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THE USE OF PRESSURE-ASSISTED LIQUID CHROMATOGRAPHY IN THE SEPARATION OF POLYNUCLEAR HYDROCARBONS

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SUMMARY

The use of pressure-assisted liquid chromatography in the separation of polynuclear hydrocarbon mixtures in used engine oils is described. Several column packings have been investigated and columns containing Corasil/C_{18} have been shown to be capable of achieving useful and reproducible separations of these materials. The detection limits of the procedure using both ultraviolet and fluorescence detectors have been determined, and the effects of temperature and solvent composition on the separations studied.

INTRODUCTION

The separation of polynuclear hydrocarbons by chromatographic techniques as a means of characterization or as a preliminary to characterization by spectroscopic techniques such as fluorimetry has been described in many publications. Separations by paper, thin-layer, column and gas–liquid chromatography have been the subject of review articles[1,2]. More recently, gas–solid chromatography[3,4] and high-pressure liquid chromatography[5–7] have been advocated as separation techniques of potential value in polynuclear hydrocarbon analysis.

Separations involving the use of pressure-assisted liquid chromatography appear to be promising in that they are free from the influence of volatility considerations. With such systems it is also possible to make direct use of the fluorescence and UV-absorbing properties of the aromatic hydrocarbons which cannot be readily utilized when gas chromatographic separations are carried out.

Our particular interest in the analysis of polynuclear hydrocarbons is to obtain forensic evidence from small amounts of used motor vehicle engine oils, which may be transferred to victims of "hit and run" accidents. It has been shown that, in use, polynuclear hydrocarbons build up in engine oils[8–10] and can provide the basis for a means of their "fingerprinting". The work we have carried out in studying this problem is, however, likely to be of more general interest and is described in this paper.

EXPERIMENTAL

Apparatus and materials

Liquid chromatograph. A Varian 4000 liquid chromatograph with a gas-pres-

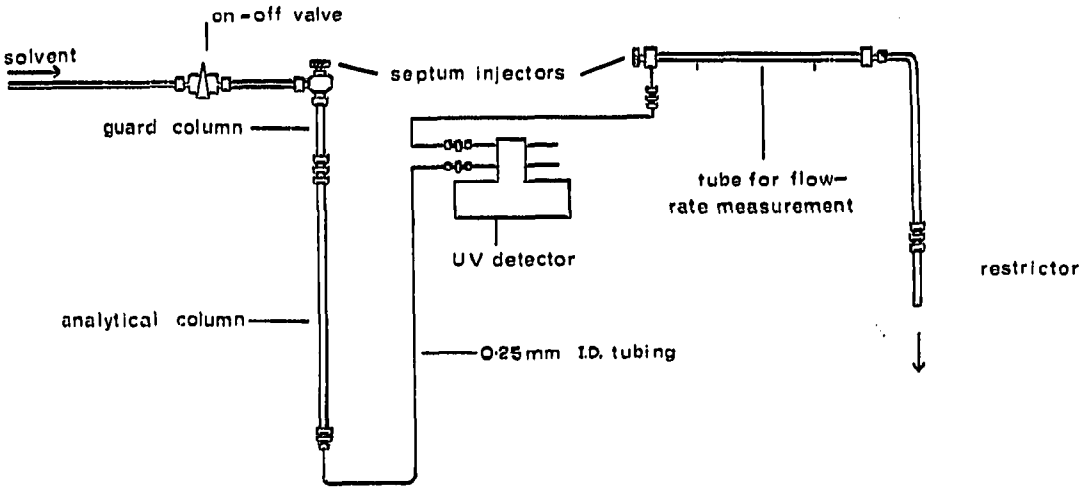


Fig. 1. Schematic diagram of a liquid chromatographic system.

surized solvent system capable of operating at pressures up to 750 p.s.i. was used. The capacity of the solvent reservoir (*ca.* 500 ml) was sufficient to allow one day's run at a single filling. A fixed-wavelength (254 nm) UV detector with a cell volume of 8 μl was used throughout this work (see Fig. 1). In some instances, an additional fluorimetric detector was connected in series.

Fluorimetric detector. A Perkin Elmer MPF 2A spectrofluorimeter with a specially constructed micro-flow cell was used as a fluorescence detector. The micro-flow cell consisted of a 5-cm length of silica tube (1 mm I.D., 2 mm O.D.) glued into a brass base designed to axially locate the tube in the cell holder at the focus of the exciting beam. A narrow-bore stainless-steel tube was brazed into the brass base to act as the solvent inlet tube. The outlet tube was attached to the other end of the silica tube using a 1/16-in. Swagelok fitting, and a PTFE ferrule, both drilled to 2 mm. The spectrofluorimeter was operated under the following conditions, designed to obtain responses from the majority of polynuclear hydrocarbons:

Excitation wavelength	350 nm
Excitation slit	40 nm bandpass
Emission monochromator	Set at the zero order position of the grating
Emission slit	40 nm bandpass

Cut-off filters were placed in the excitation and emission light paths to remove light above 400 nm and below 390 nm, respectively. Under these conditions, compounds absorbing in the region 390–310 nm and emitting above 390 nm could be detected.

Pressure source. A helium cylinder fitted with a high-pressure cylinder controller (Pressure Control Ltd., Chessington, Surrey, Model No. 7000/100/G).

Column system. Stainless-steel columns 0.9 m long and of 3.2 mm O.D. and 2.2 mm I.D. were used. These columns were sealed at the exit end with a sintered stainless-steel disc (Phase Separations Ltd.) held in place by a Swagelok stainless-steel 1/8–1/16-in. reducing union. A 15-cm guard column containing the same packing material, plugged at the entrance end with glass-wool, was attached to the analytical column

by a Swagelok 1/8–1/8-in. coupling that was completely filled with packing material. The exit end of the analytical column was connected to the UV and fluorimetric detectors in series by a piece of 0.25 mm I.D. stainless-steel tubing. The length of the tubing was dictated by the geometry of the arrangement, but was usually about 1 m. It was found necessary to have a piece of packed tubing (*ca.* 15 cm) connected after the detector to act as a restrictor so as to prevent helium bubbles from forming in the detector.

Injection system/injection technique. A standard Varian septum injection system and normal gas chromatographic septa, changed daily, were used. The injection technique, using a 5-μl syringe (SGE, Type B), was found to be important. In order to prevent septum cores blocking the needle, it was found necessary to have the whole length of the needle full of liquid at the moment of injection. Septum cores were also deposited on the top of the guard column and resulted in reduced solvent flow. They were removed about twice a week.

Flow measurement. A simple bubble flow meter was constructed from a piece of 1-mm bore glass tubing with an attached septum injector. The flow-rate was determined by injecting an air bubble and timing its rate of flow over a known distance.

Column packing materials. Samples of Porapak T (200–325 mesh), Porasil A (37–75 μm); Corasil II (37–50μm) and Corasil/C_{18} (37–50 μm) werepur chased from Waters Associates Inc.

Procedure

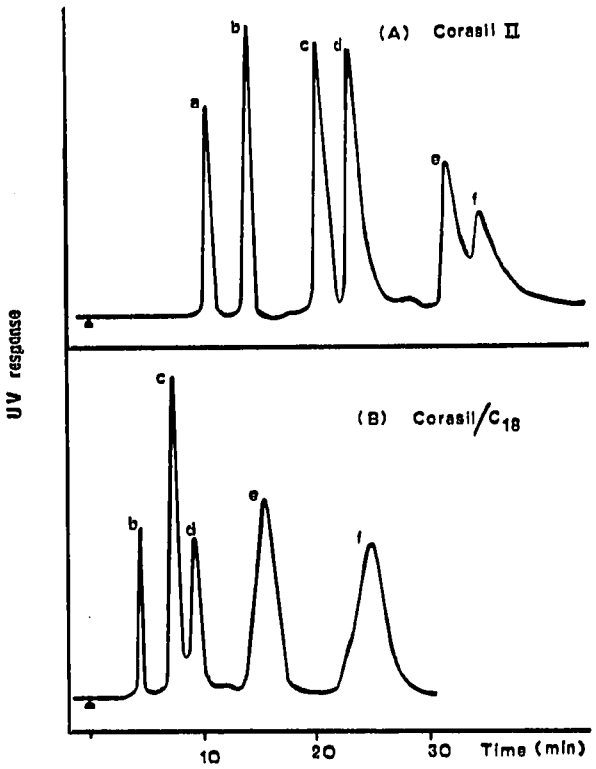
All the packings examined were dry-packed under pressure into the columns. The two adsorptive packings Corasil II and Porasil A were activated *in situ* by heating them for 16 h at 120° while purging them with dry nitrogen. The other two packings were used without modification. Preliminary screening experiments were carried out so as to establish the chromatographic properties of the columns for the polynuclear hydrocarbons. A variety of eluting solvents were used, with 2-μl aliquots of the materials in methylene chloride solution being injected. The results at this stage indicated that Porasil A and Porapak T were inferior to the other two packings.

The Corasil II and Corasil/C_{18} columns were subsequently studied in some detail using isooctane (sodium-dried) and aqueous methanol as the respective solvents. The separation of the polynuclear hydrocarbons, the influence of solvent composition on the separation and the sensitivity of the technique were determined. The effect of temperature was studied by jacketing the columns and pumping water through the jacket from a thermostatic bath. The long-term stability of the columns was also assessed by observing changes in the chromatograms obtained from standard mixtures, following periods in which the columns were used to analyze used engine oils (these were injected as 5-μl aliquots of 1.5 % solutions in cyclohexane).

RESULTS

Comparison of column packings

When Porapak T was used with methanol or 70 % isopropanol in isooctane as the solvent, very long retention times and poor separations of homologous polynuclear hydrocarbons were obtained. Improvements were achieved on Corasil II and Porasil A with isooctane as the solvent, the performance on the former column being



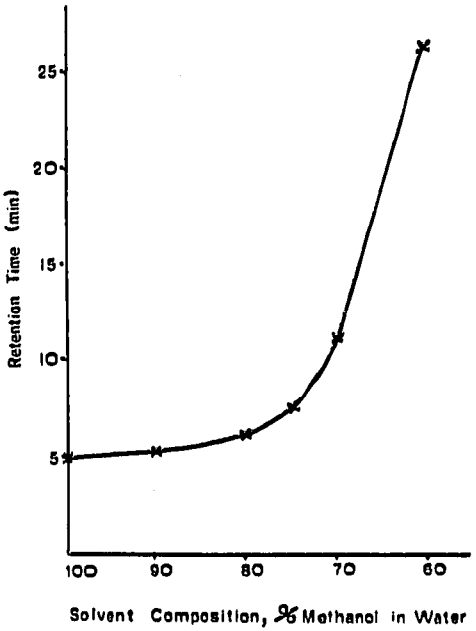


Fig. 2. Liquid chromatographic separation of aromatic hydrocarbons. a, Benzene; b, naphthalene; c, anthracene; d, fluoranthene; e, chrysene; f, perylene. See text for chromatographic conditions.

Fig. 3. Variation of the retention time of naphthalene as a function of solvent composition. Column, Corasil/C_{18}; temperature, 25°; flow-rate, 0.5 ml/min.

superior. The best results, however, were achieved on the column packed with Corasil/C_{18}. These columns displayed an efficiency of 650–1200 theoretical plates (from the anthracene peak), *i.e.*, H = 1.6–0.8 mm at a flow-rate of 0.5 ml/min. Fig. 2 compares the chromatograms obtained with mixtures of hydrocarbons eluted from a Corasil II column with isooctane and from a Corasil/C_{18} column with a 75 % solution of methanol in water.

The behaviour of Corasil II and Corasil/C_{18} columns when used to analyze used engine oils over prolonged periods (*ca.* 8 weeks) showed marked differences. The Corasil II column displayed a steady deterioration of resolution coupled with a decrease in retention times. Initially it was found possible to re-activate the column, but subsequently the deterioration became irreversible. It was not possible to use this column to characterize polynuclear hydrocarbons by retention time data over a prolonged period of use. During the same period, the Corasil/C_{18} column displayed no such deterioration.

Influence of operating conditions

The retention times of the polynuclear hydrocarbons on the Corasil/C_{18} column at a constant solvent flow-rate were found to be highly dependent upon the composi-

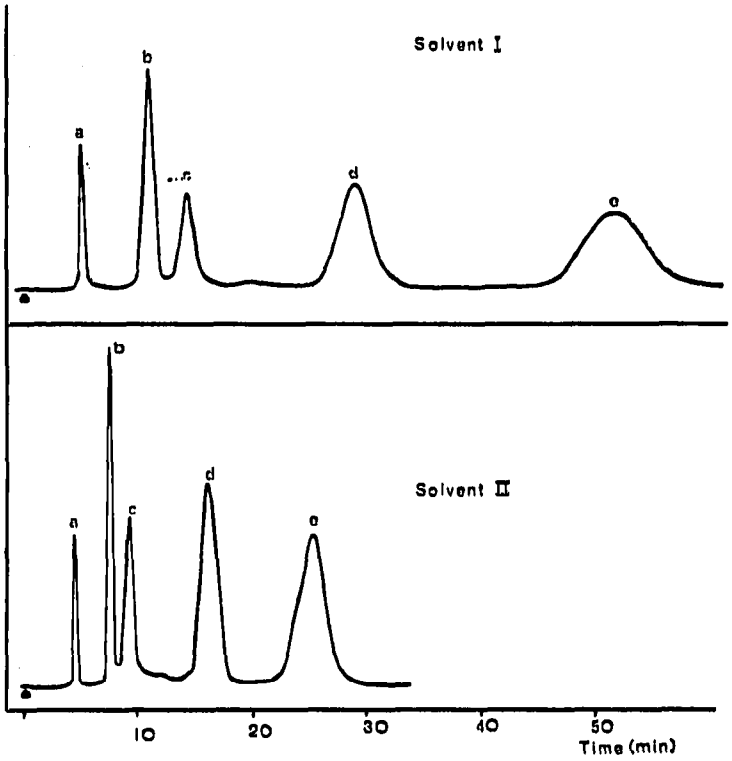


Fig. 4. Effect of solvent composition on the liquid chromatographic separation of polynuclear hydrocarbons. a, Naphthalene; b, anthracene; c, fluoranthene; d, 1,2-benzanthracene; e, 3,4-benzofluoranthene. Column, Corasil/C_{18}. Solvents: I, methanol–water (70:30); II, methanol–water (75:25); flow-rates, both 0.5 ml/min.

TABLE I

RETENTION TIME AND SENSITIVITY DATA FOR POLYNUCLEAR HYDROCARBONS

Column, Corasil/C_{18} (37–50 μm) in a 1.05 m × 2.2 mm I.D. stainless-steel column; solvent, 75% methanol + 25% water at 0.5 ml/min; pressure, 650 p.s.i.; temperature, 25°.

Compound	*Relative retention time (anthracene = 1.00)*	*Detection limits (ng)*[a]	
		UV	*Fluorescence*
Naphthalene	0.60	8	15
Acenaphthalene	0.66	15	15
Biphenyl	0.70	3	15
Fluorene	0.84	2	5
Phenanthrene	0.94	1	14
Anthracene	1.00 (8 min)	0.4	2
Fluoranthene	1.25	5	10
Pyrene	1.35	5	1
Chrysene	2.00	2	10
1,2-Benzanthracene	2.10	2	4
Perylene	3.12	4	30
3,4-Benzofluoranthene	3.15	4	5
Benzo(*a*)pyrene	3.83	8	1

[a] Considered to be the amount injected to give a peak with a height double that of the random baseline noise level.

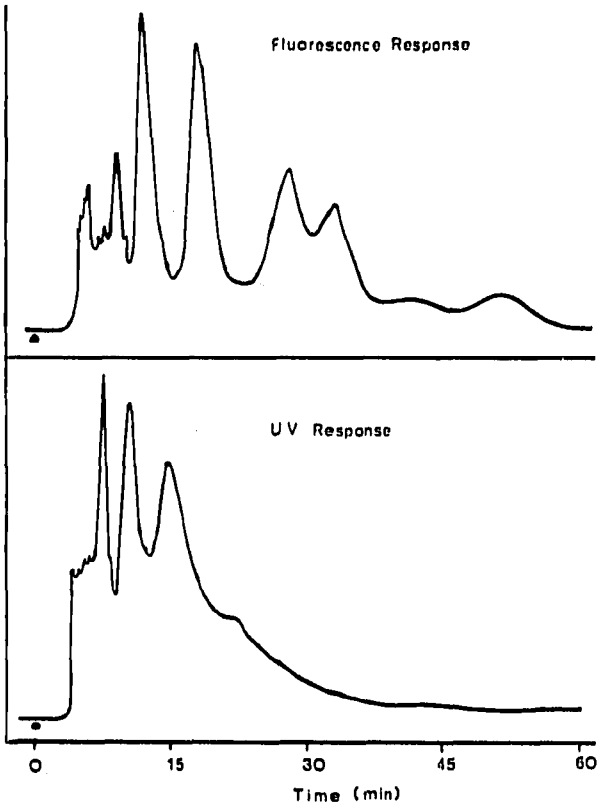


Fig. 5. Liquid chromatographic separation of a used engine oil, comparing UV and fluorescence response. Column, 1.05 m × 2.2 mm I.D. stainless steel containing Corasil/C_{18}; solvent, methanol–water (3:1); flow-rate, 0.5 ml/min; pressure, 650 p.s.i.; temperature, ambient.

TABLE II

INFLUENCE OF TEMPERATURE ON THE RELATIVE RETENTION TIME OF POLYNUCLEAR HYDROCARBONS ON A CORASIL/C_{18} COLUMN

Column and operating conditions as in Table I. Solvent flow-rate: 0.5 ml/min.

Temperature (°C)	*Relative retention time*[a]				
	1	*2*	*3*	*4*	*5*
60	0.80	1.0 (5.4 min)	1.10	1.39	1.74
50	0.77	1.0 (5.9 min)	1.14	1.49	1.96
40	0.71	1.0 (6.8 min)	1.18	1.69	2.42
25	0.60	1.0 (7.25 min)	1.25	2.10	3.15

[a] 1 = Naphthalene; 2 = anthracene; 3 = fluoranthene; 4 = 1,2-benzanthracene; 5 = 3,4-benzofluoranthene.

tion of the solvent and also the column temperature. Fig. 3 shows the variation in the retention time of naphthalene as a function of solvent composition. Fig. 4 illustrates the influence on the chromatogram of a 5 % change in the solvent composition (amounts injected were as follows: naphthalene 0.7, anthracene 0.1, fluoranthene 0.5, 1,2-benzanthracene 0.4 and 3,4-benzofluoranthene 0.6 μg) with a constant flow-rate. A solvent composition of 75 % of methanol in water was considered to give the optimum combination of analysis time and resolution. Relative retention time data when using this solvent composition, together with detection limits for the various compounds with both the UV and fluorescence detectors, are shown in Table I. Fig. 5 compares the UV and fluorescence responses on a used engine oil sample. The UV detector response to the polynuclear compounds was found to be linear over a fairly wide range. The effect of temperature on the relative retention time data is shown in Table II.

DISCUSSION

The use of the Corasil/C_{18}–aqueous methanol system is illustrative of the potential of the so-called "reversed phase" liquid chromatography. This system has the advantage over those based on liquid–solid adsorption that sample clean-up to remove water and other polar contaminants is unnecessary. The failure of the Corasil II system to maintain long-term stability when used to analyze used engine oils is probably due to irreversible adsorption of polar materials. With the very small amounts of sample used in our work, the manipulative difficulties involved in clean-up probably precludes the use of such a system. It is possible in some instances, in which a clean-up step is tolerable, however, that the high efficiencies that can be achieved with silicas[11] of small particle size may make the use of liquid–solid adsorption columns advantageous. It is well known that alumina displays superior properties compared with silica in the separation of polynuclear hydrocarbons in traditional column chromatography. Columns containing alumina have already been shown to give useful liquid chromatographic separations of the polynuclear hydrocarbons, even though the efficiencies were low[5]. We have not investigated alumina, however, as it would be expected to suffer from the same limitations as the Corasil II system.

The results show that by using a Corasil/C_{18} column it is possible to determine low levels of polynuclear hydrocarbons. The sensitivity of the UV detector to these compounds compares favourably with the response displayed when they are determined with a flame ionization detector following gas chromatographic separation. The fluorescence response obtained with the "home-made" detector is probably far from the optimum, because the full sensitivity of the spectrofluorimeter could not be utilized owing to a high back groundsignal due to scattered light and fluorescence from the solvent and quartz tubing. It is apparent from Fig. 5 that the fluorescence detector provides a useful ancillary detector, particularly in the detection of certain compounds that do not absorb strongly at 254 nm but which fluoresce strongly above 390 nm.

The elution sequence of hydrocarbons from the Corasil/C_{18} column appears to closely resemble that shown when an ODS Permaphase (Du Pont) column is used[7], although we have not been able to assess the latter packing material. The long-term stability of the Corasil/C_{18} column is such that retention time data can be used to give

qualitative information on the composition of hydrocarbon mixtures. If this is to be achieved, however, it is essential to closely control the column temperature and the solvent compostion. For our particular problem, in which we are interested in using the chromatogram as a "fingerprint", characterization of the individual components in the mixtures is unimportant. The complexity of the mixtures formed in an engine oil during use is such that a column with a far higher resolving power would be necessary to achieve characterization of the individual polynuclear hydrocarbons. The use of longer columns would greatly assist in qualitative identification but this is not feasible with the pumping system used in our work, which is already being used close to its limit.

Another possible way of achieving greater resolution would be to alter the solvent polarity, and it is obvious from Figs. 3 and 4 how marked an influence this can have. Without gradient elution facilities, however, improvements in resolution resulting from changes in solvent polarity can generally be achieved only at the expense of increased analysis time. Additional specificity could also be built into the system by operating the fluorimetric detector under conditions such that it would excite and detect only certain hydrocarbons.

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