

## 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.

Y. Watabe, T. Hattori, T. lida

### 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<sup>®</sup> 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.

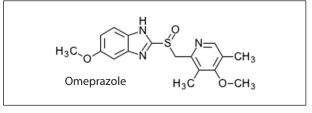


Fig. 1 Omeprazole Structure

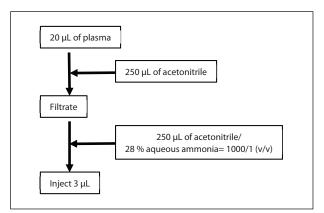


Fig. 2 Plasma Sample Pretreatment Procedure

Table 1 Analytical Conditions				
Column	: CHIRALPAK <sup>®</sup> , IC-3 (100 mm L. × 3.0 mm I.D., 3 μm)			
Mobile phase	(Not that 2.1 s of that by 5.1 km); (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	: (+) <i>m/z</i> 346.1 > 198.1 (for omeprazole)			
	(+) <i>m/z</i> 359.9 > 150.1 (for rabeprazole)			

Calibration curve was created based on human plasma samples that contained 1, 2, 10, 2 and 100  $\mu$ g/L of standard omeplazole to confirm the linearity of loaded amounts.

Fig. 3 and Fig. 4 show the MRM chromatograms for  $2 \mu g/L$  and  $20 \mu g/L$  respectively. Among the optically separated peaks, (A) is the fast-eluting isomer and (B) is the slow-eluting isomer. The linearity (r<sup>2</sup>) obtained after correcting by 1/(concentration squared) was favorable at 0.99996 for omeprazole (A) and 0.99998 for omeprazole (B).

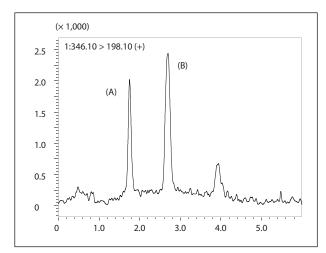


Fig. 3 Omeprazole Added to Human Plasma (2 µg/L)

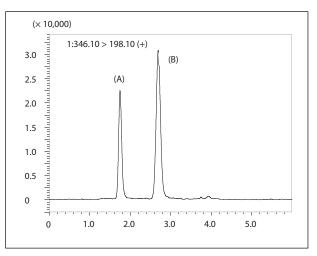


Fig. 4 Omeprazole Added to Human Plasma (20 µg/L)

The repeatability of the area values at  $2 \mu g/L$  obtained from five repetitions was favorable with RSD values of 4.4 % for both omeprazole (A) and (B). At 10  $\mu g/L$ , 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.

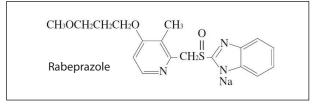


Fig. 5 Rabeprazole Structure

Calibration curve was crated based on human plasma samples that contained 0.3, 1, 3, 10 and 30  $\mu$ g/L of standard raberlazole to confirm the linearity of loaded amounts. Fig. 6 and Fig. 7 show the MRM chromatograms for 3  $\mu$ g/L and 30  $\mu$ g/L respectively. As in Fig. 3 and Fig. 4, (A) is the fasteluting 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).

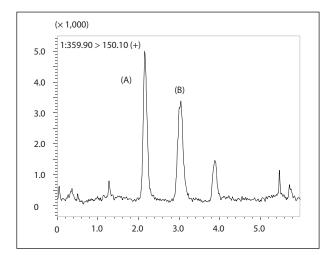


Fig. 6 Rabeprazole Added to Human Plasma (3 µg/L)

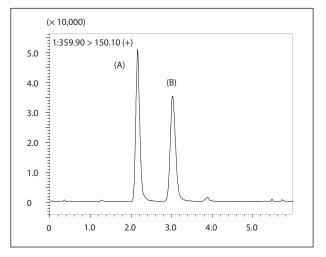


Fig. 7 Rabeprazole Added to Human Plasma (30 µg/L)

The repeatability of the area values at  $10 \mu g/L$  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 <sup>2</sup> )	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.

First Edition: Mar. 2017



Shimadzu Corporation

www.shimadzu.com/an/

#### For Research Use Only. Not for use in diagnostic procedure

This publication may contain references to products that are not available in your country. Please contact us to check the availability of these products in your country.

The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu. Company names, product/service names and logos used in this publication are trademarks and trade names of Shimadzu Corporation or its affiliates, whether or not they are used with trademark symbol "TM" or "®". Third-party trademarks and trade names may be used in this publication to refer to either the entities or their products/services. Shimadzu disclaims any proprietary interest in trademarks and trade names of other than its own.

The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to its accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject to change without notice.