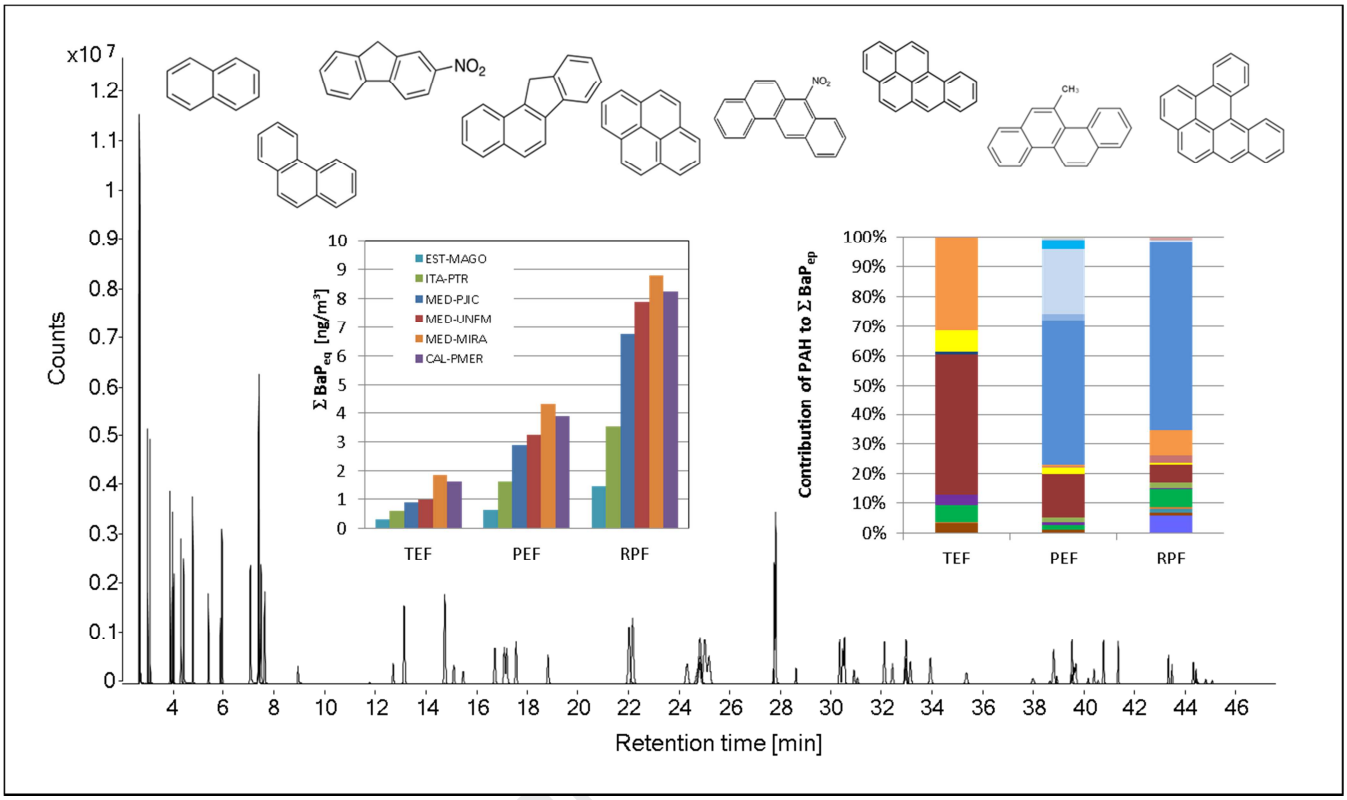
Graphical abstract



Characterization of a multianalyte GC-MS/MS procedure for detecting and quantifying polycyclic aromatic hydrocarbons (PAHs) and PAH derivatives from air particulate matter for an improved risk assessment

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Abstract

A correct description of the concentration and distribution of particle bound polycyclic aromatic hydrocarbons is important for risk assessment of atmospheric particulate matter. A new targeted GC-MS/MS method was developed for analyzing 64 PAHs including compounds with a molecular weight >300, as well as nitro-, methyl-, oxy- and hydroxyl derivatives in a single analysis. The instrumental LOD ranged between 0.03 – 0.7 pg/µL for PAHs, 0.2 – 7.9 pg/µL for hydroxyl and oxy PAHs, 0.1 – 7.4 pg/µL for nitro PAHs and 0.06 -0.3 pg/µL for methyl-PAHs. As an example for the relevance of this method samples of PM_{10} were collected at six sampling sites in Medellin, Colombia, extracted and the concentration of 64 compounds was determined. The 16 PAHs from the EPA priority list contributed only from 54 % to 69 % to the sum of all analyzed compounds, PAH with high molecular weight accounted for 8.8 % to 18.9 %. Benzo(a)pyrene equivalents (BaP_{eq}) were calculated for the estimation of the life time cancer (LCR). The LCR according to the samples ranged from 2.75×10^{-5} to 1.4×10^{-4} by a calculation with toxic equivalent factors (TEF) and 5.7×10^{-5} to 3.8×10^{-4} with potency equivalent factor (PEF). By using the new relative potency factors (RPF) recommended by US Environmental Protection Agency (U.S.EPA) the LCR ranged from 1.3×10^{-4} to 7.2×10^{-4}. Hence, it was around six times higher than the well-known TEF. The novel method enables the reliable quantification of a more comprehensive set of PAHs bound on PM and thus will facilitate and improve the risk assessment of them.

Keywords: GC-MS/MS; PAHs; nitro-, oxy-, hydroxyl-, methyl-PAHs; 302 MW PAHs; particulate matter; life time cancer risk

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Introduction

In recent years, the importance of environmental pollution like airborne particulate matter (PM) for the development of diseases has been increased evidently. PM, especially fine particles and bound chemical compounds are associated with health effects like cancer, but also allergic reactions, respiratory and cardiovascular diseases (WHO, 2005). For risk assessment the knowledge of the composition and the concentration of chemical compounds associated with air particles is essential. Polycyclic aromatic hydro carbons (PAHs) are well known to play an important role as air contaminants bound on PM.

As products of incomplete combustion of organic matter PAHs are widely distributed in the atmosphere. Main sources of PAHs are anthropogenic emission including traffic, domestic heating, biomass burning, oil refining and other industrial processes (Rehwagen et al., 2005; Samburova et al., 2017). Several PAH are worldwide known to be carcinogenic and mutagenic (IARC, 2012b).

Because of their high toxic, mutagenic, and carcinogenic potential they are relevant for human health. (U.S.EPA, 1993).

By the U.S. Environmental Protection Agency (U.S.EPA) selected 16 PAHs were as “Priority Pollutant List” (U.S.EPA, 1993). However, the number of PAHs present in the environment is significantly larger and about their carcinogenic properties exist only few studies (IARC, 2010; Samburova et al., 2017). The most well-known compound is Benzo(a)pyrene (BaP)is the most investigated compound and is classified by the International Agency for Research on Cancer (IARC) as carcinogen to human beings (Group 1) (IARC, 2012b). For this reason it has been chosen as a reference compound. However, several studies have shown that BaP as an indicator compound may not accurately predict the carcinogenic potency of whole mixtures and may underestimate their carcinogenic potency (Samburova et al., 2017, U.S.EPA, 2010). By the U.S. EPA’s Integrated Risk Information System (IRIS) program a toxic equivalent factor (TEF) approach for PAH mixtures was developed for assessing cancer risk from exposure to these compounds (Nisbet and LaGoy, 1992; U.S.EPA, 1993).

However, some studies have shown that these 16 PAHs may contribute only partially to the toxic, mutagenic and carcinogenic potential of in complex environmental samples (Müller et al., 2006). In an actual review Samburova et al. (2017) described that 16 particle-bound EPA PAHs were responsible only for 14.4 % on average (0.2 - 42 %) of the obtained BaP toxic equivalents (BaP_{eq}) in 13 studies.

In recent years PAH with a molecular weight (MW) > 300 have been studied more and more intensively. According to Durant et al. (1998) PAHs with a MW of 302 in urban airborne particles contribute up to 33 % to the total mutagenicity of the PAHs fraction. Additionally, higher mutagenic and toxic potential of isomers from Dibenzopyrene with a molecular weight of 302 were reported in several studies (Bostrom et al., 2002; Cavalieri et al., 1991; Menchini and Merli, 2012, Platt et al., 2004).

The Office of Environmental health Hazard Assessment (OEHHA, California, U.S.) proposed a potency equivalency factor (PEF) approach for PAHs where also PAH with MW > 300 are included e.g. Dibenzo(a,i)pyrene (DBaiP) and Dibenzo(a,h)pyrene (DBahP) with values of 10 (OEHHA, 1994). New studies to carcinogenicity by IARC classified DBalP as *probably carcinogenic to humans* (Group 2A) and DBaiP as well as DBahP as *possibly carcinogenic to humans* (Group 2B) (IARC, 2010). In 2010 the U.S. EPA published a draft about a new relative potency factor (RPF) approach for PAHs in mixtures with several compounds with higher RPF values than B(a)P, for example DBalP (RPF 30), Benzo(c)fluorene (BcF) (RPF 20) and Dibenz(a,c)anthracene (DBacA) (RPF 4) (U.S.EPA, 2010).

Besides these known parent PAHs also derivatives like nitro-PAH (NPAH), methyl-PAHs (MPAH),oxygenated PAHs (OPAH) and hydroxyl-PAHs (OAPAH) occur in the environment. PAHs and NPAH are mainly generated by incomplete combustion of fossil fuels and biomass and are emitted from vehicles, industries and households. Additionally, NPAHs and OPAHs are also formed by homogeneous or heterogeneous photo-oxidation reactions of PAHs with atmospheric oxidants (such as OH, NO_3 and O_3), photolysis and thermal conversions (Cochran et al., 2016; Keyte et al., 2013; Reisen and Arey, 2005; Ringuet et al., 2012a; Zimmermann et al., 2013).

Toxicological effects of NPAHs were already investigated in several studies. NPAHs can induce mutagenic/genotoxic effects, carcinogenicity, acute and chronic cytotoxic effects as well as apoptosis (Bandowe and Meusel, 2017; Benbrahim-Tallaa et al., 2012). Because of their direct-acting mutagenicity and carcinogenicity some of these derivatives can be more toxic and causing a greater threat to human health, than some parent PAHs (Kawanaka et al., 2004, 2008; Umbuzeiro et al., 2008). Even though the concentration of NPAHs in environment is lower than their related parent PAH, the toxic properties of NPAHs can be much higher (Collins et al., 1998; IARC, 2012a). The toxicological mechanisms of these effects include increased levels of reactive oxygen species, pro-inflammation, cell cycle alternations, DNA damage and DNA adduct formation (Andersson et al., 2009; IARC, 2012b; Ovrevik et al., 2013; Park and Park, 2009). The IARC Working Group on the Evaluation of Carcinogenic Risks to Humans has

classified several NPAHs as *probably carcinogenic to humans* (groups 2A) e.g. 6-nitrochrysene (6-NC) and 1-nitropyrene (1-NP), as *possibly carcinogenic to humans (*group 2B) e.g. 1,3 dinitropyrene (1,3-DNP),and 2-nitrofluorene (2-NF). 1- nitronaphthalene (1-NNAP), 2-nitronaphthalene (2-NNAP), 7-nitrobenz(a)anthracene (7-NBaA) and 6-nitrobenz(a)pyrene (6-NBaP) were grouped as *not classifiable to its carcinogenicity to humans* (group 3*)* (IARC, 2012a, Bandowe and Meusel, 2017). Already by the OEHHA (1994) and Collins et al. (1998) several NPAHs were included the PEF approach and showed partially higher toxicity than BaP (e.g. 6-NC, PEF 10).

Until now only some studies were carried out to analyze the concentration of other potent carcinogen PAHs (Alves et al., 2017; Bandowe et al., 2014; Bandowe and Nkansah, 2016; Chen et al., 2016; Huang et al., 2014; Menichini and Merli, 2012; Ringuet et al., 2012b; Samburova et al., 2017; Wang et al., 2011). Up to the present, most of the studies are analyzing 16 parent PAHs and are applying the TEF approach following Nisbet and LaGoy (1992) (Franco et al., 2017; Gao et al., 2016; Hoseini et al., 2016; Hu et al., 2018; Kang et al., 2017; Liu et al., 2017; Suman et al., 2016; Zhang et al., 2016). Due to the new understanding of a potential carcinogenicity of other compounds in PM, the analysis of 16 PAHs might lead to an underestimation of health risk (Samburova et al., 2017).

Complex mixtures of PAHs are difficult to separate by chromatography because of similar mass spectral fragmentation patterns, vapor pressures and boiling points (Manzano et al. 2012). New methods were published for analyzing PAHs in the last years. Anderson et al. (2015) presented a method with a modified ion source triple quadrupole mass spectrometer allowing to determine PAHs, including methyl-PAHs up to a MW of 302 with high sensitivity (LOD from 0.3 – 6.4 pg/µL). Tutoni et al (2016) developed a specific MRM method for analyzing NPAHs. In other studies, two runs were necessary to analyze parent PAHs and their derivatives. Electron ionization (EI-mode) was used to quantify parent PAHs and in a second run negative chemical ionization (NCI-mode) was used for OPAHs and NPAHs (Bandowe et al., 2014; Cochran et al., 2012; Karavalakis et al., 2011). Manzano et al (2012, 2013) developed a two dimensional gas chromatography method for complex PAH mixtures.

To improve a risk assessment of air particulate matter the analysis needs to be extended from 16 PAHs from the Priority Pollutant List to all (potentially) relevant PAHs and substituted PAHs. According to this background the objective of this study was to develop and establish a new method for qualitative and quantitative analysis of PAHs and their derivatives from air

particulate matter by GC-MS/MS. To proof the relevance of this method, PM_{10} samples were collected in Medellin, Colombia and the contents of PAHs were analyzed.

Methods

Sample preparation

Solutions containing 64 native compounds were prepared by combining commercially available mixtures (EPA 8310, EPA 8100) and individual PAH standards from a stock solution. The EPA 8310 polynuclear aromatic hydrocarbons mix (Sigma Aldrich, Darmstadt, Germany) contains 18 compounds as listed in Table 1 in a concentration of 2000 ng/µL of each substance, dissolved in dichloromethane (DCM). The EPA 8100 PAH additional components mix (Sigma Aldrich, Darmstadt, Germany) contains DBaeP, DBahP, 3-methylcholanthrene (3MCHO), dibenzo(c,g)carbazole (DBcgC) and benzo(j)fluoranthene (BjFL), each in a concentration of 1000 ng/µL in DCM. The other substances were purchased as native compounds as described in Table 1. A stock solution of 1 mg/mL in acetonitrile was prepared and used for preparing the calibration mix with 64 compounds.

The standard solution of all investigated compounds was prepared in ethyl acetate. The calibration curves were obtained by dilution of standard solution at seven concentration levels (1, 5, 10, 50, 100, 500, 1000 pg/µL). As internal standard the EPA 8270 Semivolatile Internal Standard Mix, (Sigma Aldrich, Darmstadt, Germany) containing 5 deuterated PAHs (naphthalene-d_8 (NAP-d_8), acenaphthene-d_{10} (ACE-d_{10}), phenanthrene-d_{10} (PHE-d_{10}), chrysene-d_{12} (CHR-d_{12}) and perylene-d_{12} (PER-d_{12})) and two additional standards dibenz(ah)anthracene-d_{14} (DBahA-d_{14})and coronene-d_{12} (COR-d_{12}) (obtained from LGC Standards, Wesel, Germany) were used. The concentration of internal standard mixtures was 1 ng/µL in each sample.

Standard reference material (SRM) from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) SRM 1649a from urban dust, SRM 1650b diesel particulate matter and SRM 2975 diesel particulate matter (industrial forklift) were used for validation of this method.

GC-MS/MS Analysis

Sample analysis was performed by an Agilent 7000A GC/MS Triple Quadrupole System coupled with a gas chromatograph 7890A (Agilent Technologies Inc. U.S.) in multiple reaction monitoring mode (MRM). A positive chemical ionization was used as ionization mode. Chemical ionization was performed using methane, helium was used as quench gas in the collision cell with a flow of 2.25 mL/min and nitrogen as collision gas with a flow of 1.5 mL/min. 1 µL of sample was injected in pulsed splitless mode with an injector temperature of 300°C, the injection pulse pressure was 40 psi until 0.2 min and purge flow to split for 200 mL/min at 1.5 min. The transfer line temperature was 280°C. A deactivated tapered borosilicate liner, repacked with a small amount of deactivated glass wool, was used as injection liner (Agilent Technologies). Chromatographic separation was performed on an Agilent J & W Select PAH column (CP 7462), 30 m x 0.25 mm x 0.15 µm with following temperature program: 70°C for 0.7 min, ramping 85°C/min to 180°C, 3°C/min to 230°C for 7 min, 28°C/min to 280°C for 10 min and finally ramping 14°C/min to 350°C (for 5 min). Total run time was 47.45 min.

Extraction of environmental samples

To evaluate the analytical method a sampling set of air particulate matter (PM_{10}) from Medellin, Colombia, was extracted and analyzed as an example. Medellin, the second largest city in Colombia is the capital of the department of Antioquia. It is located in North West of Colombia, South America (6°14'39.13'' N and 75°34'53.52'' W), in the Aburrá valley, between northern foothills of the Andes Mountains at around 1500 m over sea level. Mountains up to 3000 m over sea level are surrounding the Aburrá valley. The city and its neighboring areas have an estimated population of around 4 million people (DANE, 2015). The stations EST-MAGO and ITA-PTR are located at side roads, MED-UNFM and MED-MIRA are located at main roads, MED-PJIC is placed at the city highway and CAL-PMER is placed in the south of the Aburrá valley in the small city Caldas, were some industries exist (Fig. 1).

Samples were collected for 24 hours on the 5th of October 2015 using six PM10 High Volume Air Samplers TE-6070V (Tisch Environmental, Cleves, Ohio, USA) with a flow rate of 58 m^3/hour. PM_{10} samples were taken onto quartz microfibre filters, (QMA, size 203 x 254 mm, Whatman, UK) on six different stations located in the city of Medellin and surrounding locations in the Aburrá valley. After a sampling time filter papers were conditioned for 24 hours in a desiccator, weighted out to determine the total particulate mass and stored at -20°C.

After transport to Germany one quarter of the filter was extracted using an accelerated extraction system ASE 200 (DIONEX, GmbH, Idstein, Germany), under a pressure of 10°MPa and a temperature of 100°C. Each sample was extracted twice with DCM in sample cell of 33 mL within three static cycles of 15°min. 50°µL nonane as a keeper was spiked in each vial. The total amount of solvent was 200°mL Extracts were concentrated using a TurboVac LV evaporator (Zymark, Boston, USA) with a gentle nitrogen flow to 1 mL. A clean up procedure was performed with 3 mL aminopropyl cartridges for solid phase extraction (SPE) with 500 mg of sorbent (Thermo Fisher Scientific, Germany). SPE cartridges were conditioned first with *n*-hexane and second with DCM (2 x 2.5 mL each). 1 mL extract was applied on the SPE cartridge and eluted sequentially each with 2 x 2.5 mL 100 % DCM, 20 % DCM in *n*-hexane, 50 % DCM in *n*-hexane and finally 100 % *n*-hexane. After this procedure the extract was evaporated to dryness using a vacuum system (concentrator plus, Eppendorf,) and re-dissolved with 100 µL ethyl acetate for GC-MS/MS analysis. To correct for analyte loss during the extraction procedure 10 µL of the internal standard compound solution with a concentration of 10 ng/µL were spiked to the filter before the extraction.

Data analysis and validation

GC-MS/MS data were analyzed by MassHunter Quantitative Analysis version B.06.00 SP01 build 6.03.88 for triple Quad software (Agilent Technologies Inc. U.S.). For calibration 1 ng/µL of the internal standard mixtures were spiked in the standard sets. In GC-MS/MS MRM each compound was positively identified by retention time, quantifier product ion and one qualifier product ion.

Calibration curves were generated by measurement of a triplicate of PAH standard mix containing 64 compounds at seven concentrations. Calibration curves ranges from 1 to 1000 pg/µL. The limit of detection (LOD) was calculated following a method of Shrivastava and Gupta (2011) with equation (1)

$$LOD = 3.3 * \sigma / s \qquad (1)$$

where σ was the standard deviation of response and s the slope of the calibration curve. The limit of quantification (LOQ) was calculated as 5 times the LOD (Shrivastava and Gupta, 2011).

Precision of GC-MS/MS method for each compound was determined by analysis of triplicates of seven concentrations (1, 5, 10, 50, 100, 500, 1000 pg/µL). To determine the recovery of the

complete method a standard solution with a concentration of 2.5, 25 and 250 pg/µL, respectively, which contained all compound and internal standard mix were spiked on a quarter of blank filter (QMA Whatman UK) and extracted and analyzed as described before. The LOD and LOQ were estimated additionally in pg/m³ according the LOD and LOQ for the GC-MS/MS in pg/µL with a volume of 1400 m³ and a particle mass of 50 mg. Further, to check for potential sample contamination during laboratory procedures, blank filters were spiked only with internal standards and included in each extraction series. After extraction and analysis, the mean concentration of target compound in the blank filter were subtracted from the concentration of the real samples.

Additionally, to validate the method 20 mg of certificated standard reference material SRM 1649a - Urban Dust, SRM 1650b - Diesel Particulate Matter and SRM 2975 - Diesel Particulate Matter (Industrial Forklift) from the National Institute of Standards and Technology (NIST Gaithersburg, MD, USA), were spiked with 10 ng/µL of deuterated internal standard mix and extracted and analyzed as triplicates with the described method.

Risk assessment

The cancer risk from exposure to carcinogenic PAHs were estimated using BaP_{eq} which were calculated from the concentration of each compound and the toxic equivalent factors TEF, PEF and RPF respectively, of each individual compound (Tab.1) following equation (2) (Bandowe et al., 2014)

$$\sum BaP_{eq} = \sum_{i}^{n=i}(C_i x\ RPF_i) \qquad (2)$$

with C_i as concentration of the target compound (ng/m^3).

The life time cancer risk (LCR) was calculated using equation (3), The inhalation cancer unit risk factor of BaP (UR_{BaP}) is defined as the number of people at risk for cancer from inhalation a BaP_{eq} concentration of 1 ng/m^3 within their lifetime of 70 years. The WHO value of UR_{BaP} is 8.7×10^{-5} (WHO 2000).

$$LCR = \sum BaP_{eq}\ x\ UR_{BaP} \qquad (3)$$

According to the different toxic equivalent factors from different studies, the $\sum BaP_{eq}$ and LCR where calculated for the TEF (Nisbet and LaGoy, 1992), PEF (OEHHA, 1994) and RPF (U.S.EPA, 2010).

Results and Discussion

Characterization of GS-MS/MS method

With this method presented here it was possible to determine the concentration of parent PAHs including compounds with MW of 302 as well as several MPAH, NPAH- and OHPAH derivate in one chromatographic run, thus improving time and cost efficacy. A second run with NCI for analyzing NPAH, OPAH and OHPAH is not necessary (Bandowe et al., 2014; Cochran et al., 2012; Karavalakis et al., 2011; Tutino et al. 2016). Also the use of a very expensive GC×GC/ToF-MS system to perform a two dimensional gas chromatography like described by Manzano et al. (2012, 2013) is not required. Detailed results are shown in supplement Table S1.

For further studies about risk assessment of PAHs containing environmental samples it will be important to obtain more knowledge about the concentration of these substances because of their toxic and carcinogenic relevance. In environmental samples several isomers of PAHs are presented: e.g. BaP and BeP; DBahA, DBacA and DBajA; DBalP, DBaeP, DBaiP and DBahP. Due to their diverse chemical structure, their biological reactivity can be different.

Separating the different PAH isomers is challenging. The applied column PAH Select CP7462 from Agilent Technologies Inc. was developed to solve this problem (Oostdijk, 2010). Fig. 2 shows the total ion current chromatogram (TIC) of all 64 PAHs.

The peaks of all compounds acquired in MRM-mode are shown in Fig.3. Section 1 shows peaks for naphthalene and the internal standard NAP-d_8. The retention time of both is very close, but they can be distinguished by their *m/z* ratio. The other compounds in this time windows can be separated well by their retention time and the *m/z* ratio. 2-NNAP and 1-MF are close too, but they can be separated by their different *m/z* ratio.

Section 2 shows peaks corresponding to PHE and ANT and the internal standard PHE-d_{10} which are well separated. 9-HF and 9-FLO are eluted at the same retention time, though their different *m/z* ratio they can be identified and quantified both. Additionally, 1,5-DiHNAP is separated in this section resulting in a clear peak.

Section 3 shows clearly defined peaks of 3-NPHE and 9-NPHE, 5-NACY, 2-NF and 9-NANT, in addition with PYR and FLT. In contrast to other studies, in which separate methods have to be used to analyze parent PAHs and NPAHs (Bandowe et al., 2014; Tutino et al., 2016; Valle-Hernandez et al., 2010), the method presented here allowed to elute and to separate PAHs and their nitro derivatives in the same run. Furthermore, the three isomers BaF, BbF and BcF can be

found in this section well separated. According to the RPF approach (U.S.EPA, 2010), a potential risk factor (RPF) for benzofluorenes vary depending on their chemical structure from 0 for BaF and BbF to 20 for BcF (Tab. 1). The ability to separate these isomers will allow more accurate risk assessment of PAHs from air particles.

In Section 4 a clear separation of BghiFL, BcPHE and CPcdP by their retention time and different *m/z* ratio is demonstrated. Only few data are available on occurrence of these compounds in ambient air. Already Glatt et al. (1994a) and Giles et al. (1995) described mutagenicity and DNA adduct formation by BcPHE. It is now of increased interest because the carcinogenicity classification of BcPHE and CPcdP was upgraded to *possible carcinogenic to humans* group 2A (IARC, 2010; Menichini and Merli, 2012). CPcdP and the internal standard CHR-d_{12} are two co-eluting compounds but are identifiable by their distinct *m/z* ratio. It also shows a clearly separation of BaA and partial chromatographic separation of TRI and CHR. Furthermore, 5-MC as well as 6-MC was separated and identifiable. Because of their different classification by the IARC (group 2B and group 3, respectively), the accurate analyzes of these isomers of methylchrysene will be important. Until now, there are no data on concentration in urban air, because of the lack of resolution among methylchrysene isomers using conventional GC columns (Menichini and Merli, 2012). Closed to 5-MC, 3-NFL was eluted, but can be distinguish definite by its *m/z* ratio. The last compound included in this section is 1-NP.

Section 5 shows a clear separation of the isomers BbFL, BkFL and BjFL. Additionally, BaFL was eluted with the same transition. The analysis of these isomers will be important for an enhanced risk assessment because of their different RPF (Tab.1). All four isomers from Benzofluoranthene were evaluated for a significant carcinogenic activity (IARC, 2010). Also important is a clearly separation and identification of BaP and BeP. BaP is well known for its carcinogenic and mutagenic properties. About the properties of its isomer BeP are very few data available, however, for further risk analysis data about the concentration of BeP might be eminent. 7-NBA and 6-NC were eluted successfully. Their retention times were closed to BaFL and the internal standard PER-d_{12} but can be identified by their different transitions.

In Section 6 the chromatographic separation of isomers of dibenzoanthracenes is demonstrated. DBajA, DBacA (RPF 4) and DBahA (RPF 10, U.S.EPA, 2010) are evaluated as higher mutagenic and carcinogenic than BaP (Tab.1), which pointed out the relevance for an accurate determination of these compounds. DBahA overlapped with IcdP and DBacA eluted at the same retention time with the internal standard DBahA-d_{14}, however, with their distinct transitions these compounds can be identified clearly in MRM mode. Isomers of benzochrysene (BaC, also

known as picene), BbC and BcC were eluted and separated definite. There are not yet RPF values available, however, in several studies the mutagenic properties of BcC by formation of covalent DNA adducts was described, because it possesses both a bay region and a fjord region in its molecule (Agarwal et al., 1997; Amin et al., 2003; Giles et al., 1995, 1997; Glatt et al., 1994b). Again, the quantification of benzochrysene isomers will be important for evaluating health risks. Additionally, DBcgC was included into the list of analytes due to its new relevance. Corresponding to the 14th Report on Carcinogens (U.S.Department of Health and Human Service, 2016), DBcgC is described as carcinogen because it caused tumors in several species of animals by several different routes of exposure. DBcgC can be determined by a clear separated peak.

In earlier studies the relevance of PAH with a MW >300 were pointed out (Durant et al., 1998, (Menchini and Merli, 2012). Collins et al. (1998) showed DBalP contribute higher to the carcinogenicity of PM than BaP. These findings result in new RPF for PAHs with a MW of 302 (e.g. 30 for DBalP, U.S.EPA, 2010) and the accurate determination of these compounds in environmental samples is becoming a priority. As shown in Section 7, it was possible to separate clearly six compounds within the MW 302 group including DBalP, DBaeP, DBaiP, DBahP and DBaeFL which contribute to mutagenicity and carcinogenicity (Collins et al., 1998; U.S.EPA, 2010). COR eluted close to the internal standard COR-d_{12}, though can be distinguished by their different *m/z* ratio.

Data validation

The resulting coefficients of determination for the calibration curves (r^2) were > 0.99 and ranged from 0.9939 to 0.9999. Details for each compound are presented in Table S1. Precision for the instrumental method averaged 100.5 % ± 1.5% for all analyzed concentrations. The limit of detection (LOD) for the GC-MS/MS methods varied depending on the substance classes and ranged between 0.03 – 0.7 pg/µL for PAHs, 0.2 – 7.9 pg/µL for OHPAH and OPAH, 0.1 – 7.4 pg/µL for NPAHs and 0.06 - 0.3 pg/µL for MPAHs and are comparable to the values described for the method by Anderson et el. (2015) (Tabl. S1).

The recovery for the extraction and clean up procedure was 98 ± 4.8 % (mean and standard deviation for all compounds). The LOD and LOQ estimated in pg/m³ and ranged from 0.002 to 0.057 for parent PAHs, 0.007 to 0.54 for NPAHs, 0.004 to 0.021, for MPAHs and 0.018 to 2.75 for OPAH and OHPAH, respectively (Tab. S2).

PAH concentration from standard reference material

Three different standard reference materials, SRM 1649a, SRM 1650 and SRM 2975, received from NIST (Gaithersburg, MD, USA), were analyzed as triplicates. Results are shown in detail in the supplement material Table S2.

Concentration of PAHs can differ depending of the extraction methods. In the certificate from NIST several concentration were listed, according to different extraction procedures. NIST used different temperatures and different extraction methods. We compared the NIST values which were obtained after extraction with ASE at 100°C and 13.8 MPa with DCM as solvent. The samples were extracted with a static time of 15 min in one cycle (NIST, 2007, 2013, 2016). For the most of the compounds the concentrations achieved with the presented method were close to the NIST values (Tab. S2). For several compounds, especially NPAH and some PAH with high MW like DBalP higher concentration were found here. Different values of the SRM samples can be observed depending on the extraction method. As reported from Masala et al. (2011) higher PAH concentrations were detected than the reference NIST values because of a higher efficiency of an improved extraction method with an ASE system. Also Bergvall and Westerholm (2008) found a higher PAH concentration in SRM samples due to enhanced extraction conditions. In contrast to the NIST method the samples here were extracted twice with a static time of 15 min in three cycles. This extension of the extraction time can result in a better recovery for several compounds.

PAH concentration in Medellin and risk assessment

To evaluate the analytical method and to demonstrate its application the concentration of six environmental samples of air particulate matter (PM_{10}) from Medellin, Colombia were analyzed. The results are shown in supplement Table S3. All analyzed compound were found in the samples, with exception of 9,10 DPA. 6-NC was detectible as a peak, but lower than the detection limit.

The concentrations of these samples differed depending on their location. The total PAH of all 64 compounds ranged from 2326.4 pg/m^3 in EST-MAGO to 15751 pg/m^3 in CAL-PMER (Tab. S3, Fig.4). Approximately 70 % of the total amount are related to parent PAHs with a MW weight < 300, between 8.8 and 18.9 % are related to parent PAHs with a MW >300 and between 3 and 8 % contribute to PAH derivatives. The 16 PAHs from the EPA priority list account only

for 54 % - 69 % of the PAH concentrations, supporting the relevance for broadening the analytical scope of PAH detection.

The highest concentrations between 845 pg/m^3 and 1300 pg/m^3 were found for COR in all sampling sites with exception of EST-MAGO and ITA-PTR, sampling stations at side roads, where the COR concentration achieved 158 pg/m^3 and 480.5 pg/m^3, respectively. Concentrations from 300 pg/m^3 to 1083 pg/m^3 were found for BghiP, IcdP, BkFL, BjFL, BbFL, BaP, BeP, CHR and PYR. All these compounds are known as indicators for traffic (Nielsen, 1996) and contribute to ~ 67 % of the total amount of PAHs.

The sum concentration of NPAHs ranged between 31.1 pg/m³ in EST-MAGO and 170 pg/m³ in CAL-PMER, comparable with studies in Madrid, Spain, (Barrado et al., 2012; Barrado et al., 2013) and lower than concentration reported from megacities in China (Bandowe et al., 2014; Wang et al., 2011). NPAHs contributed to 1 % of the total PAH amount.

At the sampling station CAL-PMER the traffic related PAHs contributed only to 54 % of the total amount. High concentration of NAP and its derivatives MNAP and OHNAP were found here which result in a higher part of MPAHs (16.4%) and OHPAHs (3.9%) (Fig.4). These increased levels might originate from their industrial use (Jan et al., 2007).

For an overview on the overall carcinogenic potential of the environmental burden the sum of BaP equivalents were calculated using the TEF approach (Nisbet and LaGoy, 1992), PEF (Collins et al., 1998; OEHHA, 1994) and RPF (U.S.EPA, 2010) for all measurement points and is shown in Figure 5. Analog to higher PAH concentration from sampling stations at main roads (MED-PJIC, MED-UNFM and MED-MIRA) the sum of BaP equivalents is higher at these locations, too. Due to the different factors for the toxic potential, the sum of BaP equivalents is nearly six times higher with the calculation using the RPF, compared with the well-known TEF approach.

In order to reveal the contribution of each compound to the sum of BaP equivalents the percentage are presented in Fig. 6. Predominant compounds which contributed to the sum of BaP equivalents differ depending on the mode of calculation. Main compounds followed the TEF approach were BaP (average for all sampling points 55.6 %), DBahA (29 %), IcdP (4.8 %), BbFL (4 %), BkFL (2.3 %) and BaA (2.9 %). Major contributions according to the PEF approach were DBalP (44.8 %), DBaiP (18.9 %) and BaP (21.7 %). DBahP contributed with 4.8 % and BaA with 1.1 % to the sum of BaP equivalents. In contrast, for the calculation using the RPF approach the main compounds were DBalP (60.5 %) due to the factor 30, BaP (9.9 %),

DBahA (10.1 %). Other compounds were BcF (5.3 %), BbFL (5.4 %), BjFL (1.4 %) and CPcdP (1.5 %).

The cumulative life time cancer risk (LCR) was estimated for all sampling locations with the different factors. Corresponding to the TEF, the LCR ranged from 2.75×10^{-5} in EST-MAGO to 1.62×10^{-4} in MED-MIRA which demonstrate a higher risk for the population in all sampling places regarding a acceptable value of 1×10^{-6} to 1×10^{-5} (WHO, 2000) (Tab. 2).

Compared with other studies where also used the TEF approach for risk calculation based on 16 PAH from PM_{10} samples, the LCR value in Medellin was lower than in cities in Asia , e.g. Amritsar, India, 7×10^{-4} (Kaur et al., 2013), Dehli, India 2.9×10^{-5} to 2.3×10^{-3} (Sarkar et al., 2013), Beijing, China 1.4×10^{-4} to 5.6×10^{-3} , based on $PM_{2.5}$ samples (Bandowe et al., 2014), Hefei, China, 4.2×10^{-4} to 5.1×10^{-3} (Hu et al., 2018), similar to Northern Italy, 7×10^{-5} - 2.4×10^{-4} (Khan et al., 2018), but higher than to Balikesir, Turkey in summer 1.6×10^{-5} (Gungormus et al., 2014) and New York < 8.7×10^{-5} (Jung et al., 2010), to European Cities e.g. in Czech Republic, < 1×10^{-6} (Bulejko et al., 2016), ore Thessaloniki, Greece 1.5×10^{-5}- 1.4×10^{-6} (Manoli et al., 2016).

By calculation with the PEF approch, the LCR increased to 3×10^{-4} and with the RPF approch to $7.5 \times 10^{-4,}$ nearly six times higher than with TEF factors.

A major impact on this increased LCR by the calculation with the PEF and RPF approaches is attribute to PAHs with a MW > 300, particularly isomers from Dibenzopyrenes even though there concentrations were relatively low, in detail ranged from 2.8 pg/m³ to 170.6 pg/m³ and therefore were lower than the concentrations of BaP which ranged from 184.5 pg/m³ to 1083.8 pg/m³. Dibenzopyrenes comprise six aromatic rings and contain two reactive regions in their structure (Boström et al., 2002) . DBaeP, DBahP and DBalP can cause tumors by several routes of exposure at different tissues. Studies with several animals or cell systems demonstrate significant higher effects of DBalP than BaP at lower concentrations (U.S.Department of Health and Human Service, 2016). All of the cancer-related data for Dibenzopyrenes were positive and resulted in case of DBalP in a RPF of 30 (U.S.EPA, 2010). Hence, DBalP accounted for a high percentage to the sum of BaP equivalents and thus on a cancer risk.

Conclusion

The presented GC-MS/MS method allowed the quantification of 64 individual PAHs, including derivatives with different polarity in one run, resulting in higher time efficiency. The ability to analyze a broader spectrum of PAHs on GC-MS/MS systems enhances accurate monitoring which is necessary for an improved risk assessment of PM from ambient air. The examples from six environmental samples of air particulate matter demonstrates the relevance of new analytical methods by identifying compounds beside the 16 EPA priority PAHs that are strongly influencing the overall potential carcinogenic toxicity. These results support the argument that monitoring the 16 EPA compounds leads to an underestimation of possible risks of PAH mixtures. The used GC-MS/MS Triple Quadrupole System with the specific PAH selected column CP 7462 allowed a targeted analyzes with a very good separation of all PAH isomers with a high sensitivity.

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Table 1
List of PAH analytes with molecular weight (MW), toxic equivalent factor TEF (Nisbet and LaGoy, 1992), potency equivalent factor PEF (OEHHA, 1994), relative potency factor RPF based on tumor bioassay data (U.S.EPA, 2010) and IARC classification (IARC, 1989, 2010, 2012). CAS number and supplier

Substance	Abbreviation	MW	TEF	PEF	RPF	IARC	CAS Number	Supplier
Parent PAHs MW< 300								
Naphthalene	NAP	128.15	0.001				91-20-3	EPA 8310 [1]
Acenaphthylene	ACY	152.19	0.001				208-96-8	EPA 8310 [1]
Acenaphthene	ACE	154.08	0.001				83-32-9	EPA 8310 [1]
Fluorene	FLO	166.22	0.001			3	86-73-7	EPA 8310 [1]
Phenanthrene	PHE	178.23	0.001			3	85-01-8	EPA 8310 [1]
Anthracene	ANT	178.23	0.01			3	120-12-7	EPA 8310 [1]
Fluoranthene	FLT	202.26	0.001		0.08	3	206-44-0	EPA 8310 [1]
Pyrene	PYR	202.25	0.001			3	129-00-0	EPA 8310 [1]
Benzo(*a*)fluorene	BaF	216.09				3	238-84-6	Dr. Ehrenstorfer [2]
Benzo(*b*)fluorene	BbF	216.09				3	243-17-4	Dr. Ehrenstorfer [2]
Benzo(*c*)fluorene	BcF	216.09			20	3	205-12-9	Dr. Ehrenstorfer [2]
Benzo(*g,h,i*)fluoranthene	BghiFL	226.08				3	203-12-3	Dr. Ehrenstorfer
Benz(*a*)anthracene	BaA	228.09	0.1	0.1	0.2	2B	56-55-3	EPA 8310 [1]
Cyclopenta(*c,d*)pyrene	CPcdP	226.07			0.4	2A	27208-37-3	Dr. Ehrenstorfer [2]
Benzo(*c*)phenanthrene	BcPH	228.09				2B	195-19-7	Sigma Aldrich [1]
Triphenylene	TRI	228.09				3	217-59-4	Sigma Aldrich [1]
Chrysene	CHR	228.09	0.01		0.1	2B	218-01-9	EPA 8310 [1]
Benzo(*b*)fluoranthene	BbFL	252.09	0.1	0.1	0.8	2B	205-99-2	EPA 8310 [1]
Benzo(*k*)fluoranthene	BkFL	252.09	0.1	0.1	0.03	2B	207-08-9	EPA 8310 [1]
Benzo(*j*)fluoranthene	BjFL	252.09		0.1	0.3	2B	205-82-3	EPA 8310 [1]
Benzo(*a*)fluoranthene	BaFL	252.09				3	238-84-6	Dr. Ehrenstorfer [2]
Benzo(*e*)pyrene	BeP	252.09				3	192-97-2	Sigma Aldrich [1]
Benzo(*a*)pyrene	BaP	252.09	1	1	1	1	50-32-8	EPA 8310 [1]
Perylene	PER	264.38				3	198-55-0	Sigma Aldrich [1]
Indeno(*1,2,3-c,d*)fluoranthene	IcdFL	276.09				2B	193-43-1	Dr. Ehrenstorfer [2]
Benzo(*g,h,i*)perylene	BghiP	276.09	0.01		0.009	3	191-24-2	EPA 8310 [1]
Indeno(*1,2,3-c,d*)pyrene	IcdP	276.09	0.1	0.1	0.07	2B	193-39-5	EPA 8310 [1]
Dibenzo(*a,c*)anthracene	DBacA	278.10			4	3	215-58-7	Dr. Ehrenstorfer [2]
Dibenzo(*a,j*)anthracene	DBajA	278.10				3	224-41-9	Dr. Ehrenstorfer [2]
Dibenz(*a,h*)anthracene	DBahA	278.10	5	0.4	10	2A	53-70-3	EPA 8310 [1]
Benzo(*a*)chrysene	BaC	278.10				3	213-46-7	Sigma Aldrich [1]
Benzo(*b*)chrysene	BbC	278.10				3	214-17-5	Dr. Ehrenstorfer [2]
Benzo(*c*)chrysene	BcC	278.10					194-69-4	Sigma Aldrich [1]
Parent PAH MW> 300								
Coronene	COR	300.09				3	191-07-1	Sigma Aldrich [1]
Dibenzo(*a,l*)pyrene	DBalP	302.10		10	30	2A	191-30-0	Sigma Aldrich [1]
Dibenzo(*a,e*)pyrene	DBaeP	302.10		1	0.4	3	192-65-4	EPA 8100 [1]
Dibenzo(*a,i*)pyrene	DBaiP	302.10		10	0.6	2B	189-55-9	Sigma Aldrich [1]
Dibenzo(*a,h*)pyrene	DBahP	302.10		10	0.9	2B	189-64-0	EPA 8100 [1]
Dibenzo(*a,e*)fluoranthene	DBaeFL	302.10			0.9	3	5385-75-1	Sigma Aldrich [1]
9,10-Diphenylanthracene	9.10-DPA	330.14					1499-10-1	Sigma Aldrich [1]
Nitro PAHs								
1-Nitronaphthalene	1-NNAP	173.04				3	86-57-7	Dr. Ehrenstorfer [2].
2-Nitronaphthalene	2-NNAP	173.04				3	581-89-5	Dr. Ehrenstorfer [2]
5-Nitroacenaphthene	5-NACY	199.06		0.01			602-87-9	Dr. Ehrenstorfer [2]
2-Nitrofluorene	2-NF	211.06		0.01		2B	607-57-8	Sigma Aldrich [1]
9-Nitrophenanthrene	9-NPHE	223.06					954-46-1	Dr. Ehrenstorfer [2]
3-Nitrophenanthrene	3-NPHE	223.06					17024-18-9	Dr. Ehrenstorfer [2]
9-Nitroanthracene	9-NANT	224.06					602-60-8	Sigma Aldrich [1]
3-Nitrofluoranthene	3-NFL	247.06					892-21-7	Sigma Aldrich [1]
1-Nitropyrene	1-NP	247.06		0.1		2A	5522-43-0	Sigma Aldrich [1]
7-Nitrobenz(*a*)anthracene	7-NBaA	273.07				3	20268-51-3	Sigma Aldrich [1]
6-Nitrochrysene	6-NC	273.07		10		2A	7496-02-8	Sigma Aldrich [1]
6-Nitrobenz(*a*)pyrene	6-NBaP	297.10				3	63041-90-7	Sigma Aldrich [1]

Azareenes						
7H-Dibenzo(*c*,*g*)carbazole	DBcgC	267.10	1		194-59-2	EPA 8100 [1]
Methyl -PAH						
1-Methylnaphthalene	1-MNAP	142.07			90-12-0	EPA 8310 [1]
2-Methylnaphthalene	2-MNAP	142.07			91-57-6	EPA 8310 [1]
1-Methylfluorene	1-MF	180.09			1730-37-6	Sigma Aldrich [1]
5-Methylchrysene	5-MC	242.10	1	2B	3697-24-3	Dr. Ehrenstorfer [2]
6-Methylchrysene	6-MC	242.10		3	1705-85-7	Dr. Ehrenstorfer [2]
3-Methylcholanthrene	3-MCHOL	268.12	1.9		56-49-5	EPA 8100 [1]
Oxy and Hydroxyl PAH						
1-Hydroxynaphthalene	1-HNAP	144.05			90-15-3	Dr. Ehrenstorfer [2]
2-Hydroxynaphthalene	2-HNAP	144.05			135-19-3	Dr. Ehrenstorfer [2]
1,5-Dihydroxynaphthalene	1.5-DiHNAP	160.05			83-56-7	Sigma Aldrich [1]
9-Hydroxyfluorene	9-HF	182.07			1689-64-1	Sigma Aldrich [1]
9-Fluorenone	9-FLO	180.05			486-25-9	Sigma Aldrich [1]

Supplier: [1] Sigma Aldrich, Darmstadt, Germany
[2] Dr. Ehrenstorfer GmbH, Augburg, Germany

Table 2

Cumulative life time cancer risk (LCR) for station MED-UNFM and MED-PJIC in Medellin, calculated with Toxic equivalent factor (TEF), potency equivalent factor (PEF) and relative potency factor (RPF)

	TEF	PEF	RPF
EST-MAGO	2.75E-05	5.72E-05	1.26E-04
ITA-PTR	5.32E-05	1.40E-04	3.07E-04
MED-PJIC	7.86E-05	2.55E-04	5.89E-04
MED-UNFM	8.50E-05	2.85E-04	6.84E-04
MED-MIRA	1.62E-04	3.79E-04	7.65E-04
CAL-PMER	1.39E-04	3.42E-04	7.17E-04

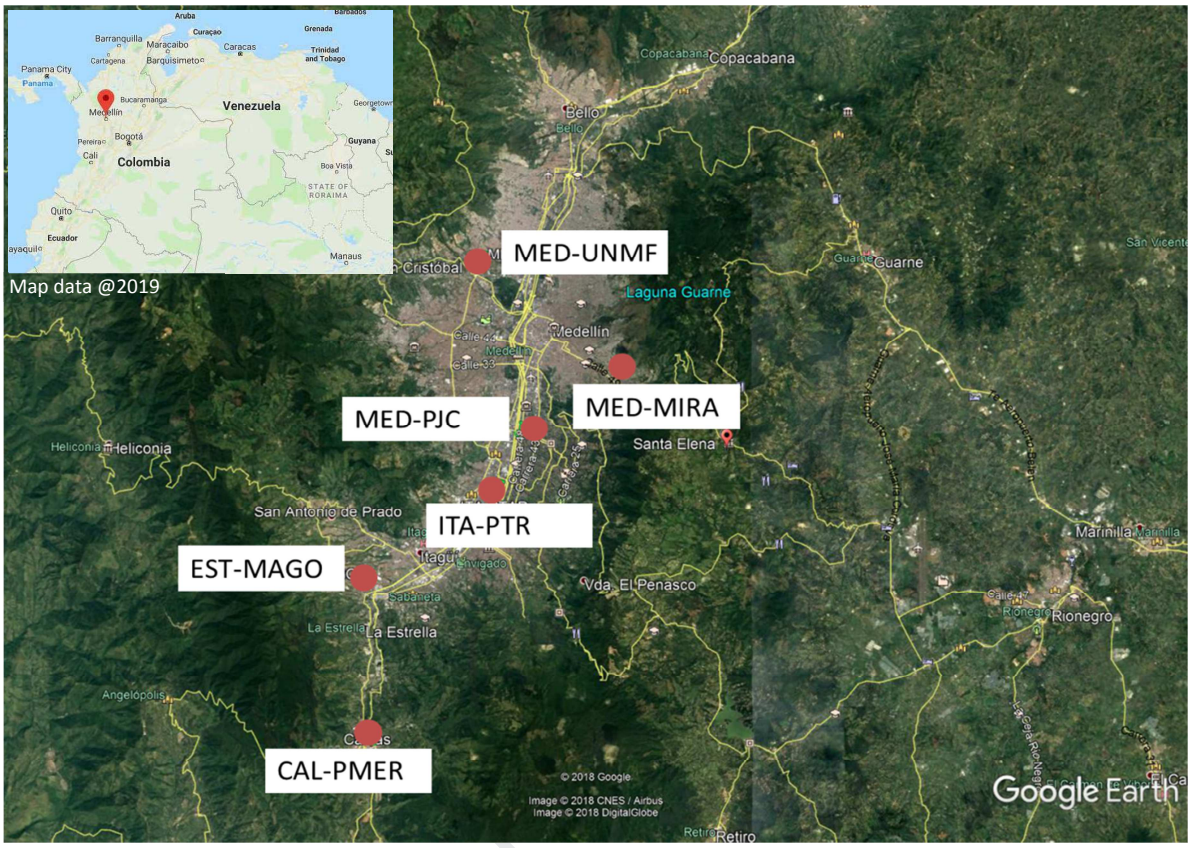

Figure 1 Sampling points in Medellin and the Aburrá valley, Colombia

Sources: Google Earth "Medellin" 6°14'39.13'' N, 75°34'53.52'' W, 13th of February, access, 16th of July, 2018
Google maps: https://www.google.com/maps/@5.1656236,-70.8201645,6z

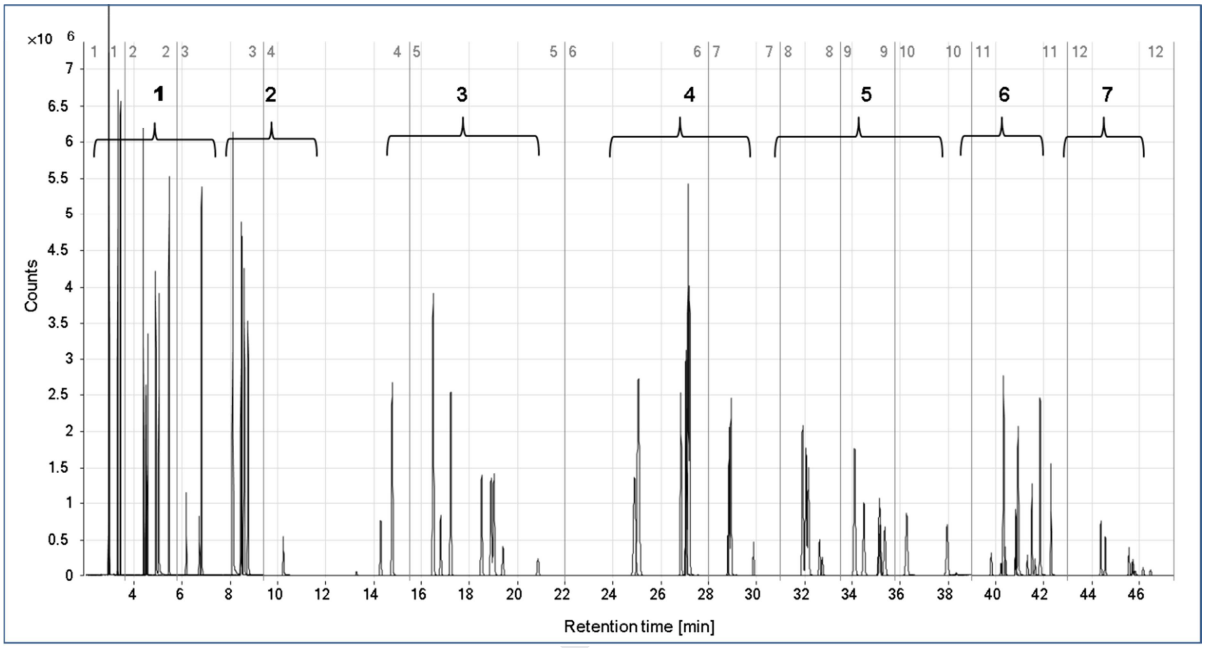

Figure 2 Total ion current chromatogram (TIC) representing 64 compounds at a concentration of 500 pg/µl and 7 internal standards at a concentration of 1000 ng/µl

Number 1 to 7 mark sections for time windows for detailed description in Fig. 3

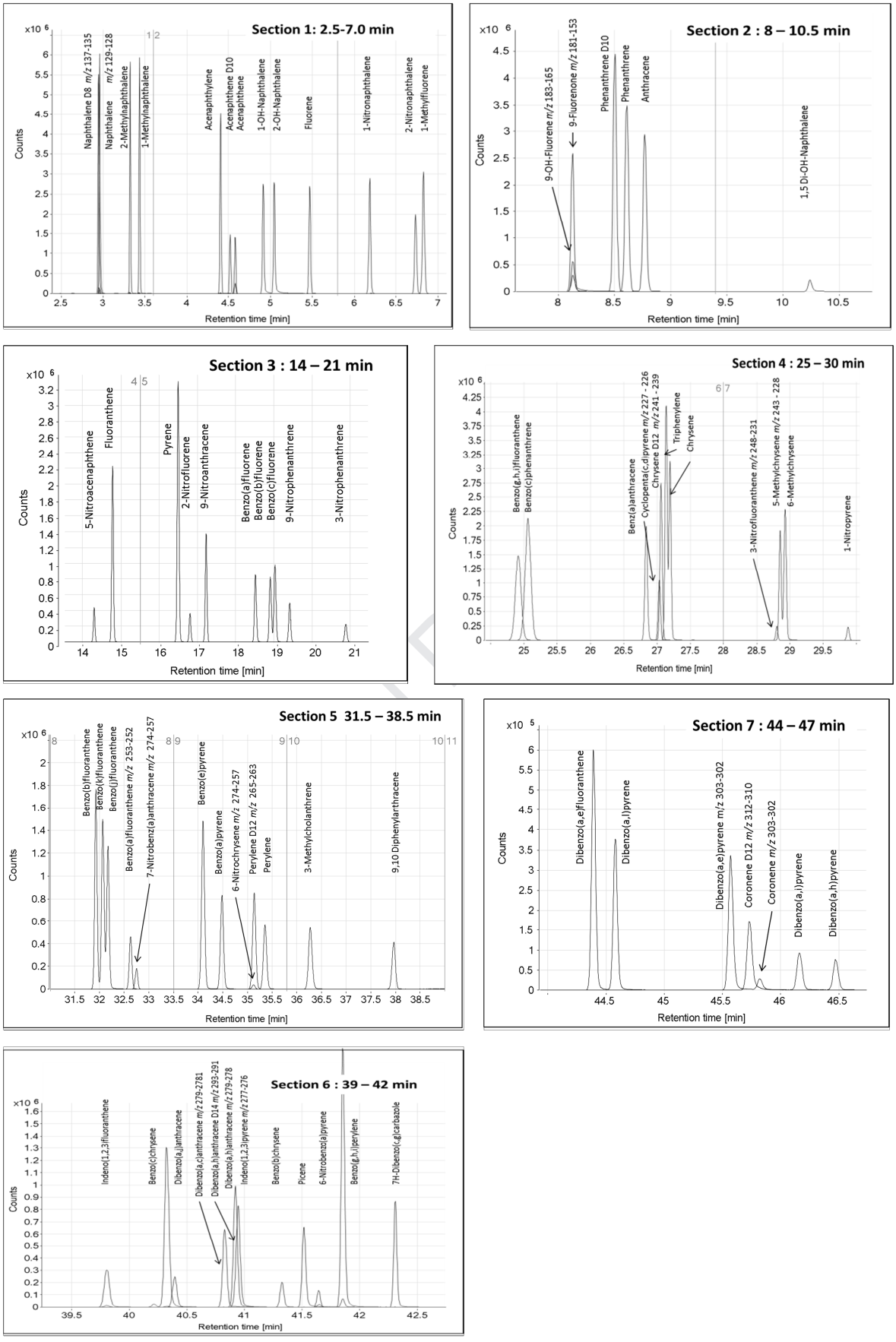

Figure 3 Peaks from MRM spectra of all analyzed compounds, sections 1-7 characterizing different time windows

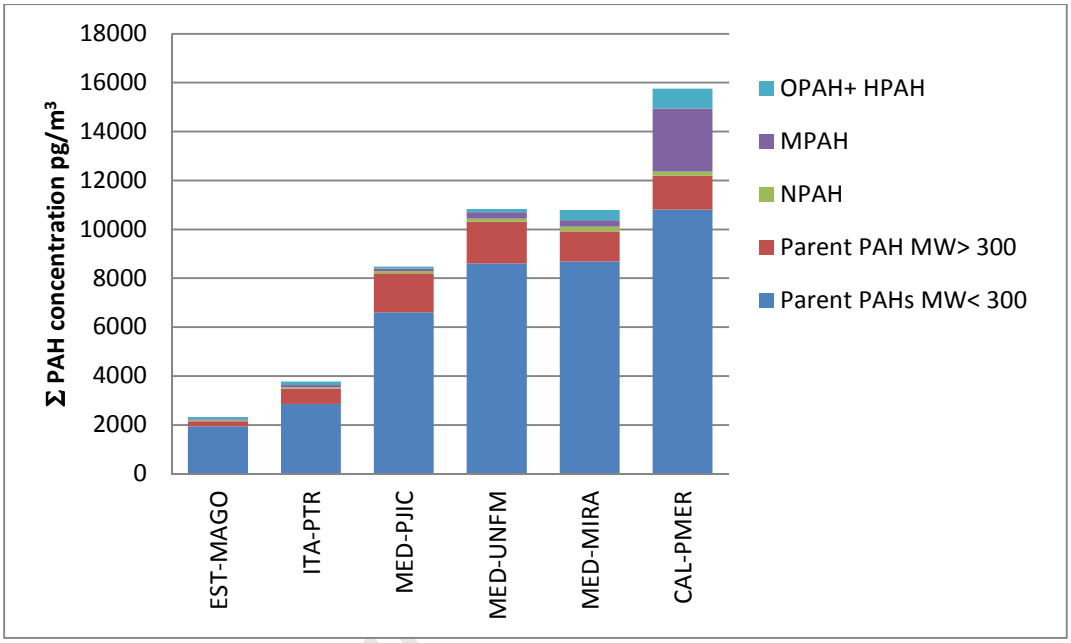

Figure 4 Contribution of different PAH groups to the total concentration of PAHs in six stations in Medellin and the Aburrá valley, Columbia

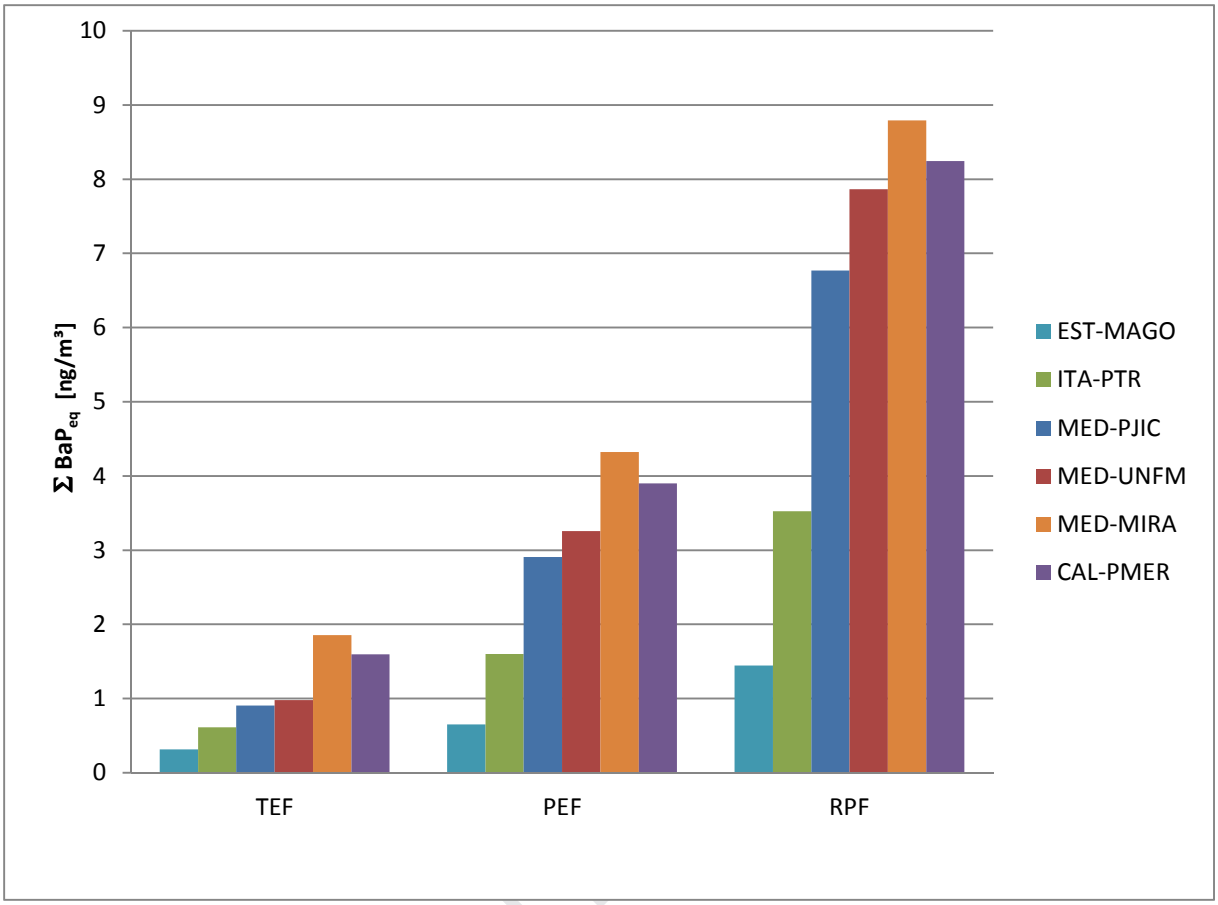

Figure 5 Sum of BaP equivalents from six samples in Medellin, calculated according toxic equivalent factor (TEF), potency equivalent factor (PEF) and relative potency factor (RPF)

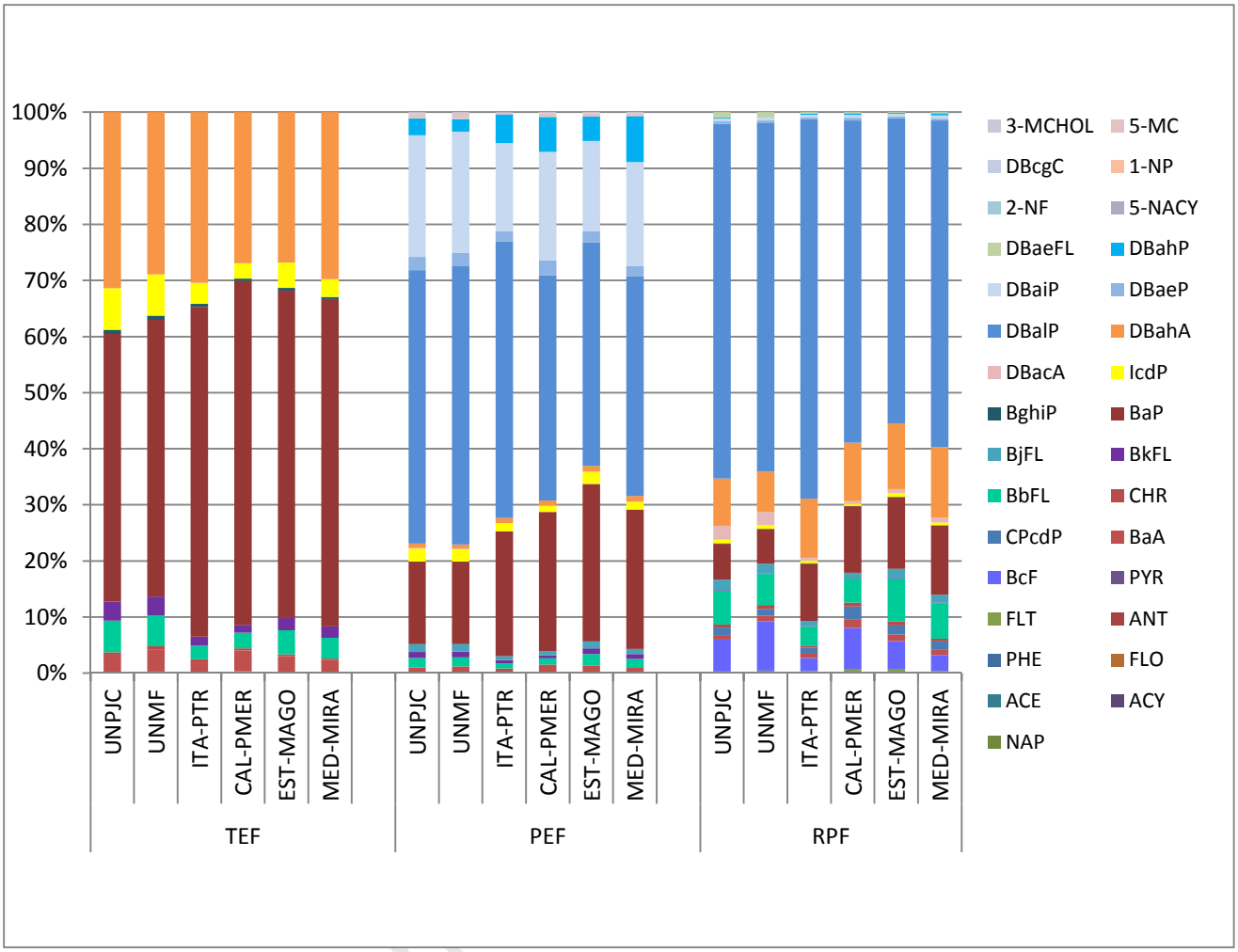

Figure 6 Contribution of each PAH with a BaP equivalent factor to the sum of BaP equivalents for samples from Medellin, calculated with toxic equivalent factor (TEF), potency equivalent factor (PEF) and relative potency factor (RPF)

Highlights

- New targeted GC-MS/MS method for analyzing 64 PAHs including derivatives in one run.
- Risk assessment based on BaP equivalents of 16 EPA PAHs leads to an underestimation
- Using new PEF and RPF the calculated life time cancer risk is up to six times higher than with the TEF
- PAHs with a molecular weight >300 contribute to around 60% to the sum of BaP equivalents