SHIMADZU
Excellence in Science

Application News

No. C213

Liquid Chromatography Mass Spectrometry

High-Sensitivity Analysis of Drugs in Ultra-Small Volume Plasma Samples Using Microflow LC/MS/MS

It is known that drugs and other xenobiotics are generally subject to metabolism in the body, which facilitates their detoxification and elimination from the body. Therefore, pharmacokinetic (PK) studies of the metabolic fate of drugs in the body are conducted by preclinical and clinical tests as part of the drug development process [(1)].

In preclinical PK studies, the concentration of drugs and their metabolites in biological samples obtained from animal experiments is analyzed by LC/MS/MS.

However, since the size of the animal model generally limits the amount of sample volume that can be taken safely, a large number of animals and a significant volume of drug may be necessary for the evaluation, and this becomes an issue from the ethical and economic point of view.

An effective approach to overcome those issues, together with the use of micro sampling technology, is the development of high sensitivity LC MS/MS methods that allows drug detection from the small amount of sample.

This article introduces an example of a high-sensitivity microflow LC/MS/MS method, for the analysis of drugs in ultra-small volume plasma samples.

D. Vecchietti, K. Matsumoto

Nexera Mikros™ Microflow Liquid Chromatography Mass Spectrometry System

This study was conducted using a Shimadzu Nexera Mikros (Fig. 1), which can be used effectively in a wide range of flow rates from the microflow region to the semi-microflow region (1 to 500 µL/min).

Fig. 1 Nexera Mikros™ Microflow LC-MS System

The Nexera Mikros can detect target components with higher sensitivity than the semi-microflow LC/MS systems in general use, which operates in the flow rate range of approximately 100 to 500 µL/min, and also offers excellent robustness and shorter analysis times in comparison with nano LC/MS system, which operates in a flow rate range of several 100 nL/min to 1 µL/min.

Fig. 2 shows the flow diagrams during trapping and during elution. Because acetonitrile is commonly used as a sample solvent for deproteinization, the peak shape in direct injection is broadened due to the elution strength of acetonitrile, and this limits the injection volume. In order to overcome this broadening of the peak by the sample solvent, the trap & elute injection method is used in the Nexera Mikros system. After the target compound is retained in the trap column (trap), the column is backflushed with the analytical mobile phase by switching a valve, and the target compound is transported to the analytical column (elute) for separation and detection.

Sample Preparation

Plasma samples (2 µL) were prepared by spiking with Verapamil and Nor-Verapamil, which are shown in Fig. 3. The spiked samples were diluted by adding 58 µL of a precipitant solution (containing acetonitrile + formic acid 0.1 % and a labelled internal standard, Verapamil-D6). After allowing the samples to stand quietly for 20 min (incubation) at a room temperature, the samples were centrifuged and the supernatant was transferred to a vial, and 5 µL of the samples was injected for analysis.

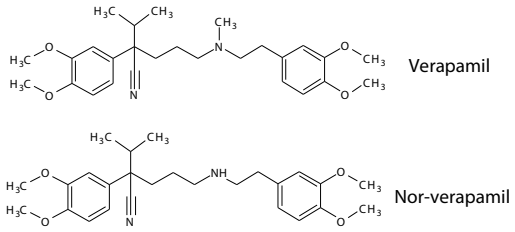

Fig. 3 Chemical Formulas of Verapamil and its Metabolite Nor-Verapamil

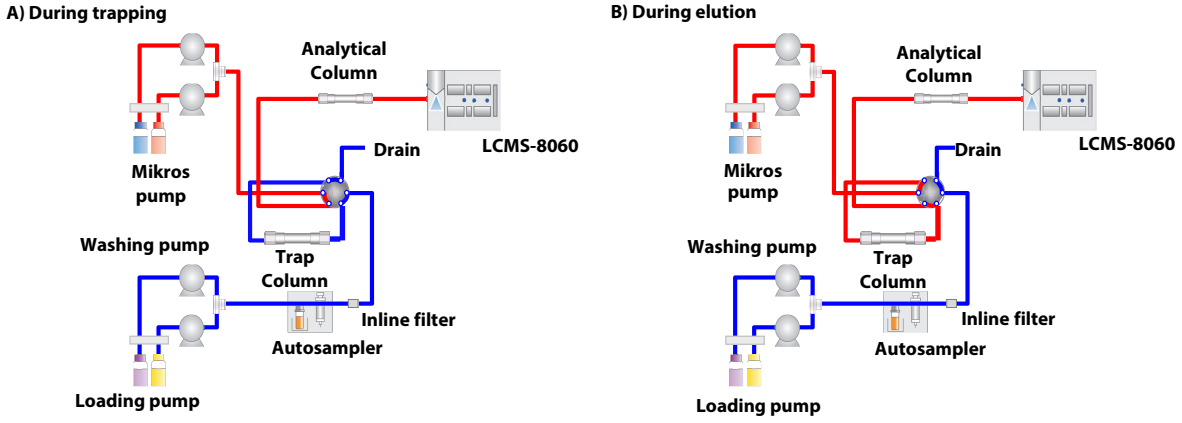

Fig. 2 Flow Diagrams of Trap and Elute System

Analytical Conditions

Table 1 and Table 2 show the conditions of HPLC using the Nexera Mikros and MS, respectively. Sample loading in the trap column was carried out using water/acetonitrile = 98/2 containing 0.1 % formic acid, and the analysis was conducted by gradient separation of the water and acetonitrile containing 0.1 % formic acid. During the analysis, 100 % acetonitrile was delivered to the trap column to wash. A comparison of microflow analysis and semi-microflow analysis was also carried out using the same device configuration.

Table 1 LC Conditions (Microflow)

Analytical column	: Shim-pack™ MC PLONAS Biphenyl (2.7 µm, 100 mm × 0.2 mm I.D.)
Trap column	: Shim-pack™ MCT LC8 (5 µm, 5 mm × 0.3 mm I.D.)
Temp.	: 40 °C
Mobile phase A	: Water + Formic acid 0.1 %
Mobile phase B	: Acetonitrile + Formic acid 0.1 %
Flow rate	: 250 µL/min (Load), 4 µL/min (Elution)
Gradient	: B Conc. 26 % (0-1 min)-95 % (4-5 min)
Injection volume	: 5 µL

Table 2 MS Conditions

Ionization	: Micro ESIMicro-ESI 8060
Probe voltage	: +2.6 kV (positive ionization)
Temp.	: Interface: no heating Desolvation line: 250 °C Heater block: 400 °C
Gas flow	: Nebulizing Gas: 1 L/min Heating gas: -- Drying gas: --
MRM	: (Quant / Qual) Verapamil (455.0 > 150.25 / 455.0 > 303.3 (165.2)) Nor verapamil (440.95 > 165 / 440.95 > 150) Verapamil D6 (461.3 > 309.3 / 461.3 > 165.25 (150.25))

Evaluation of Signal Intensity in Microflow Analysis

The effect of microflow analysis on the signal intensity of the target compound was evaluated using the Verapamil and Nor-Verapamil spiked plasma (lowest concentration calibration point, 0.5 µg/L). The sample injection volume and the linear velocity in the columns were adjusted to be the same, and the signal intensities with the microflow and semi-microflow were compared. A Micro-ESI 8060 microflow ionization unit was used as the ionization unit under the microflow condition, and a standard ESI ionization unit was used with the semi-microflow. Table 3 shows analysis conditions.

Table 3 Analysis Conditions with Microflow and Semi-Microflow LC/MS/MS

Parameter	Micro LC/MS/MS method	Semi-Micro LC/MS/MS method
Injection mode	Trap & Elute	Direct
Injection volume (µL)	5	5
Flow rate (µL/min)	4	441
Analytical column	0.2×100 mm, 2.7 µm	2.1×100 mm, 2.7 µm
Linear velocity (cm/s)	4.145	4.145
Sample concentration (µg/L)	0.5	0.5

The product described in this document has not been approved or certified as a pharmaceutical 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.

In comparison with the semi-microflow analysis, the signal intensity for Verapamil increased by 4.5 times or more and that of Nor-Verapamil increased by 3.5 times or more when the microflow was used (Fig. 4), confirming that sensitivity can be enhanced with the same sample volume by using the microflow method.

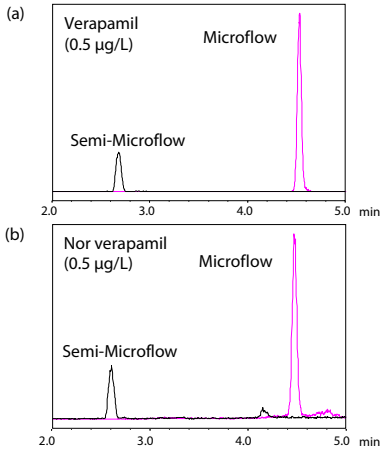

Fig. 4 Comparison of Signal Intensities of (a) Verapamil and (b) Nor-Verapamil in Microflow and Semi-Microflow LC/MS/MS (Spiked Plasma Sample at 0.5 µg/L)

Linearity of Calibration Curve

Fig. 5 shows the calibration curve (linear regression model with 1/X weighting) by the internal standard method. Calibration curves with linearity of $R^2 = 0.998$ or higher and good accuracy were obtained for both Verapamil and Nor-Verapamil (Fig. 5, Table 4).

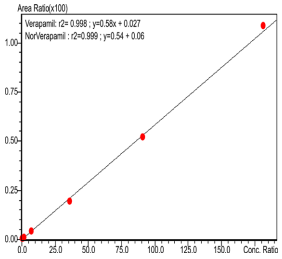

Fig.5 Calibration Curve of Verapamil

Table 4 Accuracy of Calibration Points of Microflow and Semi-Microflow LC/MS/MS

Conc. (µg/L)	Accuracy % (Verapamil)	Accuracy % (Nor verapamil)
0.5	101.4	94.2
0.9	110.7	108.4
1.8	93.9	91.2
7.3	100.5	108.5
36.3	93.5	98.1
90.8	97.3	98.9
181.6	102.6	100.7

Conclusion

Quantitation of Verapamil and Nor-Verapamil was conducted with a Nexera Mikros microflow liquid chromatography mass spectrometry system, utilizing the trap & elute system to overcome broadening of the peaks by the sample solvent.

For drug quantitation in biosamples, the use of microflow LC MS/MS method showed increased sensitivity compared to a semi micro flow method. Based on this result a reduction of initial plasma sample was possible without affecting the overall analytical performances of the method.

The use of microflow LC MS/MS technology can therefore finds a successful application in discovery-stage PK studies.

<References>

(1) Rapid Commun. Mass Spectrom. **2014**, 28, 1293–1302.

First Edition: Aug. 2020

Shimadzu Corporation

www.shimadzu.com/an/