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Method development and validation of RP-HPLC method for determination of new antipsychotic agent asenapine maleate in bulk and in pharmaceutical formulation

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ABSTRACT

A novel isocratic reverse phase liquid chromatography method for determination of Asenapine Maleate was developed and validated after optimization of various chromatographic conditions. A Hiber C18, 5 µm column having 250×4.6 mm i.d., with mobile phase containing 0.05 M potassium dihydrogen phosphate : acetonitrile [60:40, v/v, pH 2.7 adjusted with 1% o-phosphoric acid] was used. The flow rate was 1.0 mL min^{-1} and effluents were monitored at 270 nm. The retention time of asenapine was 4.2min. The linearity for Asenapine maleate was in the range of 0-150 µg mL^{-1} with coefficient of correlation 0.999. The proposed method was validated with respect to linearity, accuracy, precision and robustness.

Key words: Asenapine, RP- HPLC, Asenapt Tablets, Validation

INTRODUCTION

The chemical name of the active substance is [3aR,12bR]-rel-5-chloro-2,3,3a,12b-tetrahydro-2-methyl-1H-dibenz[2,3:6,7]oxepino[4,5-c]pyrrole-[2Z]-2-butenedioate [1:1]. The molecular formula of active substance is $C_{17}H_{16}ClNO.C_4H_4O_4$, its relative molecular mass 401.84.

Asenapine maleate is a white to off-white non hygroscopic powder, slightly soluble in water, sparingly soluble in 0.1 M HCl, soluble in methanol.The pH of a saturated asenapine solution in water is 4.2 at 23.5 °C, its pKa is 8.6. Asenapine is claimed to be a novel psychopharmacologic agent with high affinity and potency for blocking dopamine, serotonin, α-adrenergic and histamine receptors, and no appreciable activity at muscarinic cholinergic receptors. The mechanism of action of asenapine, like other atypical antipsychotics is believed to be mediated through a combination of antagonist activity at 5-HT2A and D2 receptors.

The following indications were initially applied for: treatment of schizophrenia, treatment of manic episodes associated with bipolar I disorder and its structural is shown below Fig [1].

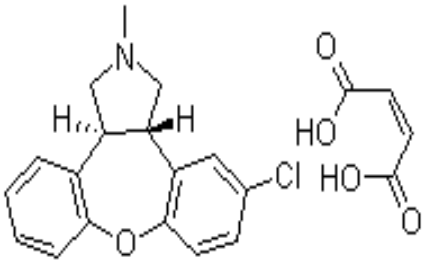

Fig [1]: structure of Asenapine

MATERIALS AND METHODS

2.1. Chemicals and Reagents

HPLC grade Acetonitrile from Merck specialties Pvt Ltd, Mumbai.Chemicals and Whatman GFC filter were used in the study.Analytically pure ASP was procured as gratis sample from Sun Pharmaceutical Pvt. Ltd., [Baroda, India]. Water HPLC grade was obtained from Rankem laboratories.Tablet formulation [Asenapt (5mg), Sun pharmaceuticals Ltd., Sikkim, India] containing labeled amount of 5 mg of asenapine sublingual tablets was purchased from local market.

2.2. Equipments

The instrument was a Water Alliance 2695 separation module, having water 2996 photodiode array detector in isocratic mode. The system was connected with the help of Empower2 software in a computer system for data collection and processing. The analytical column used is Hiber C18.

2.3. Chromatographic condition

The mobile phase consists of a mixture of 0.05N Potassium dihydrogen phosphate [pH adjusted to 2.7 with 1% o-phosphoric acid] [60 volumes] and Acetonitrile [40 volumes] was filtered through 0.45 µm nylon membrane filter before use. The injection volume was 20 µL with a flow rate 1 mL min^{-1} and detection wavelength 270 nm having ambient condition and run time 15 min.

2.4. Standard preparation

Stock solutions were prepared by accurately weighing 10 mg of ASP and transferring to 10 ml volumetric flasks containing 3 ml of methanol. The flasks were sonicated for 10 min to dissolve the solids. Volumes were made up to the mark with methanol, which gave 1000 µg mL^{-1}. Aliquots from the stock solutions were appropriately diluted with mobile phase to obtain working standards of 100 µg mL^{-1} of drug. Typical standard chromatogram of asenapine is shown in Fig [2].

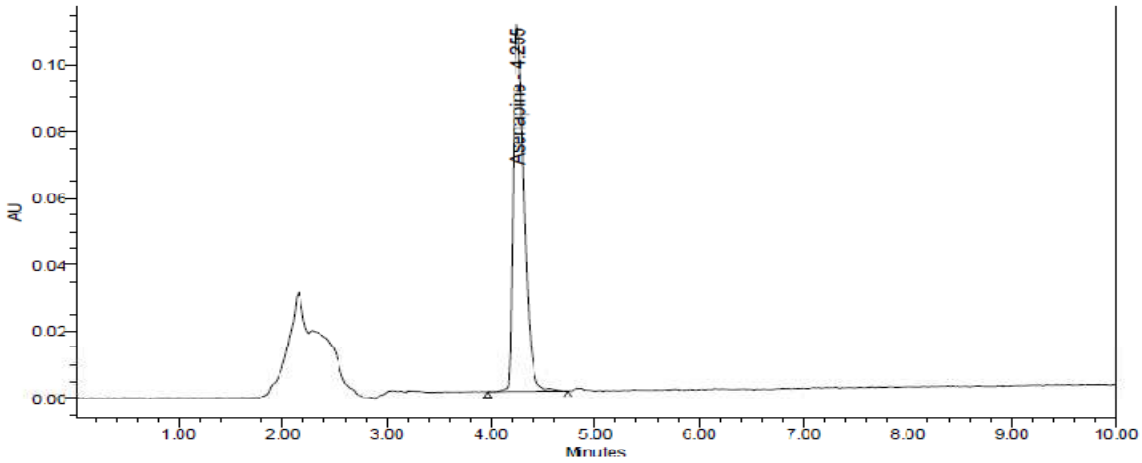

Fig [2]: standard chromatogram of Asenapine Maleate

RESULTS AND DISCUSSION

3.1. Estimation of Asenapine in tablet dosage form

The HPLC procedure was optimized with a view to develop precise and stable assay method. Asenapine Maleate was run in different mobile phase composition and different pH ranges [2.5 to 3.5] of mobile phase with different C18 columns Waters Xbridge [100 mm x 4.6 mm i.d.,5 µm] [Kromosil 150 mm x 4.6 mm i.d.,5 µm], column-Hiber [250 mm x 4.6 mm 5 µm] at ambient temperature [25º and 30º C].The flow rate was also varied from 0.5 mL to 1 mL min^{-1}. The mobile phase consists of and a mixture of 0.05N potassium dihydrogen phosphate [pH adjusted to 2.7] [60 volumes] and acetonitrile [40volumes] was filtered through 0.45 µm nylon membrane filter before use. The column used is Hiber c18, 5 µm column having 250×4.6 mm i.d.

Twenty tablets were weighed and crushed to fine powder. The powder equivalent to 25mg of Asenapine was taken in a 25 mL volumetric flask and made up with methanol. The resultant mixture was filtered through 0.45 µm nylon filter. From this filtrate 10mL of solution was pipette out into 100 ml standard flask and made up with mobile phase. The sample solution was chramatographed similar to standard solution and concentrations of asenapine in tablet samples were calculated using regression equation. Typical sample chromatogram of asenapine is shown in Fig [3]

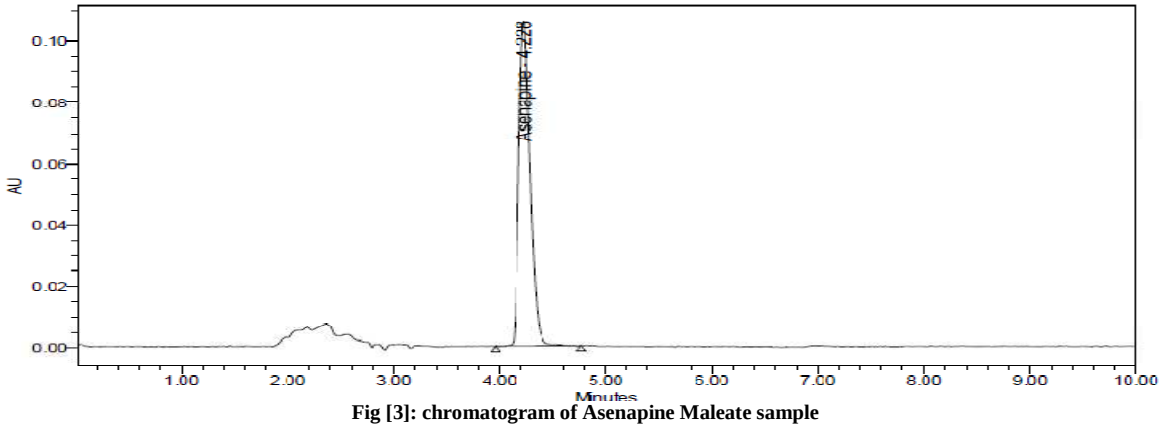

Fig [3]: chromatogram of Asenapine Maleate sample

3.2. Method Validation

The described method has been validated for the assay of Asenapine using following parameters.

3.3. Accuracy

The accuracy of the method was determined by recovery experiments. Placebo was spiked with known quantities of standard drugs at levels of 50 to150% of label claim. The recovery studies were carried out 3 times and the percentage recovery and standard deviation of the percentage recovery were calculated and presented in Table 1.

Table1: Results of accuracy studies

Sr. No	%Accuracy	Peak area	Amount Added [mg mL^{-1}]	Amount Found [mg mL^{-1}]	%Recovery	Avg %Recovery
1.	50%	396324	0.05	0.049036	98.0722	98.6280
		400555	0.05	0.04956	99.1191	
		398832	0.05	0.049346	98.69282	
2.	100%	803046	0.1	0.10003	100.0304	100.0089
		808475	01	0.10003	100.0304	
		807961	0.1	0.099967	99.966	
3.	150%	1219783	1.5	0.15092	100.6136	100.0089
		1224613	1.5	0.151518	101.012	
		1211768	1.5	0.149929	99.95253	

The mean %recovery is well within the acceptance limit, hence the method is accurate

3.4. System suitability studies

The system suitability test was carried out on freshly prepared stock solution of Asenapine to check various parameters such as column efficiency, tailing factor and number of theoretical and presented in Table 2. The values obtained were demonstrated the suitability of the system for the analysis of the drug. System suitability parameter may fall within 3% standard deviation range during routine performance of the method.

Table 2. System Suitability Studies

Sr. no	peak area	Retention Time	Theoretical plates	Tailing factor
1	797220	4.271	7542	1.49
2	804285	4.255	7693	1.49
3	804633	4.244	7391	1.52
4	815134	4.216	7405	1.49
5	812261	4.214	7414	1.52
AVG	806706.6	4.24	7489	1.502
SD	7106.898	0.024769	129.1414	0.016432
%RSD	0.880977	0.584173	1.724414	1.093986

3.5. LOD and LOQ

The LOD and LOQ of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLCmethod. The LOD is the smallest concentration of the analyte that gives a measurable response [signal to noise ratio of 3]. The LOD for Asenapine was found to be - 0.7 µg mL^{-1}.The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified [signal to noise ratio of 10]. The LOQ was 2.3 µg mL^{-1} for Asenapine Maleate respectively.

3.6. Linearity and Range

Linearity was studied by preparing standard solution at five different concentration levels. The linearity range was found to be 0-150 µg mL^{-1}. 20 µL of each solution was injected into chromatograph. Peak areas were recorded for all the chromatogram. Calibration curve was constructed by plotting peak areas [Y axis] against the amount of drug in µg mL^{-1} [X axis]. Peak area of linearity range and the parameters were calculated and presented in Table 3. The linearity curve of Asenapine was shown in Fig [4].

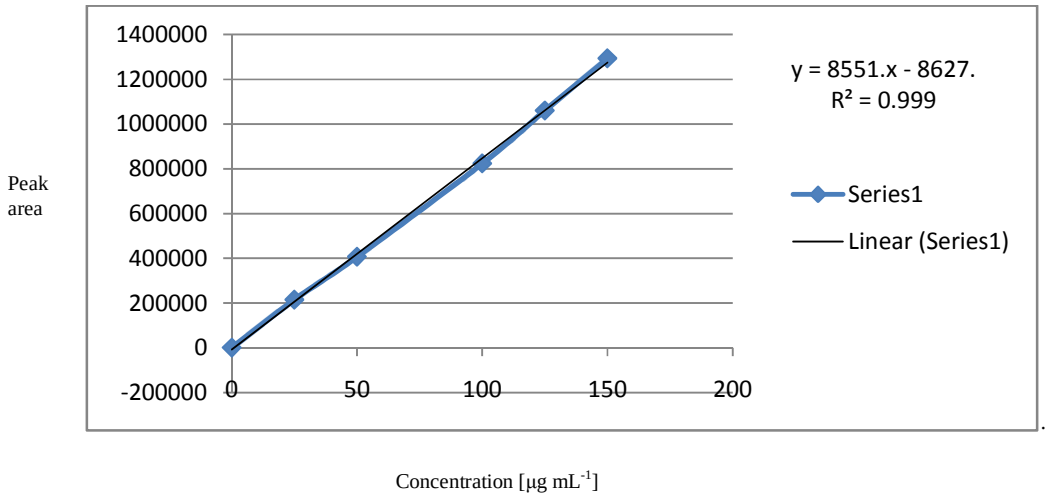

Fig [4]. The linearity curve of Asenapine concentartion vs peak area

3.7. Specificity

Specificity of the method was determined by injecting the diluted placebo. There was no interference of placebo with the principle peak, hence the developed analytical method was specific for Asenapine in tablet dosage form.

Table 3: Result of Linearity

S.No	Pipetted from stock [mL]	Volume of flask [mL]	Concentration	µg mL^{-1}	% of linearity level	Peak area
1	0	0	0		0	0
2	0.25	10	25		25	214387
3	0.5	10	50		50	406311
4	1	10	100		100	823006
5	1.25	10	125		125	1059709
6	1.5	10	150		150	1292803

3.8. Precision

3.8.1. System precision: The system precision of the method was established by six replicate injections of the standard solution containing Asenapine. The percentage RSD were calculated and presented in Table 4. From the data obtained, the developed RP-HPLC method was found to be precise.

Table 4: System precision results

S.NO	PEAK AREA
1	797220
2	804285
3	804633
4	815134
5	812261
6	815842
AVG	808229.2
SD	7369.915
%RSD	0.91186

3.8.2. Method precision

The method precision of the method was established by carrying out the analysis of Asenapine in dosage form [n=6] using the proposed method. The low value of the relative standard deviation showed that the method was precise the results obtained were presented in Table 5.

Table 5: Method Precision Result

S.NO	PEAK AREA	%ASSAY OF DOSAGE FORM
1	800739	98.9742
2	803333	99.2948
3	807949	99.8653
4	805903	99.6125
5	803440	99.3080
6	808550	99.9396
AVG	804985.7	99.4991
SD	3016.02	0.372791
.%RSD	0.374668	0.374668

Table [6] : Result of analysis of formulation

DRUG	Amount[mg/tab]	
	Label claim	Found
Asenapt	5mg	4.974mg

Table 7. Method Robustness of asenapine in Dosage Forms

Condition	Change	Retention time [Min]	%RSD
Temperature	+5ºC	4.085 4.077	0.1386
	-5ºC	4.13 4.12	0.1714
Flow rate	+0.2 mL min^{-1}	3.405 3.403	0.0141
	-0.2 mL min^{-1}	5.311 5.296	0.199

3.9 Robustness

Robustness of the method was determined by making slight change in the chromatographic condition. It was observed that there were no marked changes in the chromatograms, which. The results of robustness were presented in Table 7.

CONCLUSION

The proposed RP-HPLC method for the estimation of Asenapine in tablet dosage forms is accurate, precise, linear, robust, simple and rapid. Hence the present RP-HPLC method is suitable for the quality control of the raw material, formulation and dissolution studies.

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