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OPTIMIZED AND VALIDATED RP-HPLC METHOD FOR THE DETERMINATION OF OLANZAPINE IN PHARMACEUTICAL FORMULATIONS

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Abstract: *A simple, precise, accurate, rapid and reproducible RP-HPLC method has been developed for the determination of Olanzapine in pharmaceutical formulations. Chromatography was carried out on a reverse phase C-8 column (150 x 4.6 mm x 5 µm length), optimum separation was achieved in 15 min using a mobile phase at a flow rate of 1 mL/min and the detection was done at 260 nm. The method produced linear responses in the concentration range from 45.2-135.6 µg/mL of Olanzapine with correlation coefficients of 1, accuracy of 99.80% and precision of 1.885%. The method was found to be reproducible for analysis of the drug in pharmaceutical formulations. The results of the analysis were tested and validated statistically for various parameters according to ICH guidelines and recovery studies confirmed the accuracy of the proposed method.*

Keywords: *Olanzapine, RP-HPLC, Development, Validation, Schizophrenia.*

Introduction

Olanzapine , chemically known as 2-methyl4-(4-methyl-1-piperazinyl)-10H-thieno [2, 3-b] [1, 5] benzodiazepine, is an atypical antipsychotic drug used in the treatment of schizophrenia and other psychotic syndromes [1]. Since its introduction in 1996 in over 84 countries, several workers have reported HPLC methods for the determination of Olanzapine in plasma, serum, human breast milk and rat brain [2-12]. A few methods have also been reported using HPLC.

In the literature, there are only a few methods described for the determination of Olanzapine in pharmaceutical formulations and include non-aqueous titrimetry and UV-spectrophotometry [13] , visible spectrophotometry [14-16], and flow injection spectrophotometry [17]. A few methods have also been reported using HPLC. An HPLC method [18] with UV detection at 260 nm has been carried out with a C18 column using a mobile phase consisting of acetonitrile and aqueous tetramethylammoniumperchlorate. But, the method is poorly accurate and precise with a relative error of 1.1% and RSD of 1.8%. The same report [18] also described three more methods, capillary zone electrophoresis, derivative spectrophotometry, and linear voltammetry. Simultaneous assay of Olanzapine and fluoxetine in tablets by HPLC and HPTLC [19] has recently been reported. The LC separation was achieved on a Lichrospher 100 RP-180 C 8 column (150 mm × 4.6 mm, 5 mm) using 0.05 M KH_2PO_4 buffer (pH 5.6 adjusted with acetonitrile (50 + 50, v/v) as the mobile phase at a flow rate of 1 mL/min and ambient

temperature. Quantification was achieved by measuring UV absorption at 233 nm over the concentration range 10-70 m g/mL. The HPTLC method [20] for Olanzapine has been applied for chromatographic purity only. The reported HPLC methods are either less sensitive or have narrow linear dynamic concentration range.

The purpose of the present study is to develop a simple, sensitive, accurate and precise and time-saving HPLC method for the determination of Olanzapine in pharmaceutical formulations. The developed method has been validated by evaluation of the system suitability, specificity, linearity, limits of detection and quantification, precision and accuracy. The current method may be applied to the commercially available pharmaceutical formulations containing Olanzapine.

Materials and Methods

Drugs and Chemicals: HPLC grade acetonitrile (Origin:Merck, German) and Monobasic sodium phosphate (Analytical reagent grade, Scharlau, Spain), Sodium dodecyl sulphate(Analytical reagent grade, Scharlau, Spain), water for HPLC (Origin:PALL life sciences, India) were used for preparing the mobile phase. All other reagents used were of HPLC grade. Pure Olanzapine (Glenmark pharmaceuticals Pvt. Ltd. ;India) used as working standard without further purification. A commercial Olanzapine tablet was purchased from local market.

Instruments: A SHIMADZU SPD-20Av uv-visible detector model, an ODS reverse phase column (150 x 4.6 mm x 5 µm length), SIL 20 AC HT autosampler, CTO-10 AS vp column oven, LC-20 AT isocratic single pump with software LC solution of version 1.2 high pressure liquid chromatographic instrument was employed in the study.

Preparation of Mobile Phase

Preparation of buffer solution 1: 6.9 g of monobasic sodium phosphate was dissolved in 700 mL of water in a 1000-mL volumetric flask. Finally, water was added to make the final volume 1000 mL and pH was adjusted to 2.5 with phosphoric acid.

Preparation of buffer solution 2: 12 g of Sodium dodecyl sulfate was dissolved in 1000 mL of buffer solution 1.

Mobile Phase: A freshly prepared 50:50 v/v mixture of acetonitrile and Buffer solution 2 was used as the mobile phase. Mixed them and filtered through a filter having a nominal pore size not greater than 0.45 μm.

Preparation of standard stock solution: About 50 mg of Olanzapine working standard was dissolved in mobile phase and diluted up to 50 mL. Filtered and filtrate was collected.

Preparation of Analytical Standard Solution: 5 mL of this solution was diluted to 50 mL with mobile phase to make the concentration 100 µg/mL. Filtered through a filter having a nominal pore size not greater than 0.45 μm.

Preparation of sample solution: Twenty tablets were weighed accurately and grounded into fine powder. An amount of the powder equivalent to standard solution of Olanzapine was weighed into a 100 mL volumetric flask and 60 mL of mobile phase was added. Mixed for 15 minutes in an ultrasonic bath. Cool the sample to room temperature. Finally, mobile phase was added to make the volume 100 mL. The sample solution was further diluted with mobile phase to get required concentration. Filtered through a filter having a nominal pore size not greater than 0.45 μm. All solutions were stored at room temperature; these solutions were shown to be stable during the period of study.

Validation of the developed method: The developed method for the determination of Olanzapine was validated as per ICH guidelines (ICH 2005)[21].

System Suitability Test: Before starting validation parameters, System Suitability must be established by injecting 20 μL each for six replicate injections of system suitability solution prepared as analytical standard solution. Using six peak areas, Relative Standard Deviation (RSD %), mean tailing factor were calculated (Table 1).

Linearity and Range: Appropriate dilutions of standard stock solution (50-150µg/ml) were assayed as per the developed method for Olanzapine. To establish linearity of the proposed method, seven separate series of solutions of Olanzapine were prepared from the stock solutions and analyzed (Table 2).

Precision: Precision was done by (i) repeatability or intra-assay precision and (ii) intermediate precision.

i) **Repeatability (intra-assay precision):** Repeatability was determined from six test samples by injecting 20 μL of each sample. Duplicate injection was made for each concentration level (Table 3).

ii) **Intermediate precision:** A second analyst performed the same experiment as repeatability experiment. For determination of method precision, analyst 1 repeatability (n=6) was combined with analyst 2 precision (n=6) and expressed as method precision (n=12) (Table 4).

Accuracy: To check the accuracy of developed method and to study the interference of formulation additives analytical recovery experiments were carried out by standard addition 80%, 90%, 100%, 110% and 120% of the label claim. Accuracy was conducted by adding known amounts of Olanzapine to the sample matrix and five different concentrations of test sample were prepared. Duplicate injections were made for each concentration level (Table 5).

Robustness: The robustness of this validation was conducted by changing two different parameters (Temperature: 30◦C and 40◦C and Flow rate: 1 ml/min and 1.2 ml/min) of the method by using the same concentration of test sample of repeatability sample (Table 6).

Specificity: Specificity is determined by injecting separately blank, placebo, standard and sample solution of Olanzapine in duplicate (Fig 2, 3, 4, 5).

Results

System suitability Test

Table 1: System Suitability Test

Replicate	Peak area	Tailing Factor	%RSD		Tailing Factor		Pass/Fail
			Limit	Results	Limit	Results	
1	5712184	1.230	NMT 2.00	**0.038**	NMT 1.50	**1.22**	**Passed**
2	5715450	1.226					
3	5709523	1.221					
4	5712043	1.216					
5	5714180	1.211					
6	5710614	1.207					

Linearity and Range

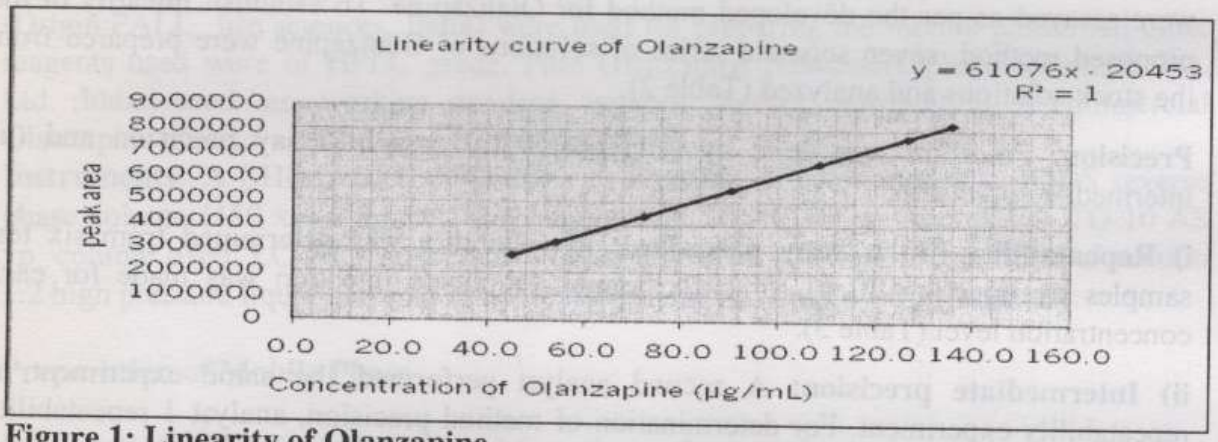

Figure 1: Linearity of Olanzapine

Table 2: Linearity and Range

% of Nominal value	Conc. of Std(µg/mL)	Peak area	Statistical Analyses	Pass/Remark
50%	45.2	2746550	Regression correlation coefficient (R^2)= 1 y-intercept = -20453 Slope of regression line = 61076	**Passed**
60%	54.2	3292143		
80%	72.3	4391145		
100%	90.4	5489316		
120%	108.5	6618909		
140%	126.6	7701134		
150%	135.6	8266544		
Lower limit of quantitation (LLOQ)				45.20 µg/mL
Upper limit of quantitation (ULOQ)				135.6 µg/mL

Precision

Table 3: Repeatability

Sample	Peak area of Sample	Average peak area of Sample	Assay, (mg/Tablet)	%RSD
1	6210988	6206629	3.84	1.884
	6202270			
2	5731741	5732712	4.00	
	5733682			
3	6098790	6097156	3.89	
	6095521			
4	6016882	6016796	4.00	
	6016709			
5	5804946	5803133	3.85	
	5801319			
6	5526234	5524071	3.97	
	5521908			
Average of Assay			3.98 mg	

Intermediated Precision

Table 4: Intermediated Precision

Analyst-1				Analyst-2		
Sample	Peak area of Sample	Average peak area of Sample	Assay, (mg/Tablet)	Peak area of Sample	Average peak area of Sample	Assay (mg/Tablet)
1	6210988	6206629	3.84	6506400	6504608	3.97
	6202270			6502816		
2	5731741	5732712	4.00	5940998	5940944	4.09
	5733682			5940890		
3	6098790	6097156	3.89	6361214	6362649	3.96
	6095521			6364084		
4	6016882	6016796	4.00	6232675	6234575	3.92
	6016709			6236474		
5	5804946	5803133	3.85	6035833	6037665	3.87
	5801319			6039496		
6	5526234	5524071	3.97	5843028	5841909	3.98
	5521908			5840790		
	RSD for analyst-1		1.884 %	RSD for analyst-2		1.852 %
RSD for 12 sample			1.885%			

Accuracy

Table 5: Accuracy

% of Nominal Value	Peak area of Sample	Average peak area of sample	%Recovery
80%	4862665	4827127	98.24
	4791588		
90%	5471860	5472486	100.50
	5473112		
100%	6186036	6186991	101.35
	6187945		
110%	6667173	6666792	98.74
	6666411		
120%	7215523	7217114	100.19
	7218704		
Mean			99.80%
RSD			1.28%

Robustness

Table 6: Robustness

Temperature (°C)	Flow rate(mL/min)	% RSD of Peak Area	Tailing Factor	Theoretical plate
30	1	1.001	1.156	6350.628
30	1.2	1.131	1.161	6368.805
40	1	1.210	1.212	6299.372
40	1.2	1.225	1.219	6301.520

Specificity

Specificity of the analyte peak was determined from that of the vehicle and blank injection. Necessary chromatograms are presented below:

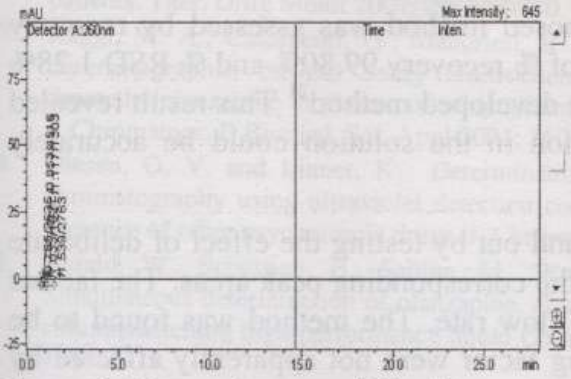

Figure 2: Chromatogram of Blank

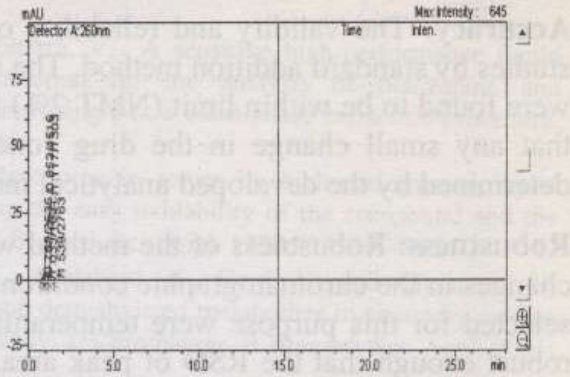

Figure 3: Chromatogram of Placebo

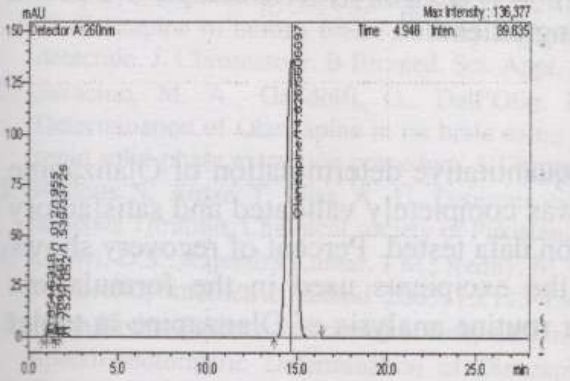

Figure 4: Chromatogram of Standard

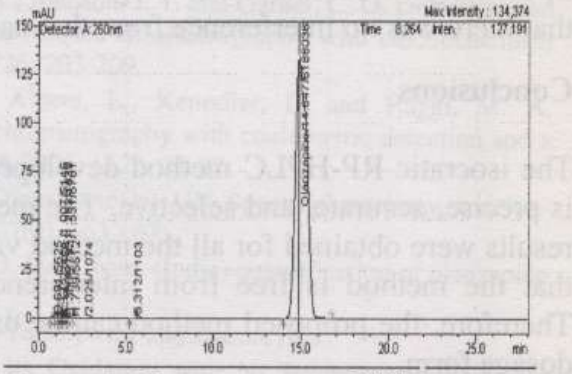

Figure 5: Chromatogram Of Sample

Discussion

System suitability: Chromatograms were automatically integrated and visually inspected for an acceptable integration. The relative standard deviation of the peak areas (RSD 0.038%), the mean tailing factor (1.22) for six system suitability injections were calculated. The system suitability parameters were within the limits[21].

Linearity and range: A good linear relationship (r^2=1) was observed between the concentration of Olanzapine and the respective ratio of peak areas. The regression curve was constructed by linear regression fitting and its mathematical expression was y=61076x-20453(where y is the ratio of peak areas of the drug to that of reference standard and x is the concentration of Olanzapine). (Fig-1) The lower limit of quantitation (LLOQ) was defined as the lowest concentration within the linear range (45.20μg/mL). The upper limit of quantitation (ULOQ) was defined as the highest concentration within the linear range (135.6μg/mL).

Precision: The repeatability and intermediate precision study of the developed method demonstrate RSD 1.884% for analyst-1 and RSD 1.852% for analyst-2 where RSD value for 12 samples was 1.885% which were not more than 2.0 % [21].That indicating the developed method has excellent repeatability and intermediate precision.

Accuracy: The validity and reliability of proposed method was assessed by recovery studies by standard addition method. The mean of % recovery 99.80% and % RSD 1.28% were found to be within limit (NMT 2%) for the developed method[21].This result revealed that any small change in the drug concentration in the solution could be accurately determined by the developed analytical method.

Robustness: Robustness of the method was found out by testing the effect of deliberate changes in the chromatographic conditions and the corresponding peak areas. The factors selected for this purpose were temperature and flow rate. The method was found to be robust enough that the RSD of peak area, tailing factor were not apparently affected by variation in the chromatographic conditions[21].

Specificity: From the chromatogram of blank, placebo, standard and sample we found that there was no interference from the inactive ingredients [21].

Conclusions

The isocratic RP-HPLC method developed for quantitative determination of Olanzapine is precise, accurate, and selective. The method was completely validated and satisfactory results were obtained for all the method validation data tested. Percent of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore, the proposed method can be used for routine analysis of Olanzapine in tablet dosage form.

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References

1. KanaKapura B., Sameer A., Mohammed A.R.; Simple and sensitive spectrophotometric determination of olanzapine in pharmaceutical formulations using two sulphonphthalein acid dyes; Journal of Food and Drug Analysis. 2009;17; 434-442.
2. Concetta, D., Gaetana, M., Vincenza, S. and Edoardo, S. Determination of olanzapine in human plasma by reversed-phase high-performance liquid chromatography with ultraviolet detection. Ther. Drug Monit. 2006; 28: 388-393.
3. Raggi, M. A., Casamenti, G., Mandrioli, R., Fanali, S., De Ronchi, D. and Volterra, V. Determination of the novel antipsychotic drug olanzapine in human plasma using HPLC with amperometric detection. Chromatographia. 2000; 51: 562-566.
4. Raggi, M. A., Mandrioli, R., Sabbioni, C., Ghedini, N., Fanali, S. and Volterra, V. Determination of olanzapine and desmethylolanzapine in the plasma of schizophrenic patients by means of an improved HPLC method with amperometric detection. Chromatographia. 2001; 54: 203-207.

5. Dusci, L. J., Hackett, L. P., Fellows, L. M. and Ilett, K. F. Determination of olanzapine in plasma by high-performance liquid chromatography using ultraviolet absorbance detection. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.2002; 773: 191-197.
6. Saracino, M. A., Koukopoulos, A., Sani, G., Amore, M. and Raggi, M. A. Simultaneous high-performance liquid chromatographic determination of olanzapine and lamotrigine in plasma of bipolar patients. Ther. Drug Monit.2007; 29: 773-780.
7. Raggi, M. A., Casamenti, G., Mandrioli, R. and Volterra, V. A sensitive high-performance liquid chromatographic method using electrochemical detection for the analysis of olanzapine and desmethylolanzapine in plasma of schizophrenic patients using a new solid-phase extraction procedure. J. Chromatogr. B Biomed. Sci. Appl.2001; 750: 137-146.
8. Olesen, O. V. and Linnet, K. Determination of olanzapine in serum by high-performance liquid chromatography using ultraviolet detection considering the easy oxidability of the compound and the presence of other psychotropic drugs. J. Chromatogr. B Biomed. Sci. Appl. 1998;714: 309-315.
9. Harald, W., Sebastian, H., Sabine, M., Werner, K., Godehard, K., Gerd, D. and Christoph, H. Simultaneous determination of olanzapine, clozapine and demethylated metabolites in serum by on-line columnswitching high-performance liquid Chromatography. J. Chromatogr. B Biomed. Sci. Appl. 2001; 759: 63-71.
10. Olesen, O. V., Poulsen, B. and Linnet, K. Fully automated on-line determination of olanzapine in serum for routine therapeutic drug monitoring. Ther. Drug Monit. 2001; 23: 51-55.
11. Kasper, S. C., Mattiuz, E. L., Swanson, S. P., Chiu, J. A., Johnson, J. T. and Garner, C. O. Determination of olanzapine in human breast milk by highperformance liquid chromatrography with electrochemical detection. J. Chromatogr. B Biomed. Sci. Appl. 1999; 726: 203-209.
12. Saracino, M. A., Gandolfi, O., Dall'Olio, R. O., Albers, L., Kenndler, E. and Raggi, M. A. Determination of Olanzapine in rat brain using liquid chromatography with coulometric detection and a rapid solid-phase extraction procedure. J. Chromatogr. A 2006; 1122: 21-27.
13. Firdous S, Aman T, Nisa A. U. Determination of Olanzapine by UV Spectrophotometry andNon-aqueous Titration, Chemical society of Pakistan, 2005, 27(2), 163 - 67.
14. Gowri, D.S.; Rajendra Kumar, J.M.; Reddy, M.V.V.N. J. Inst.Chem. (India).quantification of olanzapine by spectrophotometric method. 2003; 75:135-136.
15. Anna Krebs, Barbara Starczewska, Helena Puzanowska-Tarasiewicz and Joanna Ïled. Spectrophotometric Determination of Olanzapine by Its Oxidation with N- Bromosuccinimide and Cerium (IV) sulfate , Analytical sciences, 2006, 22(6), 829 - 33 .
16. Siva Prasad K.V, Rajendra Kumar J. M, Reddy M.V.V.N, Prabhakar G and Sankar D.G. Spectrophotometric Determination of Olanzapine in Pharmaceutical Preparations, Asian J. Chem., 2003, 15, 1127 - 30.
17. Jasinska, A.; Nalewajko, E. Batch and flow –injection methods for the spectrophotometric determination of olanzapine . Anal. Chim.Acta. 2004; 508:165-170.
18. Raggi M.A, Casamenti G, Mandrioli R, Izzo G and KenndlerE.Quantitation of olanzapine intablets by HPLC, CZE, derivative spectrometry and linear voltammetry, J. Pharm. Biomed. Anal., 2000, 23(6), 973 - 81.
19. Shah, Charmy R.; Shah, Nehal J.; Suhagia, Bhanubhai N.; Patel, Natvarlal M. J. Simultaneous Assay of Olanzapine and Fluoxetine in Tablets by Column High –Performance Liquid Chromatography and High - Performance Thin - Layer Chromatography. AOAC Intl. 2007; 90: 1573-1578..
20. Saxena, V.; Zaheer,Z.; Farooqui, M. High - Performance Thin Layer Chromatography Determination of Olanzapine in Pharmaceutical Dosage Form .Asian J. Chem.2006;18:1212 – 1222.
21. International Conference on Hormonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonised Tripartite Guideline, "Validation of Analytical Procedures": Text and Methodology Q2(R 1), Complementary Guideline on Methodology dated 06 November 1996, incorporated in November 2005, London.