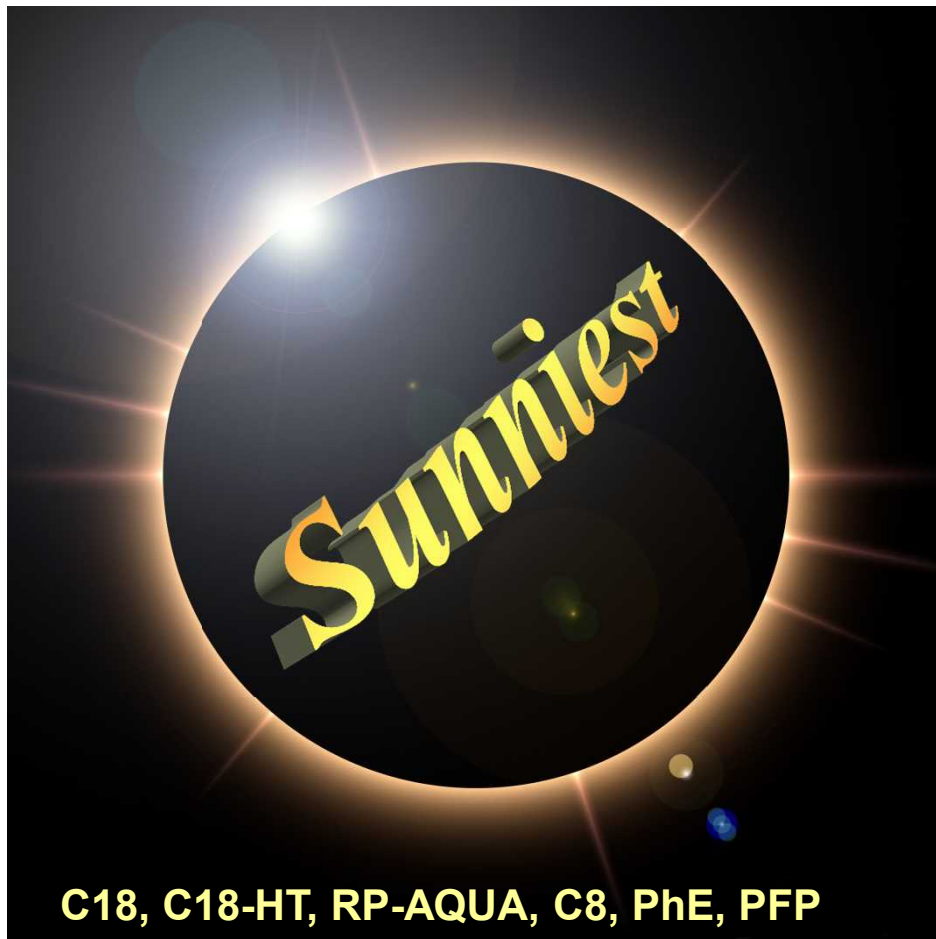


HPLC column

Sunniest



ChromaNik Technologies Inc.

Biotech AB in partnership with ChromaNik Technologies

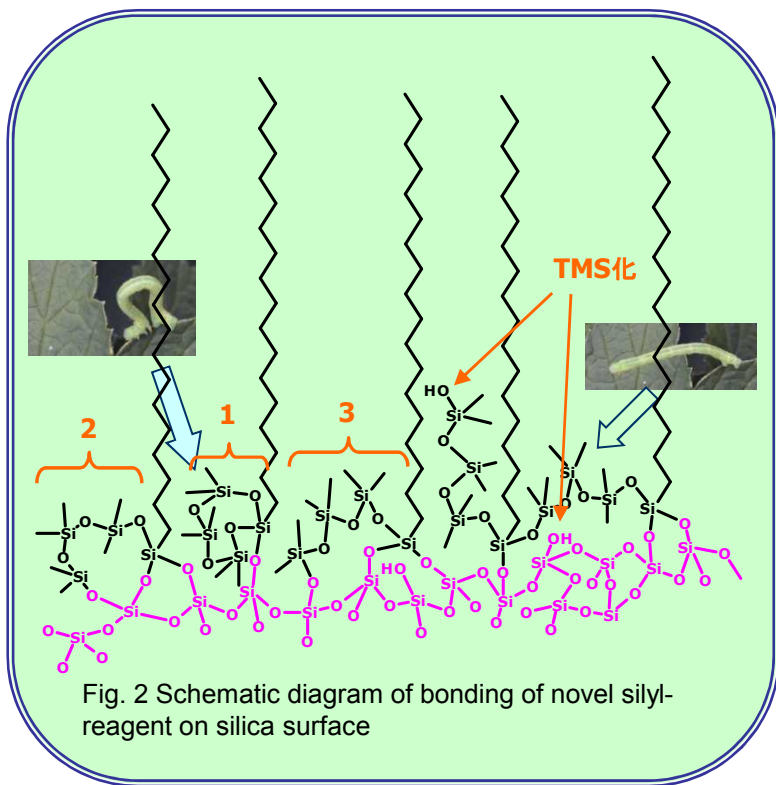
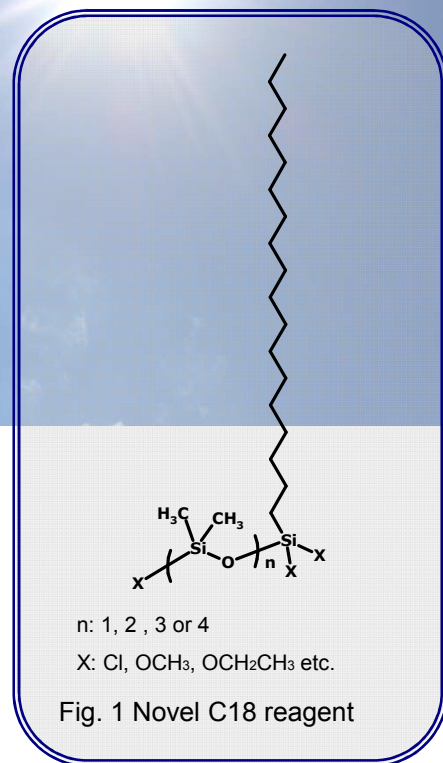
Sunniest C18, C18-HT, RP-AQUA, C8, PhE, PFP

A Novel Bonding Technique

(patent pending)

The "State of Art" trifunctional silyl-reagent was developed as shown in Fig.1. This Unique silyl-bonded reagent (HMODTS) can bond with any silanol groups on Silica Sorbent surface as shown in Fig.2. It can expand and contract by itself in Caterpillar manner. This technique can substantially minimise the contribution of residual silanol groups on Reverses phase stationary phase.

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



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 with C28 bonding offers Performance in 100% aqueous conditions, and shows enhanced retention of polar compounds.

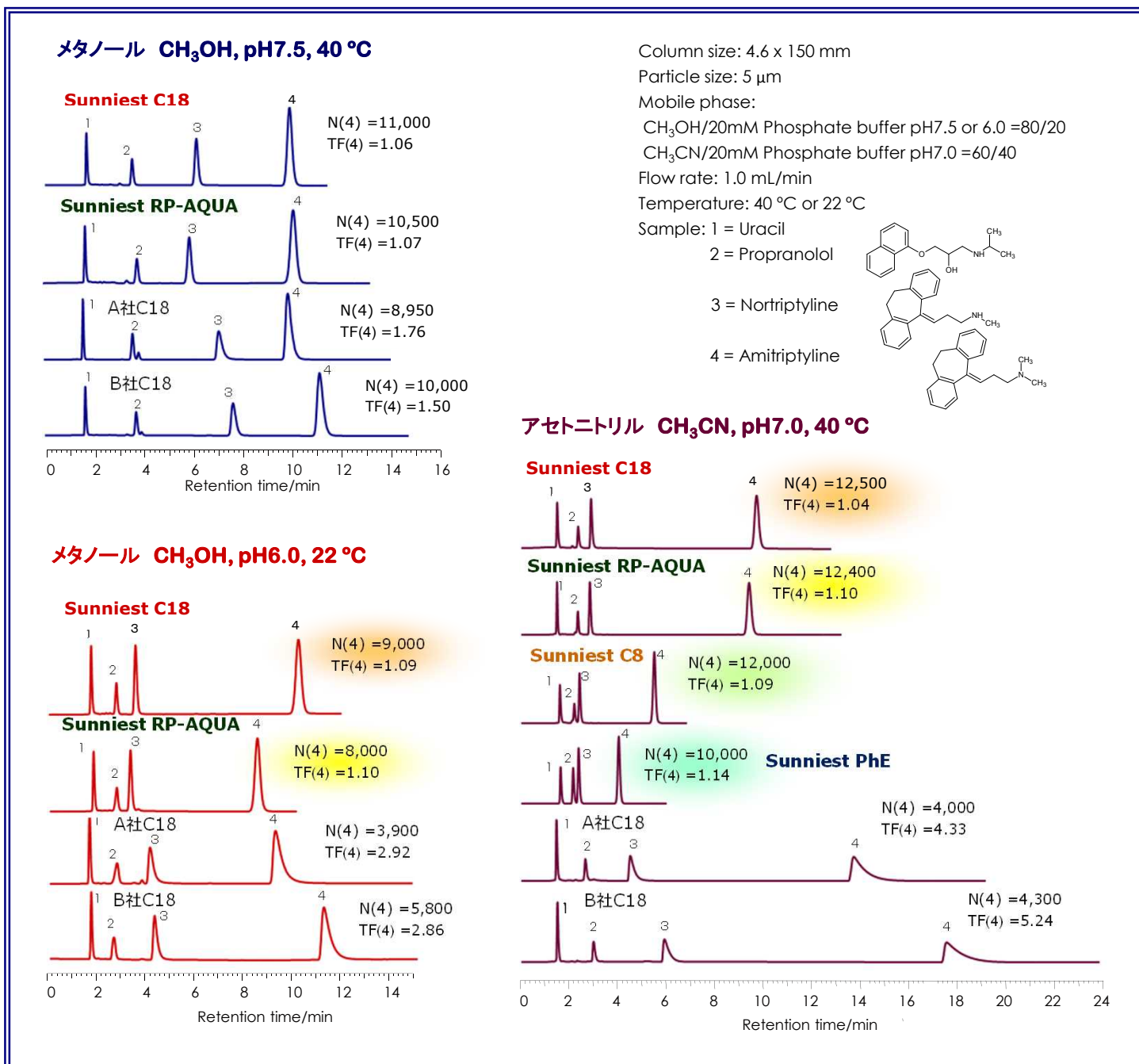
Characteristics of Sunniest

| | 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 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 |

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

◆ **Evaluation of End-capping**

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

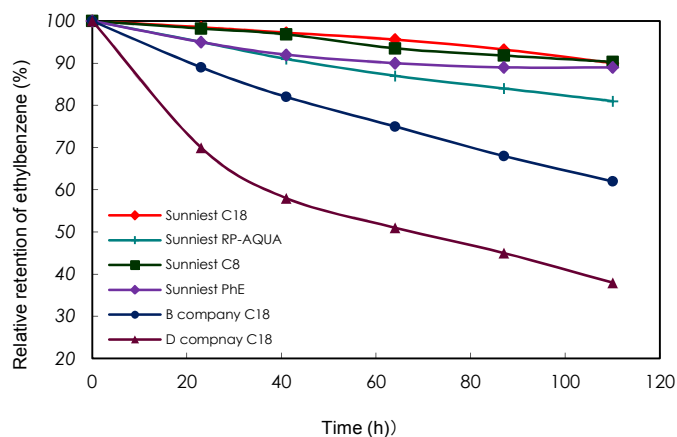


Amitriptyline is widely used to evaluate residual silanol groups on the C18 stationary phase. Peak shape of Amitriptyline was compared under 3 kinds of conditions such as methanol/phosphate buffer/40 °C, methanol/phosphate buffer/22 °C and acetonitrile/phosphate buffer/40 °C. Majority of the HPLC columns offered good peak shapes under methanol/phosphate buffer/40 °C conditions. However using Mobile phase of acetonitrile/phosphate buffer/40 °C, most of the columns(Refer column A and B) offered high extent of Tailing unlike Sunniest columns offering a symmetrical 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.

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

◆ Stability under acidic and basic pH conditions

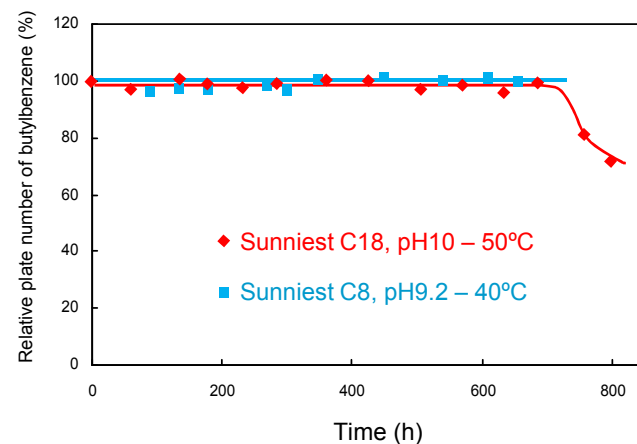


Durable test condition

Column size: 150 x 4.6 mm
Mobile phase: CH₃CN/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: CH₃CN/H₂O=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: CH₃OH/20mM Sodium borate/10mM NaOH=30/21/49 (pH10)
C8: CH₃OH/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: CH₃OH/H₂O=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 severe conditions of acetonitrile /1% trifluoroacetic acid solution (pH 1) at 80 deg C.

Our Unique HMODTS bonding technique offers significant enhancement of column life,

Considering the Sunniest RP-AQUA C28 ligand length the Sunniest RP-AQUA is less stable than Sunniest C18. However, Sunniest RP-AQUA C28 column with HMODTS bonding along with end capping offers longer column life in comparison to other RP Aqua columns.

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.

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 an alkaline standard solution for pH meter, so that its buffer capacity is high.

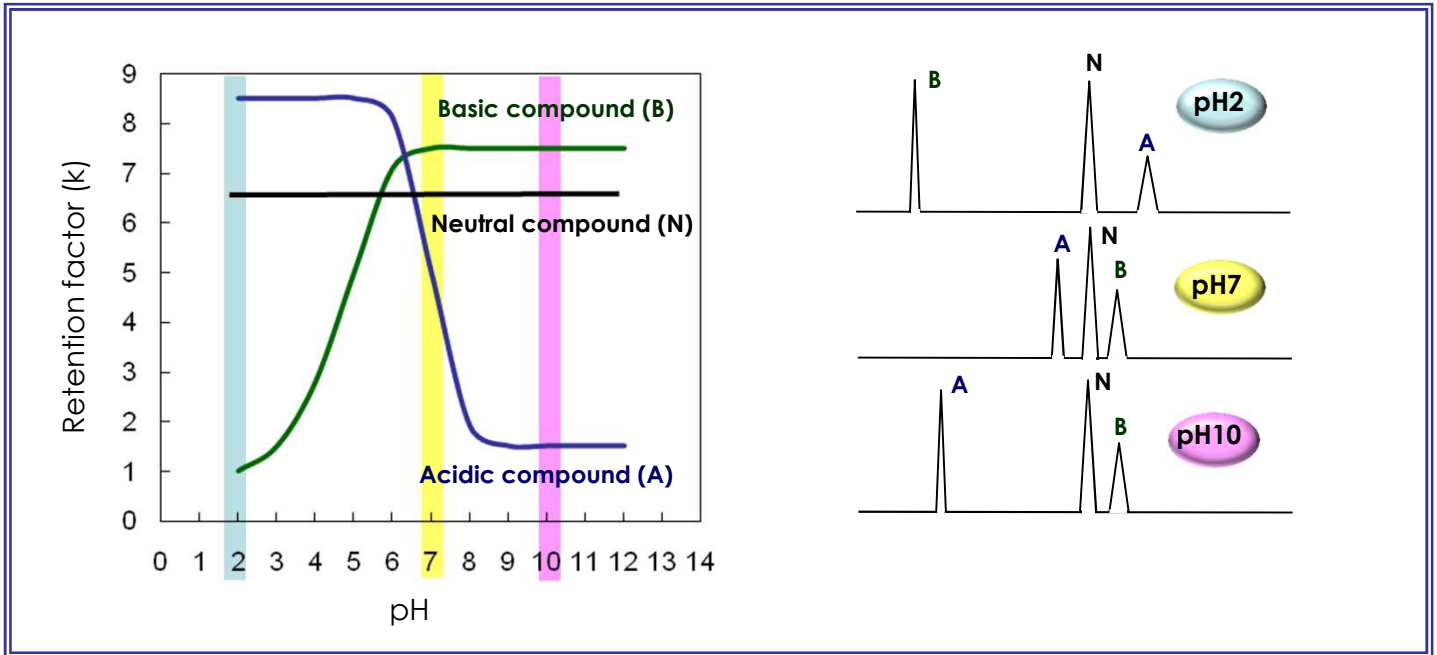
Elevated temperature of 10 °C makes column life be one third. When Sunniest C18 column is used at 40 °C, column life becomes 2,000 hours. Most of the HPLC columns stability data is offered at ambient room temperature alternate 25 °C at pH 1-10 units. At temperature of 25 °C, the column life is sixteen times longer than that at 50 °C.

★ Sunniest C18 offers performance at elevated pH and temperature. Regarding stability under basic pH condition, there are very few C18 column like Sunniest C18 & Hybrid type C18 which can sustain and offer performance under such challenging conditions of high temperature and pH. It is considered that our double end-capping & base deactivation technique leads higher stability.

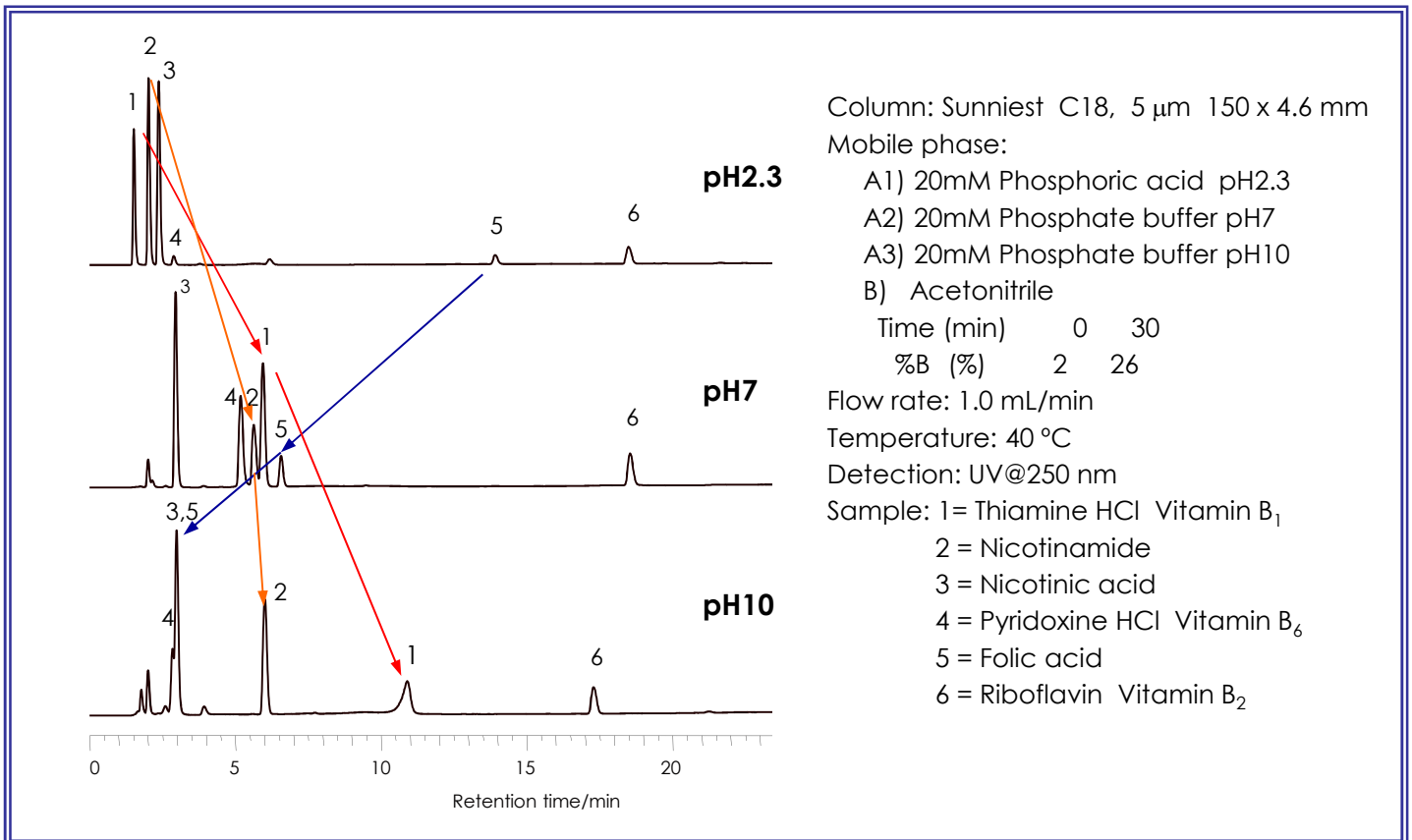
★ Sunniest C18 has operational pH Range from 1.5 to 10. Sunniest C8, Phenyl has operational pH Range 1.5 to 9 and Sunniest RP-Aqua and Pentafluorophenyl (PFP) at pH 2-8..

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

◆ Relationship between pH and retention of Acidic, Basic and Neutral compounds



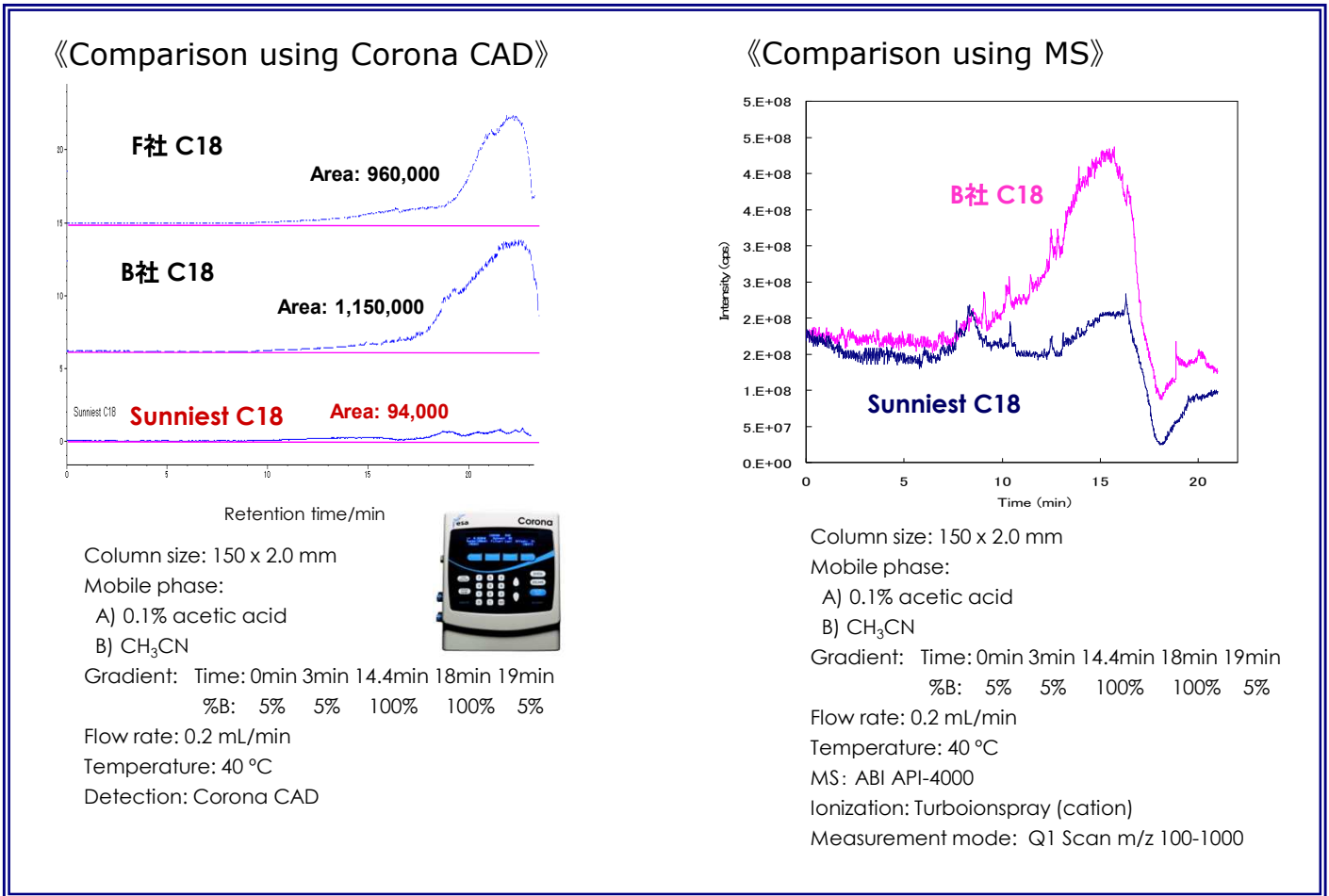
◆ pH selectivity



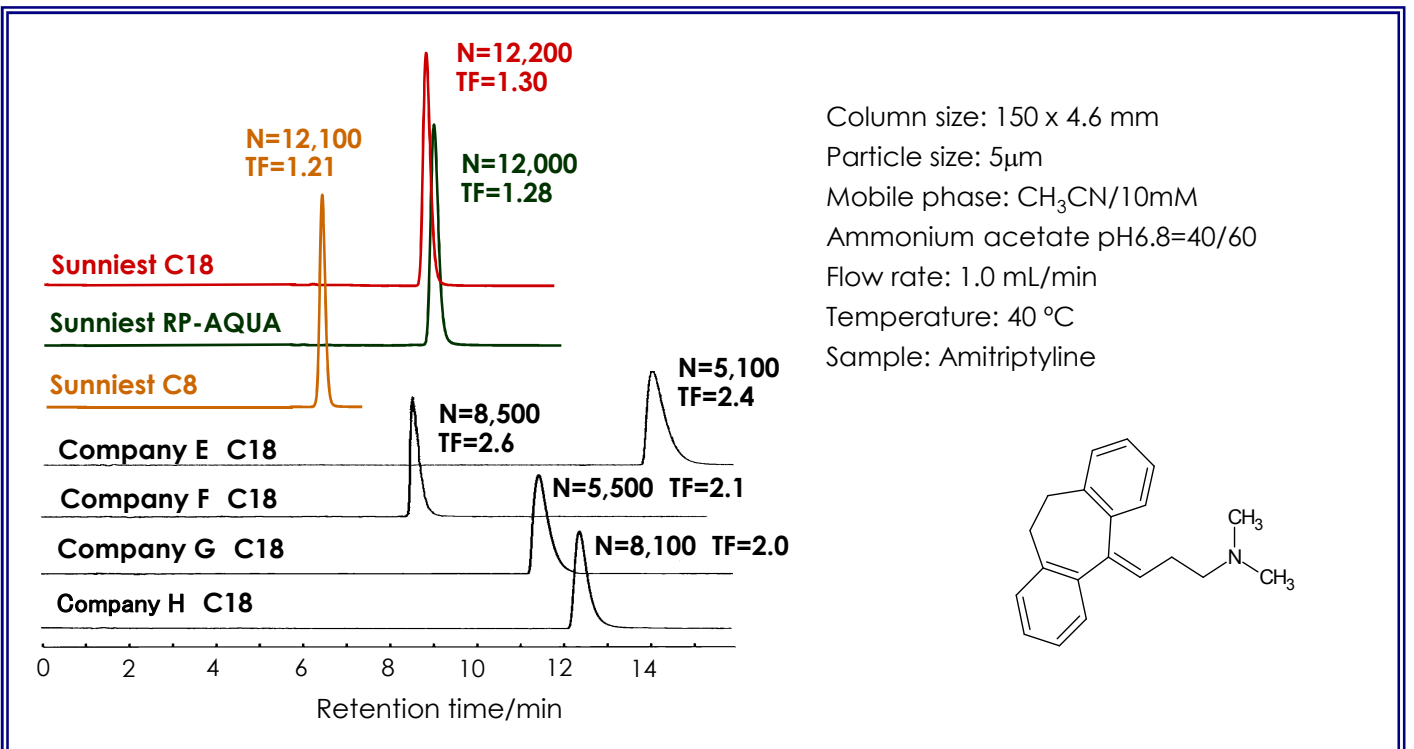
It is interesting to note that the change in pH of mobile phase can offer different selectivity of ionic compounds. Sunniest C18 can be used at the pH range from 1.5 to 10, so that a suitable analytical method can be developed using Sunniest C18 Column..

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

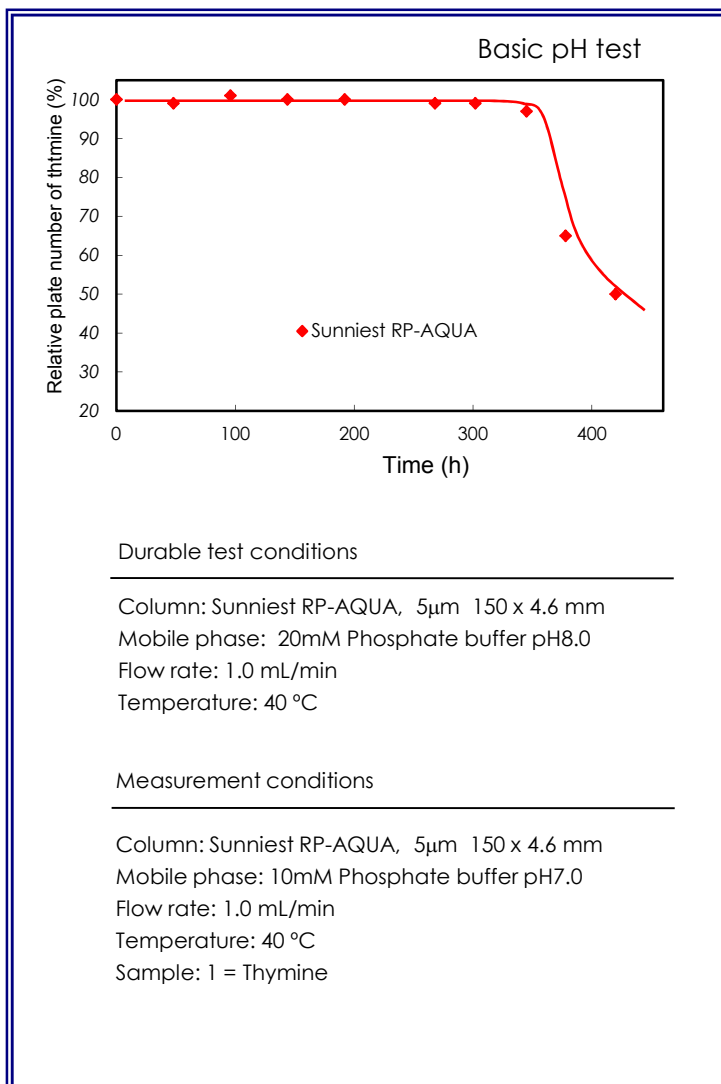
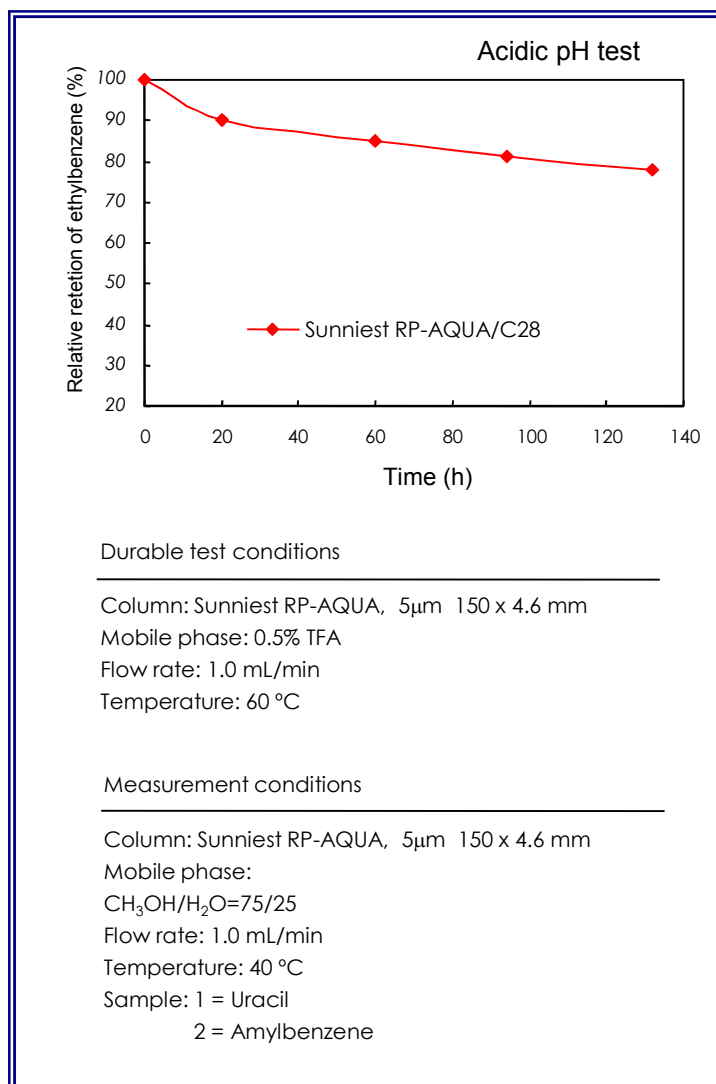
◆ **Comparison data: Bleeding from column**



◆ **Separation of antidepressants using Acetonitrile and Ammonium acetate for LC/MS**



◆ Stability of Sunniest RP-AQUA/ C28 under 100% aqueous conditions



It is important that stability is evaluated for RP Aqua columns under 100% aqueous conditions because RP-Aqua column life becomes longer with incremental contents of organic solvent in a mobile phase. Sunniest RP-AQUA/C28 column can be used under 100% aqueous conditions from pH 2 to pH 8.

★ Sunniest RP-AQUA/ C28 column can be used under 100% aqueous conditions from pH 2 to pH 8. Sunniest RP-AQUA/C28 is one of the most stable aqua type column.

★ Sunniest RP-AQUA/C28 column with HMODTS bonding along with end capping offers longer column life in comparison to other RP Aqua columns



◆ 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 "Depermeating" is more appropriate.

★ Sunniest RP-AQUA /C28 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 /C28 is less than that of a conventional C18. Expelling force (pressure) acted by capillarity on Sunniest RP-AQUA /C28 is less than atmospheric pressure. So, Sunniest RP-AQUA /C28 shows reproducible retention under 100% aqueous conditions.

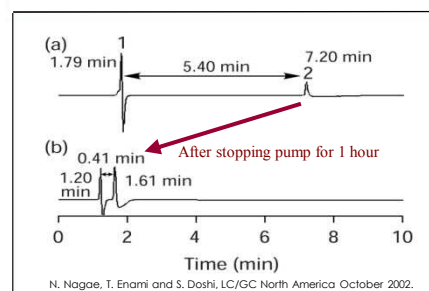


Fig. 1 Retention behavior of a C18 column under 100% aqueous mobile phase conditions

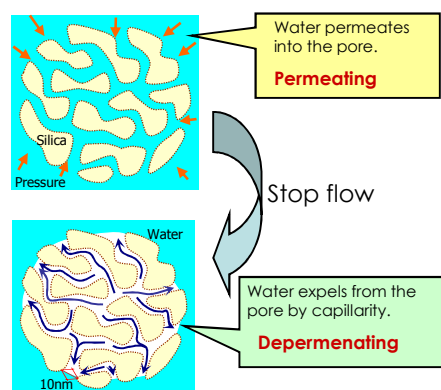
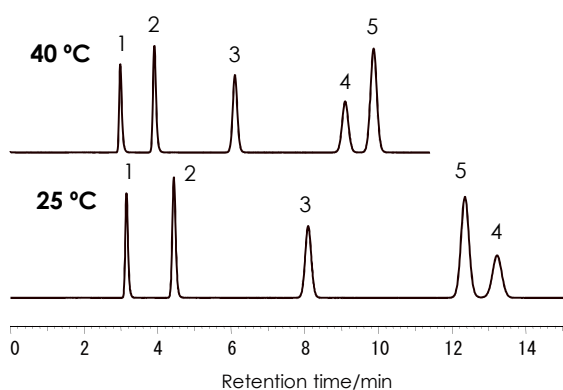


Fig. 2 Schematic diagram of C18 particle

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

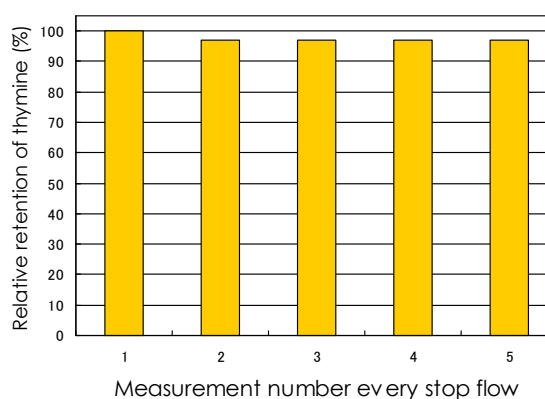
2) T. Enami and N. Nagae, American Laboratory October 2004.

◆ Separation of nucleic acid bases



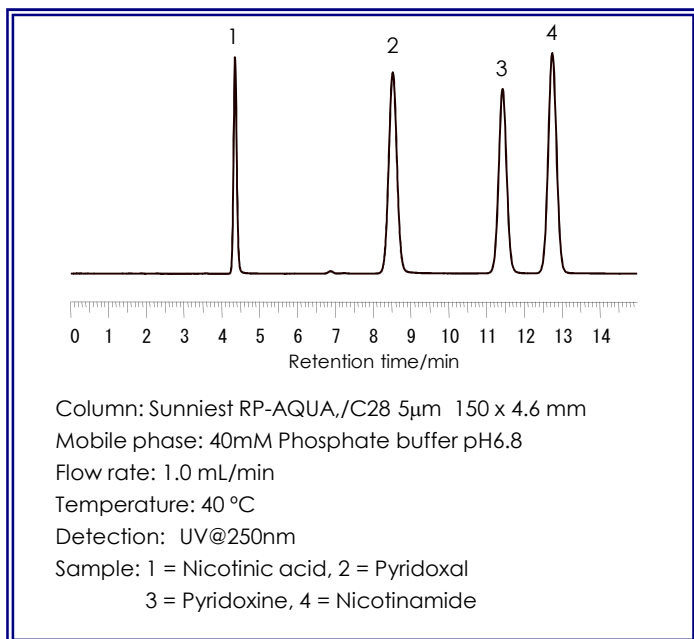
Column: Sunniest RP-AQUA,/C28 5µm 150 x 4.6 mm
Mobile phase: 10mM Phosphate buffer pH7.0
Flow rate: 1.0 mL/min
Temperature: 40 °C and 25°C
Sample: 1 = Cytosine 2 = Uracil
3 = Thymidine 4 = Uridine
5 = Thymine

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

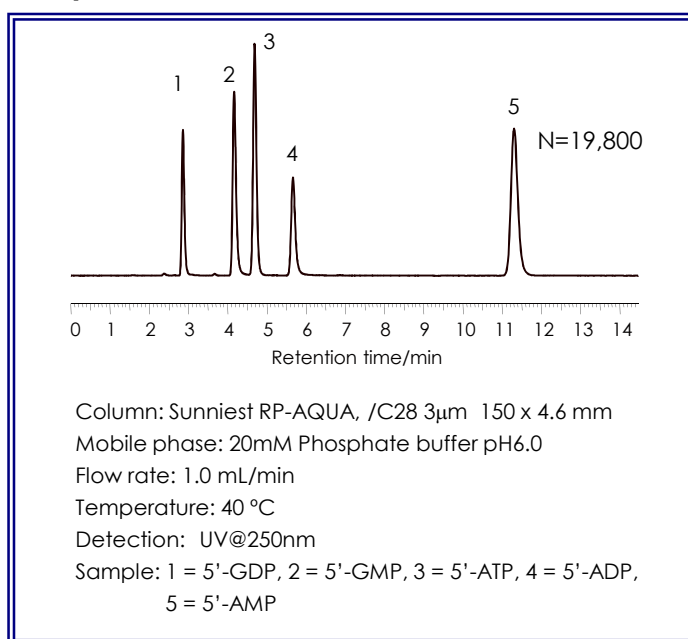


Sunniest RP-AQUA/C28 showed more than 97% of reproducibility in retention using 100% aqueous buffer as a mobile phase.

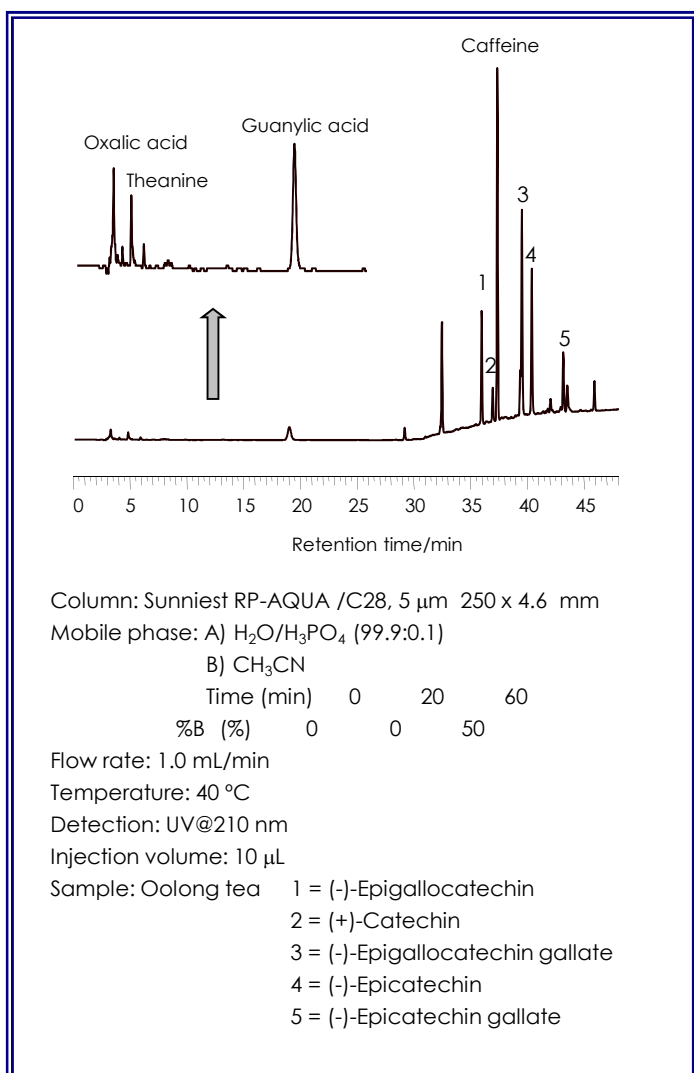
◆ Separation of water-soluble vitamins



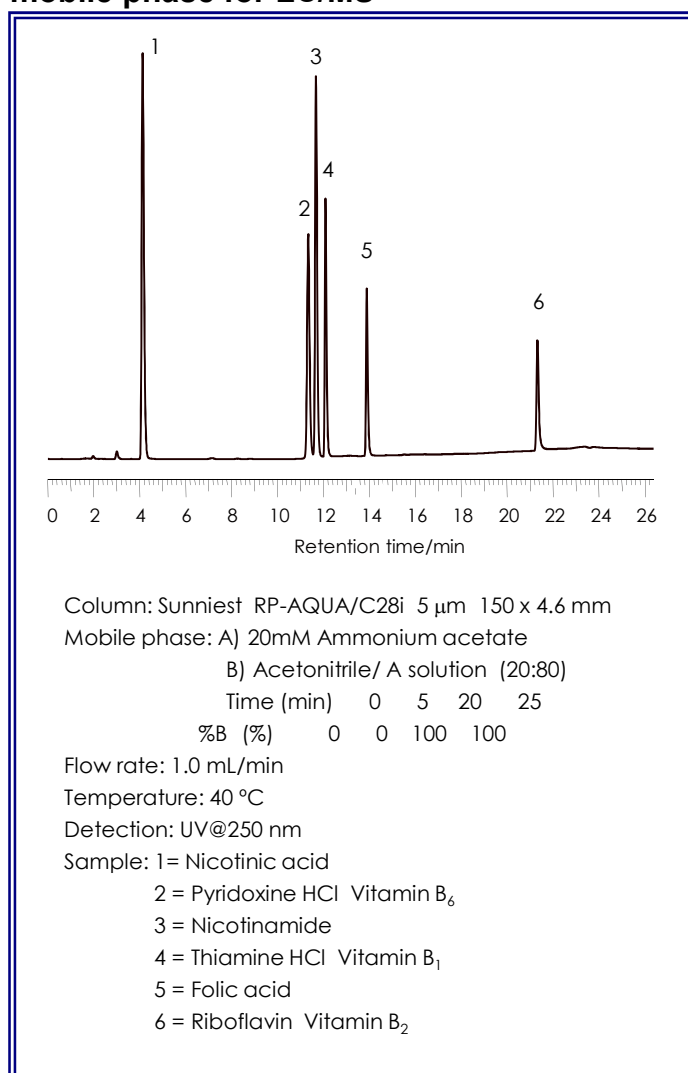
◆ Separation of nucleotides



◆ separation of Oolong tea

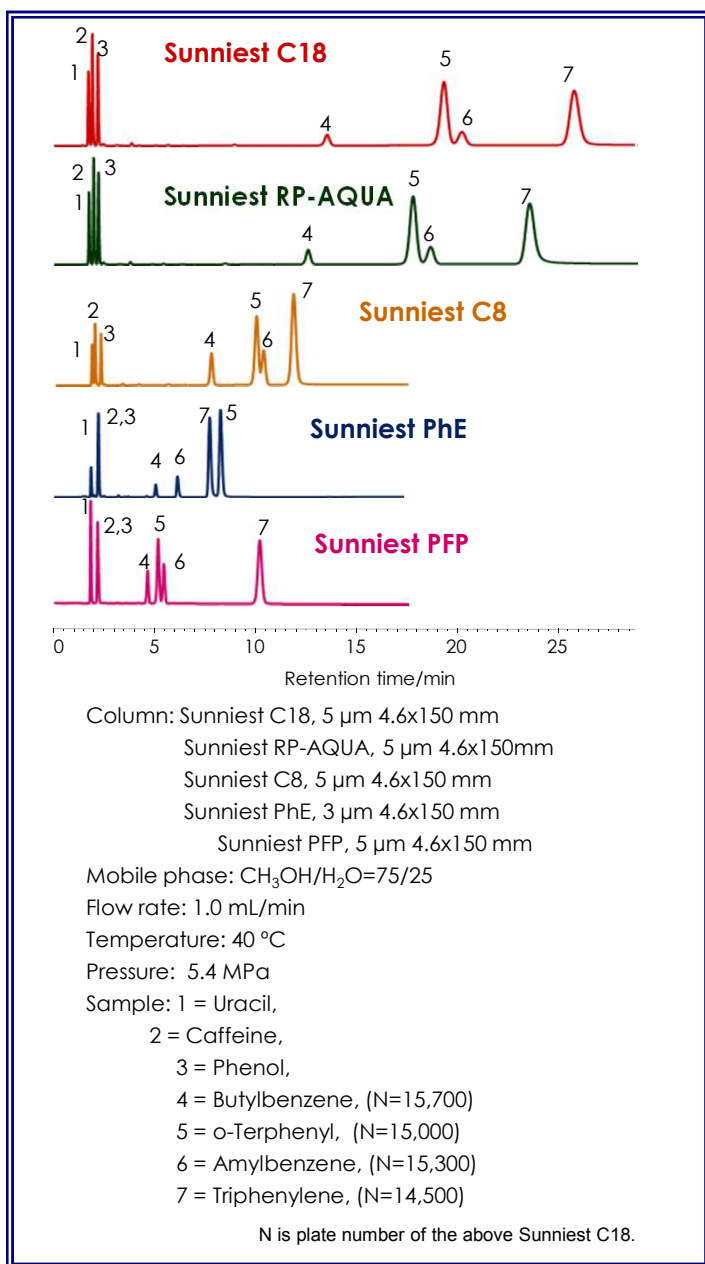


◆ Separation of water-soluble vitamins using mobile phase for LC/MS

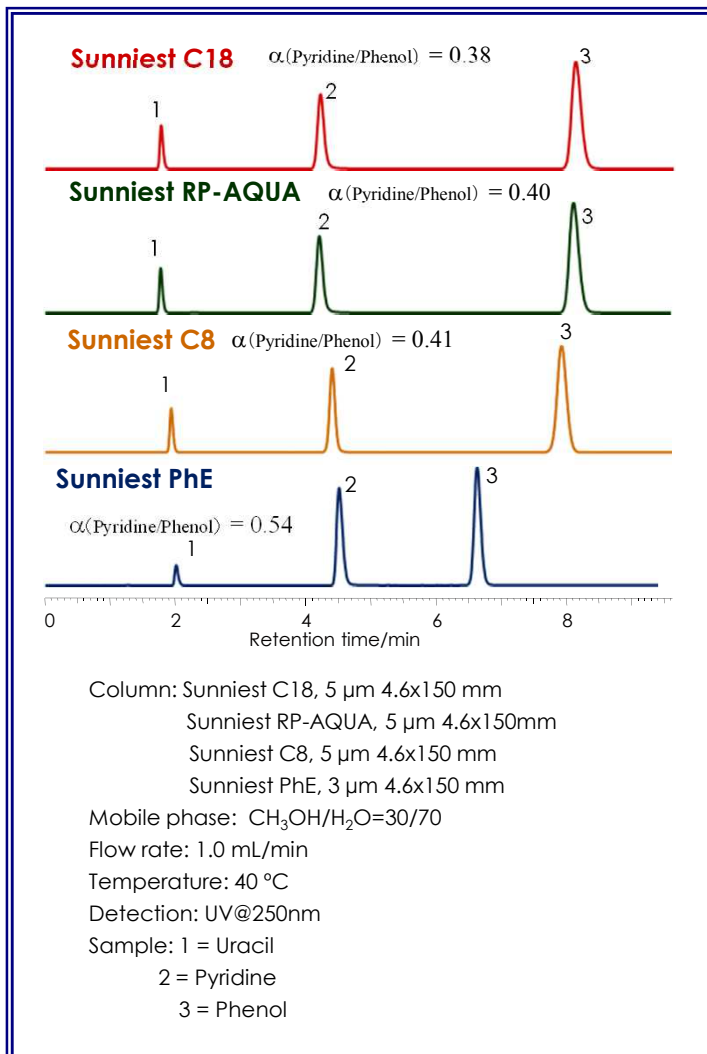


Sunniest C18,C18-HT
Sunniest RP-AQUA
Sunniest C8,PhE,PPF

◆ Separation of standard samples



◆ 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.

| | C18 | RP-AQUA | C8 | PhE | PPF |
|-------------------------------------|------|---------|------|------|------|
| 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 |
| Residual silanol activity | | | | | |
| α (Pyridine/Phenol) | 0.38 | 0.40 | 0.41 | 0.54 | ---- |

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

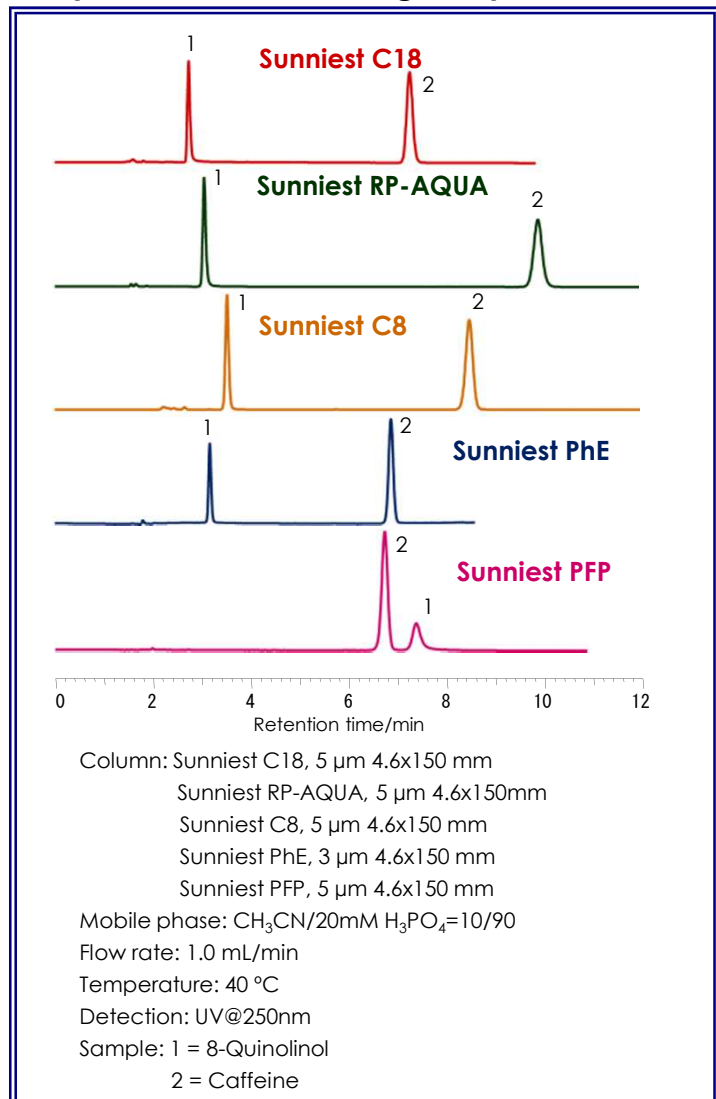


Sunniest C18

Sunniest RP-AQUA

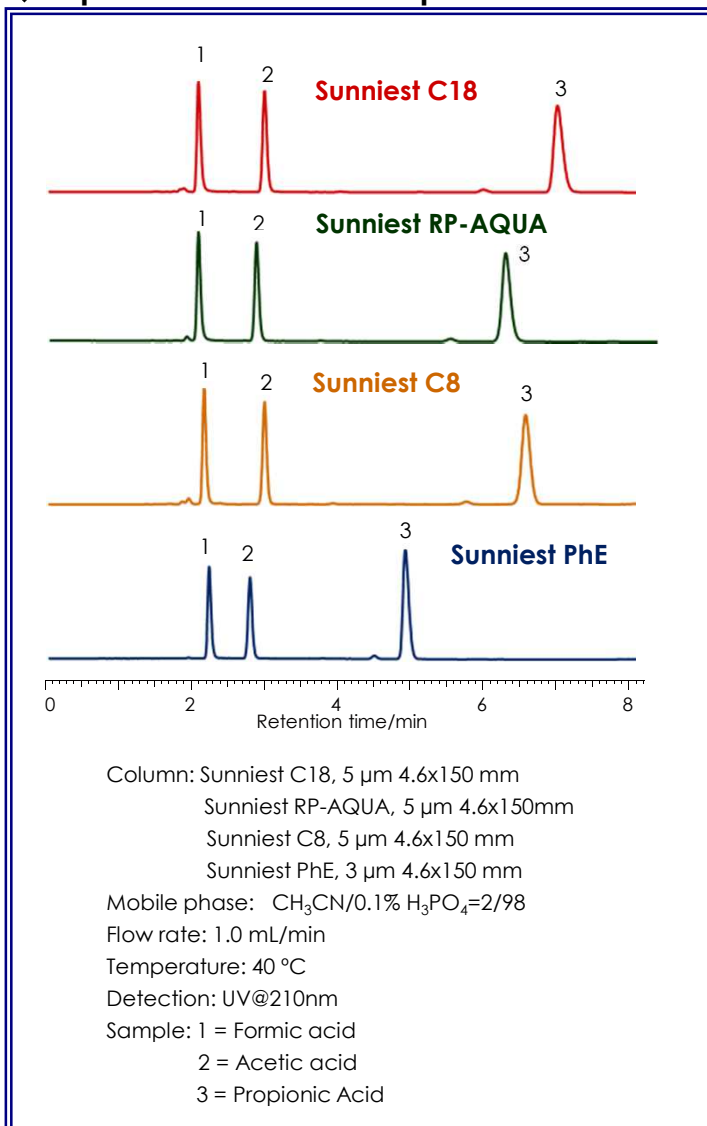
Sunniest C8

◆ Separation of a chelating compound

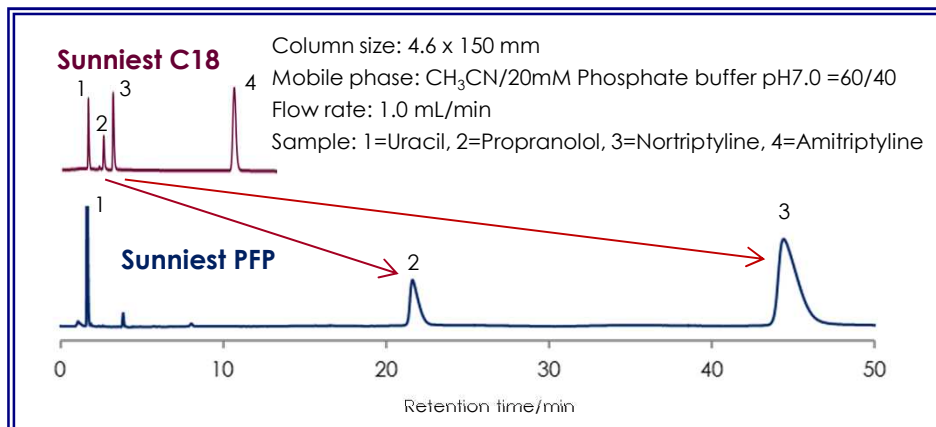


★ Sunniest C18, RP-AQUA, C8, PhE and PFP are inert for a metal chelating compound and acidic and basic compounds, so that they show symmetrical peaks of each compound.

◆ Separation of acidic compounds



◆ Retention comparison between C18 and PFP



★ PFP retains a cation such a nortriptyline much longer than a C18.





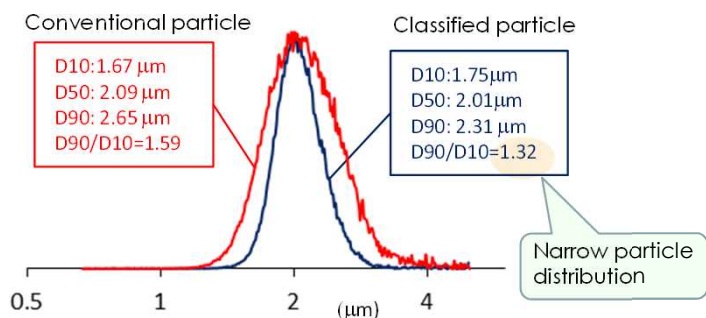
Sunniest C18-HT, 2 μm

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 grinding

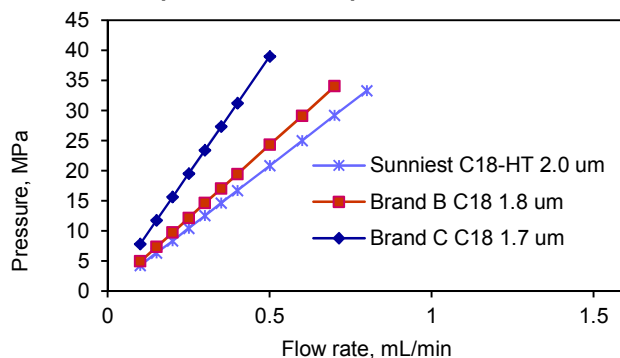
• Narrow Particle Distribution and Low Back Pressure

Measured by Coulter Counter method



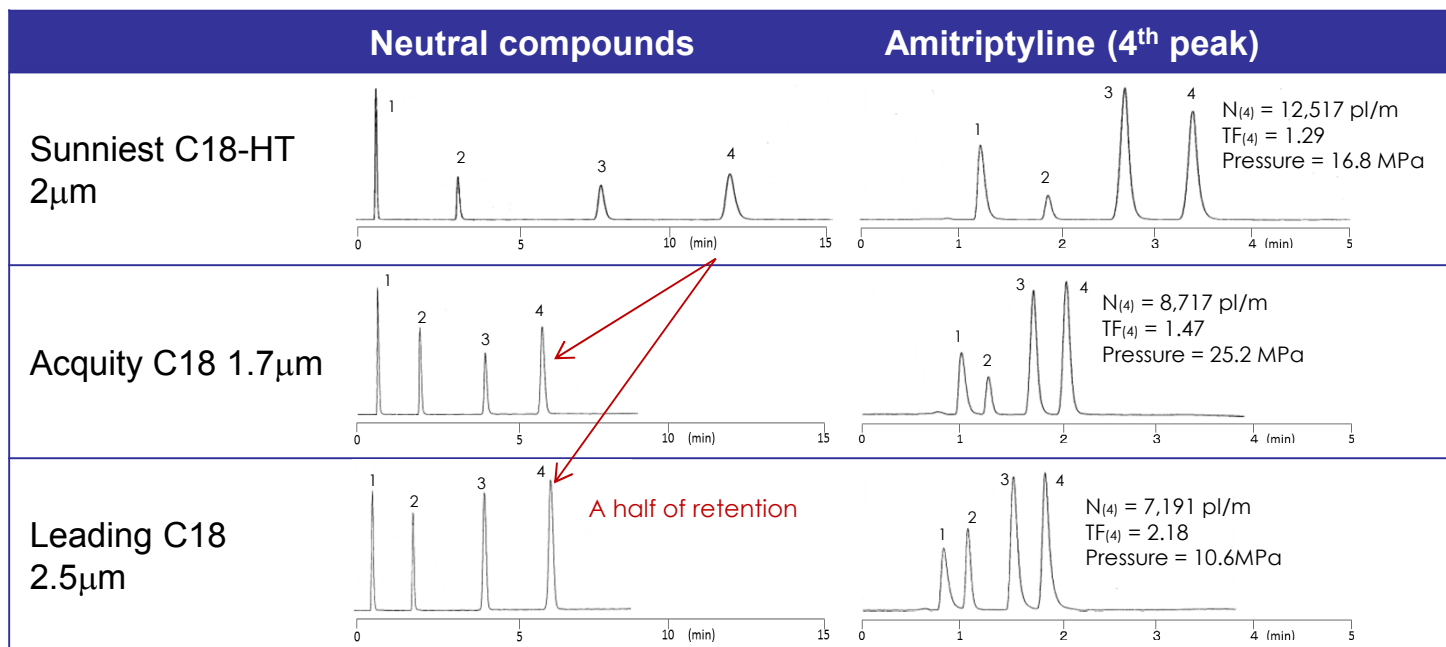
Conventional 2 μm silica gel particle was classified again. 20% volume was cut off from both sides respectively. Consequently column back pressure reduced more than 15%. Our 2 μm silica gel particle shows a half pressure to compare with the other sub-2 μm silica gel particle.

Comparison of back pressure

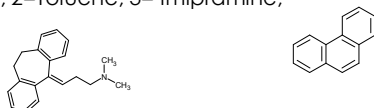


Column: Sunniest, Acquity and Zorbax
 Column dimension: 50 x 2.1 mm
 Mobile phase: Acetonitrile/water=(70/30)
 Temperature: 25 °C

• An Unique Modification Leads Good Peak Shape



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



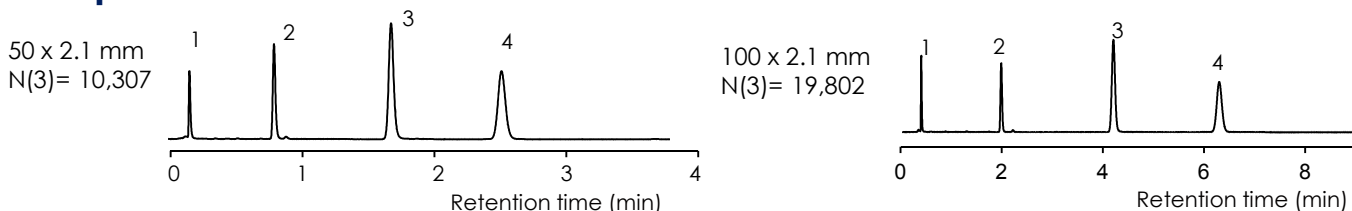
It is difficult to end-cap on sub 2 μm or 2 μm silica gel particle as well as 3 μm or 5 μm silica gel particle. Most sub 2 μm or 2 μm C18 columns show smaller plate number and larger tailing factor for a basic compound than Sunniest C18-HT. Sunniest C18-HT 2 μm shows a good peak shape for amitriptyline 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.



Sunniest C18, C18-HT

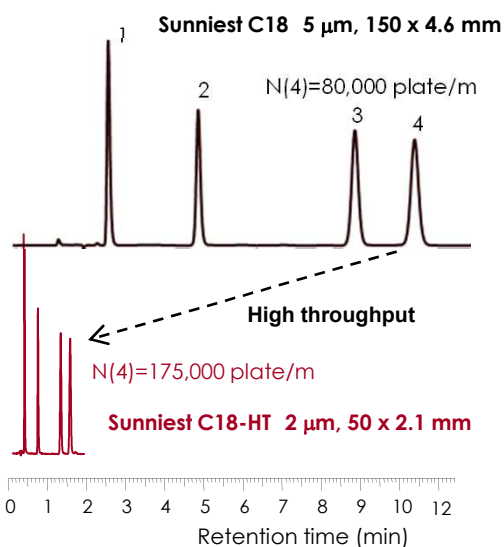


• Comparison of Plate Number

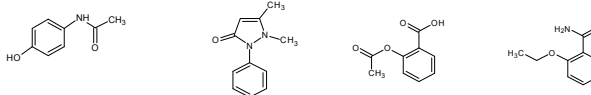


Mobile phase: CH₃CN/H₂O = 60/40
 Flow rate: 0.6 mL/min for 2.1 x 30 mm and 2.1 x 50 mm, 0.4 mL/min for 2.1 x 75 mm and 2.1 x 100 mm
 Temperature: 40 °C Detection: UV@250 nm
 Sample: 1=Uracil, 2=Toluene, 3=Acenaphthene, 4=Butylbenzene

• Separation of Analgesics

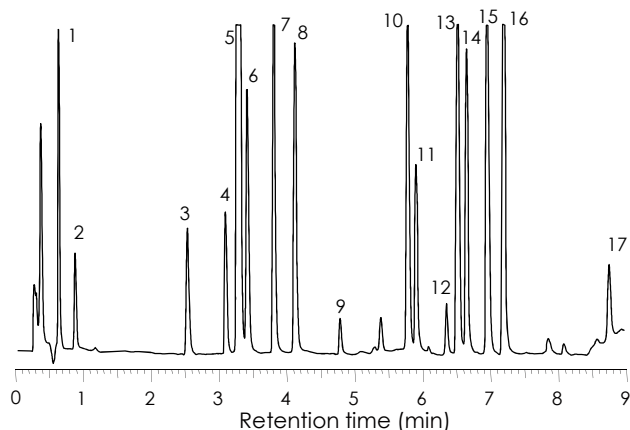


Mobile phase: CH₃CN/0.1% Formic acid = 20/80
 Flow rate: 1.0 mL/min for 150 x 4.6 mm
0.6 mL/min for 50 x 2.1 mm
 Temperature: 40 °C
 Detection: UV@230 nm
 Sample: 1=Acetaminophen, 2=Antipyrine, 3=Aspirin, 4=Ethenzamide



2 µm 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.

• Separation of Amino Acids derivatized with OPA



Column: Sunniest C18-HT 2 µm, 100 x 2.1 mm
 Mobile phase: A) 10mM Na₂PO₄ + 10mM Na₂B₄O₇ + 0.5mM Na₃
 B) Acetonitrile/Methanol/Water (45/45/10 %V)

| Time(min) | 0 | 0.2 | 7.2 | 7.8 |
|-----------|---|-----|-----|-----|
| %B | 5 | 5 | 50 | 100 |

Flow rate: 0.72 mL/min
 Temperature: 40 °C
 Detection: UV@338 nm
 Sample: 1=Aspartic acid, 2=Glutamic acid, 3=Serine, 4=Histidine, 5=Glycine, 6=Threonine, 7=Arginine, 8=Alanine, 9=Tyrosine, 10=Valine, 11=Methionine, 12=Tryptophan, 13=Pnehyalanine, 14=Isoleucine, 15=Leucine, 16=Lysine, 17=Proline

• Characteristics of Sunniest C18-HT, 2 µm

| Packings | Silica gel support | | | C18 | | | |
|-----------------|-----------------------|--------------------|---|--------------------|--------------|----------------------------|--------------------|
| | Particle size (µm) | Pore diameter (nm) | Specific surface area (m ² /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 µm 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 µm, so that it have a high capacity to resist pressure and a high operating pressure.

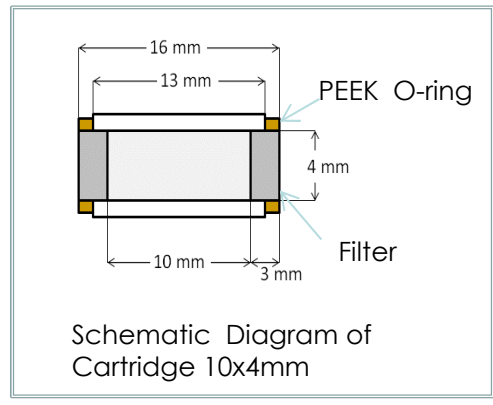


Sunniest Guard columns C18, RP-AQUA/C28, C8 PhE & PFP

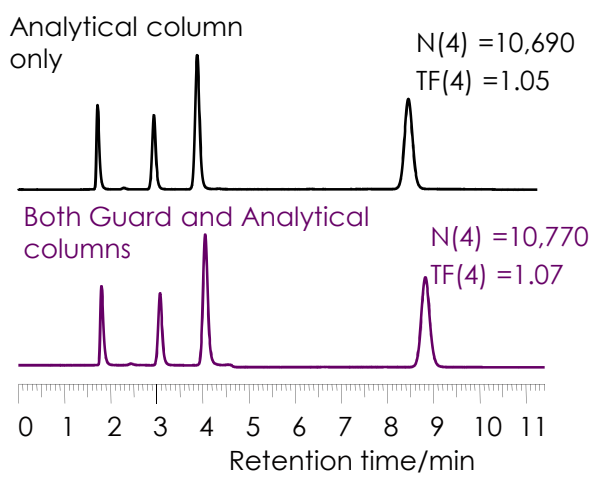
Guard Cartridge (10 x 4 mm)

Feature

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



Comparison of chromatograms



Column: Sunniest C18, 5 μm 150 x 4.6 mm
Guard cartridge 10 x 4 mm

Mobile phase:
CH₃OH/20mM Phosphate buffer pH7.5 = 80/20
Flow rate: 1.0 mL/min
Temperature: 40 °C
Pressure: 4.8 MPa, 5.6 MPa(+guard)

Sample: 1 = Uracil,
2 = Propranolol,
3 = Nortriptyline,
4 = Amitriptyline,

TF: USP tailing factor



| | Particle size | Catalog No. |
|--|---------------|-------------|
| Sunniest C18, 5 μm 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 C18, C18-HT

Sunniest RP-AQUA Sunniest C8

Sunniest PhE Sunniest PFP

*** Sunniest Ordering information**

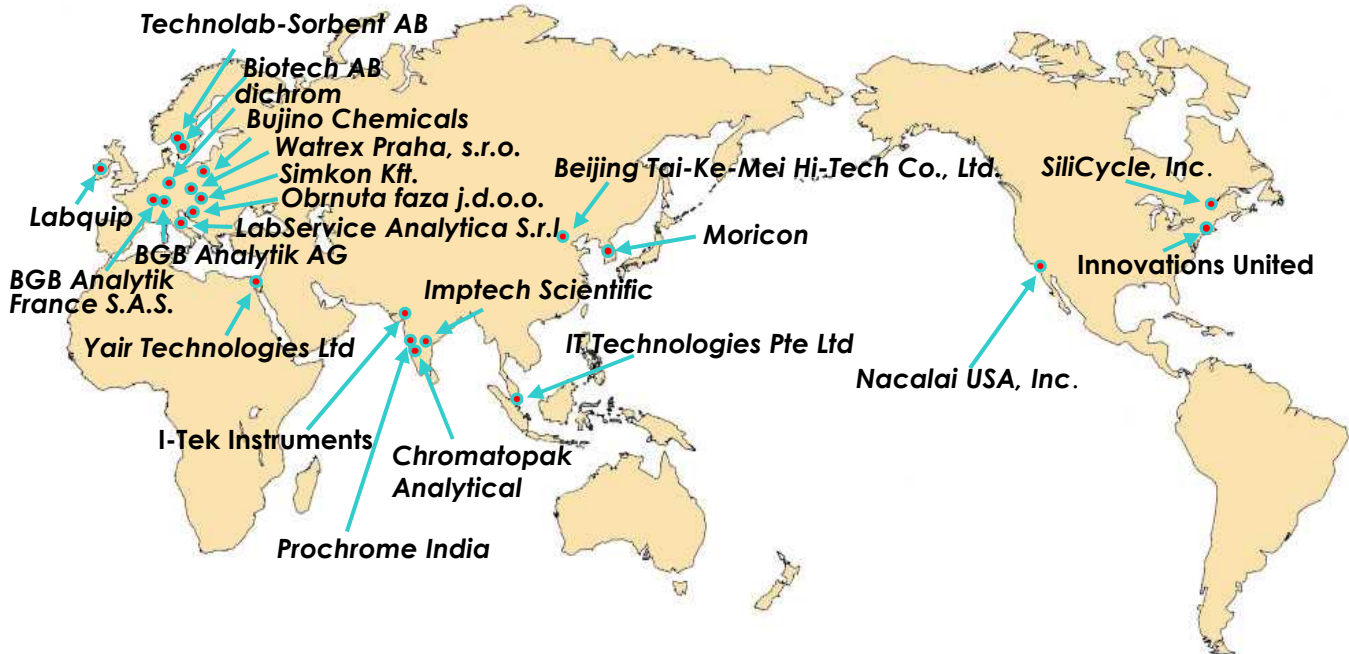
| Inner diameter [mm] | Length [mm] | Sunniest C18, 3µm | Sunniest C18, 5µm | Sunniest RP-AQUA, 3µm | Sunniest RP-AQUA, 5µm | Sunniest C8, 3µm | Sunniest C8, 5µm |
|---------------------|-------------|-------------------|-------------------|-----------------------|-----------------------|------------------|------------------|
| | | 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 [mm] | Length [mm] | Sunniest PhE, 3µm | Sunniest PhE, 5µm | Sunniest PFP, 5µm |
|---------------------|-------------|-------------------|-------------------|-------------------|
| | | 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 [mm] | Length [mm] | Sunniest C18-HT, 2µm |
|---------------------|-------------|----------------------|
| | | 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 | |

Sunniest C18
Sunniest RP-AQUA
Sunniest C8

*Our distributors in the world



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