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Application News

No. C153

Liquid Chromatograph Mass Spectrometry

Simultaneous Analysis of Antiarrhythmic Drugs in Human Blood Plasma Using the Fully Automated Sample Preparation LC/MS/MS System

During drug treatment with drugs that pose administration management difficulties, such as drugs with a narrow therapeutic range or drugs with a fine line between toxicity and effectiveness, the blood concentration of drugs in patients is measured to determine the optimal dose and method of administration for individuals based on pharmacokinetic and pharmacodynamic analysis. Application News No. C123 introduced an investigation into optimizing the analysis workflow including pretreatment by using the fully automated sample preparation LC/MS/MS system that comprises the CLAM-2000 fully automated LCMS sample preparation unit and a high performance liquid chromatograph mass spectrometer.

This article introduces a study which achieves a fast and simultaneous analysis workflow of six antiarrhythmic drugs with the fully automated sample preparation LC/MS/MS system.

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Analysis of Antiarrhythmic Drugs in Blood Plasma with Fully Automated Pretreatment

Pretreatment of blood plasma samples for analysis normally requires a process that involves deproteinization by adding an organic solvent, followed by centrifugal separation of solid components and supernatant isolation. With the fully automated sample preparation LC/MS/MS system, these preparatory steps are done automatically just by setting a blood collection tube after blood plasma separation, and LC/MS/MS analysis is continuously performed (Fig. 1). Pretreatment of the next sample can also be performed in parallel with LC/MS/MS analysis, which can greatly reduce the time required for each sample analysis.

In this analysis example, a per-sample cycle time of 7 minutes was achieved from blood plasma pretreatment to the simultaneous analysis of six antiarrhythmic drugs and metabolites using LC/MS/MS (Table 1 and Fig. 2).

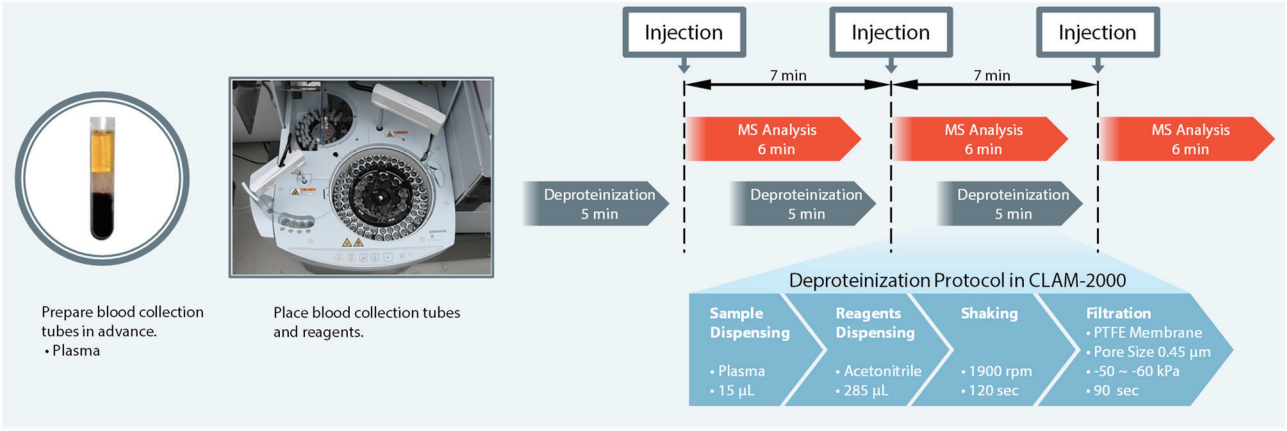


Fig. 1 Pretreatment Workflow of Blood Plasma Samples

Table 1 Antiarrhythmic Drugs and Metabolites

Compound	Molecular Formula	MRM Transition *m/z*
Amiodarone	$C_{25}H_{29}I_2NO_3$	646.0 > 58.1
Desethylamiodarone*	$C_{23}H_{25}I_2NO_3$	618.0 > 72.1
Bepridil	$C_{24}H_{34}N_2O$	367.1 > 84.1
Flecainide	$C_{17}H_{20}F_6N_2O_3$	415.0 > 301.0
Pilsicainide	$C_{17}H_{24}N_2O$	272.9 > 110.1
Cibenzoline	$C_{18}H_{18}N_2$	262.9 > 115.0
Mexiletine	$C_{11}H_{17}NO$	180.1 > 58.0

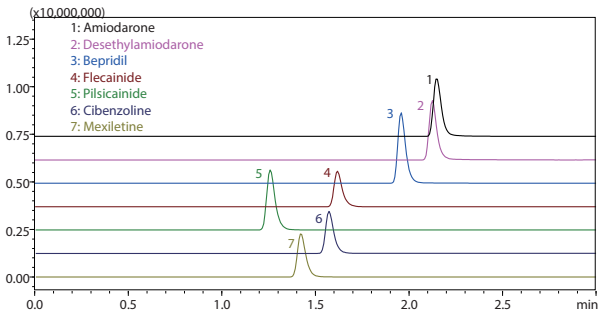


Fig. 2 Mass Chromatograms of Human Blood Plasma with Standard Additives

Validation Test of the Fully Automated Pretreatment Analysis Method

Calibration curves were created from the control blood plasma with standards added and the integrity of accuracy and precision were evaluated based on the analysis results of the QC samples (at concentrations of n = 5) (Table 2). Good linearity was obtained in the set concentration range for all antiarrhythmics. The accuracy of the QC samples in the entire range, including the quantitative lower limit, was within 100 ± 15 %. Similarly, precision (%RSD) was within 15 % and good repeatability was obtained.

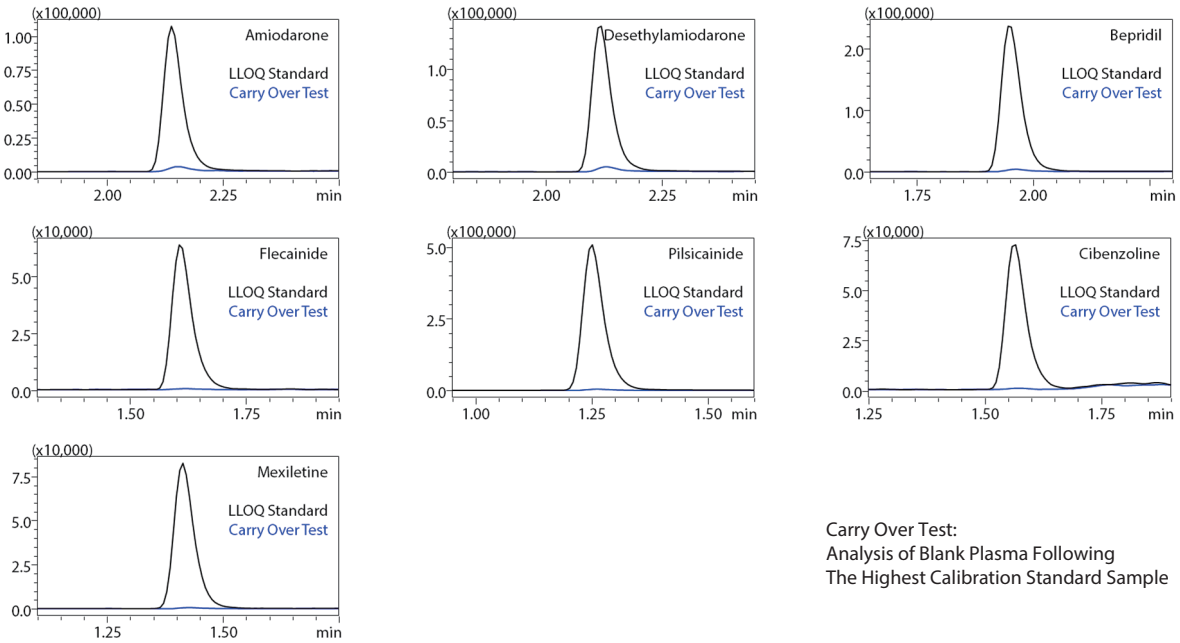
Immediately after analysis of the highest calibration standard sample, blank blood plasma was measured to check for carryover in the fully automated sample preparation LC/MS/MS system. No significant carryover was detected for any of the drugs upon comparison with the peak intensity of the lowest calibration standard sample (Fig. 3).

The above results show that the fully automated sample preparation LC/MS/MS system used in this article is capable of sufficiently reliable quantitative analysis when performing consecutive analyses of samples of wide-ranging concentrations.

Table 2 Validation Test Results for Simultaneous Analysis of Antiarrhythmic Drugs and Metabolites

Compounds	Cal. Range [ng/mL]	Correlation Coefficient R	Accuracy %				Precision %RSD, n=5			
			LLOQ	Low	Medium	High	LLOQ	Low	Medium	High
Amiodarone*1	100-3000	0.9983	98.3	100.6	99.4	103.9	4.1	2.9	3.0	2.7
Desethylamiodarone*1	100-3000	0.9987	99.2	98.9	101.1	100.3	5.3	4.2	3.6	4.2
Bepridil*2	50-1500	0.9992	100.9	100.5	96.6	103.4	4.1	3.7	2.3	1.8
Flecainide*2	50-1500	0.9987	98.1	98.7	96.7	101.4	4.7	3.3	2.4	2.4
Pilsicainide*1	100-3000	0.9987	100.4	99.6	97.3	104.8	4.0	3.0	1.8	2.0
Cibenzoline*2	50-1500	0.9987	102.4	101.4	99.1	102.9	4.2	3.4	3.0	2.4
Mexiletine*1	100-3000	0.9984	104.5	107.4	106.3	107.8	3.8	3.9	2.6	2.6

*1: 100 ng/mL for LLOQ, 250 ng/mL for Low, 1000 ng/mL for Medium, 3000 ng/mL for High
*2: 50 ng/mL for LLOQ, 125 ng/mL for Low, 500 ng/mL for Medium, 1500 ng/mL for High



Carry Over Test:
Analysis of Blank Plasma Following
The Highest Calibration Standard Sample

Fig. 3 Carryover Test Results

Table 3 Analysis Conditions (Validation Test)

System	: CLAM-2000 + Nexera + LCMS-8060		
Protocol	: Plasma disp. 15 µL - acetonitrile disp. 285 µL - shaking at 1900 rpm, 120 sec - filtration for 90 sec		
Column	: Shimadzu GLC Mastro C18 (50 mmL. × 2.1 mmI.D., 3 µm)		
Mobile Phase	: A) 0.1% Formic acid - Water, B) 0.1% Formic acid - Methanol		
Flow Rate	: 0.4 mL/min		
Time program	: B Conc. 10% (0 min) – 100% (2 – 3.5 min) – 10% (3.51 – 6 min)		
Column Temp.	50 °C	Injection Volume	: 0.2 µL
Probe Voltage	: 2.0 kV (ESI-positive mode)		
Interface Temp.	: 300 °C	DL Temp.	: 250 °C
Block Heater Temp.	: 400 °C	Nebulizing Gas Flow	: 3 L/min
Heating Gas Flow	: 10 L/min	Drying Gas Flow	: 10 L/min

Comparative Test with Manual Pretreatment

A comparative test was performed between a manual pretreatment method and the fully automated pretreatment analysis method that employs the fully automated sample preparation LC/MS/MS system. Human blood plasma for measuring the concentration of amiodarone was used.

The manual pretreatment method involved manually isolating the blood plasma, adding acetonitrile, and mixing to perform deproteinization. After centrifugal separation of this sample, the supernatant was then transferred to a vial for LC/MS/MS analysis. On the other hand, the fully automated pretreatment analysis method enabled the entire analysis process, from blood plasma isolation to LC/MS/MS analysis, to be performed completely automatically using the system described in this article (Fig. 4).

A comparison of quantitative values between the methods was performed for amiodarone and the metabolite desethylamiodarone (Fig. 5 and 6, Table 4 and 5). In the wide range of concentrations detected from the samples, there was favorable agreement between the quantitative results of the manual pretreatment method and the fully automated pretreatment analysis method. The coefficient of determination (R2) of both methods calculated from these results was 0.95 or higher (Fig. 7 and 8).

The fully automated pretreatment analysis method used by this system is a fast and low-burden analysis technique that achieves quantitative results equivalent to conventional manual pretreatment methods and we anticipate its utilization into the future.

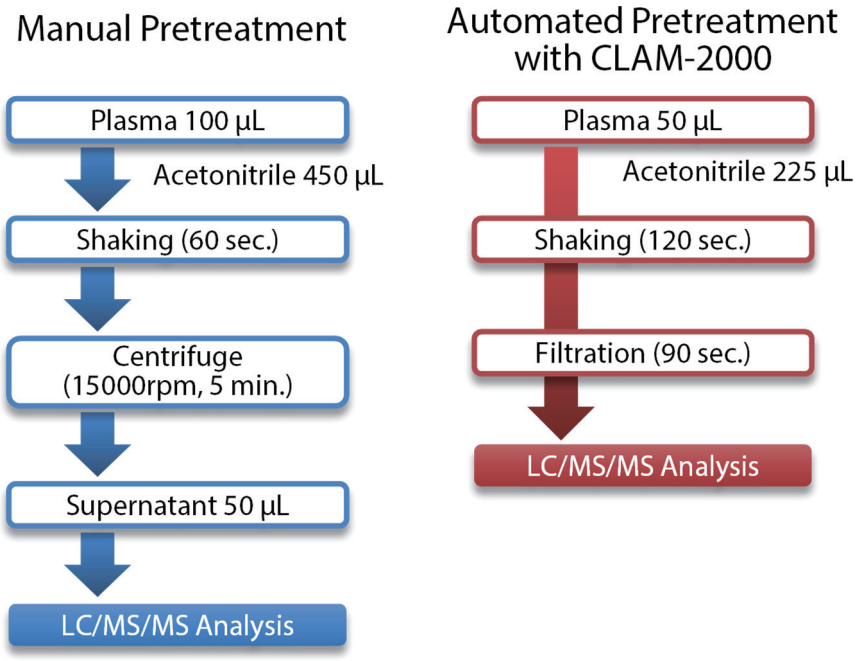


Fig. 4 Pretreatment Workflow of the Manual Pretreatment Method and Fully Automated Pretreatment Analysis Method

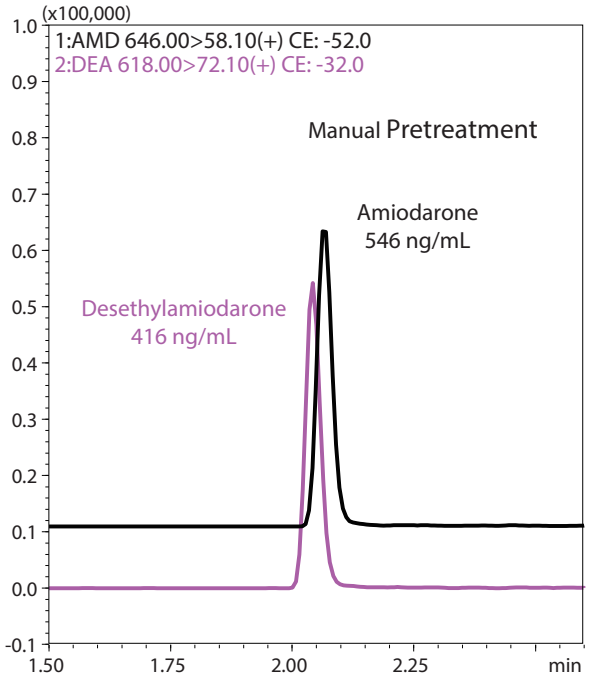


Fig. 5 Human Blood Plasma (Sample 3) Analysis Results Using the Manual Pretreatment Method

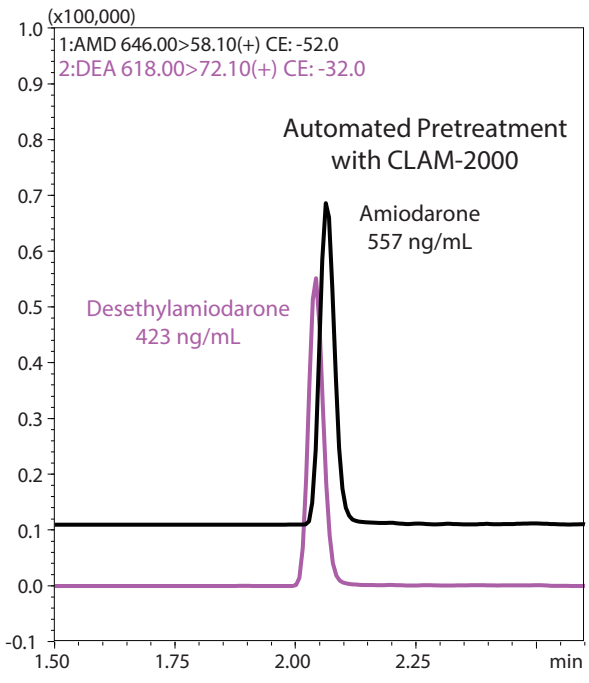


Fig. 6 Human Blood Plasma (Sample 3) Analysis Results Using the Fully Automated Pretreatment Analysis Method

Table 4 Quantitative Results of the Manual Pretreatment Method and Fully Automated Pretreatment Analysis Method (Amiodarone)

	Amiodarone		
	Manual [ng/mL]	Automated [ng/mL]	Ratio %*
Sample 1	373	411	110.2
Sample 2	399	404	101.3
Sample 3	546	557	102.0
Sample 4	205	211	102.9
Sample 5	963	895	92.9
Sample 6	1,318	1,213	92.0
Sample 7	1,271	1,229	96.7
Sample 8	1,233	1,282	104.0
Sample 9	2,259	2,208	97.7
Average			**100.0**
RSD %			**5.8**

* Automated Pretreatment / Manual Pretreatment

Table 5 Quantitative Results of the Manual Pretreatment Method and Fully Automated Pretreatment Analysis Method (Desethylamiodarone)

	Desethylamiodarone		
	Manual [ng/mL]	Automated [ng/mL]	Ratio %*
Sample 1	304	271	89.1
Sample 2	412	366	88.8
Sample 3	416	423	101.7
Sample 4	271	240	88.6
Sample 5	717	654	91.2
Sample 6	151	150	99.3
Sample 7	431	408	94.7
Sample 8	664	628	94.6
Sample 9	940	1,080	114.9
Average			**95.9**
RSD %			**8.9**

* Automated Pretreatment / Manual Pretreatment

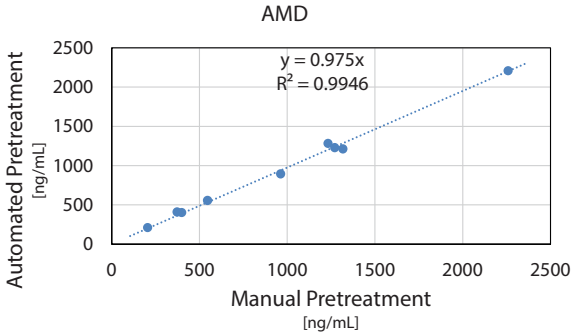


Fig. 7 Comparison of Quantitative Results for Amiodarone

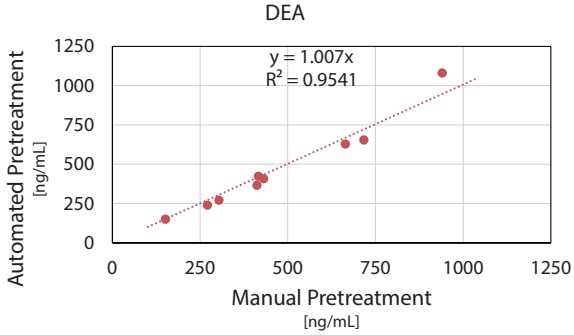


Fig. 8 Comparison of Quantitative Results for Desethylamiodarone

Table 6 Analysis Conditions (Comparative Test of Pretreatment Methods)

System	: CLAM-2000 + Nexera + LCMS-8040		
Protocol	: Plasma disp. 50 µL - acetonitrile disp. 225 µL - mixing at 1900 rpm, 120 sec - filtration for 90 sec		
Column	: Shimadzu GLC Mastro C18 (50 mmL. × 2.1 mmI.D., 3 µm)		
Mobile Phase	: A) 0.1% Formic acid - Water, B) 0.1% Formic acid - Methanol		
Flow Rate	: 0.4 mL/min		
Time program	: B Conc. 10 % (0 min) – 100 % (2 – 3.5 min) – 10 % (3.51 – 6 min)		
Column Temp.	50 °C	Injection Volume	: 0.1 µL
Probe Voltage	: 4.5 kV (ESI-positive mode)		
DL Temp.	: 250 °C	Block Heater Temp.	: 400 °C
Neb. Gas Flow	: 3 L/min	Drying Gas Flow	: 15 L/min

[Acknowledgments]
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References
- Guidance for Industry : Bioanalytical Method Validation (2001, US FDA)
- Guideline on Bioanalytical Method Validation in Pharmaceutical Development (2013, Japan MHLW)

Notes
- The product described in this document has not been approved or certified as a medical device under the Pharmaceutical and Medical Device Act of Japan. It cannot be used for the purpose of medical examination and treatment or related procedures.
- The samples described in this document were all sampled and measured at the National Cerebral and Cardiovascular Center Hospital in Japan. Permission was obtained regarding the publication of measurement data.

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