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Quantitative Determination of Olanzapine in Pharmaceutical Preparations by HPLC

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Abstract. A new high performance liquid chromatographic (HPLC) method in reverse phase was developed and validated for the determination of olanzapine (OLZ) in pharmaceutical formulations. Optimum separation was achieved in less than 10 min using a reversed phase Intersil ODS column (150 mm × 4.6 mm, i.d., particle size 5 μm), and elution was accomplished using a mobile phase (0.5 mL/min). Detection was carried out using a UV detector set at 271 nm. A rectilinear relationship between mean peak area and concentration of OLZ was observed in the range 10-200 μg/mL, with a detection limit of 3.0 μg/mL and a quantization limit of 8.0 μg/mL. Intra-day and Inter-day precision, and accuracy of the methods have been established according to the current ICH guidelines. The developed method was successfully applied to the determination of OLZ in pharmaceutical formulations. The results were statistically compared with those of the reference method by applying Student's t-test and F-test. Accuracy, evaluated by means of the spike recovery method, was in the range 97.7-102.3%, with precision (RSD) better than 2%. No interference was observed from the coformulated substances. The method was economical in terms of the time taken and the amount of solvent used.
Key words: Olanzapine, quantification, HPLC, pharmaceuticals.

Resumen. Se describe un nuevo método de cromatografía de alta resolución (HPLC) en fase inversa para la determinación de olanzapina (OLZ) en formulación de medicamentos, lográndose también su validación. La separación óptima se logró en menos de 10 min, utilizando una columna de fase inversa Intersil ODS (150 mm × 4.6 mm, d.i., y 5 μm en tamaño de partícula), y con una elusión de fase móvil de 0.5 mL/min. La detección se llevó a cabo empleando un detector UV a una longitud de onda de 271 nm. Se observó una relación lineal entre el área media del pico y la concentración de OLZ en el intervalo de 10-200 μg/mL, con un límite de detección de 3.0 μg/mL y un límite de cuantificación de 8.0 μg/mL. La precisión media y exactitud de los métodos se establecieron de acuerdo a los manuales actualizados ICH. El método desarrollado se aplicó exitosamente para la determinación de OLZ en formulaciones farmacéuticas. Los resultados se compararon estadísticamente con aquéllos del método de referencia, mediante la aplicación de las pruebas *t* y *F* del método Student. La exactitud, la cual es evaluada mediante el método de recobro de pico, alcanzó un intervalo de 97.7-102.3%, con una precisión (RSD) mejor que 2%. No se observó interferencia de las sustancias de la formulación. En cuanto al aspecto económico, el método fue ventajoso en términos del tiempo de determinación y la cantidad del disolvente empleado.
Palabras clave: Olanzapina, cuantificación, HPLC, medicamentos.

Introduction

Olanzapine (OLZ) chemically, is 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-b][1,5]benzodiazepine [1] Fig.(1). It is indicated for acute schizophrenia and psychotic conditions.

In the literature, there are only a few methods described for the determination of OLZ in pharmaceutical formulations and include non-aqueous titrimetry and UV-spectrophotometry [2], visible spectrophotometry [3-5], and flow injection spectrophotometry [6]. A few methods have also been reported using HPLC. In a method reported by Xia and Tao [7], separation and quantification were achieved on a ODS C_{18} column with mobile phase consisting of KH_2PO_4-methanol-acetonitrile (1:1:1, p^H 8.3) and UV-detection at 273 nm. The linear range was 0.01-1.0 mg/mL. An HPLC method [8] with UV detection at 260 nm has been carried out with a C_8 column using a mobile phase consisting of acetonitrile and aqueous tetramethylammonium perchlorate. But, the method is poorly accurate and precise with a relative error of 1.1% and RSD of 1.8%. The same report [8] also described three more methods, capillary zone electrophoresis, derivative spectrophotometry, and linear voltammetry. Simultaneous assay of OLZ and fluoxetine in tablets by HPLC and HPTLC [9] has recently been reported by Shah et al. The LC separation was achieved on a Lichrospher 100 RP-180 C_8 column (150 mm × 4.6 mm, 5 μm) using 0.05 M KH_2PO_4 buffer (pH 5.6 adjusted with H_3PO_4)-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 μg/mL. The HPTLC method [10] of Saxena *et al.,* for OLZ 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 was to develop a simple, sensitive, accurate and precise and time-saving HPLC method for the determination of OLZ 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 validated method was applied to the commercially available pharmaceutical formulations containing OLZ.

Experimental

Apparatus

The chromatographic system consisted of an Agilent 1100 series chromatograph equipped with an in built solvent degasser, quaternary pump, photo diode array detector with variable injector and auto sampler, and a reversed phase 5 μm Inertsil ODS column (150 mm × 4.6 mm, i.d.).

Reagents and standards

All chemicals used were of analytical reagent grade and HPLC grade acetonitrile (Merck, Ltd, Mumbai) was used. Distilled water filtered through 0.45 µm filter (Millipore) was used to prepare solutions.

Mobile phase A consisted of 0.25% ammonium acetate adjusted to pH 4.5 with trifluroacetic acid. Mobile phase B was acetonitrile. The mobile phase used was prepared by mixing mobile phase A and mobile phase B in the ratio, 70:30. The same mobile phase was also used as a diluent for the sample preparations.

Pharmaceutical grade OLZ, certified to be 99.8% pure was procured from Cipla India Ltd, Mumbai, India, and was used as received. A stock standard containing 250 µg/mL OLZ solution was prepared by dissolving accurately weighed 25 mg of pure drug in the diluent and diluting to 100 mL with the diluent in a calibrated flask.

Procedures

Chromatographic conditions

The separation was achieved at a temperature of 35 °C on the column using the mobile phase at a flow rate of 0.5 mL/ min. The detector wavelength was set at 271 nm with a sensitivity of 0.2 a.u.f.s.

Calibration graph

Working standard solutions equivalent to 10 to 200 µg/mL OLZ were prepared by appropriate dilution of stock standard solution (250 µg/mL) with the diluent solution. Ten µl aliquot of each solution was injected automatically on to the column in duplicate and the chromatograms were recorded. Calibration graph was prepared by plotting the mean peak area versus concentration of OLZ.

The concentration of the unknown was read from the calibration graph or computed from the regression equation derived using the mean peak area-concentration data.

Assay in dosage forms

Twenty tablets were weighed accurately and ground into a fine powder using agate pestle and mortar. A quantity of tablet powder equivalent 25 mg of OLZ was accurately weighed into a 100 mL calibrated flask, 60 mL of diluent solution added and content shaken for 20 min; then, the volume was diluted to the mark with the diluent and mixed well. A small portion of the extract (say 10 mL) was withdrawn and filtered through 0.2 µm filter to ensure the absence of particulate matter. The filtered solution was appropriately diluted with the diluent solution for analysis as described already.

Results and Discussion

Method development

Drug quality control, stability, metabolism, pharmacokinetics, and toxicity studies all necessitate the determination of drugs in pharmaceutical formulations and biological samples. Consequently, efficient and validated analytical methods are very critical requirements for all these investigations. Chromatographic parameters were preliminarily optimized to develop the present method for the determination of OLZ with short analysis time (<10 min). A solution of OLZ was injected in duplicate on to the column and was monitored by UV detection at 271 nm. A mobile phase consisting of 0.25% ammonium acetate (pH 4.5) and acetonitrile in the ratio 70:30 was selected after several preliminary experiments. At a flow rate of 0.5 mL/min the retention time was 7.48 min (Fig. 2). Under the described experimental conditions, the peak was well-defined and free from tailing.

Method Validation

In order to determine the adequate resolution and reproducibility of the proposed method, suitability parameters including retention time, plate number and tailing factor were investigated, and were found to be 7.48 min (Fig. 2), 5047 and 1.32, respectively, which amply indicate the method suitability.

Linearity and range

Linearity was assessed in the range of 50 to 150% of the working level concentration including working level concentration. First and last level of linearity was carried out in six replicates and other levels in duplicates. Calibration curve was constructed by plotting the mean peak area versus concentration which was linear over the concentration range 10-200 µg/mL (Fig. 3). Using the regression analysis, the linear equation, Y = 31.3774 + 21.0704 X, was obtained, where Y is the mean peak area and X concentration in µg/mL. The Linearity co-efficient of mean response of replicate determination plotted against respective concentration was found to be 0.99998.The percent y-intercept as obtained from the linearity data was less than 2%. The %

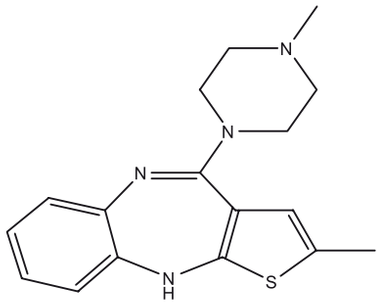

Fig. 1. Structure of Olanzapine.

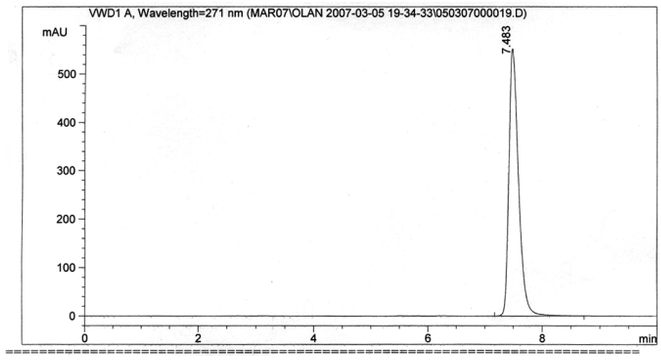

Fig. 2. Typical chromatogram.

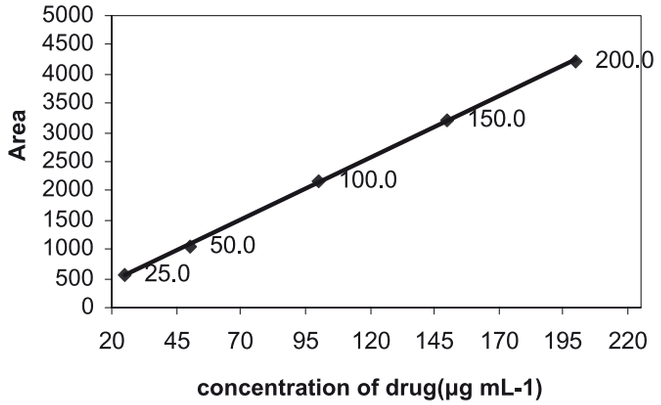

Fig. 3. Linearity curve.

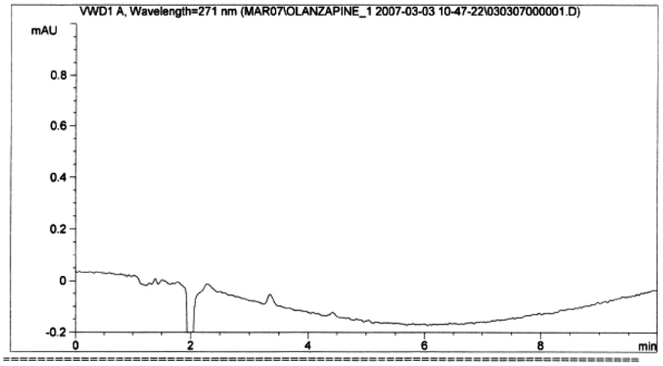

Fig. 4. Typical Chromatogram for Placebo.

RSD for peak area response of six replicates of first and last level was less than 2.0% and 1.0% for retention time.

Specificity

The specificity of an analytical method may be defined as the ability to unequivocally determine the analyte in the presence of additional components such as impurities, degradation products and matrix [11-13]. The specificity was evaluated by preparing the analytical placebo and it was confirmed that the signal measured was caused only by the analyte. A solution of analytical placebo (containing all the tablet excipients except OLZ) was prepared according to the sample preparation procedure, and injected. The resulting chromatogram is shown in Fig.4. To indentify the interference by these excipients, a mixture of inactive ingredients (placebo), standard solutions and the formulation including OLZ was chromatographed. The chromatograms did not show any other peaks, which confirmed the specificity of the method. In addition, the slope of the calibration curve for standards was compared with that prepared from formulation solutions. It was found that there was no significant difference between the slopes which indicated that excipients did not interfere with OLZ.

Detection and quantification limits

Limit of detection (LOD) and limit of quantification (LOQ) were calculated using signal-to-noise ratio method [11-13]. LOD is taken as the concentration of analyte where signal-to-noise ratio was 3, and it was found to be 3.0 µg/mL. LOQ is taken as the concentration of analyte where signal-to-noise ratio was 10, and it was found to be 8 µg/mL.

Precision

The precision of the method was evaluated in terms of intermediate precision (intra-day and inter-day) [11-13]. Three different concentrations of OLZ were analysed in seven replicates during the same day (intra-day precision) and five consecutive days (inter-day precision). Within each series, every solution was injected in triplicate. The RSD values of intra-day studies (<1%) showed that the precision of the method was satisfactory. The results of this study are given in Table 1. The inter-day precision was slightly poor with RSD values in the range 0.75-1.95%.

Precision of the injection repeatability was examined by analyzing seven injections of solutions containing OLZ at 50, 100 and 150 µg/mL, respectively. The relative standard deviations (RSD) were calculated from the peak areas and retention times. The results of this study are also given in Table 1 and found to be less than 1% and 0.5%, respectively.

Accuracy

The accuracy of an analytical method expresses the closeness between the reference value and found value [11-13]. Accuracy was evaluated as percentage relative error between the measured mean concentrations and taken concentrations. The results obtained for three concentrations are shown in Table 1 from which it is clear that the accuracy is excellent (RE <1%). The accuracy was also assessed by analyzing the synthetic mixture (prepared by adding OLZ to the placebo) and calculated the percent recovery of the active ingredient which was found to 99.12 ± 0.64, indicating that the co-formulated substances such as talc, starch, gum acacia, lactose, dextrose, hydroxyl methyl cellulose, sodium alginate and magnesium stearate did not interfere in the assay.

Table 1. Intra-day accuracy and intra-day precision.

OLZ taken, µg/mL	OLZ found*, µg/mL	Range, µg/mL	RE, %	RSD$, %	RSD@, %
50	49.6	0.67	0.80	0.78	0.32
100	99.75	0.95	0.25	0.05	0.32
150	148.9	1.30	0.73	0.26	0.15

RE. relative error; RSD. Relative standard deviation.
*Mean value of seven determinations; $ Based on peak area; @ Based on retention time.

Ruggedness and robustness

Intermediate precision of six replicate determinations on a sample was performed by four different analysts with three different instruments on different days after specifying the system suitability of the method. The %RSD of assay was less than 2.0% and the cumulative %RSD of assay of precision study and intermediate precision was also less than 2.0%.

Robustness of the method was checked by altering deliberately two critical parameters by minor variation.

1. Flow rate was changed to 0.55 mL/min.
2. pH of mobile phase was changed to 4.55.

The %RSD for peak area response was less than 2.0% and 1.0% for retention time. The cumulative %RSD of assay of precision study and robustness was also less than 2.0%.

Application of the method for the analysis of commercial formulation

The developed and validated method was applied to the determination of OLZ in three brands of tablets containing OLZ in two doses (10 and 20 mg OLZ per tablet) which are available in the local market using the procedure described earlier. Evaluation was performed using the calibration curve method since no significance difference between the slopes of the calibration curves for standards and tablet extracts was observed. The results obtained by the proposed method were statistically compared with those of the literature method [2] by applying the Student's t-test for accuracy and *F*-test for precision. As shown by the results compiled in Table 2, the calculated *t*- and *F*-values did not exceed the tabulated values, $t = 2.77$ and $F = 6.39$ at the 95% confidence level for four degrees of freedom suggesting that the proposed method and the literature method do not differ significantly with respect to accuracy and precision. The accuracy and validity of the proposed method were further ascertained by performing recovery experiments. Pre-analysed tablet powder was spiked with pure OLZ at three different levels and the total was found by the proposed method. Each determination was repeated three times. The recovery of pure drug added was quantitative (Table 3) and revealed that co-formulated substances did not interfere in the determination.

Conclusions

A simple, rapid, very accurate and precise HPLC method was developed for the determination of OLZ in pure form and in tablets. The analytical conditions and solvent system developed provided a good separation for OLZ within a short analysis time. The method was validated and demonstrated a wide linear dynamic range, a good precision and accuracy and specificity compared to the previously reported HPLC methods. Thus, the method can be proposed for routine analysis laboratories and for quality control.

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Table 2. Results of determination of olanzapine in tablets and statistical comparison with the reference method.

Formulation brand name#	Nominal amount, mg	% found* ± SD			
		Reference method	Proposed method	*t*-value	*F*-value
JOYZOL[a]	10	101.5 ± 0.51	100.9 ± 1.1	1.18	4.65
MELTOLAN[b]	20	97.2 ± 0.85	98.2 ± 1.5	1.34	3.11
OLEXA[c]	10	100.3 ± 1.01	100.9 ± 1.2	0.86	1.41

*Mean value of seven determinations.
#Marketed by: a. Wockhardt, India.; b. Alkem Ltd. ; c. Cipla Ltd.
Tabulated *t*-value at 95% confidence level is 2.77.
Tabulated *F*-value at 95% confidence level is 6.39.

Table 3. Results of recovery experiments *via* standard-addition method.

Formulation studied	OLZ in tablet, µg/mL	Pure OLZ added, µg/mL	Total found, µg/mL	Pure OLZ recovered* %
JOYZOL-10	25.2	25	50.10	99.6
	25.2	75	101.93	102.3
	25.2	125	150.58	100.3
MELTOLAN-20	24.6	25	49.50	99.6
	24.6	75	97.88	97.7
	24.6	125	151.70	101.2

*Mean value of three determinations.

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