

Development and Characterization of a New Series of Stationary Phases Exhibiting Unique Selectivity

Frank Dorman, Mike Wittrig, Roy Lautamo, Shawn Reese, Don Rhoads, Randy Romesberg, Rick Lake

Abstract

Columns containing C18 stationary phase functionalities are predominantly used for reversed phase analysis. C18 phases utilize hydrophobic interactions, such as van der Waals forces, for analyte retention. For orthogonal separations, stationary phases containing phenyl ligands are often used. Phenyl phases do not depend solely on hydrophobic interactions, but also employ pi-pi interactions to achieve alternate selectivity. Pi-pi interactions occurring with stationary phases containing phenyl groups have been shown to improve selectivity toward compounds containing aromatic moieties, unsaturation, and electron withdrawing groups. Recently, a unique biphenyl stationary phase has been developed which differs from other phenyl stationary phases. This phase has shown selectivity for sulfur containing compounds which differs significantly from other stationary phase functionalities. Several probes were used to compare the selectivity and retention capacity of phenyl-containing stationary phases. The stationary phases investigated included those which employed experimental ligands containing different numbers and orientations of phenyl groups.

Scope

Although many phenyl stationary phase functionalities exist, it is necessary to choose one type of phenyl phase that optimizes retention and selectivity for a wide range of compounds. The use of an optimized phenyl phase will provide analysts with a powerful tool for method development and orthogonal separations. This poster outlines the evaluation of several experimental phenyl-containing stationary phases for selectivity towards sulfur and electron withdrawing groups, as well as hydrophobic retention.

Experimental Stationary Phases

Phenyl containing stationary phases are now being offered by many vendors, and are often used for orthogonal separations due to their unique selectivity. Most phenyl stationary phase ligands consist of a phenyl group attached to the silica particle with an alkyl linker. While these phases exhibit alternate selectivity, overall retention capacity, especially when dealing with hydrophobic compounds, is reduced when compared to a traditional C18 stationary phase. Recently, alternate phenyl phases have been developed which employ longer alkyl linkers, or the addition of polar functionality to the linker. Another way to improve both selectivity and hydrophobic retention for a phenyl phase is to add a second phenyl group to the ligand, either through linked phenyls, or through the use of fused phenyl rings. The stationary phases evaluated for this poster (Figure 2) include traditional C18 and phenyl phases, as well as ligands employing multiple phenyl groups (biphenyl), fused rings (naphthyl and phenanthrene), the addition of polar functionality between two phenyl functional groups (diphenyl ether), and extended linkers (propyl naphthyl).

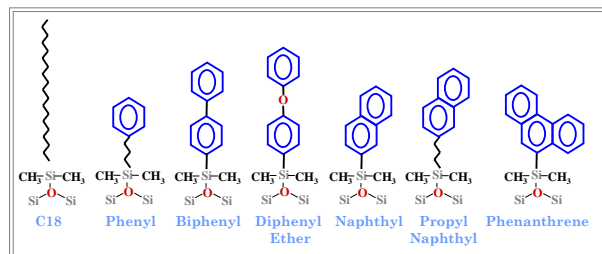


Figure 1. Structures of Stationary Phases Evaluated in this Study

Stationary Phase Evaluation with Simple Probe Compounds

Simple probe compounds were chosen to evaluate the stationary phases listed above for hydrophobic retention and selectivity for compounds containing sulfone and electron withdrawing groups. Since the probe compounds differ from one another in only one functional group, more definitive conclusions can be drawn regarding differences in selectivity and retention capacity between the stationary phases evaluated. The probe compounds were run under identical conditions for all stationary phases evaluated. Methanol was used for the organic portion of the mobile phase, since it allows analysis to more fully take advantage of pi-pi interactions with the column's stationary phase. Unless otherwise noted, analyses were performed on columns containing Pinnacle™ DB silica. The column dimensions for all stationary phases used were 5um, 150mm x 4.6mm, with the exception of the propyl naphthyl column, which had dimensions of 3um, 100mm x 4.6mm. A correction factor was applied to the results obtained from analyses on this column.

Selectivity for Sulfones

In order to evaluate stationary phase selectivity for sulfur-containing compounds, phenyl sulfone was used as a simple probe. Due to the bonding angles of the sulfone group, this group adds a hydrophobic character to compounds of which it is a member. This can make retention of sulfone containing compounds problematic when using traditional alkyl phase chemistries (e.g. C18). The biphenyl stationary phase exhibited superior retention of the phenyl sulfone probe compound. With a k' value of 2.29, the biphenyl stationary phase was the only phase to adequately retain the probe compound under these testing conditions. The diphenyl ether stationary phase retained the probe with a k' value of 1.25, which suggests that the retention mechanism of the sulfone group is based on pi-pi interactions rather than dipole-dipole interactions. As a group, the fused-ring stationary phase ligands ranked second for the retention of phenyl sulfone. Based on the difference in k' values between the naphthyl and propyl naphthyl stationary phase ligands, the addition of a propyl linker improved retention of phenyl sulfone by 34 percent, while the addition of a third fused ring only slightly improved retention of this compound (Figure 2).

Hydrophobic Selectivity

A major drawback in the use of conventional phenyl phases is reduced retention capacity of these phases for hydrophobic compounds. Biphenyl was chosen as the probe compound to evaluate hydrophobic retention. As expected, the C18 stationary phase retained this compound to the largest degree, while the phenyl phase exhibited the least retention. With a k' of 4.83, the biphenyl stationary phase exhibited a retention capacity most similar to the C18 stationary phase, which retained biphenyl with a k' of 6.17. When comparing the naphthyl, propyl naphthyl, and phenanthrene stationary phases, the addition of a propyl linker improved hydrophobic retention by 86 percent. The addition of a third fused ring only slightly improved hydrophobic retention (Figure 2).

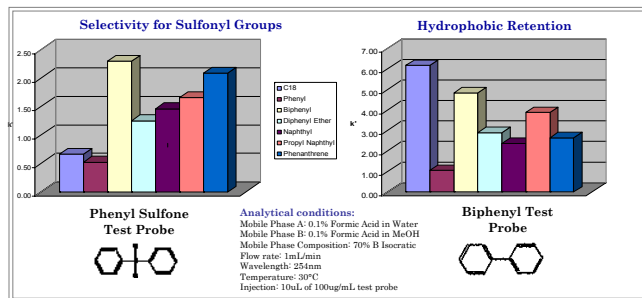


Figure 2. Evaluation of Experimental Stationary Phase Functionalities for Sulfone Selectivity and Hydrophobic Retention

Selectivity for Electron Withdrawing Groups

The experimental stationary phase functionalities in this study were evaluated for selectivity towards electron withdrawing groups, such as nitro groups and halogens. These groups are often found in pharmaceutical compounds. Benzene, nitrobenzene, and chlorobenzene were used as probe compounds for the evaluation of the experimental phenyl-containing stationary phases. The selectivity value (k') for each column for nitrobenzene and chlorobenzene was calculated. Although the C18 column exhibited the highest degree of selectivity towards the more hydrophobic chlorobenzene, the C18 phase did not resolve benzene and nitrobenzene. Among the phenyl containing stationary phases, the biphenyl and propyl naphthyl phases performed similarly. The phenanthrene stationary phase showed the extremely high selectivity toward chlorobenzene. Note that chlorobenzene did not elute from the phenanthrene column under these conditions (Figure 3).

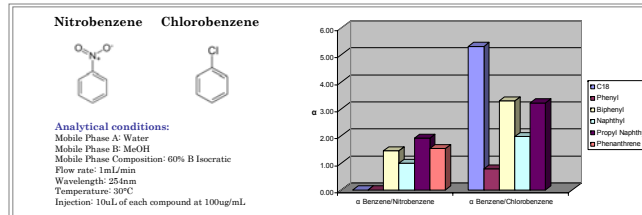


Figure 3. Evaluation of Experimental Stationary Phase Functionalities for Selectivity toward Electron Withdrawing Groups

Analysis of Pharmaceutical Compounds

Sulfur-Containing Compounds

In order to determine whether more complex compounds mirrored the retention and selectivity results obtained from the simple probe compounds, several different pharmaceutical compounds were analyzed on the experimental stationary phases. In order to evaluate selectivity toward sulfur containing compounds, several members of the sulfonamide family of drugs were analyzed on a conventional C18 and a biphenyl column. The biphenyl column was able to resolve sulfadiazine and sulfathiazole, which differ only in the substitution of a single sulfur in the form of a sulfide group (Figure 4). The selectivity of the biphenyl stationary phase towards drug compounds containing sulfur groups is further illustrated by its ability to retain the compound tenoxicam, which contains a sulfone group, and increased retention of sulfapyrazone, which contains a sulfoxide group (Figure 5). Tenoxicam and sulfapyrazone were also analyzed on the experimental phases shown in Figure 1. The biphenyl column exhibited the highest degree of selectivity for sulfapyrazone, while the phenanthrene column showed the highest retention of tenoxicam. The selectivity of the biphenyl and phenanthrene stationary phases towards compounds containing sulfone groups was established through the use of simple probe compounds, and this selectivity is also shown in the analysis of more complex pharmaceutical compounds.

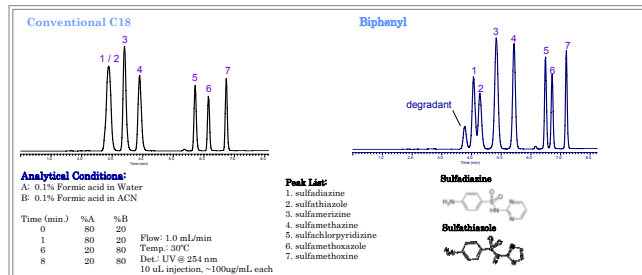


Figure 4. Sulfadiazine and sulfathiazole are resolved on the biphenyl phase, while there is no resolution for these compounds on the traditional C18 phase

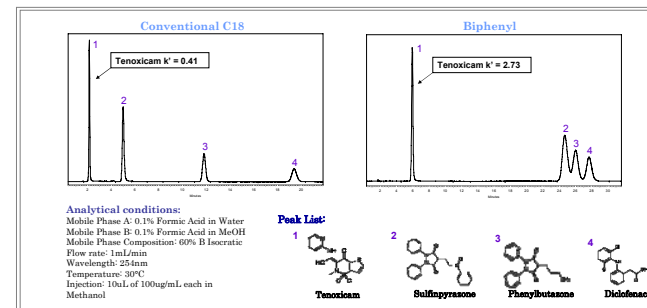


Figure 5. NSAIDs on Pinnacle DB C18 and Biphenyl. Note the biphenyl's improved selectivity toward the sulfur-containing compounds, tenoxicam and sulfapyrazone

Electron Withdrawing Groups

In Figure 3, the selectivity of the experimental phenyl-containing stationary phases toward compounds containing electron withdrawing groups was illustrated. The biphenyl stationary phase showed good retention for both the hydrophobic chlorobenzene and the more hydrophilic nitrobenzene probe compounds, while the C18 and phenyl stationary phases showed no selectivity towards nitrobenzene. Figure 6 illustrates this point in the analysis of several NSAIDs containing halogen constituents. While the C18 and biphenyl show similar selectivity toward diflunisal and flurbiprofen – both of which contain fluorine – the two stationary phases show a dramatic difference in selectivity towards the chlorinated compound, meclonamic acid. Meclonamic acid is very similar in structure to mefenamic acid, and both compounds are well-resolved on both the C18 and biphenyl stationary phases. However, the biphenyl phase retains meclonamic acid to a much higher degree, to the point that the elution order of the two compounds changes.

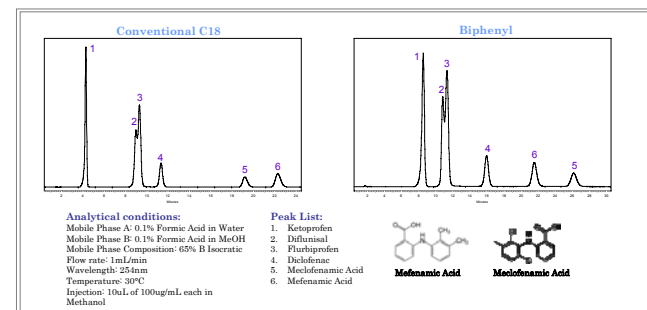


Figure 6. Halogenated NSAIDs on Pinnacle DB C18 and Biphenyl. Note the biphenyl's improved selectivity toward meclonamic acid. The elution order of mefenamic acid and meclonamic acid changes between the biphenyl and C18 stationary phase under identical conditions

Conclusion

In this study, several experimental phenyl-containing stationary phase ligands were evaluated for hydrophobic retention and selectivity toward compounds containing sulfur groups and electron withdrawing groups. The preliminary evaluation was conducted using simple probe compounds, and the findings from the preliminary evaluation were tested with more complex pharmaceutical compounds. The results from the analysis of the pharmaceutical compounds show that for the most part, the more complex compounds followed the same pattern of selectivity as the simple probe compounds. From the results of the analysis of both the simple probes and the pharmaceutical compounds, stationary phases containing multiple ring functionalities outperform traditional phenyl phase ligands which include only one phenyl ring. Additionally, the results show that the biphenyl stationary phase successfully balances retention of hydrophobic compounds with alternate selectivity toward compounds containing sulfur groups and electron withdrawing groups. This balanced retention and selectivity makes the biphenyl stationary phase valuable for method development and for use in orthogonal separations.

References

Ebbing and Gammon. General Chemistry, 7th Edition. 2002.
McMurry. Organic Chemistry, 6th Edition. 2004.