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QUANTITATION OF NITRO- AND DINITROPOLYCYCLIC AROMATIC HYDROCARBONS IN DIESEL EXHAUST PARTICULATE MATTER

William M. Draper*
University of Minnesota
Environmental Health Division
School of Public Health
Minneapolis, MN 55455

ABSTRACT--A method for routine trace analysis of nitropolycyclic aromatic hydrocarbons (nitro-PAH) in diesel exhaust particulate matter is described. Particle extracts are prefractionated by silica high pressure liquid chromatography and the appropriate band analyzed by capillary electron capture gas chromatography. With on-column injection, three dinitropyrene isomers were recovered in the range of 69-85% for fortifications of 10 ug nitro-PAH/g of soot. The high signal-to-noise ratio suggested detection limits of about 2 ug/g for these analytes. Analysis of the Bureau of Standards SRM 1650 diesel particulate sample demonstrated the methods accuracy for 1-nitropyrene determinations as well.

INTRODUCTION

Nitropolycyclic aromatic hydrocarbons occur in diesel exhaust particulate matter, coal fly ash, and atmospheric particulate polycyclic organic matter. Many of the nitro-PAH are active in standardized, short-term tests for mutagens and carcinogens (1). Nitro-PAH also are carcinogenic in a number of mammalian species (2,3). Interestingly, nitropyrene, nitrofluoranthene (4) and various dinitropyrenes (5) are capable of inducing tumors at the injection site in experimental animals.

Since diesel-powered automobiles release 0.75 to 1.5 g of soot per kilometer, several orders of magnitude higher than catalyst-equipped gasoline-powered autos, they may represent a significant contributor to urban atmospheric mutagenicity (6). As a result there has been considerable interest in modification of diesel engine operation, and in development of emission control devices, which will reduce the output of these substances to the ambient air. This is particularly true for diesel-powered machinery operated in underground mines and other enclosed occupational settings.

* Current Address: California Public Health Foundation, P.O. Box 520, Berkeley, CA 94701

Because of the high potency of specific nitro-PAH in bacterial mutagenesis assays -- the dinitropyrenes are among the most potent mutagens ever tested in the Salmonella typhimurium reversion assay (2) -- it is reasonable to propose that chemical measurement of only a few structures could provide a rough index of the composite mutagenic activity of the particle extract. Between 25 and 50% of the direct-acting mutagenicity (i.e., that not requiring mammalian liver enzymes for activity) of diesel exhaust particulate matter has been attributed to mono- and dinitropyrenes (7,8). Furthermore, chemical analysis of selected nitro-PAH mutagens may exhibit less error than bioassays, be less costly, and provide additional data useful for health risk assessment.

This report describes the development and validation of a routine procedure for the analysis of two-, three- and four-ring fused nitro-PAH by capillary electron capture gas-liquid chromatography (EC-GLC). Heretofore, these analytes have been determined by thermionic nitrogen-phosphorus detector GLC (9,10) as well as various GLC-mass spectrometry techniques (11). EC-GLC methods also have been reported (12), but with low recoveries for some of the most potent diesel particulate mutagens. The present work demonstrates that routinely available laboratory instrumentation will provide accurate and precise trace measurement of these compounds in chemically-complex soot extracts.

EXPERIMENTAL SECTION

Chemicals. Nitro-PAH were obtained commercially from Sigma (St. Louis, MO) or Aldrich (Milwaukee, WI). PAH analytical reference standards were provided by the U.S. Environmental Protection Agency (Research Triangle Park, NC). Other reagents and chemicals were commercial reagent grade and were used as received.

Nitrofluoranthenes were prepared by nitration of fluoranthene (13). One g of fluoranthene was stirred in 6 mL of glacial acetic acid at 65-70°C and one mL of concentrated nitric acid-glacial acetic acid (1:1, v/v) was added dropwise over 1.5 h. The mixture was held at 70°C for an additional 1.5 h and filtered hot to collect the product. The crude product was washed with water and recrystallized (chloroform-methanol) to remove traces of starting material, but repeated recrystallization from methanolic solvents could not resolve the isomers. Silica high pressure liquid chromatography (HPLC) on a 4.6 mm ID X 25 cm column packed with 5 um Supelcosil LC-Si (Supelco, Bellefonte, PA) (0.8 mL isooctane/min total flow rate) resolved the isomers:proton NMR spectra of the major 3- (retention volume 5.16 mL) and minor 8- (5.44 mL) nitrofluoranthene products were consistent with those in the literature (14,10). 3-Nitrofluoranthene: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.67 (d, J=8.5 Hz, 1 H), δ 7.9-8.0 (complex, 4 H), δ 7.78 (dd, J=8.7 and 7.0 Hz, 1 H), δ 7.38-7.48 (multiplets, 2 H); ms (70eV electron impact, solid probe)(rel intensity) 247 (100, M^+), 217 (34, $[M-NO]^+$), 201 (79, $[M-NO_2]^+$). 8-Nitrofluoranthene = ^{1}H-NMR ($CDCl_3$, 300 MH_z), δ 8.78 (d, J=2.0 Hz), δ 8.33 (dd, J=8.4 and 2.0 Hz), δ 8.13-7.96 (complex), δ 7.81-7.72 (complex); ms (70eV electron impact, solid probe)(rel intensity) 247 (100), 217 (34), 201 (91). The proton-containing contaminant ($CHCl_3$) was used as the internal shift reference.

Diesel Particle Samples. Diesel exhaust particulate samples were provided by the U.S. Bureau of Mines Twin Cities Research Center (Minneapolis, MN) and were collected from a Caterpillar 3304

naturally aspirated heavy-duty diesel engine (84 hp @ 1,800 RPM) coupled to a dynamometer in a test cell. The engine studied is approved for underground mine use by the Mine Safety and Health Administration. For this study, particles were collected from a 6" exhaust gas dilution tunnel after 30:1 dilution with filtered pure air on 47 mm Pallflex Teflon-coated glass fiber filters. The National Bureau of Standards standard reference diesel particulate (SRM 1650) was used for method development and a recovery study.

Particle Extraction. Diesel exhaust particulate (10 mg) or filters were transferred to 25 X 80 mm cellulose extraction thimbles and retained with a small wad of glass wool. The thimble would accomodate approximately 40 47 mm filters or up to ten 8 X 10 filters, but the Soxhlet thimble could be omitted when a larger number required extraction. The sample was extracted in the dark with 250 mL of dichloromethane (DCM) for not less than 16 hours with glass beads used in place of boiling stones. Throughout the procedure samples (and standards) were protected from room light to avoid photodecomposition of sensitive analytes. The extract was cooled, dried by passage through anhydrous sodium sulfate held in a filter paper cone, and the receiver and drying agent rinsed with an additional 25 mL of solvent.

The extract was concentrated just-to-dryness on a rotary evaporator and quantitatively transferred with 2 X 5 mL of DCM to a Luer-lock syringe fitted with a prerinsed 0.2 micron fluoropolymer filter cartridge (Acro LC 13, Gelman Sciences, Ann Arbor, MI). The filtrate was reduced to dryness under a stream of nitrogen for measurement of soluble organic fraction (SOF) weight.

SOF Fractionation. For clean-up prior to quantitative analysis the particle extract was fractionated, first on a silica Sep-pak cartridge (Waters Associates, Milford, MA) and then by silica HPLC. The silica cartridge prefractionation was intended to protect the HPLC column from polars capable of chemisorption.

The silica cartridge was first rinsed with 5 mL of DCM and the particle extract transferred to the column in 5 mL of DCM. The adsorbent was then then eluted with an additional 5 mL of DCM and the combined eluate reduced to dryness under a stream of dry nitrogen. For some studies the polar materials remaining were stripped from the cartridge with methanol.

HPLC fractionation of the SOF was carried out with an Isco (Lincoln, NE) computer-controlled solvent programmable system consisting of model 2300 pumps, a model 2301 gradient controller, an Isco V4 variable wavelength absorbance detector and a Valco C6W loop injector. A 7.8mm ID X 30 cm column packed with 10 um silica (uPorasil, Waters Associates, Milford, MA) and a 30-40 um pellicular silica guard column (Upchurch Scientific, Oak Harbor, WA) were used. About 2 mg of SOF was applied to the column or about one tenth of its reported diesel soot sample capacity (11). After injection of the sample from a 250 uL loop, the column was eluted with hexane-DCM (99:1, v/v) for 10 minutes. Between 10 and 20 minutes the solvent composition was increased linearly to 100% DCM and held for 20 minutes at which time another 5 min linear gradient returned the system to initial conditions. A total flow rate of 1.5 mL/min was routinely used thus requiring 46 min for the separation.

Prior to injecting samples the retention characteristics of the system were defined by chromatographing a mixture of naphthalene, 1-nitronaphthalene, anthraquinone and anthrone, each with a concentration of 100 ng/uL. The column effluent was monitored by absorbance at 254 nm, but the detector lamp was extinguished for fractionation of soot extracts. Nitro-PAH are collected in a single band first eluting 3 min prior to 1-nitronaphthalene (approx. 23 min) and terminating midway between anthraquinone and anthrone (approx. 35 min). The 17 mL nitro-PAH fraction was combined with 2 mL of toluene and reduced to approximately 1.5 mL under a stream of nitrogen.

Quantitation of Nitro-PAH. A Hewlett-Packard 5890A gas chromatograph (Avondale, PA) fitted with a ^{63}Ni EC detector and J & W Scientific capillary on-column injector (Rancho Cordova, CA) was used for quantitative analysis. The instrument operating conditions were as follows: detector temp, 325°C; detector purge gas, 60 mL/min argon-methane (95:5, v/v); column head pressure, 8.0 psi; carrier gas flow rate, 1.36 mL/min; detector purge flow rate, 11.8 mL/min. The GLC column was a 30 m X 0.32 mm ID fused silica capillary with a chemically-bonded 0.25 um SPB-5 silicone phase (equivalent to SE-54) (Supelco, Bellefonte, PA). The column originally had an efficiency of 2,560 effective plates/m and a 97% coating efficiency, but had been used extensively. Prior to this work the column was cleaned by rinsing with pentane and methylene chloride.

Two uL toluene samples from a total final volume of 2 mL (or 0.30 mL for optimum sensitivity) were injected onto the column. The oven temperature program required 52.3 min and consisted of the following: 120°C held for 2 min; increased to 160°C at 40°C/min; hold 1 min at 160°C; increase to 275°C at 3°C/min; hold 10 min at 275°C. Nitro-PAH were identified by retention time (t_R) or t_R relative to 1-nitropyrene and were quantitated by peak height relative to a 200 pg/uL mixed nitro-PAH external standard in toluene.

Recovery Determination and Method Validation. Ten mg samples of SRM 1650 were analyzed in triplicate. Prior to extraction 10 mg SRM 1650 soot samples were spiked by adding 1, 2.5 or 10 mL of an 0.10 ug/mL nitro-PAH mixture (DCM) to the extraction thimble. Total error, indexing both accuracy and precision, was determined by the method of McFarren (15).

RESULTS AND DISCUSSION

SOF Fractionation. Examination of the mobility of PAH and nitro-PAH on the preparative silica HPLC column revealed complete separation of these chemical classes (Table I.). Dichloromethane was selected as a carrier to ensure complete solubilization of the diesel particle extracts. Injection of 250 uL of this solvent onto the column when operating with a 99% hexane mobile phase distorted peaks for the low capacity factor PAH. PAH showed peak maxima at 8.8 to 10 min, but continued to elute until 25 min. In contrast, the nitro-PAH which are more polar eluted in compact bands. This preparative HPLC column has has been used previously for fractionation of diesel soot extracts using a more involved solvent program (16). The HPLC retention times remained very stable over a day-long period with t_R variations of 0.57 to 1.06% RSD (n=4). The high reproducibility in retention times and use of an ample retention time window ensured high recovery of the analytes. The HPLC fractionation can be carried out at higher flow rates (Table

II.) allowing collection of the nitro-PAH band in as little as 21 min. The column delivers optimum chromatographic efficiency at lower flow rates, however, and the backpressure occasionally increases to unacceptable levels at 5 mL/min.

Table I. HPLC Retention Times for PAH and Nitro-PAH Standards.[a]

Compound	Retention Time (min)[b]	
anthracene	7.8	(18.2)
phenanthrene	8.0	(22.6)
2-methylnaphthalene	8.2	(14.2)
acenaphthene	8.4	(15.8)
naphthalene	8.4	(15.8)
acenaphthylene	8.6	(21.0)
pyrene	8.8	(19.0)
fluoranthene	9.0	(20.4)
fluorene	9.0	(21.0)
9-nitroanthracene	24.4	
1-nitronaphthalene	25.6	
2-nitronaphthalene	26.0	
3-nitrofluoranthene	26.4	
1-nitropyrene	26.6	
8-nitrofluoranthene	26.8	
2-nitrofluorene	27.0	
1,3-dinitropyrene	28.2	
1,6-dinitropyrene	28.2	
1,8-dinitropyrene	29.0	
anthraquinone	31.6	
anthrone	36.2	
pyrene-4,5-dione	96.6	

[a] DCM was used as a carrier solvent and compounds were injected from a 250 uL loop. A total flow rate of 1.5 mL/min was used with the solvent program described in the text.

[b] The retention time in parentheses is that required for complete elution of the compound. Nitro-PAH and nonPAH retention markers eluted in sharp peaks.

Table II. Flow Rate Effect on Retention Standard Mobility.

Flow Rate (mL/min)	Retention Times (min) and Volumes (mL)[a]			
	Naphthalene	1-Nitronaphthalene	Anthraquinone	Anthrone
1.5	8.4 (12.6)	25.6 (38.4)	31.6 (47.4)	36.2 (54.3)
2.0	6.5 (13.0)	22.4 (44.8)	27.6 (55.2)	30.6 (61.2)
3.0	4.1 (12.3)	18.3 (54.9)	23.2 (69.6)	24.5 (73.5)
5.0	1.4 (7.0)	15.1 (75.5)	19.5 (97.5)	20.9 (105)

[a] Volumes are in parentheses

There is wide variation in the bulk composition of diesel particulate from 75% (w/w) unoxidized hydrocarbon to 65% highly oxygenated PAH in ambient air samples (16). The capacity of the HPLC column (20 mg SOF) could thus be approached for some samples. Accordingly, we limited the amount of SOF applied to the column to 2-5 mg, a level which provides abundant material for nitro-PAH analysis.

When standards were applied to the Sep-pak silica cartridge in DCM naphthalene was not retained, but quantitative elution of anthrone required an additional 5 mL of solvent. The procedure is effective in removing polar soot coextractives as evidenced by darkening of the packing and the removal of a large portion of the SOF mass. Pyrene-4,5-dione was not eluted from the cartridge under these conditions, suggesting that the majority of polar coextractives are retained -- pyrene-1,6-dione has been proposed as a marker for the boundary of the semipolar and polar fractions for particulate extracts.

Gas Chromatography. The available nitro-PAH exhibited a broad range of retention times (Table III). 8-Nitrofluoranthene eluted immediately before 1-nitropyrene on the SPB-5 bonded phase, but with extended use the column performance slowly deteriorated so that these analytes eventually converged. Separation of these compounds has been difficult with packed columns as well (11).

Table III. Gas-Liquid Chromatography Retention Times and Detector Response Factors for Nitro-PAH.

Compound	t_R (min)[a]	Relative t_R[b]	RPH-1-NP[c]	RPA-1-NP[d]
1-nitronaphthalene	9.9	0.27	1.33	1.01
2-nitronaphthalene	10.8	0.30	0.65	0.83
4-nitrobiphenyl	15.6	0.43	0.19	0.17
2-nitrofluorene	22.6	0.63	0.70	0.63
9-nitroanthracene	23.4	0.65	1.14	0.80
3-nitrofluoranthene	34.7	0.96	0.76	0.69
1-nitropyrene	36.1	1.00	1.00	1.00
1,3-dinitropyrene	45.7	1.27	0.67	1.07
1,6-dinitropyrene	47.4	1.31	0.52	0.76
1,8-dinitropyrene	48.7	1.35	0.40	0.54

[a] Retention time
[b] Retention time relative to 1-nitropyrene
[c] Peak height ratio, relative to 1-nitropyrene
[d] Peak area ratio, relative to 1-nitropyrene

On-column injection is preferred since vaporization injectors descriminate against higher boiling constituents of the SOF (11). The highest boiling analytes studied here, the dinitropyrenes, were completely resolved and had typical detector response factors. Slight variations in absolute retention time (greater than with splitless injection technique) were observed, but relative retention times were essentially constant.

Electron capture response factors ranged by a factor of six allowing lowest detection limits for the nitronaphthalenes and nitrated pyrenes in standard mixtures. The ^{63}Ni ECD was well within its linear dynamic range in spite of the relatively high signal attenuation (range 3, attenuation 4) demanded by the complex diesel soot matrix.

Method Validation. Procedural blanks exhibited little interference contributed by solvents, reagents and extraction thimbles, etc. (Table IV). A minor, but constant background signal

corresponding in retention time to 3-nitrofluoranthene was detected. This method interference, of course, is less significant when analyzing larger soot samples.

Repeated analysis of reference diesel particulate SRM 1650 produced acceptable measurements for 1-nitropyrene (Table IV). In this sample, however, an interfering cluster of unresolved peaks precluded low level measurements of 2-nitrofluorene and 9-nitroanthracene. In addition 1-nitronaphthalene measurements (not reported) were not reliable due to a highly variable interference. The potent nitro- and dinitropyrene mutagens, however, eluted in a very "clean" region of the chromatogram. It is possible that reducing the retention time window in the HPLC fractionation might remove interferences found in SRM 1650.

Table IV. Apparent Nitro-PAH Concentrations in Method Blank and Diesel Particulate SRM 1650.

	Apparent [Nitro-PAH], ug/g[a]		[Nitro-PAH], ug/g
Compound	Method Blank	SRM 1650	SRM 1650
2-nitronaphthalene	nd[b]	nd	
4-nitrobiphenyl	nd	nd	
2-nitrofluorene	nd	15 ± 1	0.27[c]
9-nitroanthracene	nd	17 ± 6	
3-nitrofluoranthene	6 ± 1	8 ± 3	
1-nitropyrene	nd	24 ± 3	19 ± 2[d]
1,3-dinitropyrene	nd	nd	
1,6-dinitropyrene	nd	nd	
1,8-dinitropyrene	nd	nd	

[a] Three replicates; ± one standard deviation
[b] Not detected
[c] Non-certified value, i.e., not based on the concordant results from two independent analytical methods
[d] Certified value, i.e., average value obtained from at least two independent methods. The listed uncertainty is two times the standard deviations for the average value

The through-the-method recovery study (Table V) demonstrated detection limits of below 10 ug/g for most of the nitro-PAH in the diesel soot matrix. At this fortification level recoveries ranged from 47 to 120% and acceptable total error was found for nitro- and dinitropyrene measurements. At higher fortification levels acceptable accuracy and precision are found for each analyte studied. Chromatograms for the SRM 1650 particle sample spiked at 10 ug/g exhibited favorable dinitropyrene signal-to-noise ratios (Figure 1.) indicating that much lower detection limits are achievable. The 69-85% recovery of the dinitropyrenes is considerably higher than has been achieved by other analytical methods (12), possibly due to the chromatographic injection technique.

Table V. Recovery of Nitro-PAH from Diesel Particulate[a]

Compound	10 ug/g Recovery, % Mean[b]	SD[c]	Total Error[d]	25 ug/g Recovery, % Mean[b]	SD[c]	Total Error[d]	100 ug/g Recovery, % Mean[b]	SD[c]	Total Error[d]
2-nitronaphthalene	69	21	73	92	18	44	78	11	44
4-nitrobiphenyl	nd	-	-	96	20	44	95	12	29
2-nitrofluorene	47	23	99	100	7	14	153	15	83
9-nitroanthracene	70	30	90	92	13	34	104	7	18
3-nitrofluoranthene	78	34	90	107[e]	14	35	115	17	49
1-nitropyrene	120	10	40	112	14	40	121	9	49
1,3-dinitropyrene	74	12	50	76	4	32	105	14	33
1,6-dinitropyrene	69	9	49	76	5	34	110	17	44
1,8-dinitropyrene	85	15	45	68	4	40	109	14	37

[a] Apparent residues in diesel particulate SRM 1650 (Table I.) are subtracted
[b] Three replicates
[c] Standard deviation
[d] Total error (15): excellent < 25; acceptable between 25 and 50
[e] Two replicates

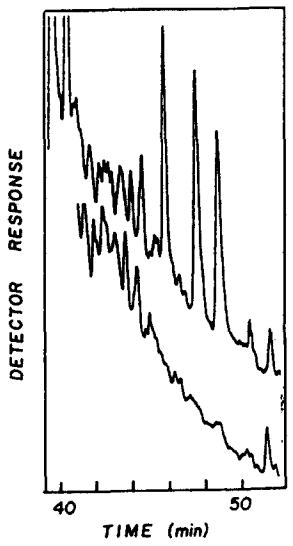

Figure 1. Chromatograms of the standard diesel exhaust particulate SRM (lower trace) and SRM 1650 fortified with 10 ug/g of 1,3-, 1,6- and 1,8-dinitropyrene (upper trace) Each sample depicted is equivalent to 66 ug of diesel particulate or 10 mg of soot in a final sample volume of 0.30 mL.

Characteristics of Diesel Particulate from a Diesel Test Cell. In order to further evaluate the utility of the present procedure additional diesel particle samples were analyzed. Unlike free particulate samples, extracts of samples collected on Teflon-impregnated glass fiber filters did not appear to require filtration.

Diesel particles collected by dilution tube sampling for this study produced considerably "cleaner" chromatograms than SRM 1650 (Figure 2.) and did not show the cluster of unresolved

peaks in the vicinity of 2-nitrofluorene and 9-nitroanthracene. Fewer coextractives also were detected in the region between 9-nitroanthracene and 1-nitropyrene. Differences in the appearance of chromatograms may be due to the method of sampling. SRM 1650 was collected from the heat exchangers of a dilution tube facility after 200 h of particle accumulation, conditions resulting in extensive artifact formation. Filter-collected particles from a dilution tube system, however, are representative of vehicular tailpipe emissions (17). Moreover, Teflon coating of the glass fiber filters, high exhaust gas dilution ratio and short sampling times minimize artifact formation.

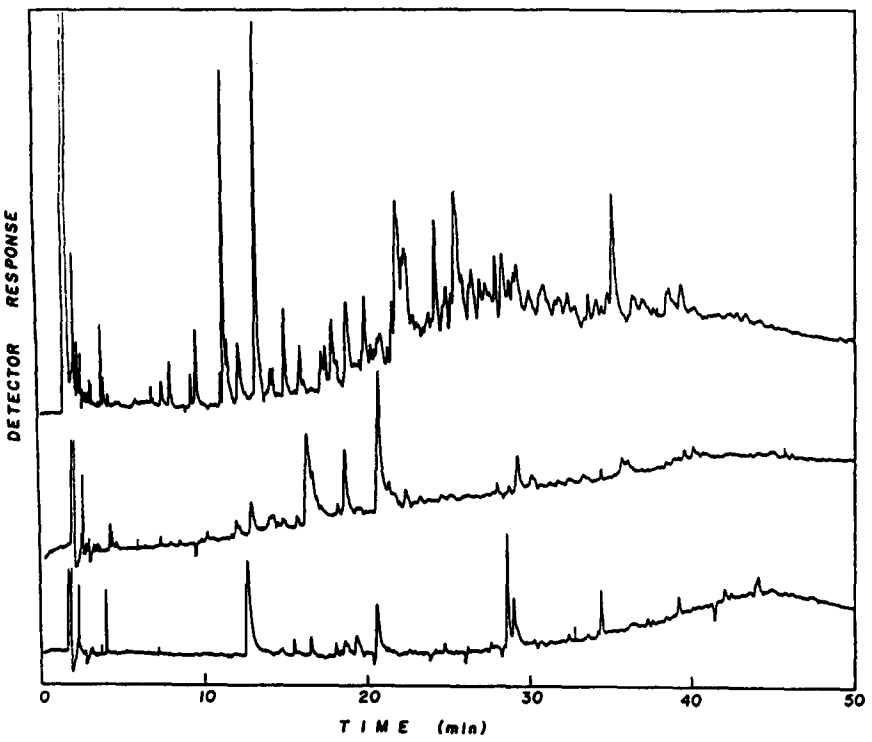

Figure 2. Chromatograms of diesel exhaust particulate from high load/moderate speed (lower trace) and moderate load/high speed (middle trace) conditions and SRM 1650 (upper trace), each equivalent to 5 ug of particulate matter.

The chemical composition of the diesel particulate from the heavy-duty mining engine was highly sensitive to operating conditions. Under 100% load the soot collected was less than 3% SOF (w/w)(Table VI.). Under reduced load and higher speed the exhaust particulate was 29% SOF (w/w) and contained higher levels of 1-nitropyrene and other nitro-PAH mutagens. The SOF which was extracted from the moderate load particulate contained a much smaller proportion of polar materials. These preliminary findings are consistent with previous reports which show that the output of mutagens is up to ten times greater at low engine loads and speeds in medium- and heavy-duty vehicles (18).

Table VI. Chemical Composition of Diesel Particulate from a Commercial, Heavy-Duty Mining Engine[a]

Parameter	High Load, Moderate Speed[b]	Low Load, High Speed[c]
particle mass (mg)	88.6	53.3
SOF mass (mg)	2.5	15.3
DCM cartridge eluate (mg)	1.2	13.6
Analyte Concentrations (ug/g)		
1-nitronaphthalene	0.77	0.47
2-nitronaphthalene	0.94	0.87
4-nitrobiphenyl	nd (<0.68)[d]	nd (<3.2)
2-nitrofluorene	0.63	8.8
9-nitronaphthalene	0.34	1.4
3-nitrofluoranthene	nd (<0.16)	nd (<0.74)
1-nitropyrene	nd (<0.12)	5.0
1,3-dinitropyrene	0.52	1.6
1,6-dinitropyrene	nd (<0.23)	nd (<1.1)
1,8-dinitropyrene	nd (<0.29)	nd (<1.3)

[a] Filter samples: dilution ratio of filtered pure air:raw exhaust was 30:1; fuel, Phillips 66 Control Fuel D-2; crankcase lubricant, SAE 30. Particles were collected on 47 mm filters with a sampling interval of <10 min.
[b] 100% load and 1,200 RPM
[c] 75% load and 1,800 RPM
[d] Not detected. Concentrations in parentheses are estimated detection limits

CONCLUSION

Mono- and dinitro-PAH are readily determined at trace levels in diesel particulate samples by capillary GLC with electron capture detector. The present method requires an on-column injection technique and prefractionation of the particle extracts by silica cartridge and preparative silica HPLC. Detection limits of 10- and 25 ug/g were demonstrated for selected nitro-PAH analytes. Detection limits of about 2 ug/g seem achievable for the dinitropyrene isomers even in SRM 1650, an extraordinarily complex soot sample. The procedure has a number of advantages including: 1) the requirement for only 10 mg of particulate; 2) attainment of precise and accurate measurements for the potent mono- and dinitropyrene mutagens; and 3) limited instrument requirements.

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