

Application News

No. C149

Liquid Chromatograph Mass Spectrometry

Developing a Chiral Amino Acid Analysis Method That Uses Column Switching

With the exception of glycine, the 20 types of amino acids that make up proteins occur as D and L optical isomers. L-amino acids occur in large quantities in the body as protein components and sources of nutrients. As for D-amino acids, despite the fact that they are much less abundant than L-amino acids, they are attracting attention in various fields as components associated with the component analysis of fermented foods, physiological functions in the central nervous system, biomarkers, and even health and beauty.

Analysis of D-amino acids is susceptible to interference by a wide variety of peptides and amino compounds, and therefore requires high sensitivity and highly selective

analysis methods for accurate measurement. Furthermore, conventional optical separation analysis of amino acids necessitated derivatization and long separation times of the amino acids.

This article introduces a rapid analysis method that employs chiral columns to achieve high separation and high sensitivity and that dispenses with derivatization [1]. This system uses two types of chiral columns alternately with high-pressure column switching valves (FCV) and allows fully automatic analysis of a wide range of D- and L-amino acids.

Y. Uno, T. Hattori

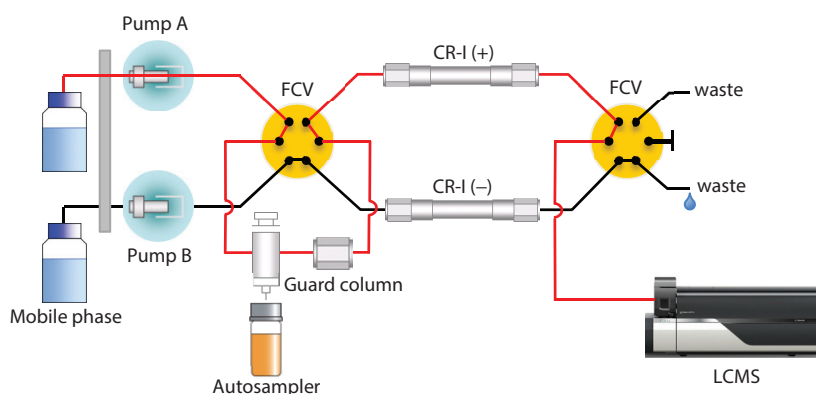


Fig. 1 Chiral Amino Acid Analysis System That Uses Column Switching

A system capable of analysis by automatically switching between two column types, CR-I (+) and CR-I (-), using two high-pressure column switching valves (FCV) was configured (Fig. 1). Pump A is connected to CR-I (+) and pump B is connected to CR-I (-). This means that even if one column is undergoing analysis, the other column can undergo stabilization without stopping mobile phase delivery.

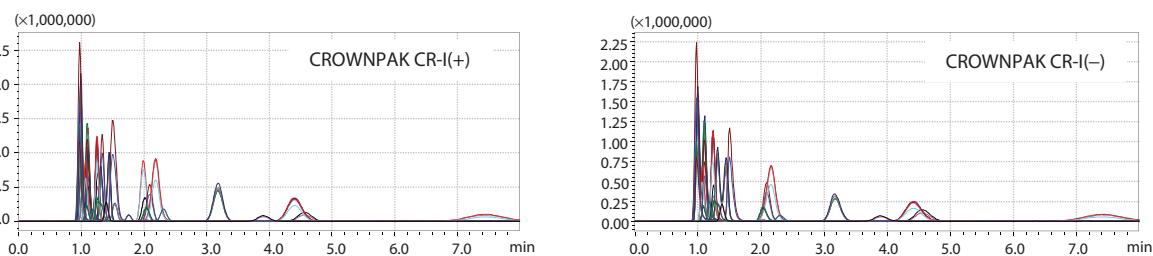


Fig. 2 MRM Chromatograms of D- and L-Amino Acids in Standard Mixed Solution (Sample Concentration: 1 ng/1 μL)

Table 1 Analysis Conditions

Column	: CROWNPAK CR-I (+) / CR-I (-) (3 mm × 150 mm, 5 μm, DAICEL Corp.)
Mobile phase	: Acetonitrile/ethanol/water/TFA = 80/15/5/0.5
Flow rate	: 0.6 mL/min
Injection volume	: 1 μL
Oven temperature	: 20 °C
Ionization mode	: ESI (Positive)
Probe voltage	: +4.0 kV
Neburizing gas flow	: 3.0 L/min
Drying gas flow	: 15.0 L/min
Heating gas flow	: 5.0 L/min
Interface temperature	: 250 °C
DL temperature	: 250 °C
Block heater temperature	: 300 °C

■ Analysis of Standard Solution

This system was employed to analyze a standard mixed solution using ¹³C₆-L-Phe as the internal standard (Fig. 2). Approximately equal area ratios were obtained with CR-I (+) and CR-I (-) for the amino acids other than Gln, Lys, Ile, *allo*-Ile, Thr, and *allo*-Thr, and this confirmed that the system is capable of separation measurement (Table 2).

With CR-I (+), L-Gln and D-Lys, D-Ile and D-*allo*-Ile, and D-Thr and D-*allo*-Thr, which each have the same MRM transition, are detected with the same peak and therefore cannot be separated.

With CR-I (-), D-Gln and L-Lys, L-Ile and L-*allo*-Ile, and L-Thr and L-*allo*-Thr, which each have the same MRM transition, are detected with the same peak and therefore cannot be separated.

However, separation measurement can be performed for these amino acids by utilizing two types of columns. For example, while D-Thr and D-*allo*-Thr cannot be separated with CR-I (+) and L-Thr and L-*allo*-Thr cannot be separated with CR-I (-), interchanging the column types allows for separation measurement (Fig. 3).

Table 2 Analysis Results of Standard Solution

	CR-I (+)		CR-I (-)		Ratio of Area (+)/(-)		CR-I (+)		CR-I (-)		Ratio of Area (+)/(-)
	RT	Ratio of Area	RT	Ratio of Area			RT	Ratio of Area	RT	Ratio of Area	
D-Ala	1.394	0.728	3.894	0.751	0.97	D-Leu	1.107	2.019	3.178	2.400	0.84
L-Ala	3.908	0.565	1.389	0.632	0.89	L-Leu	3.179	2.929	1.105	3.364	0.87
D-Arg	0.973	3.999	1.506	3.239	1.23	D-Lys	2.181	4.621 with L-Gln	7.395	1.641	2.82
L-Arg	1.499	5.633	0.981	6.718	0.84	L-Lys	7.348	1.795	2.161	5.118 with D-Gln	0.35
D-Asn	1.255	1.018	2.036	1.030	0.99	D-Met	1.259	1.704	4.554	1.859	0.92
L-Asn	2.036	0.805	1.263	0.911	0.88	L-Met	4.556	0.938	1.25	1.060	0.89
D-Asp	1.253	0.742	2.039	0.863	0.86	D-Phe	1.101	1.568	2.087	1.974	0.79
L-Asp	2.036	0.72	1.259	0.775	0.93	L-Phe	2.089	2.175	1.106	2.280	0.95
D-Cys	1.183	0.405	2.307	0.458	0.89	DL-Pro	0.957	2.756	0.971	3.105	0.89
L-Cys	2.308	0.789	1.186	0.797	0.99	D-Ser	1.222	0.224	1.756	0.253	0.89
D-Gln	1.247	2.111	2.161	3.478 with L-Lys	0.61	L-Ser	1.758	0.307	1.226	0.301	1.02
L-Gln	2.183	4.947 with D-Lys	1.239	3.686	1.34	D-Thr	1.023	1.339 with D- <i>allo</i> -Thr	1.53	0.968	1.38
D-Glu	1.246	2.972	4.426	3.262	0.91	L-Thr	1.533	0.851	1.033	1.324 with L- <i>allo</i> -Thr	0.64
L-Glu	4.388	3.506	1.24	3.731	0.94	D- <i>allo</i> -Thr	1.023	1.339 with D-Thr	1.205	0.573	2.34
Gly	2.827	0.037	2.796	0.039	0.93	L- <i>allo</i> -Thr	1.197	0.480	1.033	1.397 with L-Thr	0.34
D-His	0.967	2.797	1.099	3.917	0.71	D-Trp	1.105	2.839	1.99	3.344	0.85
L-His	1.09	3.699	0.977	2.969	1.25	L-Trp	1.988	3.458	1.111	3.510	0.99
D-Ile	0.988	4.745 with D- <i>allo</i> -Ile	1.446	2.983	1.59	D-Tyr	1.103	1.203	2.016	1.560	0.77
L-Ile	1.44	2.325	0.998	4.408 with L- <i>allo</i> -Ile	0.53	L-Tyr	2.016	1.448	1.109	1.455	1.00
D- <i>allo</i> -Ile	0.988	4.745 with D-Ile	1.313	2.926	1.62	D-Val	0.999	1.826	1.337	2.052	0.89
L- <i>allo</i> -Ile	1.308	1.844	0.998	4.101 with L-Ile	0.45	L-Val	1.331	3.170	1.008	3.251	0.97

indicates amino acids that can be separated by one column but not the other.

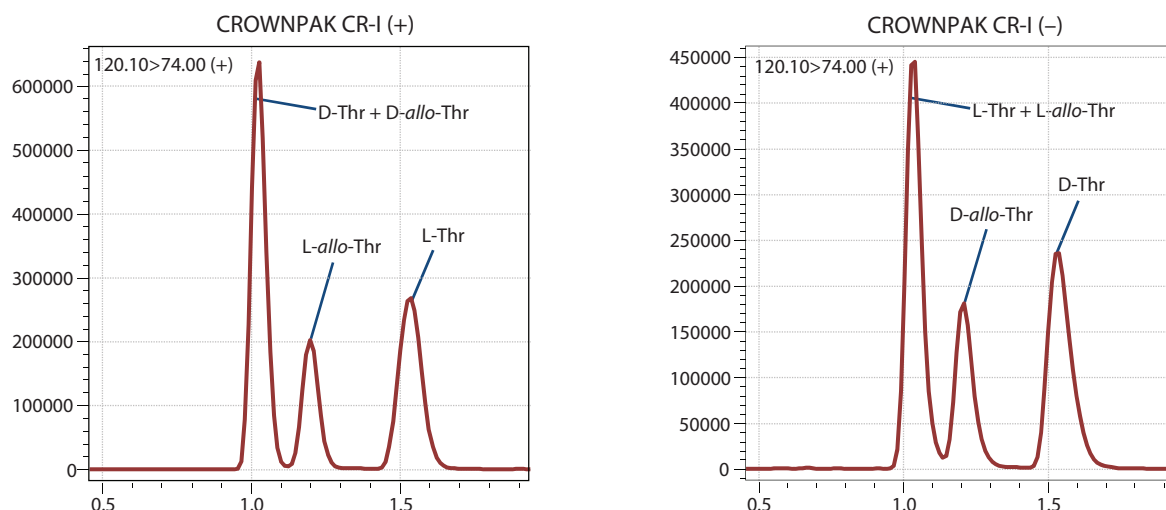


Fig. 3 Analysis Result of D/L-Threonine and D/L-*allo*-Threonine

References [1] Nakano, Y., Konya, Y., Taniguchi, M., Fukusaki, E., *Journal of Bioscience and Bioengineering*, 123, 134-138 (2016)

The analysis method presented in this edition of Application News was developed by the Fukusaki Lab in the School/Graduate School of Engineering at Osaka University.

First Edition: Apr. 2017



For Research Use Only. Not for use in diagnostic procedures.

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 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.

Shimadzu Corporation

www.shimadzu.com/an/