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Application News

No.L458

High Performance Liquid Chromatography

High Speed, High Resolution Analysis (Part 47) Analysis of Pre-Column Derivatized Amino Acids by the Nexera SIL-30AC Autosampler (Part 3)

Amino acid composition analysis has traditionally been conducted for protein quantitation and peptide structure prediction. Its use has also extended in recent years to the quantitation of such functional components as branched chain amino acids (BCAA). Previously, in Application News No. L432 and L437, we introduced examples of analysis of amino acids subjected to fluorescence derivatization using *o*-phthalaldehyde (OPA) using the SIL-30AC. Here, we introduce an example of fast analysis of amino acids in proteins.

Simultaneous Determination of 17 Amino Acids

This method utilizes the pretreatment functions of the Nexera SIL-30AC to automatically derivatize amino acid samples with OPA. The sample rack is set up with the underivatized samples in one section and empty vials in another section that will contain the sample and reagent(s) delivered by the SIL-30AC prior to injection. Table 1 shows the derivatizing reagents used with this method. Fig. 1 shows the chromatogram obtained from measurement of a standard mixture of seventeen amino acids in solution using the analytical conditions shown in Table 2. The total analysis time can be shortened by using the overlapping injection feature that was described in Application News No. L437. This feature permits derivatization and injection preparation of the sample to follow the sample that is currently being analyzed.

Table 1 Derivatization Reagents

- Mercaptopropionic Acid
  3-MercaptopropionicAcid 10 µL in 0.1 mol/L Borate Buffer (pH9.2) 10 mL
- *o* - phthalaldehyde - Ethanol Solution
  o - Phthalaldehyde 10 mg in 0.1 mol/L Borate Buffer (pH9.2) 5 mL
- Fluorenyl Methyl Chloro Formate - Acetonitrile Solution
  9-Fluorenyl Methyl Chloro Formate 4 mg in Acetonitrile 20 mL

Table 2 Analytical Conditions

Column	: YMC-Triart C18 1.9 µm (50 mm L. × 3.0 mm I.D., 1.9 µm, YMC CO., LTD.)
Mobile Phase	: A : 20 mmol/L Phosphate Potassium Buffer (pH 6.2) B : 60/40 Acetonitrile/Methanol
Time Program	: Gradient Elution
Flowrate	: 1.2 mL/min
Column Temp.	: 40 °C
Injection Volume	: 1 µL
Detection	: RF-20A$_{XS}$ Ex. at 350 nm, Em. at 450 nm → Ex. at 266 nm, Em. at 305 nm (8.5 min)
Cell Temp.	: 30 °C
Flow Cell	: Conventional Cell

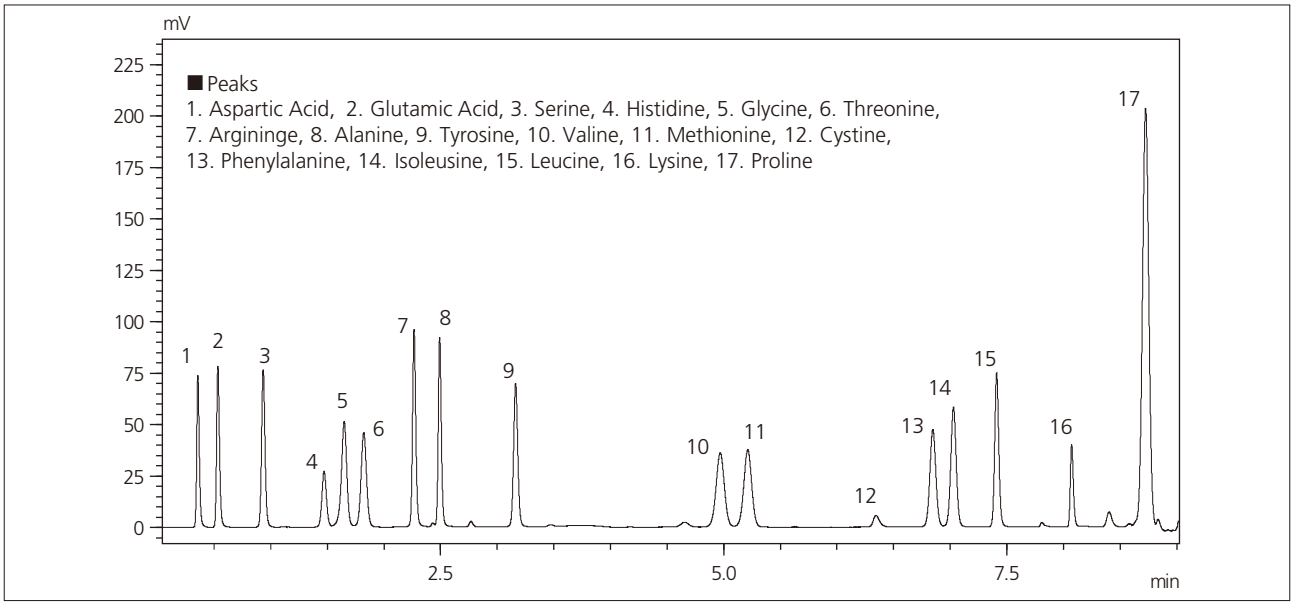

Fig. 1 Chromatogram of 17 Amino Acids in Standard Solution (25 µmol/L each)

Analysis of Angiotensin I Hydrolysate

Angiotensin is a polypeptide that exhibits vasoconstriction activity to increase blood pressure. There are four types of angiotensin, types Ⅰ - Ⅳ, that differ in the numbers and types of amino acids in their structures.
The structure of angiotensin Ⅰ consists of a total of ten amino acid residues, including aspartic acid, arginine, valine, tyrosine, isoleucine, proline, phenylalanine, leucine, and two molecules of histidine. In response to the action of angiotensin-converting enzyme (ACE), angiotensin Ⅰ is converted to angiotensin Ⅱ, which exhibits greater vasopressor activity.

After adding 500 µL of 6 mol/L hydrochloric acid (ICP grade) to 0.5 mg of angiotensin Ⅰ, the mixture was set aside for 22 hours in a reduced pressure atmosphere at 110 °C to permit complete hydrolysis.
The liquid phase was then evaporated under a stream of nitrogen gas to obtain a dry residue. The residue was then re-dissolved in 10 mL of 0.1 mol/L borate buffer for use as the sample.
The analysis results are shown in Fig. 2. Two molecules of histidine were detected while one molecule each of the other amino acids was detected, which matched the actual amino acid sequence.

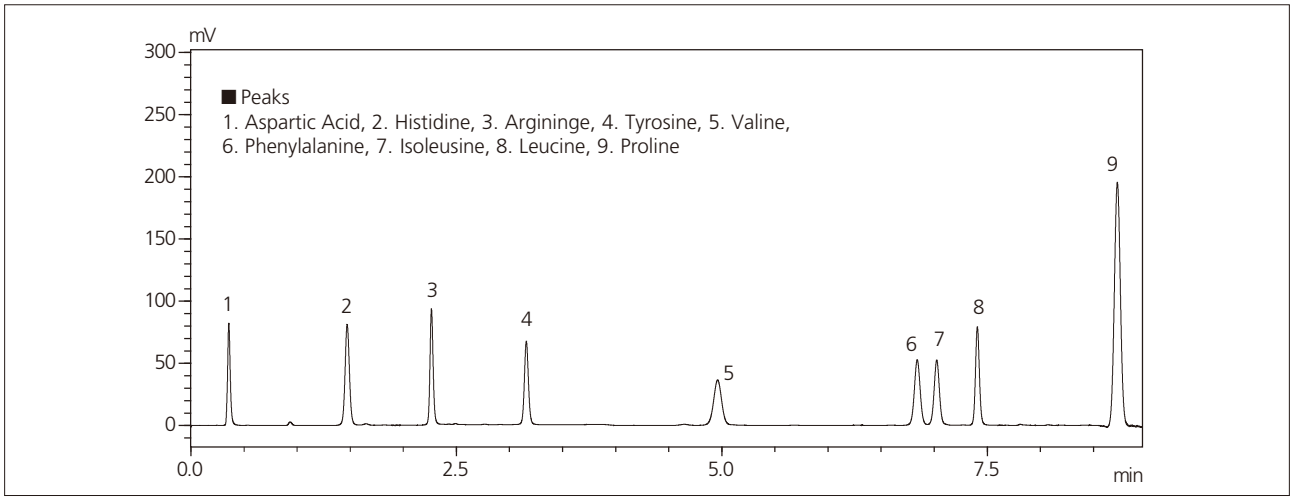

Fig. 2 Chromatogram of Angiotensin I Hydrolysate

Analysis of Bovine Serum Albumin Hydrolysate

Bovine serum albumin is a protein that is often used in biochemical experiments. In this example, after adding 500 µL of 6 mol/L hydrochloric acid (ICP grade) to 3.5 mg of bovine serum albumin, the mixture was placed in a reduced pressure atmosphere at 110 °C for 22 hours to permit complete hydrolysis.

The liquid phase was then evaporated under a stream of nitrogen gas to obtain a dry residue. The residue was then re-dissolved in 10 mL of 0.1 mol/L borate buffer for use as the sample. The results of analysis are shown in Fig. 3.

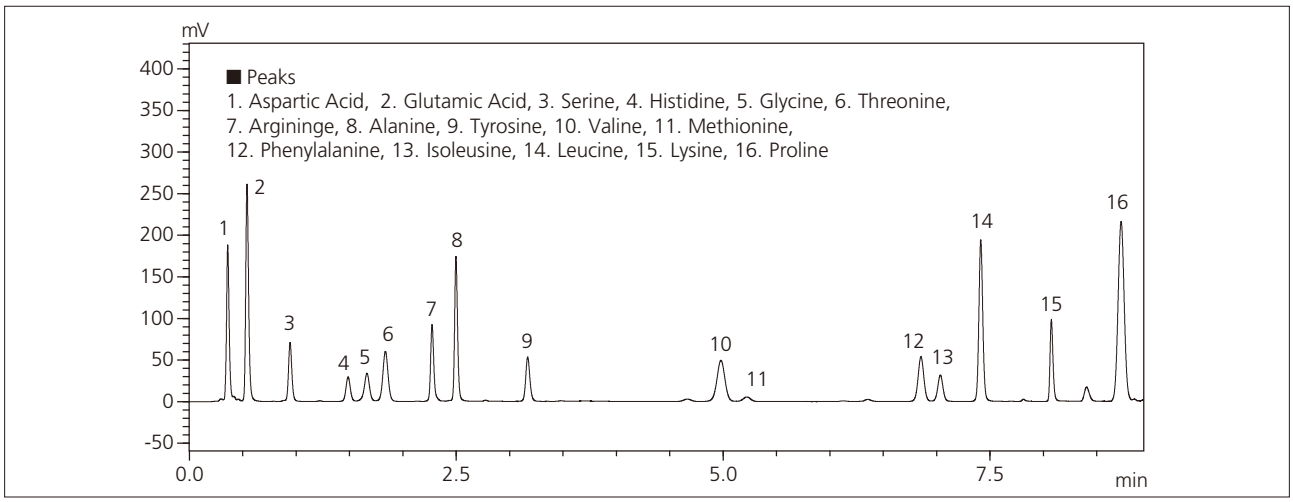

Fig. 3 Chromatogram of Bovine Serum Albumine Hydrolysate

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