Method Development, Validation and Force Degradation Stability Study of Olanzapine by UV-VIS Spectroscopy

Meenu Chaudhary*, Praveen Kumar[1], Anjali Gusain[2]

School of Pharmaceutical Sciences,
Shri Guru Ram Rai University, Dehradun, India

Abstract: A simple, unique and dependable UV-VIS spectrophotometric method used to be as soon as developed for the estimation of Olanzapine in bulk and pharmaceutical dosage forms. Water: Hydrochloric acid (9:1) was chosen as the solvent system. The λmax was discovered to be 257 nm and the response Lenier in the vary of 2-12µg/ml. The regression equation of the calibration format and correlation coefficient had been found to be Y = 0.0779+0.002 and 1.0 respectively. The %RSD values for each intraday and interday precision had been less than 1% the recovery of the drug from the pattern was once in precise ranged.

The proposed approach used to be validated for accuracy, precision, robustness, ruggedness, LOD and LOQ whilst estimating the commercial components there was no interference of excipients nd other additives. The proposed approach for balance find out about suggests that there was considerable degradation located in stress circumstance of Olanzapine. Forced degradation research (stress testing) are very necessary tool in pharmaceutical look up and development to predict long-term stability. Stress studies need o be carriedout in approach improvement to understand drug behavior however also can be carried out with approach validation for regulatory filling predict stability and measure impurities.

Key words: Olanzapine, UV-VIS Spectrophotometric, validation Study and Force Degradation study.

1. INTRODUCTION

A simple, special and reliable UV-VIS spectrophotometric method was once developed for the estimation of Olanzapine in bulk and pharmaceutical dosage forms. Water: Hydrochloric acid (9:1) was chosen as the solvent system. The λmax used to be found to be 257 nm and the response Drug balance refers to the capacity of the drug substance or product to continue to be within hooked up specification of identification, strength, fine and purity in a detailed period of time. Stability is formally defined as the time laps for the duration of which the drug product retains the equal houses characteristics that is proposed at the time of manufacture. The balance of the product is expressed as the expiry duration or technically as shelf lifestyles. [1,2]

Objective of the Stability Study:

The guidelines for stability study are given by ICH: [3,4]

Q1A (R2): Stability testing of new drug substance and products

Q1B: Stability testing: photo stability testing of new drug substance and products

Q1C: Stability testing of new dosage forms

Q1D: Bracketing and matrixing design for stability testing of new drug substances and products

Q1E: Evaluation of stability studies.

Q1F: Stability Data Package for Registration Applications in Climatic Zones III and IV

2. MATERIALS AND METHODS

Materials which are used for study of validation and force degradation study of olanzapine are given as well as following.

Table 1.1: - **List of Chemicals used for preparation of Olanzapine**

S.No.	Drug / Excipient / Solvent	Manufacturer / Supplier
1.	Olanzapine (API)	Gift Sample
2.	Hydrochloric Acid	Rankem A Grade
3.	Sodium Hydroxide	Rankem A Grade
4.	Hydrogen Peroxide (H_2O_2)	Rankem A Grade

Table No- 1.2. List of Equipment's used for preparation of Olanzapine MDTs

S. No.	Instruments / Glassware's	Manufacturer/ Supplier
1.	UV-visible double Beam Spectrophotometer	Schimadzu, Mumbai
2.	Fourier Transmission Infra-Red Spectrophotometer	Agilent
3.	Mechanical stirrer	Remi Elektrotech Ltd, Mumbai
4.	Analytical Weighing Balance	Citizone
5.	Digital Sonicator	Rivotek
6.	Digital pH meter	Systronics, Delhi
7.	Digital melting point apparatus	Perfit, Ambala cant
8.	Magnetic stirrer	Remi Elektrotech Ltd, Mumbai
9.	Volumetric Glass	Borosil A Grade
10.	Measuring Cylinder	Borosil A Grade

11.	Graduate Pippete	Borosil A Grade
12.	Bulb Pippete	Borosil A Grade
13.	Glass Beaker	Borosil A Grade
14.	Glass Road	Borosil A Grade
15.	Funnel	Borosil A Grade

METHODS:

1. IDENTIFICATION OF DRUG:-

A. Organoleptic Characteristics:-

The color, Odor, and taste of the drug were characterized and recorded.

B. Determination of Melting point:

The drug will be filled in one end fused Capillary tube and kept into digital melting point apparatus. The apparatus will operated and the temperature at which drug will start melting will be noted as melting point**.**

C. Determination The wavelength (λ_{max}) of Olanzapine in 0.1 N HCl:-Standard stock solution of Olanzapine prepared by dissolving 50 mg of Olanzapine in 50 ml of 0.1 N HCl and sonicated for 15 minutes in bath Sonicator and prepares dilution of 1 mg/1 ml i.e. 1000 µg/ml (1000 ppm) stock solution. From this stock solution prepared 10 µg/ml solutions. Scan the sample at their standard λ_{max} and determine the wavelength of Olanzapine

D. Preparation of standard plot of Olanzapine in 0.1 N HCl: -

Standard stock solution of Olanzapine will be prepared by dissolving 50 mg of Olanzapine in 50 ml of 0.1 N HCl and sonicated for 15 minutes in bath Sonicator and prepare dilution of 1 mg/1 ml i.e. 1000 µg/ml (1000 ppm) stock solution. From this stock solution we can prepare

2-12 ppm solution, scan the sample at their standard λ_{max} and prepared standard plot of olanzapine.

VALIDATION PARAMETER:

1. **Accuracy:** The accuracy of an analytical method expresses the closeness of settlement between the fee which is usual either as a traditional proper fee or an standard reference price and the fee found. This is every so often termed trueness. The accuracy information is given in table.
2. **Precision:** The precision of an analytical process expresses the closeness of settlement (degree of scatter) between a sequences of measurements acquired from more than one sampling of the equal homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision be investigated using homogeneous, authentic samples. However, if it is no longer feasible to reap a homogeneous sample it might also be investigated the usage of artificially organized samples or

a sample solution.The precision of an analytical process is usually expressed as the variance, trendy deviation or coefficient of variant of a collection of measurements.The precision data are given in ta. Precision is similarly subdivided into two parts

a. Intra-day Precision:

Intra-day precision simply means within run which assesses precision during a single analytical run.

b. Inter-day precision

Inter-day precision simply means between-run which measures precision with time, and may involve different analysts, equipment, reagents, and laboratories.

3. 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.

4. Limit of Detection

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:

$$\textbf{Limit of Detection} = \frac{\textbf{3.3} * \textbf{S.D.}}{\textbf{Slope}}$$

5. Limit of Quantitation

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 (LOQ) was calculated as per below equation:

$$\textbf{Limit of Quantitation} = \frac{\textbf{10.0} * \textbf{S.D.}}{\textbf{Slope}}$$

3. FORCE DEGRADATION STABILITY STUDY OF OLANZAPINE:

1. Hydrolytic degradation

Hydrolytic degradation usually means the cleavage of chemical bonds by the addition of water. Generally, hydrolytic degradation or scarification 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.

A. Hydrolytic Degradation of Olanzapine in Neutral Condition:

Accurately weighed 100 mg Olanzapine was taken in 100 ml volumetric flask. Then the volume was made with distilled water and refluxed for 3 Day at 60ºC. The absorbance was measured in different hour by withdrawing the required amount of sample from the reaction mixture to prepare 12µg/ml concentration and subjected for UV analysis.

B. Hydrolytic Degradation of Olanzapine in Acidic Condition

Accurately weighed 100 mg Olanzapine was taken in 100 ml volumetric flask. Then the volume was made with 0.1N HCl and refluxed for 3 day at 60ºC. Samples were withdrawn according to Protocol. From the drawn samples 12µg/ml solution were prepared and subjected for analysis. The representative UV-VIS spectrum indicates degradation after 1 day at 60°C.

C. Hydrolytic Degradation of Olanzapine in Basic Condition

Accurately weighed 100 mg Olanzapine was taken in 100 ml volumetric flask. Then the volume was made with 0.1N NaOH and refluxed for 3 day at 60ºC. Samples were withdrawn according to protocol. From the drawn samples 12µg/ml solution were prepared and subjected for analysis. The representative UV-VIS spectrum indicates degradation after 1 day at 60°C.

2. Oxidative Degradation of Olanzapine

Accurately weighed 100 mg Olanzapine was taken in 100 ml volumetric flask. Then the volume was made with 3% H_2O_2 and refluxed for 3 day at 60°C.. Samples were withdrawn according to protocol. From the drawn samples 12µg/ml solution were prepared and subjected for analysis. The representative UV-VIS spectrum indicates degradation after 1 day at 60°C.

3. Thermal Degradation of Olanzapine

Accurately weighed 1000 mg Olanzapine was taken in a covered Petridis. Then the same was kept in an oven for 7 days at 60°C Then Samples were withdrawn according to protocol. From the drawn samples 12µg/ml solution were prepared and subjected for analysis. The representative UV-VIS spectrum indicates degradation after 7 days.

Table 1.3: Conditions for Forced Degradation Studies.

Degradation Type	Experimental Conditions	Storage Conditions	Sampling Time (days)
Hydrolysis	Control API (No acid or base) 0.1M HCl 0.1 M NaOH Acid control (No API) Base control (no API)	40˚C, 60˚C	1,3,5
Oxidation	3% H_2O_2 (Peroxide)	25˚C, 60˚C	1,3,5
Photolytic	Light 1 × ICH Light 2 × ICH Light 3 × ICH	NA	1,3,5
Thermal	Heat chamber	60˚C	3,5,7

4. RESULT AND DISCUSSION:

1. IDENTIFICATION STUDY:-

A. Organoleptic Characteristics:-

Table No. – 1.4: The Organoleptic Properties of Olanzapine as well as following,

S.NO.	Organoleptic Properties	Result
1.	Color	Yellow crystalline Powder
2.	Odor	Characteristics
3.	Taste	Characteristics

B. Determination of Melting Point

Melting point of Olanzapine was found to be 195.21 ± 0.95°C (Table 1.5). From the observation of the melting point, the drug can be considered to be sufficiently pure for employing it is present investigation. Melting point in Merck Index is 190-195°C.

Table No. 1.5: - Result of Melting Point Determination of Olanzapine.

Observed Melting Point (°C)			**Mean ± S.D. (n =3)**
Sample 1	**Sample 2**	**Sample 3**	
194.66	195.40	195.58	195.21 ± 0.95

2. RESULT OF ANALYSIS:

A. Standard Curve of Olanzapine in 0.1 N HCl:-

The standard plots of Olanzapine were prepared in 0.1 N HCl. This indicate that the standard curve of Olanzapine in above media followed Beer law, R^2 values were found to be in between 1.0, the linear regression equation can be used of Olanzapine is 0.1 N HCl media.

Final Wavelength (λ_{max}) of Olanzapine is = 257 nm in 0.1N HCl Media

Table No 1.6:- Standard Plot of Olanzapine in 0.1 N HCl.

S. No.	**Concentration (µg/ml.)**	**Absorbance**			**Mean ± S.D.**
		Sample 1	**Sample 2**	**Sample 3**	
1.	Blank	0.0000	0.0000	0.0000	0.0000 ± 0.0000
2.	2.0	0.155	0.160	0.159	0.158 ± 0.002
3.	4.0	0.315	0.311	0.316	0.314 ± 0.002
4.	6.0	0.486	0.476	0.469	0.471 ± 0.004
5.	8.0	0.620	0.628	0.630	0.626 ± 0.005
6.	10.0	0.778	0.784	0.781	0.781 ± 0.003
7.	12.0	0.930	0.935	0.934	0.934 ± 0.003

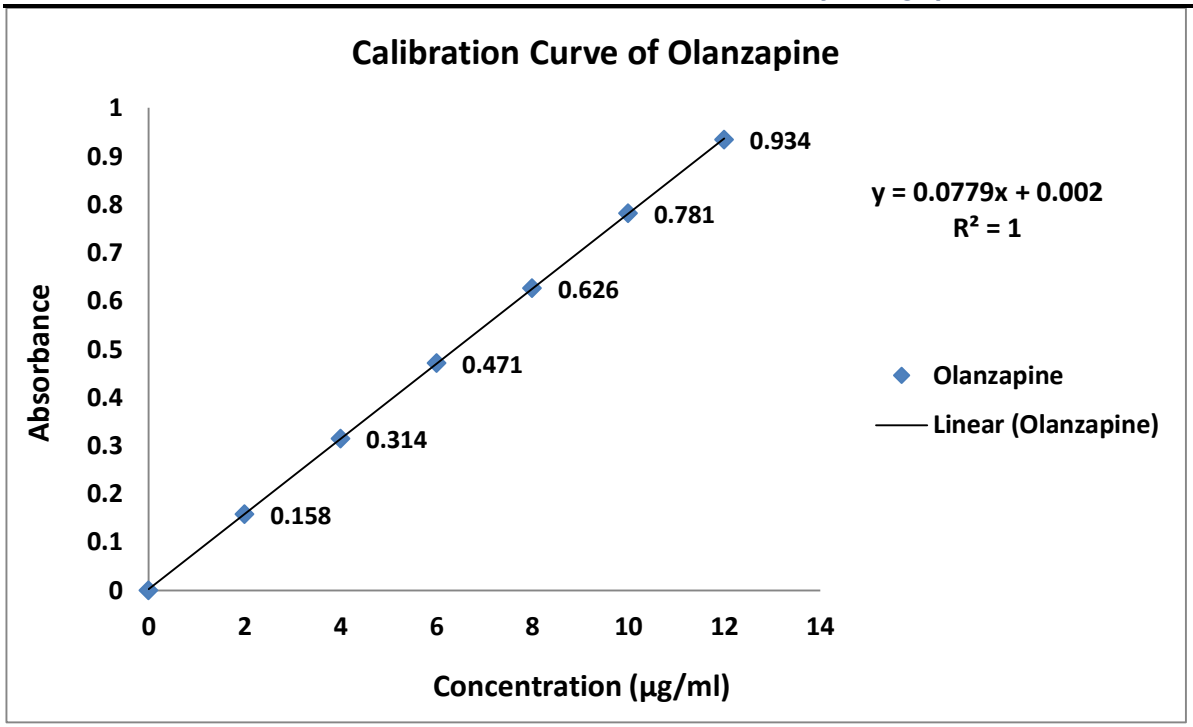

Figure 1: - Calibration Curve of Olanzapine.

Table No. 1.7:- Regression equation and correlation coefficient of Olanzapine in 0.1 N HCl.

S. No	Media	Regression Equation	Correlation Coefficient (R^2)
1.	0.1 N HCl	Y= 0.0779x + 0.002	1.0

VALIDATION RESULTS OF OLANZAPINE:

1. ACCURACY:

Table 1.8: Accuracy Data of the UV-VIS Spectrophotometric Method for Olanzapine

Sample	Concentration (µg/ml.)		Absorbance	% Recovery	Statistical Analysis
	Pure Concentration	Final Concentration			
SMP_1:80%	8	10	0.611	97.72	**Mean:-98.52 S.D.- 0.73 % RSD- 0.74**
SMP_2:80%	8	10	0.620	99.16	
SMP_3:80%	8	10	0.617	98.68	
SMP_4:100%	10	10	0.780	99.87	**Mean:-99.22 S.D.- 1.45 % RSD- 1.46**
SMP_5:100%	10	10	0.762	99.56	
SMP_6:100%	10	10	0.783	100.25	
SMp_7:120%	12	10	0.928	99.05	**Mean:-99.44 S.D.- 0.50 % RSD- 0.51**
SMp_8:120%	12	10	0.937	100.02	
SMp_9:120%	12	10	0.930	99.27	

2. PRECISION:

(a.) Repeatability:

Table 1.9: Precision Data Showing Repeatability of the UV-VIS Spectrophotometric Method for Olanzapine.

S.No.	Concentration (µg/ml.)	Absorbance	Calculated Amount (µg/ml.)	Statistical Analysis
1.	10	0.788	10.08	**Mean:- 10.06** **S.D.- 0.067** **% RSD- 0.67**
2.	10	0.780	9.98	
3.	10	0.786	10.06	
4.	10	0.781	10.0	
5.	10	0.790	10.11	
6.	10	0.794	10.16	

(b.) Intraday Precision:

Table 1.10: Intra Day Precision Data of the UV-VIS Spectrophotometric Method for Olanzapine

Concentration (µg/ml.)	Sample-1 (Abs.)	Sample-2 (Abs.)	Sample-3 (Abs.)	Statistical Analysis
10	0.782	0.783	0.782	**Mean:- 10.02** **S.D.- 0.01** **% RSD- 0.09**
10	0.790	0.779	0.785	
10	0.780	0.782	0.779	
10	0.776	0.786	0.787	
10	0.788	0.790	0.785	
10	0.784	0.781	0.791	
MEAN	**0.783**	**0.782**	**0.784**	
Cal. Amount (µg/ml.)	**10.02**	**10.01**	**10.03**	

(c.) Interday Precision:

Table 1.11: Inter Day Precision Data of the UV-VIS Spectrophotometric Method for Olanzapine

Concentration (µg/ml.)	Sample (Abs.) (Day-1)	Sample (Abs.) (Day-2)	Sample (Abs.) (Day-3)	Statistical Analysis
10	0.783	0.787	0.774	**Mean:- 10.0** **S.D.- 0.020** **% RSD- 0.20**
10	0.787	0.780	0.785	
10	0.792	0.790	0.778	
10	0.790	0.783	0.784	
10	0.781	0.780	0.775	
10	0.776	0.776	0.789	
MEAN	**0.784**	**0.782**	**0.780**	
Cal. Amount (µg/ml.)	**10.03**	**10.01**	**9.98**	

3. Ruggedness Data:

Table 1.12: Ruggedness Data of the UV-VIS Spectrophotometric Method by Different Analyst for Olanzapine.

Analyst-1				Analyst-2			
Conc^{n} (µg/ml.)	**Abs.**	**Cal. Amt. (µg/ml.)**	**Statistical Analysis**	**Conc^{n} (µg/ml.)**	**Abs.**	**Cal. Amt. (µg/ml.)**	**Statistical Analysis**
10	0.792	10.14	**Mean:- 10.5** **S.D.- 0.061** **% RSD- 0.61**	10	0.786	10.06	**Mean:- 10.0** **S.D.- 0.020** **% RSD- 0.20**
10	0.785	10.05		10	0.778	9.96	
10	0.789	10.10		10	0.784	10.03	
10	0.784	10.03		10	0.790	10.11	
10	0.779	9.97		10	0.787	10.07	
10	0.782	10.01		10	0.783	10.02	

4. Robustness Data:

Table 1.13: Robustness Data of the UV-VIS Spectrophotometric Method by Different Analyst for Olanzapine.

WATER: HCl (90:10)				WATER: HCl (85:15)			
Conc^{n} (µg/ml.)	**Abs.**	**Cal. Amt. (µg/ml.)**	**Statistical Analysis**	**Conc^{n} (µg/ml.)**	**Abs.**	**Cal. Amt. (µg/ml.)**	**Statistical Analysis**
10	0.774	9.91	**Mean:- 10.01 S.D.- 0.08 % RSD- 0.80**	10	0.783	10.02	**Mean:- 10.01 S.D.- 0.072 % RSD- 0.72**
10	0.786	10.06		10	0.775	9.92	
10	0.776	9.93		10	0.788	10.08	
10	0.790	10.11		10	0.781	10.0	
10	0.787	10.07		10	0.777	9.94	
10	0.784	10.03		10	0.789	10.10	

5. Limit of Detection (LOD) & Limit of Quantitation (LOQ): -

Table 1.14: Limit of detection and Limit of quantitation of Olanzapine by UV-VIS Spectrophotometric Method.

S.No.	Parameter	Standard Deviation	Slope	Formula	Calculation (µg/ml.)
1.	**Limit od Detection (LOD)**	0.009	0.0779	3.3*(S.D./Slope)	0.381
2.	**Limit of Quantitation (LOQ)**	0.009	0.0779	10*(S.D./Slope)	1.15

FORCE DEGRADATION STABILITY STUDY OF OLANZAPINE

1. Hydrolytic Degradation of Olanzapine:

(A.) Hydrolytic Degradation of Olanzapine in Neutral Condition:

Table 1.15: Hydrolytic Degradation of Olanzapine in Neutral Condition..

S.NO.	Name	Absorbance	Concentration (µg/ml.)	% Degradation
1.	Olanzapine	0.937	12.0	0
2.	Degradation-1	0.684	8.75	27.04
3.	Degradation-2	0.523	6.68	44.26
4.	Degradation-3	0.486	6.21	48.22
5.	Degradation-4	0.412	5.26	56.14

(B.) Hydrolytic Degradation of Olanzapine in Acidic Condition:

Table 1.16: Hydrolytic Degradation of Olanzapine in Acidic Condition..

S.NO.	Name	Absorbance	Concentration (µg/ml.)	% Degradation
1.	Olanzapine	0.937	12.0	0
2.	Degradation-1	0.511	6.53	45.0
3.	Degradation-2	0.429	5.48	54.32
4.	Degradation-3	0.386	4.92	58.92

(C.) Hydrolytic Degradation of Olanzapine in Basic Condition:

Table 1.17: Hydrolytic Degradation of Olanzapine in Basic Condition.

S.NO.	Name	Absorbance	Concentration (µg/ml.)	% Degradation
1.	Olanzapine	0.937	12.0	0
2.	Degradation-1	0.503	6.43	46.40
3.	Degradation-2	0.317	4.04	66.30
4.	Degradation-3	0.245	3.11	74.0

2. Oxidative Degradation of Olanzapine:

Table 1.18: Oxidative Degradation of Olanzapine.

S.NO.	Name	Absorbance	Concentration (µg/ml.)	% Degradation
1.	**Olanzapine**	0.937	12.0	0
2.	**Degradation-1**	0.782	10.01	16.55
3.	**Degradation-2**	0.573	7.32	38.91
4.	**Degradation-3**	0.285	3.63	69.72

3. Thermal Degradation of Olanzapine:

Table 1.19: Thermal Degradation of Olanzapine.

S.NO.	Name	Absorbance	Concentration (µg/ml.)	% Degradation
1.	**Olanzapine**	0.937	12.0	0
2.	**Degradation-1**	0.803	10.28	14.31

CONCLUSION:

The proposed technique was simple, sensitive and reliable with accurateprecision and accuracy. This approach is precise while estimating the business method barring interference of excipients and the different additives. Hence, it can be used for routine determination of Olanzapine in bulk sample. The proposed technique for Force degradation balance study indicates that there is considerable degradation located in stress condition.

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