DEVELOPMENT AND VALIDATION OF HPTLC (HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY) METHOD FOR SIMULTANEOUS ESTIMATION OF LORNOXICAM AND PARACETAMOL IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Novel analytical Chromatographic HPTLC method has been developed in presented research work for simultaneous estimation of Lornoxicam and Paracetamol in pharmaceutical dosage form. HPTLC separation was also achieved on precoated silica gel 60GF$_{254}$ using Acetone:Chloroform/Formic acid (4:4:2) as a mobile phase. Quantification was achieved with UV detection at 254 nm. The $R_f$ value of Lornoxicam was found to be 0.47±0.02 and $R_f$ value of Paracetamol was found to be 0.28±0.01. Calibration curve were linear in the range of 64-320 ng/spot for Lornoxicam and 2600-13000 ng/spot for Paracetamol. The assay results were found to be 98.85-101.32% for Lornoxicam and 99.84-101.18% for Paracetamol. The methods were found to be rapid, simple, accurate and precise. Thus the present study gives excellent method for the determination of these two drugs in combined dosage formulation without their prior separation. They enable the simultaneous determination of Lornoxicam and Paracetamol in tablet dosage form.

KEYWORDS: Lornoxicam, Paracetamol, HPTLC method, Validation.

INTRODUCTION

Lornoxicam belongs to the chemical class oxicams, which includes piroxicam, tenoxicam and meloxicam. It has non-steroidal anti-inflammatory activity. It is used to treat inflammatory...
diseases of the joints, osteoarthritis, pain after surgery, and sciatica. It works by blocking the action of cyclooxygenase, an enzyme involved in the production of chemicals, including some prostaglandins in the body.\cite{1,2} Chemically, Lornoxicam (LOR) is (3\textit{E})-6-chloro-3-[hydroxyl(pyridine-2-ylamino)methylene]-2-methyl-2,3-dihydro-4\textit{H}-thieno[2,3\textit{e}][1,2]thiazin-4-one-1,1-dioxide.\cite{3} Lornoxicam is not official in any Pharmacopoeia, but listed in the Merck Index.\cite{4} Few analytical methods for determination of lornoxicam using UV method\cite{5,6}, Bio-analytical method\cite{7}, spectro-fluorimetry method\cite{8}, LC method\cite{9,10} RP-HPLC\cite{11,12}, stability indicating HPLC method\cite{13} have been reported.

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{chemical_structure}
\caption{Chemical Structure of: [A] Lornoxicam, [B] Paracetamol.}
\end{figure}

Paracetamol is chemically 4-hydroxy acetanilide. It is a centrally and peripherally acting non-opioid analgesic and antipyretic.\cite{14,15} Paracetamol is official in I.P\cite{16}, B.P\cite{17} and USP.\cite{18} Literature survey reveals that there are UV method\cite{19,20} and HPTLC methods of Paracetamol with other drugs\cite{21,22} have been reported for the estimation of Paracetamol in Pharmaceutical formulations.

Extensive Literature survey reveals that there are some UV method, i.e. simultaneous equation method and absorbance Q ratio method\cite{23,24}, HPTLC\cite{25} RP- HPLC methods\cite{26} have been reported for simultaneous estimation of Lornoxicam and Paracetamol in combined dosage form.

This paper presents simple, rapid, economic and reproducible HPTLC method for the simultaneous estimation of both the drugs from pharmaceutical dosage form. The proposed method was optimized and validated in accordance with International Conference on Harmonization (ICH) guidelines.
MATERIALS AND METHODS

Chemicals and reagents: Lornoxicam and Paracetamol working standards were procured from Stallion Laboratories Pvt. Ltd., Ahmedabad and Dolphin Pharmaceutical Ltd, Surat. The tested pharmaceutical marketed formulation (Lornoxi-P - Lornoxicam 8mg, paracetamol 325mg) was procured from commercial pharmacy. Solvents used were Acetone, Chloroform, Formic acid, etc. Reagents were of suitable analytical grade.

Marketeted tablet preparation used was

<table>
<thead>
<tr>
<th>Trade Name</th>
<th>Company Name</th>
<th>Dose</th>
<th>Batch Number</th>
<th>Manufactured Date</th>
<th>Expiry Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lornoxi-P</td>
<td>Hetero Labs Limited</td>
<td>Lornoxicam-8mg, Paracetamol-325mg.</td>
<td>3114306</td>
<td>May2014</td>
<td>Apr2016</td>
</tr>
</tbody>
</table>

Chromatographic Conditions

The instrument used for the estimation was Camag Linomat V semi-automatic sample applicator, Camag TLC scanner 3, CATS software for interpretation of the data, Hamilton syringe and Camag twin trough chamber. The Mobile phase was Acetone: Chloroform: Formic acid (4:4:2). Chamber saturation time was 20min. Detection was performed at 254nm.

Preparation of Standard Solution

Accurately weighed quantity of 16 mg of Lornoxicam and 650 mg of Paracetamol were transferred into 25 ml volumetric flask, dissolved in methanol, sonicated for 15 min and diluted up to mark with methanol. (Solution A: 640 µg/ml of Lornoxicam and 26000 µg/ml of Paracetamol) From this solution pipetting out 1ml and diluted it upto 10 ml with methanol to produce solution B. (Solution B: 64 µg/ml of Lornoxicam and 2600 µg/ml of paracetamol).

 Determination of wavelength for measurement

From the working standard solution B 1, 2, 3, 4, 5 µl (64-320 ng/spot Lornoxicam and 2600-13000 ng/spot Paracetamol) were spotted on TLC plate under nitrogen stream using Linomat-V applicator. From Overlain spectra it was found that at 254 nm both the drug have considerable absorbance. Thus 254 nm was selected as wavelength for estimation of both the drug.

Preparation of Sample Solution

Twenty tablets were weighed and powdered. The average weight of powder was calculated. The tablet powder equivalent to 16 mg of Lornoxicam and 650 mg of Paracetamol was
transferred to a 25 ml volumetric flask, dissolved, sonicated for 15 min and diluted up to mark with methanol. The solution was filtered through Whatmann filter paper no.42 and first few ml of filtrate were discarded. From above solution pipetting out 1 ml solution and diluted up to 10 ml with methanol. From this solution sample 2 μl was spotted on activated TLC plate. The plate is scanned and the detection wavelength is 254 nm for the both drugs and peak area is measured. The concentration of each drugs are determined by using straight line equations for each drug.

RESULT AND DISCUSSION
The present study was aimed at development of rapid, convenient and cost effective HPTLC technique for determination of Lornoxicam and Paracetamol tablet dosage form. Various blends of solvent systems in varying proportions were tried as mobile phase. HPTLC separation was achieved on precoated silica gel 60GF\textsubscript{254} using Acetone:Chloroform:Formic acid (4:4:2) as a mobile phase. The R\textsubscript{f} value of Lornoxicam was found to be 0.47±0.02 and R\textsubscript{f} value of Paracetamol was found to be 0.28±0.01, respectively with saturation time of 20 minutes. The selection of wave length was based on maximum absorbance for optimum sensitivity. Quantification was achieved with UV detection at 254 nm. Calibration curve were linear in the range of 64-320 ng/spot for Lornoxicam and 2600-13000 ng/spot for Paracetamol with coefficient of correlation value 0.9963 and 0.9975 respectively. The assay results were found to be 98.85-101.32% for Lornoxicam and 99.84-101.18% for Paracetamol. The developed method was validated as per ICH Guidelines. Thus the present study gives excellent methods for the determination of these two drugs in combined dosage formulation. They enable the simultaneous determination of Lornoxicam and Paracetamol in tablet dosage form.

VALIDATION OF METHOD
1. Linearity and Range: The linearity response was determined by analyzing 6 independent levels of calibration curve in the range of 64-320 ng/spot for Lornoxicam and 2600-13000 ng/spot for Paracetamol. Peak areas of each concentration were measured and plot of peak area vs. concentration were plotted. The straight line equation was determined.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>LORNOXICAM</th>
<th>PARACETAMOL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range (ng/spot)</td>
<td>64-320</td>
<td>2600-11353</td>
</tr>
<tr>
<td>Linearity equation</td>
<td>y = 5.002x + 3029.9</td>
<td>y = 1.0419x + 8896.9</td>
</tr>
<tr>
<td>Co-relation coefficient</td>
<td>0.9963</td>
<td>0.9975</td>
</tr>
</tbody>
</table>
Figure 1: Selection Of Analytical Wavelength In Overlain Uv Absorption Spectra Of Lornoxicam (64-320 Ng/Spot) And Paracetamol (2600-13000 Ng/Spot)

Figure 2: 3d chromatogram of calibration curve of lornoxicam (64-320 ng/spot) and paracetamol (2600-13000 ng/spot).

2. ACCURACY (RECOVERY STUDY): The accuracy of the method was established using recovery technique i.e external standard addition method. The known amount of standard was added at three different levels to pre analyzed sample. Each determination was performed in triplicate. The result of recovery study is presented in table 2.

Table 2: Accuracy Results

<table>
<thead>
<tr>
<th>Tablet content taken eq. to (ng/spot)</th>
<th>Standard added (API spiked)</th>
<th>Total drugs recovered (ng/spot)</th>
<th>% Recovery of Standard added</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOR 2600</td>
<td>LOR 80%</td>
<td>115.23 4676.25</td>
<td>100.05 99.81</td>
</tr>
<tr>
<td>LOR 2600</td>
<td>LOR 100%</td>
<td>127.58 5212.63</td>
<td>99.34 100.48</td>
</tr>
<tr>
<td>LOR 2600</td>
<td>LOR 120%</td>
<td>140.84 5720.25</td>
<td>100.05 100.01</td>
</tr>
</tbody>
</table>
3. LOD & LOQ
Limit of Detection (LOD) AND Limit of Quantification (LOQ) were calculated and results found as per table 3.

Table 3: Lod & Loq

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Lornoxicam</th>
<th>Paracetamol</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOD (ng/spot)</td>
<td>20.37</td>
<td>821.72</td>
</tr>
<tr>
<td>LOQ (ng/spot)</td>
<td>61.75</td>
<td>2490.06</td>
</tr>
</tbody>
</table>

4. PRECISION: The precision expressed as standard deviation or relative standard deviation.

**Intraday precision:** Pure API of Lornoxicam and Paracetamol were taken in a ratio was analyzed at three levels of concentration for three times in a day. Peak Areas of the solutions were measured. The % RSD for Lornoxicam and Paracetamol were found.

**Interday precision:** Pure API of Lornoxicam and Paracetamol were taken in a ratio was analyzed at three levels of concentration for three consecutive days. Peak Areas of the solutions were measured, % RSD for Lornoxicam and Paracetamol were found.

Table 4: Precision Study

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration [µg/ml]</th>
<th>Intra-day Amount found [µg/ml n=3]</th>
<th>% R.S.D.</th>
<th>Inter-day Amount found [µg/ml n=3]</th>
<th>% R.S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOR</td>
<td>128</td>
<td>99.67±0.42</td>
<td>0.42</td>
<td>99.70±0.72</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>192</td>
<td>100.79±0.61</td>
<td>0.60</td>
<td>100.00±1.05</td>
<td>1.05</td>
</tr>
<tr>
<td></td>
<td>256</td>
<td>100.60±0.51</td>
<td>0.51</td>
<td>100.59±0.52</td>
<td>0.52</td>
</tr>
<tr>
<td>PCM</td>
<td>5200</td>
<td>100.23±0.43</td>
<td>0.43</td>
<td>100.36±0.71</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>7800</td>
<td>100.39±0.55</td>
<td>0.55</td>
<td>100.48±0.51</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>10400</td>
<td>99.81±0.15</td>
<td>0.15</td>
<td>99.44±0.63</td>
<td>0.63</td>
</tr>
</tbody>
</table>

5. SPECIFICITY: Specificity is carried out by taking peak purity of standard and sample of each drug and standard and sample peak spectra were overlain to check specificity of each individual drug peak and co-relation values (r) were found to be > 0.9995.

**ANALYSIS OF MARKETED FORMULATION: BY HPTLC METHOD**
Applicability of the proposed method was tested by analyzing the commercially available tablet formulation (Lornoxi-P) containing 8 mg of Lornoxicam and 325mg of Paracetamol. The results are shown in Table 5.
Table 5: Analysis Of Marketed Formulation

<table>
<thead>
<tr>
<th>Tablet content taken eq. to (ng/spot)</th>
<th>Amount found (ng/spot) (n=3)</th>
<th>Assay (% Estimated) (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOR 128</td>
<td>5200</td>
<td>129.70</td>
</tr>
<tr>
<td>PCM</td>
<td></td>
<td>5191.76</td>
</tr>
<tr>
<td></td>
<td>126.52</td>
<td>5261.5</td>
</tr>
<tr>
<td></td>
<td>127.86</td>
<td>5204.20</td>
</tr>
</tbody>
</table>

Figure 3: Peak Purity Corelation Spectra Of Lornoxicam And Paracetamol

Figure 4: Hptlc Chromatogram Of Tablet Formulation Of Lornoxicam And Paracetamol

CONCLUSION
The validated HPTLC method proved to be simple, fast, accurate, and precise and thus can be used for routine analysis of Lornoxicam and Paracetamol in tablet dosage forms.
ACKNOWLEDGEMENTS

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REFERENCES


