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Research Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPTLC METHOD FOR DETERMINATION OF OXCARBAZEPINE IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT

A sensitive, selective, precise and stability indicating High Performance Thin Layer Chromatography (HPTLC) for analysis of Oxcarbazepine (OXC) was developed to resolve drug response from that of their degradation products. The method employed TLC aluminium plates precoated with silica gel 60 F_{254} as the stationary phase. The solvent system consisted of Ethyl Acetate: Methanol (6:4v/v). This system was found to give compact spot for Oxcarbazepine (R_f value 0.64±0.01). The drug was subjected to stress test conditions like acid, alkali, neutral hydrolysis and oxidative degradation. The spot for product of degradation was well resolved from the drug. Densitometric analysis of drug was carried out in the fluorescence mode at 366 nm. The linear regression data for the calibration plots showed good linear relationship with R^2=0.999 in the concentration range of 100-350ng/band. The result indicates that the drug was susceptible to degradation, to different extent in different conditions.

Keywords: HPTLC; Oxcarbazepine; Stability-indicating; Degradation; Validation; Densitometry

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 Fig. 1.

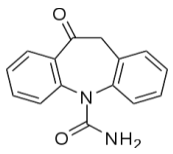

Fig. 1: Chemical Structure of OXC

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]

Spectroscopic methods have been reported in the literature for determination of Oxcarbazepine in pharmaceuticals[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, no stability indicating HPTLC method for estimation of Oxcarbazepine has been reported. A very viable alternative for stability indicating analysis of Oxcarbazepine is HPTLC. The advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering analysis time and cost per analysis.

The focus of the present study was to develop an accurate, specific, reproducible and stability indicating method for the determination of low levels of Oxcarbazepine in presence of its degradation products and related impurities for assessment of purity of the bulk drug and stability of its bulk dosage forms.

MATERIALS AND METHODS

Reagents and Chemicals

Standard bulk drug of Oxcarbazepine was obtained as a gift sample from Amoli Organics Pvt Ltd., Baroda. Ethyl Acetate, methanol, hydrochloric acid, sodium hydroxide, hydrogen peroxide were of analytical grade purchased from Qualigens India Pvt. Ltd., and Loba Chemicals India Ltd. Distilled water used was of HPLC grade.

Instrumentation and Chromatographic Conditions

The samples were spotted in the form of bands of width 8 mm with a Camag microlitre 100µl syringe on precoated silica gel aluminium plate 60F-254, (20×10 cm with 250 mm thickness; E. Merck, Germany) using a Camag Linomat IV (Switzerland). The mobile phase consisted of Ethyl Acetate: Methanol (6:4v/v). Samples were applied as bands 8 mm long, at 10 mm intervals under a stream of nitrogen. The slit dimensions were 6× 0.30 mm and sensitivity was kept at auto mode. A constant application or spraying rate of 150nl/s and scanning speed 20 mm/s was employed. Linear ascending chromatogram development to distance of 8 cm was performed in 20×10 cm twin trough TLC developing chamber (Camag) at room temperature and previously saturated for 30 min with mobile phase. Subsequent to the development, TLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed on Camag TLC scanner in the fluorescence mode at 366 nm using a WINCAT's software. The source of radiation utilized was mercury lamp.

Calibration curve

Standard stock solution of Oxcarbazepine was prepared by dissolving 10mg of drug in 10ml of methanol to get a concentration of 1000µg/ml. From this solution 0.5 ml of solution was taken in 10 ml volumetric flask and diluted with methanol to get a working standard of 50µg/ml. Different volumes of stock solution 2-7µl were spotted on the TLC plate to obtain concentrations 100-350ng of Oxcarbazepine, respectively. The data of peak area versus drug concentration was treated by linear least square regression analysis and was selected as working range for the assay and recovery.

Method validation

Linearity

The linearity of response for Oxcarbazepine was assessed in the range of 100-350 ng per band for standard drug.

Accuracy and precision of the assay

Accuracy was done in terms of recovery studies and precision was measured in terms of repeatability of measurement and application. Recovery studies were carried out by standard addition method. The pre-analyzed samples were spiked with extra 80, 100 and 120% of the standard Oxcarbazepine and the mixtures were analyzed by the proposed method. The experiment was conducted in triplicate. This was done to check for the recovery of the drug at different levels in formulation.

Repeatability of measurement of peak area

Five microlitres of Oxcarbazepine (250 ng/µl) were spotted on a TLC plate, developed, dried and the spot was scanned five times without changing the plate position and % co-efficient of variance (% CV) for measurement of peak area was estimated.

Limit of detection and limit of quantification

In order to estimate the limit of detection (LOD) and limit of quantitation (LOQ), blank methanol was spotted three times. The signal to noise level was determined. LOD was considered as 3:1 and LOQ as 10:1.

Specificity

The specificity of the method was ascertained by peak purity profiling studies. Purity of the drug peak was ascertained by analyzing the spectrum at peak start, middle and at peak end. The peak purity was determined on TLC scanner 3 in the range of 200-400 nm using WINCAT's software.

Assay of marketed formulation

To determine the content of Oxcarbazepine in conventional tablets (Label claim: 300 mg per tablet), the tablets were powdered and powder equivalent to 50 mg of Oxcarbazepine was weighed and transferred to 100ml volumetric flask. The volume was then made up to mark using methanol to get a concentration of 500µg/ml. To ensure complete extraction of the drug it was sonicated for 10 min. From this solution 1ml was withdrawn and diluted to 10ml with methanol to get a working standard of 50µg/ml. Five microlitre of the above solution was spotted onto the plate followed by development and scanning. The analysis was repeated in triplicate. The possibility of excipients interference in the analysis was studied.

Accelerated degradation of Oxcarbazepine

Preparation of acid and base degradation product

From a standard stock solution of 1000µg/ml, 5ml of solution was withdrawn and added in two different 10 ml volumetric flask and diluted respectively with 0.1N HCL and 0.1N NaOH to get a concentration of 500µg/ml. From this solution 1ml was again withdrawn and further diluted with methanol to give a working standard of 50µg/ml each. This solution was allowed to stand for 24 hrs. The accelerated degradation in acidic and basic media was performed in the dark in order to exclude the possible degradative effect of light on the drug.

Preparation of hydrogen peroxide induced degradation product

From a concentration of 1000µg/ml, 5ml of solution was withdrawn and diluted to 10 ml with 3% H_2O_2 to get a concentration of 500µg/ml. From this solution 1ml was again withdrawn and further diluted with methanol to give a working standard of 50µg/ml. This solution was allowed to stand for 24 hrs.

Degradation under neutral hydrolytic conditions

From a concentration of 1000µg/ml, 5ml of solution was withdrawn and diluted to 10 ml with HPLC grade distilled water to get a concentration of 500µg/ml. From this solution 1ml was again withdrawn and further diluted with methanol to give a working standard of 50µg/ml. This solution was allowed to stand for 24 hrs.

RESULTS AND DISCUSSION

HPTLC method development and validation

The TLC procedure was optimized with a view to develop a stability indicating assay method. Both the pure drug and the degraded products were spotted on the TLC plates and run in different solvent systems. Initially ethyl acetate and methanol in varying ratios were tried. The mobile phase ethyl acetate: methanol (6:4 v/v) gave good resolution, sharp and symmetrical peak with R_f value of 0.64 for Oxcarbazepine (Fig. 1). Also the spot for Oxcarbazepine was compact and not diffused. It was observed that proper drying of the TLC plate and pre-saturation of TLC chamber with mobile phase for 30 min ensure good reproducibility and peak shape of Oxcarbazepine.

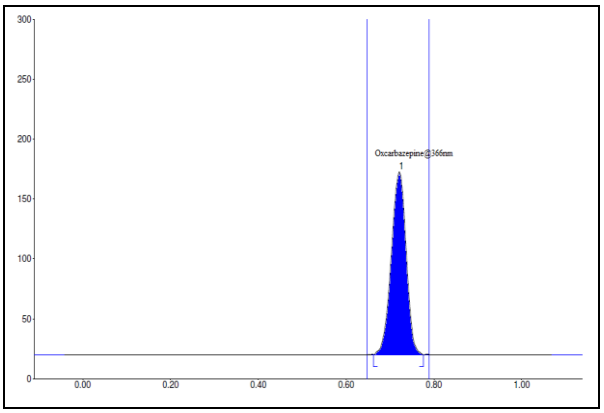

Fig. 2: Chromatogram of standard Oxcarbazepine at R_f=0.64, mobile phase ethyl acetate: methanol (6:4v/v)

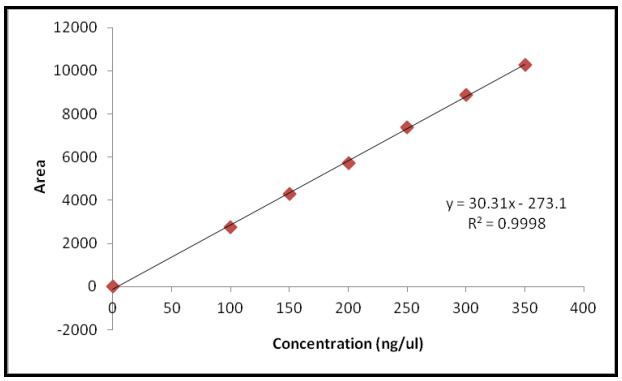

Fig. 3: Calibration curve of Oxcarbazepine

Validation

Using the optimized chromatographic conditions, the HPTLC method developed was validated in terms of linearity, LOD, LOQ, precision, accuracy and specificity.

Linearity

The analytical concentration range over which the drug obeyed Beer Lambert's law was found to be 100-350ng /band. (r^2 = 0.9909).

Precision

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. Percentage relative standard deviation (%RSD) was found to be less than 2% for within a day and day to day variations, which proves that method is precise.

Limit of Detection and Limit of Quantitation

The LOD, with a signal-to-noise ratio of 3:1, was found to be 100ng per spot. The LOQ, with a Signal-to-noise ratio of 10:1 was found to be 300ng per band.

Recovery studies

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 80%, 100% and 120%. Known amounts of standard Oxcarbazepine were added to pre-analyzed samples and were subjected to the proposed HPTLC method.

Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found within limit, indicating the non interference of any other peak of degradation product, impurity or matrix.

Solution Stability

The solution stability of Oxcarbazepine was carried out by leaving the standard stock solution in tightly capped volumetric flask at room temperature for 24 hrs and calculated for %RSD of assay of Oxcarbazepine for the study period during mobile phase and solution stability experiments,

Assay of marketed formulation

A single spot at R_f of 0.64 was observed in the chromatogram of the drug samples extracted from conventional tablets. There was no interference from the excipients commonly present in the conventional tablets. The drug content was found to be 98.94% with a % R.S.D. of less than 2. It may, therefore, be inferred that degradation of Oxcarbazepine had not occurred in the marketed formulations that were analyzed by this method. The low % R.S.D. value indicated the suitability of this method for routine analysis of Oxcarbazepine in pharmaceutical dosage forms.

Accelerated degradation

Acid and base induced degradation product

The chromatograms of the acid and base degraded samples for Oxcarbazepine showed additional peak at **R_f** value of 0.47 and 0.71, respectively. The concentration of the drug was found to be changing from the initial concentration indicating that Oxcarbazepine undergoes degradation under acidic conditions. The base spot was obtained under basic conditions due to formation of salt.

Table 1: Recovery Studies

Level of recovery (%)	Amount taken (ng/band)	Amt of std added (ng/band)	Total amount recovered (ng/band)	% Recovery*
80	100	120	221	100.5
100	100	150	255	102.0
120	100	180	285	101.7

*Average of three determinations

Table 2: Summary of validation parameters

Parameter	Data
Detection Wavelength (nm)	366
Beer's Law Limit (ng/band)	100-350
Regression equation	30.31x-273.1
Correlation Coefficient (r^2)	0.9998
Accuracy	101.4
Limit of detection (ng)	100
Limit of quantitation (ng)	300
Specificity	Specific
Precision (%RSD)	1.75

Table 3: Degradation study results for Oxcarbazepine bulk drug

S. No.	Type of Degradation	Condition	% of Oxcarbazepine Degraded
1	Acid	0.1N HCL, kept at R.T for 24 hours	19.8%
2	Base	0.1N NaOH, kept at R.T for 24 hours	22.8%
3	Water	Distilled Water, kept at R.T for 24 hours	11.5%
4	Hydrogen Peroxide	3% H_2O_2, kept at R.T for 24 hours	26.6%

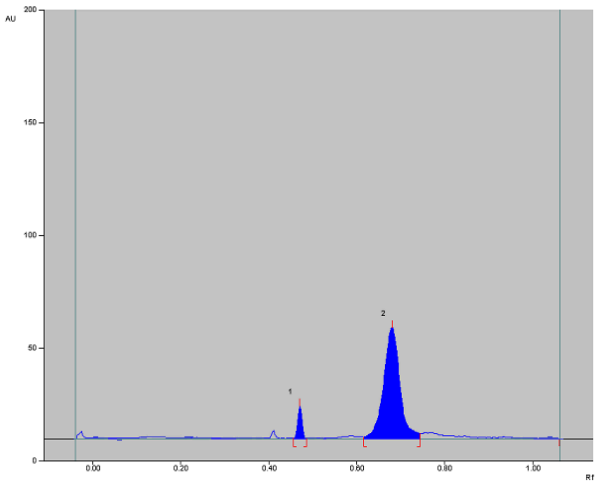

Fig. 4: A) Densitogram of acid (0.1 N HCl, kept at R.T for 24 hrs) treated OXC; peak 1 (degraded) (R_f: 0.47), peak 2 (OXC) (R_f: 0.63).

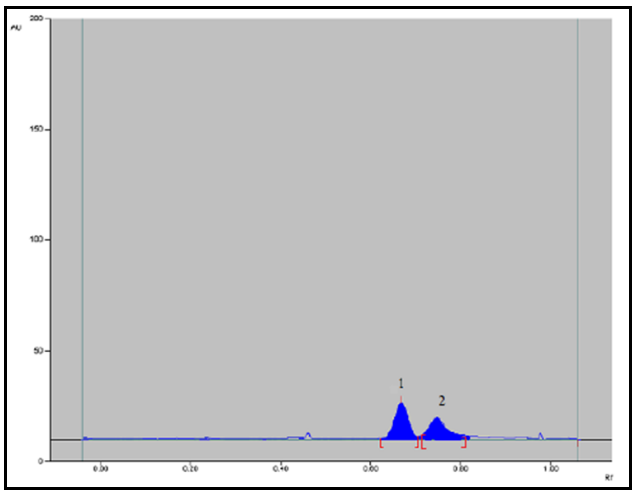

Fig. 4 B) Densitogram of base (0.1 N NaOH kept at R.T for 24 hrs) treated OXC; peak 1 (OXC) (R_f: 0.63), peak 2 (degraded) (R_f: 0.71)

Hydrogen peroxide induced degradation product

The samples degraded with hydrogen peroxide did not show additional peak. But reduction in peak area was noted.

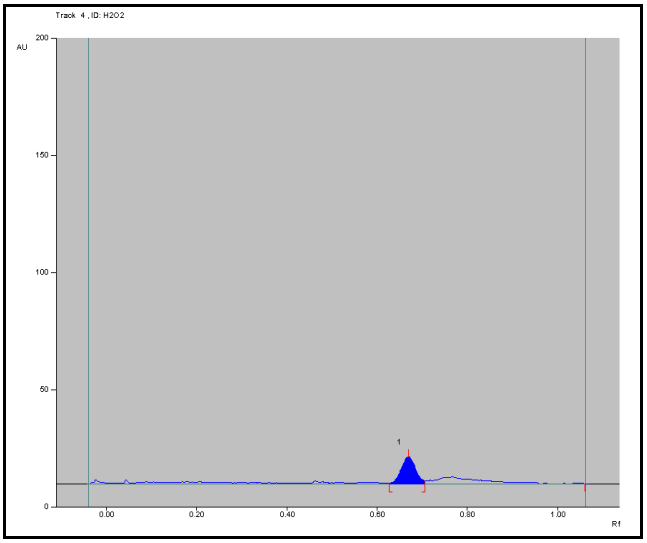

Fig. 5: Densitogram of oxidative degradation (3% w/v, kept at R.T for 24 hrs) treated Oxcarbazepine (R_f: 0.63).

Neutral hydrolytic degradation product

The sample degraded under neutral hydrolytic conditions showed reduction in peak area at **R_f** =0.63

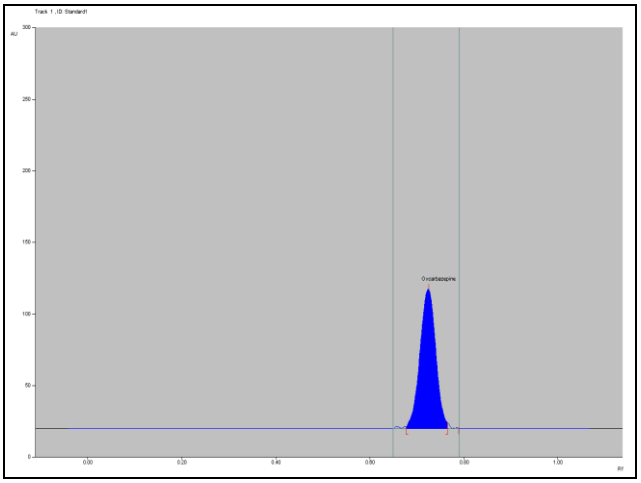

Fig. 6: Densitogram of neutral degradation (in distilled water kept at R.T for 24 hrs) of Oxcarbazepine; peak 1 (Oxcarbazepine) (R_f: 0.63).

On plate stability

After the chromatogram development the analyzed plates of drug sample and formulation were checked for stability for 6hrs to detect the presence of additional spots if any. There was no indication of compound instability in the sample solution.

CONCLUSION

The developed HPTLC technique is precise, specific, accurate and stability indicating one. This proves that the method is reproducible and selective for the analysis of Oxcarbazepine as bulk drug and in pharmaceutical formulations. The method can be used to determine the purity of the drug available from various sources by detecting the related impurities. It may be extended to study the degradation kinetics of Oxcarbazepine. As the method could effectively separate the drugs from their degradation products it can be employed as a stability indicating one.

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