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DETERMINATION OF BENZO[*a*]PYRENE AND OTHER POLYAROMATIC HYDROCARBONS IN PETROLEUM OILS BY DIRECT LIQUID CHROMATOGRAPHY

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SUMMARY

A high-speed liquid chromatographic (LC) method is used for direct determination of benzo[*a*]pyrene (B[*a*]P) and other polyaromatic hydrocarbons in petroleum oils. High efficiency liquid-liquid chromatographic columns were developed on a microsilica support modified by a highly selective solvent for aromatic hydrocarbons, *i.e.*, dimethyl sulphoxide. Usually a fluorescence spectrometric detector is used. For B[*a*]P determination, analysis time is *ca.* 20 min and the detection limit is 5 µg per litre of oil, for a direct oil injection of 2 µl.

INTRODUCTION

Numerous methods have been reported for the isolation and determination of benzo[*a*]pyrene (B[*a*]P) and other polyaromatic hydrocarbons (PAHs) in environmental or petroleum samples. Some authors, such as Klimisch and Ambrosius¹, Boden² and Nakagawa *et al.*³, focused on the determination of B[*a*]P, but most of the others were also concerned with different PAHs. Dong *et al.*⁴ and Fox and Staley⁵ analyzed PAHs in suspended particulate matter, Lao *et al.*⁶ and Lee *et al.*⁷ sampled and analyzed industrial effluents, emissions from coke ovens, coal tar and airborne particulates.

In 1971 Catchpole *et al.*⁸ reviewed carcinogenicity and its relation to the PAH content of petroleum. For the same purpose, Lafontaine and Limasset⁹ studied B[*a*]P in petroleum oils. Analyses for PAHs in creosote or anthracene oils have been carried out by Popl *et al.*¹⁰, and in bituminous materials by Grant and Meiris¹¹. Many studies have recently been published on PAH measurement in the environment. For example, Basu and Saxena^{12,13} studied drinking water, and Lee *et al.*¹⁴ investigated oil polluted waters.

All the methods published use extraction and concentration steps before liquid or gas chromatographic determinations of B[*a*]P and PAHs in the concentrate.

In connection with PAH analysis in lubricating oils, we wished to develop an efficient and simple method for B[*a*]P and other PAHs. Columns filled with microsilicas, bonded or not, cannot be used for B[*a*]P and other PAHs in raw oil samples.

Silica columns require the use of hexane or heptane as eluents and a low water content, which is not easy to control. Moreover we have observed that retention of PAHs with five or more rings was insufficient for their isolation when present at low levels in lubricating oils (*cf.*, Table I). Bonded silica (C_{18}) columns are easier to use but lead to an elution order which does not follow the ring number. However, high efficiency is obtained and PAH synthetic mixtures show very good resolution^{3-5,15}. With complex hydrocarbon mixtures, such as lubricating oils, the results were very disappointing.

We therefore decided to explore the capabilities of liquid-liquid chromatographic columns. Two problems had to be solved: choice of the liquid phase and preparation of high-efficiency liquid-liquid chromatographic columns.

After several trials with polar solvents, we decided to use dimethyl sulphoxide (DMSO). This solvent is often used for liquid-liquid extraction of PAHs in oils or other samples⁸ and is known for its highly selective solvent properties¹⁶. Its behaviour with petroleum samples is well known at our Institute where an industrial process for selective aromatic extraction by DMSO has been developed¹⁷.

The main difficulty with liquid-liquid chromatography is to maintain good efficiency when a solvent is deposited on microsilica or other adsorbents. Engelhardt and Weigand¹⁸ used β,β' -oxydipropionitrile (ODPN) on silica. Viricel and Lemar¹⁹ studied liquid chromatographic phenomena on microsilica particles impregnated with polar stationary phases such as ODPN or Carbowax 20M. For PAH separation, Vespalec²⁰ used tricyanoethoxypropane on silica columns, Grimmer and Böhnke²¹ chose dimethylformamide (DMF) on Sephadex LH-20 and Jentoft and Gouw²² tried several polar solvents (DMSO, DMF, Carbowax) on a gas chromatographic support. In all cases, column efficiency was poor and did not allow direct analysis of B[a]P and other PAHs. We observed the same behaviour.

We have now developed a new simple method for preparing efficient liquid-liquid chromatographic columns for polar solvents, and have used a DMSO column with fluorescence detection for B[a]P and some other PAHs in lubricating oils. For some PAHs such as coronene, the fluorescence was very slight and UV detection at 290 nm was used.

EXPERIMENTAL

Materials

Microsilica particles (LiChrosorb Si 60, Merck) were used. The mean diameter of these non-spherical particles was 5 μm and porosity was *ca.* 550 m^2/g .

Dimethyl sulphoxide (E. Merck, Darmstadt, G.F.R.) was freshly distilled and dehydrated on activated molecular sieves (5 \AA). The elution solvent was *n*-heptane (pure grade; J. T. Baker, Phillipsburgh, N.J., U.S.A.) freed from aromatic traces by filtration in large columns filled with activated silica gel.

Standard samples of PAHs were commercial products (Fluka, Buchs, Switzerland; Aldrich, Milwaukee, Wisc., U.S.A.) used without purification. Reference solutions were prepared in *n*-heptane and stored at 4°, protected from light.

Apparatus

A Varian Model 8500 liquid chromatograph was equipped with a Valco Model

7000 sample injection valve and connected to a DuPont Model 836 variable wavelength fluorescence detector or to an LDC Spectromonitor I, variable wavelength UV detector. Signals were recorded on a Sefram Servotrace strip-chart recorder. Stainless-steel columns (30 cm \times $\frac{1}{4}$ in. O.D.) fitted with Swagelok connectors were used.

Column preparation

Silica particles were dehydrated by heating in a vacuum for 1 h, and then loaded under pressure in the column. A solution of DMSO in *n*-heptane was forced through the column. The concentration of DMSO and volume depended on the silica:DMSO ratio required for the separation. After this process the column is ready to be used and operating conditions can be adjusted. The procedure is patented.

HPLC operating conditions

The mobile phase was generally *n*-heptane + 0.35% DMSO. The flow-rate was 90 ml/h and column pressure was 70 bars. When the UV fluorescence detector was used, the excitation filter band was set from 325 up to 385 nm, and the emission filter band at > 408 nm. For UV detection, the wavelength was 290 nm. Oil samples were diluted with *n*-heptane in ratios from 2 to 10, and the volume of solution injected through the valve ranged between 10 and 30 μ l. The analysis time was 20 min for B[a]P and 25 min for coronene.

RESULTS AND DISCUSSION

It is well known that the concentration of the stationary phase in liquid-liquid chromatography is closely related to elution time. We observed the same relationship when the DMSO content of silica was modified to obtain the best separation of PAHs and more especially of B[a]P.

Optimization of DMSO deposit on silica

The capacity factor (k') of several aromatic hydrocarbons, defined by the ratio

TABLE I

CAPACITY FACTORS (k') OF AROMATIC COMPOUNDS FOR DIFFERENT DMSO/SILICA WEIGHT RATIOS

Compound	DMSO/Silica $\times 100$				
	0 (+ water)	15	25	33	45
Benzene	0.50	0.60	0.42	0.49	0.52
Naphthalene	0.94	1.09	0.92	1.04	1.16
Anthracene	1.64	1.90	1.76	2.00	2.37
Benz[a]anthracene	2.71	4.09	4.27	4.40	5.71
Benzo[a]pyrene	3.28	5.27	5.09	5.54	6.89
1,3,5-Trimethylbenzene	0.60	0.45	0.27	0.27	0.26
2,6-Dimethylnaphthalene	1.02	0.82	0.64	0.64	0.63
9-Methylanthracene	1.71	1.54	1.72	1.72	1.74

$(t_R - t_0)/t_0$ where t_R is the retention time and t_0 the dead time, was determined and the results are collected in Table I. To emphasize the selective effect of DMSO, k' values are also given for microsilica alone (the water content was not determined) with *n*-heptane as eluent. DMSO/silica ratios were determined by the analysis of the effluent solution (*n*C₇ + DMSO) and its volume.

A ratio of 15% is obtained by using *n*-heptane as eluent after column preparation under pressure. These columns are stable for several weeks of extensive use. A ratio of 45% is obtained by using as eluent *n*-heptane saturated by DMSO. The chromatogram of a synthetic PAH mixture is given in Fig. 1. Column efficiency measured on B[a]P is 15,000 plates for a column length of 30 cm, and HETP is 2×10^{-2} mm.

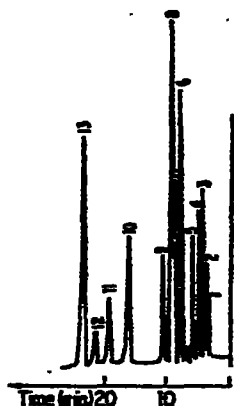


Fig. 1. Chromatogram of synthetic PAH mixture on DMSO/silica (ratio 45%). Eluent: *n*-heptane + 0.35% DMSO. UV detection at 254 nm. Peaks: 1 = dodecylbenzene; 2 = 1,3,5-trimethylbenzene; 3 = benzene; 4 = 2,6-dimethylnaphthalene; 5 = naphthalene; 6 = 9-methylanthracene; 7 = anthracene; 8 = phenanthrene; 9 = pyrene; 10 = benzo[a]anthracene; 11 = benzo[a]pyrene; 12 = perylene; 13 = benzo[*g,h,i*]perylene.

Figs. 2 and 3 show the chromatograms obtained for a petroleum distillation cut 320–480° with DMSO/silica ratios of 15 and 45% and using UV fluorescence detection. When the ratio increases from 15 to 45, B[a]P resolution gets better and benzo[*b*]fluoranthene is resolved from B[a]P.

We conclude that if the DMSO content of the column is increased, the PAH with the highest ring number has the longest retention time, and the most highly alkyl-substituted PAH has the shortest retention time. These trends are the same as those observed for liquid-liquid extraction by DMSO.

Choice of detection modes

We use two detection modes, *i.e.*, UV absorption or UV fluorescence. Both can be employed at different wavelengths. For low contents of B[a]P and some other PAHs, we chose fluorescence detection. For higher contents, or if the compounds gave no fluorescence response, UV absorption detection was preferred.

Fluorescence detection. The UV fluorescence detector is highly sensitive and selective^{4,5,23}. Only PAHs which are more condensed than anthracene exhibit fluorescence characteristics. So with this detection mode, the response of other aromatic compounds is almost totally absent.

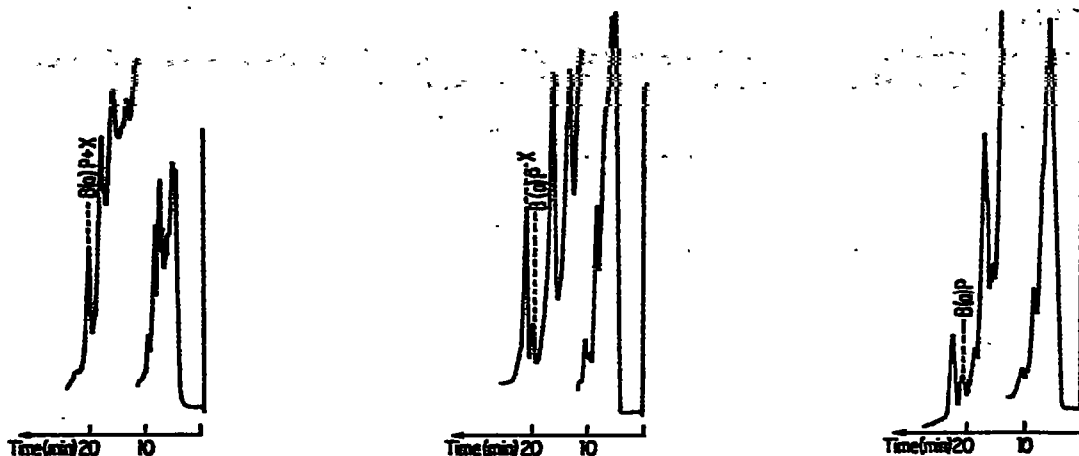


Fig. 2. Spectrum of distillation cut (320–480°) on DMSO/silica (ratio 15%). Eluent: *n*-heptane. UV spectrofluorescence detection. X = benzo[*b*]fluoranthene.

Fig. 3. Spectrum of distillation cut (320–480°) on DMSO/silica (ratio 45%). Eluent: *n*-heptane + 0.35% DMSO. UV spectrofluorescence detection.

Fig. 4. Spectrum of furfural refined product. Conditions as in Fig. 3.

With the spectrofluorimetric method, the optimum excitation wavelength for B[*a*]P determination in solution is $\lambda_{ex} = 368$ nm. Calculations are done at the maximum in the emission spectrum, $\lambda_{em} = 406$ nm or 429 nm.

With a DuPont liquid detector, filters of large wavelength intervals are recommended to obtain enough energy. Therefore, the best wavelengths for sensitivity and selectivity of B[*a*]P response are λ_{ex} 325–385 nm and $\lambda_{em} > 408$ nm. Under these conditions, some highly condensed PAHs show almost no response. For example, coronene gives no response. Table II gives the response factors of several PAHs relative to the B[*a*]P response.

UV absorption detection. Variable-wavelength UV detection provides a detection mode with variable selectivity. At $\lambda = 254$ nm, non-selective detection of aromatic hydrocarbons is obtained, and nearly representative spectra are obtained from monoaromatic compounds up to chrysene. These detection conditions are not very useful in oil studies because detector responses are saturated up to anthracene, and for more condensed compounds they do not allow a very sensitive detection. For example, the detection limit for B[*a*]P is 15 mg per litre of oil for 2 μ l injections.

Wavelengths between 290 and 298 nm make for good extinction of slightly condensed PAHs and give a good fingerprint of PAHs with four rings and more. Higher wavelengths (> 300 nm) lead to a very selective detection mode, with high relative response factors between aromatic hydrocarbons, even with the same ring number. At about 380 nm, B[*a*]P has the same response as at 254 nm. However, with the extinction of non-condensed aromatic hydrocarbons, the detection limit is increased up to 2 mg per litre of oil for 10 μ l injections.

*Benzo[*a*]pyrene detection*

Although the measurement of B[*a*]P remains important, the concept of B[*a*]P

TABLE II

WEIGHT RESPONSE FACTORS OF SOME PAHs RELATIVE TO B[a]P WITH UV FLUORESCENCE AND UV ABSORBENCY DETECTION MODES

Compound	UV Fluorescence Exc. 325-385 nm Em. > 408 nm	UV 254 nm
Anthracene	0.07	0.64
Dimethylbenz[a]anthracene	0.20	0.58
Pyrene	0.001	0.63
Benz[a]anthracene	0.025	0.84
Triphenylene	~0	2.41
Fluoranthene	0.24	0.30
Chrysene	0.03	0.68
Tetracene	~0	1.16
Benzo[a]pyrene	1	1
Perylene	0.95	0.15
Benzo[g,h,i]perylene	0.24	0.34
Dibenz[a,c]anthracene	~0	1.53
Coronene	~0	2.10

as a single tracer of carcinogenesis is highly criticized. With our method, the same performances obtained for B[a]P determination could also be reached for the other PAHs separated during the same analysis as for B[a]P.

Performances for B[a]P determination. Experimental conditions are detailed in Table III. Under these conditions, the limit of detection is 5 µg per litre of oil (ca. 5 ppb) and the detector response is linear between $5 \cdot 10^{-9}$ and 10^{-6} g. This range of linearity allows direct B[a]P determination in oil between 5 µg/l (≈ 5 ppb) up to ca. 500 mg/l (≈ 500 ppm).

TABLE III

EXPERIMENTAL CONDITIONS FOR HPLC DETERMINATION OF B[a]P IN OIL SAMPLES

Column	30 cm × 6.35 mm O.D.
packing	LiChrosorb silica, 5 µm (E. Merck)
pressure	70 bars
eluent	n-heptane + 0.35% DMSO, flow-rate 90 ml/h
Detection	UV fluorimeter (DuFont), excitation filter: from 325 up to 385 nm emission filter: >408 nm
Analysis time	20 min

For lower levels of B[a]P, a concentration step by liquid-liquid DMSO extraction can be applied, and for higher concentrations, solvent dilution of samples is necessary before injection.

B[a]P determination in crude-oil cuts. In these studies we attempted to locate B[a]P in the distillation range. Consequently we analyzed distillation cuts between 350° and 530°. Table IV gives the results. Total B[a]P content is found between 400° and ca. 480°, confirming the calculation of boiling points of B[a]Ps from the boiling point at reduced pressure and from the retention time in gas chromatographic analysis. At atmospheric pressure, we obtained values of the boiling point ranging from 460 to 475°. Fig. 3 shows the general spectrum obtained for crude-oil cuts.

TABLE IV
 PRESENCE OF B[a]P IN DISTILLATION PETROLEUM CUTS

Distillation cut temperature range (°C)	Weight balance of the cut (%)	B[a]P content of the cut (µg/l)	Contribution of B[a]P in crude oil (µg/l)
340-480	30	10^2	≈ 300
480-535	16	< 100	< 15
			Total ≈ 300
150-400		< 5	
400-450		$1.6 \cdot 10^2$	
450-480		≈ 10^2	

If the final boiling point of the cut studied is higher than 500°, it is not generally possible to attain the lower detection limit because the presence of heavier alkyl PAHs does not allow the detector response to return to zero.

The composition of crude oils is so complex that we wanted to confirm the accuracy of B[a]P determination by the direct liquid chromatographic method described here. For this purpose B[a]P was determined by UV spectrofluorimetry after thin-layer chromatography (TLC) or after semipreparative liquid chromatography.

Comparison of B[a]P determinations by direct liquid chromatography and by UV spectrofluorimetry-TLC. These comparisons were achieved on crude-oil cuts (Figs. 3-5), on furfural refined oils (Fig. 4) and on oils from different processes (Figs. 6, 7).

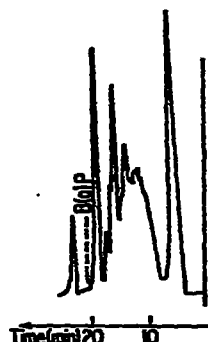
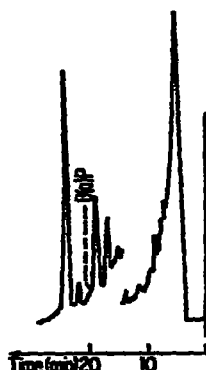
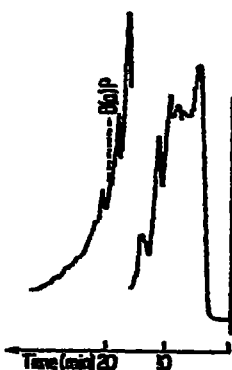


Fig. 5. Spectrum of distillation cut (480-535°). Conditions as in Fig. 3.

Fig. 6. Spectrum of hydrocracked oil. Conditions as in Fig. 3.

Fig. 7. Spectrum of hydrofinished oil. Conditions as in Fig. 3.

Several methods have been reported for the determination of B[a]P in oily samples, using extraction, fractionation and UV spectrofluorimetry. We used a method⁹ in which each determination requires *ca.* 8 h. There are three fractionation steps (1-3) to isolate potential benzo[a]pyrene before quantitative measurement by

UV spectrofluorimetry⁴: (1) liquid chromatography on an alumina column to isolate a raw PAH fraction; (2) liquid-liquid extraction by cyclohexane-nitromethane to extract a very condensed PAH fraction; (3) TLC with continuous development on 30% acetylated cellulose plates to recover only the B[a]P band; (4) titration of B[a]P by UV spectrofluorimetry at $\lambda_{\text{ex}} = 368$ nm, recording of the emission spectrum and estimation of B[a]P at a maximum of ca. 406 nm.

The method was applied to several samples already studied. Results are collected in Table V. For some samples, there is good agreement. For others, especially at B[a]P contents greater than 1 mg/l, the indirect method gives low values. With this method we observed some losses due to adsorption of B[a]P on alumina liquid chromatographic supports and partial extraction of B[a]P by nitromethane. A recovery of only 50% B[a]P added to an oil sample was sometimes obtained.

TABLE V
B[a]P CONTENT OF DIFFERENT OIL SAMPLES

Sample	Reference	B[a]P content ($\mu\text{g/l}$ of oil)	
		Direct liquid chromatography	Isolation and UV spectrofluorimetry
<i>Crude oil fractions</i>			
>340°		300*	133
340-480° cut	A	10 ³	
480-525° cut	B	<100	
<i>Solvent-refined oil</i>			
Food, 310-500°	C	1.17·10 ³	0.5·10 ³
Furfural refined product	D	~30	—
Furfural extract	E	2.93·10 ³	2.5·10 ³
<i>Hydrocracked oil</i>	F	<5	**
<i>Hydrofinished oil</i>	G	<5	**
<i>Lubricating motor oil</i>			
before use	H	**	**
after use	I	5.1·10 ³	4·10 ³
<i>High severity solvent-treated oil</i>		20	25

* Calculated from A and B.

** None detected.

In liquid chromatographic (LC) methods a higher value could be obtained when B[a]P is not eluted alone. So we decided to trap the peak corresponding to B[a]P, in a crude-oil cut (340-480°). The LC fraction obtained was submitted to UV spectrofluorimetric characterization at low temperatures (77°K) on FICA 50 MK II apparatus under the following conditions: $\lambda_{\text{ex}} = 368$ nm; excitation slit, 7.5 nm; emission slit, 0.5 nm. Spectra of the B[a]P LC fraction and of a B[a]P solution (20 $\mu\text{g/l}$) in *n*-heptane give the same absorption maxima. So B[a]P is eluted alone in the direct LC method described.

Determination of other PAHs

The performance of these liquid-liquid chromatographic columns is clearly demonstrated in Fig. 1 for a synthetic PAH mixture. Table VI gives capacity factors for pure PAHs.

TABLE VI

CAPACITY FACTORS (k') FOR PAHs ON DMSO/SILICA COLUMN (45% RATIO)

	k'
<i>Non-alkylated PAHs</i>	
Benzene	0.52
Naphthalene	1.16
3 rings	
Anthracene	2.37
Phenanthrene	2.52
4 rings	
Pyrene	3.13
Chrysene	5.00
Triphenylene	5.63
Benz[a]anthracene	5.71
5 rings	
Benzo[a]pyrene	6.89
Benzo[e]pyrene	7.42
Anthanthrene	7.10
Perylene	8.00
Dibenz[a,c]anthracene	12.77
>5 rings	
Benzo[g,h,i]perylene	8.47
Coronene	9.40
<i>Alkylated PAHs</i>	
1,3,5-Trimethylbenzene	0.26
n-Butylbenzene	0.26
1,3,5-Trisopropylbenzene	0.13
2,6-Dimethylnaphthalene	0.63
9-Methylanthracene	1.94
Diisoamylanthracene	0.53
Dimethylbenz[a]anthracene	2.74
<i>Naphthenic PAHs</i>	
Acenaphthene	0.86
Fluorene	1.39
2,3-Benzofluorene	2.86
Fluoranthene	3.58
Benzo[k]fluoranthene	7.04
Benzo[b]fluoranthene	7.36
Indeno[1,2,3,c,d]pyrene	8.82
<i>Hydrogenated PAHs</i>	
Dihydro-9,10-anthracene	1.42
Octahydroanthracene	0.24
<i>Phenyls</i>	
Diphenyl	1.16
Paraterphenyl	2.28
<i>Sulphurated PAHs</i>	
Dibenzothiophene	2.37
<i>Nitrogenated PAHs</i>	
Carbazole	1.74
Acridine	4.68
5,7-Dimethylbenz[a]acridine	3.10

Several applications in the oil industry are illustrated by the chromatograms shown. Thus examples are given for oils obtained after other standard industrial treatments such as hydrocracking (Fig. 6, sample F) or hydrofinishing (Fig. 7, sample G). The boiling point range for F is 320–500°. For G the initial boiling point is 360°, the final boiling point is not determined, but 80% distilled at 540°. The F and G spectra look quite similar and contain some very well resolved peaks (which we are studying at present). This type of spectrum was obtained every time there were heating phenomena (for example, crankcase motor oil, tobacco smoke extract) (Fig. 9).

Fig. 8 reproduces chromatograms of a motor oil before and after use (samples H and I).

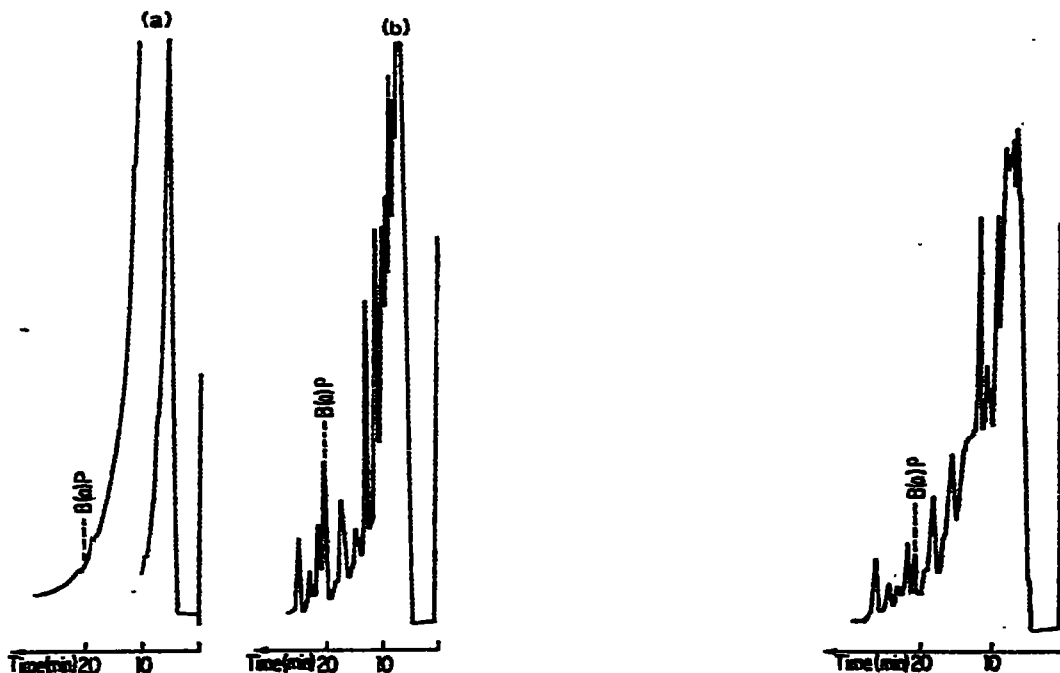


Fig. 8. Spectra of a lubricating motor oil before (a) and after (b) use. Conditions as in Fig. 3.

Fig. 9. Spectrum of a concentrate of tobacco smoke. Conditions as in Fig. 3.

CONCLUSION

The method described here is for the direct determination of B[a]P and other PAH in oily samples, with a final boiling point below 500°. It is also suitable for other kinds of samples related to the environment, such as cigarette smoke concentrate, water pollutants or engine emissions.

Although B[a]P is a highly carcinogenic compound, its measurement does not evaluate the carcinogenicity of hydrocarbon samples. In fact, legislation tends to take into account B[a]P and other PAHs such as fluoranthene, benzo[*b*]- and benzo[*k*]-fluoranthene, benzo[*g,h,i*]perylene and indeno[1,2,3,*c,d*]pyrene. Except for fluo-

ranthene, all these compounds can be determined by the method described here; determination of fluoranthene is possible if the sample does not contain a high concentration of PAHs with three and more aromatic rings.

Research is currently being undertaken at our Institute to correlate the PAH content of oil with mutagenic characteristics²⁴. This new type of liquid-liquid chromatography can be used for semi-preparative fractionation, giving fractions which may be analyzed in detail and evaluated for mutagenic properties.

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