Application Note

430024X

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

Introduction

Collagen, one of the proteins has been recently attracting attention from the viewpoint of health and beauty, since collagen is a main constituent of corium which gives skin vitality and keeps skin soft without wrinkles. Amino acid structure of collagen is known as the arrangement of glycine sequencing at every 3 residue and contains other amino acids such as proline, hydroxyproline and alanine. In addition as specific amino acids for collagen, there are hydroxyproline and hydroxylysine. In order to measure the constituent amino acids of proteins or peptides such as collagen, sample preparation such as hydrolysis and so on needs to be implemented. DAB-Label kit available from JASCO for amino acid derivatization using Dabsyl chloride as reagent includes the tools and reagents for vapor-phase hydrolysis by hydrochloric acid for proteins or peptides, and by using such DAB-Label kit, collagen can be hydrolyzed and amino acids can be derivatized with Dabsyl chloride in order to analyze the constituent amino acids of collagen.

In this report, collagen was hydrolyzed with DAB-Label kit and derivatized amino acids were separated by Ultra High-performance Liquid Chromatography (UHPLC) using UV detector.

Keyword: UHPLC, collagen, amino acids, Dabsyl chloride, precolumn derivatization, 1.8 µm, C18 column, UV 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:	20mM Sodium acetate buffer (pH6.0)/Acetonitrile (85/15)
Column oven:	X-LC 3067CO	Eluent B:	Acetonitrile
Autosampler:	X-LC 3159AS	Gradient condition:	(A/B), 0 min (95/5)→ 2.5 min (95/5)→ 7.5 min (80/20)→ 11 min (45/55)→ 11.5 min (15/85)→12 min (15/85)→ 12.05 min (95/5) 1cycle; 15 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:	22 dabsyl-amino acids 2.0 nmol/mL each

The procedure for hydrolysis and Dabsyl derivatization is shown in Fig. 1.

(1) Dilute the sample with ultrapure water.
(2) Put 20 µL of sample into sample tube and evaporate by centrifugal dryer.
(3) Set up sample tube in vessel* for hydrolysis and add 0.3 mL of 6M hydrochloric acid to the vessel.
(4) Heat at 110ºC for 24 hours under reduced pressure.
(5) Remove residual chlorine by vacuum pump after hydrolysis.
(6) Dilute the sample with buffer solution for Dabsyl derivatization.
(7) Weigh 20 µL of sample.
(8) Add 40 µL of Dabsyl derivatization reagent* and agitate.
(9) Heat at 70ºC for 12 minutes(while agitating arbitrarily).
(10) After cooling, add 440 µL of buffer solution* for dilution and agitate.

* is included in DAB-Label kit,

Fig. 1. Procedure for hydrolysis and Dabsyl derivatization

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Result

Chromatogram of 22 constituents of Dabsyl amino acids in standard mixture is shown in Fig. 2. 22 constituents of amino acids were clearly separated within 11.5 minutes with gradient condition for sufficient separation of hydroxyproline and hydroxylysine that are specific amino acids for collagen.

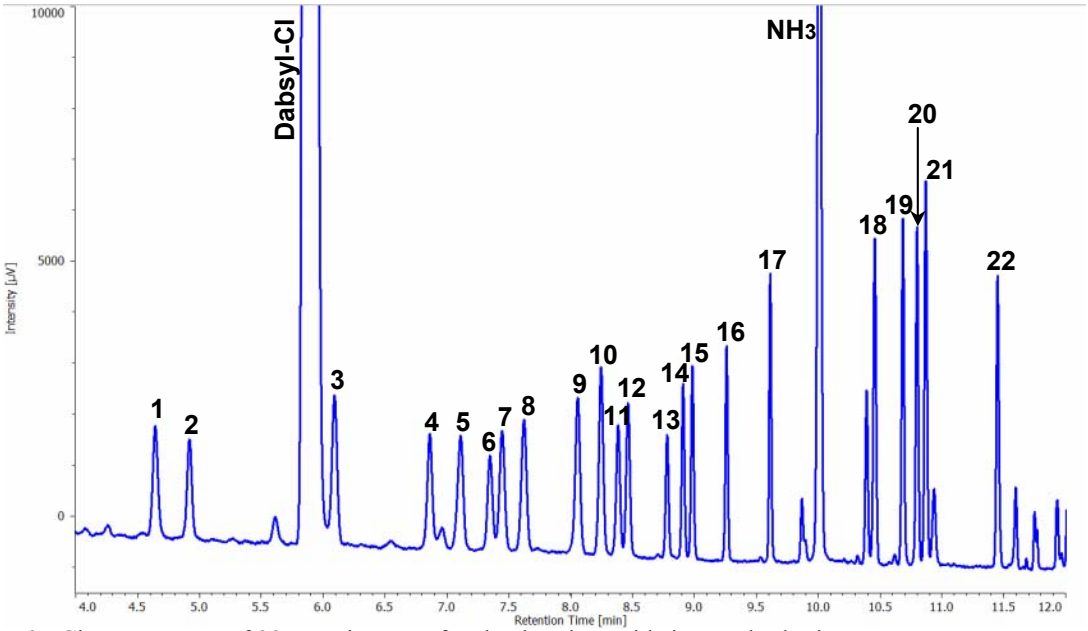

Fig. 2. Chromatogram of 22 constituents of Dabsyl amino acids in standard mixture
1: Aspartic acid, 2: Glutamic acid. 3: Hydroxyproline, 4: Serine, 5: Threonine, 6: Arginine, 7: Glycine, 8: Alanine, 9: Proline, 10: Taurine, 11: Valine, 12: GABA, 13: Methionine, 14: soleucine, 15: Leucine, 16: Phenylalanine, 17: Cystine, 18: Hydroxylysine, 19: Ornithine, 20: Lysine, 21: Histidine, 22: Tyrosine

Chromatogram of amino acids derivatized with Dabsyl after hydrolysis of functional drink containing collagen is shown in Fig. 3. Amino acids including GABA and ornithine contained in the drink were detected successfully.

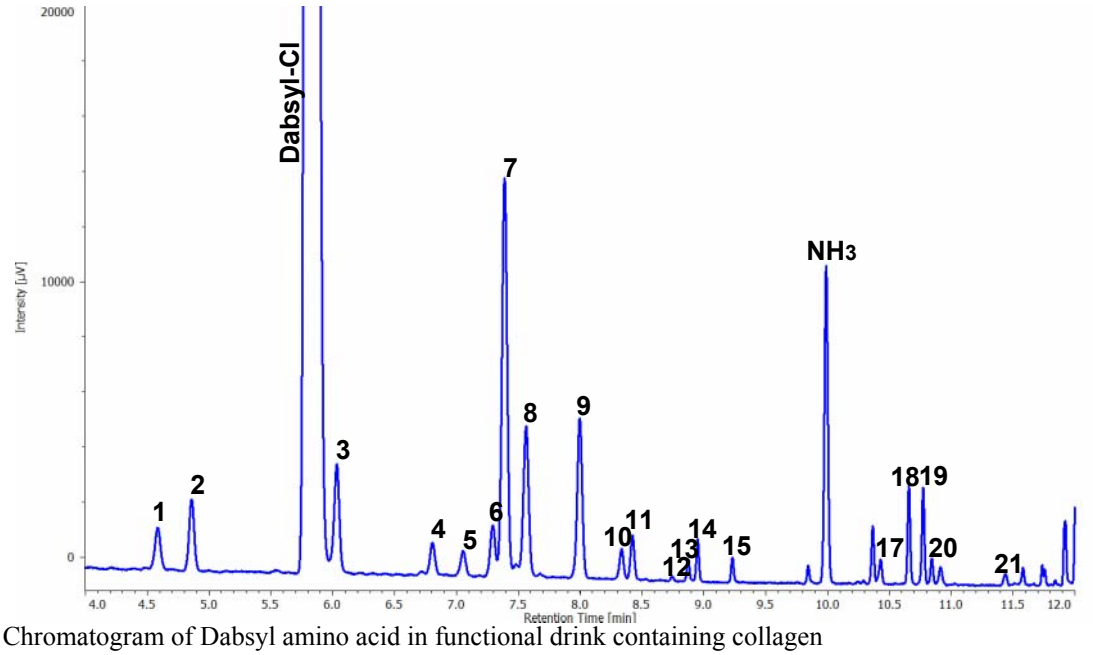

Fig. 3. Chromatogram of Dabsyl amino acid in functional drink containing collagen
The peak numbers are the same as in Fig. 2.
Preparation: Hydrolysis and Dabsyl derivatization were implemented according to the procedure shown in Fig. 1.

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