



Analysis of Amino Acids in Seasonings by Automated Pre-Column Derivatization HPLC

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Food

Abstract

Using the automated pre-column derivatization method, both primary and secondary amino acids were analyzed simultaneously. Compared with post-column derivatization or manual pre-column derivatization methods, this method has some advantages, such as simple system configuration, low running costs and good stability of quantitative data.

This application brief describes the application of amino acid analysis in Japanese traditional seasonings.

Analyzed Compounds

Normal protein amino acids: • aspartic acid (Asp) • glutamic acid (Glu) • serine (Ser) • histidine (His) • glycine (Gly) • threonine (Thr) • alanine (Ala) • arginine (Arg) • tyrosine (Tyr) • valine (Val) • methionine (Met) • phenylalanine (Phe) • isoleucine (Ile) • leucine (Leu) • lysine (Lys) • proline (Pro).

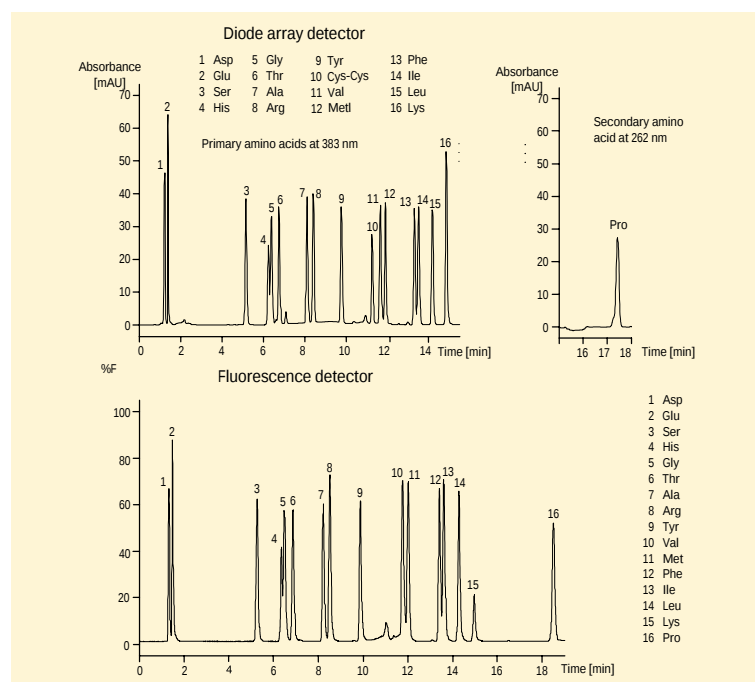


Figure 1
Chromatogram of amino acid stand solution, 250 pmol/μl each

Conditions

Column 250 mm × 2 mm i.d.

Agilent amino acid analysis column

Mobile Phase

A = 20 mM acetate buffer

(pH 7.2)/0.018 % TEA/

0.3 % THF/0.01 mM EDTA

B = 100 mM acetate buffer

(pH 7.2)/CH₃CN/CH₃OH = 10/20/20

Temperature 40 °C

Injection vol 1 μl

Diode array detector

A—338/10 nm, reference 390/20 nm

B—262/16 nm, reference 324/8 nm

Fluorescence detector

primary amino acid, Ex 340 nm, Em 450 nm

secondary amino acid, Ex 262 nm,

Em 305 nm



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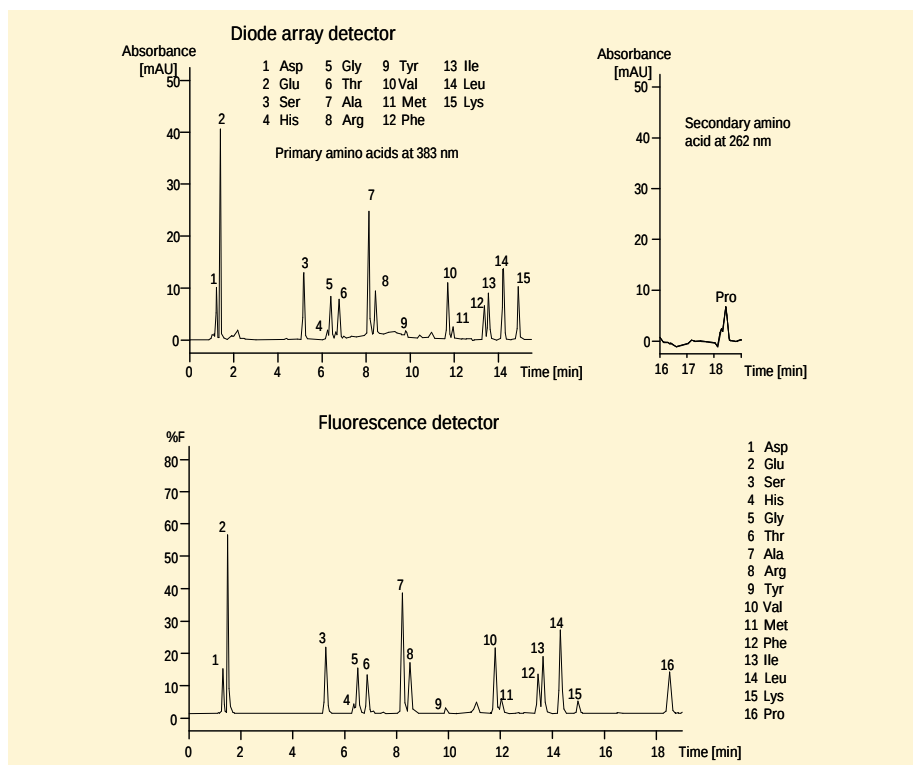


Figure 2
Chromatogram of amino acids in soy sauce

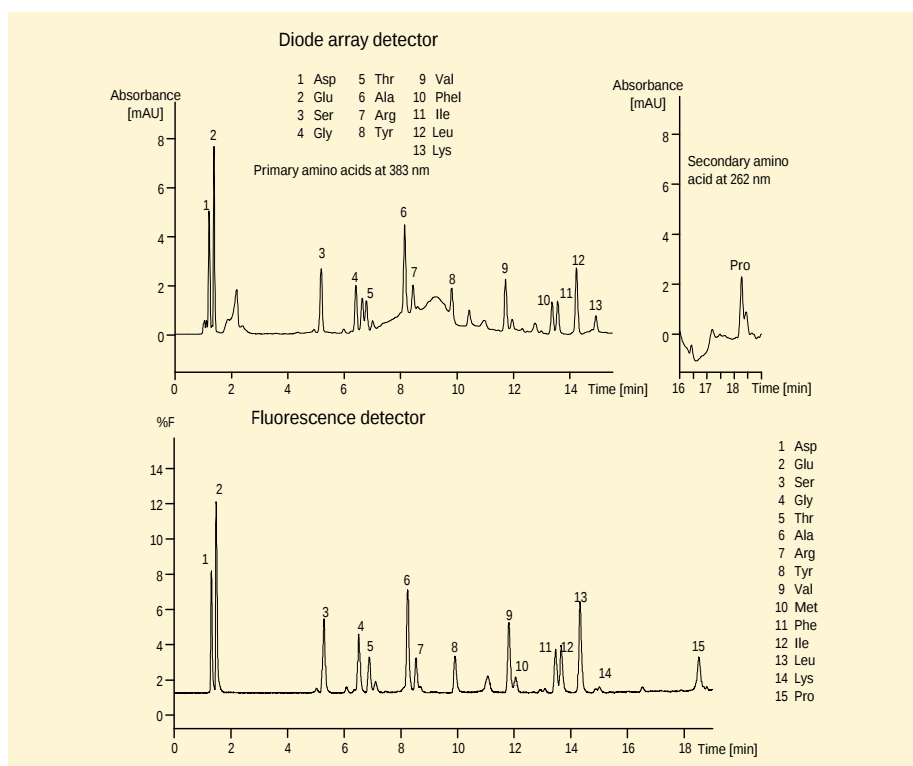


Figure 3
Chromatogram of amino acids in mirin

Sample

Soy sauce and mirin
(Japanese traditional seasonings).

Method performance

Limit of Detection =
< 0.5 pmol/μl with DAD,
except for proline.

Limit of Detection =
< 10 pmol/μl with DAD,
for proline.

Equipment

Agilent 1100 Series

- degasser
 - binary pump
 - autosampler
 - thermostatted column compartment
 - diode array detector
 - fluorescence detector
- Agilent ChemStation + software

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