

STRESS DEGRADATION STUDIES AND DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF ASENAPINE MALEATE

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ABSTRACT

A simple and accurate RP-HPLC method was developed for the estimation of Asenapine maleate in bulk and tablet dosage form. Isocratic elution at a flow rate of 1ml/min was employed on a C18 column. The mobile phase consists of methanol, n-butanol and glacial acetic acid in the ratio 60:20:20 v/v. The UV detection wavelength was 270 nm. The retention time for asenapine maleate was 1.9 min with an asymmetry of 0.95. The method was validated for several parameters such as accuracy, precision, robustness as per ICH guidelines. Linearity was observed in the range of 10-100 µg/ml with correlation coefficient of 0.998. The mean recovery were found to be in the range of 98.9-100.2% and the % RSD for interday and intraday was found to be 0.98 and 0.53 respectively. To investigate on the stability of drug, forced degradation studies were performed under different stress conditions recommended by International Conference on Harmonization (ICH). Stress degradation methods are used to understand the degradation chemistry of drug. The results of the study show that the proposed RP-HPLC method was simple, rapid, precise, accurate and stability indicating and hence can be successfully used for the routine analysis of Asenapine maleate in bulk and pharmaceutical formulation.

Keywords: Asenapine maleate, Stress degradation, Validation, ICH, Linearity

INTRODUCTION

The use of analytical sciences in the discovery, development and manufacture of pharmaceuticals is wide-ranging. From the analysis of minute amounts of complex biological materials to the quality control of the final dosage form, the use of analytical technology covers an immense range of techniques and disciplines. Analytical methods are developed according to ICH guidelines. Forced degradation or accelerated degradation is a process whereby the natural degradation rate of a product or material is increased by the application of an additional stress. Forced degradation studies are used to identify reactions which may occur to degrade a processed product. Usually conducted before final formulation, forced degradation uses external stresses to rapidly screen material stabilities. Stability testing provides information about how the quality of bulk drug or drug products varies with time under the influence of various environmental factors such as temperature, humidity, and light and helps to determine recommended storage conditions and establish retest periods and shelf lives⁽¹⁾. The objective of the present work was to develop a simple, sensitive, precise, accurate and stability indicating RP-HPLC method for the determination of Asenapine maleate after performing different stress conditions as per ICH Guidelines.

Asenapine maleate is chemically (3aRS,12bRS)—chloro-2-methyl-2,3,3a,12b-tetrahydro-1H-dibenzo[2,3:6,7]oxepino[4,5-c]pyrrole(2Z)-2-butenediate is a atypical antipsychotic drug^{2,3} (figure1). It was approved by USFDA in August 2009. It is an antagonist at 5-HT_{2A}, dopamine and α-adrenergic receptors and has high affinity for dopamine (D₂) and serotonin(5-HT_{2A}) receptors and its efficacy is mainly mediated through the combination of antagonist activity at D₂ and 5-HT_{2A} receptors. It is indicated for treatment of various psychotic conditions like schizophrenia and bipolar disorders in adults^{4,5,6} and mainly works by controlling the psychotic symptoms through antagonism of selected dopamine and serotonin receptors in the CNS^{7,8}.

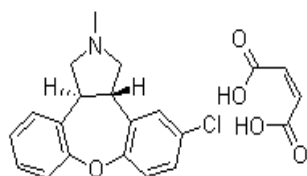


Fig. 1: Structure of Asenapine maleate

MATERIALS AND METHODS

Chemicals and reagents

All the chemicals and solvents used were of AR and HPLC grade. Asenapine tablets of 10mg strength were procured from the local pharmacy.

Instrumentation

The liquid chromatographic system consisted of shimadzu HPLC System consisting of RP- C₁₈ column, variable wavelength programmable UV Visible detector SPD-20A and a rheodyne injector with 20µl fixed loop.

Chromatographic conditions

The mobile phase consisting of methanol, n-butanol, and glacial acetic acid were degassed and filtered using a 0.45µm membrane filter. The composition of the mobile phase on the retention time of Asenapine was thoroughly investigated. The concentrations of the methanol:n-butanol:glacial acetic acid (60:20:20) were optimized to give symmetric peak with short runtime. The eluent were monitored at 270 nm, flow rate was 1.0mL/min, with ambient temperature, and runtime was 6 min. The volume of injection loop was 20µl, prior to injection of the drug solution the column was equilibrated for at least 30 min with the mobile phase flowing through the system.

Preparation of standard stock solution

50 mg of Asenapine maleate was weighed accurately and transferred to a 50ml volumetric flask. It was dissolved in HPLC grade methanol. The solution was sonicated for 10min and made up the volume with HPLC grade methanol. The resulting solution was filtered through a 0.45µm membrane filter and used as standard stock solution of 1mg/ml.

Development of calibration curve

Appropriate aliquots of standard asenapine stock solution were taken in different volumetric flasks and the resultant solution was diluted up to the mark with the mobile phase to obtain a final concentration of 10, 20, 30, 40, 50, 60,70,80,90, and 100ug/ml. Each of the solutions was injected using isocratic mode with UV detection at 270nm and flow rate of 1ml/min. From the resultant chromatogram obtained, peak area was used to plot the calibration curve.

Validation

Validation can be defined as (ICH) Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics^{10,11}.

The method was validated for several parameters like linearity, accuracy, precision, Robustness, Limit of detection (LOD), and Limit of quantification (LOQ) according to ICH guidelines.

Linearity

The linearity of the analytical method was its ability to elicit test results which are directly proportional to analyte concentration in samples within a given range.

Precision

Precision of the method was performed as intraday precision and interday precision. The intraday and interday precision studies were carried out by estimating the corresponding responses of the analysis on the same day and on three different days.

Accuracy

Accuracy of the proposed method was determined using recovery studies. The recovery studies were carried out by adding different amounts (80%, 100%, 120%) of the pure drug to the pre-analyzed formulation. The solutions were prepared in triplicates and the % recovery was calculated.

System Suitability parameters

To ensure the validity of the analytical procedure, a system suitability test was established. System suitability parameters for the developed HPLC method were determined by injecting six replicates of the standard solution (30µg/ml). Parameters such as a number of theoretical plates (N), tailing factor and retention time were calculated.

Robustness

The drug solution was subjected to small, deliberate changes like flow rate, wavelength and pH. The results obtained were not affected by varying the conditions and were in accordance with the results in original conditions.

LOQ and LOD

Limit of detection (LOD) is the lowest amount of analyte in the sample that can be detected. Limit of quantification (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined by suitable precision and accuracy. LOQ and LOD were determined using the following equation. $LOQ = 10s/m$, $LOD = 3.3s/m$ where s is the standard deviation of the response and m is the slope of the related calibration curve.

Stress degradation studies of Asenapine maleate

Intentional degradation was attempted to stress conditions of photolytic degradation, acid hydrolysis (using HCl), base hydrolysis (using NaOH) and oxidative degradation (using H₂O₂) of asenapine to evaluate the ability of the proposed method to separate drug from its degradation products. Drug concentration of 1 mg/ml was used in all the degradation studies. After completion of the degradation processes, the solutions were neutralized and diluted with mobile phase⁽¹²⁾.

Acid & alkaline degradation

Forced degradation in acidic media was performed by taking an aliquot of stock solution in 10ml volumetric flask and diluted up to the mark with 1.5N HCl to obtain a final concentration of 100mcg/ml. Similarly, forced degradation in alkaline media was performed using 1.5N NaOH.

Oxidative degradation

Oxidative degradation was performed by taking an aliquot of stock solution in 10ml volumetric flask and diluted up to the mark with 3%w/v of hydrogen peroxide to obtain a final concentration of 100mcg/ml.

Photolytic degradation

Contents of capsule in powder form and solutions form were prepared and exposed to light to determine the effects of light irradiation on the stability of drug in solution and in the solid state. Approximately 50 mg of drug was spread on a glass dish in a layer that was less than 2 mm thick and a solution of same drug (1mg/ml) was prepared in mobile phase. Control samples, which were protected with aluminum foil, were also placed in the dark cabinet and exposed concurrently.⁽¹³⁾

Estimation of Asenapine maleate in pharmaceutical dosage form

Twenty tablets were weighed and powdered. An accurately weighed portion of the powder equivalent to 50mg of asenapine was transferred to 50ml of volumetric flask, dissolved in methanol, sonicated and finally made up the volume with methanol. The resulting solution was filtered through the 0.45µm membrane filter to give a final concentration of 1mg/ml. Appropriate solutions were prepared by taking suitable aliquots of the clear filtrate and diluting them with mobile phase.

RESULTS AND DISCUSSION

The proposed method provides a simple, accurate, economical and convenient method for the analysis of Asenapine maleate using HPLC. By the proposed method, the retention time of Asenapine maleate was found to be 1.9 minutes. The resulting chromatogram obtained is shown in Figure 2.

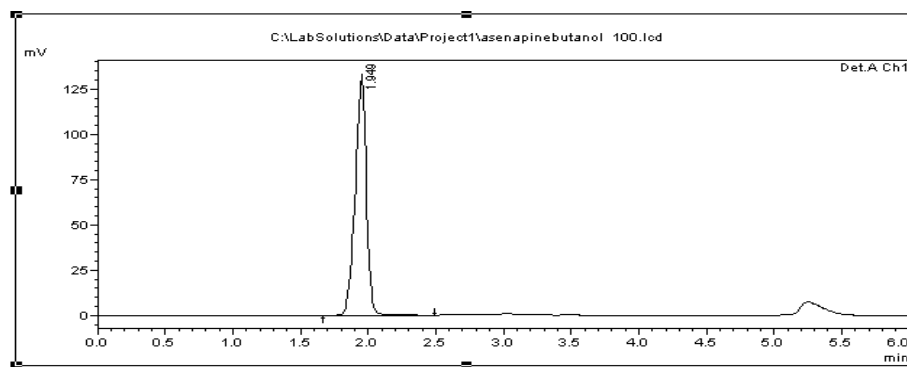


Fig. 2: Chromatogram of Asenapine maleate

Linearity was obeyed in the concentration range of 10-100µg/ml and the correlation 0.998. The regression equation of Asenapine maleate concentration over its peak area ratio was found to be $Y = 7727x - 6525$,

where Y is the peak area ratio and X is the concentration of Asenapine maleate (µg/ml). The calibration curve obtained is shown in figure 3 and Linearity data are shown in Table I.

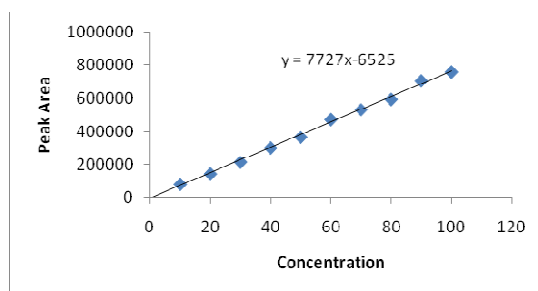


Fig. 3: Calibration curve of Asenapine maleate

The intraday and interday precision studies were carried out and the percent relative standard deviation (% RSD) was calculated and it was found to be 0.53 and 0.98 respectively, which is within the acceptable criteria of not more than 2.0. The results are shown in Table I.

Table I: Results of precision studies

Precision studies	Mean	Standard deviation	%RSD
Intra-day	215534	1148.5	0.53
Inter -day	219242	2182.6	0.98

Table II: system suitability parameters

Parameters	Results
Retention time	1.9 min
Asymmetric factor	0.95
Number of Theoretical plates	3601
Tailing factor	1.1

The System suitability parameters like number of theoretical plates (N) was found to be 3601, tailing factor 1.1 and asymmetry factor 0.95, which indicates efficient performance of column. The results are shown in Table II.

The drug solution was subjected to small, deliberate changes like flow rate, wavelength and pH. The results obtained were not affected by varying the conditions and were in accordance with the results in original conditions. This shows the method was robust. The limit of detection and limit of quantification was found to be 5.46 μ g/ml and 16.57 μ g/ml respectively, indicates the sensitivity of the method. Satisfactory recovery in the range of 98.9-100% is obtained by the proposed method. A good percentage of recovery indicates that the proposed method is accurate.

Asenapine shows sufficient degradation by acid hydrolysis in 1N HCL, the major degradation product formed at retention time 3 and 6.8 minutes. In the same manner Asenapine shows sufficient alkali hydrolysis in 1N NaOH, degradation peaks are formed at retention time 3 minutes (Figure 3&4)

Sufficient degradation of Asenapine is observed in oxidation with 3% H₂O₂. Additional peaks are formed at 3 and 3.2 minutes indicates the degradation. Studies are shown that Asenapine is unstable in light and sufficient degradation was observed in light after 2 days in direct sunlight, degradation peak was observed at 3 minutes. (Figure 5&6)

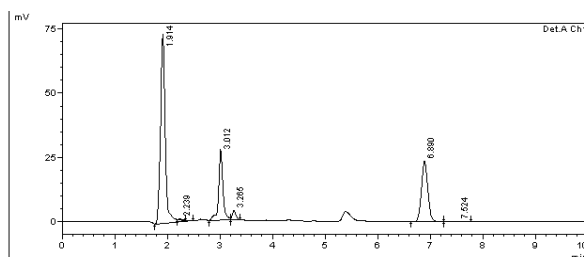


Fig. 3: Acid degradation

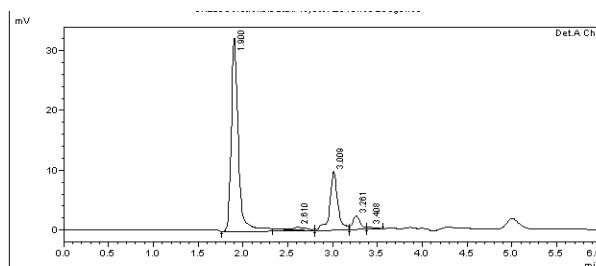


Fig. 4: Base degradation

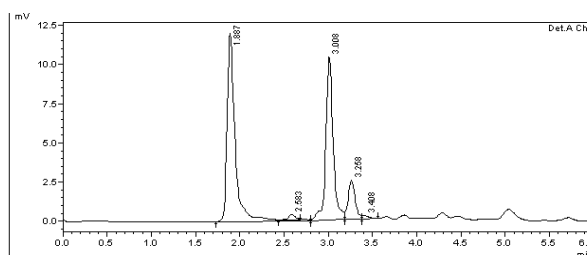


Fig. 5: Oxidative degradation

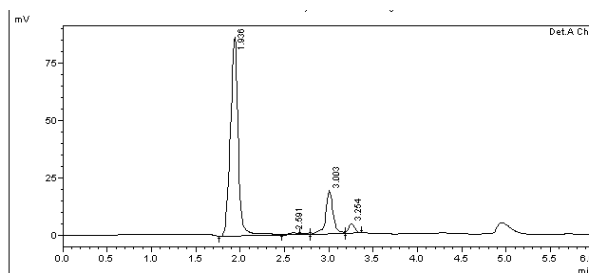


Fig. 6: Photolytic degradation

Estimation of Asenapine maleate in pharmaceutical dosage form was carried out and got 98.7% as assay value. The result of assay obtained was found to be in good agreement with the labeled claim, indicating the absence of interference of the excipients. The summary of the developed method is shown in Table III.

Table III: Validation parameters

Parameter	Results
Linearity range	10-100µg/ml
Correlation coefficient	0.998
Regression equation	Y= 7727x-6525
Retention time	1.9min
Theoretical plates	3601
Tailing Factor	1.1
Asymmetric factor	0.95
Mean% recovery(accuracy)	98.2-100.2%
Limit of detection(µg/ml)	5.46 µg/ml
Limit of Quantification(µg/ml)	16.57µg/ml
Precision (%RSD)	Interday(0.98), Intraday (0.53)

CONCLUSION

The developed HPLC method was found to be simple, precise, accurate and reproducible for the estimation of Asenapine maleate in bulk and pharmaceutical dosage forms. Hence this method can be conveniently adopted for routine quality control analysis of Asenapine maleate bulk and its pharmaceutical dosage forms. The proposed method is specific without any interference of excipients and hence can be used for the routine analysis of Asenapine maleate in bulk and in pharmaceutical formulation. A forced degradation study by HPLC was also performed to develop a stability indicating method, under various conditions like acid hydrolysis, alkali hydrolysis, oxidation and photolytic degradation. The drug was extensively degraded under all the performed conditions.

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