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

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MEASUREMENT OF POLYCYCLIC AROMATIC HYDROCARBONS IN SOILS AND SEDIMENTS BY PARTICLE-BEAM/HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY

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ABSTRACT

A tentative 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 measurable by current EPA methods.

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ACRONYMS AND ABBREVIATIONS

GC/MS	gas chromatograph/mass spectrometer
HP	Hewlett-Packard
LC	liquid chromatograph
LC/MS	liquid chromatograph/mass spectrometer
MS	mass spectrometer
MW	molecular weight
PAHs	polycyclic aromatic hydrocarbons
PB	particle beam
PFTBA	perfluorotributylamine
RSD	relative standard deviation
SRM	standard reference material
THF	tetrahydrofuran
TIC	total ion chromatogram
UV	ultraviolet
EI	electron ionization
HPLC	high performance liquid chromatography

SECTION 1

INTRODUCTION

Polycyclic aromatic hydrocarbons (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. These 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: dibenzo(a,e)pyrene, dibenzo(a,h)pyrene, and dibenzo(a,i)pyrene (1). The fourth add-on analyte was another MW 302 PAH isomer, dibenzo(a,l)pyrene.

The instrument performance characteristics of the PB LC/MS system were investigated with respect to the target PAHs. Specific parameters considered were chromatography, detection limits and precision, response range, spectral quality, and the ability to analyze for PAHs in "real world" 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 on spiked soil samples and on a standard reference material (SRM). A preliminary draft method is presented in Appendix B.

SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

Low molecular weight PAHs (MW<220) cannot be measured accurately with the PB instrument. However, PAHs with MW>220 can be measured with good accuracy and precision. The PB 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.

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%). 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%. 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 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. For best results over a wide concentration range, polynomial curve fits should be used.

The 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.

SECTION 3

EXPERIMENTAL

Chromatographic separations employed a Hewlett-Packard (HP) 1090L liquid chromatograph (LC) with a 250-mm x 4.6-mm I.D. 5-μm C18 column (Vydac 201TP54). An ultraviolet (UV) filter photometric detector was used to monitor the column effluent in some cases. The column was at room temperature and a flow rate of 0.4 mL/min was used. The mobile phase programs used to separate the analytes are described later in this section. The LC was controlled by a local user interface.

The LC system was coupled to an HP 5988A mass spectrometer (MS) by an HP 59980A PB interface. The interface was operated with a desolvation chamber temperature of 45° C. The PB probe was kept at a distance of 0.5 mm from the ion source of the MS. The PB interface was tuned by setting the nebulizer capillary position and helium flow to maximize the response of the m/z 302 ion of 10 ng dibenzo(a,h)pyrene. This was done by using single-ion monitoring under flow-injection conditions at the mobile phase composition corresponding to the retention time of dibenzo(a,h)pyrene.

The HP 5988A MS used in this study had a modified ion source. First, a stainless steel plug was inserted into the GC inlet of the source. Second, the instrument manufacturer enlarged the diameter of the PB inlet to the source. Source temperature was either 280° C or 300° C for the analysis done here. A typical MS operating pressure of 1.2×10^{-5} torr was measured by a Bayard-Alpert ion gauge tube. The MS was run in electron ionization mode with an electron energy of 70 eV and an emission current of 300μA. The electron multiplier was a Galileo channeltron. The ion source was tuned with perfluorotributylamine (PFTBA) by maximizing the m/z 219 ion with solvent at 0.4 mL/min (methanol/THF, 95:5). PFTBA was introduced through a reservoir on the PB transfer tube. 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 was concentrated. The second procedure was more detailed. It consisted of using Method 3540 of the SW-846 followed by solvent exchange into cyclohexane. The cyclohexane extract was then cleaned up using Method 3630 followed by solvent exchange into acetonitrile.

Two objectives were considered for the liquid chromatographic separation method used on the target PAHs. First, a mobile phase and column were selected to effect separation of most target analytes in 30 to 40 minutes. 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 minutes. The mobile phase program is listed in Table 1.

TABLE 1. LIQUID CHROMATOGRAPHIC MOBILE PHASE PROGRAM

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

SECTION 4

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.

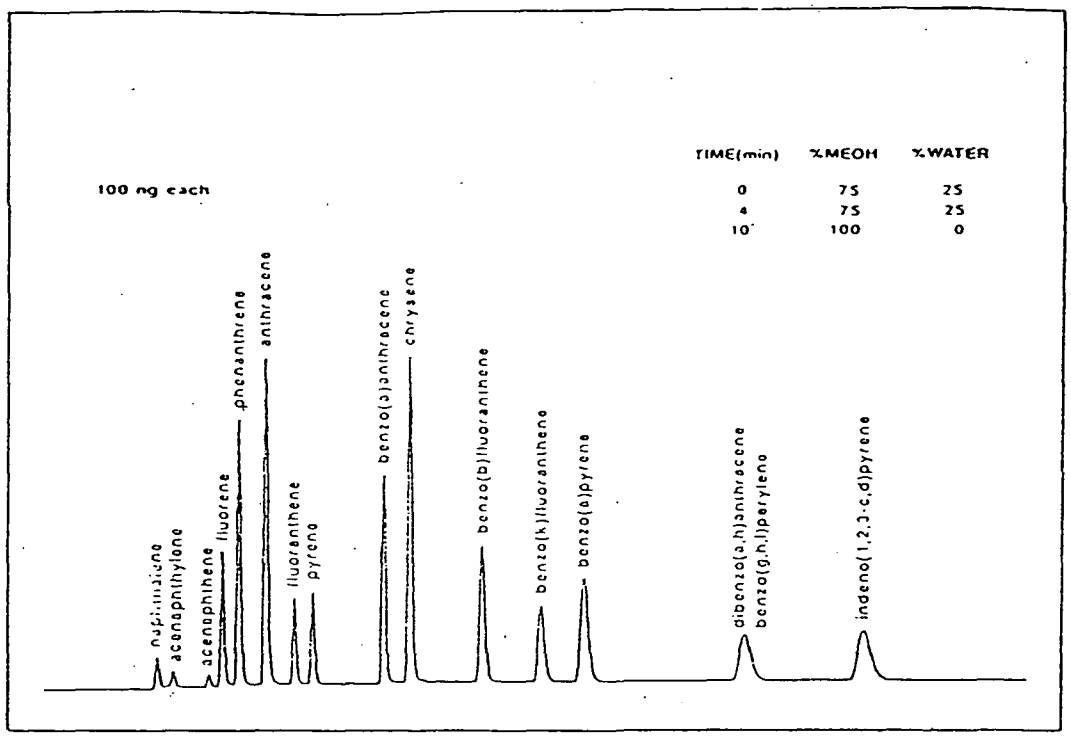
INSTRUMENT PERFORMANCE

Detection Limits and Precision

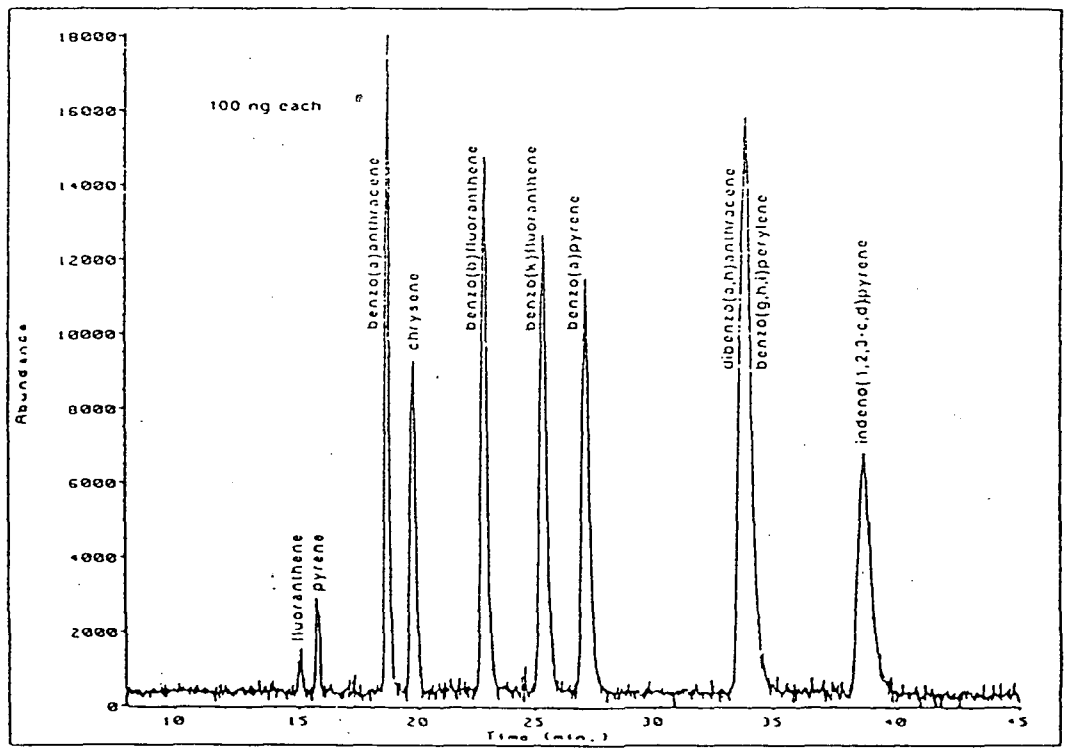
The estimated instrument detection limits and precision of the PB LC/MS system for those PAHs investigated in this study are shown in Table 2. The detection limits were determined from full-scan extracted ion chromatograms at the 25-ng level. These values are 3 times the standard deviation of seven replicates.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

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



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.

Response Characteristic and Calibration

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. This observation is consistent with the notion that at low analyte concentrations smaller particles are produced in the interface and that smaller particles are not transported through the interface as efficiently as larger particles. 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. Table 3 lists response factors from a typical calibration curve.

TABLE 3. SIX-POINT CALIBRATION CURVE

	RESPONSE FACTORS x 10^3						
Compound	25 ng	50 ng	100 ng	250 ng	500 ng	1,000 ng	%RSD
benzo(a)anthracene	6.64	6.80	6.36	7.00	7.71	10.9	22.4
chrysene	4.09	3.65	3.52	3.33	4.00	4.89	14.3
benzo(b)fluoranthene	8.68	11.1	13.1	16.4	19.3	24.7	37.7
benzo(k)fluoranthene	6.39	6.74	6.42	6.71	8.00	11.9	27.8
benzo(a)pyrene	7.93	8.92	9.49	11.4	13.6	20.3	38.2
dibenzo(a,l)pyrene	4.32	5.59	6.98	8.45	10.4	12.6	38.3
dibenzo(a,h)anthracene	2.13	2.43	2.38	2.73	3.55	5.43	39.9
benzo(g,h,i)perylene	3.88	4.01	4.29	5.24	6.90	10.6	44.6
indeno(1,2,3-c,d)pyrene	5.41	6.34	5.64	6.07	8.21	12.9	38.4
dibenzo(a,e)pyrene	3.74	4.98	5.47	4.27	5.52	9.11	34.3
dibenzo(a,i)pyrene	3.88	3.86	3.60	3.59	3.87	6.49	26.6
dibenzo(a,h)pyrene	4.35	4.15	3.55	3.18	3.49	4.34	13.0

The enhancement of analyte signal in PB LC/MS systems by mobile additives (e.g., ammonium acetate) or coelution of the analyte with other compounds has been reported (2). The coelution effect is potentially detrimental to quantitative analysis because the extent of signal enhancement is dependent on a number of variables. Some of the factors reported to contribute to signal enhancement include the relative concentrations of the target analytes and the coeluting substance, molecular structure of the coeluting substance, and desolvation chamber temperature (2,3). Other factors not explicitly reported may be operating.

We investigated the use of perylene-d12 as an internal standard. Perylene-d12 coelutes with benzo(b)fluoranthene under the chromatographic conditions employed. A standard solution containing both benzo(b)fluoranthene and perylene-d12 exhibited no signal enhancement for .

benzo(b)fluoranthene when compared with a standard solution that did not contain perylene-d12. A mitigating factor in this observation was the fact that benzo(b)fluoranthene and perylene-d12 were present in the standard solution at the same concentration, 100 ng/μL, well above detection limits. It has been reported that coelution effects can be negligible when the coeluters are present in similar amounts at levels well above detection limits (3).

Analysis of a PAH contaminated soil showed little practical difference in quantitative results between external and internal standard calibration (see Table 5). The relative percent difference between internal standardization results and external standardization results were within expected measurement precision (see Table 8) for most of the target PAHs detected. That these results were in good agreement was somewhat unexpected because of the potential for coelution effects.

Also unexpected was good agreement between results obtained by fluorescence detection and those obtained by PB LC/MS using external standardization (Table 5). The analyzed samples were complex with all target PAHs coeluting with one or more matrix components (e.g., non-target PAHs or other coextracted substances). PB LC/MS analyte signal from the samples would be expected to be subject to coelution effects whereas the external standard solutions would not. Even so, external standardization gave reasonably accurate results as compared with fluorescence detection. From this limited data set, it appears that coelution effects do not present a significant problem for quantitative analysis. However, because of the limited number of samples examined, we cannot conclude that coelution effects are not a potential source of error.

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. Table 4 displays the range of the retention times observed over a 4.5 hour period during which six standards were analyzed. During the analysis of the standards, the room temperature gradually increased, shortening the analysis times.

Spectral Quality

The PB LC/MS mass spectra of the 12 target analytes are displayed in Appendix A. One of these, dibenzo(a,e)pyrene, is also shown in Figure 2. This figure displays spectral features common to all of the PAHs studied. One, 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 of one half as large as the molecular ion and (M-nH)+ ions. Spectra A and B of Figure 2 display some of the spectral variations we have observed with the PAHs on this system. The spectra were obtained under similar conditions but at different times. It can be seen that in spectra B, the doubly charged ions have a greater relative abundance than in spectra A. The reason for this anomaly is not known at this time but may be related to local ion source pressure. Examination of mass spectra for dibenzo(a,e)pyrene over the width of the eluting peak shows the doubly charged ions to be more abundant by as much as 40% on the peak upslope compared with the doubly charged ion abundance at the peak apex. Doubly charged ion abundance on the peak downslope was unchanged from apex values. This phenomenon appears in all the spectra of the PAHs examined but is more pronounced in the heavier ones.

TABLE 4. RETENTION-TIME STABILITY OF THE PB LC/MS ANALYSIS OF PAHs

Compound	Retention Time Change (min)	Difference (min)	Relative % Difference
benzo(a)anthracene	11.04 to 10.89	0.15	1.4
chrysene	11.85 to 11.63	0.22	1.9
benzo(b)fluoranthene	13.56 to 13.28	0.28	2.1
benzo(k)fluoranthene	14.93 to 14.49	0.44	3.0
benzo(a)pyrene	15.96 to 15.45	0.51	3.3
dibenzo(a,l)pyrene	16.10 to 15.73	0.37	2.3
dibenzo(a,h)anthracene	17.96 to 17.43	0.53	3.0
benzo(g,h,i)perylene	19.11 to 18.56	0.55	2.9
indeno(1,2,3-c,d)pyrene	20.25 to 19.62	0.63	3.2
dibenzo(a,e)pyrene	21.04 to 20.51	0.53	2.6
dibenzo(a,i)pyrene	26.79 to 26.18	0.61	2.3
dibenzo(a,h)pyrene	28.55 to 27.72	0.83	3.0

Performance on Soil Extracts

Figure 3 is a total ion chromatogram (TIC) of a PAH contaminated soil from The Dalles, OR. The soil was extracted by using the acetonitrile sonication as described in the experimental section. A stack plot of four selected ions is illustrated in Figure 4. Note the presence of several peaks at mass 326. Examination of spectra from these peaks indicate them to be PAHs. Table 5 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 found on a separate LC system with fluorescence detection. Examination of Table 5 reveals agreement between PB quantitative results and results obtained by fluorescence detection. Table 6 lists some selected nontarget compounds tentatively identified in this soil sample. Compounds listed in Table 6 illustrate PB capabilities for the identification of unknowns.

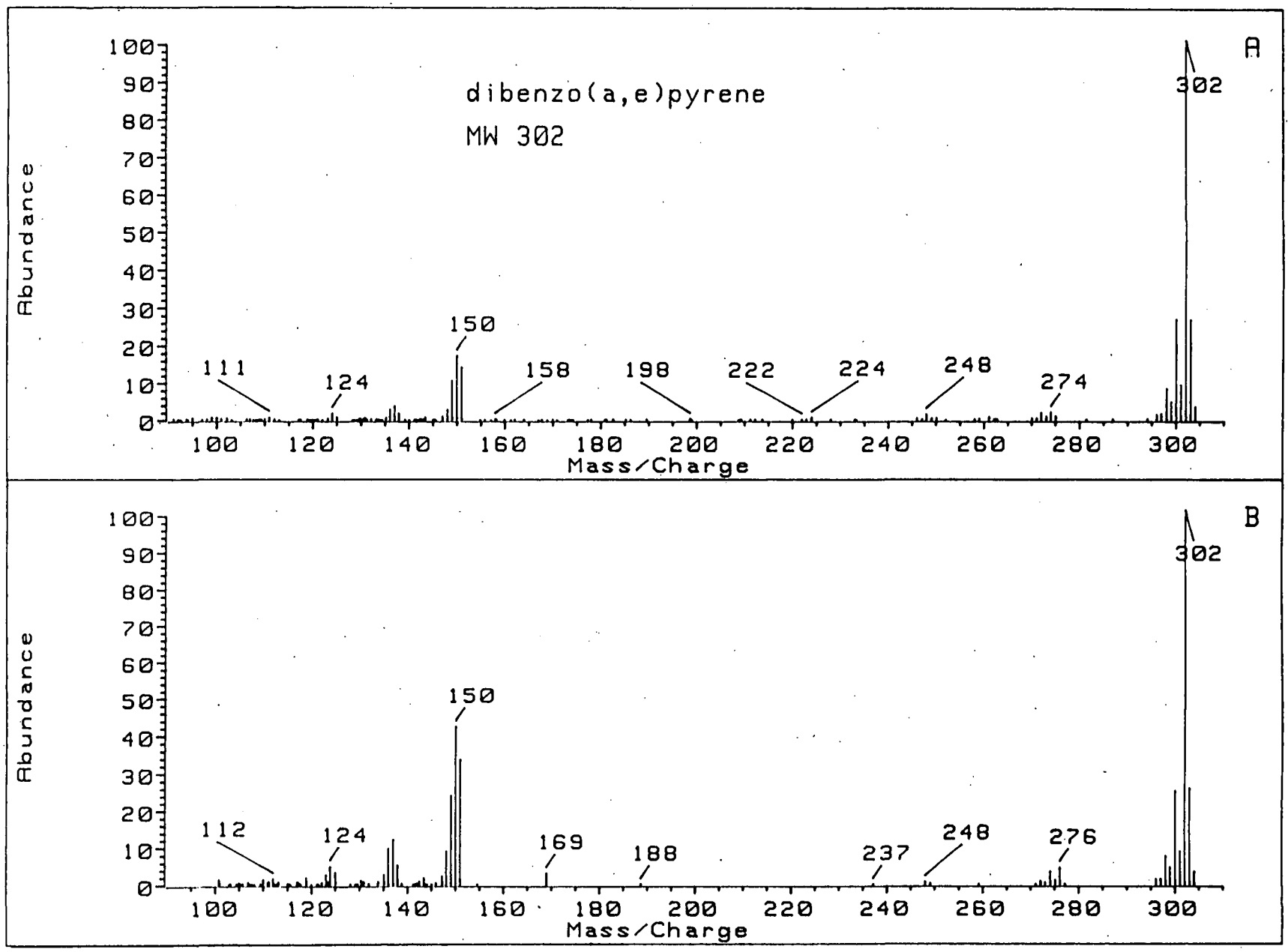


Figure 2. Comparative Particle Beam EI Mass Spectra.

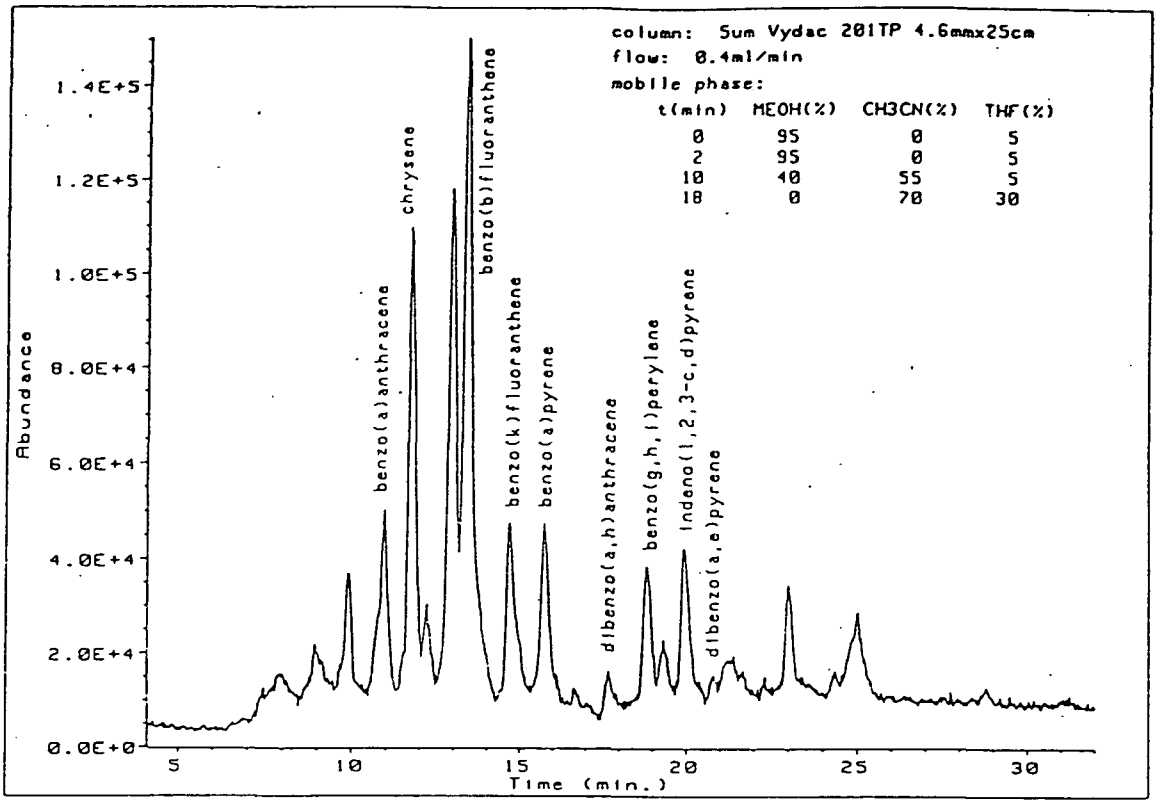


Figure 3. Particle Beam TIC of a PAH Contaminated Soil.

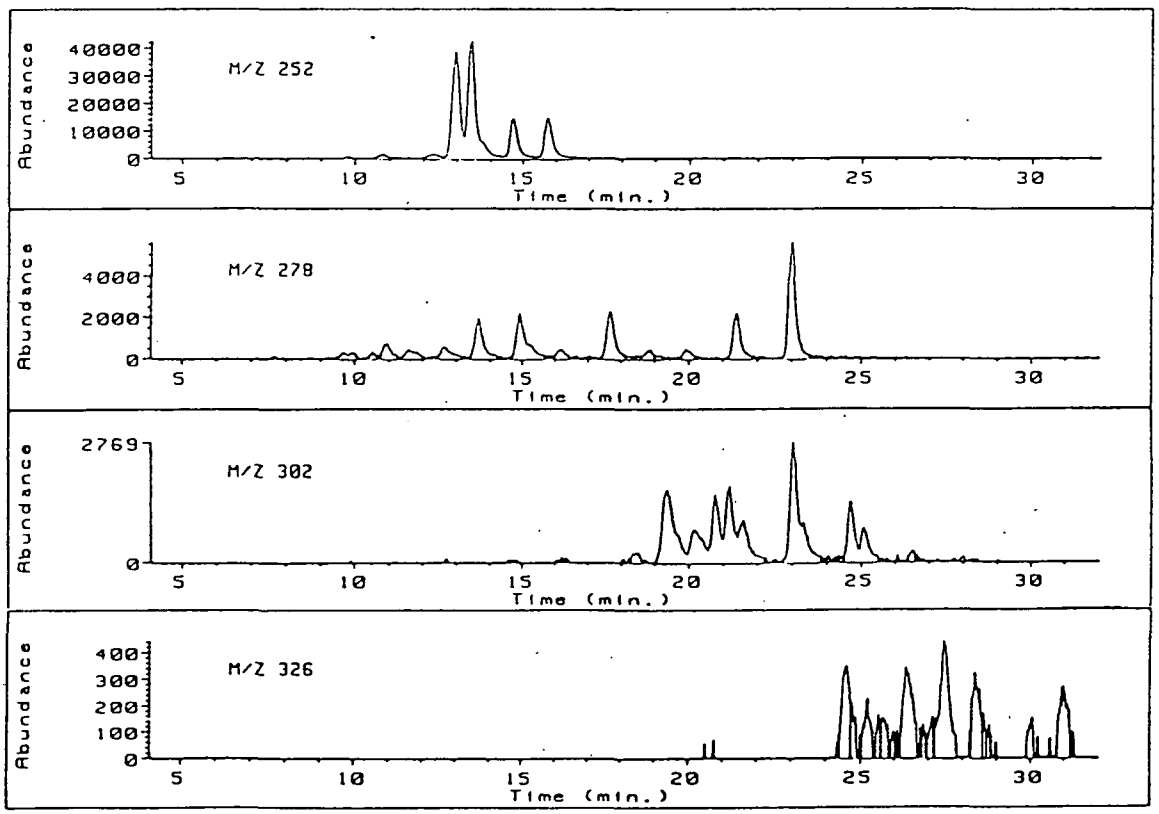


Figure 4. Selected Ion Chromatograms of a PAH Contaminated Soil.

TABLE 5. COMPARISON OF PB LC/MS QUANTIFICATION METHOD VS. FLUORESCENCE FOR PAH TARGET ANALYTES

			Soil Extract (μg/g)		
RT (min)	m/z	Compound	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

TABLE 6. TENTATIVELY IDENTIFIED, SELECTED NONTARGET COMPOUNDS IN SOIL SAMPLE

RT (min)	Base m/z	Tentative Identification
7.91	217	benzo(a)carbazole
9.78	254	binaphthalene
9.90	228	triphenylene
10.76	253	(chrysene/etc.)nitrile
12.23	234	benzonaphthothiophene isomer
12.95	252	benzopyrene/fluoranthene isomer
15.05	266	methyl-substituted 252 isomer
16.69	268	methyl cholanthrene isomer
19.30	268	methyl cholanthrene isomer
19.38	302	dibenzopyrene/fluoranthene isomer
21.22	302	dibenzopyrene/fluoranthene isomer
21.40	278	278 PAH isomer
22.35	284	dinaphthothiophene isomer
23.00	278	picene
23.03	302	dibenzopyrene/fluoranthene isomer
24.30	300	coronene
27.45	326	dibenzoperylene isomer

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 blank soils and the 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 analyzed with the PB instrument. Recoveries were calculated by using integrated quantitation ion abundances and a six point external calibration. Table 7 lists the target analyte recoveries as percentages of the spiked amount and the standard deviations in percent recovery. 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 the 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.

TABLE 7. PAH SPIKE RECOVERIES (PERCENT)

	0.5 µg/g (n=2)		2.5 µg/g (n=3)	
Compound	Mean Recovery	Relative % Difference	Mean Recovery	Standard Deviation
benzo(a)anthracene	108	2.5	76	12
chrysene	131	12	100	18
benzo(b)fluoranthene	88	1.5	71	14
benzo(k)fluoranthene	112	1.0	83	15
benzo(a)pyrene	63	11	59	13
dibenzo(a,l)pyrene	41	1.0	42	13
dibenzo(a,h)anthracene	122	8.0	86	19
benzo(g,h,i)perylene	96	5.0	70	15
indeno(1,2,3-c,d)pyrene	96	4.5	72	15
dibenzo(a,e)pyrene	74	8.5	79	14
dibenzo(a,i)pyrene	84	ND	80	19
dibenzo(a,h)pyrene	31	ND	50	16

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 8 along with estimated method detection limits. Method detection limits were estimated from observed instrument detection limits (Table 2). Values were adjusted for concentration/dilution factors imposed by the sample preparation scheme (SW-846 Method 3540 and Method 3630): A 20-µL injection, a 1-mL final extract volume, and a 10-g sample size. Final values were corrected with the observed recoveries, multiplied by a factor of two, and rounded to two decimal places. The method detection limits are estimates and have not been experimentally verified.

TABLE 8. 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	15
dibenzo(a,h)pyrene	0.11	45	16

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 analyte amounts were obtained by integrated quantitation ion areas and a six point external calibration. In addition, the extracts were analyzed by HPLC with UV diode array detection for comparative purposes. The results are listed in Table 9 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 8. 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. A PB total ion chromatogram of one of the SRM extracts is shown in Figure 5.

TABLE 9. RESULTS OF SRM ANALYSIS

Compound	Certified Value (μg/g)	HPLC/UV (μg/g)	PB with Clean-up (μg/g)	PB w/o 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

w/o = without
NA = certification not available
NF = not found
PB = particle beam

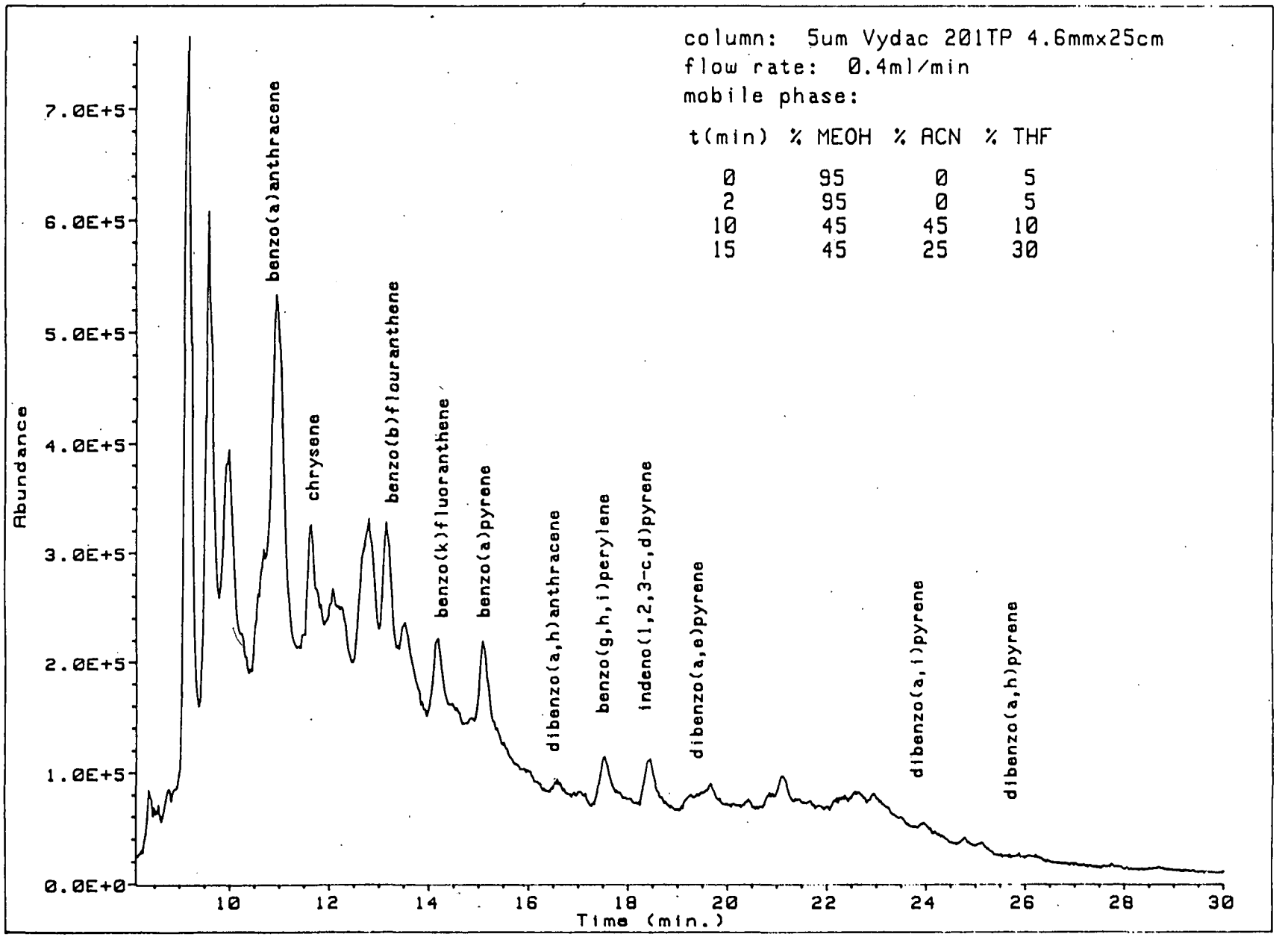


Figure 5. Particle Beam TIC of a PAH/Standard Reference Material.

REFERENCES

1. 51 Federal Register 5561, February 1986.

2. Bellar, T. A., T. D. Behymer, and W. L. Budde; J. Am. Soc. Mass Spectrom, 1, 92-98 (1990).

3. Bajic, S., D.R. Doerge, and C.J. Miles; An Investigation of Ion Abundance Enhancements for the Particle Beam LC/MS Analysis of Ethylenethiourea (ETU) in Food Samples; Proceedings of the 39th ASMS Conference on Mass Spectrometry and Allied Topics, Nashville, TN, May 19-24, 1991.

APPENDIX A

Particle Beam EI Mass Spectra

of

Polycyclic Aromatic Hydrocarbons

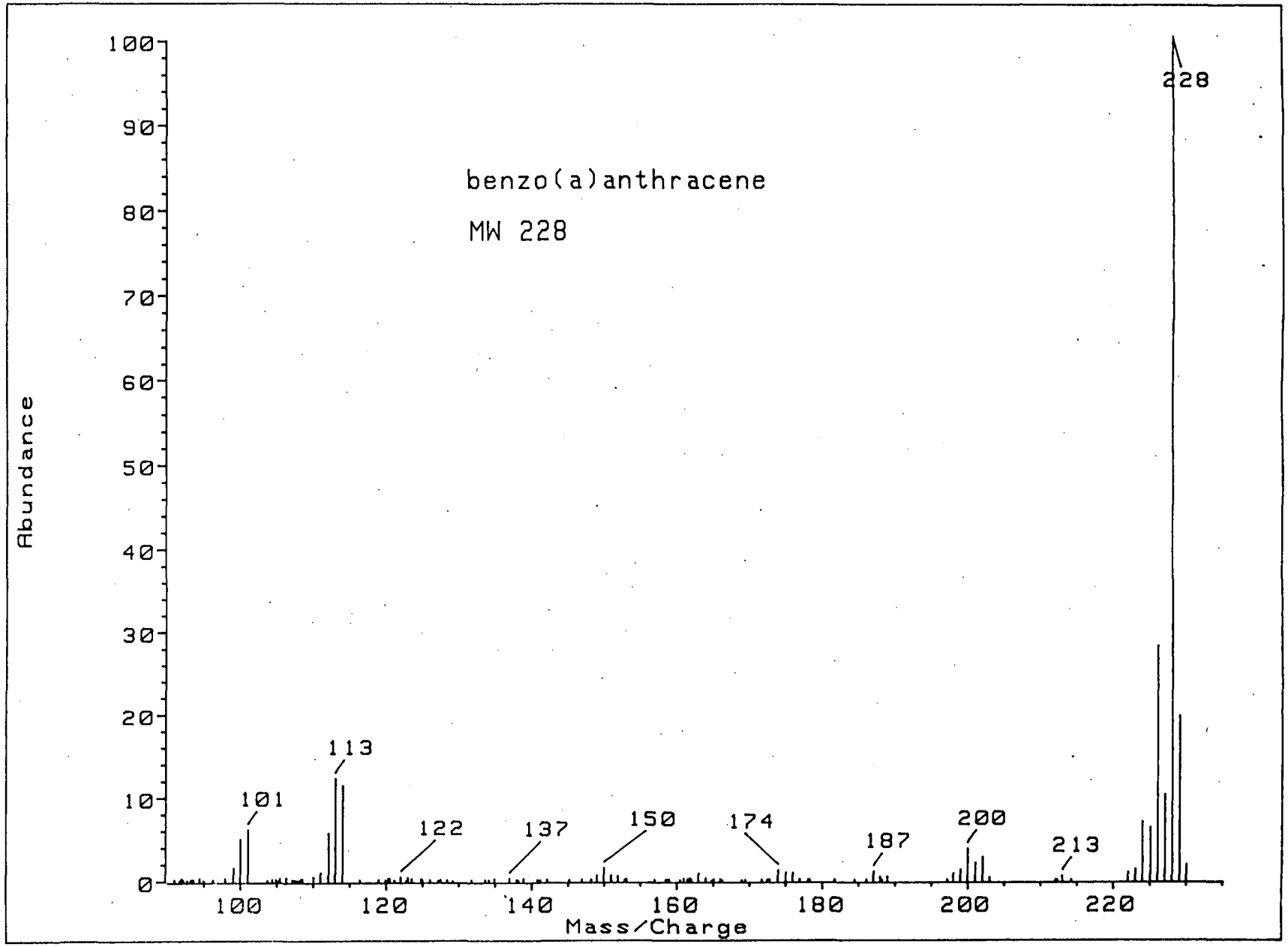


Fig. A-1. Particle Beam EI Mass Spectrum of Benzo(a)anthrancene.

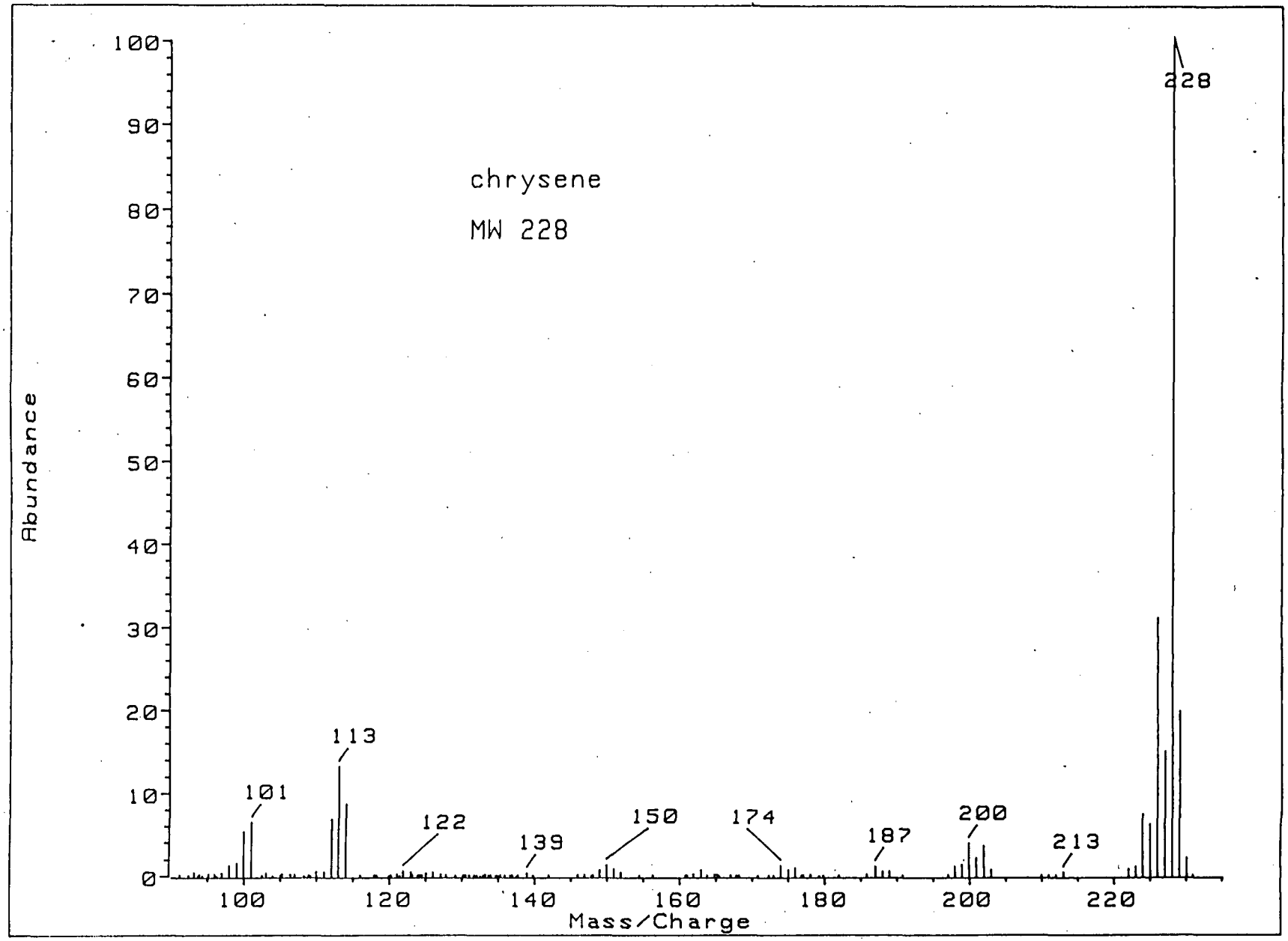


Fig. A-2. Particle Beam EI Mass Spectrum of Chrysene.

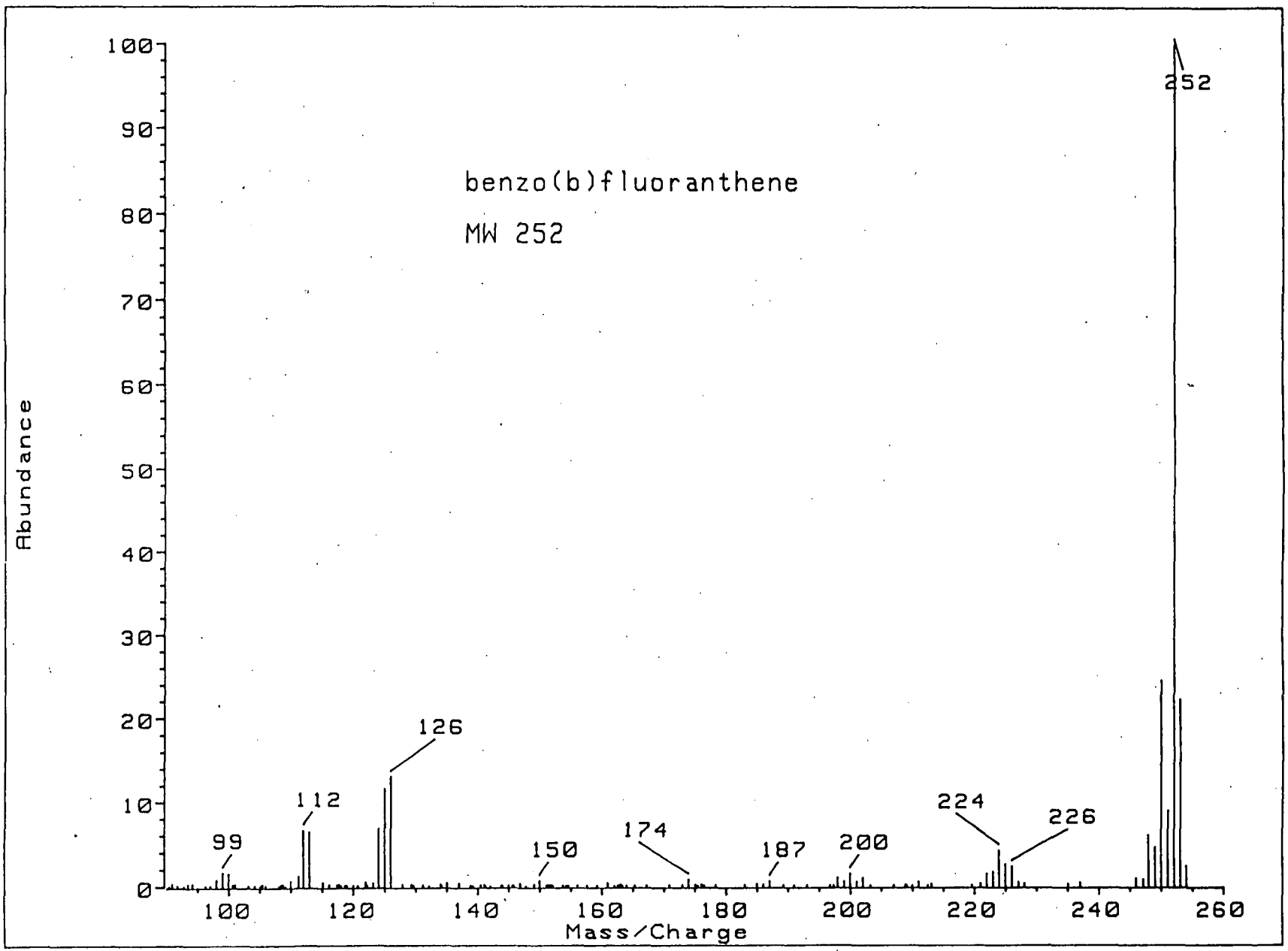


Fig. A-3. Particle Beam EI Mass Spectrum of Benzo(b)fluoranthene.

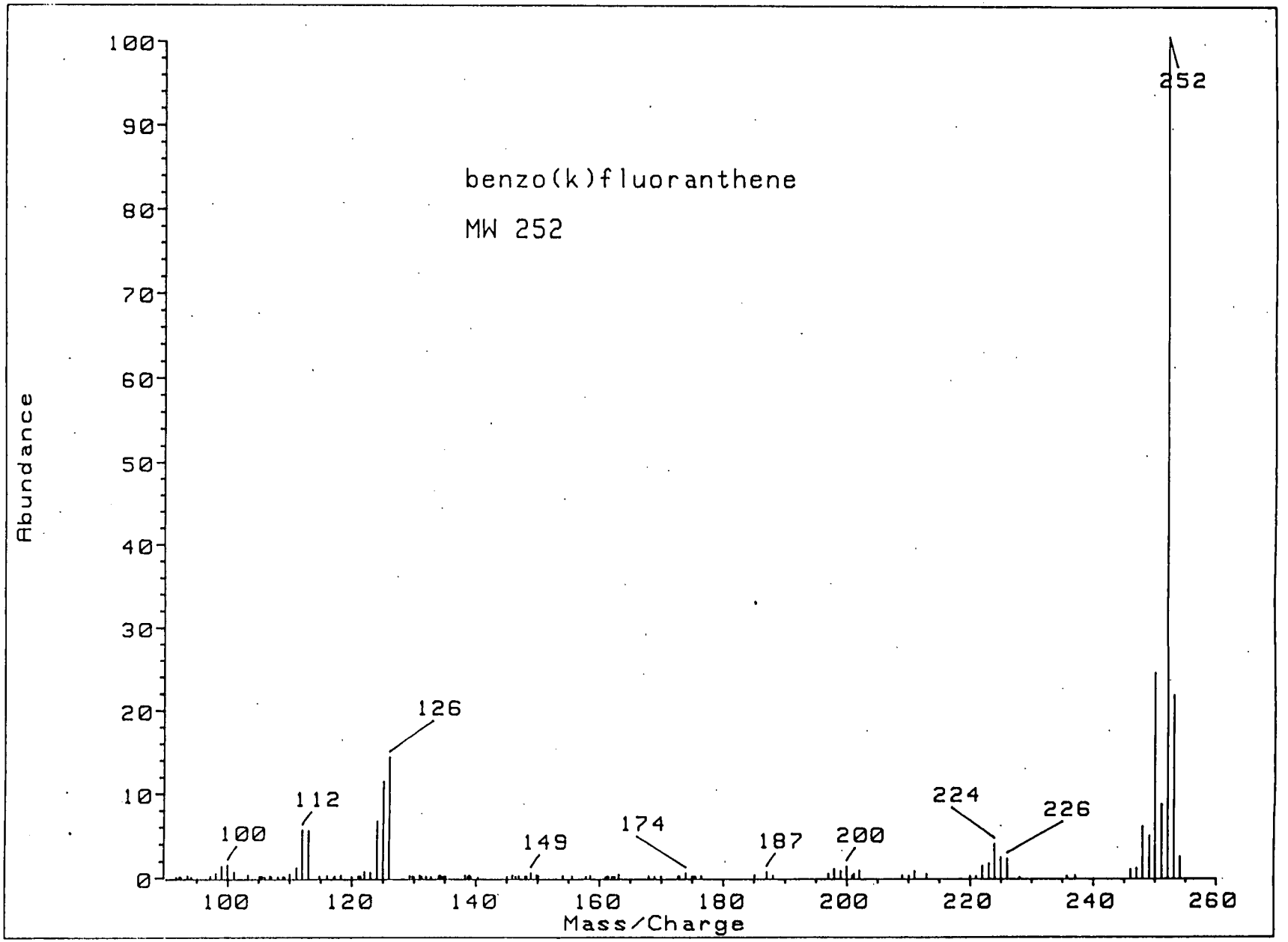


Fig. A-4. Particle Beam EI Mass Spectrum of Benzo(k)fluoranthene.

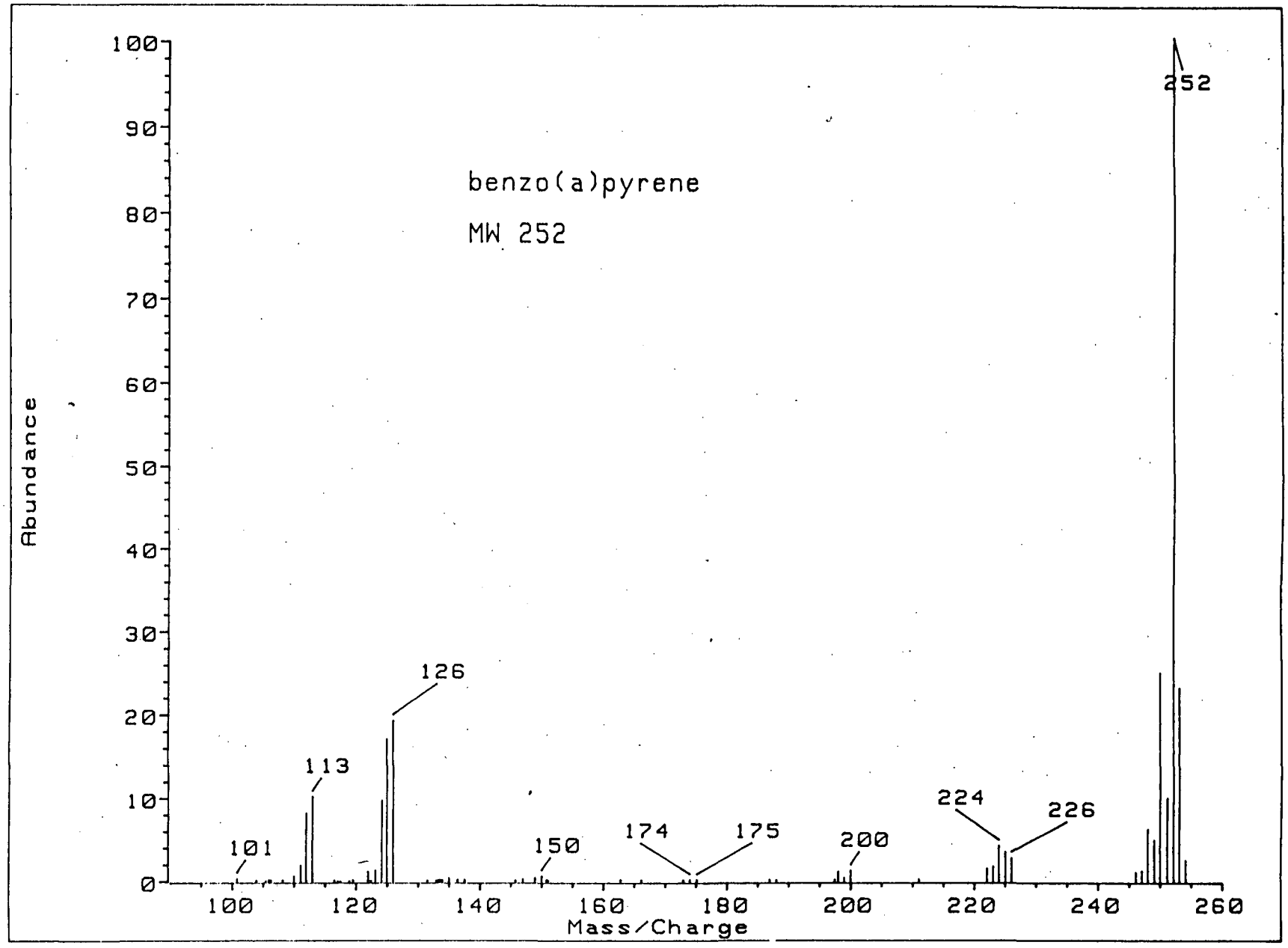


Fig. A-5. Particle Beam EI Mass Spectrum of Benzo(a)pyrene.

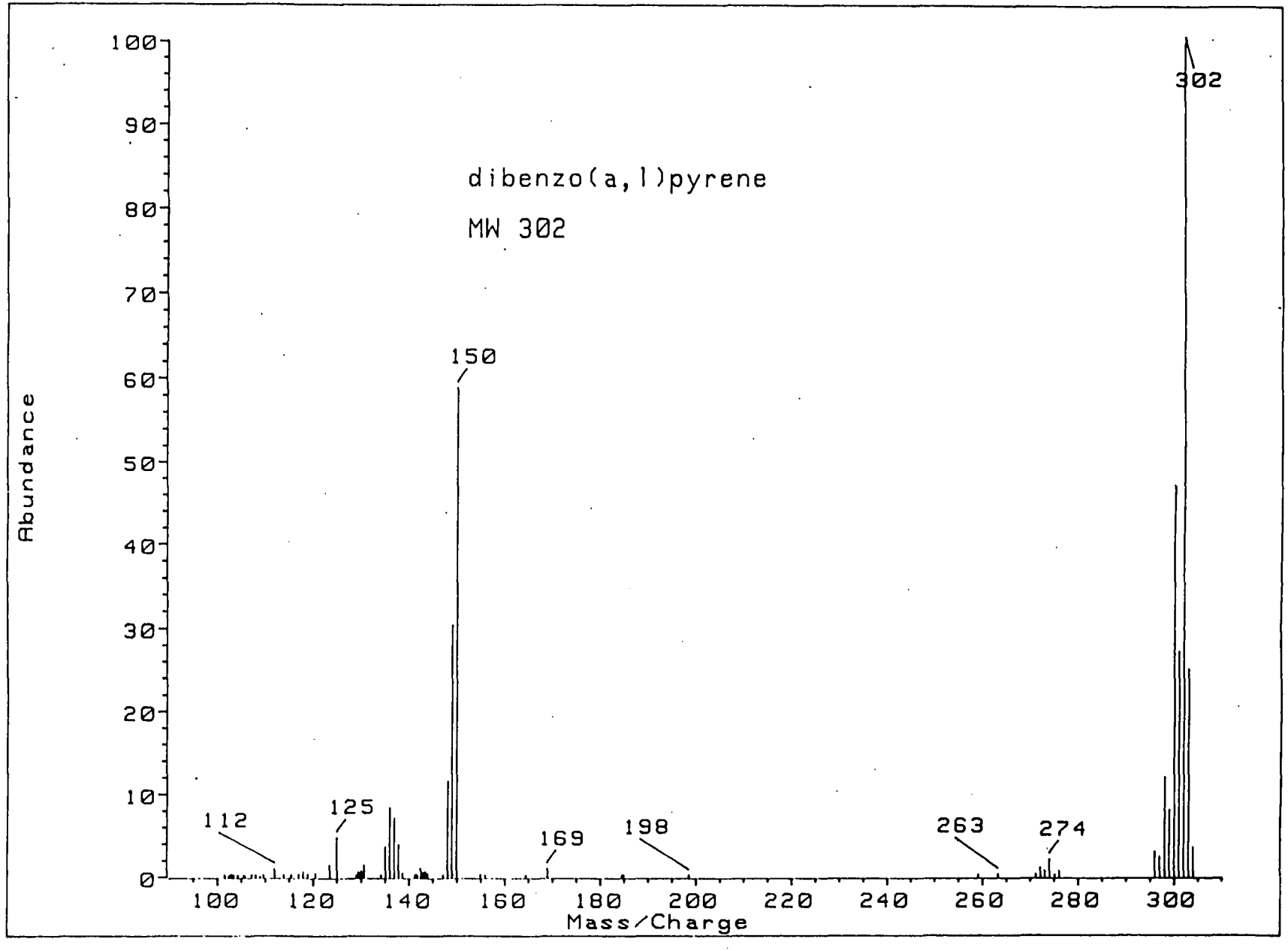


Fig. A-6. Particle Beam EI Mass Spectrum of Dibenzo(a,l)pyrene.

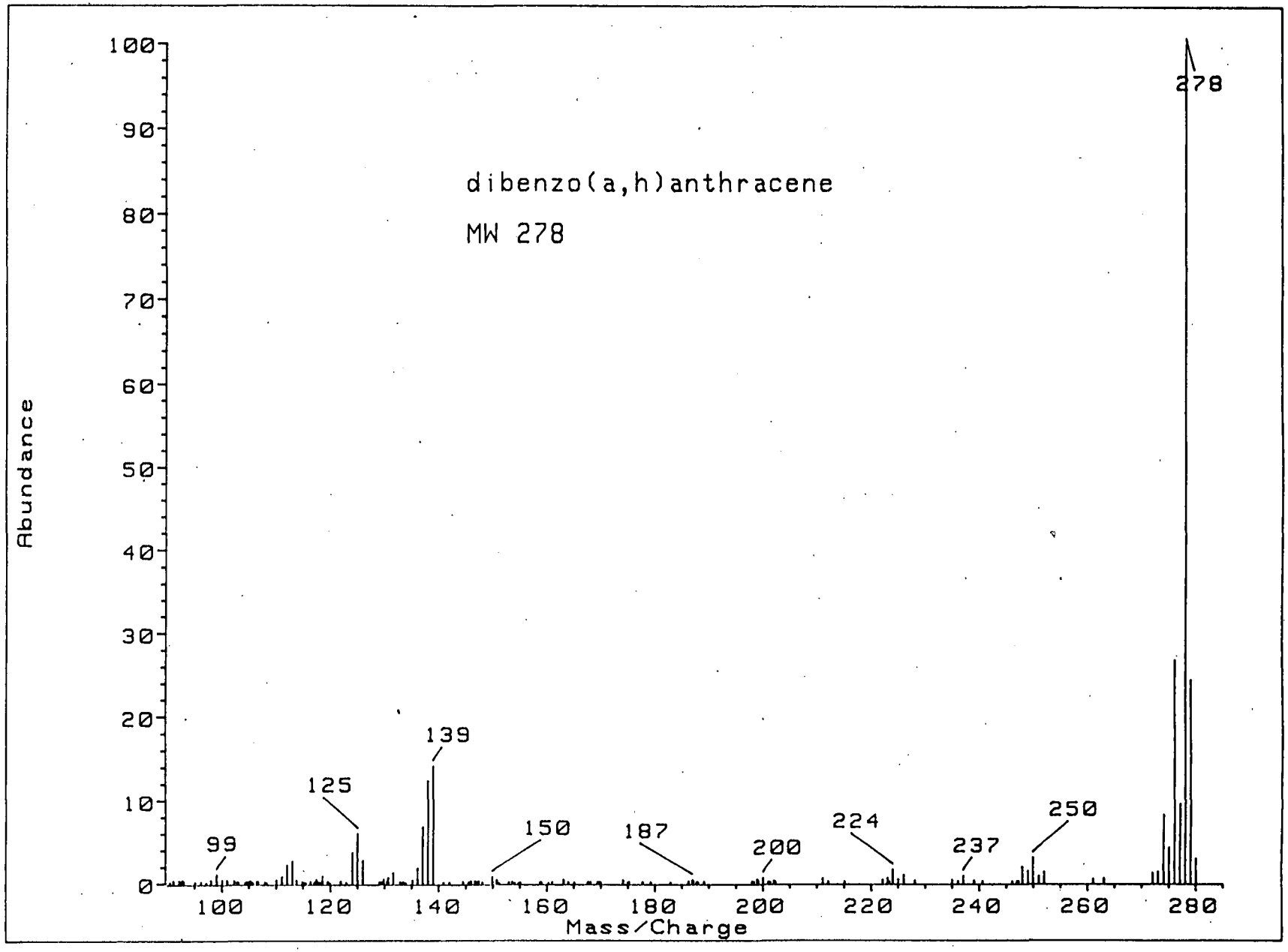


Fig. A-7. Particle Beam EI Mass Spectrum of Dibenzo(a,h)anthracene.

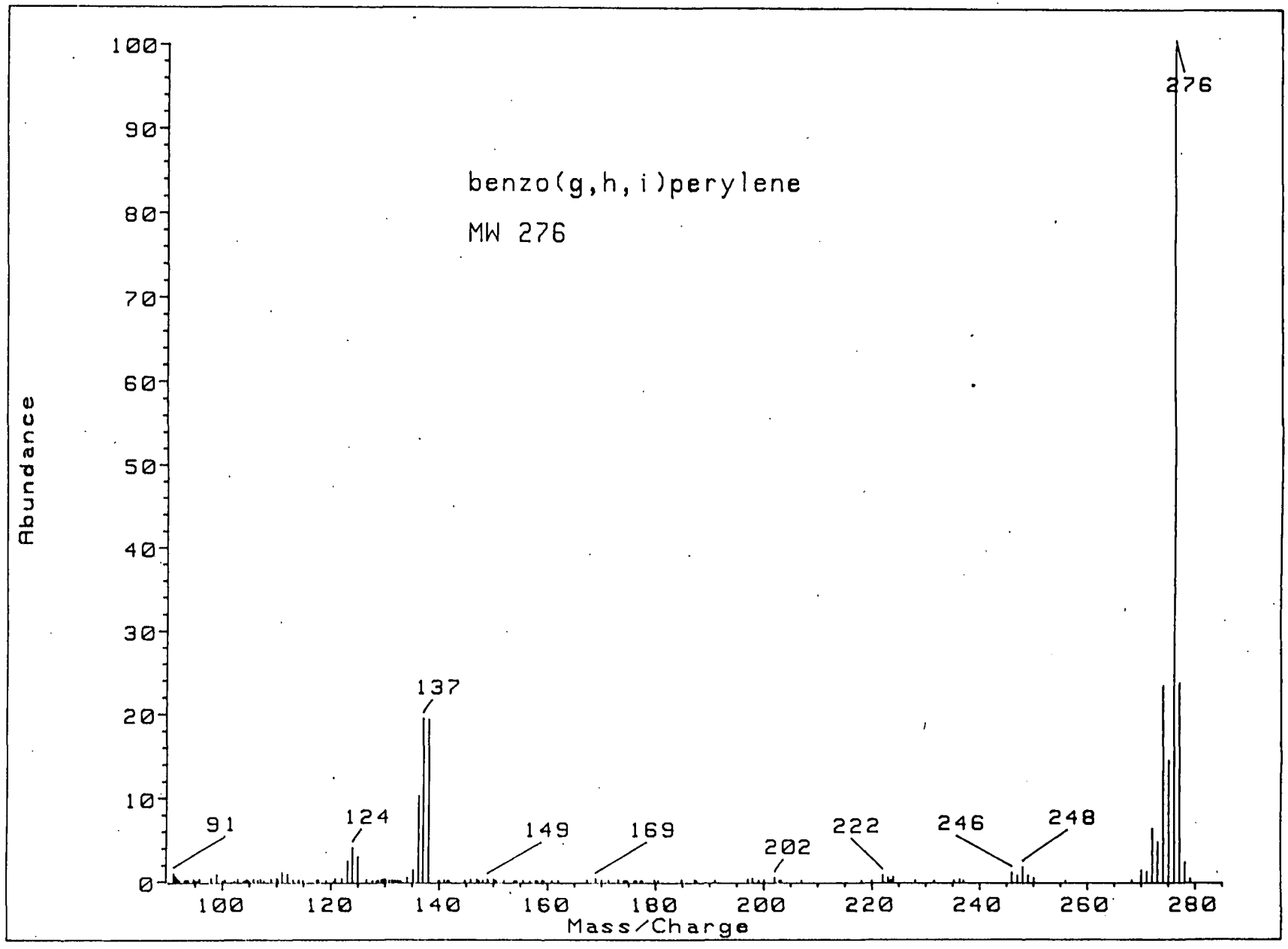


Fig. A-8. Particle Beam EI Mass Spectrum of Benzo(g,h,i)perylene.

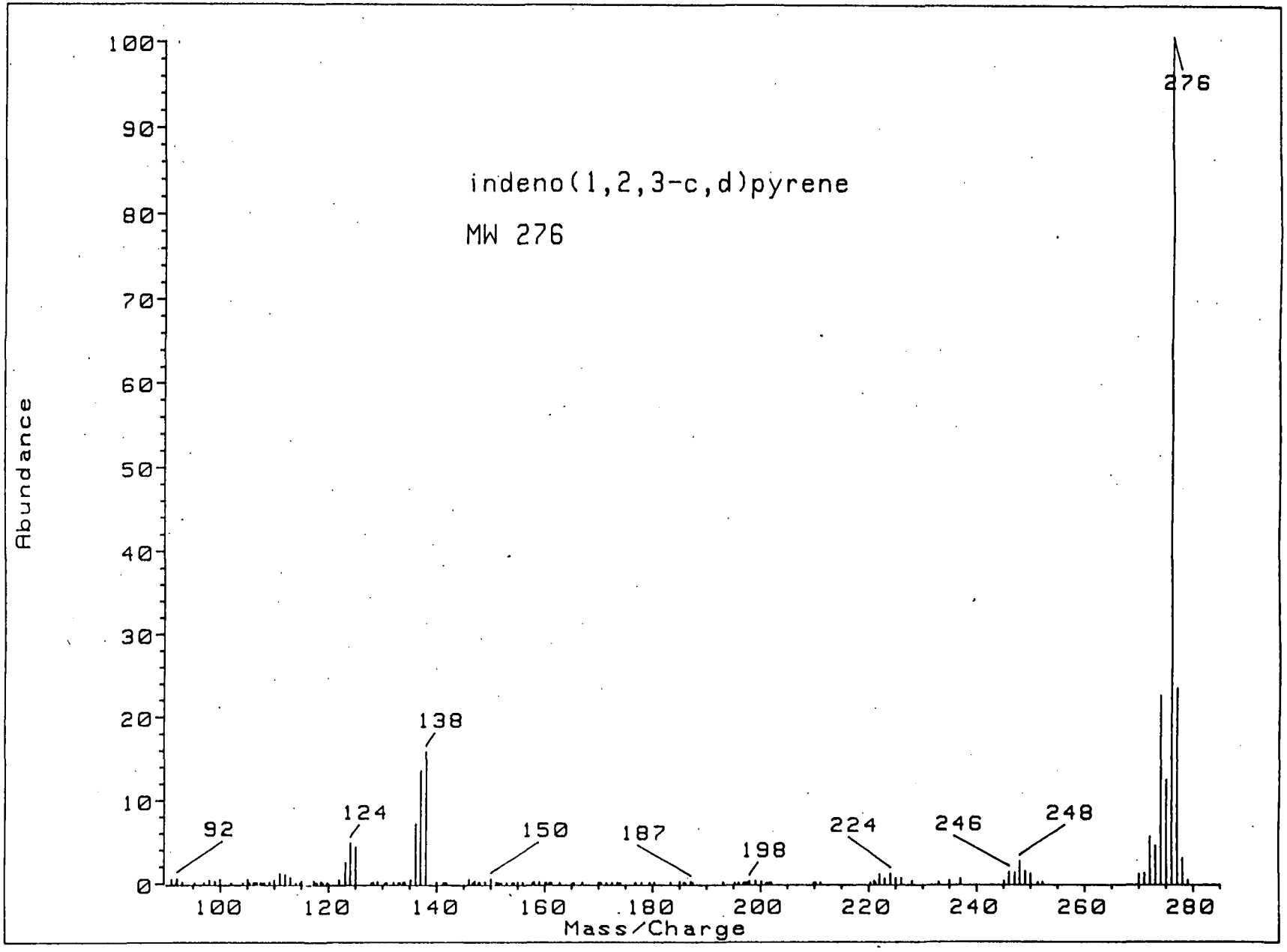


Fig. A-9. Particle Beam EI Mass Spectrum of Indeno(1,2,3-c,d)pyrene.

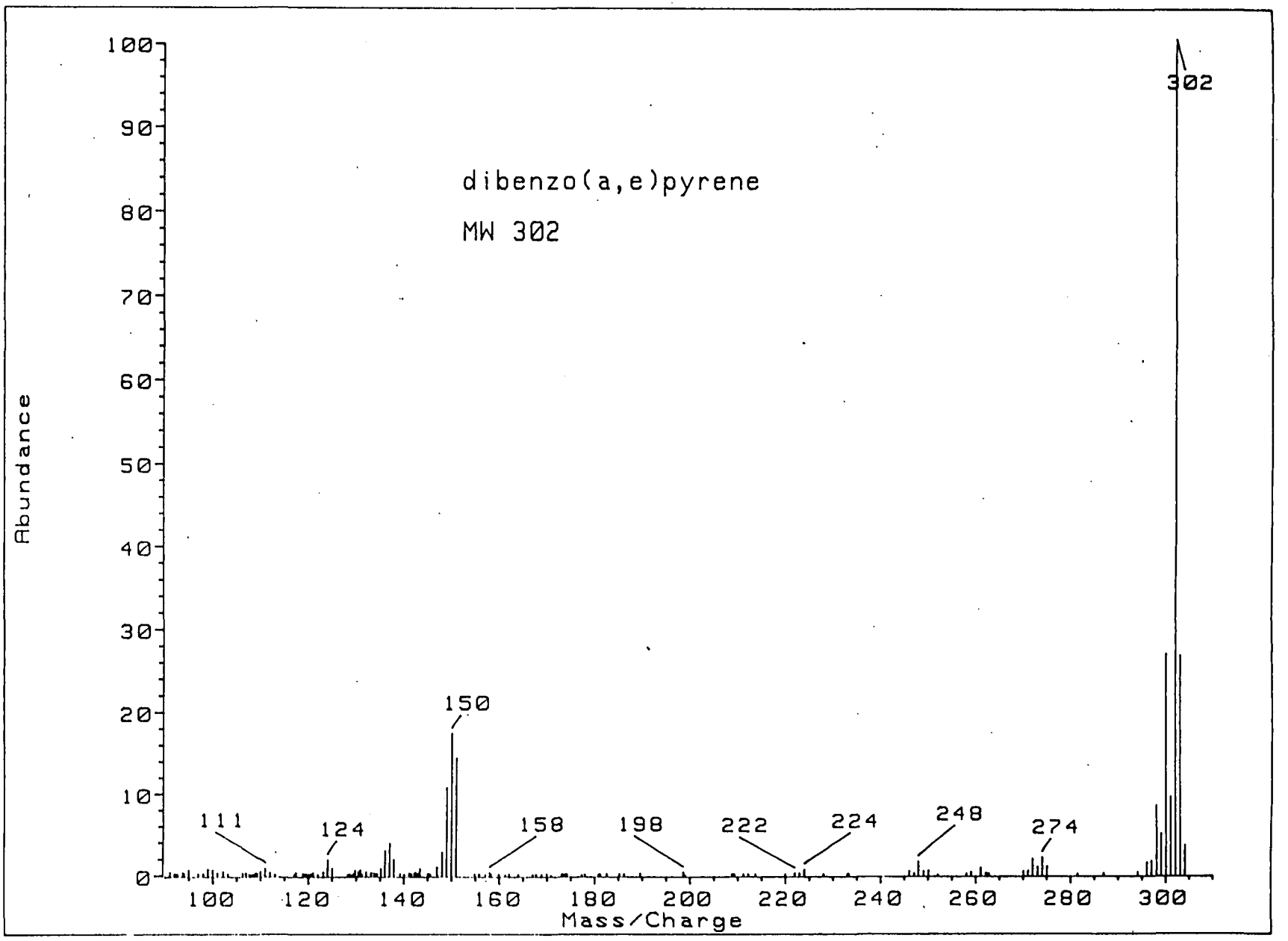


Fig. A-10. Particle Beam EI Mass Spectrum of Dibenzo(a,e)pyrene.

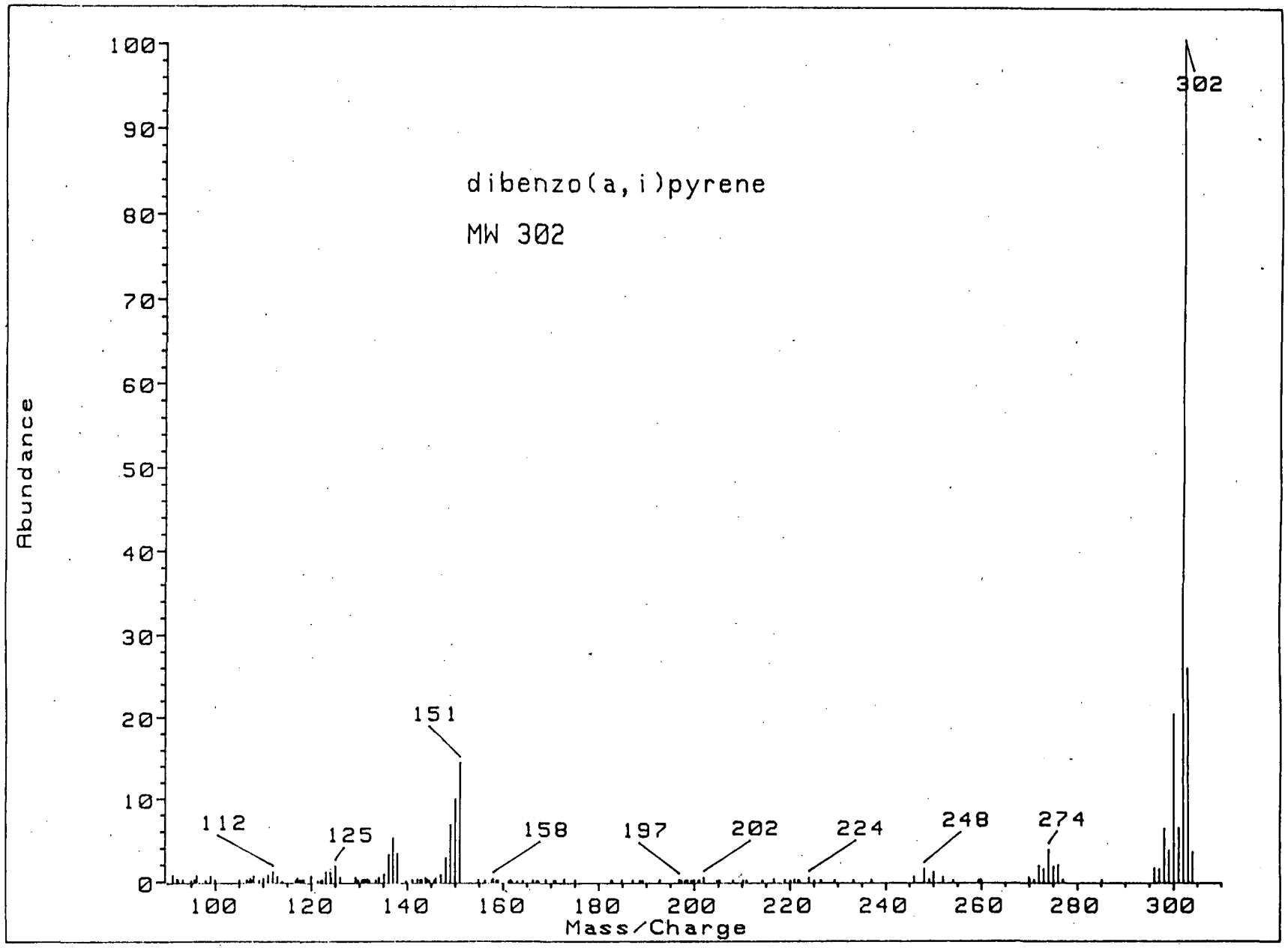


Fig. A-11. Particle Beam EI Mass Spectrum of Dibenzo(a,i)pyrene.

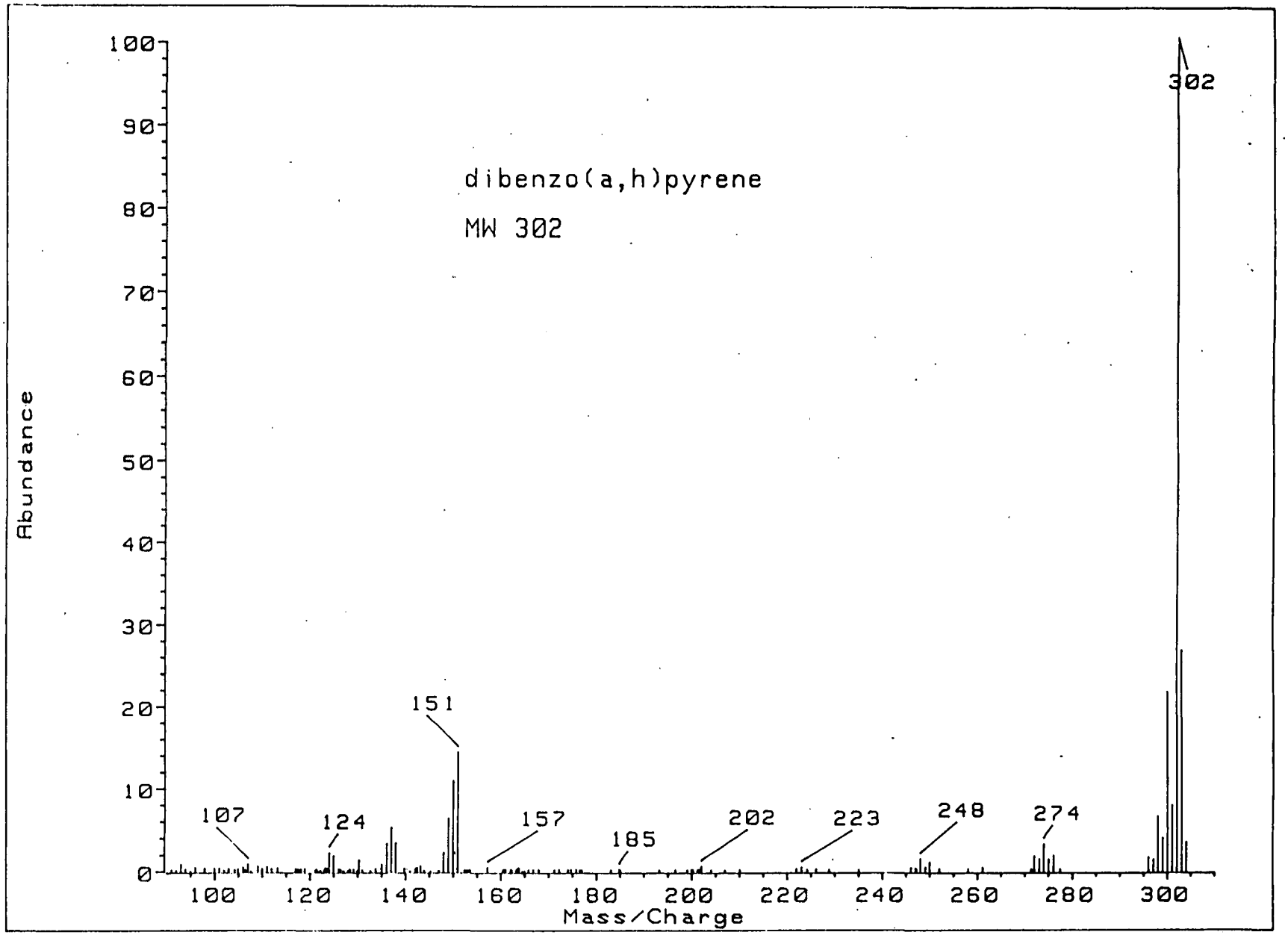


Fig. A-12. Particle Beam EI Mass Spectrum of Dibenzo(a,h)pyrene.

APPENDIX B

PRELIMINARY DRAFT METHOD

PARTICLE BEAM/LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY ANALYSES OF POLYCYCLIC AROMATIC HYDROCARBONS

1.0 SCOPE AND APPLICATION

1.1 This method is used to determine the concentration of certain polycyclic aromatic hydrocarbons (PAHs) in extracts prepared from soils and sediments. Table B-1 lists the nominal retention times of the target PAHs along with their quantitation ion and secondary ion.

TABLE B-1. CHARACTERISTIC IONS FOR PARTICLE BEAM/ LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY OF POLYCYCLIC AROMATIC HYDROCARBONS

Compound	Retention Time (min)	Quantitation Ion	Secondary Ion
benzo(a)anthracene	10.95	228	113
chrysene	11.67	228	113
benzo(b)fluoranthene	13.20	252	126
benzo(a)pyrene	15.20	252	126
dibenzo(a,l)pyrene	15.45	302	150
dibenzo(a,h)anthracene	16.27	278	139
benzo(g,h,i)perylene	17.66	276	138
indeno(1,2,3-c,d)pyrene	18.56	276	138
dibenzo(a,e)pyrene	19.31	302	150
dibenzo(a,i)pyrene	24.36	302	151
dibenzo(a,h)pyrene	26.13	302	151

a) see 6.2 for liquid chromatographic conditions

2.0 SUMMARY OF METHOD

2.1 This method provides particle beam (PB) liquid chromatography/mass spectrometry (LC/MS) conditions for detecting certain PAHs. Prior to using this method, appropriate sample extraction techniques must be used. A 20-µl aliquot of the extract is injected into a liquid chromatograph (LC), and compounds in the effluent are passed through a PB interface and subsequently analyzed by a quadrupole mass spectrometer (MS) operated in the electron ionization (EI) mode.

3.0 INTERFERENCES

3.1 Raw LC/MS data from all blanks, samples, and spikes must be evaluated for interferences. Determine if the source of interference originates in the preparation or clean-up of the samples and correct the problem.

3.2 Contamination by carryover can occur whenever high-level and low-level samples are sequentially analyzed. To reduce carryover, a solvent blank should be analyzed following an unusually concentrated sample.

3.3 The chromatographic conditions described allow for a unique resolution of the specified PAH compounds covered by this method. Other PAH compounds, in addition to matrix artifacts, may interfere.

4.0 APPARATUS AND MATERIALS

4.1 Particle beam/liquid chromatograph/mass spectrometer system.

4.1.1 Liquid chromatograph: An analytical system complete with a ternary gradient pumping system and all necessary accessories.

4.1.2 Reverse-phase column: C-18, 5-μm particle-size diameter, in a 250-mm x 4.6-mm I.D. stainless steel column (Vydac No. 201TP54 or equivalent).

4.1.3 Particle beam interface: Any PB type interface that meets all analysis criteria may be used (HP 59980A or equivalent).

4.1.4 Mass Spectrometer: Capable of producing full-scan EI type mass spectra when interfaced to an LC system.

4.1.5 Data system: A computer system must be interfaced to the MS. The system must allow the continuous acquisition and storage of all mass spectra obtained during the chromatographic program on machine-readable media. The computer must also have the ability to integrate extracted ion profiles for quantitation purposes and the ability to match acquired spectra against a spectral library for compound identification. Provisions for fitting response data to second or third order curves may also be necessary.

5.0 REAGENTS

5.1 Methanol: HPLC quality

5.2 Acetonitrile: HPLC quality

5.3 Tetrahydrofuran: HPLC quality

5.4 Stock standard solution:

5.4.1 Prepare individual 1.0-mg/mL solutions of dibenzo(a,e)pyrene and dibenzo(a,i)pyrene in benzene or toluene. Next, prepare a 0.5-mg/mL solution of dibenzo(a,h)pyrene in benzene or toluene. Finally, prepare 1.0-mg/mL solutions in acetonitrile for the remainder of target compounds listed in Table 1. Mix 1.0 mL dibenzo(a,h)pyrene standard along with 0.5 mL of each of the other target compound solutions prepared above. Bring this solution up to 10.0 mL with acetonitrile giving a final concentration of 50 μg/mL of each compound. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

5.4.2 Transfer the stock standard solution into a Teflon-sealed screw-cap bottle. Store at 4° C and protect from light. The stock standard should be checked frequently for signs of degradation or evaporation, especially just prior to using it to prepare calibration standards.

5.4.3 The stock standard solution must be replaced after one year, or sooner if comparison with check standards indicates a problem.

5.5 Calibration standards: Calibration standards at a minimum of five concentration levels should be prepared through dilution of the stock standard with acetonitrile. One of the concentration levels should be at a concentration near, but above, the method detection limit. The remaining concentration levels should correspond to the expected range of concentrations found in real samples or should define the working range of the PB LC/MS system. Calibration standards must be replaced after six months, or sooner if comparison with check standards indicates a problem.

5.6 PB LC/MS tuning compound: Perfluortributylamine should be introduced through the PB interface into the MS with solvent flow on. The MS should be tuned to maximize mass 219.

5.7 Internal standards: The use of internal standards is optional. To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Coelution effects on the use of internal standards have not been clearly established.

5.7.1 Prepare calibration standards at a minimum of five concentration levels for each analyte as described in Paragraph 5.5.

5.7.2 To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with acetonitrile.

5.7.3 Analyze each calibration standard according to Section 6.0.

6.0 PROCEDURE

6.1 Extraction and Cleanup: See SW-846 Methods 3540 and 3630. Method 3540 is followed by a solvent exchange into cyclohexane and Method 3630 is followed by a solvent exchange into acetonitrile.

6.2 LC conditions: Using the column described in Paragraph 4.1.2, follow the conditions recommended below.

Time (min)	% ACN	% MEOH	% THF
0	0	95	5
2	0	95	5
10	45	45	10
15	25	45	30

Mobile phase flow rate = 0.4 mL/min
Column temperature = ambient

6.3 PB conditions: A desolvation chamber temperature of 45° C is recommended. The nebulizer and helium flow should be set to maximize the response of the m/z ion of 10 ng dibenzo(a,h)pyrene with 25% acetonitrile, 45% methanol, and 30% tetrahydrofuran as the mobile phase.

6.4 Recommended MS conditions:

scan range: 100-500
scan time: 0.5 to 1.0 scans/sec
source temperature: 280° C to 300° C
electron energy: 70eV
emission current: 300 μA
tuning: maximize m/z 219 of perfluorotributylamine

6.5 Calibration:

6.5.1 Refer to Method 8000 of the SW-846 for proper calibration procedures. Use the areas from each for the proper quantitation ions listed in Table 1 to quantify each of the PAHs. The procedure for internal or external standard calibration may be used. Use Table 3 for guidance in selecting the lowest point on the calibration curve.

6.5.2 Assemble the necessary PB LC/MS apparatus and establish operating parameters equivalent to those indicated in Section 6.2 through 6.4. By injecting 20 μl of the calibration standards, establish the sensitivity limit of the MS and the linear range of the analytical systems for each compound. In case of a nonlinear response over the concentration range required for analysis, the analyst may apply a more appropriate model (i.e., point-to-point calibration or

polynomial curvefits). A curve will be considered nonlinear when the relative standard deviation in response factors is 20 or greater.

6.5.3 Before using any cleanup procedure, the analyst should process a series of calibration standards through the procedure to confirm elution patterns and the absence of interferences from the reagents.

6.6 Daily calibration: A calibration standard at mid-level concentration containing all target analytes must be performed every 20 samples during analysis. Compare the response factor data from the standards every 20 samples with the average response factors from the initial calibration if it is considered linear. If another calibration model is used, then the response factor from the mid-point chosen for the daily calibration should be compared with the response factor from the initial calibration of that same concentration. In either case, the daily calibration can have no more than 20% relative difference for any of the compounds or a new calibration curve must be constructed.

6.7 PB LC/MS analysis:

6.7.1 Table B-1 summarizes the estimated retention times of the PAHs determined by this method. Figure B-1 is an example of the separation achievable using the conditions given in Paragraph 6.2.

6.7.2 If internal standard calibration is to be performed, add the internal standard to the sample prior to injection. Inject 20 µL of the sample extract into the LC. Re-equilibrate the LC column at the initial gradient conditions for at least 10 minutes between injections.

6.7.3 Using either the internal or external calibration procedure (Method 8000), determine the quantity of each component peak, in the sample chromatogram, that corresponds to the compounds used for calibration purposes. See Section 7.8 of Method 8000 for calculation equations.

6.7.4 Using either a self-created mass spectral library or a computer library, confirm the identity of each component in the sample.

6.7.5 If the peak area exceeds the linear range of the system, dilute the extract and reanalyze.

6.7.6 If interferences prevent measurement of the peak area, further cleanup is required.

7.0 METHOD PERFORMANCE

7.1 This method was tested using a sandy loam soil spiked at levels of 0.5 μg/g and 2.5 μg/g in triplicate. The recovery results for the analysis of the extracts are displayed in Table B-2. One of the low-level extracts was a two-phase mixture and was not used to calculate the percent recovery. 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 these 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.

7.2 Table B-3 presents the detection limits, precision, and accuracy for this method. Method detection limits were estimated from observed instrument detection limits. Values were adjusted for concentration/dilution factors imposed by the sample preparation scheme, assuming use of a 20-μL injection 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. The recovery data are pooled and treated as a single data set to generate overall method precision and accuracy values.

7.3 Method performance was also tested by analyzing an SRM obtained from the National Research Council of Canada and designated as HS-3. In addition, the extracts were analyzed by HPLC with UV diode array detection for comparative purposes. The SRM (marine sediment) was extracted and cleaned-up using the silica gel procedure in triplicate. Table B-4 lists the results of the SRM analysis. The PB results failed to meet acceptance criteria ($p \pm 2s$) for only one analyte, benzo(k)fluoranthene. The HPLC/UV analysis failed acceptance criteria for two of the target analytes. In general, results obtained from PB analysis and HPLC/UV were in agreement and agreed with certified values.

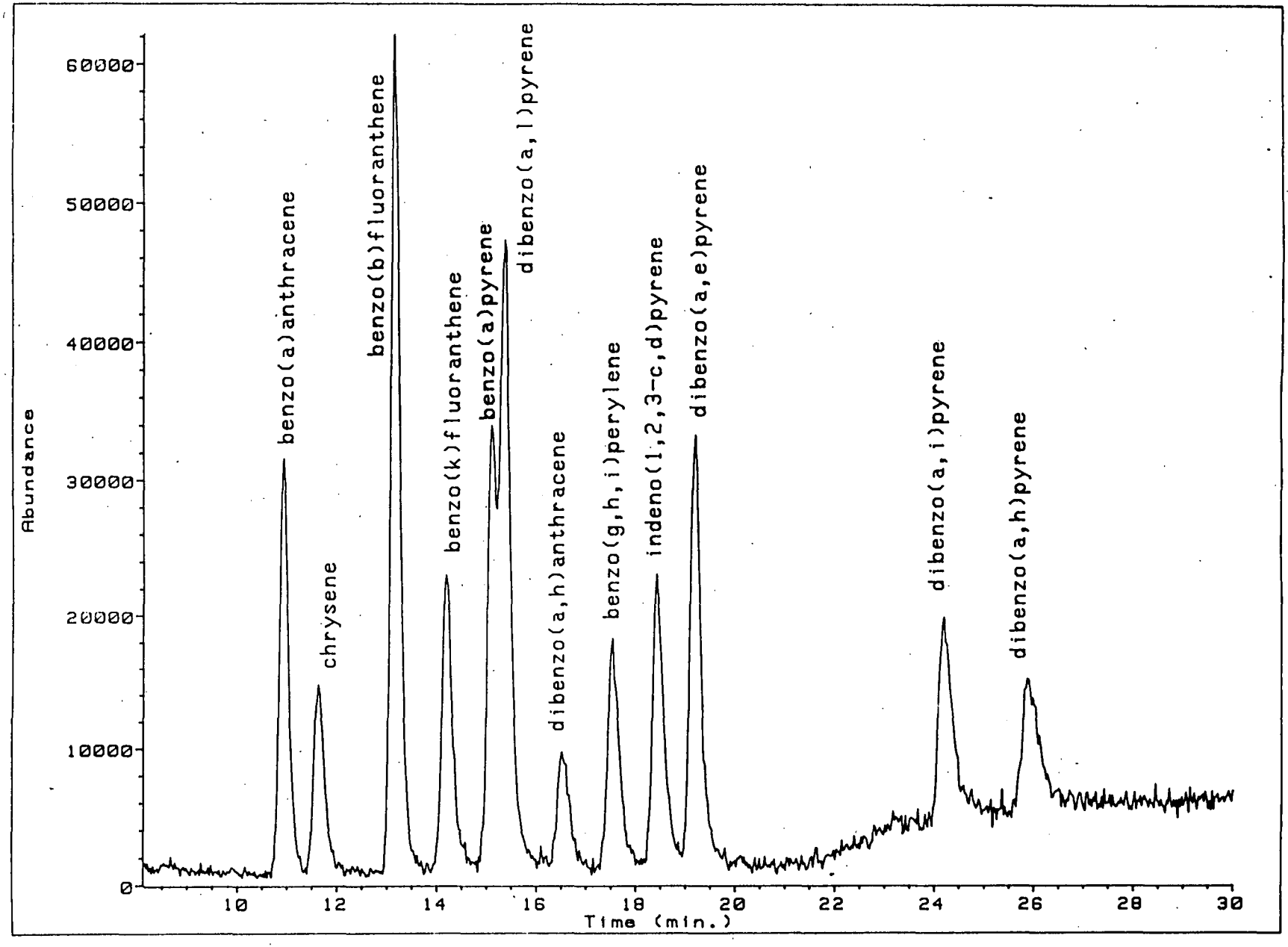


Figure B-1. Particle Beam Liquid Chromatography/Mass Spectrometry of Polynuclear Aromatic Hydrocarbons.

TABLE B-2. PAH SPIKE RECOVERIES

Compound	0.5 µg/g (n=2)		2.5 µg/g (n=3)	
	Mean Recovery (%)	Standard Deviation	Mean Recovery (%)	Standard Deviation
benzo(a)anthracene	108	2.5	76	12
chrysene	131	12	100	18
benzo(b)fluoranthene	88	1.5	71	14
benzo(k)fluoranthene	112	1.0	83	15
benzo(a)pyrene	63	11	59	13
dibenzo(a,l)pyrene	41	1.0	42	13
dibenzo(a,h)anthracene	122	8.0	86	19
benzo(g,h,i)perylene	96	5.0	70	15
indeno(1,2,3-c,d)pyrene	96	4.5	72	15
dibenzo(a,e)pyrene	74	8.5	79	14
dibenzo(a,i)pyrene	84	ND	80	19
dibenzo(a,h)pyrene	31	ND	50	16

TABLE B-3. 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	15
dibenzo(a,h)pyrene	0.11	45	16