GC MS/MS METHOD FOR POLYCYCLIC AROMATIC HYDROCARBONS ANALYSIS IN AMBIENT AIR $PM_{2.5}$

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ABSTRACT

A large number of studies have been dedicated to the development of methods for qualitative and quantitative determination of polycyclic aromatic hydrocarbons (PAHs), present in ambient air particulate matter (PM). However, due to the complex matrix and low atmospheric concentrations of PAHs, their assessment is still a challenge for the scientific community. In general, chromatographic techniques, such as GS/MS, are the commonly employed analytical methods for determination of PAHs. A mass spectrometer is typically utilized in full scan or selected ion monitoring (SIM) modes. However, the complexity of the samples and the presence of numerous isomers of alkylated and high molecular mass compounds, could affect the sensitivity and selectivity of mass spectrometric (MS) analysis in SIM mode and be a source of serious experimental errors. To reduce such errors, it is required to include an additional clean-up step and/or to use more selective and even specific detection techniques.

This study proposes a GC MS/MS method in multiple reaction monitoring (MRM) mode for quantification of 16 U.S. Environmental Protection Agency priority PAHs plus coronene, perylene and benzo[e]pyrene in PM with aerodynamic diameter below 2.5 μm. Prior to analysis collected samples were processed through an optimized sample preparation procedure: i) ultrasonicated extraction with an appropriate solvent, ii) drying and cleaning via column chromatography and iii) concentration by nitrogen purging. The accuracy of the method was evaluated by spiking Whatman® QM-A quartz-fiber filters with different working standards containing PAHs analytes. Linearity was assessed based on the coefficient of determination and Fisher test. LODs and LOQs varied, respectively in the range of 0.29 - 0.69 pg m^{-3} and 0.87 - 2.09 pg m^{-3}. The suggested method is compared to a previously developed SIM method and has proven to be more superior.

Keywords: GC MS/MS, selected ion monitoring, multiple reaction monitoring, particulate matter, method validation.

INTRODUCTION

Air pollution is one of the largest environmental risks - it can harm vegetation and ecosystems, and is also detrimental to human health, contributing to chronic and serious other diseases, such as trachea, bronchus and lung cancers, aggravated asthma and lower respiratory infections [1]. Pollutants with the strongest evidence for public health concern include particulate matter (PM), carbon monoxide (CO),

ozone (O_3), nitrogen dioxide (NO_2) and sulfur dioxide (SO_2) [2]. As opposed to atmospheric trace gases which have the same chemical and physical properties wherever they occur, PM represents a complex mixture with widely varying particle sizes, composition and chemical properties [3, 4]. Therefore, airborne PM is the pollutant having by far the largest impact upon human health and it is categorized as the most harmful pollutant in the atmosphere [3, 5, 6].

PM is generally classified into three main groups considering their aerodynamic properties, i.e. coarse particles (aerodynamic diameter less than 10 μm, PM_{10}), fine particles (aerodynamic diameter less than 2.5 μm, $PM_{2.5}$), and ultrafine particles (aerodynamic diameter less than 100 nm or 0.1 μm, $PM_{0.1}$) [4, 7]. Research on PM shows that the particles that have major impact on human health are those with an aerodynamic diameter less than 10 μm and especially those with an aerodynamic diameter less than 2.5 μm [7]. The latter can penetrate deeper into the respiratory tract, leading to deterioration of chronic respiratory diseases such as asthma, bronchitis, and emphysema, and further be absorbed into the bloodstream causing intoxication of other body systems [7 - 10]. However, the toxicity of PM depends not only on their size, but also on their surface properties, chemical composition, and multi-species interactions. Main chemical components in PM fractions include inorganic salts, iron compounds, trace metals and minerals resulting from erosion and destruction of rocks, soils and constructions, elemental carbon, organic carbon and organic compounds [3, 11 - 14].

Because of their high toxicity and diverse health effects, the main focus of many researches is directed toward PM organic composition [14 - 17]. Acknowledged as strongly harmful organic compounds present in PM are aldehydes, ketones, benzene, dioxins, polycyclic aromatic hydrocarbons (PAHs) and their derivatives [18]. Determination of chemical composition of $PM_{2.5}$ is valuable for deriving information about pollution sources and their contribution to ambient air pollution levels, which in turn would be propitious to pollution control and environmental protection. For Bulgaria, studies on the chemical composition of $PM_{2.5}$ are extremely relevant, as so far such have been done only for the city of Sofia and do not cover significant classes of pollutants, incl. PAHs. From this class of pollutants only one representative i.e. benzo[a]pyrene, is regularly measured in the atmospheric air in a relatively small number of places.

PAHs are hydrophobic, lipophilic, toxic and persistent organic compounds ubiquitously spread in the environment, by-products of incomplete combustion or pyrolysis of organic materials and fossil fuels [19 - 25]. Due to their expressed toxic, carcinogenic and mutagenic properties PAHs have been in the spotlight of many researches and have been broadly studied in different matrixes [19, 20, 23 - 25]. Currently, sixteen PAHs are considered to be of greatest concern by the U.S. Environmental Protection Agency (USEPA) and these are quantified in most of the exposure and environmental studies. The EU Directive 2004/107/EC proposed benzo[a]pyrene as a marker for the carcinogenic risk of PAHs in ambient air and set a target value of 1 ng m^{-3} as a total content in the PM_{10} fraction, averaged over a calendar year. Additionally, it advises the monitoring of other relevant PAHs such as benzo[a]anthracene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k] fluoranthene, indeno[1,2,3-c,d]pyrene and dibenzo[a,h] anthracene. However, the existing EU reference method, i.e. EN 15549:2008, allows evaluation only of benzo[a] pyrene as part of the PM_{10} fraction. Thus, adoption of national standards, ISO methods or development of new methods for analysis of a larger number of PM bound PAHs is required.

A vast number of studies have been dedicated to the development of methods for qualitative and quantitative determination of PAHs, present in ambient air PM [19, 26 - 30]. However, analysis of PAHs in ambient air PM is extremely challenging due to the complex matrix, low atmospheric concentrations and volatile nature of the targeted species and require specific pretreatment, extraction, and clean-up procedures before the final instrumental detection. For the collection of airborne PAHs, large volumes of air must be sampled in order to concentrate them on a suitable sorbent material, since their concentration in air is relatively low (of the order of ng m^{-3}) [22]. Most commonly used sampling media for PM associated PAHs include quartz-fiber filters (QFFs), glass fiber filters, Teflon coated glass fiber and Teflon membrane filters [21]. The advantages of QFFs, i.e. higher purity, leading to lower blank levels and higher thermal stability, enabling baking at elevated temperatures for decontamination prior sampling, make them the preferred sampling media [31, 32]. Another

challenging step in PAHs analysis is to extract all compounds of interest with sufficiently good efficiency due to large variations in their physicochemical properties [22]. Various extraction approaches (soxhlet extraction [33 - 35], microwave assisted extraction - MAE [36 - 38], ultrasound assisted extraction - UAE [39, 40], pressurized liquid extraction - PLE [10, 41, 42], etc.), are applied to recover PAHs from the solid phase. Soxhlet extraction is one of the most popular techniques, due to its high extraction efficiency, availability and lower cost [19, 21]. However, this technique is known to be time consuming (8 - 48 h) and requires large amounts of solvent (300 - 500 mL) [21]. The newer extraction protocols are aimed at reducing the volume of solvents used, as well as the time required for sample preparation. Good recoveries have been reported for protocols including PLE [10], MAE [36], UAE [39], cold fiber solid phase microextraction [43]. Solvent-free methods are the new emerging techniques, which rely on the collection of the airborne PAHs on sorbent or filter materials for subsequent direct release by thermal desorption into the analytical instrument [21]. Although these techniques look promising, the data of their efficiency considering the analysis of particle-bound PAHs is still scarce [44].

Due to the variety of matrix interferences, usually a sample clean-up step is applied prior to instrumental analysis. The methodologies used for this purpose may vary widely and depend on the subsequent instrumental method used. Instrumental techniques such as gas chromatography (GC) and high-performance liquid chromatography (HPLC) coupled with suitable detection techniques, have been extensively applied for PAHs analysis [19]. HPLC is usually combined with fluorescence (FLD) or ultraviolet (UV) detectors. HPLC-FLD is much more sensitive and selective than HPLC-UV regarding PAHs determination, but unfortunately some PAHs do not exhibit fluorescence or have low fluorescence detection response (i.e. benzo[ghi] perylene, acenaphthylene) [45, 46]. Although HPLC offers the advantage of shorter run times, qualitative and quantitative determination of PAHs in ambient air samples is generally carried out using GC system because of its greater selectivity, resolution and sensitivity compared to that achieved with HPLC [19, 21, 45, 47]. GC is commonly combined with mass spectrometer (MS) [32, 42, 48] and flame-ionization detector (FID) [49, 50]. GC/MS system is usually preferred over GC-FID, since it's more accurate for the quantification of PAHs, because interferences from coeluting compounds are minimized by the selective nature of the detector [45, 51]. The extensive literature survey indicates that GC/MS is the most utilized system for PM bound PAHs analysis. GC/MS operated in the selected ion monitoring (SIM) mode has advantages over full-scan mode, since it provides sensitive results and even detection of ultra-trace levels, as it reduces matrix effects and interference, and thus greatly simplifies the extracts cleaning step. In our previous study, a methodology for qualitative and quantitative determination of 19 PAHs in PM utilizing GC/MS system operated in SIM mode was developed [18]. The elaborated method was validated in terms of several analytical parameters and successfully applied to urban PM_{10} samples. It is worth noting that although the aforementioned method demonstrated good selectivity and sensitivity in PAHs analysis, for some PM samples, especially those sampled during winter, matrix interferences were observed. Reduction of these effects could be achieved either by improving the sample clean up step or by employing more selective or specific detection technique. In order not to overburden the already labor-intensive sample preparation, our choice was the development of a more selective technique, since GC/MS system available in the laboratory contains MS detector with three quadrupoles connected in series and provides the possibility to perform the so-called tandem mass spectroscopy (GC MS/MS). Comparing triple quadrupole analyzer with single quadrupole analyzer, the former is more specific since it allows generation of product ions from preselected precursor ion, thus providing more accurate quantification and confirmation of compounds of interest in a complex matrix [51, 52].

The objectives of the current study are: *i)* to improve the previously developed GC/MS method in SIM mode via utilizing additional fragmentation step of PAHs of interest, and thus realizing multiple reaction monitoring mode (MRM), or so called GC MS/MS for quantification of 16 USEPA priority PAHs plus coronene, perylene and benzo[e]pyrene in $PM_{2.5}$; *ii)* to validate the developed GC MS/MS method, in order to demonstrate that it is suitable for the quantification of the selected compounds; *iii)* and to compare the results obtained by the application of the developed GC MS/MS method with those from accredited laboratory.

EXPERIMENTAL

Materials

For qualitative and quantitative assessment of 19 PAHs under consideration, the following certified reference materials (CRMs) were used: i) CRM48905 (Supelco) containing 16 PAH compounds, i.e. naphthalene (Naph), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), anthracene (Ant), phenanthrene (Phe), fluoranthene (Fla), pyrene (Pyr), benzo[a]anthracene (BaA), chrysene (Chr), benzo[a] pyrene (BaP), benzo[b]fluoranthene (BbF), benzo[k] fluoranthene (BkF), benzo[g,h,i]perylene (BghiP), indeno[1,2,3-c,d]pyrene (IndP), dibenzo[a,h] anthracene (DahA) with concentration 2000 μg mL^{-1} each in dichloromethane:benzene (1:1), supplied by Sigma-Aldrich; ii) CRMs (Dr. Ehrenstorfer) of coronene (Crn), perylene (Per) and benzo[e]pyrene (BeP) in acetonitrile (ACN) with concentration of 10 μg mL^{-1} purchased from LGC; iii) CRM46955 (Supelco) Internal standard (IS) mixture containing naphthalene-d8 (d8-Naph), acenaphthene-d10 (d10-Ace), phenanthrene-d10 (d10-Phe), chrysene-d12 (d12-Chr), perylene-d12 (d12-Per) with concentration 2000 μg mL^{-1} each in dichloromethane (DCM), supplied by Sigma-Aldrich; iv) CRMs (Dr. Ehrenstorfer) of anthracene-d10 (d10-Ant), fluoranthene-d10 (d10-Fla), benzo[a]pyrene-d12 (d12-BaP), benzo[a]anthracene-d12 (d12-BaA) used as IS as well; v) CRMs (Dr. Ehrenstorfer) of fluorene-d10 (d10-Flu) and pyrene-d10 (d10-Pyr) in ACN with concentration of 10 μg mL^{-1} and 100 μg mL^{-1}, respectively, purchased from LGC and used as recovery standards (lab surrogates). All PAH compounds discussed in the current study are described in Table 1, as the chemical structures of the investigated 19 analytes are illustrated in Fig. 1.

HPLC and GC grade solvents, i.e. DCM, toluene (Tol) and other reagents and materials, i.e. glass wool (silanized), sodium sulfate anhydrous (puriss. p.a. grade) and surface-deactivated via silanization amber vials to minimize potential PAHs sorption, were purchased from Sigma-Aldrich.

Extraction and clean up

All blank and sample filters were prepared for subsequent analysis through a previously optimized sample preparation procedure, which consist of ultrasonicated extraction with an appropriate organic solvent [18]. Shortly, the filters were spiked with 200 μL of 100 pg μL^{-1} of the two lab surrogates and immersed in 10 mL of organic solvent, i.e. DCM, and sonicated for 30 min in an ultrasonic bath at ambient temperature. The obtained extracts were subsequently dried and cleaned via column chromatography technique, as the column was packed with glass wool and 0.5 g of anhydrous sodium sulphate. The cleaned extracts were further concentrated by nitrogen purging to about 300 - 500 μL, as prior to the concentration step a few drops of Tol were added to each sample as PAH keeper. The cleaned and concentrated extracts were spiked with 300 μL of 100 pg μL^{-1} IS mixture (See Table 1 for list of IS) and diluted to exactly 1000 μL prior to GC MS/MS analysis.

GC MS/MS analysis

GC MS/MS system (Thermo Scientific Trace 1300/TSQ 8000) was employed for PAHs identification and quantification. A capillary column containing 5 % diphenyl, 95 % dimethylpolysiloxane TG-5ms (30 m × 0.25 mm × 0.25 μm) was used for separating PAHs. Helium with high purity (99.999 %) was used as a carrier gas at a constant flow of 1.2 mL min^{-1}. A volume of 1 μL was injected in splitless mode at inlet temperature of 280°C. Initial column temperature was 60°C with 1 min hold, then increased up to 120°C at ramp rate of 20°C min^{-1}, held for 1 min, and then increased up to 300°C at ramp rate of 10°C min^{-1} and held for 15 min at the final temperature. The mass spectrometer was operated in electron impact ionization mode at 70 eV. The ion source and transfer line temperatures were 250°C and 270°C, respectively. PAHs quantification was carried out in MRM mode and argon (99.999 %) was used as collision gas. In order to determine the retention times of PAHs of interest and to select precursor ions for MRM optimization, full scan (m/z = 40 - 350) was performed. Then, product ion spectra were acquired by collision-induced dissociation with argon. Collision energies (CEs) from 0 to 50 eV were applied. The product ions selected were the ions with the highest m/z ratio (increase in selectivity) and abundance (increase in sensitivity). With this procedure the MRM method with three transitions per each PAH was developed, two transitions for qualification (MRM1 and MRM2) and a transition for quantification (MRM3).

Table 1. Summary of PAHs of interest (including internal and recovery standards) and MRM transitions with corresponding collision energies (CE).

№	Compound	Abbreviation	Formula	MW	IS Group	MRM Method					
						MRM1	CE	MRM2	CE	MRM3	CE
1	Naphthalene - d8	*d8-Naph*	$C_{10}D_8$	136	1	136.1 > 84.1	25	136.1 > 108.1	20	136.1 > 134.1	15
2	Naphthalene	*Naph*	$C_{10}H_8$	128	1	128.1 > 78.0	20	128.1 > 102.0	20	128.1 > 127.1	15
3	Acenaphthylene	*Acy*	$C_{12}H_8$	152	2	152.1 > 126.0	25	152.1 > 150.1	30	152.1 > 151.1	20
4	Acenaphthene - d10	*d10-Ace*	$C_{12}D_{10}$	164	2			164.2 > 160.1	30	164.2 > 162.1	15
5	Acenaphthene	*Ace*	$C_{12}H_{10}$	154	2	153.1 > 151.1	30	153.1 > 152.1	20	154.1 > 153.1	15
6	Fluorene - d10	*d10-Flu*	$C_{13}D_{10}$	176	2	174.1 > 170.1	30	174.1 > 172.1	20	176.2 > 174.1	20
7	Fluorene	*Flu*	$C_{13}H_{10}$	166	2	165.1 > 115.0	25	165.1 > 164.1	20	166.1 > 165.1	15
8	Phenanthrene - d10	*d10-Phe*	$C_{14}D_{10}$	188	3	188.1 > 158.1	35	188.1 > 160.1	20	188.1 > 184.1	30
9	Phenanthrene	*Phe*	$C_{14}H_{10}$	178	3	178.1 > 152.1	20	178.1 > 176.1	25	178.1 > 177.1	15
10	Anthracene - d10	*d10-Ant*	$C_{14}D_{10}$	188	4	188.1 > 158.1	35	188.1 > 160.1	20	188.1 > 184.1	25
11	Anthracene	*Ant*	$C_{14}H_{10}$	178	4	178.1 > 151.1	30	178.1 > 152.1	20	178.1 > 176.1	25
12	Fluoranthene - d10	*d10-Fla*	$C_{16}D_{10}$	212	5			212.1 > 208.1	35	212.1 > 210.1	20
13	Fluoranthene	*Fla*	$C_{16}H_{10}$	202	5	202.1 > 152.1	30	202.1 > 200.1	30	202.1 > 201.1	20
14	Pyrene - d10	*d10-Pyr*	$C_{16}D_{10}$	212	3			212.1 > 208.1	35	212.1 > 210.1	25
15	Pyrene	*Pyr*	$C_{16}H_{10}$	202	3	202.1 > 199	45	202.1 > 200.1	35	202.1 > 201.1	20
16	Benzo[a]anthracene - d12	*d12-BaA*	$C_{18}D_{12}$	240	6			240.2 > 212.2	25	240.2 > 236.1	30
17	Benzo[a]anthracene	*BaA*	$C_{18}H_{12}$	228	6	228.1 > 202.1	25	228.1 > 226.1	30	228.1 > 227.2	15
18	Chrysene - d12	*d12-Chr*	$C_{18}D_{12}$	240	7	240.2 > 212.1	25	240.2 > 236.1	30	240.2 > 238.1	15
19	Chrysene	*Chr*	$C_{18}H_{12}$	228	7	228.1 > 202.1	25	228.1 > 226.1	30	228.1 > 227.1	15
20	Benzo[b]fluoranthene	*BbF*	$C_{20}H_{12}$	252	8	252.1 > 226.1	25	252.1 > 250.1	30	252.1 > 251.2	20
21	Benzo[k]fluoranthene	*BkF*	$C_{20}H_{12}$	252	8	252.1 > 226.1	25	252.1 > 250.1	30	252.1 > 251.2	20
22	Benzo[e]pyrene	*BeP*	$C_{20}H_{12}$	252	8	252.1 > 226.1	30	252.1 > 250.1	30	252.1 > 251.2	15
23	Benzo[a]pyrene - d12	*d12-BaP*	$C_{20}D_{12}$	264	8			264.2 > 236.2	25	264.2 > 260.2	35
24	Benzo[a]pyrene	*BaP*	$C_{20}H_{12}$	252	8	252.1 > 226.1	25	252.1 > 250.1	30	252.1 > 251.2	20
25	Perylene - d12	*d12-Per*	$C_{20}D_{12}$	264	9			264.2 > 236.2	25	264.2 > 260.2	35
26	Perylene	*Per*	$C_{20}H_{12}$	252	9	252.1 > 226.1	25	252.1 > 250.1	30	252.1 > 251.2	15
27	Indeno[1,2,3-cd]pyrene	*IndP*	$C_{22}H_{12}$	276	8	276.1 > 273.1	50	276.1 > 274.1	35	276.1 > 275.1	20
28	Dibenzo[a,h]anthracene	*DahA*	$C_{22}H_{14}$	278	8	278.1 > 274.1	50	278.1 > 276.1	30	278.1 > 277.2	20
29	Benzo[ghi]perylene	*BghiP*	$C_{22}H_{12}$	276	8	276.1 > 272.1	50	276.1 > 274.1	40	276.1 > 275.1	20
30	Coronene	*Crn*	$C_{24}H_{12}$	300	8			300.1 > 298.1	45	300.1 > 299.1	25

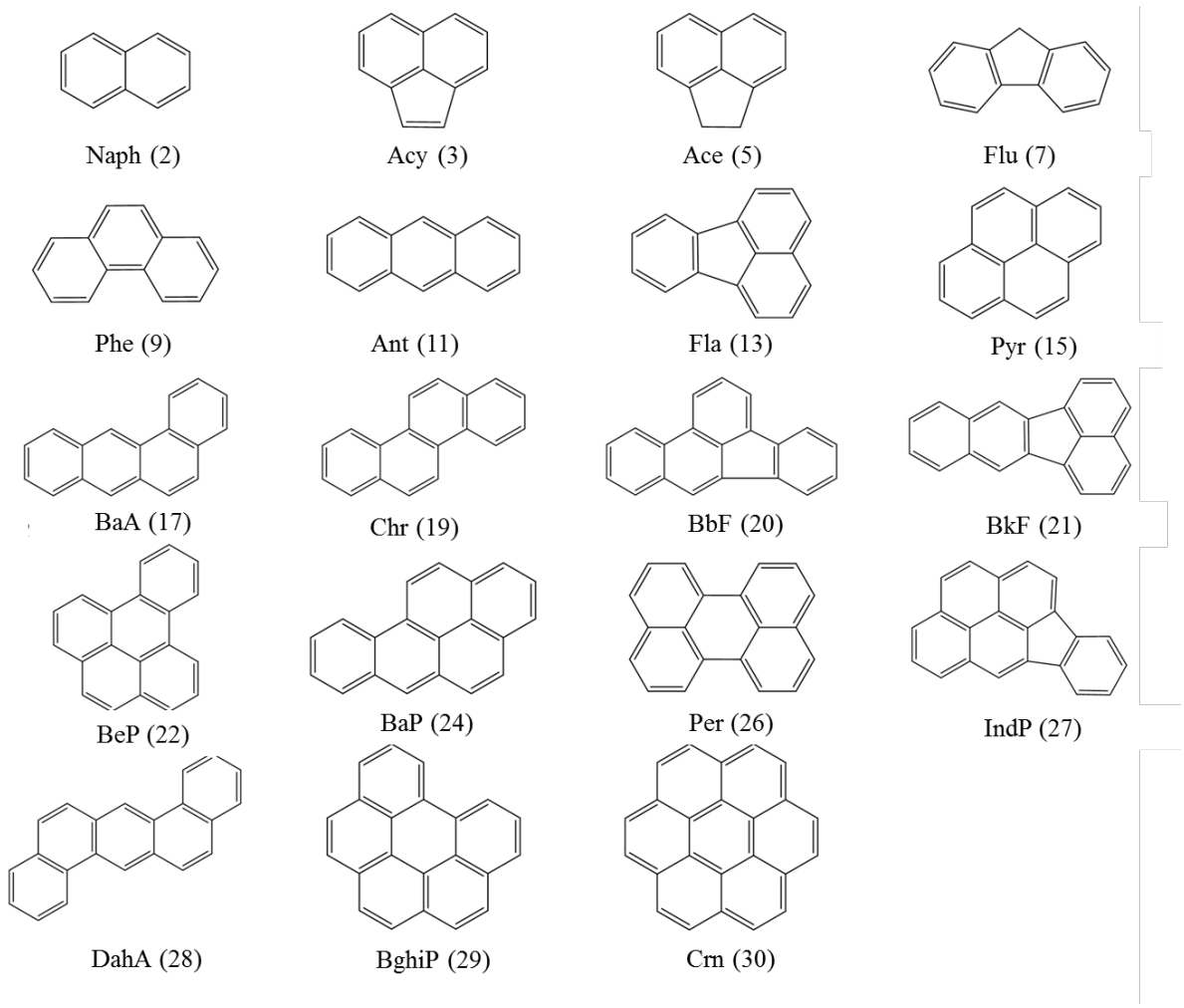


Fig. 1. Chemical structure of the investigated PAHs (numbering according to Table 1).

Data analysis

To confirm the identity of each individual PAH contained in the samples, the ratios of the intensities of the confirmation transitions (MRM1 and MRM2) to the quantification transition (MRM3) were used. The identity of each peak was confirmed by comparing the experimental ratios of the samples with the theoretical ratios of the reference standards. Briefly, the following deviations were accepted for confirmation: ± 20 % (for relative intensities greater than 50 %); ± 25 % (for relative intensities of 20 % - 50 %); ± 30 % (for relative intensities of 10 % - 20 %,) and ± 50 % (for relative intensities lower than 10 %). Furthermore, the ratio of the chromatographic retention time of the analyte to that of the internal standard, i.e. the relative retention time of the analyte, shall correspond to that of the calibration solution at a tolerance of ± 0.5 %. These criteria are in accordance with the 2002/657 European Commission Decision [53].

Quantification of the analytes of interest was performed by the internal standard method using a ten-point (each point analyzed in triplicate) calibration graph in the concentration range of 0.1 - 100 ng mL^{-1}. All calibration standards were prepared by appropriate dilution of the stock standards in DCM and contained recovery standards, i.e. d10-Flu and d10-Pyr. The amount of the IS in the prepared calibration solutions was 30 ng mL^{-1} each.

Method validation

The objective of analytical method validation is to demonstrate that it is suitable for its intended purpose [54].

The word "validation" implies that something has been proven to be true, useful, and acceptable as a standard. The International Organization for Standardization defines validation as confirmation by verification and the provision of objective evidence that certain requirements for a particular purpose have been met [55]. To ensure the applicability of the developed method during the analysis of real samples several analytical parameters were evaluated, i.e. linearity, sensitivity, selectivity, limit of detection (LOD), limit of quantification (LOQ), accuracy (in terms of trueness and precision).

Linearity of the derived in the current study calibration curves was initially evaluated based on the coefficient of determination (R^2). However, sole use of R^2 is not recommended as a means to demonstrate linearity and therefore appropriate statistical methods also should be applied to evaluate linear relationship [56, 57]. In this study the analysis of variance (ANOVA) was utilized for testing the statistical significance of the regression model and acceptability of the linearity of the calibration function. The used criterion was at a given level of significance (in this case 0.05), or Significance F to be lower than 0.05.

In the current study sensitivity was estimated based on the change in the analytical response divided by the corresponding change in analyte concentration and expressed by the slope of the calibration curve, while the selectivity of the method was assessed by comparing the separation of the chromatographic peaks of investigated PAHs, i.e. native and deuterated, in the MRM chromatograms of spiked blanks and real $PM_{2.5}$ sample extracts.

The determination of LOD and LOQ could be carried out by several approaches [57], as the one employed herein was a function of the standard deviation (SD) of the intercept versus the slope of the calibration curve, as follows:

$$LOD = 3.3 \times \frac{SD\ of\ the\ intercept\ of\ the\ regression}{slope\ of\ regression\ curve}, pg\ \mu L - 1 \quad (1)$$

$$LOQ = 10 \times \frac{SD\ of\ the\ intercept\ of\ the\ regression}{slope\ of\ regression\ curve}, pg\ \mu L - 1 \quad (2)$$

Trueness and precision are estimated via spiking blank filters with the compounds of interest, including the two recovery standards. Spikes were made at three different concentration levels, covering the calibration range (5 pg μL^{-1}, 40 pg μL^{-1} and 80 pg μL^{-1}), with five replications per concentration. The recoveries of the target compounds were used as an indication of the trueness of the method, whereas precision was represented as the relative standard deviation (RSD) of the concentrations of the five replicates at each concentration level. For trace level analysis, acceptable recoveries are in the range 60 % - 120 % [53, 58].

As a final "check" of the elaborated method, interlaboratory study was performed. The results obtained by the current protocol are compared with those from accredited laboratory of the Institute for Medical Research and Occupational Health, Environmental Hygiene Unit, located in Zagreb, Croatia. Objects of analysis were two: one ready to use quality control (QC) sample containing PAHs of interest with concentration 50 ppb each; and two identical $PM_{2.5}$ samples, from parallel sampling. The $PM_{2.5}$ samples were collected in accordance with EN 12341:2014 on Whatman® QM-A quartz filters, 47 mm for 24 h, using certified air sampler DadoLAB with flow rate of 2.3 N m^3 h^{-1}, which corresponds to approximately 55 m^3 sampled air for 24 h. All collected samples were stored at 3°C - 4°C until analysis. Prior to sampling all quartz filters were baked at 500°C utilizing a muffle furnace for at least 5 h, cooled down to room temperature and weighed to the nearest 0.0001 g. After sampling, the $PM_{2.5}$ loaded filters are conditioned as recommended by EN 12341:2014 and weighed to determine the mass of $PM_{2.5}$ and respectively its concentration in ambient air. The averaged $PM_{2.5}$ mass and concentration from the parallel sampling are 1.01 (SD = 0.01) mg and 18.30 (SD = 0.20) µg m^{-3}, respectively.

Each laboratory performed their own protocol for extraction and quantification of PAHs. The extraction method employed in this study is described in details elsewhere [18]. The methodology utilized by the accredited laboratory is as follows: the $PM_{2.5}$ sample is extracted via ultrasonic extraction in hexane, as prior to extraction the surrogate standard solution of d12-Per is added to the sample and laboratory blanks. After concentration of the extracts to dryness, deuterated ISs are added and then the mixture is dissolved in 1 mL of hexane and analyzed through GC MS/MS.

RESULTS AND DISCUSSION

Method development

The development of the MRM method involved the following main steps: *i*) precursor ion selection; *ii*) identification of product ions; and *iii*) optimization of MRM transitions. In order to identify the retention times of compounds of interest and their precursor ions, an analysis of a standard solution, containing all PAHs, including deuterated internal standards and lab surrogates, was carried out in Full Scan mode under the previously established optimal chromatographic conditions. The obtained chromatogram was processed with specialized software (AutoSRM), which provided information on the intensity of ions in the mass spectrum of a given compound and based on the highest m/z ratio and intensity, the precursor ion was selected. Further, the selected precursor ions were subjected to collision-induced dissociation in the second quadrupole at varying collision energies (typically between 0-50 eV). The results obtained from this step gave information about the generated product ions from a specific precursor ion (intensity, area, used collision energy). For each compound three product ions were selected based on the highest m/z ratio (increasing selectivity) and intensity (increasing sensitivity). The transitions identified in the last stage, which would be used in the MRM mode, were subjected to optimization, which includes determination of the optimal collision energy for a given transition. In fact, the tracking of three product ions for each analyte in combination with the precursor ion is the fingerprint assuring the specificity of the MRM method.

With this procedure, MRM method with three transitions for each PAH was developed, a quantitative transition (MRM3) and two qualitative transitions (MRM1 and MRM2). The parameters of the developed MRM method are summarized in Table 1.

Ion ratio stability

Confirmation of compounds detected in samples was carried out through monitoring the specific product ions selected per compound, and more precisely through tracking the ion ratio between qualifier and quantifier ions. Stability of ion ratios is essential for any mass spectrometer in a routine laboratory setting in order to safeguard against false positive results [59]. In order to determine how stable the measurements are, ion ratio of each analyte and its potential deviation from the initially determined value was calculated.

In the developed method, all compounds had three transitions (MRM1, MRM2 and MRM3) except for Crn, and the three ions had been monitored in the samples, blanks, and standards. Throughout the complete series of calibration curves and analyzed blanks and $PM_{2.5}$ samples, the ion ratios (MRM1/MRM3 and MRM2/MRM3) were calculated. The average value of the calculated ratios and the relative standard deviations for a time period of two years and more than 300 injections are shown in Table 2. The ion ratio precision is within the acceptable limits as stated by the 2002/657 European Commission Decision and demonstrated accurate confirmation in both samples and standard injections across the concentration range [53].

Method validation

The first aspect of any analytical method validation are the selectivity/specificity establishment and assessing the linearity of the calibration range. Selectivity of SIM has been previously demonstrated [18]. However, as mentioned earlier, some samples (those collected in winter), exhibited high number of interferences, expressed as coeluting peaks and high background noise levels at elevated GC temperatures. This, in turn, resulted in poor peak identification and quantitation, especially at the lowest calibration levels. The improved selectivity of the developed MRM method can be demonstrated via comparing chromatograms of samples collected in winter in the urban area of Burgas and analyzed utilizing both methods (SIM and MRM, Fig. 2). As can be seen in Fig. 2 the coeluting with Ace and Flu peaks in SIM mode had disappeared in MRM mode and the mentioned PAHs are not interfered from other components. A comparison between Full Scan, SIM and MRM modes is represented in Fig. 3. The intensive peaks of different organic compounds extracted from the sample disguise PAHs of interest in Full Scan mode, while in SIM mode most of those interfering compounds are eliminated, but higher background noise is observed for retention times above 20 min. The latter could be due to accumulation of sample deposits in the column/injector and/or matrix effects which are highly probable since winter samples are prone to have much more “impurities”. In MRM these disadvantages are overcome, and background noise is significantly reduced. This is further confirmed by the

Table 2. Ion Ratio variation.

Compound	Quantifier ion (MRM3)	Qualifier Ions (MRM1 and MRM2)	Average Ion Ratio	RSD, %	Allowed deviation [53]
Naph	127.1	78.0	0.141	12.8	30
		102.0	0.255	9.5	25
Acy	151.1	126.0	0.126	10.9	30
		150.1	0.288	7.0	25
Ace	153.1	151.1	0.432	9.4	25
		152.1	0.841	9.0	20
Flu	165.1	115.0	0.085	12.5	50
		164.1	0.575	12.2	20
Phe	177.1	152.1	0.227	16.7	25
		176.1	0.301	11.3	25
Ant	176.1	151.1	0.886	11.6	20
		152.1	0.591	13.7	20
Fla	201.1	152.1	0.049	15.8	50
		200.1	0.466	11.8	25
Pyr	201.1	199.0	0.231	8.0	25
		200.1	0.500	7.3	20
BaA	227.2	202.1	0.055	18.6	50
		226.1	0.443	14.3	25
Chr	227.1	202.1	0.065	18.6	50
		226.1	0.533	12.8	20
BbF + BkF*	251.2	226.1	0.045	21.2	50
		250.1	0.474	15.4	25
BeP	251.2	226.1	0.044	19.1	50
		250.1	0.422	13.8	25
Bap	251.2	226.1	0.055	21.1	50
		250.1	0.509	16.4	20
Per	251.2	226.1	0.028	16.4	50
		250.1	0.469	17.3	25
IndP	275.1	273.1	0.357	18.6	25
		274.1	0.522	15.9	20
DahA	277.2	274.1	0.178	21.7	30
		276.1	0.554	14.7	20
BghiP	275.1	272.1	0.115	9.3	30
		274.1	0.537	9.7	20
Crn	299.1	298.1	0.627	8.8	20

**Sum of BbF and BkF*

comparison of signal-to-noise ratios of these two modes via analyzing PAH compounds with concentration 1ng mL^{-1}, the results are listed in Table 3. It is evident that the signal-to-noise ratios of individual PAHs gained with MRM mode are superior to those of SIM.

In Table 4 are summarized linearity, evaluated based on coefficient of determination, R^2, together with the validation parameters directly calculated from calibration curve (i.e. LOD, LOQ and sensitivity). For comparison purposes validation parameters of previously developed

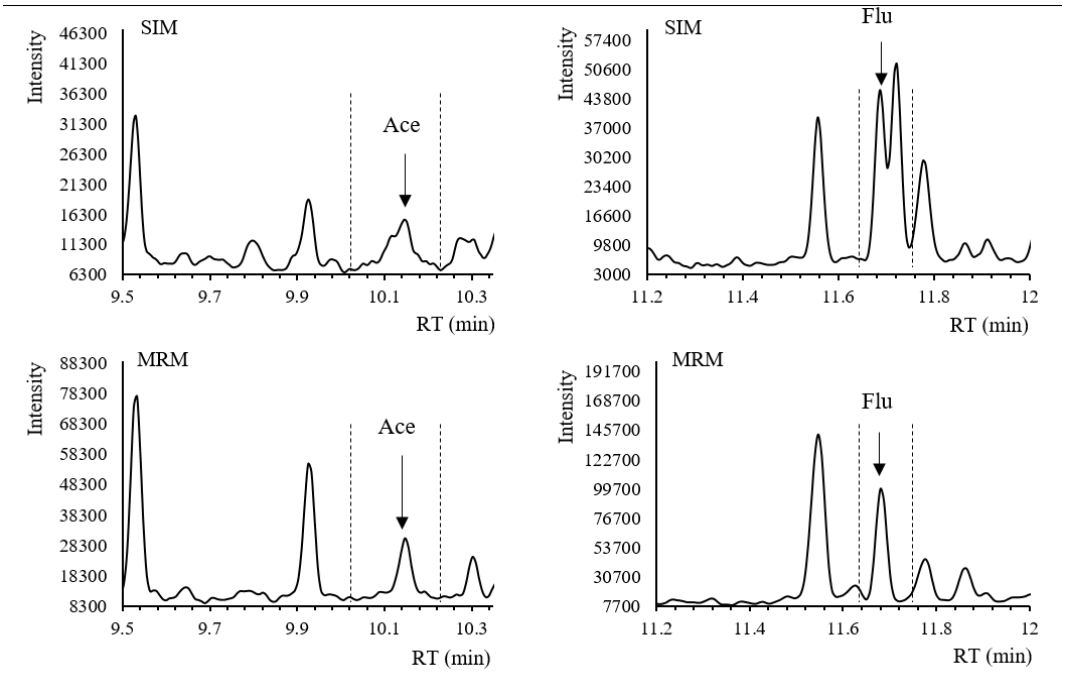


Fig. 2. Comparison of SIM and MRM modes.

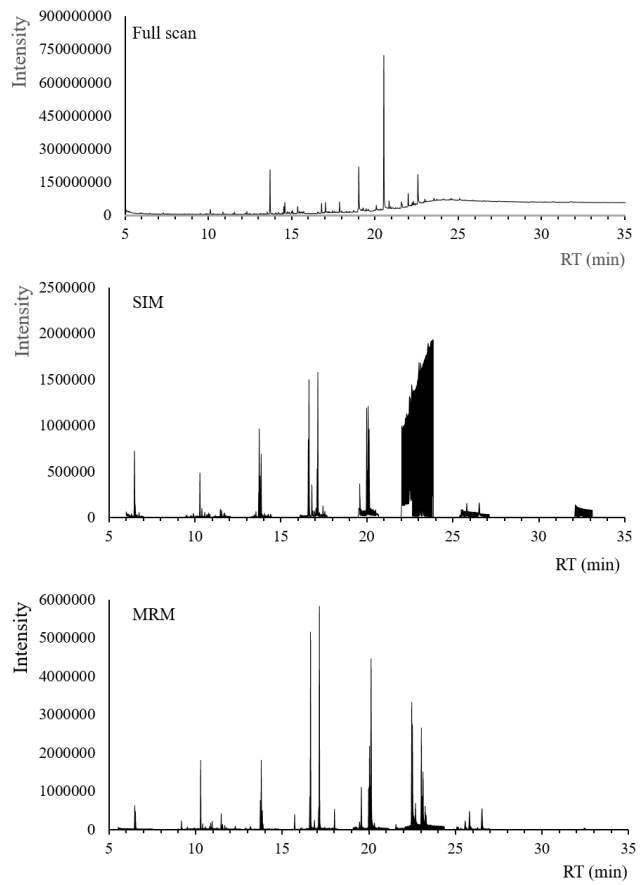


Fig. 3. Comparison between Full Scan, SIM and MRM modes of a real sample collected in an urban area of Burgas.

Table 3. Signal-to-noise ratios of PAHs determined using SIM and MRM modes.

Compound	Signal-to-noice ratios at level 1ng mL^{-1}	
	SIM	MRM
Naph	138	976
Acy	130	774
Ace	84	547
Flu	101	922
Phe	61	975
Ant	108	372
Fla	155	820
Pyr	45	885
BaA	89	272
Chr	55	345
BbF + BkF*	14	137
BeP	22	106
BaP	17	95
Per	18	154
IndP	< 10	169
DahA	25	131
BghiP	82	246
Crn	60	227

**Sum of BbF and BkF*

SIM method are also included [18]. It can be seen that in the studied concentration range, the calibration graph is certainly linear with a coefficient of determination above 0.999 for all investigated PAHs. The analysis of variance (ANOVA) for all PAHs under consideration indicates that Significance $F < 0.05$, which means that the relationship is statistically significant at the chosen significance level of 0.05.

The presented in Table 4 LOD and LOQ values of the investigated PAHs are expressed in pg μL^{-1}. Both LOD and LOQ values of the developed GC MS/MS instrumental method were calculated through equations (1) and (2), respectively, as LOD values of the analytes varied in the range of 0.016 - 0.038 pg μL^{-1}, while LOQ values were in the range of 0.048 - 0.115 pg μL^{-1}. LOD and LOQ of entire method, assuming that 55.0 m^3 of air (at standard conditions) are sampled for 24 h, varied in the range of 0.29 - 0.69 pg m^{-3} and 0.87 - 2.09 pg m^{-3}, respectively. Apparently, the elaborated MRM method is distinguished by lower LOD and LOQ values compared to previously developed by us SIM method (Table 4, [18]) and other proposed in the literature methods [42, 48, 60, 61].

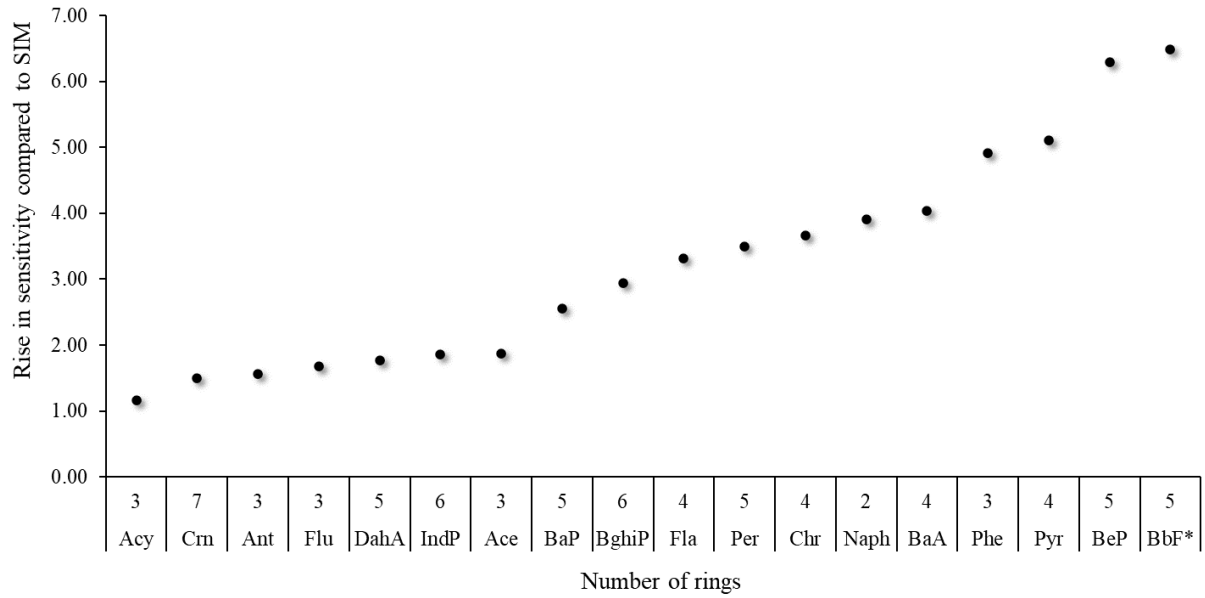
Considerable differences are noticeable in the sensitivity of the developed MRM method. The lowest sensitivity is registered for deuterated recovery standards as found in Naydenova et.al [18]. Generally, considering PAHs of interest, the rise in sensitivity in this instrumental method compared to the SIM method is doubled for 3-ring PAHs and risen up to six times for 5-ring PAHs (Fig. 4). The rise in sensitivity is expected since background and matrix noises are reduced as MRM allows only selected product ions to pass through the third quadrupole, which in turn results in higher signal-to-noise rations as stated earlier.

The accuracy in terms of trueness (recovery) and precision (RSD) of the extracted filters are summarized in Table 5. Accuracy data of PAHs studied at 40 and 80 ppb concentration levels are within acceptable limits, i.e.

Table 4. Validation parameters.

Compound	MRM					SIM [18]			
	Linearity (R^2)	Significance F	LOD pg μL^{-1}	LOQ pg μL^{-1}	Sensitivity	Linearity (R^2)	LOD pg μL^{-1}	LOQ pg μL^{-1}	Sensitivity
Naph	0.9995	3.9×10^{-31}	0.025	0.077	4.124	0.9998	0.013	0.040	1.055
Acy	0.9995	2.5×10^{-31}	0.025	0.075	1.683	0.9995	0.024	0.073	1.452
Ace	0.9996	5.3×10^{-32}	0.023	0.069	1.942	0.9998	0.013	0.040	1.039
Flu	0.9998	8.7×10^{-35}	0.016	0.048	1.985	0.9982	0.045	0.138	1.181
Phe	0.9994	2.4×10^{-30}	0.028	0.085	5.225	0.9997	0.019	0.058	1.063
Ant	0.9997	1.1×10^{-33}	0.018	0.055	1.600	0.9995	0.023	0.070	1.021
Fla	0.9995	4.2×10^{-31}	0.025	0.077	3.888	0.9993	0.027	0.083	1.173
Pyr	0.9997	1.2×10^{-32}	0.021	0.063	4.433	0.9977	0.051	0.155	0.868
BaA	0.9996	2.0×10^{-29}	0.022	0.068	3.347	0.9992	0.030	0.091	0.828
Chr	0.9996	4.9×10^{-31}	0.022	0.066	2.903	0.9989	0.036	0.109	0.793
BbF + BkF*	0.9991	9.9×10^{-32}	0.020	0.060	12.407	0.9984	0.049	0.148	1.625
BeP	0.9995	8.0×10^{-28}	0.028	0.086	11.722	0.9970	0.059	0.178	1.863
BaP	0.9993	1.9×10^{-23}	0.038	0.115	3.409	0.9981	0.047	0.143	1.336
Per	0.9993	1.9×10^{-23}	0.038	0.115	4.797	0.9968	0.070	0.213	1.371
IndP	0.9995	2.4×10^{-29}	0.027	0.082	1.765	0.9968	0.060	0.183	0.952
DahA	0.9995	9.7×10^{-30}	0.026	0.078	1.816	0.9975	0.053	0.161	1.023
BghiP	0.9992	8.1×10^{-28}	0.033	0.101	4.218	0.9991	0.032	0.097	1.435
Crn	0.9998	4.1×10^{-32}	0.019	0.057	2.592	0.9990	0.036	0.109	1.723

**Sum of BbF and BkF*



**Sum of BbF and BkF*

Fig. 4. Rise in sensitivity of MRM mode compared to previously developed SIM mode.

Table 5. Accuracy and precision data for investigated PAHs.

№	Compound	5ppb		40ppb		80ppb	
		Recovery, %	RSD, %	Recovery, %	RSD, %	Recovery, %	RSD, %
1	Naph	92.4	16.3	75.3	15.9	118.9	14.2
2	Acy	50.6	11.4	48.7	11.8	46.6	9.1
3	Ace	85.4	2.4	70.6	10.2	66.1	5.8
4	Flu	80.6	16.5	110.5	4.4	99.1	4.9
5	Phe	81.6	24.2	69.1	10.2	65.5	8.5
6	Ant	91.0	9.5	76.3	9.6	77.3	6.6
7	Fla	85.6	12.4	66.9	7.8	67.8	6.8
8	Pyr	75.3	9.7	63.3	13.4	68.7	12.1
9	BaA	83.4	15.9	72.6	11.0	72.8	8.6
10	Chr	103.3	16.2	80.4	11.3	77.3	8.5
11+12	BbF + BkF*	116.6	13.7	105.5	17.1	101.7	9.5
13	BeP	115.3	19.1	90.7	15.1	88.3	9.8
14	BaP	103.3	9.2	87.9	14.1	84.3	3.9
15	Per	86.4	9.3	89.9	9.5	87.6	4.3
16	IndP	95.2	22.0	81.7	12.4	79.7	4.6
17	DahA	90.8	23.6	85.2	11.4	82.5	4.9
18	BghiP	77.1	18.2	73.1	13.7	71.8	6.4
19	Crn	52.9	20.1	61.3	18.5	62.0	7.1

**Sum of BbF and BkF*

Table 6. Interlaboratory comparison.

Compound	QC sample			Real $PM_{2.5}$ sample		
	Burgas	Zagreb	RSD, %	Burgas	Zagreb	RSD, %
	Concentration, pg μL^{-1}			Concentration, pg μL^{-1}		
Pyr	44.94	62.66	18.03	8.57	8.76	6.82
BaA	49.67	61.24	12.87	5.86	5.60	8.29
Chr	50.52	57.37	7.18	8.00	8.26	7.27
BbF + BkF*	98.66	121.20	12.69	22.75	28.37	13.04
BeP	46.64	30.55	25.89	6.18	8.03	16.22
BaP	51.97	49.44	3.02	11.63	10.12	7.88
IndP	48.13	60.08	12.34	16.53	16.09	3.03
DahA	62.47	62.64	2.75	3.35	2.72	14.35
BghiP	49.25	59.99	11.47	11.05	16.09	21.46

**Sum of BbF and BkF*

trueness 60.0 % - 120.0 % and precision ≤ 20.0 %. An exception at these two concentration levels is observed only for Acy, characterized by a trueness outside of the permissible values, although relatively good precision is observed. With regards to accuracy data at the lowest concentration level of 5 ppb, quite low and outside of the permissible recoveries for Acy and Crn, and unacceptable precision for IndP, DahA and Crn are registered. In fact, largest deviations of precision are observed for the lowest concentration level, probably due to evaporation losses. Generally, lower recoveries are noticed for low molecular mass PAHs (with some exceptions), since they have higher vapor pressure and are prone to easily evaporate.

Interlaboratory Study

Results from the interlaboratory comparison are shown in Table 6. It should be mentioned that the laboratory of the Institute for Medical Research and Occupational Health, Environmental Hygiene Unit, located in Zagreb, Croatia, is accredited in the analysis of ambient air PAHs, i.e. Pyr, BaA, Chr, BbF, BkF, BeP, BaP, IndP, DahA, BghiP.

Accuracy results, expresses as RSD between the two pairs of experimental data amongst the participating labs, are within the acceptable limits and even better for $PM_{2.5}$ sample. In as much as B[a]P is used as a marker for the carcinogenic risk of PAHs in ambient air according to Directive 2004/107/EC it could be highlighted that it is quantified with high accuracy in both, the QC and $PM_{2.5}$ samples [62].

As a result of the conducted study, it can be summarized that the ameliorated method for qualitative and quantitative analysis of $PM_{2.5}$ bound 19 PAHs is distinguished by the following advantages: it allows analysis of extremely limited amounts of sample (approximately 2 - 3 mg), short extraction times with sufficient recovery rates and precision, low consumption of solvents and reagents, simplified sample preparation, as meanwhile provides high sensitivity, selectivity and low detection and quantification limits.

CONCLUSIONS

In the current study an optimized procedure for qualitative and quantitative determination of 16 USEPA priority PAHs plus coronene, perylene and benzo[e] pyrene in $PM_{2.5}$ samples is elaborated via utilizing GC MS/MS method in MRM mode. The proposed methodology is validated in terms of several analytical parameters and combines advantages as analysis of tremendously small quantities of sample, short extraction times, low consumption of solvents and reagents, simplified sample preparation as meanwhile provides high sensitivity, selectivity, accuracy and low detection and quantification limits. The proven linear dynamic range of the optimized method is 0.1 - 100 pg μL^{-1}

with $R^2 > 0.999$ and Significance $F < 0.05$. In regard to selectivity and sensitivity, MRM mode has proven to be superior to SIM mode. Accuracy data of the derived analytical method are within acceptable limits for the studied concentration range, i.e. trueness 60.0 - 120.0 % and precision < 20.0 % (except for Acy and Crn). Interlaboratory comparison showed that the results of the analyzed QC and real $PM_{2.5}$ samples are also within the eligible limits.

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