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Der Pharma Chemica, 2012, 4 (2):644-649
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Development and validation of UV spectrophotometric method for the estimation of asenapine maleate in bulk and pharmaceutical formulation

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ABSTRACT

A simple, accurate, precise and sensitive UV spectrophotometric method was developed for the determination of Asenapine maleate in bulk and pharmaceutical dosage form. The solvent used is methanol and the wavelength corresponding to maximum absorbance of the drug was found at 270nm. Beers law was observed in the concentration range of 10- 60µg/ml with correlation coefficent 0.9997. The linear regression equation obtained by least square regression method were y=0.0132X-0.0016, where y is the absorbance and x is the concentration of the pure drug solution. The method was validated for several parameters like accuracy, precision as per ICH guidelines. The values of relative standard deviation and % recovery were found to be satisfactory, indicating that the proposed method is precise and accurate and hence can be used for the routine analysis of Asenapine maleate in bulk and pharmaceutical formulation.

Key words: Asenapine Maleate ,UV spectrophotometry, ICH.

INTRODUCTION

Asenapine maleate is chemically (3aRS,12bRS)—chloro-2-methyl-2,3,3a,12b-tetrahydro-1*H*-dibenzo [2,3:6,7]oxepino[4,5-c]pyrrole(2Z)-2-butenediate[1],[2]is a typical antipsychotic drug[2],[3] (**figure1**). It was approved by USFDA in Agust 2009. It is an antagonist at 5-HT , dopamine and α-adrenergic receptors and has high affinity for dopamine($D_{2)}$ and serotonin($5\text{-}HT_{2A)}$ receptors and its efficacy is mainly mediated through the combination of antagonist activity at D_2 and $5\text{-}HT_{2A}$ receptors. It is indicated for treatment of various psychotic conditions like schizoprenia and bipolar disorders in adults[4],[5],[6] and mainly works by controlling the psychotic symptoms through antagonism of selected dopamine and serotonin receptors in the CNS[7],[8].

Literature survey reveals that only an LC-MS study has been reported for the quantification of asenapine in human plasma.[9] No UV spectrophotometric method has been reported for the estimation of Asenapine. The objective of the present work was to develop a simple, sensitive, precise and accurate UV spectrophotometric method for the determination of Asenapine maleate in bulk and pharmaceutical formulations as per ICH Guidelines.

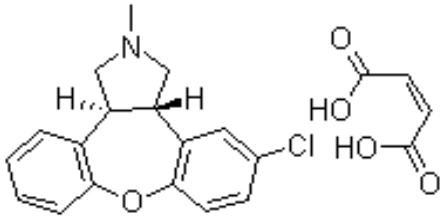


FIGURE 1: Structure of Asenapine Maleate

MATERIALS AND METHODS

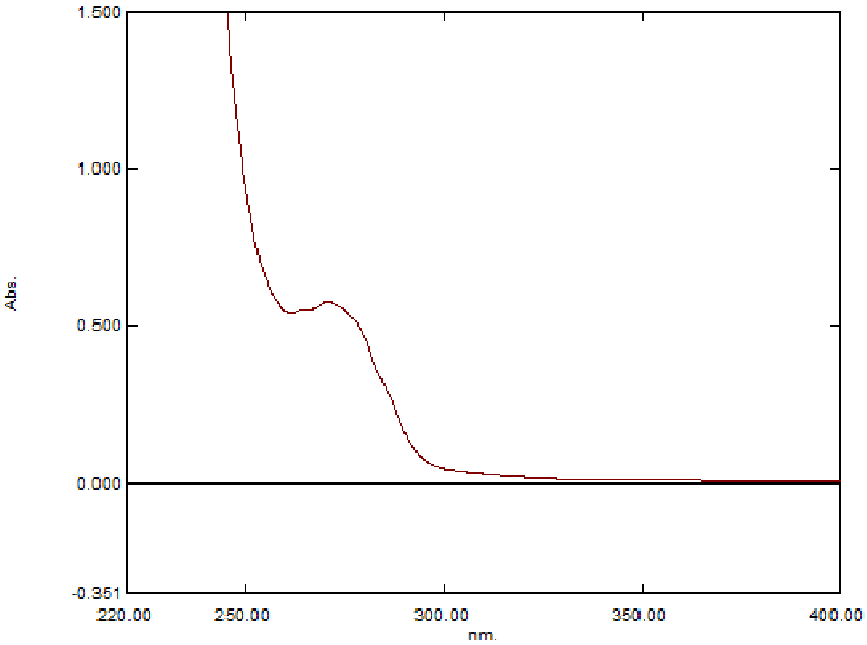
Instrumentation
A Shimadzu UV –Visible spectrophotometer model 1800 with 1cm matched quartz cells were used for measuring the absorbance.

Chemicals and reagents
Asenapine pure drug was obtained as a gift sample. Tablets of 10mg strength were procured from the local market under the commercially available brand name Asenapt. All the chemicals used were of analytical grade.

Determination of maximum wavelength (λmax)
Preparation of stock solution-Standard stock solution of Asenapine was prepared by dissolving accurately weighed 100mg of Asenapine in methanol in a 100ml volumetric flask to give a concentration of 1000µg/ml. From this, 10ml of the solution was transferred to a 100ml volumetric flask and made up the volume with methanol to give a concentration of 100µg/ml which is the standard stock solution.

From the above stock solution, pipetted out 2ml and 3ml in to 10ml volumetric flask and finally made up the volume with methanol to produce a concentration of 20µg/ml and 30µg/ml respectively. The samples was then scanned in UV spectrophotometer from a range of 200-400nm against methanol as blank and the wavelength corresponding to maximum absorbance in methanol was found at 270nm**(Figure 2)**

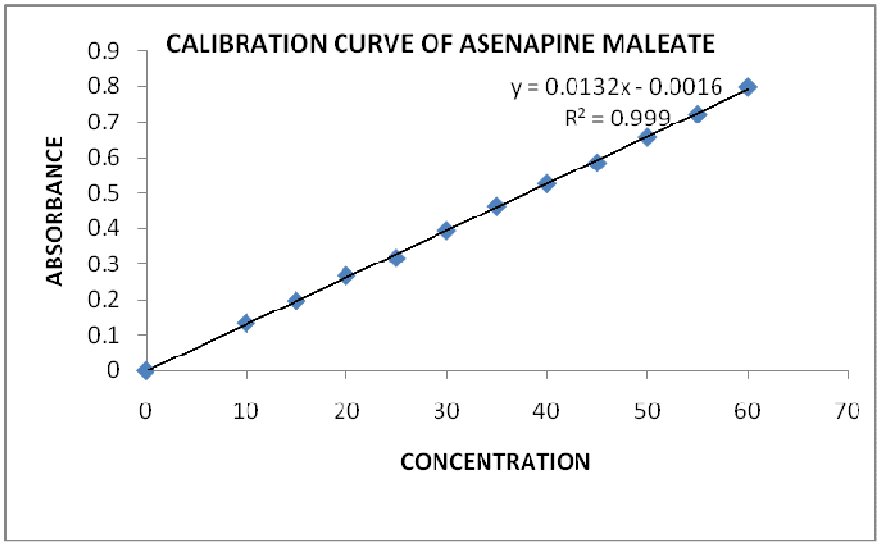
FIGURE2: UV Spectrum of Asenapine maleate in methanol



Preparation of standard calibration curve

For the preparation of standard calibration curve, concentration of 10-60µg were prepared by pipetting out 1,1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5 and 6ml from the 100µg/ml solution in to a 10ml volumetric flask and made up the volume with methanol. The absorbance of each solution was measured at 270nm against methanol as blank. Calibration curve of the drug was then plotted by taking the absorbance obtained on y-axis and the concentration of the solution on x-axis **(Figure3).** The curve showed linearity in the range of 10-60µg/ml with correlation coefficient 0.9997.

FIGURE 3: Calibration curve of Asenapine Maleate



VALIDATION

Validation can be defined as (ICH) Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics[10].

The method was validated for several parameters like linearity, accuracy, precision, Ruggedness, Robustness, Limit of detection(LOD), Limit of quantification(LOQ) according to ICH guidelines**.**

Linearity

The linearity of the analytical method was its ablity 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 analysed. The drug showed linearity in the range of 10-60µg/ with correlation coefficient 0.9997. Linearity data are shown in **Table1.**

TABLE 1:Linearity table of Asenapine

Concentration(µg/ml)	Absorbance
10	0.134
15	0.198
20	0.268
25	0.318
30	0.395
35	0.465
40	0.518
45	0.587
50	0.659
55	0.724
60	0.800

Precision

Precision studies were carried out to ascertain the reproduciblity of the proposed method. Repeatablity was determined by preparing six replicates of same concentration of the sample and the absorbance was measured. 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 was reported as %RSD. The precision result showed a good reproducibility **(Table 2)** with percent relative standard deviation less than 2. The results of intraday and interday precision studies are shown in (**Table 3 andTable4).**

TABLE 2: Precision results showing repeatability

Concentration (µg/ml)	Absorbance	Statistical Analysis
20	0.268	
20	0.268	Mean- 0.268
20	0.269	SD - 0.000753
20	0.267	% RSD- 0.28
20	0.268	
20	0.269	

TABLE 3: Intraday precision

Concentration(µg/ml)	Absorbance1	Absorbance2	Absorbance3	Average %RSD
20	0 .269	0.268	0.268	
20	0 .268	0 .269	0.267	
20	0 .268	0 .267	0.268	
20	0 .267	0 .269	0.265	
20	0 .266	0 .268	0.266	
20	0 .267	0 .266	0.266	
% RSD	0.42	0.43	0.48	0.44

TABLE 4: Interday precision

Concentration(µg/ml)	%RSD			Average
	DAY1	DAY2	DAY3	%RSD
20	0 .38	0.56	0.67	0.53

Accuracy

Accuracy of the proposed method was determined using recovery studies. The recovery studies were carried out by adding different amounts (80%,100%,120%) of the pure drug to the pre-analysed formulation. The solutions were prepared in triplicates and the % recovery was calculated. The results are shown in (**Table 5).**

TABLE 5: Accuracy readings of Asenapine maleate

Labelled claim (mg)	Level of Addition (%)	Amount of pure drug added (mg)	% Recovery	Statistical Analysis		
				MEAN	SD	%RSD
10	80	8	100.8			
10	80	8	100.0	99.93	0.9016	0.90
10	80	8	99.0			
10	100	10	101.2			
10	100	10	99.60	100.4	0.8	0.79
10	100	10	100.4			
10	120	12	98.10			
10	120	12	100.0	99.17	0.9724	0.98
10	120	12	99.44			

Ruggedness

Ruggedness was determined by carrying out analysis by two different analyst and the respective absorbance was noted and the results was indicated as % RSD **(Table6).**

TABLE 6: Results showing Ruggedness

Analyst 1		
Concentration(µg/ml)	Absorbance	Statistical analysis
20	0.268	
20	0.267	Mean- 0.2675
20	0.268	SD - 0.00104
20	0.266	% RSD-0.38
20	0.267	
20	0.269	
Analyst 2		
20	0.268	
20	0.267	
20	0.268	Mean – 0.2671
20	0.268	SD - 0.00098
20	0.266	% RSD – 0.36
20	0.266	

Robustness

Analysis was carried out at two different temperatures, room temperature and at 18^0c to determine the robustness of the method and the respective absorbance was measured. The results was indicated as %RSD **(Table7).**

TABLE7: Results showing robustness

Room temperature		
Concentration(µg/ml)	Absorbance	Statistical analysis
20	0.268	
20	0.267	Mean – 0.2673
20	0.268	SD - 0.000816
20	0.266	%RSD - 0.30
20	0.268	
20	0.267	
Temperature 18^0c		
20	0.267	
20	0.268	Mean – 0.2665
20	0.265	SD - 0.00104
20	0.266	%RSD – 0.39
20	0.267	
20	0.266	

LOQ and LOD

Limit of detection (LOD) is the lowest amount of analyte in the sample that can be detected. Limit of quantification (LOQ)is the lowest amount of analyte in the sample that can be quantitatively determined by suitable precision and accuracy. LOQ nd LOD was 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 values of LOQ and LOD were found to be 4.26 and 1.40µg/ml respectively.

The results of various parameters of the developed method are shown in **TABLE 8.**

TABLE 8: Summary of the method developed

Parameter	Result
Absoption maxima	270nm
Beers law range	10-60µg/ml
Correlation coefficient	0.9997
Regression equation	0.0132x-0.0016
Slope	0.0132
Intercept	-0.0016
Accuracy	98-101.2%
Precision (%RSD)	Intraday(0.44), interday(0.53)
LOD, µg/ml	1.40
LOQ,µg/ml	4.26

Quantification in dosage form

To analyse the concentration of drug in the pharmaceutical formulation, Twenty tablets were accurately weighed and powdered. Tablet powder equivalent to 100mg was accurately weighed and transferred to a 100ml volumetric flask, dissolved in methanol, sonicated, and finally made up the volume with methanol. The solution was centrifuged for the excipients to settle down and the resulting solution was filtered using whatsmann filter paper no1 .The solution was suitably diluted so as to obtain a concentration in the linearity range and the absorbance was measured at 270nm against methanol as blank. The result of analysis are shown in (**Table 9)**.

TABLE 9: Quantification in dosage form

Formulation	Label claim(mg)	Estimated amount of drug (mg)	% Labelled claim
Asenapt	10	9.84	98.4%

RESULTS AND DISCUSSION

The proposed method provides a simple , accurate, economical and convinient method for the analysis of Asenapine maleate using UV spectrophotometry. The wavelength corresponding to maximum absorbance in methanol was found at 270nm. Beers law was obeyed in the concentration range of 10-60µg/ml with correlation coefficient 0.9997. Acurracy of the proposed method was determined by the recovery studies, and good %recovery (98-101.2%) of the drug obtained indicate that the method is accurate. The method was found to be precise as %RSD values for interday and intraday was found to be less than 2. The method was also found to be rugged and robust as the % RSD values were found to be less than 2. The limit of detection and limit of quantification of the proposed method was found to be 1.40and 4.26 µg/ml indicating that the method developed is sensitive. The results of assay obtained was found to be in good agreement with the labeled claim, indicating the absence of interference of the excipients.

CONCLUSION

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

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