John V. Goodpaster,[1,2] *Ph.D.; Samuel B. Howerton,*[1] *B.S.; and Victoria L. McGuffin,*[1] *Ph.D.*

Forensic Analysis of Commercial Petroleum Products Using Selective Fluorescence Quenching

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ABSTRACT: A novel method for the forensic analysis of commercial petroleum products is presented. In this approach, the petroleum sample is extracted with nitromethane and then separated by capillary liquid chromatography with laser-induced fluorescence detection. The addition of selective fluorescence quenching agents allows the sample to be profiled by the distribution of alternant and nonalternant polycyclic aromatic hydrocarbons (PAHs). In preliminary studies, the quenching behavior of nitromethane and diisopropylamine was established by using a standard mixture of sixteen PAHs ranging in size from two to six aromatic rings. Subsequent examination of new and used motor oil demonstrated that characteristic differences arise in the PAH content, which may allow for the unique identification of oil from a particular engine or vehicle. In addition, three brands of petrolatum jelly were successfully distinguished. Although a number of alternant alkylated and heterocyclic PAHs were found in all petrolatum samples, there were significant differences in the relative concentrations of alternant as well as nonalternant PAHs. This allowed for clear differentiation of the samples through qualitative inspection of their chromatograms as well as quantitative statistical correlation techniques.

KEYWORDS: forensic science, fluorescence, fluorescence quenching, polycyclic aromatic hydrocarbons, capillary liquid chromatography, petroleum, petrolatum jelly, motor oil

Commercial petroleum products have become progressively more important in the fields of criminalistics and trace evidence. This is due not only to the severity of the crimes in which this type of evidence is found, but also to the wealth of information available from appropriate chemical analysis of these materials. For example, the analysis of petroleum mixtures can provide circumstantial links between motor oil and a particular vehicle, fossil fuels and a fire of suspicious origin, crude oil and a site of environmental contamination, or petrolatum jelly and a crime scene involving sexual assault.

In all of these cases, the petroleum-based samples can be profiled by qualitative and quantitative analysis of the polycyclic aromatic hydrocarbons (PAHs). PAHs consist of fused aromatic rings in many and varied configurations, which can be divided into two classes: alternant and nonalternant. To distinguish between these classes, each carbon atom in the aromatic structure is labeled, alternately skipping an atom between labels. Alternant PAHs possess a structure in which no two atoms of the same type (labeled or unlabeled) are adjacent. Examples include anthracene, pyrene, and other PAHs that consist solely of six-membered rings (Fig. 1). Nonalternant PAHs have a structure in which such labeling results in two adjacent atoms of the same type. Examples include fluorene and fluoranthene, which contain one five-membered ring in addition to six-membered rings (Fig. 1). Such subtle changes in structure can cause large differences in the physical and chemical properties of PAH isomers (1–3). The ability to differentiate between these isomers in petroleum products is important as their distribution can indicate the formation conditions and history of the sample (4), thereby creating a more characteristic profile for comparison with other samples.

To date, various techniques have been utilized to determine PAHs in petroleum products (2,5–8). Among these techniques, luminescence is especially beneficial because of its high sensitivity and selectivity for PAHs (9). Spectra may be obtained by scanning the excitation and emission wavelengths independently or synchronously (10), or by acquiring multiwavelength excitation-emission matrices (11). In addition, fluorescence or phosphorescence lifetime measurements can provide further information for the identification and characterization of PAHs (12,13). These luminescence techniques, alone or in combination, have been applied to the selective determination of PAHs in petrolatum jellies, lubricants, and motor oils (8,13–18).

However, these techniques are not always sufficiently selective for the analysis of PAHs in complex samples of forensic interest. Luminescence spectra in the solution phase exhibit a loss of vibrational fine structure when compared to the gas or solid phase. This loss of structure arises predominantly from collisions of the excited-state PAH with solvent molecules (19,20). Although alternant PAH isomers often display some structure in their solution-phase emission spectra (Fig. 2*A*), nonalternant PAHs usually do not (Fig. 2*B*) (19–21). As a result of their rather featureless spectra, identification of unknown PAHs can be difficult. One approach that can address this challenge is the use of laser-induced fluorescence with selective fluorescence quenching agents. While quenching is generally thought to be detrimental in fluorescence spectroscopy, it can be used to analytical advantage if invoked in a carefully designed and controlled manner. This approach can provide valuable photophysical and photochemical information about individual PAHs that can be used for their classification or identification. It can also be used to profile complex petroleum mixtures based on the absence or presence of various PAH isomers (2,3).

[1] Department of Chemistry, Michigan State University, East Lansing, MI.
[2] Current address: Analytical Chemistry Division, National Institute of Standards and Technology, Gaithersburg, MD.

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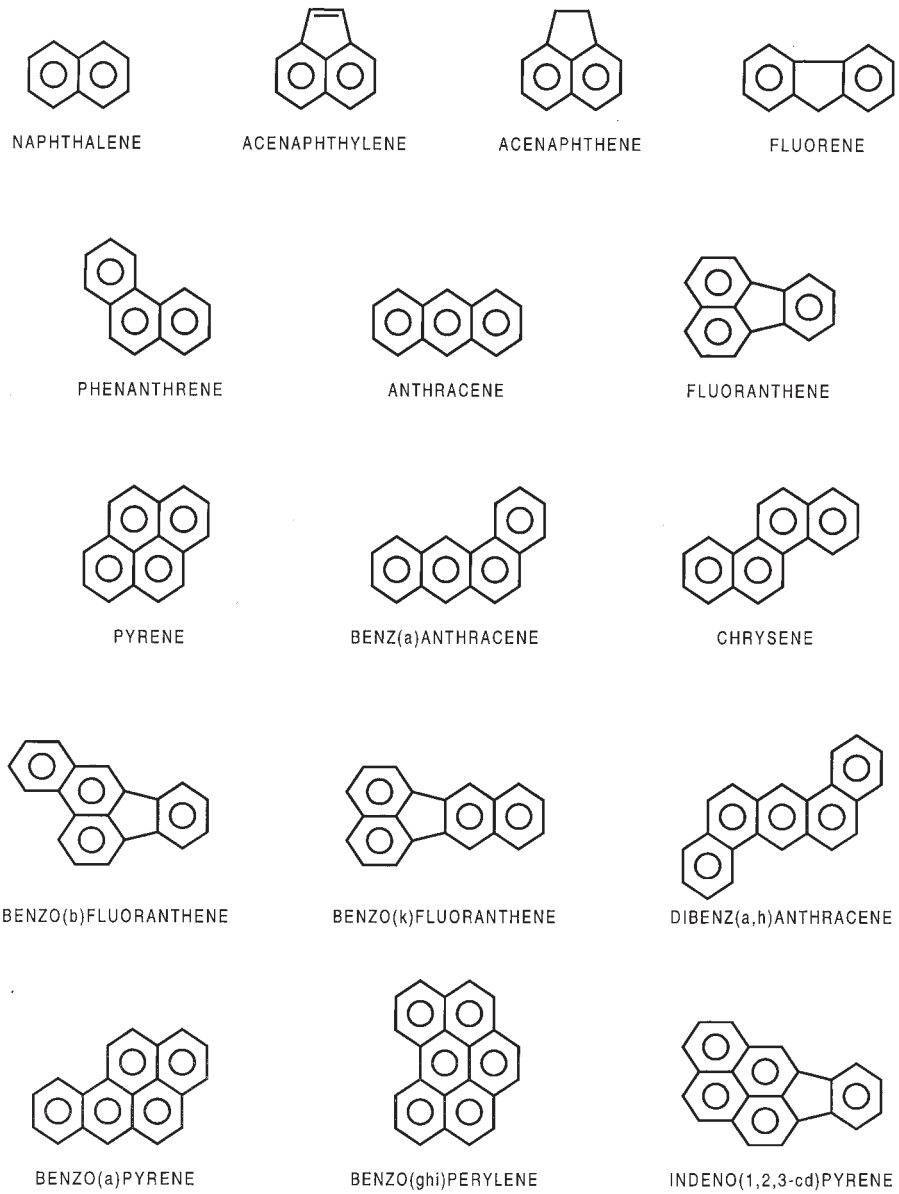

FIG. 1—*Structures of alternant and nonalternant polycyclic aromatic hydrocarbons.*

Although there are many mechanisms for fluorescence quenching, the dynamic quenching process is most useful for analytical applications (3). In this process, an excited-state fluorophore collides with a ground-state quencher to form a transient complex or exciplex. These complexes often involve substantial charge transfer between the fluorophore and quencher and, thus, can facilitate energy transfer to the quencher. As the exciplex dissociates, both fluorophore and quencher return to the ground state via nonradiative pathways, so that the excess energy is dissipated through vibrational relaxation and external conversion. Because the complex must be formed during the excited-state lifetime, dynamic quenching is diffusion controlled and is dependent on the concentrations of the fluorophore and quencher. This type of quenching is described by the Stern-Volmer equation (19,20)

$$\frac{P_f^\circ}{P_f} = 1 + K_d C_q \qquad 1$$

where P_f° and P_f represent the fluorescence power in the absence and presence, respectively, of the quencher at molar concentration C_q. A graph of the fluorescence power ratio (P_f°/P_f) as a function of the quencher concentration is linear with a slope equal to the Stern-Volmer constant (K_d) and an intercept of unity. The Stern-Volmer constant is a direct measure of the efficiency of the fluorescence quenching process.

Although a large number of quenchers for PAHs have been identified, only a few have been characterized in sufficient depth and detail to permit routine use in forensic applications (3). Initial studies by Sawicki et al. (22) showed that nitromethane, which acts as an electron acceptor, selectively quenches the fluorescence of alternant PAHs. Subsequent studies by Acree et al. demonstrated that this so-called "nitromethane selective quenching rule" is broadly applicable, including unsubstituted PAHs as well as those with various functional groups and heterocyclic rings (23–26). A quantitative study by Ogasawara et al. (27) revealed that the Stern-Volmer quenching constants of nitromethane are 33 to 100 times greater for alternant than for nonalternant isomers. In contrast, recent investi-

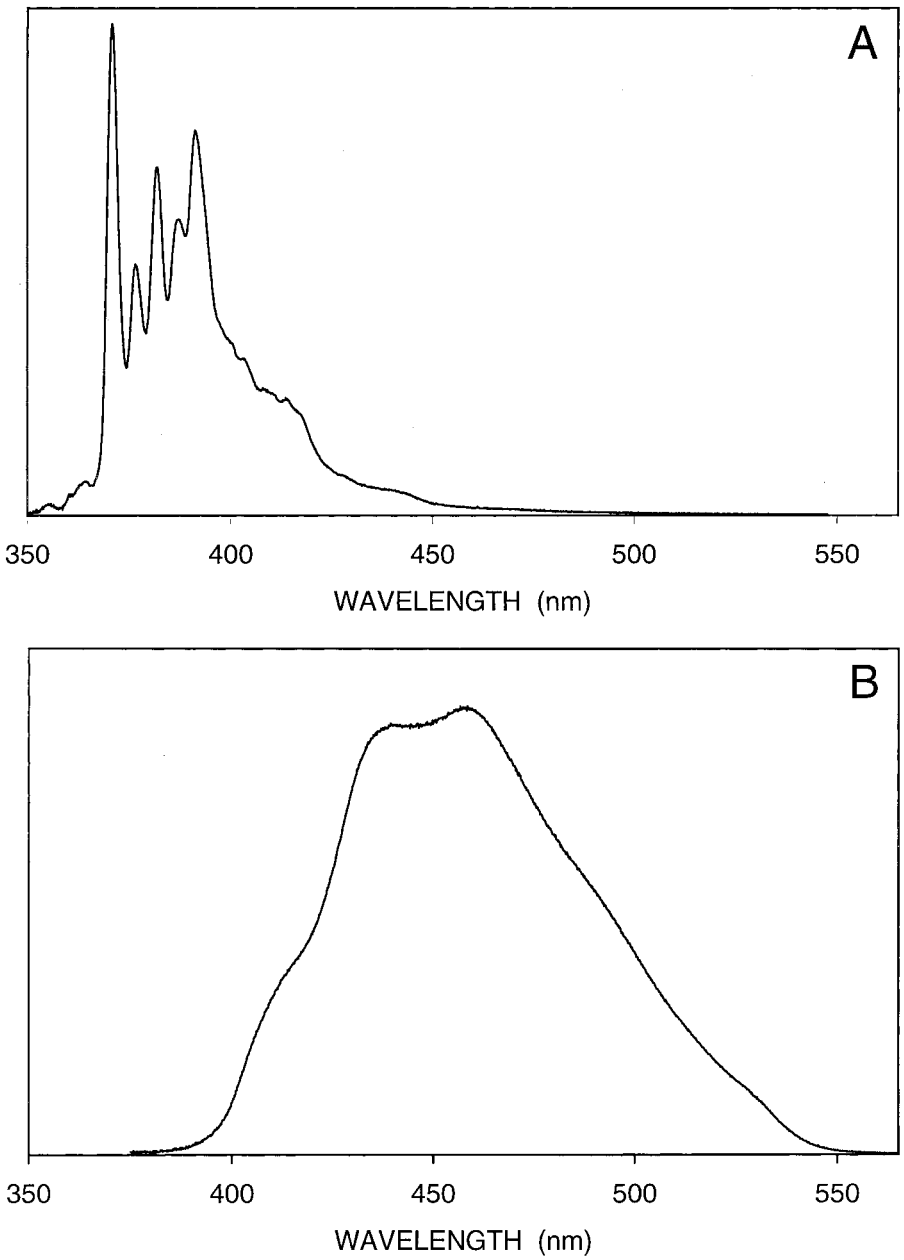

FIG. 2—*Fluorescence spectra showing the characteristic vibrational fine structure for alternant and nonalternant polycyclic aromatic hydrocarbons. Laser-induced fluorescence detection: 325 nm excitation, 350 to 564 nm emission, 2 nm resolution. Solutes: (A) 10^{-5} M pyrene in methanol, (B) 10^{-5} M fluoranthene in methanol.*

gations by Goodpaster and McGuffin (28) demonstrated that aliphatic amines, which act as electron donors, are selective quenchers for nonalternant PAHs. The Stern-Volmer quenching constants of diisopropylamine are typically 15 to 45 times greater for nonalternant than for alternant isomers.

In this study, laser-induced fluorescence with selective fluorescence quenching is combined with high-efficiency capillary liquid chromatography for the analysis of petroleum-based samples. This experimental approach provides unparalleled separation efficiency as well as detection sensitivity and specificity for particular PAH isomers. A wide range of information is acquired from a sample that can be used to identify individual PAHs, including chromatographic retention time, fluorescence emission spectra, and Stern-Volmer quenching constants. In addition, this approach provides many ways to profile the distribution of PAHs in a sample, including chromatograms at individual fluorescence wavelengths, chromatograms at integrated fluorescence wavelengths, chromatograms with fluorescence quenching of alternant PAHs by nitromethane, and chromatograms with fluorescence quenching of nonalternant PAHs by diisopropylamine. Through study of the relative distribution of PAHs, the formation conditions of an unknown petroleum mixture can be deduced and can aid in its classification and identification. This approach may help to determine if a known and unknown petroleum sample share a common source through qualitative and quantitative comparison of their resultant chromatograms.

Experimental Methods

Chemicals

A standard mixture (EPA 610, Supelco) consisting of sixteen alternant and nonalternant PAHs (Fig. 1) ranging in concentration from 98 to 1990 μg/mL was obtained. This mixture was volumetrically diluted with spectroscopic-grade nitromethane (EM Science) to yield a 10% (v/v) solution prior to injection. A reference sample of motor oil (Pennzoil™, 5W30), a sample of the same oil brand after 1371 miles of highway use, and three commercial formulations of petrolatum jellies (Vaseline™, Meijer™, and Smart Choice™ brands) were also obtained.

Two quenchers were chosen for these studies based upon their previously reported selectivity for alternant and nonalternant PAHs. Nitromethane (EM Science) was volumetrically diluted with high purity, spectroscopic-grade methanol (Baxter Healthcare, Burdick and Jackson Division) to yield a 2% (v/v) solution. Diisopropylamine (Aldrich) was volumetrically diluted with high purity, spectroscopic-grade acetonitrile (Baxter Healthcare, Burdick and Jackson Division) to yield a 50% (v/v) solution. High purity, spectroscopic-grade methanol (Baxter Healthcare, Burdick and Jackson Division) was used as the mobile phase for liquid chromatography.

Sample Preparation

For the motor oil samples, 20 mL portions of oil were extracted five times with 20 mL portions of spectroscopic-grade nitromethane (EM Science) in order to isolate the polycyclic aromatic compounds (5–7). The nitromethane was then removed by using a rotary evaporator (Büchi/Brinkmann, Rotavapor-R), yielding a brown, oily residue. This residue was redissolved in 2 mL of nitromethane and analyzed by capillary liquid chromatography.

Weighed portions (~10 g) of the petrolatum jelly samples were dissolved in 20 mL of spectroscopic-grade hexane (Baxter Healthcare, Burdick and Jackson Division). The hexane solutions were extracted five times with 20 mL portions of nitromethane. The nitromethane was then evaporated, yielding approximately 25 mg of yellow residue. This residue was redissolved in 2 mL of nitromethane before chromatographic analysis.

Instrumentation

Each of the samples was analyzed on the system shown in Fig. 3. A reciprocating piston pump (Beckman Instruments, Model 114M) was used to deliver the methanol mobile phase at a nominal flow rate of 1.0 μL/min. The sample was introduced by means of a valve with a fixed volume of 1.0 μL (Valco Instruments, Model ECI4W1), which was subsequently split 1:23 to provide an injection volume of approximately 43 nL. The sample constituents were then separated on a fused-silica capillary column (Hewlett-Packard, 200 μm i.d., 320 μm o.d., 1.5 m length) that was packed with a 5 μm octadecylsilica stationary phase (Shandon, Hypersil C18, 115 000 theoretical plates), as described previously (29). The column was immersed in a water bath maintained at 24°C to minimize the effect of temperature fluctuations on the separation. The column effluent was combined and thoroughly mixed with the quencher solution, which was delivered by a syringe pump (PE/Applied Biosystems, Model 140) at a nominal flow rate of 1.0 μL/min.

The PAHs were then detected by laser-induced fluorescence in a fused-silica capillary flow cell (Polymicro Technologies, 75 μm i.d., 360 μm o.d). A helium-cadmium laser (Melles Griot, Model 3074-40M, 325 nm, 32 mW) was used to irradiate the entire cross section of the flow cell. Fluorescence emission was collected orthogonal to the incident radiation and was collimated and filtered to remove stray light. The resulting emission was then refocused onto the entrance slit of a 0.34 m Czerny-Turner monochromator (Instruments SA, Model 340E, 300 groove/mm grating) and detected by a charge-coupled device (Instruments SA, Model (A) TECCD-2000 × 800-7). The CCD detector was thermoelectrically cooled and maintained at a temperature of −40°C. Instrument control and data acquisition were provided by a commercially available electronic interface (Instruments SA, Model CCD 2000) and the associated software (Instruments SA, Spectramax for Windows, Version 3.1). This fluorescence detection system had a detection limit of 3×10^{-9} M (2.3 ppb) quinine sulfate, a linear range of 10^5, and a spectral range of 300 nm with 1 nm resolution (30).

Data Analysis

As PAHs emit over a wide range of wavelengths, the fluorescence detector response was integrated over the range of 350 to 564

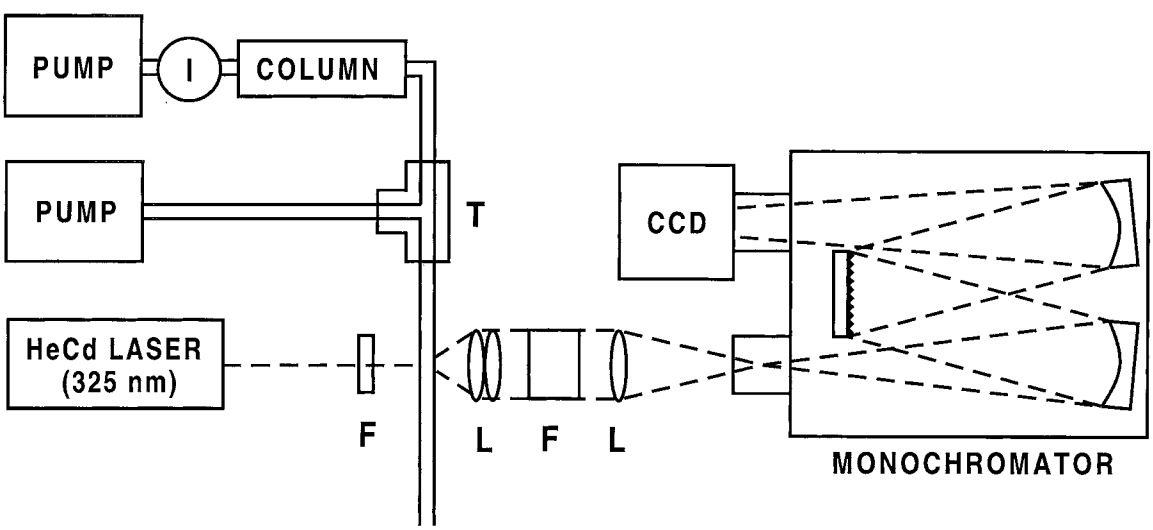

FIG. 3—*Schematic diagram of the experimental system for capillary liquid chromatography with laser-induced fluorescence and fluorescence quenching detection.* I = *injection valve,* T = *mixing tee,* L = *lens,* F = *filter,* CCD = *charge-coupled device.*

nm and the resultant area was displayed as a function of time. The time axes of all chromatograms were normalized in order to ensure that the known PAHs had the same retention times in each chromatogram. The chromatograms were then exported as ASCII files into the statistical analysis software (Jandel, SigmaStat, Version 1.02). The chromatograms were compared with one another by using the product moment correlation method (31,32). This method can be used to establish the extent of similarity between two chromatograms, both of which are regarded as independent variables. This parametric method assumes that the association (if any) is linear and that the residuals are normally distributed with constant variance. The resulting scatter plot shows the relationship between the relative peak heights or concentrations of the PAHs in the two samples. The correlation coefficient *(r)* of this plot quantifies the degree of similarity, and the corresponding *P*-value expresses the statistical reliability of the results.

Results and Discussion

In the discussion that follows, a standard mixture of PAHs and five petroleum samples are analyzed by capillary liquid chromatography without and with selective fluorescence quenching. The retention time, fluorescence emission spectrum, and observed quenching behavior are used to deduce the identity of each component. In addition, the chromatograms obtained with either nitromethane or diisopropylamine allow for profiling of the mixtures based on their alternant and nonalternant PAH content. This approach can help to establish the relative similarity and dissimilarity of two samples without specific identification of their components.

Standard PAH Mixture

A chromatogram of the standard mixture of PAHs (EPA 610) with laser-induced fluorescence detection is shown in Fig. 4*A*. The identity of each PAH was confirmed by comparison of the retention time and fluorescence spectrum with authentic standards (33,34). Of the sixteen known components in this sample, only eleven are fluorescent with excitation at 325 nm. Several of the smaller PAHs, including naphthalene, acenaphthylene, acenaphthene, fluorene, and phenanthrene, are not excited efficiently at this wavelength. The remainder of the PAHs, however, are readily detected in spite of the relatively small mass injected (0.42 to 0.85 ng).

A chromatogram of the standard after addition of nitromethane is shown in Fig. 4*B*. It is immediately evident that the nonalternant PAHs (fluoranthene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, and indeno(1,2,3-*cd*)pyrene) substantially retain their original fluorescence intensity. In contrast, the alternant PAHs (anthracene, pyrene, benz(*a*)anthracene, chrysene, benzo(*a*)pyrene, dibenz(*a,h*) anthracene, and benzo(*ghi*)perylene) are significantly quenched. This observation is consistent with the previously reported Stern-Volmer constants of 0.07 and 0.64 M^{-1} for the representative nonalternant PAHs fluoranthene and benzo(*b*)fluoranthene, and 94 and 61 M^{-1} for the representative alternant PAHs pyrene and benzo(*a*)pyrene (30). It is also noteworthy that benzo(*k*)fluoranthene appears to be more highly quenched than the other nonalternant PAHs in Fig. 4*A*. This behavior is in accord with differences in the electron-donating ability of the aromatic system to the nitromethane quencher (27,35). In fact, the gas-phase ionization energy (36) of benzo(*k*)fluoranthene (8.167 eV) is substantially less than that of fluoranthene (8.466 eV) and benzo(*b*)fluoranthene (8.410 eV), which suggests that it is a better electron donor. Rather, benzo(*k*)fluoranthene is more analogous to the alternant PAHs benz(*a*)anthracene (8.111 eV) and chrysene (8.261 eV), which is reflected in the quenching behavior.

Chromatograms of the PAH standard after addition of diisopropylamine are shown in Fig. 4*C*. In general, the nonalternant PAHs are moderately quenched and the alternant PAHs are unaffected. This observation is consistent with the previously reported Stern-Volmer constants of 17.1 and 21.2 M^{-1} for the representative nonalternant PAHs fluoranthene and benzo(*b*)fluoranthene, and 1.2 and 0.47 M^{-1} for the representative alternant PAHs pyrene and benzo(*a*)pyrene (28). Benzo(*k*)fluoranthene is an interesting exception to this general trend, as it is relatively unquenched by diisopropylamine. Its behavior, again, is more similar to the alternant PAHs benz(*a*)anthracene and chrysene than to the other nonalternant PAHs fluoranthene and benzo(*b*)fluoranthene.

Automotive Engine Oil

Chromatograms of a sample of unused Pennzoil™ motor oil are shown in Figs. 5*A* to *C*. None of the PAHs in this sample could be identified from the standard sixteen-component mixture. All of the PAHs exhibited relatively low retention times and featureless emission spectra. Such lack of vibrational structure tends to suggest nonalternant character, as discussed previously. However, the observed quenching behavior is indicative of alternant character. All PAHs were significantly quenched upon addition of nitromethane (Fig. 5*B*), whereas only slight quenching was seen for some PAHs upon addition of diisopropylamine (Fig. 5*C*). Taken together, these observations suggest two possible explanations. First, PAHs that are heavily alkylated tend to lose vibrational fine structure in their emission spectra, even if their parent structure is alternant (e.g., dimethylbenz(*a*)anthracene) (34). Despite this lack of structure, these compounds would be expected to behave as alternant PAHs and be quenched by nitromethane. Second, alternant PAHs with nearly circular arrangements of rings (e.g., benzo(*c*) phenanthrene and benzo(*c*)chrysene) also lack vibrational spectral detail due to the inherent flexibility of their nonplanar structures, but preserve their alternant quenching behavior (34). Given the low retention times of the compounds in unused motor oil, it seems more likely that they are small, highly alkylated alternant PAHs rather than the larger cyclic isomers.

This explanation is supported by the dramatic changes that were observed in the motor oil after use in an automobile. The results for an identical sample of oil after 1371 miles of use are shown in Figs. 6*A* to *C*. A number of the components from the unused motor oil are detected in this sample and are denoted by an asterisk (*). However, the overall chromatogram is decidedly more complex and includes numerous PAHs of higher molecular weight. In particular, a number of PAHs from the standard mixture are found at relatively high levels including both alternant (anthracene, pyrene, benz(*a*) anthracene, benzo(*a*)pyrene, benzo(*ghi*)perylene) and nonalternant (fluoranthene, benzo(*k*)fluoranthene) isomers.

Although the remaining PAHs in the chromatogram cannot be identified from the standard sixteen-component mixture, in some cases their general structure and alternant/nonalternant class can be deduced from their emission spectra and quenching behavior. Specific identifications are not possible because of the large number of isomers and the lack of vibrational fine structure in their fluorescence spectra. For example, Peak (a) has a retention time and spectrum consistent with methylated PAHs having an angular arrangements of four rings (i.e., isomers of methylchrysene and/or methylbenz(*a*)anthracene). In addition, this peak is quenched upon addition of nitromethane but is not affected by diisopropylamine

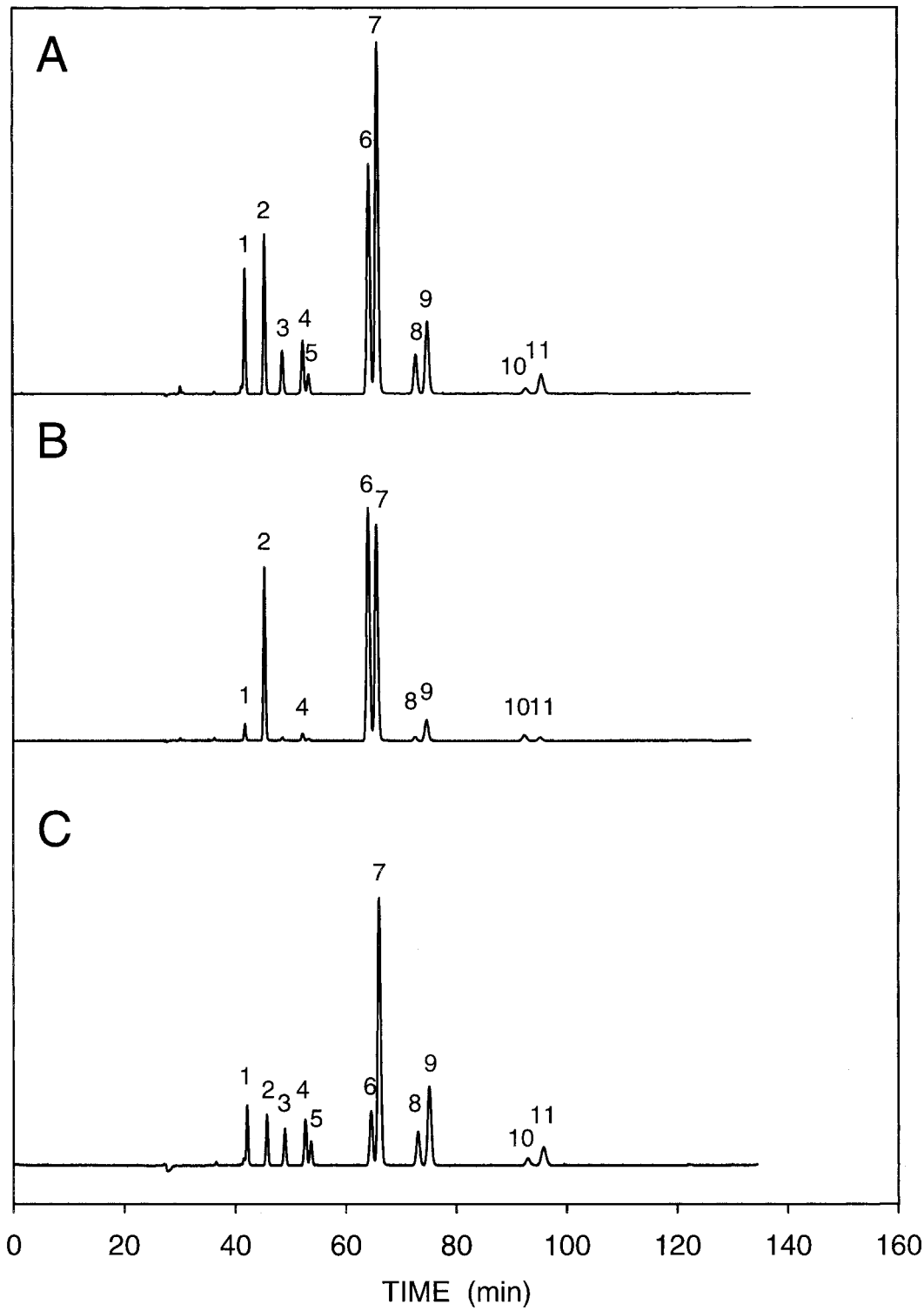

FIG. 4—*Chromatograms of standard polycyclic aromatic hydrocarbons (EPA 610) with post-column addition of (A) 100% methanol, 1.0 μL/min, (B) 2% v/v nitromethane in methanol, 1.0 μL/min, (C) 50% v/v diisopropylamine in acetonitrile, 1.0 μL/min. Column: 1.5 m × 200 μm i.d. fused-silica capillary, packed with 5 μm Shandon Hypersil C18. Mobile phase: methanol, 1.0 μL/min, 24 °C. Laser-induced fluorescence detection: 325 nm excitation, 350 to 564 nm emission, 2 nm resolution. Solutes: (1) anthracene, (2) fluoranthene, (3) pyrene, (4) benz*(a)*anthracene, (5) chrysene, (6) benzo*(b)*fluoranthene, (7) benzo*(k)*fluoranthene, (8) benzo*(a)*pyrene, (9) dibenz*(a,h)*anthracene, (10) indeno(1,2,3-*cd*)pyrene, (11) benzo*(ghi)*perylene.*

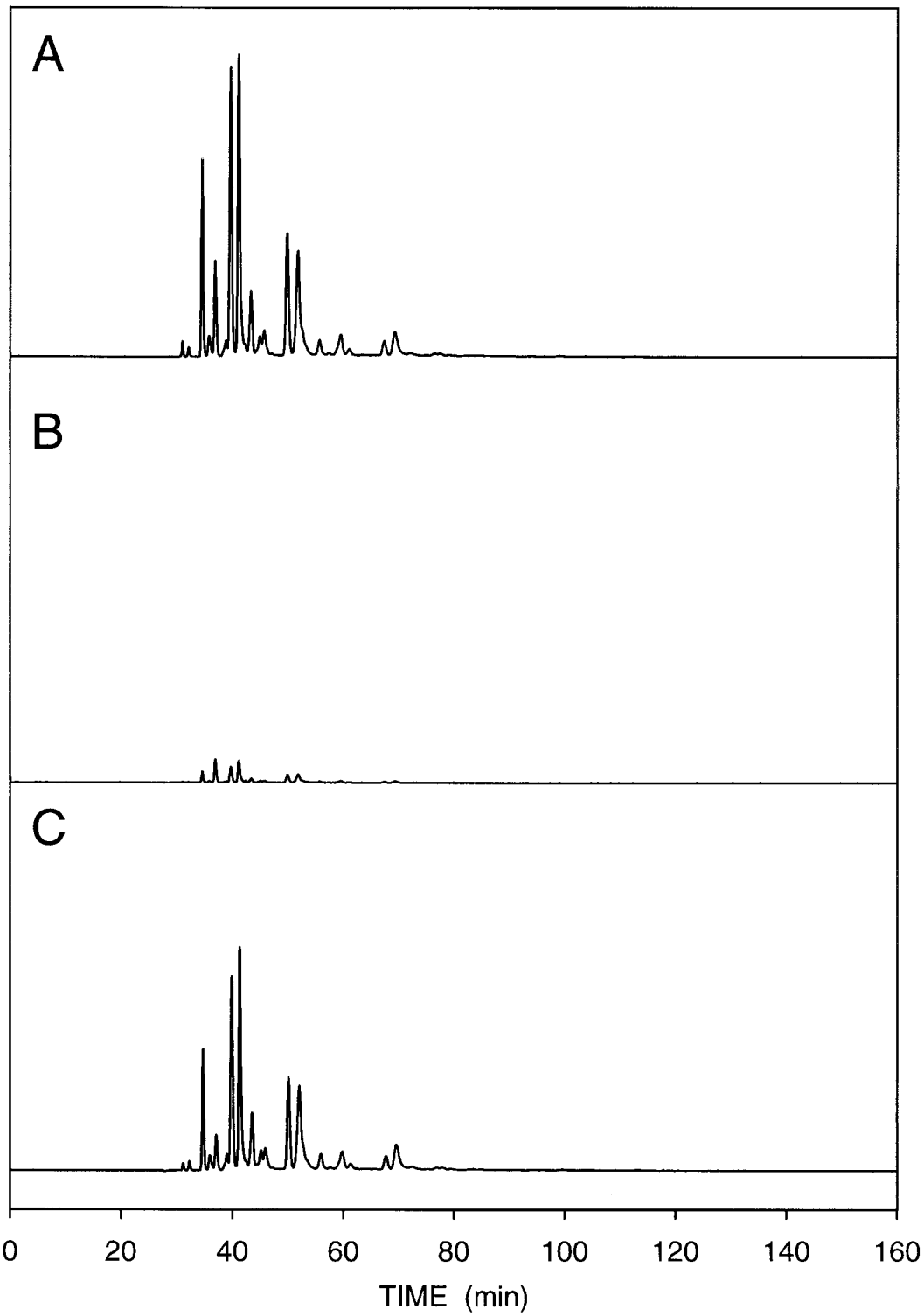

FIG. 5—*Chromatograms of unused Pennzoil™ motor oil (5W30) with postcolumn addition of (A) 100% methanol, 1.0 μL/min. (B) 2% v/v nitromethane in methanol, 1.0 μL/min, (C) 50% v/v diisopropylamine in acetonitrile, 1.0 μL/min. Other experimental conditions and solutes as described in Fig. 4.*

(see Figs. 6*B* and 6*C,* respectively). This confirms that the overall structure of this PAH is alternant in character.

There are two likely sources of new PAHs in motor oil after use, the first being reactions of highly alkylated PAHs to form unsubstituted or methylated PAH isomers. In particular, highly alkylated PAHs are less stable and form at lower temperatures over longer time scales (e.g., during formation of crude oil) (4). In contrast, PAHs that are devoid of side chains form rapidly at high temperatures (e.g., during exposure to high engine temperatures) (4). Such high temperature conditions must be sustained over a long time period in order to form the most stable isomers. The PAH isomers that are most stable contain alternant, clustered arrangements of aromatic rings (e.g., pyrene), followed by angular arrangements (e.g., benz(*a*)anthracene) and linear arrangements (e.g., anthracene). Fi-

nally, nonalternant PAHs (e.g., fluoranthene) tend to form at lower temperatures and the number of nonaromatic rings increases with reaction time (4). A wide variety of such reactions is possible at the low and high temperatures typical of automobile engines. This allows for the formation of a number of PAHs, whose identity and distribution may be reflective of the particular engine, the operating conditions, and the motor oil used.

The second possible source of unsubstituted PAHs in used motor oil is contamination by fuel or its combustion products from the engine cylinders. Gasoline and diesel fuels are known to contain al-

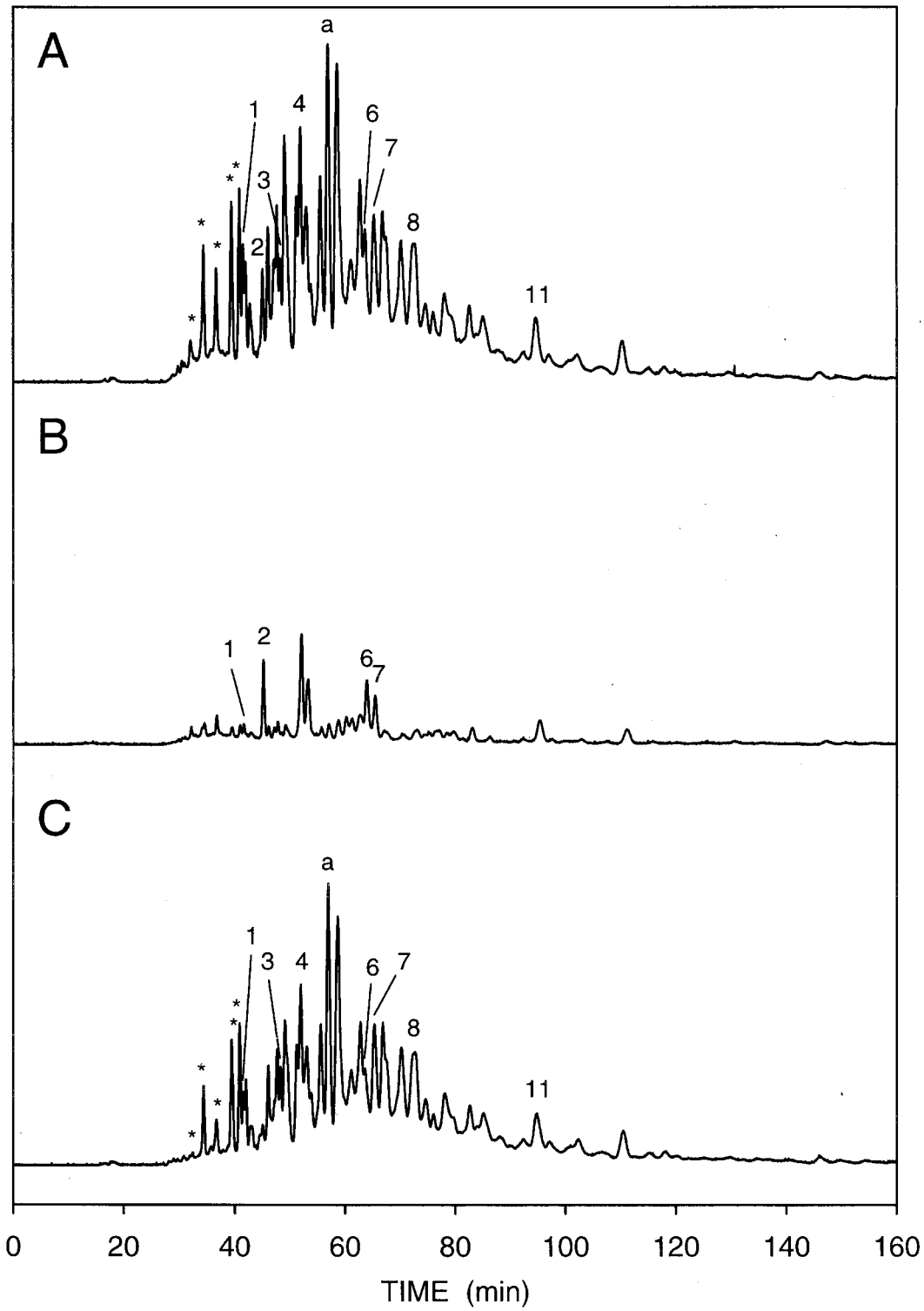

FIG. 6—*Chromatograms of used Pennzoil™ motor oil (5W30) with postcolumn addition of (A) 100% methanol, 1.0 μL/min, (B) 2% v/v nitromethane in methanol, 1.0 μL/min, (C) 50% v/v diisopropylamine in acetonitrile, 1.0 μL/min. Solutes: (*) residual peaks from unused oil, (1) anthracene, (2) fluoranthene, (3) pyrene, (4) benz*(a)*anthracene, (a) consistent with methylchrysene and methylbenz*(a)*anthracene isomers, (7) benzo*(k)*fluoranthene, (8) benzo*(a)*pyrene. Other experimental conditions and solutes as described in Fig. 4.*

ternant and nonalternant PAHs that are highly soluble in oil (34). In addition, the distribution and identity of these PAHs may differ by type or even brand of fuel as well as the combustion temperature of the engine. Therefore, the process of driving should impart a number of characteristics to the motor oil that could be used for its unique identification and comparison to a reference sample, regardless of the source of detected PAHs.

Petrolatum Jelly

A chromatogram of Vaseline™ brand petrolatum jelly is shown in Fig. 7*A*. After comparison of retention times and reference spectra, two PAHs that are present in the standard mixture (fluoranthene and pyrene) have been successfully identified at trace levels in this sample. Fluoranthene appears to be coeluting with another PAH whose emission, while shifted to shorter wavelengths, is similarly unstructured. This would imply that, like fluoranthene, this compound is nonalternant (21). These conclusions are confirmed by the quenching behavior. For example, upon addition of nitromethane (Fig. 7*B*), no decrease is observed for the unknown/fluoranthene peak whereas the pyrene peak is completely quenched. The reverse trend is seen in Fig. 7*C,* where addition of diisopropylamine causes a significant decrease in the unknown/fluoranthene peak but has little effect on the pyrene peak.

Four main groups of PAHs were identified in the Vaseline™ sample. As discussed previously, Group (a) has retention times and spectra consistent with alkylated PAHs having angular arrangements of four rings (i.e., isomers of methylchrysene and/or methylbenz(*a*)anthracene). These PAHs are quenched upon addition of nitromethane but are not affected by diisopropylamine (Figs. 7*B* and 7*c,* respectively). This confirms that the overall structure of these PAHs is alternant in character. Group (b) has structured emission spectra centered at approximately 360 nm. This group remains as yet unidentified, but shares the same quenching behavior as Group (a) and, therefore, can be tentatively identified as alternant. Group (c) has emission spectra that are differentiable from Group (a) and are consistent with heterocyclic PAHs having angular arrangements of four to five rings (i.e., isomers of benzacridine and dibenzacridine). These PAHs also demonstrate alternant character in their quenching behavior. Group (d) is the major component of this extract, and has a retention time and slightly structured emission spectrum consistent with highly alkylated fluoranthene, benzo(*b*)fluoranthene, or a larger dibenzofluoranthene isomer. Furthermore, like fluoranthene and benzo(*b*)fluoranthene, this component shows no change upon addition of nitromethane and a marked decrease in intensity upon addition of diisopropylamine, which supports the inference of a nonalternant structure.

A number of similarities and differences can be seen in the results for different brands of petrolatum jelly. For example, the chromatograms obtained for a Meijer™ brand product are shown in Figs. 8*A* to *C*. Major similarities include the presence of fluoranthene and pyrene, as well as a number of PAHs that are assigned to Groups (a), (b), and (c), as described above. In addition, the retention times for these latter PAHs correspond to those seen in the Vaseline™ sample, indicating that a number of the same PAHs are present in both samples. Major differences include a larger number of components, the presence of a small amount of anthracene, and the lack of any large, nonalternant PAH such as Peak (d) in Fig. 8*A*. Indeed, upon addition of nitromethane (Fig. 8*B*), the vast majority of components in this sample are rendered undetectable, with only the fluoranthene peak clearly remaining. Conversely, only fluoranthene is affected upon addition of diisopropylamine (Fig. 8*C*), implying that the remainder of the PAHs are alternant in structure.

The results for the final brand of petrolatum (Smart Choice™) are shown in Figs. 9*A* to *C*. This sample also possesses unique characteristics such as the presence of pyrene in the absence of any other standard PAH. Furthermore, no peaks corresponding to Group (b), as described above, could be found. The presence of peaks assigned to Groups (a) and (c) demonstrates that a number of the same alkylated and heterocyclic PAHs appear in these samples, but their relative distribution varies. Finally, the quenching studies shown in Figs. 9*B* and 9*C* indicate that this sample contains no detectable levels of nonalternant PAHs.

Statistical Correlation Analysis

The results discussed above demonstrate that different brands of petrolatum jellies can be easily discriminated on the basis of the presence or absence of various alternant and nonalternant PAHs. It is important to note that complete separation and identification of all PAHs is not necessary for profiling of these mixtures. Successful differentiation can be achieved through qualitative comparisons of the chromatograms obtained without and with selective quenching agents that correspond to different populations of PAH isomers within the sample. More quantitative comparison of the chromatograms can be obtained through statistical methods such as the product moment correlation method (31,32). When samples are derived from exactly the same origin, the relative peak heights or concentrations of PAHs in each sample are identical and the resulting correlation coefficient (r) is equal to 1.00. When samples are of similar or related origin, many of the same PAHs may be present but at different concentrations. This results in an intermediate degree of correlation with typical values of r in the range of 0.50 to 0.90. Finally, when samples are of distinctly unrelated origin, the disparate distribution of PAHs will result in little or no correlation with typical values of r less than 0.50. In all cases, valid conclusions can be drawn about the identity or origin of the samples when the P-value for the product moment correlation is less than 0.05, corresponding to the 95% confidence limit. For this study, the calculated P-values ranged from 4.9×10^{-220} to 2.8×10^{-2} and, thus, have statistical significance.

Table 1 summarizes the results of the product moment correlation for the three petrolatum jelly samples examined with fluorescence detection alone (see Figs. 7*A,* 8*A,* and 9*A*). It is apparent that there is little correlation between the Vaseline™ and the Meijer™ or Smart Choice™ samples, despite the common PAHs found in each sample ($r = 0.159$ and 0.142, respectively). As many of the PAHs in the more complex Meijer™ and Smart Choice™ samples are not found in the Vaseline™ sample, these samples present the unique challenge of profiling with limited information for which this correlation method is well suited. The Meijer™ and Smart Choice™ samples show a rather high degree of correlation ($r =$

TABLE 1—*Correlation coefficient* (r) *of the product moment method for chromatograms of petrolatum jelly obtained by using laser-induced fluorescence detection.*

Sample	Vaseline™	Meijer™	Smart Choice™
Vaseline™	1.000	0.159	0.142
Meijer™	0.159	1.000	0.931
Smart Choice™	0.142	0.931	1.000

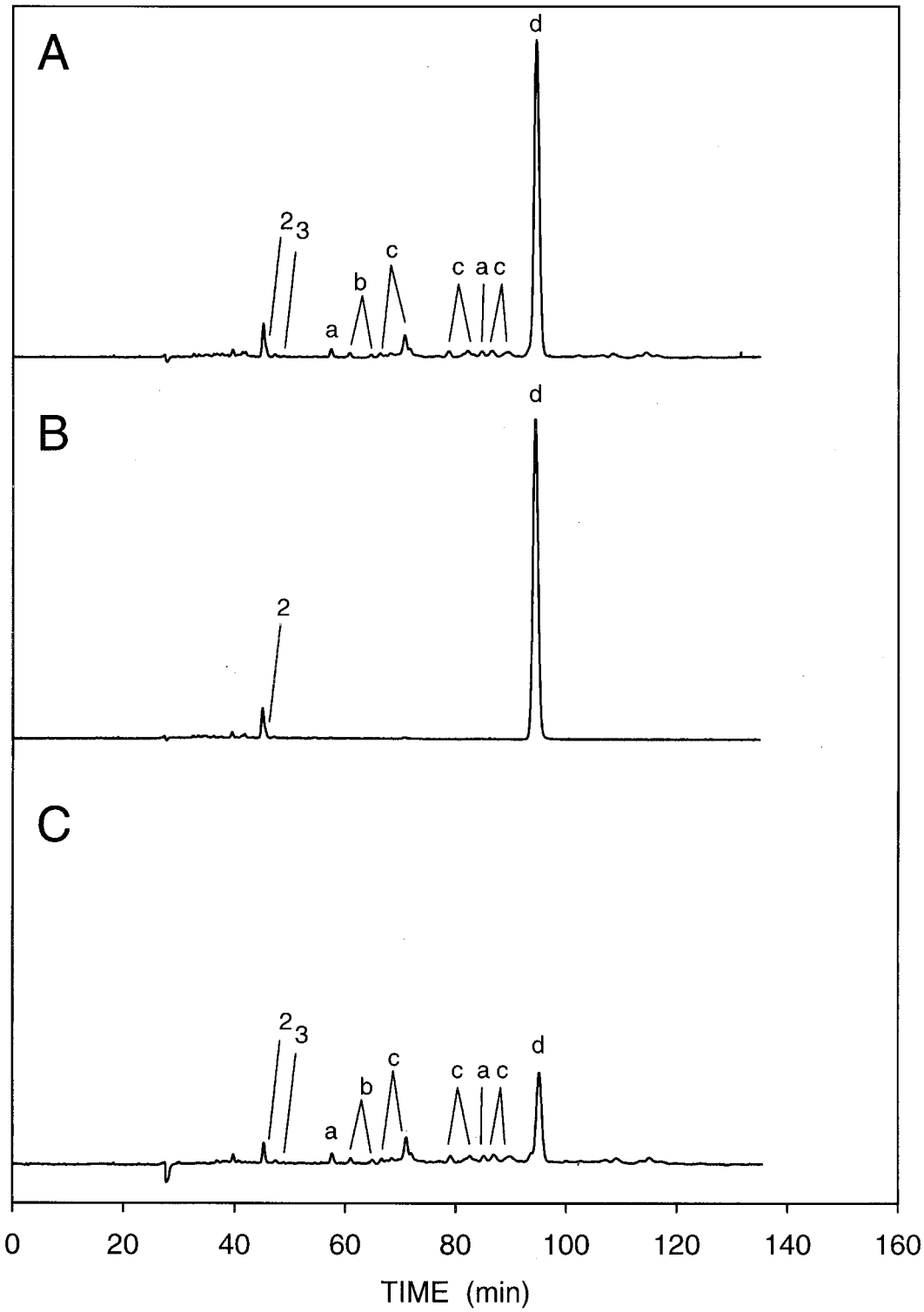

FIG. 7—*Chromatograms of Vaseline™ petrolatum jelly with postcolumn addition of (A) 100% methanol, 1.0 μL/min, (B) 2% v/v nitromethane in methanol, 1.0 μL/min, (C) 50% v/v diisopropylamine in acetonitrile, 1.0 μL/min. Solutes: (2) fluoranthene, (3) pyrene, (a) consistent with methylchrysene and methylbenz*(a)*anthracene isomers, (b) unknown alternant PAHs, (c) consistent with benzacridine and dibenzacridine isomers, (d) consistent with an alkylated fluoranthene, alkylated benzo*(b)*fluoranthene, or dibenzofluoranthene isomer. Other experimental conditions and solutes as described in Fig. 4.*

0.931), which is consistent with the similar appearance of their chromatograms in Figs. 8*A* and 9*A*. In addition, this reflects a similarity in their overall composition, petroleum source, and manufacturing conditions. However, slight variations in their components allow for differentiation of these samples (see below).

PAH profiling using statistical correlation methods becomes even more versatile and powerful when combined with selective fluorescence quenching. Table 2 summarizes the results of the product moment correlation for the three samples with fluorescence quenching by nitromethane (see Figs. 7*B,* 8B, and 9*B*). As

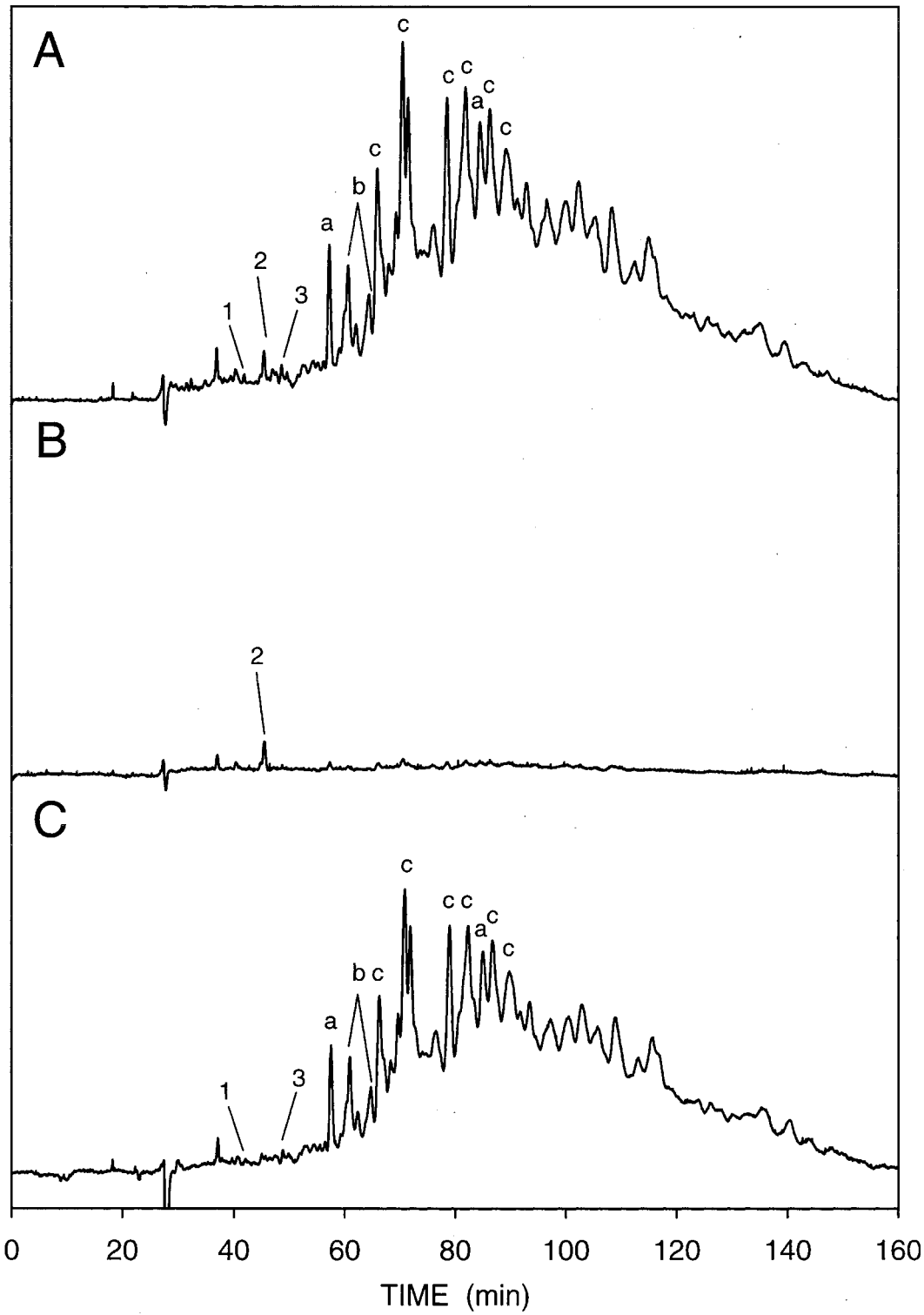

FIG. 8—*Chromatograms of Meijer™ petrolatum jelly with post-column addition of (A) 100% methanol, 1.0 μL/min, (B) 2% v/v nitromethane in methanol, 1.0 μL/min, (C) 50% v/v diisopropylamine in acetonitrile, 1.0 μL/min. Solutes: (1) anthracene, (2) fluoranthene, (3) pyrene, (a) consistent with methylchrysene and methylbenz(*a*)anthracene isomers, (b) unknown alternant PAHs, (c) consistent with benzacridine and dibenzacridine isomers. Other experimental conditions and solutes as described in Fig. 4.*

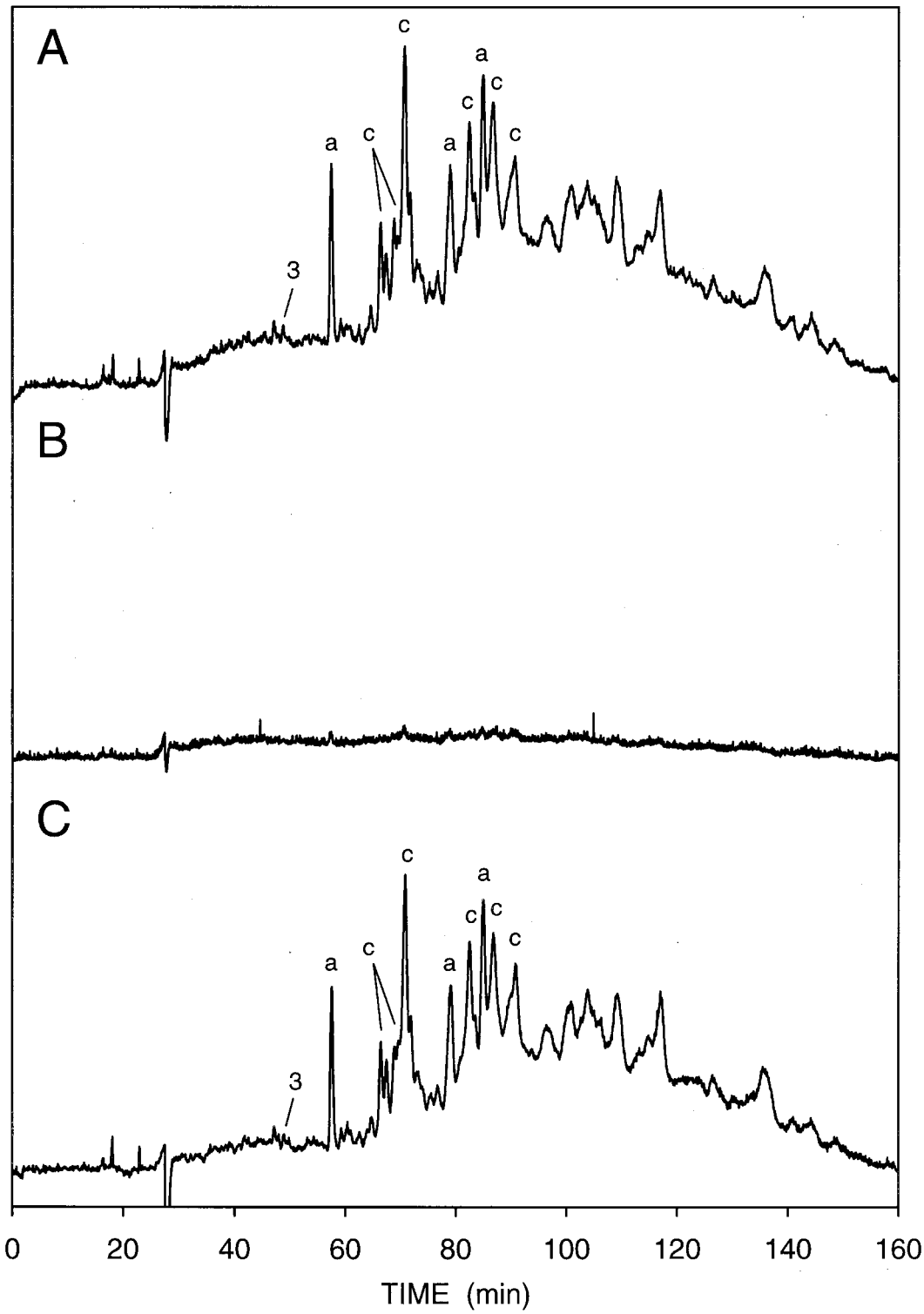

FIG. 9—*Chromatograms of Smart Choice™ petrolatum jelly with post-column addition of (A) 100% methanol, 1.0 μL/min, (B) 2% v/v nitromethane in methanol, 1.0 μL/min, (C) 50% v/v diisopropylamine in acetonitrile, 1.0 μL/min. Solutes: (3) pyrene, (a) consistent with methylchrysene and methylbenz*(a)*anthracene isomers, (c) consistent with benzacridine and dibenzacridine isomers. Other experimental conditions and solutes as described in Fig. 4.*

TABLE 2—*Correlation coefficient (r) of the product moment method for chromatograms of petrolatum jelly obtained by using laser-induced fluorescence detection with selective quenching by nitromethane.*

Sample	Vaseline™	Meijer™	Smart Choice™
Vaseline™	1.000	0.146	0.100
Meijer™	0.146	1.000	0.895
Smart Choice™	0.100	0.895	1.000

TABLE 3—*Correlation coefficient (r) of the product moment method for chromatograms of petrolatum jelly obtained by using laser-induced fluorescence detection with selective quenching by diisopropylamine.*

Sample	Vaseline™	Meijer™	Smart Choice™
Vaseline™	1.000	0.395	0.374
Meijer™	0.395	1.000	0.936
Smart Choice™	0.374	0.936	1.000

the alternant PAHs are selectively quenched, this correlation discriminates on the basis of the distribution of nonalternant PAHs in the samples. When viewed on this basis, the Vaseline™ sample is still distinctly different from the Meijer™ or Smart Choice™ brands. In fact, the degree of correlation decreases, as the many alternant PAHs that exist in common between these samples are diminished in the quenched chromatograms. This behavior is also seen for the Meijer™ and Smart Choice™ samples, whose nonalternant content is limited to a small amount of fluoranthene in the Meijer™ sample and no detectable nonalternant PAHs in the Smart Choice™ sample.

Table 3 summarizes the results of the product moment correlation with fluorescence quenching by diisopropylamine (see Figs. 7*C,* 8*C,* and 9*C*). As the nonalternant PAHs are selectively quenched, this correlation discriminates on the basis of the distribution of alternant PAHs in the samples. In all cases, the correlation between samples based on the alternant PAHs is larger than that for either the nonalternant PAHs (Table 2) or the unquenched chromatograms (Table 1). These results show that the samples are most similar in their alternant character.

Summary

Fluorescence and selective fluorescence quenching appear to provide complementary information for profiling PAHs in complex samples. For example, unquenched fluorescence emission offers broad-based information about the possible identities of unknown PAHs. In contrast, fluorescence quenching by nitromethane allows selective discrimination of the nonalternant PAHs and quenching by diisopropylamine allows selective discrimination of the alternant PAHs. Only when all of these profiles show a high degree of correlation can it be confidently concluded that two forensic samples are of the same origin. In this study, comparison of chromatograms without and with selective quenchers successfully distinguished three different brands of petrolatum jelly, based largely upon the distribution of nonalternant PAH isomers. In addition, the effect of normal use on motor oil was shown to impart a characteristic profile that may be used to identify the source of such samples. This approach may also be applied to the analysis of arson evidence. The presence of petroleum-based accelerants would be expected to alter the distribution of alternant and nonalternant PAHs as well as the degree of alkylation when exposed to the high temperatures of a fire. Finally, because of the small sample volumes (nanoliter) of capillary liquid chromatography and the inherent sensitivity (ppb) of laser-induced fluorescence detection, this approach should be suitable for the small amounts of petroleum-based materials that are generally obtained for forensic analysis.

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Additional information and reprint requests:
Victoria L. McGuffin, Ph.D.
Department of Chemistry
Michigan State University
East Lansing, MI 48824-1322