

Columns for HPLC/UHPLC



**FlexFire 300 SEC**

**FlexFire 120 SEC**



## FlexFire 300 SEC

## FlexFire 120 SEC

### SEC (size exclusion chromatography) column for easy analysis of high molecular weight compounds

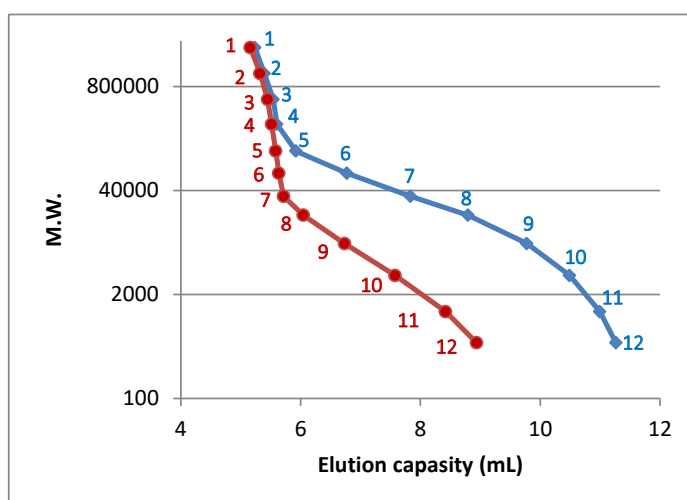
The FlexFire 300 SEC and 120 SEC columns are designed to separate high molecular weight compounds such as proteins and antibodies, as well as low-molecular weight compounds, based on their "size" in solution. These SEC columns are mainly used for isocratic elution and the methods are easy to construct. The results obtained are simple and ideal for the analysis of biological macromolecules such as intact proteins and antibodies.

### Specifications

	Flex Fire 300 SEC	Flex Fire 120 SEC
Particle Size	5 µm	5 µm
Chemistry	Diol	Diol
Surface Area	170 m <sup>2</sup> /g	340 m <sup>2</sup> /g
Pore Volume	1.4 mL/g	1.0 mL/g
Pore Diameter	30 nm	12 nm
Carbon	6 %	9 %
End-cap	No	No
pH Range	pH2-10	pH2-10
Temperature	< 80 °C	< 80 °C
Pressure	< 300 bar (4,351 psi)	< 300 bar (4,351 psi)

The FlexFire series uses a newly developed silica gel based material.

With its higher purity and higher strength than conventional materials, the FlexFire series provides sharp peaks, improved sensitivity, and better alkali resistance, all of which are currently required.



### ■ Polystyrene calibration curve

#### Conditions:

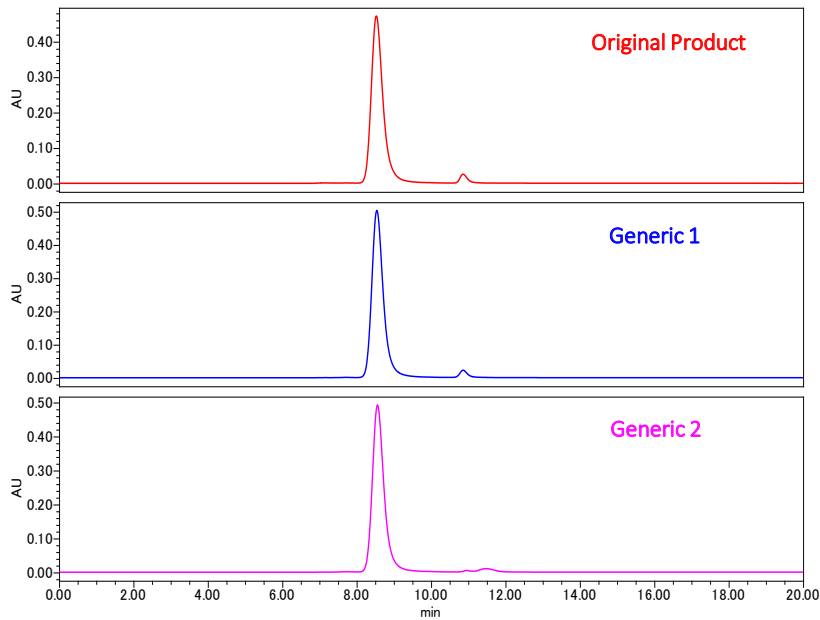
Column: FlexFire 300 SEC, 5 µm  
FlexFire 120 SEC, 5 µm  
Size: 7.8 x 300 mm  
Mobile phase: THF  
Flow rate: 1.0 mL/min  
Temperature: 30 °C  
Detection: UV254 nm

System: Waters alliance e2695

#### Sample: Polystyrene (M.W.)

1. 2,460,000
2. 1,170,000
3. 554,000
4. 271,000
5. 125,000
6. 66,000
7. 34,000
8. 19,700
9. 8,670
10. 3,460
11. 1,220
12. 498

## Analysis of anti-CD20 monoclonal antibody (Rituximab)



### Conditions;

Column: FlexFire 300 SEC, 5  $\mu\text{m}$  (4.6x250 mm)

Mobile phase: 100 mM  $\text{HCOONH}_4$

Flow rate: 0.3 mL/min

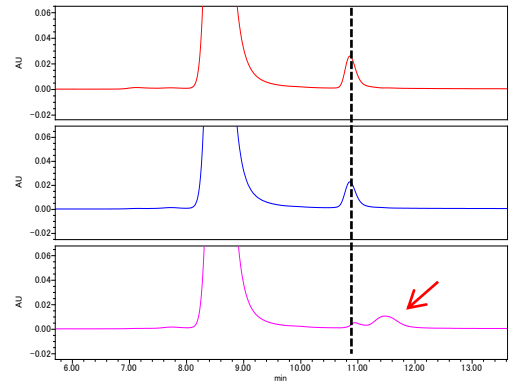
Temperature: 30  $^{\circ}\text{C}$

Detection: UV280 nm

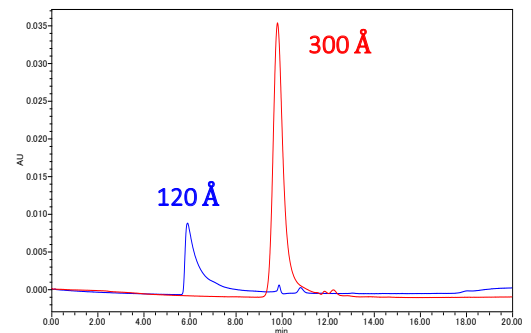
Sample: Anti-CD20 monoclonal antibody

Injection volume: 10  $\mu\text{L}$

System: Waters alliance e2695



A peak was detected in Generic 2 that was not present in the other formulations.

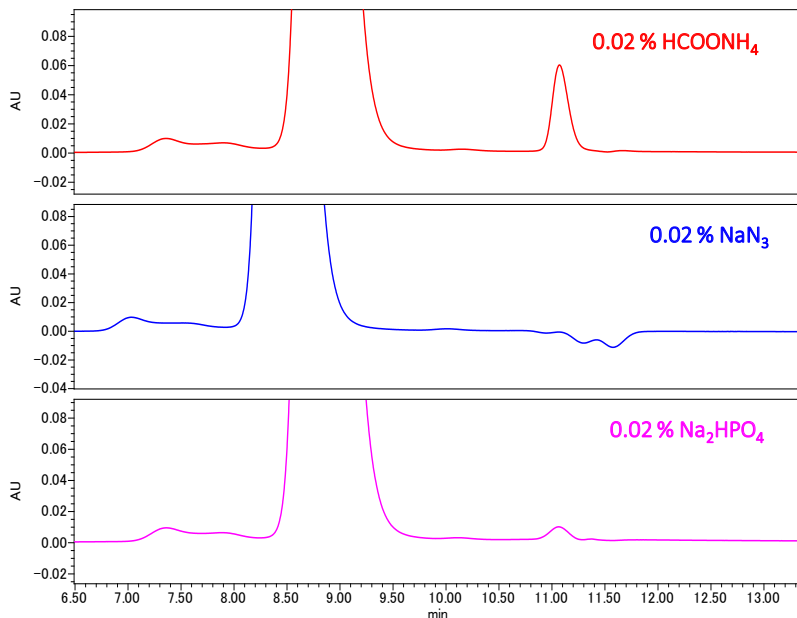


The choice of pore size should be based on the molecular weight of the compound.

The FlexFire 300 SEC is best for proteins, antibodies and subunits. The FlexFire 120 SEC is best for nucleic acids and peptides.

The data above is a comparison of NISTmAb (250  $\mu\text{g}/\text{mL}$ ) at 300  $\text{\AA}$  and 120  $\text{\AA}$ .

## Effect of mobile phase type on peak



### Conditions;

Column: FlexFire 300 SEC, 5  $\mu\text{m}$  (4.6x250 mm)

Mobile phase: 0.02 %  $\text{HCOONH}_4$

0.02 %  $\text{NaN}_3$

0.02 %  $\text{Na}_2\text{HPO}_4$ , pH7.0

Flow rate: 0.3 mL/min

Temperature: 30  $^{\circ}\text{C}$

Detection: UV280 nm

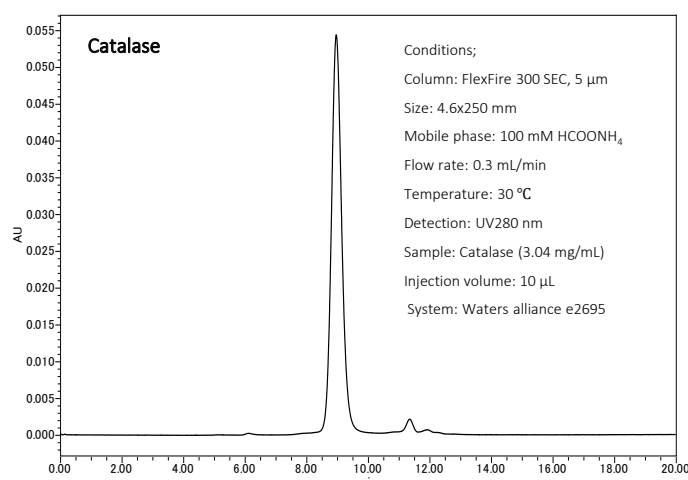
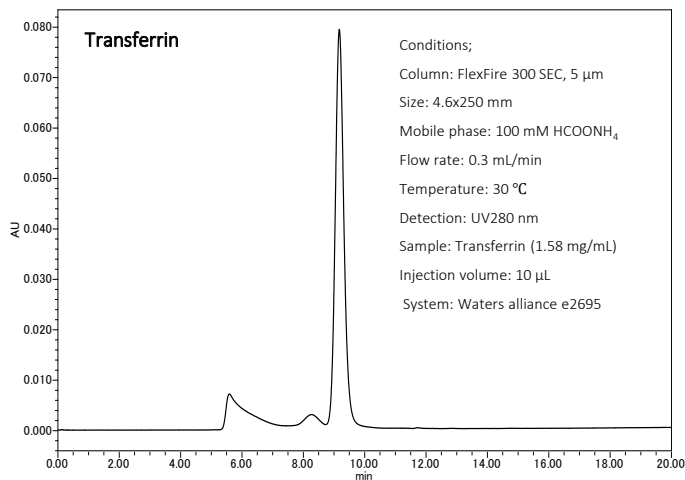
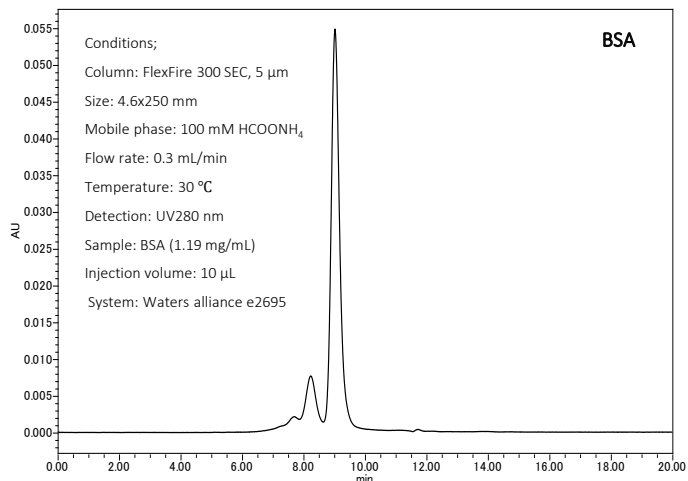
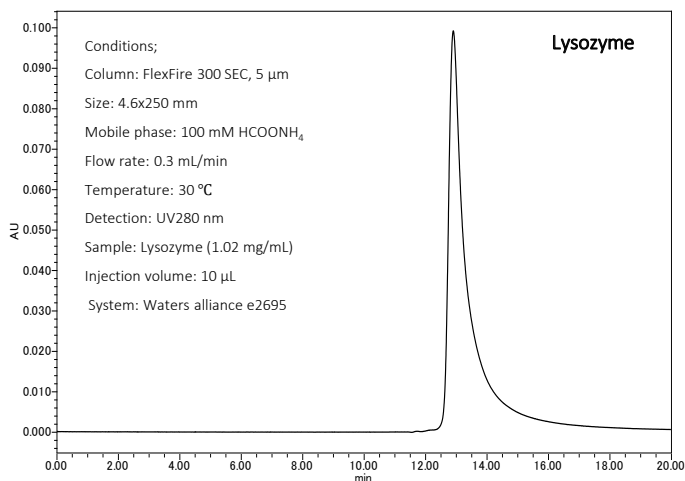
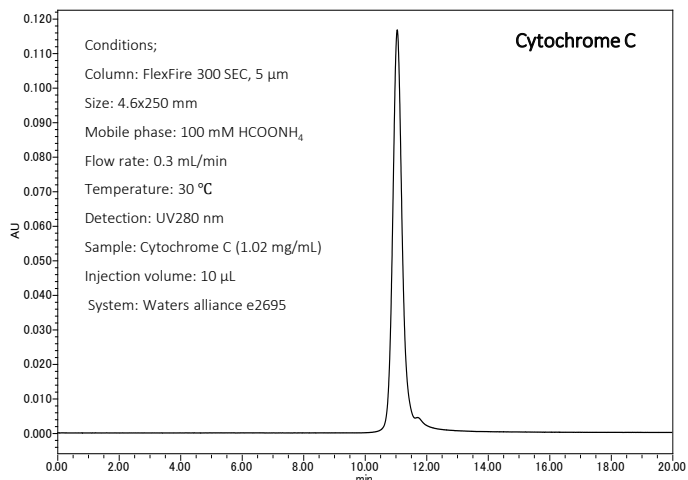
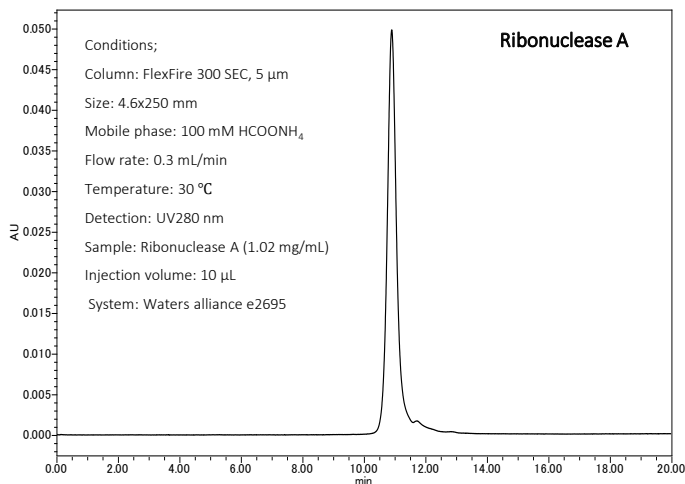
Sample: Anti-CD20 monoclonal antibody

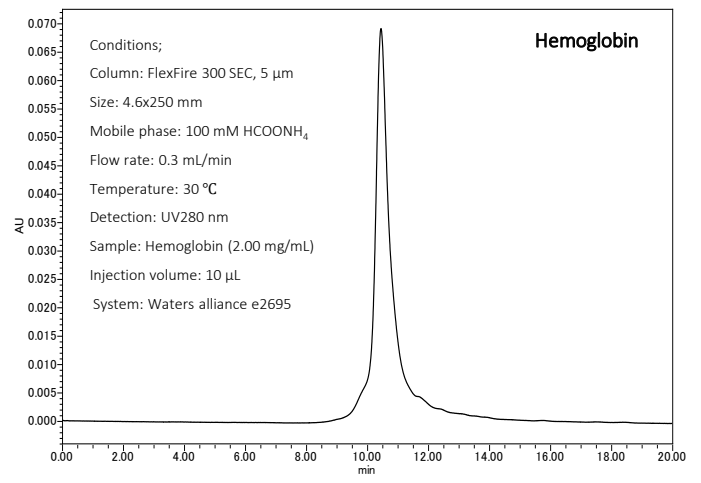
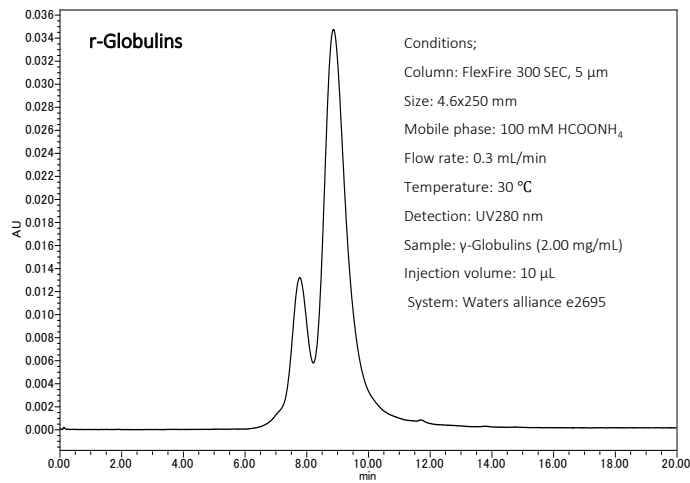
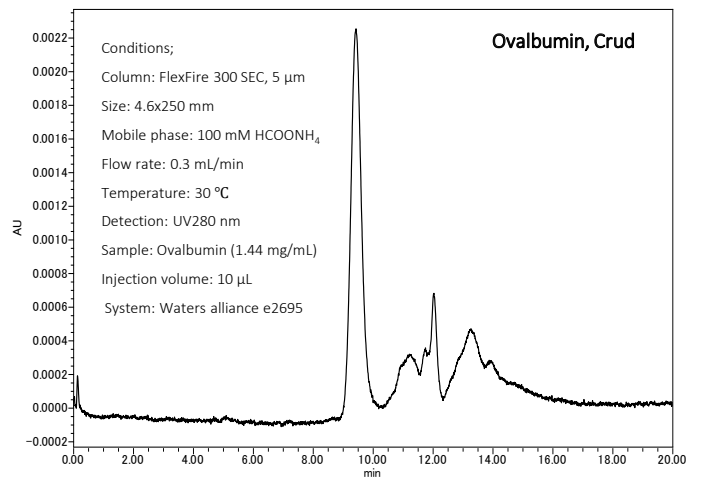
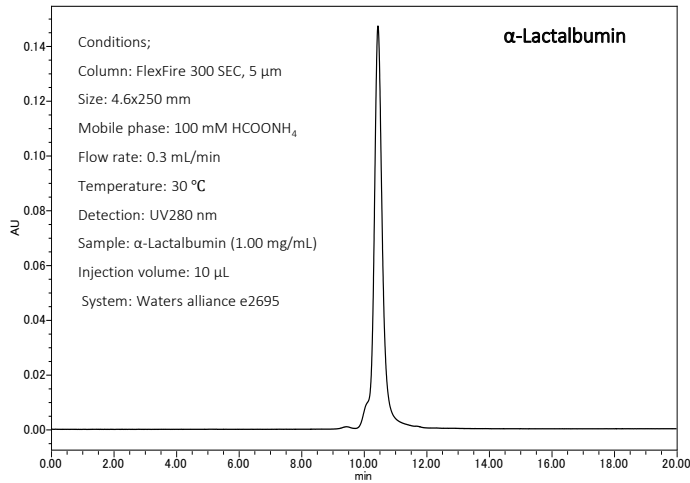
Injection volume: 10  $\mu\text{L}$

System: Waters alliance e2695

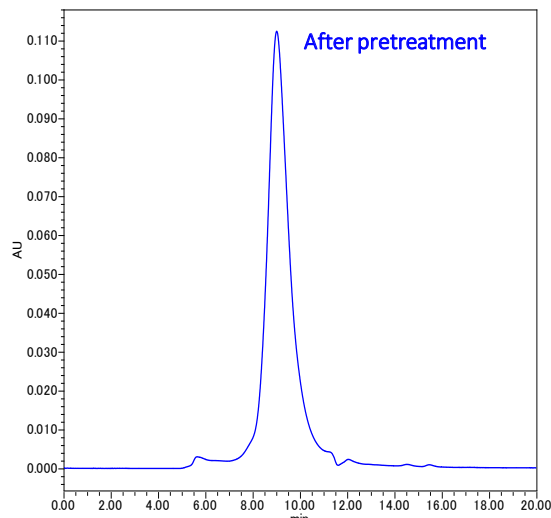
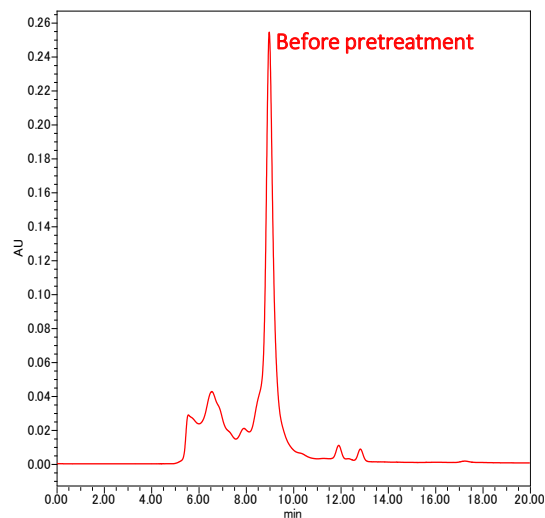
SEC columns, which are often used with phosphate buffer, can give good results with ammonium formate. It also avoids the risk of precipitation when the detector is LC/MS or CAD.

# Applications





## Analysis of IgG antibodies in human serum

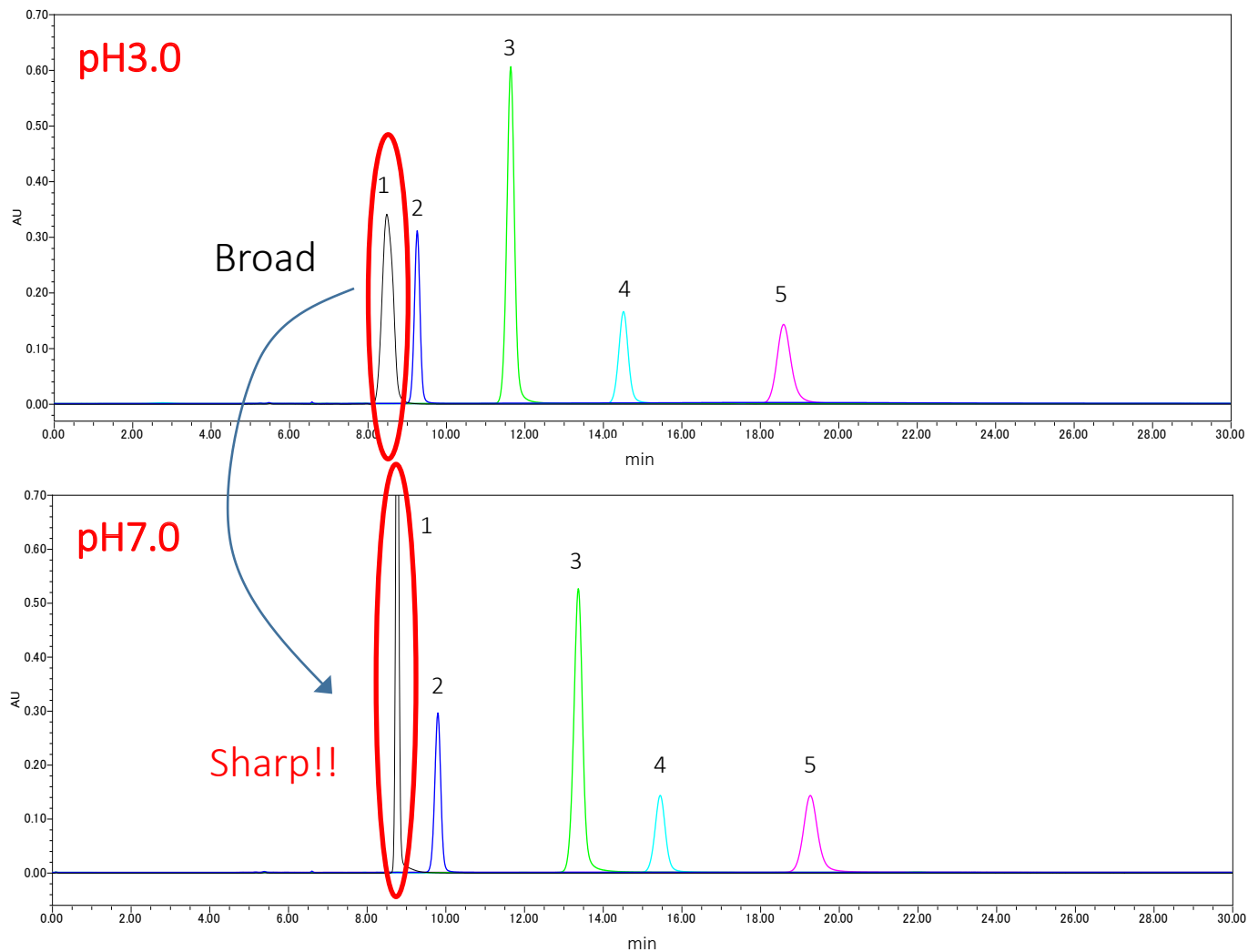


If you know your target, you can use a purification kit before analysis by SEC column.

Conditions;  
 Column: FlexFire 300 SEC, 5 μm    Size: 4.6x250 mm  
 Mobile phase: 100 mM HCOONH<sub>4</sub>    Flow rate: 0.3 mL/min  
 Temperature: 30 °C    Detection: UV280 nm  
 Sample: Serum "COVID-19" positive patient  
 Injection volume: 10 μL    System: Waters alliance e2695

Pretreatment: MonoSpin ProA from G.L. Science Co. was used to purify the antibodies.  
 \*MonoSpin is a registered trademark of G.L. Science Corporation.

## FlexFire 120 SEC for HILIC separations



### Conditions;

Column: FlexFire 120 SEC, 5  $\mu$ m 4.6x250 mm

Mobile phase: Acetonitrile/ HCOONH<sub>4</sub>, pH3.0 or 7.0

Flow rate: 0.5 mL/min

Temperature: 30 °C

Detection: UV260 nm

Sample: 1. Thymine (0.53 mg/mL)

2. Uracil (0.22 mg/mL)

3. Uridine (1.06 mg/mL)

4. 5-FluoroCytosine (0.47 mg/mL)

5. Adenine (0.16 mg/mL)

Injection volume: 2.0  $\mu$ L

System: Waters alliance

The FlexFire SEC series can also be used as HILIC columns, and peak shapes can be improved by changing pH.

In addition to baseline stability under a wide range of conditions from acidic to alkaline, it is suitable for HILIC mode analysis of both neutral and basic compounds.

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