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A Novel Bonding Technique Using a Polyfunctional Silyl-Reagent for Reversed-Phase Liquid Chromatography

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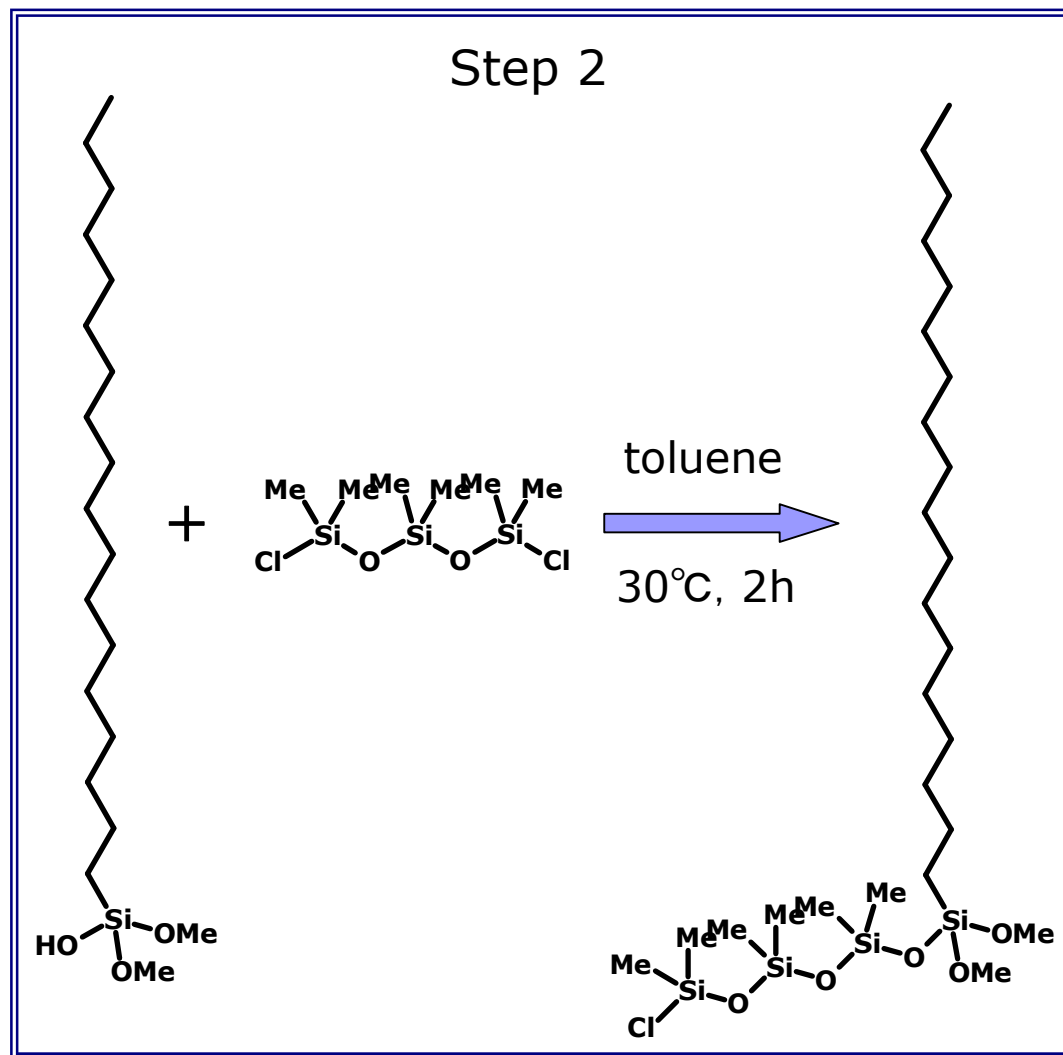
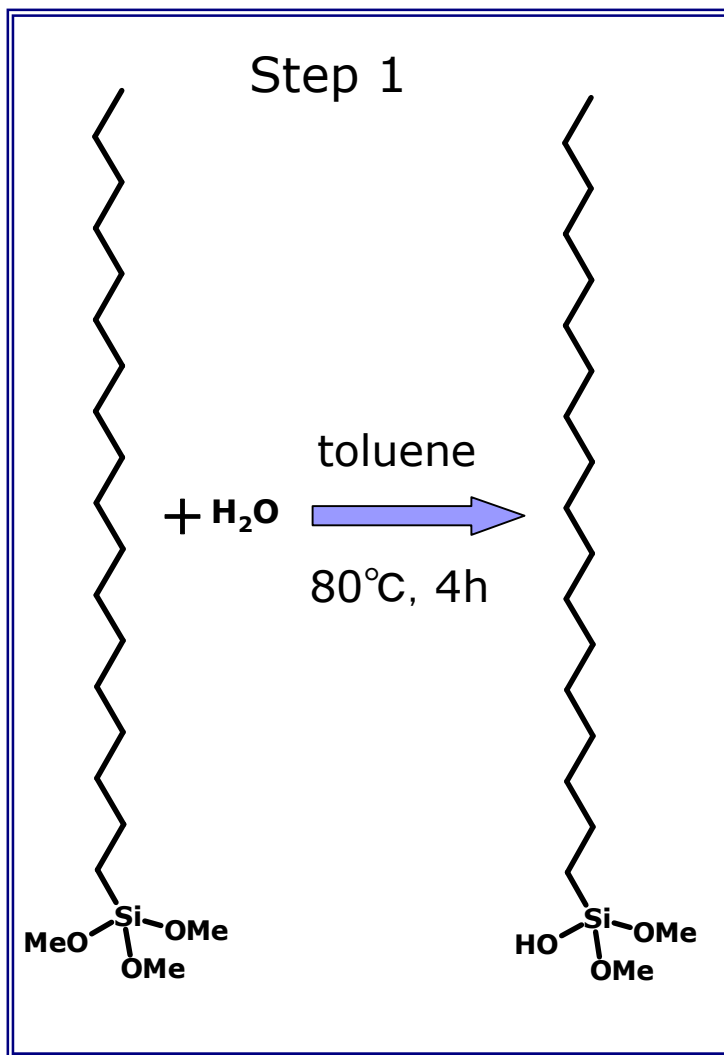
ABSTRACT

Reversed- phase LC columns have been improved by a pure silica, a new end-capping reagent, bonding technology and a hybrid silica particle et al. and are widely used now. Most of reversed phase silica materials are monomerically or polymerically bonded with alkyl chain, then end-capped with trimethylsilane or hexamethyltrisiloxane et al. In this study, polyfunctional silyl-reagent (I) was synthesized with octadecyltrimethoxysilane and hexamethyldichlorotrisiloxane, polyfunctional silyl-reagent (II) was synthesized with octadecyltrichlorosilane and bis(triethoxysilyl)ethane. The former reagent is called hexamethyloctadecyltetrasiloxane (HMODTS). Silica gel, which was bonded with these reagents and finally end-capped with trimethylchlorosilane, was

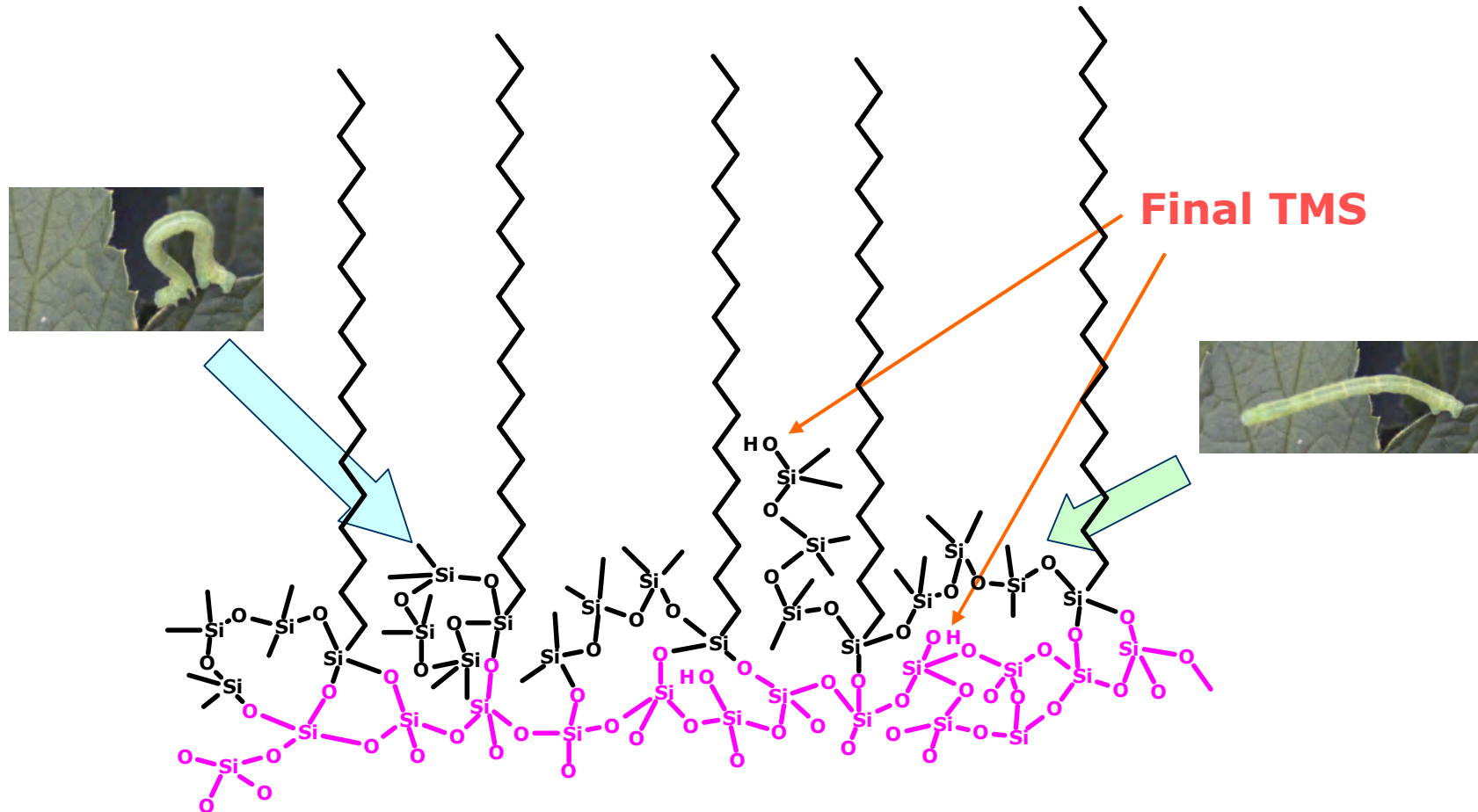
evaluated to separate acidic and basic compounds. Stability of these phases was evaluated under acidic and basic pH at high temperature. These phases showed symmetrical peaks of both acidic and basic compounds such as formic acid and amitriptyline. Especially a symmetric peak of amitriptyline was obtained even if both acetonitrile and ammonium acetate were used as a component of a mobile phase although most of C18 columns show a terrible tailing peak of amitriptyline at the same conditions. In the case of silyl-reagent (II), column life was more than 250 hours from pH 2 to pH 8 at 80 degree Celsius. A novel bonding technique using a polyfunctional silyl-reagent could make effect of residual silanol groups the least.

C18 silyl-reagent 1 (HMODTS) Patent pending

(Sunniest C18) Hexamethyloctadecyltetrasilane



Bonding state of HMODTS on silica



An Arm of HMODTS 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.

Characteristics of Sunniest C18

Used Silica gel:

12 nm, 340 m²/g, 5 μm

Carbon content after bonding HMODTS:

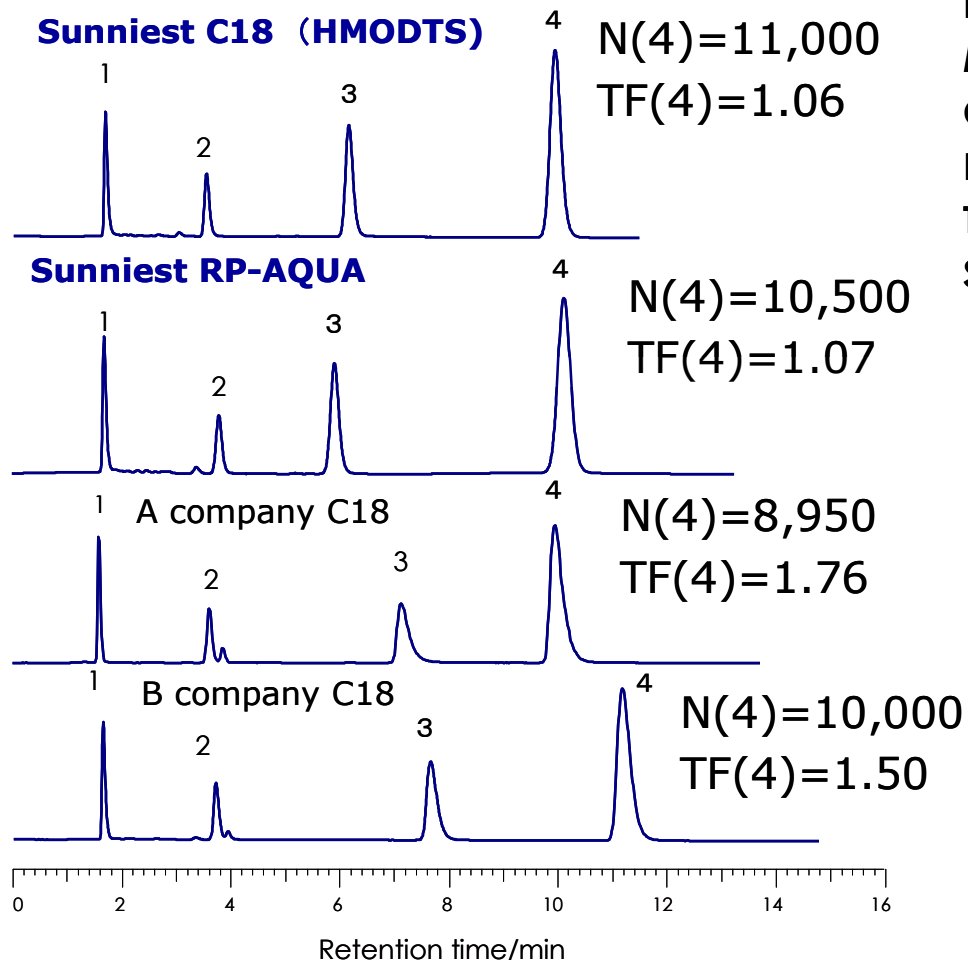
16.1%

Carbon content after final end-capping:

16.3%

Evaluation of end-capping Comparison of amitriptyline peak I

CH₃OH, pH7.5, 40 °C



Column size: 150 X 4.6 mm

Particle size: 5µm

Mobile phase:

CH₃OH/20mM Phosphate buffer pH7.5=80/20

Flow rate: 1.0 mL/min

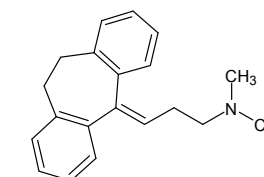
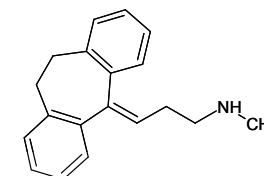
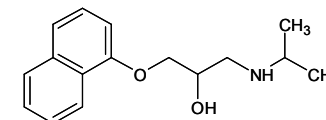
Temperature: 40 °C

Sample: 1 = Uracil

2 = Propranolol

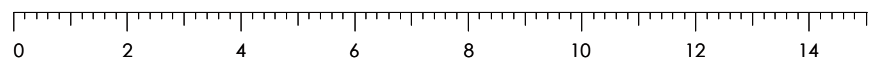
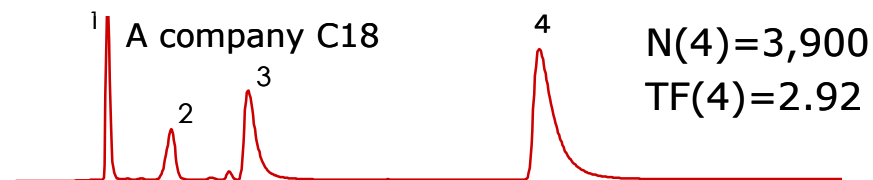
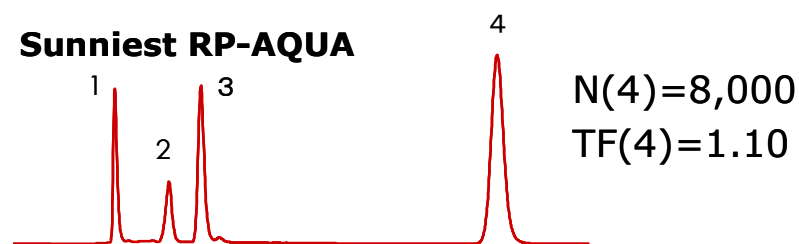
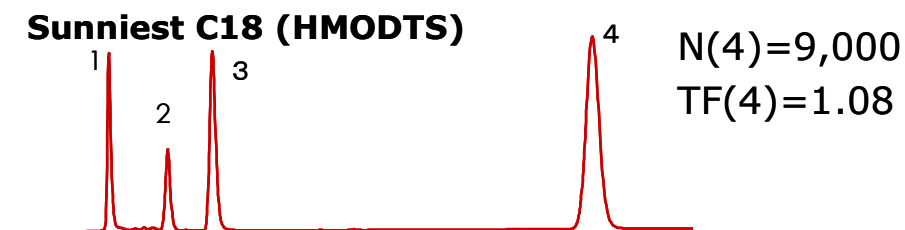
3 = Nortriptyline

4 = Amitriptyline



Evaluation of end-capping Comparison of amitriptyline peak II

CH₃OH, pH6.0, 22 °C



Retention time/min

Pittcon 2009

Column size: 150 X 4.6 mm

Particle size: 5µm

Mobile phase:

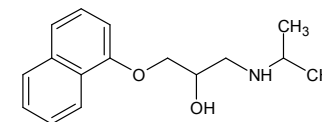
CH₃OH/20mM Phosphate buffer pH6.0=80/20

Flow rate: 1.0 mL/min

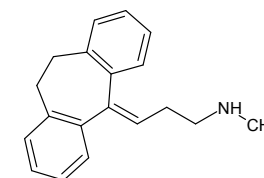
Temperature: 22 °C

Sample: 1 = Uracil

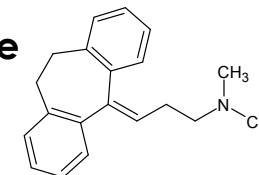
2 = Propranolol



3 = Nortriptyline



4 = Amitriptyline



Evaluation of end-capping Comparison of amitriptyline peak III-A

CH₃CN, pH7.0, 40 °C

Column size: 150 X 4.6 mm

Particle size: 5µm

Mobile phase:

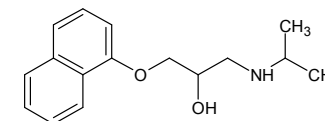
CH₃CN/20mM Phosphate buffer pH7.0=60/40

Flow rate: 1.0 mL/min

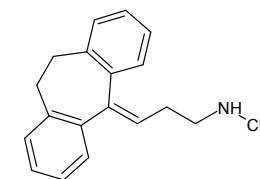
Temperature: 40 °C

Sample: 1 = Uracil

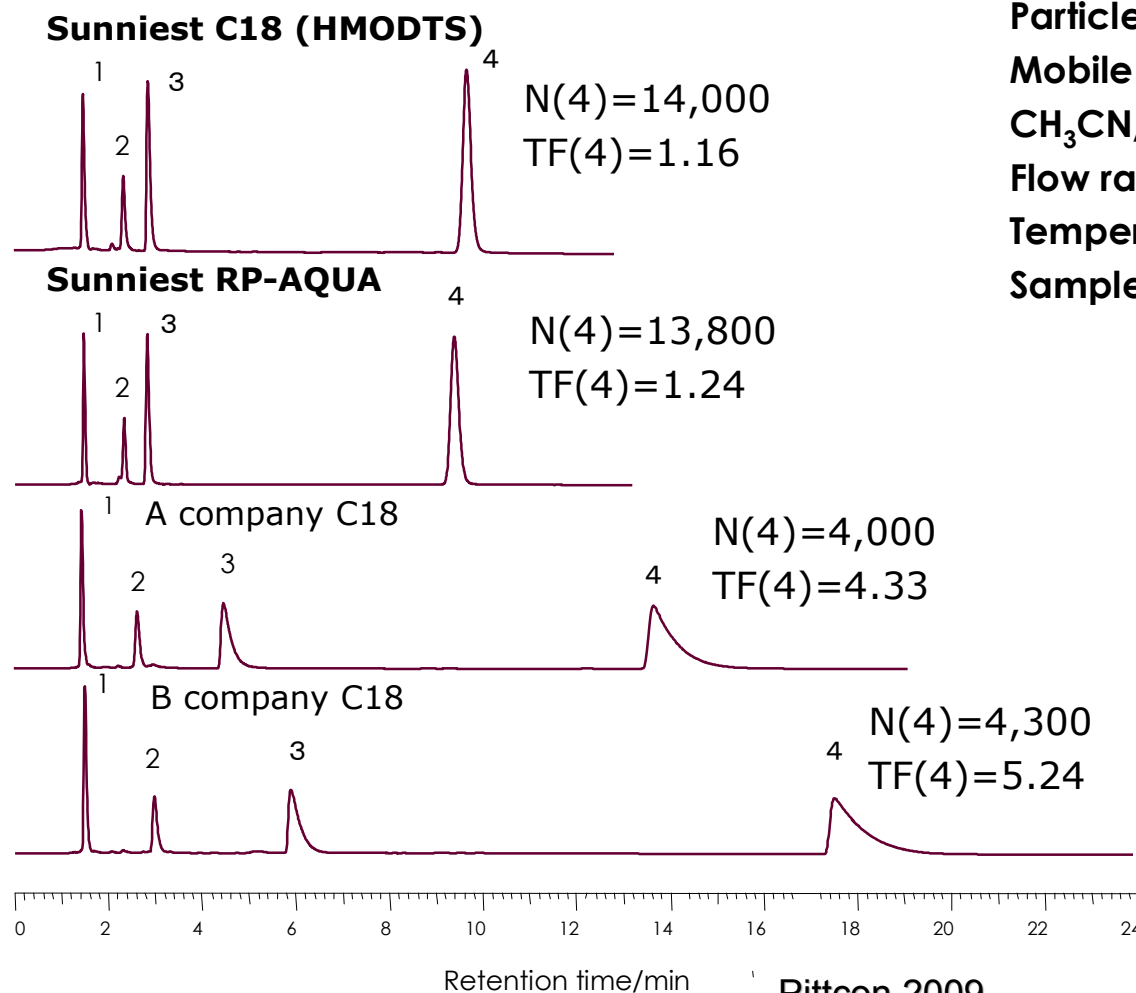
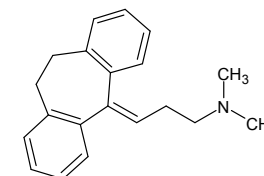
2 = Propranolol



3 = Nortriptyline



4 = Amitriptyline



Evaluation of end-capping

Comparison of amitriptyline peak II-B

Column	TF	N
Sunniest C18(HMODTS)	1.16	14,000
Sunniest RP-AQUA	1.24	13,800
D1	5.19	3,300
D2	2.19	14,200
AT	3.25	5,300
S1	1.74	8,300
W1	1.97	10,600
WS2	1.59	10,100
W3	1.33	10,000
Japanese company A1 C18	3.07	8,500
Japanese company A2 C18	2.52	9,200
Japanese company B1 C18	2.23	50
Japanese company B2 C18	2.01	10,900
Japanese company B3 C18	7.75	3,600

Column	TF	N
Japanese company C1 C18	2.14	8,700
P1	1.09	9,500
M1	2.01	11,200
Japanese company D1 C18	1.30	12,000
Japanese company D2 C18	2.92	8,000
Japanese company D3 C18	2.70	6,100
Japanese company E1 C18	0.99	11,400
Japanese company F1 C18	3.44	6,700
Japanese company G1 C18	1.71	10,000
Japanese company G2 C18	2.15	11,500
Japanese company H1 C18	11.1	2,100
Japanese company I1 C18	3.77	7,400
A1	3.28	5,900

Column size: 150 X 4.6 mm

Particle size: 5 μ m

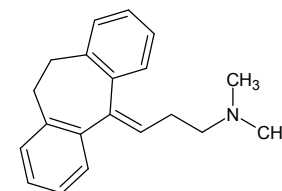
Mobile phase:

**CH₃CN/20mM Phosphate
buffer pH7.0=60/40**

Flow rate: 1.0 mL/min

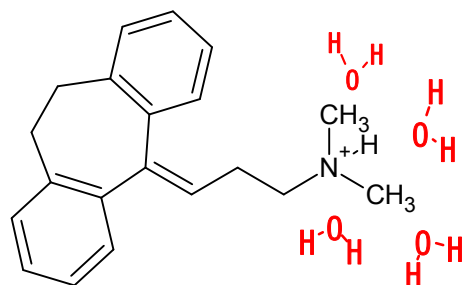
Temperature: 40 °C

Sample: Amitriptyline

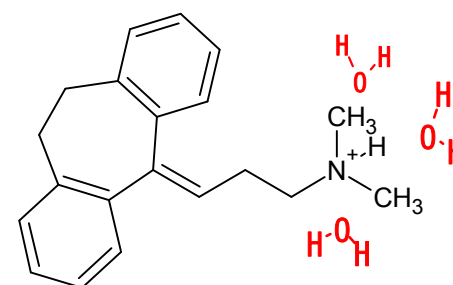


Change of water solvation

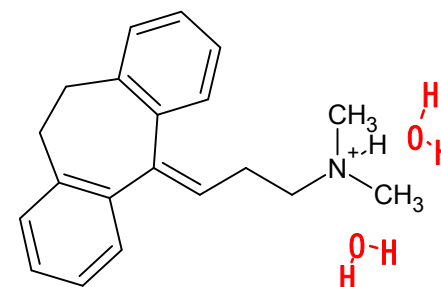
Why does peak tailing occur under different conditions although the same column is used?



Methanol 40°C



Methanol 22°C



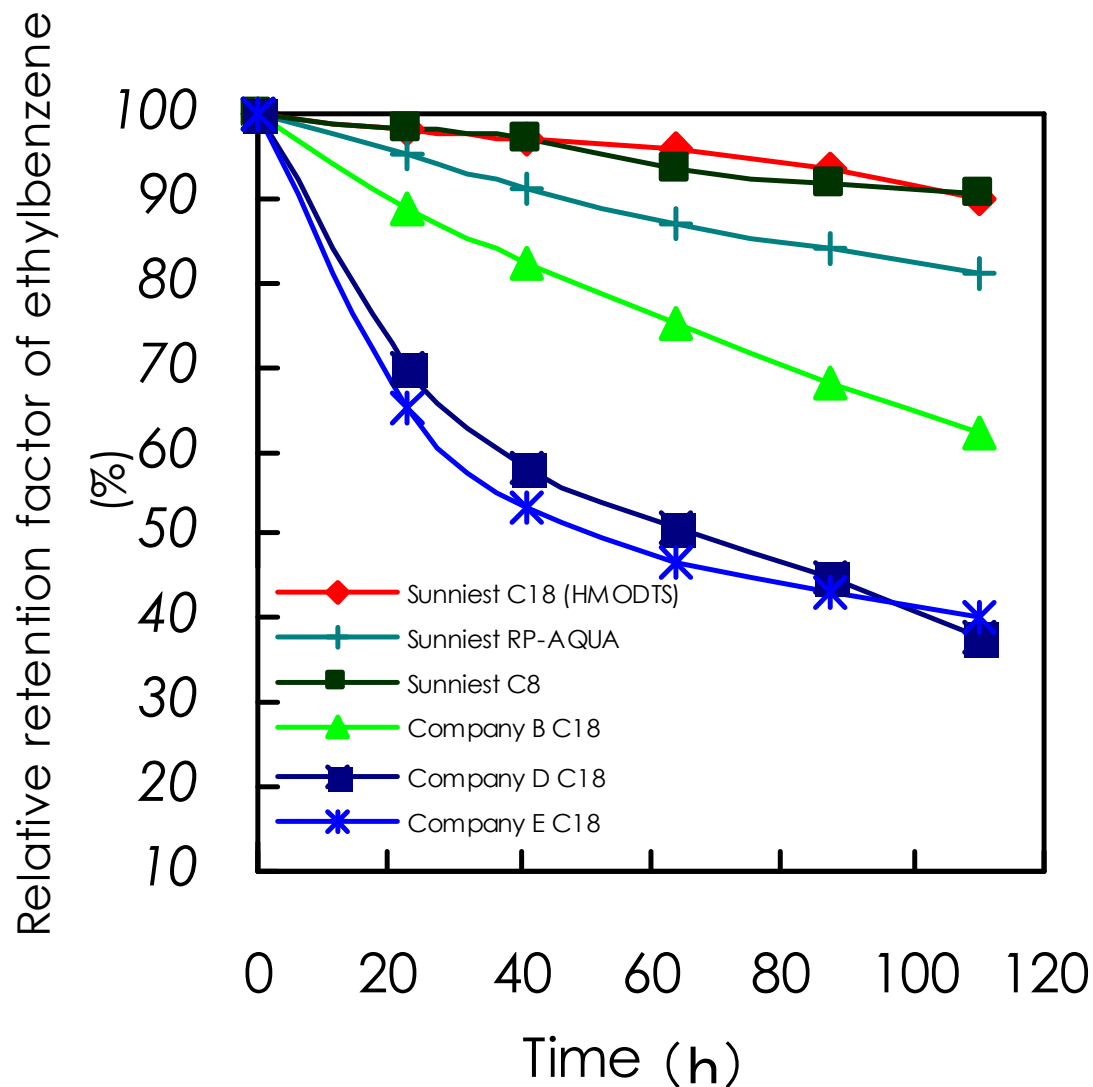
Acetonitrile 40°C

Water solvation volume on a cation changes under conditions such as methanol or acetonitrile, 40°C or 22°C.



The less water solvation, the more peak tailing.

Stability under acidic conditions



Test conditions

Column size: 150 x 4.6 mm

Mobile phase:

CH₃CN/1.0% TFA, pH1=10/90

Flow rate: 1.0 mL/min

Temperature: 80 °C

Measurement conditions

Column size: 150 x 4.6 mm

Mobile phase:

CH₃CN/H₂O=60/40

Flow rate: 1.0 mL/min

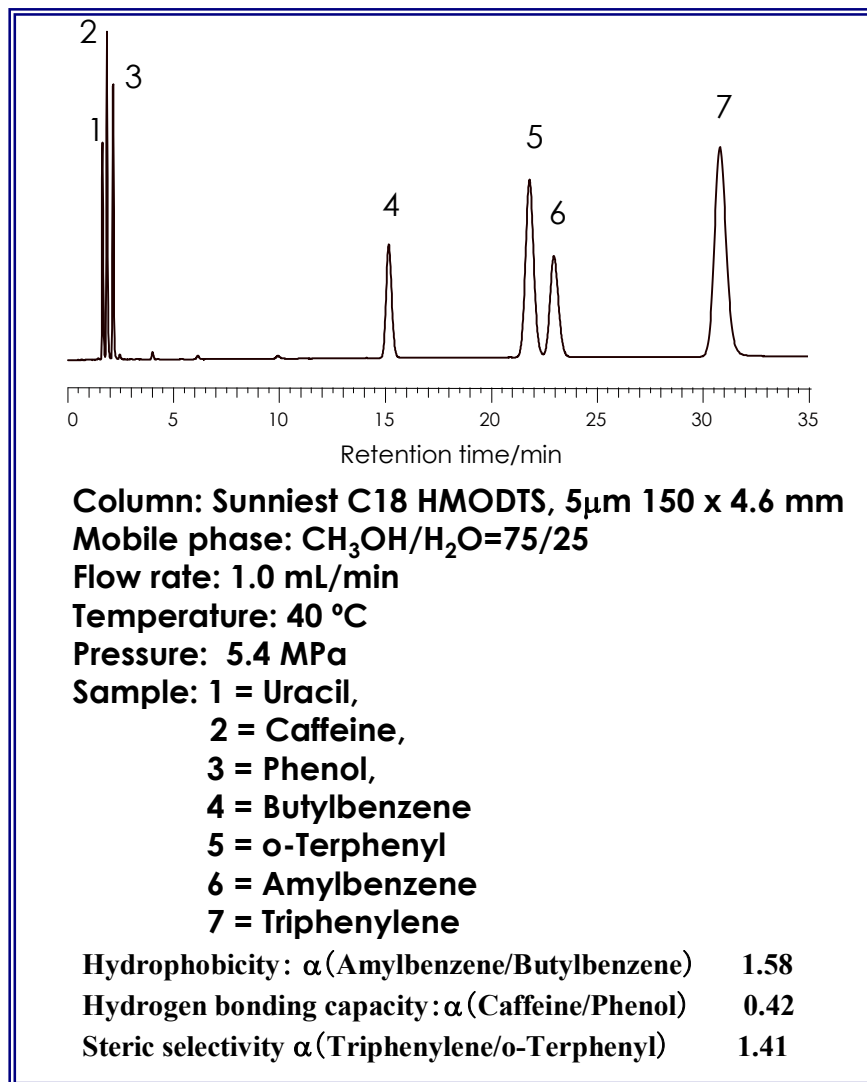
Temperature: 40 °C

Sample: 1 = Uracil

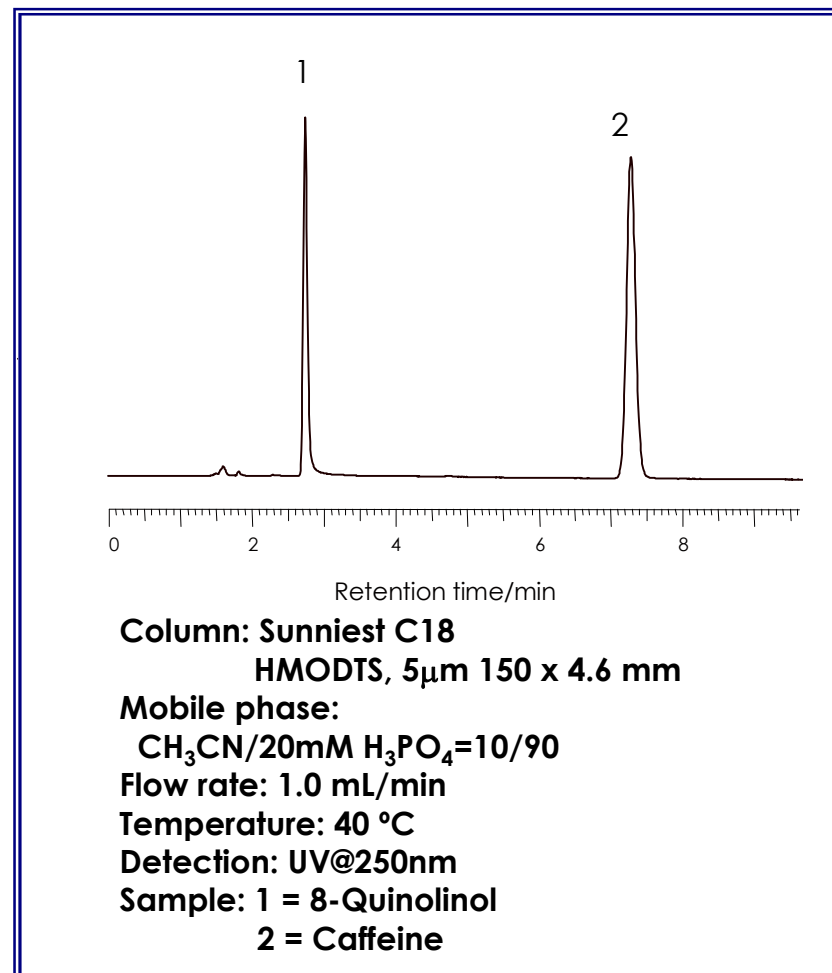
2 = Ethylbenzene

Separation of standard samples I

Neutral samples

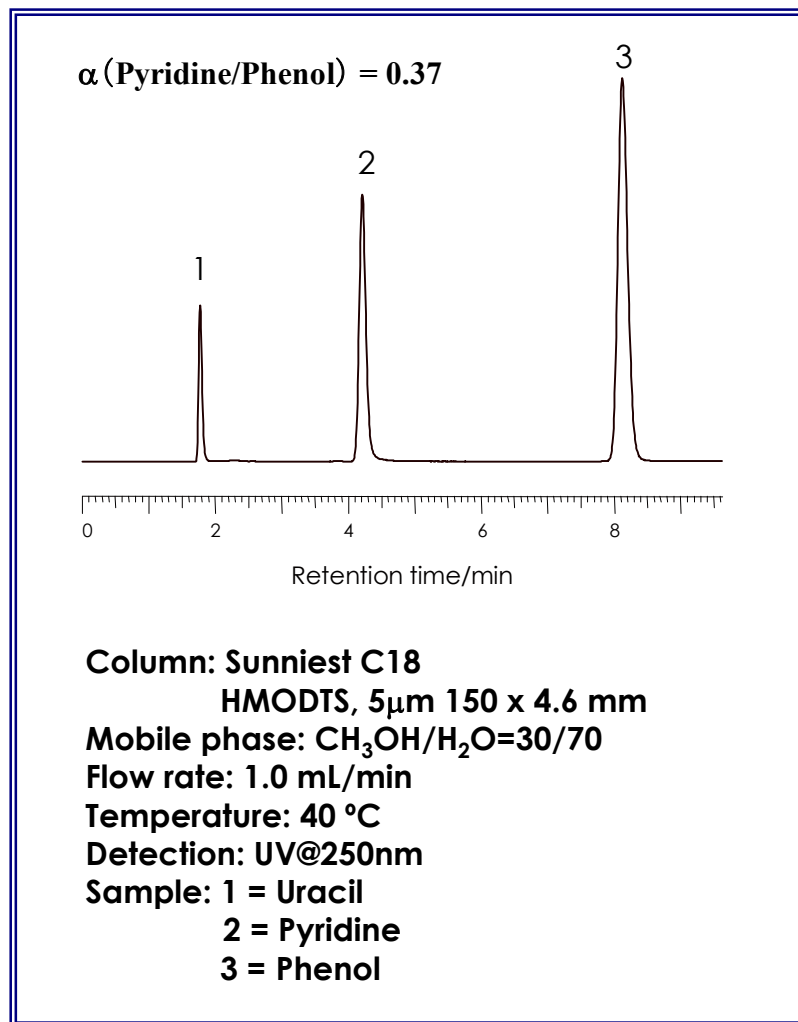


Chelating sample

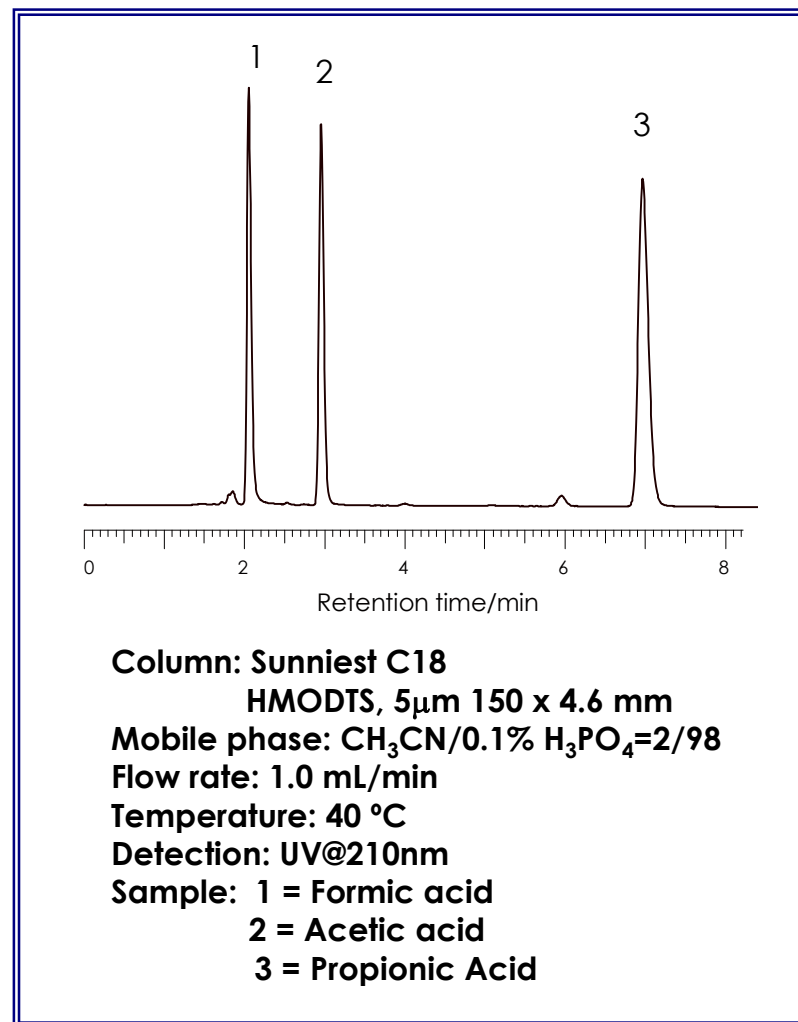


Separation of standard samples II

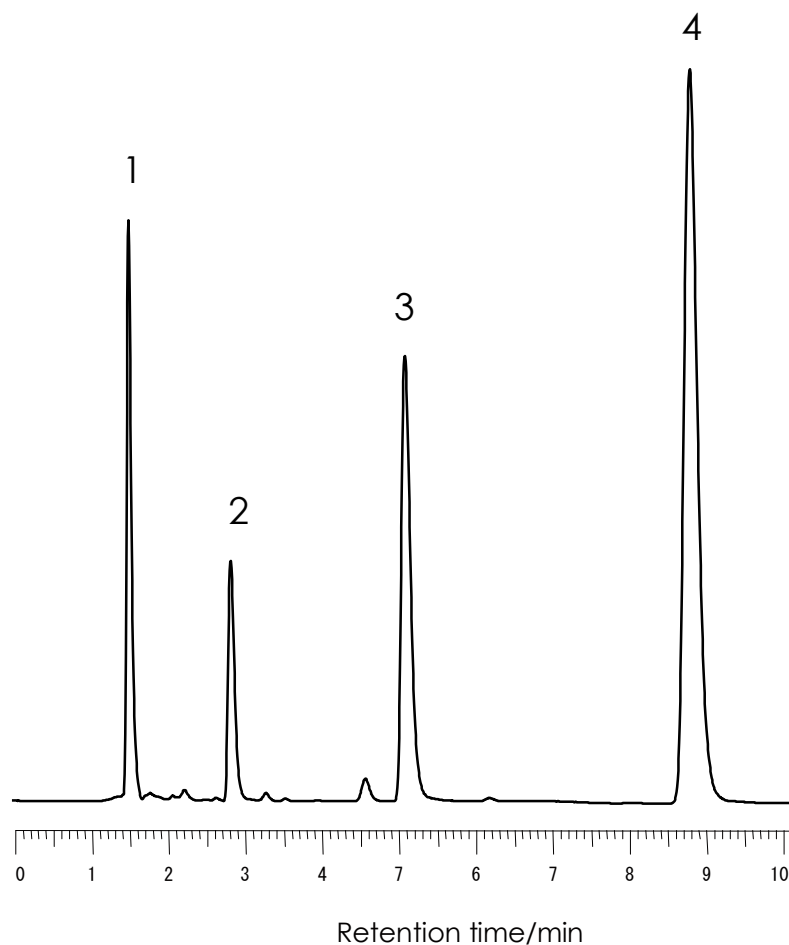
Pyridine and Phenol



Acidic samples



Separation of basic compounds using mobile phase for LC/MS



Column: Sunniest C18 HMODTS, 5 μ m 150 x 4.6 mm

Mobile phase:

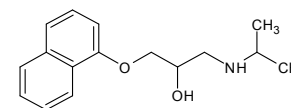
CH₃CN/10mM Ammonium acetate pH6.8=40/60

Flow rate: 1.0 mL/min

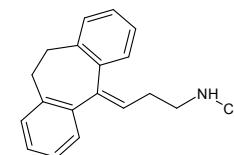
Temperature: 40 °C

Sample: 1 = Uracil,

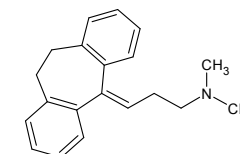
2 = Propranolol,



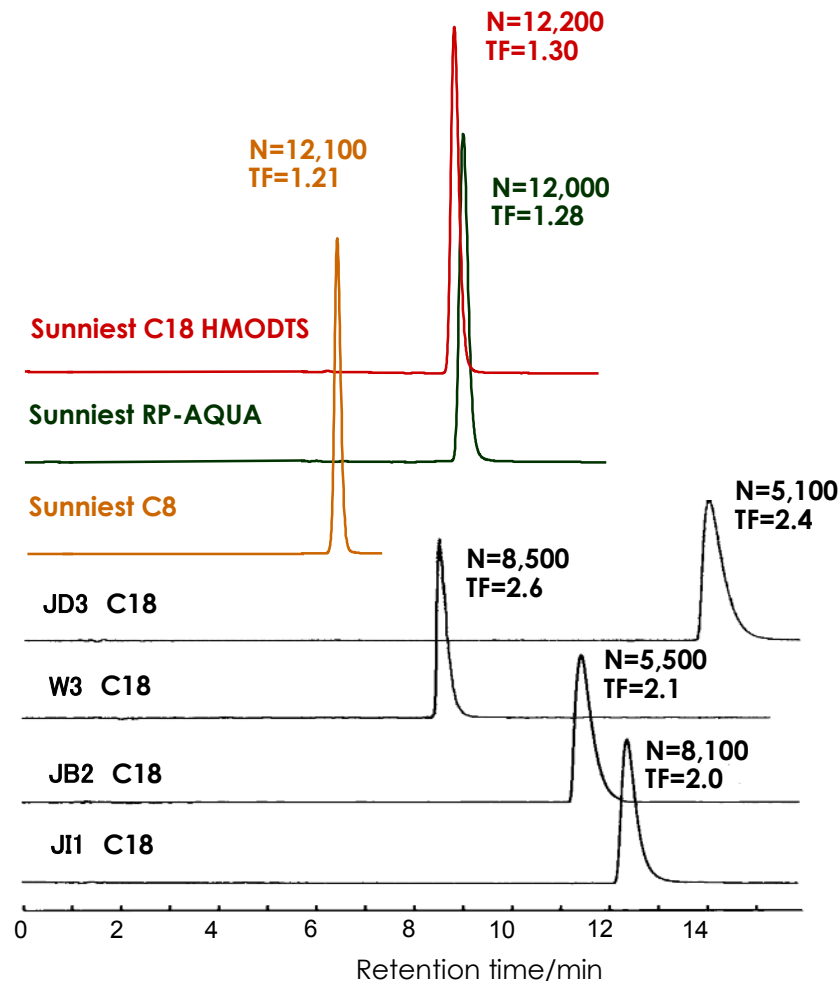
3 = Nortriptyline,



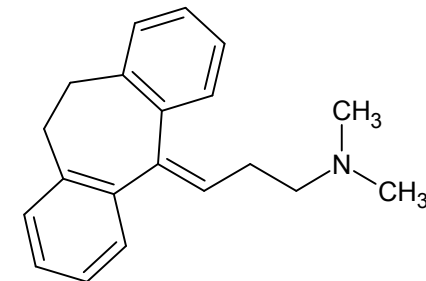
4 = Amitriptyline



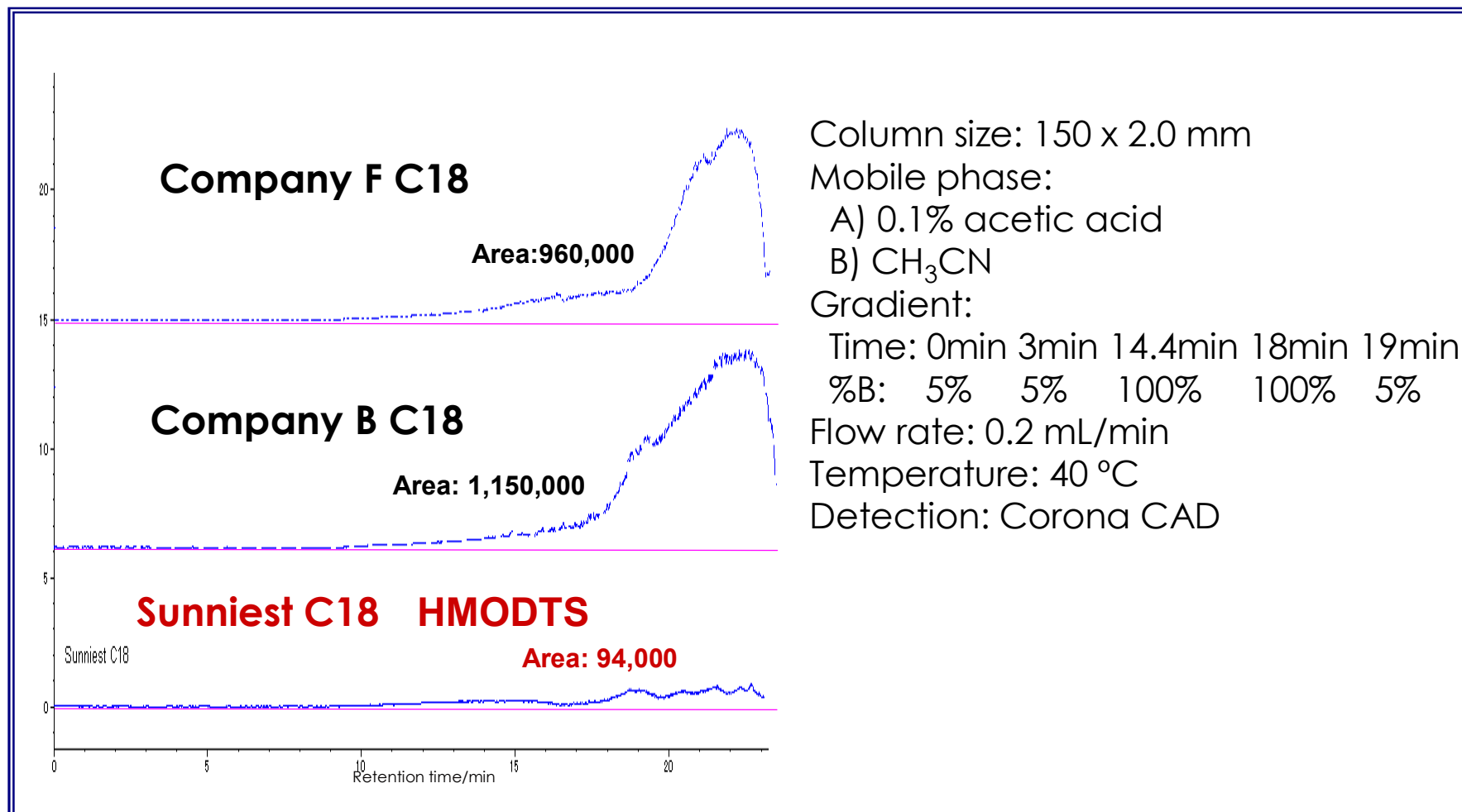
Comparison of amitriptyline peak using mobile phase for LC/MS



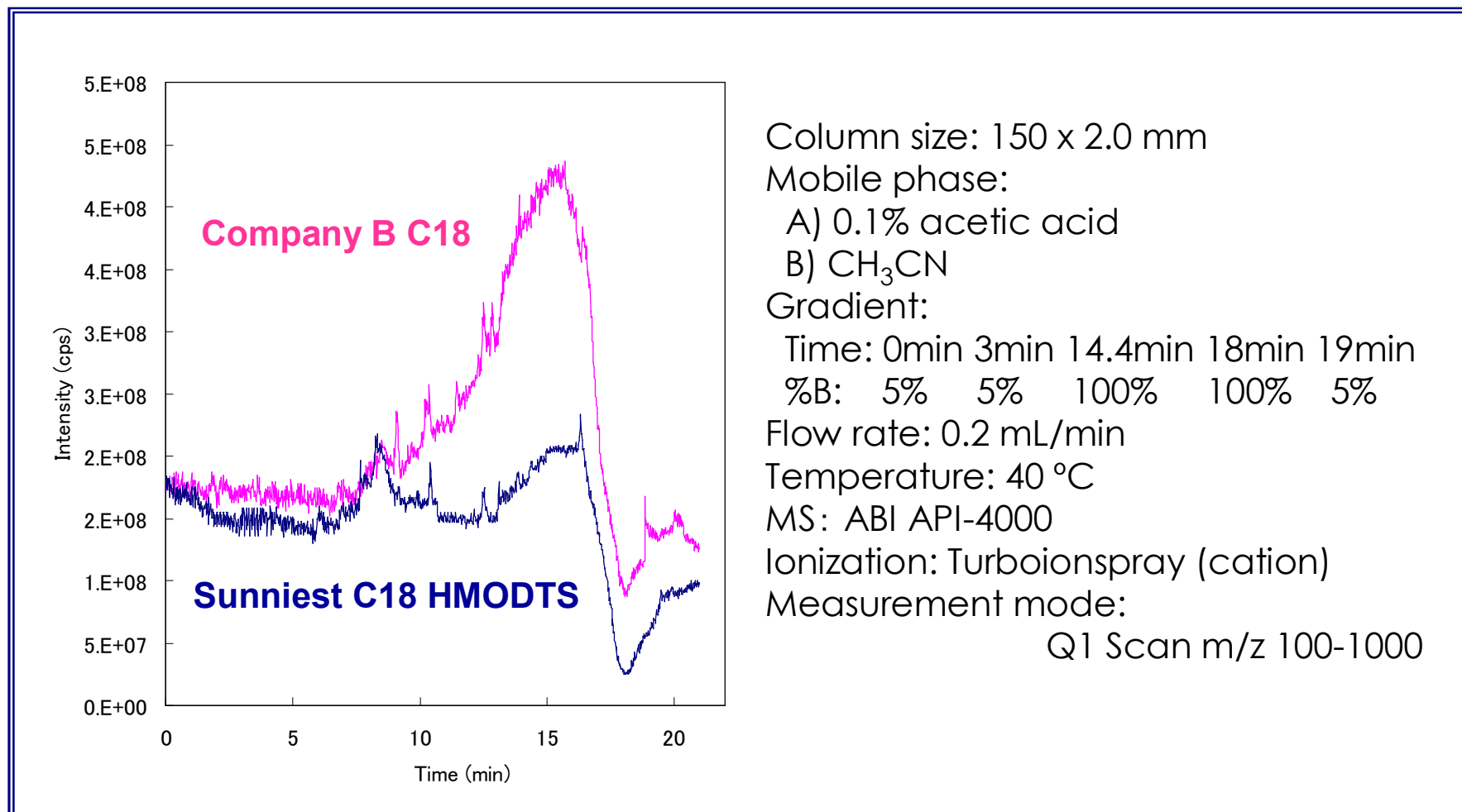
Column size: 4.6x150 mm
 Particle size: 5µm
 Mobile phase: CH₃CN/10mM Ammonium acetate
 pH6.8=40/60
 Flow rate: 1.0 mL/min
 Temperature: 40 °C
 Sample: Amitriptyline



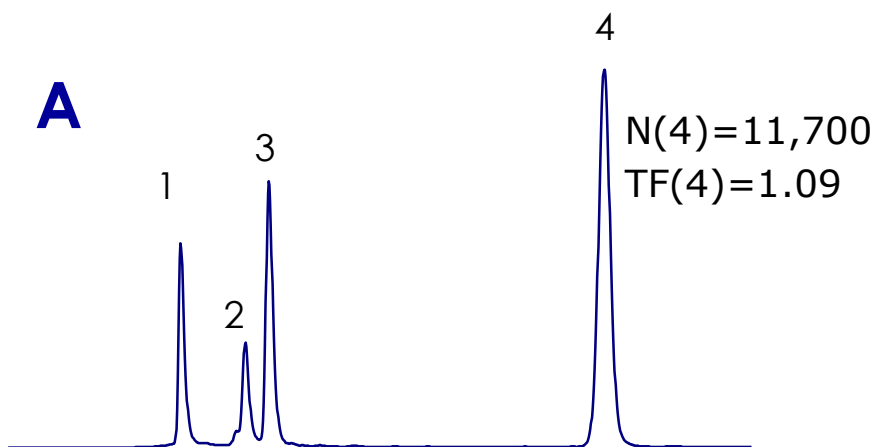
Bleeding Test I



Bleeding Test II



Synthesis of Octyl group (C8, HMOTS) Separation of amitriptyline



Column: Sunniest C8 HMOTS, 5 μ m 150 x 4.6 mm

Mobile phase:

A) CH₃CN/20mM Phosphate buffer pH7.0=60/40

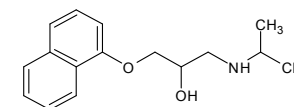
B) CH₃CN/10mM Ammonium acetate pH6.8=40/60

Flow rate: 1.0 mL/min

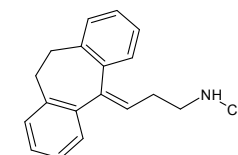
Temperature: 40 °C

Sample: 1 = Uracil,

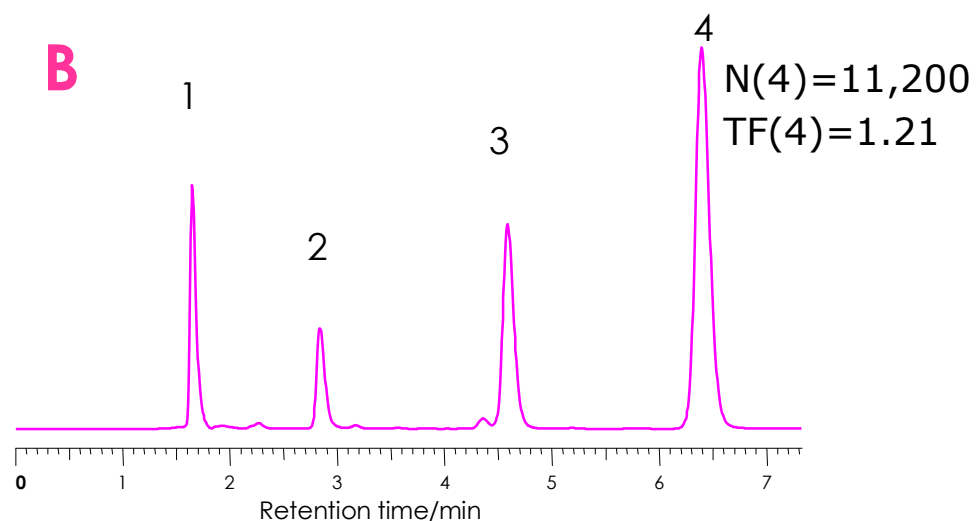
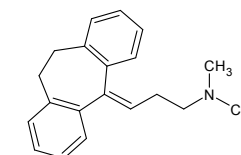
2 = Propranolol,



3 = Nortriptyline,

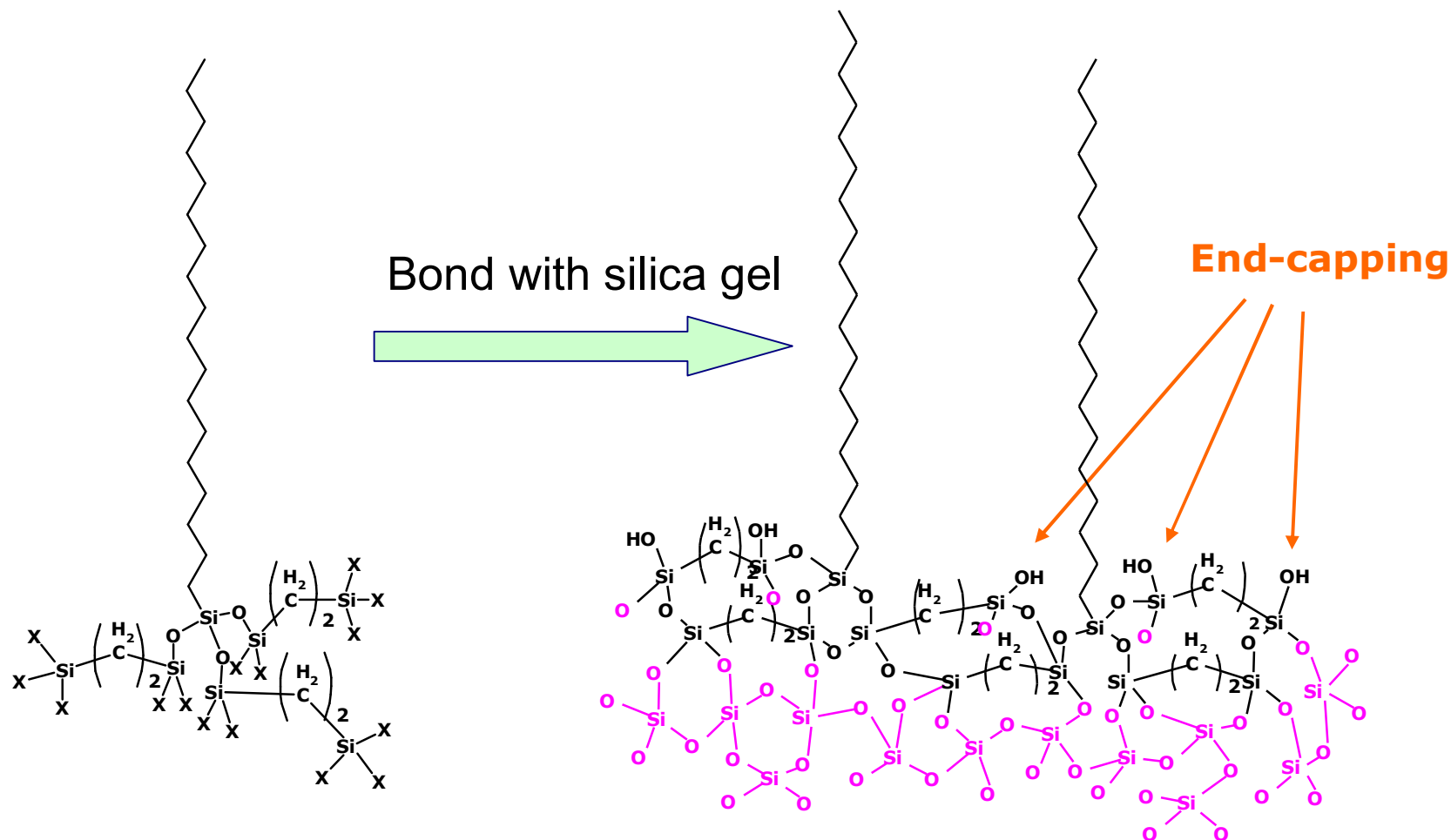


4 = Amitriptyline



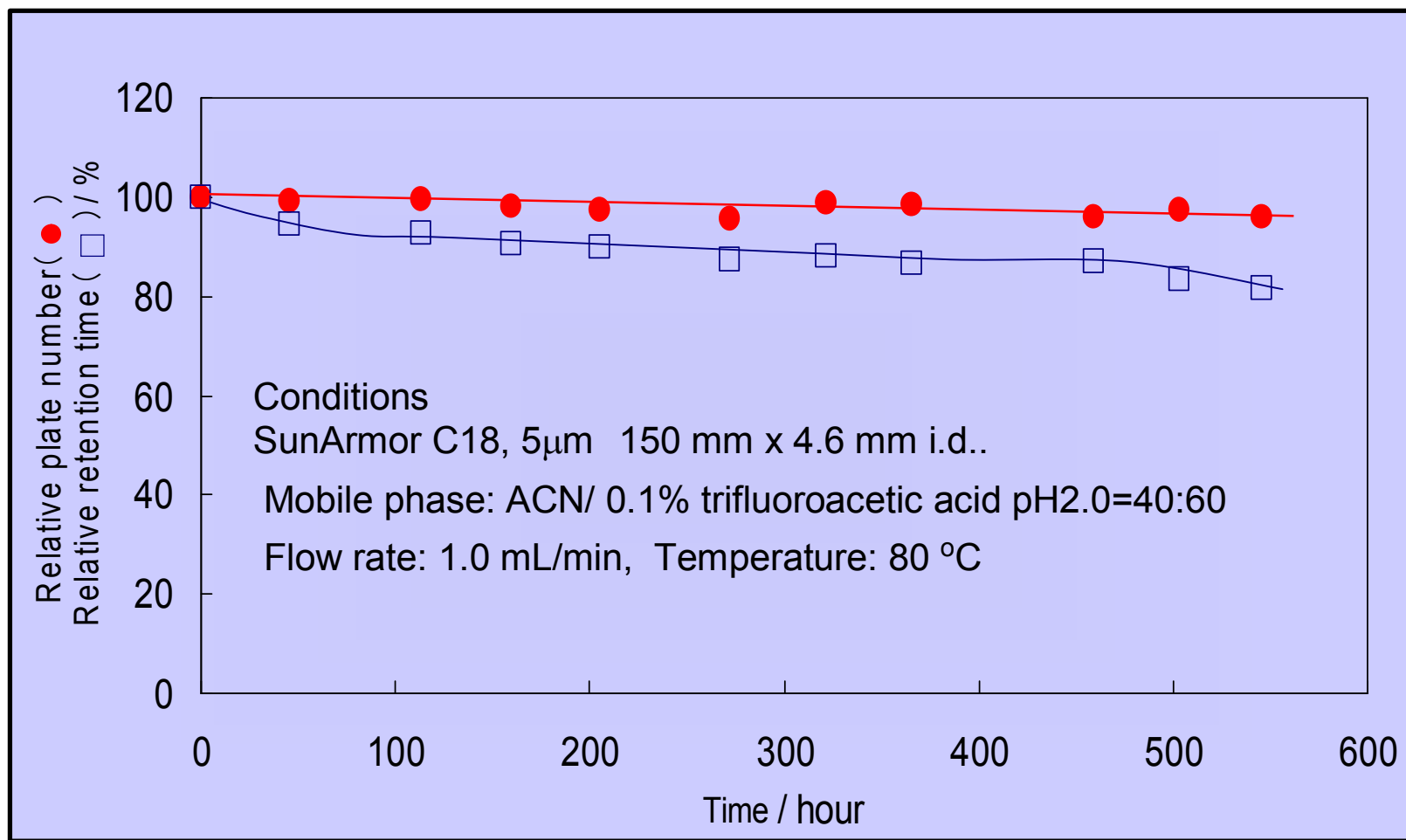
C18 silyl-reagent 2 Patent pending

(SunArmor C18)

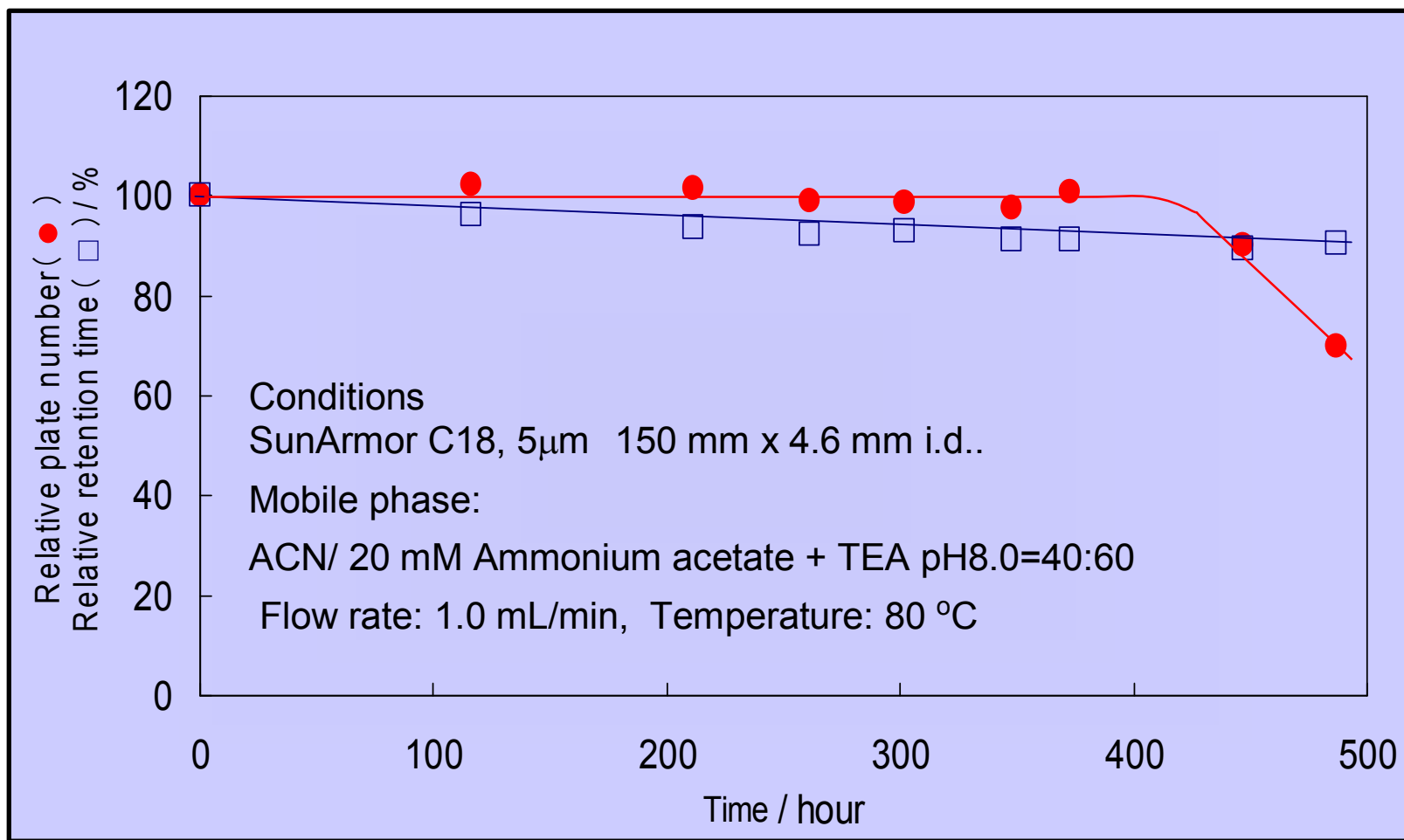


Used Silica gel: 12 nm, 340 m²/g, 5 μm

Stability at 80°C-pH 2



Stability at 80°C-pH 8.0



Conclusions

- Polyfunctional silyl-reagents were developed using C18 silyl reagent and end-capping reagent such as octadecyltrimethoxysilane, hexamethyldichlorotrisiloxane and octadecyltrichlorosilane, bis(triethoxysilyl)ethane.
- Functional group of HMODTS can bond with any silanol groups on silica surface.
- There is the least effect of residual silanol groups on proposed C18 stationary phase. And basic compounds can be separated well without any restriction concerned with a mobile phase.