Application Note

430030X

Analysis of amino acids using on-line pre-column derivatization with OPA with Rapid Separation High Performance Liquid Chromatography

Introduction

The high selectivity and high sensitivity measurement of amino acids requires the derivatization as amino acids do not absorb in UV-Vis region or fluorescence. Pre-column and post-column derivatization methods can be used for the analysis of amino acids. In the case of pre-column derivatization, the sample is derivatized in the autosampler and then separated in a C18 or similar column and detected. On the other hand, in the case of post-column derivatization, the sample is separated using an ion-exchange column or similar first, then mixed with reaction solution for derivatization and then detection.

JASCO offers several analysis systems. 1. Pre-column derivatization system using orthophtalaldehyde (OPA) for fluorescence detection, 2. Pre-column derivatization system using Dabsyl chloride for absorbance detection, 3. Post-column derivatization system using OPA for fluorescence detection and 4. Post-column derivatization system using ninhydrin for absorbance detection.

The pre-column derivatization method has many advantages such as a simple system configuration, wide selectivity of derivatization reagents, and offers a high sensitive measurement.

In this application, 19 amino acids were measured using OPA pre-column derivatization through the automated pre-column derivatization function of autosampler. The RHPLC conditions drastically reduced the measurement time.

Keyword: RHPLC, Fluorescence detector, OPA pre-column derivatization, amino acid, C18

Experimental Condition

Column:	X-PressPak V-C18 (2.0 mmI.D. x 50 mmL, 2 µm)
Eluent A:	1M citrate buffer (pH 5.8) 3.5 mL in 1 L of H_2O
Eluent B:	1M citrate buffer (pH 5.8) 3.5 mL in 1 L of $CH_3CN/C_2H_5OH/H_2O$ (30/30/40)
Gradient condition(A/B):	1cycle; 12 min
	0 min(90/10)→0.2 min(90/10)→2.2 min(72/28)→2.5 min(72/28)→
	4.6 min(42/58)→5.0 min(42/58)→6.1 min(23/77)→6.15 min(0/100)→
	7.0 min(0/100)→7.05 min(90/10)
Flow rate:	0.6 mL/min
Column temp.:	40 ˚C
Detection:	Fluorescence detection (Ex; 345 nm, Em; 455 nm, Gain; x10)
Injection volume:	1 µL
Standard sample:	19 amino acids 20 nmol/mL each in 0.01 M hydrochloric acid

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Result

The 19 standard amino acids were distinctly separated as shown in Figure 1.

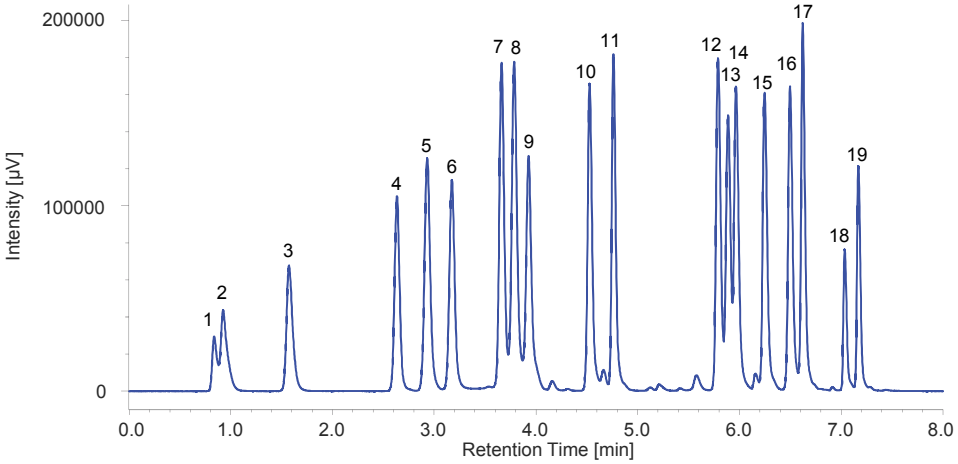

Fig.1 Chromatogram of standard mixture of amino acids

1: Cysteic acid, 2: Aspartic acid, 3: Glutamic acid, 4: Asparagine, 5: Serine, 6: Histidine, 7: Arginine, 8: Glycine, 9: Threonine, 10: Alanine, 11: Tyrosine, 12: Methionine, 13: Valine, 14: Tryptophan, 15: Phenylalanine, 16: Isoleucine, 17: Leucine, 18: Ornithine, 19: Lysine

The chromatogram of soy sauce is shown in figure 2 with the sample preparation details below.

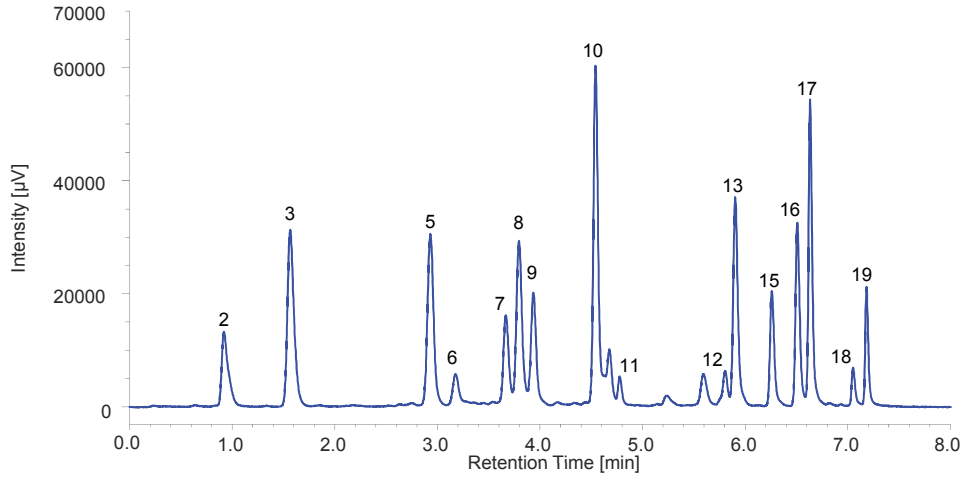

Fig.2 Chromatogram of soy source

2: Aspartic acid, 3: Glutamic acid, 5: Serine, 6: Histidine, 7: Arginine, 8: Glycine, 9: Threonine, 10: Alanine, 11: Tyrosine, 12: Methionine, 13: Valine, 15: Phenylalanine, 16: Isoleucine, 17: Leucine, 18: Ornithine, 19: Lysine

* Pretreatment method of the sample:

1) Soy source was diluted 10000 times with 0.01 M HCl.
2) Filtered with 0.2 μm membrane filter
3) Injected into HPLC

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The chromatogram of an ornithine-containing soft drink is shown in figure 3 with the sample preparation details below.

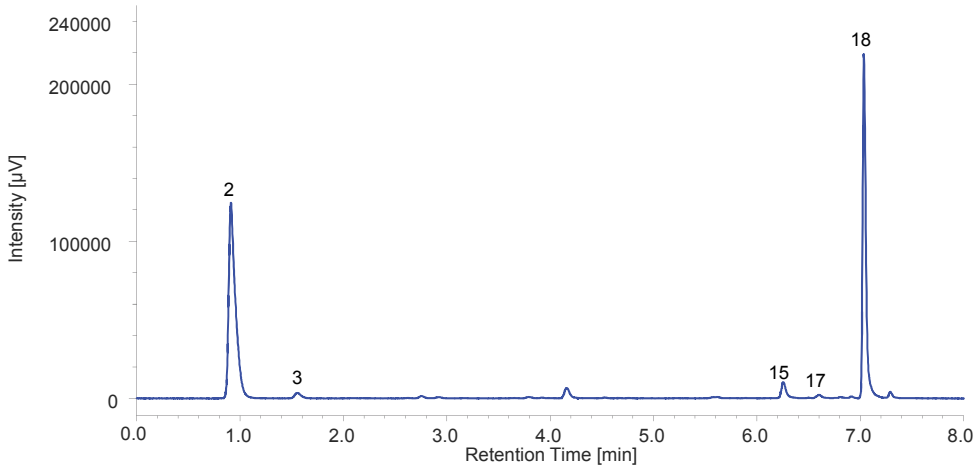

Fig. 3 Chromatogram of ornithine-containing soft drink
2: Aspartic acid, 3: Glutamic acid, 15: Phenylalanine, 17: Leucine, 18: Ornithine

* Pretreatment method of the sample:

1) Ornithine-containing soft drink was diluted 100 times with 0.01 M HCl.
2) Filtered with 0.2 μm membrane filter
3) Injected into HPLC

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