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Preparation and comparison of a pentafluorophenyl stationary phase for reversed-phase liquid chromatography

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ABSTRACT

A pentafluorophenyl stationary phase was prepared for reversed-phase liquid chromatography. The amount of organic moiety bonded on a silica support was determined from thermogravimetric curves of modified silica gel. The specific surface area of the gel was obtained from nitrogren sorption measurements at 77 K. The retention behaviour of solutes on a column packed with this gel was examined and compared with that on a commercially available phenylmethyl column. The capacity factors of the solutes were normalized to unit surface area of the support (k'/S_{BET}) and compared with the k'/S_{BET} values for 52 different solutes in an isoeluotropic system. It was observed that the pentafluorophenyl stationary phase exhibited specific fluorine–fluorine interactions and showed enhanced retention not only of fluorine-containing compounds but also of some other halogen-containing solutes.

INTRODUCTION

Although reversed-phase high-performance liquid chromatography (RP-HPLC) is more widely used than the normal-phase mode and improvements are still being made, the former has generally been considered to be less selective than the latter with respect to changes in retention due to steric factors associated with the solutes and changes in the type of solvents in the mobile phase. The major factor in retention in RP-HPLC is solvophobic interaction[1], which is less polar and gives lower steric selectivity compared with polar adsorption in normal-phase chromatography.

The present practice in RP-HPLC is that a preliminary attempt at separation on a C_{18} stationary phase with a certain composition of an aqueous mobile phase is followed by a change in the eluent composition in order to optimize the k' values and the separation. The use of tetrahydrofuran and solvents other than methanol or acetonitrile was shown to be useful[2–4]. If the desired separation is not obtained, one can try other commercially available stationary phases such as C_8 or phenyl. Organic functional groups other than octadecylsilyl (ODS) have been bonded to silica. An aryl ether bonded phase was prepared and used for the RP-HPLC of nitroaromatic

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compounds[5]. Amino acids and peptides were separated using a bonded peptide stationary phase[6]. Several other organic bonded phases have been tried in attempts to increase the selectivity of the stationary phase by using very long alkyl[7], organomercury(II) species[8], alkyl, aryl, aralkyl and alicyclic structures[9] and cyclodextrin[10] in the stationary phase.

Recently, highly fluorinated RP-HPLC stationary phases have been introduced[11–16] but few studies have been reported.

Berendsen *et al.*[12] directly compared a heptadecafluorodecyldimethyl (HFD) support with its hydrocarbonaceous analogue, a decyl (C_{10}) bonded phase. Only eleven solutes were used in the study. Billiet *et al.*[11] used a wider variety of the test solutes but compared an HFD column with an octadecyl-bonded silica (ODS) column. The chromatographic difference between decyl- and an octadecyl-bonded phases was concluded to be insignificant. They found the HFP-bonded phase to have an increased selectivity over the octadecyl-bonded phase for esters, ketones and fluoro-substituted solutes. Sadek and Carr[17] extended the number of solutes tested in order to compare directly the chromatographic characteristics of the HFD- and C_{10}-bonded phases. In addition, two other fluorinated phases, heptafluoroisopropoxypropyldimethyl (HFIPD) and pentafluorophenydimethyl (PFP), were evaluated under the same mobile phase conditions as for the HFD and C_{10} phases in an attempt to compare all four phases qualitatively. They found that the selectivity of the solutes is completely different on the hydrocarbon and fluorocarbon decyl columns.

In this work, we prepared a pentafluorophenyl-bonded stationary phase for RP-HPLC. The silica gel was modified with pentafluorophenyldimethylchlorosilane. The purpose was to examine the retention behaviour of the solutes on a column packed with a pentafluorophenyl-modified silica gel stationary phase and to compare it with a commercially available phenylmethyl phase in RP-HPLC. We used a phenyl phase as a reference to eliminate the effects of phenyl groups present in the stationary phase. This paper describes some details of the chromatograhic properties of the PFP phase. Both specific effects and the general separation power were considered.

EXPERIMENTAL

Equipment

Chromatographic separations were carried out with a Varian 8500 solvent delivery system, a Model 7125 sample injector (Rheodyne, Berkely, CA, U.S.A.) and a Varian Model 635 UV–VIS monitor. The gel was packed in a 120 × 4. 0 mm I.D. stainless-steel tube by Bio-Separation Technologies (Budapest, Hungary). The thermal studies were performed with a MOM (Budapest, Hungary) Derivatograph-3427. The BET surface area of silica gels was determined with a sorptometer made in the Department for Physical Chemistry, Technical University of Budapest (Hungary).

Materials

A reference phenylmethyl phase column (5 μm; 120 × 4.0 mm I.D.) was obtained from Bio-Separation Technologies. Separon SGX Si-100 (5 μm) was purchased from Laboratorní Přístoje (Prague, Czechoslovakia). Pentafluorophenyldimethylchlorosilane was obtained from Fluka (Buchs, Switzerland). Methanol was of liquid chromatographic grade (Merck, Darmstadt, F.R.G.). All fluorinated solutes

were kindly provided by Chemical Works of Budapest (Hungary). All other chemicals were obtained from commercial sources and used without purification.

Chromatography

The test solutes were dissolved in methanol at a concentration of 0.1 mg/ml. Typically 10 μl were injected. The flow-rates were usually 1 ml/min. Sample peaks were detected at 254 nm.

Preparation of pentafluorophenyl stationary phase packing

The preparation of the PFP stationary phase packing was based on Unger's procedure[18]. A 10-g amount of dried silica gel was mixed of pentafluorophenyldimethylchlorosilane in anhydrous toluene and refluxed in a sealed flask. The reaction was complete within 8 h. The PFP-modified silica gel was filtered and washed successively with toluene, methanol, water and methanol. The solid phase was kept at 353 K for several hours under vacuum. Residual hydroxy groups were deactivated by treating the solid material with 10% of trimethylchlorosilane in toluene. A 4-g amount of this deactivated PFP-bonded silica gel for packing was suspended and sonicated in 50 ml of isopropyl alcohol. The supernatant was decanted after standing for 20 min. The packing material was again resuspended in 40 ml of isopropyl alcohol and transferred to the packing reservoir. The packing pump used was a Haskel 122. Packing was performed at 47 MPa with methanol as the pressurizing and washing solvent. A 120 × 4.0 mm I.D. stainless steel column was used.

Analysis of prepared PFP- and reference phenylmethyl-modified silica gels

The amount of organic moiety bound on the silica support was determined from the thermogravimetric analysis (TGA) curves of modified silica gels[19]. The specific surface areas of the gels (S_{BET}) before and after grafting were obtained from nitrogen sorption measurements at 77 K.

Preparation of isoeluotropic system

The term isoeluotropic system was introduced by Schoenmakers *et al.*[20] for mobile phases of different composition that yield (on average) equal retention times on a given RP-HPLC stationary phase. The retention of the examined solutes was expressed in terms of their capacity factor (k'). In order to eliminate the influence of the specific surface area of the investigated support, we used k'/S_{BET} instead of k'. The validity of this approach has been demonstrated[21–23]. The eluotropic strength of such a mixture can be expressed as the volume fraction of the corresponding binary mixture

TABLE I

CHARACTERISTICS OF SILICA GELS USED

Functional group bonded	*Particle size (μm)*	*Specific surface area, S_{BET} (m^2/g)*	*Mean pore diameter (nm)*	*Surface concentration μmol/m^2*	*Carbon (%)*
Phenylmethylhydroxysilyl	5	397	10	3.6	13.0
Pentafluorophenyldimethylsilyl	5	389	10	3.5	12.5

TABLE II

SUMMARY OF RETENTION DATA OBTAINED ON THE REFERENCE PHENYLMETHYL AND ON THE PENTAFLUOROPHENYL COLUMNS

The values of ln k'_0, slope, their standard errors (S.E.) and correlation coefficient (r) were obtained using eqn. 1.

Solute	*Phenylmethyl column*					*Pentafluorophenyl column*				
	ln k'_0	*S.E.*	*Slope*	*S.E.*	*r*	*ln* k'_0	*S.E.*	*Slope*	*S.E.*	*r*
Aniline	2.419	0.243	−4.546	0.448	−0.981	1.973	0.362	−4.208	0.668	−0.953
m-Cresol	3.836	0.245	−6.214	0.452	−0.989	3.421	0.287	−5.702	0.530	−0.983
Nitrobenzene	4.546	0.260	−6.271	0.480	−0.988	3.864	0.402	−5.785	0.741	−0.968
Methyl benzoate	6.021	1.065	−8.145	1.969	−0.901	4.957	0.319	−6.978	0.588	−0.986
Bromobenzene	5.975	0.298	−7.783	0.551	−0.99	5.241	0.327	−7.117	0.604	−0.986
p-Xylene	6.493	0.341	−8.265	0.629	−0.988	5.901	1.058	−7.282	1.952	−0.881
4-Chloro-3-nitro-α,α,α-trifluorotoluene	7.071	0.349	−9.228	0.643	−0.99	6.673	0.374	−8.677	0.690	−0.987
2-Chloro-α,α,α-trifluorotoluene	7.593	0.626	−9.696	1.105	−0.981	7.269	2.378	−8.74	4.972	−0.779
n-Propylbenzene	7.683	0.608	−9.441	1.073	−0.981	7.108	0.599	−8.921	1.057	−0.979

of methanol in water (φ_M). When another stationary phase is used the retention will change. However, this effect can be nullified by changing the mobile phase composition.

Let us consider the capacity factor per unit specific surface area of the gel, $k'/S_{BET} \times 10^3 = 12.87$, obtained for bromobenzene in the binary mixture methanol–water (55:45) ($\varphi_M^{Ph} = 0.55$) on a reference phenylmethyl (Ph) column. Using a PFP stationary phase the same k'/S_{BET} value is obtained with the mobile phase methanol–water (49:51) ($\varphi_M^{PFP} = 0.49$). The value of φ_M^{PFP} that corresponds to φ_M^{Ph} will be different for different solutes. The RP-HPLC system phenylmethyl/methanol (φ_M^{Ph})–water and PFP phase/methanol (φ_M^{PFP})–water can be referred to as an isoeluotropic system. An arbitrary solute is than expected to yield similar k'/S_{BET} values in both systems.

RESULTS AND DISCUSSION

Analysis of stationary phase

The amount of organic moiety bound on the silica support was determined by TGA. The maximum surface concentrations of the bonded Si–aryl groups are given in Table I. The specific surface areas of the gels were obtained from nitrogen sorption measurements at 77 K. It was assumed that the starting silica had a specific surface area of 535 m^2/g (Laboratorní Přístoje). These measurements (see Table I) demonstrate a considerable reduction in S_{BET} values on the modified silica supports of *ca.* 25–28% of the starting gel.

Polarity of columns

Retention data of ten solutes were obtained on two columns, one filled with phenylmethyl stationary phase (as reference) and the other with the pentafluorophenyl (PFP) phase. We used mixtures of methanol and water at 5% composition intervals, from 40 to 70% methanol. In Table II. $\ln k'_0$ values and standard errors of the $\ln k'$ data are presented, using the following linear equation to relate $\ln k'$ with the mobile phase composition, φ:

$$\ln k' = \ln k'_0 - p_2\varphi \tag{1}$$

where φ is the volume fraction of organic solvent in the water–organic solvent mixture, k'_0 represents the capacity factor of a solute with pure water as a mobile phase and p_2 is a constant for a given solute–eluent combination. Ln k'_0 and p_2 were obtained from least-squares approximation of the generally convex $\ln k'$ *vs.* φ curve by a straight line over the interval examined. Table II also gives standard errors of $\ln k'$ from the straight line. It can be seen from Table II that the values obtained for $\ln k'_0$ on the PFP column are considerably smaller than those obtained with the phenylmethyl column, wich illustrates that with the same mobile phase composition, the retention will decrease on the PFP phase. In order to elute a solute from the PFP column with the same k' as observed on the phenylmethyl column, a mobile phase must be used that contains less methanol and, hence, more water. Consequently, PFP behaves as a less retentive stationary phase than the phenylmethyl stationary phase. We tried to exclude other factors (silica gel, pore volume, carbon content, type of chlorosilane modifier and column length that could play a dominant role in the separation of solutes by

TABLE III

ANALYSIS OF THE SELECTIVITY OF DIFFERENT PHASES FOR 52 DIFFERENT TYPES OF SOLUTES IN THE ISOELUOTROPIC SYSTEM

Least-squares analysis of the data was used. The values of the intercept (*a*), slope (*b*) and correlation coefficient (*r*) were obtained using eqn. 2.

Solutes	*a*	*b*	*r*
Fluorinated (16 compounds)	−0.92	1.39	0.87
Haogenated (26 compounds)	−1.50	1.30	0.87
Halogenated other than fluorinated (10 compounds)	−0.45	1.15	0.91
Polar (19 compounds)	0.24	0.85	0.92
Apolar (7 compounds)	0.58	1.07	0.85

RP-HPLC. We guessed that this effect might be due to the fluorination because the perfluorocarbon molucules have both a relatively high dipole moment of the C–F groups of 189 D and low polarizability indices compared with those of hydrocarbons.

Specific effect

The retention behaviour of PFP bonded silica gel was examined and compared with commercially available phenylmethyl-bonded silica gel. The capacity factors of the solutes were normalized to unit surface area of the support, k'/S_{BET}, for 52 very different solutes in an isoeluotropic system were calculated from an anisoelutropic system. We compared the k'/S_{BET} value of bromobenzene ($k'/S_{BET} \times 10^3 = 12.87$) in a binary system of methanol ($\varphi_M^{Ph} = 0.55$)–water (55:45) on a reference phenylmethyl phase. Using the PFP phase the same retention values could be achieved with the mobile phase methanol ($\varphi_M^{PFP} = 0.49$)–water (49:51). Systematic increases in selectivity on the PFP column would be manifested by a slope of the plot of k'/S_{BET} of the PFP phase *versus* that of the phenylmethyl phase (reference) of greater than unity. We applied least-squares analysis of the data.

$$k'/S_{BET_{PFP}} = a + bk'/S_{BET_{Ph}} \quad (2)$$

The 52 solutes that were examined were divided into five groups according to their chemical properties. The results are given in Table III. With fluorinated and halogenated compounds the slopes are greater than unity, for the non-apolar group they are about unity, which is evident from the isoeluotropic system, but for the polar groups they are less than unity. The correlation coefficients are only 0.85–0.92 because of scatter of the data points about the line. We conclude that the PFP phase shows an improved selectivity for halogenated and particularly fluorinated compounds.

We defined the specific effect on the PFP phase in the isoeluotropic system as the deviation from the line of

TABLE IV

SPECIFICITY OF THE PENTAFLUOROPHENYL COLUMN FOR FLUORINATED COMPOUNDS

Specificity for solute *i* eluted from the PFP phase relative to the isoeluotropic system with the phenylmethyl (Ph) phase was obtained using eqn. 4.

Solute	$k'/S_{BET_{PFP}} \times 10^3$ *[methanol–water (44:51)]*	$k'/S_{BET_{Ph}} \times 10^3$ *[methanol–water (55:45)]*	S_i
Bromobenzene	12.877	12.877	0.00
3-Amino-α,α,α-trifluorotoluene	7.118	6.221	0.897
2-Hydroxy-α,α,α-trifluorotoluene	7.327	6.221	1.106
3-Amino-α,α,α-trifluorotoluene	8.374	6.549	1.825
4-Hydroxy-α,α,α-trifluorotoluene	9.631	6.549	3.082
2-Chloro-5-amino-α,α,α-trifluorotoluene	12.877	10.258	2.619
4-Chloro-3,5-dinitro-α,α,α-trifluorotoluene	15.075	13.097	1.978
2-Chloro-3,5-dinitro-α,α,α-trifluorotoluene	16.540	14.514	2.026
α,α,α-Trifluorotoluene	20.620	14.079	6.541
2-Chloro-5-nitro-α,α,α-trifluorotoluene	23.030	19.208	3.822
4-Chloro-3-nitro-α,α,α-trifluorotoluene	25.020	17.244	7.776
2,4-Dichloro-5-amino-α,α,α-trifluorotoluene	26.171	20.191	5.980
4-Chloro-α,α,α-trifluorotoluene	27.427	20.628	6.799
2-Chloro-α,α,α-trifluorotoluene	32.452	22.030	10.422
3-Bromo-α,α,α-trifluorotoluene	39.362	25.757	13.605
2,4-Dichloro-α,α,α-trifluorotoluene	48.156	33.833	14.323
5-Bromo-2-chloro-α,α,α-trifluorotoluene	52.553	40.818	11.735

$$k'/S_{BET_{PFP}} = k'/S_{BET_{Ph}} \quad (3)$$

In this case, specificity should be defined for a solute *i* as

$$S_i = k'/S_{BET_{PFP}} - k'/S_{BET_{Ph}} \quad (4)$$

TABLE V

SPECIFICITY OF THE PENTAFLUOROPHENYL COLUMN FOR HALOGENATED COMPOUNDS

Specificity for solute *i* eluted from the PFP phase relative to the isoeluotropic system with the phenylmethyl (Ph) phase was obtained using eqn. 4.

Solute	$k'/S_{BET_{PFP}} \times 10^3$ *[methanol–water (49:51)]*	$k'/S_{BET_{Ph}} \times 10^3$ *[methanol–water (55:45)]*	S_i
Bromobenzene	12.877	12.877	0.00
2-Chloroaniline	4.606	4.584	0.022
2,4-Dinitrochlorobenzene	7.224	9.275	−2.051
Chlorobenzene	11.829	10.913	0.916
1,4-Dichlorobenzene	17.377	17.244	0.133
1,3-Dichlorobenzene	18.216	17.462	0.754
Bromotoluene	19.052	20.736	−1.684
1,2-Dichlorobenzene	20.309	18.334	1.973
1,2,5-Trichlorbenzene	29.095	28.814	0.281
1,2,4-Trichlorobenzene	29.523	28.814	0.709

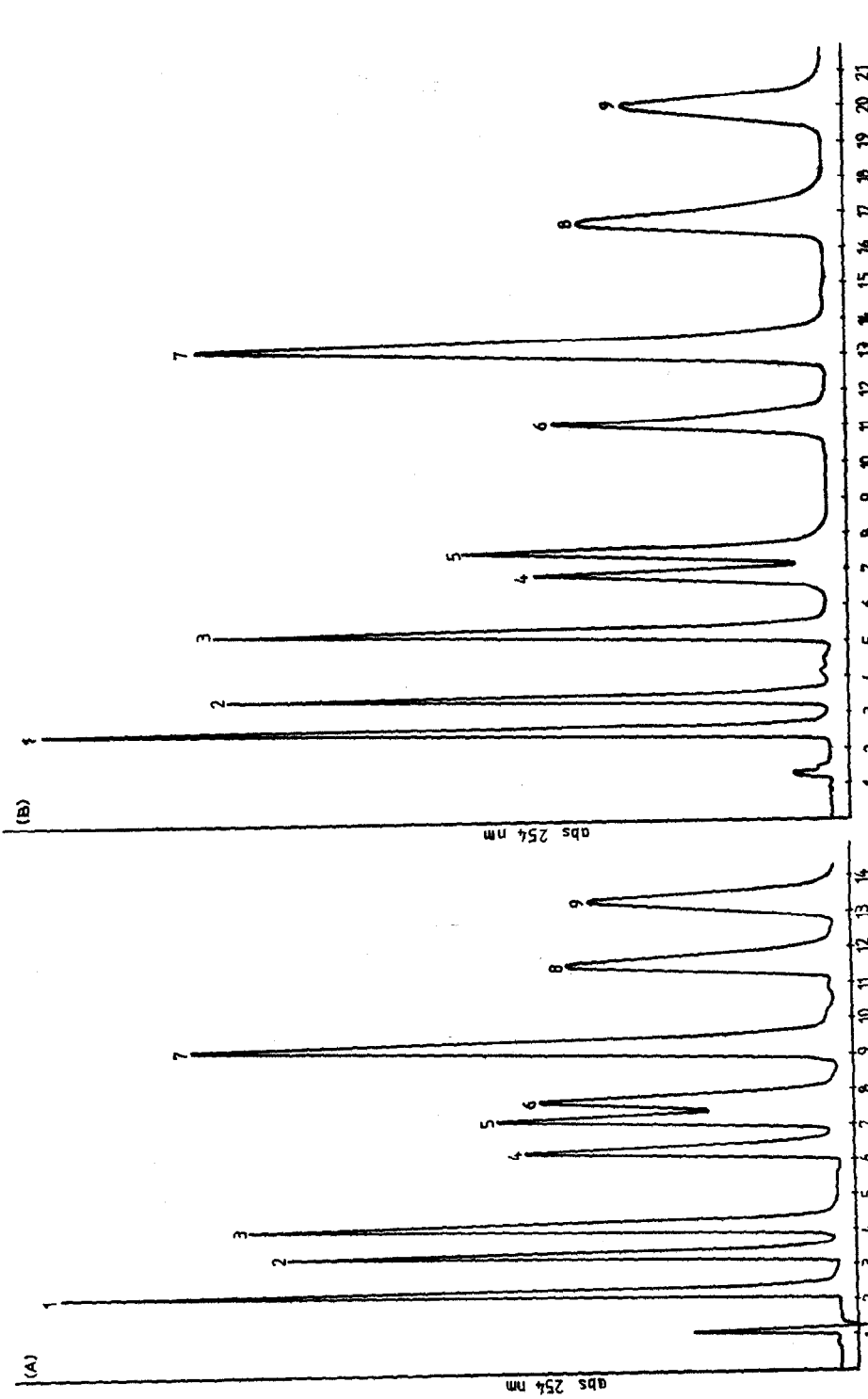

Fig. 1. (A) Separation of test solutes on phenylmethyl phase. Mobile phase, methanol–water (55:45, v/v); flow-rate, 1 ml/min; detection, UV (254 nm); temperature ambient. (B) Separation of test solutes on PFP phase. Conditions as in A, except mobile phase, methanol–water (49:51, v/v). Peaks: 1 = phenol; 2 = 2-chloroaniline; 3 = 2-amino-α,α,α-trifluorotoluene; 4 = chlorobenzene; 5 = bromobenzene; 6 = α,α,α-trifluorotoluene; 7 = 4-chloro-3-nitro-α,α,α-trifluorotoluene; 8 = 2-chloro-α,α,α-trifluorotoluene; 9 = 3-bromo-α,α,α-trifluorotoluene.

TABLE VI

SPECIFICITY OF THE PENTAFLUOROPHENYL COLUMN FOR POLAR COMPOUNDS

Specificity for solute *i* eluted from the PFP phase relative to the isoeluotropic system with the phenylmethyl (Ph) phase was obtained using eqn. 4.

Solute	$k'/S_{BET_{PFP}} \times 10^3$ *[methanol–water (49:51)]*	$k'/S_{BET_{Ph}} \times 10^3$ *[methanol–water (55:45)]*	S_i
Bromobenzene	12.877	12.877	0.000
p-Nitrophenol	1.570	2.182	−0.612
o-Nitrophenol	2.513	4.348	−1.835
o-Nitroamiline	2.827	3.054	−0.227
m-Nitroaniline	3.035	3.492	−0.457
p-Nitroaniline	4.291	5.019	−0.728
1,4-Dinitrobenzene	4.291	5.456	−1.65
1,2-Dinitrobenzene	5.967	8.731	−2.764
Nitrobenzene	6.909	7.203	−0.294
2,4-Dinitrofluorobenzene	7.013	9.603	−2.590
2,4-Dichlorobenzene	7.224	9.275	−2.051
Phenol	2.721	2.399	0.322
m-Cresol	4.397	3.709	0.688
Anisole	6.176	7.311	−1.135
1-Naphthol	7.746	8.293	−0.547
Aniline	2.198	2.182	0.016
2-Chloroaniline	4.606	4.584	0.022
Benzaldehyde	3.977	5.020	−1.043
Acetophenone	6.070	5.894	0.176

because this result is positive when the specific interaction between the solute and the PFP phase causes a specific retardation. The fluorinated solutes show a strong specific effect on the PFP phase (see Table IV), but the specificity is not limited to only these molecules. We observed specific interactions between the chlorinated compounds and the fluorinated phase (see Table V). It can be seen from Table IV that the PFP phase preferentally retains the fluoro-substituted solutes *vs.* the phenylmethyl phase. We guess that this is due to the "like prefers like" effect. Sadek and Carr[17] generally found stronger retardation of more polar, non-ionizable groups (NO_2, OH, etc.) (see Table VI) for nitro-substituted solutes, but for other groups our results are the same. A clear illustration of the specific effect occurring in the PFP system is provided by the chromatograms in Fig. 1.

Two chromatograms were obtained in an isoeluotropic system. The chromatogram in Fig. 1A corresponds to a methanol–water (55:45) mobile phase on the phenylmethyl column and Fig. 1B shows the elution of the same mixture of substances on the isoeluotropic PFP system with methanol–water (49:51) mobile phase. The solutes are numbered in order of appearance. It is evident that the PFP phase (B) is the better stationary phase for fluorinated ad chlorinated substances. The order of appearance of solutes did not change but the retention times in Fig. 1B are is longer than those in Fig. 1A. We observed a strong specific retardation for fluorinated and mild specificity for chlorinated compounds.

Stationary phase with a pentafluorophenyl functional group have been shown to have some merit such as increased retentivity and widely different selectivity for

halogenated substances *vs.* the phenylmethyl phase. It can be a good alternative to a phenyl phase, and the complementary use of such stationary phases with commercially available phases will increase the capability of RP-HPLC.

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