

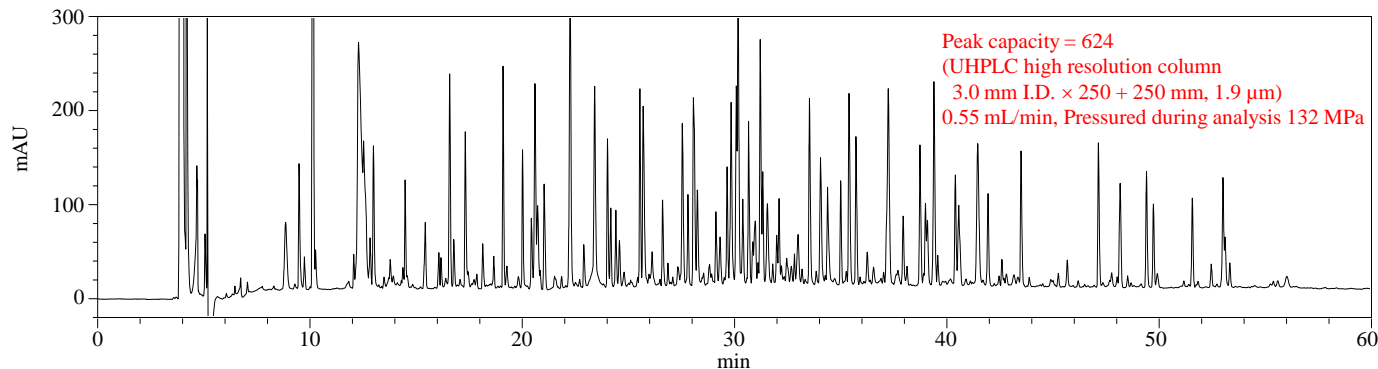
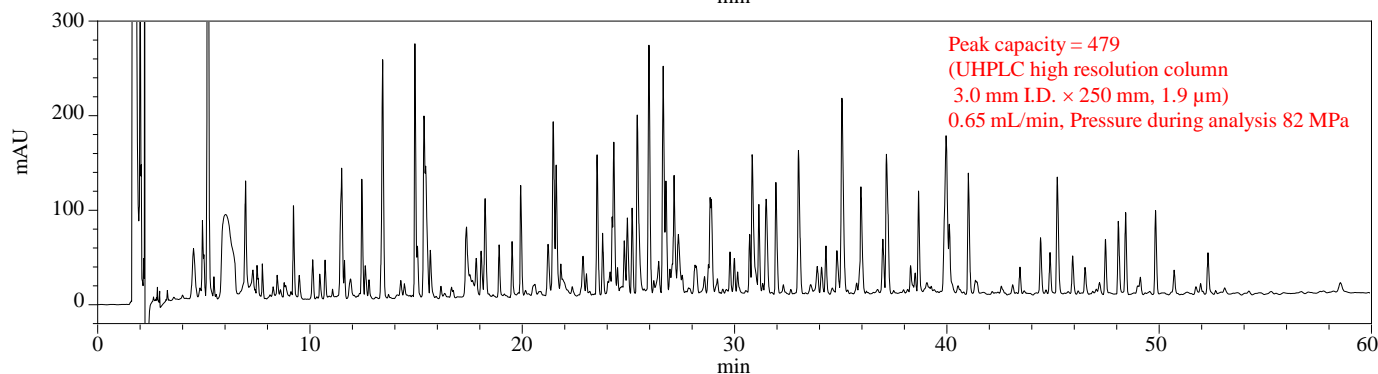
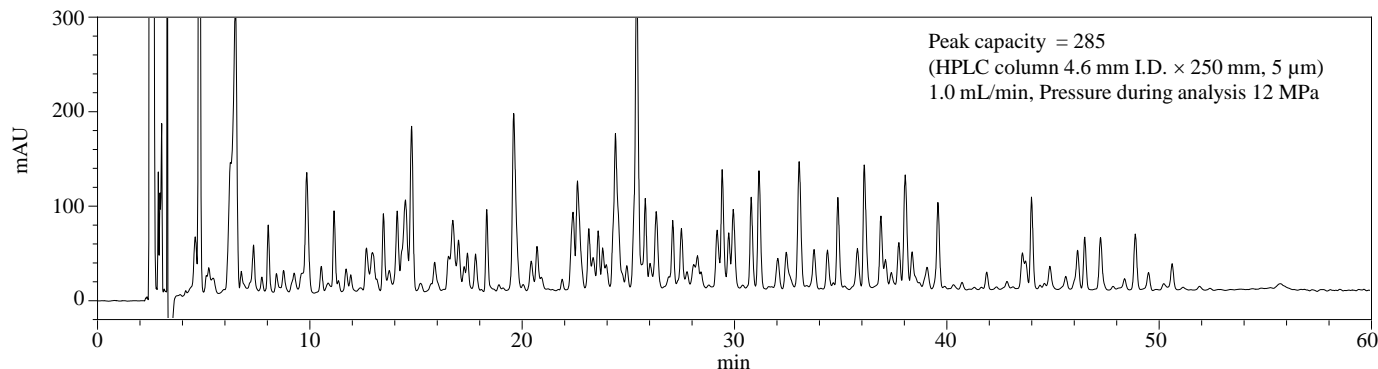
High-Resolution Analysis of BSA Digest by UHPLC(2 columns connected in series) AS/LC-032

Peptide mapping is a method to analyze the changes of protein-constituting amino acids by separating peptide fragments formed by digesting proteins with enzymes using HPLC, etc. and then, comparing the chromatographic patterns.

Recently, this method is also applied for the quality assessment of biopharmaceuticals. As many peptide peaks will emerge, it is important that high resolution can be achieved.

In Application Sheet (AS/LC-031), the result obtained by the high-resolution high-speed analysis using Hitachi ultra high-speed liquid chromatograph, ChromasterUltra Rs, with LaChromUltra II C18 high resolution column for UHPLC (3.0 mm I.D. × 250 mm, 1.9 μm) was introduced. This time, an example of the analysis in which two columns were connected in series for the purpose of achieving even higher resolution is introduced here. BSA digest was used as the model sample as it was in AS/LC-031.

Analysis Example of BSA (Bovine Serum Albumin) Digest



Analyzed by connecting two UHPLC high resolution columns in series, the peak capacity about 2.2 times higher than that by a HPLC column was obtained. The advantage of the highly pressure resistant system and column with a pressure resistance of 140 MPa was again demonstrated here.

| <Analytical Conditions for HPLC Column> | <Analytical Conditions for UHPLC Column> |
|---|---|
| Column : LaChrom II C18 (5 μm) 4.6 mm I.D. × 250 mm | Column : LaChromUltra II C18 (1.9 μm) 3.0 mm I.D. × 250 mm LaChromUltra II C18 (1.9 μm) 3.0 mm I.D. × 250+250 mm |
| Eluents : A) 0.05 % TFA / H ₂ O (v/v) B) 0.05 % TFA / CH ₃ CN (v/v) 5 % B (0 min) → 45 % B (60 min) | Eluents : A) 0.05 % TFA / H ₂ O (v/v) B) 0.05 % TFA / CH ₃ CN (v/v) 5 % B (0 min) → 45 % B (60 min) |
| Flow rate : 1.0 mL/min | Flow rate : 0.65 mL/min, 0.55 mL/min |
| Column temperature : 40°C | Column temperature : 40°C |
| Detection wavelength : UV 214 nm (DAD) | Detection wavelength : UV 214 nm (DAD) |
| Injection vol. : 10 μL | Injection vol. : 5 μL |

Main system configuration: ChromasterUltra Rs DAD system
(6170 Binary Pump, 6270 Autosampler, 6310 Column Oven, 6430 Diode Array Detector, Organizer)

NOTE: These data are an example of measurement; the individual values cannot be guaranteed.