Selective and Sensitive Analysis of 1-Nitropyrene in Diesel Exhaust Particulate Extract by Multidimensional HPLC

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Key Words

Column liquid chromatography
Nitro-PAHs
Diesel particulate extract
Column switching

Summary

A multicolumn (MC) HPLC method for the determination of 1-nitropyrene (1-NP) at trace-levels via on-line reduction to 1-aminopyrene and fluorescence detection is presented. On the first column, packed with a pyrenebutyric acid amide stationary phase, the nitro-derivatives of PAHs are strongly retained and separated from other matrix components. The nitro-PAHs-containing fractions are transferred onto a RP_{18}-column via stepwise gradient elution and finally separated according to their various lipophilicities and sizes. To increase the overall selectivity and sensitivity of the multidimensional method (MD-HPLC system) post-column, on-line reduction of the nitro-PAHs to the respective amino-PAHs via a short catalysis column is performed thus enhancing the sensitivity significantly (to low pg levels). The applicability of this method for the determination of trace amounts of 1-NP in real samples (diesel particulate extracts) is demonstrated.

Introduction

Nitro-substituted polyaromatic hydrocarbons (nitro-PAHs) have been observed in ambient air, carbon black, toners, surface and ground waters and particulate exhaust emissions of diesel engines. In diesels they are formed during combustion by reaction of PAHs with oxides of nitrogen [1, 2]. As many nitro-PAHs are suspected to be mutagenic and/or carcinogenic, the determination of these compounds in environmental samples is of considerable interest [3–6]. 1-nitropyrene (1-NP), the major nitroarene observed in diesel exhaust particulate extracts, has been shown to cause mutations without enzymatic activation and is therefore a powerful direct acting mutagen. 1-NP is able to generate active species which bind to DNA; *in vivo* it is metabolized to compounds which are themselve mutagenic [6]. Nitroreduction in salmonella and Chinese hamster ovary cells in closely related to mutation to mutation induction by 1-NP and 1,8-dinitropyrene, which is up to 40-times more mutagenic than 1-NP in suspension cultures of *salmonella typhimurium* TA1538 [7]. Pitts et al have found 1-NP to be responsible for 20–27 % of the mutagenicity of a diesel exhaust particulate extract [8]. 1-NP has therefore become a benchmark compound for analytical studies.

Diesel exhaust particulate extract represents a very complex matrix which contains 1-NP and other nitroarenes only in trace amounts. Thus, the analysis involves several pretreatment and fractionation steps to isolate the PAHs, especially the nitro-PAHs. Fractionation is performed by, for example solvent-solvent extraction, adsorption chromatography on different adsorbents by thin layer and column chromatography, separation via gel chromatography or supercritical-fluid extraction [9]. In the development of the fractionating scheme, the recovery of mass as well as of mutagenicity should be considered because of the poor recovery mutagenic material by several adsorbents [10]. In the final analytical step high efficiency GC [11–16] or HPLC [17–21] methods for quantifying the PAHs and nitro PAHs are often used. Owing to the complexity of the samples, there is also a need for sensitive and selective detection of nitro-PAHs to exclude false signals from coeluting and interfering substances. Separation via GC is often coupled with MS, ECD, NPD, flame ionization, thermal energy analyzer, tandem MS, negative ion, atmospheric-pressure ionization MS and chemiluminescense detection [22–25]. For HPLC, the common detection principles are UV, reductive electrochemical, photoreduction/fluorescence, chemical reduction/fluorescence, chemiluminescence, differential pulse detection and negative ion, atmospheric-pressure ionization MS [24, 26–32]. Robbat [33] reported the evaluation of a nitrosyl-

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specific gasphase, chemiluminescent detector. Usually, determination of nitro-PAHs is carried out by capillary GC-MS. However, decomposition of the thermally unstable compounds occuring either at the GC-MS interface or in the injection system represents a source of errors which can be overcome by HPLC as an alternative high efficiency separation.

To enhance the number of separable peaks, multi-dimensional HPLC-methods have become quite popular, applications to environmental trace analysis are numerous. Previously, we reported on a two-dimensional column switching HPLC-system for determining 1-NP in diesel exhaust particulate extracts [36]. In continuation of this work [34–36, 27], we have developed an improved method for 1-NP analysis with higher selectivity and sensitivity by changing the detection principle. Although 1-NP is UV-active, it lacks inherent fluorescence. Unlike 1-NP, the reduced compound 1-aminopyrene is not only UV active but also fluorescent. Instead of the formerly used UV/VIS detection via a diode array detector, we employed on-line reduction of the 1-NP to the corresponding, fluorescent 1-aminopyrene. Using fluorescence detection, selectivity and sensitivity for 1-nitropyrene are increased substantially.

Experimental

Apparatus

The column-switching system consisted of two pumps: model 410; column and solvent (mobile phase) switching unit: model Tracer MCS 670; programmer: Model Anacomp 220 (all models Kontron, Switzerland); injector: Rheodyne 7125 (50 µm loop), fluoresence detector: RF 350 (Ex =354 nm, Em = 433 nm, Shimadzu); recorder: Servogor RE 511 (Goerz, Austria) and a water bath as column oven (80 °C).

Columns

Column 1: 250 × 4 mm ID stainless steel column packed with „pyrenebutyric acid amide phase" (PBA) on Lichrosorb Si 100, 7 µm, chemical bonding of the PBA phase see [36].

Column 2: 125 × 4 mm ID RP_8 5 µm (Seibersdorf).

Column 3: 30 × 4.6 mm ID packed with Rh-Pt catalyst on alumina 5 µm, material from S.B. Tejada; see S. B. Tejada [29].

Chemicals

1-NP was from Aldrich (Germany), water was bidistilled in a quartz apparatus (Leopold, Austria), methanol used for chromatography was HPLC grade (Lichrosolv, Merck, Germany), other solvents were of p.a. quality. 1-pyrene butyric acid was from Eastman-Kodak (USA), aminopropyltriethoxysilane from Dynamit Nobel (Germany).

Collection and Extraction of Diesel Exhaust Particulates

Tests wit diesel engine A were carried out with a vehicle of 1135 kg inertial weight on a chassis dynamometer. The vehicle was tested under US-test procedures [37] a) Federal test procedure (FTP) 75, b) FTP 72 and c) Highway fuel economy test (HWFET) as described in the Federal Register (1975) [38].

Tests with diesel engine B were performed on a stationary test stand, running modes 1 to 6 of an ECE R-49 13-mode-cycle (Mode 1 = low idle, mode 2 to 6 = intermediate speed and in mode 2 10 % load, in mode 3 25 % load, mode 4 50 % load, mode 5 75 % load and mode 6 100 % load) [39].

The diesel engine exhausts were diluted with filtered air in a dilution tunnel and the particulates collected on a Teflon-coated glass fiber filter at a maximum temperature of 52 °C. These loaded particulate filters were weighed, refluxed with toluene for at least 1 h, hot filtered and toluene distilled off to less than 2 ml. With these approximately 2 ml a dimethylsulfoxide – cyclohexane separation was performed as described by Grimmer and Boehnke [40]. The more polar compounds (PAHs and substituted PAHs) were thus concentrated in cyclohexane and separated from non-polar compounds (paraffins). The PAH-containing cyclohexane fraction was transferred to a silica gel column (20 mm I.D., filled with 15 g silica 100–200, 70–150 mesh). Less polar compounds were removed with 50 ml predistilled cyclohexane and the moderately polar compounds (PAHs) eluted with 70 ml cyclohexane – toluene (9 : 1) and, after that, the more polar fraction (including nitro-PAGs) eluted with 100 ml cyclohexane - diethyl ether (1 : 1), which was evaporated down to about 1.5 ml. These diesel soot extracts were finally transferred to standard GC vials and dried under nitrogen, followed by redissolution in different volumes of methanol (250–1200 µl). Several samples were not completely soluble in methanol, therefore all samples were centrifuged before injection. Extracts containing more than 20 ng 1-NP per injection were further diluted and re-analyzed.

Results and Discussion

It has been shown by Posch et al. [27] that coupling of a „pyrenebutyric acid amid phase" (PBA) with a common RP_{18} packing provides good results for analysis of nitro-PAHs in complex matrices. A medium polar fraction of organic solubles of diesel particulate extract cannot be adequately separated either on a PBA-phase, based predominantly on π-π-interaction phenomena or on a RP_{18} column based on lipophilicity and size factors. However, combination of these two HPLC columns (equal separation principles) via a column-switching set-up gives satisfactory results [36].

The immobilized π-base PBA and the nitro-derivatives of PAHs, which are π-acids, are able to form relativley

strong charge transfer complexes resulting in very high k' values for these compounds. Similar π-base phases, based on charge transfer complexation with nitro-aromatic solutes were discussed by Siggia and Mourey [41].

In the first step of column switching, the π-acid compounds of the organic extract are separated from weak π-acids and other substances on the PBA-column. In a second step, a zone cut of the effluent eluted from column 1, containing the nitro-arenes, is transferred onto the second column having a different chromatographic selectivity. To avoid intolerable peak-broadening due to the relatively large transfer volume from column 1 loaded (injected) onto the second column, a so-called "effluent mixing" technique is used. Working in the reversed phase mode the transfer effluent fraction from column 1 is diluted with plain water resulting in a strong reduction in the eluting strength of the mixed "injection solvent". Thus, a significant on-column focusing effect of analytes on the top of column 2 occurs although the total amount injected onto column 2 increases substantially. After this so called "on column concentration" effect the final analysis and separation of the transferred compounds is by a step gradient-elution on column 2.

In continuation of previous work, we made a few modifications to obtain a more selective and sensitive determination of nitroaromatics, in particular 1-NP. Firstly, we employed another PBA-phase, which contained a larger amount of bonded pyrenebutyric acid resulting in an increased retention of nitro-PAHs. Therefore, the group type separation of nitro-PAHs from other organic compounds in diesel particulate extract was enhanced by the forced charge-transfer complexation processes. Instead of the formerly used, step gradient-elution of analytes from the PBA-phase with MeOH/water mixtures of 65:35 – 80:20 – 90:10 and finally MeOH, we performed only isocratic elution using straight methanol for 22 min. After "effluent mixing" with water and eluent transfer the following separation of some nitro-PAHs was carried out on a RP_8 column (column 2).

Changing the final detection method for 1-NP represents the most important development compared to the previously used method. In the literature several different methods for on-line reduction of nitro-PAHs

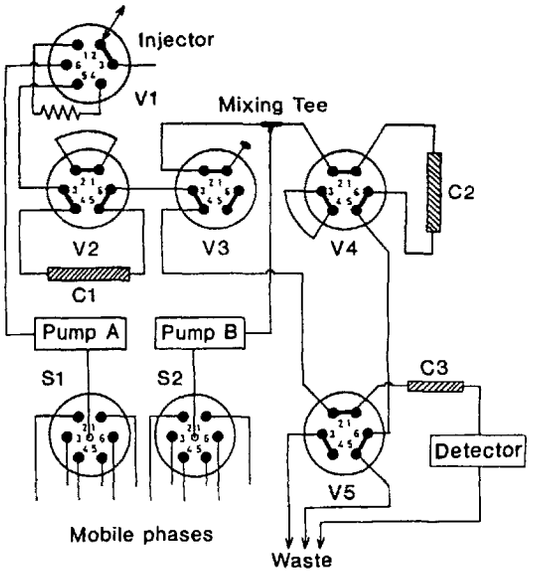

Figure 1

Multicolumn HPLC set-up. V1–V5: high-pressure six-port switching-valves; S1 and S2: low pressure selector valves; C1–C3: thermostated columns, see Figure 2; M1–M3: mobile phases, see Figure 2.

Time (min)	11	14	20
Column 1	M 1		
Column 2		M 2	M 2 + M 1	M 3
Pump A (ml/min)	1.5	0.75	1.5
Pump B (ml/min)	1.5	0.75	1.5

COLUMNS	MOBILE PHASES
C 1: PBA (250 x 4 mm I.D.)	M 1: MeOH
C 2: RP-18 (125 x 4 mm I.D.)	M 2: H_2O
C 3: Rh-Pt Catalyst (30 x 4.6 mm I.D.)	M 3: MeOH - H_20 = 80 - 20

Figure 2

Tim table for valve switching scheme corresponding to multicolumn HPLC.

are discussed. After photo and electrochemical reduction, chemical reduction via short columns containing different catalysts is often used for determination of nitroarenes or quinones. It has been shown by MacCrehan and May [30] that on-line reduction of nitro derivatives of PAHs occurs using a column packed with a mixture of zinc powder and silica and a mobile phase consisting of acetonitrile and water buffered with ammonium acetate. In contrast to the catalyst columns described by Tejada et al [29], the powdered zinc is consumed and therefore the reducing column has a finite lifetime. The latter columns are packed beds of noble-metal catalysts. After testing various substances, the column containing about 1 % Pt/Rh on 5 µm Spherisorb 5 AY alumina proved the best and catalyst is not consumed during reduction. Therefore, it has an almost indefinite lifetime. Usui [42] recently reported reducing quinones using a platinum-black catalyst with stable reduction activity using methanol, ethanol, 2-propanol and small amounts of water as mobile phase; Goetze and co-worker used a reduction column packed with 5 % Pt on alumina [43]. Shino described a high-performance chromatography system using a mobile phase saturated with H_2 followed by fluorescence detection after post-column reduction by a platinum oxide column for determining endogenous vitamin K in plasma [44]. Of various possibilities we chose Pt/Rh on alumina for on-line reduction of nitro-PAHs using an aqueous mobile phase. Varying excitation and emission frequencies varies the selectivity of the whole multi-

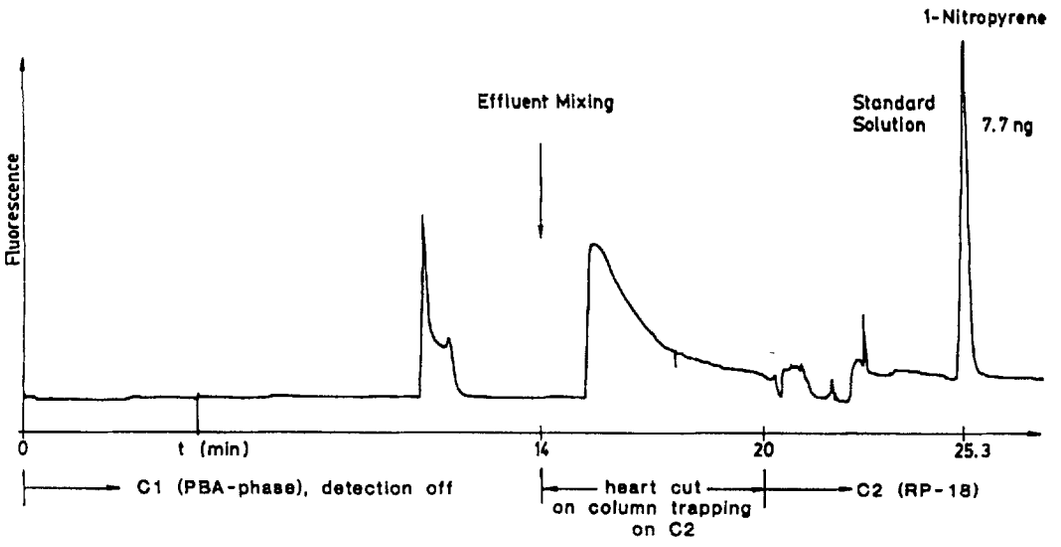

Figure 3

Multicolumn HPLC chromatogram of 1-nitropyrene standard solution. Conditions as Figure 2. Fluoresence detection Ex = 354 nm, Em = 433 nm.

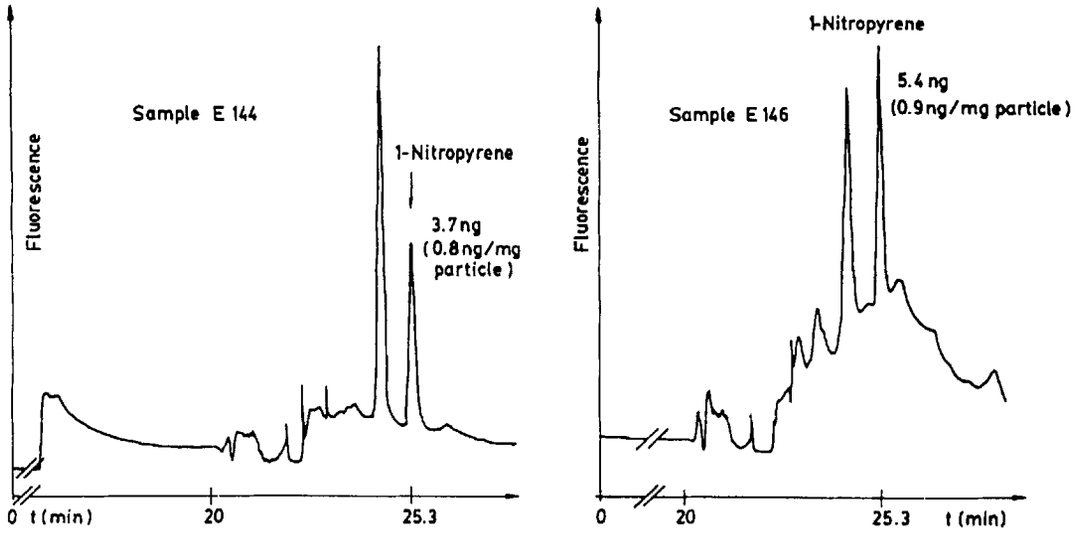

Figure 4

Multicolumn HPLC chromatogram of two representative diesel exhaust particulate extracts. Conditions as Figure 3.

dimensional HPLC-system. Several compounds have unique spectral properties that are useful in confirming identification. Therefore, misleading analytical results by interfering substances with other spectral features are unlikely. To remove dissolved oxygen from the mobile phase and to stabilise the activity of the catalyst we degassed the mobile phase continuously with helium and inserted another reducing column between pumps and injector.

The final valve-switching set-up used for determination of 1-NP is shown in Figure 1. The corresponding time diagram giving an overview of all variables, including mobile phase changes and step gradients during a total run, is shown in Figure 2.

Determination of 1-NP in Diesel Particulate Extracts

Working with real samples, it is recommended that a reliable time window of cutting and transferring the appropriate effluent fraction from column 1 to column 2 is chosen. Owing to possible effects caused by various matrices (type and total amount of compounds injected on column 1) the retention volume of the selected nitro-derivatives on the PBA-phase may show small differences (overloading effects). Furthermore, the capacity factors on both columns depend on the column temperature [45]; therefore, the columns should be thermostated to obtain accurate results (see Figure 1). A chromatogram of a 1-NP-containing, standard solution is shown in Figure 3; Figure 4 represents two authentic pre-separated diesel exhaust particulate extracts containing different amounts of 1-NP and background materials.

Using this system for 1-NP on a routine basis and for diesel engine evaluation and engineering as above (see Experimental and Table I) the data summarized in Tables II and III have been measured.

The results for engine A show a reduction of 1-NP when using exhaust gas recirculation (EGR), which is most significant in the Highway fuel economy test (see Table II and Figure 5A). Using an oxidation catalyst with engine A significant reductions of 1-NP are observed in all cases (see Figure 5A). Results for

Table I. Diesel engine description.

Engines	A	B
Displacement (l)	1.8	4.0
Number of Cylinder	4	4
Aspiration	naturally aspirated	turbo charged
Injection type	direct	direct

Table II. 1-Nitropyrene emission of engine A.

without EGR / without CAT			
test	mg particles / filter	ng 1-NP / filter	ng 1-NP / km
HWFET	117.59	854	603
FTP 72 / 1	39.38	66	164
/ 2	54.57	40	72
FTP 75 / 1	44.07	75	152
/ 2	64.00	41	72
/ 3	59.13	90	203
with EGR / without CAT			
test	mg particles / filter	ng 1-NP / filter	ng 1-NP / km
HWEFT	84.57	412	2.98
FTP 72 /1	37.88	86	168
/ 2	36.48	25	46
FTP 75 / 1	39.15	72	141
/ 2	34.00	37	67
/ 3	41.42	66	144
with EGR / with CAT			
test	mg particles / filter	ng 1-NP / filter	ng 1-NP / km
HWEFT	39.86	85	64
FTP 72 /1	20.53	12.8	25
/ 2	14.40	4.4	12
FTP 75 / 1	17.00	8.4	26
/ 2	14.20	3.6	9
/ 3	19.81	10.4	21

EGR = exhaust gas recycling; CAT = catalyst; HWFET = higway fuel economy test;
FTP = federal test procedures.

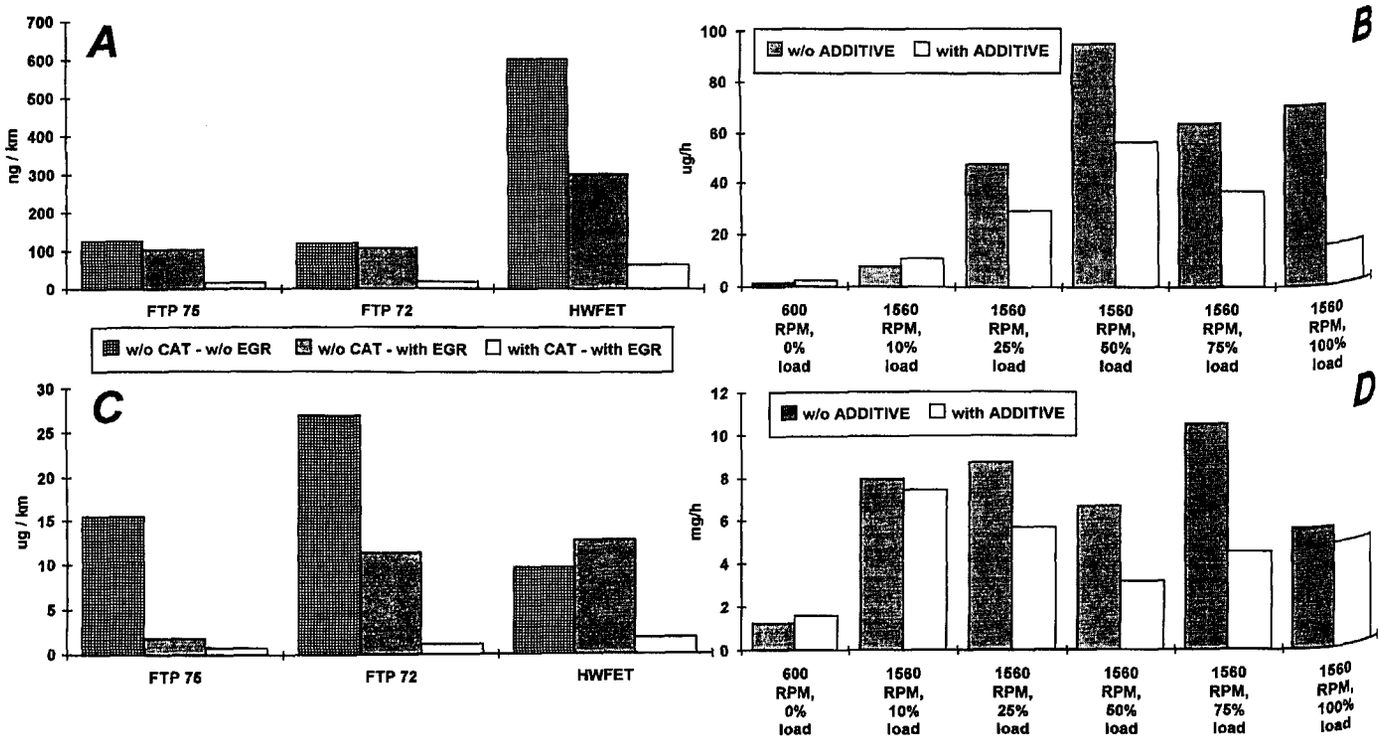

Figure 5

A: 1-NP emission (ng km^{-1}) of diesel engine A (1.8 L DI/NA).
B: 1-NP emission (µg h^{-1}) of diesel engine B (4.0 L DI/TC).
C: Pyrene emision (µg km^{-1}) of diesel engine A (1.8 L DI/NA).
D: Pyrene emision (mg h^{-1}) of diesel engine B (4.0 L DI/TC).

Table III. 1-Nitropyrene emission of engine B (µg h^{-1}).

		without additive			with additive		
RPM	% load	1. test	2. test	average	1. test	2. test	average
600	0	0.6	2.2	1.4 ± 1.13	1.5	3.4	2.5 ± 1.34
1560	10	7.1	9.1	8.1 ± 1.41	7.3	14.8	11.1 ± 5.30
1560	25	48.8	46.9	47.9 ± 1.34	29.0	30.1	29.6 ± 0.78
1560	50	102.6	86.7	94.7 ± 11.24	52.2	61.2	56.7 ± 6.36
1560	75	47.2	79.9	63.6 ± 23.12	20.1	53.6	36.9 ± 23.69
1560	100	75.0	65.3	70.2 ± 6.86	16.7	14.2	15.5 ± 1.77

Table IV. Pyrene emission of engine A (µg km^{-1}).

test	w/o EGR – w/o CAT	with EGR – w/o CAT	with EGR – with CAT
HWFET	9.7	12.8	1.9
FTP 72 / 1	36.1	16.1	1.3
/ 2	17.7	6.7	1.2
FTP 75 / 1	23.7	3.0	1.9
/ 2	10.2	1.0	0.4
/ 3	18.2	2.4	0.6

EGR = exhaust gas recycling; CAT = catalyst; HWFET = highway fuel economy test;
FTP = federal test procedures.

Table V. Pyrene emission of engine B ($\mu g\ h^{-1}$).

		without additive			with additive		
RPM	% load	1. test	2. test	average	1. test	2. test	average
600	0	1301	1229	1265 ± 51	1210	1994	1602 ± 554
1560	10	9119	6855	7987 ± 1601	8824	6049	7437 ± 1962
1560	25	10573	6931	8752 ± 2575	5102	6246	5674 ± 809
1560	50	6515	6848	6683 ± 235	2917	3464	3191 ± 387
1560	75	10087	10930	10509 ± 596	3360	5708	4534 ± 1660
1560	100	4400	6830	5615± 1718	3969	5667	4818 ± 1201

engine B show maximum 1-NP emission at half engine load (see Table III and Figure 5B), therefore no relationship between 1-NP- and NOx-emission for the engine exists (NOx-formation increases with increasing engine load).
Furthermore it was found, that with a fuel additive a significant reduction in 1-NP-emission can be achieved. Comparing 1-NP-emmissions of both engines with the corresponding pyrene-emissions (see Tables IV and V and Figures 5C and 5D, data obtained from AVL), it becomes obvious that no relationship between those two PAH-emissions exists. These observations confirm that 1-NP-emmissions are real engine emissions and not artifacts arising during particulate sampling processes, see also [46, 47, 15, 16].

Criteria for Evaluating and Validating Total Multidimensional HPLC Method

- check retention data of 1-NP on column 1
- fix time window for zone cut of effluent fraction to be transferred
- check retention data of 1-NP on column 2
- check reduction rate of 1-NP to 1-aminopyrene and its detection as 1-aminopyrene (1-AP)
  determine linearity of quantification of 1-NP (as 1-AP) in the range 1–20 ng per injection (typical correlation coefficient found > 0.998)
- check reproducibility with standard mixture containing low and high concentrations of 1-NP (C.V. = 3 %, n = 6)
- check reproducibility with real samples (C:V. = 3 %, n = 4)
- check day-to-day-reproducibility (± 4 %)
- check overall recovery (sample pre treatment + MD-HPLC) 72 ± 8 %
- determine detection and determination limit (20 pg corresponding to approx. 10 ppb)

Conclusion

The method described shows high selectivity due to its multidimensionality based on a two dimensional LC-including sample pretreatment a three dimensional LC-system paired with chemical specifity via the on-line, post-column reduction process of nitro-PAHs to the respective amino-PAHs. The apparatus requirements and the time-controlled, valve-switching set-up respectively are based on standard hardware units and work on a routine basis. Using this flexible MD-HPLC system and adjusting the time table appropriately, the method is applicable to quantitative analysis of nitro-PAHs, in particular 1-nitropyrene, in diesel particulate extracts but also to extracts of other sources like, for example, road dust.

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