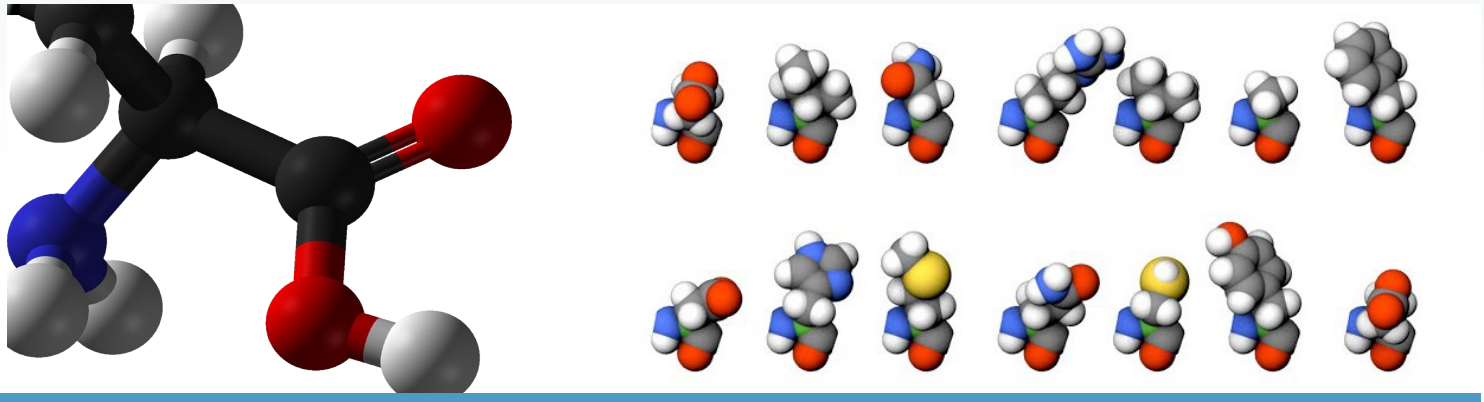
Application Note



Analysis of Protein Hydrolysate Amino Acids using OPA Post-column Derivatization by Quaternary Low Pressure Gradient System

Introduction

Amino acid analysis has been applied to several categories such as food, medicine, protein science and metabolome study, and is an important measurement technique.

An amino acid analysis system using a low pressure gradient unit with OPA post column derivatization provides excellent repeatability and good separation in a short analysis time.

The analysis results of food analysis and amino acid composition analysis of protein using this amino acid analysis system are reported.

PU-2089 Quarternary Gradient HPLC Pump

Keywords: Amino acids to organize proteins, Quaternary low pressure gradient, OPA, Post-column derivatization, Fluorescence detector

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Experimental

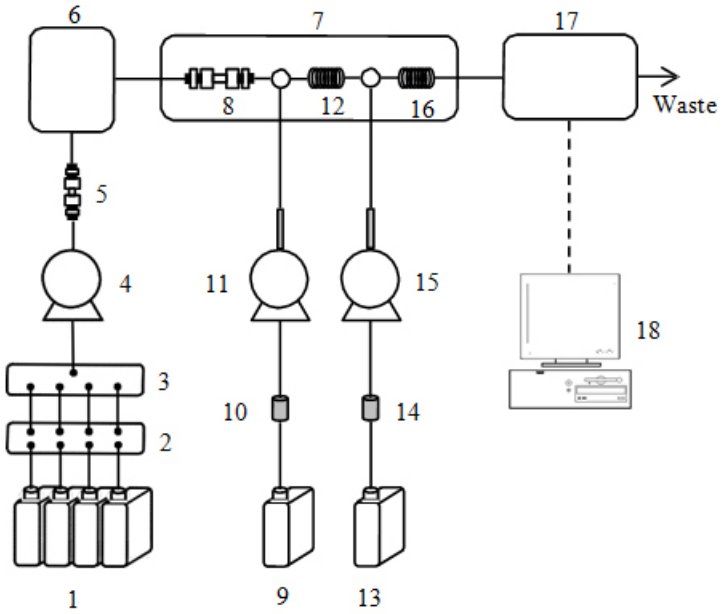
Equipment

Eluent Pump:	PU-2089
Reagent Pump:	PU-2085 x2
Column Oven:	CO-2065
Autosampler:	AS-2057
Detector:	FP-2025

Conditions

Column:	AA pak Na-LG (6.0mmIDx50mmL,5 µm)
Ammonia Filter:	AEC pak Na-LG (4.6mmIDx35mmL)
Eluent:	Amino Buffer Na-LG(1st~4th)
Reagent:	Amino Reagent Na-LG (Hypo, OPA)
Eluent Flow Rate:	0.5mL/min
Reagent Flow Rate:	0.5mL/min each
Column Temperature:	60°C
Reaction Temperature:	60°C
Wavelength:	Ex.345nm,Em.455nm,Gainx10
Injection Volume:	10 µL
Standard Sample:	20 amino acids 50nmol/mL eachin 0.2N citric buffer (pH2.2)

Schematic Diagram



1: Eluent (Amino Buffer Na-LG(1st~4th))
2: Degasser
3: Low pressure gradient unit
4: Eluent pump
5: Ammonia filter (AECpak Na-LG)
6: Autosampler
7: Column oven
8: Column (AApak Na-LG)
9: Reagent 1 (Amino Reagent Na-LG Hypo)
10: Airtrap 1
11: Reagent pump 1(Hypo)
12: Reaction coil 1
13: Reagent 2 (Amino Reagent Na-LGOPA)
14: Airtrap 2
15: Reagent pump 2 (OPA)
16: Reaction coil 2
17: Fluorescence detector
18: Chromatography data system(ChromNAV)

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Results and Discussion

Figure 1 shows the chromatogram of the standard mixture of 20 kinds of amino acids.The sample was well separated within 45 minutes (1 cycle 60 min).

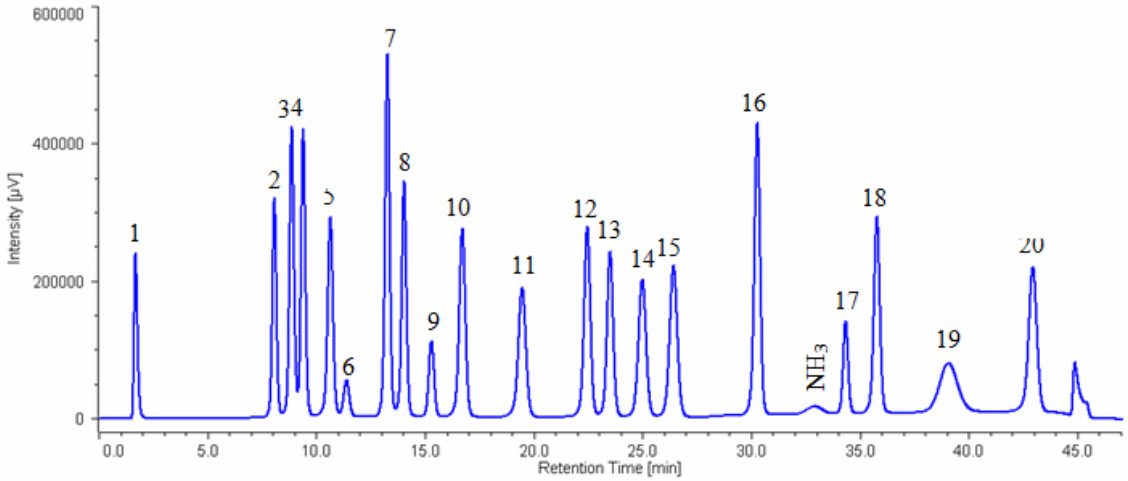

Figure 1. Chromatogram of the amino acid standard mixture (500 pmol each)
1:Cysteic acid,2:Asparatic acid,3:Threonine,4:Serine, 5: Glutamicacid, 6:Proline, 7: Glycine,8: Alanine
9:Cystine,10:Valine,11: Methionine,12:Isoleucine,13:Leucine,14: Tyrosine,15: Phenylalanine,16:GABA
17:Lysine,18:Histidine,19:Tryptophan, 20: Arginine

Figure 2 shows the chromatogram of a sports drink while figure 3 shows the chromatogram of white wine containing GABA.

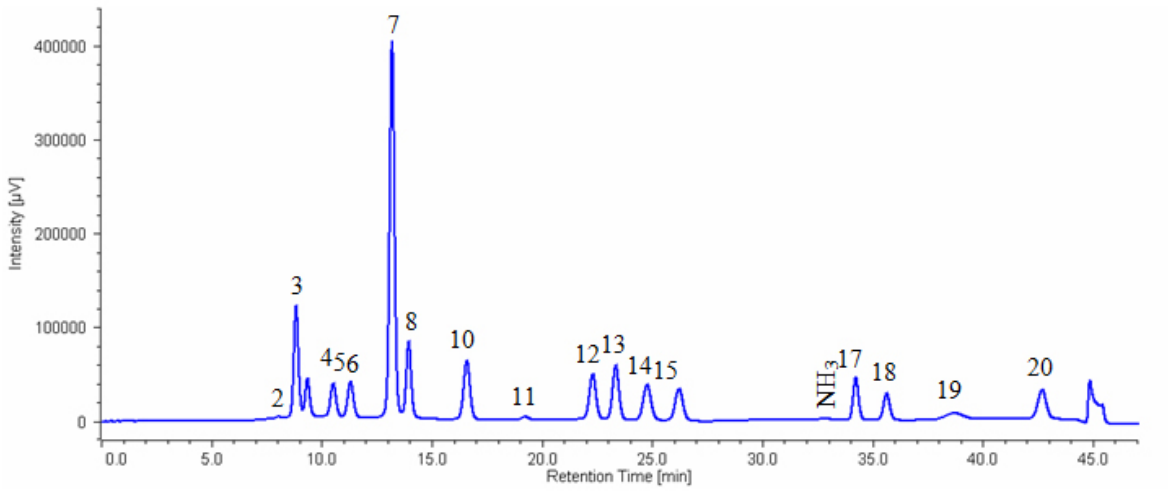

Figure 2. Chromatogram of a sports drink
2: Asparatic acid, 3: Threonine, 4:Serine, 5:Glutamicacid, 6:Proline, 7:Glycine, 8: Alanine, 10: Valine,
11: Methionine,12: Isoleucine,13: Leucine,14: Tyrosine,15: Phenylalanine, 17: Lysine, 18:Histidine,
19: Tryptophan, 20: Arginine

Sample preparation:
Sports drink was diluted 150-fold by 0.2 N citric acid buffer (pH2.2) and then filtrated using 0.45 μm membrane filter.

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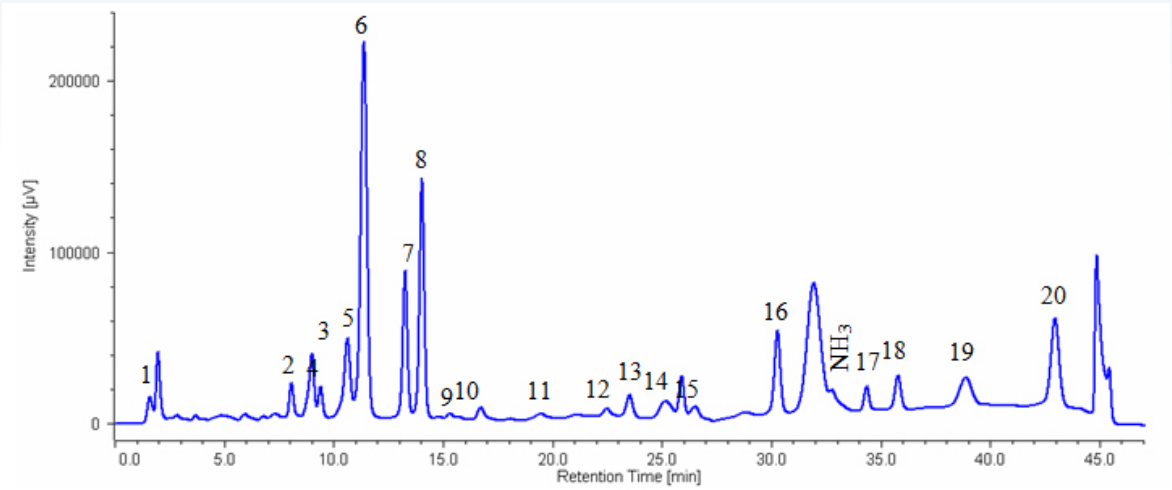

Figure 3. Chromatogram of white wine containing GABA.
1:Cysteic acid, 2:Asparatic acid, 3:Threonine, 4:Serine, 5: Glutamicacid, 6:Proline, 7: Glycine, 8: Alanine
9: Cystine,10: Valine,11: Methionine,12: Isoleucine,13: Leucine,14: Tyrosine,15: Phenylalanine, 16:GABA
17: Lysine,18: Histidine,19: Tryptophan, 20: Arginine

Sample preparation: White wineincluding GABA was diluted 10-fold by 0.2N citric acid buffer (pH 2.2) and then filtrated using 0.45 μmmembrane filter.

Figure 4 shows the chromatogram of hydrolyzed myoglobin (horse skeletal muscle).
Table 1 shows the results of amino acid composition compared with theoretical value (referred to Japan Bio chemistry DataBook I ,Tokyo Kagaku Dojin). It was confirmed that the amino acid composition calculated from this measurement was in good agreement with the theoretical value.

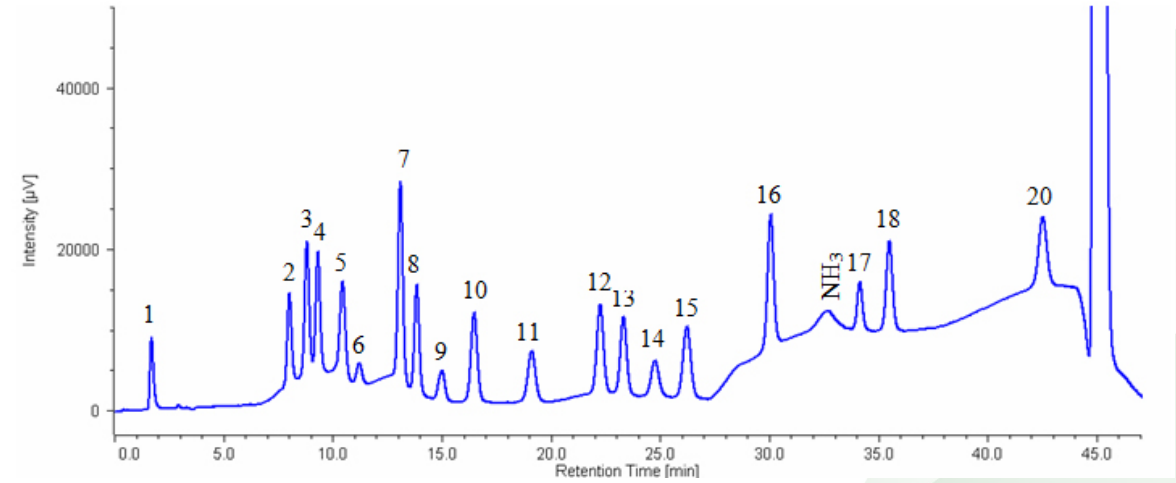

Figure 4 Chromatogram of hydrolyzed myoglobin (horse skeletal muscle).
1:Cysteic acid,2:Asparatic acid,3:Threonine,4:Serine, 5: Glutamicacid, 6:Proline, 7: Glycine,8: Alanine
9:Cystine,10:Valine,11: Methionine,12:Isoleucine,13:Leucine,14: Tyrosine,15: Phenylalanine,16:GABA
17:Lysine,18:Histidine,20:Arginine

Samplepreparation:
1. Myoglobin was diluted to 200μg/mL by ultra pure water. 2.The solution filled a 20 μL sample tube and then dried up by centrifugal evaporator. 3. Sample tube was set in vessel for hydrolysis and 0.3 mL of hydrochloric acid was added to thevessel. 4. The sample was heated for 24 hours under vacuumed condition.
5. The sample was hydrolyzed and the residual chlorine was removed using vacuum pump.
6.500μL of 0.2N citric acid buffer (pH2.2) was added to the sample and agitated.

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Table 1 Comparison of amino acid composition of myoglobin between the measured and theoretical value.

Amino Acids	Measured Values	Theoretical Values
Asp	10.2	10
Thr	7.2	6
Ser	5.4	6
Glu	16.2	18
Pro	4.7	4
Gly	13.5	13
Ala	15.4	17
Val	7.0	8
Met	2.2	2
Ile	8.6	8
Leu	18.3	17
Tyr	2.8	2
Phe	7.4	7
Lys	19.5	19
His	11.9	12
Arg	2.7	2

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