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RP-HPLC Method Development, Validation and Stability Indicating Study of Olanzapine in Tablet Dosage Form

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Abstract

RP-HPLC method was developed for evaluation of Olanzapine in pharmaceutical formulation. The separation was conducted by using mobile phase consisting of buffer (KH_2PO_4): acetonitrile in the ratio of 60:40.The wavelength was found at 257.0nm and it is freely soluble in 0.1 NHCL, methanol, acetonitrile and benzene and slightly soluble in ethyl alcohol. The linearity of method was investigated in the range of 5-25 µg/ml and R.S.D was found 1.994. The aim of this research study was to develop and validate simple, accurate, precise, sensitive and cost effective RP-HPLC method for quantitative evaluation of Olanzapine drugs and to develop a validated Stability Indicating RP-HPLC method for determination of Olanzapine drug in pharmaceutical formulations which are critical for the quality control laboratories.

Keywords: RP-HPLC, Olanzapine, Stability Indicating RP-HPLC

Introduction

Simultaneous estimation of drug combination is generally done by separation using chromatographic methods like HPLC, GC and HPTLC etc. These methods are accurate and precise with good reproducibility, but the cost of analysis is quite high owing to expensive instrumentation, reagent and expertise. Hence it is advisable to develop simpler and cost effective method for simultaneous estimation of drugs for routine analysis of formulation. Spectrophotometric analysis fulfills such requirement where the simultaneous estimation of the drug combination can be done with similar effectiveness as that of chromatographic methods. [1,2] Most of the drugs in multicomponent dosage forms can be analyzed by HPLC method for the reason that of the several advantages like rapidity, specificity, accuracy, precision and ease of automation in this method. HPLC method eliminates tiresome extraction and isolation procedures.

As opposed to normal-phase HPLC, reversed-phase chromatography is based mainly dispersive forces (hydrophobic or Vander Waals interactions). The polarities of mobile and stationary phases are reversed, such that the surface of the stationary phase in RP-HPLC is hydrophobic and mobile phase is polar, where mainly water-based solutions are employed. Reversed-phase HPLC is by far the most admired mode of chromatography. Almost 90% of all analyses of low-molecular-weight samples are performed out by using RP HPLC. Less polar (more hydrophobic) analytes are more attracted and spend more time linked with the hydrophobic bonded phase, therefore, they are eluted at last.[3, 4, 5]

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Stability testing allows the establishment of suggested storage conditions, retest periods, and eventually product shelf-life and expiry dating. In pharmaceutical field stability studies finds an application in drug development programs [6,7].
Olanzapine is chemically,2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-b][1,5] benzo-diazepine. It is an atypical antipsychotic, approved by the U.S. Food and Drug Administration (FDA) for the treatment of schizophrenia and bipolar disorder.It is classified as a thienobenzodiazepine. Olanzapine is an antagonist at types 1, 2 and 4 dopamine receptors. Olanzapine's antipsychotic effect is due to antagonism at dopamine and serotonin type 2 receptors, with greater activity at serotonin 5-HT 2 receptors than at dopaminetype 2 receptors. Antagonism at muscarinic receptors. H1 receptors, and alpha (1) - receptors also occurs with olanzapine[8,9, 10].
The aim of this research study is to develop and validate simple, accurate, precise, sensitive and cost effective RP-HPLC method for quantitative evaluation of Olanzapine drugs and to develop a validated Stability Indicating RP-HPLC method for determination of Olanzapine drug in pharmaceutical formulations which are critical for the quality control laboratories.

Material and Methods

Chemical and reagents

Olanzapine was procured from Scan research laboratories, Bhopal, Methanol (AR Grade), Acetonitrile (HPLC), Methanol (HPLC), and Water (HPLC) procured from Merck Ltd., India.

Instruments

A Lab India 3000 + spectrophotometer with 1cm quartz cells. The HPLC system consisted of a waters pump, a U.V. Visible detector, a Thermo C18 (250 X 4.60 mm), 5µm column, a Lichrocart, HPLC guard cartridge system and a data ace software.

Methods

Selection of Wavelength suitable wavelength for the HPLC analysis was determined by recording UV spectrum in the range of 200-400 nm for olanzapine. Suitable wavelength selected was 257 nm

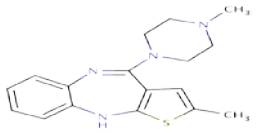

Fig. 1: Chemical structure of Olanzapine

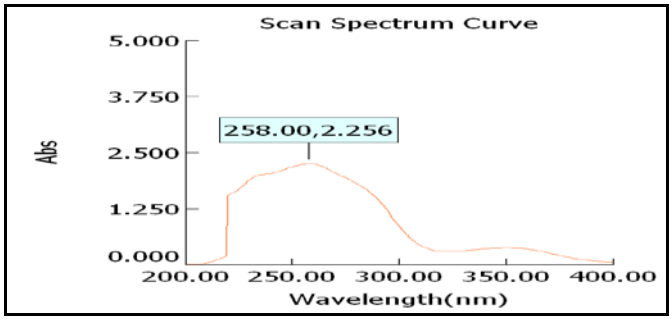

Fig. 2: UV Spectra of Olanzapine

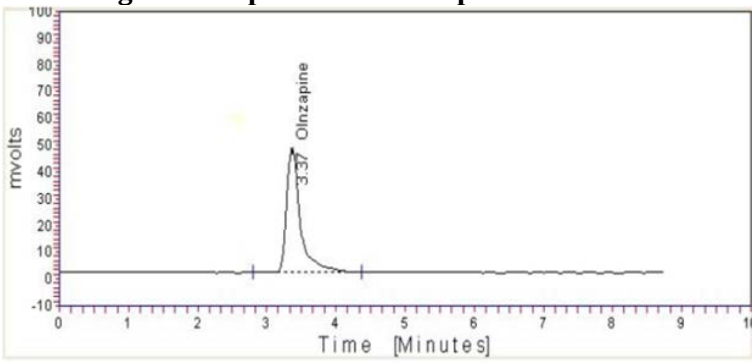

Fig. 3: Typical chromatogram for the tablet formulation

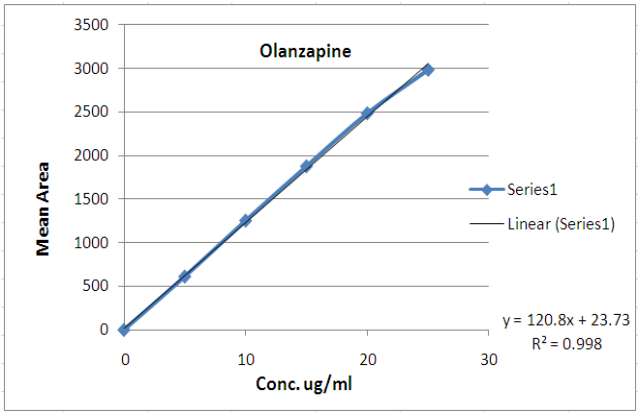

Fig. 4: Calibration Curve

Chromatographic conditions

The developed method uses a reverse phase C18 column, Phenomena Gemini (C18 250 X 4.60 mm ×5µm), mobile phase consisting of buffer (KH_2PO_4): acetonitrile in the ratio of 60:40. The mobile phase was set at a flow rate of 1.0 ml/min and the volume injected was 20 µl for every

injection. The detection wavelength was set at 257 nm.

Preparation Mobile Phase

1.75 gm KH_2PO_4 in 1000 ml of water add 1 ml of TEA and adjust the pH 6 with OPA .The mobile phase was filtered through 0.45 µm filter paper to remove particulate matter and then degassed.

Preparation of Standard Stock Solution:-

10mg of Olanzapine was weighed accurately and transferred to separate 10ml volumetric flask, and the volume was adjusted to the mark with the Water: Acetonitrile (50:50 v/v) to give a stock solution of 1000ppm.

Preparation of Working Standard Solution:-

From stock solutions of Olanzapine 1 ml was taken and diluted up to 10 ml from this solution 0.5, 1.0, 1.5, 2.0, 2.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 100 ml with Water : ACN (50:50 v/v) , gives standard drug solution of 5, 10, 15, 20, 25 µg/ ml concentration.

Preparation of the Calibration Curves of the Drug:-

Standard drug solutions were injected 3 times and the mean peak area of drug was calculated and plotted against the concentration of the drug. The regression equation was found out by using this curve. The calibration curve is shown in Figure 4

Analysis of tablet formulation

Assay of tablet formulation

For analysis of the tablet formulation, weight equivalent to weight 10 mg of Olanzapine was transferred to 10 ml volumetric flask and dissolved in mobile phase. The solution was shaking vigorously for 20 mins and filtered through Whattman filter paper no. 41, then volume was made up to mark with mobile phase. From the above solution 1 ml of solution was taken and diluted to 10 ml with mobile phase to get a solution containing 100 µg/ml. From the above solution 1 ml of solution was taken and diluted to 10 ml with mobile phase to get a solution containing 10 µg/ml of Olanzapine. The amounts of Olanzapine in tablet formulation were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with tablet formulation. Result is shown in (Table no.1).

Table 1: Assay Analysis for Olanzapine in tablet Formulation

Std Conc. µg/ml	Olanzapine
	10
Rep-1	10.10
Rep-2	10.05
Rep-3	10.08
% found *	
Rep-1	101
Rep-2	99.504
Rep-3	100.298
Mean	10.076
SD	0.747
% RSD	0.742

Results and Discussion

METHOD DEVELOPMENT

A Reverse phase HPLC method was developed considering the system suitability parameters i.e. tailing factor (T), the number of theoretical plates (N), run time and the cost effectiveness. The optimized method developed resulted in the elution of Olanzapine at 4.560 min. Figures 3&4 represent chromatograms of blank and standard solution (10µg/ml) respectively. The total run time is 10 minutes. System suitability tests are an integral part of method development and validation. System suitability tests are used to ensure adequate performance of the chromatographic system. System suitability parameters were evaluated for six replicate injections of the standard at working concentration. The results are given in Table 2.

Table 2: System suitability studies results

System suitability Parameter →	RT	AUC	Theoretical plates	Tailing factor
Rep-1	2.375	1251.23	3078	1.18
Rep-2	2.374	1250.458	3056	1.20
Rep-3	2.375	1256.658	3098	1.15
Mean	2.374667	1252.782	3077.333	1.176667
S.D.	0.000577	3.378835	21.00794	0.025166

Table 3: Calibration data for Olanzapine

Conc. µg/ml	0	5	10	15	20	25
Rep.	0	0	0	0	0	0
1	0	609.517	1251.23	1863.78	2483.691	2979.817
2	0	610.258	1250.458	1865.589	2486.985	2980.715
3	0	615.547	1256.658	1898.564	2478.985	2985.855
Mean	00	611.774	1252.782	1875.978	2483.22	2982.129
S.D.	00	3.288452	3.378835	19.58124	4.020715	3.257899
S.D%	000	0.537527	0.269707	1.043789	0.161915	0.109247

Method validation

Authentication of the investigative method is the process that starts by laboratory studies in which the requirements of the performance properties of method are met for the intended analytical application. To the validation of analytical procedures RP-HPLC method developed was validated according to International Conference on Harmonization (ICH) and USP guidelines. Various parameters or criteria are used for the method of validification, such as linearity, accuracy, precision, system suitability, etc.

System Suitability Parameters

Separation variables (Table No.2) were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, three replicates of working standard of Olanzapine 10 µg/ml was injected separately. Peak report and column performance report were recorded for all chromatogram.

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyst in the sample. Standard solutions contained 5 to 25 µg/ ml of olanzapine. Linearity solutions were injected in triplicate. The equations of the calibration curve was y = 120.8x+23.73, the calibration graphs were found to be linear in the aforementioned concentrations with correlation coefficients 0.998. The results are mentioned in the Table 2 & calibration curve Fig 4.

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed (Table no.4).

Precision

(A)Repeatability

Standard dilutions were prepared and three replicates of each dilution were analyzed in same day for repeatability and results were subjected to statistical analysis. (Table no.5). Standard dilutions were prepared and three replicates of each dilution were analyzed in different days and by different analysts. Statistical analysis was carried out.

(B) Intermediate Precision

(a) Day to Day

The statistical analysis method was carried out and the data is presented in (Table no 6).

Robustness

As per ICH norms, small, but deliberate variations, by altering the pH and concentration of the mobile phase were made to check the method capacity to remain unaffected. The effect of change in pH of mobile phase, flow rate, mobile phase ratio on the retention time, theoretical plates, area under curve and percentage content of Olanzapine was studied. Results are shown in (Table no.7).

Stability Study

Forced Degradation studies

In order to determine whether the method is stability indicating, forced degradation studies were conducted on olanzapine powder and the analysis was carried out by HPLC with a U.V. detector.

20µl of each of forced degradation samples were injected at regular intervals (Shown in Table 8)

Acid degradation

50 mg of olanzapine sample was taken into a 50 ml round bottom flask, 50 ml of 0.1 M HCl solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10

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µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of Olanzapine.

Base degradation

50 mg of olanzapine sample was taken into a 50 ml round bottom flask, 50 ml of 0.1 M NaOH solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of Olanzapine.

Hydrolytic degradation

50 mg of olanzapine sample was taken into a 50 ml round bottom flask, 50 ml of water was added and the contents were mixed well and kept for constant stirring for 48 h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of Olanzapine.

Oxidative degradation

50 mg of olanzapine sample was taken into a 50 ml round bottom flask, 50 ml of 3% hydrogen peroxide solution was added, and contents were mixed well and kept for constant stirring for 24 hr at room temperature. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of Olanzapine.

Thermal degradation

50 mg of olanzapine sample was taken in to a petri dish and kept in oven at 50ºC for 4 weeks.

Table 4: Results of Accuracy studies for Olanzapine

Level of Recovery (%)	80	100	120
	Olanzapine	Olanzapine	Olanzapine
Amount present (mg)	10 10 10	10 10 10	10 10 10
Amount of Std. added (mg)	8 8 8	10 10 10	12 12 12
Amount recovered (mg)	7.98 8.01 8.00	10.05 10.00 9.98	11.95 12.01 11.95
% Recovery	99.75 100.12 100.00	100.50 100.00 99.80	99.58 100.08 99.58

Table 5: Repeatability data of Tablet Formulation

Drug	Label claim	Amount found*	Label claim (%)	S.D.	% RSD
Olanzapine	10 mg	9.95	99.50	0.254	0.125

Table 6: Intra-day and Inter-day Precision

Intra-day Precision		Inter-day Precision	
	% Label Claim		% Label Claim
	Olanzapine		Olanzapine
After 1hr	99.20	First day	97.50
After 2hr	99.10	Second day	97.00
After 3hr	99.00	Third day	96.80
After 4hr	98.85		
After 5hr	98.70		
After 6hr	98.30		
Mean	98.97	Mean	97.1
SD	0.198746	SD	0.360555
% RSD	0.200814	% RSD	0.371324

Table 7: Robustness of Formulation

Compound	% RSD in Normal	Changed Condition n= 6	
Temperature		- 5 °C	+ 5 °C
Olanzapine	0.54	0.69	0.52
Flow rate		(- 10%)	(+10%)
Olanzapine	0.41	0.48	0.89
Mobile phase ratio		- 2 %	+ 2 %
Olanzapine	0.31	0.77	0.15

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Table 8: Forced degradation studies of Olanzapine

Stress conditions	Drug recovered (%)	Drug decomposed (%)
Standard drug	99.90	0
Acidic hydrolysis	83.26	16.64
Alkaline hydrolysis	89.65	10.25
Oxidative degradation	91.23	8.67
Thermal degradation	98.89	1.01

Conclusion

The developed stability indicating HPLC method for quantitative estimation of olanzapine in bulk and pharmaceutical dosage forms is fast, simple, accurate, and more precise. Validation of this method was accomplished, getting results meeting all requirements. Thus, the developed HPLC method can be used for routine quality control tests.

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