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

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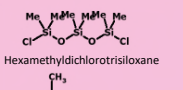
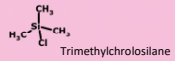
An End-capping has been recognized to be an important factor for a silica based reversed phase column. In this study, not only bonding with an end-capping reagent but also conversion of silanol groups to siloxane bond by heating were evaluated as an end-capping.

Double end-capping

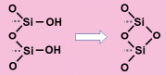


Hexamethyldichlorotrisiloxane



Trimethylchrolosilane

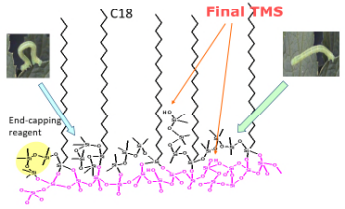
High temperature end-capping



Conversion from silanol groups to siloxane bond

- Low bleeding
- High stability

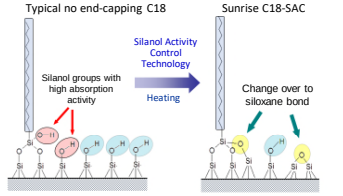
End-capping with hexamethyldichloro-trisiloxane and TMS on C18 silica



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.

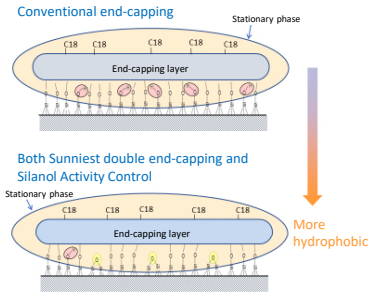
We named this end-capping method as Sunniest double end-capping.

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

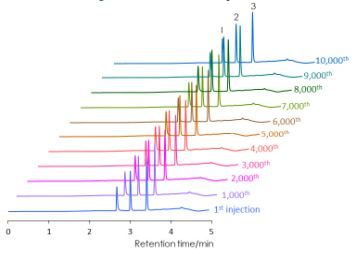


A basic compound shows no tailing on Sunrise C18-SAC because hydrated silanol groups don't make a basic compound tailing as well as silica column on HILIC mode shows no tailing for a basic compound.

Comparison of 2 kinds of end-capping



Stability under basic pH condition

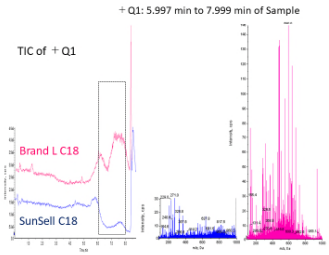


Column: SunShell C18 2.6 µm, 50 x 2.1 mm
Mobile phase: A) 0.1% trifluoroacetic acid pH 2.0
B) Acetonitrile
Gradient program:

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

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

Bleeding test using LC/MS

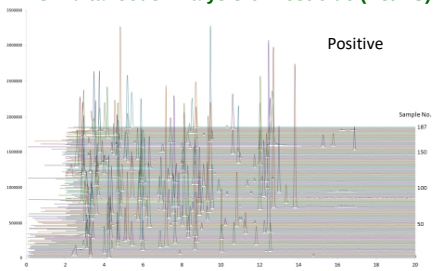


Column size: 50 x 2.1 mm
Mobile phase: A) 0.1% acetic acid
B) Acetonitrile
Gradient program:

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

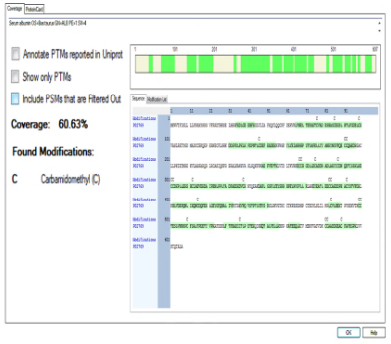
Flow rate: 0.4 mL/min
Temperature: 40 ºC
MS： ABI API-4000
Ionization: Turboionspray (cation)
Measurement mode: Q1 Scan m/z 100-1000

Simultaneous Analysis of Pesticide (LC/MS)



Column: SunShell C18 2.6 µm, 100 x 2.1 mm
Mobile phase: A) 0.5 mM Ammonium acetate in H_2O
B) 0.5 mM Ammonium acetate in CH_3OH
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 ºC
Detection: LC/MS/MS (QTRAP®4500: ESI, MRM)
Injection volume: 5 µL (STD 10ppb)

IDA measurement using SunShell C18, 2.6 µm 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 µg on column
Detection: QTRAP5500
Detection mode: IDA measurement
HPLC：Ultimate 3000 RSLC nano
Trap column：
Acclaim PepMap 100, 3 µm, 20 x 0.075 mm i.d.
Analytical column:
SunSehll C18, 2.6 µm, 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.