

METHOD DEVELOPMENT AND VALIDATION OF REVERSE PHASE-HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF OLANZAPINE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

Objective: To develop and validate reverse phase-high performance liquid chromatographic method for estimation of olanzapine in bulk and tablet dosage form.

Methods: Chromatographic analysis was performed on XTerra C18 (150×3.5 mm inner diameter, 5 μm) column using a mobile phase consisting of buffer (potassium dihydrogen phosphate) and methanol (45:55% v/v) with a flow rate of 0.6 ml/minutes. The detection was carried out at 247 nm.

Results: The calibration curve of olanzapine was linear in the range of 30-70 μg/ml. The mean % assay of marketed formulation was found to be 100.2%, and % recovery was observed in the range of 98-102%. Relative standard deviation for the precision study was found <2%.

Conclusion: The developed method is simple, precise and rapid, making it suitable for estimation of olanzapine in bulk and tablet dosage form.

Keywords: Olanzapine, Reverse phase-high performance liquid chromatographic, Validation.

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INTRODUCTION

Olanzapine is a second-generation neuroleptic drug approved by Food and Drug Administration used for the treatment of psychiatric patients suffering from schizophrenia to bipolar disorders. It is chemically (2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno-[2,3b] [1,2] benzodiazepine. As per the literature review, olanzapine was estimated by few methods like spectrophotometric [1-5] liquid chromatography-mass spectrometry (MS) [6,7], high performance liquid chromatographic (HPLC) [8,9] capillary zone electrophoresis [10] and gas chromatography-MS [11]. Therefore, there is a need for a reliable, sensitive and rapid method for its analysis in bulk and pharmaceutical preparations. The objective of the work is to develop reverse phase-HPLC (RP-HPLC) method for estimation of olanzapine in tablet dosage form with the simple, rapid, accurate and economical method and validated for system suitability, linearity, accuracy, precision, and robustness as per ICH guidelines [12]. The method has been satisfactorily applied to the determination of olanzapine in pharmaceutical preparations. Chemical structure of olanzapine is shown in Fig. 1.

METHODS

Instrumental and analytical conditions

Chromatographic separation was performed at ambient temperature on a reverse phase XTerra C18 column (150 mm × 3.5 mm i.d., 5 μm particle size). The mobile phase used in this analysis consists of a mixture of buffer and methanol in the ratio of 45:55. The mobile phase was filtered, degassed before use. The flow rate was adjusted to 0.6 ml/minutes, and the detector wavelength was set at 247 nm. The injector volume of standard and sample was 20 μl. The solution was injected and chromatograms were recorded.

Reagents and chemicals

All the chemicals and reagents used were HPLC grade. Potassium dihydrogen phosphate (AR grade) is used for preparing buffer solution and adjusting the pH to 5.4 with sodium hydroxide (AR grade), HPLC

grade methanol was used for mobile phase preparation. Commercial samples of olanzapine tablets were purchased from the local pharmacy store.

Preparation of mobile phase

Accurately weigh 2.0 g of potassium dihydrogen phosphate in 1000 ml volumetric flask and diluted to mark with HPLC water. Mix 450 ml (45%) of buffer and 550 ml (55%) of methanol and degassed in a digital ultra sonicator for 10 minutes and then filtered through 0.45 μ filter under vacuum filtration and adjust the pH to 5.4 with sodium hydroxide. The mobile phase was used as the diluent.

Preparation of standard stock solution

The standard stock solution was prepared by transferring 10 mg of olanzapine into 10 ml of clean dry volumetric flasks add about 7 ml of diluent and sonicate to dissolve and make the volume up to the mark with diluent. Further pipette out 0.5 ml of above olanzapine stock solution into 10 ml volumetric flask and dilute to the mark with diluent. The standard chromatogram is shown in Fig. 2.

Preparation of sample solution

A total of 20 tablets were weighed and crushed into a fine powder. The quantity of powder equivalent to 10 mg of olanzapine was accurately weighed and transferred into 10 ml volumetric flask, diluent was added to it and sonicate to dissolve for 10 minutes and filtered through 0.45 μ membrane filters. Then, the volume was made up to 10 ml with diluent. Further pipette out 0.5 ml of above olanzapine stock solution into 10 ml volumetric flask and makeup to the mark with diluent. Sample chromatogram is shown in Fig. 3.

Method validation

This method was validated according to ICH guidelines to establish the performance characteristics of a method to meet the requirements for the intended application of the method. They were tested using the optimized chromatographic conditions and instruments.

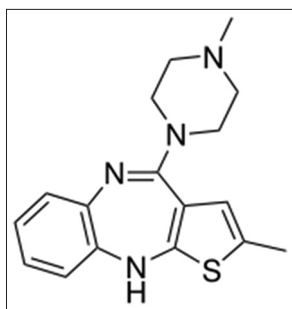


Fig. 1: Structure of olanzapine

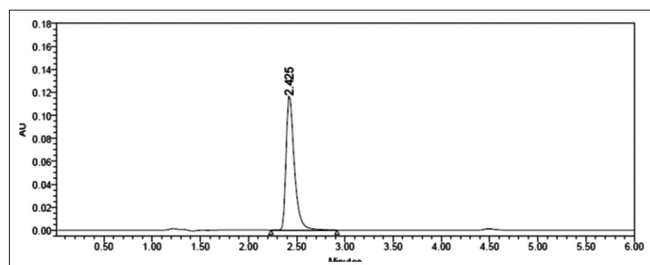


Fig. 2: Chromatogram of standard olanzapine

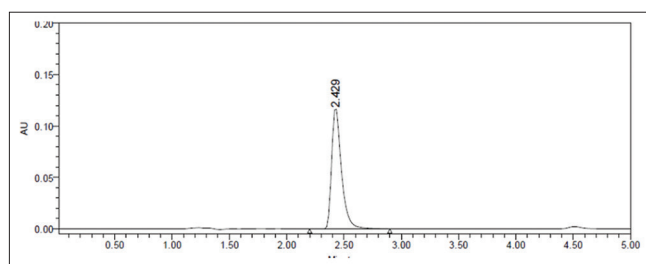


Fig. 3: Chromatogram of sample olanzapine

Specificity

It is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. Excipients that are commonly used were spiked into a pre-weighed quantity of drugs. Appropriate dilutions were injected into chromatographic system, and the quantities of the drugs were determined. The chromatogram did not show any other peaks, which confirmed the specificity of the method. The blank chromatogram is shown in Fig. 4.

System suitability

System performance parameters of HPLC method were determined by injecting standard solutions. Parameters such as a number of theoretical plates (N), tailing factor, and retention time were determined. From system suitability studies it is observed that % relative standard deviation (RSD) values are within the limit, i.e., not more than two which indicates good performance of the system. Chromatogram is shown in Fig. 2, and results are tabulated in Table 1.

Linearity

A series of solutions were prepared using olanzapine working standard solution at a concentration levels from 30 to 70 µg/ml and the peak area response of all solutions are measured. A graph was plotted against the concentration (µg/ml) on X-axis versus area/response on Y-axis. The detector response was found to be linear with a correlation coefficient of 0.999. Linearity results are tabulated in Table 2 and Fig. 5.

Precision

The precision of the method expresses the closeness of agreement between a series of measurement obtained from multiple sampling of

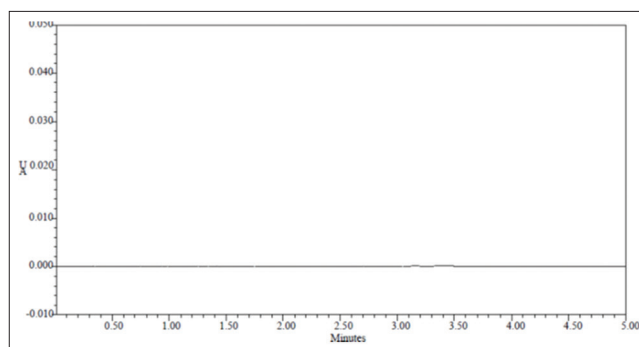


Fig. 4: Chromatogram of blank

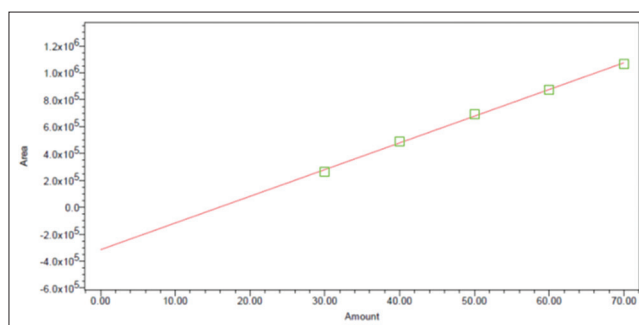


Fig. 5: Linearity graph of olanzapine

the same homogenous sample under prescribed conditions. Precision studies were performed, and the results are reported in term of RSD. The repeatability studies were conducted by estimating response of five different concentrations of olanzapine and reported in terms of % RSD. The results are tabulated in Table 3 and 4.

Accuracy (% recovery)

Accuracy of the method was determined by calculating the recovery of olanzapine by the spiked method. A known quantity of olanzapine was added to a pre-determined sample solution, and the amount of olanzapine was estimated by measuring peak areas. Mean % recovery values are within the limit (limit is 98-102%). Accuracy data were presented in Table 5.

Robustness

Robustness is a measure of its capacity to remain unaffected by small, but deliberate variation in the method parameters and gives an indication of its reliability during normal the robustness was performed for the flow rate variations from 0.5 ml/minutes to 0.7 ml/minutes and mobile phase ratio variation from more organic phase to less organic phase ratio for olanzapine. The method is robust only in less flow condition and the method is robust even by change in the mobile phase $\pm 5\%$. The standard and samples of olanzapine were injected by changing the conditions of chromatography. There was no significant change in the parameters such as resolution, tailing factor, asymmetric factor, and plate count. The method passed robustness test with well % RSD. Robustness data were presented in Table 6a and b.

Limit of detection (LOD)

Preparation of 50 µg/ml solution

Accurately weigh and transfer 10 mg of olanzapine working standard into a 10 ml clean dry volumetric flask add about 7 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (stock solution.) Further pipette out 0.5 ml of olanzapine of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent.

Table 1: System suitability parameters

S. No.	Peak name	RT	Area	Height (μ V)	USP plate count	USP tailing
1	Olanzapine	2.425	695226	117709	4159.0	1.5
2	Olanzapine	2.429	694341	115422	4059.3	1.4
3	Olanzapine	2.426	694434	117204	4059.3	1.5
Mean \pm SD			694667 \pm 486.6			
% RSD			0.07			

RT: Retention time, USP: United States of Pharmacopeia, SD: Standard deviation, RSD: Relative standard deviation

Table 2: Linearity of olanzapine

S. No.	Linearity level	Concentration (ppm)	Area
1	I	30	264840
2	II	40	491451
3	III	50	690307
4	IV	60	873311
5	V	70	1065958
Correlation coefficient			0.999

Table 3: Precision results for olanzapine

S. No.	Peak name	RT	Area (mV.s)	Height (mV)
1	Olanzapine	2.423	696977	117834
2	Olanzapine	2.424	696531	117366
3	Olanzapine	2.424	696214	117666
4	Olanzapine	2.424	695535	117576
5	Olanzapine	2.423	696708	117805
Mean \pm SD			696393 \pm 553.8	
% RSD			0.08	

RT: Retention time, SD: Standard deviation, RSD: Relative standard deviation

Table 4: Intermediate precision (ruggedness) results of olanzapine

S. No.	Peak name	RT	Area (mV.s)	Height (mV)
1	Olanzapine	2.423	693078	117646
2	Olanzapine	2.424	693338	117177
3	Olanzapine	2.424	695080	117534
4	Olanzapine	2.424	694843	117535
5	Olanzapine	2.423	695336	117665
Mean \pm SD			694335 \pm 1047.5	
% RSD			0.15	

RT: Retention time, SD: Standard deviation, RSD: Relative standard deviation

Preparation of 1.6% solution at specification level (0.08 μ g/ml solution)

Pipette 1 ml of 10 μ g/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluent. Further pipette 1.6 ml of above-diluted solution into a 10 ml of volumetric flask and dilute up to the mark with diluents.

Calculation of S/N ratio

Average baseline noise obtained from blank: 46 μ V

Signal obtained from LOD solution (1.6% of target assay concentration): 127 μ V

$$S/N = 127/46 = 2.76.$$

Acceptance criteria

S/N ratio value shall be 3 for LOD solution.

Limit of quantification (LOQ)

Preparation of 5.6% solution at specification level (0.28 μ g/ml solution)

Pipette 1 ml of 10 μ g/ml from above stock solution into a 10 ml of volumetric flask and dilute up to the mark with diluent. Further, pipette

Table 5: Accuracy results for olanzapine

% concentration (at specification level)	Area	Amount added (mg)	Amount found (mg)	% recovery	Mean recovery
50	1048287	15.0	15.20	101.38	100.2%
100	1376694	20.0	19.99	99.86	
150	1715480	25.0	24.88	99.49	

Table 6a: Robustness results for olanzapine

Flow rate (ml/min)	USP plate count	USP tailing
0.5	4187.6	1.5
0.6	4092.5	1.4
0.7	4084.7	1.4

USP: United States of Pharmacopeia

Table 6b: Robustness results for olanzapine

Change in organic composition in the mobile phase	USP plate count	USP tailing
5% less	4194.5	1.4
Actual*	4092.5	1.4
5% more	3097.0	1.4

*Results for actual mobile phase composition (45:55), Buffer: Methanol have been considered from assay standard, USP: United States of Pharmacopeia

5.6 ml of above-diluted solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Calculation of S/N ratio

Average baseline noise obtained from blank: 46 μ V.

Signal obtained from LOD solution (5.6% of target assay concentration): 463 μ V.

$$S/N = 463/46 = 10.0.$$

Acceptance criteria

S/N ratio value shall be 10 for LOQ solution.

CONCLUSION

In this investigation, a simple, sensitive, precise, and accurate RP-HPLC method was developed and validated for the quantitative estimation of olanzapine in bulk drug and tablet dosage form. This method was simple since diluted samples are directly used without any preliminary chemical derivatization or purification steps. Linearity was observed in the concentration range of 30-70 μ g/ml. The % RSD values were within two which indicate that the method was found to be precise. Specificity experiment shows that there is no interference of excipients with the main peaks, which confirmed the specificity of the method. The RP-HPLC method is more sensitive, accurate and precise compared to the spectrophotometric methods. This method can be used for the routine determination of olanzapine in bulk drug and in pharmaceutical dosage forms.

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