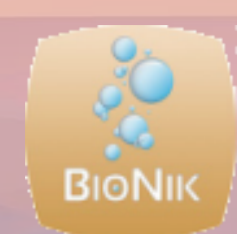


Evaluation of Bidentate End-capping Silylation Reagents for HPLC



BioNik Inc.

www.bionikinc.com

N. Nagae^a, T. Tsukamoto^a, R. Koyama^a, E. Shearer^b
 [a] ChromaNik Technologies Inc. Namiyoke, Minato-ku, Osaka Japan
 [b] BioNik Inc. Obuchi, Fuji, Shizuoka Japan

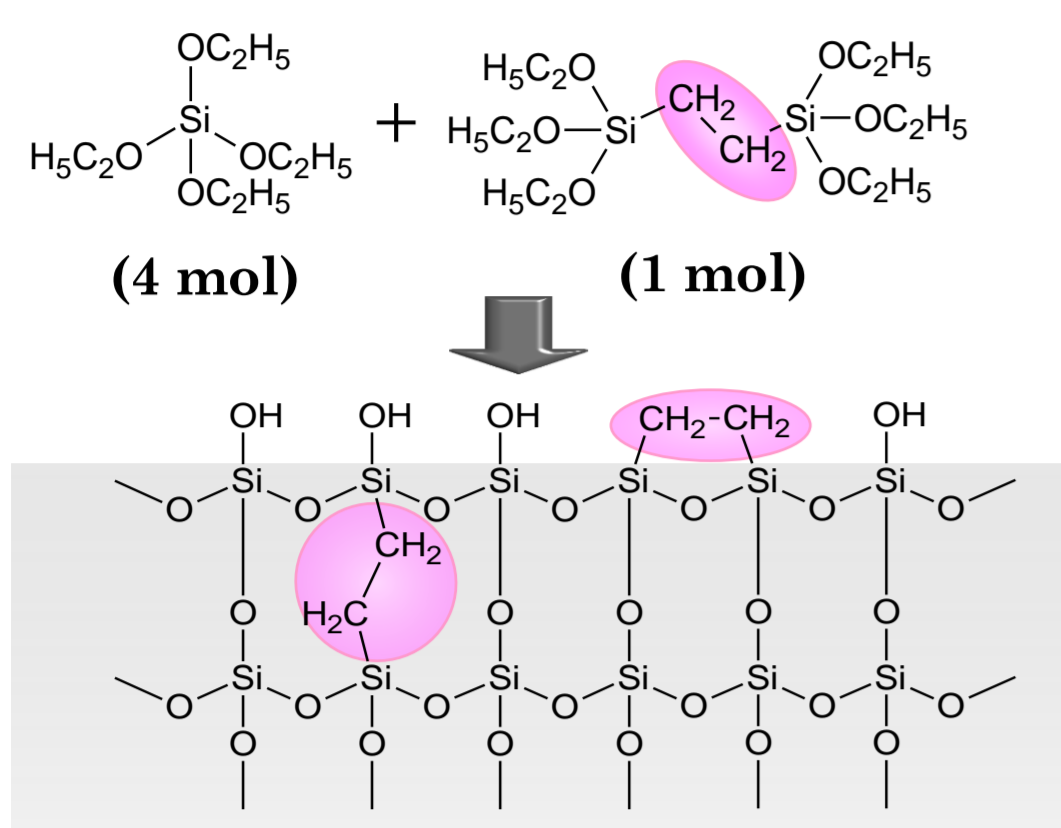


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3 types of based silica gel

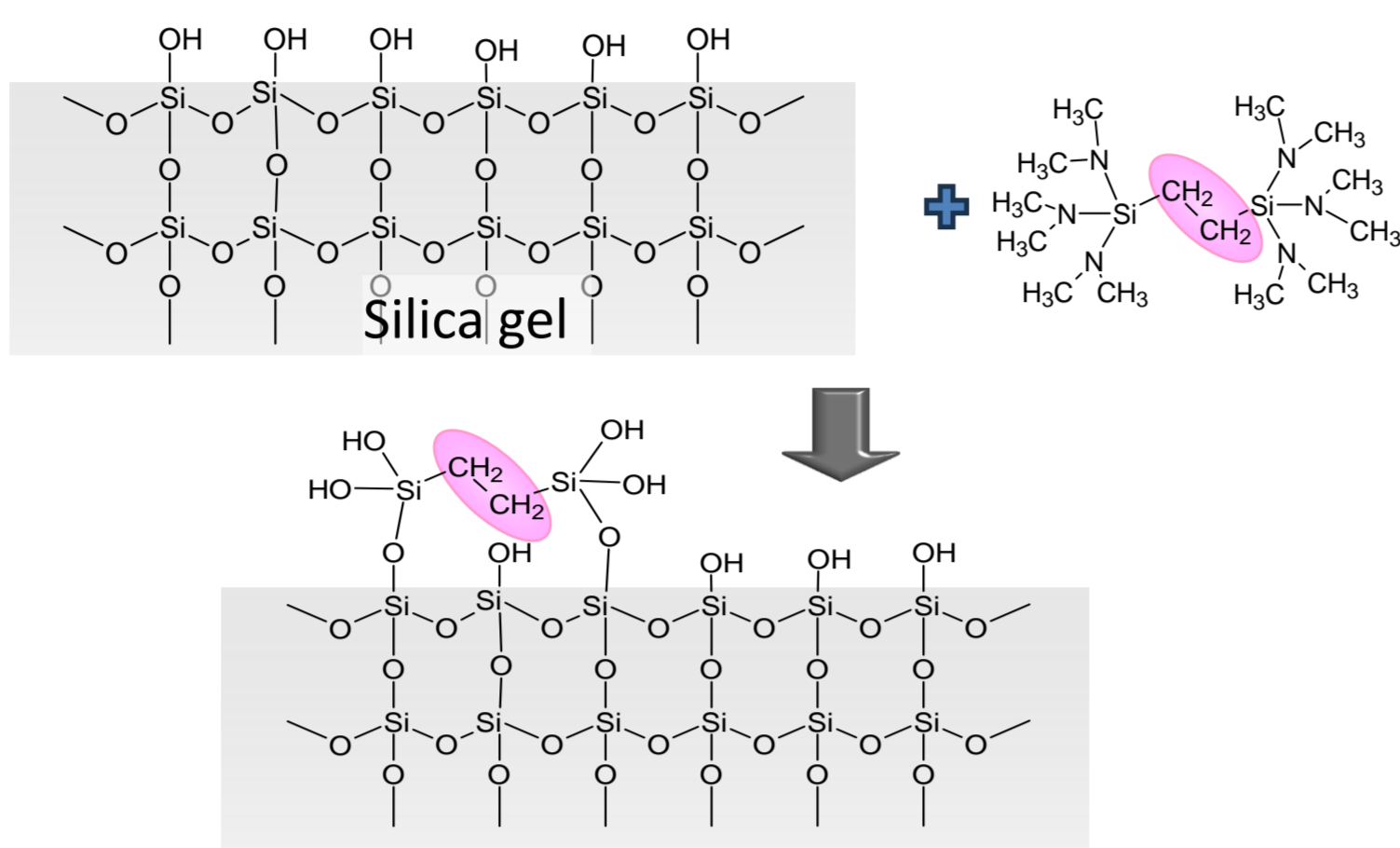
1. Hybrid ①

Ethylene cross-linked silica gel

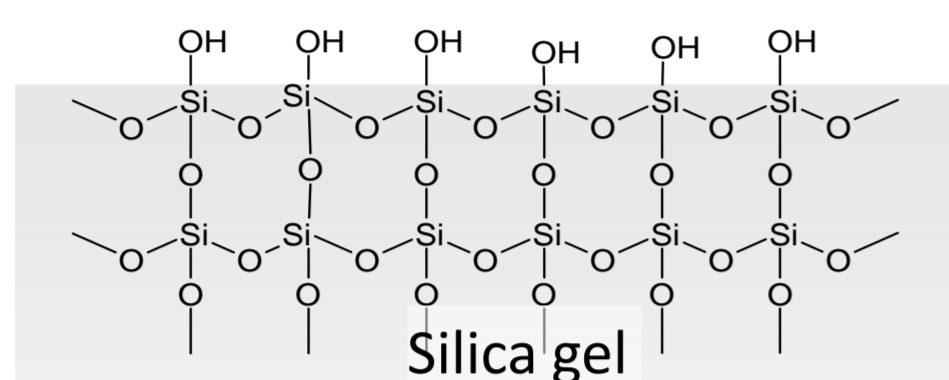


2. Hybrid ②

Ethylene chains bonded to the silica gel surface



3. Silica gel

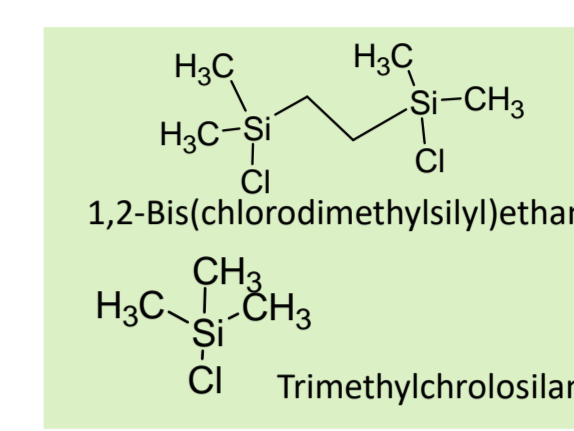
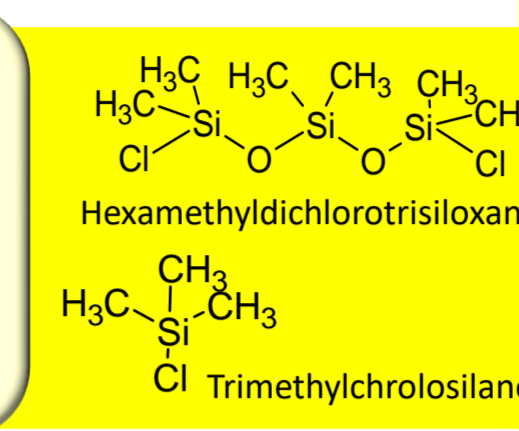


End-capping after C18 bonding

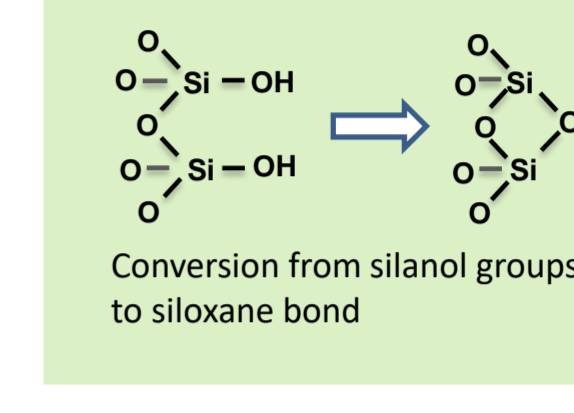
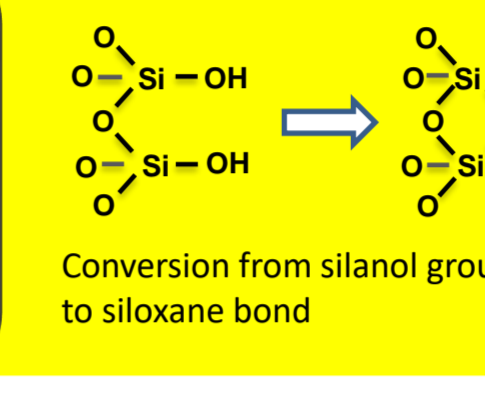


2 types of end-capping reagents

Double end-capping



Elevated temperature end-capping



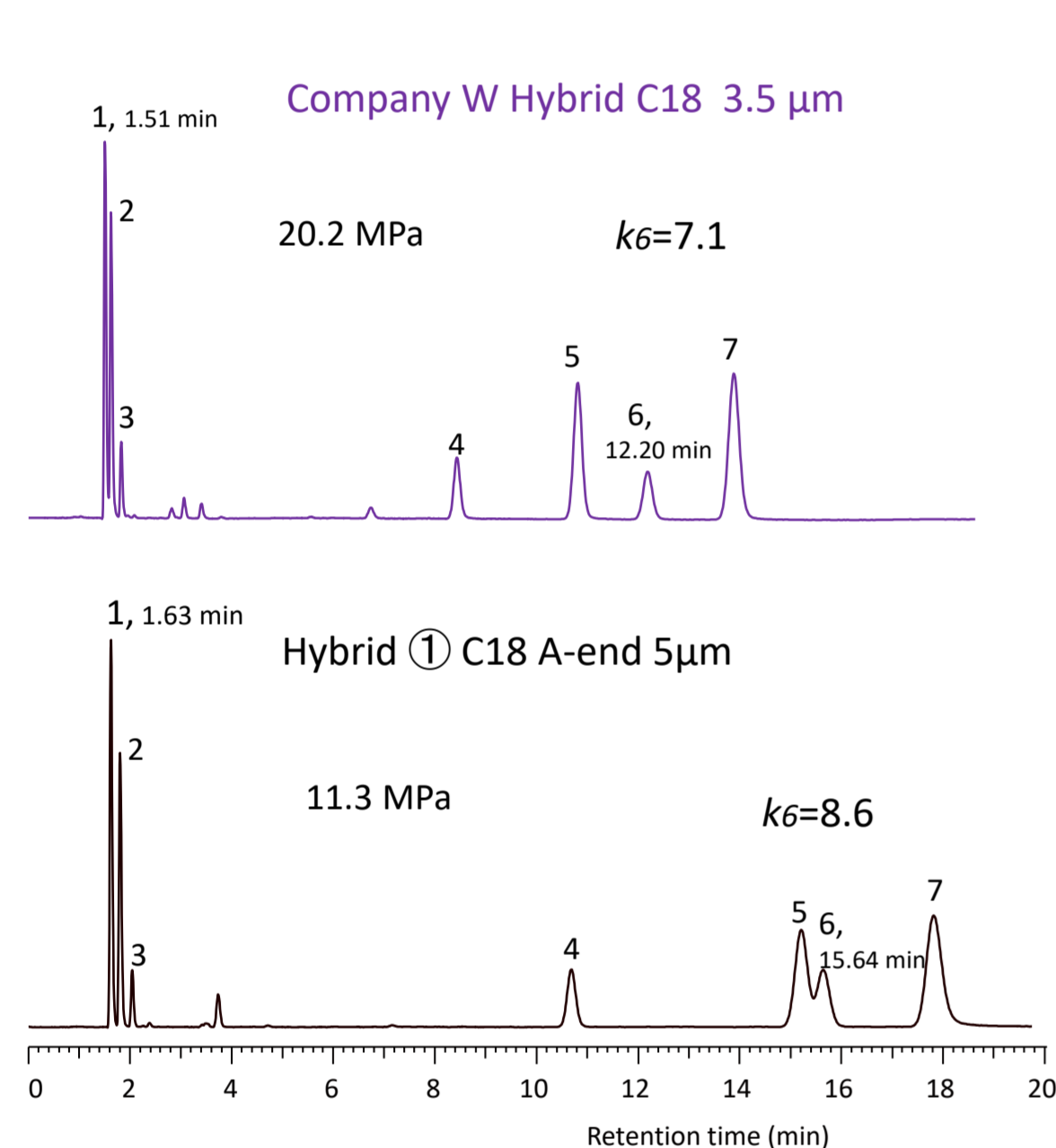
A-type end-capping

B-type end-capping

1,5-Dichlorohexamethyltrisiloxane 1,2-Bis(chlorodimethylsilyl)ethane

★ Six types of C18 packing materials were prepared and compared for hydrogen bonding, hydrophobicity, steric selectivity, peak shape for basic compounds, and durability against acidity and alkalinity.

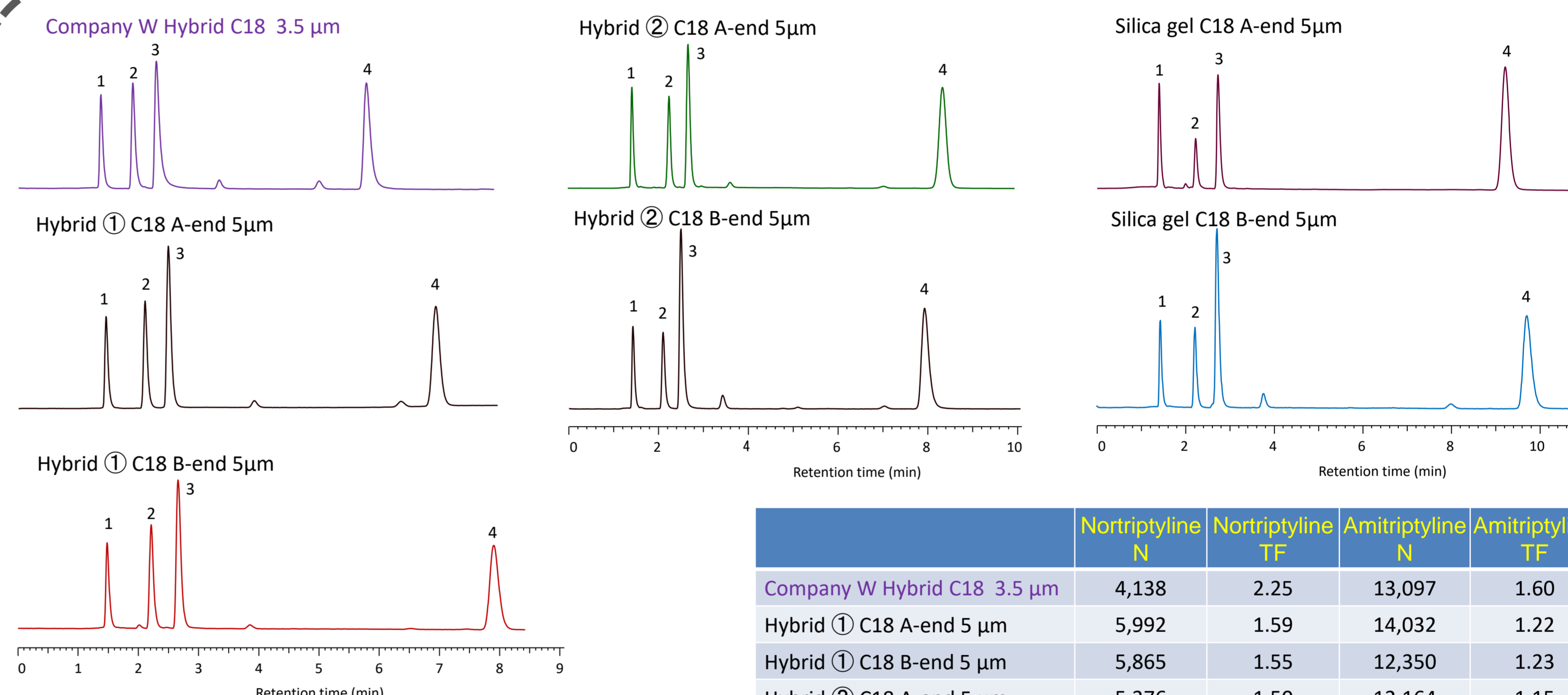
Tanaka Index, specific surface area and carbon loading



Column: Shown in figure
 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)	specific surface area (m ² /g)	Carbon loading
Company W Hybrid C18 3.5 μm	0.38	1.54	1.33	185	17.4%
Hybrid ① C18 A-end 5 μm	0.43	1.55	1.19	209	15.9%
Hybrid ① C18 B-end 5 μm	0.43	1.55	1.23	209	16.0%
Hybrid ② C18 A-end 5 μm	0.40	1.54	1.35	332	17.0%
Hybrid ② C18 B-end 5 μm	0.40	1.56	1.39	332	17.2%
Silica gel C18 A-end 5 μm	0.42	1.58	1.41	330	15.3%
Silica gel C18 B-end 5 μm	0.43	1.60	1.29	330	15.9%

Peak shape of basic compounds



Column: Shown in figure
 Column size: 150 x 4.6 mm
 Mobile phase: Acetonitrile/20mM phosphate buffer pH7.0=60/40
 Flow rate: 1.0 mL/min
 Temperature: 40 °C
 Detection: UV@250 nm
 Sample: 1=Uracil, 2=Propranolol, 3= Nortriptyline, 4=Amitriptyline

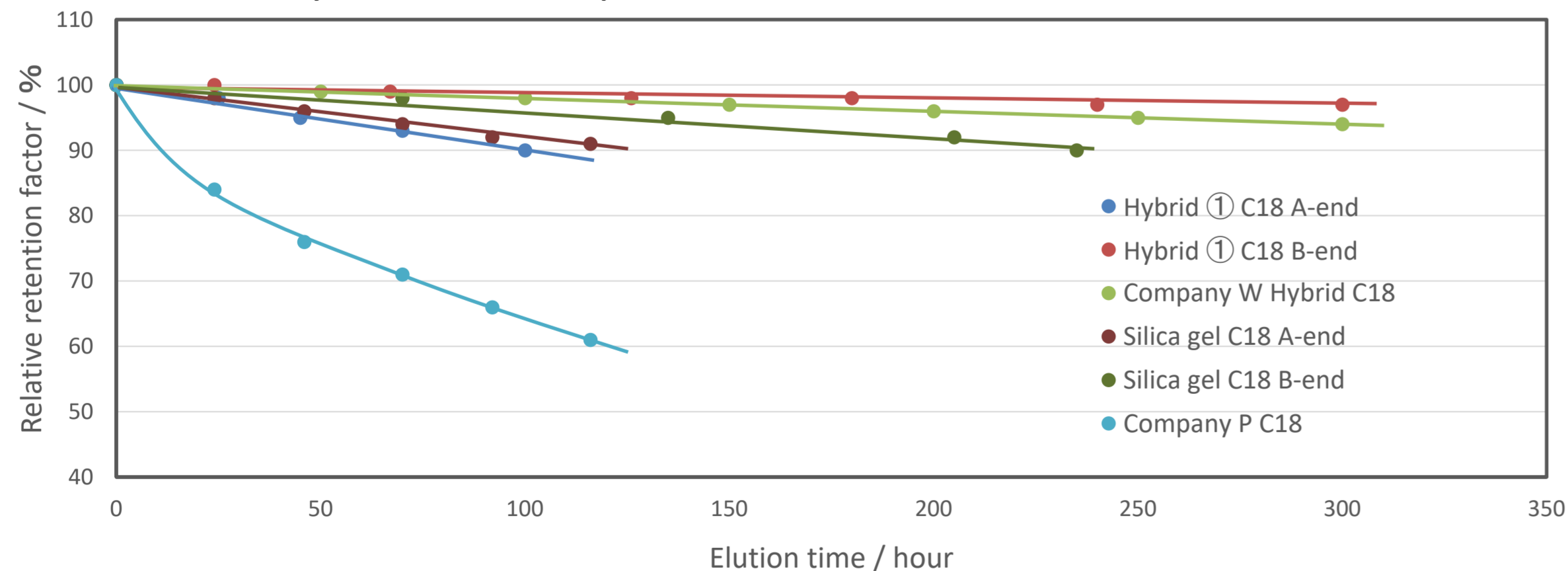
	Nortriptyline N	Nortriptyline TF	Amitriptyline N	Amitriptyline TF
Company W Hybrid C18 3.5 μm	4,138	2.25	13,097	1.60
Hybrid ① C18 A-end 5 μm	5,992	1.59	14,032	1.22
Hybrid ① C18 B-end 5 μm	5,865	1.55	12,350	1.23
Hybrid ② C18 A-end 5 μm	5,376	1.50	12,164	1.15
Hybrid ② C18 B-end 5 μm	4,818	1.63	11,343	1.43
Silica gel C18 A-end 5 μm	6,323	1.51	12,922	1.14
Silica gel C18 B-end 5 μm	5,451	1.54	12,724	1.34

N: Theoretical plate, TF: Tailing factor

★ Under neutral conditions using acetonitrile as the mobile phase, basic compounds are prone to tailing. With bidentate end-capping, both types A and B showed less tailing. The results were more symmetrical than Company W Hybrid C18, which was used for comparison.

Durability under acidic and basic pH conditions

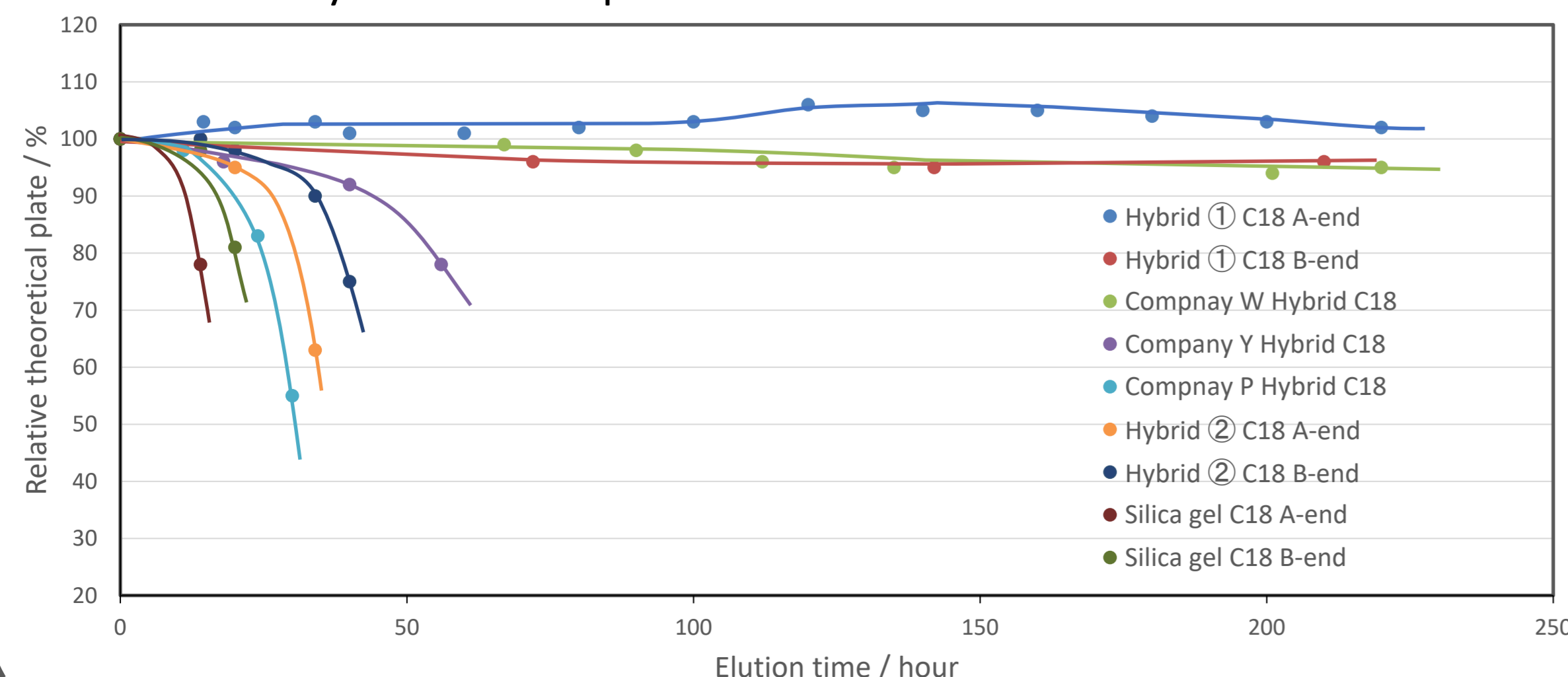
Durability under acidic pH condition



Durable test condition
 Column size: 50 x 2.1 mm
 Mobile phase: Acetonitrile/1.0% TFA, (pH 1)=10/90
 Flow rate: 0.4 mL/min
 Temperature: 80 °C

Measurement condition
 Column size: 50 x 2.1 mm
 Mobile phase: Acetonitrile/water =60/40
 Flow rate: 0.4 mL/min,
 Temperature: 40 °C
 Sample: 1 = Uracil (t₀), 2 = Butylbenzene

Durability under basic pH condition



Durable test condition
 Column size: 150 x 4.6 mm
 Mobile phase: Methanol/50mM potassium phosphate pH 11.5=10/90
 Flow rate: 1.0 mL/min
 Temperature: 40 °C

Measurement condition
 Mobile phase: Acetonitrile/water=70/30
 Flow rate: 1.0 mL/min
 Temperature: 40 °C
 Sample: 1 = Butylbenzene

★ For comparison, the test results of hybrid C18 from companies W, Y, and P are also shown.

Conclusions

- ✓ Six types of packing materials were synthesized using three types of silica base materials and two types of bidentate end-capping reagents after C18 bonding.
- ✓ Comparison of hydrogen bonding, hydrophobicity, and steric selectivity in columns packed with six types of packing materials showed that the end-capping reagent had little effect, while the silica base material had a greater effect.
- ✓ The degree of tailing of the peaks of basic compounds was compared. As a result, no significant difference was observed among the six types of packing materials, and peaks with less tailing were obtained. Company W hybrid C18, used as a reference, showed tailing of basic compounds (especially nortriptyline).
- ✓ In both durability tests under acidic and basic pH conditions, packing materials using B type 1,2-bis(chlorodimethylsilyl)ethane for end-capping were more durable than A type 1,5-dichlorohexamethyltrisiloxane. It was presumed that this is because the B type end-capping reagent is more hydrophobic, making it difficult for water molecules, which cause hydrolysis and lead to deterioration, to approach the packing silica surface.
- ✓ The durability under basic pH condition was greatly affected by the silica base material, and the silica with ethylene chains crosslinked into the silica skeleton showed the highest durability, which was equivalent to that of Hybrid C18 from company W.