e-ISSN: 2249 – 7781
Print ISSN: 2249 – 779X

International Journal of Pharmaceutical Research & Analysis
www.ijpra.com

Research Article

STABILITY INDICATING HPLC ASSAY METHOD DEVELOPMENT FOR DETERMINATION OF OLANZEPINE

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ABSTRACT
A simple, precise and specific reverse phase high performance liquid chromatographic method has been developed and validated for the determination of Olanzepine in tablet. It was found that the excipient in the tablet dosage forms does not interfere in the quantification of active drug by proposed method. The HPLC separation was carried out by reverse phase chromatography on The Agilent HPLC 1200 series, with Open Lab software, PDA and VWD detector were used for quantitative estimation of OLZ. Stationary phase consisted of Xterra RP C18 (150 x 4.6mm, 5µm), SPD–10 UV detector and LC 10 ADVP Pumps. Auto-injector with 15 µl injection volume with a Mobile phase comprised of buffer consisting 0.138 % w/v of sodium dihydrogen phosphate monohydrate in Ultra pure water (pH was adjusted to 6.8 with NaOH) and Acetonitrile in the ratio 50:50 at flow rate 1.0 mL/min. The detection was monitored at 270 nm. The calibration curve for Olanzepine e was linear from 25-150 µg/mL. The inter-day and intra-day precision was found to be within limits. Accuracy (recoveries: 99.8-103.2%) and reproducibility were found to satisfactory. The method was found to be specific against excipients interference and stress condition. Stress testing showed degradation product and impurity were well-separated from the parent compound, conforming stability-indicating capacity of the method.

Keywords: HPLC, Validation, Stability Indicating method, ICH Guideline.

INTRODUCTION

Analysis is important in every product but it is vital in medicines as it involves life. The assurance of quality is achieved through analysis of drug product. [1] Today, absorption spectroscopy and HPLC are the most valuable analytical techniques for pharmaceuticals. They will probably remain a useful tool in the future despite further advances in analytical chemistry because of several overwhelming advantages for the solution of many problems. [2-5]. These advantages include speed, simplicity, specificity and sensitivity. According to ICH guidelines on impurities in new drug products, identification of impurities below 0.1% level is not considered to be necessary, unless the potential impurities are expected to be unusually potent or toxic. Impurities present in excess of 0.1% should be identified and quantified by selective methods.

A stability-indicating method is an analytical method that accurately quantities the active ingredients without interference from degradation products, process impurities, excipients, or other potential impurities. [6-8] A method that accurately quantities significant degradants may also be considered stability-indicating. Stability-indicating assay method according to 1987 US-FDA guideline was defined as the quantitative analytical methods that are based on the characteristic structural, chemical or biological properties of each active ingredient of a drug product and that will distinguish each active ingredient from its degradation products so that the active ingredient content can be accurately measured. [9] This definition in the draft guideline of 1998 has been modified as validated quantitative analytical methods that can detect the changes with time in the chemical, physical, or microbiological properties

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of the drug substance and drug product, and that are specific so that the contents of active ingredient, degradation products, and other components of interest can be accurately measured without interference. [10-12] Thus, the major changes brought in the new guideline are with respect to (i) introduction of the requirement of validation, and (ii) the requirement of analysis of degradation products and other components, apart from the active ingredients(s).

There are two types of stability- indicating assay methods (SIAM):

(i) Specific SIAM - defined as a method that is able to measure unequivocally the drug(s) in the presence of all degradation products, excipients and additives, expected to be present in the formulation.

(ii) Selective SIAM - defined as a method that is able to measure unequivocally the drug(s) and all degradation products in the presence of excipients and additives, expected to be present in the formulation. [13] By these definitions, it means that a 'Selective SIAM' is a procedure of both types-'Specific SIAM' as well as 'Selective SIAM'. In case of the former, the method is not fully separative to all components, but does separate the drug equivocally. [14-15] This normally is a situation where efforts fail to separate degradation products when they are large in number. Selective SIAM' is that of more importance with respect to new drugs, but for old and established drugs, where significant body of information exists; the use of 'Specific SIAM' can do an equally good job during stability sample analysis.[16] SIAM separating all types of possible degradation products should normally be developed through stress testing under different ICH suggested conditions. In case, however, it is not possible to develop a 'Selective SIAM' due to the complex nature of degradation, one can target for a method that takes into account degradation products only formed under accelerated and long-term storage conditions. [17-19]

Olanzapine has a higher affinity for 5-HT2 serotonin receptors than D2 dopamine receptors. It is highly soluble and highly permeable drug moiety.Like most atypical antipsychotics, compared to the older typical ones; Olanzapine has a lower affinity for histamine, cholinergic miscarrying and alpha adrenergic receptors. [20] The mode of action of Olanzapine involves antagonism at serotonin receptors**.**

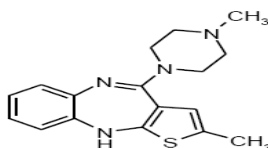

Fig 1: Structure of Olanzapine.

MATERIALS AND METHODS

CHEMICALS & REAGENTS

Olanzapine was supplied by Glenmark Pharmaceuticals Ltd. Mumbai. Label claim of Olanzapine is 50 mg.

Chemicals and Reagents:

a) Acetonitrile - HPLC grade

b) Sodium dihydrogen phosphate monohydrate - AR grade

c) Orthophosphoric acid - AR grade

d) Double Distilled Water

e) Sodium hydroxide – AR Grade

INSTRUMENTS

The Agilent HPLC 1200 series, Waters HPLC with Empower software waters 2996 PDA, 2487, waters 2695 separation module PDA were used for quantitative estimation of PRA in pharmaceutical dosage form.

EXPERIMENTAL WORKS

Chromatographic system:

The Agilent HPLC 1200 series, with Open Lab software, PDA and VWD detector were used for quantitative estimation of OLZ. [21] Mobile phase comprised of buffer consisting 0.138 % w/v of sodium dihydrogen phosphate monohydrate in Ultra pure water (pH was adjusted to 6.8 with NaOH) and Acetonitrile in the ratio 50:50. A reversed phase Xterra RP C18, 5µm (150×4.6mm) with pore size of 100 A0 was used for chromatographic studies. [22-25] The column was maintained at 40°C and injection volume of 10 µl was used. The mobile phase used for the studies consist of Phosphate Buffer: Acetonitrile (60:40) v/v and the pH adjusted to 2.0 with Orthophosphoric acid. [26-28] The mobile phase was filtered through 0.45µ membrane filter and degassed by sonication. The flow rate was adjusted to 1.0 mL/min and detection was carried out on wavelength 210 nm.

Optimized chromatographic conditions for the assay

Stationary Phase :Xterra RP C18(150 x 4.6mm, 5µm

Flow Rate 1.0 mL/min

Detection 270nm

Pump Mode Isocratic

Injection Volume 15µl

Run Time 5min

Column Temperature 25°C

Thermostat 25°C

Retention Time About 3.5 min

Preparation of diluent:

Acetonitrile and HPLC grade water was mixed in the ratio of 50:50.

Preparation of mobile phase:

An accurately weighed quantity of 1.38 g of Sodium dihydrogen phosphate monohydrate was dissolved in 1000 mL of Ultra pure water, mixed well and pH was adjusted to 6.8 with sodium hydroxide. Prepared buffer and acetonitrile was mixed in the different ratios. Shaked and mixed well then sonicated for 5 min.

Preparation of Olanzapine Standard Solution:

An accurately weighed quantity about 50.0 mg Olanzapine reference standard was transferred into 50.0 mL volumetric flask. About 5 mL acetonitrile was added, sonicate for 5 min to dissolved and volume was made up to the mark with diluent. A 5 mL portion of this solution was transferred into 50 mL volumetric flask and volume was made up to the mark with diluent. (Concentration: 100 µg/mL).

Selection of detection wavelength

About 50.0 mg of Olanzapine reference standard was transferred to 50.0 mL volumetric flask. To it 5 mL of acetonitrile was added and sonicated to dissolve. Then volume was made up to mark with acetonitrile (1000 µg/ mL). From above solution 5 mL was further diluted to 100 mL with acetonitrile (50 µg/ mL). This solution was scanned in the UV range from 400-200 nm. Spectrum recorded is shown in **Fig.No. 2.**

Study of system suitability parameters

The standard solution was prepared by earlier mentioned procedure. After equilibration of column with mobile phase, five replicate injections of 15 µL solution were injected. The chromatograms were recorded and the peak response i.e. peak area was measured. [29] The RSD of five replicate injections should not be more than 2.0%. Tailing factor for Olanzapine peak should not be more than 2.0. Number of theoretical plates should not be less than 3000.

Study of Beer-Lamberts law

Aliquots of standard solution were diluted in range 2.5 mL to 7.5 mL in a series of 50.0 mL volumetric flask and volume was made up to mark with diluent to obtain concentration ranging from 24.981 µg/mL to 74.944 µg/mL for Olanzapine.

Preparation of Sample solution for assay:

Accurately weighed quantity of about 50 mg Olanzapine (OLZ) sample was transferred into 50 mL volumetric flask. About 5 mL of acetonitrile was added, sonicated for 5 minutes with intermittent shaking. Sample was allowed to cool at room temperature. Volume was made up to the mark with diluent and mixed. A 5 mL portion of this solution was further diluted to 50 mL with diluent. The procedure was repeated six times. [30] The optimized chromatographic parameters were applied to check the presence of unknown peaks in diluents (blank), standard solution and sample solution of OLZ. A 15 µL injection of blank, standard solution and sample solution was injected and chromatograms recorded are shown in **Fig. No. 3, 4 and 5** respectively. [31]

Recovery study

It was carried out by standard addition method. Standard drug at three different levels i.e. 80%, 100% and 120% added in fixed amount of placebo. The amount of drugs was quantified as per the test method. The percentage recovery was calculated from the amount recovered and actual amount added.

Validation of proposed HPLC method

Accuracy

Accuracy of the proposed method was ascertained on the basis of recovery studies performed by standard addition method.

Precision

Precision of any analytical method is expressed as SD and %RSD of series of replicate measurements. Precision of estimation of Olanzapine by HPLC method was ascertained by replicate analysis of homogeneous samples of tablets.

Specificity

Calculated the average weight of 20 tablets and crushed it into fine powder. Sample equivalent to 50 mg of Olanzapine was transferred to five different 100 mL volumetric flasks. [31-35] About 50 mL of diluent was added and sonicated for 20 minutes with occasional swirling. Cool at room temperature. The samples were then exposed to various stress condition as follows, (a) 5 mL of 5N HCL was added and heated at 70°C for 2 min (b) 2.5 mL of 2N NaOH was added then add 2.5 mL of 2N HCL (c) 5 mL of 50% peroxide was added. (d) Samples were exposed at 105°C for 8 hrs and (e) Control sample. [36-38] After exposing the samples, diluted it up to mark with diluent and mixed. Filter through 0.45 Nylon filter paper. Pipette out 5 mL of filtered solution into 50 mL volumetric flask and diluted up to mark with diluent.

Interference study

Specificity of the method was evaluated by injecting the blank, placebo and control sample solution prepared as per the proposed method into HPLC system to check for the interference if any at the retention time of PRA. [39-40] There was no interference from the blank and placebo at the retention time and peak purity of the drug.

Linearity and Range

From the data obtained under study of Beer-Lamberts law, a graph was plotted as peak area vs. concentration of drug (µg/mL) which was observed to be linear over the range of 80-120% of labeled claim.

Ruggedness

The ruggedness of the proposed method has been verified by analyzing the six samples of same batch used for method precision by two different analysts using two different instruments, on different days.

Robustness

To evaluate the robustness, the following small deliberate variations made in the method such as change in flow rate, change in wavelength, change in column temperature, change in mobile phase composition and change in pH of mobile phase, and analyzed the sample in triplicate.

Stability of analytical solution

The sample and standard preparations were stored at room temperature and tested against freshly prepared standard preparation.

Table 1: Observation and result for accuracy of OLZ sample

Sample No.	Amount found (mg)	Area (mAU)	% Recovery
Acc. 50%-1	49.4992	42874441	101.5
Acc. 50%-2	50.0494	43338102	101.6
Acc.50% -3	49.4148	42783911	102.3
Acc. 50%-4	49.6294	42978586	101.5
Acc. 50%-5	49.8107	43130302	102.7
Acc. 50%-6	48.9854	42416842	99.5
Acc. 100% -1	98.3251	85098429	98.4
Acc. 100% -2	99.8544	86482759	101.2
Acc. 100% -3	98.6013	85386287	100.0
Acc. 150% -1	150.7442	130557070	102.4
Acc. 150% -2	147.4286	127654467	99.9
Acc. 150% -3	147.8804	128088831	100.3
Acc. 150% -4	149.8313	129752907	100.8
Acc. 150% -5	150.5742	130330650	101.8
Acc. 150% -6	145.7029	126162921	98.5
	Mean	**100.8**	
	SD	**1.35**	
	% RSD	**1.34**	

Table 2: Results for Specificity Studies of OLZ

Sr No	Experiment	Degradation Condition	% Assay	% Degradation w.r.t. control	Peak Purity
1	Control	--	98.3	--	1.000
2	Acid Degradation	0.1N HCl - 60°C/ 1hr	89.5	8.8	1.000
3	Base Degradation	0.1N NaOH-60°C/1hr	87.5	10.5	1.000
4	Peroxide Degradation	3% H_2O_2- 60°C/ 1hr	85.2	13.1	1.000
5	Thermal Degradation	60°C – 8 hours	96.5	1.8	1.000
6	Water Degradation	60°C/ 1hr	95.0	3.5	1.000

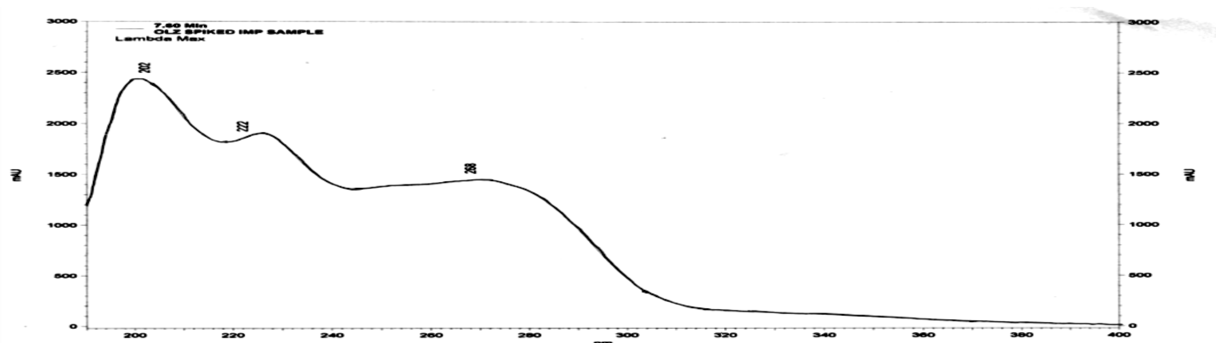

Figure 2: UV spectrum of Olanzapine

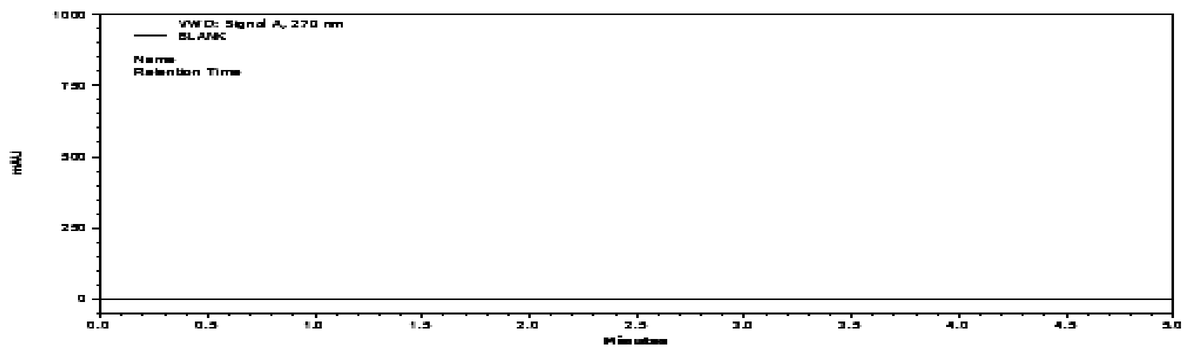

Figure 3: Chromatogram of blank

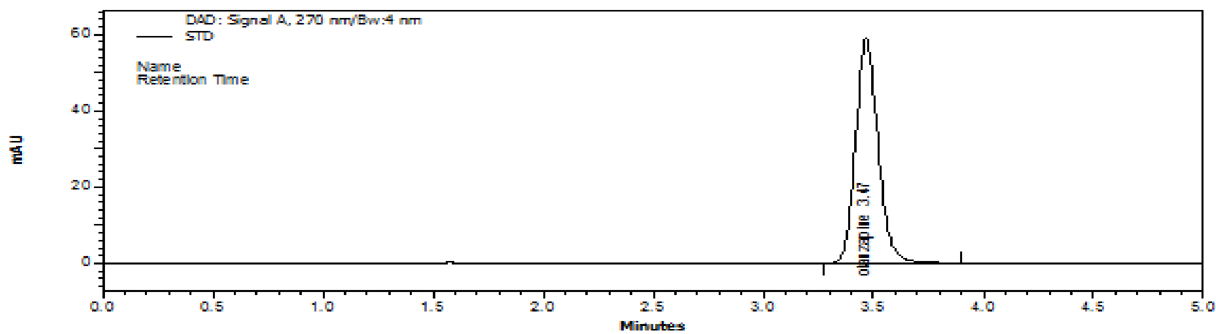

Figure 4: Chromatogram of Standard

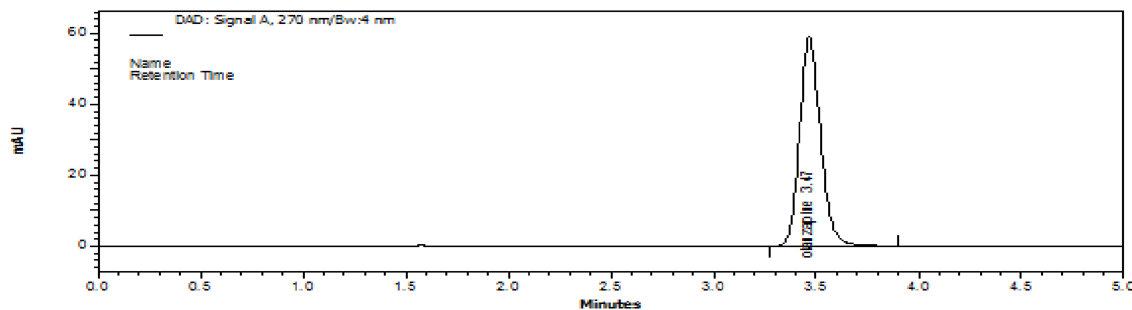

Figure 5: Chromatogram of Sample

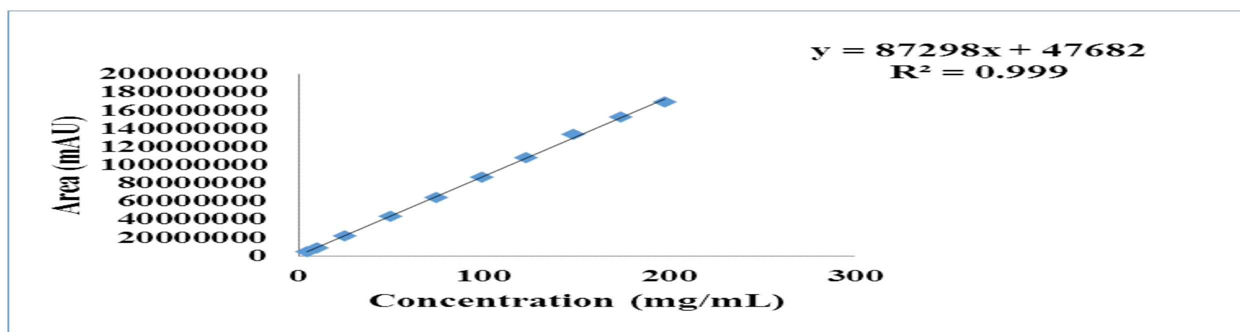

Figure 6: Study of linearity of OLZ

DISCUSSION

In conclusion, the results obtained by RP-HPLC method for estimation of OLZ were found to be accurate, precise and reliable. [41-42] The method was found to be linear over the concentration range of 5 µg/mL to 200 µg/mL and accurate over the concentration range of 50 µg/mL to 150 µg/mL. The method is cost effective due to run time and rapid for estimation of OLZ. The method was found to be rugged, robust and reproducible hence can be used for the routine quality control analysis of OLZ.

CONCLUSION

From all above studies, we can conclude that the proposed HPLC method can be used successfully for determination of Assay in Olanzapine and can also be useful in detecting the degradation of unknown impurities. Further this method may be applied to preparative HPLC for qualification of unknown impurities which might be generated during forced degradation studies. The results obtained by HPLC method for determination of Olanzapine are reliable, accurate and precise. The method can be employed for routine quality control analysis of Olanzapine in tablet dosage form.

ACKNOWLEDGEMENT

The authors are very grateful to all Management and Principal of RKDF College of Pharmacy for encouragement to carry out the work as well as for providing the facilities to carry out the research work

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Cite this article:
Abhay Kumar*, Ketkee Mandawar, Seema Sahu, Dr. Nishi Prakash Jain. Stability Indicating HPLC Assay Method Development for Determination of Olanzepine. *International Journal of Pharmaceutical Research & Analysis,* 2024, 14(2), 76-83.

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