Phenyl-bonded stationary phases

Introduction

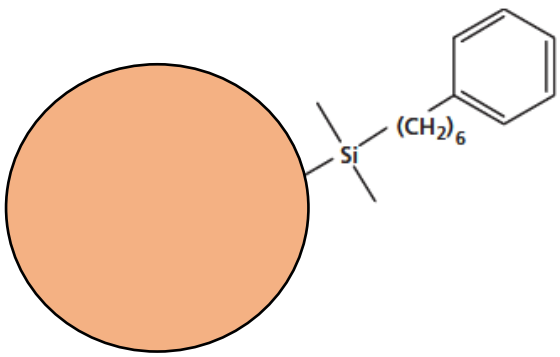
With the explosive increase in the number of new large-scale peptide purification processes, more and better tools are needed.

Distinguishing between the correctly formed target peptide molecule and the mishappened ones, often only differing in a single amino acid, is difficult. The separation power of the conventional alkyl chain-bonded reversed phase silica types (C18, C8 bonded) mostly utilize hydrogen bond interactions as the means of separation.

For stronger, better or **ALTERNATIVE** separation, secondary interactions can be employed or if available different separation modes should be explored.

A rather powerful alternative separation is the π–π interaction.

The chemistry



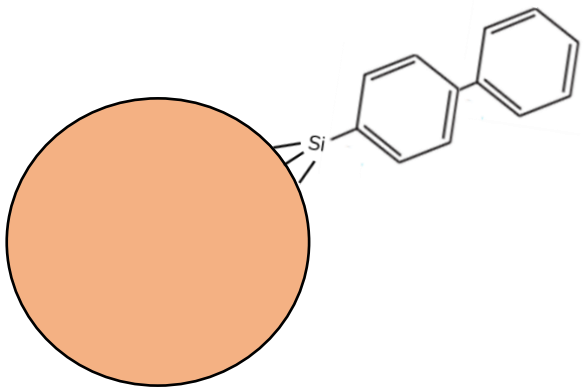
Phenyl Hexyl stationary phase

SP-100-10-C6Ph-HP

Hydrophobic interaction due to the alkyl group and π–π interaction due to the phenyl group can be expected.

	Specifications
Pore size [nm]	8.5 – 11.5
Surface area [m^2/g]	400 – 480
Pore volume [mL/g]	1.00 – 1.20
D90/D10	≤ 1.60
Total carbon [%]	16.0 – 21.0

* Except for carbon, the physical properties of bonded silica gel are the values of bare silica gel.



BiPhenyl stationary phase

SP-100-10-BiPh-LP

SP-100-10-BiPh-HP

A stronger π–π interaction can be expected by having two phenyl groups.

	Specifications
Pore size [nm]	8.5 – 11.5
Surface area [m^2/g]	400 - 480
Pore volume [mL/g]	1.00 – 1.20
D90/D10	≤ 1.60
Total carbon [%]	13.0 or 17.0

* Except for carbon, the physical properties of bonded silica gel are the values of bare silica gel.

Inquiry

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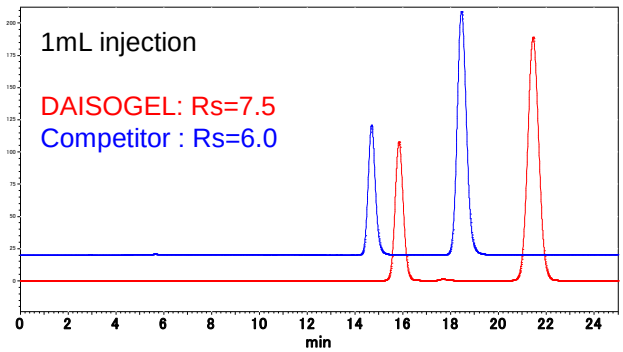
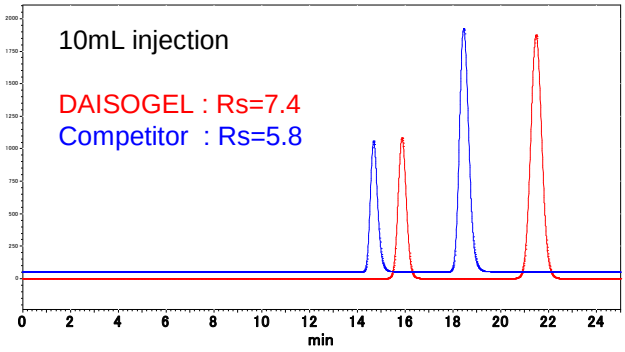
Phenyl-bonded stationary phases

Resolution (*SP-100-10-C6Ph*)

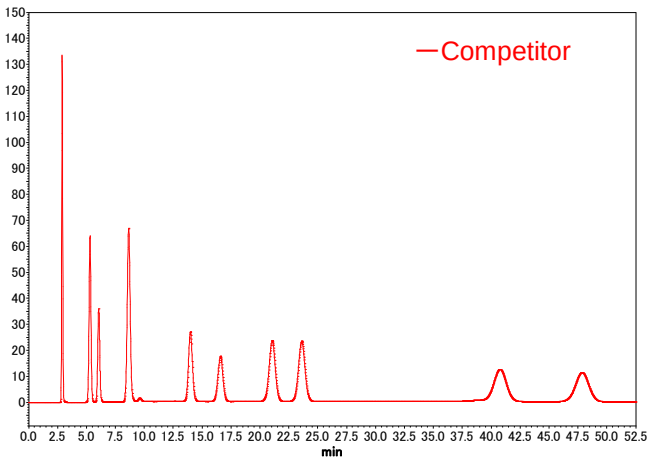
Column size	: 4.6x250 mm
Mobile phase	: MeOH :H_2O=50:50
Sample	: ①Nitrobenzene ②*m*-Dinitrobenzene
Flow rate	: 1.0 mL/min
Temp	: 4 0 ℃
Detector	: UV254 nm

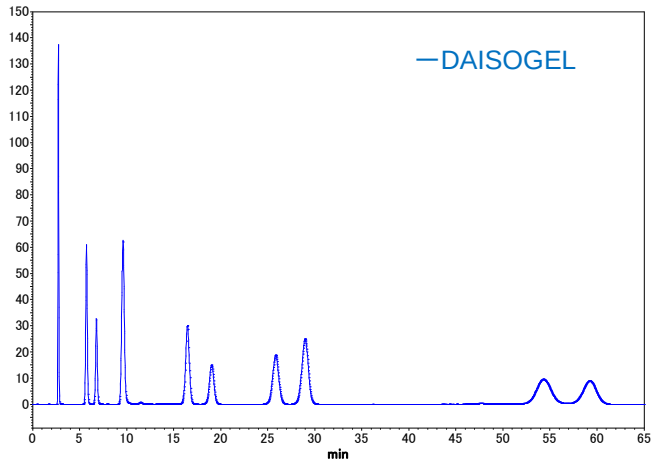
Higher resolution was shown than competitor. And there was no change in resolution when the injection volume was increased.





10 standard test (*SP-100-10-C6Ph*)





Column size	: 4.6 x 250 mm
Mobile phase	: MeOH :H_2O=50:50
Sample	: 1) Uracil 2) Caffeine 3) Phenol 4) 2-Ethylpyridine 5) Benzene 6) Methyl benzoate 7) N, N-Dimethylaniline 8) Toluene 9) 3-Phenylacetylacetone 10) Naphthalene
Flow rate	: 1.0 mL/min
Temp	: 40 ℃
Detector	: UV254 nm

		Competitor	DAISOGEL
Hydrophobicity	α(T/B)	1.87	1.91
Hydrogen bonding	α(CA/Ph)	0.76	0.74
Surface polarity	α(MB/T)	0.66	0.62
Ion exchange 1	α(EP/B)	0.52	0.50
Ion exchange 2	α(DA/T)	0.88	0.88
Retention factor	kN	15.50	20.54

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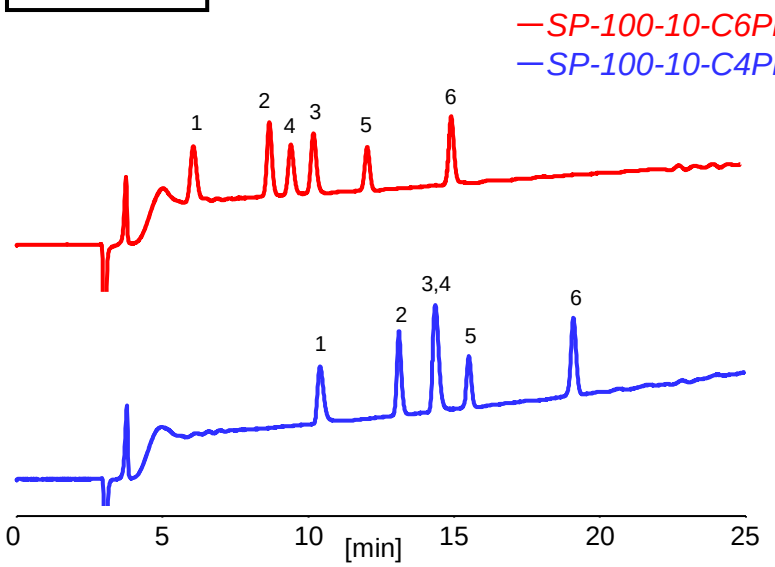
Phenyl-bonded stationary phases

Alkyl chain length (*SP-100-10-C6Ph / SP-100-10-C4Ph*)

Osaka Soda has an original Phenyl phase with a shorter linked than PheHex: a C4 linker chain. Occasionally, the ButylHexyl shows different separation patterns compared to PheHex due to the dominance of π–π interaction over hydrogen bond interaction.

1st case



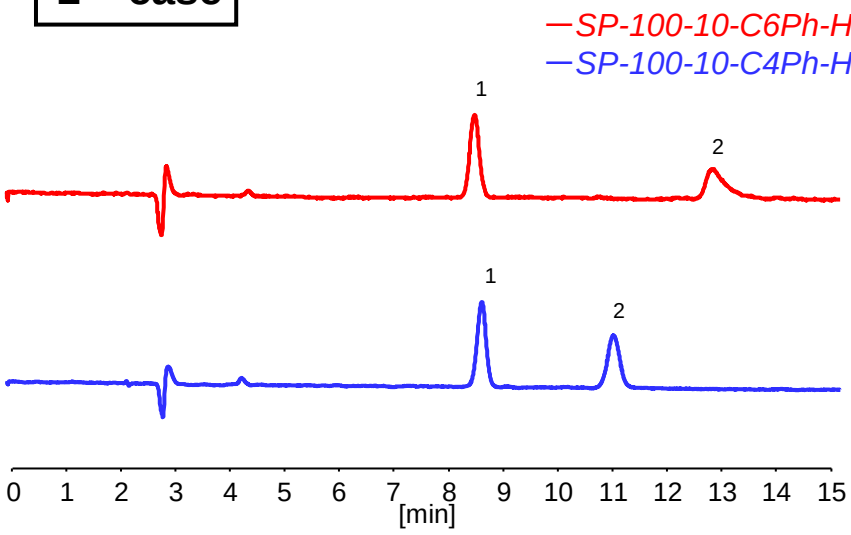
Peptide standards test

1. Bradykinin
2. Angiotensin II
3. Neurotensin
4. Angiotensin I
5. Oxytocin
6. Leu-Enkephalin

50ppm each in H_2O

Column size	: 4.6x250 mm
Mobile phase	: A) 0.1 vol% HCOOH, H2O / CH3CN = 90 / 10 B) 0.1 vol% HCOOH, H2O / CH3CN = 50 / 50
	: B 0 % (0 min) -> 50 % (30 min) -> 0 % (30.1 min) Gradient
Flow rate	: 1.0 mL/min
Temp	: 4 0 ℃
Detector	: UV254 nm

2nd case



Salicylic acid test

1. Phenol
2. Salicylic acid

Column size	: 4.6 x 250 mm
Mobile phase	: 0.1 vol% H3PO4 in 30% CH3CN
Flow rate	: 1.0 mL/min
Temp.	: 35 ℃
Detector	: UV254 nm

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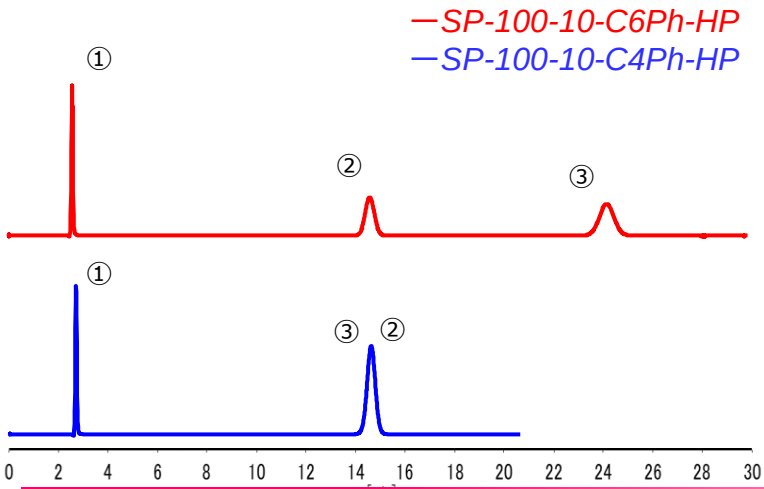
Phenyl-bonded stationary phases

Alkyl chain length (*SP-100-10-C6Ph / SP-100-10-C4Ph*)

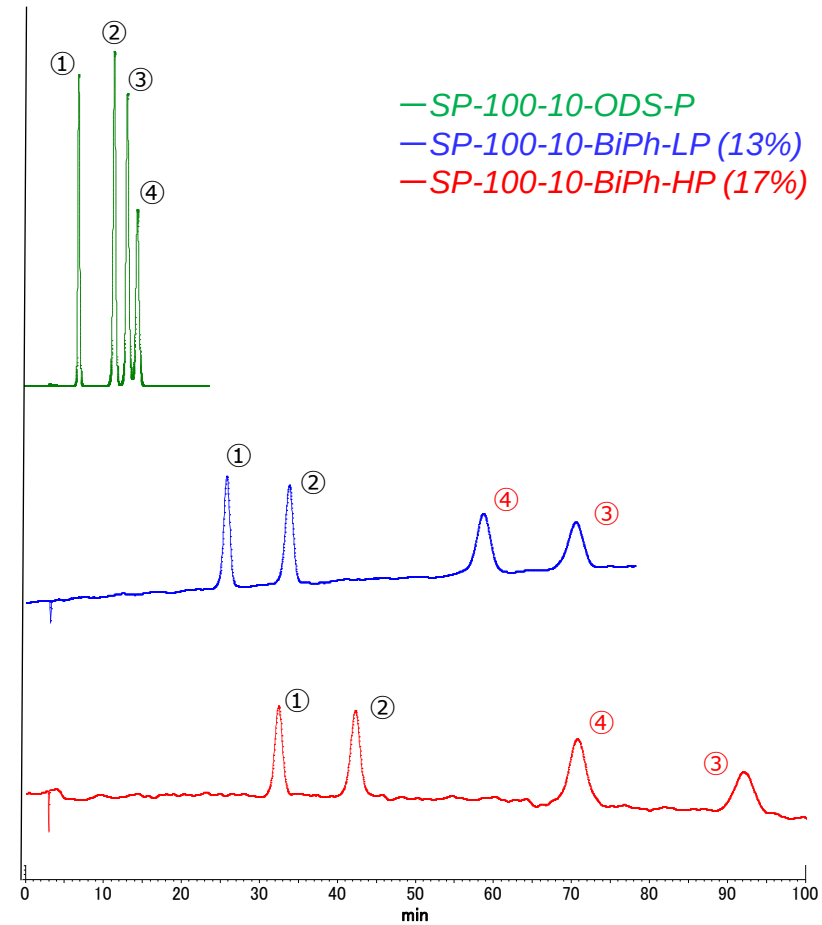
3rd case



Column size	: 4.6 x 250 mm
Mobile phase	: MeOH :H_2O=80:20
Sample	: ① Uracil
	② o-Terphenyl
	③ Triphenylene
Flow rate	: 1.0 mL/min
Temp.	: 35 ℃
Detector	: UV254 nm

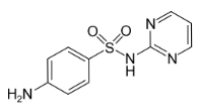
In this last case, the PheHex silica is the one able to differentiate between the two isomers.

Sulfonamide test (*SP-100-10-BiPh-LP/HP*)

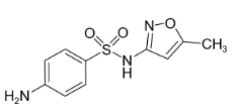


Column size	: 4.6 x 250 mm
Mobile phase	: MeOH:50mM NH_4NO_3=20:80
Sample	:

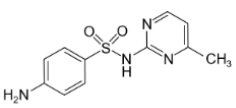
①Sulfadiazine



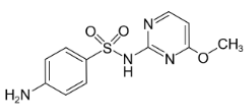
②Sulfamethoxazole



③Sulfamerazine



④Sulfamonomethoxine



Flow rate	: 1.0 mL/min
Temp	: 40 ℃
Detector	: UV254 nm

Very different separation profile was shown between C18 and BiPhenyl.

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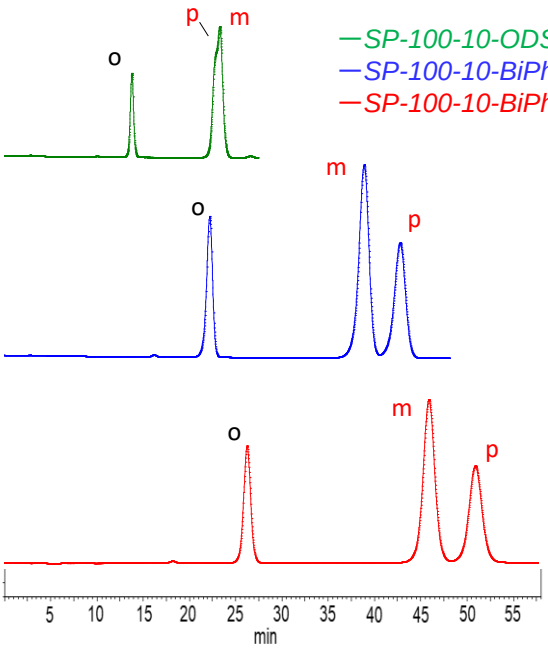
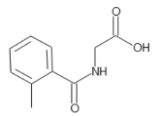
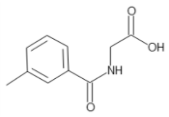
Phenyl-bonded stationary phases

Regioisomers *o-*, *m-*, *p-* (*SP-100-10-BiPh-LP/HP*)

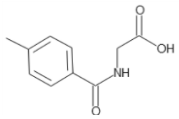


Column size : 4.6 x 250 mm
Mobile phase : MeOH:25mM Na_3PO_4=25:75
Sample :
①*o*-Methylhippuric acid ②*m*-Methylhippuric acid





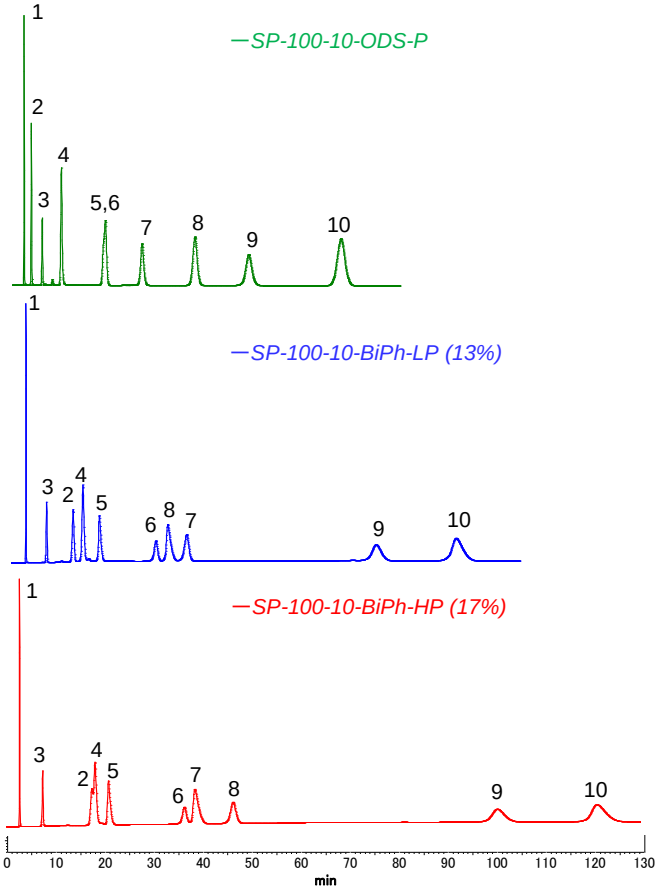
③*p*-Methylhippuric acid



Flow rate : 1.0 mL/min
Temp : 40 ℃
Detector : UV230 nm

BiPhenyl phase is expected to separate aromatic isomers

10 standard test (*SP-100-10-BiPh-LP/HP*)



Column size : 4.6x250 mm
Mobile phase : MeOH :H_2O=50:50
Sample : 1) Uracil
2) Caffeine
3) Phenol
4) 2-Ethylpyridine
5) Benzene
6) Methyl benzoate
7) N, N-Dimethylaniline
8) Toluene
9) 3-Phenylacetylacetone
10) Naphthalene
Flow rate : 1.0 mL/min
Temp : 40 ℃
Detector : UV254 nm

		C18	BPh 13%	BPh 17%
Hydrophobicity	α(T/B)	2.10	1.93	1.97
Hydrogen bonding	α(CA/Ph)	0.40	2.27	3.13
Surface polarity	α(MB/T)	1.00	0.92	0.94
Ion exchange 1	α(EP/B)	0.46	0.78	0.85
Ion exchange 2	α(DA/T)	0.69	1.13	1.22

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