DEVELOPMENT OF VALIDATED HPTLC METHOD FOR SIMULTANEOUS DETERMINATION OF LEVOFLOXACIN HEMIHYDRATE AND CEPPODOXIME PROXETIL IN SYNTHETIC MIXTURE AND TABLET DOSAGE FORM

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ABSTRACT
Simple, precise and accurate method is described for the direct determination of Levofloxacin hemihydrate and Cefpodoxime proxetil in combined dosage form without prior separation. The method was based on HPTLC separation of the two drugs followed by the densitometry measurements of their spots at 237 nm for both drugs. The separation was carried out on silica gel 60GF254 using mobile phase Butanol: Ethyl Acetate: hexane: triethylamine(2:6:4:0.1). The linearity for Levofloxacin hemihydrates and Cefpodoxime proxetil were found to be be 125-750 ng/spot and 100-600 ng/spot with r² 0.9989 and 0.9992. The Rf values were 0.66 and 0.33 for Levofloxacin hemihydrates and Cefpodoxime proxetil respectively. Developed method has been validated according to ICH guidelines. Percentage recoveries were found to be 99.89-101.59% for Levofloxacin hemihydrate and 98.96-102.01% for Cefpodoxime proxetil respectively. The proposed method was applied successfully for the determination of the two drugs in synthetic mixture and pharmaceutical dosage form.

KEYWORDS: Levofloxacin hemihydrate, Cefpodoxime Proxetil, HPTLC and Method Validation.

INTRODUCTION
Levofloxacin hemihydrate, chemically named as [(S)-9-fluoro-2, 3-dihydro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-7H-pyrido [1, 2, 3-de]-1, 4-benzoaxine-6-carboxylic acid hemihydrate], is third generation fluoroquinolones class antimicrobial agent. It is yellowish white to yellow colour powder. It is freely soluble in methanol, slightly soluble in water and GAA.

Figure 1: structure of Levofloxacin hemihydrate
Cefpodoxime Proxetil, chemically named as (RS)-1(isopropoxycarbonyloxy) ethyl (+)-(6R,7R)-7-[2-(2-amino-4-thiazolyl)-2-[(Z) methoxyimino] acetamido]-3-methoxy methyl-3-cephem-4-carboxylate, is third generation Cephalosporin class antimicrobial agent. It is White to light brownish-white powder. It is freely soluble in Dehydrated alcohol, Acetonitrile, Methanol and very slightly soluble in water.

Referring to Literature survey, there is no published HPTLC method for simultaneous determination of Levofloxacin hemihydrate and Cefpodoxime proxetil in combination. So, present work aims to develop a simple, precise and accurate method for the direct determination of Levofloxacin hemihydrate and Cefpodoxime proxetil in combined dosage form. The proposed method is optimized and validated as per ICH guidelines.

MATERIALS AND METHODS

CHEMICAL AND REAGENTS: Levofloxacin hemihydrate and Cefpodoxime proxetil were procured from Kamron laboratory and tested pharmaceutical formulation GLEVO-POD (200 mg CEF and 250 mg LEVO) were procured from local market. Butanol, Ethyl Acetate, hexane, triethylamine and methanol were of suitable Analytical Grade.

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 Butanol: Ethyl Acetate: hexane: triethylamine (2:6:4:0.1 v/v/v/v). Chamber saturation time was 20min. Detection was performed at 237nm.

Preparation of Standard Solution: 125 mg of Levofloxacin hemihydrate (LEVO) and 100 mg Cefpodoxime proxetil (CEF) were dissolved and diluted with methanol upto 10ml. From this, 0.1 ml solution was taken and diluted upto 10ml with methanol.

Preparation of Sample Solution: 20 Tablets (GLEVO-POD) were weighed powdered. Powder equivalent to 125 mg of LEVO and 100 mg of CEF were transferred into 10ml Volumetric flask and diluted upto the mark with methanol.
RESULTS AND DISCUSSION:

Literature survey revealed that there was no any reported HPTLC method for simultaneous estimation of Levofloxacin hemihydrate and Cefpodoxime proxetil. The present study was aimed at development of speedy and cost effective HPTLC technique for determination of LEVO and CEF in tablet dosage forms. Various blends of solvent systems in varying proportions were tried as mobile phase. However, mobile phase consisting Butanol: Ethyl Acetate: hexane: triethylamine in the ratio of (2:6:4:0.1 v/v/v/v) was found to be more suitable with Rf values 0.66 of LEVO and 0.33 for CEF, respectively with saturation time of 20 minutes. The selection of wave length was based on maximum absorbance for optimum sensitivity. The drugs showed good linearity in the range of 125-750ng/spot for LEVO and 200-600ng/spot for CEF with coefficient of correlation value 0.9989 and 0.9992, respectively. From the recovery studies, the accuracy results were 99.89-101.59% for LEVO and 98.96-102.01% for CEF and were found to be highly accurate.

VALIDATION OF THE METHODS 57:

1. Linearity and Range: Linearity was found in the range of 125-750ng/spot for LEVO and 100-600ng/spot for CEF. The drug peak area was calculated for each concentration level and a graph was plotted of drug concentration against the peak area. Calibration parameters are given in table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Levofloxacin hemihydrates</th>
<th>Cefpodoxime proxetil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range (ng/spot)</td>
<td>125-750</td>
<td>100-600</td>
</tr>
<tr>
<td>Linearity equation</td>
<td>y = 17.64x + 5900.6</td>
<td>y = 18.694x + 1414.6</td>
</tr>
<tr>
<td>Co-relation coefficient</td>
<td>0.9989</td>
<td>0.9992</td>
</tr>
</tbody>
</table>

*LEVO- Levofloxacin hemihydrate ,CEF- Cefpodoxime proxetil*
2. **Precision:** The precision expressed as standard deviation or relative standard deviation.

   a. **Intraday precision:** Combined dosage form was analyzed at three levels of concentration of the assay for three times in a day. Peak Area of the solutions was measured. The % RSD for LEVO and CEF was found to be 0.0610 % and 0.4202 %, respectively.

   b. **Interday precision:** Combined dosage form was analyzed at three levels of concentration of the assay for three consecutive days. Peak Area of the solutions was measured. The % RSD for LEVO and CEF was found to be 0.237% and 0.173%, respectively.

3. **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 table 2.

   **TABLE 2: SPECIFICITY DATA**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Co-relation r (s,m)</th>
<th>Co-relation r (m,e)</th>
<th>Peak purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEVO</td>
<td>0.999884</td>
<td>0.999049</td>
<td>Pass</td>
</tr>
<tr>
<td>CEF</td>
<td>0.999623</td>
<td>0.999087</td>
<td>Pass</td>
</tr>
</tbody>
</table>

*LEVO- Levofoxacin hemihydrate, CEF- Cefpodoxime proxetil*

3. **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 preanalysed sample. Each determination was performed in triplicate. The result of recovery study is presented in Table 3.

**TABLE 3: ACCURACY RESULTS**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Assay level</th>
<th>Tablet content taken (ng/spot)</th>
<th>Standard added (ng/spot)</th>
<th>Total drug recovered (ng/spot)</th>
<th>% Recovery of standard added</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LEV</td>
<td>CEF</td>
<td>LEV</td>
<td>CEF</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>250</td>
<td>200</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>80%</td>
<td>250</td>
<td>200</td>
<td>200</td>
<td>160</td>
</tr>
<tr>
<td>3</td>
<td>100%</td>
<td>250</td>
<td>200</td>
<td>250</td>
<td>200</td>
</tr>
<tr>
<td>4</td>
<td>120%</td>
<td>250</td>
<td>200</td>
<td>300</td>
<td>240</td>
</tr>
</tbody>
</table>

*LEVO- Levofloxacin hemihydrate  
CEF- Cefpodoxime proxetil

**TABLE 4: ASSAY RESULT OF MARKETED FORMULATION**

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Formulation</th>
<th>LEVO Content (ng/spot)</th>
<th>CEF Content (ng/spot)</th>
<th>Assay (% label claim) (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLEVO-POD</td>
<td>Tablet</td>
<td>250</td>
<td>200</td>
<td>99.076 98.97</td>
</tr>
</tbody>
</table>

*LEVO- Levofloxacin hemihydrate  
CEF- Cefpodoxime proxetil

![Figure 4: HPTLC chromatogram of marketed formulation](image_url)

Figure 4: HPTLC chromatogram of marketed formulation
CONCLUSION:
The validated HPTLC method proved to be simple, less expensive, fast, accurate, and precise and thus can be used for routine analysis of Levofloxacin hemihydrate and Cefpodoxime proxetil in bulk and pharmaceutical dosage forms.

REFERENCES:
3. Indian Pharmacopoeia; Vol-II. Govt. of India; Indian Pharmacopoeial Commission; Ministry of Health and Family Welfare; Ghaziabad; 2010.
5. ICH guidelines, validation of analytical procedures Q2A; ICH Harmonized Tripartite Guidelines, 1996.

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