Development of RP-HPLC methods for the Estimation of Cefixime in Bulk Drugs and in Pharmaceutical Dosage Forms

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Two simple and sensitive reverse phase high performance liquid chromatographic methods (A and B) have been developed for the estimation of cefixime in bulk form and in pharmaceutical formulations. The quantification was carried out using a RP silica column and RP C18 column in isocratic mode for methods A and B respectively. The mobile phase was made up of acetonitrile and water in the ratio of 3 : 2 (v/v). Beclomethasone dipropionate was used as an internal standard. The detection was carried out at 254 nm and the linearity was found to be in the range of 3–15 µg/mL. The methods were duly validated by evaluation of the required parameters.

Key Words: RP-HPLC, Cefixime, Pharmaceutical dosage forms.

INTRODUCTION

Cefixime (CFX) is a semi-synthetic, cephalosporin antibiotic for oral administration. Chemically, it is (6R and R)-7-[2-(2-amino-4-thiazolyl)glyoxylamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7^2-(Z)-[O-(carboxymethyl)oxime]trihydrate. Its empirical formula is $C_{16}H_{13}N_5$-$Na_2O_7S_2$ and molecular weight is 507.50 as trihydrate. CFX is highly stable in the presence of β-lactamase enzymes. As a result, many organisms resistant to penicillins and some cephalosporins due to the presence of beta-lactamases may be susceptible to CFX. Literature survey reveals that very few HPLC methods[1, 2] were reported for CFX. The proposed method was accurate and precise for the estimation of cefixime in bulk as well as in pharmaceutical formulations.

EXPERIMENTAL

An isocratic high performance liquid chromatograph (Schimadzu) with two LC-10AS pumps, variable wavelength programmable UV-Visible detector SPD-10A, chromatopac integrator CR6A, 20 µL Rheodyne 7125 loop injector and RP silica column or RP C18 column (250 × 4.6 mm i.d., particle size 10 µm) were used. Cefixime and beclomethasone dipropionate were gift samples from Cipla Labs. Acetonitrile (Qualigens) used in the experiment was of AR grade. Triple distilled water was used throughout the experiment.

Chromatographic conditions: The chromatographic column used was 250 × 4.6 mm Techsphere silica or Techsphere ODS with 10 µm particles. Both acetonitrile and water were filtered through 0.45 µm membrane filter and sonicated before use. The HPLC equipment was operated at ambient temperature. The attenuation was set at 6. The range was set at 0.002 AUFS (met A) or 0.001 AUFS (met B) with chart speed of 5 mm/min. The flow rate of the mobile phase was maintained at 1.0 mL/min. Detection was carried out by UV detector at 254 nm and the injection volume was 20 µL.

Preparation of internal standard solution: About 100 mg of beclomethasone dipropionate reference standard was dissolved in 100 mL of HPLC grade methanol to get 1 mg/mL solution. It was further diluted with mobile phase to prepare an internal standard of 100 μg/mL. The solution was sonicated for 30 min.

Assay procedure for methods A and B: About 100 mg of pure sample of CFX was weighed accurately and dissolved in 100 mL of HPLC grade water to get 1 mg/mL solution. It was further diluted to prepare a standard solution of 100 μg/mL. The solution was sonicated for 30 min. Subsequent dilutions of this solution were made after addition of beclomethasone dipropionate (100 μg/mL) as an internal standard (IS) to get concentrations of 3–15 μg/mL of CFX and 10 μg/mL of IS in each dilution. The solutions prepared as above were filtered through 0.45 μm membrane filter and then 20 μL of filtrate was injected five times into the column (Techsphere silica for method A and Techsphere ODS for method B) at a flow rate of 1.0 mL/min. The ratio of drug peak area to that of internal standard for each of the drug concentrations was calculated. The regression of the drug concentration over the ratio of drug peak area to that of internal standard was obtained. This regression equation was used to estimate the amount of CFX in tablet dosage forms.

Estimation of CFX in tablet dosage forms: About 20 tablets were pulverized and the powder equivalent to 100 mg of CFX was weighed, dissolved in 100 mL of HPLC grade water. The insoluble portion was filtered through a 0.45 μm membrane filter. The filtrate was further diluted to prepare a solution of 100 μg/mL and sonicated for about 30 min. The procedure followed was same as that of standard assay procedure. The mean peak area ratios of the drug to the internal standard of five determinations were calculated and the drug content in the tablets was quantified using the standard graph of the pure sample.

RESULTS AND DISCUSSION

The present study was carried out to develop two simple, rapid, accurate and precise HPLC methods for the analysis of CFX in pharmaceutical dosage forms. The retention times for CFX and internal standard (beclomethasone dipropionate) were 3.54 and 5.52 min, respectively for method A and 1.64 and 6.22 min for method B. Each of the samples injected five times and the same retention times were observed in all cases. The ratio of the peak area of the CFX to peak area of internal standard for different concentrations set up as above were calculated and the average values for five such determinations are shown in Table-1.

TABLE-1
CALIBRATION OF THE PROPOSED METHODS

Drug concentration (μg/mL)	Mean peak area ratio of method A (n = 5)	Mean peak area ratio of method B (n = 5)
3.0	0.210	0.152
6.0	0.425	0.310
9.0	0.640	0.450
12.0	0.847	0.598
15.0	1.074	0.743

Regression equation (from 3.0 to 15.0 μg/mL).
$Y = 0.00387X + 0.00418$ (r = 0.9999) [Method A],
$Y = 0.00254X - 0.00182$ (r = 0.9999) [Method B].

Typical chromatograms were shown in Fig. 1. The peak areas of both the drug and internal standard were reproducible as indicated by low coefficient of variation (0.2479 for method A and 0.3638 for method B).

Model chromatogram of CFX   Model chromatogram of CFX
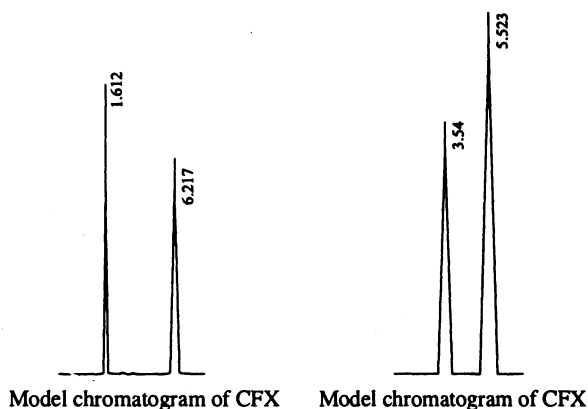
A good linear relationship (r = 0.9999) was observed between the concentration of the CFX and the respective ratio of peak areas. The calibration equation was found to be Y = 0.00387X + 0.00418 for method A and Y = 0.00254X − 0.00182 for method B (where Y is the ratio of peak area of drug to that of internal standard, X = concentration of CFX). The intra-day and inter-day variations of the methods were determined using five replicate injections of three different concentrations, which were prepared and analyzed on the same day and three different days over a period of two weeks, a low coefficient of variation was observed (Table-2). This shows that the present HPLC method is highly precise.

TABLE-2
PRECISION OF THE PROPOSED METHOD A

| Concentration of CFX (µg/mL) | Observed concentration of CFX (µg/mL) | | | |
| | Intra-day | | Inter-day | |
	Mean (n = 5)	% CV	Mean (n = 5)	% CV
6.0	6.02	0.38	·6.04	0.53
9.0	8.99	0.27	9.01	1.22
12.0	12.04	0.64	12.05	0.32

To ensure the reliability and accuracy of the method, recovery studies were carried out by mixing a known quantity of drug with preanalyzed sample and contents were reanalyzed by the proposed method. The values are shown in Tables 3 and 4. About 99.9% of CFX could be recovered from the preanalyzed samples indicating the high accuracy of the proposed HPLC methods.

The drug content in the tablets was quantified using the proposed analytical method. The mean amount of CFX in two different brands of tablet dosage forms is shown in Table-5. The absence of additional peaks in the chromatogram

indicates the non-interference of the common excipients used in the tablets. The tablets were found to contain 99.89–99.98% of the drug. It can be concluded that the proposed HPLC methods were sensitive and reproducible for the routine analysis of CFX in pharmaceutical dosage forms.

TABLE-3
PRECISION OF THE PROPOSED METHOD B

Concentration of CFX (µg/mL)	Observed concentration of CFX (µg/mL)			
	Intra-day		Inter-day	
	Mean (n = 5)	% CV	Mean (n = 5)	% CV
6.0	6.01	0.33	6.08	0.35
9.0	8.98	0.17	9.05	0.78
12.0	11.99	0.56	12.04	0.97

TABLE-4
RESULTS OF RECOVERY STUDY

Amount of drug added (µg)	Recovery from drug solution (µg)	Recovery from method A (µg)	Recovery from drug solution (µg)	Recovery from method B (µg)
3.0	3.02	2.99	3.03	3.01
9.0	9.01	9.02	9.02	8.99
15.0	14.98	15.01	14.99	15.04

TABLE-5
ASSAY OF CFX IN TABLET DOSAGE FORMS

Pharmaceutical formulation used	Labelled amount of drug (mg)	Mean (± s.d.) amount (mg) of method A	Mean (± s.d.) amount (mg) of method B	Mean (± s.d) amount (mg) found by the reference method
Tablet I	200	200.33 ± 0.66	199.18 ± 0.81	200.12 ± 0.46
Tablet II	200	199.87 ± 0.19	200.42 ± 0.46	200.12 ± 0.22

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