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Gas chromatographic–mass spectrometric determination of nitro polycyclic aromatic hydrocarbons in airborne particulate matter from workplace atmospheres contaminated with diesel exhaust

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Abstract

A method is described for the determination of 1-nitropyrene and 2-nitrofluorene in low volume ambient air samples (1–150 m^3). This method is based on acetone extraction by sonication followed by solid-phase fractionation on silica gel, nitroreduction, derivatization with heptafluorobutyric anhydride and analysis with gas chromatography–mass spectrometry. On-line zinc reduction of nitroarenes was compared with a newly developed reduction by sodium hydrosulfide hydrate. This method was validated by the analysis of air samples collected from the atmosphere of workplaces associated with the use of diesel engines, samples from chassis dynamometer studies, and standard reference materials (1587 and 1650).

1. Introduction

Exposure to diesel exhaust may represent a health hazard. More specifically, chronic exposure to relatively low levels of diesel exhaust may be a risk factor in the development of lung cancer [1–3]. Because of the presumed health hazards methods for accurate exposure assessment are urgently needed [2]. Nitro polycyclic aromatic hydrocarbons (nitro-PAHs) could be useful as tracers of airborne diesel exhaust particulate matter and markers of toxicity because of their characteristic appearance in diesel exhaust [4]. 1-Nitropyrene (1-NP) is one of the most abundant nitro-PAHs that have been identified in diesel exhaust [5–22]. Apart from its presence in diesel exhaust, 1-NP was also reported to occur in other combustion processes such as coal and wood combustion, in photocopier toner and during aluminium manufacturing [6,23–27]. However, the levels in these combustion emissions (expressed as μg 1-NP/g particles) are usually much lower than diesel engine emissions [6]. Lower levels of nitro-PAHs have also been observed in the exhaust of gasoline fueled engines [6,28]. In addition, 1-NP has been reported to be non-detectable in cigarette smoke, bitumen fumes and coke oven emissions [29,30]. Relatively high levels of 1-NP were observed in grilled chicken [31]. However, in grilled and smoked sausage and fish that contained considerable amounts of PAHs, 1-NP

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was not detected [32]. Its isomers 2- and 4- but not 1-NP were reported as products of atmospheric transformations [33]. 1-NP appearing in the ambient atmosphere is believed to be primarily originating from combustion (e.g. diesel exhaust emission) sources [33,34].

2-Nitrofluorene (2-NF) is present in the particulate phase of diesel exhaust emissions as well as in the gas phase [4,12]. Besides in diesel exhaust [12,13,17,20,22,28,35–38], 2-NF has been reported to be present in ambient air samples [39,40] and in river sediments [41] and it was tentatively identified in grilled sausage and chicken [31,32].

From a toxicological perspective there are arguments that would support the use of 1-NP as a marker for the genotoxic properties of diesel exhaust: 1-NP is the most abundant representative of a group of strong direct acting mutagens in these emissions. It has been suggested to use 1-NP as an indicator of the overall direct acting mutagenicity of diesel exhaust derived particulate extracts [42,73]. 1-NP is associated with the particulate phase of diesel exhaust and would therefore also be associated with constituents that could play a role in its carcinogenic potential since these constituents are known to be located in the particulate phase of diesel exhaust [3].

The availability of reliable and sensitive techniques of determination of 1-NP in low volume air samples is a basic requirement for the use of this compound as a tracer of diesel exhaust exposure and/or marker constituent for its genotoxicity. We have developed a method consisting of a simple sample work-up and reliable gas chromatographic–mass spectrometric (GC–MS) determination that can be applied for routine analysis. This method was developed and validated for workplace air samples. Analysis of nitro-PAHs has been reported using reductive electrochemical detection [8,17,43,44], flame-ionization detection [16,20,22,38,45], electron capture detection [10,38,46,47], nitrogen selective detection [13,38,48], thermal energy analysis [10,15,20], electron ionization mass spectrometry [5,16,21,22,26,32,45], positive and negative ion chemical ionization mass spectrometry [16,19,21], negative ion atmospheric pressure ionization mass spectrometry [49–52], methane enhanced negative ion mass spectrometry [53], high resolution mass spectrometry [35], MS–MS [12,36,54], and Fourier transform infrared spectrometry [55]. High-performance liquid chromatography (HPLC) has been used with UV [5,56,57], chemiluminescence [15,16,57], and fluorescence detection [5,13,28,40,42–44,55,57].

1-NP was detected with most of these techniques, while 2-NF was detected only with fluorescence detection [5,13,39,40,43,58], flame ionisation detection [20,31,35,38], electron capture detection [17,41], chemiluminescence detection [15], and mass spectrometry [12,22,32,35,36].

The analysis of nitro-PAHs using gas chromatography may be troublesome because 1-NP may decompose during the GC analysis [59]. Nitroreduction and derivatization have been introduced to improve chromatographic qualities of the analyte. This pretreatment provides excellent chromatographic behaviour, higher detection sensitivity [19,38,60] and selectivity, such as demonstrated in the sensitive detection of arylamines in biological samples [61–63]. Nitroreduction is usually conducted after fractionation of the sample [42–44]. Metals used for on-line reduction of 1-NP include zinc, cadmium and tin [28,43,44,64]. For batchwise reduction, $NaBH_4$ and KBH_4 catalyzed with copper(II)chloride have been reported [38,40].

The objective of this study was to develop an analytical method for simple routine analysis of nitro-PAHs in low-volume ambient air samples. We have focused on the determination of 1-NP and 2-NF in samples that were collected in work environments contaminated with diesel exhaust.

2. Materials and methods

2.1. Chemicals

2-Aminofluorene (2-AF, 98%), 1-aminopyrene (1-AP, 99%) and 1-NP (97%) were supplied by Aldrich Europe (Bornem, Belgium). 2-Nitropyrene (≥98%), 4-nitropyrene (≥99%), 2-aminofluoranthene (99%), and 3-aminofluoranthene (98%) were obtained from

Chemsyn (Lenexa, MO, USA). 1-Nitro-[$^2H_9$]pyrene (1-N[$^2H_9$]P, >99%) and 2-fluoro-7-nitrofluorene (2-F-7-NF, >99.8%), used as internal standards throughout the analysis, were obtained from Sigma (St. Louis, MO, USA) and Chemsyn, respectively. Heptafluorobutyric anhydride (HFBA), 2-nitrofluorene (2-NF, 98%) and granular zinc (mesh size ca. 590 μm) were supplied by Janssen Chimica (Geel, Belgium). Glass beads (160–250 μm) were obtained from Tamson (Zoetermeer, Netherlands). Sodium hydrosulfide hydrate (NaSH, 27% water) was obtained from Aldrich (Steinheim, Germany). Sep-Pak Si cartridges were supplied by Millipore (Bedford, MA, USA). Sodium hydroxide pellets and ethanol (abs) were obtained from Merck (Darmstadt, Germany). 2-Amino-7-fluorofluorene (2-A-7-FF) was obtained by reduction of 2-F-7-NF with Raney Nickel according to Litvinenko and Grekov [65]. All organic solvents used were HPLC-grade (Lab-Scan, Dublin, Ireland). Demineralized (demi) water (tap water treated in a Milli RO system, Millipore) and aqua pure (demi water treated in a Nanopure system, Barnstead, Boston, MA, USA) were used. Other chemicals used were of the highest purity available.

2.2. Air and diesel exhaust samples

Total suspended particulate matter (TSPM) was sampled using a high-volume sampler (type Gromoz, Agricultural University, Wageningen, Netherlands) equipped with an open face sampler head with an entrance opening diameter of 140 mm. Air was sampled at a flow rate of ca. 1.0 m^3/min. Respirable suspended particulate matter (RSPM) was collected using a cyclone with a 50% cut-off diameter of ca. 5 μm at 0.050 m^3/min [66]. This fraction slightly overestimates the respirable fraction such as defined in the European Standard EN481. For RSPM sample collection a medium-volume sampler (consisting of an oil-free vacuum pump, a gas meter and a restriction valve) was used. The operation of high- and medium-volume samplers was kept under surveillance continuously. The flow rate was checked every 15 min and adjusted if necessary. Sampling times varied from 2–10 h per filter.

The inhalable suspended particulate matter (ISPM) was sampled in the breathing zone of railroad workers using small-size air sampling pumps equipped with IOM sampler heads (Institute of Occupational Medicine, Edinburgh, Scotland). Air was sampled at a flow of ca. 0.0020 m^3/min using an electronically flow-controlled personal air sampling pump. The air volumes of these samples ranged from 0.5 to 1 m^3.

Particulate matter was collected on PTFE coated polystyrene membrane filters (TE 38, Schleicher and Schüll, Dassel, Germany). The filters were placed in an exsiccator prior to weighing. This procedure was repeated after collecting the air samples. Gravimetric determinations were conducted using a Mettler AE 163 analytical balance (Mettler-Toledo, Zürich, Switzerland).

Diesel exhaust particulate samples were obtained from the TNO Road Vehicle Research Institute (Delft, Netherlands). Briefly, samples from heavy duty and light duty vehicles (HDV and LDV) were collected on two Pallflex TA60A20 filters (Pallflex, Putnam, CO, USA), placed in series, under constant pressure sampling conditions. In the case of LDV samples, the filters were combined with a back-up Amberlite XAD_2 adsorbent cartridge for collection of volatile PAHs. The filter and adsorbent extracts were combined prior to analysis.

2.3. Apparatus

For automated reduction of nitro-PAHs an HPLC system consisting of a Spectra Physics SP 8800 ternary HPLC pump and a Spectra-Physics SP 8875 autosampler equipped with a 50-μl loop were used. Final analysis of derivatized amino-PAHs was performed on a Varian 4300 GC equipped with a Varian 8100 autosampler and a Varian Saturn 1 IonTrap MS detector. In the interlaboratory comparison at the Agricultural Research Department of the State Institute of Quality Control of Agricultural Products (RIKILT-DLO, Wageningen, Netherlands), a VG Autospec EQ high-resolution (HR) mass

spectrometer (VG Instruments, Altrincham, England) equipped with an HP5890 gas chromatograph and an HP7673A autosampler (Hewlett-Packard, Palo Alto, CA, USA) were used.

2.4. Reference materials

Standard Reference Material (SRM) 1587 consisting of a methanolic solution of seven nitro-PAHs with certified concentrations for 1-NP and 2-NF (Table 1) produced by the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA), was kindly supplied by the TNO Institute of Environmental Sciences (Delft, Netherlands). The NIST SRM 1650, a diesel exhaust particulate matter with a certified 1-NP content, was supplied by CN Schmidt (Amsterdam, Netherlands). This particulate sample was collected from several diesel engines with direct fuel injection and would be representative for heavy duty engines. The extract of a particulate sample from a Yanmar diesel engine (single cylinder; 4 kW full load) fueled with CEC-RF03-A-84 reference fuel was used for reproducibility testing. The soot was collected on a Gelman glass fibre filter type A/E (Gelman, Ann Arbor, MI, USA) at 100 to 130°C at the beginning of collection (for more details see [67]).

2.5. Filter extraction

The filters loaded with TSPM were cut into pieces. The filter pieces were extracted in 25 ml of acetone by sonication for 15 min. The extract was pipetted into a large glass tube and reduced in volume under N_2 at 50°C. The filter was extracted twice more with 10 ml of acetone by sonication for 5 min. The three resulting extracts were combined, reduced in volume to about 5 ml under N_2 at 50°C and centrifuged for 5 min at 600 *g*. The solvent was collected by pipetting into a pre-weighed glass tube and reduced in volume under N_2 at 50°C. The remaining residue was washed with 5 ml of acetone and centrifuged for 5 min at 600 *g*. The acetone washing was added to the concentrated acetone extract and the solvent was subsequently removed under N_2 at 50°C. The amount of dry extract was determined gravimetrically. The dry extract was dissolved in acetone, sonicated and divided in four pre-weighed glass tubes. Each of the portions corresponded to a sampled air volume of ca. 25–150 m^3. The four portions were evaporated to dryness under N_2 and weighed. Two portions were used for nitro-PAH analysis. The third and fourth portions were stored at −20°C for future analysis. All the dry extracts were stored at −20°C in the dark, until further analysis. When analyzing RSPM, ISPM, and diesel exhaust samples derived from HDV, the whole filters were extracted thrice with 10 ml of acetone. The RSPM samples were divided into two equal portions in the same way as described above. One of these was analyzed. The other portion was kept at −20°C in the dark for future analysis.

Table 1
Certified concentrations of 1-NP and 2-NF in the standard reference materials used

Material	Concentration (μg/g)	
	1-NP	2-NF
SRM 1587	8.95 ± 0.28	9.67 ± 0.39
SRM 1650	19 ± 2	0.27[a]

[a] Not certified.

2.6. Fractionation

Extracts of air and diesel exhaust samples, as well as standard solutions in dichloromethane, were fractionated into non-polar, intermediately polar and polar fractions through solid-phase extraction with Sep-Pak Si cartridges on a vacuum manifold [8]. After activation of the cartridge with 5 ml of dichloromethane, 200 μl of sample or standard solution was loaded onto the cartridge. It was flushed with air until all of the solvent had passed through the cartridge. Non-polar compounds were washed from the cartridge with 3 ml of *n*-hexane and discarded. The intermediately polar fraction containing the com-

pounds of interest was eluted from the cartridge with 5 ml of dichloromethane. The solvent was removed under N_2 at 30°C until dryness.

2.7. On-line reduction (method A)

The fractions containing the nitro-PAHs were dissolved in 200 μl methanol and pipetted into glass inserts in crimp-cap vials. Nitro-compounds were reduced (overnight) to their amino analogues on a stainless steel 40 mm × 3 mm I.D. reduction column, filled with granular zinc and glass beads (1:1, w/w). The column was placed at 40°C in the column oven of an HPLC system. The mobile phase consisted of 90% methanol, 10% aqua pure with 15 m*M* glacial acetic acid and 5 m*M* sodium chloride at a flow of 1 ml/min. Aliquots of 50 μl of fractionated sample solutions in methanol were injected into the HPLC system. Fractions of 5 ml of mobile phase effluent containing the amino-PAH were collected using a fraction collector (Pharmacia, Uppsala, Sweden). The fraction collector was kept in the dark in a N_2 atmosphere to prevent light induced oxidation of amino-PAHs. To prevent the formation of H_2, after the reduction column a back pressure was created by installing a stainless steel 150 mm × 4.6 mm I.D. column containing a C_{18} stationary phase. This resulted in a considerable extension of the time needed to process each sample, but was preferred over a loss in reduction efficiency because of gas formation in the reduction column. Between the pump and the autosampler an oxygen scrubber, consisting of a stainless steel 150 mm × 4.6 mm I.D. column filled with zinc, was placed to prevent oxidation of the zinc and thus extending the lifetime of the reduction column. Amino-PAHs were extracted from the mobile phase effluent after reduction of the volume to ca. 0.5 ml under N_2 at 50°C by adding 2 ml of toluene and 0.5 ml of a 0.3 *M* sodium hydroxide solution to neutralize the acetic acid. After 5 min of centrifugation at 600 *g* the toluene layer was collected and dried under N_2 at 50°C.

The amino-PAHs were derivatized with HFBA to HFB-amino-PAHs by redissolving the residue of the aforementioned toluene layer in 100 μl of *n*-hexane. Next, 20 μl of HFBA were added, mixed and kept at 40°C for 30 min. The samples were cooled in cold tap water and 1 ml of *n*-hexane and 0.5 ml of aqua pure were added to remove the excess of HFBA. After centrifugation for 5 min at 600 *g*, the water layer was carefully removed. The remaining *n*-hexane was removed under N_2 at 40°C until dryness. The residue was redissolved in 50 μl of isooctane and placed in tapered inserts in screw-cap vials for analysis by GC–MS.

2.8. Reduction using NaSH (method B)

The nitro-PAHs were reduced by a simple one-tube reduction step adopted from Hisamatsu and co-workers [55]. Briefly, an aliquot of 0.5 ml of ethanol and 0.5 ml of a 10% aqueous NaSH solution were added to the dried fractionated samples and mixed. After 0.5 h incubation at 100°C, 250 μl of 1.2 *M* aqueous sodium hydroxide solution and 3 ml of *n*-hexane were added. After 3 min centrifugation at 1250 *g*, the *n*-hexane layer was collected and dried under N_2 at 40°C. The dried residue of the *n*-hexane layer was dissolved in 100 μl of *n*-hexane. An aliquot of 20 μl of HFBA was added and the sample was kept at 60°C for 30 min. After cooling in cold tap water, 3 ml of *n*-hexane were added to dilute the sample and 100 μl of aqua pure were added to neutralize the excess of HFBA. After 3 min centrifugation (1250 *g*) the water layer was carefully removed and the remaining *n*-hexane layer was dried under N_2 at 40°C. The residue was dissolved in 40 μl of isooctane and placed in a tapered insert in a screw cap vial for analysis by GC–MS.

2.9. Analysis by GC–MS

Aliquots of 1 μl of sample followed by 0.5 μl of solvent wash (separated by an air gap) were injected on-column in a septum equipped programmable injector (SPI) at 5 μl/s with a 0.1 min post-injection "hot needle" time. The column system consisted of a 2.5 m × 0.53 mm I.D. deactivated fused-silica retention gap (different brands) and a 30 m × 0.25 mm I.D. DB-5MS

coated ($d_f = 0.25$ μm) fused-silica capillary column (J and W Scientific, Folsom, CA, USA). The carrier gas used was helium at a column head pressure of 96 kPa. The SPI was programmed from a 1-min hold at 95°C to a final temperature of 280°C at 90°C/min, with a 10-min hold at 280°C. Separation was achieved programming the GC oven temperature from a 1-min hold at 95°C to a final temperature of 200°C at 20°C/min, followed by 5°C/min to a final temperature of 280°C with a 5-min hold at 280°C. The transfer line and ion source were kept at temperatures of 290°C and 235°C, respectively. The mass spectrometer was operated in the EI mode at an electron ionization energy of 70 eV. A mass range of m/z 150–450 was scanned at a rate of 1 scan/s between 10 and 11 min and between 15.5 and 17 min after injection when the compounds of interest and their internal standards eluted from the column. HFB derivatives of 2- and 4-aminopyrene and 2- and 3-aminofluoranthene have an identical mass spectrum as the HFB derivative of 1-AP. These compounds were analyzed in order to verify the identity of the peak with the same retention time as the derivatized 1-AP standard. The counter-expertise analysis parameters were essentially the same, except that the injection was splitless at 280°C and the oven temperature was kept at 95°C for 3 instead of 1 min. The column length was 60 instead of 30 m. The HRMS was operated in the EI + mode at an electron ionization energy of 35 eV. A mass range of m/z 377.1–396.1 was scanned between 22 and 27 min after injection and a mass range of m/z 413.1–423.1 was scanned between 29 and 36 min after injection at a rate of ca. 3 scans/s and a resolution of 6000.

2.10. Preparation of standards and calibration

Stock solutions and dilutions of the nitro compounds and internal standards where made in ethanol. In the case of the analysis of RSPM (method B) *n*-hexane was used for further dilution of the stock. The final concentrations were 0 to 100 pg/μl on column (method A) and 0 to 900 pg/μl on column (method B). Just before fractionation, to each dilution internal standard was added with a final concentration of 50 pg/μl on column (method A) and 120 and 350 pg/μl (method B) for RSPM and TSPM samples, respectively. Standards used for calibration and the SRM 1650 and 1587 were analyzed in duplicate. Ca. 3 mg of SRM 1650 was used. Of SRM 1587 a dilution was prepared corresponding to a concentration of 100 pg/μl on-column.

2.11. Determination of reduction recovery

The recovery of the on-line zinc reduction was evaluated by comparison of the plots of peak areas vs. concentration after injection of several dilutions of the nitro or amino analogues of the compounds of interest. Dilutions were prepared in such a way that final concentrations of 100 to 1000 pg on column were tested. The ratio of the slopes, acquired through linear regression analysis, of the nitro and amino analogues provided accurate determinations of the reduction recovery.

For method B ethanol solutions of the nitro and amino analogues and of 1-N[$^2H_9$]P and 7-F-2-NF of similar concentrations were prepared. Nitro or amino analogues were mixed with internal standard solutions for each one of final dilutions ranging from 60 to 540 pg on column. These samples were treated with NaSH as if they were samples.

2.12. Estimation of the limit of determination

The limit of determination was estimated by testing the intra-day repeatability of the analysis of a diesel exhaust particulate sample extract spiked with 1-NP and 2-NF in the range 1.25–25.0 ng/g particulate matter. This sample was collected from the inside of a front wheel cover of a diesel fueled LDV using a soft brush. It was used as a surrogate diesel exhaust sample, containing a mixture of soot particles from various mobile sources. In the sample 1-NP and 2-NF were not detected. Spiked extracts were fractionated, and reduced, in triplicate, prior to GC–MS analysis, in duplicate. The reduction was conducted using method B.

2.13. Calculations

The dry acetone extract weights were adjusted for the contribution of solvent and filter residues. The correction factor was determined by extraction of blank filters, evaporating the solvent under N_2 and calculating the dry extract weight per mg filter and per ml acetone. A calibration curve was constructed by plotting the ratio of the peak areas of 1-NP and the internal standard vs. the 1-NP amount in the solution. The 1-NP levels that were calculated from the calibration curve were corrected for the 97% purity of the 1-NP reference. The same was done for 2-NF. Results of duplicate analysis were calculated as arithmetic means. Differences between the results of the analysis of the series of TSPM samples were evaluated in a two-tailed paired Students' t-test.

3. Results

In Fig. 1 the mass spectra of reduced and derivatized 1-NP, 2-NF and the respective internal standards 1-N[$^2H_9$]P and 7-F-2-NF are presented. The quantitations in the samples were based on the molecular ions.

During the fractionation on Sep-Pak Si recoveries of 1-NP and 2-NF were excellent ($100.4 \pm 3.6\%$ and $95.2 \pm 1.0\%$, respectively). The recovery of the on-line zinc reduction (method A) amounted to ca. 102% for 1-NP and ca. 93% for 2-NF. In Table 2 the results of recovery

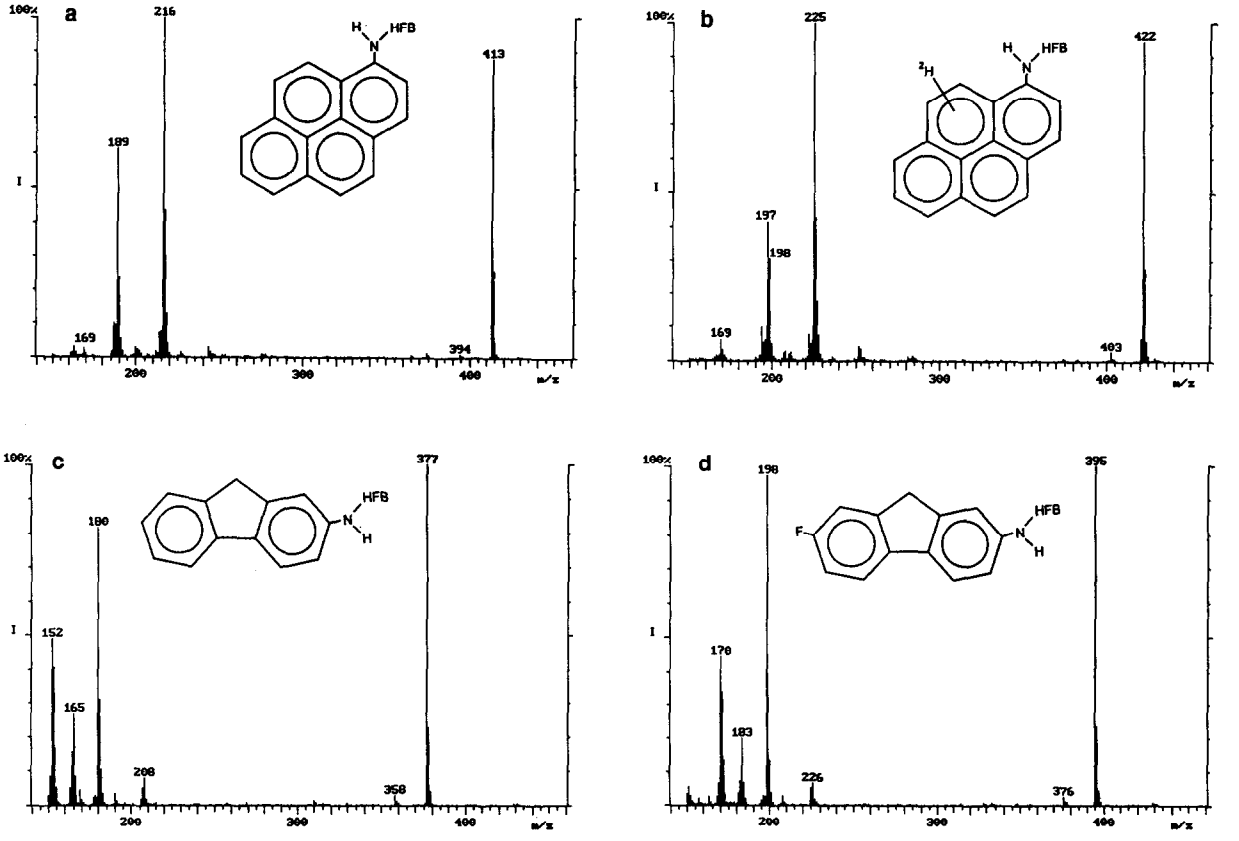

Fig. 1. Electron ionization mass spectrum of reduced and derivatized 1-NP (a), 1-N[$^2H_9$]P (b), 2-NF (c), and 7-F-2-NF (d). The molecular ions are m/z 413, 422, 377, and 395, respectively. The fragments $[M-197]^+$ (m/z 216, 225, 180 and 198, respectively) were formed through loss of COC_3F_7. The fragments $[M-224]^+$ (m/z 189, 198, 153, and 171, respectively) were formed through loss of $CNHCOC_3F_7$.

Table 2
Recovery of 1-NP and 2-NF after reduction with NaSH

Concentration of 1-NP and 2-NF (pg/μl on column)	Recovery (%)	
	1-NP	2-NF
60	108.3 ± 15.0	97.3 ± 3.9
120	114.8 ± 12.4	87.8 ± 8.5
270	104.0 ± 2.7	90.5 ± 4.2
540	106.5 ± 3.3	93.1 ± 1.0

Determinations in triplicate (mean ± S.D.).

tests of reductions using NaSH (method B) are presented. The mean recoveries for 1-NP and 2-NF were 108.4 ± 4.6 and 92.2 ± 4.0%, respectively. In Table 3 the extraction efficiencies from the aqueous solution, directly after the NaSH reduction, are presented for four different organic solvents. Hexane was selected to be used because of the excellent recovery of 1-NP. The recoveries for 2-NF were moderate and not much dependent on the choice of solvent.

Table 3
Extraction efficiencies directly after the NaSH reduction

Solvent	Extraction efficiency (%)	
	2-NF	1-NP[a]
Dichloromethane	70.6 ± 2.6	71.3 ± 11.8
Toluene	57.7 ± 5.0	67.0 ± 21.0
Hexane	64.4 ± 7.2	96.8 ± 8.4
Ethyl acetate	66.5 ± 4.3	81.0 ± 6.9

Determinations in triplicate (mean ± S.D.).
[a] 7F-2-NF was used as internal standard.

Table 5
Dilutions of 2-NF and 1-NP spiked into a diesel exhaust extract and analyzed in triplicate

Concentration on column (pg/μl)	R.S.D. (%)	
	2-NF	1-NP
10	11.1	9.7
20	6.9	4.7
40	1.1	3.8
80	1.9	4.1
120[a]	0.3	3.0
160	1.8	2.8
200	2.2	1.7

[a] Analyzed in duplicate.

The SRM 1587 was analyzed on four different occasions (see Table 4) showing good reproducibility and accuracy for both 1-NP and 2-NF when compared to the certified values. Two portions of SRM 1650 were analyzed in duplicate resulting in a 1-NP concentration of 18.0 ± 0.4 μg/g (mean ± S.D.). This was within 5.5% of the certified value.

Table 5 presents the results of repeatability tests for the analysis from diesel exhaust derived particulate extracts spiked with 1-NP and 2-NF. The relative standard deviations of determinations in triplicate remained well below 10%,

Table 4
Repeatability of the analyses of SRM 1587

Day	Concentration (μg/g)		Deviation from certified values (%)	
	1-NP	2-NF	1-NP	2-NF
1	8.98	9.13	0.3	−5.6
2	10.96	8.57	22.5	−11.4
3	7.58	9.58	−15.3	−0.9
4	8.23	8.68	−8.0	−10.2
Mean	8.94 ± 1.46	8.99 ± 0.46	−0.1	−7.0

Determinations in duplicate.

down to amounts of 20 pg 1-NP and 2-NF injected on column.

Fig. 2 shows the GC–MS total ion chromatogram and single-ion chromatograms of 1-NP and 1-N[$^2H_9$]P. The results of the analysis of ca. 3 mg SRM 1650 and of a workplace sample are presented.

The results of the duplicate analysis (methods A and B) and of the counter-expertise (B′) of 36 samples of TSPM derived from 12 different workplace atmospheres and a reference sample are presented in Table 6. In more than half of the samples analyzed, 1-NP was detected. Most of the samples with non-detectable 1-NP were outdoor samples. Only in three out of 22 indoor samples 1-NP could not be detected using method A. Using method B this number was 2. In a supplementary single analysis on the GC–HRMS

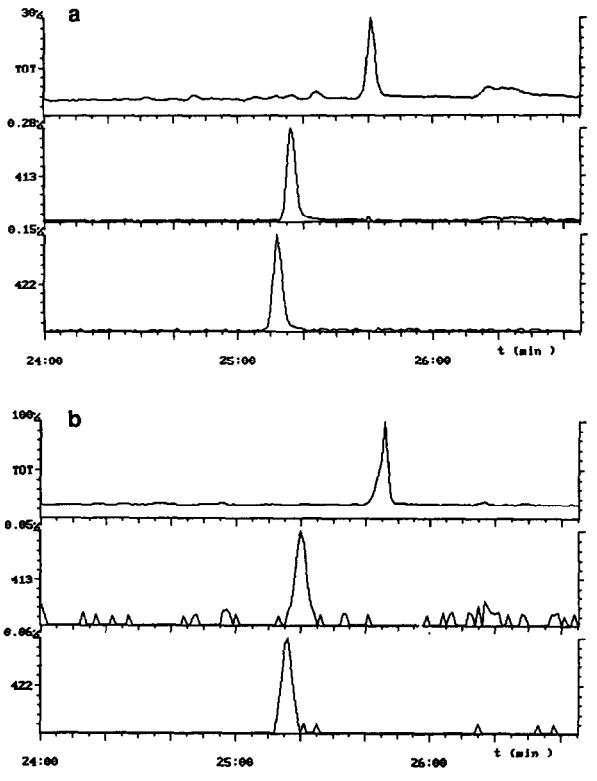

Fig. 2. GC–MS total ion chromatogram and single-ion chromatograms at m/z 413 and m/z 422 of 1-nitropyrene and 1-nitro[$^2H_9$]pyrene, respectively. Analysis of SRM 1650 (a) and a TSPM sample collected in a concrete manufacturing facility in the vicinity of an operating fork lift truck (b).

system 1-NP was detected in all of the indoor samples and all of the outdoor samples, except for the sample collected at the farm. The outdoor samples containing detectable 1-NP levels were collected on a river vessel close to exhaust pipes of the main ship's engine and power supply. In addition to these samples, 1-NP was detected using method B in outdoor atmospheres at a level of 36 pg/m^3 on a busy street crossing in a city centre. On the airport platform a relatively high level of 1-NP was detected using method A. The high level of 1-NP that was determined using method A was not reconfirmed in the analysis of this particular sample using methods B and B′. It may have been caused by contamination of the sample. Therefore, this observation was excluded from further (statistical) analysis. The two series of results obtained from the analysis of the TSPM samples were evaluated in a paired two-sided t-test. We did not observe a difference in 19 analyzed 1-NP levels using either method A or B. These results correlated significantly ($r = 0.85$, $p < 0.0001$). The interlaboratory comparison of the analysis of a series of 21 samples (B–B′) resulted in a correlation coefficient of 0.92 ($p < 0.0001$).

In Table 7 the results of the GC–HRMS analyses are expressed as the 1-NP content of the dust (in μg/g dust) and of the soluble organic fraction (SOF) (in μg/g SOF). The lowest levels of 1-NP were observed in outdoor samples. The SOF and TSPM of the samples collected in the city centre contained more than tenfold higher amounts of 1-NP than the sample collected in a forest area.

Extracts of RSPM derived from different workplace atmospheres were analyzed using method B. In Table 8 the results of these analysis are presented. In ten of the samples 1-NP was detected. Nine of these samples originated from indoor workplaces associated with the use of fork lift trucks. One of the samples was collected outdoors on a river vessel. We did not detect 2-NF in any of the RSPM samples.

We have identified 1-NP in two samples out of a series of ten low-volume ISPM samples (0.7–0.9 m^3) collected in the breathing zones of railroad workers during an 8 h working period.

Table 6
Air concentrations of 1-NP as determined from extracts of TSPM collected at workplaces associated with the use of diesel engines

Facility	Sources of diesel exhaust	Sample volume (m^3)	1-NP		
			A (ng/m^3)	B (ng/m^3)	B′ (ng/m^3)
River vessel	Ship's engine	385	0.093	ND[a]	0.031
		325	ND	0.11	0.10
		365	ND	ND	0.021
Repair shop for trains	Train engines	345	0.39	0.16	0.28
		155	0.44	0.42	0.34
		275	ND	0.24	0.26
		465	0.24	0.29	0.39
Army driving lessons	Armoured cars	345	ND	ND	0.012
		150	ND	ND	0.015
Flower auction	Trucks	340	0.11	0.086	0.080
		395	0.16	ND	0.082
Farming	Tractor	127	ND	ND	ND
Gardening	Passing traffic	420	ND	0.034	0.036
Airport platform	Platform vehicles[b]	225	0.45	ND	0.042
		260	ND	ND	0.045
		275	ND	ND	0.037
Concrete manufacturing	Fork lift trucks	135	0.45	0.64	0.61
		150	0.70	0.85	0.71
Chemical plant	Fork lift trucks	425	0.17	0.11	0.12
		400	0.38	0.25	0.56
		450	0.11	0.095	0.11
		405	0.25	0.39	0.29
Aluminium rolling	Fork lift trucks	200	0.78	1.6	1.2
		210	1.6	0.93	1.2
		265	1.2	0.77	0.98
		205	0.59	0.62	0.87
Galvanization workshop	Fork lift trucks	465	0.15	0.088	0.071
		360	0.19	0.14	0.15
		135	ND	0.12	0.14
		230	ND	ND	0.044
Grass verge maintenance	Lawn mowers	215	ND	ND	0.0066
River vessel	Ship's aggregate	605	0.33	0.45	0.79
Reference	Unknown	427	ND	ND	0.0017

(A) On-line zinc reduction based method; (B) SHH reduction based method with EI/ITD detection and (B′) HRGC–MS detection. Determinations A and B in duplicate.
[a] ND = Not detected.
[b] Lift platforms, power supplies, trucks, pull-offs.

The 1-NP levels were 1.84 and 2.90 ng/m^3. The 1-NP content of the dust was 2.74 and 0.87 $\mu g/g$ ISPM, respectively. In the other eight samples 1-NP was not detected.

Results of the analysis of particulate matter sampled directly from the tail pipe of vehicles tested on a chassis dynamometer are presented in Table 9. A modern technology heavy duty engine emitted the highest levels of 1-NP per km in an urban driving pattern. The light duty vehicle equipped with an oxydation catalyst emitted much lower levels of 1-NP per km in

Table 7
1-NP content in extracts of TSPM collected at workplaces associated with the use of diesel engines as determined after reduction with NaSH (mean ± S.D.)

Source	1-NP		N
	SOF (μg/g)	TSPM (μg/g)	
Ship's aggregate	11.9	7.6	1
Fork lift trucks (light duty)	6.8 ± 5.6	3.2 ± 2.8	12
Fork lift trucks (heavy duty)	6.1 ± 2.3	1.2 ± 1.4	2
Train engines	6.0 ± 2.6	1.3 ± 0.8	4
Trucks	2.2 ± 0.13	0.36 ± 0.035	2
Ship's engine	2.0 ± 0.94	0.97 ± 0.57	3
Platform vehicles	1.4 ± 0.45	0.60 ± 0.23	3
Passing traffic	1.2	0.49	1
Armoured cars	0.40 ± 0.025	0.11 ± 0.0014	2
Lawn mowers	0.33	0.099	1
Unknown (reference)	0.076	0.034	1

three different driving patterns. We did not investigate the influence of the catalyst on these emission levels.

4. Discussion

We have presented an accurate and simple method for the detection and quantification of 1-NP and 2-NF associated to airborne particulates derived from various sources, mainly diesel fuel combustion. We have demonstrated that the 1-NP content of fresh and aged diesel exhaust particulates can be determined from low-volume air samples.

The sonication technique that we have used yields quantitative extractions from SRM 1650. Supercritical fluid extraction did not provide significantly higher yields of 1-NP than extraction by sonication [22]. Acetone was selected as an

Table 8
1-NP content in extracts of RSPM collected at workplaces associated with the use of diesel engines as determined after reduction with NaSH

Facility	Sources of diesel exhaust	Sample volume (m^3)	1-NP	
			RSPM (μg/g)	(ng/m^3)
Repair shop for trains	Train engines	23.3	4.7	0.48
		1.4	10.6	3.9
		4.5	4.5	0.79
Concrete manufacturing	Fork lift trucks	21.8	1.9	0.63
		18.3	4.2	0.82
Chemical plant	Fork lift trucks	20.3	5.6	0.39
		20.0	7.0	0.31
Aluminium rolling	Fork lift trucks	21.3	7.7	1.0
		23.8	5.4	0.71
River vessel	Ship's aggregate	30.3	9.1	0.72

Table 9
Determinations of 1-NP in exhaust derived from trucks during simulated driving cycles

Vehicle	Driving pattern	1-NP	
		μg/g dust	μg/km
Heavy duty	Sub urban	1.94	2.13
	Sub urban	1.94	3.28
	Urban	3.39	6.48
	Motorway	1.94	0.87
	Motorway	2.73	1.37
Light duty[a]	European Driving Cycle (cold start)	–[b]	0.32
	European Driving Cycle (hot start)	–[b]	0.22
	US '75 Driving Cycle	–[b]	0.36

[a] With oxydation catalyst.
[b] Amount of sampled particulate matter not determined.

extraction solvent because it very efficiently extracts nitro-PAHs from diesel exhaust particulates [68]. Because of lack of accurate methods of spiking diesel exhaust soot samples the efficiency of the extraction procedure could not be determined.

The fractionation on silica provides sufficient clean-up for samples of aged diesel exhaust particulate matter including the SRM 1650. When analyzing particles sampled directly from the tailpipe on a chassis dynamometer further fractionation may be needed.

Zinc reduction may be used in an on-line reductive treatment of nitro-PAHs from environmental samples combined with HPLC and/or GC–MS analysis [64]. In practice, on-line reduction may be troublesome and unreliable because of variations in the quality of the reductive column and the complexity of the laboratory equipment. We have also tested some other materials such as tin chloride and copper borium hydride [38,69] for catalytic reduction of nitro-PAHs. These methods did not meet the requirements regarding the reduction efficiency or caused practical problems during separation of the amino-PAHs. We have selected the batch-wise NaSH reduction because of the excellent recoveries of 1-NP and 2-NF, its good reproducibility, simplicity, and low cost.

For determination of the repeatability of the newly developed 1-NP analysis (method B), the test sample was analyzed repeatedly. The inter-day coefficient of variation amounted to 7.7% ($n = 7$). For the series of TSPM samples the repeatability was 10.8% for method A and 8.4% for method B (based on a series of seventeen TSPM samples with detectable 1-NP levels for both methods). The reproducibility as calculated from the interlaboratory comparison of the analysis of a series of 21 TSPM samples (B–B′) amounted to 16.6%.

The results of the analysis of spiked diesel exhaust particulate samples demonstrated an excellent repeatability for both compounds, indicating a limit of determination down to 10 pg on-column. This corresponds to a level of 1-NP and 2-NF as low as ca. 1 ng/g particulate matter (with a relative standard deviation of ca. 10%).

The peak designated as 1-NP signal was not attributable to known nitropyrene isomers or nitrofluoranthenes. The HFB derivatives of the corresponding amino analogues were injected as standards. The retention order was 2-NF < 2-F-7-NF ≪ 3-nitrofluoranthene < 4-nitropyrene < 2-

nitrofluoranthene ≤ 1-N[$^2H_9$]P ≤ 1-NP < 2-nitropyrene.

On average particulate emissions from the light duty engines such as used in power supplies and in fork lift trucks contained higher levels of 1-NP than heavy duty engines with (mostly) direct fuel injection. This is consistent with the observation that indirect injected engines (LDVs) tend to have higher 1-NP emissions than direct injected engines (HDVs) [70]. The 1-NP content of the dust and of the SOF observed in this study (cf. Table 7) was lower than the levels that were observed in freshly emitted diesel exhaust particles, reported in the 1980's [6–11,17].

We detected 2-NF in a sample collected in the vicinity of a power supply of a river vessel. The 2-NF content was ca. 25 times smaller than the 1-NP content in the same sample. The sample may have contained a considerable amount of freshly emitted diesel exhaust particles, resulting in a higher level of more volatile compounds such as 2-NF.

The occurrence of 2-NF has been reported several times in fresh (diluted) samples collected on a filter directly from the exhaust of a diesel engine running on a chassis dynamometer. These samples have shown condensation of lower molecular weight hydrocarbons from the gas phase onto the particles [71], suggesting that the occurrence of 2-NF in these samples may be associated with the specific sampling conditions in a dilution tunnel [72]. Paschke and co-workers [22] have also identified 2-NF in particulate matter scraped directly from the exhaust pipe of a bus engine (sample collection procedure not specified). This material may have contained nitro-PAHs that were adsorbed onto particles deposited in the tail pipe or formed by conversion of deposited PAHs by passing nitrogen oxides. We did not find reports of mass spectral based evidence of the recovery of 2-NF from ambient particles. So far, the only reports have been based on fluorescence detection [39].

The analysis of 1-NP from dilution tunnel samples derived from HDV and LDV showed that the method needs to be improved further. In the series of HDV samples 1-NP was detected in five out of seven samples, whereas in the series of LDV samples only three out of sixteen samples showed detectable amounts of 1-NP. We estimate that improvements in sample work-up and/or detection may result in a higher score of samples with detectable 1-NP levels.

We have demonstrated the accurate identification and quantification of levels of 1-NP from low-volume air samples and diesel exhaust samples using a non-laborious work-up and a current GC–MS technique. The method shows good reproducibility and may be used for routine analysis of nitro-PAHs from workplace air samples containing diesel exhaust combustion products.

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