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Column switching for the high-performance liquid chromatographic analysis of polynuclear aromatic hydrocarbons in petroleum products

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ABSTRACT

Contact with polynuclear aromatic hydrocarbons which are ubiquitous pollutants can lead to the induction of various cancers. The measurement of these substances is thus of prime importance. However, the complexity of many samples in which they may be found results in a long and difficult analysis. High-performance liquid chromatography, when used in conjunction with column-switching procedures, can realise the separation of specific analytes in complex mixtures such as petroleum products.

The benefits are achieved by concentrating the analytical effort only on compounds of interest, while disregarding the rest. Optimum separations occur when different retention mechanisms are combined within a configuration. However, the incompatibilities that arise due to the range of solvents used can present problems. To overcome these we have investigated the combination of cyclodextrin and reversed-phase bonded phases. The employment of these differing retention mechanisms permits the rapid determination of polynuclear aromatic hydrocarbons with no sample preparation or pretreatment, hence the analysis time is reduced significantly as is the risk of contamination of the laboratory personnel and environment.

INTRODUCTION

Recently, it has become necessary to analyse very complex mixtures such as environmental, biological, food and petroleum samples for low levels of potentially harmful substances. Tighter national and international regulations covering a wide range of consumer products, industrial emissions etc., have increased the need for the accurate analyses of a wide range of multicomponent mixtures.

High-performance liquid chromatography (HPLC) is often the method of choice in many analytical laboratories. However, due to the large number of components in a complex sample, these samples may be difficult to analyse by orthodox liquid chromatographic techniques. The problems associated with analysing complex samples have been shown clearly by Davis and Giddings [1]. In their paper it was estimated that a column efficiency of around 200 000 theoretical plates was required

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to give a 90% probability that a twenty-component mixture would be fully resolved. As a petroleum sample may contain many hundreds, if not thousands of different components, the difficulties that arise can clearly be seen.

The resolving power of HPLC may be enhanced significantly by the introduction of column-switching techniques. These are chromatographic techniques aimed at the determination of the concentration of a specific analyte in a complex matrix. The technique provides the optimum efficiency for separations of the component of interest, whilst simultaneously minimising analysis time by decreasing the time spent in separating the components of a sample that are of no analytical interest. The separation is developed by both chromatographic and mechanical means. This is achieved by using more than one column connected by switching valves so that different parts of the sample may follow different paths through the column configuration. Column switching strategies have been applied to a wide range of analytical problems including polynuclear aromatic hydrocarbons (PNAs) [2–4], pharmaceutical agents [5,6] and biological metabolites [7,8].

PNAs are common environmental pollutants derived from a number of natural and man-made sources, including the 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 [9,10], and leads to considerable analytical effort being focused on this group [4]. The determination of these compounds in petroleum products causes significant analytical problems due to the complex nature of the matrix. The analysis of petrochemicals such as crude, processed and synthetic oils present interesting problems to the analyst. There is a wide variety of materials with very different properties present. Additionally, there are important safety considerations due to the flammable nature and toxicity of the matrix. The analytical problem is further complicated by the addition of various performance improvers and additives, notably to fuels and lubricating oils. The presence of a wide range of high-molecular-weight polymers and small, charged additives presents the analyst with a complex problem. By using column-switching techniques, to improve the resolving power of separation techniques, many otherwise difficult analyses may be attempted.

A selective method for the determination of benzo[*a*]pyrene in aviation fuel, based on a two-column switching procedure, has been published previously by the authors [3]. In this paper an improved method is described that permits the simultaneous analysis of a range of different PNAs. The application of the procedure for the analysis of lubricating oil base stocks of differing complexity is used to illustrate the operation. The use of three-column systems to realise the separation of very complex mixtures is discussed. The method published previously is based on the use of a cyclodextrin column to produce the initial fractionation of the sample. It is interesting to note that while cyclodextrin columns have been used successfully to separate PNAs [11,12], the conditions used here, *i.e.* relatively high organic modifier percentages, would not result in a separation of the group. This is highly advantageous as it simplifies the operation of the system. As the PNAs elute as one unresolved peak only one heart-cut operation is required to transfer the group onto the next column, be it either an analytical or further fractionation column. The mechanism whereby the PNAs are retained is thought to be by inclusion formation, the gamma cyclodextrin, which is the largest of the three cyclodextrin-bonded phases available has a cavity

internal diameter of about 0.95 nm, this compares to benzo[*a*]pyrene which has an estimated size of 0.88 nm across the long axis. Benzo[*a*]pyrene is thus able to enter the cavity of the γ- and β-cyclodextrins, but would be excluded from the α-cyclodextrin (0.57 nm) [13]. Within cavity π–π interactions between the cyclodextrin and the PNA determine the stability of the inclusion complex. The mobile phase modifier has a greater affinity for the cavity than the PNAs and is therefore able to displace them. At concentrations above 25–35% acetonitrile little interaction between the cavity and the PNAs occur.

EXPERIMENTAL

Materials

The PNA standards were 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 β- and γ-cyclodextrin columns (250 × 4.6 mm I.D.) were packed by Astec (U.S.A.) and supplied through Technicol (Stockport, U.K.). The Vydac C_{18} 201TP54 (250 × 4.6 mm I.D.) was also obtained from Technicol. Standard PNA solutions were made up in acetonitrile and stored in the dark to avoid photo-induced degradation. As the PNAs used were either confirmed or suspected carcinogens, the appropriate safety precautions were taken when handling the compounds or their solutions. The samples of petroleum products were obtained from commercial sources. Two lubricating oil base stocks were used. The first, sample 1, contains approximately 30% aromatic compounds while the second, sample 2, contains approximately 40%.

Equipment

The chromatography was carried out using the following equipment. A Waters series 6000 HPLC pump was used, the mobile phase being generated by a modified Micromeritic gradient former. All solvents were filtered through a 2-μm Millipore filter under negative pressure and continuously degassed with helium. To perform on-line dilution, a Kontron Series 420 pump was used. The solvents were mixed using a stainless-steel low dead volume 'T' piece. 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. The emission and excitation wavelengths were chosen as the best compromise between instrumental noise, limits of detection for PNAs and the greatest discrimination between the analytes and the interferences. Excitation and emission slits were set at 5 nm. A Midas Plus chromatographic data station, Comus Instruments (Hull, U.K.) was used to control the column-switching system and to acquire, process and store the data. A Goerz BBC SE 120 chart recorder was used to record the chromatograms. A schematic diagram of the column configuration has been previously presented [3]. The 'T' piece and second pump required for on-line dilution were placed before the second cyclodextrin fractionation column.

Column switching configuration

The configuration consists of three analytical columns (C2 to C5), typically a

Vydac C_{18} and two Astec cyclodextrin columns connected in a serial fashion by the use of switching valves, four Rheodyne Model 7000 switching valves with pneumatic actuators (V1 and V3 to V5), a sample injection valve (V2), two pumps and a fluorescence detector.

This very flexible system allows a sample to be analysed on any of the three columns or to allow a sample to be fractionated on either the first or second column with the fraction of interest analysed on the second or third column. In addition pre-concentration is made possible by the inclusion of a guard column (C1) operated in conjunction with the first switching valve (V1).

The valves are all controlled by pneumatic actuators that, in turn, are themselves controlled by the computerised system. Thus, this allows complex column-switching procedures to be performed without operator intervention.

Chromatographic technique

Sample fractionation. The petroleum sample, after filtration, was fractionated using a β-cyclodextrin pre-column (C2), with an isocratic mobile phase of acetonitrile–water (40:60). The timing for the heart cut was determined through the use of a range of standard compounds. For the simple lubricating fuel a 45-s heart cut beginning at 3.75 min was found to be appropriate. If, due to the complexity of the sample, it was found necessary to include a second fractionation step, a γ-cyclodextrin column (C3), was used. This column was equilibrated with acetonitrile–water (10:90). To ensure the analyte was retained strongly at the head of the second column water was mixed, on-line, via a T-piece with the eluent from the first column just prior to the second column. A makeup flow-rate of 1 ml min^{-1} was found to be sufficient. The sample was then fractionated using the conditions outlined above. In this case however, a heart cut of 65 s beginning at 4.24 min was more appropriate.

Heart-cut analysis. The heart cut was analysed on the 25-cm Vydac C_{18} column (C4), using a 40-min linear gradient from 30 to 100% acetonitrile at 1 ml min^{-1}. 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 disperson. The peaks found in the sample were identified by a comparison between the unknown and standard retention times.

RESULTS AND DISCUSSION

The refined products of crude petroleum are complex mixtures that are used either as sources of power, heat, lubrication or building, and more recently, as base stocks for chemicals. Due to the complexity of the product and the inadequacies in analytical techniques, the petroleum industry uses a host of semi-empirical measurements, or customer acceptance tests. While in practice these empirical tests have generally sufficed to ensure the quality of each petroleum product, the use of more selective techniques is required to allow an assessment to be made as to the environmental or health impact of the sample or to allow a particular facet of the product's performance to be studied. The PNAs, in addition to their environmental impact play an important role in the modification of a product's performance characteristics.

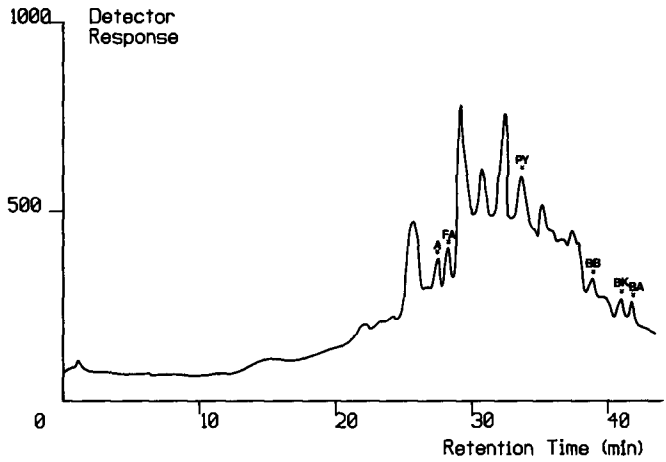

Fig. 1. Separation of sample 1 using a two-column, cyclodextrin–C_{18} column-switching configuration. A = Anthracene; FA = fluoranthene; PY = pyrene; BB = benzo[*b*]pyrene; BK = benzo[*k*]pyrene; BA = benzo[*a*]pyrene.

Fig. 1 shows a separation of the simple base oil sample (30% aromatic) using a two-column technique. The initial fractionation on the cyclodextrin column produces a fraction of considerably lower complexity that can be analysed on the final Vydac C_{18} column. From this chromatogram it is possible to identify specific PNAs.

If, however, the complexity of the sample is further increased, the resolving power of the system fails to separate the sample and a broad peak results. This occurs because even after fractionation the resulting fraction is still too complex. A reduction in the length of the heart-cut window would achieve a fraction of lower complexity but also would exclude a number of the PNAs. The problem may be overcome using one of two approaches. Firstly, it is possible to incorporate an off-line sample pre-treatment step either before or after the fractionation step using a solid-phase extraction column or a further LC separation. Alternatively, it is possible to incorporate a third column within the column-switching configuration. It is preferable to use a third column instead of an off-line technique so as not to negate the advantages of an enclosed analytical system, namely improved safety and analytical performance. The sample would thus undergo two separate fractionation procedures and as two different columns are used the mechanism of fractionation will be different in each case. To maintain the efficiency of the separation it is necessary to ensure that at each fractionation procedure the fraction of interest is reconcentrated at the head of the next column. This is usually achieved by equilibrating the second column with a solvent mixture of lower eluting strength than that which will carry the fraction on to the column. This is, however, difficult to achieve when using the cyclodextrin columns due to the low density of the bonded phase. The amount of organic modifier necessary to elute the fraction suppresses any reconcentration that may occur. Without reconcentration the PNAs pass on to the second column where they begin to undergo separation. The final fraction which would be required to include all the PNAs will also include a large proportion of interfering material. In addition to the

time required for the heart cut on to the analytical column will be such that reconcentration does not occur on this column either.

To achieve the necessary reconcentration it has been found necessary to mix water with the mobile phase immediately before the second column. This has the effect of reducing the percentage of the organic modifier in the solvent stream, thus allowing the necessary reconcentration to occur at the head of the second column. The separation on the second cyclodextrin column results in a fraction of suitable complexity, containing all the PNAs within a tight band, that can be switched rapidly to the analytical column. A separation achieved by this procedure can be seen in Fig. 2.

The mechanism whereby the PNAs are separated from the interferences is unclear. Deviations from the expected retention behaviour of benzo[*a*]pyrene have been reported [14]. At the concentrations of organic modifier used in this study, there will be only minimal retention of PNAs due to inclusion formation. The lack of retention, as compared to the majority of the interferences is advantageous as it allows the rapid elution of the PNAs, as a group and within a small time period.

The quantitation of a component in a complex sample poses many problems. The use of an internal standard may not be appropriate due to either the highly selective nature of the separation or as it may not be fully resolved from either the analytes or the interferences. In this study, to obtain quantitative information, external standards have been used. Each sample was followed by a blank sample and every fifth sample was a calibration blend. It must nonetheless be understood that the information obtained is only of a semi-quantitative nature. When the sample matrix is extremely complex even highly selective column-switching procedures do not result in complete baseline separation and therefore the integration conditions will be different. This leads to inaccuracies in the determination of peak areas and hence analytical concentrations. Table I contains the semi-quantitative data for samples 1 and 2.

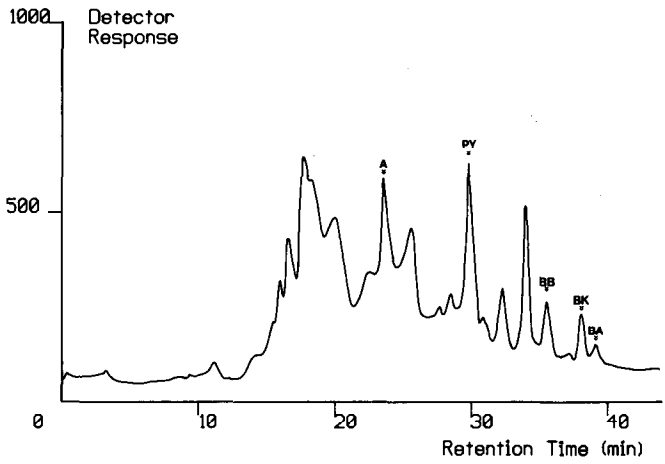

Fig. 2. Separation of sample 2 using a three-column, cyclodextrin–cyclodextrin–C_{18} column-switching configuration with on-line dilution between the first and second cyclodextrin columns. Abbreviations as in Fig. 1.

TABLE I

SEMI-QUANTITATIVE DATA FOR PNAs DETECTED
Mean result, $n = 3$.

Analyte	Sample 1 ($\mu g\ l^{-1}$)	Sample 2 ($\mu g\ l^{-1}$)
A	40.0	144.0
FA	17.5	Not detected
PY	11.8	35.2
BB	Trace	5.7
BK	Trace	4.2
BA	Trace	Trace

Work is currently in progress to automate the system further by incorporating a degree of 'intelligent processing' to permit the computer program to modify the protocol to obtain the best result with minimal input from the operator. Additionally it is hoped to study the retention and fractionation mechanisms of the cyclodextrin columns more closely.

CONCLUSIONS

Significant advantages over off-line and other on-line techniques are achieved by using a column-switching strategy. 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. The use of a three-column system enables the analysis of very complex mixtures within a closed environment. The use of cyclodextrin columns to fractionate the sample is highly advantageous and results in the rapid production of a greatly simplified matrix. On-line dilution may be succesfully used to realise the development of complex column-switched systems in which many different columns are used and to maintain the system efficiency by ensuring that reconcentration, where necessary, does occur.

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