Acta Poloniae Pharmaceutica – Drug Research, Vol. 71 No. 2 pp. 249–264, 2014 ISSN 0001-6837
Polish Pharmaceutical Society

STABILITY-INDICATING HPLC METHOD FOR THE DETERMINATION OF CEFQUINOME SULFATE

AGNIESZKA DOŁHAŃ*, ANNA JELIŃSKA and MONIKA MANUSZEWSKA

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Poznan University of Medical Sciences, Grunwaldzka 6, 60-780 Poznań, Poland

Abstract: A novel and sensitive stability-indicating RP-HPLC method for the quantitative determination of cefquinome sulfate has been developed. Chromatographic separation and quantitative determination were performed using a high-performance liquid chromatograph with UV detection. As the stationary phase a LiChroCART RP-18 column (5 μm particle size, 125 mm × 4 mm, Merck, Darmstadt, Germany) was used. The mobile phase consisted of 10 volumes of acetonitrile and 90 volumes of an 0.02 M phosphate buffer (pH = 7.0). The flow rate of the mobile phase was 1.0 mL/min. The eluents were monitored by a UV-VIS detector at 268 nm. The drug was subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal degradation. Significant degradation was found under basic, oxidizing stress and UV light. The developed method was validated with respect to linearity, accuracy, precision and robustness.

Keywords: cefquinome sulfate, degradation, RP-HPLC, stability-indicating, validation

Cefquinome sulfate is a veterinary, parenteral, fourth-generation cephalosporin. A significant enhancement of activity and an extension of the antibacterial spectrum were achieved by the introduction of a methoxyimino–aminothiazolyl moiety into the acyl side chain of cephalosporins, which made them resistant to inactivation by β-lactamases (1–3).

Fourth-generation cephalosporins have a broad spectrum of antibacterial activity against Gram-positive and Gram-negative bacteria, including *Pseudomonas aeruginosa* and *Enterobacteriaceae* (4–6). Those compounds are also easily transported across the blood-brain barrier (7–14). Fourth-generation cephalosporins are used to treat infections of the urinary tract, lungs, skin and soft tissues as well as in post-operative prophylaxis (7, 15).

Cefquinome sulfate is an aminothiazolyl cephalosporin with a broad spectrum of activity against the majority of strains found in animal infections (16) such as *Actinobacillus spp., Haemophilus spp., Clostridium spp., Corynebacterium, Erysipelothrix rhusiopathiae, Proteus spp., Salmonella spp., Streptococcus spp., Pasteurella spp., Staphylococcus spp., Pseudomonas aeruginosa, Escherichia coli* and *Enterobacteriaceae*. Cefquinome sulfate is often applied in the treatment of *meningitis-mastitis-agalactiae* (MMA) (17, 18).

Pharmaceutical dosage forms should be stable during preparation, administration and action. ß-Lactam antibiotics (penam analogues, cephalosporins and carbapenems) are susceptible to degradation both in aqueous solutions (19–27) and in the solid state (28–38). Determination of cephem analogs is the result of different physical and chemical factors activity.

The guidelines of the International Conference on Harmonization (ICH) require the development of stability-indicating assay methods (SIAMs) appropriate for the determination of drugs after stability analysis (Q1A-R2) (39).

During stress tests, the effect of temperature and air humidity should be determined in solid state. For solutions, the effect of temperature, light, oxidizing agent, buffer pH and infusion liquid need to be analyzed. The impact of biochemical processes on the formation of metabolites has to be considered as well. Finally, the chemical structure and toxicity of principal degradation products, impurities and metabolite(s) should be established.

The aim of this work was to develop and validate an HPLC method with UV detection suitable

* Corresponding author: e-mail: agnieszka_dolhan@wp.pl; phone:+4861 8546650, fax: +48 618546652

for the identification, determination, and stability study of cefquinome sulfate.

EXPERIMENTAL

Chemicals

Cefquinome sulfate was obtained from Bepharm Pharmaceuticals, China. All other chemicals and solvents (acetonitrile, disodium hydrogen phosphate, orthophosphoric acid) were obtained from Merck KGaA (Germany) and were of analytical grade. High-quality pure water was prepared using an Exil SA 67120 purification system (Millipore, Molsheim, France). Acetanilide 98.5% grade (Sigma-Aldrich, USA) was used as an internal standard.

Equipment

The LC system used for method development, forced degradation studies and method validation was from Shimadzu (Japan) and was composed of LC-6A pump, C-R6A CHROMATOPAC interface and UV-Vis Shimadzu SPD-6AV detector. The Rheodyne injection valve had 50 µL volume. Photostability studies were carried out using a Suntest CPS$^+$ device (Atlas®) with a Solar ID65 filter, USA. Thermal stability studies were performed in a Wamed KBC – 125W heat chamber, Poland.

Chromatography

The chromatographic column used was LiChroCART 125 mm × 4 mm (5 µm), No. 019297 (Merck, Darmstadt, Germany). The mobile phase consisted of 10 volumes of acetonitrile and 90 volumes of an 0.02 M phosphate buffer (pH = 7.0) and was also used as a diluent. The flow rate of the mobile phase was 1.0 mL/min. The eluents were monitored by a UV-VIS detector at 268 nm. The injection volume was 50 µL.

Preparation of standard solutions

Fifty milligrams of cefquinome was accurately weighed into 25 mL volumetric flask, dissolved and diluted to 25.0 mL with the mobile phase (standard solution).

Twenty milligrams of acetanilide was accurately weighed into a 100 mL volumetric flask, dissolved and diluted to 100.0 mL volume with acetonitrile (internal standard solution).

Specificity/application of stress (forced degradation study)

The degradation of cefquinome sulfate in aqueous solutions was studied at 313 K in hydrochloric acid (0.1 M) and in sodium hydroxide (0.1 M). The ionic strength of all solutions was adjusted to 0.5 M with a solution of sodium chloride (4.0 M). Degradation was initiated by dissolving an accurately weighed 5.0 mg of cefquinome sulfate in 25.0 mL of the solution equilibrated to 313 K in a stoppered flask. At specified time intervals, samples of the solutions were withdrawn and instantly cooled with a mixture of water and ice.

In order to perform oxidative degradation, 5.0 mg of cefquinome sulfate was accurately weighed and dissolved in 5.0 mL of the mobile phase, to which 20.0 mL of a 3% H_2O_2 solution was added and kept at room temperature for 20 min. Samples of reaction solutions were withdrawn and instantly cooled with an ice/water mixture.

Thermal degradation was involved weighing 5.0 mg of cefquinome sulfate into a 5 mL vial and placing it in a heat chamber at 373 K (RH = 0%). After 1 week the vial was removed, cooled to room temperature and the content was dissolved in the mobile phase. The so obtained solution was transferred into measuring flasks and diluted with the mobile phase to 25.0 mL.

UV degradation was conducted by weighing 5.0 mg of cefquinome sulfate and exposing it to sunlight for 48 h (1.2×10^{-6} lux/h. The samples were dissolved and diluted with the mobile phase to 25.0 mL.

Method validation

The HPLC method was validated with respect to specificity, linearity, precision, accuracy and robustness, according to the ICH guidelines (38).

Precision

The precision of the method was determined by injecting six samples 20 mg/mL in triplicate on the same day. The %RDS area of cefquinome was calculated.

Sensitivity

LOD (limit of detection) = 3.3σ/S and LOQ (limit of quantitation) = 10σ/S, (σ = the standard deviation of the response, S = the slope of the calibration curve) were determined from the regression equation for cefquinome sulfate.

Linearity and range

Method linearity was evaluated in the concentration range 0.034 – 0.1 mg/mL (50 – 150% of the nominal concentration of cefquinome sulfate during the degradation studies). Samples of each solution were injected three times and each series comprised 6 experimental points.

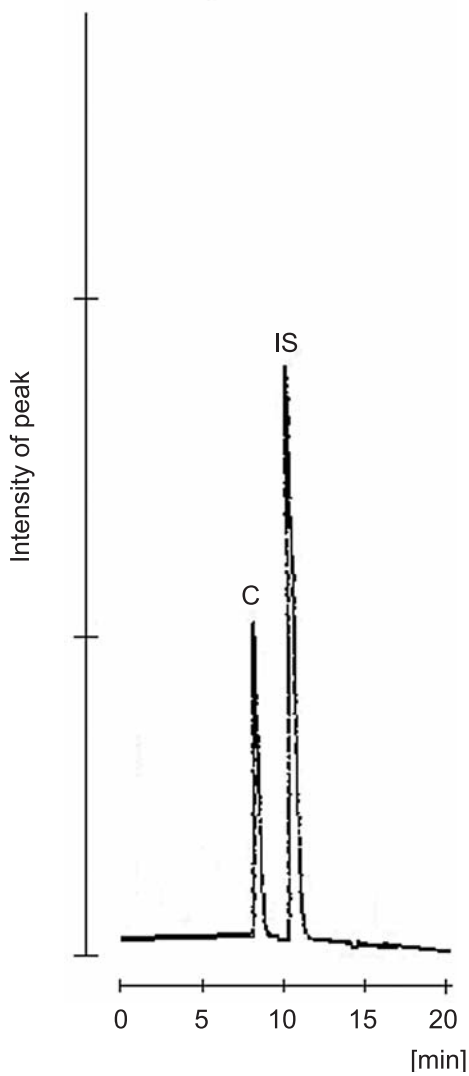

Figure 1. The HPLC chromatogram of a blank sample of cefquinome sulfate (C – cefquinome sulfate, IS – internal standard)

Accuracy

The accuracy of the method was determined by recovering cefquinome sulfate from the placebo. At three levels (80, 100 and 120%) of the nominal concentration of cefquinome sulfate during the degradation studies, three samples were prepared for each recovery level. The solutions were analyzed and the percentage of recovery was calculated.

Robustness

To determine the robustness of the method, the experimental conditions were changed and the purity and shape of the peak was evaluated. The following parameters were altered: the composition of the mobile phase (content of acetonitrile 5% and 20%), the mobile phase flow rate (flow rate 0.8 and 1.4 mL/min), wavelength of absorption (258 nm), temperature (20 and 30°C), the pH of the phosphate buffer (6 and 8). The influence of each parameter on the retention time, resolution, area and peak shape was evaluated (Table 1).

Solution stability and mobile phase stability

The solution stability of cefquinome sulfate in the assay was examined by leaving the test solutions in tightly capped volumetric flasks at room temperature and at 4°C for 24 h. The samples were assayed against a freshly prepared standard solution. The stability of the mobile phase was evaluated by determining the samples against a freshly prepared reference standard solutions at 0 and 24 h.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

A satisfying resolution of cefquinome sulfate and its degradation products formed under various

Table 1. Results of robustness studies.

Parameter	Retention time [min]	Area of peak	Shape of peak	Purity [%]
Optimal	8.54	946484	High, sym.	100.00
ACN = 5%	8.16	943662	High, asym.	100.00
ACN = 20%	1.68	934631	Asym.	95.68
pH = 6	1.69	940015	High, sym.	100.00
pH = 8	8.86	941895	High, asym.	100.00
f = 0.8 mL/min	10.5	1141293	High, asym.	100.00
f = 1.4 mL/min	5.84	929714	High asym.	100.00
λ = 258 nm	8.08	937399	High, asym.	100.00
T = 20°C	8.67	934385	High, sym.	99.99
T = 30°C	7.62	1028480	High, asym.	99.94

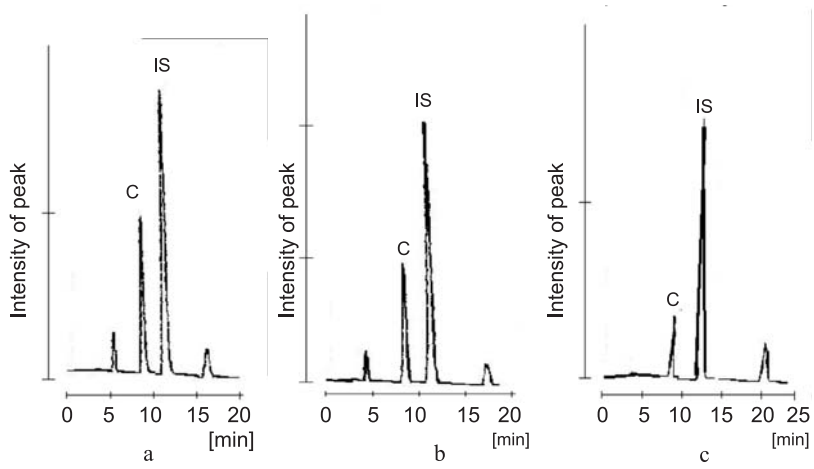

Figure 2. Chromatograms for cefquinome sulfate (C) after basic hydrolysis (a), sunlight (b) and xidizing stress (c), IS – internal standard.

Table 2. Precision studies (n = 6).

A_{cefq}/A_{is}	
0.322	SD = 0.003 RDS = 0.95%
0.315	
0.315	
0.318	
0.321	
0.320	

A = peak area, cefq = cefquinome sulfate, is = internal standard

stress conditions was achieved when the stressed samples were analyzed by using an RP C-18 column and a mobile phase composed of 10 volumes of acetonitrile and 90 volumes of phosphate buffer (pH 7). Detection was carried out at 268 nm. The mobile phase flow rate was 1.0 mL/min. The retention time of cefquinome sulfate was 8.54 min, and the internal standard (acetanilide) 10.65 min (Fig. 1). The purity of the sample peak was 100%.

Method validation

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

Photodiode array detection was used to demonstrate the specificity of the method and to evaluate the homogeneity of the cefquinome sulfate peak. The peak purity values were more than 98.5% at 268 nm, what proves that the degradantion products did not interfere with the main peak.

The linearity of the method was determined in a range 50–150% of the assay concentration. The calibration plots were linear in the concentration range 0.03 – 0.1 mg/mL (n = 13, r = 0.9963) The calibration curves were described by the equation y = ac + b, y = (4.816 ± 0.252)c, b = 0.014 ± 0.018. The b values were not statistically significant.

The intra-day and inter-day precision values were calculated for concentration 6.67×10^{-2} mg/mL of cefquinome sulfate (Table 2). The RDS value was 0.95% and proved that the method was precise.

The recovery test was performed at three levels (80, 100 and 120%) of the nominal concentration of cefquinome sulfate .The recovery values ranged from 99.10 to 101.31% for each concentration, which proved that the method was accurate (Table 3).

Under the chromatographic conditions applied, the LD of cefquinome was 6.14 10^{-3} mg/mL and the QL was 18.61×10^{-3} mg/mL. The robustness of the procedure was evaluated by changing the composition of the mobile phase, its flow rate (0.8–1.2 mL/min), temperature (20–30°C (±1°C)) and pH (6.0–8.0). The effect of those changes on retention time, peak resolution, shape and area was evaluated. It was found that those alternations did not effect those parameters. Modifications of the mobile phase composition (organic to inorganic component ratio) resulted in significant changes in peak retention time.

Results of forced degradation experiments

Cefquinome sulfate, similarly to other cephalosporins, is vulnerable to degradation under

Table 3. Recovery studies (n = 3).

Spiked concentration (mg/mL)	Measured concentration ± SD (mg/mL)	Recovery (%)
0.0534 (~80%)	0.054 ± 0.002	101.31
0.0667 (~100%)	0.066 ± 0.001	99.10
0.0804 (~120%)	0.080 ± 0.001	99.38

Table 4. Results of forced degradation studies.

Stress conditions and time studies	Degradation [%]	Peak purity [%]
Acidic 0.1M HCl/313K/90 min	8.2 9	8.87
Basic 0.1M NaOH/313K/3.5 min	75.0	99.56
Oxidizing 3% H_2O_2/298K/20 min	92.4	99.76
Thermal /373K/ 1 week	18.4	98.65
Sunlight 48 h	25.2	100.0

the influence of physical and chemical factors. It was observed that 8–93% of cefquinome sulfate was degredated during acidic hydrolysis, basic hydrolysis, oxidation, UV irradiation and exposure to an increased temperature (Table 4).

CONCLUSIONS

This HPLC method for assay cefquionome sulfate was successfully developed and validated for its intended purpose. The method was shown to be specific, linear, precise, accurate and robust. Cefquinome sulfate was found to be very susceptible to basic hydrolysis, oxidizing and photolysis (Fig. 2).

REFERENCES

1. Marshall W.F., Blair J.E.: Mayo Clin. Proc. 74, 187 (1999).
2. Dürckheimer W., Adam F., Fischer G., Kirrstetter R.: Adv. Drug Res. 17, 61 (1988).
3. Neu H.C.: Lancet 320, 252 (1982).
4. Kariyama T., Karasawa T., Nakagawa S., Yamamoto E.: Oral Microbiol. Immunol. 17, 285 (2002).
5. Climo M.W., Markowitz S.M., Williams D.S., Hale-Cooper C.G., Archer G.L.: J. Antimicrob. Chemother. 40, 59 (1997).
6. Neu H.C., Chin N.X., Huang H.B.: Antimicrob. Agents Chemother. 37, 566 (1993).
7. Zając M., Pawełczyk E., Jelińska A.: Drugs used in infections and invasive deseases (Polish). in Medicinal chemistry (Polish). pp. 467–468, 490–506, Scientific Publisher of Poznań Medical Academy, Poznań 2006.
8. Janiec W.: Pharmacodynamics of drugs used in infections and invasive deseases (Polish). in Pharmacodynamics (Polish). Vol. 2, pp. 1087–1095, 1114–1123, PZWL, Warszawa 2008.
9. Yang Q., Wang H., Chen M., Ni Y., Yu Y., Hu B., Sun Z et al.: Int. J. Antimicrob. Agents 36 507 (2010).
10. Barber M.S., Giesecke U., Reichert A., Minas W.: Adv. Biochem. Eng. Biotechnol. 88, 179 (2004)
11. Kambaroudis A.G., Papadopoulos S., Christodoulidou M., Gerasimidis T.: Surg. Infect. 11 535 (2010).
12. Bonfiglio G., Marchetti F, Chemotherapy 46, 229 (2000).

13. Garau J.: Diagn. Microbiol. Infect. Dis. 31, 478 (1998).
14. Wilson W.R.: Diagn. Microbiol. Infect. Dis. 31, 473 (1998).
15. Hordo Ł.: Aptekarz Polski (Polish) 38, 16 (2009).
16. Murphy S.P., Erwin M.E., Jones R.N.: Diagn. Microbiol. Infect. Dis. 20, 49 (1994).
17. Morioka A., Asai T., Ishihara K., Kojima A., Tamura Y., Takahashi T.: J. Vet. Med. Sci. 67, 207 (2005).
18. Limbert M., Isert D., Klesel N., Markus A., Seeger K., Seibert G., Schrinner E.: Antimicrob. Agents Chemother. 35, 14 (1991).
19. Conannon J., Lovitt H., Ramage M, Tai L.H., McDonald C., Sunderland V.B.: Am. J. Hosp. Pharm. 43, 3027 (1986).
20. Aki H., Niiya T., Iwasel Y., Goto M., Kimura T.: J. Therm. Anal. Calorim. 77, 423 (2004).
21. Patel G., Rajput S.: Acta Chromatogr., 23(2), 215 (2011).
22. Ikeda Y., Ban J., Ishikawa T., Hashiguchi S., Urayama S, Horibe H.: Chem. Pharm. Bull. 56, 1406 (2008).
23. Jelińska A., Dobrowolski L., Oszczapowicz I.: J. Pharm. Biomed. Anal., 35, 1273 (2004).
24. Sugioka T., Asano T., Chikaraishi Y., Suzuki E., Sano A., Kuriki T., Shirotsuka M., Saito K.: Chem. Pharm. Bull. 38, 1998 (1990).
25. Fubara J.O., Notari R.E.: J. Pharm. Sci. 87, 1572 (1998).
26. Zając M., Cielecka-Piontek J., Jelińska A.: J. Pharm. Biomed. Anal. 43(2), 445 (2007).
27. Cielecka-Piontek J., Jelińska A.: React. Kinet. Mech. Cat. 102, 37 (2011).
28. Ovcharova G.D., Nachevara R.N.: Antibiotiki 29, 166 (1984).
29. Pawełczyk E., Hermann T., Zając M et al.: Pol. J. Pharmacol. Pharm. 1, 47 (1988).
30. Medenecka B., Jelińska A., Zając M., Bałdyka K., Juszkiewicz K., Oszczapowicz I.: Acta Pol. Pharm. Drug Res. 66, 563 (2009).
31. Jelińska A., Medenecka B., Zając M, Knajsiak M.: Acta Pol. Pharm. Drug Res. 65, 262 (2008).
32. Zając M., Jelińska A., Zalewski P.: Acta Pol. Pharm. Drug Res. 62, 89 (2005).
33. Jelińska A., Dudzińska I., Zając M., Oszczapowicz I, Krzewski W.: Acta Pol. Pharm. Drug Res. 62, 18 (2005).
34. Zając M., Jelińska A., Dobrowolski L., Oszczapowicz I.: J. Pharm. Biomed. Anal. 21, 32, 1181 (2003).
35. Jelińska A., Zając M., Gostomska J., Szczepaniak M.: Farmaco 58, 309 (2003).
36. Jelińska A., Zając M., Jakubowska M.: React. Kinet. Catal. Lett. 73, 325 (2001).
37. Cielecka-Piontek J., Zając M., Jelińska A.: J. Pharm. Biomed. Anal., 46, 52 (2008).
38. Zalewski P., Cielecka-Piontek J., Jelińska A.: Central European Journal of Chemistry, 10, 121 (2012).
39. ICH Validation of Analytical Procedures, Metodology Q2B, International Conference on Harmonization, IFPMA, Geneve 2000.

Received: 30. 04. 2013