Evaluation of Optimized C30 Phase for Separation of Structurally Related Isomers

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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. However, in many cases a C30 column shows a tailing peak. This tailing is considered to be a reason why C30 ligand density is too high. We have reported optimization for a pore diameter of the superficially porous silica and a ligand density of C30 phase [1]. In this study, the optimized C30 phase was evaluated to compare with C18 phase or the other C30 phase.

Regarding separation of cis and trans-vitamin K1, the optimized C30 phase showed better separation than the other C30 phase while a C18 phase could not separate them. The higher temperature, the worse separation of isomers. The optimized C30 phase could separate cis and trans-vitamin k1 at over 30 degree Celsius although the other C30 phase couldn't.

This C30 phase was applied for separation of some structural related isomers, and showed higher resolution of isomers than conventional C18 phase.

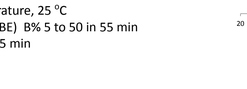
Optimization of C30 phases bonded on Core shell silica

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 1: 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
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.



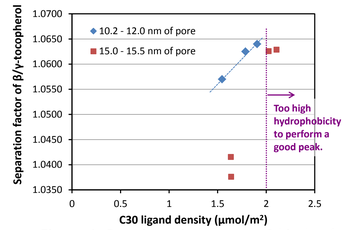


Figure 1: Relationship between 2 kinds of ligand density and separation factor.

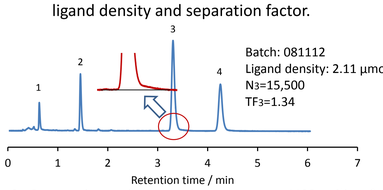


Figure 2: Comparison of theoretical plate and USP tailing factor.
Column dimension, 100 x 2.1mm; mobile phase, acetonitrile/water = 60/40; flow rate, 0.3 mL/min; temperature, 30 °C; peak, 1 = uracil, 2 = ethylbenzoate, 3= acenaphthene, 4 = butylbenzene.

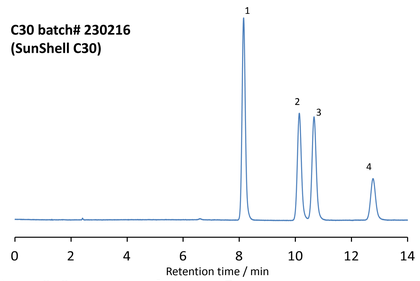


Figure 3: Separation of vitamin E.
Column, C30 batch# 230216, 2.6 µm 150 x 3.0 mm;
mobile phase, methanol/water = 97/3; flow rate, 0.43 mL/min; temperature, 25 °C;
detection, UV295 nm, sample, 1 = δ-tocopherol, 2 = γ-tocopherol, 3 = β-tocopherol, 4 = α-tocopherol.

Table 2: Separation factor of vitamin E

	SunShell C30, 2.6 µm 150 x 3.0 mm
Separation factor of β/γ-tocopherol	1.064
Resolution of β/γ-tocopherol	2.01

*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.

Comparison of C30 phases

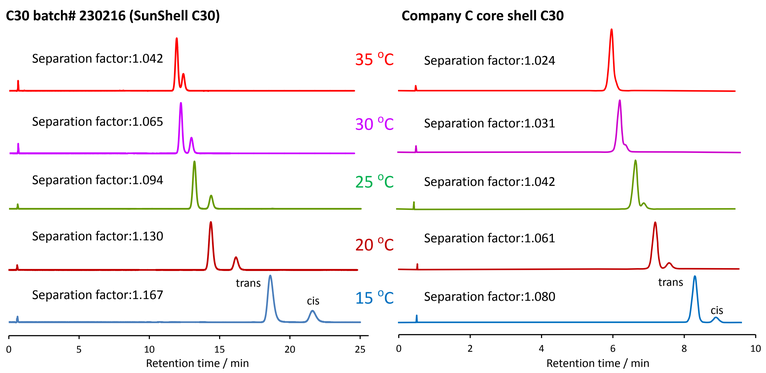


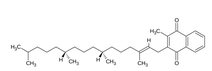
Figure 4: Separation of vitamin K1 isomers.

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).

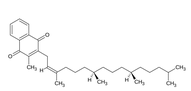
Table 3: Separation factor and resolution of vitamin K1

	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)



Applications of C30 phase

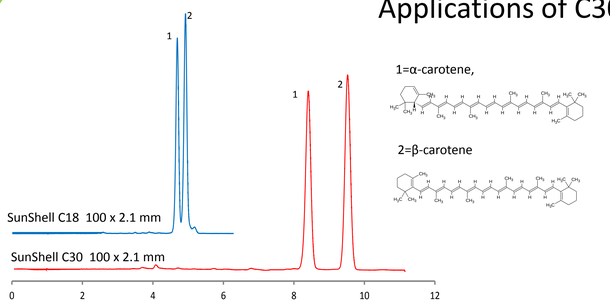


Figure 5: Separation of Carotenes
Column size: 100 x 2.1 mm, Mobile phase: 80/20=CH3CN/CHCl3
Flow rate: 0.2 mL/min, Temperature: 30 ºC, Detection:UV450nm
Sample: 1=a-carotene, 2=b-carotene

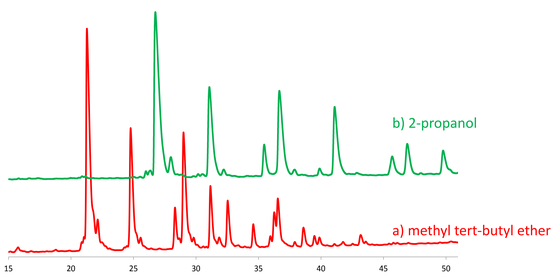


Figure 6: Chromatogram of linseed oil using MTBE or 2-propanol

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

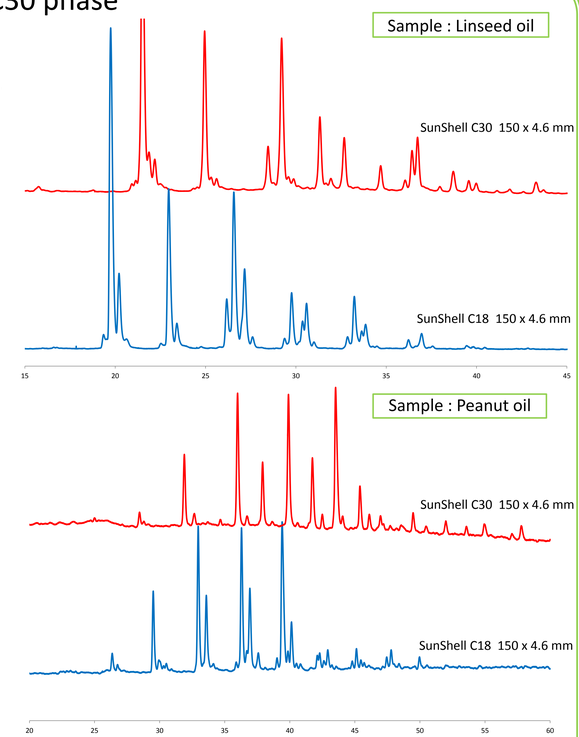


Figure 7: Separation of oil samples

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

Conclusion

- C30 phase could separate β-tocopherol and γ-tocopherol although C18 phase could not separate such isomers.
- 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.
- C30 phase showed both longer retention time of carotenes and better separation of carotenes than C18 phase.
- C30 phase showed better separation for oil sample and shorter analysis time using methyl tert-butyl ether as mobile phase than using 2-propanol.
- C30 phase showed different selectivity comparing with C18 phase when oil samples were separated.