

Analysis of Dabsyl Amino Acids using HPLC

Introduction

The pre-column derivatization using HPLC for amino acids analysis has prevailed as a simultaneous separation method of multiple components using a selective, high-sensitivity detector and a reversed phase column.

Many results of amino acids analysis using dabsyl chloride (4-(Dimethylamino)azobenzene-4'-sulfonyl chloride:DABS-Cl) as a reagent for pre-column derivatization have been reported because the amino acids derivatized by dabsyl chloride are more stable than those by other derivatization agents. Also a simply a UV/VIS detector can be used with high sensitivity. Here the standard mixture of amino acids was measured by the system using dabsyl chloride

Keyword: HPLC, Amino acids, DABS-Cl, DAB Label, 5.0 μm , Dabsylpak II, UV/VIS detector



Jasco PU-2080, DG-2080-53, LG-2080-02, AS-2055, CO-2060, UV-2070

Experimental Equipment:

Pump:	PU-2080
Degasser:	DG-2080-53
Mixer:	LG-2080-02
Column oven:	AS-2055
Autosampler:	CO-2060
Detector:	UV-2070

Conditions:

Column:	Dabsylpak II (4.6 mmID x 150 mmL, 5 μ m)
Pre-column:	Dabsylpak II-P (4.6 mmID x 35 mmL, 5 μ m)
Eluent A:	20 mM Sodium acetate (pH6.0)
Eluent B:	Acetonitrile
Gradient condition:	(A/B), 0 min (78/22, 3 min (78/22), 25 min (70/30), 40 min (40/60), 40.1 min (20/80), 45 min (20/80), 45.1 min (78/22), 1 cycle: 55 min
Flow rate:	1.0 mL/min
Column temp.:	45 $^{\circ}$ C
Wavelength:	465 nm
Injection volume:	20 μ L
Standard sample:	Amino acids mixture 40 pmol each, Type H

Results

Figure 1 shows the chromatogram of standard mixture of amino acids. 17 kinds of amino acids were separated in 45 minutes.

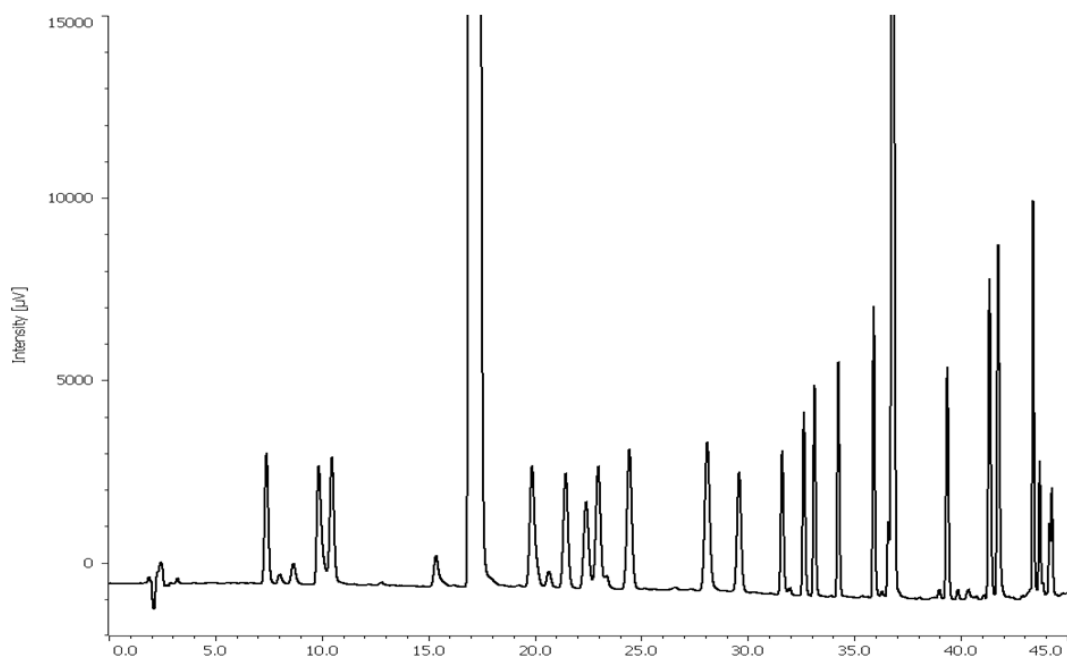


Figure 1. Chromatogram of standard mixture of amino acids. Aspartic acid, Glutamic acid, Serine, Threonine, Arginine, Glycine, Alanine, Proline, Valine, Methionine, Isoleucine, Leucine, Phenylalanine, Cystine, Lysine, Histidine, Tyrosine

Advantages of Dabsylation

- By using the kit (DAB Label), the procedure of intricate preparation of derivatization can be avoided.
- Amino acid composition of 0.5 µg of proteins or peptides can analyzed with good accuracy and reproducibility.
- 17 kinds of the derivatized amino acids can be separated within 45 minutes.

DABS-Cl reacts with α-amino groups, ε-amino groups, phenolic hydroxyl groups and imidazole groups. Figure 2 shows a reaction formula with α-amino group.

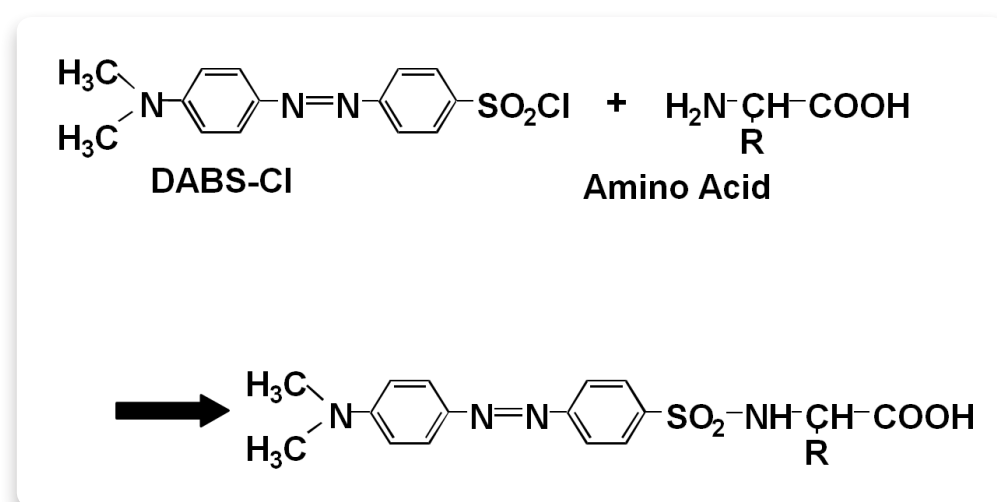


Figure 2. Reaction formula with α-amino group.