Fresh
Bioscience
... present science

The NEXT Generation of Silica

Sensitive HPLC and UHPLC Columns

- Ultra high efficiency
- Robustness and perfect reproducibility
- Exceptional Performance
- Kinds of Formats for applications

SenSil 110 UHPLC Columns

For small molecular applications

Allantoin was analyzed using a column packed with unmodified silica gel. In this analysis, the new silica gel gives good results without using a buffer. The new silica gel provides the best performance.

What's SenSil?

The SenSil series is a new product released by Fresh Bioscience in 2020. SenSil was created for exploring high sensitivity when the analysis coupled with mass spectrometry, there is no normal size that has been introduced in this series. All of them are designed from a microbore column to nano column, with an inner diameter range from 2.0 to 0.02 mm, and method transfer is facilitated with particle sizes of 5 μm, 2.6 μm and 1.6 μm. A major point of the SenSil series is based on new generation silica gel, which is different from the conventional one, it significantly succeeded in increasing mechanical strength and reducing impurities.

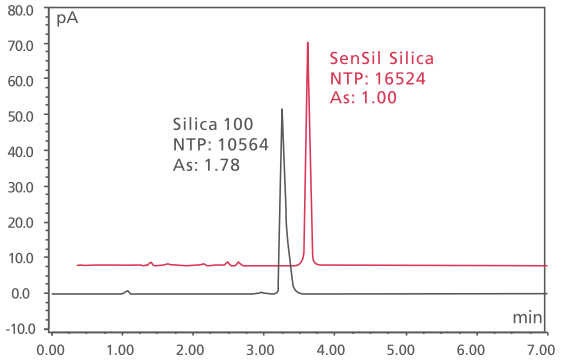


Fig.1 Comparison of materials

Analytical conditions:

Column:	SenSil Silica, 3 μm
	Exsil Silica 100, 3 μm
Dimension:	4.6 x 150 mm
Mobile phase:	Acetonitrile/Water=90/10
Flow rate:	1.0 mL/min
Temperature:	40 °C
Detection:	CAD
Sample:	Allantoin
Injection volume:	1.0 μL
System:	Thermo Fisher SCIENTIFIC UltiMate 3000

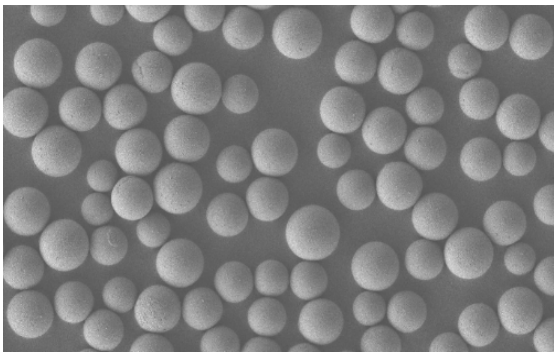


Fig.2 TEM image of 3 particle size silica gel

Flexibility

Six types of chemistry were available as reversed-phase columns and HILIC columns on January 2020, after that we released a unique 1,000 angstrom pore size C4 and traditional wide pores with reverse phase functions in 2021, We also have plans to release special functional groups.

In this art, the SenSil series provides a great deal of flexibility for separation by adequate chemistry functionality along with three particle sizes.

Durability

Additional all columns greatly improved pressure and pH durability. They can stand up with UHPLC system pressure limitation, and the pH is stable in the range of pH 1-10 on average. Sometimes aqueous ammonia or triethylamine can also be added to the mobile phase, which make it possible to improve the peak shape and find new separation pattern.

(The durability is show in Fig.3)

Table 1 SenSil series line up and properties

	SenSil C18	SenSil C18-AQ	SenSil C18 +	SenSil C8	SenSil C30	SenSil HILIC
Particle size	1.6 µm, 2.6 µm, 5 µm	1.6 µm, 2.6 µm, 5 µm	1.6 µm, 2.6 µm, 5 µm	1.6 µm, 2.6 µm, 5 µm	1.6 µm, 2.6 µm, 5 µm	1.6 µm, 2.6 µm, 5 µm
Chemistry	Octadecyl	Octadecyl	Octadecyl	Octyl	Triacontyl	
Surface area	340 m2/g	340 m2/g	340 m2/g	340 m2/g	340 m2/g	340 m2/g
Pore volume	1.0 mL/g	1.0 mL/g	1.0 mL/g	1.0 mL/g	1.0 mL/g	1.0 mL/g
Pore size	110Å	110Å	110Å	110Å	110Å	110Å
Carbon Load	22%	8.5%	12%	12%	11%	
End-cap	Y	Y	Y	Y	Y	N
pH	1-10	1-9	1-10	1-10	1-10	1-5
Max. Temp.	60	60	60	60	60	60
Pressure range	1.6 µm: 1,000 bar (=100 Mpa=14,500 psi)					
	2.6 µm: 600 bar(=60 Mpa=8,702 psi)					
	5 µm: 300 bar (=30 Mpa=4,351 psi)					

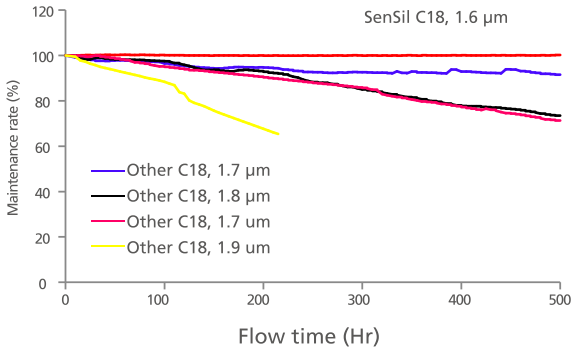


Fig.3 Retention time maintenance rate with respect to liquid flow time

Conditions:

Column: SenSil C18, 1.6 um
Dimension: 2.0 x 50 mm
Mobile phase: Acetonitrile/10 mM NH4OH, pH 10.5 =60/40
Flow rate: 0.5 mL/min
Temperature: 40 °C
Detection: UV at 254 nm
Sample: 1. Uracil (0.01 mg/mL)
2. Naphthalene (0.1 mg/mL)

We compared the durability of SenSil C18 and competitor C18 columns under alkaline conditions. With SenSil C18, the retention time did not change even after 500 hours. Columns that cannot withstand liquidity decrease with retention time.

Column selection from bond density

Fig.4 shows the bond density of the SenSil series. In this graph, C18-AQ and C30 have low bond density, it is stable to use in a 100% aqueous mobile phase.

C18 and C8 are highly versatile columns that engage in hydrophobic interaction, which is the basis of reverse phase chromatography.

C18+ silica surface contains a positive charge which shows great contribution to acids and bases peak shape by ionic interaction.

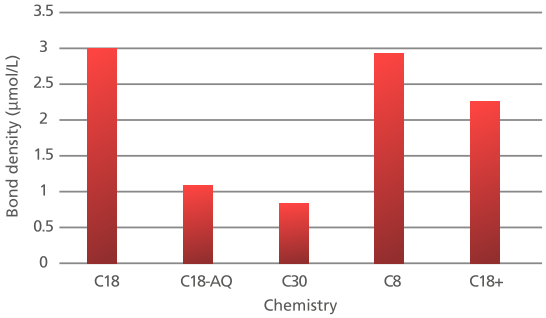


Fig.4 Bond density of each chemistry

UHPLC Method Transfer

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

For example, research and development require rapid results. In such a case, by selecting a particle size of 1.6 µm, the purpose can be achieved with a time reduction of more than half the planned time.

After that, the method can be transferred to 5 µm in the quality control department, and perfect inspection can be performed at low cost.

And why does SenSil only have 2.0 mm ID smaller columns? The answer is very simple. Nowadays, it is meaningful for many labs to directly shoot a valuable sample with nanoscale injection amount.

Moreover, it is an era that environment consideration is frequently questioned. The theme is to release products that consider not only analysis but also before and after.

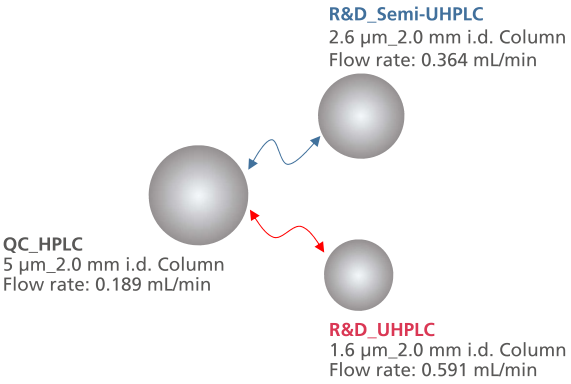
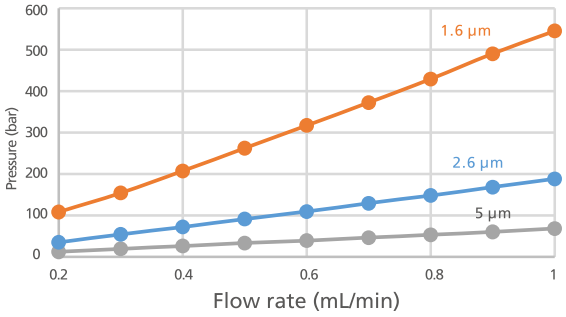
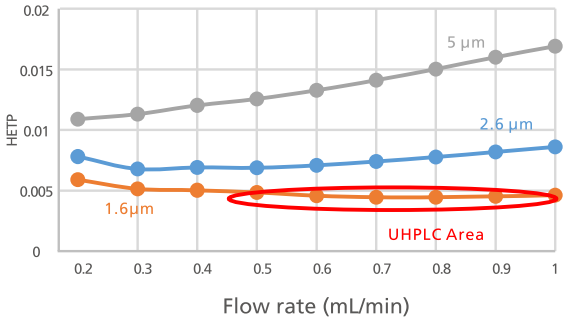


Fig.6 Image model of method transfer

Conditions:

Column:	SenSil C18
Dimension:	2.0 x 50 mm
Mobile phase:	Acetonitrile/Water=60/40
Flow rate:	0.2 mL/min～1.0 mL/min
Temperature:	40 °C
Detection:	UV at 254 nm
Injection volume:	0.16 µL
Sample:	Naphthalene





To the best of our knowledge, the transfer conditions can be easily determined using the software provided by the system manufacturer. However, since the concept of the transfer method is different, it is necessary to decide a certain amount of rules.

And most importantly, the SenSil series has the same physical properties of silica gel, so you won't betray it!

SenSil C18 has the ability to support various applications. In this analysis example, very simple analysis conditions are constructed only by adding formic acid. By selecting a particle size of 1.6 μm, polyphenol analysis, which has required a lot of time, was completed in just 4 minutes.

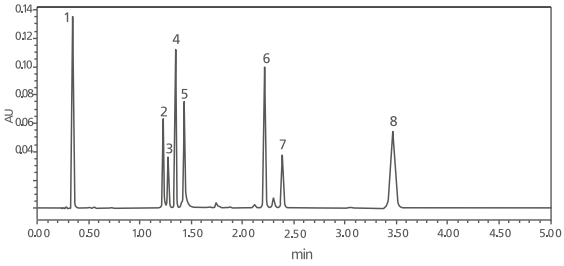


Fig.6 Image model of method transfer

Conditions:

Column: SenSil C18 , 1.6 μm
Dimension: 2.0 x 50 mm
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	6
1.28	0.5	45	55	6
3.60	0.5	45	55	6
3.61	0.5	80	20	6

Temperature: 40 °C
Detection: UV at 260 nm
Injection volume: 0.2 μL
Sample:
1. Puerarin
2. Baicalin
3. Resveratrol
4. Daidsein
5. Quercetin
6. Biochanin A
7. Curcmin
8. Ipriflavone

Reproducibility is paramount when using 100 % aqueous mobile phase.

When the pump is stopped, the mobile phase in the pores will escape. And once again, the compound will not be retained. However, SenSil C18-AQ can achieve reproducibility by repeating this operation. This is because SenSil C18-AQ controls the bond density so that this phenomenon does not occur.

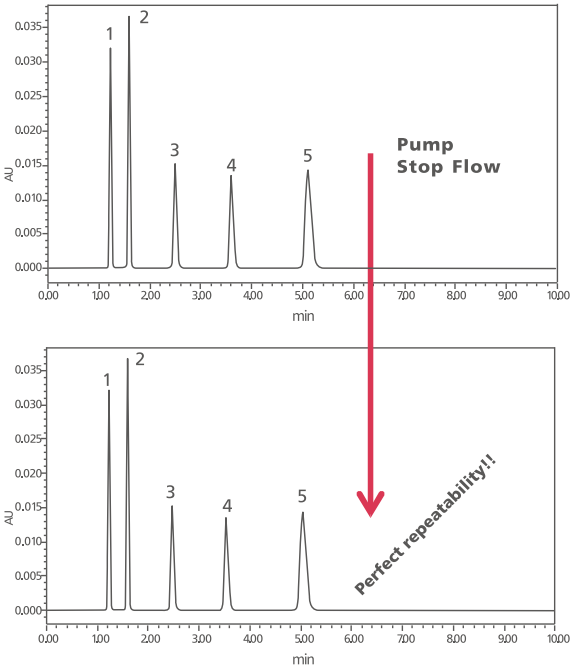


Fig.8 Stop-flow test under 100 % aqueous mobile phase conditions

Conditions:

Conditions: SenSil C18-AQ, 2.6 μm
Column: 2.0 x 100 mm, Stainless
Mobile Phase: 10 mM HCOONH4
Flow rate: 0.3 mL/min
Temperature: 40 °C
Detection: UV at 260 nm
Injection volume: 0.2 μL
Sample:
1. Cytosine (53 μg/mL)
2. Uracil (50 μg/mL)
3. Guanine (52 μg/mL)
4. Thymine (50 μg/mL)
5. Adenine (50 μg/mL)

SenSil C30 can be used with 100% aqueous mobile phase, just like SenSil C18-AQ. SenSil C30 has an unparalleled separation potential. C18 is a very effective alternation when the goal cannot be achieved.

In Fig. 9, it is compared with other company's C30 column. Even with the same C30, SenSil C30 is very good at retaining and separating highly polar compounds.

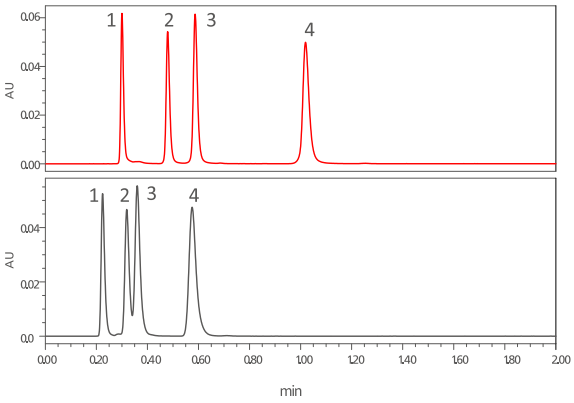


Fig.9 Analysis of dNTP with 100% aqueous mobile phase

Conditions:

Column:	SenSil C30, 1.6 μm Other column, 2 μm ※Columns for analysis of highly polar compounds
Dimension:	2.0 x 50 mm
Mobile phase:	25 mM Ammonium phosphate, pH 7.0
Flow rate:	0.5 mL/min
Temperature:	40 °C
Detection:	UV at 260 nm
Sample:	1. dCTP 2. dTTP 3. dGTP 4. dATP
Injection volume:	0.2 μL

SenSil C8 has an ideal update compared to the previous C8. As Fig.11 shows, SenSil C8 produces a sharp peak in pesticide analysis. As a result, Fig. 11 shows that S/N is advantageous.

C8 is often used to save time instead of C18. However, SenSil C8 is as versatile as C18 and can be a tool as a main column. In particular, SenSil C8 will be very effective as switching from C18 column in LC/MS.

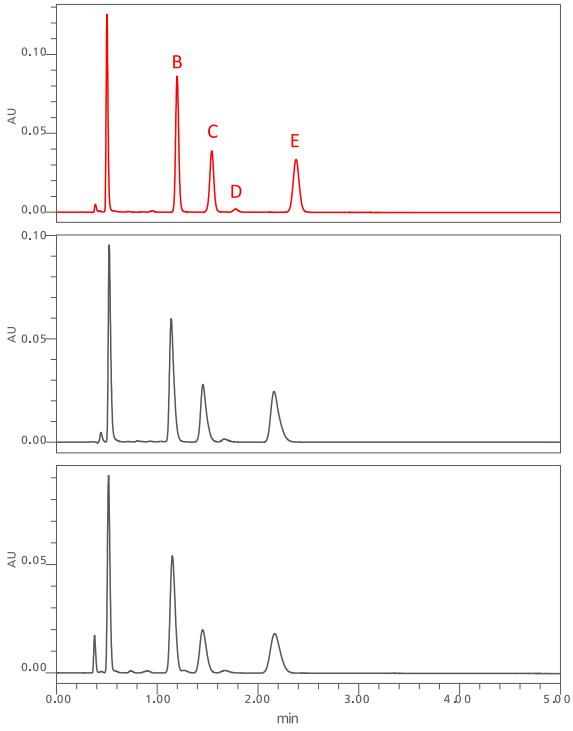


Fig.10 Comparison of C8 columns (with pesticides)

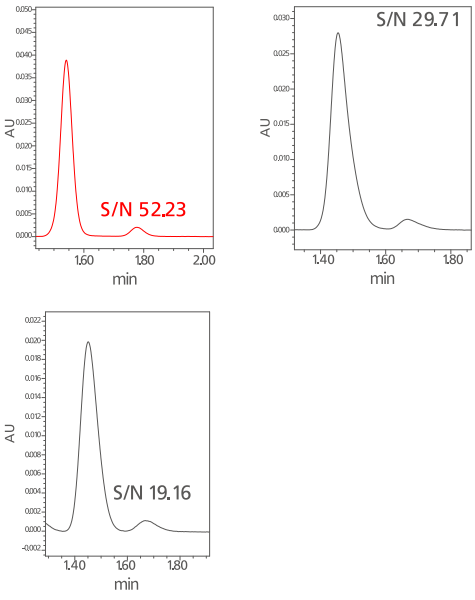


Fig.11 S/N comparison of pesticide D

Conditions:

Column:	SenSil C8, 2.6 μm Exsil 100 C8, 3 μm Other C8, 3 μm
Dimension:	2.0 x 50 mm
Mobile phase:	Acetonitrile/0.1% H3PO4=45/55
Flow rate:	0.3 mL/min
Temperature:	40 °C
Detection:	UV at 254 nm
Injection volume:	0.2 uL

The peak shape will have a significant effect on C18+ in the next section.

The positive charged surface C18 column is not well recognized. We have done a lot of research on the C18+ column. As a result, the idea that "the C18+ column has a short retention" was changed due to Ion-exclusion chromatography. Naturally, the retention capacity is small, but there are compounds that can be seen. For example, as shown in Fig. 11, S/N is higher than that of C18 column. In addition, peaks that were not visible at C18 can be clearly seen at C18+. This is very effective for the analysis of impurities and metabolites using LC/MS.

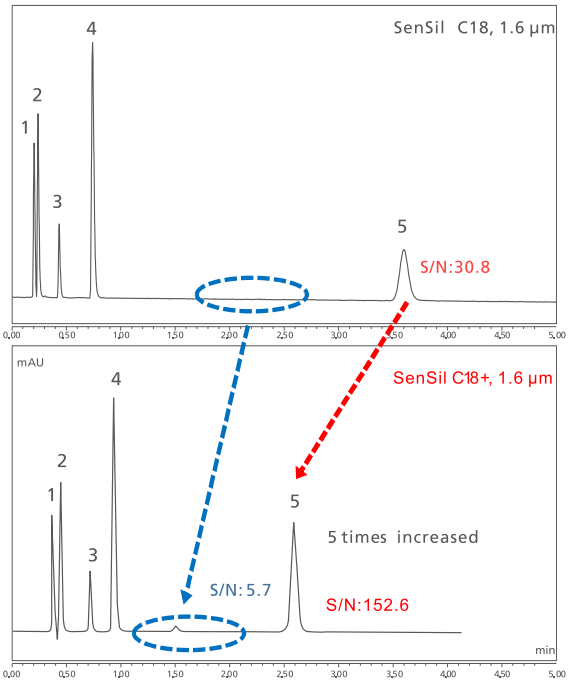


Fig.12 S/N comparison (C18 vs C18+)

Sample:
1. Uracil
2. Caffeine
3. Phenol
4. Amitriptyline
5. Naphthalene

Analysis conditions are described in the QA report.

SenSil HILIC has no linking group. That is, the silica gel carrier is directly involved in the separation. Mainly suitable for analyzing basic compounds under acidic conditions. It is useful when reverse phase columns performed not well or when you want to increase highly polar compounds retention.

Moreover, SenSil HILIC can be used as a normal phase column. This makes it possible to analyze compounds using UHPLC, such as oils with high hydrophobic.

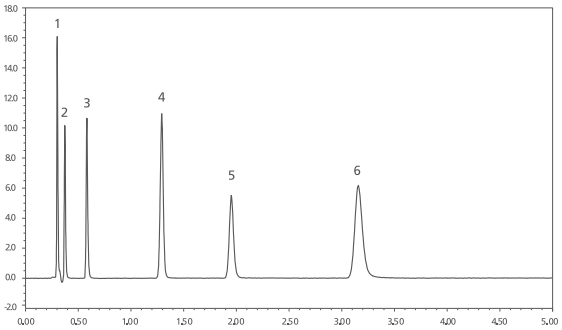


Fig.13 Analysis of basic compounds using SenSil HILIC

Conditions:

Column:	SenSil HILIC, 1.6 µm
Dimension:	2.0 x 50 mm
Mobile phase:	Acetonitrile/10 mM HCOONH , pH 3.0=90/10
Flow rate:	0.5 mL/min
Temperature:	40 °C (ForcedAir)
Detection:	UV at 254 nm
Acquisition rate:	20 Hz
Injection volume:	0.08 µL
Sample:	1. Acetoaminophen (0.9 µg/mL)
	2. Uracil (8.0 µg/mL)
	3. Bromehexine (48 µg/mL)
	4. Paroxetine (0.5 mg/mL)
	5. Phenylephrine (1.1 mg/mL)
	6. Metformin (0.24 mg/mL)

Ordering information

SenSil™ C18 Columns

PACKING	FORMAT	I.D. X LENGTH	PART NO.
C18, 1.6 µm	LC/MS	2.0 x 30 mm	S611SAO
	LC/MS	2.0 x 50 mm	S611SBO
	LC/MS	2.0 x 75 mm	S611SCO
	LC/MS	2.0 x 100 mm	S611SDO
	LC/MS	2.0 x 150 mm	S611SFO
C18, 2.6 µm	LC/MS	2.0 x 30 mm	S621SAO
	LC/MS	2.0 x 50 mm	S621SBO
	LC/MS	2.0 x 75 mm	S621SCO
	LC/MS	2.0 x 100 mm	S621SDO
	LC/MS	2.0 x 150 mm	S621SFO
C18, 5 µm	LC/MS	2.0 x 50 mm	S651SBO
	LC/MS	2.0 x 75 mm	S651SCO
	LC/MS	2.0 x 100 mm	S651SDO
	LC/MS	2.0 x 150 mm	S651SFO
	LC/MS	2.0 x 250 mm	S651SGO

SenSil™ C18-AQ Columns

C18-AQ, 1.6 µm	LC/MS	2.0 x 30 mm	S612SAO
	LC/MS	2.0 x 50 mm	S612SBO
	LC/MS	2.0 x 75 mm	S612SCO
	LC/MS	2.0 x 100 mm	S612SDO
	LC/MS	2.0 x 150 mm	S612SFO
C18-AQ, 2.6 µm	LC/MS	2.0 x 30 mm	S622SAO
	LC/MS	2.0 x 50 mm	S622SBO
	LC/MS	2.0 x 75 mm	S622SCO
	LC/MS	2.0 x 100 mm	S622SDO
	LC/MS	2.0 x 150 mm	S622SFO
C18-AQ, 5 µm	LC/MS	2.0 x 50 mm	S652SBO
	LC/MS	2.0 x 75 mm	S652SCO
	LC/MS	2.0 x 100 mm	S652SDO
	LC/MS	2.0 x 150 mm	S652SFO
	LC/MS	2.0 x 250 mm	S652SGO

SenSil™ C18+ Columns

C18+, 1.6 µm	LC/MS	2.0 x 30 mm	S613SAO
	LC/MS	2.0 x 50 mm	S613SBO
	LC/MS	2.0 x 75 mm	S613SCO
	LC/MS	2.0 x 100 mm	S613SDO
	LC/MS	2.0 x 150 mm	S613SFO
C18+, 2.6 µm	LC/MS	2.0 x 30 mm	S623SAO
	LC/MS	2.0 x 50 mm	S623SBO
	LC/MS	2.0 x 75 mm	S623SCO
	LC/MS	2.0 x 100 mm	S623SDO
	LC/MS	2.0 x 150 mm	S623SFO
C18+, 5 µm	LC/MS	2.0 x 50 mm	S653SBO
	LC/MS	2.0 x 75 mm	S653SCO
	LC/MS	2.0 x 100 mm	S653SDO
	LC/MS	2.0 x 150 mm	S653SFO
	LC/MS	2.0 x 250 mm	S653SGO

SenSil™ C8 Columns

PACKING	FORMAT	I.D. X LENGTH	PART NO.
C8, 1.6 µm	LC/MS	2.0 x 30 mm	S614SAO
	LC/MS	2.0 x 50 mm	S614SBO
	LC/MS	2.0 x 75 mm	S614SCO
	LC/MS	2.0 x 100 mm	S614SDO
	LC/MS	2.0 x 150 mm	S614SFO
C8, 2.6 µm	LC/MS	2.0 x 30 mm	S624SAO
	LC/MS	2.0 x 50 mm	S624SBO
	LC/MS	2.0 x 75 mm	S624SCO
	LC/MS	2.0 x 100 mm	S624SDO
	LC/MS	2.0 x 150 mm	S624SFO
C8, 5 µm	LC/MS	2.0 x 50 mm	S654SBO
	LC/MS	2.0 x 75 mm	S654SCO
	LC/MS	2.0 x 100 mm	S654SDO
	LC/MS	2.0 x 150 mm	S654SFO
	LC/MS	2.0 x 250 mm	S654SGO

SenSil™ C30 Columns

C30, 1.6 µm	LC/MS	2.0 x 30 mm	S615SAO
	LC/MS	2.0 x 50 mm	S615SBO
	LC/MS	2.0 x 75 mm	S615SCO
	LC/MS	2.0 x 100 mm	S615SDO
	LC/MS	2.0 x 150 mm	S615SFO
C30, 2.6 µm	LC/MS	2.0 x 30 mm	S625SAO
	LC/MS	2.0 x 50 mm	S625SBO
	LC/MS	2.0 x 75 mm	S625SCO
	LC/MS	2.0 x 100 mm	S625SDO
	LC/MS	2.0 x 150 mm	S625SFO
C30, 5 µm	LC/MS	2.0 x 50 mm	S655SBO
	LC/MS	2.0 x 75 mm	S655SCO
	LC/MS	2.0 x 100 mm	S655SDO
	LC/MS	2.0 x 150 mm	S655SFO
	LC/MS	2.0 x 250 mm	S655SGO

SenSil™ HILIC Columns

HILIC, 1.6 µm	LC/MS	2.0 x 30 mm	S616SAO
	LC/MS	2.0 x 50 mm	S616SBO
	LC/MS	2.0 x 75 mm	S616SCO
	LC/MS	2.0 x 100 mm	S616SDO
	LC/MS	2.0 x 150 mm	S616SFO
HILIC, 2.6 µm	LC/MS	2.0 x 30 mm	S626SAO
	LC/MS	2.0 x 50 mm	S626SBO
	LC/MS	2.0 x 75 mm	S626SCO
	LC/MS	2.0 x 100 mm	S626SDO
	LC/MS	2.0 x 150 mm	S626SFO
HILIC, 5 µm	LC/MS	2.0 x 50 mm	S656SBO
	LC/MS	2.0 x 75 mm	S656SCO
	LC/MS	2.0 x 100 mm	S656SDO
	LC/MS	2.0 x 150 mm	S656SFO
	LC/MS	2.0 x 250 mm	S656SGO

Contact your Fresh Bioscience office or distributor for current or Local prices

SenSil 300 UHPLC Columns

Next-Gen Bio-UHPLC Column for large molecules

Introduction

We released "SenSil BT Column" in July 2020. The SenSil BT Columns are made by a 300Å silica gel substrate for biopharmaceutical analysis. The wide pores target low molecular compounds to hundreds of thousands of high molecular compounds, leading to the characterization of the primary structure.

The SenSil wide pore series can perform from detection of intact proteins and mAbs to fragmented peptides only on this column. The process does not require cumbersome column and mobile phase changes. In addition, SenSil 300 series offer a flexible particle size, 1.6, 2.6, 5 µm, which is suit for both UHPLC and HPLC.

Retention and separation due to different pore sizes

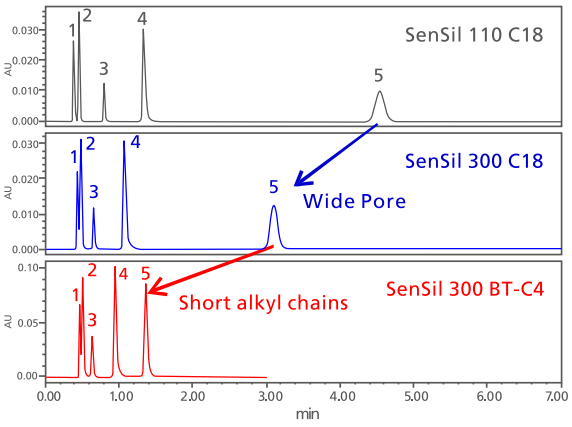
The physical properties differ greatly between the pore size of 110Å and 300Å. Large pores lead to small surface area, shorter wide pores. Common butyl bounded on this substrate result in less retention and high recovery.

Specification of comparison target column

	SenSil 300 BT-C18
Particle size	1.6, 2.6, 5 µm
Chemistry	Octadecyl
Surface area	170 ㎡/g
Pore volume	1.4 mL/g
Pore diameter	300Å
Carbon Load	15%

	SenSil 300 BT-C8
Particle size	1.6, 2.6, 5 µm
Chemistry	Octyl
Surface area	170 ㎡/g
Pore volume	1.4 mL/g
Pore diameter	300Å
Carbon Load	8%

	SenSil 300 BT-C4
Particle size	1.6, 2.6, 5 µm
Chemistry	Butyl
Surface area	170 ㎡/g
Pore volume	1.4 mL/g
Pore diameter	300Å
Carbon Load	5%



Conditions:

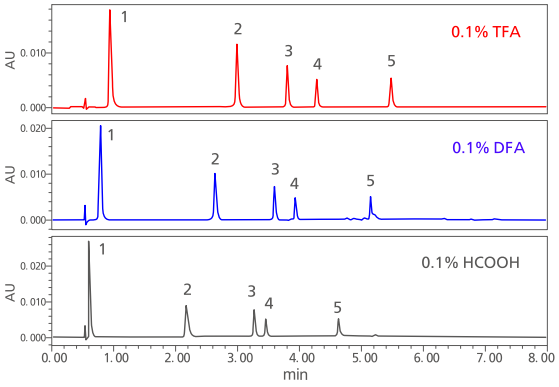
Column: SenSil 110 C18, 2.6 µm
SenSil 300 C18, 2.6 µm
SenSil 300 BT-C4, 2.6 µm
Dimension: 2.0 x 50 mm
Mobile phase: Acetonitrile/10 mM HCOONH4 =40/60
Flow rate: 0.3 mL/min
Temperature: 40 °C
injection volume: 0.1 µL
System: Waters ACQUITY UPLC H-Class PLUS
Sample:
1. Uracil
2. Caffeine
3. Phenol
4. Amitriptyline
5. Naphthalene

Retention comparison with SenSil QC samples

Obtained retention varies with surface area and carbon content. If the retention is extremely strong, it may take less time to move to a column with wide pores or a column with short chain alkylation rather than the difference in alkyl chains in the same series.

Analysis of peptides

Columns with a pore size of 110Å class are often used for the analysis of low molecular weight peptides, but nowadays, biopharmaceutical analysis is drawing attention, and there is a demand for columns that can be used for a wide range of analysis from low molecular weight to high molecular weight.



Conditions:

Column: SenSil 300 C4, 2.6 µm
Dimension: 2.0 x 50 mm
Mobile phase: A) Water + 0.1% TFA
B) Acetonitrile + 0.1% TFA
A) Water + 0.1% DFA
B) Acetonitrile + 0.1% DFA
A) Water + 0.1% HCOOH
B) Acetonitrile + 0.1% HCOOH

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
8.50	0.3	100	0	6

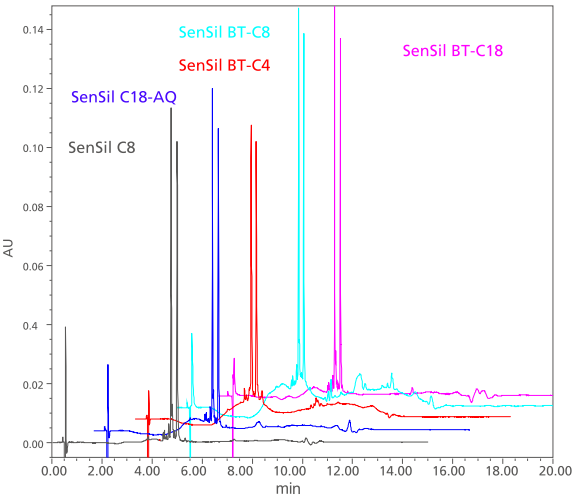
Temperature: 40 °C
Detection: UV at 260 nm
Injection volume: 0.2 µL
Sysytem: Waters ACQUITY UPLC H-Class PLUS
Mixer: 100 µL
Sample: 1. Gly-Tyr (0.20 mg/mL)
2. Val-Tyr-Val (0.21 mg/mL)
3. Methionine_Enkephalin (0.18 mg/mL)
4. Angiotensin II (0.17 mg/mL)
5. Insulin (0.20 mg/mL)

Peptide analysis in different mobile phases

In the analysis of small peptides, even if it is not TFA mobile phase, Analysis is possible. Measurement in negative mode of LC/MS If contamination of the ion source is a concern, use 0.1% formic acid mobile phase analyzing can help you minimize risk.

Analysis of cyclic peptides

There are multiple types of peptides, including dipeptides, tripeptides, oligopeptides and polypeptides. Among them, cyclic peptides are known to have excellent physical properties such as metabolic stability and membrane permeability. It can be solved in the same way as chain peptides in analysis.



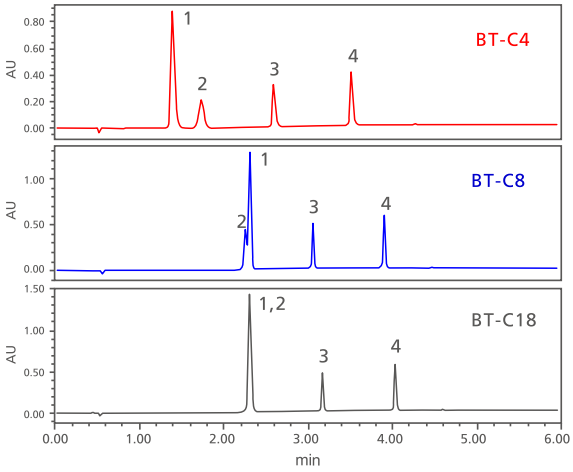
Conditions:

Column: SenSil 300 BT-C18, 2.6 µm
SenSil 300 BT-C8, 2.6 µm
SenSil 300 BT-C4, 2.6 µm
SenSil 110 C18-AQ, 2.6 µm
SenSil 110 C8, 2.6 µm
Dimension: 2.0 x 50 mm
Mobile phase: A) Water + 0.1% TFA
B) Acetonitrile + 0.1% TFA

Gradient:

min	mL/min	%A	%B	Curve
0.00	0.3	100	0	
4.76	0.3	60	40	6
8.42	0.3	20	80	6
8.50	0.3	100	0	6

Temperature: 40 °C
Sample: Colistin (0.51 mg/mL)
Injection volume: 0.5 µL
System: Waters ACQUITY UPLC H-Class PLUS
Mixer: 100 µL



Conditions:

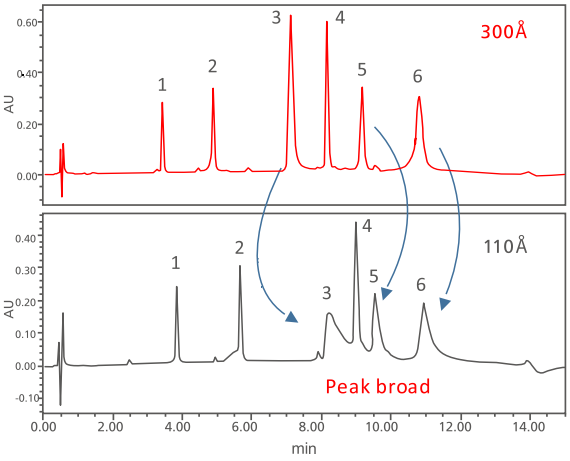
Column: SenSil BT-C4, 2.6 μm
SenSil BT-C8, 2.6 μm
SenSil BT-C18, 2.6 μm
Dimension: 2.0 x 50 mm
Mobile phase: A) Water + 0.1% TFA
B) Acetonitrile + 0.1% TFA
Gradient:

min	mL/min	%A	%B	Curve
0.00	0.3	100	0	
4.76	0.3	60	40	6
8.42	0.3	20	80	6
8.50	0.3	100	0	6

Temperature: 40 °C
Injection volume: 0.5 μL
System: Waters ACQUITY UPLC H-Class PLUS
Mixer: 100 μL
Sample: Cyclo (-RGDSP)
Cyclo (-GRGESP)
Cyclo (-RGDfK)
Cyclo (-RGDfC)

Analysis of proteins

Unlike the peptides described in the previous section, the molecular weight of proteins becomes large at once. Molecules that do not fit in the pores cause peak broadening and peak collapse. The sharp peaks that cannot be obtained with a 110Å pore size column can be obtained with a 300Å column.

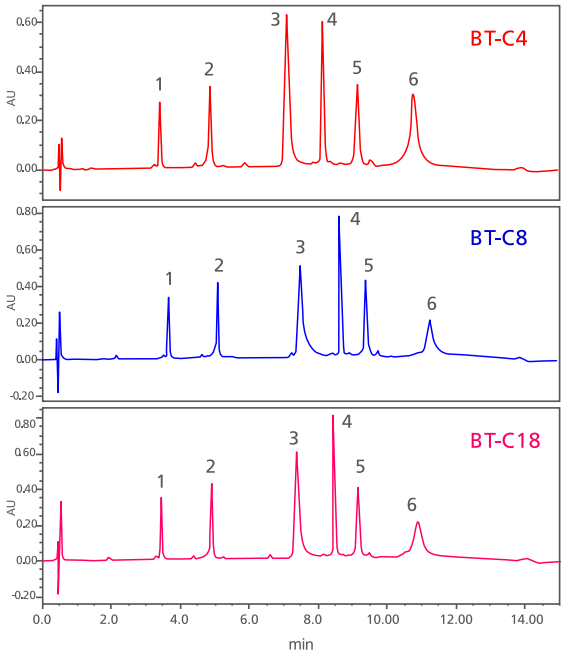


Conditions:

Column: SenSil 300 BT-C4, 2.6 μm
SenSil 110 C18-AQ, 2.6 μm
Dimension: 2.0 x 50 mm
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	
12.60	0.3	40	60	6
12.63	0.3	80	20	6

Temperature: 40 °C
Detection: UV at 210 nm
Injection volume: 10 μL
System: Waters ACQUITY UPLC H-Class PLUS
Mixer: 100 μL
Sample: 1. Ribonuclease A (13 KDa)
2. Cytochrome C (11 KDa)
3. BSA (67 KDa)
4. Myoglobin (14 KDa)
5. Enolase (46 KDa)
6. Phosphorylase B (97 KDa)



Conditions:

Column: SenSil 300 BT-C4, 2.6 µm
SenSil 300 BT-C8, 2.6 µm
SenSil 300 BT-C18, 2.6 µm
Dimension: 2.0 x 50 mm
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	
12.60	0.3	40	60	6
12.63	0.3	80	20	6

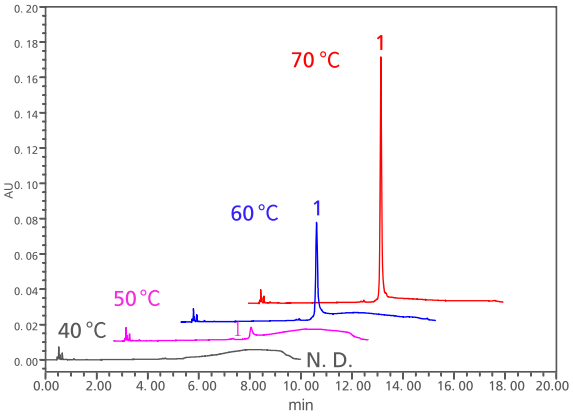
Temperature: 40 °C
Detection: UV at 210 nm
Injection volume: 10 µL
System: Waters ACQUITY UPLC H-Class PLUS
Mixer: 100 µL
Sample: Ribonuclease A (13 KDa)
Cytochrome C (11 KDa)
BSA (67 KDa)
Myoglobin (14 KDa)
Enolase (46 KDa)
Phosphorylase (97 KDa)

Analysis of mAb

Monoclonal antibodies have received a great deal of attention as antibody drugs. In addition to identifying proteins of biological origin, it is expected to treat diseases for which there is no effective treatment such as cancer and infectious diseases.

This monoclonal antibody can also be analyzed by HPLC and UHPLC and is widely used for product stabilization and quality control. Also, the analytical conditions vary depending on the type of column.

Here, we will introduce what the SenSil 300 series can do in monoclonal antibody analysis.



Conditions:

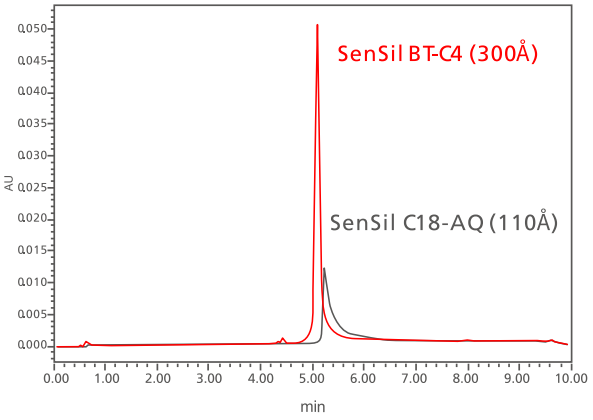
Column: SenSil 300 C4, 2.6 µm
Dimension: 2.0 x 50 mm
Mobile phase: A) Water + 0.1% TFA
B) Acetonitrile + 0.1% TFA
Gradient:

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, 50 °C, 60 °C, 70 °C
Detection: UV at 280 nm
Sample: Intact Mouse IgG1 (5.0 mg/mL)
Injection volume: 1.0 µL
System: Waters ACQUITY UPLC H-Class PLUS
Mixer: 100 µL

Comparison with 110Å column

The molecular weight of Intact Mous IgG1 used in this data is approximately 150 kDa. For compounds with large molecular weight and structure, matching pore size is important.



Conditions:

Column: SenSil 300 BT-C4, 2.6 µm
SenSil 110 C18-AQ, 2.6 µm
Dimension: 2.0 x 50 mm
Mobile phase: A) Water + 0.1% TFA
B) Acetonitrile + 0.1% TFA

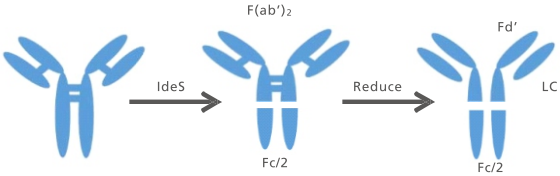
Gradient:

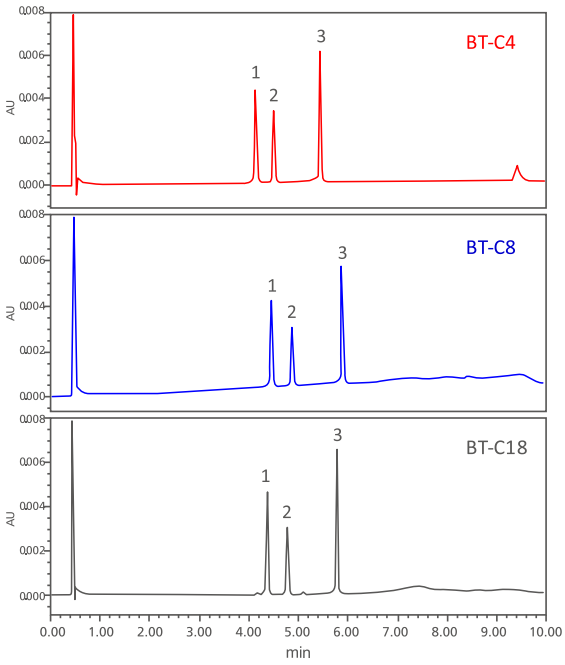
min	mL/min	%A	%B	Curve
0.00	0.3	80	20	
8.40	0.3	40	60	6
8.42	0.3	80	20	6

Temperature: 70 °C
Detection: UV at 280 nm
Sample: Intact Mouse IgG1 (5.0 mg/mL)
Injection volume: 1.0 µL
System: Waters ACQUITY UPLC H-Class PLUS
Mixer: 100 µL

Fragmentation of monoclonal antibodies

Monoclonal antibodies can be fragmented (lower molecular weight) by enzymes. Three fragments have been obtained by reducing F(ab')2 and Fc/2 obtained by IdeS digestion.





Conditions:

Column: SenSil 300 BT-C4, 2.6 µm
SenSil 300 BT-C8, 2.6 µm
SenSil 300 BT-C18, 2.6 µm
Dimension: 2.0 x 50 mm
Mobile phase: A) Water + 0.1% TFA
B) Acetonitrile + 0.1% TFA

Gradient:

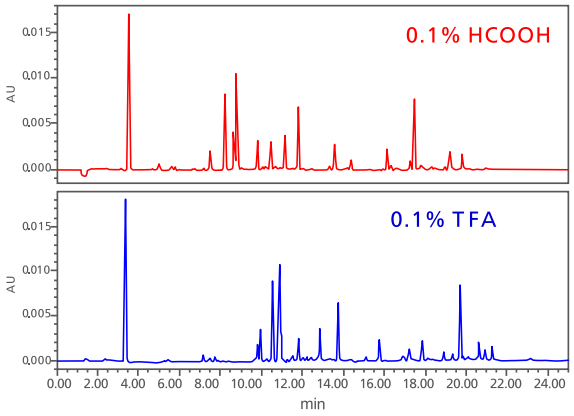
min	mL/min	%A	%B	Curve
0.00	0.3	100	0	
4.76	0.3	40	60	6
8.42	0.3	80	20	6
8.50	0.3	100	0	6

Temperature: 70 °C
Detection: UV at 260 nm
Injection volume: 1.0 µL (0.25 ug)
Sysytem: Waters ACQUITY UPLC H-Class PLUS
Mixer: 100 µL
Sample: mAb subunit
1. Fc/2 2. LC 3. Fd'

Intact protein/mAb peptide mapping

The fragment obtained by IdeS digestion/reduction of the monoclonal antibody is reduced to around 25 KDa. It is possible to further reduce the molecular weight by digesting the intact protein or mAb with an enzyme such as trypsin.

BSA



Conditions:

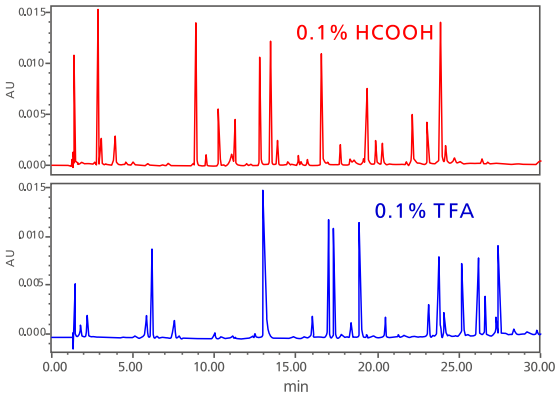
Column: SenSil 300 BT-C4, 2.6 µm
Dimension: 2.0 x 150 mm
Mobile phase: A) Water + 0.1% HCOOH
B) Acetonitrile + 0.1% HCOOH
A) Water + 0.1% TFA
B) Acetonitrile + 0.1% TFA

Gradient:

min	mL/min	%A	%B	Curve
0.00	0.3	100	0	
30.0	0.3	50	50	6
30.1	0.3	100	0	6

Temperature: 40 °C
Detection: UV at 280 nm
Sample: Tryptic digest of BSA
Injection volume: 10 µL
system: Waters ACQUITY UPLC H-Class PLUS
Mixer: 100 µL

mAb



Conditions:

Column: SenSil 300 BT-C4, 2.6 µm
Dimension: 2.0 x 150 mm
Mobile phase: A) Water + 0.1% HCOOH
B) Acetonitrile + 0.1% HCOOH
A) Water + 0.1% TFA
B) Acetonitrile + 0.1% TFA

Gradient:

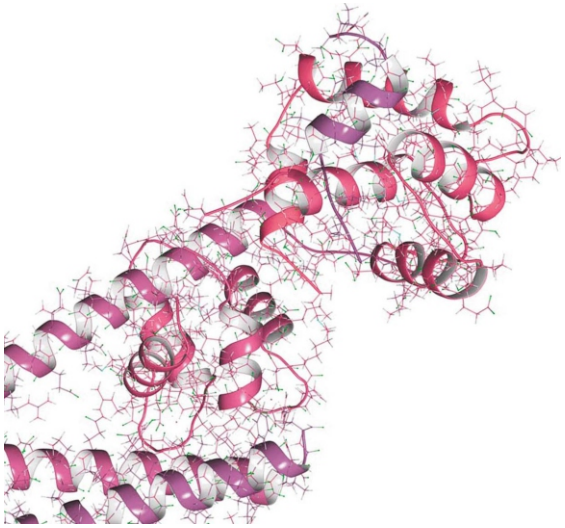
min	mL/min	%A	%B	Curve
0.00	0.3	100	0	
30.0	0.3	50	50	6
30.1	0.3	100	0	6

Temperature: 70 °C
Detection: UV at 280 nm
Sample: Tryptic digest of mAb
Injection volume: 10 µL
system: Waters ACQUITY UPLC H-Class PLUS
Mixer: 100 µL

Ordering information

SenSil™ 300 BT-C4 Columns

PACKING	FORMAT	I.D. X LENGTH	PART NO.
BT-C4, 1.6 µm	Microbore	2.0 x 30 mm	S713BAO
	Microbore	2.0 x 50 mm	S713BBO
	Microbore	2.0 x 75 mm	S713BCO
	Microbore	2.0 x 100 mm	S713BDO
	Microbore	2.0 x 150 mm	S713BFO
BT-C4, 2.6 µm	Microbore	2.0 x 30 mm	S723BAO
	Microbore	2.0 x 50 mm	S723BBO
	Microbore	2.0 x 75 mm	S723BCO
	Microbore	2.0 x 100 mm	S723BDO
	Microbore	2.0 x 150 mm	S723BFO
	Microbore	2.0 x 250 mm	S723BGO
BT-C4, 5 µm	Microbore	2.0 x 50 mm	S753BBO
	Microbore	2.0 x 75 mm	S753BCO
	Microbore	2.0 x 100 mm	S753BDO
	Microbore	2.0 x 150 mm	S753BFO
	Microbore	2.0 x 250 mm	S753BGO



SenSil™ 300 BT-C18 Columns

PACKING	FORMAT	I.D. X LENGTH	PART NO.
BT-C18, 1.6 µm	Microbore	2.0 x 30 mm	S711BAO
	Microbore	2.0 x 50 mm	S711BBO
	Microbore	2.0 x 75 mm	S711BCO
	Microbore	2.0 x 100 mm	S711BDO
	Microbore	2.0 x 150 mm	S711BFO
BT-C18, 2.6 µm	Microbore	2.0 x 30 mm	S721BAO
	Microbore	2.0 x 50 mm	S721BBO
	Microbore	2.0 x 75 mm	S721BCO
	Microbore	2.0 x 100 mm	S721BDO
	Microbore	2.0 x 150 mm	S721BFO
	Microbore	2.0 x 250 mm	S721BGO
BT-C18, 5 µm	Microbore	2.0 x 50 mm	S751BBO
	Microbore	2.0 x 75 mm	S751BCO
	Microbore	2.0 x 100 mm	S751BDO
	Microbore	2.0 x 150 mm	S751BFO
	Microbore	2.0 x 250 mm	S751BGO

SenSil™ 300 BT-C8 Columns

PACKING	FORMAT	I.D. X LENGTH	PART NO.
BT-C8, 1.6 µm	Microbore	2.0 x 30 mm	S712BAO
	Microbore	2.0 x 50 mm	S712BBO
	Microbore	2.0 x 75 mm	S712BCO
	Microbore	2.0 x 100 mm	S712BDO
	Microbore	2.0 x 150 mm	S712BFO
BT-C8, 2.6 µm	Microbore	2.0 x 30 mm	S722BAO
	Microbore	2.0 x 50 mm	S722BBO
	Microbore	2.0 x 75 mm	S722BCO
	Microbore	2.0 x 100 mm	S722BDO
	Microbore	2.0 x 150 mm	S722BFO
	Microbore	2.0 x 250 mm	S722BGO
BT-C8, 5 µm	Microbore	2.0 x 50 mm	S752BBO
	Microbore	2.0 x 75 mm	S752BCO
	Microbore	2.0 x 100 mm	S752BDO
	Microbore	2.0 x 150 mm	S752BFO
	Microbore	2.0 x 250 mm	S752BGO

SenSil 1,000 UHPLC Columns

For Intact Protein, Antibody and ADC

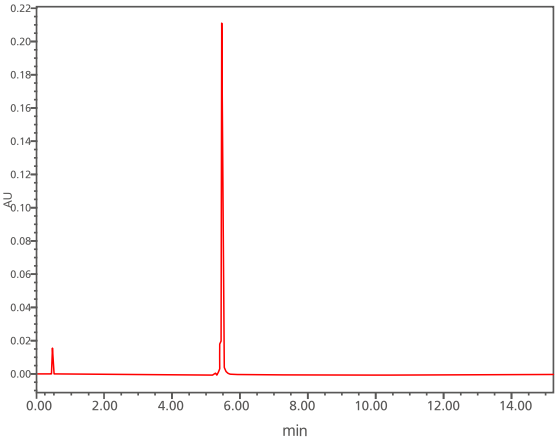
From intact protein and antibody analysis to post-digestion peptide analysis

SenSil mAb-C4 was developed based on 1,000Å silica gel. With this single column, you can analyze intact proteins and monoclonal antibodies, and even analyze peptides after digestion. Matching the molecular weight of the compound with the pore size of the silica gel base material enables more sensitive analysis.

Specifications of SenSil mAb-C4

	mAb-C4
Functional	Butyl
Surface area	24 m2/g
Pore diameter	1,000Å
Pore volume	1.4 mL/g
Carbon Load	0.6%
End-cap	Yes
pH range	1 -10
Temperature range	5-80 °C
Particle size	1.6, 2.6, 5 μm

Analysis of Nist monoclonal antibody (NISTmAb) RM 8671



Conditions:

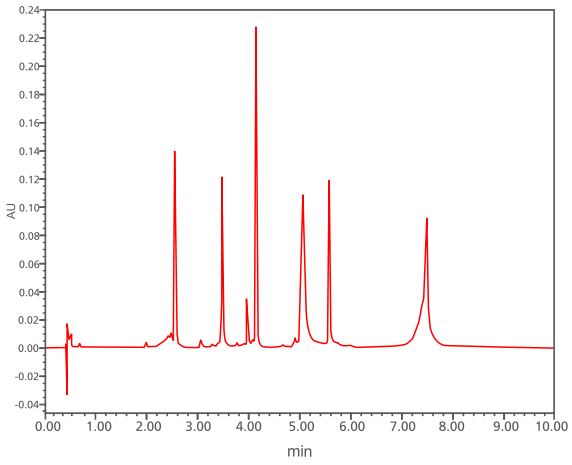
Column: SenSil mAb-C4, 2.6 um
Dimension: 2.0 x 50 mm
Mobile phase: A) Water + 0.1% TFA
B) Acetonitrile + 0.1% TFA

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: 70 °C
Detection: UV at 280 nm
Sample: NISTmAb (0.83 mg/mL)
Injection volume: 0.5 uL
System: Waters ACQUITY UPLC H-Class PLUS
Mixer: 100 uL

Analysis of 6 Protein mixtures



Conditions:

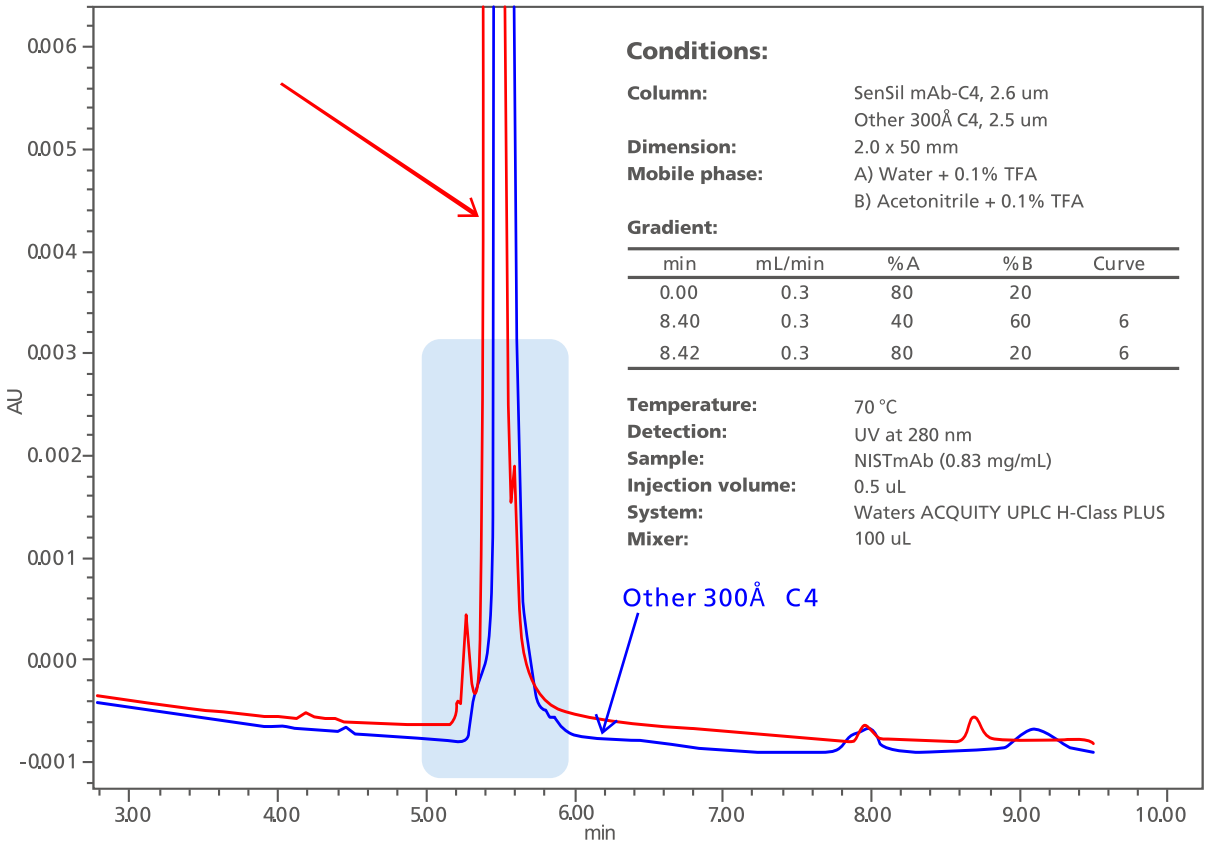
Column: SenSil mAb-C4, 2.6 um
Dimension: 2.0 x 50 mm
Mobile phase: A) Water + 0.1% TFA
B) Acetonitrile + 0.1% TFA

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: 70 °C
Detection: UV at 280 nm
Injection volume: 0.2 uL
System: Waters ACQUITY UPLC H-Class Plus
Mixer: 100 uL
Sample:
1. Ribonucleses A
2. Ctochrome C
3. Lysozeme
4. BSA
5. Myoglobin
6. Obalbumin

Easier to discover more fractions!



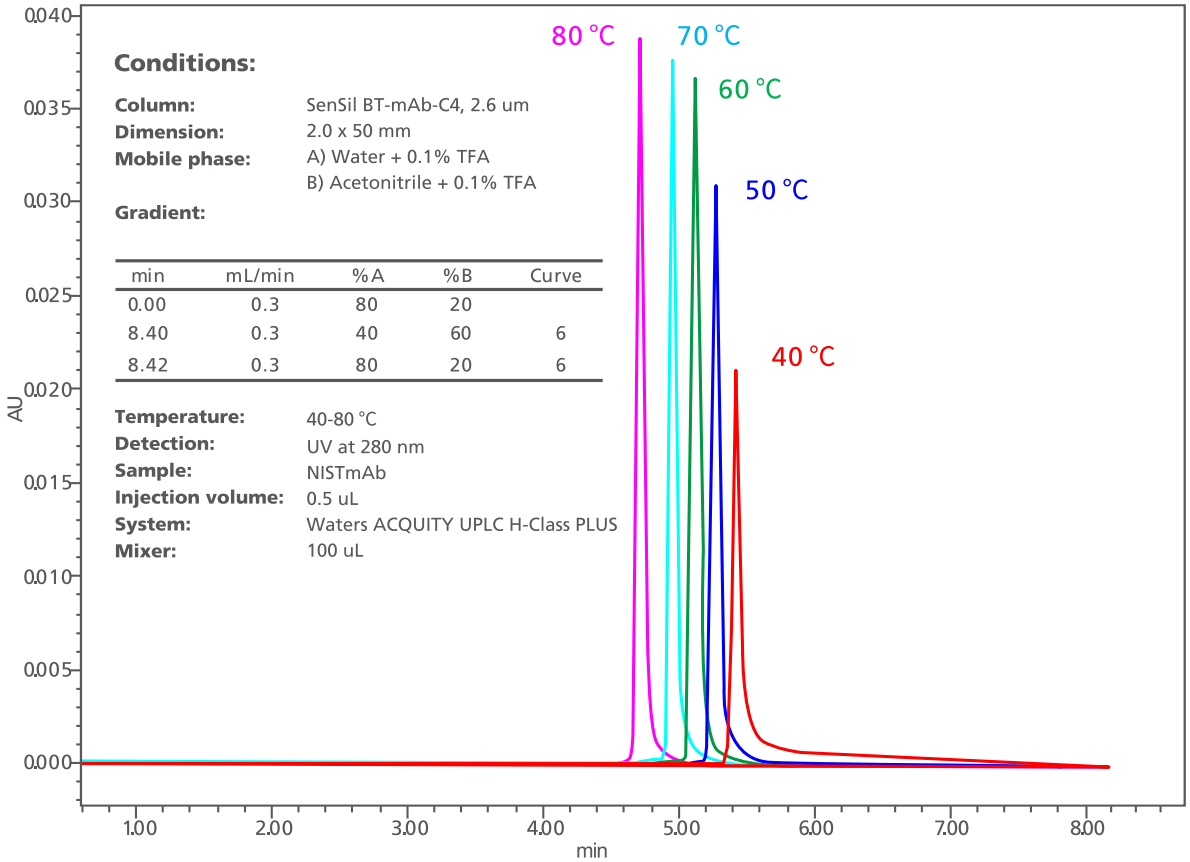
Conditions:

Column:	SenSil mAb-C4, 2.6 um
	Other 300Å C4, 2.5 um
Dimension:	2.0 x 50 mm
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:	70 °C
Detection:	UV at 280 nm
Sample:	NISTmAb (0.83 mg/mL)
Injection volume:	0.5 uL
System:	Waters ACQUITY UPLC H-Class PLUS
Mixer:	100 uL



Conditions:

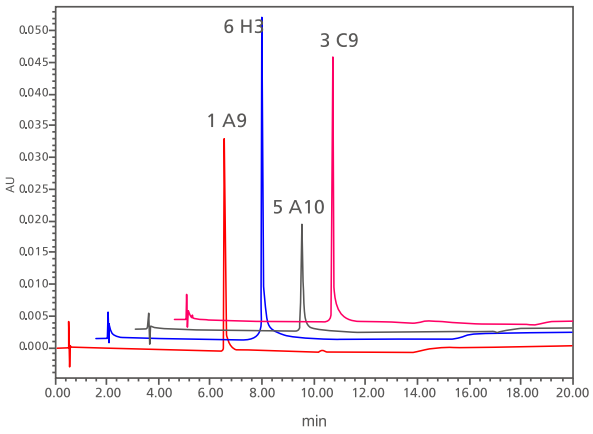
Column:	SenSil BT-mAb-C4, 2.6 um
Dimension:	2.0 x 50 mm
Mobile phase:	A) Water + 0.1% TFA
	B) Acetonitrile + 0.1% TFA

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:	40-80 °C
Detection:	UV at 280 nm
Sample:	NISTmAb
Injection volume:	0.5 uL
System:	Waters ACQUITY UPLC H-Class PLUS
Mixer:	100 uL

Anti-SARS-CoV-2 (COVID-19) Antibody



Conditions:

Column: SenSil mAb-C4, 5 um
Dimension: 2.0 x 50 mm
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: 70 °C
Detection: UV at 280 nm
Injection volume: 10 uL
System: Waters ACQUITY UPLC H-Class PLUS
Mixer: 100 uL
Sample: [1A9] SARS-CoV/SARS-CoV-2 (COVID-19) spike antibody
[6H3] SARS-CoV/SARS-CoV-2 (COVID-19) nucleocapsid antibody
[5A10] SARS-CoV/SARS-CoV-2 (COVID-19) NSP8 antibody
[3C9] SARS-CoV/SARS-CoV-2 (COVID-19) ORF7a antibody

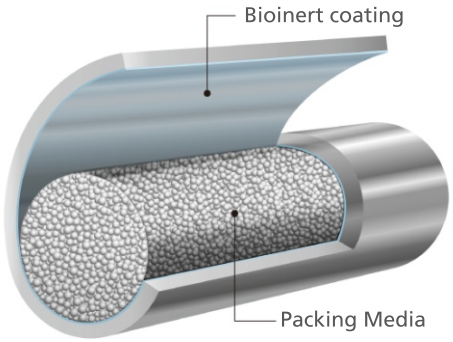
SenSil mAb-C4 has wide selectivity under all conditions (composition, pH, temperature). This selectivity facilitates method development for characterization and saves time by selecting UHPLC with 1.6/2.6 µm particle size.

Bioinert column hardware

We've minimized the need for priming with a new titanium infused biocompatible hardware and frit that doesn't interfere with protein or peptide integrity!

Titanium UHPLC hardware is designed to curtail:

- Unwanted secondary interactions
- Problematic carryover
- Recovery issues between injection to detection



Ordering information

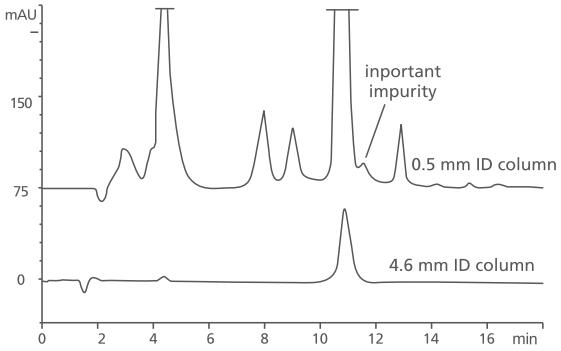
SenSil™ BT-mAb-C4 Columns			
PACKING	FORMAT	I.D. X LENGTH	PART NO.
BT-mAb-C4, 1.6 µm	Microbore	2.0 x 30 mm	S912BAO
	Microbore	2.0 x 50 mm	S912BBO
	Microbore	2.0 x 75 mm	S912BCO
	Microbore	2.0 x 100 mm	S912BDO
	Microbore	2.0 x 150 mm	S912BFO
BT-mAb-C4, 2.6 µm	Microbore	2.0 x 30 mm	S922BAO
	Microbore	2.0 x 50 mm	S922BBO
	Microbore	2.0 x 75 mm	S922BCO
	Microbore	2.0 x 100 mm	S922BDO
	Microbore	2.0 x 150 mm	S922BFO
BT-mAb-C4, 5 µm	Microbore	2.0 x 50 mm	S952BBO
	Microbore	2.0 x 75 mm	S952BCO
	Microbore	2.0 x 100 mm	S952BDO
	Microbore	2.0 x 150 mm	S952BFO
	Microbore	2.0 x 250 mm	S952BGO

SenSil micro-UHPLC Columns

For low amount sample analysis

Introduction to micro-HPLC

Micro-UHPLC plays a crucial role in the modern laboratory by expanding the boundaries of conventional UHPLC. Today micro-UHPLC is used more or less on routine basis for a wide range of applications ranging from biotechnology to environmental analysis, e.g. peptide mapping by capLC-ESI-MS or analysis of important low-abundant metabolites in plasma.



Keys to defining a "well-characterized" micro-UHPLC column by traditional UHPLC terms are:

Selectivity

The micro-UHPLC column must be able to separate the target compound(s) from other compounds present, e.g. to monitor synthetic products such as peptides or oligonucleotides for identity and purity by separating important impurities from major products. Resolving minor impurities such as deamidation products or oxidized methionine variants place the strongest demands on micro-UHPLC column selectivity. In such cases, where high resolution between major product and impurities seldom is obtained high column efficiency plays a crucial role or achieving sufficient resolution.

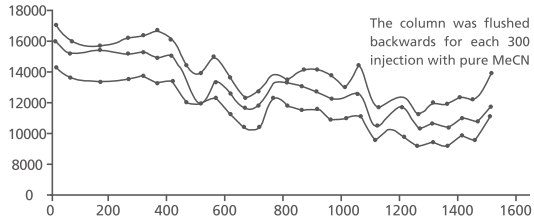
Furthermore, in situations where the total sample amount is too small to allow detection of target compounds (e.g., important impurities or metabolites) with conventional UHPLC instruments, the use of a micro-UHPLC instrument is a necessity. Such an example is shown below.

Stability

Although the robustness of micro-UHPLC columns cannot match the robustness of their big brothers, the selectivity and efficiency must be constant over numerous injections to ensure robust and reliable assays. Only micro-UHPLC columns which are physically and chemically stable and which maintain their chromatographic properties over certain period of time are acceptable for methods used on routine basis. By proper sample preparation and the use of guard columns/column switching, micro-UHPLC columns can last for several hundreds injections.

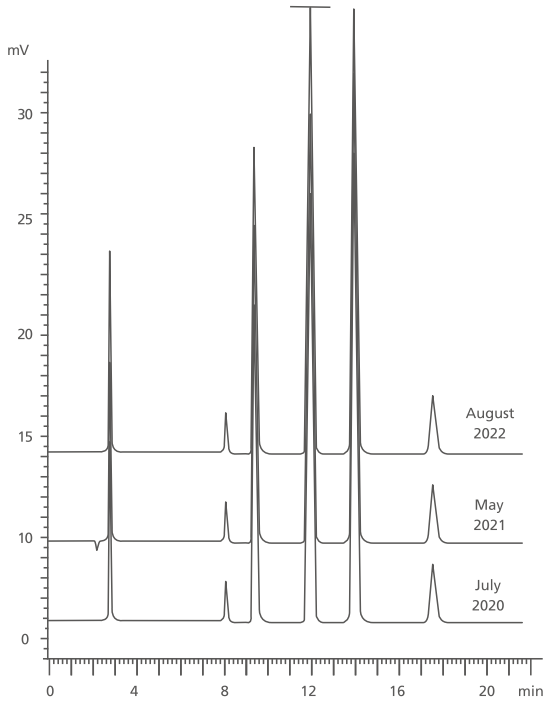
Conditions:

Column:	3 µ Reprosil-pur C18-AQ 120Å
Dimension:	0.3 x 150 mm
Mobile phase:	MeCN-water(70:30)
Flow Rate:	3 µL/min
Temp:	30 °C
Injection Volume:	50 nL
Detector:	UV at 254 nm
Sample:	naphthalene, biphenyl, fluorene



Reproducibility

Micro-UHPLC column selectivity and efficiency should remain the same when used micro-columns are replaced with new ones. A big advantage of micro-UHPLC columns is that several thousands columns can be made from the same batch of packing material, which eliminate problems with "batch-to-batch"-variations. Chromatogram shows three 3.0 µ Reprosil C18 120Å (0.3 x 150 mm) columns packed and tested over a period of 2 years, which clearly illustrates the high micro-UHPLC column reproducibility achievable.



Advantages of using micro-UHPLC columns

Improved Limit of Detection!

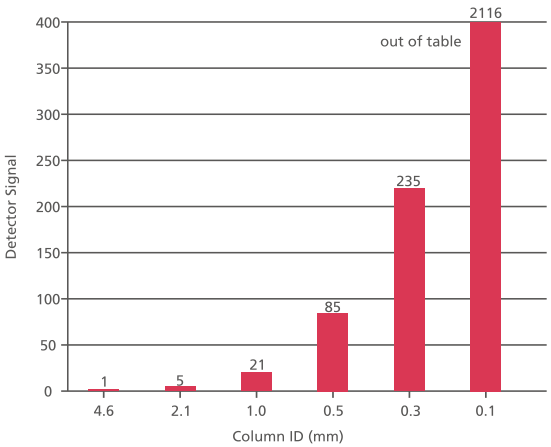
When only limited sample amounts are available for analysis, SenSil micro-UHPLC columns offer lower limits of detection (LOD) when connected to concentration-sensitive detectors such ESI-MS or when post-column flow-splitting is applied. For example, a 0.5 mm ID column gives 85 x lower LOD than a 4.6 mm ID column. In many applications, the sample amount is simply too small to be detected with conventional UHPLC instruments.

Reduced Waste

SenSil micro-UHPLC columns offer a drastic reduction in organic solvents consumption compared to conventional sized UHPLC columns. For example, a 1.0 mm ID column will reduce the mobile phase waste by a factor of 21 compared with a standard 4.6 mm ID column.

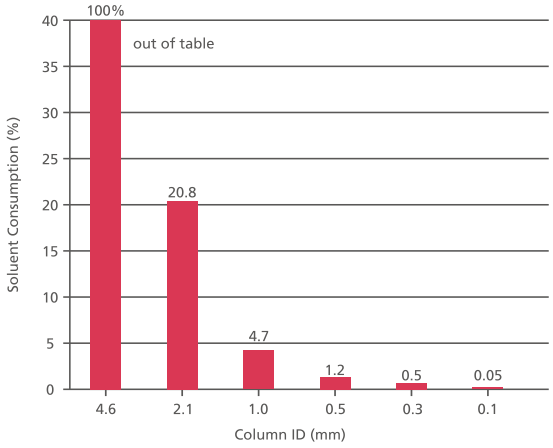
High-Temperature Compatibility

The low thermal mass of micro-UHPLC columns is ideal for exploiting temperature to modulate the chromatography, e.g. preheating of the mobile phase is simplified or often unnecessary. Higher column temperatures increase the mass transfer rate, thereby improving column efficiency and, in general, peaks are sharpened and resolution improved. Higher temperatures usually give reduced retention and thus less organic modifier is required, and the analysis can often be performed at a higher volumetric flow rate with no loss in resolution.



Reduced Column Length-Faster Separations

Many separations can be performed on shorter columns and higher flow-rates without sacrificing the baseline resolution. Furthermore, when using highly selective detectors such as the MS baseline resolution is often not required. However, being able to separate the analyte(s) from the void volume/matrix is preferred, especially when analysing complex matrixes such as biological samples, due to possible suppression effects in the ion source. The SenSil micro-UHPLC columns are therefore offered in 3 cm length for 1.6 µ particles and 5 cm length for 2.6 µ particles. Such short narrow-bore columns offer extremely low solvent consumption per analysis.



Excellent chromatographic performance

SenSil micro-UHPLC column facts:

Fully Validated Columns
High Reproducibility
High Robustness
More than 250,000 plates/m on routine basis

The SenSil range of micro-UHPLC columns are packed with materials from leading reputable manufacturers of stationary phases on the market. A wide range of bonding chemistries combined with ultra-pure silica particles provides excellent chromatographic performance for acidic, basic and neutral molecules. The SenSil columns are supplied with stainless steel screens or titanium frits to ensure high biocompatibility.

Our packing procedures enable us to pack the SenSil micro-UHPLC columns with high efficiency. For example, we routinely obtain 150,000-180,000 and 300,000-350,000 plates/m on columns packed with 2.6 µ SenSil C18 and 1.6 µ SenSil C18, respectively.

Column Validation

We guarantee that each column is carefully examined and tested before it leaves us. The columns are tested in a commercially available micro-UHPLC system to insure that you as a customer can reproduce our results. Each column is delivered with a Certificate of Analysis showing the test results.

Types of micro-UHPLC columns

Column ID (mm)	Column Category	Flow Rate Range (µL/min)	Optimum Flow Rate (µL/min)[a]	Analyte Capacity[b]	Required Amount of Pept./Proteins (fmole)	Bed volume 15 cm column (µL)
1.0	micro	20-200	50	-10 µg	-1,000	120
0.75	micro	10-100	25	-5 µg	-500	66
0.5	micro	5-50	10	-2.5 µg	-250	30
0.3	micro	2-20	3	-1 µg	-100	10
0.15	micro	0.5-5	1	-250 ng	-25	2.5

a For true low dead-volume systems

b Based on 1.6 µ SenSil 110Å C18-AQ

SenSil microbore columns

The SenSil microbore columns provide high efficiencies and are available with a variety of packings. The columns are packed in robust 1/8" O.D. glass-lined stainless steel tubing offering smooth internal surface with high biocompatibility and the possibility of operating the column at elevated temperatures. Standard 1/16" end fittings provide simple, universal and robust connections.

Microbore UHPLC columns offer a convenient compromise between sensitive capillary columns and highly robust conventional-bore columns. A 1.0 mm ID column provides more than a twenty-fold increase in sensitivity over a 4.6 mm ID column when injecting the same amount (mass) of sample. This increase in sensitivity can be critical for accurate quantification of sample limited applications. Moreover, the solvent waste is markedly reduced, offering a greener environment in the laboratory and economical benefits when using expensive solvents.

Typical flow rates: 1.0 mm ID: 20 - 200 µL/min

Wetted parts: quartz, stainless steel 316

End fittings: female 1/16" connections in both ends

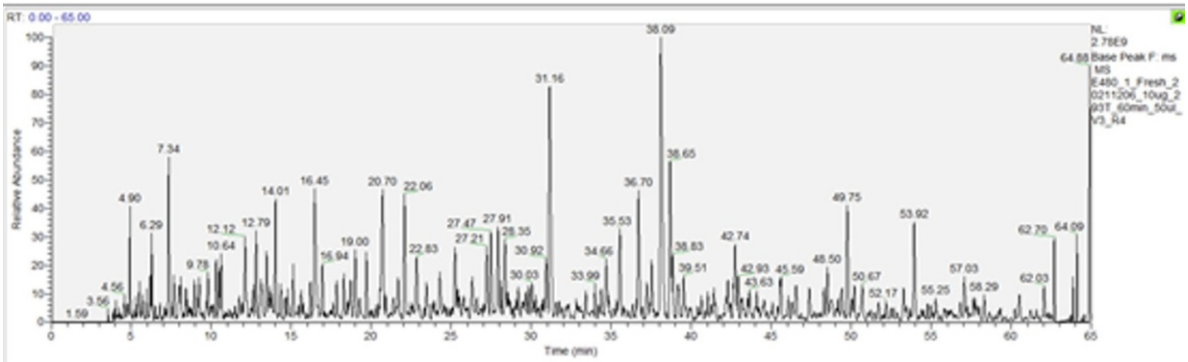
SenSil capillary columns

Capillary UHPLC columns offer high sensitivity due to their low dispersion characteristics. For example, a 0.3 mm ID column provides about 200-fold increase in sensitivity over a 4.6 mm ID column when injecting the same amount (mass) of sample. The analytes elute in smaller peak volumes, resulting in higher response for concentration-sensitive detectors (UV, fluorescence, ESI-MS). This increase in sensitivity is often a necessity for accurate quantification of sample limited applications.

The SenSil capillary UHPLC columns are ideal for use with detectors requiring very low flow rates, such as ESI-MS. This feature, combined with low dispersion characteristics, has led to a steadily increasing acceptance of such columns in applications where limited sample amounts lead to problems in detection sensitivity. This is relevant in the areas of pharmacokinetics, trace analysis and in particular the expanding fields of bioanalytical and proteomic analysis.

The SenSil capillary UHPLC columns provide high efficiencies and are available with a variety of packings. The capillary columns are packed in robust glass-lined stainless steel. Standard 1/16" end fittings provide simple, universal and robust connections.

Typical flow rates:	0.3 mm ID: 2 - 20 µL/min
	0.5 mm ID: 5 - 50 µL/min
Wetted parts:	quartz, PEEKsil
End fittings:	female 1/16" connections (1/32" is optional) in both ends



The chromatogram of SenSil C18-AQ, 1.6 µm, 150 x 1.0 mm ID column was carried out with method on paper below:
Bian, Y., Zheng, R., Bayer, F.P. et al. Robust, reproducible and quantitative analysis of thousands of proteomes by micro-flow LC–MS/MS. Nat Commun 11, 157 (2020).

Ordering information

SenSil™ BT-C18-AQ Columns

PACKING	FORMAT	I.D. X LENGTH	PART NO.
C18-AQ, 1.6 µm	Micro	0.15 x 30 mm	S612BAT
	Micro	0.15 x 50 mm	S612BBT
	Micro	0.15 x 75 mm	S612BCT
	Micro	0.15 x 100 mm	S612BDT
	Micro	0.15 x 150 mm	S612BFT
	Micro	0.3 x 30 mm	S612BAS
	Micro	0.3 x 50 mm	S612BBS
	Micro	0.3 x 75 mm	S612BCS
	Micro	0.3 x 100 mm	S612BDS
	Micro	0.3 x 150 mm	S612BFS
	Micro	0.5 x 30 mm	S612BAR
	Micro	0.5 x 50 mm	S612BBR
	Micro	0.5 x 75 mm	S612BCR
	Micro	0.5 x 100 mm	S612BDR
	Micro	0.5 x 150 mm	S612BFR
	Micro	0.75 x 30 mm	S612BAQ
	Micro	0.75 x 50 mm	S612BBQ
	Micro	0.75 x 75 mm	S612BCQ
	Micro	0.75 x 100 mm	S612BDQ
	Micro	0.75 x 150 mm	S612BFQ
C18-AQ, 2.6 µm	Micro	0.3 x 30 mm	S622BAS
	Micro	0.3 x 50 mm	S622BBS
	Micro	0.3 x 75 mm	S622BCS
	Micro	0.3 x 100 mm	S622BDS
	Micro	0.3 x 150 mm	S622BFS
	Micro	0.3 x 250 mm	S622BGS

SenSil™ BT-HILIC Columns

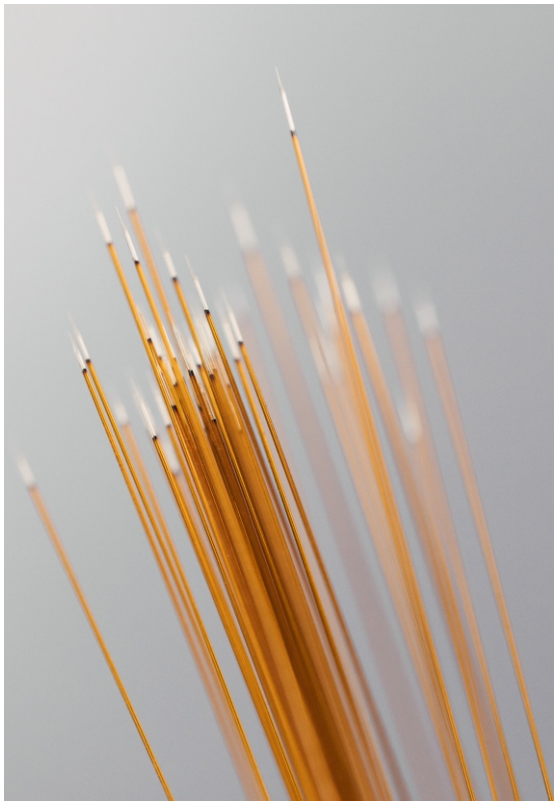
PACKING	FORMAT	I.D. X LENGTH	PART NO.
HILIC, 1.6 µm	Micro	0.15 x 30 mm	S616BAT
	Micro	0.15 x 50 mm	S616BBT
	Micro	0.15 x 75 mm	S616BCT
	Micro	0.15 x 100 mm	S616BDT
	Micro	0.15 x 150 mm	S616BFT
	Micro	0.3 x 30 mm	S616BAS
	Micro	0.3 x 50 mm	S616BBS
	Micro	0.3 x 75 mm	S616BCS
	Micro	0.3 x 100 mm	S616BDS
	Micro	0.3 x 150 mm	S616BFS
	Micro	0.5 x 30 mm	S616BAR
	Micro	0.5 x 50 mm	S616BBR
	Micro	0.5 x 75 mm	S616BCR
	Micro	0.5 x 100 mm	S616BDR
	Micro	0.5 x 150 mm	S616BFR
	Micro	0.75 x 30 mm	S616BAQ
	Micro	0.75 x 50 mm	S616BBQ
	Micro	0.75 x 75 mm	S616BCQ
	Micro	0.75 x 100 mm	S616BDQ
	Micro	0.75 x 150 mm	S616BFQ
HILIC, 2.6 µm	Micro	0.3 x 30 mm	S626BAS
	Micro	0.3 x 50 mm	S626BBS
	Micro	0.3 x 75 mm	S626BCS
	Micro	0.3 x 100 mm	S626BDS
	Micro	0.3 x 150 mm	S626BFS
	Micro	0.3 x 250 mm	S626BGS

Other packing materials and particle sizes may be available. Visit our website or call Fresh Bioscience Customer Service for assistance.

SenSil nano-UHPLC Columns

For ultra-low amount sample analysis

Nano-UHPLC combined with nano-ESI-MS is the cutting-edge technology within miniaturized separation techniques and it is rapidly becoming the dominating analysis tool within proteomics/peptidomics. The SenSil nano-UHPLC columns provide high efficiencies and reproducibility and they are available with a variety of packings. The columns are packed in PEEK-coated fused silica capillaries (PEEK-SIL), which are flexible and robust for practical use. Standard 1/16" end fittings provide simple, universal and robust connections.



The SenSil nano-UHPLC columns offer ultra-high sensitivity due to their low dispersion characteristics. A 75 µm I.D. column provides a 3760-fold increase in sensitivity over a 4.6 mm I.D. column when injecting the same amount (mass) of sample. The analytes elute in smaller peak volumes, resulting in higher response for concentration-sensitive detectors (UV fluorescence, ESI-MS). This increase in sensitivity is often a necessity for accurate quantification of sample limited applications.

Typical flow rates:	100 µm ID: 0.25 - 2.5 µL/min
	75 µm ID: 0.1 - 1 µL/min
Wetted parts:	fused silica, stainless steel 316
End fittings:	female 1/16" (1/32" is optional) connections in both ends.

Types of Nano-UHPLC columns

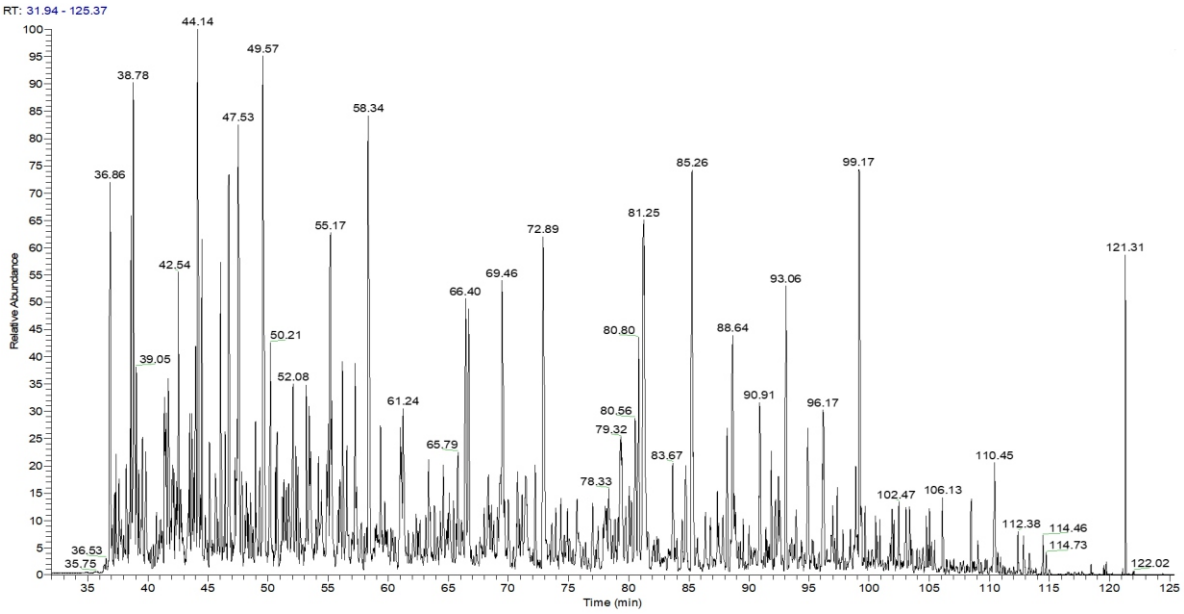
Column ID (mm)	Column Category	Flow Rate Range (µL/min)	Optimum Flow Rate (µL/min)[a]	Analyte Capacity[b]	Required Amount of Pept./Proteins (fmole)	Bed volume 15 cm column (µL)
0.1	nano	0.25-2.5	0.5	-100 ng	-2	2
0.075	nano	0.1-1	0.3	-50 ng	-1	1.1
0.050	nano	0.05-0.5	0.1	-25 ng	-0.5	0.5
0.020	nano	0.01-0.1	0.02	-4 ng	-0.1	0.1

a For true low dead-volume systems

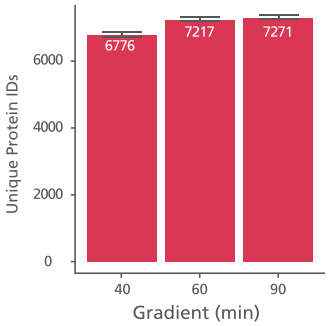
b Based on 1.6 µ SenSil 110Å C18-AQ

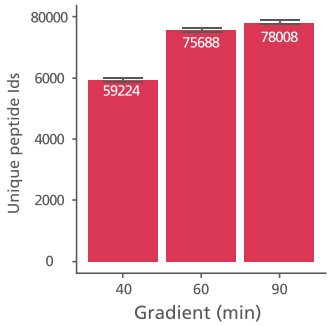
Deep preteome coverage

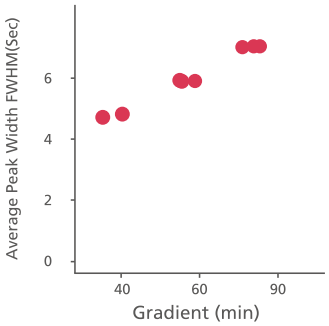
Maximise the number of identifications that you can achieve from a single shot sample analysis



Identified unique proteins, peptides and average Full Width at Half Maximum (FWHM) from 200 ng Hela Tryptic Digest injection on an SenSil C18-AQ, 1.6 µm, 250 mm x 75 µm ID column using different gradient lengths. Samples were run on a M-class UPLC coupled to a Bruker timsTOF Pro, dia-PASEF acquisition. Data analyzed using DIA-NN.







Ordering information

SenSil™ C18-AQ Columns

PACKING	FORMAT	I.D. X LENGTH	PART NO.
C18-AQ, 1.6 µm	Nano	0.020 x 40 mm	S612PXY
	Nano	0.020 x 50 mm	S612PBY
	Nano	0.020 x 75 mm	S612PCY
	Nano	0.020 x 100 mm	S612PDY
	Nano	0.020 x 150 mm	S612PFY
	Nano	0.020 x 250 mm	S612PGY
	Nano	0.050 x 75 mm	S612PCW
	Nano	0.050 x 100 mm	S612PDW
	Nano	0.050 x 150 mm	S612PFW
	Nano	0.050 x 250 mm	S612PCV
	Nano	0.075 x 75 mm	S612PDV
	Nano	0.075 x 100 mm	S612PEV
	Nano	0.075 x 125 mm	S612PFV
	Nano	0.075 x 150 mm	S612PGV
	Nano	0.075 x 250 mm	S612PJV
	Nano	0.075 x 500 mm	S612PKV
	Nano	0.075 x 600 mm	S612PBP
	Nano	0.1 x 50 mm	S612PCP
	Nano	0.1 x 75 mm	S612PDP
	Nano	0.1 x 100 mm	S612PEP
	Nano	0.1 x 125 mm	S612PFP
	Nano	0.1 x 150 mm	S612PGP
	Nano	0.1 x 250 mm	S612PJP
	Nano	0.1 x 500 mm	S612PLP
	Nano	0.1 x 800 mm	S612PLP

SenSil™ C18-AQ Emitter Columns

PACKING	FORMAT	I.D. X LENGTH	PART NO.
C18-AQ, 1.6 µm	Nano	0.020 x 40 mm	S612EXY
	Nano	0.020 x 50 mm	S612EBY
	Nano	0.020 x 75 mm	S612ECY
	Nano	0.020 x 100 mm	S612EDY
	Nano	0.020 x 150 mm	S612EFY
	Nano	0.020 x 250 mm	S612EGY
	Nano	0.050 x 75 mm	S612ECW
	Nano	0.050 x 100 mm	S612EDW
	Nano	0.050 x 150 mm	S612EFW
	Nano	0.050 x 250 mm	S612EGW
	Nano	0.075 x 75 mm	S612ECV
	Nano	0.075 x 100 mm	S612EDV
	Nano	0.075 x 125 mm	S612EEV
	Nano	0.075 x 150 mm	S612EFV
	Nano	0.075 x 250 mm	S612EGV
	Nano	0.075 x 500 mm	S612EJV
	Nano	0.075 x 600 mm	S612EKV
	Nano	0.1 x 50 mm	S612EBP
	Nano	0.1 x 75 mm	S612ECP
	Nano	0.1 x 100 mm	S612EDP
	Nano	0.1 x 125 mm	S612EEP
	Nano	0.1 x 150 mm	S612EFP
	Nano	0.1 x 250 mm	S612EGP
	Nano	0.1 x 500 mm	S612EJP
	Nano	0.1 x 800 mm	S612ELP

Other packing materials and particle sizes may be available. Visit our website or call Fresh Bioscience Customer Service for assistance.

Fresh Bioscience offers...

Custom Packed Columns

In addition to the range of micro-UHPLC column dimensions previously listed, we also manufacture tailor-made columns that might be required for your particular application. For 0.1-0.5 mm ID columns, other column hardware materials such as PEEKsil are also available on request. Please contact your local distributor or us for further details about our custom packing service.

Batch Reservation Service

For challenging applications/validated methods we also offer a batch reservation service, which completely eliminates batch related reproducibility concerns. Based on your projected column usage, we will reserve the quantity of packing material you need and use it each time you order a new column.

Warranty

SenSil UHPLC columns are warranted to be free from defects in materials or workmanship. Fresh Bioscience will promptly replace any defective column unless such defects are attributed to customer abuse, misuse or neglect. Please contact your local distributor or us for further information.

Technical Support

For technical support, please contact your local distributor or Fresh Bioscience ltd.

Care and Use of SenSil Columns

Installation and testing

Remove the column from its container and retain the container for storing the column when not in use. The flow direction during the column packing process is indicated on the column tag. Operate the column with the mobile phase flowing in this direction. Before connecting the column outlet to the detector, flush the column with mobile phase, this will prevent small particles, settled on the column frits during shipping and handling, from being washed into the detector. With high performance columns, significant efficiency will be lost if long lengths of large I.D. tubing are employed. For optimum performance, we recommend the following connecting tubing dimensions:

Column ID (mm)	Flowrate (µL/min)	Tubing ID (µm)
0.075 / 0.1 *	0.2 / 0.3	25
0.3	3	< 75
0.5	10	< 125
1.0	40	< 200

* nano-LC columns require a dedicated nano-injector even when using column-switching for sample loading (max. 0.1 mm bore).

Filters and guard columns can further reduce column performance if not properly selected and maintained. After connecting to the HPLC system, begin to pump an appropriate mobile phase to equilibrate the column. Enclosed along with your new SenSil column you will find a performance test chromatogram (Certificate of Analysis) generated on your column. The mobile phase used for the separation of the test mixture is the shipping solvent.

Care and maintenance

The following guidelines will be helpful for most columns prepared with rigid silica-based packings.

Pressure: The column backpressure depends on the packing material and the mobile phase used. With extended use a gradual increase in pressure is usually seen, however, a sudden increase in pressure signals a plugging problem that should be corrected (see column cleaning). However, do not exceed a maximum pressure of 1,000 bars on your column. Usually, this is not a relevant problem for most users, as most HPLC pumps have a maximum pressure below this limit. Exceptions are packings with pore size of 300Å and larger.

Temperature / column oven

The hardware of SenSil Columns tolerates temperatures up to 150 °C. However, at high temperatures bonded phases can be lost over time and a decrease in efficiency and peak symmetry might be observed due to dissolution of the silica particles. Column temperatures above 60 °C are not recommended for silica-based particles. We recommend using a column oven to assure reproducible retention times.

Filters and guard columns

Column life is improved with in-line filters or guard columns. Contact our technical personnel for help in choosing guard protection.

Mobile phase solvents

All common UHPLC grade organic solvents can be used with your SenSil Column. Buffers made from acetate, formate, citrate and phosphate salts can be used up to 0.2 M without adverse effects. As long as the appropriate pH range is not exceeded, organic modifiers and ion pair reagents can be used. However, some ion-pair reagents could be difficult to flush from the column, and columns used with these reagents should be dedicated to the particular analysis involved. Limit the use of strong bases, and avoid strong acids. Do not mix solutions that might precipitate or gel in the column or in the system. The pH range for your column should usually not exceed 1.5-10.0.

Column lifetime

The lifetime of your SenSil Column is highly dependent on the sample and the employed conditions and cannot be generalized. Maximize column lifetime by making sure that samples and mobile phases are clean and particle free, and by using a guard column and/or filters.

Column storage

When storing your SenSil Column, flush it with acetonitrile/methanol after cleaning and seal it. Do not store columns containing buffers, salt solutions, acidic mobile phases or tetrahydrofuran.

For technical support or application:

support@freshbioscience.com

For more information:

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