A Novel End-capping Concept for High Performance Liquid Chromatography

高效液相色谱的新型封端概念

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Importance of End-capping for Reversed Phase LC

反相LC色谱柱封端的重要性

Stationary Phase 固定相

C18 (ODS)

C8

C4

TMS（三甲基氯硅烷）

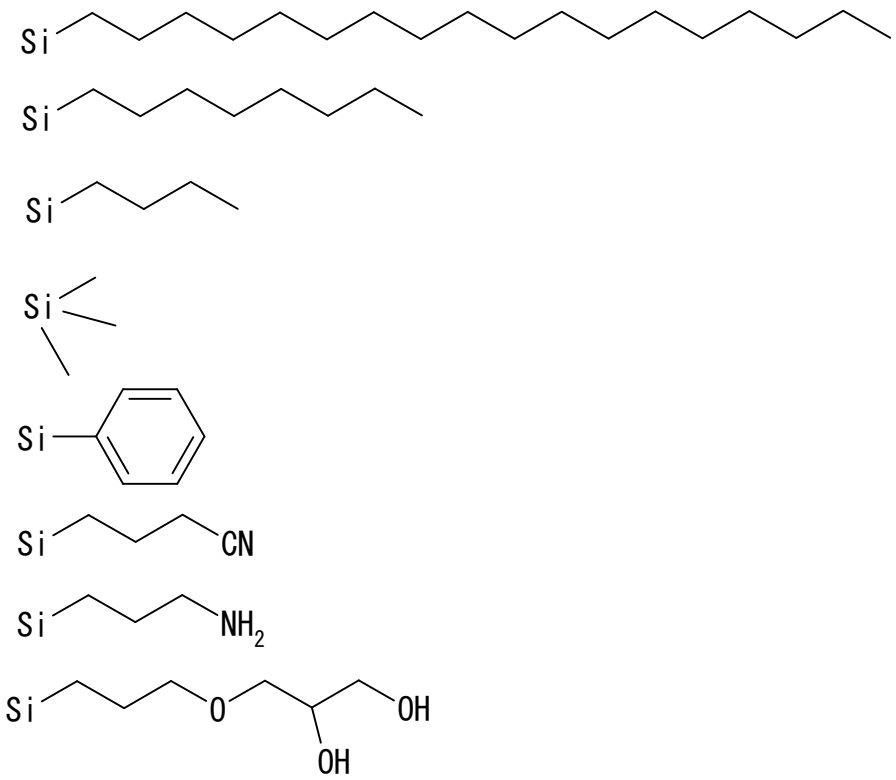
Ph（苯基）

CN（氰基丙基）

NH2（氨基丙基）

Diol（二醇基）

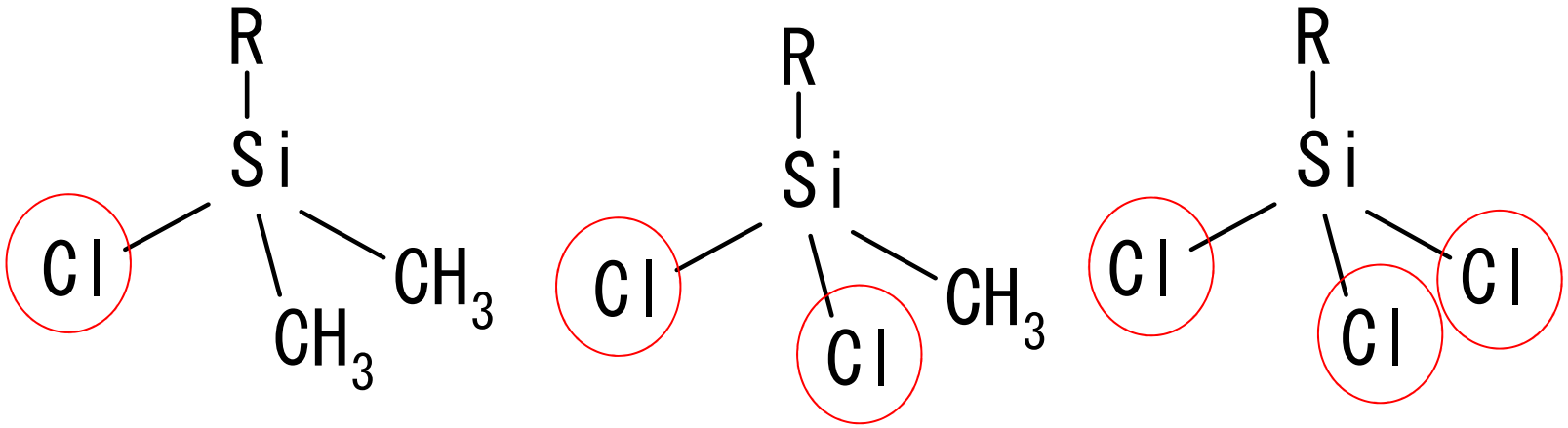
C30



Functionality of reagent(mono, di, tri)
试剂的功能

甲硅烷基化试剂的类型

单官能团	双官能团	三官能团
Monofuntional	Difuntional	Trifuntional



Cl与二氧化硅表面上的Si-OH和Si-O-Si硅氧烷键反应

然而，三官能团试剂在所有三个点位都不能结合硅氧烷

ODS（C18）

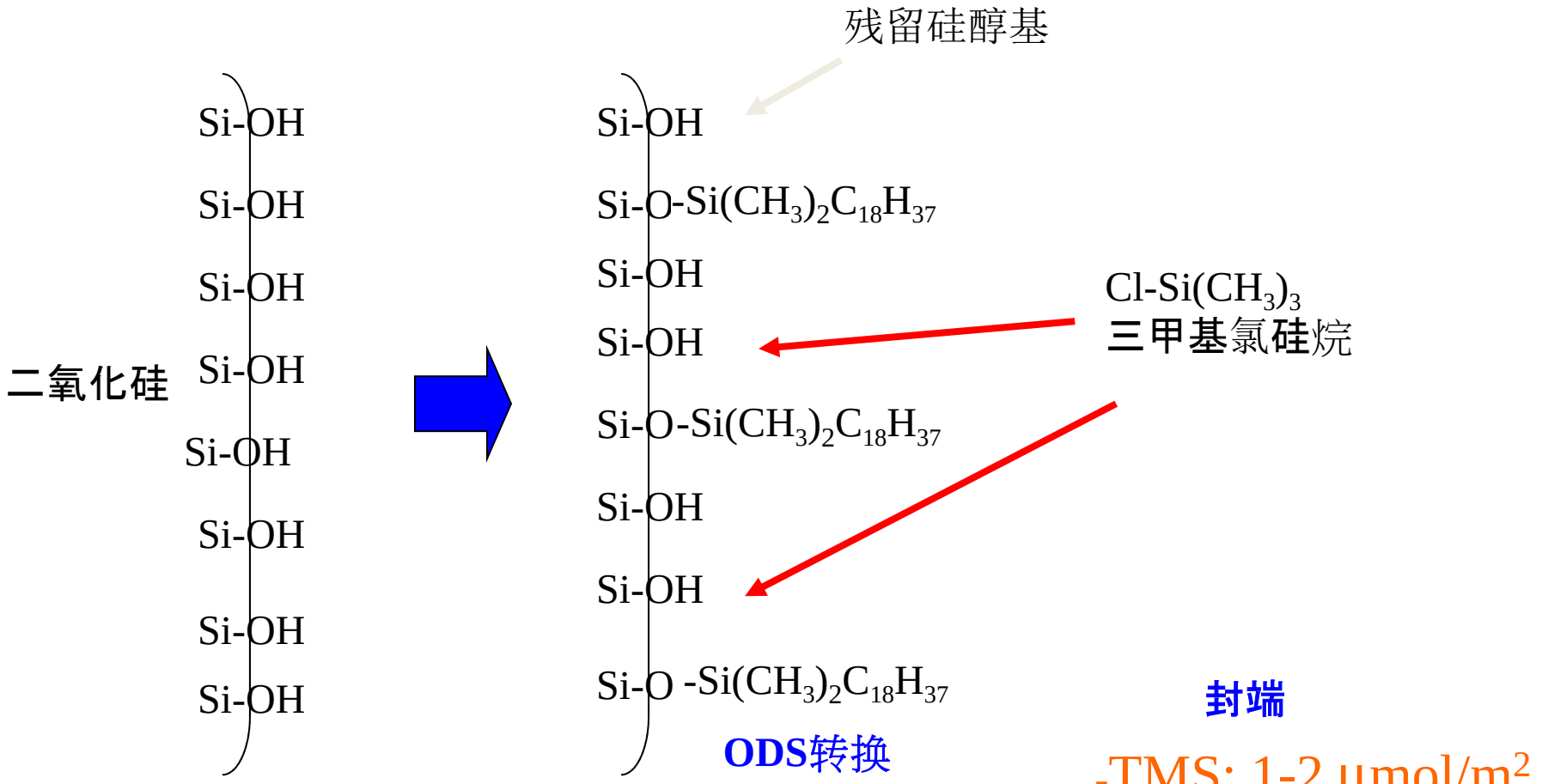
C18（十八烷基甲硅烷基ODS）与硅胶表面结合

$\equiv Si\text{-}OH + Cl\text{-}Si(CH_3)_2C_{18}H_{37}$

（二氧化硅表面）

$\equiv Si\text{-}O\text{-}Si(CH_3)_2C_{18}H_{37} + HCl$

End-capping 封端



Ratio of residual silanol group
残留硅醇基的比例

二氧化硅表面的硅醇基的量为8～9μmol/ m^2

C18键合后，仍有2/3硅醇基残留

C18键**合密度**～3 μmol/m^2

作为顶尖的封端技术，C18键合和TMS等封端后

1980年代　1/2

1990年代　2/5

2000年代　1/3　　**硅烷醇基残留**

1/3残留硅醇基的作用几乎被封端剂阻断

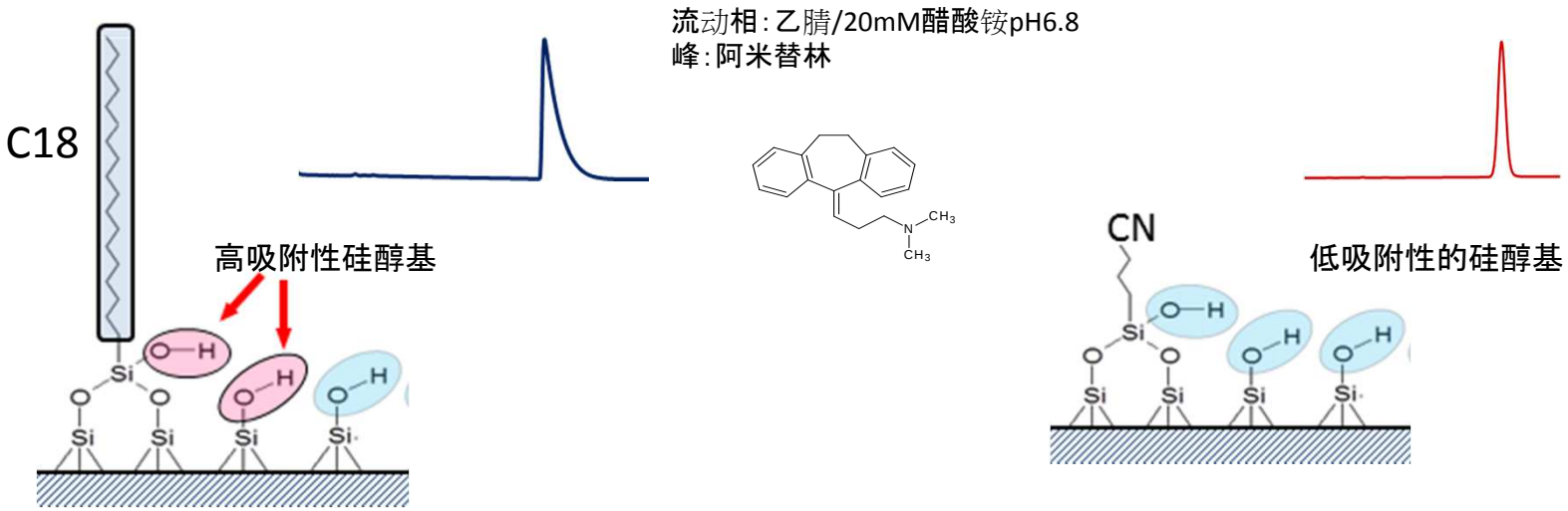
Interaction of silanol group
硅醇基的作用

1. Adsorption 吸附　　Si-OH
2. Ion exchange 离子交换　Si-O^-　阳离子(-N^+)
3. Ion exclusion 离子排斥　Si-O^-　阴离子(-COO^-)
4. Hydrogen bond 氢键　　Si-OH

Problems with unhydrated silanol group
未水合的硅醇基所引起的问题

对于反相如C18，碱性化合物拖尾起因于硅醇基

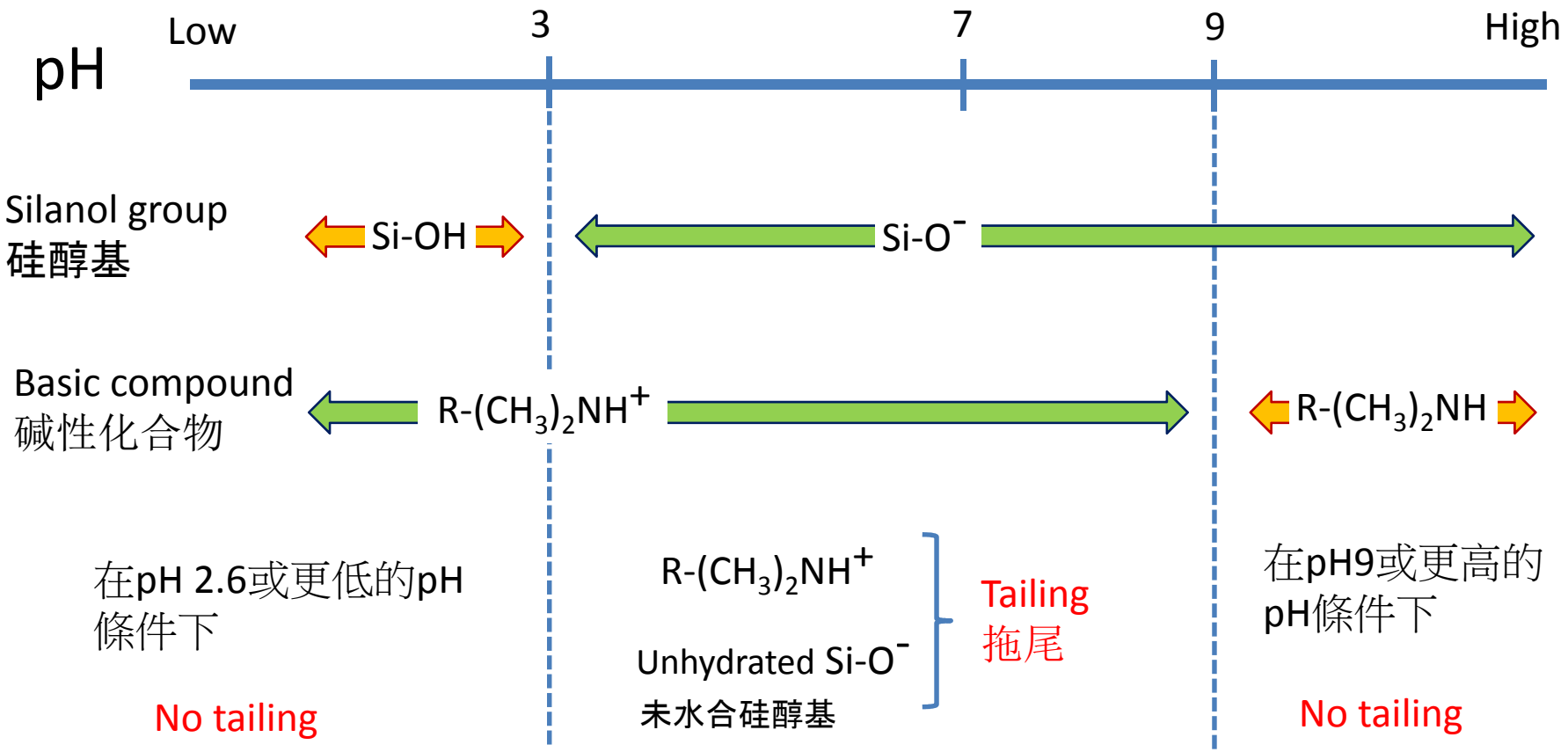
在Cyano（CN）等的反相中，碱性化合物不因硅醇基而拖尾



O–H 在由有机溶剂和水组成的流动相中，由于疏水基团(C18)的影响，难以水合的硅醇基

O–H 较少受疏水基团影响并且充分水化的硅醇基

How tailing occurs
拖尾如何发生



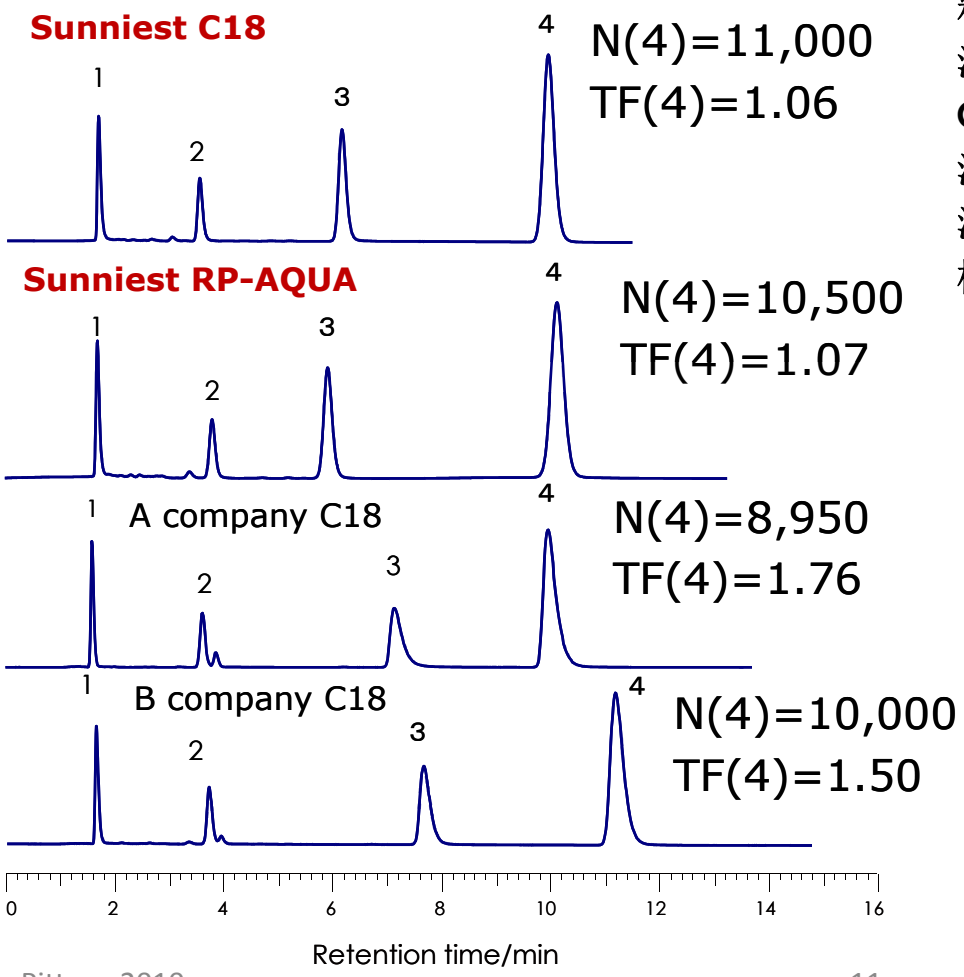
The difference between acetonitrile and methanol
乙腈与甲醇的差异

Acetonitrile 乙腈: 氢键相互作用弱，
硅醇基的影响较大

Methanol 甲醇: 氢键相互作用强，
将甲醇放置在硅烷醇基团周围.
硅醇基的影响降低

Comparison of amitriptyline peak I
阿米替林峰的比较

CH_3OH,甲醇,pH7.5, 40 ℃



柱尺寸：**150×4.6mm**

粒径：**5微米**

流动相：

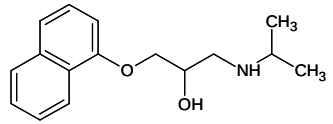
CH3OH / 20mM磷酸盐缓冲液pH7.5 = 80/20

流量：**1.0mL / min**

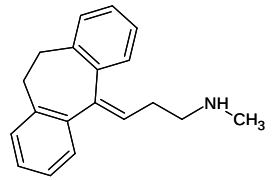
温度：**40℃**

样品：**1 =尿嘧啶**

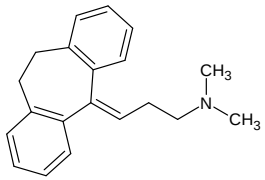
2 =普萘洛尔



3 = Nortriptyline

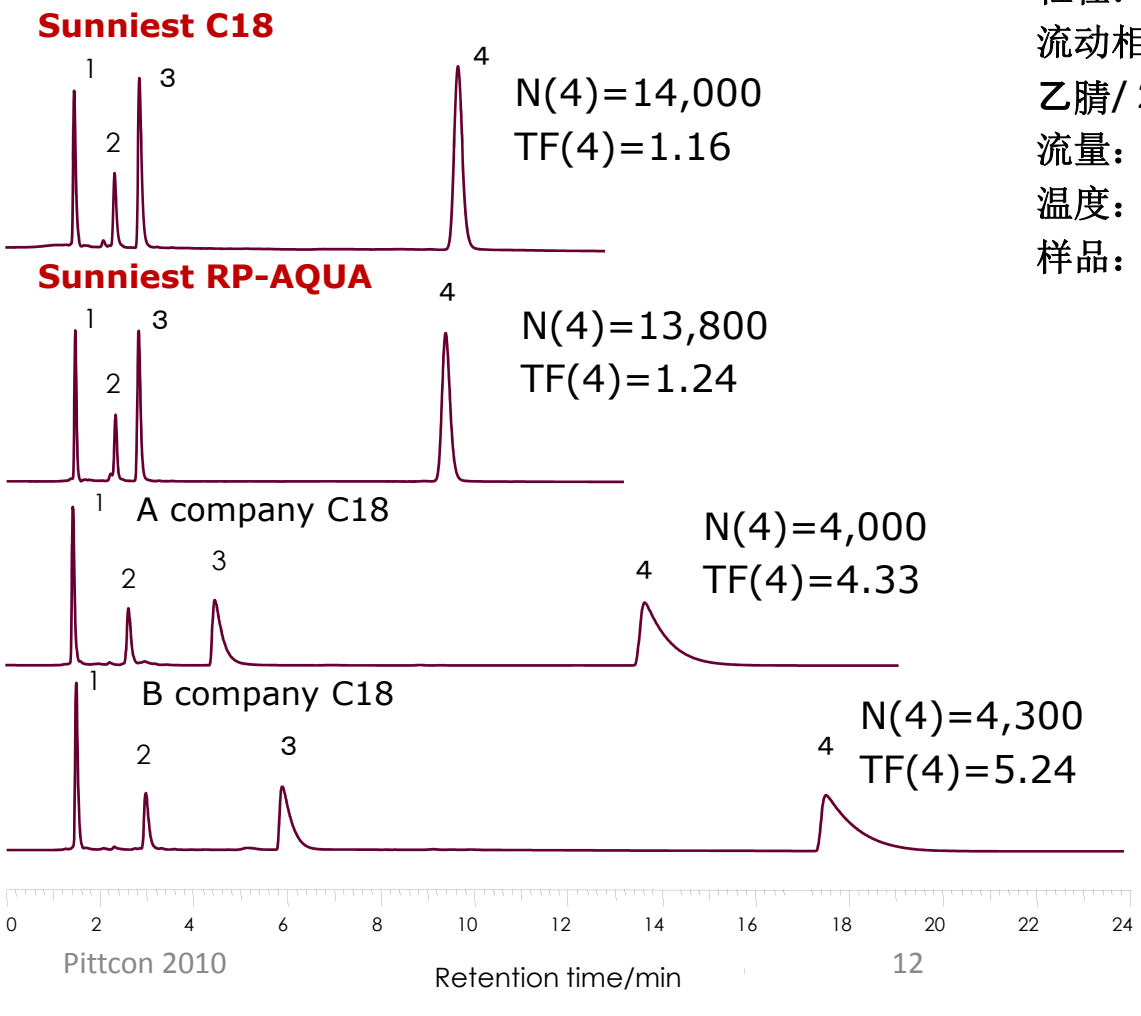


4 =阿米替林

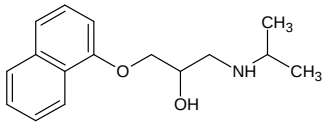


Comparison of amitriptyline peak II
阿米替林峰的比较

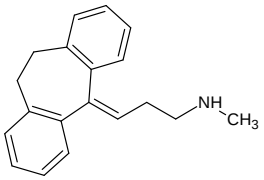
CH_3CN,乙腈, pH7.0, 40 °C



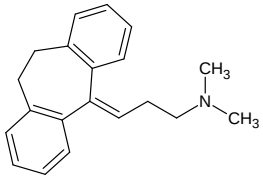
柱尺寸：**150×4.6mm**
粒径：**5微米**
流动相：
乙腈/ 20mM磷酸盐缓冲液pH7.0 = 60/40
流量：**1.0mL / min**
温度：**40℃**
样品：**1 =尿嘧啶**
2 =普萘洛尔



3 = Nortriptyline

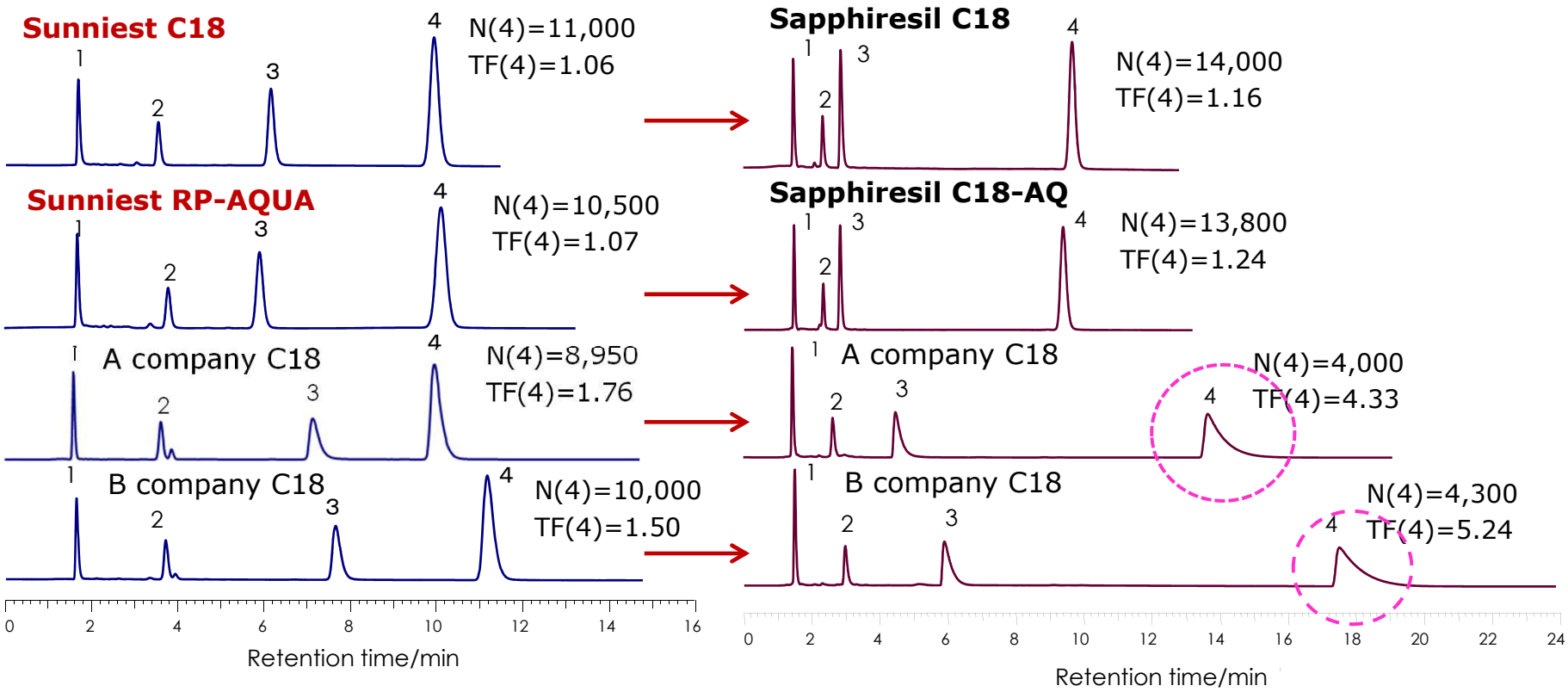


4 =阿米替林



The difference between acetonitrile and methanol
乙腈与甲醇的差异

Methanol 甲醇 → Acetonitrile 乙腈

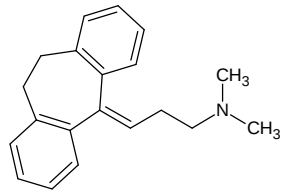


Comparison of amitriptyline peak III
阿米替林峰的比较

色谱柱	拖尾因子	理论塔板数
Sunniest C18	1.16	14,000
Sunniest RP-AQUA	1.24	13,800
Acclaim PolarAdvantage II	5.19	3,300
Acclaim 120 C18	2.19	14,200
ACE C18	3.25	5,300
Ascentis C18	1.74	8,300
Atlantis T3 C18	1.97	10,600
SunFire C18	1.59	10,100
X-Bridge C18	1.43	10,000
Cadenza 5CD-C18	3.07	8,500
Unison US-C18	2.52	9,200
CAPCELLPAK C18 MG II	2.01	10,900
CAPCELLPAK C18 MG III	7,75	3,600

色谱柱	拖尾因子	理论塔板数
Develosil ODS-UG-5	2.14	8,700
Gemini-NX C18	1.29	9,500
Hiber Purospher STAR RP-18e	2.01	11,200
Inertsil ODS-4	1.30	12,000
Inertsil ODS-SP	2.92	8,000
Inertsil ODS-3	2.70	6,100
L-column ODS	1.56	10,400
Shim-pack VP-ODS	3.44	6,700
TSKgel ODS-100V	1.71	10,000
TSKgel ODS-100Z	2.15	11,500
Wakopak Navi C18-5	11.1	2,100
YMC-Pack Pro C18	3.77	7,400
ZORBAX Eclipse Plus C18	3.28	5,900

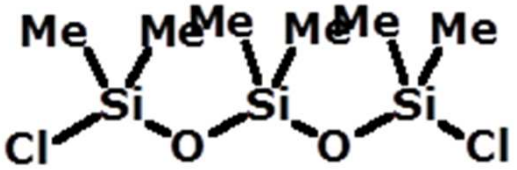
柱尺寸：**150×4.6mm**
粒径：**5微米**
流动相: **乙腈/ 20mM磷酸盐缓冲液pH7.0 = 60/40**
流量：**1.0mL / min**
温度：**40℃**
样品：阿米替林



Our end-capping method
我们的封端方法

1)Special end-capping reagent

特殊封端剂

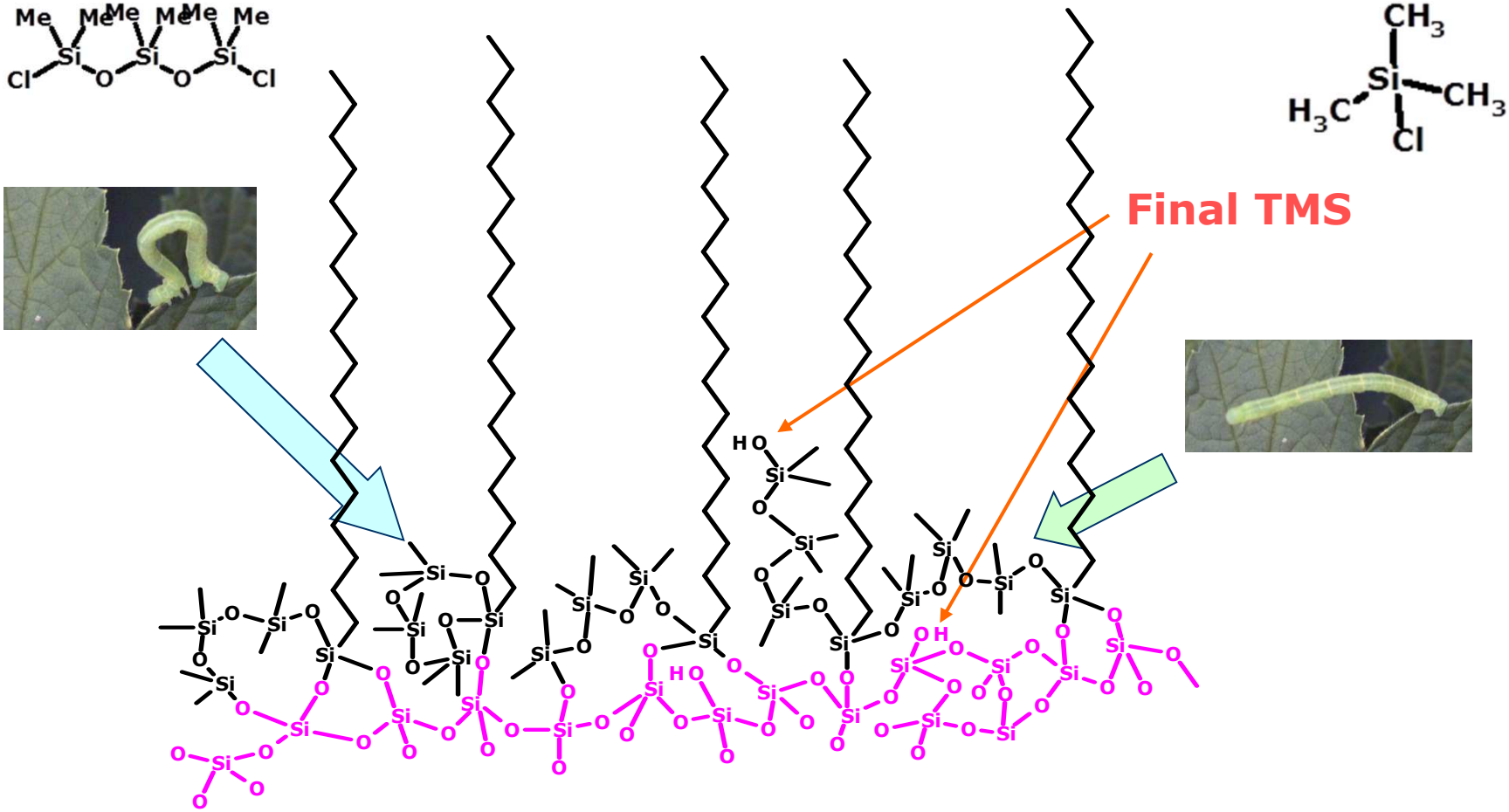


1)High reaction temperature

反应温度高

End-capping of Sunniest and SunShell
Sunniest和SunShell的封端

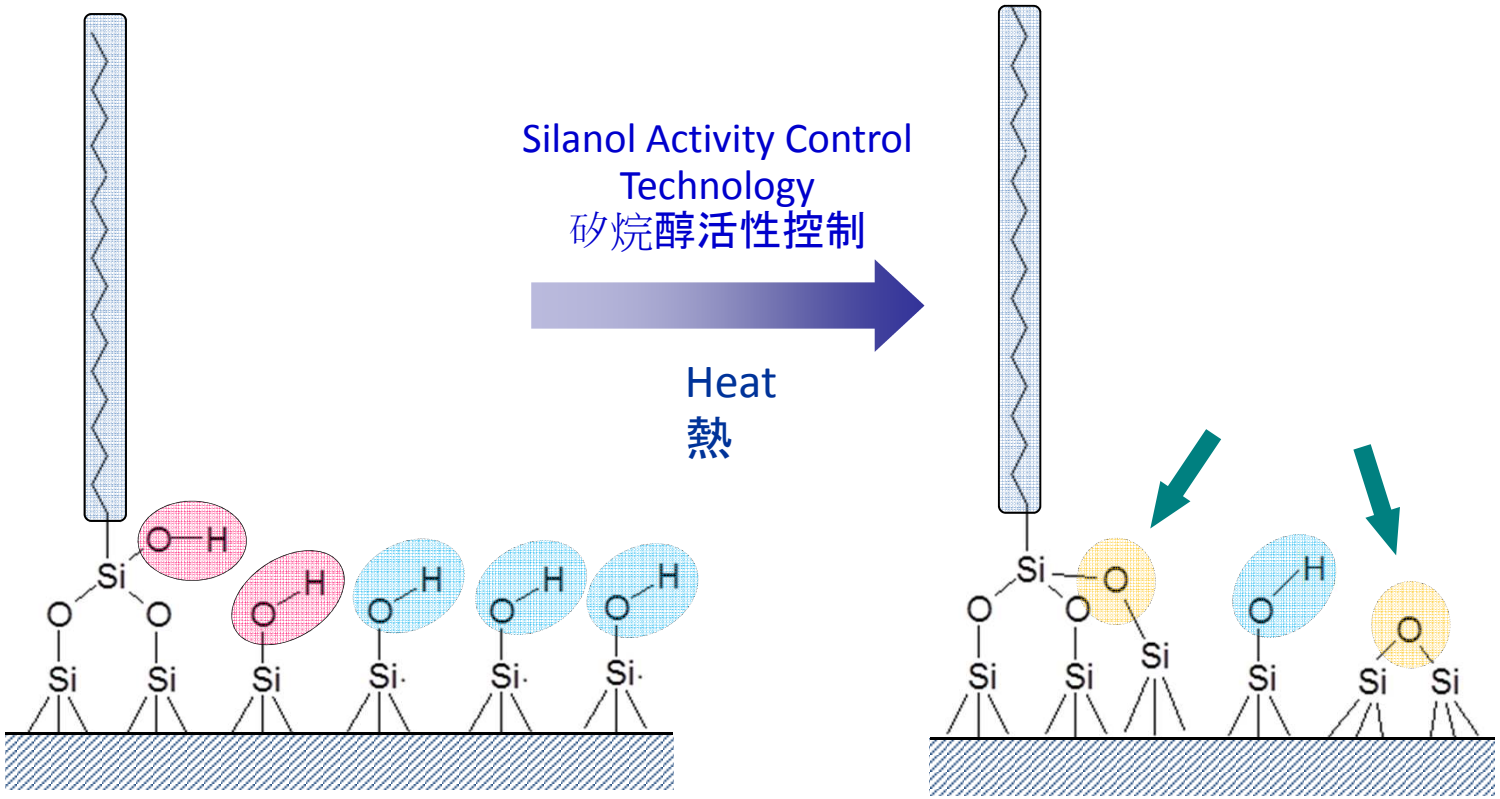
hexamethyldichrolotrisiloxane



Silanol Activity Control
硅醇基活性控制

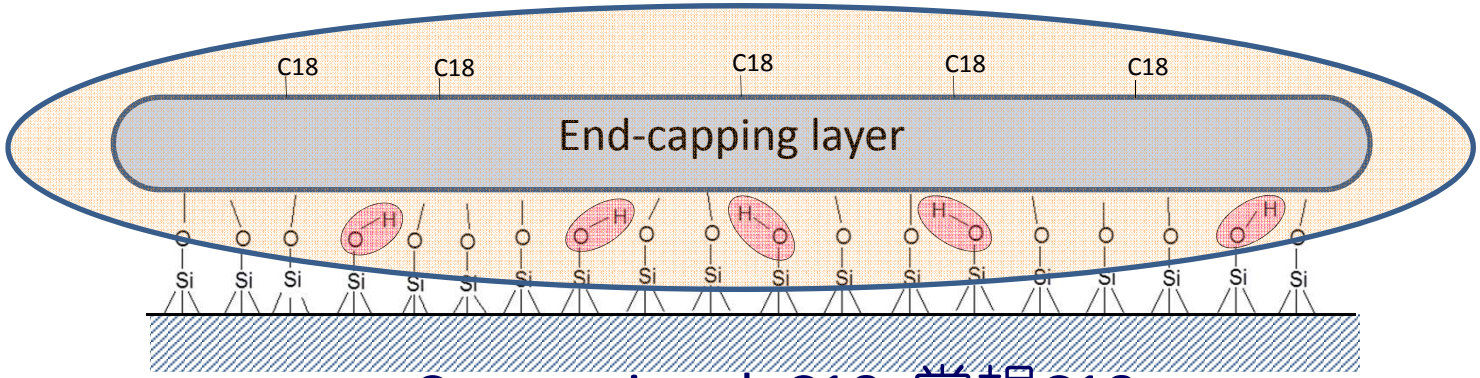
Typical no endcapping C18

Sunrise C18-SAC



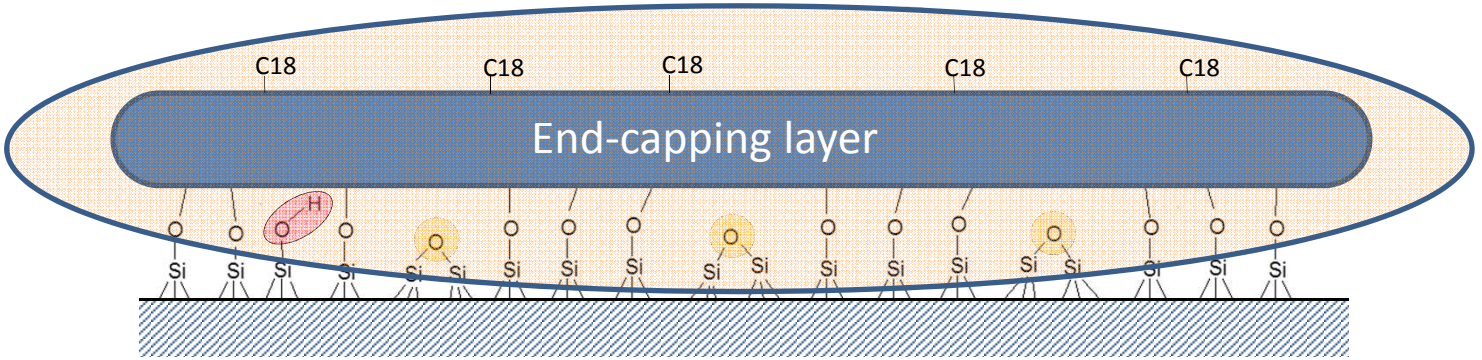
The amount of residual silanol groups
残留硅醇基的量

Conventional end-capping



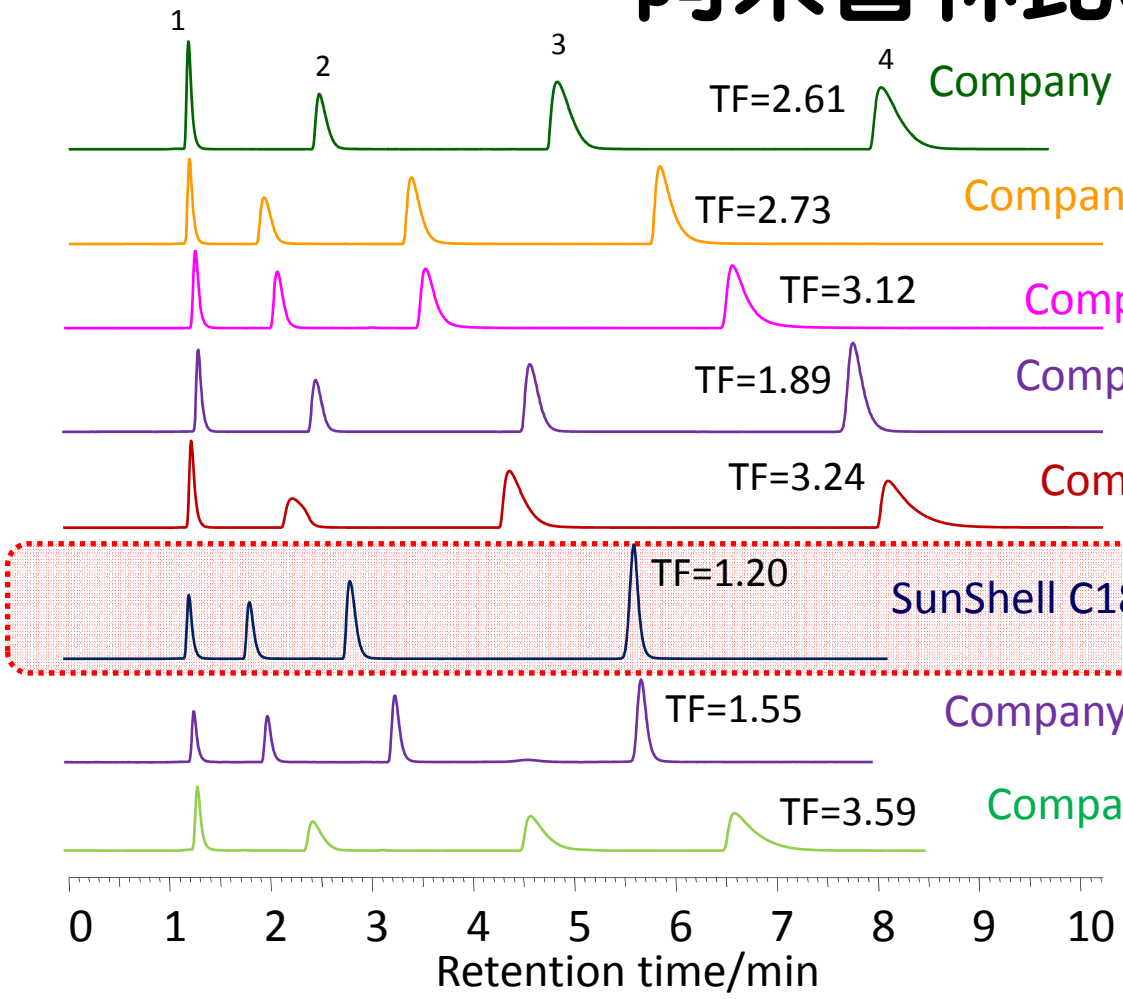
Conventional C18 常規C18

Both end-capping with hexamethyldichrolotrisiloxane and heating



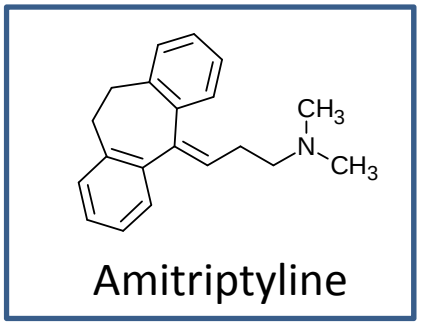
Sunniest C18 and SunShell C18

Comparison of Amitriptyline
阿米替林比较



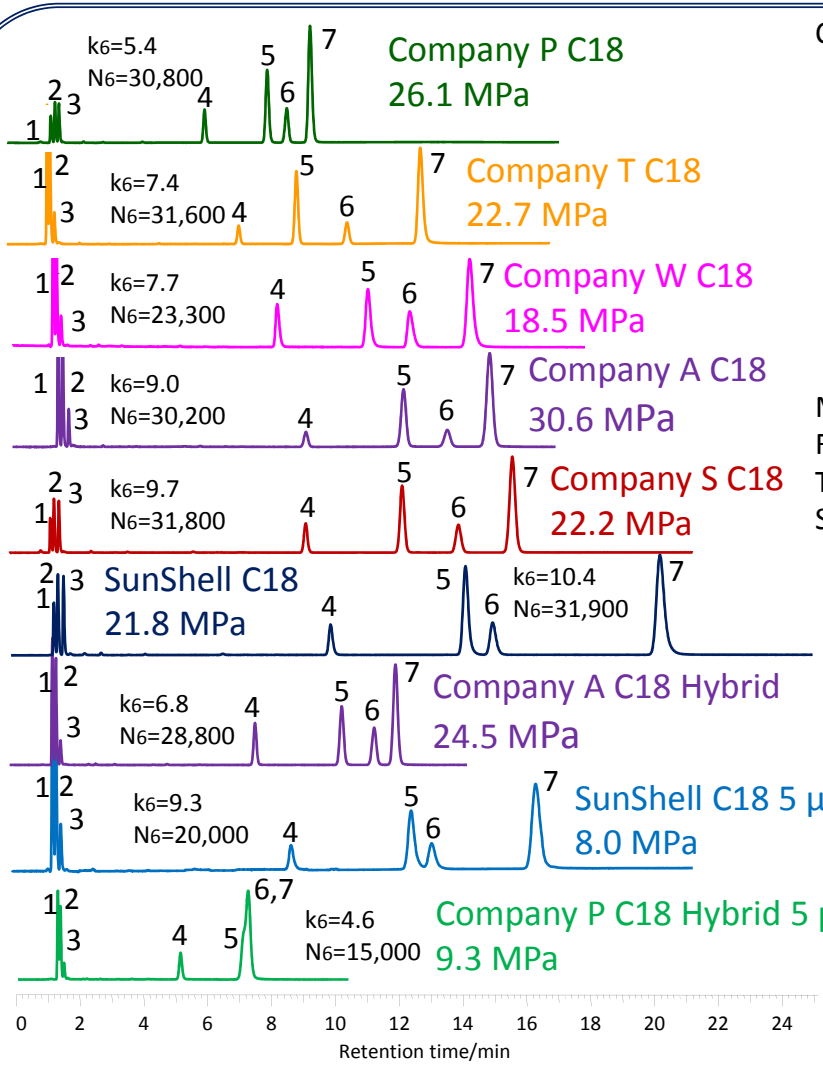
Mobile phase: **Acetonitrile/10mM ammonium acetate pH6.8**=(40:60)
Column dimension: 150 x 4.6 mm, Flow rate: 1.0 mL/min, Temp.: 40°C

Sample: 1=Uracil, 2=Propranolol, 3= Nortriptyline, 4=Amitriptyline



Amitriptyline

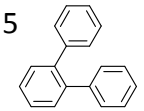
Comparison of core shell C18s 核壳C18比较

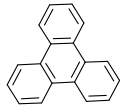


Column:

Company P C18, 2.6 µm 150 x 4.6 mm (26.1 Mpa, 30,800 plate)
Company T C18, 2.6 µm 150 x 4.6 mm (22.7 Mpa, 31,600 plate)
Company W C18, 2.7 µm 150 x 4.6 mm (18.5 Mpa, 23,300 plate)
Company A C18, 2.7 µm 150 x 4.6 mm (30.6 Mpa, 30,200 plate)
Company S C18, 2.7 µm 150 x 4.6 mm (22.2 Mpa, 31,800 plate)
SunShell C18, 2.6 µm 150 x 4.6 mm (21.8 Mpa, 31,900 plate)
Company A C18 Hybrid, 2.7 µm 150 x 4.6 mm (24.5 Mpa, 30,200 plate)
SunShell C18, 5 µm 150 x 4.6 mm (21.8 Mpa, 31,900 plate)
Company P C18 Hybrid, 5 µm 150 x 4.6 mm (26.1 Mpa, 30,800 plate)

Mobile phase: CH_3OH/H_2O=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

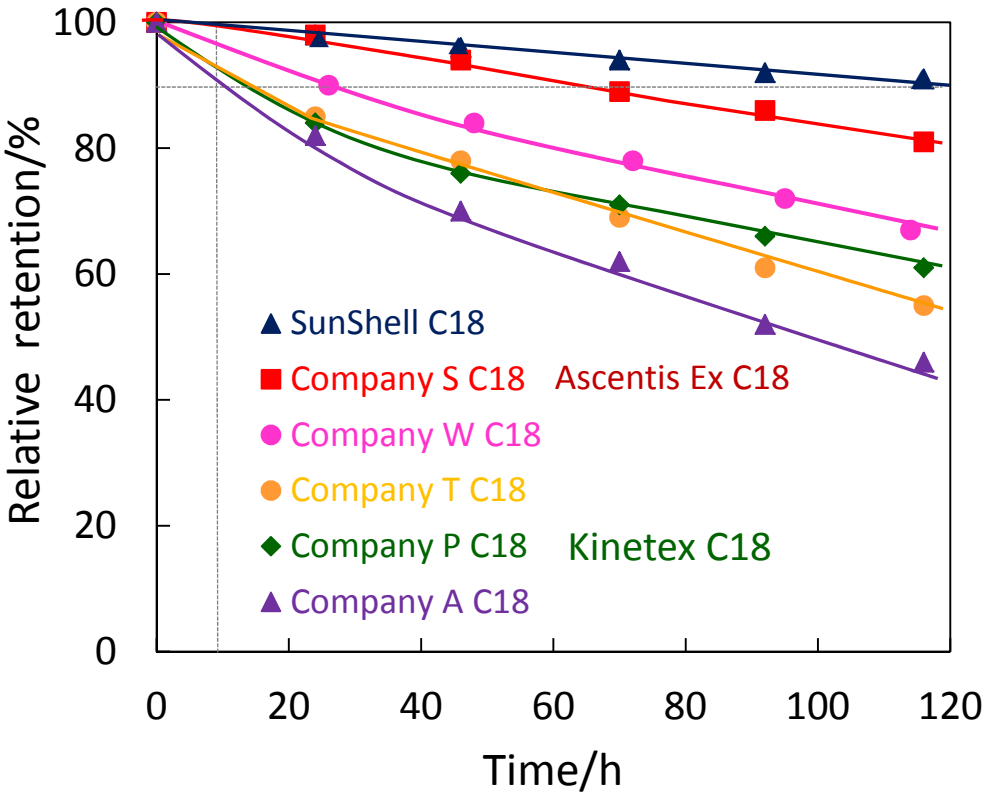
5 

7 

	氢键性能 (Caffeine/Phenol)	疏水 (Amylbenzene/Butylbenzene)	立体选择性 (Triphenylene/o-Terphenyl)
Company P C18	0.48	1.54	1.20
Company T C18	0.35	1.56	1.50
Company W C18	0.38	1.59	1.32
Company A C18	0.42	1.57	1.25
Company S C18	0.44	1.60	1.31
SunShell C18	0.39	1.60	1.46
Company A C18 Hybrid	0.42	1.58	1.19
SunShell C18 5 µm	0.42	1.59	1.35
Company P C18 Hybrid 5 µm	0.40	1.45	1.02

Stability test under acidic pH condition

在酸性条件下的耐久性（加速测试）

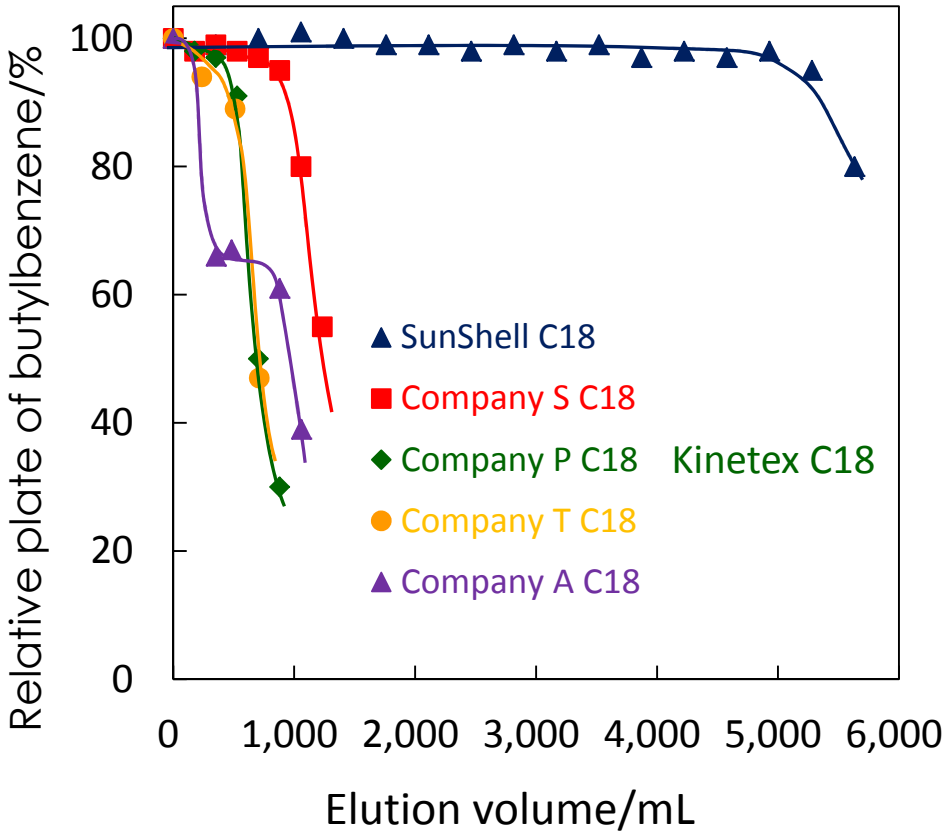


耐久性的测试条件
柱尺寸：50 x 2.1 mm
流动相：乙腈/1.0%TFA, pH1 = 10/90
流量：0.4mL/min
温度：80℃

测量条件
柱尺寸：50 x 2.1 mm
流动相：乙腈/水= 60/40
流量：0.4mL/min
温度：40°C
样品：1 =尿嘧啶
2 =丁基苯

Stability test under basic pH condition

在碱性条件下的耐久性



耐久性的测试条件
柱尺寸：50 x 2.1 mm
流动相：乙腈/ 20mM硼酸钠/ 10mM NaOH = 30/21/49 (pH10)
流量：0.4mL/min
温度：50°C

测量条件
柱尺寸：50 x 2.1 mm
流动相：乙腈/水= 70/30
流量：0.4mL/min
温度：40°C
样品：1 =丁基苯

Summary
摘要

*Residual silanol groups in C18 stationary phase make basic compounds be tailing, so that an end-capping is very important for separation of basic compounds.
*End-capping makes stability increase under acidic and basic pH conditions.
*HPLC column manufacturers have their own end-capping skill. ChromaNik has the best end-capping skill.

* C18固定相中的残留硅醇基使碱性化合物拖尾，因此封端对于分离碱性化合物非常重要。
*封端将增加在酸性和碱性条件下的稳定性。
* HPLC色谱柱制造商各有自己的封端技术，ChromaNik具有最佳的封端技术。