APPLICATION NOTE 73372

Analysis of cefprozil and related impurities by reversed-phase liquid chromatography with UV detection

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Application benefits

- The method for impurities quantification of cefprozil monohydrate according to Ph. Eur. monograph was successfully implemented on a Thermo Scientific™ Hypersil GOLD™ aQ column.
- The method is suitable for drug substance impurity analysis, without any adjustment.
- The Thermo Scientific™ Vanquish™ Core HPLC system provides excellent repeatability for retention time and peak area, as well as peak symmetry.

Goal

To demonstrate the European Pharmacopoeia based analysis of impurities in cefprozil with the new Vanquish Core HPLC system.

Introduction

Cefprozil is a second generation, broad-spectrum oral cephalosphorin antibiotic. It belongs to β-lactam antibiotics with anti-bacterial activity.[1] Cefprozil works by inhibiting bacterial septum and cell wall synthesis formation due to the binding to penicillin-binding proteins (PBPs) in the bacterial cytoplasmic membrane.[2] It is used to treat respiratory tract, skin, and ear infections, acute and chronic bronchitis, and other bacterial infections.[1]

Cefprozil consists of a mixture of the two diastereoisomers (i.e., Z- and E- isomers), and the Z-isomer is mainly responsible for the antibiotic activity.[1] The chemical structure of cefprozil is presented in Figure 1.

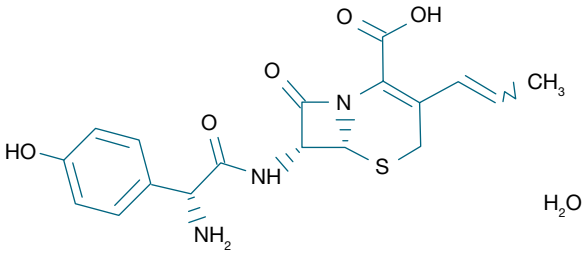

Figure 1. Chemical structure of cefprozil

Levels of impurities present in drug substances or formulation are strictly regulated by authorities such as the International Council for Harmonisation (ICH) and the U.S. Food and Drug Administration (FDA).[3] Based on the recommended daily dose of 1000 mg cefprozil,[4] reporting, identification, and quantitation limits of cefprozil impurities according to ICH guidelines are, in order, 0.05%, 0.1%, and 0.15%[5].

In this work, a method for the analysis of cefprozil related impurities is performed using a Vanquish Core high performance liquid chromatography (HPLC) system. The method is based on the cefprozil monohydrate European Pharmacopoeia (Ph. Eur.) monograph[6] and is suitable for impurity analysis for batch-release or stability evaluation. The Vanquish Core HPLC system delivers excellent retention time and peak area precision, whereby reliable results are generated.

Experimental

Chemicals

- Deionized water, 18.2 MΩ·cm at 25 °C, Thermo Scientific™ Barnstead™ GenPure™ xCAD Plus Ultrapure Water Purification System (P/N 50136149)
- Fisher Scientific™ acetonitrile, Optima™ LC/MS grade (P/N A955)
- Fisher Scientific ammonium phosphate, monobasic (P/N 10744914)
- Hydrochloric acid, fuming, 37% (purchased from a reputable vendor)
- Ph. Eur. reference standard: Cefprozil CRS batch 1 (Catalog code Y0001371)
- Ph. Eur. reference standard: Cefprozil for peak identification CRS batch 2 (Catalog code Y0001367)
- Ph. Eur. reference standard: Cefprozil impurity mixture CRS batch 2 (Catalog code Y0001368)
- Ph. Eur. reference standard: Cefprozil impurity A CRS batch (Catalog code Y0001372)

Sample handling

- Fisherbrand™ Mini Centrifuge (P/N 12-006-901)
- Thermo Scientific™ Orion™ 3 Star pH Benchtop Meter (P/N 13-644-928)
- Fisherbrand Mini Vortex Mixer (P/N 14-955-152)
- Thermo Scientific™ Finpipette™ F1 Variable Volume Single-Channel Pipettes: 100–1000 µL, 10–100 µL, 1–10 µL (P/N 4641100N, 4641070N, 4641030N)
- Vials (amber, 2 mL), Fisher Scientific (P/N 15508760)
- Snap Cap with Septum (Silicone/PTFE), Fisher Scientific (P/N 10547445)

Instrumentation

- Thermo Scientific™ Vanquish™ Core HPLC system consisting of:
  - Thermo Scientific™ Vanquish™ System Base (P/N VC-S01-A)
  - Thermo Scientific Vanquish Quaternary Pump C (P/N VC-P21-A)
  - Thermo Scientific Vanquish Split Sampler CT (P/N VC-A12-A)
  - Thermo Scientific Vanquish Column Compartment C (P/N VC-C10-A-03)
  - Thermo Scientific Vanquish Diode Array Detector CG (P/N VC-D11-A) with standard flow cell, 13 µL (P/N 6083.0510)

Sample preparation

Five separate solutions (test solutions *a* and *b* and reference solutions *b, c,* and *f*) were prepared as described in the Ph. Eur. monograph.

Test solution *a*

Test solution *a* was prepared by dissolving 0.125 g *cefprozil chemical reference substance (CRS)* in 1 mL of 103 g/L HCl solution and then diluting to 25.0 mL with solvent A (refer to mobile phase preparation section).

Test solution *b*

Test solution *b* was prepared by diluting the test solution *a* in a ratio of 1 to 15 with solvent A.

Reference solution *b*

Reference solution *b* was prepared by dissolving 5 mg *cefprozil for peak identification CRS* in 0.05 mL of 103 g/L HCl solution and adding 1 mL of solvent A. The *cefprozil for peak identification CRS* contains impurities B, H, and M, and the cefprozil Z- and E-isomers.

Reference solution *c*

Reference solution *c* was prepared by dissolving 3 mg *cefprozil CRS* and 6 mg *cefprozil impurity mixture CRS* in 2 mL of 103 g/L HCl solution and then diluting to 50 mL with solvent A. The *cefprozil impurity mixture CRS* contains impurities D and F.

Reference solution *f*

Reference solution *c* was prepared by dissolving 10.0 mg *cefprozil impurity A CRS* in 100.0 mL water. Then, 1.0 mL of the solution was further diluted to 10.0 mL with water.

Sample matrix preparation

The sample matrix was prepared by mixing 40 µL of 103 g/L HCl solution with 960 µL of solvent A, which was then diluted with solvent A in a ratio of 1 to 15.

Mobile phase preparation

Solvent A was prepared by dissolving ammonium dihydrogen phosphate (11.5 g) in 1000 mL water. The solution pH was measured as 4.39 and the solution was used without further pH adjustment. Solvent B was prepared by mixing acetonitrile (500 mL) with solvent A of 500 mL.

Chromatographic conditions

Table 1. HPLC conditions

Parameter	Value
Column	Thermo Scientific™ Hypersil GOLD™ aQ (4.6 × 250 mm, 5 µm) P/N 25305-254630*
Solvent A	0.1 M aqueous ammonium dihydrogen phosphate at pH 4.4
Solvent B	50/50 (v/v) 0.1 M aqueous ammonium dihydrogen phosphate at pH 4.4/ACN
Gradient	Time (min) %B 0 19 8 19 20 64 25 64 25.2 19 45 19
Flow rate	1.0 mL/min
Column temperature	40 °C (forced air with passive pre-heater)
Sampler temperature	4 °C
Injection volume	10 µL
Needle wash solvent	Acetonitrile/water 50/50 (v/v)
Needle wash mode	Before draw
UV detector parameters	Detection at 230 nm 3D scan 190–300 nm Data collection rate 10 Hz Response time 0.5 s

*The column meets specifications, such as length, inner diameter, particle size, and the bonded phase (i.e., end-capped octadecylsilyl silica gel), of the Ph. Eur. monograph.

Chromatography Data System

Thermo Scientific™ Chromeleon™ 7.3 Chromatography Data System (CDS) was used for data acquisition and processing.

Results and discussion

System suitability test

The system suitability test was performed with the reference solution *c* injected on the Hypersil GOLD aQ column. The reference solution *c* consists of a mixture of *cefprozil CRS* (comprised of cefprozil Z- and E-isomers) and *cefprozil impurity mixture CRS* (containing impurities D and F). The requirement for system suitability is that the resolution R_s between the cefprozil Z-isomer and the impurity F is greater than 1.4. Figure 2 shows a chromatogram obtained for reference solution *c.*

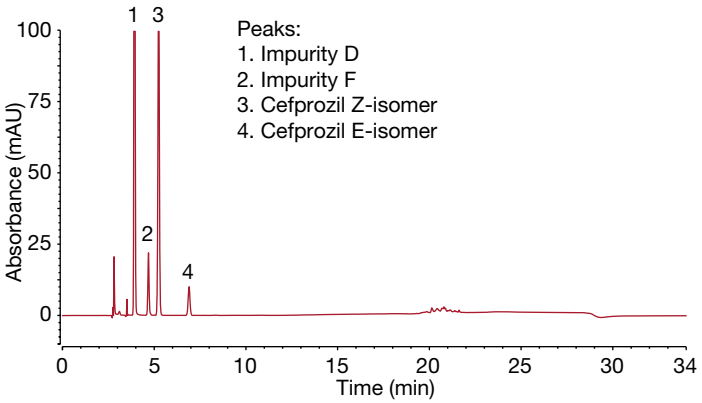

Figure 2. Chromatogram of reference solution *c* for system suitability test, showing a baseline separation of impurity F and cefprozil Z-isomer (R_s of 4.25). Peaks due to cefprozil Z- and E-isomers, and impurities D and F were assigned by comparing to the chromatogram provided by the Ph. Eur. *with cefprozil impurity mixture CRS.*

According to the monograph, peaks were assigned by comparing the chromatogram in Figure 2 to the one supplied with *cefprozil impurity mixture CRS.*[7] Retention times of impurities D and F, and cefprozil Z- and E-isomers were found to be 3.9, 4.7, 5.2, and 6.9 min, respectively. The cefprozil Z-isomer and the impurity F were well separated (R_s of 4.25), outperforming the Ph. Eur. acceptance criteria. In addition, the peak asymmetry values were in the range 0.87–1.02 (refer to Table 2 in the section "Method performance test"), meeting the Ph. Eur. criteria (i.e., 0.8–1.5).

Determination of cefprozil-related impurities

Impurities in cefprozil were quantified by peak area relative to the active pharmaceutical ingredient (API) cefprozil Z-isomer. The cefprozil standard solution (i.e., test solution *b*) was prepared by diluting test solution *a* (consisting of *cefprozil CRS*) in a ratio of 1 to 15, so that the UV absorbance of the API (i.e., cefprozil Z-isomer, around 1100 mAU) fell within the linear range of the detector. A chromatogram of the cefprozil standard is shown in Figure 3. Cefprozil Z- and E-isomers were well resolved from all impurities. The cefprozil isomers peaks were pure, as indicated by the Peak Purity Match values, 985 for isomer Z and 1000 for isomer E. A low-level impurity (indicated with *) eluted in front of cefprozil Z-isomer; however, the peak pair of isomer Z and impurity * was still sufficiently resolved (R_s 1.9). A total of 26 impurities were detected, accounting for 1.1% of the total relative area of cefprozil solution. Six impurities (i.e., impurities 1, 2, 3, 4, 5, 6) showed a relative area of >0.05%, which is the reporting threshold based on the ICH Q3A guideline. Two of the six (i.e., impurity 1 and 3) showed a relative area of >0.1%, the identification threshold. To identify these two impurities (i.e., impurities 1 and 3), two reference solutions (i.e., reference solutions *b* and *f*) were analyzed. By comparison of the injections of reference solutions *b* and *f,* impurities 1 and 3 were identified as impurities A and B. Table 1 lists retention times and relative peak areas for cefprozil Z- and E-isomers, as well as the six impurities. In addition, the two identified impurities above the corresponding threshold were indicated in the parentheses (Impurities A and B from the monograph).

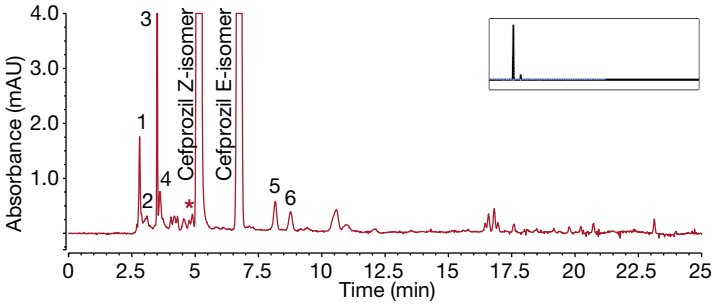

Figure 3. Chromatogram of cefprozil test solution *b,* diluted with test solution *a* (consisting of *cefprozil CRS*) in a ratio of 1 to 15. The chromatogram was subtracted from the chromatogram of the sample matrix. For the preparation of the sample matrix, refer to experimental section. All impurities above the reporting threshold are labelled with numbers according to the elution order. By comparison of the injections of reference solutions *b* and *f*, impurities 1 and 3, found above the identification threshold, were identified as impurities A and B as stated in the monograph, respectively.

Table 1. Retention time (RT) and relative area for all impurities in cefprozil solution, found above the reporting threshold, and API isomers (n = 3). The two identified impurities above the corresponding threshold were indicated in the parentheses (Impurities A and B from the monograph).

	Average RT (min)	Average relative area (%)
Impurity 1 (Impurity A)	2.81	0.14
Impurity 2	3.09	0.06
Impurity 3 (Impurity B)	3.50	0.28
Impurity 4	3.61	0.09
Cefprozil Z-isomer	5.16	89.23
Cefprozil E-isomer	6.74	9.67
Impurity 5	8.17	0.07
Impurity 6	8.77	0.06

Method performance test

Repeatability tests were performed for cefprozil Z- and E-isomers, and two identified impurities (i.e., impurities A and B). Three individual reference solutions with pure peaks were injected, resulting in clear peak area integration. This enabled accurate determination of the peak area precision to characterize the quantification performance of the method. The reference solution *b* (the standard for impurity B) and the reference solution *c* (the standard for cefprozil Z- and E-isomers) were injected seven consecutive times, the reference solution *f* (the standard for impurity A) three consecutive times. Table 2 summarizes the quantification performance result. Excellent precision of retention times and peak areas, as well as peak asymmetry were achieved on the Vanquish Core HPLC system. The relative standard deviation (RSD) of retention times and areas were less than 0.1% and 0.4%, respectively, for all cefprozil isomers and related impurities.

Table 2. Precision of RT and peak area for cefprozil and related impurities, along with peak asymmetry (n = 7).

Name	Average RT (min)	RT RSD (%)	Area RSD (%)	Average asymmetry (Ph. Eur.)
Impurity A* (impurity 1)	2.82	0.00	0.04	1.08
Impurity B (impurity 3)	3.51	0.04	0.29	0.98
Cefprozil Z-isomer	5.24	0.06	0.36	1.00
Cefprozil E-isomer	6.89	0.07	0.33	0.99

*Indicates the result of three consecutive injections (n = 3).

Conclusion

- The quantification of cefprozil-related impurities based on the Ph. Eur. method was successfully demonstrated.
- The Vanquish Core HPLC system delivered high precision in retention time and area.
- The Hypersil GOLD aQ column provided sufficient separation of impurity F and cefprozil Z-isomer, outperforming the criteria for system suitability.

References

1. Rai, B.P.; Tewari, N.; Nizar, H.; Prasad, M.; Joseph, S. Commercial synthesis of cefprozil: Development and control of process impurity, *Org. Process Res. Dev.* **2014,** *18,* 662–664.
2. Kong, K.-F.; Schneper, L.; Mathee, K. Beta-lactam antibiotics: From antibiosis to resistance and bacteriology, *APMIS* **2010,** *118,* 1–36.
3. Pilaniya, K.; Chandrawanshi, H.K.; Pilaniya, U.; Manchandani, P.; Jain, P.; Singh, N. J. *Adv. Pharm. Technol. Res.* **2010,** 302–310.
4. Barriere, S.L., Update on new oral cephalosporins, *Antimicrobic newsletter* **1992,** *8,* 21–24.
5. International Conference on Harmonization (ICH). Q3A (R2): Impurities in new drug substances. **2008.**
6. European Directorate for the Quality of Medicines & HealthCare; European Pharmacopeia (Ph.Eur.) Online, 9th edition **2018** (9.4), monograph 2342; Cefprozil monohydrate.
7. Cefprozil impurity mixture CRS batch 2: European Directorate for the Quality of Medicines & Health Care; European Pharmacopoeia (Ph.Eur.); 7, Allée Kastner CS 30026, F-67081 Strasbourg (France).

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