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

Norikazu Nagae Ph.D, Kouji Yamamoto, Chiaki Kadota
ChromaNik Technologies Inc.
Email: info@chromanik.co.jp
Website: http://chromanik.co.jp
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 hexamethylene-trisiloxane et al. In this study, polyfunctional silyl-reagent was synthesized with octadecyltrimethoxysilane 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

Step 1

```
+ H₂O
```
toluene

```
80°C, 4h
```

Step 2

```
+ Hexamethyloctadecyltetrasilane
```
toluene

```
30°C, 2h
```

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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

CH$_3$OH, pH 7.5, 40 °C

Sunniest C18 (HMODTS)

Column size: 150 X 4.6 mm
Particle size: 5 μm
Mobile phase:
CH$_3$OH/20mM Phosphate buffer pH 7.5=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 ºC

Sunniest C18 (HMODTS)

1 2 3 4
N(4)=9,000
TF(4)=1.08

Sunniest RP-AQUA

1 2 3 4
N(4)=8,000
TF(4)=1.10

A company C18

1 2 3 4
N(4)=3,900
TF(4)=2.92

B company C18

1 2 3 4
N(4)=5,800
TF(4)=2.86

Column size: 150 X 4.6 mm
Particle size: 5 µm
Mobile phase: CH₃OH/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

Retention time/min

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

CH$_3$CN, pH7.0, 40 ºC

Sunniest C18 (HMODTS)

1.  
2.  
3.  
4.  

N(4) = 14,000
TF(4) = 1.16

Sunniest RP-AQUA

1.  
2.  
3.  
4.  

N(4) = 13,800
TF(4) = 1.24

A company C18

1.  
2.  
3.  
4.  

N(4) = 4,000
TF(4) = 4.33

B company C18

1.  
2.  
3.  
4.  

N(4) = 4,300
TF(4) = 5.24

Column size: 150 X 4.6 mm
Particle size: 5 µm
Mobile phase:
CH$_3$CN/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

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<td>A1</td>
<td>3.28</td>
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- **Column size:** 150 X 4.6 mm
- **Particle size:** 5 μm
- **Mobile phase:** CH$_3$CN/20mM Phosphate buffer pH7.0=60/40
- **Flow rate:** 1.0 mL/min
- **Temperature:** 40 ºC
- **Sample:** Amitriptyline

![Amitriptyline structure](image)
Stability under acidic condition

Test condition
- Column size: 150 x 4.6 mm
- Mobile phase: CH$_3$CN/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$_3$CN/H$_2$O =60/40
- Flow rate: 1.0 mL/min
- Temperature: 40 ºC
- Sample: 1 = Uracil
- 2 = Ethylbenzene

![Relative retention factor of ethylbenzene](chart)
Stability under basic pH condition at 50 ºC

Durable test condition

Column: Sunniest C18 HMODTS, 5 μm
150 x 4.6 mm
Mobile phase: CH₃OH/20mM Sodium borate /10mM NaOH=30/21/49 (pH10)
Flow rate: 1.0 mL/min
Temperature: 50 ºC

Measurement condition

Column: Sunniest C18 HMODTS, 5 μm
150 x 4.6 mm
Mobile phase: CH₃OH/H₂O=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 B₃
        2 = Nicotinamide
        3 = Nicotinic acid
        4 = Pyridoxine HCl  Vitamin B₆
        5 = Folic acid
        6 = Riboflavin  Vitamin B₂
Comparison of 4 kinds of mobile phase

Column: Sunniest C18 HMODTS, 5 μm 150 x 4.6 mm
Mobile phase:
A) CH₃OH/20mM Phosphate buffer pH7.5 = 80/20
B) CH₃OH/20mM Phosphate buffer pH6.0 = 80/20
C) CH₃CN/20mM Phosphate buffer pH7.0 = 60/40
D) CH₃CN/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: CH₃CN/10mM Ammonium acetate  
\[ pH 6.8 = 40/60 \]  
Flow rate: 1.0 mL/min  
Temperature: 40 °C  
Sample: Amitriptyline
Bleeding Test I

Column size: 150 x 2.0 mm
Mobile phase:
A) 0.1% acetic acid
B) CH₃CN
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

Company FC18
Area: 960,000

Company BC18
Area: 1,150,000

Sunniest C18 HMODTS
Area: 94,000
Bleeding Test II

Column size: 150 x 2.0 mm
Mobile phase:
A) 0.1% acetic acid
B) CH$_3$CN
Gradient:
Time: 0min 3min 14.4min 18min 19min
%B: 5% 5% 100% 100% 5%
Flow rate: 0.2 mL/min
Temperature: 40 °C
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

Pressure (MPa)

Concentration of organic solvent (%)

- Methanol
- Acetonitrile

Column: Sunniest C18 HMODTS, 2 μm 50 x 2.0 mm
Mobile phase: CH₃OH/H₂O, CH₃CN/H₂O
Flow rate: 0.5 mL/min
Temperature: 40 ºC

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Evaluation of amitriptyline on 2 μm particle

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

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

Comparison of plate number

Comparison of tailing factor

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

Sunniest C18 HMODTS
5 μm, 150 x 4.6 mm

Mobile phase:
CH₃CN/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

Sunniest C18 HMODTS
2 μm, 50 x 2.0 mm

N(4)=162,000 plate/m
N(4)=80,000 plate/m
Conclusions

- Polyfunctional silyl-reagents were developed using C18 silyl reagent and end-capping reagent such as octadecyltrimethoxysilane, 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.