Evaluation of C30 Phase Bonded on Superficially Porous Silica

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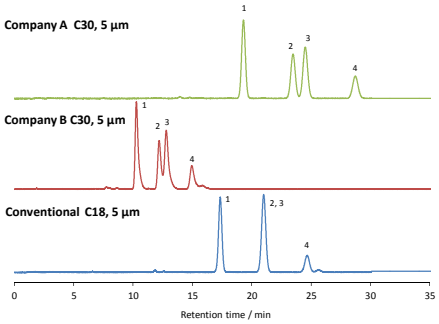
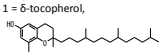
A long alkyl group like C30 (triacontyl group) phase has been known to be more suitable than a conventional C18 phase for separation of hydrophobic structurally related isomers such as vitamin E or vitamin K1. In this study, separation factor of beta-tocopherol and gamma-tocopherol which were structurally related isomers was evaluated to vary both a pore diameter of the superficially porous silica and a ligand density of the C30 group. Regarding a pore diameter, 12 nm showed the largest separation factor of beta and gamma-tocopherol among 10nm, 12 nm and 16 nm. Regarding a ligand density, the higher a ligand density, the larger a separation factor of beta and gamma-tocopherol. However, when a ligand density was too high, much high hydrophobicity caused peak tailing and a drop of theoretical plate. The most suitable ligand density existed for the highest resolution. Finally separation of cis and trans-vitamin K1 was compared and the same result as separation of beta and gamma-tocopherol was obtained.

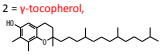
The problem of totally porous C30 column



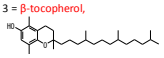
Column dimension, 250 x 4.6 mm;
mobile phase, methanol/water = 97/3;
flow rate, 1.0 mL/min;
temperature, 30 °C;
detection, UV295 nm;
sample,
1 = δ-tocopherol,



2 = γ-tocopherol,



3 = β-tocopherol,



4 = α-tocopherol.

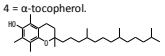


Table 1: Physical properties and separation factor and resolution of vitamin E

	Company A C30, 5 µm	Company B C30, 5 µm	Conventional C18, 5 µm
Specific surface area (m^2/g)	300	200	300
Pore diameter (nm)	13	20	13
Carbon loading (%)	18	20	18
Ligand density (µmol/m^2)	1.9	3.7	3.0
Theoretical plate	16,500	6,100	15,800
USP tailing factor	1.02	1.63	1.04
Separation factor of β/γ-tocopherol	1.048	1.062	---
Resolution of β/γ-tocopherol	1.30	0.85	---

*When the ligand (C30) density is too high, peak tailing occurs more and a theoretical plate is low. High ligand density on the particle surface is one of reasons of a low theoretical plate and a tailing peak.

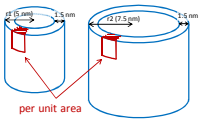
Evaluation of C30 phases bonded on Core shell silica

Bonding method of C30 phase
1) Triacontyltrichlorosilane was bonded on core shell silica at reflux in toluene.
2) As an end-capping, trimethylchlorosilane was reacted at reflux in toluene.

Table 2: Physical properties and separation factor of vitamin E and K1

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 (µmol/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
Separation factor of cis /trans vitamin K1 [c]		1.0856	1.0829	1.0944	1.0692	1.1054	1.0758	1.1174
Volume of 1.5 nm thickness inside pore [d] (µL/m^2)		1.279	1.292	1.313	1.350	1.350	1.355	1.355
Ligand (C30) density [e] (mol/L)		1.400	1.200	1.455	1.215	1.500	1.208	1.555

a: Measured by Quantachrom Autosorb.
b: Mobile phase, methanol/water=97/3; temperature, 25 °C.
c: Mobile phase, methanol/water=96/4; temperature, 25 °C.
d: Postulated a pore as a cylinder with a same diameter, listed as the volume of per square meter.
e: Ligand density in the volume of 1.5 nm thickness inside of pore.



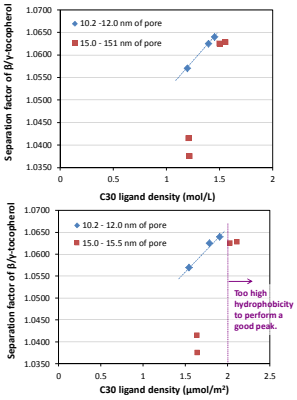


Figure 1: Relationship between 2 kinds of ligand density and separation factor.

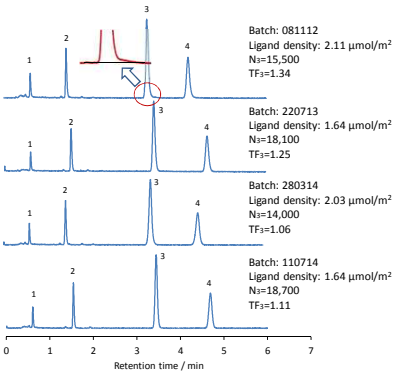


Figure 2: Comparison of theoretical plate and USP tailing factor.
Column dimension, 100 x 2.1mm; mobile phase, acetonitrile/water = 60/40; flow rate, 0.3 mL/min; temperature, 30 °C; peak, 1 = uracil, 2 = ethylbenzoate, 3= acenaphthene, 4 = butylbenzene.

*Regarding a ligand density, number of moles per volume is considered to be better than number of moles per surface area. The higher a ligand density, the larger the separation factor of β/γ-tocopherol. However, more than 2 µmol/m^2 of ligand density caused the low theoretical plate and the tailing peak.

Separation of Vitamin E and K1 isomers

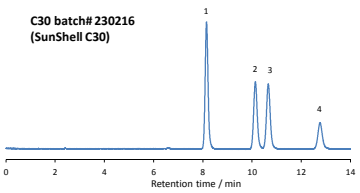


Figure 3: Separation of vitamin E.
Column, C30 batch# 230216, 2.6 µm 150 x 3.0 mm; mobile phase, methanol/water = 97/3; flow rate, 0.43 mL/min; temperature, 25 °C; detection, UV295 nm, sample, 1 = δ-tocopherol, 2 = γ-tocopherol, 3 = β-tocopherol, 4 = α-tocopherol.

Separation factor of β/γ-tocopherol: 1.064
Resolution of β/γ-tocopherol: 2.01

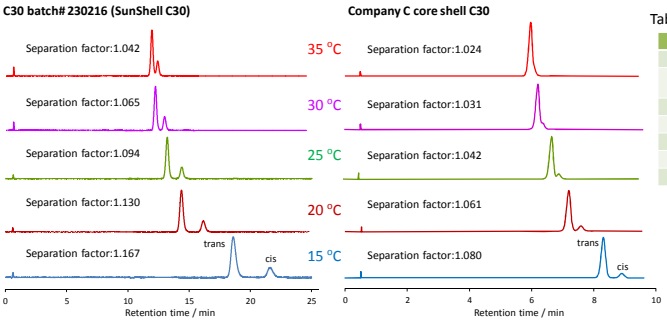
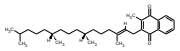


Figure 4: Separation of vitamin K1 isomers.

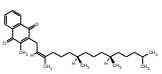
Table 3: Separation factor and resolution of vitamin K1

	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

Vitamin K1 (trans)



Vitamin K1 (cis)



Column, C30 batch# 230216 (SunShell C30), 2.6 µm 100 x 2.1 mm, company C core shell C30, 2.6 µm 100 x 2.1 mm; mobile phase, methanol/water = 96/4; flow rate, 0.35 mL/min; temperature is described in a figure; detection, UV250 nm; sample, vitamin K1 isomers (trans and cis).

Conclusion

* C30 phase could separate β-tocopherol and γ-tocopherol although C18 phase could not separate such isomers.

* The higher C30 ligand density, the larger separation factor of β-tocopherol and γ-tocopherol. However, high hydrophobicity on the particle surface made packing state bad. As a result, more than 2 µmol/m2 of ligand density caused a low theoretical plate and a tailing peak.

* C30 phase bonded inside a pore with 12 nm diameter showed not only high ligand density per volume but also no too high hydrophobicity on the particle surface in order to perform good separation (high theoretical plate and no tailing). Proposed C30 phase (batch# 230216) showed better separation of vitamin K1 isomers than company C C30.