

1. Column for Macromolecule

Proteins and monoclonal antibodies (mAbs) have molecular weights of tens to hundreds of kilodaltons. In order to fit the requirements of your analysis for these compounds, the FlexFire WP (Wide Pore) series have a lineup of four types at 300 Å and one monoclonal antibody dedicated column (1000 Å), for a total of five types. This wide range of options allows users to select the best column to fit their needs.

Here we introduce the usefulness of wide pore columns, targeting even larger macromolecules than in the previous report “Monoclonal Antibody Analysis”.

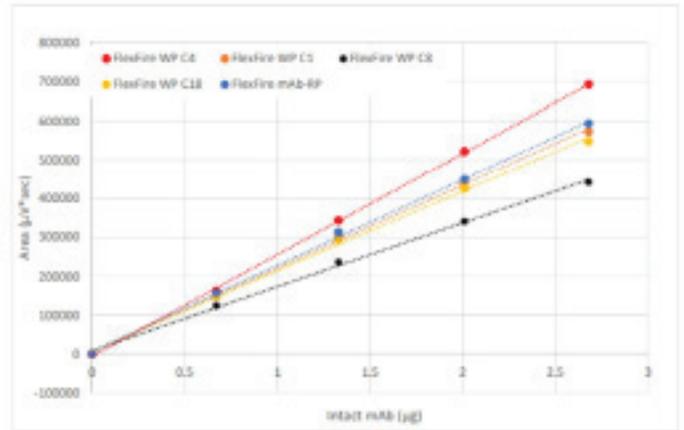
2. FlexFire WP Series Specifications

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

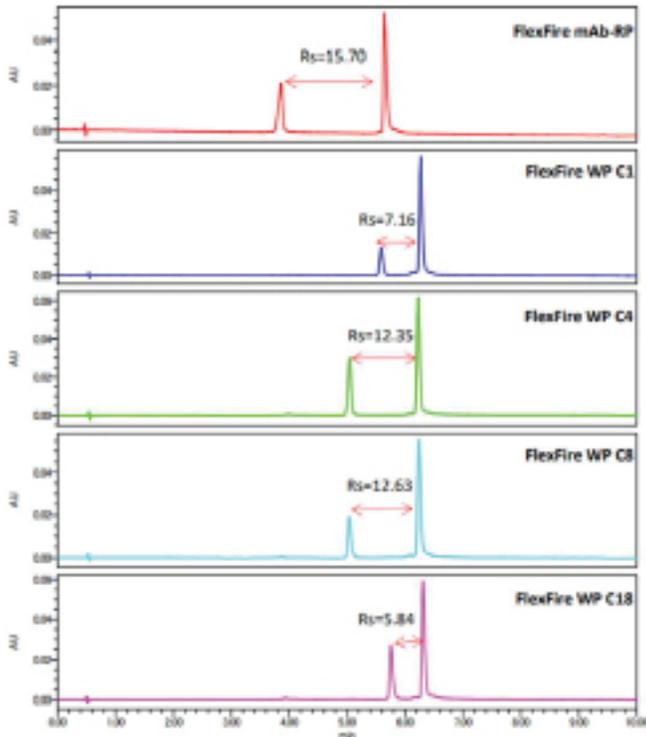
3. Recovery Rate of mAbs

The figure and table show the recovery rate of mAbs(M.W.150K) by each column. Columns with alkyl chains of C4 or less provide better linearity and higher recovery rate.

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



4. Comparison of Separation of Myoglobin and Unknown Compounds



Conditions:

Column: FlexFire WP Series, 2.6µm (3.0x50mm)
 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	G
8.42	0.3	80	20	G

Temperature: 40°C

Detection: UV280nm

Sample: Myoglobin

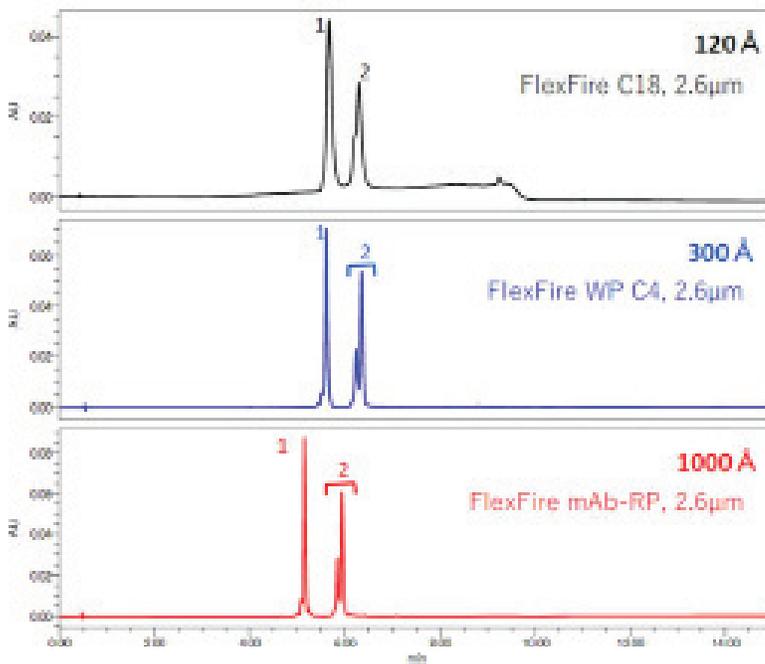
Injection volume: 0.3µL

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

Separation of myoglobin and unknown compounds

All columns give good results of separation of myoglobin and unknown compounds. Since a high degree of separation is obtained with FlexFire mAb-RP, WP C8, and WP C4, it is effective in separating multiple components.

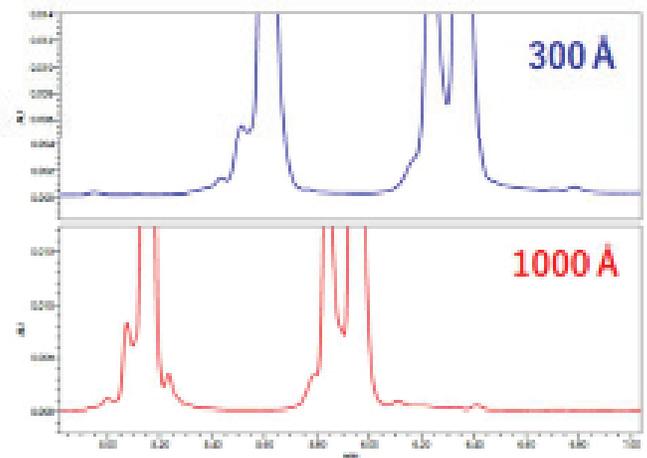
5. Analysis of Protein



Conditions;
 Column: FlexFire C18, 2.6µm (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

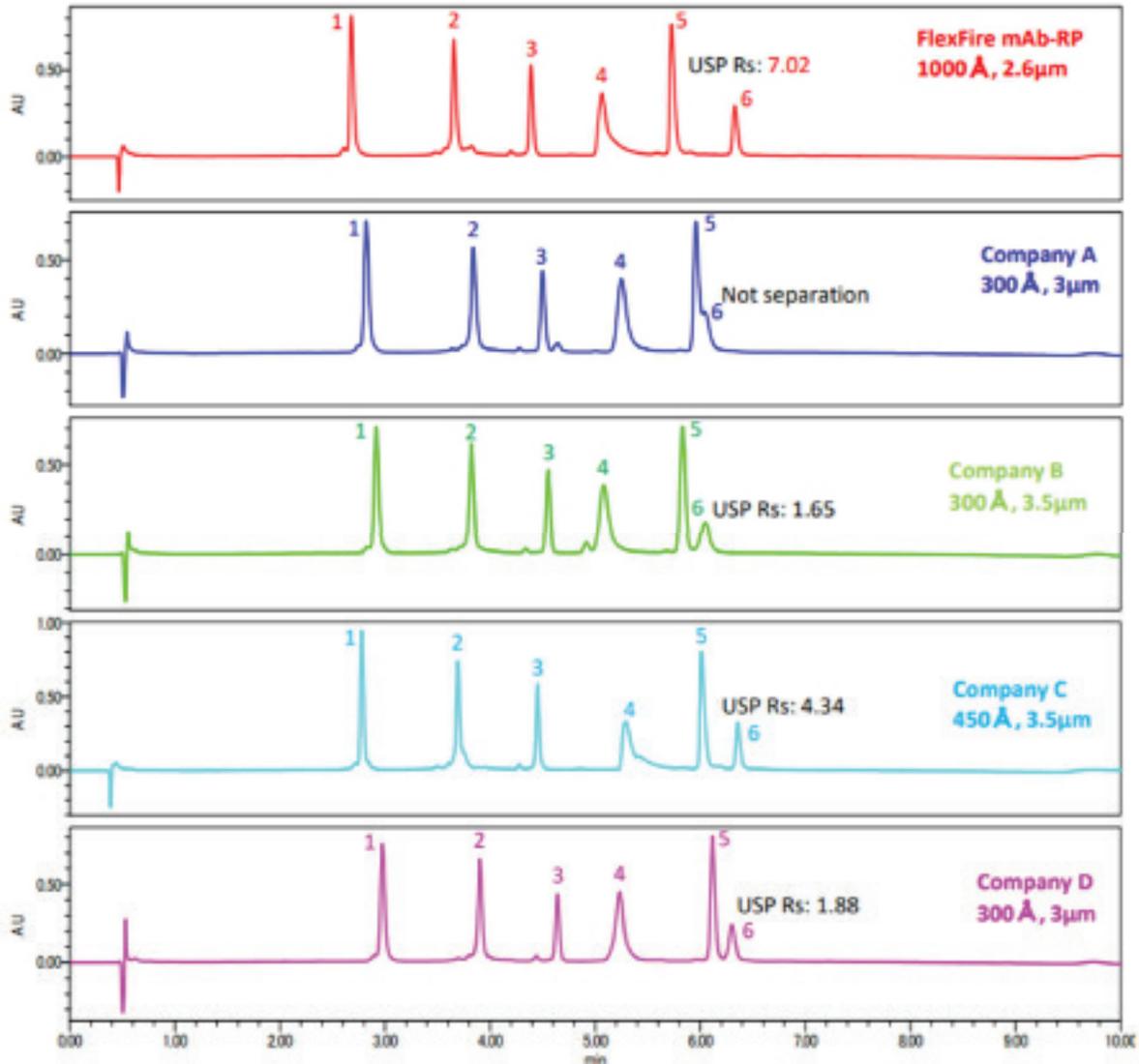
Temperature: 40°C
 Detection: UV280nm
 Sample: 1. α-Lactalbumin (0.34mg/mL)
 2. β-Lactoglobulin (1.00mg/mL)
 Injection volume: 2.0µL
 System: Waters ACQUITY UPLC H-Class PLUS
 Mixer: 100µL



Analysis of Lactalbumin

Compared to a column with pore size 120Å, those with pore size 300Å and 1000Å presented better peak shapes. Especially the column with pore size 1000Å achieved clearer peak separation.

6. Separation Comparison of Multi-component Proteins.



Conditions:

Column: FlexFire mAb-RP, 2.6 μ m (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 $^{\circ}$ C

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: 2.0 μ L

System:

Waters ACQUITY UPLC H-Class PLUS

Mixer:

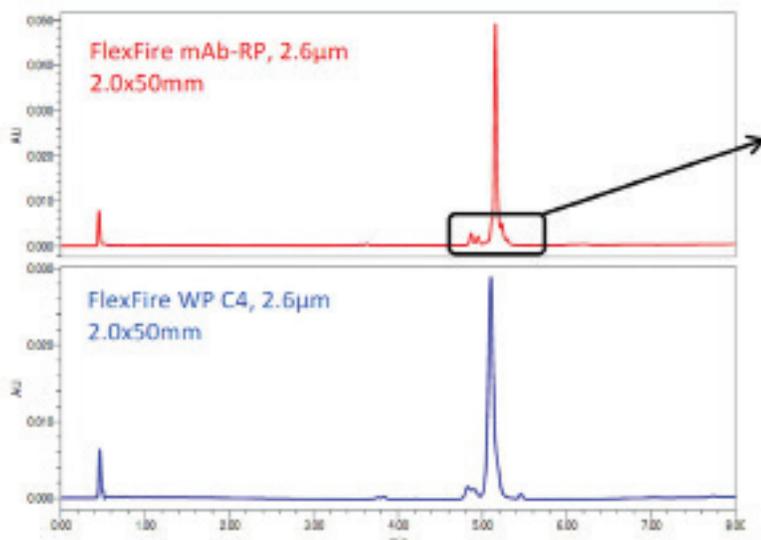
100 μ L

Comparison of separation between myoglobin and catalase

Even though it is possible for a column with a pore size of 300 \AA to separate at MW 10kDa-60kDa, for macromolecules with MWs of 200 kDa larger pore sizes such as 1000 \AA and 450 \AA achieved better separation.

By using FlexFire mAb-RP, it can be expected not only to separate between macromolecules but also to detect unknown compounds contained in protein samples.

7. Monoclonal Antibody Analysis



Conditions:

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

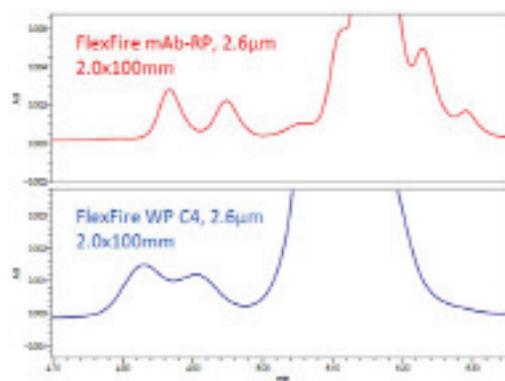
Mobile phase: A) Water + 0.1%TFA B) Acetonitrile + 0.1%TFA

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°C
Detection: UV280nm
Sample: NISTmAb
Injection volume: 1.0 μ L

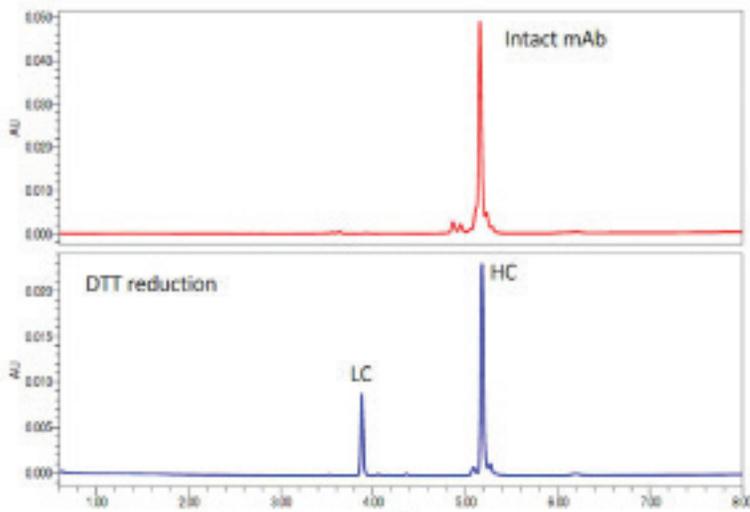
System: Waters ACQUITY UPLC H-Class PLUS
Mixer: 100 μ L



Monoclonal antibody analysis example

In the previous technical report “Antibody Analysis with UHPLC-MS”, we focused on using columns with a 300Å pore size. In this report, we found columns with a pore size of 1000Å produce sharper peaks and enabled us to gain new insights.

You can find analysis examples of a column with pore size 300Å in the technical report “Antibody Analysis with UHPLC-MS”.



Conditions:

Column: FlexFire mAb-RP, 2.6 μ m (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°C
Detection: UV280nm
Sample: NISTmAb, Reduced NISTmAb
Injection volume: 1.0 μ L

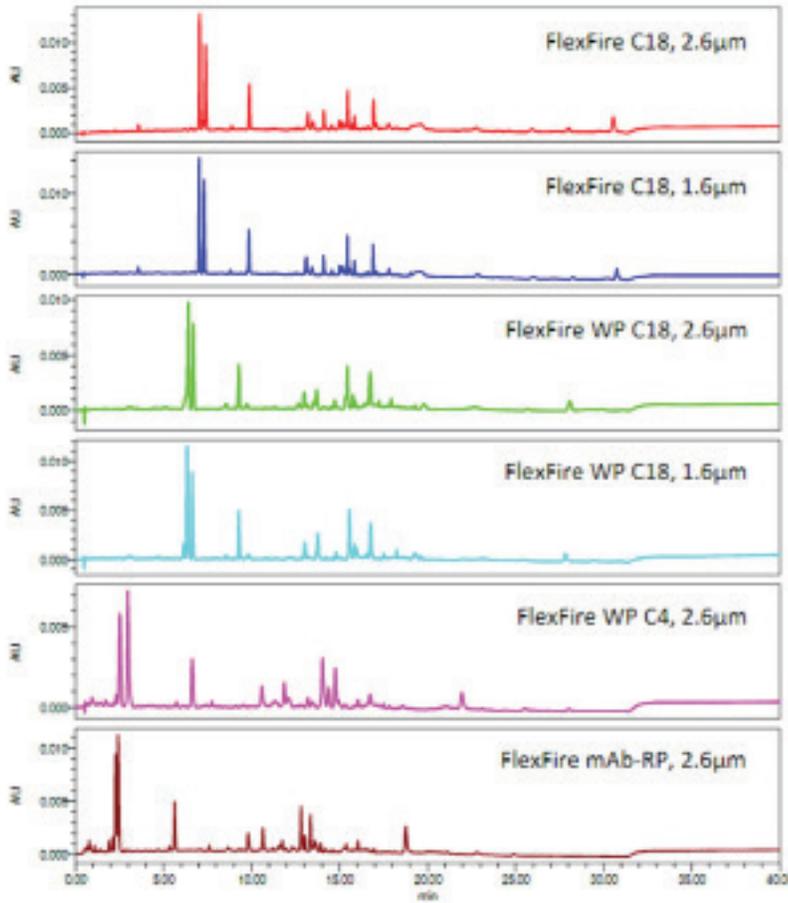
System: Waters ACQUITY UPLC H-Class PLUS
Mixer: 100 μ L

Analysis of mAb reductant

The reductant was made by mixing 20 mM DTT with NISTmAb in 1: 1, and the reaction was carried out at 40 °C-2 hrs.

You can find analysis examples of a column with pore size 300Å in the technical report “Antibody Analysis with UHPLC-MS”.

8. Peptide Mapping



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:

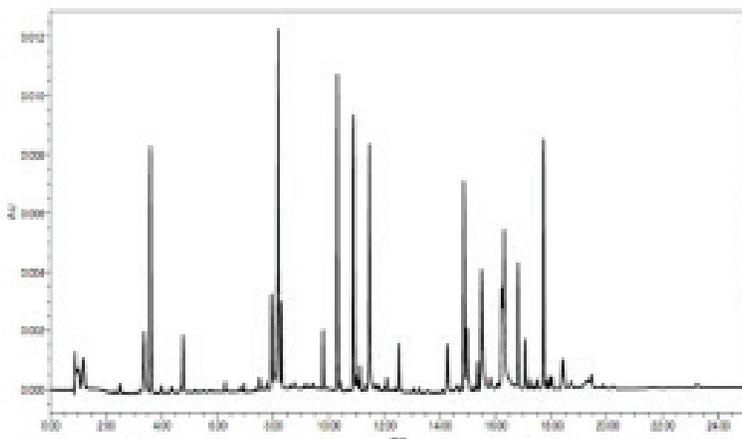
min	mL/min	%A	%B	Curve
0.00	0.3	80	20	
8.40	0.3	40	60	6
8.42	0.3	80	20	6

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

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

“Thermo Scientific TM SMART Digest TM Kit (Thermo Fisher Scientific)” was used for protein digestion. If your samples include only digested peptides, a standard column with a pore size of 120Å can be an option. However, you can get better results if you match the pore size of the column to the molecular weight

8-1. mAb Tryptic Digest



Conditions:

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

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

Gradient:

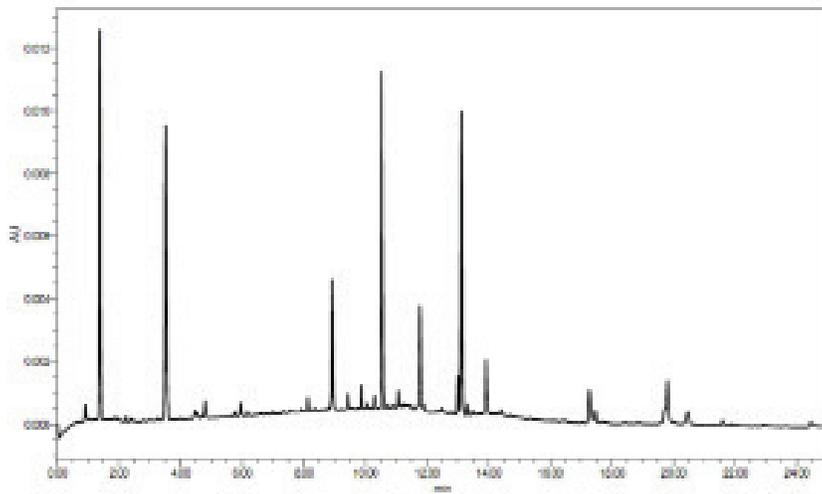
min	mL/min	%A	%B	Curve
0.00	0.3	80	20	
8.40	0.3	40	60	6
8.42	0.3	80	20	6

Temperature: 60°C
 Detection: UV280nm
 Sample: NISTmAb Digest
 Injection volume: 1-8µL

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

Monoclonal antibodies frequently have molecular weights near 150 kDa. In “7. Monoclonal antibody analysis”, the utility of using mAbs 1000Å pore size for intact mAbs was revealed. You can use the same column for both intact analysis and digest analysis in peptide mapping.

8-2. Tryptic Digest of Hemoglobin



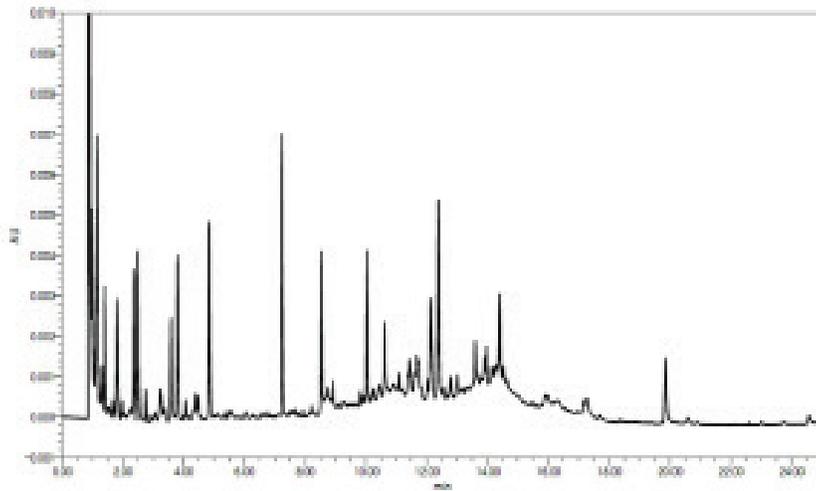
Conditions:
 Column: FlexFire mAb-RP, 2.6µm (2.0x50mm)
 Mobile phase: A) Water + 0.1%TFA B) Acetonitrile + 0.1%TFA

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°C
 Detection: UV280nm
 Sample: Hemoglobin Digest
 Injection volume: 1.0µL

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

8-3. Tryptic Digest of ADH



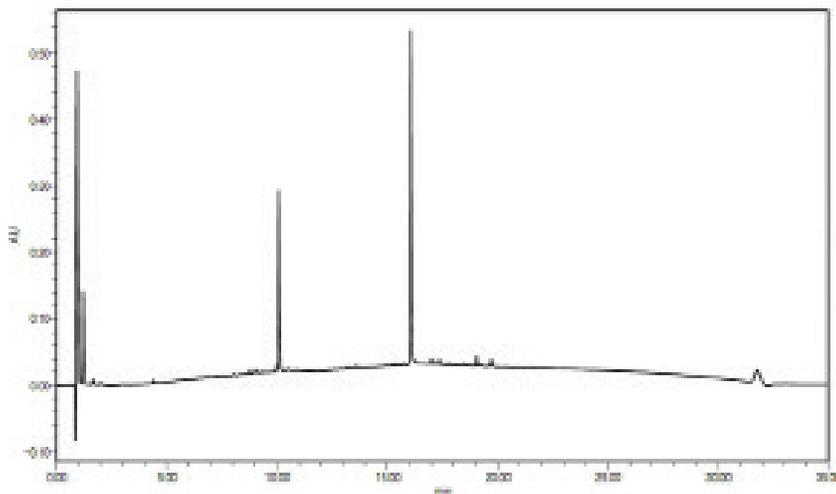
Conditions:
 Column: FlexFire mAb-RP, 2.6µm (2.0x50mm)
 Mobile phase: A) Water + 0.1%TFA B) Acetonitrile + 0.1%TFA

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°C
 Detection: UV280nm
 Sample: ADH Digest
 Injection volume: 1.0µL

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

8-4. Analysis of Synthetic RNA



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

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°C
 Detection: UV280nm
 Sample: Insulin Digest
 Injection volume: 1.0µL

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

■ Contact Us



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