RESEARCH ARTICLE

A Novel Stability Indicating Method Development and Validation for Simultaneous Estimation of Oxcarbazepine and Olanzapine by RP-HPLC in Pharmaceutical Dosage Form

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ABSTRACT
A novel stability-indicating RP-HPLC method was developed and validated for the simultaneous estimation of Oxcarbazepine and Olanzapine in pharmaceutical dosage forms. The method utilized a Waters X-Terra RP-18 column with a mobile phase composition of acetonitrile and 0.1% triethylamine (30:70), pH adjusted to 2.5 using orthophosphoric acid. The flow rate was maintained at 1 mL/min, and detection was carried out at 272 nm. The method demonstrated excellent linearity for Oxcarbazepine (37.50–225.00 µg/mL, $R^2 = 0.99989$) and Olanzapine (5.00–30.00 µg/mL, $R^2 = 0.99973$), along with high precision and accuracy (%RSD < 1.0). Robustness was confirmed under varied conditions, and forced degradation studies validated the method's specificity, with no significant interference from degradants. The proposed method is reliable, reproducible, and suitable for routine analysis of Oxcarbazepine and Olanzapine in combined dosage forms.

Keywords; RP-HPLC, Oxcarbazepine, Olanzapine, Stability-indicating method, Method validation
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INTRODUCTION

Oxcarbazepine (Fig.1) is a second-generation antiepileptic drug used for the treatment of partial seizures in adults and children aged two years and older. It works by inhibiting voltage-sensitive sodium channels, stabilizing hyperexcited neural membranes, and suppressing repetitive neuronal firing. Regular monitoring of serum sodium levels is advised during prolonged therapy due to the risk of severe hyponatremia. Additionally, oxcarbazepine may influence thyroid hormone levels, leading to hypothyroxinemia in some cases.[1-3] Its favorable pharmacokinetics and lower risk of severe hypersensitivity reactions compared to carbamazepine make it a preferred choice in specific populations.[4-6]

Olanzapine (Fig.1)is an atypical antipsychotic primarily used to treat schizophrenia and bipolar I disorder, including acute manic or mixed episodes and maintenance therapy. It modulates dopamine and serotonin activity by antagonizing D2 and 5-HT2A receptors. Olanzapine has a black-box warning for increased mortality in elderly patients with dementia-related psychosis, and it is not approved for this population.[7-9] Combination therapy with fluoxetine is approved for the treatment of depressive episodes in bipolar I disorder.[10] Previous studies have developed and validated RP-HPLC methods for estimating individual drugs, such as oxcarbazepine and olanzapine, in bulk and dosage forms.[11,12] The current aim is to develop and validate an RP-HPLC method for the simultaneous estimation of oxcarbazepine and olanzapine.

MATERIALS AND METHODS

Instrumentation

The instruments used in this study included a Waters Alliance HPLC system (e2695) with Empower 2.0 software, an Eutech pH meter, a Sartorius weighing balance, and a UV-1700 UV/VIS spectrophotometer.

Chemical used

The chemicals and reagents used in the study included Acetonitrile (HPLC grade, Rankem), Formic Acid (AR grade, Analytical Reagents), HPLC grade Water (Milli-Q or equivalent), and Ortho Phosphoric Acid (AR grade, Analytical Reagent). These high-purity reagents ensured the reliability and accuracy of the analytical method.

Preparation of Standard and Sample Solutions

To prepare the standard solution, 150 mg of oxcarbazepine and 20 mg of olanzapine were dissolved in a diluent in a 100 mL volumetric flask, sonicated, and diluted to the mark (stock solution). From this, 5 mL was diluted to 50 mL with

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Figure 1: Chemical structure of oxcarbazepine and olanzapine

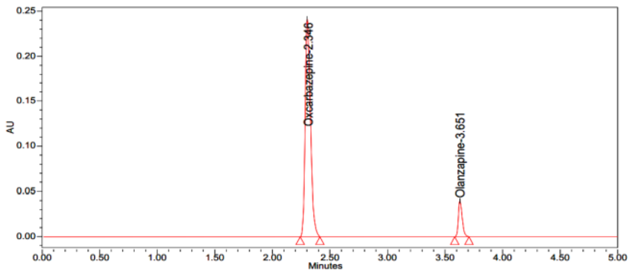


Figure 2: Optimized chromatogram of Oxcarbazepine and Olanzapine

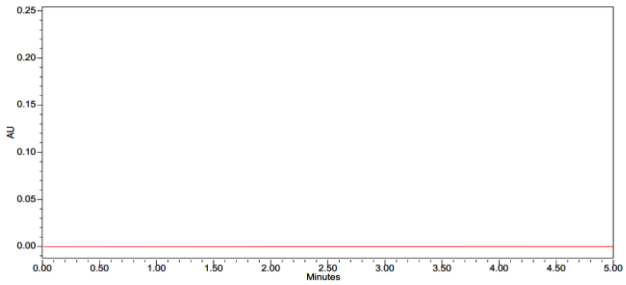


Figure 3: Chromatogram of blank

Table 1: Chromatographic Trials for Method Optimization

Trial No.	Stationary Phase	Mobile Phase Composition	Observation
1	Luna Phenyl Hexyl	Acetonitrile: 0.1% Formic acid (20:80)	Plate count is not within the limit
2	(250x4.6mm, 5µ)	Acetonitrile: 0.1% Formic acid (30:70)	Splitting of the peak is observed
3	Waters X-Terra RP-18	Acetonitrile: 0.1% Formic acid (40:60)	Unknown peaks are observed
4	(250mmx4.6, 5µm)	ACN : 0.1% TEA pH-2.5/OPA (10:90)	Baseline is not good
5		ACN : 0.1% TEA pH-2.5/OPA (20;80)	Response of the peaks are very high
6		ACN : 0.1% TEA pH-2.5/OPA (30:70)	This method is suitable for validation

diluent to achieve 150 ppm oxcarbazepine and 20 ppm olanzapine. For the sample solution, 21.8 mg of oxcarbazepine and 10.2 mg of olanzapine were dissolved in a diluent in a 10 mL flask, sonicated for 30 minutes, and diluted to the mark (stock solution). From this, 1 mL was diluted to 10 mL, filtered through a 0.45-micron filter, and analyzed.

Method Development

Multiple trials were conducted using different columns and mobile phase ratios. Luna Phenyl Hexyl (250x4.6mm, 5µ) and Waters X-Bridge Phenyl were tested with varying ratios of Acetonitrile and aqueous modifiers (0.1% TFA pH-2.5/OPA). Observations included system suitability and plate count issues, broad and unknown peaks, and suboptimal peak height, indicating the need for further optimization. (Table 1).

Method Validation

The method validation process entails performing a sequence of tests grounded in the analytical method. The purpose of these tests is to assess and record the method's performance characteristics and evaluate its suitability for a particular analytical objective. The performance characteristics of LC methods encompass specificity, selectivity, precision, linearity, robustness, recovery, Minimum detectable concentration, Minimum quantifiable concentration, and ruggedness.[13-16]

Specificity

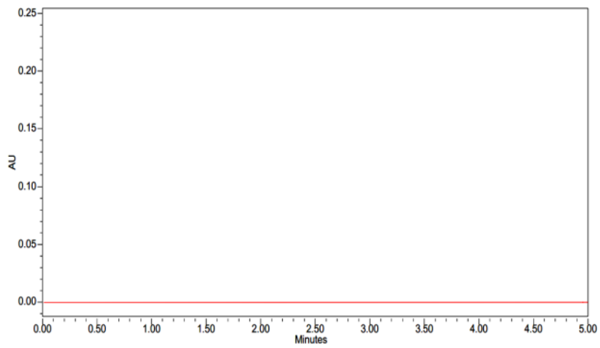


Figure 4: Chromatogram of placebo

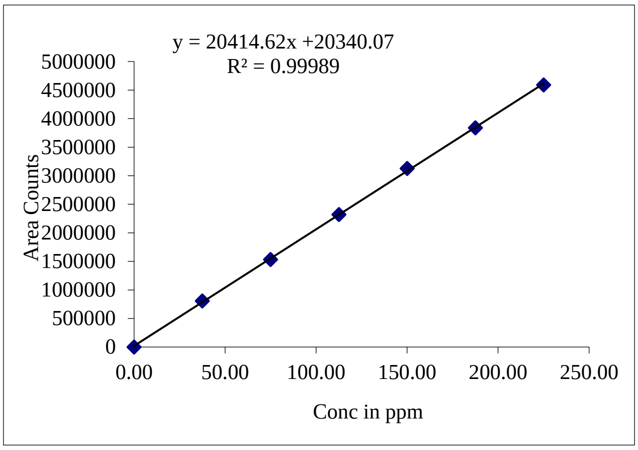
An analytical method must be capable of identifying a target analyte exclusively among controls and known impurities to assess specificity. Chromatograms were acquired for standards, blanks, and samples to accomplish this.

Linearity, Precision, Accuracy and Robustness

From the stock, a series of 37-225 µg/mL for oxcarbazepine and 5.00-30.00. µg/mL for olanzapine solutions were made, diluted, and injected three times into the HPLC system; the chromatograms were obtained from each of them. Every chromatogram had a recorded peak area; the mean peak area was computed. The study of precision under varied experimental conditions like different equipment, labs, and days was termed intermediate precision. The method accuracy and recovery rate were assessed at 50%, 100%,

Table 2: Results of linearity for Oxcarbazepine & Olanzapine

S.No	Oxcarbazepine		Olanzapine	
	Conc.(µg/ml)	Peak area	Conc.(µg/ml)	Peak area
1	37.50	806027	5.00	103956
2	75.00	1533258	10.00	208457
3	112.50	2320982	15.00	311024
4	150.00	3127416	20.00	416524
5	187.50	3840379	25.00	502856
6	225.00	4590828	30.00	618475
Regression equation	y = 20414.62x +20340.07		y =20437.80x + 2189.00	
Slope	20414.62		20437.80	
Intercept	20340.07		2189.00	
R^2	0.99989		0.99973	



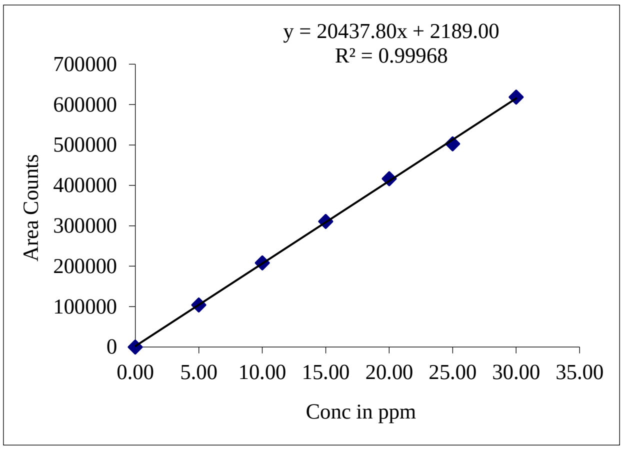


Figure 5: Calibration curve for Oxcarbazepine and Olanzapine

Table 3: System precision table of Oxcarbazepine & Olanzapine

S.No	Oxcarbazepine		Olanzapine	
	Conc. (µg/ml)	Area	Conc. (µg/ml)	Area
1.	150	3121662	20	415236
2.	150	3134094	20	411574
3.	150	3131557	20	413258
4.	150	3117461	20	416256
5.	150	3128950	20	413859
6.	150	3124123	20	417248
Mean	3126308		414572	
S.D	6321.12		2081.38	
%RSD	0.20		0.50	

Table 4: Method Precision for Oxcarbazepine & Olanzapine

S. No.	Area for Oxcarbazepine	Area for Olanzapine
1	3112367	411874
2	3131542	412305
3	3105690	416524
4	3129720	413254
5	3106541	417541
6	3153164	410637
Average	3123171	413689
Standard Deviation	18500.87	2741.85
%RSD	0.59	0.66

and 150% concentrations. Flow rate, organic phase ratio, and temperature were modified to test robustness, and the mean %RSD was analyzed.

Forced degradation studies

These conditions included acidic and alkaline hydrolysis (using 2N HCl and 2N NaOH, respectively), Peroxide (with 10% hydrogen peroxide), thermal stress (in a hot air oven at 105°C), photolytic exposure (under UV light), and simple hydrolysis. Each treatment was applied to oxcarbazepine and olanzapine and maintained for specified durations, such as 30 minutes for chemical exposures and up to 6 hours for thermal stress

RESULTS AND DISCUSSION

Method Optimization

HPLC with a PDA detector was used to optimize the chromatographic technique for estimating oxcarbazepine and olanzapine together. The optimized settings used a Waters X-Terra RP-18 (250mmx4.6, 5µm) for effective separation. The 30:70 mobile phase of acetonitrile and 0.1% TEA pH-2.5/OPA provided optimum resolution and peak symmetry. The detection wavelength was 272 nm for maximum analyte sensitivity. Set the flow rate to 1 mL/min, injection volume to 10 µL, and run time to 5 minutes. (Fig.2)

Specificity

While the peaks of the analytes were apparent, and there were no interferences in the chromatograms, the findings of

Table 5: Accuracy results of Oxcarbazepine & Olanzapine

%Conc Level	Oxcarbazepine			Olanzapine		
	Area	% Recovery	Mean Recovery	Area	% Recovery	Mean Recovery
50%	1570941	100.5	99.7	207458	100.1	98.9
	1556471	99.6		203652	98.3	
	1549355	99.1		203864	98.4	
100%	3141728	100.5	100.1	417844	100.8	99.8
	3128659	100.1		412035	99.4	
	3115074	99.6		411456	99.3	
150%	4683214	99.9	99.9	612547	98.5	98.5
	4695370	100.1		614542	98.8	
	4679666	99.8		610854	98.2	

Table 6: Robustness results of Oxcarbazepine & Olanzapine

Parameter	Condition	Oxcarbazepine Retention Time (min)	Oxcarbazepine %RSD	Olanzapine Retention Time (min)	Olanzapine %RSD
Flow Rate Change	Less flow (0.9ml)	2.528	0.38	3.793	1.12
	Actual (1.0ml)	2.346	0.20	3.651	0.50
	More flow (1.1ml)	2.202	0.45	3.464	0.46
Organic Phase Change	Less Org (27:73)	2.685	0.41	3.952	0.20
	Actual (30:70)	2.342	0.20	3.656	0.50
	More Org (33:67)	2.075	0.42	3.230	99.2

Table 7: Assay of Oxcarbazepine & Olanzapine

Brand	Drug	Avg sample area (n=2)	Std. Conc. (µg/ml)	Sample Conc. (µg/ml)	Label amount (mg)	% assay
Oxcarb-150	Oxcarbazepine	3121267	150	150	150	99.8
Olanzapine	Olanzapine	414901	20	20	20	100.2

Table 8: Forced Degradation results for Oxcarbazepine and Olanzapine

Stress conditions	Oxcarbazepine		Olanzapine	
	% Assay	% Deg	% Assay	% Deg
Control	100	0	100	0
Acid	87.8	12.2	88.2	11.8
Alkali	84.6	15.4	85.5	14.5
Peroxide	86.7	13.3	98.8	1.2
Reduction	96.3	3.7	89.9	10.1
Thermal	90.2	9.8	97.2	2.8
Photolytic	97.7	2.3	95.8	4.2
Hydrolysis	97.3	2.7	97.8	2.2

the chromatograms also demonstrated that the approach was selective. Chromatograms of specificity are shown in Figures 3 and 4.

Linearity

The linearity results for Oxcarbazepine and Olanzapine showed excellent correlation with concentrations ranging from 37.50–225.00 µg/mL and 5.00–30.00 µg/mL, respectively (Table 2). Regression equations for Oxcarbazepine ($y = 20414.62x + 20340.07$, $R^2 = 0.99989$) and Olanzapine ($y = 20437.80x + 2189.00$, $R^2 = 0.99973$) confirm the method's accuracy and reliability (Figure 5).

System Precision and Method Precision

The system precision results for Oxcarbazepine (150 µg/mL) and Olanzapine (20 µg/mL) showed consistent peak areas with %RSD values of 0.20 and 0.50, respectively, indicating excellent precision (Table 3).

The method precision for Oxcarbazepine and Olanzapine, evaluated by RP-HPLC, showed consistent results with average areas of 3123171 and 413689, respectively. The %RSD values of 0.59 for Oxcarbazepine and 0.66 for Olanzapine confirm the method's precision for quantitative analysis (Table 4).

Accuracy

The % mean recovery for Oxcarbazepine at 50%, 100%, and 150% levels was 99.7%, 100.1%, and 99.9%, respectively, indicating excellent accuracy. Similarly, Olanzapine showed % mean recoveries of 98.9%, 99.8%, and 98.5% at the corresponding levels, validating the method's accuracy and reliability (Table 5).

Robustness

The robustness study demonstrated consistent retention times and %RSD values for Oxcarbazepine and Olanzapine under varied flow rates (0.9–1.1 mL/min) and organic phase compositions (27:73 to 33:67), with %RSD values below 1.12, confirming the method's robustness (Table 6).

Assay

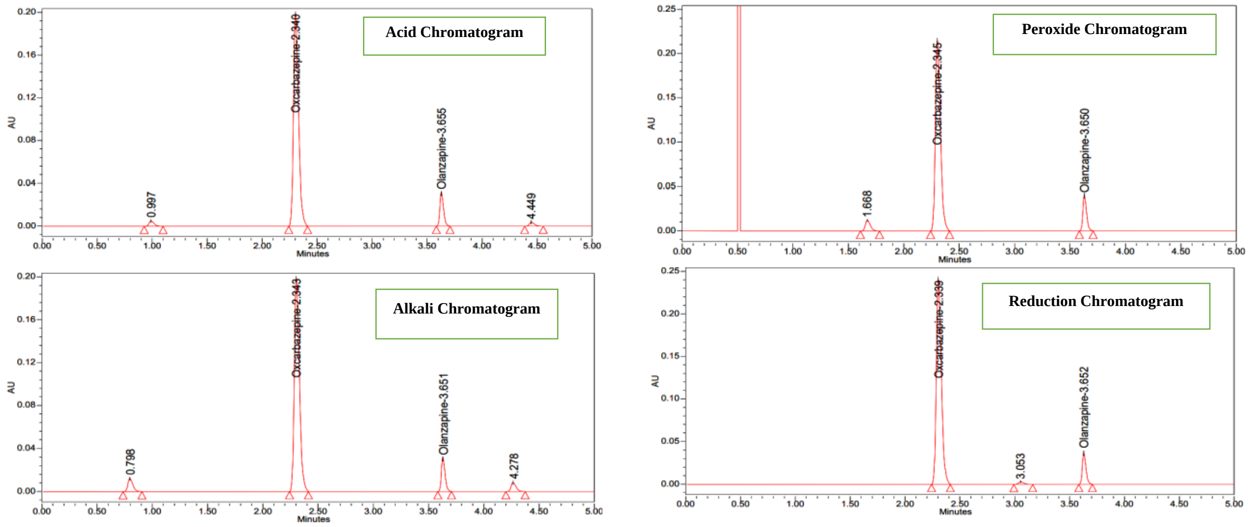


Figure 6: Chromatogram of Reduction degradation

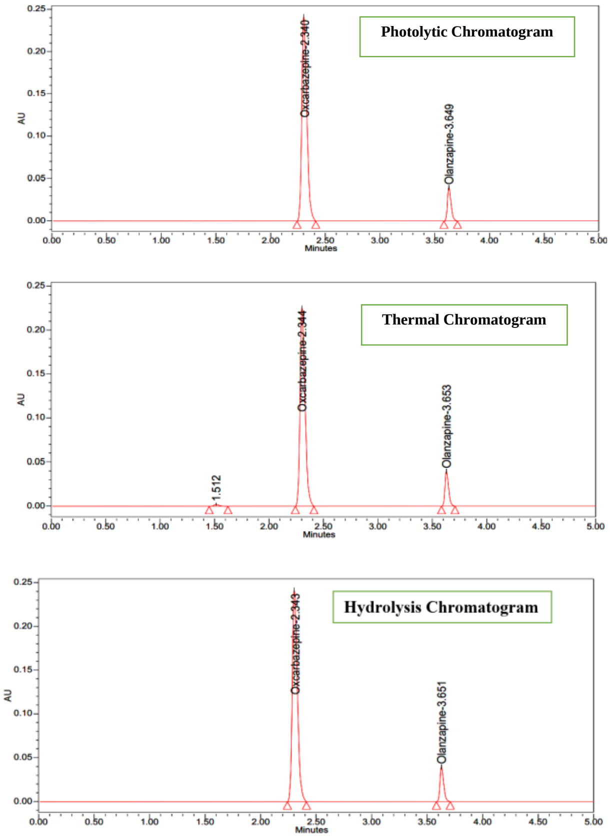


Figure 7: Chromatograms observed during Stress study

The assay results for Oxcarbazepine & Olanzapine showed % assay values of 99.8% and 100.2%, respectively, indicating compliance with the labeled amounts. (Table 7).

Forced Degradation studies

Forced degradation studies for Oxcarbazepine & Olanzapine showed varied % assay and % degradation under different stress conditions (acid, alkali, peroxide, reduction, thermal, photolytic, and hydrolysis). Purity angles remained below their thresholds, indicating no significant co-eluting impurities, ensuring method specificity. The highest degradation was observed under peroxide stress for both compounds. (Table 8, Figure 6,7).

CONCLUSION

The developed RP-HPLC method for the simultaneous estimation of Oxcarbazepine and Olanzapine in pharmaceutical formulations was successfully validated according to ICH guidelines. The method exhibited excellent linearity, precision, and accuracy, with robust performance under varied chromatographic conditions. Forced degradation studies confirmed the method's specificity and suitability for stability-indicating analysis. With its simplicity, reliability, and reproducibility, the method is highly suitable for routine quality control and stability studies of pharmaceutical formulations containing these two active ingredients. This work provides a significant contribution to pharmaceutical analysis, ensuring precise and efficient drug quantification.

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