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QUANTITATIVE DETERMINATION OF ASENAPINE MALEATE USING REVERSE PHASE-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A novel isocratic reverse-phase high performance liquid-chromatography method for determination of asenapine maleate was developed and validated after optimization of various chromatographic conditions. Samples were separated on a waters x-terra C_{18} (100 mm × 4.6 mm, 3.5 µ) analytical column. The mobile phase used was acetonitrile: 0.1M phosphate buffer (p^H 3.2) 65:35%v/v operated at 30 °C column oven temperature was pumped at a flow rate of 1.0 mL min^{-1} and the column eluents were monitored at a wavelength of 272 nm. When sample was injected into the Finnigan surveyor high performance liquid-chromatography system through Finnigan surveyor auto-sampler injector, separation was achieved within 5.0 min. The present method was demonstrated and it was validated with the acceptable values for selectivity, linearity (within the expected concentration range (10–50 µg mL^{-1}; $r^2 > 0.999$)), recovery (>95%), precision (%RSD < 2.0), sensitivity (limit of detection: 1.85 µg mL^{-1} and lower limit of quantification: 2.34 µg mL^{-1}), robustness, and ruggedness.

KEYWORDS: **Asenapine maleate, pharmaceutical dosage form, RP-HPLC, Validation.**

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INTRODUCTION

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 atypical antipsychotic drug[1] (Figure1). It is an antagonist at 5-HT, dopamine and α-adrenergic receptors and has high affinity for dopamine (D2) and serotonin (5-HT2A) receptors and its efficacy is mainly mediated through the combination of antagonist activity at D2 and 5-HT2A receptors. It is indicated for the treatment of various psychotic conditions like schizophrenia and bipolar disorders in adults and mainly works by controlling the psychotic symptoms through antagonism of selected dopamine and serotonin receptors in the CNS[2].

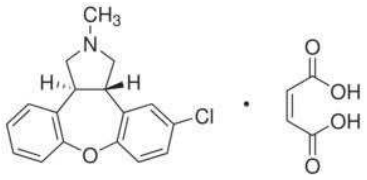

Figure 1
Structure of Asenapine maleate

Literature survey reveals that only UV spectrophotometric[3], HPLC[4] methods had been reported for the estimation of Asenapine in bulk and pharmaceutical dosage form. The objective of the present work was to develop a simple, sensitive, precise and accurate RP-HPLC method for the determination of Asenapine maleate in bulk and pharmaceutical formulations as per ICH guidelines[5-6].

EXPERIMENTAL:

Chemicals and reagents

Pure Asenapine maleate was gifted by Manus Aktteva, Ahmedabad. Tablets of 10mg strength were procured from the local market under the commercially available brand name Sycrest®[7]. Acetonitrile (Merck), water (Millipore), methanol (Merck), used were –HPLC grade and sodium dihydrogen phosphate, O-phosphoric acid were of analytical grade obtained from S.D.Fine chemicals Ltd.

Instrumentation

The estimation was carried out using a basic level isocratic system. The Finnigan surveyor liquid chromatographic system consisted of the following components: Finnigan surveyor LC pump, Photo diode array detector and Finnigan surveyor auto sampler plus. Chromatographic analysis was performed using X caliber on a Waters X-terra C_{18} (100 mm × 4.6 mm, 3.5 µ).

Optimization of Chromatographic conditions[8]

Optimization of mobile phase was performed based on resolution, asymmetry factor and peak area obtained. Mobile phase was prepared using various combinations of polar and non-polar solvents. Phosphate buffer (0.1M) pH was varied from 4.0 to 3.0 using 1% O-phosphoric acid. The buffer was prepared by dissolving sodium dihydrogen phosphate in water and filtered using membrane filter 0.45µ. This solution was then mixed with required quantity of acetonitrile and the solution was sonicated for 15 mins before use. Different columns were employed to obtain a better chromatogram with acceptable parameters. The flow rate was optimized from 1.0 to 2.5 ml/min. The column oven temperature was set at 30°C and auto sampler temperature was set at 10°C after optimization.

Preparation of standard solution of asenapine maleate

A stock solution of asenapine maleate was prepared by accurate weighing 10 mg of drug, transferring to 10 ml volumetric flask, dissolving in 5 ml of methanol and diluting with Milli Q water. Appropriate aliquot (0.1mL) of this solution was further diluted with 10 ml of methanol to obtain final standard solution of 10 µg/ml of asenapine maleate. Resultant solution was filtered through Whatman filter paper (#72) and then used. Further aliquots were prepared and diluted to 10 ml to obtain final concentration of 50, 40, 30, 20, and 10 µg/ml of asenapine maleate to construct calibration curve. A reverse phase C-18 column was equilibrated with the prepared mobile phase. Mobile phase flow rate was maintained at 1.0 ml/min and effluents were monitored at 272 nm. The sample was injected using autosampler with an injection volume of 10 µL and the total run time was 5.0 min. The chromatograms were developed and peak area was determined for each concentration of drug solution.

Preparation of analytical blank

Mobile phase without the drug was taken as the analytical blank and was injected into the column and signal obtained was noted. Blank chromatogram is represented in Figure 2.

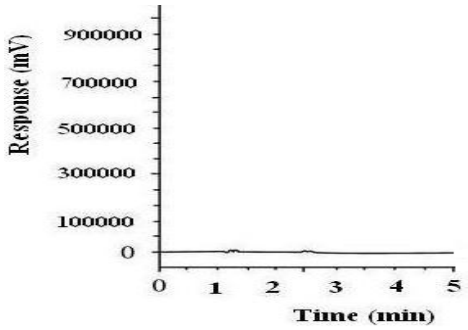

Figure 2
Chromatogram of analytical blank

METHOD VALIDATION

Specificity The specificity of the RP- HPLC method was determined by comparing chromatogram of standard and sample solution. Representative chromatogram is represented in Figure 3. The parameters like retention time (Rt), resolution (Rs), tailing factor (Tf) and theoretical plates were calculated. Results of system **s**uitability are recorded in Table 1.

Table 1
System suitable parameters of proposed method

S.No.	System suitable parameters	
1	λmax	272nm
2	Retention time	3.06
3	Theoretical plates	5061
4	Tailing factor	1.52
5	LOD	1.85
6	LOQ	2.34
7	%RSD	1.2

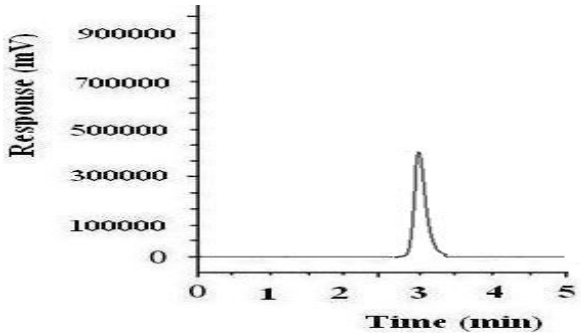

Figure 3
Representative chromatogram of Asenapine

Sensitivity

The sensitivity of the method was determined with respect to LOD and LOQ. The LOD and LOQ were separately determined by using 3.3(S/s) and 10(S/s) (where S- standard deviation of area response and s-slope) respectively based on the standard calibration curve. The proposed method had shown LOD and LOQ values as 1.85 and 2.34 respectively.

Linearity

Calibration standards ranging from 10 to 50µg/ml were prepared by subsequent dilution of the above stock solution with mobile phase. Calibration curve of asenapine maleate (Figure 4) was constructed by plotting peak area versus applied concentration of asenapine maleate and regression equation was computed. The results are represented in Table 2.

Table 2
Linearity of asenapine maleate

Concentration(µg/ml)	Area
10	224890
20	435980
30	658930
40	879630
50	1105380

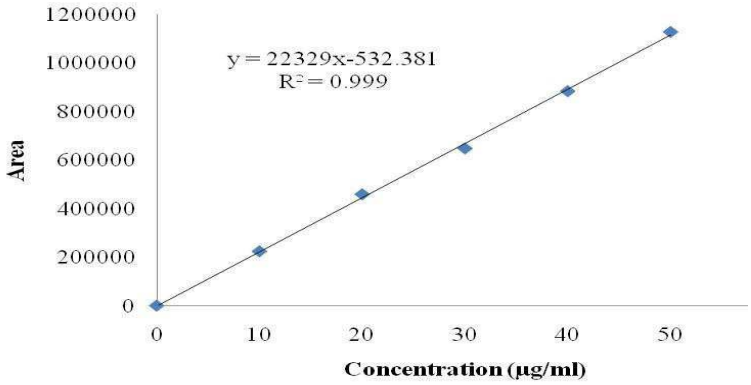

Figure 4
Calibration curve of Asenapine maleate

Precision

Intra-day and inter-day precision of the assay sample containing asenapine maleate (45 µg/mL) were analyzed six times in the same day (intraday) and for three consecutive days by different analysts. The results are shown in the Table 3.

Table 3
Precision data of the proposed method (n* =6)

Analyte	Intra day Concentration (µg/mL)	Area	%RSD	Inter day Concentration (µg/mL)	Area	%RSD
Asenapine maleate	45	996450	1.19	45	989980	1.22

***Mean of six determinations**

Accuracy

Accuracy of the method was tested by carrying out recovery studies at three different spiked levels (80%, 100%, and 120%) on the basis of the label claim. At each level, three determinations were performed and results were obtained. The results are shown in the Table 4.

Table 4
Recovery studies of asenapine maleate

Analyte	% type of level	Formulation (mg)	Amount of drug added (mg)	Amount recovered (mg)	% Drug recovered	%RSD
Asenapine maleate	80%	10	8	7.88	98.5	0.05
	100%	10	10	9.59	95.9	0.09
	120%	10	12	11.86	98.8	0.009

Robustness

Robustness was evaluated in terms of pH sensitivity and change in organic phase of the mobile phase. pH was changed by ± 0.5 and the organic phase composition was varied by ± 5%. The results are represented in Table 5.

Table 5
Robustness studies of asenapine maleate

	Chromatography Conditions								
	Flow rate			Temperature			% Organic Phase		
	1.0	1.2	1.4	25°C	30°C	35°C	-2%	0%	2%
Area	265659	222637	202376	214145	209640	211967	211967	222637	216923
%RSD	0.53	0.79	1.94	0.15	0.19	0.14	0.15	0.12	0.18

Analysis of asenapine maleate from tablets

Sample solution containing tablet powder equivalent to asenapine concentration in standard solution was prepared. The triturated tablet powder equivalent to 10 mg of asenapine was sonicated with 5 ml methanol for 20 mins. The volume was made upto 100 ml with water and the solution was filtered

using Whatman filter paper (#72). The sample solution was chromatographed (Figure 5) similar to the standard solution and concentrations of asenapine in tablet samples were calculated by using regression equation. The results are represented in Table 6.

Table 6
Analysis of pharmaceutical formulation (n*=6)

Analyte	Label claim (mg/tablet)	Amount found mg/tablet	%RSD
Asenapine maleate	10	9.59	1.1

Mean of six determinations

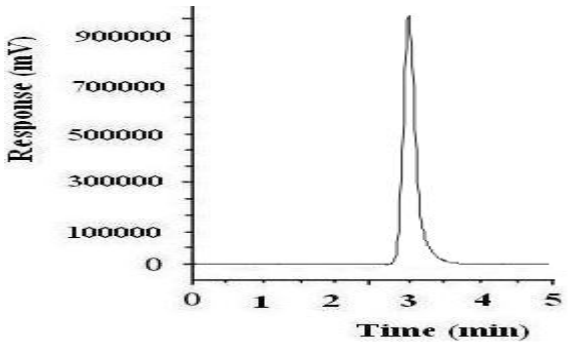

Figure 5
Chromatogram of Asenapine sample

RESULTS AND DISCUSSION

An attempt was made to develop a simple and accurate RP-HPLC method to determine Asenapine in bulk and pharmaceutical formulation. The mobile phase consisting of a 0.1M phosphate buffer (pH = 3.2 ± 0.05 with O-Phosphoric acid) and acetonitrile in the ratio (35:65% v/v) was employed. The chromatographic separation was carried on a Waters Xterra C18 column at a flow rate of 1.0 mL/min with PDA detector at 272 nm. The run time has set at 5 min for the HPLC system and was found to be the best for the analysis. The retention time of Asenapine maleate was found to be 3.06 min with tailing factor 1.52 and number of theoretical plates 5061. The peak areas of the drug were reproducible as indicated by the low coefficient of variation. The % RSD for both the tablet analysis and recovery studies was less than 2%. Hence, the proposed method is indicating high degree of precision and accuracy for both tablet analysis and recovery studies. The LOD and LOQ values are 1.85 and 2.34 µg/mL respectively. The results of the robustness study also indicated that the method is robust and is unaffected by small and deliberate variations in the chromatographic system.

CONCLUSION

The developed RP-HPLC method is simple, accurate, precise and robust method with all the acceptable parameters and can be employed successfully for the estimation of asenapine maleate in bulk and pharmaceutical formulation.

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