Polycyclic Aromatic Hydrocarbons (PAHs) and their Derivatives (O-PAHs, N-PAHs, OH-PAHs): Determination in Suspended Particulate Matter (SPM) – a Review

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Abstract
The aim of this paper is the presentation of the current state-of-the-art about the determination of polycyclic aromatic hydrocarbons (PAHs) and their oxidized forms originating from Suspended Particulate Matter (SPM) samples. The influence of SPM on health is twofold. SPM, as composed of small particles, is dangerous for the respiratory system. Additionally, SPM is a carrier of many hazardous compounds, particularly PAHs. Recently, several researches focus on the derivatives of PAHs, particularly nitro-, oxy- and hydroxy-PAHs, which are more dangerous than the parent PAHs. Both gas and high-performance liquid chromatography with various detection techniques are used to analyze both PAHs and their oxidized forms. Due to the appearance of these compounds in the environment, at a very low level, an analyte concentration step has to be applied prior to analysis. If GC and HPLC techniques are chiefly used as analytical tools for these analyses, the spectrum of analyte concentration procedures is very broad. Many analyte concentration techniques are proposed: from classic liquid-solid extractions, including Soxhlet technique, pressurized liquid extraction (ASE) or microwave oven (MWE) and sonic supported extraction to SPE techniques applications. However, one should remember that PAH determination methods are tools for solving the main problem, i.e., the evaluation the health hazard connected to the presence of SPM in air. Thus, the main drawback of several papers found in this review, i.e., the lack of information concerning limit of detection (LOD) of these methods makes their applicability very limited.

Keywords PAHs · nitro-PAHs · oxy-PAHs · Analytical procedures for PAHs · Air pollution · SPM
1 Introduction

Smog is a serious problem in many cities all over the world (Musa Bandowe et al. 2019). The word ‘smog’ comes from a combination of two English words – smoke and fog (Schwartz Cowan 1997). This is an unnatural phenomenon, formed mainly due to windless weather – artificial fog composed of residues after incomplete combustion of many types of fuels (Abbasi and Keshavarzi 2019). The main component of smog is suspended particulate matter, defined as aerosols of liquid drops or solid particles. Suspended particulate matter can be of natural origin (e.g., sea salt) or anthropogenic (e.g., dust from fuel combustion processes) (Reizer 2016). In the second case, Suspended Particulate Matter (SPM) is a measure of the air contamination. The size can classify SPM. Dust with particle diameter less than 10 μm is called PM10, and with particle diameter less than 2.5 μm, PM2.5. Many studies also mentioned PM4 with particle diameter 4 μm and PM1 with particle diameter 1 μm (Kic 2018; Boac et al. 2009).

The influence of SPM on health is twofold. Tiny particles of SPM can be dangerous for the respiratory system. The PM10 fraction is called the ‘chest fraction’ because of presenting danger on chest organs – heart, bronchi (Brown et al. 2013). Much smaller PM2.5 can penetrate pulmonary alveoli, and is called ‘respiratory fraction’ (Boldeo et al. 2006). The SPM is also a carrier of many hazardous compounds, formed in the incomplete combustion of many types of fuel processes. Heavy metal (e.g., cadmium, nickel) are adsorbed mainly on PM2.5 (Deng et al. 2006). They can penetrate the blood and can cause diseases typical of exposure to these metals.

Polycyclic aromatic hydrocarbons (PAHs) are among the substances that receive particular attention. PAHs are also adsorbed on suspended particulate matter. PAHs are a group of more than 10,000 compounds (Kurnia et al. 2018). PAHs can be divided by their molecular weight into low molecular weight (LMW) and high molecular weight (HMW) subtypes. LMW PAHs form a group of compounds with two- or three-ring structure, while HMW PAHs contain four or more rings (Saldarriga-Noena et al. 2018). Many studies report their carcinogenicity (Hayakawa 2018; IARC 2016), toxicity (Bandowe et al. 2019) and mutagenicity (Bandowe et al. 2014; Umbuzeiro et al. 2008) functions. The carcinogenic properties of PAHs increase with increasing molecular weight. While HMW PAHs are more carcinogenic, LMW PAHs are characterized by increased toxicity (Kim et al. 2013). For various reasons, the United States Environmental Protection Agency (USEPA) lists 16 compounds belonging to the PAH group as recommended for environmental monitoring (Keith 2014; USEPA 2008); however, the number of PAHs which are present in the air cause more danger (Mueller et al. 2019). Furthermore, these compounds can be converted into much more toxic oxidized forms of PAHs, when the conditions are favorable (Mueller et al. 2019; Ringuet et al. 2012). Nitro- and oxy-PAHs are suspected to be more toxic than parent forms due to their direct action as mutagens, but the level of knowledge about the carcinogenicity of these compounds is still under study (de Oliveira Galvao et al. 2018). Also, the acute toxicity is different for the original PAHs and their derivatives. For example, the LD50 for fluorene is 16,000 mg/kg (rat, oral), while for nitrofluorene it is 1,600 mg/kg (Zaciara 2007).

Particulate matter (PM) has become a major research issue receiving increasing attention because of its significant negative impact on human health. Besides the particle’s morphological characteristics, the chemical composition plays an important role on adverse health effects of PM (Walgraeve et al. 2010). Most of the PAHs with low vapor pressure in the air are adsorbed on PM particles, due to the fact that even though PAHs show low
solubility in water, they are highly lipophilic. When dissolved in water or adsorbed on particulate matter, PAHs can undergo photodecomposition when exposed to ultraviolet light from solar radiation. In the atmosphere, PAHs can react with pollutants such as ozone, nitrogen oxides, and sulfur dioxide, yielding diones, nitro- and dinitro-PAHs, and sulfonic acids, respectively. PAHs may also be degraded by some microorganisms in the soil (Srogi 2007). Table 1 presents a review of publications presenting the latest developments in the determination of PAHs in particulate matter.

As it may be seen while analyzing the table, recently many qualitative and quantitative studies of PAHs in various environmental matrices have been successfully carried out. Nevertheless, this type of analysis still presents many problems, especially at the stage of sample preparation. Due to the widely varying physical and chemical properties of PAHs, their measurement is often difficult and costly. Methodology for sampling, analysis, and emission estimation will need to be harmonized in order to properly assess current ambient concentrations, the effect of future control measures, and to refine any further action which may be required to adequately assess their human health impacts. Therefore, continuous research is needed to improve the current methods and their technical parameters.

The table above summarized the latest progress in this domain. The table data are focused on recent advances in PAH extraction techniques from environmental matrices, utilizing novel sample preparation approaches and adsorbents. Most of the novel extraction techniques are variations of the conventional SPE approach; however, liquid-liquid extraction (LLE) based approaches have also been reported. A lot of progress has been made in the field of sorbent development for micro and miniaturized SPE sample preparation techniques. Metal-organic frameworks, covalent organic frameworks, zeolitic imidazole frameworks, graphene, graphene oxide and carbon nanotubes are some typical examples of novel extraction sorbents that have been implemented as such, or after surface modification for the extraction of PAHs from environmental samples. At the same time, ionic liquids, polymeric ionic liquids and deep eutectic solvents have been implemented in liquid phase microextraction approaches as extraction solvents and in solid-phase extraction approaches for the surface modification of a plethora of adsorbents (Manousi and Zachariadis 2020; Nascimento et al. 2019; Jiping et al. 2020).

The selection of the extraction technique, as well as the extraction sorbent can be based on the needs of the analysis (e.g., selectivity) and the laboratory equipment. Matrix solid phase extracton (MSPE) and dispersive solid phase extraction (d-SPE) are some examples of simple, rapid and environmentally friendly extraction procedures, that have recently gained a lot of attention, due to their convenience in sample handling. The application of these techniques has rapidly increased, and a wide variety of sorbents have been evaluated. Other novel miniaturized extraction forms, including SBSE, PT-SPE and FPSE have also become popular during the last years. These techniques have been evaluated in less extend compared to SPE, MSPE and d-SPE methods; however, due to their ease in operation, they are considered useful alternatives that enrich the toolbox of analytical chemists (Sun et al. 2020; Lisowski and Zarzycki 2013).

Recently, the research on new analytical techniques for the trace nitro-PAHs took special attention (Sun et al. 2020). As the sample matrix is complex and the nitro-PAHs are at trace levels in the samples, the requirements for sample treatment are very high. Many novel adsorption materials such as carbon nanotubes / magnetic carbon nanotubes, graphene oxide, aptamers, molecularly imprinted polymers (MIPs), electro spun nanofibers, and many newly emerging extraction solvents including ionic liquids, supramolecular solvents, and deep eutectic solvents have good application prospects in the extraction of trace nitro-PAHs in the various samples because of their environmentally friendly and
| Authors          | Title                                                                 | Aim                                                                                                                                                                                                 |
|-----------------|----------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Ravindra et al. (2001) | Health Risk Assessment of Urban Suspended Particulate Matter with Special Reference to Polycyclic Aromatic Hydrocarbons: A Review | This paper reviews the literature on PAHs in conjunction with the particulate matter from a global perspective. Airborne suspended particulate matter is an important marker of air quality. The term ‘particulates’ includes organic and inorganic matter, nitrogen compounds, sulphur compounds, polycyclic aromatic hydrocarbons (PAHs), several heavy metals, and radionuclides. |
| Srogi (2007)    | Monitoring of environmental exposure to polycyclic aromatic hydrocarbons: a review | The scope of this review is to give an overview of PAH concentrations in various environmental samples and to discuss the advantages and limitations of applying these parameters in the assessment of environmental risks in ecosystems and human health. There is an increasing trend to use the behavior of pollutants (i.e., bioaccumulation) as well as pollution-induced biological and biochemical effects on human organisms to evaluate or predict the impact of chemicals on ecosystems. The emphasis in this review is, therefore, placed on the use of bioaccumulation and biomarker responses in air, soil, water and food, as monitoring tools for the assessment of the risks and hazards of PAH concentrations for the ecosystem, as well as on its limitations. |
| Walgraeve et al. (2010) | Oxygenated polycyclic aromatic hydrocarbons in atmospheric particulate matter: molecular characterization and occurrence | This paper presents a comprehensive review focusing on the analysis, fate, and behavior of oxygenated PAHs in the atmosphere. The first part of the paper briefly introduces the main sources and atmospheric pathways of oxygenated PAHs, available physicochemical properties, and their health effects. The second and main part of this paper gives a thorough discussion on the entire analytical sequence necessary to identify and quantify oxygenated PAHs on atmospheric PM. |
| Kim et al. (2013) | A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects | This review offers an overview of PAH properties, fates, transformations, human exposure, and health effects (acute and chronic) associated with their emission to the atmosphere. Biomarkers of PAH exposure and their significance are also discussed. |
| Authors                  | Title                                                                 | Aim                                                                                                                                 |
|-------------------------|----------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|
| Lammel (2015)           | Polycyclic aromatic compounds in atmosphere – a review identifying   | This article aims to identify polycyclic aromatic compounds studied in research and monitoring programs in the atmospheric environment and to identify research needs. |
|                         | research needs                                                       |                                                                                                                                 |
| Abdel-Shafy and Mansour (2016) | A review on polycyclic aromatic hydrocarbons: source, environmental | The aim of this review is to discuss the PAHs impact on the environment and on the magnitude of the human health risks posed by such substances. They also contain essential information on concentrations, burdens, and fate of polycyclic aromatic hydrocarbons (PAHs) in the atmosphere. The primary anthropogenic sources of PAHs and their effect on the concentrations of these compounds in the air are discussed. The fate of PAHs in the air, their persistence, and the main mechanisms of their losses are presented. Health hazards associated with PAH air pollution are stressed. |
|                         | impact, effect on human health and remediation                        |                                                                                                                                 |
| Lawal (2017)            | Polycyclic aromatic hydrocarbons: a review                            | In this paper, the recent literature is reviewed describing polycyclic aromatic hydrocarbons (PAHs) in air, water, soil and sediment, waste sludge, biomonitoring, and toxicity. Aspects of sampling, sample preparations such as extraction of PAHs are discussed and analytical methods used are also reviewed. Developments in direct measurement techniques, such as ultraviolet absorption spectrometry and synchronous luminescence, are noted. This review also discusses the microbial PAH-remediation and PAH-degradation, emphasizing on biological and physicochemical factors influencing the biodegradation. |
| Bandowe and Meusel (2017) | Nitrate polycyclic aromatic hydrocarbons (nitro-PAHs) in the          | The authors present an extensive review of published literature on the sources, formation, physicochemical properties, methods of determination, occurrence, concentration, transport, fate, (eco) toxicological, and adverse health effects of nitro-PAHs. They also make suggestions and recommendations about data needs and future research directions on nitro-PAHs. |
|                         | environment - a review                                               |                                                                                                                                 |
| Authors                          | Title                                                                 | Aim                                                                                                                                                                                                 |
|---------------------------------|-----------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Oliveira et al. (2019)          | Children environmental exposure to particulate matter and polycyclic   | This work aims to critically review the available information on children’s environmental exposure to PM and PAHs in indoor and outdoor air of school environments and their total exposure to PAHs by assessing PAH biomarkers of exposure. Pollutant levels and their main sources are presented and discussed regarding the school geographical location, and the reported concentrations are compared to the existing national and international guidelines. The primary health evidence of children’s exposure to PM and PAHs at schools is also reviewed. |
|                                 | aromatic hydrocarbons and biomonitoring in school environments:        |                                                                                                                                                                                                     |
|                                 | A review on indoor and outdoor exposure levels, major sources and     |                                                                                                                                                                                                     |
|                                 | health impacts                                                        |                                                                                                                                                                                                     |
| Munyeza et al. (2019)           | A review of monitoring of airborne polycyclic aromatic hydrocarbons:   | In this review, the Authors focus on the status of the monitoring of polycyclic aromatic hydrocarbons (PAHs) in ambient air as well as in living (indoor) and working environments in Africa from 2000 to 2018. |
|                                 | An African perspective                                                |                                                                                                                                                                                                     |
| Manousi and Zachariadis (2020)  | Recent Advances in the Extraction of Polycyclic Aromatic Hydrocarbons | This review focuses on the recent advances in the extraction techniques of PAHs from environmental matrices, utilizing novel sample preparation approaches and adsorbents.                                      |
|                                 | from Environmental Samples, Review                                    |                                                                                                                                                                                                     |
| Sun et al. (2020)               | Advances in analysis of nitrated polycyclic aromatic hydrocarbons     | In this review, the sources, toxicity, contamination status, physico-chemical properties, particularly the advances of their sample preparation and quantification methods in various matrices in the past decade are summarized. The research is devoted to nitrated polycyclic aromatic hydrocarbons (nitro-PAHs) being the derivatives of PAHs. They are trace pollutants commonly found in the environment and have been widely detected in various matrices. Nitro-PAHs are direct mutagens and carcinogens to humans, so they have attracted increasing public attention. In order to accurately assess the adverse effects of nitro-PAHs on human health, it is necessary to analyze them in different samples. |
|                                 | in various matrices                                                   |                                                                                                                                                                                                     |
non-toxic or low toxic features, their low cost, and their easy availability. There is an increasing interest in various miniaturized systems like microfluidic paper-based analytical device (m-PAD), micro total analysis system (m-TAS), surface-enhanced Raman scattering (SERS) devices, surface plasmon resonance (SPR) system, and other miscellaneous biosensors, as well as electric-field driven separation devices including diverse forms of electrophoresis and microchip capillary electrophoresis. All these techniques have potential applications in the future for nitro-PAHs analysis, especially in their real-time or on-site analysis (Yang et al. 2019; Kaneta et al. 2018).

The aim of this paper is a presentation of the current state-of-the-art about the determination of PAHs and their oxidized forms originating from Suspended Particulate Matter samples.

2 PAHs and their Oxidized Forms

PAHs are a group of compounds, which contain condensed aromatic rings. It is a group of more than 10,000 compounds, but the National Institute of Standard and Technology (NIST) reported 660 structural formulas of PAHs in NIST Special Publication 922/2011 (Kubiak 2013). PAHs are formed by incomplete combustion of many types of fuels, particularly diesel fuel (Abbasi and Keshavarzi 2019). When they are adsorbed on suspended particulate matter, they are one of the main components of smog. Many studies report their harmful impact on human health.

PAHs in the human body are converted in reaction with cytochrome P450 into phenols and dihydriodols. Then, part of dihydriodiol is converted by additional oxidation into dihydriodiol epoxides (Moorthy et al. 2015). The metabolites of PAH have been determined as biomarkers to evaluate the exposure of humans to PAHs. The urinary 1-hydroxypyrene has been used as a biological indicator for evaluating exposure. The presence of PAHs metabolites (1-hydroxypyrene and naphthol) was identified in the wastewater effluent amongst anti-androgenic chemicals (Rostkowski et al. 2011). The routes of intake of nitro-PAHs by humans and animals from the environment are inhalation, oral ingestion, and dermal contact. Several test systems, e.g., bacterial strains (Ames test using Salmonella), mammalian and fish cell lines, biological molecules (DNA) plants and rats and mouses in-vivo studies have been used to study the carcinogenicity, mutagenicity/genotoxicity of nitro-PAHs in environmental media (soil, sediments, atmospheric particulates (Abbas et al. 2018). According to scientific studies and the International Agency for Research on Cancer (IARC) Working Group on the Evaluation of Carcinogenic Risks to Humans, several nitro-PAHs have been classified into carcinogenic groups (Bandowe and Meusel 2017). The level of toxicity of some nitro-PAHs was determined as much higher than the level of toxicity of benzo[a]pyrene (Collins et al. 1998).

Both the USEPA and the European Environmental Agency recognize the necessity of monitoring the content of PAHs in the air. Benzo[a]pyrene was adopted in the 1980 s as a standard compound in air quality monitoring and research, as a determinant of mischievousness. Already in the 90 s many studies were conducted in which the concentration of PAHs in the air was determined. The concentration of PAHs and benzo[a]pyrene strongly depended on the sampling sites (urban, rural, industrial, traffic air).

Sapota (Sapota 2002) pointed out that the recommendations of American and European agencies are different – the American one recommends detailed analysis of all 16 priority PAHs, and the European agency recommends the measurement of benzo[a]pyrene only.
Recently, more research focused on oxidized forms of PAHs – nitro-, oxy- and hydroxy-derivatives. New research also mentions that heterocyclic thio-PAHs are noteworthy compounds (Bandowe and Meusel 2017). Many studies describe the toxicity of oxidized PAH forms as higher than the non-substituted PAHs (de Oliveira Galvao et al. 2018; Bandowe and Meusel 2017; Idowu et al. 2019). The derivatives of PAHs can be formed in two ways. One is a primary emission from combustion processes. The second way is the photochemical reactions between PAHs and other contamination (nitrogen oxides, sulfur oxides) or ozone in the atmosphere (Cordeiro and Corio 2009; Drotikova et al. 2020). Some forms of nitro-derivatives of PAHs are indicator products of certain chemical transformations (Mulder et al. 2019). But in contrast to PAHs, there are no regulations about PAH derivative concentrations in the environment. Only Swedish and Danish environmental protection agencies included nitro-PAHs in their environmental monitoring studies (Brorström-Lunden et al. 2008) and California EPA lists several nitro-PAHs which should be regularly monitored (OEHHA 2005). Oxy- and nitro-PAHs are not included in existing monitoring programs (Lammel et al. 2020). Additionally, the occurrence of PAH derivatives can provide information about parent PAH sources alongside methods such a chemical mass balance (CMB) or isomer ratio analysis (Ozaki et al. 2020). Figure 1 shows possible ways of oxidation or nitration on the example of pyrene and benzo[a]pyrene.

The main problem is the lack of sufficiently sensitive methods of tracking the course of PAH reactions in the atmosphere (Lammel 2015). Reactions between PAHs and different

![Fig. 1 a) Possible way of photochemical oxidation of pyrene. b) Different ways of nitration and oxidation of PAHs (adapted from Lu et al. 2011). (adapted from Cordeiro and Corio 2009)](image-url)
radicals in the atmosphere can occur both with and without sunlight. Figure 2 shows ways of PAH transformation (example of naphthalene).

3 Sample Preparation and Analytical Procedures for PAHs and Derivatives Originating from SPM

A majority of PAHs and their oxidized forms can be analyzed by the same analytical techniques. Figure 3 shows the scheme of determination of PAHs and their derivatives.

3.1 PAHs

The sampling may be performed with a variety of samplers, both low- and high-volume sampling rates and even passive samplers. Sampling media include quartz fiber, glass fiber, Teflon-coated glass fiber, and Teflon membrane filters (Szulejko et al. 2014). Glass and quartz fiber filters (GFF and QFF) are mainly annealed at high temperatures (400-550 °C) for several hours to lower blank levels associated with the new filters and weighed before the sampling. The previously weighed filters are reweighed after sample collection; then, the filters are protected and stored at a temperature below 15 °C. The generally used technique is gas chromatography with mass spectrometry (GC/MS), but (ultra)high performance liquid chromatography ((U)HPLC) with the reversed-phase system is also used (Singh et al. 2019). Fluorescence detectors, diode arrays, and mass spectrometry are the most often used detectors. Detailed conditions for GC/MS analysis are given by Mueller et al. (2019). They used GC/MS to analyze 64 PAHs and their derivatives, achieving a low limit of detection (LOD). They proposed using methane as a chemical ionization, helium as a quench gas, and nitrogen as the collision gas. Their

![Fig. 2 Reactions of naphthalene with atmospheric radicals during day- and nighttime. (adapted from Vione et al. 2004)](image_url)
total run time was 47.45 min, with a selected temperature program. Also, Mulder et al. (2019) present a well-described analytical procedure using GC/MS. Wilson et al. (2017) suggest using normal-phase liquid chromatography with aminopropyl stationary phase. Some differences can be observed in the sample preparation processes. Barrado et al. (2012; 2013) used extraction with microwave oven (MWE) following a 40-min program with temperature gradation to final temperature of 120 °C and power range of 900 W. They proposed using dichloromethane as an extraction solvent. The efficiency of this process was reported as exceeding 80%. Crimmins and Baker (2006) proposed sonic supported extraction. Samples were sonicated for 30 min in dichloromethane and then stored for 48 h at -20 °C. After that, the extract was evaporated to 200 µL under nitrogen flow. The samples were analyzed by the GC/MS. Jakovljević et al. (2018) used extraction with a mixture of toluene-cyclohexane (7:3) in a sonic bath. Then, the samples were evaporated to dryness and re-dissolved in acetonitrile. The samples were analyzed by HPLC with fluorescence detector. Umbuzeiro et al. (2008) used dichloromethane in Soxhlet extraction. Similar procedures are described by Lammel et al. (2020). This
process lasted for 20 h at 40 °C. After extraction, the solvent was evaporated to almost dryness and the sample was re-dissolved in a small amount of dichloromethane.

Abbasi and Keshavarzi (2019) proposed a similar procedure. After Soxhlet extraction, they used silica gel column to pre-concentrate and clean-up extract, and then the extract was fractionated to 4 fractions with different polarities. Almost identical procedure was proposed by Mulder et al. (2019) except that dichloromethane and acetone/hexane (1:1) were used for extraction. Albinet et al. (2006) proposed using pressurized liquid extraction (ASE) with dichloromethane as a solvent. Samples were supplemented with chemical standards and twice extracted by ASE, and then dried under Na₂SO₄. Similar procedure was used by Wilcke et al. (2014), and then by Bandowe et al. (2014). Oleagotia et al. (2019) extracted PAHs with a microwave and acetonitrile as a solvent. (U)HPLC with ultraviolet and fluorescence detection was used as an analytical technique. Han et al. (2019) proposed a similar procedure of sample extraction, and then they fractionated the extract of the PAHs, nitro-PAHs, oxy-PAHs and OH-PAHs using SPE cartridges. PAHs are eluted by a mixture of n-hexane and dichloromethane (ratio 1:1 v/v). This fraction contains beyond PAHs also nitro- and oxy-PAHs. GC-EI-MS were used to determine PAHs. Comparison of the most frequently used sampling and analytical methods for PAHs is given in the summarizing table after the Section 3.1-3.3.

3.2 Nitro-PAHs

Samples for PAHs and nitro-PAHs analyses can be collected simultaneously using the methods described earlier for PAHs. Nitro-PAHs, as well as the other PAHs, can be analyzed by the GC or (U)HPLC coupled with different detectors. Various methods of ionization can be used: negative ion chemical ionization (NICI), electron impact (EI), atmospheric pressure ionization (APCI), atmospheric pressure photo-ionization (APPI) with instruments operated in selective ion monitoring (SIM), or multiple reaction monitoring mode (MRM). Fluorescence, chemiluminescence and photodiode array detection methods can be used besides mass spectroscopy. Considering that nitro-PAHs can decompose at higher temperatures, Sun et al. (2020) point out that HPLC analysis is a better method for nitro-PAH analysis. Regardless of the method of determination, the most important element is the preparation of samples, taking into account the low levels of nitro-PAH concentrations. Zhao et al. (2020a) describe exactly the procedure of sample preparation. They extracted sample filters with dichloromethane by Soxhlet at 45 °C. Next, the samples were evaporated to about 1 mL and purified through a column with silica gel and alumina. Then, the samples were eluted by a column with hexane and a mixture of hexane and dichloromethane (DCM) (1:1 v/v). The eluates (hexane/DCM) were collected and evaporated again. Next, the samples were analyzed by CG/MS with electron impact ionization (EI).

Almost identical method was used by Zhao et al. (2020b). Oliveira Galvao et al. (2018) and Kitanovski et al. (2020) proposed using GC-MS to analyze both nitro- and oxy-PAHs. Also, Gao et al. (2018), Mueller et al. (2019) and dos Santos et al. (2019) presented a similar procedure. The possibility of measurements at a room temperature (where the nitro-PAHs are more stable) is the main advantage in using (U)HPLC in comparison to GC. C-18 Columns (Schauer et al. 2004; Mirivel et al. 2010; Nyiri et al. 2016) or phenyl-hexyl column (Garcia-Alonso et al. 2012) can be used for the separation of small, volatile molecules and large unstable molecules. Several different methods of sample preparation were presented. Zielinska and Sammy (2006) used the conventional method of sample preparation by Soxhlet extraction, sonication and pressurized fluid extraction (PFE). They
mentioned dichloromethane, toluene, or benzene as the most commonly used solvents. PFE is a time saving, and easy to use method, with low consumption of the solvent. Bamford et al. (2003) and Ringuet et al. (2012) also prepared samples by means of PFE method. Simultaneous analysis of both PAHs and nitro-PAHs with the same procedure of the extraction by pressurized liquid extraction and dichloromethane as an extraction solvent was reported by Bandowe et al. (2014). Also, Han et al. (2019) proposed a determination of PAHs and nitro-PAHs from a single sample. In this research, GC-NICI-MS was applied for the determination of nitro-PAHs, while to determine the other PAHs the authors proposed a slightly different method. An efficient analysis of both PAHs and nitro-PAHs was also proposed by Pham et al. (2019). HPLC with fluorescence detector (HPLC-FL) was used as a basic analytical method. The need of a separation of PAHs and nitro-PAHs before the analysis was indicated by Schauer et al. (2004). PAHs and nitro-PAHs were separated before the analysis using a glass column with silica. PAHs were eluted with cyclohexane/ dichloromethane (in 1:1 ratio) fraction.

In turn, Bamford et al. (2003) used SPE and mixture of hexane and dichloromethane to separate PAHs and nitro-PAHs. This method was also used by Cochran et al. (2012). Nyiri et al. (2016) applied ultrasonic assisted extraction of PAHs and their oxy- and nitro-derivatives with hexane as a solvent. Tang et al. (2016) and Fujiwara et al. (2014) applied dichloromethane as a solvent in the ultrasonic extraction of PAHs and nitro-PAHs. Wang et al. (2019) applied a novel method such as Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF-MS) with magnetic nanocomposites as matrix. This method has significant limitations in the analysis of small molecules (it is applied to analyze proteins and macromolecules), but an application of the right matrix (the authors used Fe₃O₄/ZIF-8 magnetic nanocomposites) allowed to successful analysis of nitro-PAH. The proposed analytical procedures for nitro-PAHs are summarized in the Table 3, right after Section 3.1-3.3.

3.3 O-PAHs

In order to properly describe the analytical methods of O-PAHs, it is necessary to divide that group of compounds into hydroxy-PAHs and oxy-PAHs. Molecules belonging to the hydroxyl group require different preparation before the analysis. Cochran et al. (2012) indicate a necessity of sample derivatization prior to GC-MS analysis of hydroxy-PAHs. Hydroxy-PAHs require an inactivating hydroxyl group before the GC-MS analysis in contrast to PAHs, nitro-PAHs and oxy-PAHs, which can be analyzed without the derivatization. In this case, the mixture of bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) was used as a derivatization agent at 70 °C for 6 h, and complete transformation of hydroxyl group was achieved. Similar procedure of OH-PAHs derivatization was proposed by Berger-Brito et al. (2018). Richter-Brockmann et al. (2019) proposed a transformation of OH-PAH (3-OH-BaP) into methyl ether using dimethyl sulfoxide, sodium hydroxide, and methyl iodide. After the reaction, the ether was extracted with cyclohexane using SPE. Then, they proposed GC-APLI-MS analysis. When the LC-MS method is applied, there is no need for derivatization of hydroxy-PAHs (Han et al. 2019). Barrado et al. (2012) applied low-temperature ultrasonication with methanol as an extraction solvent. The extraction procedure based on ethyl acetate was proposed by O’Connel et al. (2013); then, the GC and LC-MS techniques were used for quantification of 24 O-PAHs. Oxy-PAHs can be analyzed together with nitro-PAHs and PAHs without derivatization (Albinet et al. 2006). Han et al. (2019) proposed analysis of both nitro- and
oxy-PAHs simultaneously. Also, Lammel et al. (2020) proposed a similar analytical procedure of both nitro- and oxy-PAHs, using GC-APCI-MS. Analytical investigation of oxy-PAHs requires slightly different conditions of temperature than PAHs (Souza et al. 2014). Analytical procedures for O-PAH were collected and presented in Table 4. Please note that the table addresses not only PM-related air contaminations, but also reflects similar research devoted to soil, sediments, and urine analysis.

Sample preparation methods can be divided into two groups: adsorption techniques and extraction techniques using various extractants. The main analytical tools are GC and HPLC. The advantages and disadvantages of the presented techniques are difficult to assess due to the lack of the key value, which is LOD. For PAHs, the best method seems to be high volume sampler + ASE, for nitro-PAH, PSE with dichloromethane, and for O-PAH, ASE. HPLC is a better technique for hydroxy-PAH analysis as it does not require sample derivatization.

As it may be seen in Tables 2, 3 and 4, a variety of methods and their modifications have been proposed towards efficient analysis of PAHs, nitrated PAHs, oxygenated forms of PAHs (oxy-PAHs), and hydroxy-PAHs (OH-PAHs) in environmental samples. These tables summarize research on new sample treatment methods, sample analysis methods, and method performance for PAHs and their derivatives in various matrices. Not only were the samples extracted and purified by various methods, but the extracts were also analyzed by various techniques, such as gas chromatography, single and triple quadrupole mass spectrometry in electron ionization and negative ion chemical ionization with selective ion monitoring and reaction selective monitoring, as well as liquid chromatography and triple quadrupole mass spectrometry. Each group of analytes was treated by different analysis modes, such as GC-EI-SIM for PAH, GC-NICI-SRM for nitro-PAH, GC-EI-SRM for oxy-PAH, and LC-ESI-MS / MS for OH-PAH. The proposed instrumental techniques are very sensitive, ensuring detection of very low concentrations, even at the level of pg.

4 Summary

Analytical methods for basic PAHs are well developed, while in the case of derivatives (nitro-, oxy-, hydroxy-PAHs) there are still open questions. The main problem in the analysis of derivatives is their concentration in the air (much lower than basic compounds), their multiplicity (it is possible to form several derivatives from one basic compound), and the increased reactivity of some of them, which requires special treatment. Nevertheless, the analysis is similar for both PAHs and PAH derivatives, the main differences being in the sample preparation techniques.

PAHs can be successfully analyzed using GC/MS and HPLC (both in the reversed and normal phase system). The preparation of the environmental samples, usually taken on glass or quartz fiber filters, involves the extraction of compounds. To this goal, various techniques are used, such as Soxhlet extraction, and microwave and ultrasonic extraction.

In the case of nitro-derivatives of PAHs, it is possible to analyze them simultaneously with PAHs. These compounds can also be analyzed by GC and HPLC methods, but some research indicates that nitro-derivatives may be decomposed at higher temperatures. Therefore, HPLC is considered to be a better method for nitro-derivatives analysis in comparison with GC.

The hydroxy-PAHs analytics require the derivatization prior to the GC analysis. The –OH groups must be blocked before the GC analysis is started. Most often, this is done...
Table 2  Comparison of analytical procedures for PAHs

| Number of PAHs analyzed | Sample treatment and preparation methods | Sample matrix    | Sample analysis method | Method performance | Ref.                  |
|------------------------|------------------------------------------|------------------|------------------------|--------------------|----------------------|
| 12                     | Gilair pump (styrene-divinylbenzene adsorption tube, GFF) | Gas and particle | HPLC-FL                | No data            | Viau et al. (2000)   |
| 12                     | High volume active sampler (GFF), Ultrasonic extraction (hexane) | Particle         | HPLC-DAD/FL            | No data            | Hassanien et al. (2001) |
| 39                     | High volume active sampler (GFF), Ultrasonic extraction (hexane) | Particle         | GC-MS                  | No data            | Barakat (2002)       |
| 15                     | Vacuum pump (GFF, PUF and XAD-2 resin) Soxhlet extraction (DCM with n-hexane) | Gas and particle | GC-FID                 | LODs: 0.029-0.064 ng/m$^3$ LOQs: 0.087-0.192 ng/m$^3$ | Hassan and Khoder (2012) |
| 28                     | High volume air sampler (PUF and QFF) ASE | Gas and particle | GC-MS                  | No data            | Khairy and Lohmann (2013) |
| 15                     | Low volume air sampler (PUF and QFF) Soxhlet extraction (DCM-hexane) | Gas and particle | HPLC-FL                | No data            | Abdallah and Atia (2014) |
| 16                     | Low volume air sampler (PUF and QFF) Ultrasonic extraction (DCM) | Gas and particle | GC-MS                  | No data            | Haiba and Hassan (2018) |
| 10                     | Battery operated pumps (GFF) Soxhlet extraction (DCM) | Particle         | HPLC-FL                | No data            | Fanou et al. (2006)  |
| 13                     | GFF | Gas and particle | HPLC-FL                | No data            | Fanou et al. (2011)  |
| 25                     | Mini-pump (GFF) Soxhlet extraction (acetone-hexane) | Particle         | GC-MS                  | No data            | Muendo et al. (2006)  |
| 15                     | Weighed on filter papers Soxhlet extraction (DCM) | Particle         | GC-MS                  | No data            | Lisouza et al. (2011)  |
| 22                     | Medium volume air sampler (QFF) Soxhlet extraction (DCM-acetone) | Particle         | GC-MS                  | No data            | Ladji et al. (2007)  |
| 16                     | High volume active sampler (QFF) Soxhlet extraction (DCM-acetone) | Particle         | GC-MS                  | No data            | Ladji et al. (2009)  |
| Number of PAHs analyzed | Sample treatment and preparation methods                                      | Sample matrix | Sample analysis method | Method performance | Ref.                      |
|-------------------------|------------------------------------------------------------------------------|---------------|------------------------|--------------------|--------------------------|
| 25                      | Medium volume sampler (QFF) Soxhlet extraction (DCM-acetone)                 | Particle      | GC-MS                  | No data            | Moussaoui et al. (2010)  |
| 18                      | High volume air sampler (QFF) Soxhlet extraction (DCM-acetone)               | Particle      | GC-MS                  | No data            | Ladji et al. (2014)      |
| 10                      | High volume active sampler (PUF, GFF) Soxhlet extraction (n-hexane)          | Gas and particle | HPLC-FL               | LODs: 0.02-0.5 ng/m³ | Nizzetto et al. (2008)   |
| 32                      | Personal aerosol sampler (GFF) Ultrasonic extraction (DCM)                   | Gas and particle | GC-MS                  | No data            | Titcombe and Simcik (2011) |
| 30                      | High volume sampler (PUF, GFF) ASE (hexane-acetone)                          | Gas and particle | GC-MS                  | LOD: 0.005-0.009 ng/m³ | Arinaitwe et al. (2012)  |
| 11                      | Dual impactor (Teflon coated GFF) Ultrasonic extraction (benzene-ethanol)    | Particle      | HPLC-FL               | No data            | Taylor and Nakai (2012)   |
| 11                      | Dual impactor (Teflon coated GFF) Ultrasonic extraction (benzene-ethanol)    | Particle      | HPLC-FL               | LOD: 0.001-0.04 ng/ml | Taylor et al. (2015)      |
| 20                      | IVL sampler (Teflon filter) Soxhlet extraction (DCM-hexane)                  | Particle      | GC-FID                 | No data            | Safo-Adu et al. (2014)   |
| 17                      | Low volume air sampler (QFF) Soxhlet extraction (acetone-hexane)              | Particle      | GC-MS                  | LODs: 0.02-0.67 ng/m³ | Bortey-Sam et al. (2015) |
| 14                      | Low volume air sampler (QFF) Ultrasonic extraction (DCM-acetone)             | Particle      | HPLC-FL               | LODs: 0.09-1.06 ng/m³ | Hassine et al. (2014)     |
| 6                       | High volume air sampler (PUF, QFF) ASE                                       | Gas and particle | GC-MS                  | No data            | Garrison et al. (2014)    |
| 8                       | Dual E-sampler (QFF) Soxhlet extraction (DCM with n-hexane)                  | Particle      | HPLC-UV               | LODs: 0.001-0.0305 mg/L | Moja et al. (2014)        |
### Table 2 (continued)

| Number of PAHs analyzed | Sample treatment and preparation methods | Sample matrix | Sample analysis method | Method performance | Ref. |
|-------------------------|------------------------------------------|---------------|------------------------|--------------------|------|
| 15                      | High volume air sampler (QFF)            | Particle      | GC-MS                  | LODs: 0.40–38 ng/m³ | Liu et al. (2014) |
|                         | Soxhlet extraction (DCM)                 |               |                        |                    |      |
| 13                      | High volume air sampler (PUF, QFF)       | Gas and particle | GC-MS            | No data           | Osman and Younis (2015) |
|                         | Soxhlet extraction (hexane)              |               |                        |                    |      |
| 20                      | Low volume air sampler (QFF)             | Particle      | GC-MS                  | No data           | Salaudeen et al. (2017) |
|                         | Ultrasonic extraction (DCM)              |               |                        |                    |      |
| 34                      | High volume air sampler (QFF)            | Particle      | GC-MS                  | No data           | Barhoumi et al. (2018) |
|                         | ASE                                      |               |                        |                    |      |
| 15                      | High volume sampler (GFF)                | Particle      | HPLC-FL                | No data           | Kalisa et al. (2018)  |
|                         | Ultrasonic extraction (benzene-ethanol) |               |                        |                    |      |
| Number of nitro-PAHs analyzed | Analytical procedures for nitro-PAHs                                                                 | Sample matrix                                                                 | Analysis method                                                                 | Method performance                                                                 | Ref.                                                                 |
|------------------------------|------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|---------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|----------------------------------------------------------------------|
| 8                            | LPE with dichloromethane, then SPE purified by using aminopropyl SPE cartridges                     | Diesel particulate matter                                                      | GC-EM-MS (gas chromatography electron monochromator-mass spectrometry)          | Recoveries: 4.9-100%; LODs: 0.033-5.11 ng/mg                                      | Havey et al. (2006)                                                    |
| 8                            | No treatment                                                                                         | Soil, motor oil, particles (ashes), urine, and saliva                          | Atmospheric pressure solid analysis probe (ASAP)-Q-TOFMS.                       | Identification and semi-quantitation                                               | Carrizzo et al. (2015)                                                 |
| 16                           | SE with n-hexane-dichloromethane (50:50,v/v)                                                         | Airborne particulates                                                          | HPLC-FL (5% PteAl_2O_3 reduction column); LOD: 0.06-1.25 mg/L                   | Delhomme et al. (2007)                                                            |                                                                     |
| 16 and other PAHs            | SE (dichloromethane-methanol) then SPE purification                                                 | Airborne particulate matter                                                    | GC-MS                                                                            | LOD: 1-10 pg                                                                     | Cochran et al. (2012)                                                  |
| 9 and other PAHs.            | LPE with hexane-methylene chloride (1:4,v/v) and cleaned up using HLB cartridge                    | Airborne particulate matters                                                   | HPLC-APPI-MS/MS; LOD: 3 pg except 2NFl, Recovery: 95.6-109%                     | Lung and Liu (2015)                                                              |                                                                     |
| 6                            | UAE with dichloromethane                                                                            | Particulate matter                                                             | HPLC- UVD                                                                       | No data                                                                          | Lan et al. (2014)                                                     |
| 20 nitro-PAHs and 4 oxy-PAHs | PUF samples: SE with hexane-acetone (1:1, v/v); Other samples: MAE with hexane-acetone (1:1, v/v) | Air samples                                                                    | GC-MS;                                                                          | No data                                                                          | Li et al. (2015)                                                     |
| 5                            | SE with dichloromethane                                                                             | Ultrafine airborne particles (PM1.0)                                            | GC-ECD;                                                                         | No data                                                                          | Garcia et al. (2014)                                                  |
| No. | Methodology | Samples | Analytical Procedures | LODs | Recoveries | References |
|-----|-------------|---------|-----------------------|------|------------|------------|
| 12  | UAE with dichloromethane-methanol-toluene (1:1:1; v/v/v) and cleaned-up with a glass column filled with silica | Air particulate matter, soot | HPLC on-line reduction, and fluorescence detection; | LODs: 0.03-0.5 mg/L; Recoveries: 70-90% | Schauer et al. (2004) |
| 17  | PSE with dichloromethane | Ambient air samples (on both gas and particulate phases) and standard reference material 1649a (urban dust) | GC-NICI-MS | LODs: 0.03-0.07 pg; Recoveries: 14-84% | Albinet et al. (2006) |
| 11  | UAE with n-hexane | PM2.5 | LC-APCI-MS/MS | LOD: 21-83 pg/m³; LOQ: 83-167 pg/m³ | Nyiri et al. (2016) |
| 8   | MAE/UAE | Airborne particulate matter and tree bark | (U)HPLC-MS/MS (APCI) | No data | Fujiwara et al. (2014) |
| 28  | PSE with dichloromethane and purified with amino-propyl SPE cartridge | Air and diesel particulate reference materials | GC-MS | No data | Bamford et al. (2003) |
### Table 4  Comparison of analytical procedures for O-PAHs

| Number of O-PAHs analyzed | Sample treatment and preparation methods | Sample matrix | Analysis method | Method performance | Ref. |
|---------------------------|------------------------------------------|---------------|----------------|-------------------|------|
| 21 oxy-PAHs               | Soxhlet extraction                       | Diesel particulate reference material | GC-MS          | Oxy-PAH LOD: 5-36 pg Derivatized hydroxy-PAHs LOD: 1-21 pg | Cochran et al. (2012) |
| 11 hydroxy-PAHs           |                                          |               |                |                   |      |
| 10                        | Microwave assisted extraction (MAE) with acetonitrile/methyl chloride (9:1) | Sediments     | GC-MS, HPLC-UV-FLD | LOD with derivatization: 90-440 ng/g Without derivatization: 180- > 10 000 ng/g | Berger-Brito et al. (2018) |
| 1 (3-OH-BaP)              | Extraction with toluene Conversion into a methyl ether | Human urine   | GC-APLI-MS     | LOD: 0.6 fg       | Richer-Brockmann et al. (2019) |
| 9 oxy-PAHs                | ASE with acetone and HEX (1:1 v/v ratio) | Sediments     | GC-EI-MS/MS (O-PAHs) LC-ESI-MS/MS (OH-PAHs) | Oxy-PAHs LOD: 0.01-0.1 ng/g Hydroxy-PAHs LOD: 0.003-0.006 ng/g | Han et al. (2019) |
| 5-hydroxy-PAHs            |                                          |               |                |                   |      |
| 2 (OH-PAHs)              | Low-temperature ultrasonication with methanol | Air particulate matter | HPLC-FL | No data | Barrado et al. (2012) |
| 24                        | No treatment                             | Reference material | LC-APCI-MS GC-EI-MS | LC LOD: 0.18-36 ng/ml GC LOD: 2.6-26 ng/ml | O’Connell et al. (2013) |
| 9                         | ASE with dichloromethane                 | Ambient air samples | GC-NICI-MS | LOD: 0.01-2.60 pg | Albinet et al. (2006) |
| 5                         | Soxhlet extraction                       | Ambient air samples | GC-APCI-MS/MS | No data | Lammet et al. (2020) |
| 3                         | Soxhlet extraction with dichloromethane | Air aerosols   | GC-MS         | LOD: 0.0016-0.0030 ng/m³ | Souza et al. (2014) |
wwwby wa reaction with a mixture of BSTFA and TMCS, or by a conversion of the hydroxyl groups to methyl ethers. When using liquid chromatography, there is no need to derivatize the samples. Even due to the increased polarity of these derivatives in relation to the parent compounds, it is not quite simple to separate these compounds; some research points to methanol as a good extractant for O-PAHs. During the analysis of O-PAHs, slightly different temperature conditions are required than in the analysis of PAHs. Therefore, it is difficult to determine all these compounds together; however, some research indicates the possibility of simultaneous determination of nitro- and oxy-derivatives.

It should be noticed that the PAH-derivatives analysis is a topic that recently drew much attention from scientists (Lawal 2017; Tran et al. 2020). The growing number of papers published on this subject shows that this topic is alive and still requires filling some knowledge gaps. Recently, the topic of researching indoor air quality, especially related to education, has emerged (Soggiu et al. 2020; Tripathy-Lang 2021; Reinmuth-Selzle et al. 2017). As regarding air pollution children are a very sensitive group, this topic should be further developed. At the same time, it is worth considering the sampling system, as well as several techniques for reducing the impact of the matrix. Moreover, the knowledge about PAH-derivatives formation mechanisms is significant and should be continuously developed. The interest in this topic is encouraged, along with the resulting increase in awareness not only of scientists, but also decision-makers.

5 Conclusions

The number of analytical methods concerning analysis of PAHs and its derivatives are impressive. However, one should remember that PAH determination methods are tools only for solving the main problem, i.e., an evaluation the health hazards associated with the presence of SPM in air. Thus, methods suitable for reaching this goal should be based on the application of GC or HPLC techniques equipped with relatively high accuracy, but using commonly available detectors. Due to the appearance of these compounds in the environment at very low concentrations, an analyte concentration step has to be applied prior to the analysis. These methods should be relatively simple but repeatable and assuring method LOD below the expected concentrations of PAHs and their derivatives in the environment.

According to the authors’ opinion, there is need for two types of analytical methods: the standard method for environmental monitoring, and a second one suitable for detecting new potential PAH derivatives. The second group of mentioned methods should be based on HPLC or GC but equipped with very sensitive and reliable detectors such as Quadrupole – Time of Flight (Q-TOF). These techniques should be mainly aimed at qualitative analysis.

The main problem when using the GC technique is the need to derivatize OH-PAHs prior to analysis. In our opinion, there is a need to develop methods of simultaneous concentration and derivatization of the sample in order to avoid multi-stage processes. Moreover, a main drawback of several papers, found by authors in this review, i.e., the lack of information concerning LOD of these methods, makes applicability of these very limited.

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**Data Availability** Data sharing not applicable in this article as no datasets were generated during the current study.

**Declarations**

**Competing Interest** The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

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