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Development and validation of HPLC method for determination of Oxcarbazepine in bulk and pharmaceutical formulation

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ABSTRACT

A rapid, reliable, sensitive, selective, precise and accurate High Performance Liquid Chromatography method for analysis of Oxcarbazepine in pharmaceutical formulations. Separation was achieved with a reversed-phase Waters (alliance) HPLC C18, 150 X 4.6, 5µ, employing UV detection at215nm. The solvent system consisted of mixed buffer and Acetonitrile in the ratios (55: 45 v/v) at a flow rate of 1.0 ml/min. The run time was 10.0 min and the retention time was 3.871min. The linear regression data for the calibration plots showed good linear relationship with r^2=0.999 in the concentration range of 7.5-45µg/ml. The developed method was validated for accuracy, precision, robustness, detection and quantification limits as per the ICH guidelines. The wide linearity range, accuracy, sensitivity, short retention time and composition of the mobile phase indicated that this method is better for the quantification of Oxcarbazepine

KEYWORDS

HPTLC;
Oxcarbazepine;
Validation.

INTRODUCTION

Oxcarbazepine, [OXC; 10, 11-Dihydro-10-oxo-5*H*-dibenz [*b, f*] azepine- 5-carboxamide] is an anticonvulsant and mood stabilizing drug, used primarily in the treatment of epilepsy and bipolar disorder. Oxcarbazepine is structurally a derivative of Carbamazepine, adding an extra oxygen atom on the dibenzazepine ring. Its chemical structure is shown in Figure 1. This difference helps reduce the impact on the liver of metabolizing the drug, and also prevents the serious forms of anemia occasionally associated with Carbamazepine. Aside from this reduction in side effects, it is assumed to have the same mechanism as Carbamazepine - sodium channel inhibition - and is generally used to treat the same conditions. Oxcarbazepine has recently been found associated with a greater enhancement in mood and reduction in anxiety symptoms than other drugs employed to treat epilepsy[1,2]. Oxcarbazepine is a relatively new antiepileptic drug, is a 10-keto analogue of carbamazepine with a similar the rapeutic profile, but with less adverse effects and less clinical relevant pharmacokinetic drug interactions[3-5]. Oxcarbazepine is indicated as first-line drug in monotherapy orpolytherapy for the treatment of partial seizures with or without secondarily generalized tonic–clonic epileptic seizures[6-8]. Spectroscopic methods have been reported in the literature for determination of Oxcarbazepine in pharmaceuti-

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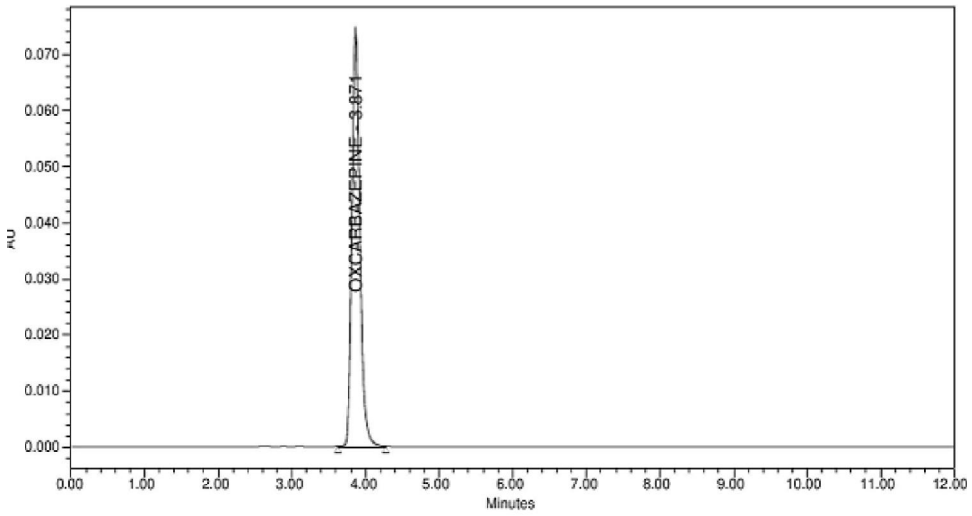

	Peak Name	RT	Area	Height	% Area	USP Tailing	USP Plate Count
1	OXCARBAZEPINE	3.871	574234	74527	100.00	1.24	5995

Figure 1 : Chromatogram of Oxcarbazepine

cals[3-8]. Considering the biological significance of OXC, several quantitative analytical procedures have been reported in the literature for its determination and such methods include liquid chromatographic methods HPLC[10-32], HPTLC[9], GC[33-34], atmospheric pressure chemical ionization liquid chromatography/mass spectrometry[35], HPLC/MS[36], LC-electron spray mass spectrometry[37], LC-MS/MS[38], micellar electro kinetic chromatography[39] and voltammetry[40]. However till now, Development and Validation of Hplc Method for Determination of Oxcarbazepine Bulk and Pharmaceutical Formulation no method for estimation of Oxcarbazepine has been reported. A very viable alternative for Pharmaceutical analysis of Oxcarbazepine is HPLC. The advantage of HPLC is that several samples can be run simultaneously using a small quantity of mobile phase like HPLC, thus lowering analysis time and cost per analysis. The focus of the present study was to develop an accurate, specific, reproducible and determination of low levels of Oxcarbazepine in presence of its Bulk and Pharmaceutical Formulation of the bulk drug.

MATERIALS AND METHODS

Oxcarbazepine sample was obtained from. Rantus Pharma Pvt. Ltd Hyderabad. Oxcarbazepine tablet was purchased from local market. The solvents used Sodium dihydrogen orthophosphate and disodium hydrogen phosphate (HPLC grade), Acetonitrile (AR grade), these chemicals were purchased from Merck Chemicals (Tirupati, (AP) India).

Selection of mobile phase

Chromatographic separation studies were carried out Waters HPLC 2 2695 series consisting 4 pump, C-18, column on the working standard solution of Oxcarbazepine (10µg/ml). Initially, trials were carried out using Mixed Phosphate Buffer and Acetonitrile in various proportions along with varying pH, to obtain the desired system suitability parameters. After several trials, Mixed Phosphate Buffer: Acetonitrile (pH adjusted to 6.5 with Sodium dihydrogen orthophosphate and disodium hydrogen phosphate) (55: 45 v/v), was chosen as the mobile phase, which gave good resolution and acceptable peak parameters.

Chromatographic conditions

Column	: Symmetry C18, 150 X 4.6, 5µ.
Flow Rate	: 1.0 ml/min
Wave length	: 215 nm
Column temperature	: 35°C
Injection volume	: 20 µL
Diluent	: Mobile Phase

Elution type : Isocratic
Needle wash solution : Water: Acetonitrile (90:10)

Preparation of standard stock solution

30mg of Oxcarbazepine reference standard was weighed accurately and transferred in 100ml volumetric flask. Drug was dissolve in Mixed Phosphate Buffer and Acetonitrile (55: 45 v/v) and volume was made up to 100ml with same solvent. So as to get the concentration 100µg/ml. 1ml standard stock solution of Oxcarbazepine was then diluted in 10ml Mixed Phosphate Buffer and Acetonitrile (55: 45 v/v) to get working standard solution 10µg/ml.

Preparation of mobile phase

Mobile phase was prepared by Mixed Phosphate Buffer and Acetonitrile (pH adjusted to 6.5 with Sodium dihydrogen orthophosphate and disodium hydrogen phosphate) (55: 45v/v), filtered through 0.45µ membrane filter paper and then sonicated on ultra sonic water bath for 30min.

Selection of detection wavelength

From the standard stock solution further dilutions were done using Mixed Phosphate Buffer and Acetonitrile (55: 45 v/v) and scanned over the range of 200 - 400nm and the spectra was obtained. It was observed that Oxcarbazepine showed considerable absorbance at 215 nm.

Chromatogram of Oxcarbazepine

The column was saturated with the mobile phase (indicated by constant back pressure at desired flow rate). Standard solution of Oxcarbazepine was injected to get the chromatogram. The retention time for Oxcarbazepine was found to be 3.871 min. Chromatogram of Oxcarbazepine is shown in (Figure 1).

Validation of analytical methods

The validation for HPLC method development was performed using parameters like Linearity, Precision, Accuracy, Limit of detection (LOD), Limit of quantification (LOQ) and Robustness.

Linearity

The standard stock solution containing 100µg/ml of Oxcarbazepine to prepare range of standard solutions containing six different concentrations of analyte. The linearity of the relationship between peak area and concentration was determined by analyzing six standard solutions over the concentration range 7.5-45.0µg/ml. The results obtained are shown in (TABLE 1). The peak areas were plotted against the corresponding concentrations to obtain the calibration curve (Figure 2).

TABLE 1 : Linearity of Oxcarbazepine

%	Conc (mcg)	Area
25	7.5000	574234
50	15.0000	1010782
75	22.5000	1502883
100	30.0000	2030026
125	37.5000	2516727
150	45.0000	2993945

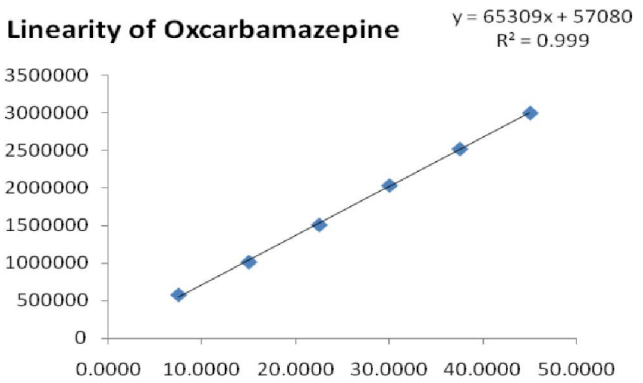

Figure 2 : Linearity of Oxcarbazepine

Precision

The precision of the method was demonstrated by intraday and inter-day variation studies. In the inter day studies, 3 different concentrations 15.0, 30.0 and 45.0µg/ml were injected in stabilized chromatographic conditions and were analyzed in triplicate. The percentage RSD was calculated. The result obtained for intraday variations are shown in (TABLE 3 & 4). In the inter day variation studies, 15.0, 30.0 and 45.0µg/ml were injected in stabilized chromatographic conditions and were analyzed. This procedure was repeated once a day for three consecutive days. The percentage RSD was calculated. The result obtained for inter-day variations are shown in (TABLE 2& 3).

Accuracy

To check accuracy of the method, recovery stud-

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TABLE 2 : System precision

S No	Name	RT	Area
1	Injection-1	3.886	2027045
2	Injection-2	3.89	2059092
3	Injection-3	3.891	2057057
4	Injection-4	3.889	2025460
5	Injection-5	3.89	2057659
6	Injection-6	3.893	2058954
Avg		3.890	2047545
Std Dev		0.002	16518.3
% RSD		0.060	0.81

TABLE 3 : Method precision

S No	Name	RT	Area
1	Solution-1	3.885	2027821
2	Solution-2	3.887	2033908
3	Solution-3	3.889	2039021
4	Solution-4	3.892	2055464
5	Solution-5	3.895	2059910
6	Solution-6	3.897	2054389
Avg		3.891	2045086
Std Dev		0.005	13219.8
% RSD		0.120	0.65

TABLE 4 : Accuracy of Oxcarbazepine

50		100		150	
S No	Area	S No	Area	S No	Area
Injection-1	1009978	Injection-1	2029965	Injection-1	2998940
Injection-2	1010023	Injection-2	2030756	Injection-2	2999923
Injection-3	1010143	Injection-3	2031019	Injection-3	2997984
Avg	1010048	Avg	2030580	Avg	2998949
amt Recoverd	49.69	amt Recoverd	99.91	amt Recoverd	147.53
%Recovery	99.38	%Recovery	99.91	%Recovery	98.35

TABLE 5 : Raggedness of Oxcarbazepine day-1

S No	Name	RT	Area
1	Injection-1	3.885	2027821
2	Injection-2	3.887	2033908
3	Injection-3	3.889	2039021
4	Injection-4	3.892	2055464
5	Injection-5	3.895	2059910
6	Injection-6	3.897	2054389
Avg		3.891	2045086
Std Dev		0.005	13219.8
% RSD		0.120	0.65

ies were carried out by mixing standard drug solution to pre analyzed sample solution at three different levels 50%, 100% and 150%. Basic concentration of sample chosen was 15.0µg/ml of Oxcarbazepine bulk drug solution to which 30.0and 45.0 µg/ml of Oxcarbazepine tablet solution was added. These solutions were injected in stabilized chromatographic conditions in triplicate to obtain the chromatograms. The drug concentrations of Oxcarbazepine were calculated by using linearity equation. The results obtained are shown in (TABLE -4).

ASSAY

Standard préparation

Transfer 10 ml of standard stock solution in to 100 mL volumetric flask and make up to volume With diluent.

Sample preparation

Transfer sample quantitatively equivalent to 40 mg of Oxcarbazepine in to 100 mL volumetric flask add 100 mL of diluent, sonicate to dissolve for 10 minutes and dilute to volume with diluent. Further filter the solution through filter paper. Dilute 10 ml of filtrate to 100 ml with mobile phase.

Procedure

Inject 20 µL of blank solution, standard solution, and sample solution record the chromatogram. And calculate percentage of assay the results are shown in TABLE 8.

Assays result

Oxcarbazepine = 99.14%

Limit of detection (LOD)

LOD is calculated from the formula:

$$DL = \frac{3.3\sigma}{S}$$

Where, σ = standard deviation of response for the lowest conc. In the range, S = slope of the calibration curve, LOD = Oxcarbazepine: 0.8346 µg/ml

TABLE 6 : Raggedness of Oxcarbazepine day-2

S No	Name	RT	Area
1	Injection-1	3.884	2013265
2	Injection-2	3.886	2015099
3	Injection-3	3.886	2016901
4	Injection-4	3.889	2020154
5	Injection-5	3.891	2025464
6	Injection-6	3.894	2028043
Avg		3.888	2019821
Std Dev		0.004	5888.3
% RSD		0.096	0.292

TABLE 7 : Raggedness of Oxcarbazepine day-1&-2

S No	Name	RT	Area
1	Injection-1	3.885	2027821
2	Injection-2	3.887	2033908
3	Injection-3	3.889	2039021
4	Injection-4	3.892	2055464
5	Injection-5	3.895	2059910
6	Injection-6	3.897	2054389
7	Injection-7	3.884	2013265
8	Injection-8	3.886	2015099
9	Injectoion-9	3.886	2016901
10	Injection-10	3.889	2020154
11	Injection-11	3.891	2025464
12	Injection-12	3.894	2028043
	AVG	3.890	2032453.25
	STDEV	0.00423	16409.690
	%RSD	0.11	0.81

TABLE 8 : Assay of Oxcarbazepine

2099255	30	10	100	100	99.7	656	100.000	Result
2130453	100	100	65.2	10	100	300		99.14 %

Limit of quantification (LOQ)

The quantitation limit (QL) may be expressed as:

$$QL = \frac{10\sigma}{S}$$

LOQ = Oxcarbazepine: 2.5292µg /ml.

RUGGEDNESS

The ruggedness of test method is demonstrated by carrying out precision studies with different analysts and on different days. % of RSD on Day-1& Day-2. The % of RSD of areas from six injections should not be more than 2.0%. The results shown in TABLE 5 & 6.

ROBUSTNESS

Robustness was performed by injecting the Oxcarbazepine standard solution in to the HPLC by altering the flow rate and column oven temperature from the normal chromatographic conditions. The results are tabulated in (TABLE 9). Summary of validation parameters or Oxcarbazepine.

RESULTS AND DISCUSSION

Validation parameter pxcarbazepine

Linearity Equation	Y=65309x + 57080
(r2)	0.999
Range	7. 5 – 45.0µg/ml
Precision (% RSD)	
Intraday	0.65%
Inter day	0.292%
Accuracy (% recovery)	99.38%, 99.91%, 98.35%
LOD	0.8346µg/ml
LOQ	2.5292µg/ml

TABLE 9 : Robustness of the Oxcarbazepine

Peak Name	RT	Area	Height	%Area	USP Tailing	USP Plate Count
OXZ	4.831	2489023	248888	100.00	1.35	5740
F1&F2	3.246	1669915	218367	100.00	1.22	4159
OXZ	3.888	2041440	236261	100.00	1.29	4773
T1&T2	3.887	2034407	235407	100.00	1.29	4775

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The developed method was found to be precise as the %RSD values for intraday and inter-day were found to be less than 2%. Good recoveries (98% to 102%) of the drug were obtained at each added concentration, indicating that the method was accurate. The method was also found to be specific indicated by the %recoveries ranging from 98% to 102%. The LOD and LOQ were found to be 0.8346µg/ml and 2.5292µg/ml indicating the sensitivity of the method. The method was also found to be robust as indicated by the % RSD values which are less than 2%.

CONCLUSION

All the above factors lead to the conclusion that the proposed method as accurate, precise, simple, sensitive, robustness and cost effective and can be applied successfully for the estimation of Oxcarbazepine bulk and pharmaceutical formulation.

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