NOMURA CHEMICAL

Concept

What's FlexFire ?

FlexFire's Flex expresses Flexible, and Fire expresses the strength of fire. The logo was created with the motif of the phoenix as a symbol of the fire.

Forty years have passed since Nomura Chemical started "Develosil". We made a big decision in this memorable year. It was a renewal of silica gel. Making new silica gel is not easy. However, by reviewing all the know-how and user feedback that we have accumulated over the past 40 years, we were able to understand what was needed.

I hope that the FlexFire series born from this will set a new history for Nomura Chemical.

Get started with FlexFire! !

Transfer from the Develosil series

The FlexFire series can be easily transferred from those who use the Develosil ODS series, HG / UG series, XG series, and HSR series to those who develop new methods. In addition, differences in the degree of separation that occur during transfer are supported by the method development technology that we have cultivated so far.

New Silica-gel

What is the difference between the previous products and the FlexFire series?
-It differs in the silica gel selected as shown above.

Fig. 1 shows the results of comparing the performance of conventional silica gel and new silica gel. As shown in this figure, we were able to obtain silica gel with uniform particles and few impurities.

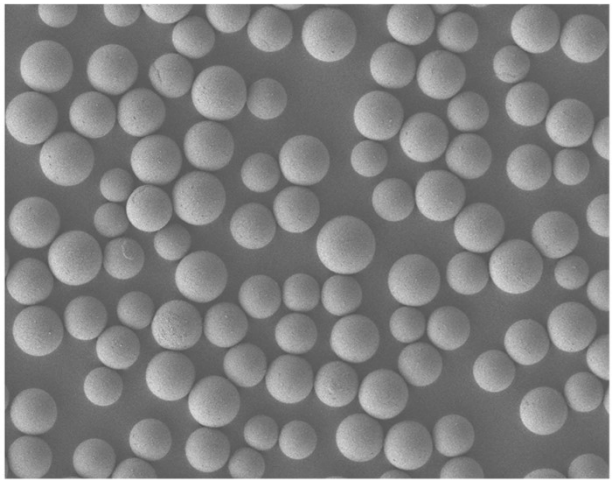
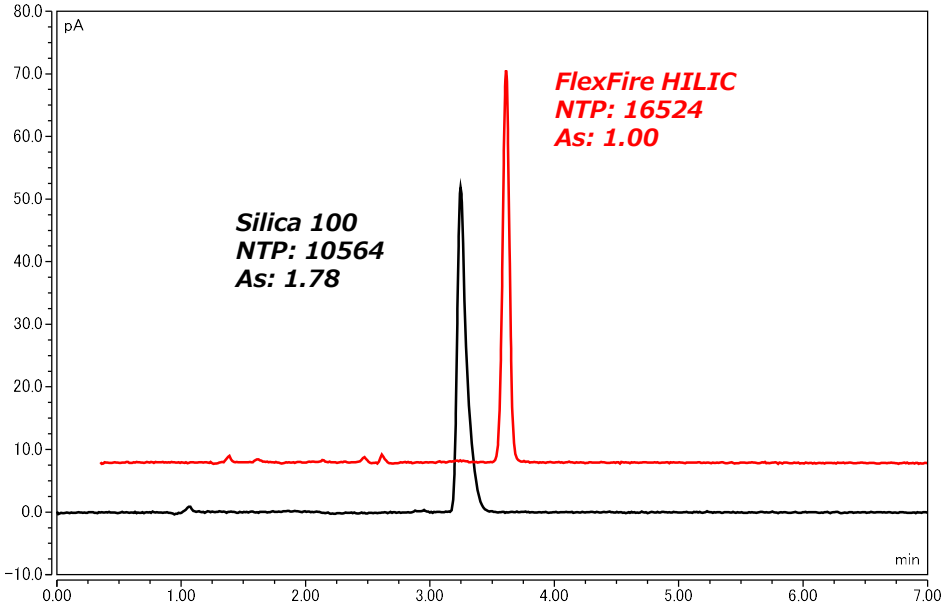


Fig.2 TEM image of silica gel for FlexFire (1.6μm-5μm)



Analytical conditions;

Column:	FlexFire HILIC, 3μm (4.6x150mm)
	Develosil Silica 100-3, 3μm (4.6x150mm)
Mobile phase:	Acetonitrile/Water=90/10
Flow rate:	1.0mL/min
Temperature:	40℃
Detection:	CAD
Sample:	Allantoin
Injection volume:	1.0μL
System:	Thermo Fisher SCIENTIFIC UltiMate 3000

Fig.1 Comparison of materials

System matching

"Is your system HPLC? or UHPLC?" With the FlexFire series, the grain size can be selected according to the system. For any system, the FlexFire series is the most effective for your system.

Φ4.6mm and Φ1.0mm columns have been added to the FlexFire series to suit many systems.

【FlexFire series and system matching example】

Category	Compatible i.d.	Compatible particle size	Verification system
HPLC	Φ4.6mm	2.6μm, 5μm	Waters alliance
UHPLC	Φ2.0mm Φ1.0mm	1.6μm, 2.6μm, 5μm	Waters H-Clss PLUS Shimadzu Nexra X3 Agilent 1290 Infinity II Thermo Vanquish H

This table shows the results based on our own verification in normal use. Φ4.6mm can be used in UHPLC systems, but the flow cell and piping need to be replaced.

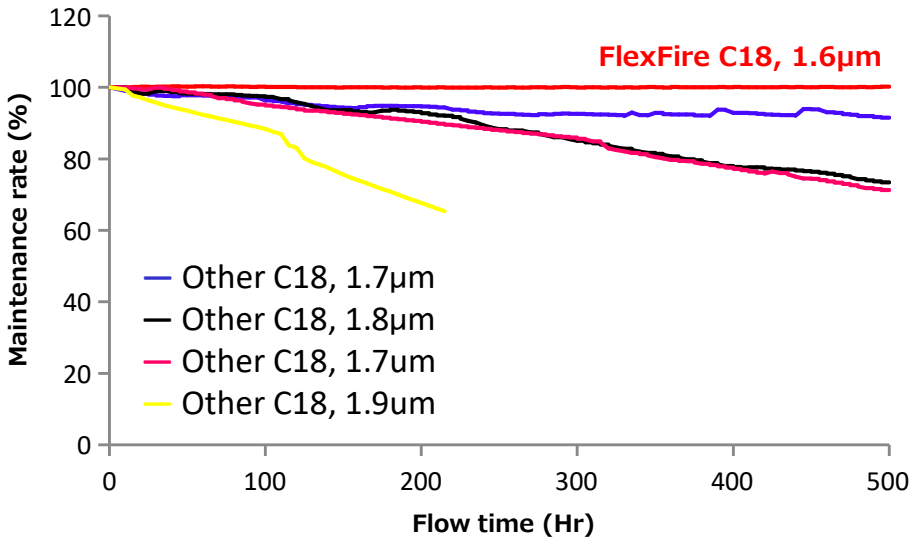
FlexFire's Line up

Mode	Product name	Particle size			Pore diameter	Surface area	Pore volume	Carbon
		1.6μm	2.6μm	5μm	(nm)	(m^2/g)	(mL/g)	(%)
Reversed phase	FlexFire C18	○	○	○	11	340	1.0	22
	FlexFire AQ C18	○	○	○	11	340	1.0	8.5
	FlexFire C8	○	○	○	11	340	1.0	12
	FlexFire C1	○	○	○	11	340	1.0	5.5
	FlexFire C8	○	○	○	11	340	1.0	12
	FlexFire C30	○	○	○	11	340	1.0	11
Reversed phase (Wide Pore)	FlexFire WP C18		○	○	30	170	1.4	15
	FlexFire WP C8		○	○	30	170	1.4	7
	FlexFire WP C4		○	○	30	170	1.4	5
	FlexFire WP C1		○	○	30	170	1.4	3
	FlexFire mAb-RP		○	○	100	24	0.8	1.3
SEC	FlexFIre 120SEC		○	○	11	340	1.0	6
	FlexFIre 300SEC		○	○	30	170	1.4	9
HILIC	FlexFIre HILIC	○	○	○	11	340	1.0	—

This list is as of November 2021. The pH range has now been updated to pH 1-10 (except for HILIC). The FlexFire series will be further updated, such as WPC4, which targets high molecular compounds such as proteins and monoclonal antibodies.

And one of the big decisions is that the FlexFire series has only a 2.0mm ID column. This is a future-ready reform with the spread of UHPLC systems.

Durability and bond density



Conditions;

Column:	FlexFire C18, 1.6um 2.0x50mm
Size:	Acetonitrile/10mM NH_4OH, pH10.5=60/40
Mobile phase:	0.5mL/min
Flow rate:	40℃
Temperature:	UV254nm
Detection:	
Sample:	1.Uracil (0.01mg/mL)
	2.Naphthalene (0.1mg/mL)
Injection volume	0.16uL

Fig.3 Retention time maintenance rate with respect to liquid flow time

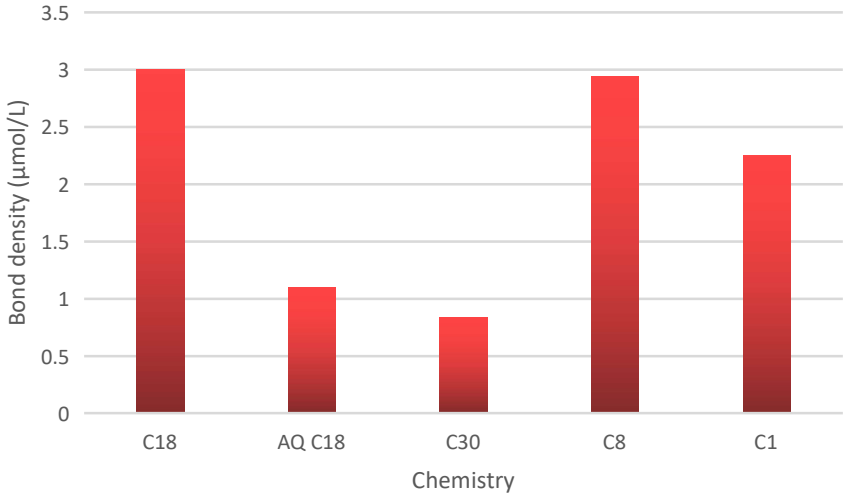


Fig.4 Bond density of each chemistry

Silica-gel undergoes cleavage of the bonded phase under acidic conditions. And, the silica gel dissolves under alkaline conditions. However, the new silica-gel substrate of the FlexFire series has improved its strength. Figure 3 demonstrates that FlexFire is sufficiently durable at pH 10.5.

Until now, the durability changed according to the bond density, but tough silica gel has sufficient durability even for low-density chemistry.

UHPLC Method Transfer

The FlexFire series is available in particle sizes of 1.6µm, 2.6µm and 5µm. The particle size can be selected according to the system. And method transfer from 5µm to 1.6µm can be performed easily.

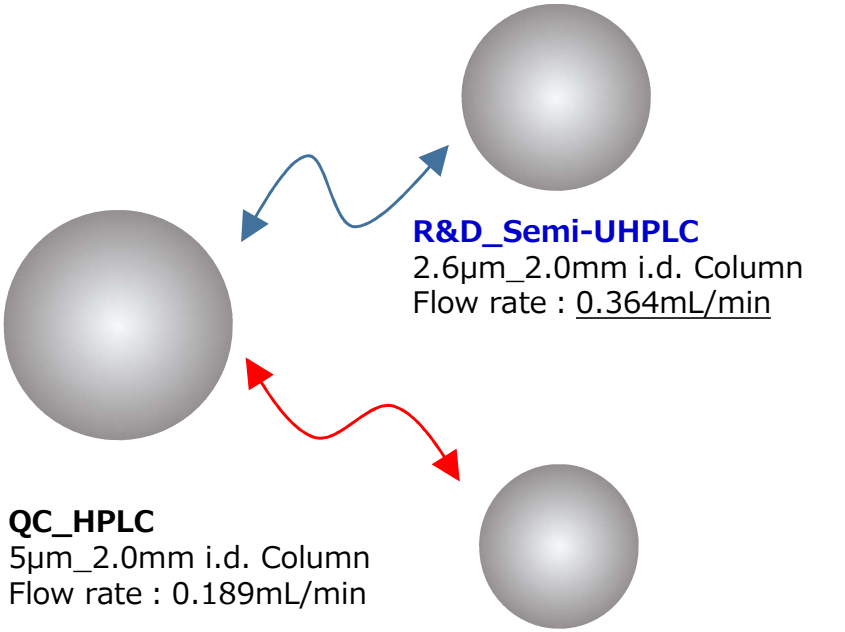
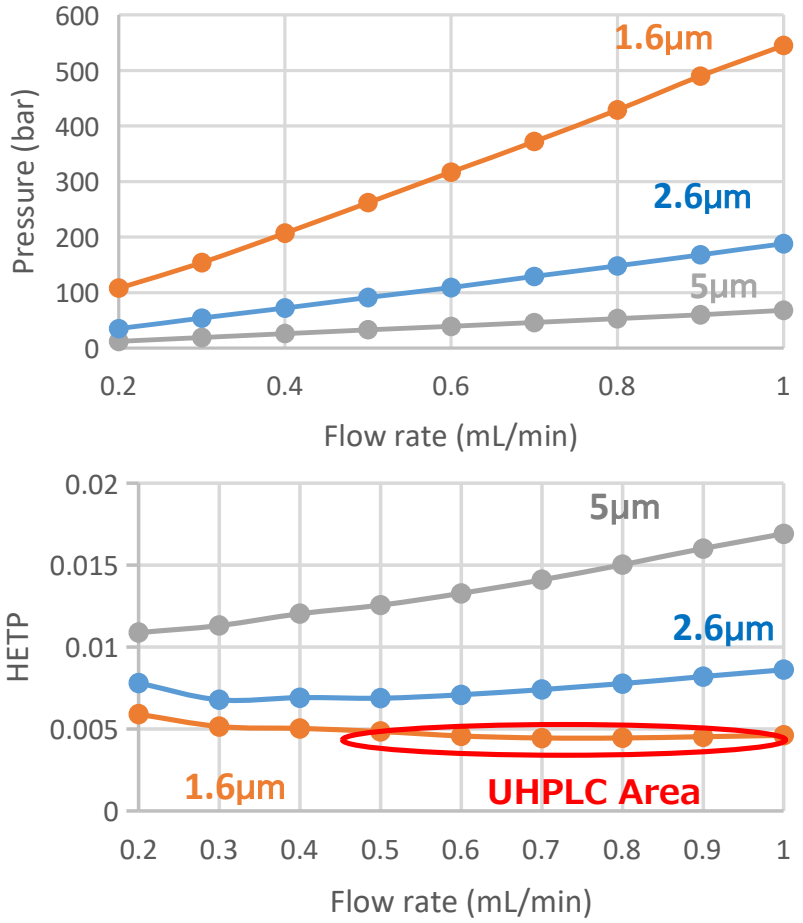


Fig.5 Image model of method transfer

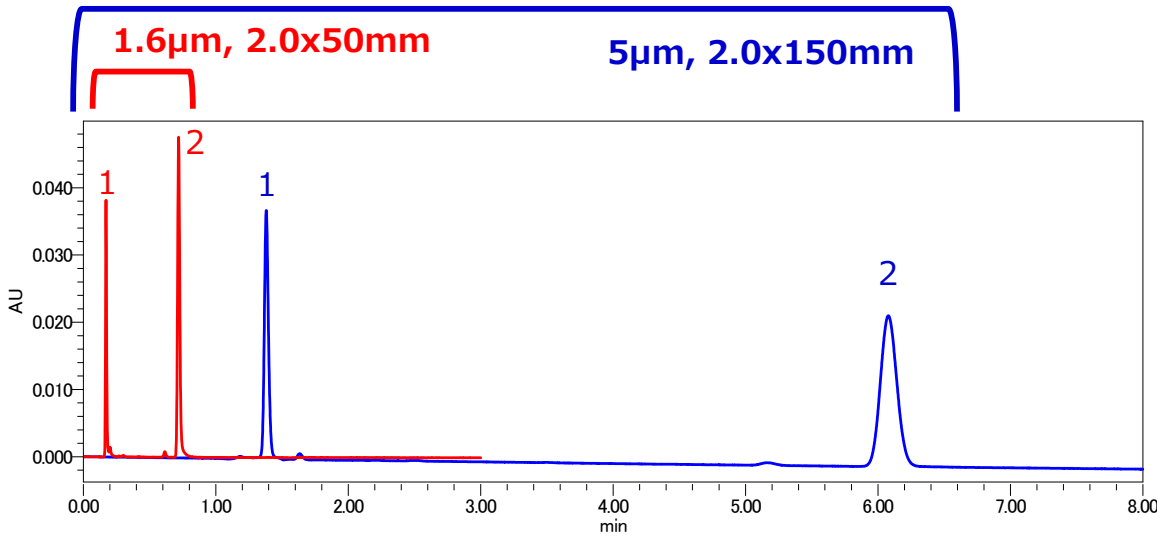


Conditions;

Column:	FlexFire C18 (2.0x50mm)
Mobile phase:	Acetonitrile/Water=60/40
Flow rate:	0.2mL/min～1.0mL/min
Temperature:	40℃
Detection:	UV254nm
Sample:	Naphthalene
Injection volume:	0.16µL

Fig.6 Pressure and HETP vs Flow Rate for FlexFire C18

Example of method transfer



Conditions:

Column	FlexFire C18, 5µm (2.0x150mm)
	FlexFire C18, 1.6µm (2.0x50mm)
Mobile phase:	Acetonitrile/Water=60/40
Flow rate:	5µm: 0.189mL/min
	1.6µm: 0.591mL/min
Temperature:	40℃
Detection:	UV254nm
Sample:	1.Uracil
	2.Naphthalene
Injection volume:	5µm: 0.3µL
	1.6µm: 0.1µL
System:	Waters ACQUITY UPLC H-Class PLUS

Fig.5 Example simple method transfer

The advantage of UHPLC method transfer is a significant reduction in analysis time.

In this example, about 8 minutes were reduced to 0.8 minutes by method transfer.

Moreover, method transfer can be easily performed with the attached software.

Comparison of insulin peak shapes

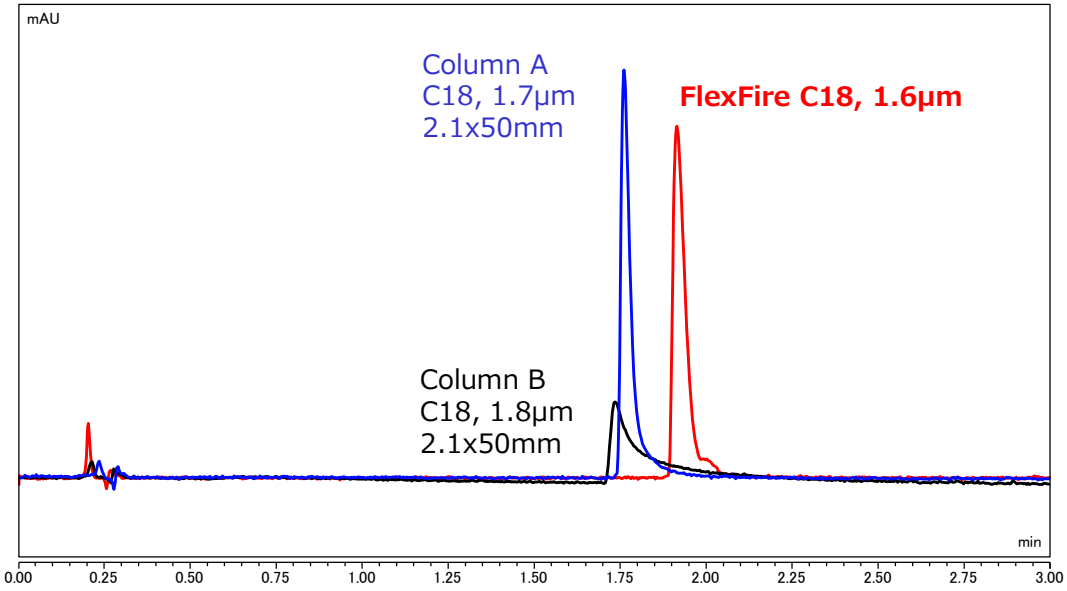


Fig.6 Comparison of Insulin peak shapes

Insulin was analyzed using a 0.1% formic acid mobile phase. The peak shape of insulin differs depending on the column. Insulin uses a TFA mobile phase to significantly improve the peak shape, but the introduction to LC / MS is severe.

Conditions:

Column: FlexFire C18, 1.6µm (2.0x50mm)

Mobile phase: A) Water + 0.1%HCOOH

B) Acetonitrile + 0.1%HCOOH

Gradient:

min	mL/min	%A	%B	Curve
0.00	0.5	80	20	5
5.04	0.5	40	60	5
5.05	0.5	80	20	5

Temperature: 40℃

Detection: UV280nm

Sample: Insulin, Human, recombinant(0.97mg/mL)

Injection volume: 0.3µL

System: Thermo Fisher SCIENTIFIC Vanquish_H

Mixer: 10µL

Comparison of insulin peak shapes with Various mobile phases

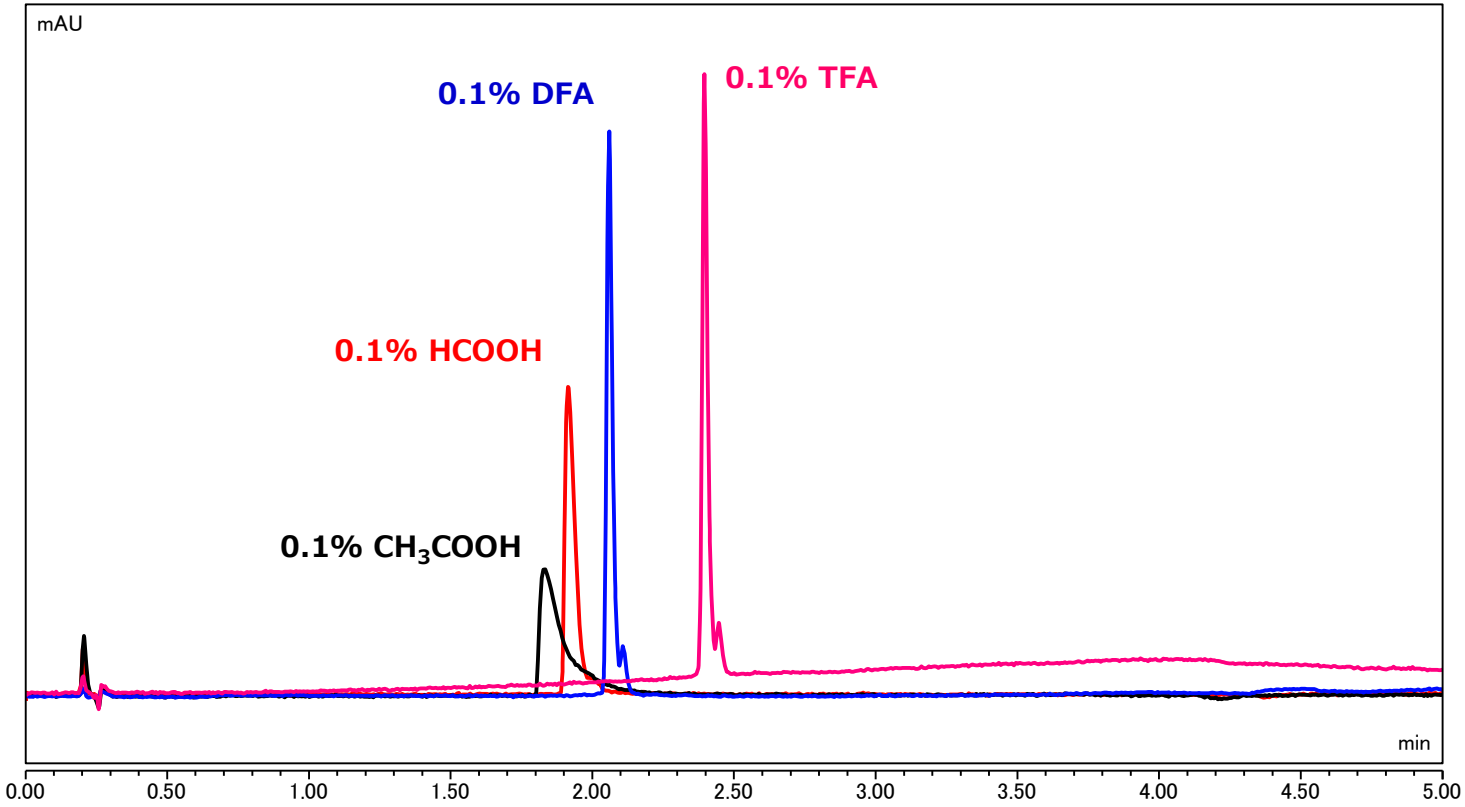
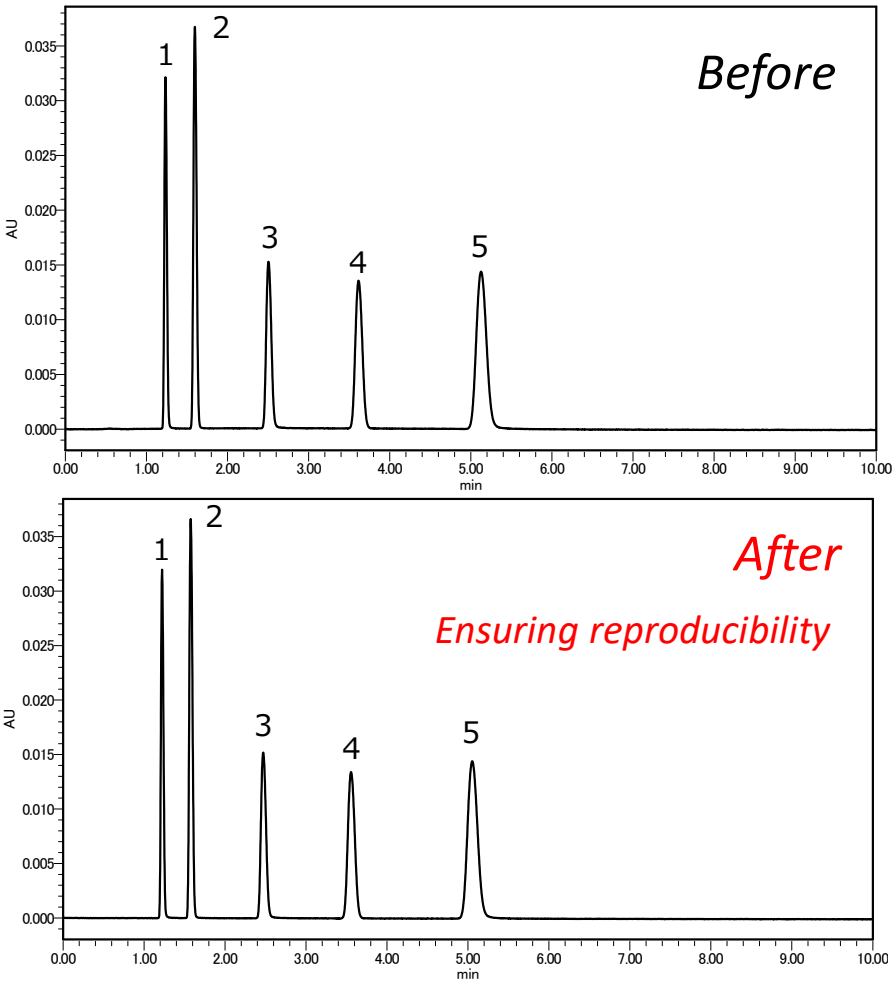


Fig.7 Comparison of Insulin peak shapes with various mobile phase

If good results are not obtained with 0.1% formic acid, it is desirable to switch to DFA or TFA. If ion suppression or contamination of the ion source affects, it is necessary to reduce the effect of the ion pair reagent by mixing with formic acid or the like.

Conditions that can be used with 100% aqueous mobile phase



Pomp Flow ➡ Stop (30min) ➡ Flow

Fig.8 Stop-flow test under 100% aqueous mobile phase conditions

Conditions:

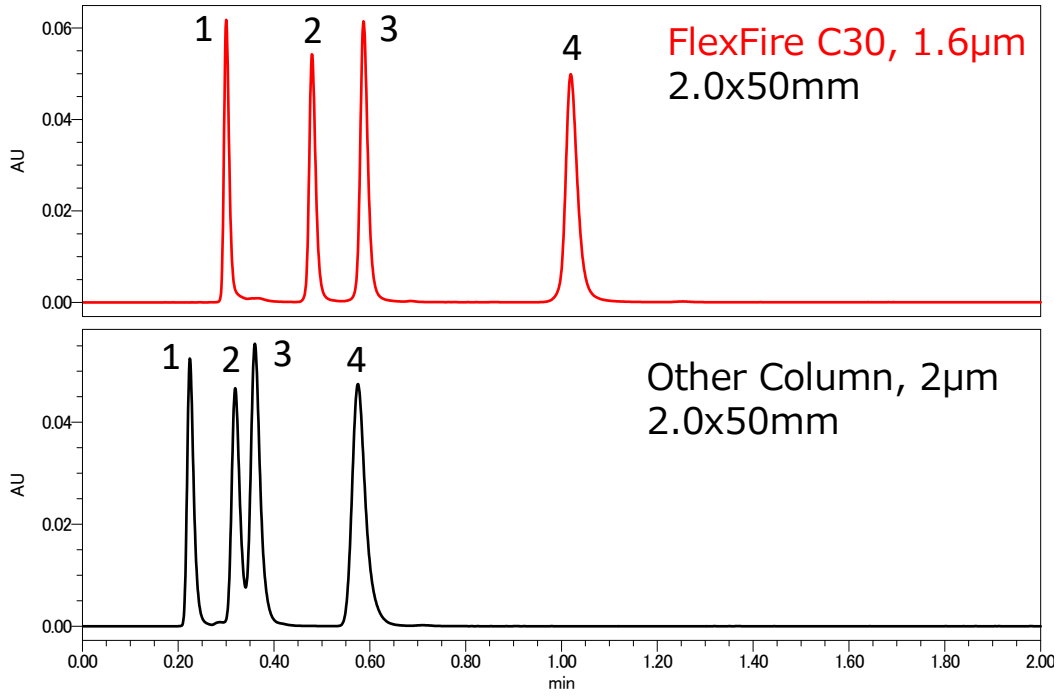
Column:	FlexFire AQ C18, 2.6μm
Size:	2.0x100mm, Stainless
Mobile Phase:	10mM $HCOONH_4$
Flow rate:	0.3mL/min
Temperature:	40℃
Detection:	UV260nm
Sample:	1.Cytosine (53μg/mL)
	2.Uracil (50μg/mL)
	3.Guanine (52μg/mL)
	4.Thymine (50μg/mL)
	5.Adenine (50μg/mL)
Injection volume:	0.2μL

The most important thing to consider with 100% aqueous mobile phase is reproducibility.

The mechanism is very simple. By stopping the pump, the mobile phase taken into the pores escapes. Then, even when the liquid sending is started again, the mobile phase is not taken into the pores.

This can be avoided by controlling the bond density. FlexFire AQ C18, C30, C1 controls the bond density so that it can be used in 100% aqueous system.

Overwhelming holding power with 100% aqueous mobile phase



The 100% aqueous mobile phase has a significant effect on improving the separation of the front part.

In particular, the longer the alkyl chain, the stronger the retention of the hydrophobic compound.

That is, a column that can be used with an aqueous 100% mobile phase can separate low polar compounds from high polar compounds in a well-balanced manner.

Conditions;

Column:	FlexFire C30, 1.6μm (2.0x50mm)
	Other column, 2μm (2.0x50mm)
	※Columns for analysis of highly polar compouds
Mobile phase:	25mM Ammonium phosphate, pH7.0
Flow rate:	0.5mL/min
Temperature:	40℃
Detection:	UV260nm
Sample:	1.dCTP 2.dTTP 3.dGTP 4.dATP
Injection volume:	0.2μL

Fig.9 Analysis of dNTP with 100% aqueous mobile phase

S/N up with short alkyl chain

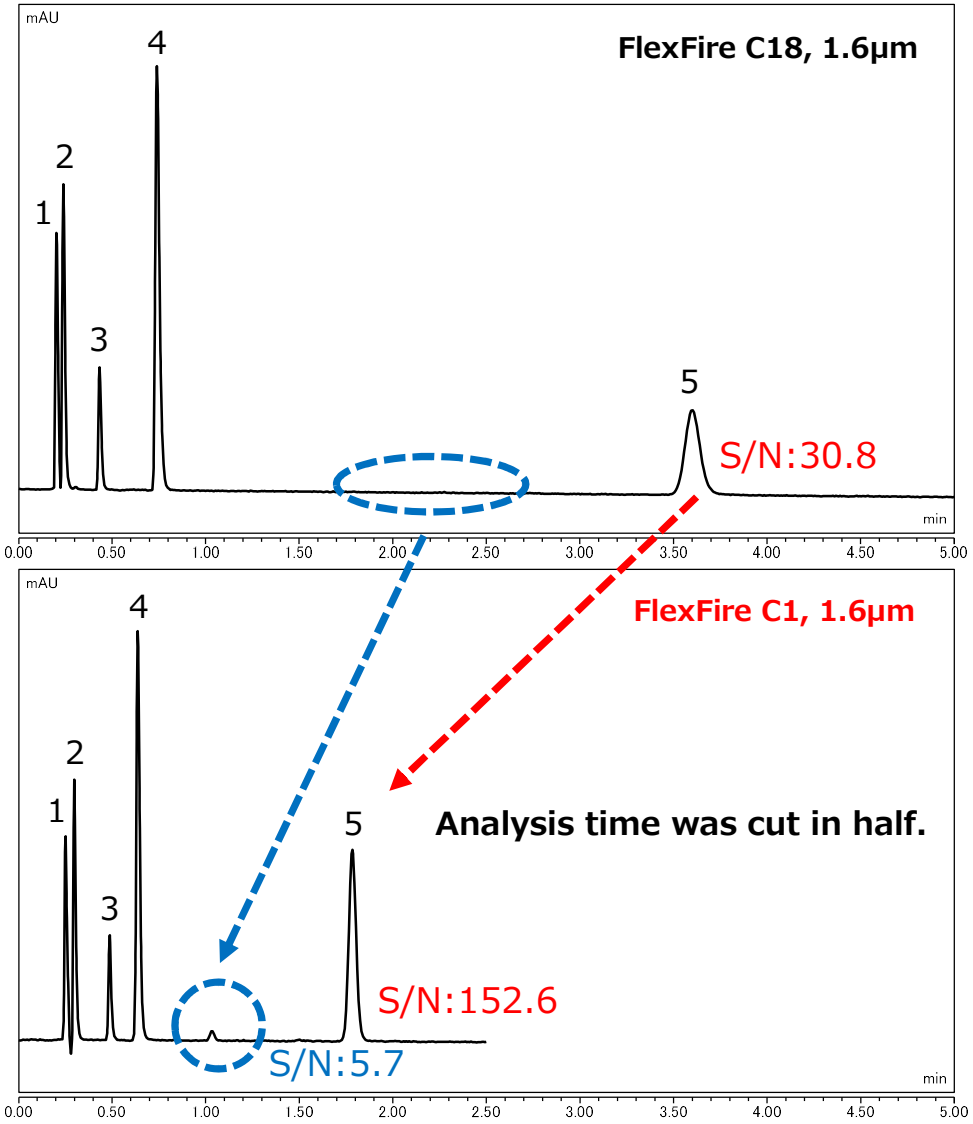


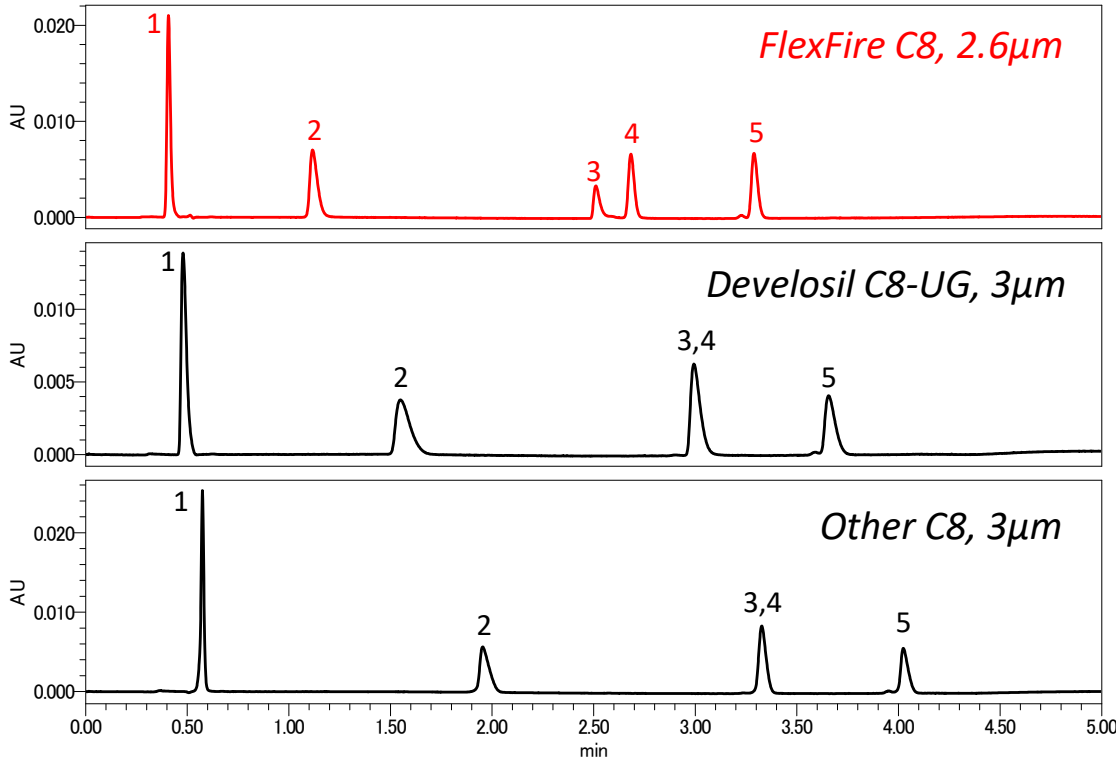
Fig.10 S/N comparison (C18vs C1)

Perhaps some people do not know the existence of C1. C1 is, so to speak, the last point of the reversed phase. Isn't C1 an image that is weak and unsuitable for separation? C1 does not have a short retention, and can be very good if it is considered to have low adsorption.

Now, does the data right here look like that? The retention and separation of the front part is not inferior to C18. An immediate understanding is the retention of hydrophobic compounds (Naphthalene). However, S / N has overwhelmingly high C1. And the peak that was not C18 can be confirmed in C1.

This has a very good effect on metabolic analysis. The combination of UHPLC methods and low adsorption further accelerates analysis time savings.

C8 column for sharp peaks



Conditions;

Column:	FlexFire C8, 2.6µm (2.0x50mm) Develosil C8-UG, 3µm (2.0x50mm) Other C8, 3µm (2.0x50mm)
Mobile phase:	A) Water + 0.1%HCOOH
Flow rate:	B) Acetonitrile + 0.1%HCOOH

Gradient:

min	mL/min	%A	%B	Curve
0.00	0.3	90	10	
2.93	0.3	75	25	6
2.96	0.3	90	10	6

Detection:	UV260nm
Sample:	1. Gly-Tyr 2. Val-Tyr-Val 3. Angiotensin II 4. Met-Enk 5. Leu-Enk
Injection volume:	0.2uL
System:	Waters ACQUITY UPLC H-Class PLUS

Fig.11 Separation comparison of Peptide

FlexFire C8 was developed for retention and separation. It is particularly excellent in separation among C8 columns of the same class. When the peak stands sharply, the S / N tends to be high. If the separation at C18 is insufficient, there is a good chance that it can be improved by changing to C8.

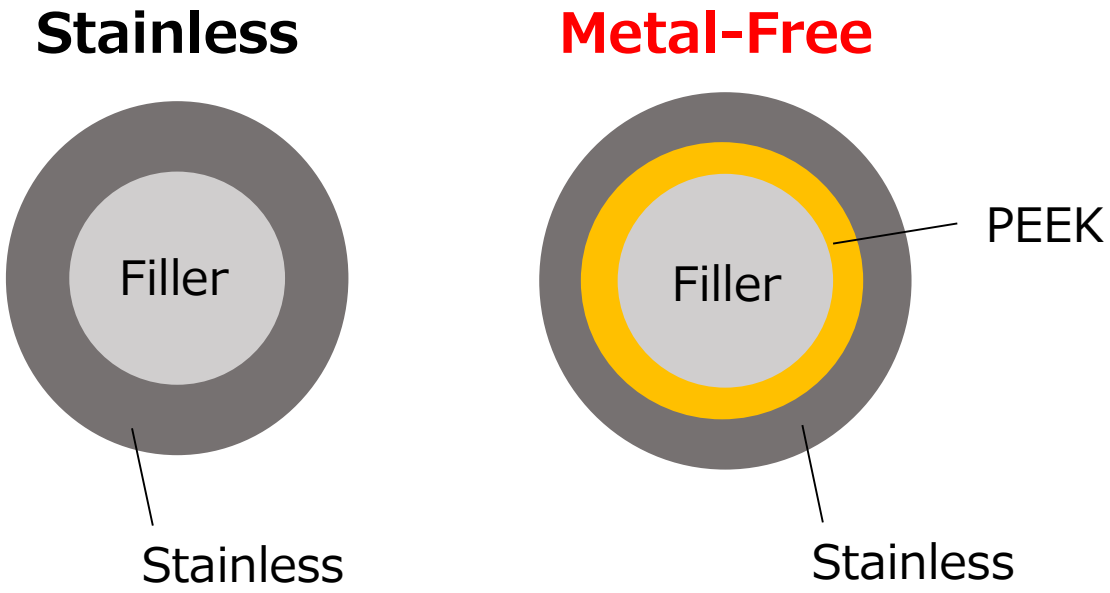
Metal-Free column

Metal free column was added from FlexFire series. Compounds containing phosphorus groups adsorb to metals. Until now, the use of a phosphate buffer improved the peak shape, but it had a major problem.

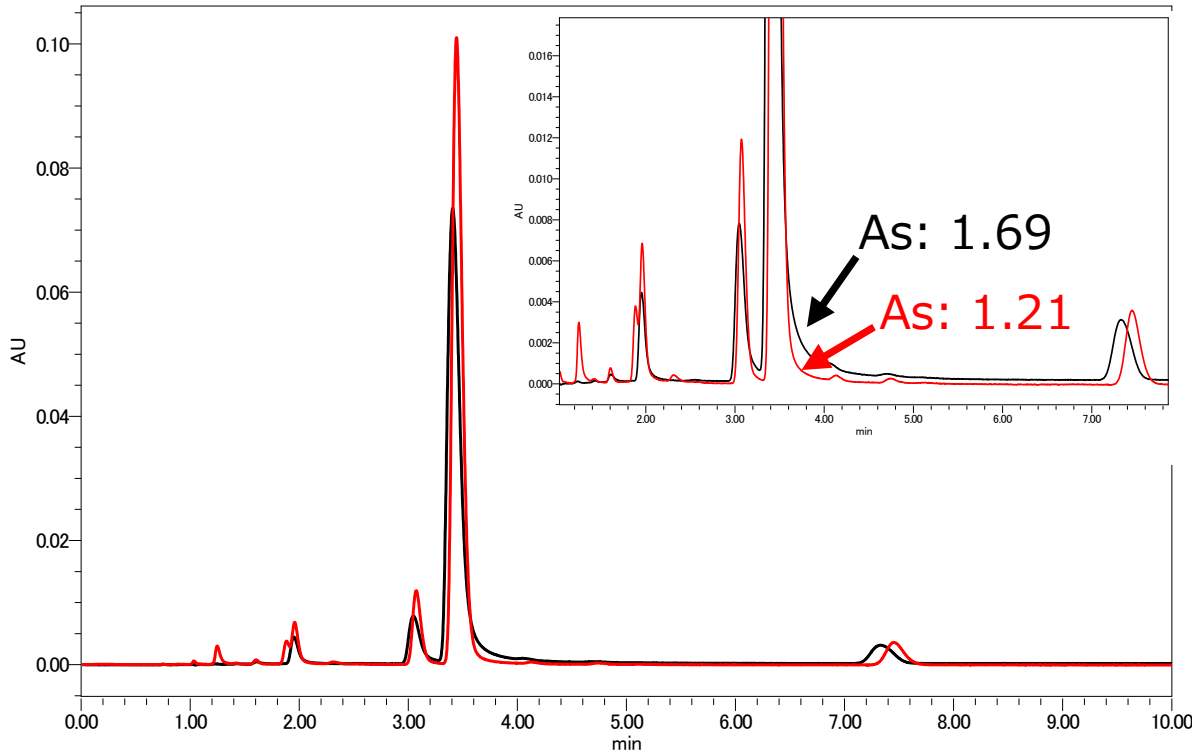
It cannot be introduced into LC / MS.

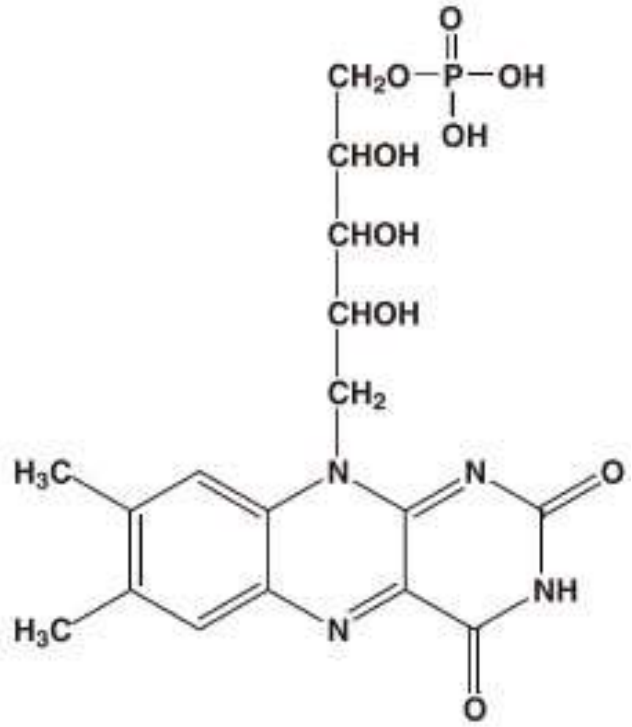
So we looked at metal-free columns and could get very good results with volatile buffers.

FlexFire's metal-free columns have a PEEK tube built into a stainless steel tube. This ensures high durability even under high pressure of UHPLC columns such as 1.6µm particle size.



Example using metal-free column (Flavin mononucleotide)



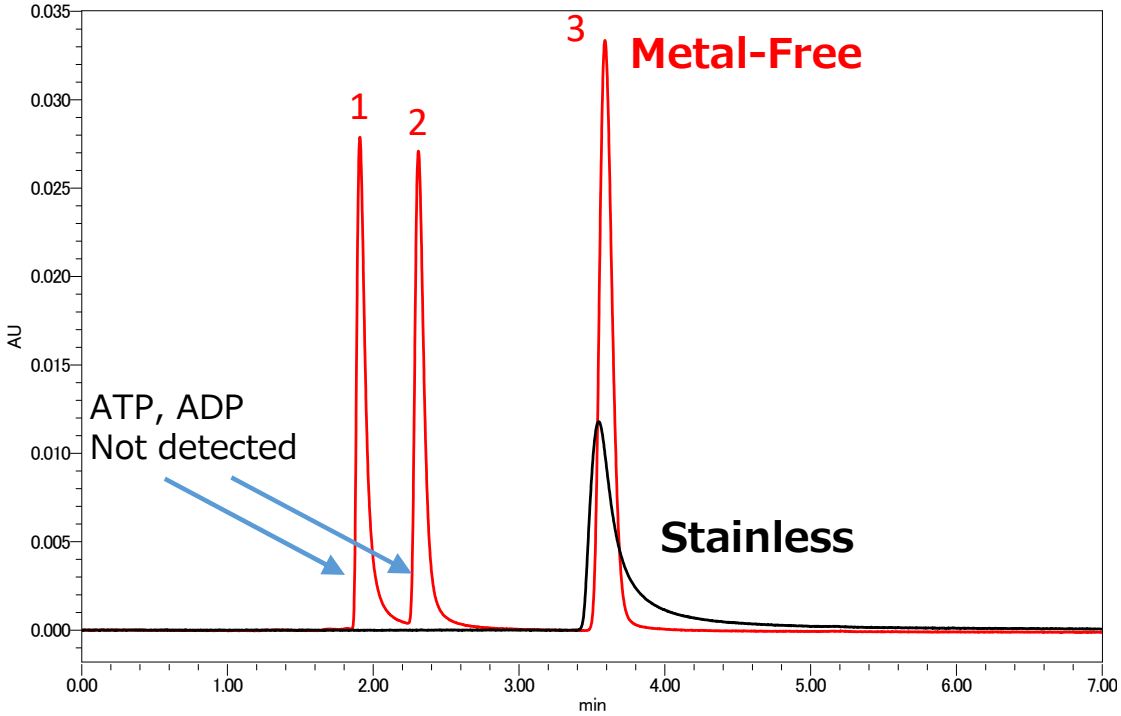


Flavin mononucleotide

Conditions;

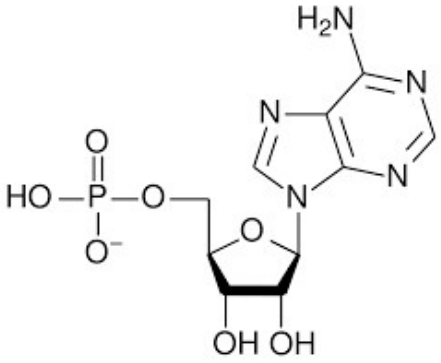
Column:	FlexFire AQ C18, 2.6µm (2.0x100mm) : Stainless
	FlexFire AQ C18, 2.6µm (2.0x100mm) : Metal Free
Mobile phase:	Acetonitrile/25mM $HCOONH_4$=10/90
Flow rate:	0.3mL/min
Temperature:	40℃
Detection:	UV254nm
Sample:	Flavin mononucleotide (0.52mg/mL)
Injection volume:	0.2µL
System:	Waters ACQUITY UPLC H-Class PLUS

Example using metal-free column (AMP, ADP, ATP)

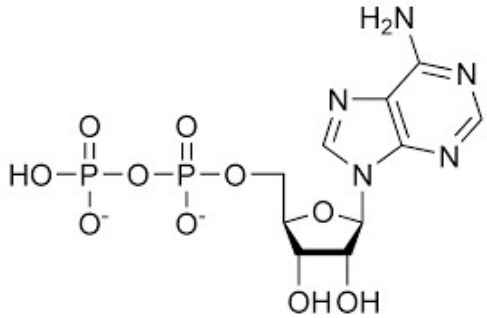


Conditions:

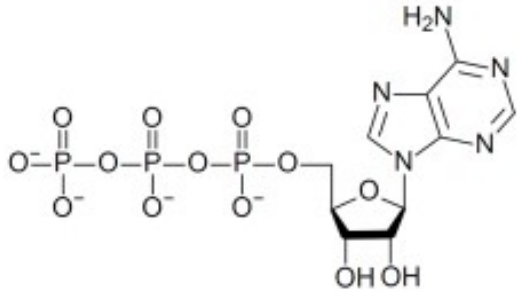
Column:	FlexFire AQ C18, 2.6µm (2.0x100mm) : Stainless
	FlexFire AQ C18, 2.6µm (2.0x100mm) : Metal Free
Mobile phase:	10mM $HCOONH_4$
Flow rate:	0.3mL/min
Temperature:	40℃
Detection:	UV260nm
Sample:	1. ATP (0.16mg/mL)
	2. ADP (0.17mg/mL)
	3. AMP (0.16mg/mL)
Injection volume:	0.2µL
System:	Waters ACQUITY UPLC H-Class PLUS



AMP

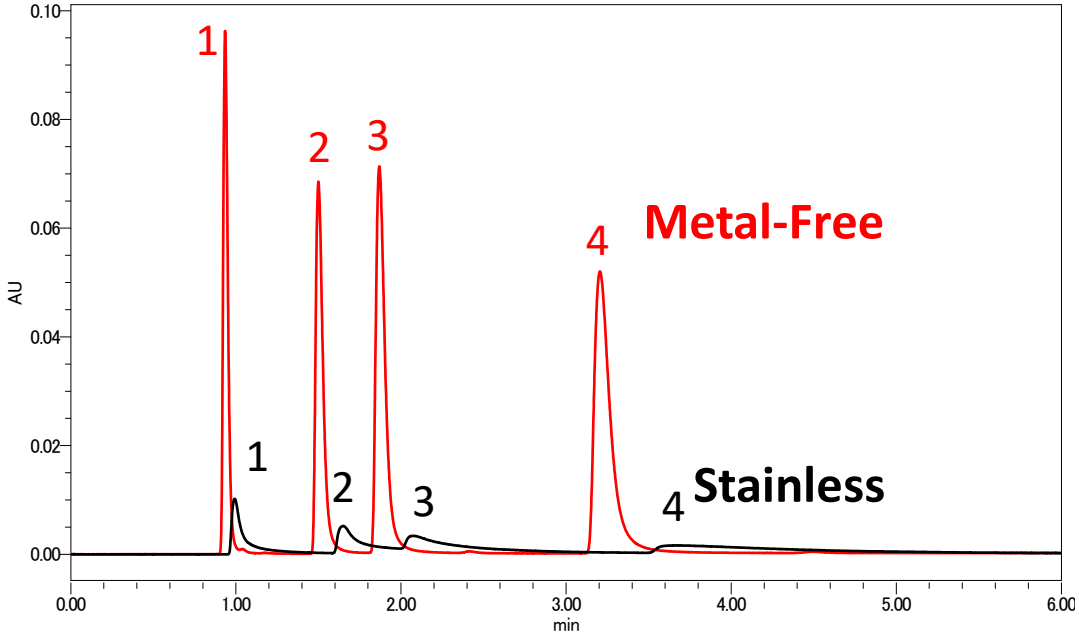


ADP



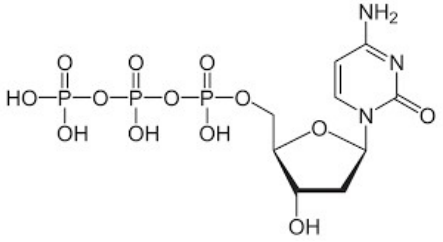
ATP

Example using metal-free column (dNTP)



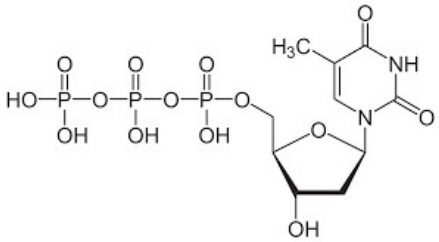
Conditions:

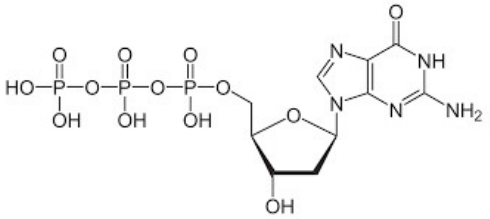
Column:	FlexFire AQ C18, 2.6μm (2.0x100mm) : Stainless
	FlexFire AQ C18, 2.6μm (2.0x100mm) : Metal Free
Mobile phase:	10mM $HCOONH_4$
Flow rate:	0.3mL/min
Temperature:	40℃
Detection:	UV260nm
Sample:	1. dCTP (13μM)
	2. dTTP (13μM)
	3. dGTP (13μM)
	4. dATP (13μM)
Injection volume:	0.2μL
System:	Waters ACQUITY UPLC H-Class PLUS



dCTP

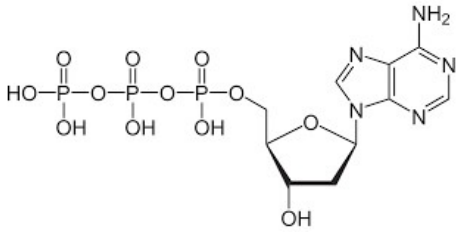
dTTP





dGTP

dATP



Analysis of biopolymer compounds

In recent years, biopharmacy has grown into a very large market. In particular, nucleic acids and antibodies have grown remarkably, and with the spread of corona-virus, they have become familiar keywords to the general public.

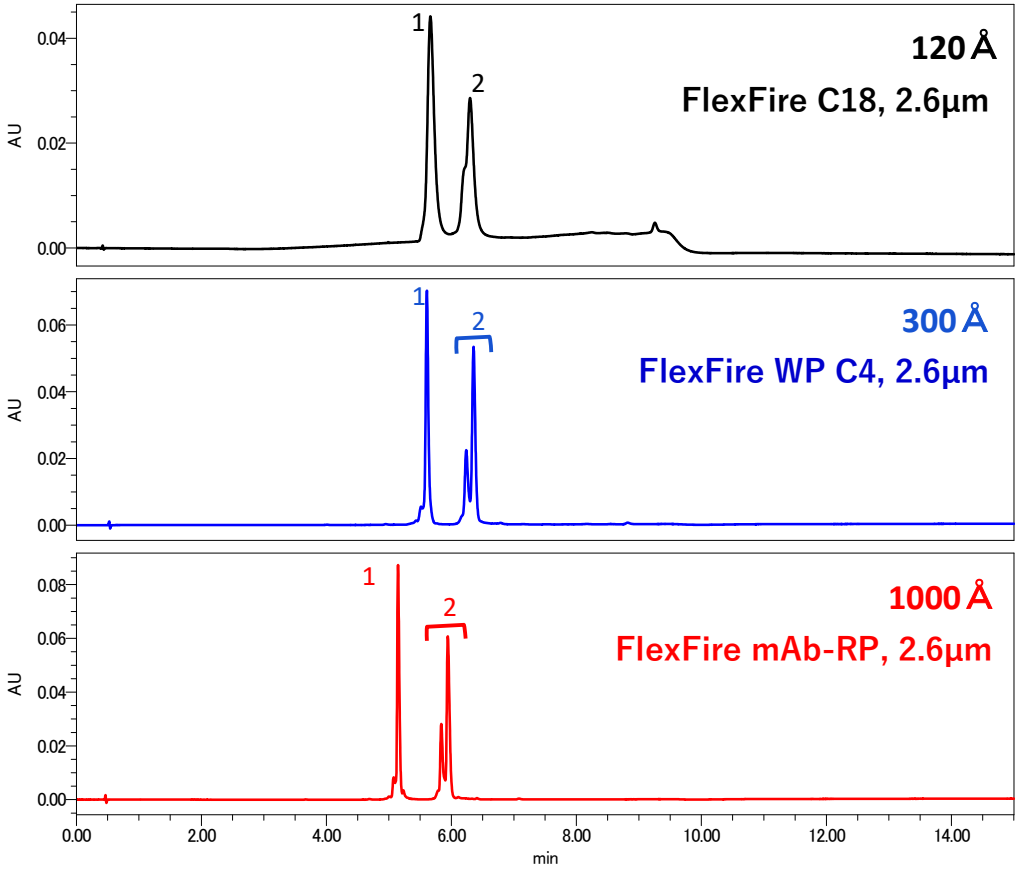
In addition, HPLC / UHPLC is essential for these analyses, and manufacturers strive daily for better results.

We have succeeded in developing and commercializing a new wide pore column in the FlexFire series.

FlexFire series lineup

	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)

Reasons for wide pore columns



The peak becomes clearer as the pore size increases. Especially at 1000 Å, you can get a clearer peak than the standard 300 Å.
And this 1000Å silica gel substrate is rare in the industry.

Conditions;

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

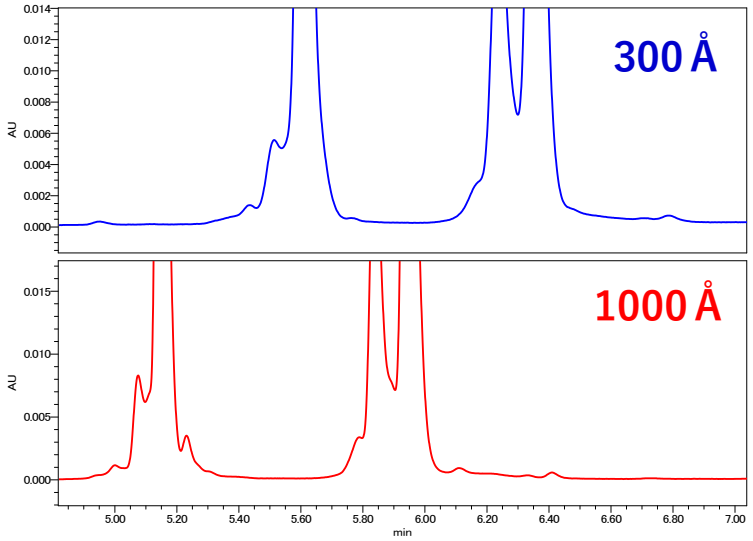
Mobile phase: A) Water + 0.1%TFA B) Acetonitrile + 0.1%TFA

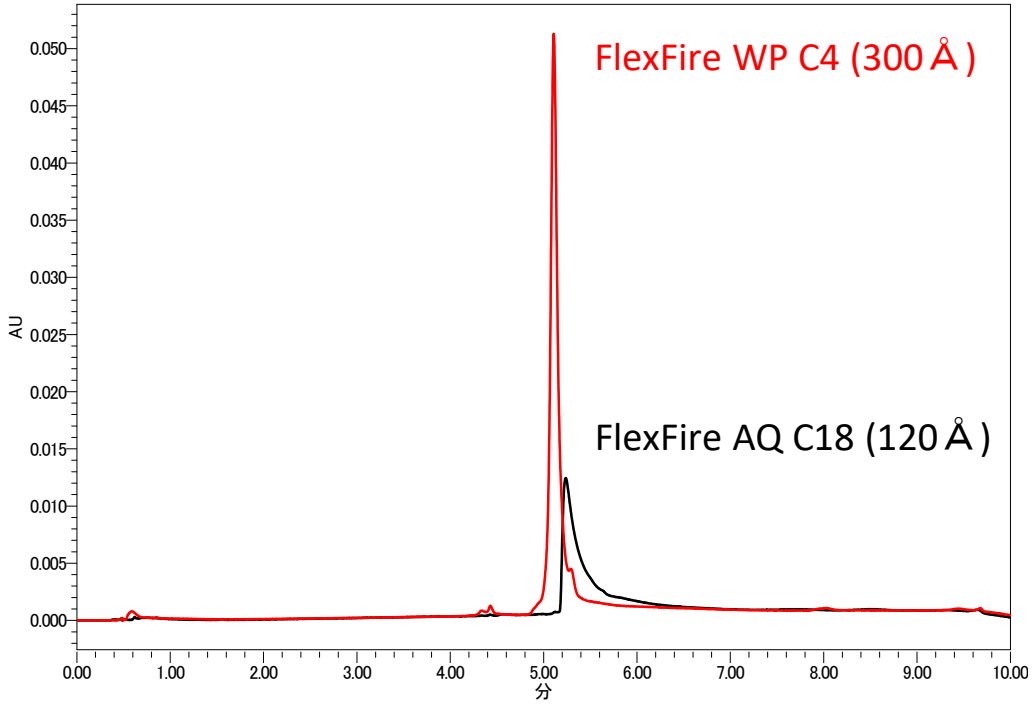
Gradient:

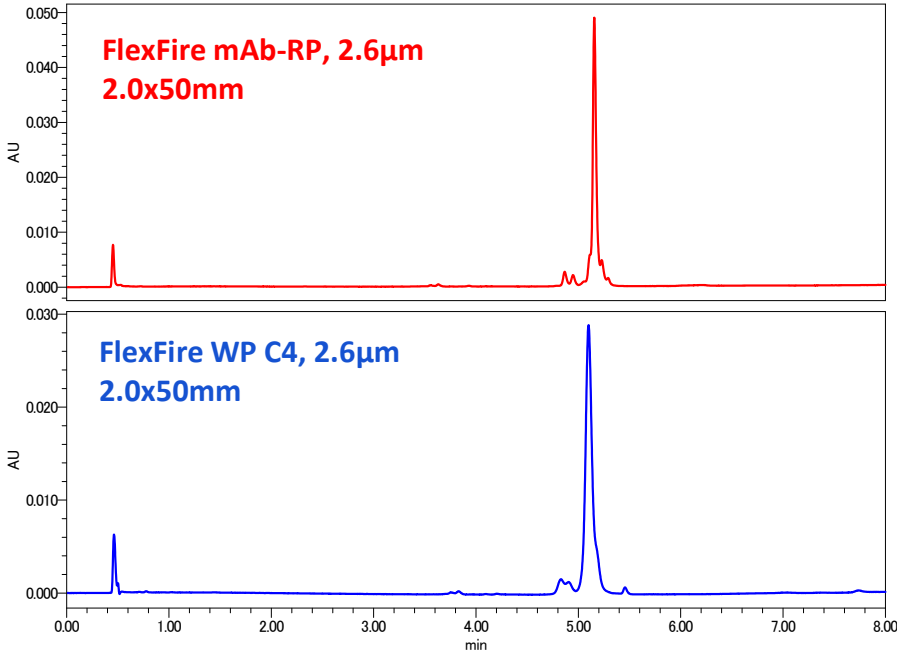
min	mL/min	%A	%B	Curve
0.00	0.3	80	20	
8.40	0.3	40	60	6
8.42	0.3	80	20	6

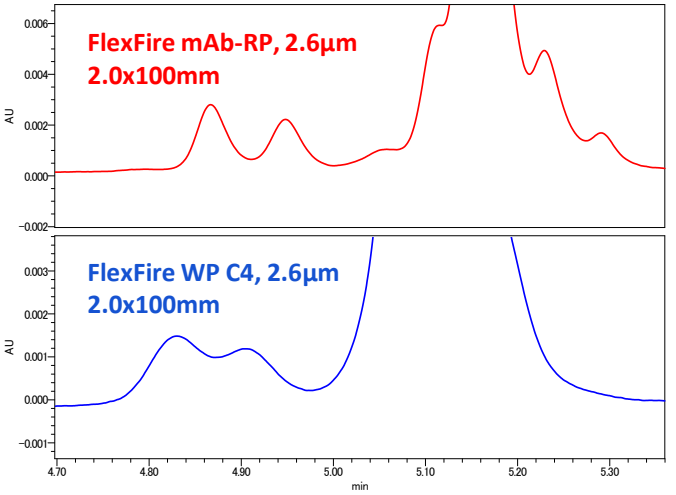
Temperature: 40℃
Detection: UV280nm
Sample: 1. α-Lactalbumin (0.34mg/mL)
2. β-Lactoglobulin (1.00mg/mL)
Injection volume: 2.0uL

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









Conditions:

Column: FlexFire WP C4, 2.6µm (2.0x50mm)
FlexFire AQ C18, 2.6µm (2.0x50mm)

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

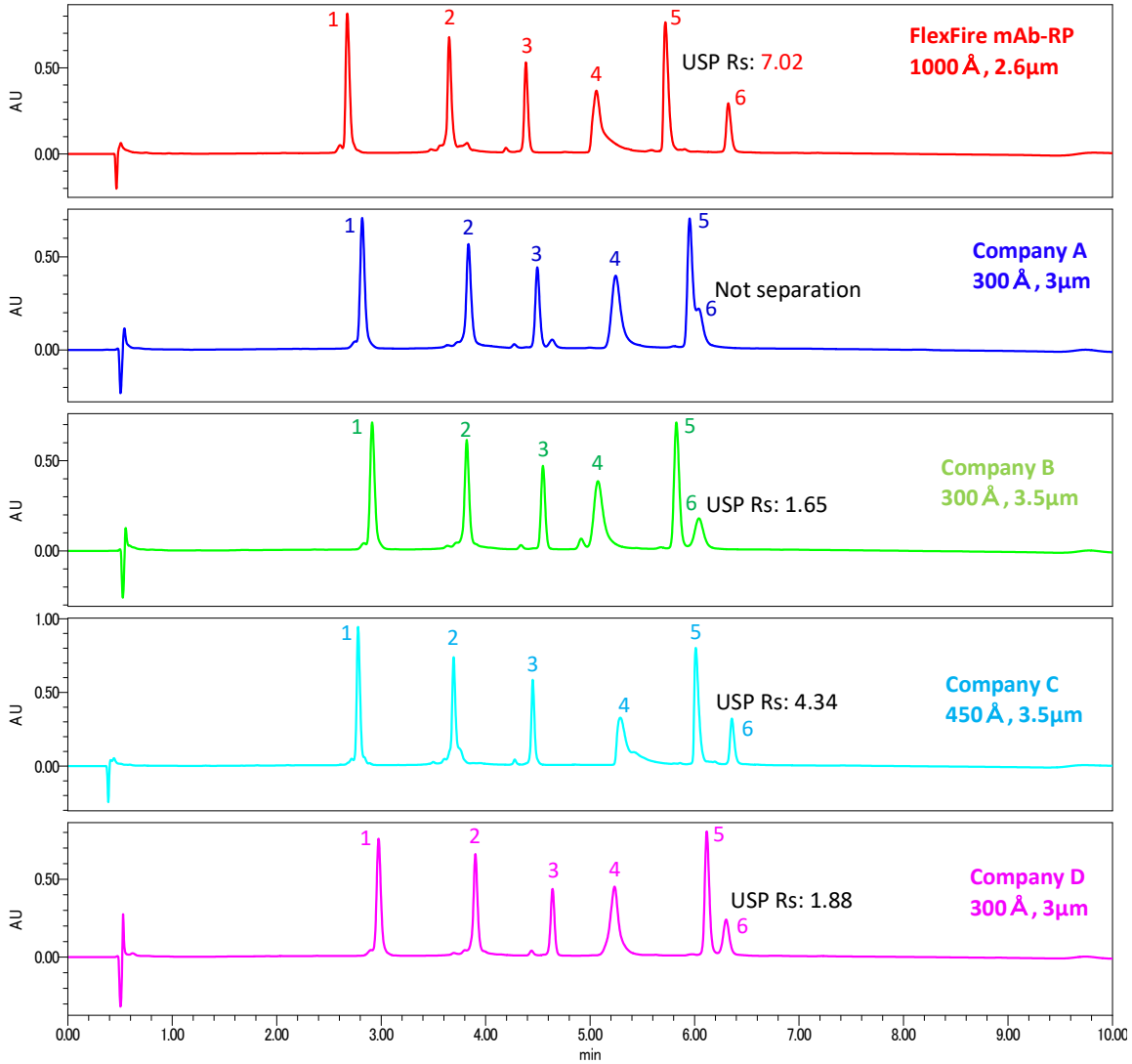
Gradient:

Time	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: 70℃
Detection: UV280nm
Sample: 1. Intact Mouse IgG1 (5.0mg/mL)
Injection volume: 1.0µL
System: Waters ACQUITY UPLC H-Class PLUS
Mixer: 100µL

Wide pore columns are very important for the analysis of compounds with very large molecular weights such as antibodies.
Especially for large molecular weight (100KDa-), 1000Å is effective.

Separation of catalase (220KDa)



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

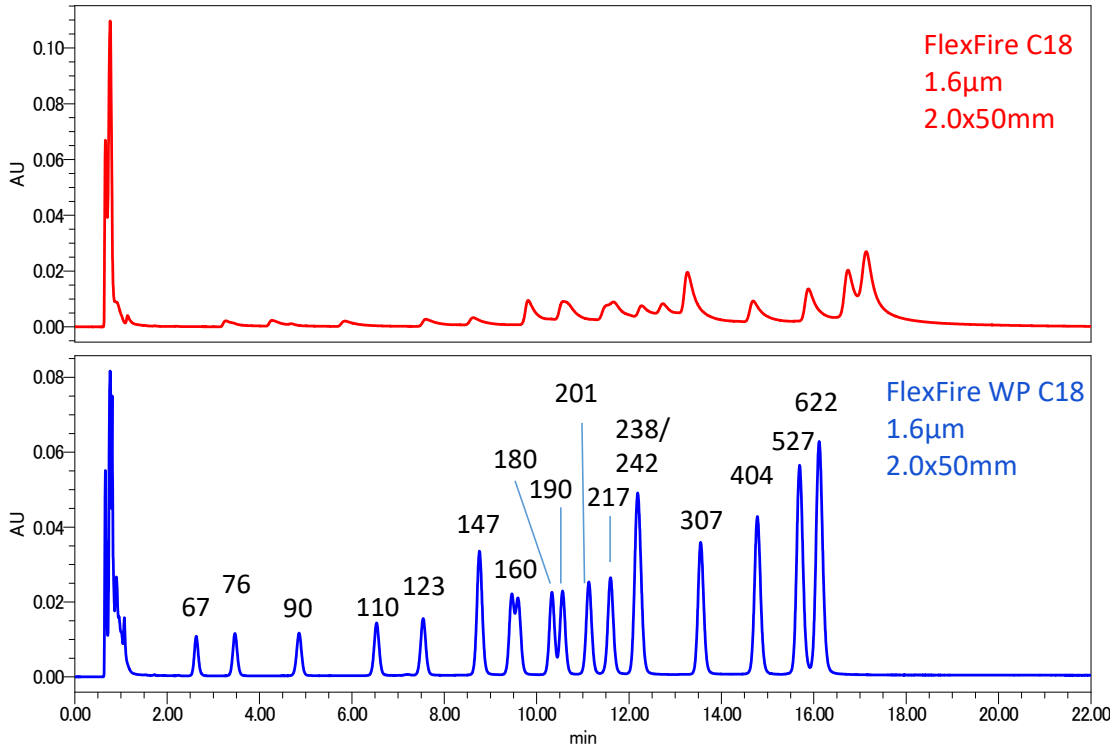
System: Waters ACQUITY UPLC H-Class PLUS

Mixer: 100μL

When catalase with a larger molecular weight is separated and compared with other proteins, the larger the pore size, the better the separation.

Separation example by wide pore column

pBr322 MspI Digest



Conditions:

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

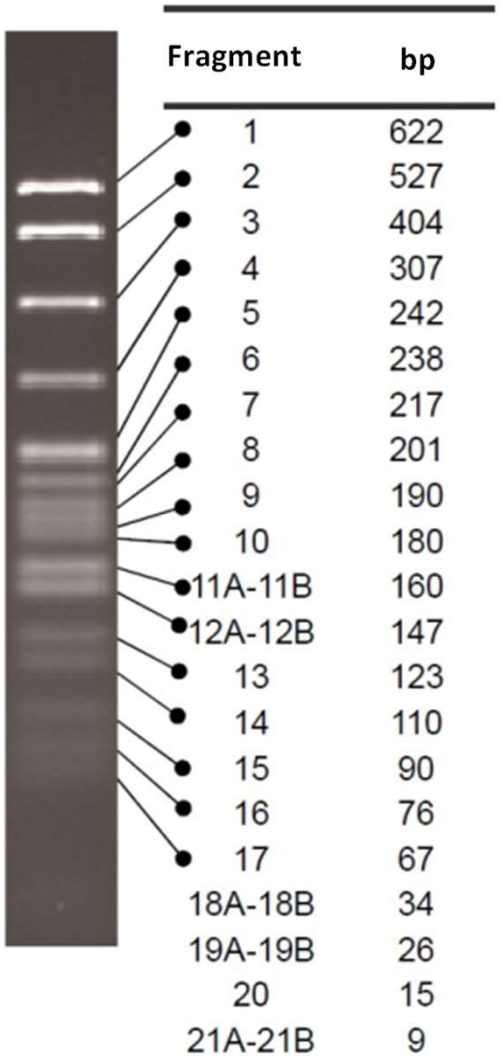
Mobile phase: A) 0.1M TEAA, pH7.0 B) 0.1M TEAA, pH7.0/ACN=80/20

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

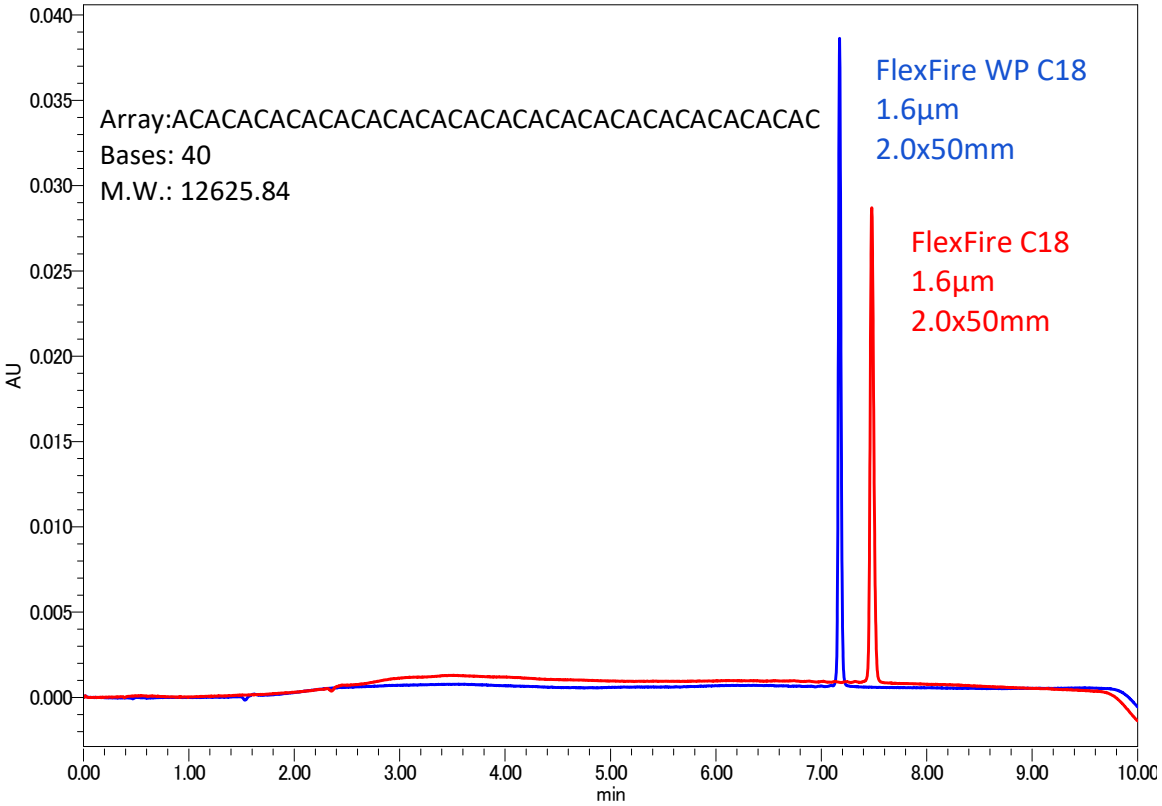


3% Agarose 21

EtBr Staining

Separation example by wide pore column

Synthesized 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

Nucleic acids are not as large in molecular weight as proteins. Therefore, it is possible to analyze even with a normal pore column. However, we have found that using a wide pore column for the analysis of nucleic acids derived from the human body gives very good sensitivity.

Finally

Do you notice?
Most applications are conditions that can be implemented in LC / MS. We can derive the best analytical conditions for many users.
At present, there is a shift from low molecular weight compounds to high molecular weight compounds. We have already developed columns applicable to these compounds. Check the details on the homepage.

We cover all the processes ourselves. This is rare worldwide. That's why we can spend time on a lot of research.

Get started with FlexFire! !