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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF SOME SUBSTITUTED FLUORENES

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SUMMARY

High-performance liquid chromatographic studies of a series of fluorenes substituted at positions 2-, 2,7-, and 2,5-, were carried out in the normal- and reversed-phase partition and adsorption modes of liquid chromatography. The capacity ratios for these derivatives have been determined using UV detection as a monitor, and the observed behavior of the derivatives has been discussed. Studies in reversed-phase mode were carried out using two columns which differed from each other in the extent of carbon loading. An appropriate choice of solvents allowed reversed-phase columns to be used as deactivated silica adsorption columns, and a normal partition column to be used in the reversed-phase mode. Several different chromatograms have been included which illustrate some typical separations of the fluorene compounds that have been carried out.

INTRODUCTION

In a recent paper we described the behavior of a series of biphenyl derivatives in the three most commonly used modes of high-performance liquid chromatography (HPLC)[1]. These derivatives had been synthesized as part of a program to develop specific and sensitive radioimmunoassay (RIA) procedures for chemical carcinogens containing the biphenyl skeleton[2,3]. Fluorene is a compound structurally related to biphenyl in which the 2- and 2′-positions (in the biphenyl numbering system) have been connected by a methylene bridge, thus creating a conformationally rigid planar system. This basic structure is common to several carcinogens such as 2-acetamidofluorene and 2,7-dinitrofluorene.

During the development of a RIA procedure for 2-acetamidofluorene[4] we found it necessary to synthesize a variety of fluorene-based derivatives. In this paper we report our observations on the behavior of these derivatives in several HPLC systems.

EXPERIMENTAL

Chromatography equipment

The instrument used in these studies was a Varian 4200 series liquid chromatograph (Varian Assoc., Palo Alto, Calif., U.S.A.) which was equipped with two 250-ml syringe pumps and a solvent programmer for gradient elution analysis. Samples which eluted from a column were monitored with a Varian Variscan (Model 635) UV–Vis detector and recorded on a Hewlett-Packard Model 7127A recorder (Hewlett-Packard, Avondale, Pa., U.S.A.).

Columns

The pre-packed columns used in this study included the following: (1) Partisil 10-PXS, 10-μm silica (25 cm $\times$ 4.6 mm) from Whatman, Clifton, N.J., U.S.A.; (2) Partisil 10-PAC, bonded cyanopropyl on 10-μm silica (25 cm $\times$ 4.6 mm) from Whatman; (3) Bondapak CN, bonded cyanopropyl on 10-μm silica (30 cm $\times$ 3.9 mm) from Waters Assoc., Milford, Mass., U.S.A.; (4) Partisil 10-ODS, bonded octadecyl on 10-μm silica (25 cm $\times$ 4.6 mm) from Whatman: (5) ODS-silica "heavy phase load" (25 cm $\times$ 4. 6 mm) from Universal Scientific Company, Atlanta, Ga. U.S.A.

Solvents and chemicals

Organic solvents used in these studies were distilled-in-glass grade and purchased from Burdick & Jackson Labs., Muskegon, Mich., U.S.A. Before use, each solvent was filtered through a Millipore MF-Millipore 0.22-μm GSWP, or Fluoropore 0.45-μm HAWP filter, as applicable, (Millipore, Bedford, Mass., U.S.A.). Water was distilled and filtered through Millipore reverse osmosis system RO15. Tetrahydrofuran (THF) and dimethylformamide (DMF) used for the preparation of samples were distilled before use.

Compounds obtained from commercial or other sources were: 2,7-dinitrofluorene, 2-nitrofluorene, 2-aminofluorene, and fluorene (Aldrich, Milwaukee, Wisc., U.S.A.); 2-acetamidofluorene (Eastman, Rochester, N.Y., U.S.A.). The 1-, 3-, 5-, and 7-hydroxy-2-acetamidofluorene derivatives were gifts from Dr. Elizabeth Weisburger at the National Cancer Institute (Bethesda, Md., U.S.A.). All other chemicals were synthesized in our laboratory according to standard procedures. Information concerning synthesis may be obtained from the authors. Purity and structure were established by elemental analysis and standard physicochemical techniques.

Methodology and operating conditions

All of the compounds used were injected onto columns in THF solution. Samples were prepared at concentrations such that 1–2-μl portions injected onto a column gave an adequate detector response. Compounds were monitored at or near the wavelength of maximum UV absorption. For example, many of the nitro compounds responded better in the 300–320 nm range. Samples were stored at 4° in the dark, and were prepared fresh at biweekly intervals.

All studies were carried out at ambient temperature. Replicate injections of each compound were made using a 10-μl Hamilton syringe. Retention times were measured in centimeters on the chart paper and results were expressed as capacity

ratios (k'). In normal adsorption and normal partition chromatographic studies fluorene was the reference compound used for the calculation of t_0. Acetone was used as a reference for reversed-phase studies.

RESULTS AND DISCUSSION

Normal adsorption chromatography

Capacity ratios for some of the fluorene derivatives examined on a silica adsorption column have been summarized in Table I. Not all of the compounds are discussed individually, but a few features of Table I are mentioned here.

TABLE I

CAPACITY RATIOS FOR SOME FLUORENE DERIVATIVES ON A NORMAL ADSORPTION COLUMN

Column: Partisil 10-PXS (25 cm × 4.6 mm). Conditions: flow-rate, 60 ml/h; chart speed: 1 in./min; UV absorption at 254 nm (or 300 resp. 320 nm if no reasonable absorption at 254 nm); 0.5 a.u.f.s.; ambient temperature. —: The compound was strongly retained in this solvent system. Solvent systems: A, 50% hexane in 2-propanol–dichloromethane (30:70); B, methanol–dichloromethane (1:99); C, methanol–chloroform (3:97).

Compound	k'		
	A	*B*	*C*
Fluorene	0.00	0.00	0.00
2-Heptafluorobutyramidofluorene	0.03	0.06	0.06
2-Nitrofluorene	0.04	0.01	0.00
2-Trifluoroacetamidofluorene	0.11	0.16	0.13
2,7-Dinitrofluorene	0.11	0.03	0.03
7-Nitro-2-heptafluorobutyramidofluorene	0.11	0.12	0.03
2-Nitrofluorenone	0.15	0.08	0.05
7-Nitro-2-trifluoracetamidofluorene	0.17	0.21	0.14
2-Nitro-5-aminofluorene	0.33	0.13	0.13
2-Aminofluorene	0.46	0.33	0.17
7-Nitro-2-aminofluorene	0.63	0.29	0.20
7-Amino-2-trifluoroacetamidofluorene	0.87	0.37	0.54
2,7-Diaminofluorene	1.15	0.66	0.51
2-Acetamidofluorene	1.43	0.66	0.42
7-Nitro-2-acetamidofluorene	1.62	0.64	0.45
2,7-Diacetamidofluorene	2.87	—	2.19
7-Amino-2-acetamidofluorene	2.07	0.95	0.82

Binary solvent systems B (methanol–dichloromethane) and C (methanol–chloroform) are less retentive and generally give faster elution times for the compounds than does the ternary system A (hexane–2-propanol–dichloromethane). Fluorene, a reference used for this series, elutes first in all three of the systems used in this mode, while the diamide, 2,-7-diacetamidofluorene elutes last in all three systems. There are, as was noted in the biphenyl series[1], some minor differences in the elution order of any two given compounds in different solvent systems.

If one examines the monosubstituted series of compounds, one can see that the simple amine 2-aminofluorene elutes ahead of its carcinogenic acetylated deriva-

tive 2-acetamidofluorene. However, the compounds 2-trifluoroacetamidofluorene and 2-heptafluorobutyramidofluorene are less polar than the amine and elute earlier than the parent compound. The observed k' values also indicate the slightly less polar nature of the heptafluorobutyryl group compared to that of the trifluoroacetyl group.

Resolution of several synthetic mixtures of fluorene derivatives is illustrated in Figs. 1 and 2. A mixture of the aminonitro derivative 7-nitro-2-aminofluorene and some related derivatives is resolved in the chromatogram in Fig. 1. 7-Nitro-2-trifluoroacetamidofluorene elutes prior to the parent amine. Reduction of the nitro group gives the derivative 7-amino-2-trifluoroacetamidofluorene which elutes later than the parent amine. The derivatives 7-nitro-2-acetamidofluorene and 7-amino-2-acetamidofluorene elute in that order, but both of these compounds elute later than 7-nitro-2-aminofluorene. In Fig. 2, 7-nitro-2-aminofluorene is shown in admixture with 2,7-dinitrofluorene and the reduced 2,7-diaminofluorene. The non-polar dinitro derivative elutes first, followed by monoamino and diamino compounds.

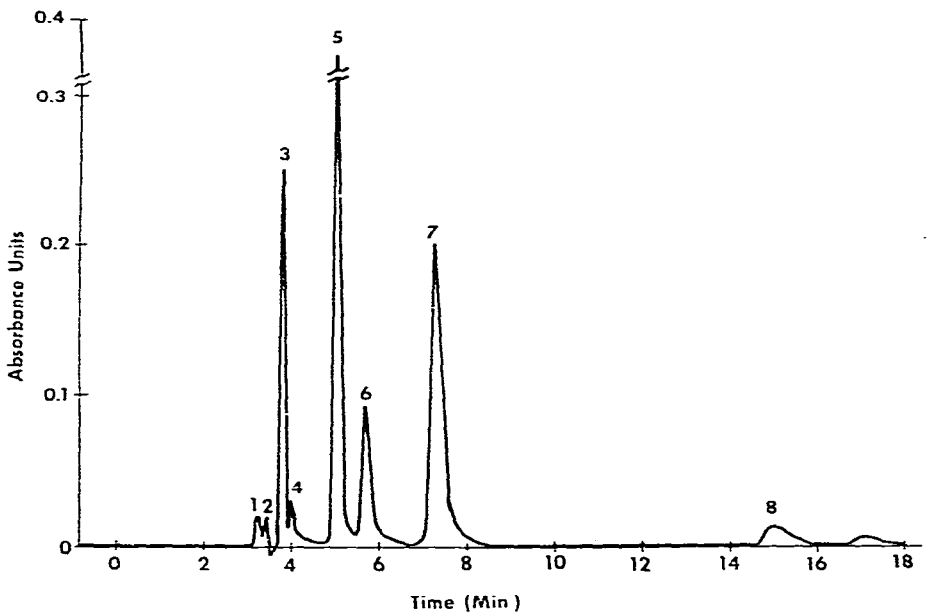

Fig. 1. HPLC of some fluorene derivatives. Peaks: 1 and 2 = unidentified; 3 = 7-nitro-2-trifluoroacetamidofluorene; 4 = unidentified; 5 = 7-nitro-2-aminofluorene; 6 = 7-amino-2-trifluoroacetamidofluorene; 7 = 7-nitro-2-acetamidofluorene; 8 = 7-amino-2-acetamidofluorene. Conditions: column, Partisil 10-PXS (25 cm × 4.6 mm); flow-rate, 60 ml/h; chart speed, 0.5 in./min; solvents, (A) hexane and (B) 2-propanol–dichloromethane (30:70), initial 45% B with 5% B/min gradient started at 9 min; UV detection at 254 nm, 0.5 a.u.f.s.

Under appropriate solvent conditions, a reversed-phase column can be used as a deactivated silica gel column in the normal adsorption mode. We have examined a group of the fluorene based derivatives which do not elute in the reversed-phase systems in a non-aqueous solvent system consisting of hexane and propanol. Two columns were used in the study. Column A was a "heavy phase load" ODS column (*ca.* 15%) while the Partisil ODS column (Column B) has a phase loading of

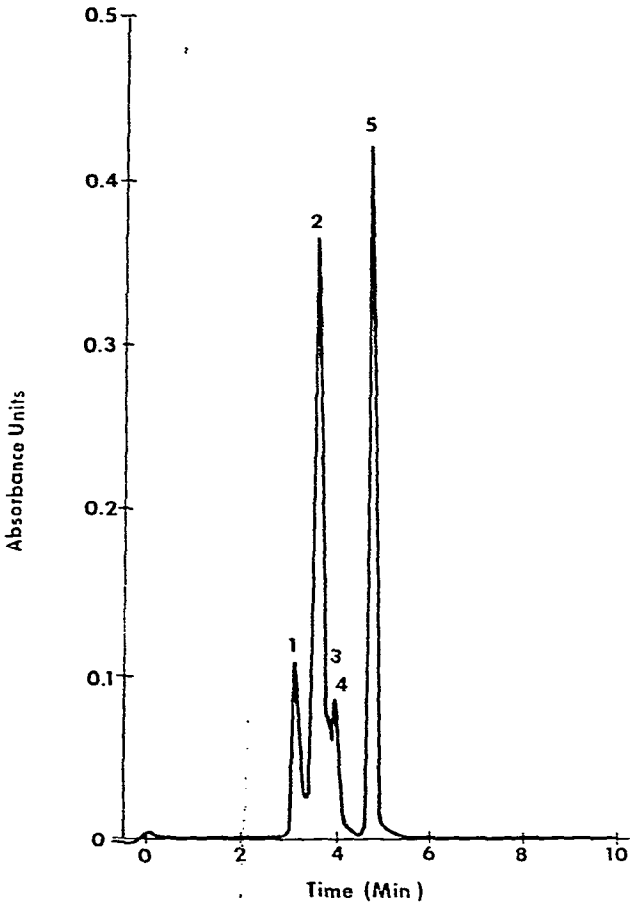

Fig. 2. HPLC of some fluorene derivatives. Peaks: 1 = 2,7-Diacetamidofluorene; 2 = 7-nitro-2-aminofluorene; 3 and 4 = unidentified; 5 = 2,7-diaminofluorene. Conditions: column, Partisil 10-PXS (25 cm × 4.6 mm); flow-rate, 60 ml/h; chart speed, 0.5 in./min; solvent, methanol–dichloromethane (3:97); UV detection at 254 nm, 0.5 a.u.f.s.

approximately 5%. Capacity ratios for the group of compounds eluted in a hexane–propanol system are given in Table II. In this system, Column A is less retentive and a larger number of the compounds elute from Column A than from Column B. This observation would be expected on the basis of the heavier phase load on Column A, which results in fewer available hydroxyls (through silanization) for use as adsorption sites. Column B has more available hydroxyls, which permit a more adsorption-like behavior. Thus, retention times are longer and k' values are higher. Figs. 3 and 4 are examples of some separations of fluorene derivatives which can be effected using the reversed-phase columns in the adsorption mode.

Normal partition chromatography

Capacity ratios for compounds eluted from a normal partition "cyano" type column are summarized in Table III. In general, these data parallel those for normal adsorption chromatography given in Tables I and II. As expected, a few discrepancies

TABLE II

CAPACITY RATIOS FOR FLUORENE DERIVATIVES ON ODS-SILICA REVERSED-PHASE COLUMNS USED AS NORMAL ADSORPTION COLUMNS

Columns: A, Universal scientific "heavy phase load" ODS-Silica (25 cm × 4.6 mm); B, Partisil 10-ODS (25 cm × 4.6 mm). Conditions: flow-rate 50 ml/h; chart speed 1 in./min; UV absorbance at 254 nm, 0.5 a.u.f.s. (see legend to Fig. 1); ambient temperature; pressure 150 p.s.i.; solvent 2-propanol–hexane (5:95). —: The compound was strongly retained in this system.

Compound	*k'*	
	A	*B*
Fluorene	0.00	0.00
2-Trifluoroacetamidofluorene	0.00	0.32
2-Heptafluorobutyramidofluorene	0.00	0.18
7-Nitro-2-heptafluorobutyramidofluorene	0.00	0.62
7-Nitro-2-trifluoroacetamidofluorene	0.03	1.20
2-Nitrofluorene	0.05	0.15
2-Nitrofluorenone	0.14	0.71
2,7-Dinitrofluorene	0.20	0.42
2-Nitro-5-aminofluorene	0.23	2.92
7-Amino-2-trifluoroacetamidofluorene	0.33	—
2-Aminofluorene	0.41	—
2-Acetamidofluorene	0.95	—
7-Nitro-2-aminofluorene	1.59	—

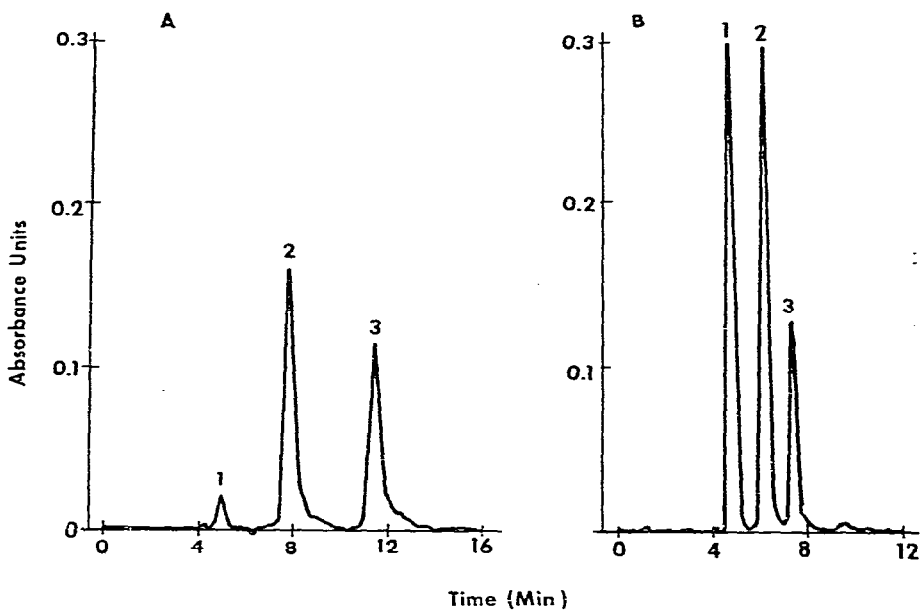

Fig. 3. HPLC of some fluorene derivatives. Peaks: A: 1 = unidentified; 2 = 7-nitro-2-heptafluorobutyramidofluorene; 3 = 7-nitro-2-trifluoroacetamidofluorene. B: 1 = 2-Nitrofluorene; 2 = 2,7-dinitrofluorene; 3 = 2-nitrofluorenone. Conditions: column, Partisil 10-ODS (25 cm × 4.6 mm); flow-rate, 50 ml/h; chart speed, 0.25 in./min; solvent, 2-propanol–hexane (3:97); UV detection at 320 nm, 0.5 a.u.f.s.

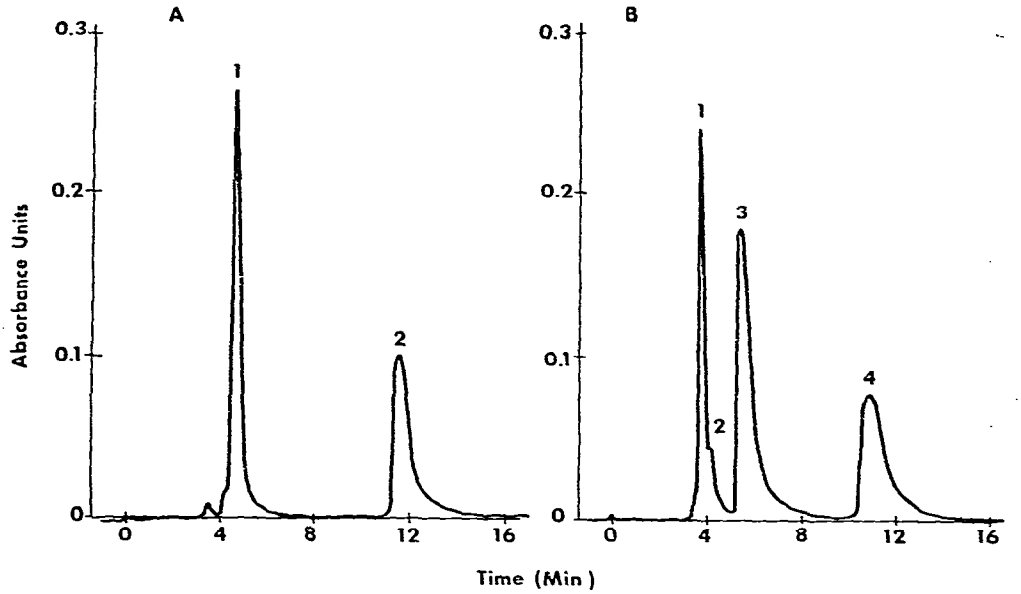

Fig. 4. HPLC of some fluorene derivatives. Peaks: A: 1 = 2-nitro-5-aminofluorene; 2 = 7-nitro-2-aminofluorene. B: 1 = 2-Trifluoroacetamidofluorene; 2 = unidentified; 3 = 2-aminofluorene; 4 = 2-acetamidofluorene. Conditions: column, ODS "heavy load" column (25 cm × 4.6 mm); flow-rate, 50 ml/h; chart speed, 0.25 in./min; solvent, 2-propanol–hexane (1:99); UV detection at 254 nm, 0.5 a.u.f.s.

TABLE III

CAPACITY RATIOS FOR SOME FLUORENE DERIVATIVES ON A NORMAL PARTITION COLUMN

Column: Partisil 10-PAC (25 cm × 4.6 mm). Conditions: flow-rate, 60 ml/h; chart speed, 1 in./min; UV detection at 254 nm, 0.5 a.u.f.s. (see legend Fig. 1); temperature ambient, pressure 400 p.s.i.: —: The compound was strongly adsorbed in this system. Solvent systems: A, 50% hexane in 2-propanol–dichloromethane (30:70); B, 50% hexane in methanol–dichloromethane (10:90); C, methanol–chloroform (3:97); D, 3% methanol–dichloromethane (3:97).

Compound	*k'*			
	A	*B*	*C*	*D*
Fluorene	0.00	0.00	0.00	0.00
2-Nitrofluorene	0.06	0.08	0.04	0.15
2-Heptafluorobutyramidofluorene	0.06	0.17	0.09	0.02
2,7-Dinitrofluorene	0.12	0.17	0.04	0.00
2-Trifluoroacetamidofluorene	0.17	0.42	0.22	0.11
7-Nitro-2-heptafluorobutyramidofluorene	0.15	0.47	0.14	0.10
2-Nitrofluorenone	0.17	0.19	0.09	0.00
7-Nitro-2-trifluoroacetamidofluorene	0.27	0.64	0.28	0.13
2-Nitro-5-aminofluorene	0.52	0.54	0.22	0.11
2-Aminofluorene	0.99	0.63	0.36	0.37
7-Nitro-2-aminofluorene	1.13	0.85	0.35	0.21
2-Acetamidofluorene	1.97	1.50	0.64	0.51
7-Amino-2-trifluoroacetamidofluorene	2.01	2.52	0.86	0.51
7-Nitro-2-acetamidofluorene	2.47	1.91	0.81	0.52
7-Amino-2-acetamidofluorene	4.77	6.88	6.39	2.56
2,7-Diaminofluorene	5.45	3.29	1.14	0.71
2,7-Diacetamidofluorene	—	—	—	—

do occur in the order of elution of some of the compounds. For example, while 2-nitrofluorene elutes near the solvent front ahead of 2-nitrofluorenone in solvent systems A, B, and C, it elutes after the nitroketone in system D. Most of the compounds elute rapidly in systems C and D. Only 7-amino-2-acetamidofluorene, 2,7-diaminofluorene, and 2,7-diacetamidofluorene have k' values greater than one in system C, and in system D only the mono- and diacetyl compounds have k' values greater than one.

Fig. 5 illustrates the resolution of two mixtures on a Partisil 10-PAC column. In Panel A, 2-aminofluorene is shown relative to three of its amide derivatives. The order of elution is that predicted from the k'-values for these compounds given in Table II. Resolution of three of the disubstituted fluorenes is illustrated in Panel B. Since the nitro group is common to all three compounds, the elution order is determined by the polarity of the amide groups.

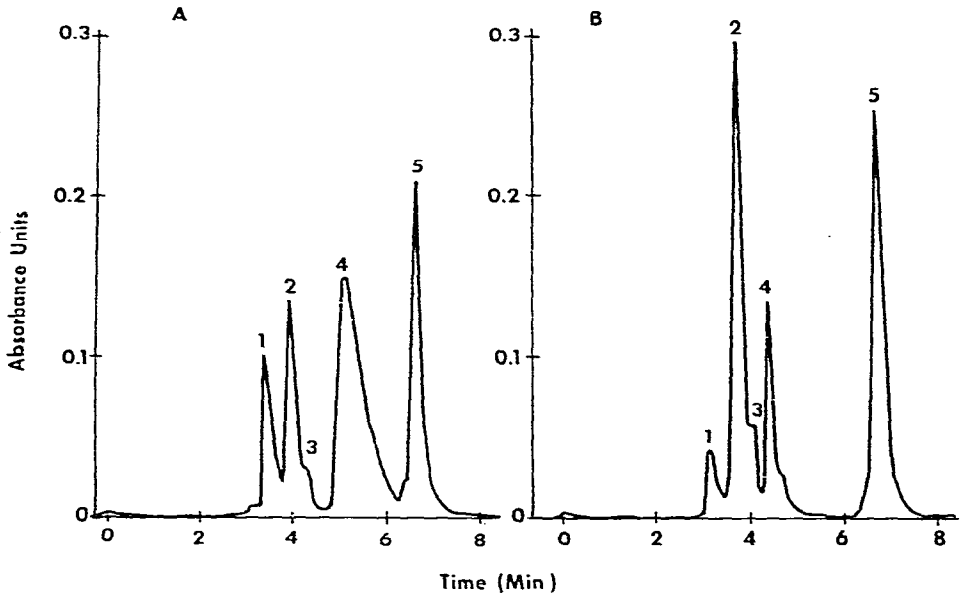

Fig. 5. HPLC of some fluorene derivatives. Peaks: A: 1 = 2-heptafluorobutyramidofluorene; 2 = 2-trifluoroacetamidofluorene; 3 = unidentified; 4 = 2-aminofluorene; 5 = 2-acetamidofluorene. B: 1 = Unidentified; 2 = 7-nitro-2-heptafluorobutyramidofluorene; 3 = unidentified; 4 = 7-nitro-2-trifluoroacetamidofluorene; 5 = 7-nitro-2-acetamidofluorene. Conditions: column, Partisil 10-PAC (25 cm × 4.6 mm); flow-rate, 60 ml/h; chart speed, 0.25 in./min; solvent, 50% hexane in 2-propanol–dichloromethane (30:70); UV detection at 254 nm, 0.5 a.u.f.s.

Reversed-phase partition chromatography

The results of capacity ratio determinations for a series of fluorene derivatives on a Partisil 10-ODS reversed-phase column are presented in Table IV. Generally, compounds elute faster in the acetonitrile–water solvent system than in the methanol–water solvent system. In the following discussion, we have described the behavior of several groups of compounds in our reversed-phase systems.

The monosubstituted nitro compound, 2-nitrofluorene, elutes late in either solvent system indicating the non-polar character of the nitro group. Reduction of

TABLE IV

CAPACITY RATIOS FOR SOME FLUORENE DERIVATIVES ON A REVERSED-PHASE PARTITION COLUMN

Column: Partisil 10-ODS (25 cm × 4.6 mm). Conditions: flow-rate (methanol–water) 70 ml/h and (acetonitrile–water) 60 ml/h; chart speed, 1 in./min; UV detection at 254, 0.5 a.u.f.s. (see legend to Fig. 1); ambient temperature. —: The compound gave inconsistent results or was strongly retained in this solvent system.

Compound	*k'*	
	Methanol–water (75:25)	*Acetonitrile–water (70:30)*
7-Hydroxy-2-acetamidofluorene	0.64	0.13
5-Hydroxy-2-acetamidofluorene	0.71	0.16
2,7-Diacetamidofluorene	1.04	0.19
3-Hydroxy-2-acetamidofluorene	1.06	0.31
7-Amino-2-acetamidofluorene	1.41	0.20
1-Hydroxy-2-acetamidofluorene	1.71	0.46
2-7-Diaminofluorene	1.74	—
7-Amino-2-trifluoroacetamidofluorene	1.90	2.16
2-Nitro-5-aminofluorene	1.90	0.91
2-Acetamidofluorene	2.10	0.67
7-Nitro-2-aminofluorene	2.34	1.28
2-Aminofluorene	2.54	—
2-Trifluoroacetamidofluorene	2.69	1.19
2-Nitrofluorenone	2.70	1.15
7-Nitro-2-acetamidofluorene	2.85	1.04
2,7-Dinitrofluorene	2.90	1.53
7-Nitro-2-trifluoroacetamidofluorene	3.17	1.13
2-Nitrofluorene	3.81	1.62
2-Heptafluorobutyramidofluorene	6.29	2.10
7-Nitro-2-heptafluorobutyramidofluorene	7.71	2.39

the nitro compound 2-aminofluorene results in a decrease in k'; that is, the amine elutes faster than the nitro compound. We have noted, however, that the monoamine, as well as the diamine, 2,7-diaminofluorene, gives inconsistent results in acetonitrile–water and is not reported in this solvent system.

The effect of the acetyl and substituted acetyl groups upon the retention times and k' values of the amine is opposite that observed in adsorption mode (Tables I and II). The more polar acetyl compound elutes prior to the amine while the heptafluorobutyryl and trifluoroacetyl compounds elute later than the amine. A separation of the three monoamides and the diamide 2,7-diacetamidofluorene is shown in Fig. 6.

The four ring hydroxylated compounds 7-, 5-, 3-, and 1-hydroxy-2-acetamidofluorene are important metabolites of the known carcinogen 2-acetamidofluorene[5]. Several groups have reported different liquid chromatographic systems which resolve the four hydroxy metabolites and the parent compound 2-acetamidofluorene[6–8]. Capacity ratios for these compounds in our reversed-phase systems are also given in Table IV. Figs. 7 and 8 illustrate separations of these compounds in two isocratic systems and in one gradient system. In the solvent systems which we have examined, as in those examined by the earlier investigators, resolution of the 7- and 5-hydroxy metabolites is difficult.

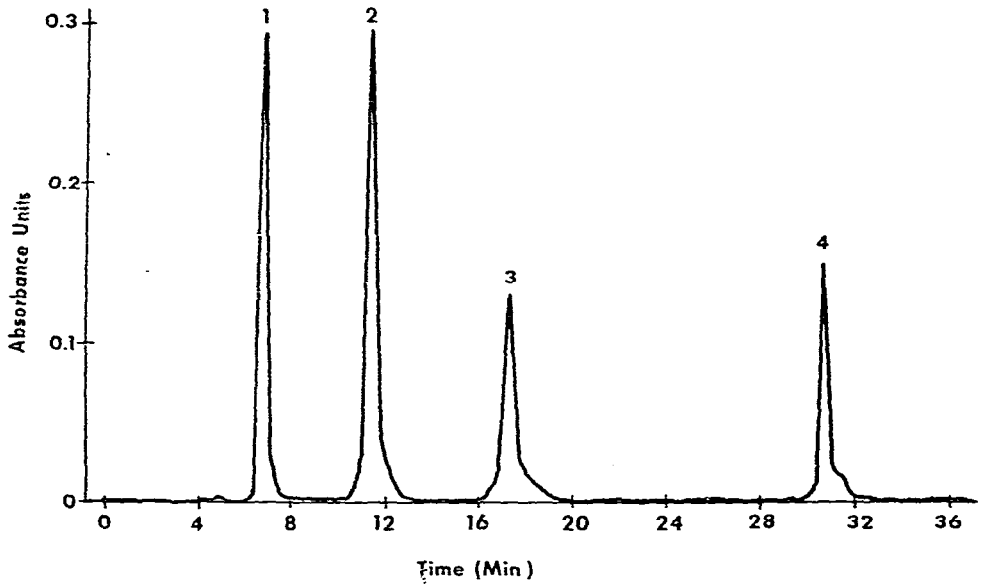

Fig. 6. HPLC of some fluorene derivatives. Peaks: 1 = 2,7-diacetamidofluorene; 2 = 2-acetamidofluorene; 3 = 2-trifluoroacetamidofluorene; 4 = 2-heptafluorobutyramidofluorene. Conditions: column, Partisil 10-ODS (25 cm × 4.6 mm); flow-rate, 60 ml/h; chart speed, 0.25 in./min; solvents, (A) water, (B) acetonitrile, initial 60% B with 2% B/min gradient started at 20 min; UV detection at 280 nm, 0.5 a.u.f.s.

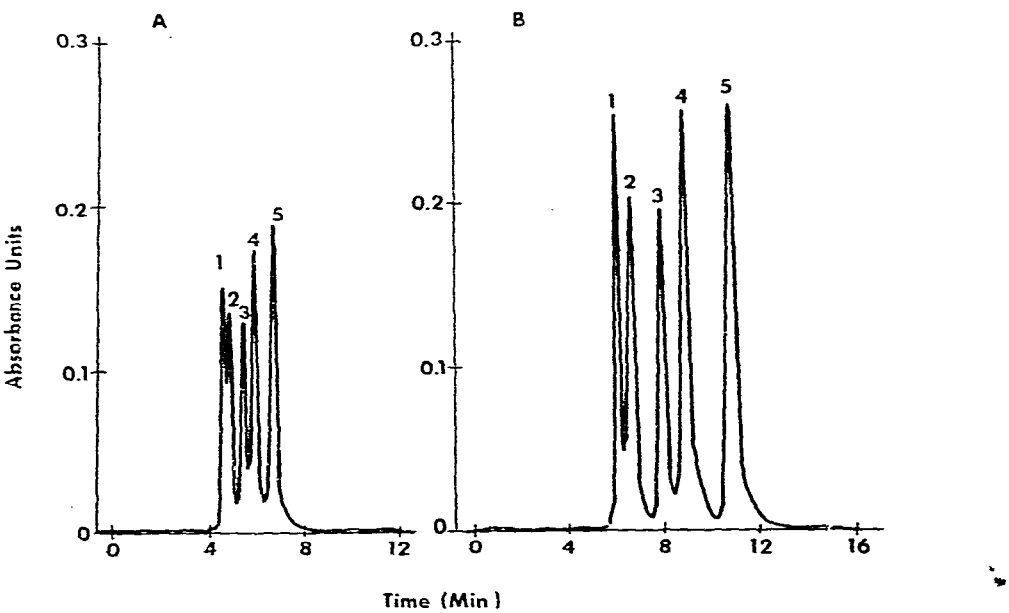

Fig. 7. HPLC of some fluorene derivatives. Peaks: 1 = 7-hydroxy-2-acetamidofluorene; 2 = 5-hydroxy-2-acetamidofluorene; 3 = 3-hydroxy-2-acetamidofluorene; 4 = 1-hydroxy-2-acetamidofluorene; 5 = 2-acetamidofluorene. Conditions: A: column, Partisil 10-ODS (25 cm × 4.6 mm) flow-rate, 60 ml/h; chart speed, 0.25 in./min; solvent, acetonitrile–water (50:50), isocratic; UV detection at 254 nm, 0.5 a.u.f.s. B: conditions as in A but with acetonitrile–water (40:60) as solvent

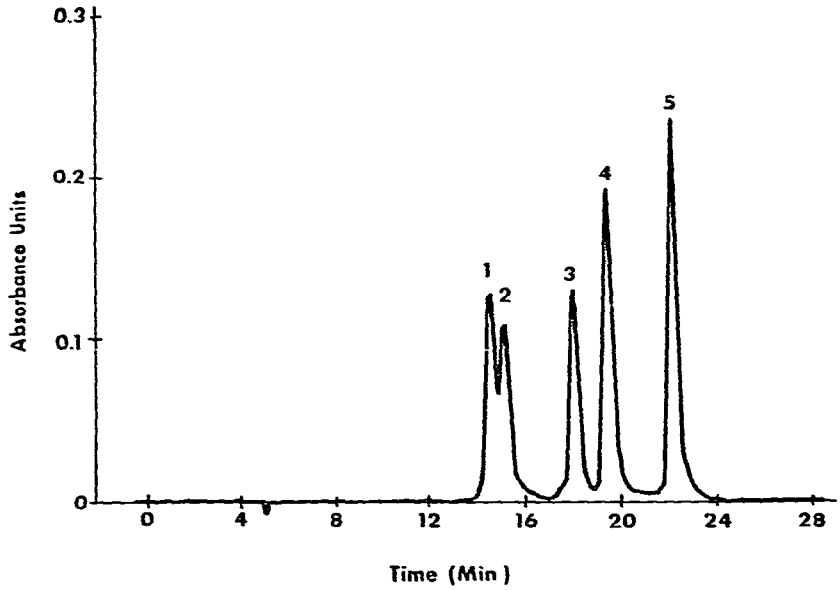

Fig. 8. HPLC of some fluorene derivatives. Peaks: 1 = 7-hydroxy-2-acetamidofluorene; 2 = 5-hydroxy-2-acetamidofluorene; 3 = 3-hydroxy-2-acetamidofluorene; 4 = 1-hydroxy-2-acetamidofluorene; 5 = 2-acetamidofluorene. Conditions: column, Partisil 10-ODS (25 cm × 4.6 mm); flow-rate, 60 ml/h; chart speed, 0.25 in./min; solvents, (A) water, (B) methanol, initial 40% B with 2% B/min gradient; UV detection at 254 nm, 0.5 a.u.f.s.

Table V contains capacity ratios for three fluorene carboxylic acids which were eluted from this column. The acids were intermediates in the synthesis of 5-hydroxy-2-acetamidofluorene[9]. As seen in the elution profile in Fig. 9, fluorenone 4-carboxylic acid elutes ahead of fluorene 4-carboxylic acid. Nitration of the latter compound gives 2-nitrofluorene 5-carboxylic acid which elutes later than either of the other two acid derivatives.

The capacity ratios for the derivatives which were eluted from the "heavy phase load" ODS column are given in Table VI. Methanol–water results in longer retention times on this column than does acetonitrile–water. One significant feature of the data in Table VI is the marked difference in retention times for 2-acetamido-

TABLE V

CAPACITY RATIOS FOR SOME FLUORENE CARBOXYLIC ACIDS ON A REVERSED-PHASE PARTITION COLUMN

Column: Partisil 10-ODS. Conditions: flow rate, 60 ml/h; chart speed, 1 in./min; UV detection at 254 nm, 0.5 a.u.f.s. (see legend to Fig. 1); ambient temperature.

Compound	*k'*	
	*Methanol–water (70:30)**	*Acetonitrile–water (60:40)**
Fluorenone 4-carboxylic acid	1.17	0.56
Fluorene 4-carboxylic acid	1.87	1.03
2-Nitrofluorene 5-carboxylic acid	2.33	1.17

* 0.1% Acetic acid added to the water.

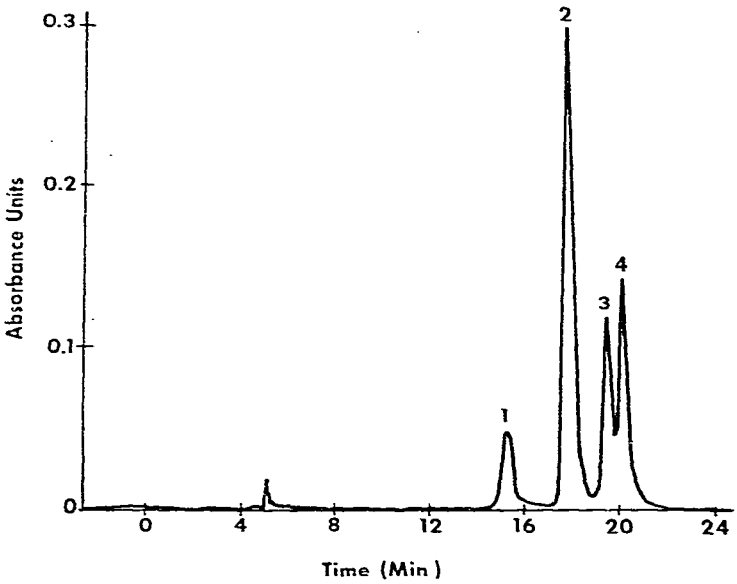

Fig. 9. HPLC of some fluorene derivatives. Peaks: 1 = fluorenone 4-carboxylic acid; 2 = fluorene 4-carboxylic acid; 3 = 2-nitrofluorene-5-carboxylic acid; 4 = 2-nitro-5-aminofluorene. Conditions: column, Partisil 10-ODS (25 cm × 4.6 mm); flow-rate, 60 ml/h; chart speed, 0.25 in./min; Solvents, (A) water, (B) methanol, 0.1% acetic acid; initial 60% B with 2% B/min gradient started at 5 min; UV detection at 300 nm, 0.5 a.u.f.s.

TABLE VI

CAPACITY RATIOS FOR SOME FLUORENE DERIVATIVES ON A "HEAVY PHASE LOAD" REVERSED PHASE COLUMN

Column: Universal Scientific ODS, "heavy phase load". Conditions: flow-rate (acetonitrile–water) 60 ml/h, (methanol–water) 70 ml/h; chart speed 1 in./min; UV absorbance at 254 nm, 0.5 a.u.f.s. (see legend to Fig. 1); ambient temperature; pressure (acetonitrile–water) 700 and (methanol–water) 1200 p.s.i. Reference compound was acetone. – : The compound was strongly retained in this system.

Compound	*k'*	
	Acetonitrile–water (70:30)	*Methanol–water (75:25)*
2-7-Diacetamidofluorene	0.00	0.10
7-Hydroxy-2-acetamidofluorene	0.03	0.00
5-Hydroxy-2-acetamidofluorene	0.09	0.09
7-Amino-2-acetamidofluorene	0.20	0.02
7-Amino-2-trifluoroacetamidofluorene	0.26	0.35
3-Hydroxy-2-acetamidofluorene	0.28	0.56
1-Hydroxy-2-acetamidofluorene	0.52	1.47
2,7-Diaminofluorene	0.62	0.02
2-Acetamidofluorene	0.71	1.98
2-Nitro-5-aminofluorene	1.14	1.53
7-Nitro-2-acetamidofluorene	1.16	4.45
2-Aminofluorene	1.20	1.76
7-Nitro-2-aminofluorene	1.44	3.26
2-Nitrofluorenone	1.56	3.26
2-Trifluoroacetamidofluorene	2.38	–
7-Nitro-2-trifluoroacetamidofluorene	2.63	–
2,7-Dinitrofluorene	2.80	6.45
2-Nitrofluorene	3.93	–
2-Heptafluorobutyramidofluorene	7.95	–
7-Nitro-2-heptafluorobutyramidofluorene	8.85	–

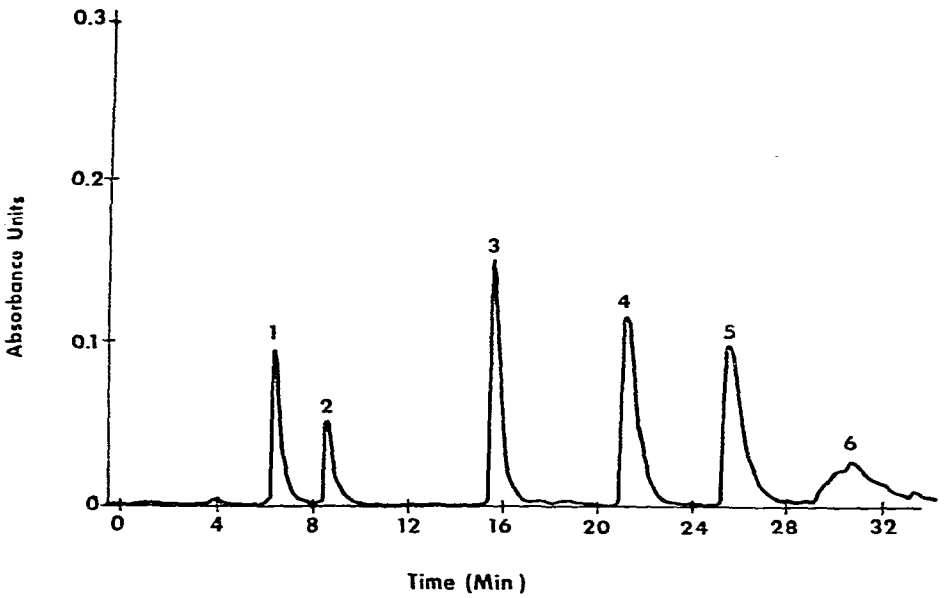

Fig. 10. HPLC of some fluorene derivatives. Peaks: 1 = 7-hydroxy-2-acetamidofluorene; 2 = 5-hydroxy-2-acetamidofluorene; 3 = 3-hydroxy-2-acetamidofluorene; 4 = 1-hydroxy-2-acetamidofluorene; 5 = 2-acetamidofluorene; 6 = unidentified peak. Conditions: column, ODS silica "heavy phase load" column (25 cm × 4.6 mm); flow-rate, 70 ml/h; chart speed, 0.25 in./min; solvents, (A) water, (B) methanol, initial 60% B with 1% B/min gradient started at 2 min; UV detection at 254 nm, 0.5 a.u.f.s.

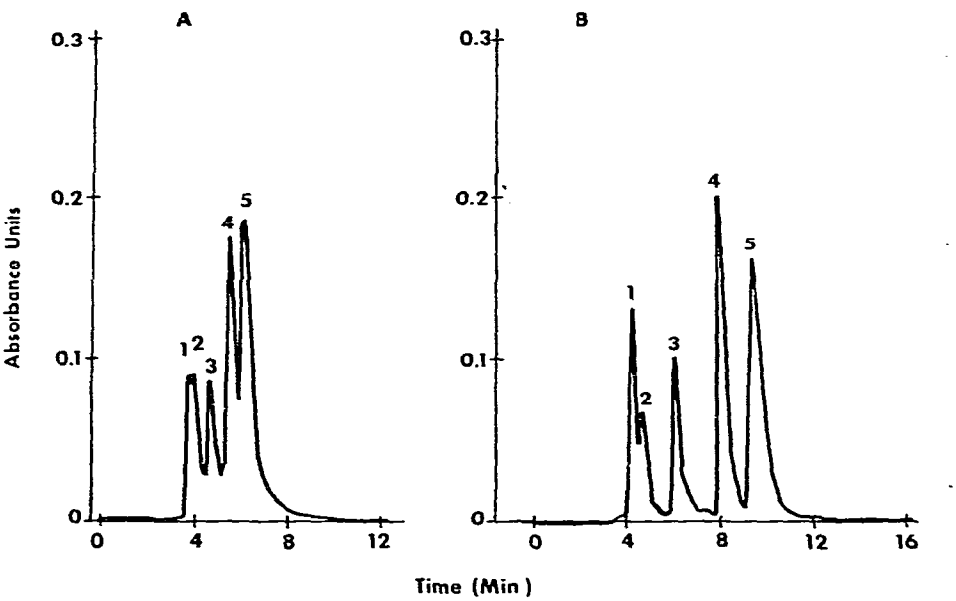

Fig. 11. HPLC of some fluorene derivatives. Peaks: 1 = 7-hydroxy-2-acetamidofluorene; 2 = 5-hydroxy-2-acetamidofluorene; 3 = 3-hydroxy-2-acetamidofluorene; 4 = 1-hydroxy-2-acetamidofluorene; 5 = 2-acetamidofluorene. Conditions: A: column, ODS-silica "heavy phase load" column (25 cm × 4.6 mm); flow-rate, 60 ml/h; chart speed, 0.25 in./min; solvent, acetonitrile–water (50:50); UV detection at 254 nm, 0.5 a.u.f.s. B: Conditions as in A but with acetonitrile–water (40:60) as solvent.

fluorene and its four hydroxylated derivatives in the methanol–water system. The observed capacity ratios suggest that these derivatives should be well resolved. Fig. 10 illustrates the resolution of a synthetic mixture of the four hydroxy metabolites and 2-acetamidofluorene under the gradient conditions indicated in the figure legend. The 5- and 7-hydroxy metabolites, only partially resolved on this column in acetonitrile–water (Fig. 11), are well resolved in the methanol–water system. Resolution is also markedly better than that observed with the Partisil 10-ODS column (See Figs. 7 and 8).

Normal partition columns may be used in the reversed-phase mode when water with either methanol or acetonitrile is employed as the mobile phase. Capacity ratios for a series of the fluorene derivatives examined on a "cyano" column under these conditions are tabulated in Table VII. The data are reasonably close to what might be predicted for a reversed-phase elution pattern. However, we have not found this column to be efficient in separating many groups of the fluorene derivatives in the two solvent systems which we examined. The hydroxylated metabolites, for example, are not well resolved in either solvent system (not shown).

TABLE VII

CAPACITY RATIOS FOR SOME FLUORENE DERIVATIVES ON A "CYANO-TYPE" COLUMN UNDER REVERSED-PHASE CONDITIONS

Column: μBondapak CN (30 cm × 3.9 mm I.D.); acetone was the reference compound. Conditions for acetonitrile–water system: flow-rate, 60 ml/h; chart speed, 1 in./min; UV absorbance at 254 nm, 0.5 a.u.f.s. (see legend to Fig. 1); ambient temperature, pressure 700 p.s.i. Conditions for methanol–water system: flow-rate 50 ml/h; chart speed 1 in./min; UV absorbance at 254 nm, 0.5 a.u.f.s.; ambient temperature; pressure 1400 p.s.i. —: The compound was retained or did not give a consistent elution pattern in this solvent.

Compound	*k'*	
	Acetonitrile–water (70:30)	*Methanol–water (80:20)*
2,7-Diacetamidofluorene	0.06	0.08
7-Hydroxy-2-acetamidofluorene	0.16	0.11
5-Hydroxy-2-acetamidofluorene	0.41	0.11
3-Hydroxy-2-acetamidofluorene	0.41	0.17
2-Acetamidofluorene	0.46	0.22
1-Hydroxy-2-acetamidofluorene	0.48	0.24
7-Nitro-2-acetamidofluorene	0.60	0.36
7-Amino-2-acetamidofluorene	1.02	0.36
2-Nitro-5-aminofluorene	1.02	0.50
7-Nitro-2-aminofluorene	1.03	0.46
2-Nitrofluorenone	1.05	1.10
2-Trifluoroacetamidofluorene	1.39	0.37
7-Amino-2-trifluoroacetamidofluorene	1.42	0.44
2-Nitrofluorene	1.39	0.50
7-Nitro-2-trifluoroacetamidofluorene	1.49	0.56
2,7-Dinitrofluorene	1.53	0.82
2-Aminofluorene	2.21	0.59
2-Heptafluorobutyramidofluorene	2.29	0.45
7-Nitro-2-heptafluorobutyramidofluorene	2.47	0.74
2,7-Diaminofluorene	—	1.68

CONCLUSION

The behavior of some fluorene derivatives on several types of chromatographic columns has been described. Solvents or solvent combinations for effecting separations of groups of these compounds are given. We do not suggest that the conditions, solvents and columns used in this study are the only ones which will carry out the observed separations. W have intended that they serve as a guide to other chromatographers in choosing chromatographic systems of their own.

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