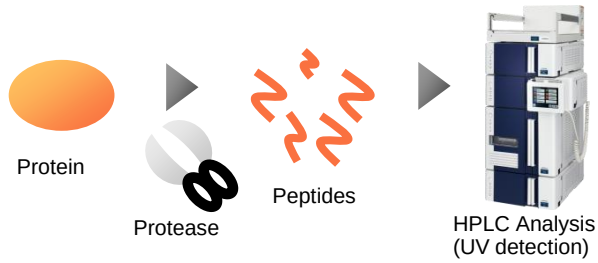
■Application for Biomedicines - Peptide Mapping Method -

Biomedicines are protein-based drugs manufactured by applying recombinant DNA technology or cell culture technology and one of the characteristics is that their raw materials are biological-origin polymers. Some guidance and guidelines have been published for the test and evaluation methods.
“Peptide mapping method,” one of the methods to identify biomedicines, is introduced here. The peptide mapping is a method developed to analyze the properties of proteins, evaluate the identity and stability, and detect the mutation. To compare the elution patterns of LC chromatograms showing many peptide peaks, it is extremely important to ensure that the repeatability for the peak retention times and areas are good.
This time, BSA and an antibody drug (IgG), a typical biomedicine, were used as model samples and the repeatability was evaluated based on the LC analysis of their digests.

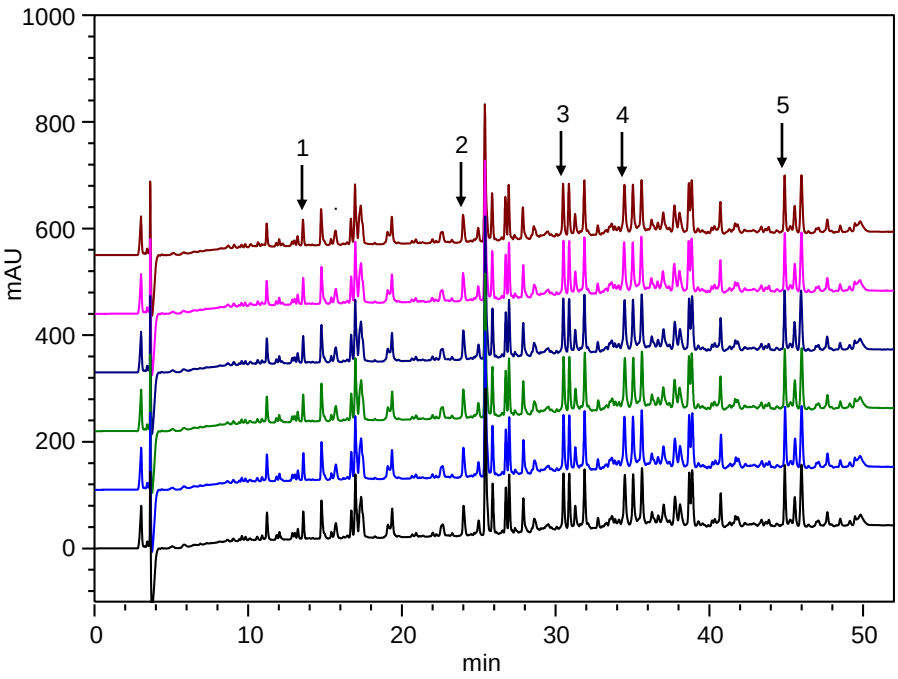
◆ Summary of Peptide Mapping Method ◆



In this method, the changes in constituting amino acids are confirmed by comparing the chromatographic patterns after the peptide fragments formed by the chemical or enzymatic digestion of proteins are separated and detected by instruments such as LC.

* Peptide mapping is a method specified based on the harmonization of three pharmacopoeias (USP, EP, JP) and is described as a reference in the JP (Japanese Pharmacopoeia) 16th edition.

■ Analysis Example of BSA (Bovine Serum Albumin) Digest



<Analysis Conditions>
Column : LaChrom C18 (5 μm) 4.6 mm I.D. × 250 mm
Eluents : A) 0.1 % TFA/H_2O(v/v)
B) 0.1 % TFA/CH_3CN(v/v)
* Gradient
Flow rate : 1.0 mL/min
Column temperature : 40°C
Detection wavelength : UV 215 nm
Injection volume : 10 μL
* A dynamic mixer is used.

<Sample Preparation>
Sample (BSA)
| ← Addition of trypsin at 1/100 of BSA by weight
Reaction 37°C, 16 hr
|
Heat treatment 90°C, 10 min
|
Centrifuge 10,000 rpm, 10 min, 3°C
|
Use the supernatant as the sample for injection

■ Peak Retention Time and Peak Area Repeatability (n=6)

[Retention Time]

Peak No.	1	2	3	4	5
Average	13.579	23.998	30.508	34.488	44.911
SD	0.009	0.012	0.012	0.018	0.012
%RSD	**0.06**	**0.05**	**0.04**	**0.05**	**0.03**

[Peak Area]

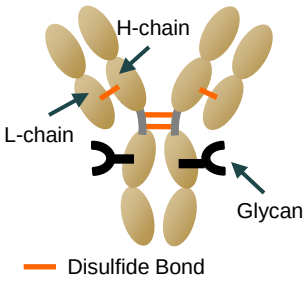
Peak No.	1	2	3	4	5
Average	313951	477180	729175	922057	814068
SD	6957	11499	17599	22397	21035
%RSD	**2.22**	**2.41**	**2.41**	**2.43**	**2.58**

When the analysis was repeated six times, the repeatability for the retention times (% RSD) was 0.06% or less and that for the peak areas (% RSD) was 2.6% or less, indicating that extremely good repeatabilities were obtained. Therefore, it was confirmed that the peptide mapping method allows the highly reliable chromatographic pattern analysis.

◆Structure of Antibody Drug (IgG)

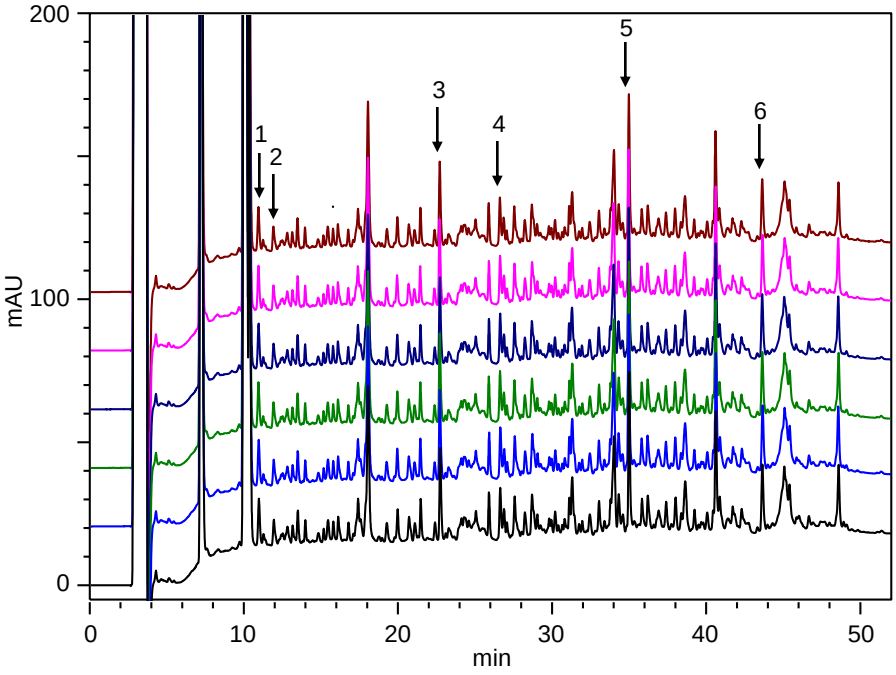
Among biomedicines, many antibody drugs prepared by making use of antibodies (immunoglobulins) are proteins having glycan structures and they are especially drawing attention for their high specificities and usefulness. While there are several types of immunoglobulins, IgG is mainly put to practical use for antibody drugs and it characteristically has a "Y"-shaped four-chain structure. Two each of the H-chain and L-chain are linked by S-S bonds (disulfide bonds) so as to form a Y-shaped heterotetramer. It is also considered that the structures of the glycans, that are linked due to post translational modifications, largely affect the activities of the medicines.

This time, IgG was subjected to a reductive alkylation and then, trypsin-digested. The fragments of the peptide was analyzed by the LC and the repeatability of the peak retention time and areas were evaluated based on the chromatographic patterns.



Basic Structure of IgG

■ Analysis Example of IgG Digest



<Analysis Conditions>

Column	: LaChrom C18 (5 μm) 4.6 mm I.D. × 250 mm
Eluents	: A) 0.1 % TFA/H_2O(v/v) B) 0.1 % TFA/CH_3CN(v/v) * Gradient
Flow rate	: 1.0 mL/min
Column temperature	: 40°C
Detection wavelength	: UV 215 nm
Injection volume	: 10 μL

* A dynamic mixer is used.

<Sample Preparation>

Sample (IgG)
↓ ← Add dithiothreitol (DTT) to make the final concentration of 10 mmol/L
Reaction 37°C, 30 min
↓ ← Add iodoacetamide (IAA) to make the final concentration of 40 mmol/L
Reaction 37°C, 30 min, light shielded
↓ ← Add cysteine (Cys) to make the concentration of 40 mmol/L
Reaction 37°C, 30 min
↓ ← Add trypsin at 1/100 of IgG by weight
Reaction 37°C, 16 hr
↓
Heat treatment 90°C, 10 min
↓
Centrifuge 10,000 rpm, 10 min 3°C,
↓
Use the supernatant as the sample for injection

(1) Reductive alkylation

■ Peak Retention Time and Peak Area Repeatability (n=6)

[Retention Time]

Peak No.	1	2	3	4	5	6
Average	10.960	11.934	22.716	26.630	34.979	43.647
SD	0.012	0.011	0.013	0.010	0.013	0.010
%RSD	0.11	0.09	0.06	0.04	0.04	0.02

[Peak Area]

Peak No.	1	2	3	4	5	6
Average	131165	80680	227672	135253	418818	200076
SD	2547	2585	6966	4474	16010	8164
%RSD	1.94	3.20	3.06	3.31	3.82	4.08

When the analysis was repeated six times, the repeatability for the peak retention times (%RSD) was 0.1% or less and that for the peak areas was 4.1% or less, indicating that extremely good repeatabilities were obtained. It was shown that the highly reliable analysis of chromatographic patterns is possible even for complex peptides such as digested IgG.

Main components of the instrument: Chromaster
5110 pump (low pressure Gr, dynamic mixer), 5210 autosampler, 5310 column oven, 5410 UV detector

Note: The data here is shown as an example of the analysis and does not warrant the performance of the instrument.