

Application Data Sheet

No.35

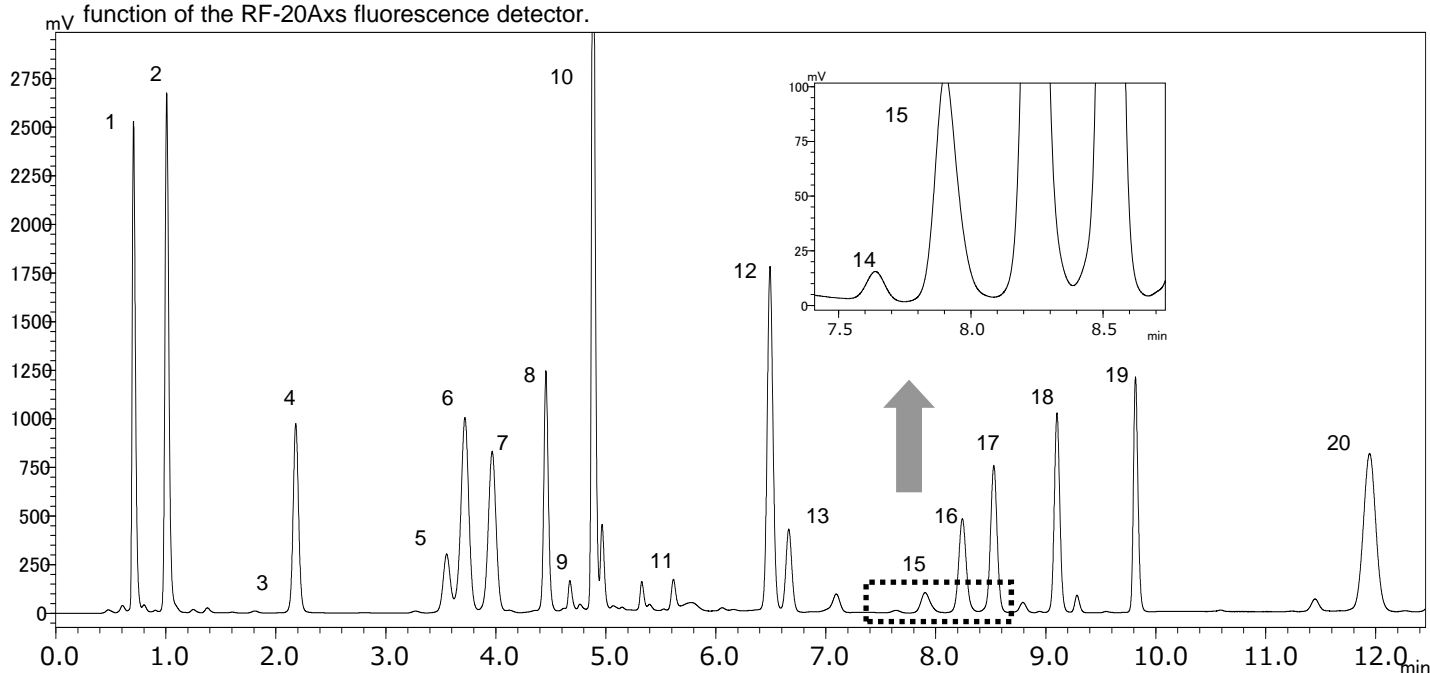
LC
Liquid Chromatograph

High-Speed Analysis of Amino Acids and Histamine in Fish Sauce via Automated OPA Pre-column Derivatization

Processed foods and fish containing a significant amount of histamine have resulted in several cases of allergic food poisoning. In Japan, no standard values have been set for histamine concentrations in foods. However, the FDA has specified levels of 50 mg/kg max. for foods in general, the EU has specified 100 mg/kg max. for marine products, and the Codex Alimentarius standard specifies 400 mg/kg max. for fish sauce.

Simultaneous Analysis of Amino Acids and Histamine in Fish Sauce

Amino acids and histamine were derivatized via o-phthalaldehyde (OPA) and chloroformic acid 9-fluorenylmethyl (FMOC) using the automatic pretreatment function of the Nexera X2 Ultra High Performance Liquid Chromatograph (UHPLC) system's SIL-30AC autosampler. A simultaneous analysis of primary amines (OPA derivatives) and secondary amines (FMOC derivatives), which differ in detection wavelength, was enabled by the wavelength switching function of the RF-20Axs fluorescence detector.



Pretreatment Procedure

1. Mix a 0.5 mol/L aqueous trichloroacetic acid solution with the sample in a 2:1 ratio (v/v ratio), and then centrifuge the mixture (10,000 rpm for 5 minutes).
2. Mix a 0.3 mol/L aqueous sodium hydroxide solution with the supernatant in a 1:3 ratio (v/v ratio).
3. Dilute the mixture 100 fold with a 0.1 mol/L boric acid buffer solution and then filter it through a 0.2 μ m rated membrane filter before proceeding with the analysis.

Column: YMC Triart C18 1.9 μ m
(75 mmL. \times 3.0 mmI.D., 1.9 μ m)
Mobile phase: A) 20 mmol/L (Potassium) Phosphate buffer (pH 6.5)
B) Acetonitrile/Methanol/Water = 45/40/15 (v/v/v)
Gradient Elution
Flow rate: 0.8 mL/min
Column temp.: 35 $^{\circ}$ C
Injection volume: 1 μ L

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

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