



# Development and Validation of Stability-Indicating Liquid Chromatographic Method for the Quantitative Determination of Oxcarbazepine in Tablet Dosage Forms

Rao KS, Belorkar N, Rao MEB

*Roland Institute of Pharmaceutical Sciences, Berhampur-760 010, Orissa, India*

*Address for correspondence: K. Srinivasa Rao; E-mail: ksrao108@gmail.com*

---

## ABSTRACT

An isocratic, reversed-phase liquid chromatographic (RP-LC) method was developed for the quantitative determination of oxcarbazepine, used in the treatment of epilepsy. This method can be also employed for the related substance determination in bulk samples. Forced degradation studies were performed on bulk sample of oxcarbazepine using acid (1.0 N hydrochloric acid), base (0.1 N sodium hydroxide), oxidation (3.0% hydrogen peroxide), heat (80°C) and photolytic degradation. The chromatographic method was fine tuned using the samples generated from forced degradation studies. Good resolution between the peaks corresponds to degradation products and the analyte was achieved on Zorbax Eclipse XDB-C18 250 X 4.6 mm, 5 µm column. The mobile phase consists of a mixture of 0.005 M KH<sub>2</sub>PO<sub>4</sub>: Methanol: Acetonitrile (70:05:25% v/v/v) effluent was monitored at 215 nm using PDA detector. The method was linear over the concentration range of 1-1000 µg/ml ( $r = 0.9999$ ) with a limit of detection and quantitation 0.1 and 0.3 µg/ml, respectively. The method has the requisite accuracy, selectivity, sensitivity and precision to assay oxcarbazepine in bulk form and pharmaceutical dosage forms. Degradation products resulting from the stress studies did not interfere with the detection of oxcarbazepine and the assay is thus stability-indicating.

**Key words:** Assay, oxcarbazepine, recovery studies, specificity, stress studies

**DOI:** 10.4103/0975-1483.57077

---

## INTRODUCTION

Oxcarbazepine is 10,11-Dihydro-10-oxo-5*H*-dibenz[*b,f*]azepine- 5-carboxamide; it 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. 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.<sup>[1,2]</sup>

A high-performance liquid chromatographic (HPLC) and spectroscopic methods were reported in the literature for the determination of oxcarbazepine in tablet formulation.<sup>[3,4]</sup> A high-performance liquid chromatographic determination of oxcarbazepine and its active metabolite in human serum and plasma was also reported in the literature.<sup>[5-6]</sup> One stability indicating liquid chromatographic method was reported in the

literature for oxcarbazepine.<sup>[17]</sup> However, the reported method's stress studies were not carried out strictly as per ICH guidelines especially photo-stability studies. On the other hand, the present method is carried out strictly according to ICH guidelines.<sup>[18,19]</sup>

According to current good manufacturing practices, all drugs must be tested with a stability-indicating assay method before release. Stress testing of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability-indicating power of the analytical procedures used. The nature of the stress testing will depend on the individual drug substance and the type of drug-product involved. Considering the susceptibility of OCZ under variety of conditions, it was felt that a HPLC method of analysis that separates the drug from the degradation products formed under ICH suggested conditions (hydrolysis, oxidation, photolysis and thermal stress) would be of general interest. These studies provide valuable information on drug's inherent stability and help in the validation of analytical methods to be used in stability studies. Attempts were made to develop a suitable single stability indicating LC method that can be used to determine the related substances and also the assay of bulk samples of OCZ. This paper deals with the development of stability indicating analytical method using the samples generated from forced degradation studies.

## EXPERIMENTAL

### Materials

Gift sample of oxcarbazepine (OCZ) was received from Alkem Laboratories, Mumbai, India. HPLC grade methanol was purchased from Fischer Scientific, India. Hydrogen peroxide was purchased from Qualigens Fine chemicals, India. High pure water was prepared by using Millipore Milli Q plus purification system. Commercial formulations Oxetol<sup>®</sup> containing 150mg of OCZ were purchased from the local market.

### Instrumentation

The LC system, used for method development, forced degradation studies and method validation was on a waters separation module 2695, with an auto injector, and waters 2696 PDA detector. The output signal was monitored and integrated using Empower software. Zorbax Eclipse

XDB-C<sub>18</sub> 250mm x 4.6 mm, 5 µm column was used.

### Chromatographic conditions

The chromatographic separation was performed using Zorbax Eclipse XDB-C<sub>18</sub> 250 X 4.6 mm, 5 µm, column. The mobile phase consists of 0.005M KH<sub>2</sub>PO<sub>4</sub>: methanol: acetonitrile (70:05:25% v/v/v). The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1 ml/min for 10 min. The column temperature was maintained at 23 ± 1°C. The eluate was monitored at 215 nm using PDA-detector. The retention time of the drug was found to be 7.0 min. The injection volume was 5 µl. Mobile phase was used as diluent during the standard and test samples preparation.

### Preparation of standard solutions

Accurately about 10 mg of the OCZ working standard was weighed and transferred into 100 ml clean, dry standard volumetric flask. To this about 70 ml of acetonitrile was added and then it was kept in an ultrasonic bath to dissolve. Then, the volume was made up to the mark with the mobile phase and mixed well. Suitable aliquots of this standard stock solution were transferred using A-grade bulb pipettes into 10-ml volumetric flasks and the solutions were made up to volume with mobile phase to give final concentrations in the range of 1-1000 µg/ml.

### Preparation of oxcarbazepine tablets for assay

OCZ tablet powder was weighed accurately to the equivalent weight of label claim and transferred into 100 ml clean, dry standard volumetric flask. To this about 70 ml of acetonitrile was added and then it was kept in an ultrasonic bath to dissolve. Then, the volume was made up to the mark with the mobile phase and mixed well. Suitable aliquot of the filtered solution was added to a 5ml volumetric flask and made up to volume with mobile phase to yield starting concentration of 0.1 mg/ml and further analyzed by using above mention chromatographic conditions.

### Forced degradation studies

To determine whether the analytical method and assay were stability-indicating, OCZ tablets and OCZ active pharmaceutical ingredient (API) powder were stressed under various conditions to conduct forced degradation studies. Intentional degradation was attempted to stress conditions of photolytic degradation, acid hydrolysis (using 1.0 N HCl), base hydrolysis (using 0.1N NaOH), oxidative

degradation (using 3.0% H<sub>2</sub>O<sub>2</sub>) and thermal treatment (heated at 80°C for two days) to evaluate the ability of the proposed method to separate OCZ from its degradation products. OCZ at a concentration of 0.5 mg/ml was used in all the degradation studies. After completion of the degradation processes, the solutions were neutralized and diluted with mobile phase.

### Acid and alkaline degradation

Forced degradation in acidic media was performed by taking an aliquot of stock solution in 10ml volumetric flask and diluted up to the mark with 1.0N HCl to obtain a final concentration of 100mcg/ml. The flask was kept aside at room temperature for 48 h and neutralized. Appropriate aliquot was taken from the above solution and diluted with mobile phase to obtain a final concentration of 10mcg/ml. Similarly, forced degradation in alkaline media was performed using 0.1N NaOH. The representative

chromatograms for acid and alkaline degradation studies are shown in Figures 1 and 2 respectively.

### Oxidative degradation

Oxidative degradation was performed by taking an aliquot of stock solution in 10 ml volumetric flask and diluted up to the mark with 3%w/v of hydrogen peroxide to obtain a final concentration of 100mcg/ml. The flask was kept aside at room temperature for two days. Appropriate aliquot was taken from the above solution and diluted with mobile phase to obtain a final concentration of 10mcg/ml. The representative chromatogram is shown in Figure 3.

### Photo stability

OCZ - API, tablet powder and solutions of both were prepared and exposed to light to determine the effects of light irradiation on the stability of OCZ in solution and

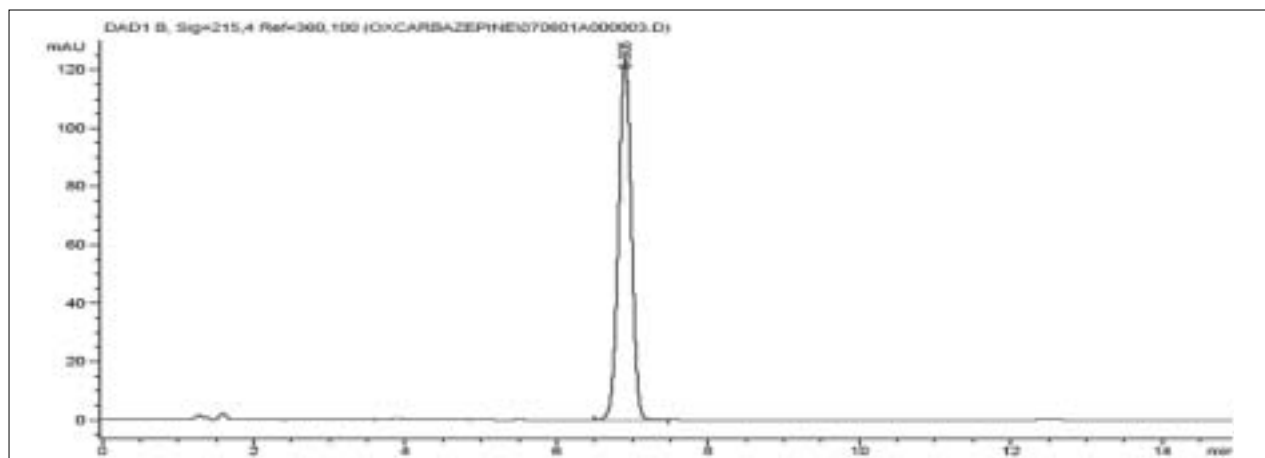


Figure 1: Typical chromatogram of acid hydrolysis degraded Active Pharmaceutical Ingredient (API)

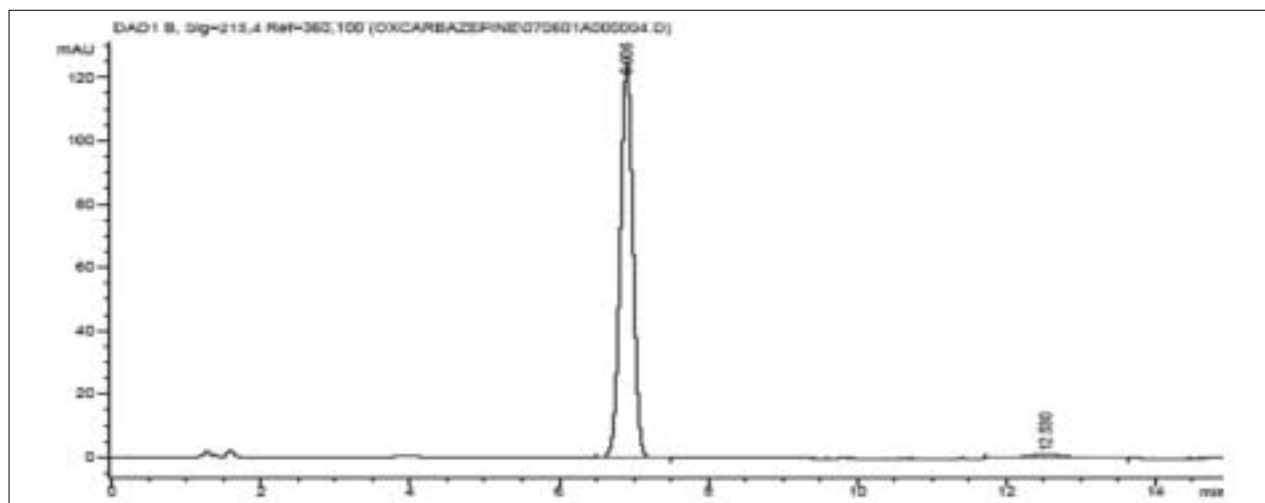


Figure 2: Typical chromatogram of alkaline hydrolysis degraded API

LC method for oxcarbazepine

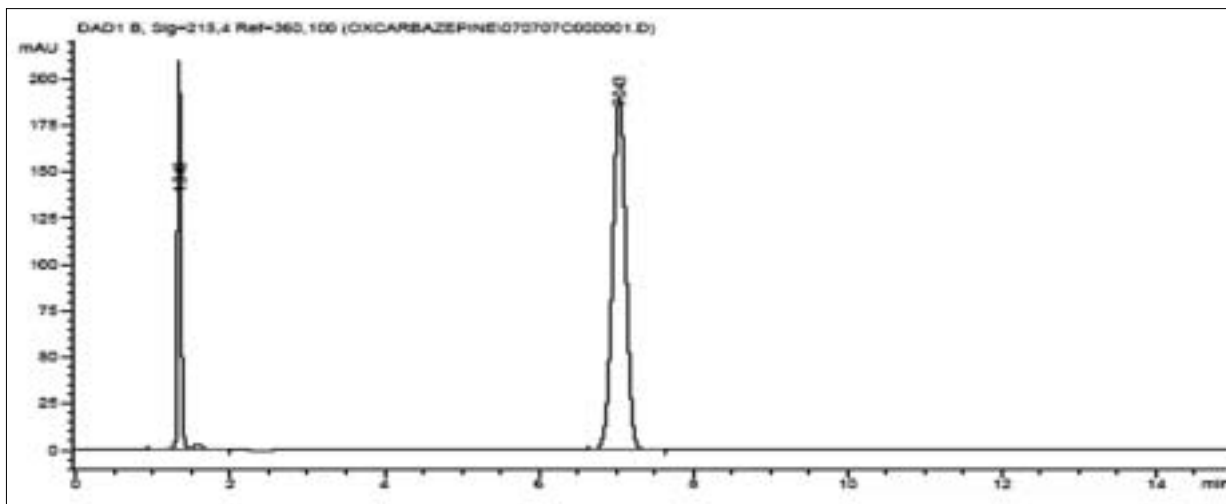


Figure 3: Typical chromatogram of oxidative hydrolysis - degraded API

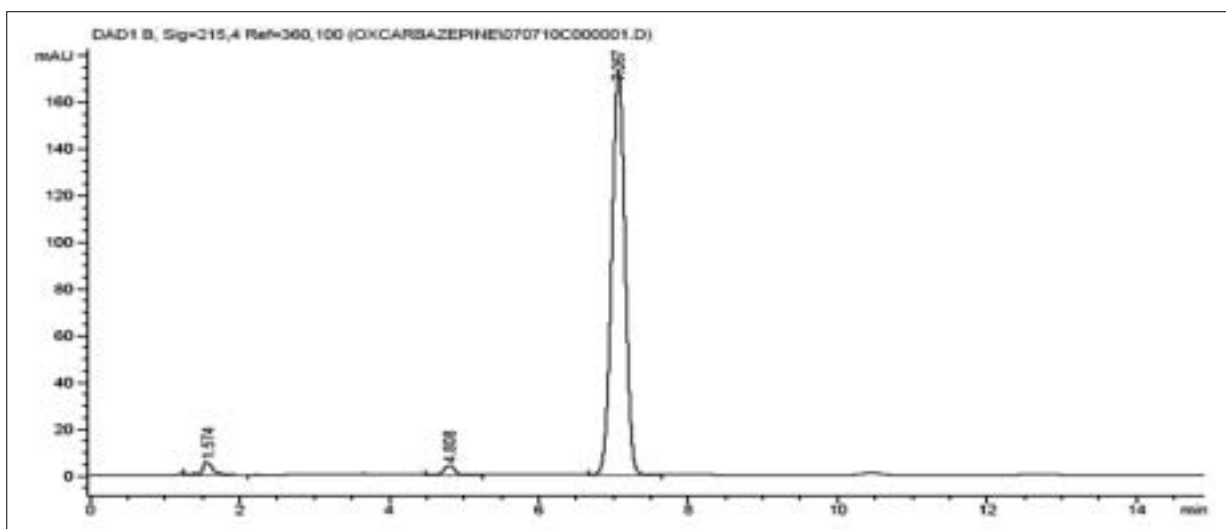


Figure 4: Typical chromatogram of photolytic treatment degraded API

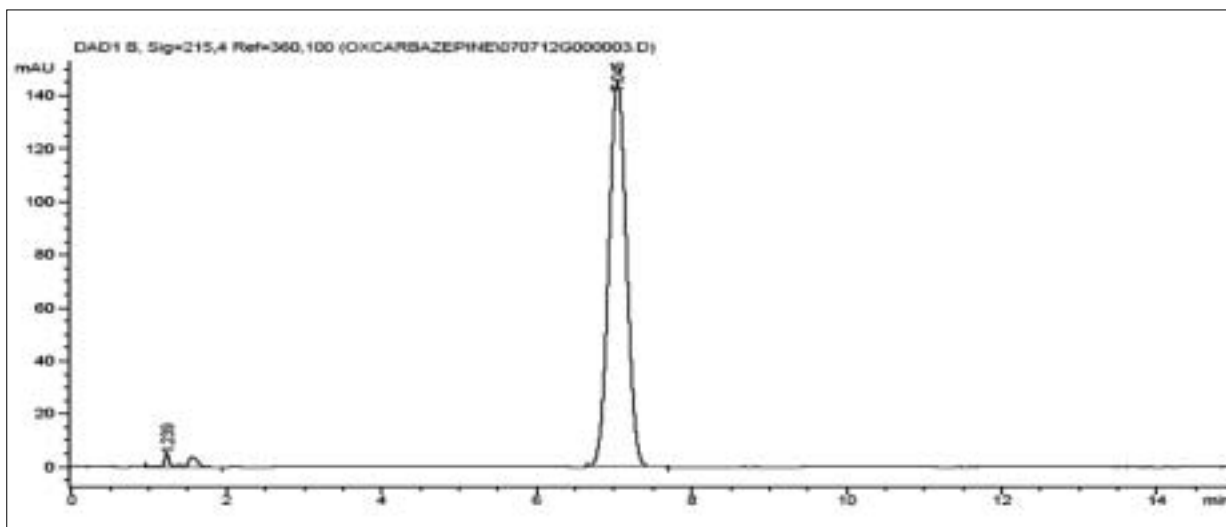


Figure 5: Typical chromatogram of heat treatment degraded API

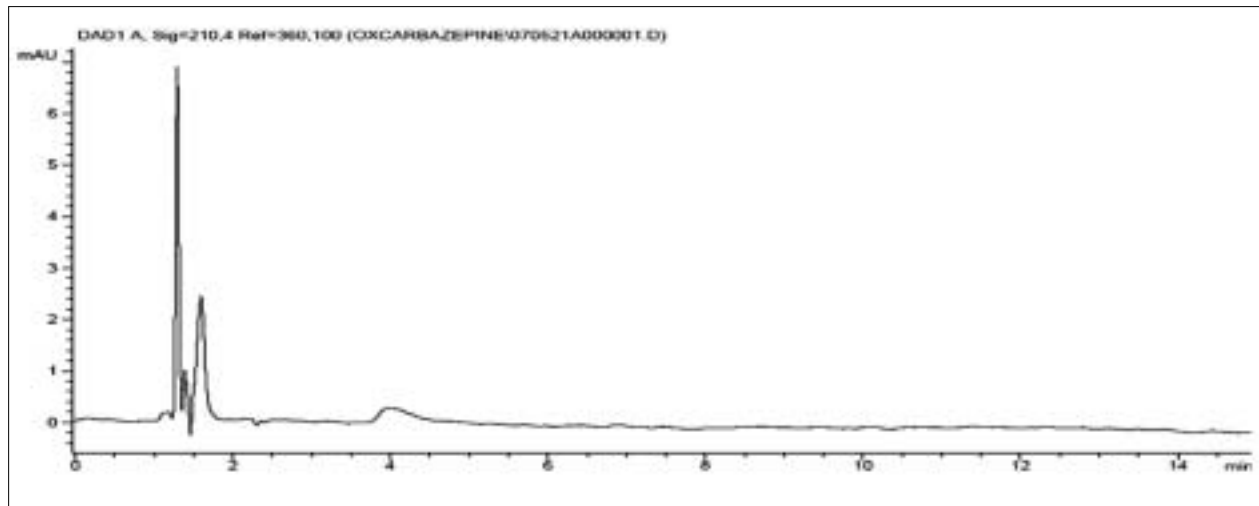


Figure 6: A representative chromatogram of the blank run

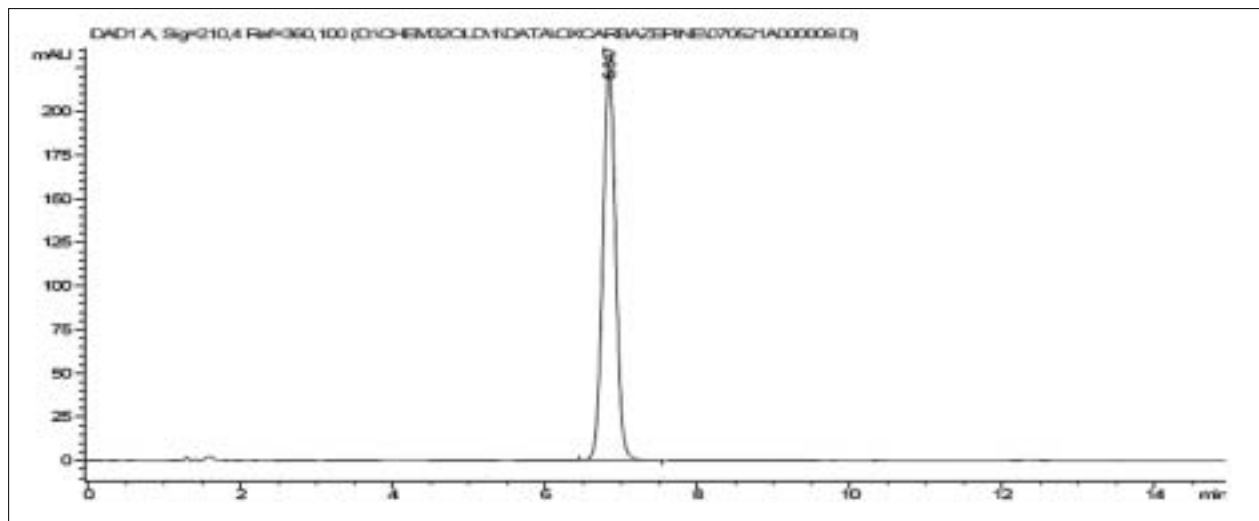


Figure 7: Typical chromatogram of OCZ in API

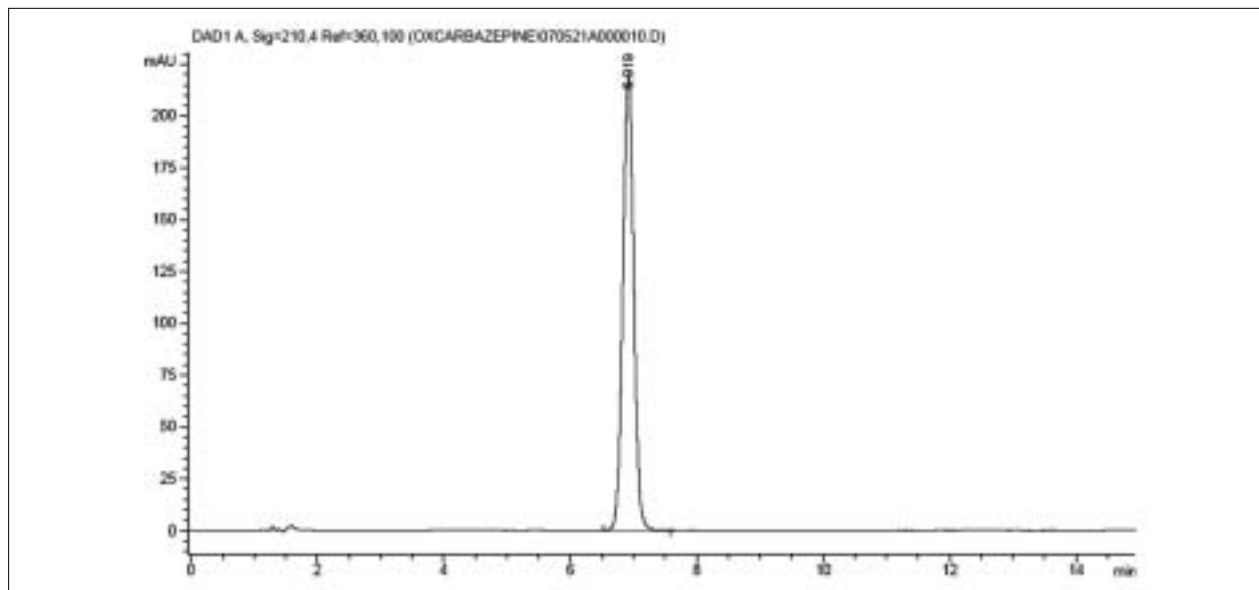


Figure 8: Typical chromatogram of OCZ in tablet dosage forms

**Table 1: Summary of forced degradation results**

Stress condition	Time (hours)	% Recovery of analyte	Retention time of analyte	Peak purity
Acid hydrolysis (1.0 N HCl at RT)	48	100.15	6.90	999
Base hydrolysis (0.1N NaOH at RT)	48	98.44	6.80	999
Oxidation (3% H <sub>2</sub> O <sub>2</sub> at RT)	48	98.14	7.04	999
Photolysis (UV light 320400 nm at RT)	40	95.96	7.06	999
Thermal treatment (80°C)	48	99.99	7.04	999

RT-room temperature; API- Active Pharmaceutical Ingredient; c mean peak area is the average of three determinations, d Peak purity values in the range 9901000 indicate a homogenous peak.

**Table 2: Accuracy data (n = 5)**

Amount of drug added (mg)	Amount of drug recovered (mg) Mean ± SD	% Drug of recovery	% RSD
120	120.49±0.41	100.41	0.34
150	147.495±0.23	98.33	0.15
180	178.908± 0.46	99.39	0.26

Data obtained from five replicates at each concentration.

**Table 3: System suitability parameters**

Parameter	Values obtained	Preferable values
Theoretical plates (N)	5623	>2500
Resolution (R)	5.2	>1.5
Tailing factor	1.31	<1.5

in the solid state. Approximately 50 mg of OCZ - API powder was spread on a glass dish in a layer that was less than 2mm thickness and a solution of API (1 mg/ml) was prepared in mobile phase. Tablet powder was also prepared in the same way. All samples for photo-stability testing were placed in a light cabinet and exposed to light for 40 h resulting in an overall illumination of  $\geq 210\text{Wh/m}^2$  at 25 °C with UV radiation at 320-400 nm. Control samples, which were protected with aluminum foil, were also placed in the light cabinet and exposed concurrently. Following removal from the light cabinet, all samples were prepared for analysis as previously described. The representative chromatogram is shown in Figure 4.

### Thermal stress studies

For thermal stress, OCZ - API, tablet powder and solutions of both were prepared and exposed to a controlled-temperature oven at 80°C for 48 h. The representative chromatogram is shown in Figure 5.

## RESULTS AND DISCUSSION

### HPLC method development and optimization

The chromatographic conditions were optimized with respect to specificity, resolution and time of analysis. The specificity of the method was established through the study of resolution factor of OCZ peak from the nearest

resolving peak. Peaks were identified using retention times compared with those of standards and the characteristic spectra were confirmed by photodiode array detection (range 200-400 nm). The chromatographic column used was Zorbax Eclipse XDB-C18 250 X 4.6 mm, 5  $\mu\text{m}$  and it was maintained at ambient temperature for the separation and the method validated for the determination of OCZ tablets. The composition and the flow rate of the mobile phase were changed to optimize the separation conditions using stressed samples and the main related substances. A mobile phase consisting of a mixture of 0.005M KH<sub>2</sub>PO<sub>4</sub>: methanol: acetonitrile (70:05:25% v/v/v) was selected, for use, after several preliminary investigatory chromatographic runs. The flow rate was maintained at 1 ml/min. Under the described experimental conditions, all peaks were well defined and free from tailing. The effects of small deliberate changes in the mobile phase composition and flow rate were evaluated as a part of testing for method robustness.

### Results of forced degradation studies

Forced degradation studies were performed for bulk drug and tablet powder, to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted to stress conditions such as acid hydrolysis (using 1.0N HCl), base hydrolysis (using 0.1N NaOH), oxidative hydrolysis (using 3.0% H<sub>2</sub>O<sub>2</sub>), thermal treatment (heated at 80°C for two days) and photolytic degradation (overall illumination of  $\geq 210\text{Wh/m}^2$  at 25°C for 40 h with UV radiation at 320-400 nm), to evaluate the ability of the proposed method to separate OCZ from its degradation products. It was observed that OCZ degrades with acidic, alkaline, oxidative and light conditions. This degradation is mainly observed in terms of loss of assay. Under the stress

degradation conditions with acid [Figure 1] and heat [Figure 5] there was no additional peaks in the chromatograms. However, under the degradation conditions with alkali [Figure 2], oxidation [Figure 3] and photolysis [Figure 4], additional peaks were observed without complete degradation of OCZ. Therefore, it may be concluded that OCZ is susceptible to alkaline, oxidative, heat treatment conditions. Table 1 indicates the extent of degradation of OCZ under various stress conditions. Photodiode array detection was used as an evidence of the specificity of the method, and to evaluate the homogeneity of the drug peak. Chromatographic peak purity data was obtained from the spectral analysis report and a peak purity value greater than 990 indicates a homogenous peak. The peak purity values for analyte peaks, in chromatograms of stressed samples were in the range of 999-1000 for drug substance, and in the range of 997-999 for tablets, indicating homogenous peaks and thus establishing the specificity of assay method. Resolution between the analyte peaks and nearest peak was more than 2.0 in all the chromatograms. Figures 15 shows the chromatograms of forced degraded samples. The degradation products were well resolved from OCZ, confirming the stability-indicating power of the method.

#### Validation of the method

The analytical method was validated with respect to parameters such as linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, selectivity, recovery and robustness/ruggedness.

##### Linearity

Linearity was established by least squares linear regression analysis of the calibration curve. The constructed calibration curves were linear over the concentration range of 1-1000µg/ml. Peak areas of OCZ was plotted against their respective concentrations and linear regression analysis was performed on the resultant curve. Correlation coefficient ( $r=3$ ) was found to be more than 0.999 with %RSD values were less than 2% across the concentration ranges studied. Typically, the regression equation was:  $y = 25.444x + 70.52$  ( $R = 0.9999$ )

##### Limit of detection and limit of quantitation

The limit of quantitation (LOQ) of the present method was found to be 0.3µg/ml with a resultant %RSD of 0.34% ( $n = 5$ ). The limit of detection (LOD) was found to be 0.1µg/ml.

##### Precision

The injection (system) precision was evaluated by performing six replicate injections of the standard OCZ

(nominally 10 mcg/ml). The method precision (intra-day repeatability) was established by performing six replicates of independently prepared samples of OCZ. The R.S.D. values were 0.18 and 0.31%, respectively.

##### Accuracy

Accuracy data for the assay following the determination of the compound of interest is summarized in Table-2. Accuracy was determined by interpolation of replicate ( $n=5$ ) peak areas of three accuracy standards of different concentration, from a calibration curve that had been prepared as previously described.

##### Specificity

The results of stress testing studies in addition to that of monitoring standard solutions of the drug in the presence of their impurities indicated a high degree of specificity of this method. The degradation product(s) of the parent compound was found to be similar for both the tablets and API powder. All the degradation products formed during forced decomposition studies were well separated from the analyte peak, demonstrating that the developed method was specific and stability-indicating.

##### Ruggedness/robustness

As recommended in the ICH Guidelines, a robustness assessment was performed during the development of the analytical procedure. The ruggedness of the method is assessed by comparison of the intra- and inter-day assay results that has been performed by two analysts. The %RSD values for intra- and inter-day assays of OCZ tablets performed in the same laboratory by two analysts did not exceed 1.8%, indicating the ruggedness of the method. In addition, the robustness of the method was investigated under a variety of conditions including changes of flow rate and column temperature. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust.

##### System suitability parameters

System suitability parameters can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system suitability are usually developed after the completion of method development and validation. (Or) The USP (2000) defines parameters that can be used to determine system suitability prior to analysis. The system suitability parameters like Theoretical plates (N), Resolution (R), Tailing factor (T), were calculated and compared with the standard values to ascertain whether the proposed RP-HPLC method for the estimation of OCZ in pharmaceutical formulations

was validated or not. The results are shown in Table 3.

### Assay

The validated method was applied to the determination of OCZ in commercially available Oxetol® tablets. Figures 6, 7 and 8 illustrates typical HPLC chromatograms obtained from blank, OCZ standard solution and from the assay of Oxetol® tablets respectively. The results of the assay ( $n = 9$ ) undertaken yielded 99.13% (%RSD = 0.054%) of label claim for OCZ. The observed concentration of OCZ was found to be  $149.41 \pm 0.2645$  mg/ml (mean  $\pm$  SD). The mean retention time of OCZ was 6.99 min. The results of the assay indicate that the method is selective for the analysis of OCZ without interference from the excipients used to formulate and produce these tablets.

### Solution stability

The R.S.D. of assay of OCZ during solution stability experiments was within 1.0%. No significant change was observed in the content of OCZ during solution stability experiments. The experimental data confirmed that sample solutions used during assay and related substance determinations were stable up to 48 h.

## CONCLUSIONS

A simple, rapid, accurate and precise stability-indicating HPLC analytical method has been developed and validated for the routine analysis of oxcarbazepine in API and tablet dosage forms. The results of stress testing reveal that the method is selective and stability indicating. The proposed method has the ability to separate the analyte from their degradation products, related substances, excipients found in tablet dosage forms and can be applied to the analysis of samples obtained during accelerated stability experiments.

## ACKNOWLEDGEMENTS

The authors are thankful to analytical research and development laboratory of International Speciality Product, Hyderabad, India for their support to carry out this project.

## REFERENCES

- Mazza M, Della Marca G, Di Nicola M, Martinotti G, Pozzi G, Janiri L, *et al.* Oxcarbazepine improves mood in patients with epilepsy. *Epilepsy Behav* 2007;10:397-401.
- Rosa AR, Cruz N, Comes M, Vieta E. Impulsivity in bipolar disorder: A randomized clinical trial of adjunctive oxcarbazepine. *Eur Neuropsychopharmacol* 2009;19:S75-6.

- Ramaa CS, Chothe PP, Naik AA, Kadam VJ. Spectrophotometric method for the estimation of Oxcarbazepine in tablets. *Indian J Pharm Sci* 2006;68:265-6
- Qi ML, Wang P, Wang LJ, Fu RN. LC method for the determination of oxcarbazepine in pharmaceutical preparations. *J Pharm Biomed Anal* 2003;31:57-62.
- Lever H, Odou P, Robert H. LC determination of oxcarbazepine and its active metabolite in human serum. *J Pharm Biomed Anal* 2002;28:517-25.
- Mandrioli R, Ghedini N, Albani F, Kennedler E. Liquid chromatographic determination of oxcarbazepine and its metabolites in plasma of epileptic patients after solid-phase extraction. *J Chromatogr B* 2003;783:25363.
- Helene B, Marylene C, Francoise B, Helene P, Jean PB, Dominique HB. Liquid chromatography-electrospray mass spectrometry determination of carbamazepine, oxcarbazepine and eight of their metabolites in human plasma. *J Chromatogr B* 2005;828:8090.
- Rouan MC, Decherf M, Le Clanche V, Lecaillon JB, Godbillon J. Automated microanalysis of oxcarbazepine and its monohydroxy and transdiol metabolites in plasma by liquid chromatography. *J Chromatogr B Biomed Sci Appl* 1994;658:167-72
- Menge G, Dubois JP. Determination of oxcarbazepine in human plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 1983;275:189-94.
- Paglia G, D'Apolito O, Garofalo D, Scarano C, Corso G. Development and validation of a LC/MS/MS method for simultaneous quantification of oxcarbazepine and its main metabolites in human serum. *J Chromatogr B* 2007;860:153-9
- Maria BS, Demetrius FN, Ismael LM, Adriano NC, Francisco EG, Fernando AF, *et al.* Simultaneous quantitative analysis of oxcarbazepine and 10,11-dihydro-10-hydroxycarbamazepine in human plasma by liquid chromatography-electrospray tandem mass spectrometry. *J Pharm Biomed Anal* 2007;45:304-11.
- Christine G, Ekkehard H. Development of a simple column-switching high-performance liquid chromatography (HPLC) method for rapid and simultaneous routine serum monitoring of lamotrigine, oxcarbazepine and 10-monohydroxycarbamazepine (MHD). *J Chromatogr B* 2007;85438-44
- Vasilios K, Marios S, Ioannis N, Dimitrios K, Chrysi G, Feras IK, *et al.* Development and validation of a high performance liquid chromatographic method for the determination of oxcarbazepine and its main metabolites in human plasma and cerebrospinal fluid and its application to pharmacokinetic study. *J Pharm Biomed Anal* 2007;43:763-8
- Manuela C, Monica B, Erica C, Carmina C, Fiorenzo A, Roberto R, *et al.* Simultaneous liquid chromatographic determination of lamotrigine, oxcarbazepine monohydroxy derivative and felbamate in plasma of patients with epilepsy. *J Chromatogr B* 2005;828:113-7
- Malgorzata K, Sebastian R, Filip B. Determination of oxcarbazepine and its metabolites in postmortem blood and hair by means of liquid chromatography with mass detection (HPLC/APCI/MS). *J Chromatogr B* 2005;825:38-46
- Loretta F, Mario F. A simple method to monitor plasma concentrations of oxcarbazepine, carbamazepine, their main metabolites and lamotrigine in epileptic patients. *Pharmacol Res* 2005;51:297-302 Pathare DB, Jadhav AS, Shingare MS. A validated stability indicating LC method for oxcarbazepine. *J Pharm Biomed Anal* 2007;43:1825-30
- Stability Testing of New Drug Substances and Products (Q1A2), ICH Harmonised Tripartite Guideline
- Validation of Analytical Procedures: Methodology (Q2B), ICH Harmonized Tripartite Guidelines.

Source of Support: Nil, Conflict of Interest: None declared.