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Stability-Indicating HPLC Method for the Determination of Oxcarbazepine in Pharmaceutical Formulation

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A simple, inexpensive and rapid stability indicating liquid chromatographic method has been developed for the quantitative determination of oxcarbazepine (OXC) in pharmaceutical formulation in presence of the degradation products. The separation was achieved by using an isocratic mobile phase consisting of the mixture of acetronitrile and water (50:50, v/v), using Hiber C_{18} (250 mm × 4.6 mm, 5 µm) column at a flow rate of 1.0 mL/min. The detection was carried out at the wavelength of 256 nm. The retention time of oxcarbazepine was about 4.0 min and it shows an excellent linearity over a range of 0.05 to 80 µg/mL (r^2 = 0.999). The drug was exposed to thermal, photolytic, hydrolytic and oxidative stress conditions and the stressed samples were analyzed by the proposed method. The degredation products were well resolved from the main peak. The percentage of the recovery of drug has been ranged from 99.83 to 102.98 % in pharmaceutical dosage form. The developed method was validated with respect to linearity, accuracy, precision, specificity and robustness. Due to its simplicity and accuracy, the method can be used for routine analysis of oxcarbazepine in bulk drug and pharmaceutical formulations.

Key Words: Column liquid chromatography, Stability indicating, Pharmaceutical preparation, Oxcarbazepine.

INTRODUCTION

Oxcarbazepine is a novel antiepileptic drug, which was developed as a second generation and follow-up compound to carbamazepine (CBZ) with similar therapeutic profile but with much less side effects. Chemically oxcarbazepine (10,11-dihydro-10-oxo-5*H*-dibenz[b,f]azepine-5-carboxamide) is a 10-keto analog of CBZ (Fig. 1) and has been registered by US FDA[1-3] in Dec 2001. Oxcarbazepine has recently been found associated with a greater enhancement in mood and reduction in anxiety symptoms than other antiepileptic drugs. Now a day oxcarbazepine is also useful in the management of trigeminal neuralgia. After oral administration it is completely absorbed and extensively metabolized to its pharmacological active 10-monohydroxy metabolite[2,3].

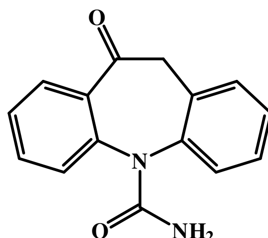

Fig. 1. Chemical structure of oxcarbazepine

Literature survey reveals that several liquid chromatographic methods were reported for the determination of oxcarbazepine and its active metabolite in human plasma and serum[4-8]. A high performance liquid chromatographic and spectroscopic method was reported for the quantitation of oxcarbazepine in tablet formulation[9,10]. But these methods utilize complex mobile phase composition and also lack of proper validation of the method[11]. Only one stability indicating high performance liquid chromatography method was reported for the determination of impurities and assay of oxcarbazepine. But the method is very complicated in terms of complex mobile phase composition and longer run time, hence the method is not feasible and economical for pharmaceutical industry. At present study attempts were made to develop simple, rapid, robust and economic method for the estimation of oxcarbazepine in the presence of degradants. It separates drugs from the degradation products under ICH suggested stress conditions (hydrolysis, oxidation, photolysis and thermal stress)[12,13]. Due to short run time and simple mobile phase composition the proposed method will be of immense help to the pharmaceutical industry for routine analysis of oxcarbazepine in quality control as well as stability studies of bulk drug and pharmaceutical formulations.

EXPERIMENTAL

Oxcarbazepine bulk drug was obtained from M/s Stadmed Private Limited (Kolkata, India) and Oxetol tablets (600 mg) were obtained from the market. HPLC-grade acetonitrile, sodium hydroxide, hydrogen peroxide and hydrochloric acid were purchased from Merck (Mumbai, India). HPLC-grade water (resistivity of 18 M.cm) generated from Milli-Q water purification system was used throughout the analysis.

The HPLC apparatus consists of a Shimadzu LC-20AT separation module and an SPD-20A UV detector (Kyoto, Japan). Detection and quantification were performed using Spinchrom software. RP-HPLC analysis was performed isocratically at room temperature using a Hiber C_{18} (250 mm × 4.6 mm, 5 μm) column. The mobile phase consisted of a mixture of acetronitrile and water (50:50, v/v) at a flow rate of 1.0 mL/min. All the samples were filtered through a 0.45 μm membrane filter and sample size of 50 μL was injected through the rheodyne injector system fitted with 50 μL fixed loop. The eluent was monitored with a UV detector set at 256 nm.

Preparation of standard solution: A stock solution of oxcarbazepine was prepared by dissolving 50 mg oxcarbazepine in a 50 mL volumetric flask with acetonitrile. The standard working solution of oxcarbazepine in the concentration range 0.05-80 μg/mL was prepared by diluting the stock solution with mobile phase.

Preparation of sample solution for assay: For sample solution, 20 tablets were accurately weighed and crushed to a fine powder. The powder sample equivalent to 600 mg oxcarbazepine was placed in a 100 mL volumetric flask. Then 80 mL of acetronitrile was poured in the flask and sonicated for 5 min. The final volume made up to 100 mL with the same, thoroughly mixed and filtered through 0.45 μ filter. This solution was further diluted by mobile phase to achieve 60 μg/mL of oxcarbazepine.

Stress study: Stress testing studies were undertaken to elucidate the intrinsic stability of the drug substance. Such testing is part of the development strategy and is normally carried out under more severe conditions than those used for accelerated testing. Forced degradation of drug substance was carried out under thermolytic, photolytic, acid/base hydrolytic and oxidative stress condition to degrade drug substance.

For acid, base and oxidative degradation, samples were individually placed into three volumetric flask and then 1N HCl, 1N NaOH and 3 % H_2O_2 were added separately into the flask. All the mixtures were heated at 80 ºC for 1 h. Acid and base treated samples were neutralized and all the three samples were then diluted by mobile phase to a concentration of 60 μg/mL.

For thermal degradation sample was exposed to heat at 80 ºC for 4 h and for photo degradation, the sample was exposed to UV lamp for 7 h. The samples were withdrawn and appropriate dilution was done to get final concentration 60 μg/mL. All the solutions were filtered through 0.45 micron filter and injected into the HPLC system to detect peaks of degradation products.

Validation of the method: The method was validated for specificity, linearity and range, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and robustness.

System suitability: This test has been performed in order to make the complete testing system suitable for the intended application. A standard containing 60 μg/mL of oxcarbazepine was injected 6 times. The parameters measured were peak area, retention time, theoretical plates and assaymetric factor.

Specificity: Specificity is the ability of the analytical method to determine the analyte (60 μg/mL) response in the presence of its degradation products under same chromatographic condition. The stability indicating capability of the method was established from the peak purity of oxcarbazepine in degraded sample.

Linearity: The linearity of the response of the drug was verified at ten concentration levels, ranging from 0.05-80 μg/mL for analyte. Linearity solutions were injected in triplicate. The calibration graph was obtained by plotting the peak area *versus* the concentration data and was treated by least-squares linear regression analysis. The slope and Y-intercept were calculated.

Limit of detection and limit of quantification: The LOD and LOQ for oxcarbazepine was determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentration.

Precision: The precision of the assay method was evaluated by six injection of three different standard solution of oxcarbazepine (0.25, 10 and 60 µg/mL) on the same day and the values of relative standard deviation were calculated to determine intra-day precision. These studies were also repeated on different days to determine inter-day precision.

Accuracy: The accuracy of the assay method was studied by recovery experiments. The recovery experiments were performed by adding known amount of the drugs in the placebo. The recovery experiments were performed at 50, 75 and 100 % of the label claim of the tablet (600 mg of oxcarbazepine). The samples were prepared in triplicate by aforementioned procedure and percentage of recoveries was calculated from the calibration curve.

Robustness: To determine the robustness of the developed method, experimental conditions were deliberately altered and the resolution between oxcarbazepine and its degradation products were evaluated. The robustness of the method was determined by analyzing sample by varying parameters *viz* detection wavelength, flow-rate and mobile phase composition of the developed method.

RESULTS AND DISCUSSION

System suitability: A suitability test was applied to check various parameters such as theoretical plates, assaymetric factor, RSD of peak area and retention time (RT) of the developed method. The results are presented in Table-1 and all the values satisfy the acceptance criteria, indicating that the system was suitable for oxcarbazepine analysis.

TABLE-1
SYSTEM SUITABILITY PARAMETERS

Sr. no.	Parameters	Results	Acceptance limit
1	Theoretical plates	3379	More than 3000
2	Asymmetric factor	1.560	Less than 2
3	RSD of peak area	1.104 %	Less than 2 %
4	RSD of RT	0.78 %	Less than 2 %

Specificity: The specificity of the developed method is illustrated in Fig. 2. The degradation was not observed in all the stress condition without base hydrolysis. The degradation peaks observed in base hydrolysis were well resolved from the analyte peak. The retention time of oxcarbazepine is about 4.0 min and no interfering peak was found at this retention time.

Linearity: Ten solutions of oxcarbazepine at concentrations ranging from 0.05-80.0 µg/mL were analyzed. The curve of the peak area response *vs.* concentration proved linear. The mean value (± RSD) of slope, intercept and correlation coefficient were 22.64 (± 1.36), 1.104 (± 0.26) and 0.999 (± 0.005), respectively.

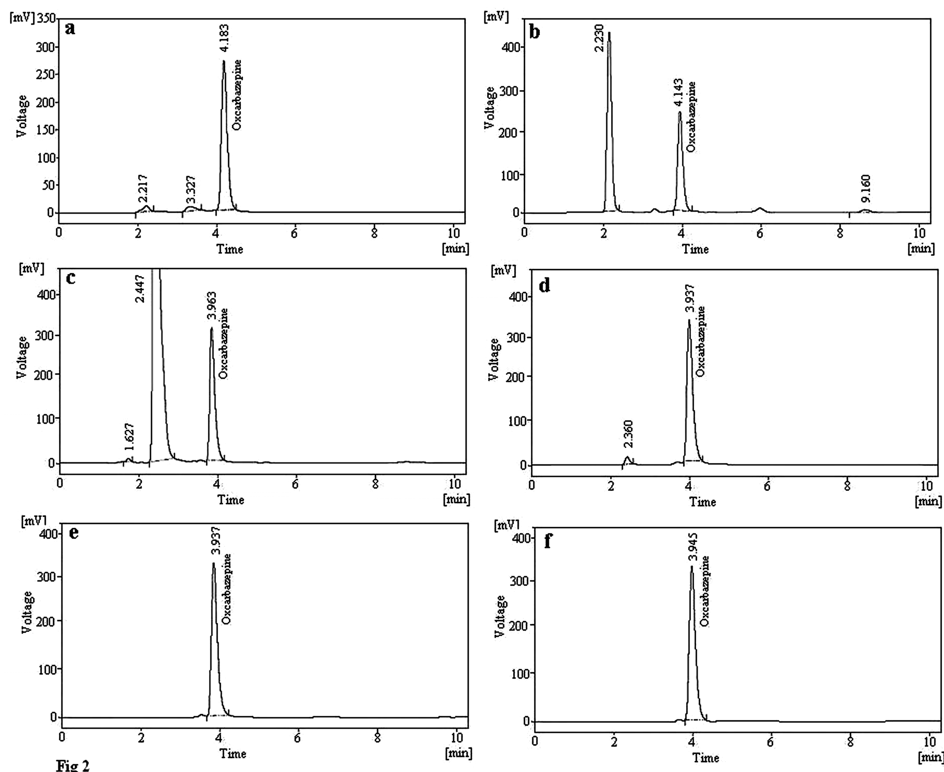

Fig. 2. LC chromatograms of oxcarbazepine (a) After acidic degradation (b) After basic degradation (c) After oxidative degradation (d) After thermal degradation (e) After photo degradation. (f) Standard solution (50 μg/mL)

Limit of detection and limit of quantification: Under the developed HPLC conditions, the LOD and LOQ for oxcarbazepine were found to be 15 and 50 ng/mL, respectively.

Precision and accuracy: The data obtained from precision experiments are given in Table-2 for intra- and inter-day precision studies. The RSD values for intra-day and inter-day precision study are < 1 %, which confirms that the method is sufficiently precise. The recovery values of oxcarbazepine ranged from 99.83 to 102.98 % and % RSD for nine determinations was 1.58.

Robustness: This test was performed to confirm that the separation was satisfactory under all the deliberate varied chromatographic conditions. In this method good separations were always achieved, indicating that the method remained selective for all components under the tested conditions.

Determination of active ingredient in tablets: The validated LC method was applied for the quantification of oxcarbazepine in tablets. Three batches of the tablets were assayed and the results are shown in Table-3. The amount of drug present in the formulation satisfies the requirement (90-110 %) of the label claim.

TABLE-2
PRECISION DATA

Actual conc. (µg/mL)	Conc. found (µg/mL) ± SD	RSD (%)
Intra-day precision		
0.25	0.2330 ± 0.002	0.920
10	10.062 ± 0.079	0.791
60	60.145 ± 0.342	0.568
Inter-day precision		
0.25	0.2290 ± 0.0014	0.623
10	9.958 ± 0.076	0.766
60	62.198 ± 0.471	0.759

TABLE-3
ASSAY RESULTS OF OXCARBAZEPINE (600 mg PER TABLET) FORMULATION PRODUCT

Batch no	Ingredient	Label claim (mg)	Found	Label claim (%)	RSD (%)
1	Oxcarbazepine	600	606.61	101.10	0.23
2	Oxcarbazepine	600	612.90	102.15	0.39
3	Oxcarbazepine	600	612.00	102.00	0.44

Conclusion

The results of the various validation studies showed that the LC method was very simple, sensitive, accurate, reproducible and stability indicating quantitative analysis of oxcarbazepine in tablets, which separates all degradants. The method was validated as per ICH guidelines and it is one of the rare studies where forced degradation studies were done under all different suggested conditions and all the products were resolved in a single isocratic run. Literature survey showed that only one stability indicating method has been published but the methods is very complicated in terms of complex mobile phase composition and longer run time (15 min). That's why the method is not feasible and economical for pharmaceutical industry. The developed method is very sensitive enough with LOD of 15 ng/mL and LOQ of 50 ng/mL. Due to simpler mobile phase and short run time, it ultimately increases the productivity of this method, thus reducing the cost of sample analysis. So the developed analytical method is more economical to pharmaceutical industry and can be used for stability testing as well as routine quality control analysis of oxcarbazepine in bulk drug and pharmaceutical formulations.

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