Application Note

High Speed Analysis of Dabsyl Amino Acids in Stout Beer using UHPLC

Introduction

There are several methods for amino acids analysis, two of which are the separation by reversed-phase column after precolumn derivatization with UV/Vis detection, and post-column derivatization after separation by ion-exchange column with fluorescence detection. The precolumn derivatization method using Dabsyl chloride as reagent is commonly used as Dabsyl chloride reacts easily with both of primary and secondary amino acids, and those derivatized amino acids are stable, assuring easy handling, good accuracy and high sensitivity. In order to enable an 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 detection.

x-lc-3080dg

Keywords: UHPLC, Amino acids. Dabsyl chloride. Precolulmn derivatization, 1.8 µm C18 column, UV/Vis detection, Aspartic acid, Glutamic acid, Serine, Threonine, Arginine, Glycine, Alanine, Proline, Taurine, Valine, GABA (χ-aminobutyric acid), Methionine, Isoleucine, Leucine, Phenylalanine, Cystine, Hydroxylysine, Ornithine, Lysine, Histidine, Tyrosine, Stout beer.

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Experimental

Equipment

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

Conditions

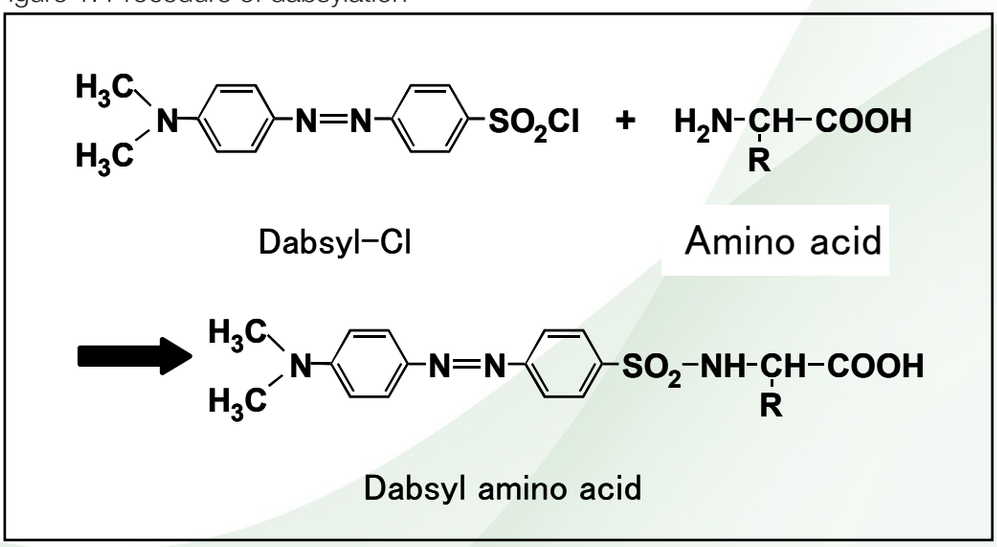
Column:	ZORBAX Eclipse Plus C18 (3.0 mmID x 50 mmL, 1.8 µm) with in-line filter
Eluent A:	20 mM Sodium acetate buffer (pH 6.0)/Acetonitrile (85/15)
Eluent B:	Acetonitrile
Gradient condition:	(A/B), 0 min(90/10) →5 min(70/30)→7.2 min(40/60)→ 7.25 min(10/90) 7.7 min(10/90)→7.75min(90/10)→ 1 cycle; 10 min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

Figure 1 shows the dabsylation procedure and in figure 2, the reaction mechanism is illustrated.

1. Dilute the sample by dabsylation buffer*
2. Take 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

Figure 1. Procedure of dabsylation



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Results

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

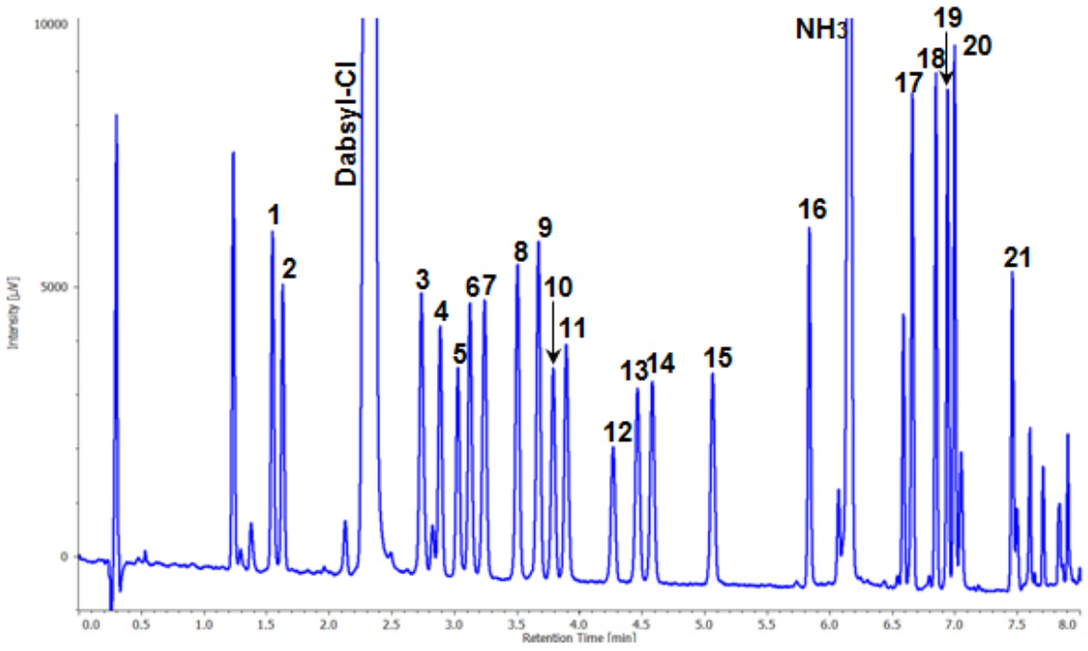

Figure 3. Chromatogram of 21 components of a dabsylated amino acid standard mixture. 1:Aspartic acid, 2: Glutamic acid, 3: Serine, 4: Threonine, 5: Arginine, 6: Glycine, 7: Alanine, 8: Proline, 9: Taurine, 10: Valine, 11: GABA (χ-aminobutyric acid), 12: Methionine, 13: Isoleucine, 14: Leucine, 15: Phenylalanine,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

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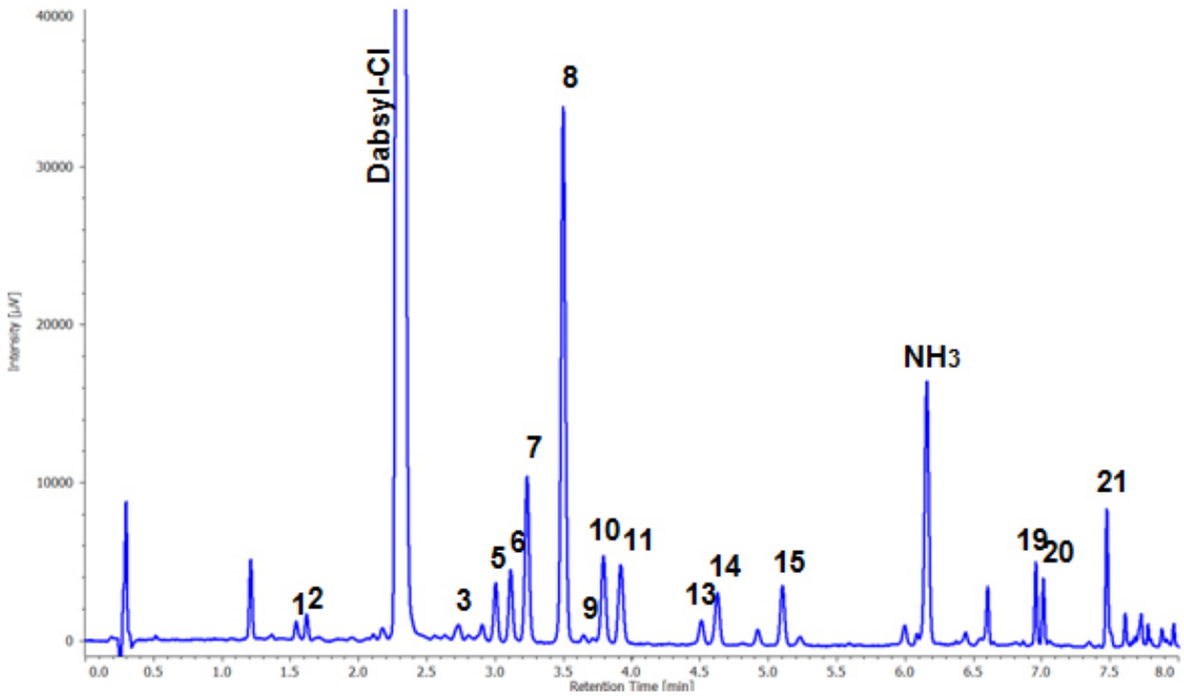
Figure 4 shows the chromatogram of dabsylated amino acids in Stout beer.



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

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