Using Aromatic Selectivity with Gemini C6-Phenyl for Difficult Separations

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Introduction

Typical alkyl-bonded reversed phase columns (C18 and C8) do not always offer the necessary selectivity needed to separate complex mixtures. In many cases challenging method development procedures such as gradients, high pH mobile phases and ion-pairing reagents may be required to obtain critical separations. The unique selectivity offered by phenyl phases can provide alternative retention characteristics, resulting in separations not achievable on typical alkyl-bonded phases. This selectivity is due in part to interactions between the π electrons of the phenyl ring of the bonded phases and the π electrons of the sample analyte, typically from the presence of aromatic groups. The π–π interaction is a type of electron donor – electron acceptor interaction that in a chromatographic system can occur between the phenyl stationary phase and the sample analyte. This interaction, which is a bit stronger than van der Waals forces and equally as important as other noncovalent interactions such as hydrogen bonding, and dipole-dipole interactions, can lead to increased retention for polar aromatic compounds versus what is typically observed for alkyl-bonded phases. The net result of such compound specific retention is a change in selectivity between different compounds, which can be utilized to perform a difficult separation where the peaks co-elute on a C18 phase. In this study we compare the retention behavior between a C18 and phenyl phase using a mixture of polar and non-polar probes with varying degrees of aromaticity in methanol and acetonitrile mobile phases.

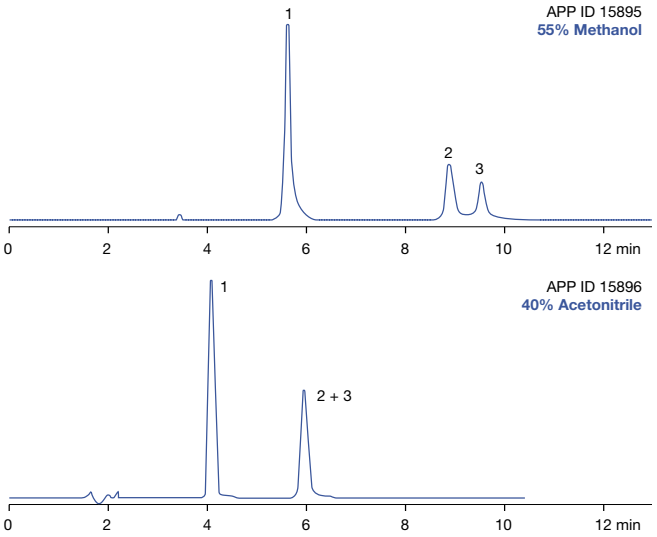
Materials and Methods

Analyses were performed using an HP 1100 LC system (Agilent Technologies, Wilmington, Delaware) equipped with a UV detector. The HPLC columns used were Gemini 5µm C18 and Gemini 5µm C6-Phenyl, 150 x 4.6mm. (Phenomenex, Torrance, California). All standards used were purchased from Sigma Chemicals (St. Louis, Missouri). Solvents were purchased from Fisher Scientific (Fairlawn, New Jersey). Isocratic HPLC runs were performed using either HPLC grade water or 20mM potassium phosphate pH 2.5, and methanol or acetonitrile was used as the organic modifier for the mobile phase. Column temperature was maintained at 30˚C and elution of peaks was monitored by UV (wavelength noted in Figures 1 and 2).

Results and Discussion

This work examined selectivity differences between a C18 bonded phase and a phenyl-bonded phase as well as the effect the organic mobile phase has on these selectivity differences. Chromatograms of Gemini 5µm C6-Phenyl comparing methanol and acetonitrile are shown in Figures 1 and 2.

In Figure 1, we compared the retention behavior of a mixture of flavonoids. When using methanol at a concentration of 55%, Kaempferol and Isorhamnetin are easily baseline resolved whereas when 40% acetonitrile is used, this pair of structurally similar compounds co-elute.



Flavonoids

Column:	Gemini 5µm C6-Phenyl
Dimensions:	150 x 4.6mm
Part No.:	00F-4444-E0-TN
Mobile Phase:	0.1% Formic Acid in water; percent organic as noted above
Flow Rate:	1.0mL/min
Temperature:	Ambient
Detection:	UV @ 254nm
Sample:	1. Quercetin 2. Kaempferol 3. Isorhamnetin

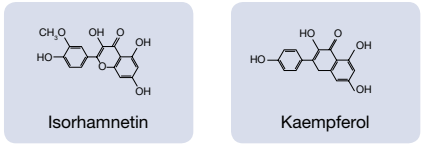


Figure 1: Flavonoids run on a Gemini C6-Phenyl Column. All conditions are the same except for the organic mobile phase used. Note the dramatic increase in retention and selectivity in methanol.

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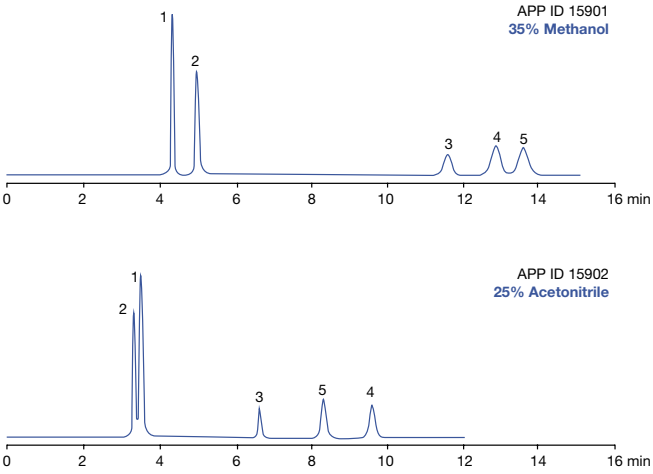
Figure 2 further demonstrates selectivity differences observed on the Gemini C6-Phenyl phase between methanol and acetonitrile mobile phases, and how such differences affect the separation of polar compounds typically found in food additives. Despite the similar elution strength of the two solvents (25% Acetonitrile versus 35% Methanol), all of the compounds elute later when methanol is used, evidence of additional interactions increasing retention. Analytes are affected by these interactions differently as is shown by the elution order change between saccharine and p-hydroxybenzoic acid (peaks 1 and 2), as well as dehydroacetic acid and methylparaben (peaks 4 and 5).



Food Additives

Column:	Gemini 5µm C6-Phenyl
Dimensions:	150 x 4.6mm
Part No.:	00F-4444-E0-TN
Mobile Phase:	20mM Potassium Phosphate pH 2.5, percent organic as noted above
Flow Rate:	1.0mL/min
Temperature:	Ambient
Detection:	UV @ 220nm
Sample:	1. Saccharin 4. Dehydroacetic Acid 2. p-Hydroxybenzoic Acid 5. Methylparaben 3. Sorbic Acid

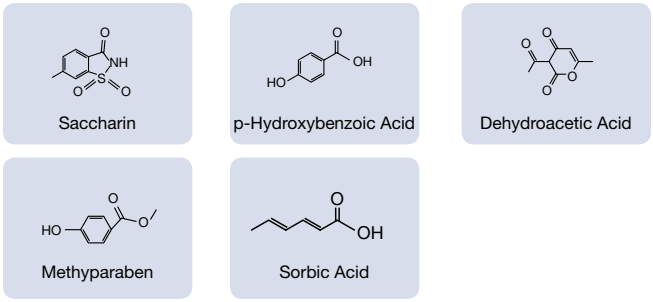


Figure 2: Food additives separation on a Gemini C6-Phenyl column. Analytes were run with the same conditions except the organic mobile phase was switched between equal elution strengths of methanol or acetonitrile. Note the change in selectivity based on the mobile phase used.

Figure 3 shows data generated with a mixture of probes (Table 1) comparing the retention (k) when using methanol verses acetonitrile. In this evaluation, the comparison of organic in the mobile phase suggests that methanol contributes to an increase in the π-π interactions of the phenyl-phase, thus allowing improved selectivity for a diverse mix of analytes. Aromatic analytes such as Sulfamethoxazole and Metoprolol exhibited strong retention differences when methanol was used whereas Nalidixic acid (non-aromatic) showed little or no change. Methanol and acetonitrile containing mobile phase was adjusted for equal eluotropic strength.

Figure 3: Methanol vs. Acetonitrile

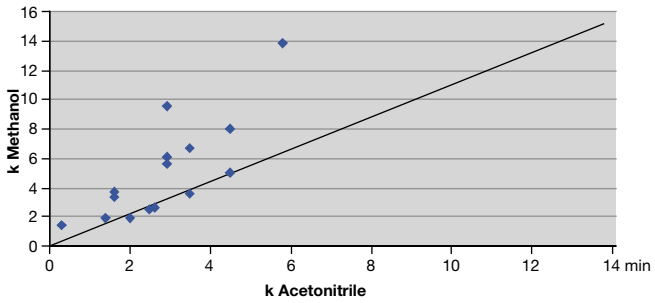


Table 1: Compounds Analyzed

Kaempferol	Sorbic acid
Isorhamnetin	Saccharin
3,4 Dihydroxyphenylacetic acid	Diazepam
Sulfamethoxazole	Ethylbenzene
Nalidixic acid	Propylbenzene
Metoprolol	Butylbenzene
Atenolol	Pentylbenzene
Methylparaben	

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Figure 4 compares the retention behavior of Gemini 5µm C6-Phenyl and Gemini 5µm C18. In this evaluation, methanol and 20mM potassium phosphate was used as mobile phase for comparing k (relative retention) of a mixture of polar and non-polar aromatic probes. The data shows that hydrophobic retention mechanisms are able to retain certain compounds such as Indomethacin, Norpseudoephedrine and Pentylbenzene where as π–π interactions combined with hydrophobic retention offer better selectivity for compounds such as Sulfamethoxazole, Saccharin, and Diphenhydramine.

Figure 4: C18 vs. Phenyl

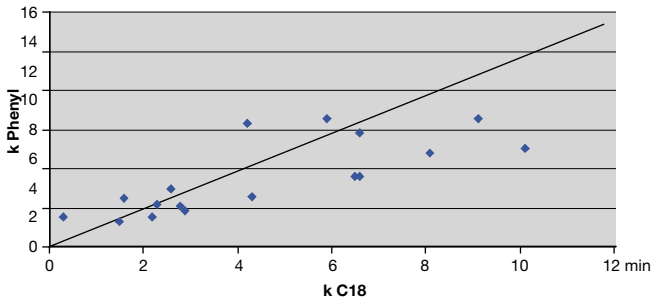

Table 2: Compounds Compared on C18 vs. Phenyl Phase

Compounds	Retained more on -
Sulfamethoxazole	Phenyl
Sulfamerazine	Phenyl
Indomethacin	C18
Diflunsial	C18
Norephedrine	C18
Norpseudoephedrine	C18
Diphenhydramine	Phenyl
Chlorpheniramine	Phenyl
Saccharin	Phenyl
p-Hydroxybenzoic acid	–
Naphthalene	C18
Benzene	–
Toluene	C18
Ethylbenzene	C18
Propylbenzene	C18
Butylbenzene	C18
Pentylbenzene	C18

Conclusions

Phenyl phases like Gemini C6-Phenyl offer differences in selectivity versus C18 columns. Much of this selectivity difference is attributable to aromatic selectivity (π-π orbital interactions between the phase and analyte); which allows one to potentially separate compounds based on differences in the aromatic structure of analytes. Further, the use of different organic mobile phases allows one to activate (with methanol) or suppress (with acetonitrile) such aromatic interactions. Modulating such interactions can selectively change the retention of a specific compound in a mixture, resulting in improved resolution. Such flexibility and utility make phenyl phases like Gemini C6-Phenyl a powerful method development tool for separations where C18 columns fail to provide the desired selectivity.

Ordering Information

Gemini™ 3µm C6-Phenyl

Part No.	Dimensions
00B-4443-B0-TN	50 x 2.0mm
00D-4443-E0-TN	100 x 4.6mm
00F-4443-Y0-TN	150 x 3.0mm
00F-4443-E0-TN	150 x 4.6mm

Gemini™ 5µm C6-Phenyl

Part No.	Dimensions
00B-4444-E0-TN	50 x 4.6mm
00F-4444-E0-TN	150 x 4.6mm
00G-4444-E0-TN	250 x 4.6mm

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