# Application Note

430022H

## Analysis of Dabsyl Amino Acids by High-performance Liquid Chromatography

### Introduction

The postcolumn derivatization with HPLC for amino acids analysis has prevailed as a simultaneous separation method of multicomponents by 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 precolumn derivatization have been reported because the amino acids derivatized by Dabsyl chloride are comparatively more stable than by other derivatization agents and for detection, 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

### Experimental

| Equipment | | Conditions | |
|---|---|---|---|
| Pump: | PU-2080 | Column: | Dabsylpak II (4.6 mmID x 150 mmL, 5 µm) |
| Degasser: | DG-2080-53 | Pre-column: | Dabsylpak II-P (4.6 mmID x 35 mmL, 5 µm) |
| Gradient unit: | LG-2080-02 | Eluent A: | 20 mM Sodium acetate (pH6.0) |
| Autosampler: | AS-2055 | Eluent B: | Acetonitrile |
| Column oven: | CO-2060 | Gradient condition: | (A/B), 0 min (78/22) → 3 min (78/22) → |
| Detector: | UV-2070 | | 25 min (70/30) → 40 min (40/60) → |
| | | | 40.1 min (20/80) → 45 min (20/80) → |
| | | | 45.1 min (78/22) 1cycle: 55 min |
| | | Flow rate: | 1.0 mL/min |
| | | Column temp.: | 45 ºC |
| | | Wavelength: | 465 nm |
| | | Injection volume: | 20 µL |
| | | Standard sample: | Amino acids mixture 40 pmol each, Type H |

### Result

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

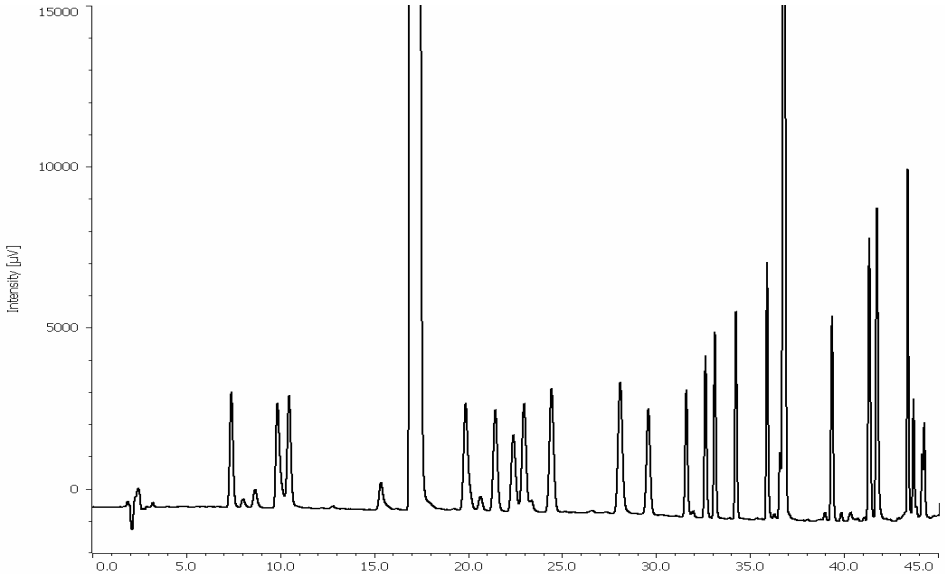


**Fig. 1.** Chromatogram of standard mixture of amino acids
1: Aspartic acid, 2: Glutamic acid, 3: Serine, 4: Threonine, 5: Arginine, 6: Glycine, 7: Alanine 8: Proline, 9: Valine, 10: Methionine, 11: Isoleucine, 12: Leucine, 13: Phenylalanine, 14: Cystine 15: Lysine, 16: Histidine, 17: Tyrosine



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**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.
- The derivatized amino acids are very stable. (for one month at room temperature)
- The derivatized amino acids can be detected by visible light at 465 nm, which allows high-sensitivity analysis without the interference from other components having UV absorption.

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

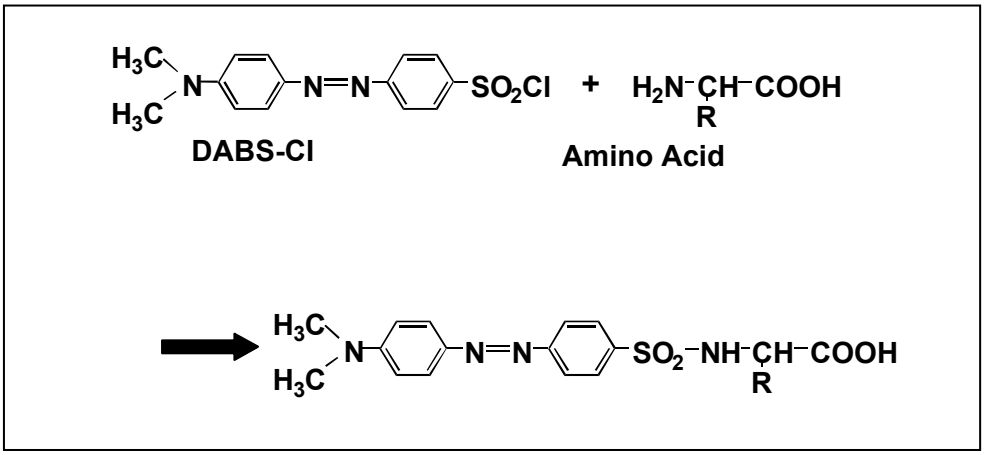


**Fig. 2.** Reaction formula with α-amino group



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