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Application News

No.L475

High Performance Liquid Chromatography

High Speed with High Resolution Analysis by Nexera-i Analysis of Cephem Antibiotics

Cephem antibiotics are a subgroup of β-lactam antibiotics which can be administered orally or by injection similar to the penicillin and carbapenem subgroups. Cephem antibiotics exhibit both a low frequency and low degree of side effects while maintaining a high degree of safety, and can therefore be applied in a wide range of cases.
Application News No. L348 introduced an example of high-speed, high-resolution analysis of cephem antibiotics using the Prominence UFLC ultra-fast HPLC system. Here, we introduce an example of high-speed, high-resolution analysis of cephem antibiotics using the new Nexera-i ultra-high-performance integrated liquid chromatograph with a PDA detector.

Analysis of Standard of Cephem Antibiotics

Cephems include about fifty types of antibiotics, but here we analyzed a standard mixture of twelve of these substances (cephradine, cephalexin, cephapirin, cephazolin, cefadroxil, cephalothin, cefamandole*, cefaclor, cefotaxime, cefoperazone, cefuroxime, cefoxitin), each at 50 mg/L. Fig. 1 shows the chromatograms acquired using the Shim-pack VP-ODS 5 µm particle size general-purpose column (upper chromatogram), and the Shim-pack XR-ODS Ⅱ 2.2 µm particle size high-speed column (lower chromatogram). The analytical conditions are shown in Table 1, and the structural formulas of the 12 compounds are shown in Fig. 2.
From the upper chromatogram in Fig. 1, the resolution of cefaclor and cephalexin (Peaks 3 and 4) detected at about six minutes is approximately 1.8, which is greater than the commonly accepted values of 1.5 to 1.7 for adequate resolution. In the lower chromatogram, the resolution of the same substances is about 1.9.
Use of the Shim-pack XR-ODS Ⅱ reduces the analysis time and mobile phase consumption to less than one fifth and a quarter of what is seen with a conventional column, respectively, while maintaining peak shape and resolution. The system backpressure under these conditions was approximately 55 MPa. The Nexera-i is operable at pressures up to 66 MPa (9570 psi), allowing high-speed analysis with small particle columns.

* Since two of the peaks that were generated were confirmed as cefamandole, they were notated as Cefamandole A and Cefamandole B, respectively.

Table 1 Analytical Conditions

Column	: Shim-pack VP-ODS (250 mm L. × 4.6 mm I.D., 4.6 µm) Shim-pack XR-ODS Ⅱ (150 mm L. × 3.0 mm I.D., 2.2 µm)
Mobile Phase	: A) 0.1 % Formic Acid in Water B) Acetonitrile
Time Program	: [VP-ODS] B. Conc. 15 % (0 min) → 55 % (35 min) → 15 % (35.01- 50 min) [XR-ODS Ⅱ] B. Conc. 15 % (0 min) → 55 % (6.95 min) → 15 % (6.96- 10 min) Ontime Injection 343 µL
Flowrate	: 0.7 mL/min (VP-ODS) 0.9 mL/min (XR-ODS Ⅱ)
Column Temp.	: 40 °C
Injection Volume	: 5 µL (VP-ODS) 2 µL (XR-ODS Ⅱ)
Detection	: LC-2040C 3D at 260 nm
Flow Cell	: High-Speed High-Sensitivity Cell

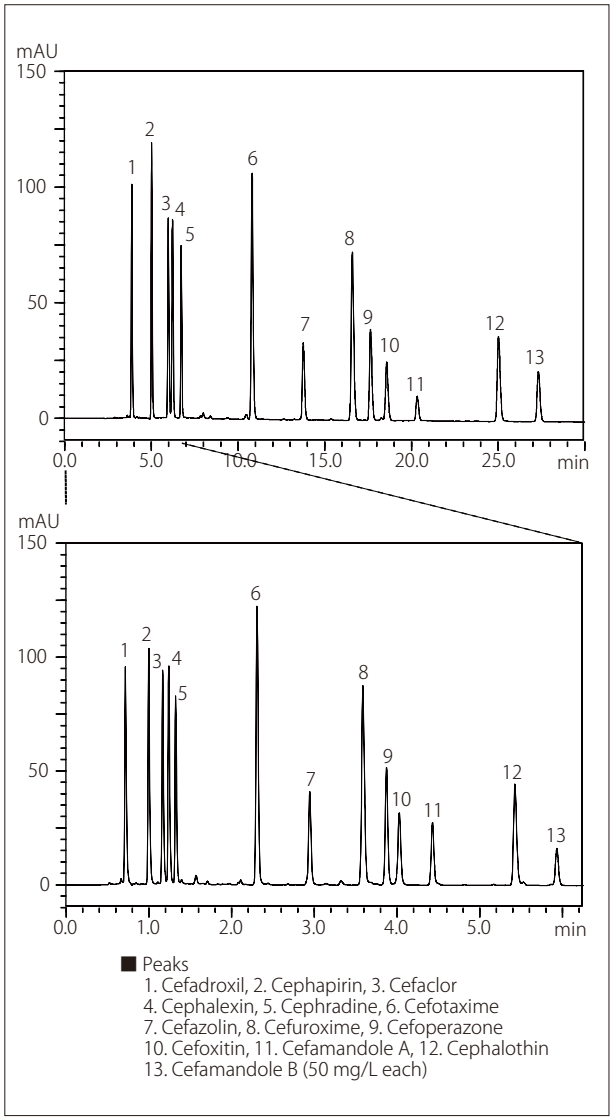

Fig. 1 Chromatograms of a Standard Mixture of 12 Cephem Antibiotics
Upper: Shim-pack VP-ODS
Lower: Shim-pack XR-ODS Ⅱ

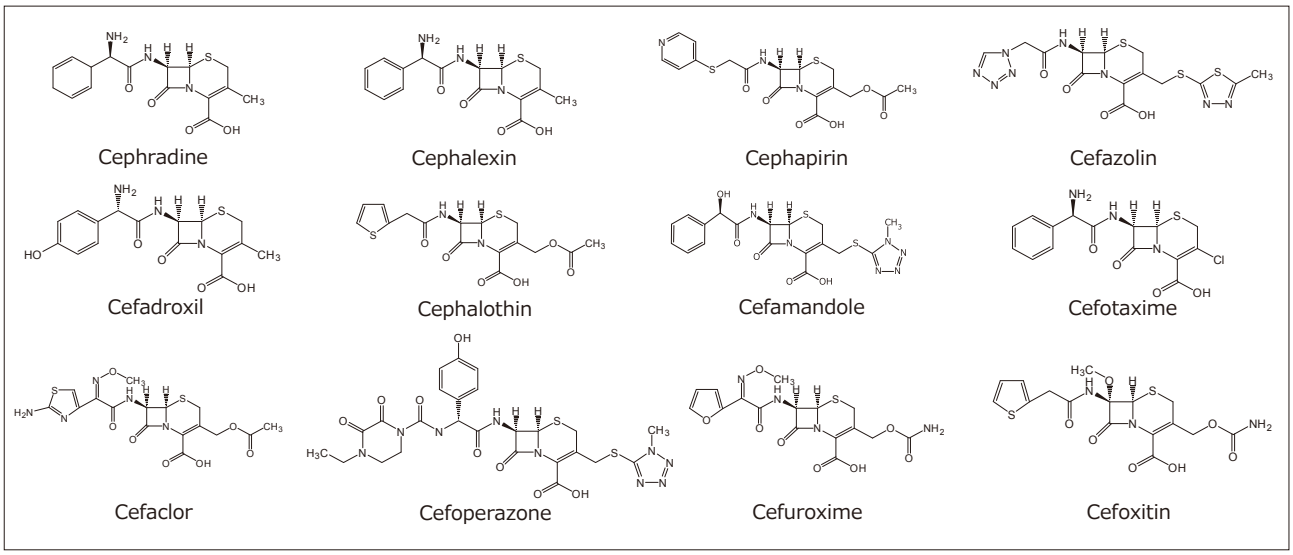

Fig. 2 Structures of 12 Cephem Antibiotics

UV-VIS Spectra

Fig. 3 shows the spectra of 12 cephem antibiotics obtained using the LC-2040C 3D.

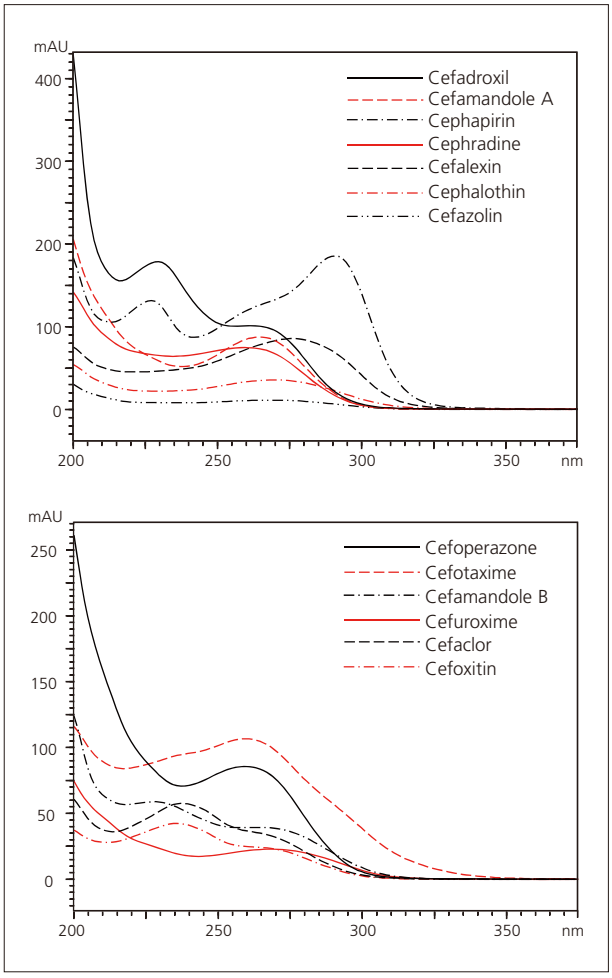

Fig. 3 Spectra of 12 Cephem Antibiotics

Repeatability

Table 2 shows the repeatability of retention time and peak area obtained from six repeat injections of a mixture of cephem antibiotics (50 mg/L each) using the Nexera-i. The analytical conditions were the same as those used with the XR-ODS Ⅱ in Table 1. Excellent repeatability of both retention time and peak area were observed.

Table 2 Repeatability of Retention Time and Peak Area of Cephem Antibiotics

ID#	Compounds	Retention Time %RSD (n=6)	Peak Area %RSD (n=6)
1	Cefadroxil	0.14	0.14
2	Cephapirin	0.09	0.17
3	Cefaclor	0.22	0.25
4	Cephalexin	0.19	0.29
5	Cephradine	0.24	0.29
6	Cefotaxime	0.07	0.10
7	Cefazolin	0.06	0.09
8	Cefuroxime	0.05	0.07
9	Cefoperazone	0.05	0.11
10	Cefoxitin	0.04	0.14
11	Cefamandole A	0.04	0.17
12	Cephalothin	0.03	0.09
13	Cefamandole B	0.03	0.21

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