High Performance Thin Layer Chromatographic Estimation of Cefuroxime Axetil in Bulk and Pharmaceutical Formulation

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Abstract
A simple, fast, precise and accurate high performance thin layer chromatographic method has been developed for the estimation of Cefuroxime axetil in dosage forms. The chromatographic separation was developed using a pre-coated silica gel 60 GF254 TLC plate as stationary phase and a chromatogram was developed using chloroform: methanol: toluene (7:4:3 v/v/v) as mobile phase. Densitometric evaluation was performed at 289 nm. Cefuroxime axetil was resolved satisfactorily at Rf values 0.72 ± 0.001. The developed method was validated for accuracy, linearity, precision, limit of detection and limit of quantification and robustness as per the ICH guidelines. The calibration curve of HPTLC data was found to be linear between 300 to 800 ng/spot for Cefuroxime axetil. The limit of detection and the limit of quantification for the Cefuroxime axetil were found to be 9.85 ng/spot and 100.95 ng/spot. The proposed method can be successfully used to determine the drug content of marketed formulation.

Key words: Cefuroxime Axetil (CA), Tablet form, HPTLC.

Introduction
Cefuroxime is a second-generation cephalosporin. Cefuroxime axetil (CA) is an ester produg of Cefuroxime, which is rendered more lipophilic by esterification of carboxyl group of the molecule by the racemic 1-acetoxyethyl bromide, thus enhancing absorption [1]. Chemical name is (RS)-1 hydroxyethyl (6R,7R)-7-[2- (2-furyl) glyoxyl-amido]-3-(hydroxyl methyl -8-oxo-5-thiazabicyclo[4.2.0]-oct-2-ene-2-carboxylate,72-(Z)-(O-methyl-oxime),1-acetate3-carbamate) used to treat or prevent infections that are proven or strongly suspected to be caused by bacteria [2] Literature survey revealed Spectrophotometric [3] RP-HPLC methods [4] were developed for Cefuroxime axetil determination in combination with other drugs. Stability indicating [5] and bioanalytical chromatographic methods have also been done [6,7]. Detailed survey of literature for Cefuroxime axetil revealed few methods have been developed on HPTLC for its determination in pharmaceutical [8,9,10]. Hence the aim of this paper is to develop a simple, sensitive, accurate and selective method through HPTLC for Cefuroxime axetil in both bulk and pharmaceutical formulations. The proposed method describes a simple, sensitive accurate and precise HPTLC method for the Cefuroxime axetil in bulk and in marketed dosage forms.

Materials and Methods
Chemical and reagents
Cefuroxime axetil was procured as a gift sample from Simpex Pharma Pvt. Ltd. Tablet was purchased from the local market (Ceftum containing Cefuroxime axetil 125 mg, marketed by GSK Pharmaceuticals, India). Chloroform, toluene, methanol, were of HPLC grade and were procured from Merck.

HPTLC instrumentation and chromatographic conditions
Chromatographic separation of drug was performed on Merck TLC plates precoated with silica gel 60F254 (10 cm x 10 cm) with 250 µm layer thickness from E Merck, Germany. The samples were applied on to plates as a band with 6 mm width with slit dimension of 5 x 0.45 mm micro using Camag 100 µl sample syringe (Hamilton, Zurich, Switzerland) with the Linomat 5 applicator (Camag Switzerland) Linear ascending development was carried out in a twin through glass chamber (10 cm x 10 cm) previously saturated with the mobile phase, chloroform: methanol: toluene (7:4:3 v/v/v) at room temperature, using 30 min of chamber saturation. The development distance was approximately 80 mm. Densitometry scanning was performed using Camag TLC Scanner 3 in the range of 200-400 nm and operated by Win cats software using deuterium lamp as source of radiation. Evaluation was by peak area with linear regression.

Preparation of standard stock solution
A standard stock solution of 100 µg/ml of CA were prepared separately using methanol as solvent.

Validation of the method
The method was validated as per ICH guidelines. The parameters checked were linearity, accuracy, precision, limit of detection, limit of quantification, robustness and specificity [11,12,13].

Calibration curve
From the working standard solution of (100 µg/ml), 1-12 µl solutions were spotted on a HPTLC plate to obtain a final concentration of 100 ng/spot to 1200 ng/spot for CA. The plates were then developed as per procedure described above and the peak areas were plotted against corresponding concentration to obtain the calibration curves.
Specificity
The specificity of the method was determined by analysis of standard drug and sample. The band for CA in the sample was identified by comparing the Rf value and the spectrum of the band compared with the band obtained from a standard drug.

Accuracy (% Recovery)
For accuracy of method, recovery studies were carried out by applying a known amount of standard CA at a level of 50%, 100% and 150% to the sample solution (standard addition method). Three determinations were performed at each level, using same chromatographic condition as describe above.

Table 1: Regression analysis of the calibration curve of Cefuroxime Axitel the proposed HPTLC method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cefuroxime Axitel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range</td>
<td>300-800 ng/spot</td>
</tr>
<tr>
<td>Slope</td>
<td>3.8801</td>
</tr>
<tr>
<td>Intercept</td>
<td>1979.1</td>
</tr>
<tr>
<td>Correlation coefficient *</td>
<td>0.9978</td>
</tr>
</tbody>
</table>

* Indicates mean of six observation

Precision (Reproducibility):
The precision of the method was verified by performing the intraday and interday precision. The intraday and interday precision of the proposed method was determined by estimating the corresponding response three times on the same day and on three different days over a period of one week for six concentration of CA (100, 300, 500, 700, 1000, 1200 ng/spot) The results are expressed in terms of relative standard deviation.

RESULTS AND DISCUSSION
Validation
To optimize the HPTLC parameters, several mobile phase were tried and satisfactory results were obtained by using the mobile phase chloroform: methanol : toluene (7:4.3 v/v/v). Quantification was achieved under UV detection at 289 nm. A sharp and symmetrical peak was resolved with an Rf of 0.73 ± 0.003. Shown in figure-1.

Linearity
The linear regression data revealed a good linear relationship over the concentration range of 300 ng/spot to 800 ng/spot with correlation coefficient \( r^2 = 0.9978 \). The results are shown in Table 1 and figure 2.

The LOD and LOQ were calculated using following equations as per International Conference on Harmonization guidelines.

\[
\text{LOD} = 3.3 \times \sigma/S, \quad \text{LOQ} = 10 \times \sigma/S
\]

Where \( \sigma \) is standard deviation of the response and S is the standard deviation of y intercept of regression lines.

Robustness
Robustness was checked by making a slight deliberate change in the experimental procedure like slight change in the mobile phase, saturation time and the values were compared with the original chromatographic conditions.

Analysis of the marketed products
To find the content of the marketed formulation, (Ceftum, Label Claim, 125 mg of Cefuroxime axetil), four tablets were weighed and average weight was determined, powdered, from this equivalent weight of 125 mg of CA was transferred into a 50 ml volumetric flask, containing 15 ml of methanol and sonicated for 30 min, filtered through Whatmann filter paper No.41 and then volume was made up to 50 ml with methanol. From this stock solution 100 ng/spot was spotted CA on a HPTLC plate and chromatogram was developed as described earlier. The analysis was repeated for three times and interference for excipients was analyzed.
Statistical data indicate that the proposed method is validated as per ICH guidelines. The developed method only one single mobile phase is sufficient for quantification of Cefuroxime axetil. The method was found to be robust since the peak area values were not significantly affected. Analysis of the marketed products was found to be precise as indicated by percent RSD not more than 2% as per ICH guidelines for interday and intraday determination. The results are shown in Table 2.

The proposed method was applied successfully to the analysis of the marketed products, providing necessary facilities to carry out the research work.

Table 2: Summary of validation parameters for Cefuroxime Axetil the proposed HPTLC method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cefuroxime Axetil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy (%) ± RSD #</td>
<td>99.38 ±0.1268</td>
</tr>
<tr>
<td>Recovery studies(%) ± RSD</td>
<td>98.23 ± 1.425</td>
</tr>
<tr>
<td>50%</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>100.89 ± 0.4125</td>
</tr>
<tr>
<td>150%</td>
<td>101.03 ± 0.1147</td>
</tr>
<tr>
<td>LOD</td>
<td>9.85 ng/spot</td>
</tr>
<tr>
<td>LOQ</td>
<td>100.95 ng/spot</td>
</tr>
<tr>
<td>Precision (%) ± RSD #</td>
<td></td>
</tr>
<tr>
<td>Inter day</td>
<td>0.2392-1.146</td>
</tr>
<tr>
<td>Intra day</td>
<td>0.3930-1.0122</td>
</tr>
</tbody>
</table>

± indicates standard deviation # Average of three trials

REFERENCE