ChromaNik Technologies Inc.

HPLC column

Sunniest C18

Sunniest RP-AQUA

Sunniest C8

Patent pending

Biotech AB in partnership with ChromaNik Technologies

ChromaNik Technologies Inc.

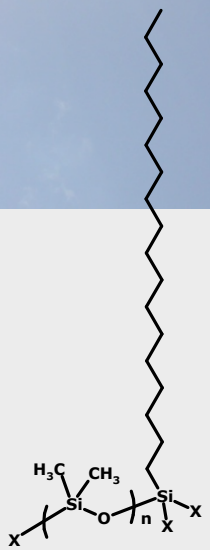
Sunniest C18
Sunniest RP-AQUA
Sunniest C8

A Novel Bonding Technique

(patent pending)

An unique trifunctional silyl-reagent was developed as shown in Fig. 1. This silyl-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).



n: 1, 2 , 3 or 4

X: Cl, OCH_3, OCH_2CH_3 etc.

Fig. 1 Novel C18 reagent

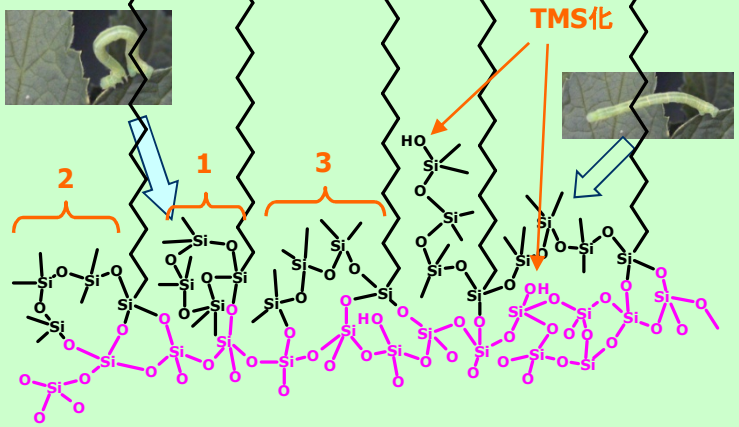


Fig. 2 Schematic diagram of bonding of novel silyl-reagent on silica surface

Features

★ Little residual silanol groups by an unique bonding technique

★Excellent stability, especially under acidic pH conditions

★ Sharp peak shape for acidic, basic and chelating compounds

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

Characteristics of Sunniest

	Particle size (μm)	Pore diameter (nm)	Specific surface area (m^2/g)	Carbon content (%)	Bonded phase	pH range
Sunniest C18	3 and 5	12	340	16	C18	1.5 - 10
Sunniest RP-AQUA	3 and 5	12	340	16	Near C18	2 - 8
Sunniest C8	3 and 5	12	340	10	C8	1.5 - 9

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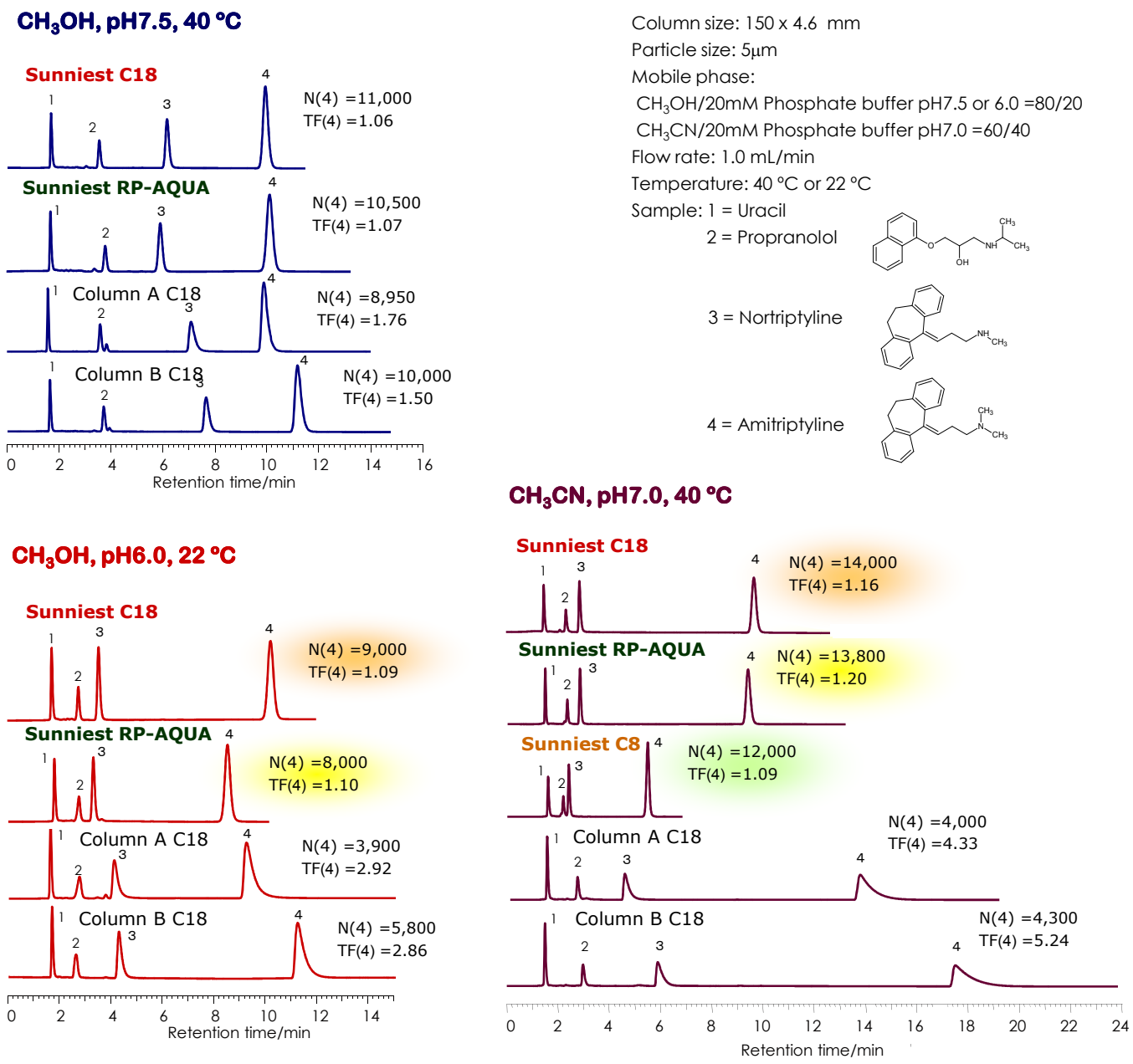
◆Evaluation of End-capping

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.

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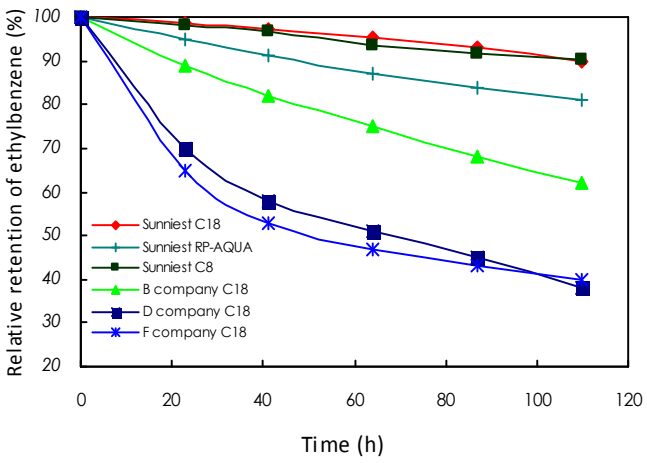
Sunniest C8

◆Stability under acidic and basic pH conditions

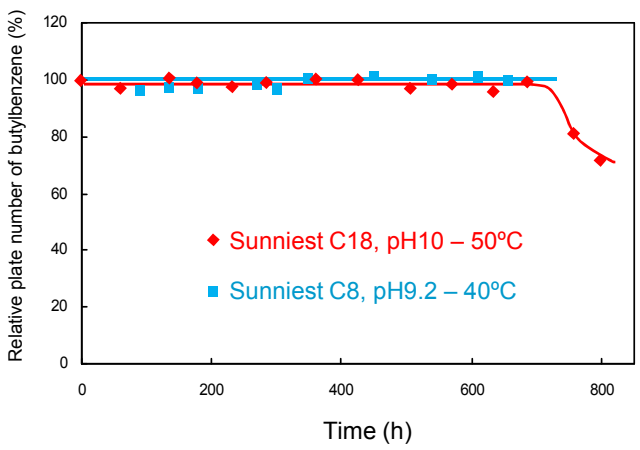


Durable test condition

Column size: 150 x 4.6 mm
Mobile phase: CH_3CN/1.0% TFA (pH1) = 10/90
Flow rate: 1.0 mL/min
Temperature: 80 °C

Measurement condition

Column size: 150 x 4.6 mm
Mobile phase: CH_3CN/H_2O=60/40
Flow rate: 1.0 mL/min
Temperature: 40 °C
Sample: 1 = Uracil
2 = Ethylbenzene



Durable test condition

Column: Sunniest C18, C8, 5 µm 150 x 4.6 mm
Mobile phase:
C18: CH_3OH/20mM Sodium borate/10mM NaOH=30/21/49 (pH10)
C8: CH_3OH/20mM Sodium borate (pH9.2) =30/70
Flow rate: 1.0 mL/min
Temperature: C18 - 50 °C, C8 - 40 °C

Measurement condition

Column: Sunniest C18, C8, 5µm 150 x 4.6 mm
Mobile phase: CH_3CN/H_2O=75/25
Flow rate: 1.0 mL/min
Temperature: 40 °C
Sample: 1 = Butylbenzene

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)]

★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.

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.

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.

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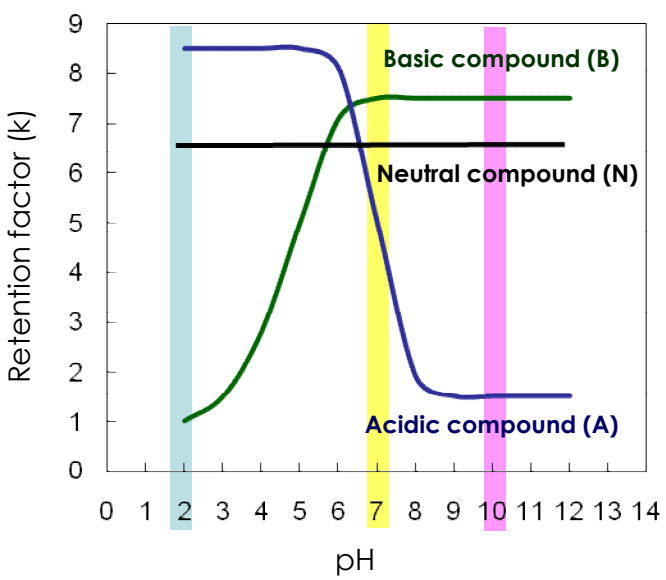
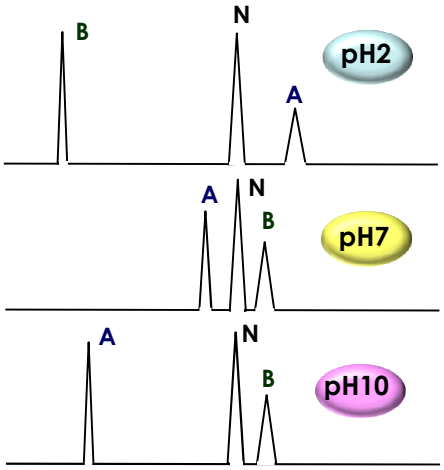
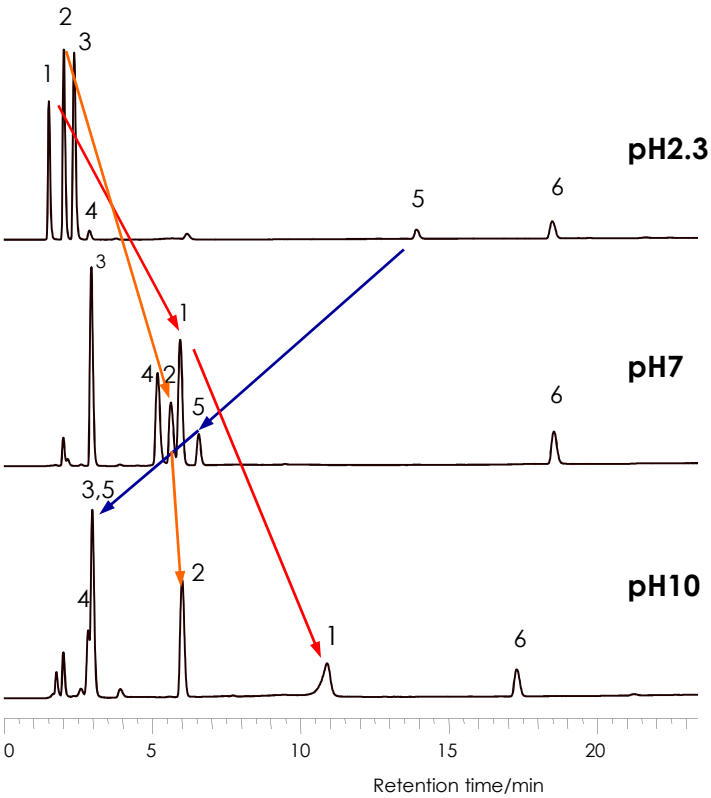
Sunniest C8

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





◆pH selectivity



Column: Sunniest C18, 5 μm 150 x 4.6 mm
Mobile phase:
A1) 20mM Phosphoric acid pH2.3
A2) 20mM Phosphate buffer pH7
A3) 20mM Phosphate buffer pH10
B) Acetonitrile

Time (min)	0	30
%B (%)	2	26

Flow rate: 1.0 mL/min
Temperature: 40 °C
Detection: UV@250 nm
Sample: 1= Thiamine HCl Vitamin B_1
2 = Nicotinamide
3 = Nicotinic acid
4 = Pyridoxine HCl Vitamin B_6
5 = Folic acid
6 = Riboflavin Vitamin B_2

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.

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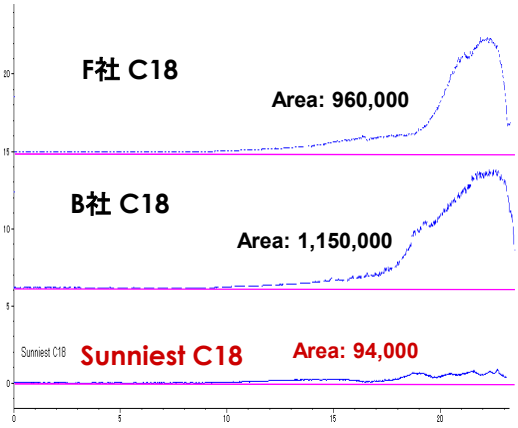
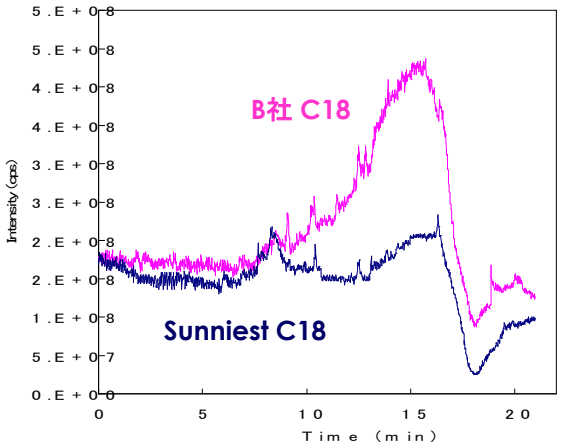
◆Comparison data: Bleeding from column

《Comparison using Corona CAD》



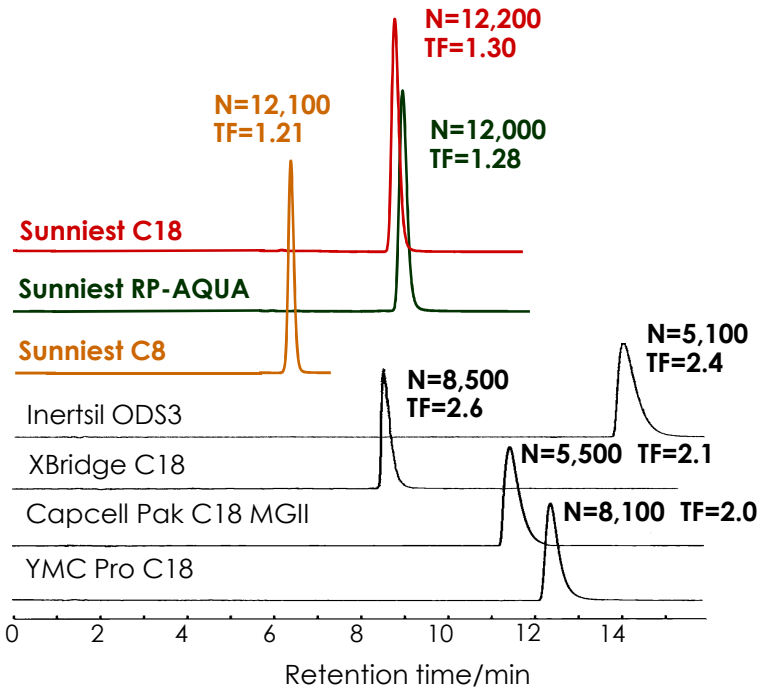
Column size: 150 x 2.0 mm
Mobile phase:
A) 0.1% acetic acid
B) CH_3CN
Gradient:
Time: 0min 3min 14.4min 18min 19min
%B: 5% 5% 100% 100% 5%
Flow rate: 0.2 mL/min
Temperature: 40 °C
Detection: Corona CAD

《Comparison using MS》

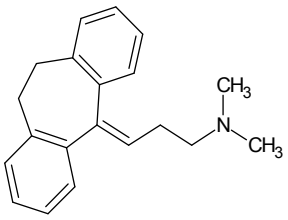


Column size: 150 x 2.0 mm
Mobile phase:
A) 0.1% acetic acid
B) CH_3CN
Gradient: Time: 0min 3min 14.4min 18min 19min
%B: 5% 5% 100% 100% 5%
Flow rate: 0.2 mL/min
Temperature: 40 °C
MS: ABI API-4000
Ionization: Turboionspray (cation)
Measurement mode: Q1 Scan m/z 100-1000

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



Column size: 150 x 4.6 mm
Particle size: 5μm
Mobile phase: CH_3CN/10mM
Ammonium acetate pH6.8=40/60
Flow rate: 1.0 mL/min
Temperature: 40 °C
Sample: Amitriptyline



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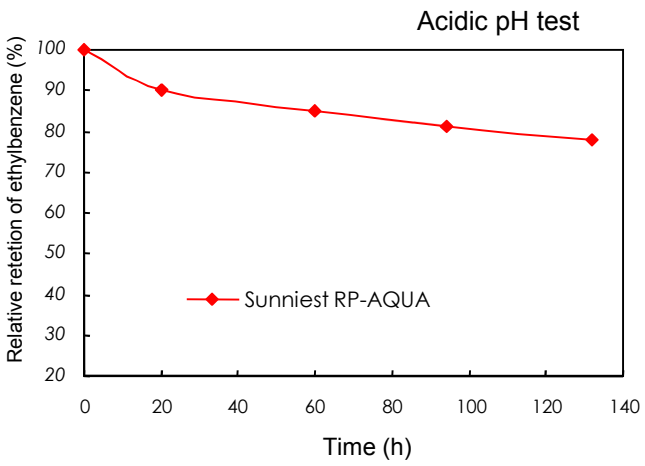
Sunniest RP-AQUA
Sunniest C8

◆Stability of Sunniest RP-AQUA under 100% aqueous conditions

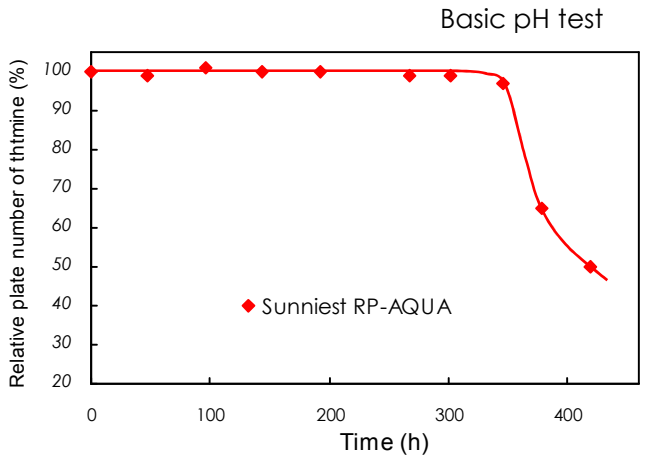


Durable test conditions

Column: Sunniest RP-AQUA, 5µm 150 x 4.6 mm
Mobile phase: 0.5% TFA
Flow rate: 1.0 mL/min
Temperature: 60 °C

Measurement conditions

Column: Sunniest RP-AQUA, 5µm 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 = Amylbenzene



Durable test conditions

Column: Sunniest RP-AQUA, 5µm 150 x 4.6 mm
Mobile phase: 20mM Phosphate buffer pH8.0
Flow rate: 1.0 mL/min
Temperature: 40 °C

Measurement conditions

Column: Sunniest RP-AQUA, 5µm 150 x 4.6 mm
Mobile phase: 10mM Phosphate buffer pH7.0
Flow rate: 1.0 mL/min
Temperature: 40 °C
Sample: 1 = Thymine

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.

★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.

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Sunniest C18
Sunniest RP-AQUA
Sunniest C8

◆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. Nagae[1-3] 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.

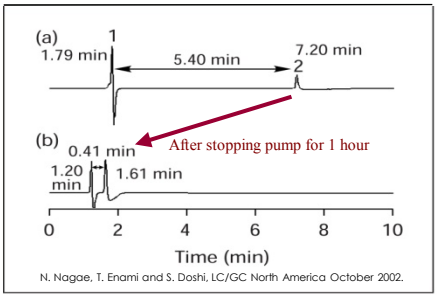


Fig. 1 Retention behavior of a C18 column under 100% aqueous mobile phase conditions

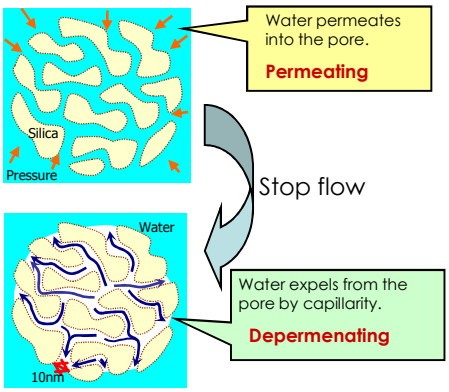
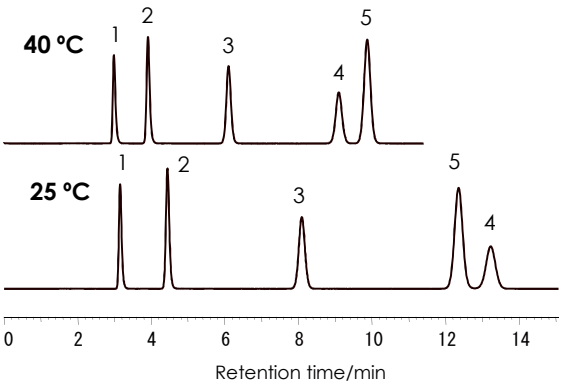


Fig. 2 Schematic diagram of C18 particle

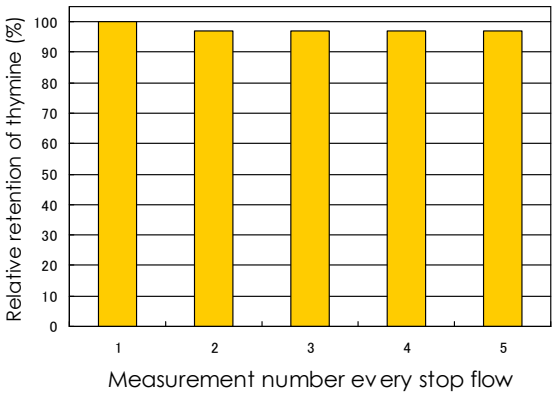
1) N. Nagae, T. Enami and S. Doshi, LC/GC North America October 2002.
2) T. Enami and N. Nagae, American Laboratory October 2004.
3) T. Enami and N. Nagae, BUNSEKI KAGAKU, 53 (2004) 1309.

◆Separation of nucleic acid bases



Column: Sunniest RP-AQUA, 5µm 150 x 4.6 mm
Mobile phase: 10mM Phosphate buffer pH7.0
Flow rate: 1.0 mL/min
Temperature: 40 °C and 25°C
Sample: 1 = Cytosine 2 = Uracil
3 = Thymidine 4 = Uridine
5 = Thymine

Change of retention of thymine at 40 °C (measurement every stop flow for 1 hour)



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

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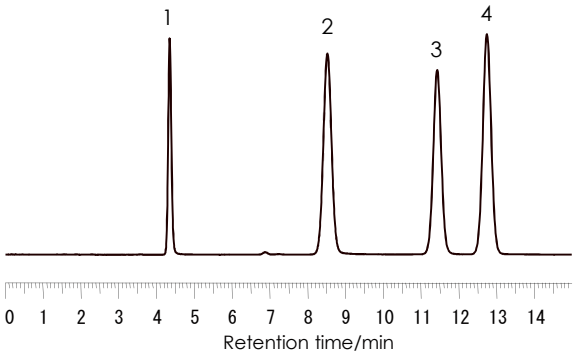
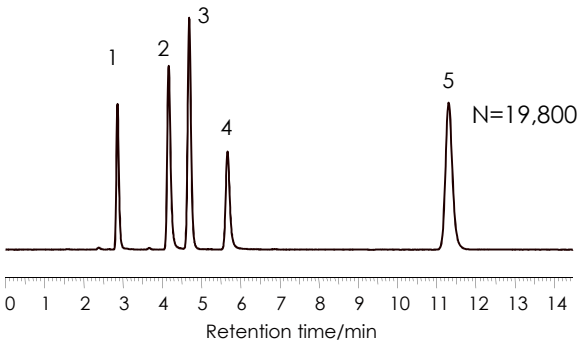
Sunniest RP-AQUA
Sunniest C8

◆Separation of water-soluble vitamins



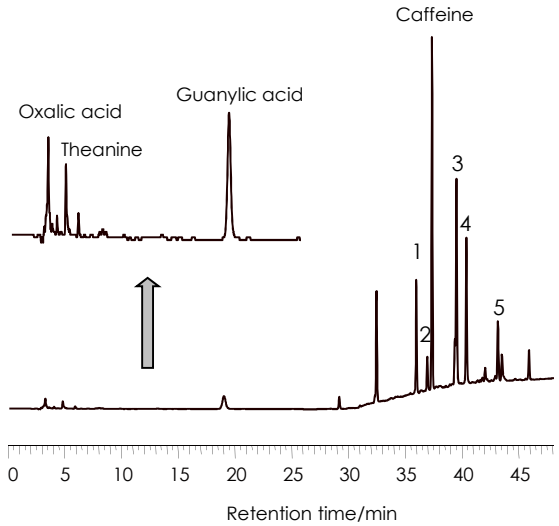
Column: Sunniest RP-AQUA, 5µm 150 x 4.6 mm
Mobile phase: 40mM Phosphate buffer pH6.8
Flow rate: 1.0 mL/min
Temperature: 40 °C
Detection: UV@250nm
Sample: 1 = Nicotinic acid, 2 = Pyridoxal
3 = Pyridoxine, 4 = Nicotinamide

◆Separation of nucleotides



Column: Sunniest RP-AQUA, 3µm 150 x 4.6 mm
Mobile phase: 20mM Phosphate buffer pH6.0
Flow rate: 1.0 mL/min
Temperature: 40 °C
Detection: UV@250nm
Sample: 1 = 5'-GDP, 2 = 5'-GMP, 3 = 5'-ATP, 4 = 5'-ADP,
5 = 5'-AMP

◆separation of Oolong tea

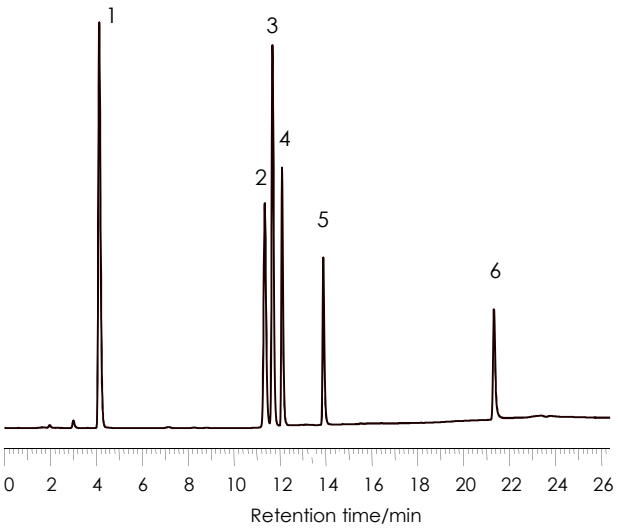


Column: Sunniest RP-AQUA , 5 µm 250 x 4.6 mm
Mobile phase: A) H_2O/H_3PO_4 (99.9:0.1)
B) CH_3CN

Time (min)	0	20	60
%B (%)	0	0	50

Flow rate: 1.0 mL/min
Temperature: 40 °C
Detection: UV@210 nm
Injection volume: 10 µL
Sample: Oolong tea 1 = (-)-Epigallocatechin
2 = (+)-Catechin
3 = (-)-Epigallocatechin gallate
4 = (-)-Epicatechin
5 = (-)-Epicatechin gallate

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



Column: Sunniest RP-AQUA, 5 µm 150 x 4.6 mm
Mobile phase: A) 20mM Ammonium acetate
B) Acetonitrile/ A solution (20:80)

Time (min)	0	5	20	25
%B (%)	0	0	100	100

Flow rate: 1.0 mL/min
Temperature: 40 °C
Detection: UV@250 nm
Sample: 1= Nicotinic acid
2 = Pyridoxine HCl Vitamin B_6
3 = Nicotinamide
4 = Thiamine HCl Vitamin B_1
5 = Folic acid
6 = Riboflavin Vitamin B_2

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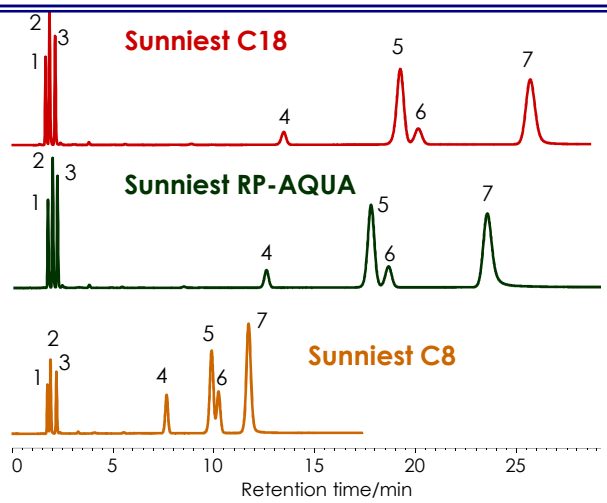
◆Separation of standard samples



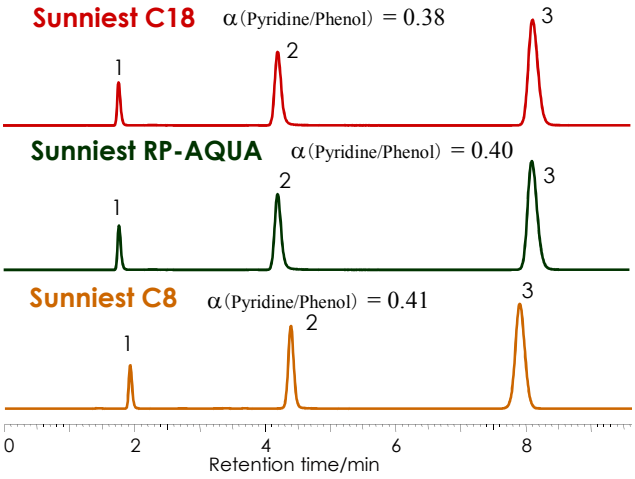
Column: Sunniest C18, 5μm 150 x 4.6 mm
Sunniest RP-AQUA, 5μ m 150 x 4.6 mm
Sunniest C8, 5μm 150 x 4.6 mm
Mobile phase: CH_3OH/H_2O=75/25
Flow rate: 1.0 mL/min
Temperature: 40 °C
Pressure: 5.4 MPa
Sample: 1 = Uracil,
2 = Caffeine,
3 = Phenol,
4 = Butylbenzene, (N=15,700)
5 = o-Terphenyl, (N=15,000)
6 = Amylbenzene, (N=15,300)
7 = Triphenylene, (N=14,500)

N is plate number of the above Sunniest C18.

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

Sunniest C18, RP-AQUA and C8 show not only high efficiency but also low column pressure.

◆Separation of pyridine and phenol



Column: Sunniest C18, 5μm 150 x 4.6 mm
Sunniest RP-AQUA, 5μ m 150 x 4.6 mm
Sunniest C8, 5μm 150 x 4.6 mm
Mobile phase: CH_3OH/H_2O=30/70
Flow rate: 1.0 mL/min
Temperature: 40 °C
Detection: UV@250nm
Sample: 1 = Uracil
2 = Pyridine
3 = 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.

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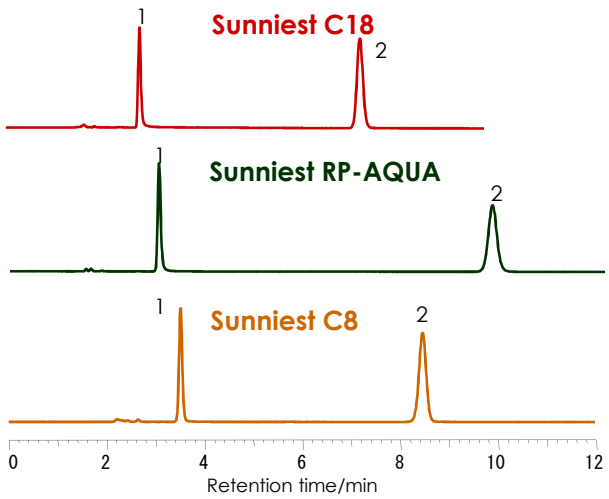
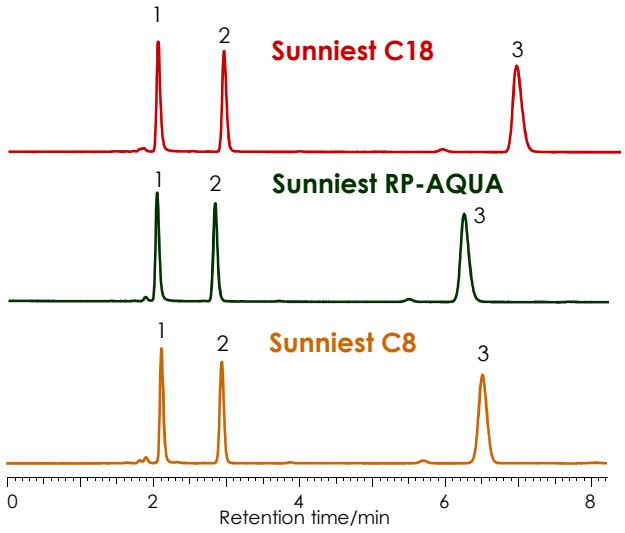
Sunniest RP-AQUA
Sunniest C8

◆Separation of a chelating compound



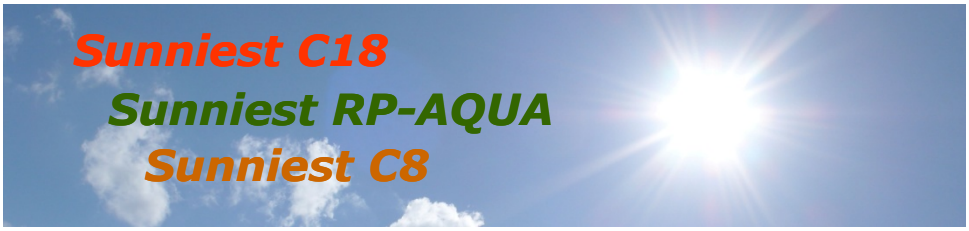
Column: Sunniest C18, 5μm 150 x 4.6 mm
Sunniest RP-AQUA, 5μ m 150 x 4.6 mm
Sunniest C8, 5μm 150 x 4.6 mm
Mobile phase: CH_3CN/20mM H_3PO_4=10/90
Flow rate: 1.0 mL/min
Temperature: 40 °C
Detection: UV@250nm
Sample: 1 = 8-Quinolinol
2 = Caffeine

◆Separation of acidic compounds



Column: Sunniest C18, 5μm 150 x 4.6 mm
Sunniest RP-AQUA, 5μ m 150 x 4.6 mm
Sunniest C8, 5μm 150 x 4.6 mm
Mobile phase: CH_3CN/0.1% H_3PO_4=2/98
Flow rate: 1.0 mL/min
Temperature: 40 °C
Detection: UV@210nm
Sample: 1 = Formic acid
2 = Acetic acid
3 = Propionic Acid

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



✻ Sunniest Ordering information

Inner diameter [mm]	Length [mm]	Sunniest C18, 3µm Catalog No.	Sunniest C18, 5µm Catalog No.	Sunniest RP-AQUA, 3µm Catalog No.	Sunniest RP-AQUA, 5µm Catalog No.	SunniestC8, 3µm Catalog No.	Sunniest C8, 5µm Catalog No.
2.0	50	EB2241	EB3241	ER2241	ER3241	EC2241	EC3241
	75	EB2251 EB2261	–	ER2251	–	EC2251	–
	100		EB3261	ER2261	ER3261	EC2261	EC3261
	150	EB2271	EB3271	ER2271	ER3271	EC2271	EC3271
3.0	150	EB2371	EB3371	ER2371	ER3371	EC2371	EC3371
	250	–	EB3381	–	ER3381	–	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	–	EB3481	–	ER3481	–	EC3481
10.0	250	–	EB3781	–	ER3781	–	EC3781
20.0	250	–	EB3881	–	ER3881	–	EC3881

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