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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF ASENAPINE MALEATE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, selective, accurate reverse-phase high performance liquid chromatographic method has been developed and validated for quantitative determination of Asenapine maleate in bulk and pharmaceutical formulations. The chromatographic separation was achieved using X Terra RP 18 (100mm × 4.6mm × 3.5µm) in isocratic mode employing Buffer (KH_2PO_4 and Tetrabutyl ammonium hydrogen sulphate pH 2.2), Acetonitrile and methanol in the ratio of 80:16:4(v/v) with a 1.2 mL/min flow rate was chosen. Detector wavelength monitored at 228 nm. The retention time was 6.1 min. The developed method is validated as per ICH Guidelines. The method is accurate (99.9-101.1%), precise (the relative standard deviations of intra and inter-day assay were 99.3% and 99.9% respectively) and linear within range 80-240µg/ml (R2=0.999) concentration and was successfully used in monitoring left over drug. The proposed method is applicable to stability studies and routine analysis of Asenapine maleate in bulk and pharmaceutical formulations.

KEYWORDS

Asenapine maleate, RP-HPLC, Development and Validation.

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INTRODUCTION

Asenapine maleate (Figure No.1) is designated chemically[1] (3aRS,12bRS)-5-Chloro-2,3,3a,12b-tetrahydro-2-methyl-1Hdibenz[2,3:6,7]oxepino[4,5-c]pyrrole (2Z)-2-butenedioate. It is it is white to off-white powder with a molecular formulae $C_{17}H_{16}ClNO{\cdot}C_4H_4O_4$ and molecular weight 401.84. It is slightly soluble in water (3.7 mg/mL) and freely soluble in methanol, ethanol and acetone. Asenapine

maleate belongs to the class Atypical Antipsychotics[2] used mainly in the treatment of Schizophrenia[3] and Bipolar Disorder[3] and in Special Populations it is used for treatment of Hepatic Impairment. Asenapine maleate has unique receptor pharmacology[4] and it acts as potent dopaminergic (D1–D4), serotonergic (5-HT2A, 5-HT2C, 5-HT6 and 5-HT7), adrenergic (α1 and α2) and histaminergic (H1) activity, but it lacks significant anti-muscarinic activity.

Literature survey revealed that few analytical methods such as HPLC[5-8,] UV-spectrophotometric[9-11] methods have been reported for the estimation of Asenapine maleate in bulk and dosage forms. LC-ESI-MS/MS[12] methods have been reported for the Metabolism and Excretion of Asenapine maleate in human plasma. Aneesh and Reshma Ghosh studied Spectrophotometric Methods for the Determination of Asenapine maleate in Pure and Pharmaceutical Dosage Forms[9], Usmangani studied "Determination of Asenapine in bulk and in Pharmaceutical formulation by RP-HPLC[5]. Aneesh T.P.A.Rajasekaran studied Stress Degradation studies and development and validation of RP- HPLC[13] method for the estimation of Asenapine maleate. The present study is aimed at developing a rapid, simple, sensitive, precise and accurate RP-HPLC method for the validation of Asenapine maleate in bulk and pharmaceutical dosage forms by conducting systematic trails as per ICH[14] guidelines.

MATERIALS AND METHODS

MATERIALS

The analysis was performed by using the analytical balance Shimadzu Libror, pH meter Control Dynamics, the HPLC used is of Water 2695 with UV detector. Column used in HPLC is XTerra RP 18 (100mm × 4.6mm × 3.5µm). For Analysis Asenapine maleate was obtained as a gift sample from MSN Labs, Hyderabad. All the chemicals used were of analytical grade. Acetonitrile, Methanol KH_2PO_4 (Rankem), Tetrabutyl ammonium hydrogen sulphate, orthophosphoricacid (Qualigens), and HPLC grade water and 0.45µ PVDF membrane filters were used. The tablet formulations were procured from local market.

METHODS[15,16]

Reverse phase chromatography was employed in this method. Separation was achieved on XTerra RP 18 (100mm × 4.6mm × 3.5µm) by using waters HPLC with UV detector and controlled by Empower 2 software. Mobile phase was filtered and degassed mixture of Methanol, Acetonitrile and Buffer prepared by adding KH_2PO_4 and Tetrabutyl ammonium hydrogen sulphate, adjusted the pH to 2.2 with Orthophosphoric acid in the ratio 4:16:80 and diluent as Methanol and water (50:50). Other parameters such as n flow rate at 1.2 ml/min, detection at 228 nm and auto sampler temperature was set at 25°C. The total LC runtime was 10 min with 10 µL injection volume. Optimized chromatographic conditions are listed in Table No.1.

Standard solution preparation

Accurately weigh about 70 mg of Asenapine maleate reference standard and transfer it into a 50ml volumetric flask. Add 30 ml of diluent and kept in an ultrasonic bath until it dissolved completely. Make up to the mark with the Diluent and mix (stock solution). Further diluted 4 ml of the stock solution into a 25 ml volumetric flask and made upto the volume with diluent. Mixed well and filtered through 0.45µm filter and injected into chromotograph. This yielded solution of 160µg/ml concentration. This reference standard solution was analyzed using the HPLC instrument conditions mentioned (Figure No.2).

Sample preparation for determination of Asenapine maleate from dosage form

Twenty tablets (Welnuf) were procured, weighed and crushed to a fine powder. Powder equivalent to 100 mg Asenapine maleate was accurately weighed into a 250 ml volumetric flask and made up to volume with mobile phase. The contents of the volumetric flask were sonicated for 30 min to enable complete dissolution of Asenapine maleate. The solution was filtered and further dilutes 4 ml of the above solution into a 25 ml volumetric flask and made upto the volume with diluent. The filtrate was diluted with mobile phase. 10 µL of these solutions were injected into the system and the peak area was recorded from the respective chromatogram (Figure No.3).

Method Validation

Validation experiments were performed to demonstrate System suitability (Table No.2), precision, linearity, Accuracy study of analytical solution and robustness.

Linearity and Range

The Linearity of detector response is established by plotting a graph to concentration versus area of Amtolmetin guacil standard and determining the correlation coefficient. A series of solution of Amtolmetin guacil standard solution in the concentration ranging from about 80µg/ml to 240µg/ml level of the target concentration were prepared and injected into the HPLC system. (Figure No.4).

Accuracy

Accuracy for the assay of Amtolmetin guacil tablets is determined by applying the method in triplicate samples of mixture of placebo to which known amount of Amtolmetin guacil standard is added at different levels (80%, 100%, and 120%) (Table No.3).

Precision

The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. Method Repeatability % RSD of Asenapine maleate is 0.7%

Robustness

The robustness of the assay method was established by introducing small changes in the HPLC conditions which included wavelength (226 and 230 nm), percentage of Buffer(PH 2.2), Acetonitrile and methanol in the ratio of 79:17:4(v/v) and flow rate (1.0 and 1.2 mL/min). Robustness of the method was studied using six replicates at a concentration level of 160 µg/mL of Asenapine maleate and found satisfactory (Table No.4).

RESULTS AND DISCUSSION

The intensive approach described in this manuscript was used to develop and validate a liquid chromatographic analytical method that can be used for assay determination of Asenapine maleate in bulk and pharmaceutical dosage form. Asenapine maleate standard having concentration 100µg/ml was scanned in UV- region between 200- 400 nm. λmax of Asenapine maleate was found to be at 228nm. Satisfactory resolution was achieved with use of a mixture of Acetonitrile and potassium dihydrogen phosphate and Tetrabutyl ammonium hydrogen sulphate buffer with PH 2.2 with UV detection at 228 nm. C8 and C18 columns were first evaluated as stationary phase for the separation of Asenapine maleate but C18 column was adopted for the analysis as it has provided a better separation of the analytes. The Asenapine maleate peak in the sample was identified by comparing with the Asenapine maleate standard and the Retention time was found to be around 6.12±0.05 minutes. System suitability parameters such as RSD for six replicate injections were found to be 0.095%, theoretical plates - 8881.6, and tailing factor - 1.20. The acceptance criteria of System Suitability is RSD should be not more than 2.0% and the method show System Suitability 0.095% which shows that the method is repeatable. The acceptance criteria of Method Repeatability is RSD should be not more than 2.0% and the method show Method Repeatability 0.7% which shows that the method is precise. The validation of developed method shows that the drug stability is well within the limits. The linearity of the detector response was found to be linear from 80 to 240µg/ml of target concentration for Asenapine maleate standard with a correlation coefficient value is greater than 0.999. The correlation coefficient of (r2) = 0.999, which shows that the method is capable of producing good response in UV-detector.

Table No.1: Optimized chromatographic conditions

S.No	Parameter	Optimized Condition
1	Chromatograph Column	X Terra RP 18 (100mm × 4.6mm × 3.5µm)
2	Mobile Phase	Methanol, Acetonitrile and Buffer prepared by adding KH_2PO_4 and Tetrabutyl ammonium hydrogen sulphate, adjusted the pH to 2.2 with Orthophosphoric acid in the ratio 4:16:80
3	Flow rate	1.2 ml/min
4	Detection	UV at 228 nm
5	Injection volume	10µl
6	Column Temperature	25^0C
7	Runtime	10 minutes

Table No.2: System suitability parameters

S.No	Parameter	Asenapine maleate
1	Calibration range (µg/ml)	80-240
2	Theoretical plates	8881.6
3	Tailing factor	1.20
4	Correlation Coefficient(r2)	0.999
5	System Suitability %RSD	0.095%

Table No.3: Accuracy study of Asenapine maleate

S.No	Conc. (%)	Drug Added (µg/mL)	Drug found (µg/mL)	% Recovery
1	80	128.6	128.2	99.70
2	100	160.3	160.1	99.9
3	120	200.6	202.8	101.10

Table No.4: Robustness study of Asenapine maleate

S.No	Parameter	Condition	Mean peak area	% RSD	% Assay
1	Flow rate (mL/min)	1.0	1235352	0.58	99.98
		1.2	1235472		
		1.4	1235512		
2	Detection wavelength (nm)	226	1235499	0.77	99.80
		228	1235460		
		230	1235392		

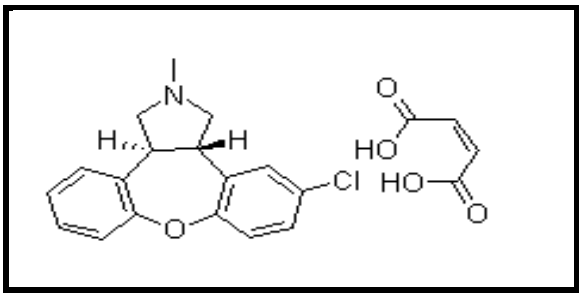

Figure No.1: Structure of Asenapine maleate

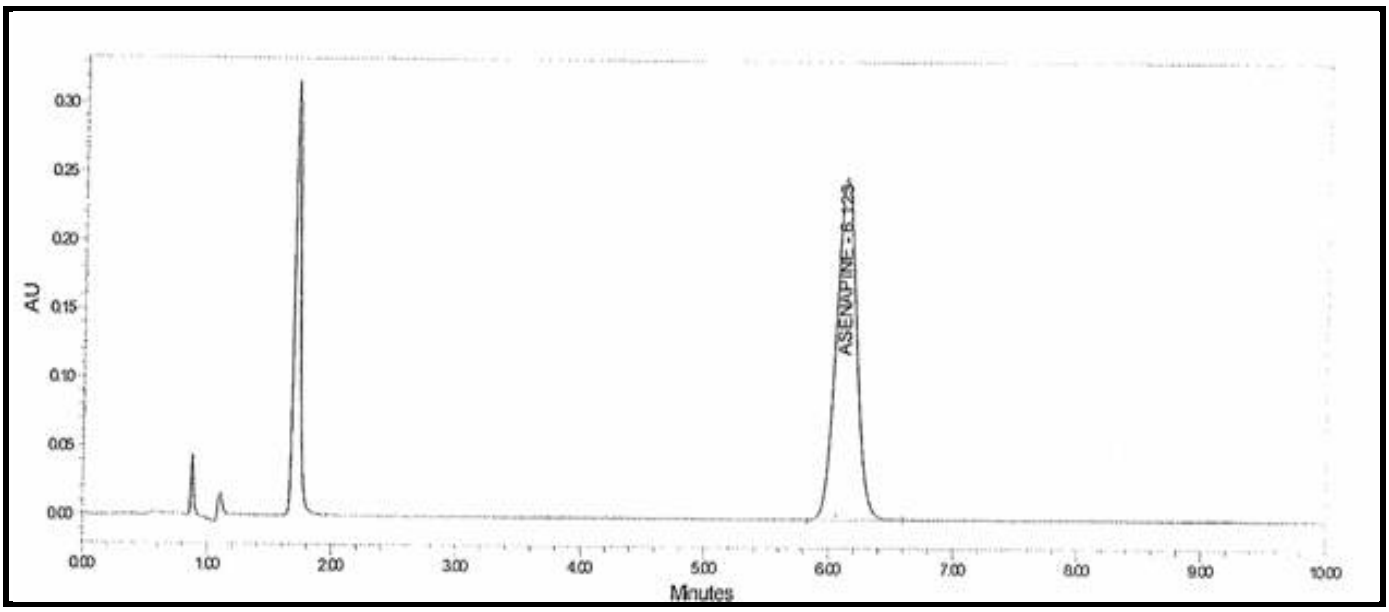

Figure No.2: Chromatogram of standard for Asenapine maleate

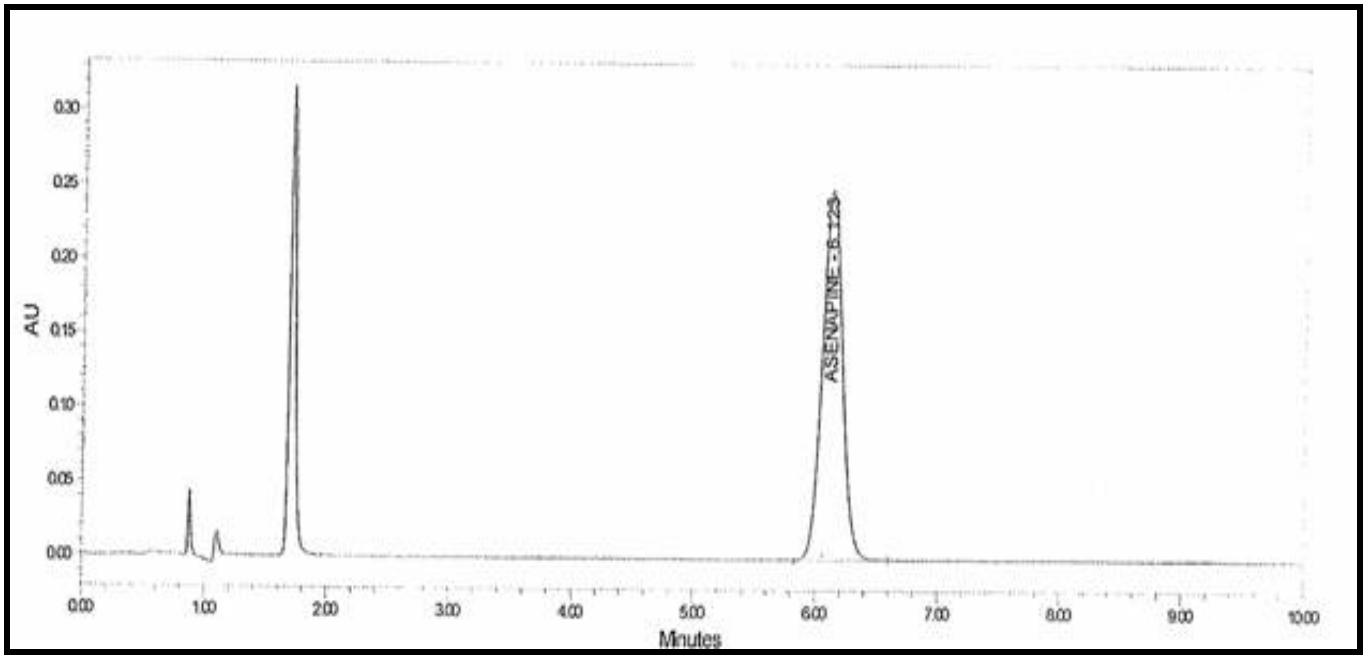

Figure No.3: Chromatogram of Sample (Welnuf) for Asenapine maleate

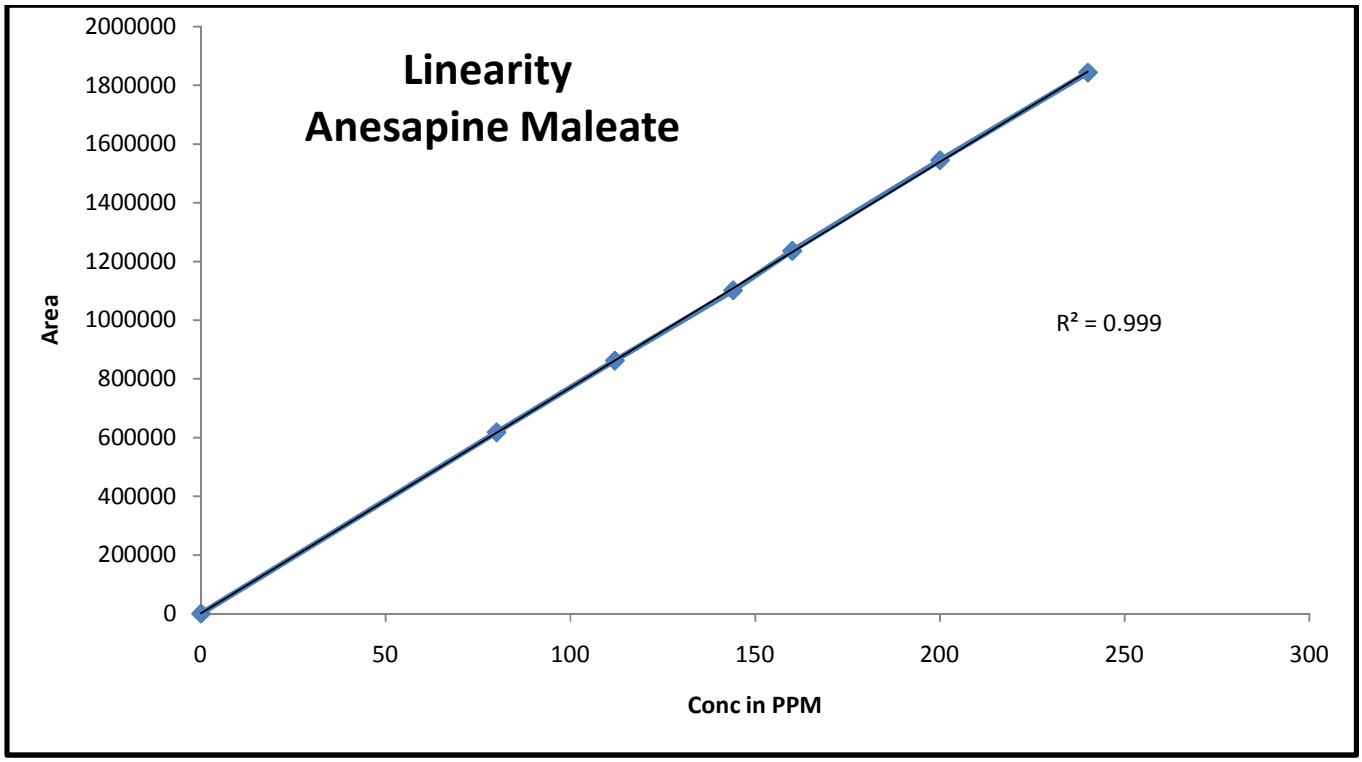

Figure No.4: Linearity of Anesapine Maleate

CONCLUSION

The validation study shows that the developed method is accurate, rapid, precise, reproducible and inexpensive with the acceptable correlation coefficient, RSD (%) and standard deviation which make it versatile and valuable. The advantages lie in the simplicity of sample preparation and the low costs of reagents used. The proposed method is simple and do not involve laborious time-consuming sample preparation the method was found to have a suitable application in routine laboratory analysis with a high degree of accuracy and precision.

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