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Application News

Ultra High Performance Liquid Chromatograph

Reliable Peptide Mapping with Nexera™ X4
-Excellent Reproducibility under Shallow Gradient Conditions-

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User Benefits

- Owing to the excellent delivery performance of Nexera X4, high retention-time reproducibility can be achieved even under long, shallow gradient conditions.
- Mass information obtained using a single quadrupole mass spectrometer can be utilized as qualitative data for each peptide fragment, thereby improving the reliability of the results.

■ Introduction

Peptide mapping is important for confirming the primary structure of proteins and for evaluating the consistency of their quality. In analyses using LC (reversed-phase chromatography), a large number of peptide fragments generated by digestion are separated using a solvent delivery approach in which the proportion of organic solvent in the mobile phase is gradually increased over an extended period through a shallow gradient. This gradual change in organic solvent enables the separation of peptides with similar hydrophobicity, but the performance of the solvent delivery pump, which precisely controls the organic solvent ratio, is critically important for achieving retention-time reproducibility. Nexera X4 (Fig. 1) inherits technologies developed in Shimadzu's Nexera series and is a next-generation ultra-high-performance liquid chromatography (UHPLC) system that achieves top-class analytical performance. Its solvent delivery pump features independently actuated plungers and a pressure feedback mechanism, enabling precise solvent delivery and excellent reproducibility even under long, shallow gradient conditions. In this article, an example is presented in which Nexera X4 was used to analyze tryptic digests of BSA. Furthermore, by combining the system with a single quadrupole mass spectrometer, the acquisition of mass information as qualitative data for each peptide fragment is also demonstrated.

Fig. 1 Nexera™ X4

■ Analytical Conditions and Target Compounds

The sample and analytical conditions are summarized in Table 1. In this article, tryptic digests of bovine serum albumin (BSA) were used as a model sample (BSA was reduced and alkylated, followed by trypsin digestion: Fig. 2). For this sample, a gradient with a shallow slope, in which the proportion of organic solvent in the mobile phase changed at 0.78% per minute, was applied.

Table 1 Analytical Conditions and Target Compounds

Sample	: Tryptic digest of BSA
Mobile phase	
Pump A	: 0.1% TFA (Trifluoroacetic acid) in water
Pump B	: 0.1% TFA in Acetonitrile
Column	: Shim-pack Scepter™ C18-120 *1 (100 mm × 2.1 mm I.D., 1.9 µm)
Injection Vol.	: 1 µL (1000 mg/L)
LC Conditions	
System	: Nexera X4
B Conc.	: 1%(0 min)→40%(50 min)→90%(50-55 min)→1%(55-85 min)
Column Temp.	: 70 °C
Flow rate	: 0.2 mL/min
Mixer	: MR180
Sample loop Vol.	: 15 µL
Detection	: 220 nm (SPD-M40 X4, STD cell)
MS Conditions	
System	: LCMS-2050
Ionization	: ESI/APCI (DUIS™), positive mode
Mode	: SCAN (*m/z* 150-2000)
Nebulizing gas flow	: 2.0 L/min
Drying gas flow	: 5.0 L/min
Heating gas flow	: 7.0 L/min
DL Temp.	: 200 °C
Desolvation Temp.	: 450 °C
Interface voltage	: +1.0 kV

*1 P/N : 227-31012-05

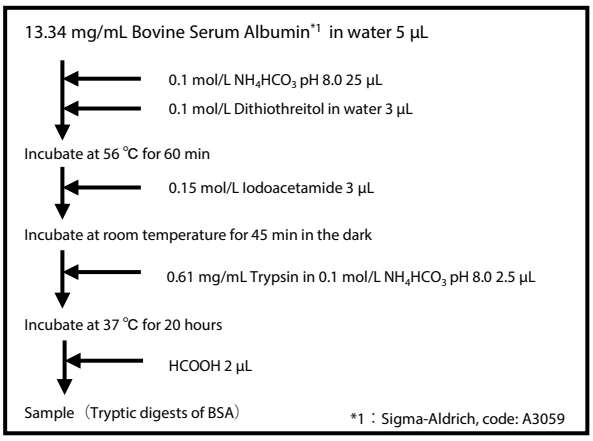

Fig. 2 Tryptic Digestion of BSA

■ Reproducibility under Shallow Gradient

To properly retain highly polar peptides in the digest and achieve sufficient separation of each peptide fragment, a gradient with a low initial organic solvent concentration (1%) was applied, in which the proportion of organic solvent in the mobile phase was increased gradually at 0.78% per minute over 50 minutes.

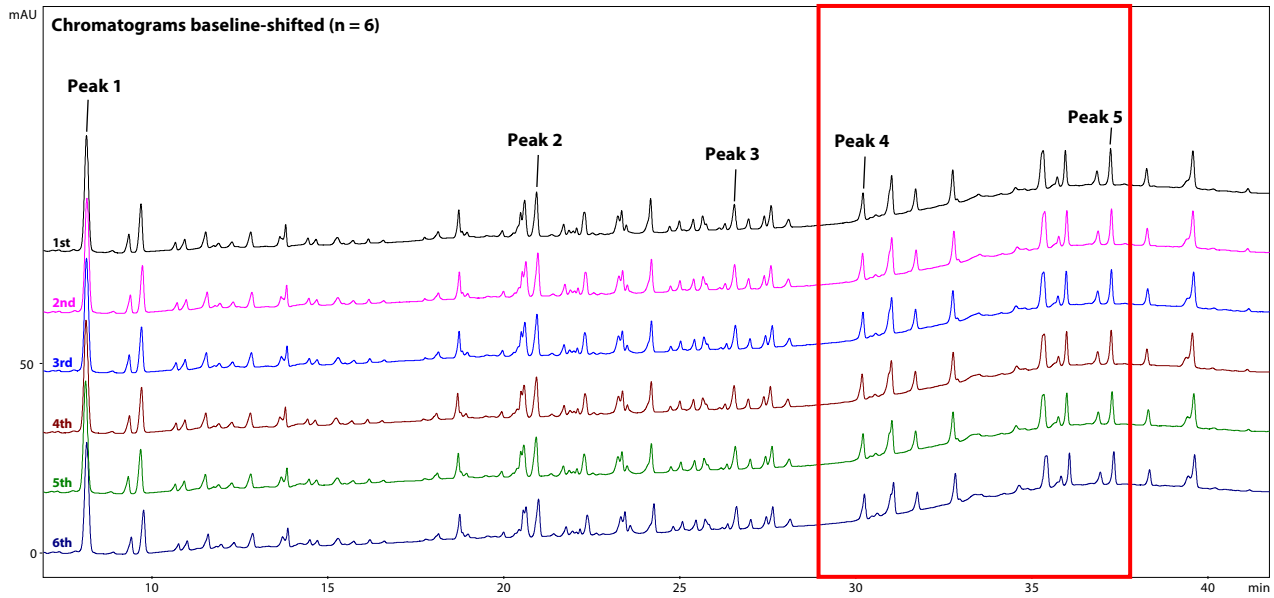

Fig. 3 UV Chromatograms for Six Replicate Analyses (n = 6)

Fig. 3 shows the chromatograms obtained from six replicate analyses, with baseline-shifted. It can be seen that the large number of peptide fragments generated by digestion were successfully separated. Peaks 1–5 in Fig. 3 represent typical peaks selected from different retention-time regions across the elution ranges of individual peptide fragments, and their retention-time reproducibility (%RSD) is summarized in Table 2. Good reproducibility was obtained for all peaks, demonstrating that the excellent solvent delivery performance of Nexera X4 ensures high reproducibility even under long, shallow gradient conditions.

Table 2 Retention-time Reproducibility (%RSD) for Six Replicate Analyses

	Retention time
Peak 1	0.16
Peak 2	0.12
Peak 3	0.09
Peak 4	0.07
Peak 5	0.08

■ Qualitative Analysis Using LCMS-2050

Fig. 4 shows the UV chromatograms and MS chromatograms corresponding to the red-boxed region in Fig. 3. The base-peak *m/z* values corresponding to each peak in the MS chromatograms are also indicated. Peaks detected in the UV chromatograms were also observed in the MS chromatograms, demonstrating that mass information can be utilized as qualitative data for each peptide fragment in addition to retention time. In peptide mapping, a large number of peaks are detected, and small variations in retention time can pose challenges for accurate identification. By combining retention-time information with mass data, which is independent of retention-time fluctuations, the reliability of the results can be improved.

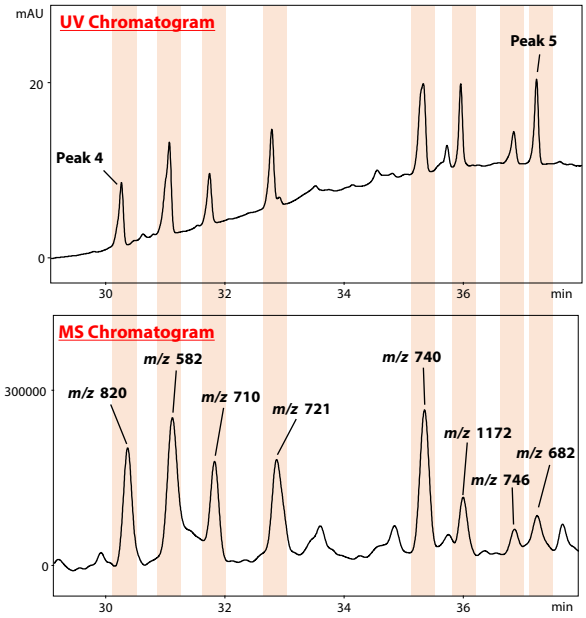

Fig. 4 Qualitative Analysis Using Mass Information

■ Conclusion

An example of peptide mapping using tryptic digests of BSA was presented. In peptide mapping, shallow gradient conditions, in which the proportion of organic solvent is gradually increased over an extended period, are used to separate the large number of peptide fragments, making the performance of the solvent delivery pump critically important for achieving retention-time reproducibility. Nexera X4 enables precise solvent delivery through independently actuated plungers and a pressure feedback mechanism, ensuring excellent retention-time reproducibility even under long, shallow gradient conditions. Furthermore, by combining the system with a single quadrupole mass spectrometer (LCMS-2050), both retention-time and mass information can be utilized as qualitative data, contributing to improved reliability of the results.

01-01125-EN First Edition: Feb. 2026

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