

High Speed Analysis of Dabsyl Amino Acids by Ultra High-performance Liquid Chromatography

Introduction

There are several methods for Amino Acids Analysis, such as separation by reversed-phase column after precolumn derivatization and detection using UV/Vis detector, or post-column derivatization after separation by ion-exchange column and detection using fluorescence detector. Among them, precolumn derivatization method using Dabsyl chloride as reagent is well known, because Dabsyl chloride reacts easily with both of primary and secondary amino acids, and derivatized amino acids are stable, assuring easy handling, good accuracy and high sensitivity. In order to enable easier derivatization method, DAB-Label kit is available from JASCO.

Here, the amino acids in Stout beer were measured using DAB-Label derivatization kit by Ultra High-performance Liquid Chromatography (UHPLC) with UV-Vis detector.

Keywords: UHPLC, Amino acids, Dabsyl chloride, Precolumn derivatization, 1.8 μ m C18 column, UV/Vis detector

Experimental

Equipment

Pump: X-LC 3185PU x 2
 Degasser: X-LC 3080DG
 Mixer: X-LC 3180MX
 Column oven: X-LC 3067CO
 Autosampler: X-LC 3159AS
 Detector: X-LC 3070UV

Conditions

Column: ZORBAX Eclipse Plus C18 (3.0 mmID x 50 mmL, 1.8 μ m) with inline filter
 Eluent A: 20 mM Sodium acetate buffer (pH 6.0)/Acetonitrile (85/15)
 Eluent B: Acetonitrile
 Gradient condition: (A/B), 0 min(90/10) \rightarrow 5 min(70/30) \rightarrow 7.2 min(40/60) \rightarrow 7.25 min(10/90) \rightarrow 7.7 min(10/90) \rightarrow 7.75 min(90/10) \rightarrow 1 cycle; 10 min
 Flow rate: 0.8 mL/min
 Column temp.: 25°C
 Wavelength: 468 nm (Cell path length: 10 mm)
 Injection volume: 1 μ L
 Standard sample: 21 dabsyl amino acids 2.0 nmol/mL each

Fig. 1 shows the dabsylation procedure and in Fig. 2, reaction mechanism of dabsylation is illustrated.

- (1) Dilute the sample by dabsylation buffer*
 - (2) Weigh it by 20 μ L.
 - (3) Add 40 μ L of dabsylation reagent and agitate.
 - (4) Warm at 70°C for 12 min. (During warming agitate several times.)
 - (5) After cooling, add 440 μ L of dilution buffer and agitate.
- * Included in DAB-Label kit

Fig. 1. Procedure of dabsylation

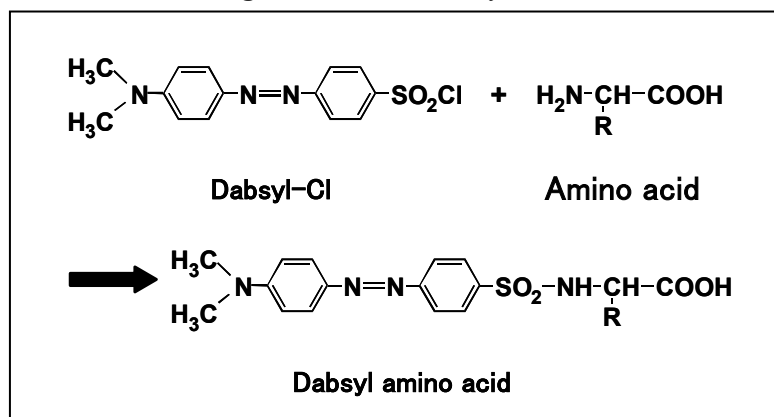


Fig. 2. Reaction mechanism of dabsylation

Result

Fig. 3 shows the chromatogram of 21 components of dabsylation amino acid standard mixture. 21 components including gamma-aminobutyric acid(GABA) - suppressive neurotransmitter of central nerve, taurine, ornithine, etc. were separated only within 7.5 minutes.

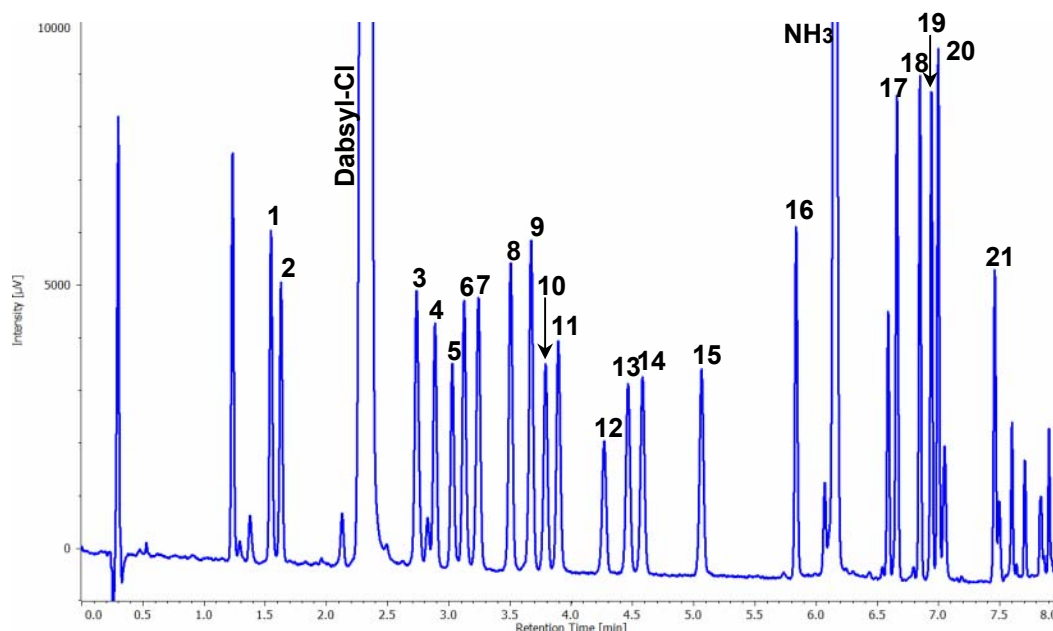


Fig. 3. Chromatogram of 21 components of dabsylated amino acid standard mixture

1: Aspartic acid, 2: Glutamic acid, 3: Serine, 4: Threonine, 5: Arginine, 6: Glycine, 7: Alanine, 8: Proline, 9: Taurine, 10: Valine, 11: GABA (γ -aminobutyric acid), 12: Methionine, 13: Isoleucine, 14: Leucine, 15: Phenylalanine, 16: Cystine, 17: Hydroxylysine, 18: Ornithine, 19: Lysine, 20: Histidine, 21: Tyrosine

Fig. 4 shows the chromatogram of dabsylated amino acids in Stout beer

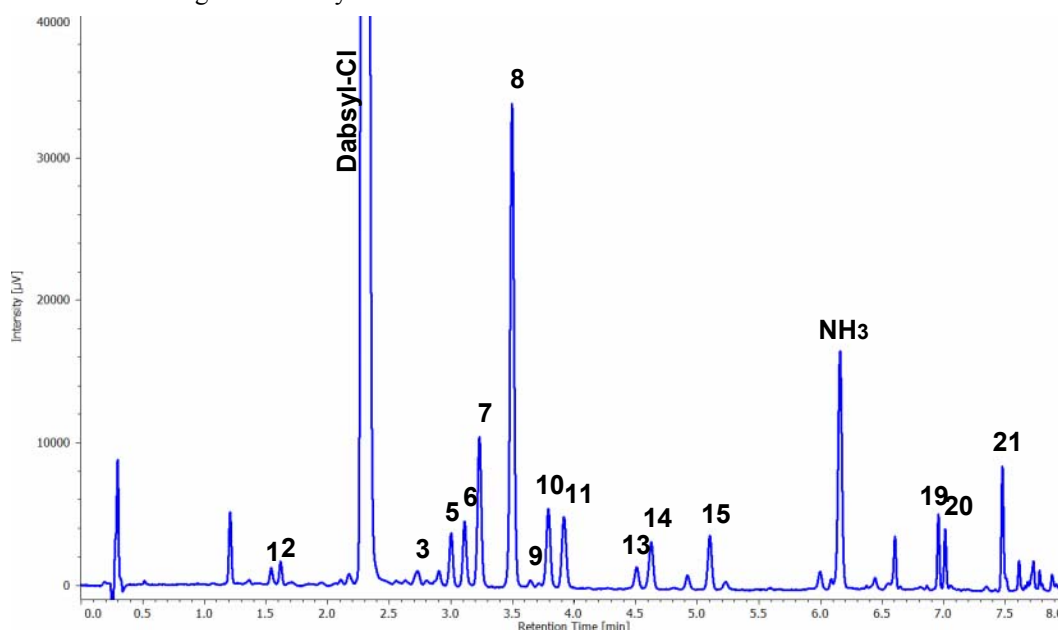


Fig. 4. Chromatogram of dabsylated amino acids in Stout beer

The peak numbers are the same as in Fig. 3. Preparation: Stout beer was diluted by 50-fold using dabsylation buffer and dabsylated according to procedure shown in Fig. 1.

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