
NOMURA
CHEMICAL
FlexFire
Marking a new history of silica gel

FlexFire series, Marking a new history of silica gel

In “FlexFire,” “Flex” stands for “flexible” and “Fire” represents the intensity of fire.

The logo was created while designing a phoenix, which is a symbol of fire, as a motif.

It has been 40 years since Nomura Chemical released “Develosil.” In this history, we made a crucial decision.

It is the upgrade of our silica gel.

It is not easy to create a new product of silica gel.

However, by reviewing all know-how and feedback from users, which have been accumulated in the past 40 years,

we were able to understand what is required.

The FlexFire Series was born from this effort,

and we hope that this series will mark a new chapter in the history of Nomura Chemical.

FlexFire

Transfer from the Develosil series

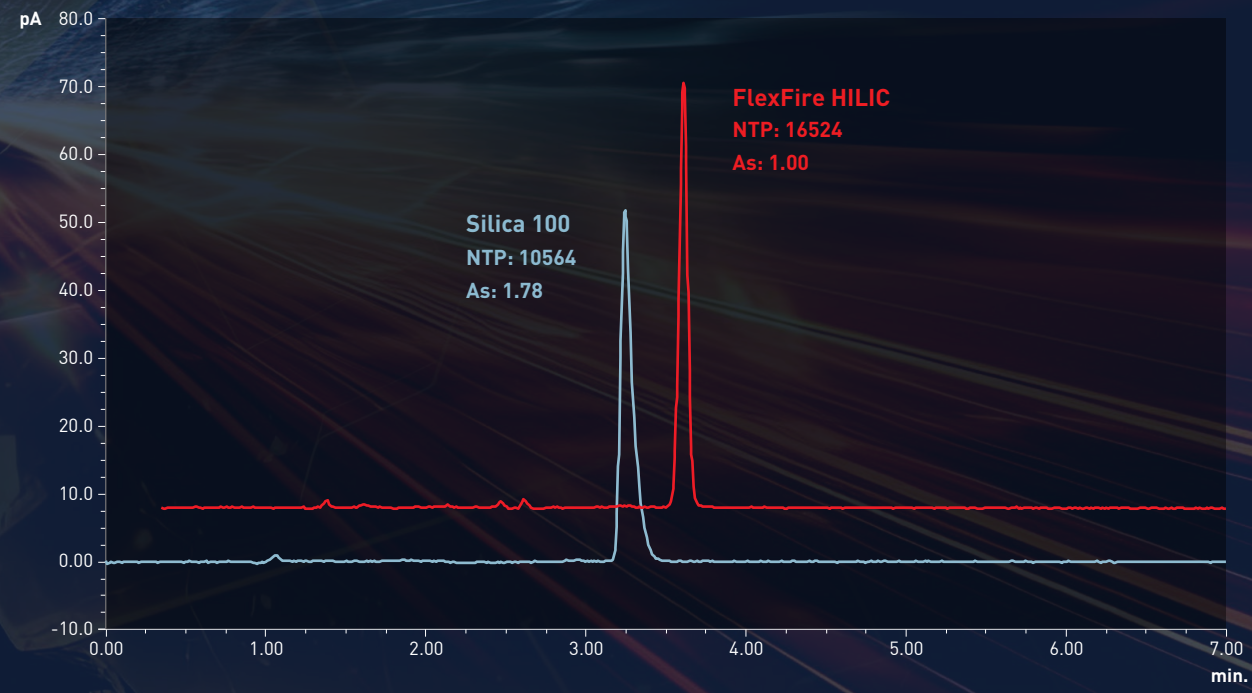
The FlexFire series can be easily transferred from those who use the Develosil ODS series, HG / UG series, XG series, and HSR series to those who develop new methods. In addition, differences in the degree of separation that occur during transfer are supported by the method development technology that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

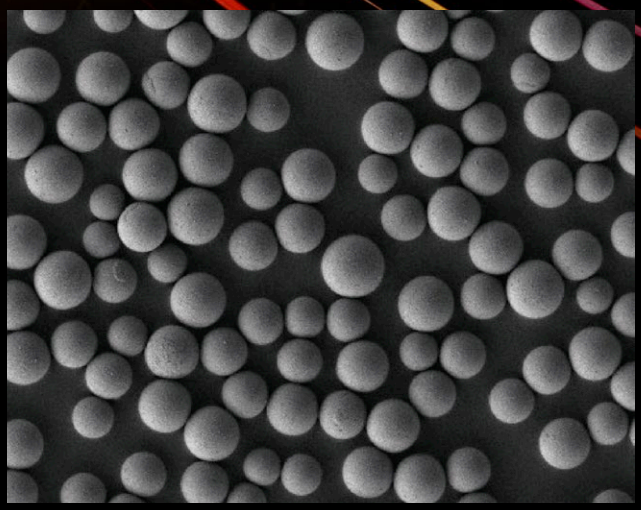


Analytical conditions;

Column:	FlexFire HILIC, 3μm (4.6x150mm)
	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 SCIENTIFICUltiMate3000

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)



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 columns have been added to the FlexFire series to suit many systems.

FlexFire series and system matching example

Category	Compatible i.d.	P article size	Verification system
HPLC	Φ4.6mm	5μm	Waters alliance
			Waters Are HPLC
			Thermo Vanquish_C
UHPLC	Φ2.0mm	1.6μm,	Waters ACQUITY UPLC H-Class
		2.6μm,	Thermo Vanquish_H
		5μm	SHIMADZU Nexra X3

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

This list is as of November 2021. The pH range has now been updated to pH 1-10 (exceptfor 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.

FlexFire series spec sheet

Product	FlexFire C18	FlexFire AQ C18	FlexFire C8	FlexFire C1	FlexFire C30	FlexFire HILIC
Chemistry	Octadecyl	Octadecyl	Octyl	Trimethyl	Triacontyl	—
Particle size (μm)	1.6, 2.6, 5	1.6, 2.6, 5	1.6, 2.6, 5	1.6, 2.6, 5	1.6, 2.6, 5	1.6, 2.6, 5
Surface Area (m²/g)	340	340	340	340	340	340
Pore Volume (mL/g)	1.0	1.0	1.0	1.0	1.0	1.0
Pore Diammeter (nm)	12	12	12	12	12	12
Carbon (%)	22	8.5	12	5.5	11	—
End cap	○	○	○	○	○	—
pH Range	pH1-11	pH1-9	pH1-11	pH1-10	pH1-10	pH1-7
Max Temperature (℃)	80	80	80	80	80	80
Max Pressure (bar)	1.6μm: 1,000bar 2.6μm: 600bar 5μm: 400bar					
USP	L1	L1	L7	L13	L62	L3

FlexFire bioseparation column

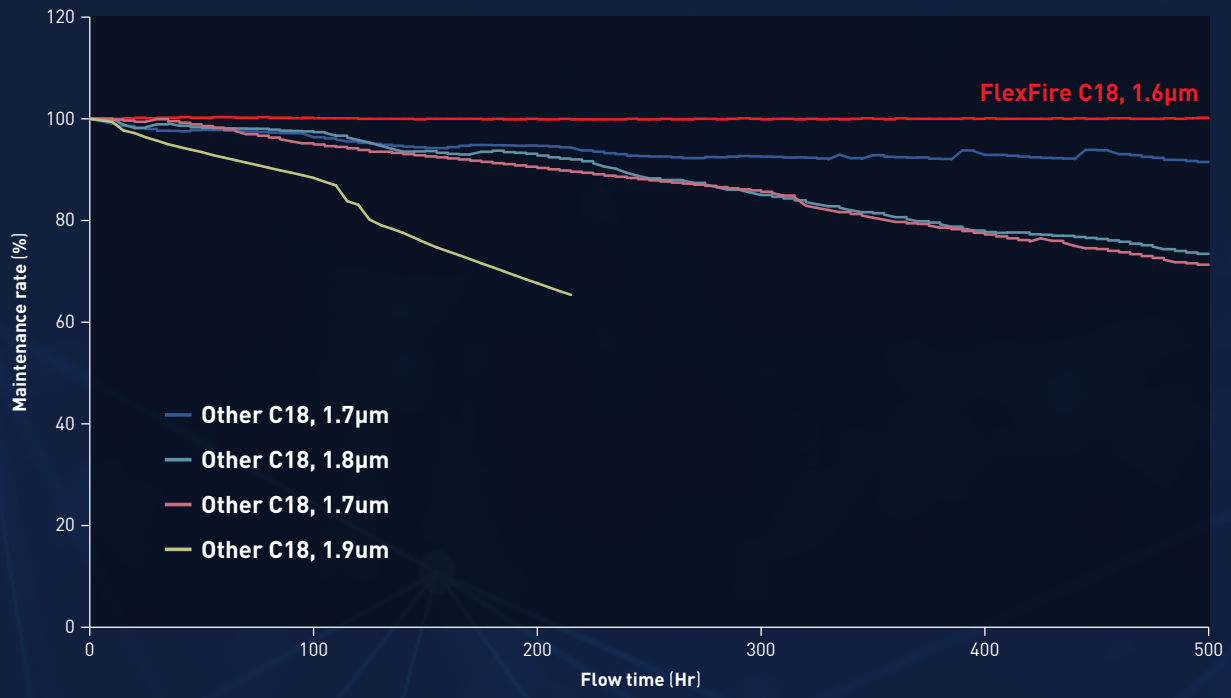
Product	FlexFire WP C18	FlexFire WP C8	FlexFire WP C4	FlexFire WP C1	FlexFire mAb-RP	FlexFire 120SEC	FlexFire 300SEC
Chemistry	Octadecyl	Octyl	Butyl	Trimethyl	Butyl	Diol	Diol
Particle size (μm)	2.6, 5	2.6, 5	2.6, 5	2.6, 5	2.6, 5	5	5
Surface Area (m²/g)	170	170	170	170	27	340	170
Pore Volume (mL/g)	1.4	1.4	1.4	1.4	0.8	1.0	1.4
Pore Diammeter (nm)	30	30	30	30	115	12	30
Carbon (%)	15	7	5	3	1.3	9	6
End cap	○	○	○	○	○	—	—
pH Range	pH1-10	pH1-10	pH1-10	pH1-10	pH1-10	pH2-10	pH2-10
Max Temperature (℃)	80	80	80	80	80	80	80
Max Pressure (bar)	2.6μm: 600bar 5μm: 400bar					5μm: 400bar	
USP	L1	L7	L26	L13	L26	L33	L33

Durability and bond density

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.

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



Analytical conditions;

Column:	FlexFire C18, 1.6um
Size:	2.0x50mm
Mobile phase:	Acetonitrile/10mM NH4OH, pH10.5=60/40
Flow rate:	0.5mL/min
Temperature:	40℃
Detection:	UV254nm
Sample:	1.Uracil (0.01mg/mL) 2.Naphthalene (0.1mg/mL)
Injection volume:	0.16uL

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.

Fig.4 Bond density of each chemistry

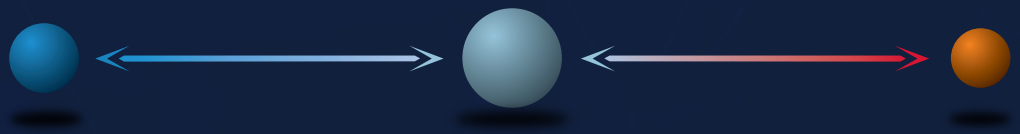
UHPLC Method Transfer

The FlexFire series is available in particle sizes of 1.6μm, 2.6μm and 5μm.

The particle size can beselected according to the system.

And methodtransfer from 5μm to 1.6μm can be performed easily.

Fig.5 Image model of method transfer

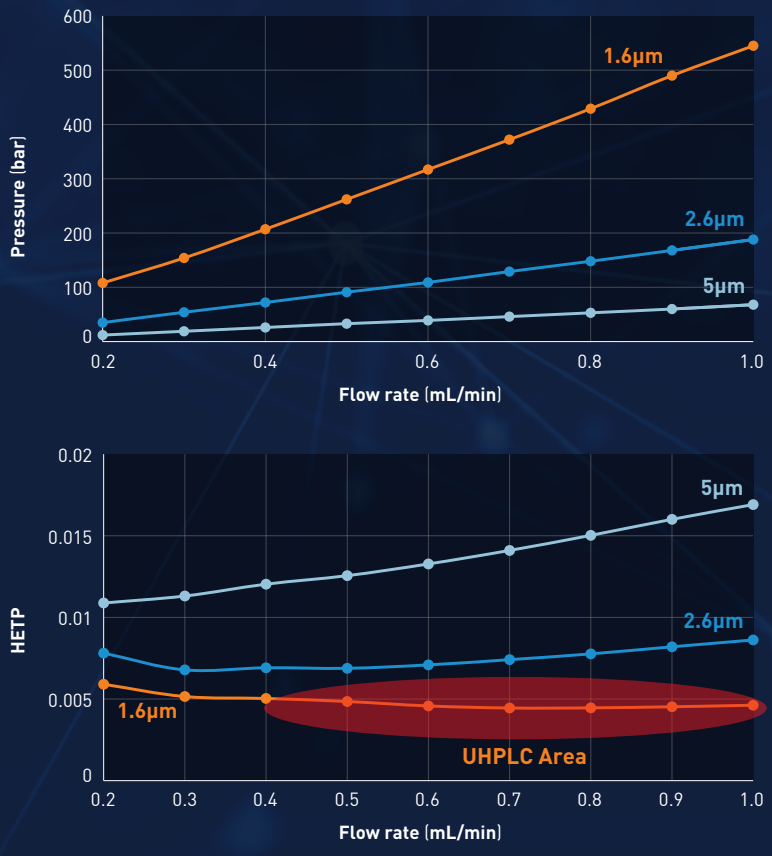


R&D_Semi-UHPLC
2.6μm_2.0mm i.d. Column
Flow rate: 0.364mL/min

QC_HPLC
5μm_2.0mm i.d. Column
Flow rate: 0.189mL/min

R&D_UHPLC
1.6μm_2.0mm i.d. Column
Flow rate: 0.591mL/min

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



Analytical conditions;

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

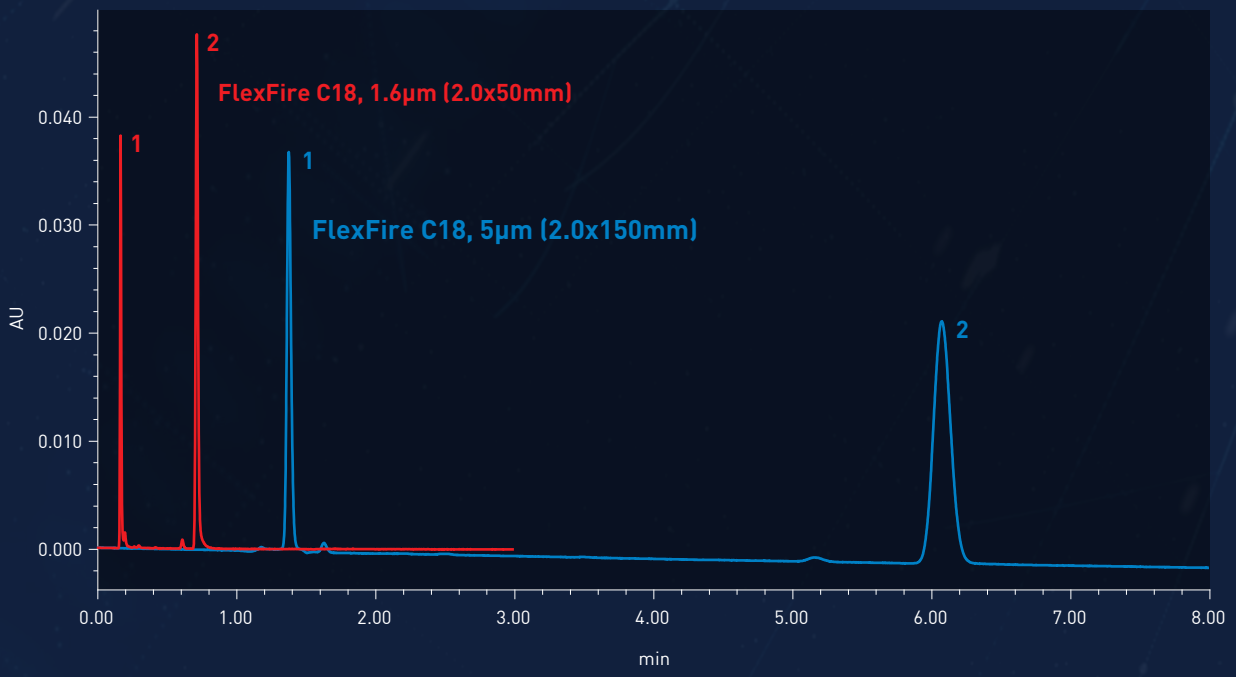
Example of method transfer

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.

Fig.7 Example simple method transfer



Analytical 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

Comparison of insulin peak shapes

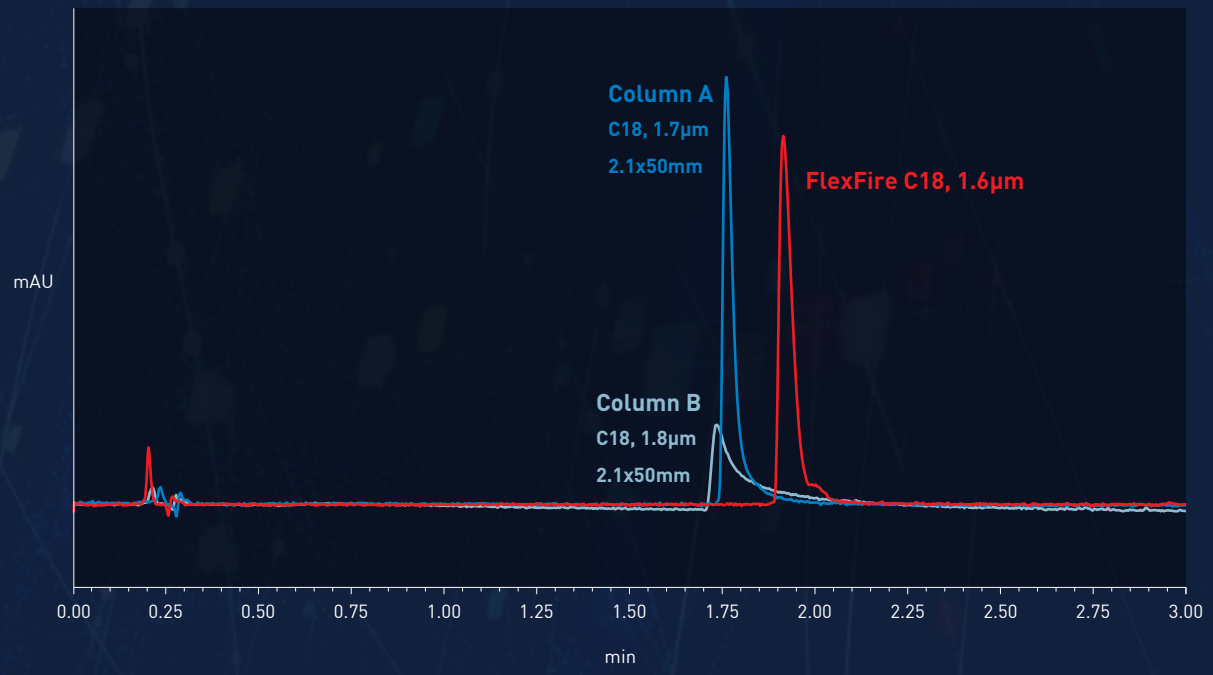
Insulin was analyzed using a 0.1% formic acid mobile phase.

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.

Fig.8 Comparison of Insulin peak shapes



Analytical 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

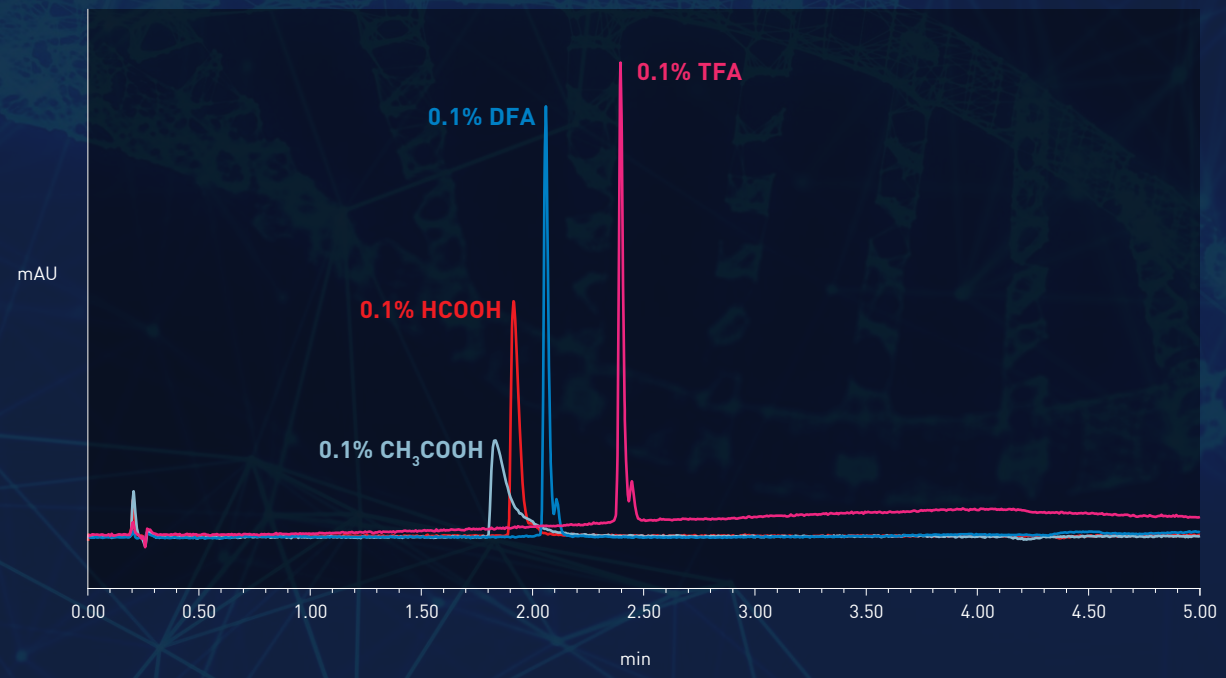
Comparison of insulin peak shapes with Various mobile phases

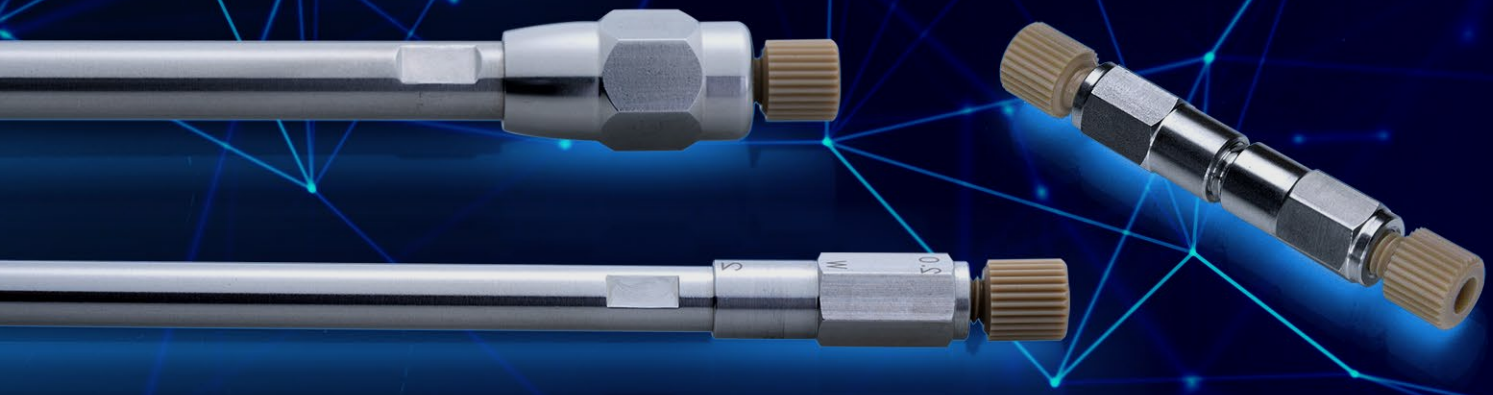
If good results are not obtained with 0.1% formic acid, it is desirable to switch to DFA or TFA.

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.

Fig.9 Comparison of Insulin peak shapes with various mobile phase





Conditions that can be used with 100% aqueous mobile phase

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

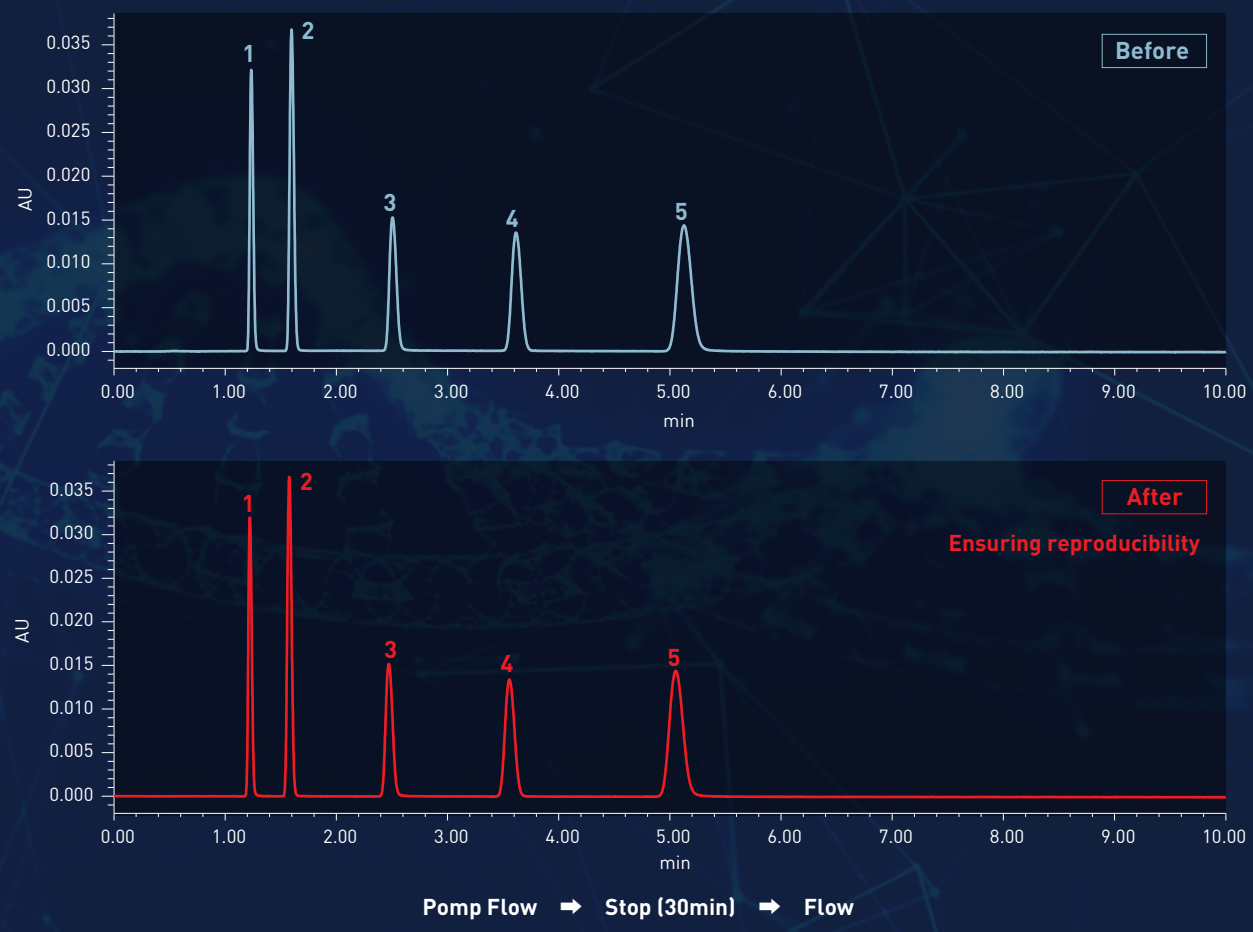
The mechanism is very simple. By stopping the pump, the mobile phase taken into the pores escapes.

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.

Fig.10 Stop-flow test under 100% aqueous mobile phase conditions



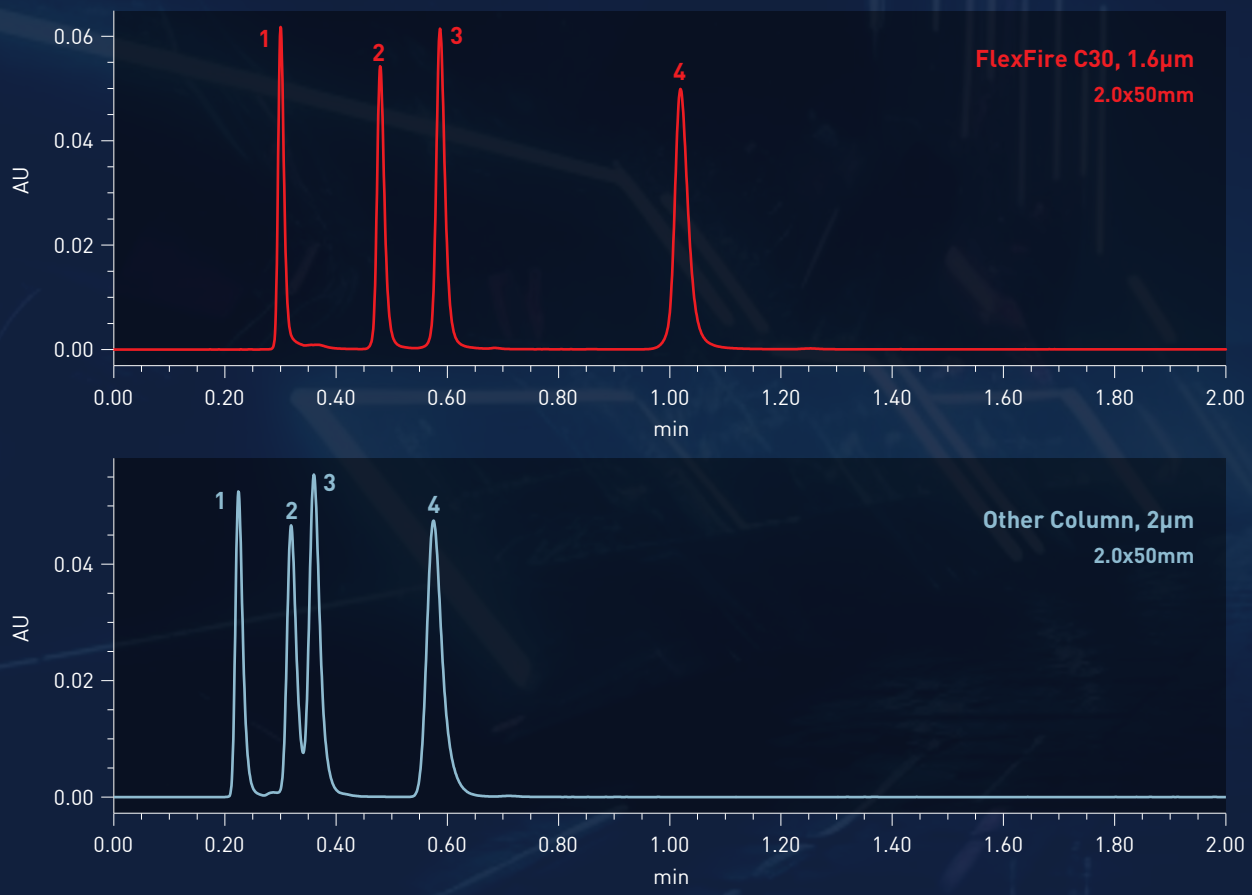
Analytical conditions;

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

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.

Fig.11 Analysis of dNTP with 100% aqueous mobile phase



Analytical 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

S/N up with short alkyl chain

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).

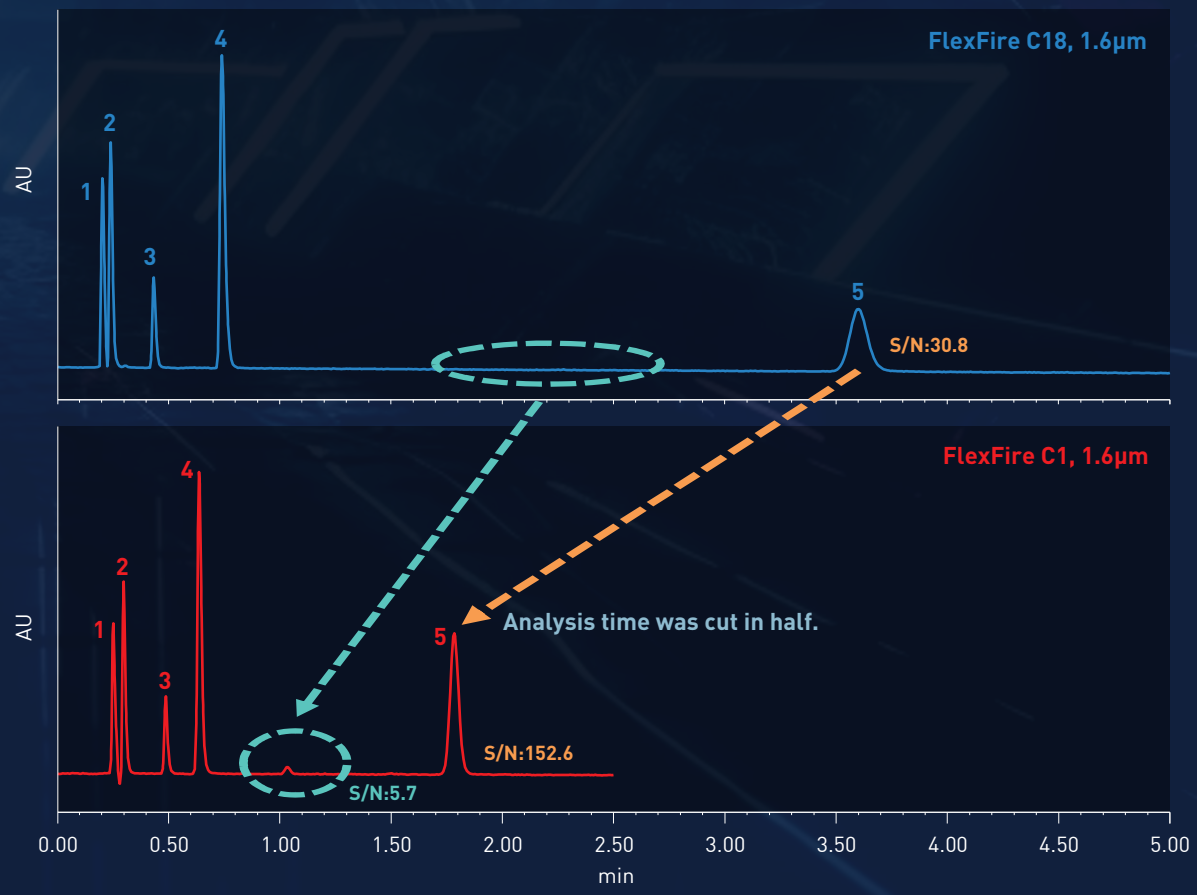
However, S / N has overwhelmingly high C1.

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.

Fig.12 S/N comparison (C18 vs C1)



C8 column for sharp peaks

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.

Fig.13 Comparison of Insulin peak shapes with various mobile phase

Analytical 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 ／ FlexFire C8, 2.6µm ／ Develosil C8-UG, 3µm ／ Other C8, 3µm

Metal-Free column

Metal free column was added from FlexFire series.

Compounds containing phosphorus groups adsorb to metals.

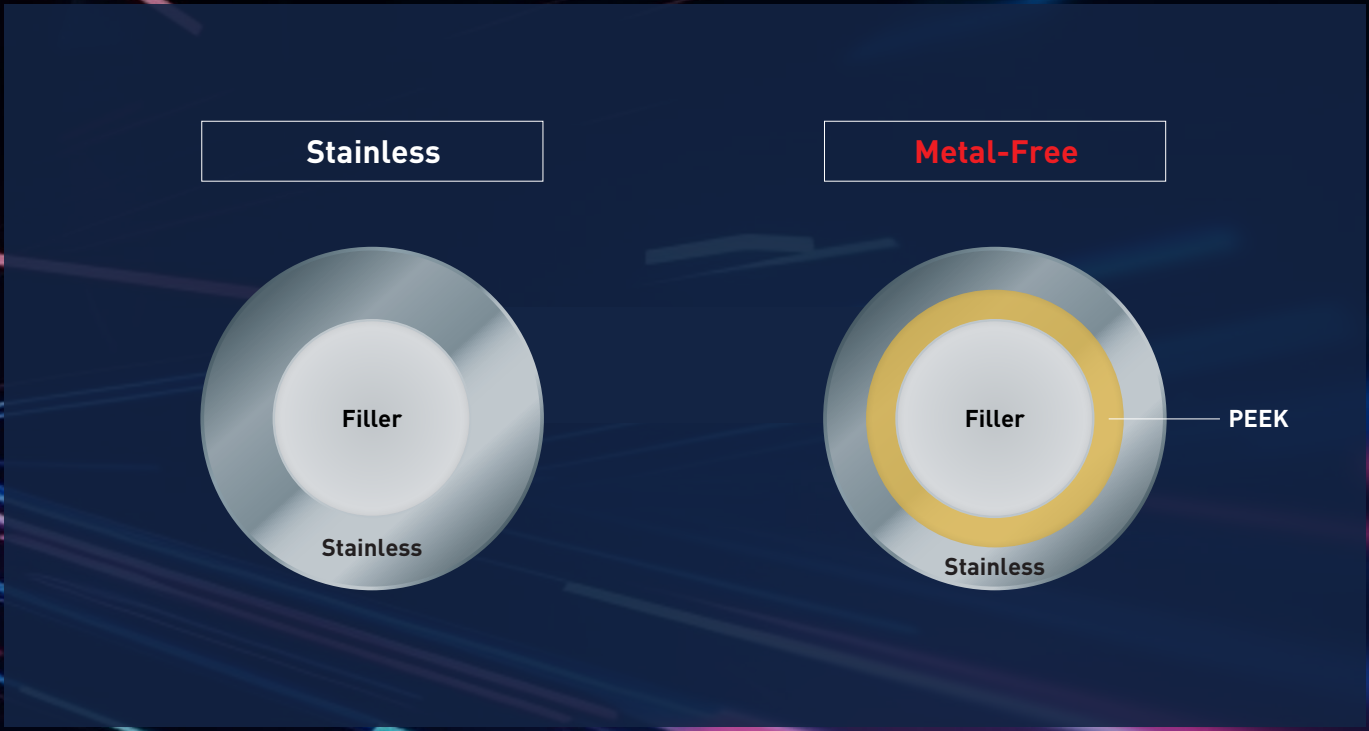
Until now, the use of a phosphate buffer improved the peak shape, but it had a major problem.

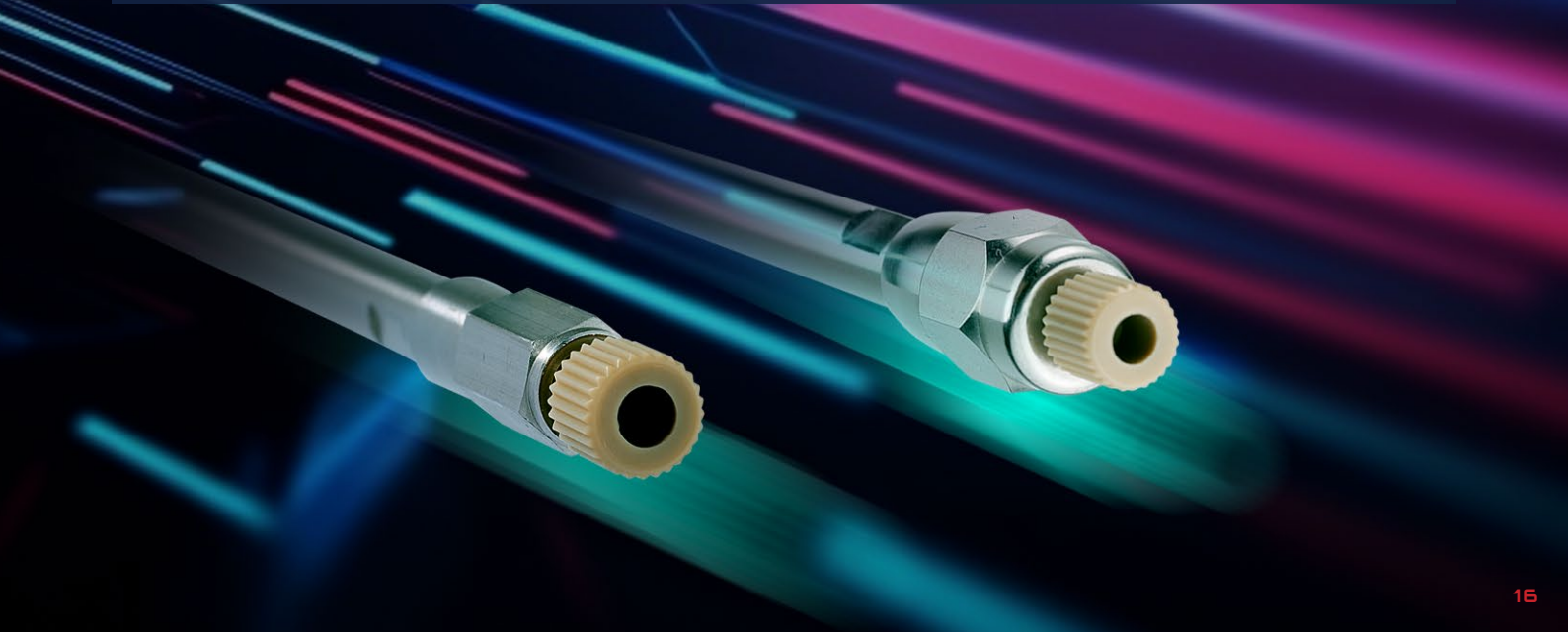
It cannot be introduced into LC / MS.

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

Develosil metal-free columns have a PEEK tube built into a stainless steel tube.

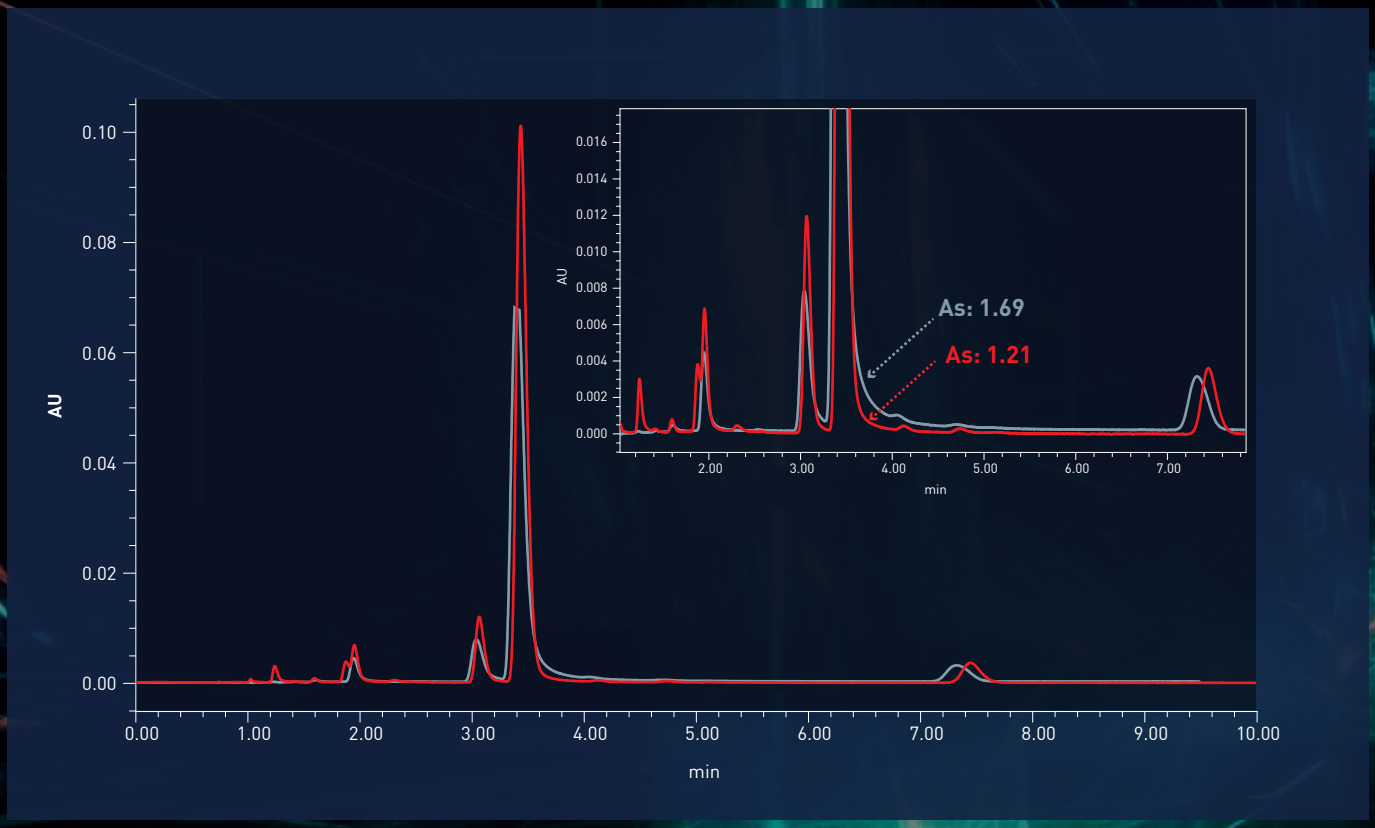
This ensures high durability even under high pressure of UHPLC columns such as 1.6μm particle size.





Example using metal-free column

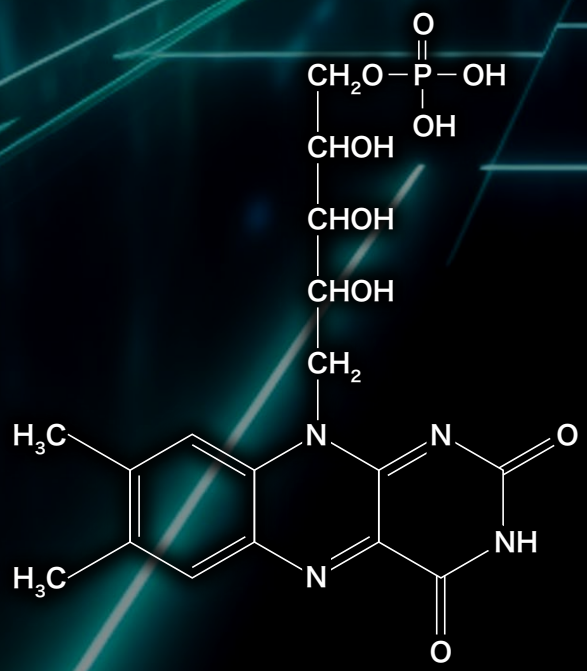
(Flavin mononucleotide)



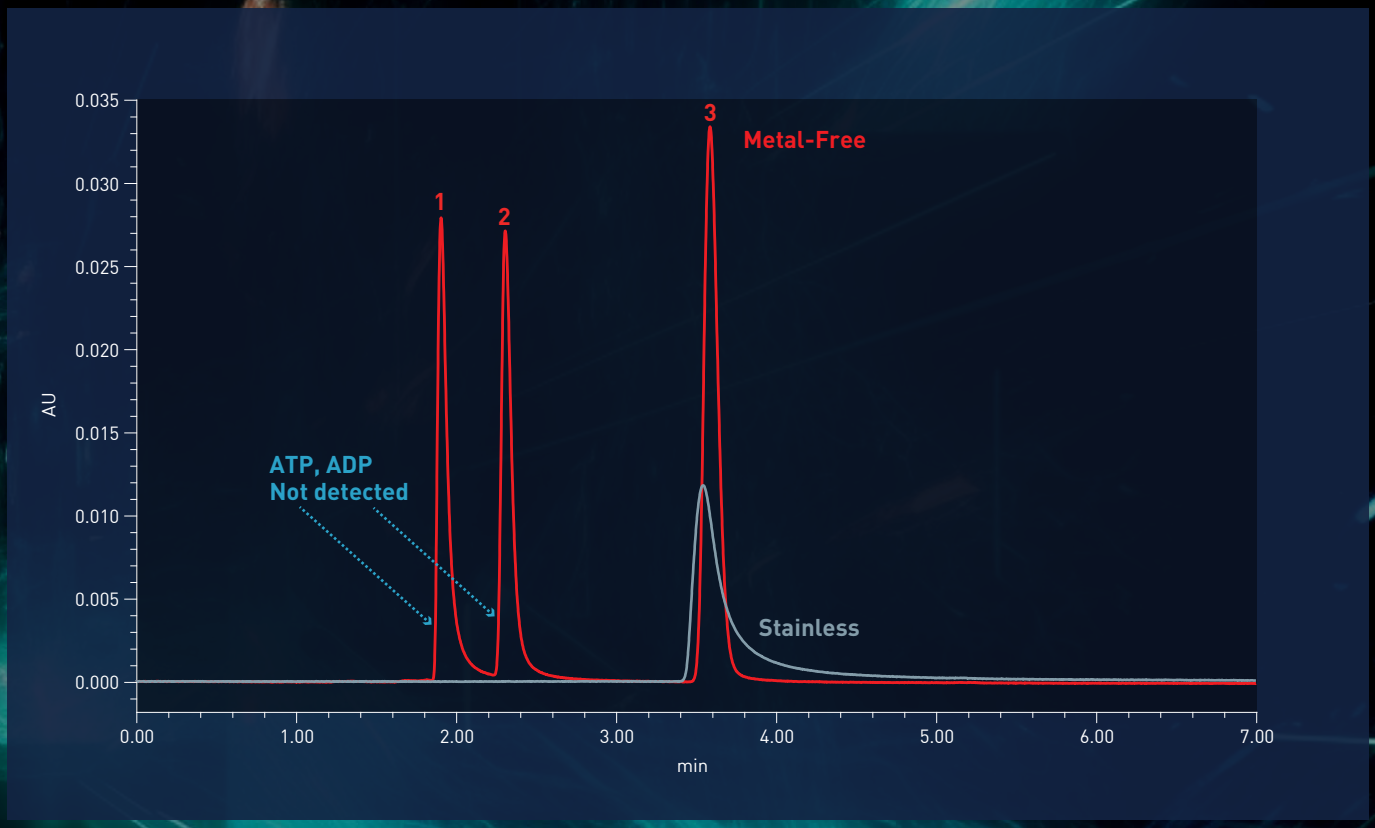
Analytical conditions;

Column:	FlexFire AQ C18, 2.6μm (2.0x100mm) : Stainless
	FlexFire AQ C18, 2.6μm (2.0x100mm) : Metal Free
Mobile phase:	Acetonitrile/25mM HCOONH4=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

Flavin mononucleotide



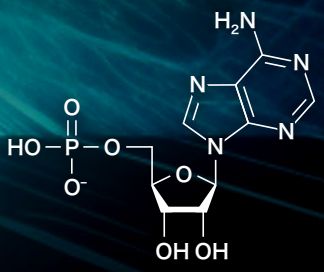
Example using metal-free column (AMP, ADP, ATP)



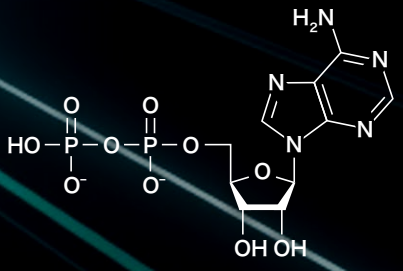
Analytical conditions;

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

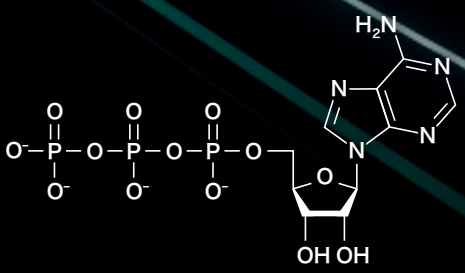
AMP



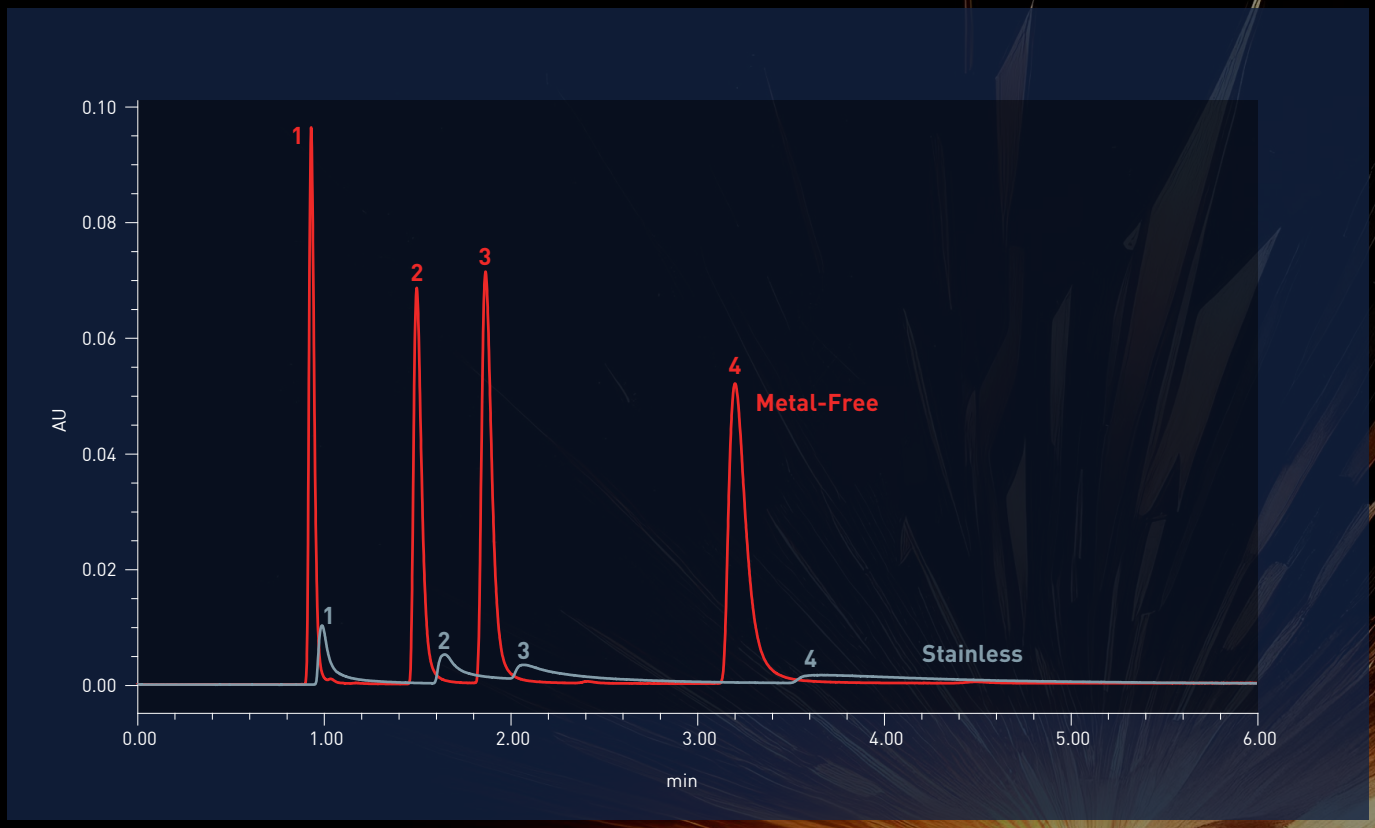
ADP



ATP



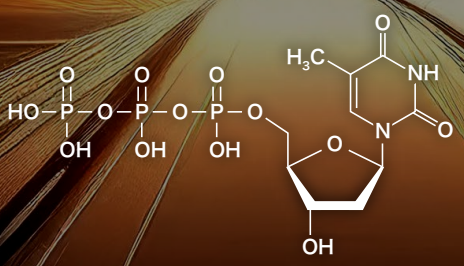
Example using metal-free column (dNTP)



Analytical conditions;

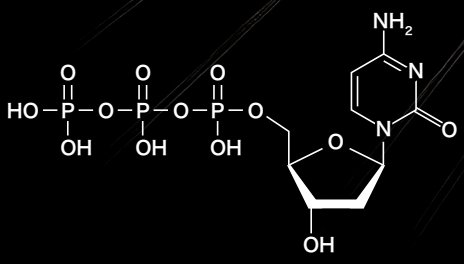
Column:	FlexFire AQ C18, 2.6µm (2.0x100mm) : Stainless
	FlexFire AQ C18, 2.6µm (2.0x100mm) : Metal Free
Mobile phase:	10mM $HCOONH_4$
Flow rate:	0.3mL/min
Temperature:	40℃
Detection:	UV260nm
Sample:	1. dCTP (13µM)
	2. dTTP (13µM)
	3. dGTP (13µM)
	4. dATP (13µM)
Injection volume:	0.2µL
System:	Waters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

dTTP

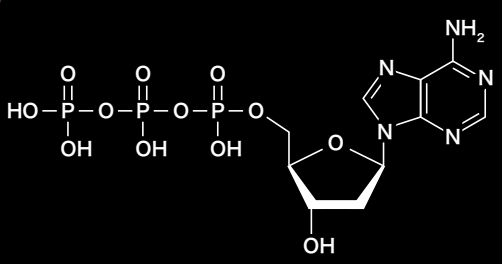


dGTP

dCTP



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.

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.

Analytical conditions;

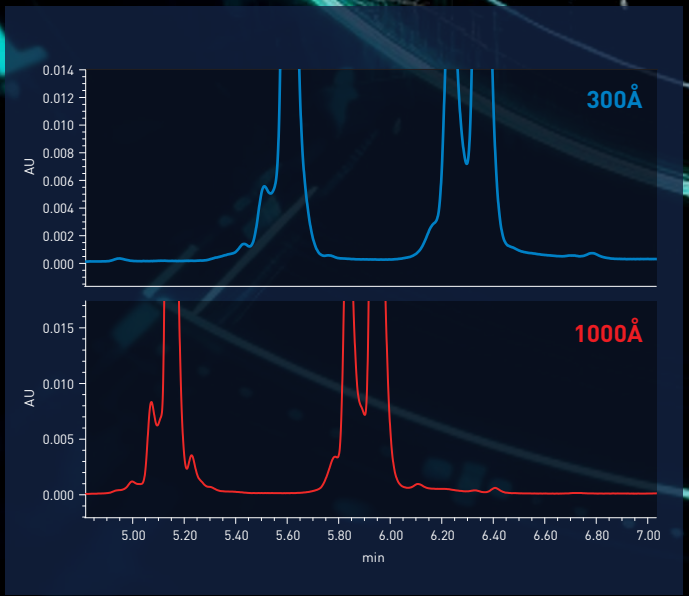
Column: **FlexFire C18, 2.6um (2.0x50mm)**
FlexFire WP C4, 2.6um (2.0x50mm)
FlexFire mAb-RP, 2.6um (2.0x50mm)

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

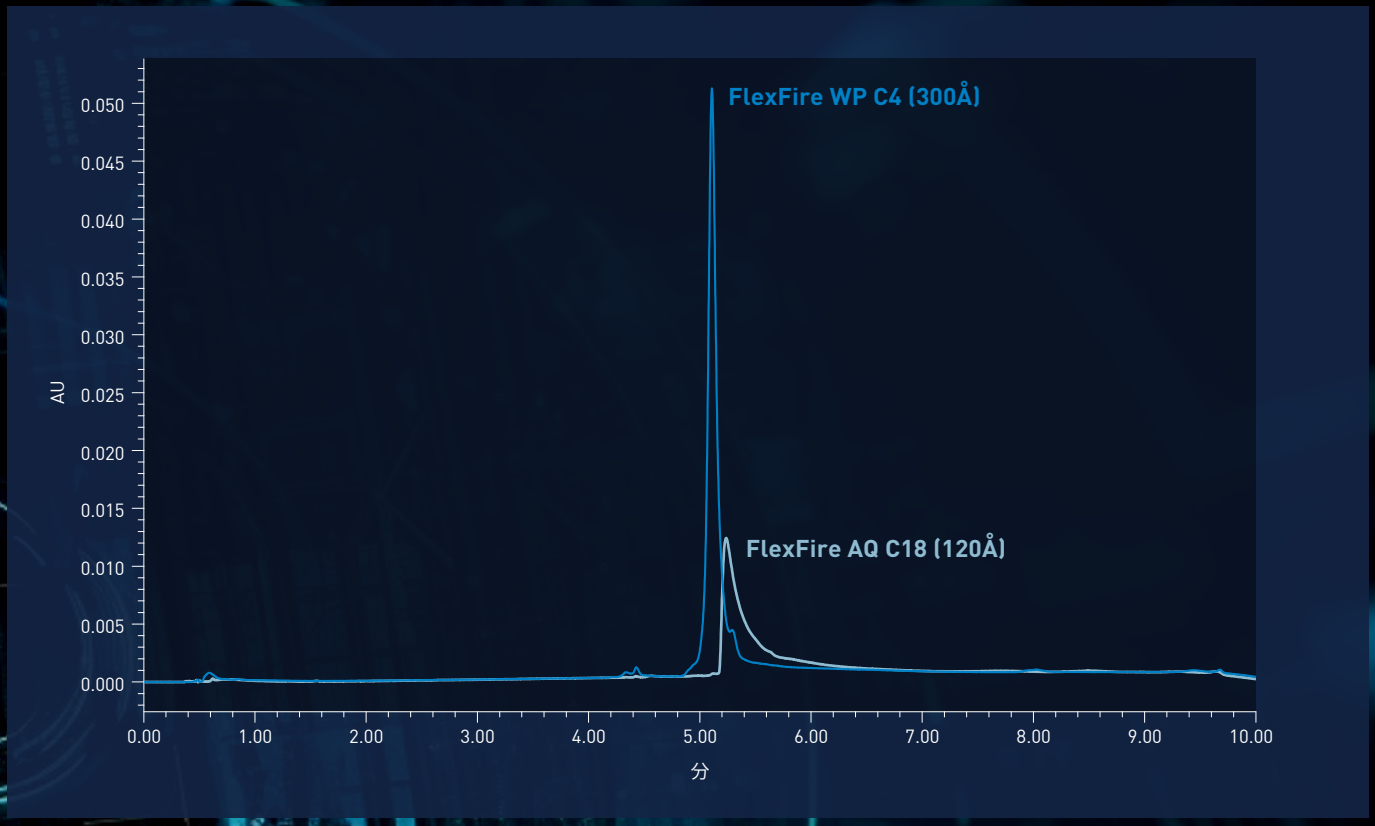
Gradient:

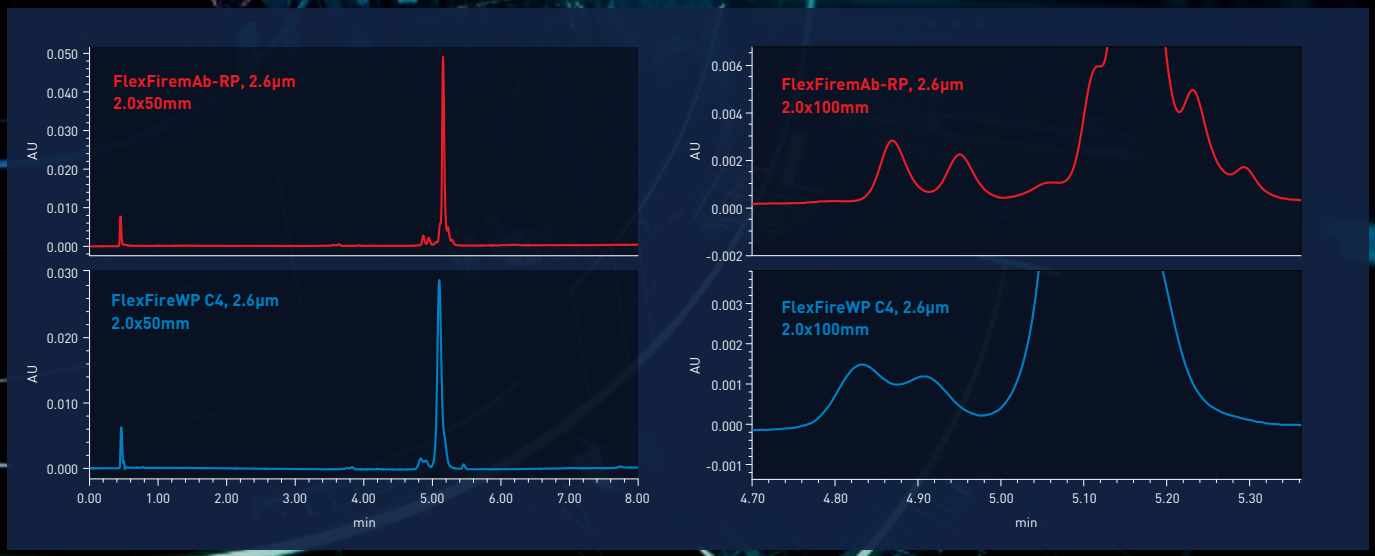
min	mL/min	%A	%B	Curve
0.00	0.3	80	20	
8.40	0.3	40	60	6
8.42	0.3	80	20	6

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



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.





Analytical 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

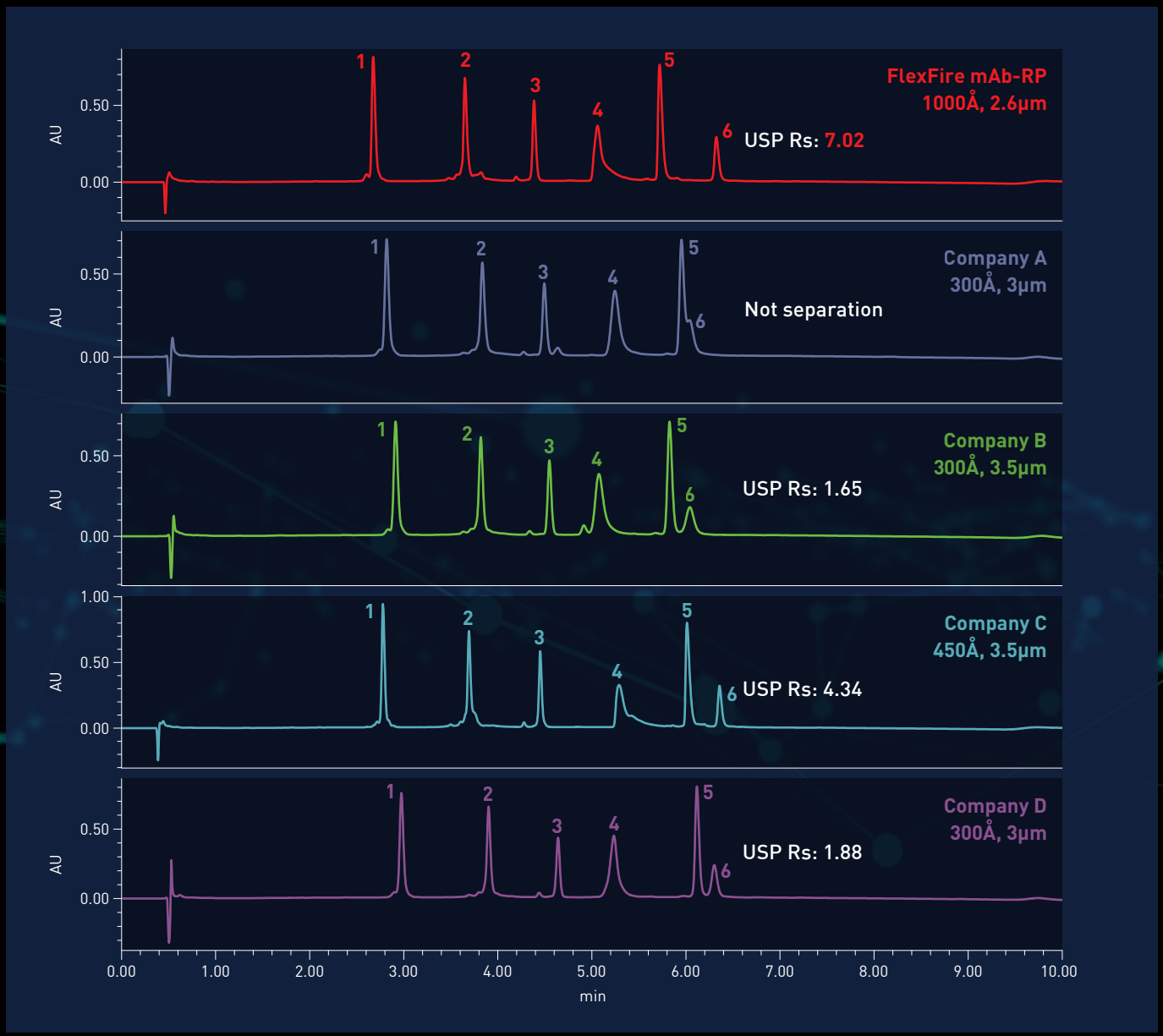
Gradient:

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

Separation of catalase (220KDa)

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



Analytical 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)

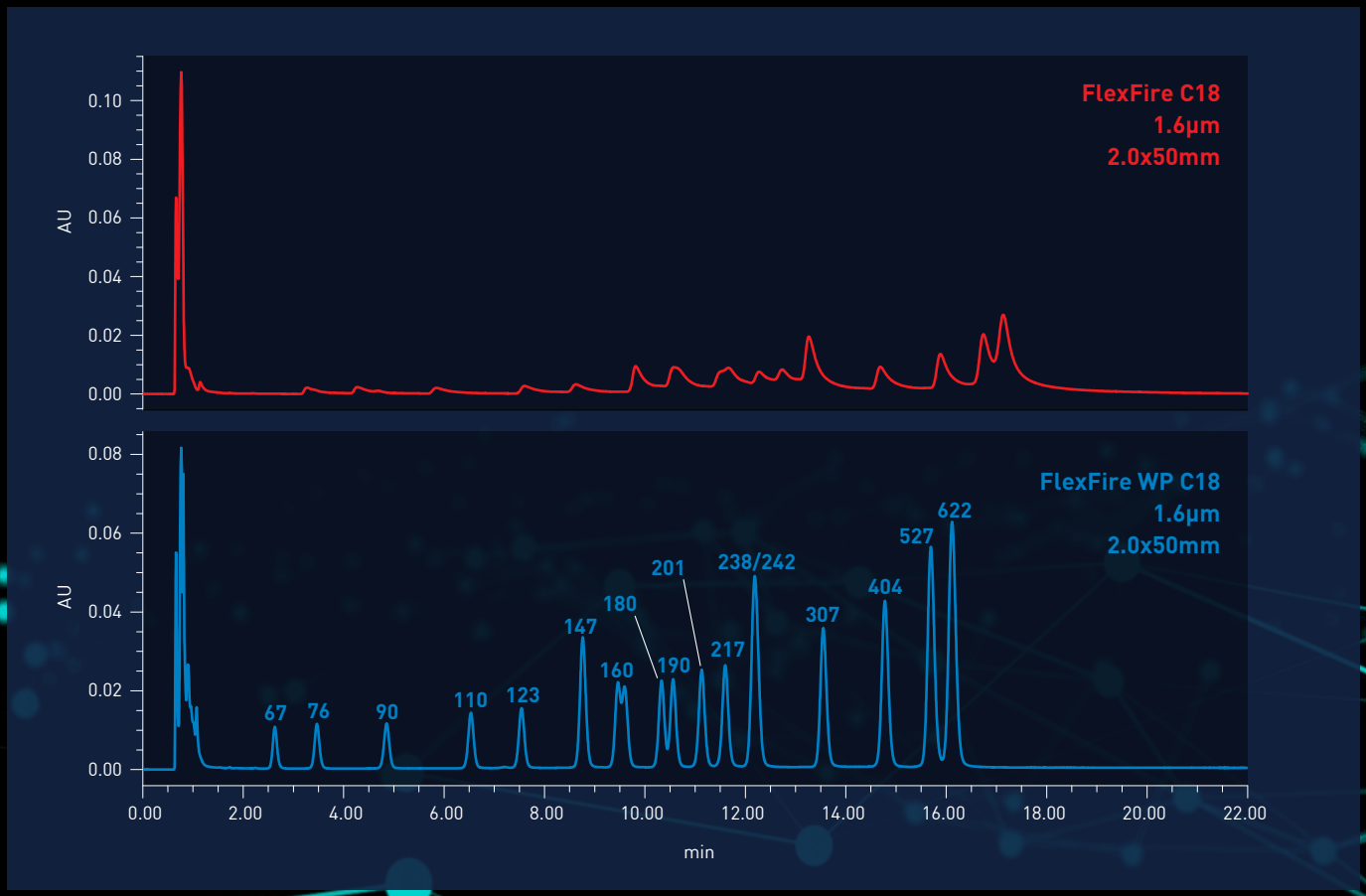
Injection volume: 2.0μL

System: Waters ACQUITY UPLC H-Class PLUS

Mixer: 100μL

Separation example by wide pore column

pBr322 MspI Digest



Analytical 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

"FlexFire WP C18, 1.6µm" is not for sale.
This column is compatible with particle sizes 2.6µm and 5µm.

EtBr Staining

Fragment	bp
1	622
2	527
3	404
4	307
5	242
6	238
7	217
8	201
9	190
10	180
11A-11B	160
12A-12B	147
13	123
14	110
15	90
16	76
17	67
18A-18B	34
19B-19B	26
20	15
21A-21B	9

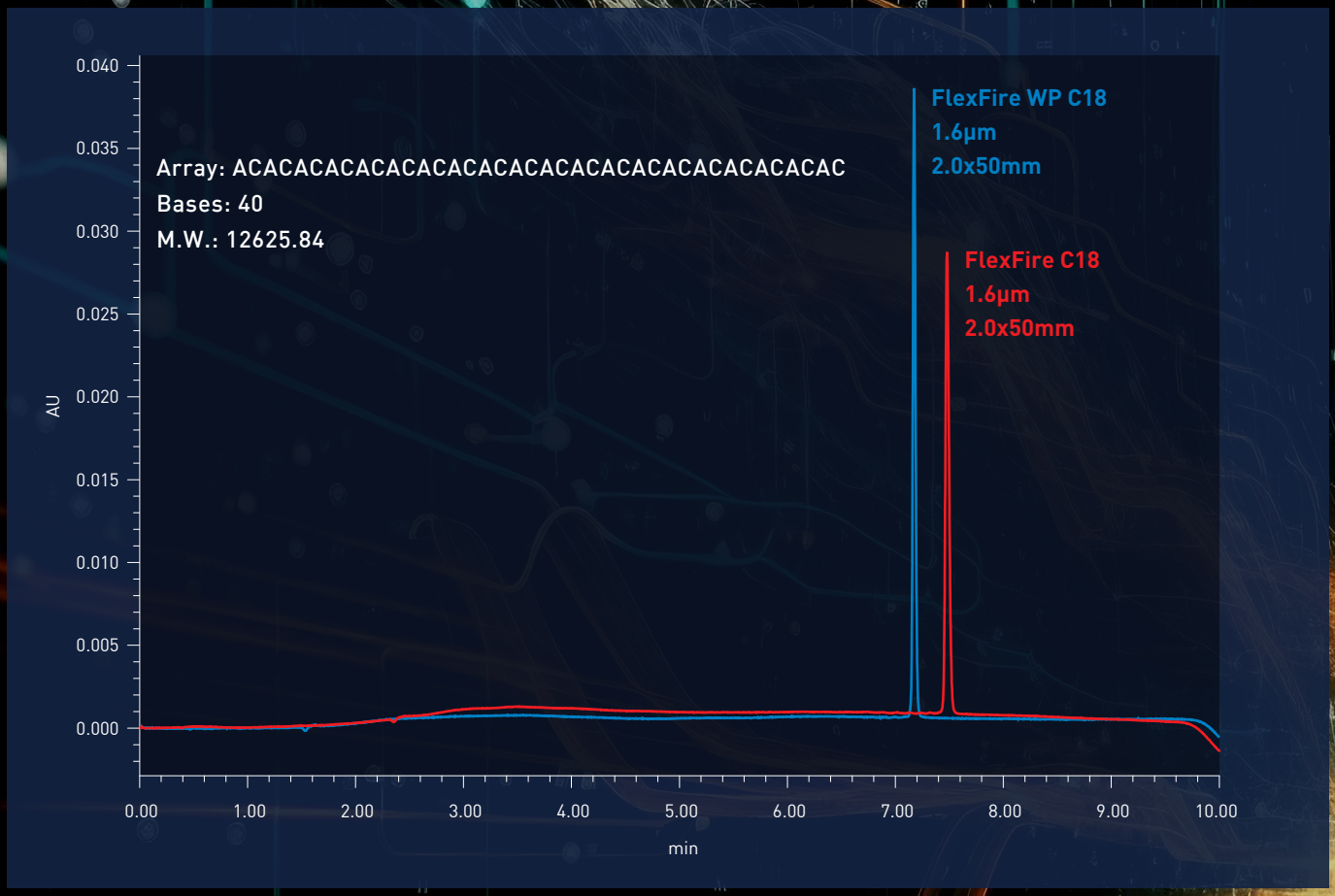
Separation example by wide pore column

Synthesized RNA

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.



Analytical 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°C
Sample: RNA
Injection volume: 0.2µL
System: Waters ACQUITY UPLC H-Class PLUS
Mixer: 100µL

"FlexFire WP C18, 1.6µm" is not for sale.
This column is compatible with particle sizes 2.6µm and 5µm.

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.

NOMURA CHEMICAL

【 CONTACT 】

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