

Application News

LC-2050C 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

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 L529A](#) 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 14 types of real food samples using the same method as in [L529A](#).

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 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.

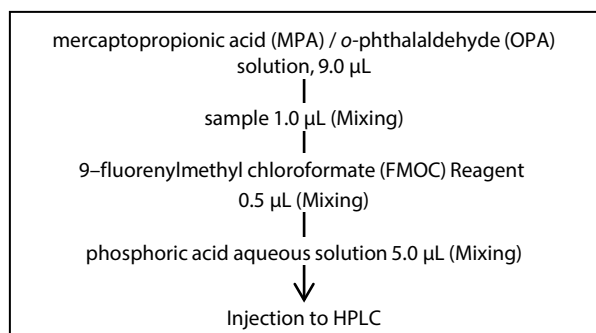


Fig. 1 Process Flow for Automatic Pre-Column Derivatization Using LC-2050C

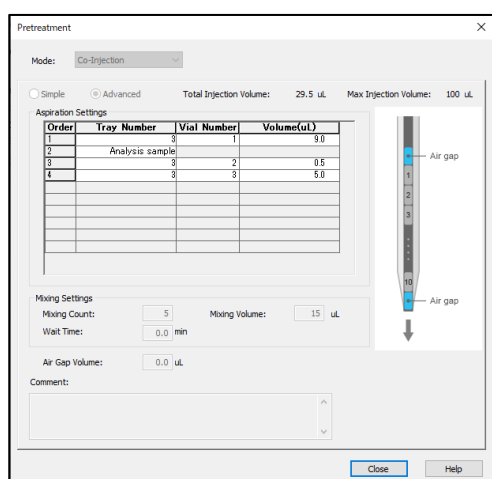


Fig. 2 Automatic Pre-Column Derivatization Setting Window

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.

Table 1 Analytical Conditions

System	: LC-2050C ^{*1}
Column	: Shim-pack™ XR-ODSII (100 mm × 3.0 mm I.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

^{*1} LC-2030C Plus system could also be used.

^{*2} P/N S228-41624-92

^{*3} 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 μ L 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 μ L of Mercaptopropionic Acid Reagent and 600 μ L OPA Reagent.
- Fmoc Reagent
Add 10 mg of 9-fluorenylmethyl 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 μ L 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 μ L 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.

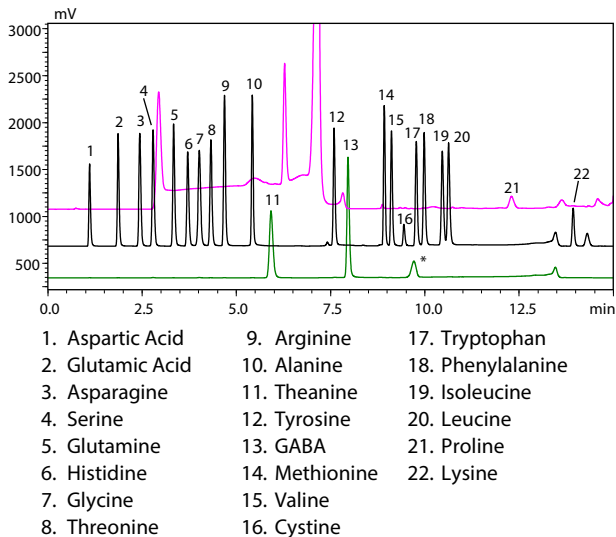


Fig. 3 Chromatograms of a Standard Sample with 22 Amino Acid Components (25 mmol/L each)

■ 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 ten samples for free amino acids (soybean extract, scallop, boiled tuna, amino acid supplement, mushroom, matcha, tomato juice, green vegetable juice, barbecue sauce, and coconut milk). Corresponding chromatograms and pretreatment protocols are shown in Fig. 4 to Fig. 31.

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)

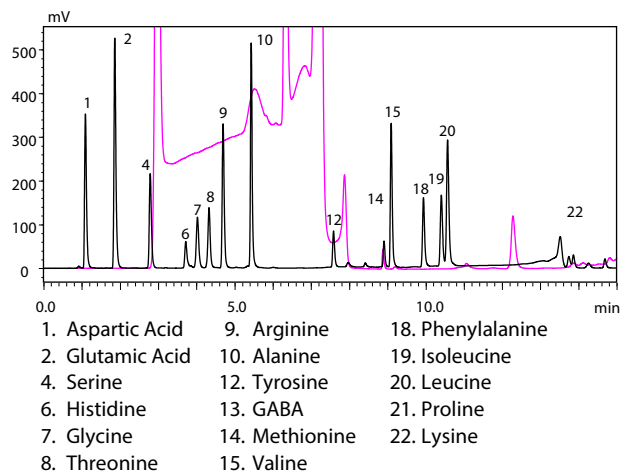


Fig. 4 Chromatograms of Brown Rice (Hydrochloric Acid Hydrolysis)

Ground brown rice 4.91 mg

- 6 mol/L HCl (500 μ L)
Seal vessel under reduced pressure
Stand at 110 $^{\circ}$ C, 22 hours
Evaporate to dryness under nitrogen atmosphere
- 10 mmol/L HCl (1000 μ L)
Dissolve with vortex mixer
Filtration (0.2 μ m, 10000 rpm $\times$ 10 minutes)
- Filtrate 50 μ L
- 10 mmol/L HCl (950 μ L)
- HPLC

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

■ Chicken Eggs

(Hydrochloric Acid Hydrolysis)

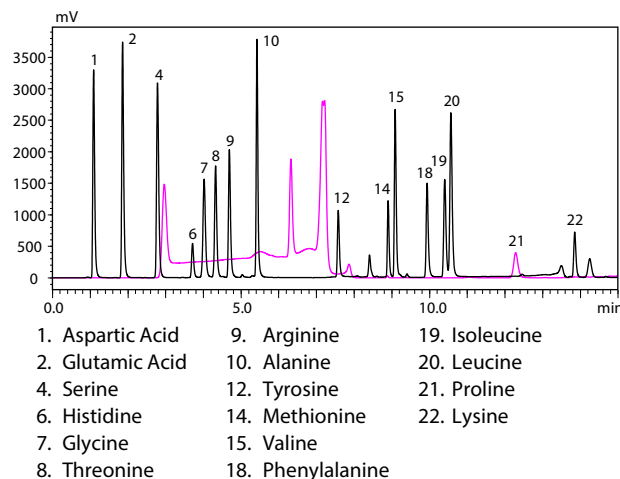


Fig. 6 Chromatograms of Chicken Eggs (Hydrochloric Acid Hydrolysis)

Stirred egg 14.26 mg

- 6 mol/L HCl (500 μ L)
Seal vessel under reduced pressure
Stand at 110 $^{\circ}$ C, 22 hours
Evaporate to dryness under nitrogen atmosphere
- 10 mmol/L HCl (1000 μ L)
Dissolve with vortex mixer
Filtration (0.2 μ m, 10000 rpm $\times$ 10 minutes)
- Filtrate 50 μ L
- 10 mmol/L HCl (950 μ L)
- HPLC

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

■ Soybean Extract (Hydrochloric Acid Hydrolysis)

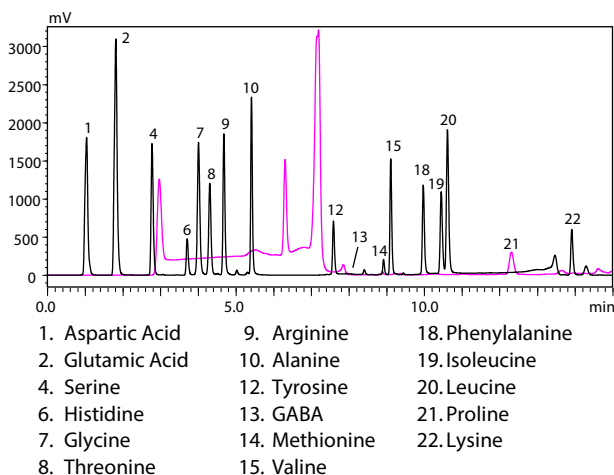


Fig. 8 Chromatograms of Soybean Extract
(Hydrochloric Acid Hydrolysis)

Soybean extract 25.72 mg

- 6 mol/L HCl (500 μ L)
- Seal vessel under reduced pressure
- Stand at 110 $^{\circ}$ C, 22 hours
- Evaporate to dryness under nitrogen atmosphere
- 10 mmol/L HCl (1000 μ L)
- Dissolve with vortex mixer
- Filtration (0.2 μ m, 10000 rpm $\times$ 10 minutes)
- Filtrate 50 μ L
- 10 mmol/L HCl (950 μ L)
- HPLC

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

■ Boiled Tuna (Hydrochloric Acid Hydrolysis Products)

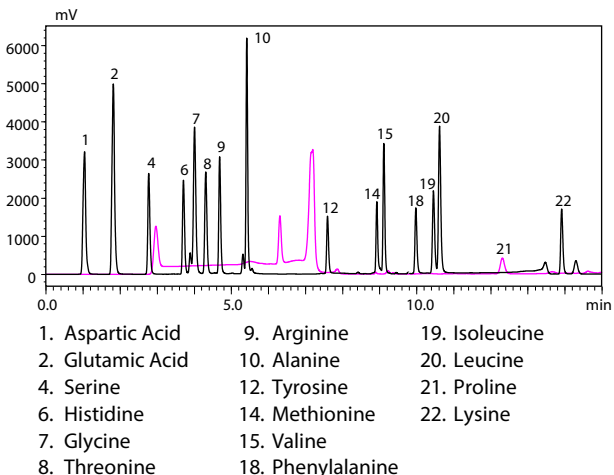


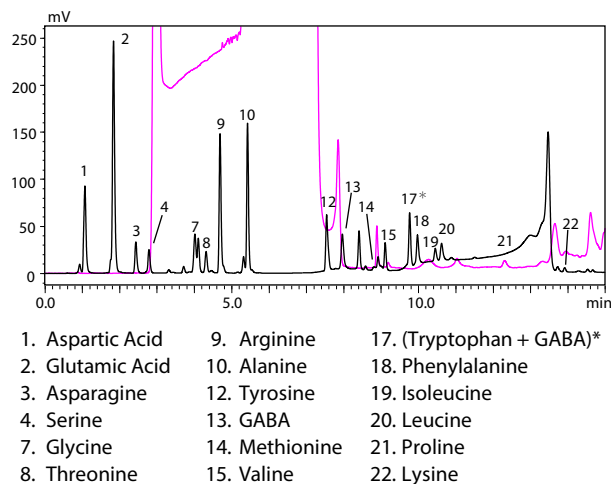
Fig. 10 Chromatograms of Boiled Tuna
(Hydrochloric Acid Hydrolysis)

Boiled tuna 8.39 mg

- 6 mol/L HCl (500 μ L)
- Seal vessel under reduced pressure
- Stand at 110 $^{\circ}$ C, 22 hours
- Evaporate to dryness under nitrogen atmosphere
- 10 mmol/L HCl (1000 μ L)
- Dissolve with vortex mixer
- Filtration (0.2 μ m, 10000 rpm $\times$ 10 minutes)
- Filtrate 10 μ L
- 10 mmol/L HCl (990 μ L)
- HPLC

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

■ Soybean Extract



* 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 $\times$ 15 minutes)
- Supernatant
- Ultrafiltration filter (3K)
- Centrifuge (10000 rpm $\times$ 20 minutes)
- Filtrate 100 μ L
- 10 mmol/L HCl (900 μ L)
- HPLC

Fig. 13 Pretreatment Protocol for Soybean Extract

■ Boiled Tuna

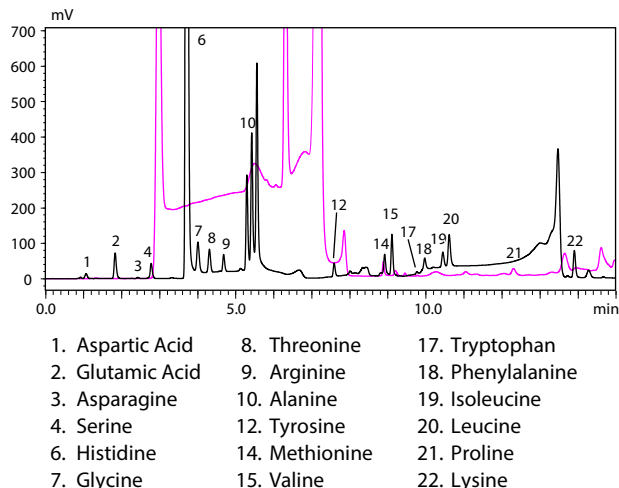


Fig. 14 Chromatograms of Boiled Tuna

Boiled tuna 4.91 g

- 10 mmol/L HCl (5 mL)
- Homogenize (1 minute)
- Centrifuge (10000 rpm $\times$ 15 minutes)
- Supernatant
- Ultrafiltration filter (3K)
- Centrifuge (10000 rpm $\times$ 20 minutes)
- Filtrate 10 μ L
- 10 mmol/L HCl (990 μ L)
- HPLC

Fig. 15 Pretreatment Protocol for Boiled Tuna

■ Scallop

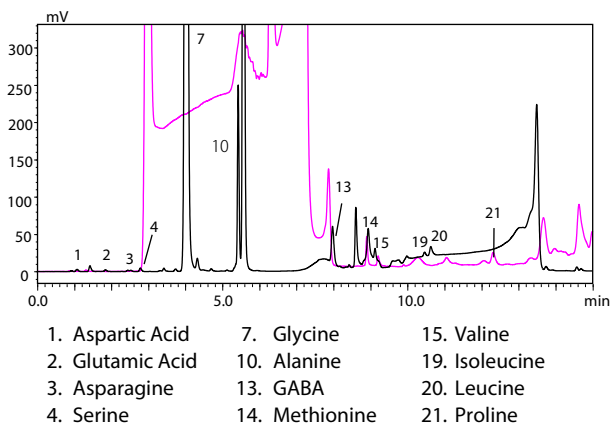


Fig. 16 Chromatograms of Scallop

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)

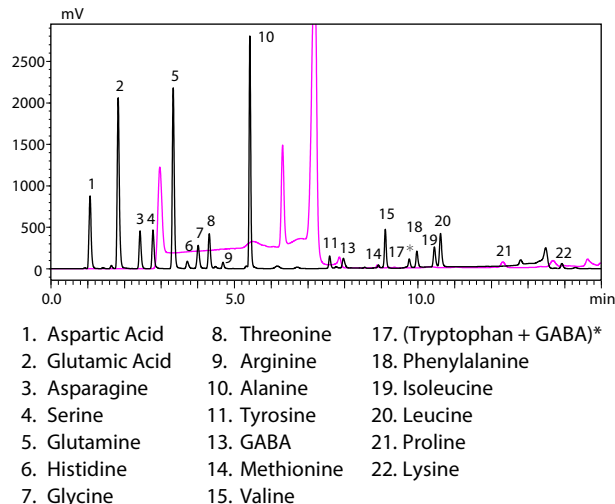
Filtrate 10 µL

10 mmol/L HCl (4990 µL)

HPLC

Fig. 17 Pretreatment Protocol for Scallop

■ Mushroom



* 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

■ Amino Acid Supplement

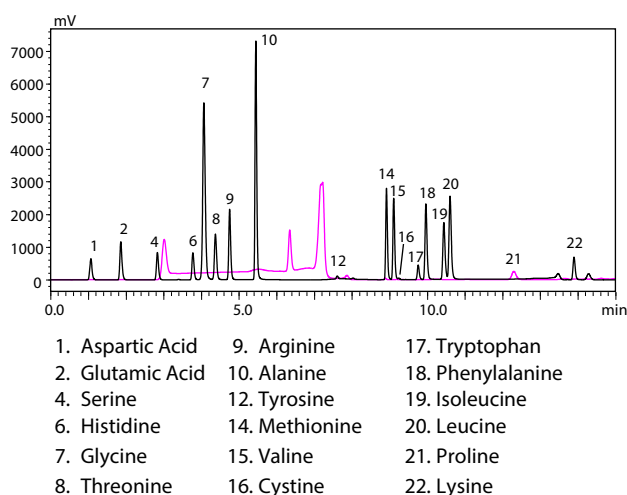


Fig. 18 Chromatograms of Amino Acid Supplement

Amino acid supplement 1.40 g

10 mmol/L HCl (25 mL)
Homogenize (1 minute)
Filtration (0.2 µm)

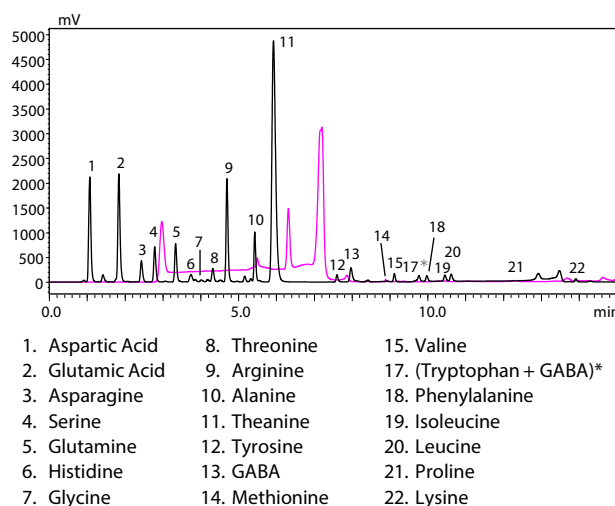
Filtrate 5 µL

10 mmol/L HCl (995 µL)

HPLC

Fig. 19 Pretreatment Protocol for Amino Acid Supplement

■ Matcha (powdered green tea)



* 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)

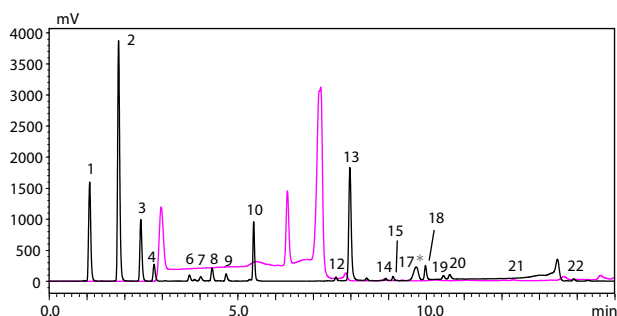
Filtrate 20 µL

10 mmol/L HCl (980 µL)

HPLC

Fig. 23 Pretreatment Protocol for Matcha

■ Tomato Juice



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

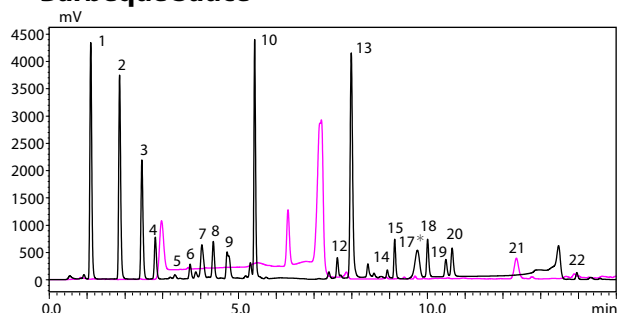
* Might include overlapping peaks for tryptophan and GABA.

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

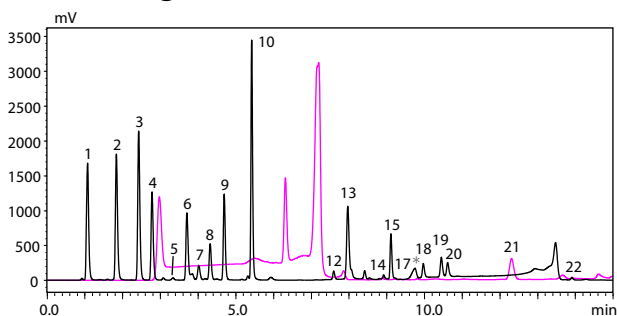
* Might include overlapping peaks for tryptophan and GABA.

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

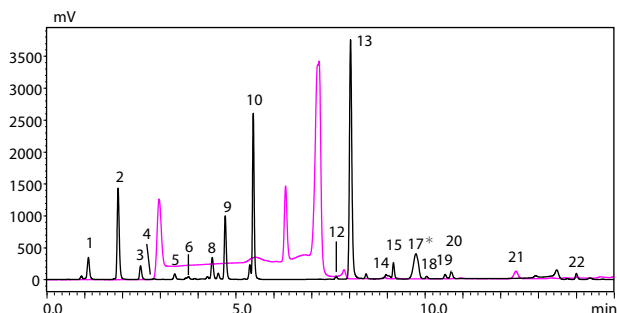
* Might include overlapping peaks for tryptophan and GABA.

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

■ Coconut Milk



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

* Might include overlapping peaks for tryptophan and GABA.

Fig. 30 Chromatograms of Coconut Milk

Coconut milk 0.53 g (0.5 mL)
 10 mmol/L HCl (9.5 mL)
 Mix with vortex
 Ultrafiltration filter (3K)
 Centrifuge (10000 rpm × 20 minutes)
 HPLC

Fig. 31 Pretreatment Protocol for Coconut Milk

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