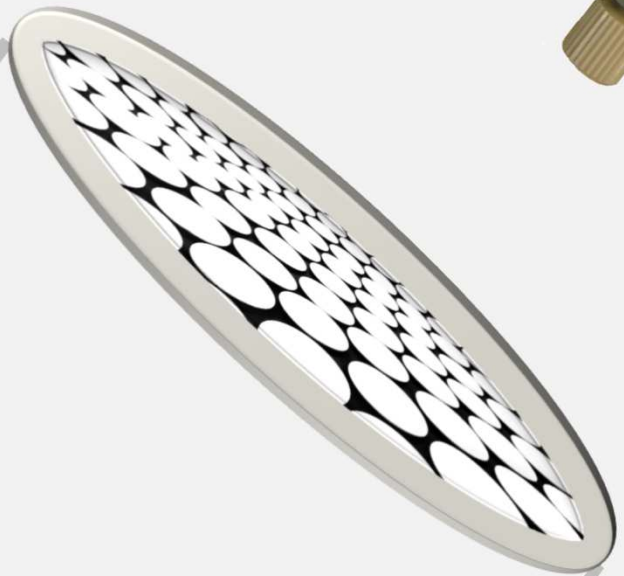
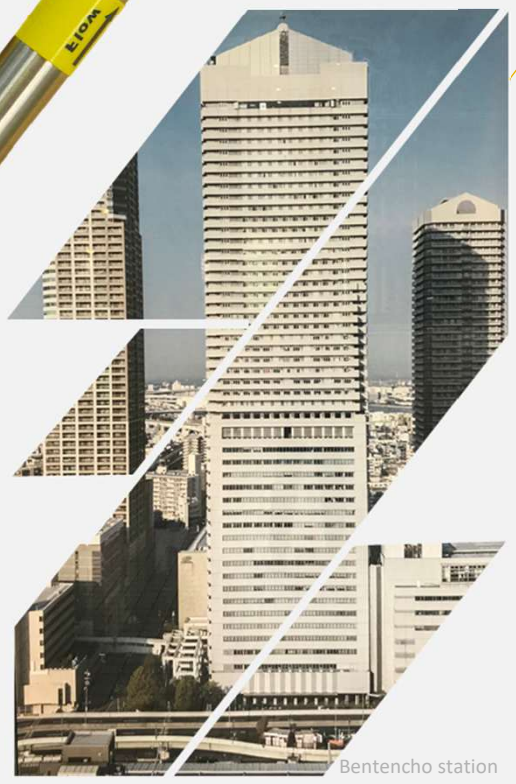
SunShell Core shell particle

Sunniest Fully porous particle

Biphenyl

UHPLC & HPLC column



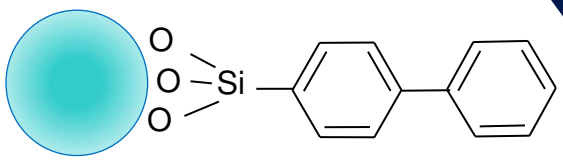


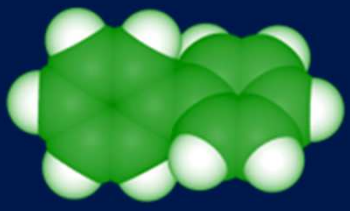
Bentencho station

ChromaNik Technologies Inc.

Biphenyl

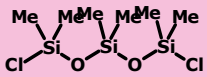
Trifuctional silyl-reagent used





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

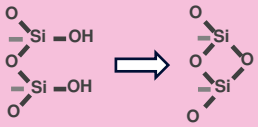
Double end-capping



Hexamethyldichlorotrisiloxane

Trimethylchrolosilane

High temperature end-capping



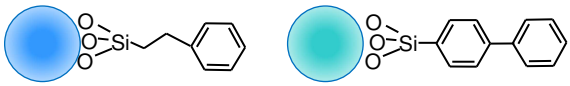
Conversion from silanol groups to siloxane bond

- ➢ Low bleeding
- ➢ High stability

Feature of Biphenyl

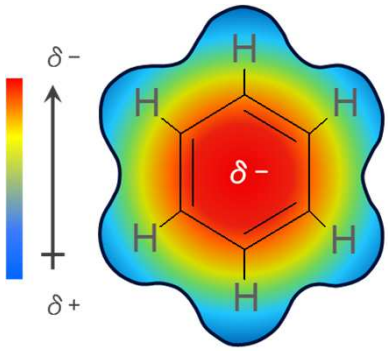
- ✓ The two benzene rings of the biphenyl group are twisted about 45 degree, and the biphenyl group shows different separation from the phenyl group alone.
- ✓ Even when an aqueous mobile phase containing no organic solvent is used, there is no decrease in retention, and separation with high reproducibility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

Interaction of L11 stationary phase (Phenyl, Biphenyl)



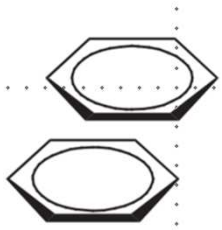
Intermolecular forces

Types of interactions		Origin of force	Feature
Electrostatic force		Charge-Charge, Permanent dipole	Works over long distances
van der Waals force	Orientation force	Permanent dipole-Permanent dipole	Attenuates at -6th power of distance and makes an important contribution only at short distances. Dispersion is dominant in non-polar molecules.
	Induced force	Permanent dipole-Induced dipole	
	London dispersion force	Induced dipole-induced dipole	
Charge transfer interaction		Electron donor-Electron acceptor	Coloring by charge transfer absorption
Hydrogen bond		Functional groups such as OH and NH	Strong specificity and directivity
Hydrophobic interaction		Non-polar groups in polar solvents	Entropy domination

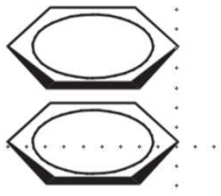


Electrostatic potential map of benzene

★As shown in the figure above, benzene has the highest electron density in the center, and the phenyl and biphenyl groups of the L11 stationary phase have a dipole-dipole interaction, which is the orientation force of the van der Waals force. It then exhibits characteristic selectivity by the π-π and CH-π interactions that are treated as part of them.

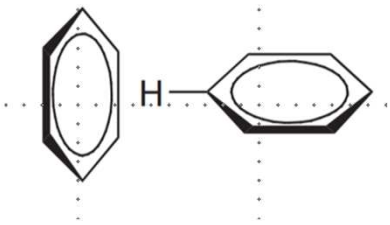


(ring-over-bond)

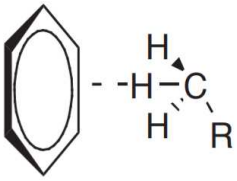


(ring-over-ring)

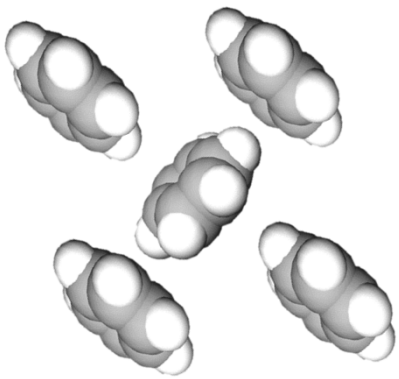
π-π stacking
π-π interaction



T-type stacking



CH-π interaction

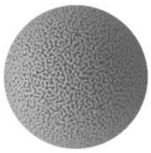


Molecular arrangement by T-type stacking

★π-π stacking and T-type stacking are known as benzene arrangement states as π-π interaction. It is presumed that T-type stacking is close to the CH-π interaction, which is the interaction between the central part of benzene and hydrogen of CH.

Specification of Biphenyl column

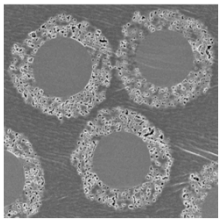
Totally porous silica 5 µm



Specification of Sunniest Biphenyl

	Fully porous silica			Bonded phase				
	Particle size (µm)	Pore size (nm)	Specific surface area (m^2/g)	Carbon loading (%)	Stationary phase	USP L line	End-capping	pH range
Sunniest Biphenyl	5	12	340	11	Biphenyl	L11	Sunniest end-capping	1.5 - 9

Core shell silica 2.6 µm



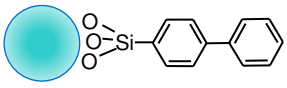
Specification of SunShell Biphenyl

	Core shell silica				Bonded phase					
	Particle size (µm)	Core size (µm)	Pore size (nm)	Specific surface area (m^2/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	Sunniest end-capping	60 MPa	1.5 - 9

Specification of other Biphenyl (cited from a brochure)

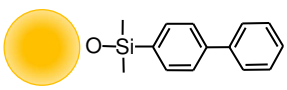
Company A Biphenyl	2.6	1.9	10	100	11	Biphenyl	L11	TMS	60 MPa	1.5 - 8.5
Company B Biphenyl	2.7	1.7	9	135	7	Biphenyldimethylsilane	L11	Yes	60 MPa	1.5 - 8.0
Company C Biphenyl	2.7	1.7	9	130	7	Biphenyldimethylsilane	L11	Yes	60 MPa	1.5 - 8.0

Bonding state using trifunctional reagents



★Sunniest Biphenyl and SunShell Biphenyl use biphenyltrichlorosilane reagents and are in the bonding state as described above. Trifunctional reagents have the characteristic of improving acid stability.

Bonding state using mono-functional reagents

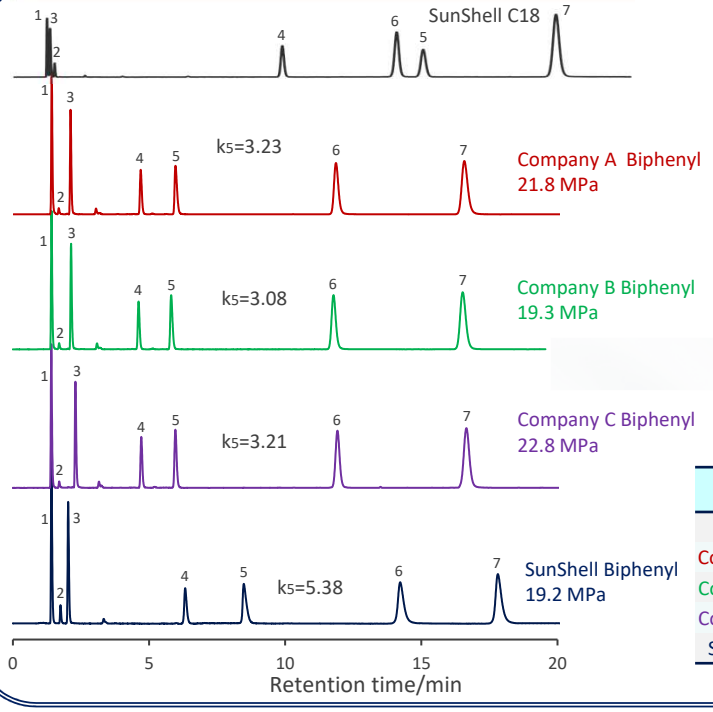


★Other companies' A, B, and C Biphenyl all use biphenyldimethylchlorosilane reagents, and the bonding state is as described above. Regarding acid stability, mono-functional reagents are inferior to trifunctional reagents.

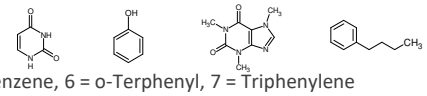
Comparison of core shell Biphenyl columns

Comparison of core shell Biphenyl phases using standard samples

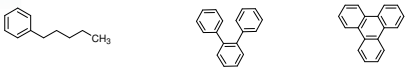
Columns for comparison
1. Kinetex Biphenyl, 2.6 µm
2. Halo Biphenyl, 2.7 µm
3. Raptor Biphenyl, 2.7 µm
4. SunShell Biphenyl, 2.6 µm



Column: Company A Biphenyl, 2.6 µm 150 x 4.6 mm
Company B Biphenyl, 2.7 µm 150 x 4.6 mm
Company C Biphenyl, 2.7 µm 150 x 4.6 mm
SunShell Biphenyl, 2.6 µm 150 x 4.6 mm
Mobile phase: CH_3OH/H_2O=75/25
Flow rate: 1.0 mL/min, Temperature: 40 ºC
Sample: 1 = Uracil, 2 = Phenol, 3 = Caffeine, 4 = Butylbenzene



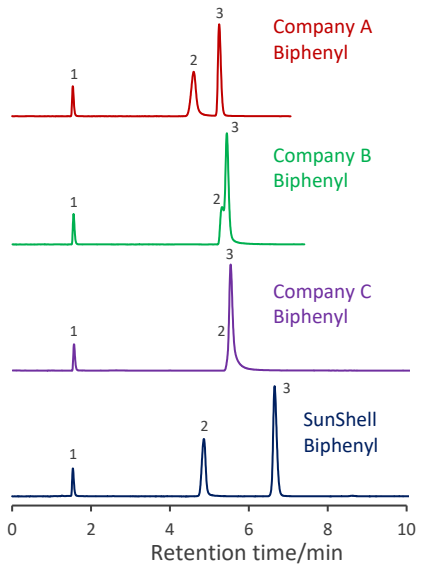
5 = Amylbenzene, 6 = o-Terphenyl, 7 = Triphenylene



	Hydrogen bond α(Caffeine/Phenol)	Hydrophobicity α(Amylbenzene/Butylbenzene)	Steric selectivity α(Triphenylene/o-Terphenyl)
SunShell C18	0.39	1.60	1.46
Company A Biphenyl	2.69	1.39	1.45
Company B Biphenyl	2.52	1.37	1.46
Company C Biphenyl	3.07	1.38	1.45
SunShell Biphenyl	1.82	1.44	1.28

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.

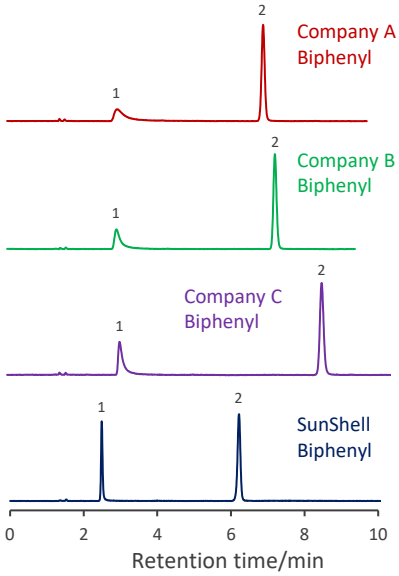
Comparison using pyridine



Column dimension: 150 x 4.6 mm
Mobile phase: CH_3OH/H_2O=30/70
Flow rate: 1.0 mL/min
Temperature: 40 ºC
Detection: UV@250nm
Sample: 1 = Uracil
2 = Pyridine
3 = Phenol

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.

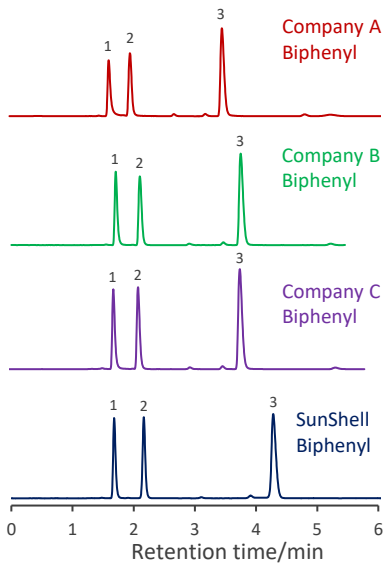
Comparison using oxine



Column dimension: 150 x 4.6 mm
Mobile phase: CH_3CN/20mM H_3PO_4=10/90
Flow rate: 1.0 mL/min
Temperature: 40 ºC
Detection: UV@250nm
Sample: 1 = 8-Quinolinol (Oxine)
2 = Caffeine

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

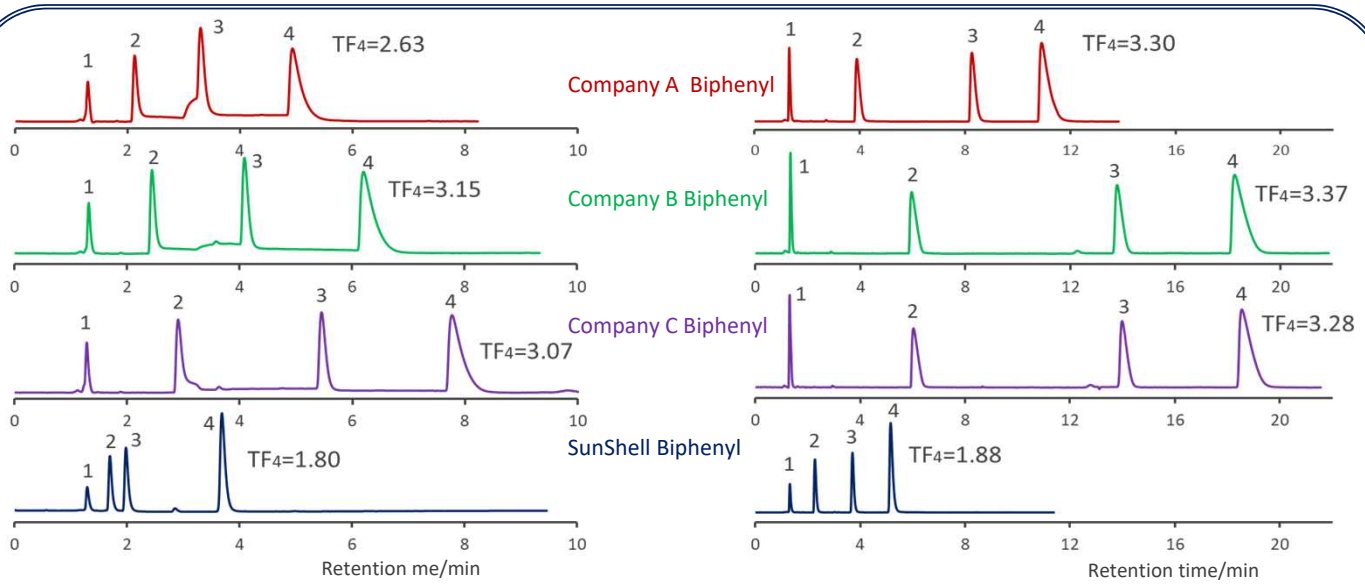
Comparison using formic acid



Column dimension: 150 x 4.6 mm
Mobile phase: CH_3CN/0.1% H_3PO_4=2/98
Flow rate: 1.0 mL/min
Temperature: 40 ºC
Detection: UV@210nm
Sample: 1 = Formic acid
2 = Acetic acid
3 = Propionic Acid

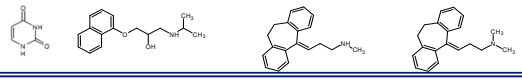
Although most acidic compounds can be eluted without problems, formic acid is a typical acidic compound that easily causes tailing. Formic acid is tailed on Company A Biphenyl.

Comparison of core shell Biphenyl phases using basic compounds



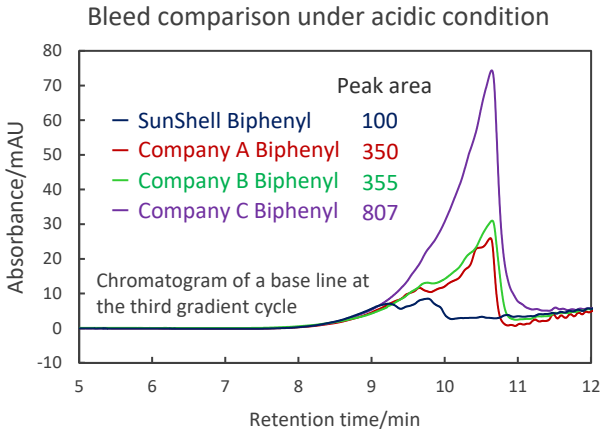
Column dimension: 150 x 4.6 mm
Mobile phase: CH_3CN/20 mM Phosphate buffer pH 7.0=60/40
Flow rate: 1.0 mL/min
Temperature: 40 ºC
Detection: UV@250 nm
Sample: 1 = Uracil, 2 = Propranolol, 3 = Nortriptyline, 4 = Amitriptyline

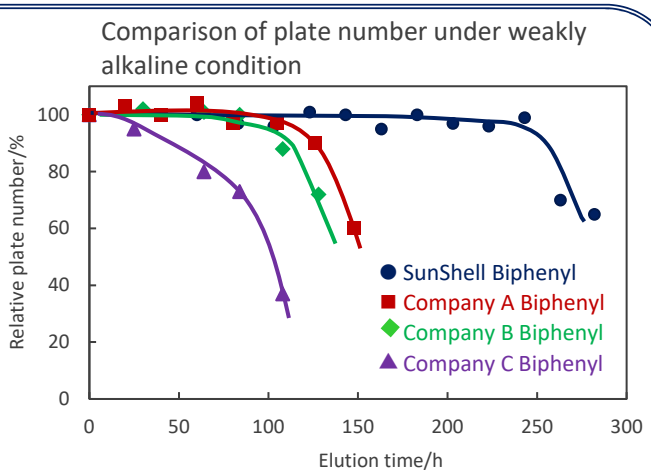
Column dimension: 150 x 4.6 mm
Mobile phase: CH_3CN/10 mM Ammonium acetate pH 6.8=40/60
Flow rate: 1.0 mL/min
Temperature: 40 ºC
Detection: UV@250 nm
Sample: 1 = Uracil, 2 = Propranolol, 3 = Nortriptyline, 4 = Amitriptyline



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 test condition
Column dimension: 50 x 2.1 mm
Mobile phase: A) 1% H_3PO_4 (pH 1.2)
B) Acetonitrile
Gradient program

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

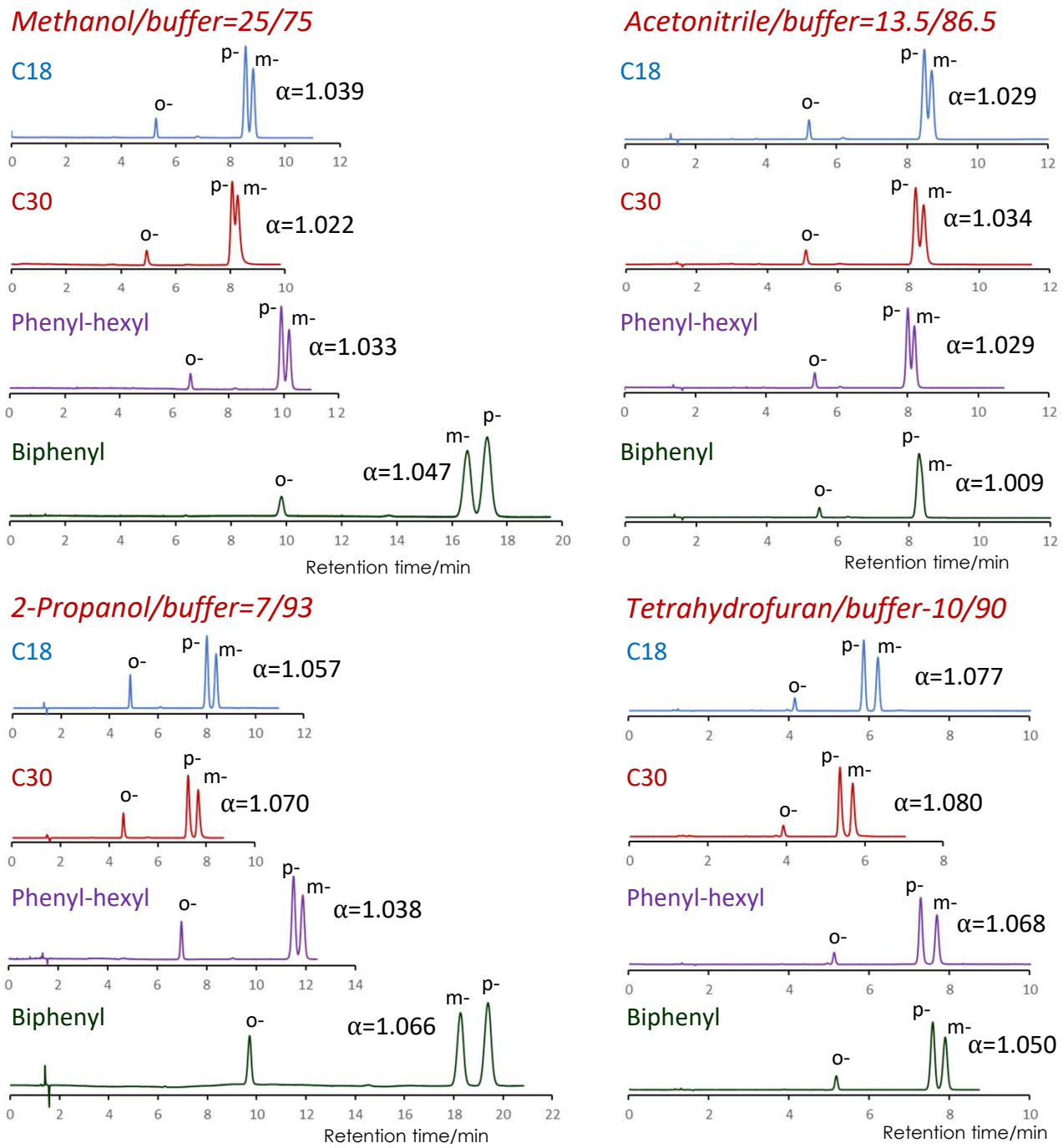
Flow rate: 0.3 mL/min
Temperature: 40 ºC
Detection: UV@250 nm

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 ºC

Measurement condition of plate number
Mobile phase: CH_3CN/H_2O=50/50
Flow rate: 0.2 mL/min
Temperature: 40 ºC
Detection: UV@250 nm
Sample: Butylbenzene

Stability under acidic condition compared baseline changes with gradient elution of 1% aqueous phosphoric acid 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 dissolved 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.

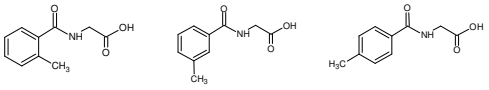
Comparison of stationary phases using isomers of methylhippuric acid



Column: SunShell C18 2.6 µm 150 x 4.6 mm
SunShell C30 2.6 µm 150 x 2.1 mm
SunShell Phenyl 2.6 µm 150 x 4.6 mm
SunShell Biphenyl 2.6 µm 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

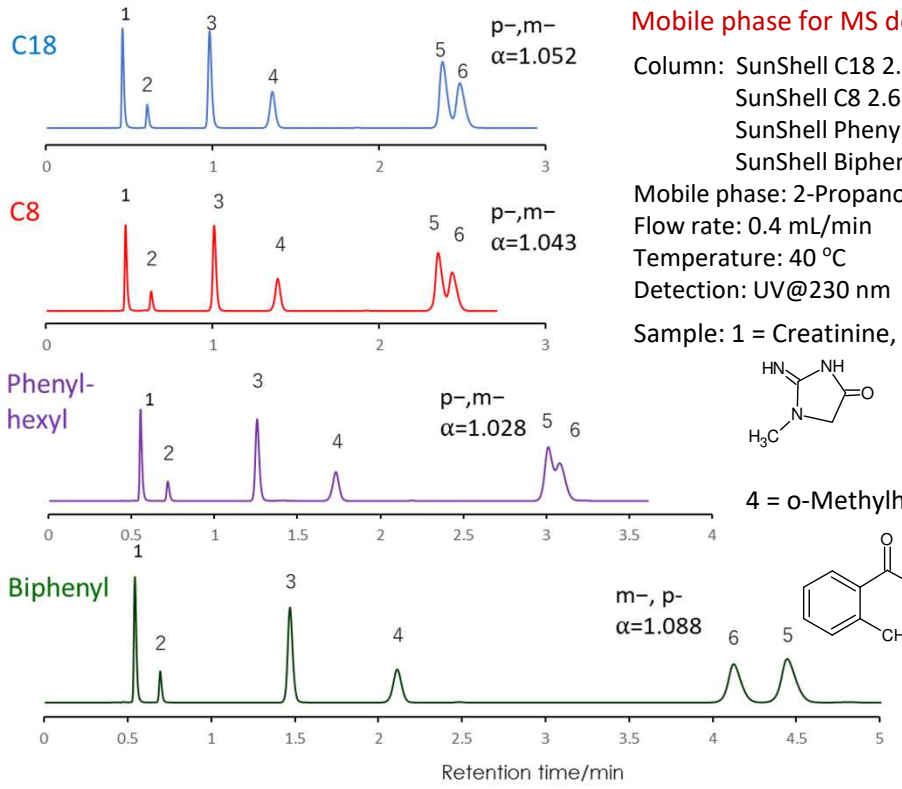
Temperature: 40 °C
Detection: UV@230 nm
Sample: *o*-, *m*-, *p*-Methylhippuric acid

ortho *meta* *para*



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 π-π 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.

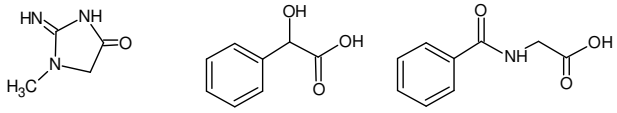
Comparison of stationary phases using creatinine, mandelic acid, hippuric acid and methylhippuric acid



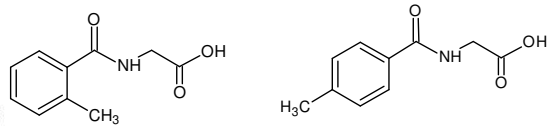
Mobile phase for MS detection

Column: SunShell C18 2.6 µm 100 x 2.1 mm
SunShell C8 2.6 µm 100 x 2.1 mm
SunShell Phenyl 2.6 µm 100 x 2.1 mm
SunShell Biphenyl 2.6 µm 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

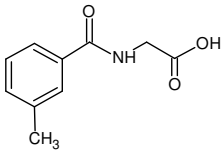
Sample: 1 = Creatinine, 2 = Mandelic aci 3 = Hippuric acid

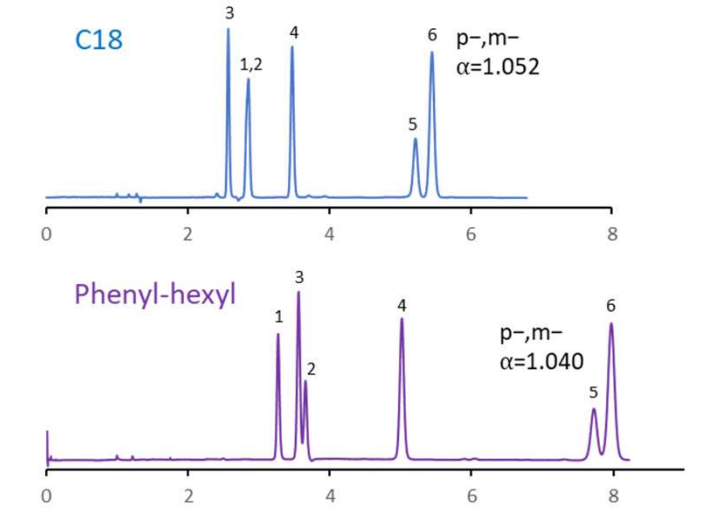


4 = o-Methylhippuric acid, 5 = p-Metylhippuric acid,



6 = m-Methylhippuric acid





Mobile phase adding ion-pair reagent

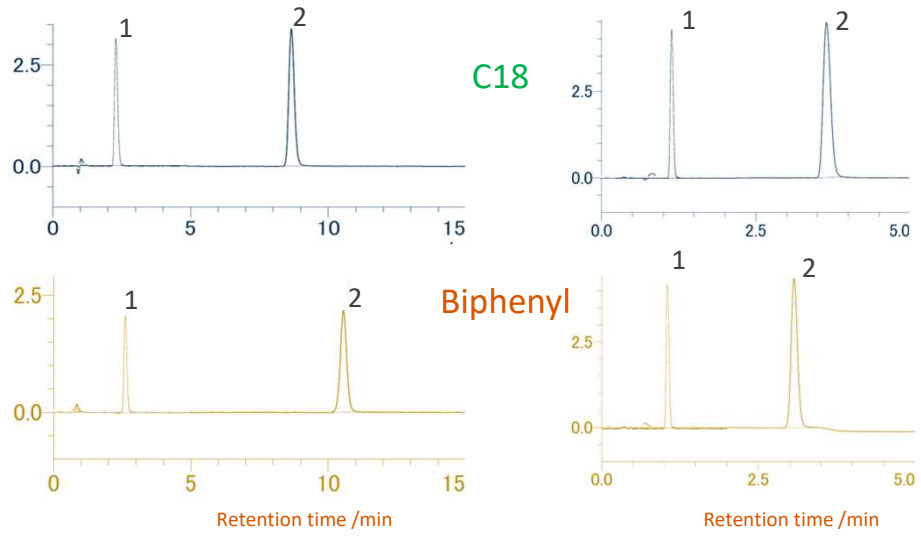
Column: SunShell C18 2.6 µm 150 x 4.6 mm
SunShell Phenyl 2.6 µm 150 x 4.6 mm
SunShell Biphenyl 2.6 µm 150 x 4.6 mm
Mobile phase: 2-Propanol:10 mM KH_2PO_4, 2 mM
Sodium 1-Octanesulfonate (pH 2.5; H_3PO_4)=10:90
Flow rate: 1.0 mL/min
Temperature: 40 °C
Detection: UV@210 nm

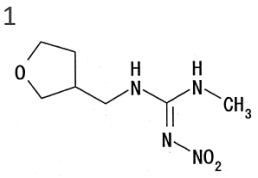
Sample: 1 = Creatinine,
2 = Mandelic aci
3 = Hippuric acid
4 = o-Methylhippuric acid,
5 = p-Metylhippuric acid,
6 = m-Methylhippuric acid

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.

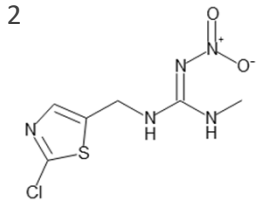
Separation of Dinotefuran and Crossianidin

Mobile phase: Methanol/water=10/90 Acetonitrile/water=10/90





Dinotefuran



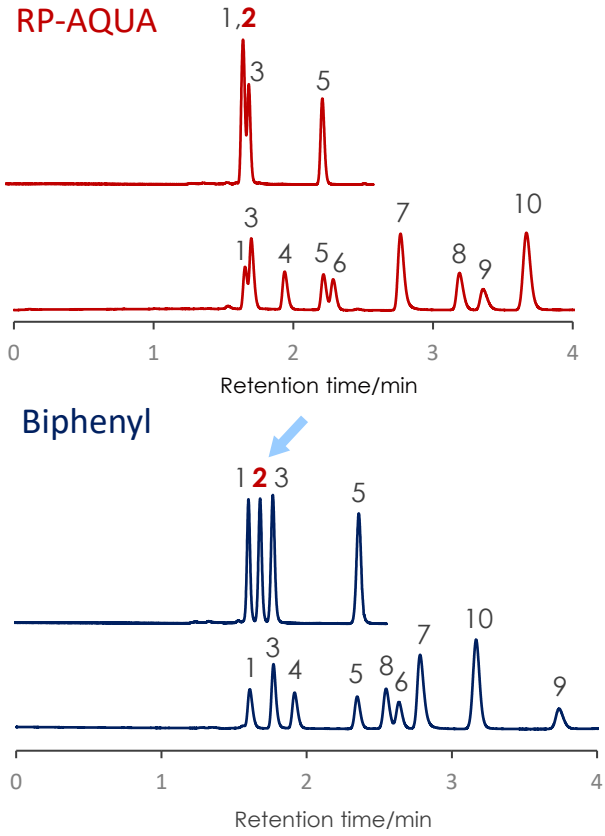
Crossianidin

Column: SunShell C18, Biphenyl 2.6 µm, 100 x 2.1 mm
Mobile phase: Methanol/water=10/90, Acetonitrile/water=10/90
Flow rate: 0.2 mL/min
Temperature: 45 ºC
Detection: UV@270 nm for dinotefuran, UV@265 nm for crossianidin
Injection volume: 5 µL

データ提供：株式会社 エスコ

Biphenyl is less hydrophobic than C18, but due to the π interaction (π-π, CH-π interaction, etc.) of the Biphenyl stationary phase, Biphenyl retains more in the methanol / water mobile phase. In the acetonitrile / water mobile phase, the retention behavior was reversed because the π interaction was weakened by the π electrons of the triple bond of acetonitrile.

Separation of Glycolic acid (organic acids)



Column: SunShell RP-AQUA, Biphenyl 2.6 µm, 150 x 4.6 mm
Mobile phase: 0.1% H_3PO_4
Flow rate: 1.0 mL/min
Column pressure: 18 MPa
Temperature: 40 ºC
Detection: UV@210nm
Injection volume: 1 µL

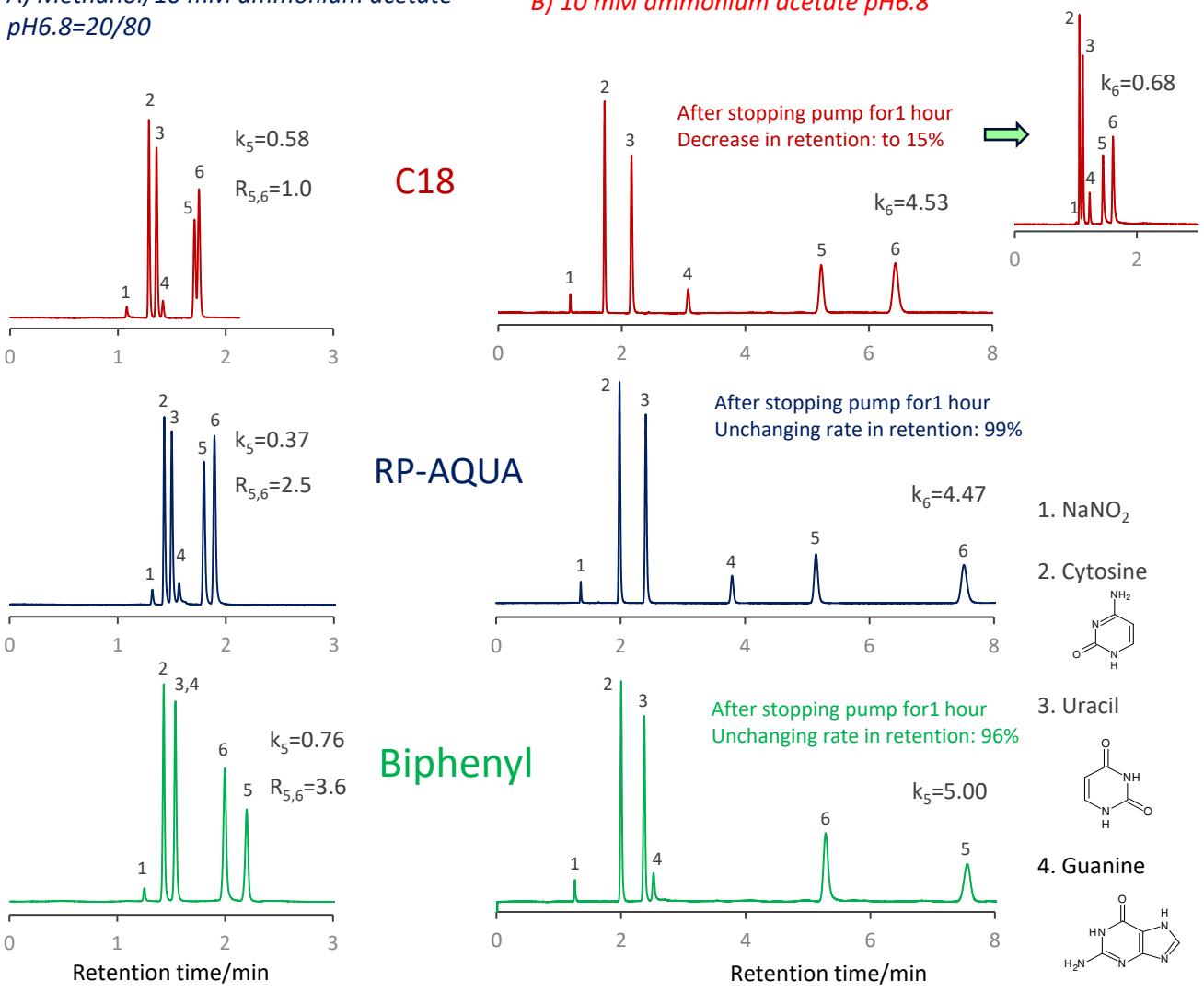
1 Tartaric acid
2 **Glycolic acid**
3 Formic acid
4 Malic acid
5 Lactic acid
6 Acetic acid
7 Maleic acid
8 Citric acid
9 Succinic acid
10 Fumaric acid

Using alkyl-based stationary phases such as C18 and RP-AQUA, the 1st and 2nd tartaric acid and the glycolic acid peak overlap, but using Biphenyl, the 1st, 2nd and 3rd tartaric acid, glycolic acid and formic acid are almost baseline separated. In addition, the order of elution of organic acids was significantly different.

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



1. $NaNO_2$
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

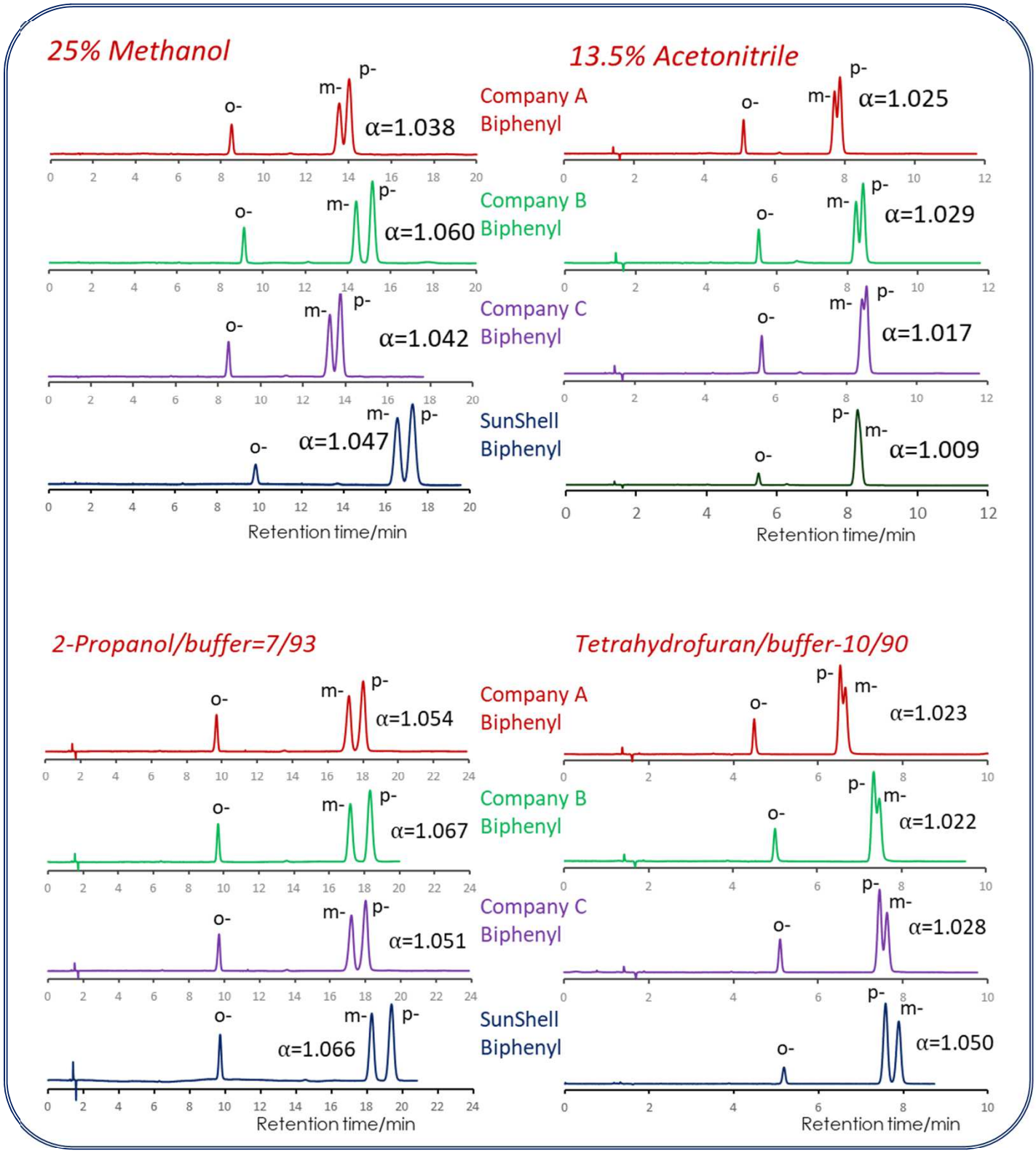
Separation of nucleic acid bases was performed on C18, RP-AQUA and Biphenyl columns. Two mobile phases were used: methanol/10 mM ammonium acetate pH 6.8=20/80 and 10 mM ammonium acetate aqueous solution. Nucleic acid bases can be separated even in the mobile phase containing 20% methanol, but the retention 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 6.



Other companies' Biphenyl and SunShell Biphenyl were compared by isomer separation of methylhippuric acid. When alcohol was used as the organic solvent for the mobile phase, the elution order was the same, and the degree of isomer separation between meta and para was slightly different for each column. The elution order was different for acetonitrile. SunShell Biphenyl was the best separation in tetrahydrofuran. While all other companies' Biphenyls are mono-functional biphenyl, SunShell Biphenyl are trifunctional biphenyl, and it is presumed that this difference was greatly reflected in the separation.

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 µm	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 µm	50	E83241	E83341	E83441	------	------	L11
	100	E83261	E83361	E83461	------	------	
	150	E83271	E83371	E83471	------	------	
	250	E83281	E83381	E83481	E83781	E83881	

Manufacturer
ChromaNik Technologies Inc.
6-3-1 Namiyoke, Minato-ku, Osaka, 552-0001 Japan
TEL: +81-6-6581-0885 FAX: +81-6-6581-0890
E-mail: info@chromanik.co.jp
URL: http://chromanik.co.jp

2112