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Development and validation of stability indicating spectrophotometric methods for determination of oxcarbazepine in pharmaceuticals

K Basavaiah*, N Rajendraprasad, M X Cijo, K B Vinay and P J Ramesh
Department of Chemistry, University of Mysore, Manasagangothri, Mysore-570 006, India

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Two simple, selective and stability indicating UV-spectrophotometric methods have been developed and validated for assay of oxcarbazepine (OXC) in bulk drug and in its dosage forms. Proposed methods are based on measurement of absorbance of OXC either in methanol (method A) or in acetonitrile (method B) at 254 nm. Calibration graphs are linear over 2-40 μg ml^{-1} in both methods with correlation coefficients (r) 0.9993 (method A) and 0.9999 (method B). Apparent molar absorptivity values were 6.73×10^3 (method A) and 6.95×10^3 l mol^{-1} cm^{-1} (method B). This study indicated that OXC was degraded in alkaline medium. Proposed methods were successfully applied to determination of OXC in tablets and results are comparable with reported method.

Keywords: Assay, Oxcarbazepine, Pharmaceuticals, Stability indicating, UV-spectrophotometry

Introduction

Oxcarbazepine [(OXC), 10,11-dihydro-10-oxo-5H-dibenzo[b,f]azepine-5-carboxamide] (Fig. 1), is a novel antiepileptic, anticonvulsant and mood stabilizing drug, used primarily in treatment of epilepsy[1] and bipolar disorders[2]. It is also used to treat anxiety and mood disorders, and benign motor tics. Several analytical methods have been reported for OXC including high-performance liquid chromatography (HPLC)[3-6], high-performance thin layer chromatography (HPTLC)[7], gas chromatography (GC)[6], microemulsion electrokinetic chromatography[8], capillary electrokinetic chromatography[9], capillary electrophoresis[10] and voltammetry[11, 12]. Visible spectrophotometric assays of OXC[13-15] have also been reported. Most of the reported methods are often time consuming, expensive, use multi or expensive reagents, cumbersome, and require expertise operational personnel. According to International Conference on Harmonization (ICH) guidelines, stress testing of drug substance should be carried out to elucidate inherent stability of active substance[16].

This study has developed two simple, inexpensive, accurate, reproducible, and stability-indicating UV spectrophotometric methods for determination of OXC in bulk drug and in tablets. Proposed methods were validated as per ICH guidelines[17].

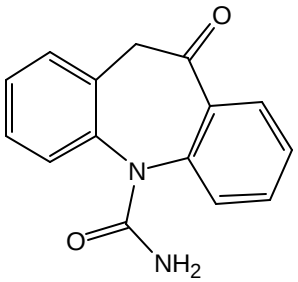

Fig. 1—Chemical structure of OXC

Experimental Section

Materials

Shimadzu Pharmaspec 1700 UV/Visible spectrophotometer was used for spectrophotometric measurements. All chemicals were of analytical reagent grade. Doubly-distilled water was used. Methanol, acetonitrile, hydrogen peroxide (H_2O_2), hydrochloric acid (HCl) and sodium hydroxide (NaOH) were purchased from Merck (Mumbai, India). OXC sample (purity 99.5%) was kindly supplied by Jubilant Life Sciences Ltd, Nanjangud, Mysore, India. Two brands of tablets [Trioptal-300 (Novartis India Ltd, Mumbai, India) and

*Author for correspondence
E-mail: basavaiahk@yahoo.co.in

Oxetol-600 (Sun Pharmaceuticals, Sikkim)] were purchased from local commercial sources.

A 0.1 N HCl was prepared by appropriate dilution of concentrated acid (sp gr, 1.18) with water. A 5% solution of H_2O_2 was prepared by diluting 9 ml of commercially available 30% reagent to 50 ml with water in a volumetric flask. A 0.1 N NaOH was prepared by dissolving required amount of pellets in water. Standard drug solutions of 80 µg ml^{-1} OXC in methanol and acetonitrile were prepared separately and used for assay in method A and method B, respectively.

Procedures

Preparation of Calibration Curve

Into a series calibration flasks (10 ml each), aliquots of standard drug solution (0.25 - 5 ml of 80 µg ml^{-1}) equivalent to OXC (2 - 40 µg ml^{-1}) were accurately transferred and volume was made up to the mark with solvent (methanol in method A; acetonitrile in method B). Absorbance of each solution was measured at 254 nm against respective solvent. Calibration curve was prepared by plotting absorbance *vs* concentration of drug. Concentration of unknown was read from respective calibration curve or computed from regression equation using Beer's law data.

Analysis of Tablets

Tablets (20) from each brand (Trioptal-300 and Oxetol-600) were weighed and crushed as fine powder. Tablet powder (equivalent to 80 mg of OXC) was transferred into a volumetric flask (100 ml). Content was shaken well with 50 ml of respective solvent (methanol in method A; acetonitrile in method B) for 20 min. Mixture was diluted to mark with same solvent. It was filtered using Whatman No 42 filter paper. First 10 ml portion of filtrate was discarded and a subsequent portion was diluted (conc., 80 µg ml^{-1}) and subjected to analysis.

Analysis of Placebo Blank and Synthetic Mixture

A placebo blank (starch, 10; acacia, 15; hydroxyl cellulose, 10; sodium citrate, 10; talc, 20; magnesium stearate, 15; and sodium alginate, 10 mg) was prepared by combining all components to form a homogeneous mixture. Placebo blank (5 mg) was accurately weighed and its solution was prepared as that prepared for tablets, and then subjected to analysis.

A synthetic mixture was prepared by adding OXC (20 mg) to placebo. Extraction procedure for tablets was applied by taking required quantity of synthetic mixture to prepare OXC (80 µg ml^{-1}) solutions. Three different volumes of resulting synthetic mixture solution (equiv to 10, 20 and 30 µg ml^{-1} OXC in both methods) were subjected to analysis.

Forced Degradation Study

A 5 ml aliquot of standard OXC (80 µg ml^{-1}) was taken in triplicate in a volumetric flask (10 ml) and mixed with 5 ml of 0.1 N HCl (acid hydrolysis) or 0.1 N NaOH (alkaline hydrolysis) or 5% H_2O_2 (oxidative degradation) and boiled for 2 h at 80°C on a hot water bath. Solution was cooled to room temperature (RT) and diluted to the mark with either methanol (method A) or acetonitrile (method B). In thermal degradation, solid drug was kept in Petri dish in oven at 100°C for 24 h. After cooling to RT, OXC (8 mg) was weighed and transferred to a 100 ml volumetric flask, dissolved in and diluted up to the mark with respective solvent. Stock solutions of drug (80 µg ml^{-1}) in each method were exposed to UV radiation of wavelength 254 nm and of 1.4 flux intensity for 48 h in a UV chamber. Finally, absorbance of all resulted solutions (80 µg ml^{-1} in OXC) obtained from acid and alkaline hydrolysis, oxidative degradation, thermal and UV degradation of OXC, was measured at 254 nm against respective solvent as blank in each case.

Validation

Intra-day and Inter-day Accuracy and Precision

Three different concentrations of OXC within the range of study (10, 20 and 30 µg ml^{-1} in method A; 15, 20 and 25 µg ml^{-1} in method B) were analyzed in seven replicates during same day (intra-day precision) and five consecutive days (inter-day precision). For inter-day precision, each day analysis was performed in triplicate and pooled-standard deviation was calculated as[18]

$$S_p = \sqrt{\frac{(X_i - \overline{X}_1)^2 + (X_j - \overline{X}_2)^2 + (X_k - \overline{X}_3)^2}{N - k}} \quad \ldots(1)$$

where X_i, X_j and X_k are individual concentrations of OXC found in each set ($\overline{X}_1$, $\overline{X}_2$ and $\overline{X}_3$) are mean values found for data sets 1, 2 and 3, respectively, and N is total number of measurements from k sets. Accuracy was evaluated as percentage relative error (%RE) between found and taken concentrations as

$$\% RE = \frac{(OXC_T - OXC_F)}{OXC_T} \times 100 \quad \ldots(2)$$

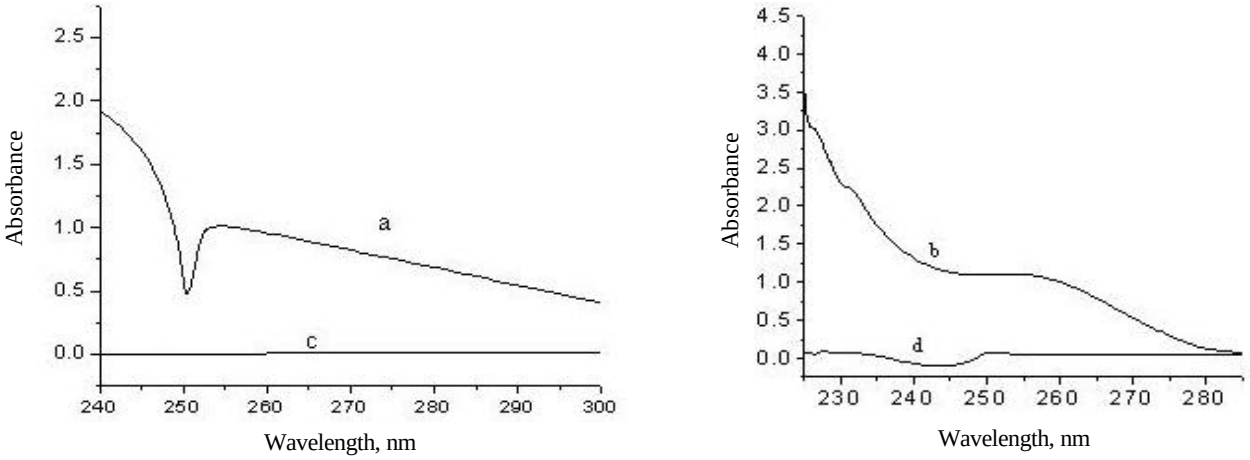

Fig. 2—Absorption spectra of: a) OXC (40 µg ml^{-1}) in methanol; b) OXC (40 µg ml^{-1}) in acetonitrile; c) methanol; and (d) acetonitrile

where subscripts T and F refer to taken and found, respectively.

Assessment of Accuracy by Recovery Experiment

Accuracy of methods was determined by calculating %RE of OXC. In both methods, different aliquots of pure drug solution (equivalent to 5, 10 and 15 µg ml^{-1} OXC) were spiked separately into tablet solutions containing 10.23 and 10.36 µg ml^{-1} OXC, in method A and method B, respectively, and then analysis was performed.

Determination of Limits of Detection (LOD) and Limits of Quantification (LOQ)

A replicate measurement of absorbance of respective solvent (methanol in method A; acetonitrile in method B) at 254 nm was made (n = 5) and standard deviation value was calculated. LOD and LOQ were calculated[17] as LOD = 3.3 S/b and LOQ = 10 S/b, where S is standard deviation obtained from absorbance values measured for either methanol (method A) or acetonitrile (method B) at 254 nm, and b is slope obtained from respective calibration graph.

Ruggedness

A triplicate analysis was performed by four different analysts, using four different instruments for three different amounts (10, 20 and 30 µg ml^{-1} in method A; 15, 20 and 25 µg ml^{-1} in method B) of OXC. Concentration of OXC in each case was calculated. Precision as RSD (relative standard deviation) was evaluated.

Results and Discussion

Spectral Characteristics

OXC solution either in methanol (method A) or acetonitrile (method B) showed absorption maximum at 254 nm, and at this wavelength methanol or acetonitrile had insignificant absorbance (Fig. 2). Therefore, further investigation for analysis of OXC was carried out at 254 nm and used as analytical wavelength (λmax).

Method Validation

Linearity, sensitivity, LOD and LOQ

A linear correlation was found between absorbance at λ_{max} and concentration of OXC. Graphs are described by regression equation: Y = a + bX (where Y = absorbance of drug solution; a = intercept; b = slope and X = concentration of drug in µg ml^{-1}). Slope (b), intercept (a) and correlation coefficient (r) for each system were evaluated by Beer's law limits. Molar absorptivity and Sandell sensitivity values[19] of both methods were calculated. LOD and LOQ were also calculated (Table 1). High values of molar absorptivity (ε), low values of Sandell sensitivity and LOD revealed that proposed methods are highly sensitive.

Precision and Accuracy

To check repeatability and system suitability of proposed methods, assays were repeated seven times within day (intra-day precision) and five times on five different days (inter-day precision). Assays were performed for three levels of analyte. RSD (%) values were $\leq$ 1.07% (intra-day) and $\leq$ 2.50% (inter-day), indicating high precision of methods. Accuracy of methods was evaluated as RE (%) between measured mean concentrations and taken concentrations for OXC. RE (%) values of $\leq$ 3.08% demonstrate high accuracy of proposed methods (Table 2).

Table 1—Sensitivity and regression parameters

Parameter	Method A	Method B
λ_{max}, nm	254	254
Linear range, μg ml^{-1}	2-40	2-40
Molar absorptivity(μ), L $mol^{-1}cm^{-1}$	6.73×10^3	6.95×10^3
Sandell sensitivity*, μg cm^{-2}	0.038	0.036
Limit of detection (LOD), μg ml^{-1}	0.39	0.33
Limit of quantification (LOQ), μg ml^{-1}	1.18	1.00
Regression equation, Y**		
Intercept (a)	0.0129	-0.0030
Slope (b)	0.0258	0.0281
Regression coefficient (r)	0.9993	0.9999

*Limit of determination as weight in μg per ml of solution, which corresponds to an absorbance of A = 0.001 measured in a cuvette of cross-sectional area 1 cm^2 and l = 1 cm. **Y=a+bX, where Y is absorbance, X is concentration in μg/ml, a is intercept and b is slope

Table 2—Evaluation of intra-day and inter-day accuracy and precision

Method	OXC taken, μg ml^{-1}	Intra-day accuracy and precision (n=7) OXC found±CL, μg ml^{-1}	%RE	%RSD	Inter-day accuracy and precision (n=5) OXC found±CL, μg ml^{-1}	%RE	%RSD
A	10.0	10.30±0.08	3.00	0.87	10.31±0.28	3.08	2.22
	20.0	20.21±0.04	1.07	0.22	20.32±0.34	1.59	1.36
	30.0	29.53±0.03	1.58	0.11	30.65±0.78	2.16	2.04
B	15.0	14.65±0.15	2.35	1.07	15.28±0.38	1.85	2.00
	20.0	20.12±0.15	0.60	0.82	19.78±0.39	1.11	1.58
	25.0	24.90±0.17	0.40	0.75	25.27±0.39	1.08	1.25

%RE, percent relative error; %RSD, relative standard deviation; and CL, Confidence limits were calculated from CL = ± $tS/\sqrt{n}$ (value of t is 2.45 and 2.77 for six and four degrees of freedom respectively; at 95% confidence level, S = standard deviation and n = number of measurements).

Table 3—Method ruggedness expressed as intermediate precision (% RSD)

Method	OXC taken, μg ml^{-1}	Ruggedness Inter-analysts (%RSD), (n=4)	Inter-instruments (%RSD), (n=4)
A	10.0	0.85	2.48
	20.0	0.62	1.85
	30.0	0.74	2.14
B	15.0	0.92	2.72
	20.0	0.42	2.04
	25.0	0.57	2.54

Selectivity

In analysis of placebo blank, absorbance value was nearly same as that for solvent in both methods, suggesting non-interference by inactive ingredients added to prepare placebo. Effect of matrix in assay of OXC was checked using a synthetic mixture. An aliquot (4 ml) of resulting OXC extract prepared by using synthetic mixture was assayed by following general procedure (n = 3), and yielded recovery (%) values of 98.45-103.5 of OXC. These results pronounced non-interference from matrix added to prepare synthetic mixture.

Ruggedness

Method ruggedness was demonstrated having the analysis done by four analysts, and also by a single analyst performing analysis on four different instruments in same laboratory. RSD (%) in both instances were 0.42 - 2.72%, indicating acceptable ruggedness (Table 3).

Application to Tablet Analysis

OXC tablets were analyzed by developed methods and by a reported method[14], which involved addition of Folin-Ciocalteu's (F-C) reagent to OXC in alkaline

Table 4—Results of analysis of tablets by proposed methods and comparison of results with reference method

Tablet brand name	Nominal amount mg/tablet	Found* (% of label claim ± SD)		
		Reference method	Method A	Method B
Trioptal 300	300	102.6±1.48	103.2±1.25 t = 0.69 F = 1.40	101.0±0.68 t = 2.34 F = 4.74
Oxetol 600	600	102.7±1.56	102.3±1.10 t = 0.48 F = 2.01	103.6±1.28 t = 1.00 F = 1.49

*Mean value of 5 determinations; Tabulated t-value at 95 % confidence level and for four degrees of freedom is 2.77. Tabulated F-value at 95 % confidence level and for four degrees of freedom is 6.39.

Table 5—Results of recovery study *via* standard-addition method

Tablets studied	Method A				Method B			
	OXC in tablet, μg ml^{-1}	Pure OXC added, μg ml^{-1}	Total found μg ml^{-1}	Pure OXC recovered %±SD*	OXC in tablet, μg ml^{-1}	Pure OXC added, μg ml^{-1}	Total found μg ml^{-1}	Pure OXC recovered %±SD*
Oxetol 600	10.23	5.00	15.41	103.6±0.72	10.36	5.00	15.35	99.74±0.42
	10.23	10.00	20.94	107.1±1.26	10.36	10.00	20.71	103.5±0.96
	10.23	15.00	25.47	101.6±0.56	10.36	15.00	25.56	101.3±0.76

*Mean value of three determinations

Table 6—Results of stability indicating study by forced degradation study

Parameters studied	OXC taken μg ml^{-1}	Method A		Method B	
		OXC Found* μg ml^{-1}	%Recovery of OXC±SD	OXC found* μg ml^{-1}	%Recovery of OXC±SD
Acid hydrolysis	30.0	31.48	104.9±1.12	31.21	104.0±1.25
Alkaline hydrolysis	30.0	56.48	188.3±2.13	56.58	188.6±2.35
Oxidative degration	30.0	31.17	103.9±1.05	31.21	104.0±2.22
Thermal degradation	30.0	31.21	104.0±1.03	29.86	99.53±0.59
UV degradation	30.0	31.24	104.2±0.89	29.89	99.64±1.05

*Mean value of 3 determinations

medium followed by absorbance measurement at 760 nm. Results were compared by Student's t-test and variance-ratio F-test[20]. Calculated t- and F- values did not exceed tabulated values of 2.77 (t) and 6.39 (F) at 95% confidence level and for four degrees of freedom (Table 4), indicating close similarity between proposed methods and reported method with respect to accuracy and precision.

Recovery Study

To further ascertain accuracy and reliability of proposed methods, recovery experiments were performed *via* standard-addition procedure. Pre-analyzed tablet powder was spiked with pure OXC at three different levels and total was found by proposed methods. Each determination was repeated three times. Recovery (%) of pure OXC added was within permissible limits, indicating absence of inactive ingredients in assay (Table 5).

Stability Indicating Property

Stability indicating property of drug was studied by a forced degradation study. OXC was subjected to acid,

base and H_2O_2 induced degradation, thermal and UV degradation. Study was performed by measuring absorbance of OXC solution only after subjecting to forced degradation. Recovery (%) of OXC was calculated in each case. Results revealed no change in absorbance of OXC solution resulting from acid hydrolysis, H_2O_2 induced degradation, thermal and UV degradation when compared with pure OXC solution (Table 6). There was significant change in absorbance of base induced OXC solution, confirming that OXC is susceptible to base hydrolysis.

Conclusions

OXC was subjected to stress studies under various ICH-recommended conditions. Drug undergoes extensive degradation under alkaline conditions and stable to acidic, photolytic, and oxidative stress conditions. Methods were validated for linearity, precision, accuracy, selectivity and ruggedness. Application of these methods for analysis of OXC in tablet dosage forms showed that there was no interference of excipients in determination. This study is a typical example of development of a stability indicating assay, established following ICH guidelines. Methods can be used to determine purity of drug available from various sources and in stability studies.

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