■ High-speed High-resolution Analysis of BSA Digest by UHPLC

Peptide mapping is an analysis method in which peptide fragments generated from the digestion of protein with an enzyme are separated by LC, etc. and the chromatographic patterns are compared so as to confirm changes in the constituent amino acids. Recently, this method is also applied to the quality assessment of biopharmaceuticals. As many peptide peaks will emerge, it is extremely important that the analysis is performed with high resolution as well as with good peak retention time and area reproducibilities.
This time, the high-resolution and high-speed analysis result obtained by Hitachi ultra high speed chromatograph ChromasterUltra Rs is introduced here. BSA digest used as the model sample was separated by using a HPLC column and UHPLC high resolution column and the peak capacities were compared.

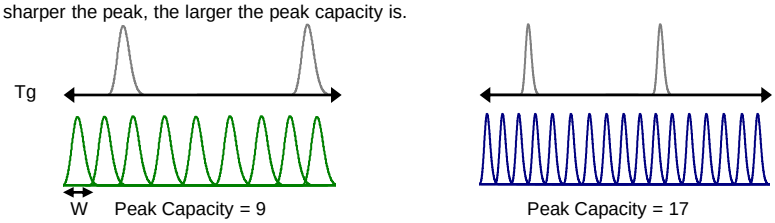
(Optional, includes the parts prepared by a customer)

ChromasterUltra Rs

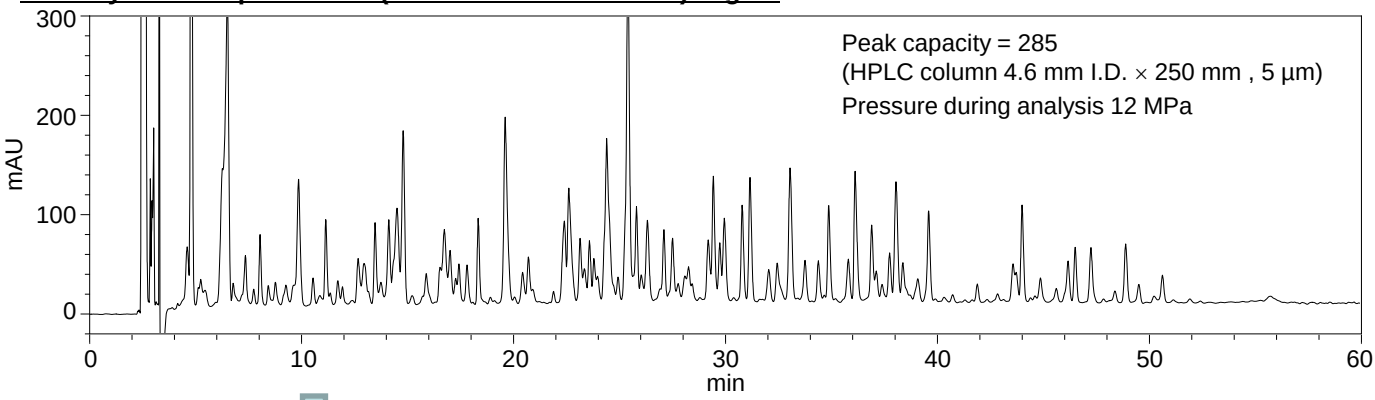
◆Peak Capacity◆

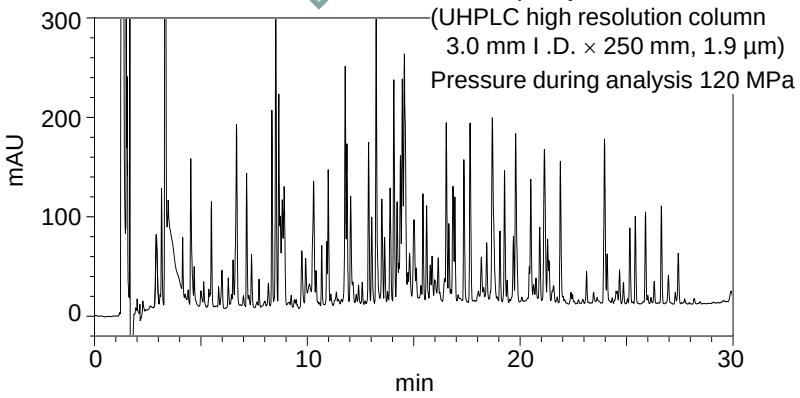
Peak capacity = 1+Tg / W(4σ)

This shows "how many peaks with the peak width of W can be contained in the gradient time of Tg." W is the mean of the widths of all detected peaks. This is used as the scale for the resolution of gradient elution and the sharper the peak, the larger the peak capacity is.



■ Analysis Example of BSA (Bovine Serum Albumin) Digest





LaChromUltra II C18 column, with the adoption of inorganic-organic composite silica material having improved physical and chemical durability compared to the conventional silica gel, achieves high pressure resistance.
As a result, a high resolution column (1.9 µm, 250 mm) for UHPLC which can provide the number of theoretical plates of 50000/column was included in the lineup.
By using this column, ca.1.4 times greater peak capacity could be obtained with ½ of the analysis time compared to the analysis by a HPLC column. By using the system with the pressure resistance of 140 MPa and a high pressure resistant column, it was possible to perform the analysis shown here.

<Analytical Conditions for HPLC Column>
Column : LaChrom II C18 (5 µm) 4.6 mm I.D. × 250 mm
Eluents : A) 0.05 % TFA / H_2O (v/v)
B) 0.05 % TFA / CH_3CN (v/v)
5 % B (0 min) → 45 % B (60 min)
Flow rate : 1.0 mL/min
Column temp. : 40°C
Detection wavelength : UV 214 nm (DAD)
Injection vol. : 10 µL

<Analytical Conditions for UHPLC Column>
Column : LaChromUltra II C18 (1.9 µm) 3.0 mm I.D. × 250 mm
Eluents : A) 0.05 % TFA / H_2O (v/v)
B) 0.05 % TFA / CH_3CN (v/v)
5 % B (0 min) → 45 % B (30 min)
Flow rate : 0.85 mL/min
Column temp. : 40°C
Detection wavelength : UV 214 nm (DAD)
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.