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Note

Phthalimidopropylsilane —A new chemically bonded stationary phase for the determination of polynuclear aromatic hydrocarbons by high-pressure liquid chromatography

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In recent years the technique of high-pressure liquid chromatography (HPLC) has been increasingly used for the separation of polynuclear aromatic hydrocarbons (PAH). Although adsorbents such as alumina[1] and silica gel[2,3] have given promising separations of some PAH, there is now a tendency for workers in this field to use chemically bonded phases, particularly reverse phases such as octadecylsilane (ODS)[4–6]. Although a wide range of siloxane bonded phases has been studied already[7], this paper describes the preparation and performance of a new packing material, 3-phthalimidopropyltrichlorosilane chemically bonded to silica gel, which was developed during our studies on the separation and analysis of PAH compounds in foodstuffs.

EXPERIMENTAL

Apparatus

A Waters Assoc. Model 6000 pumping system was used for the packing of columns, and as the solvent delivery system, whilst the detector used was the Aminco Fluoromonitor filter fluorescence instrument, fitted with an 18-μl flow cell.

Materials

Microparticulate silica gel, Partisil 5 (Whatman, Maidstone, Great Britain) and 3-phthalimidopropyltrichlorosilane and methyltrichlorosilane (Silar Laboratories, Richmond, Great Britain) were used.

Preparation of the stationary phase (PPS)

Silica gel was refluxed with 2 *N* hydrochloric acid for 2 h, washed firstly with water until neutral, then twice with methanol. Finally, the silica gel was dried overnight at 100°. The treated dry silica gel (7 g), contained in a dry flask, was refluxed with 25 ml dry xylene and 10 ml 3-phthalimidopropyltrichlorosilane for 2 h. At the end of this period, 2 ml of methyltrichlorosilane were added and the refluxing continued for a further 30 min to ensure that as few active sites as possible remained. After cooling, the phthalimidopropylsilane (PPS) was transferred to a large centrifuge

bottle with xylene, washed three times with xylene to remove unreacted silanes, then washed three times with methanol. On the final wash, about 90% of the PPS was allowed to settle, and the cloudy supernatant layer of methanol removed. This ensured the removal of any "fines" produced during the silanising process. The material was then dried overnight at 100°.

Column packing procedure

The column used was 25 cm × 4.6 mm I.D. stainless-steel tubing. The end fitting and the injection assembly have been described elsewhere[8]. Packing was carried out using a modified balanced density technique[9] with a thick slurry in 10 ml of tetrachloroethane–tetrabromoethane (40:60). The slurry was pumped into the column using methanol from two Waters Assoc. 6000 pumps combined, to give an initial maximum flow-rate of 20 ml/min. During the whole of the packing period of 30 min, the pressure was maintained at 5000 p.s.i.

RESULTS AND DISCUSSION

Table I shows the elution of a number of PAH compounds relative to benzo[*a*]pyrene on the 25-cm PPS column, and on a 20-cm ODS–Partisil 5 column. The solvent system used with both columns was methanol–water (90:10) at 20°. With a flow-rate of 1.5 ml/min, benzo[*a*]pyrene elutes in 11.5 min from the PPS column and in 8.8 min from the shorter ODS column. Under the conditions described, the HETP for benzo[*a*]pyrene on the PPS column was 0.09 mm with a capacity factor,

TABLE I

ELUTION OF SOME PAH COMPOUNDS RELATIVE TO BENZO[*a*]PYRENE

Conditions: mobile phase, methanol–water (90:10) at a flow-rate of 1.5 ml/min. Retention times of benzo[*a*]pyrene were 8.8 and 11.5 min on ODS and PPS, respectively.

Compound	*Rel. retention time*	
	ODS	*PPS*
Fluoranthene	0.40	0.48
Benz[*a*]anthracene	0.57	0.55
9,10-Dimethylanthracene	0.59	0.61
Benzo[*ghi*]fluoranthene	0.61	0.75
12-Methylbenz[*a*]anthracene	0.76	0.64
7-Methylbenz[*a*]anthracene	0.79	0.73
Benzo[*cd*]fluoranthene	0.87	0.82
Benzo[*e*]pyrene	0.88	1.07
Perylene	0.92	1.20
Benzo[*k*]fluoranthene	0.93	0.82
Benzo[*a*]pyrene	1.00	1.00
Dibenz[*ah*]anthracene	1.20	0.93
Indeno[1,2,3-*cd*]pyrene	1.47	1.45
Benzo[*ghi*]perylene	1.50	1.86
2,3-Dihydro-3-methyl-1H-benzo[*a*]cyclopent[*h*]anthracene (3-methylcholanthrene)	1.62	1.07
Dibenzo[*def,mno*]chrysene (anthanthrene)	1.80	1.86

k', of 4.1, which is very similar to the figures obtained for the ODS column with a HETP of 0.08 mm and a k' of 3.9.

Fig. 1 shows the separation of the same four PAH compounds on both columns, whilst Fig. 2 shows the traces obtained with an extract of mussels which had been subjected to a preliminary clean-up and modified separation stage[10] before injection.

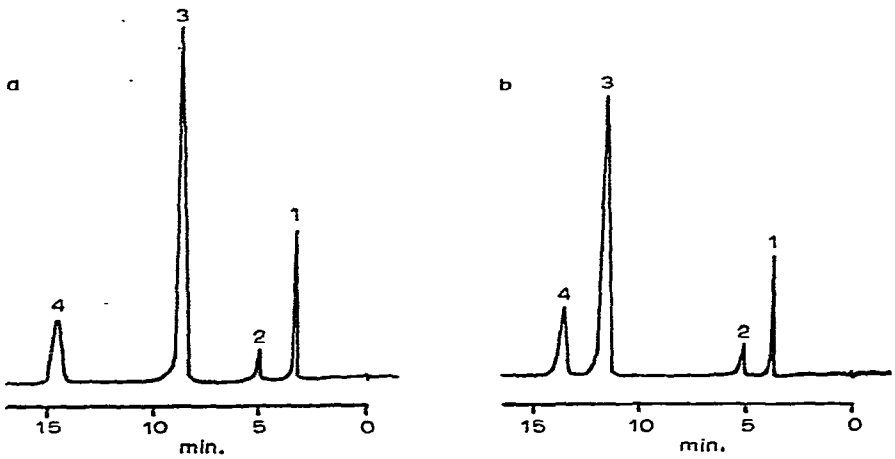

Fig. 1. Separation of PAH standards on (a) ODS and (b) PPS columns. 1 = Fluoranthene 9.6 ng, 2 = benz[*a*]anthracene 5.6 ng, 3 = benzo[*a*]pyrene 10.0 ng, 4 = 3-methylcholanthrene 11.3 ng. Conditions: mobile phase, 90% methanol at a flow-rate of 1.5 ml/min; detection, fluorescence; temperature, 20°.

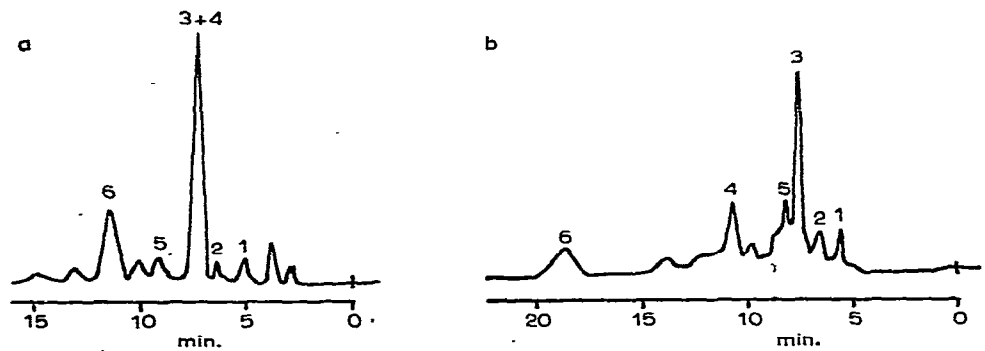

Fig. 2. Extract of mussels chromatographed on (a) ODS and (b) PPS columns. 1 = Benz[*a*]anthracene, 2 = 12-methylbenz[*a*]anthracene, 3 = benzo[*k*]fluoranthene, 4 = perylene, 5 = dibenz[*ah*]anthracene, 6 = benzo[*ghi*]perylene. Conditions as for Fig. 1.

Some useful separations have been achieved using the PPS column. Of particular note are the two PAH compounds benzo[*k*]fluoranthene and perylene which we have observed to be present in some mussels (Fig. 2). Although not separated by ODS[5], they are readily separated by the PPS column. Relative retentions differ on the two columns, thus whilst some PAH (3-methylcholanthrene and dibenz[*ah*]anthracene) are eluted earlier using the PPS column packing, others are eluted later (perylene and

benzo[*ghi*]perylene). Benzo[*a*]pyrene and benzo[*e*]pyrene are eluted in reverse order on the PPS column as compared with the ODS column. Examination of Table I will reveal other PAH separations which have been improved by using the PPS column. The new packing material has been used routinely in this laboratory alongside the ODS material for the estimation of PAH in seafoods, and has shown no sign of deterioration over a period of three months.

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