

Evaluation of Retention Behavior and Stability of Novel Trifunctional Biphenyl Phase



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Norikazu Nagae¹, Tom Toyasusukamoto¹, Ryuji Koyama¹, Scott Silver²

ChromaNik Technologies Inc. Namiyoke, Minato-ku, Osaka Japan²

Pyvot, 1040 1st Avenue, Suite 330 New York, NY

*Corresponding author email: nagae@chromanik.co.jp

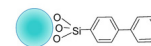
For more information email: scott@pyvot.tech



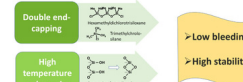
ChromaNik Technologies Inc.
www.chromanik.co.jp

Biphenyl columns are currently available from several HPLC column manufacturers. Their biphenyl phases are only mono-functional. In this study, tri-functional biphenyl stationary phase was modified on a core shell silica and double end-capping was done at high reaction temperature. Tri-functional biphenyl stationary phase was compared with mono-functional biphenyl stationary phase not only for measurement of hydrogen bond capacity, hydrophobicity and steric selectivity but also for a peak shape of a metal chelating compound and a basic compound. Furthermore stability of each biphenyl stationary phase was evaluated under both acidic and basic pH conditions. Although phenyl stationary phase shows higher hydrogen bond capacity than alkyl stationary phases, biphenyl stationary phase showed the highest hydrogen bond capacity. Such a high hydrogen bond capacity led to unique separation selectivity when separating *o*-, *m*-, *p*-methylhippuric acid and vanillin and DNP-aldehydes. Proposed trifunctional biphenyl stationary phase showed the most stable under both acidic and basic pH conditions.

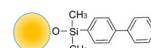
SunShell Biphenyl, Tri-functional Biphenyl



Feature of end-capping of SunShell Biphenyl



Company A, B and C Biphenyl, Monofunctional Biphenyl

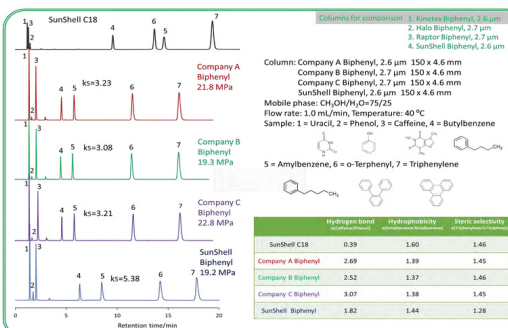


Specification of SunShell

SunShell Biphenyl	Core shell silica				Bonded phase			
	Particle size (μm)	Core size (μm)	Shell size (nm)	Specific surface area (m ² /g)	Carbon loading (%)	Stationary phase	End-capping	Maximum pressure (MPa)
SunShell Biphenyl	2.6	1.6	9	100	5	Biphenyl	Yes	60 MPa

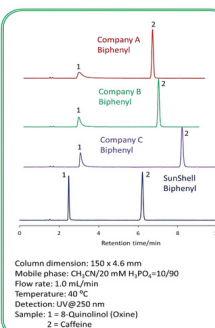
Specification of other Biphenyl (copied from a brochure)								
Company A Biphenyl	2.6	—	10 (effective)	200 (effective)	11	Biphenyl	Yes	60 MPa
Company B Biphenyl	2.7	—	9	100	7	Biphenyl	Yes	60 MPa
Company C Biphenyl	2.7	—	9	100	7	Biphenyl	Yes	60 MPa

Comparison of core shell Biphenyl phases using standard samples



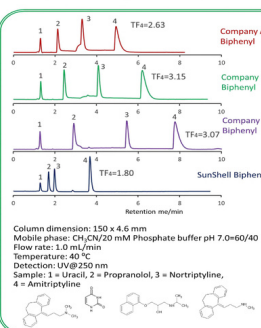
Compared with other company's core shell type Biphenyl, Biphenyls from companies A, B and C showed similar separation patterns. It was confirmed that SunShell Biphenyl has a higher retention of amylbenzene No. 5 and a larger separation factor (hydrophobicity in the table) for the difference of one carbon 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 oxine



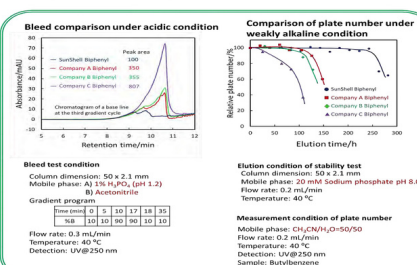
8-Quinololinol (oxine) is a metal chelating compound, and if there are metal impurities in the packing material, its peak becomes a tailing peak.

Comparison using basic compounds



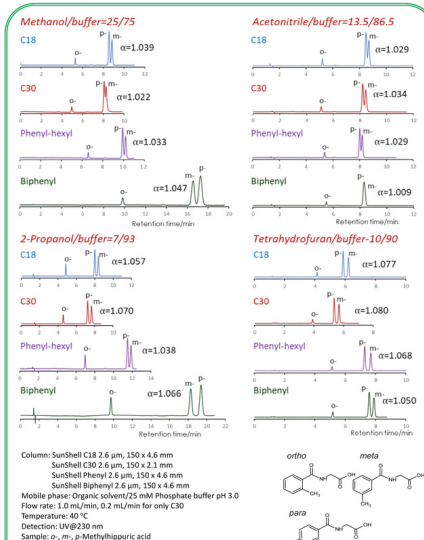
Tailing of basic compounds (amitriptyline) occurs more easily in the acetonitrile/buffer mobile phase than in the methanol/buffer mobile phase. A mixture of acetonitrile and 20 mM phosphate buffer was used as a mobile phase to compare with core-shell Biphenyl. Biphenyl from other companies had a terrible tailing for basic compounds.

Comparison of stability



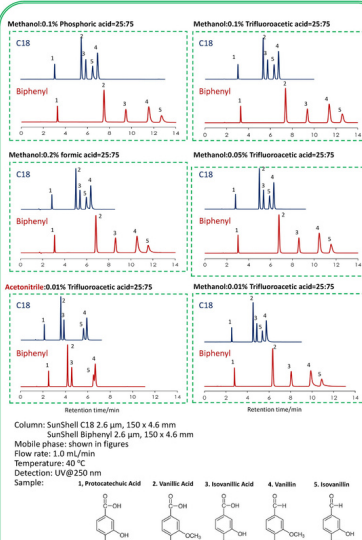
Stability under acidic condition compared baseline changes with gradient elution of 1% aqueous phosphoric acid solution and acetonitrile. Biphenyl groups are cut off from the silica surface under acidic condition and elute out of the column as the amount of organic solvent increases. Baseline variability 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 detected, 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



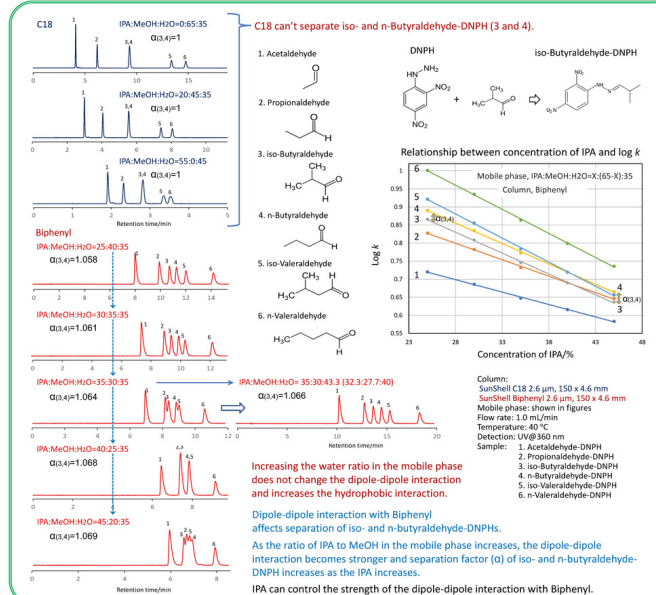
When an alcohol was used as an organic solvent in the mobile phase, Biphenyl showed the longest retention time of all stationary phases and also reversed the elution order of *m*- and *p*-methylhippuric acid.

Separation of vanillin and isovanillin using C18 and Biphenyl column



When an alcohol was used as an organic solvent in the mobile phase, Biphenyl showed long retention time and reversed an elution order of vanillin and isovanillin to compare with C18.

Separation of 6 kinds of DNP-aldehyde using C18 and Biphenyl column (2,4-Dinitrophenylhydrazine = DNPH)



Increasing the water ratio in the mobile phase does not change the dipole-dipole interaction and increases the hydrophobic interaction. Dipole-dipole interaction with Biphenyl affects separation of iso- and n-butylaldehyde-DNPHs. As the ratio of IPA to MeOH in the mobile phase increases, the dipole-dipole interaction becomes stronger and separation factor (α) of iso- and n-butylaldehyde-DNPH increases as the IPA increases. IPA can control the strength of the dipole-dipole interaction with Biphenyl.