Study of Secondary Interaction Based on Residual Silanol Groups for Reversed-Phase Liquid Chromatography

ChromaNik Technologies Inc.

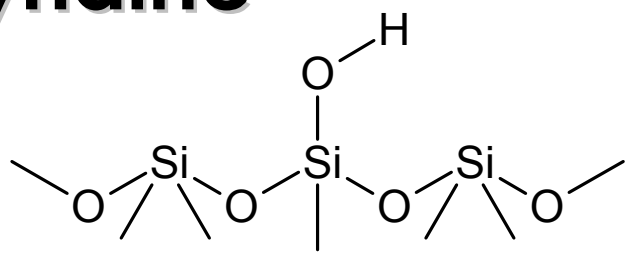
Norikazu Nagae Ph.D

abstract

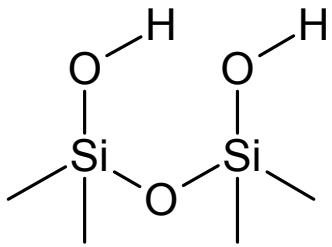
It has been said that residual silanol groups on C18 silica stationary phase yielded peak tailing for basic compounds. A lot of efforts have been exerted for reducing effect of residual silanol groups by an end-capping or embedding a polar group in an alkyl chain. Consequently, a sharp peak of a basic compound has been obtained although retention of a basic compound has been getting little. On the other hand, on hydrophilic interaction chromatography (hilic) mode, silica column is recently used under a mixture of organic solvent and buffer solution, which is the same as a mobile phase used for reversed-phase liquid chromatography. In this mode, a peak shape of a basic compound is good not tailing. In other words a silanol group on a silica stationary phase is not a cause for tailing of a basic compound, at least on hilic mode.

We found that a silanol group controlled its activity by the heat treatment didn't make a basic compound tailing even if it existed on the C18 reversed-phase. Secondary interaction based on residual silanol groups for reversed-phase liquid chromatography was evaluated using C18 stationary phase with silanol groups controlled its activity. It was observed that retention of basic compounds and polar compounds was several times larger than that using a conventional end-capped C18 silica stationary phase, furthermore it dropped as pH value of a mobile phase decreased and salt concentration in a mobile phase increased while retention of neutral compounds didn't change completely. It is considered that an ion exchange interaction occurred clearly by residual silanol groups on C18 silica stationary phase. As a result, a residual silanol group controlled its activity could change selectivity of basic compounds without peak tailing.

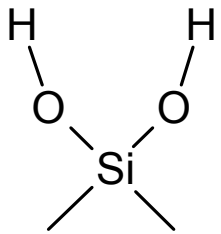
Form of silanol groups and peak shape of pyridine



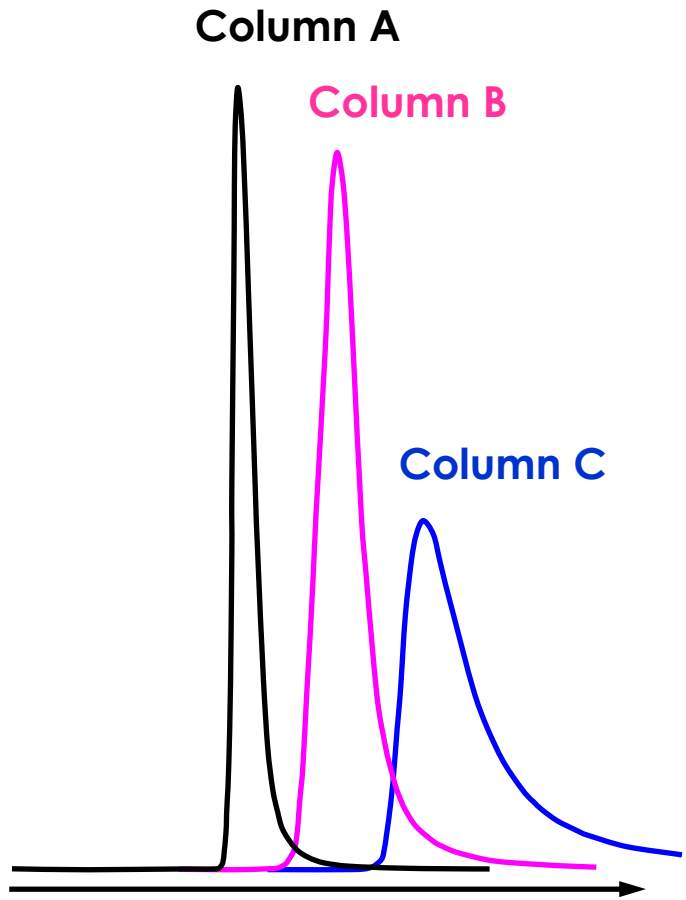
Isolated Silanol



Vicinal Silanol



Geminal Silanol



Pyridine peak

60% CH_3OH/H_2O

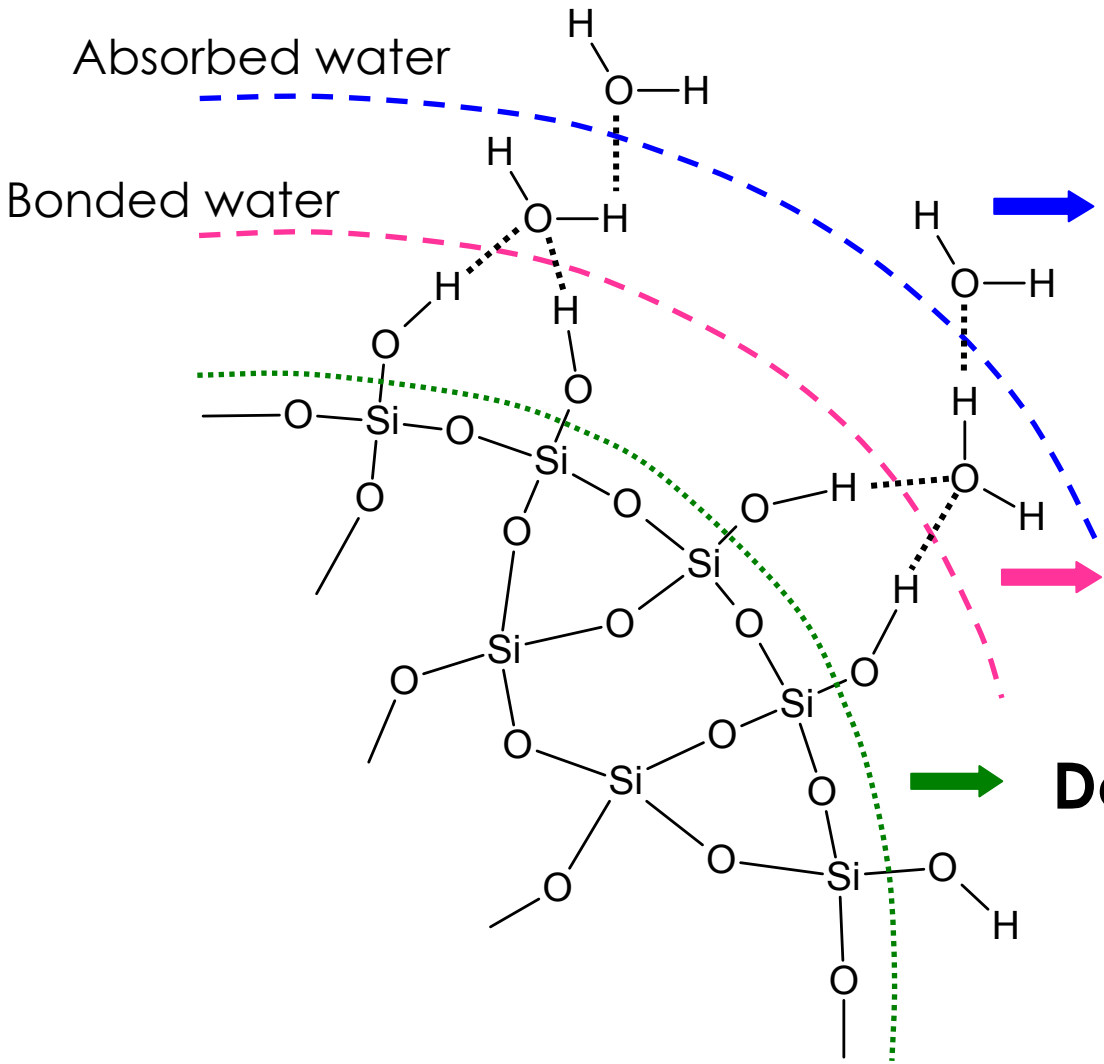
Our hypothesis

- Peak tailing of a basic compound is caused by the state of silanol groups, not by existence of silanol groups on silica surface.
- Silanol groups near a strong hydrophobic site don't occur an ion-exchange interaction etc. rapidly.

Consequence

- Decrease density of alkyl groups and make hydrophobicity low.
- Control activity of silanol groups near only hydrophobic site

Variation of water on silica surface



Desorption of absorbed water (105 ~ 150 °C)

Desorption of bonded water (400 °C)

Desorption of silanol group (800 °C)

Change of silanol groups by heat treatment

- Condensation of silanol group starts from near 100 °C.
- Vacuum heat treatment makes an amount of silanol group low by water deprivation and siloxane produces.
- Under 400 °C, siloxane reverts silanol groups by rehydration.
- Over 400 °C, rehydration do not occur.

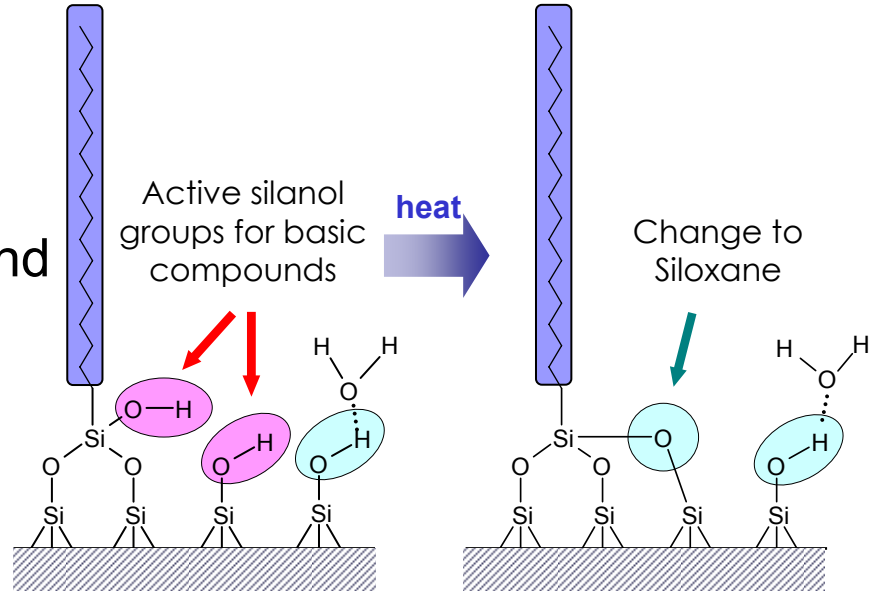
Control of silanol group

After heat treatment

Siloxane near hydrophobic site (C18) is kept at the same state and no silanol goup produces by rehydration.

Prevention of tailing for basic compound

Siloxane away from hydrophobic site is rehydrated. Consequently silanol group produces and it is unaffected by hydrophobic site.

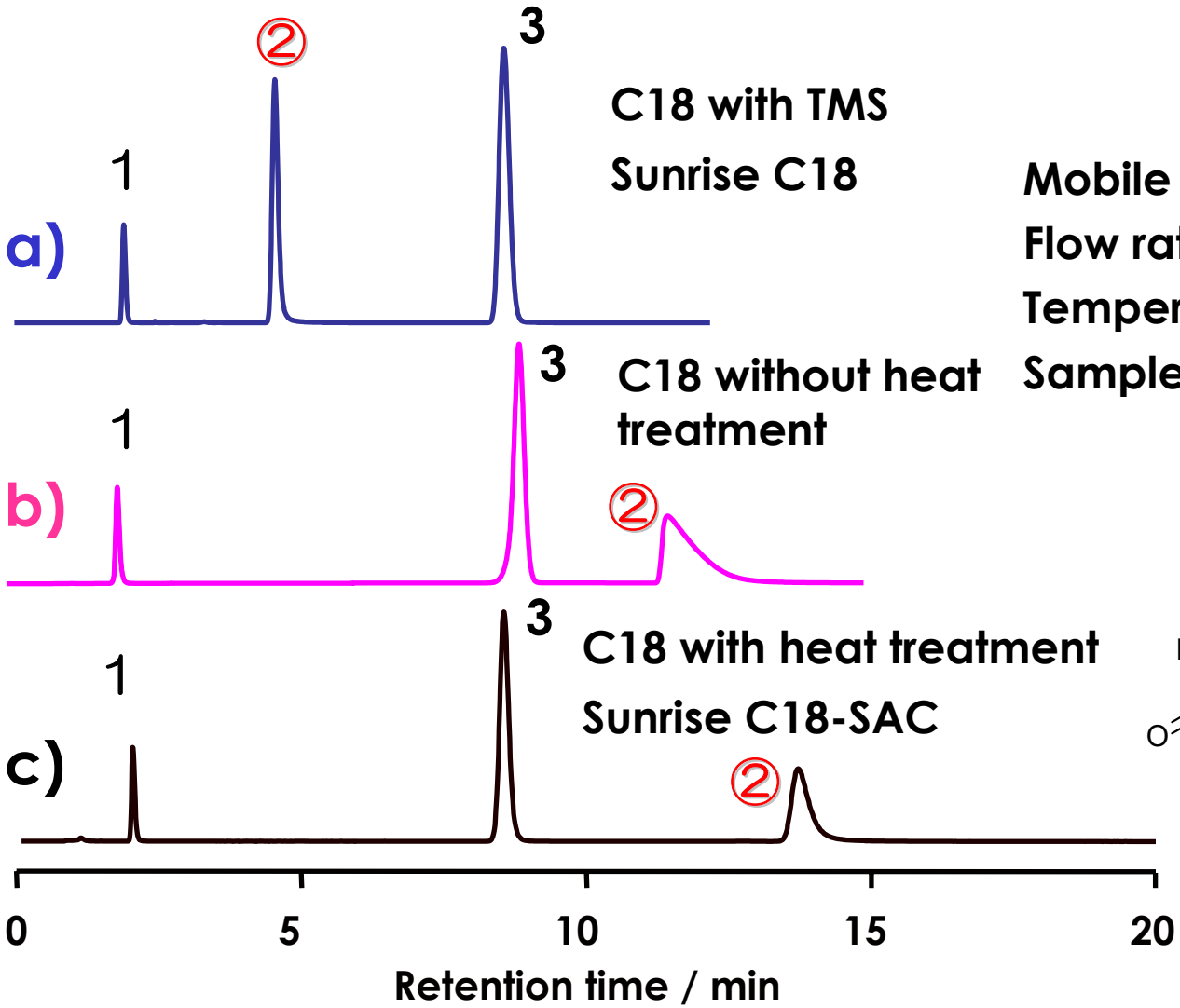


Increase of retention of polar and basic compounds, in spite of no change of retention of a neutral compound

Experiment

- **Silica gel**
  - Pore diameter: 12 nm, Specific surface area: 340 m^2/g, Particle size: 5 μm
- **Bonded Octadecylsilane**
  - Carbon content: 14%
  - As reference, prepareC18 phase with end-capping (TMS)
- **Heat treatment in a vacuum**
  - Temperature: 200 °C, Treatment time: 48 hours
  - As reference, prepare C18 phase without heat treatment
- **Evaluation**
  - Column: Sunrise C18-SAC (with heat treatment)
    Sunrise C18 (with end-capping)
  - Column dimension: 150 mm x 4.6 mm i.d.
  - Mobile phase: acetonitrile or methanol/buffer solution

Comparison of pyridine peak shape



Mobile phase: 30% CH_3OH/H_2O

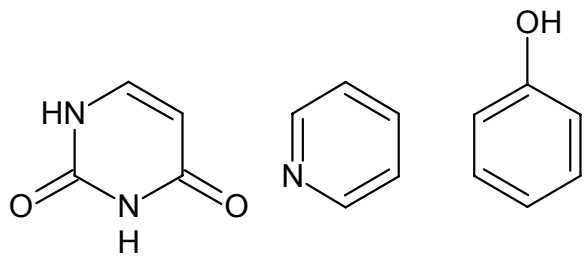
Flow rate: 1.0 mL/min

Temperature: 40 °C

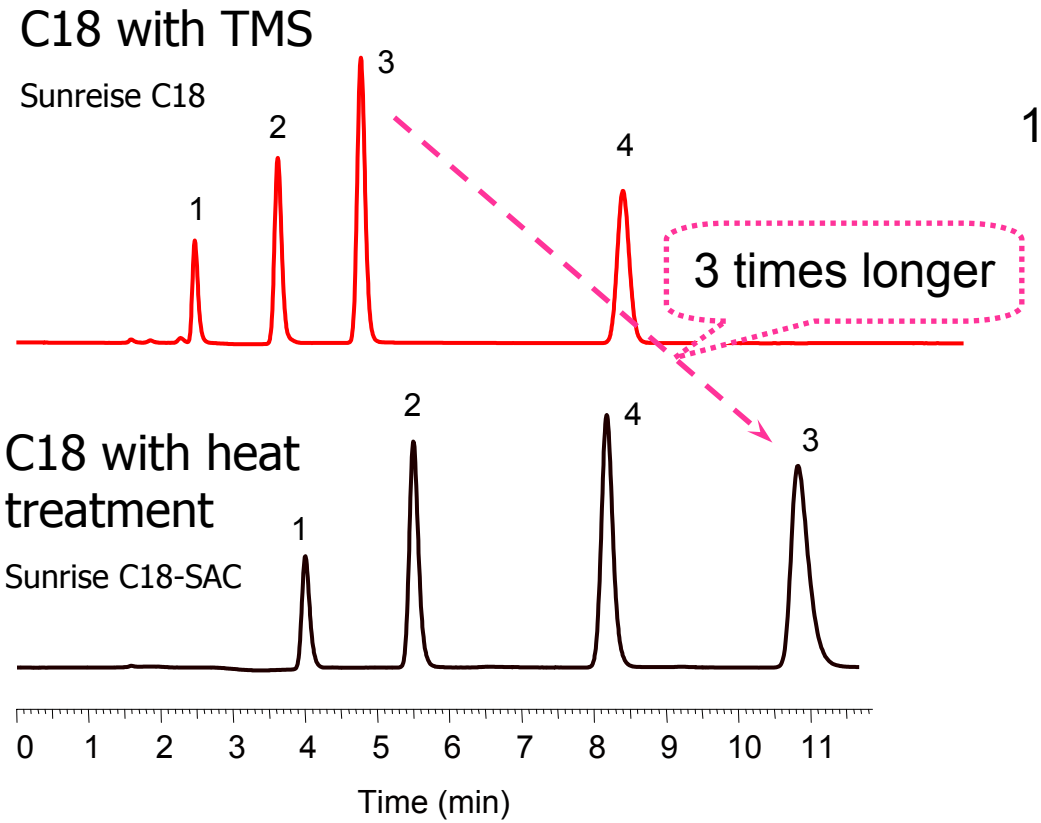
Sample: 1=Uracil

②=Pyridine

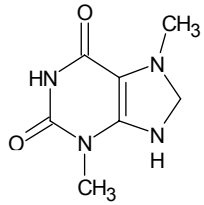
3=Phenol



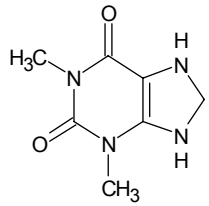
Separation of xanthine



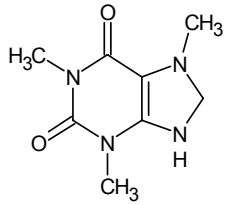
1= Theobromine



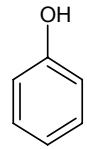
2= **Theophylline**



3= Caffeine



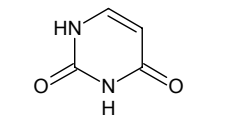
4= **Phenol**

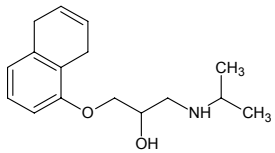


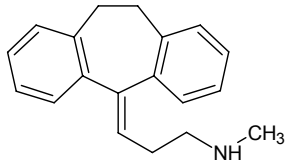
Column dimension: 150 x 4.6 mm
Mobile phase: Methanol/20mM Phosphate buffer (pH4.5)=(30:70)
Flow rate: 1.0 mL/min
Temperature: 40 °C

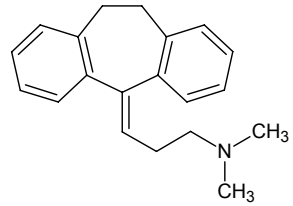
Separation of antidepressants

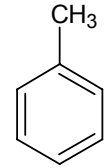
1=Uracil **2=Propranolol** **3=Nortriptyline** **4=Amitriptyline** **5=Toluene**











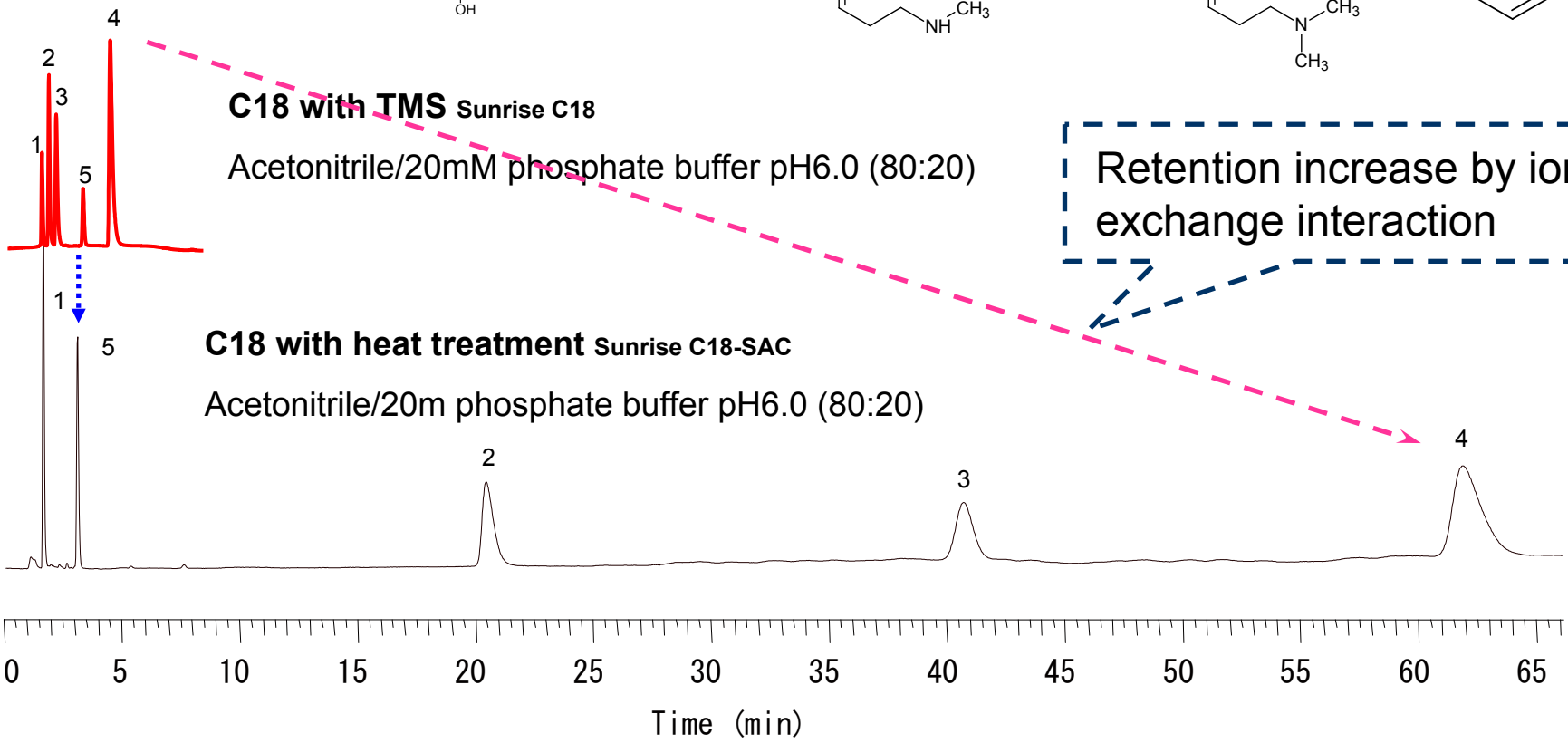
C18 with TMS Sunrise C18

Acetonitrile/20mM phosphate buffer pH6.0 (80:20)

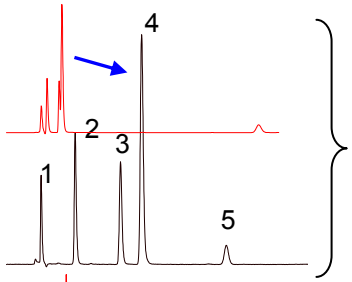
Retention increase by ion-exchange interaction

C18 with heat treatment Sunrise C18-SAC

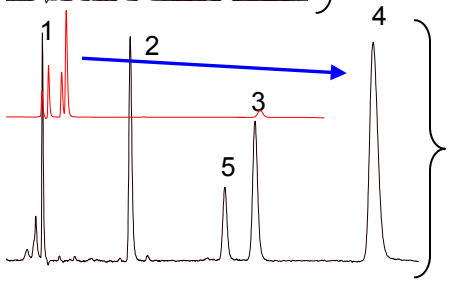
Acetonitrile/20m phosphate buffer pH6.0 (80:20)



Control of retention by mobile phase pH

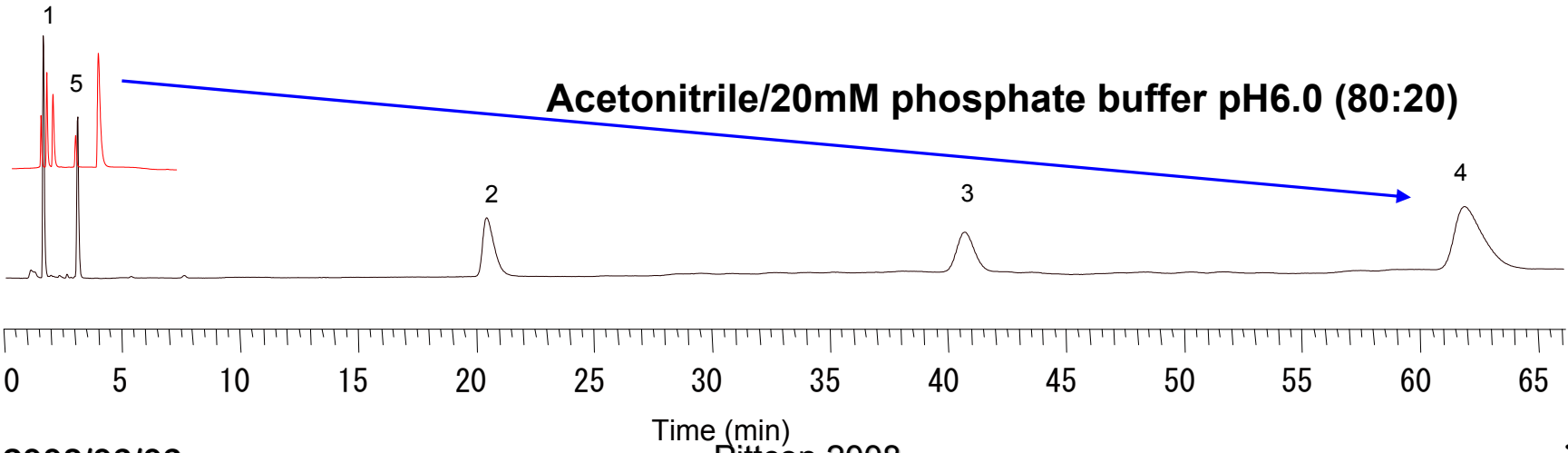


Acetonitrile/20mM phosphate buffer pH3.0 (50:50)

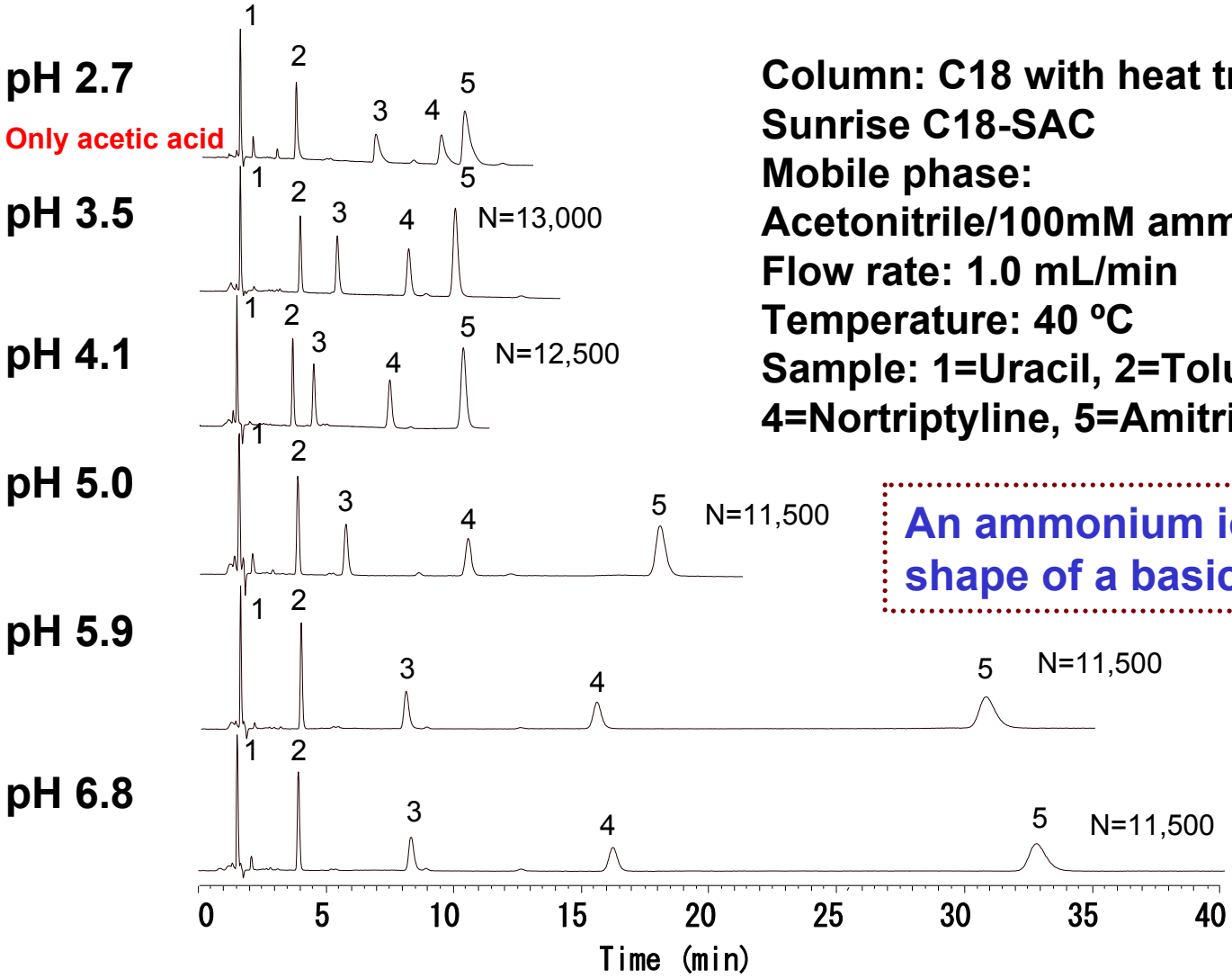


Acetonitrile/20mM phosphate buffer pH4.5 (50:50)

Column: C18 with TMS, Sunrise C18, C18 with heat treatment, Sunrise C18-SAC
Flow rate: 1.0 mL/min
Temperature: 40 ºC
Sample: 1=Uracil, 2=Propranolol, 3=Nortriptyline, 4=Amitriptyline, 5=Toluene



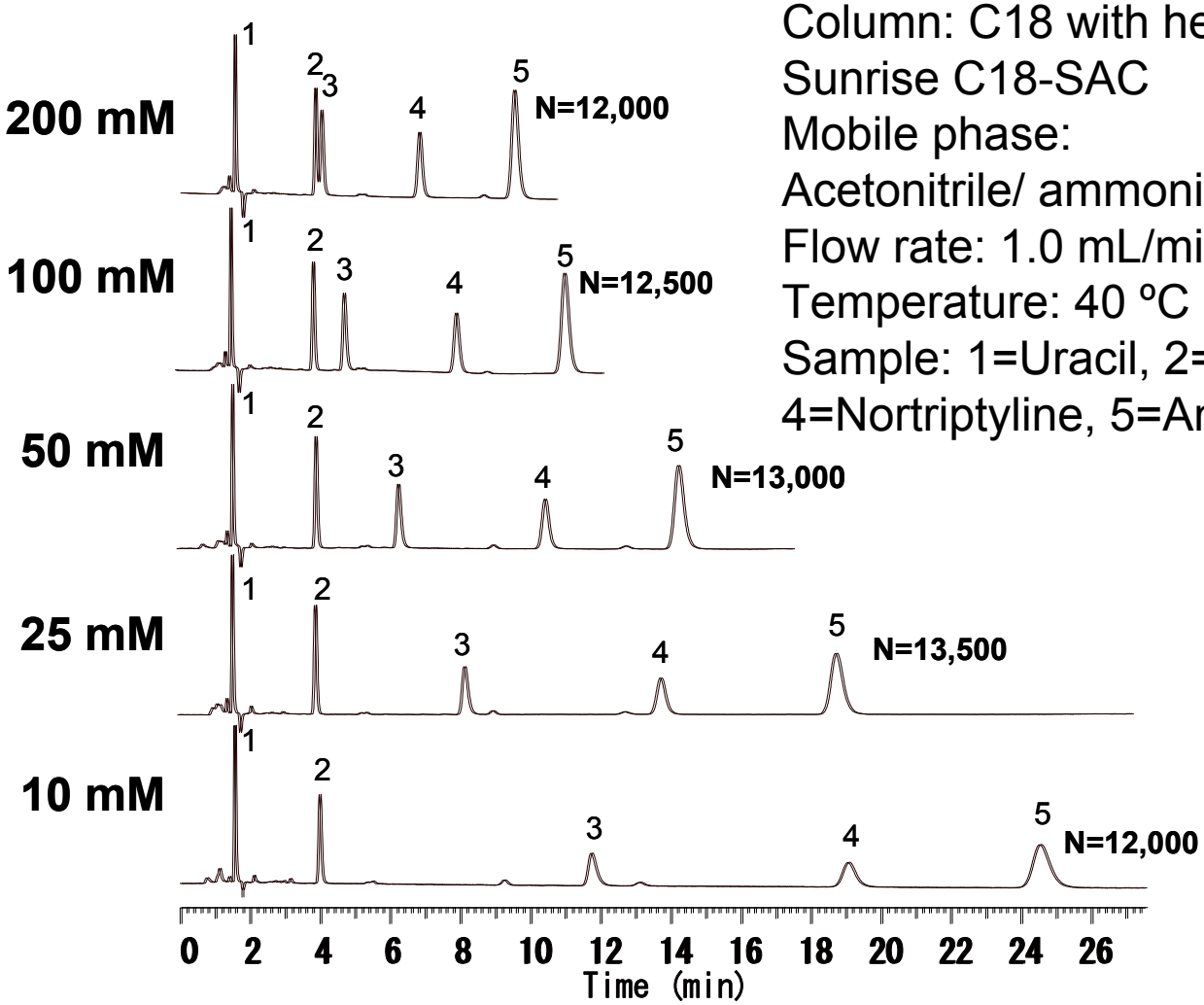
Separation of basic compounds with ammonium acetate: Effect of pH



Column: C18 with heat treatment, Sunrise C18-SAC
Mobile phase: Acetonitrile/100mM ammonium acetate(70:30)
Flow rate: 1.0 mL/min
Temperature: 40 ºC
Sample: 1=Uracil, 2=Toluene, 3=Propranolol, 4=Nortriptyline, 5=Amitriptyline

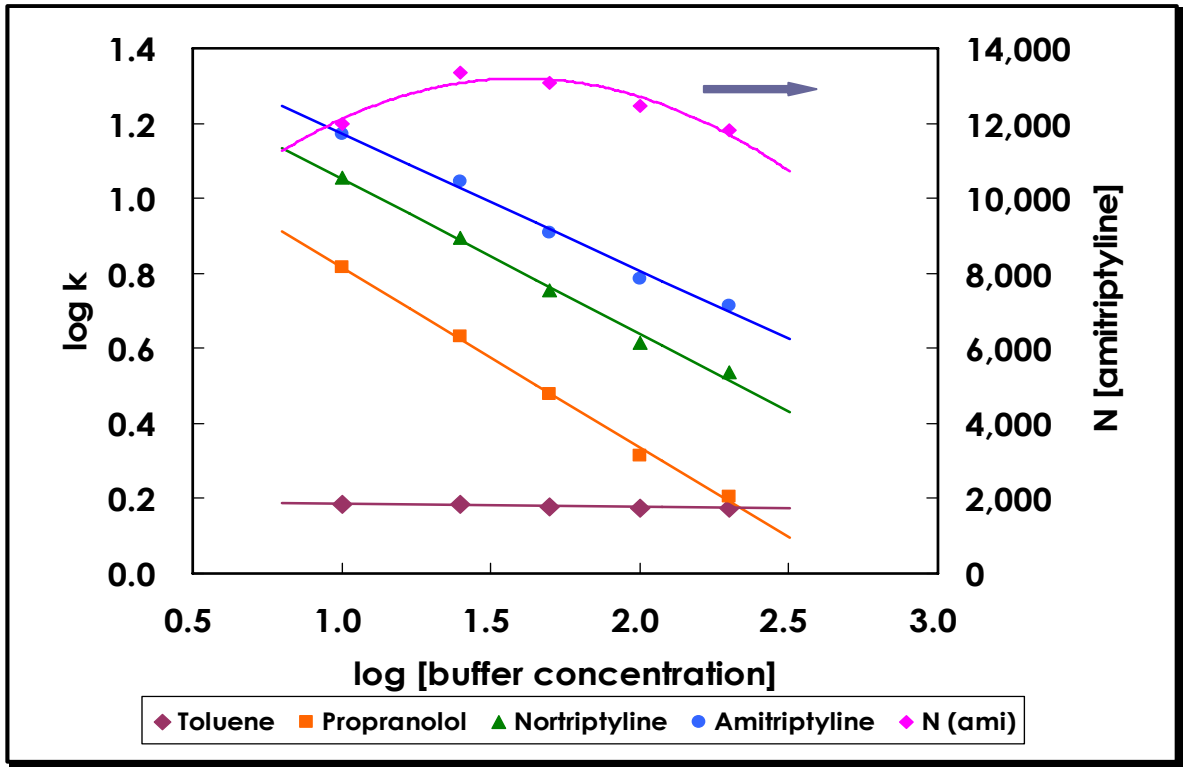
An ammonium ion makes a peak shape of a basic compound sharp.

Separation of basic compounds with ammonium acetate: Effect of salt concentration



Column: C18 with heat treatment,
Sunrise C18-SAC
Mobile phase:
Acetonitrile/ ammonium acetate (70:30)
Flow rate: 1.0 mL/min
Temperature: 40 ºC
Sample: 1=Uracil, 2=Toluene, 3=Propranolol,
4=Nortriptyline, 5=Amitriptyline

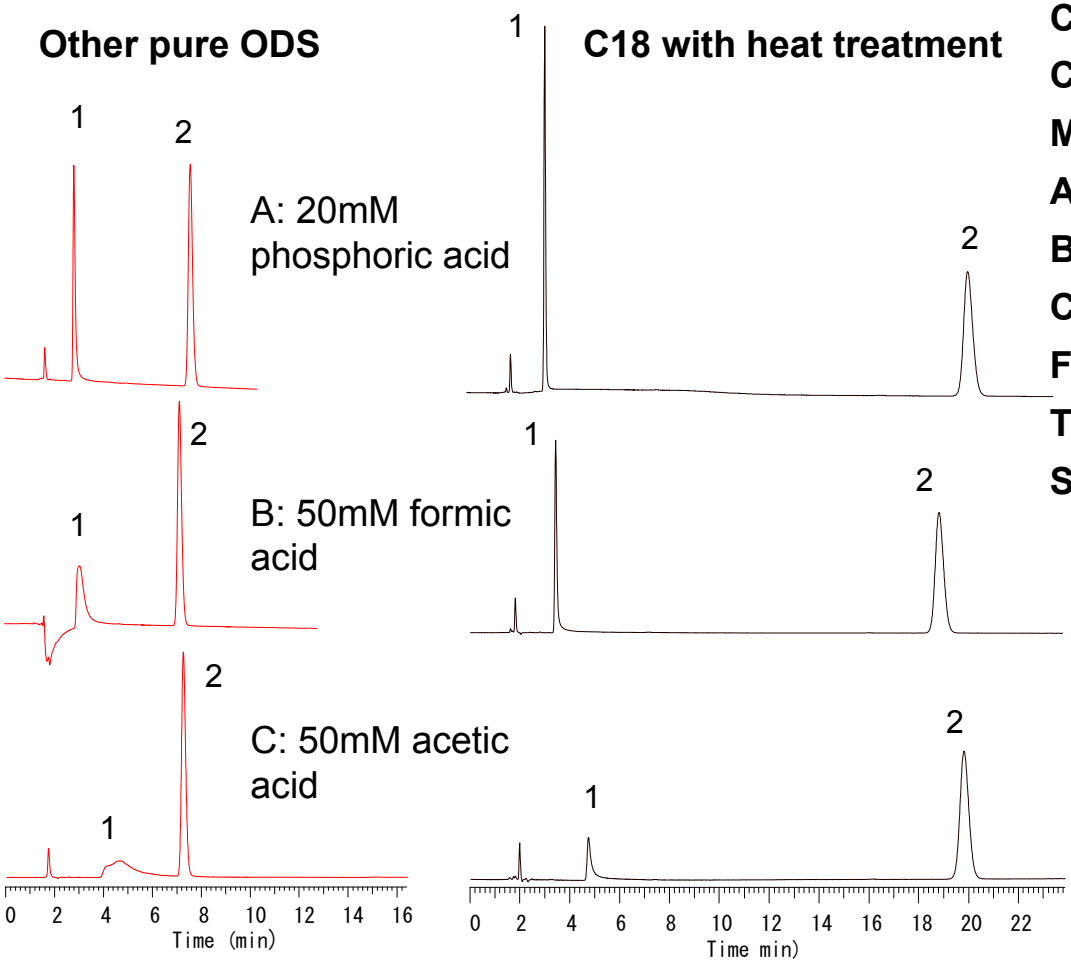
Relationship between buffer concentration and retention



$$\log k = -\frac{z_B}{z_A}\log[A] + \frac{z_B}{z_A}\log[\overline{A}] + \log\frac{V_r}{V_m} + \frac{1}{z_A}\log K_A^B$$

A : ion in eluent, B : ion of sample, k : retention of B, K : selection coefficient
Z_A, Z_B : charge, V_r, V_m : resin volume and mobile phase volume in column

Separation of metal chelating compound



Column: Other pure ODS 5μm

C18 with heat treatment, Sunrise C18-SAC,

Mobile phase:

A)Acetonitrile/20mM phosphoric acid (10:90)

B)acetonitrile/50mM formic acid (10:90)

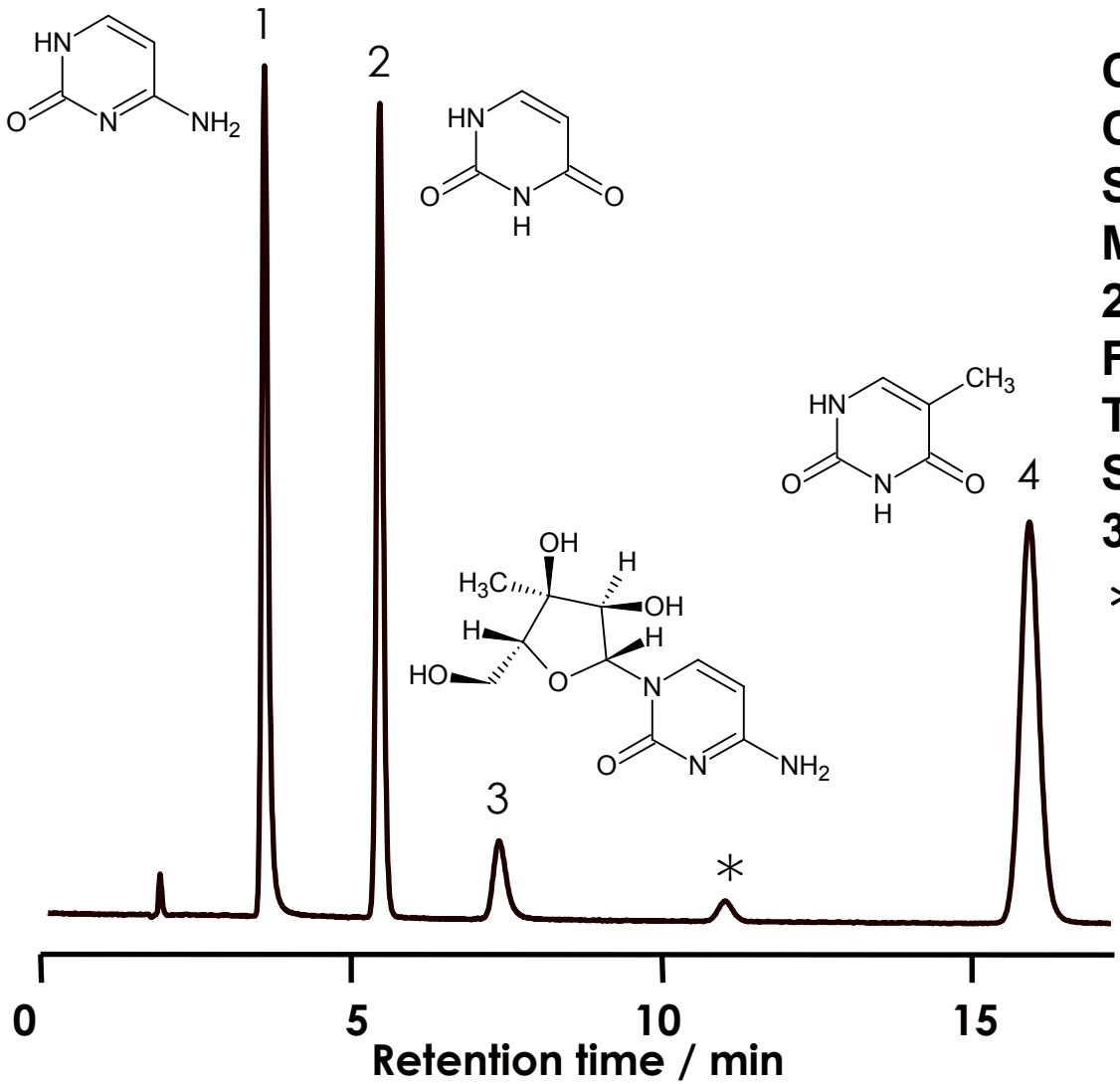
C)acetonitrile/50mM acetic acid (10:90)

Flow rate: 1.0 mL/min

Temperature: 40 ºC

Sample: 1=8-Quinolinol, 2=Caffeine

Separation of nucleic acid bases



Column:
C18 with heat treatment,
Sunrise C18-SAC
Mobile phase:
20mM Phosphate buffer pH4.5
Flow rate: 1.0 mL/min
Temperature: 25 ºC
Sample: 1=Cytosine, 2=Uracil,
3=Cytidine, 4= Thymine,
＊=impurity

【Conclusions】

- Residual silanol group in proposed C18 phase made retention of polar compounds including basic compounds high.
- Silanol group controlled its activity by heat treatment did not make peak of basic compounds tailing.
- Furthermore, ion-exchange interaction was recognized and retention of basic compounds could be changed by pH or salt concentration in mobile phase.
- Proposed C18 phase was effective to separation of a metal chelating compound. It is surmised that hydration or change of state of silanol groups contributed to suppress influence of a metal impurity on silica support.