Validated RP-HPLC Method for Estimation of Asenapine in Bulk and Tablet Dosage Form

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Abstract: A simple and selective LC method is described for the determination of ASENAPINE dosage forms. Chromatographic separation was achieved on a C18 column using mobile phase consisting of a mixture of Triethylamine Buffer: Acetonitrile (50:50) with detection of 220nm. Linearity was observed in the range 15-45 µg /ml for ASENAPINE ($r^2$=0.997) for the amount of drug estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Keywords: Reverse Phase- High Performance Liquid Chromatography (RP-HPLC), Asenapine, $r^2$ correlation coefficient.

INTRODUCTION

Asenapine [1, 2] is a serotonin, dopamine, nor adrenaline, and histamine antagonist in which asenapine possesses more potent activity with serotonin receptors than dopamine. Chemically it is known as (2Z)-but-2-enedioic acid;17-chloro-4-methyl-13-oxa azatetracyclo[12.4.0.0²,⁶.0⁷,¹²]octadeca-1(14),7,9,11,15,17-hexaene. The chemical structure of asenapine was given in Fig.1

Fig-1: Structure of Asenapine

As per the literature review, several methods were there for the determination of its pharmacological action. Asenapine was estimated individually by few methods like UV [4, 5] spectroscopy, HPLC [6] and LC-MS [7, 8]. But there was no stability indicating RPHPLC Method. So the aim of present work was to develop and validated stability indicating RP-HPLC method for the determination of Asenapine in bulk and tablet dosage form.

MATERIALS AND METHODS

Chemicals and Reagents

Asenapine was obtained as gift sample from Chandra laboratories, Hyderabad. Acetonitrile, water used was of HPLC grade.

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Standard preparation
Weigh accurately 10 mg of ASENAPINE in
100 ml of volumetric flask and dissolve in 100ml of
mobile phase and make up the volume with mobile
phase From above stock solution 30 µg/ml of
ASENAPINE is prepared by diluting 3ml to 10ml with
mobile phase. The chromatogram of standard
Asenapine solution was shown in Fig.2.and the average
Retention time was found to be about 3.075.

Sample preparation
10 tablets (each tablet contains 5mg of
ASENAPINE was weighed and taken into a mortar and
crushed to fine powder and uniformly mixed. Tablet
stock solutions of ASENAPINE (100µg/ml) were
prepared by dissolving weight equivalent to 5 mg of
ASENAPINE and dissolved in sufficient mobile phase.
After that filtered the solution using 0.45-micron
syringe filter and Sonicated for 5 min and dilute to
100ml with mobile phase. Further dilutions are prepared
in 5 replicates of 30µg/ml of ASENAPINE was made
by adding 3 ml of stock solution to 10 ml of mobile
phase.

Validation [9-11]

System suitability
A standard solution of Asenapine working
standard was prepared as per procedure and was
injected five times into the HPLC system. The system
suitability parameters were evaluated from standard
chromatograms obtained by calculating the % RSD
retention time, tailing factor, theoretical plates, peak
areas from five replicate injections are within range and
results shown in Table.1.

Linearity
To demonstrate the linearity of assay method,
inject 5 standard solutions with concentrations of about
15µg/ml to 45 µg/ml of Asenapine. Plot a graph to
concentration versus peak area. Correlation co-efficient
was found to be 0.997 and linearity plot was shown
Fig.3.and results were in Table.2.

Accuracy
Three concentrations of 75%, 100%, and 125%
are injected in triplicate manner and % recovery was
calculated as 100.5%. The results were in Table.3.

Precision

Repeatability
Six working sample solutions are injected and the % amount was calculated and % RSD was found to be 0.92.

Intermediate precision
Six working sample solutions are injected on
the next day of the preparation of samples and %
amount was calculated and % RSD was found to be
0.92. The Results were shown in Table.4

Robustness
To demonstrate the robustness of the method,
prepared solution as per test method and injected at
different variable conditions like using different
conditions like Temperature and wavelength shown in
Table.5

Limit of Detection (LOD)
LOD is the lowest level of concentration of
analyte in the sample that can be detected, though not
necessarily quantitated. It is calculated by using this
formula,

\[
LOD = 3.3\sigma/S
\]

Where, \(\sigma\) = the standard deviation of the response
\(S\) = the slope of the calibration curve

Limit of Quantitation
LOQ is the lowest concentration of analyte in a
sample that may determined with acceptable accuracy
and precision when the required procedure is applied. It
is calculated by using this formula,

\[
LOQ = 10\sigma/S
\]

Where,
\(\sigma\) = Standard deviation of the response,
\(S\) = Slope of calibration curve.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capacity Factor</td>
<td>(k &gt; 2)</td>
</tr>
<tr>
<td>Injection precision</td>
<td>RSD (&lt; 1%) for (n \geq 5)</td>
</tr>
<tr>
<td>Resolution</td>
<td>(R &gt; 2)</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>(A_{t} \leq 2)</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>(N &gt; 2000)</td>
</tr>
</tbody>
</table>
Table-2: linearity of ASENAPINE

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Conc.(µg/ml)</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>1170177</td>
</tr>
<tr>
<td>2</td>
<td>22.5</td>
<td>1697912</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>2407163</td>
</tr>
<tr>
<td>4</td>
<td>37.5</td>
<td>2834924</td>
</tr>
<tr>
<td>5</td>
<td>45</td>
<td>3306552</td>
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</tbody>
</table>

Table-3: Accuracy data for ASENAPINE

<table>
<thead>
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<th>Recovery level</th>
<th>Accuracy</th>
<th>ASENAPINE</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Amount taken(mcg/ml)</td>
<td>Area</td>
</tr>
<tr>
<td>50%</td>
<td>15</td>
<td>1176833</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1178517</td>
</tr>
<tr>
<td>100%</td>
<td>30</td>
<td>2490174</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>2426700</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>2415495</td>
</tr>
<tr>
<td>150%</td>
<td>45</td>
<td>3292362</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>3285307</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>3297269</td>
</tr>
</tbody>
</table>

Table-4: Results for Method precision of ASENAPINE

<table>
<thead>
<tr>
<th>ASENAPINE</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.No.</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>avg</td>
</tr>
<tr>
<td>stdev</td>
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<tr>
<td>%RSD</td>
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Table-5: Result of Robustness study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ASENAPINE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time(min)</td>
<td>Area</td>
</tr>
<tr>
<td>Flow 1.0ml/min</td>
<td>3.671</td>
</tr>
<tr>
<td></td>
<td>2.696</td>
</tr>
<tr>
<td>Wavelength 252nm</td>
<td>3.081</td>
</tr>
<tr>
<td></td>
<td>3.086</td>
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</table>

Fig-2: Chromatogram of ASENAPINE standard

CONCLUSION
In conclusion, a simple, selective, sensitive and accurate stability indicating RP-HPLC method was developed and validated for the analysis of Asenapine. Further method was found to be linear, precise, accurate and robust. The degradation studies reveal the stability of the drug. Hence the proposed method can be safely and successfully used for the estimation of Asenapine in routine analysis.

ACKNOWLEDGEMENT
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REFERENCES
1. Available from Drugs.com/ monograph/asenapine maleate.html (accessed on 28/1/12).