Validated stability-indicating high-performance thin-layer chromatographic method for estimation of cefpodoxime proxetil in bulk and in pharmaceutical formulation according to International conference on harmonization guidelines

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ABSTRACT

Aim: A simple, selective, precise, and stability-indicating high-performance thin-layer chromatographic (HPTLC) method for analysis of cefpodoxime proxetil both in bulk and in pharmaceutical formulation has been developed and validated. Materials and Methods: The method employed HPTLC aluminum plates precoated with silica gel 60 RP-18 F254 as the stationary phase. The solvent system consisted of toluene:methanol:chloroform (4:2:4 v/v). The system was found to give compact spot for cefpodoxime proxetil (Rf value of 0.55 ± 0.02). Densitometric analysis of cefpodoxime proxetil was carried out in the absorbance mode at 289 nm. Results: The linear regression analysis data for the calibration plots showed good linear relationship, with r2 = 0.998 ± 0.0015 with respect to peak area in the concentration range of 100–600 ng per spot. The mean value ± SD of slope and intercept were 3.38 ± 1.47 and 986.9 ± 108.78 with respect to peak area. The method was validated for precision, recovery, and robustness. The limits of detection and quantification were 3.99 and 12.39 ng per spot, respectively. Cefpodoxime proxetil was subjected to acid and alkali hydrolysis, oxidation, and thermal degradation. The drug undergoes degradation under acidic and basic conditions, indicating that the drug is susceptible to both acid and base. The degraded product was well resolved from the pure drug, with significantly different Rf value. Statistical analysis proves that the method is repeatable, selective, and accurate for the estimation of the investigated drug. Conclusion: The proposed HPTLC method can be applied for identification and quantitative determination of cefpodoxime proxetil in both bulk drug and pharmaceutical formulation.

KEY WORDS: Cefpodoxime proxetil, degradation, High performance thin layer chromatography (HPTLC), stability, validation

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closely related to cefmetazole and cefpodoxime proxetil contaminants in pharmaceutical manufacturing environments, development and validation of isomer-specific Reverse Phase-HPLC method, spectrophotometric determination of cefpodoxime proxetil in swab samples, and determination of cefpodoxime proxetil in pharmaceutical formulations by densitometry thin layer chromatography (TLC).

To the best of our knowledge, there are no publications related to the stability-indicating chromatographic determination of cefpodoxime proxetil by high-performance thin-layer chromatographic (HPTLC) in pharmaceutical dosage forms. The International Conference on Harmonization (ICH) guidelines entitled 'Stability Testing of New Drug Substances and Products' requires that stress testing be conducted to elucidate the inherent stability characteristics of the active substance. Susceptibility to oxidation is one of the required tests. Hydrolytic and photolytic stability are also required to be tested. An ideal stability-indicating method is one that quantifies the drug and also resolves its degradation products. In HPTLC, the consumption of mobile phase per sample is quite low than HPLC. This saves cost per analysis as well as analysis time. HPTLC facilitates repeated detection (scanning) of the chromatogram with the same or different parameters. The HPTLC technique is most suited for testing the impurity profile of drug substances and content uniformity as per compendial specifications.

The aim of this work was to develop an accurate, specific, and repeatable stability-indicating method for the determination of cefpodoxime proxetil in the presence of its degradation products as per ICH guidelines.

Materials and Methods

Chemicals and reagents

Cefpodoxime proxetil was supplied as a gift sample from Torrent Pharmaceutical Ltd., Ahmedabad, India. All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, India.

HPTLC instrumentation

The samples were spotted in the form of bands of 6 mm width with a CAMAG microliter syringe on aluminum plates precoated with silica gel 60 RP-18 F\textsubscript{254} (10 × 10 cm, with 250 mm thickness; E. Merck), using a CAMAG Linomat 5 applicator. The plates were prewashed with methanol and activated at 60°C for 5 min prior to chromatography. The slit dimension was kept at 6.00 × 0.45 mm (micro), and 20 mm/s scanning speed was employed. The mobile phase consisted of chloroform:methanol:toluene (4:2:4 v/v); 10 ml of mobile phase was used. Linear ascending development was carried out in a 10 × 10 cm twin-trough glass chamber (CAMAG, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 30 min at room temperature (25 ± 2°C). The length of the chromatogram run was approximately 8 cm. Before the development, the TLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed on a CAMAG TLC scanner 3 and was operated by winCATS software.

Preparation of standard solution and linearity study

An accurately weighed quantity of 10 mg cefpodoxime proxetil was transferred to a 10 ml volumetric flask and dissolved in methanol. The volume was made up to the mark with the same solvent to obtain a concentration of 1000 ng/μl. Aliquots of 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 μl of standard solutions of cefpodoxime proxetil were applied on the TLC plate with the help of a microliter syringe, using a Linomat 5 sample applicator to obtain the concentrations of 100, 200, 300, 400, 500, and 600 ng per spot. The standard curves were evaluated for within-day and day-to-day reproducibility. Each experiment was repeated six times.

Method validation

Precision

Repeatability of the sample application and measurement of peak area were carried out using six replicates of the same spot (400 ng per spot of cefpodoxime proxetil). The intra- and inter-day variation for the determination of cefpodoxime proxetil was carried out at three different concentration levels of 500, 400, and 500 ng per spot.

Limit of detection (LOD) and limit of quantification

In order to determine the limits of detection and quantification, cefpodoxime proxetil concentrations in the lower part of the linear range of the calibration curve were used. Cefpodoxime proxetil solutions of 100, 120, 140, 160, 180, and 200 ng per spot were prepared and applied in triplicate. The limit of quantification (LOQ) and limit of detection (LOD) were calculated using the equations LOD=3.3 × N/B and LOQ=10 × N/B, where N is the standard deviation of the peak areas of the drugs (n=5), and B is the slope of the corresponding calibration curve.

Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for cefpodoxime proxetil in the sample was confirmed by comparing the R\textsubscript{i} values and spectra of the spot with that of the standard. The peak purity
of cefpodoxime proxetil was assessed by comparing the spectra at three different levels, i.e., peak start (S), peak apex (M), and peak end (E) positions of the spot.

**Ruggedness**

Ruggedness of the method was performed by spotting 400 ng of cefpodoxime proxetil by two different analyst keeping same experimental and environmental conditions.

**Accuracy**

Recovery study was carried out by adding extra 320 (80%), 400 (100%), and 480 ng (120%) of the drug standards of cefpodoxime to the prestudied sample of 400 ng, and the mixtures were reanalyzed by the proposed method. At each level six determinations were performed. This was done to check the recovery of the drug at different levels in the formulations.

**Robustness**

Small changes in the mobile phase composition were introduced and the effects on the results were examined. Mobile phases having different composition of chloroform:methanol:toluene (±2:4 v/v) were tried out and chromatograms were run. The amount of mobile phase, temperature, and relative humidity was varied in the range of ±5%. The plates were prewashed with methanol and activated at 60 ± 5°C for 2, 5, and 7 min prior to chromatography. Time from spotting to chromatography and from chromatography to scanning was varied from 0, 20, and 40 min.

**Application of proposed method to tablet formulation**

To determine the concentration of cefpodoxime proxetil in tablets (labeled claim: 200 mg per tablet), the contents of 20 tablets were weighed, the mean weight was determined, and they were then finely powdered. The powder equivalent to 10 mg of cefpodoxime proxetil was weighed. The drug from the powder was extracted with methanol. To ensure complete extraction of the drug, it was sonicated for 30 min. The volume was made up to 10 ml and the solution was filtered using 0.45 μm filter (Mill filter, Milford, MA). The above solution (400 ng per spot) was applied on a TLC plate followed by development and scanning as described in experimental section. The analysis was repeated in triplicate.

**Forced degradation of cefpodoxime proxetil**

**Acid- and base-induced degradation**

Ten milligrams of cefpodoxime proxetil was separately dissolved in 10 ml of methanolic solution of hydrogen peroxide (6.0%, v/v). The solution was kept for 8 h at room temperature in the dark in order to exclude the possible degradative effect of light. The resultant solution was applied on a TLC plate in triplicate (0.4 μl each, i.e., 400 ng per spot). The chromatograms were run as described in Experimental Section.

**Photochemical degradation**

The photochemical stability of the drug was also studied by exposing the stock solution to direct sunlight for 24 h. The resultant solution (0.4 μl, i.e., 400 ng per spot) was applied on a TLC plate and chromatograms were run as described in Experimental Section.

**Dry heat-degradation product**

The powdered drug that was stored at 55°C for 8 h under dry heat condition showed no significant degradation. In all degradation studies, the average peak areas of cefpodoxime proxetil after application (400 ng per spot) of three replicates were obtained.

**Results and Discussion**

**Development of optimum mobile phase**

The TLC procedure was optimized with a view to developing a stability-indicating assay method. Initially, toluene: Methanol (4:2 v/v) gave good resolution, with Rf value of 0.55 for cefpodoxime proxetil, but a typical peak nature was missing. Finally, the mobile phase consisting of toluene:methanol:chloroform (4:2:4 v/v) gave a sharp and well-defined peak at Rf value of 0.55 [Figure 2]. Well-defined spots were obtained when the chamber was saturated with the mobile phase for 30 min at room temperature.
**Calibration curve**

The linear regression data for the calibration curves showed good linear relationship over the concentration range of 100–600 ng/spot. The linear regression equation was found to be \( Y = 4.358X + 125.7 \) \( (r^2 = 0.995) \).

**Validation of method**

**Precision**

The precision of the developed HPTLC method was expressed in terms of percentage relative standard deviation (%RSD). The results reveal the high precision of the method (Table 1).

**LOD and LOQ**

Detection limit and quantification limit was calculated by the method as described in Experimental Section. The LOQ and LOD were found to be 3.99 and 12.11, respectively. This indicates that the method has adequate sensitivity.

**Recovery studies**

The proposed method, when used for extraction and subsequent estimation of cefpodoxime proxetil from the pharmaceutical dosage form after over-spotting with 80%, 100%, and 120% of additional drug, afforded good recovery of cefpodoxime proxetil. The amounts of drug added and determined as well as the percentage recovery are listed in Table 2.

**Specificity**

The peak purity of cefpodoxime proxetil was assessed by comparing the spectra at peak start, peak apex, and peak end positions of the spot, i.e., \( r^2 (S, M) = 0.999 \) and \( r^2 (M, E) = 0.9988 \). Good correlation \( (r^2 = 0.99) \) was also obtained between standard and sample spectra of cefpodoxime proxetil [Figure 3]. The results of specificity shows that the excipients from the tablet formulation do not interfere in the study.

**Robustness of the method**

The standard deviation of the peak areas and \( R_s \) values were calculated for each parameter and %RSD was found to be less than 2%. The low values of %RSD values (Table 3) indicate the robustness of the method.

**Analysis of the marketed formulation**

A single spot at \( R_s 0.55 \) was observed in the chromatogram of the drug samples extracted from the tablets. There was no interference from the excipients commonly present in the tablet. The percentage drug content and %RSD were calculated. The low %RSD value indicates the suitability of this method for routine analysis of cefpodoxime proxetil in pharmaceutical dosage forms.

**Force degradation**

The chromatogram of the acid-degraded samples for cefpodoxime proxetil showed additional peaks at \( R_s \) values of 0.10 and 0.44 [Figure 4] and the base-degraded drug showed peaks at 0.03, 0.15, and 0.17 [Figure 5]. The spot of the degraded product was well resolved from the cefpodoxime proxetil spot. In both cases, the concentration of the drug was changed from

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**Table 1: Intra-day and inter-day precision of HPTLC method**

| Drugs | Conc. (ng/spot) | Intra-day | Inter-day |
|-------|----------------|-----------|-----------|
|       | % amount found* | %RSD      | % amount found* | %RSD |
| CEF   | 300            | 97.22     | 0.45      | 95.76 | 1.80 |
|       | 400            | 99.84     | 0.35      | 98.7  | 0.37 |
|       | 500            | 97.74     | 0.28      | 96.23 | 0.24 |

HPTLC: High-performance thin-layer chromatographic, CEF: Cefpodoxime proxetil. *Mean of three estimations

**Table 2: Recovery study**

| Drug/label claim (mg tablet) | Initial amt. (ng/spot) | Added amt. (ng/spot) | Amt. of standard drug added (%) | Drug recovered | %RSD |
|-----------------------------|------------------------|----------------------|---------------------------------|----------------|------|
| CEF/200                     | 400                    | 0                    | 98.40                           | 1.02           |
|                             | 400                    | 320                  | 80                              | 97.39          | 1.80 |
|                             | 400                    | 400                  | 100                             | 103.84         | 0.98 |
|                             | 400                    | 480                  | 120                             | 103.72         | 1.33 |

CEF: Cefpodoxime proxetil. *Mean of three estimations at each level

**Table 3: Robustness of the method**

| Parameter                        | SD of peak area \( R_s \) value | %RSD          |
|----------------------------------|---------------------------------|---------------|
| Mobile phase composition         | 44.52/0.02                      | 0.90/0.98     |
| Mobile phase volume              | 39.82/0.04                      | 0.81/1.2      |
| Development distance             | 32.37/0.02                      | 0.65/0.96     |
| Activation of TLC plate          | 39.84/0.01                      | 0.81/0.75     |
| Duration of saturation           | 29.79/0.02                      | 0.70/0.86     |
| Time from spotting to chromatography | 21.16/0.03                     | 0.50/1.02     |
| Time from chromatography to scanning | 23.34/0.02                     | 0.43/0.97     |

\( n=6 \), TLC: Thin layer chromatography

**Figure 3: A typical overlain spectrum of standard drug and drug extracted from tablet**
the initial concentration, indicating that cefpodoxime proxetil undergoes degradation under acidic and basic conditions. This indicates that the drug is susceptible to acid–base hydrolysis. The chromatograms of hydrogen peroxide–degraded [Figure 6], photo-degraded [Figure 7], and dry heat–degraded [Figure 8] samples of cefpodoxime proxetil showed only the spots of the pure drug. The lower Rf values of degraded components indicate that they were less polar than the analyte itself. The results are listed in Table 5.

**Conclusion**

The developed HPTLC method was precise, specific, accurate [Table 4], and stability-indicating, and was validated based on ICH guidelines. Statistical analysis proves that the method is repeatable and selective for the analysis of cefpodoxime proxetil as bulk drug as well as in pharmaceutical formulations.

### Table 4: Summery of validation parameter

| Parameter data                              | Cefpodoxime proxetil |
|---------------------------------------------|----------------------|
| Linearity range (ng per spot)               | 100–600              |
| Correlation coefficient                     | 0.998                |
| Limit of detection (ng per spot)            | 3.99                 |
| Limit of quantification (ng per spot)       | 12.11                |
| Recovery (n=6)                              | 100.83               |
| Ruggedness (%RSD)                           |                      |
| Analyst I (n=6)                             | 1.13                 |
| Analyst II (n=6)                            | 1.63                 |
| Precision (%RSD)                            |                      |
| Repeatability of application (n=6)          | 0.96–1.29            |
| Inter-day (n=6)                             | 0.56–1.50            |
| Intra-day (n=6)                             | 0.21–1.62            |
| Robustness                                  | Robust               |
| Specificity                                 | Specific             |

The developed HPTLC method was precise, specific, accurate [Table 4], and stability-indicating, and was validated based on ICH guidelines. Statistical analysis proves that the method is repeatable and selective for the analysis of cefpodoxime proxetil as bulk drug as well as in pharmaceutical formulations.

**Figure 4:** HPTLC chromatogram of acid (0.1 N HCl, 8 h, RT)–treated cefpodoxime proxetil; peak 1 (impurity) (Rf: 0.10), peak 2 (impurity) (Rf: 0.44), peak3 (cefpodoxime proxetil) (Rf: 0.55)

**Figure 5:** HPTLC chromatogram of base (0.5 N NaOH, 8 h, RT)–treated cefpodoxime proxetil; peak 1 (impurity) (Rf: 0.03), peak 2 (impurity) (Rf: 0.15), peak 3 (cefpodoxime proxetil) (Rf: 0.17), peak 4 cefpodoxime proxetil (Rf: 0.56)

**Figure 6:** HPTLC chromatogram of hydrogen peroxide (20% w/v, 8 h, RT)–treated cefpodoxime proxetil; peak 1 (cefpodoxime proxetil) (Rf: 0.55)

**Figure 7:** HPTLC chromatogram of photo-degraded (24 h) cefpodoxime proxetil; peak 1 (cefpodoxime proxetil) (Rf: 0.55)
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The method can be used to determine the purity of the drug available from various sources by detecting the related impurities. As the method separates the drug from its degradation products, it can be employed as a stability-indicating test.

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