

for HPLC



FlexFire Standard Series
FlexFire WP Standard Series

NOMURA CHEMICAL CO., LTD.



FlexFire Standard Series

Smooth method transfer to UHPLC with new standard !!

It is no exaggeration to say that a column with a particle size of 5µm and an inner diameter of 4.6Φ is the standard for HPLC analysis. With the spread of UHPLC, this 4.6Φ method is the first step towards transferring the UHPLC method. Until now, the FlexFire series has been composed only of an inner diameter of 2.0Φ, but in order to carry out research and development ⇔ quality control more smoothly, we have lined up a particle diameter of 5µm and an inner diameter of 4.6Φ.

Spec of FlexFire Standard Series

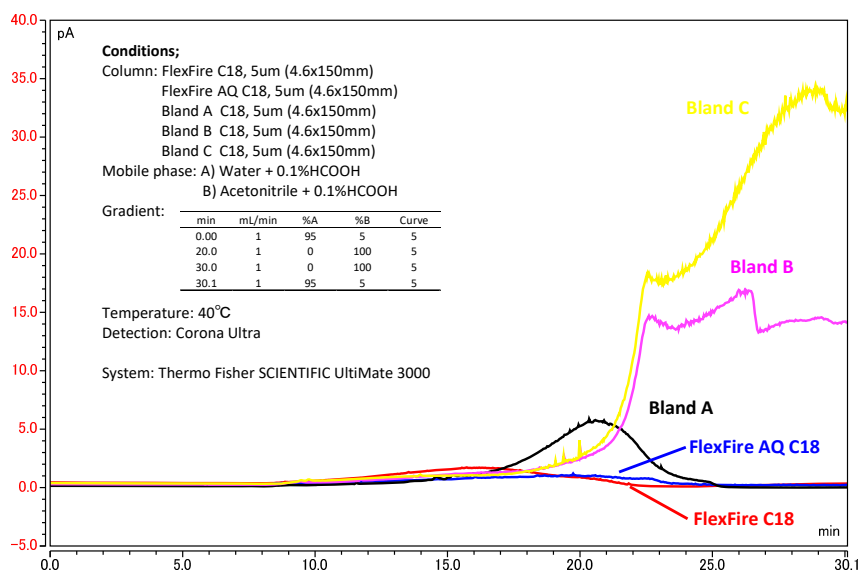
	FlexFire C18	FlexFire AQ C18	FlexFire C8	FlexFire C1	FlexFire C30	FlexFire HILIC
Particle size	5µm	5µm	5µm	5µm	5µm	5µm
Chemistry	Octadecyl	Octadecyl	Octyl	Trimethyl	Triacetyl	—
Surface area	340m ² /g	340m ² /g	340m ² /g	340m ² /g	340m ² /g	340m ² /g
Pore volume	1.0mL/g	1.0mL/g	1.0mL/g	1.0mL/g	1.0mL/g	1.0mL/g
Pore diameter	11nm	11nm	11nm	11nm	11nm	11nm
Carbon	22%	8.5%	12%	5.5%	11%	—
End-cap	○	○	○	○	○	—
pH	pH1-10	pH1-9	pH1-10	pH1-9	pH1-10	pH1-5
Temperature	~80°C	~80°C	~80°C	~80°C	~80°C	~60°C
Max pressure	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)	5µm: 300bar (=30Mpa=4,351psi)

The FlexFire series is a low bleed column !!

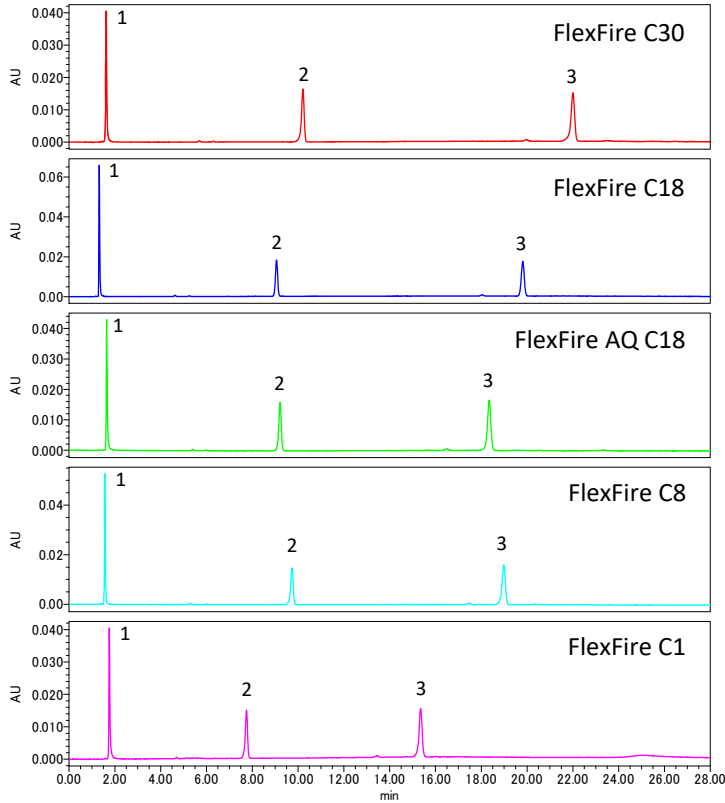
The more sensitive the detector, the greater the effect of column bleed on the S/N and quantitative analysis results.

In addition, this effect becomes more pronounced when the ligand binding density is low and the end capping process is weak.

Specifically, columns that can be used from 100% water system called "AQ" tend to cause bleeding, but FlexFire series can suppress this bleeding..



Pharmaceutical analysis UHPLC method transfer



Conditions;
Column

FlexFire C30, 5um (4.6x150mm)
FlexFire C18, 5um (4.6x150mm)
FlexFire AQ C18, 5um (4.6x150mm)
FlexFire C8, 5um (4.6x150mm)
FlexFire C1, 5um (4.6x150mm)

Mobile phase:

A) 25mM HCOONH₄ B) Acetonitrile

Gradient:

min	mL/min	%A	%B	Curve
0.00	1.0	50	50	
22.54	1.0	5	95	6
42.29	1.0	5	95	6
42.61	1.0	50	50	6

Temperature:

40°C

Detection:

UV254nm

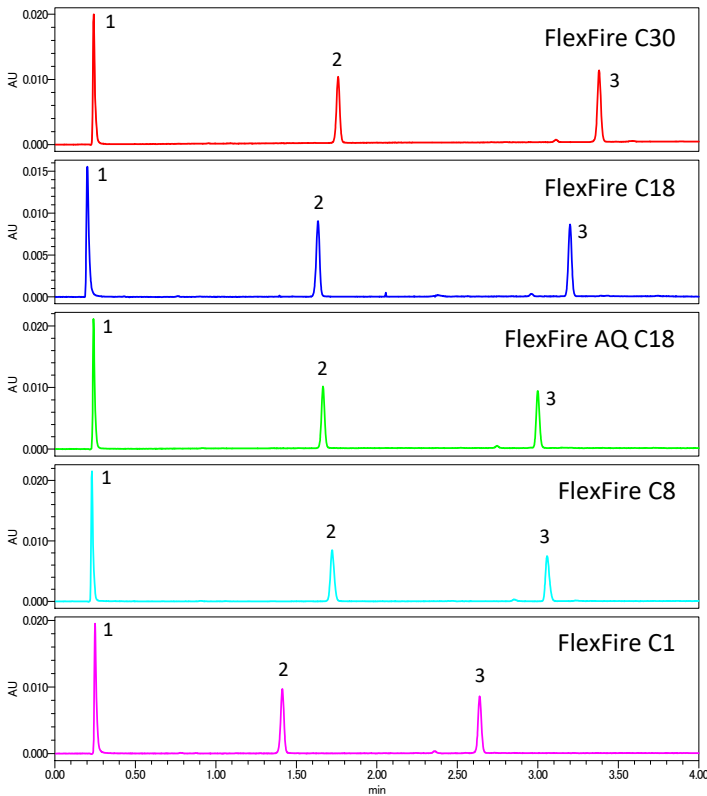
Sample:

1.Favipilavir (1.01mg/mL)
2.Nelfinavir Mesylate Hydrate (1.04mg/mL)
3.Ivermectin (1.07mg/mL)

Injection volume: 2.0uL

System:

Waters alliance



Conditions;
Column:

FlexFire C30, 1.6um (2.0x50mm)
FlexFire C18, 1.6um (2.0x50mm)
FlexFire AQ C18, 1.6um (2.0x50mm)
FlexFire C8, 1.6um (2.0x50mm)
FlexFire C1, 1.6um (2.0x50mm)

Mobile phase:

A) 25mM HCOONH₄ B) Acetonitrile

Gradient:

min	mL/min	%A	%B	Curve
0.00	0.5	50	50	
2.84	0.5	5	95	6
5.33	0.5	5	95	6
5.37	0.5	50	50	6

Temperature:

40°C

Detection:

UV254nm

Sample:

Favipilavir (0.1mg/mL)
Nelfinavir Mesylate Hydrate (0.1mg/mL)
Ivermectin (0.1mg/mL)

Injection volume: 0.2uL

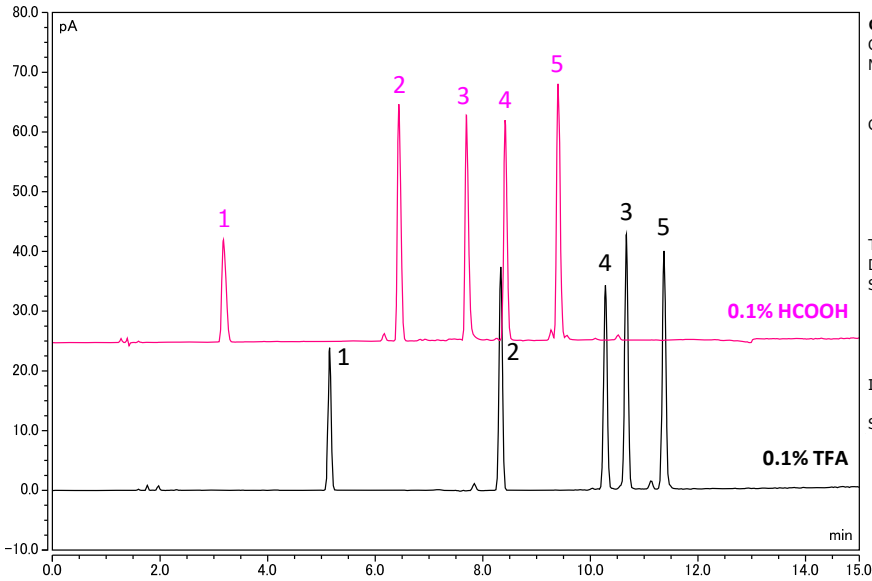
System:

Waters ACQUITY UPLC H-Class Plus
100uL

The addition of the FlexFire Standard series enables quick quality control ⇔ research and development.

Since this data is constructed from UHPLC using method conversion software, the total time including analysis can be significantly reduced.

Analysis of Peptide



Conditions;

Column: FlexFire C18, 5um (4.6x150mm)
 Mobile phase: A) Water + 0.1%TFA B) Acetonitrile + 0.1%TFA
 A) Water + 0.1%HCOOH B) Acetonitrile + 0.1%HCOOH

Gradient:

min	mL/min	%A	%B	Curve
0.0	1.0	95	5	5
15.0	1.0	50	50	5
25.0	1.0	50	50	5
25.1	1.0	95	5	5

Temperature: 40°C

Detection: CAD

Sample:

1. Gly-Tyr
2. Val-Tyr-Val
3. Angiotensin II
4. Met-Enk
5. Leu-Enk

Injection volume: 2.0µL

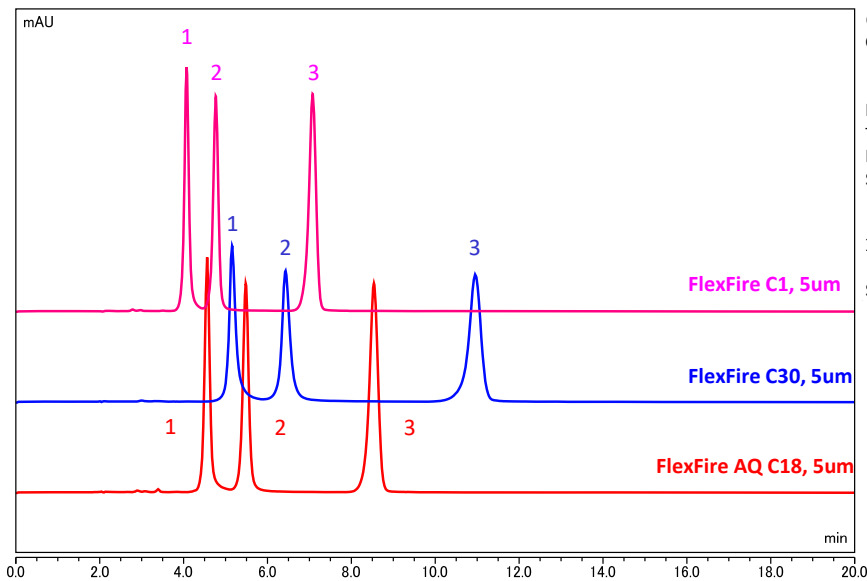
System:

ThermoFisher SCIENTIFIC UltiMate 3000

It may be possible to replace the conditions previously analyzed with ion pair reagents with formic acid for analysis.

In particular, LC / MS may limit the use of TFA, so choosing formic acid as the first method avoids the risk of reduced sensitivity.

Nucleic acid analysis



Conditions;

Column: FlexFire AQ C18, 5um (4.6x150mm)
 FlexFire C30, 5um (4.6x150mm)
 FlexFire C1, 5um (4.6x150mm)

Mobile phase: 25mM HCOONH₄ + Inactivatig agent (0.1%)

Temperature: 40°C

Detection: UV260nm

Sample:

1. ATP
2. ADP
3. AMP

Injection volume: 5.0µL

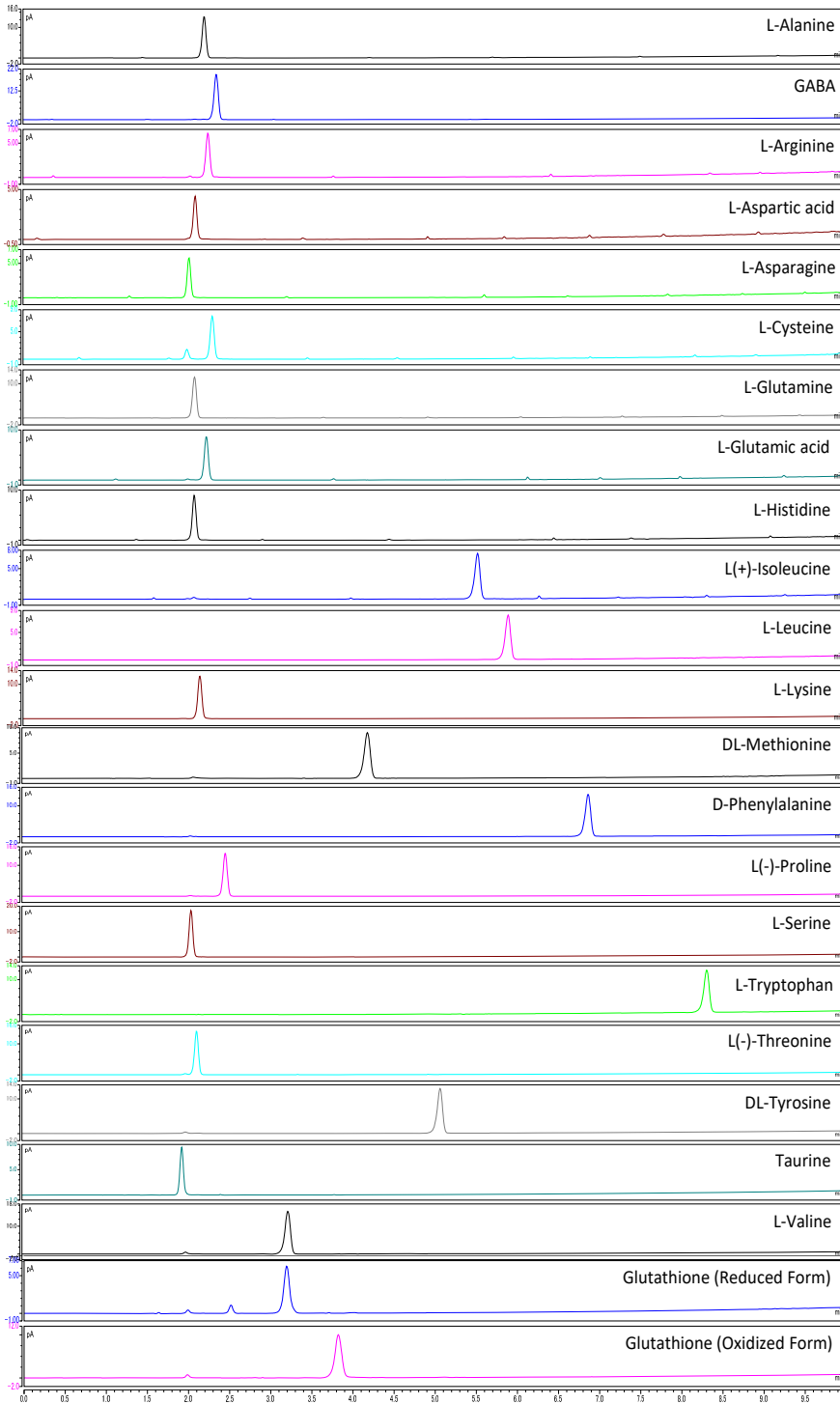
System:

ThermoFisher SCIENTIFIC UltiMate 3000

Compounds with phosphate groups, such as AMP, tend to use phosphate buffers, but in recent years these compounds have been found to adsorb to metals contained in column hard and fillers.

Therefore, by making it metal-free, these compounds can use formic acid buffer solution. In this data, a commercially available inactive agent is added to the mobile phase. As a result, adsorption in the flow path can be reduced, and results equivalent to those of a metal-free column can be obtained.

Analysis of Amino acids

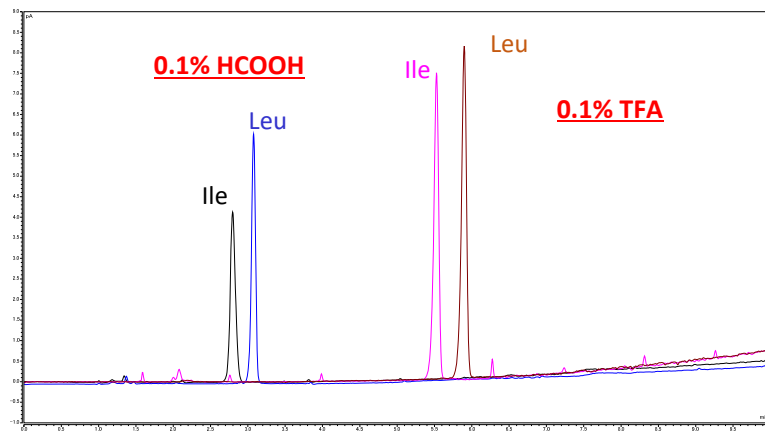


Conditions;
 Column: FlexFire AQ C18, 5µm 4.6x150mm
 Mobile phase: A) Water + 0.1% TFA
 B) Acetonitrile + 0.1% TFA
 Gradient:

min	%A	%B	Curve
0.0	95	5	5
30.0	5	95	5
30.1	95	5	5

 Flow rate: 1.0mL/min
 Temperature: 40°C
 Injection volume: 0.2µL (L(+)-Isoleucine, L-Leucine: 0.1µL)
 Detection: CAD
 Sample: 01. L-Alanine (1.04mg/mL)
 02. GABA (1.06mg/mL)
 03. L-Arginine (1.02mg/mL)
 04. L-Aspartic acid (1.00mg/mL)
 05. L-Asparagine (0.98mg/mL)
 06. L-Cysteine (1.02mg/mL)
 07. L-Glutamine (1.00mg/mL)
 08. L-Glutamic acid (1.10mg/mL)
 09. L-Histidine (1.00mg/mL)
 10. L(+)-Isoleucine (1.00mg/mL)
 11. L-Leucine (0.98mg/mL)
 12. L-Lysine (1.06mg/mL)
 13. DL-Methionine (1.02mg/mL)
 14. D-Phenylalanine (0.98mg/mL)
 15. L(-)-Proline (1.06mg/mL)
 16. L-Serine (1.00mg/mL)
 17. L-Tryptophan (1.02mg/mL)
 18. L(-)-Threonine (1.00mg/mL)
 19. DL-Tyrosine (0.98mg/mL)
 20. Taurine (1.02mg/mL)
 21. L-Valine (1.00mg/mL)
 22. Glutathione (Reduced Form) (1.02mg/mL)
 23. Glutathione (Oxidized Form) (1.02mg/mL)
 System: ThermoFisher SCIENTIFIC UltiMate 3000

Since amino acids are difficult to detect with a UV detector, it is necessary to take some measures such as derivatization. However, it takes a long time to measure to carry out derivatization, but in this report, by using a CAD detector, derivatization is not required and detection by direct injection is possible. In addition, although 0.1% TFA is used in this report, analysis is possible even with 0.1% formic acid, and the separation of leucine and isoleucine, which are indicators in amino acid analysis, is also good.



Separation comparison of leucine and isoleucine

Conditions;
 Column: FlexFire AQ C18, 5µm 4.6x150mm
 Mobile phase: A) Water + 0.1%TFA or 0.1%HCOOH
 B) Acetonitrile + 0.1%TFA or 0.1%HCOOH
 Gradient:

min	%A	%B	Curve
0.0	95	5	5
30.0	5	95	5
30.1	95	5	5

 Flow rate: 1.0mL/min
 Temperature: 40°C
 Detection: CAD
 Sample: L(+)-Isoleucine (1.00mg/mL)
 L-Leucine (0.98mg/mL)
 Injection volume: 0.1µL
 System: ThermoFisher SCIENTIFIC UltiMate3000



FlexFire WP Standard Series

New wide pore column for biopharmacy analysis

FlexFire WP Standard series developed for HPLC with 4.6 Φ inner diameter and 5 μ m particle diameter WP Standard series has been added. This series was developed targeting biopharmacy analysis which has become very popular in recent years.

It has succeeded in higher sensitivity and lower adsorption than conventional wide pore columns, and can be applied to a wide range of polymer compounds from intact to subunits.

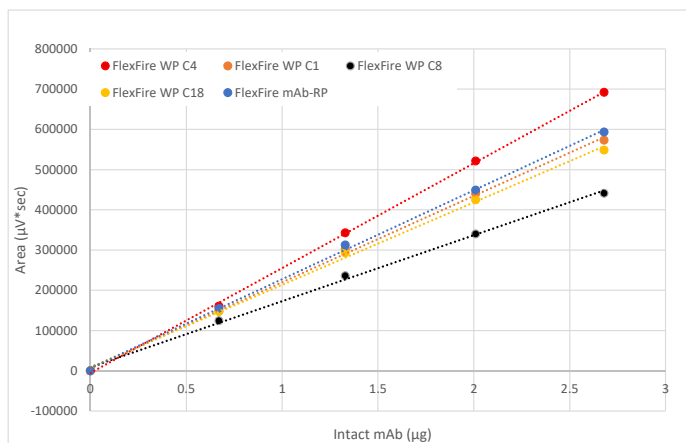
Spec of FlexFire WP Standard

	FlexFire WP C4	FlexFire WP C18	FlexFire WP C8	FlexFire WP C1
Particle size	5 μ m	5 μ m	5 μ m	5 μ m
Chemistry	Butyl	Octadecyl	Octyl	Trimethyl
Surface area	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
Pore diameter	30nm	30nm	30nm	30nm
Carbon	5%	15%	7%	3%
End-cap	O	O	O	O
pH	pH1-10	pH1-10	pH1-10	pH1-10
Temperature	~80 $^{\circ}$ C	~80 $^{\circ}$ C	~80 $^{\circ}$ C	~80 $^{\circ}$ C
Pressure range	5 μ m: 300bar (=30Mpa=4,351psi)	5 μ m: 300bar (=30Mpa=4,351psi)	5 μ m: 300bar (=30Mpa=4,351psi)	5 μ m: 300bar (=30Mpa=4,351psi)

mAb recovery rate

With conventional columns, fillers with long alkyl chains tend to have lower recovery rates, but with the FlexFire WP series, higher recovery rates can be obtained with all fillers.

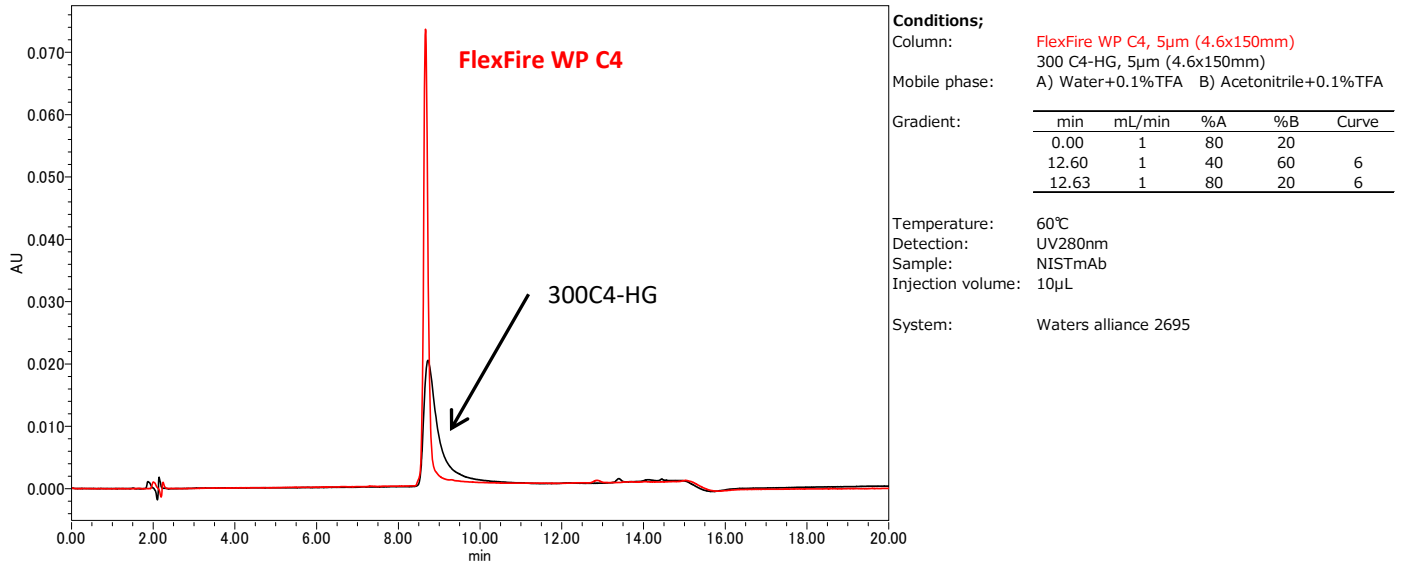
Column	R ²	Recovery (%)
FlexFire WP C1	0.99886	104
FlexFire WP C4	0.99961	103
FlexFire WP C8	0.99795	89.8
FlexFire WP C18	0.99823	98.1
FlexFire mAb-RP	0.99886	101



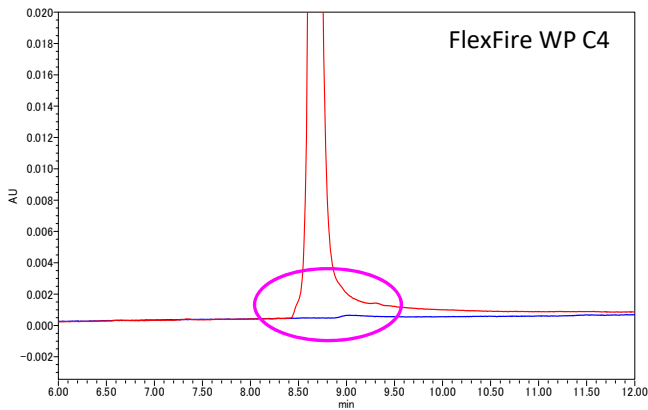
Improved sensitivity and low adsorption

Compared to conventional silica gel, the FlexFire series uses silica gel that has improved strength and reduced impurities. It's also packed with refined particles for unprecedented performance.

Analysis of Intact mAb



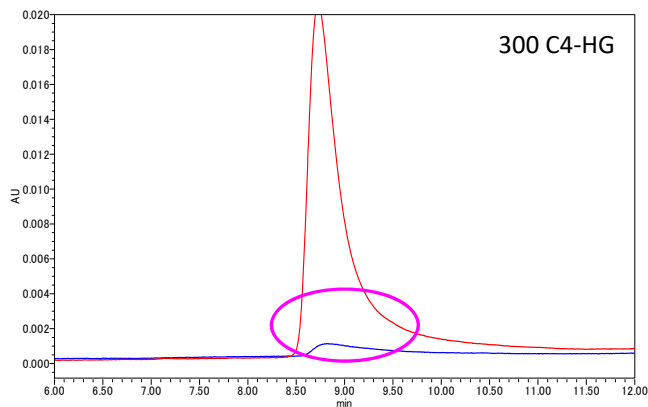
Carryover



Carryover has a great impact on quantitative analysis because what is adsorbed on the syringe of the autosampler will be reflected in the next analysis.

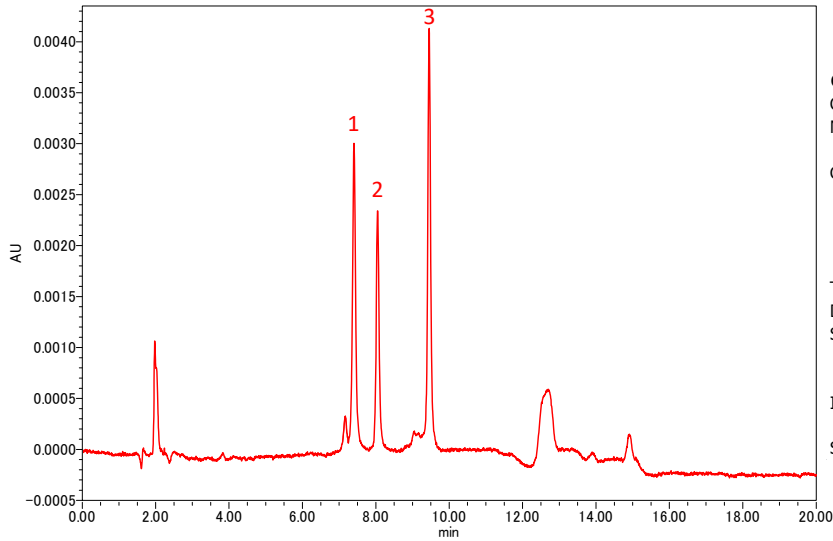
As a countermeasure, the cleaning solvent and the number of cleanings are important.

In addition to this, in recent years, there is a factor that causes adsorption on the column, and in order to solve this, the number of cases where a metal-free column is used has increased.



However, here it can be confirmed that there is carryover due to the difference in filler. Thus, it is important that not only the system and column hardware but also the filler itself has few residual metals and impurities.

Application



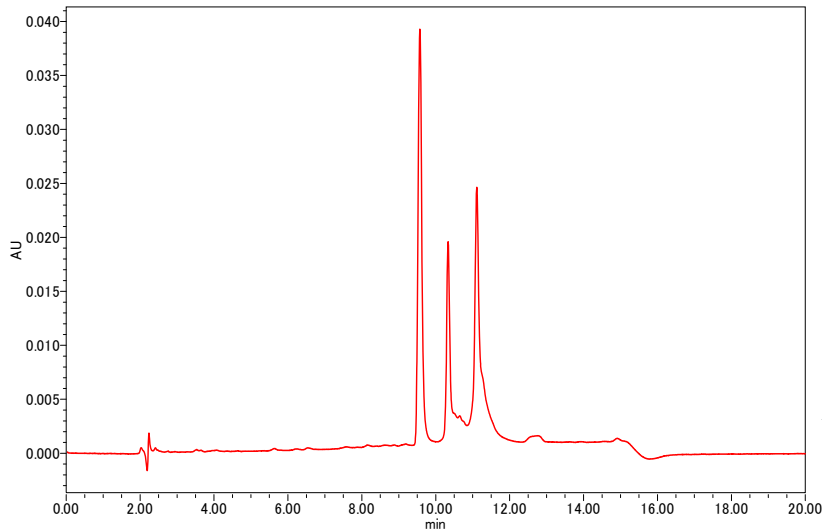
Analysis of mAb subunit

Conditions;

Column: FlexFire WP C4, 5 μ m (4.6x150mm)
 Mobile phase: A) Water+0.1%TFA B) Acetonitrile+0.1%TFA

Gradient:	min	mL/min	%A	%B	Curve
	0.00	1	80	20	
	12.60	1	40	60	6
	12.63	1	80	20	6

Temperature: 60°C
 Detection: UV280nm
 Sample: 1.Fc/2
 2.LC
 3.Fd'
 Injection volume: 10 μ L
 System: Waters alliance 2695



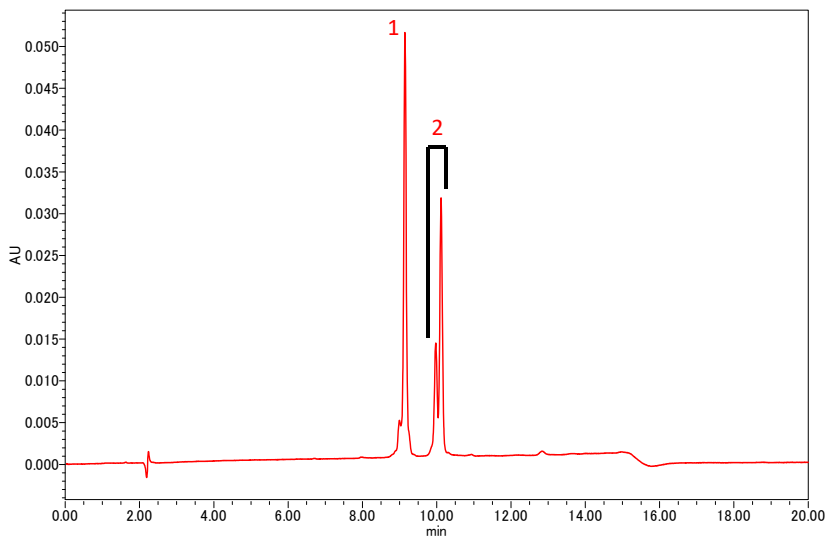
Analysis of Hemoglobin

Conditions;

Column: FlexFire WP C4, 5 μ m (4.6x150mm)
 Mobile phase: A) Water+0.1%TFA B) Acetonitrile+0.1%TFA

Gradient:	min	mL/min	%A	%B	Curve
	0.00	1	80	20	
	12.60	1	40	60	6
	12.63	1	80	20	6

Temperature: 60°C
 Detection: UV280nm
 Sample: Hemoglobin
 Injection volume: 10 μ L
 System: Waters alliance 2695



Analysis of Lactalbumin

Conditions;

Column: FlexFire WP C4, 5 μ m (4.6x150mm)
 Mobile phase: A) Water+0.1%TFA B) Acetonitrile+0.1%TFA

Gradient:	min	mL/min	%A	%B	Curve
	0.00	1	80	20	
	12.60	1	40	60	6
	12.63	1	80	20	6

Temperature: 60°C
 Detection: UV280nm
 Sample: 1. α -Lactalbumin (0.25mg/mL)
 2. β -Lactoglobulin (0.58mg/mL)
 Injection volume: 10 μ L
 System: Waters alliance 2695

Selection of guard column

Two types of guard columns can be selected for the FlexFire series.

Column type	Holder	Coupler	Material
Filling type	×	○	Various fillers
Filter type	○	×	Sintered filter



Filling type: i.d. 4.0mm~6.0mm

The same filler as the analytical column can be selected for this guard column. A coupler is required to connect to the analysis column.



Filter type: i.d. 1.0mm~6.0mm

Since it is mounted directly on the column, there is no need to worry about dead volume. The holder can be used semi-permanently. Only the filter can be replaced.

The prices listed in the price list are retail prices as of February 2021 and are subject to change without notice. The listed price does not include consumption tax.

■ お問い合わせ/Contact us



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Develosil USA

Develosil USA
10060 Carroll Canyon Rd. Ste. 100 San Diego, CA 92131
Phone: 858-800-2433
Web: <https://develosil.us/>

FlexFire STD Series

Order information

Category	Product Name	Code
HPLC	FlexFire C18, 5um 4.6x35mm	301-546035W
HPLC	FlexFire C18, 5um 4.6x50mm	301-546050W
HPLC	FlexFire C18, 5um 4.6x75mm	301-546075W
HPLC	FlexFire C18, 5um 4.6x100mm	301-546100W
HPLC	FlexFire C18, 5um 4.6x150mm	301-546150W
HPLC	FlexFire C18, 5um 4.6x250mm	301-546250W
HPLC	FlexFire C30, 5um 4.6x35mm	302-546035W
HPLC	FlexFire C30, 5um 4.6x50mm	302-546050W
HPLC	FlexFire C30, 5um 4.6x75mm	302-546075W
HPLC	FlexFire C30, 5um 4.6x100mm	302-546100W
HPLC	FlexFire C30, 5um 4.6x150mm	302-546150W
HPLC	FlexFire C30, 5um 4.6x250mm	302-546250W
HPLC	FlexFire C8, 5um 4.6x35mm	303-546035W
HPLC	FlexFire C8, 5um 4.6x50mm	303-546050W
HPLC	FlexFire C8, 5um 4.6x75mm	303-546075W
HPLC	FlexFire C8, 5um 4.6x100mm	303-546100W
HPLC	FlexFire C8, 5um 4.6x150mm	303-546150W
HPLC	FlexFire C8, 5um 4.6x250mm	303-546250W
HPLC	FlexFire C1, 5um 4.6x35mm	304-546035W
HPLC	FlexFire C1, 5um 4.6x50mm	304-546050W
HPLC	FlexFire C1, 5um 4.6x75mm	304-546075W
HPLC	FlexFire C1, 5um 4.6x100mm	304-546100W
HPLC	FlexFire C1, 5um 4.6x150mm	304-546150W
HPLC	FlexFire C1, 5um 4.6x250mm	304-546250W
HPLC	FlexFire HILIC, 5um 4.6x35mm	305-546035W
HPLC	FlexFire HILIC, 5um 4.6x50mm	305-546050W
HPLC	FlexFire HILIC, 5um 4.6x75mm	305-546075W
HPLC	FlexFire HILIC, 5um 4.6x100mm	305-546100W
HPLC	FlexFire HILIC, 5um 4.6x150mm	305-546150W
HPLC	FlexFire HILIC, 5um 4.6x250mm	305-546250W
HPLC	FlexFire AQ C18, 5um 4.6x35mm	306-546035W
HPLC	FlexFire AQ C18, 5um 4.6x50mm	306-546050W
HPLC	FlexFire AQ C18, 5um 4.6x75mm	306-546075W
HPLC	FlexFire AQ C18, 5um 4.6x100mm	306-546100W
HPLC	FlexFire AQ C18, 5um 4.6x150mm	306-546150W
HPLC	FlexFire AQ C18, 5um 4.6x250mm	306-546250W

FlexFire STD Series

Order information

Category	Product Name	Code
Guard column	FlexFire C18, 5um 4.6x10mm	301-546010W
Guard column	FlexFire C30, 5um 4.6x10mm	302-546010W
Guard column	FlexFire C8, 5um 4.6x10mm	303-546010W
Guard column	FlexFire C1, 5um 4.6x10mm	304-546010W
Guard column	FlexFire HILIC, 5um 4.6x10mm	305-546010W
Guard column	FlexFire AQ C18, 5um 4.6x10mm	306-546010W
Guard Filter	Develosil Guard Filter (1Holder+1Filter)	GF28126
Exchange Filter	3Filters	GF28155

FlexFire WP STD Series

Order information

Category	Product Name	Code
HPLC	FlexFire WP C18, 5um 4.6x35mm	308-546035W
HPLC	FlexFire WP C18, 5um 4.6x50mm	308-546050W
HPLC	FlexFire WP C18, 5um 4.6x75mm	308-546075W
HPLC	FlexFire WP C18, 5um 4.6x100mm	308-546100W
HPLC	FlexFire WP C18, 5um 4.6x150mm	308-546150W
HPLC	FlexFire WP C18, 5um 4.6x250mm	308-546250W
HPLC	FlexFire WP C8, 5um 4.6x35mm	309-546035W
HPLC	FlexFire WP C8, 5um 4.6x50mm	309-546050W
HPLC	FlexFire WP C8, 5um 4.6x75mm	309-546075W
HPLC	FlexFire WP C8, 5um 4.6x100mm	309-546100W
HPLC	FlexFire WP C8, 5um 4.6x150mm	309-546150W
HPLC	FlexFire WP C8, 5um 4.6x250mm	309-546250W
HPLC	FlexFire WP C4, 5um 4.6x35mm	310-546035W
HPLC	FlexFire WP C4, 5um 4.6x50mm	310-546050W
HPLC	FlexFire WP C4, 5um 4.6x75mm	310-546075W
HPLC	FlexFire WP C4, 5um 4.6x100mm	310-546100W
HPLC	FlexFire WP C4, 5um 4.6x150mm	310-546150W
HPLC	FlexFire WP C4, 5um 4.6x250mm	310-546250W
HPLC	FlexFire WP C1, 5um 4.6x35mm	311-546035W
HPLC	FlexFire WP C1, 5um 4.6x50mm	311-546050W
HPLC	FlexFire WP C1, 5um 4.6x75mm	311-546075W
HPLC	FlexFire WP C1, 5um 4.6x100mm	311-546100W
HPLC	FlexFire WP C1, 5um 4.6x150mm	311-546150W
HPLC	FlexFire WP C1, 5um 4.6x250mm	311-546250W
Category	Product Name	Code
Guard column	FlexFire WP C18, 5um 4.6x10mm	308-546010W
Guard column	FlexFire WP C8, 5um 4.6x10mm	309-546010W
Guard column	FlexFire WP C4, 5um 4.6x10mm	310-546010W
Guard column	FlexFire WP C1, 5um 4.6x10mm	311-546010W
Guard Filter	Develosil Guard filter (1Holder + 1Filter)	GF28126
Exchange filter	3Filters	GF28155