Validated RP- HPLC Method for the Assay of Etoricoxib (A Non-Steroidal Anti-Inflammatory Drug) in Pharmaceutical Dosage Forms

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Abstract: A simple, accurate, sensitive and reproducible reverse phase high performance liquid chromatographic method has been developed for the quantitative determination of Etoricoxib in pharmaceutical dosage forms. The assay was performed on Hypersil ODS C-18 (250 x 4.6 mm., 5μm particle size) column using acetonitrile and potassium dihydrogen phosphate buffer (pH 4.2) (46:54 % v/v) as mobile phase with UV detection at 280 nm (flow rate 1.2 ml/min). Bromhexine was used as an internal standard. Quantization was achieved by measurement of the peak area ratio of the drug to the internal standard. The limit of detection (LOD) and the limit of quantification (LOQ) were 0.0704 μg ml⁻¹ and 0.2134 μg ml⁻¹ respectively. Each analysis required no longer than 10 minutes. The calibration curve was linear over the concentration range from 0.5-85.0 μg ml⁻¹. The retention times of Etoricoxib and Bromhexine were found to be 3.083 and 7.631 minutes respectively. The proposed method was validated according to the ICH guidelines and can be used successfully to analyse marketed formulations.

Key words: Etoricoxib, Bromohexine, Acetonitrile, RP-HPLC, ICH.

Introduction

Etoricoxib¹ (ETC) (Figure 1) is a new non-steroidal anti-inflammatory drug (NSAID) which is chemically 5-chloro-6’-methyl-3-[4-(methylsulphonyl) phenyl]-2,3’-bipyridine. It inhibits the synthesis of prostaglandins by inhibiting the activity²,³ of the enzyme, cyclooxygenase-2. It has highest COX-2 selectivity and better safety profile. It is active at low dose and has less gastric toxicity⁴.
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**Figure 1.** Chemical Structure of Etoricoxib (ETC).

Etoricoxib is preferred over conventional NSAIDs as they may lead to serious gastrointestinal complications such as ulcer, severe bleeding and perforation resulting in hospitalization and even death\(^5\). It is mainly used for the osteoarthritis, rheumatoid arthritis and acute gouty arthritis\(^6-8\). ETC administered as a tablet is rapidly and completely absorbed and available; the absolute bioavailability is estimated to be 100% and is metabolized extensively via oxidation 6' -methyl hydroxylation and 1'-N-oxidation and the metabolites are excreted largely in the urine\(^9\). A high fat meal decreased the rate of absorption without effecting the extent of absorption of ETC; therefore it can be dosed irrespective of food. The dose proportionality of oral ETC in healthy volunteers as well as the Single- and Multiple-Dose Pharmacokinetics of ETC was also studied\(^10-11\).

ETC was determined in human plasma\(^12-15\) using HPLC-MS/MS. HPLC\(^16-17\), spectrophotometric\(^18\) and capillary zone electrophoresis\(^19\) were also reported for the determination of ETC. ETC was determined in serum and synovial fluid of patients with inflammatory arthritis by ultra performance liquid chromatography–inductively coupled plasma mass spectroscopy and quadrupole time-of-flight mass spectrometry and also by HPTLC\(^21\) method using chloroform: methanol : toluene (4:2:4 v/v) mobile phase. Matthews et al\(^22\) isolated and characterized the photolytic products using HPLC-UV-fluorescence, HPLC-MS/MS, NMR and HPLC-NMR showing that Etoricoxib undergoes a photocyclization reaction when irradiated with UV light (254 nm) leading to the formation of two major isometric photocyclazation products. In the present study the authors proposed a relatively simple, reliable reproducible RP-HPLC method for the determination of Etoricoxib in pharmaceutical dosage forms and validated as per the ICH\(^23\) guidelines.

**Experimental**

**Chemicals and Reagents**

Pharmaceutical grade Etoricoxib (ETC) drug was supplied as gift sample from Ranbaxy Research Laboratories Ltd. (Haryana, India). Etoricoxib is available in India as tablets with brand names ETOZOX (Cipla), KINGCOX (Cadila HC) and ETROBAX (Ranbaxy) (60, 90 and 120 mg per tablet) and were purchased from the local market. HPLC grade acetonitrile (Merck) was used and all other chemicals were of analytical grade.
Instrument used
Quantitative HPLC was performed on a binary gradient HPLC with Shimadzu LC-10AT and LC-10AT VP Series HPLC pumps, with a 20µL sample injection loop (manual) and SPD 10AT series UV-Visible detector. The output signal was monitored and integrated using Shimadzu Class-VP Version 6.12 SP1 Software. A Hypersil ODS C₁₈ column (250mm × 4.6mm, 5µm) was used for separation. Afcoset analytical electronic balance was used.

Selection of Mobile Phase
The mobile phase acetonitrile: potassium dihydrogen phosphate buffer (0.05 M) was prepared 46:54 % v/v at a flow rate of 1.2 ml/min produces peaks with good resolution for Etoricoxib and Bromohexine (Internal standard).

Preparation of standard drug solution
Stock solution of Etoricoxib (ETC) (1mg/ml) was prepared by dissolving 25 mg of Etoricoxib in 25 ml of volumetric flask containing 10 ml of mobile phase. The solution was sonicated for about 20 minutes and then made up to volume with mobile phase. Working standard solutions of Etoricoxib were prepared by suitable dilution of the stock solution with mobile phase. Similarly stock solution of internal standard was prepared by dissolving 25 mg of Bromohexine in 25 ml of volumetric flask containing 10 ml of mobile phase, sonicated for 20 min. then made up to the volume with mobile phase.

Linearity
A series of standard solutions of Etoricoxib were prepared by taking suitable aliquots of drug solution (0.5-85 µg ml⁻¹) from the stock solution and spiked with internal standard solution (Bromohexine, IS) (10 µg ml⁻¹) and the volume was made up to 10 ml with mobile phase in a 10 ml volumetric flask. 20 µl of each of ETC solution spiked with internal standard (Bromohexine, 10 µg/mL) were injected into the HPLC system thrice and the chromatograms were recorded. A typical chromatogram of ETC was shown in Figure 2. The peak area obtained for ETC and the internal standard solutions were recorded and the peak area ratio of ETC to internal standard was calculated for all the solutions. A calibration curve was constructed by taking concentration of ETC on x-axis and the corresponding peak area ratios on y-axis (Figure 3).

![Figure 2. Representative Chromatogram of pure Etoricoxib (20 µg ml⁻¹) (Rt = 3.083 mins) with Bromohexine (IS) (10 µg ml⁻¹) (Rt = 7.631 mins).](image-url)
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Figure 3: Calibration curve of Etoricoxib.

Assay procedure for marketed formulations

Twenty tablets were weighed, finely powdered and powder equivalent to 25 mg of ETC was weighed accurately and extracted with mobile phase in a 25ml volumetric flask and sonicated. This solution was filtered through 0.45µm filter paper and diluted further with the mobile phase as per the requirement. All determinations were carried out in triplicate. 20 µl of the above solutions from different brands of formulation spiked with internal standard were injected into the HPLC system and the chromatograms were recorded (Figure 4 and 5). The assay results are shown in Table 1.

Figure 4. Representative Chromatogram of Etoricoxib Formulation (ETOZOX - 60 mg) (20 µg ml$^{-1}$) (Rt = 3.101 mins) with Bromohexine (IS) (10 µg ml$^{-1}$) Rt = 7.652 mins).

Figure 5: Representative Chromatogram of Etoricoxib Formulation (ETROBAX - 60 mg) (20 µg ml$^{-1}$) (Rt = 3.097 mins) with Bromohexine (IS) (10 µg ml$^{-1}$) (Rt = 7.645 mins).
Table 1: Analysis of commercial formulation.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Tablet brand</th>
<th>Labeled amount (mg)</th>
<th>Amount obtained by proposed method* (mg) ± SD</th>
<th>% Recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ETOZOX</td>
<td>60</td>
<td>59.79 ± 0.0156</td>
<td>99.65</td>
<td>0.252</td>
</tr>
<tr>
<td></td>
<td>ETROBAX</td>
<td>60</td>
<td>59.84 ± 0.0231</td>
<td>99.73</td>
<td>0.354</td>
</tr>
<tr>
<td>2</td>
<td>KINGCOX</td>
<td>60</td>
<td>59.68 ± 0.0534</td>
<td>99.47</td>
<td>0.113</td>
</tr>
</tbody>
</table>

*Each value is average of five determinations ± Standard deviation (SD).

Method validation

Precision
The precision (Inter-day and Intra-day precision) was ascertained by actual determination of three replicates (n=3) at three different levels (20, 40 and 60 µg/ml) of ETC with 10 µg/ml internal standard. 20 µl of these solutions were injected in to the HPLC system and the peak area ratio of ETC to the internal standard was noted and the percent relative standard deviation was calculated (Table 2).

Table 2: Intra-day and inter-day precision for Etoricoxib (n = 3).

<table>
<thead>
<tr>
<th>Conc. (µg/mL)</th>
<th>Intra-day precision</th>
<th>Inter-day precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean peak area Ratio (Drug / I.S)</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>20</td>
<td>2.01331 ± 0.00153</td>
<td>0.0759</td>
</tr>
<tr>
<td>40</td>
<td>3.99566 ± 0.00252</td>
<td>0.0629</td>
</tr>
<tr>
<td>60</td>
<td>6.05766 ± 0.0153</td>
<td>0.0252</td>
</tr>
</tbody>
</table>

*Each value is average of three determinations ± Standard deviation (SD).

Accuracy
To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, 120%) of ETC sample solutions to the pre-analyzed ETC formulation solution (within the linearity range) spiked with the internal standard and the percentage recovery values were calculated (Table 3).
Table 3: Accuracy study of Etoricoxib (ETC).

<table>
<thead>
<tr>
<th>Spike level %</th>
<th>Pure drug</th>
<th>Formulation</th>
<th>Concentration (µg ml⁻¹)</th>
<th>Total conc. (µg ml⁻¹)</th>
<th>% Recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>16</td>
<td>20</td>
<td>36</td>
<td>99.48</td>
<td>Mean = 99.733%</td>
<td>SD = 0.219622</td>
</tr>
<tr>
<td>80</td>
<td>16</td>
<td>20</td>
<td>36</td>
<td>99.87</td>
<td>% RSD = 0.2202</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>20</td>
<td>20</td>
<td>40</td>
<td>98.94</td>
<td>Mean = 99.523%</td>
<td>SD = 0.505799</td>
</tr>
<tr>
<td>100</td>
<td>20</td>
<td>20</td>
<td>40</td>
<td>99.79</td>
<td>% RSD = 0.5082</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>24</td>
<td>20</td>
<td>44</td>
<td>99.97</td>
<td>Mean = 99.840%</td>
<td>SD = 0.353412</td>
</tr>
<tr>
<td>120</td>
<td>24</td>
<td>20</td>
<td>44</td>
<td>100.11</td>
<td>% RSD = 0.35398</td>
<td></td>
</tr>
</tbody>
</table>

Results and Discussion

Etoricoxib obeys beer’s law in the concentration range of 0.5-85 µg/ml with regression equation 0.101 X - 0.006 and correlation coefficient 0.999. The LOD and LOQ were found to be 0.0704 and 0.2134 µg/mL. The marketed formulations were analyzed and the percentage recovery values were in between 99.47-99.65 % (Table 1). The method is precise (Table 2) as well as accurate (Table 3) as the RSD is less than 2%. The theoretical plates are found to be 5384 (>2000) and the tailing factor is 1.1 (<1.5).

Conclusion

The proposed method was found to be simple, precise, accurate, rapid and economical for the determination Etoricoxib in pharmaceutical dosage forms. The sample recoveries in all formulations were in good agreement with their respective label claims suggesting that no interference of excipients from the formulation. Hence this method can be conveniently adopted for routine analysis of Etoricoxib.

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References

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