Acta Chromatographica 26(2014)2, 267–282
DOI: 10.1556/AChrom.26.2014.2.6

Development and Validation of a Stability-Indicating RP-LC Method for the Estimation of Process-Related Impurities and Degradation Products of Oxcarbazepine in Pharmaceutical Formulation

P.S. REDDY[1,2,*], K.S. BABU[2], AND N. KUMAR[1]

[1]Dr. Reddy's Laboratories Ltd., IPDO, Bachupally, Hyderabad-500072, A.P, India
[2]Department of Chemistry, S. K. University, Anantapur-515055, A.P., India
E-mail: sunilpsr@yahoo.com

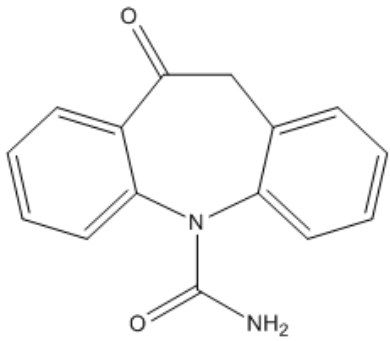
Summary. A stability-indicating gradient reverse-phase liquid chromatographic method was developed for the quantitative determination of process-related impurities and forced degradation products of oxcarbazepine in pharmaceutical formulation. The method was developed by using Inertsil cyano (250 × 4.6 mm) 5 µm column with mobile phase containing a gradient mixture of solvent A (0.01 M sodium dihydrogen phosphate, pH adjusted to 2.7 with orthophosphoric acid and acetonitrile in the ratio of 80:20 *v*/*v*) and B (50:40:10 *v*/*v*/*v* mixture of acetonitrile, water, and methanol). The flow rate of mobile phase was 1.0 mL min^{-1}. Column temperature was maintained at 25°C and detection wavelength at 220 nm. Developed reverse-phase high-performance liquid chromatography (RP-HPLC) method can adequately separate and quantitate five impurities of oxcarbazepine, namely imp-A, imp-B, imp-C, imp-D, and imp-E. Oxcarbazepine was subjected to the stress conditions of oxidative, acid, base, hydrolytic, thermal, and photolytic degradation. Oxcarbazepine was found to degrade significantly in acid, base, and oxidative stress conditions. The degradation products were well resolved from oxcarbazepine and its impurities. The developed method was validated as per International Conference on Harmonization (ICH) guidelines with respect to specificity, linearity, limit of detection and quantification, accuracy, precision, and robustness.

Key Words: development, validation, stability-indicating, LC–MS, degradation, oxcarbazepine

Introduction

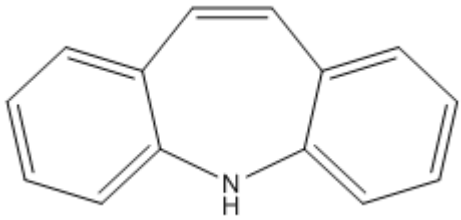
Oxcarbazepine is an antiepileptic drug available as tablet and suspension formulation for oral administration. Its chemical designation is 10, 11-dihydro-10-oxo-5*H*-diben (b, f) azepine-5-carboxamide *(Fig. 1)*. Its empirical formula is $C_{15}H_{12}N_2O_2$, which corresponds to a molecular weight of 252.27 [1, 2].

In the literature survey, there were very few LC assay methods that have been reported for determination of oxcarbazepine in pharmaceutical preparation [3, 4]. Estimation of oxcarbazepine and its metabolites in biological fluids by high-performance liquid chromatography (HPLC) and liquid chromatography–mass spectrometry (LC–MS) has been performed [5–11]. Only one method was reported for the determination of oxcarbazepine and its related compounds by reverse-phase liquid chromatography (RP-LC) [12], but the method deals with only three impurities present in active pharmaceutical ingredient, and forced degradation studies were not performed properly. In a reported method, no degradation was observed under acid hydrolysis, hydrolytic, and oxidative stress conditions, while oxcarbazepine degrades significantly in acid, base hydrolysis and oxidation and moderate degradation under hydrolytic stress condition. To establish the stability-indicating property of the method, possible degradation products should be formed under stress studies, and all degradation products should be well resolved from each other and known impurities. Besides the reported known impurities in this method, we have observed two other potential impurities in our drug product. Thus far to our present knowledge, no stability-indicating HPLC method was reported for the estimation of all five impurities of oxcarbazepine in pharmaceutical formulation. Hence, we have developed a new stability-indicating RP-LC method that can adequately separate and quantitate five impurities of oxcarbazepine, namely, imp-A, imp-B, imp-C, imp-D, and imp-E *(Fig. 1)* in pharmaceutical dosage forms. Force degradation studies of oxidative, photolytic, hydrolytic, thermal, acid and base hydrolysis stress were performed on the placebo and drug products to show the stability-indicating nature of the method. The developed LC method was validated with respect to specificity, LOD, LOQ,

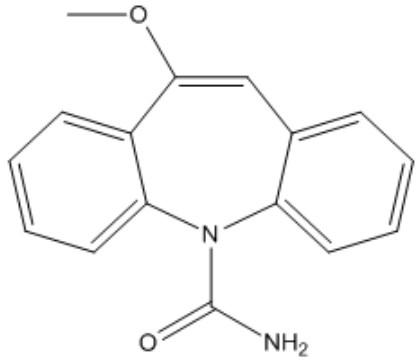


Oxcarbazepine [10, 11-dihydro-10-oxo-5*H*-dibenz (b, f) azepine-5-carboxamide]

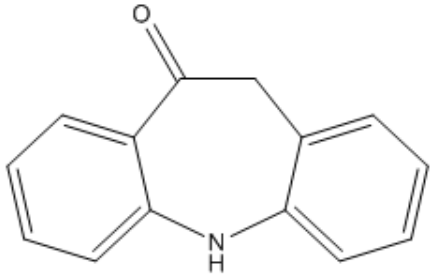
Fig. 1. Structures and chemical names of Oxcarbazepine and its impurities



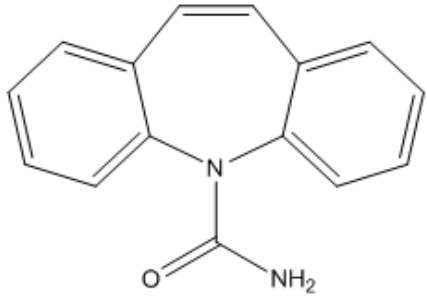
Impurity A [5*H*-dibenz (b, f) azepine]



Impurity B [10-methoxy-5*H*-dibenz (b, f) azepine-5-carboxamide]

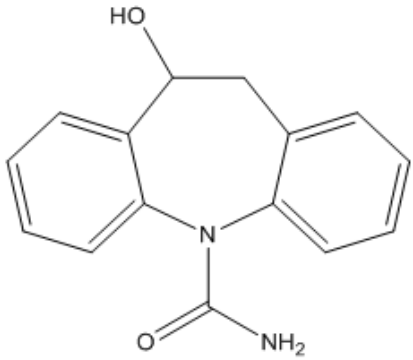


Impurity C [10, 11-dihydro-10-oxo-5*H*-dibenz (b, f) azepine]



Impurity D [5-carbamoyl-5*H*-dibenz (b, f) azepine]

Fig. 1. (continued)



Impurity E [10,11-dihydro-10-hydroxy-5*H*-dibenz (b, f) azepine-5-carboxamide]

Fig. 1. (continued)

linearity, precision, accuracy, and robustness. These studies were performed in accordance with established International Conference on Harmonization (ICH) guidelines [13–15].

Experimental

Chemicals, Equipments, and Solutions

Samples of oxcarbazepine tablets and its impurities imp-A, imp-B, imp-C, imp-D, and imp-E were supplied by Dr. Reddy's laboratories limited, Hyderabad, India. The HPLC grade acetonitrile, methanol, and analytical grade sodium dihydrogen phosphate and ortho-phosphoric acid were purchased from Merck, Mumbai, India. High purity water was prepared by using Millipore Milli-Q Plus water purification system (Millipore, Milford, MA, USA).

The chromatographic analysis was performed using Waters Alliance 2695 separation module (Waters Corporation, Milford, USA) equipped with 2489 UV/visible detector or 2998 PDA detector (for specificity and forced degradation studies), degasser, quaternary pump, and autosampler system. The output signals were monitored and processed using Empower 2 software. Cintex digital water bath was used for hydrolysis studies. Photostability studies were carried out in photostability chamber (Sanyo, Leicestershire, UK). Thermal stability studies were performed in a dry air oven (Cin-

tex, Mumbai, India). The pH of the solutions was measured by a pH meter (Mettler-Toledo, Switzerland).

Acetonitrile was used as diluent. An impurity identification solution containing oxcarbazepine (1000 μg mL^{-1}) and all five impurities (each 2.0 μg mL^{-1}) were prepared by dissolving appropriate amount in diluent.

A standard stock solution of oxcarbazepine (200 μg mL^{-1}) was prepared by dissolving the appropriate amount of compound in the diluent. Working solution of 2 μg mL^{-1} was prepared from the stock solution for the determination of impurities.

Chromatography

The method was developed using Inertsil cyano (250 × 4.6 mm) 5 μm column with mobile phase containing a gradient mixture of solvent A (0.01 M sodium dihydrogen phosphate, pH adjusted to 2.7 with ortho-phosphoric acid and acetonitrile in the ratio of 80:20 v/v) and B (50:40:10 $v/v/v$ mixture of acetonitrile, water, and methanol). The mobile phases were filtered through nylon 0.45 μm membrane filters and degassed. The gradient program (time (min)/%B) was set 0/0, 5/5, 15/5, 40/70, 50/70, 51/0, and 70/0. The flow rate of the mobile phase was 1.0 mL min^{-1}. The column temperature was maintained at 25°C, and the eluted compounds were monitored at the wavelength of 220 nm. The injection volume was 10 μL.

Analysis of Dosage Forms

To determine the impurities in drug product, 20 tablets were weighed and crushed into a clean and dry mortar-pestle. Tablet powder equivalent to 100 mg of oxcarbazepine was dissolved in 70 mL of diluent with sonication for 15 min and diluted to 100 mL with diluent to give a solution containing 1000 μg mL^{-1} drug.

Force Degradation Study

Forced degradation studies were performed at 1000 μg mL^{-1} concentration of oxcarbazepine on tablets to provide an indication of the stability-indicating property and specificity of proposed method. Peak purity test was carried out for the oxcarbazepine peaks by using PDA detector on stress samples. All the solutions used in forced degradation studies were prepared by dissolving the drug product in small volume of stressing

agents. After degradation, these solutions were diluted with diluent to yield stated oxcarbazepine concentration of about 1000 μg mL^{-1}. Conditions employed for performing the stress studies were as follows [13–15]:

Acid hydrolysis of the drug product was carried out in presence of 1 N hydrochloric acid at 80°C temperature for 45 min. Sample was prepared for analysis as previously described under sample preparation.

Base hydrolysis of the drug product was carried out in presence of 1 N sodium hydroxide at 80°C temperature for 10 min. Sample was prepared for analysis as previously described under sample preparation.

To study the effect of oxidizing conditions, sample was prepared and weighed accurately equivalent to 100 mg oxcarbazepine tablet powder and dissolved in 20 mL diluent. Then 5 mL of 1% H_2O_2 was added, and the mixture was kept at 80°C temperature for approximately 1 h and then diluted to 100 mL with diluent.

To study the effect of temperature, equivalent to 100 mg oxcarbazepine tablet powder was stored at 105°C for 12 h. Sample was dissolved in 70 mL of diluent and diluted to 100 mL with diluent.

To study the effect of neutral (water) hydrolysis, equivalent to 100 mg oxcarbazepine tablet powder was dissolved in 20 mL diluent. Then, 10 mL of water was added to the sample, and the mixture was kept at 80°C temperature for 12 h. The solution was then left to reach ambient temperature and diluted to 100 mL with diluent.

Susceptibility of the drug product to light was studied [13]. Tablet powder for photo stability testing was placed in a photostability chamber and exposed to white florescent lamp with an overall illumination of 1.2 million lux hours and near UV radiation with an overall illumination of 200 watt/m^2 h^{-1} at 25°C. Following removal from the photostability chamber, sample was prepared for analysis as previously described under sample preparation.

Results and Discussion

Method Development and Optimization

During the development of the method, it has been observed that imp-E formed in acid hydrolysis and oxidative stress study. The retention time of impurity has been identified by injecting impurity standard solution in chromatographic system. To confirm mass number of impurity, LC–MS method has been developed.

LC–MS Method for Peak Identification

The method was developed using Inertsil cyano, 250 × 4.6 mm, 5 μm column as stationary phase. The mobile phase containing a gradient mixture of solvent A (0.01 M formic acid, pH 2.7) and B (acetonitrile, water, and methanol in the ratio of 60:20:20 *v*/*v*/*v*). Acetonitrile was used as diluent. The gradient program (time (min)/%B) was set 0/15, 5/20, 15/20, 35/75, 45/80, 46/15, and 55/15. Prior to use, mobile phase was mixed thoroughly and degassed. The mobile phase pumped at 1.0 mL min^{-1}. The column temperature was maintained at 25°C. The injection volume for sample was 10 μL.

Oxidative degraded sample (sample treated with 1% H_2O_2 at 80°C for 1 h) and acid hydrolysis sample (sample treated with 1 N HCl at 80°C for 45 min) were analyzed with LC–MS method. The *m*/*z* ratio for imp-E was 255 *(Fig. 2)*.

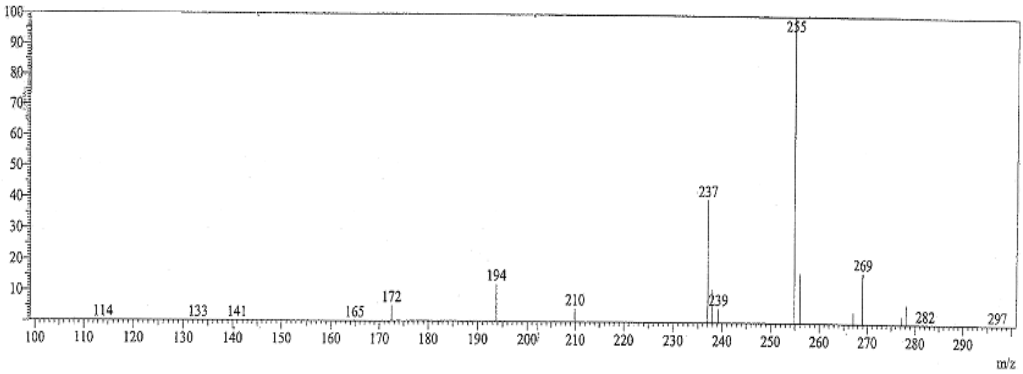


Fig. 2. Mass spectrum of Impurity E

Stability-Indicating RP-HPLC Method for Sample Analysis

The main objective of the chromatographic method was to separate closely eluting compounds imp-E and oxcarbazepine and to elute all impurities with a shorter run time and symmetrical peak shape. The blend containing 1000 μg mL^{-1} of oxcarbazepine and 2.0 μg mL^{-1} of each impurity was used for separation. An isocratic method was employed using 0.01 M sodium dihydrogen orthophosphate (pH 3.0), acetonitrile, and methanol in the ratio of 60:25:15 as mobile phase, waters symmetry shield C18 (250 mm × 4.6 mm) 5 μm column with flow rate of 1.0 mL min^{-1} on HPLC equipped with photo diode array detector. Resolution between imp-E and oxcarbazepine was less than 1.5 and imp-A retained strongly. To increase the resolution and reduce

the run time, an attempt was made with gradient elution with mobile phase 0.01 M sodium dihydrogen orthophosphate buffer (pH 2.7) as solvent-A and mixture of acetonitrile and methanol in the ratio of 70:30 as solvent-B. Resolution between imp-E and oxcarbazepine was more than 2.5. During forced degradation study, some unknown degradation products co-eluted with imp-E and imp-A. To resolve all degradation products from each other and from known impurities, mobile phase compositions, gradient program, and column stationary phase were further optimized, and final chromatographic conditions are described below.

Inertsil cyano (250 mm × 4.6 mm) 5 μm was used as the stationary phase. The mobile phase A consists of 0.01 M sodium dihydrogen phosphate, pH adjusted to 2.7 with ortho-phosphoric acid and acetonitrile in the ratio of 80:20 v/v and mobile phase B contained a mixture of acetonitrile, methanol, and water in the ratio of 50:10:40 $v/v/v$. The flow rate was 1.0 mL min^{-1} with a gradient program of (time (min)/%B) 0/0, 5/5, 15/5, 40/70, 50/70, 51/0, and 70/0. The column temperature was maintained at 25°C, and detection was monitored at 220 nm. The injection volume was 10 μL. Using the optimized conditions, all impurities and degradation products were well separated from each other and oxcarbazepine, and the typical relative retention times of imp-A, imp-B, imp-C, and imp-D were about 0.70, 1.44, 1.72, 2.99, and 3.83, respectively. The developed method was determined to be specific for oxcarbazepine and all impurities.

Forced Degradation Study

Forced degradation of oxcarbazepine was performed to provide an indication of the stability-indicating properties and specificity of the method. Placebo interference was evaluated in duplicate by analyzing the placebo prepared as per test method. No peak due to placebo was detected at the retention time of oxcarbazepine and its impurities. All force degradation samples were analyzed at 1000 μg mL^{-1} concentration of oxcarbazepine using PDA detector to ensure the homogeneity and purity of oxcarbazepine peak. The purity of oxcarbazepine was unaffected in the presence of its impurities and degradation products. The mass balance (% assay + % sum of all degradants + % sum of all impurities) results were calculated and found to be more than 98.1% *(Table I)*, thus confirming the stability-indicating power of the developed method.

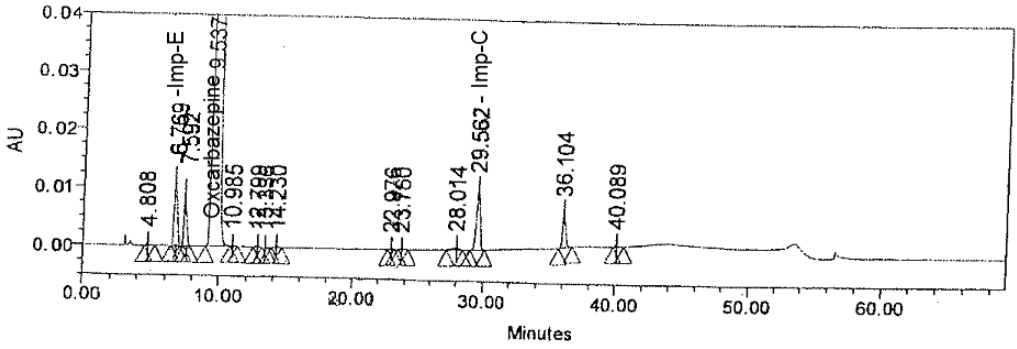
Oxcarbazepine was found unstable in acid hydrolysis. On heating the drug product in 1 N HCl at 80°C for 45 min, 6.02% degradation was observed and significant rise was seen in imp-C (1.98%), imp-E (0.60%), and

an unknown degradation product (0.72%) at RRT 0.80 *(Fig. 3A)*. Results of forced degradation study are reported in *Table I*.

Table I. Summary of forced degradation results

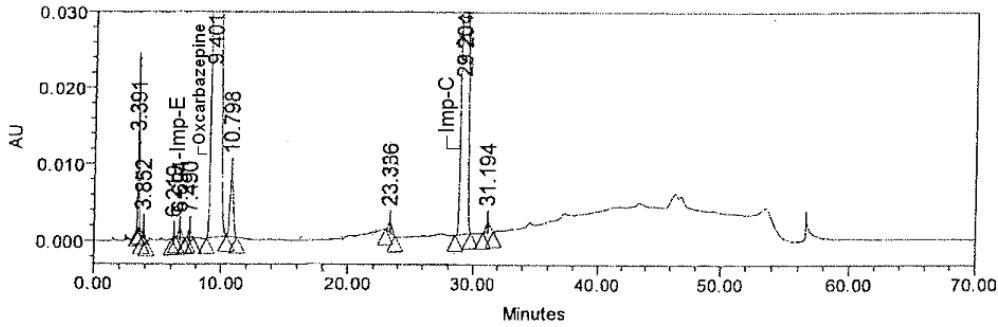
Stress condition	% Impurity						% Degradation	% Assay of active substance	Mass balance (%)
	Imp-A	Imp-B	Imp-C	Imp-D	Imp-E	MUSI[a]			
Acid hydrolysis	ND	ND	1.98	ND	0.60	0.72	4.37	94.7	99.1
Base hydrolysis	ND	ND	7.19	ND	0.03	0.25	9.18	89.3	98.5
Oxidation degradation	0.02	ND	0.37	ND	0.59	1.42	3.97	94.1	98.1
Thermal degradation	ND	ND	ND	ND	0.05	ND	0.08	99.3	99.4
Water degradation	ND	ND	0.69	ND	0.03	ND	0.80	98.2	99.0
Photolytic degradation	ND	ND	ND	ND	0.05	ND	0.07	99.7	99.8
Humidity degradation	ND	ND	ND	ND	0.05	ND	0.07	99.1	99.2

[a]MUSI = maximum un-specified impurity;
ND = not detected.

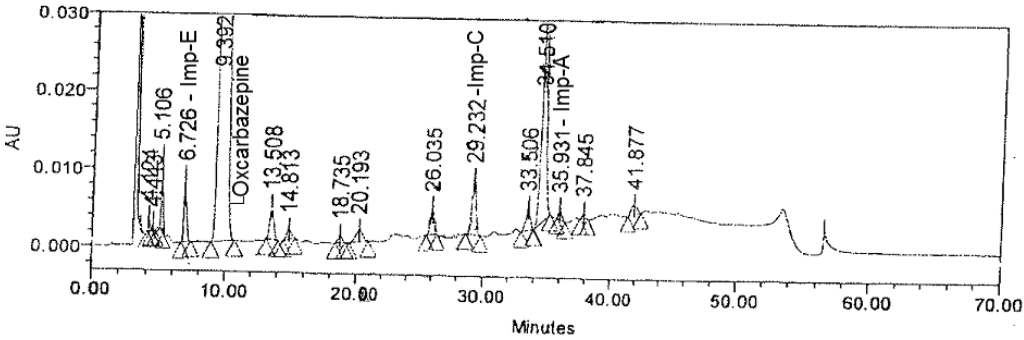


(A)

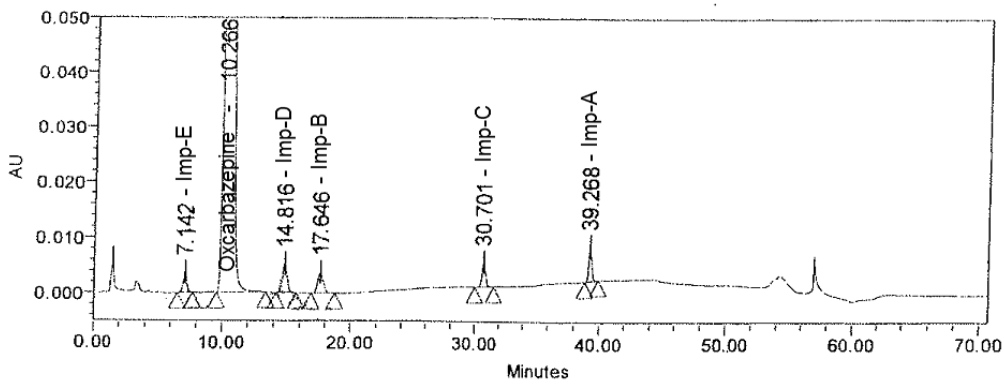
Fig. 3. Typical chromatograms of (A) acid degradation sample



(B)



(C)



(D)

Fig. 3. Typical chromatograms of (B) base degradation sample, (C) oxidative degradation sample, and (D) oxcarbazepine test spiked with its impurities

Oxcarbazepine was found to be highly susceptible in alkali. Major degradation (9.18%) of the drug was observed in 1 N NaOH at 80°C for 10 min and lead to the formation of imp-C (7.19%), imp-E (0.03%), and an unknown

degradation product (0.25%) at RRT 1.15 *(Fig. 2B)*. Results of forced degradation study are reported in *Table I*.

The drug product was found to be labile to hydrogen peroxide. In 1% H_2O_2 at 80°C for 1 h, the drug was found degrade about 3.97% and formed imp-C (0.37%), imp-E (0.59%), and maximum unknown degradant (1.42%) at RRT 3.67 *(Fig. 2C)*. Results are reported in *Table I*.

The drug product was found stable under dry heat for 12 h at 105°C, and no single maximum degradation product was more than 0.05%. Results of forced degradation study are reported in *Table I*.

The drug product was found to be slightly unstable in neutral (water) hydrolysis at 80°C temperature for 12 h. In water hydrolysis, drug product was found degrade about 0.80% and lead to the formation of imp-C (0.69%) and imp-E (0.03%) *(Table I)*.

Drug product was exposed to white florescent lamp with an overall illumination of 1.2 million lux hours and near UV radiation with an overall illumination of 200 $W/m^2\ h^{-1}$. Analysis of photo degradation sample showed no additional peak in the chromatogram, indicating that oxcarbazepine is stable in photolytic stress conditions *(Table I)*.

Validation of the Method

The proposed method was validated as per ICH guidelines [13–15]. The following validation characteristics were addressed: specificity, accuracy, precision, limit of detection and quantification, linearity, range, and robustness.

System suitability was determined before sample analysis from six replicate injections of the standard solution containing 2 $\mu g\ mL^{-1}$ of oxcarbazepine. The acceptance criteria were less than 5% relative standard deviation (RSD) for peak areas, USP tailing factor less than 2.0 for oxcarbazepine peak from standard solution. All critical parameters tested met the acceptance criteria *(Table II)*.

Table II. System suitability test results

Parameters	Specification	Observed values	
		Precision	Intermediate precision
Area (% RSD, $n = 6$)	$\leq$5.0%	0.5	0.7
USP tailing	$\leq$2.0	1.1	1.0

To assess specificity, forced degradation study was performed on drug product, and all degradation products formed under stress study were well

resolved from oxcarbazepine peak. Placebo interference was evaluated by analyzing the placebo equivalent amount present in sample, prepared as per test method. No peak due to placebo was detected at the retention time of oxcarbazepine and its impurities.

The precision of method was verified by repeatability and intermediate precision. Repeatability was checked by injecting six individual preparations of oxcarbazepine tablets spiked with its five impurities; imp-A, imp-B, imp-C, imp-D, and imp-E at 0.20% level (0.20% of impurities with respect to 1000 μg mL^{-1} oxcarbazepine). The intermediate precision of the method was also evaluated using different analyst and different instrument and performing the analysis on different days. The relative standard deviation of the areas of each impurity peak was calculated and found to be less than 4.9% in repeatability and less than 5.0% in intermediate precision study, which confirms the good precision of the method. The % RSD values are presented in *Table III*.

Table III. Linearity and precision data

Parameter	Imp-A	Imp-B	Imp-C	Imp-D	Imp-E
LOD (μg mL^{-1})	0.089	0.038	0.034	0.031	0.046
LOQ (μg mL^{-1})	0.254	0.107	0.102	0.092	0.109
Linearity range (μg mL^{-1})	0.257–3.902	0.107–4.280	0.102–4.077	0.092–4.574	0.109–4.361
Correlation coefficient	0.998	0.997	0.998	0.999	0.999
Intercept	−3389.95	−1047.05	361.32	−1390.22	1763.10
Slope	53,238.98	56,129.97	46,928.22	60,873.69	29,396.67
Bias at 100% response	−3.9	−0.9	0.8	−1.2	2.8
Precision (% RSD)	0.9	0.6	0.6	0.4	4.9
Intermediate precision (% RSD)	0.7	0.5	0.9	0.5	5.0
Precision at LOQ (% RSD)	5.6	3.8	0.1	6.0	0.3

The limit of detection (LOD) and limit of quantification (LOQ) for oxcarbazepine impurities 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. Precision study was also carried out at the LOQ level by injecting six individual preparations of oxcarbazepine impurities and calculated the % RSD of the area. Results are reported in *Table III*.

Linearity test solutions were prepared by diluting the stock solutions to the required concentrations. The solutions were prepared at six concentration levels from LOQ to 200% of specification level (LOQ, 0.05%. 0.10%, 0.20%, 0.30%, and 0.40% for all five impurities). Calibration curves were plotted between the responses of peak versus analyte concentrations. The correlation coefficient obtained was greater than 0.997. The coefficient correlation, slope, and *y*-intercept of the calibration curve are reported in *Table III*. The above results showed an excellent correlation between peak area and concentration of imp-A, imp-B, imp-C, imp-D, and imp-E.

Accuracy of the method for imp-A, imp-B, imp-C, imp-D, and imp-E was evaluated in triplicate using five concentration levels LOQ, 1.00, 2.00, 3.00, and 4.00 μg mL^{-1}. The percentage recovery of imp-A, imp-B, imp-C, imp-D, and imp-E in oxcarbazepine samples varied from 93.3% to 104.8%. The LC chromatogram of spiked sample at 0.2% level of all five impurities in oxcarbazepine tablets sample is shown in *Fig. 2D*. The recovery values for oxcarbazepine impurities are presented in *Table IV*.

Table IV. Evaluation of accuracy study

Amount spiked[a]	% Recovery[b]				
	Imp-A	Imp-B	Imp-C	Imp-D	Imp-E
LOQ	93.3 ± 3.7	102.8 ± 2.5	98.1 ± 0.1	98.4 ± 4.7	104.0 ± 0.3
50%	103.3 ± 2.0	93.9 ± 3.1	104.8 ± 2.0	100.2 ± 2.5	102.7 ± 2.8
100%	98.2 ± 1.2	97.4 ± 2.4	104.6 ± 1.0	101.5 ± 0.5	104.6 ± 0.5
150%	97.3 ± 0.7	99.4 ± 1.3	103.7 ± 1.6	101.3 ± 1.8	102.4 ± 0.8
200%	97.7 ± 1.2	100.4 ± 1.0	104.2 ± 1.1	102.3 ± 0.6	104.6 ± 1.1

[a]Amount of five impurities spiked with respect to 0.2% specification level individually.

[b]Mean ± % RSD for three determinations.

To determine the robustness of the developed method, experimental conditions and the relative retention time of each impurity were deliberately altered, and system suitability parameters for oxcarbazepine standard were recorded. The variables evaluated in the study were pH of the mobile phase buffer, column temperature, flow rate, and % organic in the mobile

phase. The flow rate of the mobile phase was 1.0 mL min^{-1}. To study the effect of flow rate on the separation, flow was changed to 0.8 and 1.2 mL min^{-1}. The effect of the percent organic strength was studied by varying organic solvent by ±10% in solvent A and B. The effect of pH of mobile was studied at 2.6 and 2.9 instead of 2.7 in solvent A. The effect of the column temperature was studied at 20 and 30°C instead of 25°C. In all the deliberate varied chromatographic conditions (flow rate, column temperature, pH of mobile phase buffer, and composition of organic solvent), all analytes were adequately resolved and elution order remained unchanged. The tailing factor for oxcarbazepine peak from standard solution was less than 1.3, and RSD for peak areas was less than 2.2% *(Table V)*.

Table V. Robustness results of HPLC method

Variation in chromatographic condition	Relative retention time (RRT)[a]					Observed system suitability parameters	
	Imp-A	Imp-B	Imp-C	Imp-D	Imp-E	USP Tailing ≤2.0	% RSD ≤5.0 (n = 6)
Column temperature, 20°C	3.76	1.76	2.98	1.47	0.68	1.2	0.4
Column temperature, 30°C	3.88	1.71	3.02	1.44	0.70	1.0	0.1
Flow rate, 0.8 mL min^{-1}	3.33	1.73	2.69	1.45	0.69	1.1	0.7
Flow rate, 1.2 mL min^{-1}	4.28	1.72	3.25	1.44	0.69	1.1	1.0
Acetonitrile, 90% (mobile phase A)	3.67	1.80	2.98	1.50	0.67	1.1	0.4
Acetonitrile, 110% (mobile phase A)	3.74	1.67	3.05	1.42	0.69	1.1	0.5
Acetonitrile, 90% (mobile phase B)	4.01	1.73	3.10	1.45	0.69	1.2	1.0
Acetonitrile, 110% (mobile phase B)	3.86	1.68	3.02	1.42	0.71	1.3	0.2
Methanol, 90% (mobile phase B)	4.07	1.64	3.17	1.41	0.71	1.1	1.0
Methanol, 110% (mobile phase B)	4.24	1.67	3.25	1.42	0.70	1.2	1.7
Mobile phase buffer, pH 2.6	4.14	1.67	3.20	1.42	0.70	1.2	1.0
Mobile phase buffer, pH 2.9	2.51	1.46	2.16	1.46	0.59	1.2	2.2

[a]Relative retention time of each impurity with respect to oxcarbazepine.

The solution stability of oxcarbazepine and its impurities was determined by leaving test solution and standard solutions in tightly capped volumetric flasks at room temperature for 6 and 48 h, respectively, and measured the amount of five impurities at every 70 min against freshly prepared standard solution. The stability of mobile phase was also determined by freshly prepared solutions of oxcarbazepine and its impurities at 24 h interval for 48 h. The mobile was not changed during the study. During solution and mobile phase stability study, the variability in the estimation of all five impurities was within ±15% during solution stability and mobile phase stability. The results from solution stability and mobile phase stability experiments confirmed that mobile phase was stable up to 48 h and sample solution and standard solutions were stable up to 2 and 48 h, respectively.

Conclusions

A simple and efficient reverse-phase HPLC method was developed and validated for quantitative analysis of oxcarbazepine impurities in pharmaceutical dosage forms. The method was found to be precise, accurate, linear, robust, and rugged during validation. Satisfactory results were obtained from the validation of the method. The method is stability-indicating and can be used for routine analysis of production samples and to check the stability of the oxcarbazepine tablets.

Acknowledgments

The authors are thankful to the management of Dr. Reddy's Laboratories Ltd., Hyderabad for providing facilities to carry out this work.

References

[1] http://www.rxlist.com/trileptal-drug.htm
[2] S.M. Grant and D. Fauld, Drug, **43**, 873 (1992)
[3] M.L. Qi, P. Wang, L.J. Wang, and R.N. Fu, J. Pharm. Biomed. Anal., **31**, 57 (2003)
[4] K.S. Rao, N. Belorkar, and M.E.B. Rao, J. Young Pharm., **1**, 270 (2009)
[5] A. Kumps, J. Liq. Chromatogr., **7**, 1235 (1984)
[6] M. Klys, S. Rojek, and F. Bolechala, J. Chromatogr. B, **825**, 38 (2005)
[7] G. Paglia, O. D'Apolito, D. Garofalo, C. Scarano, and G. Corso, J. Chromatogr. B, **860**, 153 (2007)
[8] M.D.L.LS. Cavazos, V.T.D.L. Cruz, and N.W.D. Torres, J. Liquid Chromatogr. Rel. Tech., **28**, 693 (2005)
[9] M. Bhatt, S. Shah, and Shivprakash, Biomed. Chromatogr., **25**, 751 (2011)
[10] H.H. Maurer, C. Kratzsch, A.A. Weber, F.T. Peters, and T. Kraemer, J. Mass Spectro., **37**, 687 (2002)

[11] G. Srinubabu, B.V. Ratnam, A.A. Rao, and M.N. Rao, Chem. Pharm. Bull., **56**, 28 (2008)
[12] D.B. Pathare, A.S. Jadhav, and M.S. Shingare, J. Pharm. Biomed. Anal., **43**, 1825 (2007)
[13] ICH Q1B, Photostability Testing on New Drug Substances and Products, 1996
[14] ICH Q1A (R2), Stability Testing of New Drug Substances and Products, 2003
[15] ICH Q2 (R1), Validation of Analytical Procedures: Text and Methodology, 2005

Accepted by DA