

## Shimadzu Application Handbook: Analysis of amino acids with YMC-Triart

Shimadzu provides an application handbook for liquid chromatography.

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Within this huge application collection, YMC-Triart C18 is recommended for amino acid analysis:

- 1. Analysis of OPA derivatized amino acids**
- 2. High-speed analysis of amino acids and histamine in fish sauce**
- 3. Analysis of OPA and FMOC derivatized amino acids**

**Use YMC-Triart C18 for the analysis  
of free and derivatized amino acids!**

Amino acids  
YMC-Triart C18

Shimadzu recommends  
YMC columns

Date: 16.05.2017  
Author: Anna Bergmann

## 1 Analysis of pre-column derivatized amino acids

On pages 12-13, a method for the fast analysis of derivatized **amino acids in proteins** with fluorescence detection is described (Application News No. 458). The amino acids were automatically derivatized with *o*-phthalaldehyde (OPA). The UHPLC column used for the separation was **YMC-Triart C18 (TA12SP9-0503PT)**. After method development with a standard mixture of 17 derivatized amino acids, the method was transferred to real samples: Analysis of angiotensin I hydrolysate and bovine serum albumin hydrolysate.

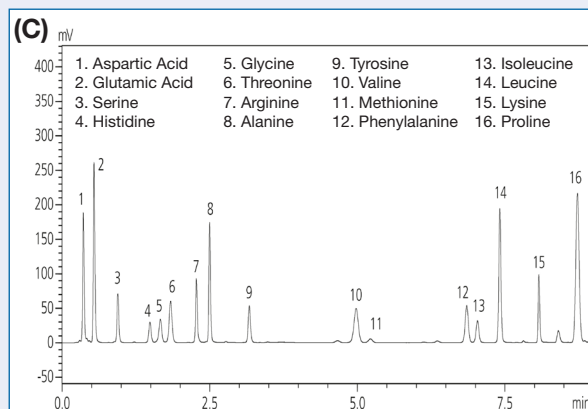
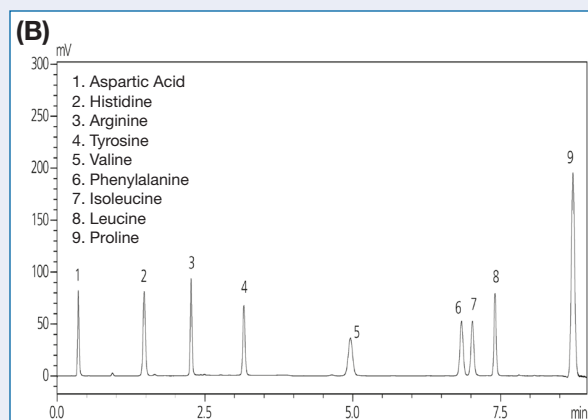
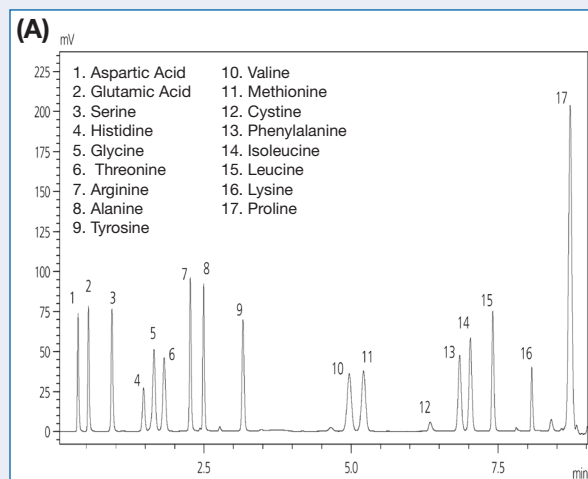


Figure 1: Analysis of 17 derivatized amino acids with YMC-Triart C18: (A) standard mixture, (B) angiotensin I hydrolysate, (C) BSA hydrolysate.

Table 1: Analytical conditions

<b>Column</b>	YMC-Triart C18 1.9 µm particle size, 12 nm pore size 50 × 3.0 mm ID
<b>Part No.</b>	TA12SP9-0503PT
<b>Mobile phase</b>	A: 20 mM potassium phosphate buffer (pH 6.2) B: 60/40 acetonitrile/methanol
<b>Elution</b>	Gradient
<b>Flow rate</b>	1.2 mL/min
<b>Temperature</b>	40°C
<b>Detection</b>	Fluorescence, Ex. at 350 nm / Em. at 450 nm Ex. at 266 nm / Em. at 305 nm (8.5 min)
<b>Injection</b>	1 µL

## 2 High-speed analysis of derivatized amino acids and histamine in fish sauce

A method for the simultaneous analysis of derivatized **amino acids and histamine in fish sauce** is described on page 55 (Application Data Sheet 35). Foods which contain significant amounts of histamine can cause allergic food poisoning.

The amino acids and histamine are derivatized with OPA and 9-fluorenylmethyl chloroformate (FMOC). The used UHPLC column for amino acid separation was **YMC-Triart C18 (TA12SP9-L503PT)**.

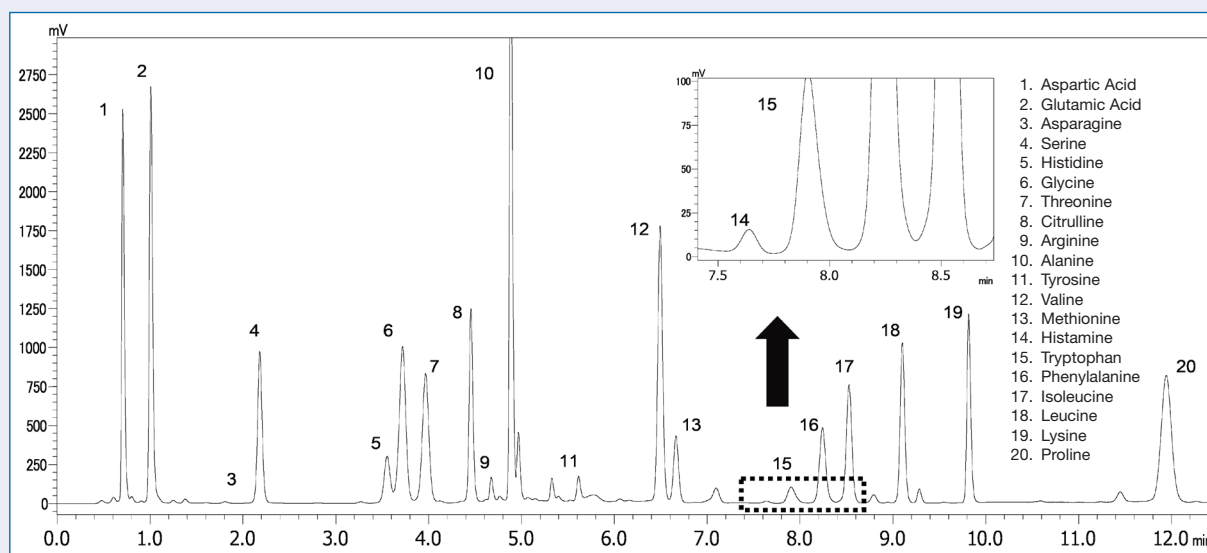


Figure 2: Simultaneous analysis of derivatized amino acids and histamine in fish sauce.

Table 2: Analytical conditions

<b>Column</b>	YMC-Triart C18, 1.9 µm particle size, 12 nm pore size, 75 × 3.0 mm ID
<b>Part No.</b>	TA12SP9-L503PT
<b>Mobile phase</b>	A: 20 mM potassium phosphate buffer (pH 6.5) B: 45/40/15 acetonitrile/methanol/water
<b>Elution</b>	Gradient
<b>Flow rate</b>	0.8 mL/min
<b>Temperature</b>	35°C
<b>Detection</b>	Fluorescence
<b>Injection</b>	1 µL

Amino acids  
YMC-Triart C18

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YMC columns

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### 3 Analysis of OPA and FMOC derivatized amino acids

The third application note (Application News No.L432) is shown on pages 58-59. It describes a method for the simultaneous determination of 22 amino acids derivatized with OPA and FMOC. The amino acid derivatives were separated with an **YMC-Triart C18 UHPLC column (TA12SP9-L503PT)**. The two derivates (OPA-AA and FMOC-AA) are detectable at different wavelengths. As a result of the automatic wavelength switching feature of the detector, simultaneous determination was possible. After method development with a standard mixture of 22 amino acids, a **commercially available soft drink** was analysed.

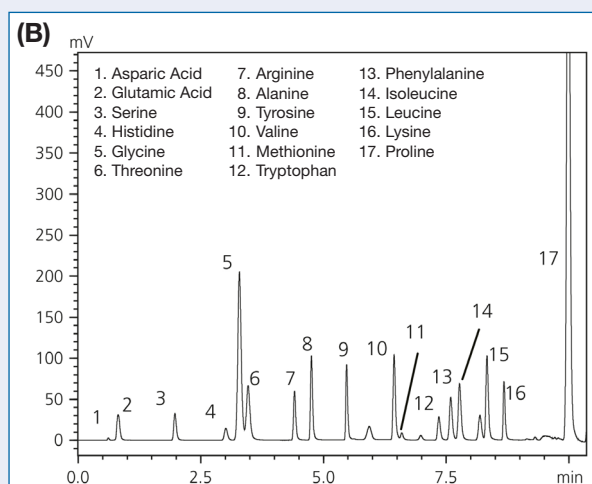
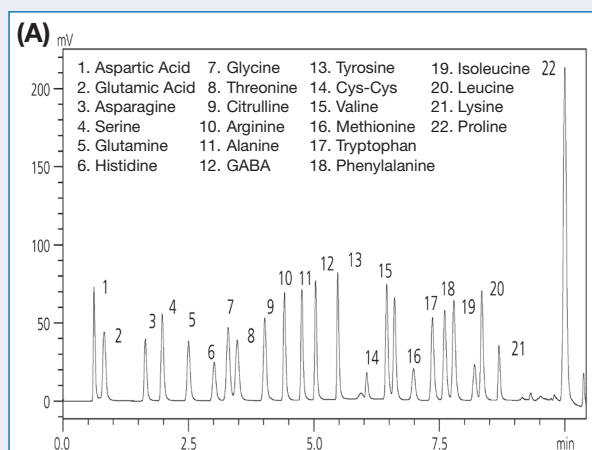


Table 3: Analytical conditions

<b>Column</b>	YMC-Triart C18 1.9 µm particle size, 12 nm pore size 75 × 3.0 mm ID
<b>Part No.</b>	TA12SP9-L503PT
<b>Mobile phase</b>	A: 20 mM potassium phosphate buffer (pH 6.9) B: 45/40/15 acetonitrile/methanol/water
<b>Gradient Elution</b>	11% B → 13% (0-3 min) → 31% (5 min) → 37% (7.5 min) → 70% (10 min) → 100% (10.5 → 13.5 min) → 11% (14 min)
<b>Flow rate</b>	0.8 mL/min
<b>Temperature</b>	35°C
<b>Detection</b>	Fluorescence, Ex. at 350 nm / Em. at 450 nm Ex. at 266 nm / Em. at 305 nm (9 min)
<b>Injection</b>	1 µL

Figure 3: Analysis of OPA and FMOC derivatized amino acids  
(A) standard mixture, (B) commercially available soft drink.