# Application Note

Date: No. 430014H-E

# Amino acids using precolumn derivatization with DABS-Cl

A small amount of amino acids of protein and peptide can be quantitatively analyzed by vapor-phase hydrolyzing with hydrochloric acid, derivatizing with DABS-Cl (Dimethylamino azobenzene-sulfonyl chloride) and separated on reversed phase HPLC. DAB-Label is a kit to facilitate the troublesome work for derivatization. The obtained DAB-Labeled amino acids provide good stability and can be stored for quite a while.

Fig.1 shows the outline of the amino acid analysis by using DAB-Label, Fig. 2 shows the reaction formula of DABS-Cl and amino acid and Fig.3 shows the chromatogram of the derivatized representative amino acid samples.

*Keywords: 1.Amino acid, 2.STD mixture, 3.ODS, 4.Vis, 5.DABS-Cl*

**Conditions:**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Column: | Dabsylpak | | | | | | | | |
| Precolumn: | Dabsylpak-P | | | | | | | | |
| Eluent A: | 50mM NaH2PO4(pH6.6)/DMF(96/4) | | | | | | | | |
| Eluent B: | CH3CN | | | | | | | | |
| Time(min) | 0.0 | 9.0 | 14.0 | 16.0 | 18.0 | 22.0 | 23.0 | 24.0 | 25.0 |
| A(%) | 85 | 70 | 59 | 49 | 46 | 36 | 10 | 10 | 85 |
| B(%) | 15 | 30 | 41 | 51 | 54 | 64 | 90 | 90 | 15 |
| 1Cycle 40.0min | | | | | | | | | |
| Wave length: | 465nm | | | | | | | | |
| Flow rate | 1.0ml/min | | | | | | | | |
| Column Temp: | 40 degree celsius | | | | | | | | |
| Sample: | Amino Acid Std. Soln. Type H (Wako Pure Chem) 50pmol/ul Histamine 500pmol/ul | | | | | | | | |
| Injection volume: | 20ul | | | | | | | | |

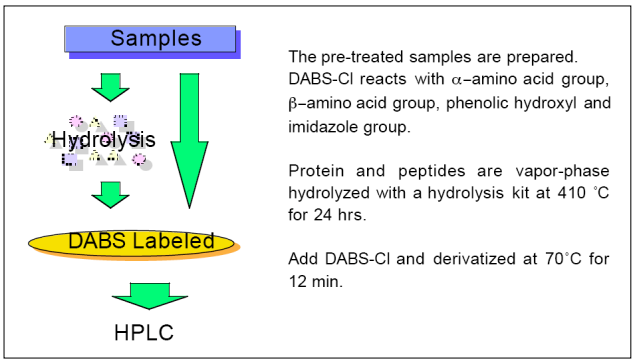


Fig.1 Outline of amino acid analysis by using DAB-Label

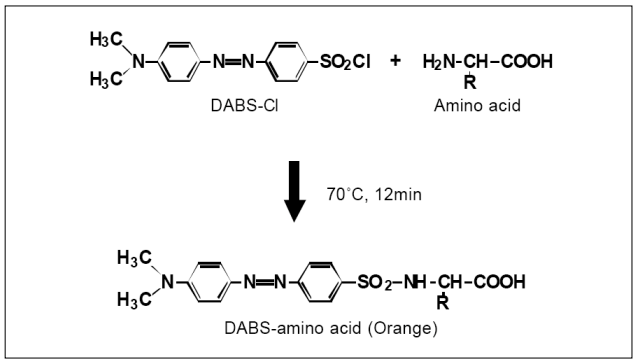


Fig. 2 Reaction formula of DABS-Cl and amino acids

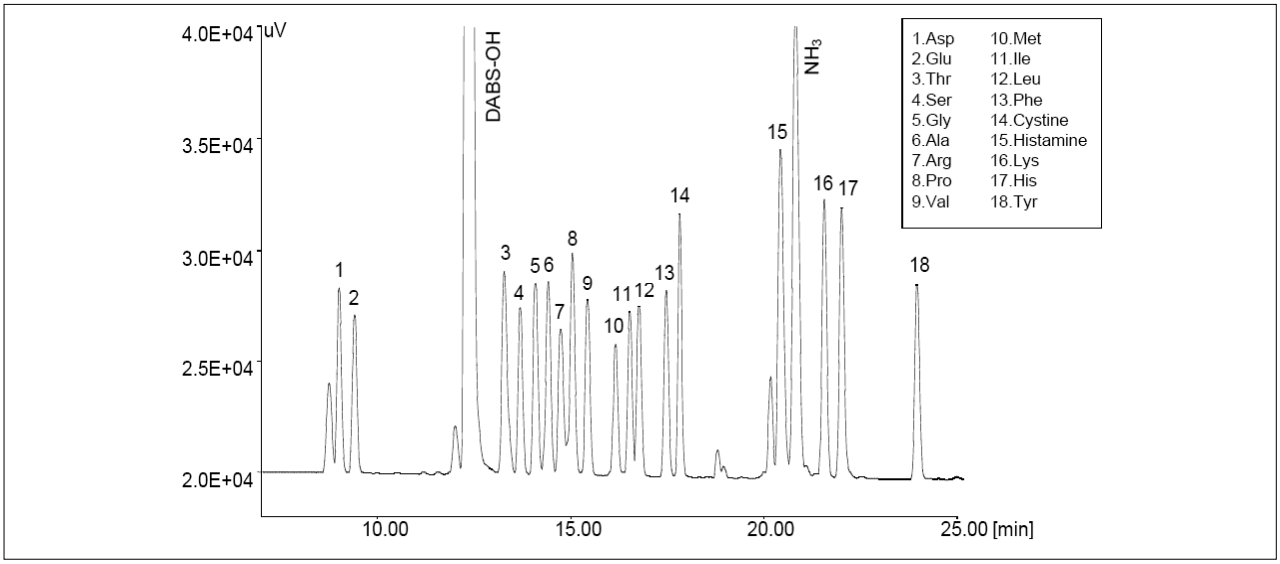


Fig.3 Chromatogram of the derivatized representativ amino acid samples

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