Scholars Academic Journal of Pharmacy (SAJP)
Abbreviated Key Title: Sch. Acad. J. Pharm.
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A Unit of Scholars Academic and Scientific Society, India
www.saspublisher.com

ISSN 2347-9531 (Print)
ISSN 2320-4206 (Online)

Pharmaceutical Analysis

Validated RP-HPLC Method for Estimation of Asenapine in Bulk and Tablet Dosage Form

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Original Research Article

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Article History
Received: 06.04.2018
Accepted: 17.04.2018
Published:30.05.2018

DOI:
10.21276/sajp.2018.7.5.1

Abstract: A simple and selective LC method is described for the determination of ASENAPINE dosage forms. Chromatographic separation was achieved on a C_{18} column using mobile phase consisting of a mixture of Triethylamine Buffer: Acetonitrile (50:50) with detection of 220nm. Linearity was observed in the range 15-45 µg /ml for ASENAPINE (r^2 =0.997) for the amount of drug estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Keywords: Reverse Phase- High Performance Liquid Chromatography (RP-HPLC), Asenapine, r^2 correlation coefficient.

INTRODUCTION

Asenapine [1, 2] is a serotonin, dopamine, nor adrenaline, and histamine antagonist[3] in which asenapine possesses more potent activity with serotonin receptors than dopamine. Chemically it is known as (2Z)-but-2-enedioic acid;17-chloro-4-methyl-13-oxa azatetracyclo[12.4.0.0^{2},^{6}.0^{7},^{12}]octadeca-1(14),7,9,11,15,17-hexaene.The chemical structure of asenapine was given in Fig.1

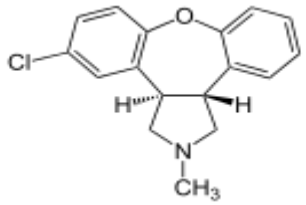

Fig-1: Structure of Asenapine

As per the literature review, several methods were there for the determination of its pharmacological action. Asenapine was estimated individually by few methods like UV [4, 5] spectroscopy, HPLC [6] and LC-MS [7, 8]. But there was no stability indicating RPHPLC Method. So the aim of present work was to develop and validated stability indicating RP-HPLC method for the determination of Asenapine in bulk and tablet dosage form.

MATERIALS AND METHODS

Chemicals and Reagents

Asenapine was obtained as gift sample from Chandra laboratories, Hyderabad. Acetonitrile, water used was of HPLC grade.

Instrumentation

A water HPLC system with LC solutions software with a PDA detector and was ZODIAC column used for analysis.

Chromatographic conditions

An HPLC system which is operated using software, LC solutions, fitted with ZODIAC column and PDA detector (at 220nm) was used for the analysis. Isocratic run with flow rate 1ml/min was preferred for resolving the drug.

Preparation of mobile phase

A mixture (50:50) of Triethylamine and Acetonitrile was used as mobile phase.

Standard preparation

Weigh accurately 10 mg of ASENAPINE in 100 ml of volumetric flask and dissolve in 100ml of mobile phase and make up the volume with mobile phase From above stock solution 30 µg/ml of ASENAPINE is prepared by diluting 3ml to 10ml with mobile phase. The chromatogram of standard Asenapine solution was shown in Fig.2.and the average Retention time was found to be about 3.075.

Sample preparation

10 tablets (each tablet contains 5mg of ASENAPINE was weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of ASENAPINE (100µg/ml) were prepared by dissolving weight equivalent to 5 mg of ASENAPINE and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 30µg/ml of ASENAPINE was made by adding 3 ml of stock solution to 10 ml of mobile phase.

Validation [9-11]

System suitability

A standard solution of Asenapine working standard was prepared as per procedure and was injected five times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms obtained by calculating the % RSD retention time, tailing factor, theoretical plates, peak areas from five replicate injections are within range and results shown in Table.1.

Linearity

To demonstrate the linearity of assay method, inject 5 standard solutions with concentrations of about 15µg/ml to 45 µg/ml of Asenapine. Plot a graph to concentration versus peak area. Correlation co-efficient was found to be 0.997 and linearity plot was shown Fig.3.and results were in Table.2.

Accuracy

Three concentrations of 75%, 100%, and 125% are injected in triplicate manner and % recovery was calculated as 100.5%. The results were in Table.3.

Precision

Repeatability

Six working sample solutions 100ppm are injected and the % amount was calculated and % RSD was found to be 0.92.

Intermediate precision

Six working sample solutions are injected on the next day of the preparation of samples and % amount was calculated and % RSD was found to be 0.92. The Results were shown in Table.4

Robustness

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like Temperature and wavelength shown in Table.5

Limit of Detection (LOD)

LOD is the lowest level of concentration of analyte in the sample that can be detected, though not necessarily quantitated. It is calculated by using this formula,

$$LOD = 3.3\sigma/S$$

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

Limit of Quantitation

LOQ is the lowest concentration of analyte in a sample that may determined with acceptable accuracy and precision when the required procedure is applied. It is calculated by using this formula,

$LOQ = 10\sigma/S$

Where,

σ = Standard deviation of the response,

S = Slope of calibration curve.

Table-1: Acceptance Limits for System Suitability Test [32]

Parameter	Limit
Capacity Factor	$k' > 2$
Injection precision	RSD < 1% for n ≥ 5
Resolution	$R_s > 2$
Tailing factor	$A_s \leq 2$
Theoretical plates	$N > 2000$

Table-2: linearity of ASENAPINE

S.No.	Conc.(µg/ml)	Area
1	15	1170177
2	22.5	1697912
3	30	2407163
4	37.5	2834924
5	45	3306552

Table-3: Accuracy data for ASENAPINE

Recovery level	Accuracy ASENAPINE			
	Amount taken(mcg/ml)	Area	%Recovery	Average % Recovery
50%	15	1176833	100.66	100.5
	15	1178517		
	15	1178517		
100%	30	2490174	101.54	
	30	2426700		
	30	2415495		
150%	45	3292362	99.55	
	45	3285307		
	45	3297269		

Table-4: Results for Method precision of ASENAPINE

ASENAPINE		
S.No.	Rt	Area
1	3.107	2073796
2	3.025	2036834
3	3.085	2078955
4	3.078	2075109
5	3.098	2063159
6	3.079	2075519
avg	3.07867	2067229
stdev	0.02863	15823.2
%RSD	0.92983	0.76543

Table-5: Result of Robustness study

Parameter	ASENAPINE	
	Retention time(min)	Area
Flow		
1.0ml/min	3.671	2589974
1.4ml/min	2.696	1891623
Wavelength		
252nm	3.081	2113775
256nm	3.086	2154238

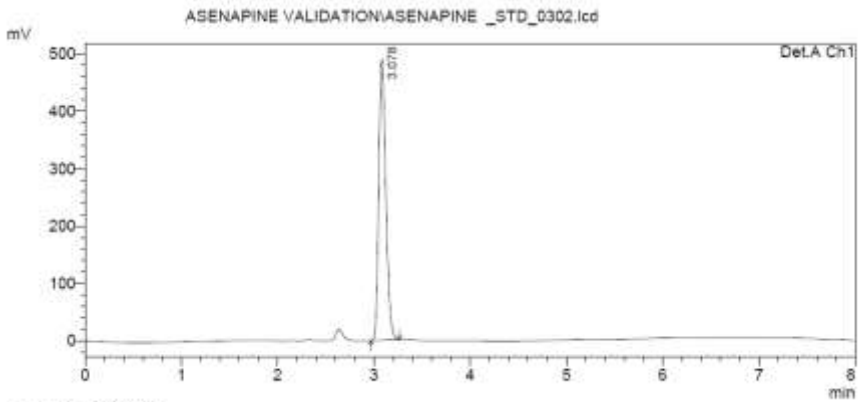

Fig-2: Chromatogram of ASENAPINE standard

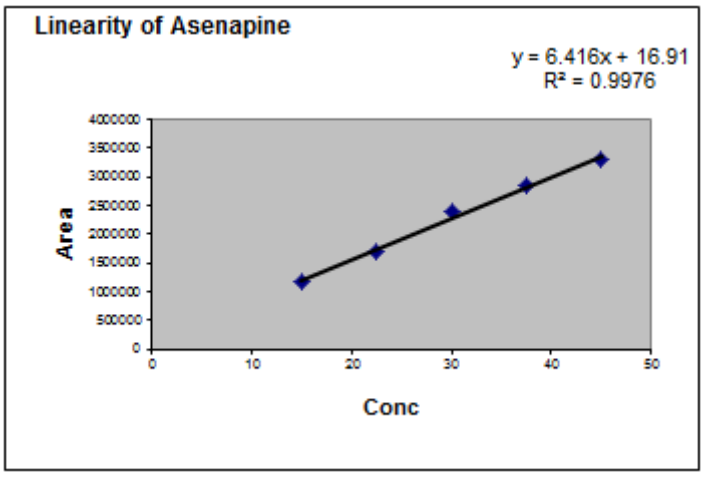

Fig-3: Linearity graph of ASENAPINE

CONCLUSION

In conclusion, a simple, selective, sensitive and accurate stability indicating RP-HPLC method was developed and validated for the analysis of Asenapine. Further method was found to be linear, precise, accurate and robust. The degradation studies reveal the stability of the drug. Hence the proposed method can be safely and successfully used for the estimation of Asenapine in routine analysis.

ACKNOWLEDGEMENT

Authors are very thankful to chairman and secretary of J B educational institutions, Hyderabad, for guidance encouragement and providing laboratory facilities

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