

## High Speed Separation of Amino Acids Using Pre-column Derivatization by a UHPLC (X-LC<sup>®</sup>) 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  $\mu$ m 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/ $\mu$ L each.

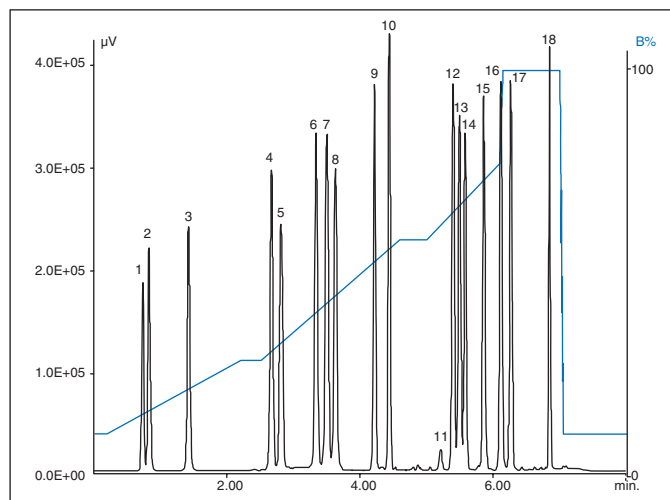
The method was applied to the analysis of cooking wine. The wine was purchased at a grocery store, diluted 10 times with water, and then filtered with a 0.2  $\mu$ m membrane filter.

### 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.



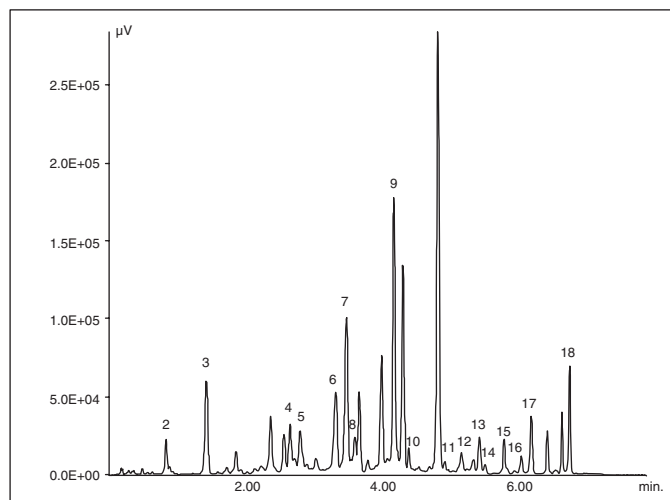
**Figure 1 X-LC chromatogram of a standard mixture of 18 amino acids (each 20 pmol)**

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  $\mu$ L; reagent volume, reaction volume, 20  $\mu$ L; reaction time, 30 sec; mixing time, 2 min; injection volume, 1  $\mu$ L Chromatographic conditions: Eluent A=1.0 M citrate buffer (pH 5.8) 3.5 mL in 1 L of H<sub>2</sub>O, Eluent B=1.0 M citrate buffer (pH 5.8) 3.5 mL in 1 L of CH<sub>3</sub>CN/C<sub>2</sub>H<sub>5</sub>OH/H<sub>2</sub>O(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  $\mu$ m), 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 <sub>3</sub>          | 0.423 | 1.806     |
| Asp                          | 0.429 | 1.899     |
| Glu                          | 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 <sub>4</sub> <sup>+</sup> | 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<sub>3</sub> = 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



**Figure 2 X-LC chromatogram of the cooking wine**

The wine was diluted 10 times with water, and then filtered with 0.2  $\mu\text{m}$  membrane filter. The other conditions are the same as in the Figure 1 caption.