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Original Article

DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING UV SPECTROSCOPIC METHOD FOR OLANZAPINE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, specific and reliable UV-VIS spectrophotometric method was developed for the estimation of Olanzapine in bulk and pharmaceutical dosage forms. Water: hydrochloric acid (9:1) was chosen as the solvent system. The λ_{max} was found to be 258nm and the responses were linear in the range of 5-40µg/ml. The regression equation of the calibration graph and correlation coefficient were found to be y = 0.059x + 0.171 and 0.998 respectively. The %RSD values for both intraday and interday precision were less than 1%. The recovery of the drug from the sample was ranged between 98.31% and 99.68%. The proposed method was validated for accuracy, precision, robustness, ruggedness, LOD and LOQ. While estimating the commercial formulation there was no interference of excipients and other additives. Hence this method can be used for routine determination of Olanzapine in bulk and their pharmaceutical dosage forms. The proposed method for stability study shows that there was appreciable degradation found in stress condition of Olanzapine.

Keywords: Olanzapine, UV-VIS Spectrophotometric, Stability study, Degradation.

INTRODUCTION

Olanzapine (trade name Zyprexa or in combination with fluoxetine Symbyax) is an atypical antipsychotic, approved by the U.S. Food and Drug Administration (FDA) for the treatment of schizophrenia and bipolar disorder.[1, 2, 3] Olanzapine is structurally similar to clozapine, but is classified as athienobenzodiazepine. The Olanzapine formulations are manufactured and marketed by the pharmaceutical company Eli Lilly and Company; the drug went generic in 2011. Sales of Zyprexa in 2008 were $2.2B in the US alone, and $4.7B in total.[4] Pharmacological research has demonstrated that Olanzapine has nanomolar receptor affinity for dopamine D1 –D5, serotonin 5HT, 2A / 2B/ 2C, 5HT3 and 5HT6 receptors.

In addition Olanzapine is a potent antagonist of α1 -adrenergic and histamine H1 receptors 1, 2, 3. It is important to note that the atypical antipsychotics offer many clinical benefits in the treatment of schizophrenia compared to traditional antipsychotics such as phenothiazines and butyrophenones, also called 'classical neuroleptic agents" and have emerged as first line therapy for schizophrenia.[5] UV Spectrophotometric method was developed and validated as per ICH guidelines.[6] Spectrophotometric method is generally preferred especially by small-scale industries as the cost of the equipment is less and the maintenance problems are minimal. The method of analysis is based on measuring the absorption of a monochromatic light by colorless compounds in the near ultraviolet path of spectrum (200-380 nm) [7-9]

Structure of Olanzapine

The chemical name of Olanzapine is 2-methyl-4-(4-methyl-1-piperazinyl)-10*H*-thieno[2,3-b][1,5]benzodi -azepine

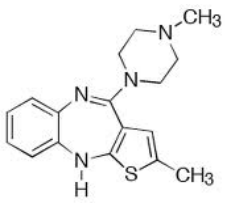

Fig. 1: Structure of Olanzapine

Some important properties

Chemistry

Olanzapine can be synthesized in a various chemical pathway. Olanzapine can be prepared starting from malononitrile and propionaldehyde: this is the most reliable synthetic pathway.

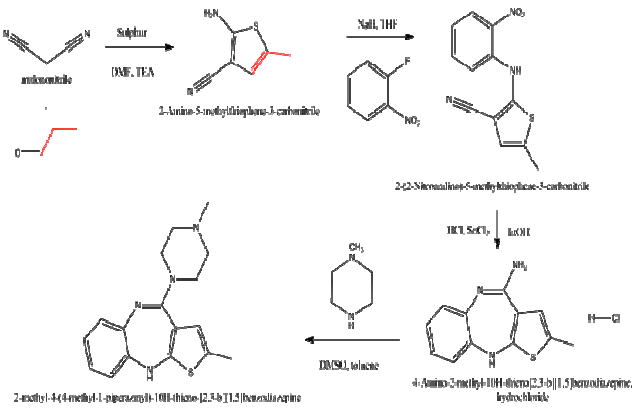

Fig. 2: Schematic diagram of Olanzapine synthesis

MATERIALS AND METHODS

Apparatus: UV spectrophotometer was used having 1 cm optical path length.

Method

Determination of Working Wave Length

In order to determine the wave length of maximum absorption (λ_{max}) of the drug, different concentrations of Olanzapine (5-40µg/ml) in water: hydrochloric acid (9:1) were scanned using UV-VIS spectrophotometer within the wave length region of 200-400 nm against water and HCl as blank. The resulting spectra were shown in

Fig. 8.1 and the absorption curve showed characteristic absorption maxima at 258 nm for Olanzapine.

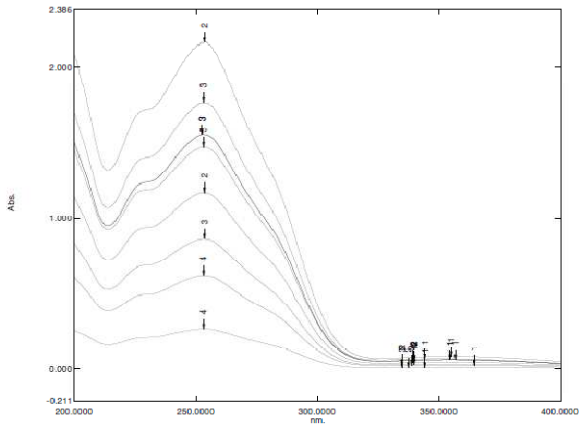

Fig. 3: Overlay UV-VIS Spectra of Olanzapine

Preparation of Calibration Curve

For preparation of calibration curve of Olanzapine, a stock solution of 1000µg/ml was prepared. From it different concentrations ranging from 5-40µg/ml prepared and were scanned at 258 nm in UV-VIS spectrophotometer.

Then the respective absorbances were noted, which are given in table no 8.2 A calibration curve was plotted using these readings taking concentration on X- axis and absorbance on Y-axis. From the calibration curve it was found that it shows linearity in the range of 5-40µg/ml with regression coefficient 0.998.

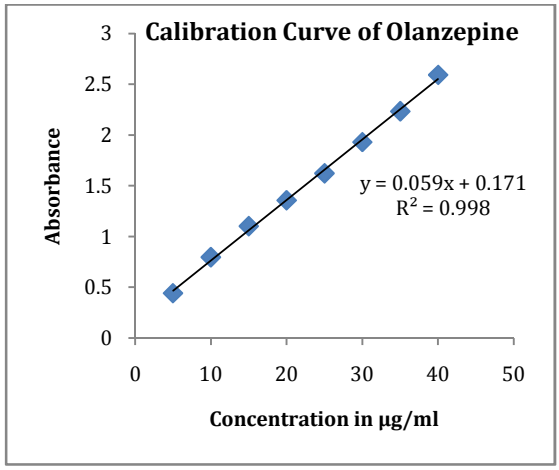

Fig. 4: Calibration Curve of Olanzapine

Optical characteristics of Olanzapine

The optical characteristics like Beer's Law Limit, Sandell's Sensitivity, Standard Deviation, % Relative Standard Deviation, Correlation Coefficient, Regression equation, Slope, Intercept, absorption Maxima are given below

Table 1: Some important properties of Olanzapine

Properties	Specifications
Formula	$C_{17}H_{20}N_4S$
Form	Powder
Color	Yellow
Molecular mass	312.439
Melting point	195 °C (383 °F)
Solubility in water	Practically insoluble in water mg/ml (20 °C)
Storage temperature	2-8°C
pH sensitivity	The water solubility is pH dependent and increase at low pH. The solubility is 87.4 mg/ml at pH 5, 0.193 mg/ml at pH 7 and 0.017 mg/ml at pH 9
pKa	Strongest acidic 14.17. strongest basic 7.24

Table 2: Optical Characteristics of Olanzapine

Beer's Law Limit	5-40 µg/ml
Sandell's Sensitivity (µg/cm2/0.001absorbance unit)	0.01128
Standard Deviation	0.0077
% Relative Standard Deviation	0.0704
Correlation Coefficient	998
Regression equation (Y)	Y=0.059X+0.171
Slope(a)	0.059
Intercept(b)	0.171
Absorption Maxima	258 nm

Validation parameters

Accuracy: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness. The accuracy data are given in table 3.

Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. The precision data are given in table 4. Precision is further subdivided into two parts:

Intra-day Precision

Intra-day precision simply means within run which assesses precision during a single analytical run. The intra-day precision data are given in table 5.

Inter-day precision

Inter-day precision simply means between-run which measures precision with time, and may involve different analysts, equipment, reagents, and laboratories. The inter-day precision data are given in table 6.

Robustness/ Ruggedness

The definition for robustness/ruggedness applied is the robustness/ruggedness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness can be described as the ability to reproduce the (analytical) method in different laboratories or under different circumstances without the occurrence of unexpected differences in the obtained results, and a robustness test as an experimental set-up to evaluate the robustness of a method. The term ruggedness is frequently used as a synonym.

Several definitions for robustness or ruggedness exist which are, however, all closely related. Robustness/ Ruggedness data are given in table 7 and table 8.

Limit of Detection and Limit of Quantitation

LOD: The Limit of Detection (LOD) of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value determined with statistical method by using Statistical formula. The limit of Detection (L.O.D.) was calculated as per below equation:

$$LOD = \frac{3.3 \text{ X } S.D}{Slpoe}$$

LOQ: The Limit of quantification (LOQ) of an individual analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with statistical method by using statistical formula. The limit of Quantification (L.O.Q.) was calculated as per below equation:

$$LOQ = \frac{10 \text{ X } S.D}{Slpoe}$$

The limit of detection (LOD) and limit of quantification (LOQ) data are given in table 9.

Assay and control of impurities

Assay procedures are intended to measure the analyte present in a given sample. In the context of this document, the assay represents a quantitative measurement of the major component(s) in the drug substance. For the drug product, similar validation characteristics also apply when assaying for the active or other selected component(s). The same validation characteristics may also apply to assays associated with other analytical procedures (e.g., dissolution). Testing for impurities can be either a quantitative test or a limit test for the impurity in a sample. Either test is intended to accurately reflect the purity characteristics of the sample. Different validation characteristics are required for a quantitative test than for a limit test. The assay data are given in table 10.

Stability Studies

Hydrolytic degradation

Hydrolytic degradation usually means the cleavage of chemical bonds by the addition of water. Generally, hydrolytic degradation or saccharification is a step in the degradation of a substance. This can be performed in three conditions i.e. neutral medium, acidic medium and basic medium.

Hydrolytic Degradation of Olanzapine in Neutral Condition:

Accurately weighed 10 mg bulk drug was taken in 10 ml volumetric flask. Then the volume was made with distilled water and refluxed for 5 h at 60ºC. The absorbance was measured in different hour by withdrawing the required amount of sample from the reaction mixture to prepare 25µg/ml concentration and subjected for UV analysis. The required data are given in table11.

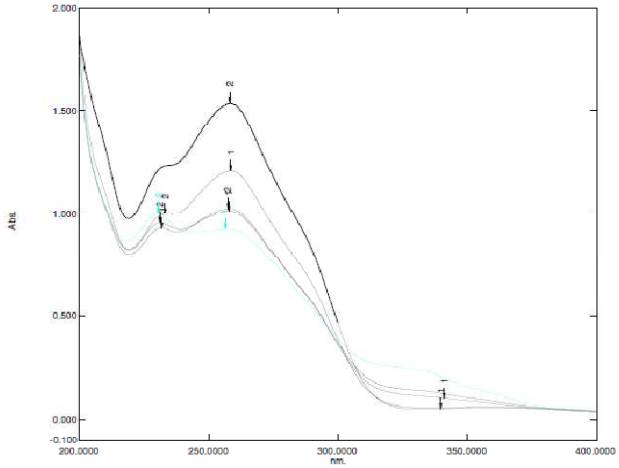

Fig. 5: UV-VIS spectrum of Olanzapine in Neutral Degradation

Hydrolytic Degradation of Olanzapine in Acidic Condition

Accurately weighed 10 mg bulk drug was taken in 10 ml volumetric flask. Then the volume was made with 0.1N HCl and refluxed for 5 h at 60ºC. Samples were withdrawn according to protocol. From the drawn samples 25µg/ml solution were prepared and subjected for analysis. The representative UV-VIS spectrum indicates degradation after 5 hr at 60⁰C. The required data are given in table12.

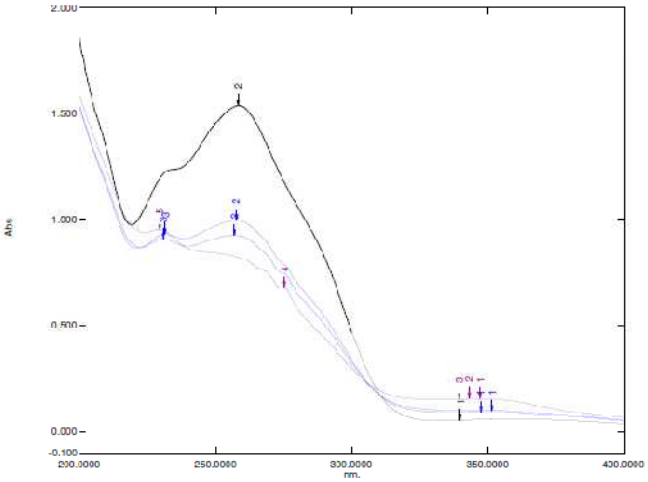

Fig. 6: UV-VIS spectrum of Olanzapine in Acidic Degradation

Hydrolytic Degradation of Olanzapine in Basic Condition

Accurately weighed 10 mg bulk drug was taken in 10 ml volumetric flask. Then the volume was made with 0.1N NaOH and refluxed for 5 h at 60ºC. Samples were withdrawn according to protocol. From the drawn samples 25µg/ml solution were prepared and subjected for analysis. The representative UV-VIS spectrum indicates degradation after 5 hr at 60⁰C. The required data are given in table13.

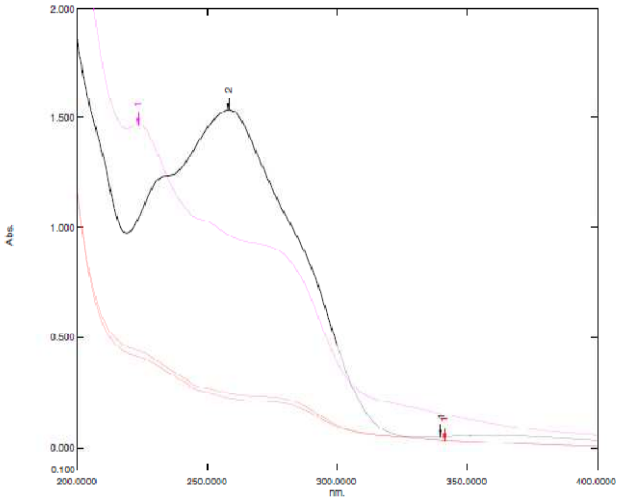

Fig. 7: UV-VIS spectrum of Olanzapine in Basic Degradation

Oxidative Degradation of Olanzapine

Accurately weighed 10 mg bulk drug was taken in 10 ml volumetric flask. Then the volume was made with 3% H_2O_2 and refluxed for 5 h at 60ºC. Samples were withdrawn according to protocol. From the drawn samples 25µg/ml solution were prepared and subjected for analysis. The representative UV-VIS spectrum indicates degradation after 5 hr at 60^{0}C. The required data are given in table 14.

Thermal Degradation of Olanzapine

Accurately weighed 100 mg bulk drug was taken in a covered Petridis. Then the same was kept in an oven for 7 days at 60ºC. Then Samples were withdrawn according to protocol. From the drawn samples 25µg/ml solution were prepared and subjected for analysis. The representative UV-VIS spectrum indicates degradation after 7 days.

RESULTS AND DISCUSSION

The objective of the present work was development and validation of UV spectral study and degradation of Olanzapine using UV spectrophotometer. The UV Spectra for Olanzapine were recorded at the wavelength of 258nm (λ max).

The method was found to be simple and the accuracy, precision, intra-day precision, inter-day precision, repeatability and assay were performed and the results were tabulated below. With this study the degradation pattern likes hydrolytic (Acidic, Alkaline and Neutral) and oxidative degradation were also studied and results were given below:

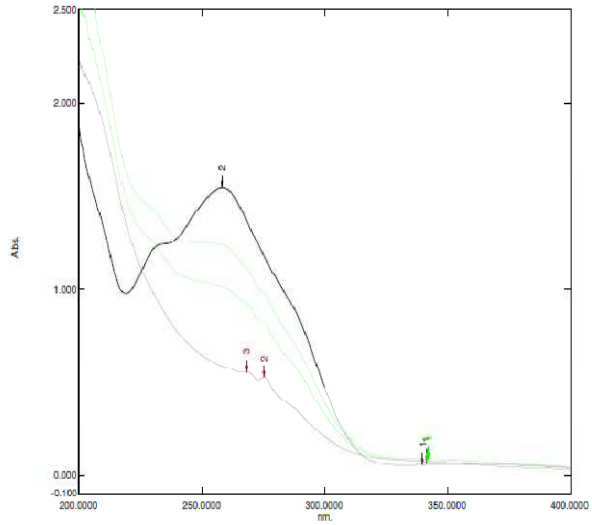

Fig. 8: UV-VIS spectrum of Olanzapine in Oxidative Degradation

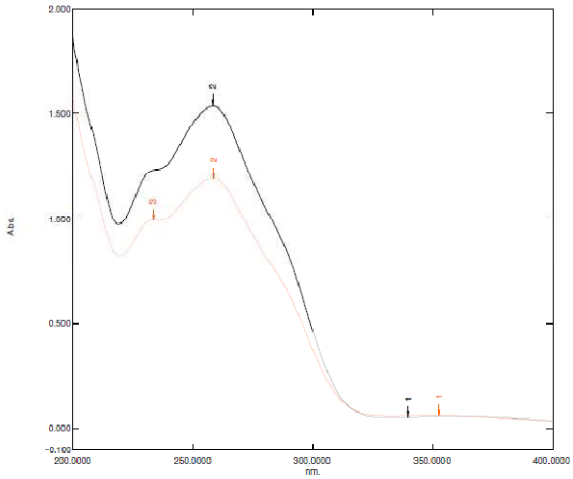

Fig. 9: UV-VIS spectrum of Olanzapine in Thermal Degradation

Table 3: Accuracy Data of the UV-VIS Spetrophotometric Method for Olanzapine

Samples	Concentration (µg/ml)		% Recovery	Statistical Analysis
	Pure	Formulation		
S_1:80%	16	20	98.72	Mean = 98.31
S_2:80%	16	20	97.36	S.D = 0.1846
S_3:80%	16	20	98.85	% R.S.D = 0.2160
S_4:100%	20	20	100.08	Mean = 99.13
S_5:100%	20	20	98.25	S.D = 0.2510
S_6:100%	20	20	99.06	% R.S.D = 0.3449
S_7:120%	24	20	100.43	Mean = 99.68
S_8:120%	24	20	99.26	S.D = 0.895
S_9:120%	24	20	99.37	% R.S.D = 0.885

Table 4: Precision Data Showing Repeatability of the UV-VIS Spetrophotometric Method for Olanzapine

S. No.	Concentration (µg/ml)	Absorbance	Calculated amount (µg/ml)	Statistical Analysis
1	20	1.358	20.11	
2	20	1.361	20.16	Mean=20.03
3	20	1.349	19.96	S.D=0.170

4	20	1.354	20.05	%RSD=0.568
5	20	1.347	19.93	
6	20	1.352	20.01	

Table 5: Intra Day Precision Data of the UV-VIS Spetrophotometric Method for Olanzapine

Conc.(µg/ml)	Absorbance1	Absorbance2	Absorbance3	Statistical Analysis
20	1.337	1.360	1.363	
20	1.345	1.352	1.378	Mean=20.07
20	1.341	1.350	1.371	S.D=0.012
20	1.331	1.342	1.370	%R.S.D=0.156
20	1.346	1.357	1.374	
20	1.348	1.359	1.379	
Mean	1.341	1.353	1.372	
Calc.Amt.	19.83	20.03	20.35	

Table 6: Inter Day Precision Data of the UV-VIS Spetrophotometric Method for Olanzapine

Conc.(µg/ml)	Abs.(Day 1)	Abs.(Day 2)	Abs.(Day 3)	Statistical Analysis
20	1.325	1.364	1.381	
20	1.328	1.344	1.388	Mean =20.10
20	1.351	1.358	1.382	S.D =0.046
20	1.330	1.371	1.377	%R.S.D =0.577
20	1.355	1.338	1.380	
20	1.321	1.356	1.384	
Mean	1.335	1.355	1.382	
Calc. Amt.	19.72	20.06	20.52	

Table 7: Ruggedness Data of the UV-VIS Spectrophotometric Method by Different Analyst for Olanzapine

Analyst-1				Analyst-1			
Conc. (µg/ml)	Abs.	Calc. Amt. (µg/ml)	Statistical Analysis	Conc. (µg/ml)	Abs.	Calc. Amt. (µg/ml)	Statistical Analysis
20	1.317	19.42	Mean=19.49	20	1.329	19.62	Mean=19.81
20	1.336	19.74	S. D=0.037	20	1.341	19.83	S.D=0.048
20	1.324	19.54	%RSD=0.124	20	1.338	19.77	%RSD=0.162
20	1.322	19.50		20	1.349	19.96	
20	1.311	19.32		20	1.339	19.79	
20	1.318	19.44		20	1.348	19.94	

Table 8: Robustness Data of the UV-VIS Spectrophotometric Method by Different Solvent Composition for Olanzapine

Water: HCl (92:08)				Water: HCl (88:12)			
Conc. (µg/ml)	Abs	Calc. Amt. (µg/m)	Statistical Analysis	Conc. (µg/ml)	Abs	Calc. Amt. (µg/m)	Statistical Analysis
20	1.342	19.84	Mean=19.94	20	1.367	20.27	Mean=19.97
20	1.364	20.22	SD=0.092	20	1.378	20.45	SD=0.117
20	1.321	19.49	%RSD=0.308	20	1.326	19.57	%RSD=0.391
20	1.335	19.72		20	1.318	19.44	
20	1.357	20.10		20	1.365	20.23	
20	1.368	20.28		20	1.344	19.88	

Table 9: Limit of detection and Limit of quantitation of Olanzapine

S. No.	Parameters	S.D	Slope(b)	Formula	Calculation(µg/ml)
1	LOD	0.0077	0.059	3.3(S. D/b)	0.4306
2	LOQ	0.0077	0.059	10(S. D/b)	1.305

Table 10: Assay Data of Olanzapine Formulations

Formulation	Labeled claim (mg/tab.)	Observed Amount(+S.D)mg	% Recovery	%R.S.D
Oleanz 5	5	4.79 + 0.063	95.80	0.621
Zyprexa	5	4.92 + 0.069	98.40	0.087

Table 11: Hydrolytic Degradation of Olanzapine in Neutral Condition

S. No..	Name	Absorbance	Conc.	%Degradation
1	Drug	1.646	25	0
2	Degradation1	1.216	17.71	29.16
3	Degradation2	0.984	13.77	44.92
4	Degradation3	0.957	13.32	46.72
5	Degradation4	0.849	11.49	54.04

Table 12: Hydrolytic Degradation of Olanzapine in Acidic Condition:

S. No.	Name	Absorbance	Conc.	%Degradation
1	Drug	1.646	25	0
2	Degradation1	1.025	14.47	42.12
3	Degradation2	0.891	12.20	51.20
4	Degradation3	0.783	10.37	58.52

Table 13: Hydrolytic Degradation of Olanzapine in Basic Condition

S. No.	Name	Absorbance	Conc.	%Degradation
1	Drug	1.646	25	0
2	Degradation1	0.918	12.66	49.36
3	Degradation2	0.453	4.77	80.92
4	Degradation3	0.417	4.16	80.36

Table 14: Oxidative Degradation of Olanzapine

S. No.	Name	Absorbance	Conc.	%Degradation
1	Drug	1.646	25	0
2	Degradation1	1.354	20.05	19.80
3	Degradation2	0.991	13.89	44.44
4	Degradation3	0.574	6.83	72.68

Table 15: Thermal Degradation of Olanzapine

S. No.	Name	Absorbance	Conc.	%Degradation
1	Drug	1.646	25	0
2	Degradation1	1.368	20.28	18.88

CONCLUSION

The proposed method was simple, sensitive and reliable with good precision and accuracy. This method is specific while estimating the commercial formulation without interference of excipients and the other additives. Hence, it can be used for routine determination of Olanzapine in bulk sample and pharmaceutical formulation. The proposed method for stability study shows that there is appreciable degradation found in stress condition.

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