Application Note
No. 430020X-E

High Speed Separation of Amino Acids Using Pre-column Derivatization by a UHPLC (X-LC®) System and its Application to Wine Analysis

Introduction

Amino acid analysis is becoming more important in a variety of application fields, ranging from food analysis to protein science. A number of separation and detection methods are currently used. Among them, a combination of pre-column derivatization with OPA (o-Phthalaldehyde) and separation on a C18 column with fluorescence detection. This is generally preferred due to the simplicity and high sensitivity of the method.
We examined the usefulness of an X-PressPak V-C (2.0 mm I.D. x 50 mm L.) packed with a 2 μm diameter packing material for the ultra-high speed separation of amino acids. The results were examined to determine whether the performance of the column and the chromatography system exceeds those of conventional HPLC.

Experimental

The UHPLC system utilized in this experiment was a JASCO X-LC system consisting of two of a 3185PU pump, a 3080DG degasser, a 3180MX mixing unit, a 3067CO column oven, a 3120FP fluorescence detector, a 3159AS autosampler, and a chromatography data system.
The standard solution was prepared by mixing the amino acid standard (type H, WAKO, Japan), cysteic acid, and tryptophan to be a concentration of 20 pmol/μL each.
The method was applied to the analysis of cooking wine. The wine was purchased at a grocery store, diluted 10 times with water, and then filtered with a 0.2 μm membrane filter.

Results and Discussion

Figure 1 shows an X-LC chromatogram of a standard mixture of 18 amino acids (20 pmol each). The UHPLC system provides an analysis time 5 times shorter than conventional HPLC without sacrificing the resolution between each peak.
Table 1 shows the reproducibility of retention times and peak areas. Relative standard deviations of the retention times are between 0.048 and 0.429%, and those of peak areas are between 0.546 and 1.9%. These results consider the X-LC system to be an excellent separation technique.
Figure 2 shows an X-LC chromatogram of the wine sample. Eighteen amino acids were clearly separated from unidentified peaks.

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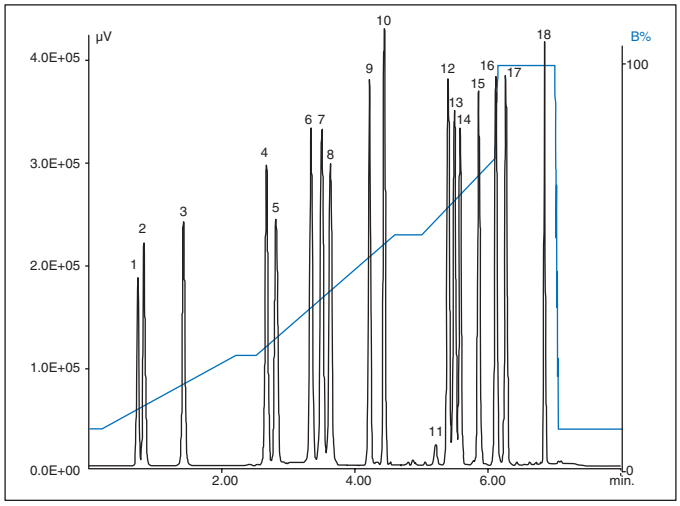

Figure 1 X-LC chromatogram of a standard mixture of 18 amino acids (each 20 pmol)
Peaks: 1=Cysteic acid, 2=Aspartic acid, 3=Glutamic acid, 4=Serine, 5=Histidine, 6=Arginine, 7=Glycine, 8=Threonine, 9=Alanine, 10=Tyrosine, 11=Ammonium, 12=Methionine, 13=Valine, 14=Tryptophan, 15=Phenylalanine, 16=Isoleucine, 17=Leucine, 18=Lysine Conditions of pre-column derivatization: sample solution=amino acid standard solution (type H)+cysteic acid+Tryptophan (20 nmol/mL each), derivatization reagent=0.4 M borate buffer (pH 9.0) OPA(1% MeOH solution)/2-mercaptoethanol (1/0.5/0.01), autosampler reaction conditions=sample volume, 100 μL; reagent volume, reaction volume, 20 μL; reaction time, 30 sec; mixing time, 2 min; injection volume, 1 μL Chromatographic conditions: Eluent A=1.0 M citrate buffer (pH 5.8) 3.5 mL in 1 L of H_2O, Eluent B=1.0 M citrate buffer (pH 5.8) 3.5 mL in 1 L of $CH_3CN/C_2H_5OH/H_2O$(30/30/40), A/B = 90/10(0 min) - 90/10(0.2 min) - 72/28(2.2 min) - 72/28(2.5 min) - 42/58(4.6 min) - 42/58(5.0 min) - 23/77(6.1 min) - 0/100(6.15 min) - 0/100(7.0 min) - 100/0(7.05 min), injection to injection time = 10 min, flow rate = 0.6 mL/min, column = X-PressPak V-C18(2.0 mmI.D. x 50 mmL., 2 μm), column temperature = 40°C, detection wavelength = Ex, 345 nm; Em, 455 nm
The blue line indicates the gradient profile (B%)

Table 1 Reproducibility of retention times and peak areas (%RSD)

Amino Acid	Rt	Peak Area
Cys-SO_3	0.423	1.806
Asp	0.429	1.899
Giu	0.338	1.047
Scr	0.161	0.604
His	0.164	0.701
Arg	0.149	0.683
Gly	0.114	0.869
Thr	0.104	0.859
Ala	0.080	0.584
Tyr	0.074	0.782
NH_4^-	0.071	2.290
Mct	0.061	0.584
Val	0.060	0.998
Trp	0.060	0.546
Phe	0.058	0.651
Ileu	0.054	0.911
Lcu	0.055	0.601
Lys	0.048	0.743

Cys-SO_3 = Cysteic acid, Asp = Aspartic acid, Glu = Glutamic acid, Ser = Serine, His = Histidine, Arg = Arginine, Gly = Glycine, Thr = Threonine, Ala = Alanine, Tyr = Tyrosine, Met = Methionine, Val = Valine, Trp = Tryptophan, Phe = Phenylalanine, Ileu = Isoleucine, Leu = Leucine, Lys = Lysine

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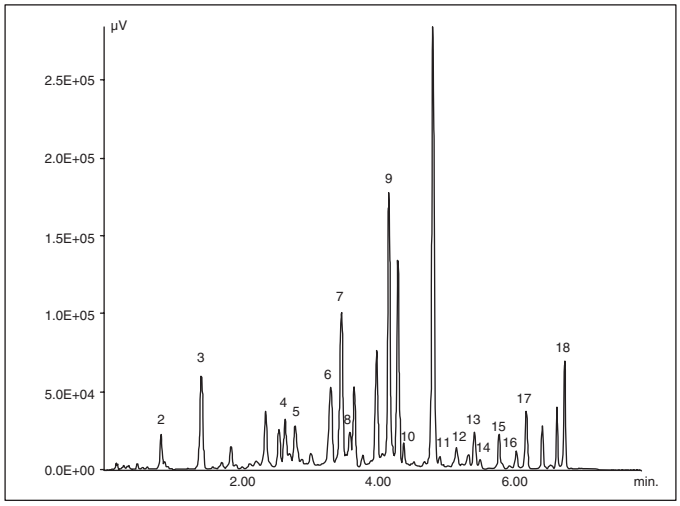

Figure 2 X-LC chromatogram of the cooking wine
The wine was diluted 10 times with water, and then filtered with 0.2 μm membrane filter. The other conditions are the same as in the Figure 1 caption.

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