Separation Behavior of Various Organic Compounds on Branched-Polyfluoroalkylsilane Coated SilicaGel Columns

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Abstract

Fluorinated compounds are separated using fluorinated columns. The elution order depends on the number of fluorine atoms in the solutes. The addition of a masking agent of adsorption point to the mobile phase improves the separation of nitrogen-containing compounds, giving rise to symmetric peaks. Better recognition of geometrical isomers and epimers is achieved on fluorinated packings, which is attributed to the less solvophobic interaction than on an octadecyl siloxane column and the rigid molecular structure of the fluorocarbon chain. Using 2,2,2-trifluoroethanol as a mobile phase, cresol isomers are separated on fluorinated columns.

Introduction

Many fluorine-containing materials have been produced as main products or byproducts in the course of fluorine chemistry and industrial production processes. The separation and purification of fluorine-containing compounds are therefore required in many scientific and technological fields. For this purpose, various fluorocarbon-bonded packings have been developed for gas chromatography (GC) and liquid chromatography (LC) (1,2). However, these materials did not exhibit high separation efficiency in high-performance liquid chromatography (HPLC) applications, probably due to metallic impurities in the silicagels and nonoptimum analytical conditions (3,4).

Using high-purity, homogeneous spherical silicagels, the preparation conditions of the branched fluorocarbon-bonded silicagel particles has been established (5). In this paper, the separation conditions of many different types of geometrical isomers are examined using the optimized branched-type fluorocarbon-bonded silicagel packings.

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Experimental

Chemicals

All test samples were reagent grade and used as received. Acetonitrile, methanol, THF, and water were HPLC grade. 2,2,2-Trifluoroethanol (TFEO), purchased from Central Glass Co. Ltd. (Tokyo, Japan), was used as a mobile phase without further purification. Heptafluorobutylamine hydrochloride (HFBA) was prepared by the neutralization of heptafluorobutylamine (PCR, Florida) with hydrochloric acid.

Columns for HPLC

Two commercially available fluorinated silicagel columns and a trial octadecyl siloxane (ODS) column made from the same batch of silicagels were used. The fluorinated columns were Fluofix® 120N and 120E (NEOS, Kobe, Japan), which were synthesized according to a previously reported method using the high-purity silicagels (average diameter, 5 µm; average pore diameter, 12 nm) (5). Fluofix 120E and ODS columns were endcapped with trimethylsilane, whereas Fluofix 120N was not endcapped.

HPLC equipment

The LC system consisted of a Waters (Milford, MA) Module-1 within a high-performance pump, a variable wavelength ultraviolet detector, an autosampler, and a column temperature controller, combined with a Waters Millennium 2010J chromatographic control system.

Table I. Separation of Fluorinated Benzenes*

Sample	*k*	α
Monofluorobenzene	2.0	
		1.15
1,4-Difluorobenzene	2.3	
		1.13
1,2-Difluorobenzene	2.6	
		1.00
1,3-Difluorobenzene	2.6	
		1.23
1,2,4-Trifluorobenzene	3.2	
		1.13
1,3,5-Trifluorobenzene	3.6	
		1.19
1,2,4,5-Tetrafluorobenzene	4.3	
		1.12
1,2,3,4-Tetrafluorobenzene	4.8	
		1.31
Pentafluorobenzene	6.3	
		1.43
Hexafluorobenzene	9.0	

* Conditions: column, Fluofix 120N (150 × 4.6-mm i.d.), mobile phase; MeCN–water (40:60); detection, absorption at 254 nm.
† $\alpha = k_2/k_1$

Table II. Separation of Positional Isomers of Fluorobenzaldehyde*

Sample	Fluofix 120E *k*	Fluofix 120E α	ODS *k*	ODS α
2-Fluorobenzaldehyde	9.2		17.4	
		1.10		1.00
4-Fluorobenzaldehyde	10.1		17.4	
		1.08		1.14
3-Fluorobenzaldehyde	10.9		19.8	

* Conditions: column, Fluofix 120E (150 × 4.6-mm i.d.); mobile phase, MeCN–water (10:90); detector, absorption at 254 nm.

Table III. Separation of Positional Isomers of Fluoroanisole*

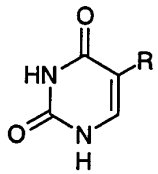
Sample	Fluofix 120E *k*	Fluofix 120E α	ODS *k*	ODS α
2-Fluoroanisole	2.2		5.0	
		1.18		1.34
4-Fluoroanisole	2.6		6.7	
		1.15		1.19
3-Fluoroanisole	3.0		8.0	
		1.10		0.84
2,4-Difluoroanisole	3.3		6.7	

* Conditions: column, Fluofix 120N (250 × 4.6-mm i.d.); mobile phase, MeOH–water (50:50); detection, absorption at 254 nm.

HPLC measurement conditions

The flow rate was fixed at 1 mL/min, and the column temperature was maintained at 40°C. All samples were dissolved in suitable organic solvents at a concentration of approximately 0.1%, and the injection volume was 5 µL. Uracil (Wako Pure Chemical Industries, Osaka, Japan) was used for estimation of the column void volume t_0 (min). Retention factor k was obtained using the equation $(t_r - t_0)/t_0$, where t_r is the retention time (min) of the solute. The separation factor α was obtained by the equation k_2/k_1 where k_i is retention factor of compound i.

Table IV. Separation of Uracil Derivatives*

	Substituent *R*	t_r/min
	NH_2	3.1
	H	3.6
	F	4.6
	CH_3	5.5
	Cl	6.5
	Br	7.2

* Conditions: column, Fluofix 120E (250 × 4.6-mm i.d.); mobile phase, 10mM NaH_2PO_4–1mM tetrabutylammonium hydrosulfate (pH 6.4); detection, absorption at 254 nm.

Table V. Separation of Caffeine and Phenol*

Sample	Fluofix *k*	Fluofix α	Fluofix *As*	ODS *k*	ODS α	ODS *As*
Phenol	2.18		1.38	2.67		1.79
		1.85				0.42
Caffeine	4.04		2.01	1.10		2.39

* Conditions: column, Fluofix 120N (150 × 4.6-mm i.d.); mobile phase, MeOH–water (15:85); detection, absorption at 254 nm.
† *As* is the asymmetry factor at 10% peak height; $As = b/a$, where a is the front peak width and b is the back peak width.

Table VI. Separation Behavior of Chlorophenols and Cresols Isomers*

Sample	*k*	α
Phenol	6.7	
		1.21
4-Chlorophenol	8.1	
		1.06
3-Chlorophenol	8.6	
		1.07
2-Cresol	9.2	
		1.05
2-Chlorophenol	9.7	
		1.04
3-Cresol	10. 1	
		1.06
4-Cresol	10.7	

* Conditions: column, three Fluofix 120N (250 × 4.6-mm i.d.) columns connected in series; mobile phase, TFEO–water (20:80); detection, absorption at 270 nm.

Results and Discussion

Separation of fluorine-containing compounds

Table I shows that 10 fluorine-substituted benzene isomers were eluted in the order of increasing number of fluorine atoms attached to the benzene ring. These positional isomers were separated with a separation factor α of more than 1.1 except for 1,2- and 1,3-difluorobenzenes. Tables II and III show the separation of the fluorinated compounds that the ODS column fails to separate. The elution order of fluoroanisoles on Fluofix is according to the number of fluorine atoms, but on ODS, it is random. This reflects the general principle of "like dissolves like" and suggests the very strong oleophobic properties of the Fluofix stationary phase. Six uracil derivatives are separated by the recognition of substituents as shown in Table IV. They are eluted in the order of the atomic size of halogen atoms. Anti-cancer agents are separated by Fluofix as shown in Figure 1A and 1B. Industrially produced perfluoropentane contains some isomers that can only be separated by GC using a special carbon column. High-purity normal perfluoropentene cannot be obtained by ordinary distillation, but clear separation can be achieved by HPLC using Fluofix as shown in Figure 1C. Fluofix can separate isomers by the recognition of not only fluorine but also bromine, as illustrated in Figure 1D.

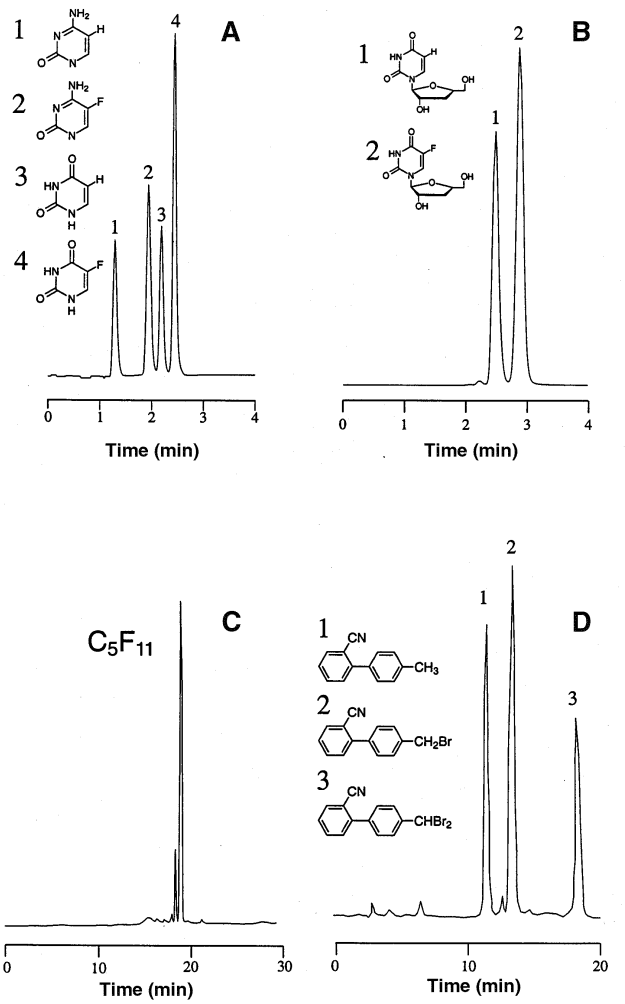


Figure 1. Separation of fluorinated anti-cancer agents (A,B), perfluoropentane isomers (C), and bromine-containing biphenyl derivatives (D). Conditions (A): column, Fluofix 120N (150 × 4.6-mm i.d.); mobile phase, 0.1% tetrabutylammoniumchloride–0.1% acetic acid; detection, absorption at 254 nm. Conditions (B): column, Fluofix 120N (150 × 4.6-mm i.d.); mobile phase, MeOH–water (5:95); detection, absorption at 254 nm. Conditions (C): column, four Fluofix 120N (150 × 4.6-mm i.d.) columns connected in series; mobile phase, THF; detection, refractive index; temperature, 30°C. Conditions (D): column, Fluofix 120N (150 × 4.6-mm i.d.); mobile phase, THF–water (35:65); detection, absorption at 254 nm.

Separation of nitrogen-containing compounds

Caffeine and phenol are used to investigate the property of HPLC packings having hydrogen bondings caused by residual silanols (6). Table V shows the elution behavior of caffeine and phenol on Fluofix and ODS columns. The elution order of these compounds on Fluofix is opposite to that on ODS. Caffeine, which contains several nitrogen atoms, shows a larger asymmetry factor A_s on Fluofix packings than on ODS packings. From ^{29}Si-NMR measurements, it was confumed that the extent of

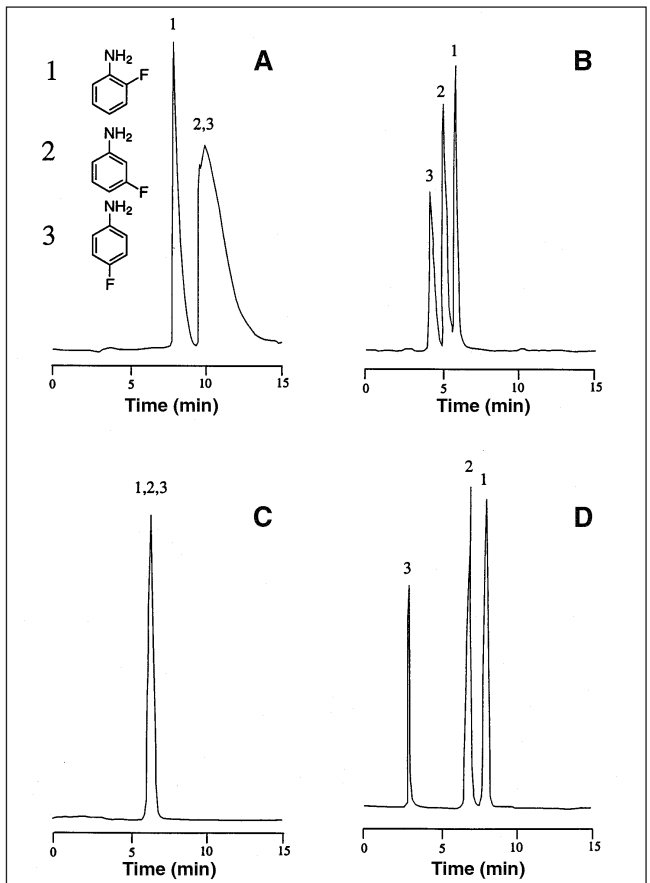


Figure 2. Separation of fluoroanilines. Conditions (A): column, Fluofix 120E (150 × 4.6-mm i.d.); mobile phase, MeOH–0.1% trifluoroacetic acid (10:90); detection, absorption at 254 nm. Conditions (B): column, no end-capping ODS (150 × 4.6-mm i.d.); mobile phase, MeOH–0.1% trifluoroacetic acid (10:90); detection, absorption at 254 nm. Condition (C): column, Fluofix 120N (150 × 4.6-mm i.d.); mobile phase, MeOH–0.1% ammoniumchloride (20:80); detection, absorption at 254 nm. Conditions (D): column, Fluofix 120N (150 × 4.6-mm i.d.); mobile phase, MeOH–0.1% HFBA (10:90); detection, absorption at 254 nm.

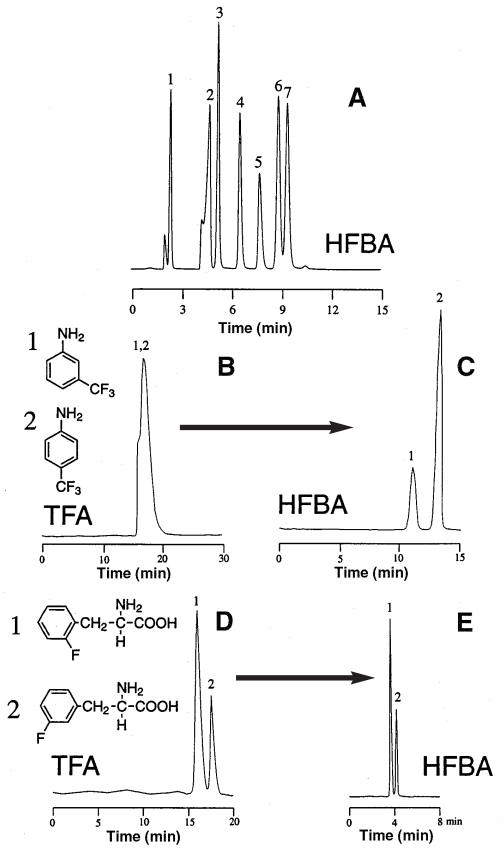


Figure 3. Separation of basic compounds. Peaks: 1, 4-fluoroaniline; 2, 3-fluoroaniline; 3, 2-fluoroaniline; 4, 3,4-difluoroaniline; 5, 2,4-difluoroaniline; 6, 2,6-difluoroaniline; 7, 2,5-difluoroaniline. Conditions: column, Fluofix 120N (150 × 4.6-mm i.d.); detection, absorption at 254 nm. Mobile phases: MeOH–0.1% HFBA (20:80) (A); MeOH–0.1% TFA (40:60) (B); MeOH–0.1% HFBA (40:60) (C); MeOH–0.1% TFA (10:90) (D); MeOH–0.1% HFBA (10:90) (E).

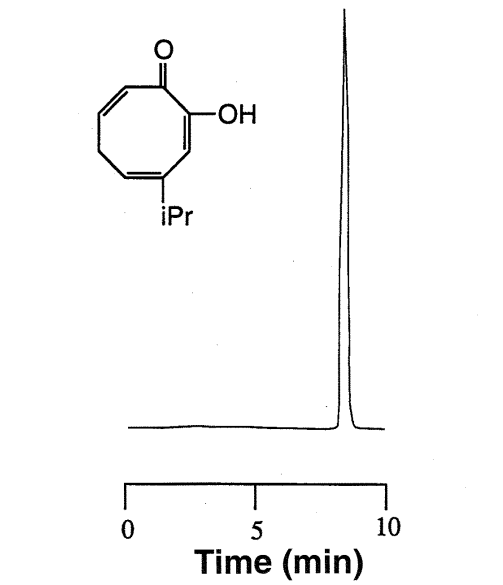


Figure 4. Separation of hinokitiol(B-tujaplicine). Conditions: column, Fluofix 120E (150 × 4.6-mm i.d.); mobile phase, MeCN–0.1% EDTA • Na_2–20mM H_2PO_4 (25:75); detection, absorption at 210 nm.

endcapping on Fluofix was the same as that on ODS packing (7). It therefore seems that the late retention and large A_s value of caffeine are caused not by residual silanols but by the surface property of fluorocarbon-bonded silicagels. This is clearly shown in Figure 2. The chromatogram obtained from Fluofix 120E, which was fully endcapped, has long tailing for fluorinated aniline isomers (Figure 2A), but no such tailing is observed on the non-endcapped ODS (Figure 2B). These results show that the origin of the tailing is not attributable to the residual silanols on packings. The addition of ammonium chloride to the mobile phase makes the tailing disappear, but the fluoroaniline isomers cannot be separated (Figure 2C). By changing the additive from ammonium chloride to HFBA, the 3 isomers were completely separated in the opposite elution order of ODS, as shown in Figure 2D. Seven mono- and difluoroanilines were separated by adding HFBA to the mobile phase, as shown in Figure 3A. Improvement of the separations of some nitrogen-containing compounds by changing the acidic additive (TFA) to a fluorinated basic addititive (HFBA) is clearly shown in Figure 3B–3E. Another example of the masking of the adsorption point on the surface of fluorinated packing is shown in Figure 4. Because hinokitiol has a strong tendency to coordinate with metals, it

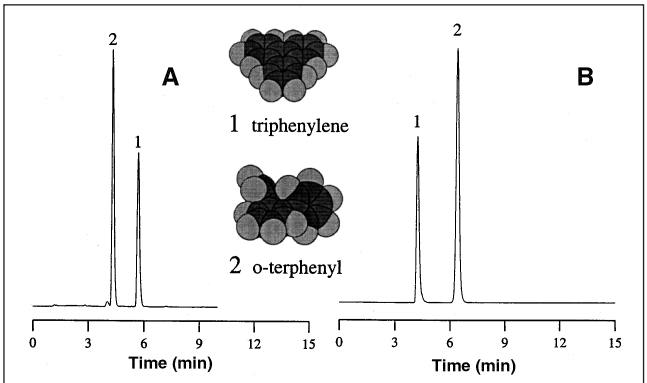


Figure 5. Recognition of steric structure. Conditions (A): column, Fluofix 120N (150 × 4.6-mm i.d.); mobile phase, MeOH–water (60:40); detection, absorption at 254 nm. Conditions (B): column, ODS (150 × 4.6-mm i.d.); mobile phase, MeOH–water (90:10); detection, absorption at 254 nm.

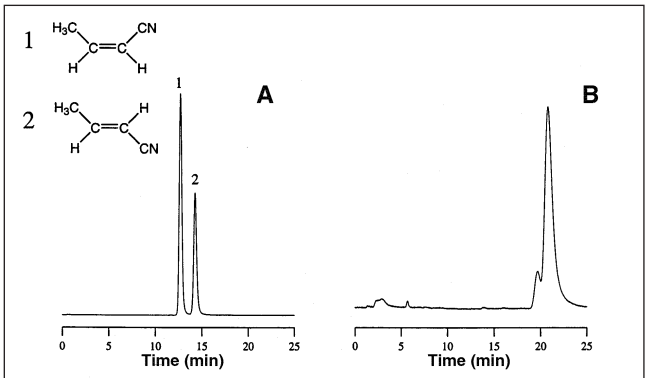


Figure 6. Separation of geometrical isomers. Conditions (A): column, Fluofix 120N (150 × 4.6-mm i.d.); mobile phase, water; detection, absorption at 210 nm. Condisitons (B): column, ODS (150 × 4.6-mm i.d.); mobile phase, water; detection, absorption at 210 nm.

often has long tailing on ordinary ODS columns due to the metallic impurity and residual silanols in the silicagel packings (8,9). As shown in Figure 4, the addition of EDTA to a mobile phase gave a symmetrical peak without tailing. From these results, it is concluded that the origin of long tailing for nitrogen-containing compounds reported in a previous paper (3) is not in the residual silanols arising from incomplete endcapping but in the surface property of fluorinated packings arising from the large oleophobicity of fluorocarbon.

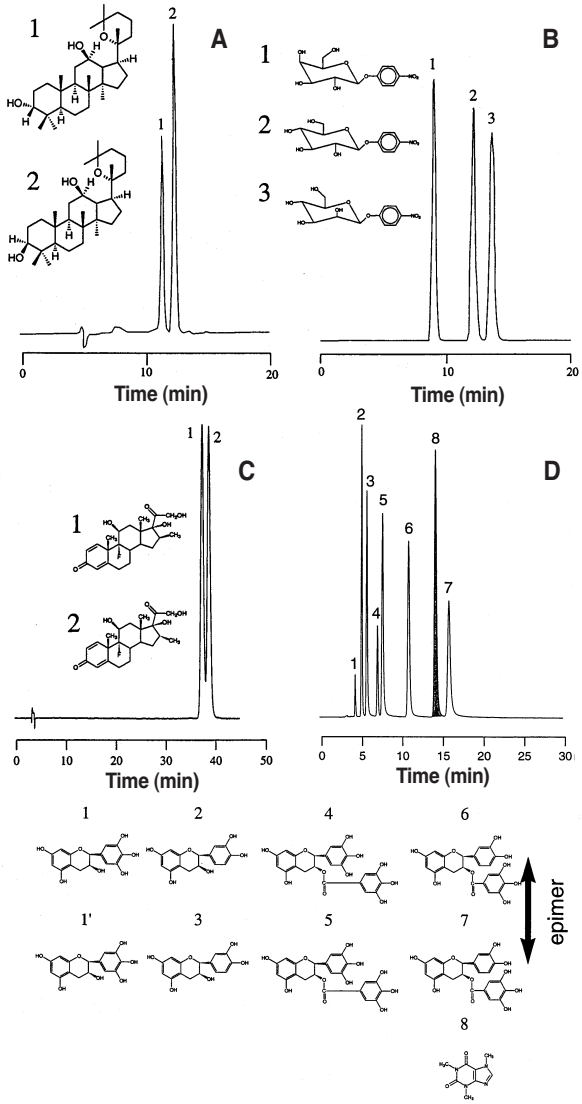


Figure 7. Separation of epimers. Peaks (A): 1, 3β-panaxadiol; 2, 3α-panaxadiol. Conditions (A): column, Fluofix 120N (250 × 4.6-mm i.d.); mobile phase, MeCN–water (60:40); detection, refractive index. Peaks (B): 1, *p*-nitrophenol β-D-galactopyranoside; 2, *p*-nitrophenol β-D-glucopyranoside; 3, *p*-nitrophenol β-D-mannopyranoside. Conditions (B): column, Fluofix 120N (150 × 4.6-mm i.d.); mobile phase, MeOH–water (2:98); detection, absorption at 254 nm. Peaks (C): 1, betamethasone; 2, dexamethasone. Conditions (C): column, Fluofix 120N (250 × 4.6-mm i.d.); mobile phase, MeCN–water (20:80); detection, absorption at 240 nm. Peaks (D): 1, gallocatechin; 1', epigallocatechin; 2, catechin; 3, epigallocatechingallate; 4, epicatechin; 5, gallocatechingallate; 6, epicatechingallate; 7, catechingallate; 8, caffeine. Conditions (D): column, Fluofix 120N (250 × 4.6-mm i.d.); mobile phase, MeOH–0.1% H_2PO_4 (14:86); detection, absorption at 254 nm.

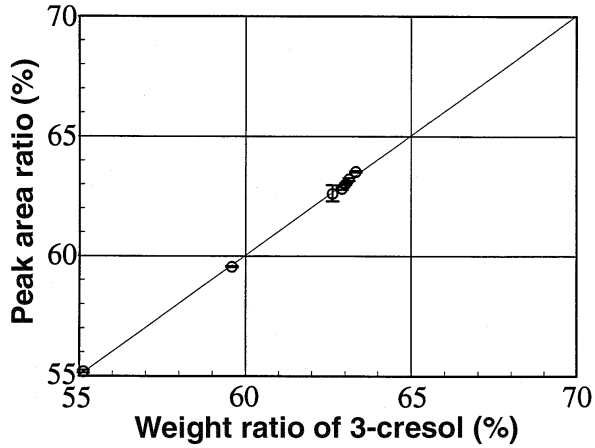


Figure 8. Relationship between initial weight ratio and HPLC peak area ratio of 3- and 4-cresol mixture. Conditions: column, three Fluofix 120N (250 × 4.6-mm i.d.) columns connected in series; mobile phase, TFEO; detection, absorption at 254 nm.

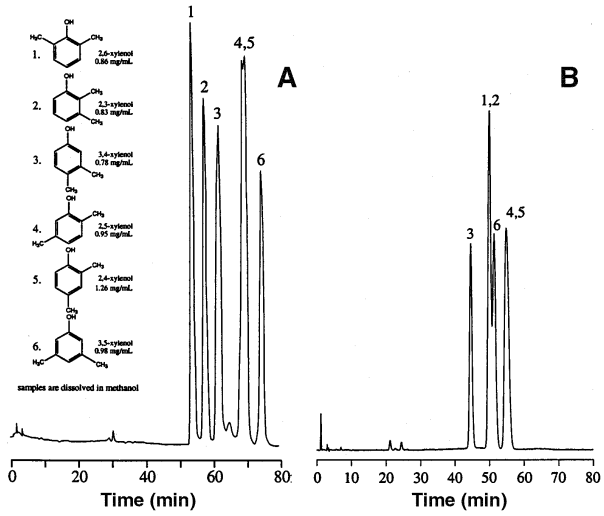


Figure 9. Separation of xylenol isomers. Conditions (A): column, Fluofix 120N (250 × 4.6-mm i.d.); mobile phase, MeOH–water (10:90); detection, absorption at 254 nm. Conditions (B): column, ODS (250 × 4.6-mm i.d.); mobile phase, MeOH–water (25:75); detection, absorption at 254 nm.

Table VII. Separation of Xylenol Isomers

Sample	Fluofix 120N *k*	Fluofix 120N α	ODS *k*	ODS α
2,6-Xylenol	18.9		21.5	
		1.10		1.00
2,3-Xylenol	20.2		21.5	
		1.08		0.89
3,4-Xylenol	21.9		19.2	
		1.08		1.24
2,5-Xylenol	23.6		23.9	
		1.04		1.00
2,4-Xylenol	24.6		23.9	
		1.06		0.93
3,5-Xylenol	26.0		22.2	

Recognition of molecular structure

Geometrical isomer

Figure 5 demonstrates that the recognition of the steric structure on the fluorinated packings is different from that on a hydrocarbon packings. Planar polyaromatic molecules such as triphenylene usually are eluted later than steric polyaromatic molecules on the ODS column (10). In contrast, on Fluofix, large planar polyaromatic compounds are eluted faster and almost at the time of void volume. This is attributed to the difference in interaction between the solute and the stationary phases providing the fluorinated packings with the unique separation character for structural isomers (11). The separation of geometrical isomers, which cannot be accomplished on the ODS column, is shown in Figure 6. It seems that this separation characteristic is attributed to the less solvophobic interaction in comparison with long chain hydrocarbon column and the rigid molecular structure of the fluorocarbon chain in fluorinated silanes.

Epimer

Separations of epimers are summarized in Figure 7. Figures 7A and 7B show typical samples (panaxdiols and PNP-sugars, respectively) that cannot be separated by an ordinary ODS column. Hormones such as betamethasone and dexamethasone can also be separated (Figure 7C). Natural products containing many different epimers extracted from green tea are separated by using a simple mobile phase system on Fluofix (Figure 7D). Caffeine is eluted after 8 acidic compounds that are catechins. This indicates that fluorocarbon-bonded packings may discriminate basic compounds from acidic compounds.

Positional isomer of various phenols

Isomers of chlorophenol and cresol have been baseline separated using TFEO as a mobile phase, as shown in Table VI. It is reported that ordinary ODS columns cannot separate 3- and 4-cresol. Conversely, the use of TFEO as a mobile phase with Fluofix can separate 3 cresol isomers completely. An example of the quantitative analysis of the mixture is shown in Figure 8. The peak area ratio is linearly proportional to the initial weight ratio with an experimental error of less than 0.1%.

Xylenol is used as the starting material for phenol polymers or intermediate for medicine or agricultural herbicides. Isomers of xylenol derivatives can be analyzed by capillary GC (12) but cannot be separated by HPLC. Six xylenol isomers that cannot be separated on an ODS column are separated with sufficiently large separation factor α on the Fluofix column, as shown in Figure 9 and Table VII. These results indicate that fluorinated packings will become a new tool for the study of phenol polymers originated from xylenols.

Conclusion

Fluorinated compounds such as fluorobenzenes, fluorobenzaldehydes, and fluoroanisoles were separated using a fluorinated column. The elution order depended on the number of fluorine atoms in the solutes. Halogen-substituted compounds were also separated much better on the Fluofix column than on the ODS column. The addition of a masking agent of the adsorption point, such as HFBA or EDTA, to the mobile phase improved the separation of nitrogen-containing compounds, giving rise to symmetric peaks. It was demonstrated that the tailing originated from the surface property of fluorinated packings caused by the large oleophobicity of fluorocarbon. Better recognition of geometrical isomers and epimers was achieved on fluorinated packings, which was attributed to the less solvophobic interaction than ODS and the rigid molecular structure of the fluorocarbon chain. It was demonstrated that the fluorinated packings had a high selectivity for geometrical isomers of phenols. Seventeen chlorophenol isomers of EPA 604 method standard sample were well separated with a short retention time using an acidic mobile phase and gradient method. Using TFEO as a mobile phase, cresol isomers were separated on the fluorinated column. Xylenol isomers were also separated using a simple mobile phase of methanol–water. Polyphenols such as flavonoids were also separated with sharp peaks and a high durability under extreme eluting conditions.

Acknowledgments

An acknowledgment is due to the late Dr. Hiroyuki Hatano of the International Institute of Technology Analysis for his helpful advice and discussions on HPLC applications. The authors also thank Dr. Ben Zhong Tang of the Hong Kong University of Scientific & Technology for reading and correcting this manuscript.

References

1. P. Varughese, M.E. Gangoda, and R.K. Gilpin. Applications of fluorinated compounds as phases and additives in chromatography and their uses in pharmaceutical analysis. *J. Chromatogr. Sci.* **26:** 401–405 (1988).
2. N.D. Danielson, L.G. Beaver, and J. Wangsa. Fluoropolymers and fluorocarbon bonded phases as column packings for liquid chromatography. *J. Chromatogr.* **544:** 187–99 (1991).
3. P.C. Sadek and P.W. Carr. Study of solute retention in reversed-phase high performance liquid chromatography on hydrocarbonaceous and three fluorinated bonded phases. *J. Chromatogr.* **288:** 25–41 (1984).
4. G.E. Berendsen, K.A. Pikaart, L. de Galan, and C. Olieman. *Anal. Chem.* **52:** 1990–93 (1980).
5. T. Monde, T. Kamiusuki, T. Kuroda, K. Mikumo, T. Ohkawa, and H. Fukube. High-performance liquid chromatographic separation of phenols on a fluorocarbon-bonded silica gel column. *J. Chromatogr. A* **722:** 273–80 (1996).
6. K. Kimata, K. Iwaguchi, S. Onishi, K. Jinno, R. Eksteen, K. Hosoya, M. Araki, and N. Tanaka. Chromatographic characterization of silica C_{18} packing materials. Correlation between a preparation method and retention behavior of stationary phase. *J. Chromatogr. Sci.* **27:** 721–28 (1989).
7. T. Kamiusuki, T. Monde, K. Yano, T. Yoko, and T. Konakahara. *Chromatographia* **49:** 649–56 (1999).
8. Y. Ohtsu, Y. Shiojima, T. Okumura, J. Koyama, K. Nakamura, O. Nakata, K. Kimata, and N. Tanaka. Performance of polymer-coated silica C_{18} packing materials prepared from high-purity

silica gel. The suppression of undesirable secondary retention processes. *J. Chromatogr.* **481:** 147–57 (1989).
9. J. Koyama, J. Nomura, Y. Ohtsu, O. Nakata, and M. Takahashi. *Chem. Lett.* **1990:** 687–690 (1990).
10. K. Jinno, S. Shimura, N. Tanaka, K. Kimata, J.C. Fetzer, and W.R. Biggs. *Chromatographia* **27:** 285–90 (1989).
11. K. Jinno and H. Nakamura. *Chromatographia* **39:** 285–93 (1994).
12. *Catalog and Technical Reference*, J&W Scientific, Folsom, CA, 1997.

Manuscript accepted August 10, 1999.