Abstract Number: 2080 – 14P

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

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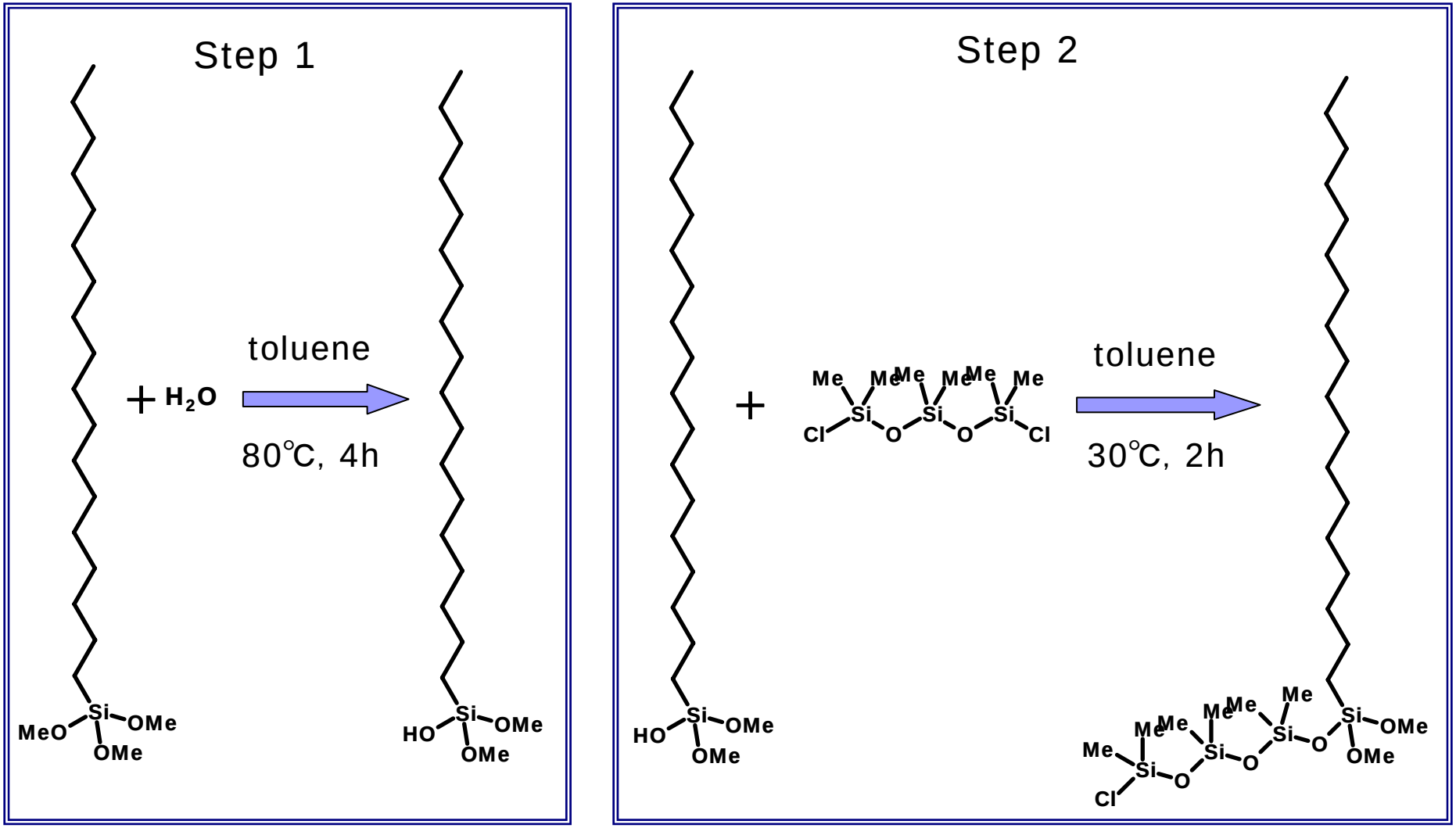
ABSTRACT

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 hexamehyltrisiloxane et al. In this study, polyfunctional silyl-reagent was synthesized with octadecyltrimethoxylsilane and hexamethyldichlorotrisiloxan. This reagent is called hexamethyloctadecyltetrasiloxane (HMODTS). C18 silica gel, which was bonded with this reagent and finally end-capped with trimethylchlorosilane, was evaluated to separate acidic and basic compounds. Stability of this phase was evaluated under both acidic and basic pH at high temperature. This phase showed symmetrical peaks of

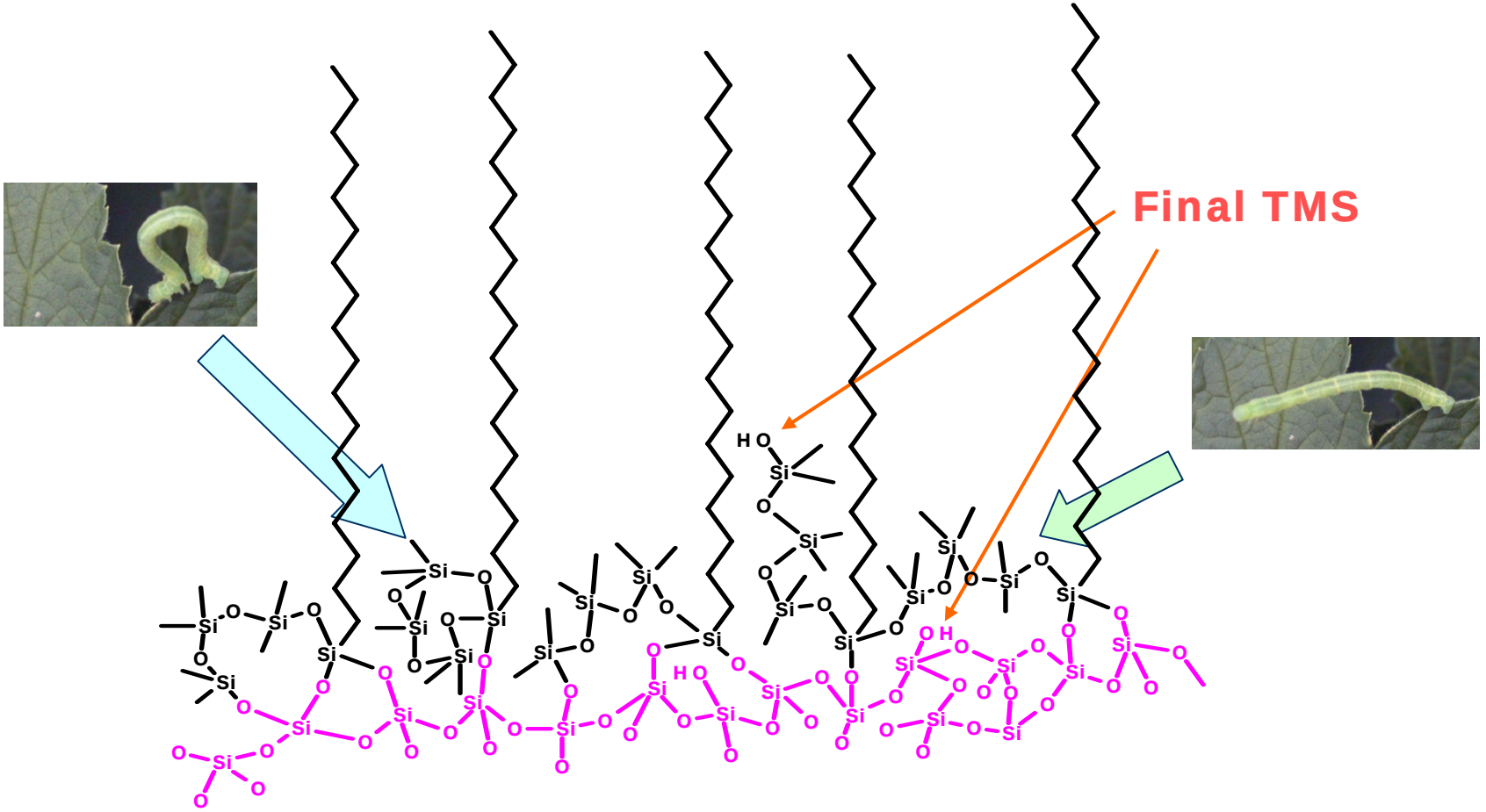
both acidic and basic compounds such as formic acid and amitriptyline. Especially a symmetric peak of amitriptyline was obtained even if both acetonitrile and ammonium acetate were used as a component of a mobile phase although most of C18 columns showed a terrible tailing peak of amitriptyline at the same conditions. Column life was more than 500 hours from pH 1.5 to pH 10 at 50 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 m^2/g, 5 μm

Carbon content after bonding HMODTS:

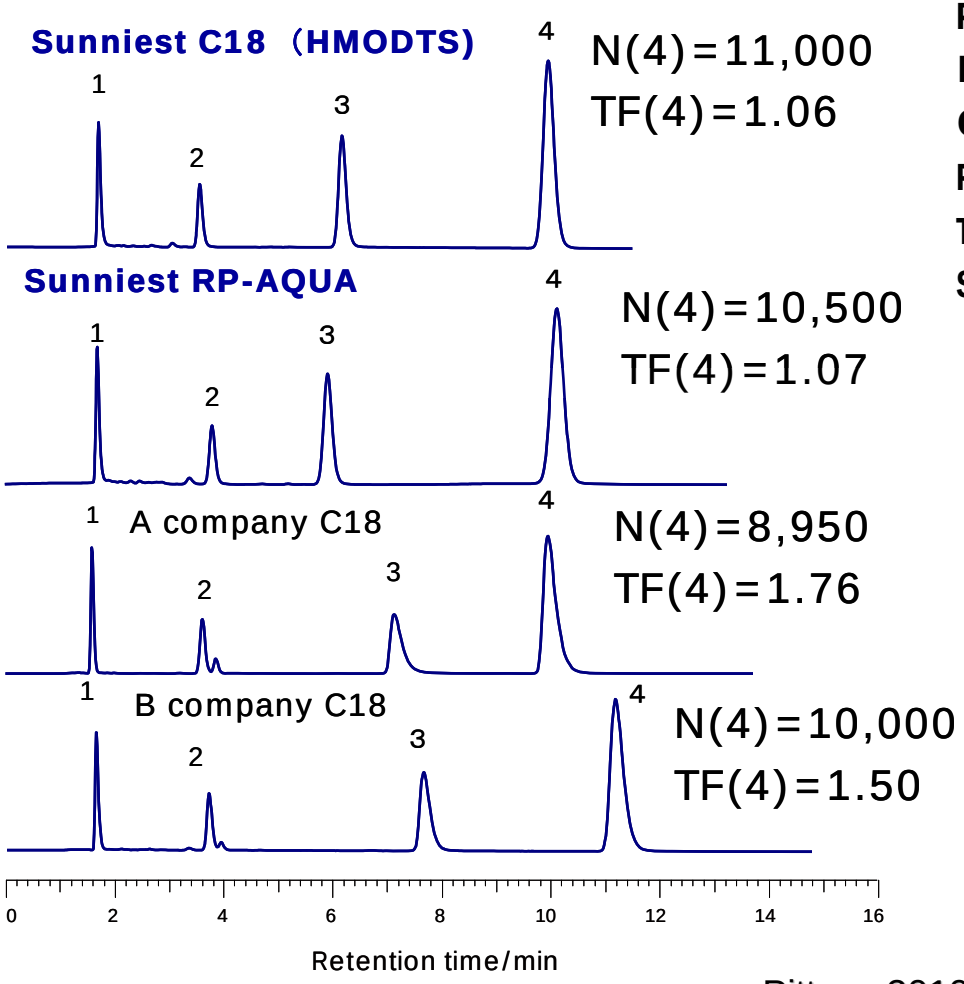
16.1%

Carbon content after final end-capping:

16.3%

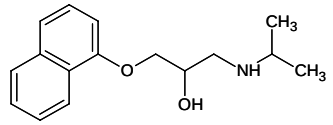
Evaluation of end-capping
Comparison of amitriptyline peak I

CH_3OH, pH7.5, 40 ºC

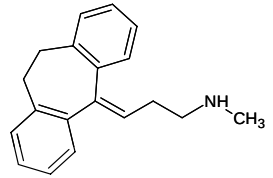


Column size: 150 X 4.6 mm
Particle size: 5 μm
Mobile phase:
CH_3OH/20mM Phosphate buffer pH7.5=80/20
Flow rate: 1.0 mL/min
Temperature: 40 ºC
Sample: 1 = Uracil

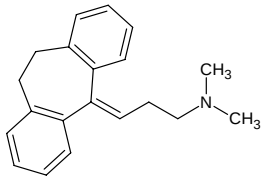
2 = Propranolol



3 = Nortriptyline

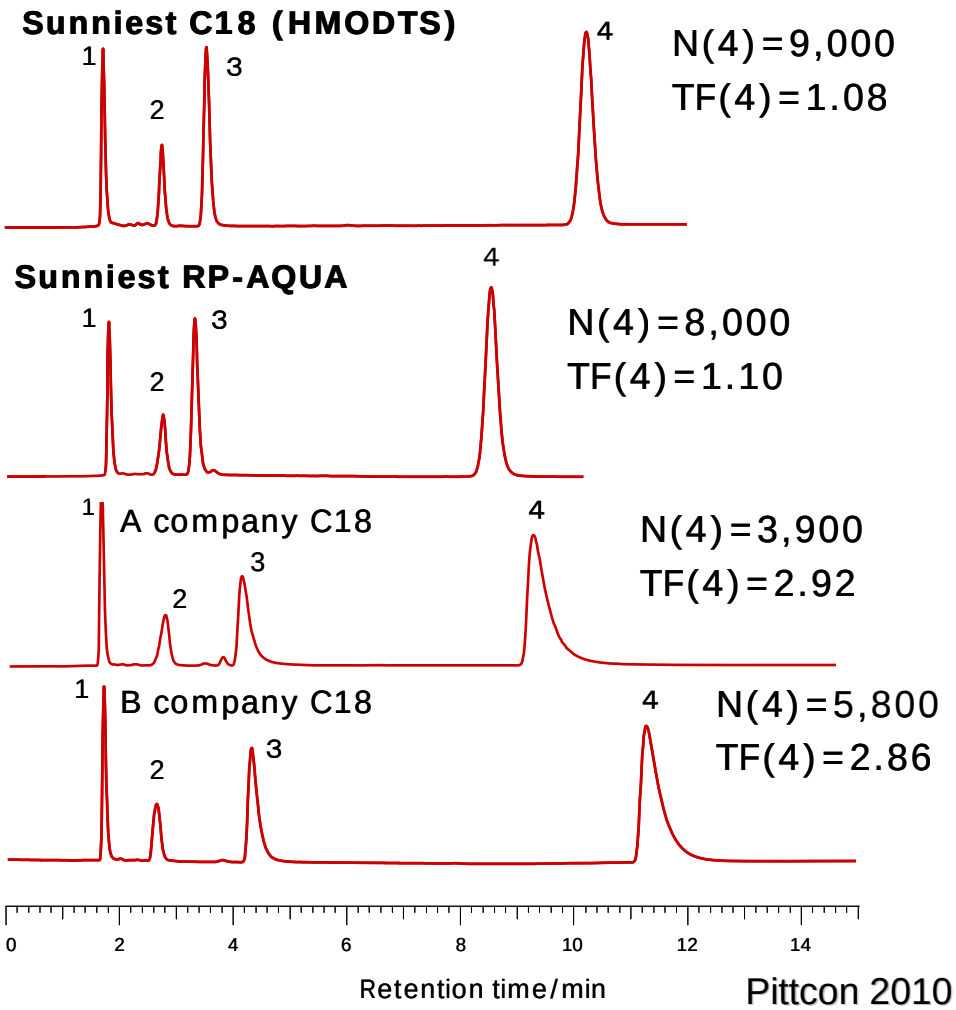


4 = Amitriptyline

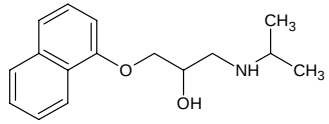


Evaluation of end-capping
Comparison of amitriptyline peak II

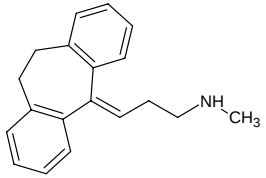
CH_3OH, pH6.0, 22 ºC



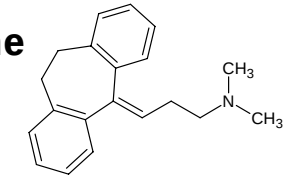
Column size: 150 X 4.6 mm
Particle size: 5 μm
Mobile phase:
CH_3OH/20mM Phosphate buffer pH6.0=80/20
Flow rate: 1.0 mL/min
Temperature: 22 ºC
Sample: 1 = Uracil
2 = Propranolol



3 = Nortriptyline

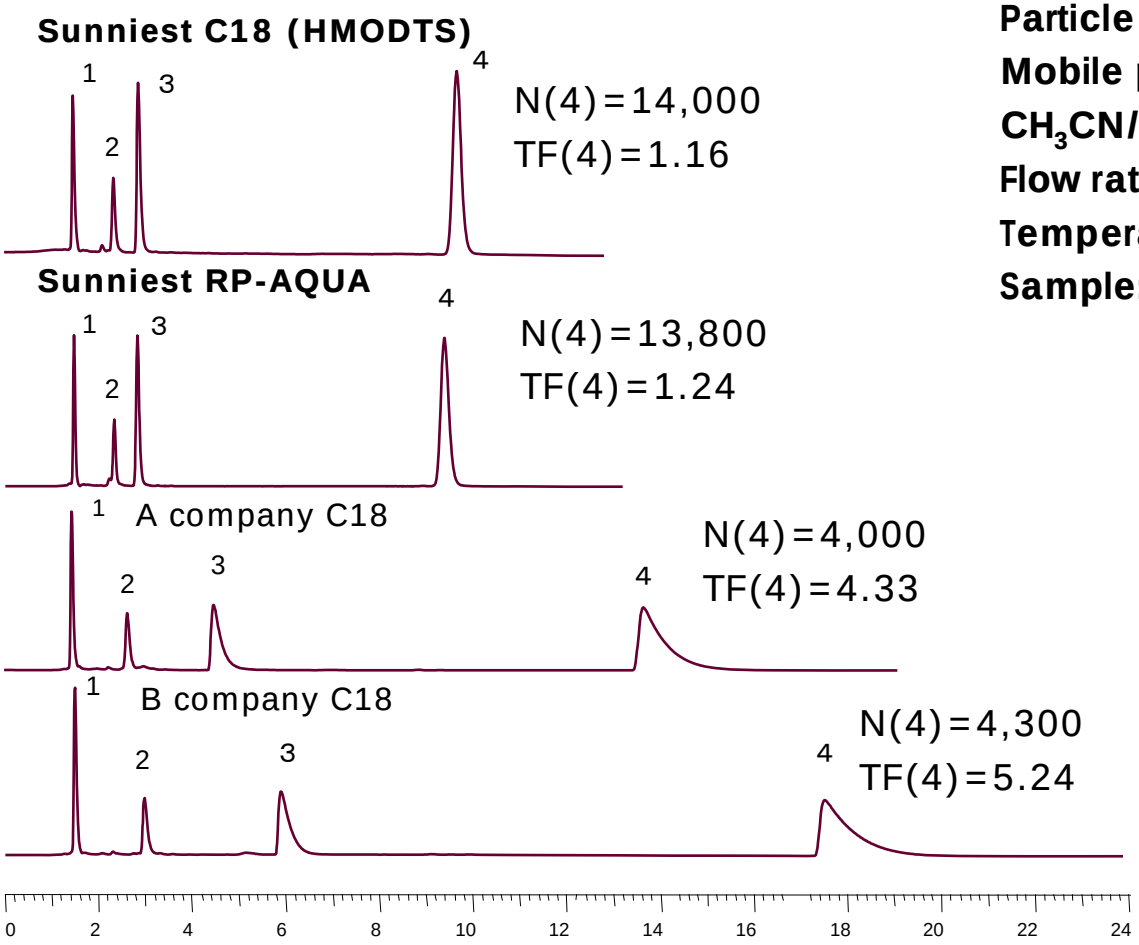


4 = Amitriptyline



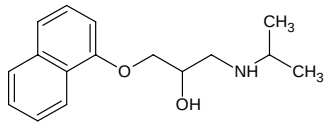
Evaluation of end-capping
Comparison of amitriptyline peak III-A

CH_3CN, pH7.0, 40 ºC

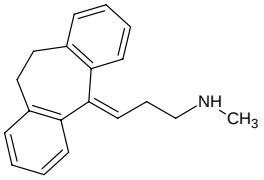


Column size: 150 X 4.6 mm
Particle size: 5 μm
Mobile phase:
CH_3CN/20mM Phosphate buffer pH7.0=60/40
Flow rate: 1.0 mL/min
Temperature: 40 ºC
Sample: 1 = Uracil

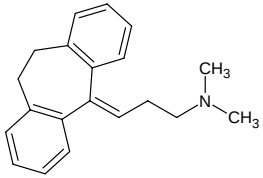
2 = Propranolol



3 = Nortriptyline



4 = Amitriptyline

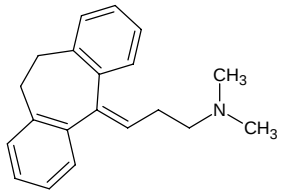


Evaluation of end-capping
Comparison of amitriptyline peak III-B

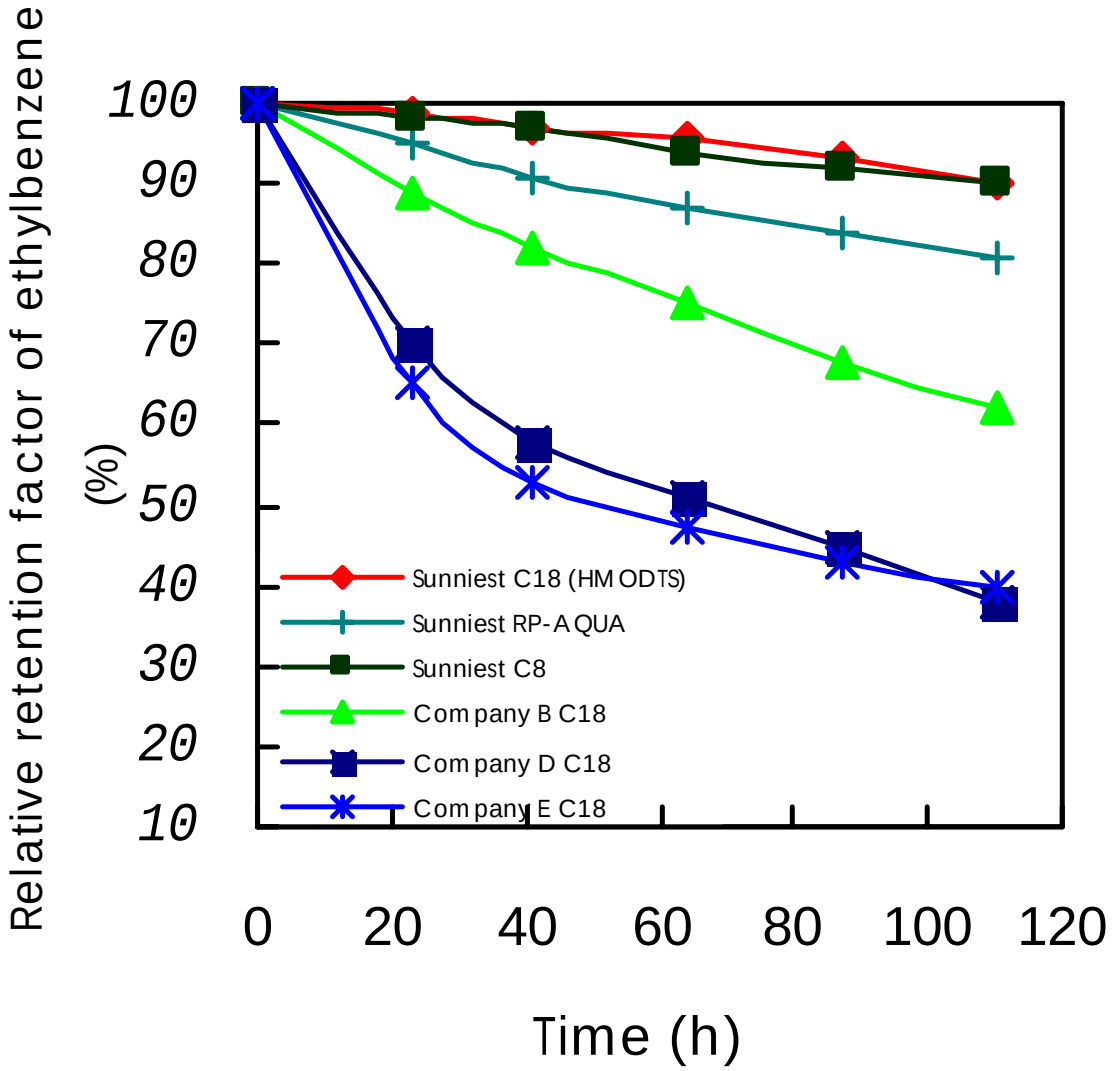
Column	TF	N
Sunniest C18(HMODTS)	1.16	14,000
Sunniest RP-AQUA	1.24	13,800
D1	5.19	3,300
D2	2.19	14,200
AT	3.25	5,300
S1	1.74	8,300
W1	1.97	10,600
WS2	1.59	10,100
W3	1.33	10,000
Japanese company A1 C18	3.07	8,500
Japanese company A2 C18	2.52	9,200
Japanese company B1 C18	2.23	50
Japanese company B2 C18	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 μm
Mobile phase:
CH_3CN/20mM Phosphate buffer pH7.0=60/40
Flow rate: 1.0 mL/min
Temperature: 40 ºC
Sample: Amitriptyline



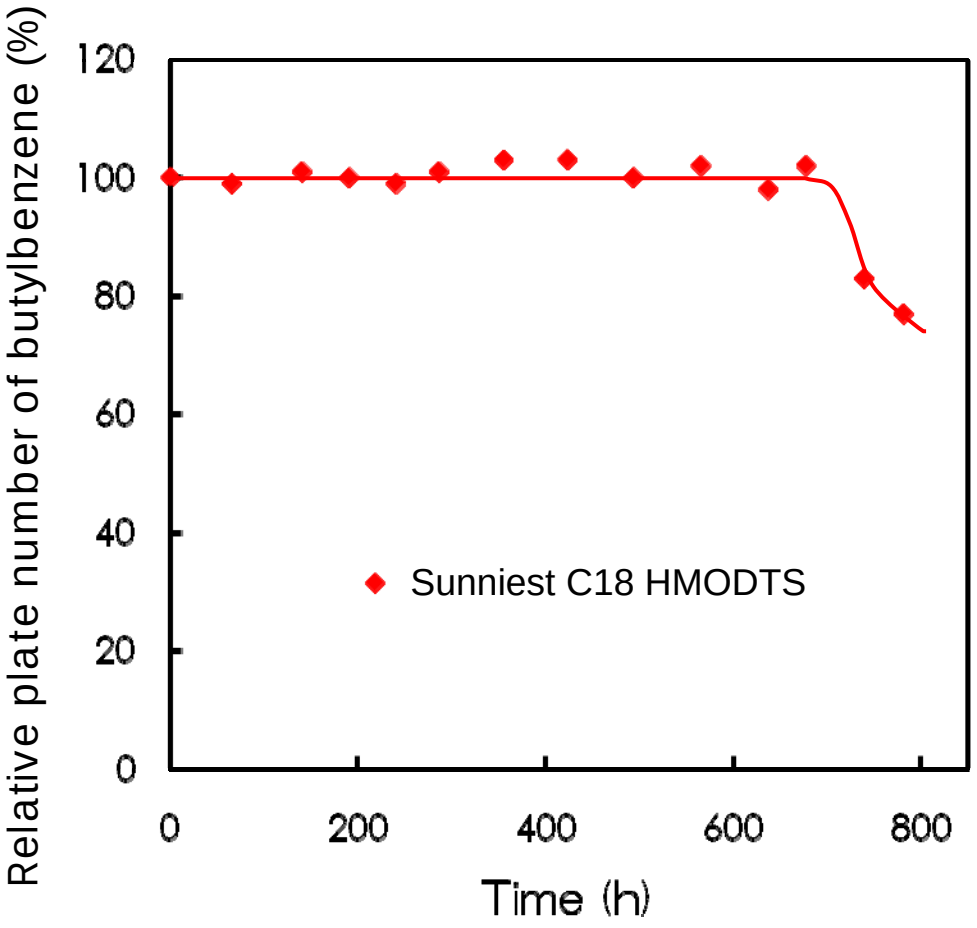
Stability under acidic condition



Test condition
Column size: 150 x 4.6 mm
Mobile phase:
CH_3CN/1.0% TFA, pH1=10/90
Flow rate: 1.0 mL/min
Temperature: 80 ºC

Measurement condition
Column size: 150 x 4.6 mm
Mobile phase:
CH_3CN/H_2O=60/40
Flow rate: 1.0 mL/min
Temperature: 40 ºC
Sample: 1 = Uracil
2 = Ethylbenzene

Stability under basic pH condition at 50 ºC



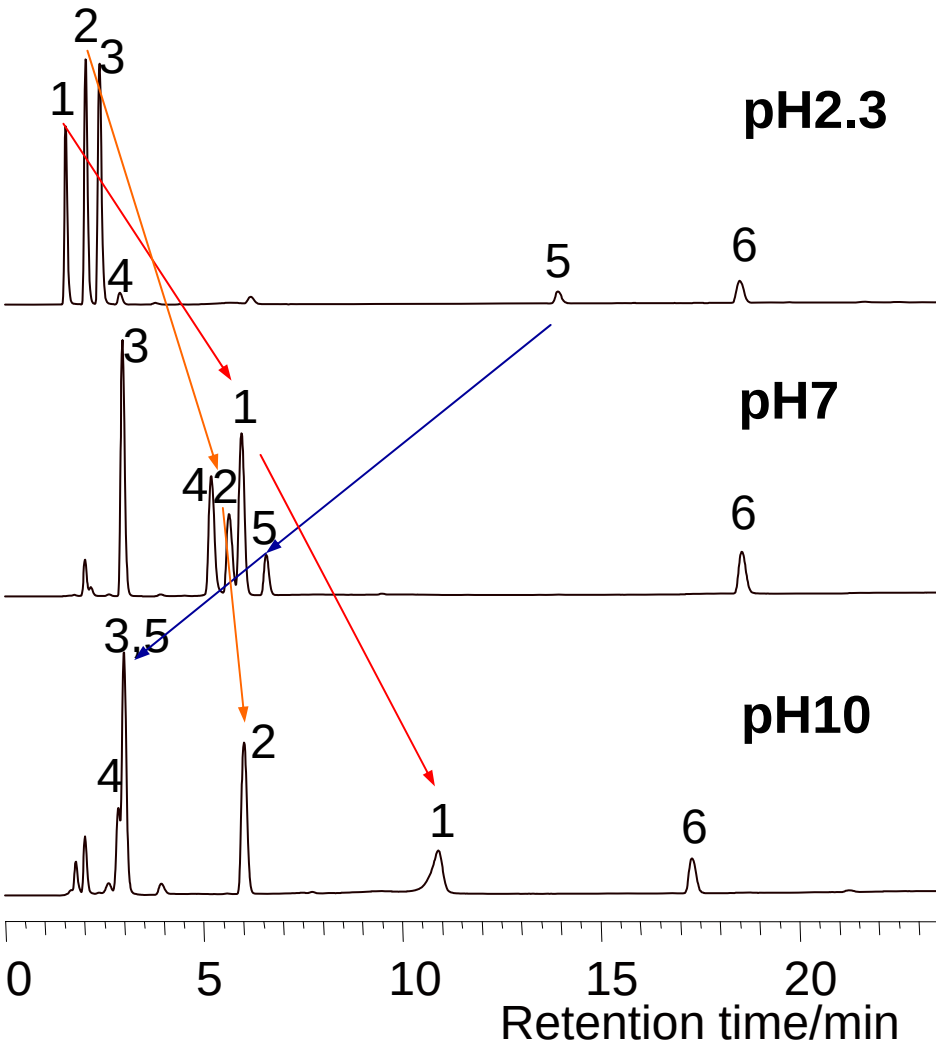
Durable test condition

Column: Sunniest C18 HMODTS, 5 µm
150 x4.6 mm
Mobile phase: CH_3OH/20mM Sodium borate /10mM NaOH=30/21/49 (pH10)
Flow rate: 1.0 mL/min
Temperature: 50 ºC

Measurement condition

Column: Sunniest C18 HMODTS, 5µm
150 x 4.6 mm
Mobile phase: CH_3OH/H_2O=75/25
Flow rate: 1.0 mL/min
Temperature: 40 ºC
Sample: 1 = Butylbenzene

pH selectivity

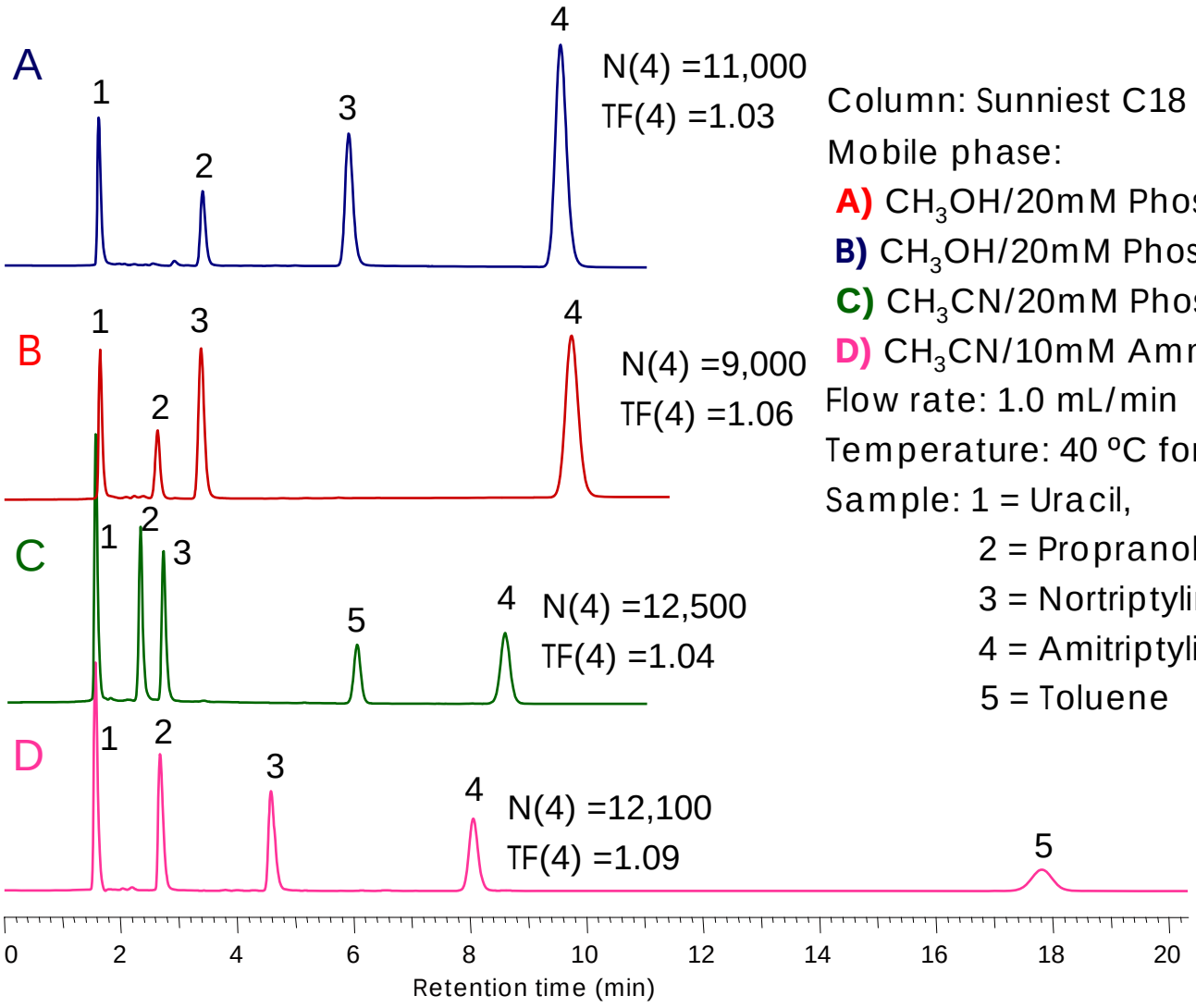


Column: Sunniest C18 HMODTS, 5 μm,
150 x 4.6 mm
Mobile phase:
A1) 20mM Phosphoric acid pH2.3
A2) 20mM Phosphate buffer pH7
A3) 20mM Phosphate buffer pH10
B) Acetonitrile

Time (min)	0	30
%B (%)	2	26

Flow rate: 1.0 mL/min
Temperature: 40 ºC
Detection: UV@250 nm
Sample: 1= Thiamine HCl Vitamin B_1
2 = Nicotinamide
3 = Nicotinic acid
4 = Pyridoxine HCl Vitamin B_6
5 = Folic acid
6 = Riboflavin Vitamin B_2

Comparison of 4 kinds of mobile phase



Column: Sunniest C18 HMODTS, 5 μm 150 x 4.6 mm

Mobile phase:

A) CH_3OH/20mM Phosphate buffer pH7.5 = 80/20

B) CH_3OH/20mM Phosphate buffer pH6.0 = 80/20

C) CH_3CN/20mM Phosphate buffer pH7.0 = 60/40

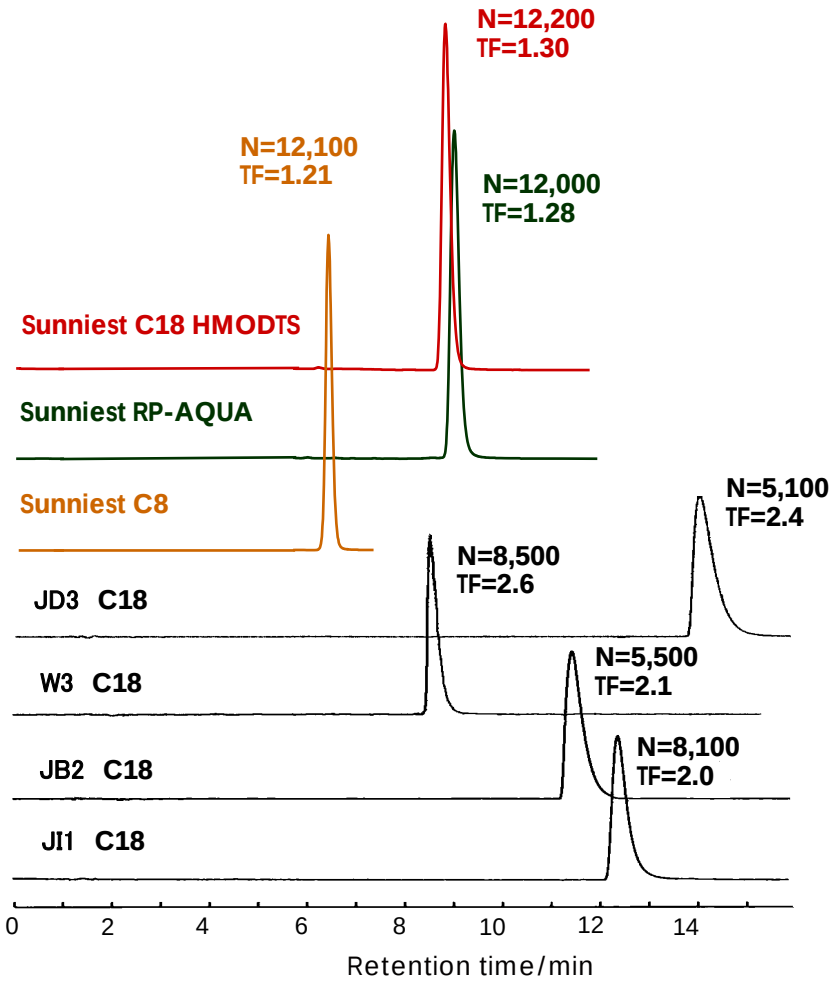
D) CH_3CN/10mM Ammonium acetate pH6.8 = 40/60

Flow rate: 1.0 mL/min

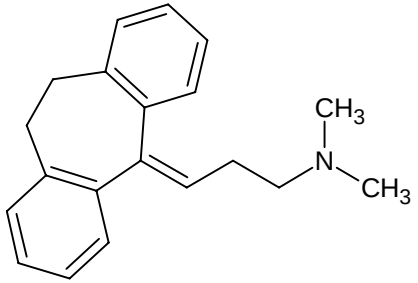
Temperature: 40 ºC for **A**, **C** and **D**, 22 ºC for **B**

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

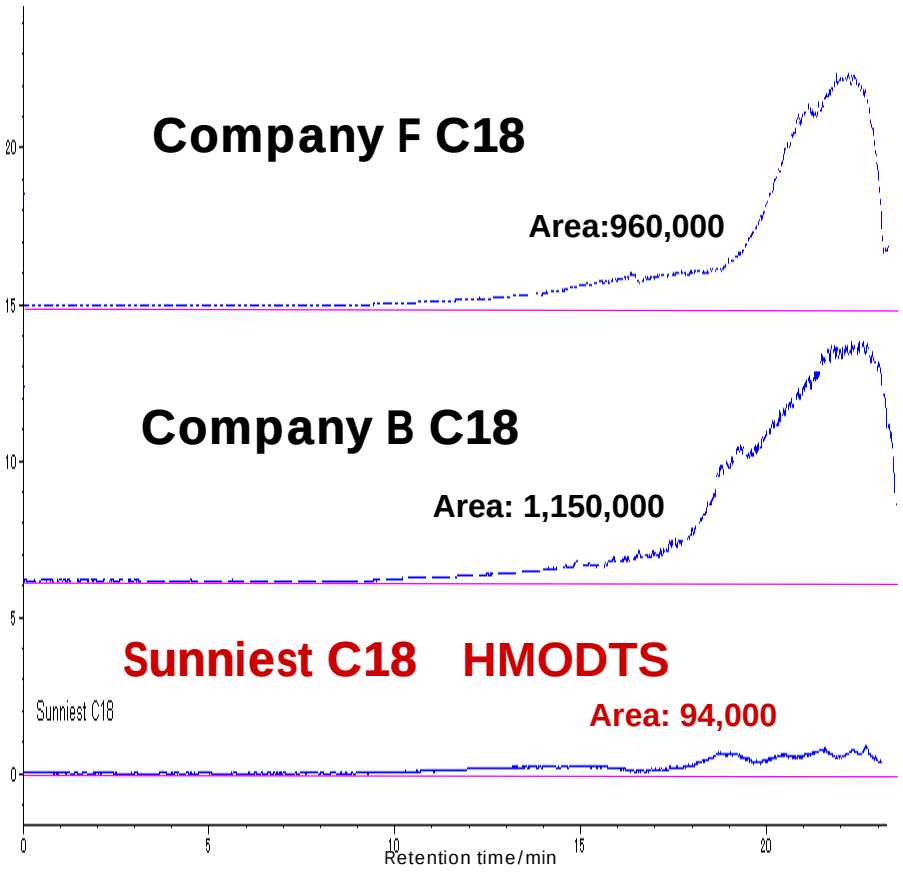
Comparison of amitriptyline peak using mobile phase for LC/MS



Column size: 150 x 4.6 mm
Particle size: 5 μm
Mobile phase: CH_3CN/10mM Ammonium acetate pH6.8=40/60
Flow rate: 1.0 mL/min
Temperature: 40 ºC
Sample: Amitriptyline



Bleeding Test I

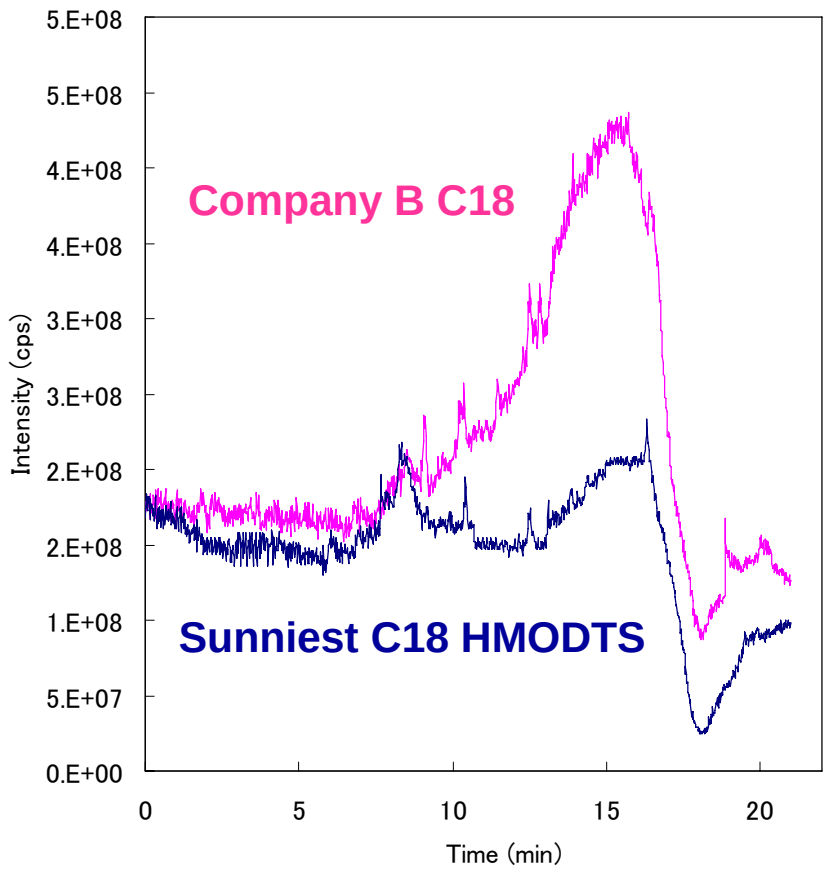


Column size: 150 x 2.0 mm
Mobile phase:
A) 0.1% acetic acid
B) CH_3CN
Gradient:

Time:	0min	3min	14.4min	18min	19min
%B:	5%	5%	100%	100%	5%

Flow rate: 0.2 mL/min
Temperature: 40 ºC
Detection: Corona CAD

Bleeding Test II



Column size: 150 x 2.0 mm
Mobile phase:
A) 0.1% acetic acid
B) CH_3CN
Gradient:

Time:	0min	3min	14.4min	18min	19min
%B:	5%	5%	100%	100%	5%

Flow rate: 0.2 mL/min
Temperature: 40 ºC
MS: ABI API-4000
Ionization: Turboionspray (cation)
Measurement mode:
Q1 Scan m/z 100-1000

2 μm particle
Narrow particle distribution

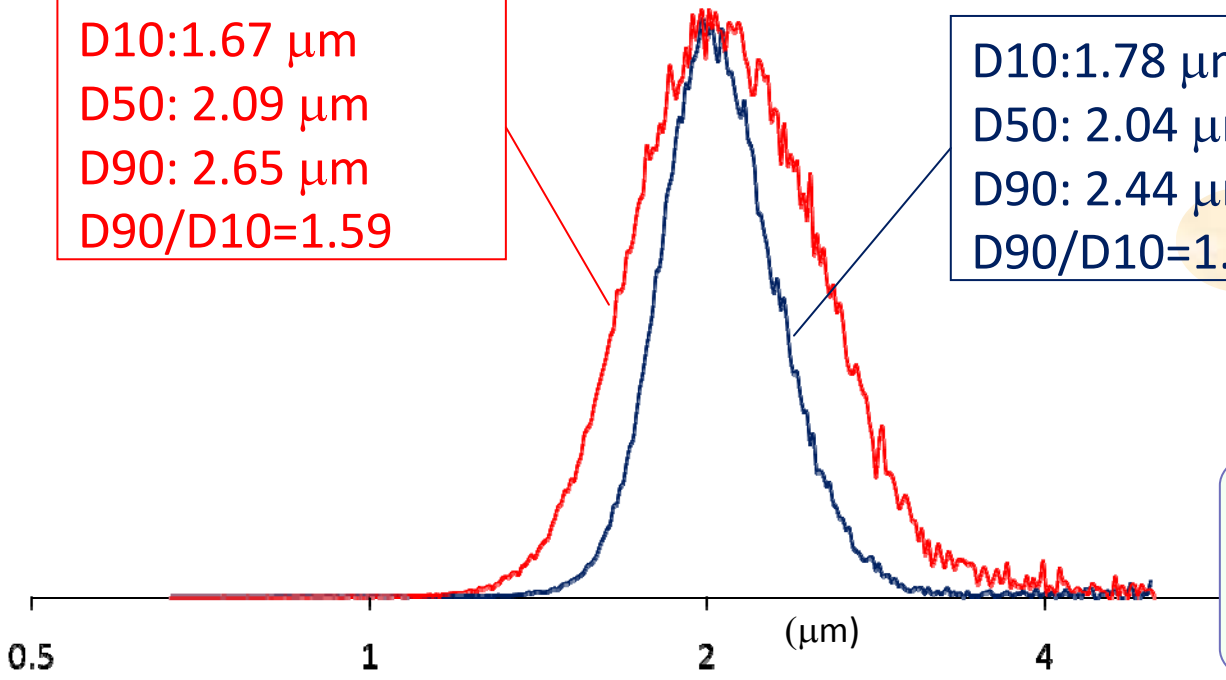
Measured by Coulter Counter method

Conventional particle

D10:1.67 μm
D50: 2.09 μm
D90: 2.65 μm
D90/D10=1.59

Particle used in this study

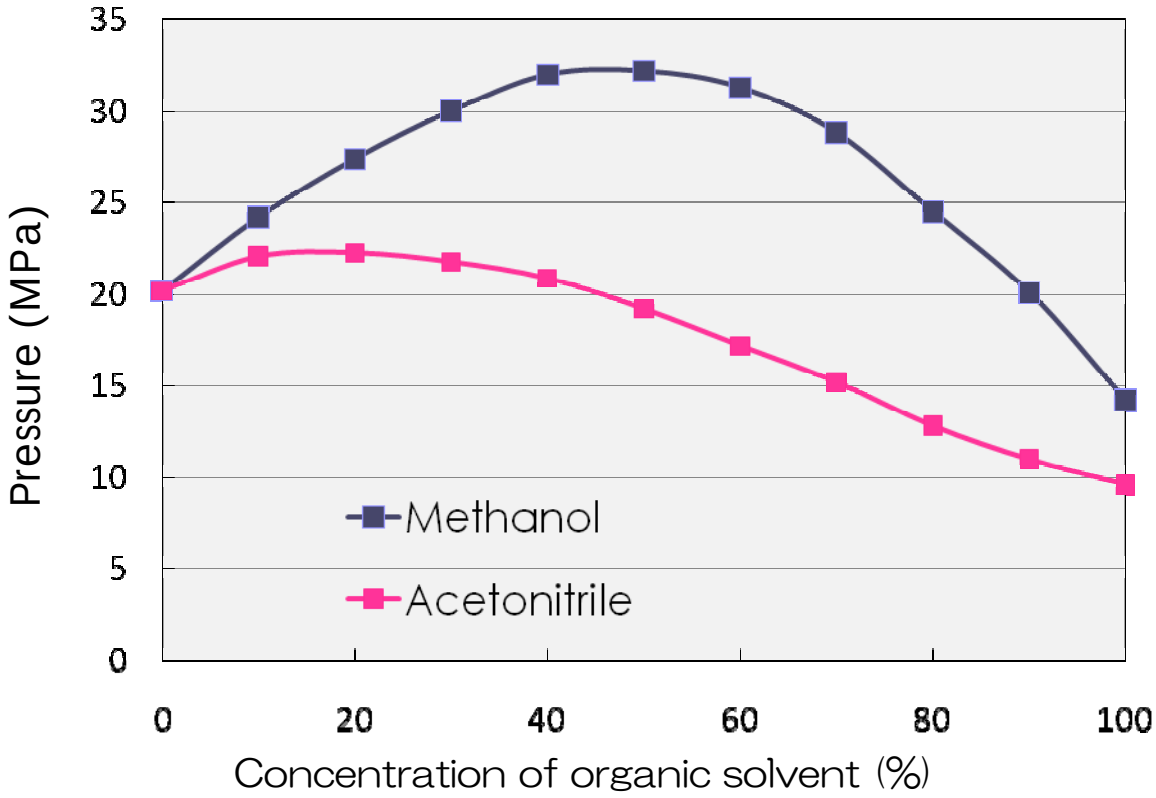
D10:1.78 μm
D50: 2.04 μm
D90: 2.44 μm
D90/D10=1.37



The narrowest particle distribution

20% volume was cut off from both sides respectively.

Column pressure using methanol or acetonitrile and water



Column: Sunniest C18 HMODTS , 2 μm 50 x 2.0 mm

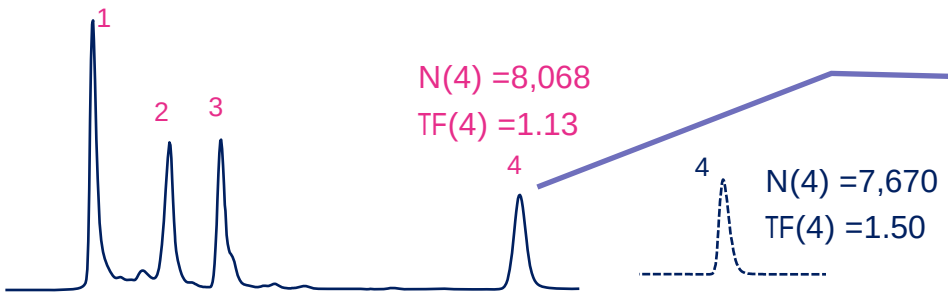
Mobile phase: CH_3OH/H_2O, CH_3CN/H_2O

Flow rate: 0.5 mL/min

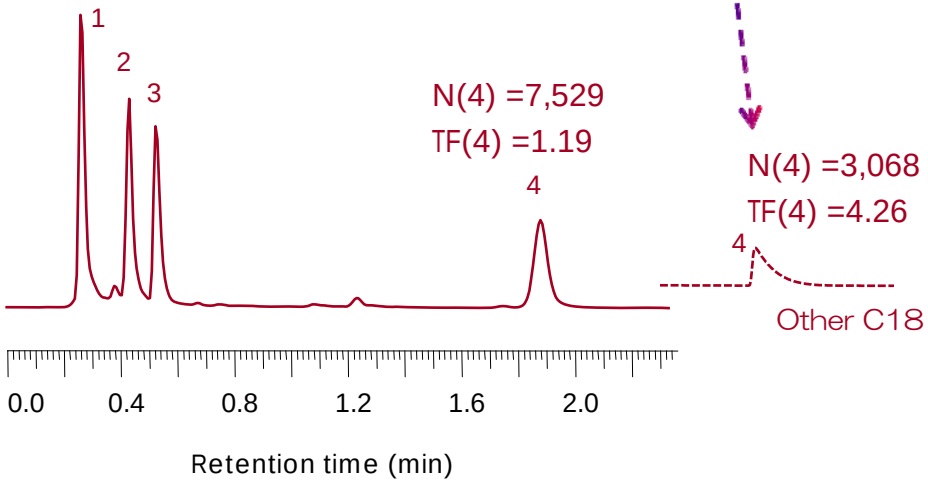
Temperature: 40 ºC

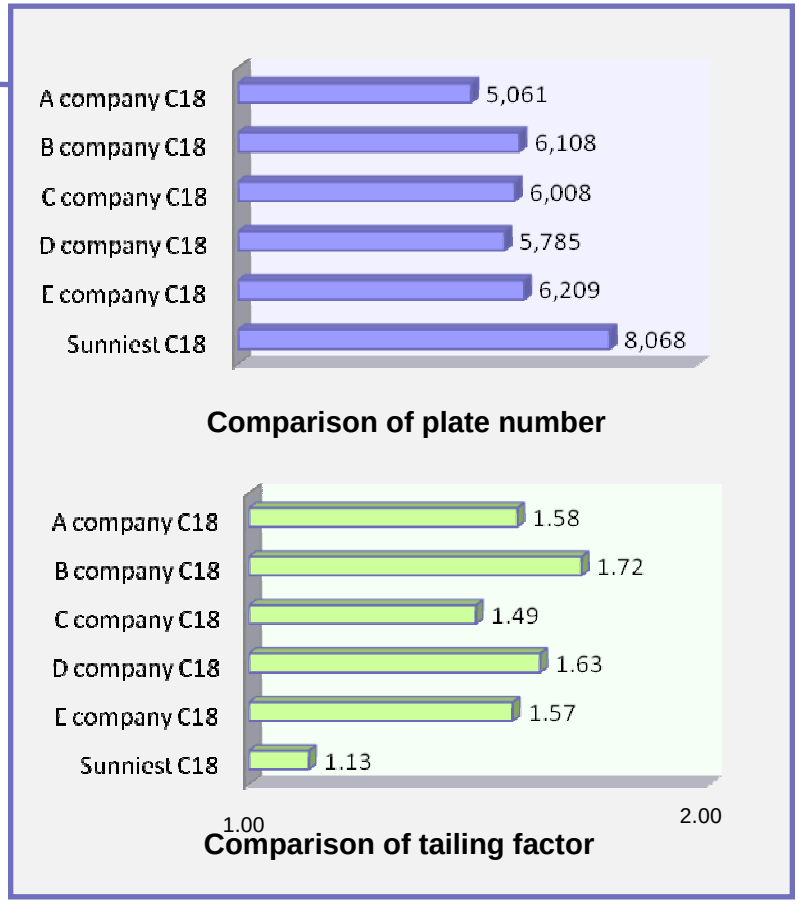
Evaluation of amitriptyline on 2 μm particle

A Methanol/20 mM phosphate buffer pH 7.5 = (80:20)



B Acetonitrilel/20 mM phosphate buffer pH 7.0 = (60:40

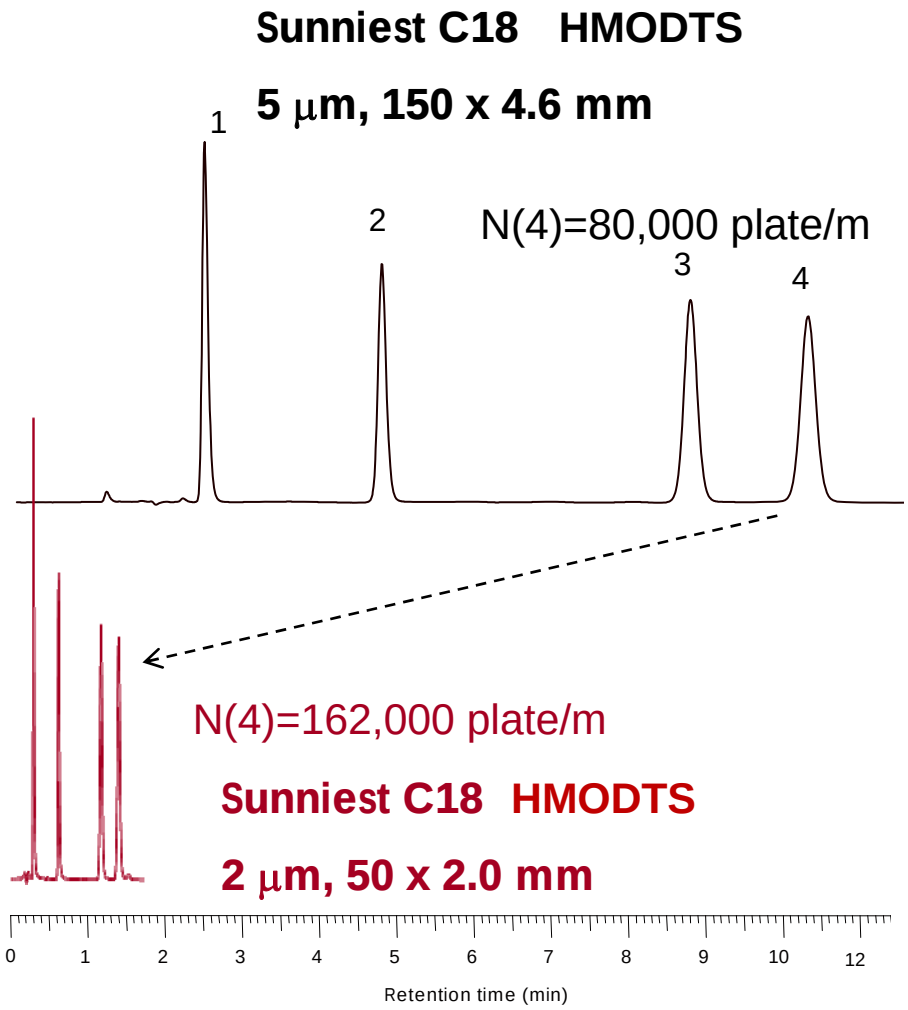




Column: Sunniest C18 HMODTS, 2 μm 50 x 2.0 mm, Temperature: 40 ºC

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

High throughput separation of analgesics



Mobile phase:

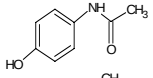
CH_3CN/0.1% Formic acid = 20/80

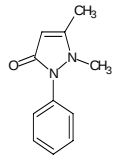
Flow rate: 1.0 mL/min for 150 x 4.6 mm ,

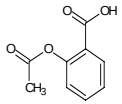
0.6 mL/min for 50 x 2.0 mm

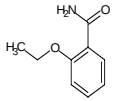
Temperature: 40 ºC

Detection: UV@230 nm

1  Acetaminophen

2  Antipyrine

3  Aspirin

4  Ethenzamide

Conclusions

- Polyfunctional silyl-reagents were developed using C18 silyl reagent and end-capping reagent such as octadecyltrimethoxylsilane, hexamethyldichlorotrisiloxane(HMODTS).
- 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.
- 2 μm particle was applied and same result was obtained.