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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF OLANZAPINE IN FORMULATED PRODUCT

Navya Khandelwal[1], Tapeesh Bharti[2*], Chatrasal Singh Rajput[2] and R P S Rathore[1]

[1]Department of Quality Assurance, B.N College, Udaipur, India.

[2]Jubilant Chemsys Ltd., B-34, Sector 58, Noida, U P India.

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***Correspondence for Author**
Tapeesh Bharti
Jubilant Chemsys Ltd.,
B-34, Sector 58, Noida,
U P India.

ABSTRACT

A novel approach was carried out to develop and validate a rapid and selective analytical method by using Reverse Phase Ultra Performance Liquid Chromatographic (RP-UPLC) technique for the analysis of Olanzapine in raw materials, their pharmaceutical dosage forms. The developed analytical UPLC method is superior in technology to conventional HPLC method with respect to speed, resolution, solvent consumption and cost of analysis. Elution time for the separation was 3.5 min in reverse phase mode and ultra violet detection was carried out at 254 nm. Efficient separation was achieved on Water Acquity BEH C18 column (50 × 2.1 mm, 1.7µm) UPLC column using 0.1% v/v TFA and acetonitrile as organic solvent in a gradient. The olanzapine sample was first dissolved in ethanol and the final dilution done by using water:acetonitrile (50:50 v/v) as diluent. The method showed excellent recoveries for all drugs in bulk. The test solution was found to be stable in diluent for 72 h when stored at RT (i.e 25 ^{o}C) . Recovery data were in the range 96.74 to 101.35%. The developed UPLC method was validated with respect to linearity, precision, accuracy, stability and specificity**.** The method was economical in terms of the time taken and the amount of solvent used. To the best of our knowledge, a validated reverse phase analytical method for the analysis of olanzapine by using UPLC technique disclosed in this investigation was not published elsewhere.

KEYWORDS: Olanzapine, UPLC, new method development, validation.

INTRODUCTION

Olanzapine is an atypical antipsychotic that belongs to thienobenzodiazpine class, used for the treatment of schizophrenia and bipolar disorder. Olanzapine has the chemical name 2-

Methyl-4-(4-methyl-1-piperazinyl)-10*H*-thieno[2,3-*b*][1,5]benzodiazepine (Figure 1). In literature many analytical methods are specified for the determination of Olanzapine. Some UV-spectrophotometry,[1] visible spectrophotometry,[2-9] kinetic spectrophotometry,[10] capillary zone electrophoresis and linear voltammetry,[11] Titrimetry[12] have been reported for the quantification of Olanzapine in pharmaceuticals. High performance thin layer chromatography (HPTLC) has been used to quantify Olanzapine in pharmaceutical. High performance liquid chromatography[13] (HPLC) with UV detection has been used for the determination of the drug in human blood serum and blood plasma.[14-19] The reported HPLC methods are more time consuming, complex mobile phase mixtures, use high flow rate of analysis, lack of sensitivity and peak symmetry. However there was some report available on the estimation of Olanzapine by UPLC method.[20] The purpose of the present study was to develop a simple, sensitive, accurate and precise and time-saving UPLC method for the determination of Olanzapine. The developed method has been validated by evaluation of the system suitability, specificity, linearity, limits of detection and quantification, precision and accuracy. Hence RP UPLC method was developed for the quantitative determination of olanzapine by using Water Acquity BEH C18 column (50 × 2.1 mm, 1.7µm) UPLC column.

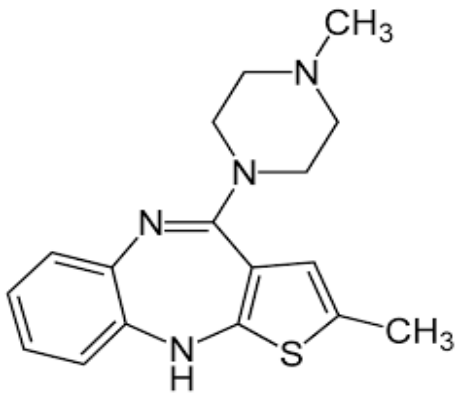

Fig.1. Structure of Olanzapine.

MATERIAL AND METHODS

Materials and Reagents

A well-characterized working standard of Olanzapine was procured from Jubilant Life sciences India (Purity 98.9 %). Commercially available Olanzapine purchased from local pharmacy. Ethanol, Trifluoroacetic acid and Acetonitrile HPLC Grade were obtained from Sigma Aldrich. Water was prepared by using Millipore Milli Q Plus water purification system.

Chromatographic conditions

Chromatography separation was performed on Waters Acquity UPLC with photodiode array detector. The output signal was monitored and processed using masslynx software. The chromatographic column is Water Acquity BEH C18 column (50 × 2.1 mm, 1.7µm). The mobile phase of 0.1% TFA and acetonitrile in the ratio 80:20v/v at a flow rate of 0.6 ml/min. The detection was monitored at the Wavelength of 254nm. The injection volume was 1.0µL and the chromatographic runtime of 3.5min was used.

Preparation of solutions

Preparation of Buffer

Buffer was prepared by dissolving 500 µl TFA in 500 ml Milli-Q water, mixed well, filter through 0.2µ 6,6 Nylon membrane filter paper.

Preparation of mobile phase

The mobile phase was prepared by mixing Buffer: Acetonitrile in a ratio (80:20) and filtered through 0.2µ 6, 6 Nylon membrane filter paper.

Preparation of the Olanzapine Standard & Sample Solution

Standard Solution Preparation

Weighed and Accurately transferred 101.12 mg of Olanzapine standard in a 100 ml volumetric flask and added a little quantity of ethanol then sonicated to dissolve it completely and the volume was made up to the mark with the water and Acetonitrile in a ratio (50:50). Further 5.00 ml of the above solution was diluted up to 20.00 ml the water and Acetonitrile in a ratio (50:50).

Sample Solution Preparation

Each 10 tablets containing 5mg of olanzipine were weighed and powdered. To prepare 250µg/ml concentration of sample solution, a quantity of powder equivalent to 5mg (86.98mg) was weighed accurately and transferred to 10.0 ml volumetric flask. The sample was initially dissolved in diluent (Ethanol) and then sonicated to dissolve it completely. The volume was made up to mark with the water and acetonitrile in a ratio (50:50) and filter through 0.2µm nylon filters. Then 5ml of this solution was further diluted to get the final concentration ratio of 250 µg/ml.

RESULTS AND DISCUSSION

Method Development

Different chromatographic conditions were experimented to achieve efficiency of the chromatographic system. Parameters such as mobile phase selection, wavelength of detection, column selection, column temperature optimization. Several proportions of buffer and solvents were evaluated in order to obtain suitable composition of the mobile phase. Choice of retention time and run time were the major tasks while developing the method.

Method validation

Based on International Conference on Harmonization (ICH) guidelines, the method is validated with regard to system suitability, linearity, accuracy, precision, robustness and sensitivity as follows.

Precision

The precision of the method was evaluated by carrying out six independent assay of test sample against a qualified reference standard and measurements of peak area response of standard preparation was found to be 0.33. The results are summarized in table 1 and Figure 2, Figure 3.

Table 1: Results of precision (System and Method)

S.No	Replicate	RT	Standard Area	Sample	Sample Area
1	Replicate-1	1.86	25106.35	Test-1	24041.41
2	Replicate-2	1.86	25153.34	Test-2	24020.58
3	Replicate-3	1.86	25232.52	Test-3	24080.83
4	Replicate-4	1.86	25215.81	Test-4	24156.37
5	Replicate-5	1.86	25303.04	Test-5	24035.55
6	Replicate-6	1.86	25320.80	Test-6	24106.00
	Average	1.86	25221.98		**24073.46**
	SD	0.00	83.15		**51.47**
	%RSD	0.00	0.33		**0.21**

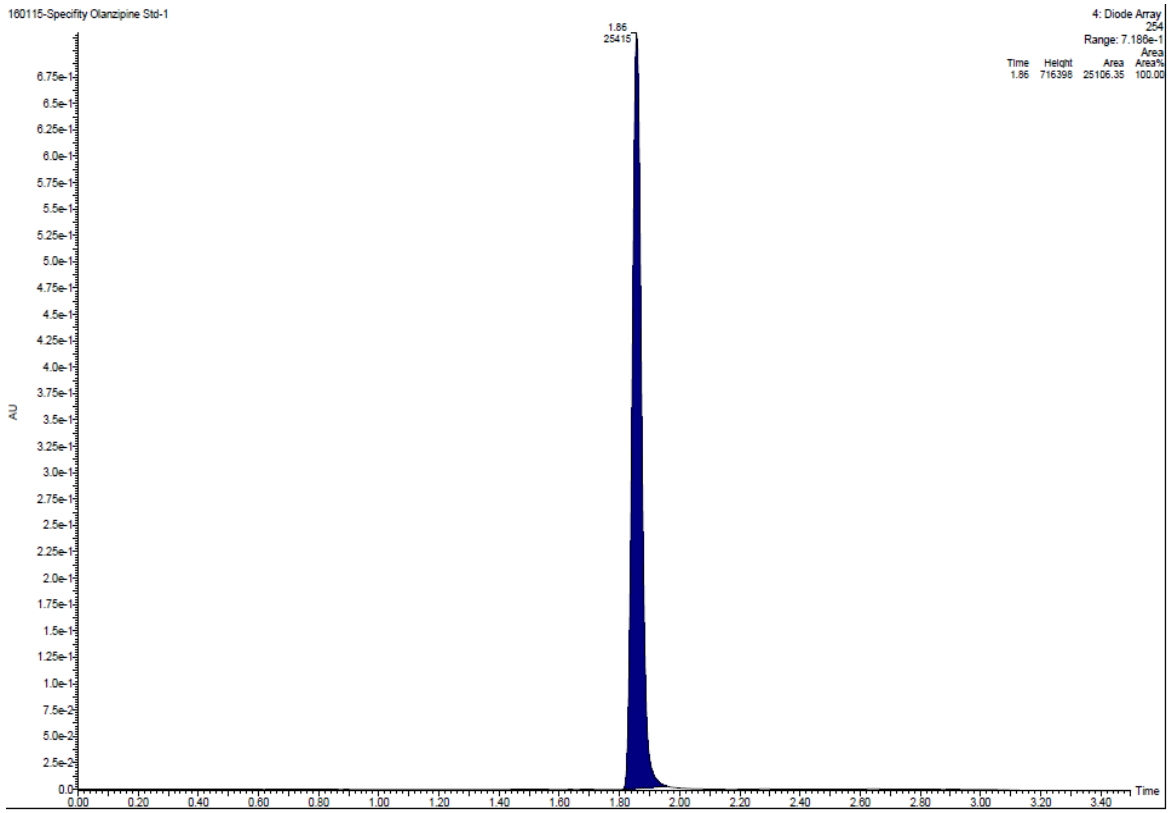

Figure 2: Chromatogram of Olanzapine Standard solution

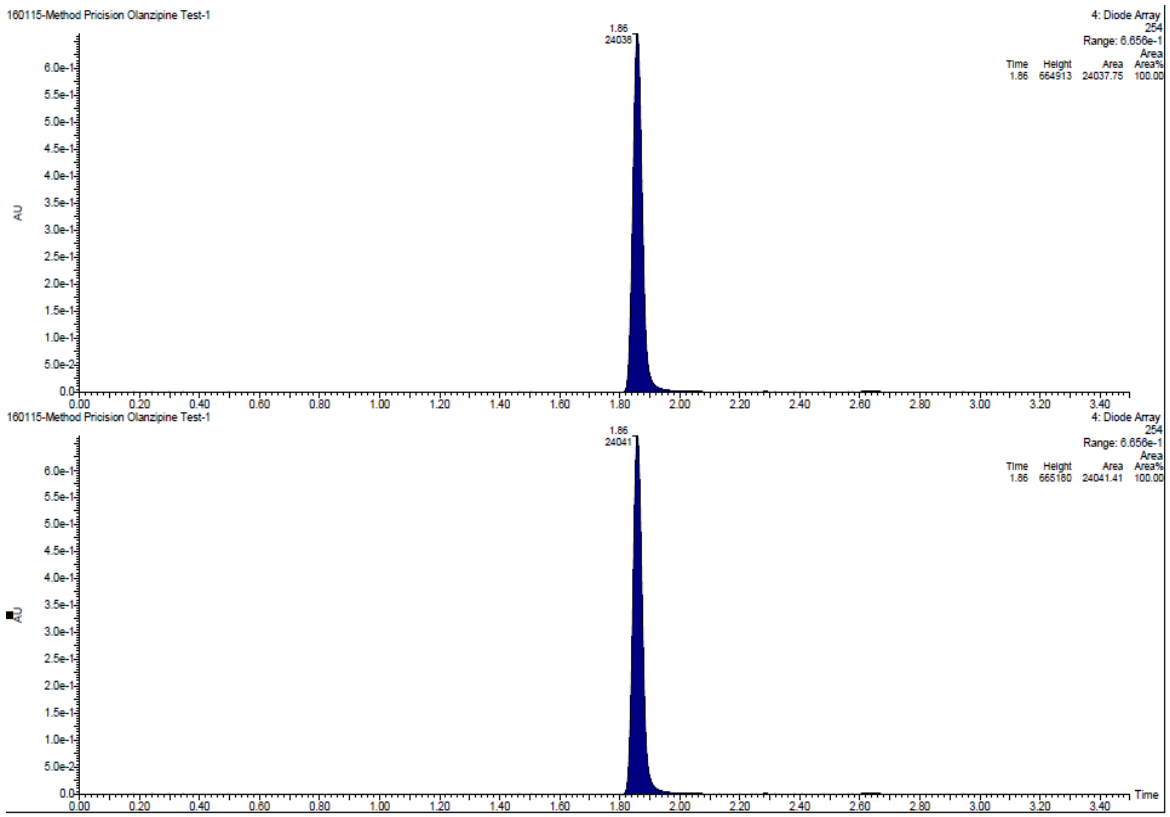

Figure 3: Chromatogram of Olanzapine Sample solution

Specificity

The specificity of the method was determined by comparing the chromatograms obtained from the sample containing Olanzapine standard stock with those obtained from test sample of olanzapine and blank of that. The specificity study revealed at the absence of interference of impurities with the drug since no extra peak appeared at the Retention Time of drug.The relative standard deviation for six replicate measurements of peak area response of standard preparation was found to be 1.16.

Reproducibility (Intermediate Precision)

This was performed by assaying the six samples of Olanzapine against qualified reference standard. The %RSD for the relative standard deviation for assay obtained from 6 precision samples was found to be 0.11 and peak area response of six replicate injections of standard solution of Olanzapine was found to be **0.23** %.

Accuracy

Recovery of the assay method for Olanzapine was established by three determinations of test sample using tablets at 50%, 100% and 150% of concentration. Each solution was injected thrice (n=3) into UPLC system and the average peak area was calculated from which Percentage recoveries. All the individual recoveries were found to be between 96.64 to 101.35%. All individual recoveries levels were found to be within 0.02 to 0.14% (%RSD). The results are summarized in table-3.

Table-3: Results of Accuracy

Level	Sample area	Average area	Sample Wt. (mg)	Amount added (µg)	Amount recovered (µg)	% Recovery	Average % recovery	SD	% RSD
50%	13430	13420	43.49	434.90	441.09	101.42	101.35	0.14	0.14
	13399				440.07	101.19			
	13431				441.12	101.43			
100%	26176	26166	86.98	869.80	859.71	98.84	98.80	0.07	0.07
	26145				858.69	98.72			
	26176				859.71	98.84			
150%	38389	38391	130.47	1304.70	1260.83	96.64	96.64	0.02	0.02
	38386				1260.73	96.63			
	38398				1261.12	96.66			

Linearity

Linearity was assessed in the range of 25, 50, 75, 100, 125 and 150 % of the working level concentration including working level concentration. All levels of linearity were carried out

in six replicates. Calibration curve was constructed by plotting the mean peak area versus concentration which was linear over the concentration range. The Linearity co-efficient of mean response of replicate determination plotted against respective concentration was calculated. The results are summarized in table-4 and Figure 4.

Table-4: Linearity Data

Level	Concentration (ppm)	Area
25%	62.5	5247.42
50%	125	11913.48
75%	187.5	18975.35
100%	250	25081.15
125%	312.5	32110.95
150%	375	39917.50

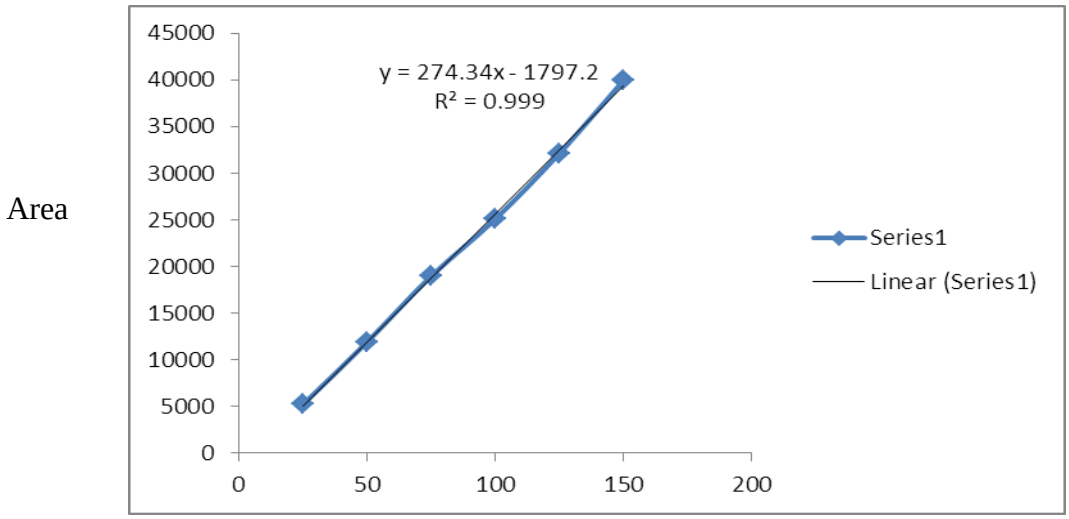

Figure 4

Stability of Solution

The % Cumulative RSD for standard area and % assay of sample at initial hr and at predetermined time intervals at room temperature was found to be **0.80.** The Assay method is also reproducible, gives stability up to 72 hrs.

Hours	Sample		RT	Area	Average Area	% Assay
0 Hr	1		1.87	26760	26755	99.6
	2		1.87	26751		
2.5 Hr	1		1.86	26620	26574	98.6
	2		1.86	26528		
Day 2	1		1.86	26355	26350	98.1
	2		1.86	26345		
Day 3	1		1.86	26268	26261	97.8
	2		1.86	26255		

Average			**1.86**		**26485**	**98.52**
SD			0.01			**0.78**
%RSD			0.01			**0.80**

Robustness

Robustness is the capacity of a method to remain unaffected by small deliberate variations in method parameters. Such as change in flow rate (±0.10 ml/min), buffer content (±10%), temperature (±5℃).

CONCLUSION

The new, gradient RP-UPLC method proved to be simple, linear, precise, accurate, robust, rugged and rapid. The developed method was capable of giving faster elution, maintaining good separation more than that achieved with conventional HPLC. The short retention time of 1.86 min allows the analysis of a large number of samples in a short period of time and is therefore more cost-effective for routine analysis in the pharmaceutical industries. It is suitable for rapid and accurate quality control of olanzapine in tablet formulations.

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