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## Research Article

### Development And Validation of a Stability-Indicating RP-HPLC Assay Method for Atazanavir Sulfate in Bulk Drug and Capsule Dosage Form, and Its Application In Dissolution Studies

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#### ARTICLE INFO

#### ABSTRACT

This research aimed to develop and validate a stability-indicating RP-HPLC method for the quantitative estimation of Atazanavir sulfate in both bulk drug and capsule dosage forms. The method's specificity was established through stress tests, demonstrating its ability to accurately quantify Atazanavir sulfate in the presence of potential degradation products. Validation parameters including linearity, accuracy, precision, and robustness were assessed in accordance with ICH guidelines, confirming the method's reliability and suitability for routine pharmaceutical analysis. Additionally, the developed RP-HPLC method was applied to conduct a dissolution study of Atazanavir sulfate capsules, providing valuable insights into its dissolution behavior under specified conditions. The dissolution method was validated for linearity, precision, and accuracy, further supporting its applicability for dissolution testing purposes. Results from the stability-indicating RP-HPLC method revealed the drug's stability under various stress conditions, with significant degradation observed only in base degradation. The dissolution study highlighted the drug's consistent release profile over the specified time intervals. Overall, the developed methods offer reliable and specific means for the quantitative analysis and dissolution testing of Atazanavir sulfate, contributing to pharmaceutical quality control and stability assessment. These methods are poised to facilitate routine analytical procedures in pharmaceutical laboratories, ensuring the quality, safety, and efficacy of Atazanavir sulfate-based products.

**Keywords:** Atazanavir sulfate; RP-HPLC method; Dissolution study; Stability indicating; Pharmaceutical analysis

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## 1. Introduction

The efficacy of antiretroviral medications, crucial in managing HIV infections, hinges on the stability and integrity of their formulations. Among these medications, Atazanavir Sulfate (AS) holds a significant position as a potent HIV protease inhibitor. Despite its therapeutic efficacy, ensuring the stability of AS in pharmaceutical formulations remains a pressing concern. The integrity of these formulations is vital to maintain therapeutic efficacy, necessitating the development of robust analytical methods characterized by high specificity and sensitivity [1].

High-Performance Liquid Chromatography (HPLC) has emerged as a cornerstone technique in pharmaceutical analysis, renowned for its precision in quantifying pharmaceutical compounds. For AS, the development of a stability-indicating RP-HPLC assay method is imperative. Such a method not only facilitates the accurate determination of AS concentration in bulk drug and capsule dosage forms but also enables the differentiation of AS from potential degradation products, thereby ensuring the integrity and quality of pharmaceutical formulations [2].

Moreover, dissolution studies play a pivotal role in pharmaceutical development by providing crucial insights into drug release kinetics. Understanding the dissolution behavior of AS from capsule dosage forms is essential for optimizing formulations and ensuring consistent drug delivery, ultimately enhancing therapeutic outcomes.

This research aims to address these challenges by developing and validating a stability-indicating RP-HPLC assay method for AS in both bulk drug and capsule dosage forms. Additionally, the application

of this method in dissolution studies aims to elucidate the release kinetics of AS from capsule formulations. Through comprehensive validation and dissolution investigations, this study seeks to provide a robust analytical framework for the quality control of AS formulations, thereby contributing to advancements in HIV therapeutic strategies and pharmaceutical sciences [3].

## 2. Material and Method

### 2.1 Materials

Atazanavir (ATV) sourced from Anant Pharmaceuticals Pvt. Ltd. constituted the primary active pharmaceutical ingredient utilized in this study. High-quality solvents, including water of Milli-Q grade or equivalent, acetonitrile (HPLC Gradient Grade, Rankem), and methanol (HPLC Gradient Grade, Merck), were instrumental in preparing the mobile phase and standard solutions. Additionally, reagents such as triethylamine (For Chromatography, Merck) and ortho-phosphoric acid (GR Grade, Merck) were employed for method optimization and adjustment of pH levels. For filtration purposes, 0.45 $\mu$  Teflon + Glass membrane filters (Make mdi-Cat.No. SYTG0602MNXX104) or their equivalents were utilized to ensure sample purity and consistency. These meticulously selected materials and reagents were integral to the successful development and validation of the stability-indicating RP-HPLC assay method for Atazanavir Sulfate in both bulk drug and capsule dosage forms [4].

### 2.3 HPLC Method Development and Optimization

The development of the RP-HPLC assay method for Atazanavir Sulfate (AS) involved several key steps to ensure accurate and reliable quantification.

Initially, a standard solution of AS was employed for method development trials, allowing for optimization of parameters. Concurrently, degraded samples, generated through systematic forced degradation studies, were utilized to refine the method as a stability-indicating assay [5].

#### ***Selection of Stationary Phase:***

Considering the reversed-phase HPLC mode and the molecular structure of AS, a C18 bonded phase column, specifically the Zorbax Eclipse XDB C18 (150 mm X 4.6 mm, 3.5 $\mu$ m), was chosen to facilitate optimal separation [6].

#### ***Selection of Mobile Phase:***

The mobile phase composition was meticulously selected based on solubility assessments and literature surveys. Ultimately, a mixture of Buffer Solution pH 6.5 and acetonitrile in a ratio of 40:60 v/v was determined as the optimal mobile phase for achieving satisfactory chromatographic separation.

#### ***Selection of Detector and Detection Wavelength:***

A UV-visible 2487 detector was chosen for its reliability and ease of wavelength adjustment. The detection wavelength was set at 250 nm after assessing the absorption characteristics of AS, ensuring maximum sensitivity [7].

#### ***Optimization of Chromatographic Parameters:***

Optimization of the mobile phase strength involved evaluating various buffer-to-acetonitrile ratios. The selected mobile phase composition effectively resolved peak tailing, with a flow rate of 1.0 mL/min yielding satisfactory system suitability parameters and retention time (3.9 min).

#### ***System Suitability:***

System suitability tests were conducted to verify the proper functioning of the analytical system. The % RSD for area response, tailing factor, and theoretical plates of AS peak met predefined acceptance criteria, ensuring the reliability of the method.

#### ***Estimation of Atazanavir Sulfate in Capsule Dosage Form by Proposed Method:***

The proposed method was applied to estimate AS content in capsule dosage forms. This involved injecting equal volumes of blank, standard solutions, and sample solutions into the HPLC system. The % assay of AS in the sample was calculated using a predefined formula, incorporating parameters such as peak area, sample weight, standard weight, and label claim [8].

### **2.3 Method Validation**

#### ***Specificity:***

Specificity, a pivotal aspect of method validation, delineates the ability to discern the analyte unequivocally amidst potentially confounding components. In the context of assay development for Atazanavir Sulfate (AS), specificity entails demonstrating the method's resilience against interference from impurities or excipients. To assess specificity comprehensively, various preparations including blanks, standards, and samples were meticulously prepared. Additionally, placebo preparations and solutions spiked with known impurities, such as Pyridinyl benzaldehyde lactose acetal (PBLA), Pyridinyl benzaldehyde (PB), 5-Hydroxymethyl-2-furaldehyde (5-HMF), and Dealkyl Atazanavir, were employed. Subsequent HPLC analysis facilitated the evaluation of identification, peak purity, and interference, ensuring the method's selectivity and reliability [9].

Identification parameters necessitated the comparison of retention times between standard and sample peaks. The absence of peaks at the retention time of AS in blank, placebo, and known impurity preparations was imperative to ascertain interference-free analysis. Moreover, peak purity assessments were conducted, wherein purity angles were evaluated against predefined thresholds, ensuring the integrity of standard and sample peaks. These stringent criteria collectively ensured the specificity and robustness of the developed RP-HPLC assay method for AS, vital for its accurate quantification in pharmaceutical formulations [10].

#### **2.4 Forced Degradation Studies**

For the assessment of the stability-indicating nature of the analytical method, forced degradation studies were conducted on capsules and placebos under various stress conditions, in accordance with ICH Q1A (R2) guidelines. These stress conditions encompassed acid/base hydrolysis, oxidation, thermal, and photolytic degradation [11].

##### ***Photo Degradation:***

The powder equivalent to 500 mg of Atazanavir was prepared from capsules and placebos, then transferred to volumetric flasks and diluted with diluent. Subsequently, the sample solutions were exposed to UV and white light for 1.2 million lux hours. After exposure, the solutions were filtered and further diluted for analysis.

##### ***Thermal Degradation:***

Similarly, Atazanavir powder was prepared and transferred to volumetric flasks, then subjected to 60°C in a hot air oven for 2 hours. Post-thermal exposure, the solutions were filtered, diluted, and analyzed.

##### ***Acid and Alkali Degradation:***

For acid and alkali degradation studies, Atazanavir powder was prepared and treated with 5N HCl and 5N NaOH, respectively. The solutions were then neutralized, filtered, diluted, and analyzed following the same procedure as described above.

##### ***Peroxide Degradation:***

In peroxide degradation studies, Atazanavir powder was mixed with 30% hydrogen peroxide solution, followed by incubation at 60°C. After cooling, the solutions were made up to volume, filtered, diluted, and analyzed.

The purity of the Atazanavir peak, as demonstrated by the PDA, served as the primary acceptance criterion for forced degradation studies. The absence of impurities or degradation products at the retention time of Atazanavir peak indicated the stability-indicating nature of the analytical method under the tested stress conditions [12].

#### **2.5 Linearity**

To assess the linearity of the RP-HPLC assay method for Atazanavir Sulfate (AS), a series of standard solutions were prepared at different concentration levels. These solutions were generated by diluting known volumes of an intermediate stock solution with the diluent, resulting in concentrations spanning the desired range. The concentrations and corresponding volumes used for each level are detailed in Table 1. Following preparation, a concentration (ppm) versus area graph was constructed, and regression analysis was performed to determine the correlation coefficient ( $r^2$ ), y-intercept, and slope of the regression line. The linearity of the method was deemed satisfactory if the correlation coefficient ( $r^2$ )

was not less than 0.999, indicating a strong linear relationship between concentration and detector response. Additionally, the % limit of the y-intercept was assessed to ensure its proximity to the corresponding y-coordinate of the working level, thereby confirming the method's reliability and accuracy across the tested concentration range [13].

### **2.6 Accuracy (By Recovery Study)**

The accuracy of the RP-HPLC assay method for Atazanavir Sulfate (AS) was evaluated through a recovery study spanning the concentration range of 50% to 150% of the sample concentration. To achieve this, calculated amounts of Atazanavir sulfate API were added to placebo formulations to attain three different levels: 50%, 100%, and 150% of the sample concentration. S

For each sample, the designated amounts were weighed and transferred into 250 mL volumetric flasks. Subsequently, 180 mL of diluent was added, followed by stirring, sonication, and dilution to volume. After filtration through a 0.45 $\mu$  Teflon + Glass membrane filter, the filtrate was further diluted, and triplicate samples at each level were prepared and injected into the HPLC system. Chromatograms were recorded, and % recovery was calculated from the peak area of the drug.

The acceptance criteria for accuracy assessment necessitated the fulfillment of system suitability criteria. Furthermore, the mean recovery for the concentration range of 50% to 150% should fall within the range of 98.0% to 102.0%. Additionally, individual recoveries for each level within this concentration range were expected to be within the same range, ensuring the method's accuracy and reliability across the specified concentration levels [14].

### **2.7 Precision**

#### ***System Precision:***

System precision was evaluated by injecting the standard solution (450ppm) six times, and chromatograms were recorded. The retention time and area of each determination were measured, and the percent relative standard deviation (% RSD) was calculated. The acceptance criterion for system precision stipulated that the % RSD of the peak area from six replicate injections should not exceed 2.0% [15].

#### ***Method Precision (Repeatability):***

Method precision, also known as repeatability, assesses the agreement among individual test results when the method is applied repeatedly to homogeneous samples. Six replicate injections of assay concentration (450ppm) of both standard and sample solutions were analyzed. The percentage assay of the sample in comparison to the label claim was determined, and % RSD of the assay results was calculated. The acceptance criterion for method precision required that the % RSD for % assay of six independent samples for Atazanavir should be  $\leq$  2.0%.

#### ***Intermediate Precision (Ruggedness):***

Intermediate precision was evaluated by analyzing the standard solution (450ppm) and sample solution (450ppm) on different days. The % assay and % RSD were calculated, and system suitability criteria were ensured. The acceptance criteria for intermediate precision specified that the RSD for % assay of six independent samples should be  $\leq$  2.0%, and the absolute mean difference for % assay from method precision and intermediate precision should be  $\leq$  2.0%. This comprehensive assessment ensured

the reliability and consistency of the analytical method across different conditions and time points [16].

## 2.8 Robustness

The robustness assessment of the analytical method aimed to verify its resilience to minor variations in method parameters, including flow rate, wavelength, column temperature, and pH of the mobile phase. Each parameter was intentionally altered within predefined limits, as detailed in Tables 6.6 to 6.9. For each modified condition, the standard solution (450ppm) was injected six times, and chromatograms were recorded.

System suitability criteria had to be met for each variation. Additionally, the absolute difference in % assay value under each modified condition was compared to the original condition. It was mandated that this difference should remain within  $\pm 2.0\%$ , indicating that the method's performance was consistent despite parameter variations.

Variations included changes in flow rate by  $\pm 0.1\text{mL}/\text{min}$ , wavelength by  $\pm 5\text{nm}$ , column temperature by  $\pm 5^\circ\text{C}$ , and pH of the mobile phase by  $\pm 0.2$  units. These systematic evaluations ensured the method's robustness and reliability under normal operating conditions [17].

## 2.9 Dissolution Study of Atazanavir Sulfate by Developed RP-HPLC Method

In the dissolution study of Atazanavir sulfate using the developed RP-HPLC method, solutions were meticulously prepared to ensure accuracy and reliability. A stock solution of Atazanavir sulfate was first created by accurately weighing 90 mg of the substance and transferring it into a 50 mL volumetric flask. Diluent was added, and the

solution was sonicated until complete dissolution. This stock solution served as the reference standard for further preparations.

From this stock solution, a standard solution was prepared by pipetting 5 mL of Atazanavir sulfate into a 25 mL volumetric flask and diluting it with dissolution medium to the required concentration. Similarly, sample preparations were conducted by weighing and transferring a capsule into the dissolution vessel, following by running the dissolution process under specified parameters. Aliquots were withdrawn at designated time intervals, filtered, and then injected into the chromatographic system for analysis.

The dissolution media, consisting of 0.025 M Hydrochloric acid, was prepared according to standard protocols. The dissolution parameters were optimized in alignment with the Official Generic Drug (OGD) guidelines outlined in the USP. This included the use of a USP Type II (Paddle) apparatus with a sinker, a media volume of 1000 mL, a rotation speed of 50 rpm, and a temperature of  $37^\circ\text{C}$  ( $\pm 0.5^\circ\text{C}$ ). Sampling time points were set at 5, 10, 15, 20, 30, and 45 minutes, along with a recovery phase. These optimized conditions ensured the efficacy and accuracy of the dissolution study [18].

## 3. Result and Discussion

### 3.1 Stability Indicating RP-HPLC Method Development and Optimization

The selection of an appropriate detection wavelength is crucial for the accurate and sensitive analysis of Atazanavir sulfate. In our study, the spectrum of Atazanavir sulfate standard solution (6 ppm) was scanned, revealing a prominent absorption peak at 250.0 nm. This wavelength was selected as

the detection wavelength for further experimentation due to its optimal sensitivity and specificity.

#### ***Trial 1:***

The initial chromatographic analysis was conducted using a Zorbax Eclipse XDB C18 column with a particle size of 3.5 $\mu$ . The mobile phase composition, consisting of a buffer at pH 6.5 mixed with acetonitrile (50:50), resulted in the elution of the Atazanavir peak at a retention time of 7.895 minutes. While the peak purity was satisfactory, adjustments to the mobile phase composition were necessary to reduce the retention time (Figure 1).

#### ***Trial 2:***

In the subsequent trial, the mobile phase composition was modified to buffer at pH 6.5 mixed with acetonitrile (45:55), resulting in a reduced retention time of 5.327 minutes. Despite achieving the desired reduction in retention time, further optimization of the mobile phase composition was warranted (Figure 2).

#### ***Trial 3:***

Refinement of the chromatographic conditions led to the optimization of the method. The mobile phase composition was adjusted to buffer at pH 6.5 mixed with acetonitrile (40:60), significantly reducing the run time to 7 minutes. Under these conditions, the Atazanavir peak was eluted at a retention time of 3.969 minutes, demonstrating peak purity and favorable chromatographic performance. Moreover, the absence of interference between Atazanavir sulfate and known impurities was confirmed (Figure 3).

#### ***System Suitability:***

The system suitability test confirmed the robustness of the chromatographic system under the optimized conditions. The tailing factor was determined to be 1.1, and the theoretical plates were calculated to be 5896, meeting the acceptance criteria. Additionally, the % RSD for the area of six replicate injections ranged from 0.14%, indicating excellent reproducibility and precision (table 2).

These results collectively demonstrate the successful development and optimization of a stability-indicating RP-HPLC method for the accurate and reliable analysis of Atazanavir sulfate. The optimized method parameters provide a robust framework for future analyses and applications in pharmaceutical research and quality control.

### **3.2 Assay Results of Atazanavir Sulfate in Capsule Dosage Form**

The assay results for Atazanavir sulfate in the capsule dosage form were obtained using the proposed method. Chromatograms of the standard solution and sample solution of Atazanavir sulfate were recorded, and the peak areas were measured. In Table 3 and Figure 4, the assay results are summarized, showing the peak areas of the standard and sample solutions along with the calculated % assay. The mean % assay was found to be 99.1%, indicating that the method accurately quantifies the amount of Atazanavir sulfate in the capsule dosage form. This result falls within the acceptance criteria, demonstrating the reliability and effectiveness of the developed RP-HPLC method for the determination of Atazanavir sulfate in pharmaceutical formulations.

### **3.3 Method Validation**

#### ***3.3.1 Specificity***

The RP-HPLC method's specificity was evaluated to ensure accurate detection of Atazanavir sulfate amid potential interferences. Chromatograms of blank, placebo, standard, sample, and spiked sample solutions showed no interfering peaks. Parameters like retention time, symmetry, theoretical plates, purity angle, and threshold met acceptance criteria. Thus, the method accurately quantifies Atazanavir sulfate in complex matrices, affirming its specificity (table 4).

### **Linearity**

System suitability tests for linearity demonstrated satisfactory tailing factor (1.1) and theoretical plate count (5070). The % RSD for the area of six replicate injections ranged within 0.16%, indicating good precision. Linearity was confirmed across the concentration range of 50% to 150%, as shown in Table 7.6. The correlation coefficient ( $r^2$ ) was found to be 1.000, indicating a strong linear relationship between concentration and response. The slope of the calibration curve was calculated as 6132.180, with a y-intercept of -0.74. The method exhibited excellent linearity, meeting the acceptance criteria, and ensuring accurate quantification of Atazanavir sulfate over the specified concentration range (Table 5 and figure 5).

### **Accuracy**

System suitability tests for accuracy demonstrated a satisfactory tailing factor (1.1) and theoretical plate count (4983), with a % RSD of 0.06 for the area of six replicate injections. Accuracy was assessed through recovery studies using the standard addition method. As shown in Table 7.8, the mean recovery percentages for Atazanavir sulfate at three different levels (50%, 100%, and 150%) were found to be close to 100%, with a mean recovery of 101.6%. The

% RSD for mean recovery was 0.35, indicating good precision. These results are within the acceptance criteria range of 98.0% - 102.0%, confirming the accuracy of the proposed method. Thus, the method accurately quantifies Atazanavir sulfate without interference from excipients (table 6).

### **Precision**

System precision was evaluated by analyzing the peak area of Atazanavir sulfate from six replicate injections. The results indicated a % RSD of 0.19%, which falls within the acceptance criterion of 2.0%. This demonstrates that the system precision meets the validation requirement.

For method precision, six replicate injections were performed for the assay of Atazanavir sulfate on a single batch. The % RSD for the assay values was found to be 0.40%, complying with the acceptance criterion of 2.0%. This confirms the method's precision.

Intermediate precision was assessed by analyzing the assay of Atazanavir sulfate on different days. The % RSD of the assay values from six determinations demonstrates a value of 0.36%, within the acceptance limit of 2.0%. Additionally, pooled data from two analysts reveals an average % RSD of 0.39%. The absolute mean difference between method precision and intermediate precision is also within the acceptable range of 2.0%, confirming the method's ruggedness.

In summary, the precision parameters, including system precision, method precision, and intermediate precision, meet the method validation criteria, demonstrating the robustness and reliability of the developed RP-HPLC method for the estimation of Atazanavir sulfate.



## Robustness

The robustness of the developed RP-HPLC method for the estimation of Atazanavir sulfate was evaluated by deliberately varying certain method parameters and assessing their impact on the % assay. From the results presented in Table 7, it is evident that the absolute difference in % assay under each modified condition remained within  $\pm 2.0\%$ . Modifying the flow rate by  $\pm 0.1$  mL/min resulted in absolute differences of 0.8% and 0.4% in % assay, both within the acceptable range. Changing the wavelength by  $\pm 5$  nm led to absolute differences of 1.1% and 1.7% in % assay, which fall within the specified limits. Altering the buffer pH by  $\pm 0.2$  units caused absolute differences of 1.5% and 2.0% in % assay, remaining within the acceptable range. Varying the column temperature by  $\pm 5^\circ\text{C}$  resulted in an absolute difference of 0.0% and 0.1% in % assay, demonstrating the robustness of the method. Overall, the proposed RP-HPLC method exhibited robustness, as evidenced by the minimal impact of deliberate variations in method parameters on the % assay of Atazanavir sulfate.

### 3.4 Forced Degradation Study

The forced degradation study was conducted to assess the stability of Atazanavir sulfate under various stress conditions, including acid, base, peroxide, thermal, and photolytic degradation. Chromatograms obtained from the study depicted in Figure 6 were analyzed to evaluate the degradation profile of the drug.

In acid degradation, no additional peaks were observed, indicating the drug's high stability under acidic conditions. However, in base degradation, the appearance of additional peaks in the chromatogram indicated degradation of the drug by approximately

18.2%. Conversely, hydrogen peroxide degradation resulted in a degradation of approximately 11.4%, with additional peaks observed in the chromatogram.

Thermal degradation showed no additional peaks, suggesting the drug's stability under thermal stress. Similarly, in photolytic degradation under various conditions such as ambered, ambered with foil, and clear, no additional peaks were observed, indicating stability under these conditions as well.

The results of the forced degradation study are summarized in Table 8 showcasing the % assay, extent of degradation achieved, and purity parameters under each stress condition. Overall, Atazanavir sulfate demonstrated stability under acid, thermal, hydrogen peroxide, photolytic ambered, photolytic ambered with foil, and photolytic clear degradation conditions, but instability under base degradation, as per ICH guidelines.

## Conclusion

In conclusion, the developed stability-indicating RP-HPLC method offers a reliable and specific means for the quantitative analysis of Atazanavir sulfate, suitable for pharmaceutical quality control and stability assessment purposes. Through thorough validation in accordance with ICH guidelines, the method has demonstrated robustness, accuracy, and reliability across various parameters such as specificity, linearity, accuracy, precision, and robustness. Furthermore, its successful application in the dissolution study of Atazanavir sulfate in capsule dosage form underscores its suitability for pharmaceutical analysis. Overall, this method stands as a valuable tool for ensuring the quality, safety, and efficacy of

Atazanavir sulfate-based pharmaceutical products, contributing to regulatory compliance and patient welfare.

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#### Conflict of interest

The authors declare no conflict of interest regarding the publication of this research article.

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**Table 1: Preparation of linearity samples**

Linearity Level%	Volume taken from Linearity stock solution (mL)	Total volume (mL)	Concentration(ppm)	
			Atazanavir sulfate	Atazanavir
50	5	50	225	198
80	4	25	360	316
100	5	25	450	395
120	6	25	540	474
150	15	50	675	593

**Table 2: Result of System suitability**

<b>Tailing factor</b>	<b>1.1</b>
<b>Theoretical plates</b>	<b>5896</b>
<b>S. No.</b>	<b>Area</b>
1	2579512
2	2573821

3	2578679
4	2580212
5	2582800
6	2574221
<b>Mean</b>	<b>2578207.5</b>
<b>% RSD</b>	<b>0.14</b>

**Table 3: Result of assay by proposed method**

<b>Parameters</b>	<b>Atazanavir sulfate</b>
Standard area	2579782
	2576128
	2575559
	2576360
	2576747
	2573320
Mean area	2576316
Sample area	2624612
	2657451
Mean area	2641031.5
% Assay	99.1

**Table 4: Result of specificity**

<b>Component</b>	<b>Retention Time</b>	<b>Symmetry factor</b>	<b>Theoretical plates</b>	<b>Purity angle</b>	<b>Purity threshold</b>
Blank	-	-	-	-	-

Placebo	-	-	-	-	-
Standard solution	3.561	1.5	8089	0.271	0.360
Sample solution	3.614	1.5	8103	0.404	0.443
Spiked sample solution	3.558	1.5	8163	0.267	0.381

**Table 5: Results of linearity**

Level (%)	Concentration (ppm)	Response		
		1	2	Mean
50	193.972	1178663	1178825	1178744
80	310.355	1868119	1861740	1864930
100	387.944	2383901	2394334	2389118
120	465.533	2816416	2813679	2815048
150	581.916	3533387	3583155	3558271
<b>Co-relation coefficient (r<sup>2</sup>)</b>				<b>1.000</b>
<b>SLOPE</b>				<b>6132.180</b>
<b>Y-INTERCEPT</b>				<b>-0.74</b>
<b>WORKING LEVEL AREA</b>				<b>2389118.0</b>
<b>%LIMIT OF Y-INTERCEPT ( ± 5 OF WORKING LEVEL)</b>				<b>-0.74</b>

**Table 6: Result of Accuracy**

Level (%)	Theoretical concentration (mcg/mL)	% Recovery	Mean recovery%
50	171.916	101.6	102.0
	172.661	102.1	
	172.261	102.4	

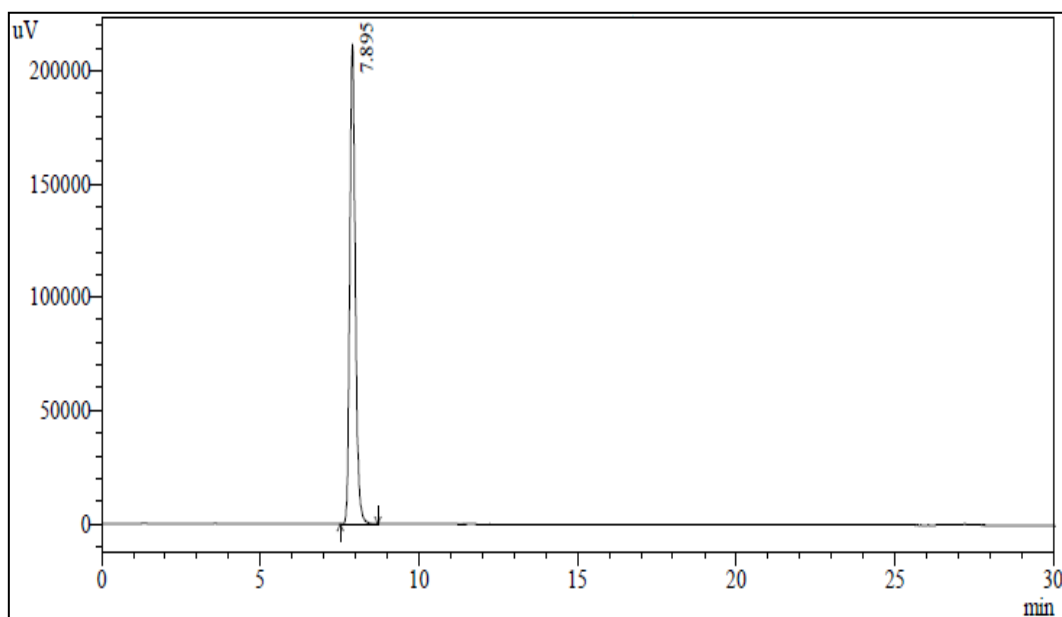
100	345.156	101.8	101.6
	345.039	101.4	
	345.343	101.7	
150	517.804	100.7	101.3
	518.355	101.8	
	523.886	101.3	
<b>Mean recovery</b>			<b>101.6</b>
<b>%RSD</b>			<b>0.35</b>

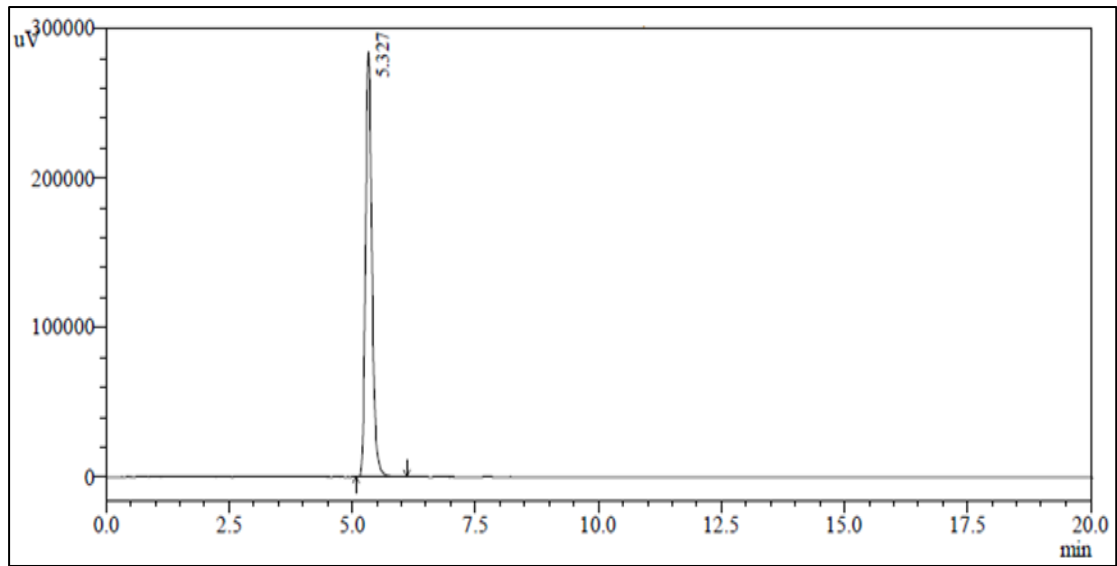
**Table 7: Result of Robustness**

Parameters	Values	Retention Time	Tailing factor	Theoretical plates	% RSD of standard area	% Assay	Absolute difference
Control	As per method	3.791	1.1	4511	0.49	99.6	-
Flow rate ( $\pm 0.1$ mL/min)	0.9 mL/min	4.30	1.3	2475	0.06	98.4	0.8
	1.1 mL/min	3.541	1.4	2316	0.22	99.2	0.4
Change in Wavelength( $\pm 5$ nm)	245 nm	3.896	1.2	2959	0.11	100.7	1.1
	255 nm	3.851	1.2	3186	0.11	101.3	1.7
Buffer pH( $\pm 0.2$ unit)	pH-6.3	3.791	1.2	4840	0.30	98.1	1.5
	pH-6.7	3.809	1.0	3572	0.26	97.6	2.0
Column temperature ( $\pm 5^\circ\text{C}$ )	25 $^\circ\text{C}$	3.712	1.2	4779	0.17	99.6	0.0
	35 $^\circ\text{C}$	3.781	1.2	5423	0.10	99.7	0.1

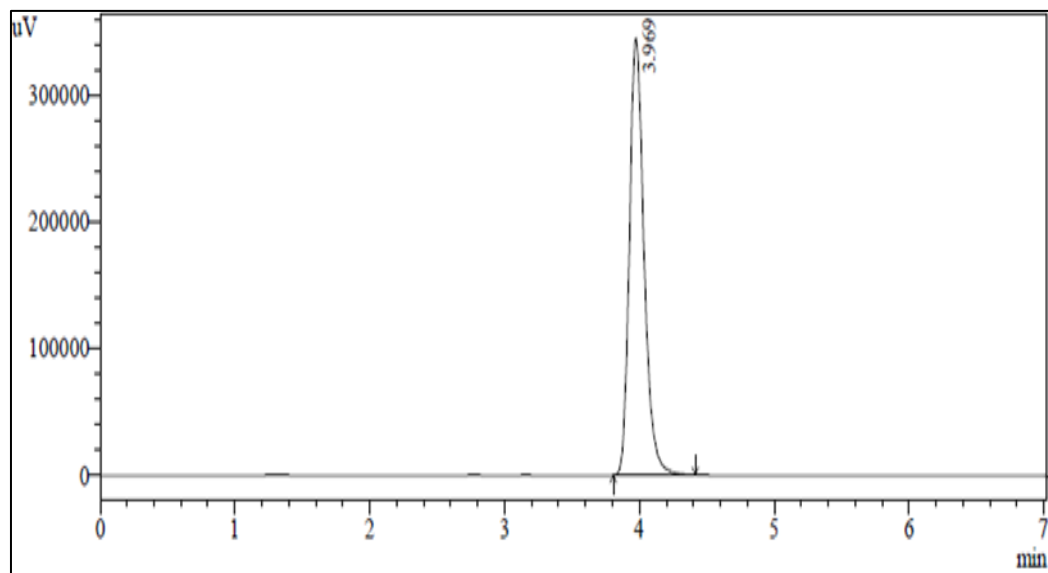
**Table 8: Results of force degradation study**

Conditions	% Assay	Degradation achieved	Purity angle	Purity threshold
Control	99.2	-	0.170	0.381
Acid degradation	96.3	2.9	0.183	0.386
Base degradation	81.0	18.2	0.200	0.398
Control	98.1	-	0.203	0.399
Peroxide degradation	92.8	5.3	0.185	0.390
Thermal degradation	97.5	0.6	0.101	0.272
Control	98.6	-	0.208	0.274
Photolytic (Ambered)	97.4	1.2	0.208	0.275
Photolytic(Ambered+foil)	98.2	0.4	0.214	0.275
Photolytic (Clear)	87.2	11.4	0.213	0.277

**Fig.1: Trial 1**

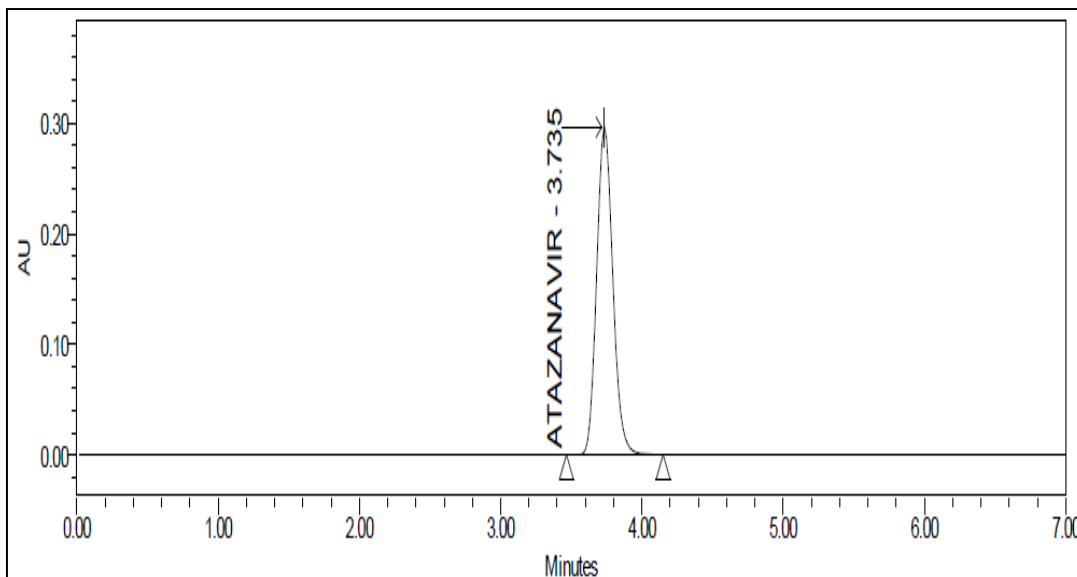


**Fig. 2: Trial 2**

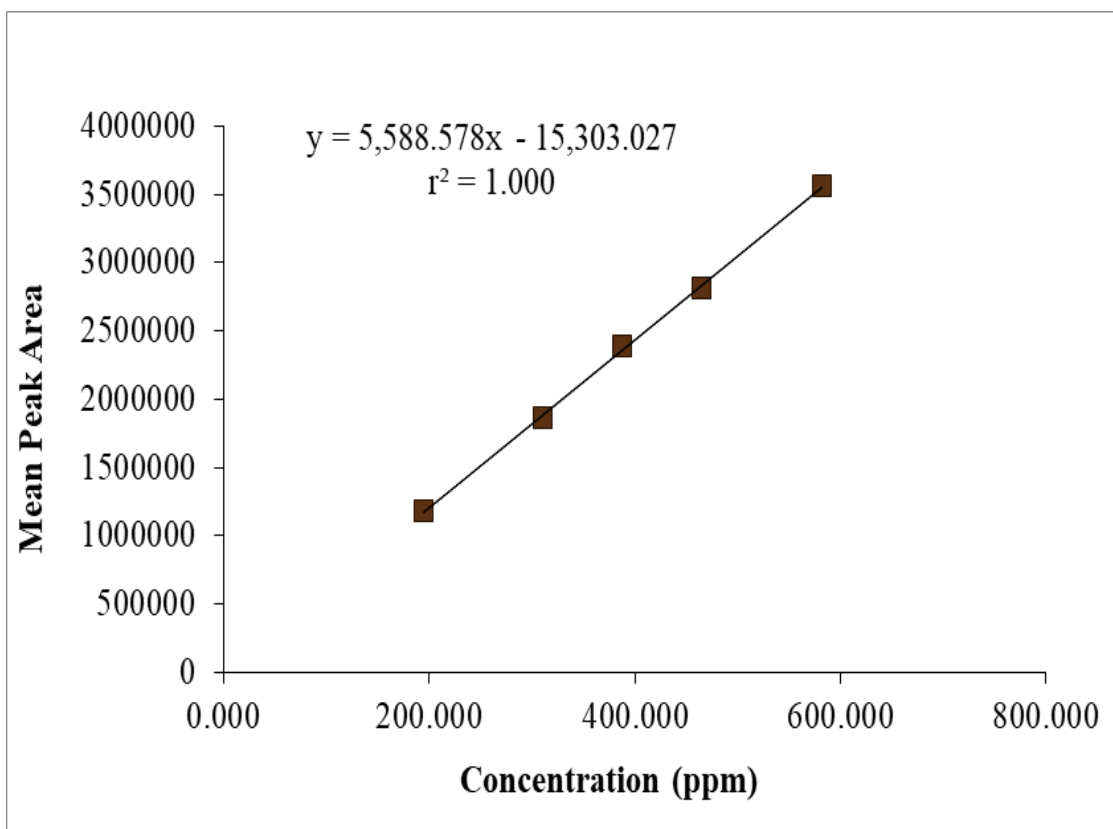


**Fig.3: Trial 3**

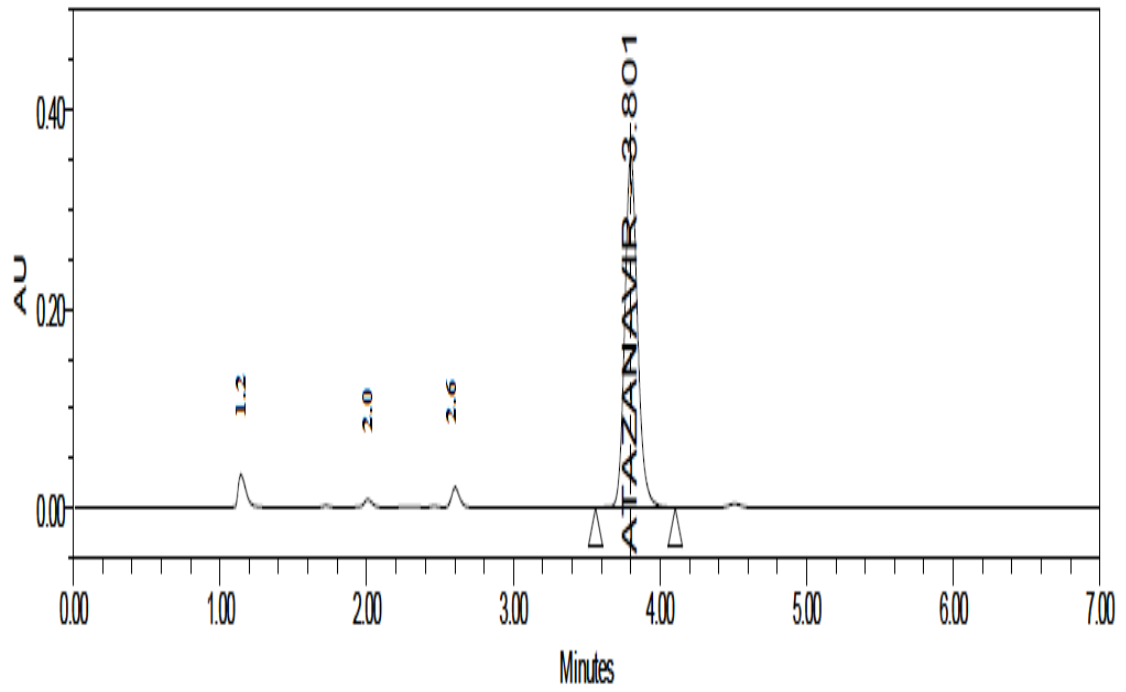




**Fig 4: Chromatogram of standard solution of Atazanavir sulfate**



**Fig 5: Calibration curve of Atazanavir sulfate**



**Fig 6: Chromatogram of photolytic clear degradation sample**