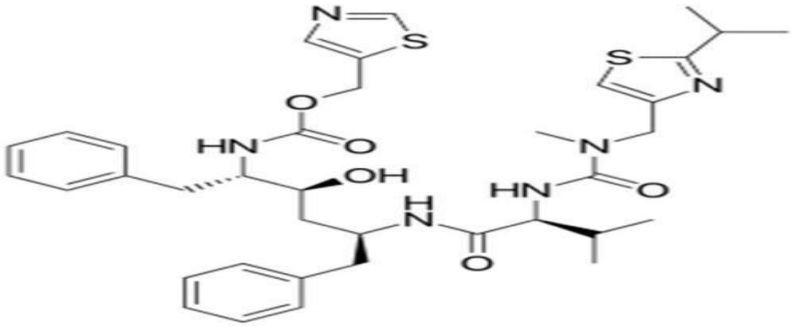

Fig 2: Chemical Structure of Ritonavir

Introduction to HPLC

HPLC is a chromatographic technique where it is possible to perform structural, and functional analysis, and purification of many molecules within a short time, this technique yields perfect results in the separation, and identification of amino acids, carbohydrates, lipids, nucleic acids, proteins, steroids, and other biologically active molecules. In HPLC, mobile phase passes through columns under 10 – 400 atmospheric pressure, and with a high flow rate (0.1–5 cm/sec). In this technique, use of small particles, and application of high pressure on the rate of solvent flow increases separation power of HPLC and the analysis is completed within a short time[8-9]. The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and it includes resolution, identification and quantification of a compound[10-15]. It also aids in chemical separation and purification.

METHODS AND MATERIALS

Table 3: Instruments used

S. No.	Instruments And Glass ware	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Enertech

Table 4: Chemicals used

S. No.	Chemical	Suppliers
1	Atazanavir	Sura labs
2	Ritonavir	Sura labs
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck
5	Triethylamine	Merck

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HPLC method development trails

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Atazanavir and Ritonavir working standard into a 10 ml of clean dry volumetric flasks, add about 7 ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.1 ml of the above Atazanavir and 0.3 ml of the Ritonavir stock solutions into a 10 ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water and Water: Acetonitrile and Methanol: Phosphate Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate Buffer in proportion 65:35 v/v respectively.

Optimization of Column:

The method was performed with various columns like C18 column, Symmetry and Zodiac column. X bridge C18 (4.6×150mm, 5µm) particle size was found to be ideal as it gave good peak shape and resolution at 1ml/min flow[10-12].

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used	:	Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature	:	Ambient
Column	:	X bridge C18 (4.6×150 mm, 5 µm) particle size
	:	Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 4.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra sonication.
pH	:	4.6
Mobile phase	:	Methanol: Phosphate Buffer (65:35 v/v)
Flow rate	:	1 ml/min
Wavelength	:	260 nm
Injection volume	:	10 µl
Run time	:	10 min

VALIDATION

PREPARATION OF BUFFER AND MOBILE PHASE:

Preparation of Potassium dihydrogen Phosphate (KH_2PO_4) buffer (pH 4.6):

Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 4.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra sonication.

Preparation of mobile phase:

Accurately measured 650 ml (65%) of Methanol, 350 ml of Phosphate buffer (35%) were mixed and degassed in digital ultra sonicator for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

VALIDATION PARAMETERS

SYSTEM SUITABILITY

Accurately weigh and transfer 10 mg of Atazanavir and 10 mg of Ritonavir working standard into a 10 ml of clean dry volumetric

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flasks, add about 7ml of diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.1 ml of the above Atazanavir and 0.3 ml of the Ritonavir stock solutions into a 10 ml volumetric flask and dilute up to the mark with Diluent.

Procedure: The standard solution was injected for five times and measured the area for all five injections in HPLC. The % RSD for the area of five replicate injections was found to be within the specified limits.

SPECIFICITY STUDY OF DRUG:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Atazanavir and 10 mg of Ritonavir working standard into a 10 ml of clean dry volumetric flasks, add about 7 ml of diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.1 ml of the above Atazanavir and 0.3 ml of the Ritonavir stock solutions into a 10 ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Sample Solution:

Take average weight of one tablet and crush in a mortar by using pestle and weigh 10 mg equivalent weight of Atazanavir and Ritonavir sample into a 10 ml clean dry volumetric flask and add about 7 ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.1 ml of the above Atazanavir and 0.3 ml of the Ritonavir stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

% ASSAY =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

PREPARATION OF DRUG SOLUTIONS FOR LINEARITY:

Accurately weigh and transfer 10 mg of Atazanavir and 10 mg of Ritonavir working standard into a 10 ml of clean dry volumetric flasks, add about 7 ml of diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).

Preparation of Level – I (6 ppm of Atazanavir & 18 ppm of Ritonavir):

Pipette out 0.06 ml of Atazanavir and 0.18 ml of Ritonavir stock solutions was taken in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (8 ppm of Atazanavir & 24 ppm of Ritonavir):

Pipette out 0.08 ml of Atazanavir and 0.24 ml of Ritonavir stock solutions was taken in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (10 ppm of Atazanavir & 30 ppm of Ritonavir):

Pipette out 0.1 ml of Atazanavir and 0.3 ml of Ritonavir stock solutions was taken in a 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (12 ppm of Atazanavir & 36 ppm of Ritonavir):

Pipette out 0.12 ml of Atazanavir and 0.36 ml of Ritonavir stock solutions was taken in a 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (14 ppm of Atazanavir & 42 ppm of Ritonavir):

Pipette out 0.14 ml of Atazanavir and 0.42 ml of Ritonavir stock solutions was taken in a 10 ml of volumetric flask dilute up to

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the mark with diluent.

Procedure:
Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

PRECISION REPEATABILITY

Preparation of Atazanavir and Ritonavir Product Solution for Precision:
Accurately weigh and transfer 10 mg of Atazanavir and 10 mg of Ritonavir working standard into a 10 ml of clean dry volumetric flasks, add about 7 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.1 ml of the above Atazanavir and 0.3 ml of the Ritonavir stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent. The standard solution was injected for five times and measure the area for all the five injections in HPLC. The % RSD for the area of five replicate injections was found to be within the specified limits.

INTERMEDIATE PRECISION:
To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining the same conditions.

Procedure:
DAY 1:
The standard solution was injected for six times and measure the area for all the six injections in HPLC. The % RSD for the area of six replicate injections was found to be within the specified limits.

DAY 2:
The standard solution was injected for six times and measure the area for all the six injections in HPLC. The % RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy:
For preparation of 50% Standard stock solution:
Accurately weigh and transfer 10 mg of Atazanavir and 10 mg of Ritonavir working standard into a 10 ml of clean dry volumetric flask, add about 7 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.05 ml of the above Atazanavir and 0.15 ml of the Ritonavir stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent.

For preparation of 100% Standard stock solution:
Accurately weigh and transfer 10 mg of Atazanavir and 10 mg of Ritonavir working standard into a 10 ml of clean dry volumetric flasks add, about 7 ml of diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.1 ml of the above Atazanavir and 0.3 ml of the Ritonavir stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent.

For preparation of 150% Standard stock solution:
Accurately weigh and transfer 10 mg of Atazanavir and 10 mg of Ritonavir working standard into a 10 ml of clean dry volumetric flask, add about 7 ml of diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.15 ml of Atazanavir and 0.45 ml of Ritonavir from the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluents.

Procedure:
Inject the three replicate injections of individual concentrations (50%, 100%, 150%) under the optimized conditions. Record the chromatograms and measure the peak responses. Calculate the amount found and amount added for Atazanavir and Ritonavir and

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calculate the individual recovery and mean recovery values.

ROBUSTNESS:

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for the variation of results.

For preparation of Standard solution:

Accurately weigh and transfer 10 mg of Atazanavir and 10 mg of Ritonavir working standard into a 10 ml of clean dry volumetric flask and add about 7 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.1 ml of the above Atazanavir and 0.3 ml of the Ritonavir stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent.

Effect of Variation of flow conditions:

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, the remaining conditions are same. 10 µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of mobile phase organic composition:

The sample was analyzed by variation of mobile phase i.e., Methanol and Phosphate Buffer was taken in the ratio and 50:50, 40:60 instead (65:35), remaining conditions are same. 10 µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION OF SYSTEM SUITABILITY

Chromatographic conditions: The method was performed with various C18 column, Symmetry and Zodiac column. X Bridge C18 (4.6 × 150mm), 5 µm particle size was found to be ideal as it gave good peak shape and resolution at 1 ml/min flow, equilibrated with Methanol and Phosphate buffer (pH- 4.6) (65:35% v/v) as a mobile phase. Run time was 10 min and here the peaks were separated and showed better resolution. Conditions of optimized chromatography are shown in table No. 5.

Table No. 5: Optimized Chromatographic Conditions

Mobile Phase	Methanol and Phosphate buffer (pH- 4.6) (65:35% v/v)
Wavelength	260 nm
Flow Rate	1 ml/min
Run Time	10 min
Temperature	Ambient
Injection Volume	10 µl
Column	X Bridge C18 (4.6 × 150mm), 5 µm particle size

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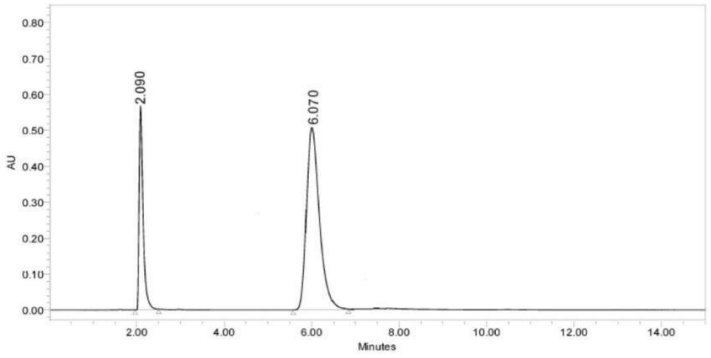

Fig 3: Optimized Chromatogram for Ritonavir (Rt=2.090 min) & Atazanavir (Rt= 6.070 min)

Specificity: There were no other components present at the elution time for Atazanavir and Ritonavir. As seen in the figure 3, a blank chromatogram is obtained.

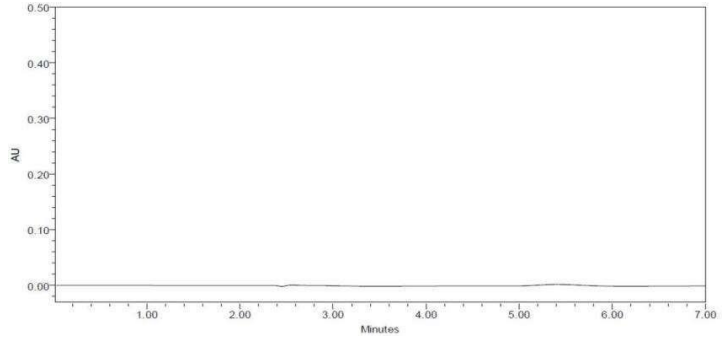

Fig 4: Chromatogram showing blank (mobile phase preparation)

Linearity: The linearity range was found to be 20-100µg/ml of Atazanavir, 10-50 µg/ml of Ritonavir and chromatograms are shown in Table no- 6.

Table 6: Linearity study of Atazanavir

Concentration (µg/ml)	Average Peak Area
20	8040807
40	14318417
60	21087985
80	27913928
100	34584741

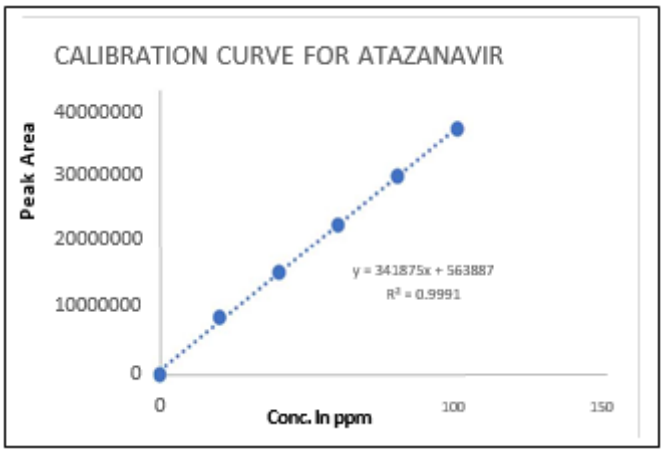

Fig 5: Calibration Graph for Atazanavir

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Table 7: Linearity study of Ritonavir

Concentration (µg/ml)	Average Peak Area
10	1010252
20	2049374
30	3072706
40	3921068
50	4952813

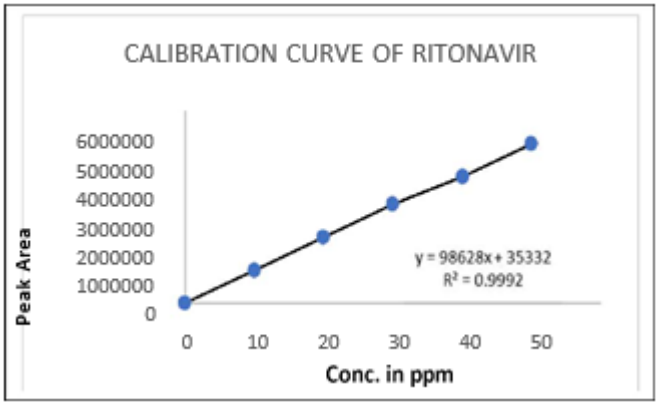

Figure 6: Calibration Graph for Ritonavir

Table 8: Analytical performance parameters of Atazanavir and Ritonavir

PARAMETERS	ATAZANAVIR	RITONAVIR
Slope (m)	34187	98628
Intercept	56388	35332
Correlation Coefficient	0.999	0.999

ASSAY:

Table 9: Assay Results

S. No.	Name of the compound	Label Claim	Amount Taken (from Combination tablet)	%Purity
1.	Atazanavir	100 mg	20 mg	100.1 %
2.	Ritonavir	50 mg	10 mg	100.1 %

PRECISION:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Table 10: Results of method precision for Atazanavir

S. No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Atazanavir	6.061	3582264	567917	5568.0	1.0	2.5
2	Atazanavir	6.062	3586491	517719	5359.2	1.1	2.5
3	Atazanavir	6.063	3598154	567933	5565.5	1.0	2.5
4	Atazanavir	6.064	3564125	517733	5355.2	1.1	2.5
5	Atazanavir	6.064	3569412	562173	5568.0	1.0	2.5

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Mean			3580089				
Std. Dev			13609.81				
% RSD			0.380153				

Table 11: Results of method precision for Ritonavir:

S. No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Ritonavir	2.084	3569412	567917	5568.0	1.0
2	Ritonavir	2.083	3465125	517719	5359.2	1.1
3	Ritonavir	2.082	3598154	567933	5565.5	1.0
4	Ritonavir	2.081	3586491	517733	5355.2	1.1
5	Ritonavir	2.080	3582694	567917	5568.0	1.0
Mean			3560375			
Std. Dev			54225.61			
% RSD			1.523031			

Table 12: Results of Intermediate precision Day- 1 for Atazanavir

S. No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Atazanavir	6.061	15481579	567917	5568.0	1.0	2.5
2	Atazanavir	6.062	15369852	517719	5359.2	1.1	2.5
3	Atazanavir	6.063	15248454	567933	5565.5	1.0	2.5
4	Atazanavir	6.064	15874692	517733	5355.2	1.1	2.5
5	Atazanavir	6.064	15236547	567933	5568.0	1.0	2.5
6	Atazanavir	6.064	15217547	567133	5359.2	1.1	2.5
Mean			15404779				
Std. Dev.			251289.4				
% RSD			1.6				

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Table 13: Results of Intermediate precision Day- 1 for Ritonavir

S. No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Ritonavir	2.081	3481579	567917	5568.0	1.0
2	Ritonavir	2.082	3458121	517719	5359.2	1.1
3	Ritonavir	2.083	3426581	567933	5565.5	1.0
4	Ritonavir	2.084	3465712	517733	5355.2	1.1
5	Ritonavir	2.085	3451476	567917	5568.0	1.0
6	Ritonavir	2.085	3452106	567514	5359.2	1.1
Mean			3455929			
Std. Dev			18188.92			
% RSD			0.5			

Table 14: Results of Intermediate precision Day 2 for Atazanavir

S. No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Atazanavir	6.061	15481579	567917	5568.0	1.0	2.5
2	Atazanavir	6.062	15369852	517719	5359.2	1.1	2.5
3	Atazanavir	6.063	15248454	567933	5565.5	1.0	2.5
4	Atazanavir	6.064	15874692	517733	5355.2	1.1	2.5
5	Atazanavir	6.064	15236547	567933	5568.0	1.0	2.5
6	Atazanavir	6.064	15217547	567133	5359.2	1.1	2.5
Mean			15404779				
Std. Dev			251289.4				
% RSD			1.6				

Table 15: Results of Intermediate precision Day 2 for Ritonavir

S. No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Ritonavir	2.081	3481579	567917	5568.0	1.0
2	Ritonavir	2.082	3458121	517719	5359.2	1.1
3	Ritonavir	2.083	3426581	567933	5565.5	1.0

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4	Ritonavir	2.084	3465712	517733	5355.2	1.1
5	Ritonavir	2.085	3451476	567917	5568.0	1.0
6	Ritonavir	2.085	3452106	567514	5359.2	1.1
Mean			3455929			
Std. Dev.			18188.92			
% RSD			0.5			

ACCURACY:

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

Table 16: The accuracy results for Ritonavir

% Concentration (at specification Level)	Area	Amount Added (ppm)		% Recovery	Mean Recovery
50 %	1543793	15	15.2	101.9	100.9 %
100 %	3035883	30	30.4	101.4	
150 %	4451005	45	44.7	99.4	

Table 17: The accuracy results for Atazanavir

Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50 %	1084420	30	30.07	100.2	99.6 %
100 %	2096069	60	59.6	99.4	
150 %	3112684	90	89.3	99.3	

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

a) LIMIT OF DETECTION

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD = 3.3 × σ / s

Where, σ = Standard deviation of the response, S = Slope of the calibration curve

Result: Ritonavir: 1.9 µg/ml**, Atazanavir:** 2.60 µg/ml

b) LIMIT OF QUANTITATION

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be

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quantitatively determined.

LOQ = 10×σ/S

Where, σ = Standard deviation of the response, S = Slope of the calibration curve

Result: Ritonavir: 3.5 µg/ml, **Atazanavir:** 6.3 µg/ml

ROBUSTNESS

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1 ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Ritonavir and Atazanavir. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±10%.

Table 18: Results for Robustness Atazanavir

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Flow rate of 1.0 ml/min	2029854	6.068	5359.2	1.1
Flow rate of 0.9 ml/min	1738319	7.101	5999.1	1.2
Flow rate of 1.1 ml/min	1638304	5.007	5989.2	1.1
Less aqueous phase	1973724	7.108	5387.2	1.1
More aqueous phase	2102838	5.008	5938.1	1.1

Table 19: Results for Robustness of Ritonavir

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical Plates	Tailing factor
Flow rate of 1.0 ml/min	3425413	2.088	5568.2	1.0
Flow rate of 0.9 ml/min	3425282	3.111	5922.2	1.2
Flow rate of 1.1 ml/min	3517879	1.880	5868.8	1.2
Less aqueous phase	3175485	3.101	5836.2	1.2
More aqueous phase	3365431	1.881	5282.6	1.1

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP- HPLC method was developed for the quantitative estimation of Atazanavir and Ritonavir in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatization or purification steps. Ritonavir is soluble in water. It dissolves in methanol at 50 mg/ml to yield a clear to hazy, colourless solution. It is very slightly soluble in ether and benzene. Atazanavir was found to be soluble in 100% ethanol, methanol or water (50 mg/ml), soluble in chloroform; practically insoluble in ether, benzene and soluble in DMSO, and dimethyl formamide. Methanol and Phosphate buffer pH 3.4 (65:35 v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The % RSD values were within 2 and the method was found to be precise. The results expressed in tables for RP-HPLC method are promising. The RP-HPLC method is more

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sensitive, accurate and precise compared to the other spectrophotometric methods. This method can be used for the routine determination of Atazanavir and Ritonavir in bulk drug and in pharmaceutical dosage forms.

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