HPLC Analysis of the Large Polycyclic Aromatic Hydrocarbons in a Diesel Particulate

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Key Words

Column liquid chromatography
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Summary

High performance liquid chromatography (HPLC) was used to separate the large polycyclic aromatic hydrocarbons in a diesel particulate extract. Identification of individual peaks was made using a photodiode array UV/visible detector to collect their absorbance spectra. Comparisons between standard compounds and the peaks were made using both retention times and spectra. Compounds of up to 10 rings were identified.

Introduction

The analysis of materials for polycyclic aromatic hydrocarbons (PAHs) is of great importance because of increasing concern over the mutagenicity of several of these compounds. The level of mutagenic activity of a particular PAH is very dependent on structure, so that similar isomers can range from being highly active to totally inactive. Determination of the PAHs and their levels in a material could be used to estimate its mutagenicity. Many analytical techniques have been used for PAH analysis, but many of the more common ones suffer from limitations when applied to the larger PAHs (those of over 24-ring carbons). The very low volatility of the large PAHs makes gas chromatography analysis impossible. The large number of isomers for any particular carbon number limits mass spectroscopy because of the identical molecular weights and similar fragmentation patterns that isomers yield. High-performance liquid chromatography (HPLC), when coupled to a photodiode array detector, can both separate these molecules and differentiate the isomeric types.

Each isomeric PAH has a unique, characteristic UV/visible absorbance spectrum, since the spectrum is generated by absorbance of photons with energies matching the electronic transitions in the molecule. The relative energies of the transitions are determined by the overall shape and size of the aromatic conjugation in the molecule [1, 2]. These transitions yield a pattern of absorbance maxima and minima whose relative intensities and shapes easily identify a PAH. Thus, the large, isomeric PAHs naphtho[8,1,2-bcd]perylene and dibenzo[cd,lm]perylene have quite different UV/visible spectra because the relative locations of the seven rings are different (Fig. 1). When this ability to spectrally differentiate isomers is coupled to the separating capabilities of HPLC, a very powerful technique for PAH analysis results.

In the past, analyses for the large PAHs in many complex mixtures have been done using various methods. The materials examined include carbon blacks, coal tars, chimney soot, and automobile and other airborne particulates [3–7]. All of these analyses utilized either HPLC or thin-layer chromatography for separation and separate secondary analyses for characterization. The ancillary methods used included UV/visible, fluorescence, and mass spectroscopies. These types of characterization can be very definitive, but also are very slow and tedious. The correlation of data from various independent analyses adds to the time needed to characterize the sample. On-line detection of the HPLC column eluent by use of a full-spectrum UV/visible absorbance detector would be as accurate in characterization, but would be many times faster and allow simultaneous comparison of both spectral and chromatographic data.

The analysis of PAHs in automobile or diesel engine particulates has mainly centered on the small PAHs of five or fewer rings [8–10]. The primary reason for this emphasis has been that these small PAHs are known to contribute to the mutagenic activity of engine particulates [11]. The primary means for analyzing these smaller PAHs have been either GC/MS or HPLC with single wavelength absorbance or fluorescence monitoring. A secondary reason for the

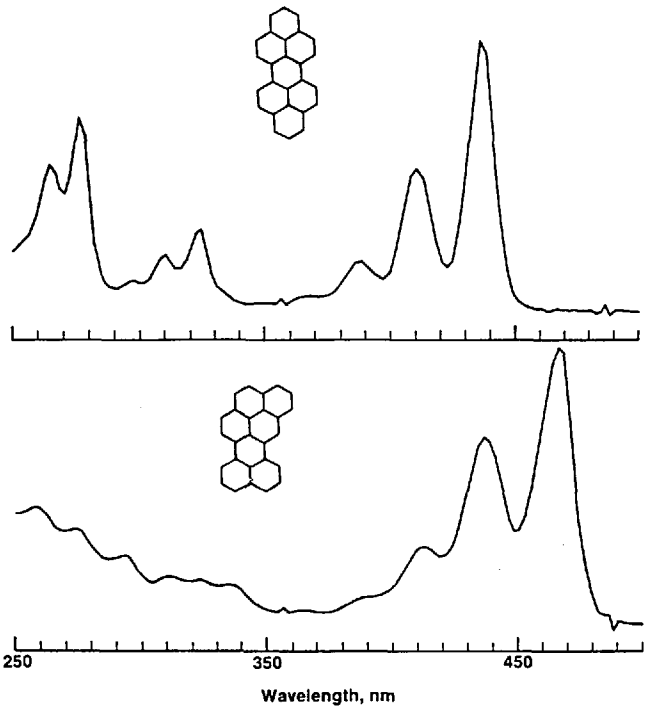

Fig. 1

Spectra of the two $C_{26}H_{14}$ isomers, dibenzo[cd,lm]perylene and naphtho[8,1,2bcd]perylene.

small PAHs being the focus of the analyses is the paucity of standard compounds of the large PAHs. This shortage prevents both identification of the large PAHs actually occurring in a sample and evaluation of the potential mutagenic activity associated with the large isomers. The identification of large PAH isomers present in samples was, until recently, limited to only the few available standard compounds. The best possible identification, in most cases, relied on matching sample spectra to spectra from the original syntheses of these compounds in the organic chemistry literature. This approach normally utilized the chromatography only as a separating tool and not as a separate independent piece of datum, since retention time comparisons could not be made. This is much less desirable than comparison with standards run under identical analytical conditions. A significant change in this situation has been the recent increase in the available standard PAHs and use of a coupled HPLC/full-spectrum photodiode array detector [12, 13]. As part of a program to analyze materials for large PAHs, we report here the examination of a diesel particulate.

Experimental

A 20-g sample of diesel exhaust particulate (collected in the exhaust pipe of a Volkswagen engine operated at 4000 rpm) was extracted with a Soxhlet apparatus using 300 mL of methylene chloride [14]. The resulting tar (1.4g) was mixed with silica gel. This mixture was added to the top of a column, 1.8cm i.d. × 20cm, packed with 200-mesh silica and sequentially eluted with n-hexane, and 15% methylene chloride in methanol. The aromatic fraction was determined to be the second of the four fractions collected because of its intense blue-violet fluorescence when exposed to UV radiation.

A reversed-phase HPLC separation of the PAH fraction was performed on a Vydac 218TP5 octadecyl-bonded phase column (0.46cm i.d. × 25cm, Separations Group, Hesperia, CA) after first drying an aliquot of the sample (containing approximately 12mg of PAHs) and then redissolving it in 250μL of 1:1 methanol/methylene chloride. A 25μL sample was injected with a Valco C6U loop injector. A 45-minute linear gradient from 85:15 to 10:90 methanol/methylene chloride was used. A flow rate of 2.75mL/min was used. All solvents were HPLC grade (Burdick and Jackson Laboratories, Muskegon, MN) and used as received. The standard PAHs were obtained from a variety of commercial sources or were synthesized [12, 13]. The standards were dissolved in 1:1 methanol/methylene chloride at concentrations of approximately 10ng/25μL.

The detector system used was a Hewlett-Packard (HP) 1040A photodiode array detector. Data collection, as well as control of the detector, was accomplished with an HP-85 microcomputer with MCR-2 software (Informetrix, Seattle, WA). The eluent was monitored every 0.25 second and the spectra of the peaks at their maxima were stored on flexible discs using an HP 82901M disc drive. An HP 7470 plotter was used to display the data. The range of stored spectra was 250–600nm with a spectral resolution of 2nm. (All spectra shown have small baseline "peaks" at 358 and 488nm due to electronic differences between those photodiodes and the remainder of the array.) Storage of a spectrum was either performed manually or by triggering a peak detection subroutine in the system software. (When a minima in the second derivative curve of the profiling wavelength signal was seen, which indicated a peak maximum, a spectrum was stored.) The chromatographic monitoring wavelength was 305nm, which was found to be a good wavelength for many large PAHs.

Results and Discussion

The chromatogram obtained for the extract and structural assignments for the identified peaks are shown in Fig. 2. The large PAHs represented 0.1 to 0.2wt% of the total PAH content. Only those peaks which could be matched both chromatographically and spectrally to standards were considered to be confirmed. In addition to the PAH structures shown, a total of 47 other PAHs of 6–12 rings were run for comparison, although they were not seen in the sample. Spiking of aliquots of the sample with known compounds was done for the retention time comparisons. Spectral comparisons were made by overlaying the stored spectra from the diesel extract with those from separate data files collected from chromatograms of standard compound mixtures. The names of the compounds identified are listed in Table I. As examples of the spectral comparisons used for identification purposes, two pairs of spectra from peaks and standard compounds are shown in Figs. 3 and 4. Excellent spectral and retention time agree-

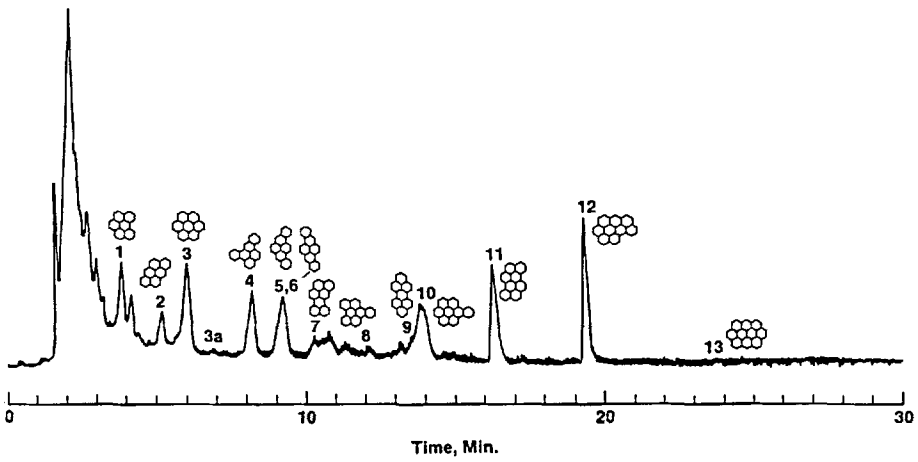

Fig. 2

Chromatogram of the diesel particulate extract, monitored at 305nm. Other conditions as given in the experimental section.

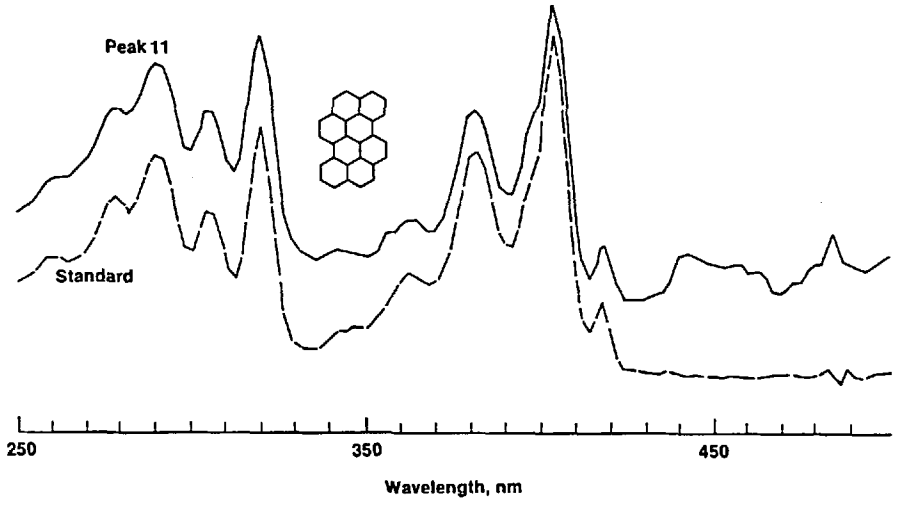

Fig. 3

Comparison spectra of Peak 11 (——, retention time = 16.26min) and benzo-[pqr]naphtho[8,1,2bcd]perylene (– –, retention time = 16.32min).

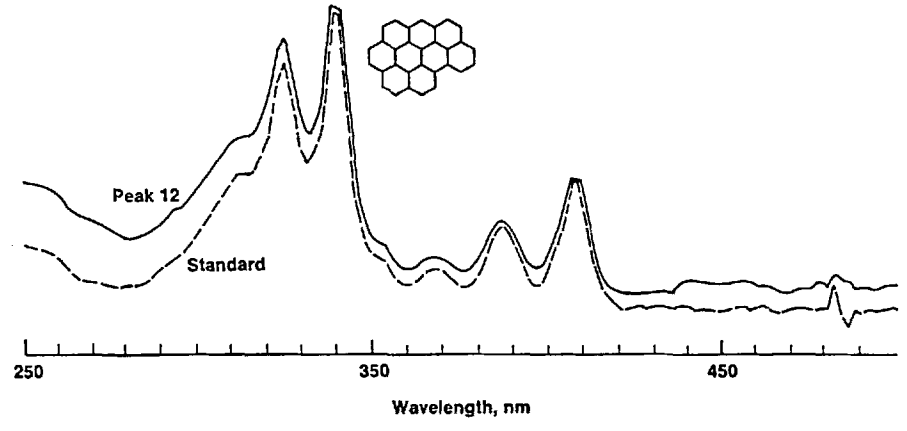

Fig. 4

Comparison spectra of Peak 12 (——, retention time = 19.23min) and naphtho-[8,1,2abc]coronene (– –, retention time = 19.21min).

Table I. Compounds found in the diesel extract

Peak	Name
1	benzo[ghi]perylene
2	dibenzo[def,mno]chrysene
3	coronene
3a	methyl coronene
4	naphtho[1,2,3,4def]chrysene
5	benzo[rst]pentaphene
6	dibenzo[b,def]chrysene
7	naphtho[8,1,2bcd]perylene
8	naphtho[1,2,3,4ghi]perylene
9	dibenzo[cd,lm]perylene
10	benzo[a]coronene
11	benzo[pqr]naphtho[8,1,2bcd]perylene
12	naphtho[8,1,2abc]coronene
13	ovalene

ments are seen in each case. The spectral range of the detector is also important in analysis for large PAHs because many distinct spectral features are present in the upper wavelength region (>400nm).

The last identified component, ovalene (Peak 13), is not seen as a distinct peak at 305nm. The optimum wavelengths for its detection, however, are not good ones for many of the other compounds found. A single wavelength profile does not show all of the peaks seen with a full-spectrum detector. This highlights another advantage of the photodiode array detector; it can more easily detect peaks than conventional UV detectors because all wavelengths are monitored.

All of the PAHs of six or more rings were found to be highly fused types, and no linear or nonalternant (PAHs with five-member rings) types were seen. This contrasts dramatically with the smaller PAHs seen in this particulate extract. Of the four-ring PAHs found, the linear-type PAHs chrysene and benz(a)anthracene are major components, and there was an appreciable amount of fluoranthene. The total amount of large PAHs found was approximately 20μg. Several peaks of low intensity were seen, but the spectra could not be matched. These could possibly be small amounts of linear or nonalterant PAHs.

Little work has been performed to determine the mutagenicity of the larger PAHs. Some have been shown to be inactive (coronene and ovalene), but one – dibenzo[cd,lm]-perylene – has very weak activity [15] and the two dibenzopyrene isomers (Peaks 5 and 6) have moderate activity. The absolute levels of these large PAHs are small, so their effect on sample mutagenicity should be minimal when compared to the influences of the large levels of the smaller, more active PAHs. However, their presence in particulate matter necessitates determination of their mutagenic activities so that the source of the activity in the total particulate can be better pinpointed.

Conclusion

A diesel particulate extract was examined for large PAHs. The use of reversed-phase HPLC, with a photodiode array detector for spectral monitoring of the eluent, allowed identification of several six-ring and larger PAHs. Only fused PAHs were found. No linear types or those with five-membered rings were found.

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