VALIDATED HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF DICLOXACILLIN SODIUM AND CEPPODOXIME PROXETIL IN TABLET DOSAGE FORM

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ABSTRACT
This research paper describes development and validation of HPTLC (high performance thin layer chromatography) method for simultaneous estimation of Cefpodoxime Proxetil (CEF) and Dicloxacillin Sodium (DCX) in tablet dosage forms. Separation was achieved on aluminum plates pre-coated with 0.2 mm layers of silica gel 60F254 using chloroform: methanol:10% trifluoroacetic acid (9:1.4:0.2, v/v/v) as mobile phase. Densitometric quantification was achieved with UV detection at 235 nm. The Rf values of Dicloxacillin and Cefpodoxime were 0.42 and 0.70, respectively. The method was validated as per ICH guidelines. The method has linear response over the concentration range 100–2000 ng/spot and 250–5000 ng/spot with mean recovery of 99.75% and 99.43%, for Dicloxacillin and Cefpodoxime, respectively. In conclusion the developed method was found to be simple, precise, accurate, specific, sensitive and applicable for the routine simultaneous estimation of Cefpodoxime Proxetil (CEF) and Dicloxacillin Sodium (DCX) in tablets.

KEYWORDS: High performance thin layer chromatography (HPTLC), Cefpodoxime Proxetil, Dicloxacillin Sodium.

INTRODUCTION
Cefpodoxime proxetil (CEF), chemically 1-(isopropoxy carbonyloxy) ethyl(6R,7R) -7- [2-(2-amino – 4 - thiazolyl) - (z) – 2 - (methoxy imino) acetamido] – 3 – methoxy methyl – 3 –
Cephem - 4 carboxylate (fig.1B), is a third generation cephalosporin, used in infections of the respiratory tract, urinary tract, skin and soft tissues.\(^1\)\(^-\)\(^2\) It is official in IP\(^3\) and USP\(^4\) which describe liquid chromatography method for its estimation. Various UV, HPTLC and HPLC methods are reported for estimation of Cefpodoxime alone.\(^5\)\(^-\)\(^9\) Literature survey reveals RP-HPLC, HPTLC, UV-visible spectrophotometric methods for the simultaneous estimation of CEF in combination with other drugs.\(^10\)\(^-\)\(^14\) In 1997 (2S,5R,6R)-6- [3 - (2, 6 – dichloro phenyl) – 5 – methyl -1,2 – oxazole –4- amido]-3,3- dimethyl -7- oxo-4-thia-1 azabicyclo heptane-2-carboxylic acid (fig. 1A), is a penicillinase resistant penicillin, used in the treatment of bacterial infections such as pneumonia and bone, ear, skin and urinary tract infection.\(^15\)\(^-\)\(^16\) It is official in BP,\(^17\) IP\(^18\) and USP.\(^19\) Literature survey also revealed several UV spectrophotometric methods and RP-HPLC methods estimation of DCX in combination with other drugs.\(^20\)\(^-\)\(^25\) A deep literature survey indicates some UV spectrophotometric\(^26\)\(^-\)\(^27\) and RP-HPLC\(^28\)\(^-\)\(^29\) methods for simultaneous estimation of DCX and CEF both in tablet dosage form. So far no HPTLC method has been reported for simultaneous estimation of both drugs in combined dosage form. HPLTC is most flexible, reliable, and cost-efficient separation technique which is being aimed for the rapid analysis of large numbers of compounds. So the aim of the present investigation was to develop a simple, precise, accurate and specific HPTLC method for the simultaneous estimation of both drugs in pharmaceutical formulation.

Fig 1: Structure of (A) Dicloxacillin Sodium (DCX) and (B) Cefpodoxime Proxetil (CEF).
MATERIAL AND METHODS

Instruments
A Camag HPTLC system comprising of Camag Linomat V automatic sample applicator, Hamilton syringe (100 μl), Camag TLC Scanner 3, Camag WinCATS software, Camag Twin-trough chamber (10×10 cm) and ultrasonicator were used during study.

Material and Reagents
Dicloxacillin sodium and Cefpodoxime Proxetil standard were procured as a gift samples from Indica Laboratories, Gujarat (India). Tablets containing 200 mg of CEF and 500 mg of DCX (Zedocef DXL 200, Macleods Pharma) were purchased from local pharmacy. Pre-coated Silica gel 60F254 TLC plates (10×10 cm, layer thickness 0.2 mm, E. Merck, Mumbai, India) were used as a stationary phase. All reagents used were of analytical grade and purchased from s.d. Fine Chemicals, Mumbai, India.

Preparation of Standard Stock Solution
10 mg of each CEF and DCX were accurately weighed, dissolved and diluted with methanol and make up to the final concentration of 100 μg/ml of each drug.

Preparation of Sample Stock Solution
Twenty tablets were accurately weighed and finely powdered. An accurately weighed tablet powder equivalent to 10 mg of CEF and 25 mg of DCX was transferred to 100 ml conical flask and mixed with 30 ml of methanol. The solution was sonicated for 20 min. Then the solution was filtered through Whatman filter paper No. 41 and residue was washed thoroughly with methanol. The filtrate and washings were combined and appropriately diluted to get 100 μg/ml of CEF 250 μg/ml of and DCX.

METHODOLOGY
The plates were previously prewashed with methanol and activated in an oven at 50° for 5 min. The chromatographic conditions used were TLC plates (10×10 cm) precoated with silica gel 60F254 as stationery phase and chloroform: methanol:10%triflouroacetic acid (9:1.4:0.2 v/v/v) as mobile phase. The chamber saturation time kept was 45 min at temperature 25°C and mobile phase is migrated to the distance of 80 mm. The source of radiation used was the deuterium lamp. Standard solutions of CEF and DCX were spotted and plate was developed. Densitometric scanning was performed using CAMAG TLC scanner 3 in reflectance mode at
235 nm with Win CATS software. The slit dimensions were length 5 mm, width 0.45 mm and the scanning rate was 10 mm/s.

Aliquots of 1, 2, 5, 10, 20, 30 μl of standard solution (100μg/ml) of both CEF and DCX were applied on the TLC plate and analyzed as above mentioned chromatographic conditions. The standard calibration curves were prepared and regression equations were calculated. Sample solution was spotted on the plate and analyzed as per above chromatographic conditions. The analysis was repeated in triplicate. The content of the drug was calculated from the peak areas recorded. The developed method was validated in terms of linearity, accuracy, precision, limit of detection, limit of quantification and specificity as per ICH guidelines.\[^{30}\]

**RESULT AND DISCUSSION**

Several mobile phase compositions were tried to accomplish good resolution between CEF and DCX. Better separation was attained for CEF and DCX with $R_f$ values of 0.70 and 0.42, respectively using chloroform: methanol: 10% trifluoroacetic acid (9:1.4:0.2, v/v/v) as mobile phase (fig.2).

![Densitogram of standard spot DCX and CEF with $R_f$ at 0.42 and 0.70, respectively by TLC at 235 nm.](image-url)
The linear regression data showed that the method was linear in the range of 100-3000 ng/spot for CEF and DCX. The correlation coefficients ($r^2$) value obtained for CEF and DCX were 0.9989 and 0.9981, respectively suggest that the method is linear in the range 100-3000 ng/spot for both (Table 1).

The limit of detection (LOD) and limit of quantification (LOQ) were determined using the equations as per ICH. LOD and LOQ were found to be 27.08 and 82.07 ng/spot and 27.36 82.90 ng/spot for CEF and DCX, respectively. The values for LOD and LOQ showed that the proposed method is sensitive for the determination of CEF and DCX (Table 1).

**Table 1: Summary of Validation Parameters by Proposed Method.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CEF</th>
<th>DCX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention Factor (Rf)</td>
<td>0.42</td>
<td>0.70</td>
</tr>
<tr>
<td>Linearity &amp; Range (ng/spot)</td>
<td>100 - 3000</td>
<td>100 – 3000</td>
</tr>
<tr>
<td>Regression équation (Y=mx+c)</td>
<td>$Y = 6.215X + 272.9$</td>
<td>$Y = 1.715X+132.6$</td>
</tr>
<tr>
<td>Slope (m)</td>
<td>6.215</td>
<td>1.715</td>
</tr>
<tr>
<td>Intercept (c)</td>
<td>272.9</td>
<td>132.6</td>
</tr>
<tr>
<td>Correlation coefficient ($r^2$)</td>
<td>0.9989</td>
<td>0.9981</td>
</tr>
<tr>
<td>Limit of detection (LOD) (ng/spot)</td>
<td>27.08</td>
<td>27.36</td>
</tr>
<tr>
<td>Limit of Quantification (LOQ) (ng/spot)</td>
<td>82.07</td>
<td>82.90</td>
</tr>
<tr>
<td>Repeatability (%RSD, n=6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repeatability of application</td>
<td>0.71</td>
<td>0.59</td>
</tr>
<tr>
<td>Repeatability of measurement</td>
<td>0.38</td>
<td>0.47</td>
</tr>
<tr>
<td>Precision (%RSD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interday Precision (n=3)</td>
<td>0.91 - 1.28</td>
<td>0.82 - 1.13</td>
</tr>
<tr>
<td>Intraday Precision (n=3)</td>
<td>0.51 - 1.01</td>
<td>0.32 - 0.97</td>
</tr>
<tr>
<td>Specificity</td>
<td>Specific</td>
<td>Specific</td>
</tr>
<tr>
<td>% Assay ± SD (n=3)</td>
<td>99.89 ± 0.90</td>
<td>99.69 ± 1.05</td>
</tr>
</tbody>
</table>

Represents the number of replicates, ng/spot is nanogram per spot, RSD represents relative standard deviation and SD represents standard deviation

Repeatability of sample application was assessed by spotting 5 μl of drug solution 6 times on a TLC plate followed by development of plate and recording the peak area for 6 spots. The % RSD for peak area values of CEF and DCX were found to be 0.71 and 0.59, respectively. Repeatability of measurement of peak area was determined by scanning the same spot for six times without changing the position of the plate and % RSD of peak area of CEF and DCX were found to be 0.38 and 0.47, respectively. The intra-day precision was determined by analyzing three standard solutions (200, 500 and 1000 ng/spot) for 3 times on the same day while inter-day precision was determined by analyzing corresponding standards three times.
daily for 3 days over a period of one week. The inter-day and intra-day coefficients of variation for CEF and DCX were found to be in the range of 0.91-1.28 % and 0.51-1.01% and 0.82-1.13% and 0.32-0.97%, respectively. The low values of % RSD indicate that the method is precise (Table 1).

The accuracy (% recovery) of the method was studied by standard addition method. The mean % recoveries with standard deviation obtained for CEF and DCX, were 99.43 % ± 1.01 and 99.75 % ± 0.54, respectively. The results of recovery studies shown in Table 2 revealed that there is no interference of excipients during analysis of both drugs and the method is accurate.

Table 2: Recovery Data CEF and DCX by Proposed Method.

<table>
<thead>
<tr>
<th>Level</th>
<th>Amount of Sample Taken (ng/spot)</th>
<th>Amount of Standard Added (ng/spot)</th>
<th>Amount of Standard Recovered (ng/spot)</th>
<th>Mean % Recovery ± SD (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I (50 %)</td>
<td>200</td>
<td>100</td>
<td>98.45</td>
<td>98.45 ± 0.61</td>
</tr>
<tr>
<td>II (100 %)</td>
<td>200</td>
<td>200</td>
<td>199.34</td>
<td>99.67 ± 0.92</td>
</tr>
<tr>
<td>III (150 %)</td>
<td>200</td>
<td>300</td>
<td>300.51</td>
<td>100.17 ± 1.49</td>
</tr>
<tr>
<td>DCX</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I (50 %)</td>
<td>500</td>
<td>250</td>
<td>247.80</td>
<td>99.12 ± 0.81</td>
</tr>
<tr>
<td>II (100 %)</td>
<td>500</td>
<td>500</td>
<td>501.60</td>
<td>100.32 ± 0.45</td>
</tr>
<tr>
<td>III (150 %)</td>
<td>500</td>
<td>750</td>
<td>748.65</td>
<td>99.82 ± 0.36</td>
</tr>
</tbody>
</table>

Represents number of replicates, SD is standard deviation, ng/spot is nanogram per spot.

The specificity of the method was ascertained by analyzing standard and sample solutions of both drugs. The spots of CEF and DCX in samples were confirmed by comparing the Rf and spectra of the sample spot with that of standard. The peak purity of DCX and CEF was assessed by comparing their respective spectra at the peak start, middle and peak end position of the spot. The r (S, M) = 0.9995 & 0.9996 and r (M, E) = 0.9992 & 0.9994 for CEF and DCX indicates that the method is specific. The comparative spectrum of CEF and DCX standard and sample is shown in fig. 3 & 4.
Fig. 3: Overlain spectra of standard and sample spot of CEF.

Fig. 4: Overlain spectra of standard and sample spot of DCX.
The proposed HPTLC method was successfully applied to determine CEF and DCX in their tablet dosage form. The mean % Assay with SD for CEF and DCX obtained is 99.89% ± 0.90 and 99.69% ± 1.05, respectively. The assay results obtained for the marketed formulation were in accordance with label claim.

A simple, accurate, precise, specific and sensitive high performance thin layer chromatography method has been developed and validated for simultaneous estimation of CEF and DCX in tablets. It does not suffer from interference from common excipients present in the pharmaceutical formulation and can be conveniently adopted for routine quality-control analysis CEF and DCX in tablet dosage form.

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