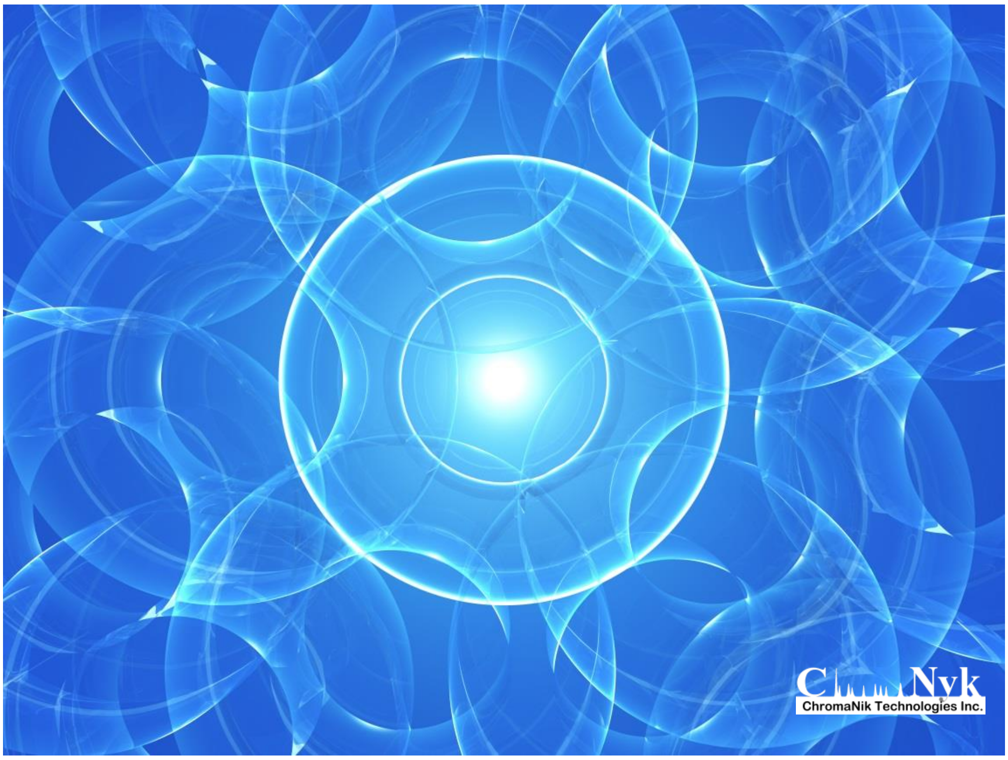
ChromaNik Technologies Inc.

C18, C18-WP, HFC18-16, HFC18-30,RP-AQUA, C8, PFP, Phenyl, C8-30, C4-30, HILIC-Amide and 2-EP

2.6 μm and 5 μm HPLC column

SunShell



Core Shell Particle

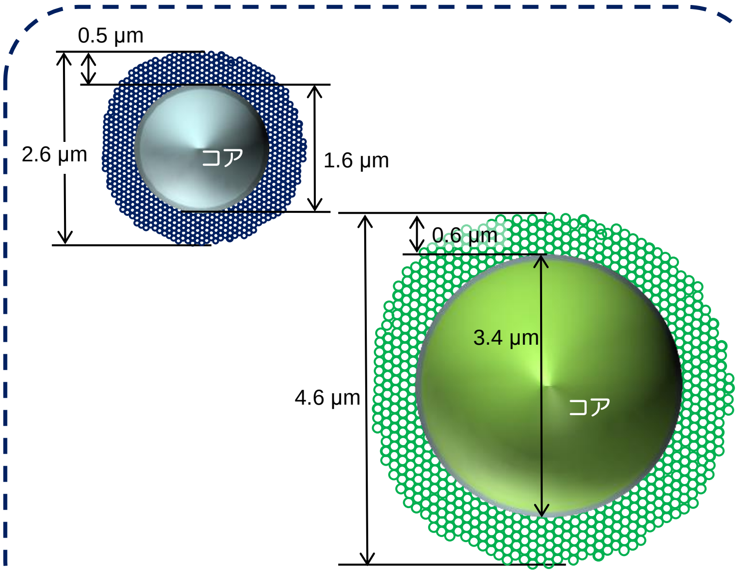
"SunShell " is a core shell silica column made by ChromaNIk Technologies.

The next generation to Core Shell particle

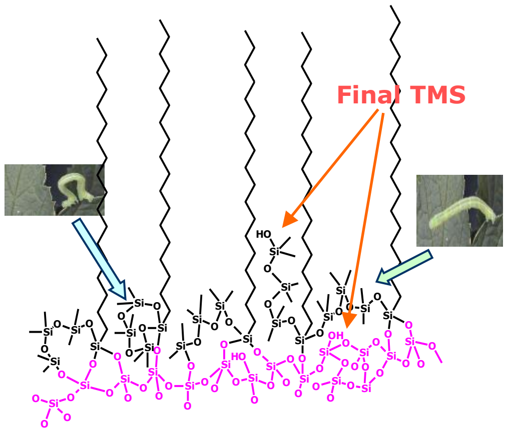
Superficially porous silica

Features of SunShell 2.6 µm and 5 µm

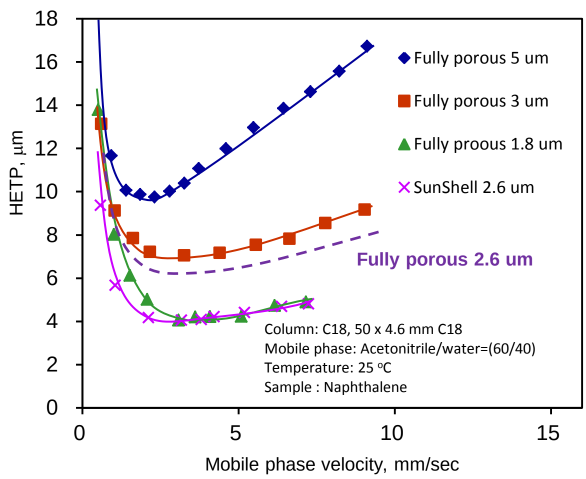
*1.6 µm and 3.4 µm of core and 0.5 µm and 0.6 µm of superficially porous silica layer
*Same efficiency and high throughput as a Sub 2 µm and 3 µm particle
*Same pressure as a 3 µm and 5 µm particle
*Same chemistry as Sunniest technology (reference figure 1)
*Good peak shape for all compounds such as basic, acidic and chelating compounds
*High stability (pH range for SunShell C18, 1.5 to 10) * Low breeding



Schematic diagram of a core shell silica particle, 2.6 µm 4.6 µm



Schematic diagram of bonding of SunShell C18

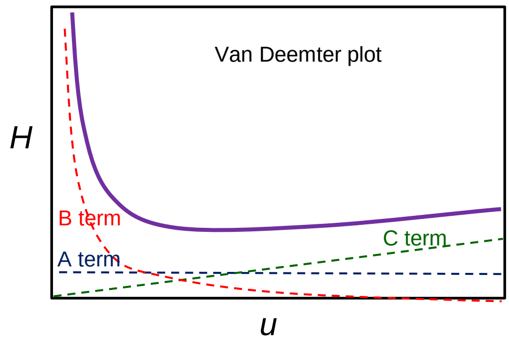


Comparison of plate height plots

Van Deemter Equation

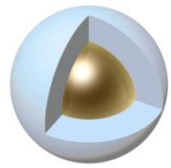
$$H = Ad_p + B\frac{D_m}{u} + C\frac{d_p^2}{D_m}u$$

A term : Eddy diffusion(dp is particle diameter)
B term : Longitudinal diffusion
(Dm is diffusion coefficient)
C term : Mass transfer



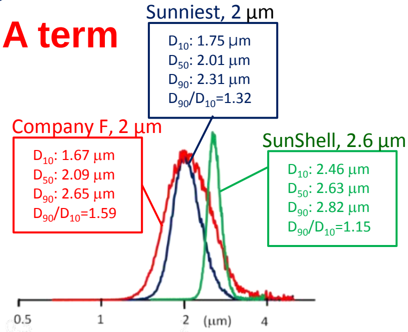
SunShell C18 shows same efficiency as a sub 2 µm C18. In comparison between fully porous 2.6 µm and core shell 2.6 µm (SunShell), SunShell shows lower values for A term, B term and C term of Van Deemter equation. The core shell structure leads higher performance to compare with the fully porous structure.

Why does a 2.6 µm core shell particle show the same performance as a sub 2 µm particle?

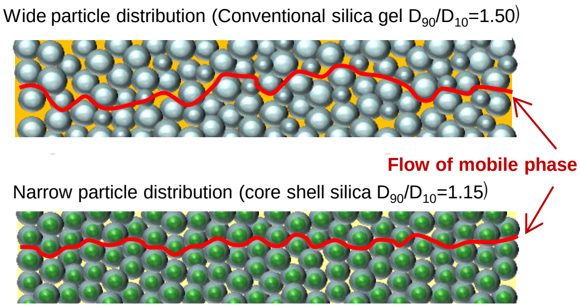


All terms in Van Deemter Equation reduce.

A term



Comparison of Particle Size Distribution

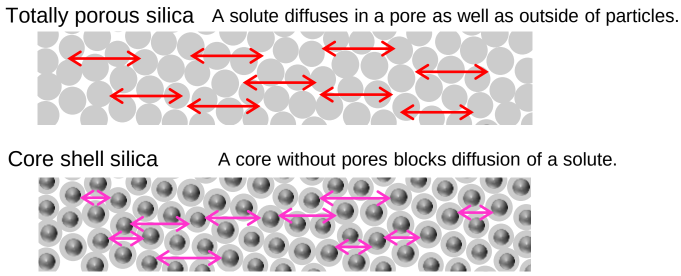


Packing state of core shell and fully porous silica

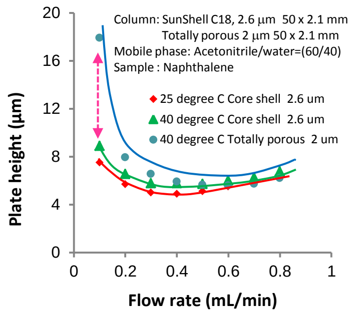
The size distribution of a core shell (SunShell) particle is much narrower than that of a conventional totally porous particle, so that the space among particles in the column reduces and efficiency increases by reducing Eddy Diffusion (multi-path diffusion) as the A term in Van Deemter Equation.

B term

Diffusion of a solute is blocked by the existence of a core, so that a solute diffuses less in a core shell silica column than in a totally porous silica column. Consequently B term in Van Deemter Equation reduces in the core shell silica column.

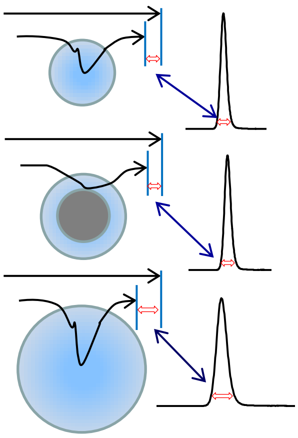


Difference of longitudinal diffusion



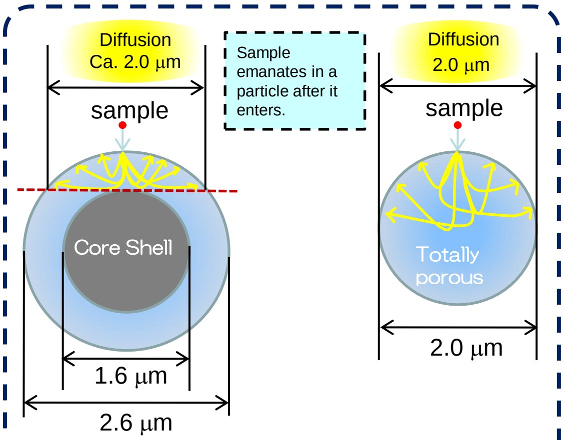
Plot of Flow rate and Plates height

C term



Comparison of diffusion path

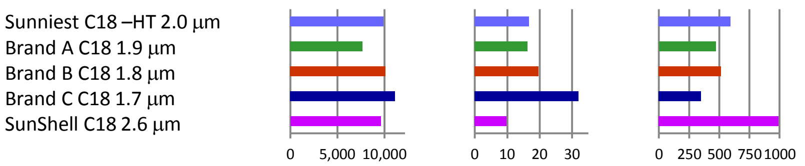
As shown in the left figure, a core shell particle has a core so that the diffusion path of samples shortens and mass transfer becomes fast. This means that the C term in Van Deemter Equation reduces. In other words, HETP (theoretical plate) is kept even if flow rate increases. A 2.6 µm core shell particle shows as same column efficiency as a totally porous sub-2 µm particle.
The right figure shows that a diffusion width of a sample in a 2.6 µm core shell particle and a 2 µm totally porous particle. Both diffusion widths are almost same. The 2.6 µm core shell particle is superficially porous, so that the diffusion width becomes narrower than particle size. Same diffusion means same efficiency.



Diffusion of sample in core shell and totally porous silica

Comparison of Performance by Plate/Pressure

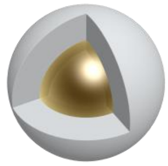
	Plate	Back press. (MPa)	Plate/back press.
Sunniest C18 –HT 2.0 µm	9,900	16.7	593
Brand A C18 1.9 µm	7,660	16.3	470
Brand B C18 1.8 µm	10,100	19.6	515
Brand C C18 1.7 µm	11,140	32.0	348
SunShell C18 2.6 µm	9,600	9.7	990



Column: 50 x 2.1 mm C18, Mobile phase: Acetonitrile/water=(70/30), Temperature: 25 °C

Under a constant back pressure condition, SunShell C18 showed more than 2 times higher performance to compare with totally sub-2µm porous C18s.

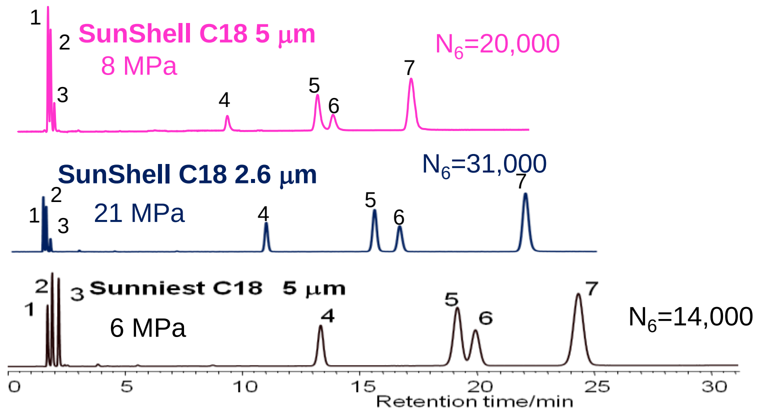
SunShell C18, 2.6 µm, 5 µm



Characteristics of SunShell C18

	Core shell silica			C18 (USP L1)				
	Particle size	Pore diameter	Specific surface area	Carbon content	Bonded phase	End-capping	Maximum operating pressure	Available pH range
SunShell C18	2.6 µm	9	150	7	C18	Sunniest end-capping	60 MPa or 8,570 psi	1.5 - 10
SunShell C18	4.6 µm	9	90	5.5	C18	Sunniest end-capping	60 MPa or 8,570 psi	1.5 - 10

Comparison of retention and plate using HPLC



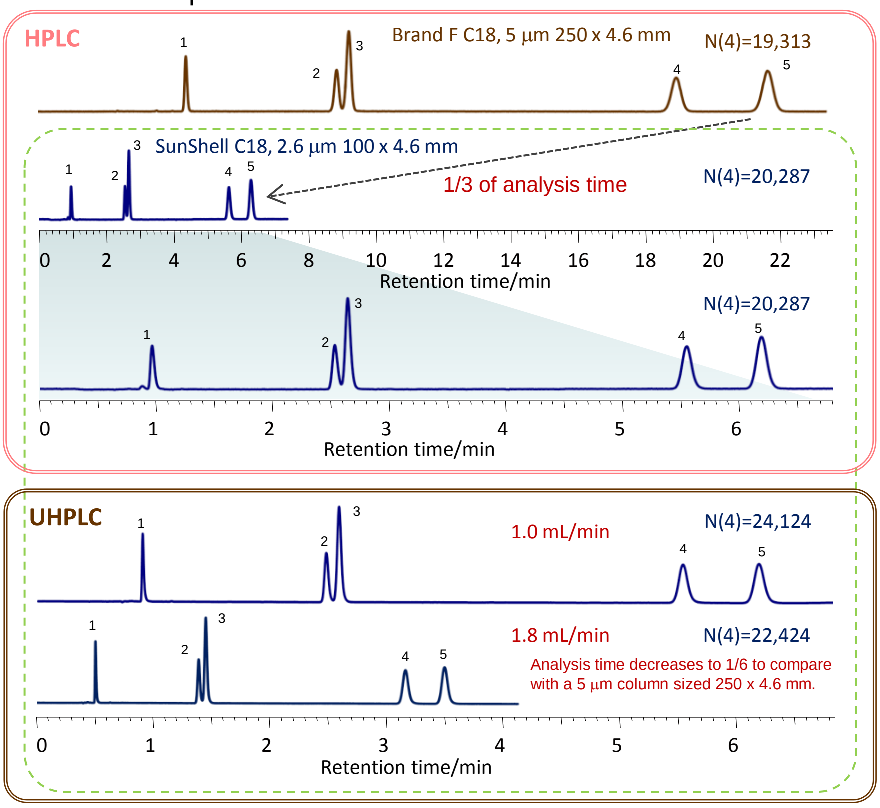
Column size: 150 x 4.6 mm
Mobile phase: CH_3OH/H_2O=75/25
Flow rate: 1.0 mL/min
Temperature: 40 ºC
Sample: 1 = Uracil
2 = Caffeine
3 = Phenol
4 = Butylbenzene
5 = o-Terphenyl
6 = Amylbenzene
7 = Triphenylene
HPLC: Hitachi LaChrom ELITE
(Tubing, 0.25 mm i.d.)

	Totally porous silica Sunniest C18, 5 µm		Core shell silica SunShell C18, 2.6 µm		Core shell silica SunShell C18, 5 µm	
Specific surface area	340 m²/g		150 m²/g		90 m²/g	
Packings weight (150x4.6mm)	1.5 g		2.7 g		3.2 g	
Surface area in a column	510 m²/g (100%)		405 m²/g (79%)		288 m²/g (56%)	
	Retention time (t_R)	Retention factor (k)	Retention time (tR)	Retention factor (k)	Retention time (t_R)	Retention factor (k)
1) Uracil	1.70	0	1.34	0	1.30	0
6) Amylbenzene	19.96	10.74	16.56	11.36	13.43	9.33
Relative value of Amylbenzene	100%	100%	83%	106%	67%	87%

There is a little difference of k between totally porous and core shell particles.

Examples of transfer from a conventional 5 µm column to SunShell column

Isocratic separation



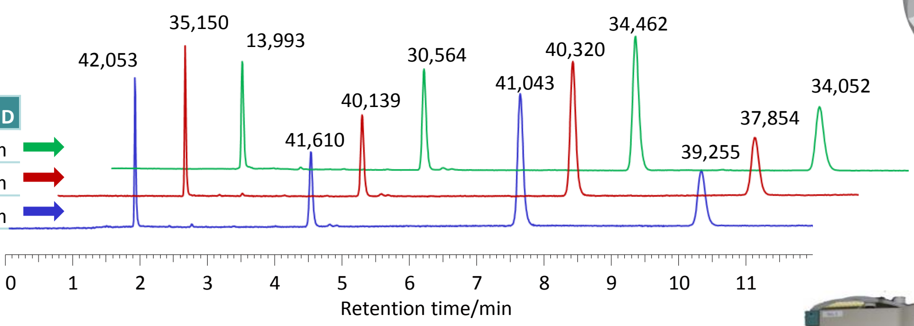
Column:
Brand F C18, 5 µm 250 x 4.6 mm
SunShell C18, 2.6 µm 100 x 4.6 mm
Mobile phase:
CH_3CN/20mM Phosphoric acid = 45/55
Flow rate: 1.0 mL/min,
1.8 mL/min at the lowest chromatogram
Temperature: 25 ºC
Pressure: 9.5 MPa for Brand F C18 5 µm
13.4 MPa for SunShell C18 2.6 µm
Detection: UV@230 nm

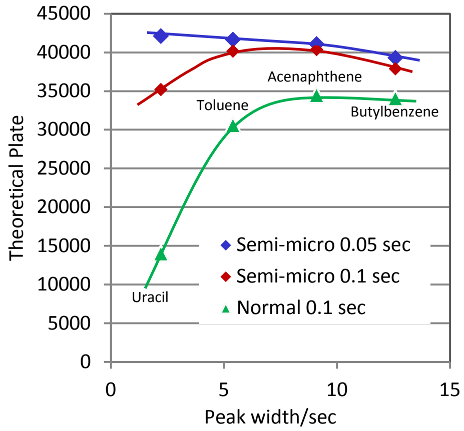
Sample: 1 = Benzydamine
2 = Ketoprofen
3 = Naproxen
4 = Indomethacin
5 = Ibuprofen

HPLC: Hitachi LaChrom ELITE (Tubing, 0.25 mm i.d.)
UHPLC: Jasco X-LC

Comparison between normal and semi-micro HPLC

Flow cell	Response	Sampling	Tubing ID
Normal	0.1 sec	0.4 sec	0.25 mm
Semi-micro	0.1 sec	0.4 sec	0.13 mm
Semi-micro	0.05 sec	0.05 sec	0.13 mm





Relationship between Peak width and theoretical plate

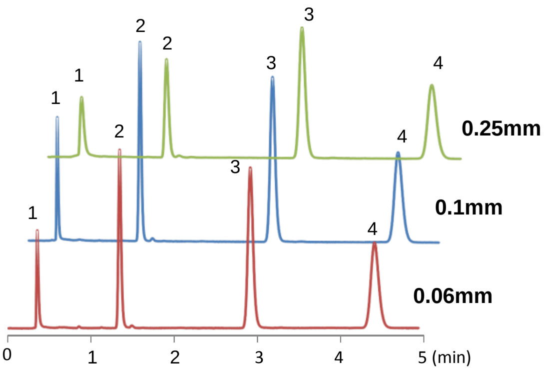
Comparison of chromatograms

Column:
SunShell C18, 5 µm 250 x 4.6 mm
Mobile phase:
CH_3CN/H_2O= 70/30
Flow rate: 1.0 mL/min
Temperature: 40 °C
Pressure: 6.7 MPa
Detection: UV@250 nm
Sample: 1 = Uracil
2 = Toluene
3 = Acenaphthene
4 = Butylbenzene

HPLC: Hitachi LaChrom ELITE

Semi-micro HPLC derives near 100% performance of a core shell column. Even if normal HPLC is used, it derives 80% performance except for a narrow peak whose width is less than 5 second

Effect of inner diameter of tubing



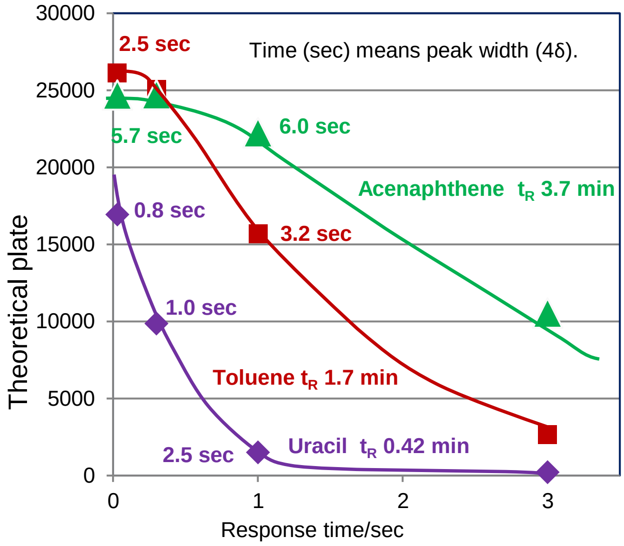
Average of theoretical plate (n=3)

Inner diameter of tubing	0.06mm	0.1mm	0.25mm
Peak (1)	792	785	246
Peak (2)	7790	7652	3535
Peak (3)	10704	10345	7998
Peak (4)	10113	9772	7689

Column: SunShell C18, 2.6 µm 50 x 2.1 mm
Mobile phase: CH_3CN/H_2O=60/40
Flow rate: 0.3 mL/min Temperature: Ambient
Tube length: 30 cm (Peek, from the column to the flow cell)
Instrument: X-LC(JASCO) Response time: 0.01 sec

The above theoretical plate was compared changing the inner diameter of tubing between a column and a flow cell of the detector. A tubing with a large inner diameter has a large dead volume, so that it makes the peak width be wide. As a result, theoretical plate decreases. I recommend to use the tubing with 0.1 mm or less than 0.1 mm inner diameter for core shell columns.

Effect of response time of detector



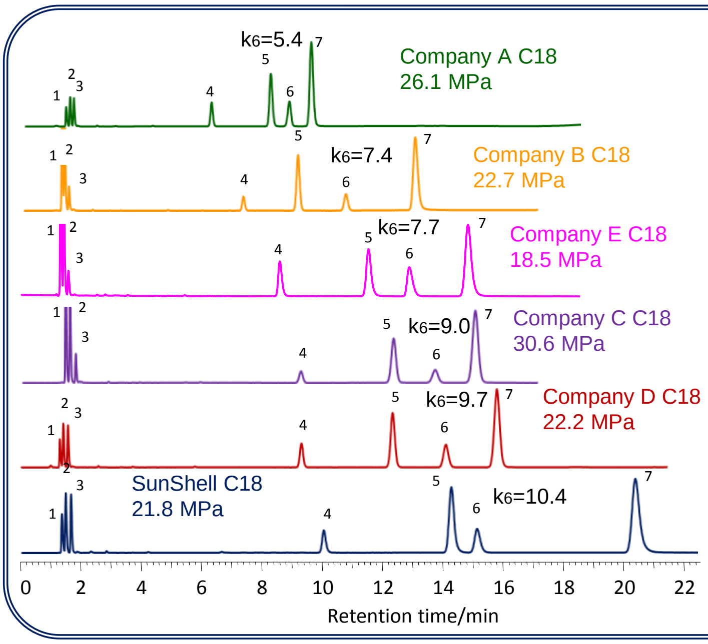
Column: SunShell C18, 2.6 µm 100 x 4.6 mm
Mobile phase: CH_3CN/H_2O=60/40
Flow rate: 1.8 mL/min Temperature: Ambient
Sample: Toluene Tube: i.d.0.1㎜ x 20 cm Peeksil
Instrument: X-LC(JASCO)

The response time of a detector is important. Regarding uracil, the real peak width is less than 0.8 sec. When the peak width is less than 1 sec, 0.03 sec of response time is needed. Furthermore, the sampling rate of an integrator should be set to be 0.1 sec.

Comparison of core shell columns

Used columns
1. Kinetex C18, 2.6 µm
2. Accucore C18, 2.6 µm
3. PoroShell C18 EC, 2.7 µm
4. Ascentis Express C18, 2.7 µm
5. Cortecs C18, 2.7 µm
6. SunShell C18, 2.6 µm

Comparison of standard samples between core shell C18s

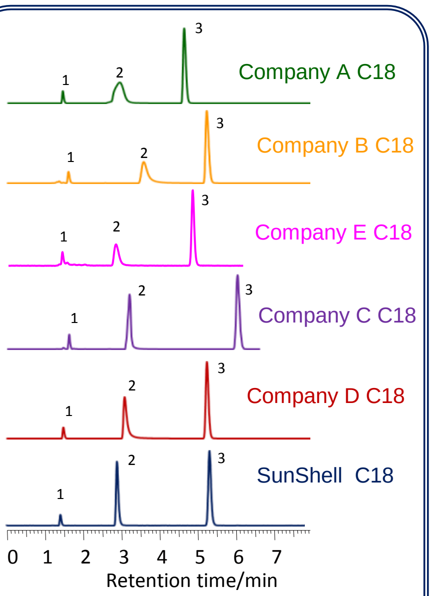


Column: Company A C18, 2.6 µm 150 x 4.6 mm (26.1 Mpa, 30,800 plate)
Company B C18, 2.6 µm 150 x 4.6 mm (22.7 MPa , 31,600 plate)
Company W C18, 2.7 µm 150 x 4.6 mm (18.5 MPa , 23,300 plate)
Company C C18, 2.7 µm 150 x 4.6 mm (30.6 MPa , 30,200 plate)
Company D C18, 2.7 µm 150 x 4.6 mm (22.2 MPa , 31,800 plate)
SunShell C18, 2.6 µm 150 x 4.6 mm (21.8 MPa , 31,900 plate)
Mobile phase: CH_3OH/H_2O=75/25
Flow rate: 1.0 mL/min, Temperature: 40 ºC
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)
Company A C18	0.48	1.54	1.20
Company B C18	0.35	1.56	1.50
Company E C18	0.38	1.59	1.32
Company C C18	0.42	1.57	1.25
Company D C18	0.44	1.60	1.31
SunShell C18	0.39	1.60	1.46

Retention of standard samples and back pressure were compared for five kinds of core shell type C18s. Company A C18 showed only a half retention to compare with SunShell C18. Steric selectivity becomes large when ligand density on the surface is high. SunShell C18 has the largest steric selectivity so that it has the highest ligand density. This leads the longest retention time.

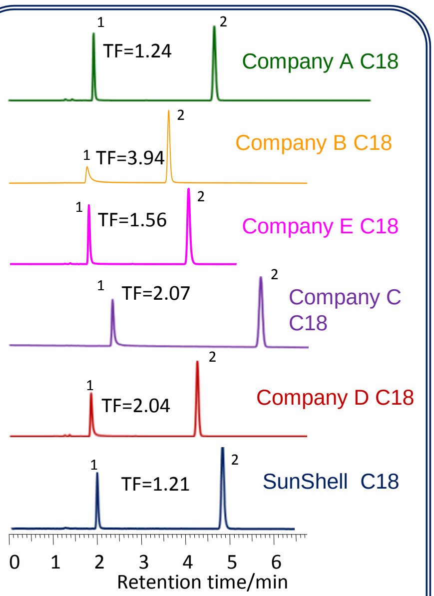
Comparison of pyridine



Column dimension: 150 x 4.6 mm
Mobile phase: CH_3OH/H_2O=30/70
Flow rate: 1.0 mL/min
Temperature: 40 ºC
Detection: UV@250nm
Sample: 1 = Uracil
2 = Pyridine
3 = Phenol

Residual silanol groups make pyridine be tailing under methanol/water mobile phase condition. SunShell C18 shows a sharp peak for pyridine.

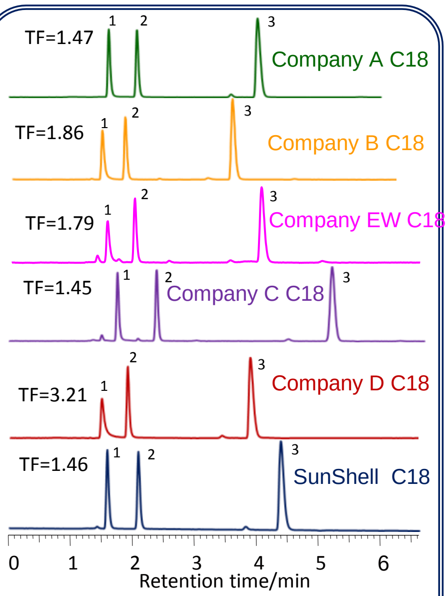
Comparison of Oxine



Column dimension: 150 x 4.6 mm
Mobile phase: CH_3CN/20mM H_3PO_4=10/90
Flow rate: 1.0 mL/min
Temperature: 40 ºC
Detection: UV@250nm
Sample: 1 = 8-Quinolinol (Oxine)
2 = Caffeine

8-Quinolinol (Oxine) is a metal chelating compound. Metal impurities in the core shell particle leads the tailing for oxine peak.

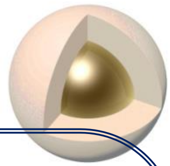
Comparison of formic acid



Column dimension: 150 x 4.6 mm
Mobile phase: CH_3CN/0.1% H_3PO_4=2/98
Flow rate: 1.0 mL/min
Temperature: 40 ºC
Detection: UV@210nm
Sample: 1 = Formic acid
2 = Acetic acid
3 = Propionic Acid

Formic acid is used as an indicator for a acidic inertness. SunShell and Company A and C C18 show a sharp peak.

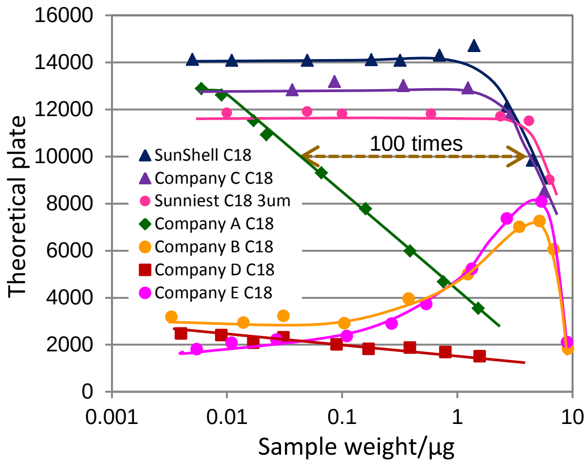
Loading capacity of amitriptyline as a basic compound



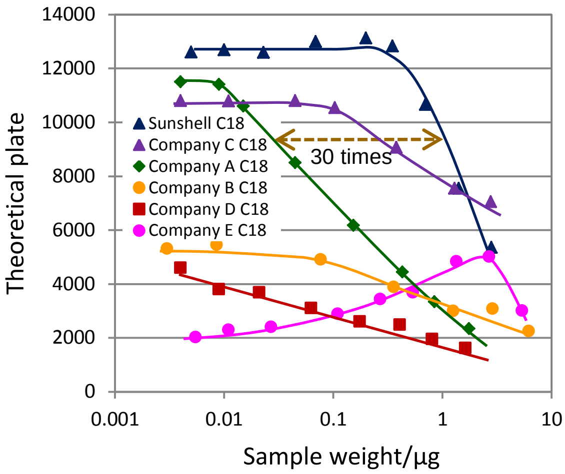
Amitriptyline overlords much more at acetonitrile/buffer mobile phase than methanol/buffer. Three kinds of core shell C18s were compared loading capacity of amitriptyline at three different mobile phases.

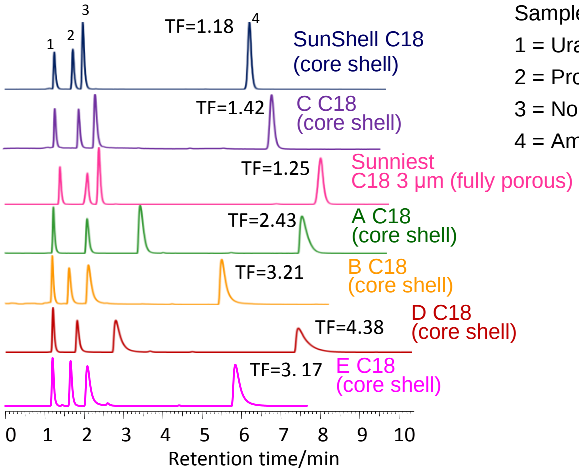
Common condition: Column dimension,150 x 4.6 mm, flow rate; 1.0 mL/min, temperature; 40 °C

Mobile phase: Acetonitrile/**20mM phosphate buffer pH7.0**=(60:40)

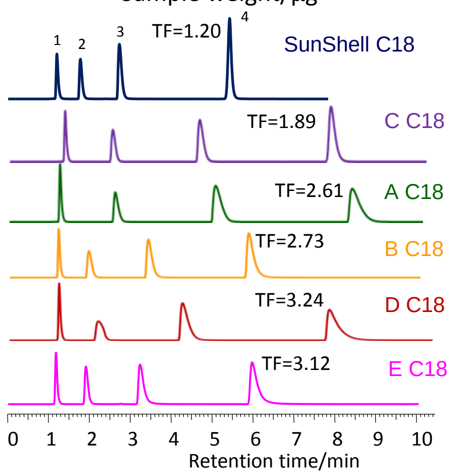


Mobile phase: Acetonitrile/**10mM acetate ammonium pH6.8**=(40:60)

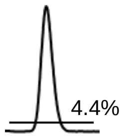




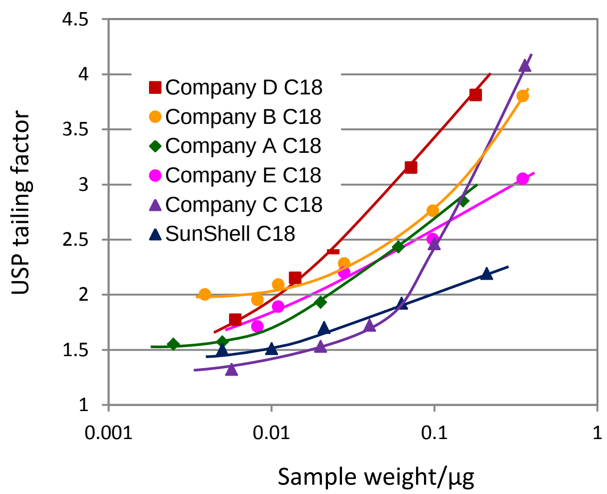
Sample:
1 = Uracil (0.07µg)
2 = Propranolol (1.53µg)
3 = Nortriptyline (0.32µg)
4 = Amitriptyline (0.32µg)

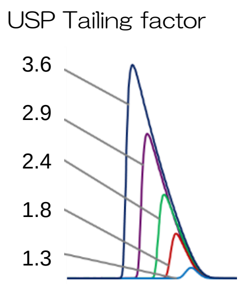


Theoretical plate was calculated by 5σ method using peak width at 4.4% of peak height.



Mobile phase: Acetonitrile/**0.1% formic acid**=(30:70)



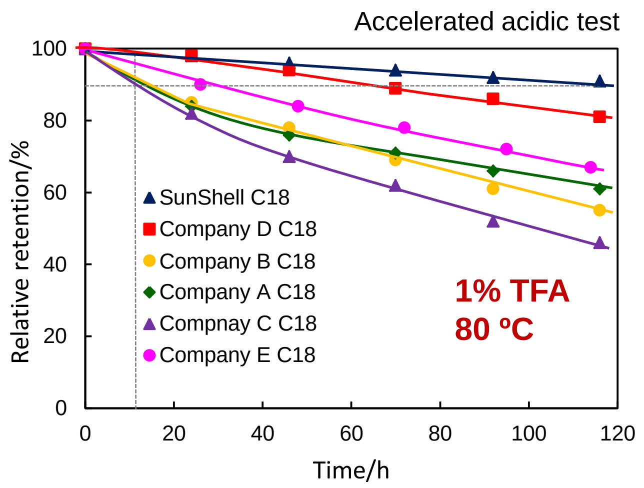


Amitriptyline overloads at low weight when acetonitrile/0.1% formic acid mobile phase. A peak is shifted forward under overloading.

Comparison column
1. Kinetex C18, 2.6 µm
2. Accucore C18, 2.6 µm
3. PoroShell C18 EC, 2.7 µm
4. Ascentis Express C18, 2.7 µm
5. Cortecs C18 2.7 µm
6. SunShell C18, 2.6 µm

All columns are core shell type. All columns sized 150 x 4.6 mm except for company E show 38,000 to 40,000 plates for a neutral compound. However regarding a basic compound like amitriptyline, SunShell C18 and company C C18 showed a good peak, while Company A, B and D C18 showed a poor peak. Company A C18 overloaded at more than 0.01 µg of amitriptyline while SunShell C18 overloaded at more than from 0.3 to 1 µg of amitriptyline. Surprisingly loading capacity of company A C18 was only one hundredth to compare with SunShell C18 under acetonitrile/20mM phosphate buffer pH7.0=(60:40) mobile phase. Company D C18 always showed poor peak of amitriptyline.

◆Evaluation of Stability



Durable test condition

Column size: 50 x 2.1 mm
Mobile phase: CH_3CN/1.0% TFA, pH1=10/90
Flow rate: 0.4 mL/min
Temperature: 80 ºC

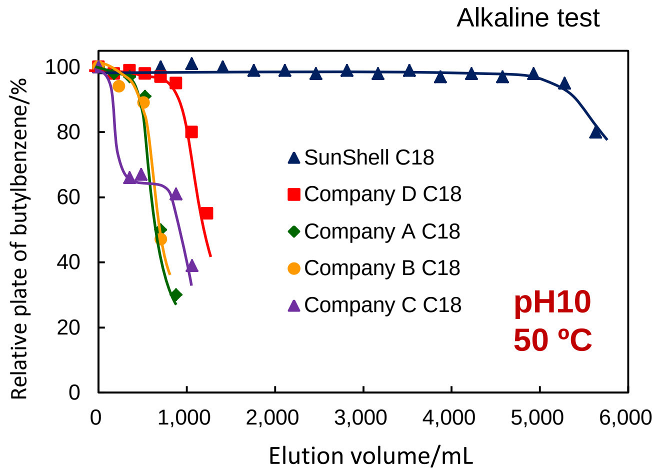
Measurement condition

Column size: 50 x 2.1 mm
Mobile phase: CH_3CN/H_2O=60/40
Flow rate: 0.4 mL/min
Temperature: 40 ºC
Sample: 1 = Uracil (t_0)
2 = Butylbenzene

Stability under acidic pH condition was evaluated at 80 ºC using acetonitrile/1% trifluoroacetic acid solution (10:90) as mobile phase. 100% aqueous mobile phase expels from the pore of packing materials by capillarity and packing materials doesn't deteriorate. 10% acetonitrile in a mobile phase allows an accurate evaluation.[1-3)]

★Sunshell C18 has kept 90% retention for 100 hours under such a severe condition. SunShell C18 is 5 to 10 times more stable than the other core shell C18.

1) N. Nagae, T. Enami and S. Doshi, LC/GC North America October 2002.
2) T. Enami and N. Nagae, American Laboratory October 2004.
3) T. Enami and N. Nagae, BUNSEKI KAGAKU, 53 (2004) 1309.



Durable test condition

Column Size: 50 x 2.1 mm
Mobile phase:
CH_3OH/20mM Sodium borate/10mM NaOH=30/21/49 (pH10)
Flow rate: 0.4 mL/min
Temperature: 50 ºC

Measurement condition

Column Size: 50 x 2.1 mm
Mobile phase: CH_3CN/H_2O=60/40
Flow rate: 0.4 mL/min
Temperature: 40 ºC
Sample: 1 = Butylbenzene

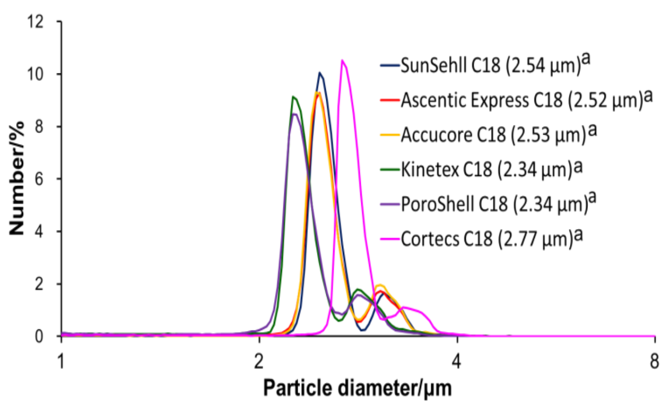
Stability under basic pH condition was evaluated at 50 ºC using methanol/Sodium borate buffer pH 10 (30:70) as mobile phase. Sodium borate is used as a alkaline standard solution for pH meter, so that its buffer capacity is high.

Elevated temperature of 10 ºC makes column life be one third. The other company shows stability test at ambient (room temperature). If room temperature is 25 ºC, column life at room temperature (25 ºC) is sixteen times longer than that at 50 ºC.

★ SunShell C18 is enough stable even if it is used under pH 10 condition. Regarding stability under basic pH condition, there is little C18 column like SunShell C18 except for hybrid type C18. It is considered that our end-capping technique leads high stability.

★ SunShell C18 can be used at the pH range from 1.5 to 10.

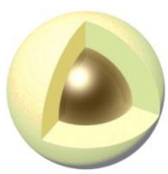
◆Comparison of particle size



*Measured using Beckman Coulter Multisizer 3 after C18 materials were sintered at 600 degree Celsius for 8 hours. The measured value of each sintered core shell silica is considered to be different from that of the original core shell silica.

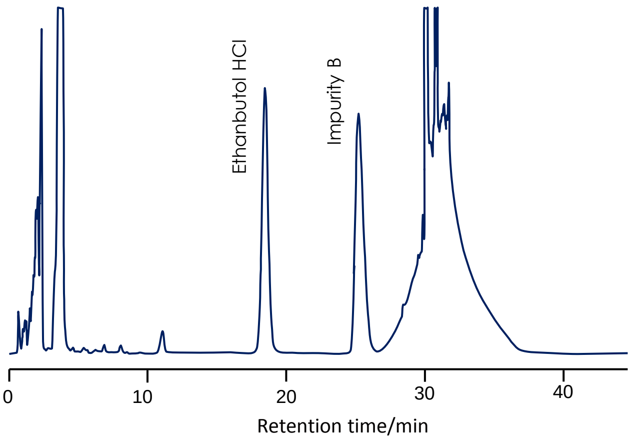
a. Median particle size

Comparison column
1. Kinetex C18, 2.6 µm (pH 1.5 to 10)
2. Accucore C18, 2.6 µm (pH 1 to 11)
3. PoroShell C18 EC, 2.7 µm (pH 2 to 9)
4. Ascentis Express C18, 2.7 µm (pH 2 to 9)
5. Cortecs C18 2.7 µm (pH 2 to 8)
6. SunShell C18, 2.6 µm (pH 1.5 to 10)



SunShell

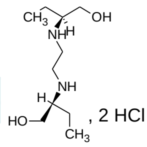
Ethambutol Hydrochloride



Column: SunShell C18, 2.6 µm 100 x 4.6 mm
Mobile phase: A) Methanol/water (50/50 V/V)
B) Methanol

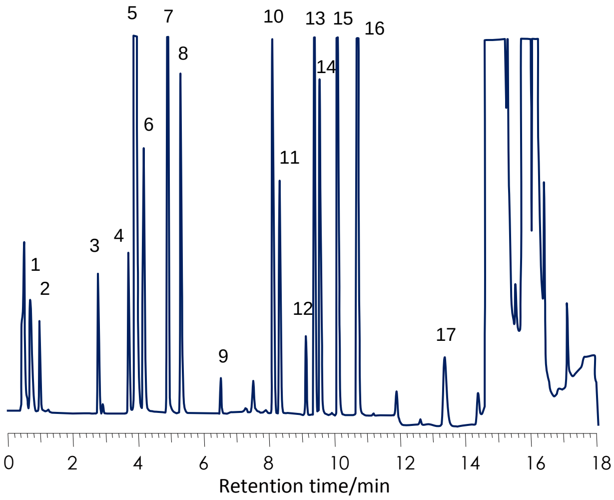
Time (min)	0	30	35	37	38
%B	29	29	100	100	29

Flow rate: 1.0 mL/min
Temperature: 40 ºC
Detection: UV 215 nm
Injection volume: 10 µL



Ethambutol Hydrochloride

Amino Acids derivatized with OPA and FMOC

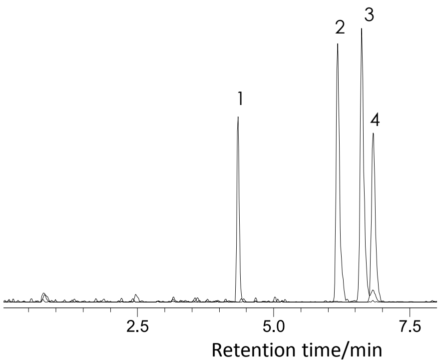


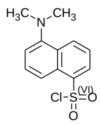
Column: SunShell C18 2.6 µm, 150 x 2.1 mm
Mobile phase: A) 10mM Na_2PO_4 + 10mM $Na_2B_4O_7$ + 0.5mM NaN_3 (pH7.8)
B) Acetonitrile/Methanol/Water (45/45/10 %V)

Time (min)	0	0.4	12.8	13.8
%B	5	5	50	100

Flow rate: 0.61 mL/min
Temperature: 40 ºC
Detection: UV@338 nm
Sample: 1=Aspartic acid, 2=Glutaminc acid, 3=Serine, 4=Histidine, 5=Glycine, 6=Threonine, 7=Arginine, 8=Alanine, 9=Tyrosine, 10=Valine, 11=Methionine, 12=Tryptophan, 13=Pnehylalanine, 14=Isoleucine, 15=Leucine, 16=Lysine, 17=Proline

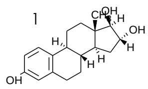
Dansylated estrogen hormones





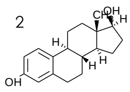
Dansyl chloride

1



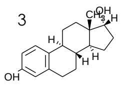
Estriol,

2



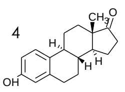
17Beta-estradiol,

3



17Alpha-estradiol

4



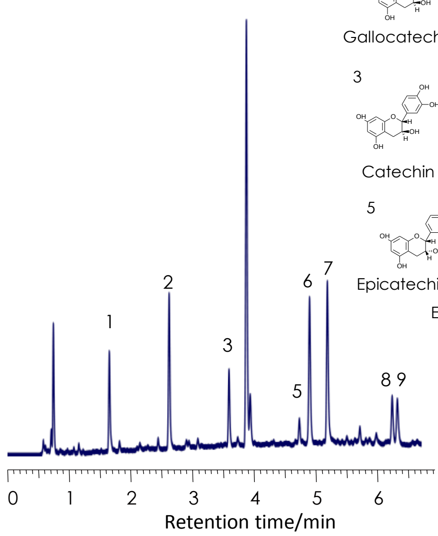
Estrone

Column: SunShell C18 2.6 µm, 100 x 2.1 mm
Mobile phase:
A) H_2O with 0.1% formic acid.
B) CH_3CN with 0.1% formic acid.
Gradient program:
0 - 0.5 min: 10% B
0.51 – 3.0 min: 10 - 72% B
3.01 - 6.0 min: 72% B
6.01 - 7.0 min: 72 - 100% B
7.01 - 10.0 min: 100% B

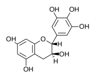
Flow rate: 0.45 mL/min.
Temperature: 40 ºC
Detection: MS(sim), m/z, 522.20, 506.20, 504.20
Samples: 1. Dansylated estriol, 2. Dansylated 17beta-estradiol,
3. Dansylated 17alpha-estradiol , 4. Dansylated estrone

Courtesy of Department of Chemistry & Biochemistry, The University of Texas at Arlington

Oolong tea

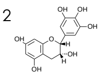


1



Gallocatechin

2

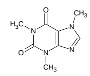


Epigallocatechin

3

Catechin

4

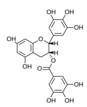


Caffeine

5

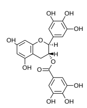
Epicatechin

6



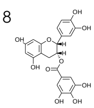
Epigallocatechin gallate l

7



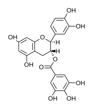
Gallocatechin gallate

8



Epicatechin gallate

9



Catechin gallate

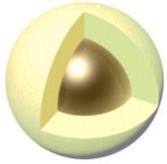
Column: SunShell C18 2.6 µm, 75 x 4.6 mm
Mobile phase:
A) 0.1% Phosphoric acid
B) CH_3CN
Gradient program

Time	0 min	7.5 min	10 min
%B	2%	25%	25%

Flow rate: 1.0 mL/min,
Temperature: 25 ºC
Detection: UV@250 nm
Sample: Oolong tea

SunShell C18-WP, RP-AQUA, C8, Phenyl, PFP, 2.6 µm

(Pentafluoropheny)

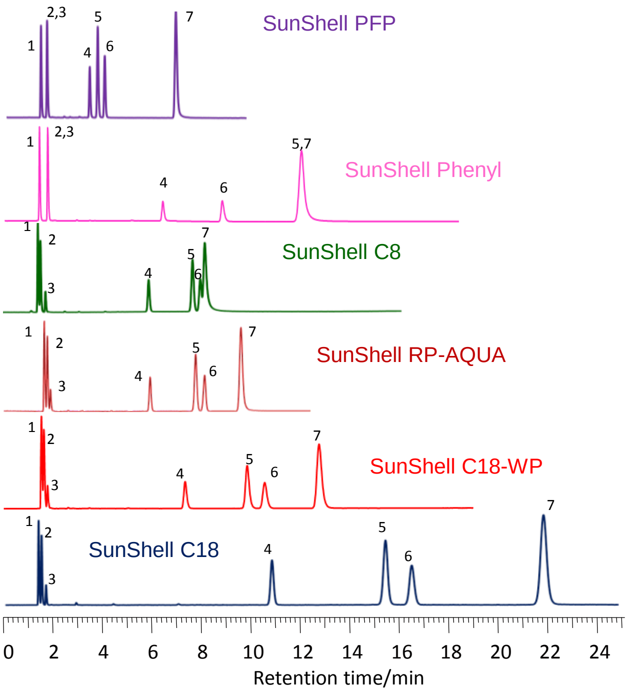


◆ Characteristics of SunShell

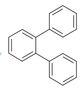
	Core shell silica			Bonding phase					
	Particle size	Pore diameter	Specific surface area	Carbon content	Bonded phase	USP L line	End-capping	Maximum operating pressure	Available pH range
SunShell C18	2.6 µm	9nm	150 m²/g	7%	C18	L1	Sunniest endcapping	60 MPa	1.5 - 10
SunShell C18-WP	2.6 µm	16 nm	90 m²/g	5%	C18	L1	Sunniest endcapping	60 MPa	1.5 - 10
SunShell RP-AQUA	2.6 µm	16 nm	90 m²/g	4%	C28	Equivalent to L62	Sunniest endcapping	60 MPa	2 - 8[a)]
SunShell C8	2.6 µm	9nm	150 m²/g	4.5%	C8	L7	Sunniest endcapping	60 MPa	1.5 - 9
SunShell Phenyl	2.6 µm	9nm	150 m²/g	5%	Phenylhexyl	L11	Sunniest endcapping	60 MPa	1.5 - 9
SunShell PFP	2.6 µm	9nm	150 m²/g	4.5%	Pentafluorophenyl	L43	TMS endcapping	60 MPa	2 - 8

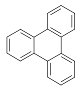
a) Under 100% aqueous condition

◆ Comparison of standard samples



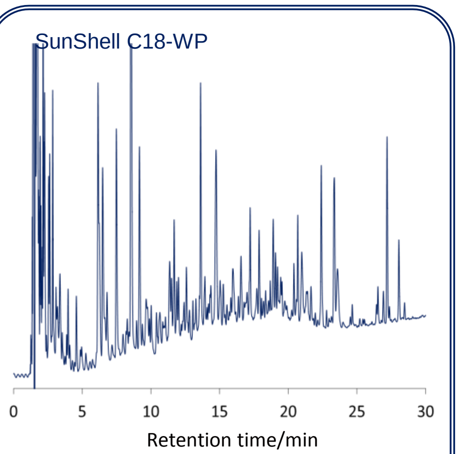
Column: SunShell C18, C18-WP, RP-AQUA, C8, Phenyl, PFP, 2.6 µm 150 x 4.6 mm
Mobile phase: CH_3OH/H_2O=75/25
Flow rate: 1.0 mL/min
Temperature: 40 ºC
Sample: 1 = Uracil
2 = Caffeine
3 = Phenol
4 = Butylbenzene
5 = o-Terphenyl
6 = Amylbenzene
7 = Triphenylene





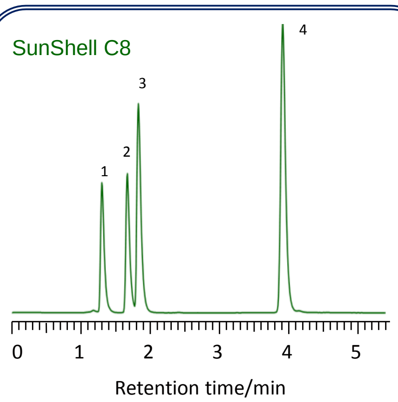
	Hydrogen bonding (Caffeine/Phenol)	Hydrophobicity (Amylbenzene/Butylbenzene)	Steric selectivity (Triphenylene/o-Terphenyl)
PFP	1.00	1.31	2.38
Phenyl	1.00	1.48	1.01
C8	0.32	1.46	1.08
RP-AQUA	0.52	1.52	1.30
C18-WP	0.40	1.55	1.35
SunShell C18	0.39	1.60	1.46

Separation of peptides



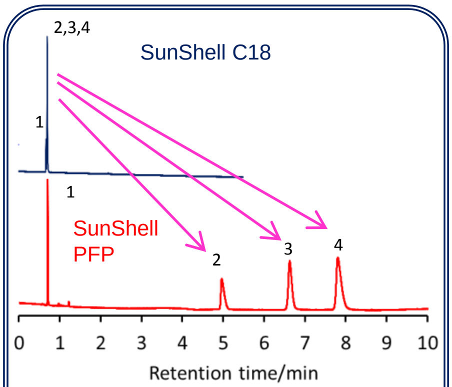
Column: SunShell C18-WP, 2.6 µm (16 nm) 150 x 4.6 mm,
Mobile phase: A) 0.1% TFA in Acetonitrile/water(10:90)
B) 0.1 % TFA in Acetonitrile
Gradient program: %B 5% – 50% in 50 min
Flow rate: 1.0 mL/min , Temperature: 25 ºC,
Detection: UV@210 nm,
Sample: Tryptic digest of BSA

Separation of amitriptyline using C8



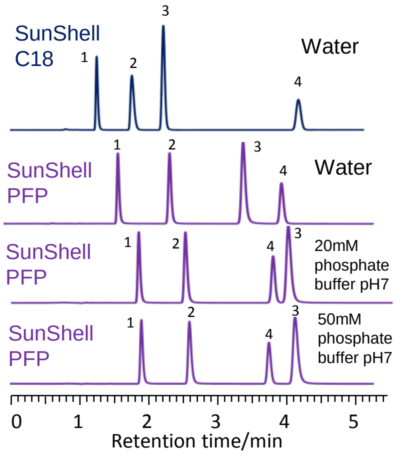
Column: SunShell C8, 2.6 µm 150 x 4.6 mm
Mobile phase:
CH_3CN/20mM phosphate buffer H7.0=60/40
Flow rate: 1.0 mL/min
Temperature: 40 ºC
Detection: UV@250nm
Sample: 1 = Uracil, 2 = Propranolol,
3 = Nortriptyline, 4 = Amitriptyline

Separation of basic compounds



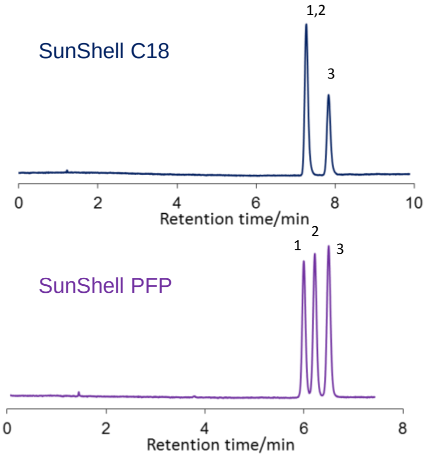
Column: SunShell C18, 2.6 µm 150 x 4.6 mm
SunShell PFP, 2.6 µm 150 x 4.6 mm
Mobile phase:
CH_3CN/10mM phosphate buffer pH7.0=80/20
Flow rate: 1.8 mL/min
Temperature: 25 ºC
Sample: 1 = Uracil, 2 = Propranolol,
3 = Nortriptyline, 4 =Amitriptyline

Separation of xanthines



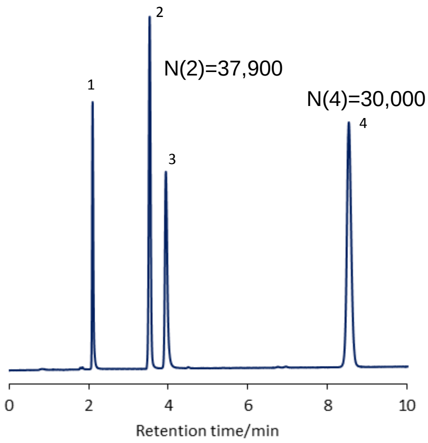
Column: SunShell C18, PFP, 2.6 µm 150 x 2.1 mm
Mobile phase: CH_3OH/water or buffer=30/70
Flow rate: 0.3 mL/min
Temperature: 25 ºC
Detection: UV@250nm
Sample: 1 = Theobromine
2 = Theophyline
3 = Caffeine
4 = Phenol

Separation of cresol isomers



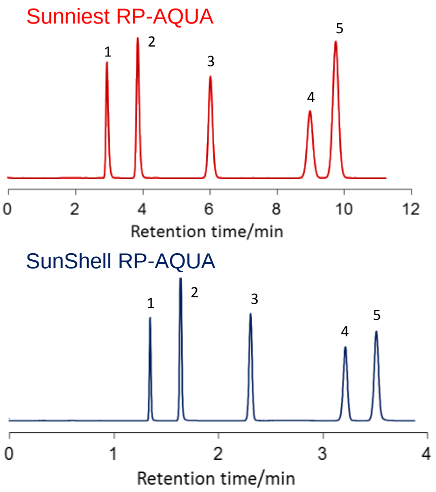
Column: SunShell C18, PFP, 2.6 µm 150 x 4.6 mm
Mobile phase: CH_3OH/H_2O=40/60
Flow rate: 1.0 mL/min
Temperature: 25 ºC
Detection: UV@250nm
Sample: 1 = p-Cresol
2 = m-Cresol
3 = o-Cresol

Separation of nucleotides



Column:
SunShell RP-AQUA, 2.6 µm 150 x 4.6 mm
Mobile phase: 20mM Phosphate buffer pH6.0
Flow rate: 1.0 mL/min
Temperature: 25 ºC
Detection: UV@250nm
Sample: 1 = 5'-GDP
2 = 5'-ATP
3 = 5'-ADP
4 = 5'-AMP

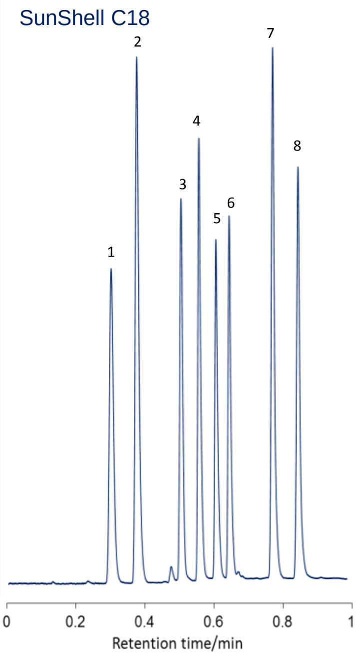
Separation of nucleic acid bases



Column:
Sunniest RP-AQUA, 5 µm 150 x 4.6 mm
SunShell RP-AQUA, 2.6 µm 150 x 4.6 mm
Mobile phase:
10mM Phosphate buffer pH7.0
Flow rate: 1.0 mL/min for Sunniest
1.5 ml/min for SunShell
Temperature: 24 ºC
Sample: 1 = Cytosine, 2 = Uracil, 3 = Thymidine,
4 = Uridine, 5 = Thymine

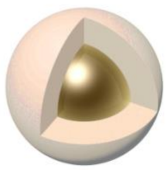
	Plate(5)	Resolution (4,5)
Sunniest	14,000	1.98
SunShell	30,000	3.79

High-throughput separation

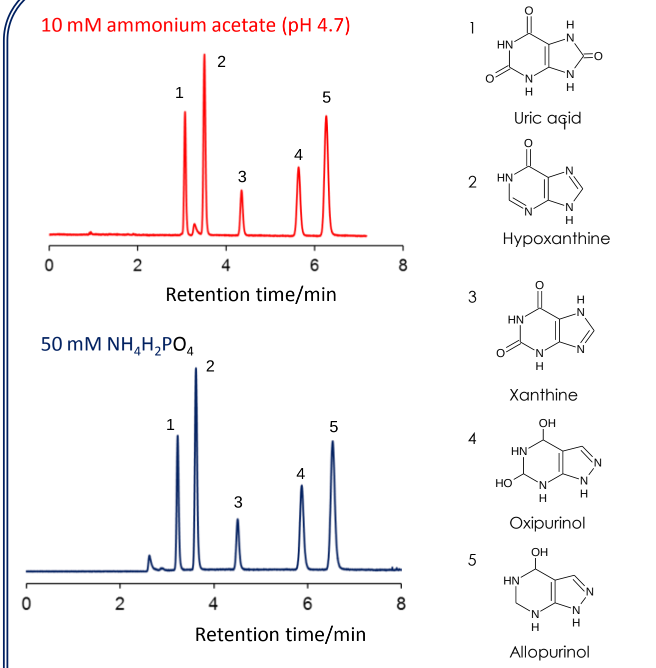


Column: SunShell C18, 30 x 3.0 mm.
Mobile phase: A) Water, B) Acetonitrile; Gradient (Acetonitrile %), 0.00 min - 35%, 0.40 min - 100%, 0.80 min - 100%, 0.85 min - 35%, 1cycle; 1.8min, (High-pressure gradient).
Flow rate: 1.0 mL/min.
Temperature: 40 ºC.
Injection Volume: 1 µL.
Wavelength: 200 - 500nm, CH-9, 215 - 500nm (Max Abs.).
Sample: Mixture of ultraviolet absorbers,
1 = 2,2',4,4'-Tetrahydroxybenzophenone,
2 = Ethyl *p*-aminobenzoate,
3 = 2, 4-Dihydroxybenzophenone,
4 = 2,2'-Dihydroxy-4-methoxybenzophenone,
5 = 2,2'-Dihydroxy-4,4'-dimethoxybenzophenone,
6 = 2-Hydroxy-4-methoxybenzophenone,
7 = 2-(2'-Hydroxy-5'-metylphenyl) benzotriazole,
8 = 4-tert-Butylphenyl salicylate.
Courtesy of Jasco.

A peak width is just one second!!

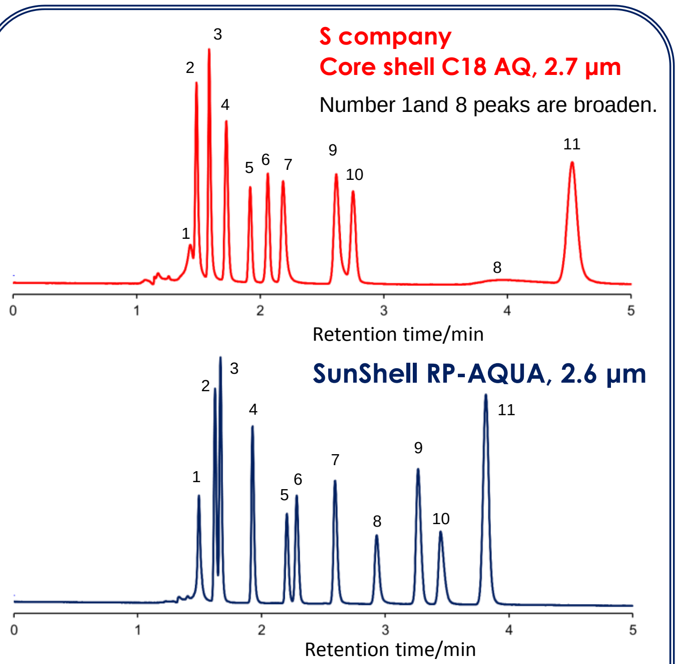


Purine analogue



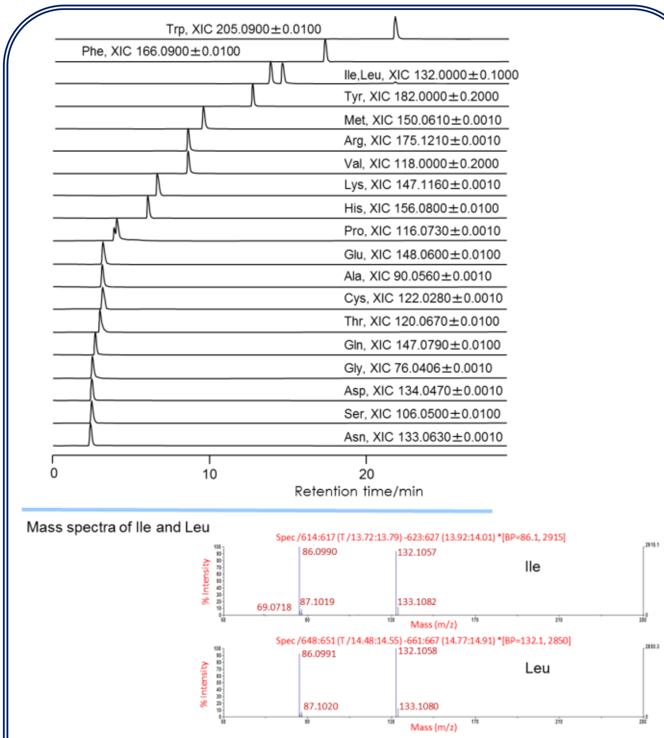
Column: SunShell RP-AQUA, 2.6 µm 100 x 4.6 mm
Mobile phase: 50 mM $NH_4H_2PO_4$ or 10 mM ammonium acetate (pH 4.7)
Flow rate: 1.0 mL/min
Temperature: Ambient
Detection: UV@250 nm
Sample: 1 = Uric acid, 2 = Hypoxanthine, 3 = Xanthine, 4 = Oxipurinol, 5 = Allopurinol

Organic acid



Column dimension: 150 x 4.6 mm
Mobile phase: 0.1% H_3PO_4
Flow rate: 1.0 mL/min
Temperature: 40 °C
Detection: UV@210nm
Sample:
1 = Oxalic acid, 2 = Tartaric acid, 3 = Formic acid, 4 = Malic acid,
5 = Lactic acid, 6 = Acetic acid, 7 = Diglycolic acid, 8 = Maleic acid,
9 = Citric acid, 10 = Succinic acid, 11 = Fumaric acid.

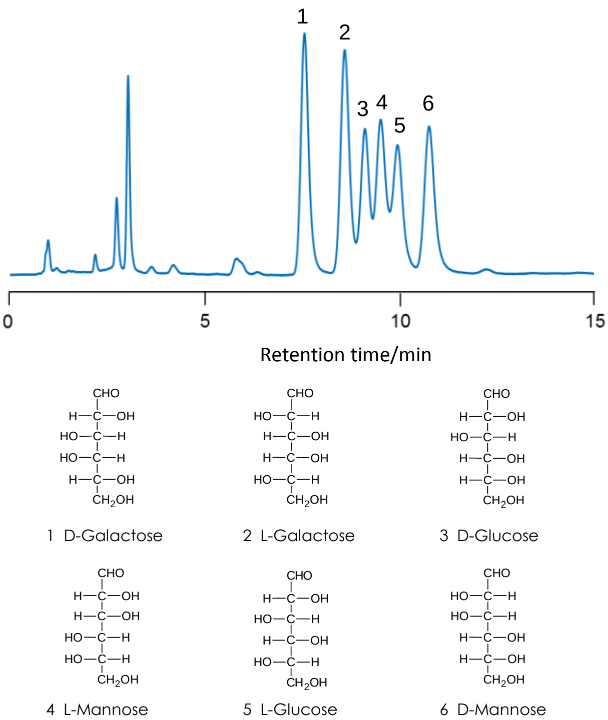
Amino acids (LC/MS)



Column: SunShell RP-AQUA, 2.6 µm, 150 x 2.1 mm
Mobile phase: A) 5 mM HFBA, B) 5 mM HFBA in CH_3CN / H_2O (9/1)
%B 0% to 20% in 20 min (HFBA: Heptafluorobutyric acid)
Flow rate: 0.2 mL / min
Temperature: 40 °C
Detection: MS (NanoFrontier LD) ESI Positive,
Extracted ion chromatogram (EIC)

Courtesy of Dr Takeo Kaneko, Hokohama National University

Monosaccharides derivatized with L-Tryptphan



Column: SunShell RP-AQUA, 2.6 µm 100 x 4.6 mm
Mobile phase: 5 mM Phosphate and 25 mM tetraborate (pH 9.6)
Flow rate: 1.0 mL/min
Temperature: 20 °C
Detection: UV@220 nm
Sample: Monosaccharides derivatized with L-Tryptphan

Courtesy of Dr Shuji Kodama, Tokai University

SunShell 2.6 µm
HFC18-16, HFC18-30, C8-30, C4-30

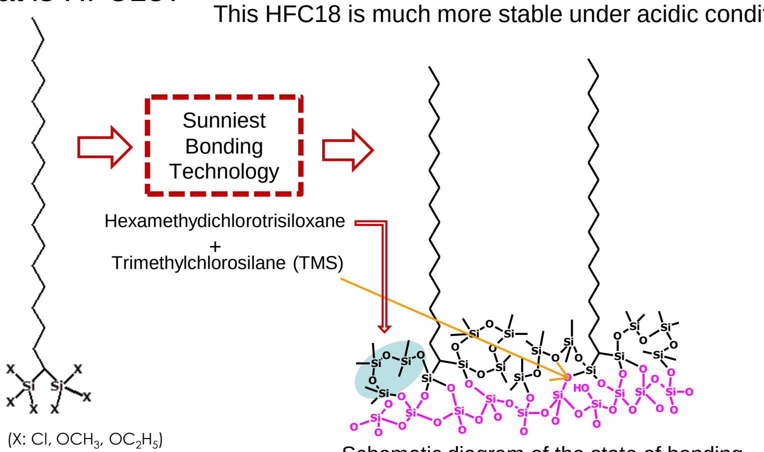
For separation of peptides and proteins

Characteristics of SunShell

	Core shell silica			Bonding phase						
	Particle size	Pore diameter	Specific surface area	Stationary phase	Carbon content	Ligand density	End-capping	Maximum operating pressure	Available pH range	USP L line
SunShell C18-WP	2.6 µm	16 nm	90 m²/g	C18	5 %	2.5 µmol/m²	Sunniest endcapping	60 MPa or 8,570 psi	1.5 - 10	L1
SunShell HFC18-16	2.6 µm	16 nm	90 m²/g	C18	2.5%	1.2 µmol/m²	Sunniest endcapping	60 MPa or 8,570 psi	1.5 – 9	L1
SunShell HFC18-30	2.6 µm	30 nm	40 m²/g	C18	1.3%	1.2 µmol/m²	Sunniest endcapping	60 MPa[a] or 8,570 psi[a]	1.5 - 9	L1
SunShell C8-30	2.6 µm	30 nm	40 m²/g	C8	1.2%	2.5 µmol/m²	Sunniest endcapping	60 MPa[a] or 8,570 psi[a]	1.5 – 9	L7
SunShell C4-30	2.6 µm	30 nm	40 m²/g	C4	0.9%	3 µmol/m²	Sunniest endcapping	60 MPa[a] or 8,570 psi[a]	1.5 – 9	L26

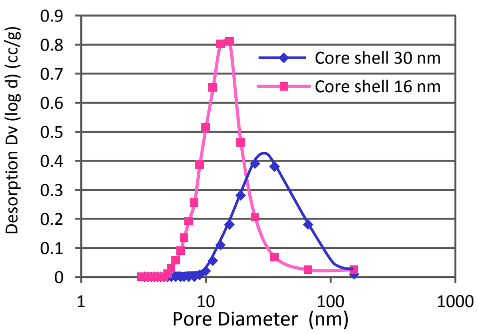
a: 50MPa, 7141psi for 4.6 mm i.d. column

What is HFC18? Hexa-Functional C18 has six functional groups.
This HFC18 is much more stable under acidic condition.

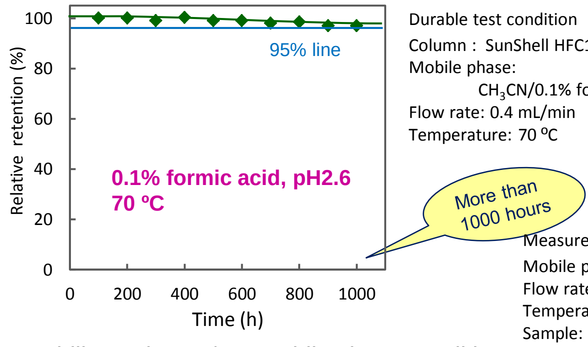


Schematic diagram of reagent

Schematic diagram of the state of bonding on silica surface



Pore distribution of core shell particle

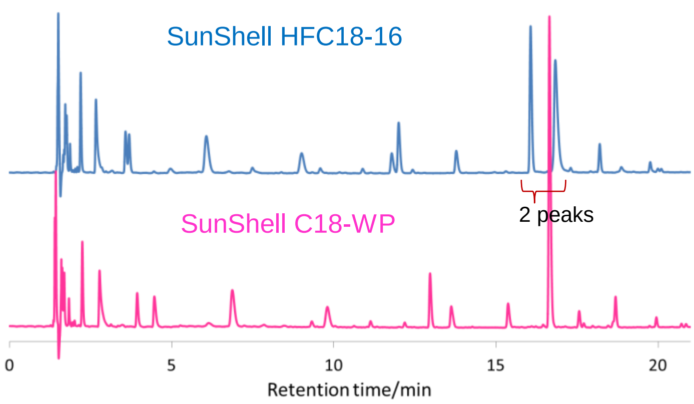


Stability under LC/MS mobile phase condition

Durable test condition
Column : SunShell HFC18-16 2.6 µm, 50 x 2.1 mm
Mobile phase:
CH_3CN/0.1% formic acid, pH2.6=40/60
Flow rate: 0.4 mL/min
Temperature: 70 °C

Measurement condition
Mobile phase: CH_3CN/H_2O=60/40
Flow rate: 0.4 mL/min
Temperature: 40 °C
Sample: 1 = Uracil
2 = Butylbenzene

Separation of peptides



Column: SunShell HFC18-16, 2.6 µm (16 nm) 150 x 4.6 mm
SunShell C18-WP, 2.6 µm (16 nm) 150 x 4.6 mm
Mobile phase: A) 0.1% TFA in Acetonitrile/water(10:90)
B) 0.1 % TFA in Acetonitrile

Gradient program:

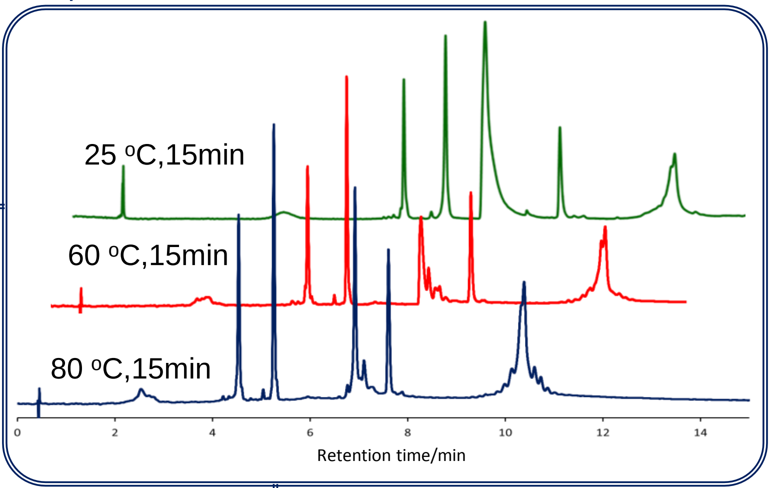
Time	0 min	5 min	40 min
%B	5%	5%	50%

Flow rate: 1.0 mL/min
Temperature: 25 °C
Detection: UV@210 nm
Sample: Tryptic digest of cytochrome C

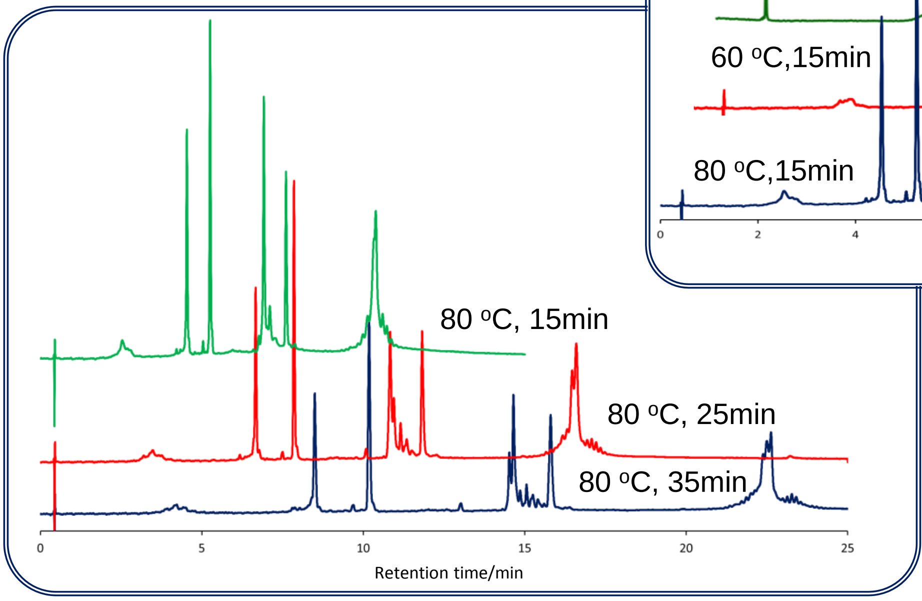
SunShell 2.6 µm HFC18-16, HFC18-30, C8-30, C4-30

For separation of peptides and proteins

Comparison of column temperature



Comparison o0f gradient time

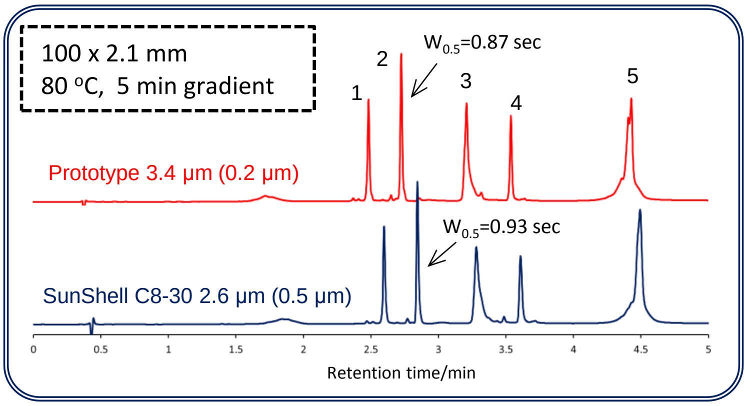


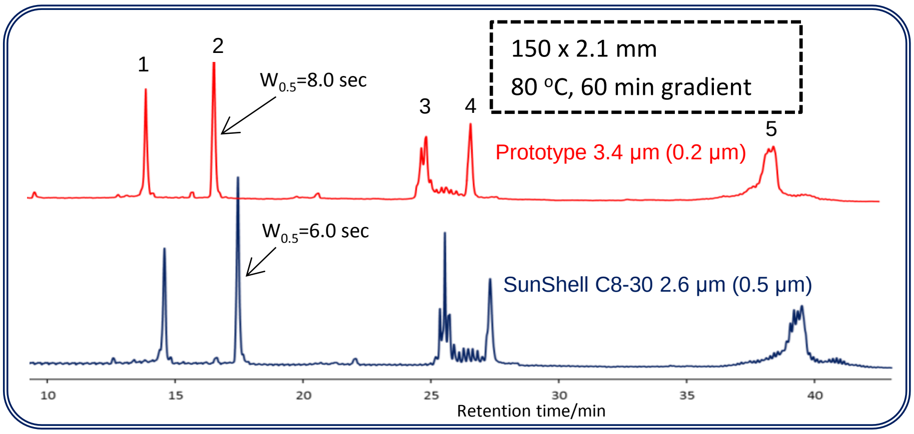
Column:
SunShell C8-30, 2.6 µm (30 nm) 100 x 2.1 mm,
Mobile phase: A) 0.1% TFA in water
B) 0.08 % TFA in acetonitrile
Gradient program: Time 0 min 15, 25, 35 min
%B 20% 65%
Flow rate: 0.5 mL/min ,
Temperature: 25 °C 60 °C or 80 °C
Detection: UV@215 nm,
Sample:1 = Cytochrome C, 2 = Lysozyme, 3 = BSA,
4 = Myoglobin, 5 = Ovalbumin

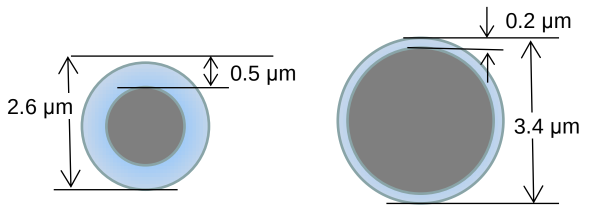
A macromolecule compound like a protein diffuses very slowly, so that an elevated temperature makes a peak be shaper and improves separation. BSA peak seemed to be tailing at 25 degree Celsius. BSA, however, was separated several peaks at 80 degree Celsius. Furthermore separation of proteins was improved by a long gradient time. Although one peak of BSA was obtained under 15 minute gradient elution at 25 degree Celsius, more than 7 peaks from BSA were obtained under 35 minute gradient elution at 80 degree Celsius.

Comparison of thickness of porous layer

Column:
SunShell C8-30, 2.6 µm (30 nm, 0.5 µm layer) 100 or 150 x 2.1 mm,
Sunshell C8-30, 3.4 µm (30 nm, 0.2 µm layer) 100 or 150 x 2.1 mm (prototype)
Mobile phase: A) 0.1% TFA in water, B) 0.08 % TFA in acetonitrile
Gradient program: Time 0 min 5 or 60 min
%B 20% 65%
Flow rate: 0.5 mL/min ,
Temperature: 80 °C,
Detection: UV@215 nm,
Sample:1 = Cytochrome C, 2 = Lysozyme, 3 = BSA, 4 = Myoglobin, 5 = Ovalbumin







SunShell C8-30, 2.6 µm
Specific surface area: 40m^2/g

Sunshell C8-30, 3.4 µm
Specific surface area: 15m^2/g

It has been said that the thin porous layer on a core shell particle had an advantage over separation of macromolecule compounds such as proteins. Indeed regarding high-throughput separation under 5 minute gradient elution, SunShell C8-30 3.4 µm with 0.2 µm thickness porous layer showed shaper peaks than 2.6 µm with 0.5 µm thickness porous layer. However, under long gradient elution at 80degree Celsius, a reversed phenomenon is observed. The difference of a peak width is bigger than the value due to the difference of a particle size. As a result, the thickness of the porous layer has nothing with the peak width of a protein under such a condition. Regarding retention time, the wider the surface area, the longer the retention time. In other words, the partition interaction on a 0.5 µm porous layer material worked for longer time, so that BSA and ovalbumin was separated much better on 0.5 µm porous layer material than 0.2 µm porous layer material.

SunShell 2-EP, 2.6 μm

For Supercritical fluid Chromatography

2.6 μm core shell column shows only one third of back pressure to compare with 1.7 μm fully porous column although both show almost same efficiency. By such low back pressure, a difference of density of supercritical fluid between an inlet and an outlet of the column is reduced. Consequently, . 2.6 μm core shell column performs a superior separation for SFC.

Characteristics of SunShell 2-EP

	Core shell silica							
	Particle size	Pore diameter	Specific surface area	Carbon content	Bonded phase	End-capping	Maximum operating pressure	Available pH range
SunShell 2-EP	2.6 μm	9 nm	150 m^2/g	2.5%	2-Ethylpyridine	no	60 MPa or 8,570 psi	2 – 7.5

Comparison between SunShell 2-EP and 1.7 μm fully porous 2-EP

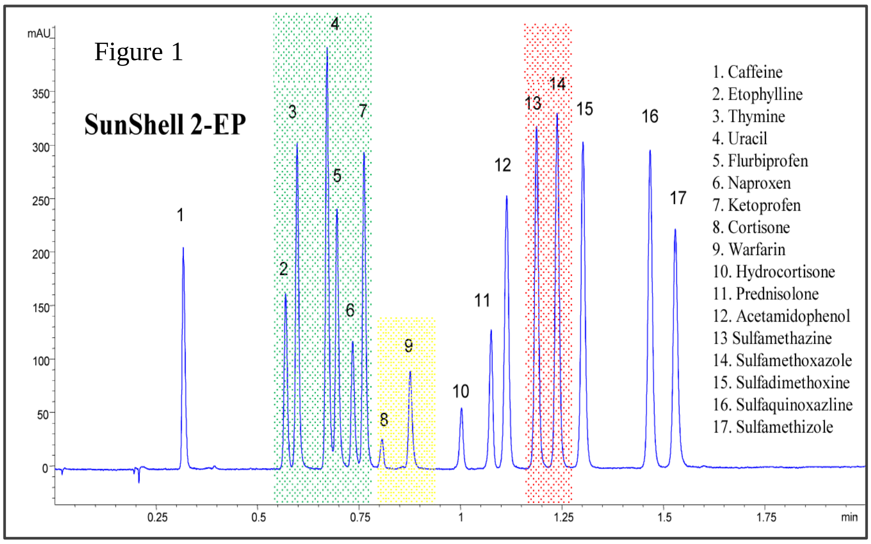


Figure 1: Chromatogram of the separation for he 17-component mix using the Sun Shell 2-EP 150 x 3.0 mm column. A methanol gradient of < 2 minutes was used on the Agilent 1260 Infinity SFC system. SFC conditions: flow rate: 4.0mL/min; outlet pressure 160 bar; column temperature 55^{0}C. Gradient program: 5.0-7.5% in 0.20 min, then 7.5-20% in 1.3 min and held at 20% for 0.2 min.

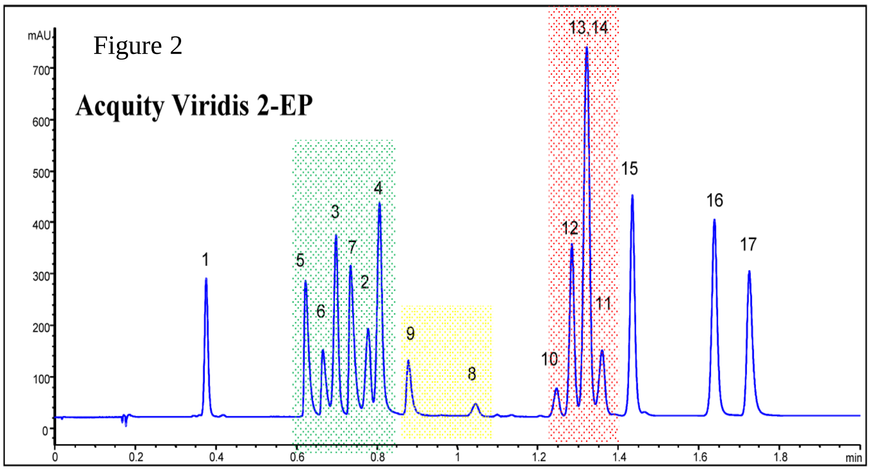
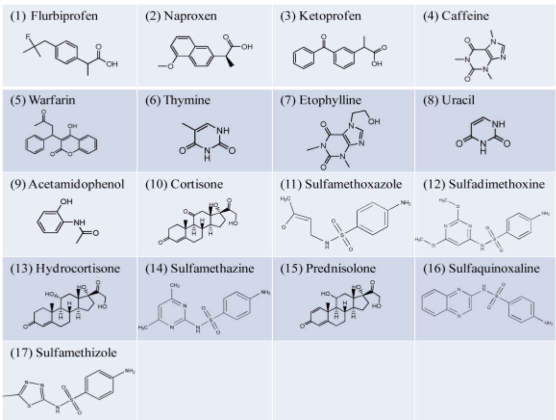
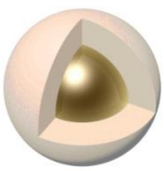


Figure 2: Chromatogram of the separation for the 17-component mix using Acquity UPC^2 Viridis 2-EP 100 x 3.0 mm column. 16 of the 17 components were resolved. A methanol gradient of < 2 minutes was used on the Agilent 1260 Infinity SFC system. SFC conditions: flow rate 3.5 mL/min; outlet pressure 160 bar; and column temperature 70^{0}C. Gradient program: 5.0-12.5% in 1.0 min, 12.5% for 0.25 min, then 12.5-20% in 0.75 min.



Courtesy of Pfizer Inc.



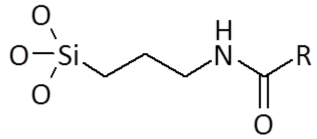
SunShell HILIC-Amide, 2.6 µm

For Hydrophilic Interaction Chromatography

Characteristics of SunShell HILIC-Amide

	Core shell silica			Amide (USP L68)				
	Particle size	Pore diameter	Specific surface area	Carbon content	Bonded phase	End-capping	Maximum operating pressure	Available pH range
SunShell HILIC-Amide	2.6 µm	9 nm	150 m^2/g	3%	Amide	no	60 MPa or 8,570 psi	2 - 8

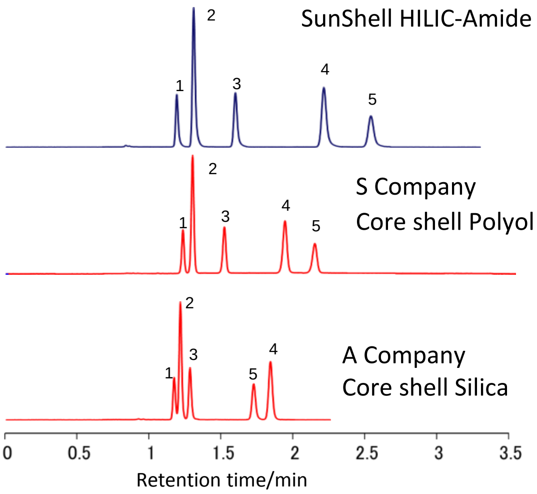
Stationary phase of HILIC-Amide



R: Hydrophilic group

Stationary phase of SunShell HILIC-Amide consists of AMIDE and HYDROPHILIC GROUP, so that this stationary phase is more polar than an individual group. High speed separation is leaded by core shell structure that derives high efficiency and fast equilibration.

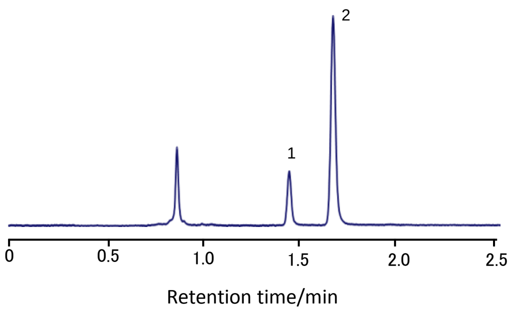
Separation of Nucleic acid bases: Comparison of the other core shell hilic columns



Column:
SunShell HILIC-Amide, 2.6 µm 100 x 4.6 mm,
Coreshell polyol, 2.7 µm 100 x 4.6 mm,
Core shell Silica, 2.7 µm 100 x 4.6 mm
Mobile phase:
Acetonitrile/20 mM ammonium acetate(pH4.7) = 8/2
Flow rate: 1.0 mL/min
Temperature: 40 °C
Detection: UV@250 nm
Sample: 1 = Thymine, 2 = Uracil, 3 = Uridine, 4 = Cytosine, 5 = Cytidine

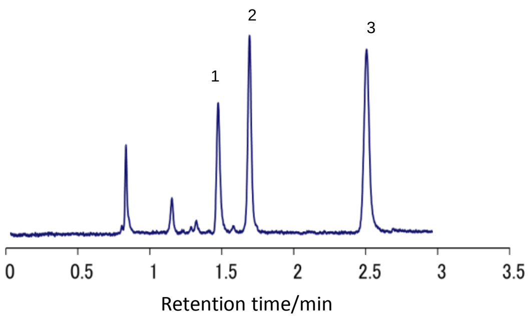
Regarding retention of cytidine, SunShell HILIC-Amide showed 30% higher retention factor than S core shell polyol.

Separation of Cyanuric acid and Melamine



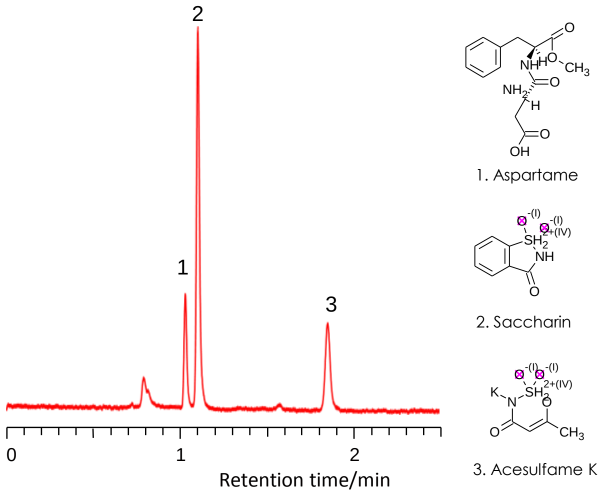
Column: SunShell HILIC-Amide, 2.6 µm 100 x 4.6 mm
Mobile phase:
Acetonitrile/5 mM phosphate Buffer (pH6.9) =75/25
Flow rate: 1.0 mL/min
Temperature: 40 °C
Detection: UV@220 nm,
Sample: 1 = Cyanuric acid, 2 = Melamine

Separation of water- soluble vitamins



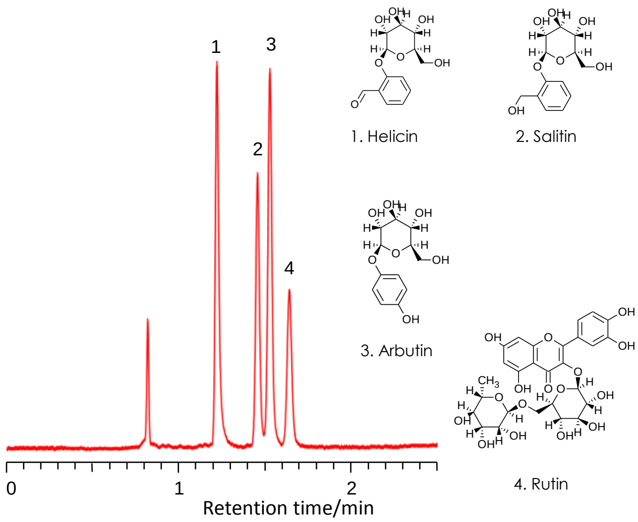
Column: SunShell HILIC-Amide, 2.6 µm 100 x 4.6 mm
Mobile phase:
Acetonitrile/25 mM phosphate buffer (pH2.5) =8/2
Flow rate: 1.0 mL/min
Temperature: 40 °C
Detection: UV@250 nm,
Sample: 1 = Nicotinic acid, 2 = Ascorbic acid, 3 = Pyridoxine

Artificial sweeteners



Column:
SunShell HILIC-Amide, 2.6 µm, 100 x 4.6 mm
Mobile phase:
Acetonitrile: 25 mM phosphate buffer (pH2.5) =8:2
Flow rate: 1.0 mL/min ,
Temperature: Ambient
Detection: UV@215 nm
Sample: 1 = Aspartame, 2 = Saccharin, 3 = Acesulfame K

Glycoside



Column:
SunShell HILIC-Amide, 2.6 µm, 100 x 4.6 mm
Mobile phase:
Acetonitrile:25 mM phosphate Ammonium (pH4.9) =8:2
Flow rate: 1.0 mL/min
Temperature: Ambient
Detection: UV@215 nm
Sample: 1 = Helicin, 2 = Salicin, 3,= Arbutin, 4 = Rutin

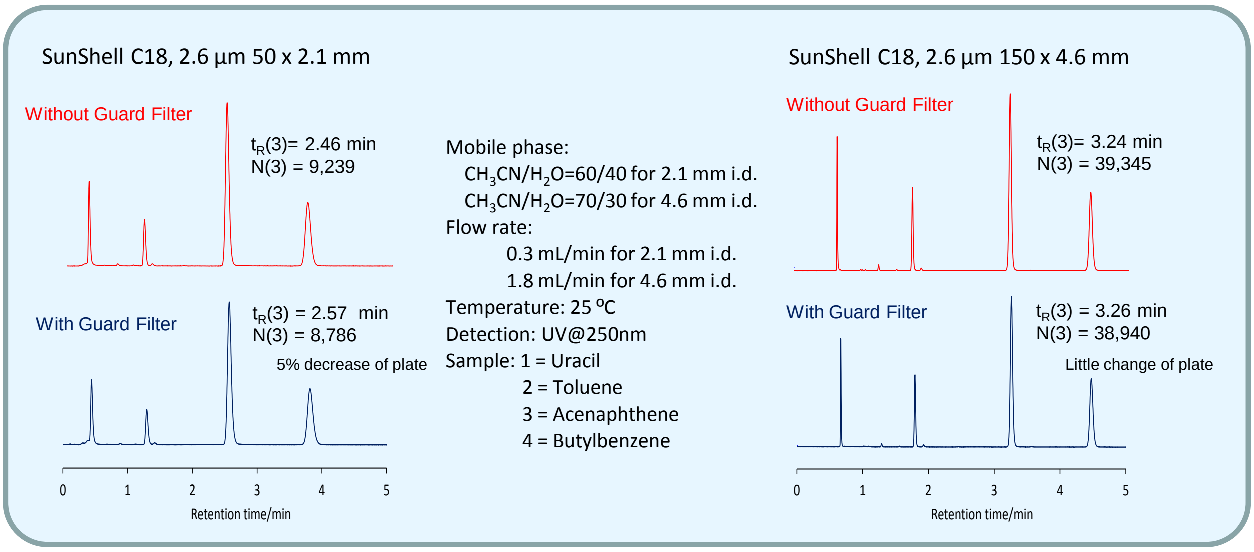
SunShell RP Guard Filter

＜Cartridge Type,　Bonded with C18 and End-Capped with TMS＞

Available as a guard column for reversed phase

- ✓ The filter is made of porous glass sized 4 mm i.d. and 4 mm thickness.
- ✓ Pore diameter is 2 μm.
- ✓ Low dead volume structure
- ✓ Back pressure on glass filter is ca. 0.1 MPa at 1.0 mL/min of flow rate.
- ✓ Upper pressure limit is more than 60 MPa
- ✓ Available for 2.1 mm i.d to 4.6 mm i.d. column

Evaluation of SunShell RP Guard Filter



Price of SunShell RP Guard Filter

Name	quantity	Part number	Photo
SunShell RP Guard Filter Starter Kit	Holder: 1 piece, RP Guard filter : 1 piece Tubing: 1piece, Nut: 2 pieces, Ferrule: 2 pieces (pressure Max: 9000 psi , 62MPa)	CBGAKN	
SunShell RP Guard Filter For exchange	5 pieces	CBGAAC	
SunShell RP Guard Filter Holder	1 piece	CBGAAH	

Ordering information of SunShell

	Inner diameter (mm)	1.0	2.1	3.0	4.6	USP category
	Length (mm)	Catalog number	Catalog number	Catalog number	Catalog number	
SunShell C18, 2.6 µm	30	------	CB6931	CB6331	CB6431	L1
	50	CB6141	CB6941	CB6341	CB6441	
	75	------	CB6951	CB6351	CB6451	
	100	CB6161	CB6961	CB6361	CB6461	
	150	CB6171	CB6971	CB6371	CB6471	
	250	------	------	CB6381	CB6481	
SunShell C18, 5 µm	150	------	------	CB3371	CB3471	
	250	------	------	CB3381	CB3481	
SunShell C8, 2.6 µm	30	------	CC6931	CC6331	CC6431	L7
	50	------	CC6941	CC6341	CC6441	
	75	------	CC6951	CC6351	CC6451	
	100	------	CC6961	CC6361	CC6461	
	150	------	CC6971	CC6371	CC6471	
SunShell PFP, 2.6 µm	30	------	CF6931	CF6331	CF6431	L43
	50	------	CF6941	CF6341	CF6441	
	75	------	CF6951	CF6351	CF6451	
	100	------	CF6961	CF6361	CF6461	
	150	------	CF6971	CF6371	CF6471	
SunShell C18-WP, 2.6 µm	30	------	CW6931	CW6331	CW6431	L1
	50	------	CW6941	CW6341	CW6441	
	75	------	CW6951	CW6351	CW6451	
	100	------	CW6961	CW6361	CW6461	
	150	------	CW6971	CW6371	CW6471	
SunShell RP-AQUA, 2.6 µm	30	------	CR6931	CR6331	CR6431	Equivalent to L62
	50	CR6141	CR6941	CR6341	CR6441	
	75	------	CR6951	CR6351	CR6451	
	100	CR6161	CR6961	CR6361	CR6461	
	150	CR6171	CR6971	CR6371	CR6471	
SunShell Phenyl, 2.6 µm	30	------	CP6931	CP6331	CP6431	L11
	50	------	CP6941	CP6341	CP6441	
	75	------	CP6951	CP6351	CP6451	
	100	------	CP6961	CP6361	CP6461	
	150	------	CP6971	CP6371	CP6471	
SunShell HILIC-Amide, 2.6 µm	30	------	CH6931	CH6331	CH6431	L68
	50	------	CH6941	CH6341	CH6441	
	75	------	CH6951	CH6351	CH6451	
	100	------	CH6961	CH6361	CH6461	
	150	------	CH6971	CH6371	CH6471	
SunShell 2-EP, 2.6 µm	30	------	CE6931	CE6331	CE6431	
	50	------	CE6941	CE6341	CE6441	
	75	------	CE6951	CE6351	CE6451	
	100	------	CE6961	CE6361	CE6461	
	150	------	CE6971	CE6371	CE6471	
SunShell HFC18-16, 2.6 µm	50	------	CG6941	CG6341	CG6441	L1
	100	------	CG6961	CG6361	CG6461	
	150	------	CG6971	CG6371	CG6471	
SunShell HFC18-30, 2.6 µm	50	------	C46941	C46341	C46441	L1
	100	------	C46961	C46361	C46461	
	150	------	C46971	C46371	C46471	
SunShell C8-30, 2.6 µm	50	------	C36941	C36341	C36441	L7
	100	------	C36961	C36361	C36461	
	150	------	C36971	C36371	C36471	
SunShell C4-30, 2.6 µm	50	------	C26941	C26341	C26441	L26
	100	------	C26961	C26361	C26461	
	150	------	C26971	C26371	C26471	

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