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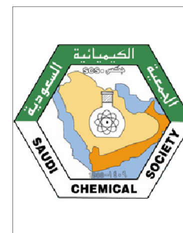

ORIGINAL ARTICLE

Development and validation of indirect visible spectrophotometric methods for oxcarbazepine in pure and the tablet dosage form

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Abstract Three simple, accurate and highly sensitive indirect spectrophotometric methods have been developed for the determination of oxcarbazepine (OXC) in both pure and in pharmaceutical preparations. The methods are based on the oxidation of oxcarbazepine by a known excess of cerium(IV) in acid medium. This was followed by the determination of unreacted cerium(IV), which oxidizes leuco dyes to colored dyes in the same acid medium. In method A, an unreacted cerium(IV) oxidizes leuco crystal violet to crystal violet dye which is measured at 580 nm. A bluish-colored malachite green with a maximum absorption at 610 nm is developed in method B. In method C, cerium(IV) oxidizes leuco xylene cyanol FF to blue colored xylene cyanol FF having absorption maximum at 610 nm. In all these methods, the amount of cerium(IV) reacted corresponds to the amount of OXC and the absorbance is found to decrease linearly with OXC concentration. Beer's law was obeyed in the concentration range of 0–2.5, 0–2.0 and 0–2.5 μg ml^{-1} for methods A, B and C, respectively, and the corresponding molar absorptivity values are 3.86×10^4, 4.41×10^4 and 2.16×10^4 l $mol^{-1}cm^{-1}$. All variables have been optimized and the results were statistically compared with those of a literature method by employing the student's *t*-test and *F*-test. No interference was observed from excipients normally added to the tablets.
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1. Introduction

Oxcarbazepine (OXC), is chemically known as 10, 11-dihydro-10-oxo-5H-dibenz (*b,f*) azepine-5-carboxamide is a keto analog of carbamazepine and is an anticonvulsant and mood stabilizing drug. OXC is known to exert antiepileptic activity by blockade of voltage-dependent sodium channels in the brain. OXC is used to treat seizures, several types of epilepsy and in management of intractable trigeminal neuralgia. In view of its pharmacological importance, considerable work has been done for the detection and quantification of OXC. Several researchers have reported HPLC or GC (Mazzucchelli et al., 2007; Kimiskidis et al.,

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2007; Chi et al., 2006; Klys et al., 2005; Mandrioli et al., 2003; Levert et al., 2002; Rouan et al., 1994; Hartley et al., 1991; Kumps, 1984; Menge and Dubois, 1983; Noirfalise and Collinge, 1983; Von Unruh and Paar, 1985, 1986) methods for its determination in human plasma, serum, urine and saliva. Other techniques such as stripping voltammetry (Calvo et al., 2007) and spectrophotometry (Ramaa et al., 2006; Gandhimathi and Ravi, 2008) have also been reported for the assay of OXC in pharmaceutical samples. The reported chromatographic techniques (HPLC or GC) require expensive experimental set-up and are not affordable in every laboratory for routine analysis. The stripping voltammetric technique requires sophisticated instrumentation, and involvement of scrupulous experimental condition. The reported spectrophotometric methods are less accurate and less sensitive. Thus, there is a need to develop sensitive, accurate and cost-effective methods for its determination. The aim of the present study is to develop a simple, sensitive and validated spectrophotometric methods for the analysis of OXC in pure form and in pharmaceutical samples using cerium(IV), as an oxidant and leuco crystal violet, leuco malachite green and leuco xylene cyanol FF as the chromogenic reagents. The developed methods were validated for linearity, accuracy and precision.

2. Materials and reagents

2.1. Apparatus

All absorbance measurements were performed using a Systronics Model 166 digital spectrophotometer provided with 1-cm matched quartz cells. An Elico 120 digital pH meter was used for pH measurements.

2.2. Reagents and standards

All chemicals and reagents used were of analytical reagent grade and distilled water was used throughout the investigation.

2.2.1. Standard OXC solution

Pharmaceutical grade OXC was obtained from Cipla India Ltd., Mumbai, India, as a gift sample. A stock standard solution equivalent to 100 $\mu g\ ml^{-1}$ of OXC was prepared by dissolving 10 mg of the pure drug in 5 ml methanol and diluted to the volume in a 100 ml calibrated flask using distilled water. Working standard was prepared from the stock solution.

Pharmaceutical formulations of oxcarbazepine such as OXRATE 150 mg (Wockhard, India) and OXETOL 150 mg (Sun Pharma, India) were purchased from local markets.

2.2.2. Standard cerium(IV) solution (1000 $\mu g\ ml^{-1}$)

It was prepared by dissolving 0.3916 g of ammonium ceric nitrate (BDH, Anal R) in 100 ml water containing 0.5 ml of conc. HNO_3. A working standard solution was prepared by a suitable dilution of a standard solution as and when required.

2.2.3. Leuco crystal violet (LCV, 0.025%)

It was prepared by adding 250 mg of LCV (Sigma–Aldrich, Steinhein, Germany), 200 ml of water and 3 ml of 85% phosphoric acid to 1000 ml volumetric flask and shaken gently until the dye dissolved. The contents of the flask were then diluted to the mark with distilled water. The reagent was stable for more than 2 months.

2.2.4. Leuco malachite green (LMG, 0.05%)

It was prepared by adding 5 mg of LMG (Sigma–Aldrich, Steinhein, Germany), 20 ml of water and 0.5 ml of 85% phosphoric acid to 100 ml calibrated flask and shaken gently until the dye dissolved (phosphoric acid was added to dissolve the dye completely and to keep the solution stable for a longer time). The contents of the flask were diluted to 100 ml with water. The reagent was stable for more than 2 months.

2.2.5. Leuco xylene cyanol FF (LXCFF, 0.1%)

It was prepared by dissolving 100 mg of Xylene cyanol FF in 25 ml of water containing 0.7–0.8 g of zinc dust and 2 ml of 1 M acetic acid, stirred well and kept aside for 20 min, and then the resulting solution was diluted to 100 ml with water (filter if necessary).

2.2.6. Sulfuric acid

0.05 M and 0.5 M.

2.2.7. Acetate buffer (pH-4.0)

It was prepared by dissolving 13.6 g of sodium acetate trihydrate in 80 ml of water. The solution pH was adjusted to 4.0 with acetic acid, and the mixture was diluted to 100 ml with water.

2.3. Procedures

2.3.1. Preparation of calibration graph

2.3.1.1. Method A. Aliquots of pure OXC solution (0–2.5 ml; 10 $\mu g\ ml^{-1}$) were transferred into a series of 10 ml calibrated flasks. To this, 0.6 ml of cerium(IV) [50 $\mu g\ ml^{-1}$] and 0.5 ml each of 0.5 M sulfuric acid and 0.025% LCV were added, and the reaction mixture was kept in a water bath (~40 °C) for 5 min, cooled to room temperature and the contents were diluted to the mark with acetate buffer of pH-4.0 and mixed well. The absorbance was measured at 580 nm against distilled water blank.

2.3.1.2. Method B. Into a series of 10 ml calibrated flasks were placed 0–2.0 ml (10 $\mu g\ ml^{-1}$) of OXC, 0.6 ml of cerium(IV) [50 $\mu g\ ml^{-1}$], 0.5 ml of 0.5 M sulfuric acid and 0.5 ml of 0.05% LMG. The reaction mixture was kept in a water bath for 20 min (~60 °C) and cooled to room temperature. A volume of 3 ml of acetate buffer (pH-4.0) was added to each flask and then the contents were diluted to the mark with distilled water and mixed well. The absorbance of the formed malachite green was measured at 610 nm.

2.3.1.3. Method C. Accurately measured volume of the drug solution equivalent to 0–2.5 $\mu g\ ml^{-1}$ final solution of OXC was transferred into a series of 10 ml calibrated flasks. To this, 0.8 ml of cerium(IV) and 0.5 ml each of the 0.05 M H_2SO_4 and 0.1% LXCFF were added. The reaction mixture was kept in a water bath (~90 °C) for 10 min; after being cooled to room temperature, the contents were diluted to the mark with acetate buffer (pH-4.0) and mixed well. The absorbance of the formed xylene cyanol FF dye was measured at 610 nm against distilled water.

In each method, a blank was prepared similarly omitting the drug and its absorbance was measured against distilled water. The decrease in absorbance corresponding to the consumed cerium(IV) and in turn, to the drug concentration, was obtained by subtracting the absorbance of the blank

solution from that of the test solution. The calibration graph was drawn by plotting the difference in absorbance (test and blank solution) of the formed dye against the concentration of the OXC (Fig. 1). The amount of the OXC was determined from the concurrent calibration curve or regression equation.

2.4. Procedure for tablets

Twenty tablets were weighed accurately and ground into fine powder. A quantity of the powder equivalent to 10 mg of OXC was weighed accurately into a 100 ml calibrated flask and 50 ml methanol was added. The content was shaken for about 30 min; the volume was diluted to the mark with water and mixed well and filtered using a Whatman No.41 filter paper. An appropriate dilute solution was subjected to analysis by the procedures described above.

3. Results and discussion

In an acid medium, cerium(IV) quantitatively oxidizes LXCFF into xylene cyanol FF, which shows maximum absorption at 610 nm (Revanasiddappa and Kiran Kumar, 2002). In a similar reaction, cerium(IV) oxidizes leuco malachite green (LMG) to malachite green (Revanasiddappa and Dayananda, 2007). Both methods were used to determine micro amounts of cerium(IV) spectrophotometrically. In this work, known but excessive amount of cerium(IV) was utilized to oxidize OXC in sulfuric acid medium with a sufficient heating, and the unreacted cerium(IV) was determined by reacting it with LCV (method A), LMG (method B) and LXCFF (method C) in the same experimental conditions. In each method, absorbance of the formed dye was measured at specified wavelengths. This has been the basis for the determination of OXC in pure and in pharmaceutical samples.

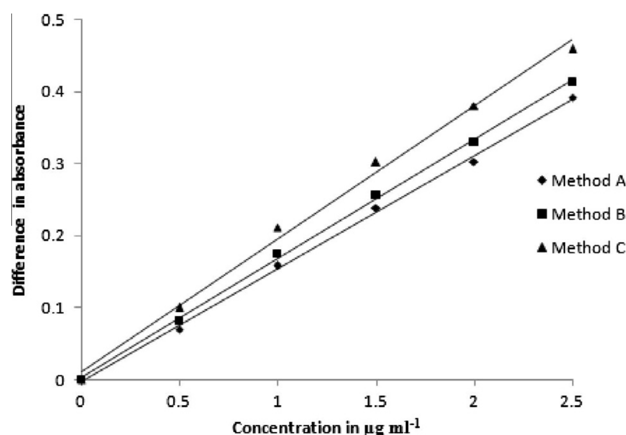


Figure 1 Calibration curves for OXC.

The reaction pathways of all the methods are shown in Scheme 1. Based on the above observations, simple spectrophotometric methods to the determination of OXC were developed and validated as per the current ICH guidelines (International Conference on Harmonization, 1996).

The various experimental parameters, which influence the formation of the colored dye, were optimized.

3.1. Effect of time

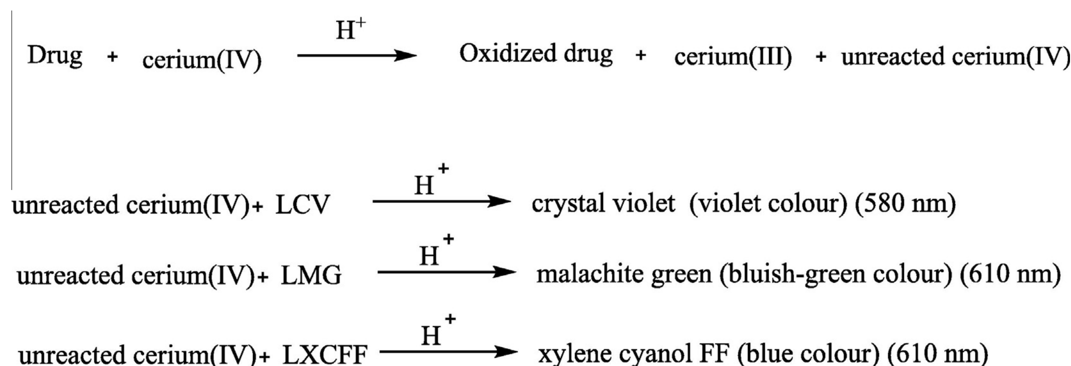
The influence of the reaction time on the absorbance of the product was studied on 1 μg ml^{-1} of OXC with different chromogenic reagents as mentioned under methods A, B and C. The optimum reaction time was found to be 5, 20 and 10 min for methods A, B and C, respectively, for the complete formation of dye.

3.2. Effects of acidity and temperature

The oxidation of LCV, LMG and LXCFF by cerium(IV) was studied. Of the various acids (sulfuric, hydrochloric and phosphoric) studied, sulfuric acid was found to be the best acid for the system. Constant absorbance readings were obtained in the 0.1–1.5 ml range of 0.5 M sulfuric acid [pH-1.0–2.3] at a temperature 40 °C for 5 min for method A or 60 °C for 20 min for method B and 0.05 M sulfuric acid [(or) pH-1.4–3.9] at 90 °C for 10 min for method C. An increase of the pH above 3.9 markedly affected the stability and sensitivity of the dye. Color development did not take place below pH-1.0 for methods A and B and pH-1.4 in method-C. Hence, a volume of 0.5 ml of 0.5 M sulfuric acid was used in methods A and B, and 0.5 ml of 0.05 M sulfuric acid in a total volume of 10 ml was used in method C.

3.3. Effects of reagent concentration and buffer media

The optimum concentration of LCV, LMG and LXCFF leading to maximum color stability was found to be 0.5 ml each of 0.025% LCV, 0.05% LMG and 0.1% LXCFF reagents per



Scheme 1 Probable reaction scheme.

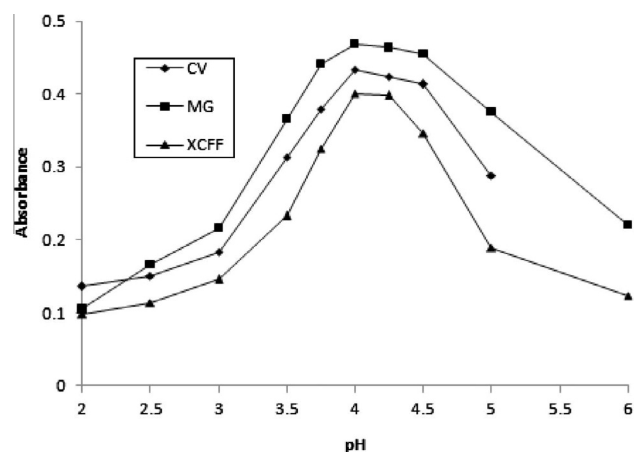


Figure 2 Effect of pH on the absorbance of CV, MG and XCFF dyes.

10 ml of the reaction mixture in methods A, B and C, respectively. The absorbance values were measured in the pH range of 3.7–4.3 for LCV and LMG (methods A and B), and in pH 3.5–4.8 for LXCFF (method C). This could be achieved by adding acetate buffer of pH-4.0 in all the methods (A, B and C). Intensity of the colored dye may be affected by a change in the above pH range. The formed colored dye was stable for more than a week in all the methods. The effect of pH on the color system is shown in Fig. 2.

3.4. Method validation

The proposed methods have been validated for linearity, sensitivity, precision, accuracy, selectivity and recovery.

3.4.1. Linearity and sensitivity

Under optimum conditions, a linear relation was obtained between the absorbance and concentration of OXC in the range 0–2.5 μg ml^{-1}. The calibration graph is described by the equation: $Y = a + bx$, where Y = absorbance, a = intercept, b = slope and x = concentration, obtained by the method of least squares. The correlation coefficient (r), intercept (a) and slope (b) for the calibration data and sensitivity parameters, such as apparent molar absorptivity and Sandell sensitivity values, the limits of detection and quantification calculated as per the current ICH guidelines (International Conference on Harmonization, 1996) are compiled in Table 1.

The limits of detection (LOD) and quantification (LOQ) were calculated according to the same guidelines using the formulas: LOD = $3.3\sigma/s$ and LOQ = $10\sigma/s$ where σ is the stan-

Table 1 Optical characteristic and statistical data of the regression equation.

Parameter	Method A	Method B	Method C
λ_{max}, nm	580	610	610
Beer's law range (μg ml^{-1})	0–2.5	0–2.0	0–2.5
Molar absorptivity (ε), (l mol^{-1}cm^{-1})	3.86×10^4	4.41×10^4	2.16×10^4
Sandell sensitivity (μg cm^{-2})	0.0065	0.0057	0.0117
Intercept (a)	−0.0019	0.0032	0.0120
Slope (b)	0.1563	0.1655	0.1843
Correlation coefficient (r)	0.999	0.999	0.997
S_a	0.0074	0.0156	0.0158
S_b	0.0035	0.0059	0.0076
LOQ(μg ml^{-1})	0.1152	0.1950	0.0464
LOD(μg ml^{-1})	0.0380	0.0644	0.0153

$Y = a + bx$, where c is the concentration of OXC in μg ml^{-1} and Y is the absorbance at the respective λ_{max}, S_a is the standard deviation of the intercept, S_b is the standard deviation of the slope.

Table 2 Evaluation of accuracy and precision.

Method	OXC taken (μg ml^{-1})	OXC found[a] (μg ml^{-1})	RE (%)	SD	SEM	RSD (%)	ROE[b] (%)
Method A	0.5	0.49	1.21	0.008	0.002	0.873	±0.872
	1.0	1.02	−1.68	0.008	0.003	0.814	±0.813
	2.0	2.01	−0.28	0.016	0.006	0.776	±0.776
Method B	0.5	0.50	−0.58	0.006	0.002	1.103	±1.102
	1.0	0.99	0.57	0.002	0.001	0.224	±0.224
	2.0	2.01	−0.26	0.005	0.002	0.271	±0.271
Method C	0.5	0.51	−0.94	0.004	0.002	0.789	±0.788
	1.0	1.02	−2.14	0.007	0.003	0.657	±0.657
	2.0	2.00	0.21	0.010	0.004	0.486	±0.486

RE: relative error; SD: standard deviation; SEM: standard error of mean; RSD: relative standard deviation; ROE: range of error.
[a] Mean value of five determinations.
[b] At the 95% confidence level for 4 degrees of freedom.

Table 3 Results of determination of OXC in tablets and statistical comparison with the reference method.

Tablet brand name	Nominal amount (mg per tablet)	Found[a] (% of nominal amount ± SD)			
		Reference method	Method A	Method B	Method C
OXRATE (Wockhard)	150 mg	98.6 ± 0.8	100.16 ± 0.42 t = 2.44, F = 1.41	99.37 ± 0.33 t = 2.14, F = 5.89	99.82 ± 0.35 t = 2.57, F = 5.31
OXETOL (Sun pharma)	150 mg	98.6 ± 0.8	99.86 ± 1.2 t = 1.252, F = 1.413	99.93 ± 0.99 t = 1.52, F = 1.53	100.2 ± 0.36 t = 2.64, F = 4.97

Tabulated t and F-values at 95% confidence level are 2.77 and 6.39, respectively.
[a] Mean value of five determinations.

Table 4 Results of recovery experiments via the standard addition technique.

Tablet brand name	OXC tablet (μg ml^{-1})	Method A: Pure OXC added (μg ml^{-1})	Method A: Total found (μg ml^{-1})	Method A: Pure OXC recovered[a] (% ± SD)	Method B: Pure OXC added (μg ml^{-1})	Method B: Total found (μg ml^{-1})	Method B: Pure OXC recovered[a] (% ± SD)	Method C: Pure OXC added (μg ml^{-1})	Method C: Total found (μg ml^{-1})	Method C: Pure OXC recovered[a] (% ± SD)
OXRATE 150 mg	1.0	0.5	1.50	100.45 ± 0.181	0.5	1.52	101.71 ± 0.193	0.5	1.506	101.18 ± 0.186
	1.0	1.0	1.99	99.66 ± 0.09	1.0	2.01	100.82 ± 0.145	1.0	2.008	100.83 ± 0.196
	1.0	1.5	2.52	101.09 ± 0.181	1.5	2.51	100.78 ± 0.097	1.5	2.489	99.26 ± 0.143

[a] Mean value of three measurements.

dard deviation (n = 5) of reagent blank determinations and s is the slope of calibration curve.

3.4.2. Accuracy and precision
To evaluate the accuracy and precision of the methods, pure drug solution at three different levels (within the working limits) was analyzed, each determination being repeated five times. The relative error (%) and relative standard deviation (%) were less than 2.0 and indicate the high accuracy and precision of the methods (Table 2).

3.4.3. Application to analysis of commercial samples
To check the validity of the proposed methods, OXC was determined in some commercial formulations. The result obtained from the determination is in close agreement between the results obtained by the proposed methods and the label claim. Statistical analysis of the results using Student's t-test for accuracy and F-test for precision revealed no significant difference between the proposed methods and the literature method (Ramaa et al., 2006) at the 95% confidence level with respect to accuracy and precision (Table 3). The calculated t- and F-values (Table 3) did not exceed the tabulated values (t = 2.77 and F = 6.39).

3.4.4. Recovery study
The accuracy and precision of the proposed methods were further ascertained by performing recovery studies. Pre-analyzed tablet powder was spiked with pure drug at three different concentrations and the total was found by the proposed methods. Each determination was repeated three times. The recovery of the pure drug added was quantitative and revealed that co-formulated substances such as talc, dextrose, alginate, acacia, etc. did not interfere in the determination. The results of recovery study are given in Table 4.

4. Conclusions

Three simple, accurate, precise and highly sensitive spectrophotometric methods were developed for the determination of oxcarbazepine in bulk drug and in tablets. The methods rely on the use of simple and cost-effective reagents in all the three methods. All methods developed are highly sensitive when compared to highly expensive techniques such as HPLC or GC and other reported spectrophotometric methods. Thus, the proposed methods can be used for routine analysis in laboratories and for quality control purposes.

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