Application Note

430012H

Analysis of Amino Acids by Automated Pre-column Derivatization with OPA

Introduction

Pre-column derivatization methods for amino acid analysis has widely been used in reversed-phase HPLC, as they offer high selectivity and sensitivity for multiple components. Many derivatization reagents are commercially available and one can choose the most suitable reagent for his/her application. Among them, orthophtalaldehyde (OPA) is one of the most commonly used reagents because it reacts with amino acids very quickly (seconds) at room temperature and derivatized amino acids can be detected by a fluorescence detector with increased sensitivity. In this application, the OPA pre-column derivatization is demonstrated by using the automated pre-column derivatization function of JASCO's autosampler for much better reproducibility than manual sampling.

Keyword : HPLC, OPA pre-column derivatization, amino acid, C18 column, fluorescence detector

Experimental

Equipment.		Conditions.	
Pump:	PU-2080	Column:	CrestPak C18S (4.6 mmID x 150 mmL, 5 µm)
Degasser:	DG-2080-54	Eluent A:	Sodium acetate buffer (Ph 6.0)/Methanol/THF (89/10/1)
Gradient Unit	LG-2080-04	Eluent B:	Methanol/THF (90/10)
Column oven:	CO-2060	Gradient condition:	(A/B), 0 min(85/15) → 7 min(80/20) → 19 min(56/44) →
Autosampler:	AS-2057		23 min(48/52) → 29 min(48/52) → 30 min(0/100) →
Detector:	FP-2020		35 min(0/100) → 35.1 ~ 60 min(85/15) 1 cycle; 60 min
		Flow rate:	1.0 mL/min
		Column temp.:	20ºC
		Wavelength:	Ex. 345 nm, Em. 455 nm, Gain x100
		Injection volume:	10 µL
		Standard sample:	18 amino acids 1 nmol/mL each in 0.01 N hydrochloric acid

Results

The 18 standard amino acids were completely separated within 35 minutes as shown in Fig. 1.

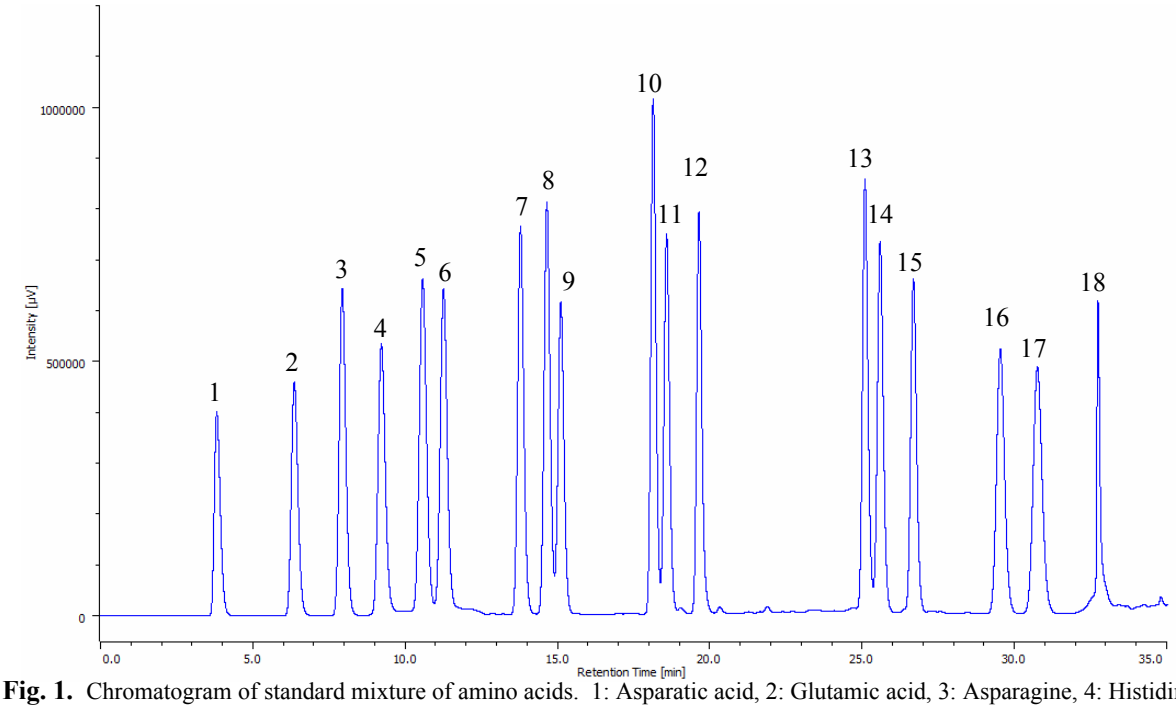

Fig. 1. Chromatogram of standard mixture of amino acids. 1: Asparatic acid, 2: Glutamic acid, 3: Asparagine, 4: Histidine, 5: Serine, 6: Glutamine, 7: Arginine, 8: Glysine, 9: Threonine, 10: Taurine, 11: Alanine, 12: Tyrosine, 13: Methionine, 14: Valine, 15: Phenylalanine, 16: Isoleucine, 17: Leucine, 18: Lysine

JASCO INTERNATIONAL CO., LTD

4-21, Sennin-cho 2-chome, Hachioji, Tokyo 193-0835, Japan
Tel: +81-42-666-1322, Fax: +81-42-665-6512

Pre-column Derivatization Function of Autosamper

The operating principle of the pre-column derivatization function of model AS-2057 autosampler is as shown in Fig. 2. By using this function, the pre-column derivatization can be performed automatically.

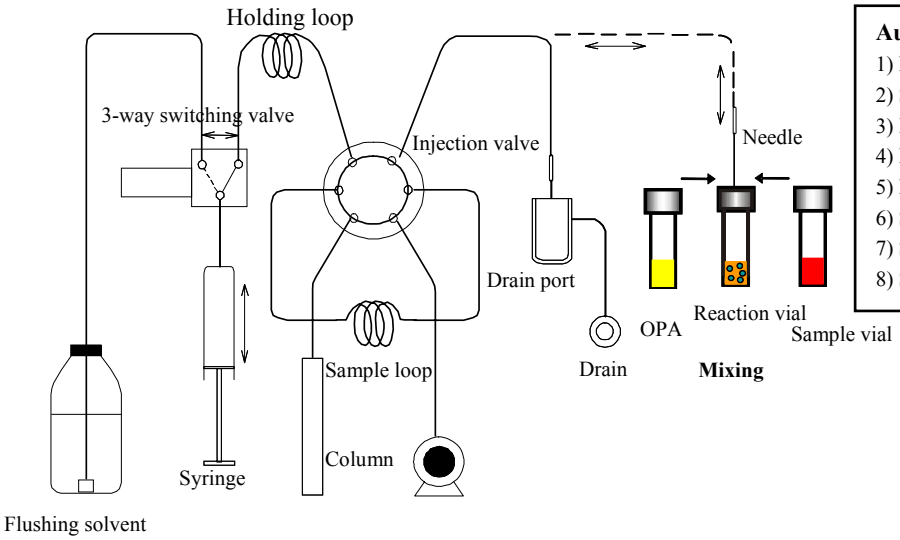

Fig. 2. Pre-column derivatization function of model AS-2057 Autosampler.

Conditions for Pre-column Derivatization

Volume of sample solution:	50 µL
Volume of reagent solution:	10 µL
Reaction time:	0.1 min
Reagent solution:	0.4 M borate/1% OPA solution/2-mercaptoethanol(1/0.5/0.01)

JASCO INTERNATIONAL CO., LTD
4-21, Sennin-cho 2-chome, Hachioji, Tokyo 193-0835, Japan
Tel: +81-42-666-1322, Fax: +81-42-665-6512