ANALYSIS OF CARCINOGENIC NITRATED POLYCYCLIC AROMATIC HYDROCARBONS - A REVIEW

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Analytical methods for the determination and monitoring of carcinogenic nitrated polycyclic aromatic hydrocarbons in various types of matrices and the use of biological markers in assessing human exposure to these substances are reviewed. Survey of occurence and biological properties of these substances together with the basic information on available reference materials are included.

Keywords: nitrated polycyclic aromatic hydrocarbons; PAN determination: NPAH biological markers.

1. INTRODUCTION

Cancer exacts substantial costs in treatment and preventive measures on a world-wide scale, as well as causing immeasurable human suffering. The approximatelly 20% cancer mortality together with the fact that environmental causes contribute to the majority of cancers, emphasizes the potential benefits of environmental detection of chemical carcinogens and raises carcinogenic substances monitoring in general and working environment to the highest priority[1,2]. Analytical measurement procedures should have a critical role in molecular epidemiology and exposure regulation, as well as in environmental monitoring[3].

According to the International Agency for Research on Cancer (IARC) in Lyon, France, nitrated polycyclic aromatic hydrocarbons (NPAH) are among those substances which can be associated with the increasing occurence of cancer[1,4,5]. The interest in these substances is steadily increasing since 1978, when Jager[6] and Pitts[7] independently discovered that PAH could react with oxides of nitrogen under conditions that can be expected in polluted air and in combustion processes[8], particularly in diesel engines[9,10]. As a result, they are widely distributed in the environment [see review[11,12,13]]. Discovery that a high proportion of the "direct-acting" mutagenicity of air and diesel particulate extracts in the Ames' bioassay is caused by NPAH, especially by 1-NP[7,8,14] further increased the interest in elucidating the environmental[15] and biological[16,17] role of these compounds. Great efforts have been made to detect this new class of environmental carcinogens in environmental samples[11]. Because of the complexity of these samples and extremely low concentration of the analytes, a number of highly selective and extremely sensitive analytical methods had to be developed and certified reference materials had to be prepared for these purposes. Attention was being paid to the development of methods suitable for various types of matrices and for the use of biological markers in assessing human exposure to these substances. This review summarizes the results of the worldwide research effort in this field.

2. OCCURENCE AND BIOLOGICAL ACTIVITIES OF NITRATED POLYCYCLIC AROMATIC HYDROCARBONS

NPAH were found especially in ambient, urban and workplace air, diesel and petrol engine exhausts, in carbon black and fotocopier toners, coal-derived synthetic fluids, fly ash, exhausts from waste incineration plants and aluminium production plants, in products of coal combustion, in natural and waste waters, sediments, cigarette smoke and some foodstuffs[11]. More than 100 different NPAH[37], number of nitrobenzopyranones[97] and nitrofluorenes[96] have been identified in various environmental samples. Some selected examples are given in Table 1 with special emphasis on papers of analytical importance. [References from this table can be used to obtain more information on analytical methods for the determination of these NPAH in given matrices].

Table 1. Occurence of selected NPAH.

Substance	Occurence and References
1-NP	TCB(11,76,78); FA(11); OE(11); DE(16,37,38,39,42, 50,52,65,67,68,69,70,71,72,75,79,81,85,86,87,88); AA(45,72,76,84,85); APE(73); CS(11); WI(85); WW(11); S(11); F(11); W(11)
1-NN	TCB(11,76); DE(67,68,70,84,86,87); AA(76,84); CS(11); W(11); S(11)
2-NN	TCB(11,76); DE(50,70,75,86,87); AA(76);
9-NA	TCB(11,76,78,84); DE(37,39,42,67,70,75,82,86,87); AA(39,72,76,84); F(35); CC(84)
3-NFA	DE(37,50,75,87); AA(6,15,77); CC(11); CS(11); W(11)
6-NBaP	TCB(11,80); OE(11); DE(7,37,46,50,74); APE(73); AA(11); W(11)

AA = ambient air; APE = aluminum plant exhaust; CC = coal combustions; CS = cigarette smoke; DE = Diesel exhaust; F = foodstuffs; FA = fly ash; OE = Otto exhaust; S = sediments; TCB = toners and carbon black; W = water; WI = waste inceneration; WW = waste water.

When analyzed in bacterial mutagenesis assays, many of NPAH have been found to be mutagenic even in the absence of exogenous metabolic activation[17]. Some of them, such as dinitropyrenes, are among the most potent mutagens ever tested[9,10,13,18]. Mutagenic and carcinogenic activity of selected NPAH is summarized in Table 2.

Table 2. Genotoxic properties of selected NPAH.

Substance	Mutagenicity	Ref	Carcinogenicity	Ref
1-NP	++	11	-/+	11,26
2-NP	++	17	-/+	17,26
4-NP	++	17	+	17,26
1,3-DNP	+++	9,10,17,18	+	17
1,6-DNP	+++	9,10,17,18	+	17
1,8-DNP	+++	9,10,17,18	+	17
1-NN	-/+	11	-	11
2-NN	-/+	11	+	11
9-NA	-/+	11	?	11
2-NF	+	10,11	+	17
3-NFA	+++	11	+	11
6-NBaP	++	11	-/+	11
2-NMNF	+++	11	++	11
7-NBA	-/+	17	-/+	17
6-NC	+	9,10	+++	17

- inactive; -/+ very weak; + weak; ++ moderate; +++ strong; ? no available data.

NPAH can enter the body by inhalation, absorption through the skin and through intestinal tract. They are probably reduced by nitro-reductases in the liver to form the methaemoglobin - inducing substances, e.g. nitroso derivatives and N-hydroxylamines[19]. However, these intermediates are further reduced to the corresponding amino aromatic compounds, which are excreted in urine in free form or after acetylation[19,20]. The cellular reduction of NPAH is catalysed by NADPH-cytochrome P-450 reductases according to the following scheme:

$$Ar\text{-}NO_2 \rightarrow Ar\text{-}NO_2^{\bullet-} \rightarrow ArN{=}O \rightarrow ArNHOH \rightarrow Ar\text{-}NH_2$$

In addition, C-hydroxylation of the aromatic ring system can occur and further metabolic derivatives of the NPAH can be formed by N-N dimerization of the reduced intermediates[21]. The toxic effects of various nitroaromatic compounds are attributed to the formation of the above mentioned free radicals as a result of enzyme activity[21]. Their most important reactions are with the cellular macromolecules, especially with proteins[22] and nucleic acids[23]. These reactions may explain the mutagenic and carcinogenic effects of NPAH independent of the effects of the corresponding APAH[10,24]. Genotoxic properties of NPAH generally depend both on the structure of the parent PAH and on the number and position of the nitrogroups[9,10,13,17,18,25,26]. The metabolic activation of NPAH to bacterial mutagens has been shown to involve nitroreduction, nitroaromatic ring oxidation, N-hydroxylarylamine O-acetylation, or, in some cases, a combination of all three pathways[13,17]. Specialized literature can be consulted for the molecular biological details of the metabolism as well as the clinical features of the nitroaromatic compounds[2-5,9-13,17-19,20-24] and a number of relevant references can be found in review[11].

3. SELECTED METHODS OF DETERMINATION OF NITRATED POLYCYCLIC AROMATIC HYDROCARBONS

3.1. Sampling

The particle-bound NPAH in the air can be collected on glass-fibre filters and subsequently desorbed with organic solvents[6,8]. The quantity of air to be sampled depends on the concentration of airborn dust and the expected amount of NPAH. If the filters are not immediately processed, it is advisable to store the exposed filters in sealed containers (e.g. in Petri dishes) in a refrigerator to eliminate loss due to evaporation or decomposition reactions. NPAH can be extracted from collected particulates using dichloromethane[6]. Cumbersome 24 hours Soxhlet extraction can be substituted by a simplified ultrasonic extraction[8]. Formation of NPAH during ambient high-volume sampling was also observed and care should be taken to avoid it[41].

Typical analysis of atmospheric NPAH involve high volume sampling on inert filter (particle phase) and adsorbent (gas phase) materials, solvent extraction of the ladden filters or adsorbents, fractionation of the extracts by liquid chromatography and quantification by GC or HPLC[96].

The blood sample for the determination of 1-NN and 2-NN is withdrawn from the arm vein, plasma is immediately separated by centrifugation and ethanol is added to cause deproteinization. NPAH present in their free forms are extracted and simultaneously preconcentrated using 2,2,4-trimethylpentane (iso-octane)[19].

Sampling procedures for other types of environmental, industrial or biological samples can be found in references in Table 1. The determination of NPAH metabolites in urine require a preliminary separation[31].

3.2. Preliminary Separation and Preconcentration

Because of the complexity of environmental, biological or air particulate samples, analytical methods for the determination of NPAH often incorporate a preliminary fractionation by liquid-liquid acid-base extraction[32,33], TLC[6,7], open bed column chromatography[6,34] or HPLC[8,16,36,37,38]. Normal-phase separations have been most successful in isolating the moderately polar NPAH from particulate extracts[14,39]. Further information on various types of preliminary separations is available[96-98].

Nowadays, SPE is most frequently used for a preliminary separation and preconcentration. For 1-NP and 1-NFA in particulate emissons of diesel engines, SPE with silica SepPak cartridge followed by capillary GC-ECD was described[52]. Similar SepPak cartridge was used for a preliminary separation of several NPAH, including 3-nitro-9-fluorenone before final RP-HPLC-PDAD determination[66]. SPE with alumina SepPak cartridge followed by RP-HPLC with chemiluminiscence, fluorimetric or electrochemical detection after on-line electrochemical reduction was used for the determination of 1-NP, 1-AP and several dinitropyrenes in car engine emissions[89]. Silica SepPak cartridge was used prior to HPLC-ED, while an NPAH preliminary separation prior HPLC-FD was achieved using semipreparative aminosilane column[8]. The NPAH metabolites (1-AP,2-AF,2-AAF, and 1-OH-2-AAF) were isolated from urine using SepPak C18 cartridges[31].

Supercritical fluid extraction of NPAH and PAH from diesel exhaust particulate matter using CO_2, $CHClF_2$ and CO_2 with added modifiers was used prior GC-MS or GC-FID. The $CHClF_2$ at 100 °C and 400 atm yielded the highest recoveries of both NPAH and PAH[94].

3.3 Spectrometric Methods

UV maxima together with extinction coefficients of selected NPAH are presented in Table 3, with more detailed information available in review[11]. ^{13}C NMR chemical shifts of the same set of substances in $CDCl_3$ (resp. in dioxan for 6-NBaP) are tabulated[11]. NMR,IR, fluorescence and mass spectra were published in a book form[61].

Fluorimetry, which is a useful tool in the detection and quantification of PAH, cannot be applied directly to NPAH, which are almost non-fluorescent. However, some NPAH are known to phosphorescence strongly at low temperatures. Absorption (in methanol) and phosphorescence (in ether-isopropanol(3:1) at 77 K) data for 22 NPAH are available[87]. Detection limits are lower than 1 ng/ml in several cases (2-NF, 9-NA, 2,7-DNF, 1,8-DNN) and between 1-5 ng/ml for many other

Table 3. UV maxima and molar absorption coefficients of selected NPAH.

Substance	max	$.10^{-4}$	max	$.10^{-4}$	max	$.10^{-4}$
1-NP	233	4.22	285	1.54	370	1.34
1-NN	213	6.12	243	1.28	327	0.51
2-NN	210	4.02	260	3.24	308	0.80
9-NA	211	2.49	245	6.42	253	6.50
3-NFA	214	3.53	254	1.91	344	0.89
6-NBaP	266	2.75	300	2.69	390	1.11
2-NMNF	217	2.39	234	3.43	376	0.88

a (nm); b ($mol.l^{-1}.cm^{-1}$).

NPAH (1-NN, 2-NN, 1,5-DNN, etc.). The spectral properties of 1-NP (a frequently used reference substance) allows its selective determination in the presence of other nitroaromatics[87]. Spectral data (NMR,MS) on nitrofluorenes[96] and nitrobenzopyranones[97] are available.

Direct coupled MS-MS has been used for the determination and identification of selected NPAH[33,47,48]. *In vivo* monitoring of environmental carcinogens using GC-MS has been reviewed[93].

3.4. Polarographic and Voltammetric Methods

Because of easy polarographic reducibility of nitro goup[53,54,55,56], trace amounts of NPAH can be determined using modern polarographic and voltammetric techniques, namely differential pulse polarography on dropping mercury electrode or differential pulse voltammetry on hanging mercury drop electrode. Because of their low polarity, adsorptive stripping voltammetry[57] is a very promising method for the determination of NPAH within the concentration range 10^{-7} to 10^{-10} $mol.l^{-1}$. So far, these techniques were used for 4-NBP[58,60] and for monitoring the efficiency of its destruction in laboratory wastes[63], their use for other NPAH is intensely investigated in our laboratories[59]. APAH, as proven metabolites of NPAH, can be determined using differential pulse voltammetry based on their anodic oxidation on either a glassy carbon rotating disk electrode or a carbon paste electrode. This approach was used for 4-ABP[60,63] and its application for other APAH is being investigated[59]. Extremely high sensitivity of modern polarographic and voltammetric techniques can be combined with highly efficient separation by HPLC, especially for the determination of APAH (see paragraph 3.5.3.).

Moreover, there is a relationship between polarographic half wave potential and mutagenicity of NPAH[62] and the knowledge of the mechanism of their polarographic reduction can give us a useful clue in elucidation of the mechanism of their metabolic transformation.

3.5. Chromatographic Methods

Chromatographic methods are the most frequently used techniques in the analysis of NPAH both in environmental and biological samples, because of their selectivity, sensitivity and a high sample throughput.

3.5.1. Thin Layer Chromatography

Detection and characterization of 7 NPAH (1-NN, 9-NA, 6-NC, 3-NFA, 1-NP, 1,6-DNP and 6-NBaP) by fluorescence quenching after TLC separation was described by Jager[6]. Silicagel plates with cyclohexan-chloroform (1:1) mobile phase are the best for general separation of NPAH from other compounds present in atmospheric samples. Celulose plates and dimethylformamide-water (2:3) mobile phase are more efficient for the separation of individual NPAH. In order to detect NPAH, which have no natural fluorescence, they were reduced on a plate with potassium borohydride solution to the corresponding APAH, which are intensely fluorescent and remain fluorescent after spraying with aniline and phenylhydrazine. (The exception is 1-NA which is quenched with phenylhydrazine). All corresponding PAH were quenched by aniline. Most nitrogen-containing heterocyclic aromatic hydrocarbons, which can be present in extracts of airborne particulates, were not quenched by aniline. However, they were quenched by phenylhydrazine. These facts can be used in the detection and characterization of trace amounts of NPAH in very complicated mixtures with the limit of detection from 500ng in the case of 1-NN to 1 ng for 1-NP, 1,6-DNP or 3-NFA. TLC was further used for a preliminary separation of NPAH[7]. TLC on silicagel plates was used for identification of some NPAH in airborne particulate matter[95].

3.5.2. Column Chromatography

The use of open bed column chromatography[6,34] for a preliminary separation of NPAH was already mentioned. Preparative column chromatography on silicagel and polystyrene-divinylbenzene copolymer was used for the separation and isolation of NPAH from diesel exhaust particulates[39].

3.5.3. High Performance Liquid Chromatography

HPLC methods have been developed with electrochemical[12,33,34] and on line reduction followed by fluorescence detection[8,46,49,50,51]. Three different approaches to HPLC determination of NPAH in air and diesel particulate extracts are presented[8], based on differential pulse, amperometric and fluorescence detection following on line reduction to APAH. The particulate extraction and fractionation procedure for each detection approach is slightly different[8]. The detection limits for 1-NP are 5ng for differential pulse polarographic detection, 60pg for DC polarographic detection after prefractionation of the sample on a SPE cartridge and 10pg for fluorescence detection after on-line reduction to 1-AP.

More than 50 NPAH have tentatively been identified in an extract of diesel exhaust particulates using high-resolution MS of directly mutagenic fractions derived from sequential fractionation of the extract by both low and high resolution liquid chromatography[39]. Two preparative chromatographic separations were employed in this study (silica-gel column chromatography followed by size exclusion chromatography on polystyrene-divinylbenzene copolymer) and the pooled eluate from gel-filtration chromatography was further divided into 21 fractions using HPLC. 38 mononitro-PAH, 4 multinitro-PAH and 14 oxygenated nitro-PAH have tentatively been identified in various HPLC fractions.

Normal phase HPLC using silica gel columns and n-hexane-benzene (3:1) eluent has been developed for isolating PAH and NPAH in complex 24-hour sample of airborne particulate matter corresponding to 2000 m^3 of sampled air[72]. The PAH fraction is then analysed by GC-FID while NPAH fraction by GC-NPD. Relative retention times of 9-NA, 10-NBA, 1-NN, 6-NBaP, 9,10-DNA and 1-NP are tabulated and mechanism of retention is discussed in this paper[72].

46 structurally related NPAH and their corresponding PAH were used to study the relationship between structure and retention time using RP-HPLC[40].

The use of HPLC-ED for monitoring the efficiency of chemical destruction of 4-ABP was described[64]. Newer approaches to HPLC analysis of NPAH are described in original papers[40,90,91,92]. Multicolumn HPLC analysis of NPAH is of particular interest[71].

3.5.4. Gas Chromatography

Probably the most powerful tool for the identification and determination of NPAH is GC-MS[16,38,42,43], with as many as

50 compounds identified in a single diesel particulate sample[39]. Other techniques for NPAH determination include GC with nitrogen selective "thermal energy analyzer"[42,44], thermoionic nitrogen-phosphorus[37,45,46], electron capture[16,45], and flame ionization[38,42] detection.

Diesel particulate extracts were analysed for NPAH by use of fused-silica capillary column GC-NPD after HPLC fractionization[37]. These samples were found to contain at least 100 different NPAH. Positive isomer identification for 17 NPAH has been made utilizing GC retention times of authentic standards and low and high resolution mass spectra and additional 45 NPAH were tentatively identified using one of the above mentioned approaches. GC-NPD retention time data and GC-MS spectral data used for the identification of these NPAH are tabulated in the quoted paper[37]. The detection limits by GC-NPD range between 0.2 and 0.5 ppm for the HPLC fractionated samples. Application of multidimensional GC to analysis of NPAH in airborne particulate matter was described[98].

Capilary GC-ECD with fused silica capilary and stationary phase DB 1701 or SE 54 was used for the determination of 1-NN, 2-NN and 4-NBP in blood and plasma of exposed persons with the limit of detection 2.5 ug/L for 1-NN and 2-NN and 10 ug/L for 4-NBP[19].

4. USE OF BIOLOGICAL MARKERS IN ASSESSING HUMAN EXPOSURE TO NITRATED POLYCYCLIC AROMATIC HYDROCARBONS

Biological markers are indicators of changes or events in human biological systems. They integrate all routes of exposure to a particular contaminant. Biological markers in an exposed individual can provide information about an original contaminant or a metabolite of a contaminant (markers of exposure), or a product of an interaction between a contaminant and some target molecule or cell (markers of effect). Successful use of biological markers requires an understanding of the fate and effects of a contaminant within a person to permit the relation of a marker data to the exposure and the establishment of relationship among biological markers of effect and exposure.

Competitive ELISA was developed for the quantification of urinary metabolites of diesel exhaust constituents using 1-NP and 2-NF as model compounds[31]. Amounts of 0.8 nmol of 2-AF, 0.3 nmol of 2-AAF and 1-OH-2-AAF and 0.006 nmol of 1-AP were shown to cause 50% competitive inhibition of antibody (4D5) binding to 6-ABP covalently bound to bovine serum albumine.

Many carcinogens, including NPAH, are metabolically activated to electrophilic metabolites that covalently bind to DNA. Adducts on DNA, if they occur at critical sites and are not repaired, can cause gene mutation, which has been shown to be an initiating step in the multistage carcinogenic process. Several methods to detect DNA chemical aducts in lymphocytes and target issues are available, including radio- or enzyme-linked immunoassays that use polyclonal or monoclonal antibodies, 32-P postlabeling, and synchronous fluorescence spectrophotometry [(see review[27] and references therein].

The first use of antibodies to detect PAH-DNA aducts involved lung tissue and peripheral white blood cells and at present antibodies are available to asses formation of DNA aducts with BaP and other PAH[27]. Immunoassays can detect frequencies as low as one adduct per 10^8 nucleotides. Assays that use monoclonal antibodies are highly specific for a given substance, but those with polyclonal antibodies, such as PAH-DNA antibodies, can react with multiple structurally related compounds and thus loose specificity. However, the highly specific monoclonal assays can be time-consuming and technically difficult. So far, this approach was not reported for NPAH[27].

In contrast with the monoclonal assays, 32-P post-labeling can be used to recognize various adducts without characterizing their chemical compositions. It can detect one adduct per 10^{10} nucleotides. The technique is generally limited to the measurement of bulky adducts that might limit the usefulness of the method to smaller contaminants, and the results of the methods are only semiquantifiable. Effective use of postlabeling method requires the synthesis of an internal standard since the efficiency of the labeling can vary according to the substance. So far, this method was used for PAH but not for NPAH[27].

Synchronous fluorescence spectrophotometry can detect one adduct per 10^7 nucleotides. It is useful only for detecting compounds that fluorescence, such as PAH or their amino derivatives, not for non-fluorescent NPAH[27]. However, fluorescent metabolites of NPAH containing amino group on an aromatic ring should be amenable to this approach.

Assays that measure protein adducts, including adducts of direct binding agents and metabolites with hemoglobin, can in some cases be a good surrogate for DNA-aducts measurements. Methods available for measuring those adducts include immunoassays, amino acid analysis by ion-exchange liquid chromatography and GC-MS with both conventional ionization and negative chemical ionization techniques[27]. GC-MS has been successfully applied to the quantitation of 4-ABP[28,29,30] which is a known metabolite of 4-NBP. SPE using an ion-exchanger folowed by GC-MS determination of 2-AF-hemoglobin adducts in blood of rats after their mild base-catalysed hydrolysis was used to measure the exposition to 2-NF[99]. Because of the three-month life span of hemoglobin, those assays reflect relatively recent exposures while DNA adducts in lymphocytes reflect exposure integrated over a longer period. Protein adducts are more abundant than their DNA counterparts and therefore provide a more sensitive measure of exposure.

Various cytogenetic techniques, chromosomal aberrations, activated oncogenes and their protein adducts, and assays that detect changes in the function of target or analogous tissues were used as markers of effect for various chemical carcinogens[27], but according to our knowledge they were not used for NPAH so far.

5. STANDARD SUBSTANCES AND CERTIFIED REFERENCE MATERIALS

NPAH represent a new class of environmental carcinogens. As a consequence the Community Bureau of Reference, Commission of European Community prepared a series of 7 nitroarenes in a purity better then 99% as certified reference materials[11]. These substances which are listed in Table 1 were selected because of their environmental and occupational importance and their biological acivities (mutagenicity and carcinogenicity). The reference materials can help to definitely identify individual compounds in the analysis of environmental samples and may also encourage toxicologists to test them in various systems. Standard reference materials SRM 1587 (mononitro-PAH in methanol), SRM 1596 (dinitro-PAH in methylene chloride), two urban particulate materials (SRM 1648 and 1649) and diesel particulate matter (SRM 1650) from National Bureau of Standards, USA, were used[8].

Synthesis of nitrofluorenes[96], nitrobenzopyranones[97], and many other NPAH[37,87] was described in connection with their identification in various environmental samples.

6. LIST OF ABREVIATIONS

2-AAF 2-acetylaminofluorene
6-ABaP ..... 6-aminobenzo(a)pyrene
4-ABP 4-aminobiphenyl
2-AF 2-aminofluorene
1-AN 1-aminonaphtalene
1-AP 1-aminopyrene
APAH ..... aminoderivatives of polycyclic aromatic hydrocarbons

BaP ..... benzo(a)pyrene
DNA ..... desoxyribonucleic acid
9,10-DNA 9,10-dinitroanthracene
2,7-DNF 2,7-dinitrofluorene
1,5-DNN 1,5-dinitronaphtalene
1,8-DNN 1,8-dinitronaphtalene
1,3-DNP 1,3-dinitropyrene
1,6-DNP 1,6-dinitropyrene
1,8-DNP 1,8-dinitropyrene
ED ..... electrochemical detection
ECD ..... electron capture detector
ELISA ..... enzyme linked imunosorbent assay
FD ..... fluorimetric detection
FID ..... flame ionization detector
GC ..... gas chromatography
HPLC ..... high performance liquid chromatography
IR ..... infrared spectroscopy
MS ..... mass spectrometry
9-NA 9-nitroanthracene
7-NBA ..... 7-nitrobenz(a)anthracene
10-NBA .....10-nitro-benz(a)anthracene
6-NBaP ..... 6-nitrobenzo(a)pyrene
4-NBP 4-nitrobiphenyl
6-NC 6-nitrochrysene
2-NF 2-nitrofluorene
1-NFA 1-nitrofluoranthene
3-NFA 3-nitrofluoranthene
2-NMNF ..... 2-nitro-7-methoxy-naphto-(2,1-b)furan
^{13}C NMR ..... carbon-13 nuclear magnetic ressonance spectroscopy
1-NN 1-nitronaphtalene
2-NN 2-nitronaphtalene
1-NP 1-nitropyrene
2-NP 2-nitropyrene
4-NP 4-nitropyrene
NPD ..... nitrogen-phosphorus detector
NPAH ..... nitrated polycyclic aromatic hydrocarbons
1-OH-2-AAF. 1-hydroxy-2-acetylaminofluorene
PDAD ..... photo diode array detector
PAH ..... polycyclic aromatic hydrocarbons
RP ..... reversed-phase
SPE ..... solid phase extraction
TLC ..... thin layer chromatography

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