

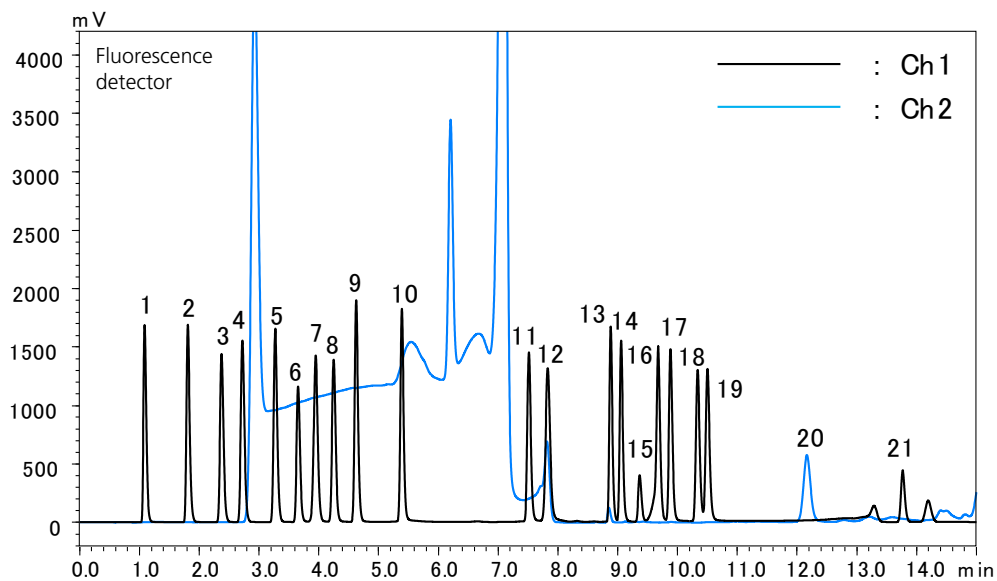
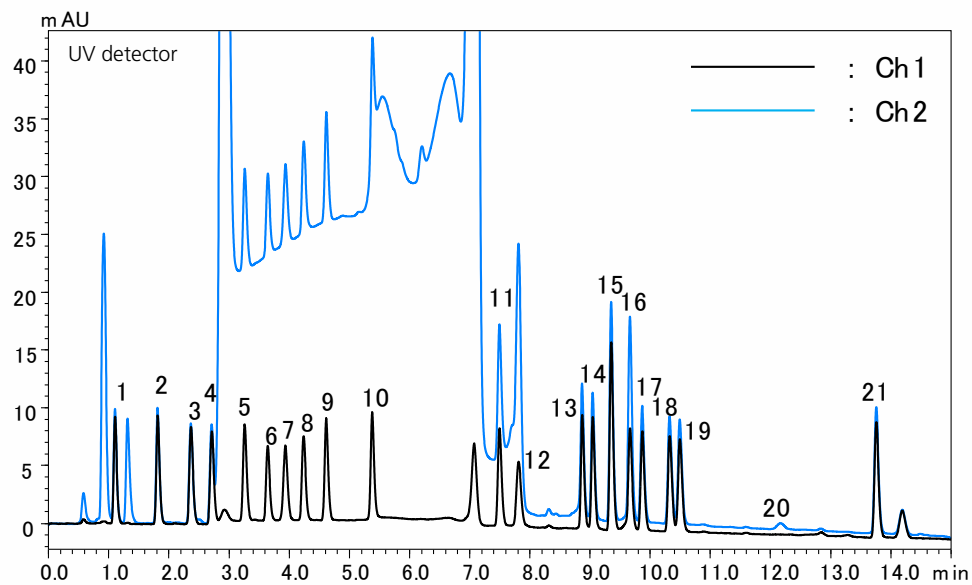
**CoreFocus**  
**Report**  
**No.279**

LC    Reversed-phase    Shim-pack Series

**Shim-pack™ XR-ODS II**

**Analysis of Amino acids by pre-column derivatization methods**

**Keywords: amino acid, gamma amino butyric acid**



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. GABA, 13. Methionine, 14. Valine, 15. Cystine, 16. Tryptophan, 17. Phenylalanine, 18. Isoleucine, 19. Leucine, 20. Proline, 21. Lysine, (Upper; UV detector : 125 µmol/L each), (Lower; Fluorescence detector : 25 µmol/L each)

Table.1 Derivatization reagent and mobile phase

<ul style="list-style-type: none"> <li>• Mercaptopropionic Acid Reagent Add 10 µL of 3-mercaptopropionic acid into 10 mL of 0.1 mol/L borate buffer.</li> <li>• 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 pure water.</li> <li>• Mercaptopropionic Acid / OPA solution Mix 300 µL of Mercaptopropionic Acid Reagent and 600 µL of OPA Reagent.</li> <li>• FMOC Reagent Dissolve 10 mg of 9-fluorenylmethyl chloroformate into 50 mL of acetonitrile.</li> <li>• Mobile Phase A: 20 mmol/L Sodium acetate buffer (pH6) Add 2.67 g of sodium acetate trihydrate and 41 µL of acetic acid into 1000 mL of pure water.</li> <li>• Mobile Phase B: Water/Acetonitrile = 100/900</li> <li>• Mobile Phase C: 20 mmol/L Sodium acetate buffer (pH5) containing 0.5 mmol/L EDTA-2Na 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.</li> <li>• Phosphoric Acid Aqueous Solution Add 0.5 mL of phosphoric acid into 100 mL of pure water.</li> </ul>
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Table.2 Analytical conditions

System	: Prominence <sup>TM</sup> -i
Column	: Shim-pack XR-ODS II (100 mm×3.0 mm I.D., 2.2 µm), P/N : 228-41624-92
Vial	: Shimadzu Vials, LC/LCMS, Glass, P/N : 227-34001-01
Mobile phase	: See the table1
Flow rate	: 1.0 mL/min
Column temp.	: 40 °C
Injection volume	: 1 µL
Detection	: i. UV (Built-in), Ch1) 350 nm Ch2) 266 nm ii. Fluorescence detector (RF-20Axs) Ch1) Ex. 350 nm, Em. 450 nm Ch2) Ex. 266 nm, Em. 305 nm

Table.3 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

Source : Application News L579 ([JP](#))

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