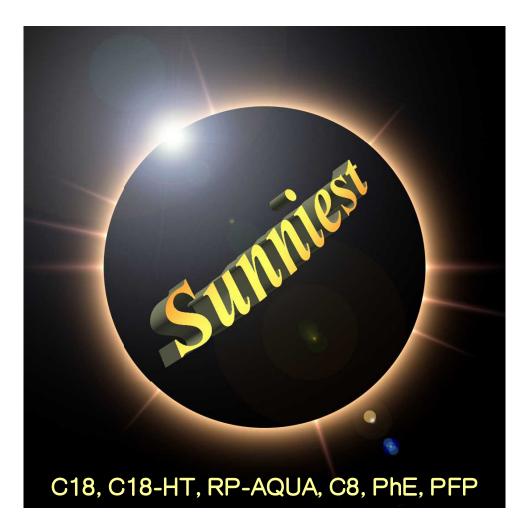




HPLC column

Sunniest



ChromaNik Technologies Inc.



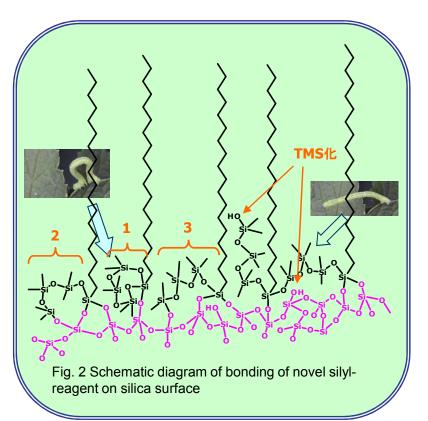
Sunniest C18, C18-HT, RP-AQUA, C8, PhE, PFP

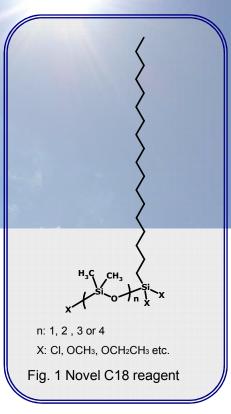
A Novel Bonding Technique

(patent pending)

An unique trifunctional silvl-reagent was developed as shown in Fig. 1. This silvl-reagent can bond with any silanol groups on silica surface as shown in Fig.2 because it can expand and contract by itself. This technique can make residual silanol groups on C18 stationary phase to be the least amount.

Finally an end-capping was done with trimethylsilyl-reagent (TMS).





Features

 \star Little residual silanol groups by an unique bonding technique

 \star Excellent stability, especially under acidic pH conditions

 \star Sharp peak shape for acidic, basic and chelating compounds

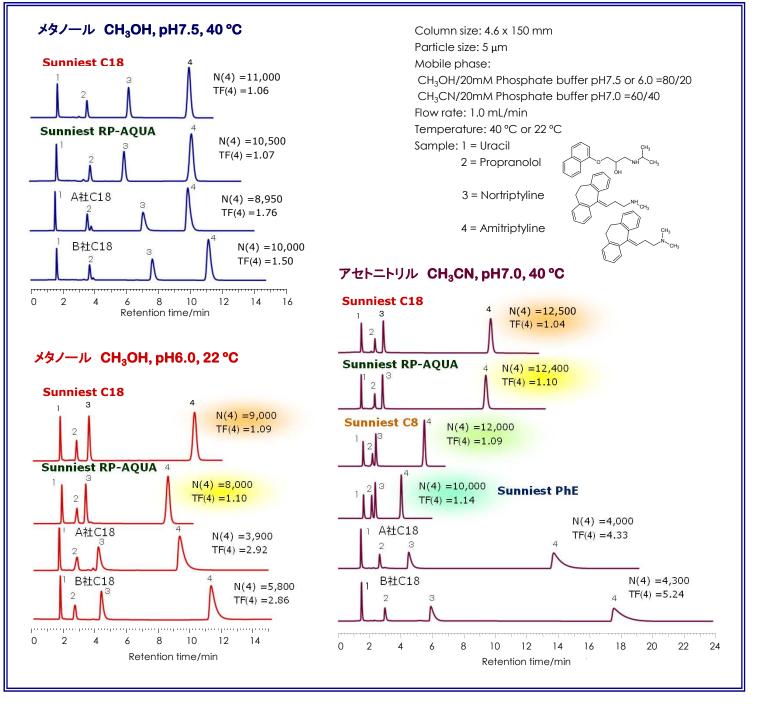
★RP-AQUA is available under 100% aqueous conditions, and shows enhanced retention of polar compounds.

	Particle size (µm)	Pore diameter (nm)	Specific surface area (m²/g)	Carbon content (%)	Bonded phase	pH range
Sunniest C18	3 and 5	12	340	16	C18	1.5 - 10
Sunniest C18-HT	2	10	340	16	C18	1.5 - 10
Sunniest RP-AQUA	3 and 5	12	340	16	C28	2 - 8
Sunniest C8	3 and 5	12	340	10	C8	1.5 - 9
Sunniest PhE	3 and 5	12	340	10	Phenylethyl	1.5 - 8
Sunniest PFP	5	12	340	10	Pentafluorophenyl	2 - 8

Characteristics of Sunniest

Evaluation of End-capping

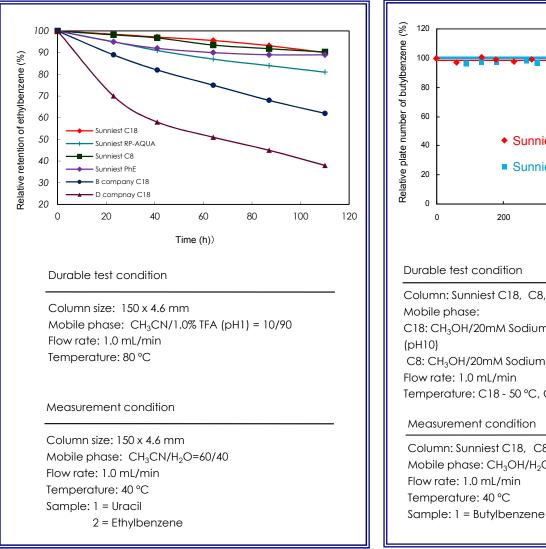
Comparison of plates number (N) and USP tailing factor (TF) of amitriptyline



Amitriptyline is widely used to evaluate residual silanol groups on the C18 stationary phase. Peak shape of amitriptyline was compared under 3 kinds of conditions such as methanol/phosphate buffer/40 °C, methanol/phosphate buffer/22 °C and acetonitrile/phosphate buffer/40 °C. Under methanol/phosphate buffer/40 °C conditions which is the most common among HPLC manufacturers, good peak shape was obtained for all columns. There were little difference of a peak shape. Under acetonitrile/phosphate buffer/40 °C, however, Sunniest columns showed a symmetrical peak, while column A and B C18 showed a terribly tailing peak.

Sunniest C18, RP-AQUA and C8 columns allow to use a wide range of mobile phase without peak tailing because of extremely low content of residual silanol groups on the stationary phase.

Stability under acidic and basic pH conditions



Stability under acidic pH conditions was evaluated at 80 °C 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⁾

 \star Sunniest C18 has kept 90% retention for 100 hours under such severe conditions.

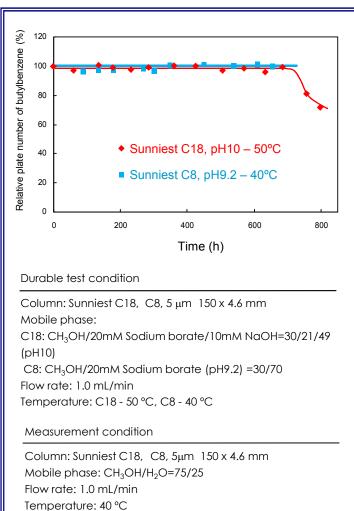
Our bonding technique can make column life be long.

Sunniest RP-AQUA is less stable than Sunniest C18. However, Sunniest RP-AQUA has more stable than other company C18 columns.



2) T. Enami and N. Nagae, American Laboratory October 2004.

3) T. Enami and N. Nagae, BUNSEKI KAGAKU, 53 (2004) 1309.



Stability under basic pH conditions was evaluated at 50 °C 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.

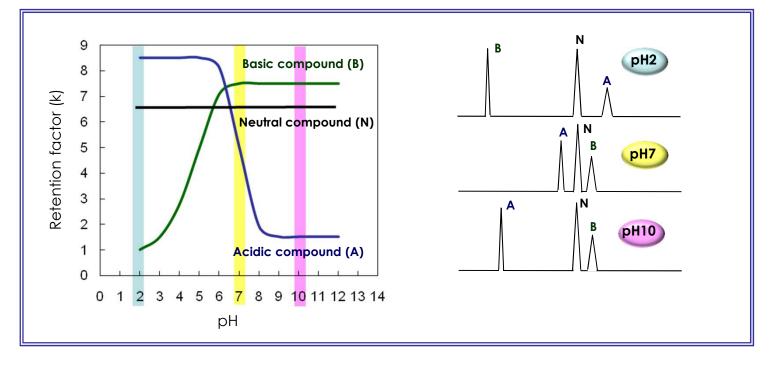
Elevated temperature of 10 °C makes column life be one third. When Sunniest C18 column is used at 40 °C, column life becomes 2,000 hours. Other company shows stability test at ambient (room temperature). If room temperature is 25 °C, column life at room temperature (25 °C) is sixteen times longer than that at 50 °C.

★ Sunniest 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 Sunniest C18 except for hybrid type C18. It is considered that our end-capping technique leads high stability.

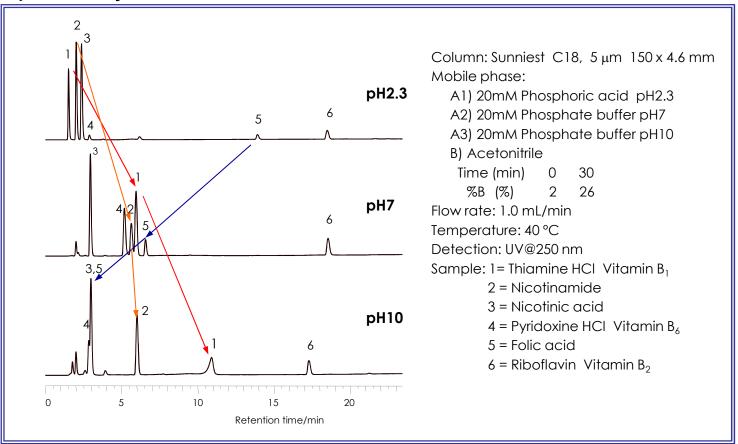
★ Sunniest C18 can be used at the pH range from 1.5 to 10. Sunniest C8 can be used at the pH range from 1.5 to 9.



Relationship between pH and retention of acidic, basic and neutral compounds



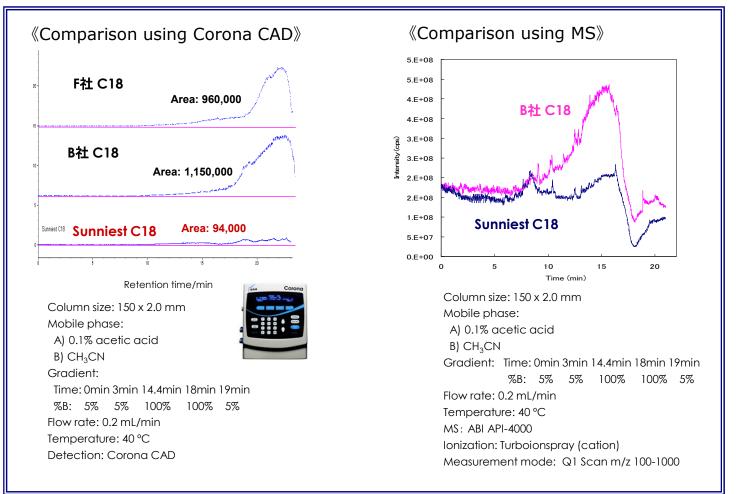
PH selectivity



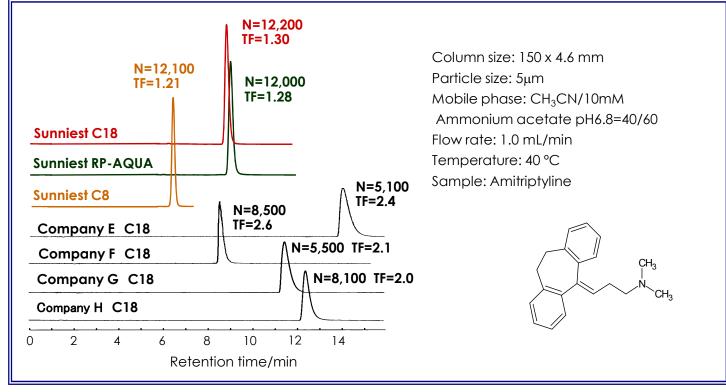
pH of mobile phase can make selectivity of ionic compounds change much. Sunniest C18 can be used at the pH range from 1.5 to 10, so that a suitable analytical method can be created using Sunniest C18.



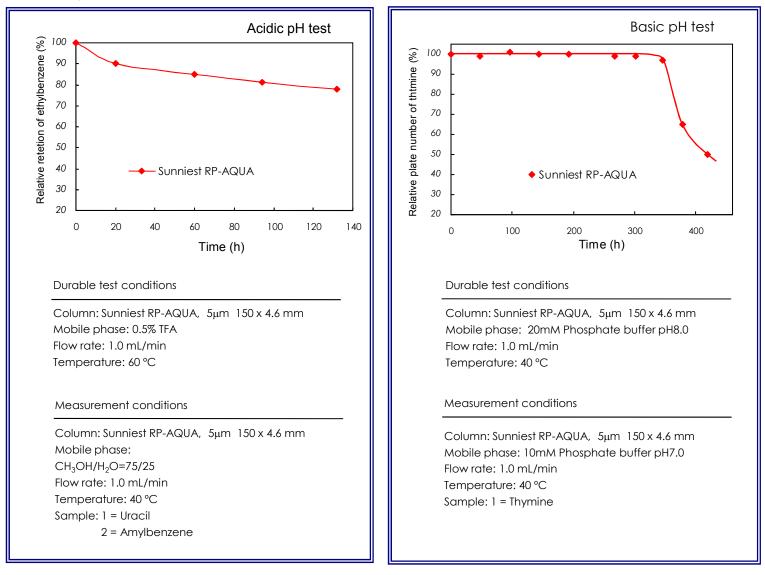
Comparison data: Bleeding from column



Separation of antidepressants using acetonitrile and ammonium acetate for LC/MS



Stability of Sunniest RP-AQUA under 100% aqueous conditions



It is important that stability is evaluated under 100% aqueous conditions for an AUQA type C18 column because column life becomes longer to increase a content of organic solvent in a mobile phase. Sunniest RP-AQUA can be used under 100% aqueous conditions from pH2 to pH8.

 \star Sunniest RP-AQUA can be used under 100% aqueous conditions from pH 2 to pH 8. Sunniest RP-AQUA is one of the most stable aqua type column.





Reproducibility of retention under 100% aqueous conditions

★ C18 and C8 reversed phases exhibit decreased and poorly reproducible retention under more than 98% aqueous conditions as shown in Fig. 1. This problem traditionally has been explained as being the result of ligand collapse or a matting effect. Nagae¹⁻³ ascertained, however, that the mobile phase was being expelled from the pores of the packing material under 100% aqueous mobile phase conditions, as Fig. 2 shows.

★ When the surface of packing materials isn't wet by water, water used as a mobile phase expels from the pore of the packing material by capillarity. This is a reason why reproducibility in retention is low under 100% aqueous conditions. Reversely pressure around the packing material makes water permeate into the pore of the packing material to overcome a force worked by capillarity.

In other words, the surface of a reversed phase like C18 isn't wet by water anytime even if water permeates into the pore of the packing material or not. So it is wrong that we say "dewetting" when water expel from the pore. Saying "Depermetating" is more suitable.

★ Sunniest RP-AQUA is a reversed stationary phase, so that it is not wet with water. However the contact angle of water on the surface of Sunniest RP-AQUA is less than that of a conventional C18. Expelling force (pressure) acted by capillarity on Sunniest RP-AQUA is less than atmospheric pressure. So, Sunniest RP-AQUA shows reproducible retention under 100% aqueous conditions.

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

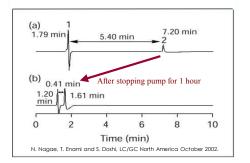


Fig. 1 Retention behavior of a C18 column under 100% aqueous mobile phase conditions

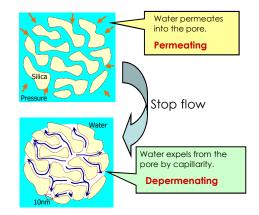
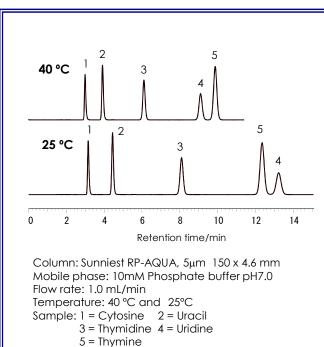
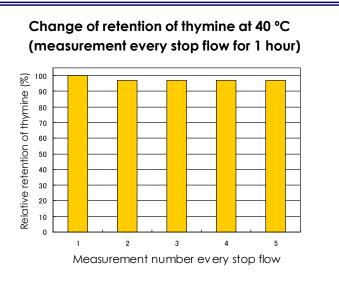


Fig. 2 Schematic diagram of C18 particle

2) T. Enami and N. Nagae, American Laboratory October 2004.

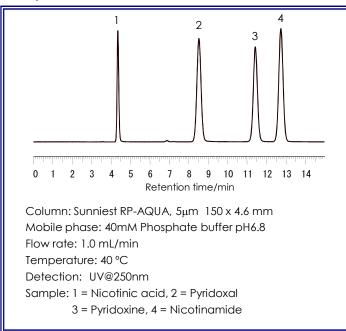


Separation of nucleic acid bases

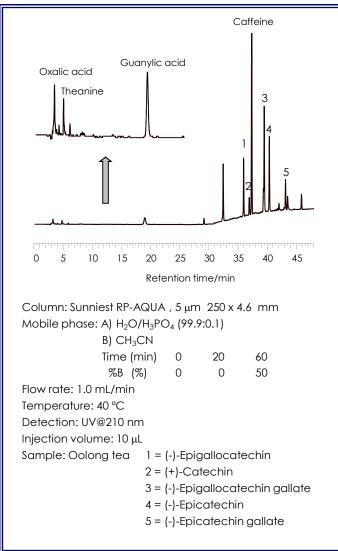


Sunniest RP-AQUA showed more than 97% of reproducibility in retention using 100% aqueous buffer as a mobile phase.

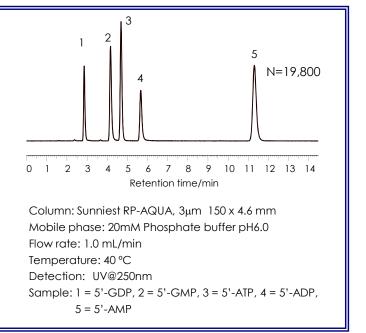
Separation of water-soluble vitamins



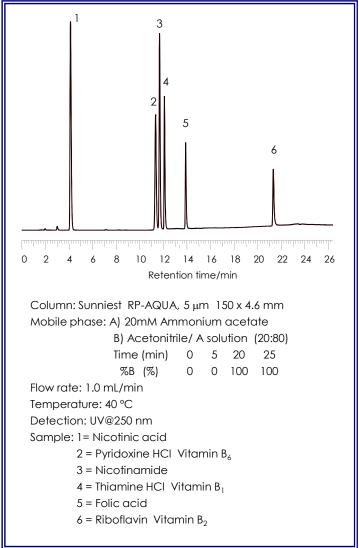
separation of Oolong tea



Separation of nucleotides

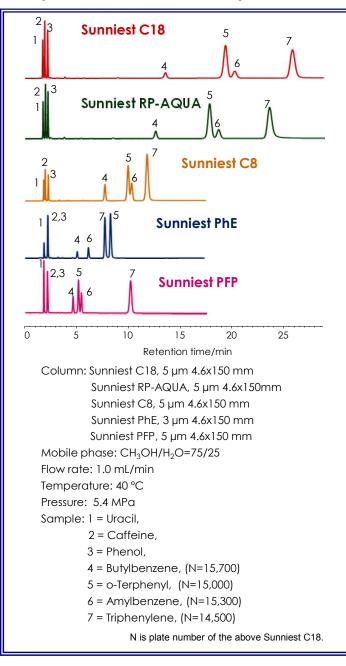


Separation of water-soluble vitamins using mobile phase for LC/MS



8

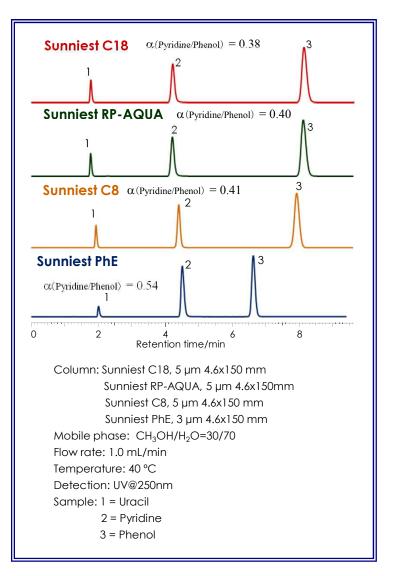
Separation of standard samples



	C18	RP-AQUA	C8	PhE	PFP
Hydrophobicity:					
α (Amylbenzene/Butylbenzene)	1.56	1.56	1.43	1.34	1.29
Hydrogen bonding capacity:					
α (Caffeine/Phenol)	0.43	0.49	0.33	1.00	1.00
Steric selectivity:					
α (Triphenylene/o-Terphenyl)	1.37	1.36	1.23	0.92	2.51

Sunniest C18 shows not only high efficiency but also low column pressure.

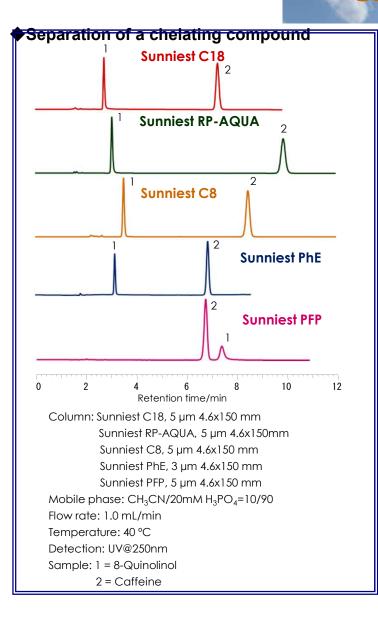
Separation of pyridine and phenol



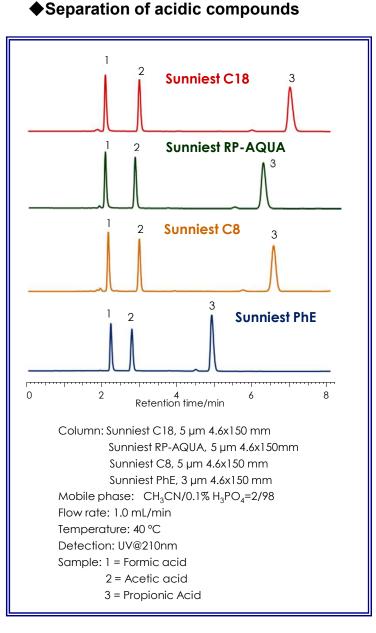
Separation factor of pyridine and phenol is said to show the amount of residual silanol groups. The lower a value of separation factor, the less an effect of residual silanol groups.

All Sunniest columns show one of the lowest value.

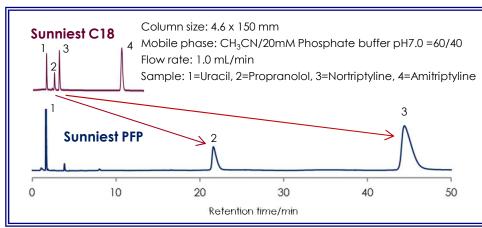




★Sunniest C18, RP-AQUA, C8, PhE and PFP are inert for a metal chelating compound and acidic and basic compounds, so that they show symmetrical peaks of each compound.



Retention comparison between C18 and PFP



 \star PFP retains a cation such a nortriptyline much longer than a C18.





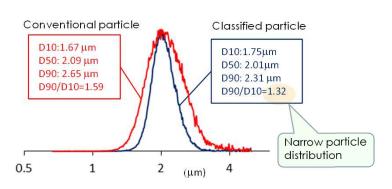
Sunniest C18-HT, 2 µm

Features

- Low back pressure and high efficiency by precisely classified particle
- High pressure packing (10,000 psi) using hard silica gels with high pressure resistant
- leads long column life without any void.
- Unique bonding technique for Sunniest
- The most suitable inner surface of column by special arinding

Narrow Particle Distribution and Low Back Pressure

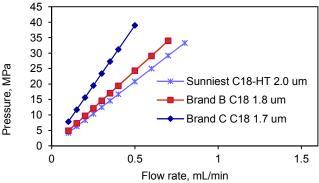
Measured by Coulter Counter method



Conventional 2 µm silica gel particle was classified again. 20% volume was cut off from both sides respectively. Consequently column back pressure reduced more than 15%. Our 2 µm silica gel particle shows a half pressure to compare with the other sub-2 μ m silica gel particle.

An Unique Modification Leads Good Peak Shape

Comparison of back pressure



Column: Sunniest, Acquity and Zorbax Column dimension: 50 x 2.1 mm Mobile phase: Acetonitrile/water=(70/30) Temperature: 25 °C

Amitriptyline (4th peak) Neutral compounds N₍₄₎ = 12,517 pl/m $TF_{(4)} = 1.29$ Sunniest C18-HT Pressure = 16.8 MPa 2μm 10 (min) 15 4 (min) 3 3 N₍₄₎ = 8,717 pl/m $TF_{(4)} = 1.47$ Pressure = 25.2 MPa Acquity C18 1.7µm 2 15 3 4 (min) 5 10 (min) 4 N₍₄₎ = 7,191 pl/m A half of retention Leading C18 $TF_{(4)} = 2.18$ Pressure = 10.6MPa 2.5µm 3 10 (min) 15 2 4 (min)

Column dimension: 50 x 2.1 mm

Mobile phase: Methanol/water=70/30 for neutral compounds

Methanol/25mM phosphate buffer (pH6.0)=80/20 for antidepressants Flow rate: 0.2 mL/min

Temperature: room temperature

Sample: Neutral compounds, 1=Uracil, 2=Toluene, 3=Biphenyl, 4=Penanthrene Antidepressants, 1=Nortriptyline, 2=Toluene, 3= Imipramine,

4=Amitriptyline

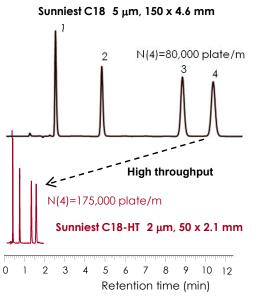
particle as well as 3 µm or 5 µm silica gel particle. Most sub 2 μ m or 2 μ m C18 columns show smaller plate number and larger tailing factor for a basic compound than Sunniest C18-HT. Sunniest C18-HT 2 µm shows a good peak shape for amitriptyline under methanol/phosphate buffer mobile phase at room temperature. Furthermore Sunniest C18-HT 2 µm shows 2 times longer retention time than the other brand columns.

It is difficult to end-cap on sub $2 \mu m$ or $2 \mu m$ silica gel



Sunniest C18-HT, 2 µm

• Separation of Analgesics

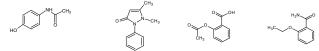


Mobile phase: CH₃CN/0.1% Formic acid = 20/80 Flow rate: 1.0 mL/min for 150 x 4.6 mm 0.6 mL/min for 50 x 2.1 mm

Temperature: 40 °C

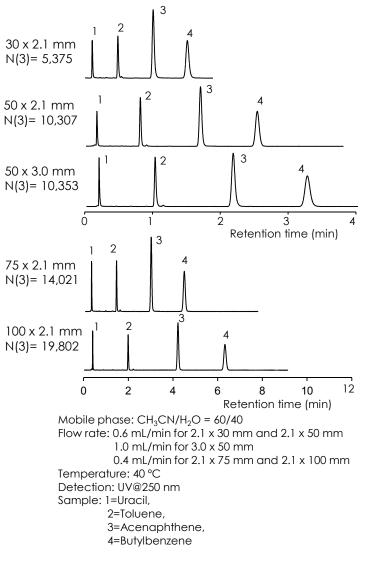
Detection: UV@230 nm Sample:

1=Acetaminophen, 2=Antipyrine, 3=Aspirin, 4=Ethenzamide



 $2 \ \mu m$ particle allows to reduce retention time because high efficiency is kept under high flow rate conditions. As shown the above chromatograms, analytical time reduced 1/8 without sacrifices of separation by using 2 μm , 50 x 2.1 mm column instead of 5 μm 150 x 4.6 mm column.

Comparison of Plate Number



- Characteristics of Sunniest C18-HT, 2 μm

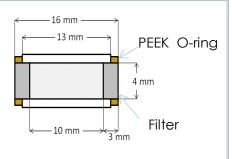
Silica gel support			C18				
Packings	Particle size (µm)	Pore diameter nm)	Specific surface area (m²/g)	Carbon content (%)	Bonded phase	Maximum operating pressure	Available pH range
Sunniest C18-HT	2.0 (Coulter counter)	10	340	16	C18	70 MPa or 10,000 psi	1.5 - 10

It is very important for 2 μ m particle to have a capacity to resist pressure because of high column back pressure. The larger a pore volume of silica gel, the weaker a capacity to resist pressure. The silica gel with 0.85 ml/g of pore volume is used for Sunniest C18-HT, 2 μ m, so that it have a high capacity to resist pressure and a high operating pressure.



Guard Cartridge (10 x 4 mm)

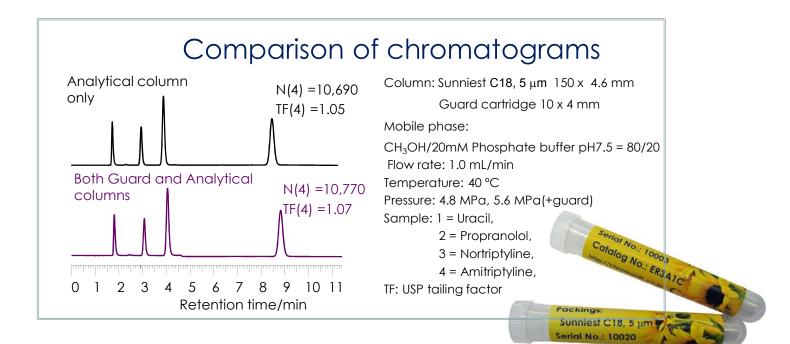
Feature *Simple structure *Low dead volume *Available for not only 5 µm column but also 3 µm column



Schematic Diagram of Cartridge 10x4mm



Photo of Cartridge and Holder



	Particle size	Catalog No.
Sunniest C18, 5 μ m Guard cartridge column (1-pak + Holder) 4 x 10 mm	5 μm	EB3A1H
Sunniest RP-AQUA, 5 μm Guard cartridge column (1-pak + Holder) 4 x 10 mm	5 μm	ER3A1H
Sunniest C8, 5 μm Guard cartridge column (1-pak + Holder) 4 x 10 mm	5 μm	EC3A1H
Sunniest C18, 5 μm Guard cartridge (4-pak) 4 x 10 mm	5 μm	EB3A1C
Sunniest RP-AQUA, 5 μm Guard cartridge (4-pak) 4 x 10 mm	5 μm	ER3A1C
Sunniest C8, 5 μm Guard cartridge (4-pak) 4 x 10 mm	5 μm	EC3A1C
Sunniest Guard cartridge holder 4 x 10 mm		HOLA1C

* Sunniest Ordering information

Inner diameter	Length	Sunniest C18, 3µm	Sunniest C18, 5µm	Sunniest RP- AQUA, 3µm	Sunniest RP- AQUA, 5µm	Sunniest C8, 3µm	Sunniest C8, 5µm
[mm]	[mm]	Catalog No.	Catalog No.	Catalog No.	Catalog No.	Catalog No.	Catalog No.
2	50	EB2241	EB3241	ER2241	ER3241	EC2241	EC3241
	75	EB2251	—	ER2251	-	EC2251	—
	100	EB2261	EB3261	ER2261	ER3261	EC2261	EC3261
	150	EB2271	EB3271	ER2271	ER3271	EC2271	EC3271
	250	EB2281	EB3281	ER2281	ER3281	EC2281	EC3281
3	50	EB2341	EB3341	ER2341	ER3341	EC2341	EC3341
	100	EB2361	EB3361	ER2361	ER3361	EC2361	EC3361
	150	EB2371	EB3371	ER2371	ER3371	EC2371	EC3371
	250	EB2381	EB3381	ER2381	ER3381	EC2381	EC3381
4.6	10	EB2411	EB3411	ER2411	ER3411	EC2411	EC3411
	50	EB2441	EB3441	ER2441	ER3441	EC2441	EC3441
	75	EB2451	—	ER2451	—	EC2451	—
	100	EB2461	EB3461	ER2461	ER3461	EC2461	EC3461
	150	EB2471	EB3471	ER2471	ER3471	EC2471	EC3471
	250	EB2481	EB3481	ER2481	ER3481	EC2481	EC3481
10	250	—	EB3781		ER3781	-	EC3781
20	50	-	EB3841	-	ER3841	-	EC3841
	150	—	EB3871	—	ER3871	—	EC3871
	250	_	EB3881	_	ER3881	_	EC3881

Inner diameter	Length	Sunniest PhE, 3µm	Sunniest PhE, 5µm	Sunniest PFP, 5µm
[mm]	[mm]	Catalog No.	Catalog No.	Catalog No.
2.0	50	EP2241	EP3241	_
	75	EP2251	—	—
	100	EP2261	EP3261	—
	150	EP2271	EP3271	—
	250	EP2281	EP3281	_
3.0	50	EP2341	EP3341	_
	100	EP2361	EP3361	—
	150	EP2371	EP3371	—
	250	EP2381	EP3381	—
4.6	10	_	EP3411	_
	50	EP2441	EP3441	EF3441
	75	EP2451	_	_
	100	EP2461	EP3461	EF3461
	150	EP2471	EP3471	EF3471
	250	EP2481	EP3481	EF3481
10.0	250	_	EP3781	_
20.0	50	_	EP3841	_
	150	_	EP3871	_
	250	—	EP3881	-

Inner diameter	Length	Sunniest C18-HT, 2µm
[mm]	[mm]	Catalog No.
2.1	20	EB1921
	30	EB1931
	50	EB1941
	75	EB1951
	100	EB1961
3.0	20	EB1321
	30	EB1331
	50	EB1341
	75	EB1351
	100	EB1361

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