極性化合物分析のための

[Second Choice]

分離ができない！
さぁ困った！次の手は？

First Choice ( )

- C18 (ODS), 5μm, 150 x 4.6 mm i.d.
- H_2O/CH_3OH 5%
  20% 80% 20min
  25mM
  - pH4.5 (KH_2PO_4) pH6.8 (KH_2PO_4/Na_2PO_4=1/1)
- 0.45μm 5~10μL

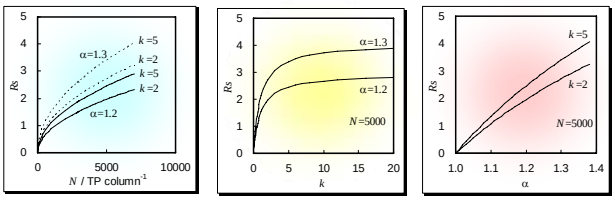
…

- !!
  - ⇨ 分離の改善
- !!
  - ( )

…

$$Rs = \frac{1}{4}\sqrt{N}\cdot\frac{\alpha-1}{\alpha}\cdot\frac{k}{1+k} \qquad Rs = \frac{1}{4}\sqrt{N}\cdot(\alpha-1)\cdot\frac{k}{1+k}$$

N α $[\alpha=k_2/k_1]$ k $[k=(Tr\text{-}T_0)/T_0]$



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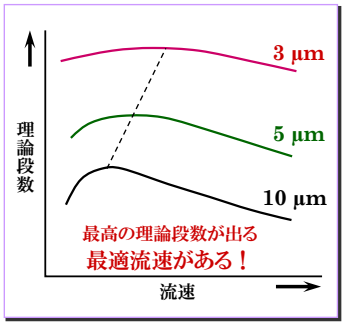
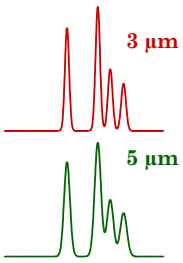
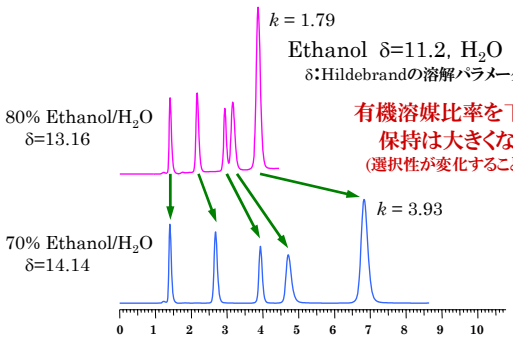
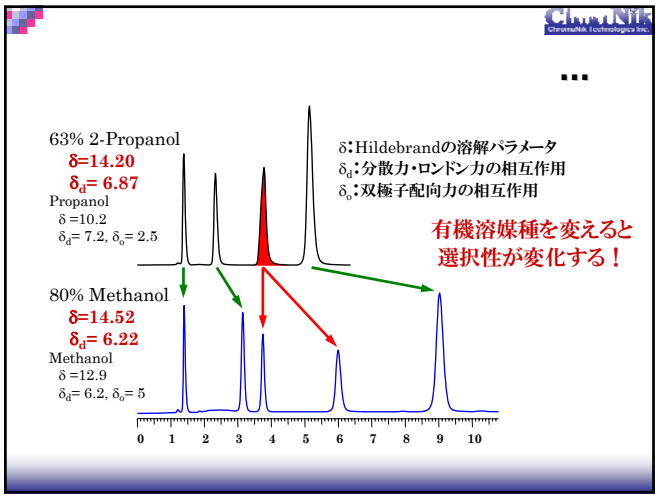


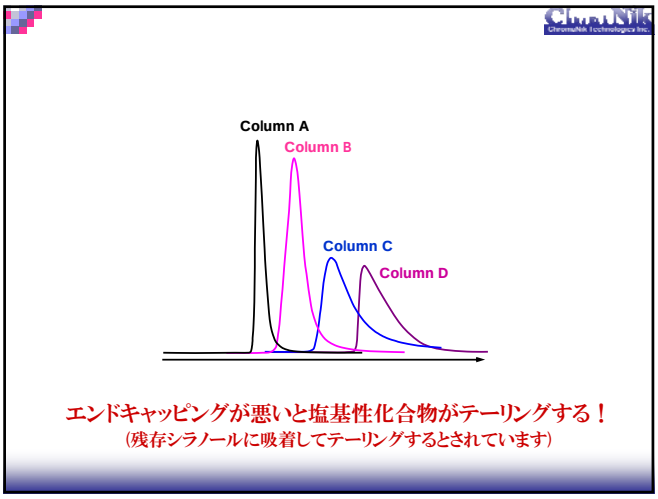


粒子径を小さくすると
理論段数は高くなる！

…







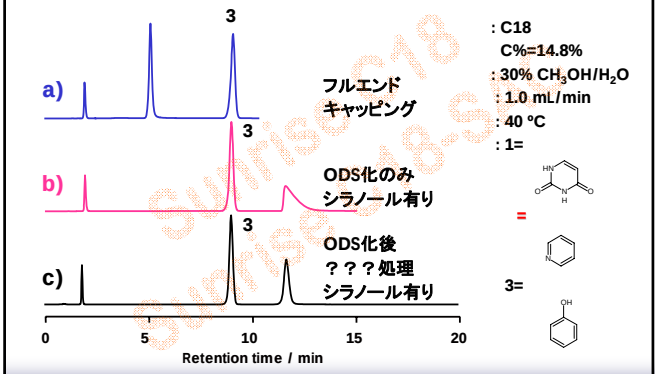
Second Choice

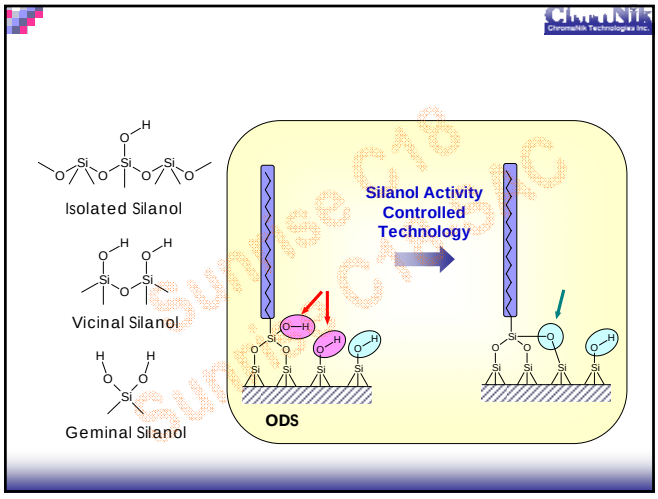
① 逆相モードのままで選択性を大幅改善

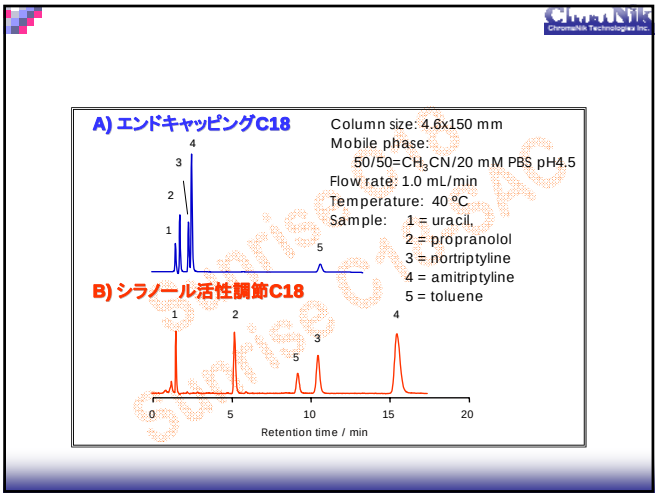
ChromaNik Sunrise C18-SAC

② 保持の小さい成分の分離の改善

ZIC®-HILIC HPLC Column







- ChromaNik
- Sunrise C18
- /

Sunrise C18-SAC

ChromaNik Sunrise C18-SAC

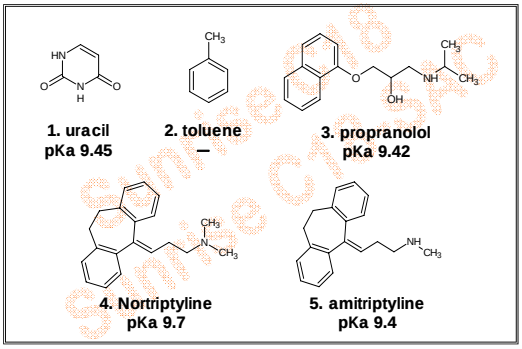
- - 12nm 340m²/g 14%
- (/)
- /

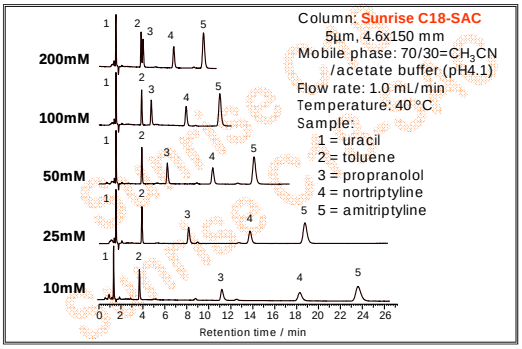
- log *k* ( ) log [*A*] ( ) z_A/z_B

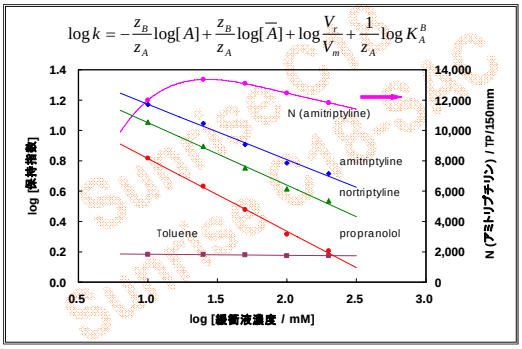
$$\log k = -\frac{z_B}{z_A}\log[A] + \frac{z_B}{z_A}\log[\overline{A}] + \log\frac{V_r}{V_m} + \frac{1}{z_A}\log K_A^B$$

- *A* *B* *k* *B*
- z_A, z_B
- V_r, V_m
- K_A^B

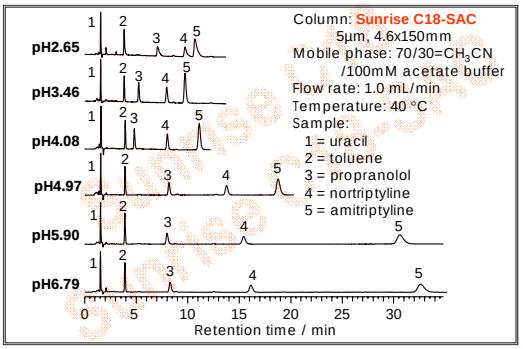
1



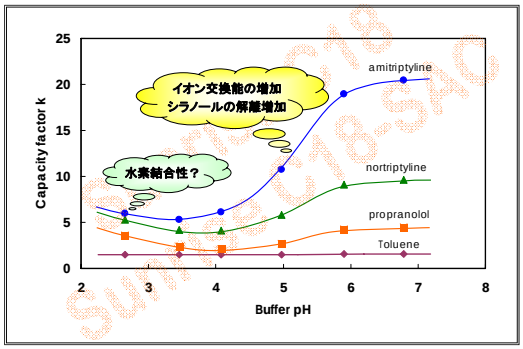




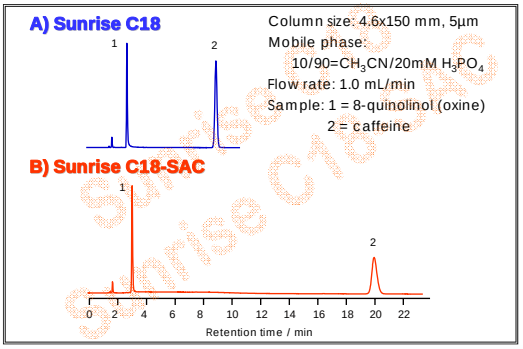
pH

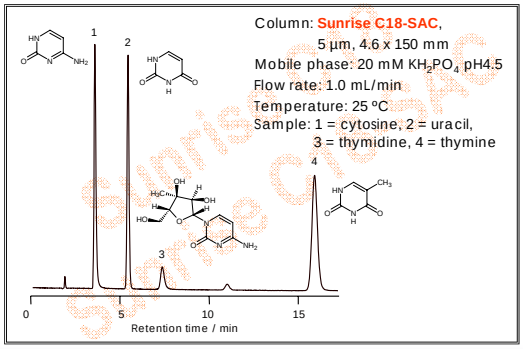


pH



8- /



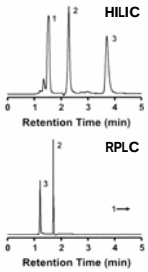


/ ...

- ...
  - C18
- ...
  - /
  - LC-MS

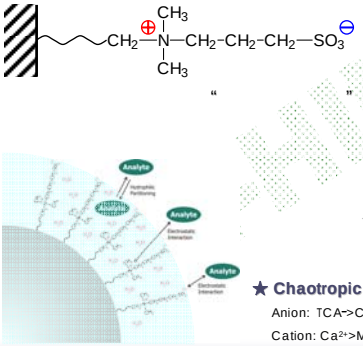
Hydrophilic Interaction Liquid Chromatography : HILIC

- /
- HILIC
- HILIC



HILIC RPLC
Mobile phase:
(HILIC) 60:40 = CH3CN/10 mM AcONH4 (pH 7)
(RPLC) 5:95 = CH3CN/10 mM AcONH4 (pH 7)
Sample: 1 = Phe-Gly-Gly-Phe, 2 = Leu-Gly-Gly,
3 = Gly-Gly-Gly

ZIC®-HILIC/*p*HILIC



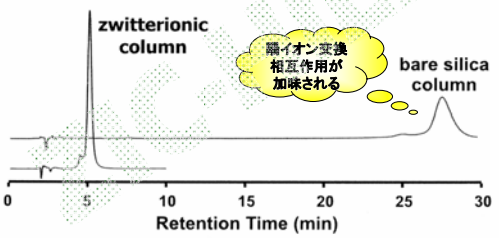
分子内塩を形成

★ Chaotropic order

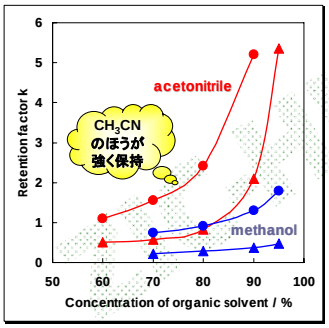
Anion: TCA⁻>ClO_4^->NO_3^->Cl^->acetate⁻>F^-> SO_4^{2-}

Cation: Ca^{2+}>Mg^{2+}>Li^+>Na^+, K^+>NH_4^+> $(CH_3)_4N^+$

Column: **ZIC®-HILIC**, bare silica column, 100 × 4.6 mm i.d.
Mobile phase: 40 % acetonitrile in CH_3COONH_4 (50 mM, pH 6.5)
Sample: bradykinin (pI 12)



HILIC

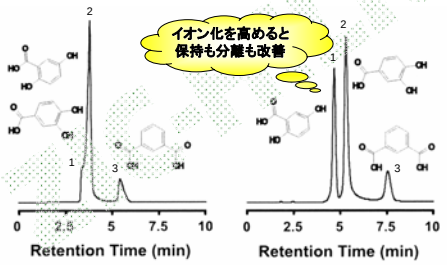


HILIC

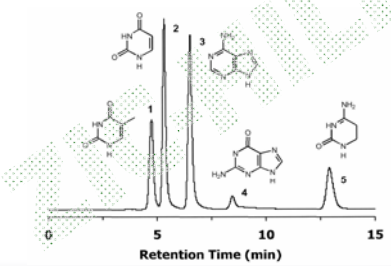
THF < acetone
< acetonitrile < IPA
< ethanol < water

Column; **ZIC®-HILIC** 150 x 4.6 mm
Mobile phase: 10 mM formic acid
Legend;
(circles) cytosine
(triangles) adenine

Column: **ZIC®-*p*HILIC**, 100 x 4.6 mm i.d.
Mobile phase: (left) 75:25 = acetonitrile/CH_3COONH_4 (17 mM, pH 6.8)
(right) 75:25 = acetonitrile/NH_4HCO_3 (17 mM, pH 9.6)
Sample: 1 = gentisic acid, 2 = protocatechuic acid, 3 = isophthalic acid



Column: **ZIC®-HILIC** 150 x 2.1 mm, 5 µm
Eluent: 80% acetonitrile/20% 25 mM CH_3COOH, 2.5 mM CH_3COONH_4
Flow rate: 0.1 mL/min, Detection: UV at 254 nm
Sample: 1 = thymine, 2 = uracil, 3 = adenine, 4 = guanine, 5 = cytosine



極性化合物のための Second Choice

- First Choice　　C18
  - First Trial Condition
  - ChromaNik Sunrise C18
- Second Choice ①
  - /
  - Sunrise C18-SAC !!
- Second Choice ② HILIC
  - C18
  - ZIC®-HILIC column !!