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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.

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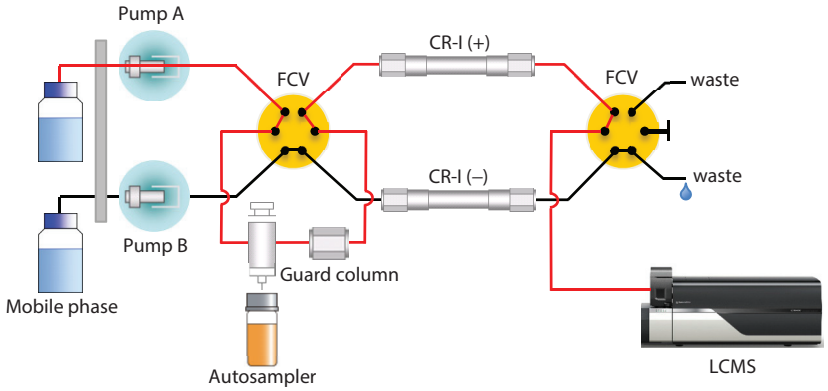

Fig. 1 Chiral Amino Acid Analysis System That Uses Column Switching

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.

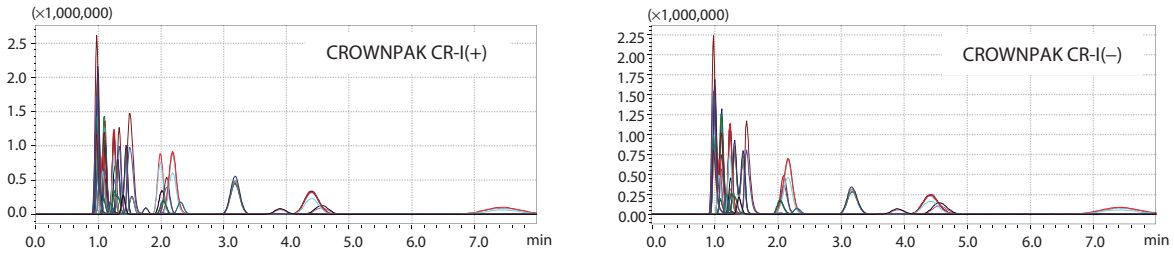

Fig. 2 MRM Chromatograms of D- and L-Amino Acids in Standard Mixed Solution (Sample Concentration: 1 ng/1 µL)

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 $^{13}C_6$-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 (+)/(–)
	RT	Ratio of Area		RT	Ratio of Area		
D-Ala	1.394	0.728		3.894	0.751		0.97
L-Ala	3.908	0.565		1.389	0.632		0.89
D-Arg	0.973	3.999		1.506	3.239		1.23
L-Arg	1.499	5.633		0.981	6.718		0.84
D-Asn	1.255	1.018		2.036	1.030		0.99
L-Asn	2.036	0.805		1.263	0.911		0.88
D-Asp	1.253	0.742		2.039	0.863		0.86
L-Asp	2.036	0.72		1.259	0.775		0.93
D-Cys	1.183	0.405		2.307	0.458		0.89
L-Cys	2.308	0.789		1.186	0.797		0.99
D-Gln	1.247	2.111		2.161	3.478	with L-Lys	0.61
L-Gln	2.183	4.947	with D-Lys	1.239	3.686		1.34
D-Glu	1.246	2.972		4.426	3.262		0.91
L-Glu	4.388	3.506		1.24	3.731		0.94
Gly	2.827	0.037		2.796	0.039		0.93
D-His	0.967	2.797		1.099	3.917		0.71
L-His	1.09	3.699		0.977	2.969		1.25
D-Ile	0.988	4.745	with D-*allo*-Ile	1.446	2.983		1.59
L-Ile	1.44	2.325		0.998	4.408	with L-*allo*-Ile	0.53
D-*allo*-Ile	0.988	4.745	with D-Ile	1.313	2.926		1.62
L-*allo*-Ile	1.308	1.844		0.998	4.101	with L-Ile	0.45

	CR-I (+)			CR-I (–)			Ratio of Area (+)/(–)
	RT	Ratio of Area		RT	Ratio of Area		
D-Leu	1.107	2.019		3.178	2.400		0.84
L-Leu	3.179	2.929		1.105	3.364		0.87
D-Lys	2.181	4.621	with L-Gln	7.395	1.641		2.82
L-Lys	7.348	1.795		2.161	5.118	with D-Gln	0.35
D-Met	1.259	1.704		4.554	1.859		0.92
L-Met	4.556	0.938		1.25	1.060		0.89
D-Phe	1.101	1.568		2.087	1.974		0.79
L-Phe	2.089	2.175		1.106	2.280		0.95
DL-Pro	0.957	2.756		0.971	3.105		0.89
D-Ser	1.222	0.224		1.756	0.253		0.89
L-Ser	1.758	0.307		1.226	0.301		1.02
D-Thr	1.023	1.339	with D-*allo*-Thr	1.53	0.968		1.38
L-Thr	1.533	0.851		1.033	1.324	with L-*allo*-Thr	0.64
D-*allo*-Thr	1.023	1.339	with D-Thr	1.205	0.573		2.34
L-*allo*-Thr	1.197	0.480		1.033	1.397	with L-Thr	0.34
D-Trp	1.105	2.839		1.99	3.344		0.85
L-Trp	1.988	3.458		1.111	3.510		0.99
D-Tyr	1.103	1.203		2.016	1.560		0.77
L-Tyr	2.016	1.448		1.109	1.455		1.00
D-Val	0.999	1.826		1.337	2.052		0.89
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.

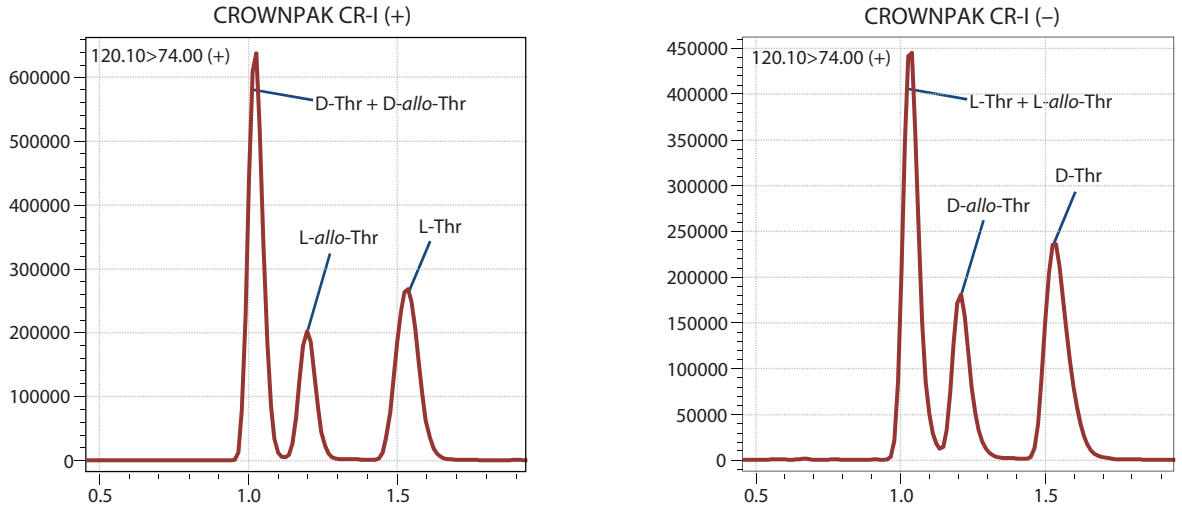

Fig. 3 Analysis Result of D/L-Threonine and D/L-*allo*-Threonine

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

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