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REVIEW ARTICLE

# Analytical Method Development and Validation of RP-HPLC for Estimation of Asenapine Maleate in Bulk drug and Tablet Dosage Form

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## ABSTRACT

A new simple, specific, sensitive, rapid accurate and precise RP-HPLC method was developed for the estimation of Asenapine in bulk and pharmaceutical formulation (sublingual Tablet). Asenapine was chromatographed on Grace Altima C18 (100mm X 4.6 mm, 5µ) in a mobile phase consisting of mixture of Buffer (Potassium Di-hydrogen Phosphate buffer, pH 4.5) and Acetonitrile (65:35 % V/V). The mobile phase was pumped at flow rate of 1.5 ml/min with detection at 220 nm. Linearity was performed with 7 levels, within the range of 50 % to 150 % of the sample concentration. The intra and inter day variation was found to be less than 2.0%. The mean recovery of the drug from the solution was 100.1%. Hence it can be applied for routine quality control analysis of Asenapine in bulk and pharmaceutical formulation.

## KEYWORDS

Asenapine, Asenapine Maleate, RP-HPLC, Assay, Estimation, Validation

## INTRODUCTION

Asenapine Maleate is an Anti-psychotic developed for the treatment of Schizophrenia and Acute Mania associated with bipolar disorder. Preliminary data indicate that it has minimal anticholinergic and cardiovascular side effects, as well as minimal weight gain. Available tablet dosage form is supplied for sublingual administration in tablets. Sublingual medication administration is way of giving someone medicine orally. Sublingual administration is when medication is placed under the tongue to be absorbed by the body. The word "sublingual" means "under the tongue. Asenapine is stable and available as Maleate salt. Each sublingual tablet contains 5mg or 10 mg Asenapine as label claim.

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It is chemically designated as (3a*RS*, 12bRS)-*rel*-5-Chloro-2, 3, 3a, 12b-tetrahydro-2-methyl-1*H* n-dibenz [2,3:6,7] oxepino [4,5-c] pyrrole, and has empirical molecular formula as $C_{21}H_{20}ClNO_5$. It is white to off-white powder with a molecular weight of 401.84 g/mol. It is freely soluble in methanol, acetone; soluble in ethanol and slightly soluble in water

Different analytical methods have been reported in the literature for by HPLC method with PDA and ESI-MS and UV as detector for estimation of Asenapine in plasma.

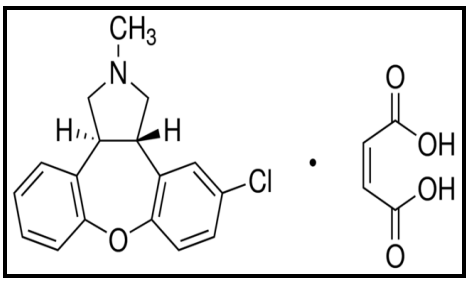


Figure 1: Structure of Asenapine Maleate

The present study was to establish a simple, reproducible and reliable HPLC method for estimation of Asenapine in bulk drug and in its formulation. The study was to develop RP-HPLC method and validate the same.

## MATERIALS AND METHODS

### Materials, Reagents and Chemicals

Asenapine maleate and its tablets were generous gift from Zydus cadila. All solvents and reagents were of analytical or HPLC grade. HPLC- grade water was prepared by using MILLI-Q water purification system. All the reagents used in this method were of analytical reagent grade.

### Experimental

#### *Selection of Wavelength*

The standard solution of 20 µg/ml of Asenapine was scanned in the range of 200- 400 nm and the wavelength was selected as 220 nm due to good response. The Linearity and Bias was passed within range 20µg/ml - 180µg/ml. so, finally 220 nm was selected as detector wavelength.

#### *Selection of Column, Mobile Phase and Diluent*

Different columns were tried for the good peak shape, like Inertsil C-8, C18, Hypersil-C18, Zorbax-C8, C18, and Grace Altima C18. Along with those, different types of mobile phase containing water- acetonitrile & water-methanol at different ratios were tried. Also, different types of mobile phase containing Buffer- Acetonitrile at different ratios and with different pH of buffers were tried. Triethylamine (TEA) addition was tried in buffer for better peak shape. At last, based on good peak shape and symmetry, 4.5 pH Potassium Di-hydrogen Phosphate buffer with TEA and Acetonitrile in the ratio of 65:35 V/V was finalized. Asenapine is more likely to soluble in solvent and at lower pH, so water-acetonitrile (60:40 V/V) at pH 3.0 was finalized based on recovery study at development stage.

#### *Buffer Preparation*

Weight accurately 3.5g of Potassium Di-hydrogen Phosphate in 1000ml of water. Add 5 ml of Triethylamine. Adjust pH of the solution to 4.5 with dilute Ortho- Phosphoric acid.

#### *Mobile Phase Preparation*

Prepare a mixture of Buffer solution and Acetonitrile in the ratio of 65:35 V/V.

#### *Chromatographic Conditions*

Column - Grace Altima C18 (100 X 4.6) mm, 5µ; Detector wavelength - 220 nm; Flow rate - 1.5 mL/minute; Injection volume - 10 µL; Column temperature - 45®C; Run time - 6 Minutes; Approximate Retention time - 2.7 Min

#### *Diluent Preparation*

Water: Acetonitrile (60:40 % V/V) mix, and adjust pH 3.0 with Acetic acid.

#### *Standard Preparation*

Transfer an accurately weighed of about 35 mg of Asenapine Maleate (which is equivalent to 25 mg of Asenapine) working standard to a 25 mL volumetric flask. Add about 25 mL of diluent and sonication to dissolve. Make volume up to the mark with diluent and mix. Further dilute 5 ml of this solution to 50ml with diluent.

#### *Sample Preparation*

Weigh accurately 20 tablets (each contains 5 mg as Asenapine) and calculate the average weight. Weight 20 intake tablets and transfer into 100 mL volumetric flask. Add about 80 mL of Diluent and sonication with occasional shaking for 30 minutes. Make volume up to the mark with Diluent and mix. Further dilute 5 ml of this solution to 50 ml with diluent. Filter the solution through 0.45 µm Millipore PVDF filter; collect the filtrate by discarding first few mL of the filtrate.

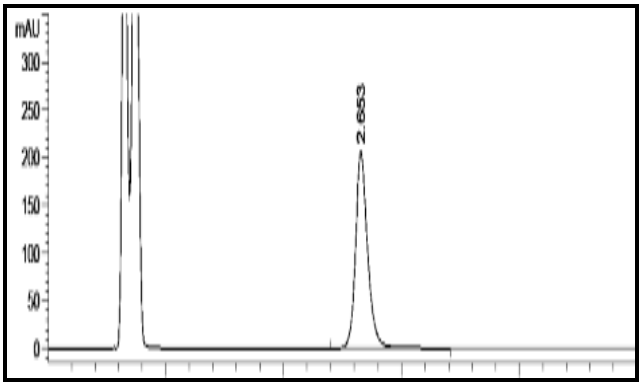


Figure 2: Chromatogram of Standard Solution

 

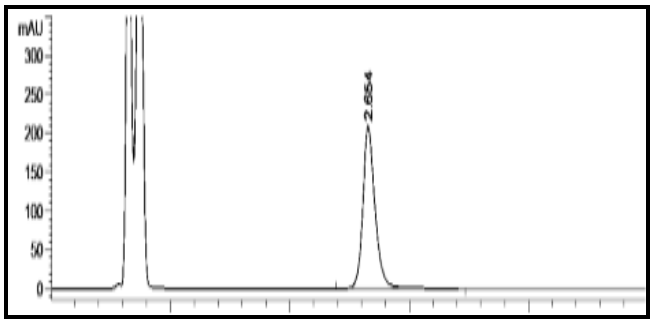


Figure 3: Chromatogram of Sample Solution

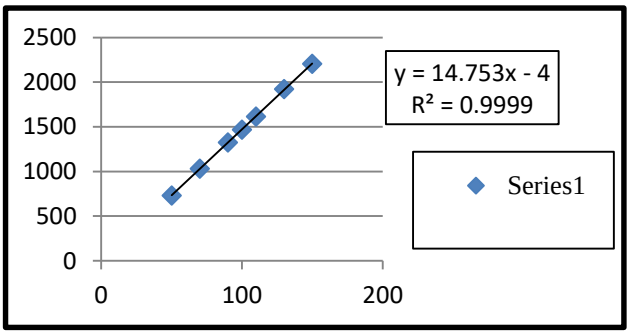


Figure 4: Linearity Curve

## Validation of Method

### *System Suitability Parameter*

Approximate retention time of Asenapine was found to be at 2.7 min. The peak shape of Asenapine was symmetrical and the asymmetry factor was less than 1.5. The proposed method was validated as per standard analytical procedure. The standard solution was repeated 6 times and the same retention time was observed in all the cases. System suitability parameters of Asenapine are mentioned in Table 1.

### *Method Precision and Intermediate Precision*

The method precision was studied along with inter-day precision. Prepared six replicates of samples at the test concentration and injected. For inter-day repeat the procedure followed for method precision on a different day by using a different column and HPLC system and using same lot of sample. Results are shown in Table 1. The RSD of six replicate determinations should not be more than 2.0%. From the data obtained, the developed HPLC method was found to be precise.

### *Linearity and Range*

Perform linearity at seven levels over the range of 50µg/ml to 150µg/ml of test concentration. A standard stock solution was prepared and further diluted to attain concentration at about 50µg/ml, 70µg/ml, 90µg/ml, 100µg/ml, 110µg/ml, 130µg/ml and 150µg/ml of the test concentration. The linearity curve was plotted between absorbance versus concentration Asenapine is shown in Figure 4 and linearity data was shown in Table 1.

Table 1: Results for System Suitability, Precision, Linearity and Range

| Validation Parameter | Asenapine | Acceptance Limits |
|---|---|---|
| Retention Time | 2.7 min (Approximate) | - |
| Asymmetry Factor | 1.2 | NMT 1.5 |
| Theoretical Plates | 4000 | NLT 2000 |
| Precision (% RSD) | 0.5 % | NMT 2.0% |
| Intermediate Precision (%RSD) | 0.4 % | NMT 2.0% |
| Range | 50% to 150% | - |
| Correlation Coefficient(r2 ) | 0.9999 | NLT 0.999 |
| Bias (% Y-Intercept) | 0.27 % | NMT 2.0 % |

### Accuracy

Accuracy was performed at 3 level 50%, 100% & 150% of the test concentration. Transfer the placebo blend equivalent to 20 Average weights of the Asenapine Maleate Tablet 5mg tablets and add to it 50%,100% and 150% of the drug with respect to the test concentration. Prepare the sample in triplicate at each level and inject each

preparation and recovery (%) & % RSD were calculated. The accuracy results data was shown in Table 2.

Table 2: Results for Accuracy

| Level | % Recovery (Average of 3 Sets) | % RSD ( Average of 3 Sets) |
|---|---|---|
| 50 % | 99.7 % | 0.4 % |
| 100 % | 100.1 % | 0.5 % |
| 150 % | 99.8 % | 0.5 % |

**Robustness**

Established the robustness of the method by making deliberate minor variations in the following method parameters.

Change the flow rate, change column temperature and change in Buffer pH. Check the effect of change in parameter on system suitability. The robustness results data was shown in Table 3.

## CONCLUSION

Based on observations and results obtained in validation study by applying the suggested procedures, for suitability of method for routine and stability analysis shall be drawn.

The observation and results obtained shall be compared with acceptance criteria and discussed in it. It is obvious that they are applicable for the determination of Asenapine should not be any interference and with good sensitivity.

The proposed validated method was successfully applied to Asenapine Maleate in bulk powder and in tablet dosage forms. The results obtained can be classified as being rapid, simple and sensitive.

Table 3: Results for Robustness study

| Parameter | Actual Condition | Change in parameter | RT for Asenapine Peak | % RSD (n=6) |
|---|---|---|---|---|
| Flow Rate | 1.5 ml/min | 1.4 ml/min | 2.85 | 0.2 |
| Flow Rate | 1.5 ml/min | 1.6 ml/min | 2.52 | 0.4 |
| Column Temperature | 45°C | 40°C | 2.74 | 0.4 |
| Column Temperature | 45°C | 55°C | 2.56 | 0.3 |
| Buffer pH | 4.5 | 4.3 | 2.81 | 0.2 |
| Buffer pH | 4.5 | 4.7 | 2.67 | 0.5 |

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