RP-HPLC–PDA Method Development and Validation for the Estimation of Oxcarbazepine in Bulk and Formulations

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Summary. A simple, precise, rapid, and accurate liquid chromatography–mass spectrometry (LC–MS) compatible reversed phase high-performance liquid chromatography–photodiode array detection (RP-HPLC–PDA) method has been developed and validated for the estimation of oxcarbazepine (OXC) in bulk and tablet formulations. The chromatographic separation was achieved on Phenomenex C18 column (150 mm × 4.6 mm, 5.0 μm particle size) using the mobile phase comprising methanol–formic acid (0.02% v/v in water) in the ratio of 50:50 (v/v) at a flow rate of 1 mL min⁻¹, and OXC was eluted at 6.4 min. Quantification and linearity were achieved at 229 nm over the concentration range of 10–50 μg mL⁻¹, and the mean percentage of assay was found to be 100.03. The method was validated for specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), stability, and robustness as per the International Conference on Harmonisation (ICH) guidelines and it is suitable to be employed in quality control.

Key Words: oxcarbazepine, HPLC, validation, LC–MS compatible, Phenomenex C18 column

Introduction

Oxcarbazepine (OXC) is a newer antiepileptic drug and is a derivative of carbamazepine adding an extra oxygen atom to the benzyl carboxamide group. Compared to carbamazepine, OXC showed improved tolerability and fewer adverse effects [1–3]. The pharmacological activity of OXC occurs primarily through its 10-monohydroxy metabolite which causes 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. It is used as monotherapy or adjunctive therapy in the treatment of partial seizures in adults with epilepsy and as adjunctive therapy in the treatment of partial seizures in children ages 4–16 with epilepsy. OXC has recently been found associated with a greater enhancement in mood and reduction in anxiety symptoms than other drugs employed to treat epilepsy [4–5].
Most of the analytical methods were published for the analysis of OXC in plasma samples by liquid chromatography–mass spectrometry (LC–MS) and high-performance liquid chromatography (HPLC) methods [6–14]. Very few HPLC and UV spectrophotometric methods were reported for the estimation of OXC in bulk and formulations [15–17]. There were also methods that described forced degradation studies that were reported with the mobile phases containing phosphate salt buffers [18, 19] and are not compatible with LC–MS detection. So, the present investigation was aimed at developing a rapid, sensitive, specific, and validated HPLC–PDA method with LC conditions compatible with MS detection.

**Experimental**

**Reagents and Materials**

Pure standard of OXC was obtained as a gift sample from Novartis India Ltd., Mumbai, India. OXETOL® tablets (Sun Pharma Ltd., Mumbai, India) were procured from the local market. All the solvents and reagents were of HPLC grade.

**HPLC Instrument**

The HPLC used was a Shimadzu Prominence HPLC with LC-20AD series binary pump systems, SIL-20A HT autosampler, PDA SPD-M20A detector and DGU-20A degasser, and LC Solutions software was used to acquire and process the data. The column used was Phenomenex C18 column (150 mm × 4.6 mm, 5.0 μ).

**Chromatographic Conditions**

The mobile phase consisted of methanol–formic acid buffer (0.02% in water) in the ratio of 50:50 (v/v), which was previously filtered through 0.45 μm membrane filter. The flow rate was optimized to 1 mL min⁻¹ which yielded a column backpressure of 93–96 kgf. The run time was set to 10 min, and the detection was carried out at 229 nm. The volume of injection was 50 μL, and the column was equilibrated for at least 30 min with the mobile phase prior to the injection of the analyte. Separations were achieved at ambient temperature.
Preparation of the Standard Solution

A stock solution was prepared by dissolving 10 mg of standard in a 10-mL volumetric flask containing about 6 mL of methanol. The solution was sonicated, and the volume was made up to the mark with methanol to obtain OXC stock solution of 1 mg mL\(^{-1}\). The working standard solutions were prepared by diluting appropriate stock solution volumes with diluent (0.02% formic acid in water) in order to get the required concentrations.

Method Validation

Linearity

The linearity of OXC responses in the concentration range of 10 to 50 \(\mu\)g mL\(^{-1}\) was determined, and the data are given in Table I.

<table>
<thead>
<tr>
<th>Linearity ((n = 3))</th>
<th>Range 10–40 (\mu)g mL(^{-1})</th>
<th>(y = 168973x - 390697)</th>
<th>(R = 0.996)</th>
<th>(R^2 = 0.998)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy ((n = 3))</td>
<td>Level of addition</td>
<td>Mean percent recovery</td>
<td>%RSD</td>
<td></td>
</tr>
<tr>
<td>80%</td>
<td>100.85</td>
<td>0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>100.02</td>
<td>0.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120%</td>
<td>98.04</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precision ((n = 6))</td>
<td>Average peak area of the standard sample (%RSD)</td>
<td>Average peak area of the assay sample (%RSD)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>System precision</td>
<td>4602908 (0.783)</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Method precision</td>
<td>-</td>
<td>4566654 (0.798)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD percent assay ((n = 3))</td>
<td>100.03 ± 0.117</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Precision

Precision was measured in terms of repeatability of application and measurement. Study was carried out by injecting six replicates of the standard and sample at a concentration of 30 \(\mu\)g mL\(^{-1}\). The data are given in Table I and shown in Fig. 3.
Accuracy

Accuracy (recovery) of the method was determined by spiking 80%, 100%, and 120% of OXC working standard at a concentration of 30 μg mL⁻¹. Samples were injected in triplicate across its range according to the assay procedure, and the data are given in Table I and shown in Fig. 2.

Robustness

Method robustness was determined by analyzing the same sample at normal operating conditions and by changing some operating analytical conditions such as flow rate and wavelength. The data are given in Table II.

Table II. Robustness data for OXC

<table>
<thead>
<tr>
<th>Chromatographic parameters</th>
<th>Retention time</th>
<th>Theoretical plates</th>
<th>Capacity factor</th>
<th>Tailing factor</th>
<th>% Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate (mL min⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.95 (−5%)</td>
<td>6.775</td>
<td>25872.26</td>
<td>3.326</td>
<td>0.978</td>
<td>99.99 ± 0.022</td>
</tr>
<tr>
<td>1.00</td>
<td>6.458</td>
<td>25186.60</td>
<td>3.191</td>
<td>0.938</td>
<td>99.72 ± 0.083</td>
</tr>
<tr>
<td>1.05 (+5%)</td>
<td>6.155</td>
<td>24115.30</td>
<td>2.74</td>
<td>0.955</td>
<td>99.76 ± 0.241</td>
</tr>
<tr>
<td>Wavelength</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>228 (−1)</td>
<td>6.308</td>
<td>4228.79</td>
<td>3.387</td>
<td>1.046</td>
<td>98.72 ± 0.006</td>
</tr>
<tr>
<td>229</td>
<td>6.308</td>
<td>4335.38</td>
<td>3.386</td>
<td>1.045</td>
<td>100.95 ± 0.020</td>
</tr>
<tr>
<td>230 (+1)</td>
<td>6.308</td>
<td>4203.85</td>
<td>3.389</td>
<td>1.049</td>
<td>99.23 ± 0.029</td>
</tr>
</tbody>
</table>

LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) values were determined by the formulae LOD = 3.3 σ/m and LOQ = 10 σ/m (where σ is the standard deviation of the responses and m is mean of the slopes of the calibration curves).

Specificity

Specificity studies were carried for both pure drug and drug product by comparing the 3D plots with blank and placebo. Peak purity tests were also carried out to show that the analyte chromatographic peak is not attributable to more than one component as the impurities are not available by purity index data. The data are shown in Figs 2 and 4.
Method Development and Validation of Oxcarbazepine

Assay

Twenty tablets were weighed individually and finely powdered. A powder blend equivalent to 10 mg of OXC was transferred to a 10-mL volumetric flask containing about 6 mL of methanol, sonicated and made up to the mark with the same. The resulting solution was filtered through 0.22-μm nylon membrane filter to obtain a stock solution of 1 mg mL⁻¹. It was further diluted with diluent to get the required concentration (30 μg mL⁻¹). The solution was injected three times into the column. From the peak area obtained, the drug content in the tablets was quantified. Results are given in Table I.

Results and Discussion

Method Development

The chromatographic conditions were optimized with a view to develop a reliable and validated RP-HPLC–PDA method for analyzing OXC in bulk and tablet dosage forms. Trials were carried out with Phenomenex C₁₈ column (150 × 4.6 mm, 5 μm) using a mobile phase of methanol–formic acid (0.02% v/v) in different ratios at a flow rate of 1 mL min⁻¹ with the detector set at 229 nm, and separations were achieved at ambient temperature. Formic acid was chosen primarily to facilitate MS detection in the later stages.

Fig. 1. Representative OXC standard chromatogram with UV spectrum and peak purity index curve
of the method applicability. All the published methods used phosphate buffers in mobile phases and are not compatible for MS detection [14]. With 70:30 (v/v) mobile phase composition, OXC was eluted at 2.5 min, and peak tailing was observed. Further trials were made with 60:40 and 50:50 proportions, and finally, a mobile phase composition of 50:50 (v/v) was chosen based on peak shape, symmetry, etc., and the OXC was eluted at 6.4 min with this mobile phase composition. A sample chromatogram of OXC under these conditions is shown in Fig. 1 along with UV spectrum and peak purity index. Peak purity index was greater than 0.9999 which indicates the absence of impurities with OXC in bulk.

**Method Validation**

This method described above had been validated as per the International Conference on Harmonisation (ICH) guidelines [20], and the results were as follows.

**System Suitability**

Five injections of 20 µg mL\(^{-1}\) standard solution were given by increasing the injection volumes from 10 µL to 50 µL. The tailing factor was less than 2%, and the theoretical plate number was well above 2000. The %RSD obtained for all the parameters was less than 2%, and all these results indicate that the present method conditions were suitable for the analysis of OXC.

**Linearity and Range**

The linearity was tested within the concentration range of 10, 20, 30, 40, and 50 µg mL\(^{-1}\) of OXC. The range of concentrations was selected based on 80–120% of the test concentration (for assay, i.e., 30 µg mL\(^{-1}\)). The value of the regression coefficient (\(R^2\)) was 0.998, and the correlation coefficient (\(R\)) was 0.996. The regression data indicated that good linearity was obtained within the concentration range tested. Data are presented in Table I.

**Accuracy**

Accuracy of the method was examined by performing recovery studies by standard addition method for drug product as the exact components are unknown. For the drug substance, the analyte peak was evaluated by 3D plot of the chromatogram in order to confirm the existence of one component at 6.4 min elution time of OXC as the impurities were not available. The recovery of the added standard to the drug product sample was calcu-
lated, and it was found to be 98.04–100.85%, and the %RSD was less than 2 which indicates a good accuracy of the method to that of the label claim. The obtained recovery results are given in Table I. The 3D plot of the standard chromatogram is shown in Fig. 2 along with those of diluent, placebo, and drug product. From the 3D plots it is clear that the peak eluting at 6.4 min was one component, free of impurities.

**Fig. 2.** 3D plots of the chromatograms for diluent (A), placebo (B), OXC standard (C), and drug product (D)

**Precision**

Precision was measured in terms of repeatability of application and measurement.
System precision: Repeatability of standard application was carried out using six replicates of the same standard concentration (30 μg mL⁻¹). The results are given in Table I and shown in Fig. 3. The %RSD of the peak area was found to be less than 1 (0.783), indicating an acceptable level of precision for the analytical system. The overlay of the chromatograms as in Fig. 3 clearly supports the statement.

Method precision: Repeatability of sample measurement was carried out in six replicates of the same sample preparations from the same homogenous blend of marketed formulation (OXETOL® tablets). The results are given in Table I. The %RSD of the peak area was found to be less than 1 (0.798).

![Fig. 3. System precision data for OXC](image)

**LOD and LOQ**

The limits of detection (LOD) and quantification (LOQ) were determined by the method based on the standard deviation (s) and the slope (m) of the calibration plot, using the formulae, LOD = 3.3 s/m and LOQ = 10 s/m. The LOD and LOQ were found to be 0.40 μg mL⁻¹ and 1.22 μg mL⁻¹, respectively. The sensitivity was found to be enough to carry out the routine analysis of OXC in bulk and formulations.

**Robustness**

Method robustness was determined by analyzing the same sample at normal operating conditions and also by changing the operating analytical conditions like wavelength of detection and flow rate of the mobile phase. Percent assay values were also estimated under these changed conditions,
and the results are given in Table II. Changes in the flow rate slightly affected the retention times of the OXC. However, parameters like capacity factor, theoretical plate number, and assay were not changed and were within the limits. Similar results were obtained with the changed wavelength. These results indicated that the method is robust in terms of changed flow rate and wavelength.

**Specificity**

Blank, placebo solution and sample solution were analyzed individually as per the method to examine interference. From the base, shifted overlay of the chromatograms in Fig. 4 and the 3D plots of diluent, standard, placebo, and formulation are shown in Fig. 2. From these figures, it can be inferred that there were no coeluting or interfering peaks where OXC eluted. This shows that the peak of analyte was pure, and excipients in the formulation did not interfere with the analyte. The peak purity indices values of the standard and sample peaks were found to be greater than 0.999, and these results were in good agreement with the above results.

![Fig. 4. Specificity data for the chromatograms of diluent (A), placebo (B), OXC standard (C), and drug product (D)](image)

**Stability of the Analytical Solution**

The stability of the standard solution was assessed by analyzing at different time intervals up to 7 days stored at 4°C. The percentage variation was found to be less than 2% to the initial concentration at different time points,
and it was observed that the solution was stable for a period of 7 days when stored at 4°C.

**Analysis of the Marketed Formulation**

The developed method was applied to the assay of OXC in the tablets. The results given in *Table I* were found to be in good agreement with the label amount, and the error of the determination did not exceed the limit.

**Conclusion**

The proposed RP-HPLC–PDA method was validated fully as per the International Conference on Harmonisation (ICH) guidelines and found to be applicable for routine quality control analysis for the estimation of OXC. The results of linearity, precision, accuracy, and specificity proved to be within the limits. The method provides selective quantification of OXC without interference from blank and placebo. The proposed method is sensitive, reproducible, reliable, rapid, and specific and also has the unique advantage of LC conditions being compatible with MS detection. Therefore, this method can be employed in quality control to estimate the amount of OXC in bulk and dosage forms.

**Acknowledgments**

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**References**


Accepted by DA