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SELECTIVE DETERMINATION OF BENZO[*a*]PYRENE IN PETROLEUM-BASED PRODUCTS USING MULTI-COLUMN LIQUID CHROMATOGRAPHY

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SUMMARY

A method is presented to enable the rapid on-line determination of benzo[*a*]-pyrene in petroleum-based products such as diesel oil and aviation fuel. A column-switching technique is employed to achieve the necessary resolution due to the complex sample matrix. Fluorescence detection is employed to further increase the selectivity towards the polynuclear aromatic hydrocarbons. A cyclodextrin bonded phase column was used to give the initial fractionation. The fraction of interest was then heart cut onto a reversed-phase C_{18} analytical column and separated using gradient elution. This technique removes, with the exception of dilution and filtration, any necessary pre-analytical step.

INTRODUCTION

Polynuclear aromatic hydrocarbons (PAHs) are common environmental pollutants derived from both natural and man-made combustion of a variety of carbon based materials. The carcinogenic nature of some of the group members, notably benzo[*a*]pyrene, has been well documented[1,2] as have both selective and general analytical techniques[2,3]. The determination of these compounds in petroleum products, and especially diesel oils, causes significant analytical problems due to the complex nature of the matrix. Diesel oil is a complex mixture of hydrocarbons with a boiling range of between 180 and 360°C. Between 15 and 30% has an aromatic nature, while the remainder is essentially aliphatic[4].

Multi-column techniques have been used to overcome the non-availability of highly efficient columns capable of resolving petroleum products. However, to achieve a final fraction of suitable complexity, incompatible techniques such as normal- and reversed-phase or size-exclusion and reversed-phase chromatography need to be used. The solvent-exchange procedures that are therefore necessary are complex and result in long analysis times and analyte loss or degradation. Sonnefeld *et al.*[5] have reported an on-line normal-/reversed-phase technique using a gas-dried intermediary diamine exchange column. May and Wise[6] have proposed a similar technique,

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although the solvent exchange was achieved using off-line solvent evaporation. The unique selectivity of the cyclodextrin bonded phases has been used to resolve the solvent incompatibility problem. The chromatographic technique involves fractionating an oil sample on a γ-cyclodextrin column. The fraction obtained from this column that contains benzo[*a*]pyrene is then passed directly to the head of a C_{18} column rather than to the detector. The fraction can then be eluted from this column and the analyte determined at a later time.

The cyclodextrins are torus-shaped polymers consisting of α-1,4 D-glucoside oligosaccharides. The external surface is ringed by hydroxyl groups giving a hydrophilic environment. The internal environment is hydrophobic and responsible for the selective nature of the column by the formation of inclusion complexes with molecules of the correct size and charge distribution[7]. Three cyclodextrins have been successfully bound to a 5-μm spherical silica base. These have been named α-, β- and γ-cyclodextrins and consist of six, seven and eight glucose monomers, respectively. The internal diameters are 0.57, 0.78 and 0.95 nm, respectively, and in all three cases the torus is about 0.78 nm deep[8]. The size of benzo[*a*]pyrene, across the wide axis, is approximately 0.88 nm suggesting that only the β- and γ-cyclodextrins will be able to form inclusion complexes. This has been confirmed[8] and benzo[*a*]pyrene has been shown to form a 2:1 analyte–cavity complex. In a recent study by Olsson *et al.*[9] the retention of a range of PAHs by monomeric and polymeric C_{18} bonded phases and by the β-cyclodextrin bonded phase were compared. While the resolving power of the cyclodextrin phase was not found to be as high as that of conventional reversed-phases, the authors did suggest that the cyclodextrin-bonded phases may offer significant advantages where the separation of PAHs of different molecular weights is required. In this study only the γ-cyclodextrin bonded phase was used.

The cyclodextrins are a relatively new group of bonded phases and have been used successfully for the separation of a number of chiral compounds such as benzo[*a*]- and benzo[*e*]pyrene, dansyl D- and L-leucine[7], and *ortho; para-* and *meta*-nitroanilines[7]. β- and γ-bonded phases have been used by Armstrong *et al.*[11] to resolve mixtures of PAHs. These separations have been achieved due to the ability of the phase to form an inclusion complex. The stability of the complex depends on the charge distribution, and the shape and size of the molecule forming the complex. There must be a hydrophobic region within the molecule that is the correct size to enter the cavity; the complex stability may be enhanced by the presence of external polar groups that are able to interact with the secondary hydroxyls. If the analyte is too large it will be unable to enter the cavity and no inclusion-derived retention will occur, although retention may still be observed due to an ionic interaction mechanism. In this mode the column acts as a high-density diol column. If the analyte is too small it will enter the cavity, but the stability of the complex is poor and the retention will be low. It is also possible to use non-polar solvents such as hexane with these phases; in this case no inclusion occurs and retention is due purely to external adsorption.

EXPERIMENTAL

Materials

Benzo[*a*]pyrene was obtained from BDH (Poole, U.K.). Chromatographic solvents were also obtained from BDH and were of HiPerSolv grade. The water used in

this study was distilled and stored in glass without further purification. The γ-cyclodextrin column (250 × 4.6 mm I.D.) was packed by Astec (U.S.A.) and supplied through Technicol (Stockport, U.K.). The Vydac C_{18} 201TP54 (250 × 4.6 mm I.D.) column was also obtained from Technicol. Standard benzo[*a*]pyrene solutions were made up in acetonitrile and stored in the dark to avoid photo-induced degradation. The oil samples were obtained from commercial sources.

Equipment

Chromatography was carried out using the following equipment. A Waters Series 6000 high-performance liquid chromatography (HPLC) pump was used, the mobile phase being generated by a modified Micrometeritic gradient former. All solvents were filtered through a 2-μm Millipore filter under negative pressure and continuously degassed with helium. A Rheodyne Model 7125 syringe loading injection valve with a 20-μl sample loop was used to introduce the sample. A Perkin-Elmer series 3000 fluorescence detector was used to monitor the eluent.

The excitation and emission wavelengths were 254 nm and 420 nm, respectively. Excitation and emission slits were set at 5 nm. A Hewlett-Packard 3390A integrator was used to determine the retention times and a Goerz BBC SE 120 chart recorder was used to record the chromatograms. A schematic diagram of the column arrangement is given in Fig. 1. Column selection was achieved using two Rheodyne Model 7000 column-selection valves. These valves were actuated manually.

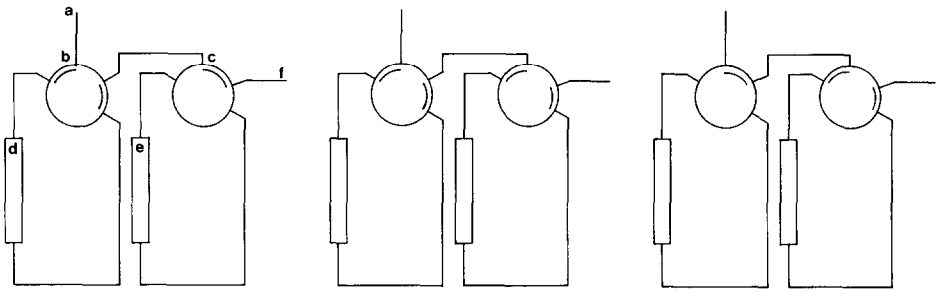

Fig. 1. Schematic of the column arrangement used in this study. Valve positions correspond to the following operations: (left): fractionation; (middle): heart cut; (right): analysis. a = Inlet from pump and sample introduction valve; b = switching valve 1, column 1 bypass; c = switching valve 2, heart cut and column 2 bypass; d = column 1, γ-cyclodextrin fractionation column; e = column 2, Vydac C_{18} analytical column; f = outlet to detector and to waste.

Chromatographic technique

Cyclodextrin fractionation column. The oil sample was fractionated, after filtration, using a cyclodextrin pre-column with an acetonitrile – water (40:60) isocratic mobile phase. The timing for the heart cut was determined by the use of a benzo[*a*]pyrene standard. The retention time was determined to be just over 4 min and did not alter significantly during this study. After the heart cut had been taken the column was cleaned using 100% acetonitrile until the baseline was re-established, as viewed on the fluorescence detector. When not in use the column was cleaned with 100% methanol and stored with this solvent.

Heart cut analysis. The heart cut was analysed on the 25-cm Vydac C_{18} column using a 40-min linear gradient from 30 to 100% aqueous acetonitrile at 1 ml min^{-1}. The valve timings, for a sample of low to medium complexity (*e.g.*, aviation fuel) are outlined in Table I.

The analytical column was equilibrated at an acetonitrile concentration 10% below that of the fractionation column, to ensure that the heart cut remained as a small plug on the head of the analytical column in order to minimize peak dispersion.

TABLE I

OUTLINE OF THE SWITCHING VALVE ACTUATION TIMINGS USED FOR AN AVIATION FUEL SAMPLE

Time (min:s)	*Action*
0:00	Column 1 in line Inject sample, start data collection
3:45	Valve 2 to position B Column 1 and 2 in line
4:30	Valve 2 to position A Column 1 in line Clean and re-equilibrate column
30:00	Valve 1 to position B Valve 2 to position B Column 2 in line Start gradient
80:00	Reset gradient
90:00	Valve 2 position A Valve 1 position A

RESULTS AND DISCUSSION

The γ-cyclodextrin column produced a suitable fraction to allow the rapid on-line determination of benzo[*a*]pyrene in petroleum samples covering a wide range of complexities. The detection limit for this technique, determined as three times the detector noise, was 0.1 ng benzo[*a*]pyrene per injection; this is equivalent to 5 ng ml^{-1}. The recovery for a 40-ng ml^{-1} benzo[*a*]pyrene standard was 98.9% using a 45-s heart cut centered on the analyte retention time. The analysis time for a light oil such as aviation fuel, including re-equilibration times was under 90 min; for a more complex sample the analysis time increases to a maximum of 120 min. If a dual pump system were used the analysis time can be reduced to under 60 min for all types of sample. This reduction may be achieved by using parallel re-equilibration and analysis. If multiple heart cuts are made, and a small, high-carbon-loaded column is used as an intermediary exchange column, a number of different PAHs may be analysed.

Fig. 2 shows a chromatogram obtained from the cyclodextrin fractionation column. The heart cut (labelled $*$) can clearly be seen in this chromatogram as a

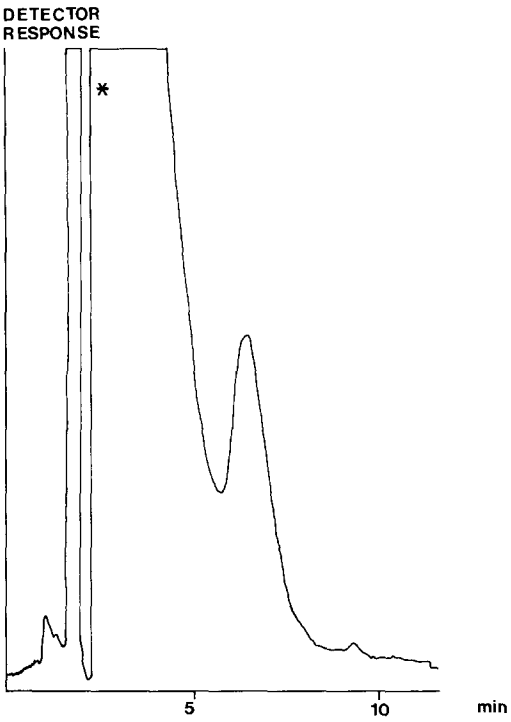

Fig. 2. Chromatogram showing a 45-s heart cut (marked *) taken from a sample of aviation fuel fractioned on a γ-cyclodextrin column. The mobile phase was acetonitrile – water (40:60), flow-rate 1 ml min^{-1}.

vacancy in the detector signal output, the rapid drop in fluorescence is due to solvent eluting from the bottom of the C_{18} column. The length of the heart cut influences the sensitivity of the analysis. A reduced heart-cut time will decrease the amount of benzo[*a*]pyrene that can be transferred to the second column but also decreases the amount of interfering substances present in the fraction. The timing and length of the heart cut was not optimized in this study, however, it would be expected that optimization of this parameter would result in a reduced minimum level of detection. This reduction could also be achieved using further fractionation columns especially when highly complex samples are to be analysed. The time of the heart cut is limited by the necessity to introduce the sample as a small plug on the head of the second column. Failure to do this leads to poor sample resolution when the final analysis is carried out. The mobile phase used to elute the benzo[*a*]pyrene fraction from the cyclodextrin column was acetonitrile–water (40:60). At this acetonitrile concentration, the organic modifier enters the cyclodextrin cavity in preference to the analyte. This has the effect of reducing inclusion formation for benzo[*a*]pyrene to a minimum and allowing it to elute in less than 4.5 min. The vast majority of interfering components are more strongly retained on the column by a mechanism, that is as yet, not fully understood. The use of lower acetonitrile concentrations would cause strong retention of benzo[*a*]pyrene and probably produce a fraction of similar complexity. However, this

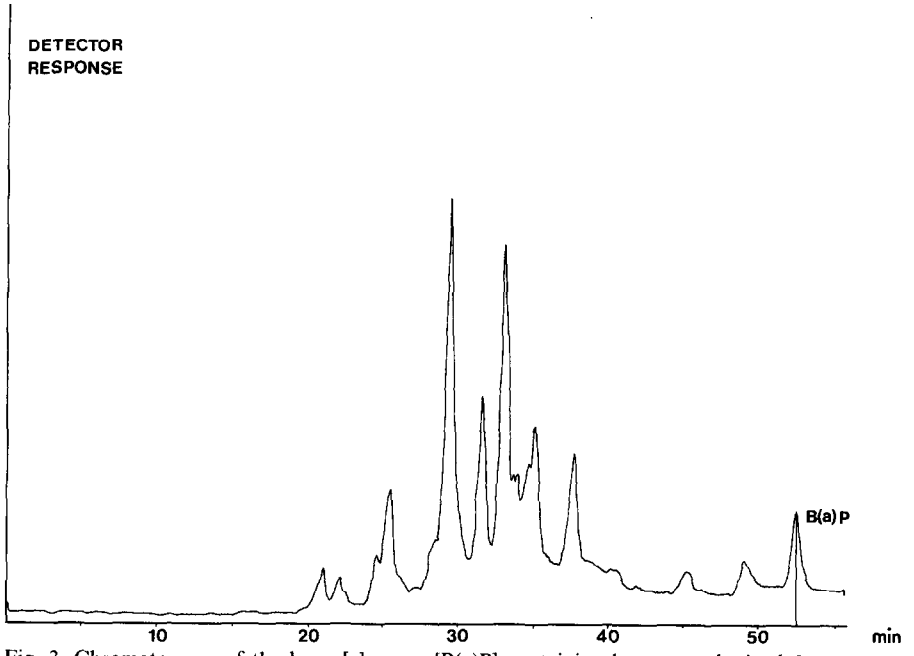

Fig. 3. Chromatogram of the benzo[*a*]pyrene [B(a)P] containing heart cut obtained from a sample of aviation fuel. The sample was spiked with 8 ng of benzo[*a*]pyrene, the analyte is labelled. The chromatographic conditions are outlined in the Experimental section.

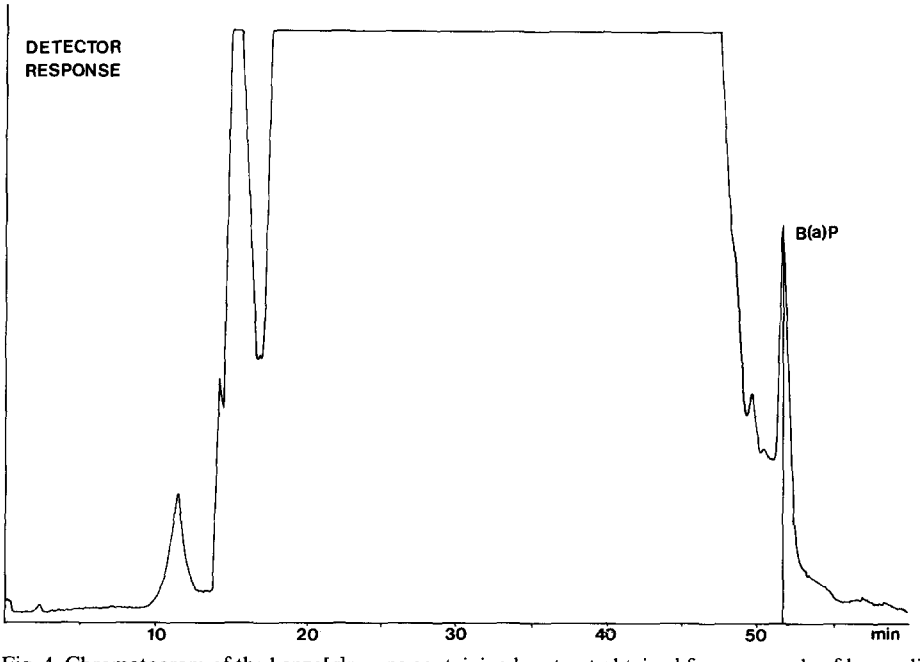

Fig. 4. Chromatogram of the benzo[*a*]pyrene containing heart-cut obtained from a sample of heavy diesel oil. The benzo[*a*]pyrene is labelled. The chromatographic conditions are outlined in the Experimental section.

would lengthen the analysis time and as the efficiency of these columns is relatively low, the heart-cut time would have to be increased significantly to maintain the high recovery. The long heart cut time, in addition to the lower acetonitrile concentrations, would mean that the sample would not be introduced as a small plug on the head of the second column and so the final resolution would be decreased.

Fig. 3 shows the final analytical chromatogram of an aviation fuel spiked with benzo[*a*]pyrene, so that the final injection contained 8 ng of the analyte. The analyte peak is baseline resolved from all interfering components suggesting that there has been a high degree of fractionation in the cyclodextrin column. This high degree of resolution allows very low levels of benzo[*a*]pyrene to be assayed.

Fig. 4 shows the final chromatogram of a heavy diesel oil. Although the benzo[*a*]pyrene has not been fully resolved, due to the far higher sample complexity, it is clearly visible and quantitation may be carried out precisely. The resolution of this sample may be improved by the incorporation of a further fractionation stage to reduce the fraction complexity without adversely affecting the recovery of benzo[*a*]-pyrene.

The method described in this paper can be used to give both quantitative and qualitative information about the amount of benzo[*a*]pyrene in various petroleum products. Significant advantages over off-line and other on-line techniques are achieved. In particular the system increases operator safety due to the reduced environment contamination and sample handling, and sample loss due to the reduced possible adsorption or volatization of the sample. The removal of any pre-analysis step, such as solid phase or solvent–solvent extraction significantly reduces the analysis time and analyst hours required for the determination. The sensivity is high due to the resolution of the sample and the almost complete transfer of the benzo[*a*]pyrene in the fraction to the analytical column. This highlights the advantages that multi-column techniques have over conventional methods. The selectivity towards the chosen analyte is high while the time spent resolving it from interferences is reduced. The limitation of this method lies in its cost and complexity, although this must be offset against the reduced analysis time and increased operator safety.

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