Original Article

VALIDATED STABILITY INDICATING HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE QUANTIFICATION OF ASENAPINE MALEATE

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ABSTRACT

Objective: A stability indicating high performance liquid chromatography method using the photodiode array detector is developed and validated for the analysis of asenapine in bulk and in its tablet formulation.

Methods: The method utilized a BDS Y Persil C18 (250 x 4.6 mm, 5 μm) column with 0.1% orthophosphoric acid and methanol (65:35 *v/v*) as mobile phase. The analysis was performed at 30 °C column temperature with a flow rate of 1 ml/min and detection at 227 nm. The method was validated for specificity, sensitivity, precision, linearity, accuracy and robustness. Asenapine was subjected to stress degradation studies under acidic, basic, oxidative, thermal and photolytic conditions.

Results: Under optimized experimental conditions, asenapine was eluted from the column at a retention time of 6.781 min. The method was linear in the range of 100-300 μg/ml. The linear regression data showed good relationship (correlation coefficient (R^2) = 0.9999). The relative standard deviation and mean recovery values were within limits. The analytical performance of the method was not affected when small variations in the experimental parameters were made. Degradation products resulting from stress degradation studies did not interfere with the detection of asenapine.

Conclusion: The proposed stability indicting high performance liquid chromatography method is sensitive, precise, accurate, robust and specific. This method can be used for quantification of asenapine in bulk drug and in tablet dosage form in the presence of its stress degradation products.

Keywords: Antipsychotic drug, Stress degradation, High performance liquid chromatography, Quantification.

INTRODUCTION

Asenapine [1-3], chemically known as (3a*RS*,12b*RS*)-*rel*-5-Chloro-2,3,3a,12b-tetrahydro-2-methyl-1*H*-dibenz[2,3:6,7]oxepino[4,5-c]pyrrole, is a second generation atypical antipsychotic agent. Asepanine is approved by Food and Drug Administration in 2009. Asenapine is long-term effective in the treatment of schizophrenia, potential to reduce the incidence of relapse, acute manic/mixed states related to bipolar I disorder.

Few reports are found in the literature for asenapine determination in pharmaceutical formulations [4-10], human plasma [11], human urine [12] and postmortem [13] samples. These include Ultra Violet (UV) spectrophotometry [4, 5], visible spectrophotometry [6], high performance liquid chromatography [7-10], liquid chromatography-mass spectrometry [11, 12] and gas chromatography-mass spectrometry [13]. None of the above reported methods is stability indicating. To the best of our knowledge three stability indicating high performance liquid chromatographic methods [14-16] are found in the literature for the quantification of asenapine in the presence of its degradation products.

The reported stability indicating methods suffers from disadvantages such as narrow range of linearity, preparation of buffer solutions, use of triple solvent system as mobile phase, less correlation coefficient value, lack of accuracy and sensitivity. Furthermore one of the three methods [15] is applied only to rat plasma sample.

Hence, the present investigation is aimed to develop and validate a simple, sensitive and accurate stability indicating high performance liquid chromatography method for the quantification of the asenapine in bulk and in its tablet formulation. The proposed have the advantages of broad linearity (upto 300 μg/ml), do not involve preparation of buffer, use of double solvent system as mobile phase and high correlation coefficient value (0.9999). The injection volume (10 μl) is less than the reported methods.

MATERIALS AND METHODS

Apparatus and HPLC conditions

A Waters 2695 alliance with binary high performance liquid chromatography (HPLC) pump equipped with the Waters 2998 photodiode array (PDA) detector and Waters Empower2 software were used in the present investigation. BDS Y Persil C18 (250 x 4.6 mm; 5 μm particle size) analytical column was used for separation and analysis of the asenapine. The column temperature was maintained at 30±1 °C. The separation was done under isocratic elution with flow rate maintained at 1 ml/min. The injection volume was 10 μl. The asenapine was analyzed using a PDA detector covering the range of 200–400 nm.

Mobile phase

The solvents and chemicals used in the preparation of the mobile phase were of HPLC grade and analytical grade, respectively. The mobile phase used was 0.1% orthophosphoric acid (Sd Fine Chemicals Ltd., Mumbai): methanol (Merck, Mumbai, India) in the ratio of 65:35 (*v/v*). The mobile phase was filtered through millipore filter before use and degassed for 15 min by sonication.

Drug standard solutions

The standard stock solution (1 mg/ml) was prepared by dissolving 100 mg of asenapine in 100 ml mobile phase. Working standard solutions equivalent to 100, 150, 200, 250, and 300 μg/ml asenapine were prepared by appropriate dilution of the stock standard solution with the mobile phase.

Tablet sample solution

Twenty tablets were weighed and crushed to a fine powder. The powder equivalent of 100 mg of asenapine was taken in a 100 ml volumetric flask containing 20 ml of mobile phase, sonicated for 20 min and made up to mark with the same diluent. The mixture was

filtered through 0.45 μm filter paper and the resultant solution was diluted properly with the mobile phase to get a final concentration of 200 μg/ml asenapine.

Stress degradation study

Stress degradation study was performed by subjecting the tablet powder to degradations such as acid, alkaline, oxidative, thermal and photolytic conditions to assess the interference of degradants. Acid, base, and oxidative degradations were performed by adding 10 ml of 0.1 N hydrochloric acid, 10 ml of 0.1 N sodium hydroxide and 10 ml of 30% peroxide solution, respectively to the sample and sonicated for 30 min. The acid degraded sample and base degraded sample are neutralized with 0.1 N sodium hydroxide and 0.1 N hydrochloric acid, respectively. Thermal degradation was performed by keeping the sample in petri dish and then placing them in an oven at 105 °C for 30 min. The photolytic study was carried out by placing the sample in petri dish and exposed to sunlight for 24 h. The degraded samples were diluted with mobile phase to get the concentration of 200 μg/ml asenapine and 10 μl aliquot of each degraded sample was injected into the HPLC system.

Analysis of asenapine in bulk

Ten μl aliquot of each working standard solution prepared in the section "Drug standard solutions" was injected automatically on to the column. The chromatograms and peak areas were recorded. The calibration curve was constructed by plotting the peak area against the concentration of the drug (μg/ml). The regression equation was derived. The concentration of unknown was determined either from the calibration curve or from the regression equation derived.

Analysis of asenapine in tablet dosage forms

Ten μl aliquot of tablet sample solution prepared in the section "Tablet sample solution" was injected automatically on to the column. The chromatograms were recorded. The peak area was calculated. The nominal content of asenapine in tablet dosage form was calculated either from the calibration curve or from the regression equation derived.

RESULTS AND DISCUSSION

Optimization of experimental parameters

Chromatographic parameters such as mobile phase composition, wavelength of detection and analytical column were optimized to achieve better efficiency of the chromatographic system. Three different analytical columns, BDS Y Persil C18 (250 x 4.6 mm, 5 μm), YMC C18 (250 x 4.6 mm, 5 μm) and Kromasil C8 (250 x 4.6 mm, 5 μm) were tested during method development. The parameters like tailing factor, retention time and plate count were taken into consideration. Based on the above said parameters BDS Y Persil C18 (250 x 4.6 mm, 5 μm) column was finalized for the analysis. Different composition of mobile phases containing a mixture (*v/v*) of 0.1 M mono potassium phosphate, methanol and 0.1 % orthophosphoric acid was evaluated in order to obtain suitable composition of the mobile phase.
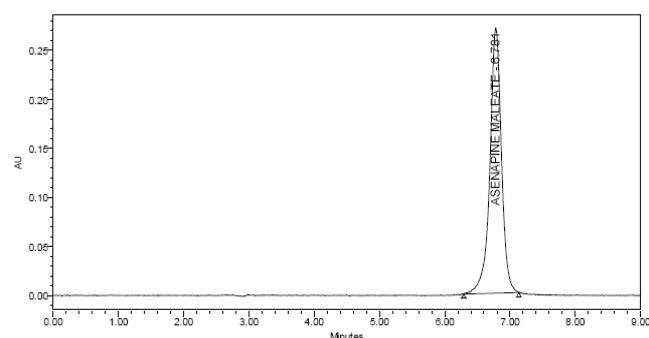

Fig. 1: Chromatogram of asenapine

Finally the mixture of 0.1 % orthophosphoric acid and methanol in the ratio of 65:35 (*v/v*) was selected as optimal as it produced well

defined and well resolved peak of asenapine at a flow rate of 1 ml/min and with column temperature of 30 °C. For the detection and quantification of asenapine, 227 nm was selected as the optimum wavelength. Under the optimized chromatographic conditions, the retention time for asenapine was found to be 6.781 min. A typical chromatogram is given in fig. 1.

Method validation [17]

System suitability

For system suitability testing, five replicates of asenapine (200 μg/ml) standard solutions were injected. The retention time, peak area, plate count and tailing factor of each replicate was established. The results of system suitability in comparison with the required limits are shown in table 1. The results are found to be within the accepted limits.

Table 1: System suitability

Parameters	Value	Recommended limits
Retention time	6.769	-
Peak area	3315183 (% RSD–0.5)	RSD ≤1
USP plate count	7832	>2000
USP tailing factor	0.94	≤ 2

RSD-Relative standard deviation, USP-United States Pharmacopeial Convention

Linearity

The developed method was tested for linearity by plotting peak area against concentration of asenapine working standard solutions. The plot of peak area *vs* the respective concentrations of asenapine were found to be linear in the concentration range of 100-300 μg/ml.

The results of linearity and regression equation for asenapine were given in fig. 2. The results show that an excellent correlation exists between peak area and asenapine concentration within the concentration range indicated above.
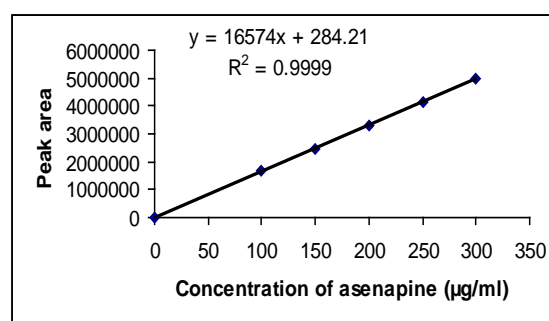

Fig. 2: Linearity curve and regression equation

Limit of quantification and detection

Limit of quantification (LOQ) and detection (LOD) of the developed method were predicted by the following formulae

(a) LOQ = 10 σ/S

(b) LOD = 3.3 σ/S

Where σ = standard deviation of response; S = slope of the calibration curve.

The LOD and LOQ for asenapine were found to be 2.4 μg/ml and 8.0 μg/ml, respectively.

Precision

The precision of the developed method was determined by the analysis of asenapine at a fixed concentration (200 µg/ml) by six replicate analyses. The precision was expressed as percent relative standard deviation (% RSD). The % RSD was found to be 0.27 %, indicating that the method is precise.

Accuracy

To study the accuracy of the developed method, recovery studies were conducted at three concentrations of 50 %, 100 %, and 150 % levels by standard addition method. The accuracy expressed as percentage recoveries was shown in table 2. The results indicated that the method is accurate.

Table 2: Accuracy of the method

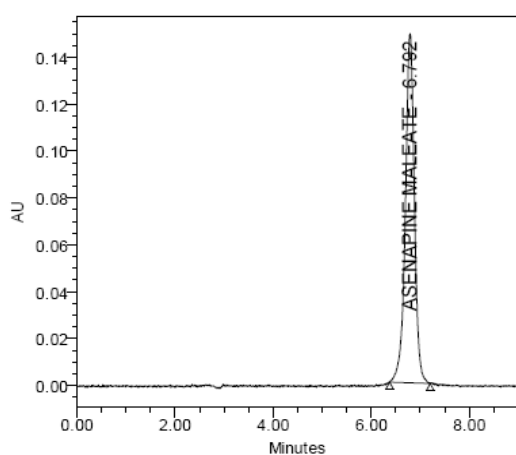| Spiked Level	Concentration of asenapine (µg/ml)		% Recovery	% Mean
	added	found		
50%	99.857	99.52	100	100
50%	99.857	99.59	100	
50%	99.857	99.78	100	
100%	200.000	200.09	100	100
100%	200.000	200.09	100	
100%	200.000	199.52	100	
150%	299.572	299.38	100	100
150%	299.572	299.29	100	
150%	299.572	298.77	100	

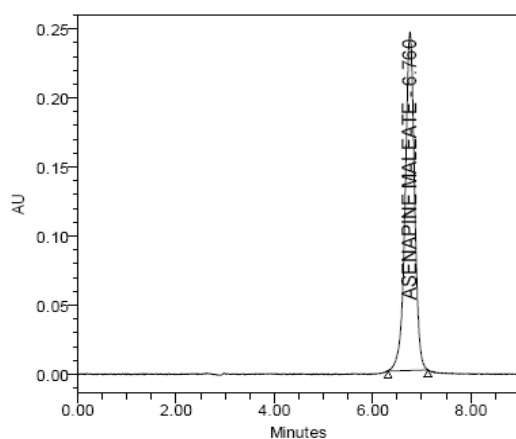Fig. 3: Chromatogram of asenapine (50 % level)

Fig. 4: Chromatogram of asenapine (100 % level)

Specificity

The specificity of the developed method was assessed through stress degradation studies. The degradation study was carried out using the tablet powder containing asenapine. The chromatograms of the stress degraded samples are shown in fig. 6-10. The samples submitted to degradation conditions showed significant alteration in the peak areas. In acid (fig. 6) and base (fig. 7) degraded samples one peak, in hydrogen peroxide (fig. 8) and photolytic (fig. 9) degradation two peaks were observed in addition to the asenapine

peak. Though the peak area was altered in dry heat degradation (fig. 10) process, no additional peak was observed. This may be due to the low concentration of the degradant. In all the stress conditions, the degradants peaks were well resolved from asenapine peak. The degradation results of various stress conditions were shown in table 3.
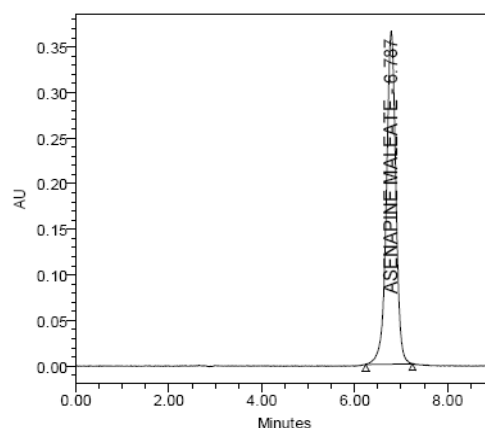

Fig. 5: Chromatogram of asenapine (150 % level)

Table 3: Stress degradation studies

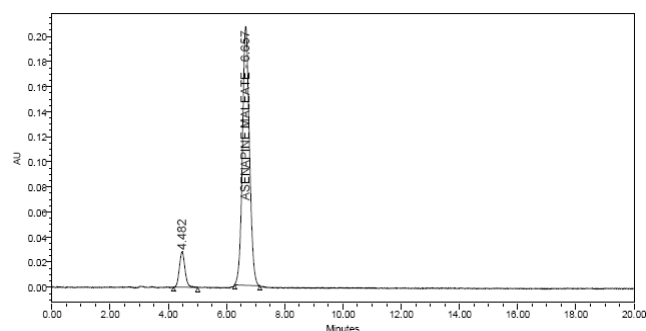| Type of degradation	Peak area	% Assay	% Degradation
Acid	3451167	94	6.0
Base	3524024	96	4.0
Peroxide	3491778	95	5.0
Heat	3475952	95	5.0
Sunlight	3486605	95	5.0
Undegraded	3315183	100	0.0

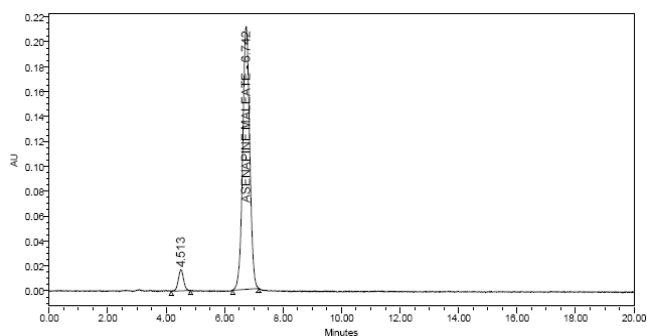

Fig. 6: Chromatogram of acid degraded sample

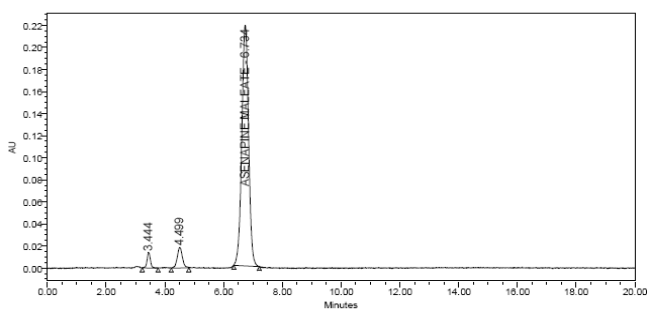**Fig. 7: Chromatogram of base degraded sample**

Fig. 8: Chromatogram of hydrogen peroxide degraded sample

Robustness

In order to confirm the robustness of the method, system suitability parameters were evaluated at different flow rate and different column temperature. Parameters such as retention time, tailing factor and plate count used to define robustness. The results demonstrated (table 4) that slight variations in method parameters had an insignificant outcome on the analysis.
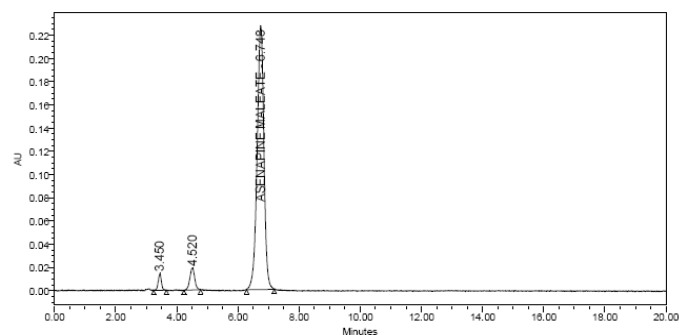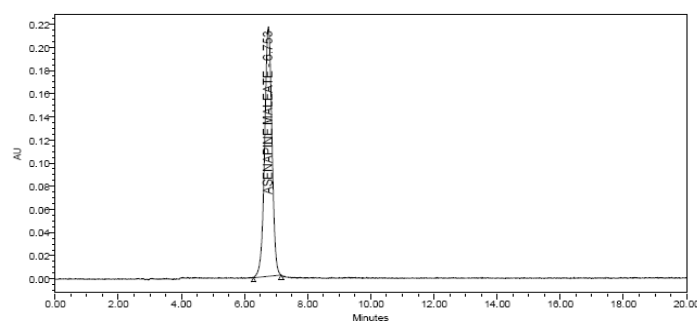

Fig. 9: Chromatogram of photo degraded sample

Fig. 10: Chromatogram of dry heat degraded sample

Table 4: Robustness of the method

Investigated parameter	Retention time	Peak area	Plate Count	Tailing
Flow 1	8.964	4457510	8804	0.95
Flow 2	8.600	4678832	6767	0.91
Temperature 1	6.805	3315625	7580	0.95
Temperature 2	6.535	3285496	8386	0.95

CONCLUSION

A stability indicating high performance liquid chromatographic method has been developed for the quantification of asenapine. The developed method was validated as per the guidelines of the International conference on harmonization. The method validation data demonstrated that the developed method is sensitive, precise, accurate, specific and robust. The method was proved to be stability indicating by the resolution of the asenapine from its stress degradation products. The method was found to be accurate for the estimation of asenapine in the tablets. Common excipients present in the tablet did not interfere with the assay. Hence, the validated high performance liquid chromatographic method can be employed in the routine analysis for the quantification of asenapine in tablet formulation.

CONFLICT OF INTERESTS

The authors have declared that no conflict of interest exists.

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