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METHOD DEVELOPMENT AND VALIDATION OF UV-SPECTROSCOPIC METHOD FOR ESTIMATION OF ASENAPINE MALEATE IN BULK AND TABLET FORMULATION

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ABSTRACT

The previous studies showed that UV method has not reported for Asenapine maleate in their pharmaceutical preparation. Therefore, the present study we developed UV method for the determination of Asenapine maleate in tablets. Asenapine maleate shows good solubility in organic solvents. Chemically asenapine maleate is (3aRS, 12bRS)-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 present work objective was to develop a validated UV Spectroscopic method for asenapine maleate drug in bulk form and in tablet dosage form. The suitable solvent selected for performing estimation of asenapine maleate by UV spectroscopic method development and validation and fixed the λ_{max} for the drug asenapine maleate. the present study successfully estimated the asenapine maleate from the formulation and performed validation studies of the drug asenapine maleate.

Keywords: Asenapine maleate, UV spectroscopic method, validation studies.

INTRODUCTION

Ultraviolet (UV) spectroscopy is a physical technique of the optical spectroscopy that uses light in the visible, ultraviolet, and near infrared ranges. The Beer-Lambert law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length. Thus, for a fixed path length, UV/VIS spectroscopy can be used to determine the concentration of the absorber in a solution. It is necessary to know how rapidly the absorbance changes with concentration.

Drug Profile

Asenapine maleate (Saphris, Sycrest- Merck) is atypical antipsychotics. the route of administration is Sub lingual. Solubility of the drug is freely soluble in methanol, acetone soluble in ethanol and slightly soluble in water. Asenapine maleate is used for the treatment of schizophrenia and bipolar mania. Exact mechanism of Asenapine and other antipsychotic agents in schizophrenia and bipolar disorder unknown; efficacy in schizophrenia may be mediated through a combination of antagonist activity at central dopamine type 2 (D₂) and serotonin type 2 (5-hydroxytryptamine [5-HT_{2A}]) receptors. Exhibits high affinity for serotonin 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT₅, 5-HT₆, and 5-HT₇ receptors; dopamine D₁, D₂, D₃, and D₄ receptors; α_1 - and α_2 -adrenergic receptors; and histamine H₁ receptors (moderate affinity for H₂ receptors). Asenapine acts as an antagonist at these receptors in vitro. Possesses no appreciable affinity for muscarinic cholinergic receptors or β -adrenergic receptors. Possesses no appreciable affinity for muscarinic cholinergic receptors or β -adrenergic receptors.

As enapine maleate is a novel psychopharmacologic agent belonging to the group dibenzoxepinopyrrolidine compounds. The active of as enapine maleate is as enapine. The proposed trade name for drug product is saphris. As enapine maleate exhibits high affinity and potency for blocking dopamine, seratotin, α -adrenergic and histamine receptors, and no appreciable activity at muscararinic and cholinergic

receptors. The molecule has two chiral centers but is being developed as the racemate. Polymorhs have been found. Asenapine maleate is the most stable form at ambient temperature as the drug substance. In addition Asenapine Maleate pseudopolymorphs were observed. Asenapine maleate has not yet been approved for marketing in any country [1-3].

Absorption: Rapidly absorbed in the sublingual, supralingual, and buccal mucosa following sublingual administration; peak plasma concentrations occur within 0.5–1.5 hours.

Volume of distribution: 70 L

Protein binding: 95% to plasma proteins (including albumin and α_1 -acid glycoprotein).

Half-life: Terminal phase half-life $(t_{{}^{1\!/}_2\!\beta})$ averages about 24 hours

Bioavailability: Administered sublingually because of the low bioavailability (<2%) and extensive first-pass metabolism observed following oral administration of tablets. Absolute bioavailability of sublingual tablets (5 mg) is 35%.

Metabolism: Metabolized mainly through direct glucuronidation by UGT1A4 and oxidative metabolism, primarily by CYP1A2 and, to a lesser extent, by CYP3A4 and CYP2D6.

Metabolites (primarily asenapine *N*-glucuronide, also *N*-desmethylasenapine and *N*-desmethylasenapine *N*-carbamoyl glucuronide) are largely inactive.

Chemical Formula: $C_{17}H_{16}CINO \cdot C_4H_4O_4$ Mol wt : 401.84

colour : A white to off-white powder

Chemical Structure

(3aRS,12bRS)-5-Chloro-2,3,3a,12b-tetrahydro-2-methyl-1dibenz[2,3:6,7]oxepino[4,5c]pyrrole(2Z)-2butenedioate(1:1).

Fig 1. Structure of Asenapine Maleate



MATERIALS AND INSTRUMENTS Drug Sample

The gift sample of pure drug asenapine maleate (Bulk powder) was received from MSN Labs, Hyderabad, India. The gift sample of asenapine maleate formulation (tablets) was received from MSN Labs, Hyderabad, India.

Chemicals and solvents

- 0.1N HCL
- Distilled Water

Instruments used

- SHIMADZU UV Pharmspec Spectrophotometer 1700
- SHIMADZU (ELB 300) Electronic balance
- SHIMADZU (BL 22OH)Electronic balance
- TOSHIBHA (India) Ultra sonicator.

METHODOLOGY

Method-A

Preparation of the stock solution

A standard stock solution containing 1000μ g/ml was prepared by dissolving 100 mg of asenapine maleate in 100ml 0.1N HCL. This stock solution used for further dilutions and by using analytical grade 0.1N HCL as solvent for estimation.

Preparation of the test solution

100 mg equivalent Asenapine maleate formulation was taken and dissolved in 100ml of 0.1N HCL by ultrasonication (for dissolving the excepients and unsoluble residual particles). Then from this 10ml solution was taken and diluted to 100ml by using 0.1N HCL. This gives concentration of 100 μ g/ml. From this solution the concentration of 10,20,30,40,50,60 μ g/ml concentration solutions were prepared [4-8].

Calibration curve preparation

Calibration curve or standard curve is a very important parameter for method validation and assay procedure for the substance. And this factor can be useful to estimate the assay value and drug content present in particular formulation of the drug. This calibration curve linearity can help to detect weather the proposed method is perfect or not.

Calibration curve data were constructed in the range of the expected concentrations of 10μ g/ml to 60μ g/ml. *Beer's* law was obeyed over this concentration range. The regression equation was determined by using

y =mX+C

Here C is concentration of analyte.

By this calibration curve we can analyse the linearity of the method and range of the method [9-13].

Procedure for the Estimation of asenapine maleate in Formulation

A commercial formulation of acenapine maleate (10 mg) tablet was obtained as gift sample from MSN Labs, Hyderabad, India. The content of 5 tablets were crushed and accurately weighed amount of the contents equivalent to 100 mg of asenapine maleate was transferred into 100 ml volumetric flask and make up the volume with 0.1N HCL and sonicate it for a while until the drug completely dissolved. The content of the flask was filtered through whatmann filter paper No.1 and 10 ml of the filtrate was diluted up to 100 ml with 0.1N HCL. And from this solution 1ml is made up to 100 ml and performs a series of dilutions from $10\mu g/ml$ to $60\mu g/ml$ and subjected to UV spectroscopy. A standard curve was prepared based on the measured absorbance values by keeping λ max of the drug as 269 nm.

Now from the calibration curve of standard asenapine maleate we can find the unknown concentration of formulation by interpolation method [14,15].

Assay Procedure

Weigh accurately about 5 tablets, powder and take 100mg equivalent quantity of Asenapine maleate and transfer into a 100ml standard flask. And dissolve the formulation in 0.1 HCL by using ultrasonication. Then pipette out 10ml of solution and make up to 100ml leads to 10μ g/ml concentration solution. This solution can be estimated in UV spectrophotometer by using 0.1 N HCL as blank at 269nm [16-19].

Percentage purity = test absorbance X dilution factor X 100 standardabsorbance

Method-B: Area under Curve Method

Area under curve method is applicable when there is no sharp peak and when broad spectra are obtained. Area calculation processing item calculation the area bound by the curve and horizontal axis. For the selection of analytical wavelength,100 μ g/ml solution of acenapine maleate was prepared by appropriate dilution of standard stock solution and scanned in the range of 200-400 nm. From the spectra of drug, area under curve in the range of 263-274 nm was selected for the analysis. The calibration curve was prepared in the concentration range of 10-60 μ g/ml at their respective AUC range. By using the calibration curve, the concentration of the sample solution can be determined by interpolation method.

RESULTS Method development parameters Selection of Solvent

Asenapine maleate is a highly lipophilic drug that is practically insoluble in water, soluble in 0.1N HCL, 0.1N NaOH ,methanol, ethanol, acetone. But it shows good solubility in 0.1N HCL and methanol. Among these, 0.1N HCL was found to be most suitable solvent for UV spectroscopic method than ethanol because of the linearity and reproducibility of the value.

Preparation of stock Solutions

A standard stock solution containing 1000μ g/ml was prepared by dissolving 100 mg of asenapine maleate in 100ml 0.1N HCL. This stock solution used for further dilutions and by using 0.1N HCL as solvent for estimation.

Fixing of wave length

After selecting the suitable solvent, the fixing of the λ_{max} for the proposed method is very important. This can be done by scanning the drug sample (ASENAPINE MALEATE) solution in 0.1N HCL in the range of 400nm-200nm and the most repeated maximum absorbance with linearity and repeatability can be fixed as λ_{max} for the drug. And in the proposed method for asenapine maleate drug shows maximum 269 nm, with more linearity, repeatability (ruggedness) and the λ_{max} was fixed as 269 nm shown in the (figure 4).

Figure 2. U.V. spectrum of ACENAPINE MALEATE drug (200-400nm)



CALIBRATION CURVE METHOD Table 1. Standard curve values

Concentration(µg/mL)	Absorbance
0	0
10	0.039
20	0.081
30	0.123
40	0.161
50	0.203
60	0.237

Calibration curve preparation

Calibration curve data were constructed in the range of the expected concentrations of $10\mu g/ml$ to $60\mu g/ml$ *Beer's* law was obeyed over this concentration range. The regression equation was found to be y = 0.004x + 0.001. The correlation coefficient (r) of the standard curve was found to be 0.999. The stock solutions and working standards were made in 0.1N HCL.

Linearity range and calibration curve is presented in Table 1 and Figure 3.

Figure 3. Standard curve of asenapine maleate



Assay Procedure

Weigh accurately about 5 tablets and take 100mg equivalent quantity of Asenapine maleate and transfer into a 100ml standard flask. And dissolve the formulation in 0.1N HCL by using ultrasonication. Then pipette out 1ml of solution and make up to 100ml leads to $100\mu g/ml$ concentration solution. This solution can be estimated in UV spectrophotometer by using 0.1N HCL as blank at 269nm. The percentage purity of the drug was found to be 99.53% w/w and amount drug was found to be around 78.87 \pm 0.218 mg/ tablet (Table 3).

 Table 2. Assay value of Acenapine Maleate

Drug	Label claim mg/tab	Amount found	%Purity
Asenapine maleate	10	9.95 ±0.21	99.5 %w/w

B.AREA UNDER CURVE METHOD

 Table 3. Area under Curve Studies of Asenapine maleate

Concentration(µg/ml)	AUC values
10	0.4586
20	0.8043
30	1.1559
40	1.6027
50	1.9531
60	2.391

Figure 4. AUC Calibration Graph of asenapine maleate



Analytical method validation

The data was statistically validated by means of least square regression method. The standard solution placed in room temperature in 0.1N HCL solution and degradation was not observed. Repeatability of values was observed over a period of 48 hours. The data was statistically validated by means of least square regression method. The detection and quantification limits were found to be 0.0122μ g/ml and 0.03832μ g/ml. The mean percentage drug estimated was 100.37 indicating the accuracy of the proposed analytical method

Beer's limit

The limits in which beers law obeyed is beers limit. In the UV method, accuracy, precision, ruggedness and robustness is should be within Beer's limit. And for asenapine maleate the beers limit range was found to be 10μ g/ml to 60μ g/ml. Within this range the drug showed accuracy, linearity, precision, ruggedness, robustness.

Molar Absorptivity

This is the important factor for determining the absorptive property of a drug in 1 mole concentration. And this value can be useful in determining the absorbance of drug in molar concentrations. This for identifying the shifts of the maximum absorbance of the drug during the method development. The molar absorptive of the asenapine maleate was found to be 6.39 x 10^{-3} L mole⁻¹ cm⁻¹.

Linearity

The linearity of the assay was determined by plotting standard calibration curves for the concentration range 10-60 μ g/ml at 269 nm using 0.1N HCL as solvent. The methods for estimation of asenapine maleate in 0.1N HCL was found to be linear in the range of concentrations 10-60 μ g/ml as suggested by the linear least square regressions (>0.999) of the standard curves.

Sample (µg/ml)	Intra-day precision	%RSD	Inter Day	%RSD
	0.102		0.105	
30	0.104	0.97	0.103	0.96
	0.103		0.104	
	0.210		0.215	
50	0.210	0.27	0.214	0.27
	0.211		0.214	

Precision and accuracy Table 4. Asenapine maleate intra-day and inter-day precision

RECOVERY STUDIES

Table 5. Recovery studies (accuracy parameter) of ASENAPINE MALEATE

Test µg/ml	Level	Amount of standard drug added µg/ml	%Recovery	Standard deviation	%RSD
	80%	8	99.8	0.141	0.141
10	100%	10	98.7	0.141	0.141
	120%	12	99.9	0.070	0.070

Method Validation Parameters of Asenapine Maleate

Table 6.	Characteristics	of Asena	pine maleate
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Parameters	Method-A	Methods-B
λ_{\max}	269 nm	264-275 nm
Beer's law limit	10-60 µg/ml	10-60 µg/ml
Molar absorptivity	$6.39 \text{ x } 10^{-3} \text{ L mole}^{-1} \text{ cm}^{-1}$	$6.39 \text{ x} 10^{-3} \text{ L} \text{ mole}^{-1} \text{ cm}^{-1}$
Regression equation $(Y = mx + c)$	y = 0.004x + 0.001	y=0.039x+0.0207
Slope (m)	0.004	0.039
Intercept (c)	0.001	0.0207
Correlation coefficient (r)	0.999	0.998
Relative standard deviation (%)	0.97	0.97
LOD Value	0.00122	0.00122
LOQ Value	0.03832	0.03832

LOD (Limit of Detection)

The LOD was found with in limit concentrations that 0.00122 $\mu g/ml.$

LOQ (Limit of Quantification)

LOQ (Limit of Quantification) Value is the minimum quantity of drug that can be quantified by the instrument and the value was found to be 0.03832 µg/ml.

Mean standard deviation for asenapine maleate at 80, 100 and 120 % was found to be0.141, 0.141 and 0.070 respectively. The low values of these statistical parameters validated the method. The value of mean percentage recovery was 97,96, 99.2 (Table 5) .This facts, together with satisfactory low values of statistical parameters, further validated the method. There was no interference of excipients in the estimation. The proposed method can be successfully employed in the routine analysis of acenapine maleate containing dosage.

DISCUSSION AND CONCLUSION

Asenapine maleate is a novel psychopharmacologic agent belonging to the group dibenzoxepinopyrrolidine compound.it exhibits high affinity and potency for blocking dopamine, seratotin, α -adrenergic and histamine receptors, and no appreciable activity at muscararinic and cholinergic receptors. The molecule has two chiral centers but is being developed as the racemate.

A method for the determination of asenapine maleate in the bulk drug and tablet formulation has been developed. From the spectrum of asenapine maleate as shown in Figure 2, it was found that the maximum absorbance is at about 269 nm in 0.1N HCL. A good linear relationship (0.999) was observed between the concentration ranges of 10-60 µg/mL. The assay of tablet was found to be 99.53%. The high percentage recovery indicates the high accuracy of the method. This demonstrates that the developed spectroscopic method is simple, accurate and reproducible. Stability of asenapine maleate is 72 hrs. The LOD of asenapine maleate was found to be0.00s122µg/ml& LOQ was found to be 0.03822µg/ml. Thus the developed method can be easily used for the routine quality control of asenapine maleate in bulk and tablet dosage form.

By AUC curve method asenapine maleate was estimated. From the spectra of drug, area under curve in the range of 263-274 nm was selected for the analysis. The calibration curve was prepared in the concentration range of 10-60 μ g/ml at their respective AUC range. By using the calibration curve, the concentration of the sample solution can be determined. LOD was found to be 0.01 μ g/ml and LOQ was found to be 0.03 μ g/ml.

CONCLUSION

This current investigation is intended to develop a method to determine the assay by U.V. Spectrophotometry

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and validate the method, for both bulk and formulation of asenapine maleate. This method involves direct analysis without any extraction steps, thus it is performed faster, simple and easier. And this method is shown accurate and précised results. By these results this method found to be rapid, simple, accurate, economic method for analysis and quality determination.ICH guide lines were followed throughout the study for method validation.