Application Note

430023X

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. Precolulmn derivatization, 1.8 μm C18 column, UV/Vis detector

Experimental

Equipment		Conditions	
Pump:	X-LC 3185PU x 2	Column:	ZORBAX Eclipse Plus C18 (3.0 mmID x 50 mmL, 1.8 μm) with inline filter
Degasser:	X-LC 3080DG		
Mixer:	X-LC 3180MX	Eluent A:	20 mM Sodium acetate buffer (pH 6.0)/Acetonitrile (85/15)
Column oven:	X-LC 3067CO	Eluent B:	Acetonitrile
Autosampler:	X-LC 3159AS	Gradient condition:	(A/B), 0 min(90/10)→5 min(70/30)→7.2 min(40/60)→ 7.25 min(10/90)→7.7 min(10/90)→7.75min(90/10)→ 1 cycle; 10 min
Detector:	X-LC 3070UV	Flow rate:	0.8 mL/min
		Column temp.:	25ºC
		Wavelength:	468 nm (Cell path length: 10 mm)
		Injection volume:	1 μL
		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 μL.
(3) Add 40 μL of dabsylation reagent and agitate.
(4) Warm at 70ºC for 12 min.(During warming agitate several times.)
(5) After cooling, add 440 μL of dilution buffer and agitate.

* Included in DAB-Label kit

Fig. 1. Procedure of dabsylation

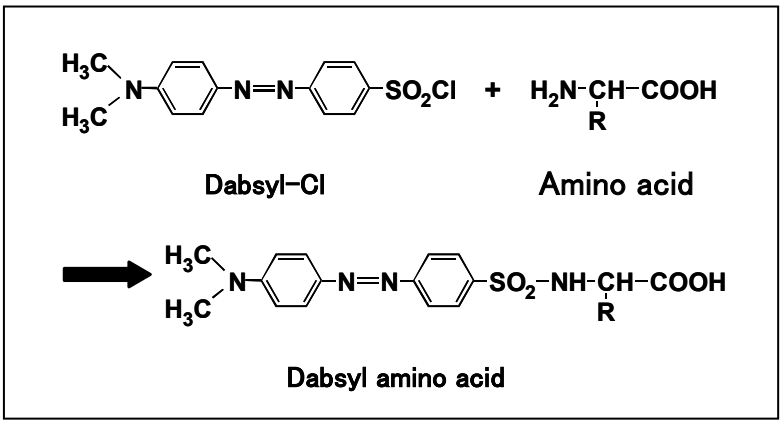

Fig. 2. Reaction mechanism of dabsylation

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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.

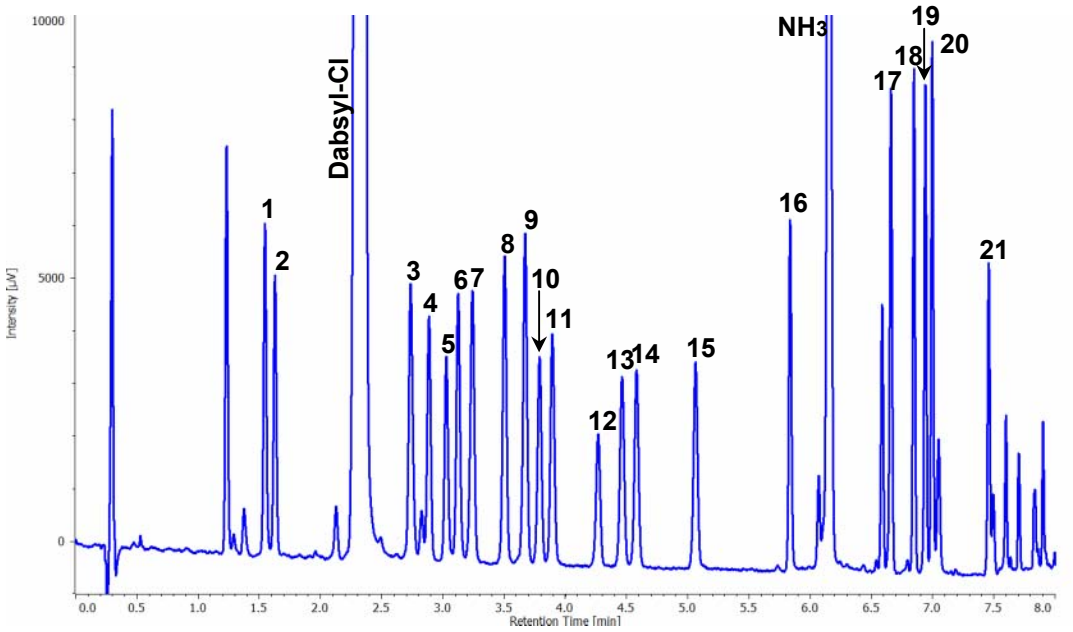

Fig. 3. Chromatogram of 21 components of dabsylated amino acid standard mixture
1: Aspartic acid, 2: Glutamic acid, 3: Serine, 4: Threonine, 5: Arginine, 6: Glycine, 7: Alanine, 8: Proline, 9: Taurine, 10: Valine, 11: GABA (χ-aminobutyric acid), 12: Methionine, 13: Isoleucine, 14: Leucine, 15: Phenylalanine,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

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

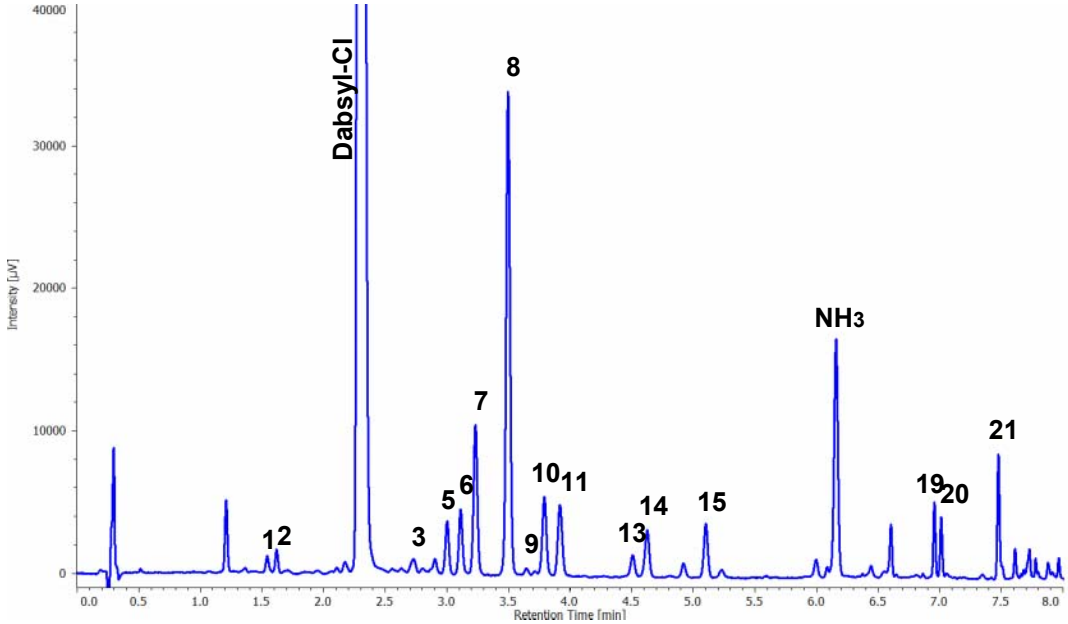

Fig. 4. Chromatogram of dabsylated amino acids in Stout beer
The peak numbers are the same as in Fig. 3. Preparation: Stout beer was diluted by 50-fold using dabsylation buffer and dabsyated according to procedure shown in Fig. 1.

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