NOMURA CHEMICAL

Technical Report No.32

Analysis of peptides/proteins and monoclonal antibodies by UHPLC part II

1. Column for polymer compounds

Proteins and monoclonal antibodies are compounds with molecular weights of tens of thousands to hundreds of thousands. A column with a pore size of about 300 Å is required to analyze these.

The FlexFire WP series has a lineup of 4 types at 300 Å and 1 type as a column dedicated to monoclonal antibodies (1000 Å), for a total of 5 types, and you can select according to your purpose.

In this report, we will introduce the usefulness of wide pore columns, targeting higher molecular compounds than the previous one.

2. Specification

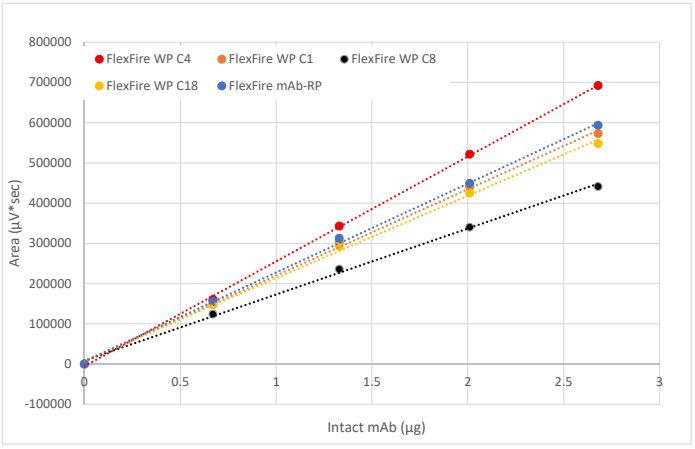
	FlexFire mAb-RP	FlexFire WP C4	FlexFire WP C18	FlexFire WP C8	FlexFire WP C1
Particle size	2.6μm, 5μm	2.6μm, 5μm	2.6μm, 5μm	2.6μm, 5μm	2.6μm, 5μm
Chemistry	Butyl	Butyl	Octadecyl	Octyl	Trimethyl
Surface area	24m^2/g	170m^2/g	170m^2/g	170m^2/g	170m^2/g
Pore volume	1.4mL/g	1.4mL/g	1.4mL/g	1.4mL/g	1.4mL/g
Pore diametter	100nm	30nm	30nm	30nm	30nm
Carbon	1.3%	5%	15%	7%	3%
End-cap	○	○	○	○	○
pH	pH1-10	pH1-10	pH1-10	pH1-10	pH1-10
Temperature	~80℃	~80℃	~80℃	~80℃	~80℃
Pressure range	2.6μm: 600bar (=60Mpa=8,702psi)	2.6μm: 600bar (=60Mpa=8,702psi)	2.6μm: 600bar (=60Mpa=8,702psi)	2.6μm: 600bar (=60Mpa=8,702psi)	2.6μm: 600bar (=60Mpa=8,702psi)
	5μm: 300bar (=30Mpa=4,351psi)	5μm: 300bar (=30Mpa=4,351psi)	5μm: 300bar (=30Mpa=4,351psi)	5μm: 300bar (=30Mpa=4,351psi)	5μm: 300bar (=30Mpa=4,351psi)

3. mAb recovery rate

The recovery rate of mAb (150KDa) in each column was calculated.

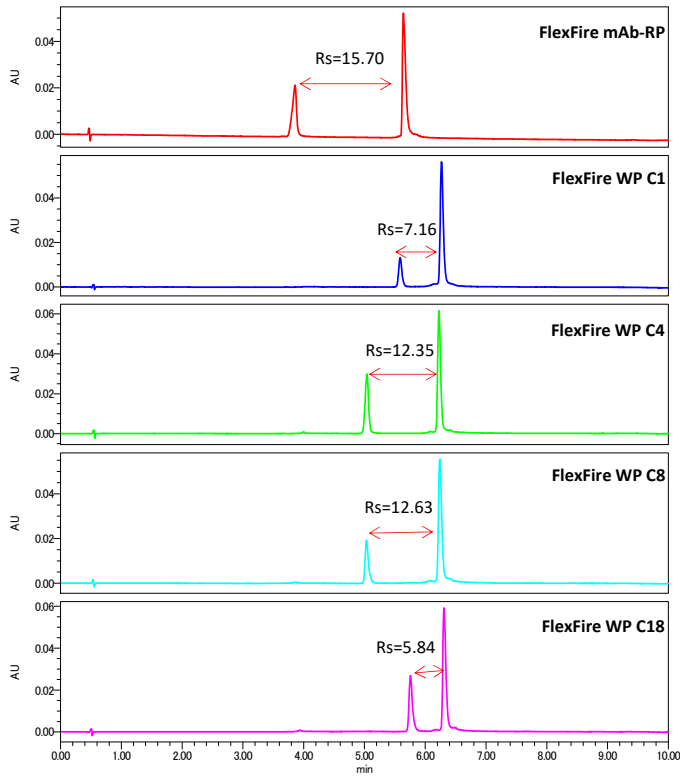
It shows a high recovery rate in every column.

Column	R^2	Recovery (%)
FlexFire WP C1	0.99886	104
FlexFire WP C4	0.99961	103
FlexFire WP C8	0.99795	89.8
FlexFire WP C18	0.99823	98.1
FlexFire mAb-RP	0.99886	101



FlexFire

4. Comparison of separation between myoglobin and impurities.



Conditions;

Column: FlexFire WP Series, 2.6μm (2.0x50mm)

Mobile phase: A) Water+0.1%TFA B) Acetonitrile+0.1%TFA

Gradient:

min	mL/min	%A	%B	Curve
0.00	0.3	80	20	
8.40	0.3	40	60	6
8.42	0.3	80	20	6

Temperature: 40℃

Detection: UV280nm

Sample: Myoglobin

Injection volume: 0.2μL

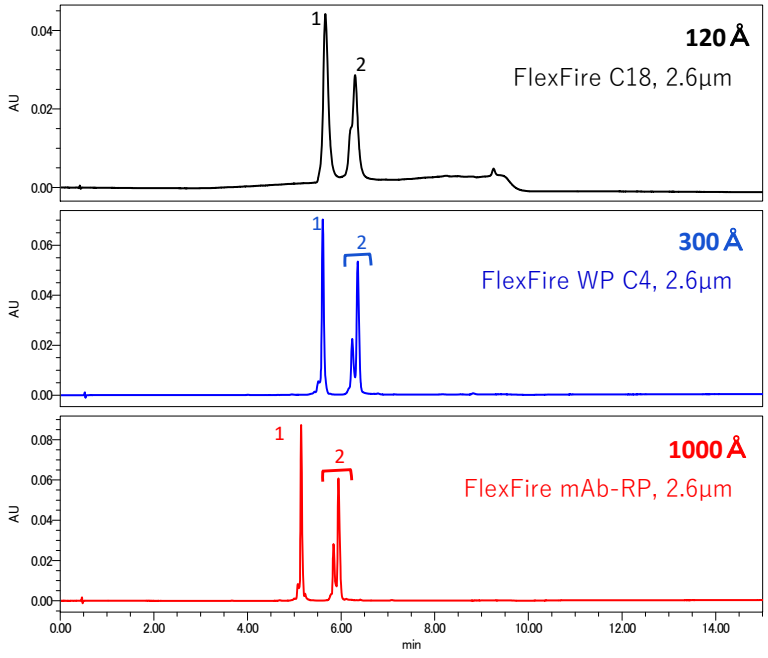
System: Waters ACQUITY UPLC H-Class PLUS

Mixer: 100μL

The separation of myoglobin and impurities is good with either column.

Since high resolution can be obtained with FlexFire mAb-RP, WP C8, and WP C4, it can be expected to be effective in separating multiple components.

5. Analysis of Lactalbumin



Conditions;

Column: FlexFire C18, 2.6um (2.0x50mm)
FlexFire WP C4, 2.6um (2.0x50mm)
FlexFire mAb-RP, 2.6um (2.0x50mm)

Mobile phase: A) Water + 0.1%TFA B) Acetonitrile + 0.1%TFA

Gradient:

min	mL/min	%A	%B	Curve
0.00	0.3	80	20	
8.40	0.3	40	60	6
8.42	0.3	80	20	6

Temperature: 40℃

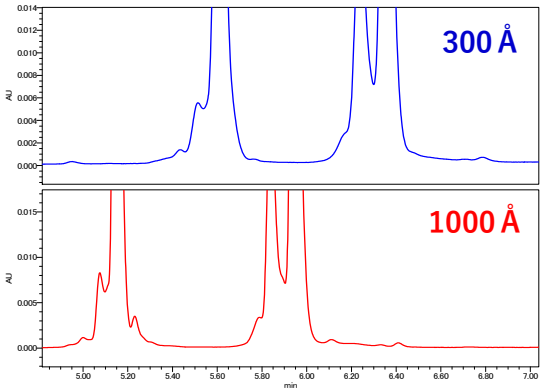
Detection: UV280nm

Sample: 1. α-Lactalbumin (0.34mg/mL)
2. β-Lactoglobulin (1.00mg/mL)

Injection volume: 2.0uL

System: Waters ACQUITY UPLC H-Class PLUS

Mixer: 100uL

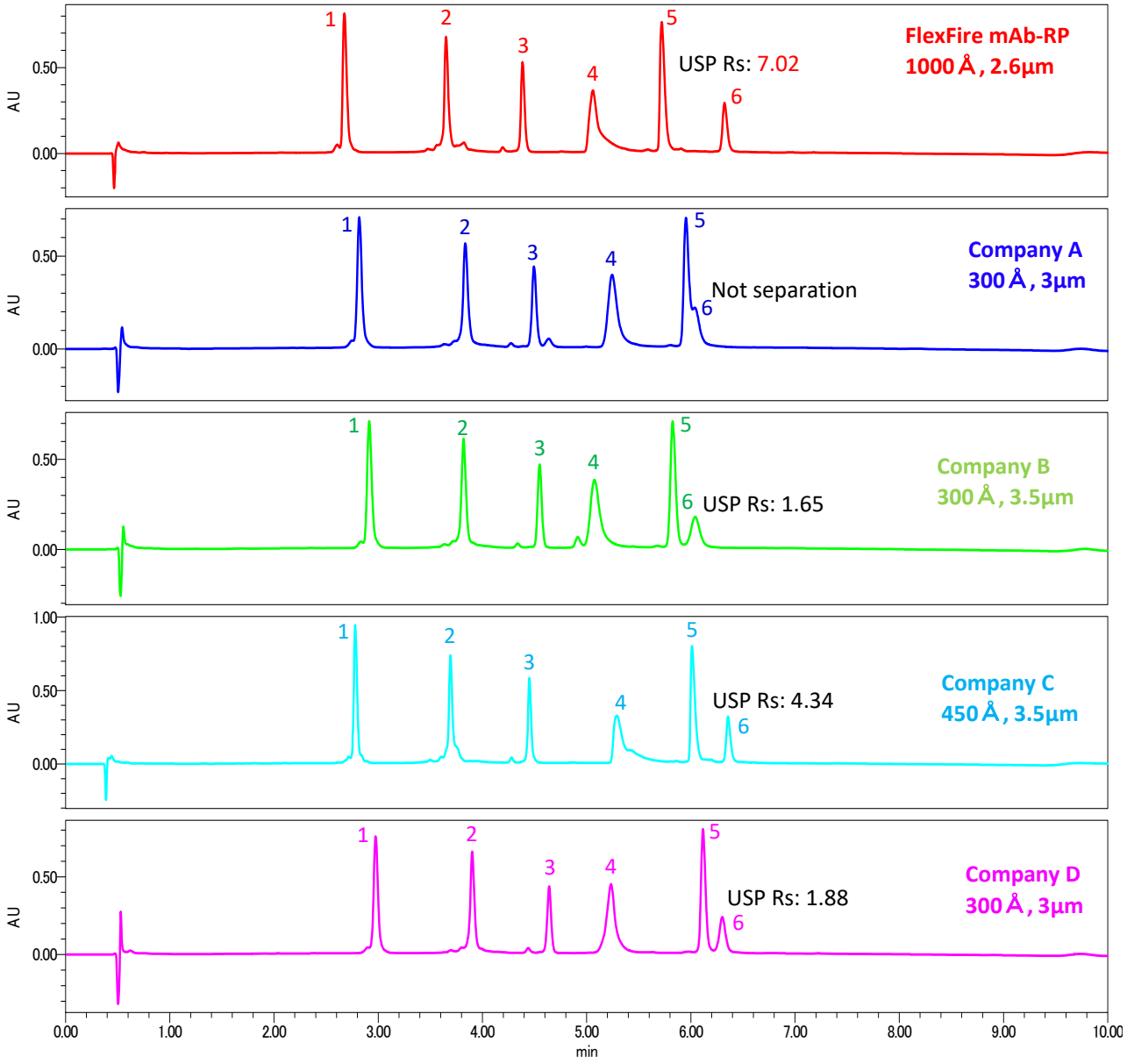


Compared to columns with a pore size of 120 Å, columns with 300 Å and 1000 Å have better peak separation and sensitivity.

Especially at 1000 Å, clearer peak separation is achieved.

FlexFire

6. Separation and comparison of multi-component proteins.



Conditions:

Column: FlexFire mAb-RP, 2.6µm (2.0x50mm)
Mobile phase: A) Water + 0.1%TFA B) Acetonitrile + 0.1%TFA

Gradient:

min	mL/min	%A	%B	Curve
0.00	0.3	80	20	
8.40	0.3	40	60	6
8.42	0.3	80	20	6

Temperature: 40℃
Detection: UV210nm
Sample: 1.Ribonuclease A (13.7KDa)
2.Cytochrome C (12.4KDa)
3.Lysozyme (14.3KDa)
4.BSA (66.3KDa)
5.Myoglobin (11.2KDa)
6.Catalase (220KDa)
Injection volume: 2.0µL

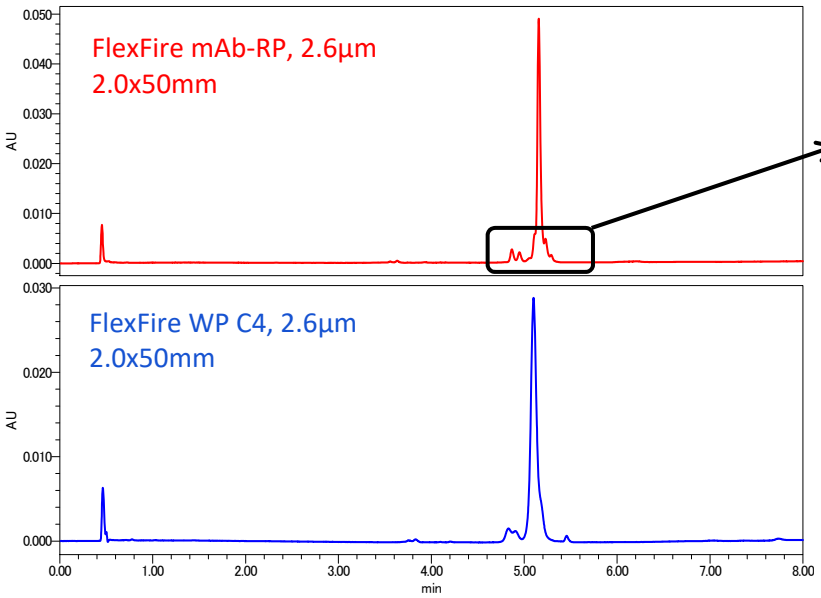
System: Waters ACQUITY UPLC H-Class PLUS
Mixer: 100µL

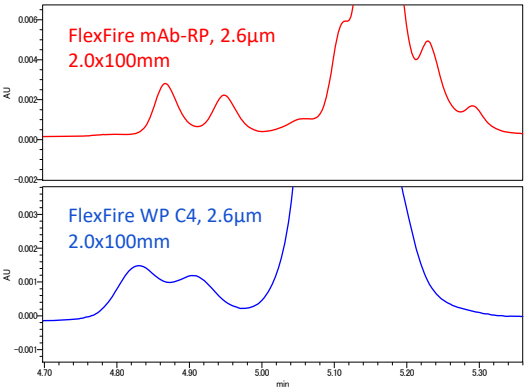
Separation with 10KDa-60KDa is possible even with a column with a pore diameter of 300Å.

It can be seen that among the 200KDa protein, the column with the larger pore size achieved the separation predominantly.

By using FlexFire mAb-RP, not only separation between macromolecules but also detection of impurities contained in proteins can be expected.

7. Analysis of mAb





Conditions;

Column: FlexFire mAb-RP, 2.6µm (2.0x50mm)
FlexFire WP C4, 2.6µm (2.0x50mm)

Mobile phase: A) Water + 0.1%TFA B) Acetonitrile + 0.1%TFA

Gradient:

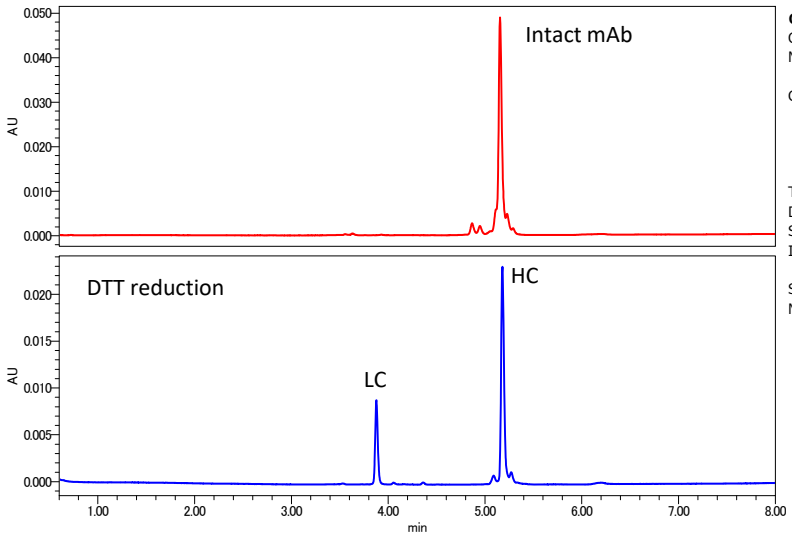
min	mL/min	%A	%B	Curve
0.00	0.3	80	20	
8.40	0.3	40	60	6
8.42	0.3	80	20	6

Temperature: 40℃
Detection: UV280nm
Sample: NISTmAb
Injection volume: 1.0µL

System: Waters ACQUITY UPLC H-Class PLUS
Mixer: 100µL

Technical Report No. 30 focused on applications with a pore diameter of 300 Å, but this report uses a column with a pore diameter of 1000 Å.
A 1000 Å column can find clearer peaks and gain new insights.

Technical Report No. 30 has an example of analysis using a 300 Å column.



Conditions;

Column: FlexFire mAb-RP, 2.6µm (2.0x50mm)

Mobile phase: A) Water + 0.1%TFA B) Acetonitrile + 0.1%TFA

Gradient:

min	mL/min	%A	%B	Curve
0.00	0.3	80	20	
8.40	0.3	40	60	6
8.42	0.3	80	20	6

Temperature: 40℃
Detection: UV280nm
Sample: NISTmAb, Reduced NISTmAb
Injection volume: 1.0µL

System: Waters ACQUITY UPLC H-Class PLUS
Mixer: 100µL

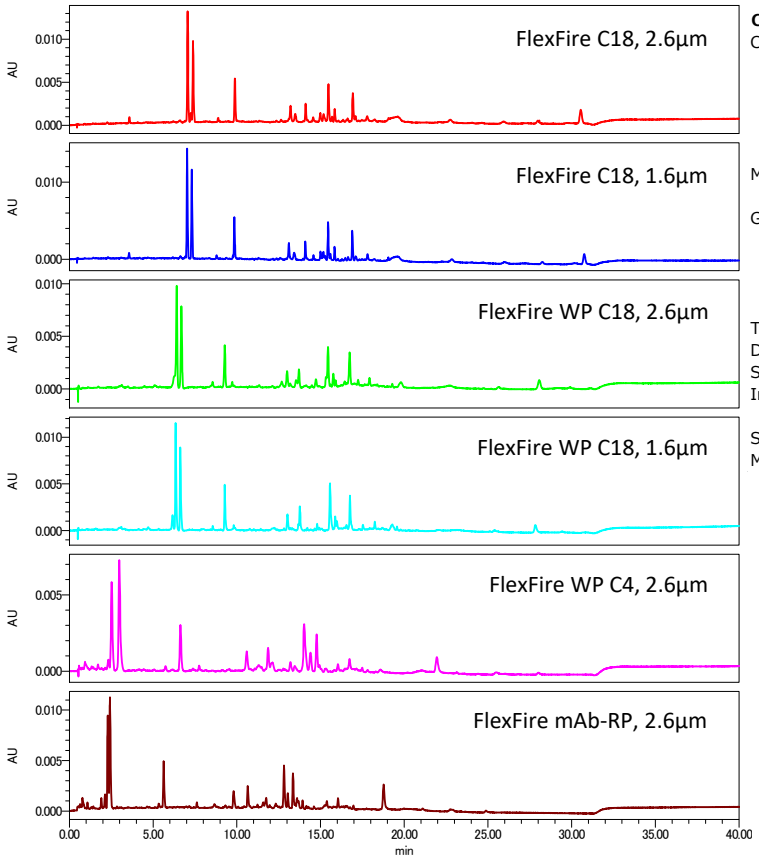
Analysis of mAb reducer

20 mM DTT was mixed 1: 1 with NISTmAb and reacted at 40 degree-2 hr.

Technical Report No. 30 has an example of analysis using a 300 Å column.

FlexFire

8. Peptide mapping (BSA digest)



Conditions;

Column: FlexFire C18, 2.6µm (2.0x50mm)
FlexFire C18, 1.6µm (2.0x50mm)
FlexFire WP C18, 2.6µm (2.0x50mm)
FlexFire WP C18, 1.6µm (2.0x50mm)
FlexFire WP C4, 2.6µm (2.0x50mm)
FlexFire mAb-RP, 2.6µm (2.0x50mm)

Mobile phase: A) Water + 0.1%TFA B) Acetonitrile + 0.1%TFA

Gradient:

min	mL/min	%A	%B	Curve
0.00	0.3	80	20	
8.40	0.3	40	60	6
8.42	0.3	80	20	6

Temperature: 40℃
Detection: UV280nm
Sample: BSA Digest
Injection volume: 1.0µL

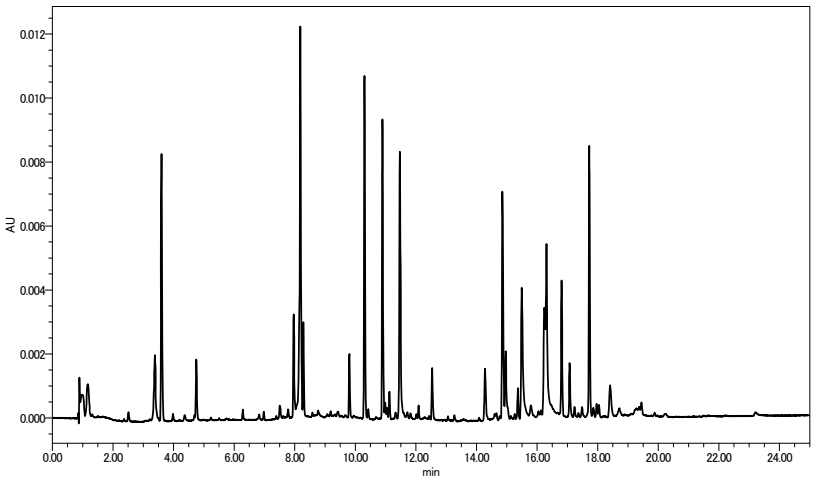
System: Waters ACQUITY UPLC H-Class PLUS
Mixer: 100µL

The Thermo Scientific ™ SMART Digest ™ Kit was used to digest the protein.

When including intact analysis, it is necessary to select a column suitable for the molecular weight, but if only peptides that have been reduced in molecular weight by digestion, a standard column such as 120 Å can be selected.

※ Thermo Scientific ™ SMART Digest ™ Kit is a registered product of Thermo Fisher Scientific Co., Ltd. 。

8-1. mAb digest



Conditions;

Column: FlexFire mAb-RP, 2.6µm (2.0x50mm)
Mobile phase: A) Water + 0.1%TFA B) Acetonitrile + 0.1%TFA

Gradient:

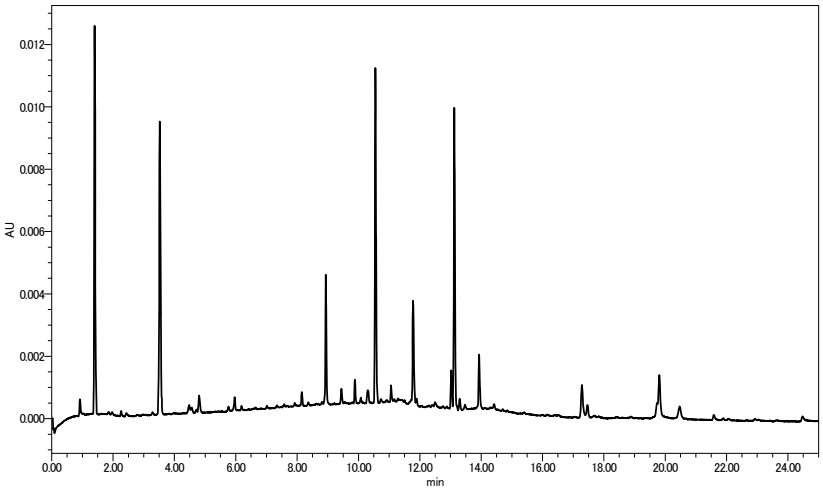
min	mL/min	%A	%B	Curve
0.00	0.3	80	20	
8.40	0.3	40	60	6
8.42	0.3	80	20	6

Temperature: 80℃
Detection: UV280nm
Sample: NISTmAb Digest
Injection volume: 1.0µL

System: Waters ACQUITY UPLC H-Class PLUS
Mixer: 100µL

mAb is a compound with a molecular weight of approximately 150 KDa.
The usefulness of intact mAb 1000Å has been found in the analysis of "7. Monoclonal antibody".
Even in peptide mapping, you can complete the process from intact analysis to digestion analysis with a single column without changing the column.

8-2. Hemoglobin digest



Conditions;

Column: FlexFire mAb-RP, 2.6µm (2.0x50mm)
Mobile phase: A) Water + 0.1%TFA B) Acetonitrile + 0.1%TFA

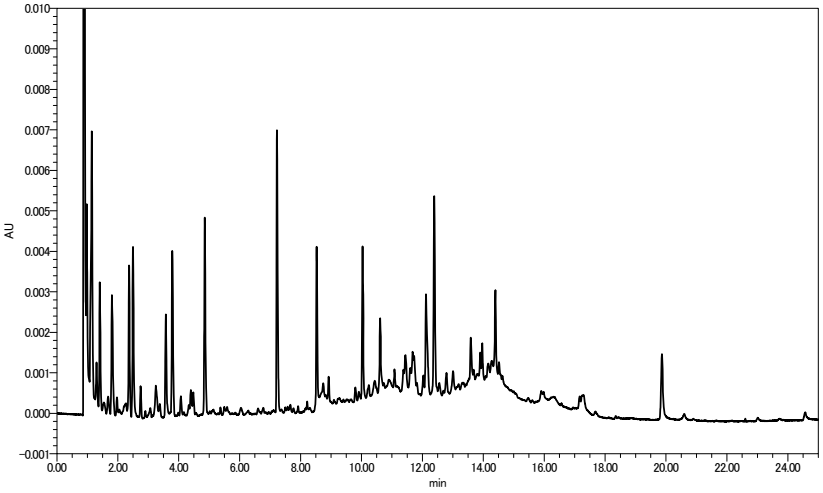
Gradient:

min	mL/min	%A	%B	Curve
0.00	0.3	80	20	
8.40	0.3	40	60	6
8.42	0.3	80	20	6

Temperature: 40℃
Detection: UV280nm
Sample: Hemoglobin Digest
Injection volume: 1.0µL

System: Waters ACQUITY UPLC H-Class PLUS
Mixer: 100µL

8-3. ADH digest



Conditions;

Column: FlexFire mAb-RP, 2.6µm (2.0x50mm)
Mobile phase: A) Water + 0.1%TFA B) Acetonitrile + 0.1%TFA

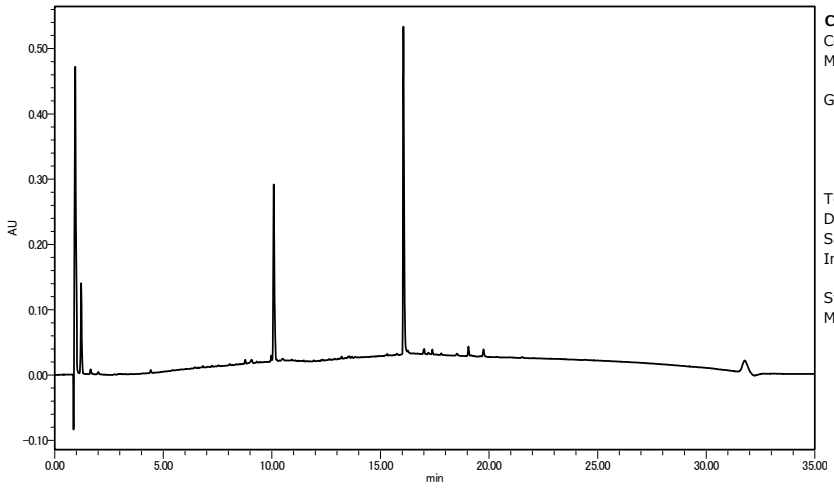
Gradient:

min	mL/min	%A	%B	Curve
0.00	0.3	80	20	
8.40	0.3	40	60	6
8.42	0.3	80	20	6

Temperature: 40℃
Detection: UV280nm
Sample: ADH Digest
Injection volume: 1.0µL

System: Waters ACQUITY UPLC H-Class PLUS
Mixer: 100µL

8-4. Insulin digest



Conditions;

Column: FlexFire mAb-RP, 2.6µm (2.0x50mm)
Mobile phase: A) Water + 0.1%TFA B) Acetonitrile + 0.1%TFA

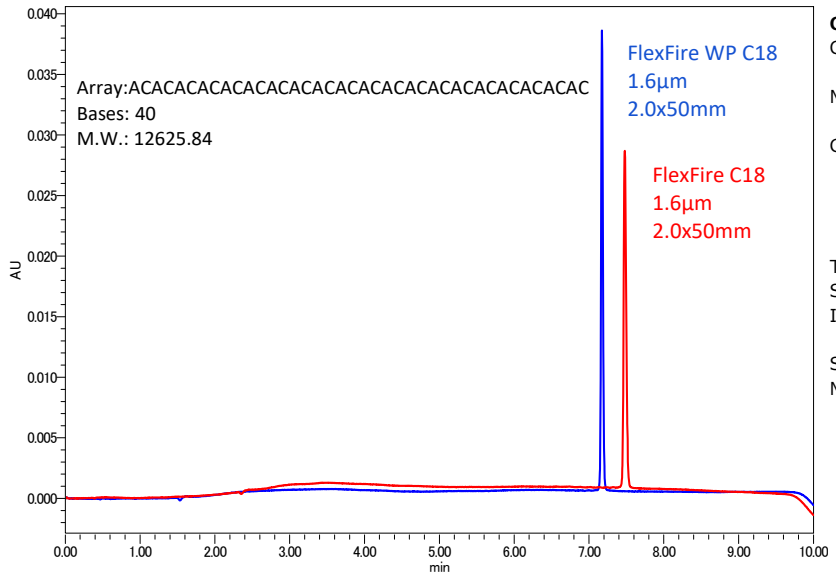
Gradient:

min	mL/min	%A	%B	Curve
0.00	0.3	80	20	
8.40	0.3	40	60	6
8.42	0.3	80	20	6

Temperature: 40℃
Detection: UV280nm
Sample: Insulin Digest
Injection volume: 1.0µL

System: Waters ACQUITY UPLC H-Class PLUS
Mixer: 100µL

9. Analysis of synthetic RNA



Conditions:

Column: FlexFire C18, 1.6µm (2.0x50mm)
FlexFire WP C18, 1.6µm (2.0x50mm)

Mobile phase: A) 100mM HFIP + 10mM TEA B) Methanol

Gradient:

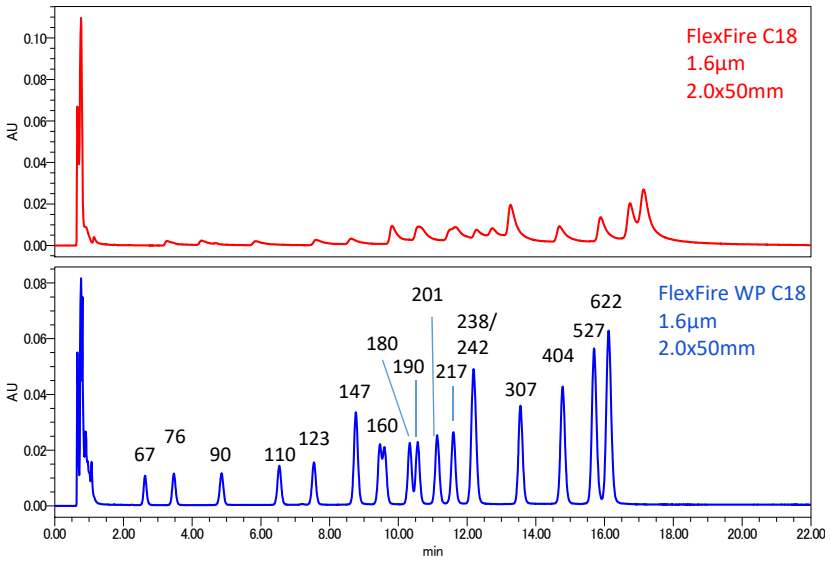
min	mL/min	%A	%B	Curve
0.00	0.3	95	5	
8.60	0.3	80	20	6
8.61	0.3	95	5	6

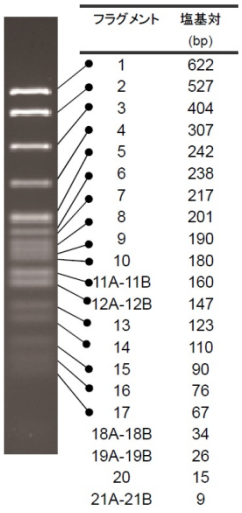
Temperature: 50℃
Sample: RNA
Injection volume: 0.2µL

System: Waters ACQUITY UPLC H-Class PLUS
Mixer: 100µL

By using a wide pore column, it can be detected more sensitively than a 120Å column.

10. Analysis of pBR322 digest





Conditions:

Column: FlexFire C18, 1.6µm (2.0x50mm)
FlexFire WP C18, 1.6µm (2.0x50mm)

Mobile phase: A) 0.1M TEAA, pH7.0 B) 0.1M TEAA, pH7.0/ACN=80/20

Gradient:

min	mL/min	%A	%B	Curve
0.00	0.2	42.5	57.5	
20.0	0.2	15.5	84.5	6
20.1	0.2	42.5	57.5	6

Temperature: 50℃
Sample: pBr322 MspI Digest
Injection volume: 10µL

System: Waters ACQUITY UPLC H-Class PLUS
Mixer: 100µL

In the analysis of polymer compounds, analysis that cannot be achieved with FlexFire C18 (120 Å) can be performed by using 300 Å “FlexFire WP C18”.

FlexFire

■お問い合わせ/Contact us

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