Newer HPTLC Method for Estimation of Cefixime, Cefpodoxime, Cefepime From Their Dosage Form

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ABSTRACT

Cefixime, cefpodoxime and cefepime are cephalosporin antibiotics used widely in the different infectious diseases. Newer HPTLC methods were developed for their estimation from individual dosage forms due to versatile applications and advantages. The HPTLC chromatogram of cefixime was developed by using a mobile phase ethylene acetate: methanol: water (4.5:5:0.5%v/v) and scanning wavelength was 292nm. The Rf value was 0.58±0.02. The mobile phase optimized for cepodoxime was methanol: ethyl acetate: toluene (1.5:3:5.5% v/v), with the Rf value 0.53±0.02. The cefixime was retained using methanol :water : chloroform (6:3:1%v/v), on a silica gel G₆₀ F₂₅₄ aluminium sheet and scanning wavelength kept at 285nm. The Rf value was 0.44. The methods were optimized validated as per guidelines and successfully applied for individual dosage form containing of each of cephalosporin.

Keyword: HPTLC, Cefixime, Cepodoxime, Cefepime.

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INTRODUCTION

HPTLC\textsuperscript{1,2} is a versatile technique that is explored for numerous drug analysis applications. It is cost effective and quicker chromatographic method when compared to others. Cephalosporins\textsuperscript{3} like cefixime, cefepime and cepodoxime were used as potential antibiotics. Cefixime is chemically, (6R,7R)-7-(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-[(carboxymethoxy)imino]acetamido]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. It is a third generation cephalosporin drug. It used as an antibiotics by inhibiting biosynthesis and arresting cell wall assembly resulting in bacterial cell death. cepodoxime is chemically (6R,7R)-7-(2Z)-2-(-1,3-thiazol-4yl)-2-(methoxyimino)acetamido]-3-(methoxymethyl)-8-oxo-5-thia-1azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. It is a third generation cephalosporin drug. It used as a antibiotics work by inhibition of bacterial cell wall synthesis. Cefepime is chemically, 1-\{[(6R,7R)-7-(2Z)-2-(2-amino-1,3-thiazol-4yl)-2-[(carboxymethoxy)imino]acetamido]-2-carboxylato-8-oxo-5 thia 1 azabicyclo 4.2.0] oct-2-ene-3-ylmethyl\}-1-methylpurrolidin-1-iun. It is a fourth generation cephalosporin drug. It provides bactericidal activity by interfering with bacterial cell wall synthesis and inhibiting cross linking of the peptidoglycan. Literature reveals few analytical works\textsuperscript{4-9} for estimation of cefepime, cepodoxime and no HPTLC method available for cefixime. This paper describes development of HPTLC for determination cefixime, cepodoxime, cefepime for the bulk and pharmaceutical dosage form.

MATERIAL AND METHOD

Solvent and chemical used

Water, methanol, toluene, chloroform, ethyl acetate were supplied by S.D.Fine chemicals PVT Ltd., Maharashtra, India.

Materials Used

Pre-coated silica gel G\textsubscript{60} F\textsubscript{254} aluminium sheets were procured from Merck, Germany.

Instruments used

Shimadzu digital electronics balance, Camag HPTLC system (with TLC scanner-3, Win CATS software and Linomat 5 as a application device.

HPTLC METHOD

Method development

Cefexime, cepodoxime, cefepime they were retained on HPTLC plate with optimized mobile phase system.

Preparation of standard solutions
A quantity of 10 mg of cefixime, cepodoxime, cefepime were weighed into individual 10 ml volumetric flasks and dissolved in methanol, further diluted to the mark (1000 µg/ml). These were used as standard stock solutions. A working standard solution containing 100 µg/ml of each was prepared.

The validation of the developed method was carried out in terms of linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ), inter and intra day precision and stability studies as per ICH guidelines.

**Linearity and range**

A 100 µl/ml solution of cefixime, cepodoxime and cefepime were used for establishing the linearity and range. Aliquots of 2 - 4.5µl of cefixime, 1- 3 µl of cepodoxime and 5- 25µl of cefepime were applied were on individual HPTLC plates. The plates were developed with respective mobile phase, scanned and peak areas were noted. The linear regression data have shown a good relation correlation coefficient over a concentration range used for three drugs.

**Accuracy**

In order to ensure the suitability and reliability of proposed methods for three drugs, recovery studies were carried out. It was done by mixing known quantities of individual standard drug with their pre analysed sample formulation and the contents were reanalyzed by proposed method. Recovery studied were carried out at 80% and 120% levels.

**Precision**

It was demonstrated by Intra-day precision, Inter-day precision, Repeatability of sample application and repeatability of sample measurement.

**Intra-day precision**

Intra-day precision was studied by different concentration in linearity range of the drugs for several times on the same days and by calculating %RSD.

**Inter-day precision**

Inter-day precision was studied by standard drug at two concentration in the linearity range of the drug for three days over a period of one week and % RSD was calculated.

**Repeatability**

Repeatability of sample application was carried by spotting 3.5 µl of drug solution is six times on precoated TLC plate followed by development of plate and %RSD was calculated for three drugs. Repeatability of measurement was determined by spotting 3.5µl of drug solution on a precoated TLC plate and developed the plate and scanned six times and %RSD was calculated.
Robustness

The robustness of the method is its ability to remain unaffected by small change in practical conditions. Here the effect of change in condition such as ratio of mobile phase (±0.5min) and saturation time (±5min) were studied to prove robustness.

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ were determined by applying decreasing amount of the drug in triplicate on the plate. The lower concentration at which the peak is detected is called limit of detection. The lowest concentration at which the peak is quantified is called limit of quantification.

Stability of chromatographic plate

When the developed chromatographic plate is exposed to atmosphere the average are likely to decompose. Hence it is necessary to study the stability of drug on plate. It was studied by scanning the plate at different time intervals and peak areas were compared with the plate area of freshly scanned plate. The developed plate was found to be stable for 8 hour for cefexime, cefpodoxime, cefepime for about 24 hour.

Analysis of formulation

Twenty tablets each containing 200mg of cefixime were taken and average weight was calculated. They were finely pulverized and the quantity of homogenized powder equivalent to 10mg of cefixime was transferred to a 100ml volumetric flask, it was dissolved with methanol, sonicated and made up to volume with methanol. The solution was filtered. Similar procedure was employed for tablets containing 200 mg of cepodoxime. For cefpime ten formulation of powder for injection (1g/10ml) of cefpime was taken and the average weight was calculated, The quantity of homogenized powder equivalent to 10mg of cefepime was transferred to a 100ml volumetric flask and up to volume with methanol Then the solution was filtered using whatmann filter paper. All these three formulation solutions were applied on individual pre coated TLC plate. After developed the plate was scanned at 292nm for cefixime, cepodoxime and 285nm for cefepime the peak areas were noted. The amount of cefixime, cepodoxime, cefepime present in their respective formulation was calculated.

RESULTS AND DISCUSSION

Novel HPTLC method has been developed for three cephalosporins cefixime, cepodoxime, cefepime for their estimation from individual dosage form and validated as per guidelines and ascertain the purpose.
The validation parameters for three drugs are listed in table 1. The typical densitogram of cefixime, cepodoxime, cefepime are shown in fig 1-3. The result of analysis cefixime, cepodoxime, cefepime in formulation by HPTLC analysis are shown in table 2.

![Figure 1: Densitogram of cefixime 200(ng/band)](image1)

![Figure 2: Densitogram of cepodoxime 100(ng/band)](image2)
Table 1. Validation parameters of cephalosporins

| Parameter                          | Cefixime (ng/band) | Cefpodoxime (ng/band) | Cefepime (ng/band) |
|-----------------------------------|--------------------|-----------------------|--------------------|
| Linearity                         | 200–450            | 100–300               | 500–2500           |
| Correlation coefficient           | 0.998              | 0.999                 | 0.998              |
| Slope                             | 11.21              | 8.124                 | 1.2                |
| Intercept                         | 593.815            | 293.581               | 85.12              |
| Precision (% RSD)                 |                    |                       |                    |
| Interday precision (n=3)          | 1.4–1.9            | 1.2–1.6               | 0.4–0.6            |
| Intraday precision (n=3)          | 0.2–0.9            | 1.4–1.6               | 0.3–0.8            |
| Repeatability of peak area (n=6)  | 0.6                | 1.4                   | 1.1                |
| Accuracy                          |                    |                       |                    |
| % recovery method (% RSD)         | 0.78–0.87          | 0.51–0.82             | 0.27–0.054         |
| Limit of detection (LOD) (ng/band)| 40                 | 60                    | 120                |
| Limit of quantification (LOQ) (ng/band) | 100             | 100                   | 250                |

Parameter Table 1. Validation parameters of cephalosporins
| Parameter                              | Limit of quantification (LOQ) (ng/band) | Parameter                              | Limit of quantification (LOQ) (ng/band) |
|---------------------------------------|----------------------------------------|---------------------------------------|----------------------------------------|
| Parameter                             | 100                                    | Parameter                             | 100                                    |
| Linearity (ng/band)                   | 200 - 450                              | Linearity (ng/band)                   | 100 - 300                              |
| Correlation coefficient               | 0.998                                  | Correlation coefficient               | 0.999                                  |
| Slope                                 | 11.21                                  | Slope                                 | 8.124                                  |
| Intercept                             | 593.815                                | Intercept                             | 293.581                                |
| Precision (ng/band)                   | 1.4 – 1.9                              | Precision (ng/band)                   | 1.2                                    |
| Interday precision (n=3)              | 0.2 – 0.9                              | Interday precision (n=3)              | 1.4 – 1.6                              |
| Intraday precision (n=3)              | 0.6                                    | Intraday precision (n=3)              | 1.4                                    |
| Repeatability of peak area (n=6)      | 0.6                                    | Repeatability of peak area (n=6)      | 1.4                                    |
| Accuracy % RSD                        | 0.78 – 0.87                            | Accuracy % RSD                        | 0.51 – 0.82                            |
| % recovery method (% RSD)             | 0.27 – 0.54                            | % recovery method (% RSD)             | 0.27 – 0.54                            |
| Limit of detection (LOD) (ng/band)    | 40                                     | Limit of detection (LOD) (ng/band)    | 60                                     |
| Limit of quantification (LOQ) (ng/band)| 100                                   | Limit of quantification (LOQ) (ng/band)| 100                                   |

Table 2. Result of formulation analysis of cephalosporins

| Label amount | Amount found | % label claim | Formulation | % RSD |
|--------------|--------------|---------------|-------------|-------|
| 200 mg/tablet| 195.2mg/tablet| 97.6          | cefixime    | 0.6   |
| 200 mg/tablet| 195.2mg/tablet| 97.6          | cefpodoxime | 0.96  |
| 1gm/10ml vial| 997.2gm/10ml | 99.7          | cefepime    | 0.37  |

CONCLUSION

Novel newer HPTLC methods were developed for the estimation of cefixime, cefpodoxime, cefepime and successfully applied to their formulation.

There were no interference found from matrix during analysis and linearity accuracy and precision of the method were good.

The method developed for cephalosporin (cefixime, cefpodoxime, cefepime) can be used in industry for routine analysis to check the amount of the drug present in pharmaceutical dosage form.

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