Analytical method development & validation of Eperisone Hydrochloride and Diclofenac Sodium in Rapisone D SR Capsules by RP-HPLC

INTRODUCTION


Figure 1: Structure of Eperisone Hydrochloride
MATERIAL AND METHODS

2.1. Apparatus

A WATERS 2690 HPLC with empower software & waters 996 PDA detector, a SYSTRONICS 119 Spectrophotometer double beam UV-Visible spectrophotometer, digital pH meter DPH 500 (Global), Vacuum filtration Unit EPCOS, a Wensar PGB 100 analytical balance & an ultrasonic bath (Frontline FS 4) was used in the study.

2.2. Reagents and Materials

DIC bulk powder was kindly gifted by Dr. Reddys laboratories, Hyderabad, Andhra Pradesh, India. EPE bulk powder was kindly gifted by Sun Pharmaceuticals Ltd., Mumbai. HPLC grade solvents Methanol & Water obtained from Mercks & Acetonitrile obtained from Molychem, Disodium hydrogen phosphate obtained from Finer chemicals Ltd., Orthophosphoric acid obtained from Mercks and Whatman filter paper no. 45 (Millipore, USA) were used in the study. RAPISONE D SR capsules containing DIC (100mg) & EPE (150mg) were purchased from local pharmacy.

2.3. Chromatographic conditions

Chromatographic separation was performed with gradient elution. Initially mobile phase was tried with Methanol: Water, Acetonitrile: Water, Acetonitrile: Methanol & then finally with Phosphate Buffer: Acetonitrile: Methanol at various pH conditions. The following optimised parameters were used as a final method for the simultaneous estimation of Diclofenac Sodium and Eperisone Hydrochloride.

<table>
<thead>
<tr>
<th>Table 1: Optimized Chromatographic Conditions</th>
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</thead>
<tbody>
<tr>
<td><strong>Column</strong></td>
</tr>
<tr>
<td><strong>Buffer</strong></td>
</tr>
<tr>
<td><strong>Mobile phase</strong></td>
</tr>
<tr>
<td><strong>Flow rate</strong></td>
</tr>
<tr>
<td><strong>Wavelength</strong></td>
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<tr>
<td><strong>Injection volume</strong></td>
</tr>
<tr>
<td><strong>Run time</strong></td>
</tr>
</tbody>
</table>

2.4. Preparation of standard stock solutions

Accurately weighed amount of 100mg of Diclofenac Sodium & 150 mg of Eperisone Hydrochloride were taken to a 100 mL cleaned and dried volumetric flask. This was then diluted with 70 mL of diluent and was sonicated. The volume was made to 100 mL with the same solvent. This was marked and labeled as Stock solution.

2.5. Preparation of sample solution (dosage form):

Take 20 RAPISONE D SR capsules & weigh them (w1). Now remove the shells of capsules & weigh empty shells (w2). Calculate the weight of the capsule contents by subtracting the empty shells weight from capsules weight \( w_s = (w_1 - w_2) \). Crush the contents into powder form. Calculate a weight equivalent to 100mg of Diclofenac Sodium & 150 mg of Eperisone Hydrochloride & take into a 100 mL volumetric flask. Add 50 mL of Mobile Phase & sonicate for 10 min. Filter under vacuum filtration unit & sonicate for 10 min. The final concentrations are 40 µg/mL of Diclofenac Sodium & 60 µg/mL of Eperisone Hydrochloride.

2.6. Validation of the proposed method

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The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines [5-10].

2.6.1. Linearity & range:
The calibration curves were plotted over a concentration range of 20-80 µg/mL for DIC and 30-120 µg/mL for EPE. Accurately measured stock solution of 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 & 0.8 mL were transferred to a series of 10 mL of volumetric flasks and diluted to the mark with mobile phase such that the final concentrations are 20, 30, 40, 50, 60, 70 & 80 µg/mL for DIC & 30, 45, 60, 75, 80, 105 & 120 µg/mL for EPE. Inject each into the chromatographic system and measure the peak area. Plot a calibration graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

2.6.2. Intra & Inter-Day Precision:
From the stock solution a concentration of 40 µg/mL of DIC & 60 µg/mL of EPE prepared by dissolving 0.4 mL in 10 mL mobile phase in a 10 mL volumetric flask. Both intra-day & inter-day precision was performed by injecting five injections at regular intervals on same day & on five alternate days over a period of 10 days. The results are reported in the form of % RSD.

2.6.3. Accuracy
The accuracy of the method was determined by calculating % recovery of EPE and DIC by analyzing 50, 100 and 150 % level sample solutions of EPE and DIC. Three injections were done for each sample & results are obtained in % mean recovery.

2.6.4. Robustness:
From stock solution 0.4 mL was diluted to 10 mL with the mobile phase to get 40 µg/mL of DIC & 60 µg/mL of EPE. Robustness performed by both flow rate variation (0.8, 1 & 1.2 mL/min) & temperature variation (20°, 25° & 30 °C). Two injections for each parameter variation were done & the acceptance criteria for tailing factor & USP plate count were determined.

2.6.5. Ruggedness:
Ruggedness performed by analyst variation. A concentration of 40 µg/mL of DIC & 60 µg/mL of EPE was prepared from stock solution & analysed by using two different analyst. Five injections were done by each analyst & the results are reported in the form of % recovery. Limit of detection and Limit of quantification

LOD and LOQ values decide about the sensitivity of the method. LOD is the lowest detectable concentration of the analyte, while LOQ is the lowest quantifiable concentration. The LOD & LOQ are calculated from the calibration curve by using the formulas (Eq.1 & Eq.2)

\[\text{LOD} = 3.3 \times \sigma / S \quad \text{Eq.1}\]
\[\text{LOQ} = 10 \times \sigma / S \quad \text{Eq.2}\]

Where, \(\sigma\) = the standard deviation and \(S\) = slope of the calibration curve

RESULTS AND DISCUSSION

The standard solutions of EPE and DIC were scanned separately in the UV range and the maximum separation of both drugs was obtained at 261 nm with good resolution. The optimized chromatogram was shown in figure 3 & system suitability parameters are given in table 2. Linear correlation was obtained between peak areas and concentrations of EPE and DIC in the concentration ranges of 30-120 µg/mL & 20-80 µg/mL, respectively. The % RSD values of EPE & DIC was found to be 0.92 & 0.45% for inter-day precision and 0.26 & 0.38% for intra-day precision respectively. Relative standard deviation was less than 2%, which indicates that proposed method is precise. The mean % recoveries were 99.03 and 100.9% for DIC and EPE, respectively, which indicates that the proposed method is highly accurate. The LOD & LOQ results indicate that the method is sensitive. The proposed method was analysed by different analysts & the results in % recovery indicates the method can be rugged. All the above results are given in table 3. The method is unaffected by small deliberate changes like change in flow rate & change in temperature & the results obtained are given in table 4. The proposed
The validated method was successfully applied to determine EPE and DIC in their capsule dosage form. The results obtained for EPE and DIC were comparable with the corresponding labeled amounts (Table 5). No interference of the excipients with the peaks of interest appeared; hence the proposed method is applicable for the routine analysis of EPE and DIC in pharmaceutical dosage forms.

3. Chromatogram of Diclofenac Sodium & Eperisone Hydrochloride

Table 2: System suitability parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Diclofenac sodium</th>
<th>Eperisone hydrochloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution</td>
<td>6.755598</td>
<td></td>
</tr>
<tr>
<td>Retention time (min)</td>
<td>2.216</td>
<td>3.682</td>
</tr>
<tr>
<td>No. of Theoretical plates</td>
<td>9440</td>
<td>10954</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.02</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Fig.4: Calibration graph of Eperisone hydrochloride

Fig.5: Calibration graph of Diclofenac sodium
### Table 3: Representation of Results of Various Parameters of EPE & DIC

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Eperisone Hydrochloride</th>
<th>Diclofenac Sodium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity &amp; Range</td>
<td>30-120ppm</td>
<td>20-80ppm</td>
</tr>
<tr>
<td>Regression equation</td>
<td>$y = 19514x - 19229$</td>
<td>$y = 5688.x - 2788.$</td>
</tr>
<tr>
<td>Correlation coeffiient</td>
<td>0.999</td>
<td>0.999</td>
</tr>
<tr>
<td>Precision (%RSD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interday</td>
<td>0.92</td>
<td>0.45</td>
</tr>
<tr>
<td>Intraday</td>
<td>0.26</td>
<td>0.38</td>
</tr>
<tr>
<td>Accuracy (%Recovery)</td>
<td>100.9%</td>
<td>99.03%</td>
</tr>
<tr>
<td>Ruggedness (%Recovery)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Analyst 1</td>
<td>0.57</td>
<td>0.77</td>
</tr>
<tr>
<td>Analyst 2</td>
<td>0.36</td>
<td>0.77</td>
</tr>
<tr>
<td>LOD</td>
<td>0.0006</td>
<td>0.0012</td>
</tr>
<tr>
<td>LOQ</td>
<td>0.0018</td>
<td>0.0038</td>
</tr>
</tbody>
</table>

### Table 4: Results of Robustness for Diclofenac Sodium & Eperisone Hydrochloride

<table>
<thead>
<tr>
<th>Robustness Parameters</th>
<th>Diclofenac sodium</th>
<th>Eperisone hydrochloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>USP plate count</td>
<td>USP tailing</td>
<td>USP plate count</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>9504.88</td>
<td>1.057</td>
</tr>
<tr>
<td></td>
<td>10962.19</td>
<td>1.535</td>
</tr>
<tr>
<td>25</td>
<td>9831.36</td>
<td>1.171</td>
</tr>
<tr>
<td></td>
<td>10974.48</td>
<td>1.578</td>
</tr>
<tr>
<td>30</td>
<td>9786.33</td>
<td>1.155</td>
</tr>
<tr>
<td></td>
<td>10942.93</td>
<td>1.552</td>
</tr>
<tr>
<td>Flow Rate (ml/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.8</td>
<td>9504.88</td>
<td>1.057</td>
</tr>
<tr>
<td></td>
<td>10962.19</td>
<td>1.535</td>
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<tr>
<td></td>
<td>10942.93</td>
<td>1.552</td>
</tr>
</tbody>
</table>

### Table 5: Application of method for the analysis of commercial formulations

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Drug</th>
<th>Mean (% w/w recovery)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAPISONE D SR capsules</td>
<td>EPE</td>
<td>100.1</td>
</tr>
<tr>
<td></td>
<td>DIC</td>
<td>99.02</td>
</tr>
</tbody>
</table>

### CONCLUSION

A RP-HPLC method was developed for the simultaneous estimation of Diclofenac Sodium & Eperisone Hydrochloride in RAPISONE D SR capsules & validated as per ICH guidelines. The developed method utilized a set of conditions which are not reported previously including a high pH mobile phase with gradient elution & does not required any special separation techniques. The method exhibited good selectivity & sensitivity. The developed method is successfully applied to the analysis of dosage form.

### ACKNOWLEDGEMENT

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### REFERENCES

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10. The International Conference on Harmonization, Q2 (R1), Validation of Analytical Procedure, Text and Methodology, 2005.


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