International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.8, No.2, pp 549-553, 2015

The Development And Validation Of A Spectrophotometric Method For A Novel Anti Psychotic Drug Asenapine Maleate

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Abstract: A simple, economic, and accurate spectrophotometric method was developed for the estimation of Asenapine Maleate (AM) in bulk dosage form. Phosphate Buffer pH 6.8 was used as a diluent. The absorptions were observed at two maxima (215 and 230nm), which were selected based on scanning spectra of AM. The linearity range was found to be 5 to 20µg/ml at 215 nm (r^2=0.992±0.0011), 230nm (r^2=0.996±0.0003) and ratio between absorbance at two wavelength (r^2=0.995±0.0014). The method was found to be simple, precise, accurate and rapid for the determination of AM in bulk dosage form.
Keywords: Asenapine Maleate, Phosphate buffer, Spectrophotometric analysis, absorbance ratio.

1. Introduction

Asenapine Maleate (AM) is atypical antipsychotics. Asenapine maleate is used for the treatment of schizophrenia and bipolar mania. Asenapine maleate is a novel psychopharmacologic agent belonging to the group dibenzoxepinopyrrolidine compounds. Asenapine maleate exhibits high affinity and potency for blocking dopamine, seratotin, α-adrenergic and histamine receptors, and no appreciable activity at muscararinic and cholinergic receptors[1]. Its molecular formula and molecular weight are $C_{17}H_{16}ClNO•C_4H_4O_4$ and 401.8g/mol, respectively. The chemical structure of AM is shown in Figure 1. Solubility of the drug is freely soluble in methanol, acetone soluble in ethanol and slightly soluble in water. Literature survey revealed that various analytical methods such as UV spectrophotometry[2-4], RP-HPLC[5-7] and GCMS[8] methods have been reported for the estimation of AM from its formulations and biological fluids.

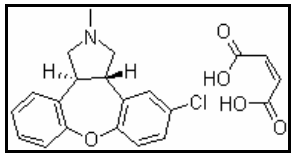

Figure 1: Structure of Asenapine Maleate

The scope of developing and validating an analytical method is to ensure a suitable method for a particular analyte to be more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation processes. A survey of literature revealed that analytical methods are not available for the AM in phosphate buffer, even though very few methods of estimation of this drug are available. Hence it is proposed to develop new methods for the assay of AM in pharmaceutical dosage forms adapting UV visible spectrophotometry. The objective of the study was to develop a simple and accurate method for the determination of AM by UV-spectrophotometry in pharmaceutical dosage forms.

2. Experimental

AM obtained from Apotex Research Pvt Ltd, Bangalore, India, were of analytical grade. Potasssium dihydrogen Phosphate (Qualigens fine chemicals, Mumbai, India) and sodium hydroxide (Qualigens fine chemicals, Mumbai, India) were of pharmaceutical or analytical grades. Quantitative estimation was performed on Labindia UV 3000+ (Labindia Maharashtra, India) and Elico SL 210 double beam UV visible spectrophotometers (Elico Pvt. Ltd, Hyderabad, India) with matched 1 cm path-length quartz cells. Absorption spectra was recorded on a fast scan speed, setting slit width to be 1 nm and sampling interval to be auto. Labindia UVWin software (Labindia, Maharashtra, India) was used along with quartz cuvette for the λmax prediction. To develop a suitable and robust method for the determination of AM, different diluents were tried based on the solubility and functional group present in the compound. Finally Phosphate buffer pH 6.8 solutions which is prepared by adding 50ml of 0.2 M potassium dihydrogen phosphate solution and 6.8ml of 0.2 M sodium hydroxide in a 200-ml volumetric flask and making it up to the mark with water, was selected due to its reproducible results. Absorbances were measured at 215 and 230nm based on the two maxima at scanning spectrum of AM. The data were collected and analyzed with LabIndia UVWin software, (Labindia, Maharashtra, India) in a computer system.

2.1.Preparations

Stock solution of AM (100 µg/mL) was prepared by dissolving 10 mg of drug in 100 ml of volumetric flask containing 40 mL of Phosphate buffer pH 6.8 . The solution was sonicated for about 15 minutes and then made up to volume with the buffer. From the stock solution, 1mL was pipetted out and transferred into the 10mL volumetric flask to get 10µg/mL of concentration. The final solutions of drug solutions were scanned and spectra obtained were analysed. From the spectrum, 215 and 230nm wavelength was selected. Then the absorbance was measured at 215 and 230nm and percentage content were calculated from the individual absorbance of 215 and 230nm and its ratio[9].

2.2.Validation

The described method has been validated for the estimation of AM using the following parameters[10]. Linearity was studied to find out the relationship of concentration with absorbance. Elevan different concentrations of AM drug (5 to 20µg/ml of drug) were employed i.e., 5, 7.5, 10, 12.5, 15 and 20µg/ml. All solutions were scanned and absorbance measured at 215 and 230nm. The calibration graph was constructed by plotting the absorbance versus the final concentration of the drug (µg/ml) and the corresponding regression equation derived. Precision was studied to find out variations in the test methods of drugs at 10µg/ml on the same day. On different days, the same solutions were scanned using different Instruments (Elico SL 210, Labindia UV 3000+) and ruggedness was determined. The precision of each method was ascertained separately from the absorbance obtained by actual determination of six replicates of a fixed amount of drug (10µg/mL). Precision and ruggedness were done on the same day and the different day respectively, and the %RSD was calculated for each. The accuracy of the method was shown by analyzing the model mixtures containing 7.5, 10, 12.5 µg/mL of sample solution of AM and along with 10 µg/mL of placebo solutions. After the measurement, the Amount found for AM and individual recoveries were calculated. Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated based on the linearity data using the formulae LOD = 3.3 X standard deviation /slope; LOQ = 10 X standard deviation /slope.

3. Results

A Spectroscopical method procedure was proposed as a suitable method for the analysis of drug AM in bulk dosage forms. A typical spectrogram of standard AM is shown in Figure 2. The two maxima were found to be 215 and 230nm. The regression equation for the method at 215 nm was found to be y = 0.0613x-0.02926 (r=0.992±0.0011), where y is absorbance, 0.0613±0.00204 is slope, -0.02926±0.017287 is intercept and r is regression coefficient and linear over Beer's range 5-20µg/ml. The regression equation for the method at 230 nm was found to be y = 0.02965x+0.01228 (r=0.996±0.0003), where y is absorbance, 0.0296±0.0001 is slope, 0.0122±0.00423 is intercept and r is regression coefficient and linear over Beer's range 5-20µg/ml. The regression equation for the method of absorption ratio at 215 and 230 nm was found to be y = 2.066x-2.426 (r=0.995±0.0014), where y is absorbance, 2.066±0.072 is slope, -2.426±1.097 is intercept and r is regression coefficient and linear over Beer's range 5-20µg/ml. The linearity graph of AM is shown in Figure 3.

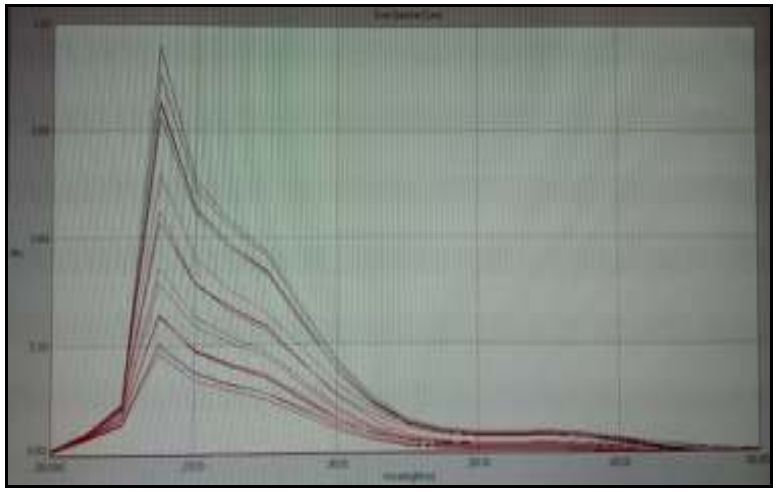

Figure 2: Spectrum of Azenapine Maleate.

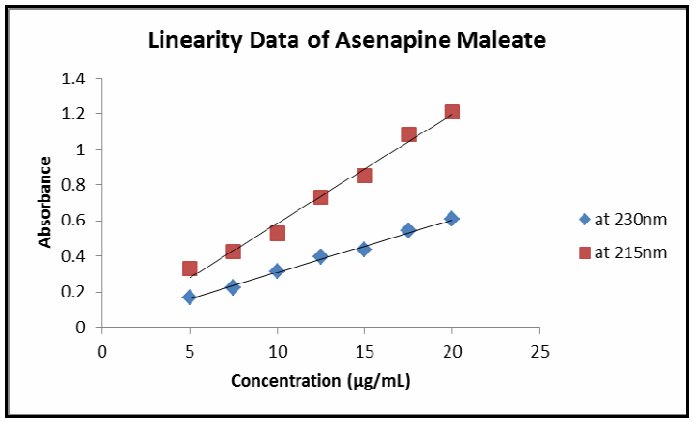

Figure 3: Linearity curve for Asenapine Maleate

The precision of the spectrophotometer system was determined using the % RSD of the absorbance for six replicate preparations of the drug. The %RSD was less than 2. Precision data are presented in Table 1. In order to verify the accuracy of the described method, recovery studies were carried out by analyzing model mixtures contained 7.5, 10 and 12.5 µg/ml of sample solution of drug AM and along with 10 µg/mL of placebo solution within the linearity ranges. The mean percentage recoveries at 215nm were found to be104.32±3.06, 100±2.422 and 108.13±0.717%w/w for 75% (7.5µg/ml), 100% (10µg/ml) and 125% (12.5µg/ml) respectively. The mean percentage recoveries at 230nm were found to be 94.56±0.85, 100±0.84 and 100.7±1.022%w/w for 75% (7.5µg/ml), 100% (10µg/ml) and 125% (12.5µg/ml) respectively. The ratio mean percentage recoveries at 215 and 230 nm were found to be 110.33±4.02, 100±2.54 and 107.38±1.43%w/w for 75% (7.5µg/ml), 100% (10µg/ml) and 125% (12.5µg/ml) respectively. The percentage content of AM was found to be 100±2.42, 100±0.84 and 100±2.54%w/w for 215nm, 230nm and its ratio respectively. Accuracy data are presented in Table 2. The percent recoveries values indicated less interference from excipients used in formulation. LOD and LOQ for AM at 215nm were found to be 0.929µg/ml and 2.817µg/ml respectively. LOD and LOQ for AM at 230nm were found to be 0.471µg/ml and 1.427µg/ml respectively. LOD and LOQ for AM at absorption ratio at two different wavelength were found to be 1.75µg/ml and 5.31µg/ml respectively.

Table 1: Data for Precision of AM

	Value at 215nm		Value at 230nm		Value of absorption ratio at 215 and 230nm	
Concentration (mcg/ml)	**Precision absorbance**	**Ruggedness absorbance**	**Precision absorbance**	**Ruggedness absorbance**	**Precision absorbance**	**Ruggedness absorbance**
10	0.524	0.52	0.31	0.34	1.690323	1.529412
10	0.516	0.534	0.314	0.35	1.643312	1.525714

10	0.541	0.54	0.315	0.345	1.71746	1.565217
10	0.53	0.528	0.321	0.348	1.65109	1.517241
10	0.535	0.518	0.32	0.347	1.671875	1.492795
10	0.52	0.521	0.318	0.342	1.63522	1.523392
Mean	0.527667	0.526833	0.316333	0.345333	1.668213	1.525629
SD	0.009438	0.008773	0.004131	0.003777	0.031398	0.023388
%RSD	1.788537	1.665245	1.305959	1.093762	1.88216	1.532998
Acceptance criteria	%RSD less than 2					
SD= Standard deviation, %RSD= percentage relative standard deviation						

Table2: Data for accuracy of Asenapine Maleate

At 215nm				
		Absorbance	**% Content**	**% Recovery**
Concentration (mcg/ml)	**Percentage Drug(%)**	**Mean ±SD**	**mean±SD**	**mean±SD**
7.5	75	0.412±0.012	78.24 ±2.295	104.32±3.060
10	100	0.527±0.0127	100±2.42	100±2.42
12.5	125	0.712±0.004	135.16±0.896	108.13±0.717
At 230nm				
		Absorbance	**% Content**	**% Recovery**
Concentration (mcg/ml)	**Percentage Drug(%)**	**Mean ±SD**	**mean±SD**	**mean±SD**
7.5	75	0.222±0.002	70.92 ±0.638	94.56±0.851
10	100	0.313±0.0026	100±0.845	100±0.845
12.5	125	0.394±0.004	125.87±1.277	100.7±1.022
Absorption ratio at 215 and 230nm				
		Absorbance ratio		**% ratio of Recovery**
Concentration (mcg/ml)	**Percentage Drug(%)**	**Mean ±SD**		**mean±SD**
7.5	75	1.857±0.070		110±4.2
10	100	1.683±0.042		100±2.54
12.5	125	1.808±0.024		107.38±1.43
SD=Standard deviation; n=3				

4. Discussion

The developed method can be used for routine analysis because the linearity found in AM is nearby to 1 that is 0.992 at 215nm; 0.996 at 230nm and 0.995 at ratio at 215 and 230nm which shows the good regression for linearity. Maximum recovery is obtained by this developed method and the mean percentage recoveries for each component are nearby 100% (within limit). So, method can be used for the routine analysis and one most important reason is that the developed method does not involve the use of expensive reagents. The spectrophotometric assay methods employed in our study indicated less interference from excipients used in formulation by the percent recoveries values. Most of the existing methods consumed expensive reagents for drug analysis. But the method we developed involves chemicals like sodium hydroxide and potassium di hydrogen ortho phosphate, and distilled water, which are very simple, economical and also easily available. And also our proposed method requires less time for the determination of AM compared to other methods, because, for other methods, as they involve the addition of reagents, they have to be kept aside for some time for the reactions to occur.

5. Conclusion

The presented method was found to be precise, sensitive and accurate. This method has simple drug preparation. The good recoveries and low coefficient of variation confirmed the suitability of proposed method for the routine analysis of AM in pharmaceuticals.

6. Acknowledgement

The authors wish to express their deep sense of gratitude to the INSPIRE, DST, Government of India, India for carrying out the work and providing necessary facilities.

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