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Application News

High Performance Liquid Chromatograph

Analysis of Amino Acids in Foods 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

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 for the research activity on functional components as well as the evaluation of the nutritional value and the taste.

Application News 01-01047 described automatic amino acid analysis using pre-column derivatization with the integrated HPLC that has pre-treatment function. This article introduces the amino acid analysis and real sample pretreatment protocols of 13 types of real food samples using the same method as in 01-01047.

■ Pre-Column Derivatization

The process flow for automatic pre-column derivatization using LC-2070C is shown in Fig. 1. Settings are entered in the autosampler pretreatment program setting windows. The setting window image is shown in Fig. 2. The MPA/OPA reagent is set as Vial 1 on Tray 3, the FMOC reagent as Vial 2, and aqueous phosphate solution as Vial 3.

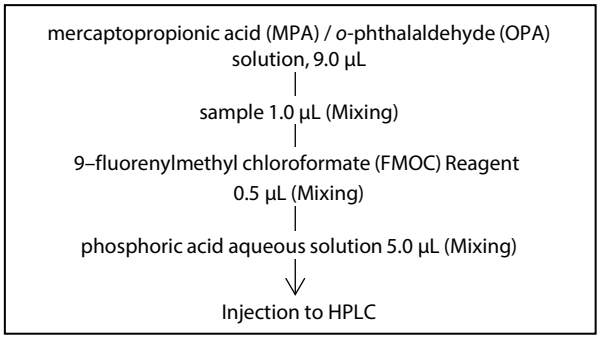


Fig. 1 Process Flow for Automatic Pre-Column Derivatization Using LC-2070C

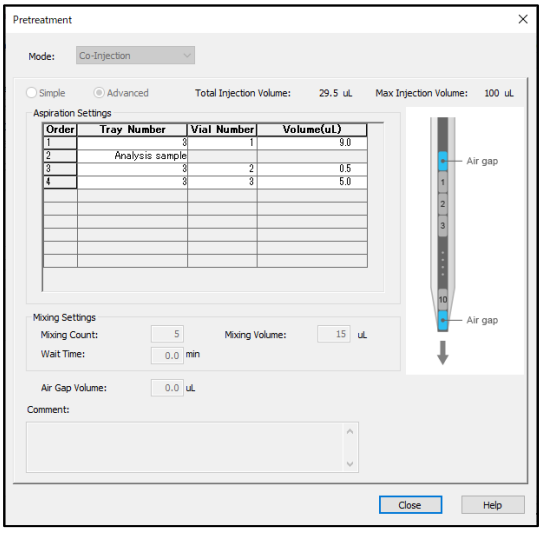


Fig. 2 Automatic Pre-Column Derivatization Setting Window

■ Analytical Conditions

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 and the gradient program in Table 2. For information about preparing the mobile phases and derivatizing agents, refer to Table 3.

Table1 Analytical Conditions

System	:	LC-2070C
Column	:	Shim-pack™ XR-ODSII (100 mm × 3.0 mmI.D., 2.2 µm) *1
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 *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 2 Gradient Program

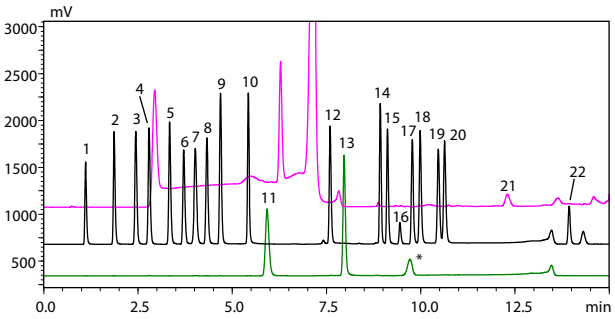
Time (min)	Module	Command	Value
0.20	Pump	B.Conc	7
1.00	Pump	B.Conc	7
4.00	Pump	C.Conc	0
5.00	Pump	B.Conc	15
5.00	Pump	C.Conc	85
7.50	Pump	B.Conc	30
7.50	Pump	C.Conc	70
12.00	Pump	B.Conc	35
12.00	Pump	C.Conc	65
14.00	Pump	B.Conc	45
14.00	Pump	C.Conc	55
14.01	Pump	B.Conc	95
14.01	Pump	C.Conc	5
17.00	Pump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

Table 3 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.

■ Analysis of Standard Amino Acids

Fig.3 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.



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

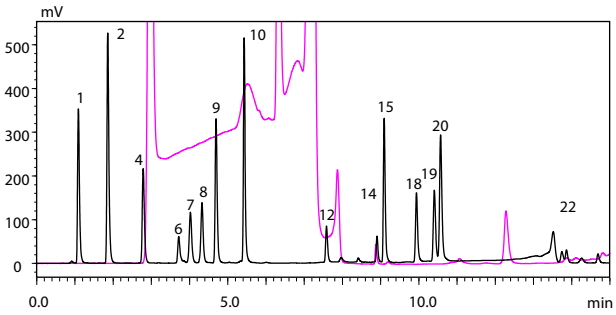
Fig. 3 Chromatograms of a Standard Sample with 22 Amino Acid Components (25 µmol/L each)

■ Analysis of Real Samples

The system was used to analyze four hydrochloric acid hydrolyzed products (brown rice, soybean extract, boiled tuna, and chicken eggs) and nine samples for free amino acids (soybean extract, scallop, boiled tuna, amino acid supplement, mushroom, matcha, tomato juice, green vegetable juice, and barbeque sauce). Corresponding chromatograms and pretreatment protocols are shown in Fig. 4 to Fig. 29.

In pre-column derivatization, the reaction might be influenced by the sample matrix due to direct addition of the derivatizing reagent to the samples. Therefore, an ultrafiltration cartridge (with molecular weight cut-off 3000) was used for deproteinization and 10 mmol/L hydrochloric acid was used as diluent for all the pretreatment protocols, to make the equal sample matrices before derivatization as much as possible.

■ Brown Rice (Hydrochloric Acid Hydrolysis)



1. Aspartic Acid
2. Glutamic Acid
4. Serine
6. Histidine
7. Glycine
8. Threonine
9. Arginine
10. Alanine
12. Tyrosine
13. GABA
14. Methionine
15. Valine
18. Phenylalanine
19. Isoleucine
20. Leucine
21. Proline
22. Lysine

Fig. 4 Chromatograms of Brown Rice (Hydrochloric Acid Hydrolysis)

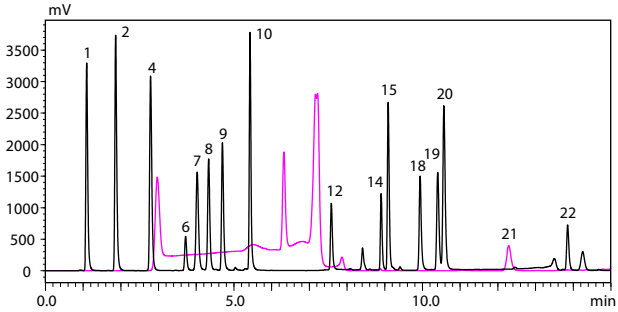
Ground brown rice 4.91 mg
- 6 mol/L HCl (500 µL)
  Seal vessel under reduced pressure
  Stand at 110 °C, 22 hours
  Evaporate to dryness under nitrogen atmosphere
- 10 mmol/L HCl (1000 µL)
  Dissolve with vortex mixer
  Filtration (0.2 µm, 10000 rpm × 10 minutes)

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

HPLC

Fig. 5 Pretreatment Protocol for Brown Rice (Hydrochloric Acid Hydrolysis)

■ Chicken Eggs (Hydrochloric Acid Hydrolysis)



1. Aspartic Acid
2. Glutamic Acid
4. Serine
6. Histidine
7. Glycine
8. Threonine
9. Arginine
10. Alanine
12. Tyrosine
14. Methionine
15. Valine
18. Phenylalanine
19. Isoleucine
20. Leucine
21. Proline
22. Lysine

Fig. 6 Chromatograms of Chicken Eggs (Hydrochloric Acid Hydrolysis)

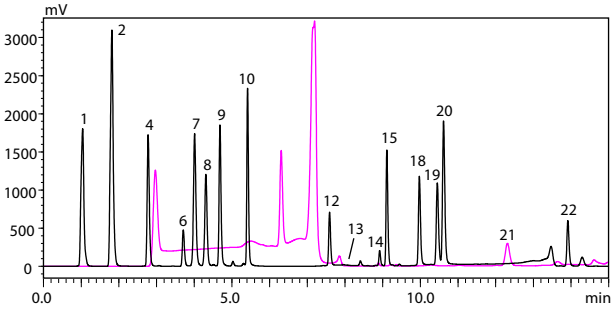
Stirred egg 14.26 mg
- 6 mol/L HCl (500 µL)
  Seal vessel under reduced pressure
  Stand at 110 °C, 22 hours
  Evaporate to dryness under nitrogen atmosphere
- 10 mmol/L HCl (1000 µL)
  Dissolve with vortex mixer
  Filtration (0.2 µm, 10000 rpm × 10 minutes)

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

HPLC

Fig. 7 Pretreatment Protocol for Chicken Eggs (Hydrochloric Acid Hydrolysis)

■ Soybean Extract (Hydrochloric Acid Hydrolysis)



1. Aspartic Acid
2. Glutamic Acid
4. Serine
6. Histidine
7. Glycine
8. Threonine
9. Arginine
10. Alanine
12. Tyrosine
13. GABA
14. Methionine
15. Valine
18. Phenylalanine
19. Isoleucine
20. Leucine
21. Proline
22. Lysine

Fig. 8 Chromatograms of Soybean Extract (Hydrochloric Acid Hydrolysis)

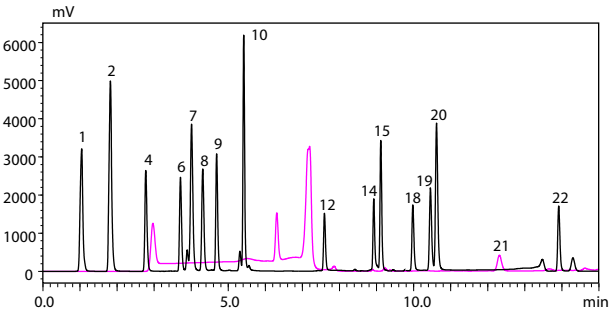
Soybean extract 25.72 mg
- 6 mol/L HCl (500 µL)
  Seal vessel under reduced pressure
  Stand at 110 °C, 22 hours
  Evaporate to dryness under nitrogen atmosphere
- 10 mmol/L HCl (1000 µL)
  Dissolve with vortex mixer
  Filtration (0.2 µm, 10000 rpm × 10 minutes)

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

HPLC

Fig. 9 Pretreatment Protocol for Soybean Extract (Hydrochloric Acid Hydrolysis)

■ Boiled Tuna (Hydrochloric Acid Hydrolysis Products)



1. Aspartic Acid
2. Glutamic Acid
4. Serine
6. Histidine
7. Glycine
8. Threonine
9. Arginine
10. Alanine
12. Tyrosine
14. Methionine
15. Valine
18. Phenylalanine
19. Isoleucine
20. Leucine
21. Proline
22. Lysine

Fig. 10 Chromatograms of Boiled Tuna (Hydrochloric Acid Hydrolysis)

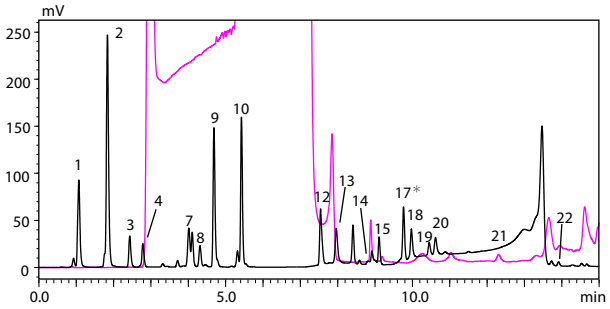
Boiled tuna 8.39 mg
- 6 mol/L HCl (500 µL)
  Seal vessel under reduced pressure
  Stand at 110 °C, 22 hours
  Evaporate to dryness under nitrogen atmosphere
- 10 mmol/L HCl (1000 µL)
  Dissolve with vortex mixer
  Filtration (0.2 µm, 10000 rpm × 10 minutes)

Filtrate 10 µL
- 10 mmol/L HCl (990 µL)

HPLC

Fig. 11 Pretreatment Protocol for Boiled Tuna (Hydrochloric Acid Hydrolysis)

■ Soybean Extract



1. Aspartic Acid
2. Glutamic Acid
3. Asparagine
4. Serine
7. Glycine
8. Threonine
9. Arginine
10. Alanine
12. Tyrosine
13. GABA
14. Methionine
15. Valine
17. (Tryptophan + GABA)*
18. Phenylalanine
19. Isoleucine
20. Leucine
21. Proline
22. Lysine

* Might include overlapping peaks for tryptophan and GABA.

Fig. 12 Chromatograms of Soybean Extract

Soybean extract 1.06 g (1 mL)
- 10 mmol/L HCl (9 mL)
  Mix with vortex mixer
  Centrifuge (10000 rpm × 15 minutes)

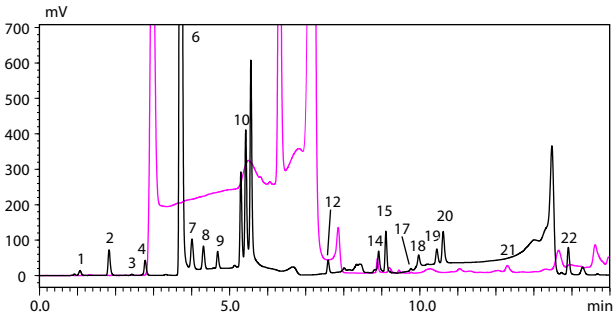
Supernatant
- Ultrafiltration filter (3K)
  Centrifuge (10000 rpm × 20 minutes)

Filtrate 100 µL
- 10 mmol/L HCl (900 µL)

HPLC

Fig. 13 Pretreatment Protocol for Soybean Extract

■ Boiled Tuna



1. Aspartic Acid
2. Glutamic Acid
3. Asparagine
4. Serine
6. Histidine
7. Glycine
8. Threonine
9. Arginine
10. Alanine
12. Tyrosine
14. Methionine
15. Valine
17. Tryptophan
18. Phenylalanine
19. Isoleucine
20. Leucine
21. Proline
22. Lysine

Fig. 14 Chromatograms of Boiled Tuna

Boiled tuna 4.91 g
- 10 mmol/L HCl (5 mL)
  Homogenize (1 minute)
  Centrifuge (10000 rpm × 15 minutes)

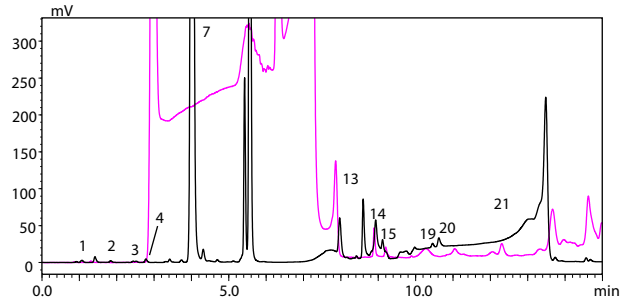
Supernatant
- Ultrafiltration filter (3K)
  Centrifuge (10000 rpm × 20 minutes)

Filtrate 10 µL
- 10 mmol/L HCl (990 µL)

HPLC

Fig. 15 Pretreatment Protocol for Boiled Tuna

■ Scallop



1. Aspartic Acid
2. Glutamic Acid
3. Asparagine
4. Serine
7. Glycine
10. Alanine
13. GABA
14. Methionine
15. Valine
19. Isoleucine
20. Leucine
21. Proline

Fig. 16 Chromatograms of Scallop

Raw scallop 1.02 g
- 10 mmol/L HCl (5 mL)
  Homogenize (1 minute)
  Centrifuge (10000 rpm × 15 minutes)

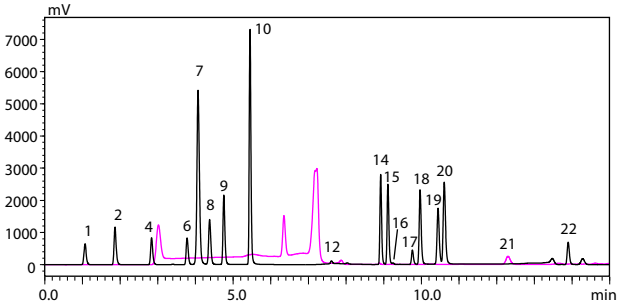
Supernatant
- Ultrafiltration filter (3K)
  Centrifuge (10000 rpm × 20 minutes)

Filtrate 10 µL
- 10 mmol/L HCl (4990 µL)

HPLC

Fig. 17 Pretreatment Protocol for Scallop

■ Amino Acid Supplement



1. Aspartic Acid
2. Glutamic Acid
4. Serine
6. Histidine
7. Glycine
8. Threonine
9. Arginine
10. Alanine
12. Tyrosine
14. Methionine
15. Valine
16. Cystine
17. Tryptophan
18. Phenylalanine
19. Isoleucine
20. Leucine
21. Proline
22. Lysine

Fig. 18 Chromatograms of Amino Acid Supplement

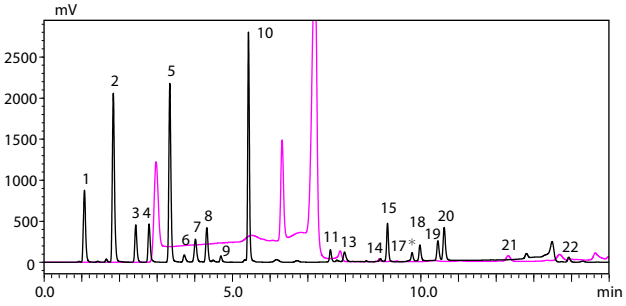
Amino acid supplement 1.40 g
- 10 mmol/L HCl (25 mL)
  Homogenize (1 minute)
  Filtration (0.2 µm)

Filtrate 5 µL
- 10 mmol/L HCl (995 µL)

HPLC

Fig. 19 Pretreatment Protocol for Amino Acid Supplement

■ Mushroom



1. Aspartic Acid
2. Glutamic Acid
3. Asparagine
4. Serine
5. Glutamine
6. Histidine
7. Glycine
8. Threonine
9. Arginine
10. Alanine
11. Tyrosine
13. GABA
14. Methionine
15. Valine
17. (Tryptophan + GABA)*
18. Phenylalanine
19. Isoleucine
20. Leucine
21. Proline
22. Lysine

* Might include overlapping peaks for tryptophan and GABA.

Fig. 20 Chromatograms of Mushroom

Sliced mushroom 2.06 g
- 10 mmol/L HCl (10 mL)
  Homogenize (1 minute)
  Centrifuge (10000 rpm × 15 minutes)

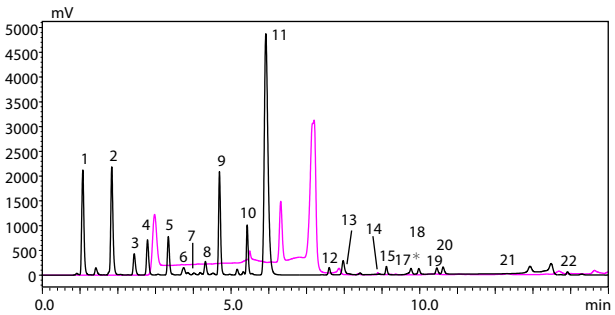
Supernatant
- Filtration (0.2 µm)

Filtrate 20 µL
- 10 mmol/L HCl (980 µL)

HPLC

Fig. 21 Pretreatment Protocol for Mushroom

■ Matcha (powdered green tea)



1. Aspartic Acid
2. Glutamic Acid
3. Asparagine
4. Serine
5. Glutamine
6. Histidine
7. Glycine
8. Threonine
9. Arginine
10. Alanine
11. Theanine
12. Tyrosine
13. GABA
14. Methionine
15. Valine
17. (Tryptophan + GABA)*
18. Phenylalanine
19. Isoleucine
20. Leucine
21. Proline
22. Lysine

* Might include overlapping peaks for tryptophan and GABA.

Fig. 22 Chromatograms of Matcha

Matcha (Powdered green tea) 0.99 g
- 10 mmol/L HCl (10 mL)
  Homogenize (1 minute)
  Put in the fridge over night
  Centrifuge (10000 rpm × 15 minutes)

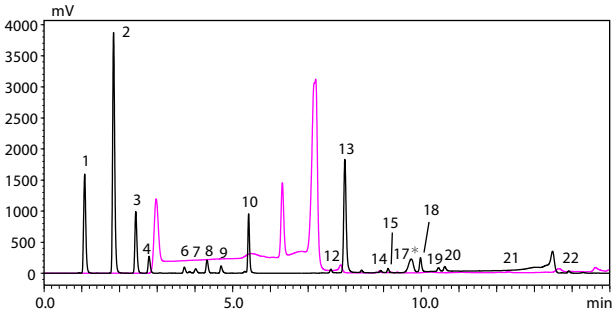
Supernatant
- Filtration (0.2 µm)

Filtrate 20 µL
- 10 mmol/L HCl (980 µL)

HPLC

Fig. 23 Pretreatment Protocol for Matcha

■ Tomato Juice



1. Aspartic Acid
2. Glutamic Acid
3. Asparagine
4. Serine
6. Histidine
7. Glycine
8. Threonine
9. Arginine
10. Alanine
12. Tyrosine
13. GABA
14. Methionine
15. Valine
17. (Tryptophan + GABA)*
18. Phenylalanine
19. Isoleucine
20. Leucine
21. Proline
22. Lysine

* Might include overlapping peaks for tryptophan and GABA.

Fig. 24 Chromatograms of Tomato Juice

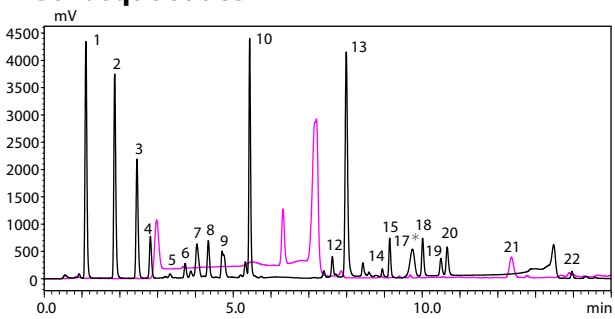
Tomato juice 0.99 g (0.5 mL)
- 10 mmol/L HCl (4.5 mL)
- Homogenize (1 minute)
- Ultrafiltration filter (3K)
- Centrifuge (10000 rpm × 20 minutes)

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

HPLC

Fig. 25 Pretreatment Protocol for Tomato Juice

■ Barbeque Sauce



1. Aspartic Acid
2. Glutamic Acid
3. Asparagine
4. Serine
5. Glutamine
6. Histidine
7. Glycine
8. Threonine
9. Arginine
10. Alanine
12. Tyrosine
13. GABA
14. Methionine
15. Valine
17. (Tryptophan + GABA)*
18. Phenylalanine
19. Isoleucine
20. Leucine
21. Proline
22. Lysine

* Might include overlapping peaks for tryptophan and GABA.

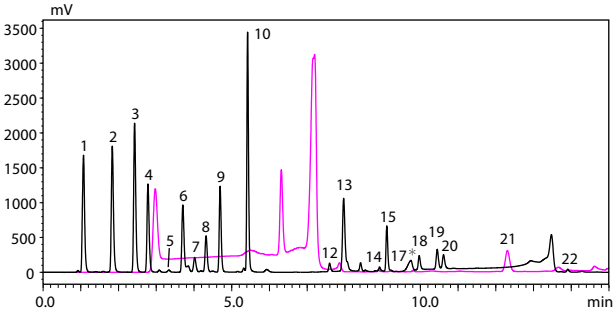
Fig. 26 Chromatograms of Barbeque Sauce

Barbecue sauce 0.23 g
- 10 mmol/L HCl (5.0 mL)
- Ultrafiltration filter (3K)
- Centrifuge (10000 rpm × 20 minutes)

HPLC

Fig. 27 Pretreatment Protocol for Barbeque Sauce

■ Green Vegetable Juice



1. Aspartic Acid
2. Glutamic Acid
3. Asparagine
4. Serine
5. Glutamine
6. Histidine
7. Glycine
8. Threonine
9. Arginine
10. Alanine
12. Tyrosine
13. GABA
14. Methionine
15. Valine
17. (Tryptophan + GABA)*
18. Phenylalanine
19. Isoleucine
20. Leucine
21. Proline
22. Lysine

* Might include overlapping peaks for tryptophan and GABA.

Fig. 28 Chromatograms of Green Vegetable Juice

Green juice 0.98 g (1.0 mL)
- 10 mmol/L HCl (4 mL)
- Homogenize (1 minute)
- Ultrafiltration filter (3K)
- Centrifuge (10000 rpm × 20 minutes)

Filtrate 200 µL
- 10 mmol/L HCl (800 µL)

HPLC

Fig. 29 Pretreatment Protocol for Green Vegetable Juice

■ Conclusion

The integrated HPLC LC-2070C is equipped with an automatic pretreatment function to analyze amino acids in food. The automatic derivatization using an autosampler enables reproducible work, reduces reagent consumption, and enables continuous analysis of multiple samples. In addition, reversed-phase separation mode can be used for precolumn derivatization, enabling high-speed analysis of 20 amino acids.

Amino acids contained in food samples containing many contaminants can be detected with high sensitivity by simple pretreatment such as dilution, filtration, and ultrafiltration. It is expected to be applied to a wide range of samples.

<Related Applications>

1. Simultaneous Analysis of Amino Acids Using Automatic Pretreatment Function of Integrated HPLC Application News No. 01-01047

01-01048-EN First Edition: Jan. 2026

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