Application Note





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

Introduction

Pre-column derivatization methods for amino acid analysis have been widely 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 the 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 offering 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.



JASCO Models AS-2055/2057

Keywords: HPLC, OPA pre-column derivativation, aminoacid, C18 column, fluorescence detector



Experimental

<u>Equipment</u>	
Eluent Pump:	PU-2080
Degasser:	DG-2080-54
Gradient Unit:	LG-2080-04
Column Oven:	CO-2060
Autosampler:	AS-2057
Detector:	FP-2020

<u>Conditions</u>	
Column:	CrestPak C18S (4.6mmlDx 150mmL,5µm)
Eluent A:	Sodium acetate buffer (Ph 6.0)/Methanol/THF(89/10/1)
Eluent B:	Methanol/THF(90/10)
Gradient condition:	(A/B), Omin (85/15), 7 min (80/20) 19min(56/44), 23 min (48/52) 29min(48/52) 30 min (0/100) 35 min (0/100) 35.1~ 60min (85/15 1 cycle; 60min
Flow rate:	1.0mL/min
Column Temperature:	20°C
Wavelength:	Ex. 345 nm,Em.455 nm,Gain x100
Injection Volume:	10 µL

18 amino acids 1 nmol/mL each in

0.01N hydrochloricacid

Results



Standard

Sample:

Figure1. Chromatogram of a standard mixture of amino acids.
1: Asparaticacid, 2:Glutamicacid, 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

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Pre-column Derivatization Function of the Autosamper

The operating principle of the pre-column derivatization function of model AS-2057 autosampler is as shown in figure2. By using this function, the pre-column derivatization can be performed automatically.



Figure 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:	0.1min
Reagent solution:	0.4Mborate/ 1% OPA solution/ 2-mercaptoethanol (1/0.5/0.01)



