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# Validated Stability-Indicating Liquid Chromatographic Method for the Determination of Atazanavir sulpahte (Anti-Retroviral Agent) in Capsules

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**ABSTRACT**

Atazanavir sulphate is an oral anti-retroviral protease inhibitor used in the treatment of HIV-1 infection. An isocratic stability-indicating high-performance liquid chromatographic method was developed and validated for the determination of Atazanavir sulphate in capsules. Reversed-phase chromatography was performed on Shimadzu model LC-20AD Prominence with SPD M 20A diode array detector using a phenomenex lichrosphere 100 C-18 (250 mm × 4.6 mm i.d., 5 µm particle size) column with 0.1% v/v acetic acid : acetonitrile (15:85, V/V) as mobile phase at a flow rate of 1.2 ml/min. The chromatographic peak for Atazanavir sulphate was observed at 2.927 min with UV detection at 254 nm. Linearity was observed in the concentration range of 1-180 µg/mL with regression equation y = 19822 x + 78985 (R2 = 0.9992). The LOD and LOQ were found to be 0.252 µg/mL and 0.769 µg/mL respectively. The percentage relative standard deviation in precision study was found to be 0.27 - 0.60 (intraday), 0.17-0.53 (inter day) and 0.26-0.27 in accuracy study (< 2%). Atazanavir sulphate was subjected to stress conditions of degradation in aqueous solutions including acidic, alkaline, oxidation, thermal and photolysis. It was found that the drug is highly resistant to alkaline and oxidation stress conditions while mild degradation was observed in acidic, thermal and photolytic study. The developed stability indicating method was validated according to ICH guidelines and found to be linear, specific, precise, accurate and robust.

**KEY WORDS:** Atazanavir sulphate, Isocratic elution, RP-HPLC, Validation, Stability-indicating.

## 1. INTRODUCTION

Atazanavir sulphate (Figure 1) is a recently introduced azapeptide inhibitor of HIV-1 Protease. It was approved by Food and Drug Administration (FDA) in 2003. Chemically Atazanavir Sulfate is (3S,8S,9S,12S)-3,12-Bis(1,1-dimethylethyl)-8hydroxy-4,11-dioxo-9-(phenylmethyl)-6-[[4-(2-pyridinyl)phenyl]methyl]- 2,5,6,10, 13 penta aza tetra decanedioic acid dimethyl ester, sulfate (1:1). Its molecular formula is $C_{38}H_{52}N_6O_7 \bullet H_2SO_4$, which corresponds to a molecular weight of 802.9 (sulfuric acid salt). Lead optimization using X-ray structural data from an inhibitor–protease complex (Priestle, 1995) led to the discovery of Atazanavir sulfate, which showed an excellent anti- viral activity with high oral bioavailability. Atazanavir sulphate selectively inhibits the virus-specific processing of viral Gag and Gag-Pol polyproteins in HIV-1 infected cells by binding to the active site of HIV-1 protease, thus preventing the formation of mature virions. Atazanavir sulphate is not active against HIV-2. Antiretroviral therapy regimens containing ritonavir-boosted atazanavir improved virological and immunological markers in adult patients with HIV-1 infection (Croom, 2009). It is used in combination with other antiretroviral agents for the treatment of HIV-1 infection, as well as post exposure prophylaxis of HIV infection.

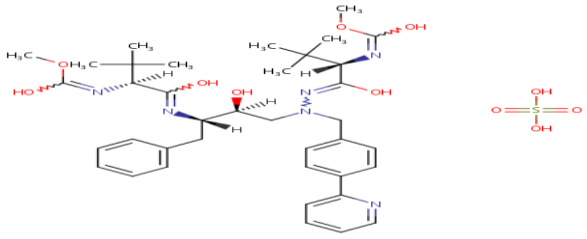

**Figure.1. Chemical structure of Atazanavir sulphate**

Analytical methods described in literature for the determination of Atazanavir sulphate in biological and other matrices include spectrophotometry (Khanage, 2010; Dey, 2010; Behra, 2011; Mittal, 2011; Nanda, 2011; Bari, 2012; Parameswara Rao, 2012), High Performance Liquid Chromatography (Dailly, 2004; Olivier, 2005; Arianna, 2006; Holland, 2006; Reyes, 2006; Cattaneo, 2008; Muller, 2010; Padmalatha, 2010; Nanda, 2011; Rao, 2011; Srinivasu, 2011; Behera, 2012; Sathish Kumar, 2012; Bhirud, 2013; Dey, 2013) and High Performance Thin Layer Chromatography (Nanda, 2011).

Quality control of pharmaceutical products requires identification and quantification of the active ingredient and its impurities for safety and efficacy reasons. Impurities and potential degradation products that may

exist in medicines can change the chemical, pharmacological and toxicological properties of the product. Very few stability indicating analytical methods were reported in the literature and since pharmacopoeias do not describe a suitable stability indicating method for the determination of Atazanavir sulphate in pharmaceutical formulations, in the present work an attempt has been made to develop a simple, rapid precise and accurate reverse phase liquid chromatographic method for the determination of Atazanavir sulphate in capsules.

## 2. MATERIALS AND METHODS

**2.1. Chemicals and reagents:** Atazanavir sulphate standard (purity 99.80%) was obtained from M/S Hetero Drugs Ltd., India. Ethanol (HPLC grade), sodium hydroxide (NaOH), hydrochloric acid (HCl), hydrogen peroxide ($H_2O_2$) and HPLC grade water were obtained from Merck, India. Acetonitrile (HPLC grade) and glacial acetic acid were procured from Qualigens. Commercial capsules (Label claim 300.0 mg) of Atazanavir sulphate marketed as ATAZOR were procured from the pharmacy store and all other chemicals were of an analytical grade and used as received.

**2.2. Instrumentation:** Chromatographic separation was achieved by using a Shimadzu Model CBM-20A/20 Alite HPLC system, equipped with SPD M20A prominence photodiode array detector on a phenomenex C18 column (250 mm × 4.6 mm, 5 µm particle size) maintained at 25 ºC.

**2.3. Chromatographic conditions:** Isocratic elution was performed using 0.1% v/v acetic acid: acetonitrile (15:85, V/V) as mobile phase with a flow rate of 1.2 mL/min. UV detection was performed at 254 nm.

**2.4. Preparation of Atazanavir sulphate stock solution:** Atazanavir sulphate stock solution (1000 µg/mL) was prepared by accurately weighing 25 mg of Atazanavir sulphate in a 25 mL volumetric flask and making up to volume with ethanol. Working solutions for HPLC injections were prepared on a daily basis from the stock solution using 0.1% v/v acetic acid: acetonitrile (15:85 V/V) as diluent. Solutions were filtered through a 0.45 µm membrane filter prior to injection and 20 µL was injected into the HPLC system.

**2.5. Validation:** The method was validated for the following parameters: linearity, limit of detection (LOD), limit of quantitation (LOQ), precision, accuracy, specificity and robustness (ICH guidelines, 2005).

**2.5.1. Linearity:** Linearity test solutions for the assay method were prepared from a stock solution at different concentration levels of the analyte (1.0-180 µg/mL). 20 µL of each solution was injected in to the HPLC system and the peak area of the chromatogram obtained was noted. The analytical curve was evaluated on three different days. The peak area vs. concentration data was analyzed with least squares linear regression. The slope and y-intercept of the calibration curve was reported.

**2.5.2. Precision:** The intra-day precision of the assay method was evaluated by carrying out 9 independent assays of a test sample of Atazanavir sulphate at three concentration levels (50, 80 and 100 µg/mL, n=3) against a qualified reference standard. The %RSD of three obtained assay values at three different concentration levels was calculated. The interday precision study was performed on three different days i.e. day 1, day 2 and day 3 at three different concentration levels (50, 80 and 100 µg/mL, n=3). The % RSD of three obtained assay values on three different days was calculated.

**2.5.3. Accuracy:** The accuracy of the assay method was studied by standard addition method. Solutions at three concentration levels (80, 100 and 120%) were prepared in triplicate from the pre analysed formulation samples and the percentage recoveries were calculated. The percentage recovery and % RSD was calculated at each level.

**2.5.4. Sensitivity/ Limit of detection (LOD) and Limit of quantification (LOQ):** The limit of detection (LOD) and limit of quantification (LOQ) were based on the standard deviation of the response and the slope of the constructed calibration curve (n=3), as described in International Conference on Harmonization guidelines Q2 (R1). Sensitivity of the method was established with respect to LOD and LOQ for Atazanavir sulphate and calculated by slope method as mentioned below.

$$LOD = 3.3 \times \sigma / S, \quad LOQ = 10 \times \sigma / S$$

**2.5.5. Robustness:** Robustness of the method was validated in terms of small deliberate variations in mobile phase composition (± 2 %), flow rate (± 0.1 mL/min), UV detection (± 2 nm) and pH (± 0.1). Each solution was injected in triplicate, peak areas observed and % RSD was calculated.

**2.5.6. Solution Stability:** The objective of stability study is to determine the time period of storage at a specified condition within which the drug product still meets its established specifications The solution stability of Atazanavir sulphate in the assay method was carried out by leaving both the sample and reference standard solutions in tightly capped volumetric flasks at room temperature for 48 hrs. Freshly prepared standard and sample solutions were injected on the initial day and the same standard and sample preparations were injected in the intervals of 6, 12, 18, 24, 48 hrs over the study period. The % RSD of the Atazanavir sulphate assay was calculated for the solution stability experiments. An additional study was carried out using the stock solution by storing it in a tightly capped volumetric flask at 5-10 ºC.

**2.6. Forced Degradation Studies/Specificity:** The study was intended to ensure the effective separation of Atazanavir sulphate and its degradation peaks or formulation ingredients at the retention time of Atazanavir

sulphate. To confirm the stability indicating nature of the analytical method, forced degradation of Atazanavir sulphate was carried out under acid/base hydrolytic, oxidative, photolytic and thermal stress conditions as per ICH recommended test conditions (ICH guidelines, 2003). All solutions for use in stress studies were prepared at an initial concentration of 1 mg/mL of Atazanavir sulphate and then diluted in mobile phase to give a final concentration of 100 µg/mL. 20 µL of each solution was injected in triplicate into the HPLC system.

**2.6.1. Acidic and Alkaline Degradation:** Atazanavir sulphate drug solution (1.0 mg/mL) was treated with 1.0 mL of 0.1 M HCl, and heated on a thermostat to $80^0$C for 30 min. The stressed sample was neutralized and diluted with mobile phase to give a final concentration of 100 µg/mL and filtered before injection.

Similarly stress studies in alkaline conditions were conducted by treating the drug solution with 1.0 mL of 0.1 M NaOH and heating on a thermostat to $80^0$C for 30 min. The stressed sample was neutralized and diluted with mobile phase to give a final concentration of 100 µg/mL and filtered before injection.

**2.6.2. Oxidative Degradation:** Oxidative stress studies were conducted by treating the drug solution with 1.0 mL of 3% $H_2O_2$ and heating on a thermostat for 30 min. The stressed sample mixture was cooled, diluted with mobile phase to give a final concentration of 100 µg/mL.

**2.6.3. Photolytic Degradation:** The drug solution for photo stability testing was exposed to UV light for 2 hours in a UV chamber (365 nm) and diluted with the mobile phase to give a final concentration of 100 µg/mL and filtered before injection.

**2.6.4. Thermal Degradation:** Thermal stress was carried out by heating the drug solution in a controlled temperature oven at $105^0$ C for 30min. The stressed sample mixture was cooled, diluted with mobile phase to give a final concentration of 100 µg/mL.

**2.7. Assay of commercial formulation:** Twenty ATAZOR capsules were procured, weighed and powder equivalent to 25 mg Atazanavir sulphate was accurately weighed into a 25 ml volumetric flask and made up to volume with ethanol. The contents of the volumetric flask were sonicated for 30 min to enable complete dissolution of Atazanavir sulphate. The solution was filtered and the filtrate was suitably diluted with mobile phase. 20 µL of this solution was injected in triplicate and the peak area was recorded from the respective chromatograms.

## 3. RESULTS AND DISCUSSION

A stability indicating reversed-phase chromatographic technique was developed to quantitate Atazanavir sulphate at 254 nm. Acetonitrile was chosen as an organic modifier in the mobile phase. Satisfactory resolution was achieved with a mixture of 0.1% v/v acetic acid: acetonitrile (15:85, V/V) as mobile phase with a flow rate of 1.2 mL/min as demonstrated in Figure 2. C18 column was adopted for the analysis because it provided a better separation of the analytes.

The present stability-indicating method for the determination of Atazanavir sulphate in pharmaceutical formulations is specific because the drug peak was well separated even in the presence of degradation products. Overall, the data demonstrated that the excipients and the degradation products did not interfere with the Atazanavir sulphate peak, indicating the specificity of the method. The complete separation of the analytes was accomplished in less than 10 min and the method can be successfully applicable to perform long-term and accelerated stability studies of Atazanavir sulphate formulations.

**3.1. HPLC Method development and optimization:** Initially the stressed samples were analyzed using a mobile phase consisting of TBHS: acetonitrile (45:55, v/v) at a flow rate of 1.0 mL/min. Under these conditions, the resolution and peak symmetry were not satisfactory, so the mobile phase was changed to 0.1 % acetic acid: acetonitrile (15:85, V/V) with a flow rate of 1.2 mL/min under which peaks were well resolved with good symmetry and sharpness. Therefore, mobile phase containing 0.1 % acetic acid: acetonitrile (15:85, V/V) at a flow rate of 1.2 mL/min was chosen as the optimized condition for the entire study.

**3.2. Method Validation:**

**3.2.1. System Suitability:** The system suitability test was performed to ensure that the complete testing system was suitable for the intended application. The parameters measured were peak area, retention time, tailing factor and theoretical plates. In all measurements the peak area varied less than 2.0%, the average retention time was 2.9 ± 0.305 minutes. The capacity factor was more than 2, theoretical plates were more than 4000 and tailing factor was less than 2.0 for the Atazanavir sulphate peak. The proposed method offers high sensitivity and Atazanavir sulphate can be detected accurately. In all the cases, the Atazanavir sulphate peak was well separated from the degradation products.

**3.2.2. Linearity:** The linearity data obtained for the calibration curve of Atazanavir sulphate was linear over the concentration range of 1.0–180 µg/mL (Table 1). A calibration curve was drawn by taking the concentration on the x-axis and the corresponding peak area on the y-axis shown in Fig.3. The regression equation was found to be y = 19822 x + 78985 with correlation coefficient 0.9992.

**3.2.3. Precision:** The precision of the method was determined by repeatability (Intra-day precision) and intermediate precision (Inter-day precision) using Atazanavir sulphate formulation solutions. Repeatability was

calculated by assaying three samples each at three different concentration levels (50, 80 and 100 µg/mL) on the same day. The inter-day precision was calculated by assaying three samples each at three different concentration levels (50, 80 and 100 µg/mL) on three different days. The % RSD range was obtained as 0.27-0.60 and 0.17-0.53 for intra-day and inter-day precision studies respectively (Table 2).

**3.2.4. Accuracy:** The accuracy of the method was established by the recovery test. A known amount of standard Atazanavir sulphate was added to aliquots of sample solutions and then diluted to yield total concentrations of 27, 30 and 33 µg/mL as described in Table 3. The solutions were prepared and injected in triplicate and the resultant % RSD was 0.27 (< 2.0 %) with a recovery of 99.70-100.5 %.

**3.2.5. Limit of Detection and Limit of Quantification:** The LOQ and LOD were determined based on the 10 and 3.3 times the standard deviation of the response, respectively, divided by the slope of the calibration curve. The LOD and LOQ were found to be 0.252 µg/mL and 0.769 µg/mL respectively.

**3.2.6. Robustnes:** The robustness of an analytical procedure refers to its ability to remain unaffected by small and deliberate variations in method parameters and provides an indication of its reliability for routine analysis. The robustness of the method was evaluated by performing the assay of Atazanavir sulphate under different analytical conditions deliberately changing from the original condition. Slight changes in mobile phase composition, flow rate, detection wavelength and pH affects the chromatographic response such as retention time and peak area as given in Table 4. The % RSD obtained for peak area was 0.69 - 1.19 (< 2.0%) indicating that the proposed method is robust.

**3.2.7. Solution Stability:** The %RSD of the assay of Atazanavir sulphate from the solution stability experiments was within 2%. The results confirm that the sample solutions used during the assay were stable up to 48 hrs at room temperature and up to 3 months at 5ºC.

**3.3. Forced degradation studies:** The stability indicating capability of the method was established from the separation of Atazanavir sulphate peak from the degraded samples. The degradation of Atazanavir sulphate was found to be very similar for both the marketed formulation and standard. Typical chromatograms obtained following the assay of stressed samples are shown in Fig. 4(a-e).

A slight decomposition (< 10 %) was observed when Atazanavir sulphate drug was exposed to acidic (4.93 %), alkaline (1.82 %), oxidation (2.29 %), thermal (5.59 %) and photolytic (3.55 %) stress conditions. During the oxidative degradation one major peak was observed at 2.214 mins without interfering the elution of drug peak (2.936 mins). Atazanavir sulphate was resistant towards oxidation and alkaline degradations. The drug has undergone mild acidic, photolytic and thermal degradations. Table 5 summarizes the data of degradation studies.

The system suitability parameters for all the degradation studies were shown in Table 5. The number of theoretical plates (N) is used to determine the performance and efficiency of the column. It is a measure of band spreading of a peak. Smaller the band spread, higher is the number of theoretical plates, indicating good column and system performance. Columns with theoretical plates ranging from 4,000 to 100,000 plates / metre are ideal for a good separation system. The theoretical plates were found to be more than 4000 and the tailing factor was less than 2.0 indicating good column efficiency and peak shape.

**3.4. Analysis of commercial formulations (Capsules):** The proposed method was applied for the determination of Atazanavir sulphate in marketed capsules and the result of the assay was found to be 99.38 - 100.03 % respectively with RSD < 2.0 %. The results indicate that the method is selective for the assay of Atazanavir sulphate without interference from the excipients used in these capsules.

**Table 1: Linearity of Atazanavir sulphate**

| S. No. | Conc. (µg/mL) | Mean Peak area ± SD | %RSD |
|---|---|---|---|
| 1 | 1 | 33601 ± 263.82 | 0.78 |
| 2 | 2 | 150695 ± 705.48 | 0.46 |
| 3 | 5 | 227914 ± 2223.50 | 0.77 |
| 4 | 20 | 416505 ± 1915.99 | 0.46 |
| 5 | 40 | 897490 ± 7018.98 | 0.78 |
| 6 | 50 | 1098523 ± 1488.50 | 0.13 |
| 7 | 80 | 1658459 ± 10249.86 | 0.61 |
| 8 | 100 | 2043830 ± 11354.35 | 0.55 |
| 9 | 150 | 3046418 ± 27190.42 | 0.89 |
| 10 | 180 | 3654938 ± 25315.89 | 0.69 |

**Table 2: Intra-day and inter-day precision studies of Atazanavir sulphate**

| S No. | Conc. (µg/ml) | Intra-day precision | | Inter-day precision | |
|---|---|---|---|---|---|
| | | Mean* ± SD | %RSD | Mean* ± SD | %RSD |
| 1 | 50 | 1016705 ± 2780.88 | 0.27 | 1059865 ± 3276.9 | 0.36 |
| 2 | 80 | 1655728 ± 10083.09 | 0.60 | 1697224± 24989.4 | 0.53 |
| 3 | 100 | 2076757 ± 6466.05 | 0.31 | 2083654 ± 15459.1 | 0.17 |

*Mean of three replicates

**Table 3: Accuracy study of Atazanavir sulphate by standard-addition method**

| Spiked Conc.(µg/mL) | Total Conc.(µg/mL) | *Measured Conc. (µg/mL) | (%) Mean Recovery* ± (%) RSD |
|---|---|---|---|
| 12 (80%) | 27 | 27.13 | 100.5 ± 0.27, 0.27 |
| 15 (100%) | 30 | 30.06 | 100.2 ± 0.26, 0.26 |
| 18 (120%) | 33 | 32.90 | 99.7 ± 0.27, 0.27 |

*Mean of three replicates

**Table 4: Robustness study of Atazanavir sulphate**

| S.No | Parameter | Condition | *Mean Peak area | *Mean Peak area±SD (%RSD) | *Assay(%) |
|---|---|---|---|---|---|
| 1 | Mobile Phase (± 2%) | 13:87 | 2065123 | 2083534 ± 17344.05 (0.83) | 99.86 |
| | | 15:85 | 2085916 | | |
| | | 17:83 | 2099565 | | |
| 2 | Flow rate (± 0.1 mL) | 1.1 | 2067260 | 2089910 ± 24889.6 (1.19) | 100.2 |
| | | 1.2 | 2085916 | | |
| | | 1.3 | 2116556 | | |
| 3 | UV detection (± 2 nm ) | 252 | 2070123 | 2085012 ± 14458.7 (0.69) | 99.9 |
| | | 254 | 2085916 | | |
| | | 256 | 2098998 | | |
| 4 | pH (± 0.1 unit) | 3.26 | 2117013 | 2094306 ± 19883.65 (0.95) | 100.4 |
| | | 3.36 | 2085916 | | |
| | | 3.46 | 2079998 | | |

**Table 5: Forced degradation studies of Atazanavir sulphate**

| Stress Conditions | *Mean Peak area | *% Drug Recovered | *% Drug Decomposed | Tailing factor | Theoretical plates |
|---|---|---|---|---|---|
| Standard Drug | 2058993 | 100 | - | 1.407 | 7554.285 |
| Acidic Hydrolysis | 1957486 | 95.07 | 4.93 | 1.683 | 7003.302 |
| Alkaline Hydrolysis | 2021519 | 98.18 | 1.82 | 1.834 | 6065.535 |
| Oxidative degradation | 2011901 | 97.71 | 2.29 | 1.440 | 7421.040 |
| Thermal degradation | 1943939 | 94.41 | 5.59 | 1.356 | 5999.263 |
| Photolytic degradation | 1985810 | 96.45 | 3.55 | 1.389 | 6989.026 |

*Mean of three replicates

**Table 6: Analysis of Atazanavir sulphate commercial formulation (Capsules)**

| S. No | Formulation | Labelled claim (mg) | *Amount found (mg) | % Label claim ± SD* |
|---|---|---|---|---|
| 1 | ATAZOR | 300 | 299.05 | 99.68 ± 0.32 |

*Mean of three replicates

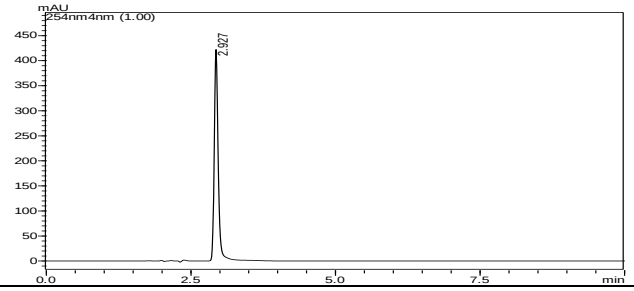


**Figure 2: Representative chromatogram of standard Atazanavir sulphate (100 µg/mL)**

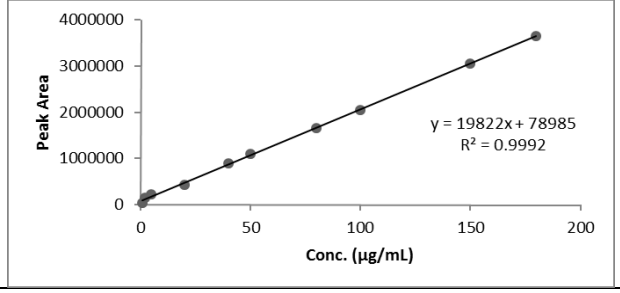


**Figure 3: Calibration Curve of Atazanavir sulphate**

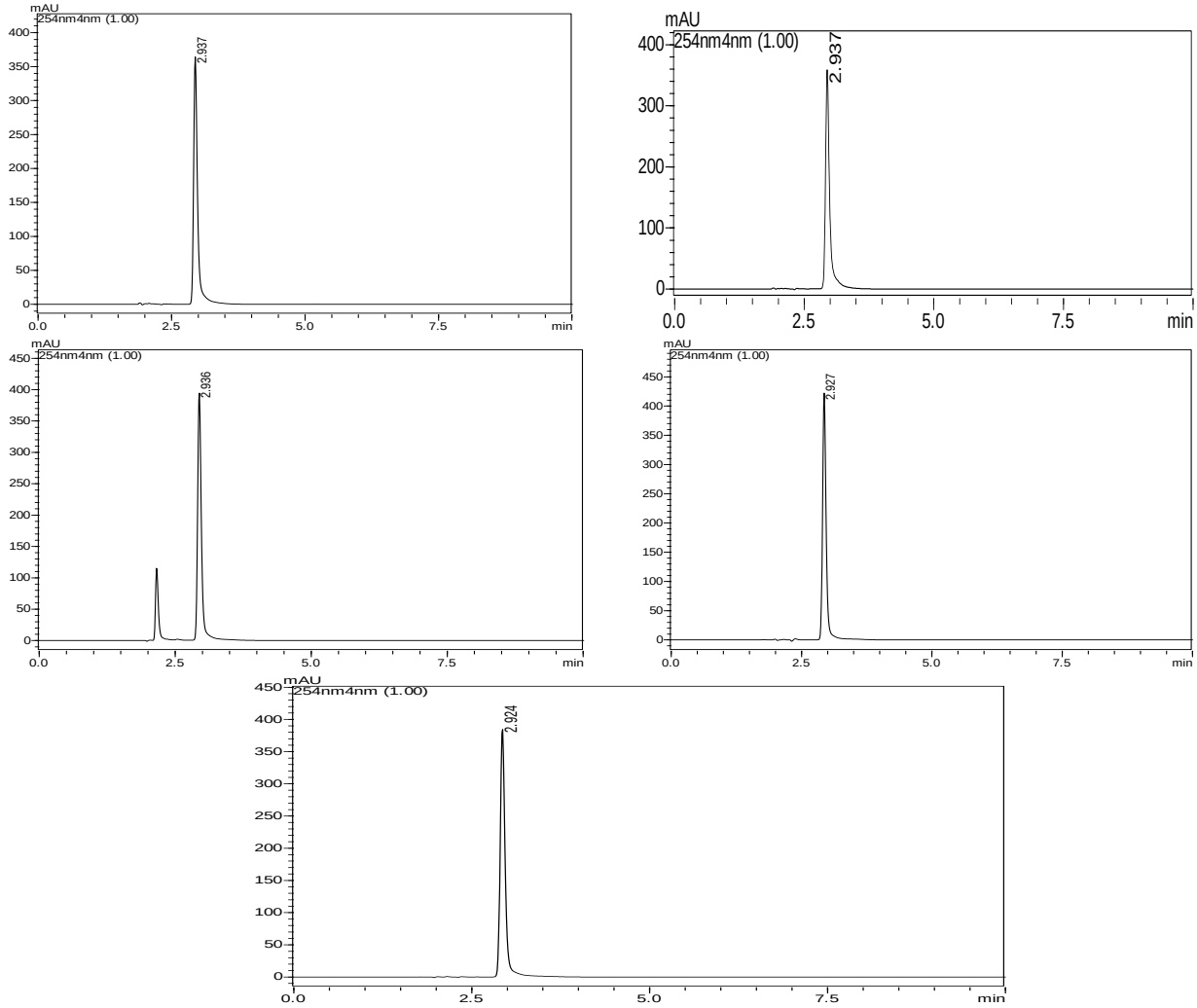


**Figure 4: Typical chromatogram of Atazanavir sulphate (100 µg/mL) a) on acidic degradation, b)on alkaline degradation, c) on oxidative degradation, d) on photolytic degradation, e)on thermal degradation**

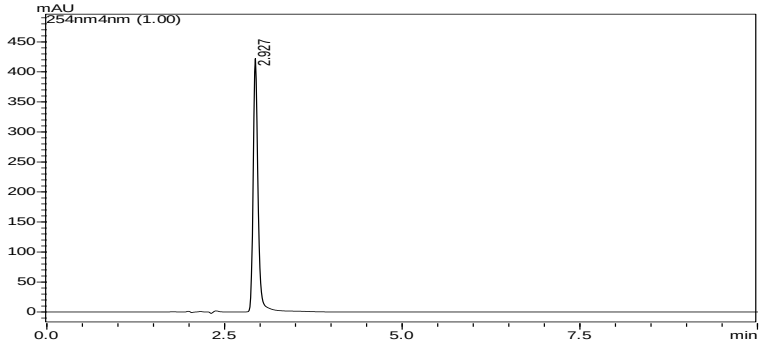


**Figure 5: Representative Chromatogram of Atazanavir sulphate (100 µg/mL) (ATAZAR® 300 mg).**

## 4. CONCLUSION

The proposed stability-indicating HPLC method was validated as per ICH guidelines and applied for the determination of Atazanavir sulphate in pharmaceutical dosage forms. The method was found to be simple, precise, accurate, robust and specific as the drug peak did not interfere with the extra peaks that eluted during the forced degradation studies. At the same time the chromatographic elution step is undertaken in a short time (< 5 min). No interference from any components of pharmaceutical dosage form or degradation products and therefore the method can be successfully applied to perform long-term and accelerated stability studies of Atazanavir sulphate formulations. In conclusion, the high sensitivity, good selectivity, accuracy and reproducibility of the proposed method is suitable for quality control analysis of complex pharmaceutical preparations containing Atazanavir sulphate.

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**REFERENCES**

Arianna L, Elisa B, Giologio P, Simple activation of the HIV protease inhibition Atazanavir in human plasma by HPLC with UV detection, J.Pharm. Biomed. Anal., 42, 2006, 500–505.

Bari NA, Kela SP, Sharma SN, Spectrophotometric simultaneous determination of Atazanavir and Ritonavir in combined tablet dosage form by ratio derivative and area under curve method, Der.Pharma.Chemica., 4, 2012, 208-213.

Behera A, Kumar SM, Chandra SS, Sankar DG, Spectrophotometric method for determination of Atazanavir sulfate in capsule dosage form, Quim.Nova, 34(8), 2011, 1349-1353.

Behera A, Sankar DG, Motera SK, Development, validation and statistical correlation of RP-LC methods for determination of Atazanavir sulfate in capsule dosage form, E.J.Chem., 9, 2012, 1778-1787.

Behera A, Sethy K, Sankar DG, Statistical correlation and simultaneous estimation of Azatanavir sulfate and Ritonavir in fixed dosage form by high performance liquid chromatography, J.Liquid Chromatogr Relat Tech., 35, 2012, 1731-1749.

Bhirud HC, Hiremath SN, Stability indicating RP-HPLC method for the determination of Atazanavir sulphate in bulk and dosage form, Drug Invention Today, 5, 2013, 81-86.

Cattaneo D, Maggiolo F, Ripamonti D, Perico N, Determination of Atazanavir in human plasma by high-performance liquid chromatography with UV detection, Journal of Chromatographic Science, 46, 2008, 485-489.

Croom KF, Dhillon S and Keam SJ, Atazanavir-A review of its use in the management of HIV-1 infection, Drugs, 69 (8), 2009, 1107-1140.

Dailly E, Raffi F, Jolliet P, Determination of Atazanavir and other anti- retroviral drugs plasma levels by high performance liquid chromatography with UV-detection, J.Chromatogr.B Analyt.Technol.Biomed.Life Sci., 813, 2004, 353–358.

Dey S, Patro SS, Suresh babu N, Murthy PN, Panda SK, Development and validation of a stability-indicating RP–HPLC method for estimation of Atazanavir sulfate in bulk, Journal of Pharmaceutical Analysis, 2013.

Dey S, Reddy YV, Reddy T, Sahoo SK, Murthy PN, Mohapatra S, Patro SS, Method development and validation for the estimation of Atazanavir in bulk and pharmaceutical dosage forms and its stress degradation studies using UV–Vis spectrophotometric method, Int. J. Pharm. Bio. Sci., 1(3), 2010, 1–8.

Holland DT, Espina Quinto R, Stefansk EK, Helms MK, Moon B, Valdez J, Connor JD, Rapid automated immunoassay for Atazanavir in plasma and cerebrospinal fluid(CSF) using the ARK ATV test: Methods validation, application and comparision with HPLC plasma method, 7th International workshop on clinical pharmacology of HIV therapy, 2006.

ICH stability testing of new drug substances and products Q1A (R2), International Conference on Harmonization, 2003.

ICH validation of analytical procedures: text and methodology Q2 (R1), International Conference on Harmonization, 2005.

Khanage SG, Deshmukh VK, Mohite PB, Dhamak VM, Appala Raju S, Development of derivative spectrophotometric estimation of Atazanavir sulfate in bulk drug and pharmaceutical dosage forms, Int.J.Pharm.& Health Sci., 1 (3), 2010, 149-154.

Mittal A, Kalia A, Malhotra D, Validated UV spectroscopic method for determination of Atazanavir sulphate in 0.025 N HCL, Impact: Pharm Analysis & Quality Assurance, 3, 2011.

Muller AC, Kanfer I, An efficient HPLC method for the quantitative determination of Atazanavir in human plasma suitable for bioequivalence and pharmacokinetic studies in healthy human subjects, Journal of Pharmaceutical and Biomedical Analysis, 53(20), 2010, 113-118.

Nanda KR, Kulkarni AA, Yadav PB, Simultaneous spectrophotometric estimation of Atazanavir sulfate and Ritonavir in tablets, Der.Pharma.Chemica., 3(3), 2011, 84-88.

Nanda KR, Yadav PB, Kulkarni AA, Dhawale ND, Deshpande AD, Stability indicating determination of Atazanavir sulfate in pharmaceutical preparation by RP-HPLC, Inventi Impact: Pharm Analysis & Quality Assurance, 3, 2011.

Nanda KR, Yadav PB, Kulkarni AA, Stability indicating validated HPTLC method for simultaneous estimation of Atazanavir sulfate and Ritonavir in pharmaceutical dosage form, Asian Journal of Research in Chemistry, 4(9), 2011, 1381-1384.

Olivier T, Clemence VM, Cedric A, Simultaneous quantitative assay of Atazanavir and 6 other HIV protease inhibitors by isocratic reversed-phase liquid chromatography in human plasma,Therap.DrugMonit., 27, 2005, 265–269.

Padmalatha M, Vanitha Prakash K, Eranna Dopadally, Validated, reversed phase high performance liquid chromatography method for the estimation of Atazanavir sulfate in pharmaceutical formulations, Oriental Journal of Chemistry, 26(1), 2010, 123-127.

Parameswara Rao K, Satynarayana MV, Naga Raju T, Ramana GV, Sensitive extraction methods for the determination of Atazanavir sulfate in bulk and in pharmaceutical formulations, RASAYAN J. Chem., 5(4), 2012, 481-485.

Priestle JP, Fassler A, Rosel J, Comparative analysis of the X-ray structures of HIV-1 and HIV-2 proteases in complex with CGP53820, an ovel pseudosymmetric inhibitor, Structure, 3, 1995, 381–389.

Rao CS, Somannavar YS, Gupta PB, Sharma HK, Hemant N, Reddy BS, Kumar HV, Bindu VH, Gradient RP-HPLC method for the determination of potential impurities in Atazanavir sulfate, Journal Of Pharmaceutical And Biomedical Analysis, 55(1), 2011, 31-47.

Reyes JMC, Hill MD, Veray G, Optimization of HPLC quantization of the protease inhibitor, Atazanavir, Ethnicity & Disease, 16, 2006, S5-8.

Sathish Kumar Konidala, Sujana K, Prameela Rani A, new validated RP-HPLC method for the determination of Atazanavir Sulphate in bulk and dosage form, Der Pharma Chemica, 4 (3), 2012, 1305-1310.

Srinivasu K, Rao JV, Raju NA, Mukkanti K, A validated RP-HPLC method for the determination of Atazanavir in pharmaceutical dosage Form, E-Journal of Chemistry, 8(1), 2011, 453-456.