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High-Performance Thin-Layer Chromatography Using Automated Multiple Development for the Separation of Heavy Petroleum Products According to Their Number of Aromatic Rings

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ABSTRACT: Automated multiple development (AMD) is a gradient elution technique for high-performance thin-layer chromatography (HPTLC), in which successive runs are performed with decreasing solvent strength and increasing developing distance. A method for obtaining separations or distributions of heavy petroleum products according to their number of aromatic rings has been developed using AMD together with caffeine-impregnated silica gel HPTLC layers and selective detection by fluorescence scanning densitometry. A gradient of dichloromethane—heptane, starting from a composition of 70:30 (v/v) to 65:35 (v/v), in 34 elution steps with a migration of 2 mm per step, over a total migration distance of 68 mm, has been used. Calibration of elution has been carried out using polycyclic aromatic compounds (PACs) with a molecular weight (MW) from 200 to 1000 atomic mass units (amu), which have been separated under the described conditions. Other parameters on AMD separation have also been discussed, such as the effect of mobile and stationary phases. The application of this technique to different heavy petroleum products is discussed.

■ INTRODUCTION

The continuous rise in the global demand for middle distillates bolsters the importance of heavy-product-based upgrading processes and, therefore, the need to develop original analytical techniques for their characterization.

Instrumental thin-layer chromatography, also known as high-performance thin-layer chromatography (HPTLC),[1,2] is especially adequate for analyzing nonvolatile, complex mixtures, such as high-boiling petroleum products.[3] Although the inherent complexity of heavy petroleum products and the finite resolution afforded by chromatographic techniques preclude molecular separation of individual components in these products, HPTLC is a well-adapted technique for quality control purposes that can be useful for hydrocarbon-type analyses, such as saturates, aromatics, resins, and asphaltenes (SARA) or distribution of polyaromatic compounds. It is at present a flexible, reliable, and cost-efficient analytical technique. The possibility of using accurate automatic systems of the sample application and different development systems in combination with low-particle-size HPTLC plates has allowed separation efficiency to be improved.[1,2] Likewise, ultraviolet (UV) and fluorescence scanning densitometry, electronic image acquisition, and more recently, online HPTLC—mass spectrometry (MS) coupling[4] are different possibilities for chromatogram evaluation.

Only minimal sample preparation is required for HPTLC. Likewise, the whole sample is analyzed because non-eluted compounds can also be scanned. This allows for the problems of irreversible adsorption, traditionally associated with column techniques, to be avoided. Other additional advantages are the possibility of scanning the same sample either under different conditions or after time because separated fractions of the sample are stored on the plate. Moreover, a high sample throughput can be achieved because HPTLC provides simultaneous and parallel separation of many samples and standards in one plate.

Charge-transfer thin-layer chromatography has been used for a long time to separate polycyclic aromatic compounds (PACs) using different electron-acceptor stationary phases. Much work, which was compiled by Cagniant,[5] has been performed on this subject. Separated PACs have molecular weights up to 300 (coronene). It was stated that, for a given elution system, migration distances for PACs depend upon the strength of the complexation between the PAC and charge-transfer stationary phase, which is a direct function of the number of aromatic rings.[5]

Caffeine-impregnated silica gel plates have been used for this purpose since 1965. In addition to the development of modern TLC technology and HPTLC plates, they became commercially available because of the work by Funk et al.,[6,7] who successfully separated six PACs in drinking water (benzo[ghi]perylene, indeno-[1,2,3-cd]pyrene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]-fluoranthene, and fluoranthene) using dichloromethane (DCM) at −20 °C using detection by fluorescence at 365 nm.

Despite being at present a robust, reliable, and flexible technology, the use of modern instrumentation together with charge-transfer plates to evaluate the distribution of PACs in petroleum products has been scarce. This was studied in a diesel fuel using a horizontal developing chamber together with caffeine-impregnated

Table 1. SARA Analysis of Some Studied Bitumens[a]

	asphaltenes (%)	saturates (%)	aromatics (%)	resins (%)
R2191 bitumen	9.5	7	63.8	19.7
R2181 bitumen	4.8	18.5	59.6	17.1
R2194 bitumen	6.6	8	63.4	22
R2195 bitumen	5	16.6	63.3	15.1

[a] TLC—FID (Iatroscan) conditions: pentane (15 min) + DCM (8 min).

HPTLC plates.[8] PAC standards up to coronene [molecular weight (MW) = 300] were separated according to their aromatic ring number and used as standards for obtaining chromatograms of a gas oil. No other papers on more complex high-boiling petroleum products were published because PAC standards with a molecular weight higher than 300 atomic mass units (amu) were not available at that time. Heavy petroleum products consist of increasingly more polar and heavy PACs than coronene in their resinic and asphaltenic fractions.

In this work, we present a method for obtaining distributions of high-boiling petroleum products according to their number of aromatic rings, which is based on HPTLC associated with the automated multiple development (AMD) gradient elution technique[2,9,10] using caffeine plates and selective fluorescence detection. Adequate PAC standards with molecular weights up to 1000 amu have been used for calibration. They include standards that show coherent spectroscopic and retention properties with regard to those found in polar fractions of heavy products.

AMD uses repeated elemental elution steps (typically of 2—3 mm) of HPTLC plates. Usually, the solvent strength of each development is lower than that of the previous step. This change in solvent strength, adjusted by mixing different proportions of up to five solvents, is combined with an increasing migration distance for each step. This results in a continuous sample reconcentration and, therefore, in extremely narrow bands, a typical peak width being about 1 mm. After each elution step, the plate is dried by vacuum. In this way, a stepwise elution gradient is formed. This results in an increased separation power with regard to other migration systems, in some cases even similar to that of HPLC.[2]

The influence of stationary and mobile phases is studied here with regard to PAC separation. Likewise, parameters affecting AMD separation of heavy petroleum products are discussed using several examples of application for different heavy petroleum products.

■ EXPERIMENTAL SECTION

Samples and Standards. Bitumens (R1141, R2191, R2181, R2194, and R2195), a heavy oil, and an asphaltene were furnished by TOTAL RM (Table 1). AAN is a Bow River bitumen from SHRP.[11,12] Samples were heated for 30 min at 150 °C in the case of bitumens and asphaltene and at 100 °C in the case of oil for homogeneization before sampling.

The following PACs were used as standards, applied either individually or in mixtures: naphthalene (≥99.7%; [91-20-3] CAS; MW, 128.17), anthracene (≥99%; [120-12-7] CAS; MW, 178.23), pyrene (≥99%; [129-00-0] CAS; MW, 202.25), benzo[k]fluoranthene (98%; [207-08-9] CAS; MW, 252.30), benzo[ghi]perylene (98%; [191-24-2] CAS; MW, 276.34), coronene (99%; [191-07-1] CAS; MW, 300.35), quinolin-65 (80%; [834884-59-2] CAS; MW, 467.62), violanthrone-79 ([85652-50-2] CAS; MW, 712.91), perylene-66 (40%; [MFCD07785507]

MDL; MW, 766.92), and violanthrone-78 ([82145-74-2] CAS; MW, 1021.41), from Sigma-Aldrich, Inc., St. Louis, MO. Figure 1 shows their structures and molecular weights.

Chromatographic Plates. HPTLC silica gel plates (10 × 20 cm; pore size, 60 Å; layer thickness, 0.20 mm) and caffeine-impregnated HPTLC silica gel glass plates (10 × 20 cm; pore size, 60 Å; layer thickness, 0.20 mm) were used from Merck (Darmstadt, Germany) and Macherey-Nagel (Düren, Germany), respectively.

Sample Application. Samples were dissolved in HPLC-grade DCM (99.5%, from Scharlau, Barcelona, Spain) and applied using the Automatic TLC Sampler 4 (Camag, Müttenz, Switzerland), as 3 or 6 mm bands. For 3 mm bands, 26 samples were applied on the same plate with a distance of 6.2 mm between tracks. For 6 mm bands, 10 samples were applied on the same plate with a distance of 14.5 mm between tracks. In both cases, two tracks were always kept free of application, as blank runs.

The first application position was 20 mm (x coordinate), and the distance from the lower edge of plate was 10 mm (y coordinate).

Typical application volumes for petroleum samples were between 0.2 and 0.8 μL, and concentrations range from 1.5 to 2.5 mg mL^{-1}. For PAC standards, application volumes were from 0.5 to 10 μL and corresponding concentrations were from 0.2 to 12 mg mL^{-1}, depending upon the standard. Each sample has been applied by triplicate.

Chromatographic Elution. An automatic multiple development (AMD2) system (Camag, Müttenz, Switzerland) was used. This equipment operates as follows: before introduction into a vacuum-tight chamber, the mobile phase for each development is automatically prepared by mixing appropriate portions from up to five different solvents. Chromatography is monitored, and the run stops when the selected developing distance is reached. The remaining solvent is withdrawn from the chamber by vacuum, and the plate is completely dried. While vacuum is released, the plate can be preconditioned via the gas phase, leaving the system ready for the next development step.

ACS reagent-grade toluene (>99.5%), HPLC-grade DCM (99.5%, stabilized with ethanol), and n-heptane (99%) were from Scharlau (Barcelona, Spain). Different binary gradients have been used.

An AMD run is defined by four parameters: gradient (initial mobile phase composition/final mobile phase composition), number of steps, distance per step (mm step^{-1}), and total developing distance (mm). The latter is given from elution starting. The final distance in the chromatogram can be obtained by adding 10 mm (y coordinate of the application point) to the total developing distance.

Fluorescence Scanning Densitometry. A TLC Scanner 3 (Camag, Müttenz, Switzerland) was used in fluorescence mode. The excitation wavelength was 365 nm using a tungsten lamp.

Reproducibility of wavelength setting (monochromator) is better than 0.2 nm, and the accuracy is better than 1 nm. Scanning is independent in both x and y directions. Reproducibility of positioning is better than 50 μm in the y direction and better than 100 μm in x direction, with a maximum scanning speed of 100 mm s^{-1}.

Camag WinCats software was used for controlling ATS4, AMD2, and TLC Scanner 3 and also for data acquisition and treatment.

■ RESULTS AND DISCUSSION

Considerations on PAC Standards. Polar, asphaltene-like fractions of heavy petroleum products are currently described as complex mixtures of molecules consisting of planar polyaromatic cores containing 4—10 fused rings and polar functional groups. Attached to the polar core are hydrophobic naphthenic rings and short aliphatic side chains.[13,14] We have used in this work PAC standards as model compounds to study their separation under AMD conditions, on caffeine-impregnated HPTLC plates.
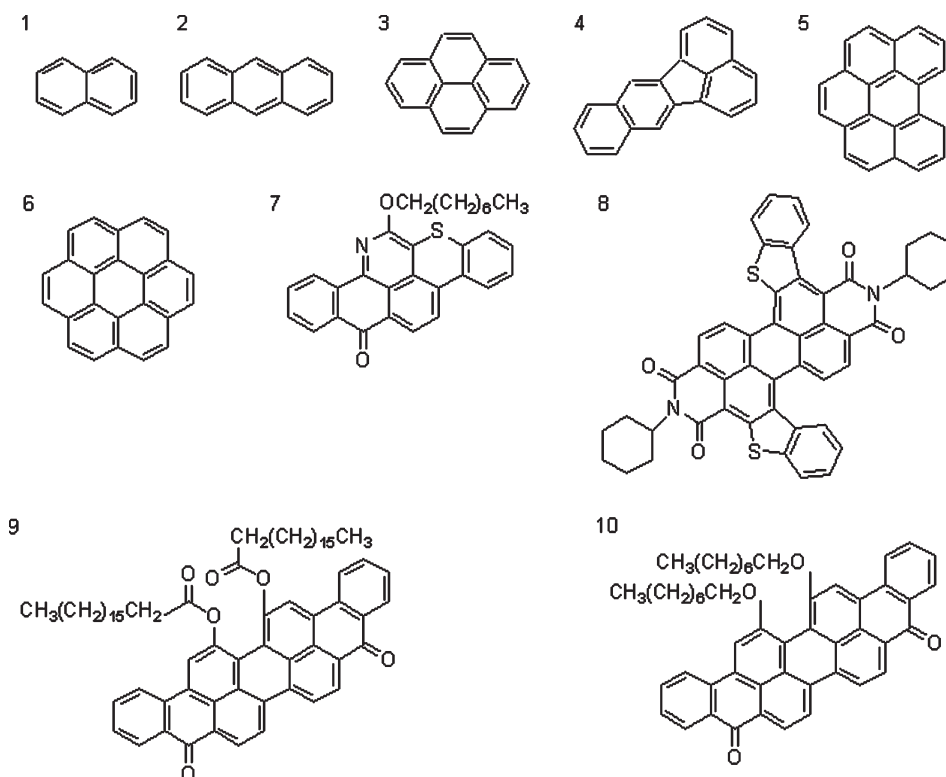
Figure 1. Chemical structures and molecular weights of PACs: 1, naphthalene, MW = 128.17; 2, anthracene, MW = 178.23; 3, pyrene, MW = 202.25; 4, benzo[*k*]fluoranthene, MW = 252.30; 5, benzo[*ghi*]perylene, MW = 276.34; 6, coronene, MW = 300.35; 7, quinolin-65, MW = 467.62; 8, perylene-66, MW = 766.92; 9, violanthrone-78, MW = 1021.41; and 10, violanthrone-79, MW = 712.91.

The selected conditions have allowed for separations or distributions of PACs in heavy petroleum products, such as bitumens, heavy oils, or asphaltenes, to be obtained. PACs are directly and selectively detected on the caffeine plates by fluorescence scanning densitometry thanks to their native fluorescence by excitation at 365 nm.

Among the studied PACs, several high-MW, highly aromatic molecules have been used as model standards for polar fractions. Four of these molecules have a MW higher than 300. Three of them (violanthrone-78, MW = 1021.4; violanthrone-79, MW = 712.9; and quinolin-65, MW = 467.4) were previously used as models for studying the kinetics of adsorption of Athabasca asphaltenes.[13,15] These molecules have been found to have specific properties similar to the properties present in bitumens. They include MW, aromaticity, naphthenic character, heteroatom content, and nature of functional groups. Another molecule that accomplishs the above requirements is perylene-66 (MW = 766.9), which has also been used here as a standard.

Two additional features of these highly aromatic, high-MW molecules support their use as standards for HPTLC separation. (1) The retention factor of these molecules (R_f = migrated distance for the sample/migrated distance for the solvent front) is similar in all cases to those of asphaltene peaks when bitumens are submitted to SARA analysis on silica gel plates, either using Iatroscan TLC—flame ionization detector (FID) or HPTLC—UV—vis. Both standards and asphaltenic peaks remain at the application point under these conditions. (2) The absorption (maxima near 280 nm) and emission characteristics of standards (500—600 nm) are coherent with those of asphaltenes in solvents (e.g., toluene), according to the literature.[13] This is also

in agreement with UV spectra directly recorded on the silica plate and with the emission signal collected from 400 nm.

Separation of PAC Standards on Caffeine-Impregnated HPTLC Plates Using AMD. Figure 2 shows retention properties of studied PACs, R_f (standard migration distance/solvent front migration distance), and migration distances (md, in mm) on silica gel and caffeine plates under two gradient AMD conditions: (1) DCM—heptane, from 70:30 (v/v) to 65:35 (v/v), 34 steps, 2 mm step^{-1}, over 68 mm (these have been the selected conditions for separating PACs either individually or in the studied petroleum products, as justified in the next subsection) and (2) toluene—heptane, from 70:30 (v/v) to 65:35 (v/v), 34 steps, 2 mm step^{-1}, over 68 mm.

The retention pattern for PACs has been evidenced to be very different when they have been developed on silica or caffeine-impregnated HPTLC plates (panels A and B of Figure 2). The studied PACs are not separated on silica gel using the DCM—heptane gradient. In this case, PACs with a MW lower than 300 migrate with the solvent front to 68 mm. On the contrary, the high-MW PACs remain uneluted at the application point. In the case of caffeine plates, PACs are distributed along the whole migration distance. High-MW PACs migrate lower distances than low-MW PACs. Migration takes place according to the number of aromatic rings, as reported in the literature,[2,5−7] because migration is a function of the strength of the charge-transfer complex formed between the aromatic compound and caffeine. Therefore, we can relate migration distance under these HPTLC conditions with the MW of PACs.

The effect of the solvent nature in the gradient can be observed in panels A and C of Figure 2. Using the same solvent percent
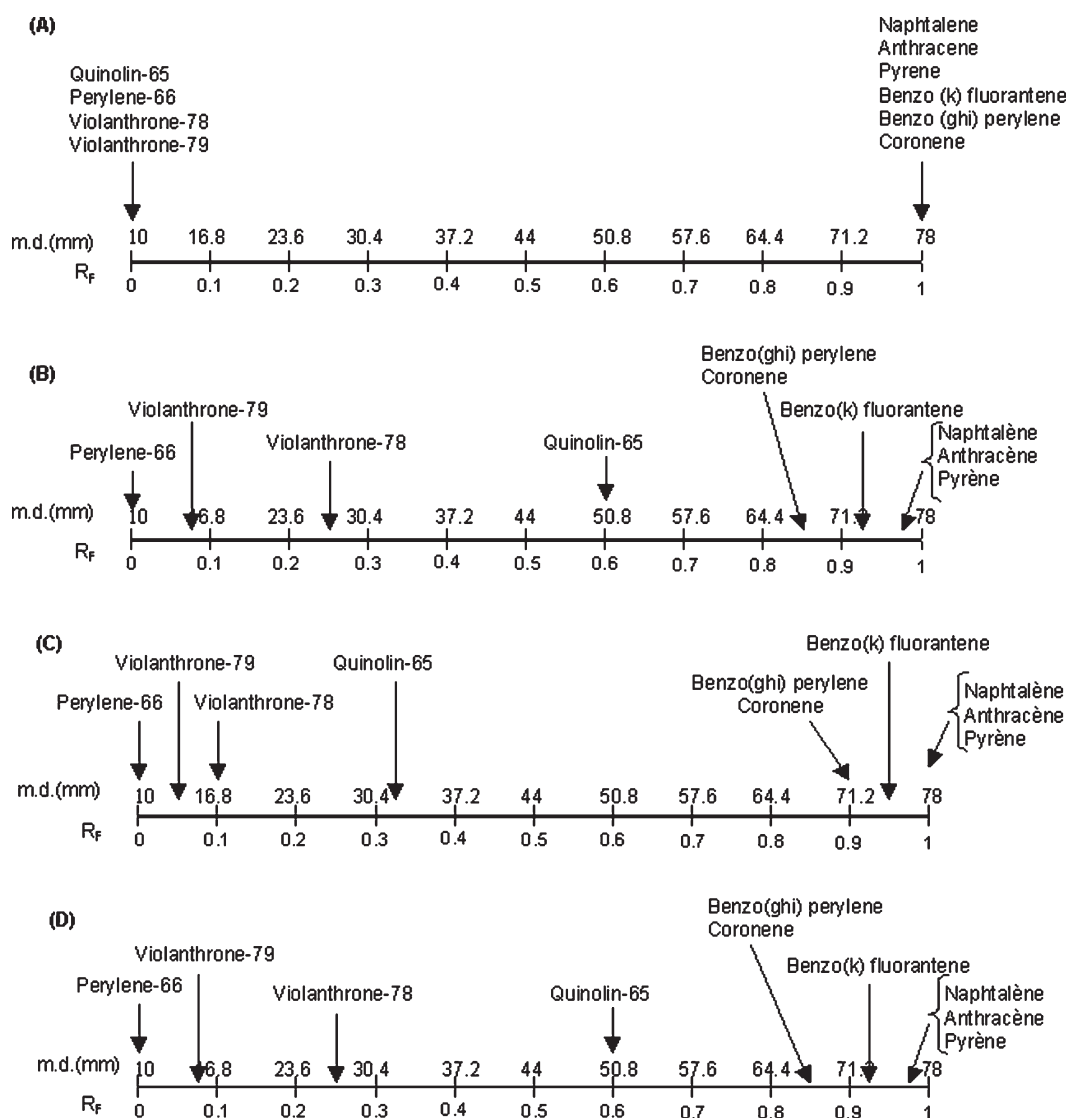
Figure 2. R_f and migration distances of individual PACs under AMD conditions: (A) on silica gel HPTLC plates using the DCM−heptane gradient, (B) on caffeine-impregnated HPTLC plates using the DCM−heptane gradient, (C) on caffeine-impregnated HPTLC plates using the toluene−heptane gradient, and (D) on caffeine-impregnated HPTLC plates using the DCM−heptane gradient applying a mixture of PAC standards (AMD conditions: gradient from 70:30 (v/v) to 65:35 (v/v), 34 steps, 2 mm step^{-1}, over 68 mm).

composition, number of steps, and distance per step as in the case of DCM−heptane, the toluene−heptane gradient provides a less convenient separation along the plate because PACs are mostly grouped in two zones in this case.

Calibration of retention using caffeine plates has been carried out by applying both pure standards and a mixture of them to evaluate whether a matrix effect produced by intermolecular interactions might take place. As can be observed in panels A and D of Figure 2, migration distances are the same for every PAC either applied one-by-one or applied as a mixture. This is in agreement with previous works on AMD, in which it has been stated that the distance that a given fraction migrates before stopping is largely independent of the sample matrix.[16] This may result in an additional advantage of application of AMD to the separation of high-boiling petroleum products because, in these products, the matrix effect by intermolecular interactions is important.

Selection of the Mobile Phase, Gradient Composition, and Step Number for HPTLC−AMD of Heavy Petroleum Products. AMD is becoming familiar in different fields of analytical

chemistry[1,2] but not in the field of fossil-fuel analysis. For this reason, a justification of the above-mentioned selected AMD gradient conditions is advisable because it illustrates the design of experiments using this technique.

The first step is to select the mobile-phase nature. Simple AMD developments (3 steps, 60 mm step^{-1}, over 60 mm) in isocratic mode, using pure DCM, pure n-heptane, and a 50 vol % mixture of DCM−heptane were first carried out to evaluate the elution power of these solvents on a representative product (R1141 bitumen). HPTLC chromatograms are depicted in Figure 3. This shows that development with pure n-heptane does not produce any elution of bitumen (Figure 3A). On the contrary, practically all of the bitumen is eluted with the solvent front when pure DCM is used as the mobile phase (Figure 3C). In the case of the 50% mixture, a part of bitumen is eluted, although most of it remains at the application point (Figure 3B). The migration distances of four individual PAC standards under each of the studied conditions are also shown in Figure 3 (see the arrows).
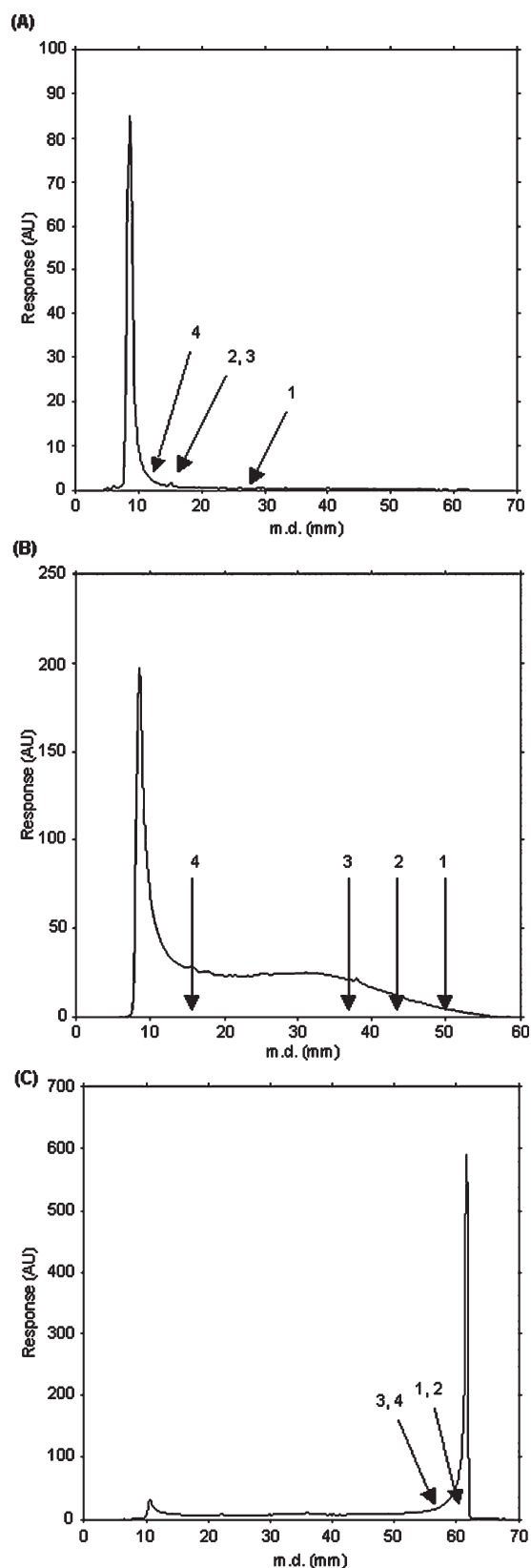
(A)

(B)

(C)

Figure 3. HPTLC chromatograms of R1141 bitumen eluted with (A) pure *n*-heptane, (B) 50:50 (v/v) DCM−heptane, and (C) pure DCM (AMD conditions: 3 steps, 60 mm step^{-1}, over 60 mm). The arrows indicate the migration distances of individual standards in each case: 1, anthracene; 2, pyrene; 3, benzo[*k*]fluoranthene; and 4, coronene.
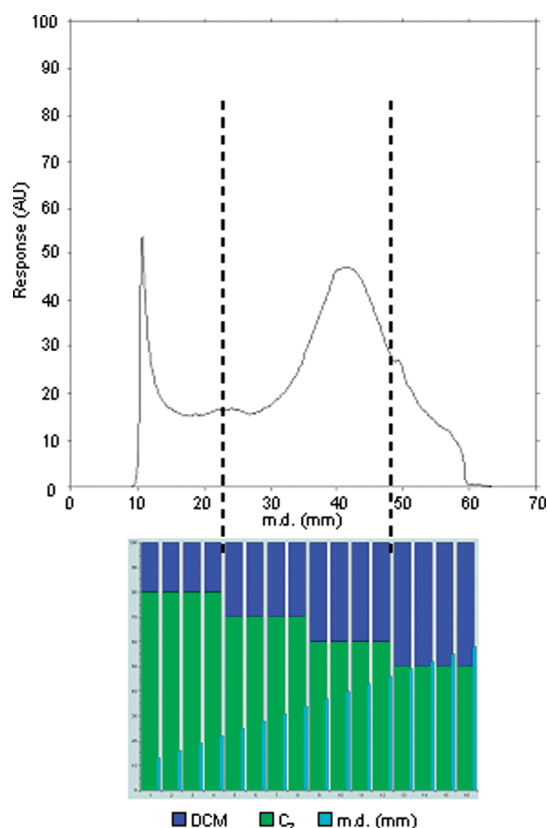

Figure 4. HPTLC chromatogram of R1141 bitumen eluted with DCM−heptane superimposed with the corresponding diagram of the gradient employed. Green, DCM vol %; blue, *n*-heptane vol %; turquoise, md of each step. Dotted lines indicate the relationship between the gradient composition and migration distances in the chromatogram (conditions: gradient starting with 80:20 and ending with 50:50, 16 steps, 3 mm step^{-1}, and 48 mm total md).

Taking into account the results shown in Figure 3, a gradient starting with 80:20 (v/v) DCM−heptane and ending with 50:50 (v/v) DCM−heptane was chosen at starting point to select the gradient conditions. Figure 4 shows the HPTLC chromatogram of R1141 bitumen using this gradient. The number of steps were increased to 16 to evaluate the influence of a increasing reconcentration. The distance per step was kept at 3 mm, and the total migration distance was 48 mm.

As shown in Figure 4, the resulting chromatogram of bitumen is not resolved. In a general AMD practice, when an unresolved chromatogram is superimposed with the corresponding diagram of the gradient employed, the region of the gradient causing the separation of given fractions can be identified (see dotted lines and a diagram of the solvent composition in Figure 4). Thus, separation can be fine-tuned and amplified for each segment of the chromatogram. Therefore, it is possible to make changes in solvent composition with predictable effects on separation. Gradients have been selected that start with 70:30 (v/v) DCM−heptane and end with 60:40 (v/v) or 65:35 (v/v) DCM−heptane.

In our case, however, the number of AMD steps is the most determinant parameter for improving bitumen separation. Figure 5 shows selected examples of the AMD chromatograms corresponding to the influence of the number of steps and distance per step on the elution of a bitumen, using a selected gradient
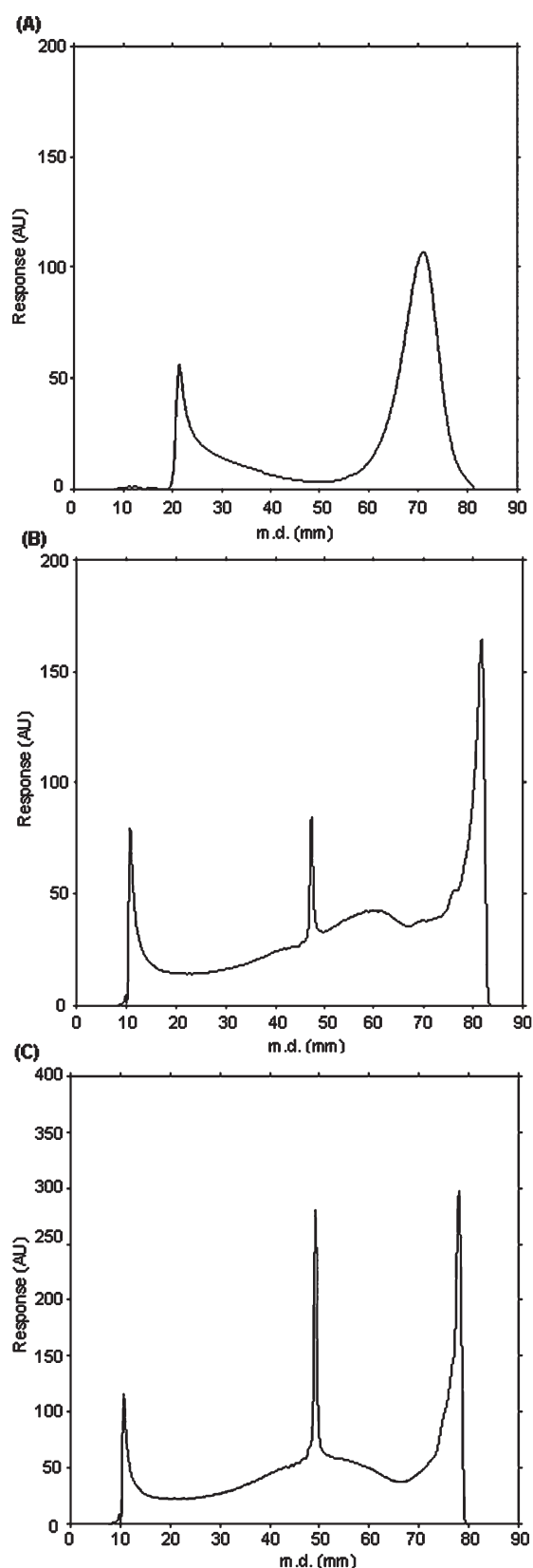
Figure 5. HPTLC chromatograms of R2191 bitumen eluted using DCM−heptane [gradient starting with 70:30 (v/v) and ending with 65:35 (v/v)] under different conditions: (A) 10 steps, 3 mm step^{-1}, (B) 24 steps, 3 mm step^{-1}, and (C) 34 steps, 2 mm step^{-1}.
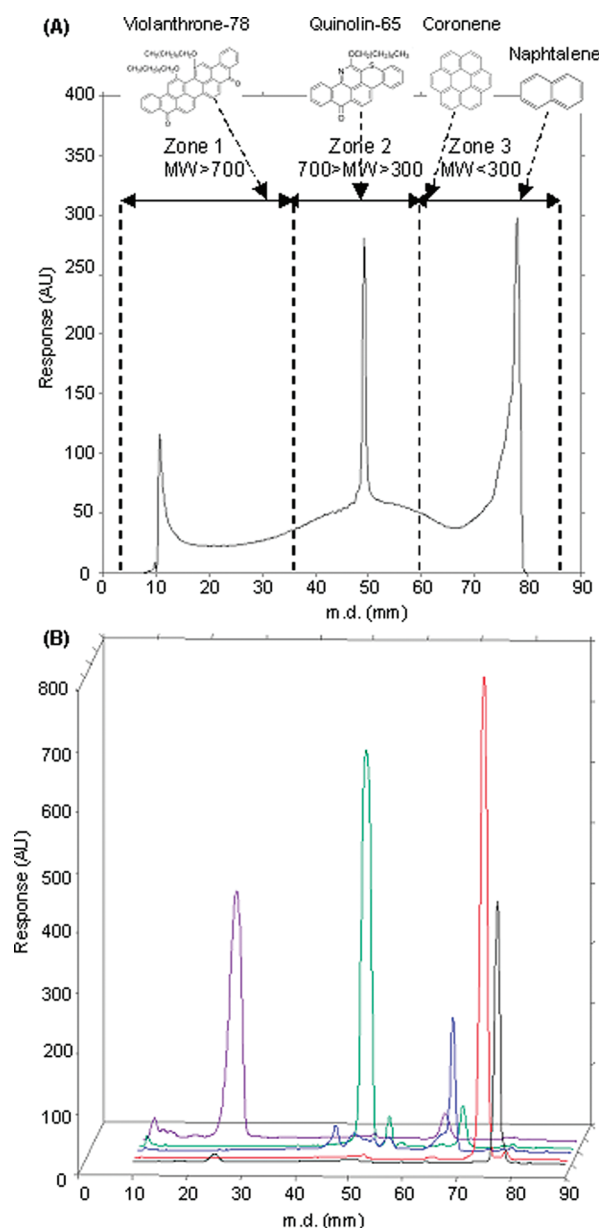

Figure 6. (A) MW zones on a HPTLC chromatogram of R2191 bitumen, eluted under the selected AMD conditions (see the text), with some PAC standards eluted near the limits of zones. (B) HPTLC chromatograms of several individual PACs eluted under the same conditions: (black line) pyrene, (red line) benzo[*k*]pyrene, (blue line) coronene, (green line) quinolin-65, and (purple line) violanthrone-78.

composition. In general, the higher the number of steps, the greater the separation efficiency. This occurs up to 34 steps for the analyzed products. No improvements in separation were obtained in experiments with 40 steps.

The number of steps has been a more important parameter than the migration distance for these products. Separation is better in the case of a migration distance of 68 mm (with 34 steps, 2 mm step^{-1}; Figure 5C) than that of 72 mm (with 24 steps, 3 mm step^{-1}; Figure 5B).

The total duration of AMD analysis ranged from 1 h (10 steps, 3 mm step^{-1}) to 4 h (24 steps) and 5 h (34 steps). Anyway, a compromise between analysis time and obtained information can
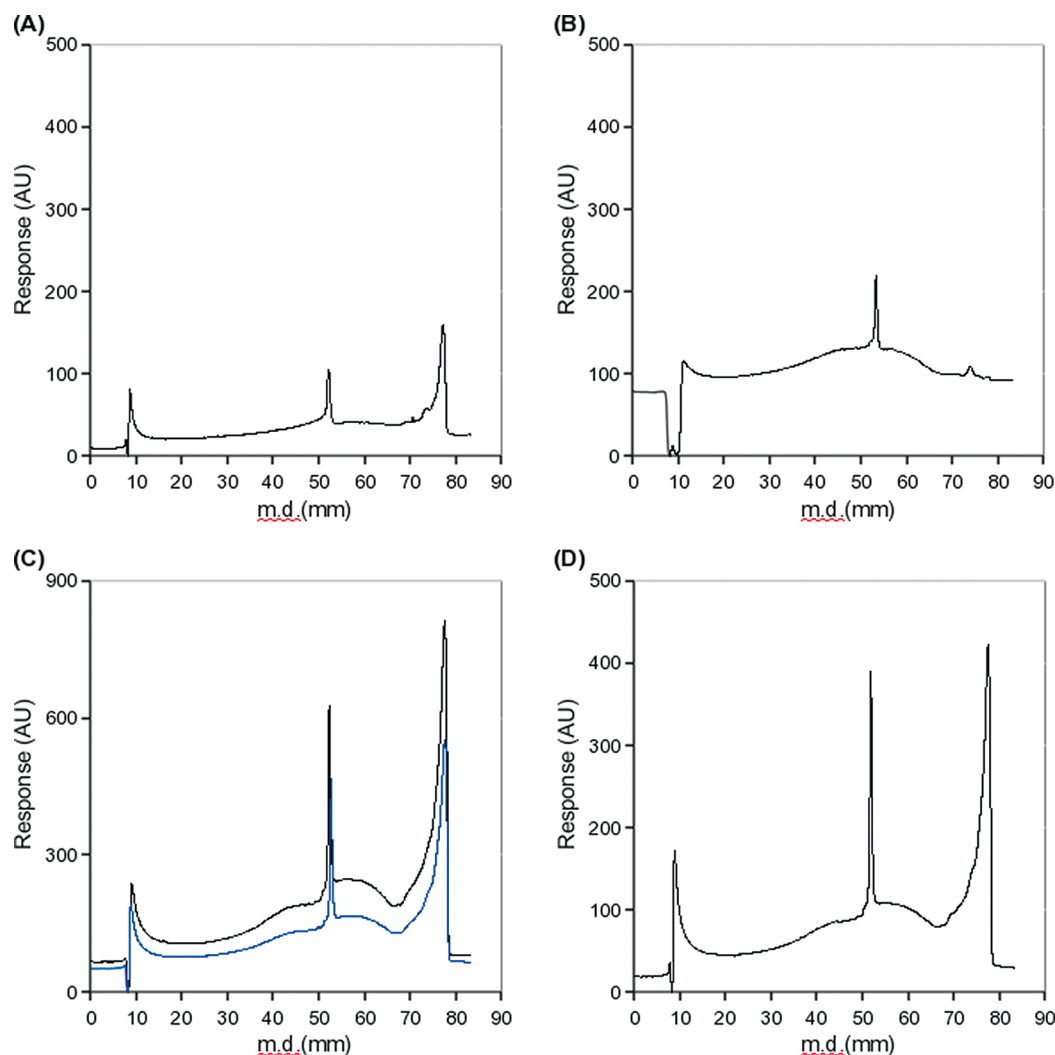
Figure 7. HPTLC chromatograms under the selected AMD conditions (see the text) of: (A) heavy oil, $3\,\mu g$; (B) asphaltene, $3\,\mu g$; (C) R2181 bitumen (black, $3\,\mu g$; blue, $1\,\mu g$); and (D) AAN bitumen, $3\,\mu g$.

be achieved for designing an AMD method. It is to be remarked that the complete program of AMD2 is fully automatic and no supervision is required during a run.

MW Zones of HPTLC—AMD Chromatograms for Distributions of Polyaromatic Compounds in Heavy Petroleum Products. Migration distances of studied PAC standards delimit several zones that correspond to different MWs of polyaromatic compounds (Figure 6). Several PAC standards can be applied on the same HPTLC plate in parallel with heavy petroleum product samples under the selected AMD conditions.

Chromatograms of several PACs are depicted in Figure 6 with that corresponding to R2191 bitumen. It is to be remarked that polyaromatic compounds are selectively detected using fluorescence by excitation at 365 nm on the caffeine-impregnated plates.

Three zones of migration corresponding to MW zones of MW > 700, 700 > MW > 300, and MW < 300 can be observed for chromatograms of heavy petroleum products.

Given the complexity of the studied products, reasonable separations have been obtained under the selected AMD conditions, although a peak-to-peak separation is not obviously possible for these samples. However, resulting chromatograms provide a clear idea of the distribution of PACs in these heavy

products according to MW in quite a similar way as GPC does to obtain a general size distribution of molecules. Therefore, both techniques can provide complementary information on heavy petroleum products and their derived conversion products. The high throughput of the HPTLC—AMD technique (26 samples per plate) is to be remarked. Other advantages of HPTLC over column-based chromatography techniques have been discussed elsewhere.[3,8]
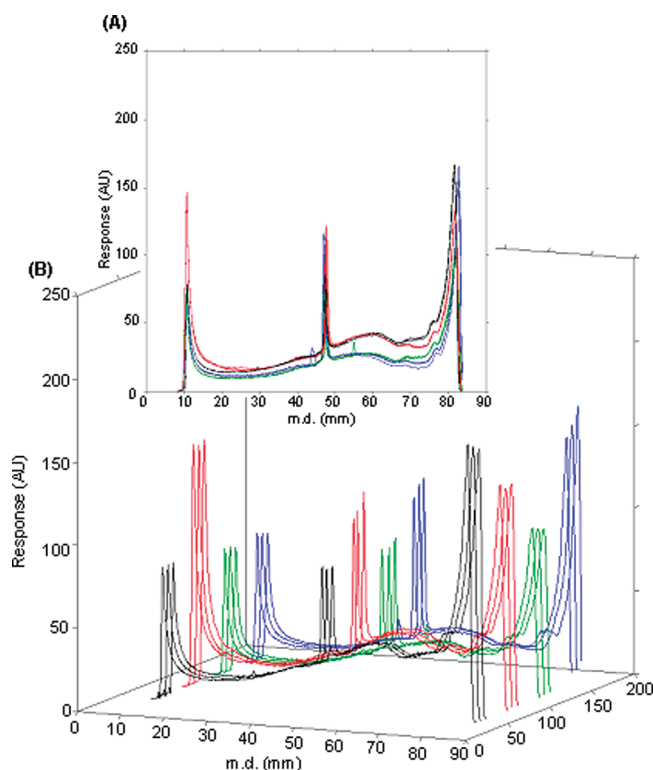
Figure 7 shows HPTLC chromatograms corresponding to the same sample load ($3\,\mu g$) of different products under the selected AMD conditions: (A) a heavy oil, (B) an asphaltene, and (C and D) two bitumens. For a given product (Figure 7C), the higher the sample load (black, $3\,\mu g$; blue, $1\,\mu g$), the higher the fluorescent signal for each peak of the chromatographic distribution.

Figure 7 shows different profiles of the PAC distribution for these products. In a heavy oil, PACs are quite uniformly distributed throughout the three MW zones. In the asphaltene, they concentrate in zone 2, while in bitumens, the concentration is higher in zone 1.

A negative peak can be observed in the point of application of chromatograms in Figure 7. This peak has a variable intensity, ranging from moderate to large, depending upon the sample

Table 2. Integration Percentages (%) of MW Zones for the PAC Distribution of Different Products (Sample Load of 3 μg)

	zone 1 (%)	zone 2 (%)	zone 3 (%)
asphaltene	7.7	77.3	15.0
heavy oil	28.0	36.7	35.4
R2181 bitumen	15.9	39.2	44.9
AAN bitumen	19.4	40.0	40.6

of application, where the heaviest and most polar PACs are also found.

Integration (Table 2) is possible because fluorescence detection by scanning densitometry shows an adequate repeatability. Figure 8 shows HPTLC chromatograms of various applications of several samples on the same plate, illustrating repeatability ($\pm$0.45 mm).

Because fluorescent responses of PACs depend upon their structure, integrations of zones must be used only for comparative purposes and must be taken with caution. Research on quantitative issues of this method is in progress. A compromise for a relatively rapid, quantitative analysis might use an average response factor per MW zone.

CONCLUSION

Despite being a suitable technique for the analysis of complex samples with polar and high-molecular-weight compounds, HPTLC has been underused in the analysis of fossil fuels. HPTLC with AMD, a gradient elution system that allows for continuous reconcentration of the sample, has led us to the method presented in this paper to separate aromatic compounds based on their number of rings, in heavy petroleum products, such as asphaltenes, bitumen, and heavy oils. AMD separation is performed using silica gel plates impregnated with caffeine. Selective detection of PACs is carried out by fluorescence scanning densitometry.

The separation power of AMD with the high throughput of HPTLC allow us to consider its future use for the development of other types of analysis in the field of fossil fuels, using other types of plates, solvent gradients, and operating conditions.

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Figure 8. (A) Frontal vision of the HPTLC chromatograms in B. (B) HPTLC chromatograms of different bitumens (by triplicate, sample load of 1 μg) on the same plate: (black) R2191, (blue) R2195, (green) R2181, and (red) R2194 (AMD conditions: DCM−heptane gradient, from 70:30 (v/v) to 65:35 (v/v); 24 steps, 3 mm step^{-1}, and 72 mm total md).

considered. It is important in the case of the asphaltene (Figure 7B). The area of this negative peak increases with the sample load.

The possibility that the negative peak is due to an artifact of the sample application system has been discarded because, in fact, it does not appear in the chromatograms corresponding to standards (Figure 6) or in blank runs. Because a fluorescence quenching of uneluted compounds should lead to negative peaks at the application point, they might be due to the quenching effect that metals have on the fluorescence of polyaromatic molecules. It is known that perylene is quenched by metals, such as Co^{2+}, Ni^{2+}, and others.[17,18] Likewise, N-PACs, such as indole, carbazole, and other derivatives, are sensitive to be quenched by different metals.[19] It is plausible to think that other heavier PACs and N-PACs may also experience quenching by metals. The latter are not eluted by the mobile phases used under the described conditions; therefore, they accumulate at the point

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