Original Article

New RP-HPLC method for the estimation of cefpirome sulfate in bulk and pharmaceutical dosage forms

Abstract

Aims: To develop and subsequently validate a simple reversed-phase high-performance liquid chromatography (HPLC) method for estimation of Cefpirome sulphate (CPS) present in pharmaceutical dosage forms. **Materials and Methods:** The proposed RP-HPLC method utilizes a LiChroCART-Lichrosphere100, C18 RP column (250 mm×4 mm×5 µm) in an isocratic separation mode with mobile phase consisting of methanol and 5mM Tetra butyl ammonium hydrogen sulphate in the proportion of 50:50 % (v/v), at a flow rate 1ml/min and the effluent was monitored at 270 nm using Doxophylline (DXP) as a internal standard. **Results:** The retention time of CPS and DXP were 2.26 min. and 3.78 minutes respectively. The described method was linear over a range of 0.5-300 µg/ml. The percentage recovery was 101.163. F-test and t-test at 95% confidence level were used to check the intermediate precision data obtained under different experimental setups; the calculated value was found to be less than critical value. **Conclusions**: The LC method described here is a very simple, sensitive, and accurate procedure for estimation of CPS. The developed and validated LC method is specific, accurate, robust and precise analysis of CPS in pure and its formulations.

Key words:
Assay, cefpirome sulfate, doxophylline, validation

Introduction

Cefpirome is a 1-[[7-[[(2-amino-4-thiazolyl) (methoxyimino) acetyl]-amino]-2carboxy -8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-en-3-yl]methyl]-6,7-dihydro-5H-1-pyrindinium hydroxide inner salt. It is a broad-spectrum semisynthetic β-lactamase-resistant fourth-generation cephalosporin having quaternary ammonium group at the 3 position of the cephem nucleus. It is used for the treatment of upper and lower urinary tract, lower respiratory tract, skin, and soft tissue infections. Cefpirome is excreted largely unchanged in the urine with a half-life of 2 hours.[1] It has an expanded spectrum of activity against *Pseudomonas sp*., *enterococci*, and *staphylococci*, as well as other Gram-positive and -negative bacteria.[2]

The proposed RP - High-Performance Liquid Chromatography (HPLC) method was validated by assessing its specificity, linearity, accuracy, precision, limits of detection and quantification, system suitability parameters, ruggedness, and robustness.

Many workers reported methods for the assay of cefpirome in pharmaceutical formulations, and in human serum.[3-8] These methods involve either complicated mobile phases, or buffers that may be corrosive to the column or flow system of HPLC. Previous investigators[9-12] have also involved various microbiological assay methods, including both microdilution and agar dilution, to quantitate cefpirome. Two studies[12,13] reported the pharmacokinetics of cefpirome in adults after quantitation of the compound in serum by using HPLC assay for which specific details were not provided.

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Materials and Methods

Chemicals and reagents

Gift sample of CPS was received from Alkem Labalories, Mumbai, India. HPLC grade methanol was purchased from Merck, India. Tetrabutylammonium hydrogen sulfate (TBHS) was purchased from Himedia, India. High pure water was prepared by using Millipore Milli Q plus purification system. Commercial formulations, Bacirom® (vial) and Forgen® (vial), containing 250 mg of CPS were purchased from the local market.

Experiment

HPLC instrumentation and conditions

Quantitative HPLC was performed on Shimadzu HPLC with LC 10 AT VP series pumps besides SPD 10 A VP ultraviolet (UV)-Visible detector. Shimadzu class VP version 6.12 SPI software was used along with LiChroCART-Lichrosphere100, C18, RP column (250 mm×4 mm×5 μm) for separation, maintained at ambient temperature, eluted with mobile phase at a flow rate of 1 ml/min for 10 minutes. The mobile phase consisted of methanol and 5 mM TBHS in the proportion of 50:50% (v/v) measurements were made with injection volume 20 μl and UV detection at 270 nm.

Preparation of mobile phase

Methanol and 5 mM TBHS (0.84885 g of TBHS was added to 500 ml of double-distilled water to make 5 mM solution of TBHS) were properly mixed in the ratio of 50:50.

Standard and sample preparation

The standard stock solution of CPS (1 mg/ml) was prepared by dissolving 25 mg each of CPS in 25 ml volumetric flask containing 10 ml of methanol and 10 ml of 5 mM TBHS. The solutions were sonicated for about 10 minutes and later diluted to desired volume with mobile phase. Standard calibration solutions of CPS having concentration in the range of 0.5 to 300 μg/ml were prepared by diluting stock solution with mobile phase. Similarly, stock solution of internal standard was prepared by dissolving 25 gm of Doxophylline in 25 ml volumetric flask containing 10 ml of methanol and 10 ml of 5 mM TBHS sonicated for 10 minutes and later diluted to desired volume with mobile phase. Working standard solutions of CPS were prepared by taking suitable aliquots of drug solution from the standard stock solution of 1 mg/ml, spiked with internal standard solution (0.1 ml from 1 mg/ml), and the volume was made up to 10 ml with mobile phase.

From the vial, equivalent to 25 mg of CPS content was transferred into a 25-ml volumetric flask containing 20 ml mixture of methanol and TBHS, ultrasonicated for 10 minutes, and then diluted up to the mark with mobile phase to yield sample stock solution. From this stock solution, suitable aliquots were taken so as to obtain a concentration in the range of linearity previously determined, and then an aliquot of the internal standard (0.1 ml from 1 000 μg/ml) was added to the sample solution prior to the dilution and the volume was further made up to the mark with mobile phase. All determinations were carried out in triplicate.

Results and Discussion

Optimization of the method

The proposed RP-HPLC method utilizes a LiChroCART-Lichrosphere100, C18 RP column (250 mm×4 mm×5 μm) in a isocratic separation mode with mobile phase methanol, and water in the proportion of 50:50% (v/v), at a flow rate of 1 ml/min and the effluent was monitored at 270 nm (λ_{max} for CPS). The retention time for CPS and DXP were 2.26 and 3.78 minutes, respectively.

Method validation

The described method has been validated for linearity, precision, accuracy, specificity, LOD and LOQ, system suitability parameters, ruggedness, and robustness.

Linearity

Least square regression analysis was carried out for the slope, intercept, and correlation coefficient [Table 1]. The linear fit of the system was illustrated graphically. The linearity range was found to be 0.5 to 300 μg/ml. Regression equation for CPS was, y=0.0735x+0.0023 (R^2=0.9998).

Accuracy

This experiment was performed at three levels, in which sample stock solutions were spiked with standard drug solution containing 80, 100, and 120% of labeled amount of the drugs (250 mg CPS) in vial, along with internal standard (10 μg/ml). Three replicate samples of each concentration level were prepared and the % recovery at each level (n=3) and mean % recovery (n=9) were determined [Table 2]. The mean recovery was 101.163%.

Precision

The precision of the proposed method was evaluated by carrying out eight independent assays of test sample. RSD (%) of eight assay values obtained was calculated. Intermediate precision was carried out by analyzing the

Table 1: Regression characteristics of the proposed HPLC method

Linearity experiment (n=5)	CPS
Range (μg/ml)	0.5-300
Mean "R" value	0.9998
Slope	0.0735
Intercept	0.0023

HPLC – High-performance liquid chromatography

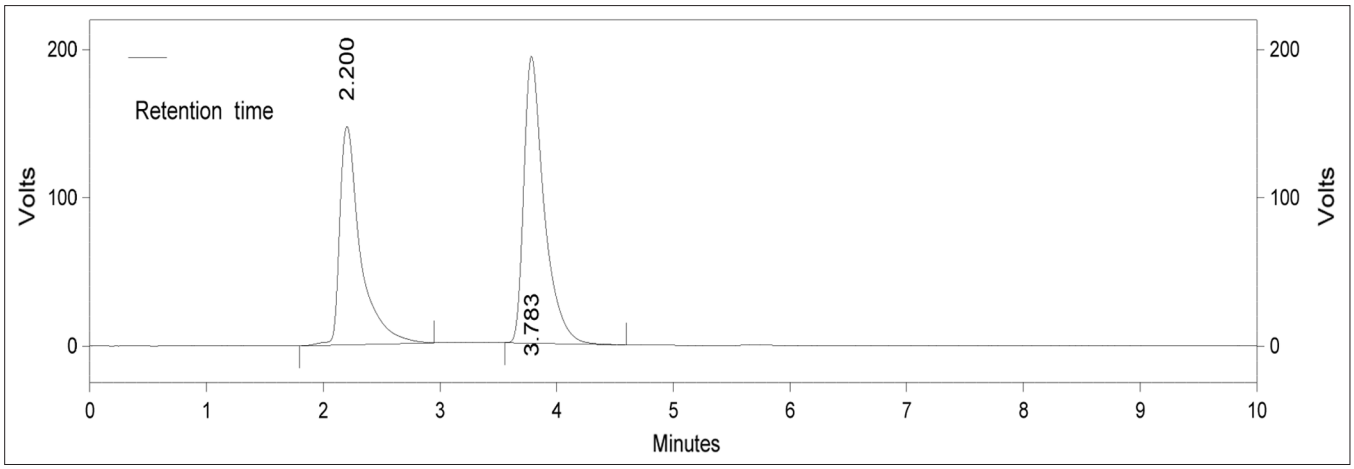

Figure 1: A typical chromatogram of CPS (10 μg/ml) and DXP (10 μg/ml) in reference standard

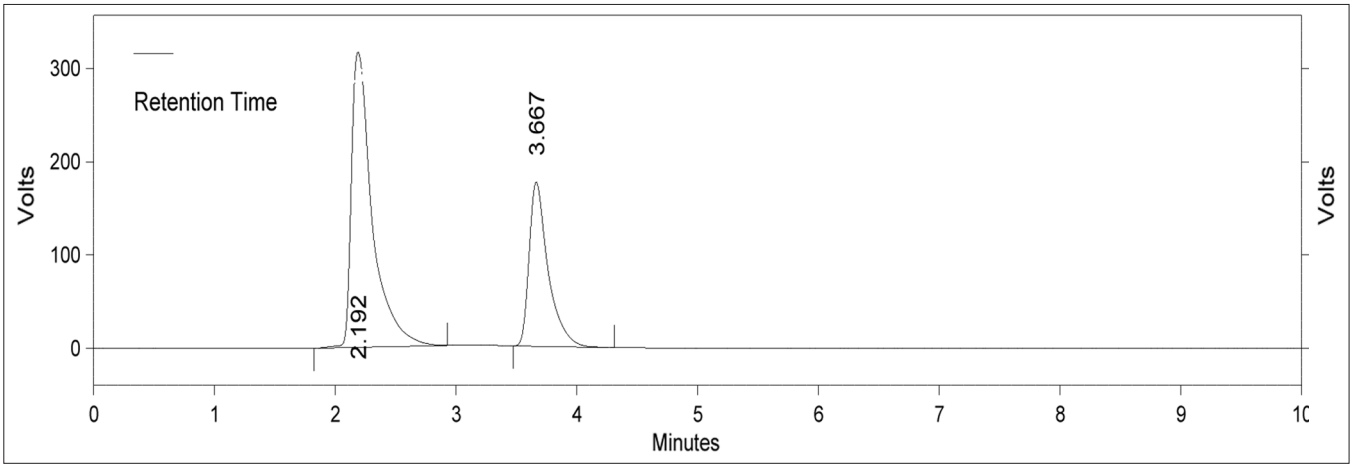

Figure 2: A typical chromatogram of CPS (20 μg/ml) and DXP (10 μg/ml) in reference standard

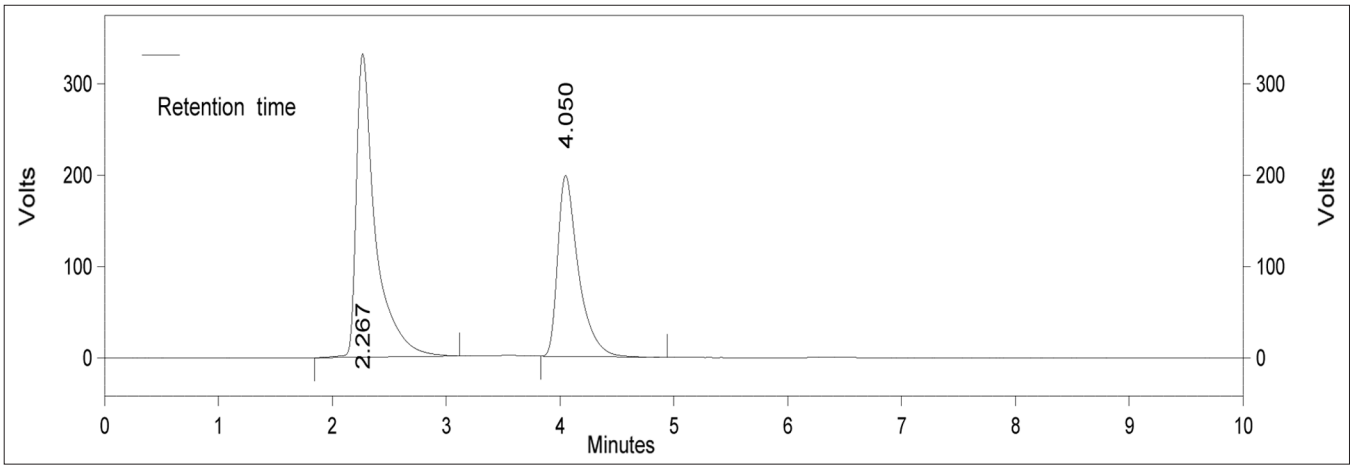

Figure 3: A typical chromatogram of CPS (20 μg/ml) (formulation) and DXP (10 μg/ml)

samples by a different analyst on another instrument. F-test and *t*-test was applied to the two sets of data at 95% confidence level, and no statistically significant difference was observed. The resultant data are presented in Table 3.

Specificity

Specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components that may be expected to present in the sample matrix. It was found that the proposed method was specific as there is no

Table 2: Results of accuracy experiment using proposed method

	CPS		
	Taken (μg)	Recovered (μg)	% Recovery
Level 1 (80)	8	8.12	101.51
Level 2 (100)	10	9.94	99.47
Level 3 (120)	12	12.3	102.51
Mean % recovery (*n*=9)		101.163±0.359	
% RSD		0.367	

interference of other active ingredients and excipients, ensuring that the peak response is due only to a single component.

LOD and LOQ

The detection and quantification limits were evaluated from calibration curves plotted in concentration range of 0.5 to 300 μg/ml. The acceptance criterion for these replicate injections was RSD not more than 30% for LOD concentration and not more than 10% for LOQ concentration. The formulae used were LOD=3.3σ/S and LOQ=10σ/S (where σ=Standard deviation of response and S=Slope of calibration curve). The standard drug solutions for each value of LOD and LOQ concentration were injected 5 times. % RSD values for the area of replicate injections were calculated. LOD and LOQ for this method were found to be 0.10 and 0.33, respectively. These values indicated that the method was very sensitive to quantify the drug.

System suitability parameters

System suitability parameters can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The system suitability parameters like Theoretical plates (N), Resolution (R), and Tailing factor (T) were calculated and compared with the standard values to ascertain whether the proposed RP-HPLC method for the estimation of CPS in pharmaceutical formulations was validated or not. System suitability is usually developed after method development and validation has been completed. The obtained value of Theoretical plates (N) in this method was 3 959, resolution between drug and internal standard was 2.08, and the tailing factor was found to be 1.558.

Robustness

The percentage recovery of CPS was good under most conditions and did not show any significant change when the critical parameters were modified. The tailing factor was always less than 2.0 and the components were well separated under all the changes carried out. Thus, the method conditions were robust.

Assay

The validated method was applied to the determination of CPS in commercially available Bacirom® (vial) and Forgen® (vial). Figures 1 to 3 illustrate typical HPLC chromatograms obtained from CPS and DXP standard solution and from the assay of Bacirom®. The observed concentration of CPS was found to be 249.03±0.512 mg (mean±SD) for Bacirom® and 248.88±0.562 for Forgen®. The results of the assay (*n*=9) undertaken yielded 99.61% (% RSD=0.2) of label claim for CPS in Bacirom® and 99.55% (% RSD=0.22) of label claim for CPS in Forgen®. The retention times for CPS and DXP were found to be 2.26 and 3.78 minutes, respectively, for standard drug and 2.267 and 4.05 minutes, respectively, for formulation. The results of the assay indicate that the method is selective for the estimation of CPS without interference from the excipients used to formulate and produce these tablets.

Table 3: Results of precision study

Precision	CPS
	Mean assay (%)/% R.S.D
Set 1 (*n*=4)	78.37/1.83
Set 2 (*n*=4)	78.73/1.69
	Calculated value/critical value
F-test	0.887/2.368
t-test	0.109/1.106

Conclusion

The LC method described here is a very simple, sensitive, and accurate procedure for estimation of CPS. The developed and validated LC method is specific, accurate, robust, and precise analysis of CPS in pure and its formulations. The method is sensitive enough for quantitative detection of the analyte in pharmaceutical preparations. The proposed method can thus be used for routine analysis, quality control, and for studies of the stability of pharmaceutical tablets containing these drugs. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non interference of formulation excipients in the estimation.

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