Acta Poloniae Pharmaceutica – Drug Research, Vol. 72 No. 3 pp. 423–427, 2015 ISSN 0001-6837
Polish Pharmaceutical Society

ANALYSIS

DEVELOPMENT AND VALIDATION OF THE STABILITY-INDICATING LC-UV METHOD FOR DETERMINATION OF CEFOZOPRAN HYDROCHLORIDE

PRZEMYSŁAW ZALEWSKI[1*], PIOTR GARBACKI[1], JUDYTA CIELECKA-PIONTEK[1], KATARZYNA BEDNAREK-RAJEWSKA[2] and ANNA KRAUSE[3]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Poznan University of Medical Sciences, Grunwaldzka 6, 60-780 Poznań, Poland
[2]Department of Clinical Pathomorphology, Faculty of Medicine I, Poznan University of Medical Sciences, Przybyszewskiego 49, 60-355 Poznań, Poland
[3]PozLab sp. z o.o. (Contract Research Organization) Parkowa 2, 60-775 Poznań, Poland

Abstract: The stability-indicating LC assay method was developed and validated for quantitative determination of cefozopran hydrochloride (CZH) in the presence of degradation products formed during the forced degradation studies. An isocratic, RP-HPLC method was developed with C-18 (250 mm × 4.6 mm, 5 µm) column and 12 mM ammonium acetate-acetonitrile (92 : 8, v/v) as a mobile phase. The flow rate of the mobile phase was 1.0 mL/min. Detection wavelength was 260 nm and temperature was 30°C. Cefozopran hydrochloride 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 cefozopran hydrochloride in pharmaceuticals and during kinetic studies.

Keywords: method validation, stability-indicating method, cefozopran hydrochloride

Cefozopran hydrochloride (Fig. 1) is a new, parenteral, fourth generation cephalosporin originally created by Takeda Chemical Industries. It was first registered for treatment in Japan in 1995. CZH has a broad spectrum of antibacterial activity against Gram positive such as *Staphylococcus aureus* and Gram negative bacteria including *Escherichia coli* and *Pseudomonas aeruginosa* (1). It is stable against various β-lactamases and has low affinities to these enzymes (2). Cefozopran hydrochloride contains imidazopyridazinium methyl group at position 3, while in position 7 it is aminothiadiazomethoxyiminoacetylamino structure (Fig. 1). Those elements are responsible for broad spectrum of antibacterial activity of CZH. It is often used for antibacterial prophylaxis in abdominal surgery and for treatment of post-operative intra-abdominal infections (IAIs) (3, 4). Its recommended dose is only 2 g per day (1 g every 12 h) because of its impact on healthcare costs, and up to 4 g per day considered only for critically ill patients. In view of its good stability in solution, 1–2 g cefozopran dissolved in 100 mL saline could be infused for 1 h every 8 h (5). Its excretion rate into urine up to 24 h after administration is 82–94% and no active anti-microbial metabolite is observed in urine (6-9). CZH is well tolerated and most of adverse effects after its administration were of a mild or moderate severity, were of short period, improved spontaneously, and recovered completely (9). Since the majority of the side effects of β-lactam antibiotics are caused by their degrada-

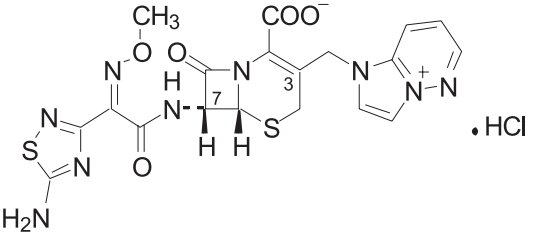

Figure 1. Chemical structure of cefozopran hydrochloride

* Corresponding author: e-mail: pzalewski@ump.edu.pl; phone 004861-854-66-49

tion products, it is vital to improve analytical methods for the determination of β-lactam analogues. Previous studies have proved that cephalosporins are susceptible to degradation in aqueous solutions (10-17) and in solid state (18-26). Developed chromatographic method for the determination of CZH had many disadvantages like significant organic solvent consumption or incompatible to HPLC-MS water phase (27-30).

The aim of this work was to develop and validate HPLC method with UV detection suitable for identification, determination, and stability study of cefozopran hydrochloride and its degradation products.

EXPERIMENTAL

Chemicals, reagents and solutions

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

All other chemicals and solvents were obtained from Merck KGaA (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).

Instrumentation

HPLC Dionex Ultimate 3000 analytical system consisted of a quaternary pump, an autosampler, a column oven and a diode array detector was used. As the stationary phase a Lichrospher RP-18 column, 5 µm particle size, 250 mm × 4 mm (Merck, Darmstadt, Germany) was used. The mobile phase composed of acetonitrile – 12 mM ammonium acetate (8 : 92, 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 260 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 cefozopran hydrochloride

Stability tests were performed according to International Conference on Harmonization Guidelines (31).

Degradation in aqueous solutions

The degradation of cefozopran hydrochloride 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 cefozopran hydrochloride in 25.0 mL of the solution equilibrated to desired temperature in stoppered flasks. At specified times, samples of the reaction solutions were withdrawn and instantly cooled with a mixture of ice and water.

Oxidative degradation

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

Thermal degradation

Samples of cefozopran hydrochloride (5.0 mg) were weighed into glass vials. In order to achieve the degradation of cefozopran hydrochloride in solid state, their samples were immersed in heat chambers at 373 K at RH = 0%, at 373 K at RH ~ 76.4% and at 353 K at RH ~ 76.4%. At specified time intervals, determined by the rate of degradation the vials were removed, cooled to room temperature and their contents were dissolved in mixture 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

Samples of cefozopran hydrochloride (5.0 mg) were accurately weighed, dissolved in 25.0 mL of water and then they were exposed to light according to ICHQ1b directions.

RESULTS AND DISCUSSION

It was observed that satisfactory resolution of cefozopran hydrochloride (retention time 4.6 min.) and their degradation products (retention time from 1.9 to 3.6 min.) formed under various stress conditions was achieved when analysis of stressed samples were performed on an HPLC system using a C-18 column and a mobile phase composed of 8 volumes of acetonitrile and 92 volumes of ammonium acetate, 12 mmol/L. The detection was carried out at 260 nm. The mobile phase flow rate was 1.0 mL/min. Typical retention times of cefozopran hydrochloride were about 4.6 min (Fig. 2). Peak asymmetry was 0.98.

Method validation

HPLC method was validated according to International Conference on Harmonization

Guidelines. The method was validated for parameters such as specificity, linearity, precision, accuracy and robustness.

Selectivity

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

The HPLC method for determination of cefozopran hydrochloride was found selective in the presence of degradation products as shown in Figure 2. Peaks were symmetrical, clearly separated from each other (Fig. 2).

Linearity

Linearity was evaluated in the concentration range 20-300 mg/L (10-150% of the nominal concentration of cefozopran hydrochloride 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 following concentration range 20-300 mg/L (n = 9, r = 0.9999). The calibration curve was described by the equation y = *ac*; y = (0.7904 ± 0.0022) *c*. The *b* value, calculated from equation y = a*c* + *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 cefozopran hydrochloride from the placebo. The recovery test was performed at three levels 50, 100 and 150% of the nominal concentration of cefozopran hydrochloride during degradation

Table 1. Intra-day, inter-day precision (n = 6) and recovery studies (n = 3).

Spiked concentration (mg/L)	Measured concentration ± S.D. (mg/L)	RSD (%)
Intra-day precision		
100.00 200.00 300.00	100.51 ± 0.22 199.32 ± 0.82 299.24 ± 1.14	0.15 0.39 0.36
Inter-day precision		
100.00	100.05 ± 0.54	0.51
Recovery studies		
Spiked concentration (mg/L)	Measured concentration ± S.D. (mg/L)	Recovery (%)
100.0 (~ 50%) 200.0 (~ 100%) 300.0 (~ 150%)	100.15 ± 0.65 199.25 ± 1.23 299.69 ± 1.51	100.15 99.62 99.90

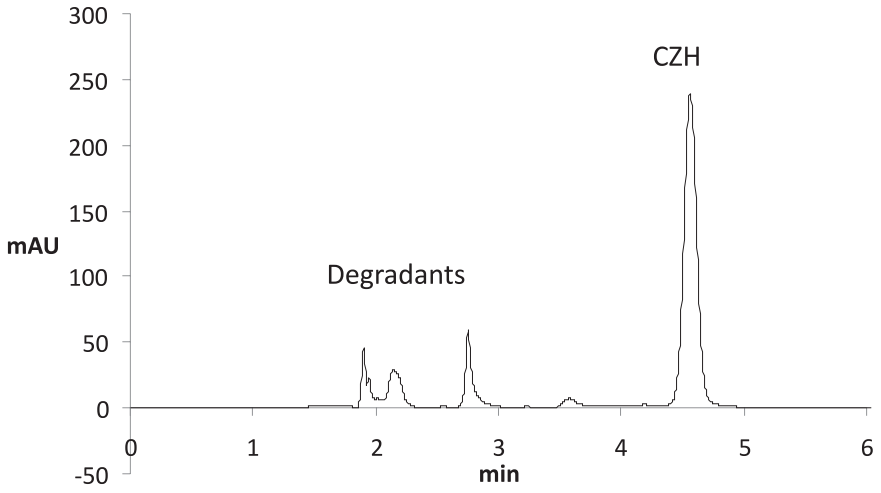

Figure 2. HPLC chromatogram of cefozopran hydrochloride (CZH) after 120 h incubation at 298 K in 1 mol/L HCl

studies. Three samples were prepared for each recovery level. The solutions were analyzed and the percentage of recoveries was calculated.

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

The intra-day and inter-day precision values of measured concentration of cefozopran hydrochloride, as calculated from linearity plots are given in Table 1. The RSD values were 0.15 and 0.51%, respectively, demonstrating that the method was precise.

Good recoveries were obtained for each concentration, confirming that the method was accurate (Table 1).

Limits of detection (LOD) and quantification (LOQ)

The LOD and LOQ parameters were determined from the regression equation of cefozopran hydrochloride: 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 cefozopran hydrochloride was 1.04 mg/L and LOQ of cefozopran hydrochloride was 3.15 mg/L.

Robustness

The robustness of the procedure was evaluated after changing the following parameters: the composition of the mobile phase (content of acetonitrile in the range 6–10%), the mobile phase flow rate (flow rate in the range 0.8–1.2 mL/min), wavelength of absorption (in the range 255–265 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 cefozopran hydrochloride.

Results of forced degradation experiments

During stability studies, degradation of 20–80% should be achieved for establishing stability-indicating nature of the assay method. In previous studies, concerning the stability of cephalosporins, it was observed that basic hydrolysis was a fast reaction (10-17). Also in the case of cefozopran hydrochloride significant degradation was observed at basic hydrolysis. Photodegradation of cefozopran hydrochloride was observed after exposition even on 1.2 million lux h (solution). It was observed that around 30% of cefozopran hydrochloride degraded under these conditions. Cefozopran hydrochloride was susceptible for degradation in solid state. At increased RH the degradation was much faster than in dry air. The results of forced degradations in various conditions are summarized in Table 2. Similar results were observed for other 4th generation cephalosporins: cefpirome sulfate (CPS) (23, 32) and cefoselis sulfate (CSS) (13, 24). CZH is more stable in solutions but easier degrades in solid state than CPS and CSS.

Table 2. Results of forced degradation studies.

Stress conditions and time studies	Degradation [%]
Acidic (1 mol/L HCl; 298 K; 77 h)	35.17
Basic (0.1 mol/ L NaOH; 298 K; 11 min)	48.76
Neutral (373 K; 65 min)	54.85
Oxidizing (3% H_2O_2; 298 K; 4 h)	23.65
Thermal (solid state; 373 K; RH~0%; 41 days)	88.68
Thermal (solid state; 373 K; RH~76.4%; 1 h)	96.83
Thermal (solid state; 353 K; RH~76.4%; 1 h)	47.61
1.2 million lux h (solution)	32.12
6.0 million lux h (solution)	83.37

CONCLUSION

The isocratic RP-LC method developed for the analysis of cefozopran hydrochloride in their pharmaceutical preparations is selective, precise and accurate. The method is useful for routine analysis due to short run time and low amounts of used solvents (acetonitrile) in mobile phase. Water phase of developed method is compatible to HPLC-MS and can be used for identification of degradation products.

Acknowledgment

This study was supported by PRELUDIUM grant from the National Science Centre Poland (UMO-2013/09/N/NZ7/01479).

REFERENCES

1. Iizawa Y., Okonogi K., Hayashi R., Iwahi T., Imada A.: Antimicrob. Agents Chemother. 37, 100 (1993).
2. Klein O., Chin N.X., Huang H.B., Neu H.C.: Antimicrob. Agents Chemother. 38, 2896 (1994).
3. Ikawa K., Nomura K., Morikawa N., Ikeda K., Ohge H., Sueda T., Taniwaki M.: J. Infect. Chemother. 14, 130 (2008).
4. Ikawa K., Morikawa N., Matsuda S., Ikeda K., Ohge H., Takesue Y., Sueda T.: Int. J. Antimicrob. Agents 30, 352 (2007).
5. Nomura K., Morikawa N., Ikawa K., Ikeda K., Fujimoto Y., Shimizu D., Taniguchi K. et al.: J. Antimicrob. Chemother. 61, 892 (2008).
6. Kita Y., Kimura Y., Yamazaki T., Imada A.: Chemotherapy 41, 121 (1993).
7. Ikawa K., Kozumi T., Ikeda K., Morikawa N., Kobayashi R.: Jpn. J. Antibiot. 62, 435 (2009).
8. Motohiro T., Hanada S., Yamada S., Sasaki H., Oki S., Yoshinaga Y., Oda K. et al.: Jpn. J. Antibiot. 47, 1589 (1994).
9. Paulfeuerborn W., Müller H.J., Borner K., Koeppe P., Lode H.: Antimicrob. Agents Chemother. 37, 1835 (1993).
10. Patel G., Rajput S.: Acta Chromatogr. 23, 215 (2011).
11. Ikeda Y., Ban J., Ishikawa T., Hashiguchi S., Urayama S., Horibe H.: Chem. Pharm. Bull. 56, 1406 (2008).
12. Jelińska A., Dobrowolski L., Oszczapowicz I.: J. Pharm. Biomed. Anal. 35, 1273 (2004).
13. Zalewski P., Cielecka-Piontek J., Jelińska A.: React. Kinet. Mech. Cat. 108, 285 (2013).
14. Sugioka T., Asano T., Chikaraishi Y., Suzuki E., Sano A., Kuriki T., Shirotsuka M., Saito K.: Chem. Pharm. Bull. 38, 1998 (1990).
15. Fubara J.O., Notari R.E.: J. Pharm. Sci. 87, 1572 (1998).
16. Zalewski P., Cielecka-Piontek J., Jelińska A.: Asian J. Chem. 25, 7596 (2013).
17. Medenecka B., Jelińska A., Zając M., Bałdyka M., Juszkiewicz K., Oszczapowicz I.: Acta Pol. Pharm. Drug Res. 66, 563 (2009).
18. Jelińska A., Medenecka B., Zając M., Knajsiak M.: Acta Pol. Pharm. Drug Res. 65 261 (2008).
19. Zając M., Jelińska A., Zalewski P.: Acta Pol. Pharm. Drug Res. 62, 89 (2005).
20. Jelińska A., Dudzińska I., Zając M., Oszczapowicz I., Krzewski W.: Acta Pol. Pharm. Drug Res. 62, 183 (2005).
21. Zalewski P., Cielecka-Piontek J., Garbacki P., Jelińska A., Karaźniewicz-Łada M.: Chromatographia 76, 387 (2013).
22. Zając M., Jelińska A., Dobrowolski L., Oszczapowicz I.: J. Pharm. Biomed. Anal. 32, 1181 (2003).
23. Zalewski P., Skibiński R., Cielecka-Piontek J.: J. Pharm. Biomed. Anal. 92, 22 (2014).
24. Zalewski P., Cielecka-Piontek J., Jelińska A.: Centr. Eur. J. Chem. 10, 121 (2012).
25. Jelińska A., Zając M., Jakubowska M.: React. Kinet. Catal. Lett. 73, 325 (2001).
26. Ikeda K., Ikawa K., Morikawa N., Ohge H., Sueda T.: J. Pharm. Biomed. Anal. 49, 1075 (2009).
27. Borner K., Borner E., Lode H.: J. Chromatogr. 615, 174 (1993).
28. Kitahashi T., Furuta I.: J. Pharm. Biomed. Anal. 34, 409 (2004).
29. Liu S.Y., Zhang D.S., Hu C.Q.: Eur. J. Med. Chem. 45, 5808 (2010).
30. Ikeda K., Morikawa N., Kuribayashi M., Ikawa K., Nomura K., Taniwaki M.: J. Pharm. Biomed. Anal. 45, 811 (2007).
31. ICH, Stability Testing of New Drug Substances and Products (Q1AR). International Conference on Harmonization, IFPMA, Geneva (2000).
32. Zalewski P., Skibiński R., Cielecka-Piontek J., Bednarek-Rajewska K. Acta Pol. Pharm. Drug Res. 71, 731 (2014).

Received: 5. 05. 2014