

Use of Folin-Ciocalteu phenol reagent and 3-methyl-2-benzothiazolinone hydrazine hydrochloride in the determination of oxcarbazepine in pharmaceuticals

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Two spectrophotometric methods are proposed for the assay of oxcarbazepine (OXC) in bulk and dosage forms using Folin-Ciocalteu phenol reagent (FCP) and 3-methyl-2-benzothiazolinone hydrazine hydrochloride (MBTH) as reagents. The first method involves addition of FCP reagent to OXC in alkaline medium followed by measurement of absorbance at 760 nm (method A), and the other involves addition of a fixed volume of MBTH after treatment of OXC with ferric chloride and measurement of absorbance at 456 nm (method B). In both methods, the amount of chromogen formed corresponds to the amount of OXC and the measured absorbance was found to increase linearly with the concentration of OXC, which is corroborated by the correlation coefficients of 0.9985 and 0.9984 for method A and B, respectively.

The systems obey Beer's law for 5–30 $\mu\text{g mL}^{-1}$ and 10–50 $\mu\text{g mL}^{-1}$ for methods A and B, respectively. The apparent molar absorptivity was calculated to be $8.06 \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$ and $3.126 \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$ for methods A and B, respectively. The limits of detection (LOD) and limit of quantification (LOQ) were calculated to be 1.6 and 5 $\mu\text{g mL}^{-1}$ for method A and 3 and 10 $\mu\text{g mL}^{-1}$ for method B. The inter-day and intra-day imprecision of the methods were found to be in the range of 1.1–1.7 and 0.9–1.1% for method A, and 1.1–1.9 and 0.6–0.9% for method B. The accuracy ranged between 98.9–99.7% and 99.3–100.1 for method A and B, respectively. No interference was observed from common pharmaceutical excipients. The methods were successfully applied to the assay of OXC in tablet preparations.

Keywords: oxcarbazepine, spectrophotometry, Folin-Ciocalteu phenol, 3-methyl-2-benzothiazolinonehydrazine hydrochloride, pharmaceuticals, validation

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Oxcarbazepine (OXC) [10,11-dihydro-10-oxo-5H-dibenz(b,f)azepine-5-carboxamide] is a synthetic antiepileptic drug (1). It is used in the treatment of partial seizures in adults and children. It is available as film coated tablets of different strengths – 150, 300 and 600 mg. A simple RP-HPLC method was reported for determining the plasma concentration of OXC and its metabolites (2). Determination of OXC and its metabolites by the LC-MS method (3) and a stability indicating RP-HPLC method for OXC were reported (4). A square wave adsorptive stripping voltage method for pharmaceuticals containing OXC (5), LC method for OXC and its metabolites from brain (6) and from cerebrospinal fluid (7) were also reported. A spectrophotometric method based on the reaction of OXC with potassium ferricyanide was reported (8), as well as HPLC method for the determination of OXC in pharmaceuticals (9). This paper reports two spectrophotometric procedures involving the reaction of OXC with two reagents, that is, Folin-Ciocalteu phenol reagent (FCP) and 3-methyl-2-benzothiazolinone hydrazine hydrochloride (MBTH).

EXPERIMENTAL

Apparatus

A Jasco model V-530 double beam UV-visible Spectrophotometer (Jasco, Japan) with 1 cm matched quartz cells was used for all absorbance measurements.

Reagents and standards

All chemicals used were of analytical purity grade and all solutions were prepared in doubly distilled water.

Folin-Ciocalteu phenol reagent (FCP). – FCP reagent (2N, Sisco Research Laboratories PVT Ltd, India), 1:2 with water, was prepared and used in method A.

3-Methyl-2-benzothiazolinone hydrazine hydrochloride (MBTH). – A 0.2% (*m/V*) solution of MBTH (99%, Himedia Laboratories PVT Ltd, Mumbai, India) was prepared in water.

Standard solution of oxcarbazepine. – Pharmaceutical grade oxcarbazepine was procured from Intas Pharmaceuticals (India). It was reported to be 99.97% pure and was used as received. A stock solution of OXC was prepared in methanol (1 mg mL⁻¹). 0.1 mg mL⁻¹ solution was used in methods A and B. Standard solutions were kept in refrigerator till use.

Procedures

Method A. – Different aliquots (0.5 mL to 3 mL) of standard 0.1 mg mL⁻¹ OXC solution were transferred into a series of 10-mL standard flasks. To each flask, 1 mL of FCP (1:2) and 1 mL of sodium carbonate (20%, *m/V*) were added and kept aside for 15 min under occasional shaking. The volume was then made up with water and absorbance of each solution was measured at 760 nm.

Method B. – Varying aliquots (1 mL to 5 mL) of standard 0.1 mg mL⁻¹ OXC solution were transferred into a series of 10-mL standard flasks. To each flask, 1 mL of MBTH

(0.2%, *m/V*) and 1 mL of ferric chloride (0.2%, *m/V*) were added and allowed to stand for 20 min under occasional shaking. The volume was then made up with water and absorbance of each solution was measured at 456 nm.

A standard graph was plotted in both methods and the unknown concentration was read from the graph or computed from the regression equation derived using Beer's law data.

Procedure for tablets. – The formulation (Intas Pharmaceuticals, India) containing 150 mg/300 mg/600 mg was extracted with 3×20 mL of methanol individually. Combined extract was filtered using Whatman filter paper and the volume was made up to 100 mL with methanol. Further dilutions were made to get 1 mg mL^{-1} OXC. Five mL of this solution was diluted to 50 mL with methanol. Appropriate volumes of this solution were taken according to the procedures described earlier for methods A and B.

Validation of the method

Various concentrations of OXC were tested to fix the linearity range of the methods; $5\text{--}30 \text{ }\mu\text{g mL}^{-1}$ for method A and $10\text{--}50 \text{ }\mu\text{g mL}^{-1}$ for method B were selected based on the correlation coefficient values (Table I). The limit of detection (LOD) and limit of quantification (LOQ) were calculated according to the current ICH guidelines (10). LOD and LOQ were calculated as 3.3 and 10 standard deviation of the blank ($n = 6$), respectively, divided by the slope of the calibration line.

Accuracy of the method was evaluated by recovery studies using the standard additives method. To a fixed and known amount of the drug in tablet powder (pre analyzed tablet containing 150 mg of OXC), pure OXC was added (50 and 100 mg) and the total amount was found by the proposed methods, from which the percentage recovery of the drug added was calculated.

Selectivity studies were performed separately by applying the proposed methods to the determination of OXC in a synthetic mixture consisting of OXC, talc, starch, lactose, calcium gluconate, calcium dihydrogen orthophosphate, sodium alginate and magnesium stearate, in the mass ratio of 15:25:30:03:05:02:07:10. OXC was extracted with 3×20 mL

Table I. Analytical and regression parameters of proposed methods

Parameter	Method A	Method B
λ_{max}	760	456
Beer's law limits ($\mu\text{g mL}^{-1}$)	5–30	10–50
Molar absorptivity ($\text{L mol}^{-1} \text{ cm}^{-1}$)	8.06×10^3	3.126×10^3
Limit of detection ($\mu\text{g mL}^{-1}$)	1.6	3
Limit of quantification ($\mu\text{g mL}^{-1}$)	5	10
Regression equation ^a		
Intercept = $a \pm \text{SD}$	0.0475 ± 0.011	0.0283 ± 0.012
Slope = $b \pm \text{SD}$	0.0100 ± 0.003	0.0683 ± 0.004
R	0.9985	0.9984

of methanol. Combined extract was filtered using Whatman filter paper and the volume was made up to 100 mL with methanol and diluted to get 1 mg mL^{-1} OXC. It was further diluted to get 0.1 mg mL^{-1} and the appropriate volume of this solution was taken according to the procedures described in methods A and method B.

The inter-day and intra-day precision of the methods was tested on three concentrations in the linear range, repeating each six times. The relative standard deviation was determined. The confidence interval was also calculated.

RESULTS AND DISCUSSION

Method development

The proposed spectrophotometric methods are indirect and are based on determination of OXC after its reaction with either FCP or MBTH and measuring the chromogen at the respective λ_{max} .

OXC reduces FCP to give an intense blue color in method A. This is due to the reduction of 1, 2 and 3 oxygen atoms of FCP reagent and the formation of molybdenum blue or tungsten blue. In method B, MBTH forms an electrophilic moiety while adding ferric chloride, which in turn couples with OXC to give green chromogen. The probable reaction of OXC with MBTH is represented in Fig. 1.

Preliminary experiments were conducted to determine the optimum concentration and volumes of FCP and MBTH to give the highest response. A volume of 1 mL of FCP (1:2) and 1 mL of 20% (*m/V*) sodium carbonate for method A and 1 mL of 0.2% (*m/V*) of ferric chloride and 1 mL of 0.2% (*m/V*) of MBTH for method B were fixed.

The optimum time required for the reaction completion in two methods was the studied and it was found that the reaction of OXC with FCP requires 15 min and OXC that of with MBTH requires 20 min. The chromogen formed by both methods was stable for not less than 2 hours.

The optical characteristics such as Beer's law limits and molar absorptivity values, together with other analytical performance characteristics such as *LOD*, *LOQ*, regression equation parameters are given in Table I.

To evaluate intra-day and inter-day precision of the methods, pure OXC was analyzed at three different concentration levels, each determination being repeated six times. The intra-day imprecision of OXC by two methods was between 0.9–1.1% for method A and 0.6–0.9% for method B. The RSD of both intra-day and inter-day were $\leq 1.9\%$ indicating good precision of the methods. Relative error ranged between 1.2 and 2.1% for both methods.

The accuracy of the method was evaluated by recovery studies by adding pure OXC to the pre-analyzed formulation. The average accuracy was found to be $99.3 \pm 0.3\%$ for method A and $99.7 \pm 0.7\%$ for method B. The accuracy of the methods was compared with the reference method (8) and is shown in Table II.

In the selectivity studies, OXC recovered was between 99.9 and 100% for method A and 99.7 and 99.9% for method B from the synthetic mixture containing additives. This

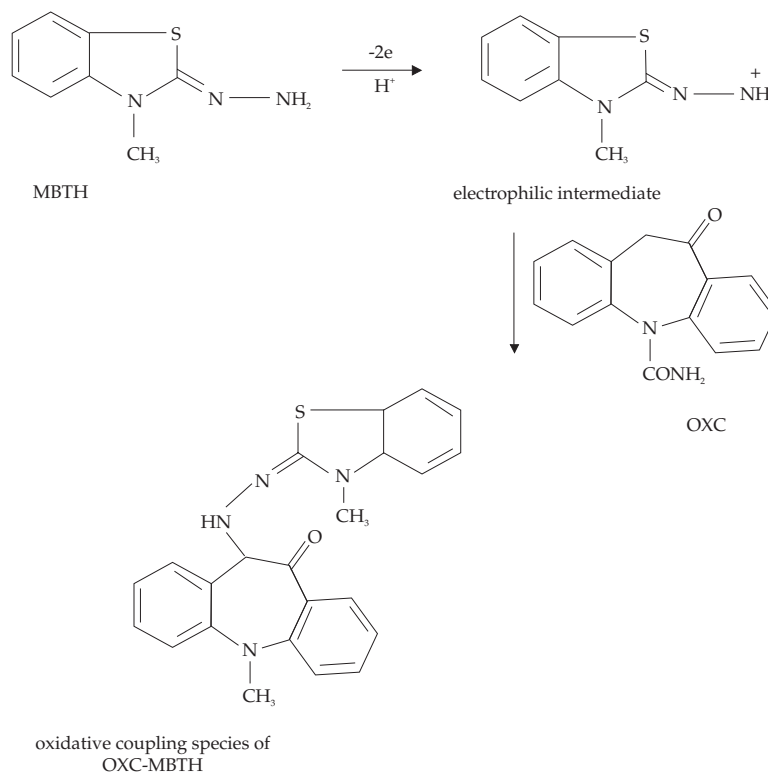


Fig. 1. Probable reaction scheme.

revealed that the additives had not interfered in the estimation of OXC by the two proposed spectrophotometric methods.

Application of the methods to tablets

The spectrophotometric methods were applied successfully to tablets containing OXC and the percent label claims were compared with those obtained by the reported methods (5, 8). A comparison of the calibration range and % label claim between the current methods and reference methods (5, 8) is shown in Table II.

Table II. Recovery study and determination of oxcarbazepine in formulations

Method	λ_{\max} (nm)	Calibration range ($\mu\text{g mL}^{-1}$)	Label claim (%) ^a			Recovery (%) ^{a,b}	Ref.
			150 mg	300 mg	600 mg		
Spectrophotometry (FCP) (method A)	456	5–30	101.3 ± 1.0	99.9 ± 1.1	99.9 ± 1.2	99.3 ± 0.3	This paper
			$t = 1.367$ $F = 2.11$	$t = 1.984$ $F = 4.32$	$t = 1.064$ $F = 1.89$	$t = 1.034$ $F = 2.68$	
Spectrophotometry (MBTH) (method B)	760	10–50	99.4 ± 0.1	100.0 ± 0.6	100.0 ± 1.2	99.7 ± 0.7	This paper
			$t = -0.948$ $F = 1.32$	$t = 1.422$ $F = 3.36$	$t = 0.991$ $F = 5.28$	$t = 1.113$ $F = 3.94$	
Reference method (spectrophotometry with potassium ferricyanide)	790	4–28	98.6 ± 0.8	98.6 ± 0.8	99.21 ± 0.53	98.5 ± 0.25	8
Adsorptive voltammetry	–	130–1300	99.1 ± 1.1	101.2 ± 0.6	101.3 ± 0.8	–	5

^a The values are mean ± SD of six determinations.

^b Standard additions of 50 and 100 mg OXC to formulations declared to contain 150 mg OXC. Tabulated t -value at 95% confidence level is 2.77; tabulated F -value at 95% confidence level is 6.39.

CONCLUSIONS

Two rapid, sensitive and accurate colorimetric methods for the determination of OXC have been developed and validated. They are rapid, do not involve complicated extraction procedures or heating and consume less time. The current spectrophotometric methods use cheap chemicals and inexpensive equipment while providing good sensitivity comparable even to the HPLC. This makes the methods highly suitable for quick routine analyses of OXC in pharmaceutical dosage forms.

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S A Ž E T A K

Upotreba Folin-Ciocalteuovog reagensa i 3-metil-2-benzotiazolinon hidrazin hidroklorida u određivanju okskarbazepina u lijekovitim pripravcima

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Predložene su dvije spektrofotometrijske metode za određivanje okskarbazepina (OXC) koje koriste Folin-Ciocalteu fenolni reagens (FCP) i 3-metil-2-benzotiazolinon hidrazin hidroklorid (MBTH). Predložene metode uspješno su primijenjene za analizu lijekovite tvari i lijekovitim pripravaka. Prva metoda uključuje adiciju FCP reagensa na OXC u lužnatom mediju i mjerenje apsorbancije pri 760 nm (metoda A), a druga adiciju istog volumena MBTH nakon obrade OXC sa željezovim(III) kloridom i mjerenje apsorbancije pri 456 nm (metoda B). U obje metode količina stvorenog kromogena proporcionalna je količini OXC, a izmjerena apsorbancija linearno raste s koncentracijom OXC, uz koeficijent korelacije 0,9985 i 0,9984 za metodu A odnosno B. Oba sustava podliježu Beerovom zakonu u koncentracijskom području $530 \mu\text{g mL}^{-1}$ i $10\text{--}50 \mu\text{g mL}^{-1}$ za metodu A odnosno B. Izračunati molni apsorpcijski koeficijent bio je $8,06 \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$ za metodu A i $3,126 \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$ za metodu B. Granice detekcije (LOD) i granice kvantifikacije (LOQ) bile su $1,6$ i $5 \mu\text{g mL}^{-1}$ za metodu A i 3 i $10 \mu\text{g mL}^{-1}$ za metodu B. Nepreciznost unutar dana i između dana bila je $1,1\text{--}1,7$ i $0,9\text{--}1,1\%$ za metodu A, odnosno $1,1\text{--}1,9$ i $0,6\text{--}0,9\%$ za metodu B. Analitički povrat bio je $98,9\text{--}99,7\%$ za metodu A i $99,3\text{--}100,1$ za metodu B. Nije primijećena interferencija uobičajenih pomoćnih tvari. Metode su uspješno upotrijebljene za određivanje OXC u tabletama.

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Ključne riječi: okskarbazepin, spektrofotometrija, Folin-Ciocalteuov reagens, 3-metil-2-benzotiazolinon hidrazin hidroklorid, lijekoviti pripravci, validacija

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