Evaluation of Durability of Hybrid Silica with Ethylene Chains

pyvot
Phone: 212-204-0075
Email: info@pyvot.tech
Web: www.pyvot.tech

BioNik Inc.
www.bionikinc.com

N. Nagae[a], T. Tsukamoto[a], R. Koyama[a], E. Shearer[b]
[a] ChromaNik Technologies Inc. Namiyoke, Minato-ku, Osaka Japan
[b] BioNik Inc. Obuchi, Fuji, Shizuoka Japan

ChromaNik Technologies Inc.
www.chromanik.co.jp

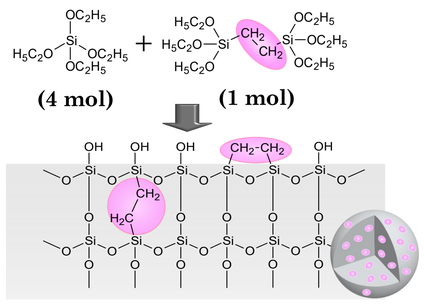
Silica-based reversed-phase columns have been widely used since the 1970s. Silica has the advantage of being mechanically strong and able to withstand high pressures, but conventional silica C18 has limited use under alkaline conditions and is only durable up to pH 8. In the 2000s, hybrid silica with ethylene chains bonded to the inside or surface of silica was developed, and patent applications were filed for these technologies. Currently, these technologies are freely available, and it is expected that these hybrid silica packings will be available on the market.

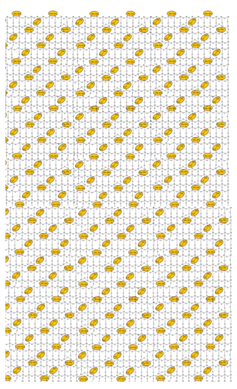
In this study, we evaluated the durability of so-called hybrid silica with ethylene chains bonded to the inside and surface of silica, and hybrid C18 bonded with C18 and end-capped. The amount of hybrid silica dissolved was measured when an alkaline mobile phase of pH 11.5 was passed through the column, and the hybrid C18 bonded with C18 and end-capped was also evaluated in the same way. The durability of hybrid silica under alkaline conditions greatly differed depending on the method of introducing and bonding ethylene chains to silica, with a difference of 100 to 1000 times the amount of dissolved silica. The difference in stability of hybrid C18 under alkaline conditions was smaller than that of hybrid silica alone, suggesting that the effect of end-capping on durability is greater than that of hybrid silica.

2 types of hybrid silica gel

1. Hybrid ①

Ethylene cross-linked silica gel





12.5 nm, 125 Å
Thickness of silica skeleton

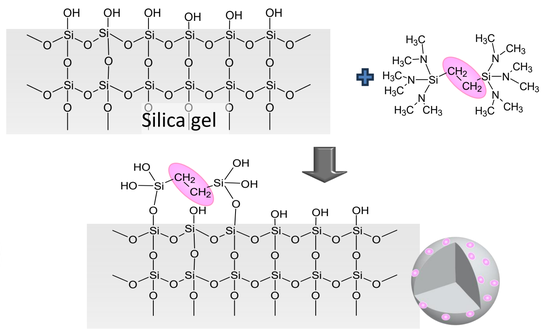
Hybrid ①
Fully hybrid silica

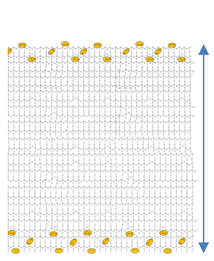
%C: 7%

(180 m²/g)

2. Hybrid ②

Ethylene chains bonded to the silica gel surface





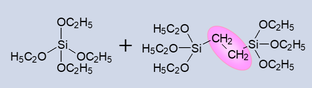
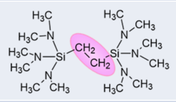
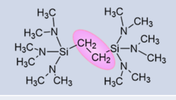
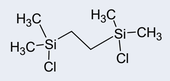
6.6 nm, 66 Å
Thickness of silica skeleton

Hybrid ②
Partially hybrid silica

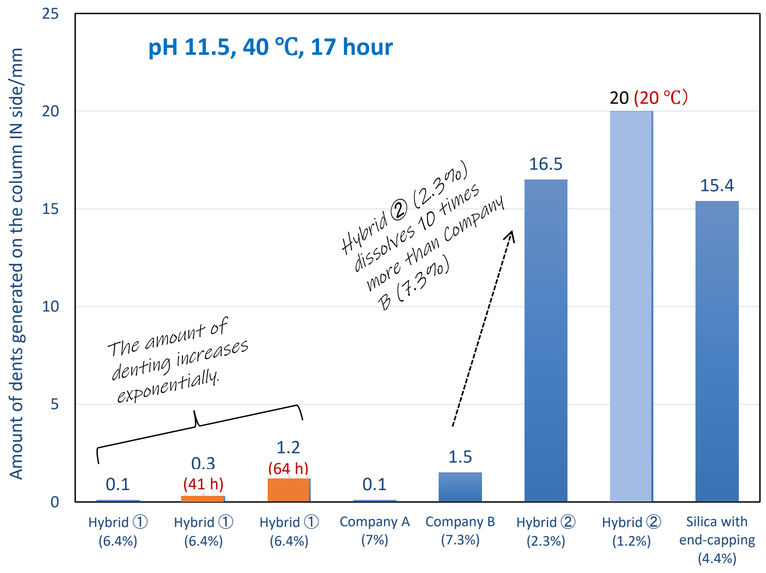
%C: 2.5%

(340 m²/g)

Hybrid silica gels used in this study

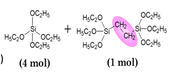
Name	Specific surface area	Pore diameter	Carbon loading	reagent
Hybrid ① (6.4%)	180 m²/g	15 nm	6.4%	
Hybrid ② (2.3%)	340 m²/g	12 nm	2.3%	Silica gel + 
Hybrid ② (1.2%)	340 m²/g	12 nm	1.2%	Silica gel + 
Silica with end-capping (4.4%)	340 m²/g	12 nm	4.4%	Silica gel + 

Comparison of durability of hybrid silica gel under alkaline conditions



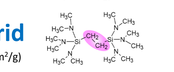
Durability test conditions (pH 11.5)
Column dimension: 100 x 2.1 mm
Mobile phase: 50 mM potassium phosphate pH 11.5
Flow rate: 0.1 mL/min
Temperature: 40 ℃
Elution time: 17 h
(The carbon loading of the ethylene chain is shown in parentheses.)

Hybrid ① (6.4%)
Company A (7%)
} **Full hybrid** (185 m²/g)

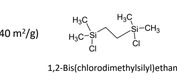


Company B (7.3%) ? Full or partial hybrid (340 m²/g)

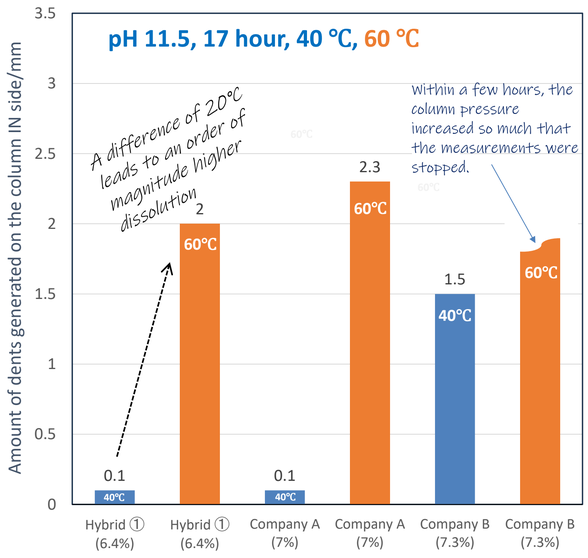
Hybrid ② (2.3%)
Hybrid ② (1.2%)
} **Partial hybrid** (340 m²/g)



Silica with only end-capping (4.4%) **No hybrid** (340 m²/g)



1,2-Bis(chlorodimethylsilyl)ethane



Durability test conditions (pH 11.5)
Column dimension: 100 x 2.1 mm
Mobile phase: 50 mM potassium phosphate pH 11.5
Flow rate: 0.1 mL/min
Temperature: 40 ℃ and 60 ℃
Elution time: 17 h

- When comparing the amount (speed) of silica dissolution when an alkaline mobile phase was passed through hybrid silica, Hybrid ② showed a value 100 to 1000 times higher than Hybrid ①.
- The amount of silica C18 dissolved in an alkaline mobile phase increases exponentially over time, and this was also the case for hybrid silica (Hybrid ①).
- When the temperature difference was 20℃, the amount of silica dissolved was about 10 times different, and a 10℃ temperature increase caused the amount of silica dissolved to increase by about three times.

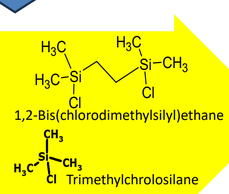
C18 bonding and end-capping

Hybrid silica gel
Hybrid① (6.4%), Hybrid② (1.2%)

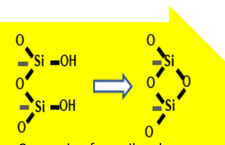
Particle size: 5 μm,

Bonding with Trifunctional C18

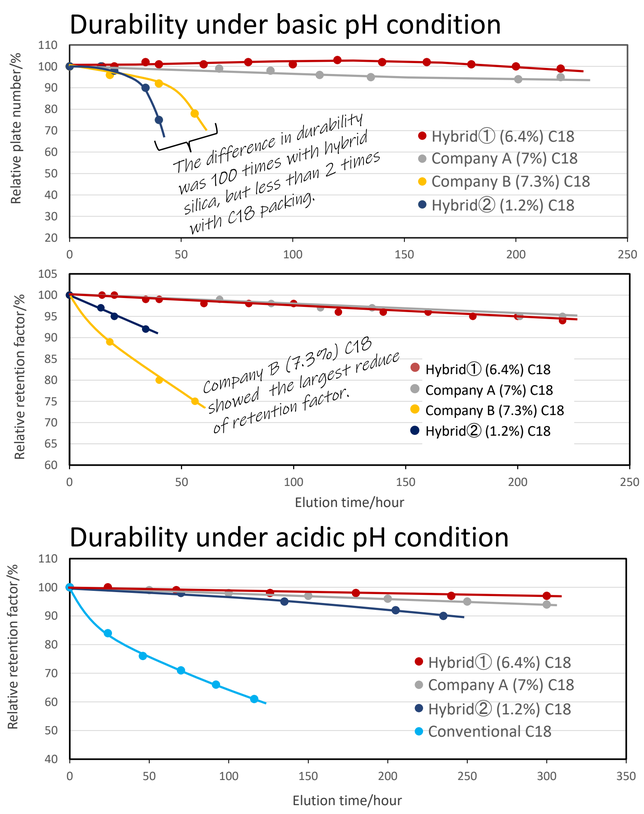
Double end-capping



Elevated temperature end-capping



Durability under acidic and basic pH conditions



Durable test condition
Column size: 150 x 4.6 mm
Mobile phase: Methanol/50mM potassium phosphate pH 11.5=10/90
Flow rate: 1.0 mL/min
Temperature: 40 ℃

Measurement condition
Mobile phase: Acetonitrile/water=70/30
Flow rate: 1.0 mL/min
Temperature: 40 ℃
Sample: 1 = Uracil (t_0), 2 = Butylbenzene

Durable test condition
Column size: 50 x 2.1 mm
Mobile phase: Acetonitrile/1.0% TFA pH 1=10/90
Flow rate: 0.4 mL/min
Temperature: 80 ℃

Measurement condition
Column size: 50 x 2.1 mm
Mobile phase: Acetonitrile/water =60/40
Flow rate: 0.4 mL/min,
Temperature: 40 ℃
Sample: 1 = Uracil (t_0), 2 = Butylbenzene

Conclusions

- ✓ It was confirmed that the durability of hybrid silica under alkaline conditions varies greatly depending on the method of bonding the ethylene chains.
- ✓ It was suggested that the durability of the C18 packing after C18 bonding and end-capping is greatly affected not only by the durability of the hybrid silica, but also by the surface treatment, especially end-capping.
- ✓ C18 using hybrid silica with ethylene chains bonded within the silica skeleton was found to have sufficient durability under mobile phase conditions from pH 1 to pH 12.

Evaluation of Bidentate End-capping Silylation Reagents for HPLC

pyvot
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Email: info@pyvot.tech
Web: www.pyvot.tech

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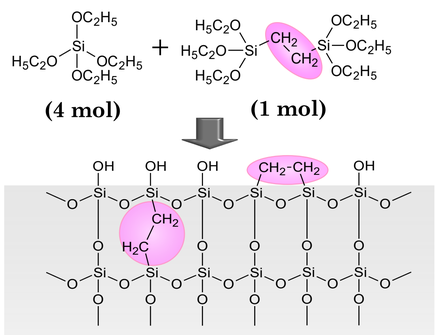
3 types of based silica gel

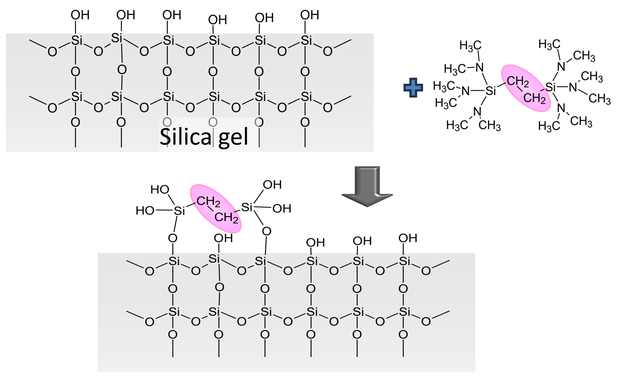
1. **Hybrid ①**

Ethylene cross-linked silica gel

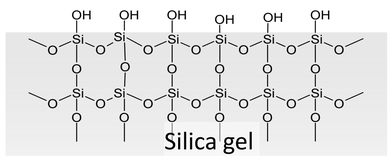


2. **Hybrid ②**

Ethylene chains bonded to the silica gel surface



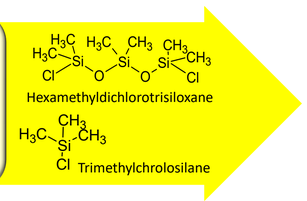
3. **Silica gel**



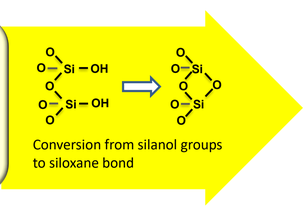
End-capping after C18 bonding

2 types of end-capping reagents

Double end-capping



Elevated temperature end-capping



A-type end-capping

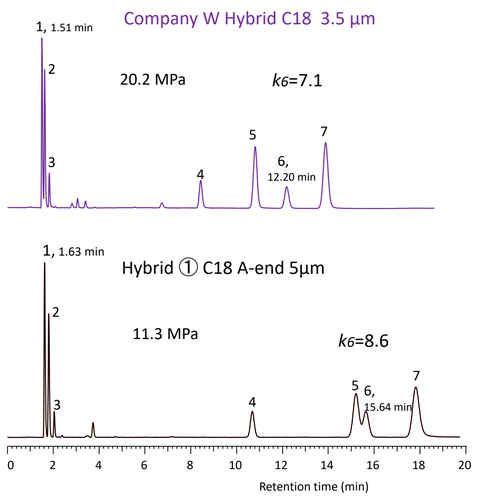
1,5-Dichlorohexamethytrisiloxane

B-type end-capping

1,2-Bis(chlorodimethylsilyl)ethane

★Six types of C18 packing materials were prepared and compared for hydrogen bonding, hydrophobicity, steric selectivity, peak shape for basic compounds, and durability against acidity and alkalinity.

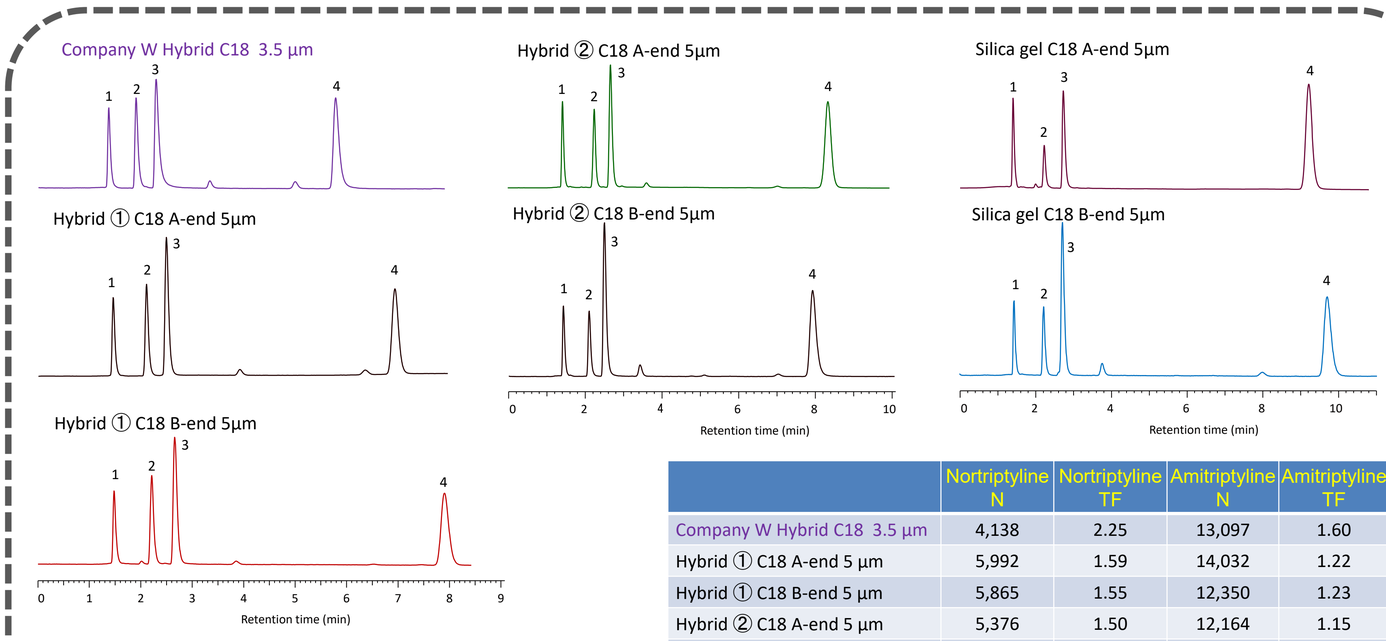
Tanaka Index, specific surface area and carbon loading



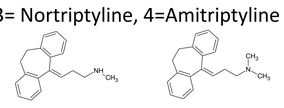
Column: Shown in figure
Column dimension: 150 x 4.6 mm
Mobile phase: CH_3OH/H_2O=75/25
Flow rate: 1.0 mL/min
Temperature: 40 °C
Sample: 1 = Uracil
2 = Caffeine
3 = Phenol
4 = Butylbenzene
5 = o-Terphenyl
6 = Amylbenzene
7 = Triphenylene

	Hydrogen bonding (Caffeine/Phenol)	Hydrophobicity (Amylbenzene/ Butylbenzene)	Steric selectivity (Triphenylene/ o-Terphenyl)	Specific surface area (m^2/g)	Carbon loading
Company W Hybrid C18 3.5 µm	0.38	1.54	1.33	185	17.4%
Hybrid ① C18 A-end 5 µm	0.43	1.55	1.19	209	15.9%
Hybrid ① C18 B-end 5 µm	0.43	1.55	1.23	209	16.0%
Hybrid ② C18 A-end 5 µm	0.40	1.54	1.35	332	17.0%
Hybrid ② C18 B-end 5 µm	0.40	1.56	1.39	332	17.2%
Silica gel C18 A-end 5 µm	0.42	1.58	1.41	330	15.3%
Silica gel C18 B-end 5 µm	0.43	1.60	1.29	330	15.9%

Peak shape of basic compounds



Column: Shown in figure
Column size: 150 x 4.6 mm
Mobile phase: Acetonitrile/20mM phosphate buffer pH7.0=60/40
Flow rate: 1.0 mL/min
Temperature: 40 °C
Detection: UV@250 nm
Sample: 1=Uracil, 2=Propranolol, 3= Nortriptyline, 4=Amitriptyline



	Nortriptyline N	Nortriptyline TF	Amitriptyline N	Amitriptyline TF
Company W Hybrid C18 3.5 µm	4,138	2.25	13,097	1.60
Hybrid ① C18 A-end 5 µm	5,992	1.59	14,032	1.22
Hybrid ① C18 B-end 5 µm	5,865	1.55	12,350	1.23
Hybrid ② C18 A-end 5 µm	5,376	1.50	12,164	1.15
Hybrid ② C18 B-end 5 µm	4,818	1.63	11,343	1.43
Silica gel C18 A-end 5 µm	6,323	1.51	12,922	1.14
Silica gel C18 B-end 5 µm	5,451	1.54	12,724	1.34

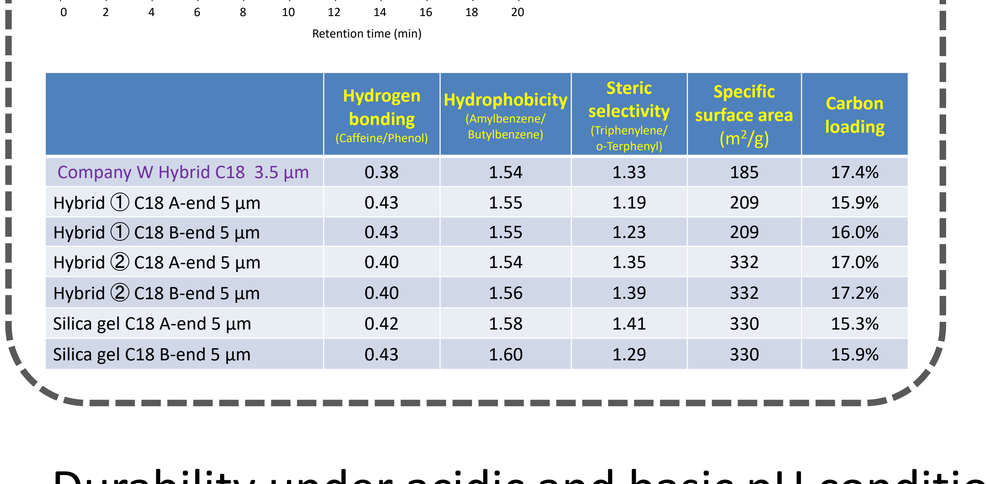
N: Theoretical plate, TF: Tailing factor

★Under neutral conditions using acetonitrile as the mobile phase, basic compounds are prone to tailing. With bidentate end-capping, both types A and B showed less tailing. The results were more symmetrical than Company W Hybrid C18, which was used for comparison.

Durability under acidic and basic pH conditions

Durable test condition
Column size: 50 x 2.1 mm
Mobile phase: Acetonitrile/1.0% TFA, (pH 1)=10/90
Flow rate: 0.4 mL/min
Temperature: 80 °C

Measurement condition
Column size: 50 x 2.1 mm
Mobile phase: Acetonitrile/water =60/40
Flow rate: 0.4 mL/min,
Temperature: 40 °C
Sample: 1 = Uracil (t_0), 2 = Butylbenzene



Durable test condition
Column size: 150 x 4.6 mm
Mobile phase: Methanol/50mM potassium phosphate pH 11.5=10/90
Flow rate: 1.0 mL/min
Temperature: 40 °C

Measurement condition
Mobile phase: Acetonitrile/water=70/30
Flow rate: 1.0 mL/min
Temperature: 40 °C
Sample: 1 = Butylbenzene

★For comparison, the test results of hybrid C18 from companies W, Y, and P are also shown.

Conclusions

- ✓ Six types of packing materials were synthesized using three types of silica base materials and two types of bidentate end-capping reagents after C18 bonding.
- ✓ Comparison of hydrogen bonding, hydrophobicity, and steric selectivity in columns packed with six types of packing materials showed that the end-capping reagent had little effect, while the silica base material had a greater effect.
- ✓ The degree of tailing of the peaks of basic compounds was compared. As a result, no significant difference was observed among the six types of packing materials, and peaks with less tailing were obtained. Company W hybrid C18, used as a reference, showed tailing of basic compounds (especially nortriptyline).
- ✓ In both durability tests under acidic and basic pH conditions, packing materials using B type 1,2-bis(chlorodimethylsilyl)ethane for end-capping were more durable than A type 1,5-dichlorohexamethyltrisiloxane. It was presumed that this is because the B type end-capping reagent is more hydrophobic, making it difficult for water molecules, which cause hydrolysis and lead to deterioration, to approach the packing silica surface.
- ✓ The durability under basic pH condition was greatly affected by the silica base material, and the silica with ethylene chains crosslinked into the silica skeleton showed the highest durability, which was equivalent to that of Hybrid C18 from company W.

Evaluation of Hybrid Silica C18 End-capped with Bidentate Silylating Reagent for HPLC

pyvot
Phone: 212-204-0075
Email: info@pyvot.tech
Web: www.pyvot.tech

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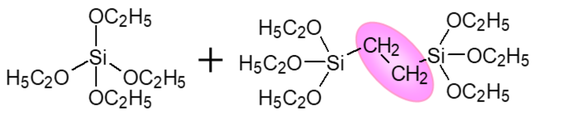
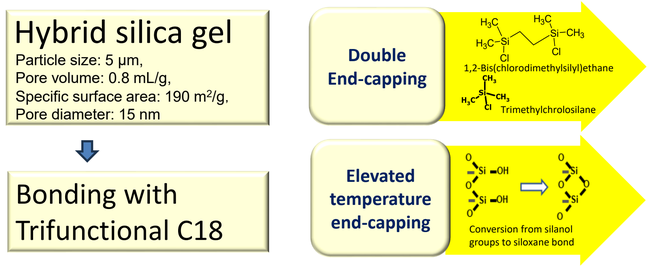
Comparison between Company W hybrid C18 and SunBridge C18 with Ethylene cross-linked silica gel And 1,2-Bis(chlorodimethylsilyl)ethane end-capping

SunBridge

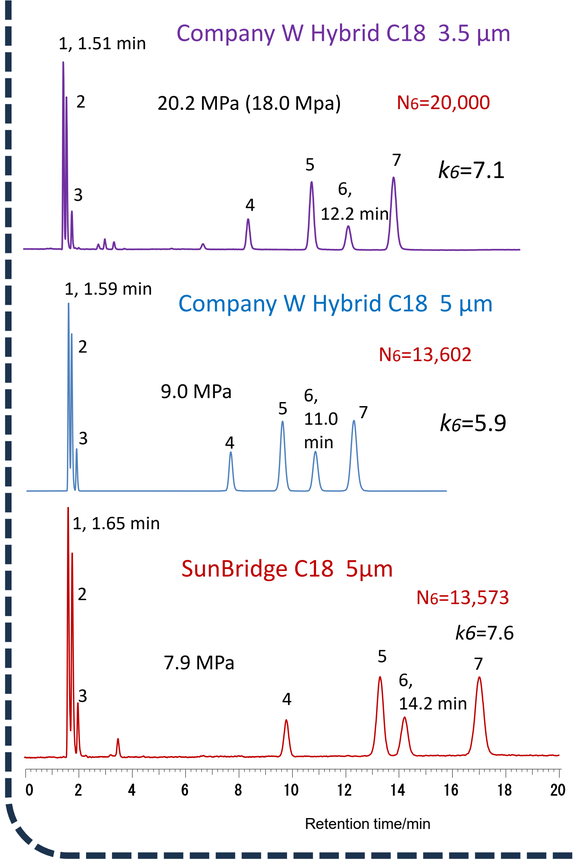


Ethylene cross-linked silica gel



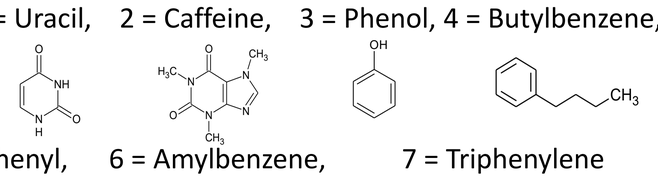
1,2-Bis(chlorodimethylsilyl)ethane end-capping

Evaluation of hydrogen bonding, hydrophobicity and steric selectivity

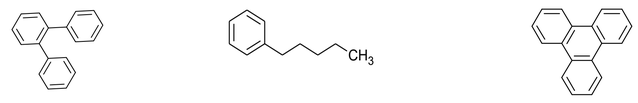


As a comparison, Hybrid C18 manufactured by W Company, which is ethylene crosslinked silica gel C18 in which an ethylene chain is incorporated into the silica skeleton, was used.

Column: Company W Hybrid C18 3.5 µm, 5 µm,
SunBridge C18 5 µm,
Column demensions: 150 x 4.6 mm
Mobile phase: CH_3OH/H_2O=75/25
Flow rate: 1.0 mL/min
Temperature: 40 °C
Sample: 1 = Uracil, 2 = Caffeine, 3 = Phenol, 4 = Butylbenzene,

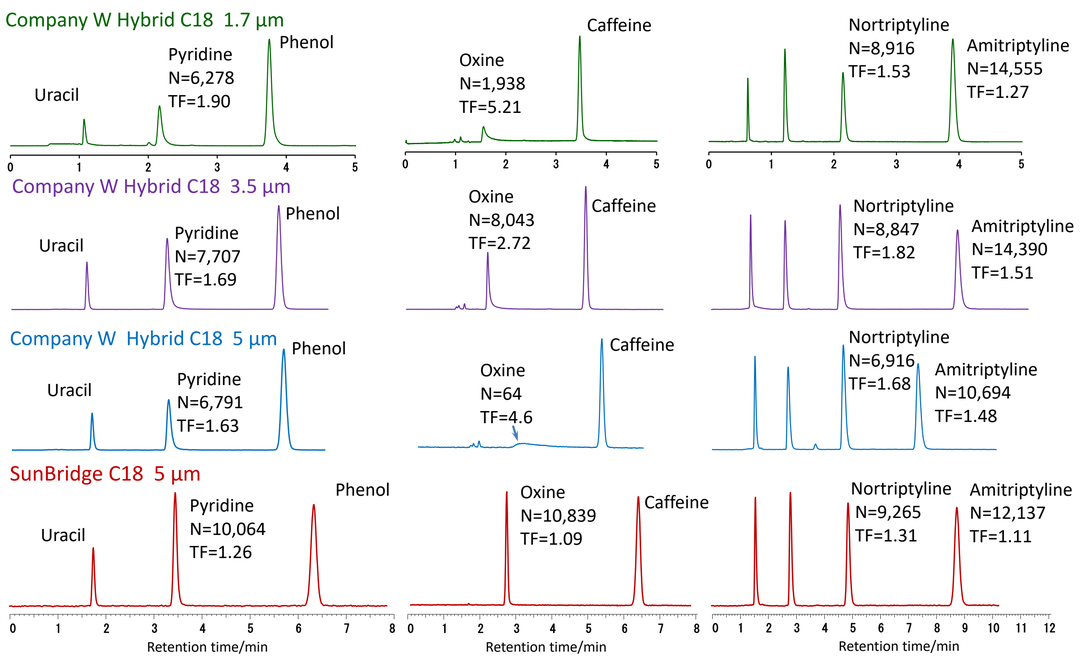


5 = o-Terphenyl, 6 = Amylbenzene, 7 = Triphenylene



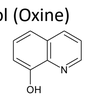
	Hydrogen bonding α(Caffeine/Phenol)	Hydrophobicity α(Amylbenzene/ Butylbenzene)	Steric selectivity α(Triphenylene/ o-Terphenyl)	Specific surface aera (m^2/g)	Carbon loading (%)
Company W Hybrid C18 3.5 µm	0.38	1.54	1.33	185	17.4%
Company W Hybrid C18 5 µm	0.39	1.52	1.33	188	18.0%
SunBridge C18 5µm	0.43	1.53	1.31	190	16.0%

Comparison of peaks for pyridine, oxine, and nortriptyline

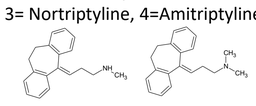


Column dimension:
150 x 4.6 mm for 3.5 and 5 µm
100 x 2.1 mm for 1.7 µm
Mobile phase: CH_3OH/H_2O=30/70
Flow rate:
1.0 mL/min for 3.5 and 5 µm
0.2 mL/min for 1.7 µm
Temperature: 40 °C
Detection: UV@250 nm
Sample: 1 = Uracil
2 = Pyridine
3 = Phenol

Column dimension:
150 x 4.6 mm for 3.5 and 5 µm
100 x 2.1 mm for 1.7 µm
Mobile phase: Acetonitrile / 20 mM H_3PO_4=10/90
Flow rate:
1.0 mL/min for 3.5 and 5 µm
0.2 mL/min for 1.7 µm
Temperature: 40 °C
Detection: UV@250 nm
Sample: 1 = 8-Quinolinol (Oxine)
2 = Caffeine

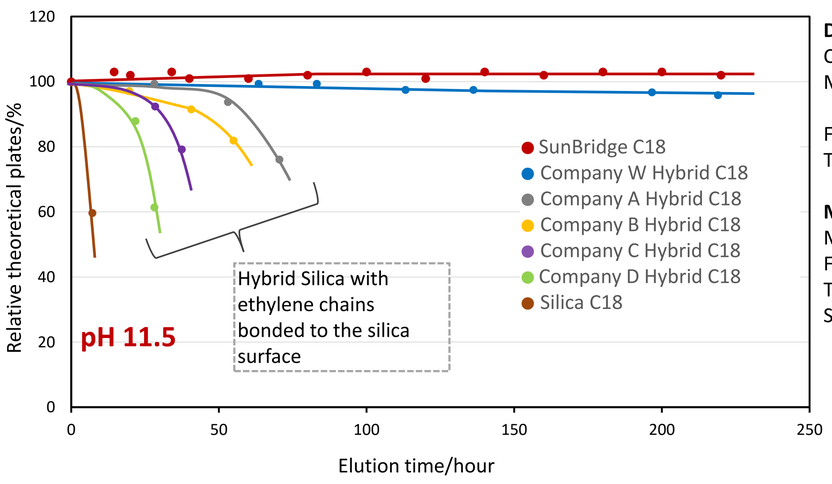


Column dimension:
150 x 4.6 mm for 3.5 and 5 µm
100 x 2.1 mm for 1.7 µm
Mobile phase: Acetonitrile/10 mM ammonium acetate pH6.8=40/60
Flow rate:
1.0 mL/min for 3.5 and 5 µm
0.3 mL/min for 1.7 µm
Temperature: 40 °C
Detection: UV@250 nm
Sample: 1=Uracil, 2=Propranolol, 3= Nortriptyline, 4=Amitriptyline



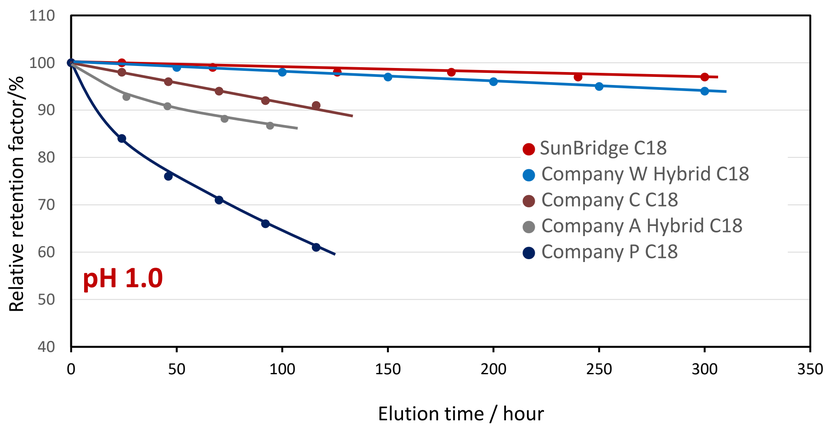
Company W Hybrid C18 showed severe tailing of metal-chelating, oxine.
Compared to Company W Hybrid C18, SunBridge C18 showed a higher number of plates and less tailing of peaks for metal-chelating and basic compounds.

Comparison of Stability under pH 11.5 and pH 1.0 conditions



Durable test condition (pH 11.5)
Column dimension: 150 x 4.6 mm
Mobile phase: Methanol/50 mM potassium phosphate pH 11.5=10/90
Flow rate: 1.0 mL/min
Temperature: 40 °C

Measurement condition
Mobile phase: Acetonitrile/water=70/30
Flow rate: 1.0 mL/min
Temperature: 40 °C
Sample: 1 = Uracil, 2 = Butylbenzene

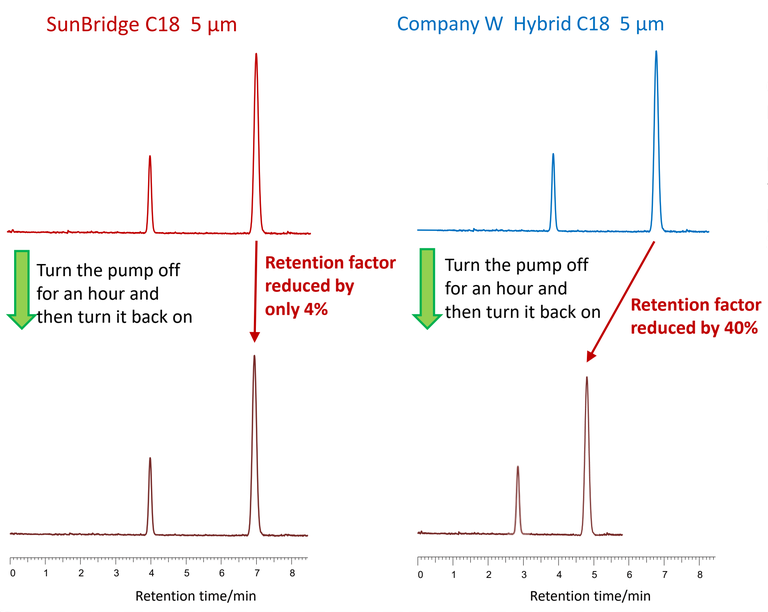


Durable test condition (pH 1.0)
Column dimension: 50 x 2.1 mm
Mobile phase: Acetonitrile/1.0% TFA pH1=10/90
Flow rate: 0.4 mL/min
Temperature: 80 °C

Measurement condition
Same as above

SunBridge C18 showed almost the same or higher stability under pH 11.5 and pH 1.0 conditions as Company W Hybrid C18, which shows an order of magnitude higher stability than other companies' hybrid C18.

Reproducibility in retention under 100% aqueous conditions



Column dimension: 150 x 4.6 mm
Mobile phase:
10 mM phosphate buffer pH 7.0
Flow rate: 1.0 mL/min
Temperature: 40 °C
Detection: UV@250 nm
Sample: 1=Uridine, 2=Adenine

Conclusions

- ✓SunBrige C18 was developed using Ethylene cross-linked silica gel and 1,2-Bis(chlorodimethylsilyl)ethane end-capping reagent.
- ✓As a comparison, Hybrid C18 manufactured by W Company, which is ethylene cross-linked silica gel C18 in which an ethylene chain is incorporated into the silica skeleton, was used.
- ✓Company W Hybrid C18 showed severe tailing of metal-chelating, oxine. Compared to Company W Hybrid C18, SunBridge C18 showed a higher number of plates and less tailing of peaks for metal-chelating and basic compounds.
- ✓SunBridge C18 showed almost the same or higher stability under pH 11.5 and pH 1.0 conditions as Company W Hybrid C18, which shows an order of magnitude higher stability than other companies' hybrid C18.
- ✓SunBridge C18 showed a excellent reproducibility in retention factor under 100% aqueous conditions while Company W Hybrid C18 showed a poor reproducibility.

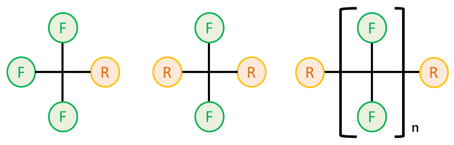
Analysis of PFAS in Tap Water Using a Pentafluorophenyl Column

Tadashi Kitta[1], Hirotake Takahashi[1],
Norikazu Nagae[2], Ryuji Koyama[2] and Tomoyasu Tsukamoto[2]
[1]Japan Food Inspection Corporation, Heiwajima, Ohta-ku, Tokyo, Japan
[2]ChromaNik Technologies Inc., Namiyoke, Minato-ku, Osaka, Japan

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are known to pose health risks due to their bioaccumulative nature and environmental persistence. In April 2026, legal restrictions setting a combined limit of 50 ng/L for PFOS and PFOA in tap and mineral water will take effect in Japan. Furthermore, the European Union has already established maximum levels for four PFAS congeners in seafood, and similar regulations are anticipated in the United States.
In this study, we developed a pentafluorophenyl (PFP) column consisting of an ethylene cross-linked hybrid silica gel and a PFP stationary phase. This column is capable of simultaneously analyzing 19 PFAS compounds, ranging from short-chain to long-chain substances. Its applicability to drinking water analysis was evaluated and compared with that of a conventional C18 column. The PFP column exhibited not only greater sensitivity for PFBA but also superior separation of PFHxS isomers compared to the C18 column.

PFAS

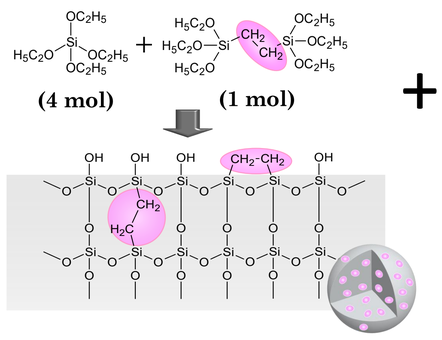
Per- and polyfluoroalkyl substances (PFAS) are defined as "fluorinated substances that contain at least one fully fluorinated methyl or methylene carbon (with no H/Cl/Br/I atoms attached).
As of January 2025, there are 14,735 PFAS with clear chemical structures and 1,915 PFAS with unclear structural information such as polymers and complex mixtures.



PFAS are heat-resistant, water-repellent, and oil-repellent, and are used in many industrial products and daily necessities. On the other hand, they are called "Forever Chemicals" because they are difficult to decompose in the environment and higher bioaccumulative. The main routes of human exposure are oral ingestion of drinking water and food, inhalation of product dust, and skin absorption through contact with products (such as cosmetics).

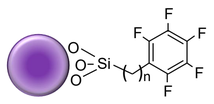
Hybrid silica PFP and C18

1. **Hybrid silica**
Ethylene cross-linked silica gel

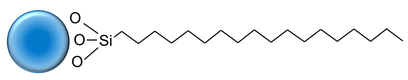


2. **Stationary phase**

PFP (SunBridge PFP-R)



C18 (SunBridge C18)



+ End-capping

● Analysis issues

When a conventional C18 column is used,
1) lack sensitivity for short-chain PFAS.
2) insufficient separation of long-chain PFAS isomers.
→ A method that can comprehensively and sensitively analyze a variety of PFASs is needed.

● Objectives

Aim to establish a comprehensive and highly sensitive method for analyzing trace PFAS in tap water using PFP columns.

Measurement conditions

HPLC	Ultimate 3000(Thermo Fisher Scientific)
Analytical column	SunBridge PFP-R 3 µm, SunBridge C18 3 µm, 100 x 2.1 mm ID
Delay column	SunBridge Delay C18 3 µm, 50 x 3.0 mm ID
Mobile phase	A) Methanol B) 2 mM Ammonium acetate
Gradient	Time (min) %B 0.0 30 1.0 30 21.0 97 24.0 97 24.1 30 28.0 30
Flow rate	0.3 mL/min
Column temperature	40 °C
Injection volume	4 µL
MS	Q Exactive Focus (Thermo Fisher Scientific)
Ion mode	ESI (-)
Resolution	70000 (at m/z 200)

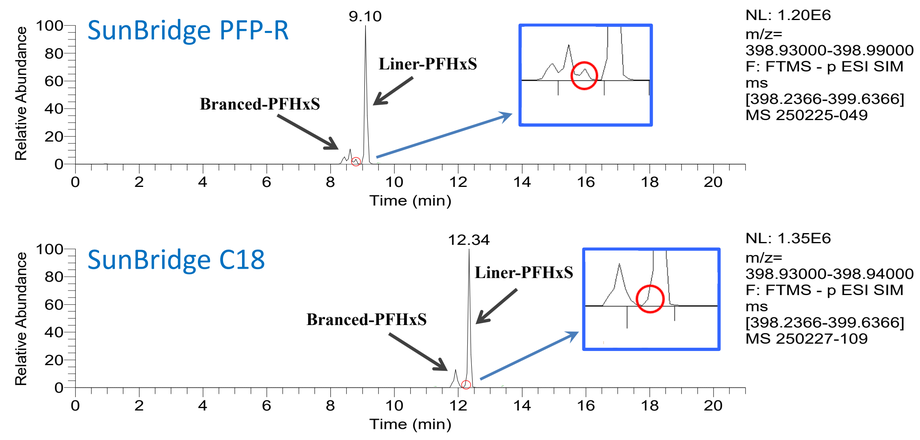
Sample pretreatment

250 mL sample
+ IS
ISOLUTE 101 (100 mg, Biotage)+ EVOLUTE PFAS (150 mg, Biotage)
+ Load → Discard
+ Water → Discard
+ 0.1% NH3 methanol → Collect
+ Acetic acid
LC/MS measurement

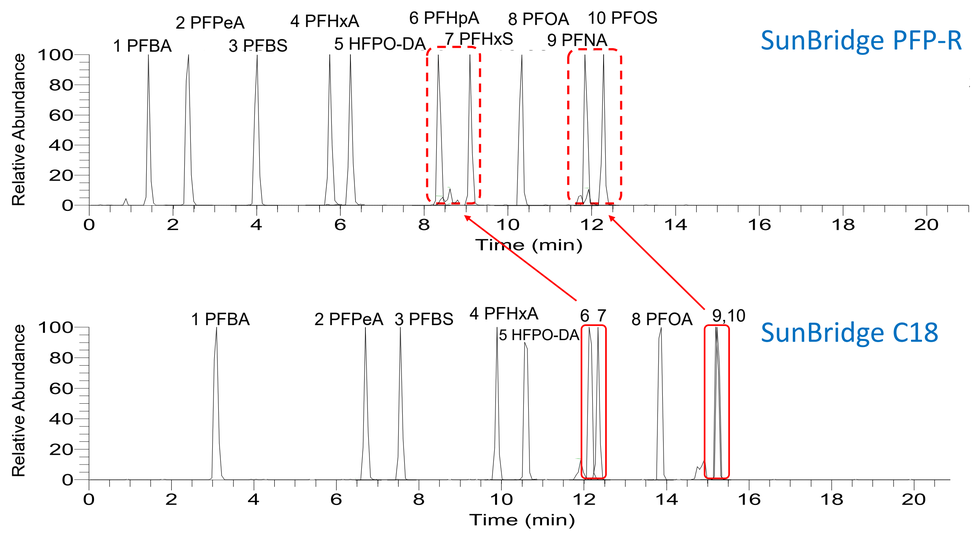
Analytes

PFOS、PFOA、PFBS、PFHxS、PFBA、PFPeA、PFHxA、PFHpA、PFNA、HFPO-DA

Comparison of isomer separation



Comparison of separation of 10 kinds of PFASs

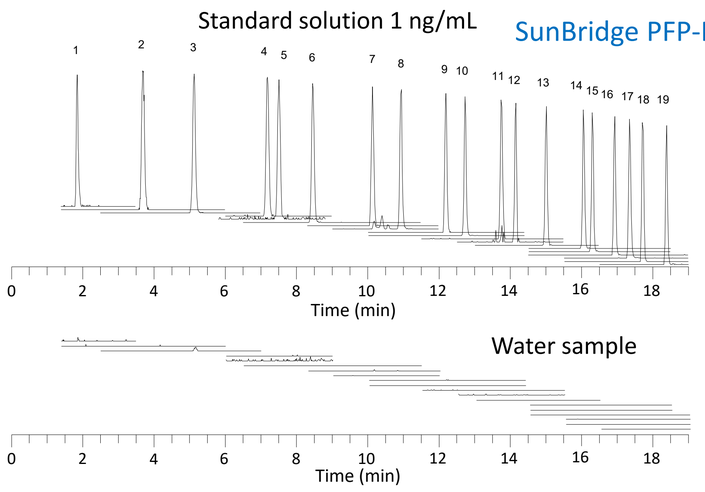


Comparison of PFBA peak intensity between PFP column and C18 column

Column	Peak intensity		
	100% MeOH	30% MeOH +70% ammonium formate	100% MeCN
SunBridge PFP-R	442,000	173,000	336,000
SunBridge C18	277,000	110,000	218,000

Isocratic separation using 100% MeOH, 100% MeCN and 30% MeOH + 70% ammonium formate.
Sample: PFBA (5 ng/mL), injection volume: 4 µL

Separation of 19 kinds of PFASs in standards and water sample



1 PFBA
2 PFPeA
3 PFBS
4 PFHxA
5 HFPO-DA
6 PFPeS
7 PFHpA
8 PFHxS
9 PFOA
10 PFHpS
11 PFNA
12 PFOS
13 PFDA
14 PFUdA
15 PFDS
16 PFDoA
17 PFTrDA
18 PFTeDA
19 FOSA

Gradient and Flow rate

Time (min)	%B	Flow rate (mL/min)
0	30	0.30
1.0	30	0.30
19.0	90	0.30
19.01	97	0.40
21.0	97	0.40
21.01	30	0.40
23.99	30	0.40
24.0	30	0.30

Other conditions are same.

Conclusions

- ✓SunBridge PFP-R exhibited not only greater sensitivity for PFBA but also superior separation of PFHxS isomers compared to SunBridge C18.
- ✓SunBridge PFP-R was able to completely separate 19 kinds of PFASs although the C18 was unable to adequately separate PFNA and PFOS.
- ✓SunBridge PFP-R is made of hybrid silica gel, so it is expected to have a longer column life than conventional PFP columns.