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Application News

No.L488

High Performance Liquid Chromatography

Peptide Mapping of Antibody Drugs by Nexera-i

Peptide mapping by HPLC is one of the important quality assurance tests used for verifying the primary structure of antibody drugs. Typically, following enzymatic digestion of the antibodies, separation is conducted using a traditional reversed phase column. Due to the large number of peaks that require separation, the use of small-particle columns and core shell columns for peptide analysis has spread in recent years.
In order to compare elution profiles for identity and mutation confirmation, a highly repeatable system is required. The Nexera-i integrated UHPLC is the ideal system for such an analysis. Here, the Nexera-i is used in the analysis of IgG (human immunoglobulin G) tryptic digest.

Analysis of IgG Tryptic Digest

For this investigation, after reduction and alkylation of IgG, tryptic enzyme digestion was used as shown in Fig. 1 for sample preparation.

Table 1 shows the analytical conditions. Here, the Aeris 1.7 µm PEPTIDE XB-C18 100 Å small-particle core-shell column and the Nexera-i integrated UHPLC system was used. Mobile phase A was 0.1 % trifluoroacetic acid (TFA) in water and mobile phase B was 0.08 % TFA in acetonitrile. To ensure proper gradient performance with TFA, an optional 300 µL mixer was used.
Fig. 2 shows the chromatogram of IgG tryptic digest, in which an extremely large number of peaks are clearly separated.

Table 1 Analytical Conditions

Column	: Aeris 1.7 µm PEPTIDE XB-C18 100 Å (150 mm L. × 2.0 mm I.D., 1.7 µm)
Mobile Phase	: A: 0.1 % trifluoroacetic acid in water B: 0.08 % trifluoroacetic acid in acetonitrile
Time Program	: B.Conc. 0 % (0 min) → 45 % (90 min) → 100 % (90.01 - 95 min) → 0 % (95.01 - 110 min)
Flowrate	: 0.2 mL/min
Column Temp.	: 60 ˚C
Injection Vol.	: 10 µL
Detection	: LC-2040C 3D at 215 nm
Flow Cell	: High-speed high-sensitivity cell

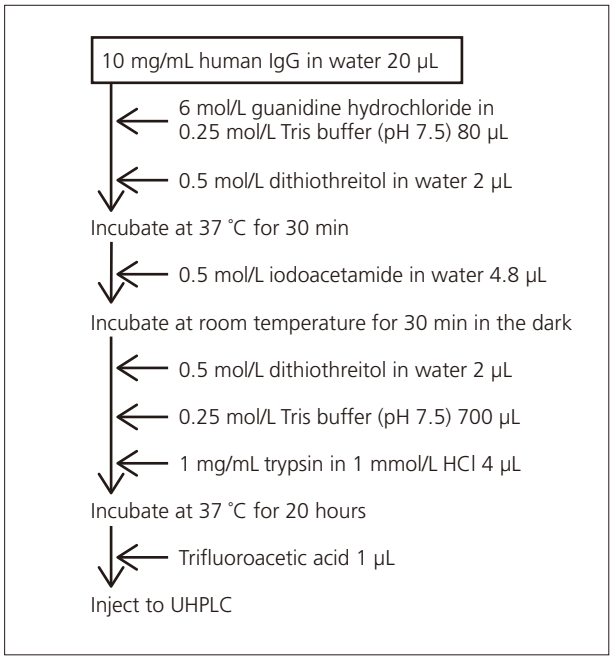

Fig. 1 Sample Preparation

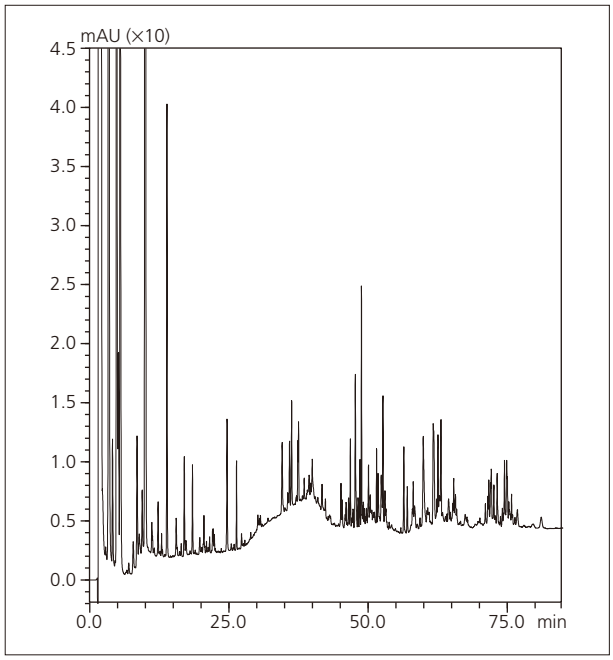

Fig. 2 Chromatogram of IgG Tryptic Digest

Repeatability

Due to the large number of peaks that must be separated when conducting peptide mapping, a gradient with a long shallow slope is required. In this analysis, the mobile phase B percentage changes from 0 % to 45 % over 90 minutes, resulting in a slope of 0.5 %/min. The optimized low-pressure gradient valve of the Nexera-i and mixer selection for use with TFA will provide repeatable delivery even with a shallow gradient.

Tables 2 and 3 show the intra-day and inter-day repeatability of retention time, respectively. Fig. 3 shows the IgG tryptic digest chromatogram intra-day repeatability. Selecting the principal peaks from the chromatogram (peaks labeled a to f), we checked their repeatability. We calculated the intra-day repeatability based on the results of six repeat analyses. The inter-day repeatability was calculated based on average of three analyses per day over a period of six days. In peptide mapping using the Nexera-i, it is clear that good intra-day and inter-day repeatability is obtained.

Table 2 Intra-day Repeatability of Retention Time (n=6)

Peak	Avg. R.T. (min)	Std. Dev. (min)	%RSD (%)
Peak a	9.929	0.027	0.271
Peak b	24.669	0.047	0.192
Peak c	36.299	0.042	0.117
Peak d	48.815	0.033	0.068
Peak e	59.864	0.032	0.054
Peak f	74.535	0.043	0.057

Table 3 Inter-day Repeatability of Retention Time (n=6)

Peak	Avg. R.T. (min)	Std. Dev. (min)	%RSD (%)
Peak a	9.907	0.016	0.159
Peak b	24.708	0.033	0.132
Peak c	36.355	0.034	0.093
Peak d	48.877	0.034	0.069
Peak e	59.901	0.027	0.046
Peak f	74.555	0.036	0.049

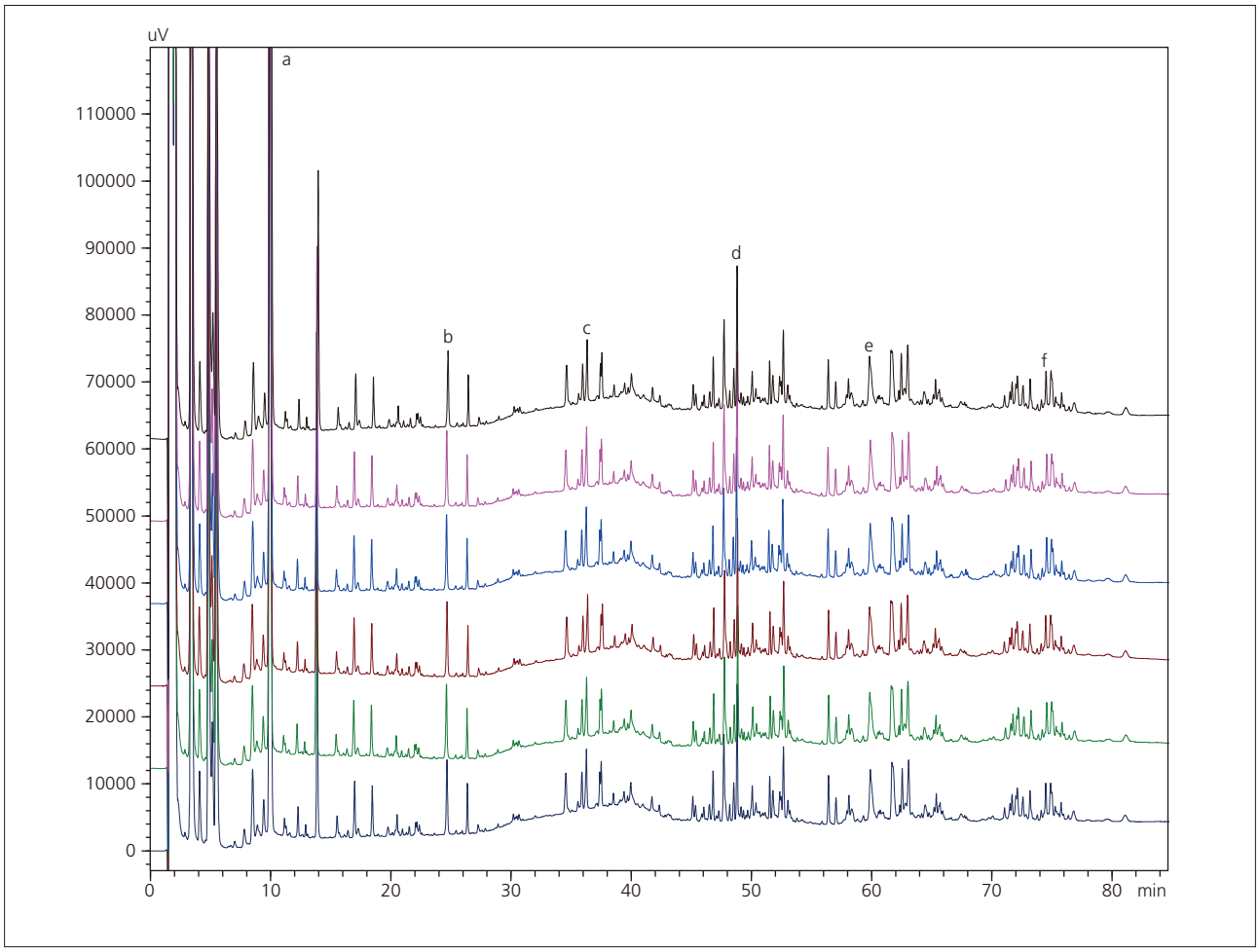

Fig. 3 Intra-day Repeatability of Chromatogram of IgG Tryptic Digest

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