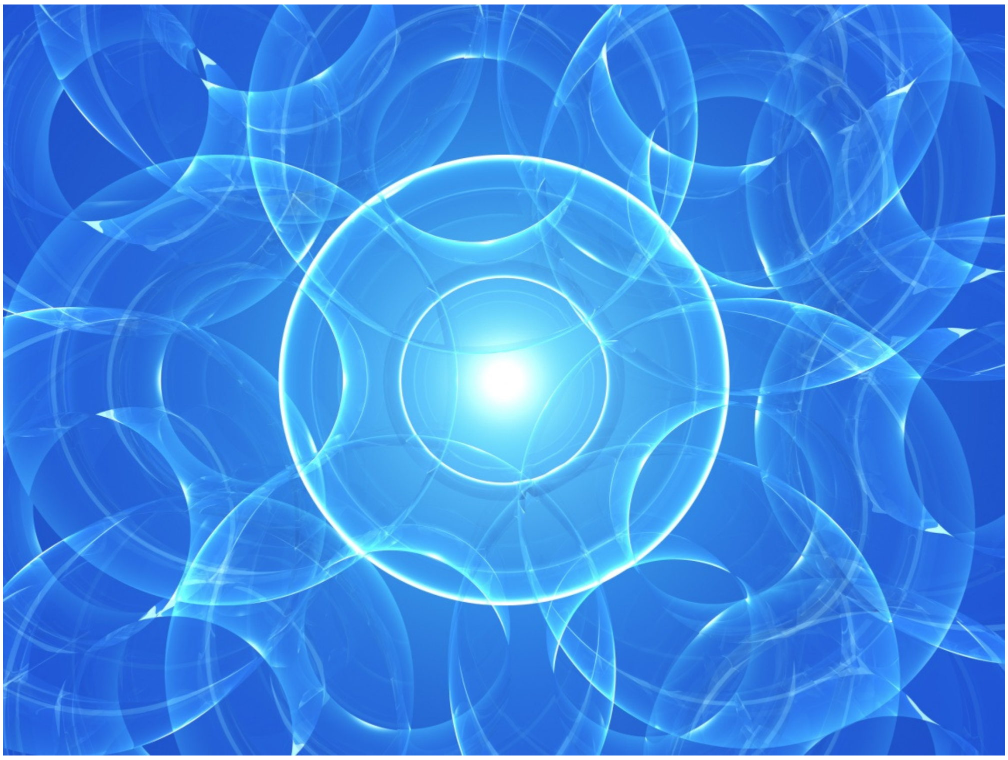
ChromaNik Technologies Inc.

BIOTECH

HPLC column C18 C8 PFP

SunShell



Core Shell Particle

ChromaNik Technologies Inc.

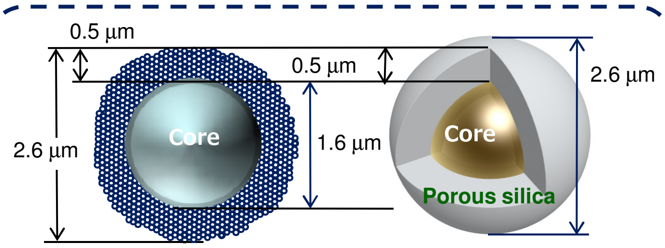
"SunShell " is a core shell silica column made by ChromaNIk Technologies.

The next generation to Core Shell particle

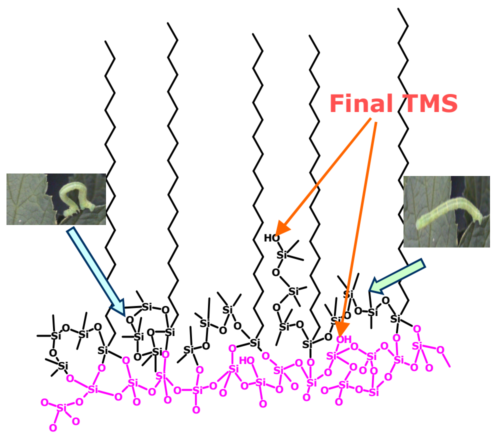
Superficially porous silica

Features of SunShell

*1.6 µm of core and 0.5 µm of superficially porous silica layer
*Same efficiency and high throughput as a Sub 2µm particle
*Same pressure as a 3 µm particle (less than a half then a sub 2µm 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

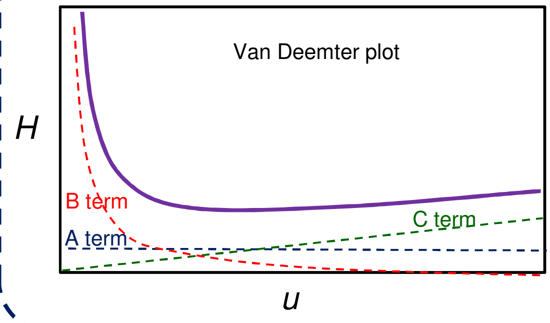


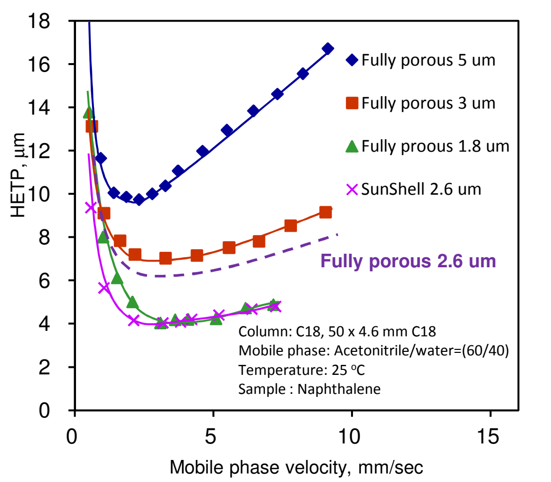
Schematic diagram of bonding of SunShell C18

Van Deemter Equation

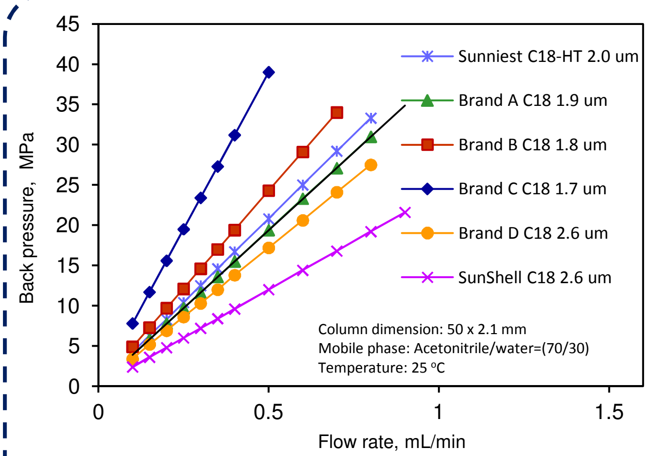
$$H = Ad_p + B\frac{D_m}{u} + C\frac{d_p^2}{D_m}u$$

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





Comparison of plate height plots

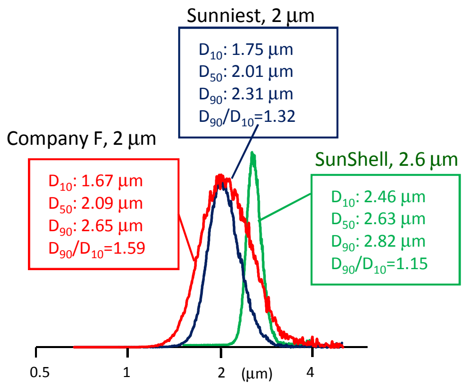


Comparison of back pressure for high throughput columns

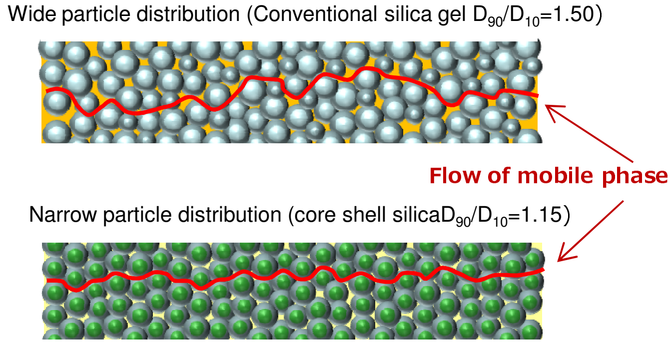
SunShell C18 shows same efficiency as a sub 2 µm C18. In comparison between fully porous 2.6 µm and core shell 2.6 µm (SunShell), SunShell shows lower values for A term, B term and C term of Van Deemter equation. The core shell structure leads higher performance to compare with the fully porous structure.

Furthermore back pressure of SunShell C18 is less than a half to compare with sub-2 µm C18s.

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

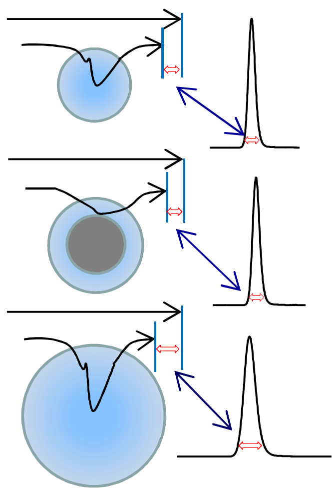


Comparison of Particle Size Distribution



Packing state of core shell and fully porous silica

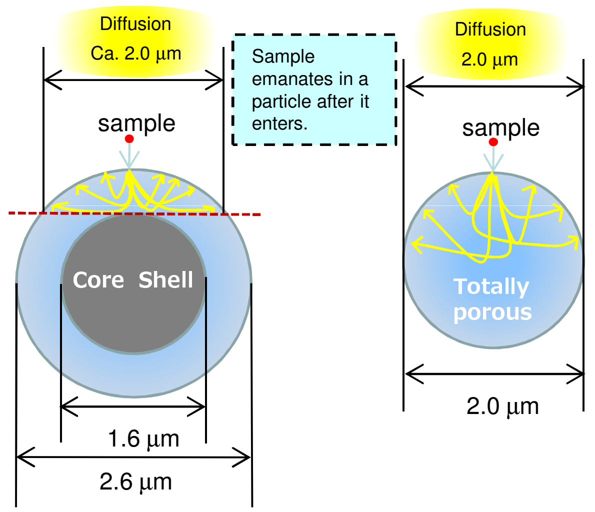
The size distribution of a core shell (SunShell) particle is much narrower than that of a conventional fully porous particle, so that the space among particles in the column reduces and efficiency increases by reducing Eddy Diffusion (multi-path diffusion) as the A term in Van Deemter Equation.



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 fully porous sub-2 μm particle.

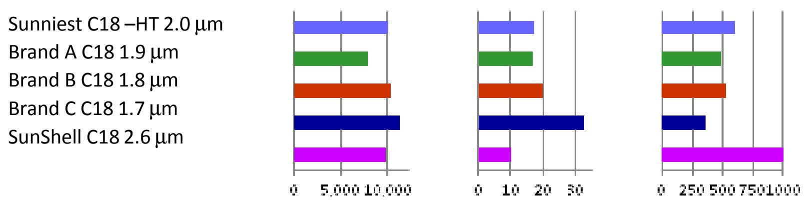
The right figure shows that a diffusion width of a sample in a 2.6 μm core shell particle and a 2 μm fully porous particle. Both diffusion widths are almost same. The 2.6 μm core shell particle is superficially porous, so that the diffusion width becomes narrower than particle size. Same diffusion means same efficiency.



Diffusion of sample in core shell and fully 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

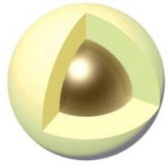


Column: 50 x 2.1 mm C18, Mobile phase: Acetonitrile/water=(70/30), Temperature: 25 ºC

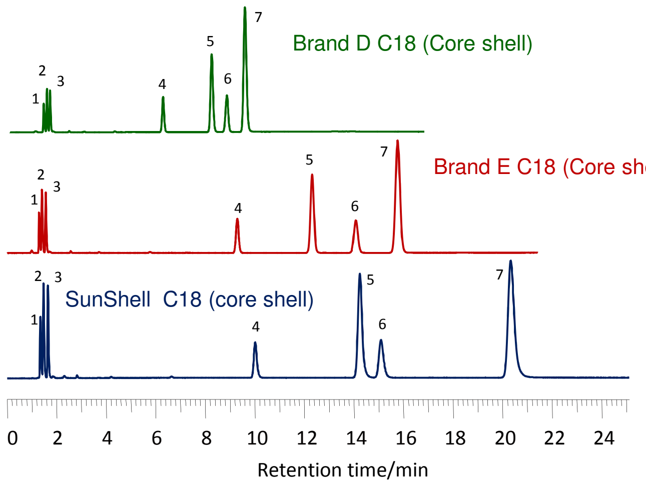
Under a constant back pressure condition, SunShell C18 showed more than 2 times higher performance to compare with fully sub-2μm porous C18s.

Characteristics of SunShell C18

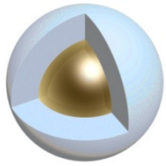
	Core shell silica			C18			
	Particle size (µm)	Pore diameter (nm)	Specific surface area (m^2/g)	Carbon content (%)	Bonded phase	Maximum operating pressure	Available pH range
SunShell C18	2.6	9	150	7	C18	60 MPa or 8,570 psi	1.5 - 10



Comparison of standard samples between core shell C18s



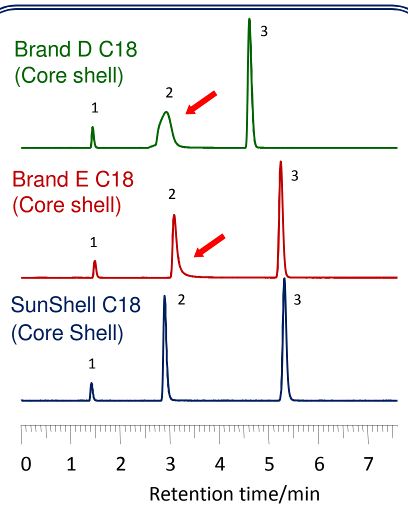
Column:
Brand D C18, 2.6 µm 150 x 4.6 mm (Core shell type)
Brand E C18, 2.7 µm 150 x 4.6 mm (Core shell type)
SunShell C18, 2.6 µm 150 x 4.6 mm
Mobile phase: CH_3OH/H_2O=75/25
Flow rate: 1.0 mL/min
Temperature: 40 °C
Sample: 1 = Uracil, 2 = Caffeine, 3 = Phenol, 4 = Butylbenzene
5 = o-Terphenyl, 6 = Amylbenzene, 7 = Triphenylene



	Hydrogen bonding (Caffeine/Phenol)	Hydrophobicity (Amylbenzene/Butylbenzene)	Steric selectivity (Triphenylene/o-Terphenyl)
Brand D C18	0.48	1.54	1.20
Brand E C18	0.44	1.60	1.31
SunShell C18	0.40	1.59	1.47

Retention of standard samples was compared for three kinds of core shell type C18s. Brand D 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.

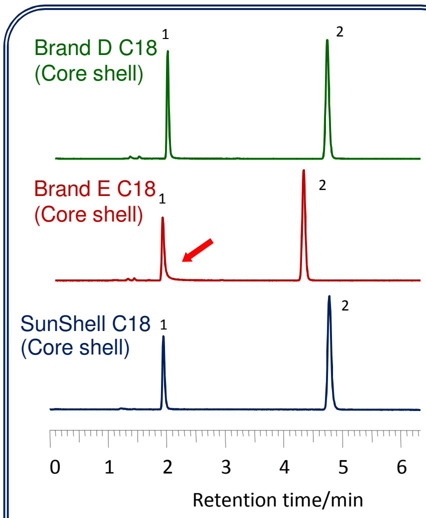
Comparison of pyridine



Column:
Brand D C18, 2.6 µm 150 x 4.6 mm
Brand E C18, 2.7 µm 150 x 4.6 mm
SunShell C18, 2.6 µm 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. Only SunShell C18 shows a sharp peak for pyridine.

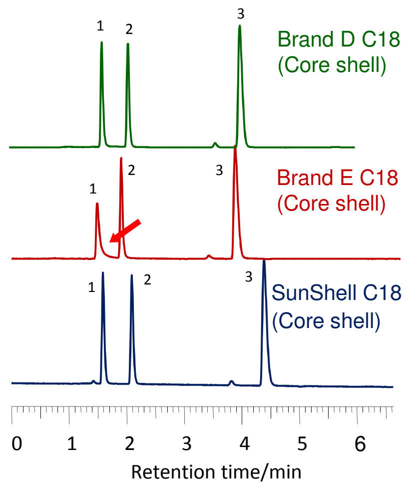
Comparison of Oxine



Column:
Brand D C18, 2.6 µm 150 x 4.6 mm
Brand E C18, 2.7 µm 150 x 4.6 mm
SunShell C18, 2.6 µm 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.

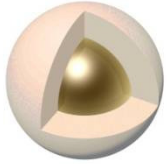
Comparison of formic acid



Column:
Brand D C18, 2.6 µm 150 x 4.6 mm
Brand E C18, 2.7 µm 150 x 4.6 mm
SunShell C18, 2.6 µm 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 brand D C18 show a sharp peak.

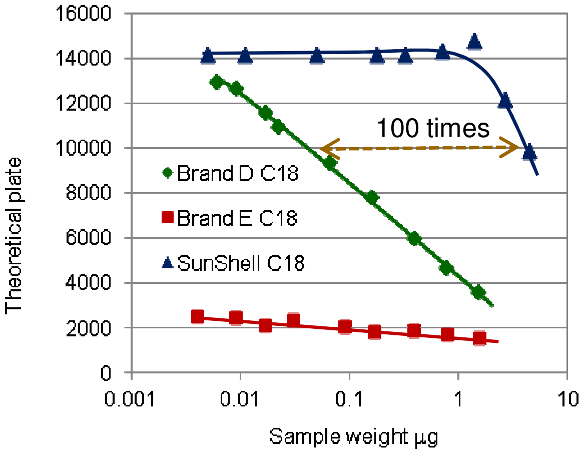
Loading capacity of amitriptyline as a basic compound



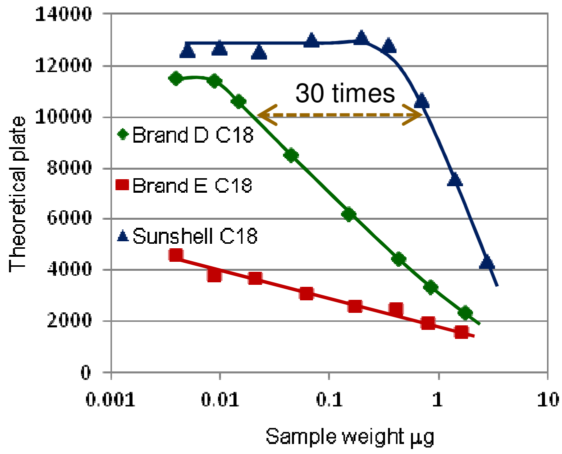
Amitriptyline overlords much more at acetonitrile/buffer mobile phase than methanol/buffer. Three kinds of core shell C18s were compared loading capacity of amitriptyline at three different mobile phases.

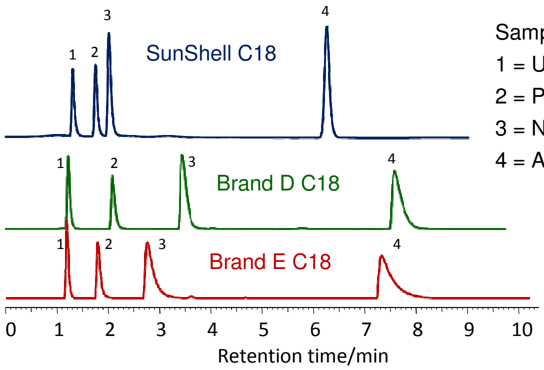
Common condition: Column dimension,150 x 4.6 mm, flow rate; 1.0 mL/min, temperature; 40 °C

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

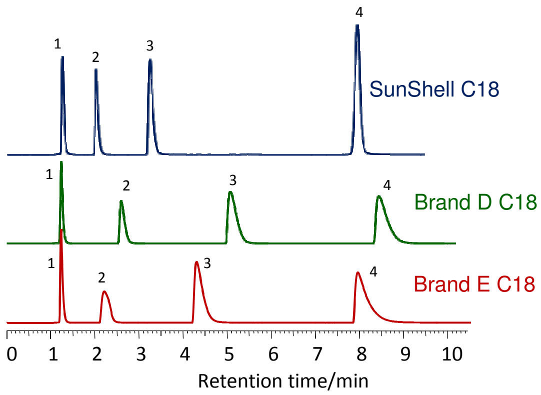


Mobile phase: Acetonitrile/**10mM acetate ammonium pH6.8**=(40:60)

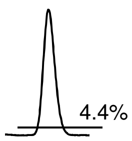




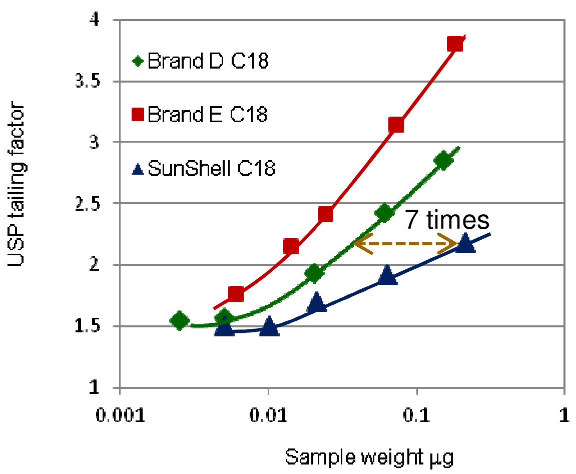
Sample:
1 = Uracil (0.07µg)
2 = Propranolol (1.53µg)
3 = Nortriptyline (0.32µg)
4 = Amitriptyline (0.32µg)



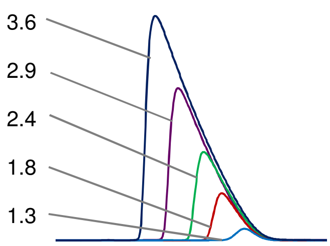
Theoretical plate was calculated by 5σ method using peak width at 4.4% of peak height.



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



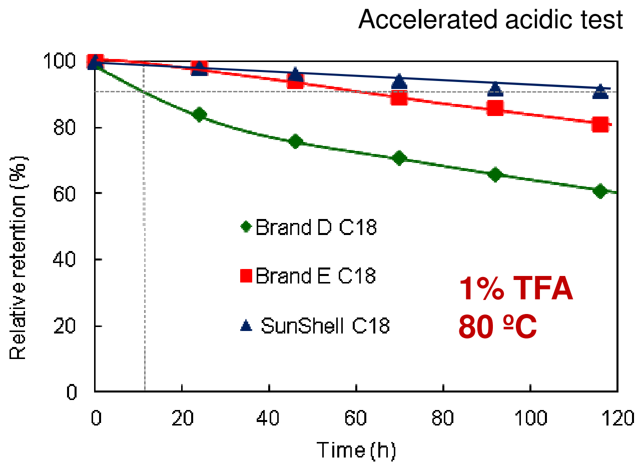
USP tailing factor



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

Brand D C18 overloaded at more than 0.01 µg of amitriptyline while SunShell C18 overloadsed at more than from 0.3 to 1 µg of amitriptyline. Surprisingly loading capacity of Brand D C18 was only one hundredth to compare with SunShell C18 under acetonitrile/20mM phosphate buffer pH7.0=(60:40) mobile phase. Brand E 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

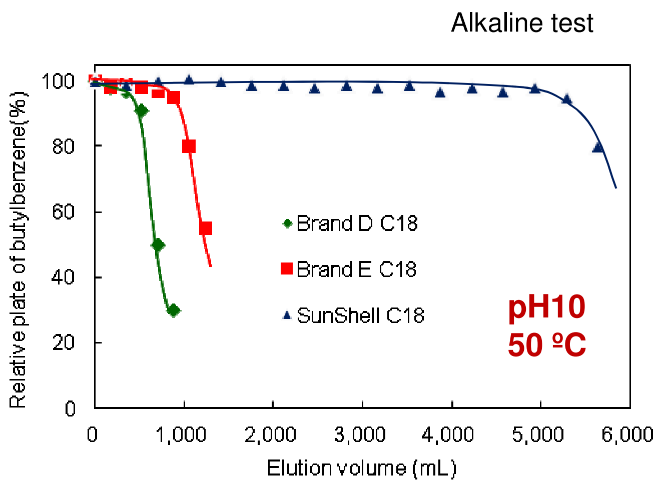
Measurement condition

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

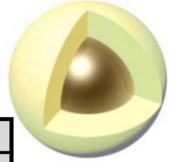
Stability under basic pH condition was evaluated at 50 ºC using methanol/Sodium borate buffer pH 10 (30:70) as mobile phase. Sodium borate is used as a alkaline standard solution for pH meter, so that its buffer capacity is high.

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.

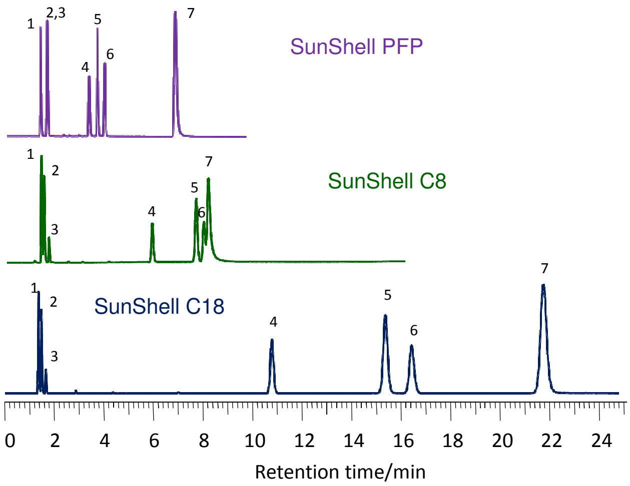
SunShell C8, PFP *(Pentafluorophenyl)*



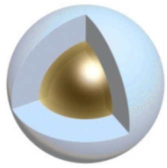
◆ Characteristics of SunShell

	Core shell silica			Bonded phase			
	Particle size (µm)	Pore diameter (nm)	Specific surface area (m²/g)	Carbon content (%)	Bonded phase	Maximum operating pressure	Available pH range
SunShell C18	2.6	9	150	7	C18	60 MPa or 8,570 psi	1.5 - 10
SunShell C8	2.6	9	150	4.5	C8	60 MPa or 8,570 psi	1.5 - 9
SunShell PFP	2.6	9	150	4.5	Pentafluorophenyl	60 MPa or 8,570 psi	2 - 8

◆ Comparison of standard samples



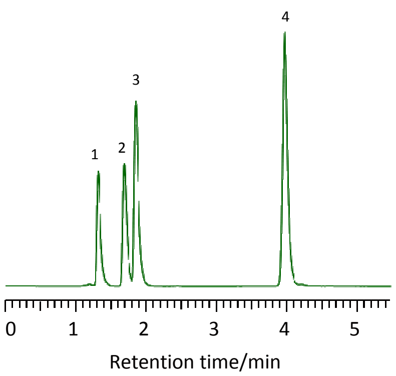
Column:
SunShell PFP, 2.6 µm 150 x 4.6 mm
SunShell C8, 2.6 µm 150 x 4.6 mm
SunShell C18, 2.6 µm 150 x 4.6 mm
Mobile phase: CH_3OH/H_2O=75/25
Flow rate: 1.0 mL/min
Temperature: 40 ºC
Sample: 1 = Uracil, 2 = Caffeine, 3 = Phenol, 4 = Butylbenzene
5 = o-Terphenyl, 6 = Amylbenzene, 7 = Triphenylene



	Hydrogen bonding (Caffeine/Phenol)	Hydrophobicity (Amylbenzene/Butylbenzene)	Steric selectivity (Triphenylene/o-Terphenyl)
SunShell PFP	1.00	1.31	2.38
SunShell C8	0.32	1.46	1.08
SunShell C18	0.39	1.60	1.46

Retention of standard samples was compared for three kinds of phases such as C18, C8 and PFP. C18 showed the highest hydrophobicity and PFP showed both the highest steric selectivity and the highest hydrogen bonding. The feature of PFP phase is to have hydrogen bonding, dipole-dipole interaction, aromatic and pi-pi interactions and hydrophobicity, which causes a different selectivity from a C18 phase.

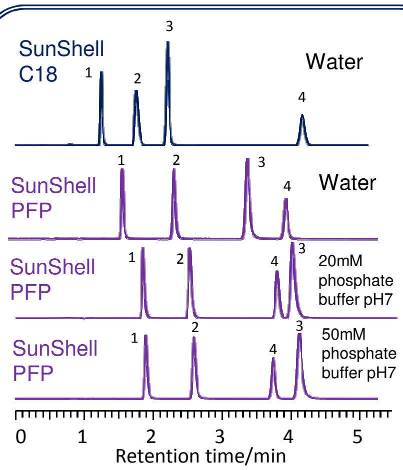
Separation of amitriptyline using C8



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

SunShell C8 showed a sharp peak for amitriptyline a well as SunShell C18.

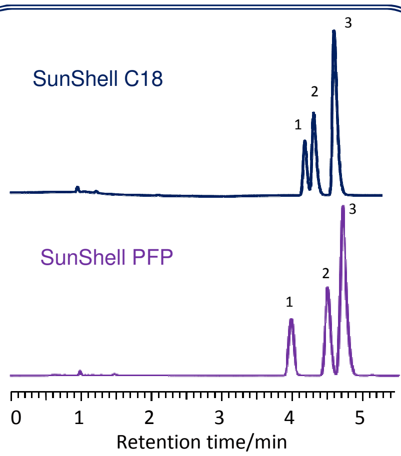
Separation of xanthines



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

SunShell PFP can retain xanthines more than SunShell C18. The higher the concentration of buffer, the longer the retention time.

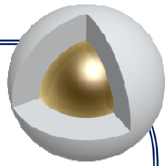
Separation of isomers of xylene



SunShell C18, PFP 2.6 µm 150 x 2.1 mm
Mobile phase:
CH_3OH/water=75:25 for SunShell C18
CH_3OH/water=60:40 for SunShell PFP
Flow rate: 0.3 mL/min
Temperature: 25 ºC
Detection: UV@250nm
Sample: 1 = o-Xylene
2 = m-Xylene
3 = p-Xylene

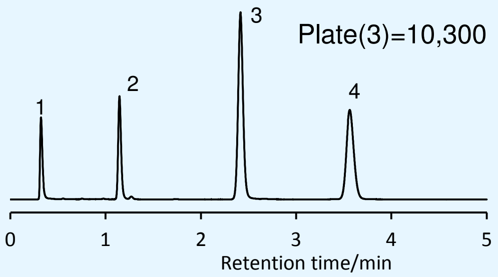
SunShell PFP showed the different selectivity from SunShell C18.

Efficiency of SunShell C18

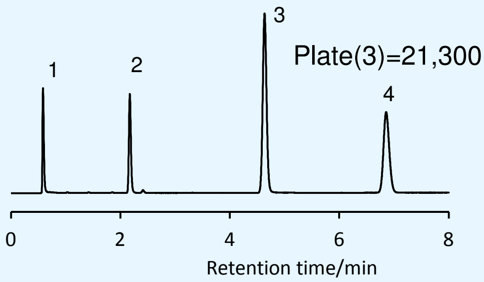


UHPLC

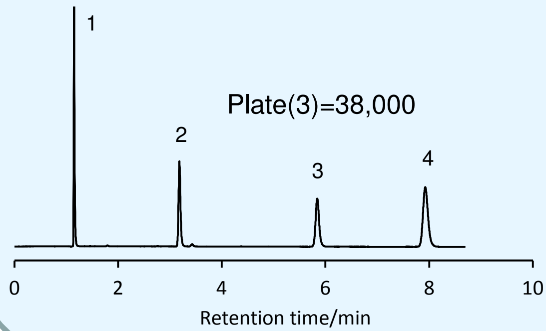
Column: SunShell C18, 50 x 2.1 mm



Column: SunShell C18, 100 x 2.1 mm



Column: SunShell C18,150 x 4.6 mm



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
Pressure: 7 MPa
Temperature: 23 °C

Sample: 1 = Uracil
2 = Toluene
3 = Acenaphthene
4 = Butylbenzene

Column: SunShell C18, 2.6 µm 100 x 2.1 mm
Mobile phase: CH_3CN/H_2O=60/40
Flow rate: 0.3 mL/min
Pressure: 12.5 MPa
Temperature: 25 °C

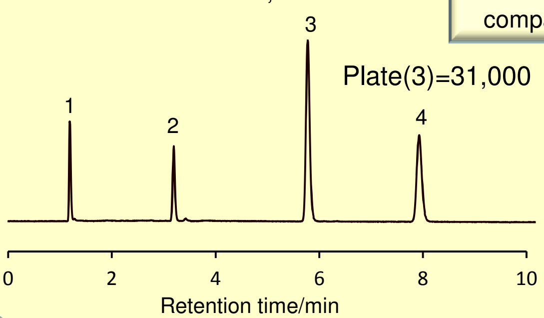
Efficiency=253,000 plate/m

Column: SunShell C18, 2.6 µm 150 x 4.6 mm
SunShell C18, 2.6 µm 100 x 4.6 mm
Mobile phase: CH_3CN/H_2O=70/30
Flow rate: 1.0 mL/min
Pressure: 14.5MPa(UHPLC),13.5 MPa(HPLC) for 150 mm
9.5MPa(HPLC) for 100 mm
Temperature: 25 °C
Sample: 1 = Uracil
2 = Toluene
3 = Acenaphthene
4 = Butylbenzene

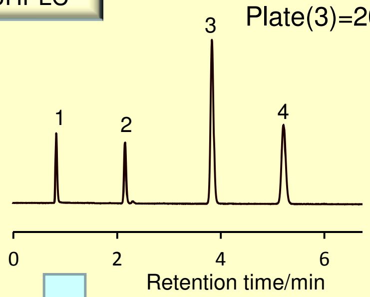
HPLC

80% efficiency to compare with UHPLC

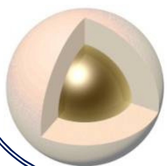
Column: SunShell C18, 150 x 4.6 mm



Column: SunShell C18, 100 x 4.6 mm



LaChrom ELITE

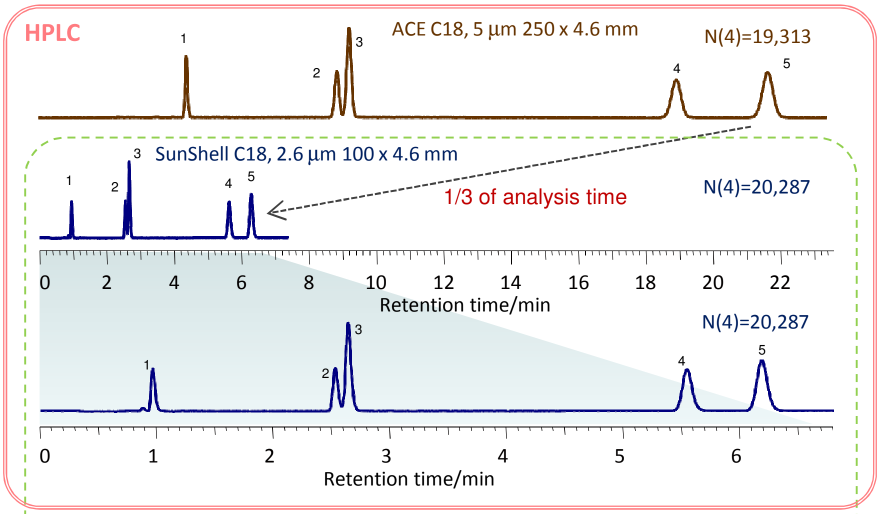


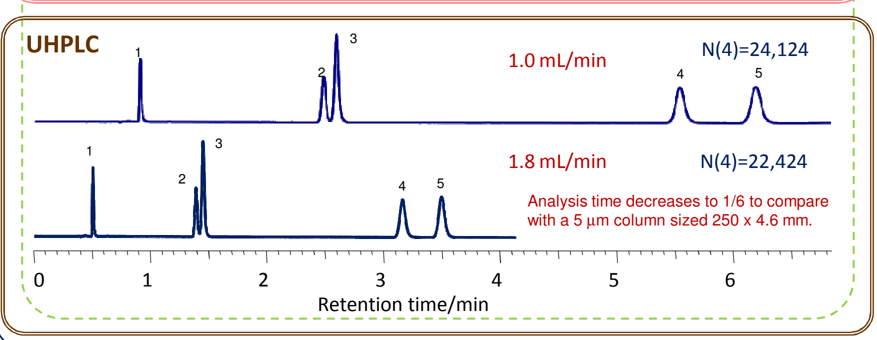
The same efficiency as 5 µm, 250 x 4.6 mm

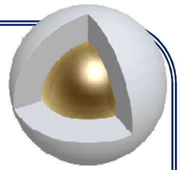
Saving 60% for analytical time and consumption of solvent

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

Isocratic separation





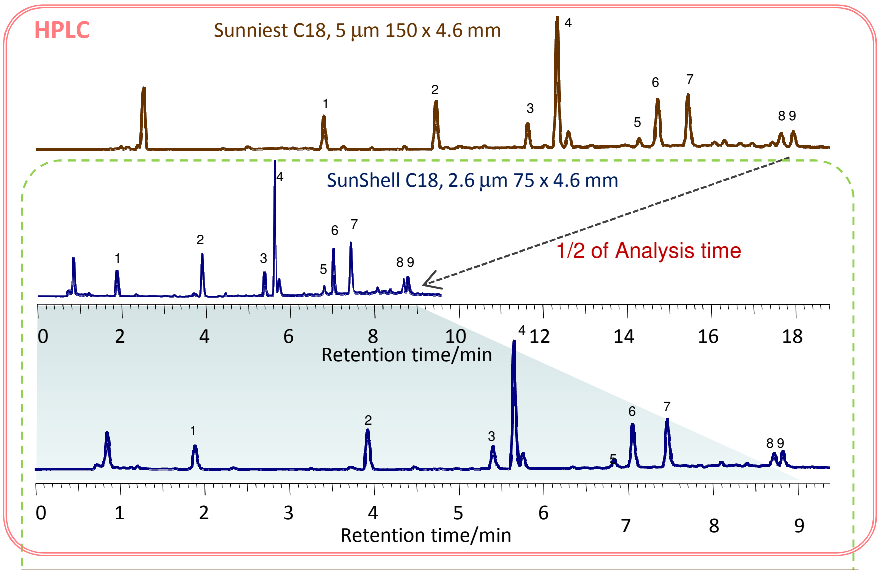


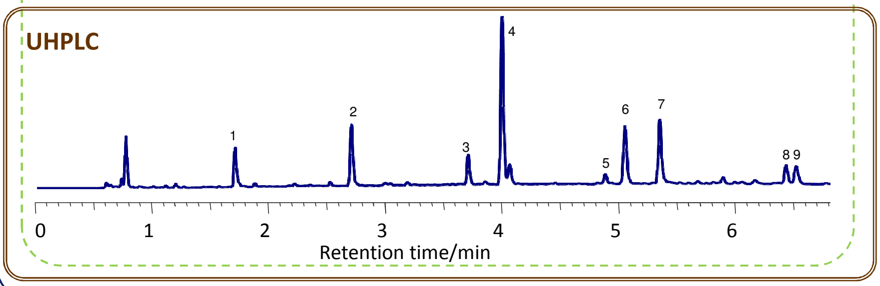
Column:
ACE C18, 5 μm 250 x 4.6 mm
SunShell C18, 2.6 μm 100 x 4.6 mm
Mobile phase:
CH_3CN/20mM Phosphoric acid = 45/55
Flow rate: 1.0 mL/min,
1.8 mL/min at the lowest chromatogram
Temperature: 25 ºC
Pressure: 9.5 MPa for ACE C18 5 μm
13.4 MPa for SunShell C18 2.6 μm
Detection: UV@230 nm

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

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

Gradient separation





Column:
Sunniest C18, 5 μm 150 x 4.6 mm
SunShell C18, 2.6 μm 75 x 4.6 mm
Mobile phase:
A) 0.1% Phosphoric acid
B) CH_3CN
Gradient program for Sunniest C18

0 min	15 min	20 min
2%	25%	25%

for SunShell C18

0 min	7.5 min	10 min
2%	25%	25%

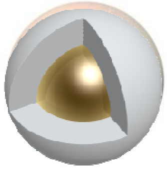
Flow rate: 1.0 mL/min,
Temperature: 25 ºC
Detection: UV@250 nm
Sample: Oolong tea
1 = Gallocatechin, 2 = Epigallocatechin, 3 = Catechin, 4 = Caffeine, 5 = Epicatechin, 6 = Epigallocatechin gallate, 7 = Gallocatechin gallate, 8 = Epicatechin gallate, 9 = Catechin gallate

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

<<Caution>>

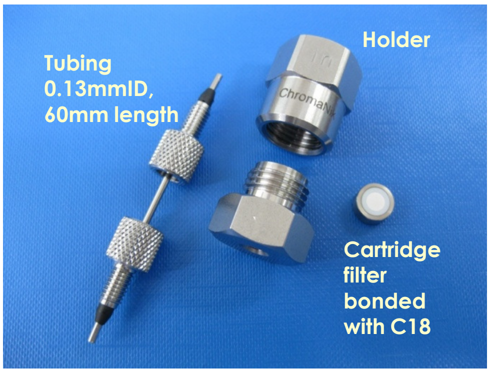
There are difference of system time lag between HPLC and UHPLC. UHPLC has much less system time lag than HPLC because of high pressure gradient system for UHPLC and low pressure gradient system for HPLC.

SunShell RP Guard Filter



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

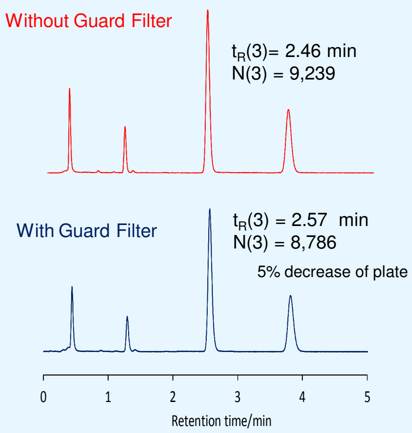
Available as a guard column for reversed phase



- ✓ 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

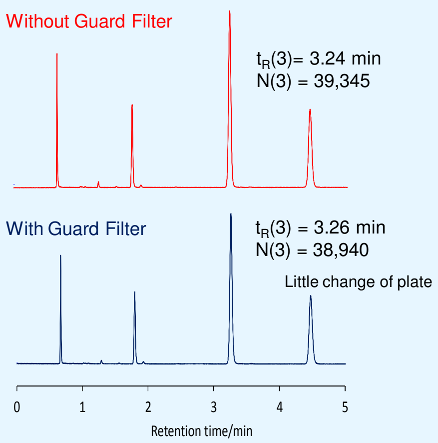
Evaluation of SunShell RP Guard Filter

SunShell C18, 2.6 µm 50 x 2.1 mm

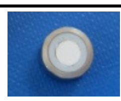
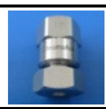


Mobile phase:
CH_3CN/H_2O=60/40 for 2.1 mm i.d.
CH_3CN/H_2O=70/30 for 4.6 mm i.d.
Flow rate:
0.3 mL/min for 2.1 mm i.d.
1.8 mL/min for 4.6 mm i.d.
Temperature: 25 ºC
Detection: UV@250nm
Sample: 1 = Uracil
2 = Toluene
3 = Acenaphthene
4 = Butylbenzene

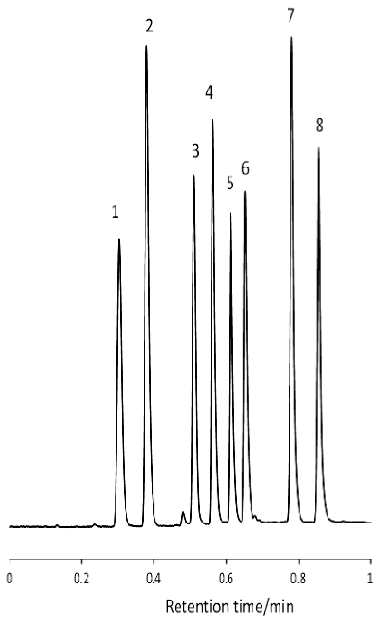
SunShell C18, 2.6 µm 150 x 4.6 mm



Price of SunShell RP Guard Filter

Name	quantity	Part number	Photo
SunShell RP Guard Filter For exchange	5 pieces	CBGAAC	
SunShell RP Guard Filter Holder	1 piece	CBGAAH	

High-throughput separation



Column: SunShell C18 30 x 3.0 mm. Mobile phase: A) Wtaer, B) Acetonitrile; Gradient (Acetonitorile %), 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.

8 kinds of compounds were separated using SunShell C18 30 x 3.0 mm column in one minute.

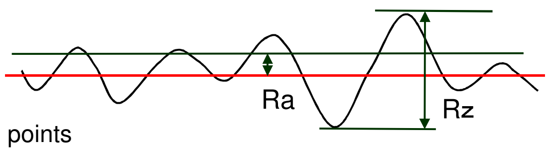
A peak width is just one second!!

Surface Roughness on Inner Surface of Column

Parameter of surface roughness

Ra: Average roughness from center line

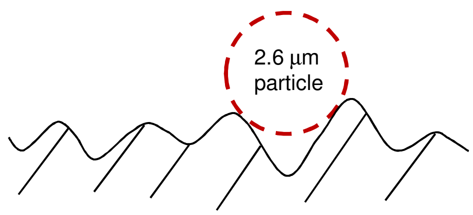
Rz: Roughness calculated from 10 points average (5 points of maximum and 5 points of minimum)



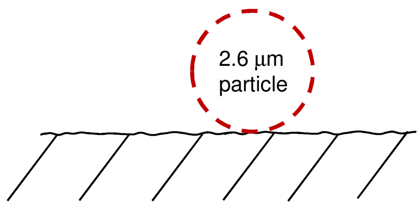
Schematic diagram of surface roughness

	G company	Y company	S1 company	S2 company	W company	ChromaNIk Technologies
Ra	0.34 µm	0.32 µm	0.37 µm	0.03 µm	0.20 µm	0.01 µm
Rz	1.88 µm	1.62 µm	1.91 µm	0.19 µm	0.90 µm	0.10 µm

It is considered that surface roughness affects column performance. Surface asperity of ChromaNik Technologies column with 2.1 mm i.d. and 3.0 mm i.d. is 1/30 to 1/20 to compare with that of GL Sciences, YMC, Shimadzu and Waters columns. ChromaNIk Technologies provides a column with a very smooth surface which is the most suitable for 2.6 µm core shell particle packings.



Inner surface of column of A, B, C companies



Inner surface of column of ChromaNik Technologies

Ordering information

	Inner diameter(mm)	2.1	3.0	4.6
SunShell C18	Length (mm)	Catalog number	Catalog number	Catalog number
	20	CB6921	CB6321	CB6421
	30	CB6931	CB6331	CB6431
	50	CB6941	CB6341	CB6441
	75	CB6951	CB6351	CB6451
	100	CB6961	CB6361	CB6461
	150	CB6971	CB6371	CB6471

	Inner diameter(mm)	2.1	3.0	4.6
SunShell C8	Length (mm)	Catalog number	Catalog number	Catalog number
	20	CC6921	CC6321	CC6421
	30	CC6931	CC6331	CC6431
	50	CC6941	CC6341	CC6441
	75	CC6951	CC6351	CC6451
	100	CC6961	CC6361	CC6461
	150	CC6971	CC6371	CC6471

	Inner diameter(mm)	2.1	3.0	4.6
SunShell PFP	Length (mm)	Catalog number	Catalog number	Catalog number
	20	CF6921	CF6321	CF6421
	30	CF6931	CF6331	CF6431
	50	CF6941	CF6341	CF6441
	75	CF6951	CF6351	CF6451
	100	CF6961	CF6361	CF6461
	150	CF6971	CF6371	CF6471

BIOTECH

Next phases, C18-WP (16 nm), RP-AQUA, Phenyl and HILIC-Amide Coming soon

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