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ABSTRACT
A reverse-phase high-performance liquid chromatographic method is developed for the estimation of Lornoxicam—Novel NSAID Drug of oxicam class. The chromatographic separation is performed using a mobile phase consisting of acetonitrile : phosphate buffer (40:60) adjusted to pH 6.0 with H₃PO₄ on a C18 (ODS ; 250 × 4.6 mm) analytical column with flow rate of 1.0 ml/min and detection at 381 nm wavelength. The elution time is 5.62 min. The calibration curve is linear in the range of 5 μg/ml to 30 μg/ml. The limit of detection is 0.69 μg/ml and the limit of quantitation is 2.10 μg/ml. The Accuracy/recoveries are 100.33% - 100.60%. The present method is successfully applied for the estimation of Lornoxicam market formulation-Tablet.

Keywords: Lornoxicam; Reverse-phase high-performance liquid chromatography; Novel NSAID; Accuracy/recoveries.

INTRODUCTION
Lornoxicam (IUPAC Name : (3E)-6-chloro-3-[hydroxy(pyridin-2-ylamino)methylene]-2-methyl-2,3-dihydro-4H-thieno[2,3-e][1,2] thiazin- 4 - one 1,1-dioxide) is a new nonsteroidal anti-inflammatory drug (NSAID) of the oxicam class with analgesic, anti-inflammatory and antipyretic properties. Lornoxicam differs from other oxicam compounds in its potent inhibition of prostaglandin biosynthesis, a property that explains the particularly pronounced efficacy of the drug. Lornoxicam is used for the treatment of various types of pain, especially resulting from inflammatory diseases of the joints, osteoarthritis, surgery, sciatica, and other inflammations. The drug is contraindicated in patients that must not take other NSAIDs, possible reasons including salicylate sensitivity, gastrointestinal bleeding and other bleeding disorders, and severe impairment of heart, liver or kidney function. Lornoxicam is not recommended during pregnancy and breastfeeding and is contraindicated during the last third of pregnancy.

Like other NSAIDs, lornoxicam inhibits prostaglandin biosynthesis by blocking the enzyme cyclooxygenase and inhibits both isoforms in the same concentration range.
that is, the ratio of COX-1 inhibition to COX-2 inhibition is 1:1. It readily penetrates into
the synovial fluid.

Literature review \cite{5-12} revealed that limited work has been reported for the
estimation of lornoxicam by HPLC method and reported HPLC methods were having on
an average 9 min retention time, that can be said as time consuming methods. Aim &
objective of this work was to develop and validate HPLC method for the estimation of
lornoxicam in bulk and tablet dosage form; with possibly less retention time than already
reported methods.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{chemstruct.png}
\caption{Chemical structure of lornoxicam}
\end{figure}

\section*{MATERIALS AND METHOD}
\subsection*{MATERIALS}
\subsubsection*{Instruments & Apparatus}
Cyberlab HPLC with C18 (ODS ; 250×4.6 mm) column; operating software LC-100,
Labindia Double beam UV-Visible spectrophotometer; operating software UV win, EIE
INSTRUMENTS PVT.LTD. Sonicator, Welltronix pH Meter. Borosil Measuring
cylinder 10 ml, 50 ml, 100 ml. Borosil Pipette 1 ml, 2 ml, 5 ml, 10ml. Borosil Volumetric
flask 10 ml, 100 ml. (all apparatus made up of glass and precalibrated)
\subsubsection*{Reagents & Chemicals}
Lornoxicam working standard was procured from Mepro Pharmaceuticals, Surendranagar, Na₂HPO₄ & NaOH Analytical Reagent Grade. HPLC Grade acetonitrile and metanol, HPLC Water from Finar Reagents, Ahmedabad, India.

METHODOLOGY

Wavelength selection for Lornoxicam

0.1N NaOH was used as a solvent for lornoxicam (working standard) wavelength scanning and as shown in below figure maximum absorbance was found at 381nm wavelength so for all mobile phases in HPLC detections done at 381nm wavelength.

![Wavelength scanning of lornoxicam with 0.1 M NaOH solvent](image)

Preparations of the solutions For HPLC

Buffer preparation: Prepare 0.02M Na₂HPO₄ solution. Adjust the pH to 6.0 by H₃PO₄

Working Standard stock preparation: Take 10.0mg lornoxicam (working standard) in 100.0ml volumetric flask. Add 10ml 0.1N NaOH to it. Then add 60 ml mobile phase to it. Make the volume 100ml by mobile phase.(100 g/ml)

Working Standard preparation: Take 10.0mg lornoxicam (working standard) in 100.0ml volumetric flask. Add 10ml 0.1N NaOH to it. Then add 60 ml mobile phase to it.
Make the volume 100ml by mobile phase. Take 2ml from it and dilute upto 10ml by mobile phase.(20 g/ml)

**Preparation of Calibration Curve:** From the working standard stock solution 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml and 3.0 ml was taken and diluted up to 10 ml with mobile phase to prepare final concentrations of 5 g/ml, 10 g/ml, 15 g/ml, 20 g/ml, 25 g/ml, 30 g/ml respectively.

**Sample/Formulation preparation:** Take equivalent weight of tablet triturate to 10.0mg lornoxicam in 100ml volumetric flask. Add 10ml 0.1N NaOH to it. Then add 60ml mobile phase to it. Make the volume 100ml by mobile phase. Take 2ml from it and dilute upto 10ml by mobile phase. The resulting solution is of 20 g/ml concentration.

**Mobile Phase Preparation:** Acetonitrile : Phosphate Buffer (40:60) at pH 6.0

**Fixed chromatographic conditions:** Stationary phase : ODS C18 Column (250×4.6mm); Mobile phase: Acetonitrile : Phosphate Buffer (40:60) at pH 6.0 ; Detection wavelength: 381 nm; Injection volume : 20 l; Temperature of column : Room temperature ; Flow rate : 1.0 ml/min.

![Chromatogram of lornoxicam (20 g/ml) in acetonitrile : phosphate buffer (40:60) at pH 6.0 [Injection volume: 20 l]](image-url)
Validation of proposed method\cite{13,14}: Validation of this method was done as per ICH guidelines.

RESULT & DISCUSSION
Specificity - The developed method is specific for particular interest of drug and have no interference in detection due to vehicles or additive materials of the formulation this is depicted by specificity as shown in below chromatograms.

![Figure 4a](image1.png)  
**Figure 4a**  
Chromatogram of lornoxicam working standard in ACN : Phosphate buffer (40:60) at pH 6.0

![Figure 4b](image2.png)  
**Figure 4b**  
Chromatogram of lornoxicam tablet in ACN : Phosphate buffer (40:60) at pH 6.0

Acceptance criteria for system suitability

<table>
<thead>
<tr>
<th>Tailing factor</th>
<th>Peak area</th>
<th>Resolution</th>
<th>Number of theoretical plates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ideal tailing factor should ≤2</td>
<td>Peak area should be &gt; 2000</td>
<td>For single drug resolution is 0 and combination of two drug ≥2</td>
<td>The number of theoretical plates should not be less than 2000</td>
</tr>
</tbody>
</table>

**TABLE 1: SYSTEM SUITABILITY DATA OF LORNOXICAM**

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Tailing factor</th>
<th>Peak area</th>
<th>Number of theoretical plates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lornoxicam</td>
<td>1.36</td>
<td>646167</td>
<td>4336.86</td>
</tr>
</tbody>
</table>
Linearity and range – Lornoxicam was found to be linear in the range of 5 µg/ml to 30 µg/ml.

LOD : 0.69 µg/ml & LOQ : 2.10 µg/ml

![Linearity Curve of Lornoxicam](image)

**Table 2: Result as Summary of Validation Parameters**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Lornoxicam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity Range (µg/ml)</td>
<td>5-30</td>
</tr>
<tr>
<td>Regression equation</td>
<td>$Y = 31922 (X) + 8131.2$</td>
</tr>
<tr>
<td>Regression coefficient</td>
<td>0.9995</td>
</tr>
<tr>
<td>Slope</td>
<td>31922</td>
</tr>
</tbody>
</table>

Accuracy - Here accuracies for Lornoxicam working standard at 50%, 100%, 150% levels were found to be 100.33 ± 0.16, 100.50 ± 0.24, 100.60 ± 0.09 respectively and which is within the limit 98.0% to 102.0%. % Relative Standard Deviations for 50%, 100%, 150% levels were found to be 0.16, 0.24, 0.09 respectively.

Precision – % RSD for Intraday, Interday and Repeatability were found to be 0.28-0.82, 0.84-0.94, 0.89

Accuracy

**Figure 5**

Linearity curve of lornoxicam

**Table 2: Result as Summary of Validation Parameters**

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<td>31922</td>
</tr>
</tbody>
</table>
## Intercept

<table>
<thead>
<tr>
<th>Accuracy (% recovery)</th>
<th>Level I 50%</th>
<th>100.33 ± 0.16</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Level II 100%</td>
<td>100.50 ± 0.24</td>
</tr>
<tr>
<td></td>
<td>Level III 150%</td>
<td>100.60 ± 0.09</td>
</tr>
<tr>
<td>Precision</td>
<td>Intra-day Precision (n=3) (%RSD) (NMT 2)</td>
<td>0.28-0.82</td>
</tr>
<tr>
<td></td>
<td>Inter-day Precision (n=3) (%RSD) (NMT 2)</td>
<td>0.84-0.94</td>
</tr>
<tr>
<td></td>
<td>Repeatability of injection (n=9) (NMT 2) (%RSD)</td>
<td>0.89</td>
</tr>
<tr>
<td>LOD</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>LOQ</td>
<td>2.10</td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>Specific</td>
<td></td>
</tr>
<tr>
<td>Assay of tablet (n=3)</td>
<td>Brand I</td>
<td>99.48</td>
</tr>
<tr>
<td></td>
<td>Brand II</td>
<td>99.12</td>
</tr>
</tbody>
</table>

### CONCLUSION

A reverse-phase high-performance liquid chromatographic method is developed for the estimation of Lornoxicam. The chromatographic separation is performed using mobile phase consisting of aetonitrile: phosphate buffer (40:60) adjusted to pH 6.0 with phosphoric acid. The proposed method utilizes isocratic elution technique at room temperature. The real advantage of the method is it’s low retention time 5.62 min, which is really good as compare to some other available methods with apx. 9-10 min. retention time. It reduces the total run time for HPLC, leads to low solvent consumption, and makes the method more economical. The method is simple, rapid, precise, and accurate for the analysis of Lornoxicam from its marketed formulations. It can be used for the routine quality control of the formulation in the pharmaceutical industry.

### ACKNOWLEDGEMENT

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REFERENCES:


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