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Application News

High Performance Liquid Chromatograph

Simultaneous Analysis of Amino Acids Using Automatic Pretreatment Function of Integrated HPLC

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User Benefits

- Automatic pre-column derivatization method enables highly selective amino acid analysis with high sensitivity.
- Proteinogenic amino acids can be analyzed within 20 minutes.
- Generally used HPLC can cover all procedures for pre-column derivatized amino acid analysis.

■ Introduction

The analysis of amino acids is necessary in various fields such as the fields of food and pharmaceuticals. A method often used in the analysis of amino acids with HPLC is post-column derivatization, and Shimadzu amino acid analysis system also uses the method. However, post-column derivatization is difficult to speed up due to the characteristics of the column which is used.

This article introduces amino analysis by automated pre-column derivatization using the automated pretreatment capability of the integrated HPLC system LC-2070C.

■ Pre-Column Derivatization

LC-2070C is equipped with automatic pretreatment functions in injection unit as standard. Using the co-injection function, solutions can be aspirated sequentially from multiple vials and mixed within the needle. Well-known derivatization reagents for amino acids are *o*-phthalaldehyde (OPA) and 9-fluorenylmethyl chloroformate (FMOC), both of which react rapidly with amino acids at room temperature. Use of this function allows derivatization to be performed automatically within the needle.

Fig. 1 shows the setting details and Fig. 2 shows the operation flow. As indicated, settings are simple even for operations involving sequential aspiration of solutions.

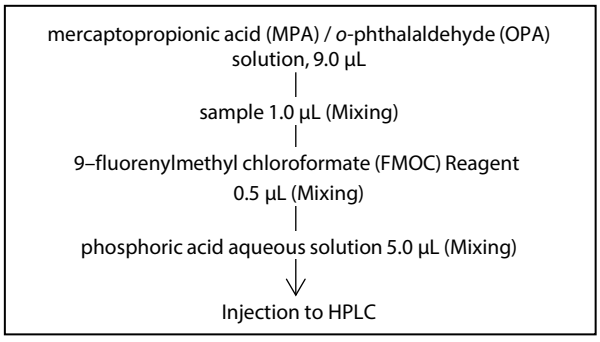


Fig. 1 Process Flow for Automatic Pre-Column Derivatization Using LC-2070C

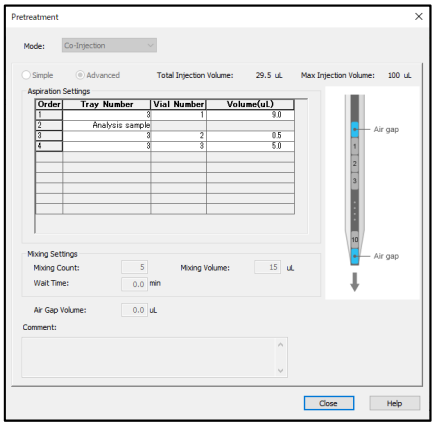


Fig. 2 Automatic Pre-Column Derivatization Setting Window

Table 1 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 Aqueous Solution : Add 0.5 mL of phosphoric acid into 100 mL of pure water.

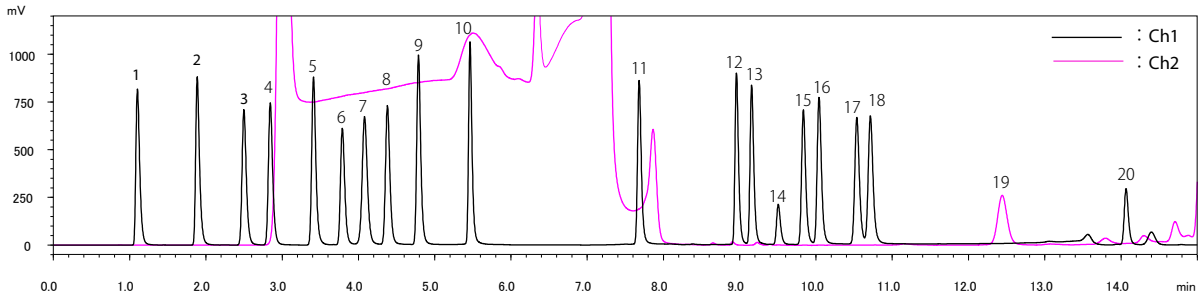
Table 2 Analytical Conditions

Column	:	Shim-pack™ XR-ODSII (100 mm × 3.0 mmI.D., 2.2 µm) *1
Mobile phase	:	See the table 1
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 *2
Detection	:	Fluorescence detector (RF-20Axs)
	:	Ch1) Ex. 350 nm, Em. 450 nm
	:	Ch2) Ex. 266 nm, Em. 305 nm

*1 P/N S228-41624-92
*2 P/N S228-15652-92

Table 3 Gradient Program

Time (min)	Module	Command	Value
0.20	Pump	B.Conc	7
1.00	Pump	B.Conc	7
4.00	Pump	C.Conc	0
5.00	Pump	B.Conc	15
5.00	Pump	C.Conc	85
7.50	Pump	B.Conc	30
7.50	Pump	C.Conc	70
12.00	Pump	B.Conc	35
12.00	Pump	C.Conc	65
14.00	Pump	B.Conc	45
14.00	Pump	C.Conc	55
14.01	Pump	B.Conc	95
14.01	Pump	C.Conc	5
17.00	Pump	B.Conc	95
17.00	Pump	C.Conc	5
17.01	Pump	B.Conc	5
17.01	Pump	C.Conc	0
19.50	Controller	Stop	



1, Aspartic Acid 2, Glutamic Acid 3, Asparagine 4, Serine 5, Glutamine 6, Histidine 7, Glycine 8, Threonine 9, Arginine 10, Alanine 11, Tyrosine
12, Methionine 13, Valine 14, Cystine 15, Tryptophan 16, Phenylalanine 17, Isoleucine 18, Leucine 19, Proline 20, Lysine

Fig.3 Simultaneous Analysis of 20 Proteinogenic Amino Acids(12.5 µmol/L Each)

■ Linearity and Repeatability

For each of the amino acids, we evaluated the linearity (r^2: coefficient of determination) using concentrations of 1.0, 1.25, 2.5, 5.0, 10, 12.5, 25 and 50 µmol/L. We also evaluated the area repeatability at 12.5 µmol/L through repeated analyses (n=6). The resulting values are listed in Table 4.

Table 4 Linearity and Area Repeatability

amino acid	Linearity (r^2)	Area (%RSD)	amino acid	Linearity (r^2)	Area (%RSD)
Asp	0.9999	0.793	Tyr	0.9998	0.966
Glu	0.9999	0.801	Met	0.9998	0.984
Asn	0.9999	0.840	Val	0.9998	0.974
Ser	0.9991	1.30	Cystine	0.9998	0.711
Gln	0.9999	0.895	Trp	0.9998	0.859
His	0.9996	0.731	Phe	0.9998	0.954
Gly	0.9996	1.08	Ile	0.9997	0.849
Thr	0.9996	0.977	Leu	0.9998	1.09
Arg	0.9998	0.865	Pro	0.9981	4.98
Ala	0.9998	1.17	Lys	0.9989	0.603

■ Analysis of Other Amino Acids

There are many amino acids which are said to contribute to health in addition to the 20 proteinogenic amino acids. A simultaneous analysis of 4 amino acids which are particularly gaining attention in recent years—citrulline, taurine, theanine, and γ-aminobutyric acid (GABA)—together with the predescribed 20 amino acids is shown in Fig. 4.

■ Analysis of Actual Samples

An example analysis of beer is shown in Fig. 5. The sample was diluted with10 mmol/L hydrochloric acid by a factor of 10, filtered through a filter with a pore size of 0.22 µm and then analyzed. The analytical conditions listed in Tables 2 and 3 were used.

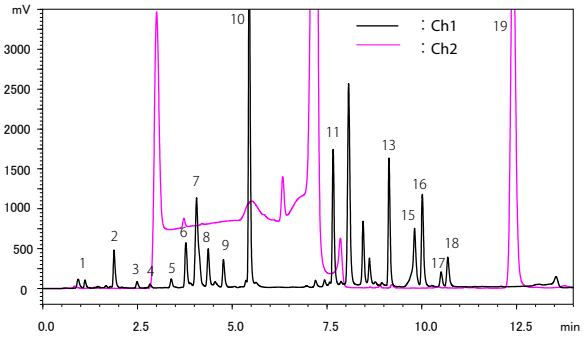


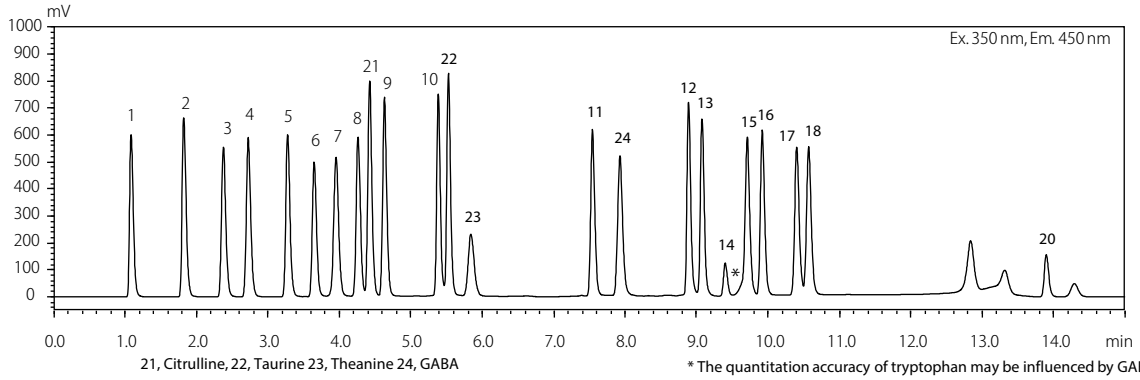
Fig.5 Chromatograms of Beer

■ Conclusion

As described, amino acids can be analyzed easily through pre-column derivatization using the automatic pretreatment function of Prominence-i. Since the derivatization is performed within the needle, the required sample and reagent volumes can be minimized. In addition, all reactants are injected into the column resulting in a highly sensitive analysis. Compared with the conventional method using an empty vial for derivatization reaction, this new method eliminates the need to prepare additional vials for the reaction, thereby simplifies analysis preparations as well.

<Related Applications>

1. Analysis of Amino Acids in Foods Using Automatic Pretreatment Function of Integrated HPLC, Application News No. 01-01048



21, Citrulline, 22, Taurine 23, Theanine 24, GABA

* The quantitation accuracy of tryptophan may be influenced by GABA

Fig.4 Analysis of 23 Amino Acids Including Functional Amino Acids (12.5 µmol/L Each)

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