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Application News

No. L517

Supercritical Fluid Chromatography

Analysis and Evaluation of Chiral Drugs in Biological Samples Using the Nexera UC-MS/MS System

As introduced in Application News No. L495, the optimization for chiral separation using supercritical fluid chromatography (SFC) starts from employing column scouting to find the column and mobile phase appropriate to separation. This article introduces an example of the selectivity and sensitivity of drug level monitoring in a biological sample and the evaluation results of the analysis method, as an application to the pharmacokinetics research of chiral separation using SFC/MS/MS, after having selected an appropriate column.

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Analysis of Omeprazole in a Plasma Sample

The applicability of human plasma matrix to SFC was evaluated taking an example of enantiomeric drug omeprazole, well-known as a proton pump inhibitor. Fig. 1 shows the chemical structure of omeprazole. Fig. 2 shows the pretreatment procedure employed for the blood plasma sample. Table 1 lists the analytical conditions. CHIRALPAK® IC-3 from Daicel Company, which exhibited good separation when utilized in Application News No. L495 was used as the column. Detection was performed using the LCMS-8050 triple quadrupole mass spectrometer.

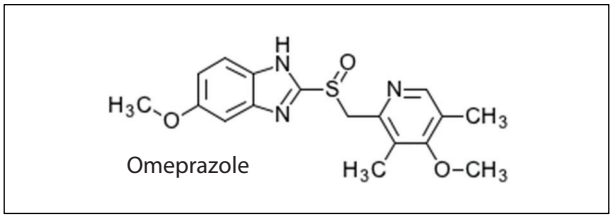

Fig. 1 Omeprazole Structure

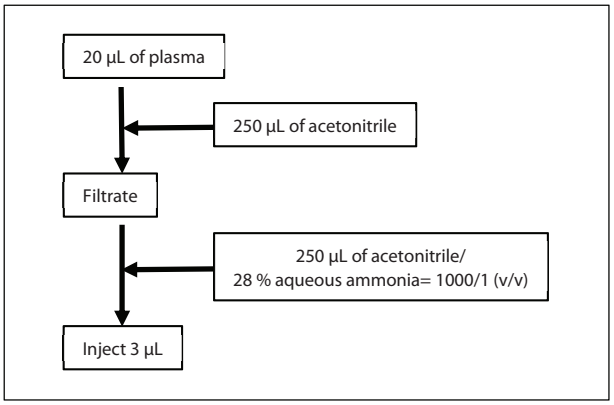

Fig. 2 Plasma Sample Pretreatment Procedure

Table 1 Analytical Conditions

Column	:	CHIRALPAK®, IC-3 (100 mm L. × 3.0 mm I.D., 3 µm)
Mobile phase	:	A) Super critical fluid of CO_2 B) Modifier: Methanol A/B = 5/1 (v/v for omeprazole, isocratic) = 4/1 (v/v for rabeprazole, isocratic)
Flow rate	:	3 mL/min
Column temp.	:	40 °C
Injection volume	:	3 µL
BPR pressure	:	10 MPa
BPR temp.	:	50 °C
Detector	:	LCMS- 8050 (ESI, MRM mode)
Make-up	:	Methanol
Make-up flow rate	:	0.1 mL/min
MRM	:	(+) *m/z* 346.1 > 198.1 (for omeprazole) (+) *m/z* 359.9 > 150.1 (for rabeprazole)

Calibration curve was created based on human plasma samples that contained 1, 2, 10, 2 and 100 µg/L of standard omeplazole to confirm the linearity of loaded amounts.
Fig. 3 and Fig. 4 show the MRM chromatograms for 2 µg/L and 20 µg/L respectively. Among the optically separated peaks, (A) is the fast-eluting isomer and (B) is the slow-eluting isomer. The linearity (r^2) obtained after correcting by 1/(concentration squared) was favorable at 0.99996 for omeprazole (A) and 0.99998 for omeprazole (B).

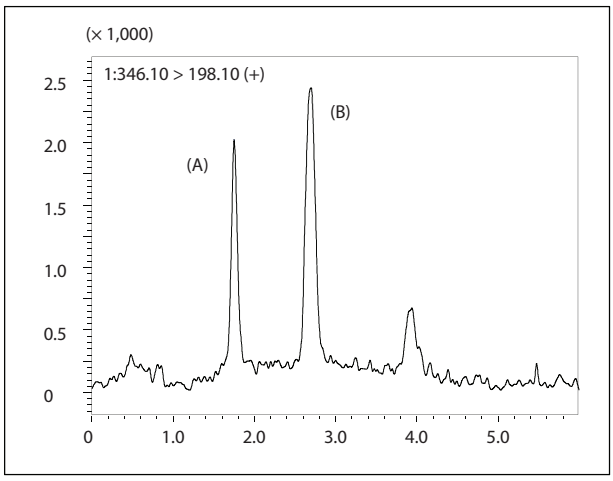

Fig. 3 Omeprazole Added to Human Plasma (2 µg/L)

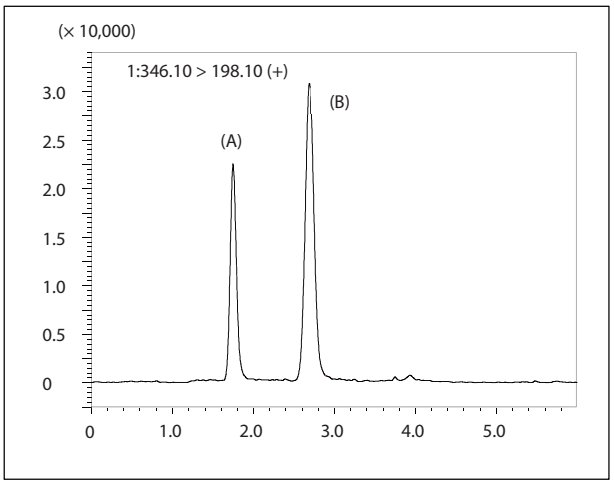

Fig. 4 Omeprazole Added to Human Plasma (20 µg/L)

The repeatability of the area values at 2 µg/L obtained from five repetitions was favorable with RSD values of 4.4 % for both omeprazole (A) and (B). At 10 µg/L, the recovery rates calculated from the results of stock solution analyses were 101.1 % and 100.5 % respectively.

Analysis of Rabeprazole in a Plasma Sample

Rabeprazole, known as a gastric acid secretion inhibitor, has a similar chemical structure to omeprazole, suggesting the possibility of successful chiral separation under similar analytical conditions including the same analytical column. Here we attempted to analyze rabeprazole in a plasma sample based on the analytical conditions used for omeprazole in the previous section. The chemical structure of rabeprazole is shown below. The structural similarity to omeprazole is easily recognized. As shown in Table 1, analysis was successful by merely changing the modifier concentration and the MRM settings.

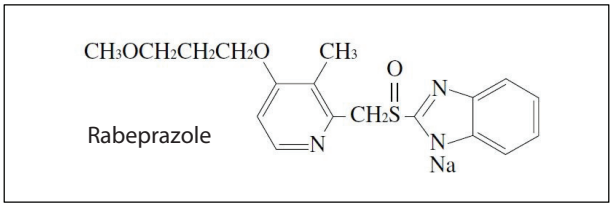

Fig. 5 Rabeprazole Structure

Calibration curve was crated based on human plasma samples that contained 0.3, 1, 3, 10 and 30 µg/L of standard raberlazole to confirm the linearity of loaded amounts. Fig. 6 and Fig. 7 show the MRM chromatograms for 3 µg/L and 30 µg/L respectively. As in Fig. 3 and Fig. 4, (A) is the fast-eluting isomer among the optically separated peaks and (B) is the slow-eluting isomer.
The linearity (r^2) obtained after correcting by 1/(concentration squared) was favorable at 0.99996 for rabeprazole (A) and 0.99999 for rabeprazole (B).

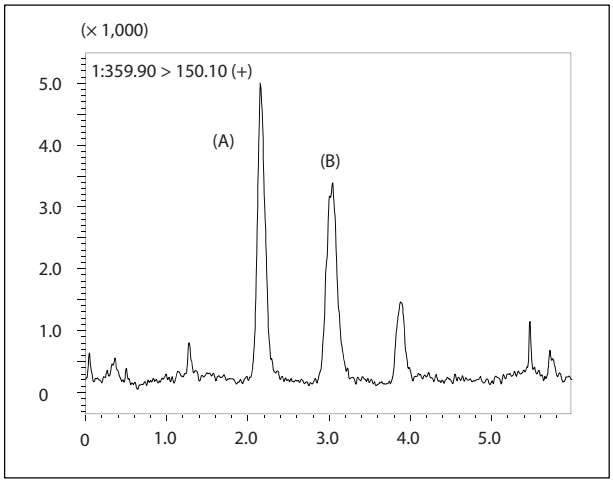

Fig. 6 Rabeprazole Added to Human Plasma (3 µg/L)

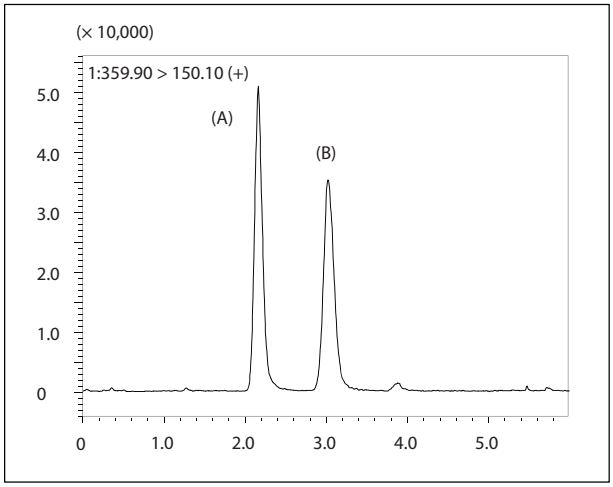

Fig. 7 Rabeprazole Added to Human Plasma (30 µg/L)

The repeatability of the area values at 10 µg/L obtained from five repetitions was favorable with RSD values of 1.8 % and 2.4 % for rabeprazole (A) and (B) respectively. The recovery rates calculated from the results of stock solution analyses were 102.5 % and 100.1 % respectively. Table 2 summarizes the linearity, peak area repeatability, and recovery rate for each compound. These results verify the applicability of this method to the practical analysis of plasma samples.

Table 2 Evaluation Results

	Linearity (r^2)	Area Repeatability (%RSD)	Recovery Rate (%) (4)
Omeprazole (A)	0.99996 (1)	4.4 (3)	101.1
Omeprazole (B)	0.99998 (1)	4.4 (3)	100.5
Rabeprazole (A)	0.99996 (2)	1.8 (4)	102.5
Rabeprazole (B)	0.99999 (2)	2.4 (4)	100.1

(1) 1 to 100 µg/L, (2) 0.3 to 300 µg/L, (3) 2 µg/L, (4) 10 µg/L

Notes: This product 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.

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