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DEVELOPMENT AND VALIDATION OF COLORIMETRY METHOD FOR ESTIMATION OF OXCARBAZEPINE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A colorimetric method has been developed and validated for estimation of Oxcarbazepinein its tablet form. Ferric chloride and potassium ferricyanide were used as the coloring agents, and a yellow green color solution was formed after reaction of the drug with ferric chloride and potassium ferricyanide. The solution absorbance was measured at 735 nm. The linearity range was in the range of 1-6µg/ml. The validation of the new proposed method was carried out on various parameters like linearity, accuracy, precision, selectivity, specificity, robustness, ruggedness, LOD and LOQ.

Keywords: Oxcarbazepine, colorimetry, ferric chloride, potassium ferricyanide, yellow green color.

INTRODUCTION

Oxcarbazepine (Fig. 1) is an Antiepileptic drug. Oxcarbazepinbe IUPAC name is 9-oxo-2-azatricyclo[9.4.0.0^ {3, 8}] pentadeca-1(11), 3(8), 4, 6, 12, 14-hexaene-2-carboxamide.Its molecular formula is $C_{15}H_{12}N_2O_2$and molecular weight is 252.268. It is soluble in acetonitrile, sparingly soluble in methanol and poorly soluble in water [1, 2]. It is the 10 keto analogue of the carbazepine and it is a neutral lipophilic compound having same mechanism of action but fewer side effects [3]. In most countries it is available in two dosage forms tablet and suspension. The exact mechanism by which Oxcarbazepine exerts its anticonvulsant effect is unknown. It is known that the pharmacological activity of Oxcarbazepine occurs primarily through its 10-monohydroxy metabolite (MHD). In vitro studies indicate an MHD-induced blockade of voltage-sensitive sodium channels, resulting in stabilization of hyperexcited neuronal membranes, inhibition of repetitive neuronal discharges, and diminution of propagation of synaptic impulses. [4] Oxcarbazepine unlike carbamazepine is not metabolized to an epoxide derivative. As the epoxide is responsible for one of the toxic effects of carbamazepine, the lack of epoxidation of oxcarbazepine is probably one reason for its better side effect profile [3]. Adverse drug reactions (ADRs) associated with Oxcarbazepine includes Dizziness, somnolence, diplopia, fatigue, nausea, vomiting, ataxia, abnormal vision, abdominal pain, tremor, dyspepsia, abnormal gait. Up to now there are bioanalytical methods developed for Oxcarbazepine but colorimetry method is not developed for estimation of Oxcarbazepine [5-7]. So here aim is to develop new colorimetric method on Oxcarbazepine. And validation will be done on Oxcarbazepine.

MATERIALS AND METHODS

Reagents and chemicals: Oxcarbazepine (pure drug) was obtained from Cipla Ltd. Goa, Ferric chloride (FC) 0.2% and Potassium ferricyanide (PF) 0.4% were obtained from loba chemicals.

***Instruments and apparatus*:** PC based Jasco V-530 UV visible spectrophotometer with 1 cm matched pair quartz cell and spectral band width of 2 nm, UV visible spectrophotometer 1601 shimadzu, and Shimadzu AU × 220 weighing balance.

Selection of reagent: Ferric chloride (FC) and potassium ferricyanide (PF) were chosen as a reagent for developing colorimetric reaction. The selection of the reagents was made on the basis of functional groups present on the structure of drug.

Optimization of reagents and condition of reaction: Quantity, concentration, temperature of reaction and sequence of addition of reagents optimized after taking several experimental trials. 1 ml of FC and PF having concentration of 0.4% and 0.2% respectively was found to be suitable for completion of the reaction. The stability of formed complex was checked after recording the absorbance of solution after an interval of 2 hours over a period of 42 hours.

Preparation of ferric chloride (FC) 0.4%: Solution of ferric chloride was prepared by dissolving 200 mg of FC in30 ml HCL. Then it was sonicated for 10 minutes and final volume was made up to 50 ml with the same solvent.

Preparation of potassium ferricyanide (PF) 0.2%: Solution of potassium ferricyanide was prepared by dissolving 100 mg of PF in 30 ml of double distilled water. It was then sonicated for 10 minutes and final volume was made up to 50 ml with the same solvent.

Preparation of standard stock solution: 10 mg of Oxcarbazepine was added in 100 ml volumetric flask. Then it was dissolved in 50 ml distilled water and sonicated for 10 minutes. The final volume was made up to 100 ml with distilled water.

Determination of wavelength of maximum absorbance (λmax): Appropriate aliquots of standard drug solution were pipetted out in 10 ml volumetric flask then1 ml of FC (0.4%) and 1ml of PF (0.2%) were added to each flask. The volume was then made up to 10 ml with double distilled water. Solutions containing 10 µg/ml of OXC were scanned separately in the range of 400-800 nm to determine the wavelength of maximum absorption for the drug. The colorimetry method developed for analysis of Oxcarbazepine and one wavelength was selected for estimation of OXC from the overlay spectrum as shown as (Fig. 2**)** the wavelength selected for the estimation of drug was 735 nm.

Procedure for plotting calibration curve: Appropriate amounts of standard drug solution were taken in to 10 ml volumetric flask. 1 ml of FC (0.4%) and 1ml of PF (0.2%) were added to each flask and the solutions were kept for 10 minutes to complete the reaction. Completion of the reaction was indicated by change in color of solution from colorless to yellow green. The volume was then made up to 10 ml with double distilled water to get the concentrations of 1, 2, 3, 4, 5 and 6µg/ml. The absorbance of green colored species was measured at 735 nm against reagent blank. Calibration curve was constructed at the same wavelength. By using microsoft Excel® software, intercept, slope, and coefficient of correlation values for calibration curve was calculated.
Calibration curve for OXC is given in (Fig. 3) absorbance values are given in (Table 1) and optical characteristics are given in (Table 2).

Analysis of tablet formulation: Marketed tablet formulation (OXC 300 mg) was analyzed by this method. Twenty tablets were weighed accurately and average weight was calculated. The tablets were triturated to fine powder. Tablet powder equivalent to 10 mg OXC was accurately weighed and dissolved in 50 ml of distilled water. The solution was sonicated for 10 minutes. Then the solution was filtered through Whatmann filter paper no. 41. And final volume was made up to 100 ml with the same solvent. Appropriate aliquots within Beer's law limit suitable concentration were prepared and analyzed**.** The concentration of OXC present in the solution was calculated by using formula: Abs = A + B* C where, A = 0.004, B = 0.042 and C = concentration of OXC**.** Results are given in (Table 3).

Method validation: [8-14] The proposed method was validated according to ICH Q_2B guidelines for the validation of analytical procedure so as to determine accuracy, precision, linearity, range, robustness, ruggedness, linearity, limit of detection, limit of quantitation and sensitivity.

Accuracy of the method: Recovery studies were performed by standard addition method to determine the accuracy of proposed method. Different concentrations of pure drug were spiked in the preanalyzed tablet samples within the analytical concentration range of the proposed method at three different set at level of 80%, 100% and 120%. The added quantities of the individual drug were estimated by above method. Accuracy was determined as percentage recovery at each concentration level. The results obtained from recovery study are given in (Table 4).

Intraday precision: The precision of the method was determined by repeated measurement of standard solution (n=6) 10 µg/ml without changing the other parameters of the method. The intraday precision of the method was determined by analyzing corresponding responses of same concentration of

drug three times on same day. And results were reported in relative standard deviation. The results are shown in (Table 5)**.**

Interday precision: The interday precision of the method was determined by analyzing corresponding responses of same concentration of drug on three different days. The reports were reported in percentage relative standard deviation (% RSD), and shown in (Table 6).

Linearity: The linearity of the response of the drug was determined at 2-10 µg/ml concentrations. The calibration curve was obtained by plotting absorbance verses concentration data, and was treated by linear regression analysis as shown in (Figure 3).

Limit of detection and limit of quantitation: The limit of detection and limit of quantitation of the drug were determined by calculating signal-to-noise ratio (3 for LOD and 10 for LOQ) using following equations nominated by International Conference on Harmonization (ICH) guideline.

LOD = 3.3 Xσ/S and LOQ = 10 Xσ/S

Where,

σ = intercept and S = slope of calibration curve

Results are shown in (Table 7).

Robustness: Robustness is the ability of the method to remain unaffected by small but deliberate changes in the conditions of analysis. Robustness was performed using 10% methanol solution and percentage relative standard deviation was calculated. Results are shown in (Table 8).

Ruggedness: Ruggedness of the method was performed by using different analyst and different instrument and percentage relative standard deviation was calculated. Results are shown in (Table 9).

Repeatability: Repeatability expresses the precision under same operating conditions over a short interval of time. Standard solutions of OXC were prepared and analyzed 6 times on same day. Results are shown in (Table 10).

RESULT AND DISCUSSION

The motif behind the proposed work was to develop an accurate, precise, reproducible and sensitive method for determination of Oxcarbazepine. For this, colored complex of OXC was formed with ferric chloride and potassium ferricyanide. This formed complex was yellow green in color and showed wavelength of maximum absorbance (λ max) 735 nm. Stability of the color as well as complex is prerequisite towards the motif. Temperature of reaction, quantity, concentration and sequence of addition of reagents were optimized after several experimental trials. The optimum quantity and concentration of ferric chloride was found to be 1 ml and 0.4% respectively. Quantity and Concentration of potassium ferricynide was optimized to 1mland 0.2% respectively. The colorimetric method so developed was found to obey Beer's-Lambert law in the concentration range of 1-6µg/ml with correlation coefficient 0.998. Result of analysis of tablet formulation showed % label claim value in the range of 100.73 % and % R.S.D. value 1.46 which shows high precision of the method. The recovery studies were also performed on the commercial formulation wherein, Oxcarbazepine shows recovery in the range of 100 to 100.12%. Recoveries obtained for the drug do not differ significantly from 100% showing that there was no interference from common excipients used in the formulation and thus indicates accuracy and reliability of the method. Interday and intraday studies show high degree of repeatability of an analytical method under normal operational conditions. Limit of detection was found to be 0.56 µg/ml and limit of quantitation was found to be 0.67 µg/ml. For testing of robustness of the method the different ratio of methanol shows adjacent absorbance. % label claim estimated value for analysis was 99.99 % and % R.S.D. was found to be 0.043 which proves the ability of the method to remain unaffected by small but deliberate change in reaction conditions. For the ruggedness by using different analyst and different equipments % lable claim estimated was fund in the range of 99.63-100.33% and % R.S.D. was found to less than 2 which indicates ruggedness of the method.

CONCLUSION

A colorimetric method was developed by using ferric chloride and potassium ferricyanide coloring reagents. Proposed method makes use of simple reagent, which an ordinary analytical laboratory can afford. The method has been statistically evaluated and results obtained were accurate, precise and insensitive and free from the interferences of other additives present in the formulation. The proposed colorimetric method can be used for determination of Oxcarbazepine in tablets. The proposed method has the advantages of simplicity, rapidity and selectivity. Low LOD and LOQ indicate that very small quantities of drug can be estimated by this method. The assay method involve less stringent control of experimental parameters such as time of analysis, the stability of the colored species, and the concentration

of the reagent. The method has been validated successfully according to using ICH Q_2B guidelines.

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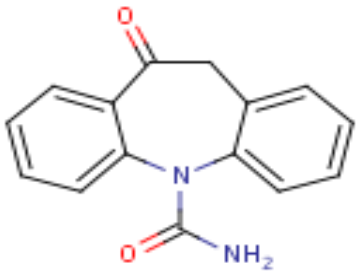

Fig1: structure of Oxcarbazepine

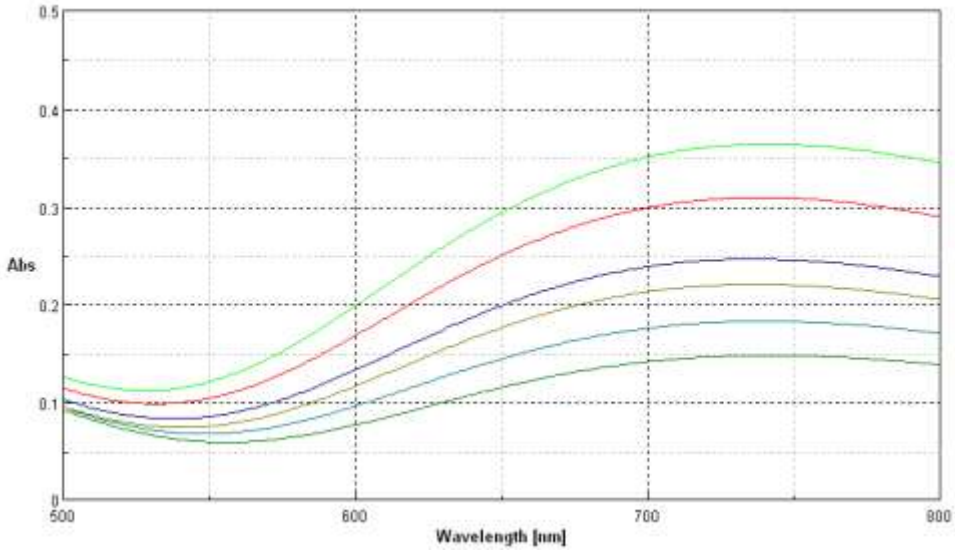

Fig 2: Overlay spectrum of OXC – reagent complex

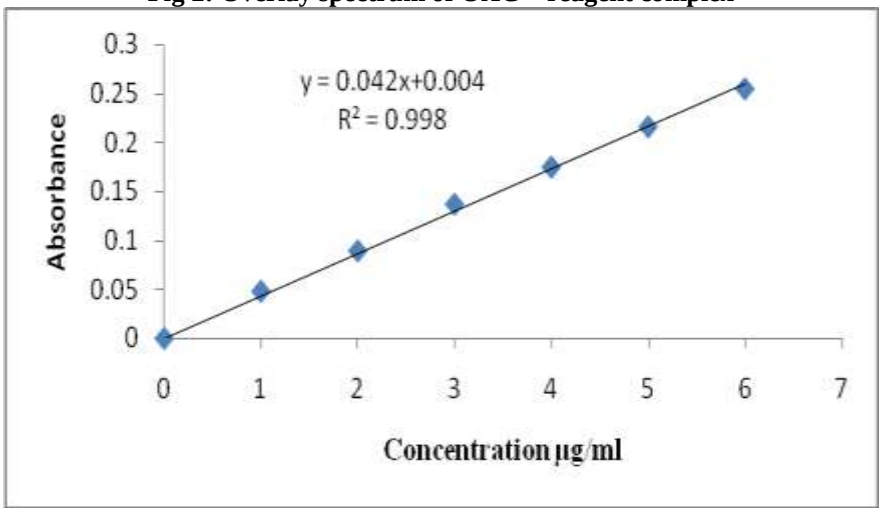

Fig 3: Calibration curve of OXC at 735 nm

Table 1: Absorbance values for calibration curve of OXC at 735 nm

Sr. No.	Concentration (µg/ml)	Absorbance
1.	1	0.048
2.	2	0.089
3.	3	0.1366
4.	4	0.1744
5.	5	0.2154
6.	6	0.2541

Table 2: Optical characteristics

Parameters	Values for OXC
Beer's law limit (µg/ml)	1-6
Correlation coefficient	0.998
Regression equation (Y*)	
Slope (B)	0.042
Intercept (A)	0.004

Y= A + B*C, where C is the concentration in µg/ml and Y is absorbance unit

Table 3: Results of analysis of tablet formulation

Analyte	% Label claim estimated*(Mean ± S. D.)	%R.S.D.
OXC	100.73 ±1.4780	1.46

**** Average of six determinations; R.S.D., relative standard deviation.***

Table 4: Results of recovery study

Level	Analyte	Label claim (mg)	% Label claim estimated*	%RSD
80%	OXC	300	100.07 ± 0.41	0.098
100%	OXC	300	100±0.43	0.10
120%	OXC	300	100.12 ± 0.38	0.092

**** Average of six determinations; R.S.D., relative standard deviation***

Table 5: Results of intraday precision

	% Label claim estimated*(Mean ± S.D.)	
T- 1	99.97 ± 0.198	0.04
T- 2	99.99 ± 0.182	0.043
T-3	99.97 ± 0.195	0.046

**** Average of six determinations; R.S.D., relative standard deviation***

Table 6: Results of interday precision

Time	% Label claim estimated (Mean ± S.D.)	%RSD
Day- 1	99.94 ± 0.08	0.02
Day- 2	99.94 ± 0.082	0.02
Day- 3	99.91 ± 0.093	1.21

**** Average of six determinations; R.S.D., relative standard deviation***

Table 7: Limit of detection and limit of quantitation

LOD (µg/ml)*	LOQ (µg/ml)*
0.56	0.67

**** Average of six determinations***

Analyte	Label claim(mg)	Tablet Analysis % Label claim estimated* (Mean ± S.D.)	% R.S.D.
OXC	300	99.99 ± 0.182	0.043

Table 8: Results of robustness

**** Average of nine determinations; R.S.D., relative Standard Deviation***

Table 9: Results of ruggedness

Parameter	Analyte	Label claim (mg)	% Label claim estimated* (Mean ± S.D.)	%R.S.D.
Analyst - 1	OXC	300	99.66 ± 1.42	1.43
Analyst - 2			100.5 ±1.3	1.4
Instrument - 1			99.63 ±1.42	1.43
Instrument - 2			100.33 ±1.34	1.34

**** Average of six determinations; R.S.D., relative Standard Deviation.***

Table 10: Results of repeatability

Analyte	Label claim(mg)	% Label claim estimated* (Mean ± S.D.)	%R.S.D.
OXC	300	100.15 ± 0.42	0.13

**** Average of six determinations; R.S.D., relative Standard Deviation.***

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