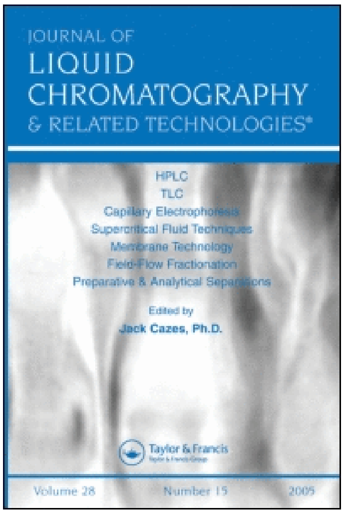

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# The Application of Perdeuterated Polycyclic Aromatic Hydrocarbons (PAH) as Internal Standards for the Liquid Chromatographic Determination of PAH in a Petroleum Crude Oil and Other Complex Mixtures

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# THE APPLICATION OF PERDEUTERATED POLYCYCLIC AROMATIC HYDROCARBONS (PAH) AS INTERNAL STANDARDS FOR THE LIQUID CHROMATOGRAPHIC DETERMINATION OF PAH IN A PETROLEUM CRUDE OIL AND OTHER COMPLEX MIXTURES

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## ABSTRACT

A sequential liquid chromatographic (LC) procedure for the determination of polycyclic aromatic hydrocarbons (PAH) in a petroleum crude oil and other complex mixtures is described. The procedure includes normal-phase LC on an aminosilane column to isolate fractions containing isomeric PAH and reversed-phase LC on a polymeric $C_{18}$ column to separate the individual PAH isomers. Appropriate perdeuterated PAH are added to the sample so that each isomeric fraction will contain one internal standard. The perdeuterated PAH are excellent internal standards for this sequential LC procedure. Perdeuterated PAH have normal-phase and reversed-phase LC retention characteristics similar to those of the parent PAH. In the normal-phase LC separation, the perdeuterated PAH elute in the same fraction as the parent PAH. In the reversed-phase LC separation, the perdeuterated PAH elute first and are generally resolved from the parent PAH. The optimized spectrofluorometric detection of each PAH analyte is accomplished by programming appropriate sets of excitation and

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emission wavelengths to correspond with the elution time of each analyte on the polymeric $C_{18}$ column. The analytical results obtained from this procedure for the analysis of a shale oil sample [Standard Reference Material (SRM) 1580] and a petroleum crude oil (SRM 1582) are compared to values obtained by gas chromatography - mass spectrometry.

## INTRODUCTION

During the past several years our laboratory has been involved in the development of liquid chromatographic (LC) methods for the determination of individual polycyclic aromatic hydrocarbons (PAH) in a variety of complex matrices (1-5). The complexity of PAH mixtures in environmental samples and oil matrices has resulted in the development of a sequential LC procedure. This procedure consists of a normal-phase isolation of fractions containing isomeric PAH on an aminosilane column followed by the reversed-phase separation of individual PAH isomers on a polymeric octadecylsilane ($C_{18}$) column.

Normal-phase LC on the aminosilane phase provides a separation of PAH based on the number of aromatic carbons in the molecule, i.e., PAH isomers elute together. Alkyl substitution on a PAH has little effect on the normal-phase LC retention, i.e., fluoranthene and pyrene (16 aromatic carbon atoms) and their alkylated homologues have similar retention characteristics (1,6). In contrast to normal-phase LC, reversed-phase LC on a polymeric $C_{18}$ stationary phase generally provides sufficient selectivity for the separation of PAH isomers including alkyl-substituted isomers (7).

Most reversed-phase $C_{18}$ separations are performed on monomeric $C_{18}$ phases; however, polymeric $C_{18}$ phases have been shown to have greater selectivity for the separation of isomeric PAH when compared to monomeric $C_{18}$ phases (6,7). The differences in chromatographic retention and selectivity for PAH on various $C_{18}$ columns are a result of the utilization of different silica substrates as supports and a variety of reagents and conditions to produce the $C_{18}$ bonded phase. Factors such as silica substrate surface area, pore size, bonded phase carbon content, and $C_{18}$ surface coverage have been found to affect the selectivity of PAH separations (8,9). Two specific examples which demonstrate the superior resolving capability of the polymeric $C_{18}$ phase for isomeric PAH are: the separation of benz[a]anthracene and chrysene and the separation of perylene and benzo[k]fluoranthene. The monomeric $C_{18}$ phase fails to adequately resolve these isomeric pairs while the polymeric $C_{18}$ phase can easily produce baseline separation of each (4,5,6).

Additional selectivity is obtained in the sequential LC procedure described in this paper by employing fluorescence detection with excitation and emission wavelengths optimized for the analyte of interest. This general procedure has been used for the quantification of selected PAH in a number of matrices including a shale oil [Standard Reference Material (SRM) 1580], (2) a number of virgin and recycled oils (3), and a coal liquid (10).

In this paper we report a modification of the LC procedure employed in this laboratory previously, to include the use of perdeuterated PAH as internal standards. This procedure was used for the determination of selected PAH in a petroleum crude oil sample as part of the analytical process for certifying this material as SRM 1582. The results obtained from the analysis of this crude oil by this LC procedure are presented and compared to results obtained from gas chromatography-mass spectrometry (GC-MS). Results are also reported for the analysis of SRM 1580, "Organics in Shale Oil".

## EXPERIMENTAL SECTION*

Petroleum crude oil samples of approximately 1.0 g size were accurately weighted into tared 25 mL flasks. Appropriate amounts of the perdeuterated internal standards phenanthrene-$d_{10}$, fluoranthene-$d_{10}$, benz[a]anthracene-$d_{12}$, and perylene-$d_{12}$, (Merck & Co., Rahway, NJ) were then added to each sample along with ~10 mL of hexane. The flasks and their contents were then sonicated for 30 minutes. Following sonication a known aliquot (~100 µl) of each sample solution was injected onto a semi-preparative aminosilane column and eluted with a mobile phase composed of 3% methylene chloride in hexane. Four isomeric PAH fractions containing PAH with 14, 16, 18 and 20 aromatic carbon atoms respectively were collected from the aminosilane column. The fraction collection was based on retention volumes, which were determined by injecting standard solutions. The collected fractions were concentrated under nitrogen and the solvent exchanged to acetonitrile prior to analysis on a reversed-phase polymeric $C_{18}$ column (Vydac 201 TP 5µm, The Separations Group, Hesperia, CA).

The PAH analytes present in each sample fraction were eluted from the polymeric $C_{18}$ reversed-phase column with a mobile phase composed of acetonitrile and water. A solvent gradient or an optimal isocratic composition of the mobile phase component was used as necessary to perform an adequate separation of the various isomers. The PAH analytes were then detected spectrofluorometrically using a detector capable of wavelength programming. (Model LS-4, Perkin Elmer Corp., Norwalk, CT).

## RESULTS AND DISCUSSION

The advantages of sequential LC with selective detection for the quantification of individual PAH in shale oil have been described previously by Hertz et al. (2) and May et al. (4). Three different modes of quantification for the analysis of the shale oil material were described by May et al. (4), i.e., external standard, internal standard and standard addition. Each mode of quantification has limitations. In the external standard method the analyst assumes that all operations such as extractions, sample transfers, dilutions, and concentrations are quantitative. Unfortunately, the determination of organic compounds in complex sample matrices is difficult; extraction is often incomplete, volatile compounds may be lost during concentration, and other undetected errors may be introduced during sample handling. The method of standard addition, the most rigorous and potentially the most accurate of the three methods, is time consuming because several samples must be processed to provide one measurement. The standard addition procedure involves spiking varying amounts of the analyte into several subsamples and plotting a response curve from which the concentration of an unspiked subsample can be interpolated.

Quantification by the internal standard method requires the selection of an appropriate compound to act as the internal standard. The internal standard must mimic the behavior of the analyte throughout the analytical procedure but can not be naturally present or coelute with other interfering species in the sample. The selection of a suitable internal standard is especially difficult when analyzing complex mixtures by highly specific analytical procedures. If a suitable internal standard is available, this method of quantification has the advantage of compensating for losses associated with sample handling without the time consuming effort of the standard addition method.

There are several reasons why the perdeuterated PAH are excellent internal standards for the quantification of PAH in complex samples by the sequential LC procedure. Perdeuterated PAH have normal-phase and reversed-phase LC retention characteristics similar to those of the parent PAH and thus will follow the PAH of interest throughout the highly selective analytical procedure. The LC retention characteristics of a number of PAH and perdueterated PAH are summarized in Table 1. In

TABLE 1

Liquid Chromatographic Retention of Polycyclic Aromatic Hydrocarbons[a]

| Compound | Number of Aromatic Carbons | $NH_2$ Column (Log Retention Index) | Polymeric $C_{18}$ Column (Log Retention Index |
|---|---|---|---|
| Phenanthrene | 14 | 3.00 | 3.00 |
| Phenanthrene-$d_{10}$ | 14 | 3.03 | 2.91 |
| Fluoranthene | 16 | 3.51 | 3.37 |
| Fluoranthene-$d_{10}$ | 16 | 3.55 | 3.31 |
| Pyrene | 16 | 3.37 | 3.48 |
| Pyrene-$d_{10}$ | 16 | 3.44 | 3.40 |
| Benz[a]anthracene | 18 | 4.00 | 4.00 |
| Benz[a]anthracene-d | 18 | 4.05 | 3.91 |
| Chrysene | 18 | 4.01 | 4.10 |
| Chrysene-$d_{12}$ | 18 | 4.06 | 4.03 |
| Perylene | 20 | 4.61 | 4.33 |
| Perylene-$d_{12}$ | 20 | 4.67 | 4.27 |
| Benzo[a]pyrene | 20 | 4.38 | 4.68 |
| Benzo[a]pyrene-$d_{12}$ | 20 | 4.49 | 4.61 |

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[a] Retention reported as logarithm of the retention index, see ref. 6 and 7.

the normal-phase LC fractionation, the perdeuterated analogues elute in the same fraction as the parent PAH since they have the same number of aromatic carbon atoms. In the reversed-phase LC separation, the perdeuterated PAH elute first and are generally completely resolved from the parent PAH (see Table 1).

In this work four perdeuterated PAH were utilized in the quantification of six PAH analytes present in the petroleum crude oil. The compounds phenanthrene-$d_{10}$, fluoranthene-$d_{10}$, benz[a]anthracene-$d_{12}$, and perylene-$d_{12}$, representing aromatic carbon numbers 14, 16, 18, and 20 respectively, were added to each sample prior to the normal-phase LC fractionation so that each fraction would contain one internal standard.

The degree of sample fractionation required to insure sufficient sample cleanup is a function of the sample complexity. The very complex shale and petroleum oils discussed in this paper are best separated into fractions which contain the PAH of only one aromatic carbon atom number. Current work in our laboratory has shown that an adequate cleanup is obtained for samples of diesel soot when these samples are divided into just two fractions: one containing 14-16 aromatic carbon PAH and the other containing 18-22 aromatic carbon PAH. The perdeuterated internal standards used for these fractions are fluoranthene-$d_{10}$ and perylene-$d_{12}$ respectively. In addition, May and Wise (5) have recently reported the use of perylene-$d_{12}$ as an internal standard for the quantification of 12 PAH in a single "total" PAH fraction from an air particulate sample.

The sequential LC procedure with perdeuterated PAH as internal standards was first evaluated by analyzing a shale oil sample (SRM 1580), which has certifed values for the concentration of several PAH. The chromatograms shown in Figure 1 are from a single reversed-phase LC analysis of a shale oil fluoranthene fraction (16 aromatic carbons). The two chromatograms illustrate the results from the two detectors used on-line; an ultraviolet (UV) fixed wavelength (254 nm) detector and a fluorescence detector. Fluoranthene-$d_{10}$, used as the internal standard in this fraction, was completely resolved from its parent PAH fluoranthene. A comparison of the two chromatograms in Figure 1 reveals the enhanced selectivity obtained from the fluorescence detector. The ability to program changes in the excitation and emission wavelengths allowed the selective monitoring of fluoranthene-$d_{10}$, fluoranthene, and pyrene during a single chromatographic analysis. One set of excitation and emission wavelengths ($\lambda_1$) was used for the detection of fluoranthene-$d_{10}$ and fluoranthene, and a second set ($\lambda_2$) for the detection of pyrene. The large, late eluting

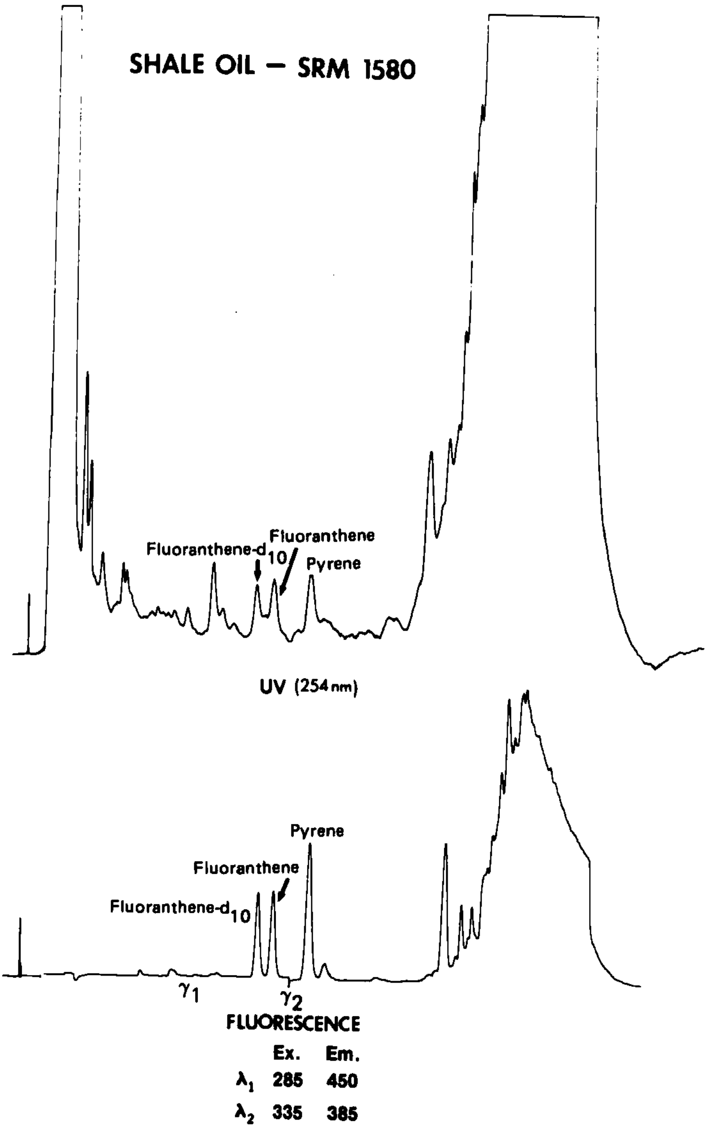

FIGURE 1 The reversed-phase LC analysis of the fluoranthene fraction (16 aromatic carbon atoms) from a shale oil sample (SRM 1580).

Mobile Phase: 45 percent acetonitrile in water isocratic until fluoranthene eluted, then gradient to 100 percent acetonitrile in 5 minutes.

TABLE 2

The Quantification of PAH in Shale Oil (SRM 1580) by Three Different Methods

| Compound | Concentration (μg/g)[a]<br>Perdeuterated[b] Internal Standard (LC) | External Standard[c] (LC) | Standard Addition[d] (GC-MS) |
|---|---|---|---|
| Fluoranthene | 51 ± 2 | 53 ± 2 | 55 ± 5 |
| Pyrene | 97 ± 6 | 107 ± 8 | 101 ± 5 |
| Benzo[a]pyrene | 26 ± 1 | 23 ± 1 | 20 ± 1 |
| Perylene | 2.5 ± 0.2 | 3.9 ± 0.6[e] | 2.8 ± 0.5 |

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[a] Uncertainty is the standard deviation of a single measurement.
[b] One sample preparation wiith triplicate analysis.
[c] Nine sample preparations with triplicate analysis.
[d] Six sample preparations with triplicate analysis.
[e] This value contains a contribution from benzo[k]fluoranthene which co-eluted with perylene under the conditions used.

envelope shown in both chromatograms of Figure 1 is the result of the elution of alkylated species with 16 aromatic carbon atoms.

In Table 2 the results obtained from the analysis of shale oil for the determination of four PAH by this procedure are compared to values obtained from two different methods. The two other procedures, LC using external standards and GC-MS using standard addition, were the basis for the original certification of this material as a Standard Reference Material. The three procedures produced statistically similar values for fluoranthene and pyrene with approximately identical precision. The value obtained for benzo[a]pyrene (BaP) by the modified procedure was somewhat higher than the values obtained from the other techniques. A comparison of the values obtained from the different procedures for perylene point out an interesting problem concerning the LC separation of isomeric PAH. A

TABLE 3

Analytical Parameters

| Analyte Compound | Internal Standard | Number of Aromatic Carbons | Fluorescence Wavelengths Used Excitation | Emission |
|---|---|---|---|---|
| Phenanthrene | Phenanthrene-$d_{10}$ | 14 | 250 | 360 |
| Fluoranthene | Fluoranthene-$d_{10}$ | 16 | 285 | 450 |
| Pyrene | Fluoranthene-$d_{10}$ | 16 | 335 | 385 |
| Benz[a]anthracene | Benz[a] anthracene-$d_{12}$ | 18 | 285 | 385 |
| Benzo[a]pyrene | Perylene-$d_{12}$ | 20 | 295 | 405[a] |
| Perylene | Perylene-$d_{12}$ | 20 | 405 | 440 |
| | | | 405 | 500 |

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[a] Perylene-$d_{12}$ monitored at Ex 405; Em 500
Benzo[a]pyrene monitored at Ex 295; Em 405

monomeric $C_{18}$ reversed-phase column was used in the original certification work (4) and consequently did not resolve perylene from benzo[k]fluoranthene (BkF). The BkF responded under the fluorescence conditions used for perylene, therefore, the external standard LC value for perylene was higher than the value generated by GC-MS. In this work, a polymeric $C_{18}$ reversed-phase column was used which provided superior resolution of perylene and BkF; thus, the perylene value obtained was comparable to the value obtained by the GC-MS technique.

The analytical parameters for the analysis of the PAH in the petroleum crude oil are summarized in Table 3. The results of these LC analyses are shown in Table 4. The chromatograms shown in Figures 2 and 3 illustrate the reversed-phase LC analysis of two of the petroleum crude oil fractions, i.e., phenanthrene fraction (14 aromatic carbons) and the perylene fraction (20 aromatic carbons) respectively. In the 20 aromatic carbon fraction following the elution of perylene-$d_{12}$ and perylene, a

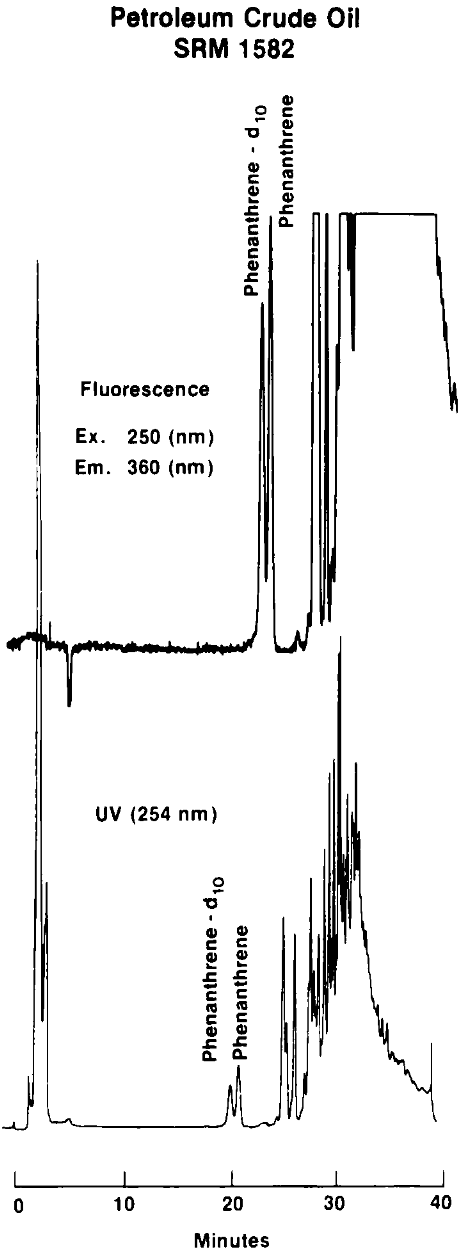

FIGURE 2 The reversed-phase LC analysis of the phenanthrene fraction (14 aromatic carbon atoms) from a petroleum crude oil sample (SRM 1582).

Mobile Phase: 45 percent acetonitrile in water isocratic until phenanthrene eluted, then gradient to 100 percent acetonitrile in 5 minutes.

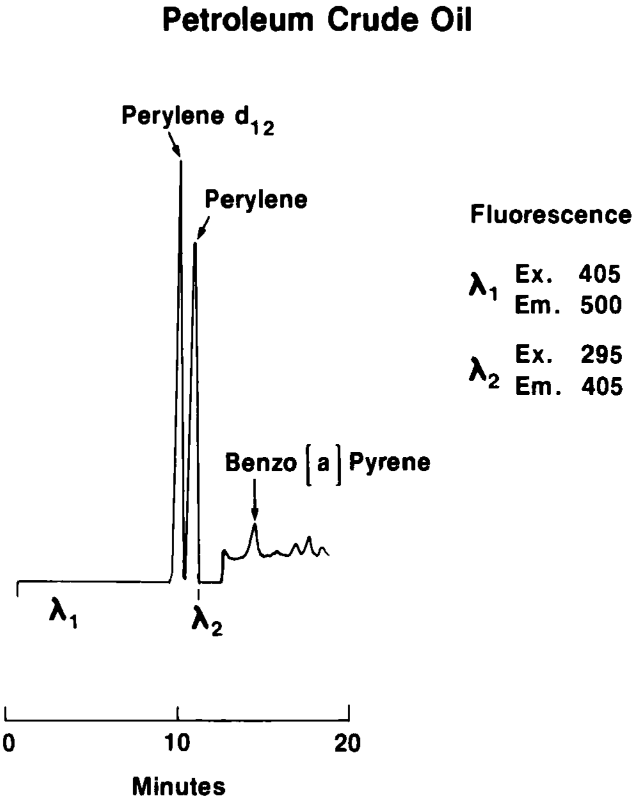

FIGURE 3 The reversed-phase LC analysis of the perylene fraction (20 aromatic carbon atoms) from a petroleum crude oil sample (SRM 1582).

Mobile Phase: 70 percent acetonitrile in water until Benzo[a]pyrene eluted, then gradient to 100 percent acetonitrile in 5 minutes.

TABLE 4

Summary of Results Obtained from the Analysis of Petroleum Crude Oil (SRM 1582) for Selected PAH

| Compound | Concentration (μg/g)[a] Sequential LC[b] | Concentration (μg/g)[a] GC-MS[c] |
|---|---|---|
| Phenanthrene | 103 ± 2 | 100 ± 7 |
| Perylene | 33 ± 1 | 30 ± 2 |
| Pyrene | 6.6 ± 0.5 | 7.6 ± 0.5 |
| Benz[a]anthracene | 3.0 ± 0.3 | 2.8 ± 0.2 |
| Fluoranthene | 2.4 ± 0.2 | 2.6 ± 0.2 |
| Benzo[a]pyrene | 1.3 ± 0.1 | 1.1 ± 0.2[d]<br>0.95 ± 0.05[e] |

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[a] Uncertainty is one standard deviation of the mean.
[b] Duplicate LC analyses of 5 samples.
[c] Duplicate GC-MS analyses of 10 samples.
[d] Determined by conventional electron impact mass spectrometry.
[e] Determined by negative ion chemical ionization mass spectrometry.

programmed wavelength change was made to optimize the detection of BaP. The optimum wavelengths for the maximum sensitivity to perylene and perylene-$d_{12}$ are excitation 405 nm and emission 440 nm. However, when measuring BaP (~1 ppm) in the same chromatographic analysis it was necessary to monitor the perylene (~30 ppm) and perylene-$d_{12}$ signals at excitation 405 nm and emission 500 nm in order to attenuate their fluorescence response (see Table 3). In Table 4 the results obtained from the analysis of the petroleum crude oil by LC and GC-MS are compared (11). The data from these two techniques were generally in reasonable agreement. Two GC-MS values are shown for BaP. The value 1.1 μg/g was obtained by conventional electron impact mass spectrometry while the value of 0.95 μg/g was generated using negative ion chemical ionization mass spectrometry.

This LC procedure using perdeuterated PAH as internal standards provides a viable alternative to the tedious and often laborious tasks of external standard or standard addition procedures. The method has been validated by use of an existing SRM (1580) and the quantitative results obtained in the certification of a petroleum crude oil as SRM 1582 compare favorably to values generated by GC-MS.

## ACKNOWLEDGMENT

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*Identification of any commercial product does not imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the material or equipment identified is necessarily the best available for the purpose.

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