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Application News

Nexera™ XR Ultra High Performance Liquid Chromatograph

High Speed Simultaneous Analysis of Amino Acids in Foods Using Automatic Pretreatment Function

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User Benefits

- The analysis time is significantly reduced compared to the post-column derivatization method.
- The analytical method is very simple and easy since the burdensome derivatization process can be performed automatically.
- Users can analyze amino acids with a simple HPLC system.

■ Introduction

Although the post-column derivatization method has been commonly used for amino acid analysis with HPLC, its disadvantages include the long analysis time due to the characteristics of the column and high cost from the complex instrument configuration. On the other hand, although pre-column derivatization enables fast analysis with simple instrument configuration, its problems include the burdensome derivatization operation and the effect of the sample matrix.

In Application News 01-00441-EN, we introduced a pre-column derivatization method for amino acid analysis using the automatic pretreatment function. With this feature, the burdensome derivatization process can be performed automatically. In this article, we introduce the examples of analyzing amino acids in various foods using the method with automatic function.

■ Automatic Pre-Column Derivatization

Nexera XR is equipped with an automatic pretreatment function that enables users to configure desired operations including sample dilution and reagent addition. For this study, we set the system to automatically mix the sample and derivatization reagent in the autosampler needle. Fig. 1 shows the flow of the derivatization, and Table 1 the preparation method for the derivatization reagents. For detailed pretreatment program parameters, please refer to Application News 01-00441-EN.

■ Analysis of Mixed Standard Amino Acid Solution

Fig. 2 shows the chromatogram of a mixed standard solution of 20 proteinogenic amino acids. The 20 components could be separated in about 14 minutes. Tables 2 to 4 on the next page show the analytical conditions.

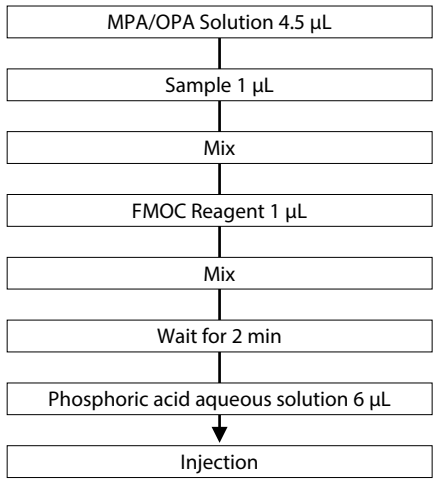
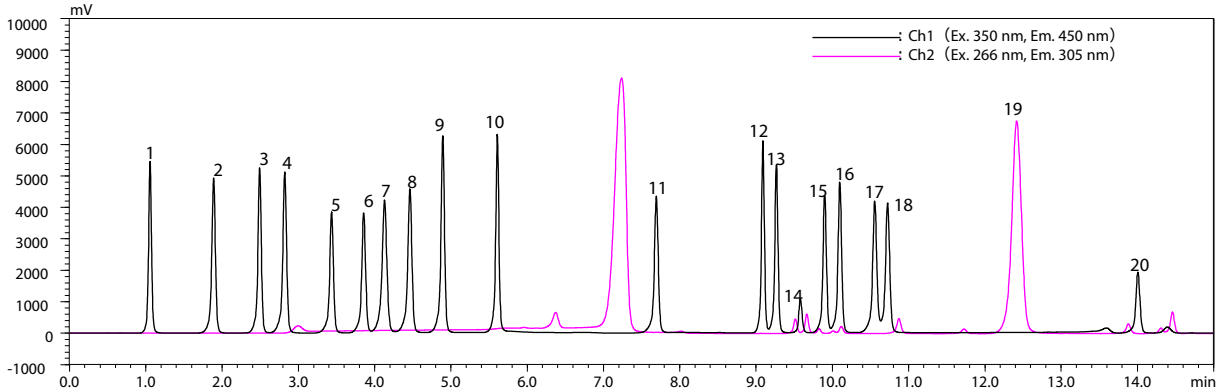


Fig. 1 Derivatization Flow with the Automatic Pretreatment Function

Table 1 Preparation of Derivatization Reagents

- 0.1 mol/L Borate buffer
  Add 0.62 g of boric acid and 0.20 g of sodium hydroxide into 100 mL of ultrapure water.
- Mercaptopropionic acid Reagent (MPA 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 ultrapure water.
- MPA / OPA Solution
  Mix 600 µL of MPA Reagent and 300 µL OPA Reagent.
- FMOC Reagent
  Dissolve 10 mg of 9-fluorenylmethyl chloroformate into 100 mL of acetonitrile.
- Phosphoric acid aqueous solution
  Add 0.5 mL of phosphoric acid into 100 mL of pure water.



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. 2 Simultaneous Analysis of 20 Proteinogenic Amino Acids (100 µmol/L each)

Table 2 Analytical Conditions

System	: Nexera XR
Column	: Shim-pack™ XR-ODS II[*1] 100 mm × 3.0 mm I.D., 2.2 µm
Mode	: Low pressure gradient
Mobile phase	: A) 20 mmol/L (Sodium) acetate buffer (pH 6) B) Water/Acetonitrile = 1 : 9 C) 20 mmol/L (Sodium) acetate buffer (pH 5) containing 0.5 mmol/L EDTA-2Na
Flow rate	: 1.0 mL/min
Column temp.	: 40 ˚C
Injection volume	: 1 µL[*2]
Sample cooler	: 4 ˚C
Detection	: Fluorescence detector (Cell temp. : 25 ˚C) Ch1) Ex. 350 nm, Em. 450 nm Ch2) Ex. 266 nm, Em. 305 nm

*1: P/N 228-41624-92, *2: P/N 227-34001-01

Table 3 Preparation of Mobile Phases

- Mobile Phase A
  Add 2.67 g of sodium acetate trihydrate and 41 µL of acetic acid into 1000 mL of pure water.
- Mobile Phase C
  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.

Table 4 Time Program

Time (min)	A.conc.	B.conc.	C.conc.
0	95	5	0
0.2	93	7	0
1	93	7	0
4	87	13	0
5	0	15	85
7.5	0	30	70
12	0	35	65
14	0	45	55
14.01	0	95	5
17	0	95	5
17.01	95	5	0
19.5	95	5	0

■ Analysis of Amino Acids in Foods

Amino acids are one of the key nutrients in food that contribute to a variety of physiological functions and also to the taste of food. We here introduce examples of the analysis of various foods (Figs. 3 to 10), and Figs. 11 to 17 show the pretreatment procedures for those analyses.

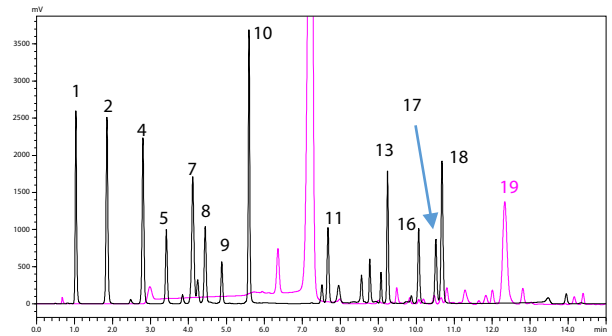


Fig. 3 Chromatogram of Sweet sake (Mirin)

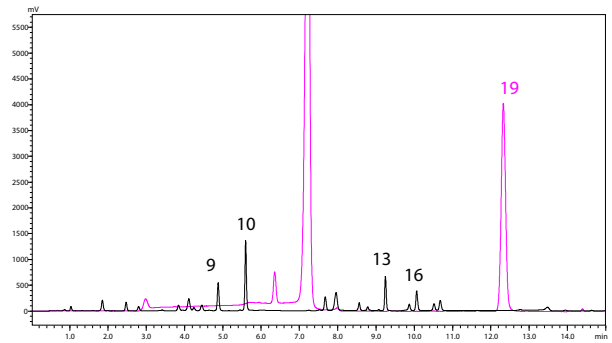


Fig. 4 Chromatogram of Beer

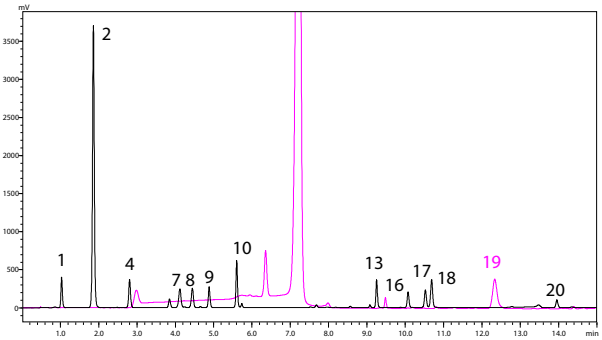


Fig. 5 Chromatogram of Dried bonito broth (Katsuo dashi)

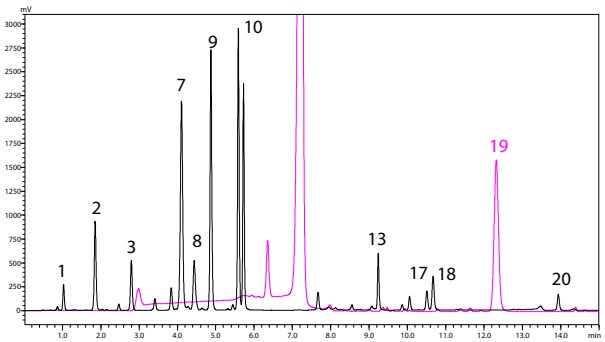


Fig. 6 Chromatogram of Cricket powder

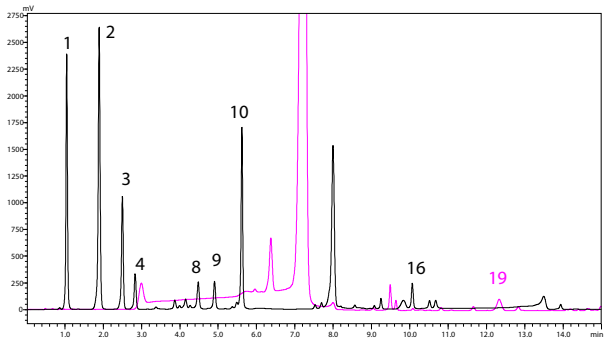


Fig. 7 Chromatogram of Ketchup

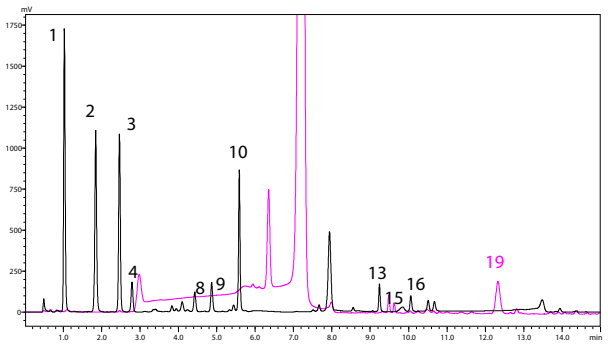


Fig. 8 Chromatogram of Worcestershire sauce

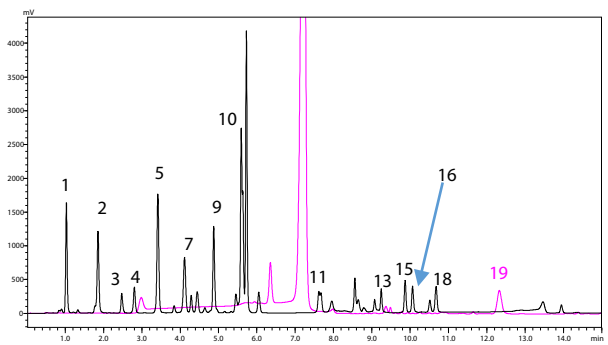


Fig. 9 Chromatogram of Soy meat

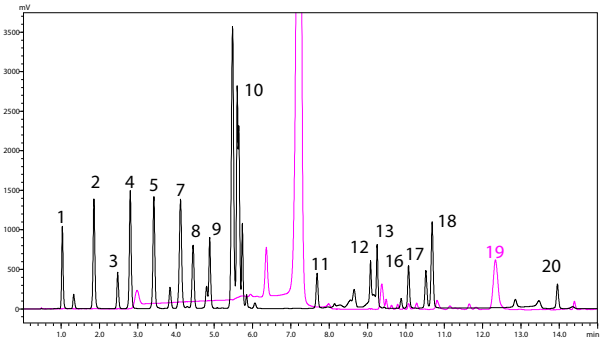


Fig. 10 Chromatogram of Chicken

- Sample
  - Filtration (0.22 µm)
- Filtration 20 µL
  - 10 mmol/L HCl 980 µL

Fig. 11 Pretreatment of Sweet sake (Mirin)

- Sample
  - Filtration (0.22 µm)
- Filtration 10 µL
  - 10 mmol/L HCl 990 µL

Fig. 12 Pretreatment of Beer

- Sample
  - Filtration (0.22 µm)
- Filtration 10 µL
  - 10 mmol/L HCl 9900 µL

Fig. 13 Pretreatment of Dried bonito broth (Katsuo dashi)

- Sample 0.599 g
  - 10 mmol/L HCl 10 mL
- Sonication for 10 min
  - Filtration (0.22 µm)
  - Ultrafiltration (50K)
    Centrifugation (10000 rpm × 10 min)
- Filtration
  - Ultrafiltration (10K)
    Centrifugation (10000 rpm × 10 min)
- Filtration 20 µL
  - 10 mmol/L HCl 980 µL

Fig. 14 Pretreatment of Cricket powder

- Samples
  - ・Worcestershire Sauce 0.698 g
  - ・Ketchup 0.504 g
    - 10 mmol/L HCl 5 mL
- Sonication for 10 min
  - Filtration (0.22 µm)
  - Ultrafiltration (50K)
    Centrifugation (10000 rpm × 10 min)
- Filtration
  - Ultrafiltration (10K)
    Centrifugation (10000 rpm × 10 min)
- Filtration 20 µL
  - 10 mmol/L HCl 980 µL

Fig. 15 Pretreatment of Ketchup and Worcestershire sauce

- Sample*1 4.339 g
  - 10 mmol/L HCl 25 mL
  - Centrifugation (4000 rpm × 20 min)
    Centrifugation (5000 rpm × 15 min)
  - Filtration (0.45 µm)
- Filtration 1 mL
  - Ultrafiltration (50K)
    Centrifugation (10000 rpm × 10 min)
  - Ultrafiltration (3K)
    Centrifugation (10000 rpm × 10 min)
- Filtration 50 µL
  - 10 mmol/L HCl 50 µL *2

Fig. 16 Pretreatment of Soy meat

*1: Soy meat was soaked in ultrapure water and allowed to stand for 24 hours. It shows the weight of sodden soy meat.
*2: Insert vial (P/N: GLCTV -104)

- Sample 4.542 g
  - 10 mmol/L HCl 50 mL
  - Centrifugation (4000 rpm × 20 min)
    Centrifugation (5000 rpm × 15 min)
  - Filtration (0.45 µm)
- Filtration 1 mL
  - Ultrafiltration (50K)
    Centrifugation (10000 rpm × 10 min)
  - Ultrafiltration (3K)
    Centrifugation (10000 rpm × 10 min)
- Filtration 100 µL
  - 10 mmol/L HCl 900 µL

Fig. 17 Pretreatment of Chicken

■ Spike-and-Recovery Test

In general, it is assumed that the derivatization efficiency is affected by the sample matrix in the pre-column derivatization method. To check the effect of the sample matrix, we conducted spike-and-recovery tests on various samples to evaluate their recovery rates. Fig. 18 shows the results. Good recovery rates were obtained for many samples, indicating that the pre-column derivatization method can be applied to various foods.

■ Conclusion

The pre-column derivatization method introduced in Application News 01-00441-EN is a very simple analytical method that enables burdensome pretreatment process to be performed automatically. In this article, we introduced the examples of analyzing various foods using the analytical method. With this method, the amino acid analysis of foods can be conducted quickly and easily.

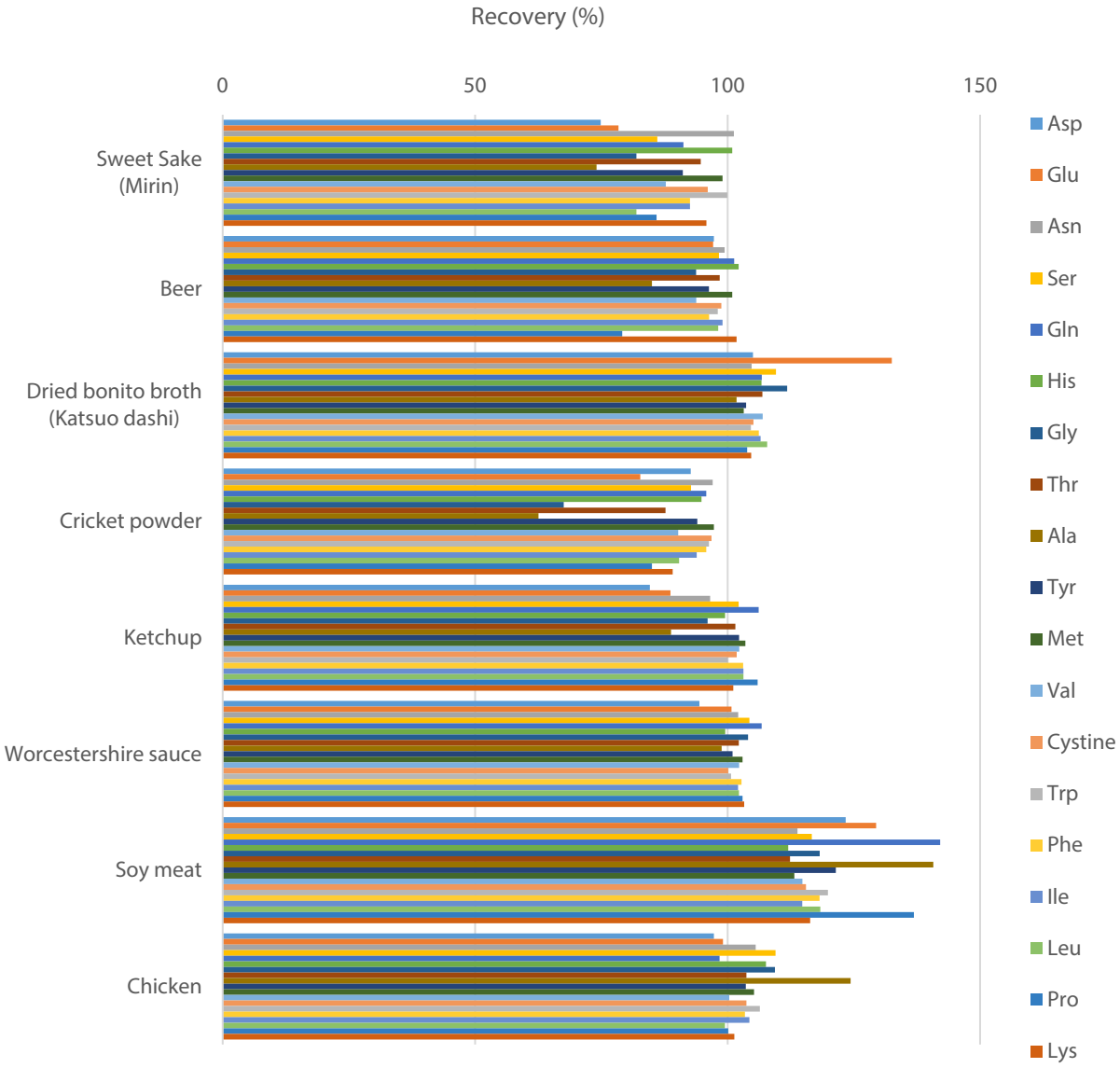


Fig. 18 Results of Spike-and-Recovery Tests

01-00446-EN First Edition: Sep. 2022

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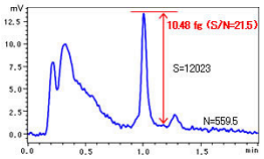

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