ABSTRACT

Background and objective: Objective of the present analytical research work was to develop and validate RP-HPLC method for the estimation of Atazanavir in bulk and dosage form. Method and Results: The RP-HPLC method for Atazanavir was developed using column Hexon C18 column (5µm, 250mm × 4.6mm) as stationary phase and Acetonitrile: Water adjusted (80;20 v/v) as mobile phase. The mobile phase was maintained at a flow rate of 0.9ml/min and volume of injection is 1 μl detection was carried out at 282 nm. The method was validated in accordance with ICH guidelines. Atazanavir were found to be linear in the concentration range of 10, 20, 30, 40, 50, 60 µg/ml respectively. The result of % assay of marketed formulation was found as 99.15-101.88 % for Atazanavir respectively. Accuracy of the method was determined by performing recovery study and the result were found in the range of 98.01-101.50 %w/w and for Atazanavir respectively. % RSD of precision study of these drugs were found less than 2 % which indicated good precision of the developed method. Conclusion: The developed HPLC method was simple, rapid, easy, accurate and precise. So, the method can be applied successfully for the routine analysis of Atazanavir in pharmaceutical industry.

KEYWORDS: Atazanavir, Acetonitrile, water, Method development, Validation.
### DRUG PROFILE

<table>
<thead>
<tr>
<th>Drug</th>
<th>Atazanavir Sulphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure</td>
<td><img src="https://via.placeholder.com/150" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>IUPAC Name</td>
<td>methyl N-[(1S)-1-{{(2S,3S)-3-hydroxy-4-[(2S)-2-[(methoxycarbonyl)amino]-3,3-dimethyl-N'-(4-(pyridin-2-yl)phenyl)methyl]butanehydrazido]-1-phenylbutan-2-yl]carbamoyl}-2,2-dimethylpropyl]carbamate</td>
</tr>
<tr>
<td>Chemical Formula</td>
<td>C_{38}H_{52}N_{6}O_{7}</td>
</tr>
<tr>
<td>Molecular Mass</td>
<td>704.8555</td>
</tr>
<tr>
<td>Melting Point</td>
<td>195-200 ºC</td>
</tr>
<tr>
<td>Physical State</td>
<td>Solid</td>
</tr>
<tr>
<td>Solubility</td>
<td>Soluble in Acetonitrile, Water, Methanol</td>
</tr>
<tr>
<td>pKa</td>
<td>11.92 &amp; 4.42 (Strongest acidic and basic respectively)</td>
</tr>
<tr>
<td>$t_{1/2}$</td>
<td>07 hrs</td>
</tr>
<tr>
<td><strong>Therapeutic Use</strong></td>
<td>It is an antiretroviral drug of the protease inhibitor (PI) class. Like other antiretroviral, it is used to treat infection of human immunodeficiency virus (HIV)</td>
</tr>
<tr>
<td><strong>Mechanism of Action</strong></td>
<td>Atazanavir 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 is not active against HIV-2. 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. Atazanavir binds to the protease active site and inhibits the activity of the enzyme. This inhibition prevents cleavage of the viral polyproteins resulting in the formation of immature non-infectious viral particles. Protease inhibitors are almost always used in combination with at least two other anti-HIV drugs. Atazanavir is pharmacologically related but structurally different from other protease inhibitors and other currently available antiretrovirals</td>
</tr>
</tbody>
</table>

### MATERIAL AND METHODS

**Raw material characterization**

**Characterization of Atazanavir Sulphate**

**Melting point determination**

Melting point was determined using digital melting point apparatus. The reference melting point of Atazanavir is 195-200 ºC.
**Determination $\lambda_{\text{max}}$ by UV**

Accurately weighed 10mg of Atazanavir Sulphate and dissolved in 100ml volumetric flask containing mobile phase Acetonitrile (ACN) and Water in the proportion of 80:20. Later, the volume was made up to the mark using abovementioned mobile phase to get concluding concentration of 100$\mu$g/ml. 1.0 ml solution from the stock solution was pipetted out and diluted to 10 ml to obtain the resultant solution 10$\mu$g/ml. The later solution was scanned using UV visible spectrophotometer in the range of 200-400nm.

**Characterization by IR spectroscopy**

IR spectrum of Atazanavir sulphate was recorded using IR spectrophotometer Jasco FTIR 4100 Japan in the range of 400-4000cm.

**Characterization of reagents and chemicals**

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Chemical</th>
<th>Grade</th>
<th>Make</th>
<th>Characterization</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Water</td>
<td>HPLC</td>
<td>--</td>
<td>As HPLC grade no further characterization performed</td>
</tr>
<tr>
<td>2</td>
<td>Acetonitrile</td>
<td>HPLC</td>
<td>Finar</td>
<td>As HPLC grade no further characterization performed</td>
</tr>
</tbody>
</table>

**Experimental Work**

**HPLC method development**

From results obtained from trial runs Acetonitrile: Water in the percentage of 80:20 was decided as mobile phase for HPLC method development.

**Preparation of standard stock solution Atazanavir Sulphate**

Precisely weighed 10mg of Atazanavir Sulphate and transferred to 100ml volumetric flask containing a mixture of Acetonitrile: Water (80:20). The volume was made up to the mark using same mixture of mobile phase to acquire the concentration of 100$\mu$g/ml. The concluding standard stock solution was filtered through 0.45$\mu$ membrane filter and degassed by ultra-sonication by performing three cycles each of 10 min.
Chromatographic circumstances set after tryout runs

Table 1: Initial chromatographic conditions.

<table>
<thead>
<tr>
<th>Chromatographic Conditions</th>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Hexon C18 (250 mm×4.6mm,5μm id)</td>
<td></td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Acetonitrile (ACN): Water (80:20 v/v)</td>
<td></td>
</tr>
<tr>
<td>Detection Wavelength</td>
<td>282 nm</td>
<td></td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.9 ml/min</td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>Ambient</td>
<td></td>
</tr>
<tr>
<td>Sample size</td>
<td>20 μl</td>
<td></td>
</tr>
<tr>
<td>Run Time</td>
<td>6 min</td>
<td></td>
</tr>
</tbody>
</table>

System suitability testing

**Preparation of working solution**

6.0ml standard stock solution (refer section 6.2.1) was withdrawn and transferred to 10 ml volumetric flask. The later diluted up to 10ml to obtain consequential solution of 60μg/ml. The resulting solution was filtered through 0.45μ membrane filter and ultra-sonicated for three cycles each of 10min. Six repeated measurements of this solution were carried out and results were documented for RT, area, tailing factor (symmetry factor) and theoretical plates. Mean, SD and %RSD were calculated for the results obtained as well as other parameters were also confirmed for satisfactoriness altitude.

- The column efficiency for DRV peak should not less than 2000 theoretical plates.
- The tailing factor for peak, should not more than 2.0.
- % CV for area shall NMT 1.5 and for RT NMT 0.5%

HPLC method validation

**Linearity and Range**

From stock solution 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 ml were pipetted out and transferred to 10 ml volumetric flasks. The each of the later aliquot was diluted up to 10ml by mobile phase to attain the consequential working solutions of 10, 20, 30, 40, 50 and 60μg/ml solutions respectively. Each of this working solution was subjected in triplicate to HPLC analysis to assess linearity between concentration and the mean peak area (of three replicate injections). Calibration curve was constructed between concentrations versus mean peak area. Results were documented for equation of line, correlation coefficient and intercept were determined.

\[ Y = mX + c \]

Where, Y- area
X- Unknown concentration
m- Slope of graph
c- Intercept

**Precision**

From the calibration range observed in the section 6.2.4.1, three QC standards were determined viz. 15, 35 and 55µg/ml as LQC, MQC and NQC in that order. The solutions for QC standards were prepared by diluting standard stock solution (100µg/ml) of 1.5, 3.5, and 5.5ml solutions up to 10ml. Area of each QC standard was recorded for intraday and interday precision in six replicates as per ICH guidelines Q2R1. Results were recorded to calculate mean, SD, %RSD. The each QC standard shall show mean peak area with %RSD within 2%.

**% Accuracy**

% Accuracy was calculated from the interpretations of precision study by means of subsequent formula. Perimeter for % accuracy is NMT 2% RSD.

\[
\% \text{ Accuracy} = \frac{\text{Mean measured conc.} - \text{Nominal conc.}}{\text{Nominal conc.}} \times 100
\]

**Robustness**

This study was planned for deliberate variations in mobile phase proportion (CAN percentage) and flow rate variation. 20µg/ml solution was selected for robustness study assess an effect of minor variations in parameters like mobile phase ratio, flow rate, etc. Three replicates for parameters given in Table were injected and mean peak area of chromatograms resulted thereof for each of the parameter was documented. The variation should not be more than 2% RSD. One factor was subjected for change at a time to determine effect.

**Table 2: Plan of variation for robustness experiment.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Standard</th>
<th>Variation 1</th>
<th>Variation 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase proportion</td>
<td>Acetonitrile: Water with (80:20)</td>
<td>82:18</td>
<td>78:22</td>
</tr>
<tr>
<td>Flow Rate (ml/min)</td>
<td>0.9 ml/min</td>
<td>1.00</td>
<td>0.8</td>
</tr>
</tbody>
</table>

**Percent recovery determination**

**Preparation of stock from API**

Exactly weighed 10mg of Atazanavir Sulphate (API) and transferred to 10 ml volumetric flask containing few ml of mobile phase and eventually the volume was made up to the mark using mobile phase. The resulting solution was filtered through 0.45µ membrane filter and ultra-sonicated for 30 min (three cycles each of 10 min). From the standard stock solution
2.0ml of stock solution was pipetted out in triplicate and kept in three different 10 ml volumetric flasks, cleaned previously and diluted up to 10ml by using mobile phase to acquire secondary solution of 10µg/ml. This solution was subjected for chromatographic analysis in given chromatographic system in triplicate and mean area calculated from the chromatogram obtained in each case and the same was documented.

**Preparation of stock from dosage form**
Twenty tablets (Atazor-200 by Emcure, Label claims 200 mg of Atazanavir sulphate,) were weighted, average weight was determined and powdered. Powder equivalent to 10mg was transferred to 100ml volumetric flasks and volume was made up to the mark using mobile phase. The resulting solution was filtered through 0.45µ membrane filter and ultra-sonicated for three cycles each of 10 min. From the stock 1.6, 2.0, 2.4ml solutions was pipetted out and diluted up to 10ml using mobile phase to obtain resultant solution of 16, 20 and 24µg/ml.

**Preparation of test solution for % recovery by spike method**
20µg/ml solution of Atazanavir sulphate (API) was spiked into each of above dilutions of 16, 20 and 24µg/ml to obtain solutions at 80%, 100% and 120% respectively. Each of these three levels was injected in triplicate and mean area for each level was determined. The mean area obtained on API injection was subtracted from the mean area of each of these three levels to obtain area corresponding to test solutions. % recovery was determined from the test and standard area using following formula.

\[
\%\ Recovery = \frac{\text{Sample area}}{\text{Standard area}} - \frac{\text{Standard dilution factor}}{\text{Sampledilution factor}} = \frac{\text{Average weight}}{\text{Label claim}} \times 100
\]

**Limit of Detection (LOD) and Limit of Quantitation (LOQ)**
LOD and LOQ were determined from the following formulae,

\[
\text{LOD} = 3.3 \times \frac{\text{SD}}{\text{S}}
\]
\[
\text{LOQ} = 10 \times \frac{\text{SD}}{\text{S}}
\]
SD is standard deviation
S is the slope of calibration curve.
RESULT AND DISCUSSION

Raw Material Characterization

Characterization of Atazanavir

Melting Point determination

Melting point was determined by using capillary method & it was found to be 195º C. The observed melting point corresponded with reference value (195-200ºC).

Determination of absorption maxima

At first, 10µg/ml solution of Atazanavir was prepared in the mixture of ACN: Water (80:20). This solution was subjected to UV analysis in qualitative mode to determine the absorption maxima (λmax). The UV spectrum obtained was as given in below and showed the absorption at different wavelengths as given in below. As shown in the maximum absorbance was noticed at 282nm. The wavelength of 282 nm was selected for quantitative determination of Atazanavir in afterward sections.

Characterization by IR Spectroscopy

IR spectroscopy is a technique used to determine functional groups present in organic molecules. IR spectrum of Atazanavir Sulphate was determined using Shimadzu FTIR 4100 Japan and the same was as given in fig.

Table: Absorbance values obtained for identification of Atazanavir Sulphate.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Wavelength in (nm)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>282</td>
<td>1.070</td>
</tr>
<tr>
<td>02</td>
<td>255</td>
<td>0.791</td>
</tr>
<tr>
<td>03</td>
<td>208</td>
<td>3.107</td>
</tr>
</tbody>
</table>

Results obtained in terms of wave numbers corresponding to different functional groups of drug were as given in Table below. Results obtained illustrated the corresponding functional groups present in Atazanavir. This confirmed the API Atazanavir Sulphate.
Characterization of chemicals and reagents

All reagents and chemicals used were of HPLC grade and as a result no auxiliary interpretation was done.

HPLC Method development

HPLC is frequently used for the quantitative estimation of drug substances and drug product as well as for studying their metabolites. In addition HPLC has turn out to be supplementary important technique for estimation of drugs in biological fluid. This supports analytical chemistry to learn pharmacodynamics, fate of drug molecule in vivo. This technique also presents advantages of estimating the constituents for the multicomponent system. This technique was engaged herein for investigation for estimation of Atazanavir Sulphate in bulk and tablet dosage form. Different parameters influencing analysis considered to be an important features for the development of analytical method. In order to set up HPLC method the different parameters were studied in further segments.
Figure: IR Spectrum of Atazanavir Sulphate.

Table: Observed wave number corresponding different functional groups of Atazanavir Sulphate.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Functional group</th>
<th>Observed wave number (cm⁻¹)</th>
<th>Reference wave number (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>C=O (amide)</td>
<td>1697.36, 1674.21</td>
<td>1680-1700</td>
</tr>
<tr>
<td>02</td>
<td>N-H</td>
<td>3364.21</td>
<td>3200-3400</td>
</tr>
<tr>
<td>03</td>
<td>O-H</td>
<td>3364.21</td>
<td>3200-3600</td>
</tr>
<tr>
<td>04</td>
<td>=C-H (Ar)</td>
<td>2963.02</td>
<td>3000-3050</td>
</tr>
<tr>
<td>05</td>
<td>C=C</td>
<td>1560.77</td>
<td>1475-1600</td>
</tr>
<tr>
<td>06</td>
<td>C-O, C-N</td>
<td>1240.23, 1147.65, 1066.64</td>
<td>1000-1300</td>
</tr>
</tbody>
</table>

Selection of mobile phase

The preferred mobile phase ACN: Water in the ratio of 80:20 was found to be suitable for separation and resolution of Atazanavir under given set of chromatographic conditions. For that reason, this mobile was selected to study various parameters as per ICH guideline Q2R1 in further segments.

System suitability Testing

To optimize the chromatographic conditions, the consequence of chromatographic variables such as proportion of mobile phase, flow rate and the column were studied. The ensuing chromatograms were recorded and the chromatographic parameters such as peak area,
resolution and theoretical plates were integrated. The circumstances obtained most outstanding resolution, symmetry factor and theoretical plates were selected for further estimation. The test was performed by six repeated measurements of standard working solution of drug. The concentration was reserved invariable at 60μg/ml for Atazanavir Sulphate. The most excellent resolution and peak shape, devoid of superfluous tailing, were obtained by use of chromatographic conditions as stated above in Table. The best resolution with rational retention time was obtained with mobile phase containing ACN: Water (80:20) with flow rate 0.9ml/min in low pressure gradient mode as shown in Table.

The representative chromatogram for system suitability testing at 282nm was as revealed in Figure. The results obtained for the system suitability testing were as depicted in Table.

![Figure: HPLC chromatogram obtained for system suitability testing.](image)

### Table: Results found in system suitability testing.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>System suitability Parameter</th>
<th>*Mean study</th>
<th>Limits</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Retention time (RT) in min</td>
<td>3.37</td>
<td>NLT 2.0 min</td>
<td>Passed</td>
</tr>
<tr>
<td>02</td>
<td>Peak Area in mV</td>
<td>1105444</td>
<td>NLT 2000</td>
<td>Passed</td>
</tr>
<tr>
<td>03</td>
<td>Theoretical plates</td>
<td>5235</td>
<td>NLT 2000</td>
<td>Passed</td>
</tr>
<tr>
<td>04</td>
<td>Tailing factor</td>
<td>1.16</td>
<td>NMT 2.0</td>
<td>Passed</td>
</tr>
<tr>
<td>05</td>
<td>% RSD of RT</td>
<td>0.46</td>
<td>NMT 0.5%</td>
<td>Passed</td>
</tr>
<tr>
<td>06</td>
<td>% RSD of Mean Area</td>
<td>0.31</td>
<td>NMT 2.0%</td>
<td>Passed</td>
</tr>
</tbody>
</table>

* *Mean study of six replicate injections, NLT (not less than), NMT (not more than).*
HPLC Method Validation

Linearity study

The linearity of an analytical procedure is its ability to draw out experimental results that are relative to the concentration of analyte in the sample. From standard stock solution, aliquots of 1.0, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0ml were taken in 10 ml volumetric flasks and diluted up to the mark with mobile phase such that to obtain concentrations of Atazanavir Sulphate in the range 10-60µg/ml. All dimensions were repeated three times for each concentration and calibration curve was constructed by plotting the peak area versus the drug concentration. The data obtained for linearity study for Atazanavir Sulphate was as illustrated in Table. The calibration curve was constructed by plotting concentration on X-axis verses mean peak area obtained for various concentrations of Atazanavir Sulphate on Y-axis and the same was as revealed in Figure. From the calibration curve plotted, the equation of straight line, slope and intercept were calculated and the data obtained for the same was as depicted in Table.

Table: Observations obtained for linearity study.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Concentration (µg/ml)</th>
<th>Mean Area*</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>10</td>
<td>194534</td>
</tr>
<tr>
<td>02</td>
<td>20</td>
<td>410880</td>
</tr>
<tr>
<td>03</td>
<td>30</td>
<td>608004</td>
</tr>
<tr>
<td>04</td>
<td>40</td>
<td>806765.71</td>
</tr>
<tr>
<td>05</td>
<td>50</td>
<td>1010193.33</td>
</tr>
<tr>
<td>06</td>
<td>60</td>
<td>1218658.91</td>
</tr>
</tbody>
</table>

The conclusion described linearity of the method for 10-60µg/ml concentration range and therefore, the method can be discover for quantitative determination of mysterious samples of Atazanavir Sulphate in the series of 10-60µg/ml.

Figure: Calibration curve of Atazanavir Sulphate.
Table: Parameters of calibration curve.

<table>
<thead>
<tr>
<th>Equation of straight Line</th>
<th>20335x - 3560</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>20335</td>
</tr>
<tr>
<td>Intercept</td>
<td>3560</td>
</tr>
<tr>
<td>Regression</td>
<td>0.999</td>
</tr>
</tbody>
</table>

**Precision**

The precision of an analytical modus operandi communicates the closeness of agreement (degree of scatter) stuck between a series of measurements obtained from abundant sampling of the identical harmonized sample under the prescribed circumstances. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, genuine illustrations. Nevertheless, if it is not likely to obtain a homogeneous sample it may be investigated using synthetically prepared samples or a sample solution. The precision was determined from the three QC standards defined previously as LQC, MQC, NQC. These QC standards were 15, 35 and 55ppm concentrations of Atazanavir Sulphate standard solutions. Six repeated measurements for each QC standard were studied for a given instrument setting. The results were recorded for area, retention time, theoretical plates, and USP symmetry factor and found to be in agreement with each other. The area for each QC standard was statistically evaluated for standard deviation and percent RSD as illustrated in Table. The results determined for this test showed percent RSD within acceptance decisive factor as per ICH guideline Q2R1. That's why, it is concluded that the projected method was precise and reproducible for quantitative estimation of Atazanavir Sulphate in the range aforesaid above.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Conc. (µg/ml)</th>
<th>Intra day</th>
<th>Inter day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Area± SD</td>
<td>%RSD</td>
<td>Mean Area± SD</td>
</tr>
<tr>
<td>01</td>
<td>15</td>
<td>295308.00</td>
<td>1.13</td>
</tr>
<tr>
<td>02</td>
<td>35</td>
<td>714189.67</td>
<td>1.13</td>
</tr>
<tr>
<td>03</td>
<td>55</td>
<td>1123353.67</td>
<td>0.77</td>
</tr>
</tbody>
</table>

**% Accuracy from the results obtained in precision experiment**

The accuracy of analytical method is the closeness of the test result obtained by the method to the true value. % Accuracy for the concentration to be analyzed should be between 98-102% w/w for Atazanavir Sulphate. It was determined on or after the results obtained for precision study. From the mean area obtained for LQC, MQC and NQC, the equivalent concentrations were calculated by means of regression equation of the calibration curve. From the data of
concentration percent accuracy (% Assay) was determined using subsequent formula and the results obtained thereof were as illustrated in Table 8. The results for percent accuracy were in compendial limit for Tramadol HCl for a selected technique. As a result, it was concluded that the aforesaid developed HPLC method passes for the test of accuracy.

\[
\% \text{ Accuracy} = \frac{\text{Mean Measured Concentration} - \text{Nominal Concentration}}{\text{Nominal Concentration}} \times 100
\]

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Nominal Concentration (μg/ml)</th>
<th>*Mean area</th>
<th>Mean measured Concentration (μg/ml)</th>
<th>% Accuracy (w/w) 98-102 %</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>35</td>
<td>294712.56</td>
<td>14.67</td>
<td>97.79</td>
<td>Passed</td>
</tr>
<tr>
<td>02</td>
<td>55</td>
<td>719345.11</td>
<td>35.55</td>
<td>101.57</td>
<td>Passed</td>
</tr>
<tr>
<td>03</td>
<td>75</td>
<td>1124671.33</td>
<td>55.48</td>
<td>100.88</td>
<td>Passed</td>
</tr>
</tbody>
</table>

**Robustness**

Robustness is persistence of analysis by way of respect to anticipated disparity in method parameter. Parameters studied for HPLC robustness assessment was as listed Table.

Robustness testing was completed by diluting the volume equivalent to 2.0ml of standard stock solution to 10ml with same mixture of mobile phase (ACN:Water) to achieve 20μg/ml solution. This solution was used to learn the upshot of purposeful distinction in method parameters on % assay of Atazanavir Sulphate. The three repeated injections were made to the prearranged set of chromatographic conditions and the results obtained were recorded in terms of mean peak area. The corresponding concentrations and percent assay in w/w was calculated for each variation executed as per Table and the results figure out thereof were as illustrated in Table. The results obtained revealed that intended discrepancy in the mobile up to 2% organic concentration to the elevated and inferior side does not appreciably influence the quantitative determination of Atazanavir Sulphate. Also, results showed that flow rate difference by 0.1 ml also demonstrated reproducibility in the assay values of Atazanavir Sulphate as per compendial standards.

**Table: Percent Assay values determined in Robustness experiment.**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Conc. (μg/ml)</th>
<th>Robustness Parameter</th>
<th>Variations</th>
<th>Mean area* (mV)</th>
<th>Mean measured Conc. (μg/ml)</th>
<th>% Assay w/w Limit (98-102%)</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>20</td>
<td>Mobile phase composition</td>
<td>82:18</td>
<td>401762</td>
<td>19.93</td>
<td>99.66</td>
<td>Passed</td>
</tr>
<tr>
<td>02</td>
<td>20</td>
<td>Mobile phase composition</td>
<td>78:22</td>
<td>409173</td>
<td>20.30</td>
<td>101.48</td>
<td>Passed</td>
</tr>
<tr>
<td>03</td>
<td>20</td>
<td>Flow Rate (ml/min)</td>
<td>1.0</td>
<td>396239</td>
<td>19.66</td>
<td>98.30</td>
<td>Passed</td>
</tr>
<tr>
<td>04</td>
<td>20</td>
<td>Flow Rate (ml/min)</td>
<td>0.8</td>
<td>411734</td>
<td>20.42</td>
<td>102.11</td>
<td>Passed</td>
</tr>
</tbody>
</table>

*Mean area of three repeated measurements.
**Percent recovery**

Percent recovery is the determination of percent purity of known drug analyte in finished product. Percent recovery study was carried out by means of spike method (standard addition method) at three different levels (80%, 100% and 120%). Permitted quantities of standard solutions enclosing analyte were added to prequantified sample (test) solutions to get 80, 100 and 120 % level test solutions.

These samples were subjected to HPLC analysis by injecting the test solutions for a prearranged chromatographic set up in triplicates and mean peak area was determined from the chromatogram (Figure) obtained. Mean area at all three levels viz. 80%, 100% and 120% was computed and it was deducted from area of equivalent standard solution (20µg/ml) to achieve valid area for sample (test) solution. Correspondent concentrations and percentage of amount recovered were calculated from regression equation. The percent assay was deduced from the regression equation as publicized in Table. Percent recovery was correspondent to pharmacopoeial limits for Atazanavir Sulphate.

![Figure: HPLC chromatogram obtained during percent recovery experiment.](image)

<table>
<thead>
<tr>
<th>Recovery Level</th>
<th>Conc. Taken (µg/ml)</th>
<th>Amount added (µg/ml)</th>
<th>Mean Area*</th>
<th>Amount Recovered (µg/ml)</th>
<th>% Recovery (w/w)</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>80 %</td>
<td>20</td>
<td>16</td>
<td>713951</td>
<td>35.28</td>
<td>98.01</td>
<td>Passed</td>
</tr>
<tr>
<td>100 %</td>
<td>20</td>
<td>20</td>
<td>810563</td>
<td>40.03</td>
<td>100.08</td>
<td>Passed</td>
</tr>
<tr>
<td>120 %</td>
<td>20</td>
<td>24</td>
<td>904567</td>
<td>44.65</td>
<td>101.5</td>
<td>Passed</td>
</tr>
</tbody>
</table>

Table: Results obtained for percent recovery experiment.
**LOD and LOQ**

The detection limit is an aspect of limit tests. It is the smallest amount of analyte in a sample that can be detected, but not necessarily quantitated under the stated experimental conditions. The quantitation limit is a characteristic of quantitative assays for low levels of compounds in sample matrices like impurities in bulk drug substances and degradation products in finished pharmaceuticals. The sensitivity of the method was estimated in terms of limit of detection and limit of quantification by using formulae viz. LOD = 3.3× δ/S and LOQ=10×δ/S (Where S was slope of calibration curve and δ is the standard deviation of area in calibration plot). LOD and LOQ were found to be 0.41µg/ml and 1.25µg/ml respectively. Accordingly, the concentration of DRV as low as 0.41µg/ml can be detected and 1.25µg/ml can be productively quantified devoid of any difficulty of contamination.

**Table: Results obtained for LOD and LOQ study.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>LOD (µg/ml)</th>
<th>LOQ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRV</td>
<td>0.41</td>
<td>1.25</td>
</tr>
</tbody>
</table>

**SUMMARY AND CONCLUSION**

The planned study was ordinary to develop a receptive, meticulous and correct HPLC method for the analysis of Atazanavir Sulphate as active pharmaceutical ingredient (API) and in Tablet dosage forms. Amalgamation of Acetonitrile (ACN) with water in dissimilar combinations was evaluated as mobile phase on a C18 stationary phase. A concoction of ACN and water in the percentage of 80:20 v/v was established to be the predominantly fitting of all union because the chromatographic peaks were enhanced, discrete, resolved and approximately free from tailing. The retention time obtained for Atazanavir Sulphate was 3.37min. System suitability parameters were studied with six repeated injections of standard solution of the Atazanavir Sulphate and the obtained parameters were found inside the acceptance standard. The peak areas of Atazanavir Sulphate were reproducible as indicated by % RSD in conventionality with the standards. A good linear relationship ($R^2 = 0.999$) was noticed among the concentration of Atazanavir Sulphate and the individual peak areas. The regression curve was plotted by linear regression fitting and its regression equation was $Y = 20335x – 3560$ Where, Y gives peak area and X is the concentration of the drug. Validation was carried out as per ICH guidelines Q2R1 for the subsequent considerations,

- Accuracy
- Precision
- Linearity and Range
Robustness
Limit of Detection
Limit of Quantitation
% Recovery

While drug (Atazanavir Sulphate) solutions including 15, 35 and 55μg/ml were analyzed by current HPLC method for studying intra and inter-day variations, low % RSD was illustrated signifying better precision of the method. High recovery appraisal found from the tablet dosage form by the anticipated method suggested that method was accurate under experimental conditions. The lack of supplementary peaks indicated non-interference of common excipients used in the manufacturing of marketed tablets dosage form. The tablet dosage form was used for looking at applicability of the method for practice investigation of Atazanavir Sulphate and found to be appropriate. The drug content in tablets was quantified using the proposed analytical method. The tablets were found to contain an average of 98.01 to 101.50% w/w of the labelled quantity of Atazanavir Sulphate.

Eventually, it was concluded that the proposed HPLC method was approachable and reproducible for the analysis of Atazanavir Sulphate in bulk and pharmaceutical tablet dosage forms with petite analysis time. As a result, we have developed accurate, precise, sensitive and pecuniary method for estimation of Atazanavir Sulphate in bulk as well as tablet dosage form.

The further study of stability testing and estimation of Atazanavir Sulphate in samples obtained from biological systems are opportunity of work than can be planned in future for this molecule.

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