

Novel Phases for HPLC Separations



The author describes several commercially available novel phases for high performance liquid chromatography separations. Novel phases can provide an alternative and complementary separation for many analyses performed on C8 or C18 columns. Many of the separations discussed in the article could not be accomplished on C8, C18, or even phenyl phases.

Reversed-phase high performance liquid chromatography (HPLC) is one of the most utilized forms of chromatography (1). However, analysts occasionally encounter difficult separations for which selectivity, ruggedness, or reproducibility are not easily obtained using traditional C8 and C18 phases. These separations might require the use of selective or novel stationary phases, such as pentafluorophenyl bonded to silica for the analysis of paclitaxel and related taxanes in the bulk drug (2) and the separation of geometrical isomers of carotene (3). These types of stationary phases separate compounds based upon selective stationary phase interactions such as steric recognition charge transfer or π - π interactions (4–6).

Novel phases also can provide an alternative and complementary separation for many analyses performed on C8 or C18 columns. In many instances, the elution order of solutes differs on novel phases, thus, providing enhanced selectivity for difficult-to-separate compounds. This complementary approach can aid in identification, proof of purity, and quantitation. The change of elution order noted on any of the novel phases also can be useful in preparative HPLC. Using a novel selective phase, such as pentafluorophenyl, it might be possible to reverse an elution order and enable the elution of minor component in front of a major component, thereby making collection and quantitation considerably easier. Using this approach, improvement in purity, isolation time, and solvent consumption can be realized for the isolation of minor components via preparative HPLC. Novel phases also offer chromatographers the flexibility to use simpler mobile phases, thereby avoiding ion-pair reagents, exotic buffer systems, extreme pH conditions, and

complex mobile preparations. In this article, I will focus on the more popular novel chemistries — polar embedded alkyl, ion-exchange embedded alkyl, fluorinated, aromatic-based, and alkyl C30 phases.

Embedded Polar and Ion-Exchange Alkyl Phases

In recent years, a number of stationary phases have been introduced commercially that use polar-embedded groups (7). These polar groups are generally incorporated in the alkyl ligand close to the surface silica. A variety of polar functional groups including amide, carbamate, urea, and ether have been “embedded.” The polar-embedded groups, in particular the amide, were originally introduced commercially for their ability to deactivate silanol interactions with basic analytes (8). Subsequently, polar-embedded phases have found application as stable phases for high aqueous mobile phases (9) and have exhibited novel properties for polar analytes (10).

Many of the amide and carbamate phases exhibit lower hydrophobicity (11) and methylene selectivity (11,12) when compared with conventionally bonded C8 and C18 stationary phases. In addition, both the amide and carbamate phases have shown enhanced selectivity toward low molecular weight acids (11). Some of this retention behavior has been attributed to the process used to make many amide phases. Some amide phases are produced via a two-step process in which an aminopropyl bonded phase is acylated to form an amide linkage (8). In this process, it is believed that not all aminopropyl groups are acylated and that unbonded amino groups can lead to mix modes of separation (12). However, the carbamate phases have been developed with one-step synthesis (13), thus, eliminating

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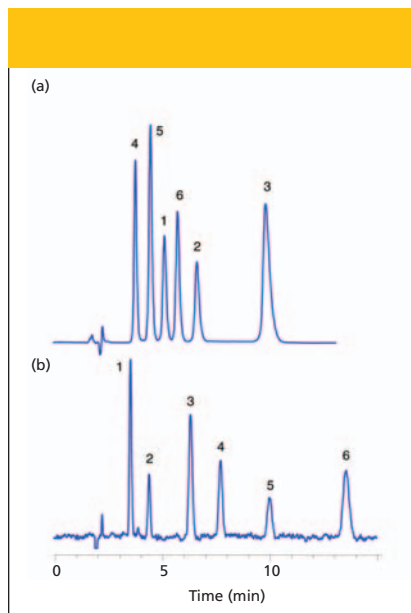


Figure 1: Catecholamine pathway on alkyl chain acid ion-exchanger phase. Mobile phase: (a) 90:10:0.1 (v/v/v) water–acetonitrile–trifluoroacetic acid; (b) 90:10 (v/v) acetonitrile–10 mM ammonium formate (pH 2.9). Column: 150 mm \times 4.6 mm, 5- μ m d_p PrimeSep 200; flow rate: 1 mL/min; detection: UV absorbance at 210 nm. Peaks: 1 = DOPA, 2 = tyrosine, 3 = phenylalanine, 4 = norepinephrine, 5 = epinephrine, 6 = dopamine. (Courtesy of SIELC Technologies.)

unbonded amino groups. In addition, hydrolysis of carbamate would result in free carboxyl groups and not free amino groups. These free carboxyl groups are unlikely to directly interact with low molecular weight acids but could pose a problem with basic analytes or matrix components. The enhanced selectivity of acidic analytes is most likely the result of the positive character of the carbamate group (11). A carbamate C8 stationary phase commercially available from Waters (Milford, Massachusetts) showed enhanced selectivity for the separation of catechin standards for the analysis of commercial preparations of *Camelia sinensis* extracts (12). Catechins are functionalized with phenol groups and phenols have been shown to have longer retention on carbamate packings (12). Another example of a different selectivity is the separations of triazine pesticides on a commercially available C14 amide stationary phase (7). The retention and separation of these triazines is substantially increased using the C14 amide when compared with a conventionally bonded C8 phase.

Recently, embedded ion-exchange alkyl phases have been introduced commercially by SIELC Technologies (Prospect Heights, Illinois) (14). These mixed-mode phases

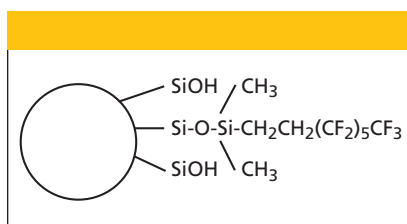


Figure 2: Typical structure of an alkyl fluorinated stationary phase based upon perfluorooctyl-1H,1H,2H,2H-dimethylchlorosilane bonding reagent.

are composed of a hydrophobic alkyl chain and either a hydrophilic acid group or a protonated base group. Both the alkyl chain and the ion-exchange functionality are contained in a single bonded-phase ligand. The ion-exchange group can be activated or deactivated depending upon the pH of the mobile phase, and if the analyte has some polar or ion-exchange character, its interaction with the column can be then pH adjusted. This type of behavior is shown in Figure 1 for catecholamine compounds. In this case, both the elution order and the retention capacity for the catecholamine mixture is altered by changing the pH and the acid modifier. The bonded phase used for the catecholamine separation is PrimeSep 200, a phase with a bonded alkyl chain containing an acid group. Mixed-mode phases also have been used successfully as a secondary column in two-dimensional (2-D) HPLC (15).

Fluorinated Stationary Phases

Perfluorinated and fluorinated stationary phases have shown novel selectivity for several compounds classes and in many cases they have proven useful as an alternative to traditional C8 and C18 phases (2–3,16). A comprehensive review, recently has been published, on perfluorinated stationary phases for HPLC (17). Table I is a partial list of many perfluorinated and fluorinated stationary phases commercially available with either alkyl chain or phenyl bonded groups. Such phases are available in different alkyl chain lengths as well as straight- and branch-chain configurations. Many of the commercially available alkyl-chain fluoro phases contain both fluorinated and unfluorinated methylene units (Figure 2) (18). These unfluorinated methylene units are generally found at the base of alkyl chain closest to the surface of the silica and can serve to stabilize the bonded alkyl chain (19). Fluorinated alkyl phases have been shown to increase selectivity for the geomet-

rical isomers of substituted phenols (18).

Yamamoto and Rokushika (20) have studied fluorinated alkyl phases using computer simulation of the molecular interaction energy based on a test set of solute molecules and various mobile-phase compositions. They prepared a C8 alkyl and two fluorinated phases bonded to the same base silica, thus, allowing for the direct comparisons between unfluorinated and fluorinated alkyl phases. Additionally, their solute test set was comprised of neutral molecules, thereby minimizing the influence of silanol group interactions. Silanol group interactions can dominate the reaction mechanism of many solutes, in particular basic compounds, such as nortriptyline. The use of neutral molecules can provide for an unconfounded explanation for the retention mechanism and allow for a clearer understanding of the role of the bonded stationary phase (6). The computer simulation provided some insight to the retention mechanism of branched and linear fluorinated alkyl stationary phases. The authors observed that the fluorinated phases used in the study exhibited lower retention for both neutral n-alkanes and polyaromatics when compared with the C8. In addition, at higher levels of methanol (90:10 [v/v] methanol–water solution) they observed that anthracene is eluted before benzene on the branched fluorinated phase. Based upon this chromatogram and the aromatic test probes, they concluded that the interaction of planar and rigid aromatic solutes differed from nonplanar aromatic solutes and the alkyl fluorinated phase displayed some shape and size selectivity. Furthermore, they concluded that the retention of the aromatic test probes at higher methanol concentrations was obstructed by the methanol molecules solvating the solute, reducing the retention of larger aromatics. The retention characteristics of alkyl fluorinated phase are complex and its eventual elucidation will require future investigations.

Perfluorinated and fluorinated phenyl stationary phases are generally contain a pentafluorophenyl group and unfluorinated alkyl chain spacer. Typically, the pentafluorophenyl group is bound to the silica surface via this alkyl spacer chain, and in many cases the unfluorinated alkyl chain is a propyl group. As with fluorinated alkyl phases the unfluorinated alkyl space groups might serve to stabilize the bonded group (19). In addi-

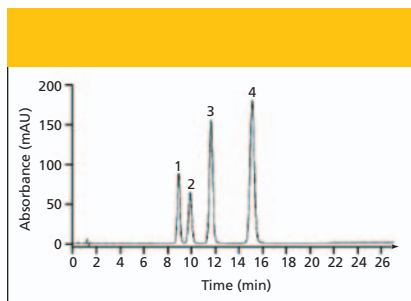


Figure 3: A separation of a mixture of nitronaphthalenes on a pentafluorophenyl bonded phase. Column: 150 mm \times 4.6 mm, 5- μ m d_p FluoroSep-RP Phenyl; mobile phase: 55:45 (v/v) methanol–water; flow rate: 1.0 mL/min; temperature: 30 $^{\circ}$ C; detection: UV absorbance at 254 nm; sample volume: 5 μ L. Peaks: 1 = 1,5-dinitronaphthalene, 2 = 1,8-dinitronaphthalene, 3 = 1-nitronaphthalene, 4 = 1,3-dinitronaphthalene. (Courtesy of ES Industries.)

tion, other modifications to the bonded phase have been made, recently, to a further pH-stabilized pentafluorophenyl phase (17). Pentafluorophenyl columns have been used to separate many complex mixtures including tocopherols (15), alkyl-substituted aromatics (21), and taxanes (2).

Przybyciel and Santangelo (22) have studied the retention behavior of mono-substituted and disubstituted nitronaphthalenes on the pentafluorophenyl phase. As part of that study, they prepared C18 and phenyl phases using the same base silica as their commercially available pentafluorophenyl. Only the pentafluorophenyl column was able to completely resolve the nitronaphthalene mixture as shown in Figure 3. Additionally, the pentafluorophenyl

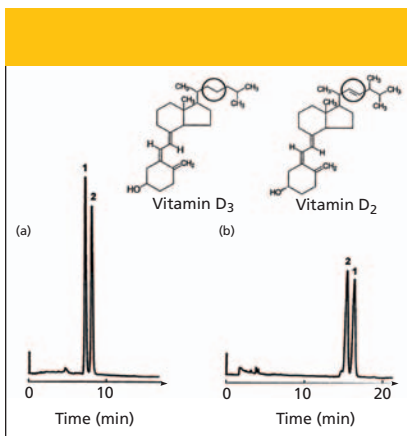


Figure 4: Separation of vitamins D₂ and D₃. Columns: (a) Cosmosil 5PYE and (b) Cosmosil 5C18-MS-II, 150 mm \times 4.6 mm, 5 μ m; mobile phase: 5:95 (v/v) water–methanol; flow rate: 1.0 mL/min; column temperature: 30 $^{\circ}$ C; detection: UV absorbance at 254 nm. Peaks: 1 = vitamin D₃, 2 = vitamin D₂. (Courtesy of Phenomenex.)

column retained every component of the mixture longer than either the C18 or phenyl column. The retention behavior of the pentafluorophenyl phase for separation of the nitronaphthalenes can suggest some π – π interactions as well as other interactions such as possible charge-transfer or electrostatic modes. The elucidation for retention mechanism for pentafluorophenyl remains largely unexplained and will require future investigation.

Aromatic-Based Stationary Phases

Phenyl bonded phases have been utilized in reversed-phase HPLC for many years (23).

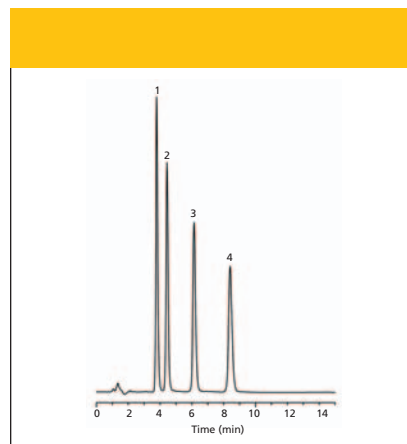


Figure 5: Contraceptive hormones on a biphenyl phase. Column: 150 mm \times 4.6 mm, 5- μ m d_p Allure Biphenyl; mobile phase: 40:60 (v/v) water–acetonitrile; flow rate: 1 mL/min; temperature: ambient; detection: UV absorbance at 220 nm; injection volume: 5 μ L; sample concentration: 100 μ g/mL each component in methanol. Peaks: 1 = b-estradiol, 2 = ethynyl estradiol, 3 = norethindrone, 4 = norgestrel. (Courtesy of Restek Corporation.)

These phenyl phases have been reported to exhibit some π – π interactions as well as steric recognition interactions (6). Bonded phenyl phases traditionally have contained either ethyl or propyl spacer chains between the silica surface and the phenyl group. These bonded ethyl-propyl phenyl phases display some π – π interactions. However, for many separations, these phases behave similarly to alkyl C8 phases. To gain unique selectivity for a phenyl phase, a new version has been developed that utilizes a hexyl spacer chain. The

Table I: Some Commercially Available Perfluorated Columns

Product Name	Supplier	Surface Area (m ² /g)	Pore Size (Å)	Column Chemistry
Chromegabond PFP/T	ES Industries	350	60	Pentafluorophenyl propyl
Epic FDD	ES Industries	230	120	Perfluorododecyl
Epic FO-LB	ES Industries	230	120	Perfluorooctyl
Epic PFP LB	ES Industries	230	120	Pentafluorophenyl
FluoroSep-RP Phenyl	ES Industries	350 and 450	60	Pentafluorophenyl alkyl chain
FluoroSep-RP Propyl	ES Industries	120	300	Perfluoropropyl
MacroSep FSP	ES Industries	-	300 and 1000	Perluorinated phenyl
MacroSep HPR	ES Industries	-	300	Perfluoroalkyl chain
Curosil PFP	Phenomenex	-	-	Perfluorophenyl
Allure PFP Propyl	Restek	-	60	Pentafluorophenyl propyl
Ultra PFP	Restek	-	100	Perfluorophenyl
Viva PFP	Restek	-	300	Pentafluorophenyl propyl
Discovery F5 HS	Supelco	-	120	Perfluorophenyl
Fluofix 120E	Thermo Electron	-	120	Perfluoroalkyl branched chain
Fluofix 300E	Thermo Electron	-	300	Perfluoroalkyl branched chain
Hypersil GOLD PFP	Thermo Electron	220	175	Pentafluorophenyl
Fluophase RP	Thermo Electron	310	100	Perfluorohexyl
Fluophase WP	Thermo Electron	100	300	Perfluorohexyl straight chain
Fluophase PFP	Thermo Electron	310	100	Perfluorophenyl

hexylphenyl stationary phase was originally developed by Blevins (24) and commercialized by Phenomenex (Torrance, California) (25). This phase is claimed to have the properties of both a phenyl and alkyl phase for many separations.

Other aromatic ligands have been bonded to silica such as 2-(2-naphthyl)ethyldimethylsilyl (NE) (5), 2-(3-pyrenyl)ethyldimethylsilyl (5) and fluorene (26). The PYE bonded phase is produced commercial by Nomura Chemical Co. (Seto, Japan) and marketed by Phenomenex. It has been reported that the 2-(3-pyrenyl)ethyldimethylsilyl phase exhibits properties that are intermediate between graphitized carbon phases and alkyl phases (27). The 2-(3-pyrenyl)ethyldimethylsilyl phase can interact via π - π as well as steric recognition mechanisms; an example of π - π behavior can be seen in the vitamin D₃-vitamin D₂ separation shown in Figure 4. There is reversal in elution order in comparison to the alkyl C18 column, and the double bond contained in vitamin D₂ can interact with the PYE phase via a π - π interaction.

A new biphenyl stationary phase recently has been commercialized by Restek Corporation (Bellefonte, Pennsylvania). This phase has been improved the separation for a number of steroid mixtures. An example of one of these steroid separations is shown in Figure 5.

Alkyl C30 Phases

Alkyl C30 phases are the longest chain of the monomeric reversed-phase HPLC phases currently available. The alkyl C30 long has been used for the unique separation of *cis-trans* carotenoid isomers in reversed-phase HPLC (28). This unique separation behavior has been attributed to the exceptional shape selectivity of the C30 phase. Solid-state nuclear magnetic resonance (NMR) spectroscopy investigations of C30 phases indicate that unique selectivity results from highly ordered alkyl chains enabling molecular shape recognition for carotenoids and tocopherols (29). Majors and Przybyciel (7) have compiled a list of commercially available alkyl C30 phases.

The C30 phase also has been applied to separations using highly aqueous mobile phases (30). These phases have been shown to be resistant to phase collapse in such conditions and the unique behavior of the C30 phase can be attributable to the higher melting point of the alkyl C30 chains.

Conclusion

Several classes of novel stationary phases including polar and ion-exchange embedded, fluorinated alkyl, fluorinated phenyl, phenyl hexyl, 2-(3-pyrenyl)ethyldimethylsilyl, biphenyl and alkyl C30 phases have been reviewed. Although these stationary phases are novel, they are commercially available from several column manufacturers. Each novel phase class has been used for a variety of separations and many of these separations have relied on a unique stationary phase-solute interaction. Many of the separations could not be accomplished on C8, C18, or even phenyl phases. The actual mechanism of interaction for many novel phases have been investigated, but a clear understanding of these interactions is still pending. Improvements, refinements and the introduction of new and better novel phases will be resulted from efforts to further elucidate these retention mechanisms. Additionally, these studies will provide the chromatographer with the knowledge and tools necessary to recognize the potential for separations that go beyond C8 and C18 stationary phases.

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