

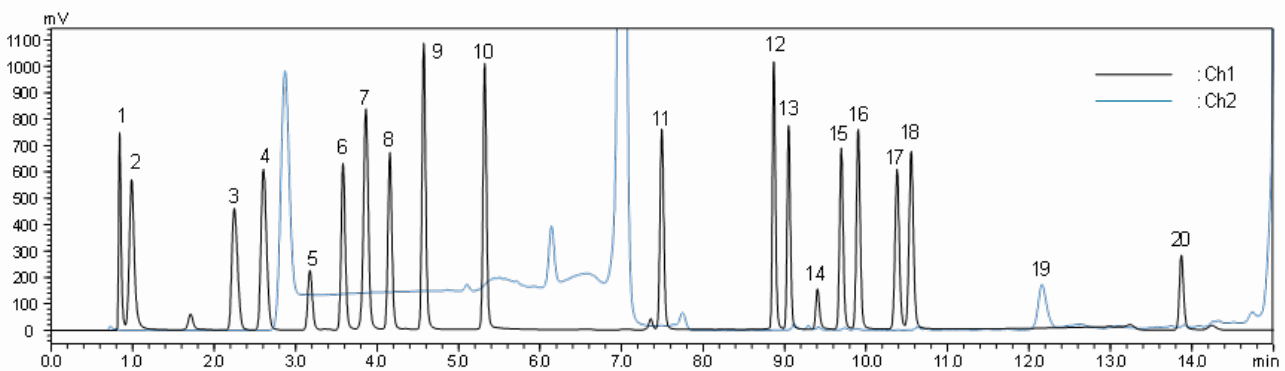
CoreFocus
Report
No.127

LC Reversed-phase Shim-pack Series

Shim-pack™ XR-ODSII

Analysis of Amino Acids Using Automatic Pretreatment Function of LC System

Keywords: primary amino acids, secondary amino acids, fluorometric derivatization



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

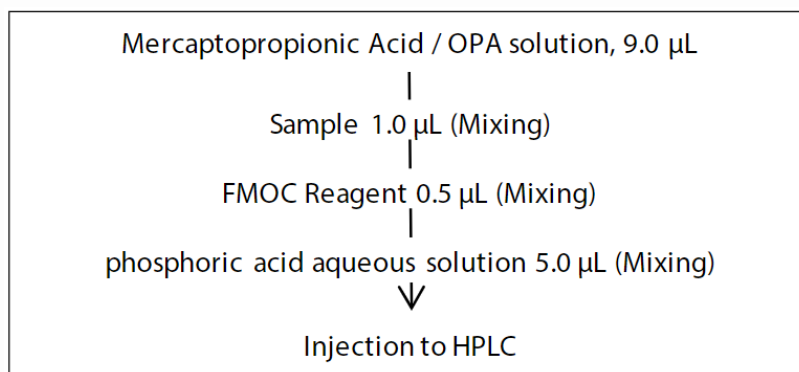
Simultaneous Analysis of 20 Proteinogenic Amino Acids

Analytical Conditions

System	: Prominence™-i (LC-2030C)
Column	: Shim-pack XR-ODSII (100 mm L. × 3.0 mm I.D., 2.2 μm), P/N : 228-41624-92
Gradient mode	: Low pressure gradient
Mobile phase	: A) 20 mmol/L Sodium acetate buffer (pH6) B) Water/Acetonitrile = 100/900 C) 20 mmol/mL Sodium acetate buffer (pH5) containing 0.5 mmol/L EDTA-2Na
Flow rate	: 1.0 mL/min
Column temp.	: 40 °C
Injection volume	: 1 μL
Detection	: Fluorescence detector Ch1) Ex. 350 nm, Em. 450 nm Ch2) Ex. 266 nm, Em. 305 nm

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



Automated Pre-Column Derivatization

Derivatization Reagents

- Mercaptopropionic Acid 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.
- Mercaptopropionic Acid / OPA solution
Mix equal volume of Mercaptopropionic Acid Reagent and OPA Reagent.
- FMOc Reagent
Dissolve 10 mg of 9 – fluorenylmethyl chloroformate into 50 mL of acetonitrile.

Source : Application News L529([JP](#), [ENG](#))

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