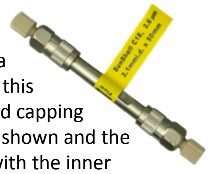
Some Hint on How to Make a Standard UHPLC Column with + 300 000 Theoretical Plates/Meter

BIOTECH AB
WWW.BIOTECH-SWEDEN.COM

Norikazu Nagae[1], Tomoyasu Tsukamoto[1], Henrik Svennberg[2]
[1] ChromaNik Technologies Inc. Namiyoke, Minato-ku, Osaka Japan
[2] Biotech AB, Innovation, Onsala, Sweden

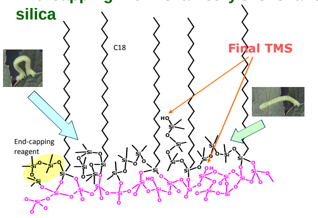
ChromaNik Technologies Inc.
www.chromanik.co.jp



The race to achieve the highest number of theoretical plate counts has been going for many years already, even though it could and should be argued that a good separation is mainly governed by good selectivity, a reasonable retention and then high column efficiency. However, the race has still been going, and this presentation will show how + 300 000 theoretical plates/meter can be achieved in a standard column for UHPLC. The importance of a good bonding and end capping technique will be discussed and hints about how this is made is revealed. The importance of base particle choice with a good heat transfer capacity will be shown and the importance of small extra column peak broadening will be emphasized. The + 300 000 theoretical plates/meter is shown in two different columns lengths with the inner diameter of 2.1 mm, 150 and 50 mm long respectively.

Effect of end-capping

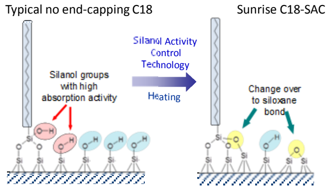
End-capping with hexamethyltrisiloxane and TMS on C18 silica



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 Sunniest end-capping.

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

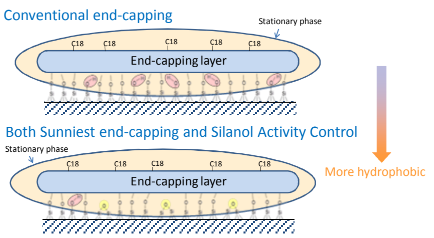


A basic compound shows no tailing on Sunrise C18-SAC because hydrated silanol groups don't make a basic compound tailing as well as silica column on HILIC mode shows no tailing for a basic compound.

O–H Non hydrated silanol group by influence of hydrophobicity of alkyl groups

O–H Hydrated silanol group without influence of alkyl groups

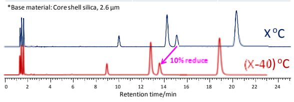
Comparison of 2 kinds of end-capping



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

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 heat)	7.7% (not cut off C18 chain by heat)
Silanol activity control	Yes	No
Retention factor (k)	10.6	9.5

*Base material: Core shell silica, 2.6 μm



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, 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

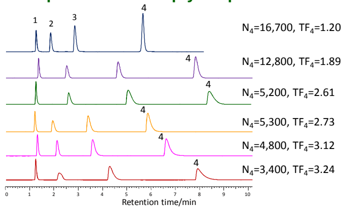
a. Retention factor of amylbenzene, mobile phase; methanol:water=75:25, 40 °C.
b. Measured after C18 packing material was pulled out of a column.
c. Measured using C18 materials sintered at 600 degree Celsius for 8 hours.

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

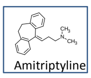
It is considered that less residual silanol group made hydrophobicity of a stationary phase high, then made retention factor large.

Both Sunniest end-capping and Silanol Activity Control

Comparison of amitriptyline peak



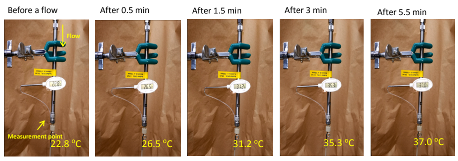
Column dimension, 150 x 4.6 mm; mobile phase, acetonitrile/10mM ammonium acetate pH6.8 = 40/60; flow rate, 1.0 mL/min; temp.: 40°C; sample, 1=uracil, 2=propranolol, 3=nortriptyline, 4=amitriptyline.

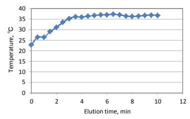


SunShell C18 showed the highest theoretical plate (N) and the lowest tailing factor. Highly-effective end-capping including heat treatment (silanol activity control) reduced tailing for a basic compound. As a consequence, high efficiency for a reversed column was achieved by highly-effective end-capping.

Effect of frictional heating

Frictional heating phenomenon

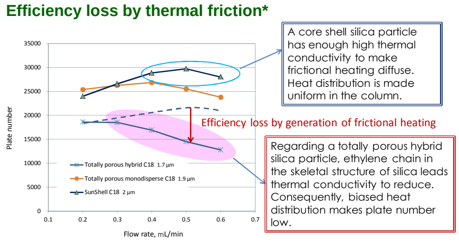




Column, core shell C18 2.6 μm, 150 x 4.6 mm; mobile phase, methanol; flow rate, 5 mL/min; column back pressure, 70 MPa; temperature, 23 °C (room temperature).

Temperature rose 14 °C at the outlet of the column by effect of frictional heating.

Efficiency loss by thermal friction*



A core shell silica particle has enough high thermal conductivity to make frictional heating diffuse. Heat distribution is made uniform in the column.

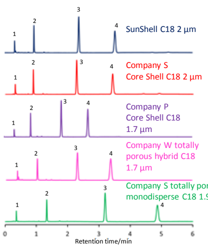
Regarding a totally porous hybrid silica particle, ethylene chain in the skeletal structure of silica leads thermal conductivity to reduce. Consequently, biased heat distribution makes plate number low.

Column dimension, 100 x 2.1 mm; mobile phase, CH_3CN/H_2O=60/40; temperature, 40 °C; sample, acenaphthene; UHPLC instrument, X-LC with 0.1 mm i.d. connecting tube (JASCO).

*Fabrice Gritti, Georges Guiochon, J. Chromatogr. A 1217 (2010) 5069.

A part of abstract
This unexpected result is accounted for by the three times smaller heat conductivity of the BEH bed (BEH 0.25 W/m/K) than that of the Kinetex bed (Kinetex 0.75 W/m/K).

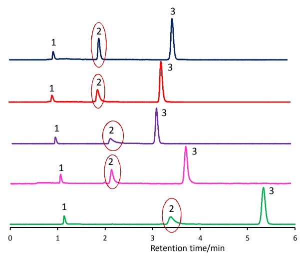
Efficiency and back pressure



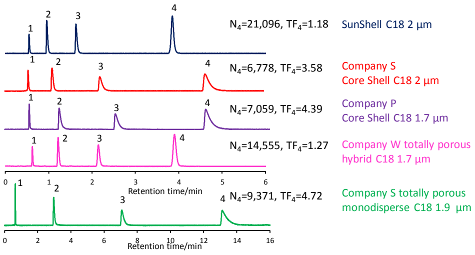
	Acenaphthene		Pressure (MPa)	Theoretical plate /pressure (MPa^{-1})
	Theoretical plate	Tailing factor		
SunShell C18	29,721	1.04	48.5	613
Company S Core Shell C18	25,533	1.19	53.5	477
Company P Core Shell C18	24,700	0.97	53.8	458
Company W totally porous hybrid C18	14,511	1.01	54.1	269
Company S totally porous monodisperse C18	26,592	1.05	43.9	605

Column, SunShell C18 2 μm 100 x 2.1 mm, company S core shell C18 2 μm 100 x 2.1 mm, company P core shell C18 1.7 μm 100 x 2.1 mm, company W C18 1.7 μm 100 x 2.1 mm, company S monodisperse C18 1.9 μm 100 x 2.1 mm; mobile phase, CH_3CN/H_2O=60/40; flow rate, 0.5 mL/min; temperature, 40 °C; sample, 1=uracil, 2=ethylbenzoate, 3=acenaphthene, 4=butylbenzene; UHPLC instrument, X-LC with 0.1 mm i.d. connecting tube (JASCO).

Peak shape and efficiency of pyridine and amitriptyline



Column dimension, 100 x 2.1 mm; mobile phase, methanol/water = 30/70; flow rate, 0.2 mL/min; temperature: 40 °C; sample, 1=uracil, 2=pyridine, 3=phenol; UHPLC instrument, X-LC with 0.1 mm i.d. connecting tube (JASCO).



Column dimension, 100 x 2.1 mm; mobile phase, acetonitrile/10mM ammonium acetate pH6.8 = 40/60; flow rate, 0.3 mL/min; temperature, 40°C; sample, 1=uracil, 2=propranolol, 3=nortriptyline, 4=amitriptyline; UHPLC instrument, X-LC with 0.1 mm i.d. connecting tube (JASCO).

Influence of frictional heating is most difficult to undergo for a core shell silica. When using a fine particle such as 2 μm or smaller than 2 μm C18 packings under high pressure condition, a core shell C18 with dense end-capping showed the highest efficiency for not only neutral compounds but also basic compounds.

Effect of extra-column volume

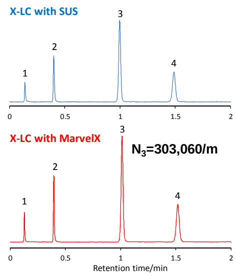
Comparison of an inner diameter of tubing in the UHPLC instrument

Connecting tube used

Instrument	Injector to column	Column to flow cell	Tube volume
X-LC with SUS	SUS 0.1 mm i.d. x 300 mm	PeekSil 0.1 mm i.d. x 200 mm	3.93 μL
X-LC with MarvelX	MarvelX 0.075 mm i.d. x 350 mm	MarvelX 0.075 mm i.d. x 150 mm	2.21 μL

*X-LC, is a UHPLC instrument manufactured by Jasco. *MarvelX is a connecting tube manufactured by IDEX.

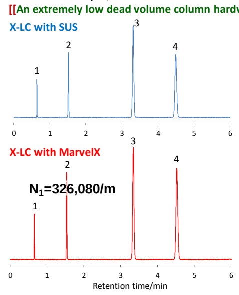
SunShell C18 2 μm, 50 x 2.1 mm



Measurement condition
Column, SunShell C18, 2 μm 50 x 2.1 mm; mobile phase, CH_3CN/H_2O=60/40; flow rate, 0.6 mL/min; temperature, RT; detection, UV@250 nm; injection volume, 0.4 μL; sample, 1=uracil, 2=ethylbenzene, 3=acenaphthene, 4=butylbenzene.

	Peak No.	SUS	Marvel X	
Efficiency	1	1208	3593	197% up
	2	7720	12625	64% up
	3	13589	15153	12% up
	4	13936	14733	6% up
Tailing factor	1	2.326	1.445	
	2	1.401	1.286	
	3	1.048	1.006	
	4	0.997	0.972	
Peak width, $h_{0.5}$ (min)	1	0.0094	0.0051	
	2	0.0107	0.0083	
	3	0.0201	0.0194	
	4	0.0297	0.0295	

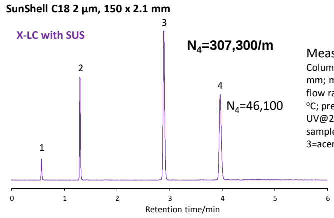
SunShell C18 2.6 μm, 150 x 4.6 mm
[[An extremely low dead volume column hardware manufactured by Tomoe Works was used.]]



Measurement condition
Column, SunShell C18, 2.6 μm 150 x 4.6 mm; mobile phase, CH_3CN/H_2O=70/40; flow rate, 1.8 mL/min; temperature, RT; detection, UV@250 nm; injection volume, 0.4 μL; sample, 1=uracil, 2=ethylbenzene, 3=acenaphthene, 4=butylbenzene.

	Peak No.	SUS	Marvel X	
Efficiency	1	41900	48912	17% up
	2	42934	44037	2.7% up
	3	38989	40899	4.9% up
	4	38125	39947	4.8% up
Tailing factor	1	1.176	1.207	
	2	1.015	1.024	
	3	1.001	1.020	
	4	1.048	1.090	
Peak width, $h_{0.5}$ (min)	1	0.0074	0.0070	
	2	0.0172	0.0170	
	3	0.0395	0.0390	
	4	0.0542	0.0533	

SunShell C18 2 μm, 150 x 2.1 mm



Measurement condition
Column, SunShell C18, 2 μm 150 x 2.1 mm; mobile phase, CH_3CN/H_2O=70/30; flow rate, 0.4 mL/min; temperature, 25 °C; pressure, 64 MPa; detection, UV@250 nm; injection volume, 0.4 μL; sample, 1=uracil, 2=ethylbenzene, 3=acenaphthene, 4=butylbenzene.

Conclusion

- End-capping done by both bonding of a silylation agent and conversion from silanol groups to siloxane bond leaded to not only high efficiency for basic compounds but also increase in retention.
- Serious losses in column efficiency was caused by frictional heating effects when very fine particles were packed into a column and it was operated at high back pressure. A core shell particle showed the least losses in column efficiency, while a totally porous hybrid particle showed large losses.
- Effect of extra-column volume is important for a column packed with very fine particles. When 0.075 mm i.d. connecting tubes were used, SunShell C18 2 μm column showed + 300,000 theoretical plates/meter.
- In case of a low dead volume column sized 4.6 mm i.d. and 150 mm length, uracil peak showed + 300,000 theoretical plates/meter even if a particle size was 2.6 μm.
- In case of 150 mm length column, efficiency was + 300,000 theoretical plates/meter even if 0.1 mm i.d. connecting tubes were used.