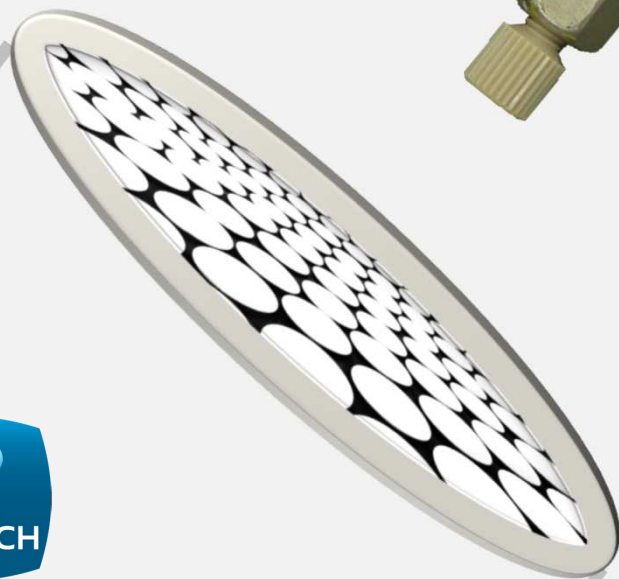


**SunShell** Core shell particle  
**Sunniest** Fully porous particle

# Biphenyl



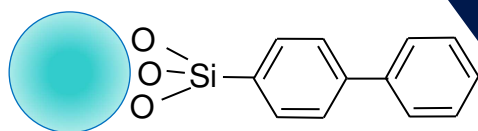
UHPLC & HPLC column



ChromaNik Technologies Inc.

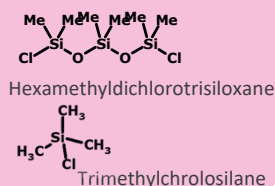
# Biphenyl

Trifunctional silyl-reagent used

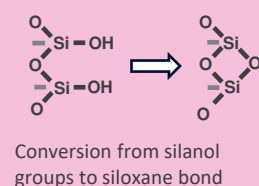


Feature of end-capping of Sunniest Biphenyl and SunShell Biphenyl (Sunniest End-capping)

**Double end-capping**



**High temperature end-capping**

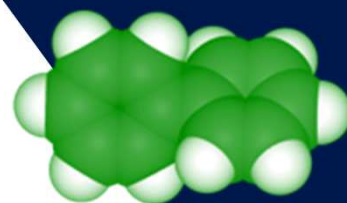


➤ **Low bleeding**

➤ **High stability**

Feature of Biphenyl

- ✓ The two benzene rings of the biphenyl group are twisted about 45 degree, and the biphenyl group shows different separation from the phenyl group alone.
- ✓ Even when an aqueous mobile phase containing no organic solvent is used, there is no decrease in retention, and separation with high reproducibility of retention time is achieved.
- ✓ Greater retention of polar compounds such as caffeine.



Specification of Sunniest Biphenyl

	Fully porous silica			Bonded phase				
	Particle size (μm)	Pore size (nm)	Specific surface area (m <sup>2</sup> /g)	Carbon loading (%)	Stationary phase	USP L line	End-capping	pH range
Sunnest Biphenyl	5	12	340	14	Biphenyl	L11	Sunnest end-capping	1.5 - 9

Specification of SunShell Biphenyl

	Core shell silica				Bonded phase					
	Particle size (μm)	Core size (μm)	Pore size (nm)	Specific surface area (m <sup>2</sup> /g)	Carbon loading (%)	Stationary phase	USP L line	End-capping	Maximum pressure	pH range
SunShell Biphenyl	2.6	1.6	9	150	5	Biphenyl	L11	Sunnest end-capping	60 MPa	1.5 - 9

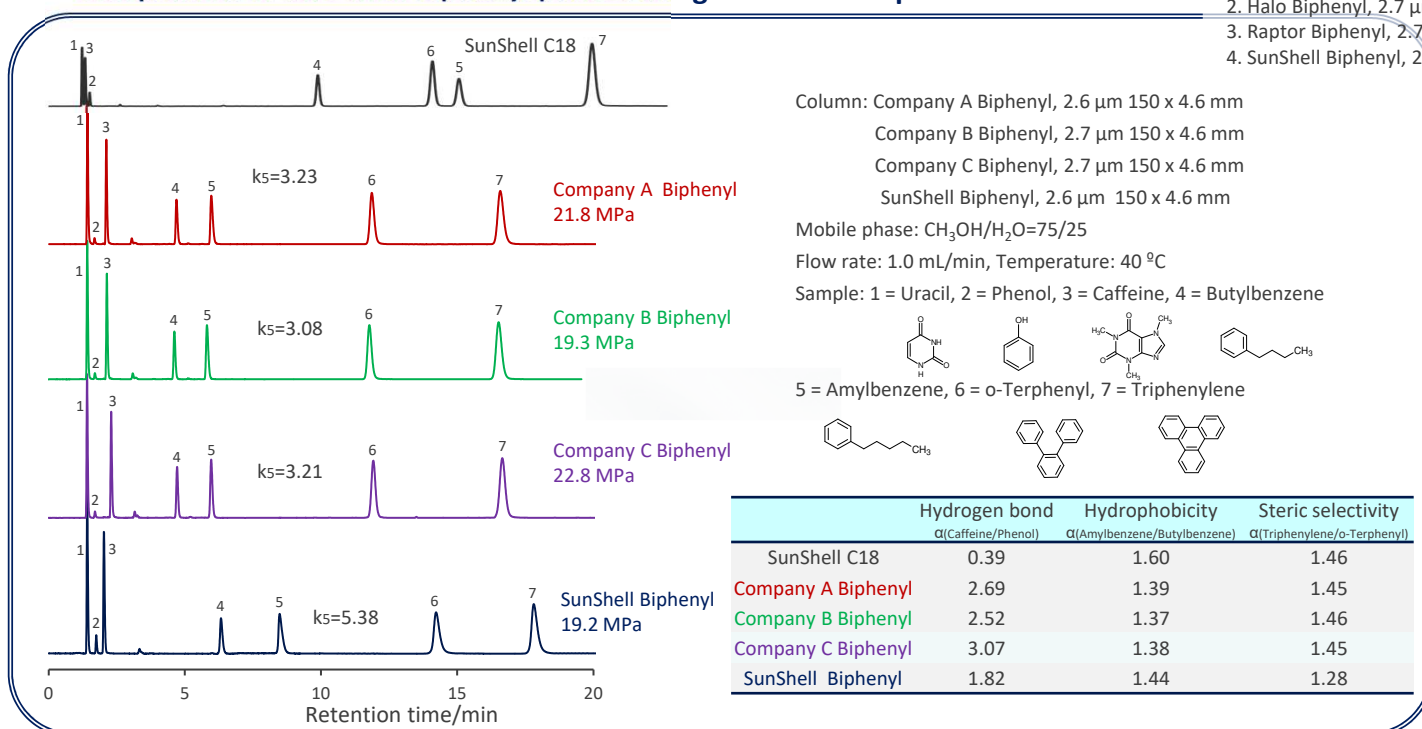
Specification of other Biphenyl (cited from a brochure)

Company A Biphenyl	2.6	---	10	100	11	Biphenyl	L11	TMS	60 MPa	1.5 - 8.5
Company B Biphenyl	2.7	---	9	150	7	Biphenyldimethylsilane	L11	Yes	60 MPa	1.5 - 8.0
Company C Biphenyl	2.7	---	9	150	7	Biphenyldimethylsilane	L11	Yes	60 MPa	1.5 - 9.0

# Comparison of core shell Biphenyl columns

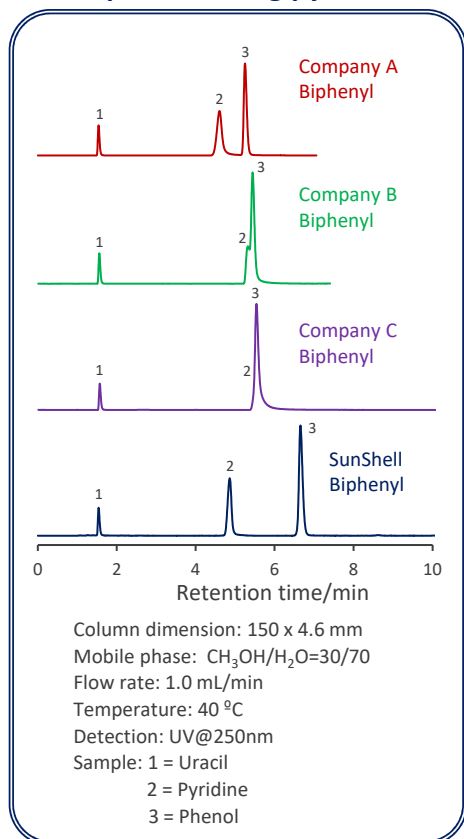
## Comparison of core shell Biphenyl phases using standard samples

- Columns for comparison
1. Kinetex Biphenyl, 2.6  $\mu\text{m}$
  2. Halo Biphenyl, 2.7  $\mu\text{m}$
  3. Raptor Biphenyl, 2.7  $\mu\text{m}$
  4. SunShell Biphenyl, 2.6  $\mu\text{m}$



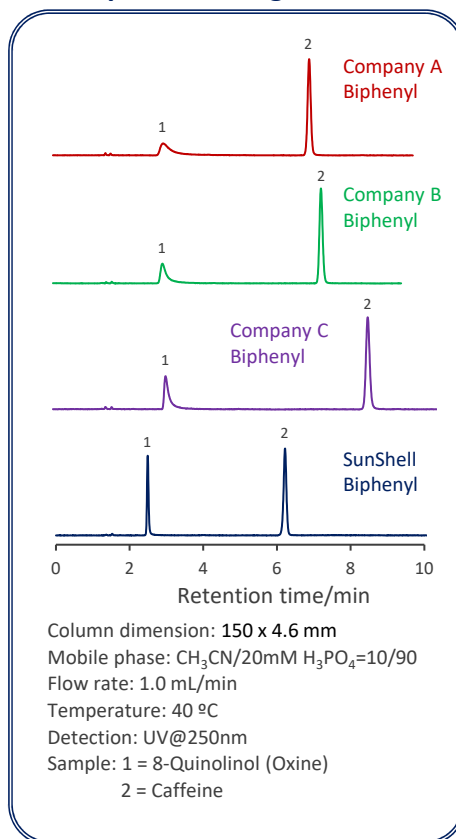
Compared with other company's core shell type Biphenyl. Biphenyls from companies A, B and C showed similar separation patterns. It was confirmed that SunShell Biphenyl has a higher retention of amylbenzene No. 5 and a larger separation factor (hydrophobicity in the table) for the difference of one carbon between butylbenzene and amylbenzene, and is more hydrophobic than other company Biphenyl. The carbon loading is 5% for SunShell Biphenyl and 7% or more for all other Biphenyls, and the high hydrophobicity of SunShell, which has the lowest carbon content, indicates high density end-capping. Moreover, the separation of standard samples is very different for biphenyl and C18.

## Comparison using pyridine



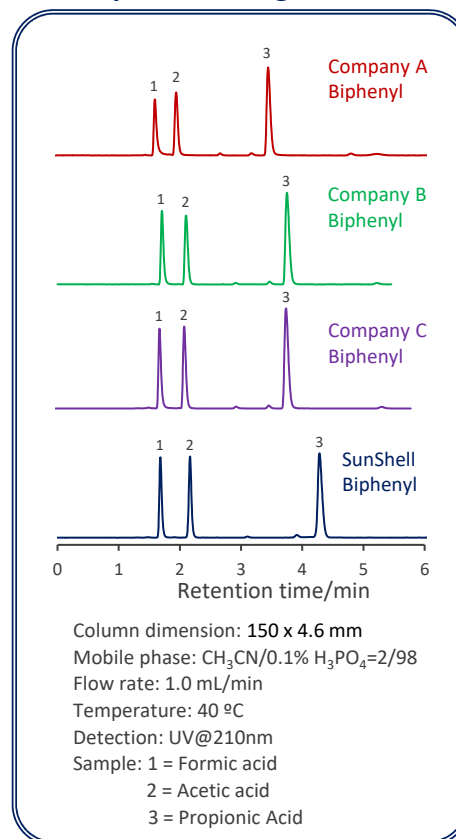
Pyridine is a compound that tends to tail due to residual silanol groups. When methanol is used as the organic solvent in the mobile phase rather than acetonitrile, tailing of pyridine is more likely to occur and the difference between brands becomes clear.

## Comparison using oxine



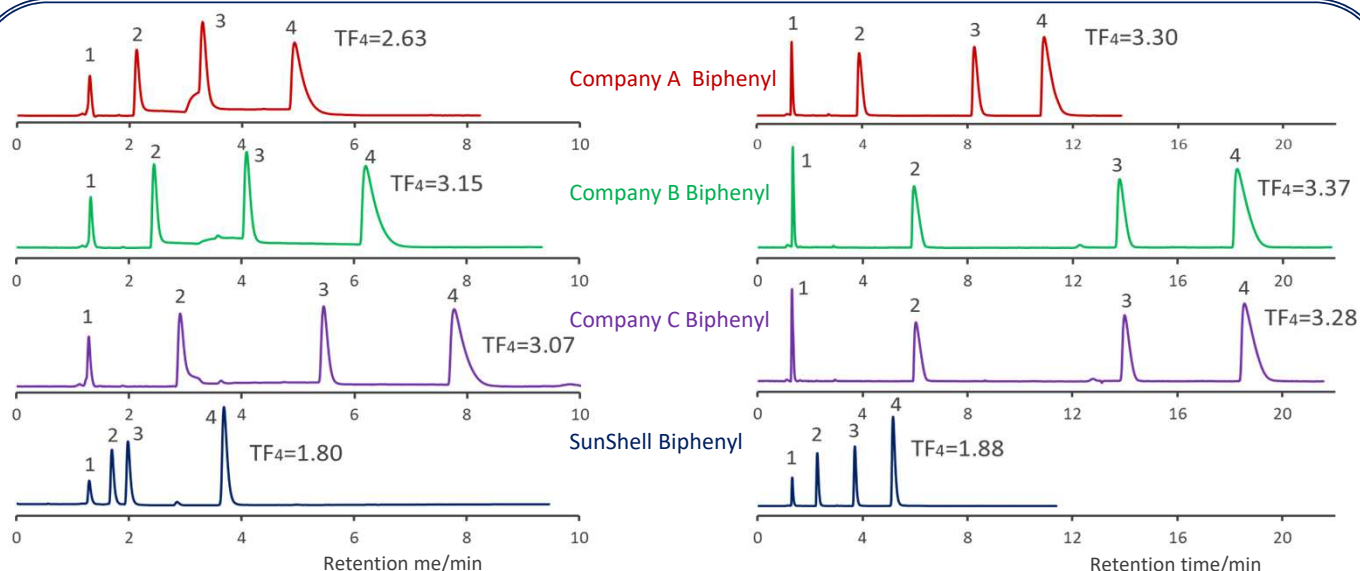
8-Quinololinol (oxine) is a metal chelating compound, and if there are metal impurities in the packing material, its peak becomes a tailing peak.

## Comparison using formic acid



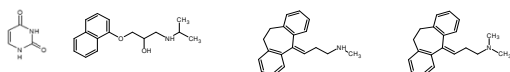
Although most acidic compounds can be eluted without problems, formic acid is a typical acidic compound that easily causes tailing. Formic acid is tailed on Company A Biphenyl.

## Comparison of core shell Biphenyl phases using basic compounds



Column dimension: 150 x 4.6 mm  
 Mobile phase: CH<sub>3</sub>CN/20 mM Phosphate buffer pH 7.0=60/40  
 Flow rate: 1.0 mL/min  
 Temperature: 40 °C  
 Detection: UV@250 nm  
 Sample: 1 = Uracil, 2 = Propranolol, 3 = Nortriptyline, 4 = Amitriptyline

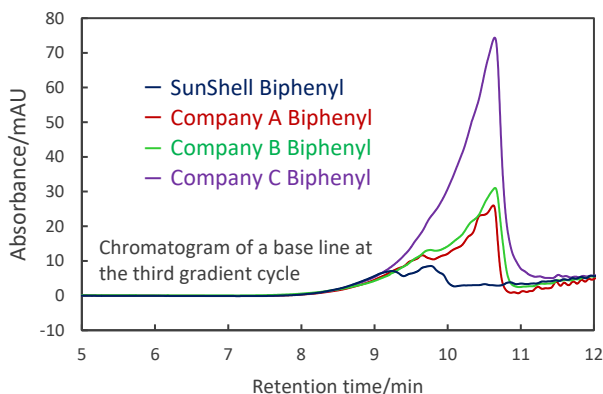
Column dimension: 150 x 4.6 mm  
 Mobile phase: CH<sub>3</sub>CN/10 mM Ammonium acetate pH 6.8=40/60  
 Flow rate: 1.0 mL/min  
 Temperature: 40 °C  
 Detection: UV@250 nm  
 Sample: 1 = Uracil, 2 = Propranolol, 3 = Nortriptyline, 4 = Amitriptyline



Tailing of basic compounds (amitriptyline) occurs more easily in the acetonitrile/buffer mobile phase than in the methanol/buffer mobile phase. A mixture of acetonitrile and 20 mM phosphate buffer and a mixture of acetonitrile and 10 mM ammonium acetate were used as mobile phase to compare with core shell Biphenyls. Biphenyl from other companies had a terrible tailing for basic compounds, and it was also confirmed that the retention time was increased, which seems to be influenced by the adsorption to residual silanol groups.

## Comparison of stability

Bleed comparison under acidic condition



### Bleed test condition

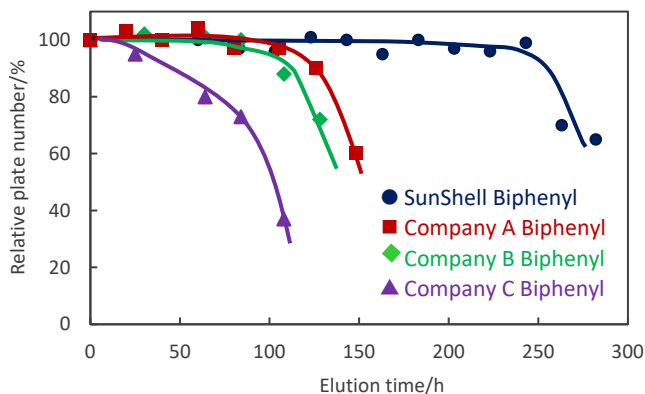
Column dimension: 50 x 2.1 mm  
 Mobile phase: A) 1% H<sub>3</sub>PO<sub>4</sub> (pH 1.2)  
 B) Acetonitrile

### Gradient program

Time (min)	0	5	10	17	18	35
%B	10	10	90	90	10	10

Flow rate: 0.3 mL/min  
 Temperature: 40 °C  
 Detection: UV@250 nm

Comparison of plate number under weakly alkaline condition



### Elution condition of stability test

Column dimension: 50 x 2.1 mm  
 Mobile phase: 20 mM Sodium phosphate pH 8.0  
 Flow rate: 0.2 mL/min  
 Temperature: 40 °C

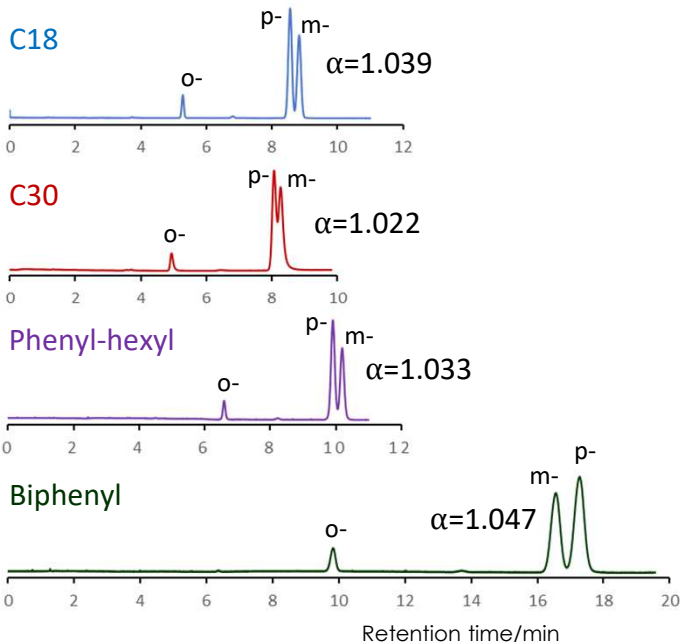
### Measurement condition of plate number

Mobile phase: CH<sub>3</sub>CN/H<sub>2</sub>O=50/50  
 Flow rate: 0.2 mL/min  
 Temperature: 40 °C  
 Detection: UV@250 nm  
 Sample: Butylbenzene

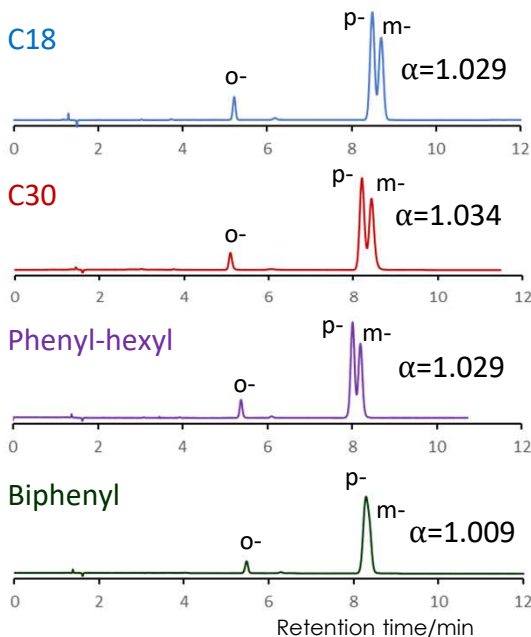
Stability under acidic condition compared baseline changes with gradient elution of 1% aqueous phosphate solution and acetonitrile. Biphenyl groups are cut off from the silica surface under acidic condition and elute out of the column as the amount of organic solvent increases. Baseline variability detected cut Biphenyl groups, with SunShell showing the least desorbed and high acid stability. In addition, under weakly alkaline condition, the silica desolved and the column-in side was dented, so the theoretical plate number of the columns were compared. Since the Biphenyl column has high reproducibility of retention time even in a 100% aqueous mobile phase (see page 6) and is effective for separating highly polar compounds, stability comparison was performed under the condition of pH 8 that does not contain an organic solvent. SunShell is more than twice as durable as other companies' columns.

## Comparison of stationary phases using isomers of methylhippuric acid

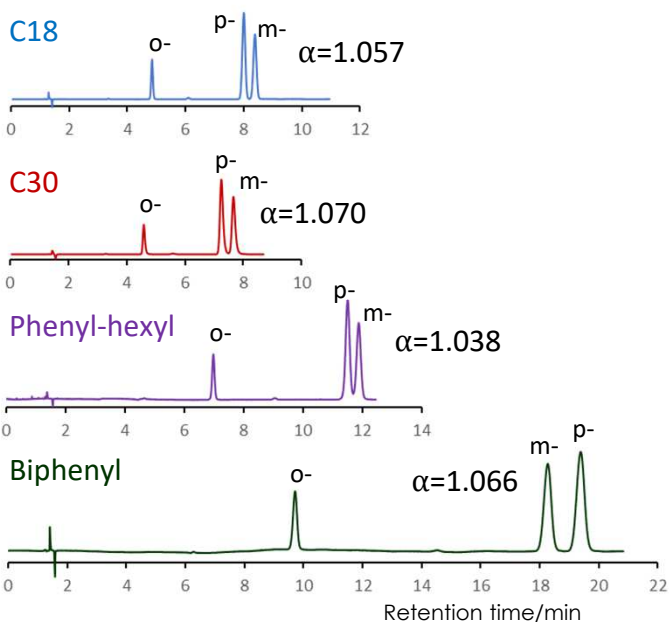
### Methanol/buffer=25/75



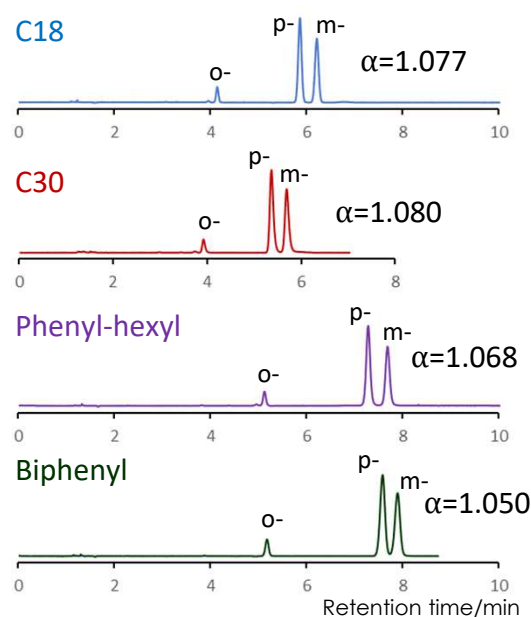
### Acetonitrile/buffer=13.5/86.5



### 2-Propanol/buffer=7/93

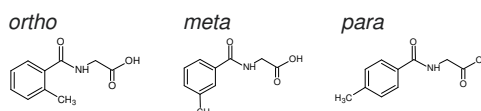


### Tetrahydrofuran/buffer=10/90



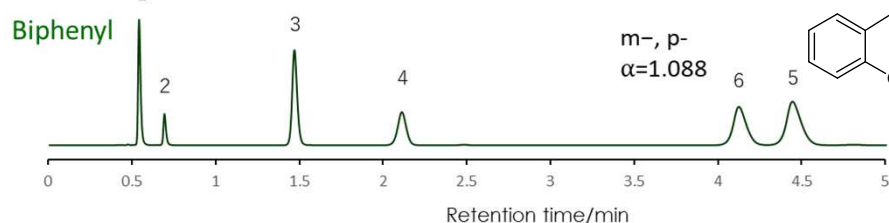
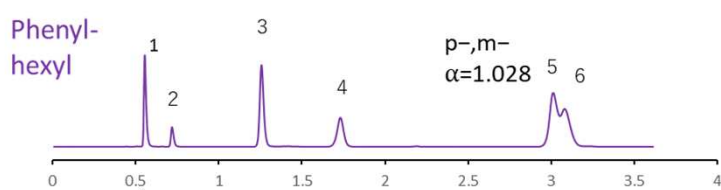
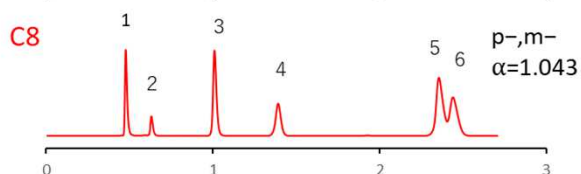
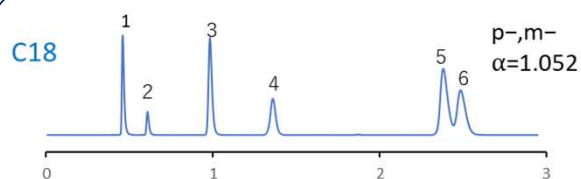
Column: SunShell C18 2.6  $\mu\text{m}$  150 x 4.6 mm  
 SunShell C30 2.6  $\mu\text{m}$  150 x 2.1 mm  
 SunShell Phenyl 2.6  $\mu\text{m}$  150 x 4.6 mm  
 SunShell Biphenyl 2.6  $\mu\text{m}$  150 x 4.6 mm  
 Mobile phase: Organic solvent/25 mM Phosphate buffer pH 3.0  
 Flow rate: 1.0 mL/min, 0.2 mL/min for only C30

Temperature: 40  $^{\circ}\text{C}$   
 Detection: UV@230 nm  
 Sample: *o*-, *m*-, *p*-Methylhippuric acid



The separation of *ortho*, *meta* and *para* isomers of methylhippuric acid was compared. The stationary phase was C18, C30, Phenyl-hexyl and Biphenyl, and the organic solvent in the mobile phase was methanol, acetonitrile, 2-propanol and tetrahydrofuran. The separation of *meta*-methylhippuric acid and *para*-methyl hippuric acid changed depending on the organic solvent, and the separation was improved in the order of acetonitrile, methanol, 2-propanol, and tetrahydrofuran. Biphenyl showed a particularly high retention when alcohol was used as the organic solvent in the mobile phase, and the elution order of *meta* and *para* was reversed from that of other stationary phases. It is considered that this is due to the high hydrogen bonding capacity obtained on comparison of separation of standard samples. When acetonitrile is used, the  $\pi$ - $\pi$  interaction between the solute and the stationary phase is weakened by the triple bond of CN in acetonitrile, so it is considered that the characteristics of Biphenyl cannot be fully exhibited. When tetrahydrofuran is used, tetrahydrofuran enters the stationary phase and a mixture of biphenyl group and tetrahydrofuran works as a stationary phase, so it is considered that the separation behavior was different from that when alcohol was used.

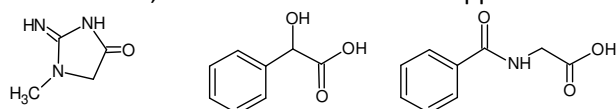
# Comparison of stationary phases using creatinine, mandelic acid, hippuric acid and methylhippuric acid



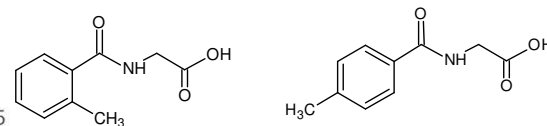
## MS検出器用移動相

Column: SunShell C18 2.6  $\mu$ m 100 x 2.1 mm  
 SunShell C8 2.6  $\mu$ m 100 x 2.1 mm  
 SunShell Phenyl 2.6  $\mu$ m 100 x 2.1 mm  
 SunShell Biphenyl 2.6  $\mu$ m 100 x 2.1 mm  
 Mobile phase: 2-Propanol/20 mM Ammonium acetate pH 6.8=3/97  
 Flow rate: 0.4 mL/min  
 Temperature: 40 °C  
 Detection: UV@230 nm

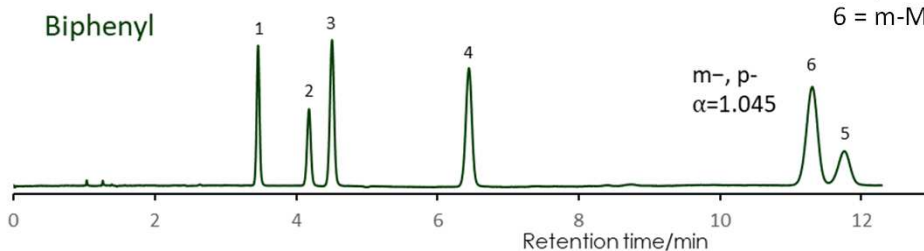
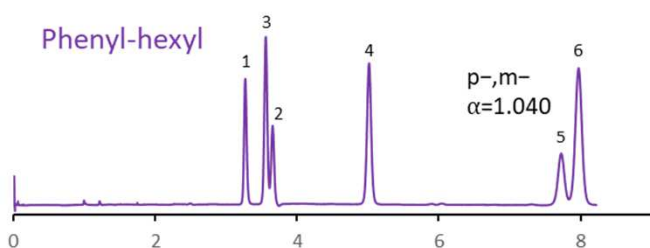
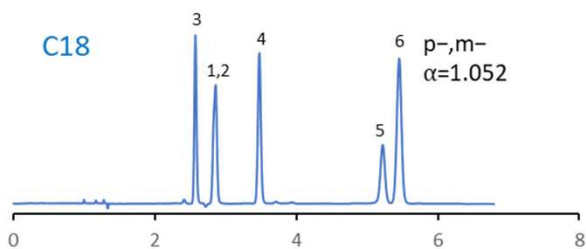
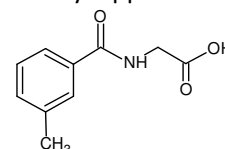
Sample: 1 = Creatinine, 2 = Mandelic acid 3 = Hippuric acid



4 = o-Methylhippuric acid, 5 = p-Methylhippuric acid,



6 = m-Methylhippuric acid



## イオンペアー試薬添加移動相

Column: SunShell C18 2.6  $\mu$ m 150 x 4.6 mm  
 SunShell Phenyl 2.6  $\mu$ m 150 x 4.6 mm  
 SunShell Biphenyl 2.6  $\mu$ m 150 x 4.6 mm  
 Mobile phase: 2-Propanol:10 mM  $\text{KH}_2\text{PO}_4$ , 2 mM Sodium 1-Octanesulfonate (pH 2.5;  $\text{H}_3\text{PO}_4$ )=10:90  
 Flow rate: 1.0 mL/min  
 Temperature: 40 °C  
 Detection: UV@210 nm

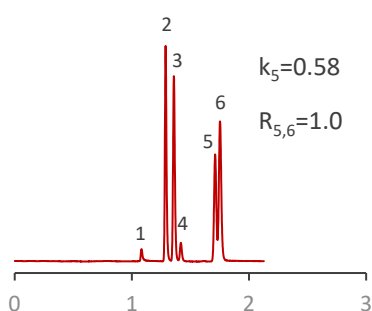
Sample: 1 = Creatinine,  
 2 = Mandelic acid  
 3 = Hippuric acid  
 4 = o-Methylhippuric acid,  
 5 = p-Methylhippuric acid,  
 6 = m-Methylhippuric acid

Phosphate buffer was used as the mobile phase for separation of methylhippuric acid isomers on the previous page, but in this comparison of the separation including creatinine, the ammonium acetate buffer mobile phase that can be applied to LC / MS and the mobile phase in which an ion pair reagent were added to a phosphate buffer solution was used. 2-Propanol was used as the organic solvent. Under both mobile phase conditions, Biphenyl showed the highest retention and the best separation. A characteristic separation of Biphenyl is that the elution order of the isomers meta-methylhippuric acid and para-methylhippuric acid is reversed to that of C18, C8 and Phenyl-hexyl.

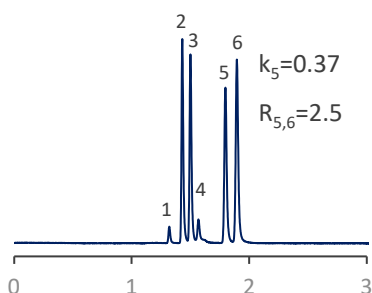
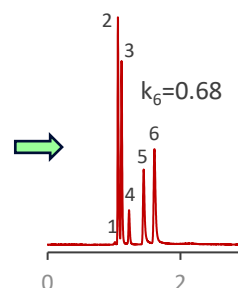
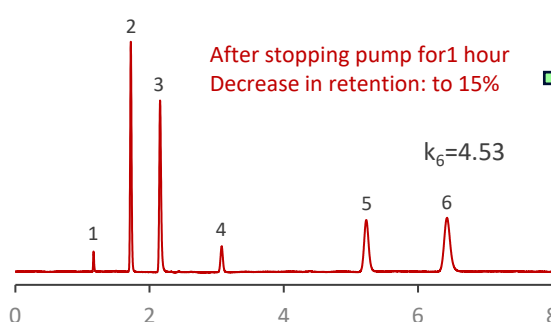
## Comparison of stationary phases using nucleic acid bases

A) Methanol/10 mM ammonium acetate pH6.8=20/80

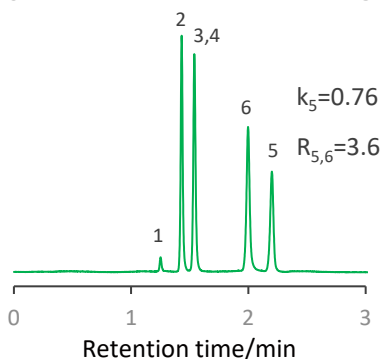
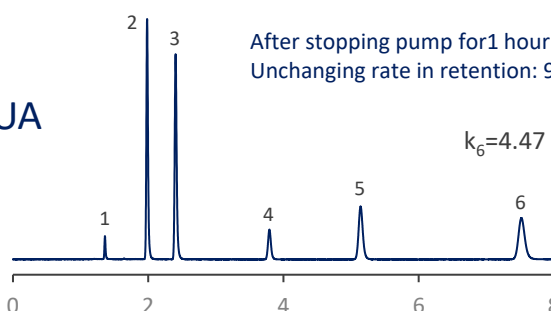
B) 10 mM ammonium acetate pH6.8



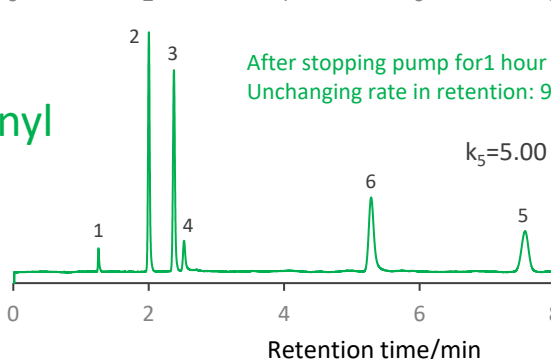
C18



RP-AQUA



Biphenyl

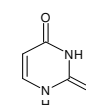


1. NaNO<sub>2</sub>

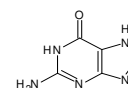
2. Cytosine



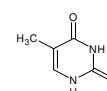
3. Uracil



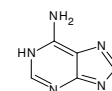
4. Guanine



5. Thymine



6. Adenine



Column: SunShell C18 2.6 μm, 150 x 4.6 mm (pore size: 9 nm)  
 SunShell RP-AQUA 2.6 μm, 150 x 4.6 mm (pore size: 16 nm)  
 SunShell Biphenyl 2.6 μm, 150 x 4.6 mm (pore size: 9 nm)  
 Mobile phase: A) Methanol/10 mM ammonium acetate pH 6.8=20/80  
 B) 10 mM ammonium acetate pH 6.8

Flow rate: 1.0 mL/min  
 Temperature: 40 °C  
 Detection: UV@250 nm

Sample: 1 = Sodium nitrite, 2 = Cytosine, 3 = Uracil, 4 = Guanine, 5 = Thymine, 6 = Adenine

Separation of nucleic acid bases was performed on C18, RP-AQUA and Biphenyl columns. Two mobile phases were used: methanol/10 mM ammonium acetate pH 6.8=20/80 and 10 mM ammonium acetate aqueous solution. Nucleic acid bases can be separated even in the mobile phase containing 20% methanol, but the retention time is shorter. In comparison of columns used this 20% methanol mobile phase, the retention factor ( $k_5$ ) of thymine at the 5th peak was highest in Biphenyl, and the resolution of the 5th and 6th peaks of thymine and adenine ( $R_{5,6}$ ) was also highest in Biphenyl. When an aqueous mobile phase containing no organic solvent was used, the retention factor of thymine eluting at last on Biphenyl was 5.00 and the adenine eluting at last on C18 and RP-AQUA were 4.53 and 4.47, respectively. Biphenyl showed the largest retention factor for the compound eluted at last. The selectivity of Biphenyl is very different from that of RP-AQUA and C18, such as the order of sample elution. It has also been confirmed that the retention time stability when using an aqueous mobile phase is as high for Biphenyl as for RP-AQUA. The retention factor on C18 decreased to 15% after stopping the flow for 1 hour, confirming low reproducibility in retention.

★ Biphenyl stationary phase has a high hydrogen-bonding capacity and is effective for retaining polar compounds.

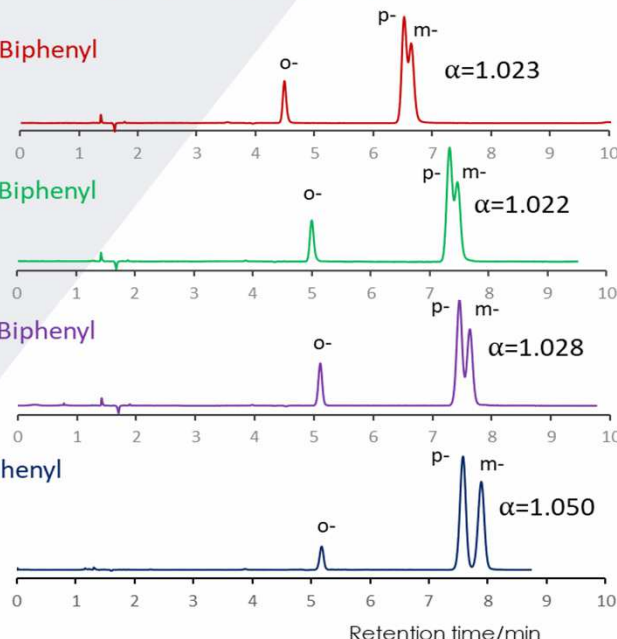
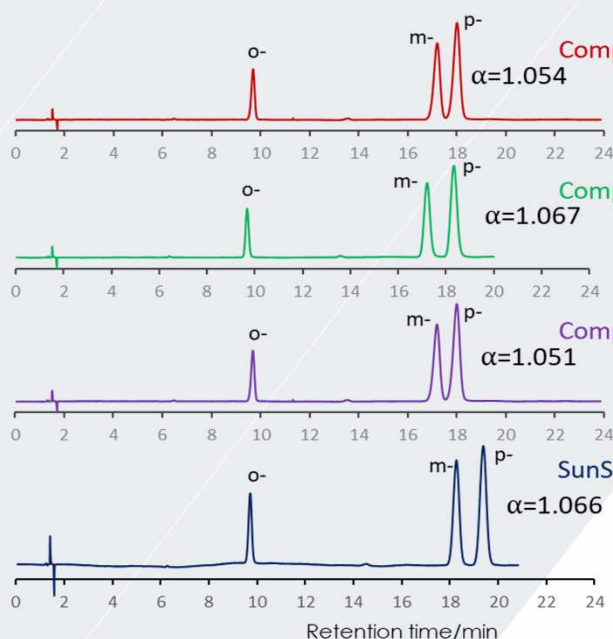
Moreover, separation with high reproducibility of retention time is achieved even with 100% aqueous mobile phase.

### Comparison of Biphenyl phases using isomers of methylhippuric acid

\* Measurement condition is the same as that in page 4.

**2-Propanol/buffer=7/93**

**Tetrahydrofuran/buffer-10/90**



#### Ordering information of SunShell Biphenyl

Packings	Inner diameter (mm)	1.0	2.1	3.0	4.6	USP category
	Length (mm)	Catalog number	Catalog number	Catalog number	Catalog number	
SunShell Biphenyl, 2.6 $\mu$ m	30	-----	C86931	C86331	C86431	L11
	50	-----	C86941	C86341	C86441	
	75	-----	C86951	C86351	C86451	
	100	-----	C86961	C86361	C86461	
	150	-----	C86971	C86371	C86471	

#### Ordering information of Sunniest Biphenyl

Packings	Inner diameter (mm)	2.0	3.0	4.6	10	20	USP category
	Length (mm)	Catalog number	Catalog number	Catalog number	Catalog number	Catalog number	
Sunniest Biphenyl, 5 $\mu$ m	50	E83241	E83341	E83441	-----	-----	L11
	100	E83261	E83361	E83461	-----	-----	
	150	E83276	E83376	E83476	-----	-----	
	250	E83281	E83381	E83481	E83781	E83881	



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E-mail: [info@biotechfluidics.com](mailto:info@biotechfluidics.com)

[www.biotechfluidics.com](http://www.biotechfluidics.com)

**Manufacturer**

**ChromaNik Technologies Inc.**

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E-mail: [info@chromanik.co.jp](mailto:info@chromanik.co.jp)

URL: <http://chromanik.co.jp>



**ChromaNik Technologies Inc.**