ELSEVIER

Journal of Chromatography A, 722 (1996) 273–280

JOURNAL OF CHROMATOGRAPHY A

High-performance liquid chromatographic separation of phenols on a fluorocarbon-bonded silica gel column

Takashi Monde*, Toshiro Kamiusuki, Tomoko Kuroda, Kayo Mikumo, Tadashi Ohkawa, Hiroyuki Fukube

Central Research Laboratory, Neos Company Ltd., No. 1-1 Ohike-machi, Kohsei-cho, Kohga-gun, Shiga, 520-32, Japan

Abstract

A branched-chain, fluorocarbonaceous, silane-bonded silica gel material for high-performance liquid chromatography (HPLC) has been developed. New columns were found to have a high ability to recognize halogen-containing molecules and separate geometrical isomers of halogen-containing phenols. The standard mixture for the EPA 604 method of analysis of many halogenous phenols was separated better than with gas chromatography. These fluorocarbon-bonded columns also separated various types of phenols without a halogen atom. Polyphenols such as flavonoids are separated with a sharp peak shape and excellent durability under extreme eluting conditions, in contrast with ordinary hydrocarbon-bonded silica gel columns.

1. Introduction

Many fluorine-containing materials have been created in the course of fluorine chemistry and its industrial production technique in the last two decades. The separation and purification of fluorine-containing compounds are required in several scientific fields. For this purpose, many fluorocarbonaceous-bonded packings have been developed for use in gas chromatography and liquid chromatography [1,2]. These packings were classified into two types: the silica gel packings treated with a fluorinated silylation agent and the organic polymers synthesized from fluorinated monomers. However, these materials did not exhibit their full separation efficiency in HPLC applications, perhaps because of metallic impurities in the silica gel or variations in the polymer gel.

The production of branched, fluorocarbon-bonded silica gel particles has been optimized, leading to a high purity and homogeneous spherical silica gel. The use of fluorocarbon-bonded silica gel HPLC columns leads to elution conditions similar to those of ordinary hydrocarbon-modified silica gel columns. Fluorocarbon-bonded silica gel has separation characteristics superior to those of HPLC columns reported previously [3,7–10].

In this paper we report the separation conditions for geometrical isomers of various types of phenols with a branched-type, fluorocarbon-bonded silica gel packing with both oleophobic and hydrophobic properties using a rigid fluorocarbon chain.

* Corresponding author.

0021-9673/96/$15.00
SSDI 0021-9673(95)00436-X

2. Experimental

2.1. Preparation of columns for HPLC

The hexafluoropropene dimer (D-2) was formed by anionic oligomerization in the presence of fluoride ions as shown in Fig. 1 (oligomerization) [4]. The allyl fluoride (FDSO) was synthesized following substitution with the perfluoro-2-methyl-2-pentylcarbanion (Fig. 1,

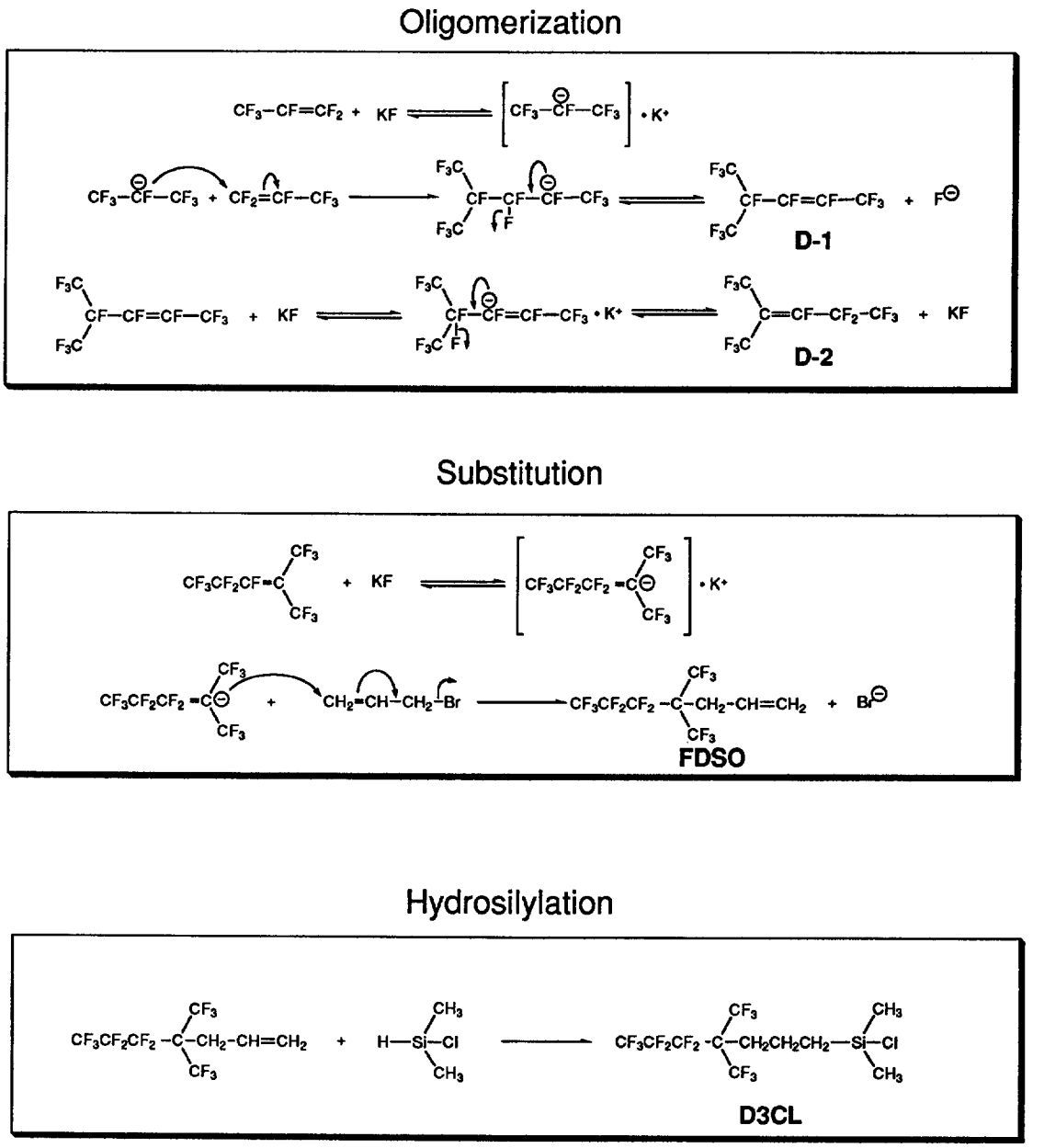


Fig. 1. Synthesis of the branched-chain polyfluorocarbon silylation agent.

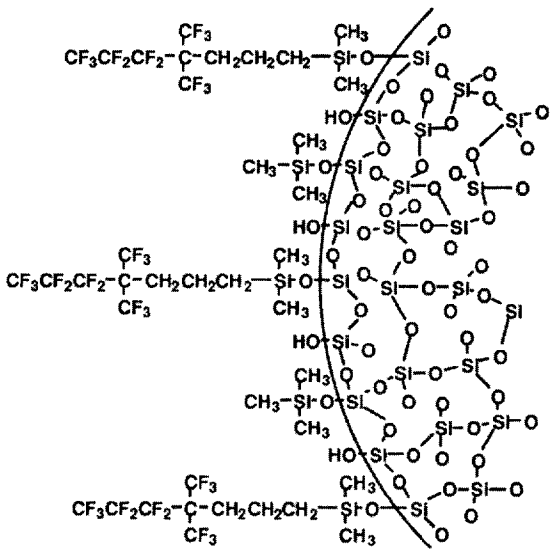
substitution) [5,6]. A branched-chain polyfluorosilane [1H,1H,2H,2H,3H,3H-tridecafluoro(4,4-dimethylheptyl)dimethylmonochlorosilane] (D3C-L) was synthesized with the procedure reported in a previous paper (Fig. 1, hydrosilylation) [7]. The preparation of silica gels treated with fluorinated and hydrocarbonaceous silylation agents was performed according to an already existing method [8–10]. All packings were made from the same silica gel. The silylation reaction with high-purity silica gel (average diameter 5 μm, average pore diameter 12 nm) was performed in dry toluene under refluxing. Fluofix 120E and other hydrocarbon-bonded silica gel packings were endcapped with trimethylsilane, except for Fluofix 120N. Columns with 4.6 mm I.D. and 150 or 250 mm long were made of stainless-steel tubing packed with a slurry technique.

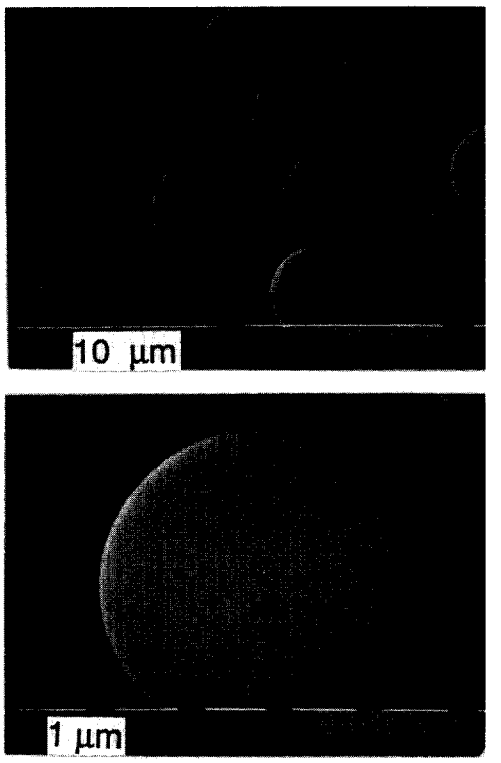
2.2. Chemicals

All test samples used were reagent grade and undistilled. Acetonitrile, methanol, tetrahydrofuran (THF) and water were purchased from Kanto Chemical (Tokyo, Japan), and the mobile-phase solvents were HPLC grade. 2,2,2-Trifluoroethanol (TFEO) purchased from Central Glass (Tokyo, Japan) was used as mobile phase without purification.

2.3. HPLC equipment

The LC system consisted of a Waters Module-1 (Milford, MA, USA) within a high-performance pump, a variable-wavelength UV detector, an autosampler, and a column temperature controller, combined with a NEC PC-9800DA micro-





(A) (B)

Fig. 2. (A) Surface model of the branched polyfluorocarbon-bonded silica gel with endcapping on the residual silanols with trimethyl silane (Fluofix 120E). (B) Scanning electron micrographs of base silica gel.

computer (Tokyo, Japan) controlled with Waters Maxima 820J chromatographic processing software.

2.4. HPLC conditions

The flow-rate was 1 ml/min, and the column temperature was maintained at 40°C. All samples were dissolved in suitable organic solvent at a concentration of about 0.1%, and the sample volume was 5 μl.

3. Results and discussion

The schematic surface model of the branched, polyfluorocarbonaceous-bonded packings with endcapping on the residual silanol and scanning electron micrographs of base silica gel are shown in Fig. 2. The base silica gel has a very smooth surface and no hollows. From elemental analysis data of Fluofix 120E, the number of bonded molecules on this surface was calculated to be 2.99 μmol/m^2. This value is nearly equal to that of ODS packings (3.00 μmol/m^2) synthesized with the same silica gel as Fluofix, and about half of the total silanols found on the silica gel surface.

Fig. 3 shows typical chromatograms on Fluofix 120N and the ODS column prepared from the same silica gel. Each column was used without endcapping to compare the different silylation agents. Both chromatograms were obtained with methanol–water (65:35, v/v) as the mobile phase and with a 150 × 4.6 mm I.D. column. Fluofix 120N retains non-polar and small molecular hydrocarbon compounds less than ODS (peak 3: ethylbenzene); in particular, polycyclic aromatic compounds (peak 2: anthracene) were eluted very fast. The retention time of fluorine-con-

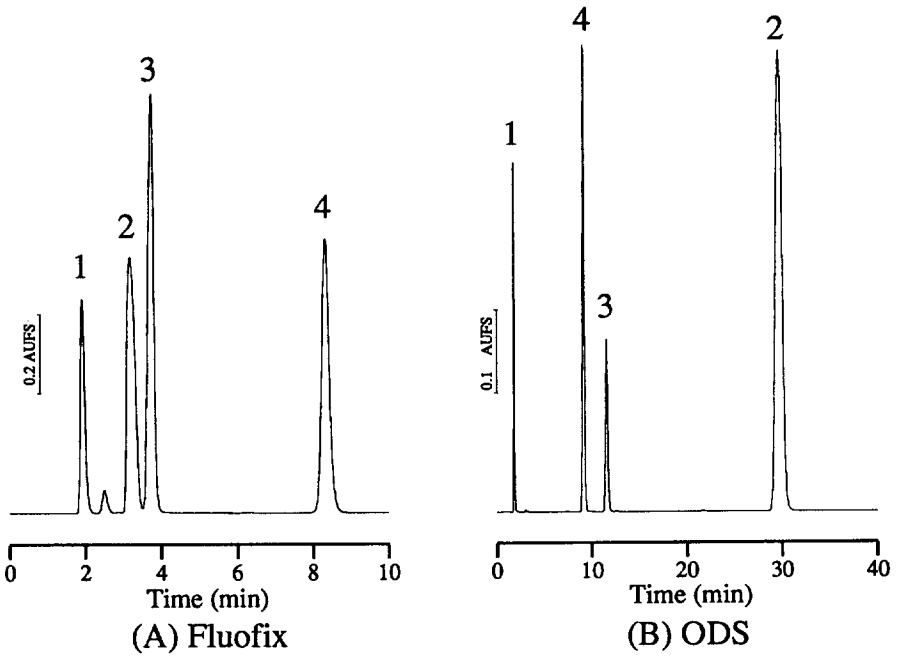


Fig. 3. Comparison of retention behavior on Fluofix and ODS column. (A) Fluofix 120N (150 × 4.6 mm I.D., 5 μm), (B) ODS (150 × 4.6 mm I.D., 5 μm). Mobile phase, methanol–water (65:35, v/v); detector, absorption at 254 nm. Peaks: 1 = uracil, 2 = anthracene, 3 = ethylbenzene, 4 = hexafluorobenzene.

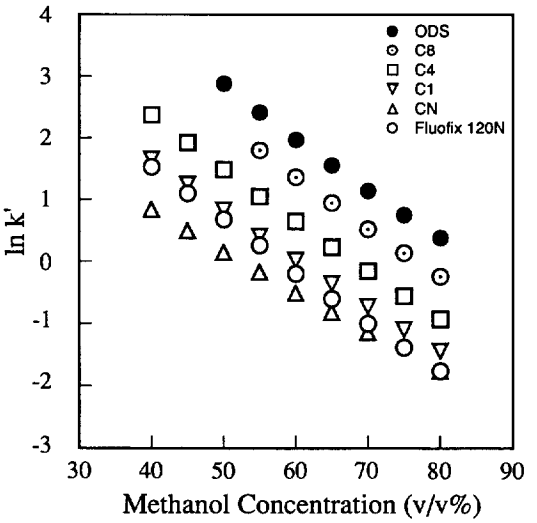


Fig. 4. Selectivity characteristics of various columns for naphthalene versus methanol concentration. Column size, 150 × 4.6 mm I.D.; detector, absorption at 254 nm.

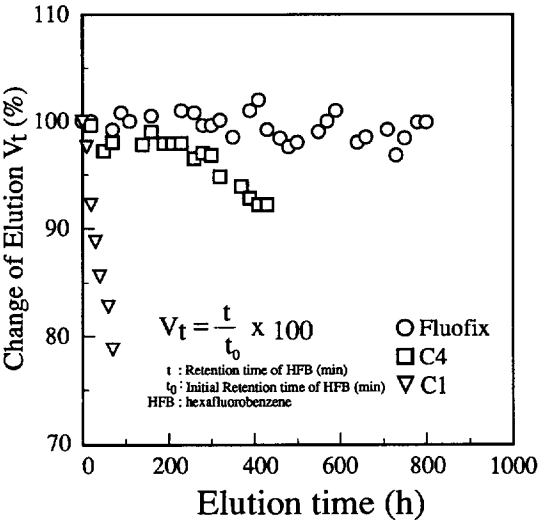


Fig. 5. The variation of elution as a function of elution time. Column, Fluofix 120N (150 × 4.6 mm I.D., 5 μm); mobile phase, methanol–0.1% trifluoroacetic acid (pH 2.1) (60:40, v/v); detector, absorption at 254 nm; sample, hexafluorobenzene; number of injections, 500 over an 800-h period.

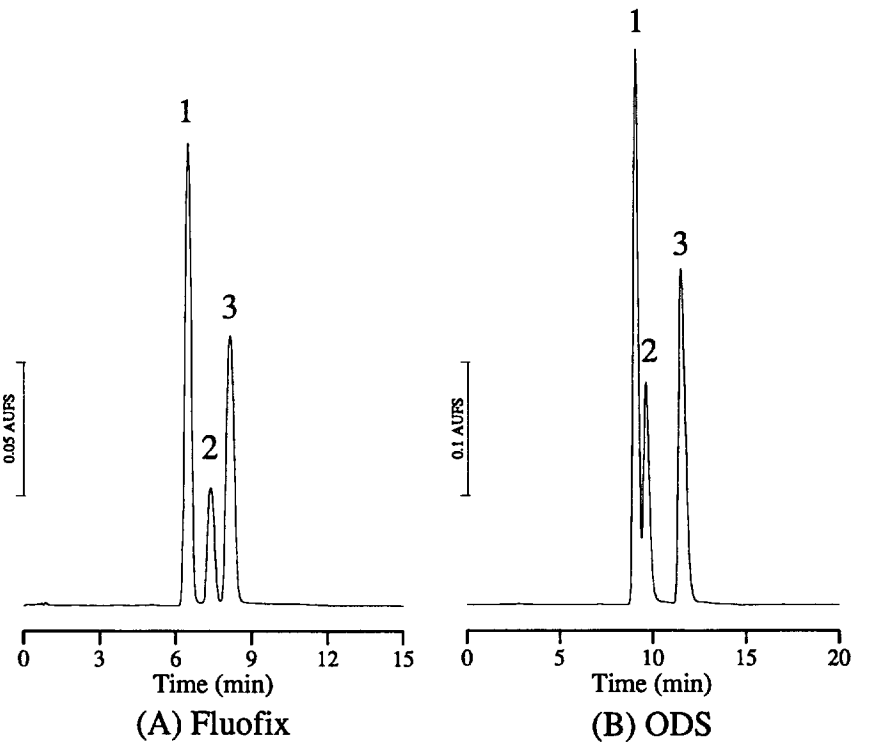


Fig. 6. Comparison of retention behavior for fluorophenol on (A) Fluofix and (B) ODS. Column: (A) Fluofix 120N (150 × 4.6 mm I.D., 5 μm), (B) Capcellpak SG120 (150 × 4.6 mm I.D., 5 μm); mobile phase, methanol–water (20:80, v/v); detector, absorption at 254 nm. Peaks: 1 = 2-fluorophenol, 2 = 4-fluorophenol, 3 = 3-fluorophenol.

taining molecules (peak 4: hexafluorobenzene) is identical to that found on the ODS material.

The selectivity characteristics of the various materials for naphthalene are shown in Fig. 4. The ln k' of naphthalene on Fluofix 120N decreases linearly with increasing methanol concentration of the eluent. It is shown that Fluofix 120N separates the neutral compounds in a reversed-phase distribution mode. The smaller retention of naphthalene on Fluofix 120N could be dependent on the oleophobic and hydrophobic character of the fluoroalkyl group on the surface of the silica gel.

The fluorocarbon-bonded column has a high durability in contrast with ordinary hydrocarbon-bonded silica gel columns, as shown in Fig. 5. The Fluofix column gave a stable retention time over a period of 800 h elution time and 500 injection cycles. With the C_1 and C_4 columns the retention time decreased rapidly for hexafluorobenzene under high-pH conditions.

A comparison of the retention time for fluorophenol on the fluorocarbon-bonded column and on ODS is shown in Fig. 6. The elution order for

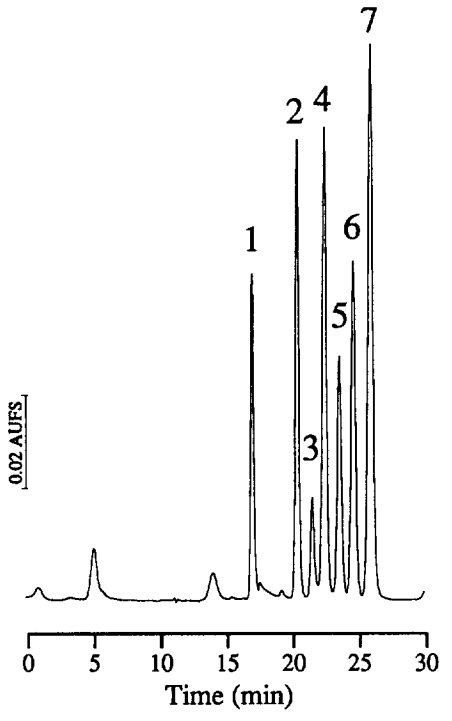


Fig. 7. Separation of chlorophenol and cresol isomers on Fluofix 120N. Column: Fluofix 120N (250 × 4.6 mm I.D., 5 μm) connected three columns in series; mobile phase, 2,2,2-trifluoroethanol–water (20:80, v/v); detector, absorption at 270 nm. Peaks: 1 = phenol, 2 = 4-chlorophenol, 3 = 3-chlorophenol, 4 = 2-cresol, 5 = 2-chlorophenol, 6 = 3-cresol, 7 = 4-cresol.

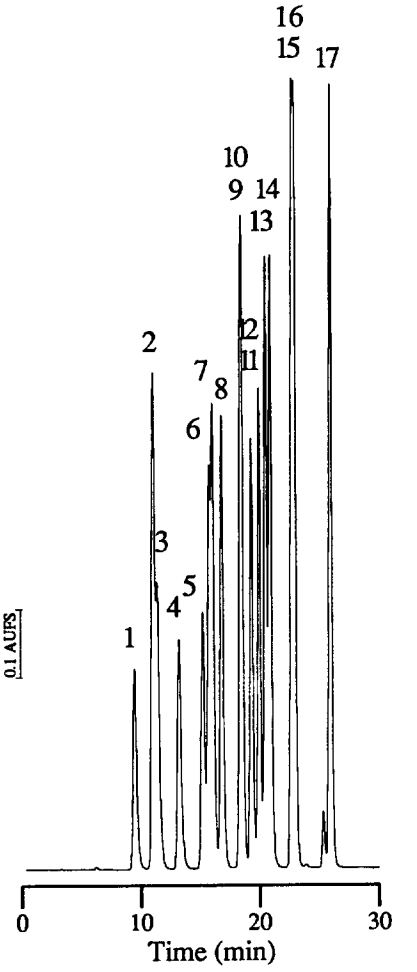


Fig. 8. Separation of chlorophenols on Fluofix 120N. Column: Fluofix 120N (150 × 4.6 mm I.D., 5 μm); mobile phase, (solvent A) acetonitrile, (solvent B) 0.1% aqueous phosphoric acid; linear gradient from 25% A to 80% A (v/v) over 50 min; detector, absorption at 230 nm. Peaks: 1 = 2-chlorophenol, 2 = 4-chlorophenol, 3 = 3-chlorophenol, 4 = 2,6-dichlorophenol, 5 = 2,3-dichlorophenol, 6 = 2,5-dichlorophenol, 7 = 2,4-dichlorophenol, 8 = 3,4-dichlorophenol, 9 = 3,5-dichlorophenol, 10 = 2,3,6-trichlorophenol, 11 = 2,4,6-trichlorophenol, 12 = 2,4,5-trichlorophenol, 13 = 2,3,4-trichlorophenol, 14 = 2,3,5-trichlorophenol, 15 = 2,3,4,6-tetrachlorophenol, 16 = 2,3,5,6-tetrachlorophenol, 17 = pentachlorophenol.

all compounds is the same on the different columns. The Fluofix column gave a shorter retention time and a better separation for the fluorophenol used, compared with the ODS column using the same elution conditions.

Isomers of chlorophenol and cresol were baseline separated, as shown in Fig. 7, using 2,2,2-trifluoroethanol as the mobile phase. A mixture of chlorophenols was partly separated with the Fluofix column, as shown in Fig. 8; they eluted in the order of their number of chlorine atoms.

Fig. 9 shows a chromatogram of standard samples of the EPA 604 method for measurement of substituted phenols. Eleven phenols were separated better than using gas chromatography [11]. The fluorinated material also allows separation of geometrical isomers and polyaromatic compounds [12].

Polyphenols such as flavonoids could be separated on the fluorinated column as shown in Fig. 10. Due to the high polarity of polyphenols, a low-pH mobile phase is necessary to separate these compounds. Under these extreme acidic conditions, the fluorinated material still gives stable retention times for polyphenols due to the chemically stable fluorocarbon on the silica gel surface. A comparison of the retention times of flavonoids on the Fluofix and ODS columns is

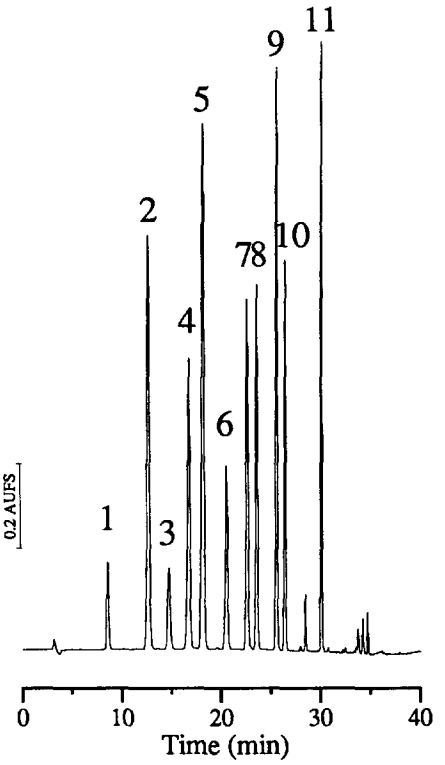


Fig. 9. HPLC analysis of the EPA 604 method standard mixture on a polyfluorocarbon-bonded silica gel column. Column: Fluofix 120N (250 × 4.6 mm I.D., 5 μm); mobile phase, (solvent A) acetonitrile, (solvent B) 0.1% aqueous trifluoroacetic acid; gradient, from 20% A to 100% A (v/v) over 30 min with concave gradient curve No. 7; detector, absorption at 230 nm. Peaks: 1 = phenol, 2 = 4-nitrophenol, 3 = 2-chlorophenol, 4 = 2-nitrophenol, 5 = 2,4-dinitrophenol, 6 = 2,4-dimetylphenol, 7 = 4-chloro-3-methylphenol, 8 = 2,4-dichlorophenol, 9 = 2-methyl-4,6-dinitrophenol, 10 = 2,4,6-trichlorophenol, 11 = pentachlorophenol.

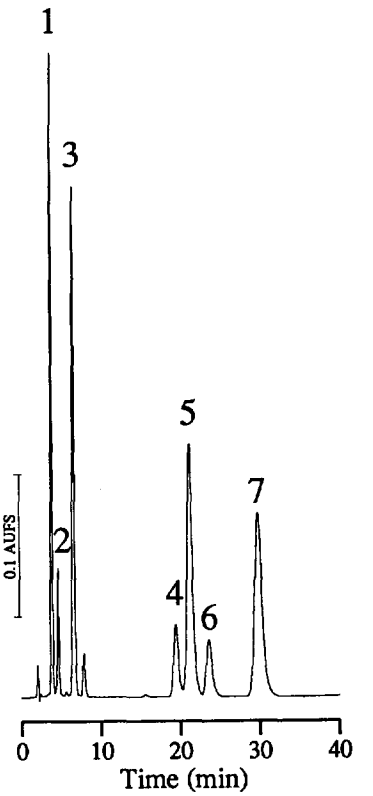


Fig. 10. Separation of flavonoids on the polyfluorocarbon-bonded silica gel column. Column: Fluofix 120N (150 × 4.6 mm I.D., 5 μm); mobile phase, THF–50 m*M* phosphate buffer (pH 2.1) (25:75, v/v); detector, absorption at 254 nm. Peaks: 1 = hesperidin, 2 = naringin, 3 = quercitrin, 4 = hesperetin, 5 = quercetin, 6 = naringenin, 7 = kaempferol.

Table 1
Retention times of flavonoids on Fluofix and ODS

Sample	Retention time (min)	
	Fluofix	ODS
Hesperidin	3.78	4.60
Naringin	4.59	5.41
Quercitrin	6.52	7.88
Hesperetin	19.42	24.58
Quercetin	21.17	27.97
Naringenin	23.79	30.28
Kaempferol	29.79	37.86

Values represent the retention time of seven flavonoids on each column. Chromatographic conditions: Fluofix 120N (150 × 4.6 mm I.D., 5 μm); mobile phase, THF–50 m*M* phosphate buffer (pH 2.7) (25:75, v/v); ODS (150 × 4.6 mm I.D., 5 μm); mobile phase: THF–50 m*M* phosphate buffer (pH 2.7) (30:70, v/v); detector, absorption at 254 nm on both columns.

shown in Table 1. Each elution condition is the limit of concentration of organic solvent given complete separation of samples. On the Fluofix column flavonoids are eluted faster than on the ODS column.

4. Conclusion

Branched polyfluorocarbonaceous silica gels used as packing materials for HPLC columns were prepared by silylation with 1H,1H,2H,2H,3H,3H - tridecafluoro(4,4 - dimethylheptyl)dimethylmonochlorosilane. It was demonstrated that the column packings have a high selectivity for the geometrical isomers of substituted phenols. Polyphenols such as flavonoids are also separated with sharp peaks and a high durability under extreme eluting conditions.

Acknowledgements

The authors would like to express their sincere thanks to Dr. Hiroyuki Hatano for his helpful discussion, and Dr. Takeo Konakahara, Dr. Kiyokatu Jinno, Dr. Noriyuki Nimura, and Dr. Fumiko Yamamoto for their helpful suggestions.

References

[1] N.D. Danielson, L.G. Beaver and J. Wangsa, J. Chromatogr., 544 (1991) 187.
[2] P. Varughese, M.E. Gangoda and R.K. Gilpin, J. Chromatogr. Sci., 26 (1988) 401.
[3] H. Itoh, T. Kinoshita and N. Nimura, J. Chromatogr. A, 662 (1994) 95.
[4] W. Brunskill, W.T. Flowers, R. Gregory and R.N. Haszeldine, Chem. Commun., 1970 (1970) 1446.
[5] I. Ikeda, M. Tsuji and M. Okahara, J. Fluorine Chem., 36 (1987) 171.
[6] W. Dmowski and R. Wozniacki, J. Fluorine Chem., 36 (1987) 385.
[7] T. Konakahara, S. Okada, T. Monde, N. Nakayama, J. Furuhashi and J. Sugaya, Nippon Kagakukai-shi, 1991 (1991) 1638.
[8] P.C. Sadek and P.W. Carr, J. Chromatogr., 288 (1984) 25.
[9] G.E. Berendsen, K.A. Pikaart and L. de Galan, Anal. Chem., 52 (1980) 1990.
[10] H.A.H. Billiet, P.J. Schoenmakers and L. de Galan, J. Chromatogr., 218 (1981) 443.
[11] Federal Register, Environmental Protection Agency, 49 (209) (1984) 65.
[12] K. Jinno and H. Nakamura, Chromatographia, 39 (1994) 285.