

A Novel End-capping for Reversed Phase for LC/MS SunShell and Sunniest column



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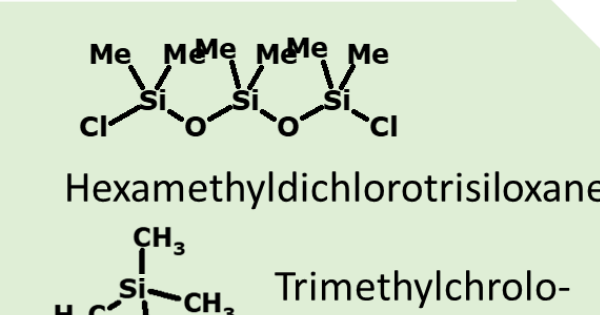


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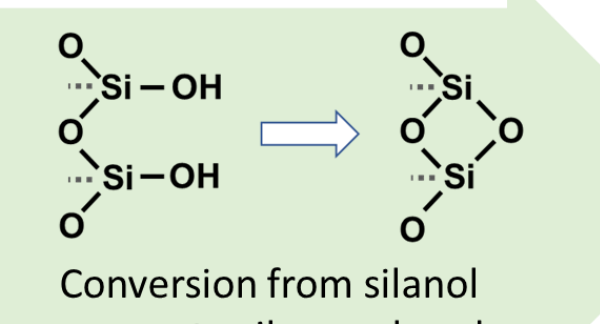
An End-capping has been recognized to be an important factor for a silica based reversed phase column. In this study, not only bonding with an end-capping reagent but also conversion of silanol groups to siloxane bond by heating were evaluated as an end-capping.

Proposed end-capping

Double end-capping



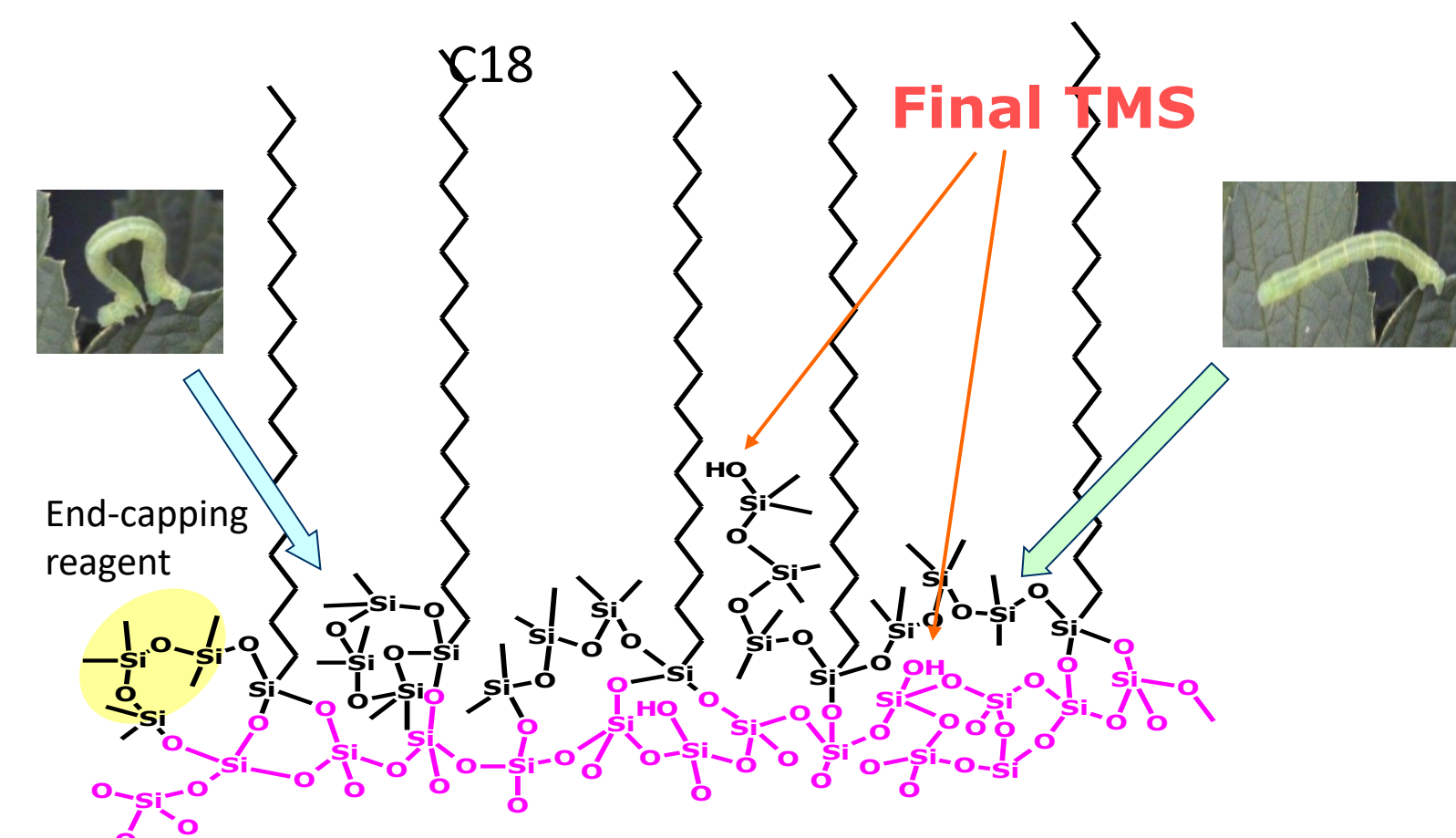
High temperature end-capping



➤ Low bleeding

➤ High stability

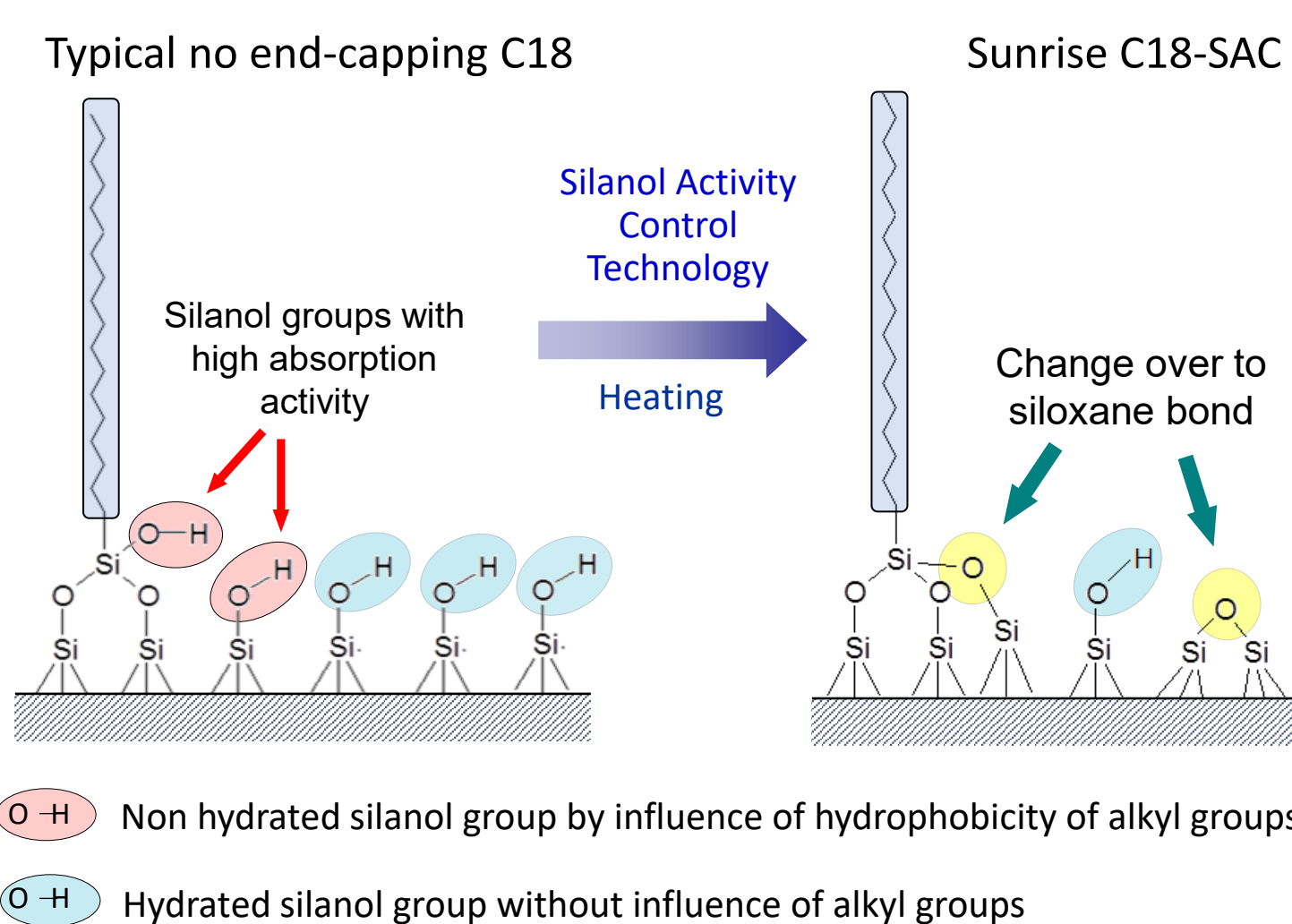
End-capping with hexamethyldichlorotrisiloxane and TMS on C18 silica



End-capping reagent 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.

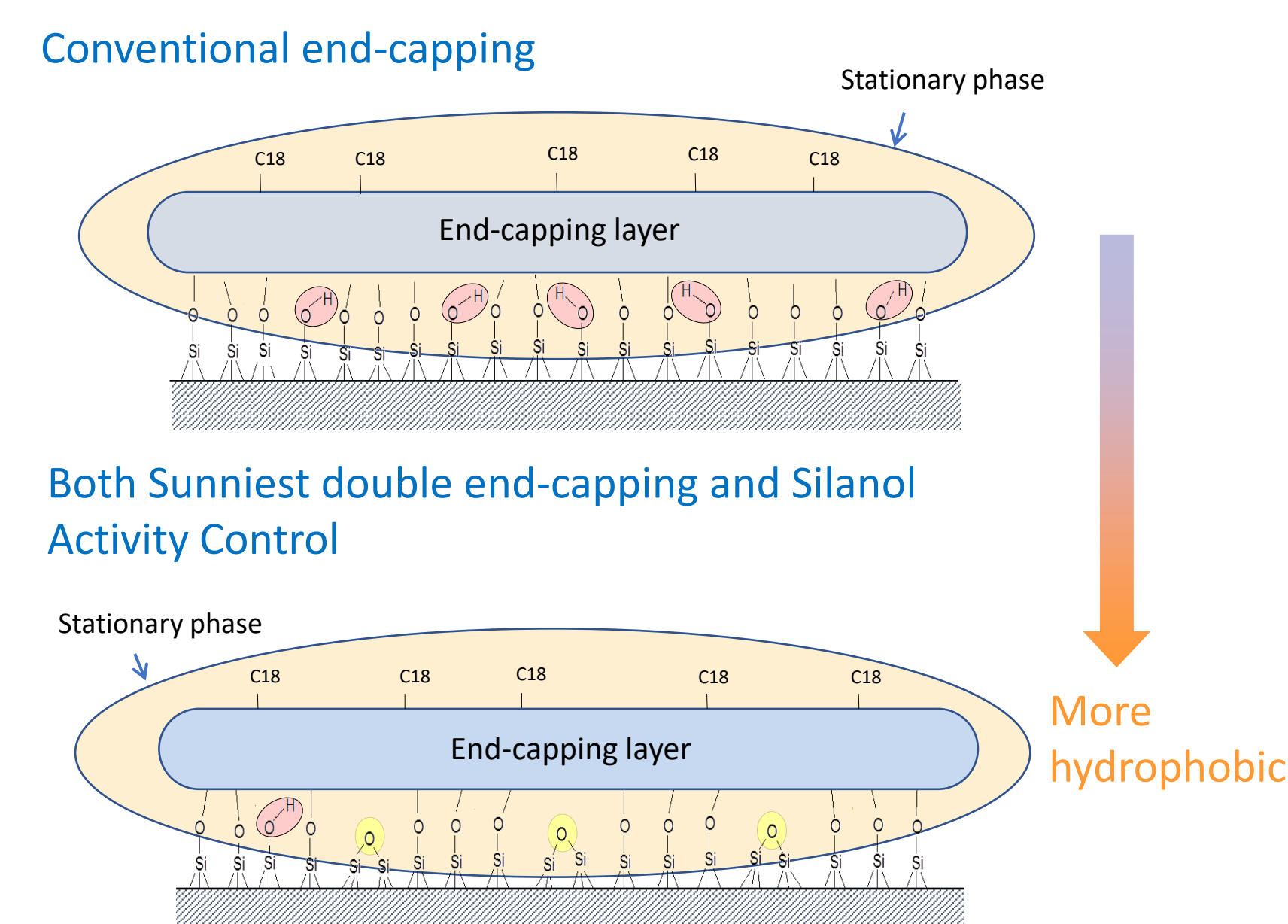
We named this end-capping method as Sunniest double end-capping.

Another end-capping with heating on C18 silica, reduce of silanol groups

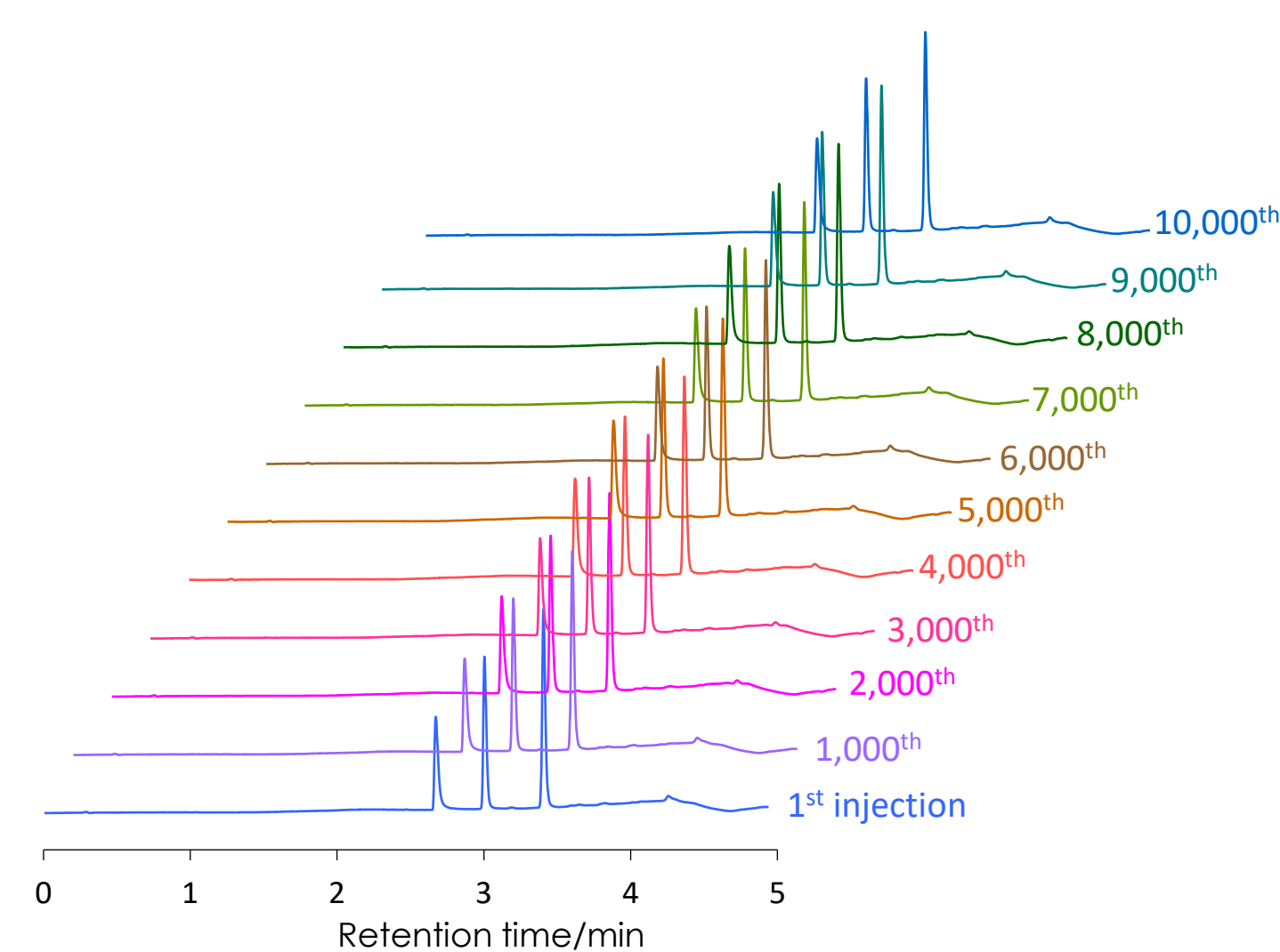


A basic compound shows no tailing on Sunrise C18-SAC because hydrated silanol groups don't make a basic compound tailing as well as silica column on HILIC mode shows no tailing for a basic compound.

Comparison of 2 kinds of end-capping



Stability under acidic pH condition

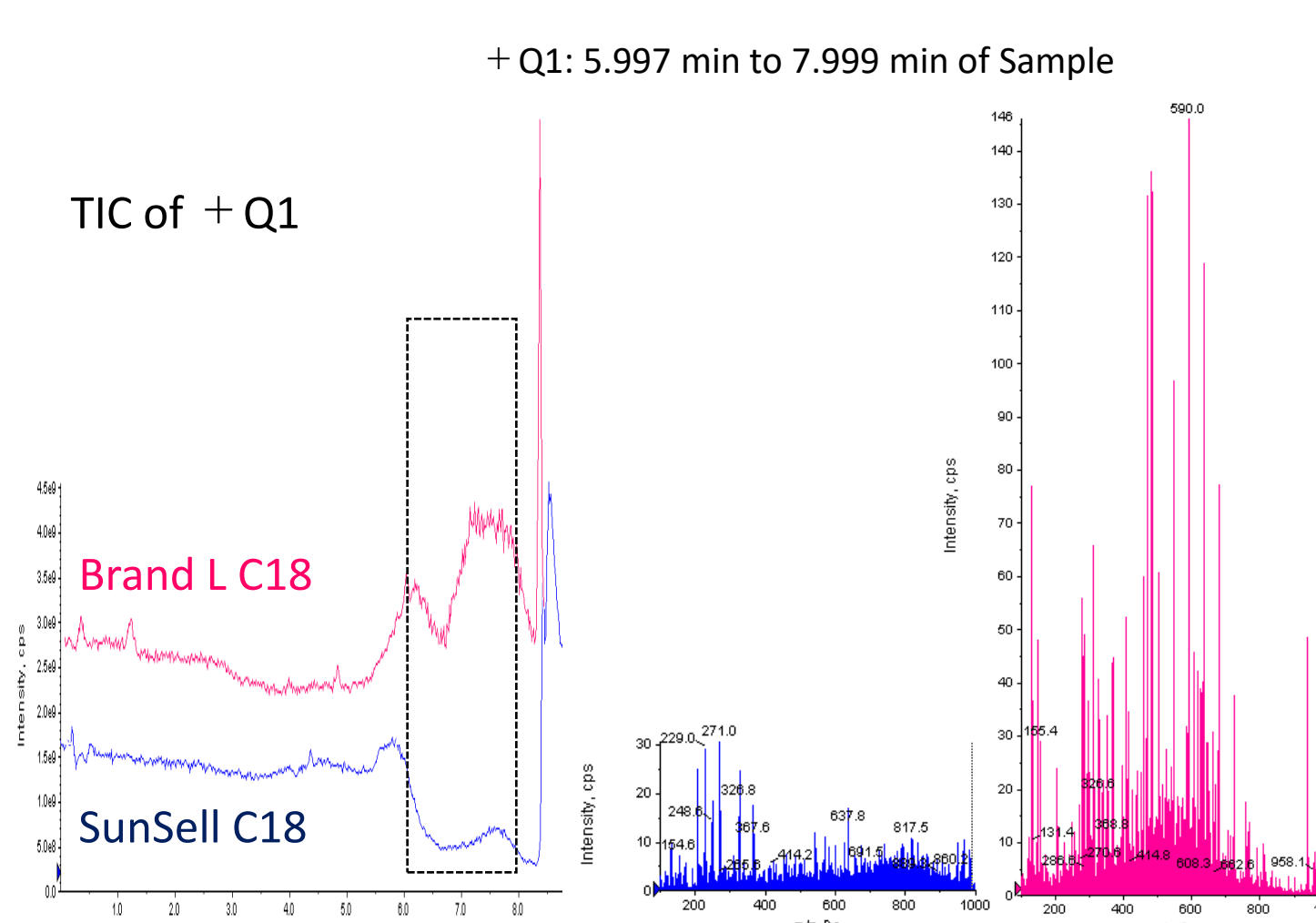


Column: SunShell C18 2.6 μ m, 50 x 2.1 mm
Mobile phase: A) 0.1% trifluoroacetic acid pH 2.0
B) Acetonitrile
Gradient program:

Time (min)	0	3	3.1	5
% B	10	90	10	10

Flow rate: 0.5 mL/min
Temperature: 40 °C
Detection: UV@270nm
Injection volume: 0.5 μ L
Sample: 1=Benzydamin (0.5 mg/mL),
2=Ketoprofen (0.04 mg/mL),
3=Indomethacin (0.05 mg/mL)

Bleeding test using LC/MS

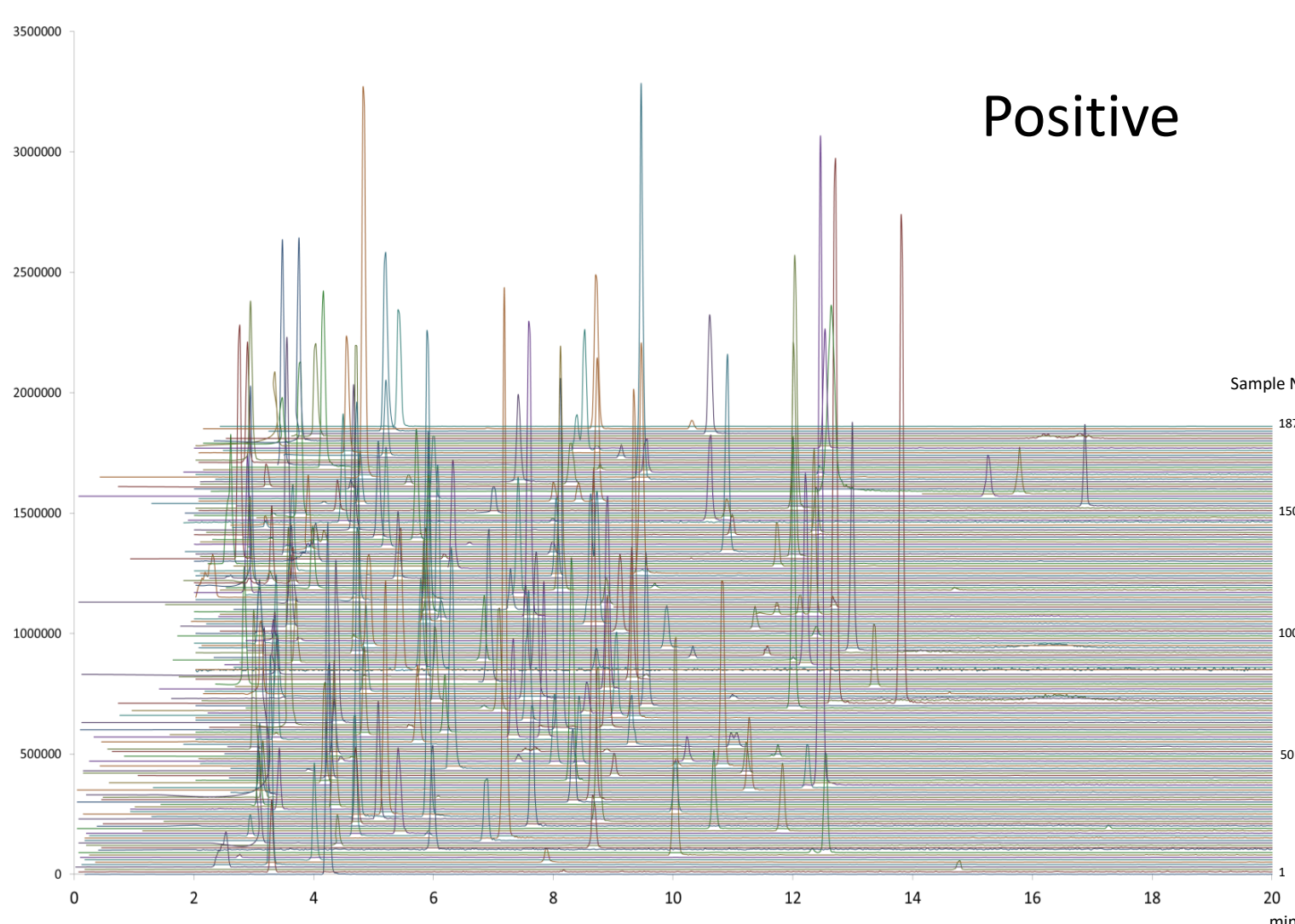


Column size: 50 x 2.1 mm
Mobile phase: A) 0.1% acetic acid
B) Acetonitrile
Gradient program:

Time (min)	0	1	5	7
% B	5	5	100	100

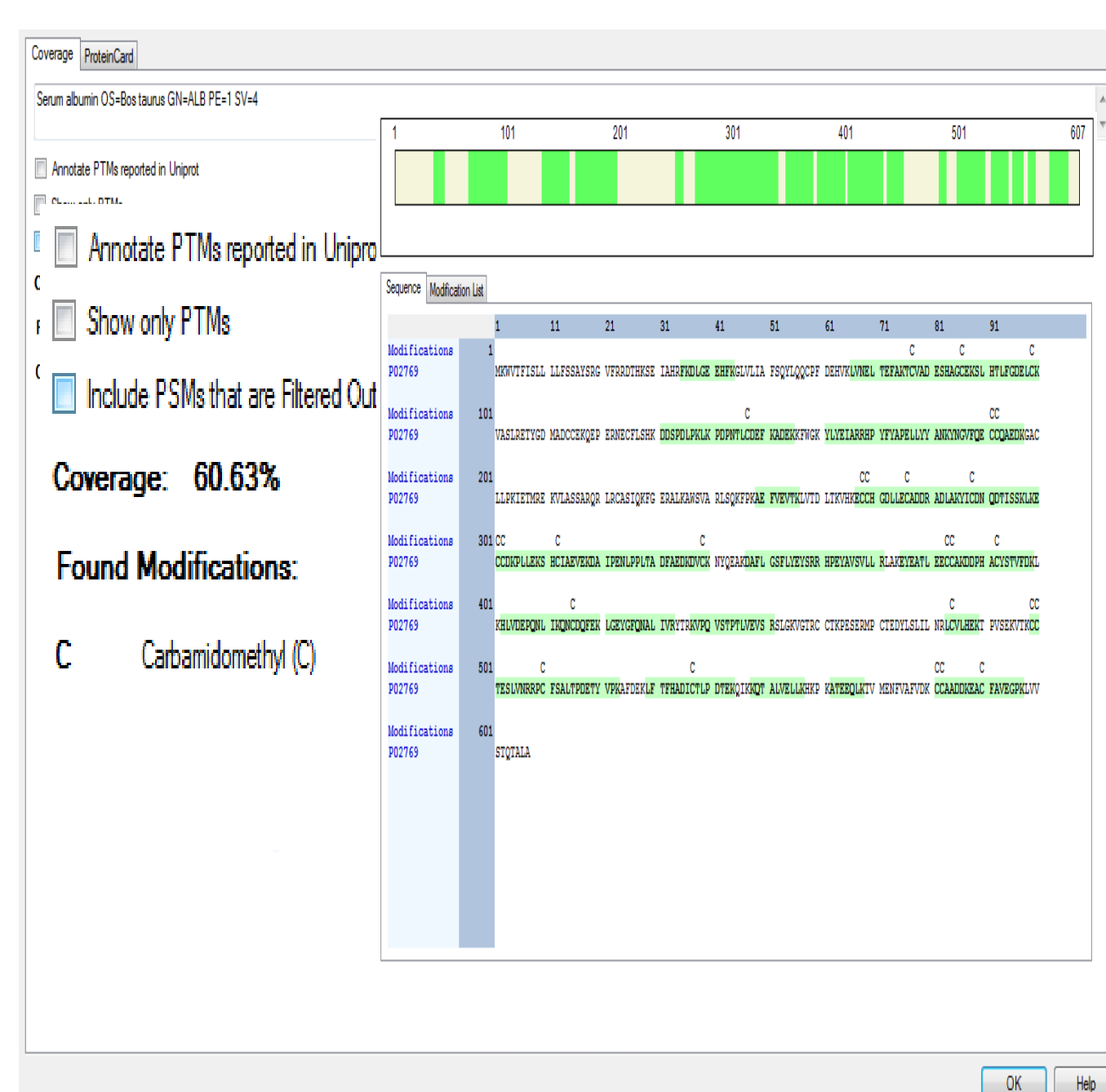
Flow rate: 0.4 mL/min
Temperature: 40 °C
MS : ABI API-4000
Ionization: Turboionspray (cation)
Measurement mode: Q1 Scan m/z 100-1000

Simultaneous Analysis of Pesticide (LC/MS)



Column: SunShell C18 2.6 μ m, 100 x 2.1 mm
Mobile phase: A) 0.5 mM Ammonium acetate in H₂O
B) 0.5 mM Ammonium acetate in CH₃OH
A/B = 95/5 – 1 min – 50/50 – 14 min – 2/98 – 5 min – 2/98 – 0.1 min – 95/5 – (Equilibrating, 10 min), v/v
Flow rate: 0.2 mL/min
Temperature: 40 °C
Detection: LC/MS/MS (QTRAP®4500: ESI, MRM)
Injection volume: 5 μ L (STD 10ppb)

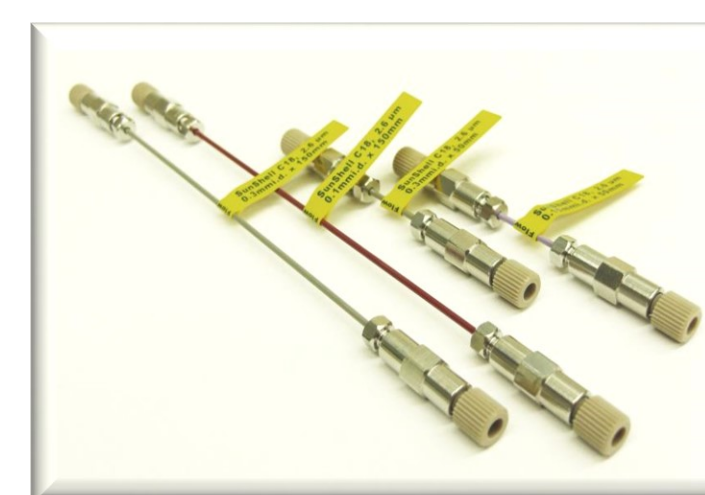
IDA measurement using SunShell C18, 2.6 μ m 150 x 0.075 mm i.d. and Nano LC/MS



After verification with the database, the sequence identification rate of BSA was over 60%, which was a higher identification rate than conventional nano-columns.

Sample: Tryptic digest of BSA, 30 μ g on column
Detection: QTRAP5500
Detection mode: IDA measurement
HPLC: Ultimate 3000 RSLC nano
Trap column: Acclaim PepMap 100, 3 μ m, 20 x 0.075 mm i.d.
Analytical column: SunShell C18, 2.6 μ m, 150 x 0.075 mm i.d.
Mobile phase: To trap column, 0.1% TFA (Sample load)
To anal. Column, A) 0.1% Formic acid, B) 0.1% Formic acid/Acetonitrile=20/80
Gradient in 25 min

Courtesy of a pharmaceutical company in Japan



Conclusion

- Hexamethyldichlorotrisiloxane was used as an end-capping reagent for a first end-capping step. Then trimethylchlorosilane (TMS) was used as an end-capping reagent for a second end-capping step.
- Silanol groups were changed to siloxane bonding by heating on C18 silica.
- Stability under acidic pH condition was improved by a proposed end-capping.