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Short communication

A validated stability indicating LC method for oxcarbazepine

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Abstract

The present paper describes the development of a stability indicating reversed phase liquid chromatographic (RPLC) method for oxcarbazepine in the presence of its impurities and degradation products generated from forced decomposition studies. The drug substance was subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal degradation. The degradation of oxcarbazepine was observed under base hydrolysis. The drug was found to be stable to other stress conditions attempted. Successful separation of the drug from the synthetic impurities and degradation product formed under stress conditions was achieved on a C18 column using mixture of aqueous 0.02 M potassium dihydrogen phosphate–acetonitrile–methanol (45:35:20, v/v/v) as mobile phase. The developed HPLC method was validated with respect to linearity, accuracy, precision, specificity and robustness. The developed HPLC method to determine the related substances and assay determination of oxcarbazepine can be used to evaluate the quality of regular production samples. It can be also used to test the stability samples of oxcarbazepine.

Keywords: Oxcarbazepine; Validation; Forced degradation; RPLC; Solution and mobile phase stability

1. Introduction

Oxcarbazepine (10,11-dihydro-10-oxo-5H-dibenz [b,f] azepine-5-carboxamide) is a 10-keto analog of carbamazepine [1] (Fig. 1), which is a new antiepileptic drug (AED) that has been registered in more than 50 countries worldwide since 1990 and recently received approval in the United States and the European Union. Oxcarbazepine is a keto analog of carbamazepine and has a more favorable pharmacokinetic profile. It is rapidly absorbed after oral administration and undergoes rapid and almost complete reductive metabolism to form the pharmacologically active 10-monohydroxy derivative [2].

A high-performance liquid chromatographic (HPLC) and spectroscopic methods were reported in the literature for the determination of oxcarbazepine in tablet formulation [3,4]. A high-performance liquid chromatographic determination of oxcarbazepine and its active metabolite in human serum and plasma was also reported in the literature [5–9]. The HPLC method for the determination of oxcarbazepine in tablet formulation [3] is applicable for quantification of oxcarbazepine in tablet formulation and is not a validated method for the quantitative determination of related substance in bulk drugs. So far to our present knowledge, no validated stability indicating analytical method for the determination of related substance in oxcarbazepine bulk drug was available in literature. Attempts were made to develop a stability indicating HPLC method for the related substance determination and quantitative estimation of oxcarbazepine. This paper deals with the forced degradation of oxcarbazepine under stress condition like acid hydrolysis, base hydrolysis, oxidation, heat and UV light. This paper also deals with the validation of the developed method for the accurate quantification of impurities and assay of oxcarbazepine.

2. Experimental

2.1. Chemicals

Samples of oxcarbazepine and its three impurities namely imp-A, imp-B and imp-C (Fig. 1) were received from Metro drugs Ltd., Hyderabad, India. Potassium dihydrogen phosphate, HPLC grade acetonitrile and methanol were purchased from Merck, Darmstadt, Germany. High purity water was prepared by using a Millipore Milli Q plus purification system.

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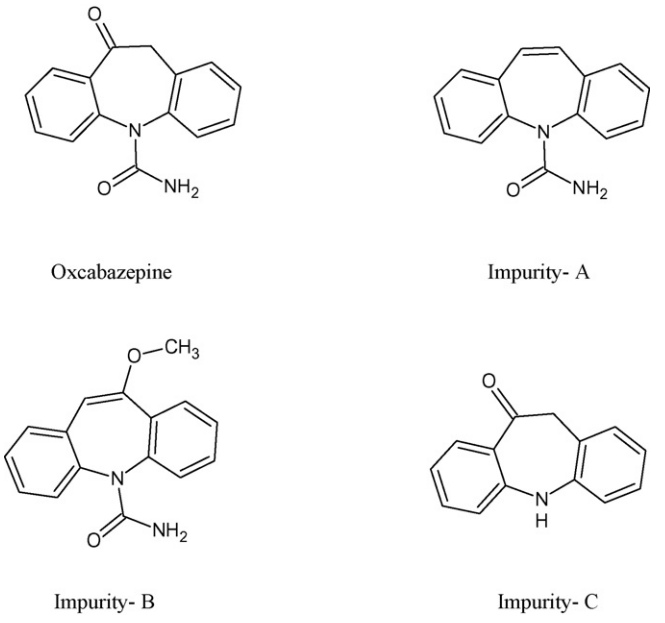

Fig. 1. Chemical structure of oxcarbazepine, imp-A, imp-B and imp-C.

2.2. Equipment

The LC system used for the method development and validation consisted of a dual piston reciprocating two LC-10AT VP pumps from Shimadzu Crop., Japan (model HPLC class 10AT), photo-diode array detector from Shimadzu Crop., Japan (model SPD-10 M VP) and auto sampler of SCL-10A series. The HPLC system was equipped with data acquisition and processing software "LC Solution" Shimadzu Crop., Japan.

2.3. Preparation of standard solutions

A stock solution of oxcarbazepine (1.0 mg/ml) was prepared by dissolving appropriate amount of substance in the mobile phase. Working solutions of 500 and 100 μg/ml were prepared from the above stock solution for the related substance determination and assay determination, respectively. A stock solution of impurity (mixture of imp-A, imp-B and imp-C) at 0.5 mg/ml was also prepared in the mobile phase.

2.4. Chromatographic conditions

The chromatographic separation was achieved on an Inertsil C18, 250 mm × 4.6 mm, 5 μm column using a mobile phase containing mixture of aqueous 0.02 M potassium dihydrogen phosphate–acetonitrile–methanol (45:35:20, v/v). The mobile phase was filtered through a nylon membrane (pore size 0.45 μm) and degassed with a helium spurge for 10 min. The flow rate of the mobile phase was 1 ml/min. The column temperature was maintained at 25 °C and the wavelength was monitored at 256 nm. The injection volume was 20 μl. The test concentration for the related substance analysis is 500 μg/ml and for assay 100 μg/ml. The standard and the test dilutions were prepared in mobile phase.

2.5. Validation of the method

2.5.1. Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities [10]. The specificity of the developed LC method for oxcarbazepine was carried out in the presence of its impurities namely imp-A, imp-B and imp-C. Stress studies were performed for oxcarbazepine bulk drug to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted to stress condition of UV light (254 nm), heat (60 °C), acid (0.5N HCl), base (0.5N NaOH) and oxidation (3.0% H_2O_2) to evaluate the ability of the proposed method to separate oxcarbazepine from its degradation product [11]. For heat and light studies, study period was 10 days whereas for the acid, base and oxidation, it was 48 h. Peak purity test was carried out for the oxcarbazepine peak by using PDA detector in stress samples. Assay studies were carried out for stress samples against qualified oxcarbazepine reference standard. Assay was also calculated for oxcarbazepine sample by spiking all three impurities at the specification level (i.e. 0.15%).

2.5.2. Precision

The precision of the assay method was evaluated by carrying out six independent assays of oxcarbazepine test sample against a qualified reference standard and calculated the % R.S.D. of assay.

The precision of the related substance method was checked by injecting six individual preparations of oxcarbazepine (0.5 mg/ml) spiked with 0.15% of imp-A, imp-B and imp-C with respect to oxcarbazepine analyte concentration. % R.S.D. of area for each imp-A, imp-B and imp-C was calculated.

The intermediate precision of the method was also evaluated using different analyst and different instrument in the same laboratory.

2.5.3. Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ for imp-A, imp-B and imp-C were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations [11]. Precision study was also carried out at the LOQ level by injecting six individual preparations of imp-A, imp-B and imp-C and calculating the % R.S.D. of the area.

2.5.4. Linearity

Linearity test solutions for the assay method were prepared from oxcarbazepine stock solutions at six concentration levels from 50 to 150% of assay analyte concentration (50, 75, 100, 125 and 150 μg/ml). The peak area versus concentration data was treated by least-squares linear regression analysis.

Linearity test solutions for the related substance method were prepared by diluting stock solutions to the required concentrations. The solutions were prepared at six concentration levels from LOQ to 200% (1.5 μg/ml) of the specification level (LOQ, 0.075, 0.15, 0.187, 0.225 and 0.3%).

Above test were carried out for 3 consecutive days in the same concentration range for both the assay and related substance

method. The % R.S.D. value for the slope and Y-intercept of the calibration curve was calculated.

2.5.5. Accuracy

The accuracy of the assay method was evaluated in triplicate at three concentration levels, i.e. 50, 100 and 150 μg/ml. The percentage of recoveries was calculated from the slope and Y-intercept of the calibration curve obtained in Section 2.5.4.

The accuracy study of impurities was carried out in triplicate at 0.075, 0.15 and 0.225% of the oxcarbazepine analyte concentration (500 μg/ml). The percentages of recoveries for impurities were calculated from the slope and Y-intercept of the calibration curve obtained in Section 2.5.4.

2.5.6. Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered and the resolution between oxcarbazepine, imp-A, imp-B and imp-C was recorded.

The flow rate of the mobile phase was 1.0 ml/min. To study the effect of flow rate on the resolution, flow was changed by 0.2 units from 0.8 to 1.2 ml/min. The effect of the column temperature on resolution was studied at 20 and 30 °C instead of 25 °C. The effect of the percent organic strength on resolution was studied by varying acetonitrile by −3 to +3% while other mobile phase components were held constant as stated in Section 2.4.

2.5.7. Solution stability and mobile phase stability

The solution stability of oxcarbazepine in the assay method was carried out by leaving both the test solutions of sample and reference standard in tightly capped volumetric flasks at room temperature for 48 h. The same sample solutions were assayed for 6 h interval up to the study period. The mobile phase stability was also carried out by assaying the freshly prepared sample solutions against freshly prepared reference standard solution for 6 h interval up to 48 h. Mobile phase prepared was kept constant during the study period. The % R.S.D. for the assay of oxcarbazepine was calculated during mobile phase and solution stability experiments.

Table 1
System-suitability report

Compound ($n = 3$)	R_t	R_S	N	T
Oxcarbazepine	4.9	–	3921	1.20
Impurity A	6.0	3.3	4715	1.25
Impurity B	7.0	2.8	5565	1.25
Impurity C	8.5	3.6	6419	1.30

n, Number of determinations; R_t, retention time; R_S, USP resolution; N, number of theoretical plates; T, USP tailing factor.

The solution stability of oxcarbazepine and its impurities in the related substance method was carried out by leaving spiked sample solutions in tightly capped volumetric flasks at room temperature for 48 h. Content of imp-A, imp-B and imp-C were determined for every 6 h interval up to the study period. The mobile phase stability was also carried out for 48 h by injecting the freshly prepared sample solutions for every 6 h interval. Content of imp-A, imp-B and imp-C were checked in the test solutions.

3. Results and discussion

3.1. Optimization of chromatographic conditions

The main objective of the chromatographic method is to separate oxcarbazepine from imp-A, imp-B and imp-C. Impurities were coeluted using different stationary phases such as C18, C8, phenyl and cyano as well as different mobile phases. The chromatographic separation was achieved on an Inertsil C18 250 mm × 4.6 mm, 5 μm column using mixture of aqueous 0.02 M potassium dihydrogen phosphate–acetonitrile–methanol (45:35:20, v/v/v) as a mobile phase. The flow rate of the mobile phase was 1.0 ml/min, at 25 °C column temperature, the peak shape of the oxcarbazepine was found to be symmetrical. In optimized chromatographic conditions oxcarbazepine, imp-A, imp-B and imp-C were separated with resolution greater than 2, typical retention time were about 4.9, 5.9, 7.0 and 8.5 min, respectively (Fig. 2). The system suitability results are given in Table 1 and the developed LC method was found to be specific

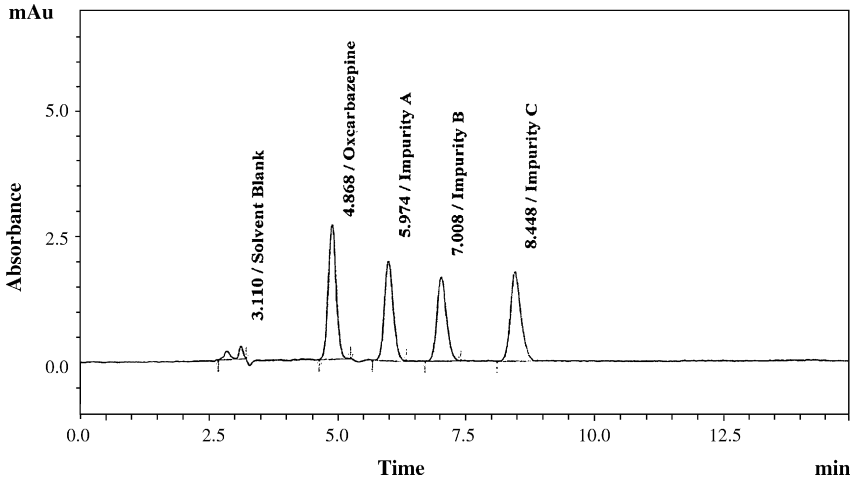

Fig. 2. HPLC resolution chromatogram of oxcarbazepine, imp-A, imp-B and imp-C.

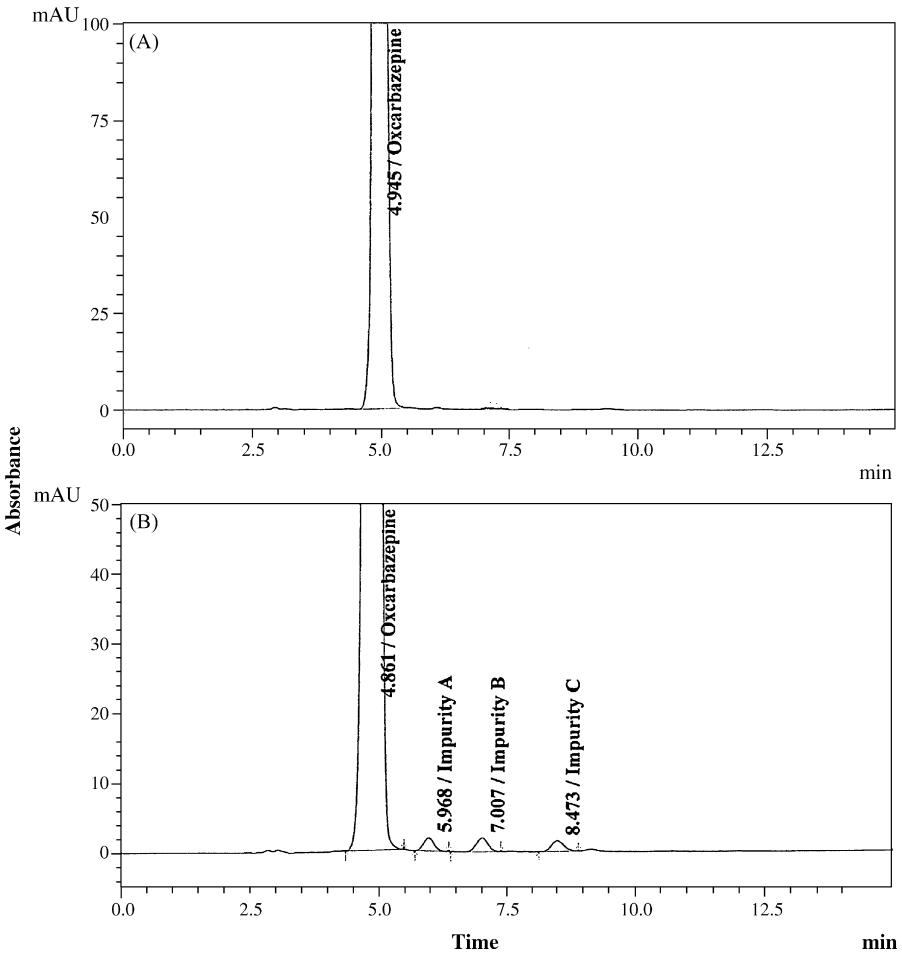

Fig. 3. HPLC chromatograms of unspiked (A) and spiked with imp-A, imp-B and imp-C at 0.15% level in oxcarbazepine sample (B).

for oxcarbazepine and its three impurities namely imp-A, imp-B and imp-C (Fig. 3).

3.2. Results of forced degradation studies

Degradation was not observed in oxcarbazepine sample when subjected to stress conditions like light, heat, acid hydrolysis and oxidation (Fig. 4). Oxcarbazepine was degraded to impurity C under base hydrolysis. Peak purity test results confirmed that the oxcarbazepine peak is homogenous and pure in all the analyzed stress samples. The assay of oxcarbazepine is unaffected in the presence of imp-A, imp-B and imp-C and its degradation products confirms the stability indicating power of the method. The summary of forced degradation studies is given in Table 2.

3.3. Precision

The % R.S.D. of assay of oxcarbazepine during the assay method precision study was within 0.8% and the % R.S.D. for the area of imp-A, imp-B and imp-C in related substance method precision study was within 3%. The % R.S.D. of the assay results obtained in the intermediate precision study was within 0.9 % and the % R.S.D. for the area of imp-A, imp-B and imp-C were well within 3%, conforming good precision of the method.

3.4. Limit of detection and limit of quantification

The limit of detection of all the impurities namely imp-A, imp-B and imp-C was achieved at 50 ng/ml for 20 μl injection volume. The limit of quantification for all three impurities

Table 2
Summary of forced degradation results

Stress condition	Time (h)	% Assay of active substance	% Mass balance (% assay + impurity)
Acid hydrolysis (0.5N HCl)	48	99.3	99.3
Base hydrolysis (0.5N NaOH)]	48	89.9	99.5
Oxidation (3% H_2O_2)	48	99.2	99.2
Thermal (60 °C)	48	99.5	99.5
UV (254 nm)	48	99.2	99.2

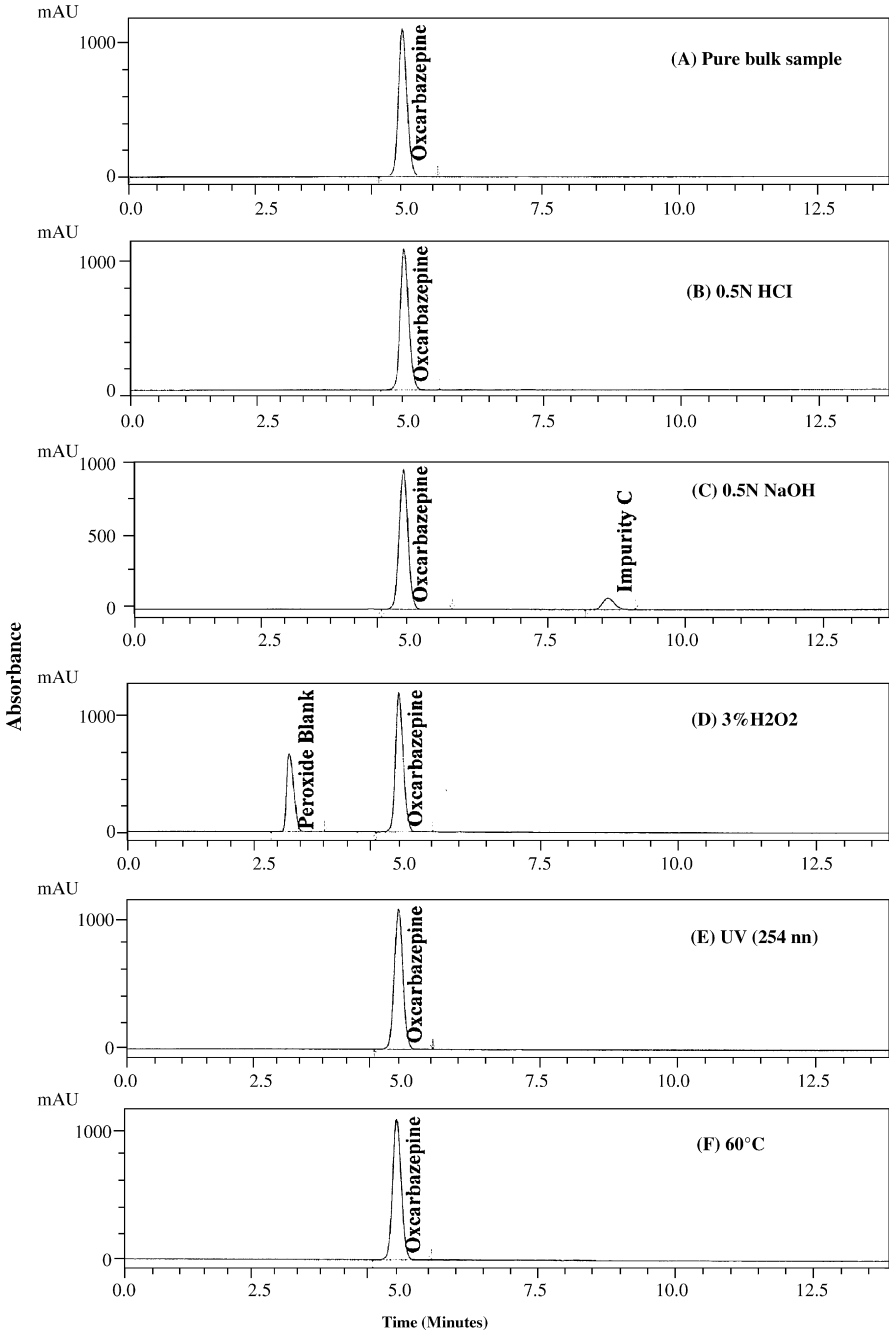

Fig. 4. Typical HPLC chromatograms of oxcarbazepine under stress condition (A) pure oxcarbazepine bulk sample, (B) 0.5N HCl, (C) 0.5N NaOH, (D) 3% H_2O_2, (E) UV (254 nm) and (F) 60 °C.

namely imp-A, imp-B and imp-C was achieved at 150 ng/ml for a 20 μl injection volume. The precision at the LOQ concentrations for imp-A, imp-B and imp-C were below 4.1%.

3.5. Linearity

The linearity calibration plot for the assay method was obtained over the calibration ranges tested, i.e. 50–150 μg/ml and correlation coefficient obtained was greater than 0.999. Linearity was checked for the assay method over the same concentration range for 3 consecutive days. The % R.S.D. values of the slope and Y-intercept of the calibration curves were 1.7 and 2.5, respectively. The result shows that an excellent correlation existed between the peak area and concentration of the analyte.

Linear calibration plot for the related substance method was obtained over the calibration ranges tested, i.e. LOQ (0.03 %) to 0.3% for impurity imp-A, imp-B and imp-C. The correlation coefficient obtained was greater than 0.998. Linearity was checked for the related substance method over the same concentration range for 3 consecutive days. The % R.S.D. values of the slope and Y-intercept of the calibration curves were 3.1 and 3.7, respectively. The above result show that an excellent correlation existed between the peak area and the concentration of imp-A, imp-B and imp-C.

Table 3
Recovery results of oxcarbazepine sample

Added (μg) ($n = 3$)	Recovered (μg)	%Recovery	% R.S.D.
50.8	50.1	98.6	0.7
101.5	100.8	100.7	0.5
150.8	150.1	99.5	0.8

n, Number of determinations.

3.6. Accuracy

The percentage recovery of oxcarbazepine in bulk drug samples was ranged from 98.6 to 100.7% (Table 3). The percentage recovery of impurities in oxcarbazepine samples varied from 97.2 to 102.5%. The HPLC chromatograms of unspiked and spiked sample at 0.15% level of all three impurities in oxcarbazepine bulk drug sample are shown in Fig. 3.

3.7. Robustness

In all the deliberate varied chromatographic conditions (flow rate, column temperature and composition of organic solvent), the resolution between critical pair, i.e. oxcarbazepine and imp-A was greater than 2.0, illustrating the robustness of the method.

3.8. Solution stability and mobile phase stability

The % R.S.D. of the assay of oxcarbazepine during solution stability experiments were within 1%. No significant changes were observed in the content of impurities namely imp-A, imp-B and imp-C during solution stability and mobile phase stability experiments when performed using the related substance method. The solution stability and mobile phase stability experiment data confirms that the sample solutions and mobile phases used during assay and the related substance determination were stable for at least 48 h.

4. Conclusion

The RPLC method developed for quantitative and related substance determination of oxcarbazepine is precise, accurate, rapid and specific. The method was fully validated showing satisfactory data for all the method validation parameters tested. The developed method is a stability indicating and can be conveniently used by quality control department to determine the related substance and assay in regular oxcarbazepine production samples and also stability samples.

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