SHIMADZU

Analysis of Amino Acids in Foods Using Automatic Pretreatment Function of Integrated HPLC

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1. Introduction

Foods contain many amino acids, including glutamic acid, which is known to provide umami flavor. Measuring the content of each amino acid is recently used in research activities on functional components, as well as in evaluating nutritional value and taste. We have previously described automatic amino acid analysis using pre-column derivatization with integrated HPLC that features pre-treatment functions (document reference L529A). This article introduces amino acid analysis and real sample pretreatment protocols for 14 types of real food samples using the same method as in L529A.

2. Pre-Column Derivatization

The process flow for automatic pre-column derivatization using LC-2050C is shown in Fig. 1. Settings are entered in the autosampler pretreatment program setting windows. The MPA/OPA reagent and FMOC reagents and aqueous phosphate solution are included in the automated injection process.

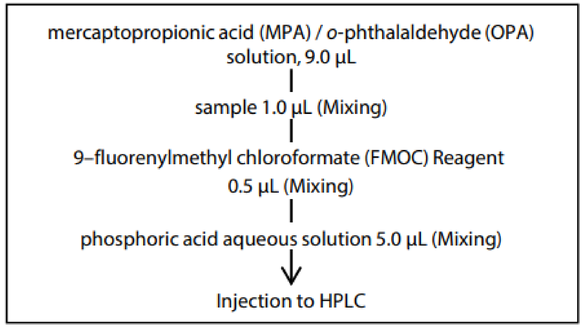

Fig. 1 Process Flow for Automatic Pre-Column Derivatization Using LC-2050C

3. Analytical Conditions

Table 1 Analytical Conditions

System	:	LC-2050C*1
Column	:	Shim-pack™ XR-ODSII (100 mm × 3.0 mmI.D., 2.2 µm) *2
Mobile phase	:	A) 20 mmol/L (Sodium) acetate buffer (pH 6)
	:	B) Water/Acetonitrile = 10:90
	:	C) 20 mmol/L (Sodium) acetate buffer (pH 5) containing 0.5 mmol/L EDTA-2Na
Mode	:	Low pressure gradient
Flowrate	:	1.0 mL/min
Column temp.	:	40 °C
Injection volume	:	1 µL
Vial	:	Shimadzu Vials, LC, 1.5 mL, Glass *3
Detection	:	Fluorescence detector (RF-20Axs)
	:	Ch1) Ex. 350 nm, Em. 450 nm
	:	Ch2) Ex. 266 nm, Em. 305 nm

The pre-column derivatization that derivatizes the target compound before injection modifies amino acids with a highly hydrophobic functional group before separation on the column. That enables separation by reversed-phase chromatography. The analytical conditions are shown in Table 1. For information about preparing the mobile phases and derivatizing agents, refer to Table 2.

Table. 2 Preparation Methods for Mobile Phases and Derivatizing Agents

- 0.1 mol/L Borate Buffer - Add 0.62 g of boric acid and 0.2 g of sodium hydroxide into 100 mL of pure water.
- Mercaptopropionic Acid Reagent - Add 10 µL of 3-mercaptopropionic acid into 10 mL of 0.1 mol/L borate buffer.
- OPA Reagent - Add 0.3 mL of ethanol into 10 mg of o-phthalaldehyde and dissolve completely. Then add 0.7 mL of 0.1 mol/L borate buffer and 4 mL of pure water.
- Mercaptopropionic Acid / OPA Solution - Mix 300 µL of Mercaptopropionic Acid Reagent and 600 µL OPA Reagent.
- FMOC Reagent - Add 10 mg of 9-fluorenylmethl chloroformate into 50 mL of acetonitrile.
- Mobile phase A - 20 mmol/L (Sodium) acetate buffer (pH 6): Add 2.67 g of sodium acetate trihydrate and 41 µL of acetic acid into 1000 mL of pure water.
- Mobile phase B - Water/Acetonitrile = 10:90
- Mobile phase C - 20 mmol/L (Sodium) acetate buffer (pH 5) containing 0.5 mmol/L EDTA-2Na: Add 0.19 g of EDTA-2Na, 2.03 g of sodium acetate trihydrate and 308 µL of acetic acid into 1000 mL of pure water.
- Phosphoric Acid Aq. Solution - Add 0.5 mL of phosphoric acid in 100 mL pure water.

4. Analytical Conditions

Fig.2 shows three chromatograms of standard amino acids. The upper and the middle show the separation of proteinogenic amino acids in two-channel detection. The lower shows the separation of theanine and γ-aminobutyric acid (GABA) at channel 1. When a sample contains tryptophan (#17) and GABA, the peak originated from GABA (*) can affect the quantitation of tryptophan.

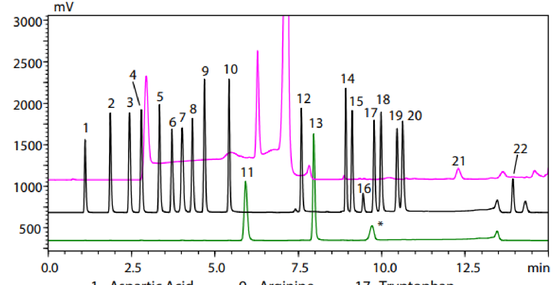

Fig. 2 Chromatograms of a Standard Sample with 22 Amino Acid Components (25 mmol/L each)

5. Analysis of Real Samples

The system analyzed four hydrochloric acid hydrolyzed products – brown rice, soybean extract, boiled tuna, and chicken eggs – and ten samples for free amino acids, including soybean extract, scallop, boiled tuna, amino acid supplement, mushroom, matcha, tomato juice, green vegetable juice, barbecue sauce, and coconut milk. In pre-column derivatization, the reaction may be affected by the sample matrix due to direct addition of the derivatizing reagent. An ultrafiltration cartridge was used for deproteinization, and 10 mmol/L hydrochloric acid was used as the diluent in all protocols to standardize sample matrices before derivatization.

6. Real Samples: Brown Rice (Hydrochloric Acid Hydrolysis)

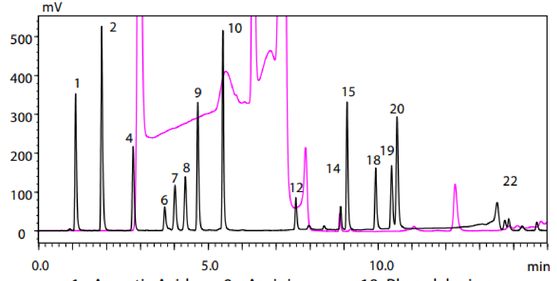

Fig. 3 Chromatograms of Brown Rice

Ground brown rice 4.91 mg
- 6 mol/L HCl (500 µL)
  Seal vessel under reduced pressure
  Stand at 110 °C, 22 hours
  Evaporate to dryness under nitrogen atmosphere
- 10 mmol/L HCl (1000 µL)
  Dissolve with vortex mixer
  Filtration (0.2 µm, 10000 rpm × 10 minutes)

Filtrate 50 µL
- 10 mmol/L HCl (950 µL)

HPLC

Fig. 4 Pretreatment Protocol for Brown Rice (Hydrochloric Acid Hydrolysis)