ChromaNik Seminar in Italy and Hungary

1) About ChromaNik Technologies (10 min)

2) Feature of Core Shell Particle and SunShell Bonding technology (30 min)

3) Comparison of Core Shell C18 Columns (Accucore, Ascentis Express, Cortecs, Kinetex, PoroShell and SunShell) (20 min)

4) Applications related Food, Proteins and Other (20min)

ChromaNik Technologies Inc.

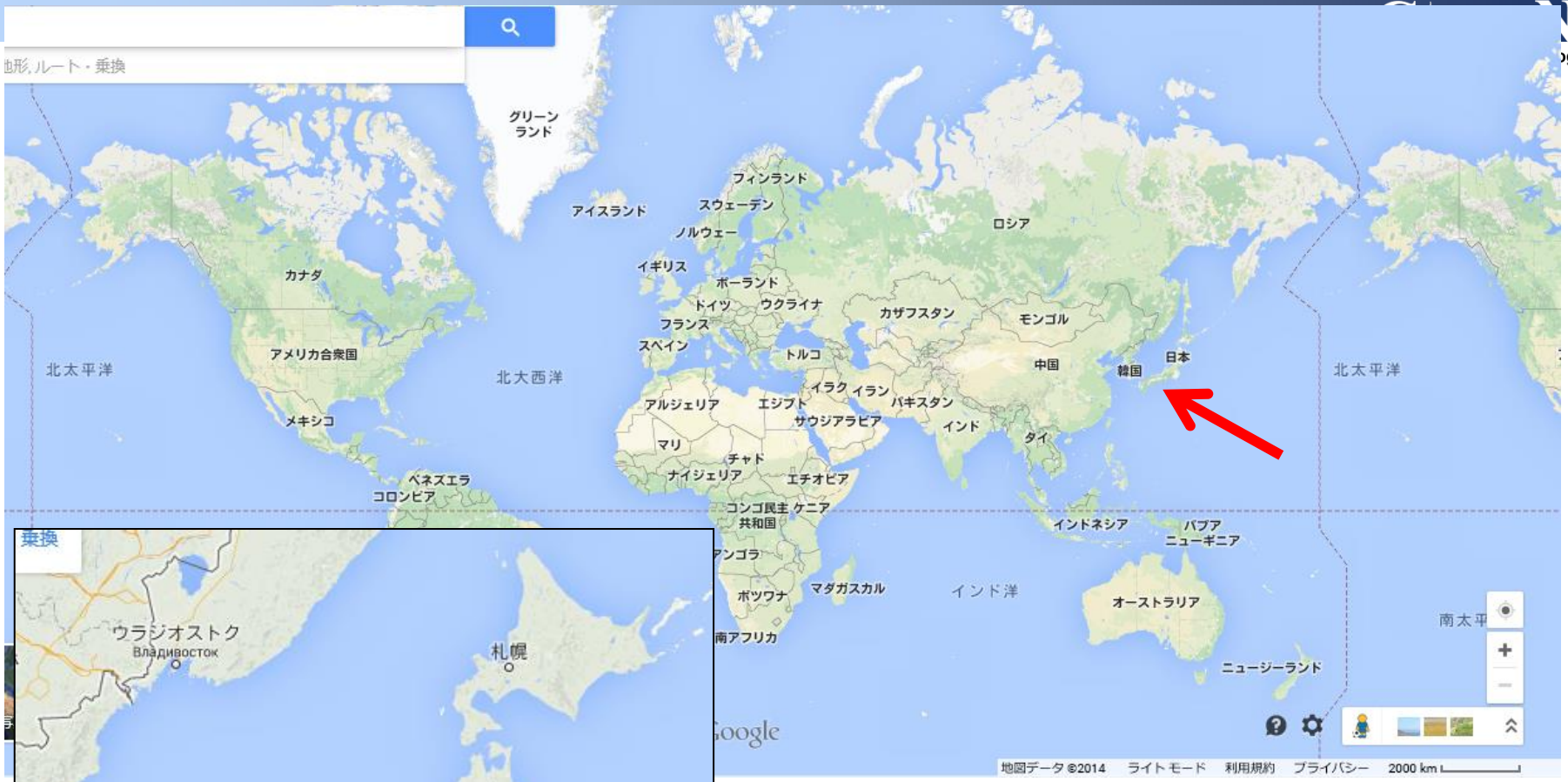
Founded in December, 2005 by Norikazu Nagae who worked for Nomura Chemical (Develosil) for 22 years.

Main products:

Sunrise C18-SAC, C18, C28 since 2007

Sunniest C18, RP-AQUA, C8, etc. since 2008

SunShell C18, C8, PFP, HILIC-Amide etc. since 2011

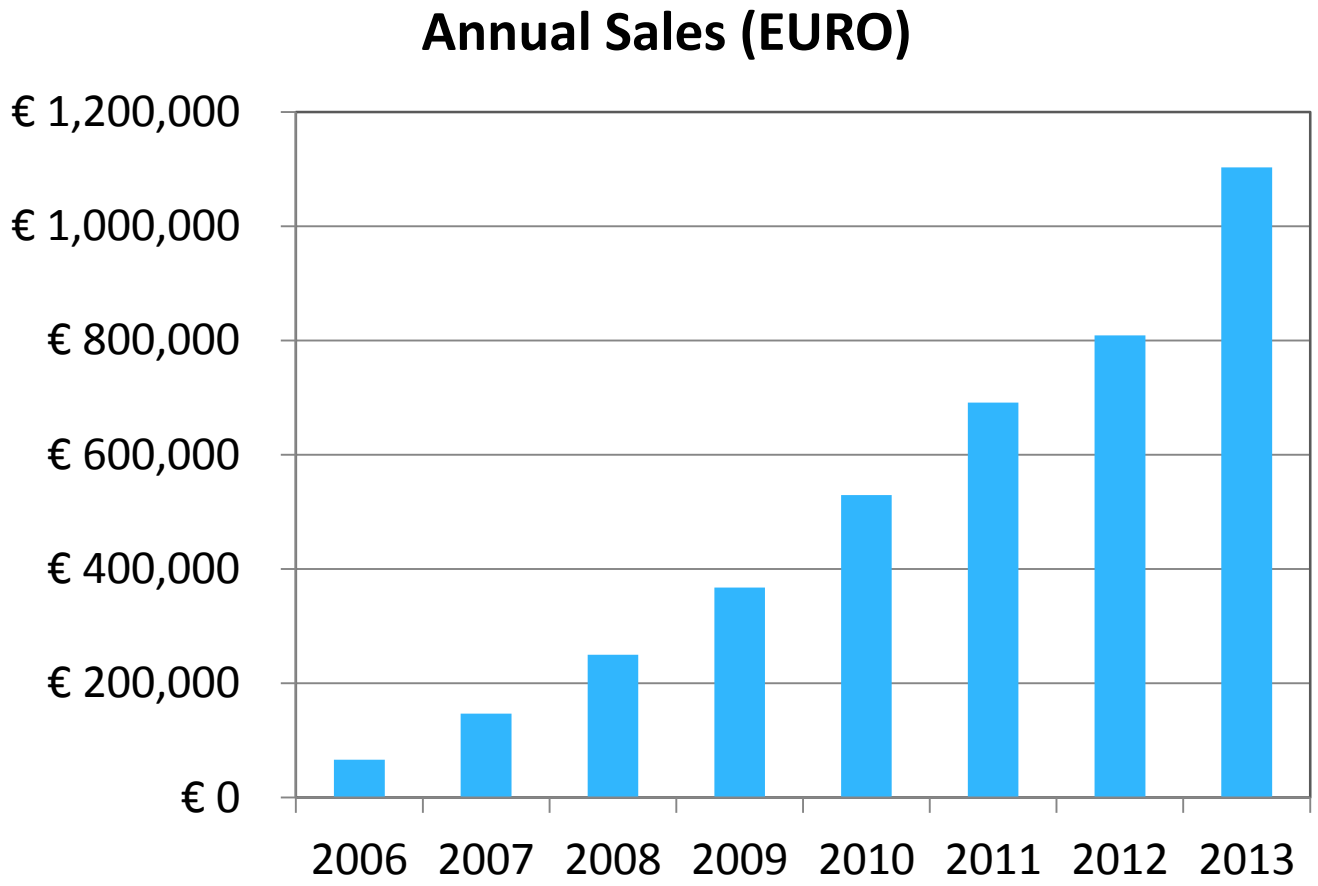


ChromaNik Technologies Inc.

Address:
6-3-1 Namiyoke, Minato-ku, Osaka, Japan

ChromaNik Technologies Inc.

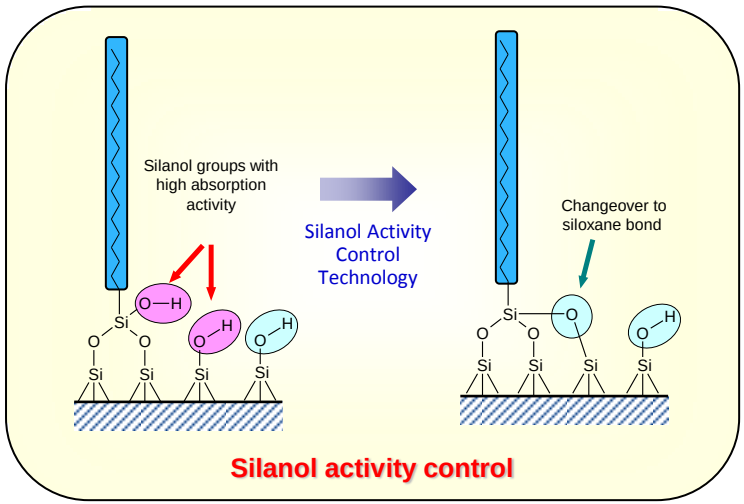
Sales by ChromaNik



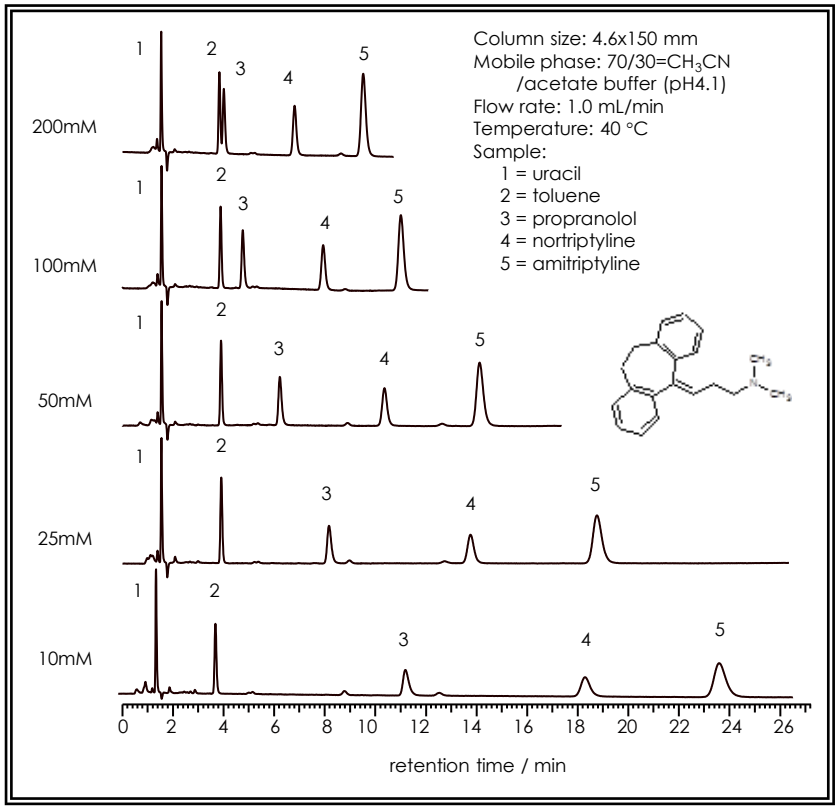
Sunrise C18-SAC
Silanol activity controlled C18

■ Silanol activity control technology

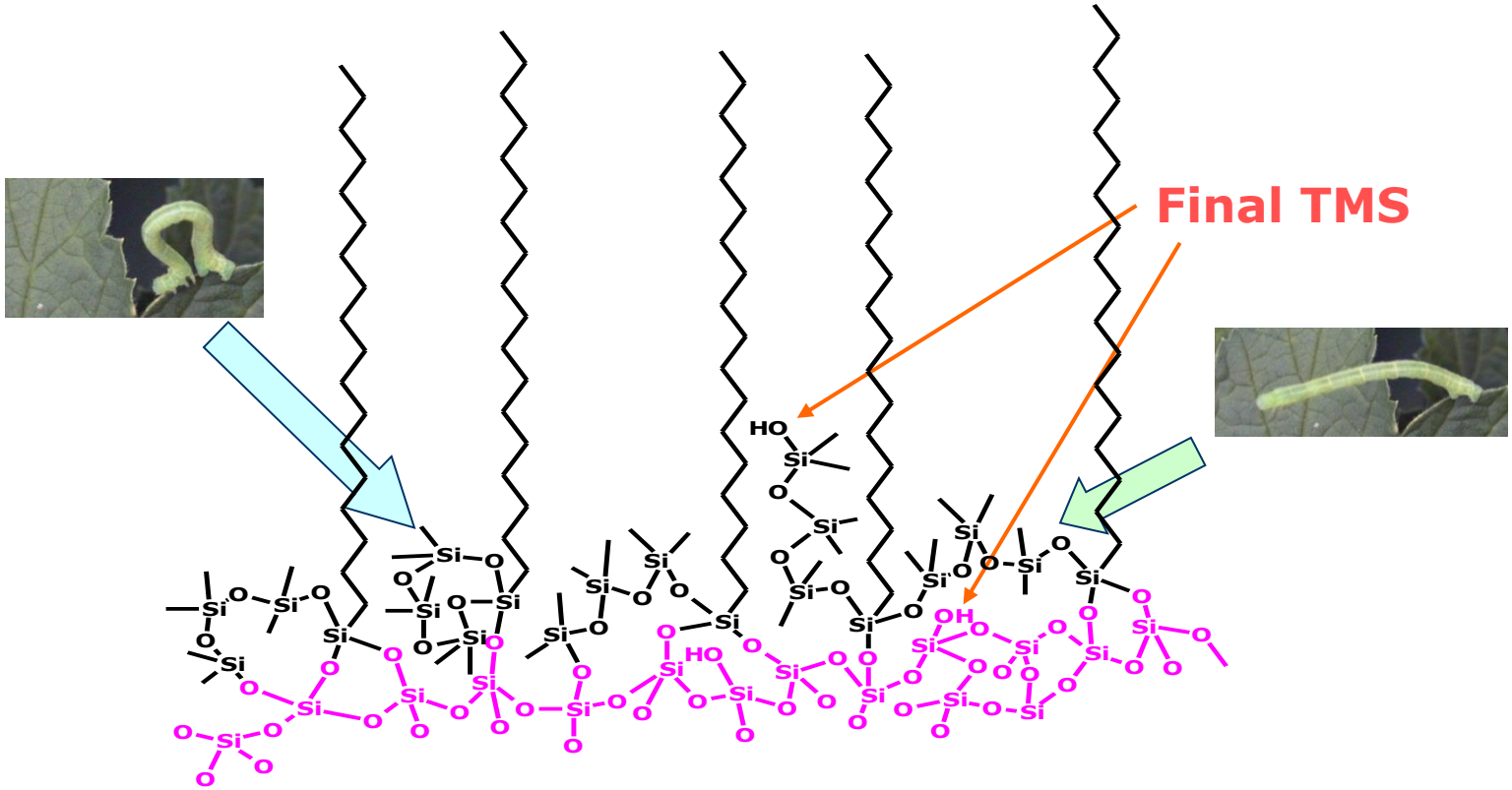
ChromaNik developed the technique that decreased only silanol groups with high absorption activity to a basic compound and remained effective sailnol groups on the stationary phase. Silanol activity control and no end-capping led the existence of silanol groups with high hydration which created a new and unique reversed-phase separation mode including hydrogen bond and ion-exchange interaction. Furthermore, silanol activity controlling, then end-capping technique improved a peak shape of a basic compound exceedingly.



■ Separation of basic compounds with ammonium acetate: Effect of salt concentration (Sunrise C18-SAC)

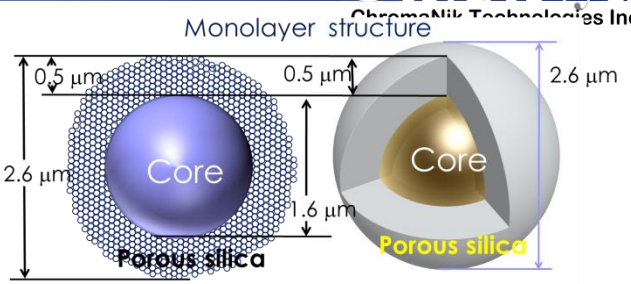


Sunniest C18
Special end-capping,
as a result high stability

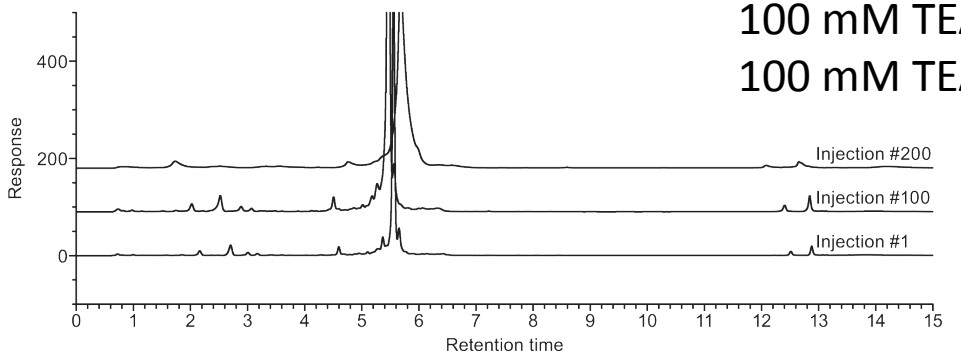


An Arm of HMODTS moves like a ***Geometrid caterpillar***, so that a functional group on the tip of the arm can bond with a silanol group which Is located anywhere.

SunShell Core shell column



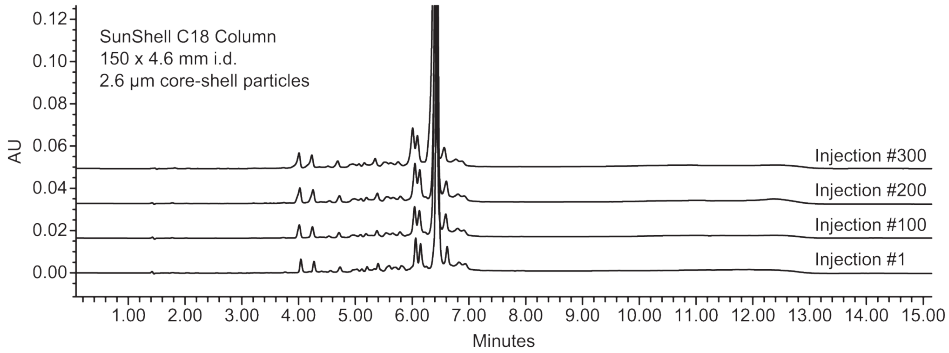
M. Biba et al. / Journal of Pharmaceutical and Biomedical Analysis 96 (2014) 54–57



100 mM TEAA in water pH7 (mobile phase A)
100 mM TEAA in acetonitrile (mobile phase B)

Kinetex C18 showed terrible peaks after 100 injections.

Fig. 1. Long-term column stability of Kinetex C18 column. IP-RPLC method conditions: Kinetex C18 column (100 mm × 3 mm i.d. and 1.7 µm core–shell particles). The mobile phase consisted of 100 mM TEAA in water (mobile phase A) and 100 mM TEAA in acetonitrile (mobile phase B). A gradient method of 10–25%B over 10 min, then 25–60%B in 2 min (to elute late eluting impurities) and 3 min post column equilibration at 10%B was used. The flow rate was 0.6 mL/min, the column temperature was 60 °C, UV detection was set at 260 nm, and injection volume was 2 µL. Atlas chromatographic system was used for system control and data processing.



SunShell C18 showed good peaks after 300 injections.

Fig. 2. Long-term column stability of SunShell C18 column. IP-RPLC method conditions: SunShell C18 column (150 mm × 4.6 mm i.d. and 2.6 µm core–shell particles). The mobile phase consisted of 100 mM TEAA in water (mobile phase A) and 100 mM TEAA in acetonitrile (mobile phase B). A standard linear gradient method of 5–25%B over 10 min with 5 min post column equilibration at 5%B was used. The flow rate was 1.5 mL/min, the column temperature was 65 °C, UV detection was set at 260 nm, and injection volume was 5 µL. Empower 2 chromatographic system was used for system control and data processing.

2) Feature of Core Shell Particle and SunShell Bonding Technology

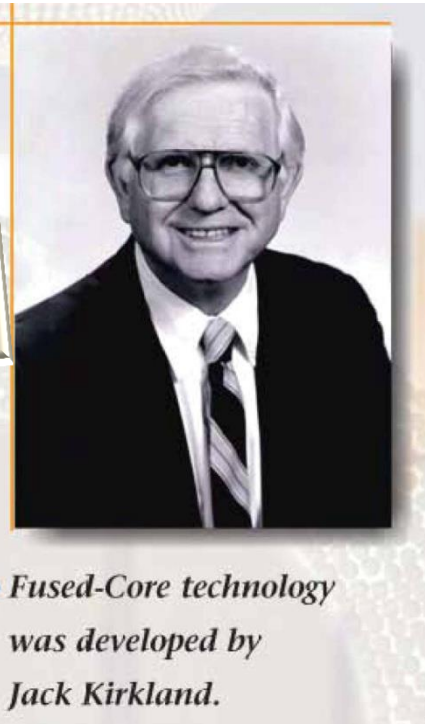
History of Core Shell Silica

Pellicullar particle presented by Kirkland in 1969.
Core diameter: 30 μm, Thickness of superficially porous layer: 0.5 μm

Superficially porous particle presented by Kirkland in 2000[1] (Poroshell)
Core diameter: 4 μm, Thickness of superficially porous layer: 0.5 μm

Superficially porous particle presented by Kirkland in 2007[2] (Halo)
Core diameter: 1.7 μm, Thickness of superficially porous layer: 0.5 μm

A dominant patent was materialized at 1967 and 1968.



• *Fused-Core technology was developed by Jack Kirkland.*

(1) J.J. Kirkland, F.A. Truszkowski, C.H. Dilks, and G.S. Engel, J. Chromatogr., A 890, 3–13 (2000).

(2) J.J. Kirkland, T.J. Langlois, and J.J. DeStefano, Am. Lab. 39, 18–21 (2007).

Current Trends in HPLC Column Usage

LCGC Europe Jan 1, 2012 By: Ronald E. Majors

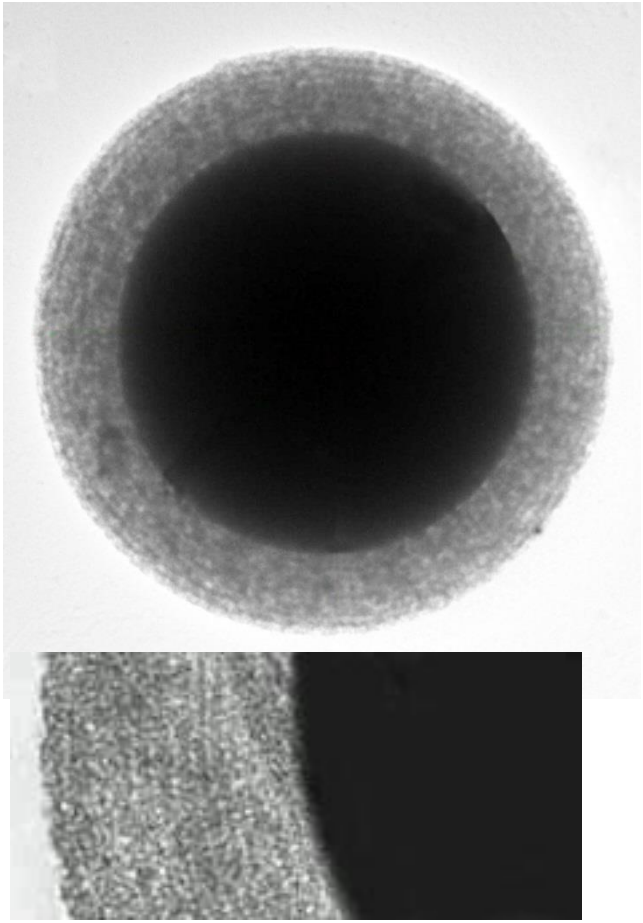
Table 14: Types of columns that will be tried in future.

Type of Column	Will Try in Future (%)
Superficially porous particle	54
Porous, sub 2 μm	49
Hybrid	34
Monolith, silica-based	31
Monolith, polymer-based	30

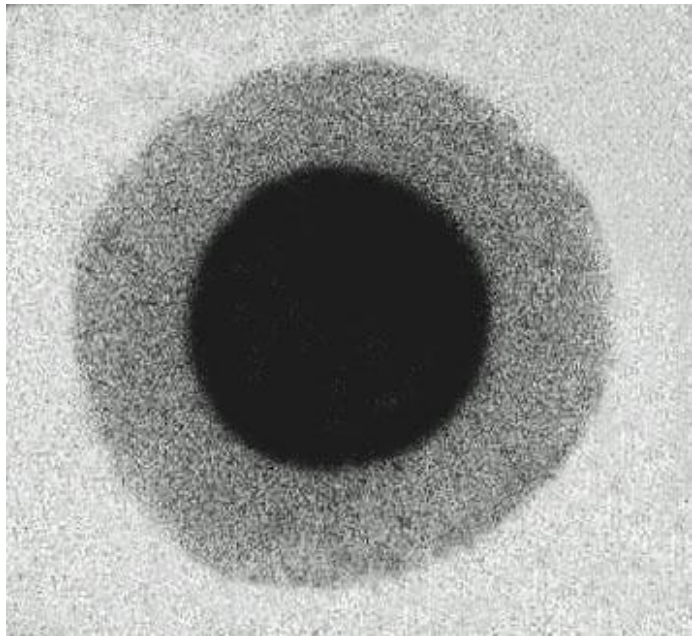
CoreShell (Solid Core) Column

- ACE UltraCore Super C18
- Accucore C18
- Aeris PEPTIDE XB-C18
- Amplus C18-30
- Ascentis Express C18
- BioShell C18
- Brownlee SPP C18
- BlueShell C18
- Capcell Core C18
- CORTECS C18
- COSMOCORE C18
- HALO C18
- Kinetex C18
- Meteoric Core C18
- NUCLEOSHELL C18
- Poroshell C18
- Raptor ARC18
- SpeedCore C18
- SunShell C18
- Coresep 100

2 kinds of typical core shell particles

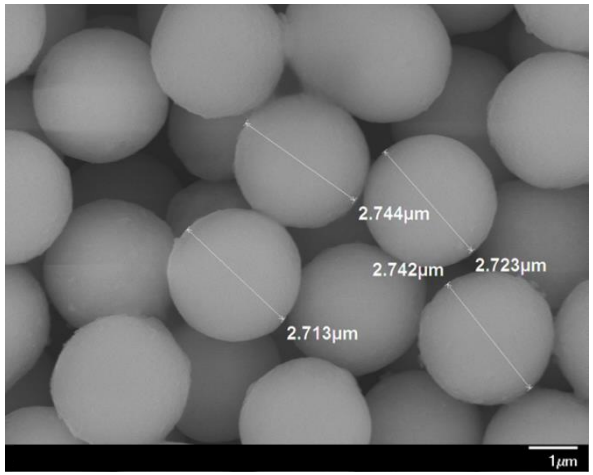


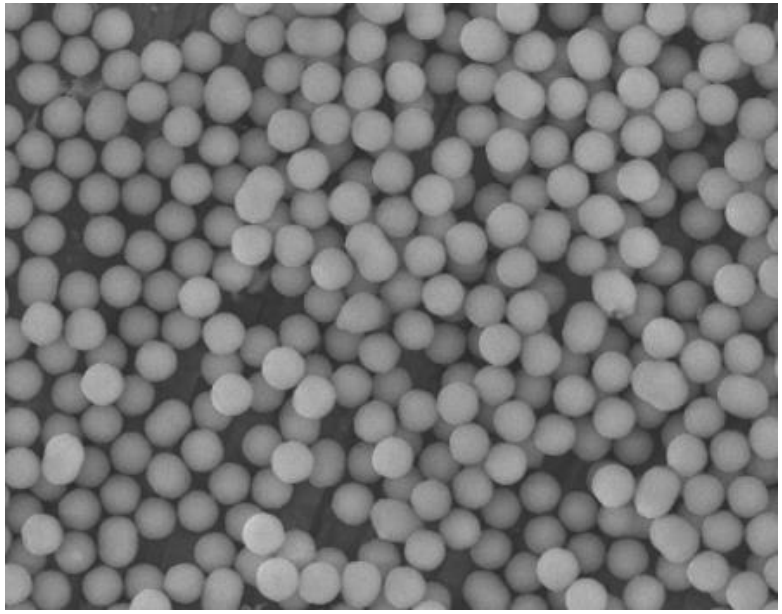
Multilayer porous silica structure using layer-by-layer method



Monolayer porous silica structure

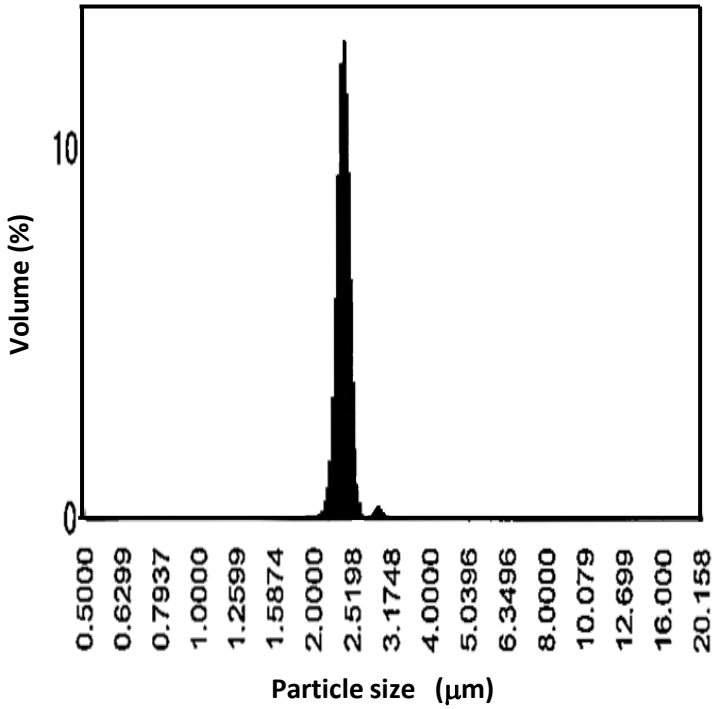
Particle distribution of A company core shell





Coulter counter

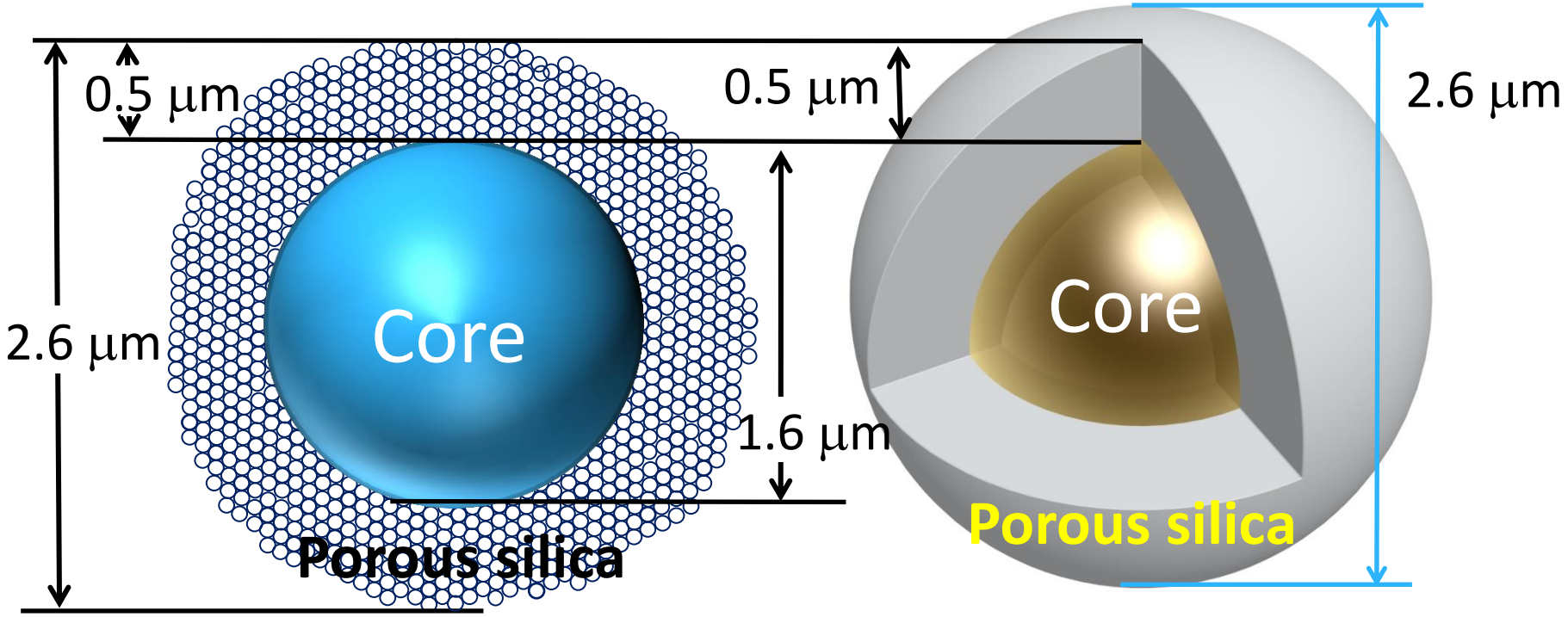
D_{90}/D_{10}=1.12



Schematic Diagram of Core Shell silica

(SunShell)

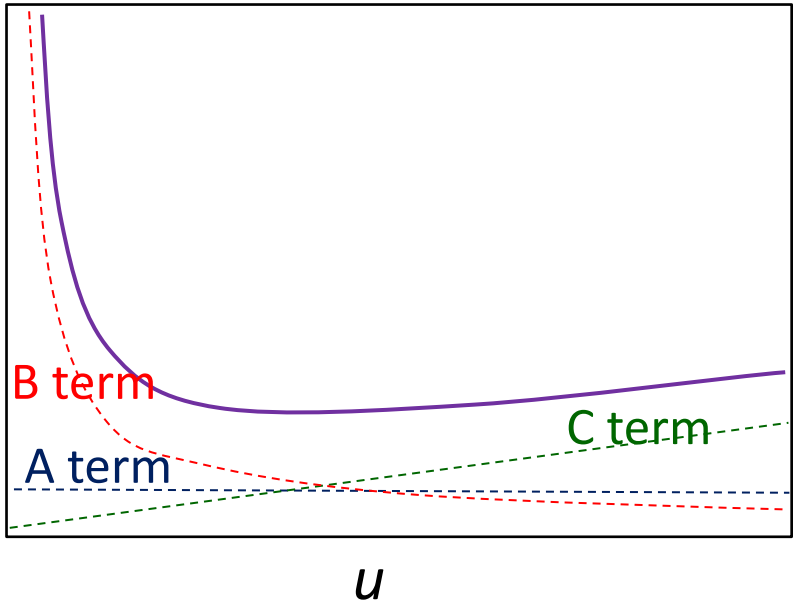
Monolayer structure



Particle diameter: 2.6 μm, Core diameter: 1.6 μm,
Thickness of porous silica: 0.5 μm
Pore volume: 0.30mL/g, Specific surface area: 150 m^2/g,
Pore diameter: 9 nm
The ratio of porous silica volume: 77%

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)

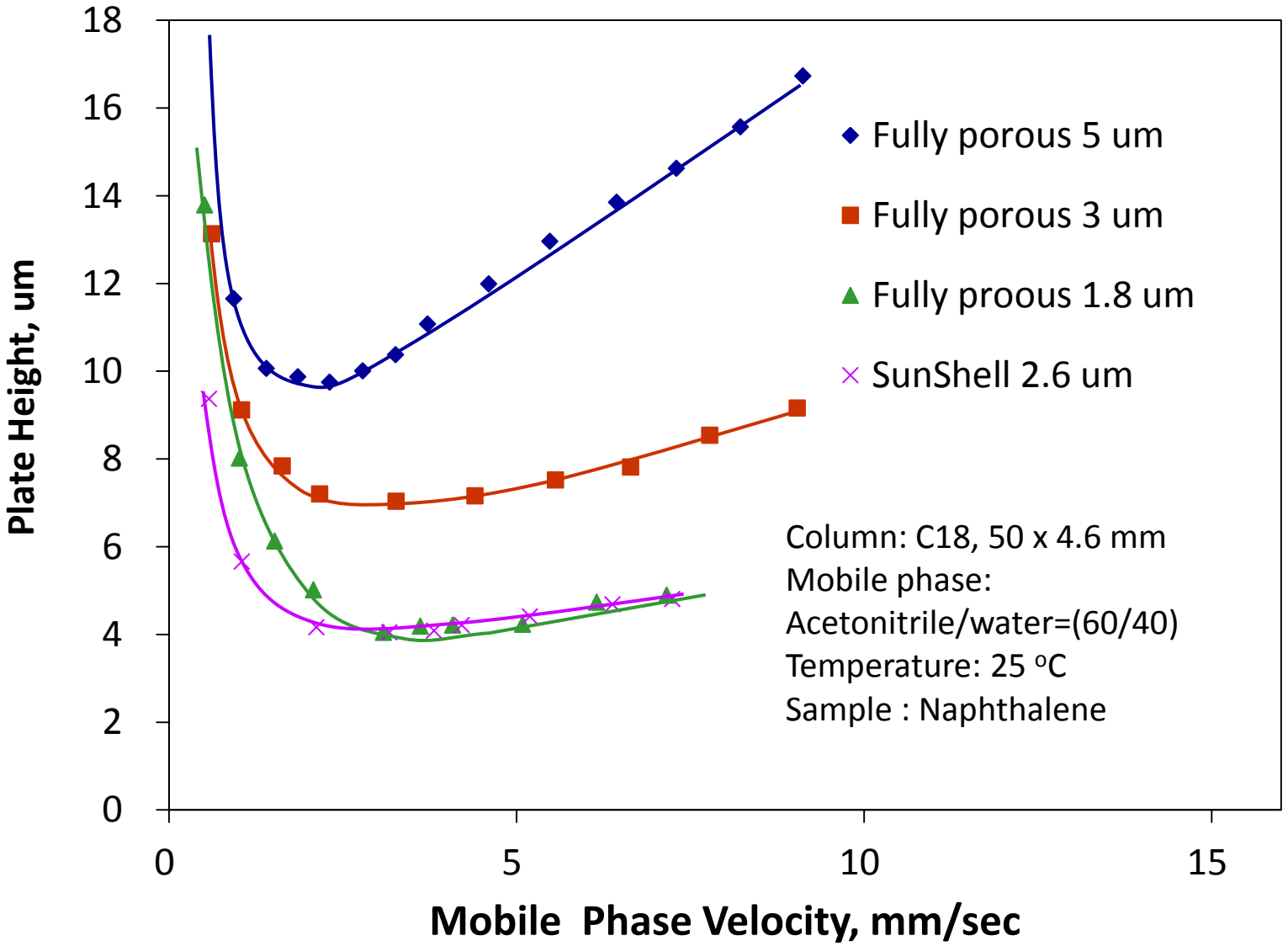
B term : Longitudinal diffusion

(Dm is diffusion coefficient)

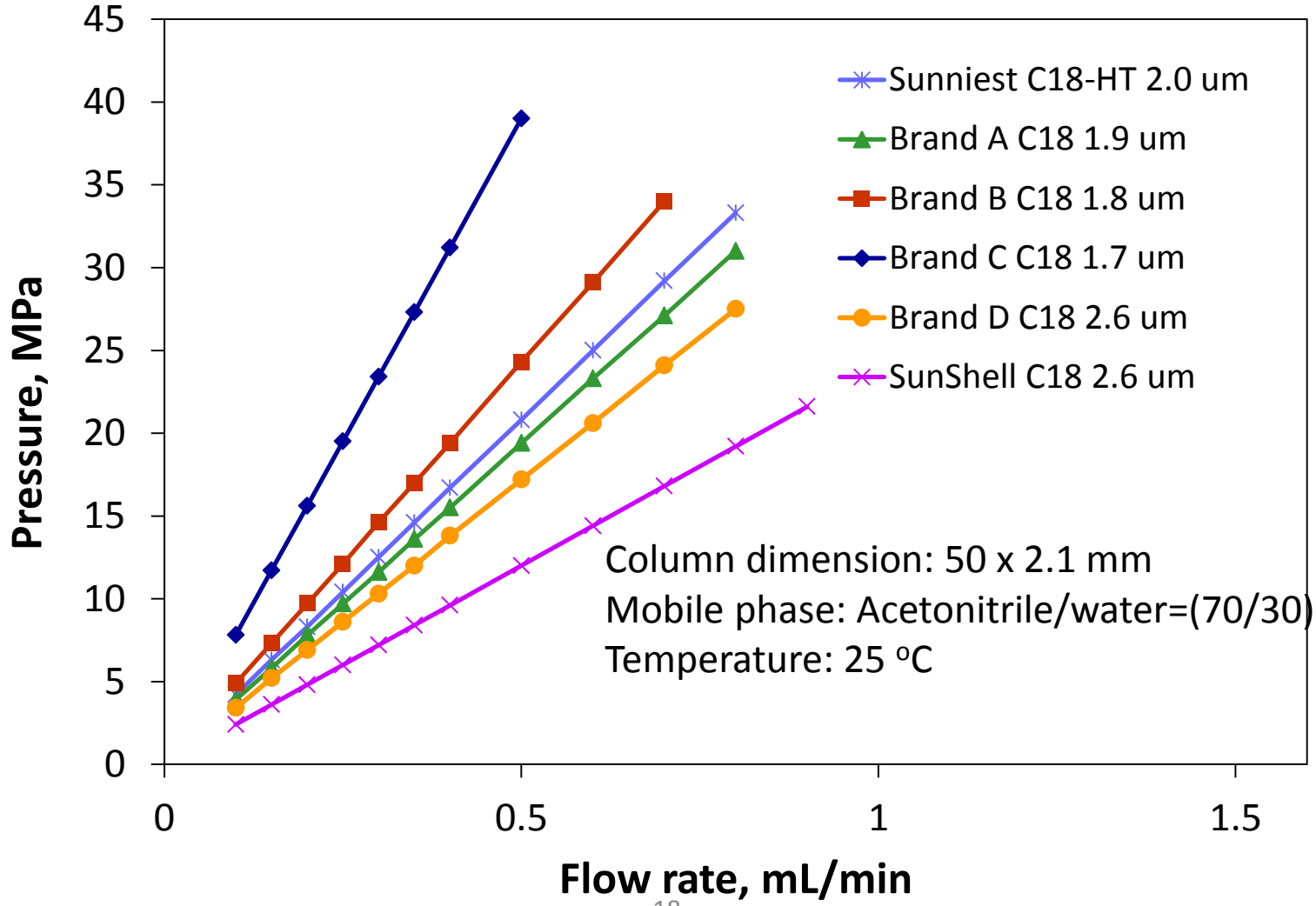
C term : Mass transfer

1. F. D. Antia and C. Horvath, *J. Chromatogr.,* 435 (1988) 1-15.

Comparison of Plate Height Plots



Comparison of Back Pressure for High Throughput Columns



Impedance time t_0/N^2

When back pressure is constant,

t_0 (no retained time) is proportional to N^2 (square plate).

$$t_0 \propto N^2$$

$$t_0 = A \cdot N^2 \qquad A = t_0/N^2$$

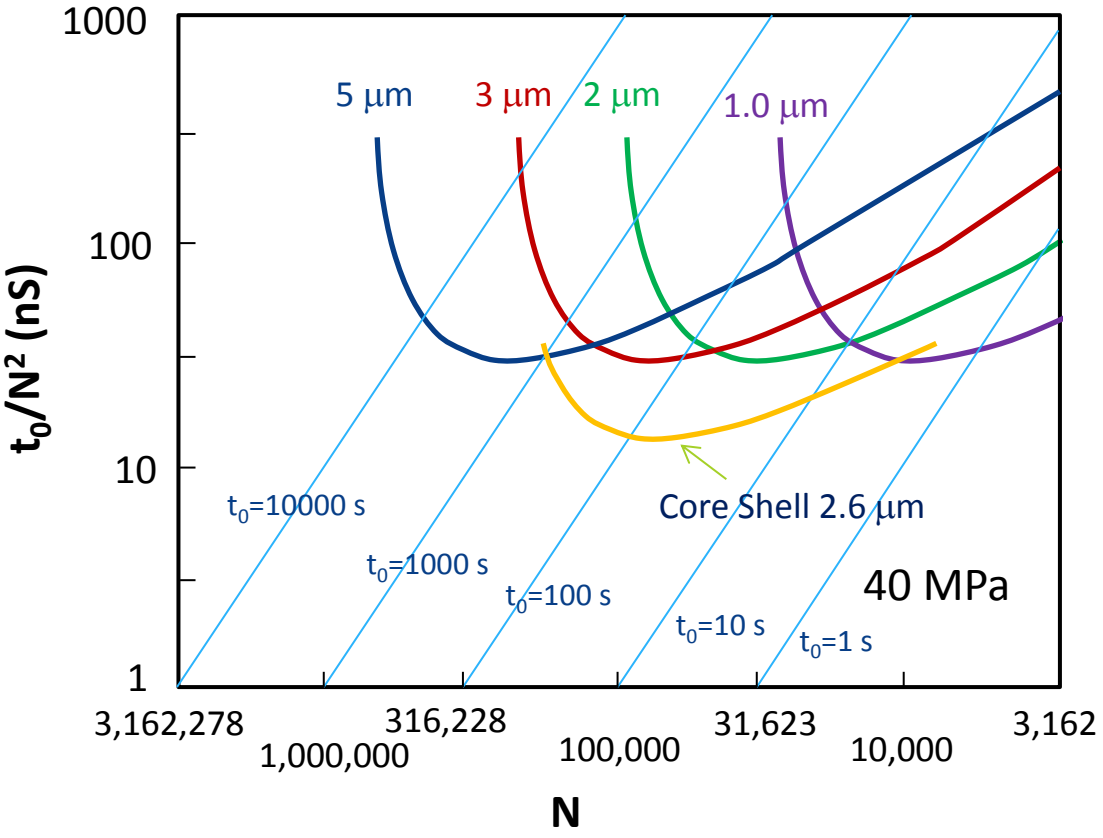
A is an impedance time.

Plate (N)	Column Length	Back pressure	Flow rate	t_0
10,000	15 cm	10 MPa	1.0 mL/min	100 S
20,000	30 cm	20 MPa	1.0 mL/min	200 S
20,000	30 cm	10 MPa	0.5 mL/min	400 S

If a back pressure is same 10 MPa, t_0 shows 4 times value when Plate becomes 2 times.

Desmet et al. Anal. Chem. 77, 4058 (2005).

Kinetic plot analysis at 40 MPa.



Calculated values were plotted for 1.0, 2.0, 3.0 , 5.0 μm fully porous particle .

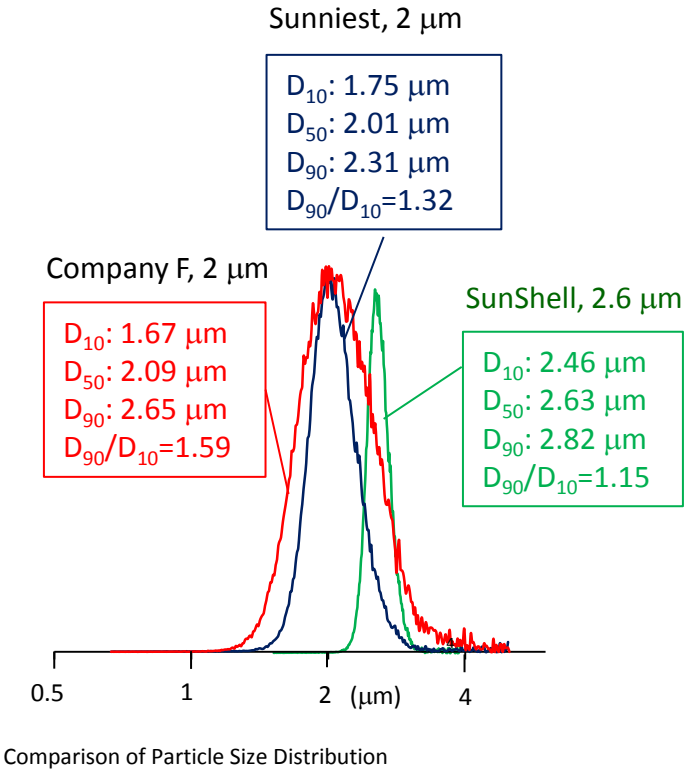
An experimental values were plotted for 2.6 μm core shell particle.

This figure means that we can separate faster using Core shell than fully porous and core shell has higher plate than fully porous at the same analysis time (t_0).

The curves for particulate columns were obtained by assuming *η=0.00046 Pa s, φ=700, Dm=2.22x10-9 m2/s, Knox equation, h=0.65v1/3+2/v+0.08v, Dp, totally porous 1.0, 2.0, 3.0 , 5.0 μm, core shell 2.6 μm.*

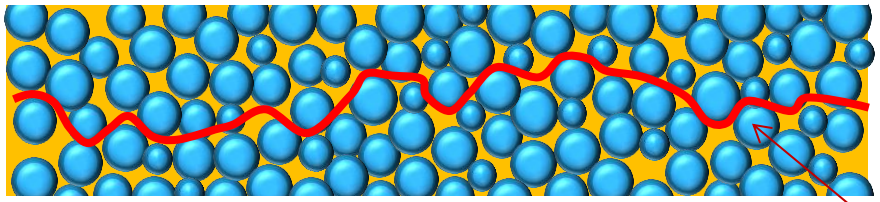
Why does a 2.6 μm core shell particle show the same performance as a sub 2 μm particle?

Narrow particle distribution



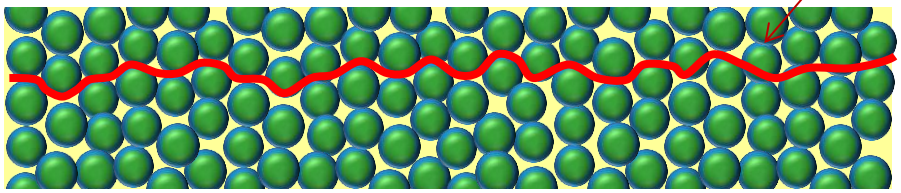
Comparison of Particle Size Distribution

Wide particle distribution (Conventional silica gel D_{90}/D_{10}=1.50)



Flow of mobile phase

Narrow particle distribution (core shell silica D_{90}/D_{10}=1.15)



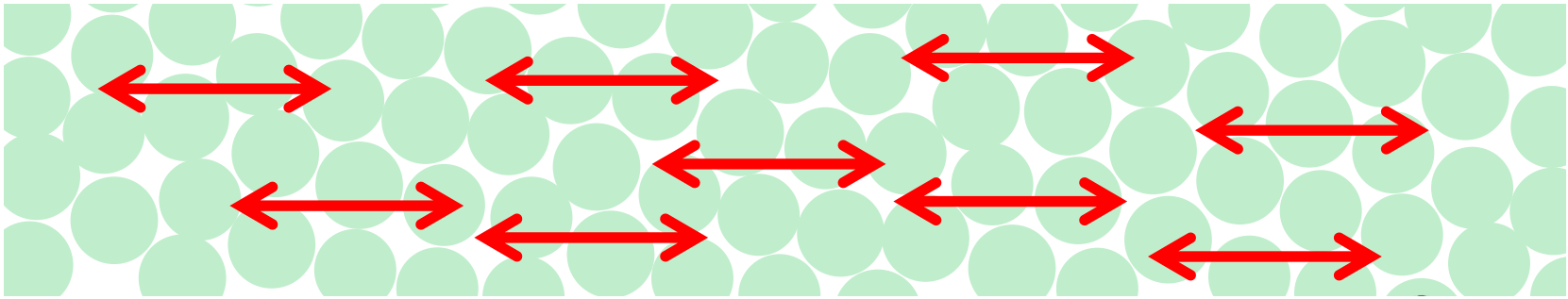
Packing state of core shell and fully porous silica

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.

Difference of diffusion in column at longitudinal direction

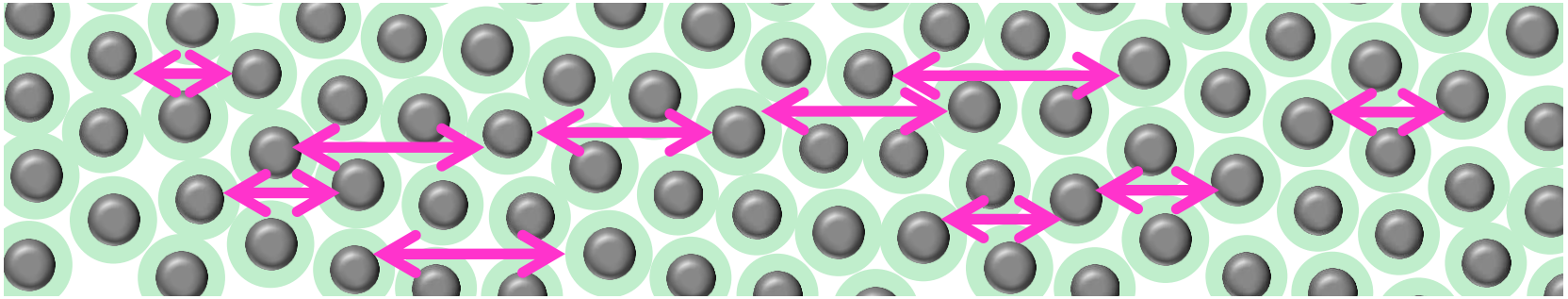
A solute diffuses both outside a particle and in a pore.

Fully porous silica



Core shell silica

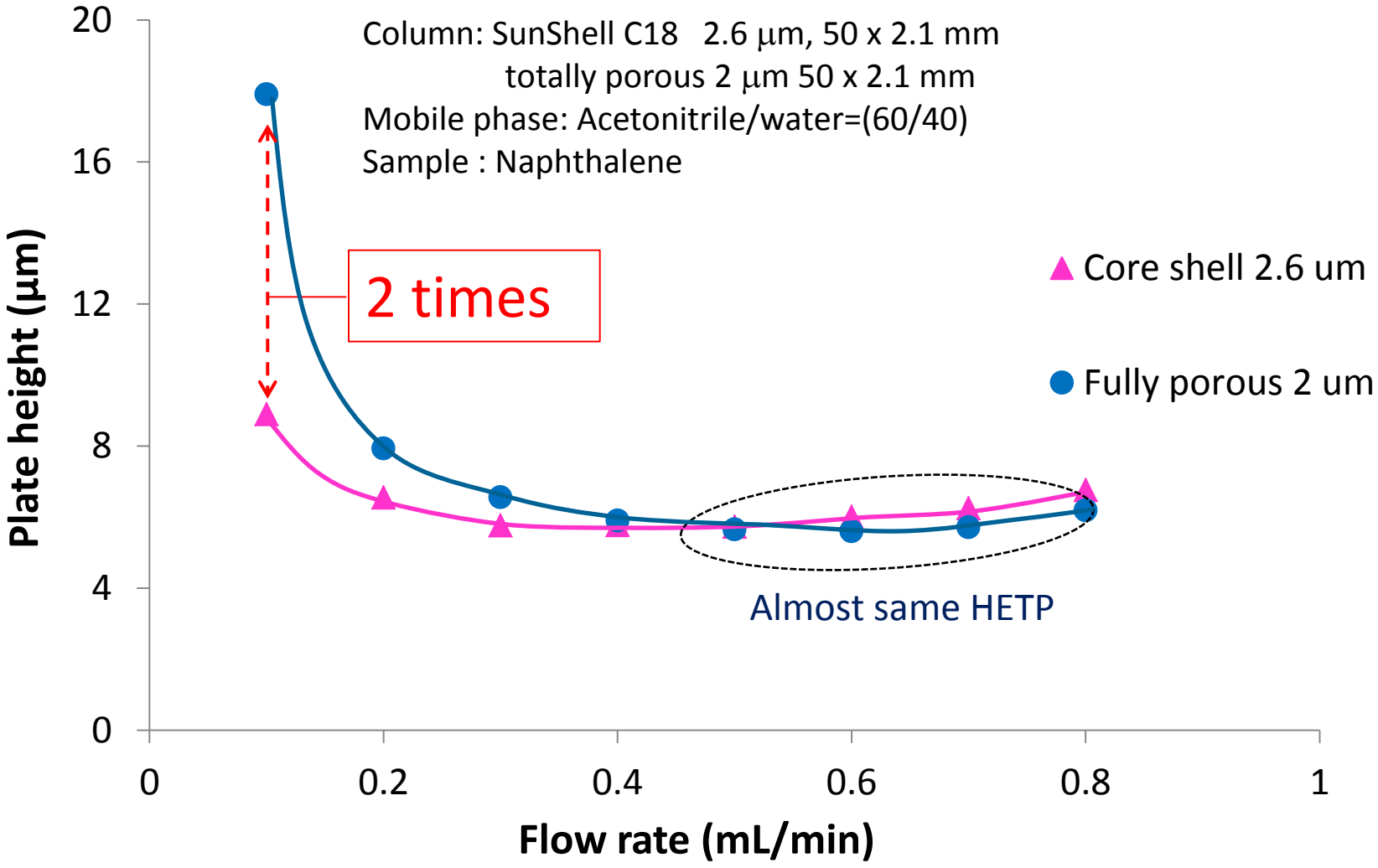
Surface ratio of core shell silica is around 30%.



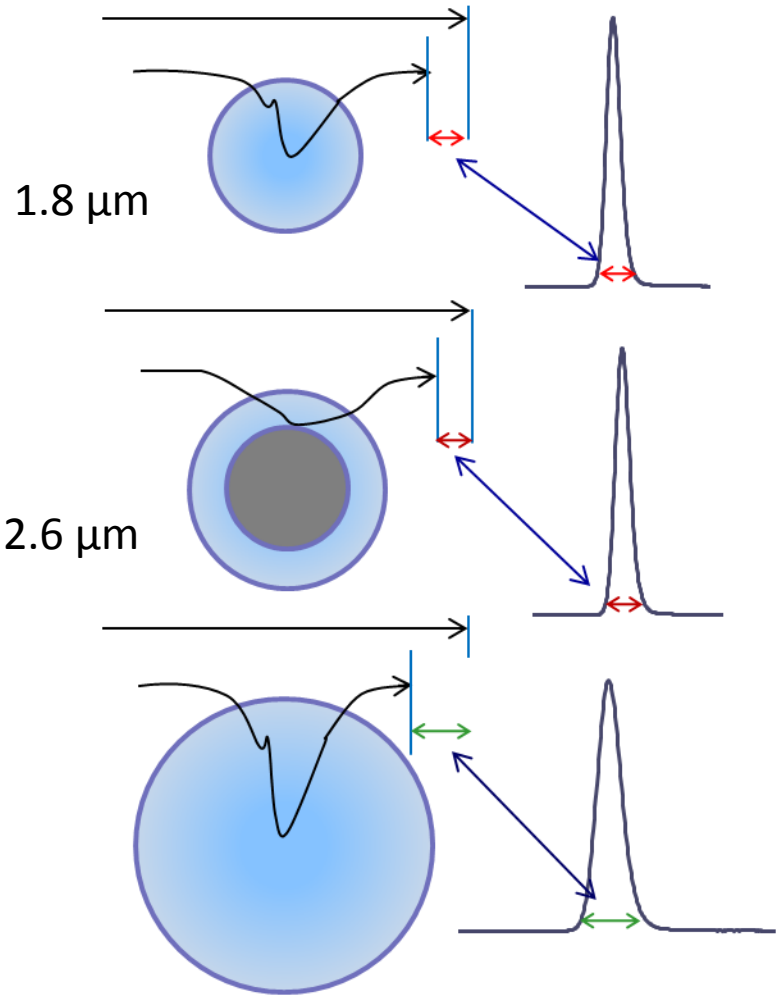
Cores block the path of diffusion of a solute.

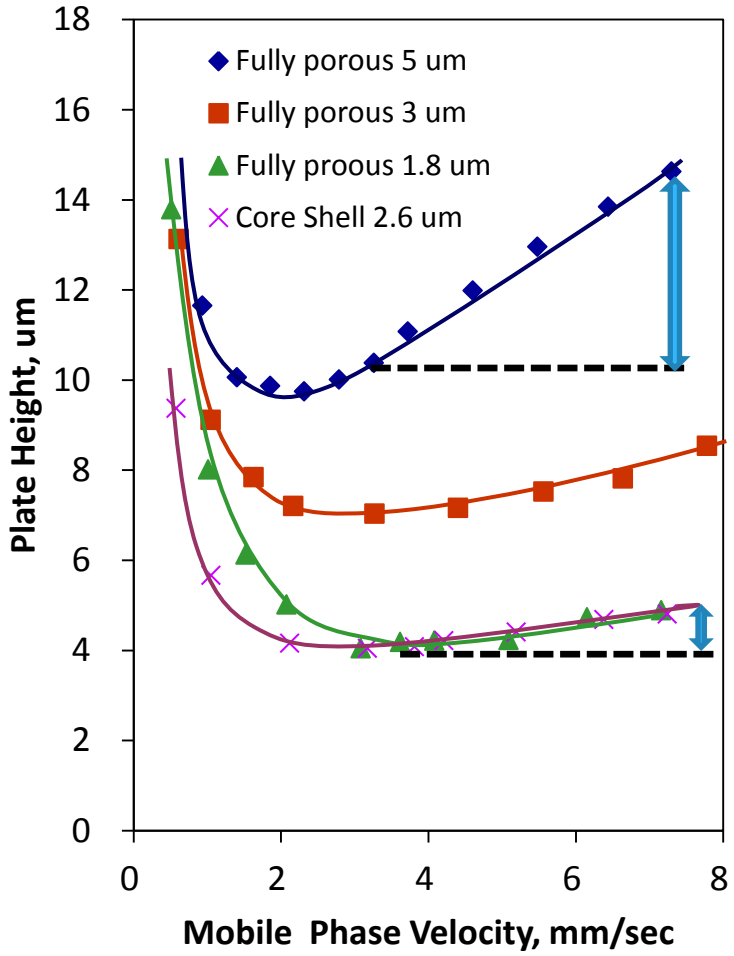
→ B term decreases to 70%.

HETP at low flow rate



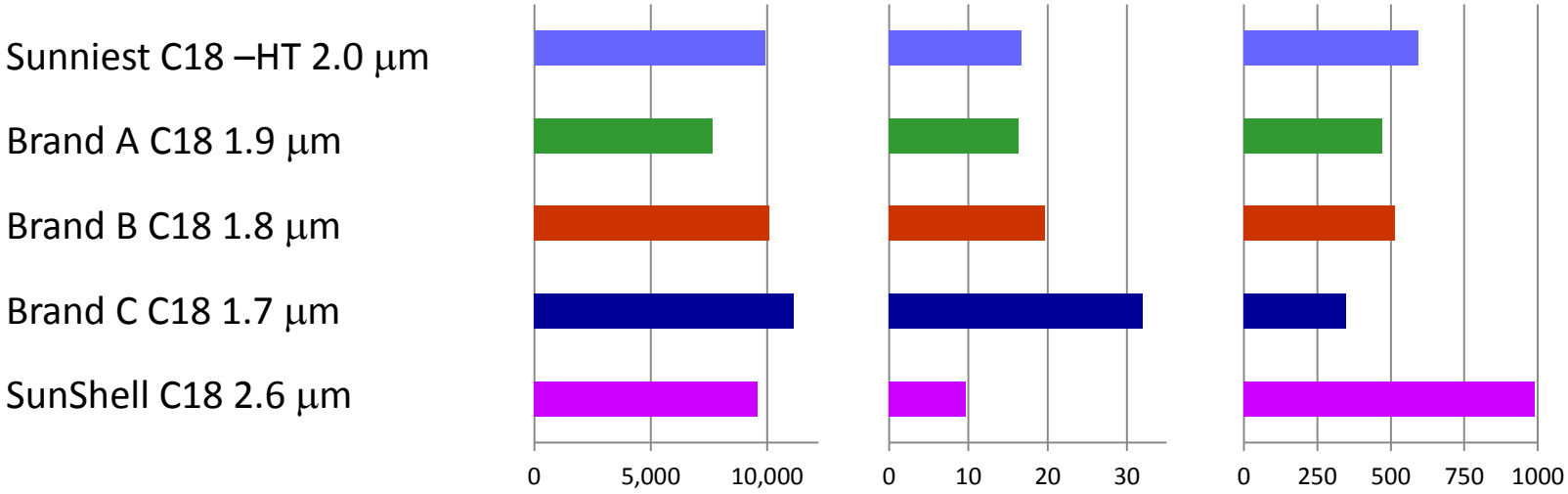
Short diffusion path by thin porous silica layer





Comparison of Performance by Plate/Pressure

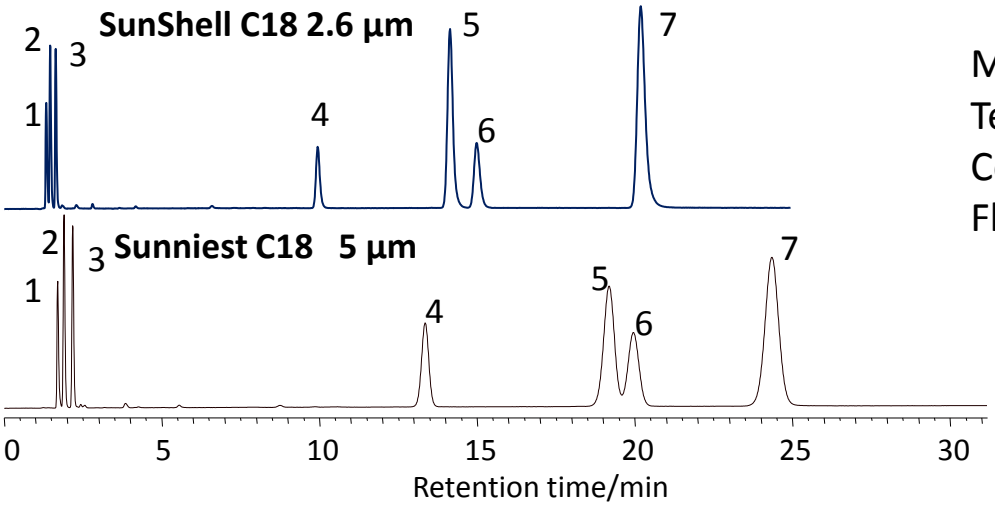
	Plates	Pressure(MPa)	Plate/pressure
Sunniest C18 –HT 2.0 μm	9,900	16.7	593
Brand A C18 1.9 μm	7,660	16.3	470
Brand B C18 1.8 μm	10,100	19.6	515
Brand C C18 1.7 μm	11,140	32.0	348
SunShell C18 2.6 μm	9,600	9.7	990



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

Comparison of retention between fully porous silica C18 and core shell silica C18s

	Fully porous silica C18 Sunniest C18 5 µm		Core shell silica C18 SunShell C18 2.6 µm		Core shell silica C18 Kinetex C18 2.6 µm	
Specific surface area	340 m^2/g		150 m^2/g		Effective 200 m^2/g	
Packing weight in the column	1.5 g (510 m^2)		2.7 g (405 m^2)		2.7 g	
	Retention time (t_R)	Retention factor (k)	Retention time (t_R)	Retention factor (k)	Retention time (t_R)	Retention factor (k)
1 = Uracil	1.70	**0**	1.35	**0**	1.36	**0**
2 = Caffeine	1.90	**0.12**	1.47	**0.09**	1.49	**0.10**
3 = Phenol	2.17	**0.28**	1.65	**0.22**	1.61	**0.18**
4 = Butylbenzene	13.35	**6.85**	10.01	**6.41**	6.19	**3.55**
5 = o-Terphenyl	19.19	**10.29**	14.24	**9.55**	8.15	**4.99**
6 = Amylbenzene	19.96	**10.74**	15.09	**10.18**	8.75	**5.43**
7 = Triphenylene	24.35	**13.32**	20.33	**14.06**	9.44	**5.94**



Mobile phase: Methanol/water(75:25)
Temperature: 40°C
Column dimension: 150 x 4.6 mm
Flow rate: 1.0 mL/min

Characteristics of SunShell (1)

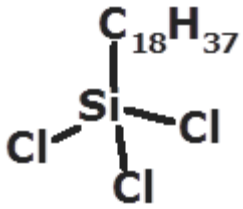
	Core shell silica			Bonded phase			
	Particle size (μm)	Pore diameter (nm)	Specific surface area (m^2/g)	Carbon content (%)	Bonded phase	End-capping	Available pH range
SunShell C18	2.6	9	150	7	C18	Sunniest endcapping	1.5 - 10
SunShell C18 5 μm	5	9	90	5.5	C18	Sunniest endcapping	1.5 - 10
SunShell C8	2.6	9	150	4.5	C8	Sunniest endcapping	1.5 - 9
SunShell PFP	2.6	9	150	4.5	Pentafluorophenyl	TMS endcapping	2 - 8
SunShell Phenyl	2.6	9	150	5	Phenylhexyl	Sunniest endcapping	1.5 - 9
SunShell RP-AQUA	2.6	16	90	4	C28	Sunniest endcapping	2 - 8[a)]
SunShell C18-WP	2.6	16	90	5	C18	Sunniest endcapping	1.5 - 10
SunShell HFC18-16	2.6	16	90	2.5	C18	Sunniest endcapping	1.5 – 9
SunShell HFC18-30	2.6	30	40	1.3	C18	Sunniest endcapping	1.5 – 9
SunShell C8-30	2.6	30	40	1.2	C8	Sunniest endcapping	1.5 – 9
SunShell C4-30	2.6	30	40	0.9	C4	Sunniest endcapping	1.5 – 9

a) This value is evaluated under 100% aqueous condition because SunShell RP-Aqua has reproducible retention under 100% aqueous condition.

Sunniest (SunShell) Bonding Technology

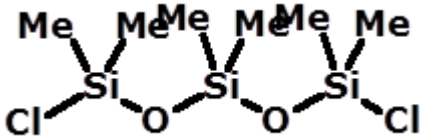
✓Trifunctional silyl-reagent,

(Octadecyltrichlorosilane)



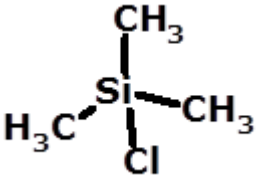
✓Difunctional silyl-endcapping reagent

(Hexamethyldichlorotrisiloxane)



✓Second silyl-endcapping reagent

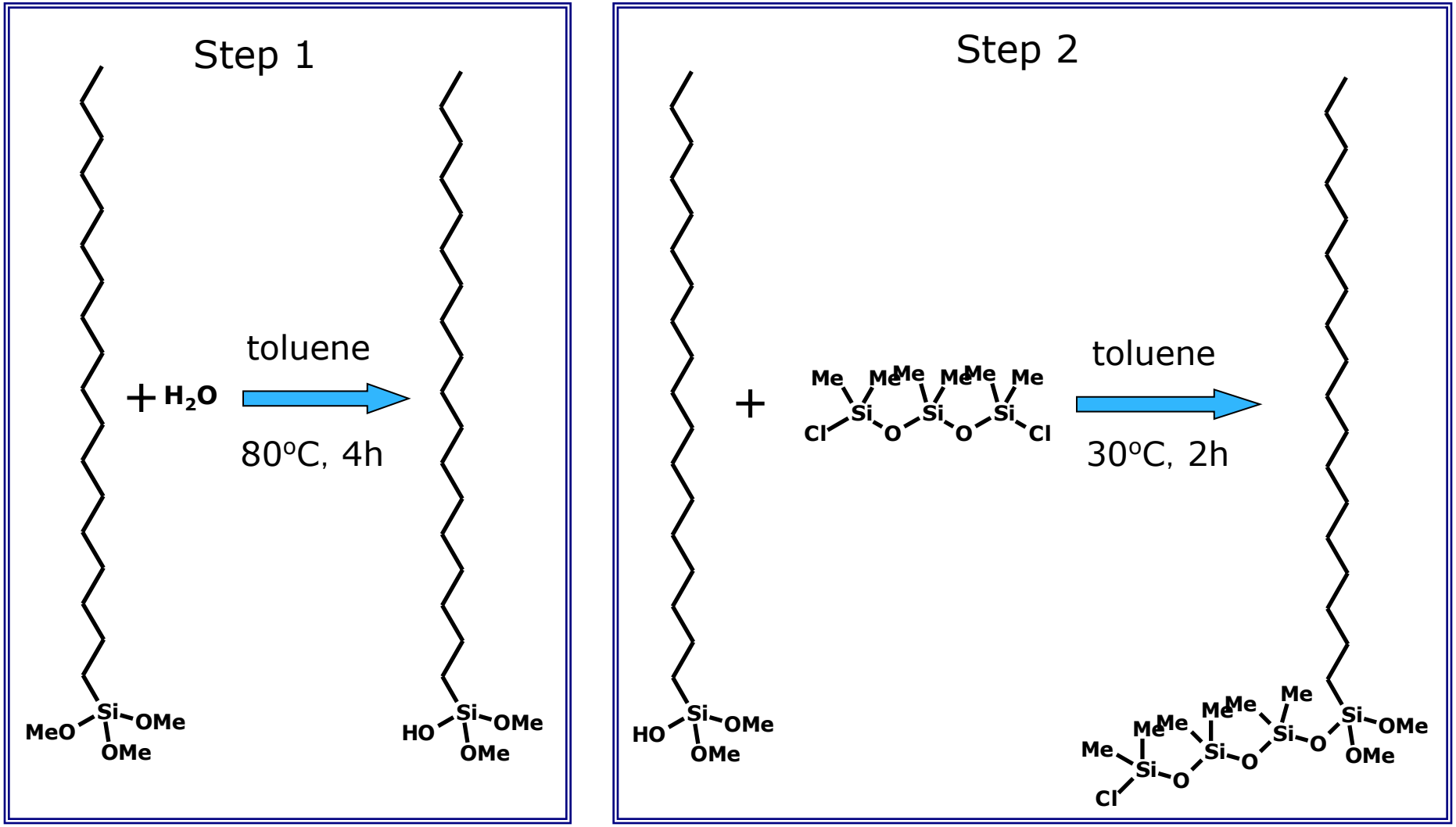
(above + Trimethylchrolosilane(TMS))



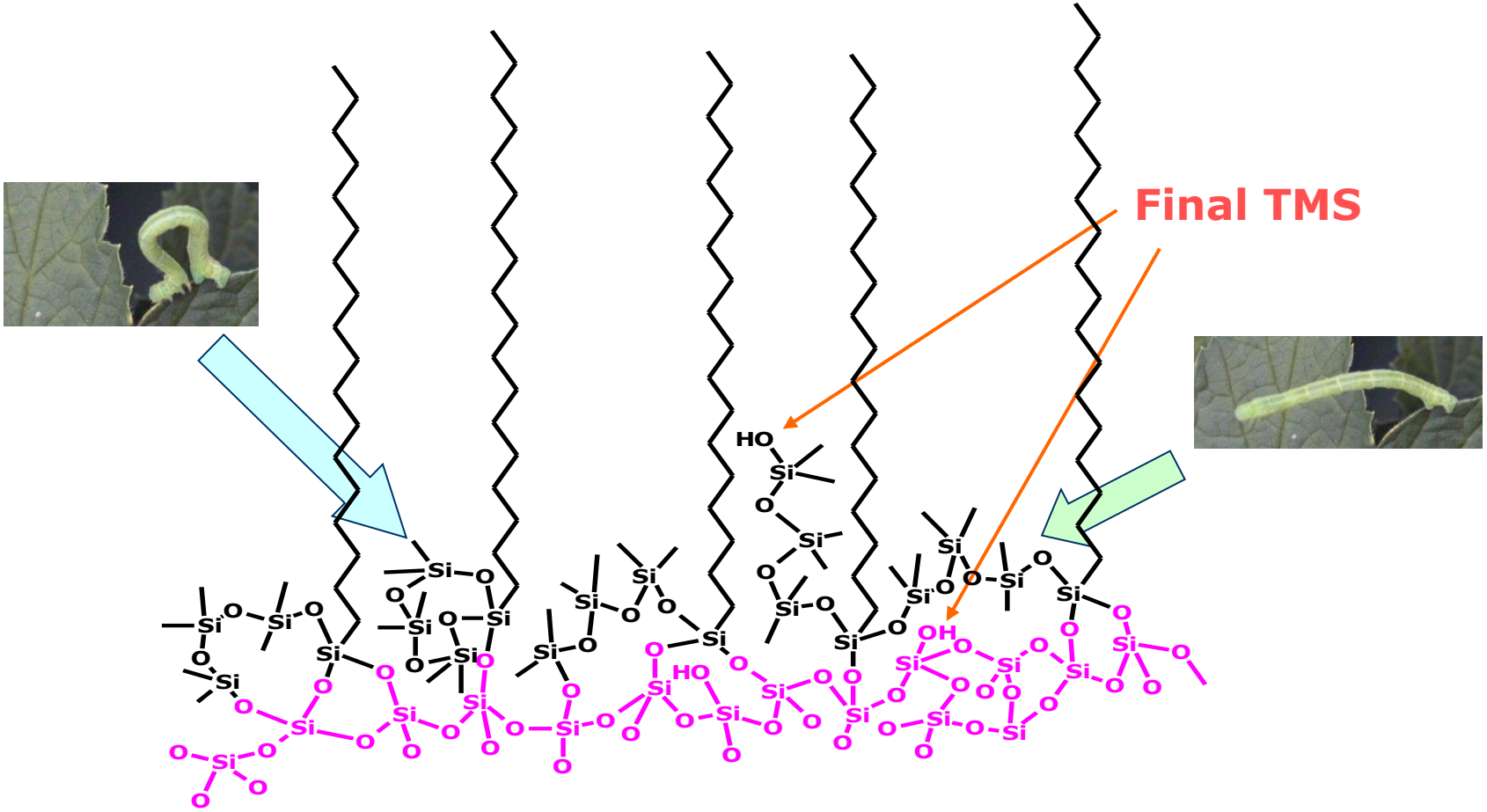
✓High reaction temperature for endcapping

C18 silyl-reagent (HMODTS)

Hexamethyloctadecyltetrasilane

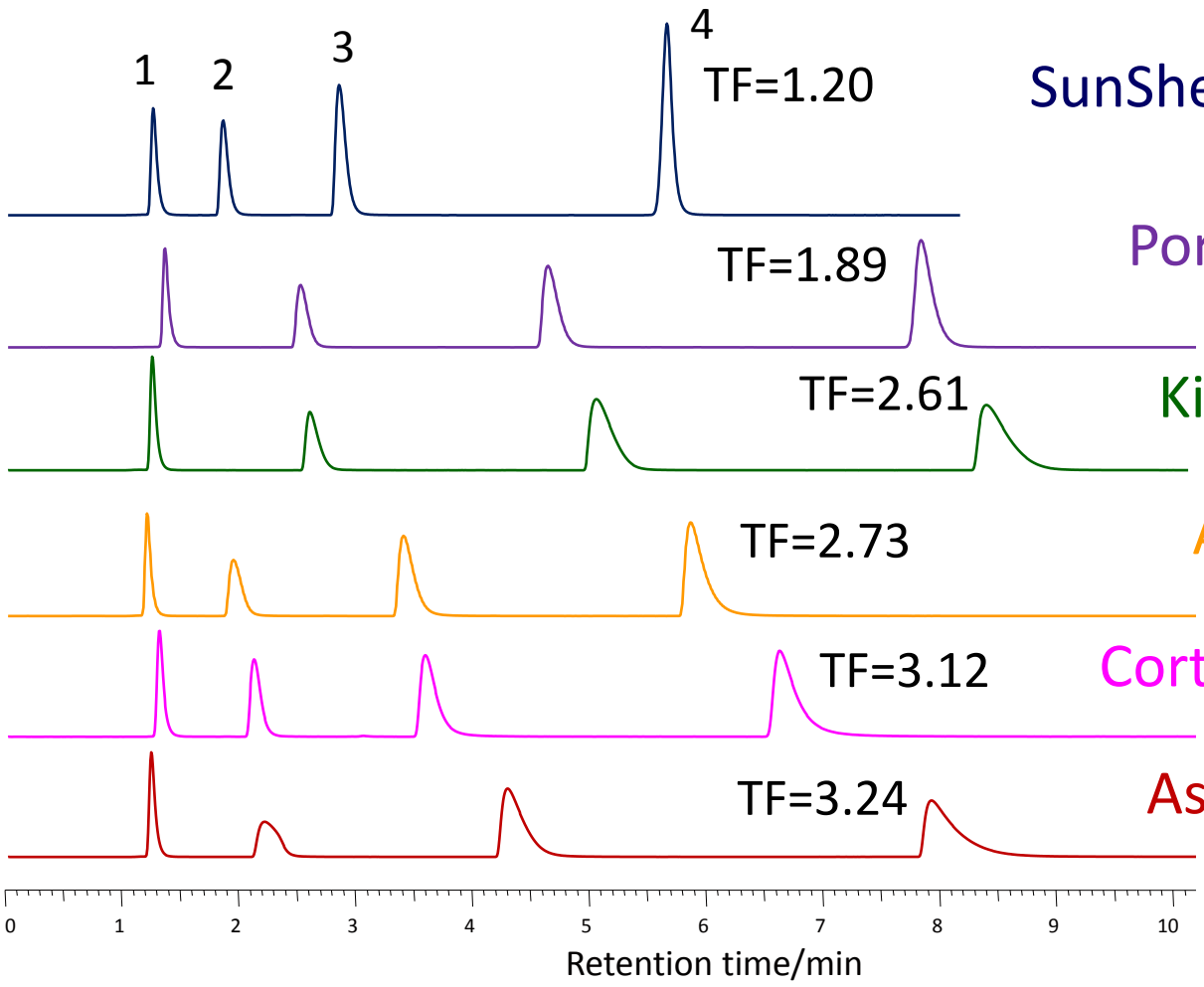


Bonding state of HMODTS on silica



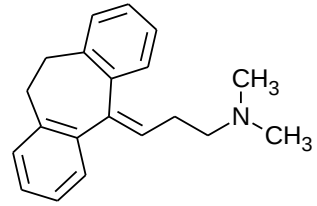
An Arm of HMODTS moves like a ***Geometrid caterpillar***, so that a functional group on the tip of the arm can bond with a silanol group which Is located anywhere.

Comparison of amitriptyline peak



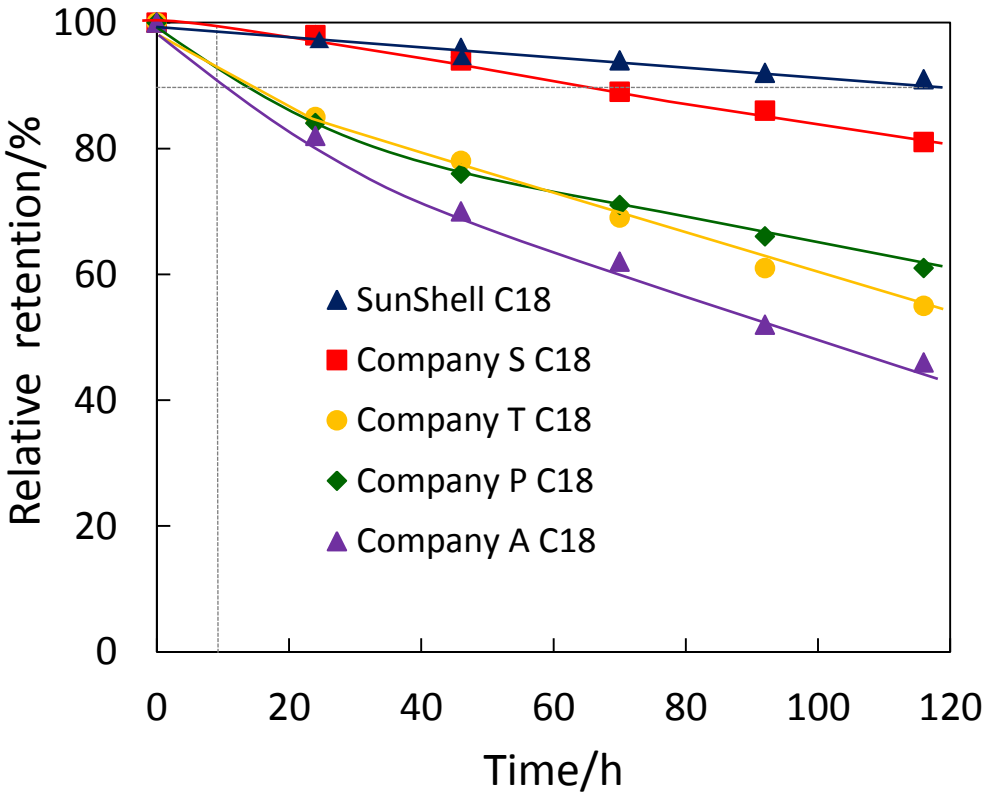
Mobile phase: Acetonitrile/**10mM ammonium acetate pH6.8**=(40:60)
Column dimension: 150 x 4.6 mm, Flow rate: 1.0 mL/min, Temp.: 40°C

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



Amitriptyline

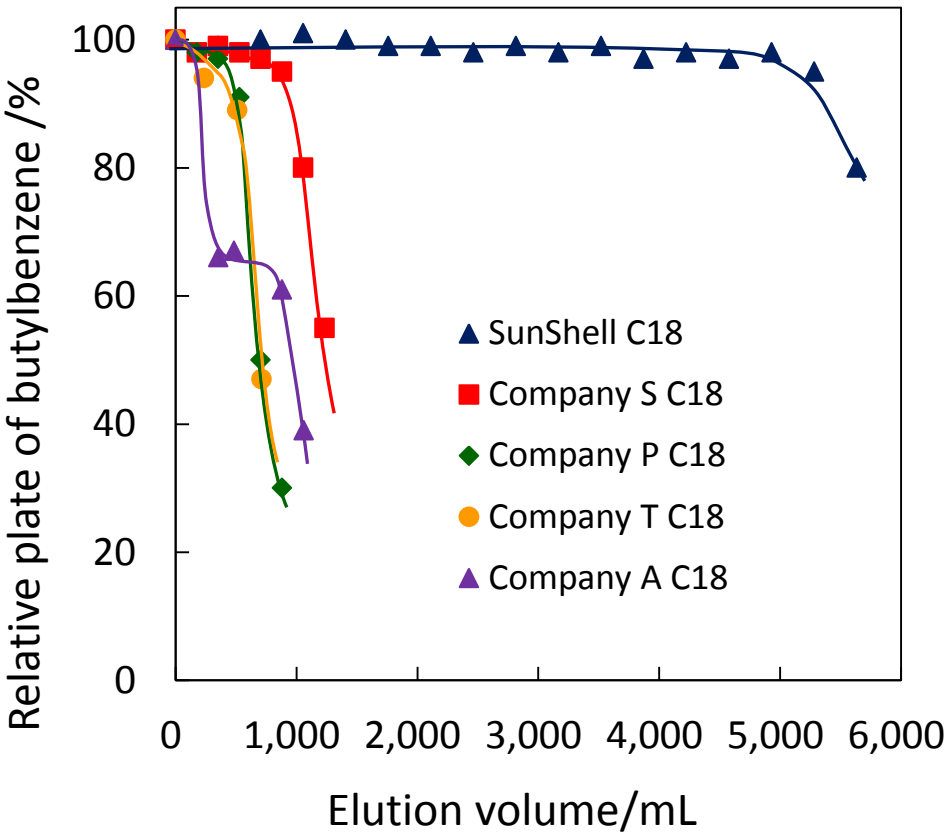
Stability under acidic pH condition



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 °C

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 °C
Sample: 1 = Uracil
2 = Butylbenzene

Stability under basic pH condition

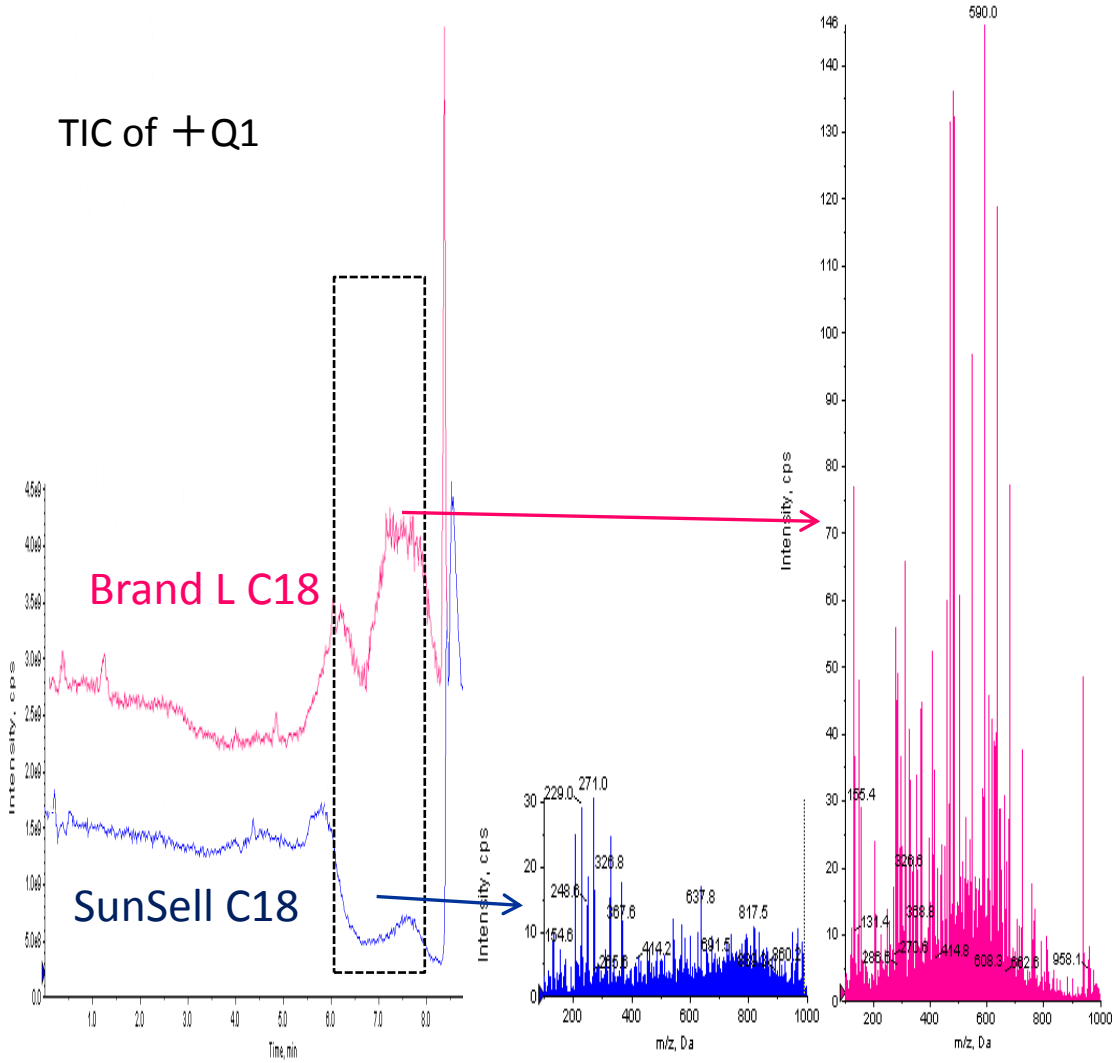


Durable test condition
Column size: 50 x 2.1 mm
Mobile phase:
CH_3OH/20mM Sodium borate/10mM NaOH=30/21/49 (pH10)
Flow rate: 0.4 mL/min
Temperature: 50 °C

Measurement condition
Column size: 50 x 2.1 mm
Mobile phase: CH_3OH/H_2O=70/30
Flow rate: 0.4 mL/min
Temperature: 40 °C
Sample: 1 = Butylbenzene

Bleeding test using LC/MS

＋Q1: 5.997 min to 7.999 min of Sample

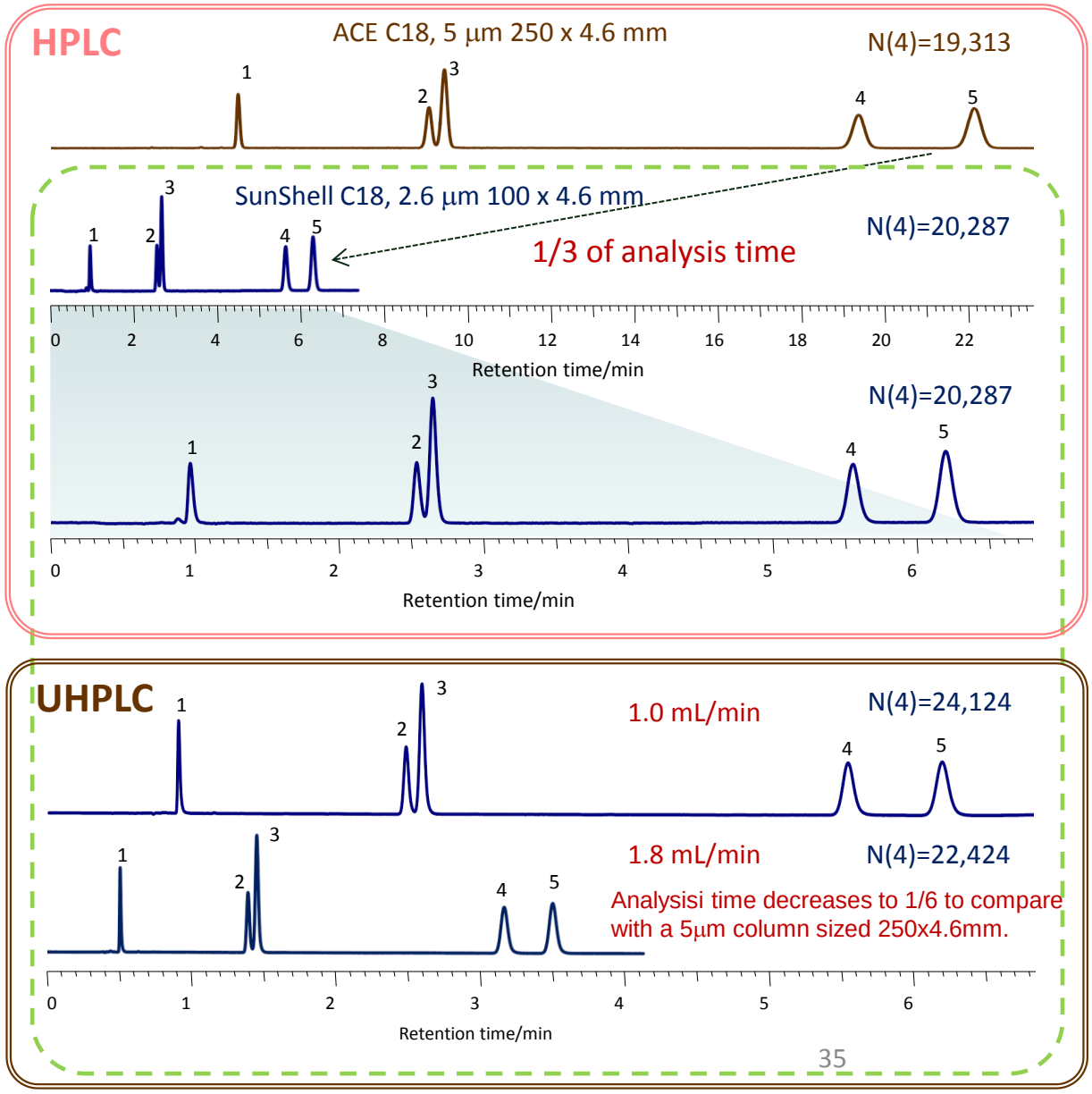


Column size: 50 x 2.1 mm
Mobile phase:
A) 0.1% acetic acid
B) CH_3CN
Gradient:

Time:	0min	1min	5min	7min
%B:	5%	5%	100%	100%

Flow rate: 0.4 mL/min
Temperature: 40 °C
MS : ABI API-4000
Ionization:
Turboionspray (cation)
Measurement mode:
Q1 Scan m/z 100-1000

Examples of transfer (isocratic separation)

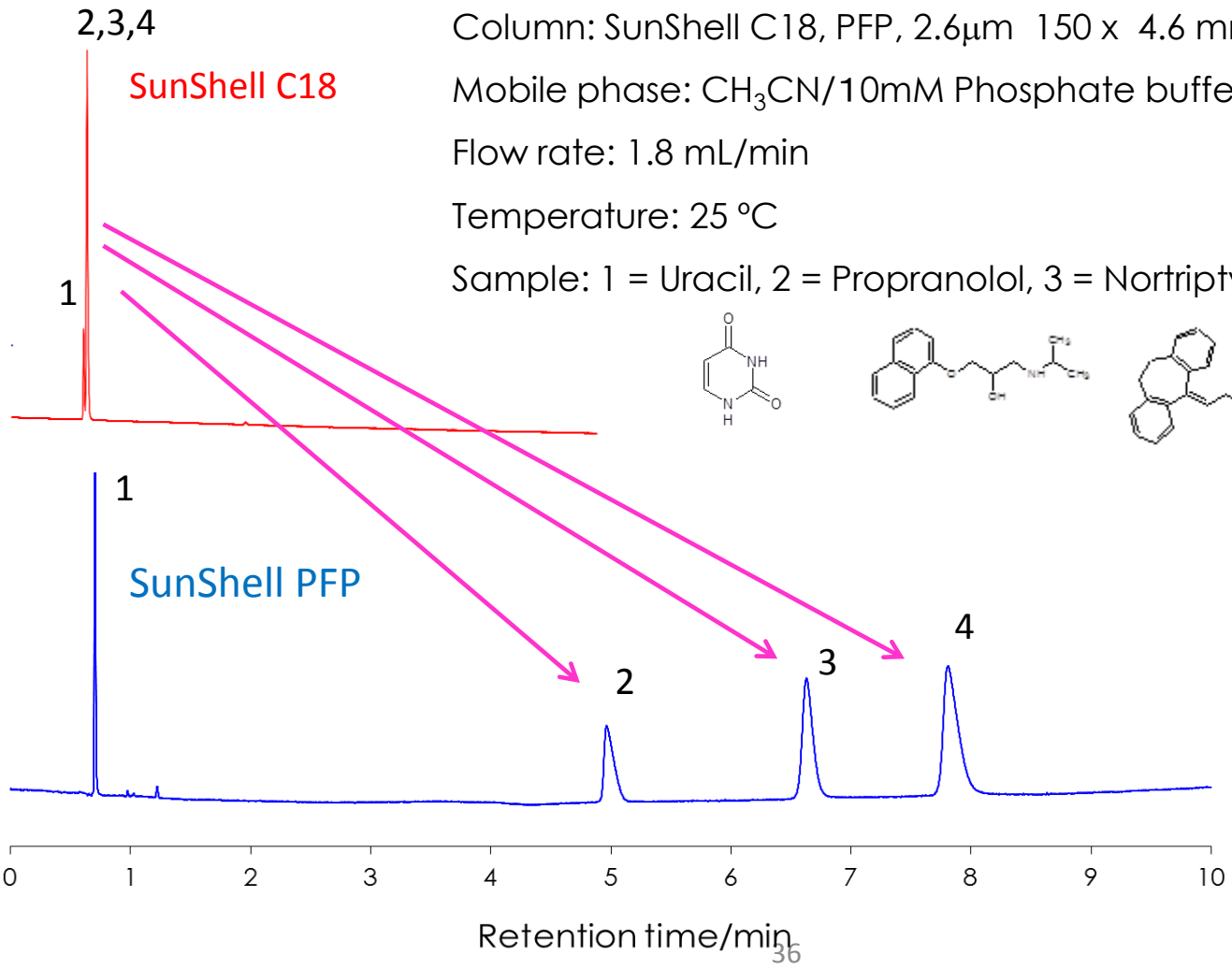


Column:
Brand F C18, 5 µm 250 x 4.6 mm
SunShell C18, 2.6 µm 100 x 4.6 mm
Mobile phase:
CH_3CN/20mM Phosphoric acid = 45/55
Flow rate: 1.0 mL/min,
1.8 mL/min at the lowest chromatogram
Temperature: 25 ºC
Pressure: 9.5 MPa for Brand F C18 5 µm
13.4 MPa for SunShell C18 2.6 µm
Detection: UV@230 nm

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

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

Retention comparison of cation between C18 and PFP phases



Column: SunShell C18, PFP, 2.6μm 150 x 4.6 mm

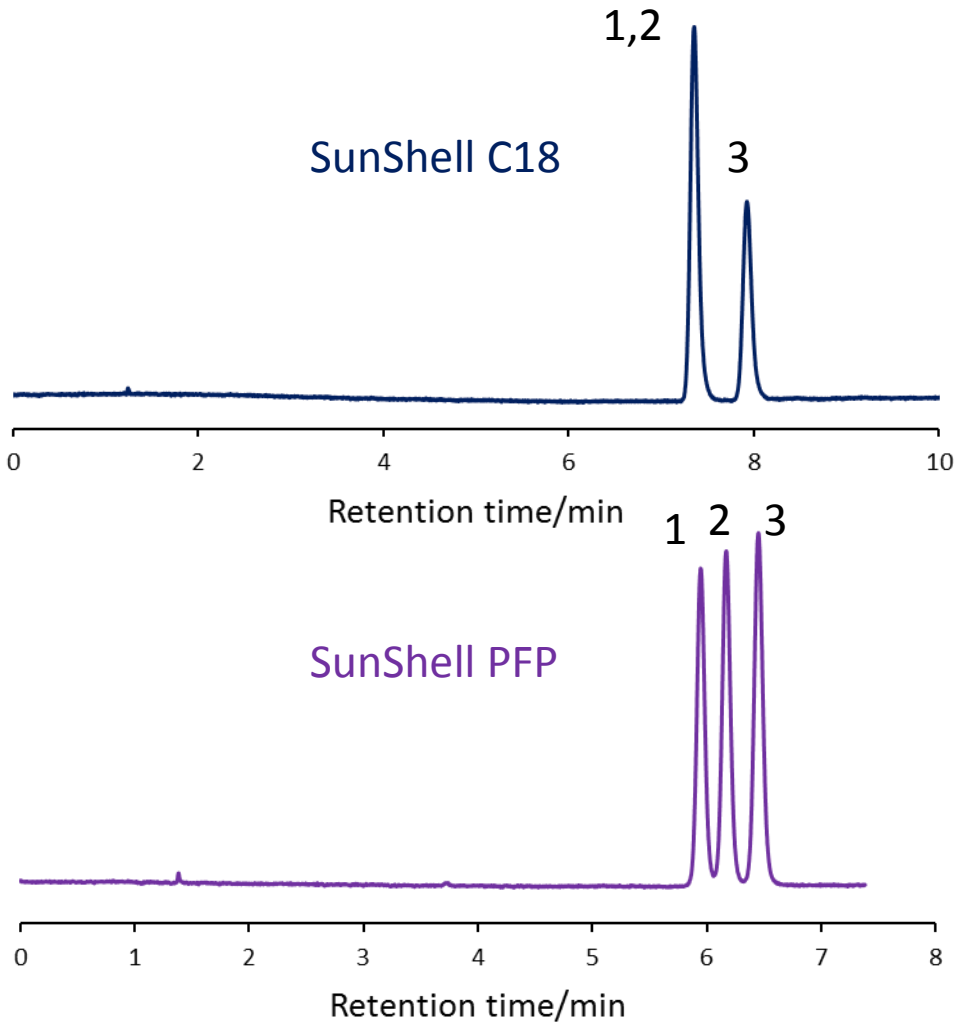
Mobile phase: CH_3CN/10mM Phosphate buffer pH7.0 = 80/20

Flow rate: 1.8 mL/min

Temperature: 25 °C

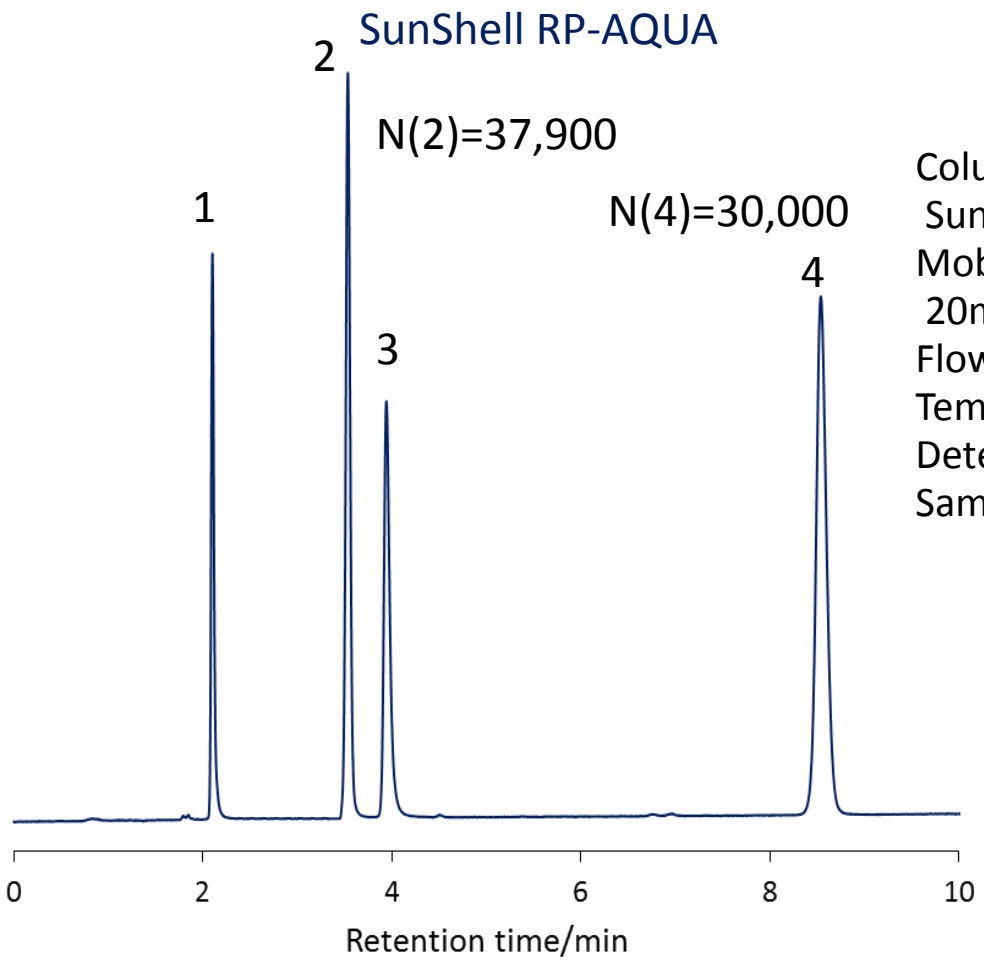
Sample: 1 = Uracil, 2 = Propranolol, 3 = Nortriptyline, 4 =Amitriptyline

Separation of isomers of cresol



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

Separation of nucleotides



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

Summary

- A core shell particle makes value of all terms of Van Deemter Equation, A , B and C be low.
- Sunniest (SunShell) bonding technology can not only decrease an effect of residual silanol groups but also increase column stability under both acidic and basic pH conditions.

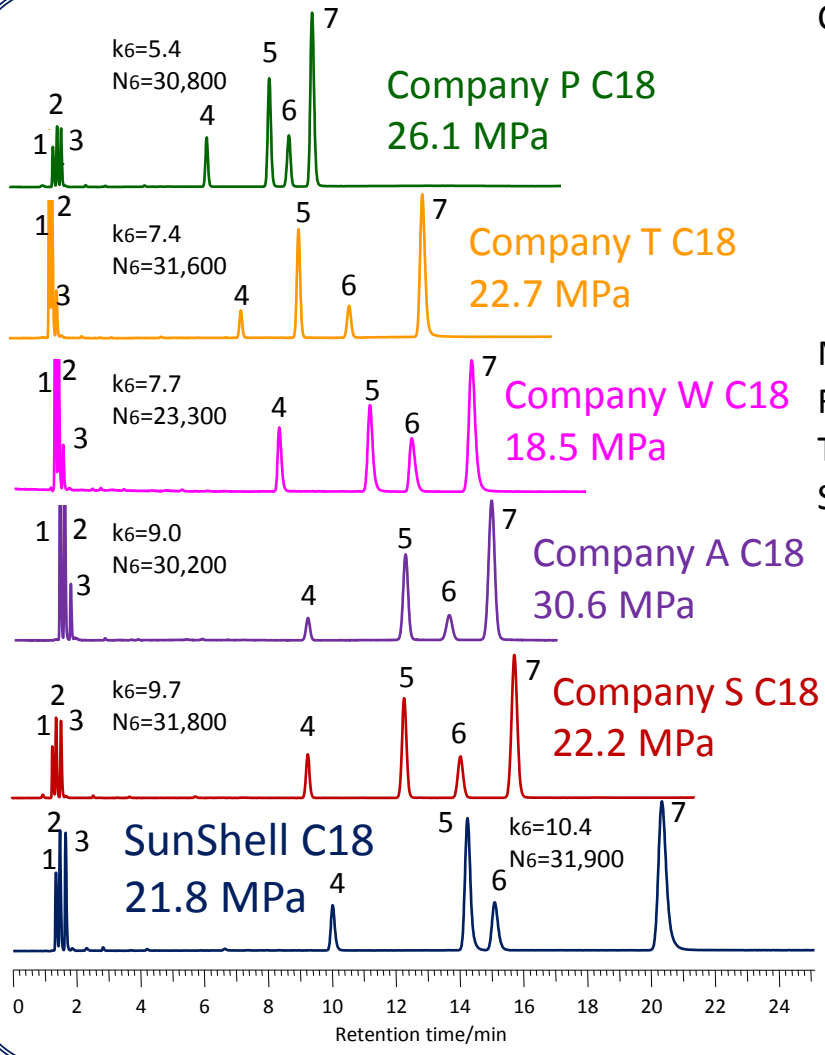
3) Comparison of Core Shell C18 Columns (Accucore, Ascentis Express, Cortecs, Kinetex, PoroShell and SunShell) (20 min)

Comparison data of 6 kinds of core shell C18 columns

Column name

1. Company P C18, 2.6 μm: Kinetex C18
2. Company T C18, 2.6 μm: Accucore C18
3. Company W C18, 2.7 μm: Cortecs C18
4. Company A C18, 2.7 μm: PoroShell C18 EC
5. Company S C18, 2.7 μm: Ascentis Express C18
6. SunShell C18, 2.6 μm

Comparison of standard samples



Column:

Company P C18, 2.6 µm 150 x 4.6 mm (26.1 Mpa, 30,800 plate)
Company T C18, 2.6 µm 150 x 4.6 mm (22.7 Mpa, 31,600 plate)
Company W C18, 2.7 µm 150 x 4.6 mm (18.5 Mpa, 23,300 plate)
Company A C18, 2.7 µm 150 x 4.6 mm (30.6 Mpa, 30,200 plate)
Company S C18, 2.7 µm 150 x 4.6 mm (22.2 Mpa, 31,800 plate)
SunShell C18, 2.6 µm 150 x 4.6 mm (21.8 Mpa, 31,900 plate)

Mobile phase: CH_3OH/H_2O=75/25

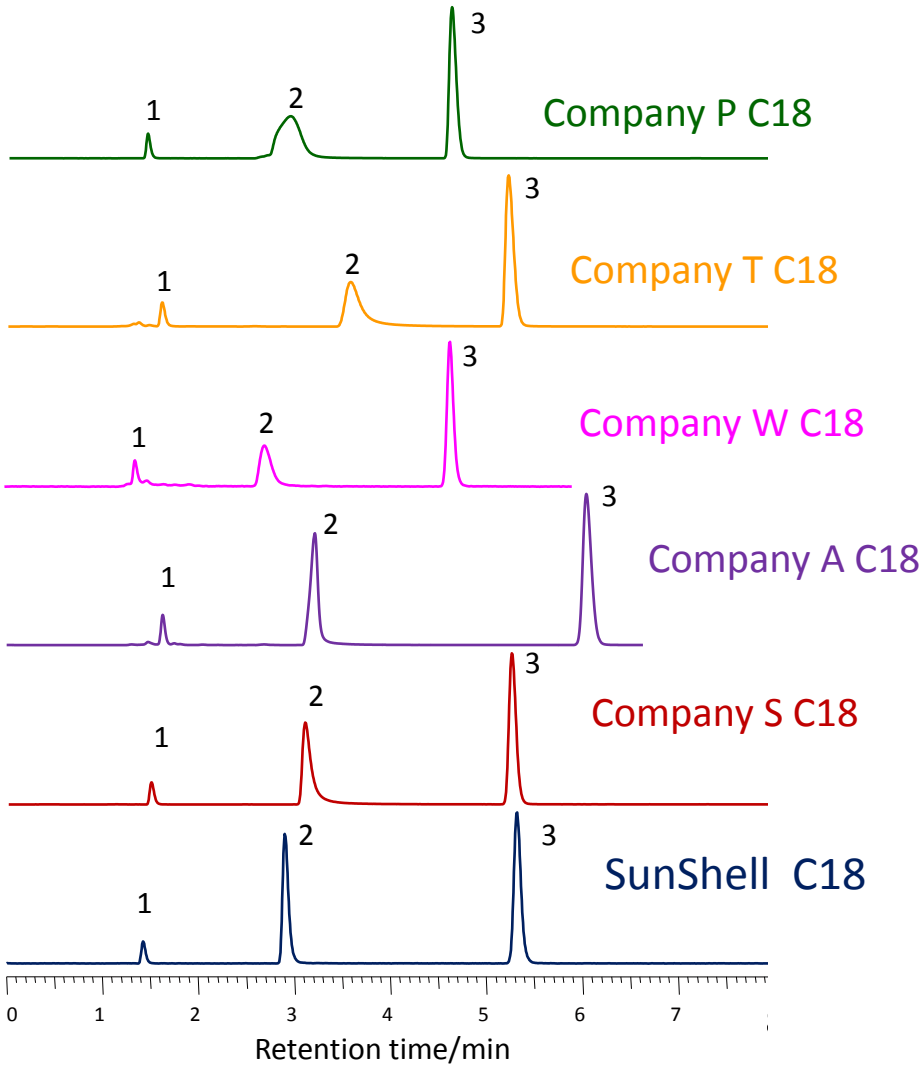
Flow rate: 1.0 mL/min

Temperature: 40 °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)
Company P C18	0.48	1.54	1.20
Company T C18	0.35	1.56	1.50
Company W C18	0.38	1.59	1.32
Company A C18	0.42	1.57	1.25
Company S C18	0.44	1.60	1.31
SunShell C18	0.39	1.60	1.46

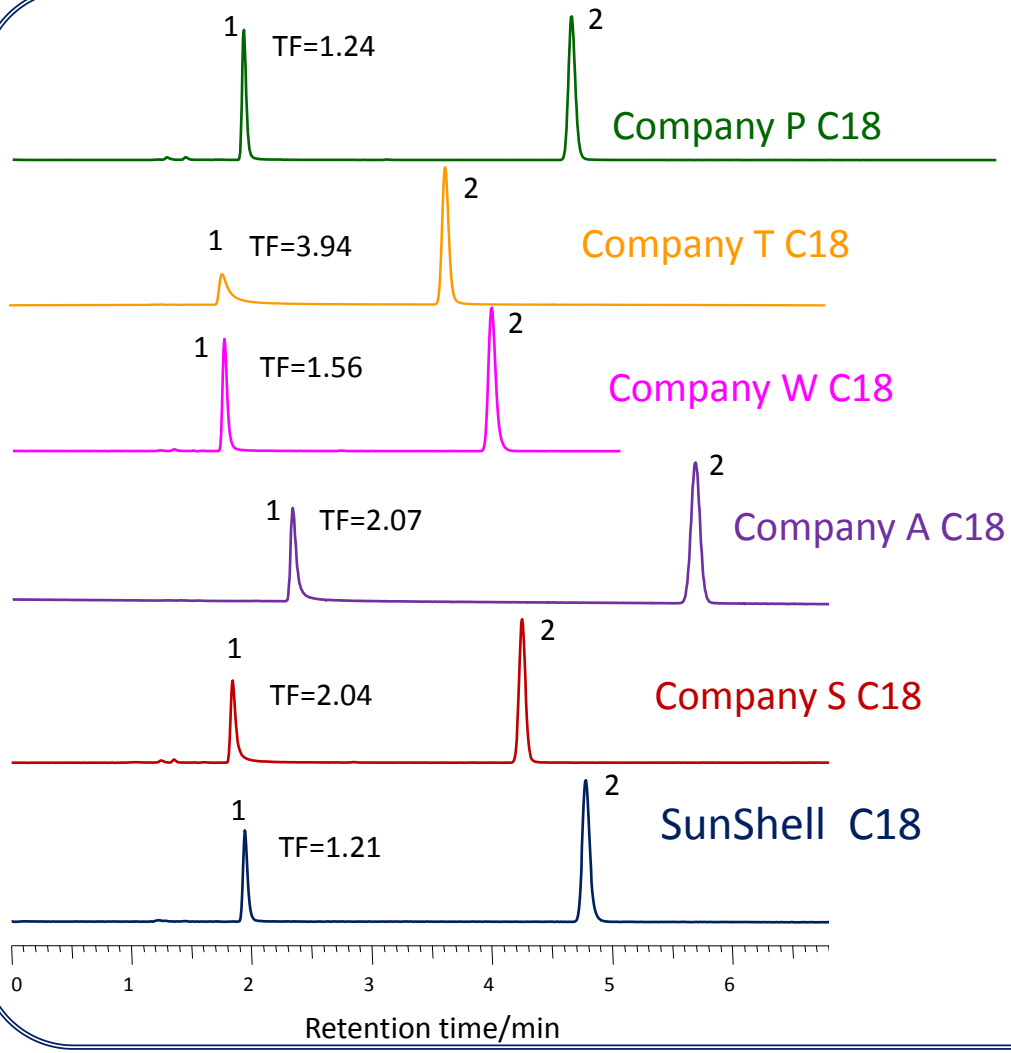
Comparison of pyridine



Column:
- Company P C18, 2.6 µm 150 x 4.6 mm
- Company T C18, 2.6 µm 150 x 4.6 mm
- Company W C18, 2.7 µm 150 x 4.6 mm
- Company A C18, 2.7 µm 150 x 4.6 mm
- Company S 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

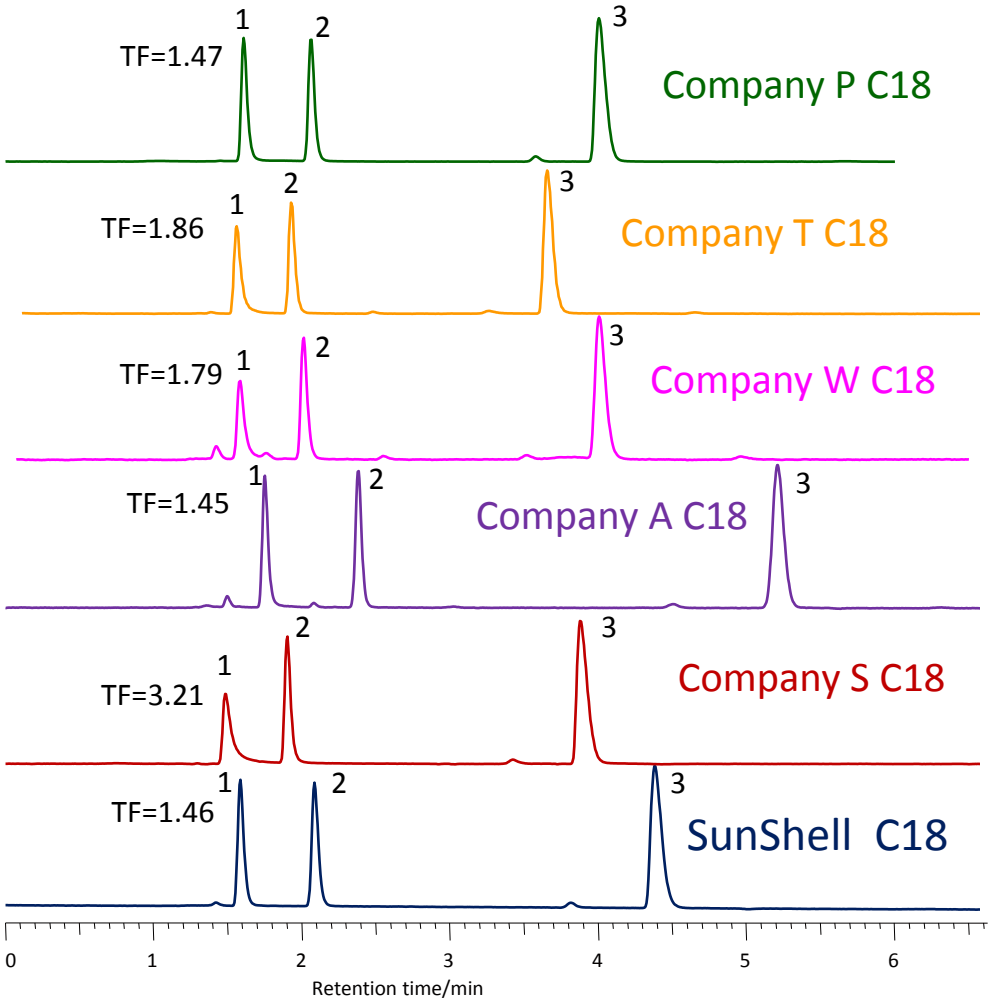
Comparison of oxine, metal chelating compound



Column:
- Company P C18, 2.6 µm 150 x 4.6 mm
- Company T C18, 2.6 µm 150 x 4.6 mm
- Company W C18, 2.7 µm 150 x 4.6 mm
- Company A C18, 2.7 µm 150 x 4.6 mm
- Company S 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

Comparison of formic acid



Column:
Company P C18, 2.6 µm 150 x 4.6 mm
Company T C18, 2.6 µm 150 x 4.6 mm
Company W C18, 2.7 µm 150 x 4.6 mm
Company A C18, 2.7 µm 150 x 4.6 mm
Company S 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

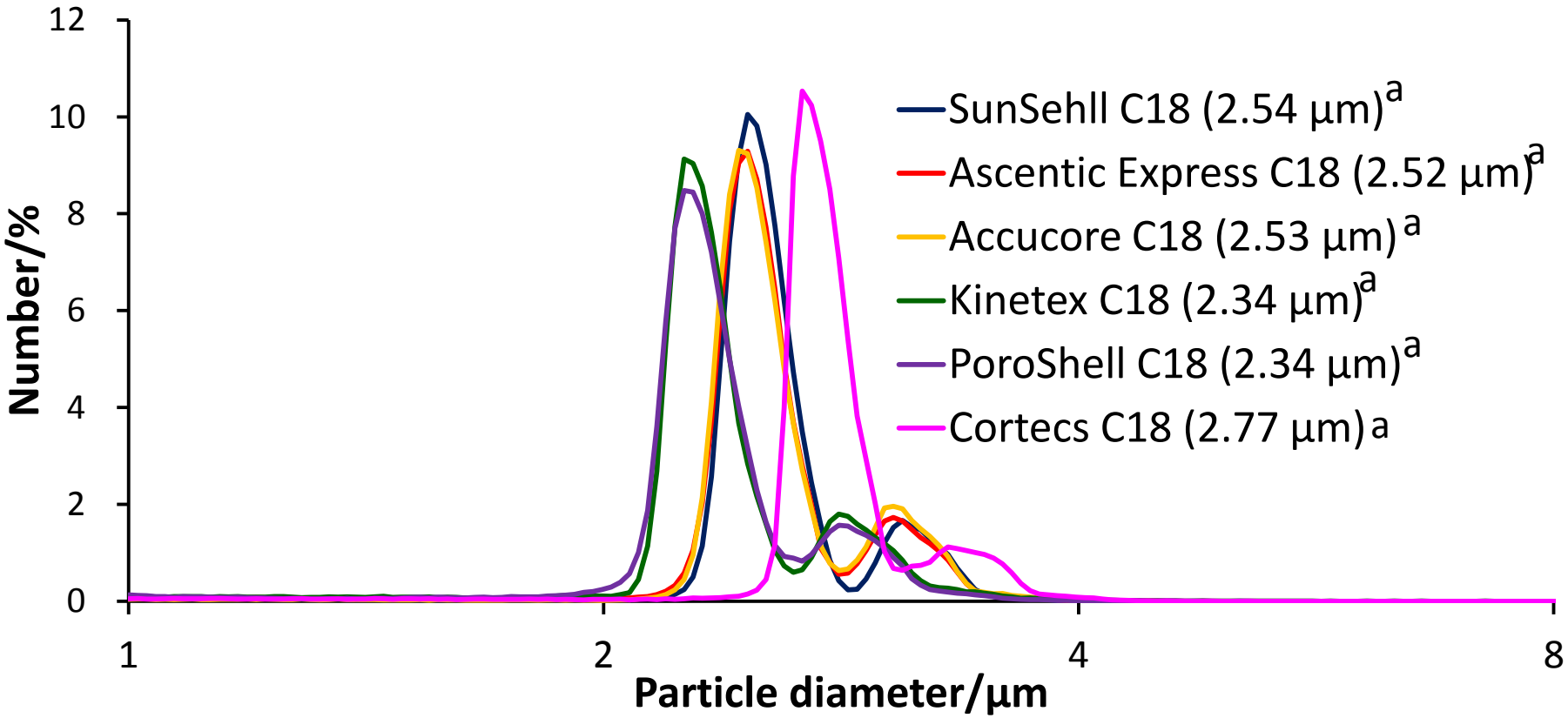
Summary of standard samples

	Pressure[a]	Retention[b]	Plate[C]	Pyridine	Oxine	Formic acid	Point
SunShell C18	○21.8	10.4	◎31,900	◎	◎	◎	14
Ascentis Express C18	○22.2	9.7	◎31,800	△	△	×	7
PoroShell C18 EC	×30.6	9.0	◎30,200	◎	△	◎	10
Cortecs C18	◎18.5	7.7	×23,300	×	○	△	6
Accucore C18	○22.7	7.4	◎31,600	×	×	△	6
Kinetex C18	△26.1	5.4	◎30,800	×	◎	◎	10

a. Mobile phase; methanol:water=75:25, 40 °C, 1mL/min, 150 x 4.6mm
b. Retention factor of amylbenzene
c. Theoretical plate of amylbenzene

◎: 3 point, ○: 2 point, △: 1 point, ×: 0 point

Particle size distribution



*Measured using Beckman Coulter Multisizer 3 after C18 materials were sintered at 600 degree Celsius for 8 hours. The value measure by Coulter Counter method is smaller than the real value because a porous material includes an electrolyte solution and the resistance value decreases.

a. Median particle size

Characteristics

	Carbon loading (%)	Specific surface area [a] (m^2/g)	Pore volume [a] (mL)	Pore diameter [a] (nm)
SunShell C18	7.3 (7)[b]	125 (150)[b]	0.261	8.34 (9)[b]
Ascentis Express C18	8.0	133 (150)[b]	0.278	8.20 (9)[b]
PoroShell C18 EC	8.5 (8)[b]	135 (130)[b]	0.414	12.3 (12)[b]
Accucore C18	8.8 (9)[b]	130 (130)[b]	0.273	8.39 (8)[b]
Cortecs C18	7.3 (6.6)[b]	113	0.264	9.32
Kinetex C18	4.9 (12 effective)[b]	102 (200 effective)[b]	0.237	9.25 (10)[b]

a. Measured after C18 materials were sintered at 600 degree Celsius for 8 hours. The measured value of each sintered core shell silica is considered to be smaller than that of the original core shell silica.

b. Value written in each brochure or literature

All data were measured in ChromaNik laboratory.

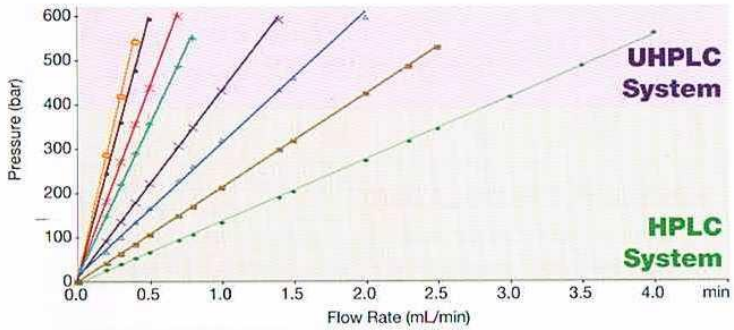
TECHNOLOGY

Choosing the Best Kinetex® Column

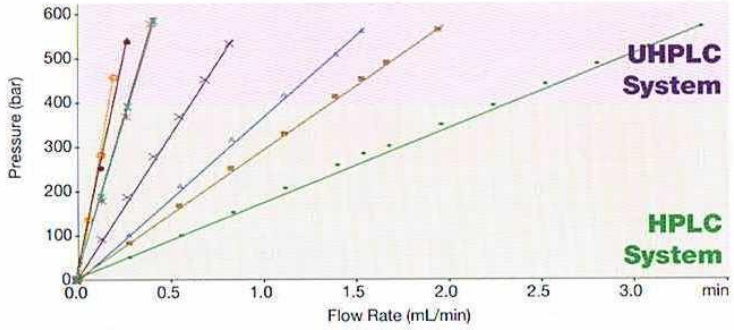
Expected Backpressure at Different Flow Rates*

There is an optimal Kinetex® column for your system and operating conditions. Use these graphs to determine the starting Kinetex® particle size and dimension for your method.

50:50 (Acetonitrile:Water)



60:40 (Methanol:Water)



Material Characteristics

Packing Material	Total Particle Size (µm)	Porous Shell (µm)	Solid Core (µm)	Pore Size (Å)	Effective Surface Area (m^2/g)	Effective Carbon Load %	pH Stability	Pressure Stability
Kinetex® C18	2.6	0.35	1.9	100	200	12	1.5 - 10	
Kinetex® PFP	2.6	0.35	1.9	100	200	9	1.5 - 8.0	600 bar
Kinetex® HILIC	2.6	0.35	1.9	100	200	0	2.0 - 7.5	
Kinetex® C18	1.7	0.23	1.25	100	200	12	1.5 - 10	
Kinetex® PFP	1.7	0.23	1.25	100	200	9	1.5 - 8.0	1000 bar
Kinetex® HILIC	1.7	0.23	1.25	100	200	0	2.0 - 7.5	

- Kinetex® 100 x 2.1 mm, 1.7 µm
- Kinetex® 50 x 2.1 mm, 1.7 µm
- Kinetex® 150 x 2.1 mm, 2.6 µm
- Kinetex® 100 x 2.1 mm, 2.6 µm
- Kinetex® 50 x 2.1 mm, 2.6 µm
- Kinetex® 150 x 4.6 mm, 2.6 µm
- Kinetex® 100 x 4.6 mm, 2.6 µm
- Kinetex® 50 x 4.6 mm, 2.6 µm

* Due to variation in system, sample and method parameters, graphs provided may not be representative of all applications. Data generated on Agilent 1200 SL.

PHENOMENEX | TEL: 310-212-0555 | FAX: 310-328-7768 | EMAIL: info@phenomenex.com

Material Characteristics

Packing Material	Total Particle Size (µm)	Porous Shell (µm)	Solid Core (µm)	Pore Size (Å)	Effective Surface Area (m^2/g)	Effective Carbon Load %	pH Stability	Pressure Stability
Kinetex® C18	2.6	0.35	1.9	100	200	12	1.5 - 10	600 bar
Kinetex® PFP	2.6	0.35	1.9	100	200	9	1.5 - 8.0	
Kinetex® HILIC	2.6	0.35	1.9	100	200	0	2.0 - 7.5	
Kinetex® C18	1.7	0.23	1.25	100	200	12	1.5 - 10	1000 bar
Kinetex® PFP	1.7	0.23	1.25	100	200	9	1.5 - 8.0	
Kinetex® HILIC	1.7	0.23	1.25	100	200	0	2.0 - 7.5	

Due to variation in system, sample and method parameters, graphs provided may not be representative of all applications.
Data generated on Agilent 1200 SL.

PHENOMENEX | TEL: 310-212-0555 | FAX: 310-328-7768 | EMAIL: info@phenomenex.com

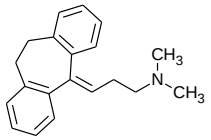
Table 1. Dimensions and material characterization of the tested columns from manufacturers' literature

Column	Type	id (mm)	Length (mm)	Particle size (μm)	Shell thickness (μm)	Pore size (Å)	Surface area (m^2/g)	Carbon load (%)	Bonding density (μmol/m^2)
Supelco Ascentis Express C_{18}	Superficially porous	4.6	100	2.7	0.5	90	150	–	3.5
Agilent Poroshell 120 EC-C_{18}	Superficially porous	4.6	100	2.7	0.5	120	130	8	3.3
Phenomenex Kinetex C_{18}	Superficially porous	4.6	100	2.6	0.35	100	200	12	2.8
Phenomenex Kinetex C_{18}	Superficially porous	2.1	100	2.6	0.35	100	200	12	2.6
Phenomenex Kinetex C_{18}	Superficially porous	2.1	100	1.7	0.23	100	200	12	2.8
Waters Acquity BEH C_{18}	Totally porous	2.1	100	1.7	–	130	185	18	3.1

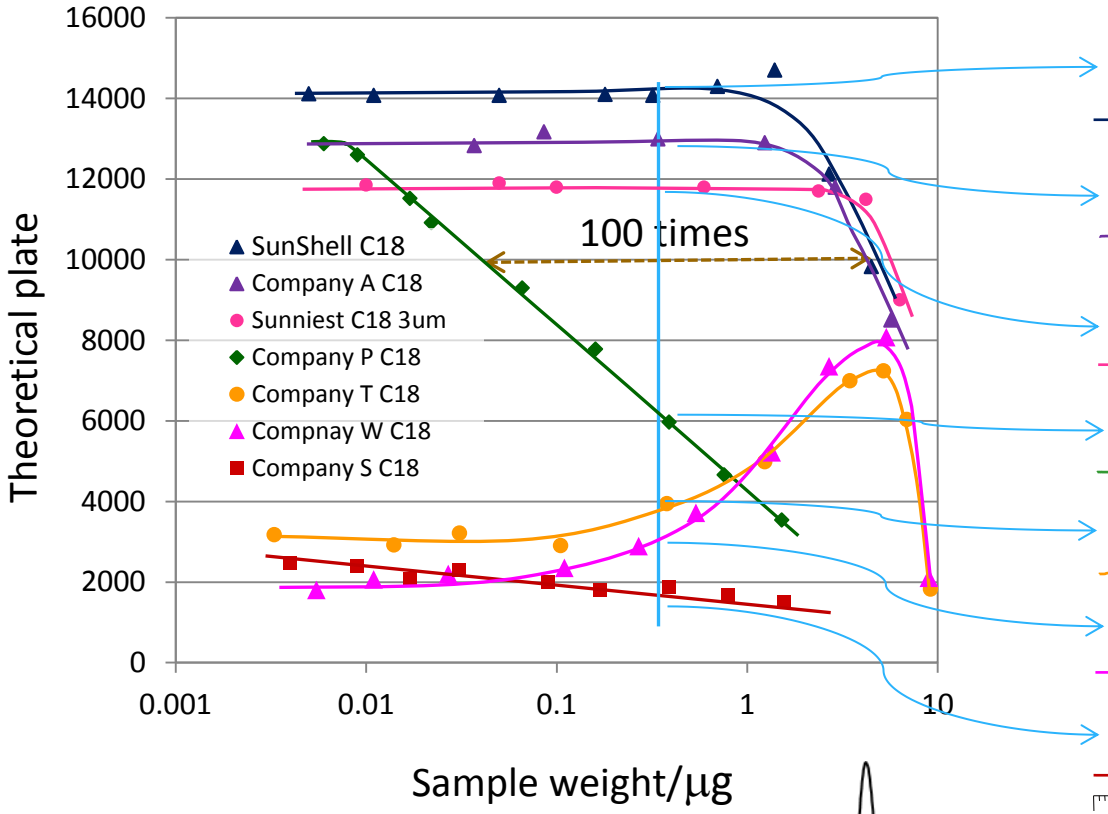
Effective surface area

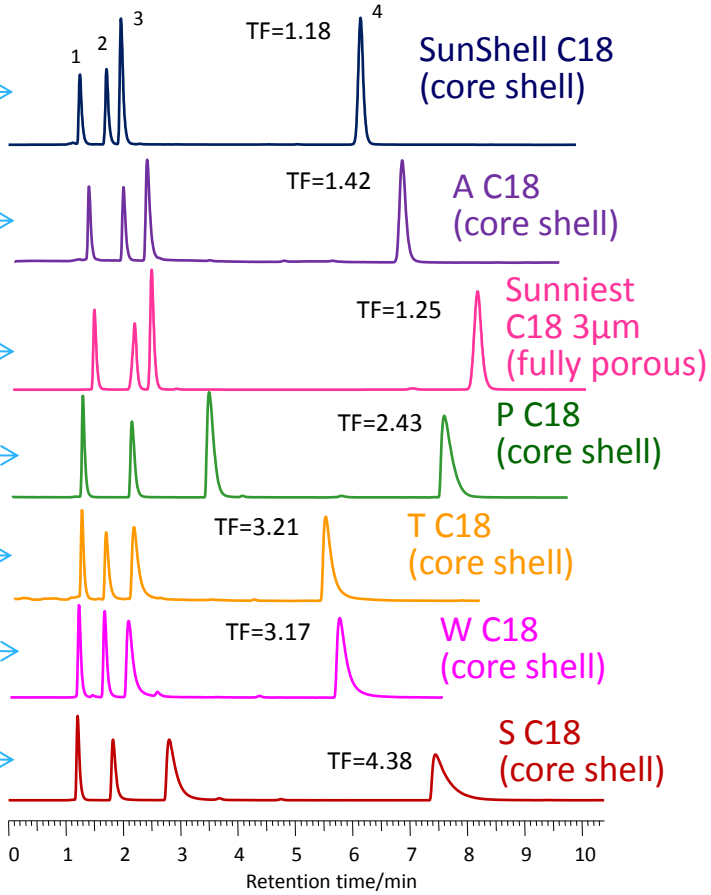
Specific surface area

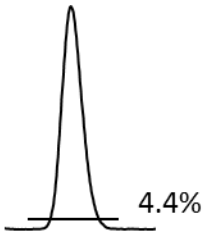
Loading capacity of amitriptyline I



Mobile phase: Acetonitrile/**20mM phosphate buffer pH7.0**=(60:40)
Column dimension: 150 x 4.6 mm, Flow rate: 1.0 mL/min, Temp.: 40°C





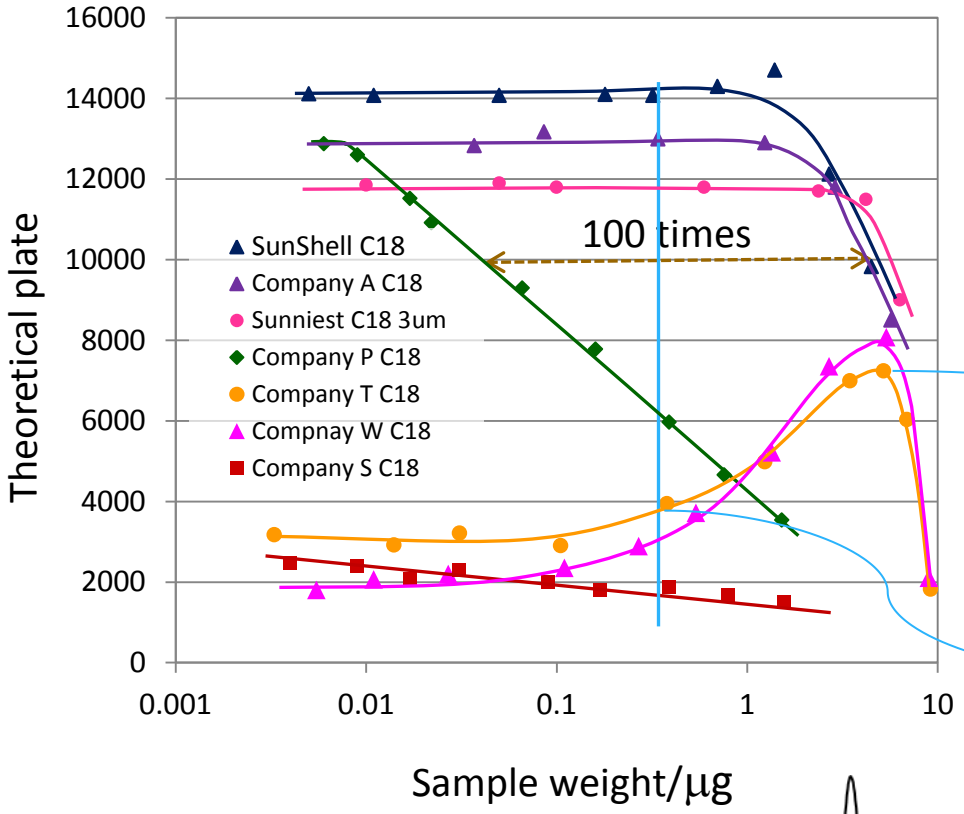


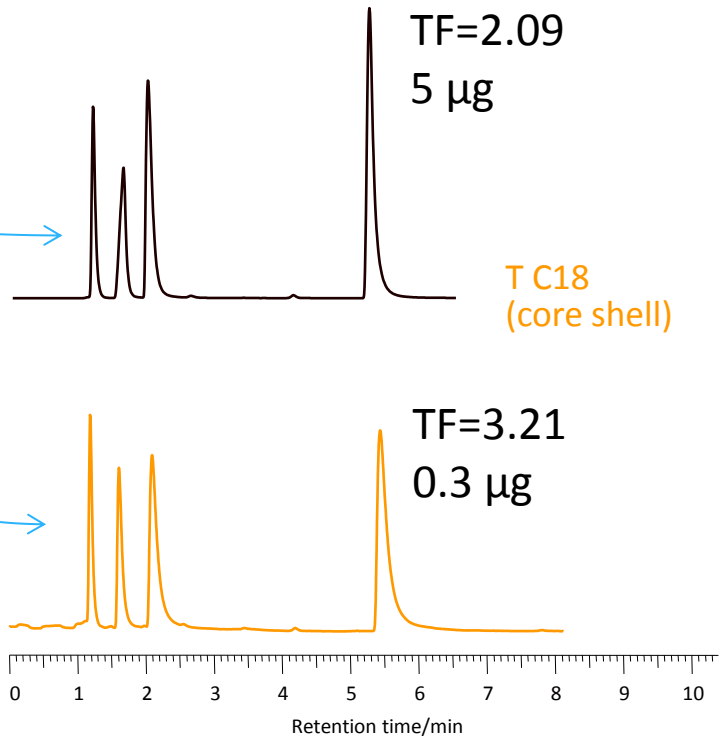
Theoretical plate was calculated by 5σ method using peak width at 4.4% of peak height.

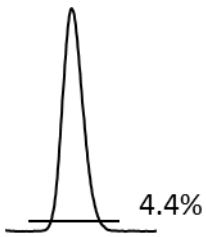
Sample: 1=Uracil, 2=Propranolol, 3= Nortriptyline, 4=Amitriptyline

Loading capacity of amitriptyline I

Mobile phase: Acetonitrile/**20mM phosphate buffer pH7.0**=(60:40)
Column dimension: 150 x 4.6 mm, Flow rate: 1.0 mL/min, Temp.: 40°C





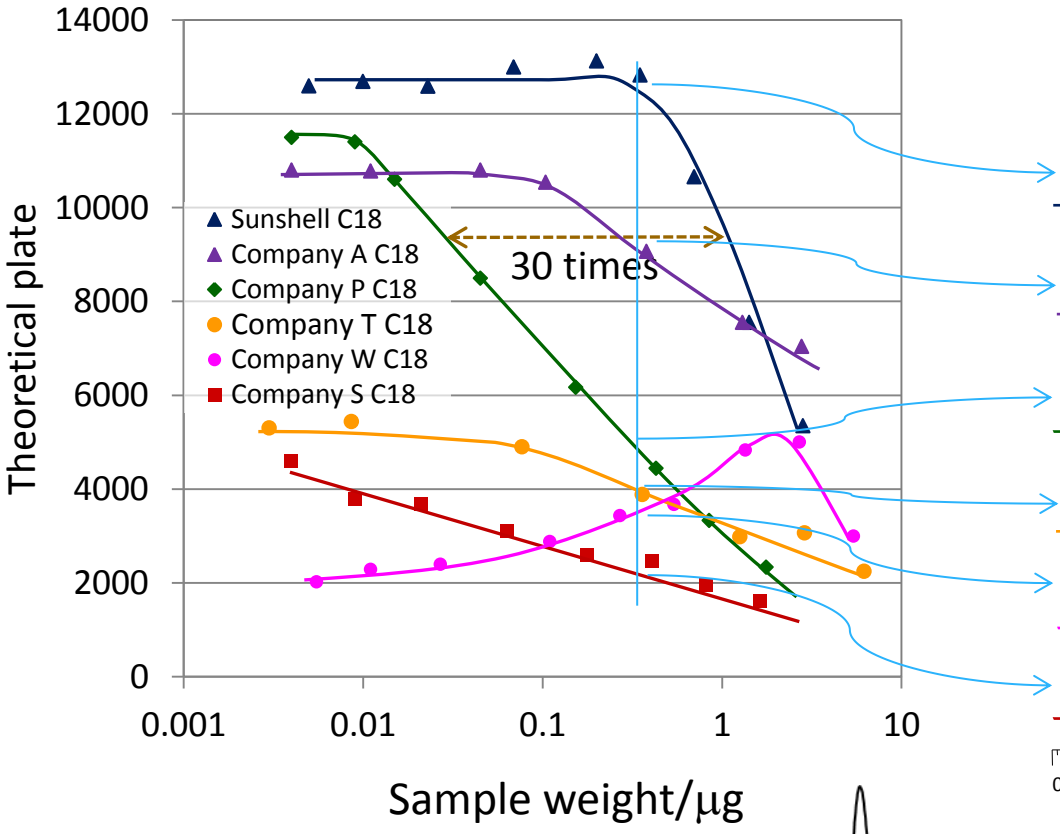


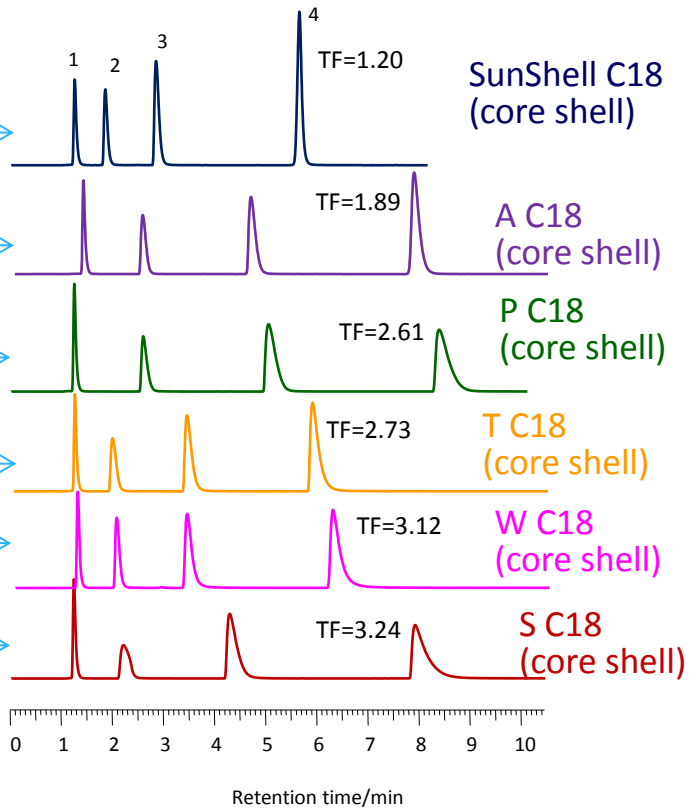
Theoretical plate was calculated by 5σ method using peak width at 4.4% of peak height.

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

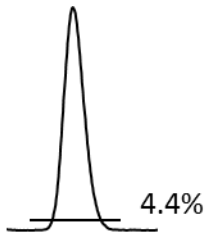
Loading capacity of amitriptyline II

Mobile phase: Acetonitrile/**10mM ammonium acetate pH6.8**=(40:60)
Column dimension: 150 x 4.6 mm, Flow rate: 1.0 mL/min, Temp.: 40°C





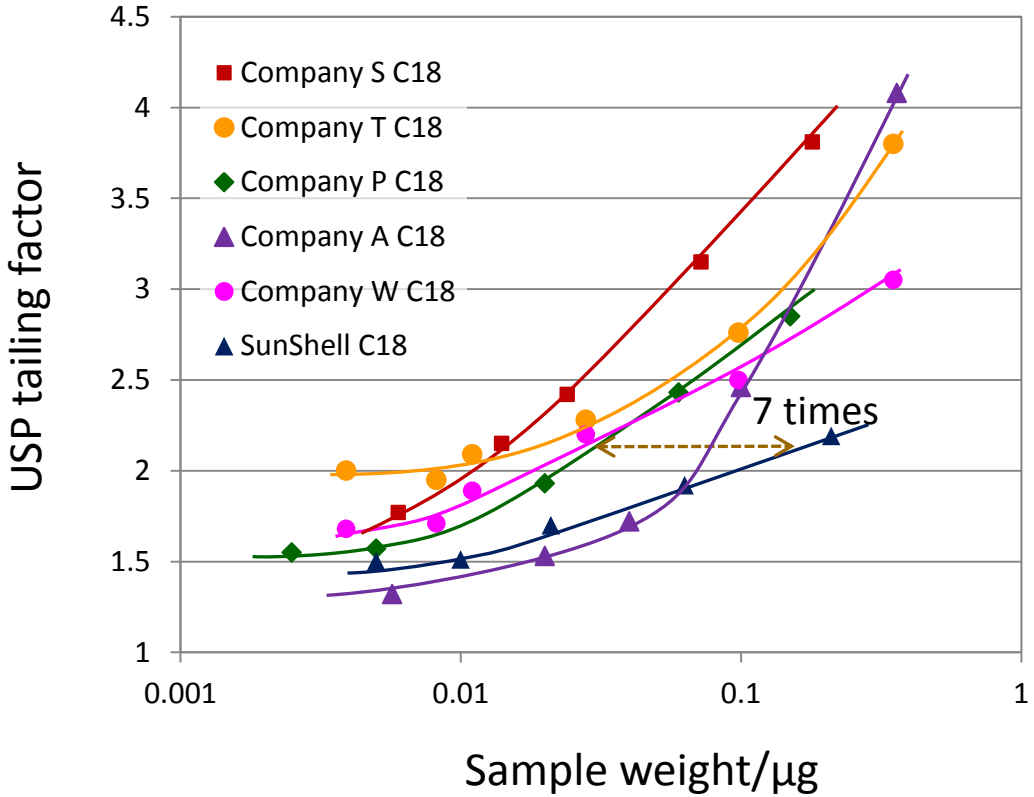
Theoretical plate was calculated by 5σ method using peak width at 4.4% of peak height.

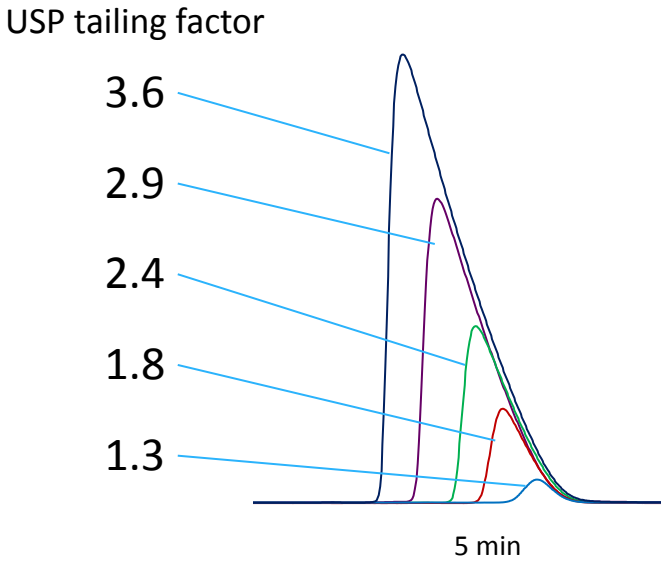


Sample: 1=Uracil, 2=Propranolol,
3= Nortriptyline, 4=Amitriptyline

Loading capacity of amitriptyline III

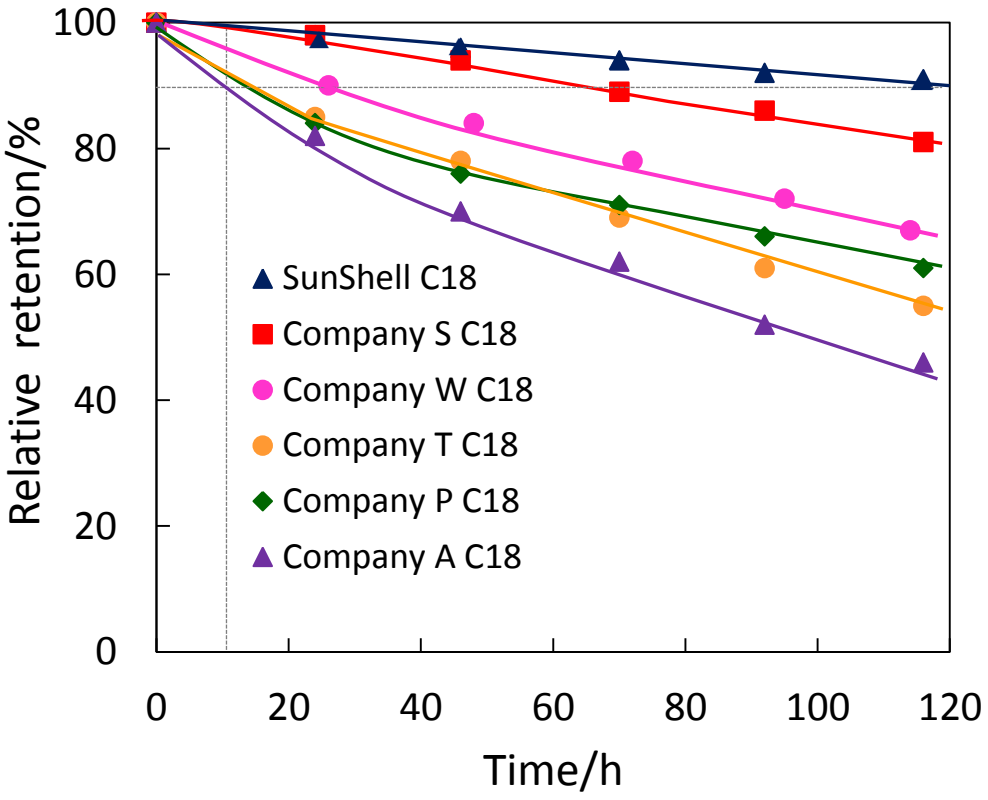
Mobile phase: Acetonitrile/**0.1% formic acid**=(30:70)
Column dimension: 150 x 4.6 mm, Flow rate: 1.0 mL/min, Temp.: 40°C





In the case of using acetonitrile /0.1% formic acid as a mobile phase, amitriptyline peak shows more tailing because a loading capacity decreases in an acidic, low-ionic-strength mobile phase.

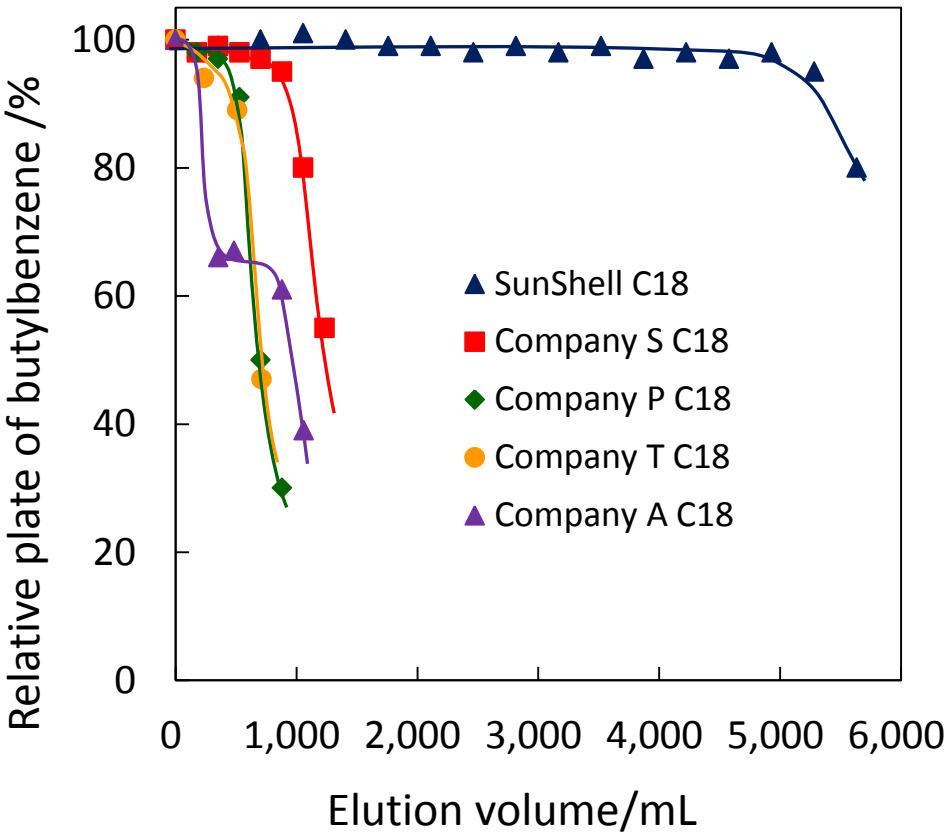
Stability under acidic pH condition



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 °C

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 °C
Sample: 1 = Uracil
2 = Butylbenzene

Stability under basic pH condition



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_3OH/H_2O=70/30
Flow rate: 0.4 mL/min
Temperature: 40 ^oC
Sample: 1 = Butylbenzene

Summary of stability

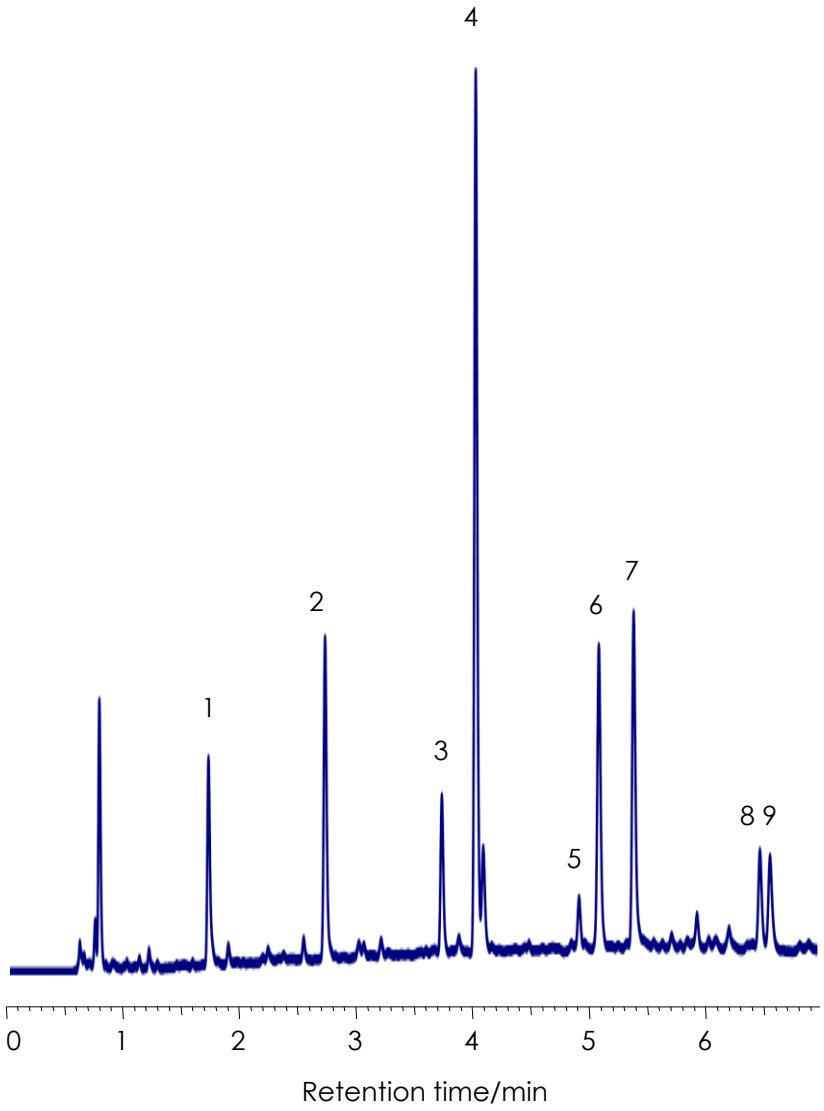
	Acidic condition pH 1	Basic condition pH 10	pH range written in each brochure
SunShell C18	◎	◎	1.5 - 10
Ascentis Express C18	○	○	2 - 9
Cortecs C18	○	not tested	2 - 8
PoroShell C18 EC	△	△	2 - 9
Accucore C18	△	△	1 - 11
Kinetex C18	△	△	1.5 - 10

Summary

- SunShell C18 showed good peaks and the highest stability.
- The value described in the brochure is not necessarily a true value.

4) Applications related Foods, Proteins and Other (20min)

Separation of Oolong tea



Column: SunShell C18 2.6 µm, 75 x 4.6 mm

Mobile phase:

A) 0.1% Phosphoric acid

B) CH_3CN

Gradient program

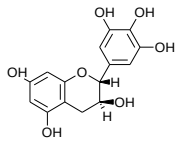
Time	0 min	7.5 min	10 min
%B	2%	25%	25%

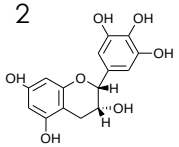
Flow rate: 1.0 mL/min,

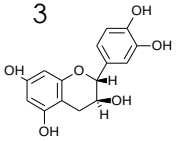
Temperature: 25 °C

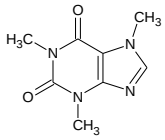
Detection: UV@250 nm

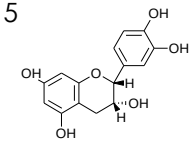
Sample: Oolong tea

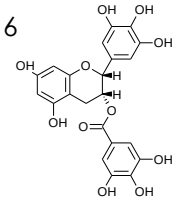
1 
Gallocatechin

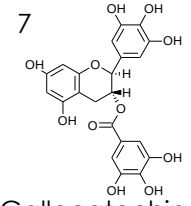
2 
Epigallocatechin

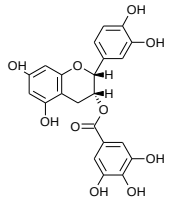
3 
Catechin

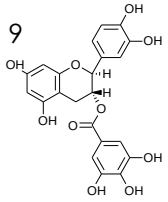
4 
Caffeine

5 
Epicatechin

6 
Epigallocatechin gallate I

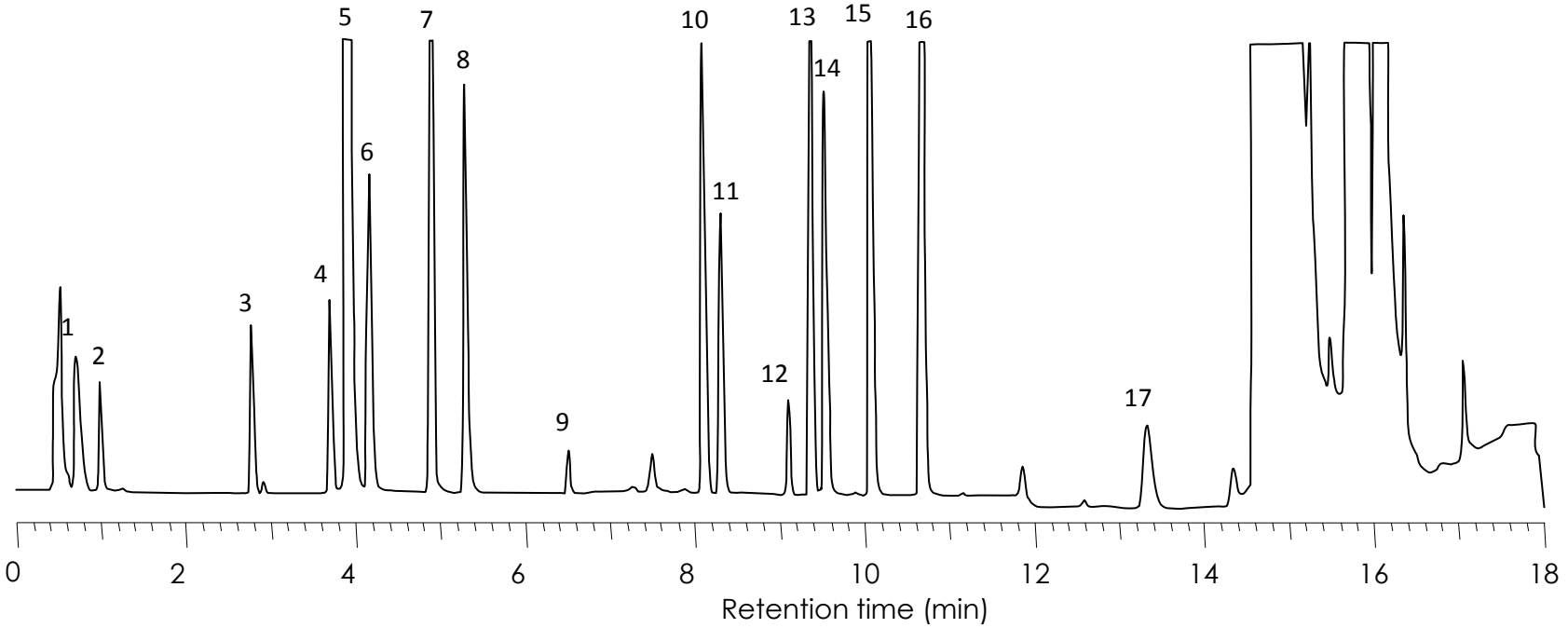
7 
Gallocatechin gallate

8 
Epicatechin gallate

9 
Catechin gallate

Amino Acids derivatized with OPA and FMOC

(*o* - Phthalaldehyde Solution, Fluorenyl Methyl Chloro Formate)



Column: SunShell C18 2.6 µm, 150 x 2.1 mm

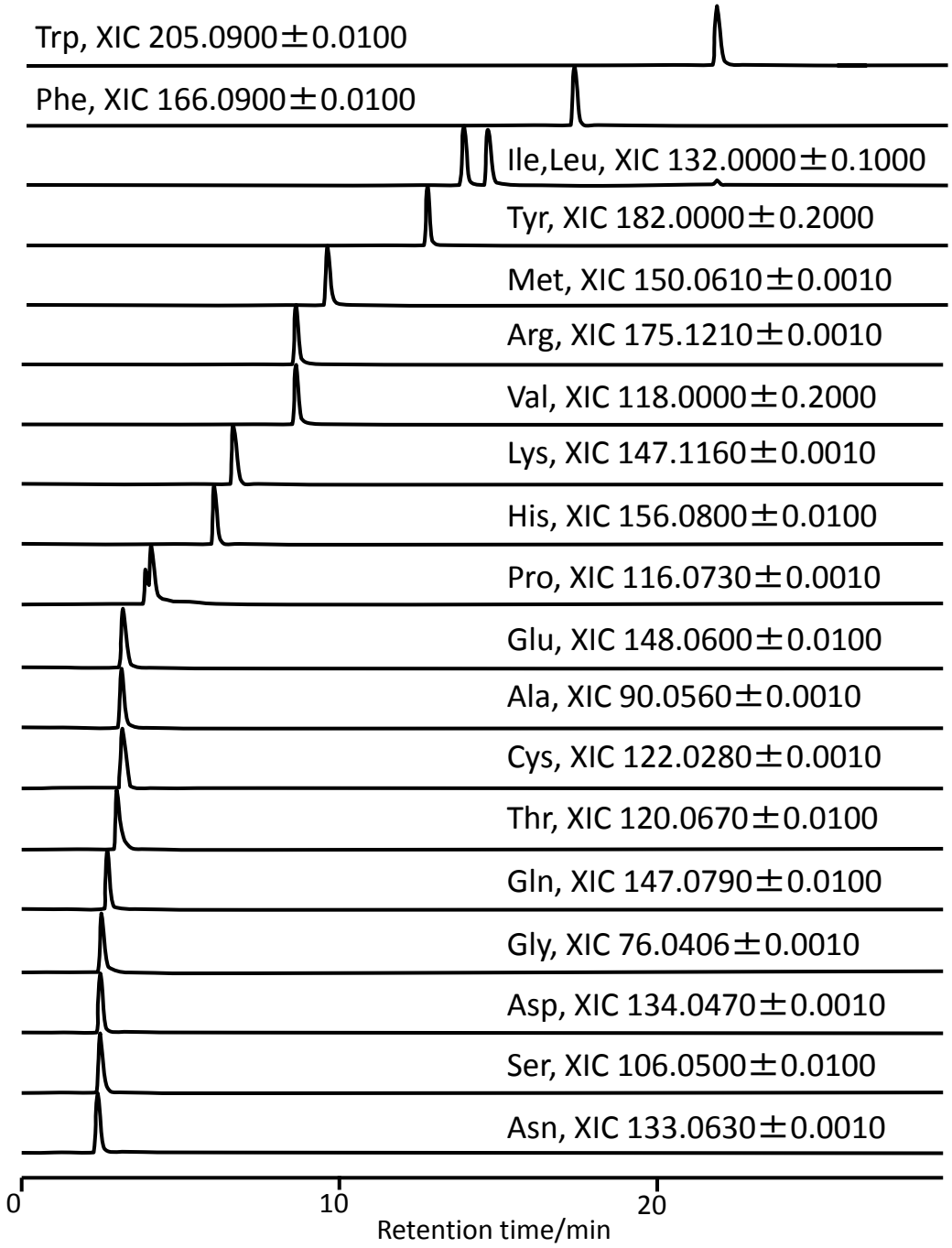
Mobile phase: A) 10mM Na_2PO_4 + 10mM $Na_2B_4O_7$ + 0.5mM NaN_3 (pH7.8)

B) Acetonitrile/Methanol/Water (45/45/10 %V)

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

Flow rate: 0.61 mL/min, Temperature: 40 °C, Detection: UV@338 nm

Sample: 1=Aspartic acid, 2=Glutaminc acid, 3=Serine, 4=Histidine, 5=Glycine, 6=Threonine, 7=Arginine, 8=Alanine, 9=Tyrosine, 10=Valine, 11=Methionine, 12=Tryptophan, 13=Pnehylalanine, 14=Isoleucine, 15=Leucine, 16=Lysine, 17=Proline



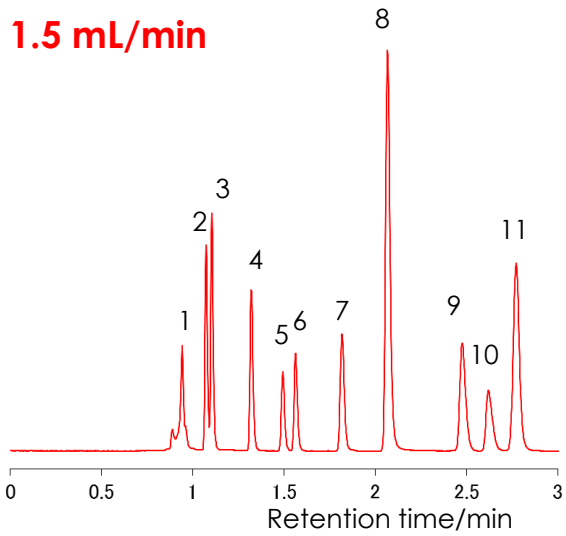
LC/MS of Amino acids

Column: SunShell RP-AQUA 2.6 µm, 2.1x150mm
Mobile phase:
A) 5 mM HFBA,
B) 5 mM HFBA in CH_3CN / H_2O (9/1)
%B 0% to 20% in 20 min
Flow rate: 0.2 mL / min
Temperature: 40 °C
Detection: MS (NanoFrontier LD) ESI Positive,
Extracted ion chromatogram (EIC)
HPLC: LaChrom Ultra

Separation of organic acids

SunShell RP-AQUA 2.6 µm,

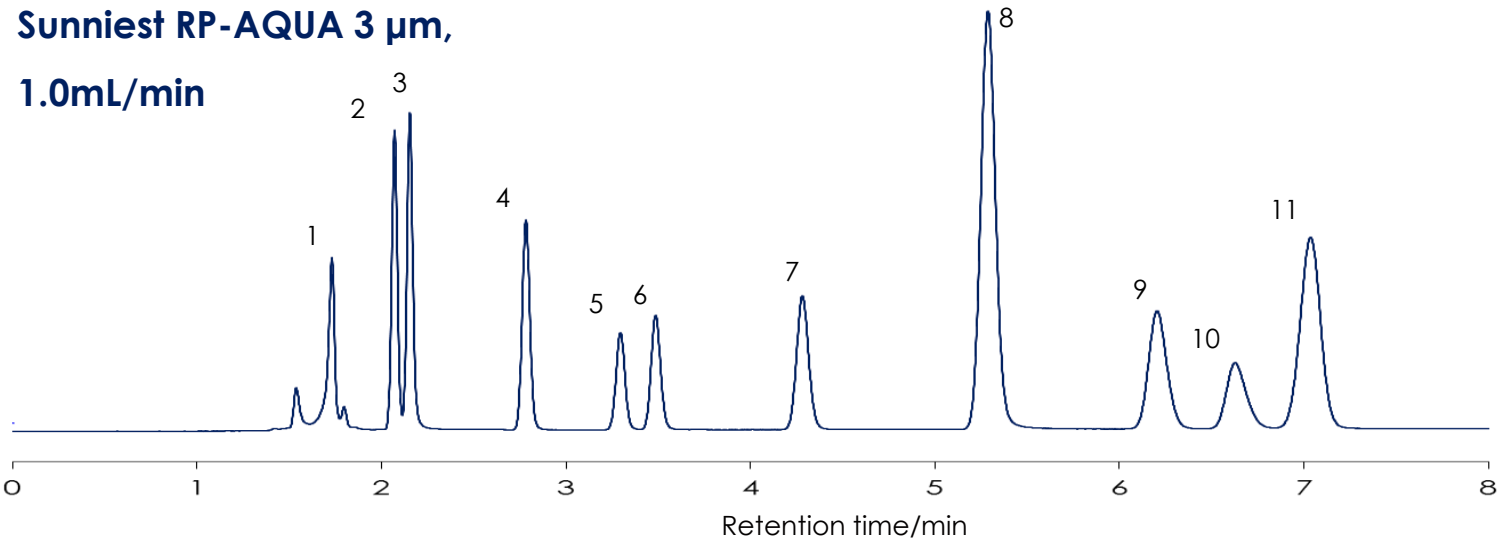
1.5 mL/min



Column dimension: 150 x 4.6 mm
Mobile phase: 0.025 M KH_2PO_4, pH2.5
Flow rate: 1.5 mL/min and 1.0 mL/min
Column pressure: 32 MPa for SunShell and 14 MPa for Sunniest
Temperature: 25 °C
Detection: UV@210nm
Sample: 1 = Oxalic acid, 2 = Tartaric acid, 3 = Formic acid,
4 = Malic acid, 5 = Lactic acid, 6 = Acetic acid,
7 = Diglycolic acid, 8 = Maleic acid, 9 = Citric acid,
10 = Succinic acid, 11 = Fumaric acid.

Sunniest RP-AQUA 3 µm,

1.0mL/min



Separation of organic acids

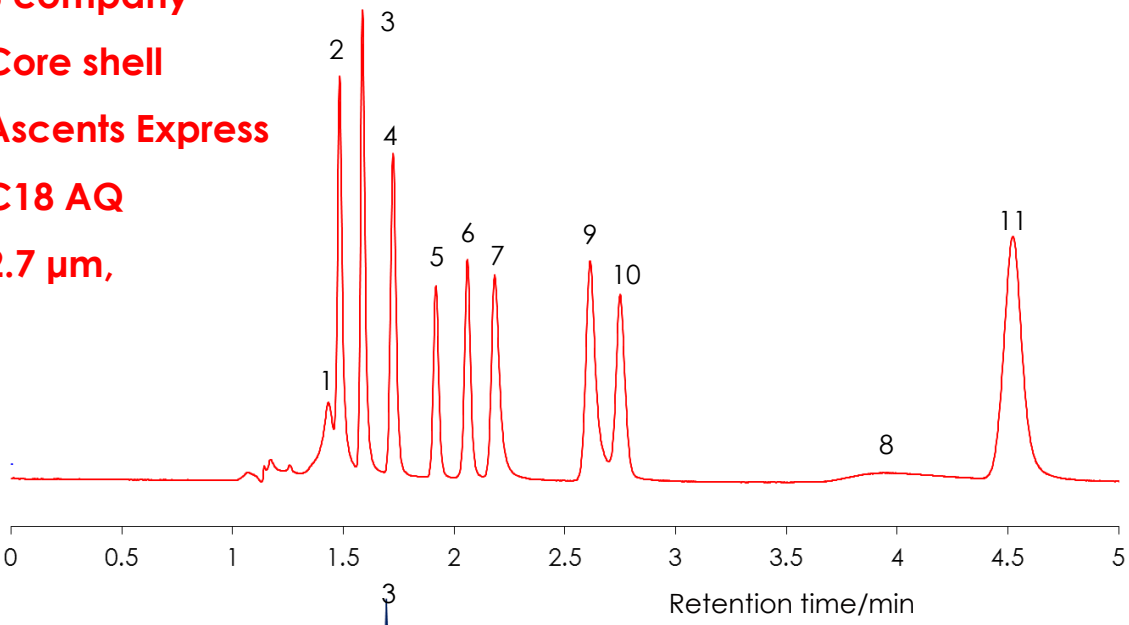
S company

Core shell

Ascents Express

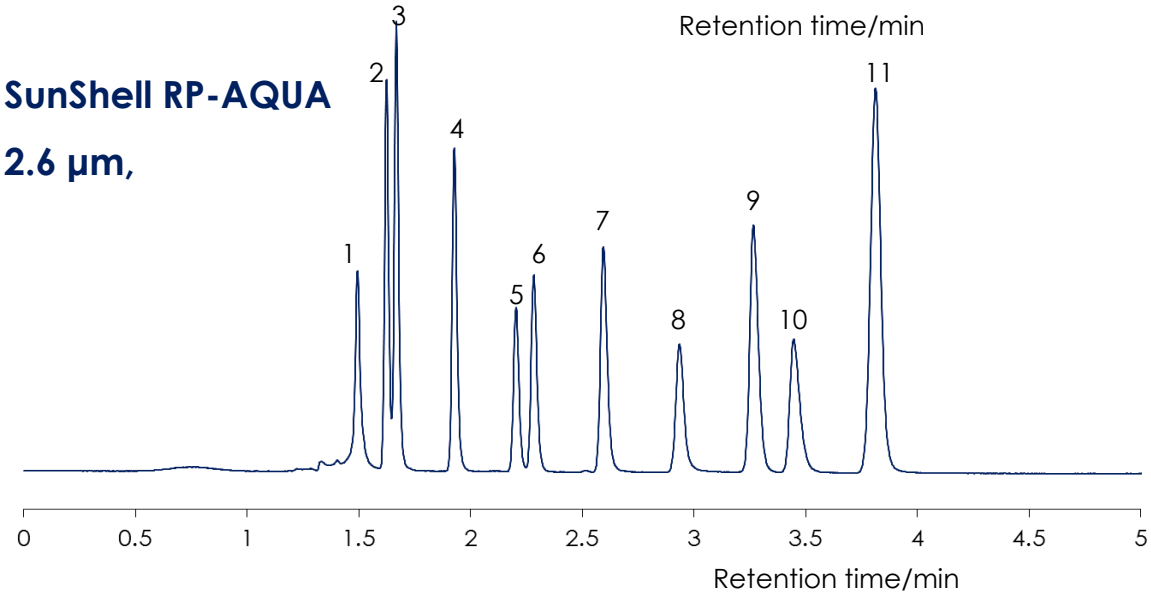
C18 AQ

2.7 µm,



SunShell RP-AQUA

2.6 µm,



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

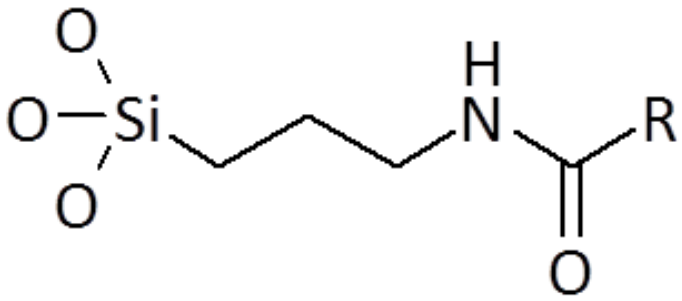
SunShell HILIC-Amide, 2.6 µm

Characteristics of SunShell HILIC-Amide

For Hydrophilic Interaction Chromatography

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

Stationary phase of HILIC-Amide

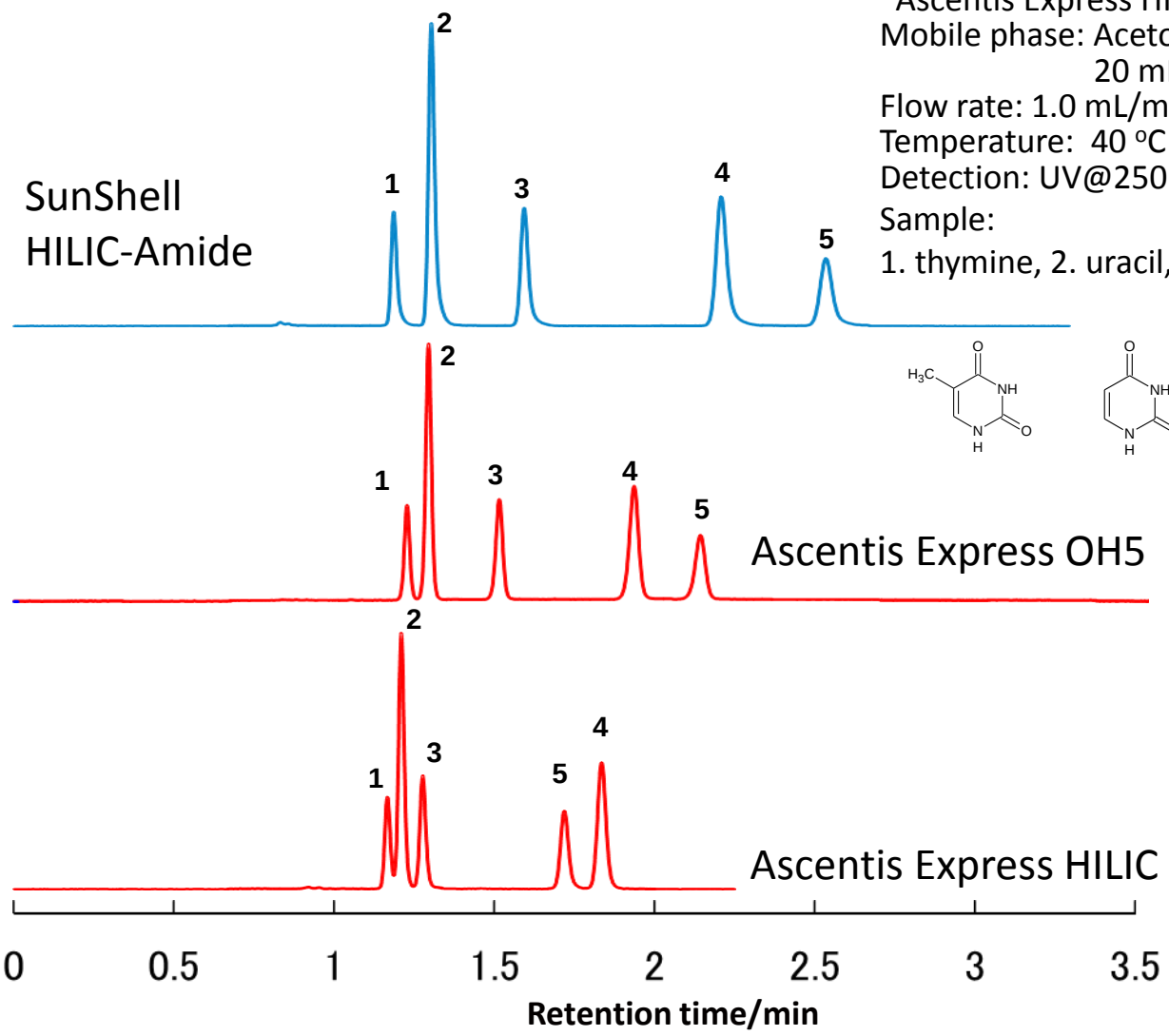


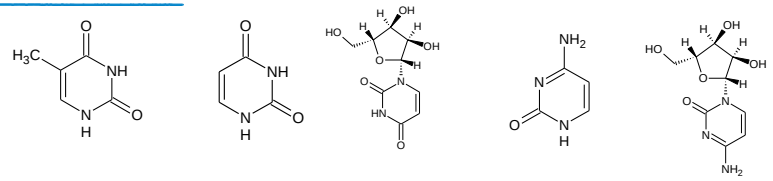
R: Hydrophilic group

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

Separation of nucleic acid bases

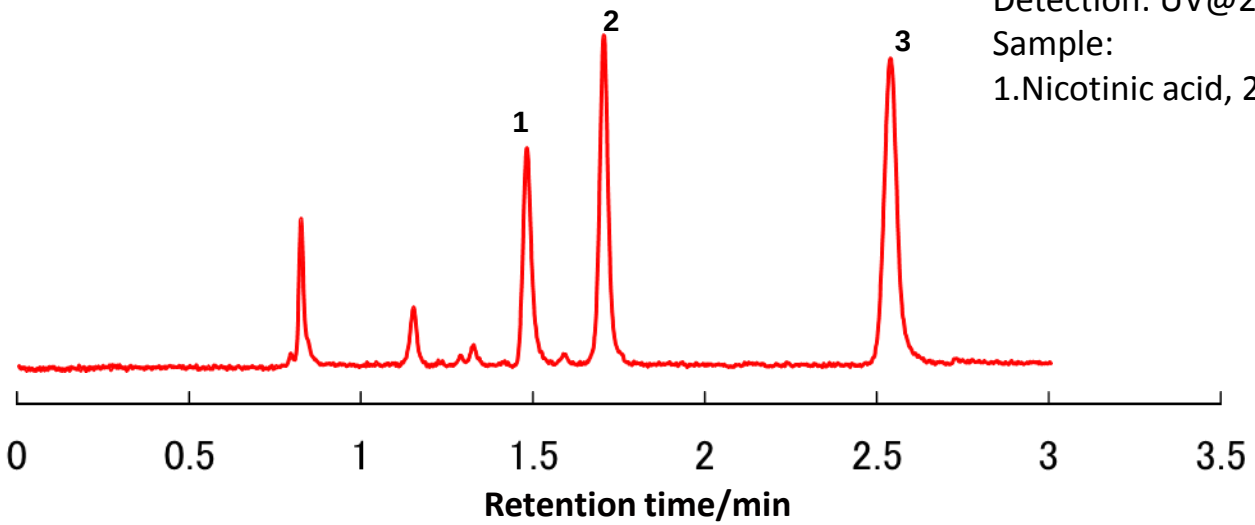
Column:
SunShell HILIC-Amide 2.6 µm : 100 x 4.6 mm,
Ascentis Express OH5 2.6 µ m : 100 x 4.6 mm
Ascentis Express HILIC 2.6 µ m : 100 x 4.6 mm,
Mobile phase: Acetonitrile :
20 mM ammonium acetate(pH4.7) =8:2
Flow rate: 1.0 mL/min
Temperature: 40 °C
Detection: UV@250 nm,
Sample:
1. thymine, 2. uracil, 3. uridine, 4. cytosine, 5. cytidine

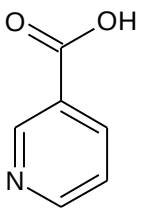




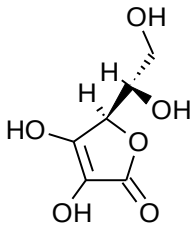
Separation of water soluble vitamins

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

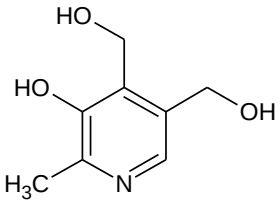




1. Nicotinic acid



2. Ascorbic acid



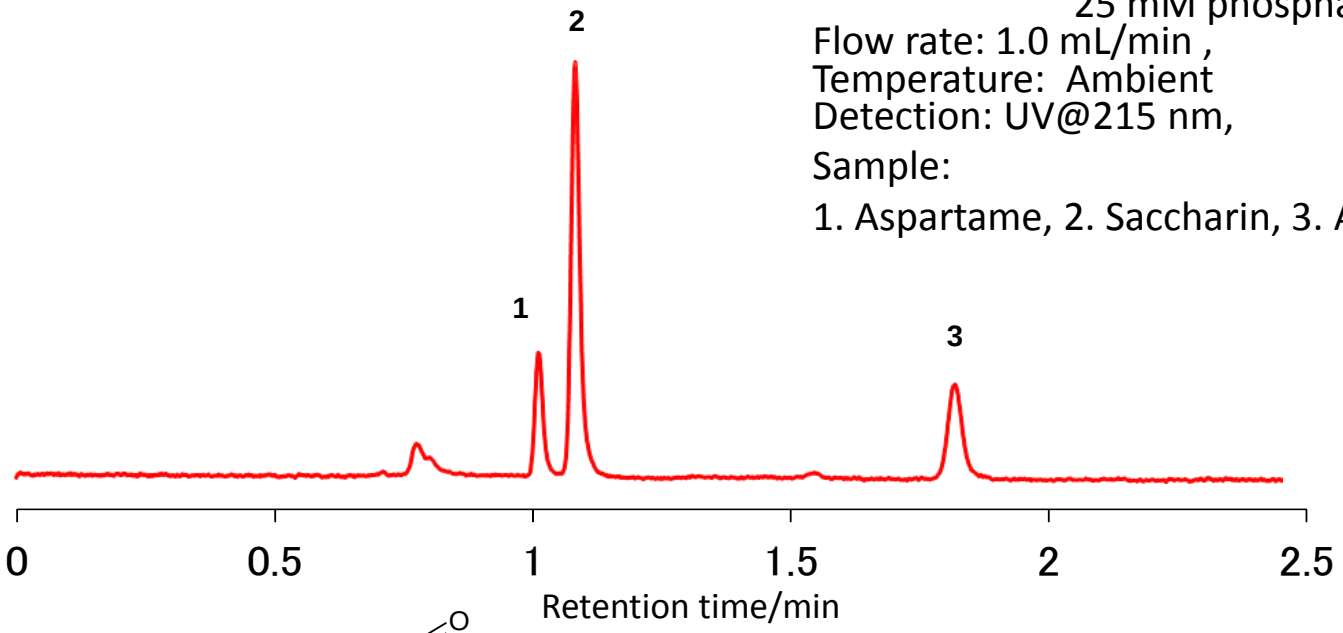
3. Pyridoxine

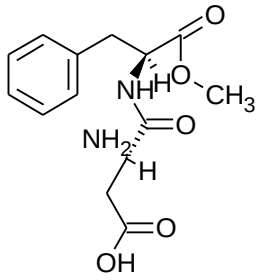
Separation of artificial sweeteners

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

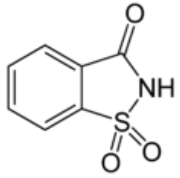
Sample:

1. Aspartame, 2. Saccharin, 3. Acesulfame K,

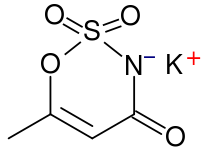




1. Aspartame

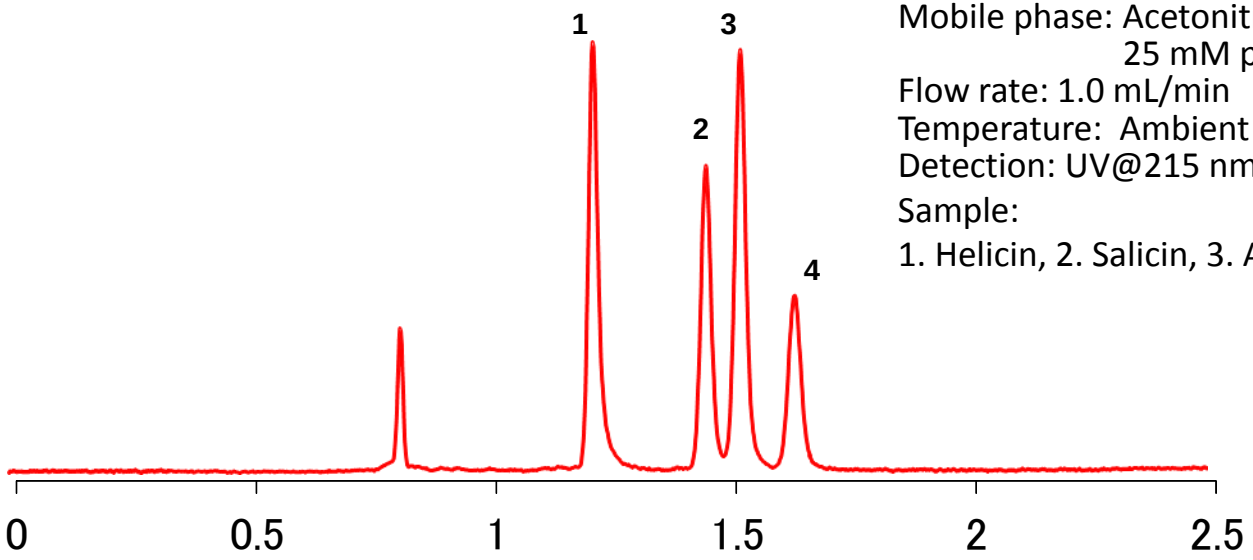


2. Saccharin

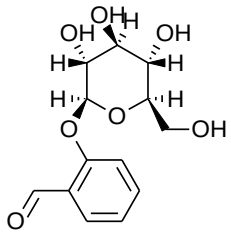


3. Acesulfame K

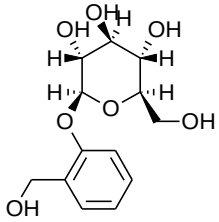
Separation of Glycosides



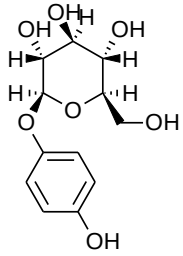
Column:
SunShell HILIC-Amide 2.6 μm : 100 x 4.6 mm,
Mobile phase: Acetonitrile :
25 mM phosphate Ammonium (pH4.9) =8:2
Flow rate: 1.0 mL/min
Temperature: Ambient
Detection: UV@215 nm
Sample:
1. Helicin, 2. Salicin, 3. Arbutin 4. Rutin



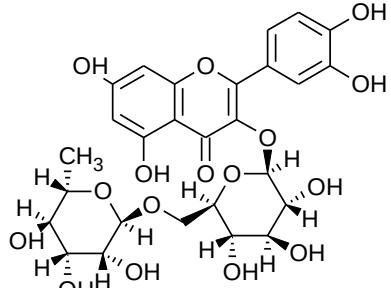
1. Helicin



2. Salitin



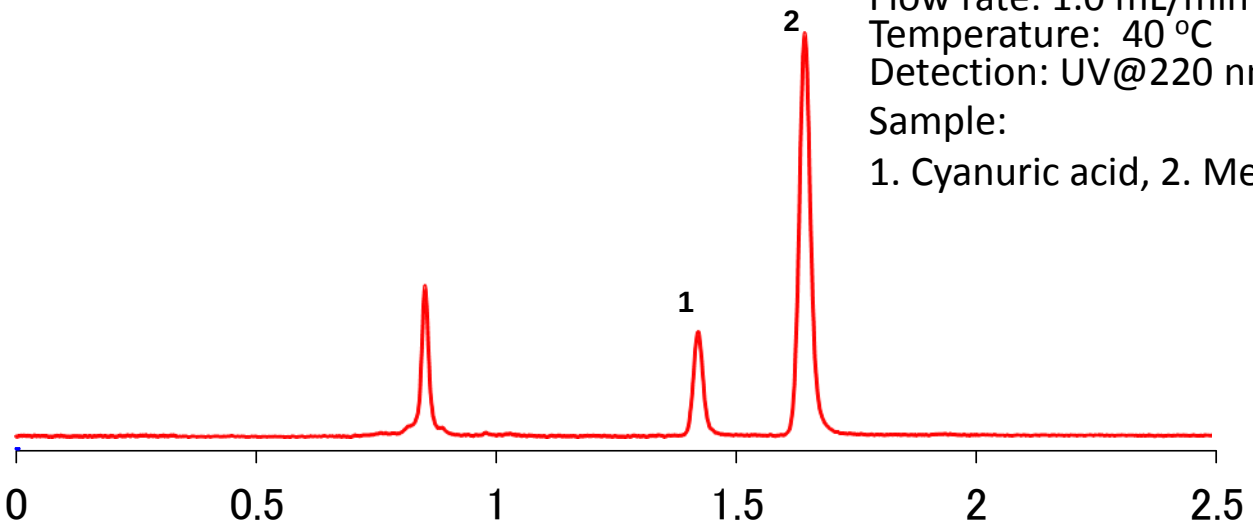
3. Arbutin

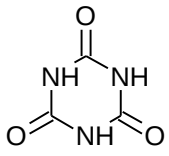


4. Rutin

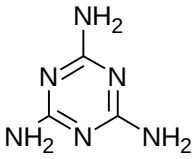
Separation of Melamine and cyanuric acid

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





1. Cyanuric acid

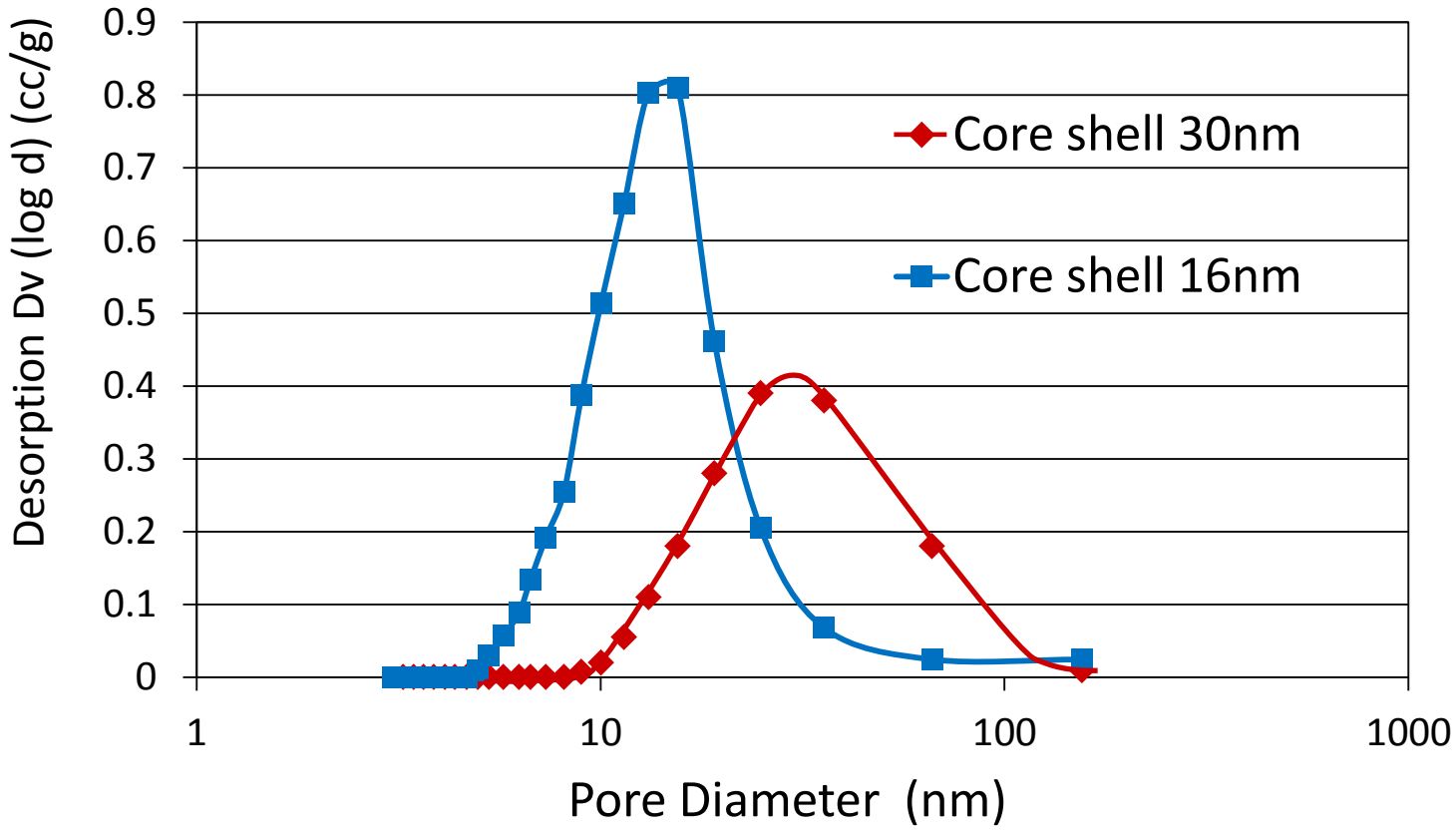


2. Melamine

List of phases for separation of high molecular weight compounds

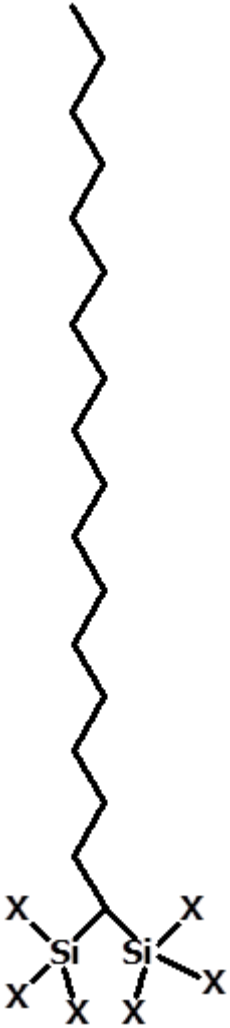
	Particle size, thickness of porous layer	Pore diameter	Surface area	Carbon loading	C18 Surface coverage	End-capping
SunShell C18-WP	2.6, 0.5 µm	16 nm	90 m^2/g	5%	2.5 µmol/m^2	Yes
SunShell HFC18-16	2.6, 0.5 µm	16 nm	90 m^2/g	2.5%	1.2 µmol/m^2	Yes
SunShell HFC18-30	2.6, 0.5 µm	30 nm	40 m^2/g	1.3%	1.2 µmol/m^2	Yes
SunShell C8-30	2.6, 0.5 µm	30 nm	40 m^2/g	1.2%	2.5 µmol/m^2	Yes
SunShell C4-30	2.6, 0.5 µm	30 nm	40 m^2/g	0.9%	3 µmol/m^2	Yes
SunShell C8-30-2 (Prototype, sell from 2015)	**3.4, 0.2 µm**	**30 nm**	**15 m^2/g**	**0.5%**	**2.5 µmol/m^2**	**Yes**

Pore size distribution of core shell particle



What is HFC18?

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



(X: Cl, OCH_3, OC_2H_5)

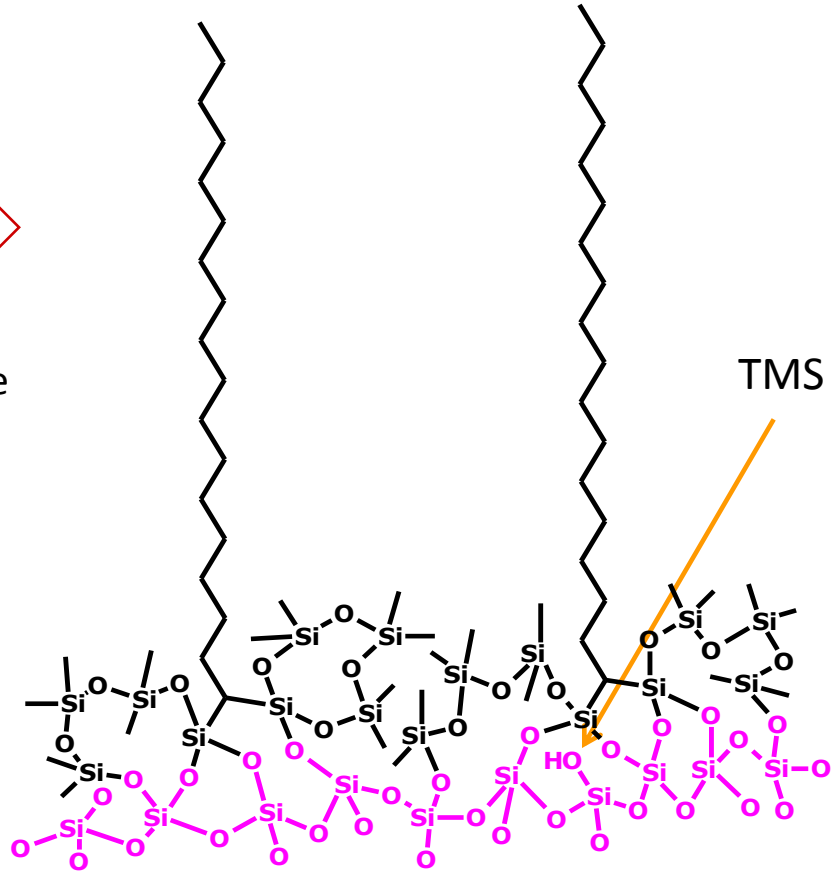
Schematic diagram of reagent

Sunniest Bonding Technology

Hexamethydichlorotrisiloxane

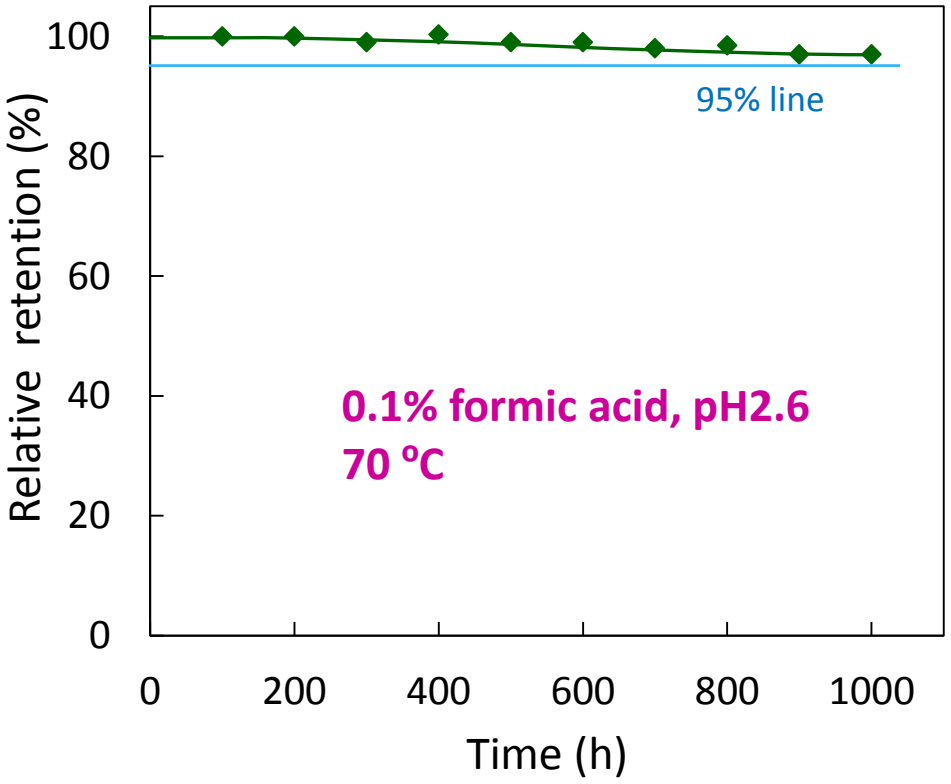
+

Trimethylchlorosilane (TMS)



Schematic diagram of the state of bonding on silica surface

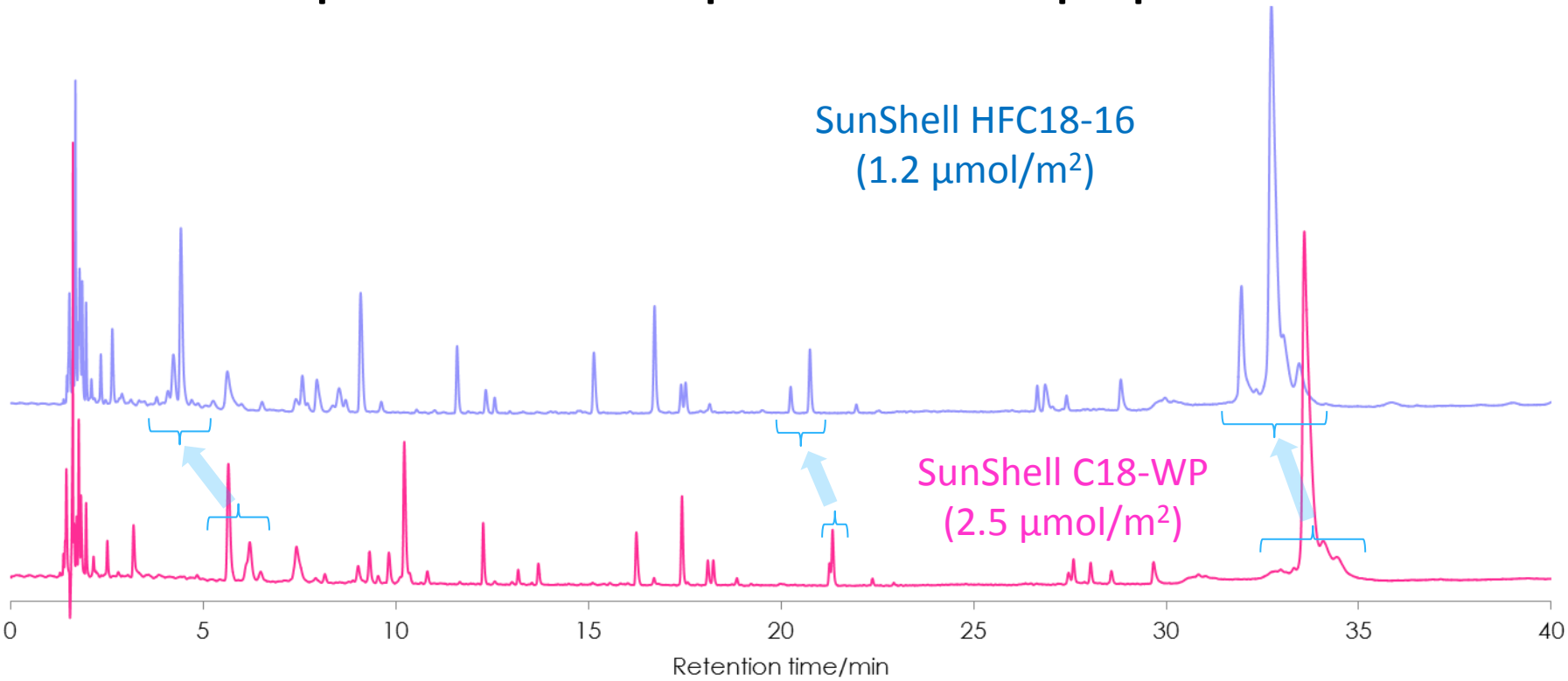
Stability of HFC18 under LC/MS mobile phase condition



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

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

Comparison of separation of peptides 1



Column: SunShell HFC18-16, 2.6 µm (16 nm) 150 x 4.6 mm,
SunShell C18-WP, 2.6 µm (16 nm) 150 x 4.6 mm

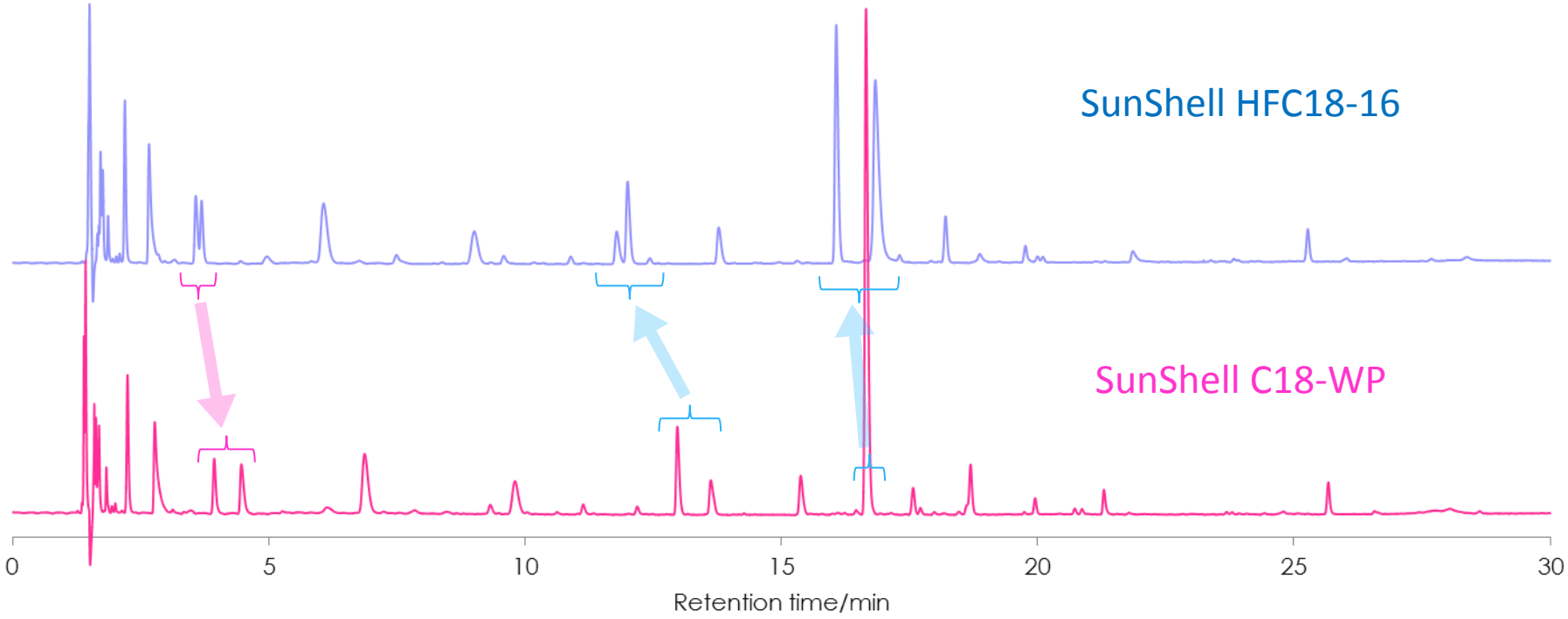
Mobile phase: A) 0.1% TFA in Acetonitrile/water(10:90)
B) 0.1 % TFA in Acetonitrile

Gradient program:

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

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

Comparison of separation of peptides 2



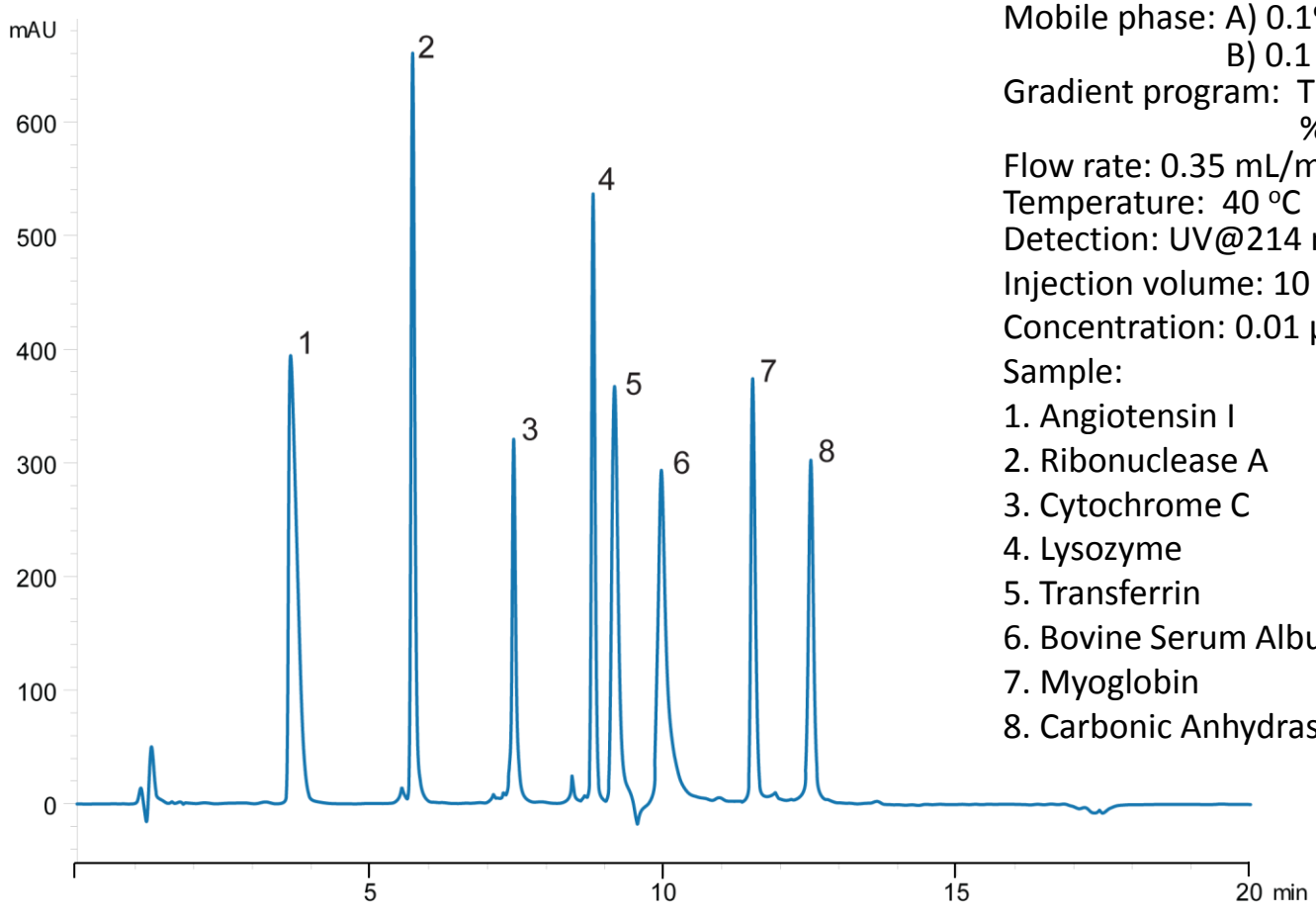
Column: SunShell HFC18-16, 2.6 µm (16 nm) 150 x 4.6 mm,
SunShell C18-WP, 2.6 µm (16 nm) 150 x 4.6 mm
Mobile phase: A) 0.1% TFA in Acetonitrile/water(10:90)
B) 0.1 % TFA in Acetonitrile

Gradient program:

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

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

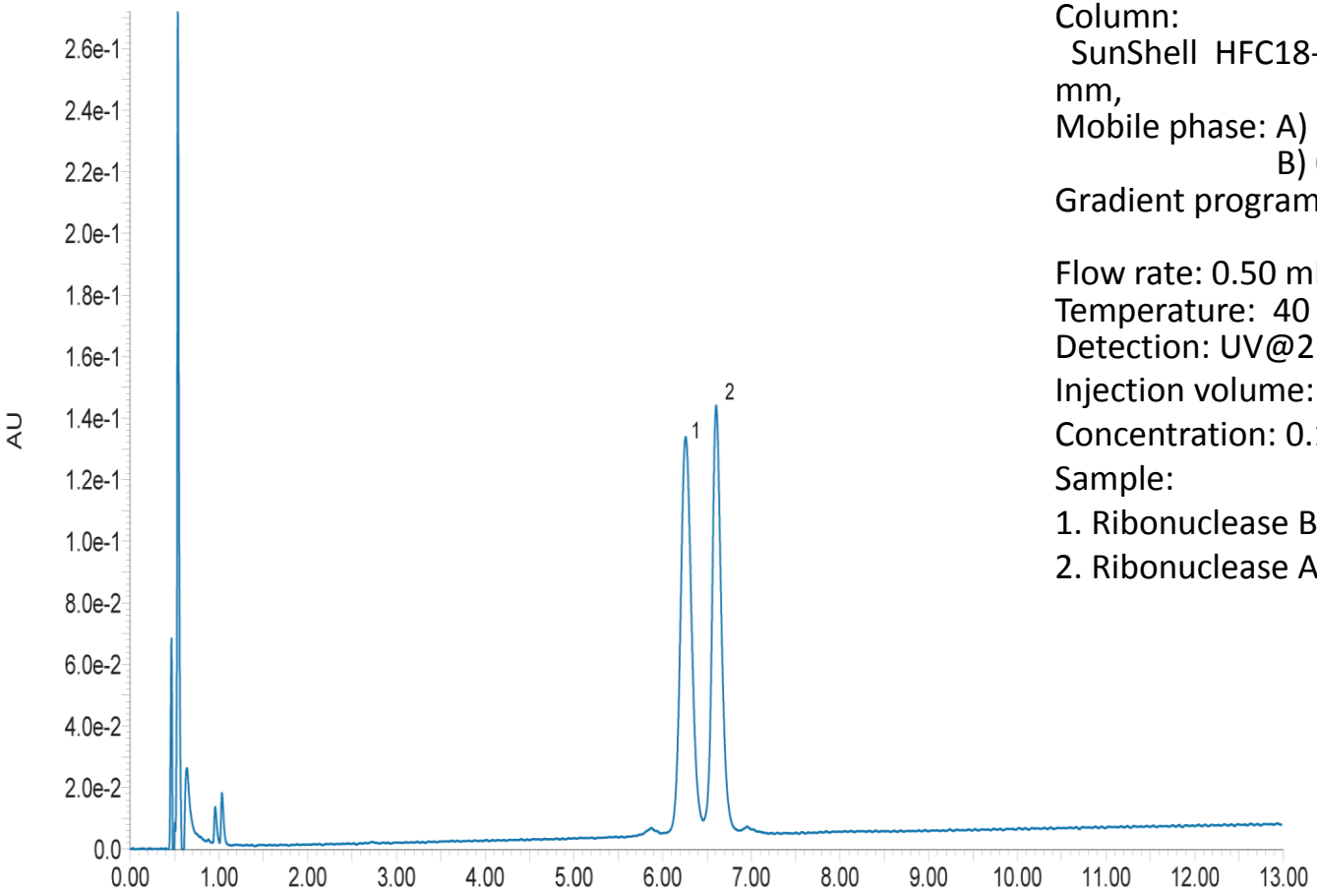
Separation of standard proteins



Column:
SunShell C8-30, 2.6 μm (30 nm) 150 x 2.1 mm,
Mobile phase: A) 0.1% TFA in water
B) 0.1 % TFA in Acetonitrile
Gradient program: Time 0 min 20 min
%B 22% 70.5%
Flow rate: 0.35 mL/min ,
Temperature: 40 °C
Detection: UV@214 nm,
Injection volume: 10 μL,
Concentration: 0.01 μg/μL each protein,
Sample:

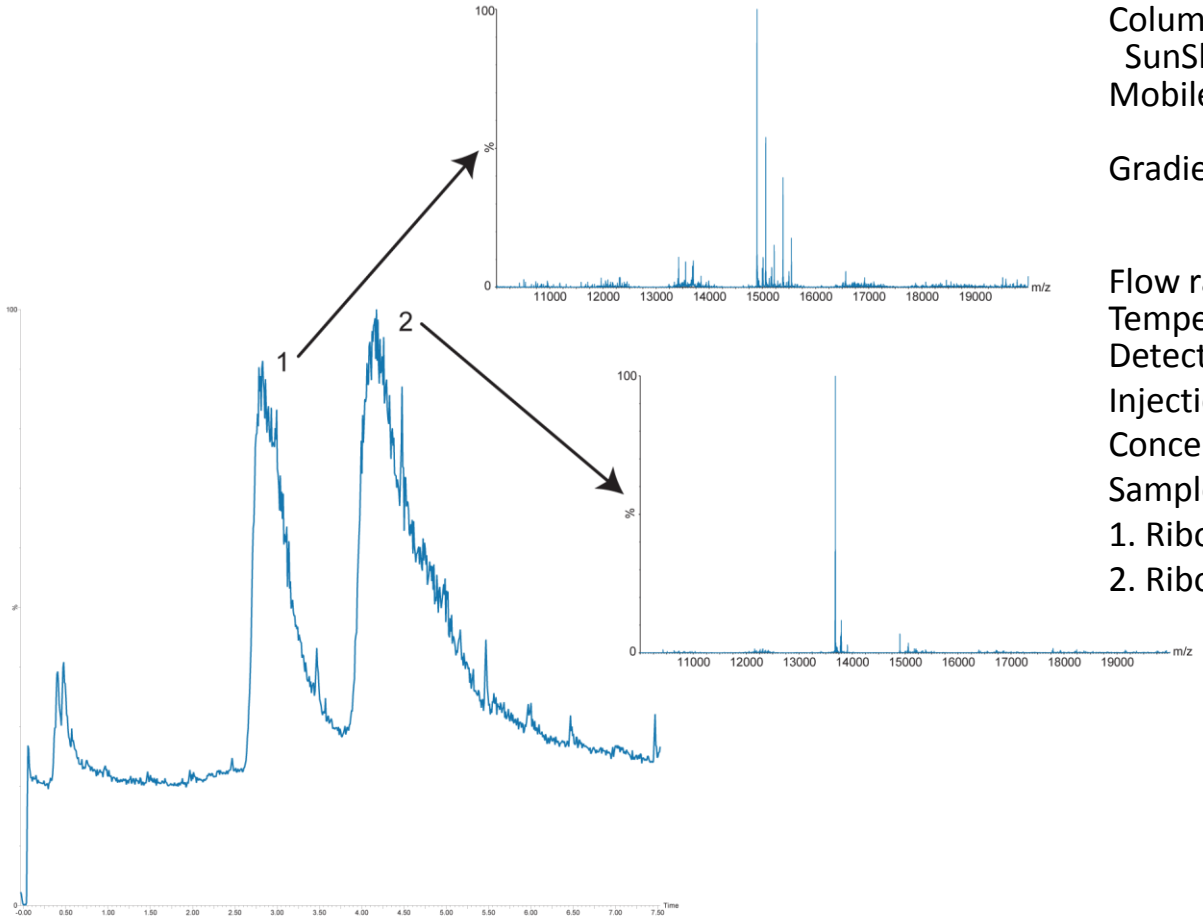
1. Angiotensin I
2. Ribonuclease A
3. Cytochrome C
4. Lysozyme
5. Transferrin
6. Bovine Serum Albumin
7. Myoglobin
8. Carbonic Anhydrase

Separation of Ribonuclease A/B



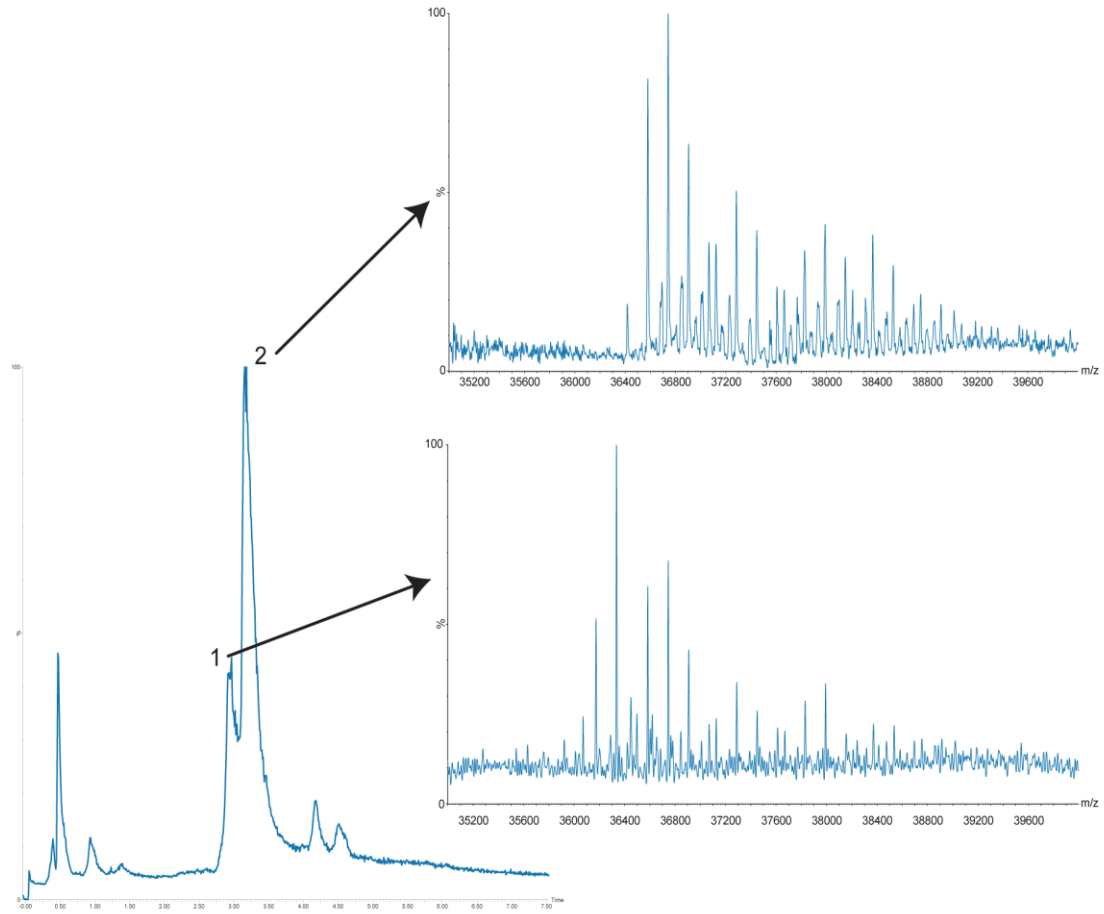
Column:
SunShell HFC18-30, 2.6 µm (30 nm) 100 x 2.1 mm,
Mobile phase: A) 0.1% TFA in water
B) 0.1 % TFA in Acetonitrile
Gradient program: Time 0 min 20 min
%B 22% 70.5%
Flow rate: 0.50 mL/min ,
Temperature: 40 °C
Detection: UV@214 nm,
Injection volume: 10 µL,
Concentration: 0.10 µg/µL each protein,
Sample:
1. Ribonuclease B
2. Ribonuclease A

Separation of Ribonuclease A/B
MS Detection



Column:
SunShell HFC8-30, 2.6 μm (30 nm) 100 x 2.1 mm,
Mobile phase: A) 0.1% Formic acid in water
B) 0.1 % Formic acid in Acetonitrile
Gradient program: Time 0 min 10 min
%B 17.5% 17.5%
Isocratic separation
Flow rate: 0.50 mL/min ,
Temperature: 60 °C
Detection: MS,
Injection volume: 10 µL,
Concentration: 0.10 µg/µL each protein,
Sample:
1. Ribonuclease B
2. Ribonuclease A

Separation of Lipase
MS Detection

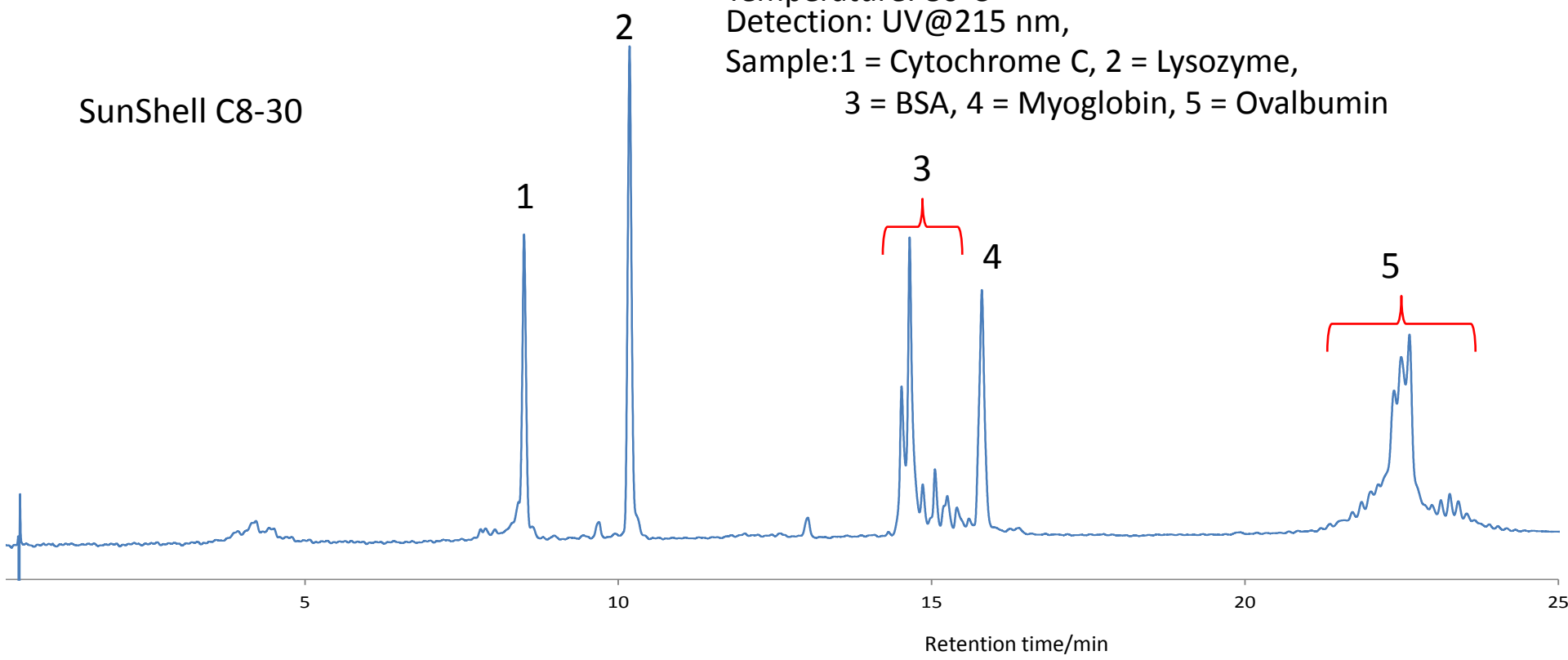


Column:
SunShell HFC8-30, 2.6 µm (30 nm) 100 x 2.1 mm,
Mobile phase: A) 0.1% Formic acid in water
B) 0.1 % Formic acid in Acetonitrile
Gradient program: Time 0 min 10 min
%B 15% 60%
Flow rate: 0.50 mL/min ,
Temperature: 60 °C
Detection: MS,
Injection volume: 10 µL,
Concentration: 0.10 µg/µL each protein,
Sample:
1. Lipase Impurity
2. Lipase

Separation of Proteins at 80 °C

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

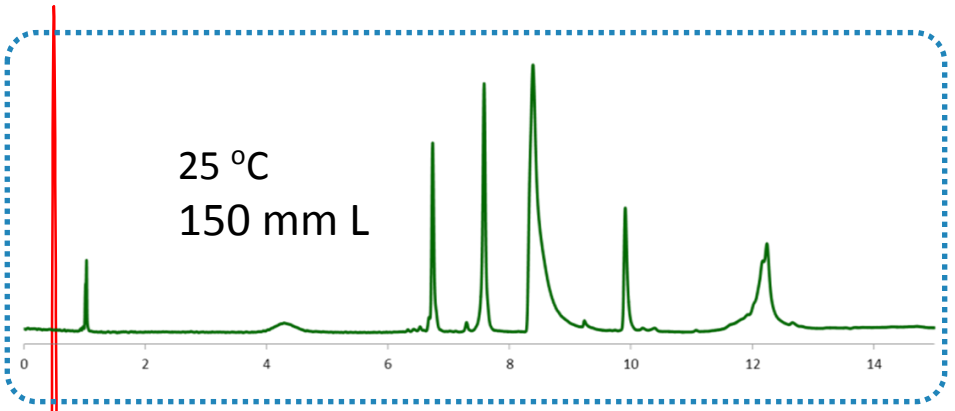
SunShell C8-30

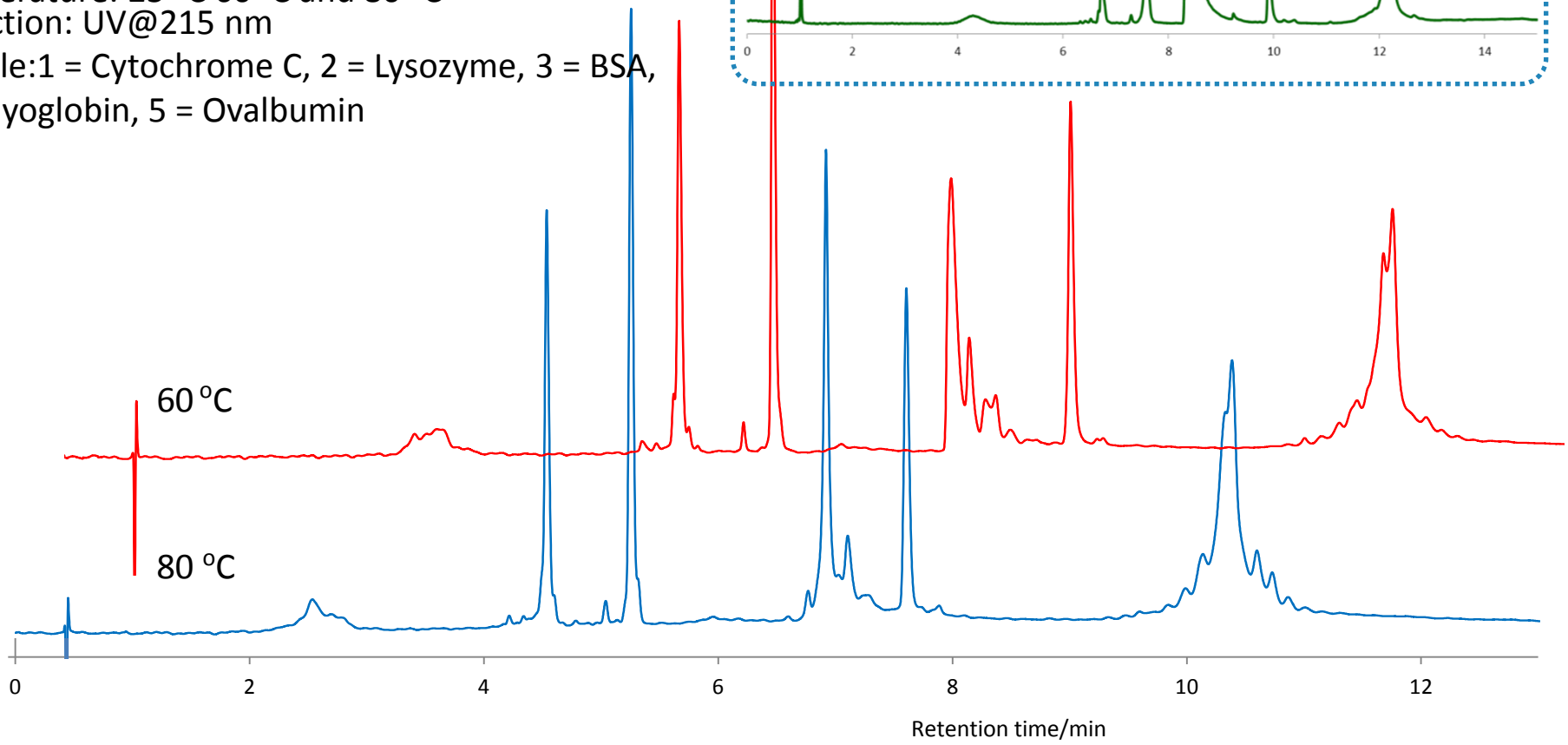


Separation of proteins Effect of temperature

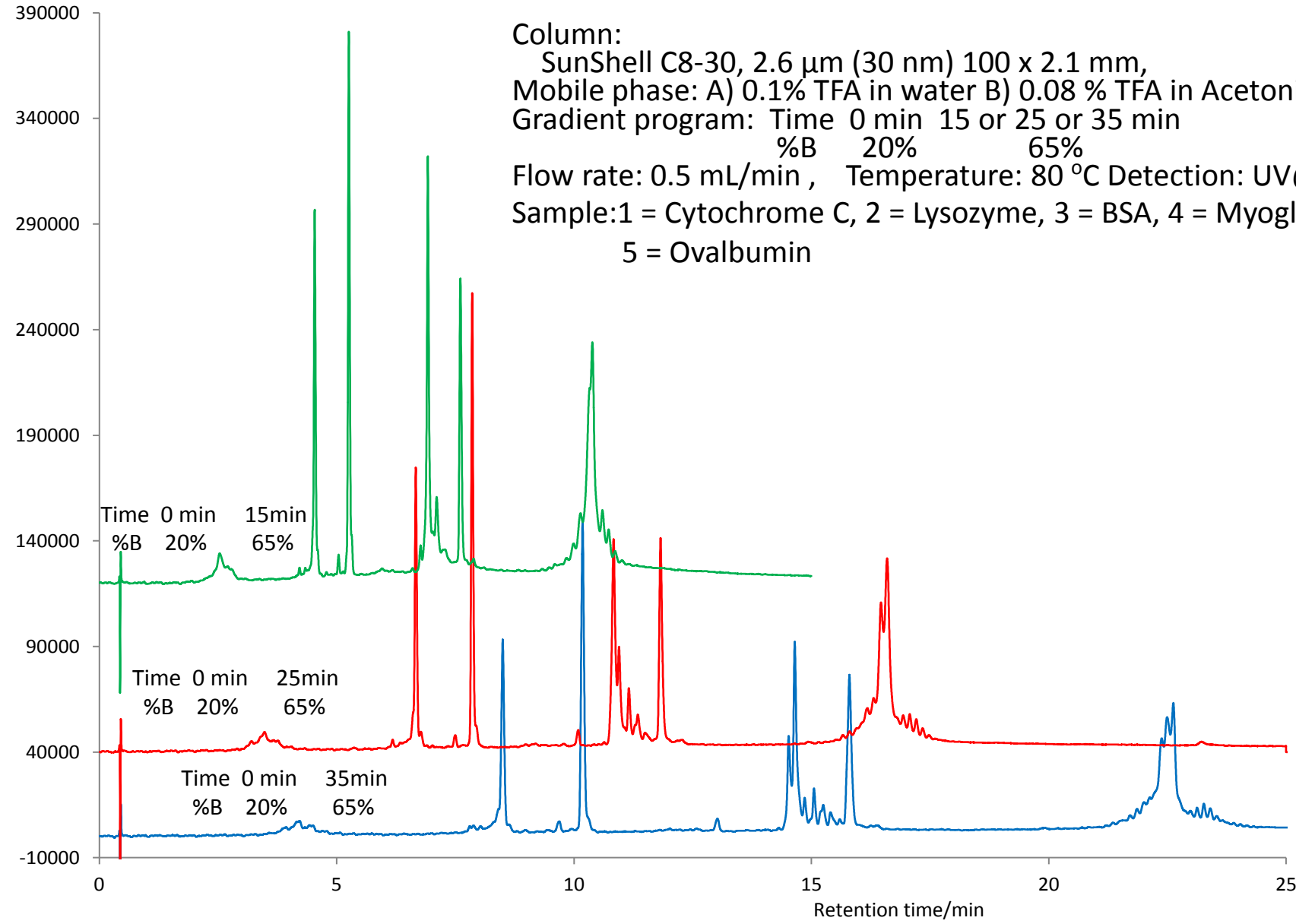
Column:
SunShell C8-30, 2.6 µm (30 nm) 100 x 2.1 mm,
Mobile phase: A) 0.1% TFA in water
B) 0.08 % TFA in Acetonitrile
Gradient program: Time 0 min 15 min
%B 20% 65%
Flow rate: 0.5 mL/min

Temperature: 25 °C 60 °C and 80 °C
Detection: UV@215 nm
Sample:1 = Cytochrome C, 2 = Lysozyme, 3 = BSA,
4 = Myoglobin, 5 = Ovalbumin





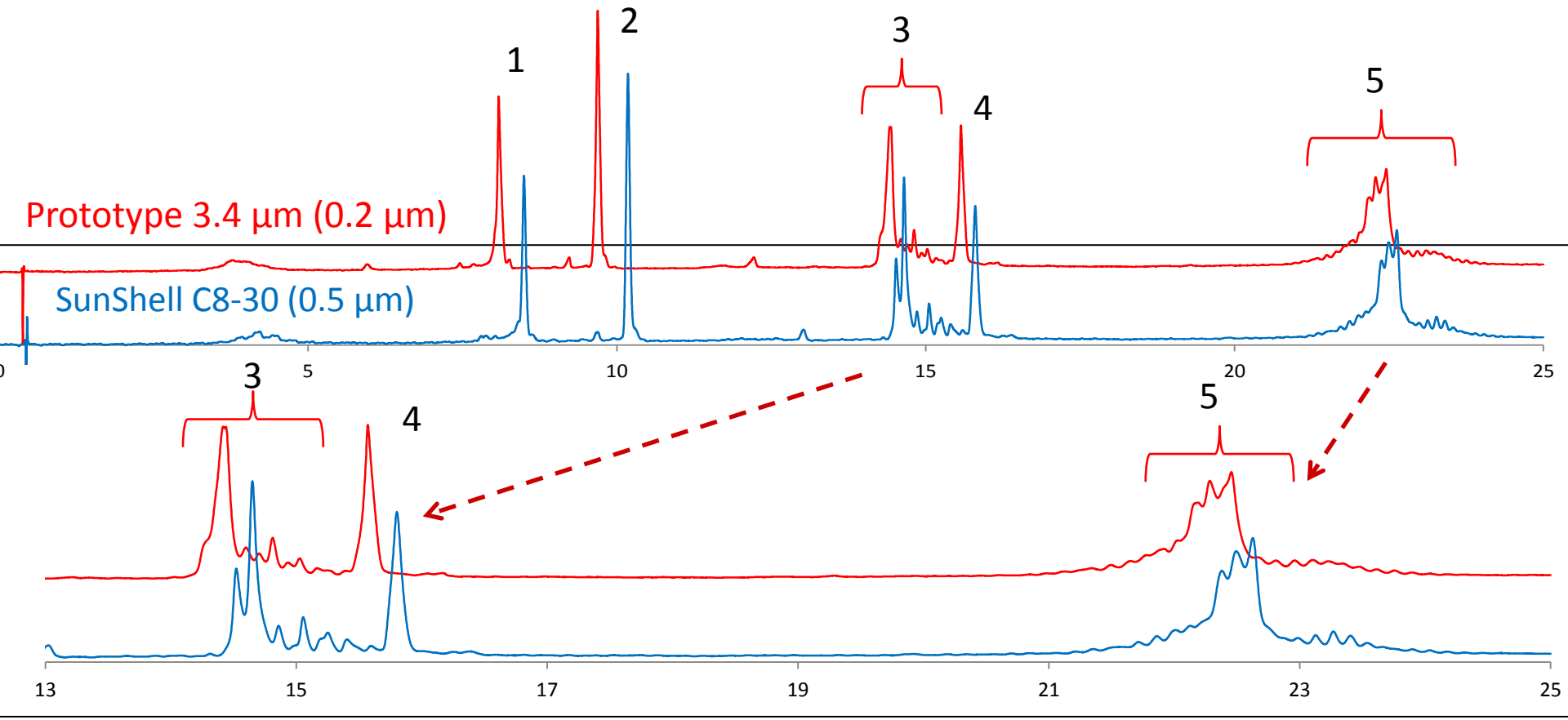
Separation of Proteins Effect of gradient time



Comparing of Protein Separation

Comparison of thickness of porous layer

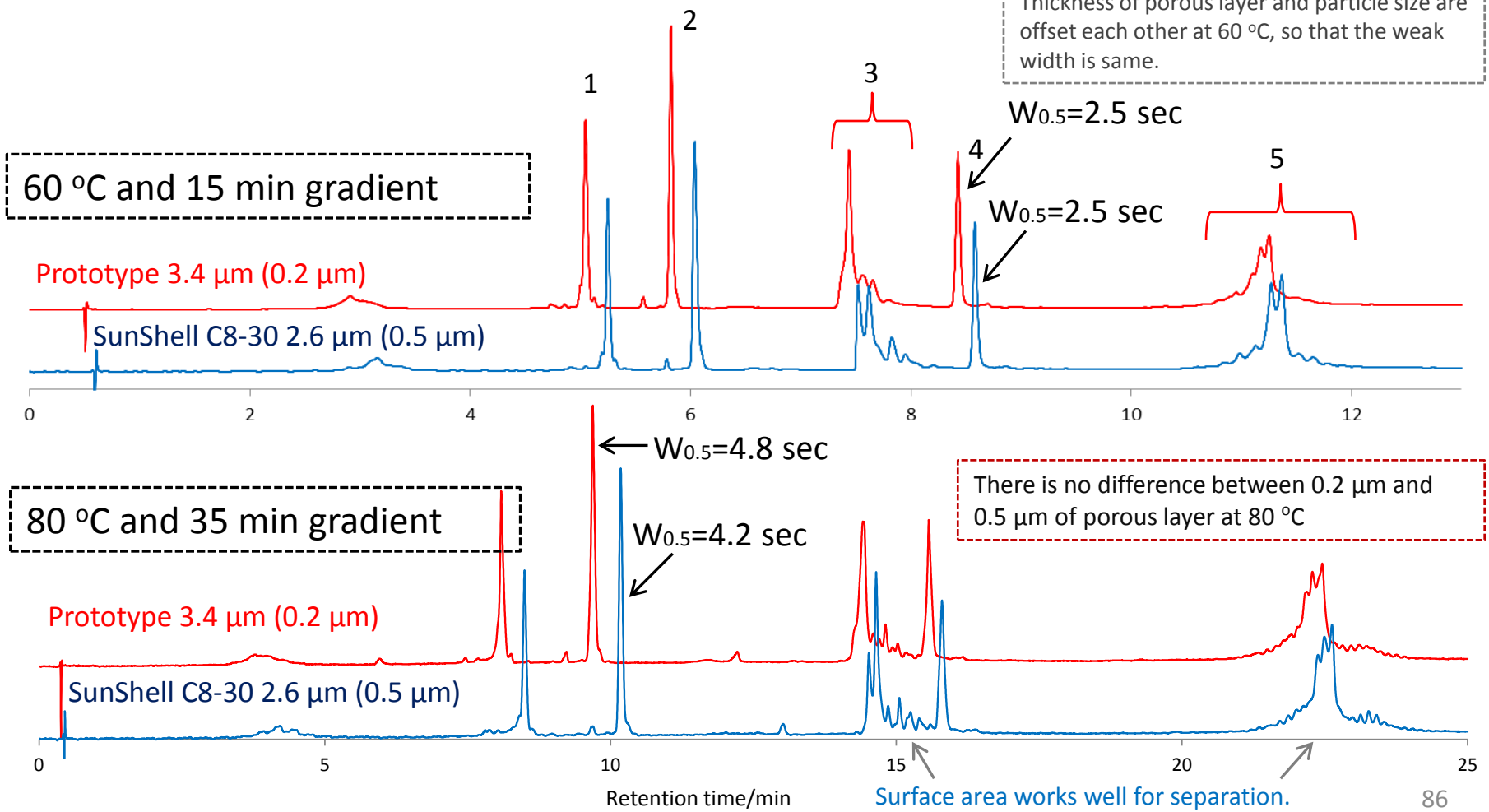
Column: SunShell C8-30, 2.6 µm (30 nm, 0.5 µm layer) 100 x 2.1 mm
Sunshell C8-30, 3.4 µm (30 nm, 0.2 µm layer) 100 x 2.1 mm (prototype)
Mobile phase: A) 0.1% TFA in water B) 0.08 % TFA in Acetonitrile
Gradient program: Time 0 min 35 min
%B 20% 65%
Flow rate: 0.5 mL/min , Temperature: 80 °C Detection: UV@215 nm,
Sample:1 = Cytochrome C, 2 = Lysozyme, 3 = BSA, 4 = Myoglobin, 5 = Ovalbumin



Separation of Proteins Comparison of thickness of porous layer

Column: SunShell C8-30, 2.6 µm (30 nm, 0.5 µm layer) 100 x 2.1 mm, Sunshell C8-30, 3.4 µm (30 nm, 0.2 µm layer) 100 x 2.1 mm (prototype)
Mobile phase: A) 0.1% TFA in water B) 0.08 % TFA in Acetonitrile
Gradient program: Time 0 min 15 or 35 min
%B 20% 65%
Flow rate: 0.5 mL/min , Temperature: 60 or 80 °C, Detection: UV@215 nm,
Sample:1 = Cytochrome C, 2 = Lysozyme, 3 = BSA, 4 = Myoglobin, 5 = Ovalbumin

Thickness of porous layer and particle size are offset each other at 60 °C, so that the weak width is same.



There is no difference between 0.2 µm and 0.5 µm of porous layer at 80 °C

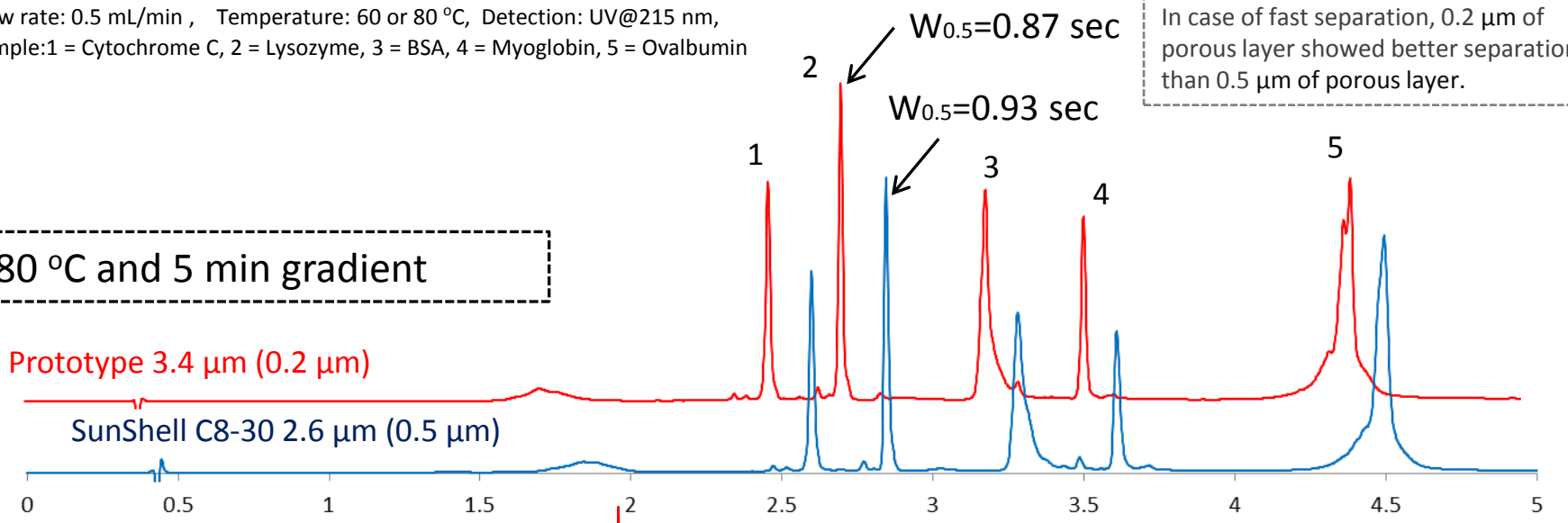
Surface area works well for separation.

Separation of Proteins Comparison of thickness of porous layer

Column: SunShell C8-30, 2.6 µm (30 nm, 0.5 µm layer) 100 x 2.1 mm, Sunshell C8-30, 3.4 µm (30 nm, 0.2 µm layer) 100 x 2.1 mm (prototype)
Mobile phase: A) 0.1% TFA in water B) 0.08 % TFA in Acetonitrile
Gradient program: Time 0 min 5 or 35 min
%B 20% 65%
Flow rate: 0.5 mL/min , Temperature: 60 or 80 °C, Detection: UV@215 nm,
Sample:1 = Cytochrome C, 2 = Lysozyme, 3 = BSA, 4 = Myoglobin, 5 = Ovalbumin

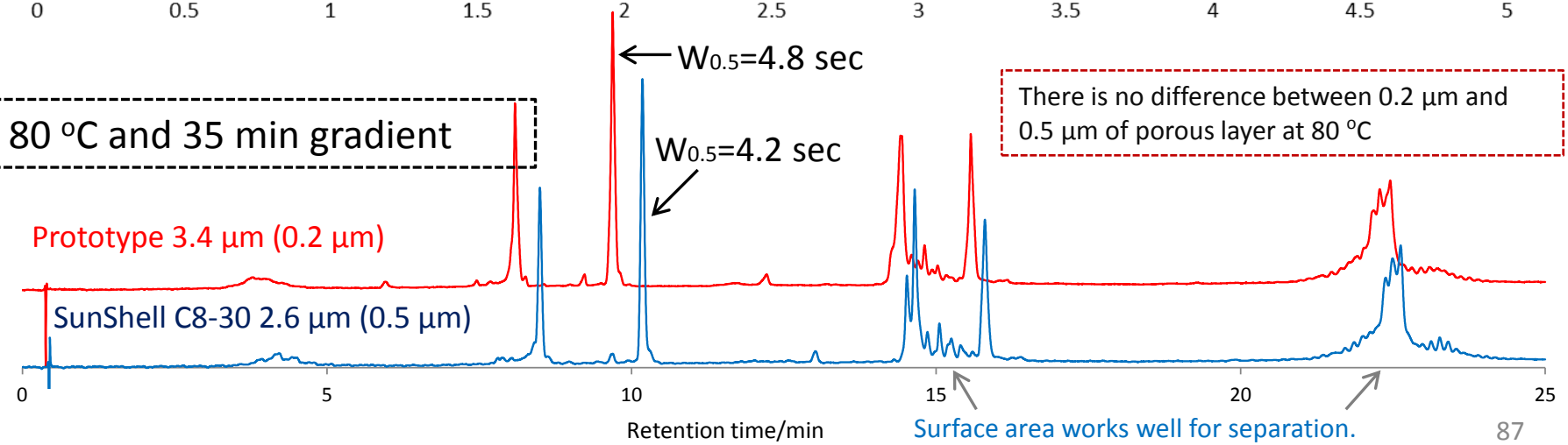
In case of fast separation, 0.2 µm of porous layer showed better separation than 0.5 µm of porous layer.

80 °C and 5 min gradient



There is no difference between 0.2 µm and 0.5 µm of porous layer at 80 °C

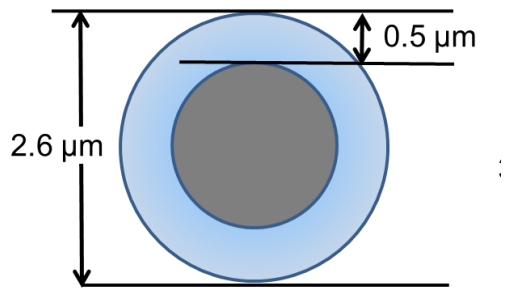
80 °C and 35 min gradient



Surface area works well for separation.

Which is better?

SunShell particle

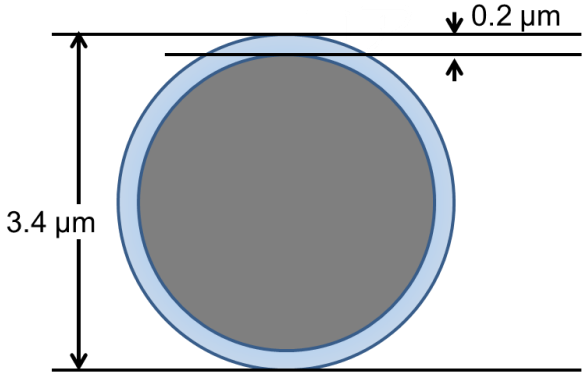


Particle size: 2.6 µm

Thickness of porous layer: 0.5 µm

Specific surface area: 40 m^2/g

Prototype particle (sales from 2015)



Particle size: 3.4 µm

Thickness of porous layer: 0.2 µm

Specific surface area: 15 m^2/g

It is said that thin layer of porous layer is suitable for separation of large biomolecules such as proteins. At more than 60 degree C, however, there is little difference of efficiency between 0.2 µm and 0.5 µm of porous layer.
Separation of proteins using 2.6 µm of particle and 0.5 µm of porous layer is better than one using 3.4 µm of particle and 0.2 µm of porous layer at 80 degree C and 35min gradient time because of a small particle.