RP HPLC Method Development for Simultaneous Estimation of Etoricoxib and Thiocolchicoside

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A simple, specific and accurate reverse phase high performance liquid chromatography (RP-HPLC) method was developed and validated for simultaneous estimation of Etoricoxib and Thiocolchicoside in bulk and solid dosage form. Separation was achieved by Zorbax C-18 analytical column having dimension (250mm * 4.6 mm i.d 5.0 µm) using methanol and water (60:40) as mobile phase and flow rate was 0.7ml/min. The detection was carried out at 283 nm wavelength using UV detector. The total chromatographic sample time per analysis was about 14.0 minutes with thiocolchicoside eluted at retention time 3.523 min and etoricoxib eluted at retention time 9.627 min. The method was validated for accuracy, precision, specificity, rapid, reliable and reproducible. LOD and LOQ value for etoricoxib and thiocolchicoside were found to be 0.332, 0.996 ppm and 0976, 0.928 ppm respectively. Regression Equation for Etoricoxib was y = 0.006x + 0.149 and regression equation for thiocolchicoside was Y = 0.030x + 0.086. As the run time was increased the retention time was decreased, so the method is simple and economical and can be adopted by regular quality control in industries and also in research laboratories.

Keywords: Etoricoxib; thiocolchicoside; RP-HPLC; validation.

1. INTRODUCTION

Etoricoxib is chemically a 5-Chloro-2-(6-Methyl Pyridine-3-yl-3-(4-methylsulfonylphenyl) pyridine. It is a non steroidal anti inflammatory drug and also used in the treatment of gout or arthritis. [1] Gout or arthritis refers to the pain that occurs when there is too much accumulation of uric acid

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in the blood. It is selective COX 2 inhibitors that decrease the GI toxicity. [2,3] There are several methods reported for the analysis of Etoricoxib in pharmaceutical dosage form as well as biological fluids and tissues that is spectroscopic methods, biological methods, HPLC etc. [3,4]

Thiocolchicoside is chemically N-[1,2-dimethoxy-
10-methylsulphanyl-9-oxo-3-[3,4,5-
trihydroxy-6-(hydroxymethyl)oxan-2-y]oxy-6,7-dihydro-5H-
benzo[a]heptalen-7-yl]acetamide. It has muscle
relaxant property and is used in the symptomatic
treatment of painful muscle spasm. It also has a
powerful convulscent activity and thus should not
be used in seizure prone individuals [5-7].

Literature Survey reveals [8-12] that there were
no specific method reported for simultaneous
estimation of etoricoxib and thiocolchicoside,
hence the current method aims for simultaneous
estimation of etoricoxib and thiocolchicoside by
simple RP-HPLC Method. This method is
validated and optimized as per ICH guidelines.

2. CHEMICALS AND REAGENTS

All the solvents used for method development
were of HPLC grade and chemicals were of
analytical grade. Methanol was obtained from
Merck, HPLC water was obtained from Ambika
Laboratories. All the solvents and solution were
filtered through membrane filter (Millipore Millex
FH, filter units, Durapore PVDF, polyethylene
0.22 µm pore size). All the solvents were
degassed before use. Pure standard of
Etoricoxib were received as gift sample form
Medipol Pharmaceuticals Pvt. Ltd Baddi and
pure standard of Thiocolchicoside were
received as a gift sample form Ashwariya Life
Sciences Pvt Ltd Baddi HP.

Instrumentation and Chromatographic
Condition: Chromatography was performed with
an Agilent Techniques 1220 compact LC
(Germany) gradient pump, a variable wavelength
detector and a rheodyne 9013 injector with 20 µl
loop, Zorbax C-18 Column (2.5cm * 4.6 mm 5µm
particles were used for chromatographic
separation under suitable condition. Detection
was carried out at 283nm and software used was
Open Lab Software .The mobile phase was a
60:40 (v/v) mixture of methanol and water. The
mobile phase was filtered through 0.45 µm
membrane filter and was filtered before use. The
flow rate was maintained at 0.7ml/min. The
column temperature was maintained at ambient
temperature. The mobile phase was used as
diluent. The UV detector was made at 283nm for
both drugs. The injection volume was 20µl and
run time was 14 min. The peak purity was
checked with PDA Detector.

Preparation of Standard Solution and
Calibration: Weighed accurately 40 mg of
thiocolchicoside working standard (stock solution
A) and 60mg of etoricoxib working standard
(stock solution B) were transferred to 100ml
volumetric flask. Sonication was used to
dissolve the content and make up the volume with
diluents (methanol and water 60:40 (v/v)). Then
1ml of stock solution A and 10 ml of stock
solution B were diluted to 100ml with diluents.
Stock solution were diluted with mobile phase to
give working standard solution containing 2 to 20
ppm of thiocolchicoside and 20 to 200 ppm of
Etoricoxib. The standard solution were injected
for construction of calibration plots by plotting
drug peak-area ratio (y) for each of drug against
concentration (x). Analysis was performed at
ambient temperature. The retention time of
etoricoxib and thiocolchicoside under these
conditions were 9.627 and 3.523 min.
respectively.

Assay Procedure: Weighed accurately 20
tablets were used to calculate the average
weight. After crushing tablet powder equivalent to
500 mg of Etoricoxib and 40 mg of
Thiocolchicoside were transferred to 250ml
volumetric flask. About 200ml of diluent was
added and was sonicated for 20 minutes with
continuous shaking (maintaining the temperature
of sonicator below 25°C. The volume was made
upto the mark with diluents and was mixed. The
solution was filtered through 0.22 µm PVDA filter,
filtrate was collected and first few milliliter of
filtrate was discarded. Five ml of that solution
was taken and was added to 100 ml diluent. A
typical chromatogram is obtained as shown in
Fig. 1.

3. METHOD DEVELOPMENT

The objective of this study was to develop
simultaneous estimation of two components
under isocratic conditions. The mobile phase
used was a 60:40 (v/v) mixture of freshly
prepared methanol and water proved to be more
effective than other mixtures used for separation.
Different flow rates 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and
0.7 ml/min. The flow rate of 0.7 ml was selected
because of better resolution of peaks. These
chromatographic conditions were found best to
provide the resolution between Etoricoxib and
Thiocolchicoside in the time of 9.627 and 3.523
min. respectively. The wavelength of detection
was 283 nm and at this wavelength no interfering compound eluted at the retention time of drugs.

**Method Validation:** The method was validated according to International Conference of Hormonization (ICH) for validation of analytical procedure. The parameters used to validate method of analysis were linearity range, accuracy, precision, limit of detection (LOD), Limit of Quantification (LOQ), specificity and robustness.

By using three series of standard solution the calibration curve was constructed. Linearity was obtained in the concentration range of 10 to 200 ppm for etoricoxib and in the range of 2 to 20 for thiocolchicoside with a correlation coefficient of 0.980 and 0.991 respectively. The equation of linear regression and statistical data is represented in Table 1. Linearity of calibration curve is also validated by high value of correlation coefficient. The limit of Detection (LOD) and Limit of Quantification (LOQ) is represented in Table 1. Low value of LOD and LOQ means that the method is sensitive. There was no interference in peaks of drug and excipients present in the marketed formulation was also determined by the specificity. Thus the proposed method was useful to quantify the etoricoxib and thiocolchicoside in different pharmaceutical formulation.

By analyzing the three concentration of bulk drug on three different ways the precision was determined. The accuracy of drug was evaluated by recovery study as evaluated in Table 3. By standard addition method recovery study was also completed. A known concentration of working standard was added to fixed concentration of pre analyzed test solution. Recovery study was also very close to 100 % which proposed the suitability and accuracy of drug product.

![Structure of Etoricoxib](image1)

![Structure of Thiocolchicoside](image2)

![Typical Chromatogram of etoricoxib and thiocolchicosde](image3)

<table>
<thead>
<tr>
<th>VWD: Signal A, 254 nm</th>
<th>Retention Time</th>
<th>Area</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>thiocolchicoside</td>
<td>3.523</td>
<td>392555809</td>
<td>52.12</td>
</tr>
<tr>
<td>etoricoxib</td>
<td>9.697</td>
<td>360678512</td>
<td>47.88</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td></td>
<td>753234321</td>
<td>100.00</td>
</tr>
</tbody>
</table>

**Fig. 1. Typical Chromatogram of etoricoxib and thiocolchicosde**
### Table 1. Statistical Data for calibration of etoricoxib and thiocolchicoside

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Etoricoxib</th>
<th>Thiocolchicoside</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity (µg/ml)</td>
<td>10 to 200</td>
<td>2 to 20</td>
</tr>
<tr>
<td>Regression Equation</td>
<td>$y = 0.006x + 0.149$</td>
<td>$y = 0.030x + 0.086$</td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>0.992</td>
<td>0.999</td>
</tr>
<tr>
<td>Slope</td>
<td>0.006</td>
<td>0.030</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.149</td>
<td>0.086</td>
</tr>
<tr>
<td>Limit of Detection (µg/ml)</td>
<td>0.332 ppm</td>
<td>0.976 ppm</td>
</tr>
<tr>
<td>Limit of Quantification (µg/ml)</td>
<td>0.996 ppm</td>
<td>0.928 ppm</td>
</tr>
<tr>
<td>Precision</td>
<td>0.08</td>
<td>0.05</td>
</tr>
<tr>
<td>System Suitability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percent RSD</td>
<td>1.94</td>
<td>1.98</td>
</tr>
<tr>
<td>Theoretical Plates</td>
<td>4910.43</td>
<td>4433.12</td>
</tr>
<tr>
<td>Asymmetry</td>
<td>1.21</td>
<td>1.11</td>
</tr>
</tbody>
</table>

### Table 2. Assay data for combined etoricoxib and thiocolchicoside formulation

<table>
<thead>
<tr>
<th>Tablet</th>
<th>Etoricoxib</th>
<th>Thiocolchicoside</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dose (mg)</td>
<td>Content/Tab (mg)</td>
</tr>
<tr>
<td>Brand 1</td>
<td>100</td>
<td>100.3</td>
</tr>
<tr>
<td>Brand 2</td>
<td>100</td>
<td>100.1</td>
</tr>
<tr>
<td>Brand 3</td>
<td>100</td>
<td>99.9</td>
</tr>
</tbody>
</table>

### Table 3. Recovery data for standard solution added to tablet formulation

#### Table 3a. Recovery of etoricoxib

<table>
<thead>
<tr>
<th>Level</th>
<th>Amount of drug added in mg</th>
<th>Amount of drug recovered</th>
<th>Recovery %</th>
<th>Mean Recovery %</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>47.98</td>
<td>47.91</td>
<td>99.91</td>
<td>100.04</td>
<td>0.290</td>
</tr>
<tr>
<td></td>
<td>48.23</td>
<td>48.29</td>
<td>100.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>48.12</td>
<td>48.20</td>
<td>100.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>61.21</td>
<td>60.92</td>
<td>99.52</td>
<td>99.93</td>
<td>0.493</td>
</tr>
<tr>
<td></td>
<td>60.32</td>
<td>60.56</td>
<td>100.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>60.11</td>
<td>60.04</td>
<td>99.88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120%</td>
<td>72.11</td>
<td>72.16</td>
<td>100.06</td>
<td>100.1</td>
<td>0.092</td>
</tr>
<tr>
<td></td>
<td>71.32</td>
<td>71.29</td>
<td>100.36</td>
<td></td>
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<tr>
<td></td>
<td>71.43</td>
<td>71.45</td>
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</tr>
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</table>

#### Table 3b. Recovery of thiocolchicoside

<table>
<thead>
<tr>
<th>Level</th>
<th>Amount of Drug Added in mg</th>
<th>Amount of Drug Recovered</th>
<th>Recovery %</th>
<th>Mean Recovery %</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>6.42</td>
<td>6.44</td>
<td>100.31</td>
<td>99.99</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>6.41</td>
<td>6.38</td>
<td>99.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.47</td>
<td>6.48</td>
<td>100.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>8.02</td>
<td>8.01</td>
<td>99.87</td>
<td>99.83</td>
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</tr>
<tr>
<td></td>
<td>8.17</td>
<td>8.16</td>
<td>99.87</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>8.19</td>
<td>8.17</td>
<td>99.75</td>
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</tr>
<tr>
<td>120%</td>
<td>9.65</td>
<td>9.63</td>
<td>99.79</td>
<td>100.06</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>9.61</td>
<td>9.64</td>
<td>100.31</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>9.60</td>
<td>9.61</td>
<td>100.10</td>
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</tr>
</tbody>
</table>
4. RESULTS AND DISCUSSION

Because of the reliability of quality control of drugs and drug product the HPLC method development has received considerable attention over the years. The purpose of this study was HPLC method development for simultaneous estimation of formulated drug product of Etoricoxib and Thiocolchicoside. The proposed method was found to be simple, statistically valid and rapid for its accuracy. In the analysis of drug there were no interfering peaks observed in the chromatogram where results that tablet and excipients do not interfere with the drug. The typical chromatogram obtained in the method development is shown in figure. The retention time (RT value) for Etoricoxib and Thiocolchicoside was found to be 9.627 and 3.523 min respectively. Linearity was obtained in the calibration curve in the range of 10 to 200 µg/ml for Etoricoxib and 2 to 20 µg/ml for Thiocolchicoside. The Correlation coefficient for Etoricoxib was found to be 0.980 and for thiocolchicoside the correlation coefficient was found to be 0.999 respectively. The linear regression equation was y = 0.006x + 0.149 for Etoricoxib and Y = 0.030x + 0.086 for thiocolchicoside respectively. The mean drug content for Etoricoxib was found to be 99.9 for Etoricoxib and drug content for thiocolchicoside was found to be 99.16 respectively. The percent relative standard deviation for Etoricoxib and Thiocolchicoside at 80%, 100% and 120% was found to be 0.290, 0.493, 0.092 and 0.41, 0.07, 0.26 respectively. The recovery test for Etoricoxib and Thiocolchicoside was found to be 94.3±1.22 respectively and mean recovery for Thiocolchicoside was found to be 98.36 ± 1.54 respectively which indicates that the proposed method of analysis is highly accurate.

5. CONCLUSION

The result obtained in the analysis has shown that the method development is simple and accurate. This method can be useful for determination of simultaneous estimation of Etoricoxib and Thiocolchicoside in pharmaceutical formulations.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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