



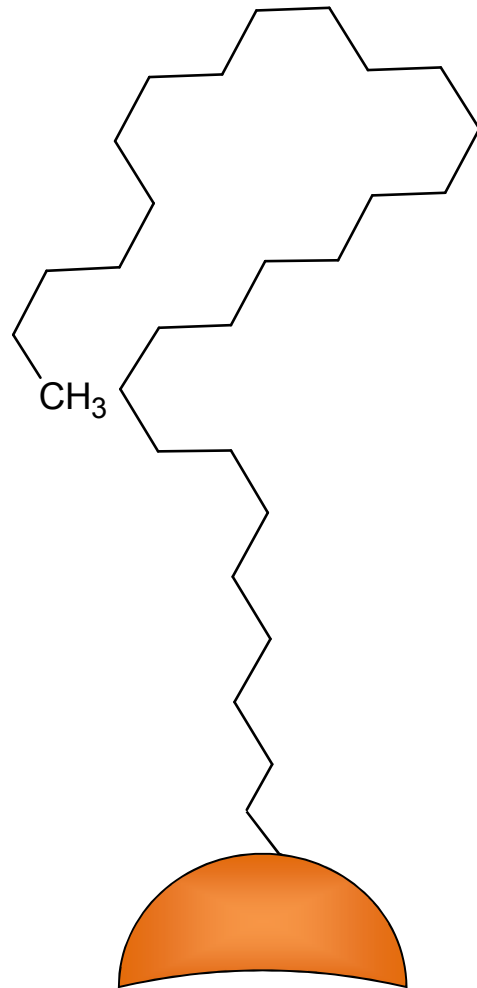
異性体，極性物質・困ったときの もう一本C30，PFP

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C30カラムの注意点



トリアコンチル (C30)

結合密度が高いほど
異性体分離に優れる

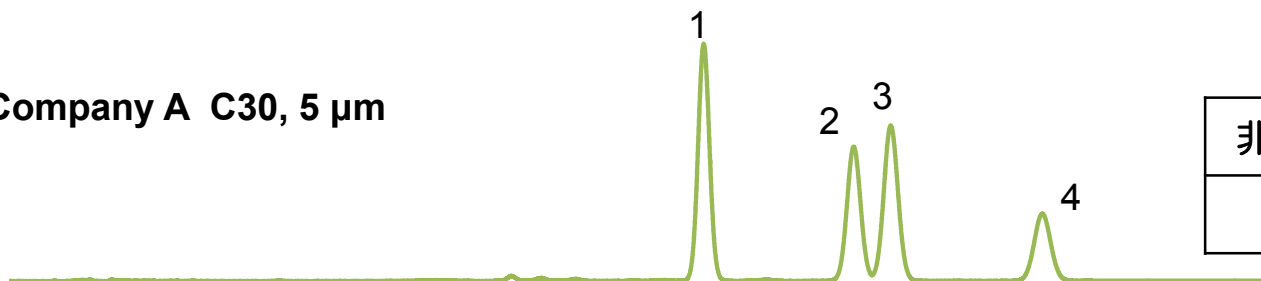


結合密度が高すぎると
ピークがテーリングする

結合密度が低いと
分離が不十分になる

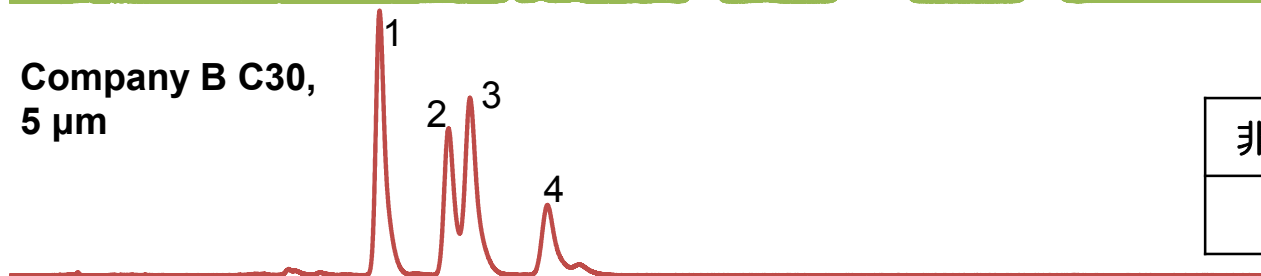
結合密度の影響

Company A C30, 5 μm



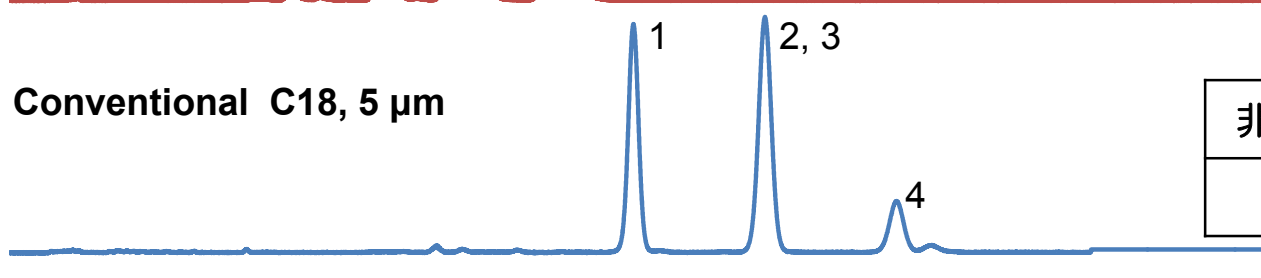
非対称性	結合密度	細孔径
1.02	1.9 μmol/m ²	13 nm

Company B C30, 5 μm



非対称性	結合密度	細孔径
1.63	3.7 μmol/m ²	20 nm

Conventional C18, 5 μm



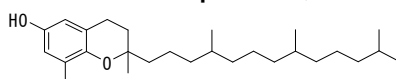
非対称性	結合密度	細孔径
1.04	3.0 μmol/m ²	13 nm

Column dimension, 250 x 4.6 mm;
mobile phase, methanol/water = 97/3
flow rate, 1.0 mL/min;
temperature, 30 °C;
detection, UV295 nm;

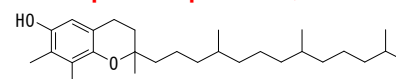
Retention time / min

sample,

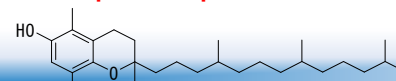
1 = δ-tocopherol,



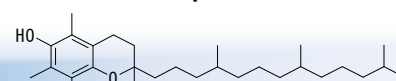
2 = γ-tocopherol,



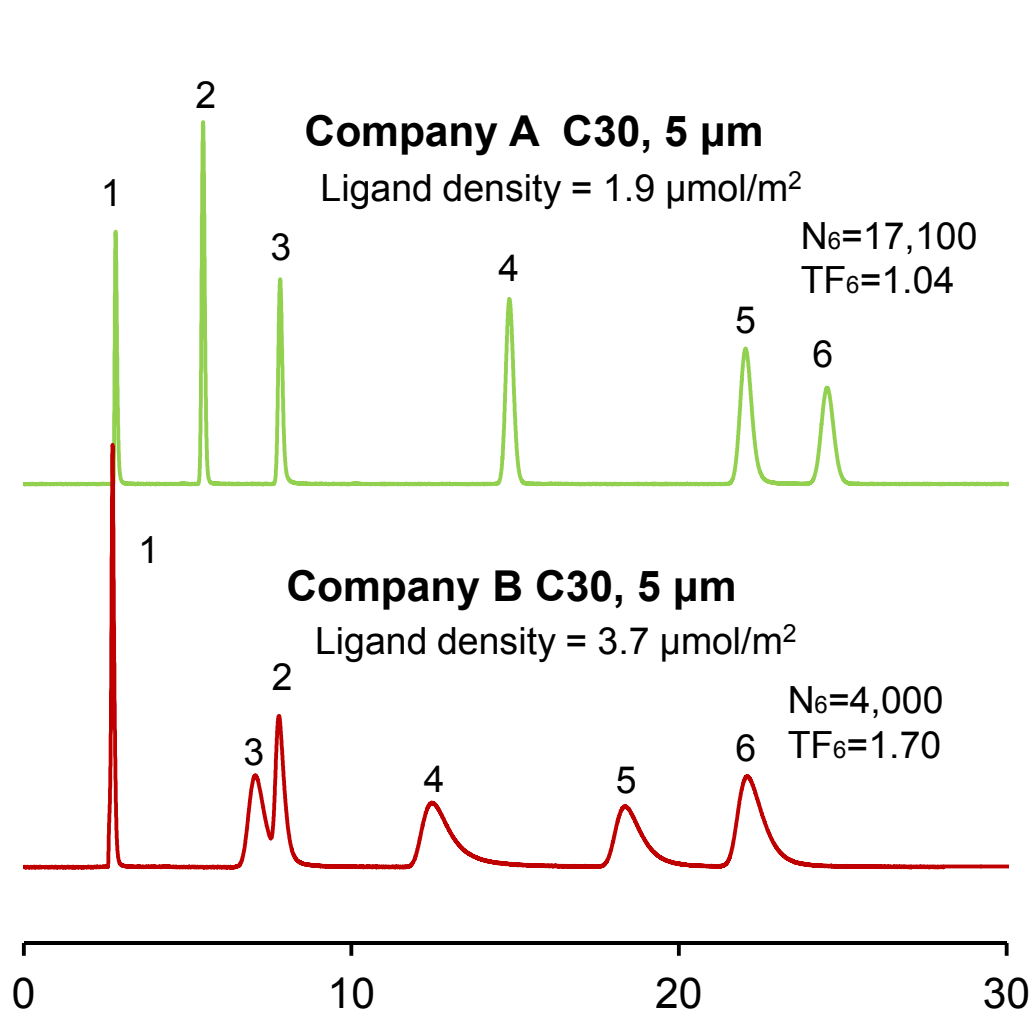
3 = β-tocopherol,



4 = α-tocopherol



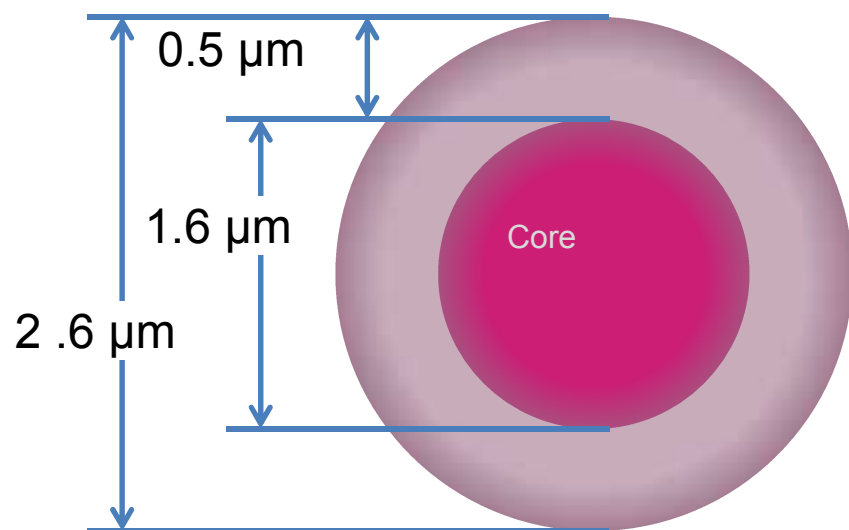
核酸塩基の分離



Column dimension, 250 x 4.6 mm
 Mobile phase: 10 mM phosphate buffer pH7.0
 Flow rate: 1.0 mL/min
 Temperature: 30 °C
 Detection: UV 254 nm
 Sample: 1 = Sodium Nitrite,
 2 = Cytosine
 3 = Uracil
 4 = Cytidine
 5 = Uridine
 6 = Thymine

移動相の有機溶媒の組成比に関わらず、結合密度の高いC30は段数が低くテーリングする。

使用した表面多孔質（コアシェル）シリカ



	細孔径	比表面積
1	10.2 nm	112 m ² /g
2	10.8 nm	106 m ² /g
3	12.0 nm	96.1 m ² /g
4	15.0 nm	79.6 m ² /g
5	15.5 nm	73.5 m ² /g

C30カラムの物性値と分離係数

Batch number		241215	241115	230216	110714	280314	220713	081112
Core shell silica	Particle diameter (μm)	2.6	2.6	2.6	2.6	2.6	2.6	2.6
	Thickness of porous layer (μm)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	Specific surface area ^a (m^2/g)	112	106	96.1	79.6	79.6	73.5	73.5
	Pore volume ^a (mL/g)	0.287	0.286	0.288	0.299	0.299	0.285	0.285
	Average pore diameter ^a (nm)	10.2	10.8	12.0	15.0	15.0	15.5	15.5
Carbon loading of only C30 (%)		6.59	5.48	6.08	4.43	5.40	4.10	5.20
Carbon loading including end-capping (%)		7.56	6.57	7.04	5.30	6.05	4.70	5.86
Ligand (C30) density ($\mu\text{mol}/\text{m}^2$)		1.79	1.55	1.91	1.64	2.03	1.64	2.11
Separation factor of β/γ -tocopherol ^b		1.0625	1.0570	1.0640	1.0376	1.0626	1.0415	1.0629
Volume of 1.5 nm thickness inside pore ^c ($\mu\text{L}/\text{m}^2$)		1.279	1.292	1.313	1.350	1.350	1.355	1.355
Ligand (C30) density ^d (mol/L)		1.400	1.200	1.455	1.215	1.504	1.210	1.557

a: Measured by Quantachrom Autosorb.

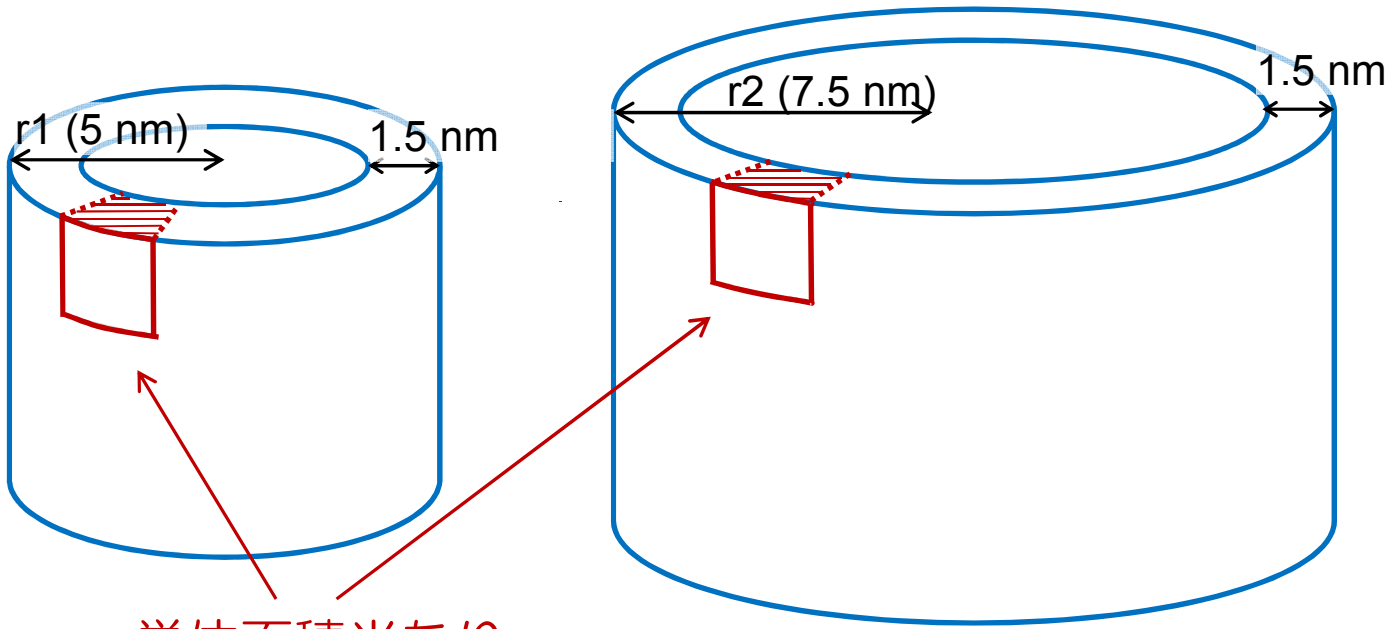
b: Mobile phase, methanol/water=97/3; temperature, 25 °C.

c: Postulated a pore as a cylinder with a same diameter, listed as the volume of per square meter.

d: Ligand density in the volume of 1.5 nm thickness inside of pore.

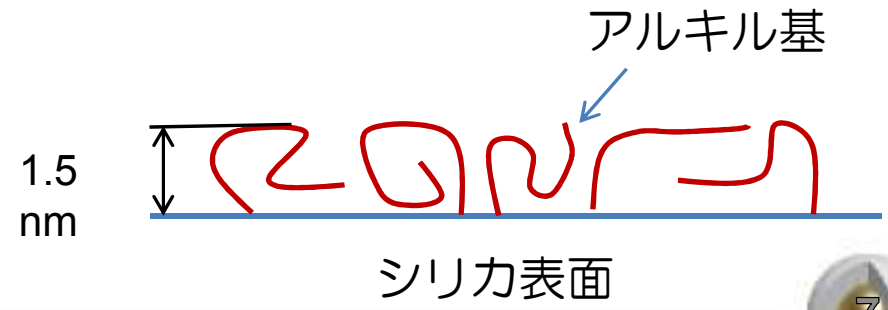
細孔表面から1.5nmの厚さの体積

Volume of 1.5 nm thickness inside pore d (L/m²)

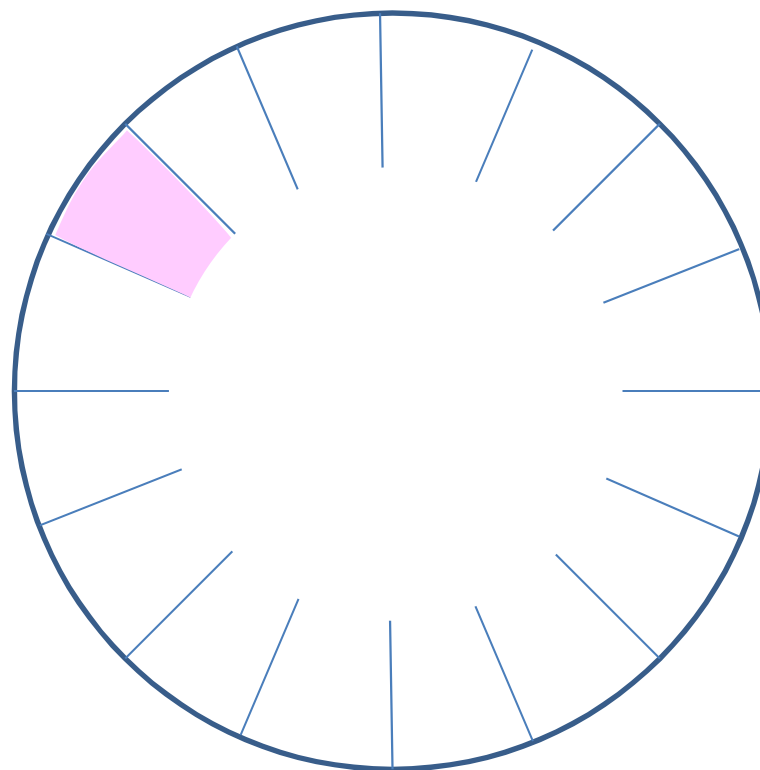
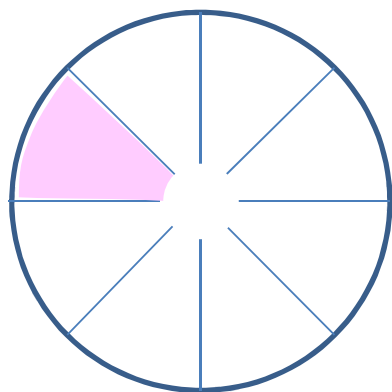


単位面積当たり

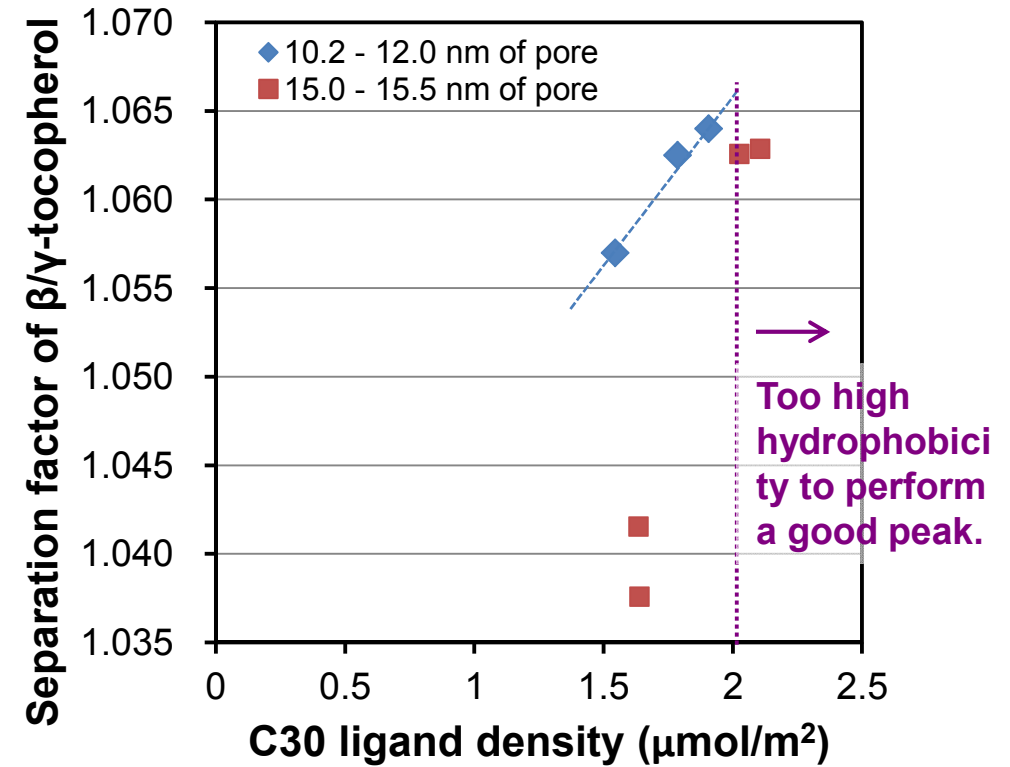
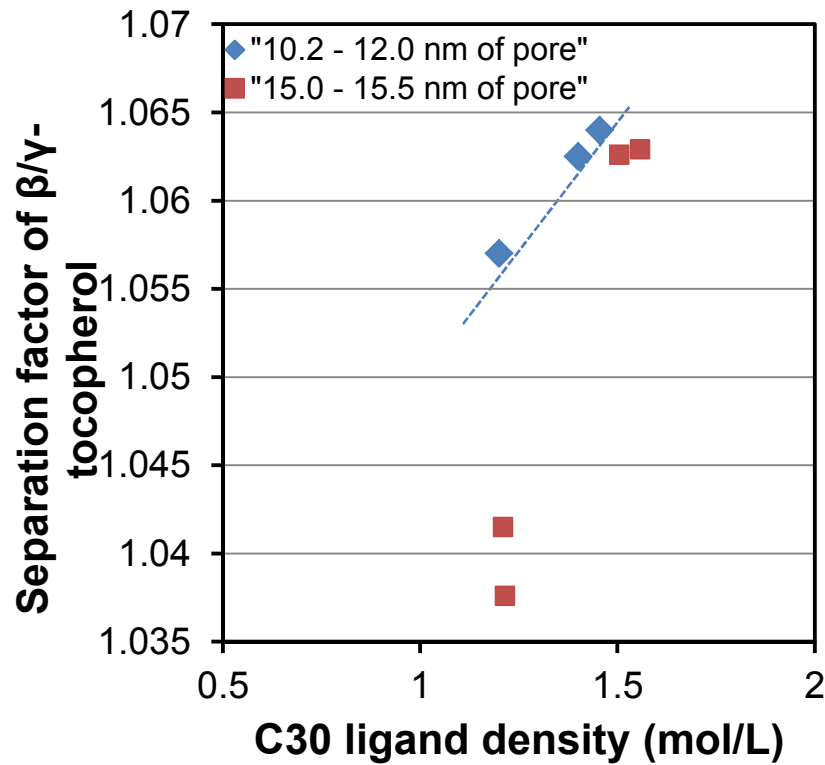
細孔を円柱と仮定・アルキル基は細孔表面から1.5nmの厚さに存在すると仮定



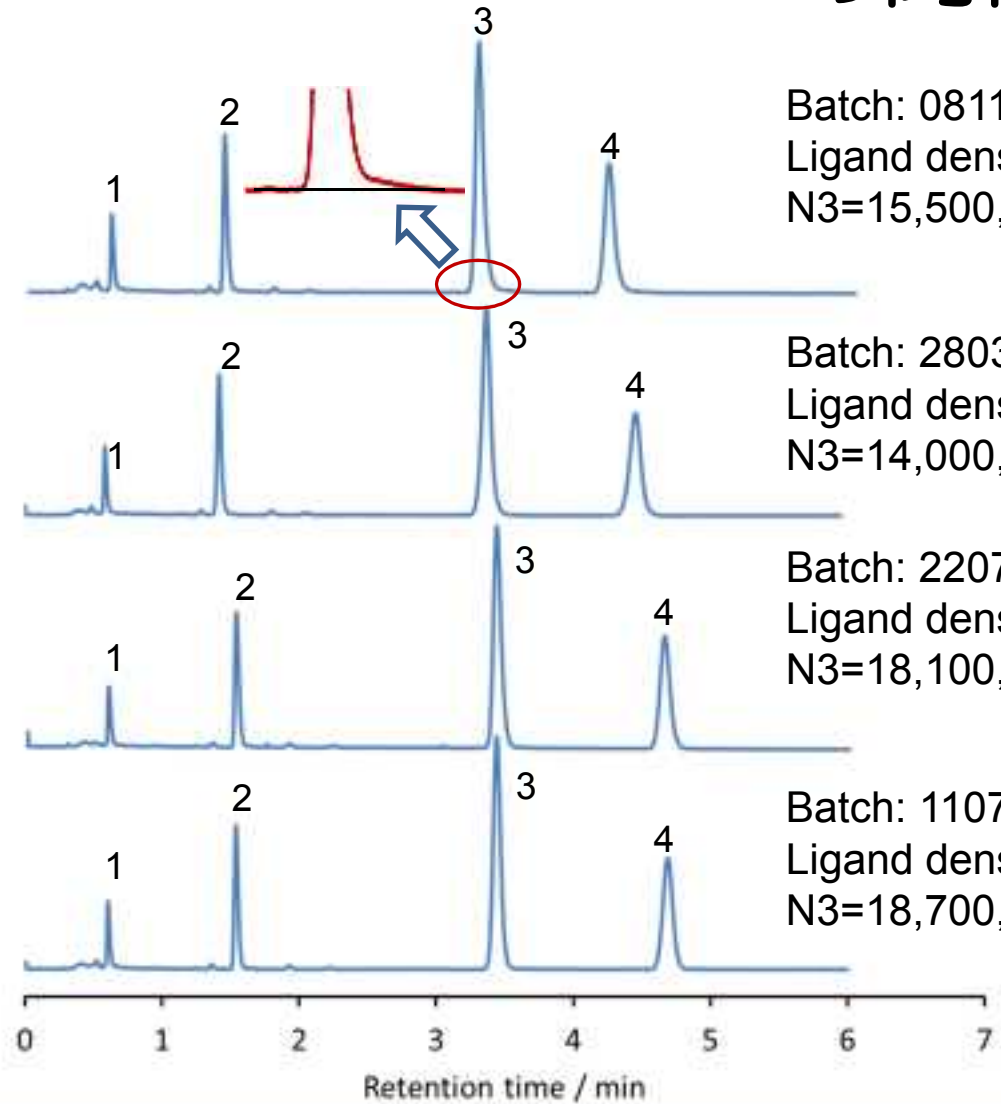
異なる細孔径での結合密度



2種類の結合密度と分離係数



結合密度と段数・テーリングファクターとの関係



Batch: 081112
 Ligand density: **2.11 $\mu\text{mol}/\text{m}^2$**
 N3=15,500, TF3=1.34

Batch: 280314
 Ligand density: **2.03 $\mu\text{mol}/\text{m}^2$**
 N3=14,000, TF3=1.06

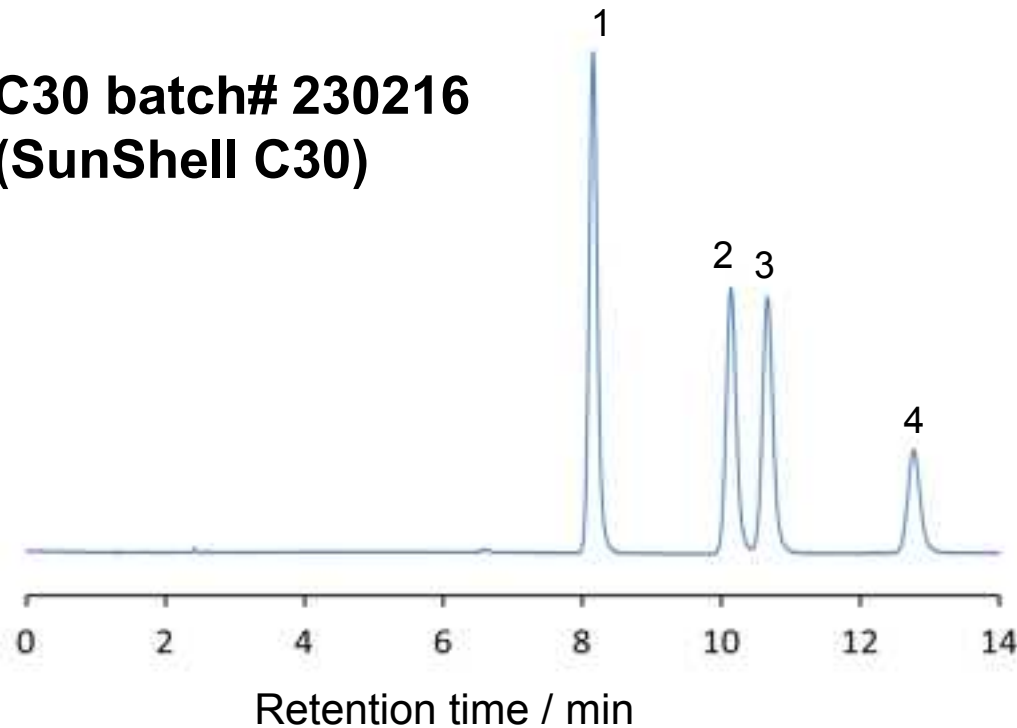
Batch: 220713
 Ligand density: **1.64 $\mu\text{mol}/\text{m}^2$**
 N3=18,100, TF3=1.25

Batch: 110714
 Ligand density: **1.64 $\mu\text{mol}/\text{m}^2$**
 N3=18,700, TF3=1.11

Column dimension: 100 x 2.1mm
 Mobile phase:
 Acetonitrile/water = 60/40
 Flow rate: 0.3 mL/min
 Temperature: 30 °C
 Sample: 1 = Uracil
 2 = Ethylbenzoate
 3 = Acenaphthene
 4 = Butylbenzene

ビタミンE異性体の分離

**C30 batch# 230216
(SunShell C30)**

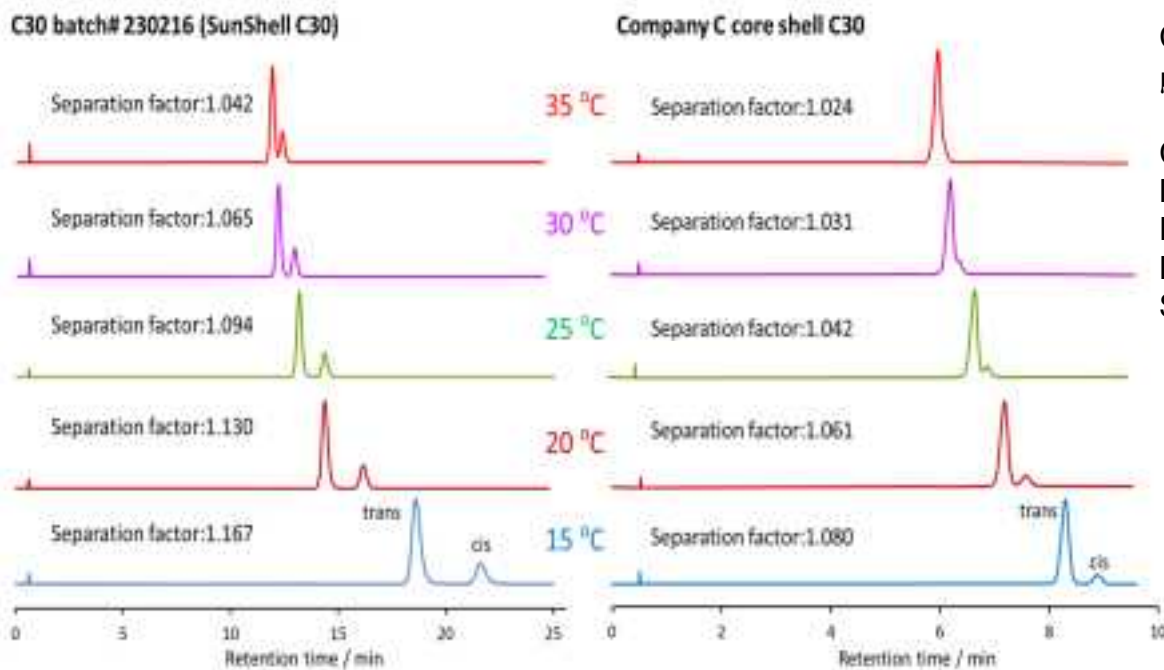


Column: C30 batch# 230216, 2.6 μ m
150 x 3.0 mm
Mobile phase: Methanol/water = 97/3
Flow rate: 0.43 mL/min
Temperature: 25 $^{\circ}$ C
Detection: UV 295 nm
Sample: 1 = δ -tocopherol
2 = γ -tocopherol
3 = β -tocopherol
4 = α -tocopherol

	Company A C30, 5 μ m 250 x 4.6 mm	Company B C30, 5 μ m 250 x 4.6 mm	SunShell C30, 2.6 μ m 150 x 3.0 mm
Separation factor of β/γ -tocopherol	1.048	1.062	1.064
Resolution of β/γ -tocopherol	1.30	0.85	2.01



ビタミンK1の異性体の分離



Column: C30 batch# 230216 (SunShell C30), 2.6 μm

Company C core shell C30, 2.6 μm

Column size: 100 x 2.1 mm

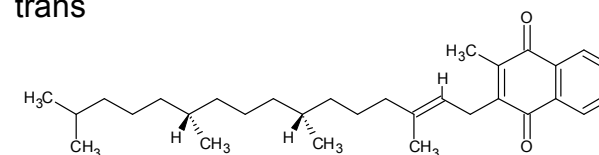
Mobile phase: methanol/water = 96/4

Flow rate: 0.35 mL/min;

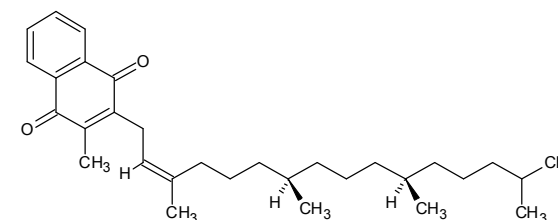
Detection: UV 250 nm;

Sample: vitamin K1 isomers (trans and cis).

trans



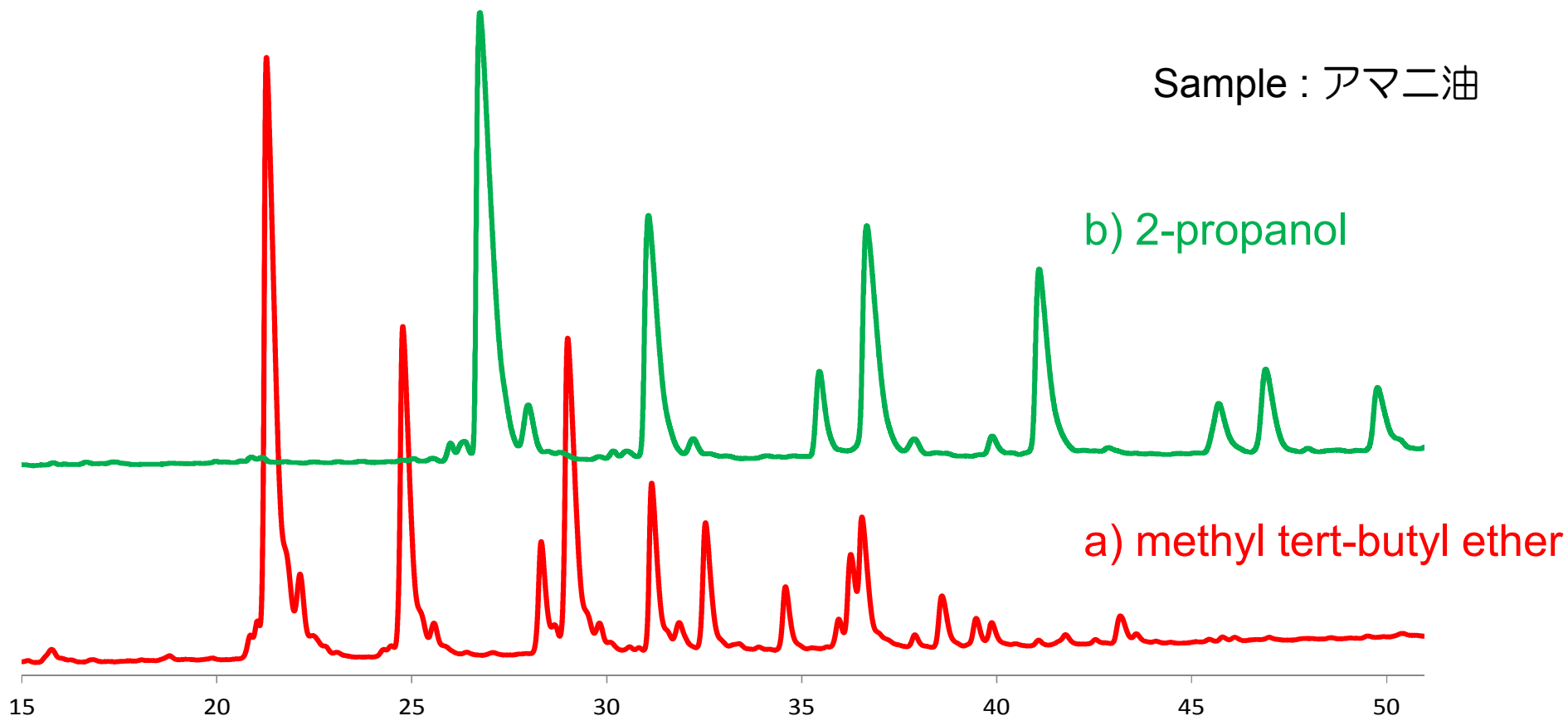
cis



	Vitamin K1 isomers (trans and cis)			
	Separation factor		Resolution	
	C30 batch# 230216	Company C C30	C30 batch# 230216	Company C C30
35 °C	1.042	1.024	1.23	---
30 °C	1.065	1.031	1.94	---
25 °C	1.094	1.042	2.88	0.91
20 °C	1.130	1.061	3.33	1.41
15 °C	1.167	1.080	3.79	1.78

溶媒の違いによる分離の変化

Sample : アマニ油



Column dimension, 150 x 4.6 mm; flow rate, 1.0 mL/min; temperature, 25 °C
 mobile phase : A:acetonitrile,, B: methyl tert-butyl ether (MTBE) B% 5 to 50 in 55 min
 mobile phase : A:acetonitrile,, B:2-propanol B% 10 to 50 in 55 min

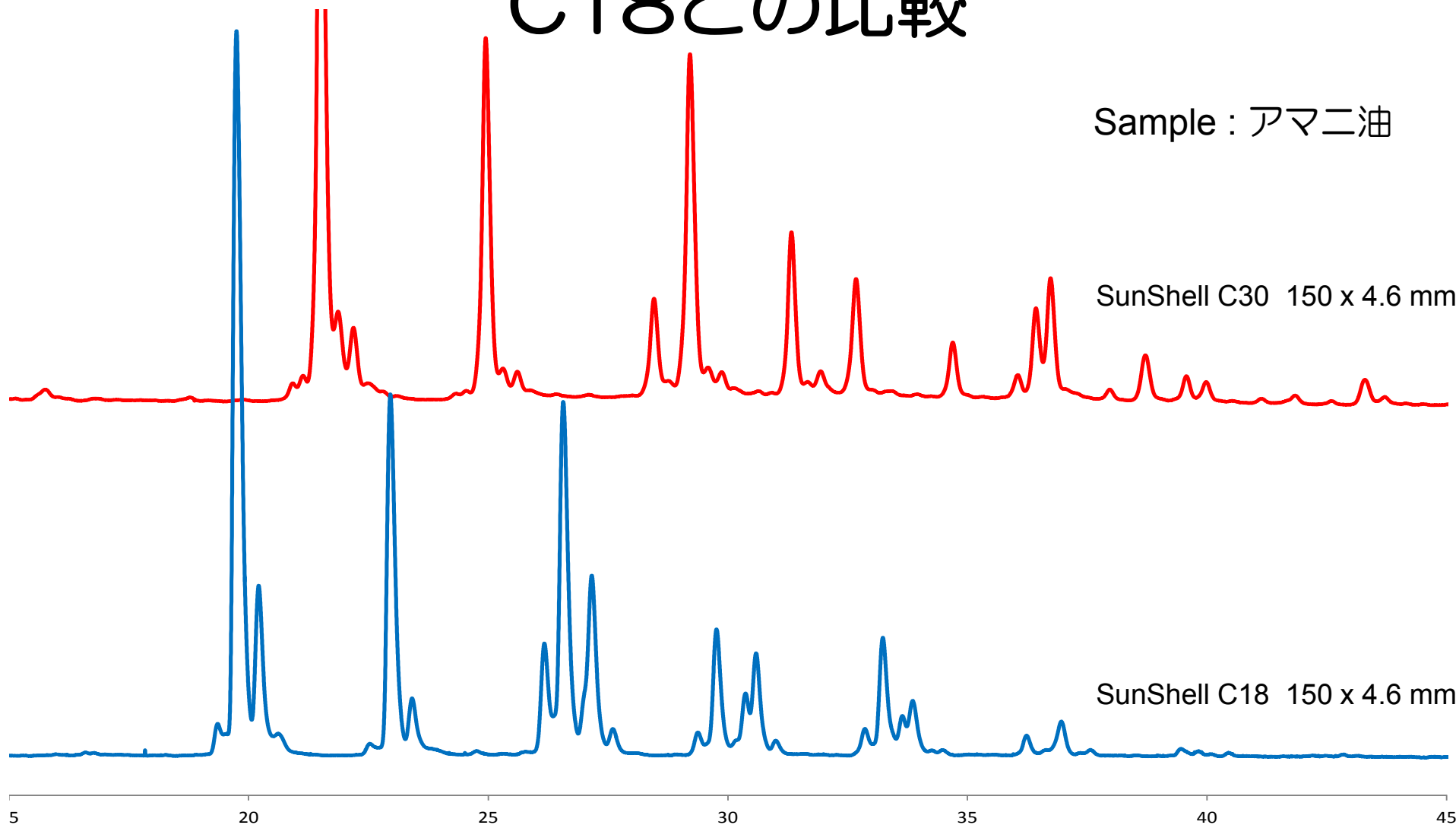


C18との比較

Sample : アマニ油

SunShell C30 150 x 4.6 mm

SunShell C18 150 x 4.6 mm

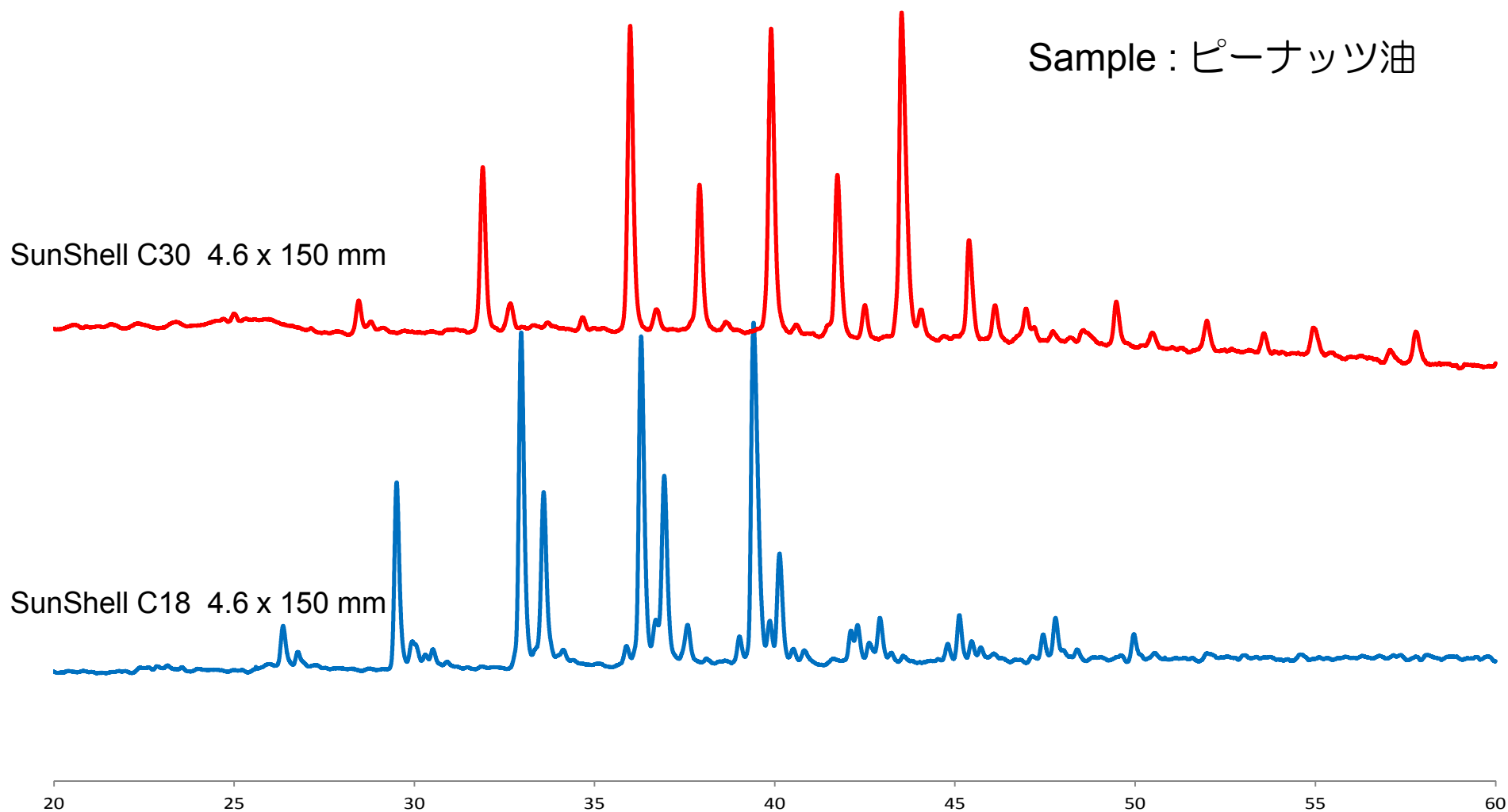


Column dimension, 150 x 4.6 mm; flow rate, 1.0 mL/min; temperature, 25 °C
mobile phase : A:acetonitrile,, B:MTBE B% 10 to 50 in 55 min



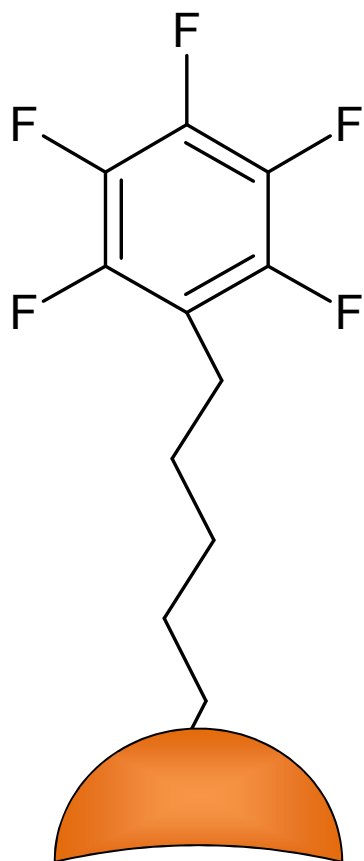
C18との比較2

Sample : ピーナッツ油



Column dimension, 150 x 4.6 mm; flow rate, 1.0 mL/min; temperature, 25 °C
mobile phase : A:acetonitrile,, B:MTBE B% 10 to 50 in 55 min

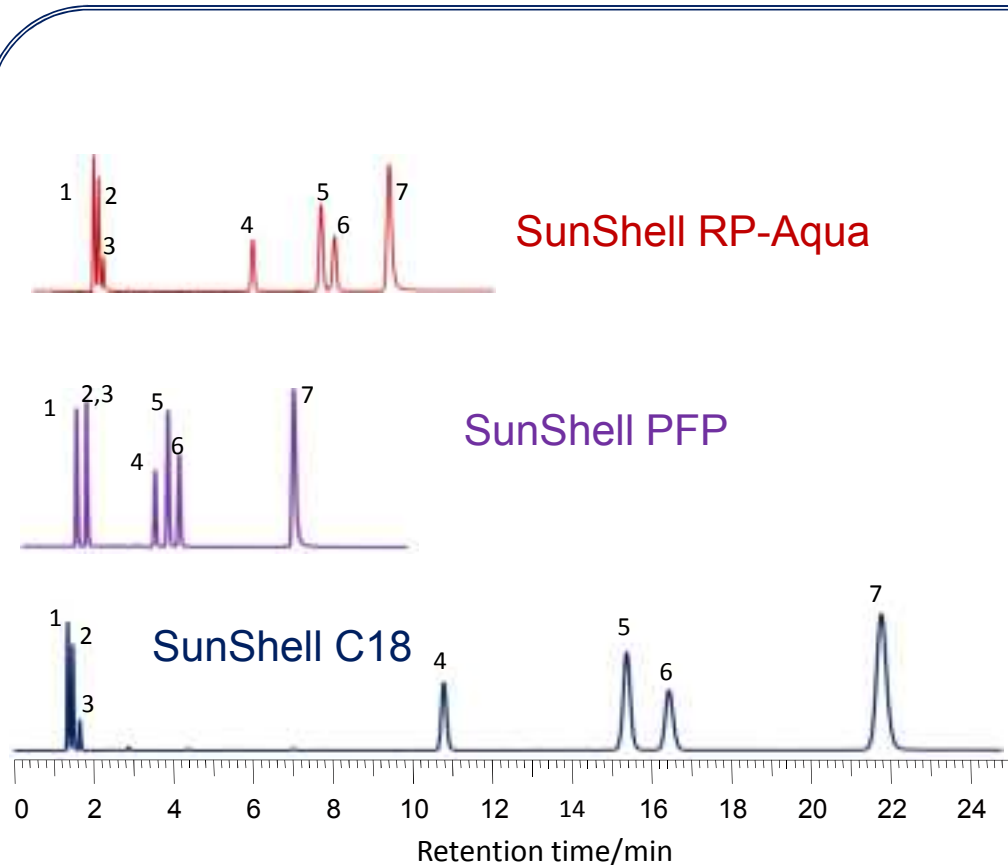
ペンタフルオロフェニル (PFP)



- 疎水性相互作用
- 水素結合性
- 双極子相互作用
- π - π 相互作用
- 静電的相互作用

- 極性化合物の保持が可能
- 立体選択性に優れている
- ハロゲン化合物の分離に有効

標準試料の分離



Column dimension: 150 x 4.6 mm

Mobile phase: CH₃OH/H₂O=75/25

Flow rate: 1.0 mL/min

Temperature: 40 °C

Sample: 1 = Uracil, 2 = Caffeine,

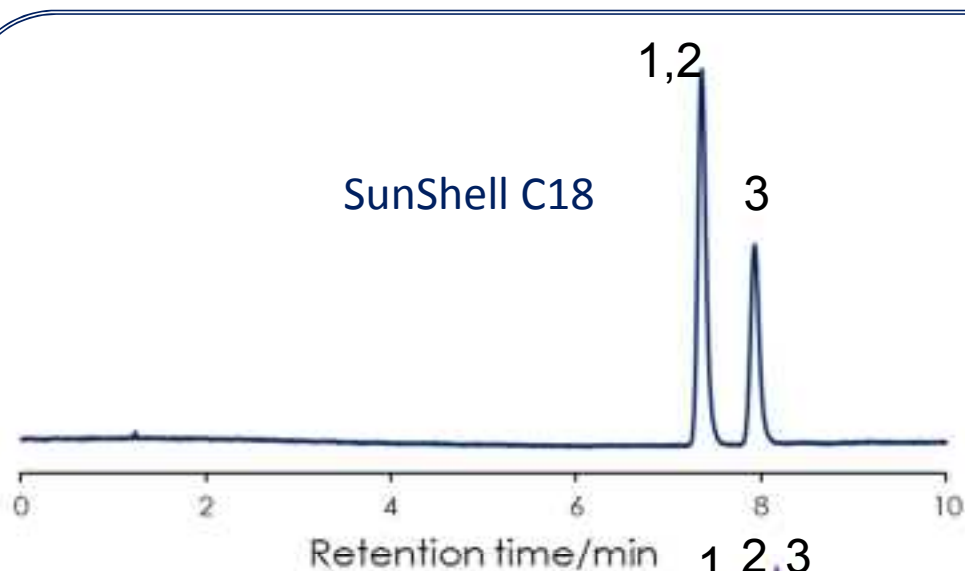
3 = Phenol, 4 = Butylbenzene

5 = o-Terphenyl, 6 = Amylbenzene,

7 = Triphenylene

	Hydrogen bonding (Caffeine/ Phenol)	Hydrophobicity (Amylbenzene/ Butylbenzene)	Steric selectivity (Triphenylene/ o-Terphenyl)
RP-Aqua	0.52	1.52	1.30
PFP	1.00	1.31	2.38
C18	0.39	1.60	1.46

クレゾール異性体の分離



Column:

SunShell C18, 2.6 μm 150 x 4.6 mm

SunShell PFP, 2.7 μm 150 x 4.6 mm

Mobile phase: $\text{CH}_3\text{OH}/\text{H}_2\text{O}=40/60$

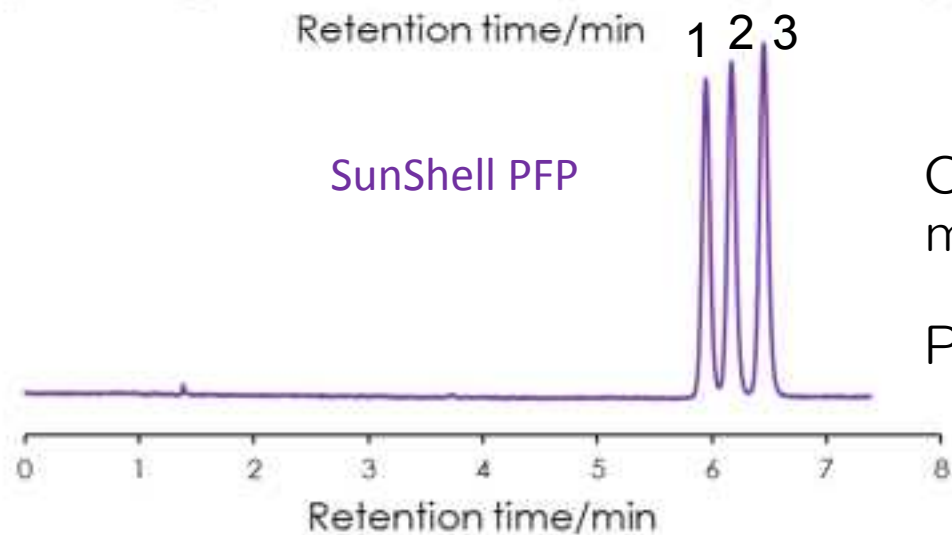
Flow rate: 1.0 mL/min

Temperature: 25 $^{\circ}\text{C}$

Sample: 1 = p-Cresol

2 = m-Cresol

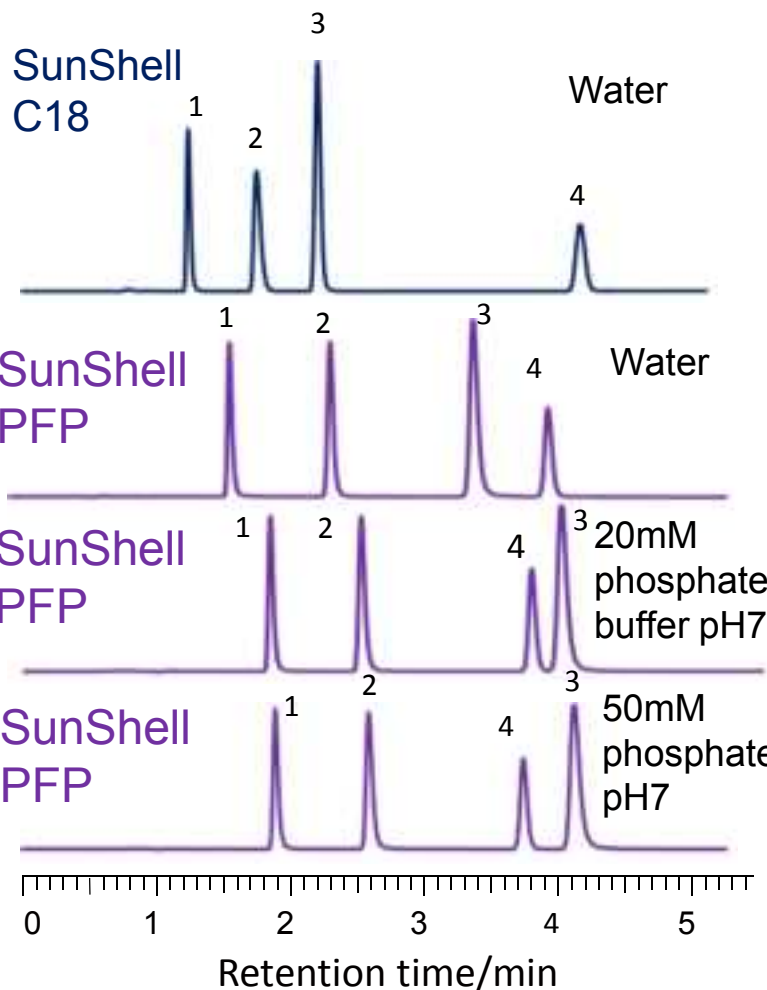
3 = o-Cresol



C18カラムではp-クレゾールと
m-クレゾールは分離不可能

PFP は分離可能

キサンチン類の分離

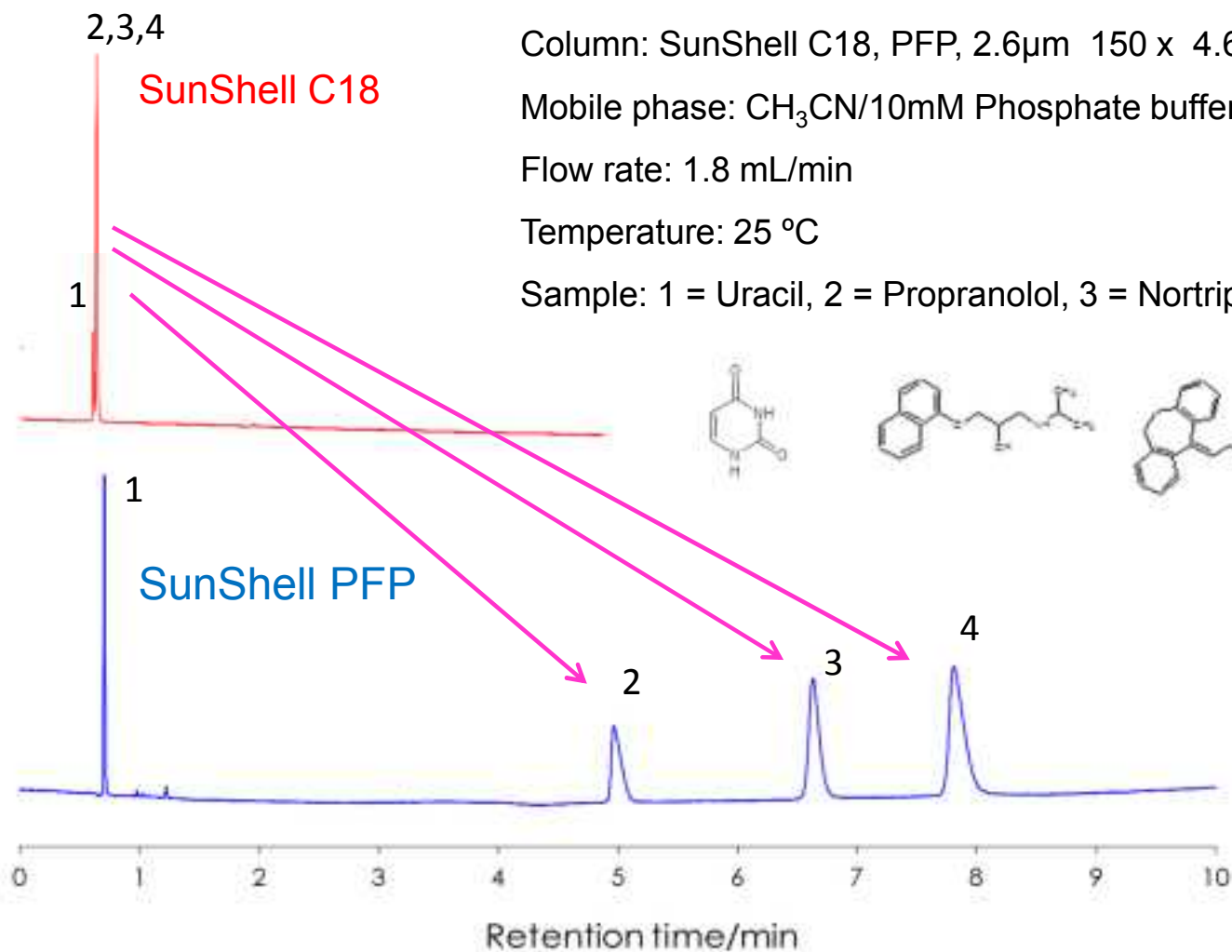


SunShell C18, PFP 2.6 μ m 150 x 2.1 mm
 Mobile phase: CH₃OH/water or buffer=30/70
 Flow rate: 0.3 mL/min
 Temperature: 25 °C
 Detection: UV@250nm
 Sample: 1 = Theobromine
 2 = Theophylline
 3 = Caffeine
 4 = Phenol

SunShell PFPはC18に比べ極性の高いカフェインを強く保持
 塩濃度の調節で分離が変化



C18とPFPによる塩基性化合物の保持比較



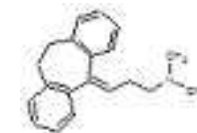
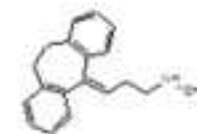
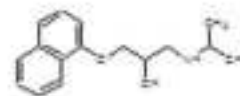
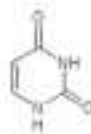
Column: SunShell C18, PFP, 2.6 μ m 150 x 4.6 mm

Mobile phase: CH₃CN/10mM Phosphate buffer pH7.0 = 80/20

Flow rate: 1.8 mL/min

Temperature: 25 °C

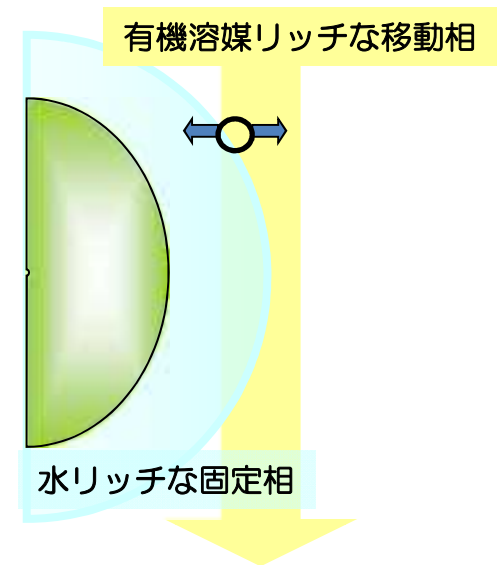
Sample: 1 = Uracil, 2 = Propranolol, 3 = Nortriptyline, 4 = Amitriptyline



PFPでHILIC・イオン交換？

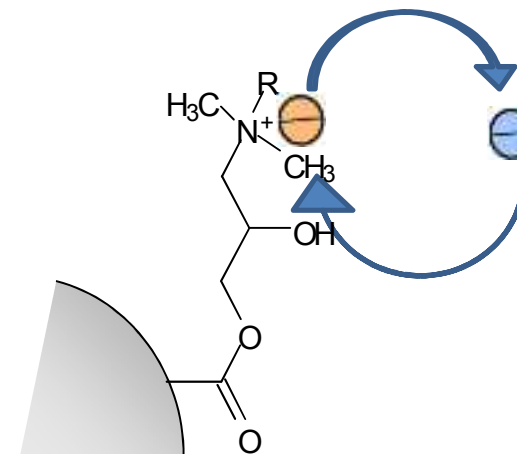
• HILICモード

- 固定相上に形成された水和層と移動相との分配
- 静電的相互作用, 水素結合



• イオン交換

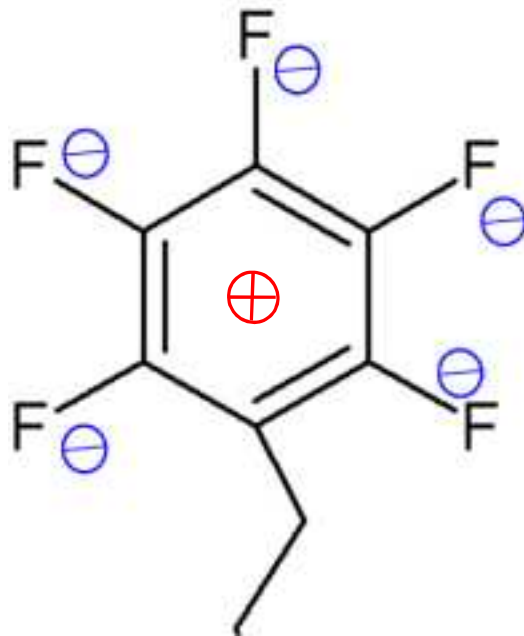
- 物質がイオン成分を取り込み、代わりに物質が持っていた他のイオン成分を放出する現象



PFPでHILIC・イオン交換

ペンタフルオロフェニル

pKa	—
分配係数(LogP)	3.54
水の溶解度 (mg/L)	50.8

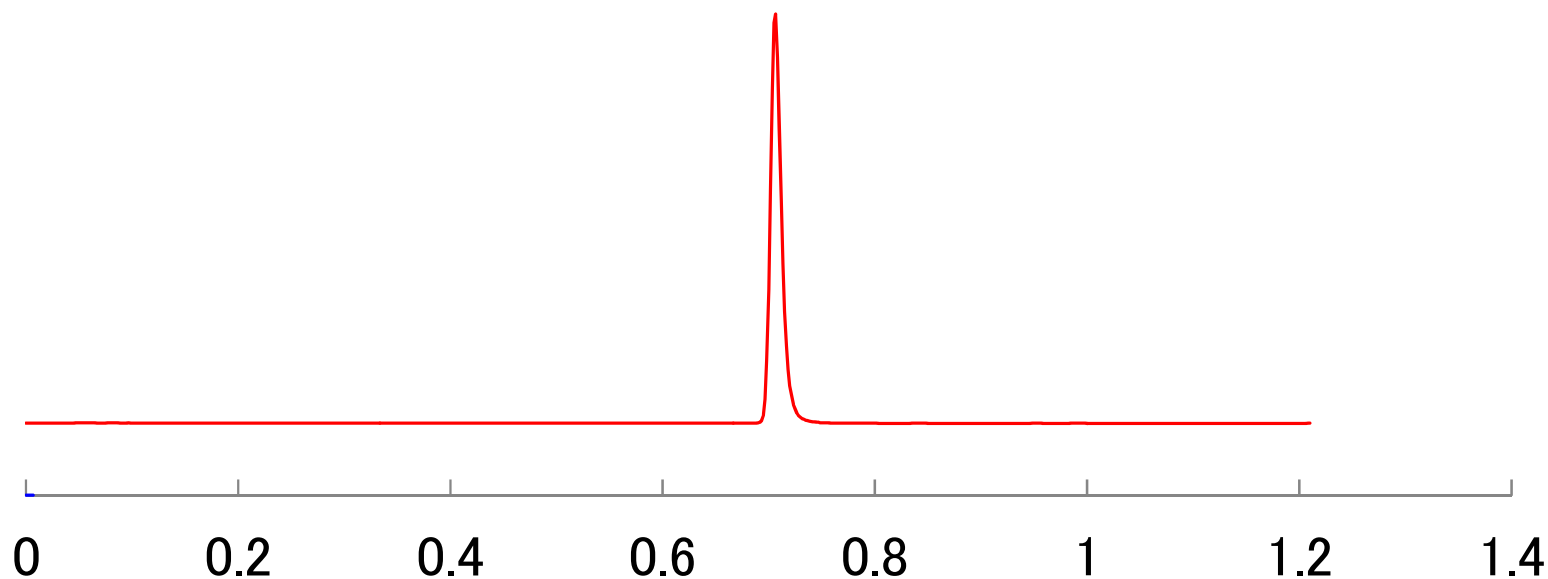


- PFPの化学的特性から水を保持するとは考えにくい
- PFP基が電化を持つ構造をしていない
- フッ素の強い電気陰性度により、電子の偏りが生じている



イオン交換に似た分離機構を示す

ウリジンの保持



Column: SunShell PFP, 2.6 μ m 150 x 4.6 mm

Mobile phase: CH₃CN/10mM Phosphate buffer pH7.0 = 80/20

Flow rate: 1.8 mL/min

Temperature: 25 °C

Sample: 1 = Uridine

ウリジンはPFPカラムに保持されない

まとめ

- 長鎖アルキル基は脂溶性化合物(ビタミンE, K1)の異性体分離に優れている。
- 細孔径により, 単位面積当たりの結合密度($\mu\text{mol}/\text{m}^2$)が同じでも, 単位体積当たりの結合密度(mol/L)は変化する。
- C30カラムは, 溶媒を低極性な溶媒に変更することで選択性が変化する
- C18カラムと比較した場合, C30カラムは異なった選択性を示す。
- PFPカラムは, 優れた立体選択性を示す。
- PFPカラムでは, HILICモード, イオン交換とは異なった相互作用が働いている