Application Note

430029H

Analysis of amino acids using on-line pre-column derivatization with OPA

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, 18 amino acids were analyzed using OPA pre-column derivatization through the automated pre-column derivatization function of autosampler.

Keyword: HPLC, Fluorescence detector, OPA pre-column derivatization, Amino acid, C18

Experimental Condition

Column:	CrestPak C18S (4.6 mmI.D. x 150 mmL, 5 µm)
Eluent A:	Sodium acetate buffer/methanol/THF(89/10/1)
Eluent B:	Methanol/THF(90/10)
Gradient condition	(A/B) : 1cycle; 60 min 0 min(85/15)→7 min(80/20)→19 min(56/44)→23 min(48/52)→29 min(48/52)→30 min(0/100)→35 min(0/100)→ 35.1 to 60 min(85/15)
Flow rate:	1.0 mL/min
Column temp.:	20 ˚C
Detection:	Fluorescence detection (Ex; 345 nm, Em; 455 nm, Gain; x100)
Injection volume:	10 µL
Standard Sample:	18 amino acids 1 nmol/mL each in 0.01 M hydrochloric acid

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Result

The 18 standard amino acids were distinctly separated as shown in Figure 1.

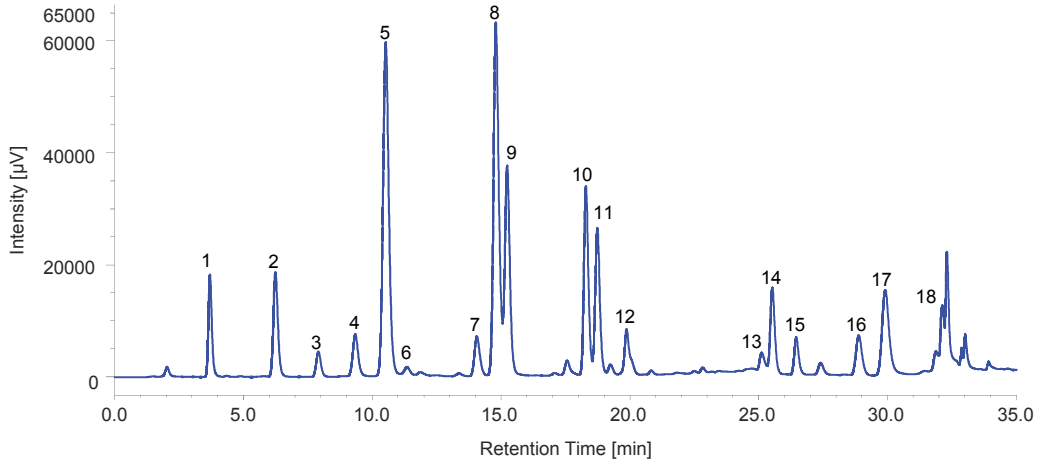

Fig.1 Chromatogram of standard mixture of amino acids

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

The chromatogram of an energy drink is shown in figure 2 with the sample preparation details below.

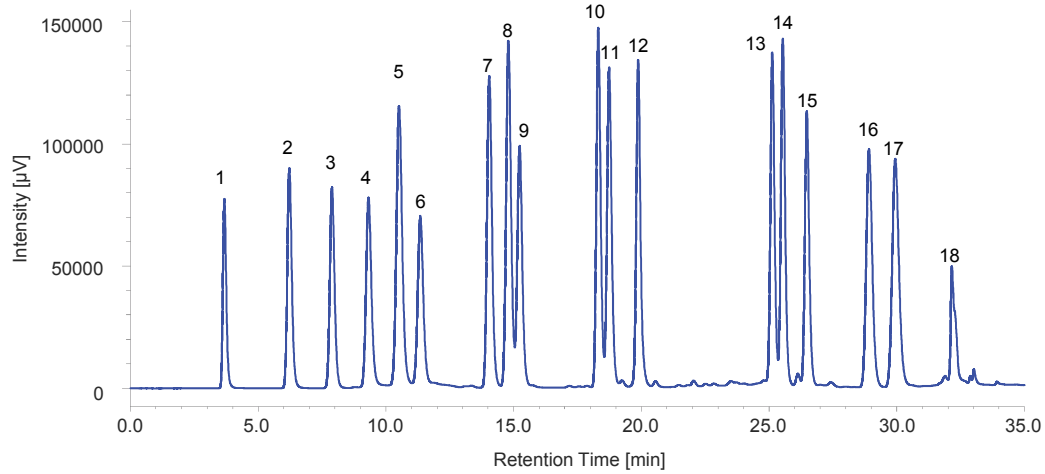

Fig.2 Chromatogram of energy drink

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

* Pretreatment method of the sample:

1) Energy 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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