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A newer RP-UFLC method development and validation of an anti-psychotic drug asenapine maleate in bulk drug and pharmaceutical formulation

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ABSTRACT

A new, simple, accurate, precise, sensitive, specific and economical Reverse Phase Ultra-Fast Liquid Chromatography (RP-UFLC) method was developed and validated for the determination of Asenapine Maleate in bulk and pharmaceutical dosage forms. An Eclipse plus C-8, 5 µm particle size column having 4.6 x 250 mm internal diameterin gradient mode, with mobile phase containing Acetonitrile: Methanol (25:75 v/ v) was used. The flow rate was 1 ml / min and effluents were monitored at 210 nm. The sample was injected into the RP-UFLC by manual injector, separation was achieved within 10 min.The retention time for Asenapine Maleate standard drug was 1.9 minute and for sample was 1.9 minute with some additional peaks. The method was validated for linearity, accuracy, precision, specificity, limit of detection, limit of quantification, and robustness. The limit of detection (LOD) and limit of quantification (LOQ) for estimation of Asenapine maleate was found to be 1.14 µg / mL and 3.46 µg / mL, respectively. Recovery studies of Asenapine maleate was found to be in the range of 98.38–101.43 %. The linearity for Asenapine Maleate was in the range of 0-25µg/ml. The correlation coefficient was found to greater than 0.998. Thus, proposed method can be successfully applied for the quantitative determination of Asenapine maleate in bulk and pharmaceutical dosage forms.

Key words: Asenapine Maleate, RP-UFLC, validation, ICH.

INTRODUCTION

Asenapine maleate (ASM) is a chemically (3aRs,12bRS)-chloro-2-methyl 2,3,3a,12b tetrahyro-1H-dibenzo[2,3:6,7]oxepinol[4,5-c]pyrrole(2Z)-2-butenediate (**Figure 1**) is an antipsychotic drug [1].Asenapine Maleate is claimed to be a novel psychopharmacologic agents with high and potency for blocking dopamine, serotonin, α-adrenergics and histamine receptors, and no appreciable activity at muscarinic receptors [2,3]. The mechanism of action of Asenapine, like other atypical antipsychotics is believed to be medicated through a combination of antagonist activity at 5-HT_2A and D_2 receptors.Literature survey reveals that few analytical methods were reported for the determination of Asenapine maleate and its related substances in biological fluids like blood, plasma, urine and pharmaceutical preparations by spectrophotometry, LC-MS and RP-HPLC [4, 5, 6].The main objective of the work is to develop, validate and apply an efficient, simple, inexpensive and a newer method for analysis of antipsychotic agents.

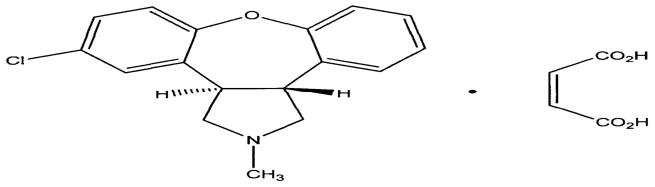

FIGURE1: Asenapine Maleate

MATERIALS AND METHODS

Instrument
RP-UFLC (Shimadzu, model SPD-M2DA2300, MADE IN JAPAN) containing PDA detector and C-8 Eclipse Plus.

Chemicals and reagents used
A reference standard of Asenapine Maleate was procured Indian Fine Chemicals, Mumbai.Acetonitrile (HPLC grade) and methanol (HPLC grade) were procured from Renkem Ltd India.

Preparation of standard drug solution
Stock solutions were prepared by accurately weighing 10 mg of ASM and transferring to 100 ml volumetric flasks containing 30 ml of mobile phase. The flasks were sonicated for 5-10 min to dissolve the solids and filtered through membrane filter 0.45µ. Volumes were made up to the mark with mobile phase, which gave 100 µg/ml. Aliquots from the stock solutions were appropriately diluted with mobile phase to obtain working standards of 20 µg/mlof drug.

Preparation of sample drug solution
The sample solutions (dosage forms) were prepared by accurately weighing drug powder equivalent 10 mg of ASM and transferring to 100 ml volumetric flasks containing 3 ml of mobile phase. The flasks were sonicated for 10 min to dissolve the solids and filtered. Volumes were made up to the mark with mobile phase, which gave 100 µg/ml. Aliquots from the stock solutions were appropriately diluted with mobile phase to obtain working standards of 20 µg/ml of drug.

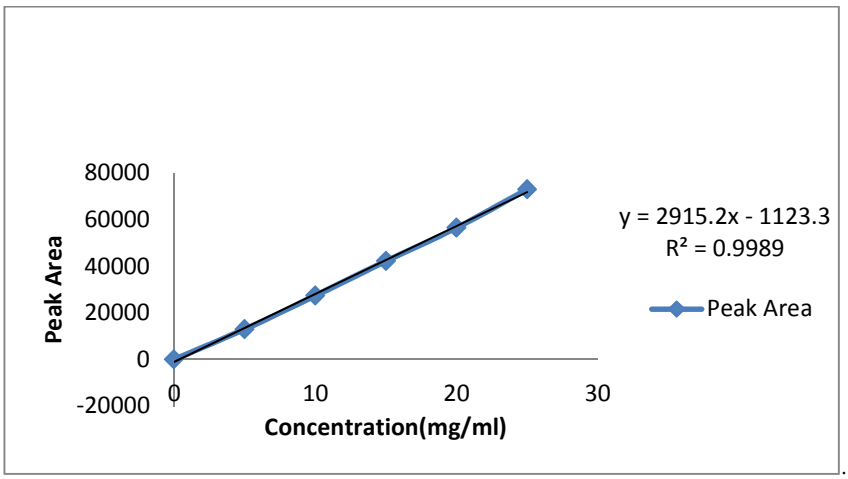

FIGURE 2: Linearity Curve of Asenapine maleate

Preparation of standard calibration curve
The study was carried out for the drug at five different concentration levels. Aliquots of standard Asenapine maleate working solutions were taken in volumetric flask and diluted with mobile phase to get the final concentration of Asenapine Maleate in the range 0-25µg/ml. All stocks and working solutions were sonicated for 5-10 minute and filtered through membrane filter (0.45 µ). Triplicate 20 µL injectors were made for each concentration and chromatograph was obtained at ambient temperature (27 ^{o}C). The calibration curve of Asenapine maleate is given in

Figure 2.
The chromatogram of same concentration of standard and sample Asenapine Maleate is shown in **Figure 3** and **Figure 4**.

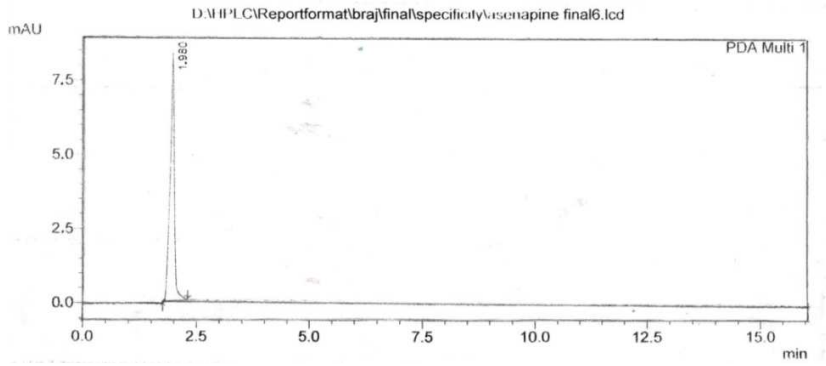

FIGURE 3: Chromatogram of Asenapine Maleate standard

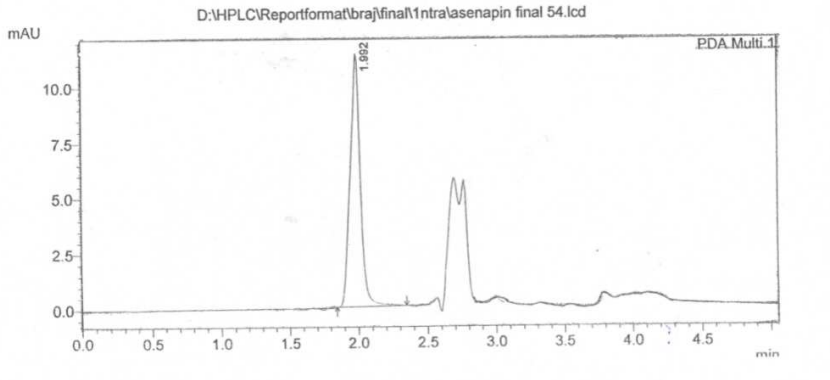

FIGURE 4: Chromatogram of Asenapine Maleate sample

Validation of the developed method

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. To establish the linearity of the proposed method, various aliquots of the standard solution of the drug were prepared from stock solution and analyzed. The linearity data are shown in **Table 1**.

TABLE 1: Linearity data of Asenapine maleate

Concentration (µg / ml)	Retention time (min)	Peak area
0	0	0
5	1.957	12940
10	1.961	27350
15	1.957	42172
20	1.980	56520
25	1.942	72921

Specificity

The specificity of the RP- UFLC method was determined by comparing chromatogram's area and retention time of standard and sample solution (**Table 2 and Table 3**).

TABLE 2: Specificity result of Standard Asenapine maleate

Concentration (µg/ml)	Retention time	Area
20	1.980	56520

TABLE 3: Specificity result of Sample Asenapine maleate

Concentration (µg/ml)	Retention time	Area
20	1.964	57862

Precision

Precision studies were carried out to ascertain the reproducibility of the proposed method. Repeatability was determined by preparing six replicates of same concentration of the sample and the absorbance was measured (**Table 4)**. Intraday precision study was carried out by preparing drug solution of same concentration and analyzing it at three different times in a day.The same procedure was followed for three different days to determine interday precision. The results were reported as %RSD and mention in **Table 5** and**Table 6**.

TABLE 4: Results of precision analysis

Concentration (µg/ml)	Peak area	Statistical Analysis
20	57862	
20	56737	Mean-5729.5
20	57861	
20	55574	SD-1010.13
20	57350	
20	58383	%RSD-1.7630

TABLE 5: Results of Intraday

Concentration (µg/ml)	Peak area 1	Peak area 2	Peak area 3	Average %RSD
20	58302	60137	9376	
20	60376	58534	60078	
20	59262	58132	61054	
20	60368	60540	58268	
20	61638	58587	61479	
20	60071	60252	60420	
%RSD	1.88	1.63	1.93	1.81

TABLE 6: Results of interday precision

Precision Conc. (µg/ml)	Peak area Day 1	Peak area Day 2	Peak area Day 3	Statistical Analysis
20	57923	58847	56334	
20	57200	56638	568521	
20	57232	57748	56840	
20	57811	59888	56341	
20	59990	59313	56434	
20	56856	58203	57531	
%RSD	1.95	1.99	0.813	1.58

Accuracy

Accuracy of the method was tested by carrying out recovery studies at three different levels (50%, 100%, and 150%) on the basis of the label claim. At each level, three determinations were performed and results obtainedwere shown in **Table8**.

TABLE 8: Accuracy readings of Asenapine maleate

Labelled claim (mg)	Level of Addition (%)	Amount of pure drug added (mg)	% Recovery	Statistical Analysis		
				Mean	SD	%RSD
10	50	5	98.73	100.45	1.222	1.216
10	50	5	101.48			
10	50	5	101.20			
10	100	10	98.56	99.76	0.855	0.857
10	100	10	100.30			

10	100	10	100.44			
10	150	15	100.47	99.65	0.910	0.913
10	150	15	98.38			
10	150	15	100.10			

Robustness

Analysis was carried out at two different temperatures, normal room temperature and at 18^{o}C to determine the robustness of the method and the respective areas was measured. The results were indicated in %RSD as shown in **Table 9**.

Table 9: Results of Robustness

Temperature 27°C			Temperature 18°C	
Concentration (µg/ml)	**Peak area**	**Statistical Analysis**	**Peak area**	**Statistical Analysis**
20	56648	Mean-55894.5 SD-1050.19 %RSD-1.87	57923	Mean-57835.33 SD-1129.391 %RSD-1.95
20	54132		57200	
20	56205		57323	
20	56206		57811	
20	55201		59990	
20	56974		56856	

LOD and LOQ

Limit of detection (LOD) is the lowest amount of analyte in the sample that can be detected and 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.The limit of detection and limit of quantitation for estimation of Asenapine maleate were 1.143µg/mL and 3.46µg/mL respectively.

The results of various parameters of the developed method are shown in **Table 10.**

TABLE 10: Characteristic parameters of Asenapine Maleate for the proposed RP-UFLC method

Parameters	RP-UFLC
Linearity range (µg / ml)	0-25
Detection wavelength (nm)	210
Mobile phase(Methanol : Acetonitrile)	75 : 25(v / v)
Retention time (min)	1.9
Regression equation (Y*)	Y = 2915.2x – 1123.3
Slope (m)	2915.2
Intercept (c)	1123.3
Correlation coefficient(r^2)	0.998
Intraday Precision (% RSD) Interday Precision (% RSD)	1.81 1.58
Limit of detection (µg / mL)	1.146
Limit of quantitation (µg / mL)	3.46

RESULTS AND DISCUSSION

An Eclipse plus C-8, 5 µm particle size column having 4.6 x 250 mm internal diameterin gradient mode, with mobile phase containing Acetonitrile: Methanol (25:75 v/ v) was used for the study. The flow rate was 1 ml / min and effluents were monitored at 210 nm. The proposed method obeyed Beer's law in the concentration range of 0-25µg/ml and correlation coefficient was found to be 0.9989. Intraday and interday precision study was performed and results showed that the developed method was found to be precise. The limit of detection and limit of quantitation for estimation of Asenapine maleate were 1.143µg/mL and 3.46µg/mL respectively. Recovery studies were carried out for the developed method by addition of known amount of standard drug solution of Asenapine maleate to pre-analyzedbulk sample solution at three different concentration levels. The resulting solutions were analyzed by the proposed methods. The recovery was in the range of 96.23 to 100.85 percentages and found to be satisfactory.

CONCLUSION

The developed method can be concluded to be simple, accurate, reliable and economical. The proposed method is specific without and interference of excipients and hence can be used for the routine analysis of Asenapine maleate in bulk and in pharmaceutical dosage form.

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