Phun with Phenyl Phases

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Introduction

Phenyl phases can provide separations not achievable on straight-chain alkyl-bonded phases (C18 or C8), nevertheless phenyl phases are often overlooked by many chromatographers. The unique selectivity is due to the interactions between the π electrons of the phenyl ring and π electrons from aromatic groups of the analytes. These interactions can lead to increased retention for polar, aromatic compounds as well as reversals in analyte elution order (compared to the elution order on a C8 or C18 phase). For the analyst who is unable to achieve a desired separation using a C18 or C8 phase, simply switching to a phenyl phase may yield the desired results.

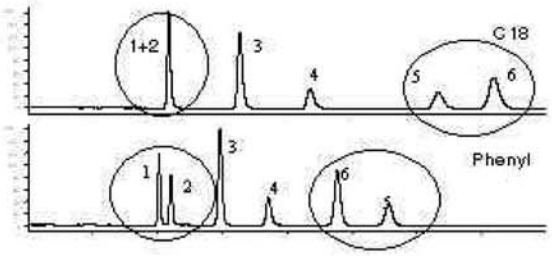
Selectivity of a phenyl phase is governed by both the chemical structure of the compounds being analyzed and the mobile phase used in the chromatographic separation.. This brief guide illustrates how best to utilize a phenyl phase and also gives some predictions regarding selectivity and retentivity of phenyl phase for a given type of analyte and running conditions.

Discussion

Comparing retention behavior of Luna® 5µ C18(2), and Luna 5µ Phenyl-Hexyl (Phenomenex, Torrance, CA) phases using acidic aromatic compounds and an acetonitrile/buffer mobile phase, we see that Luna Phenyl-Hexyl phase is less retentive for these analytes and provides different selectivity for these types of compounds under these conditions (Figure 1).

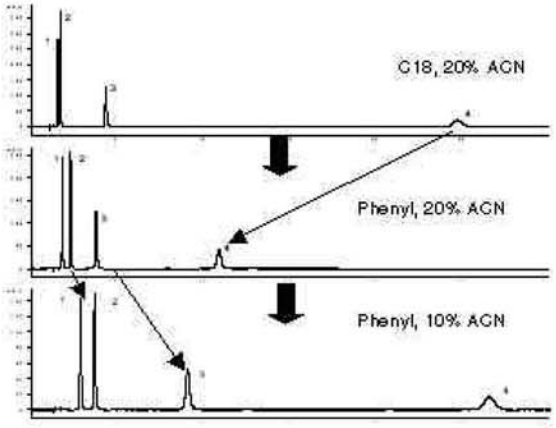
Under isocratic conditions, switching from the non-polar Luna C18(2) to the more polar Luna Phenyl-Hexyl phase reduces the retention time of the non-polar Ibuprofen peak more than the retention time of the more polar, early-eluting peak (Indoprofen). Retention between polar and non-polar peaks decreases for more polar columns. For mixtures of polar and non-polar analytes, a phenyl column will also provide adequate retention of the early-eluting peaks (peaks1 and 2) which may not be resolved on C18 phases while also reducing overall analysis time.

Figure 1. NSAIDs on Luna C18(2) and Luna Phenyl-Hexyl



Columns:	Luna 5µ C18(2) Luna 5µ Phenyl-Hexyl
Dimension:	150x4.6mm
Mobile phase:	20mM Potassium phosphate, pH 2.5 : Acetonitrile (50:50)
Flow rate:	1.5mL/min
Detection:	UV @ 254nm
Sample:	1. Indoprofen, 2. Ethyl paraben 3. Naproxen, 4. Diflunisal 5. Indomethacin, 6. Ibuprofen

Figure 2. Polar and non-polar aromatic compounds (cough and cold formulation) on Luna C18(2) and Prodigy Phenyl-3. Moving to a more polar phase increases retention of the polar peaks while reducing the run time of the late-eluting non-polar peak.

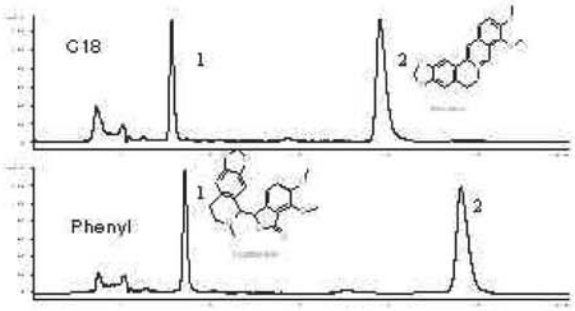


Columns:	Luna 5µ C18(2) 150x4.6mm Prodigy 5µ Phenyl-3
Dimensions:	150x4.6mm
Mobile phase:	20mM Potassium phosphate, pH 2.5 : Acetonitrile (80:20)
Flow rate:	1.5mL/min
Detection:	UV @ 210nm
Sample:	1. Pseudoephedrine, 2. Acetaminophen, 3. Guaifenesin 4. Propyl paraben

Figure 2 further illustrates this point; switching from a Luna C18(2) to a Prodigy™ 5µ Phenyl-3 (Phenomenex, Torrance, CA) the percentage acetonitrile can be reduced, increasing the resolution of the 3 early eluting peaks while still maintaining a reasonable run time. Reducing the acetonitrile to 10% on the Luna C18(2) column, the last peak, propyl paraben, is too strongly retained and thus the run time too lengthy. While these examples show the general behavior of a phenyl phase, to really appreciate phenyl selectivity you have to have the right types of analytes and running conditions. Let's first compare the effects of the use of either acetonitrile or methanol on phenyl selectivity.

In Figure 3 we compare the retention of hydrastatine and berberine, two basic aromatic components extracted from the Goldenseal plant. Using acetonitrile as the organic component, we see Luna Phenyl-Hexyl and Luna C18(2) both give roughly equivalent retention. Switching from a acetonitrile/buffer to a methanol/buffer mobile phase (Figure 4), we see a significant shift in retention behavior for the Phenyl-Hexyl phase. To be able to fully express the unique phenyl selectivity, methanol should be used as the organic component rather than acetonitrile. We can speculate that acetonitrile suppresses π-π interactions because the π electrons of the CN bond in acetonitrile compete for the phenyl "binding sites" on the stationary phase, thus limiting the amount of π-π interaction between the stationary phase and the analyte molecules.

Figure 3. Hydrastatine and berberine from Goldenseal on a Luna C18(2) and Luna Phenyl-Hexyl using acetonitrile and buffer.



Columns:	Luna 5µ C18(2) Luna 5µ Phenyl-Hexyl
Dimensions:	150x4.6mm
Mobile phase:	20mM Potassium phosphate, pH 2.5 : Acetonitrile (73:27)
Flow rate:	1.0mL/min
Detection:	UV @ 254nm
Sample:	1. Hydrastatine, 2. Berberine

Figure 4. Hydrastatine and berberine from Goldenseal on Luna C18(2) and Luna Phenyl-Hexyl phase using methanol and buffer.

Columns:	Luna 5µ C18(2) Luna 5µ Phenyl-Hexyl
Dimensions:	150x4.6mm
Mobile phase:	20mM Potassium phosphate, pH 2.5 : Methanol (50:50)
Flow rate:	1.0mL/min
Detection:	UV @ 254nm
Sample:	1. Hydrastatine, 2. Berberine

Conclusion

If we were to draw some generalities from these examples, it would be that:

1. Phenyl interaction depends on the nature of the analyte:
   - Neutral aromatics show the lowest interaction and will typically be retained less on a phenyl phase versus a C18 phase
   - Acidic aromatics may show selectivity differences, and will typically display low to moderate retention on a phenyl phase compared to a C18 phase
   - Basic aromatics will have the greatest interaction with phenyl phases. These compounds will be strongly retained on a phenyl phase, sometimes yielding greater retention than what is observed on a C18 phase.
2. The extent of the interactions with neutral, acidic, or basic aromatic analytes will be increased through the use of methanol rather than acetonitrile. Basic, aromatic analytes in a methanol mobile phase will show the greatest degree of phenyl interaction and thus will be much more retained versus linear alkyl compounds of similar hydrophobicity.

Under the right running conditions phenyl phases can provide significant selectivity differences when compared with the traditional C18 and C8 phases for the analysis of basic aromatic compounds.

Ordering Information

Order Number	Description
00F-4257-E0	Luna 5µ Phenyl-Hexyl 150 x 4.6mm
00F-4298-E0	Prodigy 5µ Phenyl-3 150 x 4.6mm

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