Studies on forced degradation of oxcarbazepine using LC-MS compatible stability indicating RP-HPLC method

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ABSTRACT

The study describes development of a stability indicating RP - HPLC - PDA method for the estimation of Oxcarbazepine in bulk form with the mobile phase compatible to LC-MS analysis. The proposed method utilizes a Phenomenex C$_{18}$ column (150 mm x 4.6 mm, 5.0 µ particle size), at ambient temperature, optimum mobile phase consisted of methanol: formic acid (0.02%) in the ratio of 50:50 (v/v). The flow rate was optimized to 1mL/min and the detection was carried at 229 nm. The drug substance was exposed to hydrolytic, oxidative, thermal and photolytic stress conditions, and the resultant samples were analysed by the proposed method.

Keywords: Oxcarbazepine, Forced degradation studies, C$_{18}$ column, PDA detection

INTRODUCTION

Oxcarbazepine (OXC) is a newer antiepileptic drug which is a derivative of Carbamazepine adding an extra oxygen atom to the benzyl carboxamide group and 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 hyper excited neuronal membranes, inhibition of repetitive neuronal discharges, and diminution of propagation of synaptic impulses. It is used as mono therapy 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].

Stability indicating HPLC methods has been reported earlier for OXC. But all of the HPLC methods involved phosphate buffer in the mobile phase which is not incompatible with mass detection [6-10]. The objective of this work was to develop a simple MS compatible analytical LC procedure, which would serve as stability indicating method for OXC in bulk form.

EXPERIMENTAL SECTION

Reagents and Materials:
Pure standard of OXC was obtained as 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 Shimazdu’s Prominence HPLC with LC-20AD series binary pump systems, SIL-20A HT auto sampler, 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 C$_{18}$ column (150 mm x 4.6 mm, 5.0 µ).
Chromatographic conditions:
The mobile phase consisted of methanol : formic acid (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 1mL/min which yielded a column back pressure of 93-96 kgf. The run time was set to 30min and the detection was carried out at 229nm. The volume of injection was 20μL and the column was equilibrated for at least 30min with the mobile phase prior to the injection of the analyte.

Preparation of the stock solution:
A stock solution was prepared by dissolving 25 mg of standard in a 5 mL volumetric flask containing about 2 ml of acetonitrile. The solution was sonicated and the volume was made up to the mark with methanol to obtain a stock solution of 5mg/ml. The working standard solutions were prepared by suitably diluting it to get the required concentrations.

Procedure for Forced Degradation Study:
Forced degradation of the drug substance was carried out under thermolytic, photolytic, acid/base hydrolytic and oxidative stress conditions [11-14]. Thermal and photo degradation of the drug substance were carried out in solid state. After the degradation stock solutions were prepared by dissolving in methanol to achieve concentration of 1mg/mL. From these solutions aliquots were diluted with the same to achieve a concentration of 10μg/mL.

Acid and Base degradation
Forced degradation in acidic and basic media was performed by taking an aliquot of stock solution (5mg/mL in acetonitrile) in 10ml volumetric flask and diluting it up to the mark with 1.0N HCl to obtain a final concentration of 100μg/mL. The flask was kept in a controlled temperature oven at 50°C for 2 days. From this, 10μL was taken and diluted with methanol to obtain a final concentration of 10μg/mL. Similarly, forced degradation in basic media was performed using 0.1N NaOH instead of 1.0N HCl.

Oxidative degradation
Oxidative degradation was performed by taking an aliquot of stock solution (5mg/mL in acetonitrile) in 10 ml volumetric flask and diluted up to the mark with 3% (v/v) of hydrogen peroxide to obtain a final concentration of 100μg/ml. The flask was kept in a controlled temperature oven at 50°C for 2 days. From this, 10μL was taken and diluted with methanol to obtain a final concentration of 10μg/mL.

Thermal degradation
For thermal stress, samples of drug substance in solid state were placed in a controlled temperature oven at 70°C for 24 hours. A stock solution of 1mg/mL was prepared in methanol from the exposed drug substance. From this, 10μL was taken and diluted with methanol to obtain a final concentration of 10μg/mL.

Photolytic degradation
For photolytic stress, a sample of drug substance in solid state was irradiated with UV radiation (overall illumination of ≥210Wh/m² at room temperature with UV radiation), for 24 hours. A stock solution of 1mg/mL was prepared in methanol from the exposed drug substance. From this, 10μL was taken and diluted with methanol to obtain a final concentration of 10μg/mL.

RESULTS AND DISCUSSION
Trials were carried out with Phenomenex C18 column (150 x 4.6 mm, 5 μ) using a mobile phase of methanol: formic acid (0.02%v/v) in different ratios at a flow rate of 1ml/min 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 of the method applicability. 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 and symmetry etc and the OXC was eluted at 6.4 min with this mobile phase composition. The results were shown in Figs 1 & 2. Peak purity index was greater than 0.9999 and indicating the absence of impurities with OXC in bulk.
Intentional degradation was attempted to various stress conditions such as acid hydrolysis (using 1N HCl), base hydrolysis (using 0.1N NaOH), oxidative hydrolysis (using 3.0% H₂O₂), thermal degradation (heated at 70°C for 24 hours) and photolytic degradation (overall illumination of ≥210Wh/m² at 25°C for 24 hours with UV radiation at 320-400 nm), to evaluate the ability of the proposed method to separate OXC from its degradation products. It was observed that OXC degrades with acidic, basic, oxidative and thermal stress conditions.
Acid degradation
Under the stress degradation conditions with acid there was an additional peak at 7.308 min in the chromatogram apart from the drug peak at 6.243 min. The % degradation was found to be 13.53%. The results were shown in Figs 3-5 and given in Table-1.

Base degradation
Under the stress degradation conditions with sodium hydroxide it was observed that complete degradation of the drug occurred. Two new peaks appeared at 4.917 min and 5.22 min respectively whose spectra differed from that of the drug. The former peak occupied an area of 31.86% while the latter occupied 68.15%. The results were shown in Figs 6-8 and given in Table-1.
Oxidative degradation

Under the stress degradation conditions with hydrogen peroxide, there were two additional peaks at 5.382 min, 18.812 min with the complete absence of the drug peak. The percentage peak areas were 17.39% and 82.61% respectively. The results were shown in Figs 9-11 and given in Table-1.
Fig 9: Overlay of chromatograms of A-blank; B-untreated OXC; C-oxygen treated OXC

Fig 10: Chromatogram of hydrogen peroxide treated OXC in methanol (10 µg/mL) along with the UV spectrum of the degradation products O₁, O₂

Fig 11: 3D plot of hydrogen peroxide treated OXC in methanol (10 µg/mL)
**Thermal degradation**
Under the stress conditions of heat, there was an additional peak at 18.913 min, apart from the drug peak at 6.373 min. The degradation was found to be 26.59%. The results were shown in Figs 12-14 and given in Table-1.

![Thermal degradation graph](image)

**Fig 12:** Overlay of chromatograms of A-blank; B-untreated OXC; C-heat treated OXC

![Thermal degradation graph](image)

**Fig 13:** Chromatogram of heat treated OXC in methanol (10µg/mL) along with the UV spectrum of the degradation products, T₁, T₂

![Thermal degradation graph](image)

**Fig 14:** 3D plot of heat treated OXC in methanol (10µg/mL)
Photo degradation
Under the stress degradation conditions with UV light, there were no additional peaks apart from the drug peak at 6.336 min. Therefore it can inferred that the drug remained stable in the given conditions. The results were shown in Figs 15-17 and given in Table-1.

Fig 15: Overlay of chromatograms of A-blank; B-untreated OXC; C-light treated OXC

Fig 16: Chromatogram of light treated OXC in methanol (10µg/mL) along with the UV spectrum of the degradation product P

Fig 17: 3D plot of light treated OXC in methanol (10µg/mL)
Photodiode array detection was used as an evidence of the specificity of the method, and to evaluate the homogeneity of the drug peak. Chromatographic peak purity data was obtained from the spectral analysis report and a peak purity value greater than 0.999 indicated a homogenous peak. The peak purity values for the peaks in chromatograms of stressed samples were in the range of 0.9999 to 1.0000 for drug substance, indicating homogenous peaks and thus establishing the specificity of the method.

Table 1: Results of forced degradation study

<table>
<thead>
<tr>
<th>Type of Stress</th>
<th>Degradation products</th>
<th>Retention Time (min)</th>
<th>% area</th>
<th>Theoretical plate #</th>
<th>Peak purity index</th>
<th>Tailing factor</th>
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<tr>
<td>Acidic / 1 N HCl/ 50°C/ 48hr/ solution</td>
<td>A₁</td>
<td>6.24</td>
<td>86.47</td>
<td>2193.80</td>
<td>1.0000</td>
<td>1.68</td>
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<td></td>
<td>A₂</td>
<td>7.31</td>
<td>13.53</td>
<td>1891.86</td>
<td>0.9998</td>
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<tr>
<td>Basic / 1 N NaOH/ 50°C/ 48hr/ solution</td>
<td>B₁</td>
<td>4.92</td>
<td>31.86</td>
<td>13323.96</td>
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<tr>
<td></td>
<td>B₂</td>
<td>5.22</td>
<td>68.14</td>
<td>2628.48</td>
<td>0.9999</td>
<td>0.00</td>
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<tr>
<td>Oxidative / 3%H₂O₂/ 100°C/ 48hr/ solution</td>
<td>O₁</td>
<td>5.38</td>
<td>17.39</td>
<td>2538.2</td>
<td>0.9999</td>
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<tr>
<td></td>
<td>O₂</td>
<td>18.81</td>
<td>82.61</td>
<td>7792.29</td>
<td>0.9999</td>
<td>1.36</td>
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<tr>
<td>Thermal / 70°C/ 24hr/ solid</td>
<td>T₁</td>
<td>6.37</td>
<td>73.45</td>
<td>2413.99</td>
<td>1.0000</td>
<td>1.17</td>
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<tr>
<td></td>
<td>T₂</td>
<td>18.91</td>
<td>26.54</td>
<td>3188.37</td>
<td>1.0000</td>
<td>1.33</td>
</tr>
<tr>
<td>Photo / RT/ 24hr/ solid</td>
<td>P</td>
<td>6.34</td>
<td>100</td>
<td>2400.83</td>
<td>1.0000</td>
<td>1.18</td>
</tr>
</tbody>
</table>

CONCLUSION

Though no attempts have been made to identify the degradation products, the described LC conditions can be used in stability indicating HPLC-PDA method for the analysis of OXC.

Acknowledgements
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REFERENCES