

A New Concept of End-capping for Reversed Phase Silica Material: Conversion of Silanol Groups to Siloxane Bond by Heating



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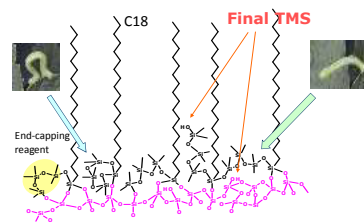


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A reversed phase column like C18 has been widely used in various fields such as pharmaceutical, medical, environmental, industrial and food area. Especially silica based C18 column has showed high performance although an end-capping must be done enough in order to decrease a peak tailing of a basic compound caused by a residual silanol group on the silica surface. An End-capping has been recognized to be an important factor for a silica based reversed phase column. In the beginning residual silanol groups after C18 bonding were reduced by bonding with trimethylsilyl reagents (TMS). This end-capping method has been improved by various end-capping reagents with not only a monofunctional group but also a difunctional group, a high reaction temperature or a long reaction time. In the past ten years some column manufacturers have developed the unique method that positive charge added on the silica surface suppressed influence of residual silanol groups against a basic compound.

In this study, not only bonding with an end-capping reagent but also conversion of silanol groups to siloxane bond by heating were tried as an end-capping. After bonding with C18 on a silica gel, a difunctional end-capping reagent like hexamethyldichlorotrisiloxane was used as a first end-capping, then trimethylchlorosilane was used as a second end-capping. These two end-capping reactions were done at over 200 degree Celsius under a nitrogen atmosphere. The C18 with the above end-capping was more hydrophobic and showed a longer retention time higher than the C18 with the above end-capping done at 40 degree Celsius lower temperature although both C18s showed a good peak shape for a basic compound. Conversion of silanol groups to siloxane bond by heating made hydrophobicity high. The peak shape of a basic compound and stability under both acidic and basic pH conditions were evaluated.

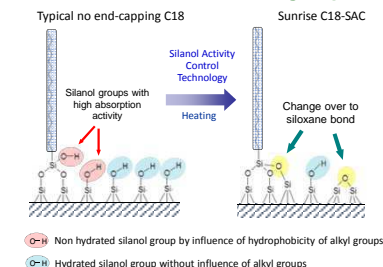
End-capping with hexamethyldichlorotrisiloxane and TMS on C18 silica



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.

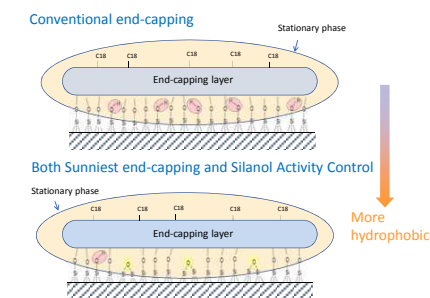
We named this end-capping method as *Sunniest end-capping*.

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



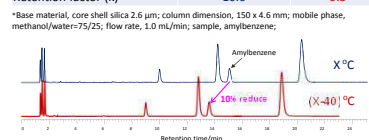
A basic compound shows no tailing on Sunrise C18-SAC because hydrated silanol groups don't make a basic compound tailing as well as silica column on HILIC mode shows no tailing for a basic compound.

Comparison of 2 kinds of end-capping



Carbon loading and retention factor (reaction at two different temperature)

End-capping reaction temperature	X °C (more than 200 °C)	(X-40) °C
Carbon loading of only C18	7.0%	7.0%
Carbon loading after end-capping	7.3% (cut off some C18 chains by heating)	7.7% (not cut off C18 chain by heating)
Silanol activity control	Yes	No
Retention factor (k)	10.6	9.5



X °C was too high to keep carbon loading of initial C18. (X-40) °C could keep carbon loading. As a result, carbon loading after end-capping at (X-40) °C was higher than that at X °C.

Regarding retention factor, a reversed result was obtained. Less silanol group made hydrophobicity of stationary phase high.

Relationship between retention factor and carbon loading

	Retention factor ^a	Carbon loading ^b (%)	Specific surface area ^{b,c} (m ² /g)
SunShell C18, 2.6 μm	10.4	7.3	125
Ascentis Express C18, 2.7 μm	9.7	8.0	133
Poroshell C18 EC, 2.7 μm	9.0	8.5	135
Cortecs C18, 2.7 μm	7.7	7.3	113
Accucore C18, 2.6 μm	7.4	8.8	130
Kinetex C18, 2.6 μm	5.4	4.9	102

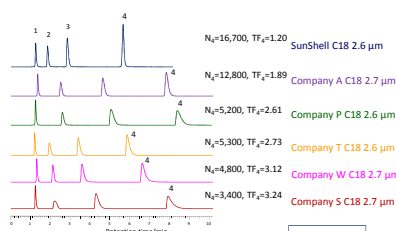
a. Retention factor of amylbenzene, mobile phase: methanol/water=75/25, 40 °C.
 b. Measured after C18 packing material was pulled out of a column.
 c. Measured using C18 materials sintered at 600 degree Celsius for 8 hours.

Both Sunniest end-capping and Silanol Activity Control

SunShell C18 showed the largest retention factor although carbon loading was not the highest.

It is considered that less residual silanol group made hydrophobicity of a stationary phase high, then made retention factor large.

Comparison of amitriptyline peak

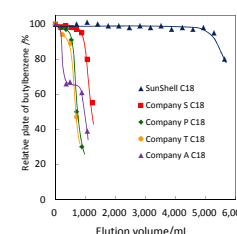


Column dimension, 150 x 4.6 mm; mobile phase, acetonitrile/10mM ammonium acetate pH6.8 = 40/60; flow rate, 1.0 mL/min; temp.: 40°C; sample, 1=uracil, 2=propranolol, 3=noritriptyline, 4=amitriptyline.



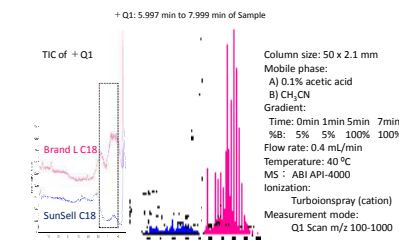
SunShell C18 showed the highest theoretical plate (N) and the lowest tailing factor. Highly-effective end-capping including heat treatment (silanol activity control) reduced tailing for a basic compound. As a consequence, high efficiency for a reversed column was achieved by highly-effective end-capping.

Stability under basic pH condition



Durable test condition
 Column size: 50 x 2.1 mm
 Mobile phase:
 CH₃OH/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:
 CH₃OH/H₂O=70/30
 Flow rate: 0.4 mL/min
 Temperature: 40 °C
 Sample: 1 = Butylbenzene

Bleeding test using LC/MS



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.
- End-capping done by both bonding of a silylation agent and conversion from silanol groups to siloxane bond led to not only high efficiency for basic compounds but also increase in retention.
- Stability under both acidic and basic pH conditions increased due to proposed end-capping.