

低吸着化のためのHPLCカラム技術と、 有機不活性管 **PS inert** の効果

Highly inert end-capping and Novel inert column hardware

ChromaNik

Original title

HPLC column technology for low adsorption and the effect of '**PS inert**' organic column hardware.

Update Date:
September 29th, 2023



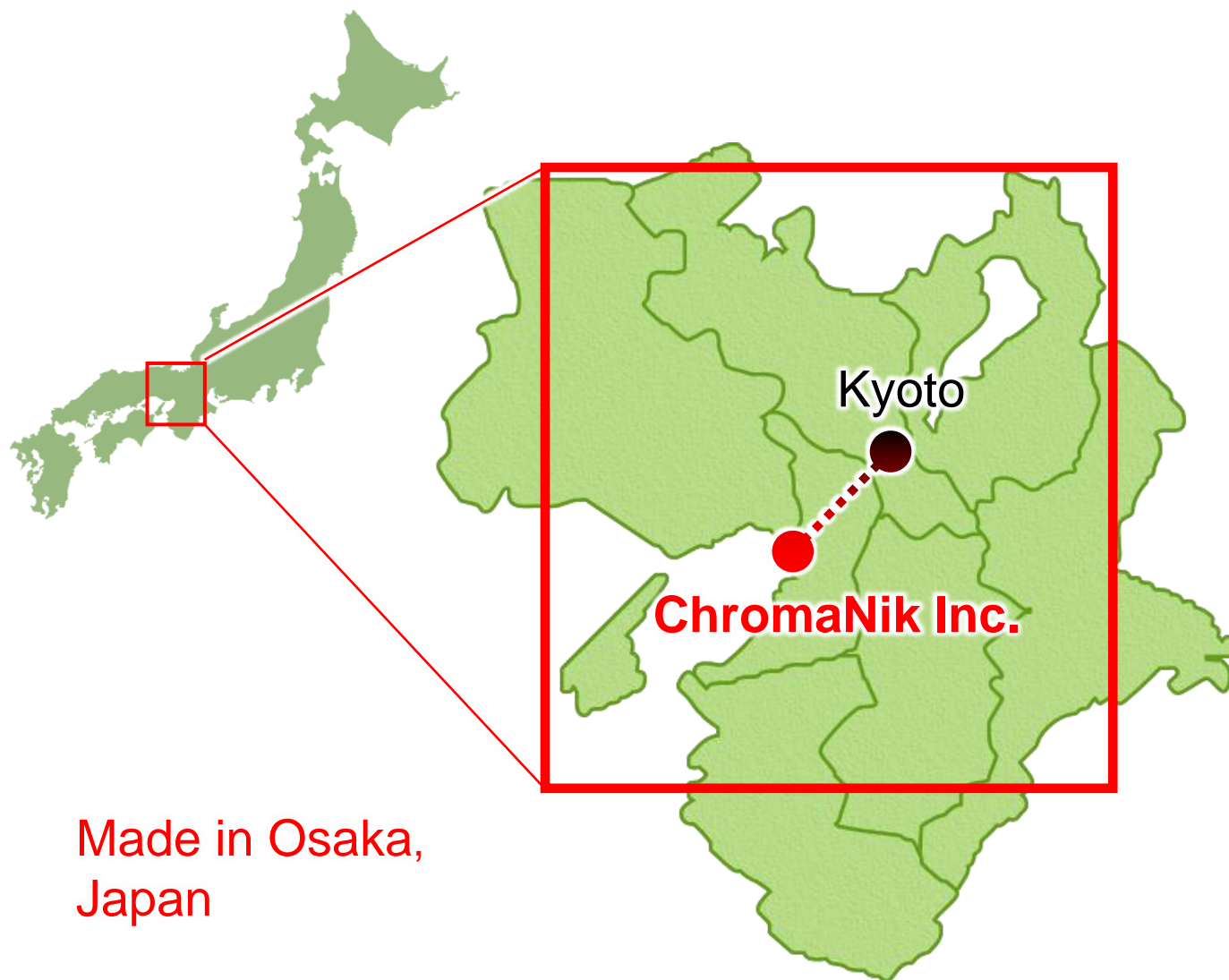
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Column Concierge

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ChromaNik Technologies Inc.



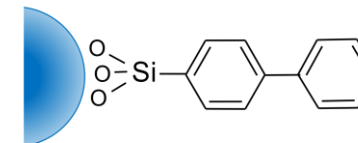
Made in Osaka,
Japan

Work

- Manufacturing of HPLC column (for RPLC, NPLC, HILIC, SFC)
- Development of new chromatographic stationary phase
- Application Development

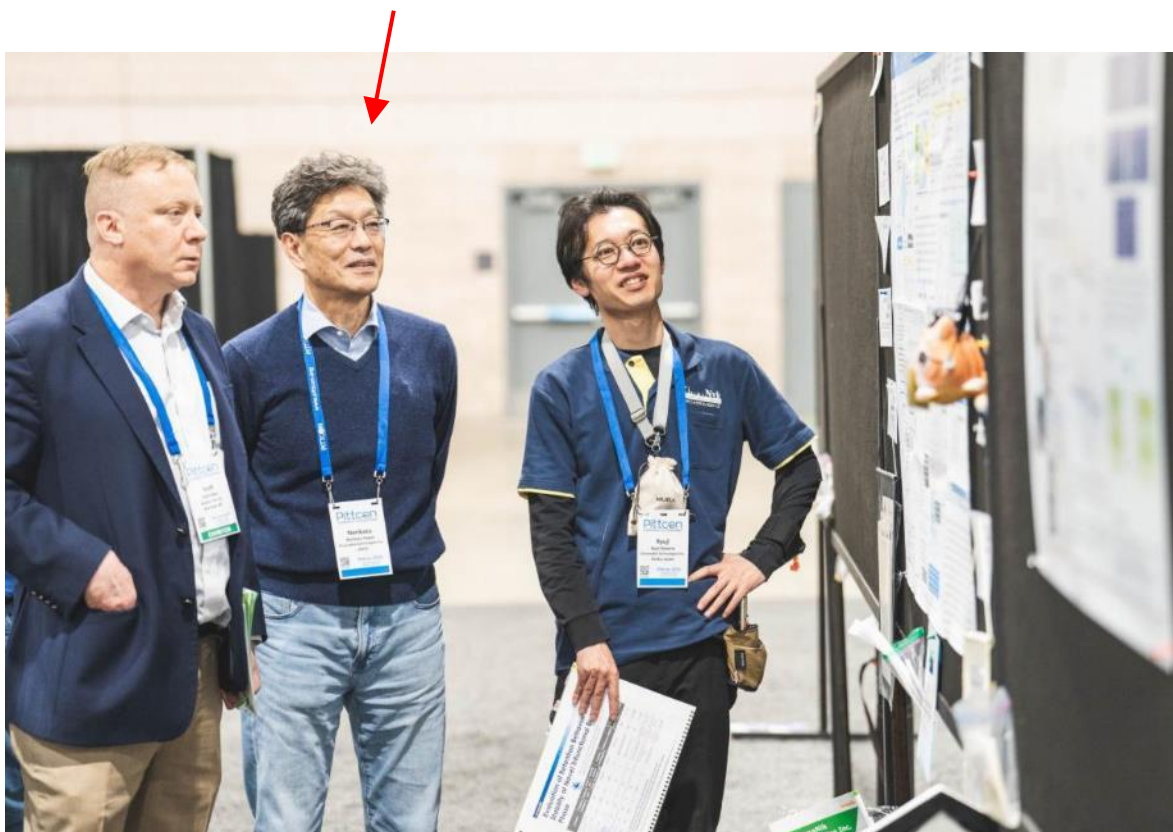
Feature

- Inertness
- High effective (core-shell)
- High separation performance



ChromaNik Technologies Inc.

Norikazu Nagae, Ph.D. (Founder)



(Photo by Pittcon-2023 – Poster session)

We have unique **end-capping** technology.

History



2005. Established ChromaNik Technologies Inc.

2006. Released ‘Sunrise’ column series

2008. Developed **Sunniest end-capping** ↘

2008. Released ‘Sunniest’ (FPP*) column series

2011. Released ‘SunShell’ (SPP*) column series

2015. Released ‘SunArmor’ column series

2022. Developed Tandem TMS end-capping

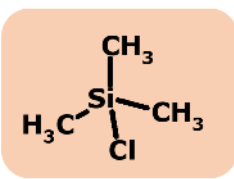
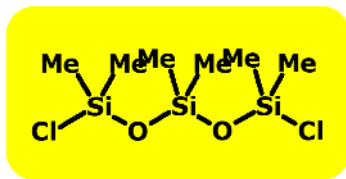
2022. Released ‘Prominert’ column series

2023. Released ‘PS inert’ column hardware

* FPP: Fully porous particle, SPP: Superficially porous particle

Overview – Sunniest end-capping

I. Dense end-capping layer form



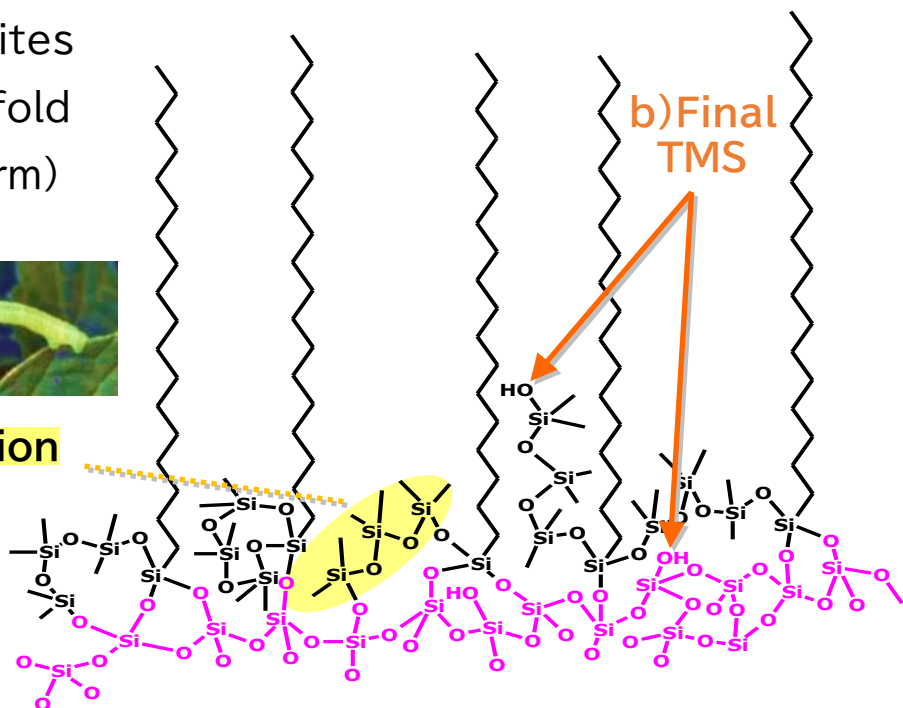
Double end-capping

▶ Key feature of end-capping layer

- Two silylation sites
- Extending scaffold (like an Inchworm)

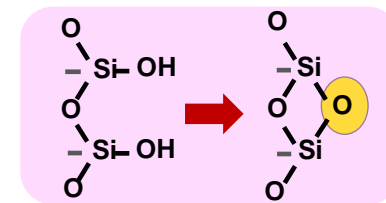


a) Twin-silylation

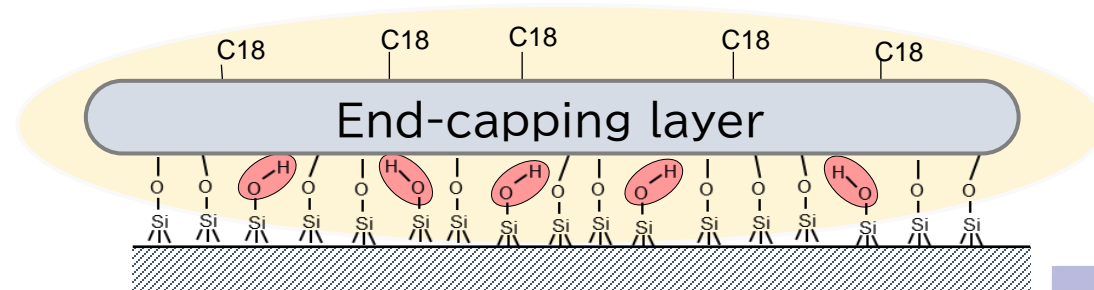


II. Siloxaneation on silica surface

& High temperature

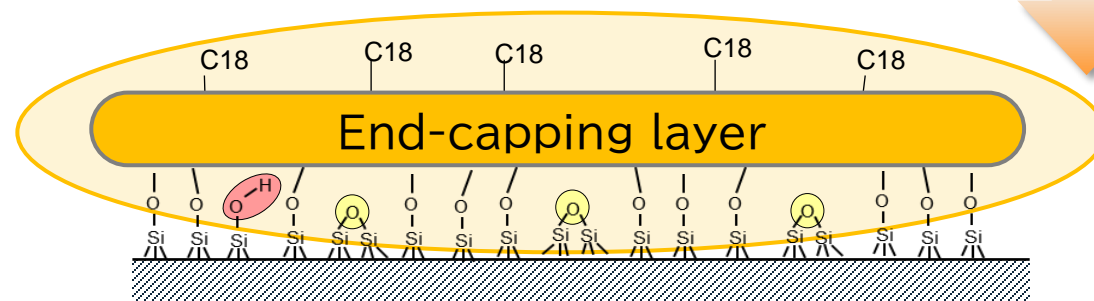


General end-capped surface

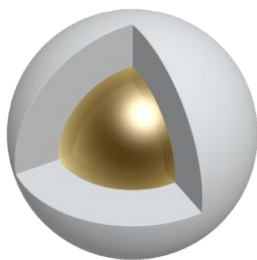


Sunniest end-capped

▶ More hydrophobic



SunShell – Highly inert & effective

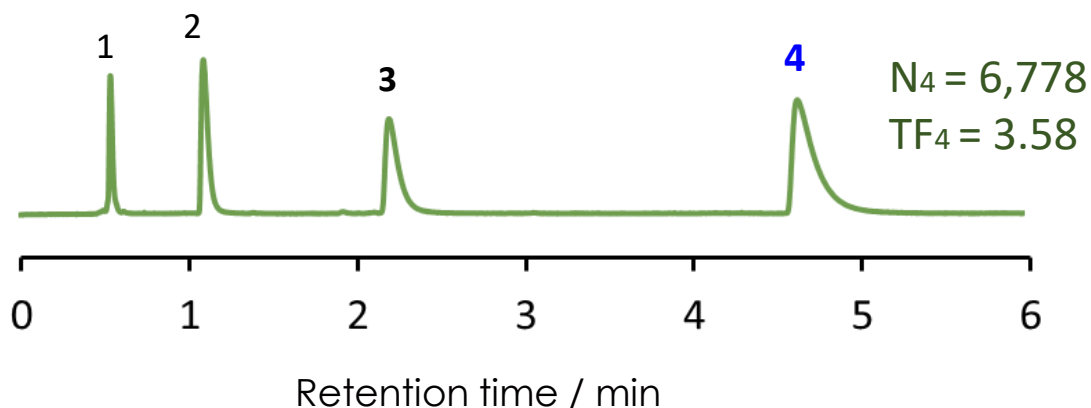


‘SunShell’ was created by the fusion of Sunniest end-capping and a superficially porous (**Core-Shell**) particle.

SunShell C18



Company B C18



Column dimension: 100 x 2.1 mm 2 μ m

Mobile phase:

CH₃CN/10 mM ammonium acetate pH 6.8 = 40/60

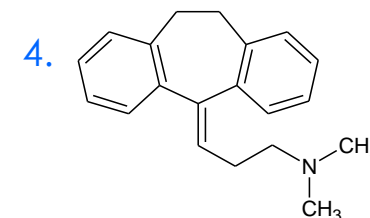
Flow rate: 0.3 mL/min

Temperature: 40 °C

Detection: UV@250 nm

Sample:

1. Uracil
2. Propranolol
3. Nortriptyline
4. Amitriptyline



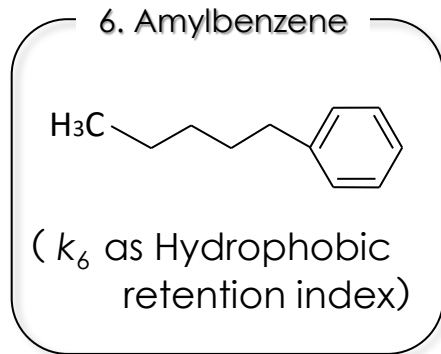
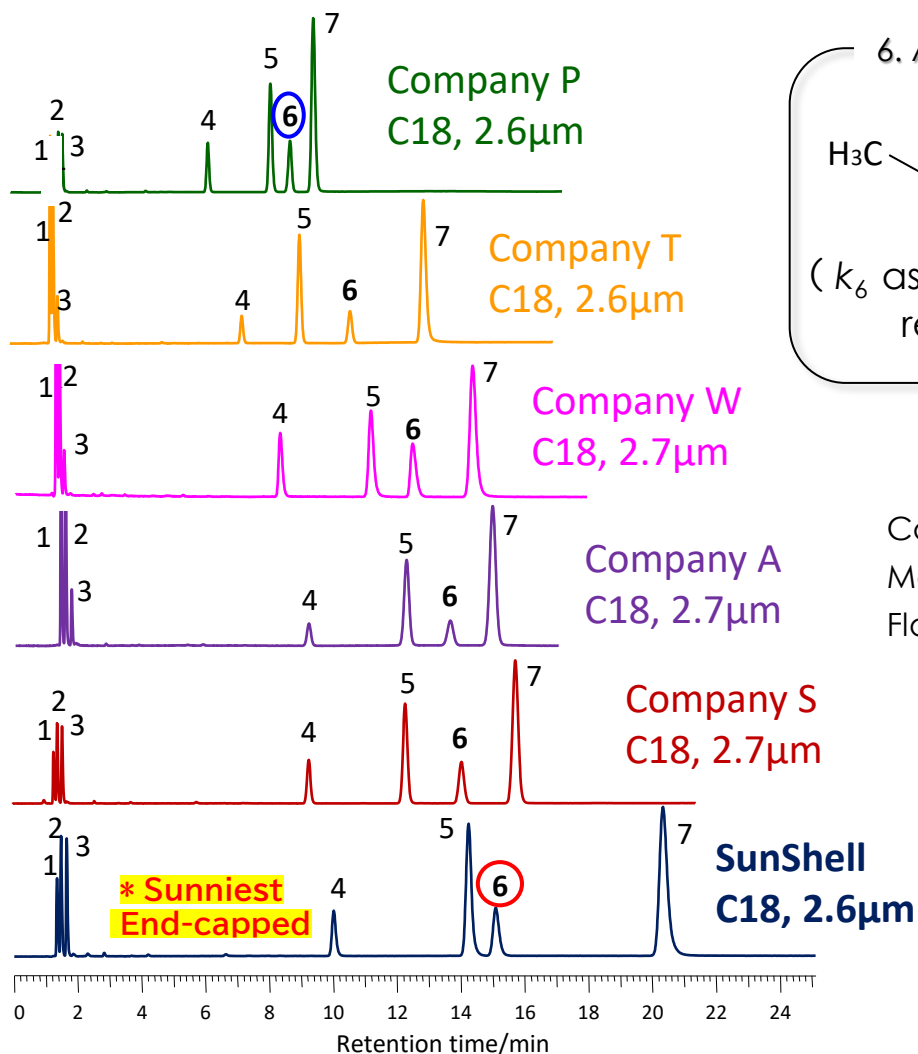
Basic compounds

(N: Theoretical plate number, TF: Tailing factor)

Comparison of core-shell columns



(k_6 : retention factor, N_6 : theoretical plate number)



Column	k_6	Carbon load (%C)	N_6	Pressure /MPa	N_6 per Pressure
Company P C18	5.4	12	30,800	26.1	1.2
Company T C18	7.4	9	31,600	22.7	1.4
Company W C18	7.7	6.6	23,300	18.5	1.3
Company A C18	9.0	8	30,200	30.6	1.0
Company S C18	9.7	7.7	31,800	22.2	1.5
SunShell C18	10.4	7	31,900	21.8	1.5

Column: Core-Shell 2.6 or 2.7 μm 150 x 4.6 mm
 Mobile phase: $\text{CH}_3\text{OH}/\text{H}_2\text{O} = 75/25$
 Flow rate: 1.0 mL/min, Temperature: 40 °C

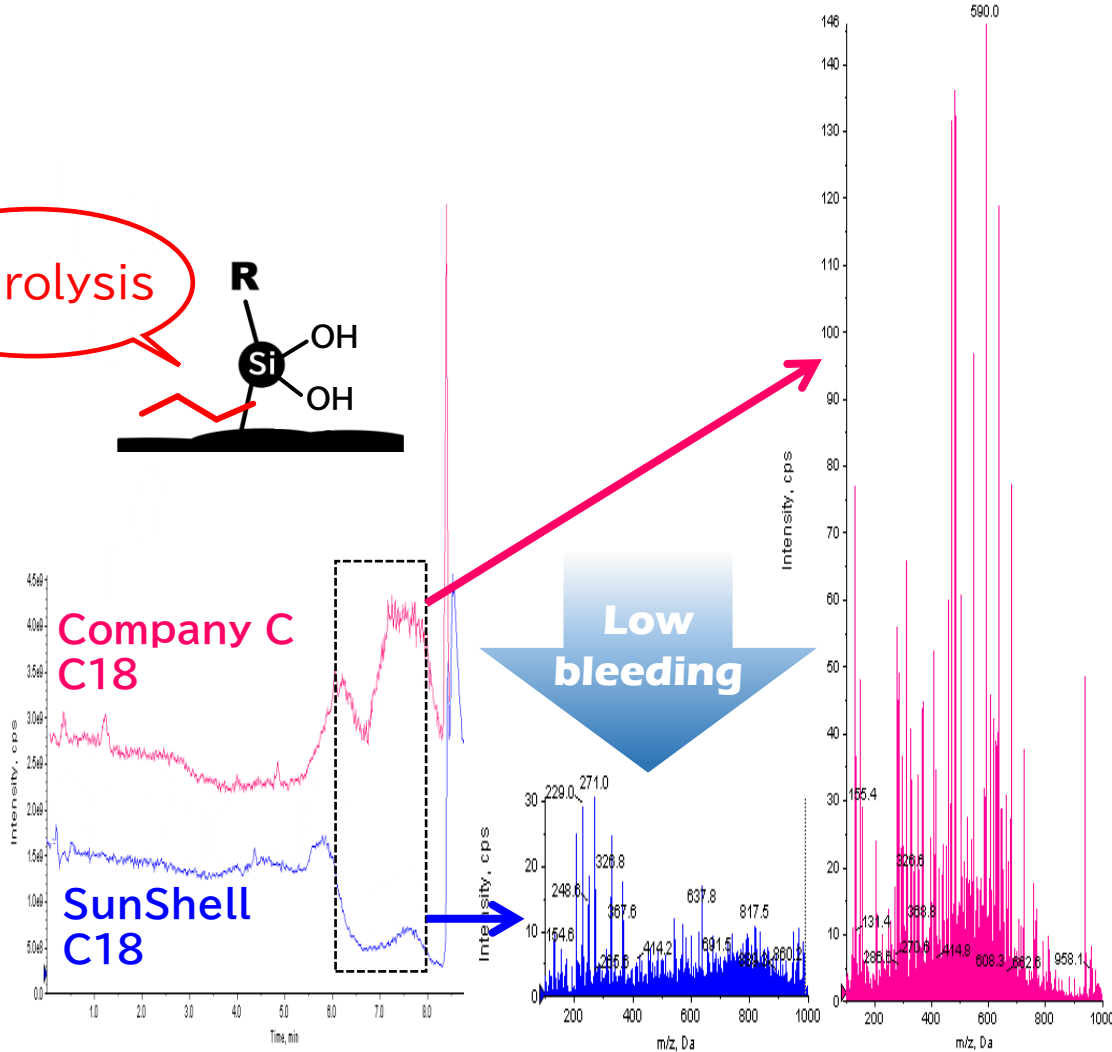
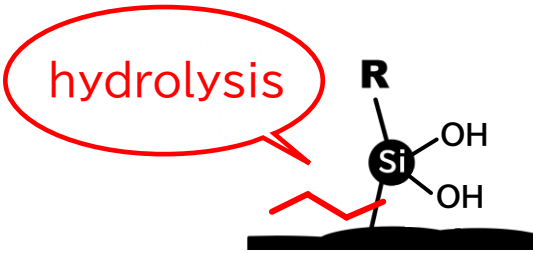
Sample :
 1. Uracil, 2. Caffeine, 3. Phenol, 4. Butylbenzene,
 5. o-Terphenyl, 6. **Amylbenzene**, 7. Triphenylene

SunShell C18 has relatively low %C, but it exhibits the **highest hydrophobicity** due to the minimal number of silanol groups.

High hydrophobicity and stability

TIC of +Q1

+Q1: 5.997 min to 7.999 min of Sample



Bleeding test using LC/MS

Column size: 50 x 2.1 mm

Flow rate: 0.4 mL/min

Mobile phase:

Temperature: 40 °C

A) 0.1% acetic acid

MS: ABI API-4000

B) CH₃CN

Ionization:

Gradient:

Turboionspray (cation)

Time: 0min 1min 5min 7min

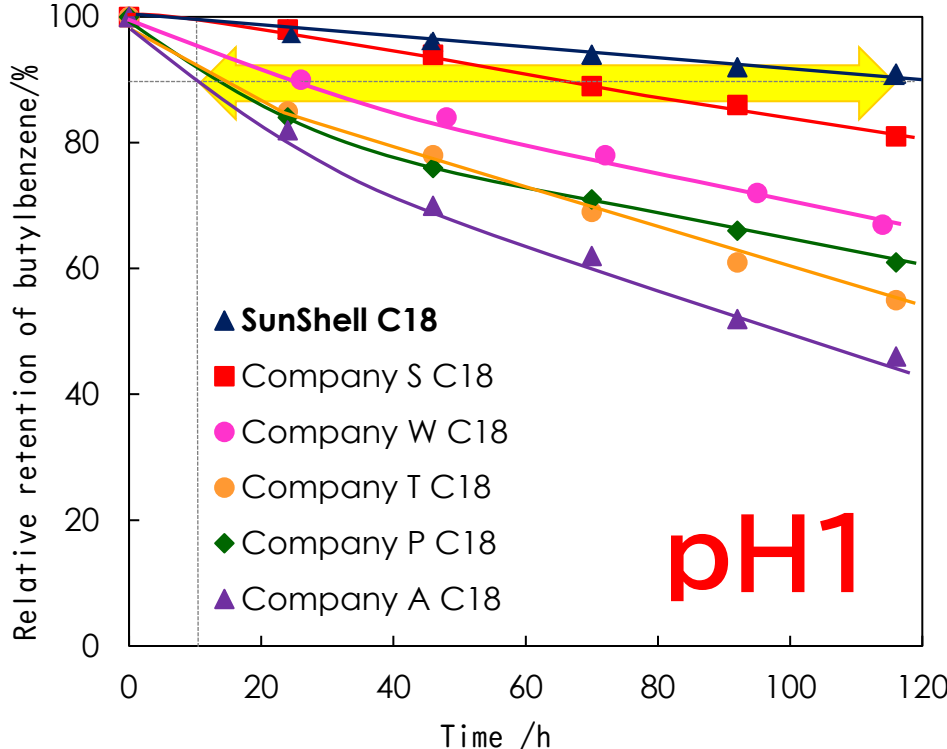
Measurement mode:

%B: 5% 5% 100% 100%

Q1 Scan m/z 100-1000

High Hydrophobization prevents hydrolysis of functional groups. SunShell improves reproducibility and S/N because of low bleeding.

Acid durability test and pH range



Column: core-shell 2.6 μm or 2.7 μm 50 x 2.1 m

Acid durability test conditions
 Mobile phase: CH₃CN/1.0% TFA (pH1) = 10/90
 Flow rate: 0.4 mL/min Temperature: 80 °C

Measurement conditions
 Mobile phase: CH₃CN/H₂O=60/40
 Flow rate: 0.4 mL/min, Temperature: 40 °C
 Sample: 1 = Uracil (t₀) 2 = Butylbenzene

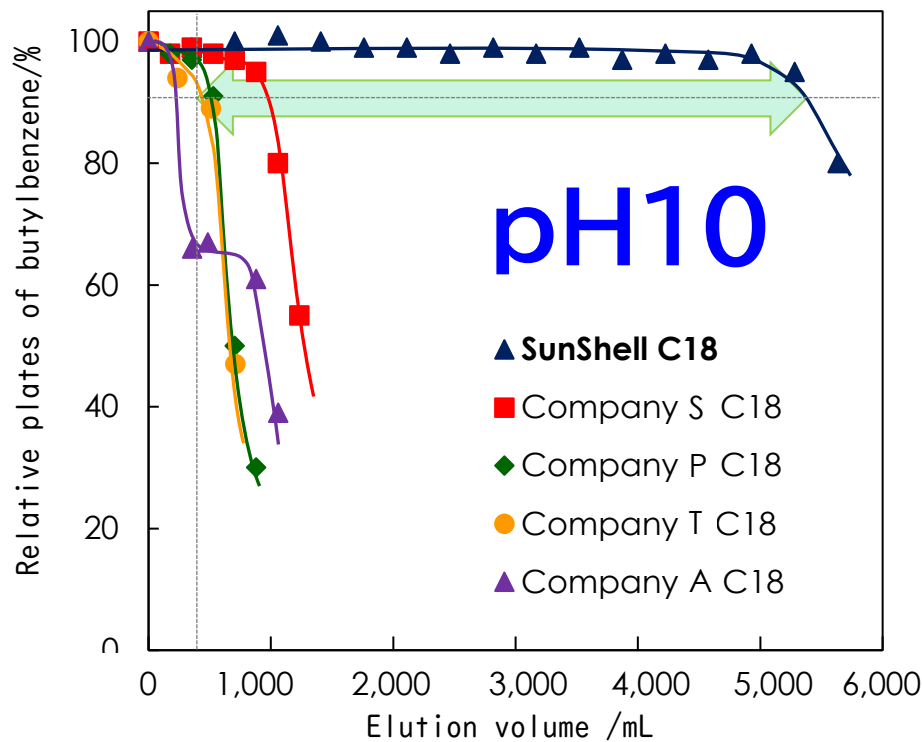
column	End-capping	pH range of use
▲ SunShell C18	Sunniest end-capping	1.5 – 10
■ Company S C18	Yes	2 – 9
● Company W C18	Yes	2 – 8
◆ Company P C18	TMS endcapping	1.5 – 10
● Company T C18	Yes	1 – 11
▲ Company A C18	Yes	2 – 9



Under acidic conditions, the functional group is cut off and relative retention decreases linearly.

SunShell showed **the least retention loss** and up to 10-fold higher stability.

Base durability test and pH range



column	End-capping	pH range of use
▲ SunShell C18	Sunniest end-capping	1.5 - 10
■ Company S C18	Yes	2 - 9
◆ Company P C18	TMS endcapping	1.5 - 10
● Company T C18	Yes	1 - 11
▲ Company A C18	Yes	2 - 9



Under basic conditions, parts of silica dissolves, a void generates and relative plates decrease.

SunShell showed **the slowest degradation** and up to 10-fold higher stability.

Column: core-shell 2.6 μm or 2.7 μm 50 x 2.1 m

Base durability test conditions
 Mobile phase: CH₃OH/20mM Sodium borate /10mM NaOH = 30/21/49 (pH10)
 Flow rate: 0.4 mL/min Temperature: 50 °C

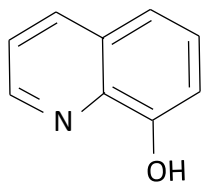
Measurement conditions
 Mobile phase: CH₃OH/H₂O=70/30
 Flow rate: 0.4 mL/min, Temperature: 40 °C
 Sample: 1 = Butylbenzene

© Inert Column hardware option

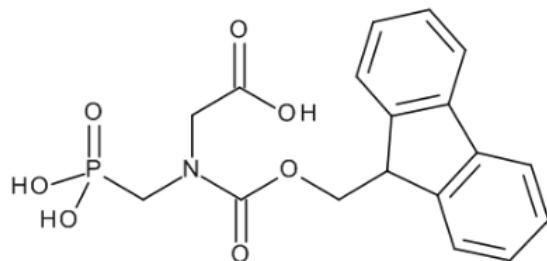


SS causes non-specific adsorption

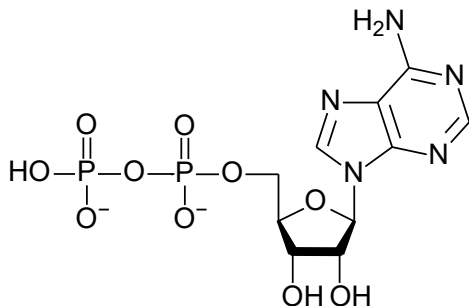
Oxine



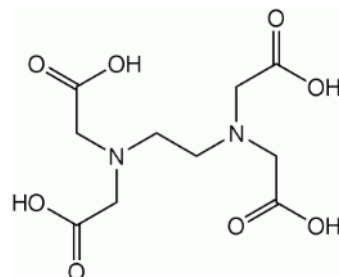
Glyphosate (- Fmoc)



ADP



EDTA

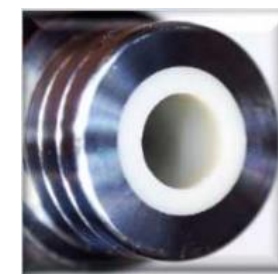


Metal chelating compounds cause low reproducibility and low sensitivity due to non-specific adsorption on SS column body.

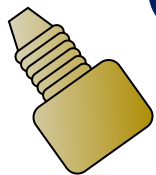
It's important to select columns that is **reduced non-specific adsorption** for LC/MS analysis.



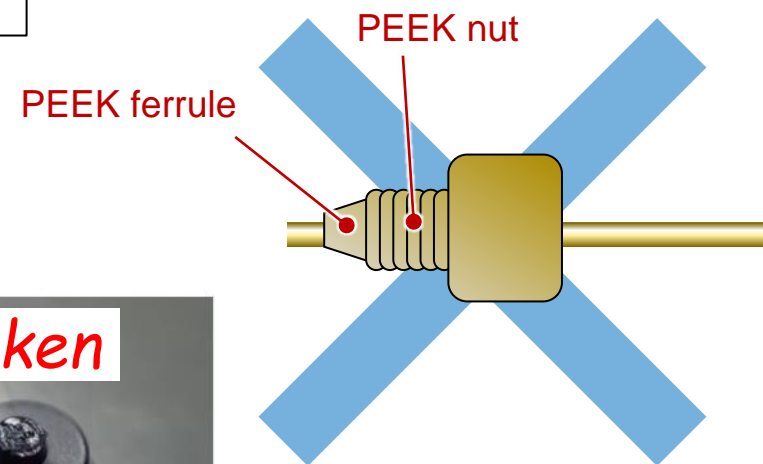
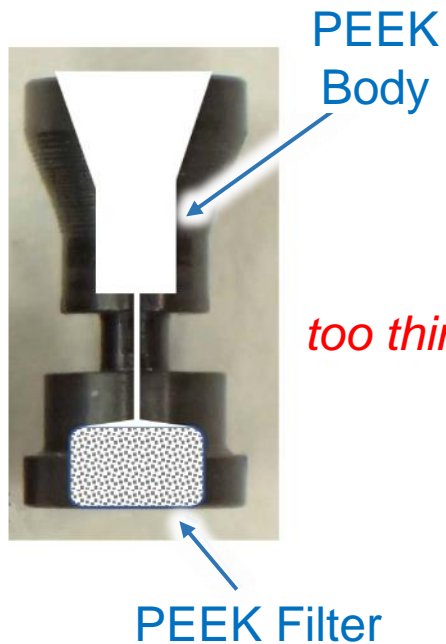
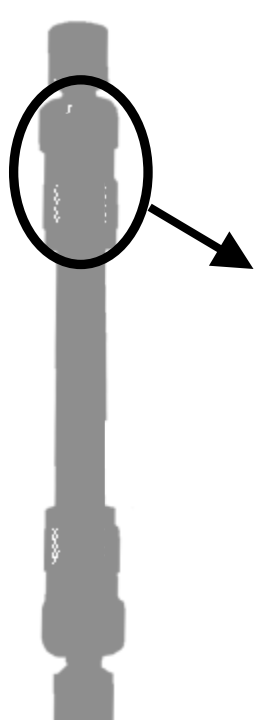
In particular, PEEK-lined stainless steel column is as known as metal-free column.



Connectable to a metal-free column?

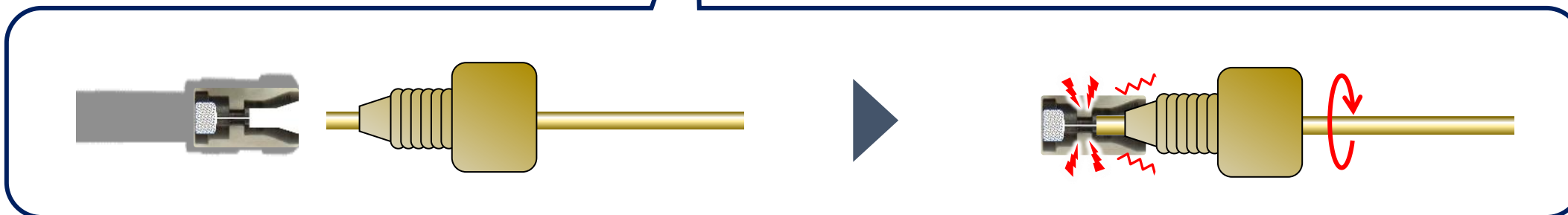


Internal structure of an old Metal-free column hardware (2.1 mm I.D.)



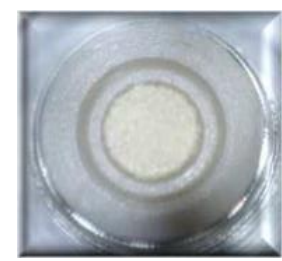
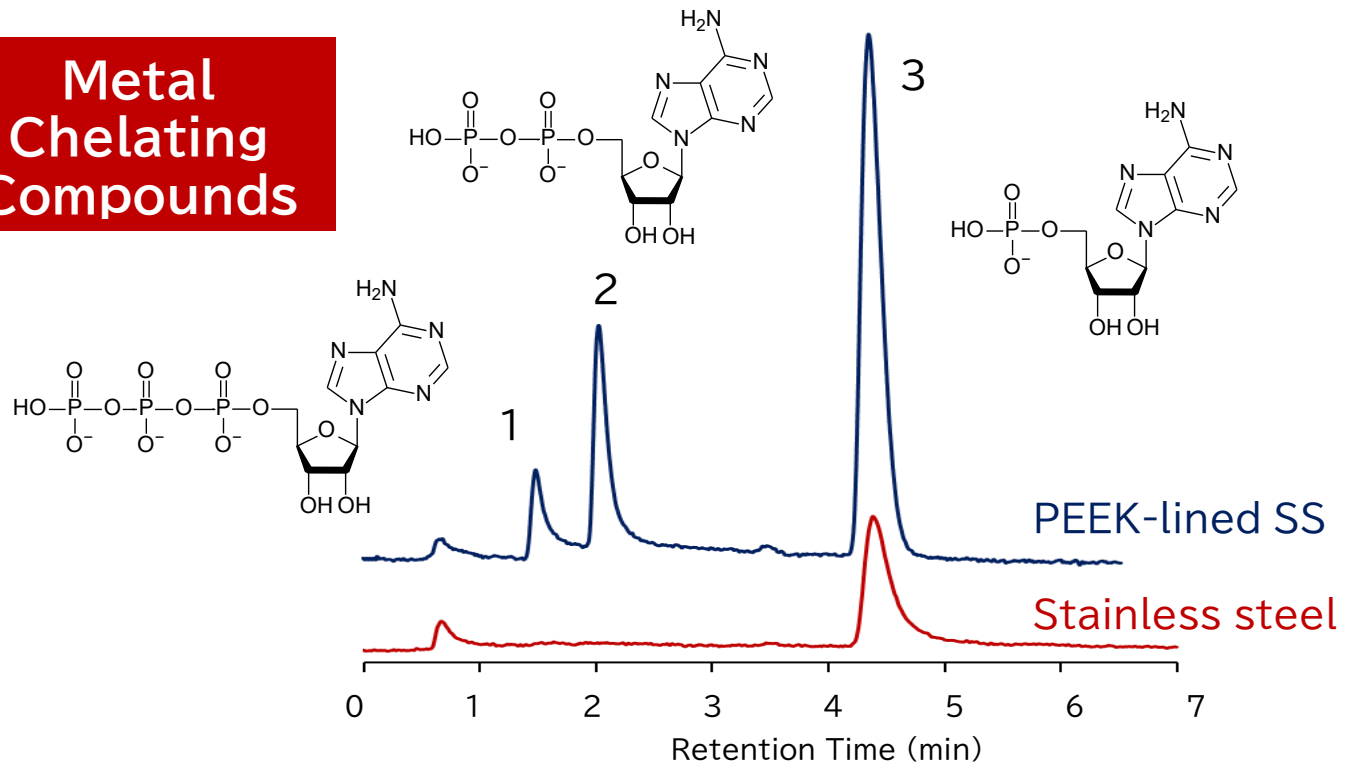
'One-piece' nut is not recommended.

When the ferrule is crimped, the body rotates with the screw and is easily broken away.

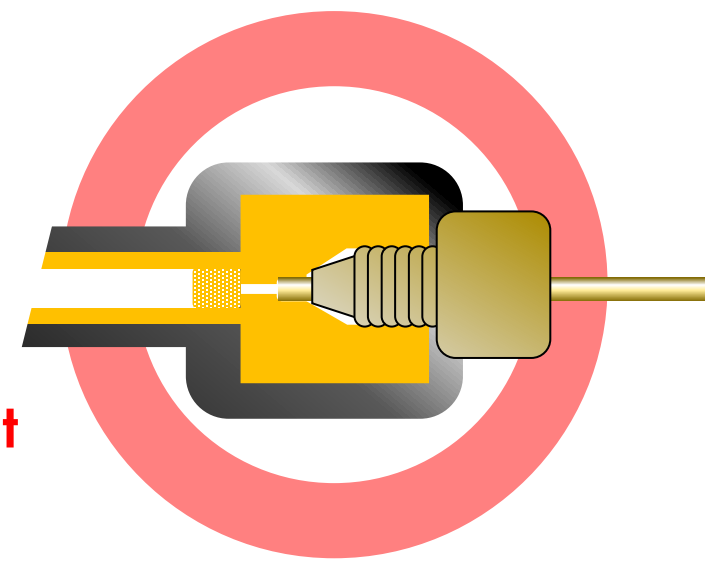


MTF – Improved metal-free column

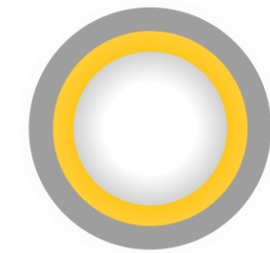
Metal Chelating Compounds



No weak point

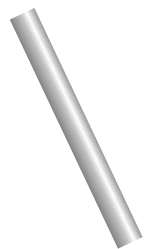


MTF



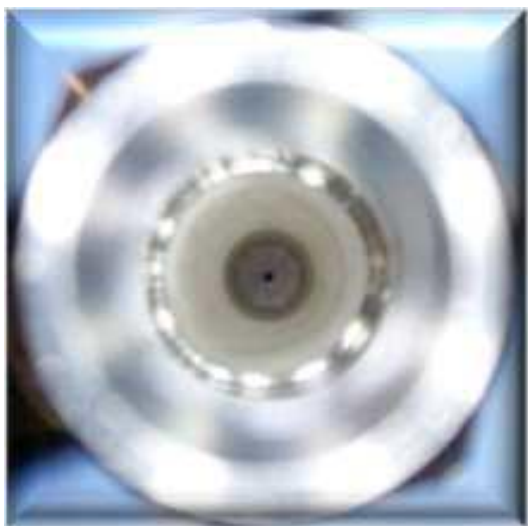
Column: Sunniest RP-AQUA 3 μ m, 50 X 2.1 mm I.D.
 Mobile phase: 10 mM HCOONH₄ Detection: UV at 250 nm
 Flow rate: 0.2 mL/min Sample: 1. 5'-ATP 2. 5'-ADP 3. 5'-AMP
 Temperature: 25 °C Injection: 1 μ L (each 25 μ g/mL)

Our metal free columns have been improved and there is **no risk of internal damage.**



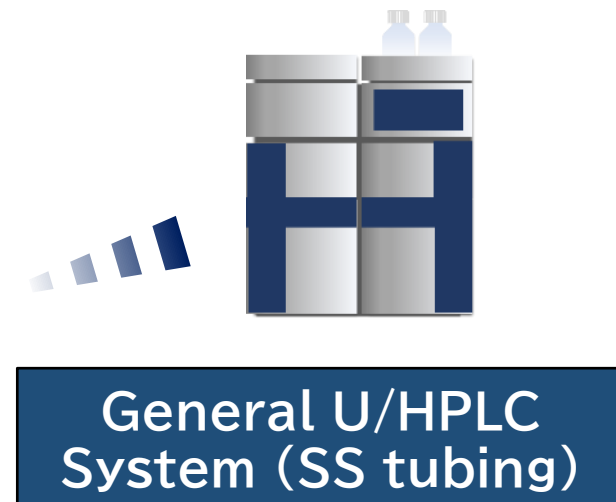
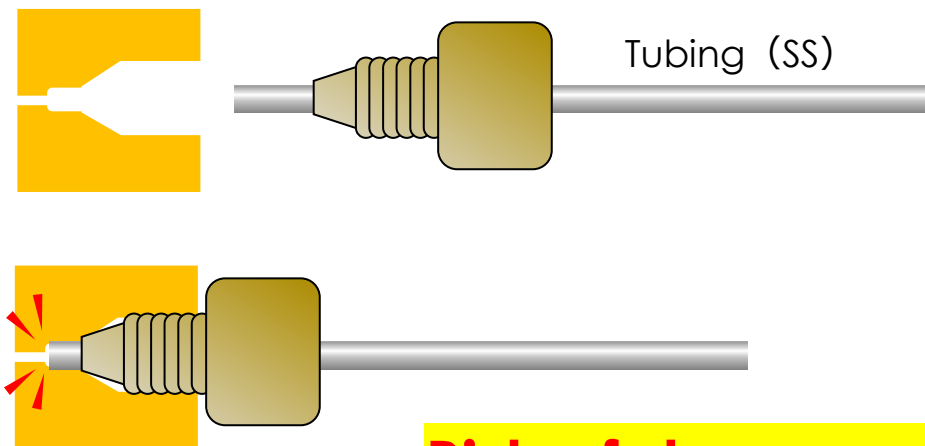
Caution when connecting SS to MTF

▶ Don't press SS tubing hard directly against PEEK parts.



Column connection of Metal-free column

Column connection (PEEK)



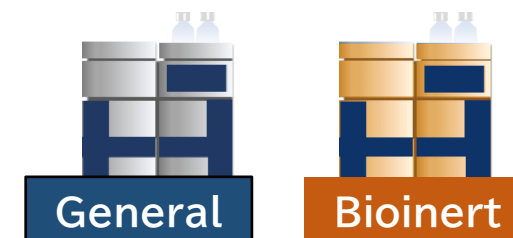
General U/HPLC System (SS tubing)

Risk of damage due to deformation

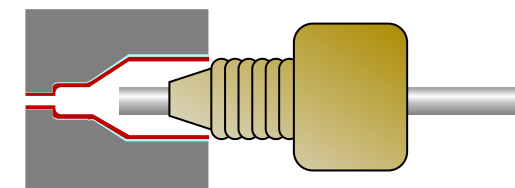
If your U/HPLC includes SS tubing, there is a better novel option.



PS inert – Novel inert hardware



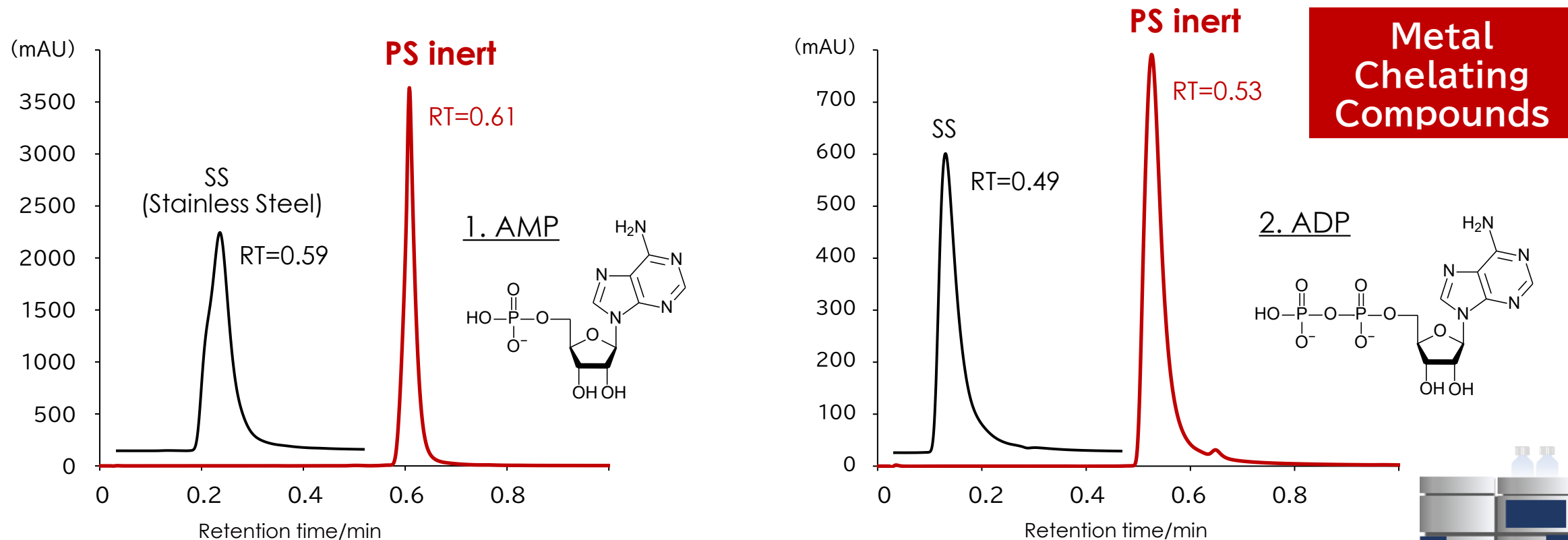
Compatible for
any U/HPLC



Prominert Surface (PS) means the organic multilayer on SS.

'PS inert' is a hydrophobic inert column hardware with PS.

Reducing non-specific adsorption

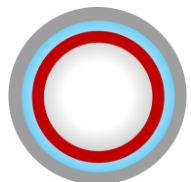


Column:
SunShell C18 2.6 μm, 100 x 2.1 mm i.d. SS / PS inert

Mobile phase:
Acetonitrile : 0.1% formic acid in Water = 1 : 99

Detection: UV@254 nm
Flow rate: 0.45 mL/min

Column Temperature: 40 °C
Injection Volume: 2 μL



Low adsorptivity & High versatility.

on HPLC (SS)

Inert column hardware line-up

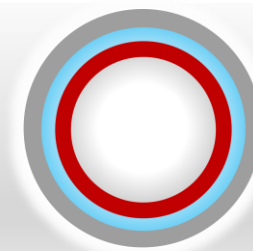
Inert option

Metal-free column



MTF

Organic inert column



PS inert

Hardware variations		
Country of manufacture	Japan	Japan
Column hardware	2.1 x 50 mm, 2.1 x 100 mm 2.1 x 150 mm, 2.1 x 250mm	2.1 x 50 mm 3.0 x 50 mm 4.6 x 50 mm 2.1 x 100 mm 3.0 x 100 mm 4.6 x 100 mm 2.1 x 150 mm 3.0 x 150 mm 4.6 x 150 mm
Compatibility	Connection by inert system (not SS) is recommended	Connection by any system (including SS) is recommended
List price* <small>*domestic price in Japan</small>	Standard column price plus ¥20,000	Standard column price plus ¥25,000
Part number (Ordering information)	□□□□□MTF (e.g. CB694MTF)	□□□□□PS (e.g. CB694PS)

When the standard part number is CB6941, add the code instead of the trailing 1.