

Analysis of Foods Using Nexera™ Post-Column Amino Acid Analysis System

Amino acids are essential nutrients that make up the proteins which form the human body. Analysis of amino acids has a long history of applying firstly to research purposes such as structural analysis and diagnoses of diseases and then to quality control of food products and drugs and other fields. Responding to increasing requests for higher speed analysis in recent years, Shimadzu Corporation developed an online pre-column method* that enables more rapid analysis. On the other hand, the post-column method is still frequently used today because of its wide range of applicability, even though it is a time-consuming technique.

Shimadzu post-column method employs an automatic analysis system that utilizes fluorescence derivatization by o-phthalaldehyde (OPA) in detection after separation of the amino acids by gradient elution using a cation exchange column. OPA derivatized amino acids have excellent sensitivity and linearity, and highly sensitive detection of proline and other secondary amino acids is also possible. Because the derivatization is carried out after separation, reaction efficiency is less prone to sample matrix effects. Therefore, this post-column method can detect amino acids with high sensitivity and high selectivity. As separation techniques, two modes are available, the Na type for analysis of proteinogenic amino acids and the Li type for analysis of free amino acids. Although simultaneous analysis of amino acids is conducted using the optimum time programs for these respective separation modes, highly accurate amino acid analysis is possible without labor-intensive preparation using a dedicated amino acid mobile phase kit and reaction reagent kit.

This article introduces amino acid analyses of various food products using the Nexera post-column amino acid analysis system, which was recently introduced in the market.

* : Please refer to Application News No. L529B.

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Analysis of Proteinogenic Amino Acids (Na Type) [Standard Sample]

Analysis of proteinogenic amino acids (Na type) enables separation and quantitation of approximately 20 types of amino acids obtained by hydrolyzing protein. The Na type method provides three separation conditions, the high resolution condition for normal analysis, a high speed condition, and a tryptophan included condition. Figs. 1 to 3 show analyses of standard amino acids under these three conditions, and Table 1 shows the analytical conditions of the Na type method.

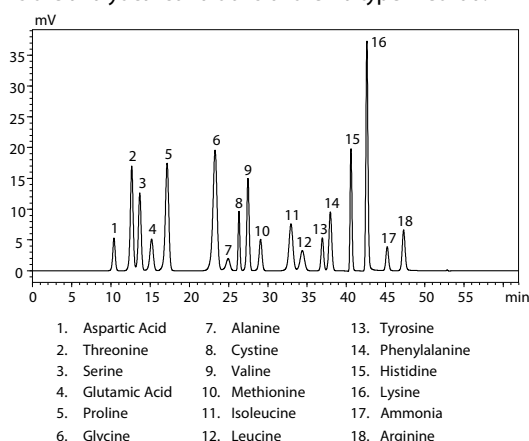


Fig. 1 Analysis of 18 Standard Amino Acids (0.1 μmol/L, Na Type High Resolution Condition)

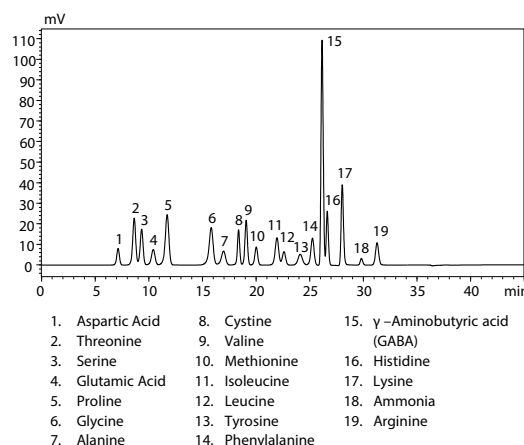


Fig. 2 Analysis of 19 Standard Amino Acids (0.1 μmol/L, Na Type High Speed Condition)

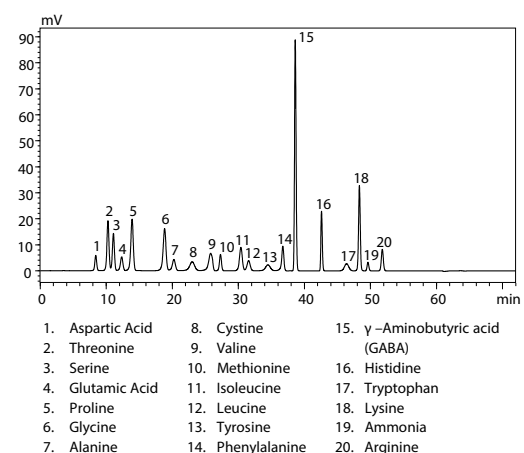


Fig. 3 Analysis of 20 Standard Amino Acids (0.1 μmol/L, Na Type Tryptophan Included Condition)

Table 1 Analytical Conditions of Na Type Method (Common)

Column	: Shim-pack™ Amino-Na (100 mm × 6.0 mm I.D., 5 μm) *1
Ammonia trap column	: Shim-pack ISC-30/S0504Na (50 mm × 4.0 mm I.D.) *2
Mobile phase	: Amino Acid Mobile Phase Kits (Na type) *3 Gradient Elution
Flow rate	: 0.4-0.6 mL/min
Column temp.	: 60 °C
Injection vol.	: 10 μL
Vial	: Shimadzu Vials, LC, 1.5 mL, Glass *4
Reaction reagent	: Amino Acid Reagent Kits *5
Flow rate of reagent	: 0.2 mL/min for each
Reaction temp.	: 60 °C
Detection	: Ex. 350 nm Em. 450 nm

*1 : P/N S228-18837-91, *2 : P/N S228-14206-91, *3 : P/N S228-21195-94, *4 : P/N S228-15652-92, *5 : P/N S228-21195-93

■ Analysis under Na Type High Resolution Condition
[Hydrochloric Acid Hydrolysis: Soybeans, Liquid Formula, Protein Powder]

The following introduces analyses using the Na type high resolution condition. Figs. 4 to 9 show analyses of 18 proteinogenic amino acids obtained by hydrochloric acid hydrolysis. Among these, 9 essential amino acids (threonine, valine, methionine, isoleucine, leucine, phenylalanine, histidine, tryptophan, lysine) must be obtained from foods, as they cannot be synthesized in the human body.

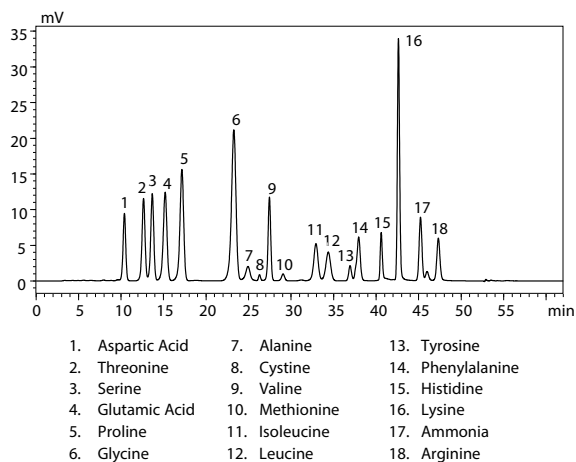


Fig. 4 Analysis of Ground Soybeans (Hydrochloric Acid Hydrolysis) (Na Type High Resolution Condition)

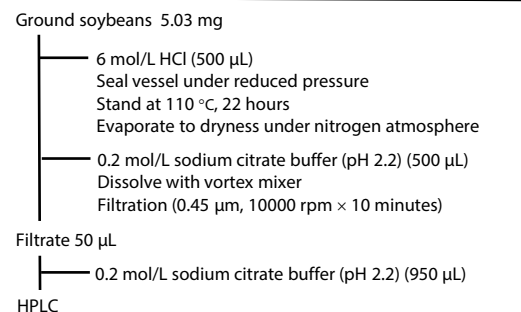


Fig. 5 Pretreatment Protocol for Ground Soybeans (Hydrochloric Acid Hydrolysis)

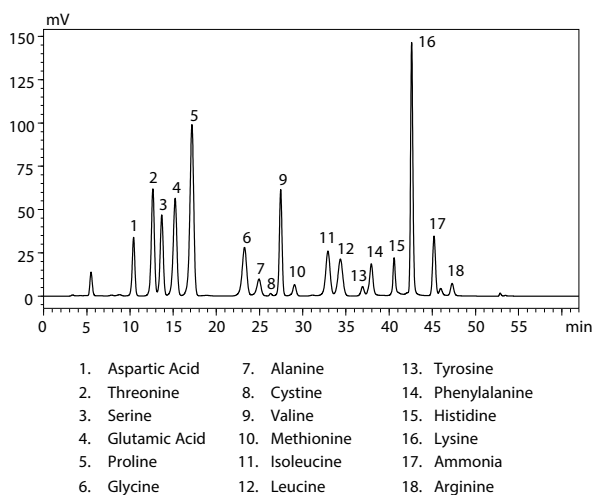


Fig. 6 Analysis of Baby Liquid Formula (Hydrochloric Acid Hydrolysis) (Na Type High Resolution Condition)

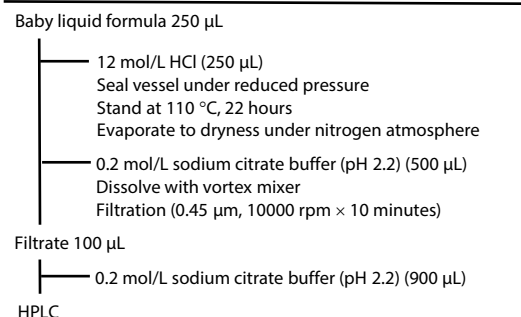


Fig. 7 Pretreatment Protocol for Baby Liquid Formula (Hydrochloric Acid Hydrolysis)

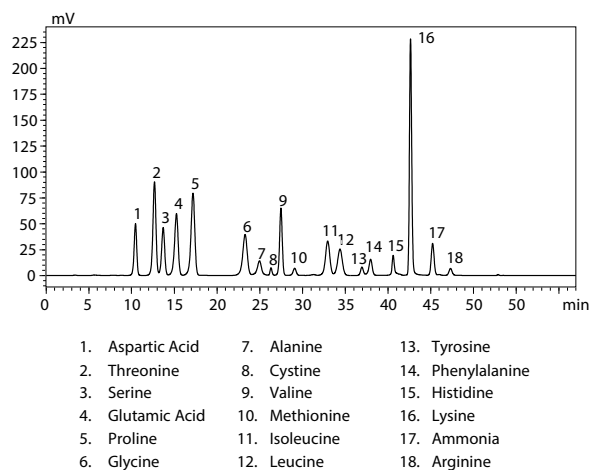


Fig. 8 Analysis of Protein Powder (Hydrochloric Acid Hydrolysis) (Na Type High Resolution Condition)

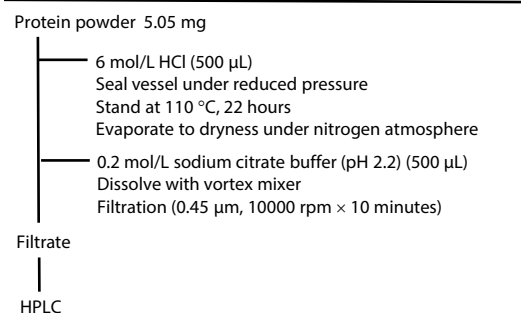
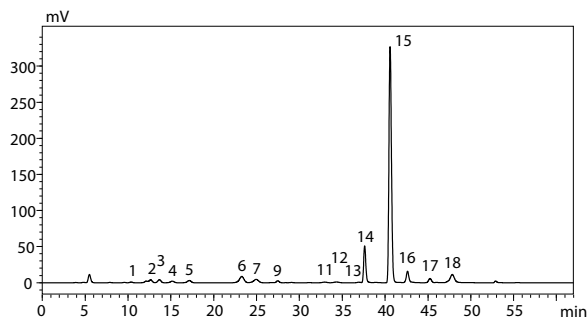


Fig. 9 Pretreatment Protocol for Protein Powder (Hydrochloric Acid Hydrolysis)

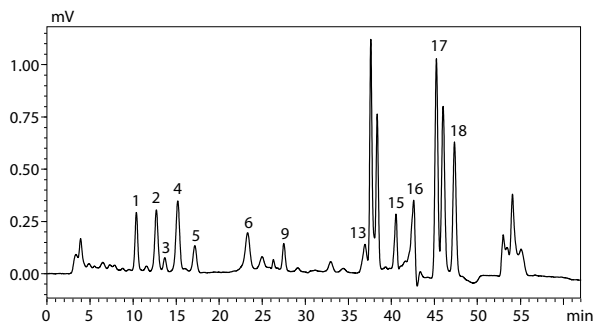
■ Analysis under Na Type High Resolution Condition
[Chicken Breast Meat, Soy Meat, Nampler (Fish Sauce), Amino Acid Supplement]

Figs. 10 to 17 show analyses of 18 free amino acids obtained by extraction from samples. Here, an ultrafiltration cartridge was used in deproteinization.



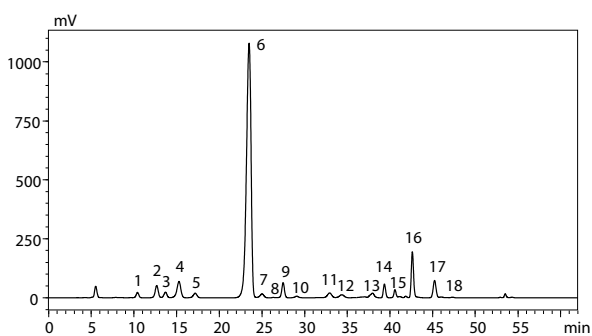
- | | | |
|------------------|-------------------|---------------|
| 1. Aspartic Acid | 7. Alanine | 15. Histidine |
| 2. Threonine | 9. Valine | 16. Lysine |
| 3. Serine | 11. Isoleucine | 17. Ammonia |
| 4. Glutamic Acid | 12. Leucine | 18. Arginine |
| 5. Proline | 13. Tyrosine | |
| 6. Glycine | 14. Phenylalanine | |

Fig. 10 Analysis of Chopped Chicken Breast Meat (Na Type High Resolution Condition)



- | | |
|------------------|---------------|
| 1. Aspartic Acid | 9. Valine |
| 2. Threonine | 13. Tyrosine |
| 3. Serine | 15. Histidine |
| 4. Glutamic Acid | 16. Lysine |
| 5. Proline | 17. Ammonia |
| 6. Glycine | 18. Arginine |

Fig. 12 Analysis of Dry Soy Meat (Na Type High Resolution Condition)



- | | | |
|------------------|----------------|-------------------|
| 1. Aspartic Acid | 7. Alanine | 13. Tyrosine |
| 2. Threonine | 8. Cystine | 14. Phenylalanine |
| 3. Serine | 9. Valine | 15. Histidine |
| 4. Glutamic Acid | 10. Methionine | 16. Lysine |
| 5. Proline | 11. Isoleucine | 17. Ammonia |
| 6. Glycine | 12. Leucine | 18. Arginine |

Fig. 14 Analysis of Nampler (Fish Sauce) (Na Type High Resolution Condition)

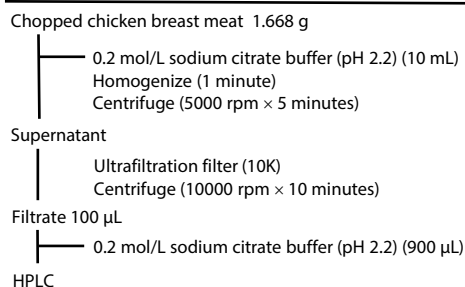


Fig. 11 Pretreatment Protocol for Chopped Chicken Breast Meat

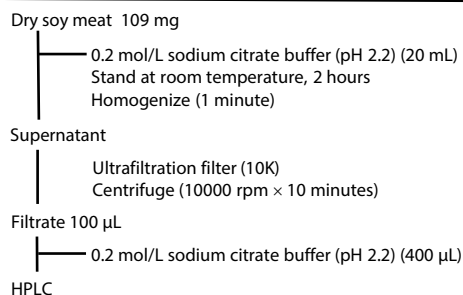


Fig. 13 Pretreatment Protocol for Dry Soy Meat

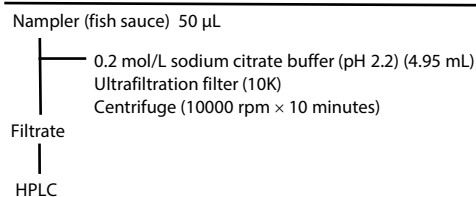
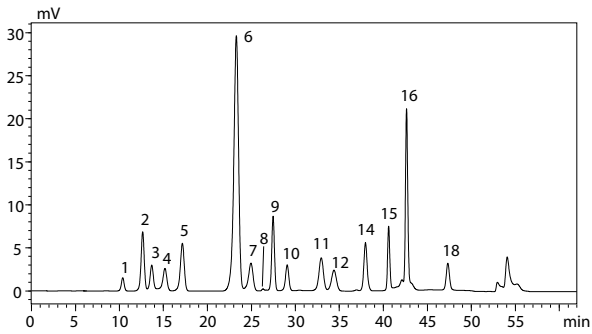


Fig. 15 Pretreatment Protocol for Nampler (Fish Sauce)



- | | | |
|------------------|----------------|-------------------|
| 1. Aspartic Acid | 7. Alanine | 14. Phenylalanine |
| 2. Threonine | 8. Cystine | 15. Histidine |
| 3. Serine | 9. Valine | 16. Lysine |
| 4. Glutamic Acid | 10. Methionine | 18. Arginine |
| 5. Proline | 11. Isoleucine | |
| 6. Glycine | 12. Leucine | |

Fig. 16 Analysis of Amino Acid Supplement (Na Type High Resolution Condition)

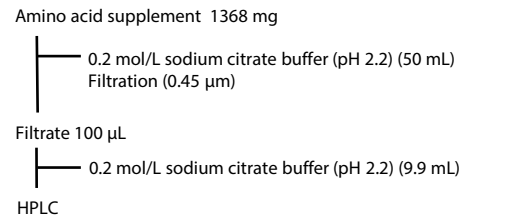
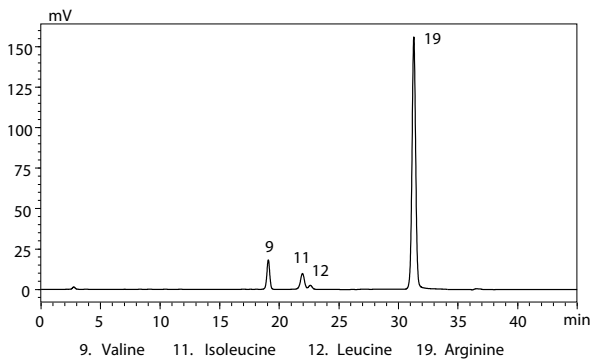


Fig. 17 Pretreatment Protocol for Amino Acid Supplement

Analysis under Na Type High Speed Condition [Sports Drink, Beer, Yogurt]

Figs. 18 to 23 show analyses under the Na type high speed condition. This separation condition has the shortest analysis time (45 min) and can be used with samples that contain comparatively few contaminants.



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|-----------|----------------|-------------|--------------|
| 9. Valine | 11. Isoleucine | 12. Leucine | 19. Arginine |
|-----------|----------------|-------------|--------------|

Fig. 18 Analysis of Sports Drink (Na Type High Speed Condition)

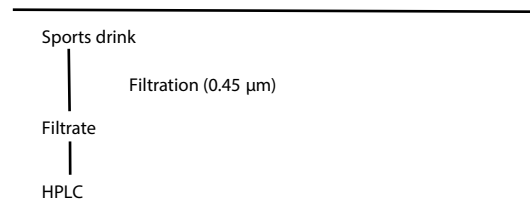
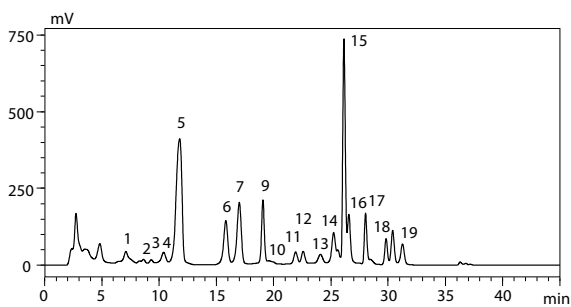


Fig. 19 Pretreatment Protocol for Sports Drink



- | | | |
|------------------|-------------------|--------------------------------|
| 1. Aspartic Acid | 9. Valine | 15. γ-Aminobutyric acid (GABA) |
| 2. Threonine | 10. Methionine | 16. Histidine |
| 3. Serine | 11. Isoleucine | 17. Lysine |
| 4. Glutamic Acid | 12. Leucine | 18. Ammonia |
| 5. Proline | 13. Tyrosine | 19. Arginine |
| 6. Glycine | 14. Phenylalanine | |
| 7. Alanine | | |

Fig. 20 Analysis of Beer (Na Type High Speed Condition)

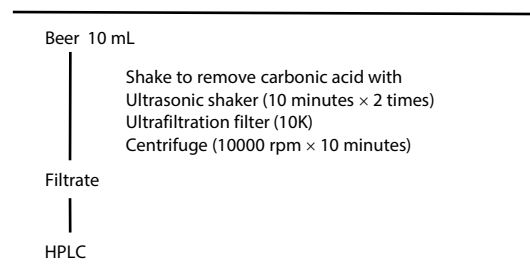


Fig. 21 Pretreatment Protocol for Beer

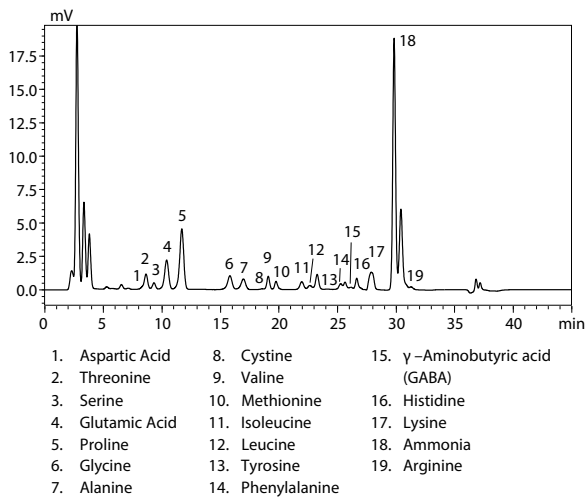


Fig. 22 Analysis of Yogurt (Na Type High Speed Condition)

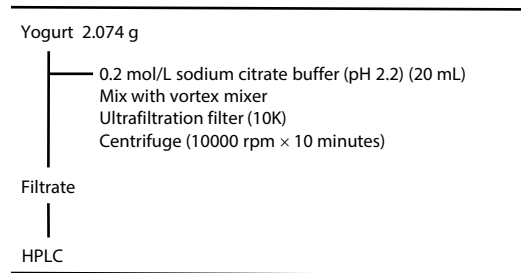


Fig. 23 Pretreatment Protocol for Yogurt

Analysis under Na Type Tryptophan Included Condition [Chicken Breast Meat (Alkali Hydrolysis), Vegetable Juice, Miso (Soybean Paste)]

Figs. 24 to 29 show analyses under the Na type tryptophan included condition. Alkali hydrolysis is used when analyzing proteinogenic amino acids containing tryptophan due to the decomposition of tryptophan by acid hydrolysis.

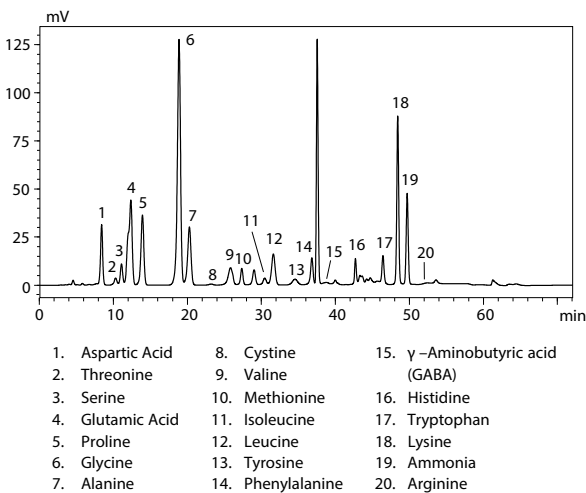


Fig. 24 Analysis of Chopped Chicken Breast Meat (Alkali Hydrolysis) (Na Type Tryptophan Included Condition)

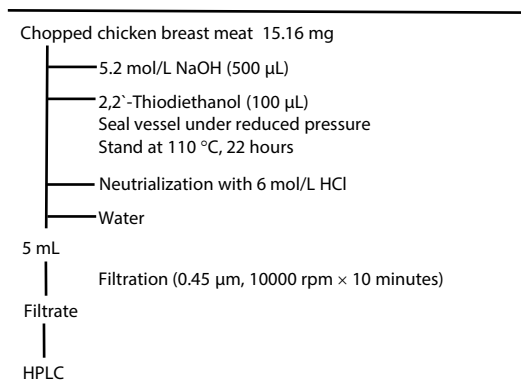


Fig. 25 Pretreatment Protocol for Chopped Chicken Breast Meat (Alkali Hydrolysis)

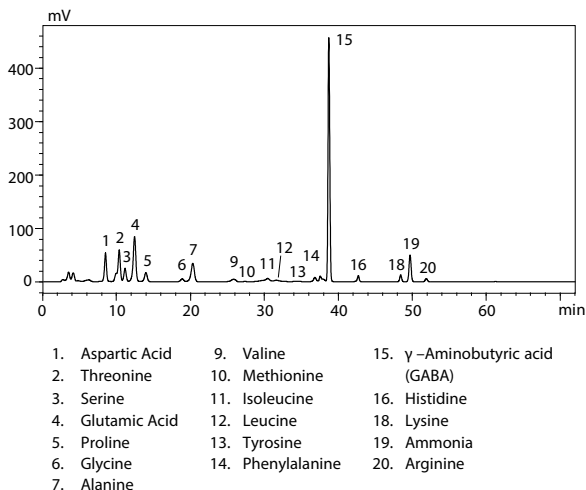


Fig. 26 Analysis of Vegetable Juice (Na Type Tryptophan Included Condition)

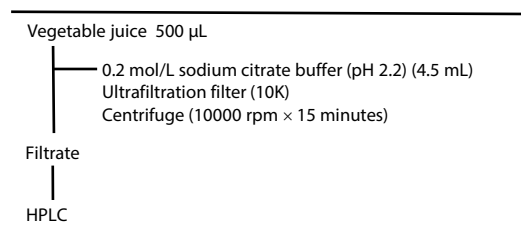


Fig. 27 Pretreatment Protocol for Vegetable Juice

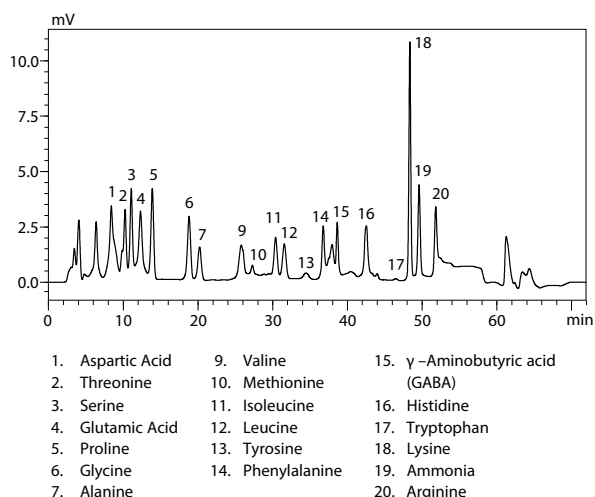


Fig. 28 Analysis of Miso (Japanese Soybean Paste) (Na Type Tryptophan Included Condition)

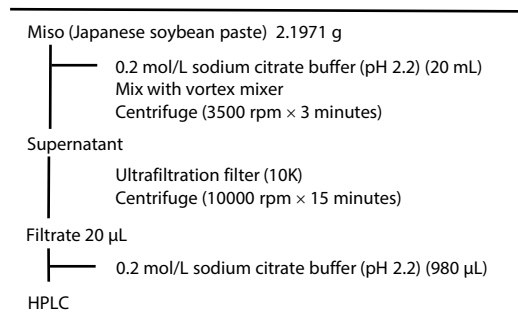


Fig. 29 Pretreatment Protocol for Miso (Japanese Soybean Paste)

Analysis of Cysteic Acid and Methionine Sulfone under Na Type Condition [Standard Sample, Chicken Breast Meat (Performic Acid Oxidation-Hydrolysis)]

Cysteine, cystine, and methionine are decomposed by hydrochloric acid hydrolysis. Therefore, in order to measure these compounds, performic acid oxidation-hydrolysis is conducted to convert cysteine and cystine to cysteic acid and methionine to methionine sulfone to be determined in the analysis.

Fig. 30 shows a standard analysis of cysteic acid and methionine sulfone, Fig. 31 shows an analysis of chopped chicken breast meat by performic acid oxidation-hydrolysis, and Table 2 shows the analytical conditions.

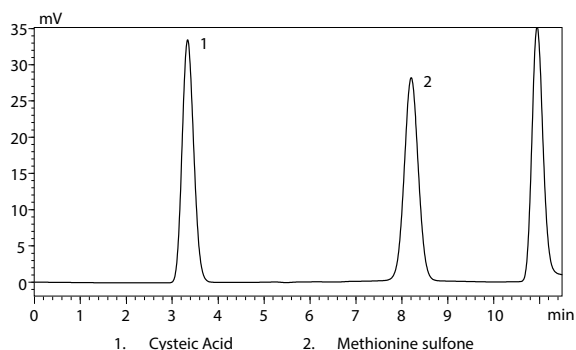


Fig. 30 Analysis of Standard Sample (Cysteic Acid, Methionine Sulfone, 0.1 mmol/L Each)

Table 2 Analytical Conditions for Cysteic Acid and Methionine Sulfone

Column	: Shim-pack Amino-Na (100 mm \times 6.0 mm I.D., 5 μ m)
Mobile phase	: A*) 67 mmol/L sodium citrate containing 7% ethanol and 0.15 mol/L perchloric acid B) 0.2 mol/L NaOH Gradient Elution
Flow rate	: 0.4 mL/min
Column temp.	: 60 $^{\circ}$ C
Injection vol.	: 10 μ L
Reaction reagent	: Amino acid reagent kits not add sodium hypochlorite to reagent A
Flow rate of reagent	: 0.2 mL/min each
Reaction temp.	: 60 $^{\circ}$ C
Detection	: Ex. 350 nm Em. 450 nm

*6 : Sodium citrate 2-hydrate 19.6 g, ethanol 70 mL, perchloric acid (60%) 14 mL into 1 L of water

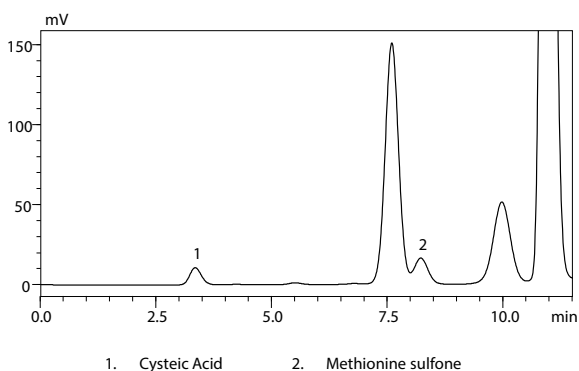


Fig. 31 Analysis of Chopped Chicken Breast Meat (Performic Acid Oxidation-Hydrolysis)

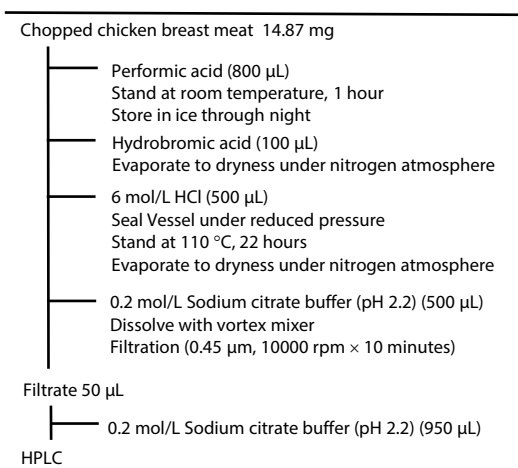


Fig. 32 Pretreatment Protocol for Chopped Chicken Breast Meat (Performic Acid Oxidation-Hydrolysis)

■ Analysis of Free Amino Acids (Li Type) [Standard Sample, Ham, Black Vinegar, Broccoli Sprout]

In free amino acid analysis (Li type), separation and quantitation of approximately 40 amino acids are possible. If the reagent kit is used, the 150 minutes analysis is conducted easily with good repeatability. Fig. 33 shows a standard analysis by the Li type method, and Table 3 shows the Li type analytical conditions. Figs. 34 to 39 are analyses of free amino acids by the Li type method. High resolution is possible with the Li type method, even with samples such as foods that contain many contaminants.

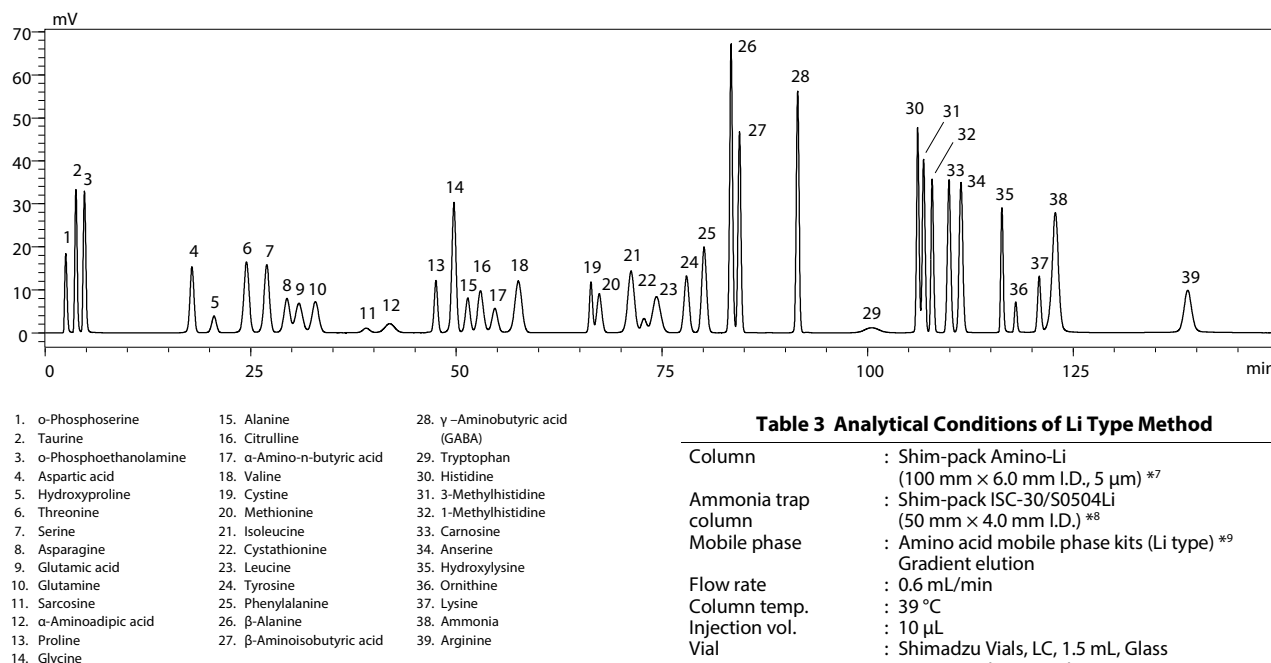


Fig. 33 Analysis of 39 Standard Amino Acids (0.1 mmol/L Each, Li Type Condition)**

** : However, the concentrations are 0.05 mmol/L for components 1-3, 12, 17, and 22, 0.25 mmol/L for component 11, and 0.026 mmol/L for component 29.

Table 3 Analytical Conditions of Li Type Method

Column	: Shim-pack Amino-Li (100 mm × 6.0 mm I.D., 5 μm) *7
Ammonia trap column	: Shim-pack ISC-30/S0504Li (50 mm × 4.0 mm I.D.) *8
Mobile phase	: Amino acid mobile phase kits (Li type) *9 Gradient elution
Flow rate	: 0.6 mL/min
Column temp.	: 39 °C
Injection vol.	: 10 μL
Vial	: Shimadzu Vials, LC, 1.5 mL, Glass
Reaction reagent	: Amino acid reagent kits
Flow rate of reagent	: 0.2 mL/min for each
Reaction temp.	: 39 °C
Detection	: Ex. 350 nm Em. 450 nm

*7 : P/N S228-18837-92, *8 : P/N S228-00821-91, *9 : P/N S228-21195-95

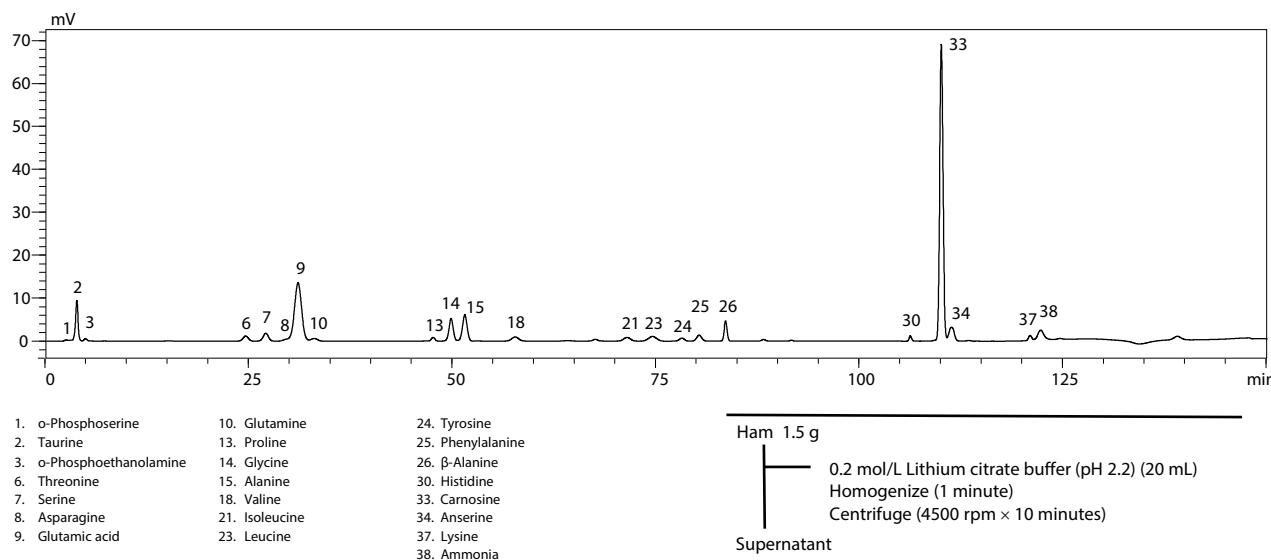


Fig. 34 Analysis of Ham (Li Type Condition)

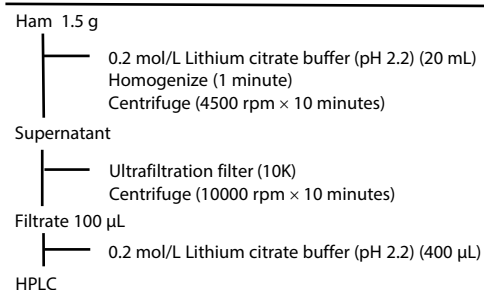
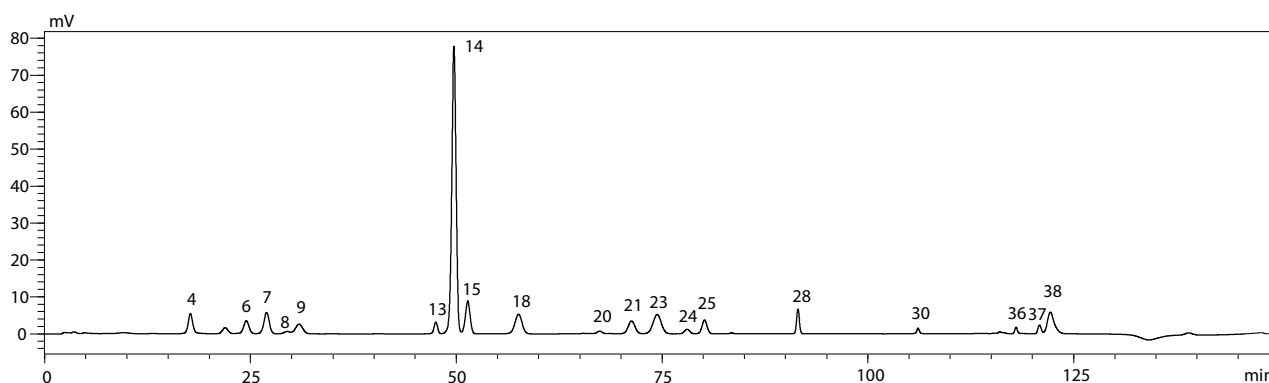


Fig. 35 Pretreatment Protocol for Ham



- | | | |
|------------------|-------------------|--|
| 4. Aspartic acid | 15. Alanine | 28. γ -Aminobutyric acid (GABA) |
| 6. Threonine | 18. Valine | 30. Histidine |
| 7. Serine | 20. Methionine | 36. Ornithine |
| 8. Asparagine | 21. Isoleucine | 37. Lysine |
| 9. Glutamic acid | 23. Leucine | 38. Ammonia |
| 13. Proline | 24. Tyrosine | |
| 14. Glycine | 25. Phenylalanine | |

Fig. 36 Analysis of Black Vinegar (Li Type Condition)

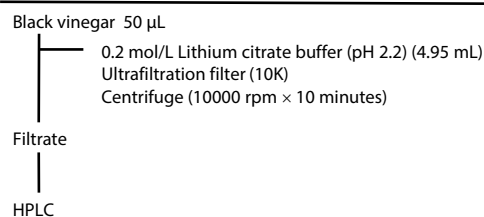
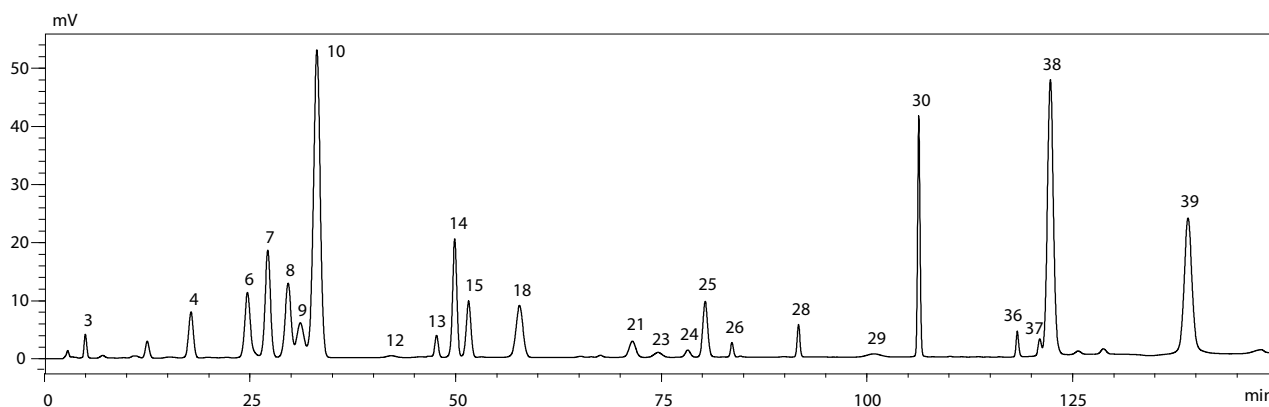


Fig. 37 Pretreatment Protocol for Black Vinegar



- | | | |
|----------------------------------|-------------------|--|
| 3. α -Phosphoethanolamine | 13. Proline | 26. β -Alanine |
| 4. Aspartic acid | 14. Glycine | 28. γ -Aminobutyric acid (GABA) |
| 6. Threonine | 15. Alanine | 29. Tryptophan |
| 7. Serine | 18. Valine | 30. Histidine |
| 8. Asparagine | 21. Isoleucine | 36. Ornithine |
| 9. Glutamic acid | 23. Leucine | 37. Lysine |
| 10. Glutamine | 24. Tyrosine | 38. Ammonia |
| 12. α -Aminoadipic acid | 25. Phenylalanine | 39. Arginine |

Fig. 38 Analysis of Broccoli Sprout (Li Type Condition)

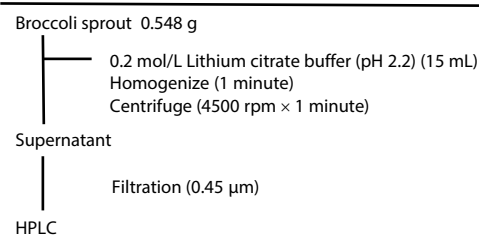


Fig. 39 Pretreatment Protocol for Broccoli Sprout

Conclusion

Analyses of various food products using the Nexera post-column amino acid analysis system were introduced.

It should be noted that the pretreatment protocols used here are just examples. When actually conducting a quantitative analysis, confirmation of the recovery rate by a recovery test prior to the analysis is recommended.

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First Edition: Oct. 2020



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