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VALIDATED STABILITY-INDICATING RP-HPLC METHOD FOR DETERMINATION OF ASENAPINE

Shyamala*, A. Swarupa, P. Anitha

Department of Pharmaceutical Analysis, Joginpally B. R. Pharmacy College, Hyd.

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.

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

Corresponding author:

Shyamala,

Department of Pharmaceutical Analysis,
Joginpally B. R. Pharmacy College,
Hyderabad,
Telangana.

QR code

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INTRODUCTION:

Asenapine [1-3] is a serotonin, dopamine, nor adrenaline, and histamine antagonist 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.

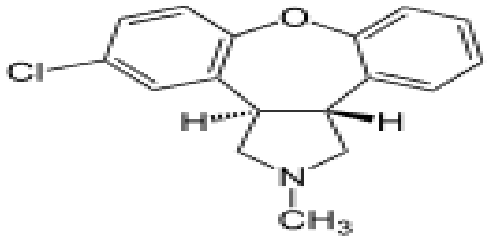

Fig.1: Structure of Asenapine

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 METERIAL:

Chemicals and Reagents:

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

Instrumentation:

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

Validation:

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 = 10σ/S

Where,

σ = Standard deviation of the response,

S = Slope of calibration curve.

Degradation Studies:

The degradation studies are carried out by following the guidelines of ICH.

Acid hydrolysis:

Asenapine is treated with 5mL of 5N Hydrochloric acid for 5Hrs at room temperature, and then neutralized with 5ml of 5N Sodium hydroxide solution.

Alkali hydrolysis:

Asenapine is treated with 5mL of 2N Sodium hydroxide for 2Hrs at room temperature, and then neutralized with 5ml of 55N Hydrochloric acid solution.

Oxidation:

Asenapine is treated with 5mL of 3% of H_2O_2 for 2Hrs at room temperature.

Thermal Degradation: Tablets were exposed to 105°C for 24Hrs.

Photolytic Degradation: Tablets were places in photolytic chamber up to achieve 1.2millian lux hrs.

***Table 1*:** Acceptance Limits for System Suitability Test.

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** **1.4ml/min**	 3.671 2.696	 2589974 1891623
Wavelength **252nm** **256nm**	 3.081 3.086	 2113775 2154238

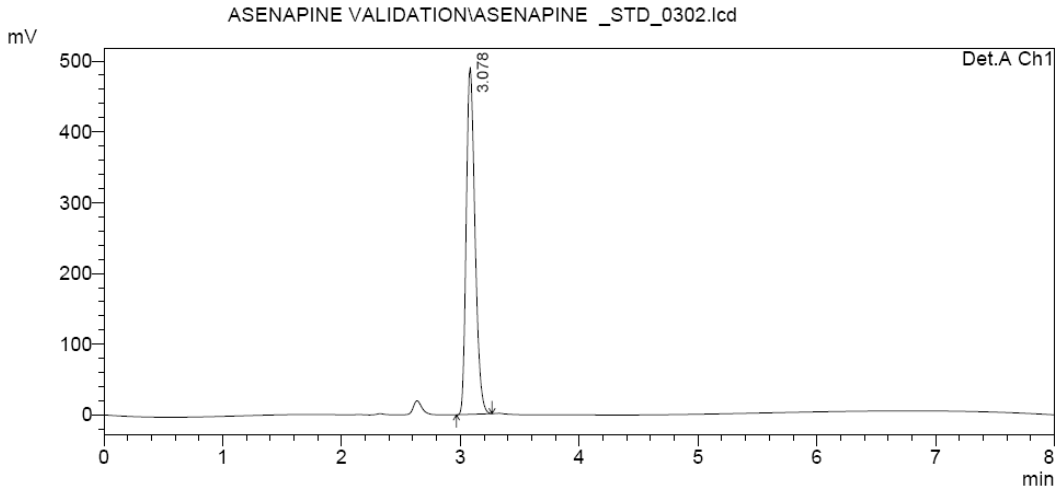

Fig. 2 : Chromatogram of ASENAPINE standard

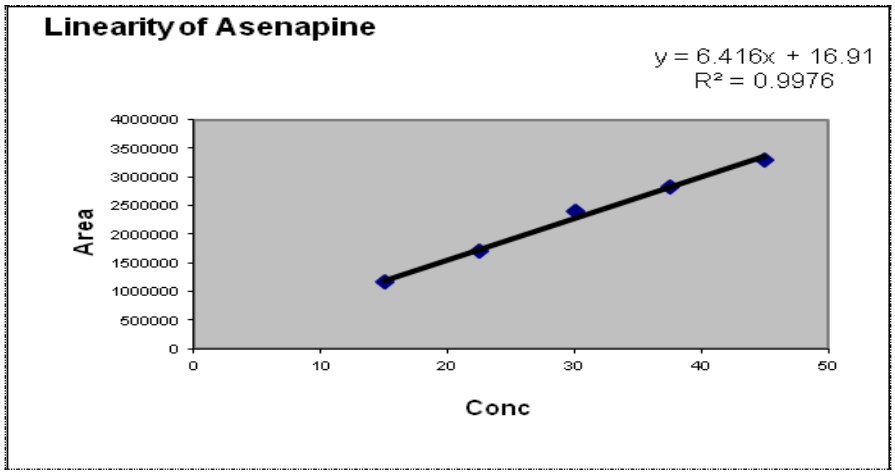

Fig. 3: Linearity graph of ASENAPINE

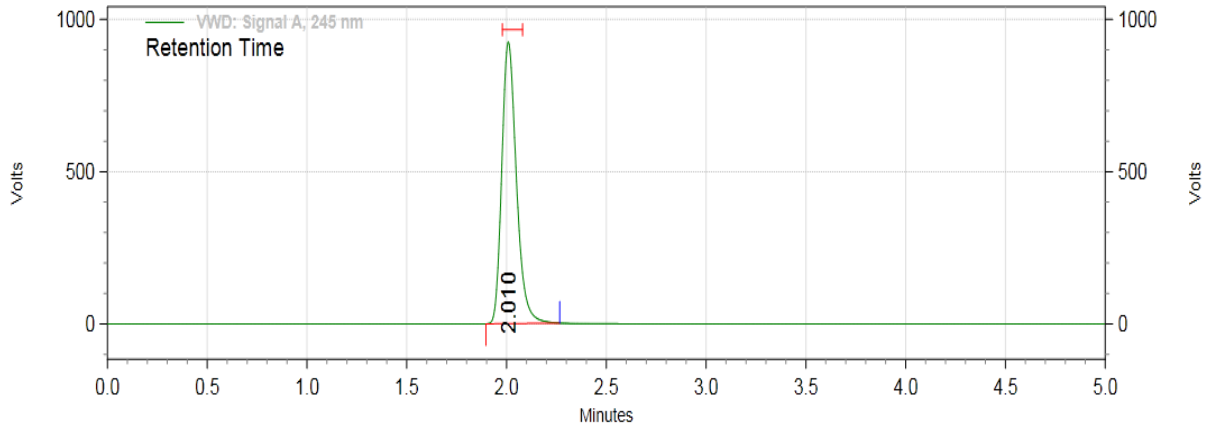

Fig. 4: Chromatogram of Acid Degradation

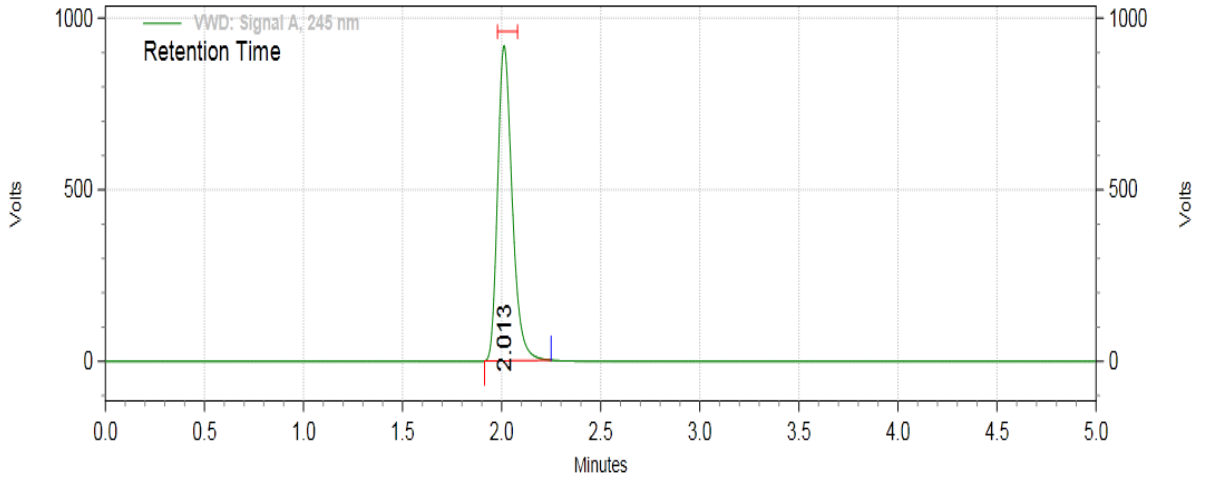

Fig. 5: Chromatogram of Alkali Degradation

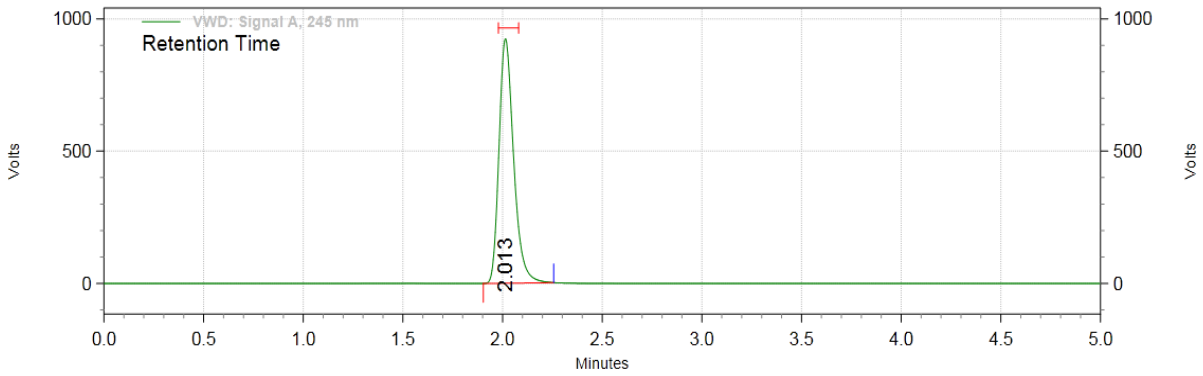

Fig. 6: Chromatogram of Peroxide Degradation

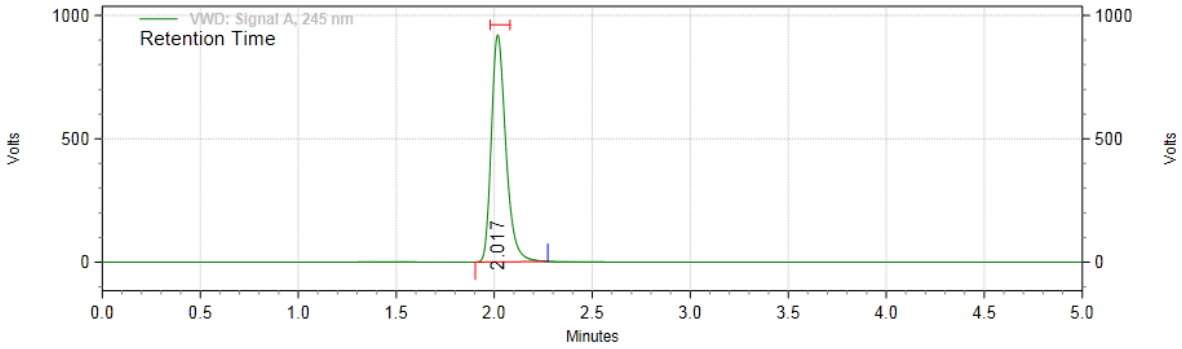

Fig. 7: Chromatogram of Thermal Degradation

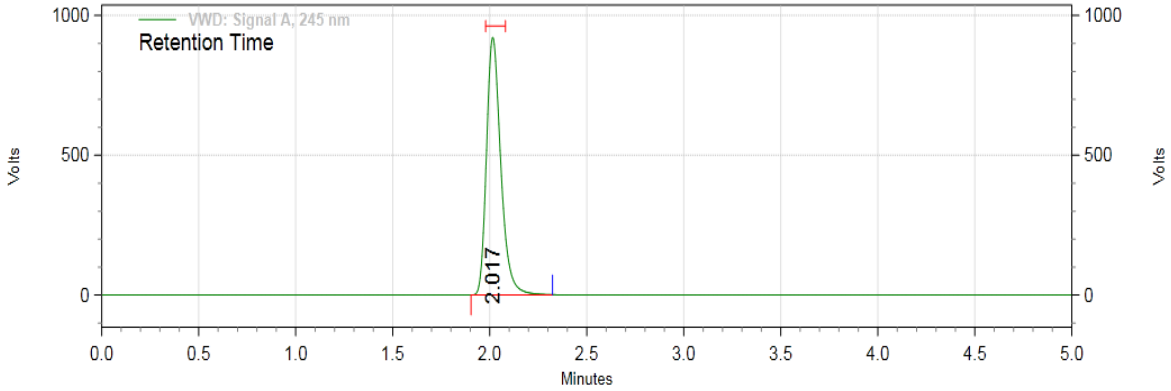

Fig. 8: Chromatogram of Photolytic Degradation

Table 6: Degradation data for Asenapine

Condition	%Assay	%Degradation	Peak Purity of main Peak
Acid Hydrolysis	97.23	2.17	0.99914
Base Hydrolysis	99.98	0.02	0.99980
Oxidation with Peroxide	99.45	0.55	0.99995
Thermal Degradation	98.40	1.60	0.99945
Photolytic Degradation	99.95	0.05	0.99965

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.

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REFERENCES:

1. Available from Drugs.com/ monograph/asenapine maleate.html(accessed on 28/1/12).
2. Available from url http://www.rxlist.com/saphris-... (accessed on 28/1/12).
3. Saphris®. Asenapine sublingual tablets. Full prescribing information. Schering Corporation, a subsidiary of Merck & Co. Inc 2010. White house Station, NJO. http://www.spfiles.com/pisaphr... [accessed January 2011].
4. Halima O. A, Aneesh T. P, Reshma Ghosh, Nathasha. R. Thomas, Development and validation of UV spectrophotometric method for the estimation of asenapine maleate in bulk and pharmaceutical formulation,Der pharma chemical, 2012,4(2):644-649.
5. Borkar AA and Gaikwad NJ: UV Spectrophotometric and RP-HPLC Estimation of Drug Asenapine in Tablet Dosage Form. Int J Pharm Sci Res 2016; 7(7): 3080-84.
6. Naga Rajan Govindarajan, Shirisha Koulagari , Archana Methuku ,Sravanthi Podhuturi, Method Development and Validation of RP-HPLC Method for Determination of New Antipsychotic Agent Asenapine Maleate in Bulk and Pharmaceutical Formulation, Eurasian J Anal Chem 2014;9(2):58–65.
7. Mohanam, V, Development and validation of Asenapine and its metabolite by Bioanalytical methods using liquid chromatography-Tandem mass spectroscopy(LC-MS).
8. Ambavaram Vijaya, BhaskarReddy, NandigamVenugopal, GajulapalleMadhavi Simultaneous determination of asenapine and valproic acid in human plasma using LC MS/MS: Application of the method to support pharmacokinetic study, Journal of Pharmaceutical Analysis Volume 3, Issue 6, December 2013, Pages 394-401.
9. ICH Q2A; guidelines on validation of analytical procedure; definitions and terminology, Federal Register 1995;60: 11260.
10. ICH Q2B; guidelines on validation of analytical procedure; Methodology, Federal Register 1996; 60: 27464.
11. International Conference on Harmonization (ICH) of Technical Requirements for the registeration Pharmaceuticals for Human use. Validation of Analytical Procedures Methodology. ICH-Q2 (R1) Geneva 1996: 1-8.