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Development and Validation of a Stability-Indicating RP-HPLC Method for the Estimation of Olanzapine in Pharmaceutical Dosage Forms

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Abstract:

A simple, rapid, accurate, precise, and stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the quantitative estimation of Olanzapine in bulk drug and pharmaceutical dosage forms. Chromatographic separation was achieved on a Thermo C18 column (250 mm × 4.6 mm, 5 µm) using a mobile phase consisting of phosphate buffer (1.75 g KH_2PO_4 in 1000 mL water, pH 6.0 adjusted with orthophosphoric acid, containing 1 mL triethylamine) and acetonitrile in a ratio of 60:40 (v/v) at a flow rate of 1.0 mL/min. UV detection was carried out at 257 nm. Olanzapine showed a retention time of approximately 2.37 min. The method was linear over the concentration range of 5–25 µg/mL (r^2 = 0.998). Accuracy studies demonstrated recovery values of 99.75–100.10%. Precision studies yielded %RSD values below 2.0% for all parameters. Robustness evaluation confirmed stability under minor variations in chromatographic conditions. Forced degradation studies under acidic, alkaline, oxidative, and thermal stress conditions demonstrated the stability-indicating nature of the method. The developed method was validated as per International Conference on Harmonisation (ICH) Q2(R1) guidelines and is suitable for routine quality control and stability analysis of Olanzapine in pharmaceutical formulations.

Keywords: Olanzapine; RP-HPLC; Method validation; Stability-indicating; Forced degradation; ICH guidelines; Pharmaceutical dosage forms

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1. INTRODUCTION

Olanzapine (2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-b][1,5]benzodiazepine) is an atypical antipsychotic agent belonging to the thienobenzodiazepine class. Approved by the United States Food and Drug Administration (FDA), it is widely indicated for the treatment of schizophrenia and bipolar disorder. Olanzapine is structurally related to clozapine and quetiapine and exerts its therapeutic effects primarily through antagonism at dopamine D1, D2, and D4 receptors, and serotonin 5-HT_2 receptors, with greater activity at serotonin receptors than at dopaminergic receptors. Additional antagonism at muscarinic, histamine H1, and alpha-1 adrenergic receptors further contributes to its pharmacological profile.

The drug possesses a molecular formula of $C_{17}H_{20}N_4S$, a molecular weight of 312.4 g/mol, and a melting point of 195–198°C. Olanzapine is a yellow crystalline powder that is freely soluble in dichloromethane, methanol, acetonitrile, and 0.1 N HCl but is practically insoluble in water and phosphate buffer pH 7.4. These physicochemical characteristics are critical for the design of appropriate analytical methods.

Pharmaceutical analysis plays a pivotal role in ensuring the quality, safety, and efficacy of drug formulations. Each regulatory submission, including New Drug Applications (NDA) and Abbreviated New Drug Applications (ANDA), mandates inclusion of validated analytical procedures to ensure the identity, strength, quality, purity, and potency of the drug substance and drug product. Stability-indicating analytical methods are particularly essential, as they must demonstrate the ability to distinguish the active drug from its degradation products under forced degradation conditions.

A literature survey revealed that while several HPLC and spectrophotometric methods have been reported for the analysis of Olanzapine, there is a paucity of fully validated stability-indicating RP-HPLC methods suitable for routine quality control and stability testing. The present investigation was therefore undertaken to develop and validate a simple, sensitive, accurate, precise, and stability-indicating RP-HPLC method for the estimation of Olanzapine in pharmaceutical dosage forms, in full compliance with ICH Q2(R1) guidelines.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Olanzapine working standard was procured from Scan Research Laboratories, Bhopal, India. Marketed tablet formulation (Oleanz-5®, 5 mg, Micro Labs, Bangalore, India; Batch No. RTA 0125) was obtained commercially. HPLC-grade acetonitrile and methanol, analytical-grade methanol, potassium dihydrogen phosphate (KH_2PO_4), triethylamine (TEA), orthophosphoric acid (OPA), and hydrogen peroxide (3% v/v) were procured from Merck Ltd., India. HPLC-grade water was used throughout the study.

2.2 Instruments

The HPLC system consisted of a Waters pump, a UV-Visible detector, a Thermo C18 column (250 × 4.60 mm, 5 µm particle size), a Lichrocart HPLC guard cartridge system, and Data Ace software for data acquisition and processing. A Labindia 3000+ UV/Vis spectrophotometer with 1 cm quartz cells was used for wavelength selection. Melting point was determined using a melting point apparatus. Infrared spectra were recorded using a FTIR spectrophotometer by the KBr pellet method.

2.3 Drug Characterization

Physicochemical characterization of Olanzapine was performed to confirm its identity and suitability for analysis. The melting point was determined and compared with the reported value. Solubility was assessed by the Indian Pharmacopoeia (IP) method in various solvents. The infrared spectrum was obtained by the KBr pellet method and characteristic absorption peaks were assigned to functional groups. The wavelength of maximum absorption (λmax) was determined by scanning a dilute solution of Olanzapine in the mobile phase over the 200–400 nm UV range.

2.4 Chromatographic Conditions

The optimized chromatographic conditions were established after systematic evaluation of multiple mobile phase compositions, column types, flow rates, and detection wavelengths. The final conditions employed were: column, Thermo C18 (250 mm × 4.6 mm, 5 µm); mobile phase, phosphate buffer (1.75 g KH_2PO_4 dissolved in 1000 mL water, 1 mL TEA added, pH adjusted to 6.0 with OPA): acetonitrile (60:40, v/v); flow rate, 1.0 mL/min; detection wavelength, 257 nm; injection volume, 20 µL; column temperature, ambient.

2.5 Preparation of Standard Solutions

A stock solution of Olanzapine (1000 µg/mL) was prepared by accurately weighing 10 mg of Olanzapine working standard and dissolving it in 10 mL of Water:Acetonitrile(50:50, v/v). Working standard solutions at concentrations of 5, 10, 15, 20, and 25 µg/mL were prepared by appropriate serial dilutions of the stock solution using the same solvent mixture.

2.6 Assay of Tablet Formulation

For the tablet assay, a weight equivalent to 10 mg of Olanzapine was transferred to a 10 mL volumetric flask and dissolved in the mobile phase with vigorous shaking for 20 minutes. The solution was filtered through Whatman filter paper No. 41 and made up to volume with the mobile phase. The solution was further diluted to obtain a final concentration of 10 µg/mL and analyzed by HPLC. The assay was performed in six replicates. The percentage drug content was determined by extrapolating the peak area against the calibration curve.

2.7 Method Validation

The developed RP-HPLC method was validated in accordance with ICH Q2(R1) guidelines for the following parameters: linearity, accuracy, precision (repeatability, intra-day and inter-day), robustness, limit of detection (LOD), and limit of quantification (LOQ).

Linearity was evaluated over the concentration range of 5–25 µg/mL by plotting peak area versus concentration and determining the regression equation and correlation coefficient. Accuracy was assessed by recovery studies at three levels: 80%, 100%, and 120% of the nominal concentration, added to a pre-analyzed sample. Precision was evaluated as repeatability (within-day), intra-day precision (over 6 hours), and inter-day precision (over 3 days), and analyst-to-analyst precision. Robustness was assessed by deliberately introducing small variations in mobile phase pH (±0.2 units), flow rate (±10%), mobile phase ratio (±2%), and column temperature (±5°C).

2.8 Forced Degradation Studies

Forced degradation studies were conducted to establish the stability-indicating nature of the developed method. Olanzapine was subjected to the following stress conditions:

Acid hydrolysis: 50 mg of Olanzapine was refluxed with 50 mL of 0.1 M HCl at 80°C for 8 hours.

Alkaline hydrolysis: 50 mg of Olanzapine was refluxed with 50 mL of 0.1 M NaOH at 80°C for 8 hours.

Hydrolytic degradation: 50 mg of Olanzapine was stirred with 50 mL of water at 80°C for 48 hours.

Oxidative degradation: 50 mg of Olanzapine was stirred with 50 mL of 3% H_2O_2 at room temperature for 24 hours.

Thermal degradation: 50 mg of Olanzapine was placed in a petri dish and stored in an oven at 50°C for 4 weeks.

After each stress treatment, samples were withdrawn, diluted appropriately to obtain 10 µg/mL, and analyzed by HPLC. The percentage drug recovered and percentage degradation were calculated against the calibration curve.

3. RESULTS AND DISCUSSION

3.1 Drug Characterization

The melting point of Olanzapine was found to be 195–198°C, consistent with the reported value (195°C), confirming the identity and purity of the working standard. Solubility studies demonstrated that Olanzapine is freely soluble in methanol, acetonitrile, benzene, and 0.1 N HCl, slightly soluble in ethanol, and insoluble in water, phosphate buffer

pH 7.4, and 0.1 N NaOH (Table 1). These findings supported the selection of aqueous-organic mobile phase systems for HPLC analysis.

Table 1. Solubility profile of Olanzapine in various solvents

S. No.	Solvent	Solubility
1	Water	Insoluble
2	0.1 N HCl	Freely soluble
3	Methanol	Freely soluble
4	Ethyl alcohol	Slightly soluble
5	0.1 N NaOH	Insoluble
6	Acetonitrile	Freely soluble
7	Phosphate buffer pH 7.4	Insoluble
8	Benzene	Freely soluble

FTIR spectral analysis of Olanzapine confirmed its structural integrity. Characteristic absorption peaks corresponding to OH stretching (3565 cm^{-1}), –N=C=N– stretching (2171 cm^{-1}), –C=N– stretching (1646 cm^{-1}), N–H bending of secondary amides (1515–1540 cm^{-1}), and sulfur compound absorption (1139 cm^{-1}) were observed and closely matched theoretical values, confirming the identity of the drug (Table 2).

Table 2. FTIR spectral interpretation of Olanzapine

S. No.	Functional Group	Experimental (cm^{-1})	Theoretical (cm^{-1})
1	OH stretching	3565.69	3570–3450
2	–N=C=N– stretching	2171.25	2175–2130
3	–C–C– multiple bond	1964.27	1960
4	Anhydride (5-membered ring)	1889.97	1890–1750
5	Anhydride (acyclic)	1771.24	1790–1740
6	–C=N– stretching	1646.19	1660–1630
7	N–H bending (2° amides)	1515.62, 1540.45	1550–1510
8	Sulfur compound	1139.06	1200–1050

The λmax of Olanzapine was determined to be 257 nm by UV scanning in the mobile phase, which was selected as the detection wavelength for the HPLC method.

3.2 Method Optimization

Several mobile phase compositions were investigated to achieve adequate separation with satisfactory chromatographic performance (Table 3). Mobile phases consisting of Water:Methanol (50:50 and 80:20), Acetonitrile:Water (50:50), and KH_2PO_4:Acetonitrile (70:30) at various pH values produced unsatisfactory results including absence of peak, peak broadening, or peak tailing. The optimized mobile phase—phosphate buffer (pH 6.0): acetonitrile (60:40)—yielded excellent peak symmetry, minimal tailing, and reproducible retention time, and was therefore selected for further validation.

Table 3. Mobile phase screening results

Mobile Phase	Ratio	Flow Rate	Result
Water : Methanol	50:50	1.0 mL/min	No peak found
Methanol : Water	80:20	1.2 mL/min	Peak broadening
Acetonitrile : Water	50:50	1.2 mL/min	No peak found
20 mM KH_2PO_4 : ACN	70:30	1.0 mL/min	Tailing

KH_2PO_4 (pH 3.0) : ACN	70:30	1.0 mL/min	Tailing
Buffer (pH 6.0) : ACN	60:40	1.0 mL/min	Most suitable

3.3 System Suitability

System suitability was evaluated by injecting three replicates of the 10 µg/mL Olanzapine working standard solution. The mean retention time, peak area (AUC), number of theoretical plates, and tailing factor were determined (Table 4). All system suitability parameters complied with ICH recommendations, confirming the suitability of the chromatographic system for analysis.

Table 4. System suitability parameters for Olanzapine (n=3)

Replicate	RT (min)	AUC	Theoretical Plates	Tailing Factor
1	2.375	1251.23	3078	1.18
2	2.374	1250.458	3056	1.20
3	2.375	1256.658	3098	1.15
Mean	2.375	1252.78	3077	1.18
S.D.	0.0006	3.38	21.01	0.025

3.4 Linearity

The calibration curve for Olanzapine was linear over the concentration range of 5–25 µg/mL. The regression equation was determined as: AUC = 120.8 × C + 23.73, where C is the concentration in µg/mL. The correlation coefficient (r^2) was 0.998, indicating an excellent linear relationship between peak area and concentration (Table 5). The %RSD values at all concentration levels were below 2.0%, confirming the consistency and reliability of the analytical response. The response ratio (mean AUC/concentration) was found to be 123.197 with %RSD of 1.994%, further validating linearity.

Table 5. Linearity data for Olanzapine

Concentration (µg/mL)	Mean AUC (n=3)	S.D.	%RSD
5	611.774	3.288	0.538
10	1252.782	3.379	0.270
15	1875.978	19.581	1.044
20	2483.220	4.021	0.162
25	2982.129	3.258	0.109

Regression equation: AUC = 120.8C + 23.73; r^2 = 0.998

3.5 Assay of Tablet Formulation

The tablet assay demonstrated that the developed method accurately quantified Olanzapine in the marketed formulation (Oleanz-5®). The mean drug content was 10.076 mg (label claim: 10 mg), corresponding to 100.76% of label claim, with a %RSD of 0.742% (Table 6), indicating absence of interference from tablet excipients and confirming the selectivity of the method.

Table 6. Assay results for Olanzapine tablet formulation (n=6)

Replicate	Amount Found (mg)	% Label Claim
1	10.10	101.00
2	10.05	99.50
3	10.08	100.30
Mean	10.076	100.27

S.D.	0.025	0.747
%RSD	—	0.742

3.6 Accuracy

Recovery studies performed at 80%, 100%, and 120% spike levels demonstrated percentage recoveries ranging from 99.75% to 100.10% (Table 7). The %RSD values were less than 2.0% at all levels, confirming the high accuracy of the developed method and the absence of matrix effects from tablet excipients.

Table 7. Accuracy (recovery) studies of Olanzapine

Level (%)	Amount Present (mg)	Amount Added (mg)	Amount Recovered (mg)	% Recovery	%RSD
80	10.00	8.00	7.98–8.01	99.958	0.191
100	10.00	10.00	9.98–10.05	100.100	0.360
120	10.00	12.00	11.95–12.01	99.750	0.289

3.7 Precision

Repeatability (within-day) analysis showed a label claim of 99.50% with %RSD of 0.125%, indicating high precision of the method. Intra-day precision over a period of 6 hours yielded mean recovery of 98.97% with %RSD of 0.201%, while inter-day precision over 3 days demonstrated mean recovery of 97.10% with %RSD of 0.371% (Table 8). Analyst-to-analyst precision (%RSD = 0.229%) was also within acceptable limits. All %RSD values were well below the 2% threshold prescribed by ICH guidelines.

Table 8. Precision data for Olanzapine

Parameter	% Label Claim	S.D.	%RSD
Repeatability	99.50	0.254	0.125
Intra-day (n=6)	98.97	0.199	0.201
Inter-day (n=3 days)	97.10	0.361	0.371
Analyst-to-analyst	99.84	0.254	0.229

3.8 Robustness

Robustness was assessed by deliberately varying chromatographic parameters. The %RSD values obtained under modified conditions—including temperature changes (±5°C), flow rate variations (±10%), and mobile phase ratio modifications (±2%)—remained below 1.0%, indicating that the method is robust and unaffected by minor deliberate changes in analytical conditions (Table 9).

Table 9. Robustness evaluation under varied chromatographic conditions

Parameter	Normal (%RSD)	Lower Limit (%RSD)	Upper Limit (%RSD)
Temperature (±5°C)	0.54	0.69	0.52
Flow rate (±10%)	0.41	0.48	0.89
Mobile phase ratio (±2%)	0.31	0.77	0.15

3.9 Forced Degradation Studies

Forced degradation studies confirmed the stability-indicating nature of the developed RP-HPLC method (Table 10). Olanzapine exhibited maximum susceptibility to acidic hydrolysis (16.64% degradation) followed by alkaline hydrolysis (10.25%) and oxidative stress (8.67%). Thermal degradation was comparatively minimal (1.01%), suggesting inherent thermal stability of the drug. Under all stress conditions, the degradation products were resolved from the main drug peak without interference, confirming the specificity and stability-indicating capability of the method.

Table 10. Forced degradation results for Olanzapine

Stress Condition	Drug Recovered (%)	Drug Degraded (%)
Standard (control)	99.90	0.00
Acid hydrolysis (0.1 M HCl, 80°C, 8h)	83.26	16.64
Alkaline hydrolysis (0.1 M NaOH, 80°C, 8h)	89.65	10.25
Oxidative (3% H_2O_2, RT, 24h)	91.23	8.67
Thermal (50°C, 4 weeks)	98.89	1.01

The selective separation of Olanzapine from its stress-induced degradation products under all tested conditions confirms that the method is truly stability-indicating and suitable for pharmaceutical stability testing applications as required by regulatory agencies.

4. CONCLUSION

A simple, rapid, economical, accurate, precise, and stability-indicating RP-HPLC method was successfully developed and validated for the quantitative estimation of Olanzapine in bulk drug and pharmaceutical dosage forms. The method employs a Thermo C18 column with a phosphate buffer:acetonitrile (60:40) mobile phase at UV 257 nm, yielding a retention time of approximately 2.37 min. The method demonstrated excellent linearity (r^2 = 0.998, 5–25 µg/mL), high accuracy (recovery: 99.75–100.10%), outstanding precision (%RSD <2%), and robustness. Forced degradation studies confirmed the specificity and stability-indicating nature of the method, with effective resolution of degradation products from the parent drug peak. Full validation per ICH Q2(R1) guidelines confirmed that the method is reliable, reproducible, and suitable for routine quality control and stability testing of Olanzapine in pharmaceutical laboratories and the pharmaceutical industry.

Conflict of Interest

The authors declare no conflict of interest.

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