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Development of an on-line coupling of liquid–liquid extraction, normal-phase liquid chromatography and high-resolution gas chromatography producing an analytical marker for the prediction of mutagenicity and carcinogenicity of bitumen and bitumen fumes

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Abstract

A fast and fully automated system for the determination of polycyclic aromatic compounds (PACs) is described. The system has been developed to produce an analytical 'marker', correlating chemical characteristics (including PAC analysis) with mutagenicity and carcinogenicity. The products of interest are bitumen fumes, bitumen and other (heavy or even residual) oil products, regardless of their boiling range. Dimethyl sulphoxide (DMSO) extractables obtained from a flow-injection analysis (FIA) system are introduced on-line in a normal-phase liquid chromatographic (NPLC) system. Here, the PACs are separated from the DMSO and possible co-extracted heavy residual species. The final step incorporates on-line gas chromatographic analysis of the three-to-six-ring PAC fraction, followed by flame-ionisation detection for quantification. It was demonstrated that data obtained from samples in the distillate lubrication-oil range correlate well with data obtained from the manual DMSO-extraction method standardised by the Institute of Petroleum as IP346.

Keywords: Bitumen; Liquid chromatography–gas chromatography; Hyphenated techniques; Instrumentation; Polynuclear aromatic hydrocarbons

1. Introduction

Worker exposure to polycyclic aromatic compounds (PACs) in bitumen fumes may pose a health hazard, because some PACs have been found to be carcinogenic. The analysis of a number of individual PACs is often used as a marker for potential mutagenicity and carcinogenicity. There are, however, indications that this is not a good approach, since only a few of the vast number of potentially hazardous PACs are quantified [1].

A fundamentally different, yet successful, approach was proposed by Van der Wiel [2]. He used dimethyl sulphoxide (DMSO) and cyclohexane to selectively extract the potentially carcinogenic PACs (those containing no, or only a few, short substituents) out of a wide range of lubricating-oil (luboil) base stocks. Total PAC contents were determined gravimetrically. Refractive-index (RI) measurements served as a yardstick for aromaticity. The

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analytical data obtained from this method proved to correlate with the available toxicological evidence based on rodent skin-painting tests. In 1980, this method was adopted by the Institute of Petroleum (IP, London, UK) as IP346 [3]. CONCAWE (the oil companies' European organization for environment, health and safety) recommends that all substances with a DMSO extract of >3% (w/w) (by IP346) should be considered carcinogenic [4].

Although simple and inexpensive with regard to equipment, the method has some drawbacks. It is a go/no-go test [4] and requires about 4 g of sample, making it incompatible with, for instance, bitumen fume sampling, where sample sizes are in the order of 1 mg. When applied to very heavy ('residual') oil fractions, large PACs (with more than seven fused aromatic rings) will also be extracted. These compounds show no or very little mutagenicity and carcinogenicity and therefore compromise the correlation. Furthermore, the method is laborious and requires several liters of solvent per analysis.

DMSO extraction followed by gas chromatographic analysis was reported for bitumen-fume condensates (BFCs) [5]. This method yielded encouraging correlations between the concentrations of three-to-six-ring PACs and the mutagenicity and carcinogenicity as determined by Ames testing [1]. Because BFCs were only available in small amounts, most of the test development and validation has been done with distillate luboils, which are a good surrogate for BFCs [1] and for which IP346 mutagenicity and often carcinogenicity data are available.

Our aim was to extend this method to a general, automated method that allowed the determination of the PAC content of oil products, regardless of their boiling range.

The use of flow injection analysis (FIA) as an extraction technique has a number of advantages in comparison with manual DMSO extraction. It is fast, easy to automate and requires only small amounts (milligrams) of sample. When working with such small amounts, gas chromatography with flame-ionisation detection (FID) can be used to determine the total amount of material extracted. At the same time, it provides an accurate way to measure exclusively the three-to-six-ring aromatics. However, gas chromatographic analysis can be adversely affected by the introduction of large amounts of DMSO and of possible co-extracted residual material. Normal-phase liquid chromatography (NPLC) can be used to separate these interfering materials from the PAC fraction of interest.

2. Experimental

2.1. Instrumentation

The experimental set-up was built around a Dualchrom 3000-series HPLC–high-resolution (HR) GC combination (Carlo Erba/CE Instruments, Milan, Italy). Dualchrom software (version 1.5, Carlo Erba) running on a 486 DX personal computer (Interscience, Breda, The Netherlands) was used to control the LC autosampler, pumps, UV detector, LC–GC interface, and the gas chromatograph. Chrom-card software (version 1.17, Carlo Erba) running on a separate personal computer (Compaq DESKPRO 4000DT model 5166) was used for data processing.

2.1.1. FIA equipment (liquid–liquid extraction)

The FIA part of the set-up consisted of a 5 m×1/16 in. O.D.×0.50 mm I.D. stainless steel (SS) coil (1 in.=2.54 cm). Two Pharmacia LKB P-500 high precision pumps (Pharmacia LKB Biotechnology, Uppsala, Sweden) were used to deliver cyclohexane (LiChrosolv, Merck, Darmstadt Germany) and DMSO (spectrophotometric grade, Janssen Chimica, Geel, Belgium) to the FIA system at a rate of 0.5 ml/min each. Samples were dissolved in cyclohexane and introduced into the cyclohexane stream using a six-port valve (Valco, Houston, TX, USA) equipped with a 9-μl loop. Depending on the amount of sample available, the latter was filled either manually (syringe) or using an autosampler (AS550, Carlo Erba). The segmentor of the FIA system was constructed out of a 1/16 in. SS tee (Valco) and will be discussed below. The separator used was of the SS–polytetrafluoroethylene (SS–PTFE) sandwich type (Free University Amsterdam, The Netherlands) [6]. The lower connections to the separator were made of 1/16 in. SS, and the upper connection (exit of cyclohexane) was of 1/16 Teflon. The separator was balanced using an 'S' series metering valve (Nupro, Willoughby, OH, USA) in

the cyclohexane exit line. This metering valve was equipped with a Kalrez seal-ring and a custom-made Vespel body-seal for use with cyclohexane.

2.1.2. Normal-phase liquid chromatography

A Phoenix 30 micro-HPLC pump with a slave pump (Carlo Erba) was used to pump a mixture of tetrahydrofuran (THF; spectroscopic grade, Merck)–*n*-octane (analytical reagent grade, Fluka, Buchs, Switzerland) at 250 μl/min through a 25 cm×2.0 mm I.D. Chromegabond RingSep NPLC Column (ES Industries, Berlin, NJ, USA). The latter features a nitroaromatic-substituted silica phase. To reduce the background of the final gas chromatographic analysis to a (constant) minimum, both THF and *n*-octane were distilled prior to use. A Micro-UVIS20 UV detector (Carlo Erba) equipped with a 1.2-μl microbore flow cell was operated at 254 and/or 300 nm for determining the time window of the fractions to be transferred to the gas chromatograph. Its outlet was connected to the LC–GC interface.

2.1.3. LC–GC interface

The standard loop-type interface with early vapour exit provided on the Dualchrom 3000 was used. The system incorporated an extra six-port valve (Valco) mounted between the UV detector and the interface, to circumvent mixing within the sample loop [7]. This mixing effect, which is caused by the parabolic Hagen-Poiseuille flow profile, would otherwise result in loss of solute material of interest [8]. 1,3-Diphenyl-1,1,3,3-tetramethyldisilazane (DPTMDS; 10 m×0.53 mm I.D.) deactivated retention gaps were obtained from BGB Analytik (Rohtenfluh, Switzerland). The first 3 m of the fused-silica capillary column used (see below) served as a retaining pre-column. All connections, including the T-piece connecting the sample valve and the carrier-gas valve to the retention gap, were made using all-glass Press-Fit connectors (Techrom, Purmerend, The Netherlands). The T-piece was placed inside the oven for reasons discussed below. Because THF caused swelling of the seat of the original early vapour exit valve (Sirai, Pioltello, Milan, Italy), the latter was replaced by a three-port Valco valve. This problem has since been recognised by Carlo Erba (similar phenomena have been observed with dichloromethane and ethyl acetate), and later versions of the Dualchrom system have been adapted by introducing a specially designed, chemically inert, heated solvent-vapour exit.

2.1.4. Gas chromatography

The capillary column used was a 30-m×0.32 mm I.D. fused-silica capillary coated with 0.25 μm 5% phenyl methylpolysiloxane (DB-5MS, J&W Scientific, Folsom, CA, USA). Helium was used as the carrier gas. The initial oven temperature of 120°C was held for 6 min. Linear programming was performed at a rate of 10°C/min up to 250°C and, thereafter, at 5°C/min. The final temperature of 350°C was maintained for 20 min. A flame-ionisation detector operated at 375°C was used for the final detection.

A schematic diagram of the entire FIA–NPLC–HRGC system is presented in Fig. 1.

2.2. Samples used for calibration, tuning and performance control

The use of these samples will be discussed in detail in the appropriate sections. All samples were dissolved in cyclohexane (spectroscopic grade, Merck).

1. A mixture of 17 individual non-substituted PACs with two to six rings (naphthalene to coronene) was used for the determination of retention time windows in the gas chromatograms. The certified PAC mixture (minus coronene) was obtained from Supelco (Bellefonte, PA, USA). Coronene was obtained from our internal sample collection (SRTCA, Amsterdam, The Netherlands) and had a purity of >98%, as measured by GC.
2. A mixture of four non-substituted PACs: naphthalene (Sigma–Aldrich), phenanthrene, coronene and benzocoronene [>98% (GC); SRTCA], was used to select the NPLC fraction to be transferred to the GC.
3. Standard sample a: anthracene oil (Cindu, Uithoorn, The Netherlands). This product consists primarily of a limited number of two–four-ring PACs and is 91% DMSO-extractable by IP346. Solutions in cyclohexane covering a wide range of concentrations were used for overall perform-

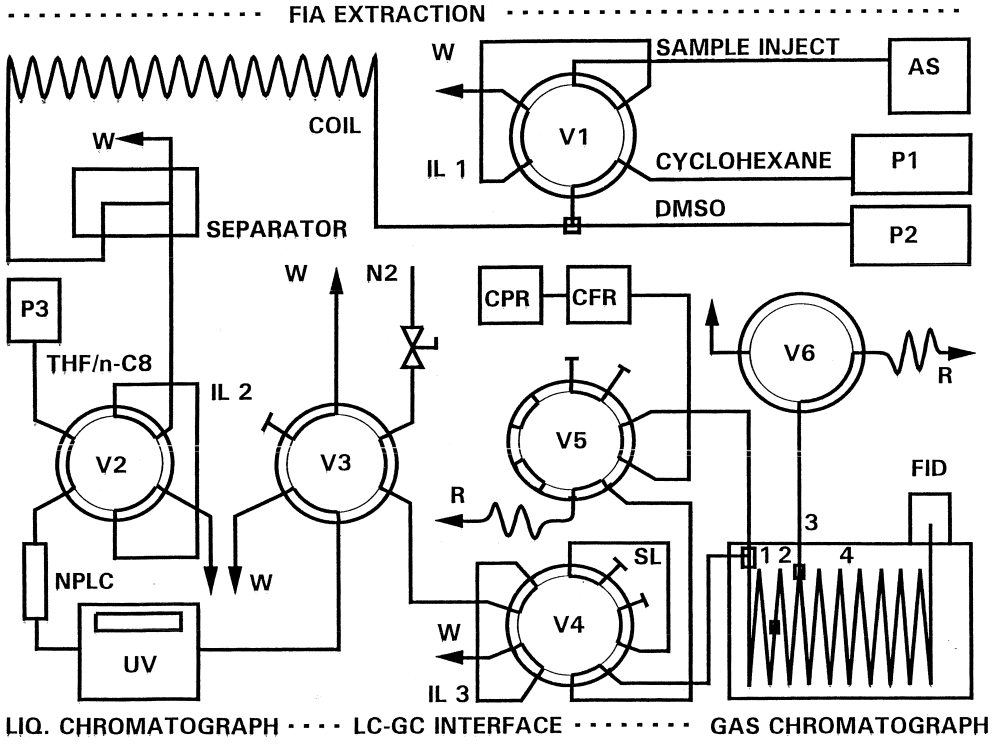

Fig. 1. Schematic diagram of the FIA–NPLC–HRGC system. AS, autosampler; P1, cyclohexane pump; P2, DMSO pump; V1, FIA, injection valve; IL1, FIA, injection loop; W, waste; P3, NPLC pump; V2, NPLC, injection valve; IL2, NPLC, injection loop; NPLC, NPLC column; UV, UV detector; V3, valve to circumvent mixing in the LC–GC, injection loop; N2, nitrogen purge of the injection loop; V4, LC–GC, transfer valve; IL3, LC–GC, injection loop; SL, standard loop; V5, carrier-gas switching valve; R, fused-silica restriction; CPR, constant-pressure regulator; CFR, constant-flow regulator; V6, early-vapour-exit valve; 1, retention gap; 2, retaining pre-column; 3, early vapour exit; 4, analytical column; FID, flame-ionisation detector.

ance control and for determining the FID response.

4. Standard sample b: luboil LVI-50 (Shell). This sample covers a broad spectrum of PACs. Approximately 0.25% (w/w) of it is DMSO-extractable. Solutions in cyclohexane covering a wide range of concentrations were used for determining the FID response.
5. Because bitumen fumes are only available in mg quantities, and bitumen fumes are similar to luboil with regard to boiling point distributions and chemical type composition [1], a series of ten distillate luboils (Shell) was used for studying correlations between the current method and IP346.

3. Results and discussion

3.1. Optimisation of the flow-injection analysis

3.1.1. Segmentation

The segmentor of the FIA system was constructed out of a 1/16 in. SS tee (Valco). DMSO was introduced into the cyclohexane stream via a piece of 0.25 mm I.D. fused silica, as depicted in Fig. 2. The actual DMSO and cyclohexane confluence point is constructed using two concentric tubes, with cyclohexane in the SS outer tube. Since DMSO has a high affinity for the walls of the SS extraction coil, this will result in immediate encapsulation of the cyclohexane (sample) stream between segments of DMSO.

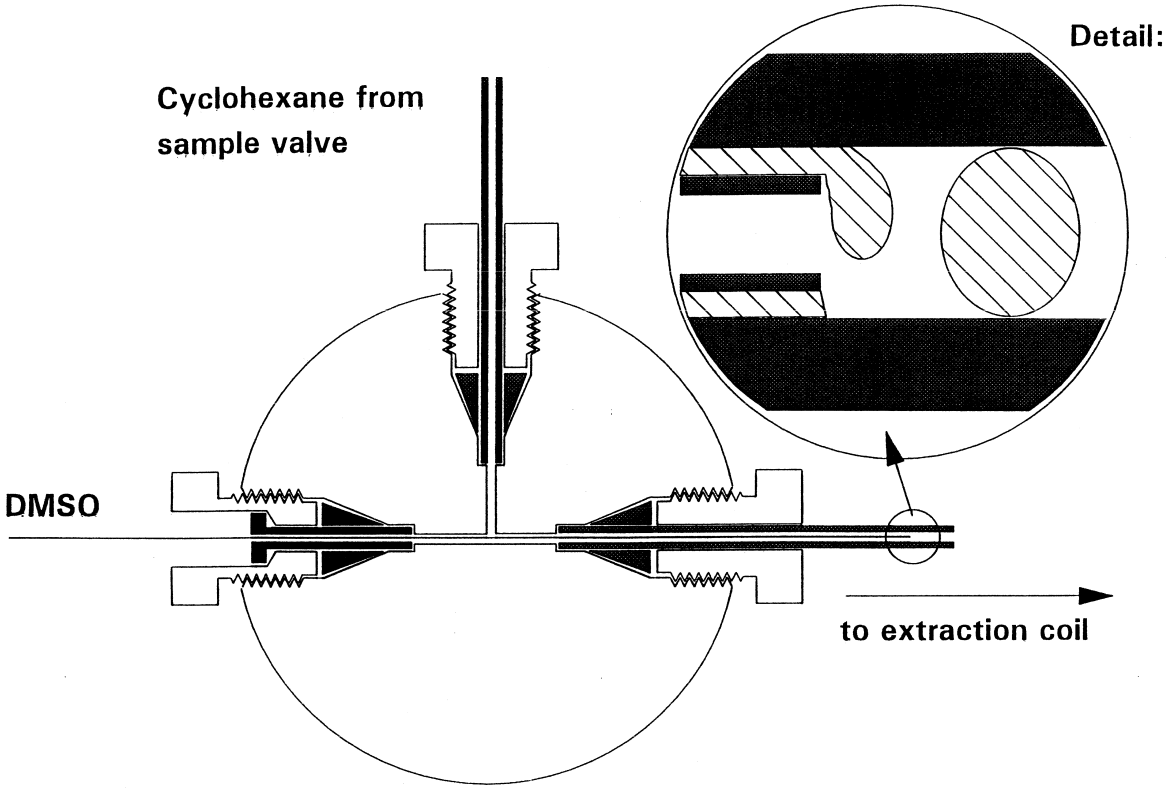

Fig. 2. Schematic diagram of the FIA segmentor.

The aim of this design was to minimise band broadening, which is crucial when working with samples of only a few milligrams, as obtained in bitumen-fume sampling. With this segmentor, we were able to create spherical cyclohexane droplets with diameters as small as 0.5 mm (i.e. the internal diameter of the coil). Glass has a similar behaviour as SS in a cyclohexane–DMSO FIA system, i.e. a high affinity for DMSO (in contrast to PTFE, which has a high affinity for cyclohexane). Indeed, we could observe the formation of a sequence of 0.5 mm cyclohexane droplets in a 1/16 in. O.D.×0.5 mm I.D. glass tube under a microscope, as depicted in Fig. 2.

Although the pumps used were of the 'pulse-free' type, this appeared to be not absolutely true. Pulses originating from the pumps' stepper-motor probably caused every sixth cyclohexane droplet to be approximately three times bigger than the other five, regardless of the flow-rate of the cyclohexane–DMSO (1:1, v/v) mixture.

3.1.2. Separation

The sandwich-type separator unit consists of an SS block and a PTFE disc, both with a groove. These are held together using a second SS block and four screws, thus creating a 1-cm×1.5 mm I.D. open tubular channel with a PTFE upper wall and an SS lower wall. The separation principle of the sandwich-type separator is based on the affinity of the construction materials (PTFE top and SS bottom) for the two different solvents and on the densities of the latter. The separator was originally designed to separate aqueous and organic phases, but should also be capable of separating DMSO and cyclohexane. DMSO has affinity for SS and is the more dense

solvent; cyclohexane has affinity for PTFE and is the less dense solvent. The separator was balanced using a metering valve (Nupro) placed in the cyclohexane exit line. Optimum balance and separation efficiency were determined by leading the DMSO and cyclohexane outlet streams into graduated cylinders and subsequent inspection of their contents. For ease of use, these cylinders were provided with a valve at the bottom and an overflow at the top.

In the original configuration, a separation of about 85% could be realized between DMSO and cyclohexane. The incomplete separation is probably caused by a small discrepancy between the volumes of the individual segments and the internal volume of the separator. In order to improve the separation, the SS lower outlet of the separator was reamed to some 3 mm, thus creating a slightly larger cavity in which the two phases could separate. After this small modification, a separation of at least 95% between the two phases could be realized. Once balanced, the separator proved to be stable for periods well in excess of one month.

The DMSO fraction containing the extracted PACs was collected in a 50-μl loop of a six-port sampling valve (Valco) for subsequent injection onto the NPLC column. The volume of the loop is a compromise: It is not large enough to contain the entire PAC fraction, but not so large as to jeopardise the subsequent LC separation. The time of injection was set to the moment the loop was filled with the maximum amount of extract. This moment was established by slightly varying the LC injection times, while striving for maximum UV response obtained from the standard sample, when using the combined FIA–NPLC–UV part of the total set-up. Once this moment was established, it proved to be stable to within 1 s during a period of several months.

3.1.3. Extraction selectivity and recovery

In order to make the extraction more selective towards the non-substituted PACs and short-alkyl-substituted PACs and, thus, applicable to a wider range of products, water was added as a modifier to the DMSO. From previous studies, we knew that optimal conditions (i.e. increased selectivity and preservation of the recoveries of non-substituted PACs) are obtained when 3.5% (v/v) water is added. Fig. 3 shows recovery results for a series of substituted and non-substituted PACs from a prototype FIA–LC–GC set-up obtained with DMSO to which no and 3.5% (v/v) water was added, vs. the calculated fraction of aromatic carbon of these PACs. Because of this, all further analyses presented in this work were carried out with 3.5% water added to the DMSO. For further details, see Sections 3.4 and 3.5 below.

The overall recoveries of a number of non-substituted two–six-ring PACs obtained with the present set-up are presented in Table 1.

3.2. Normal-phase liquid chromatography

The LC step in between FIA and HRGC served two objectives, i.e.

1. Separation of the extracted PACs from DMSO. Traces of the solvent would seriously hamper the final GC analysis by obscuring a substantial fraction of the chromatogram
2. (Rough) separation of the three–six-ring aromatics from seven+ring aromatics. Transfer of seven+ring aromatics to the GC would cause serious fouling problems because of their lack of volatility at the maximum temperature of the retention gap–GC column combination used.

Initially, we studied whether or not size-exclusion chromatography (SEC) would meet the above requirements. Using THF as the eluent, objective **1** was readily achieved with several SEC media, including cross-linked polystyrene and porous silica microspheres. Although SEC worked satisfactorily as a second separation step when analyzing BFCs, objective **2** was not met for residual samples. In a different study, we had already observed unpredictable behaviour in the separation of aromatic species using SEC [9] and a change towards NPLC was considered. A separation of aromatics according to the number of rings can be achieved when, together with a non-polar eluent, a stationary phase is used where the separation is based on interactions with aromatic π electrons. This implies, however, that the fraction containing the three–six-ring aromatics easily becomes too voluminous to be transferred to the GC, even when employing large-volume injection techniques [10]. In order to keep the transferred fraction as small as possible, we considered reducing

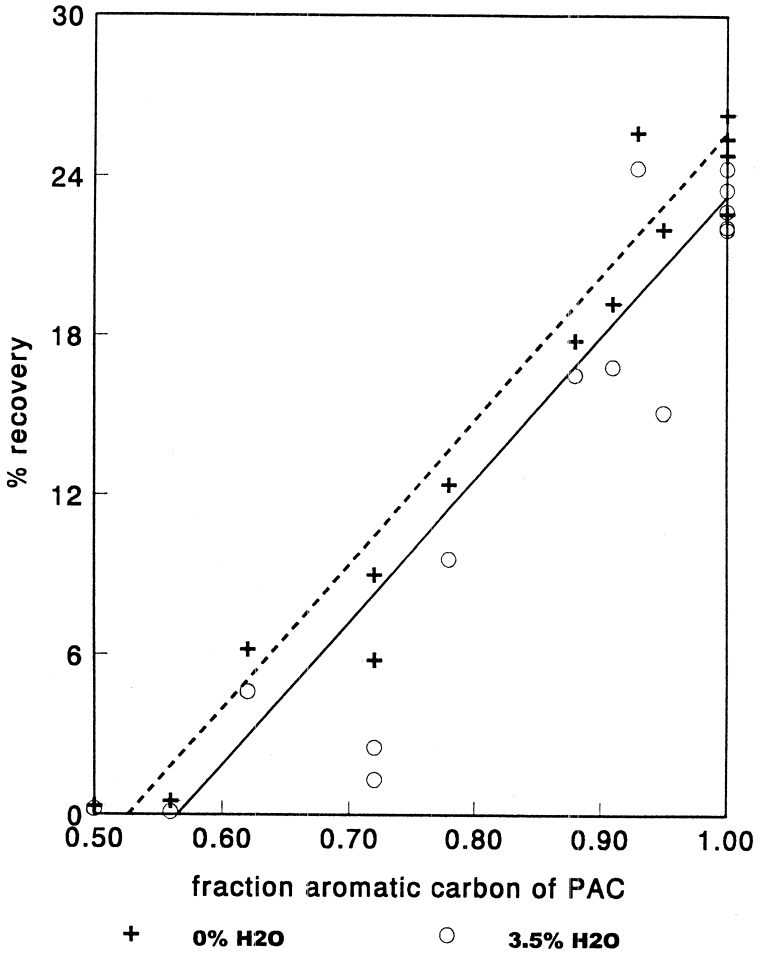

Fig. 3. PAC recovery results vs. fraction of aromatic carbon of PACs: +, 0% water added; ○, 3.5% water added.

the three–six-ring group-type separation, while preserving the separation between the three-to-six-ring aromatics and the seven+ring aromatics by adding a mobile-phase modifier. The latter could not be achieved using the frequently employed aminosilane-bonded silica phases for group-type separations. A recently released nitroaromatic-substituted silica phase, commercialised as RingSep (see Experimental), however, could successfully be used to perform the separation outlined above. Using *n*-octane with 50% (v/v) THF as modifier resulted in a separation between coronene and benzocoronene that was adequate for the present purposes (see Fig. 4). The volume of the fraction containing the three–six-ring aromatics was 608 μl (retention time, 4.40 to 6.83 min).

3.3. LC–GC transfer

The constant-flow regulator (CFR, see Fig. 1) of the GC carrier-gas control was set to a flow of 1.5 ml/min, resulting in a pressure of 64 kPa at an oven temperature of 120°C. The maximum pressure for the LC–GC transfer was set to 100 kPa using the upstream constant-pressure regulator (CPR, see Fig. 1).

The transfer of a mixture of THF–*n*-octane (50:50, v/v) at 120°C using a loop-type interface

Table 1
Percent recovery for model compounds relative to direct injection

PAC	Recovery (%) (n=5)
Naphthalene	28
Acenaphthylene	28
Acenaphthene	10
Fluorene	24
Phenanthrene	31
Anthracene	31
Fluoranthene	28
Pyrene	37
Benz[*a*]anthracene	29
Chrysene	29
Benzo[*b*]- and -[*k*]fluoranthenes	30
Benzo[*a*]pyrene	27
Indeno[1,2,3,-*cd*]pyrene	19
Dibenzo[*a*,*h*]anthracene	19
Benzo[*ghi*]perylene	25
Coronene	20
Average	26

will result in fully concurrent solvent evaporation (FCSE) of THF and partially concurrent solvent evaporation (PCSE) of *n*-octane {atmospheric boiling point (b.p.), THF 67°C, corrected for 100 kPa excess pressure 86°C; atmospheric b.p. *n*-octane 126°C, corrected 145°C [11, p. 222]}. Whereas in the NPLC step, this solvent mixture can be regarded as *n*-octane containing 50% (v/v) THF as modifier, during the LC–GC transfer, this same mixture can be regarded as THF containing 50% (v/v) *n*-octane as co-solvent.

The front end of the fraction to be transferred is fed into the (empty) injection loop of the loop-type interface by activation of the six-port valve V3 (see Fig. 1). Simultaneous activation of the two ten-port valves V4 and V5 starts the transfer of the LC fraction to the GC system. The orientation of the injection loop was chosen such that the components eluting last from the NPLC column, hence, the 'high-boiling' five–six-ring PACs were the first compo-

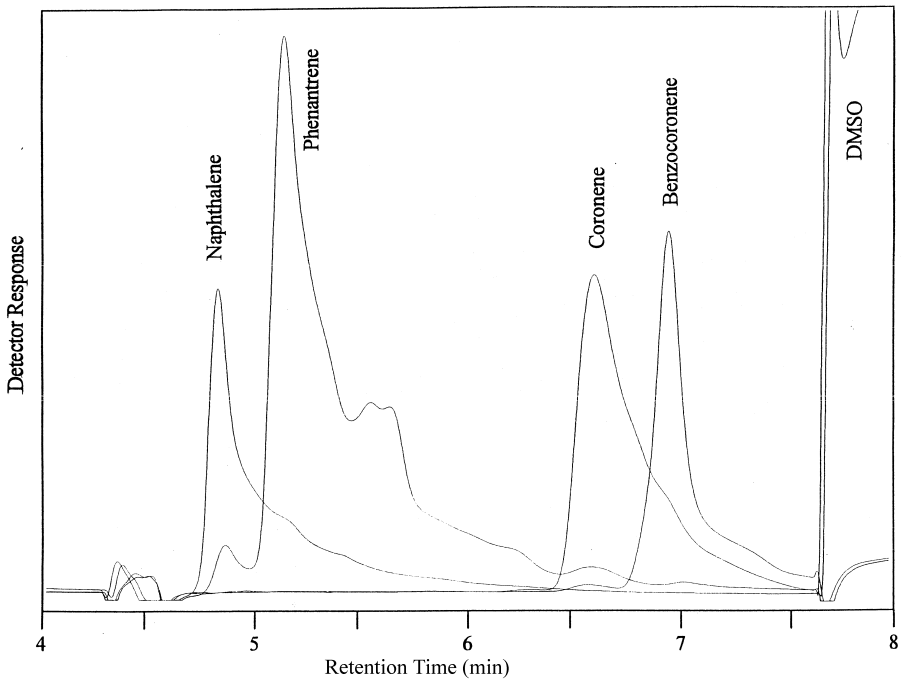

Fig. 4. NPLC separation of PACs and DMSO. The UV signal was monitored at 254 nm for the detection of naphthalene and phenanthrene and at 300 nm for coronene and benzocoronene.

nents to be transferred to the GC. The aim of this design was to prevent potential losses of analytes through the vapour exit caused by a delayed build-up of the co-solvent barrier [12]. In case the latter scenario occurs, the high-boiling PACs will still be safely reconcentrated by the phase-ratio focusing effect. More volatile PACs that really rely on co-solvent trapping will be introduced well after the build-up of an adequate barrier.

Verification of the GC-transfer conditions (temperature, length of retention gap) was performed visually by observing the evaporation process through a borosilicate glass (Pyrex) door, as described in a previous paper [7]. Presuming the use of a 10 m (0.53 mm I.D.) retention gap, the transfer temperature of 120°C was established as a condition to ensure reliable operation [i.e. no risk of penetration of (co-)solvent into the retaining pre-column, while ensuring the presence of an adequate co-solvent barrier during transfer].

The position of the T-piece connecting the sample valve and the carrier-gas valve to the retention gap was found to be important. The best results were obtained with the T-piece positioned inside the GC oven. Instead of the oscillating progress of the (main) solvent followed by violent evaporation, which is caused by delayed evaporation on the otherwise smooth and deactivated surface of the retention gap [7,11 p. 326], the site of evaporation remained stable at the very head of the retention gap. The bare fused-silica ring inside the press-fit tee seemed to act as a 'boiling bead', causing the process of evaporation to be much less violent than with the T-piece positioned outside of the oven. This also implied that the build-up of the co-solvent layer inside the retention gap became a more regular process, further enhancing the reliability of the system.

The time corresponding to the end of the transfer was established from the pressure drop occurring when the sample plug no longer prevented the carrier gas from flowing. The transfer was considered to be complete 10 s after a pressure drop of 50 kPa. The early vapour exit was closed 1 min later. The six-port valve V3 remained activated after the transfer. This resulted in flushing of the injection loop with clean solvent coming from the NPLC column, thereby minimizing sample carry-over. Valve V3 was allowed to return to its rest position 30 min after its activation. Then, a flow of 20 ml/min nitrogen emptied the, by then clean, injection loop in preparation for the next transfer.

3.4. Gas chromatography and final detection

The final gas chromatographic step served two purposes, i.e.,

1. Accurate and reproducible separation of the three–six-ring aromatic fraction from any remaining interfering materials.
2. Determination of the total (absolute) amount of extracted material within this fraction using flame-ionisation detection.

The carrier-gas control deserves some further explanation. As described above, the maximum pressure for the LC–GC transfer was set to 100 kPa. This resulted in a relatively low transfer temperature, allowing for the use of *n*-octane as co-solvent. However, since this pressure was lower than the 125 kPa required to maintain a flow of 1.5 ml/min at 350°C, this would result in a gradual change from constant-flow to constant-pressure carrier-gas control during the temperature programmed run. The maximum column head pressure observed was 76 kPa at an oven temperature of 350°C. This peculiar carrier-gas control will not affect the GC analysis in an unfavourable way; in fact, the linear gas velocity will stay close to its optimum throughout the run (28 cm/s at 120°C, decreasing to 23 cm/s at 350°C) [13]. This kind of control does not adversely affect the retention-time stability, which is crucial for the delimitation of the three–six-ring aromatic fraction.

Calibration of the FID system (mV min μg product^{-1}) was done by injecting solutions of the anthracene oil and the LVI-50 luboil in cyclohexane using the on-column injector provided on the Dualchrom system. To allow for proper on-column injection of cyclohexane solutions, the initial oven temperature was lowered to 65°C and kept at this value for 5 min. The remainder of the oven temperature programme was identical to that during the FIA–NPLC–GC operation. Injections were performed under constant-pressure conditions (52 kPa), using the constant-pressure regulator provided with the on-column injector. At the start of the first temperature ramp, the flow control of the loop-type interface (see Sections 3.3 and 3.4) took over.

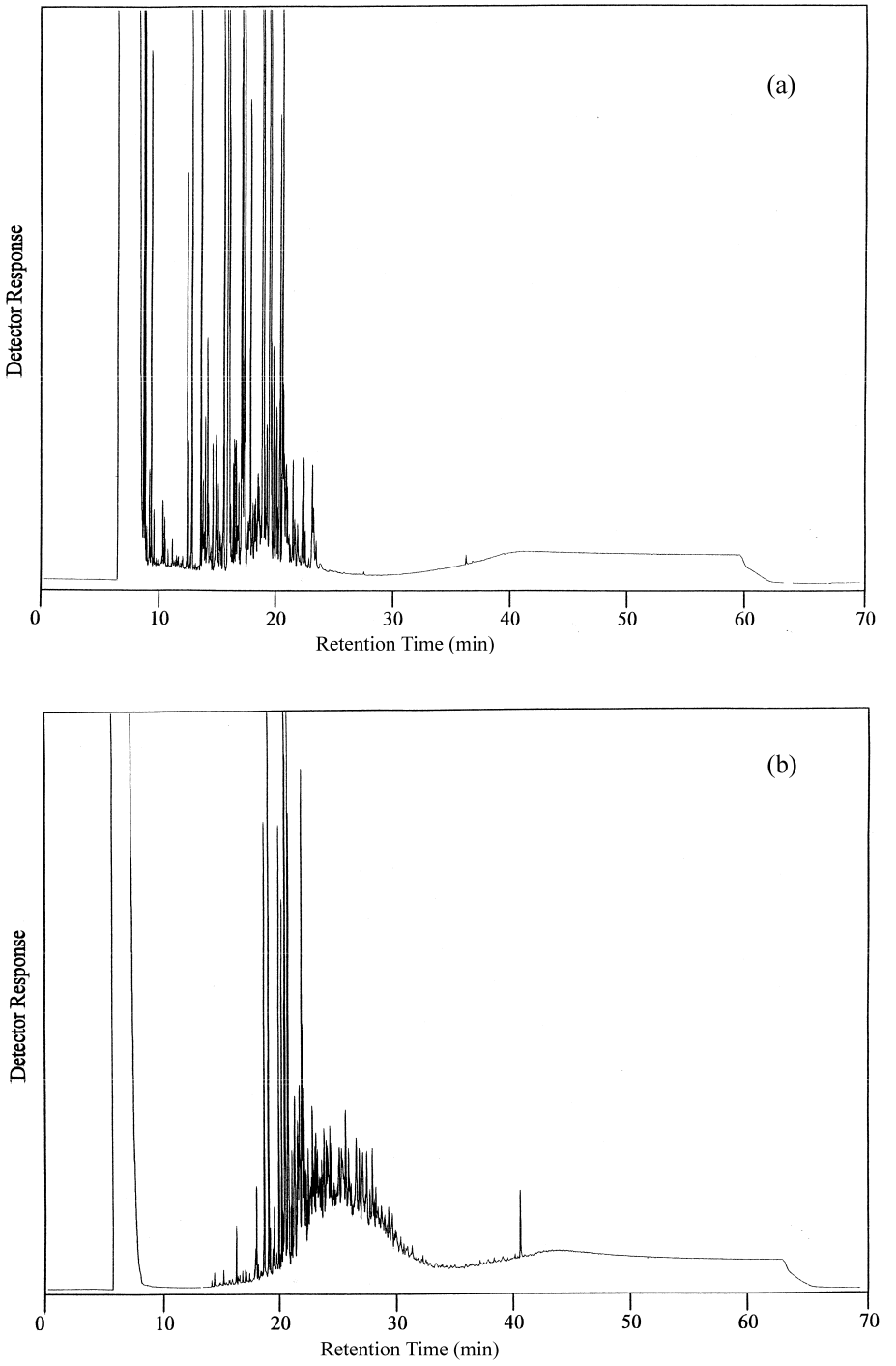

Fig. 5. (a) Typical gas chromatogram obtained from the anthracene oil. (b) Typical gas chromatogram obtained from the LVI-50 luboil. (c) Typical gas chromatogram obtained from a blank injection.

Because of the complexity of the resulting chromatograms, baseline levels were established during the initial (isothermal) part of the chromatograms, and held constant throughout the entire chromatogram. The total area of the signals resulting from the samples were subsequently calculated by subtracting the total area obtained from blank runs. In doing so, data are adequately compensated for solvent impurities and column bleed. Using this approach, the lower limit of quantification (LLQ) of the analytes is determined by the spread in total area measured for blank runs. With a measured sensitivity of the FID of 110 mV min μg product^{-1}, this results in a LLQ of 0.14 μg of extracted material. In Fig. 5, typical chromatograms obtained from the anthracene oil (Fig. 5a), the LVI-50 luboil (Fig. 5b) and a blank injection (Fig. 5c) are presented.

3.5. Correlation of results with IP346

A requirement of the IP346 determination is that the sample must be distilled ('topped') to remove the fraction boiling below 300°C, which has a negligible mutagenicity and carcinogenicity, but nevertheless can contribute to the total amount of extract. In the current set-up, this requirement is met by integrating only the fraction eluting after the time corresponding to a boiling point of 300°C. This point was established by interpolation from the retention time vs. boiling point plot obtained from the standard mixture of 17 non-substituted PACs. The fraction eluting beyond the 300°C is designated as 'the FIA–DMSO IP346 equivalent'.

With the mixture of the 17 non-substituted PACs, a system recovery of ca. 26% is measured, as compared with direct injection into the GC system (see Table 1, Section 3.1.3). This seems to be low, but, in fact, is a quite typical value found for this kind of analytical set-up [14,15]. The system recovery of methyl-substituted PACs is only slightly less (96% of that of non-substituted PACs). For the anthracene oil, a coal-tar distillate consisting of a mixture of mainly non-substituted and methyl-substituted PACs, after normalising for system recovery, the recovery equates ca. 96% of the total PACs, which is in between the 91% extract found with

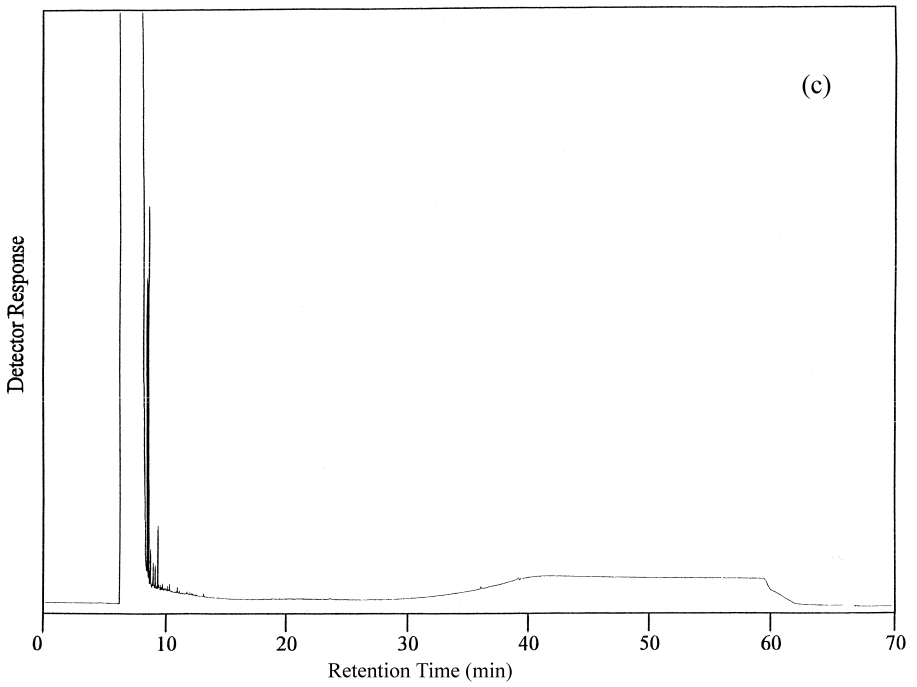

Fig. 5. (*continued*)

IP346 and the theoretical 97% recovery calculated from the distribution coefficients [2]. Hence, for this sample, which mainly consists of non-substituted and methyl-substituted PACs, both methods agree. Similarly, for the series of distillate luboils and a Bright-stock furfural extract (BFE), a rather good linear correlation was obtained between the FIA–DMSO IP346 equivalent and the results from the standard IP346 extraction (see Fig. 6). The slope of the regression line, already corrected for system recovery (IP346 over FIA–DMSO=ca. 4 as compared to ca. 1 found for the anthracene oil), indicates that this method is more selective towards non-substituted PACs than the IP346 method (see also Fig. 3). This correlation, plus results obtained on the anthracene oil, demonstrates that the FIA–DMSO approach performs very well. Results obtained from a wide range of materials ranging from bitumen fume condensates, distillate luboils to heavy residual materials, such as bitumens, and their correlation with mutagenicity and carcinogenicity will be published elsewhere [16].

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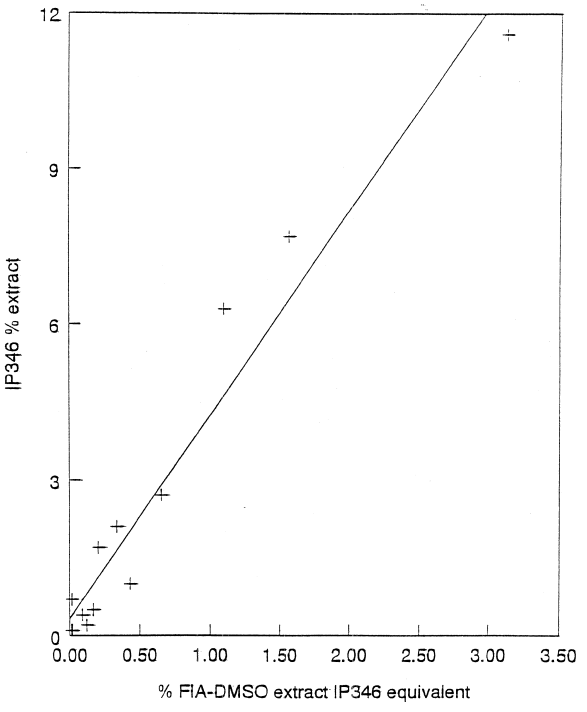

Fig. 6. Plot of FIA–DMSO IP346 equivalent (corrected) vs. IP346.

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