



Fully Automated Sample Preparation Module for LCMS CLAM<sup>™</sup>-2030 Triple Quadrupole Liquid Chromatograph Mass Spectrometer LCMS-8045/8050/8060/8060NX

# Fully Automated Simultaneous Analysis of Cephem Antibiotics in Serum by LC-MS/MS

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#### **User Benefits**

- Simultaneous analysis of 12 different cephem antibiotics is possible.
- ◆ The entire process is automated, from biological sample preparation to LC-MS/MS measurement.
- Good reproducibility is achieved with a measurement cycle time of 7 minutes.

### Introduction

Cephem antibiotics like penicillin and carbapenems are a type of  $\beta$ -lactam antibiotic used in oral medicines and injectable solutions. Cephem antibiotics are said to be effective for various illnesses because they have a high safety profile with a low frequency and degree of side effects.

Here, we introduce a simultaneous analysis method for cephem antibiotics in blood plasma by LC-MS/MS using the CLAM-2030, which enables fully-automated pretreatment of biological samples. The CLAM-2030 can be used to reduce specimen mixups and variations in procedure.

There are 50 main cephem antibiotics, of which 12 components (cephadroxil, cefapirin, cefaclor, cephalexin, cefuroxime, cefotaxime, cefoxitin, cefazolin, cepharazine, cefoperazone, cefamandole, and cephalothin) were targeted in this analysis system.

# Analytical Condition

The LC/MS system consisting of a CLAM-2030 fully-automated LCMS pretreatment instrument, a Nexera<sup>TM</sup> X2 ultra-high performance liquid chromatograph, and an LCMS-8060 triple quadrupole mass spectrometer was used (Fig. 1).

Table 1 shows the HPLC and MS analysis conditions. The trap column, analytical column, and mobile phase included in the DOSIMMUNE<sup>™</sup> immunosuppressant analysis kit were used: the sample injected into LC-MS/MS is concentrated and purified by the trap column then separated by the analytical column (Fig. 2). The three stable isotope-labeled compounds used as internal standards (IS) were from Alsachim.

Table 1 Analytical Condition

Fig. 1 LC-MS/MS System with Fully Automated Pretreatment System



Fig. 2 Flow Diagram (Trap & Elute System)

IPLC Conditions Nexera X2   Column: DOSIMMUNE trapping column   DOSIMMUNE analytical column   Mobile Phases: DOSIMMUNE mobile phase A, B   Flowrate: A/B 0.5 mL/min (for analysis)					
Time Program:	C 1.2 mL/min (for trap) 1 %B(0-0.5 min) – 30 %B(3 min) - 98 %B(4-5 min) – 1 %B(5.01-7 min)				
Column Temp.: Injection Volume:	40 °C 1 μL	40 °C 1 μL			
MS Conditions	LCMS-8060	)			
Ionization:	ESI-positive	2			
Nebulizing Gas Flow:	3 L/min				
Drying Gas Flow:	10 L/min				
Heating Gas Flow:	10 L/min				
Interface Temp.:	300 °C				
DL Temp.:	200 °C				
Heat Block Temp.:	400 °C				
Mode:	MRM				
Analyte		MRM	R.T (min)		
Cefadroxil		364.00 > 114.05	1.221		
Cefapirin		424.00 > 292.05	1.812		
Cefaclor		368.00 > 174.10	1.957		
Cefalexin		348.00 > 158.10	2.047		
Cefuroxime		442.00*2 > 364.05	2.176		
Cefotaxime		456.00 > 125.05	2.284		
Cefoxitin		445.00*2 > 215.10	2.297		
Cefazolin		455.00 > 323.10	2.315		
Cephradine		350.00 > 176.15	2.320		
Cefoperazone		646.00 > 143.15	2.939		
Cefamandole		463.00 > 158.05	3.110		
Cephalothin		414.00*2>152.00	3.520		
[2H5]-Cefaclor (C7546)*	1	373.00 > 179.15	1.941		
[ <mark>2H5</mark> ]-Cefalexin (C6695)* <sup>1</sup>		353.00 > 158.05	2.032		
[13C,2H3]-Cefotaxime (C6106)*1		460.00 > 125.05	2.269		
*1 Product Number of Alsachim		*2 [M+NH4] <sup>+</sup>			

## ■ Analysis of Cephem Antibiotic Standards

Six calibrators were prepared for 12 cephem antibiotics at 0.01, 0.05, 0.1, 0.5, 1, and 5  $\mu$ g/mL. They were analyzed six times for each point, and the results are shown in Fig. 3 and Fig. 4.

Good linearity with an R2 value of 0.998 or higher for each compound was obtained over the calibrator's concentration range. The accuracy of each calibration point was also within  $100 \pm 10$ % for each compound, and the area value reproducibility (%RSD) was better than 15%.



Fig. 3 Calibration Curves for 12 Cephem Antibiotics



Fig. 4 MRM Chromatograms of 12 Cephem Antibiotics at the Lower Limit of the Calibration

# Analysis of Cephem Antibiotics in Plasma Samples by CLAM-2030

Samples were prepared with 12 cephem antibiotics added to plasma at 0.3 and 3  $\mu\text{g/mL},$  and the internal standard was prepared to 5  $\mu$ g/mL each.

The pretreatment process of the plasma samples in the CLAM-2030 is shown in Fig. 5. First, 20 µL of 75 % isopropanol was added, then 20  $\mu L$  of plasma sample, 10  $\mu L$  of IS, and 100  $\mu L$  of methanol were added and stirred, followed by suction filtration and LC-MS/MS analysis.

The results of the assay with two concentrations of plasma samples are shown in Table 2. Pretreatment dilutes the cephem antibiotics in the plasma samples to 20/150, resulting in final concentrations of 0.04 and 0.4  $\mu$ g/mL in the measured samples, respectively. The accuracy was within 100  $\pm$  15 % for each compound, and the reproducibility of concentration values (%RSD) was better than 15 %.

# ■ Conclusion

A simultaneous analysis system for 12 cephem antibiotics was developed from automated pretreatment with CLAM-2030 to LC-MS/MS. Good linearity and accuracy were obtained for all components in the set concentration range. In addition, good results were also obtained for samples added to plasma, with accuracy within 100  $\pm$  15 % for each compound and concentration value reproducibility (%RSD) of less than 15 %.

Automatic sample preparation with the CLAM-2030 takes approximately 3 minutes and analysis time with the LCMS-8060 is 7 minutes, making it possible to obtain measurement results in a total of 10 minutes.



Conc. (µg/mL)	0.3 (Final conc. 0.04)			3 (Final conc. 0.4)		
Analyte	Measured value (µg/mL)	Accuracy%	%RSD	Measured value (μg/mL)	Accuracy%	%RSD
Cefaclor	0.28	92.4	12.1	2.89	96.3	1.8
Cefalexin	0.30	100.4	5.0	3.14	104.5	5.3
Cefotaxime	0.27	89.8	2.5	2.87	95.5	3.5
Cefazolin	0.28	94.7	5.1	2.97	99.1	2.2
Cefuroxime	0.29	96.4	5.9	2.85	95.1	3.5
Cefoperazone	0.29	98.1	1.9	2.97	99.0	4.3
Cefoxitin	0.29	96.7	5.5	2.99	99.6	5.4
Cefamandole	0.29	97.2	2.6	3.02	100.6	6.4
Cephradine	0.29	96.2	2.3	2.96	98.8	3.0
Cephalothin	0.28	93.6	2.3	2.95	98.4	4.7
Cefapirin	0.32	107.7	6.3	3.28	109.3	2.9
Cefadroxil	0.33	110.9	5.7	3.42	113.9	2.0

Table 2 Results of the Assay with Two Concentrations of Plasma Samples

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