DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING HPLC METHOD FOR DETERMINATION OF CEFPIROME SULFATE

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Abstract: The stability-indicating LC assay method was developed and validated for quantitative determination of cefpirome sulfate (CPS) in the presence of degradation products formed during the forced degradation studies. An isocratic HPLC method was developed with Lichrospher RP-18 column, 5 µm particle size, 125 mm × 4 mm column and 12 mM ammonium acetate-acetonitrile (90 : 10 v/v) as a mobile phase. The flow rate of the mobile phase was 1.0 mL/min. Detection wavelength was 270 nm and temperature was 30°C. Cefpirome sulfate as other cephalosporins was subjected to stress conditions of degradation in aqueous solutions including hydrolysis, oxidation, photolysis and thermal degradation. The developed method was validated with regard to linearity, accuracy, precision, selectivity and robustness. The method was applied successfully for identification and determination of cefpirome sulfate in pharmaceuticals and during kinetic studies.

Keywords: method validation, stability-indicating method, cefpirome sulfate

Cefpirome sulfate (CPS, Fig. 1) is a new, parenteral, fourth generation cephalosporin. CPS is effective against Gram-positive bacteria including *Staphylococcus aureus* and Gram-negative microorganisms, such as *Pseudomonas aeruginosa* (1–3). The elements responsible for such spectrum of activity are 2-amino-thiazolylmethoxyimino group in a side-chain in position 7 and cyclopentapyridine in position 3. CPS is used in treatment of various infections such as pneumonia, sepsis, urinary tract infections, and intra-abdominal infections in adult patients (4–7). The suggested administration of cef-

pirome is 1–6 g daily from two to four divided doses (7). CPS like other cephalosporins have surprisingly few serious side effects, which make it attractive for use in the treatment of a wide variety of serious infections (8). The most common adverse symptoms are nonspecific circulatory disorders (chills, tachycardia, hypertension, nausea, dyspnea, cold perspiration, weak concentration and dizziness). All adverse effects are mild or have moderate severity, are of a short period, improve spontaneously, and recovery is complete. Simultaneously, most of the side effects of β-lactams are caused by their degra-
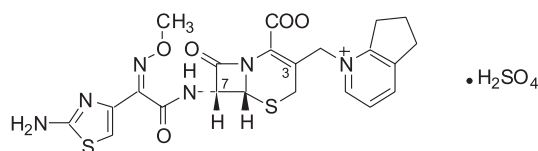

Figure 1. The chemical structure of cefpirome sulfate

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dation products thus it is so important to estimate the stability and mechanism of the degradation of this group of drugs. Stability studies are an integral part of the drug development process and are widely recognized as one of the most important procedures in pharmaceutical products registration (9–12). Previous studies confirm that cephalosporins are susceptible to degradation in aqueous solutions (13–19) and in a solid state (20–28). CPS in solution is stable in pH 4–7, slightly unstable below pH 3 and promptly degraded at pH 9 and higher (17). The degradation pathways in aqueous solutions were described (Fig. 2) (17). Developed chromatographic method for the determination of CPS had many disadvantages like significant organic solvent consumption or incompatibility to HPLC-MS water phase (29–31). The aim of this work was to develop and validate HPLC method with UV detection suitable for identification, determination, and stability study of CPS and its degradation products.

EXPERIMENTAL

Standards and reagents

CPS was obtained from CHEMOS GmbH Werner-von-Siemens Str. 3 D-93128 Regenstauf, Germany. It is a white or pale yellowish white, crystalline powder soluble in water and conforms Japanese Pharmacopeia XV standards.

All other chemicals and solvents were obtained from Merck (Germany) and were of analytical grade. High quality pure water was prepared by using the Millipore purification system (Millipore, Molsheim, France, model Exil SA 67120).

Kinetic analysis

For the kinetic study, the Dionex Ultimate 3000 analytical system consisted of a quaternary pump, an autosampler, a column oven and diode array detector was used. As the stationary phase a Lichrospher RP-18 column, 5 µm particle size, 125

Table 1. Recovery studies (n = 3).

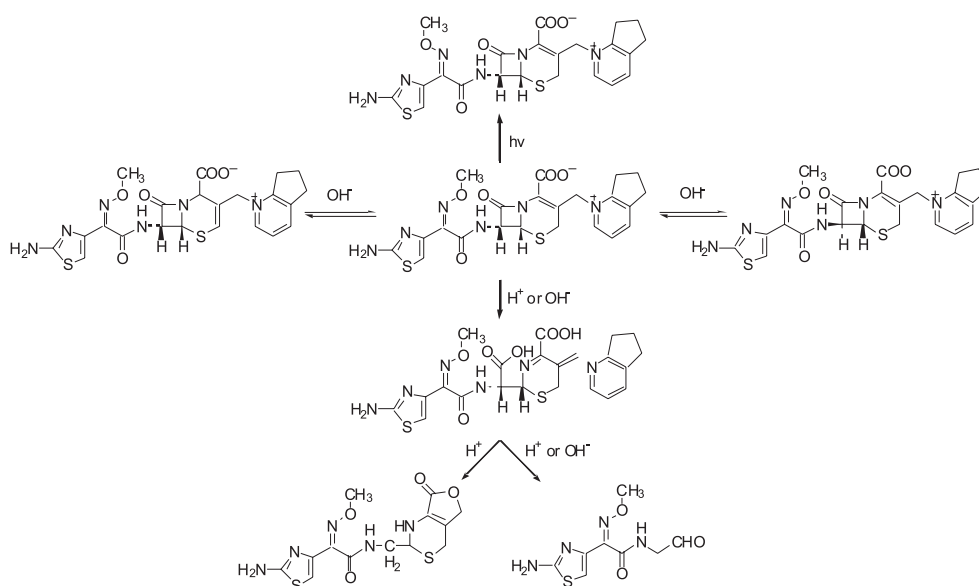| Spiked concentration (mg/L)	Measured concentration ± S.D. (mg/L) and recovery (%)
100.0 (~ 50%)	104.85 ± 0.37, 104.85
200.0 (~ 100%)	202.73 ± 6.51, 101.36
300.0 (~ 200%)	298.31 ± 1.10, 98.87

Figure 2. Degradation pathways of CPS in aqueous solutions (17)

× 4 mm (Merck, Darmstadt, Germany) was used. The mobile phase composed of acetonitrile – 12 mM ammonium acetate (10 : 90 v/v). The flow rate of the mobile phase was 1.0 mL/min and the injection volume was 10 µL. The wavelength of the DAD detector was set at 270 nm. Separation was performed at 30°C. Photodegradation stability studies were performed using Suntest CPS+ (Atlas®) with filter Solar ID65.

Procedure for forced degradation study of cefpirome sulfate

Stability tests were performed according to International Conference on Harmonization Guidelines (32).

Degradation in aqueous solutions

The degradation of cefpirome sulfate in aqueous solutions was studied in hydrochloric acid (1 mol/L) at 298 K, in sodium hydroxide (0.1 mol/L) at 298 K and in water at 373 K. Degradation was initiated by dissolving an accurately weighed 5.0 mg of CPS in 25.0 mL of the solution equilibrated to desired temperature in stoppered flasks.

Oxidative degradation

Degradation was initiated by dissolving an accurately weighed 5.0 mg of CPS in 25.0 mL solution of 3% H_2O_2 equilibrated to 298 K.

Thermal degradation

Five milligram samples of CPS were weighed into glass vials. In order to achieve the degradation in solid state, the samples were immersed in heat chambers at 393 K at RH = 0%, at 369 K at RH ~ 50.9% and at 369 K at RH ~ 90.0%. At specified time intervals, determined by the rate of degradation, the vials were removed, cooled to room temperature and their contents were dissolved in the mixture of acetonitrile and water (1 : 1 v/v). The obtained solutions were quantitatively transferred into measuring flasks and diluted with the same mixture of solvents to 25.0 mL.

UV degradation

Five milligrams of CPS were accurately weighed and dissolved in 25.0 mL of water and then exposed to light according ICHQ1b directions.

RESULTS AND DISCUSSION

It was observed that satisfactory resolution of CPS (retention time 4.59 min) and four degradation products (retention time from 2.10 to 8.05 min) formed under various stress conditions was achieved when analysis of stressed samples were performed on an HPLC system using the C-18 column and a mobile phase composed of 10 volumes of acetonitrile and 90 volumes of ammonium acetate, 12 mmol/L (Fig. 3). Shorter column (125 mm) than in other HPLC methods (250 mm) (29–31) resulted in lower amounts of organic phase wasted, while simultaneously peak asymmetry (1.351) and resolution (2.088) were still on satisfactory level.
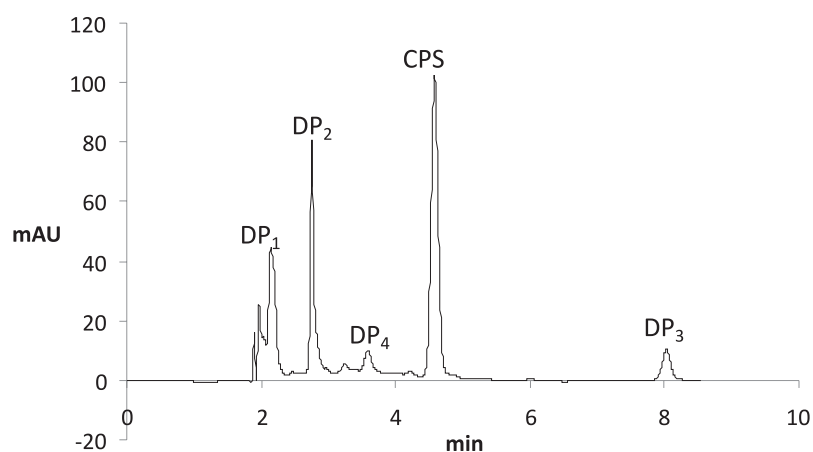

Figure 3. The HPLC chromatogram of CPS (t_R = 4.59 min) in the presence of degradation products DP (t_R from 2.10 to 8.05 min) following incubation at 363 K for 180 min

Method validation

HPLC method was validated according to International Conference on Harmonization Guidelines. The method was validated for specificity, linearity, precision, accuracy and robustness.

Selectivity

The selectivity was examined for non-degraded and degraded samples (the solutions of CPS after stress conditions of hydrolysis (acid, base and neutral), photolysis, oxidation (H_2O_2) and thermal degradation.

The HPLC method for determination of CPS was found selective in the presence of degradation products as shown in Figure 3. Peaks were symmetrical and distinctly separated from each other (Fig. 3).

Linearity

Linearity was evaluated in the concentration range of 20–300 mg/L (10–150% of the nominal concentration of CPS during degradation studies). The samples of each solution were injected three times and each series comprised 7 experimental points.

The calibration plots were linear in the concentration range 20–300 mg/L (n = 7, r = 0.9999). The calibration curve was described by the equation y = ac; y = (40346 ± 666) c. The b value, calculated from equation y = ac + b, was not significant. Statistical analysis using Mandel's fitting test confirmed linearity of the calibration curves.

Accuracy, as recovery test

The accuracy of the method was determined by recovering CPS from the placebo. The recovery test was performed at three levels: 50, 100 and 150% of the nominal concentration of CPS during degradation studies. Three samples were prepared for each recovery level. The solutions were analyzed and the percentage of recoveries was calculated. Good recoveries were obtained for each concentration, confirming that the method was accurate (Table 1).

Precision

Precision of the assay was determined in relation to repeatability (intra-day) and intermediate precision (inter-day). In order to evaluate the repeatability of the methods, six samples were determined during the same day for three concentrations

Table 2. Intra-day and inter-day precision (n = 6) studies.

Spiked concentration (mg/L)	Measured concentration ± S.D. (mg/L) and RSD (%)
Intra-day precision	
100.00	105.00 ± 0.59, 0.48
200.00	200.83 ± 0.71, 0.31
300.00	298.99 ± 1.22, 0.36
Inter-day precision	
100.00	201.99 ± 3.04, 1.43

Table 3. Results of forced degradation studies.

Stress conditions and time studies	Degradation [%]
Acidic (1 mol/L HCl; 298 K; 72 h)	37.27
Basic (0.1 mol/L NaOH; 298 K; 12 min)	56.30
Neutral (373 K; 70 min)	81.73
Oxidizing (3% H_2O_2; 298 K; 3 h)	19.34
Thermal (solid state; 393 K; RH~0%; 38 days)	69.48
Thermal (solid state; 369 K; RH~50.9%; 73.5 h)	32.91
Thermal (solid state; 369 K; RH~90.0%; 4 h)	63.00
1.2 million lux h (solution)	26.39
6.0 million lux h (solution)	94.38

of CPS. Intermediate precision was studied comparing the assays performed on two different days.

The intra-day and inter-day precision values of measured concentration of CPS, as calculated from linearity plots, are given in Table 2. The RSD values were 0.31 and 1.43%, respectively, demonstrating that the method was precise.

Limits of detection (LOD) and quantification (LOQ)

The LOD and LOQ parameters were determined from the regression equation of CPS: $LOD = 3.3\ S_y/a$, $LOQ = 10\ S_y/a$; where S_y is a standard error and a is the slope of the corresponding calibration curve.

Under applied chromatographic conditions, the LOD of CPS was 2.38 mg/L and LOQ of CPS was 7.22 mg/L.

Robustness

The robustness of the procedure was evaluated after changing the following parameters: the composition of the mobile phase; content of acetonitrile 10 ± 2%, the mobile phase flow rate 1.0 ± 0.2 mL/min; wavelength of absorption 270 ± 5 nm and temperature 30 ± 2°C. For each parameter change its influence on the retention time, resolution, area and asymmetry of peak was evaluated. No significant changes in resolution and shapes of peak, areas of peak and retention time were observed when above parameters were modified. Modifications of the composition of the mobile phase: organic-to-inorganic component ratio and pH resulted in the essential changes of retention time and resolution in determination of CPS.

Results of forced degradation experiments

In previous studies, concerning the stability of cephalosporins, it was observed that basic hydrolysis was a fast reaction (13–19). Also in the case of CPS, significant degradation was observed at basic hydrolysis. Photodegradation of CPS was observed after exposition even on 1.2 million lux h (solution). It was observed that around 26% of CPS degraded under these conditions. CPS was susceptible for degradation in solid state. At increased RH the degradation was much faster than in dry air. CPS was more stable than other 4th generation cephalosporin – cefozopran hydrochloride (33). The results of forced degradations in various conditions are summarized in Table 3.

CONCLUSION

The isocratic RP-LC method developed for the analysis of CPS in its pharmaceutical preparations is selective, precise and accurate. The method is useful for routine analysis due to short run time and low amounts of solvent (acetonitrile) used in the mobile phase. Low acetonitrile consumption is consistent with the current worldwide trend of green (sustainable) chemistry. This method can be used for determination of stability of CPS in its pharmaceutical preparations.

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