ANALYTICAL LETTERS, 17(A11), 1293-1306 (1984)

SOLVENTS AND TECHNIQUES FOR THE EXTRACTION OF POLYNUCLEAR AROMATIC HYDROCARBONS FROM FILTER SAMPLES OF DIESEL EXHAUST

KEY WORDS: diesel exhaust, solvent extraction, polynuclear aromatic hydrocarbons

George M. Breuer, Ph.D., CIH

National Institute for Occupational Safety and Health, 4676 Columbia Parkway, Cincinnati, Ohio 45226

ABSTRACT

The amounts of five polynuclear aromatic hydrocarbons--fluoranthene, pyrene, benzo(a)anthracene, benzo(k)fluoranthene, and benzo(a)pyrene--extracted by six solvents with four extraction techniques have been determined for diesel exhaust particulate collected on Teflon-coated glass fiber filters. Samples were analyzed by high pressure liquid chromatography with fluorescence detection. Toluene and methylene chloride gave higher recoveries than methanol, isopropanol, acetonitrile, and acetone for benzo(a)anthracene, benzo(k)fluoranthene, and benzo(a)pyrene. Soxhlet extraction for two hours (approximately 48 cycles) with toluene or acetonitrile was more effective than

0003-2719/84/1711-1293$3.50/0

simple mechanical agitation, ultrasonic agitation for 15 minutes, or immersion in refluxing solvent for two hours.

INTRODUCTION

Polynuclear aromatic hydrocarbons (PAH's) are found in some natural products such as coals and asphalts and are formed in the incomplete combustion of fossil fuels and organic materials. Some of them are known carcinogens, so their presence in the environment is a health concern resulting in several studies in both the community and occupational environments. A common technique for monitoring the levels of these compounds in air is to take a filter sample which is then extracted with a solvent. The extractant is analyzed gravimetrically (for total extractables) or chromatographically (for individual PAH's)[1].

The extraction process itself is a critical step in the analysis and deserves the most careful scrutiny since there are many variables to be considered, including the choices of solvent and of agitation technique. Another variable of interest is the nature of the sample since there are a variety of environments where PAH's are observed.

One type of sample of particular interest recently is diesel exhaust[2]. The high efficiency of these engines have made them increasingly popular. The exhaust contains particulate which is known to consist of a soot spherule with condensed organic material[3,4]. A previous study has shown a variation in the amount of the heavier PAH's which can be extracted by cyclohexane, acetonitrile, and methylene chloride[5]. This study has continued that previous work by studying other solvents and by determining the effect of different agitation techniques with two of those solvents. Five specific PAH's--fluoranthene (Ft), pyrene (Py), benzo(a)anthracene (BaA), benzo(k)fluoranthene (BkF), and benzo(a)pyrene (BaP)--were studied as representative compounds.

EXPERIMENTAL

Reagents

All solvents used were "Distilled in Glass" reagent grade (Burdick and Jackson, Muskegon, Mich.) except the methanol and 2-propanol which were HPLC grade (Fisher, Fair Lawn, N. J.). High purity water was produced by treatment of Cincinnati municipal water with a reverse osmosis system and Milli-Q polishing system (Millipore, Bedford, Mass.). A stock solution containing all five compounds for the HPLC analysis was prepared by weighing out known quantities of pure samples of the PAH's (Pfaltz and Bauer, Stamford, Conn.) and dissolving in a known quantity of acetonitrile in a volumetric flask, except for benzo(k)fluoranthene which was purchased as a prepared solution in acetonitrile (Nanogen Analytical Standards, Watsonville, Ca.) and an appropriate volume was added to the stock solution. Working standards at two concentrations near those observed in the samples were prepared by appropriate dilution of this stock solution.

Sampling

Samples were obtained from a mixing chamber containing diluted diesel exhaust maintained at a constant level of approximately 8 mg/m^3 respirable particulate. Samples were taken with an eight-filter multisampler which has been previously described[5]. The flow rate of 2.0 Lpm through each of the filters was controlled by orifices connected to a single pump capable of maintaining critical flow. The orifices were calibrated before each day's operation. The filters used were T60A20 Teflon-coated glass fiber filters (Pallflex, Putnam, Conn.), 37 mm diameter, contained in a standard plastic cassette with a cellulose ring to insure a seal around the edges of the filter. The cassettes were sealed with a shrinkable cellulose band.

Sampling was done for 90 minutes. The replicate sample sets for use with the different solvents were collected consecutively on the same day. Those for the extraction technique study were collected on different days.

The collected samples were protected from light and stored at -10 °C. The Teflon-coated glass fiber filter was placed in a capped glass vial which was stored in a metal can.

Desorption efficiencies using acetonitrile were previously tested for the filters by spiking known amounts of the standard solutions of the compounds onto the filters. The following efficiencies were obtained: fluoranthene, (90.8 ± 4.9)%; pyrene, (86.9 ± 3.8)%; benzo(a)anthracene, (92.1 ± 4.1)%; benzo(k)fluoranthene, (95.2 ± 2.4)%; and benzo(a)pyrene, (89.7 ± 4.8)%. The efficiencies were high and reproducible and no correction was made for desorption efficiency.

Extraction Procedures

Mechanical agitation consisted of placing the filter in a 10-mm vial with a screw cap, adding 4 mL of solvent, and using a disposable pipette to break up the filter and suspend the particulate as thoroughly as possible. Ultrasonic agitation was performed similarly, except for an additional step of placing the vial in an ultrasonic bath (Mettler Electronics, Anaheim, Ca.) for 15 minutes after mechanical disruption. Refluxing agitation was performed by placing the filter in a round bottom 25-mL flask, adding 10 mL of solvent, attaching a condenser to the ground glass neck of the flask, and refluxing for 2 hours. Soxhlet extraction was performed in the same way except the filter was held between the flask and condenser in a Bantam-ware soxhlet extractor with a 10-mm X 50-mm glass thimble having a coarse fritted glass bottom (Kontes, Vineland, N. J.). Soxhlet extraction was performed for 2 hours with a cycle time of approximately 2.5 min.

After agitation, the samples were stirred and pipetted or poured into the body of a 10-mL glass syringe with a 13 mm filter holder and a 0.2-micrometer nominal pore size Fluoropore filter (Millipore). The syringe plunger was used to drive the solvent through the filter into a glass autosampler vial. The vial was capped with a Teflon-coated septum and placed in the autosampler where it was protected from light.

Toluene and acetonitrile are immiscible with the acetonitrile/water system used with the HPLC and were removed before filtration. These extracts were taken to near dryness in a vial in a 40°C water bath by a stream of nitrogen gas. The solvent was replaced with an equal volume of acetonitrile. A set of acetonitrile extracts was also treated in this way as a test of the replacement procedure.

Analysis

Analysis was performed on a series 5000 high pressure liquid chromatograph (Varian, Palo Alto, Ca.) using a 25-cm HC-ODS column (Perkin-Elmer, Norwalk, Conn.) held at 10 °C with a water jacket and a constant temperature circulating bath (Forma Scientific, Marietta, Oh.). The PAH's were detected as they eluted by a Perkin-Elmer MPF-44B fluorometer with a microflow cell; excitation was at 295 nm and emission was at 415 nm. The slit width in the analyses was set for a band width of 16 nm. A volume of 20 microliters was injected by the Varian autosampler containing the sample vials. Vials containing standards were interspersed among the samples so that no sample was more than one vial from a standard. This permitted positive selection and quantitation of the proper peaks in the diesel exhaust extract chromatograms.

The solvent program for the filter samples started at 30% acetonitrile-70% water. This was increased to 100% acetonitrile in 45 minutes, held at 100% acetonitrile for 45 to 50 minutes, and returned to the original conditions in 5 minutes. After equilibration at 30% acetonitrile-70% water for 20 to 25 minutes, the next sample was injected. The equilibration before sample injection and the constant column temperature were essential for reproducible chromatography of the many peaks in the diesel exhaust extracts.

The output of the fluorometer was recorded on a Perkin-Elmer chart recorder. The fluorometer lamp was operated from a constant voltage supply (Sola Electronics, Elk Grove Village, Ill.) and the signal was passed through a multipole electronic filter (model 1021A, Spectrum Scientific, Newark, Del.; low-pass cut off at 0.02 Hz) to reduce the noise level of the signal.

Data Treatment

The height of the peaks in cm above a line drawn through the baseline on either side of the peak was manually measured for both the standards and the samples, and the concentration of the PAH's was calculated from the peak height. The peak heights for the standards were fit with a least squares line, and the concentrations of the samples were derived from the equation of the line. Due to the low levels encountered in the samples, the intercepts of the least squares lines were forced through zero[6]; this resulted in more consistent treatment of the values lying between the lowest standard and the detection limit.

The solution concentrations, in nanograms per milliliter (ng/mL), were then converted to air concentrations by multiplying by the solution volume (4 or 10 mL) and dividing by the air volume taken (the orifice flow rate as determined by bubble meter calibration times the sampling time; approximately 0.18 m^3) for each sample.

RESULTS AND DISCUSSION

Two sets of eight replicate samples were collected for analysis using six extraction solvents. Extraction using acetonitrile and ultrasonic agitation was done directly and also by solvent replacement to test possible loss of the PAH's during the solvent removal and addition steps. The solvent replacement results are shown in Table 1. The concentrations observed for all five compounds are the same (within 1 to 7.8%) indicating that there is no significant loss of material during the process of removing the first solvent and replacing it with the second solvent (both solvents were acetonitrile for this test). In the remaining solvent comparisons the acetonitrile results are pooled with no distinction made between the two treatments.

Five other solvents--methanol, 2-propanol, acetone, toluene, and methylene chloride--were compared with acetonitrile using ultrasonic agitation of the filters in the solvents. These data are presented in Table 2 as air concentrations (ng/m^3) for each compound and as ratios of the air concentrations using the solvent of interest to the values obtained with acetonitrile.

TABLE 1

Effect of Solvent Replacement on PAH Concentrations

Set	Solvent Replaced?	Observed Concentration, ng/m^3				
		Ft	Py	BaA	BkF	BaP
1	No	709	910	70.2	19.1	27.8
1	Yes	698	893	75.6	20.6	29.4
2	No	748	912	72.4	22.9	25.7
2	Yes	730	880	70.4	21.9	24.0

An effort was also made to use tetrahydrofuran as an extraction solvent, but standards prepared in that solvent and injected into the HPLC showed gross distortions of the peak shapes and poor resolution of peaks. No effort was made to use solvent replacement with the tetrahydrofuran.

Comparison of the concentration ratios for each compound for the different solvents shows little difference for the lighter PAH's fluoranthene and pyrene (no ratios which differ from one by more than one standard deviation). For benzo(a)anthracene the last two solvents, methylene chloride and toluene, appear to be 20 to 30 percent better than acetonitrile while acetone is 13% better. The alcohols are slightly inferior. This trend holds also for benzo(k)fluoranthene, where methylene chloride

TABLE 2

Effect of Extraction Solvent on Observed PAH Concentrations

Solvent[a]: PAH's	1	2	3	4	5	6
Ft	721±22[b]	718±6	718±76	675±25	835±200	743±100
	(1.00)[c]	1.00±.03	1.00±.11	0.94±.05	1.16±.28	1.03±.14
Py	899±15	927±124	925±41	1018±252	974±98	879±81
	(1.00)	1.03±.14	1.03±.18	1.13±.28	1.08±.11	0.98±.09
BaA	72±3	69±2	66±7	81±1	94±22	90±10
	(1.00)	0.95±.04	0.92±.11	1.13±.04	1.30±.31	1.24±.15
BkF	21±2	19±3	16±3	25±4	35±11	33±2
	(1.00)	0.92±.14	0.73±.14	1.18±.22	1.64±.51	1.56±.16
BaP	27±2	27±2	21±1	41±4	60±13	61±4
	(1.00)	1.02±.11	0.77±.08	1.53±.21	2.23±.52	2.28±.24

[a] Solvents: (1) acetonitrile, (2) methanol, (3) 2-propanol, (4) acetone, (5) methylene chloride, (6) toluene. Ultrasonic agitation was used with all solvents.

[b] Observed air concentration in ng/m^3.

[c] Ratio of air concentration to that for acetonitrile.

and toluene extract approximately 60% more material and acetone extracts 18% more. For benzo(a)pyrene, methylene chloride and toluene extract 2.2 to 2.3 times more material than acetonitrile and acetone extracts 50% more; again the alcohols are inferior. Thus methylene chloride and toluene appear to be the solvents of choice if the amount of the heavier PAH's is used as a criterion.

Other experiments were conducted to study the dependence of the amount extracted on the agitation technique. Two solvents were chosen: acetonitrile and toluene. Four extraction techniques were used: simple mechanical agitation, ultrasonic agitation, refluxing solvent, and soxhlet extraction. The results for both solvents and the five compounds analyzed are presented in Table 3 as the ratio of the air concentration obtained using mechanical, refluxing, or soxhlet extraction to that obtained using ultrasonic agitation with acetonitrile. From the data it can be seen that mechanical agitation and refluxing solvent are approximately equal in the amount extracted to ultrasonic agitation, but that soxhlet extraction is superior for all compounds except possibly pyrene. Toluene is still superior in the amount of the heavier PAH's extracted for each extraction technique.

In summary, of the solvents tested, either methylene chloride or toluene appear to be the solvent of choice for extraction of polynuclear aromatic hydrocarbons from filter samples. Of the extraction techniques studied, soxhlet extraction is pre-

TABLE 3

Effect of Extraction Technique on PAH Concentration Ratios

Technique[a]:		1	2	3	4
PAH's	Solvent				
Ft	acetonitrile	(1.00)	1.06±.04	1.06±.11	1.28±.07
	toluene	0.97±.06	0.99±.01	1.01±.02	1.30±.07
Py	acetonitrile	(1.00)	1.05±.06	0.93±.23	1.06±.21
	toluene	0.89±.02	0.90±.13	0.85±.19	1.06±.18
BaA	acetonitrile	(1.00)	1.14±.16	1.02±.11	1.45±.14
	toluene	1.28±.13	1.30±.17	1.31±.26	1.66±.20
BkF	acetonitrile	(1.00)	1.14±.25	1.27±.05	2.25±.29
	toluene	2.04±.03	2.07±.01	2.49±.09	3.03±.31
BaP	acetonitrile	(1.00)	1.26±.42	1.30±.01	2.45±.08
	toluene	2.54±.16	2.71±.29	2.90±.40	3.64±.22

[a] Techniques: (1) ultrasonic agitation, (2) simple mechanical agitation, (3) refluxing solvent, (4) soxhlet extraction. Observed air concentrations for each extraction technique with the two solvents is ratioed to that for acetonitrile with ultrasonic agitation.

ferred. Methylene chloride may have somewhat higher variability[7], although no reason for this is apparent. Benzene, although not studied here and presenting a greater health hazard to laboratory workers, may have solvent properties comparable to toluene for the PAH's and would be more easily removed during the necessary solvent replacement for HPLC analysis. Extraction of PAH's from samples other than diesel exhaust may have other optimum conditions than those found here.

ACKNOWLEDGMENTS

The author is grateful to Dr. Jerome P. Smith for many helpful discussions. Dr. Trent R. Lewis was project officer on the study maintaining the diesel engine supplying the mixing chamber. Mention of company names or products does not constitute endorsement by the National Institute for Occupational Safety and Health.

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Received March 19, 1984
Accepted March 30, 1984