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SEPARATION AND IDENTIFICATION OF MONOMETHYLATED POLYCYCLIC AROMATIC HYDROCARBONS IN HEAVY OIL

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SUMMARY

The application of high-performance liquid chromatographic fractionation using a column-switching technique to the identification of monomethylated polycyclic aromatic hydrocarbons by capillary gas chromatography and high-resolution (Shpol'skii effect) fluorescence spectroscopy is described. The column-switching technique with silica and aminosilane columns permitted the rapid fractionation of both aromatics and each aromatic ring type in heavy oils. The existence of methylphenanthrenes, methylchrysenes and methylbenz[*a*]anthracenes in Kuwait 340–500°C distillate was confirmed by the two methods.

INTRODUCTION

The determination of polycyclic aromatic hydrocarbons (PAHs) in oils, sediments and particulate extracts has attracted considerable attention for many years, because these compounds show biological activity[1,2]. For example, monomethylated PAHs are of particular interest because of their potential carcinogenic and mutagenic activities[1,2]. Therefore, various methods for quantitative and qualitative analysis of samples for PAHs have been reported[3–13].

In the determination of PAHs in oil, the usual method includes the separation of aliphatics and polar compounds from aromatics by open-column chromatography to concentrate the PAHs[3–5], then the resulting aromatics are separated into individual PAHs by capillary gas chromatography or fractionated into each aromatic ring type by high-performance liquid chromatography (HPLC) for the subsequent instrumental analysis. The time required for these methods is generally long because of the use of open-column chromatography.

In this paper, we report the application of HPLC fractionation using a column-

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switching technique to the identification of monomethylated PAHs by capillary gas chromatography and high-resolution (Shpol'skii effect) fluorescence spectroscopy. The column-switching technique permits the rapid fractionation of both aromatics and each aromatic ring type in heavy oils.

EXPERIMENTAL

Apparatus

A Model Trirotar-III liquid chromatograph (JASCO) was used with a Model R-401 refractive index (RI) detector (Waters Assoc.) and a Shimazu SPD-1 UV–visible spectrophotometric detector. Sample solution (20 μl) was injected with a Model 7120 injector (Rheodyne). Column switching was performed with two high-pressure valves. Chromatograms with the RI and UV detectors were recorded on a Model VP-6621A recorder (Matsushita Electric). Nucleosil 50-5 (300 × 4 mm I.D.) and μBondapak-NH_2 (300 × 4 mm I.D.) separation columns were used. The configuration of the system and three flow modes for the column-switching technique are presented in Fig. 1. A sample is first separated into saturates (S) and aromatics (Total A) on the silica column in flow mode I, then aromatics are backflushed and further separated into monoaromatics (1A), diaromatics (2A) and polycyclic aromatic hydrocarbons (PAHs) on the aminosilane column in flow mode II. Flow mode III is used for the direct elution of aromatics.

A Model G3000 gas chromatograph (Hitachi), equipped with a flame ionization detector and a splitless injector, was used for separating aromatics obtained by HPLC (in flow mode III). Separation was performed with an SPD-5 fused-silica

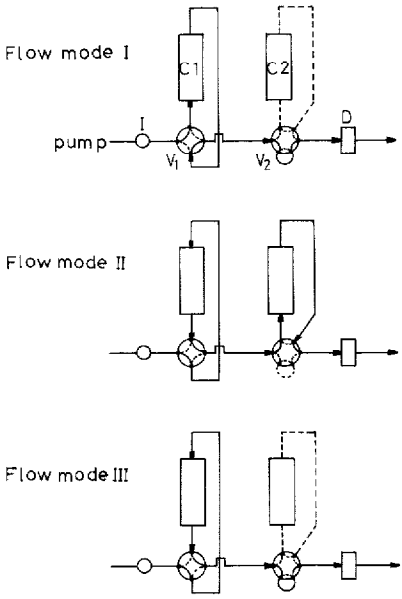

Fig. 1. Flow modes for the column-switching technique. I = Injector; V_1 and V_2 = high-pressure valves; C_1 = silica column; C_2 = aminosilane (NH_2-silica) column; D = detector.

capillary column (Supelco) (30 m × 0.25 mm I.D.); the stationary phase is slightly polar. Helium was used as the carrier gas at a flow-rate of 1.70 ml/min. Retention data were obtained with a Model D-2100 data processor (Hitachi).

High-resolution (Shpol'skii effect) fluorescence spectra of monomethylated PAHs were obtained with a laboratory-made apparatus. *n*-Alkane solutions of samples, contained in quartz tubes, were frozen by attachment to the cold head of a closed-cycle helium cryogenerator (Daikin Cryo Kelvin 202A 5L) operating at 15 K. The light from the excitation source (500-W xenon lamp; JASCO) was dispersed by a monochromator (JASCO CT-25C) and focused on the rigid solution of a sample (excitation band width 6 nm). Fluorescence was observed at 90° through a high-resolution monochromator (JASCO CT-50CS; focal distance 0.5 m, emission band width 0.08 nm) and detected with a photomultiplier (Hamamatsu R928). Aromatic compounds in HPLC fractions were identified by comparison of the high-resolution spectra of the fractions with those of reference standards recorded in this study or reported previously[2,8,10].

Samples

Taching 290–340°C and Kuwait 340–500°C distillates were used as sample.

Reagents

For the removal of polar compounds from oil, 30 g of Florisil (200–300 mesh) and 600 ml of methylene chloride–*n*-hexane (HPLC grade) (20:80) were used. Compounds insoluble in *n*-hexane in the fraction of non-polar compounds were filtered off using a polytetrafluoroethylene (PTFE) filter (Chromatodisc 25N; Kurabou) and the *n*-hexane fraction was collected. *n*-Hexane, used as a mobile phase in HPLC or as a solvent in high-resolution fluorescence spectroscopy, and *n*-octane, used in the latter, were of HPLC grade (Wako and Tokyo Kasei). PAHs of analytical-reagent grade (purity >97%) were supplied by Tokyo Kasei or Aldrich. A C_{18} cartridge (Sep-Pak C_{18}; Waters Assoc.) and methanol were used for separating high-molecular weight PAHs in HPLC fractions.

Procedure

Fig. 2 shows the flow scheme for the qualitative analysis of heavy oils. Prior to the HPLC separation, a sample (1 g) was treated with Florisil and a PTFE filter to remove polar compounds and *n*-hexane-insoluble compounds (high-molecular-weight *n*-alkanes). *n*-Hexane-soluble compounds were diluted to 25 ml and aliquots (20 μl of the solution) were further separated into each group type by HPLC with the column-switching technique. Identification of monomethylated PAHs by capillary gas chromatography and high-resolution fluorescence spectroscopy was carried out using fractions of aromatics (Total A), tricyclic aromatics (3A)-2 (mixture of anthracenes and phenanthrenes) and tetracyclicaromatics (4A)-2 (mixture of chrysenes and benz[*a*]anthracenes). The eluent (*n*-hexane) was removed from each fraction by using Model S-3 mini-evaporator (Tokyo Rikakikai).

For capillary gas chromatography, aromatics, from a double HPLC fractionation in flow mode III, were dissolved in a small amount of *n*-hexane and 2 μl of the solution were injected. The temperature of the column oven was programmed from 120 to 310°C at 3°C/min. Retention indices for each peak were calculated according to Lee *et al.*'s method[14].

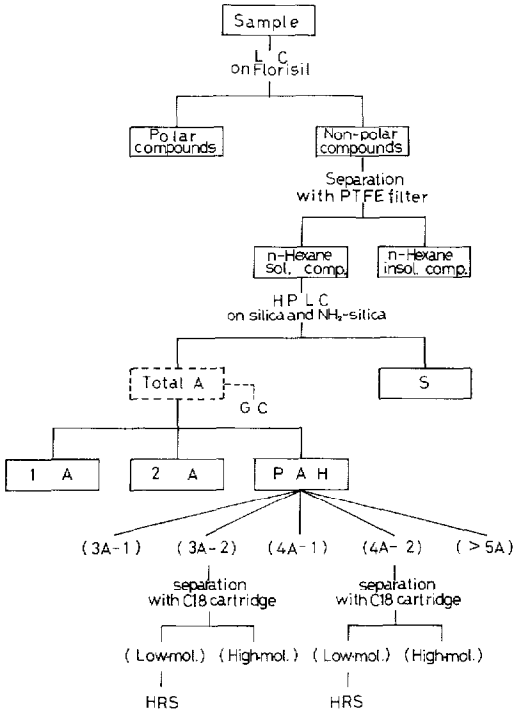

Fig. 2. Flow scheme for qualitative analysis of heavy oils. S, 1A, 2A, 3A, 4A, 5A and PAH indicate saturates, monocyclic aromatics, dicyclic aromatics, tricyclic aromatics, tetracyclic aromatics, pentacyclic aromatics and polycyclic aromatic hydrocarbons, respectively. 3A-1 and 3A-2 indicate the parent tricyclic aromatic compound and monomethylated tricyclic aromatics, respectively HRS = high-resolution fluorescence spectroscopy.

For high-resolution fluorescence spectroscopy, the 3A-2 and 4A-2 fractions were further percolated through C_{18} cartridges to remove high-molecular-weight PAHs with long alkyl chains [$>C_5$ for tricyclic aromatics and $>C_4$ for tetracyclic aromatics, judging from the percentage of retention for *n*-octylbenzene (64.5%) and *n*-butylbenzene (4.1%) and from the elution characteristics of alkylated aromatic hydrocarbons on C_{18} cartridges[15]]. Methanol (1 ml) was used as the eluent. The methanol was removed from the eluate under vacuum and monomethylated PAHs were dissolved in a suitable *n*-alkane[2,8,10] for high-resolution fluorescence spectroscopy. Tricyclic aromatics were dissolved in *n*-hexane and tetracyclic aromatics in *n*-octane.

RESULTS AND DISCUSSION

Separation by column-switching technique

Fig. 3 shows chromatograms of a high-boiling (Taching 290–340°C) petroleum distillate separated by HPLC using different columns and different techniques. The sample injected is a typical oil with a paraffinic base[16]. As Fig. 3a shows, the aminosi-

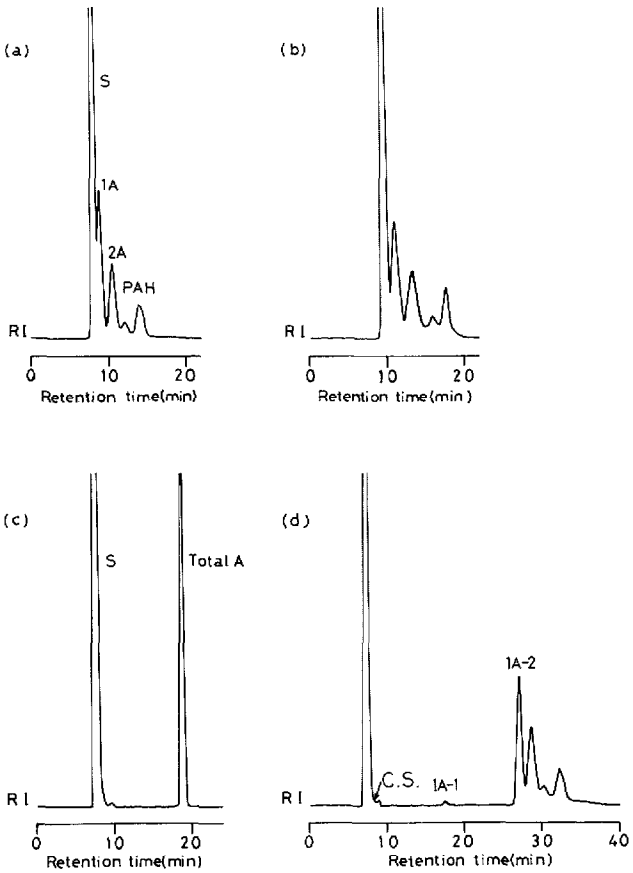

Fig. 3. HPLC traces for a Taching 290–340°C distillate. Columns or method of separation are as follows: (a) on μBondapak NH_2; (b) on μBondapak NH_2 connected with a silica column (LiChrosorb Si 100); (c) on Nucleosil 50-5, aromatics were back-flushed; (d) by the column-switching technique with Nucleosil 50-5 and μBondapak NH_2 columns. Eluent, *n*-hexane; flow-rate, 0.5 ml/min. CS = column-switching point; S = saturates; 1A = monocyclic aromatics; 2A = dicyclic aromatics; 1A-1 = monocyclic aromatics trapped between valves; 1A-2 = main band of monocyclic aromatics.

lane column does not satisfactorily separate saturates (S) from monoaromatics (1A). The use of two aminosilane columns for complete separation was reported by Grizzle and Sablotny[17]. However, this is not effective for the separation of the present kind of paraffinic oil. Fig. 3b shows that the addition of a short silica column (LiChrosorb Si 100) (50 × 4 mm I.D.) to the aminosilane column, in series, slightly increases the resolution between saturates and monoaromatics. However, the length of the silica column cannot be increased, as the resolution for ring class separation of aromatic hydrocarbons with an aminosilane column would be decreased. Better resolution between saturates and monoaromatics is obtained on the silica column, as can be seen in Fig. 3c. This advantage of the silica column and the ability of the aminosilane column to perform ring class separation was combined in the column-switching tech-

nique described here. As Fig. 3d shows, the column-switching technique permits both the separation of saturates–aromatics and separation according to ring class. Monoaromatics are separated as two peaks. The first peak (1A-1) contains early eluting monoaromatics trapped between high-pressure valves V_1 and V_2 during the elution of saturates in flow mode I. With the column-switching technique, direct elution of aromatics (Fig. 3c) is also possible in flow mode III. In spite of these improvements, monoaromatics having a large alkyl chain ($> C_{19}$) are still eluted with saturates.

Identification of monomethylated polycyclic aromatic hydrocarbons

The column-switching technique was applied to the identification of methylphenanthrene (MP), methylchrysene (MC) and methylbenz[*a*]anthracene (MBA) isomers in Kuwait 340–500°C distillate as a preparative method for capillary gas chromatography and high-resolution fluorescence spectroscopy. Fig. 4 shows a capillary gas chromatogram of aromatics (Total A in Fig. 2) obtained in flow mode III. For comparison, the chromatogram of triaromatics-2 obtained in flow mode II (fractionation was carried out five times) is also shown. The chromatogram of the aromatics shows that PAHs are clearly separated from saturated hydrocarbons.

Retention indices[14] were used for determination of MP and MC from a given chromatogram. MP and MC could be determined from the retention indices on Ultra 2 (Hewlett-Packard), reported by Radke *et al.*[18], since the characteristics of Ultra 2 are very similar (slightly polar) to those of SPD-5, as indicated in Table I. Peaks 1–4, in Fig. 4 correspond to MP. According to the retention indices, 3MP (peak 1), 2MP (peak 2), 4MP and 9MP (peak 3) and 1MP (peak 4) are determined. Distinction between 4MP and 9MP (peak 3) is impossible owing to overlapping. Similarly, 3MC (peak 8), 2MC (peak 9), 5MC (peak 10), 6MC and 4MC (peak 11) and 1MC (peak 12) are determined using retention indices. However, 2MC overlaps with 5MBA and 5MC with 4MBA, according to the retention indices on SE-52 reported by Lee *et al.*[14]

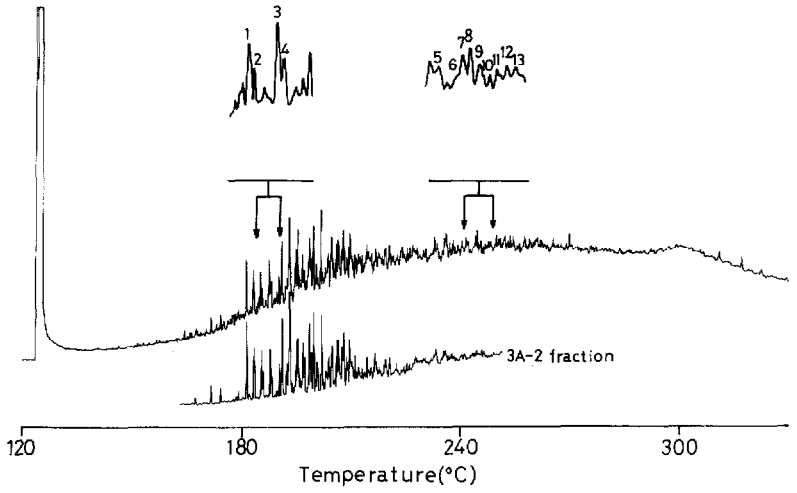

Fig. 4. Capillary gas chromatogram of aromatics from Kuwait 340–500°C distillate. Peaks 1–4 correspond to methylphenanthrene (MP) isomers and 5–13 to methylchrysene (MC) and methylbenz[*a*]anthracene (MBA) isomers.

TABLE I

RETENTION INDICES OF POLYCYCLIC AROMATIC HYDROCARBONS

Retention indices for Ultra 2 and SE-52 are taken from refs. 18 and 14, respectively.

Compound	*Retention index*		
	SPD-5	*Ultra 2*	*SE-52*
Phenanthrene	300	300	300
3-Methylphenanthrene		318.37	319.46
2-Methylphenanthrene	318.91	319.09	320.17
9-Methylphenanthrene		322.51	323.06
4-Methylphenanthrene		322.51	323.17
1-Methylphenanthrene	322.63	323.36	323.90
3,6-Dimethylphenanthrene	335.57	336.18	337.83
Fluranthene	343.18	343.52	344.01
Pyrene	350.87	350.20	351.22
Benz[*a*]anthracene	398.57		398.50
Chrysene	400	400	400
Triphenylene	400		400
11--Methylbenz[*a*]anthracene			412.72
2-Methylbenz[*a*]anthracene			413.78
1-Methylbenz[*a*]anthracene			414.37
9-Methylbenz[*a*]anthracene			416.50
3-Methylbenz[*a*]anthracene			416.63
8-Methylbenz[*a*]anthracene			417.56
6-Methylbenz[*a*]anthracene			417.57
3-Methylchrysene		416.98	418.10
5-Methylbenz[*a*]anthracene			418.72
2-Methylchrysene		418.28	418.80
12-Methylbenz[*a*]anthracene			419.39
4-Methylbenz[*a*]anthracene			419.67
5-Methylchrysene		419.56	419.68
6-Methylchrysene		420.31	420.61
4-Methylchrysene		420.31	420.87
1-Methylchrysene		422.38	422.87
7-Methylbenz[*a*]anthracene			423.14
Benzo[*e*]pyrene	452.41	453.18	450.18
Benzo[*a*]pyrene	454.11	454.83	453.44
Perylene	457.43	457.88	456.22

(Table I). Other monomethylated tetracyclic aromatic compounds (methylbenzo[*c*]phenanthrenes, -triphenylenes and -tetracenes) could also be present and eluted with MC and MBA, giving only a tentative identification of MC.

High-resolution fluorescence spectroscopy is a powerful tool for the identification of isomers of methylated PAHs[8,10]. The existence of several monomethylated PAH in sample were confirmed by this technique. The tricyclic aromatics-2 (3A-2) in Fig. 2 and tetracyclic aromatics-2 (4A-2) in Fig. 2 were obtained as illustrated in Fig. 5. Fig. 6 shows the spectrum of tricyclic aromatics-2 containing anthracenes and phenanthrenes. Owing to the selectivity of high-resolution fluorescence spectroscopy, only the last compound series is observed. In this spectrum, the existence of 9MP is shown by a strong peak. On the other hand, fluorescence from 4MP is weak. This

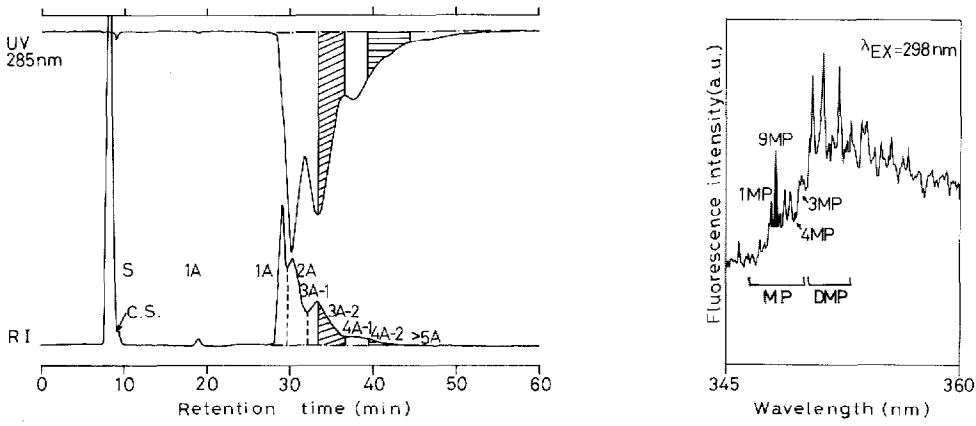

Fig. 5. Chromatograms of a Kuwait 340–500°C distillate obtained by the column-switching technique. Abbreviations as in Figs. 2 and 3. Flow-rate, 0.44 ml/min.

Fig. 6. High-resolution fluorescence spectrum of tricyclic aromatics-2 fraction. MP and DMP correspond to the methylphenanthrene and dimethylphenanthrene isomers, respectively, as reported elsewhere[19].

trend coincides with that reported by Garrigues and Ewald[8]. Although fluorescence from 1MP is also detected, that from 3MP is not clear because of overlap with the fluorescence from some of the dimethylphenanthrenes[19]. This overlapping problem may be easily eliminated by fractionating methylphenanthrenes concentrated by HPLC using an ODS column[8].

Fig. 7 shows the spectra of tetracyclic aromatics-2, containing MC and MBA, obtained by excitation at 274 and 294 nm. The spectrum obtained by selective excitation at 274 nm indicates that 1-, 2-, 3-, 4- and 6MC are contained in the tetracyclic aromatics-2. The existence of 5MC is not clear in this spectrum. On the other hand, the spectrum obtained by selective excitation at 294 nm indicates that 5-, 6-, 7- and 11MBA are also contained in the tetracyclic aromatics-2.

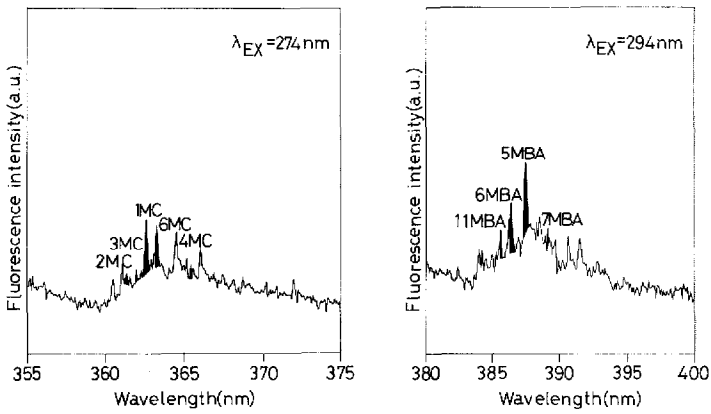

Fig. 7. High-resolution fluorescence spectrum of tetracyclic aromatics-2 fraction. MC and MBA represent methylchrysene and methylbenz[*a*]anthracene isomers, respectively.

CONCLUSION

A column-switching technique permitted the rapid fractionation of both aromatics and each aromatic ring type. It was applied as a preparative method to the identification of monomethylated PAHs by using gas chromatography and high-resolution (Shpol'skii) fluorescence spectroscopy. It was demonstrated that the separation by this technique is very reliable and is applicable to the analysis of heavy oils.

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REFERENCES

1 J. Jacob, W. Karcher and P. J. Wagstaffe, *Fresenius Z. Anal. Chem.*, 317 (1984) 101.
2 *Spectral Atlas of Polycyclic Aromatic Compounds*, Vol 1, edited by W. Karcher, R. J. Fordham, J. J. Dubois, P. G. J. M. Glaude and J. A. M. Ligthart, Reidel, Dordrecht, 1985 and Vol. 2, edited by W. Karcher, Kluwer, Amsterdam, 1988.
3 M. Popl, M. Stejskal and J. Mostecky, *Anal. Chem.*, 47 (1975) 1947.
4 D. L. Pullen and D. V. Scammells, *Fuel*, 67 (1988) 251.
5 Y. L. Tan, *Anal. Lett.*, 21 (1988) 553.
6 J. F. Mckay and D. R. Latham, *Anal. Chem.*, 44 (1972) 2132.
7 J. F. Mckay and D. R. Latham, *Anal. Chem.*, 45 (1973) 1050.
8 P. Garrigues and M. Ewald, *Anal. Chem.*, 55 (1983) 2155.
9 W. Giger and M. Blumer, *Anal. Chem.*, 46 (1974) 1663.
10 P. Garrigues, M.-P. Marniesse, S. A. Wise, J. Bellocq and M. Ewald, *Anal. Chem.*, 59 (1987) 1695.
11 T. Spitzer and W. Dannecker, *J. Chromatogr.*, 267 (1983) 167.
12 W. E. May and S. A. Wise, *Anal. Chem.*, 56 (1984) 225.
13 S. A. Wise, B. A. Benner, S. N. Chesler, L. R. Hilpert, C. R. Vogt and W. E. May, *Anal. Chem.*, 58 (1986) 3067.
14 M. L. Lee, D. L. Vassilaros, C. M. White and M. Novotny, *Anal. Chem.*, 51 (1979) 768.
15 N. Theobald, *Anal. Chim. Acta*, 204 (1988) 135.
16 W. L. Nelson, *Petroleum Refinery Engineering*, McGraw-Hill Kougakusha, Tokyo, 4th ed., 1958, p. 81.
17 P. L. Grizzle and D. M. Sablotny, *Anal. Chem.*, 58 (1986) 2389.
18 M. Radke, H. Willsch and D. H. Welte, *Anal. Chem.*, 56 (1984) 2538.
19 P. Garrigues and R. Lapouyade, in *Proceeding of the 11th International Symposium on Polycyclic Aromatic Hydrocarbons, Gaithersburg, MD, September 1987*, in press.