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Las Vegas, NV 89193-3478

Research and Development

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Project Summary

Measurement of Polycyclic Aromatic Hydrocarbons in Soils and Sediments by Particle-Beam/High-Performance/ Liquid Chromatography/Mass Spectrometry

C.M. Pace, D.A. Miller, M.R. Roby, and L.D. Betowski

A draft analytical method was developed for the measurement of certain polycyclic aromatic hydrocarbons (PAHs) in soils and sediments by particle-beam/liquid chromatography/mass spectrometry. The method applies to PAHs with a molecular weight greater than 220. Samples are prepared by SW-846 Method 3540 with optional cleanup using SW-846 Method 3630. The sample extracts are then analyzed for PAHs using a particle-beam/liquid chromatography/mass spectrometry system. Method detection limits are within the range of 0.01 to 0.10 μg/g depending on the sample size. Mean method accuracy was greater than 75 % for most of the target analytes with relative standard deviation values between 10% and 20%. An analysis of a standard reference material using this method agreed with certified values and with an analysis performed using high performance liquid chromatography (HPLC) with fluorescence detection (SW-846 Method 8310). The method shows potential as a means to measure high molecular weight PAHs not measured by current EPA methods.

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Introduction

The (PAHs) comprise a class of potentially hazardous compounds of environmental concern. The PAHs were selected for this study as part of a continuing effort to evaluate applications of particle beam (PB) liquid chromatography/mass spectrometry (LC/MS) to the measurement of pollutants in environmental samples. Initial studies determined instrument response characteristics to the EPA Method 610 target analytes. The analytes comprise 16 PAHs ranging in molecular weight from naphthalene (MW 128) to dibenzo(a,h)anthracene (MW 278).

The PB LC/MS was unsuitable for the analysis of the lower molecular weight PAHs (MW<220). Consequently, the lower molecular weight PAHs were dropped from further study, and four higher molecular weight PAHs were added as potential target analytes. The additional analytes included three MW 302 PAHs from Appendix IX (51 Federal Register 5561, February 1986): dibenzo(a,e)pyrene, dibenzo(a,h)pyrene, and dibenzo(a,i)-pyrene. The fourth add-on analyte was another MW 302 PAH isomer, dibenzo(a,l)-pyrene.

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samples. Following examination of instrument performance characteristics, a method was developed for the analysis of the target PAHs in soils and sediments. The method utilized Soxhlet extraction and silica gel column clean-up for sample preparation and the PB LC/MS for measurement. The overall method performance was evaluated for spiked soil samples and a standard reference material (SRM).

Experimental

Chromatographic separations employed a Hewlett-Packard (HP)* 1090L liquid chromatograph (LC) with a filter photometric detector and a 250-mm x 4.6-mm I.D. 5-µm C18 column (Vydac 201TP54). The column was at room temperature and a flow rate of 0.4 mL/min was used. The mobile phase program is listed in Table 1. The LC system was coupled to an HP 5988A mass spectrometer (MS) with the HP 59980A PB interface. An HP 59970 MS Chemstation data system controlled the instrument.

Two separate sample preparation schemes were used. One procedure called for a soil (or sediment) to be sonicated in acetonitrile. A portion of the sonication extract was then passed through a C-18 solid phase cartridge and subsequently concentrated. The second procedure consisted of SW-846 Method 3540 followed by solvent exchange into cyclohexane. The cyclohexane extract was then cleaned up using SW-846 Method 3630 followed by solvent exchange into acetonitrile.

Two objectives were considered for the liquid chromatographic separation of the target PAHs. First, a mobile phase and column were selected to effect separation of most target analytes in 30 to 40 min. Second, the separation had to be compatible with the PB and MS systems. For these reasons, a ternary solvent program was employed. Acetonitrile was selected because it gave the best selectivity for the later-eluting target analytes. Methanol was selected because it gave the best PB response to the target analytes. Tetrahydrofuran (THF) was selected because it reduced retention times on the last two eluting analytes and reduced the overall chromatographic run time by 15 min.

Results and Discussion

The PB LC/MS was unsuitable for the analysis of the lower molecular weight PAHs (MW < 220). Presumably, these PAHs are too volatile to pass the PB interface. Figure 1 illustrates the poor PB response to lower molecular weight PAHs by comparison with the UV response from a photometric detector connected in series with the PB interface. Accordingly, only the higher molecular weight PAHs were studied.

Instrument Performance

Detection Limits and Precision

The estimated instrument detection limits and precision of the PB LC/MS system for those PAHs investigated in this study are shown in Table 2. The detection limits were determined from full-scan extracted ion chromatograms at the 25-ng level. Detection limit values are 3 times the standard deviation of seven replicates. The precision values were calculated from the same set of seven injections at 25 ng. Considerably better detection limits can be achieved with single-ion monitoring.

Response Curves

Instrument response to PAH standard solutions covering a 50-fold concentration range (20 to 1000 ng) was nonlinear for most target PAHs (response factor RSDs > 20 percent). Response factors tended to increase with increasing concentration. On one occasion, however, a six point calibration (20 to 1000 ng) exhibited essentially linear response for most target PAHs. This occurrence was the exception and could not be reproduced. Responses over a smaller concentration range were also nonlinear but gave response factor RSDs closer to 20 percent.

Retention Times

The stability of the retention times of the target PAHs eluting from the LC column was investigated. We observed that the retention times were susceptible to small changes in column temperatures under the conditions used. Upon elevating the LC oven compartment to 37.5° C (lowest stable temperature capable by the system) drastic losses in chromatographic resolution were observed. Therefore, the analyses were carried out at ambient temperature.

Spectral Quality

The PB mass spectrum of dibenzo(a,e)-pyrene displays spectral features common to all of the PAHs studied. The molecular ion is the base peak and appears with several (M-nH)+ ions where n can be as many as six. Another prominent feature is the presence of doubly charged ions that appear at a mass to charge ratio of one half the molecular ion and (M-nH)+ ions. Spectra acquired under similar conditions but at different times show variations in the doubly charged ions relative abundance. This phenomenon appears in all the spectra of the PAHs examined but is more pronounced in the higher molecular weight PAHs.

Performance on Soil Extracts

Figure 2 is a total ion chromatogram (TIC) of a PAH contaminated soil from The Dalles, OR. The soil was extracted using acetonitrile sonication as described in the experimental section. A stack plot of four selected ions is illustrated in Figure 3. Note the presence of several peaks at mass 326. Examination of mass spectra from these peaks indicates that the components associated with the peaks are PAHs. Table 3 lists the quantities of each target compound found by internal (d12-perylene) and external standard calibration techniques. Also listed for comparison are the quantities of target compounds measured on a separate LC system using fluorescence detection. Examination of Table 3 reveals agreement between PB quantitative results and results obtained by fluorescence detection.

Method Performance

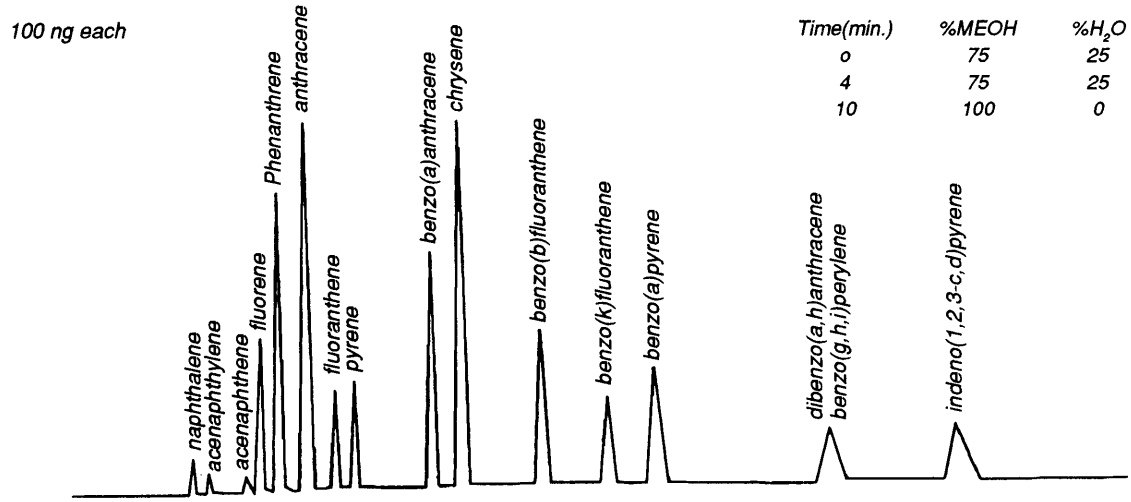
The existing SW-846 Soxhlet extraction procedure (Method 3540) was incorporated into a sample preparation scheme for the PB analysis of PAHs in soils and sediments. Because of difficulties encountered during the initial PB analysis of an acetonitrile extract of a Canadian SRM, a clean-up method was sought. Initial analysis of the SRM suggested interference from hydrocarbons. For this reason, the SW-846 silica gel clean-up (Method 3630) was employed. To evaluate overall method performance, several spiked clean soils and a Canadian SRM were analyzed.

A sandy loam soil was spiked in triplicate at two different levels, 0.5 µg/g and 2.5 µg/g. The samples were prepared as just described and the extracts were ana-

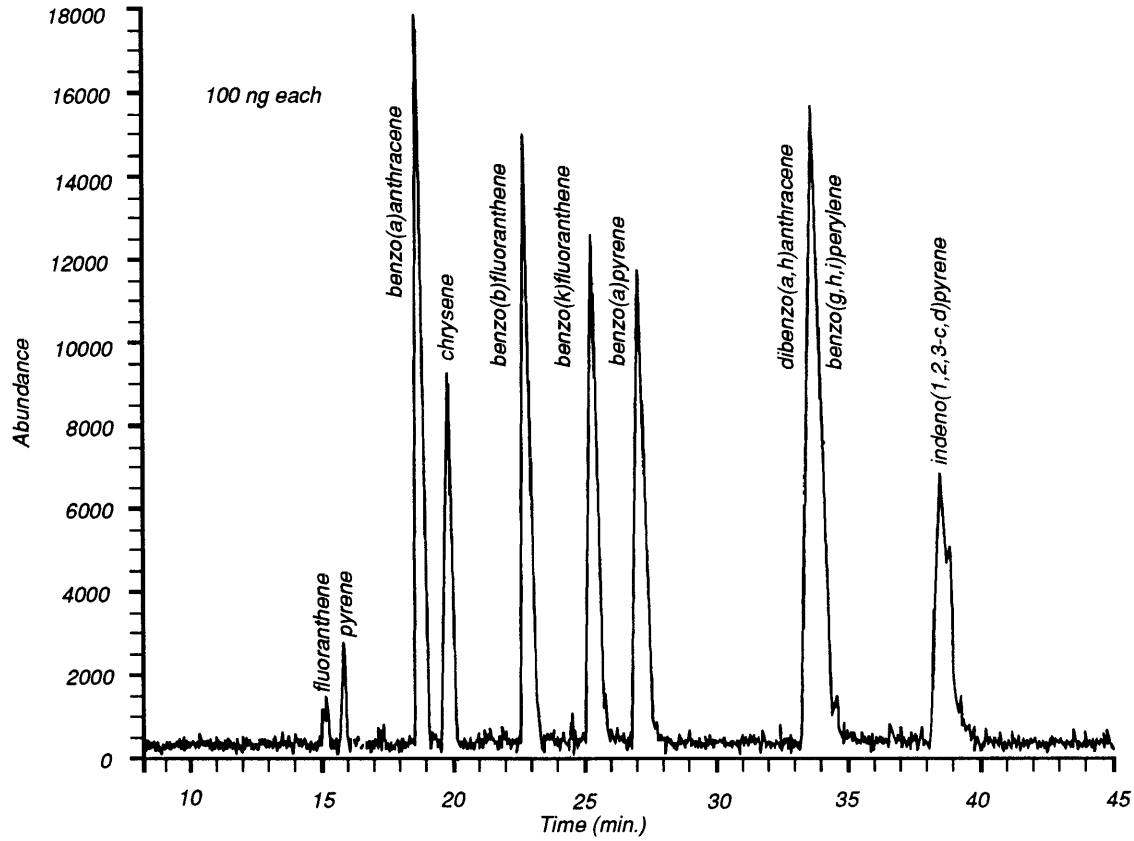
Table 1. *Liquid Chromatographic Mobile Phase Program*

Time (min)	*% Methanol*	*% Acetonitirile*	*% Tetrahydrofuran*
0	*95*	*0*	*5*
2	*95*	*0*	*5*
10	*45*	*45*	*10*
15	*45*	*25*	*30*

* Mention of trade names or commercial products does not constitute endorsement or recommendation for use.



UV Chromatogram of EPA Method 610 PAHs (254nm).



Particle Beam TIC of EPA Method 610 PAHs

***Figure 1**. Comparative chromatograms of 16 PAHs by HPLC/UV and PB LC/MS*

Table 2. *Detection Limits and Precision of the PB LC/MS for the Analysis of PAHs*

Compound	Quantitation ion	Detection limit (ng)	Precision RSD(%)
benzo(a)anthracene	228	1.8	2.4
chrysene	228	3.0	4.1
benzo(b)fluoranthene	252	1.6	2.1
benzo(k)fluoranthene	252	1.0	1.4
benzo(a)pyrene	252	2.2	2.9
dibenzo(a,l)pyrene	302	6.1	8.1
dibenzo(a,h)anthracene	278	2.4	3.1
benzo(g,h,i)perylene	276	2.4	3.2
indeno(1,2,3-c,d)pyrene	276	1.5	2.0
dibenzo(a,e)pyrene	302	2.5	3.4
dibenzo(a,i)pyrene	302	3.0	4.0
dibenzo(a,h)pyrene	302	4.8	6.3

lyzed with the PB instrument. Recoveries were calculated by using integrated quantitation ion abundances and a six point external calibration. Results from one of the low-level spikes (0.5 µg/g) were discarded. Preparation of this particular spike resulted in a two-phase extract. The two-phase extract was probably the result of incomplete solvent exchange. The data from all three high-level spikes (2.5 µg/g) were used to determine mean recovery and standard deviation although two of the higher level spikes gave significantly lower recoveries.

HPLC/UV examination of the pentane wash from the silica gel clean-up from one of the low recovery samples revealed 5% to 15% of the spiked amount for most of the target analytes had washed off the column prior to elution of the analytical fraction. This loss may have resulted from improper preparation of the silica column or from nonuniform activation of the silica gel. These results indicate the silica gel clean-up is an area of concern and a potential source of problems for overall method performance. However, losses to the column wash do not account for the low recoveries observed for dibenzo(a,h)-pyrene, as this target analyte was not found in the pentane wash. This PAH was probably not extracted efficiently with the solvent system employed.

The recovery data were pooled and treated as a single data set to generate overall method precision and accuracy values. These values are listed in Table 4 along with estimated method detection limits. Method detection limits were estimated from observed instrument detection limits. Values were adjusted for concentration/dilution factors imposed by the sample preparation scheme: a 20 µL injection, a 1-mL final extract volume, and a 10 g sample size. Final values were corrected with the observed recoveries. The method detection limits are estimates and have not been experimentally verified.

Analysis of a Standard Reference Material

An SRM was analyzed using the procedures described in this report to evaluate method performance on "real world" samples. The SRM was a marine sediment obtained from the National Research Council of Canada and designated as HS-3. The material was prepared in triplicate (5 g each) and taken through the silica gel clean-up procedure. Target

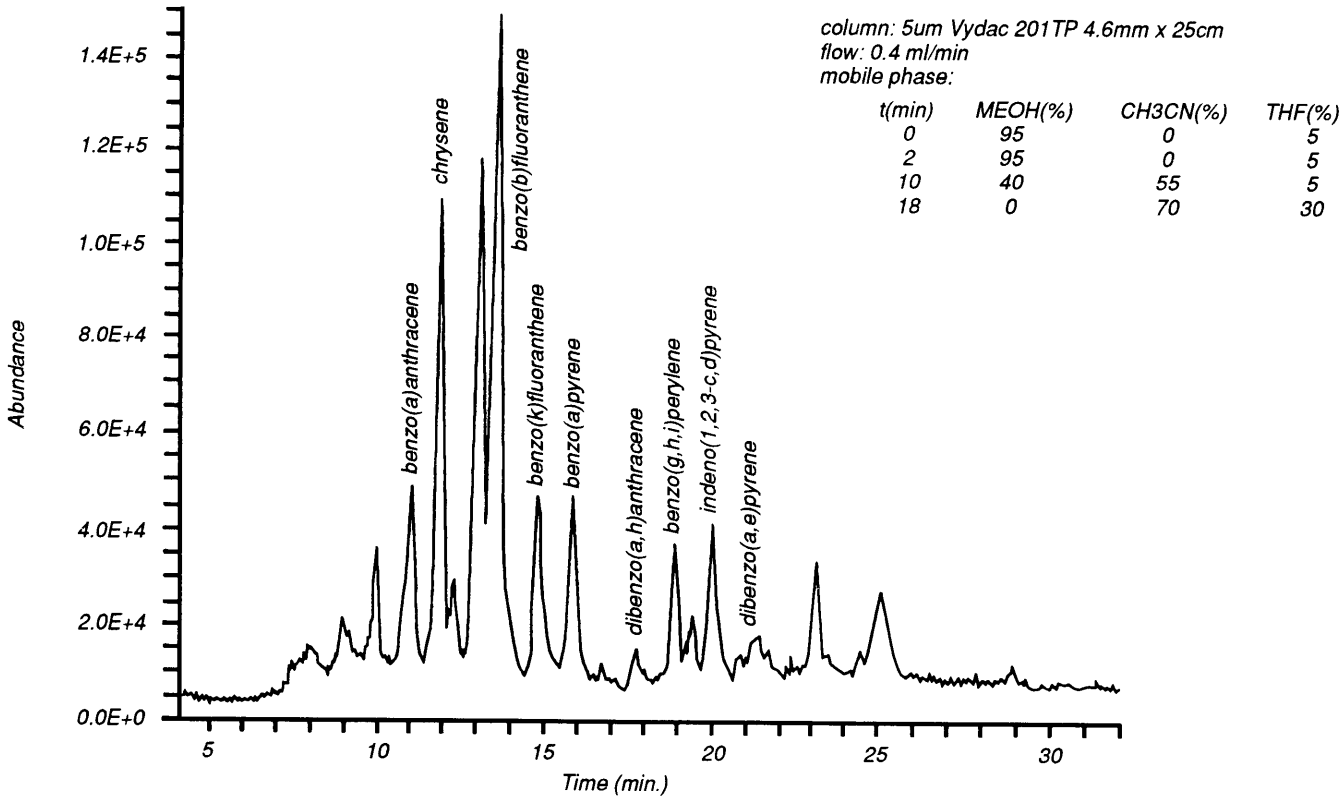


Figure 2. *Particle beam TIC of a PAH contaminated soil.*

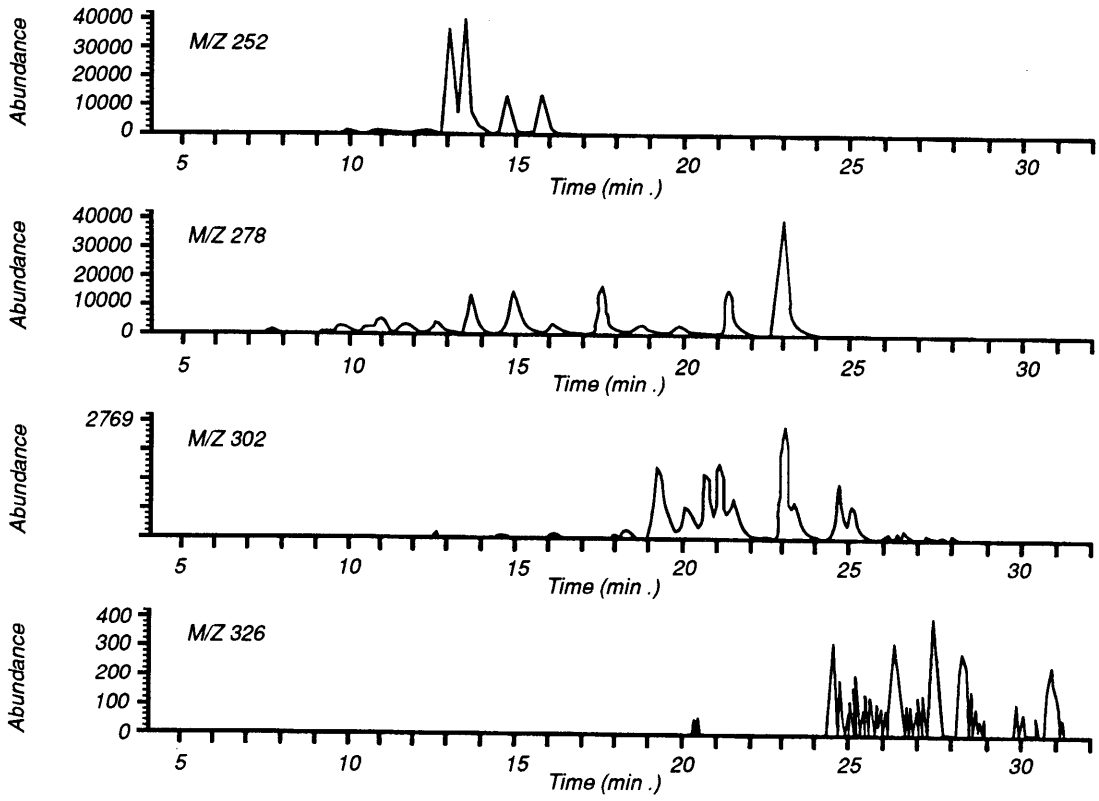


Figure 3. *Selected ion chromatograms of a PAH contaminated soil.*

analyte amounts were obtained by integrated quantitation ion areas and external calibration. In addition, the extracts were analyzed by HPLC with UV diode array detection for comparative purposes. The results are listed in Table 5 along with the certified SRM values and the initial PB results on an acetonitrile extract without clean-up.

The PB results with extract clean-up failed to meet acceptance criteria ($p \pm 2s$) for only one analyte, benzo(k)fluoranthene. Values for p and s were taken from the experimentally determined method performance parameters listed in Table 4. The HPLC/UV analysis failed acceptance criteria for two of the target analytes. The PB results on the acetonitrile extract without clean-up failed acceptance criteria for all target analytes. In this particular instance, extract clean-up appears to be essential for accurate analysis. In general, results obtained from PB analysis with extract clean-up and HPLC/UV were in agreement and agreed with certified values.

Conclusions and Recommendations

Low molecular weight PAHs (MW<220) cannot be measured accurately with the PB instrument. However, PAHs with molecular weight greater than 220 can be measured with good accuracy and precision. The instrument sensitivity to these PAHs was on the order of 1 to 10 ng in the full scan mode. Such sensitivity allows method detection limits comparable to or better than those of current GC/MS-based EPA methods.

Table 3. *Comparison of PB LC/MS Quantification Method vs. Flourescence for PAH Target Analytes*

RT (min)	m/z	Compound	Soil Extract (µg/g) IS [a]	FL [b]	EX [c]
10.94	228	benzo(a)anthracene	6.5	6.4	5.4
11.75	228	chrysene	26	21	19
13.43	252	benzo(b)fluoranthene	19	16	18
14.69	252	benzo(k)fluoranthene	5.9	6.8	7.0
15.75	252	benzo(a)pyrene	6.2	8.8	6.8
–	302	dibenzo(a,l)pyrene	–	–	–
17.67	278	dibenzo(a,h)anthracene	0.8	–	1.1
18.84	276	benzo(g,h,i)perylene	3.7	6.4	4.8
19.96	276	indeno(1,2,3-c,d)pyrene	5.6	5.2	4.5
20.77	302	dibenzo(a,e)pyrene	0.9	–	1.0
–	302	dibenzo(a,i)pyrene	–	–	–
–	302	dibenzo(a,h)pyrene	–	–	–

[a] quantitated by d12-perylene internal standard
[b] quantitated by fluorescence detection
[c] quantitated by external standards

Instrument response to PAH standard solutions covering a 50-fold concentration range (20 to 1000 ng) was nonlinear for most target PAHs (response factor RSDs > 20 %). Nonlinear response did not appear to present particular difficulties, however, provided the response was correctly modeled (i.e., point-to-point calibration or polynomial curve fits). The nonlinear response was reproducible over the course of an analytical run (24 h) and in calibration check samples gave values within 20% of initial calibration. Further, the nonlinear PB calibration gave results in agreement with HPLC/UV and HPLC/fluorescence analysis of "real world" samples.

The electron ionization (EI) mass spectra obtained from each of the target analytes were consistent with structure and comparable to reference spectra. In general, the spectra obtained from "real" samples were of sufficient quality to allow tentative identification of nontarget PAHs. However, some spectral variation was observed that did not correspond to differences in tuning and mass calibration. These variations take the form of enhanced relative abundance of the doubly charged molecular ion.

One of the potential applications of PB LC/MS emerging from these studies is the measurement of high-mass PAHs (MW>300). Current EPA methods do not measure for PAHs above mass 300. Analysis of the Canadian SRM and the PAH-contaminated soil (from The Dalles, OR) by PB LC/MS revealed the presence of eight mass 302 PAHs and five mass 326 PAHs. Evidence for PAHs above mass 326 was also obtained. These high-mass PAHs were only present at low concentrations. However, the low amount observed was probably due, in part, to poor extraction efficiency with the solvents employed (methylene chloride or acetonitrile).

We recommend that work on the application of PB LC/MS for the measurement of high-mass PAHs be pursued. This work would entail characterizing a PAH-contaminated sample for high-mass PAHs. The work would involve investigation of suitable extraction solvents, chromatographic separation of the high-mass fraction, and the identification and quantitative estimation of high-mass PAHs by PB LC/MS in combination with stop-flow fluorescence spectroscopy.

Table 4. *Method Detection Limits, Precision, and Accuracy*

Compound	MDL (μg/g)	Mean method Accuracy (n=5) (% of true value)	Standard Deviation (%)
benzo(a)anthracene	0.02	89	20
chrysene	0.03	112	23
benzo(b)fluoranthene	0.02	77	14
benzo(k)fluoranthene	0.01	95	19
benzo(a)pyrene	0.04	61	12
dibenzo(a,l)pyrene	0.14	42	9
dibenzo(a,h)anthracene	0.02	100	25
benzo(g,h,i)perylene	0.03	80	18
indeno(1,2,3-c,d)pyrene	0.02	82	18
dibenzo(a,e)pyrene	0.03	77	12
dibenzo(a,i)pyrene	0.04	81	16
dibenzo(a,h)pyrene	0.11	45	16

Table 5. *Results of SRM Analysis*

Compound	Certified Value (μg/g)	HPLC/UV (μg/g)	PB with Cleanup (μg/g)	PB without Clean-up (μg/g)
benzo(a)anthracene	14.6 ± 2.0	15.2 ± 1.5	12.1 ± 1.1	5.1
chrysene	14.1 ± 2.0	7.0 ± 0.6	19.4 ± 2.6	3.7
benzo(b)fluoranthene	7.7 ± 1.2	4.8 ± 0.6	4.4 ± 0.5	2.5
benzo(k)fluoranthene	2.8 ± 2.0	4.8 ± 0.5	5.1 ± 0.4	1.2
benzo(a)pyrene	7.4 ± 3.6	4.3 ± 0.5	3.9 ± 0.4	1.4
dibenzo(a,l)pyrene	NA	NF	NF	NF
dibenzo(a,h)anthracene	1.3 ± 0.5	0.8 ± 0.2	1.7 ± 0.4	NF
benzo(g,h,i)perylene	5.0 ± 2.0	4.1 ± 0.6	3.7 ± 0.4	0.8
indeno(1,2,3-c,d)pyrene	5.4 ± 1.3	3.6 ± 0.6	3.6 ± 0.4	0.8
dibenzo(a,e)pyrene	NA	1.2 ± 0.2	0.7 ± 0.1	NF
dibenzo(a,i)pyrene	NA	2.0 ± 0.4	0.3 ± 0.03	NF
dibenzo(a,h)pyrene	NA	NF	0.2 ± 0.06	NF

NA = certification not available
NF = not found
PB = particle beam

*U.S. Government Printing Office: 1992 — 648-080/60055