NOVEL SPECTROPHOTOMETRIC METHODS FOR THE ASSAY OF AN ANTIEPILEPTIC- OXCARBAZEPINE

H. Mallikarjuna¹, K.S. Lokesh², K.H. Shivaprasad², K.R. Venugopala Reddy¹,²*

¹Dept. of Industrial Chemistry, Sahyadri College, Kuvempu University, Shivamogga, Karnataka, India.
²Dept of Chemistry, Vijayanagara Sri Krishnadevaraya University, Bellary, 583105, Karnataka, India

ABSTRACT

New spectrophotometric methods have been developed for the assay of oxcarbazepine (OXP) in pure and pharmaceutical formulations. These methods were based on the formation of chloroform soluble ion-association complex of OXP with bromocresol purple in KCl-HCl buffers of pH-2 [Method A] and the diazocoupling of OXP with diazotized p-nitro aniline in basic medium [Method B]. The coloured species exhibited absorption maxima at 412 nm and 510 nm for method A and method B, respectively. The complexes obeyed Beer’s law over the concentration range of 0.4-6 µg/ml and 0.5-4 µg/ml with molar absorptivity values of 2.85×10⁴ and 4.24×10⁴ l/mol/cm for method A and method B, respectively. Common excipients and additives did not interfere in the assay of OXP by proposed methods. The applicability of the proposed methods was checked by analyzing various dosage forms. The results of analysis were subjected to t-test and F-test.

Key words: oxcarbazepine; spectrophotometry; assay; diazotization; formulations.

INTRODUCTION

Drugs are very important for their medicinal, intoxicating, performance enhancing or other effects in human body. The quality of the synthesized bulk drug as well as the formulations needs to be analysed accurately with the simple techniques. Analytical chemistry helps in monitoring the quality of bulk drug and their dosage forms¹. Several analytical techniques
have been employed in the quality control laboratories to penetrate chemical estimation not only of the active ingredient(s) but also the quantification of related compounds or impurities in incoming chemicals, drug materials and formulations. Various analytical techniques are used to determine the assay of drug samples in bulk and formulations. Physical and physico-chemical methods are more commonly employed. Even though the modern methods of analysis (HPLC, GLC, NMR and Mass) offer good speed, precision and accuracy, they involve sophisticated equipments which are costly and pose problems of maintenance. Hence, they may not be in the reach of most of laboratories and small scale industries, which produce bulk drugs and pharmaceutical formulations. Among various techniques, spectrophotometry still plays a significant role in the determination of compounds at micro or nanogram levels. It is simple, economically viable and easy to carry-out. The importance of a spectrophotometric method lies in the chemical reaction(s) upon which the procedures are based, rather than upon the sophistication of the instrument. Hence, spectrophotometry is generally preferred in small scale industries and most of the laboratories for routine quality assurance.

Oxcarbazepine (OXP) a novel antiepileptic drug, is the keto form of 10-hydroxy carbamazepine. This was developed as second generation and follow-up compound to carbamazepine (CBZ). OXP has similar therapeutic profile to CBZ but produces much less side effects on patients. Chemically, OXP is 10,11-dihydro-10-oxo-5H-dibenz [b,f] azepine-5-carboxamide. As a blocker of pre- and postsynaptic voltage-dependent sodium channels in the central nervous system, it has been in therapeutic use in the treatment of partial and generalized seizures, trigeminus neuralgia, affective disorders and espasticity. Clinically it has been used to treat several types of epilepsy.

Literature survey reveals the determination of OXP by chromatographic methods in pharmaceutical products and biological fluids. Because of its biological importance, efforts have been made towards the development of simple and reliable analytical techniques viz., LC, HPLC, voltammetry and UV.

The spectrophotometric technique continues to be the most preferred method for the assay of different class of drugs in pure, pharmaceutical formulations and in biological samples, because of its simplicity and reasonable sensitivity with significant economical advantages. Reported spectrophotometric methods require heating, more time for colour development, less stable and less sensitive. This prompted us to develop simple and
accurate methods for the determination of OXP in formulation, which can be used for the quality control of the product. The proposed methods are simple and sensitive for the assay of OXP.

**Experimental**

Potassium hydrogen phthalate, KCl, sodium acetate (NaOAc), p-nitro aniline, bromocresol purple (BCP), NaNO₂, NaOH, NaSO₄ anhydrous were obtained from s.d.fine chemicals Ltd., Mumbai and para nitro aniline (PNA) was obtained from Merck, India. HCl, acetic acid (AcOH), acetone, methanol carbon tetrachloride, chloroform, ethyl acetate, chlorobenzene, toluene, benzyl alcohol, isoamyl alcohol, 1,2-dichloromethane and diethyl ether solvents were obtained from s.d.fine chemicals Ltd., Mumbai. Oxcarbazepine, (OXP) was a gift sample obtained from Jubilant Life Sciences Ltd. and used as such for the assay. All the solutions were prepared and diluted with distilled water unless it is mentioned.

**Standard drug solution**

Standard solution of OXP containing 250 µgml⁻¹ was prepared by dissolving it in minimum amount of acetone and then diluted with distilled water up to the mark in a 100 ml volumetric flask. The working solution was prepared by suitable dilution of the stock solution with water as and when required. The solution was noticed to be stable at room temperature.

**Buffers**

The following buffers were prepared using the standard method⁴⁸-⁴⁰

1. KCl-HCl buffers of pH 1.0-2.2 (by mixing appropriated volume of 0.2 M each of KCl and HCl).
2. NaOAc- HCl buffers of pH 1.99-4.92 using 1M each of NaOAc and HCl.
3. NaOAc- AcOH buffers of pH 3.72-5.57 (by mixing appropriated volume of 0.2 M each of NaOAc and AcOH).
4. Potassium hydrogen phthalate- HCl buffers of pH 2.2-3.6 using 0.1M each of Potassium hydrogen phthalate and HCl.

Preparation of p- nitroaniline (PNA)

A stock solution of 0.2% of PNA was prepared by dissolving 200 mg in 1:9 HCl- H₂O mixture and solution was diluted with distilled water up to the mark in a 100 ml volumetric flask.
Recommended procedures
In order to know the effect of various parameters involved in the formation of coloured products (as described under results and discussion), a systematic study was carried out and the following procedures were recommended for the assay of OXP in pure and pharmaceutical formulations.

Analysis of bulk sample
Method A
An aliquot of the solution containing 0.4-6 µg ml\(^{-1}\) of OXP were transferred into each of 125 ml separatory funnels. A volume of 7 ml of KCl-HCl buffer of pH 2 and 3 ml of 0.5% (w/v) BCP in water was added to each separatory funnel, followed by the addition of 4 ml of chloroform. Contents were shaken well and allowed to stand at room temperature for 2 min for the separation. The separated organic phase was transferred into a 25 ml beaker. The aqueous phase was again extracted with 4 ml of chloroform. This step was repeated for the third time with 2 ml of chloroform. The successive chloroform extracts were mixed well, dried over anhydrous NaSO\(_4\) and transferred into a 10 ml volumetric flask. The absorbances of yellow coloured complex (organic layer) was recorded at 412 nm against reagent blank (Fig.1). Reagent blank was prepared by omitting the drug. The procedure was repeated for other analytical aliquots and the calibrated curve was constructed by considering the absorbances measured at each concentration levels of OXP. The amount of the drug was computed either from calibration curve or from regression equation.

Method B
Into a series of 10 ml graduated flasks, 1.4 ml of PNA and 1.4 ml of 0.5% (w/v) NaNO\(_2\) in water were added and kept aside for 5 min. Then, 1.2 ml of methanol was added to the reaction mixture and allowed to stand for 5 min. Afterwards, aliquots of standard OXP drug solution containing 0.5-4 µg ml\(^{-1}\) were transferred to series of flasks, shaken well followed by 1.2 ml 1M NaOH solution. The contents were diluted to the mark with distilled water. The absorbance was measured after 5 min at 510 nm against reagent blank (Fig.2). The amount of OXP was computed from its Beer’s law plot prepared with standard drug solution under identical conditions.
Analysis of pharmaceutical preparations

Tablets

Tablets containing OXP were weighed and finely powdered and an amount equivalent to 50 mg was transferred into a 100 ml beaker. Using the mechanical stirrer the powder was completely disintegrated in distilled water. The solution was filtered through a Whatman filter paper number 40. The residue was washed with small quantity of acetone and the solution was made up to 100 ml with distilled water. The solution was analyzed using the procedure for bulk samples. UV-Visible double beam spectrophotometer provided by Shimadzu, Japan was used for the spectrophotometric measurements.

RESULTS AND DISCUSSION

Oxcarbazepine is white to faintly orange crystalline powder. Its molecular formula is \( \text{C}_{15}\text{H}_{12}\text{N}_{2}\text{O}_{2} \) and its structure is given in Scheme I. This molecule is practically insoluble in water, very slightly soluble in methanol and soluble in acetone.

METHOD A

Extractive spectrophotometric procedures are popular for their sensitivity in the assay of drugs and hence, ion-pair extractive spectrophotometry has received a considerable attention for quantitative determination of many pharmaceutical compounds\(^{41-43}\). The ion-association complexes will be formed due to the interaction between two constituents. Ion-pair extraction spectrophotometry has been employed for quantitative assay of different pharmaceutical drugs\(^{44-49}\). Several extractive spectrophotometric methods have been established for basic nitrogenous compounds, which reacted with the acidic dyes to yield ion-pair complexes that were extractable from the aqueous solution to organic phase\(^{50-51}\). In our method, OXP reacted with BCP in acidic buffer to form chloroform- soluble yellow coloured 1:1 ion-association complex, which exhibited absorption maximum at 412 nm. Under the experimental conditions, the reagent blank showed negligible absorbance thereby permitting good analytical conditions for quantitative determination of OXP. The probable reaction mechanism for the formation of an ion-pair complex may be represented as shown in Scheme II.

METHOD B

Para nitro aniline (PNA) was used as diazotizing agent for colorimetric determination of pharmaceutical compounds. The proposed method involved the reaction of OXP with
diazotized p-nitro aniline in presence of MeOH in alkali medium to give pink coloured product with maximum absorption at 510 nm. The proposed reaction mechanism for the formation of coupled product is given in Scheme III.

Fixation of parameters:
The optimum reaction conditions were established by varying the parameter one at a time fixing others and observing the effect produced on absorbance of the coloured species\textsuperscript{52}. The effect of various parameters viz., pH of buffer solution, amount of buffer solution, concentration of dye (BCP), amount of dye, choice of extractant, effect of temperature on the coloured complex were investigated in method A.

For method B, the effects of amount of PNA, suitable acid (with its concentration) for the preparation of PNA, sodium nitrite, sodium hydroxide, time interval for the diazotization, maximum colour development and the stability of the colour product were studied at room temperature.

**FOR METHOD A**

**i) Effect of buffer/ pH**
It was observed that the formation of an ion-association complex and quantitative extraction depended on the type of buffer used and its pH. Hence, various buffers viz., KCl-HCl (pH 1.0-2.2), NaOAc- HCl (pH 1.99-4.92), NaOAc- AcOH (pH 3.72-5.57) and potassium hydrogen phthalate- HCl (pH 2.2-3.6) were tried. It was found that the KCl-HCl buffer of pH 2.0 was most suitable compared to others exhibiting maximum stability, sensitivity and reproducibility. It was also noticed that an optimum volume of 7 ml buffer was necessary for complete colour development (Fig.3). Low absorbances were observed in buffer solution of pH below 2.0 while these were not stable in buffer solution of pH above 2.0.

**ii) Effect of reagent**
For the formation of an ion-association complex of maximum stability, the effect of the reagent was investigated. It was observed that the absorbances increased up to 3.0 ml of 0.5% BCP (Fig.4) and decreased later on. Further, the complex was observed to be unstable. Hence, a volume of 3.0 ml BCP was employed for better results.
iii) Choice of extractant
Various water immiscible organic solvents viz., carbon tetrachloride, chloroform, ethyl acetate, chlorobenzene, toluene, benzyl alcohol, isoamyl alcohol, 1,2-dichloromethane and diethyl ether were tried for effective extraction of coloured species from aqueous phase. Chloroform was found to be the most suitable extractant for quantitative extraction of the complex.

iv) Sequence of addition of reagents
Experiments were carried out to study the effect of sequence of addition of reagents on stability of ion-association complex. From the results, it was concluded that there was no appreciable change in absorbance or colour of the product or stability of the complex even if the order of addition was varied.

FOR METHOD B
i) Effect of PNA on intensity of the coloured product
From investigations, it was found that 0.2% PNA prepared in 1:9 HCl-H₂O mixture gave satisfactory results. Further, maximum and constant absorbance readings were observed with 1.4 ml of 0.2% PNA (Fig.5). Intensity of the complex was affected when the volume of the reagent added was more than or less than 1.4 ml.

ii) Optimization of NaNO₂ concentration
Fig.5 revealed that the intensity of the complex increased upon the addition of 0.5% (w/v) NaNO₂ in the range of 0.5 to 1.4 ml and decreased upon further addition. Hence, a volume of 1.4 ml of reagent was used to obtain diazotized coupled product of maximum stability and sensitivity.

iii) Optimization of MeOH concentration
It was evident from Fig.5 that the maximum and constant absorbance readings were noticed with 1.2 ml of MeOH.

iv) Effect of NaOH
It was noticed from the investigations that the diazotized product was stable only in alkali medium. 1M NaOH was found to be the most suitable one as it improved the sensitivity of the reaction and gave reproducible results. Further, the experimental results indicated that a
volume of 1.2 ml of 1M NaOH was necessary for obtaining coloured product of maximum stability and intensity (Fig.6).

**Spectral characteristics**
The wavelength of absorption maximum ($\lambda_{\text{max}}$) of ion-association complex was determined by taking specified amount of OXP (within the Beer’s law limit) and the reaction product was developed following the procedure. Absorbance was recorded in the wavelength region of 300-600 nm against the reagent blank. The coloured species exhibited absorption maximum at 412 nm (Fig.1).

In method B, a pink coloured product was formed when OXP was treated with Diazotized paranitro aniline in basic medium. The coloured product exhibited absorption maximum at 510 nm against reagent blank (Fig.2).

**Precision and accuracy**
In order to determine the accuracy and precision of proposed methods, standard solutions containing three different concentrations of OXP were analyzed in five replicates. The mean results obtained are summarized in Table I. Low values of relative standard deviation and % error indicated good precision and accuracy, respectively, of the method.

**Optical characteristics of coloured species**
To find whether the coloured products formed in proposed methods obey Beer’s law or not, the absorbances of a series of solutions containing increased amounts of OXP were measured against the corresponding reagent blank at respective $\lambda_{\text{max}}$ value. Regression analysis of Beer’s law plot (Fig.7) revealed a good correlation between absorbances and concentrations of coloured products in the concentration range of 0.4-6 µg/ml$^{-1}$ and 0.5-4 µg/ml$^{-1}$ for method A and method B, respectively. Beer’s law limits, molar absorptivity and Sandell’s sensitivity values have been evaluated and are tabulated in Table I.

**Interference studies**
In order to assess the possible analytical applications of the proposed methods, the effect of some foreign substances which often accompany with these OXP in pharmaceutical products were studied by adding different amounts of foreign substances to OXP. The colour was developed following the procedure described earlier. It was observed that the talc, glucose, starch, lactose, dextrose, magnesium stearate, sodium alginate, calcium gluconate and
calcium dihydrogen orthophosphate did not interfere in the determination of OXP at the levels found in dose forms. Thus, the proposed methods were observed to be free from interference by various substances. The results are given in Table II.

**Limit of detection and quantification**

For the proposed methods, the LOD and LOQ were evaluated as per the recommendations of Analytical Method Committee\(^3\). The LOD values were evaluated and are given in Table I. Method B was observed to be more sensitive compared to method A.

**Stability of coloured spices**

The effect of temperature on stability of coloured species was studied. It was found that the coloured product was stable in the temperature range of 5-35 °C in method A. At higher temperature, the drug concentration was noticed to be increased due to evaporation of chloroform. Hence, studies were carried out at room temperature. The complex was stable for more than 8 h at room temperature.

The stability of the diazotized product formed in method B was examined at different temperatures. Reproducible results were obtained in the temperature range of 5-40 °C. The diazotized product was stable for more than 24 h at room temperature.

**Recovery studies**

In order to examine the accuracy and reproducibility of the proposed methods, recovery experiments were performed by analyzing formulations in the first instance for the active component by proposed methods. For this, known quantities of pure OXP solutions were mixed separately with definite amounts of pre-analyzed formulations and the mixtures were analyzed as before. The total amount of the drug was then determined using the proposed methods and the amount of the added drug was calculated by difference. The results were found to be 99.21-101.32% and 98.59-101.92% in method A and method B, respectively.

**Analysis of pharmaceutical formulations and statical comparison of the results with reported method\(^3\)**

The proposed spectrophotometric methods were successfully applied to the analysis of OXP in tablets. The results of analysis of tablets were compared statistically by F-test and t-test. The calculated F and t-values recorded in Table III were observed to be lower than the
theorical values at 95% confidence level thereby indicating that ther was no significant difference between repored and proposed methods.

Table I: Optical characteristics, precision and accuracy data

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values for</th>
<th>BCP</th>
<th>PNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$ (nm)</td>
<td>412</td>
<td>510</td>
<td></td>
</tr>
<tr>
<td>Beer’s law limits (µg/ml$^{-1}$)</td>
<td>0.4-6</td>
<td>0.5-4</td>
<td></td>
</tr>
<tr>
<td>Molar absorptivity $(l/\text{mol/cm}) \times 10^4$</td>
<td>2.85</td>
<td>4.24</td>
<td></td>
</tr>
<tr>
<td>Sandell’s sensitivity (ng cm$^{-2}$)</td>
<td>8.86</td>
<td>5.96</td>
<td></td>
</tr>
<tr>
<td>Correlation co-efficient (r)</td>
<td>0.9987</td>
<td>0.9975</td>
<td></td>
</tr>
<tr>
<td>Regression equation (Y)$^a$</td>
<td>-------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>Slope (b)</td>
<td>0.1107</td>
<td>0.1715</td>
<td></td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>0.0084</td>
<td>0.0124</td>
<td></td>
</tr>
<tr>
<td>Relative standard deviation (%)$^d$</td>
<td>1.13</td>
<td>1.28</td>
<td></td>
</tr>
<tr>
<td>% Error$^d$</td>
<td>1.08</td>
<td>1.32</td>
<td></td>
</tr>
<tr>
<td>Limit of detection (µg/ml$^{-1}$)</td>
<td>0.1153</td>
<td>0.1391</td>
<td></td>
</tr>
<tr>
<td>Limit of quantification (µg/ml$^{-1}$)</td>
<td>0.3842</td>
<td>0.4633</td>
<td></td>
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$^a$ Y=a+bX, where X is the concentration of the drug in µg/ml$^{-1}$.

Table II: Determination of OXP in the presence of excipients and additives.

<table>
<thead>
<tr>
<th>Excipients added</th>
<th>Amount</th>
<th>% Recovery ±% RSD$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BCP</td>
<td>PNA</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>30</td>
<td>99.81±0.84</td>
</tr>
<tr>
<td>Lactose</td>
<td>40</td>
<td>98.38±0.62</td>
</tr>
<tr>
<td>Glucose</td>
<td>40</td>
<td>99.90±0.96</td>
</tr>
<tr>
<td>Talc</td>
<td>40</td>
<td>101.07±0.88</td>
</tr>
<tr>
<td>Cellulose</td>
<td>30</td>
<td>99.44±0.96</td>
</tr>
<tr>
<td>Starch</td>
<td>30</td>
<td>100.07±0.52</td>
</tr>
<tr>
<td>Dextrose</td>
<td>30</td>
<td>99.00±0.73</td>
</tr>
<tr>
<td>Sodium alginate</td>
<td>25</td>
<td>98.82±0.62</td>
</tr>
<tr>
<td>Calcium gluconate</td>
<td>30</td>
<td>99.49±0.59</td>
</tr>
<tr>
<td>Calcium dihydrogen orthophosphate</td>
<td>30</td>
<td>98.78±0.22</td>
</tr>
</tbody>
</table>

$^a$ Average of five determinations.
Table III: Determination of OXP in pharmaceutical preparations.

| Drug       | Label claim (mg) | %Recovery* ± SD, % and comparison with the reported method\(^{36}\) | %Recovery* ± SD, % and comparison with the reported method\(^{36}\) | %Recovery* ± SD, % and comparison with the reported method\(^{36}\) |
|------------|------------------|-----------------------------------------------------------------|-----------------------------------------------------------------|-----------------------------------------------------------------
|            |                  | Reported method\(^{36}\)                                        | Method A                                                        | Method B                                                         |
| Tablets    |                  |                                                                 |                                                                |                                                                |
| Oxecarb\(^a\) | 150              | 98.97±0.496                                                    | 99.95 ± 0.67                                                   | F = 1.82; t = 1.05                                               |
|           |                  |                                                                 |                                                                  |                                                                  |
|           |                  |                                                                 | 99.12 ±0.65                                                    | F = 1.71; t = 1.32                                               |
|           |                  |                                                                 | F = 1.71; t = 1.32                                               |                                                                  |
|           |                  | 98.78±0.318                                                   | 99.33 ± 0.325                                                   | F = 1.04; t = 1.11                                               |
|           |                  |                                                                 |                                                                  |                                                                  |
|           |                  |                                                                 | 101.2±0.432                                                    | F =1.88; t = 1.66                                               |
|           |                  |                                                                 |                                                                  |                                                                  |
|           | 300              | 98.78±0.318                                                   | 99.33 ± 0.325                                                   | F = 1.04; t = 1.11                                               |
|           |                  |                                                                 |                                                                  |                                                                  |
|           |                  |                                                                 | 101.2±0.432                                                    | F =1.88; t = 1.66                                               |
|           |                  |                                                                 |                                                                  |                                                                  |
| Oxetol\(^b\) | 150              | 99.89±0.484                                                    | 99.04 ± 0.520                                                   | F = 1.15; t = 1.15                                               |
|           |                  |                                                                 |                                                                  |                                                                  |
|           |                  |                                                                 | 99.28 ±0.392                                                    | F = 1.52; t = 1.85                                               |
|           |                  |                                                                 |                                                                  |                                                                  |
|           | 300              | 99.20±1.20                                                    | 98.34 ± 0.86                                                   | F = 1.94; t = 1.18                                               |
|           |                  |                                                                 |                                                                  |                                                                  |
|           |                  |                                                                 | 100.3±0.965                                                    | F = 1.54; t = 1.12                                               |
|           |                  |                                                                 |                                                                  |                                                                  |

* Average of six determinations.

\(^{a}\) Marketed by Cipla Ltd. India.

\(^{b}\) Marketed by Sun Pharmaceutical, India.

Tabulated t-value of 95% confidence limit level is 2.57 for n = 6

Tabulated F-value of 95% confidence limit level is 5.05 for n = 6

Scheme I: Structure of oxcarbazepine
Scheme II: Probable reaction mechanism for ion-pair complex formation of OXP with BCP.

Scheme III: Probable reaction mechanism for the formation of diazo-coupled product of OXP with PNA.
Fig. 1. Absorbance spectra of (a) reagent blank and (b) ion–association complex of OXP (3 µg/ml).

Fig. 2. Absorbance spectra of (a) reagent blank and (b) the diazotized coupled product of OXP (2 µg/ml).

Fig. 3. Effect of KCl-HCl buffer on absorbances of ion-association complex of OXP with BCP.
Fig. 4. Effect of BCP on the absorbances of ion-association complex of OXP with BCP.

Fig. 5. Effect of reagents on the absorbance of OXP-PNA diazotization complex.

Fig. 6. Effect of NaOH on the absorbances of OXP - PNA diazotization complex.
CONCLUSIONS
The proposed methods make use of simple reagents, which an ordinary analytical laboratory can afford. The sensitivity in terms of molar absorptivity and precision in terms of RSD of the methods are very suitable for the determination of OXP in pure and dosage forms. The methods are found to be free from interference by common additives and excipients. The applicability of the proposed methods have been well demonstrated. In views of this, the proposed methods could be considered for the analysis of OXP in quality control laboratories.

REFERENCES