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# Optimization of sample clean-up for the GC-C-IRMS and GC-IT-MS analysis of PAHs from air particulate matter

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The optimization of sample clean-up for the analysis of air particulate matter PAHs' stable carbon isotope ratio using Solid Phase Extraction (SPE) cartridges is described in this paper. Various adsorbents, such as silica gel, alumina, florisil, commercially available for sample purification were compared. Best performance for the clean-up of 24-h air particulate matter samples was obtained with activated silica-gel columns in terms of selectivity and reproducibility. One step clean-up was optimized for concentration determination and in case of co-elutions, a second step was additionally used for carbon isotope ratio analysis. The method was subsequently validated with standard reference material and was checked for carbon isotope fractionation artefacts. No significant differences in  $\delta^{13}\text{C}$  values were found for unprocessed solutions of PAHs and solution subjected to the extraction and purification procedure. The procedure was tested on air particulate matter samples collected in three different locations in Belgium. Statistically significant differences in carbon isotope ratio of PAHs between Borgerhout location and Zelzate or Gent were noticed, confirming the differences in distribution and diagnostic ratios found during the concentration analyses and different PAH sources in these locations. The results, therefore, seem very promising for the use of  $\delta^{13}\text{C}$  of PAHs as an additional information helpful in source identification of these pollutants

Keywords: PAHs, stable carbon isotope ratio, sample clean-up, air particulate matter.

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## 1 Introduction

Polycyclic aromatic hydrocarbons are a group of pollutants commonly found in the environment. Air particulate matter bound PAHs originate mainly from incomplete combustion of organic matter. They received attention in air pollution studies because of their toxicity (carcinogenic or mutagenic potential influence) [1]. The combination of PAHs' concentration measurements with stable isotope ratio data may be of help in the identification of their sources, which is crucial for undertaking effective control measures to reduce their levels in the environment [2].

One of the conditions for a reliable analysis of isotope ratios is obtaining well separated peaks. During the chromatographic separation the isotopically heavier molecules elute slightly earlier, thus, the isotope ratios vary significantly across the widths of peaks. The beginning of the peak is strongly enriched in  $^{13}\text{C}$  whilst the end of the peak is depleted [3]. In case the compounds of interest are not well resolved from each other or from the impurities present in the sample, an artificial enrichment of the preceding peak in  $^{13}\text{C}$  would occur and the depletion of the following compound during the separate integration of peaks' areas. It is important therefore, that samples are well purified from the interferences present in the matrix. Solid phase extraction (SPE) and is often used for sample purification purposes [4]. The comparison of commercially available SPE columns for sample clean-up, loaded with various adsorbents, and the optimisation of the PAHs concentration analysis in addition to the stable carbon isotope analysis, are, therefore, the subject of this paper.

## 2 Methodology

### 2.1 Environmental samples

Samples of air particulate matter were obtained from Flemish Environmental Agency (VMM). They were collected in the period of August 2009 - February 2010. The locations of the VMM sampling stations (Gent, Zelzate and Borgerhout) represent different types of surroundings, i.e.: Gent sampling station was located in the centre of the city, at a corner of a park, thus representing an urban background, Zelzate is an suburban, industrial area located nearby a heavy steel industry (coke oven) but also a highway thus an influence from traffic may be present, Borgerhout, a district of Antwerp city, represents an urban background, influenced by a heavy traffic. Particulate matter was sampled on quartz or glass fibre filters during 24h time intervals. Filters were preheated before use at  $500\text{ }^{\circ}\text{C}$  for at least 6 h. The sampling flow was  $900\text{ L min}^{-1}$ .

## 2.2 Instrumentation

Compound specific carbon isotope analysis was performed with a Trace GC Ultra gas chromatograph (Thermo, Bremen, Germany) coupled via a combustion interface to a Delta Plus XP (Thermo) isotope ratio mass spectrometer (GC-C-IRMS). The gas chromatograph was equipped with the HP-5MS column (60m x 0.25 mm I.D. x 0.25  $\mu\text{m}$  film thickness) or DB-5MS (60 m x 0.25 mm I.D. x 0.5  $\mu\text{m}$  film thickness). The GC temperature program started at 50 °C (1.5 min hold time) and was then elevated to 180 °C at 20 °C  $\text{min}^{-1}$  and then to 300 °C at 1.5 °C  $\text{min}^{-1}$ . Injections were performed with the use of an OPTIC 3 temperature-programmable vaporizing injector (PTV) (Atas, GL International BV, Eindhoven, The Netherlands). The optimized parameters [5] were: initial temperature, 45 °C, increased to 300 °C at 5 °C  $\text{s}^{-1}$ ; the transfer time was 140 s. The transfer column flow rate was 3.6  $\text{mL min}^{-1}$ , and a vent flow rate of 230  $\text{mL min}^{-1}$  was applied. Column flow of 1.8  $\text{mL min}^{-1}$  was used and the injection volume of 100  $\mu\text{L}$ . The second detector (Ion Trap mass spectrometer) was coupled to an already existing setup of GC-C-IRMS, during this study. The effluent from the GC column was divided into two parts, one going to the oxidation reactor and further to the IRMS (around 90% of the effluent) and second (around 10% of the effluent) going through the 10 m 0.1 mm ID capillary into the IT-MS transfer line. The partition between IRMS and IT-MS detectors was determined by the physical properties (length, diameter) of the capillaries connected to the coupling piece. This allowed for the identification of the analytes by their mass spectra in addition to the information on the carbon isotope ratio. Both sets of information could, therefore, be obtained from one injection.

The IT-MS conditions were as follows: ion source temperature was 250 °C, and the transfer line was kept at 300 °C. The spectrometer was operated in total ion count (TIC) mode and electron impact (emission current 250 or 125  $\mu\text{A}$ ) was used for ionization. The mass scanning ranged from  $m/z$  40 to 550. Molecular ions were used for PAHs quantitation (peak areas).

Validation of clean-up procedure for isotopic measurements was done with the use of another GC-C-IRMS system (MAT 253, Thermo, Bremen, Germany and Agilent (Santa Clara, CA, USA) GC equipped with a standard split/splitless injector); 1  $\mu\text{L}$  sample volume was thus injected.

The carbon isotopic ratio ( $\delta^{13}\text{C}$  notation in per mille, giving deviation of a sample relative to a standard Vienna Peedee belemnite (VPDB)) was calculated with the use of Isodat software against the  $\text{CO}_2$  reference gas pulses which were injected at the beginning of each analysis. Samples were calibrated against pyrene which was used as a secondary standard. The delta value of the standard was previously measured with the use of EA-IRMS technique. The identification of PAHs, was done by comparison of the PAHs retention times with the retention times of the PAH standard (EPA610 mix, Supelco, Bellefonte, PA, USA, or 16 EPA PAHs mix, Dr. Ehrenstorfer GmbH, Augsburg, Germany), and by standard addition to the sample. From the moment of the IT-MS detector installation, the peak

identification was done based on the mass spectra comparison to the mass spectra of authentic standards and NIST mass spectra library.

### 2.3 Glassware preparation

All glassware was rinsed with acetone, soaked in hot water with detergent for about 1h, rinsed with cold, tap water and deionised water, dried at 60 °C and rinsed with acetone and *n*-hexane respectively.

### 2.4 Extraction

Extraction of PAHs from the aerosol samples was conducted by sonication. The glass or quartz fibre filters were cut in pieces, placed in Erlenmeyer flasks and into an ultrasound bath for 30 min and extracted three times with a volume of 30 ml (90 ml in total); a mixture of *n*-hexane:acetone (1:1; v:v) was used. The extracts were then filtrated using glass microfiber filters (Whatman International, Maidstone, UK) and evaporated to the volume of approximately 1 mL with the use of a rotary evaporator and a nitrogen evaporator and subsequently subjected to the further sample treatment.

Sonication extraction is generally thought to be less efficient than Soxhlet extraction [6], however, some studies show that extraction of 16 EPA PAHs by ultrasonic agitation yielded better or equal recoveries compared to traditional Soxhlet extraction [7-8]. It also offers some advantages compared to other extraction methods, such as low equipment cost, ease of operation, short extraction times which allows processing large numbers of samples relatively fast [9]. This extraction technique is commonly used for PAHs from particulate environmental samples [5-13]. A mixture of solvents used for extraction: *n*-hexane/acetone (1:1; v:v) was used previously in our laboratory [14] and by other researchers [9, 15]. It was found superior to other mixtures, such as (DCM/acetone (1:1; v:v), *n*-hexane/acetone (4:1; v:v), diethylether/ *n*-hexane (1:9; v:v)) in terms of the best recoveries [16]. The use of hydrophobic solvent, e.g. *n*-hexane can reduce the extraction efficiency in naturally moist samples; the addition of acetone, which can penetrate the pores of a wet matrix overcomes this problem [9].

### 2.5 Sample clean-up

The analytical procedure used for sample clean-up initially [14] was inefficient when extracts of 24-h high-volume sample filters were analysed (Figure 1). Large amounts of interfering peaks were seen and numerous co-elutions. In this procedure silica-gel solid phase extraction (SPE) columns (as received) were conditioned with *n*-pentane. Analytes were eluted with 10 mL of *n*-pentane followed by 10 mL *n*-pentane/DCM (1:1, v/v). These two fractions were combined and evaporated to the volume about 1 mL with the means of a rotary evaporator. The pre-cleaned extracts were then fractionated into aliphatic and aromatic fractions and three fractions were collected. The aliphatic fraction (3 mL) was eluted with

100% *n*-pentane and was discarded; the second and third fractions (2 and 4 mL, respectively) containing aromatic compounds, were eluted with *n*-pentane/DCM (9:1 and 4:6, v/v respectively). A vacuum manifold was used to increase the flow rate for the SPE. In this study, DCM present in the sample extract had to be removed before the analysis, since it can dissolve the deactivation packing of a GC liner. *n*-Pentane in the solvent mixture was thus replaced by *n*-hexane. Jang et al. 2001 [15] found negligible differences in PAH recoveries when using *n*-hexane or *n*-pentane for PAHs elution during clean-up with silica gel. All fractions were evaporated to a volume below 1 mL with. The aromatic fraction was analyzed by GC-C-IRMS for the carbon isotopic ratio and/or IT-MS for concentration and checks of peaks' purity.

The use of SPE cartridges is fast and no need for laborious preparations of columns and glassware cleaning is needed as is the case with traditional column chromatography. Low amounts of solvents are required to elute compounds of interest. We therefore focused on optimization of existing SPE procedure and testing other commercially available SPE glass cartridges for their efficiency in interferences removal. Therefore, silica gel SPE columns (1 g, Supelco, Bellefonte, PA, USA), alumina SPE columns (1 g, Macherey-Nagel, Düren, Germany), Florisil (2 g Macherey-Nagel, Düren, Germany) and cyano-silica (1 g + 0.5 g, Macherey-Nagel, Düren, Germany) were compared. In some of the setups, the SPE columns were previously activated by heating overnight at 130°C. After activation, columns were allowed to cool in a dessicator. Na<sub>2</sub>SO<sub>4</sub> was purified by heating at 400°C for at least 4 h in a shallow tray and kept in the dessicator.

**Figure 1.** Mass-to-charge signal 44 chromatogram showing the GC-C-IRMS analysis of sample collected in Zelzate. Visible, high, fronting peaks.

### 3 Results and discussion

#### 3.1 Clean-up experiments

Usually, a few sample filters were extracted and the extracts combined. Afterwards they were evaporated down to an appropriate volume and divided into equal parts, which depended on the number of columns to be compared and amount of filter extracts to be cleaned by a single column. Each part was, thus, subjected to a different cleanup procedure. For concentration determination only 1-step clean-up was optimized. The filters were sampled in various places in Belgium, both heavy traffic and remote locations, thus different PAHs levels and matrix content was expected in these samples and hence the need to combine two-three samples could not be excluded in case of low PAHs concentration for stable carbon isotope analyses. Because of different filters or amounts used on different days of clean-ups only columns used on a specific day could be compared between each other. The

performance was nevertheless highly repeatable for a specific column thus general conclusions could be drawn.

### 3.2 Qualitative evaluation of clean-up experiments

The amount of solvent necessary to remove all of the unresolved complex matrix (UCM) and *n*-alkanes from samples was investigated. The first step of clean-up in subsequent experiments consisted of alumina active, florisil active, florisil non-active, silica active, silica non-active and CN-silica non-active. Generally one to three air particulate matter filters' extracts per column were used. In the first step columns were eluted with 16 mL of *n*-hexane (in portions 3 + 3 + 10 mL). Next, 10 mL *n*-hexane/DCM (1:1, v:v) was used.

When alumina columns were used, PAHs eluted consistently already in the first 3 mL of *n*-hexane, for all other columns this amount could be discarded as it contained only UCM and *n*-alkanes. In the subsequent fraction of 3 mL of *n*-hexane, PAHs were present when non-active florisil or silica column was used. No PAHs were seen in this fraction when silica active, florisil active, or CN-silica columns were applied; 6 mL of *n*-hexane could thus be discarded for those columns. Additional 10 mL of *n*-hexane applied (16 mL in total) eluted some of the PAHs from silica active column but no PAHs were eluted from florisil active SPE cartridge. Subsequently, this amount was lowered to 3 mL in place of 10 mL. Thus, 9 mL of *n*-hexane in total could be used to elute the aliphatic fraction and UCM without eluting PAHs from active silica and florisil columns.

Remaining aromatics were eluted with 10 mL *n*-hexane/DCM (1:1, v:v). Interferences (large peaks in Figure 1) were not present in any of the eluted fractions when one filter per column was used. When silica was not activated, these components were eluted together with PAHs fraction, even if a small amount of sample matrix was used. For high sample matrix loads (>2.5 filters sampled in urban location), after a single step of clean-up, the aromatic fraction still contained the above mentioned interferences in all setups investigated. The second step of clean-up was necessary to successfully purify these samples from interferences.

For some of the clean-ups a rinse with additional 15 mL (10+5 mL) of *n*-hex/DCM (1:1, v:v) was applied, to check whether any PAHs remained on activated columns. PAHs could be seen in first 10 mL only in case of florisil active. Thus, the initial 10 mL of *n*-hex/DCM was sufficient for elution of PAHs from all studied columns except for active florisil, here, additional 10 mL of *n*-hexane/DCM (1:1, v-v) was necessary.

To conclude, in the first step of clean-up, PAHs tended to elute in the *n*-hexane elution step (aliphatics) when non-activated columns were used or alumina, despite being activated previously, whilst more than 10 mL of *n*-hexane/DCM mixture was needed to elute PAHs from a Florisil active column. No major interferences in the PAHs fraction were seen when extracts of only one filter were used per SPE column and thus samples purified in such a way could be used for PAHs concentration analyses. If pooling of few samples was needed, with the intention of measuring the  $\delta^{13}\text{C}$  of PAHs, the second step was necessary, as interferences which co-eluted with PAHs were seen in the aromatic fraction of the first step.



Activation of columns resulted in better clean-up efficiency and higher retention capacity for interferences.

The second step consisted of an active silica gel column. Here, we increased the initial volume of *n*-hexane (3 mL) to 6 mL as it was beneficial for the impurities removal prior to PAHs elution.

Fraction 2 and 3 were subsequently eluted with mixtures of solvents (*n*-hexane/DCM (9:1 and 4:6, v:v)) in amounts determined previously [14]. Samples processed in such way contained PAHs well resolved from other constituents (Figure 2). The large interferences visible after the initial clean-ups were successfully retained on the second SPE activated column. Thanks to the installation of the IT-MS, the peak of each PAH compound could be confirmed based on the mass spectra and additionally, purity of peaks could be checked in every sample analysed.

**Figure 2.** GC-C-IRMS chromatogram of air particulate matter sample (aromatic fraction) purified with a two-step clean-up using activated silica gel SPE columns.

### 3.3 Quantitative evaluation of clean-up experiments

Quantitative evaluation was conducted using an internal standard method (five point calibration curves). Deuterated standards are normally used for PAHs quantitation [13,17-18]. For stable isotope analysis, no such standards can be added because of co-elution with analytes. Instead, *p*-terphenyl was used. The internal standard was added to the sample just before injection. The recoveries of PAH standard added to the SPE columns were between 71 – 101 % (except for naphthalene, which was recovered in 55%). PAHs were eluted by applying 9 mL of *n*-hexane and 20 mL of *n*-hexane/DCM (1:1, v-v) mixture. As mentioned before, when non-activated columns were used, analytes eluted in all of the three *n*-hexane fractions. Activated florisil column seemed to adsorb irreversibly some of high MW PAHs: benzo(a)pyrene and benzo(g,h,i)perylene. Silica active gave the best performance since PAHs eluted exclusively in first 10 mL of *n*-hexane/DCM fraction except for naphthalene and very small amounts of acenaphthene and acenaphthylene.

Subsequently, the quantitative performance of SPE columns was evaluated in the presence of matrix components. One to three experiments were conducted for each column (Tables 1-5). In those tables, columns named “1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>” below each fraction refer to different clean-ups done on different days. In this way the reproducibility of each column could be assessed. The amount of PAHs eluted in each fraction was summed up and the percent recovery in each fraction calculated. For alumina active only one such clean-up was conducted, as together with previous experiments, the results were not very reproducible for this column; also the purification efficiency was worse than for other SPE columns.

**Table 1.** Percent recovery (%) of PAHs in all eluted fractions for non activated silica gel SPE column

Compound	Silica non active (%)											
	1-3 mL n-hexane		4-6 mL n-hexane		7-9 mL n-hexane		10 mL n-hex/DCM		11-20 mL n-hex/DCM		21-25 mL n-hex/DCM	
	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>
Naphthalene <sup>a</sup>	33	N.D.	44	N.D.	0	N.D.	0	N.D.	12	N.D.	11	N.D.
Acenaphthylene	0	0	45	0	16	100	26	0	7	0	5	0
Acenaphthene	8	0	23	0	0	31	18	34	51	35	0	0
Fluorene	0	0	100	0	0	100	0	0	0	0	0	0
Phenanthrene	1	0	95	0	5	90	0	9	0	0	0	1
Anthracene	0	0	69	0	9	100	22	0	0	0	0	0
Fluoranthene	0	0	92	0	8	87	0	13	0	0	0	0
Pyrene	0	0	95	0	5	91	0	9	0	0	0	0
Benz(a)Anthracene	0	0	83	0	15	65	2	35	0	0	0	0
Chrysene	0	0	83	0	17	66	0	34	0	0	0	0
Benzo(b)fluoranthene	0	0	68	0	32	45	0	55	0	0	0	0
Benzo(k)fluoranthene	0	0	68	0	32	45	0	55	0	0	0	0
Benzo(a)pyrene	0	0	79	0	21	63	0	37	0	0	0	0
Indeno (1,2,3-cd) pyrene	0	0	54	0	46	38	0	62	0	0	0	0
Dibenz(a,h)anthracene	0	0	18	0	82	0	0	100	0	0	0	0
Benzo(ghi)perylene	0	0	68	0	32	50	0	50	0	0	0	0

<sup>a</sup> Compound was not detected using other clean-ups as well

**Table 2.** Percent recovery (%) of PAHs in all eluted fractions for non activated florisil SPE column

Compound	Florisil non active (%)											
	1-3 mL n-hexane		4-6 mL n-hexane		7-9 mL n-hexane		10 mL n-hex/DCM		11-20 mL n-hex/DCM		21-25 mL n-hex/DCM	
	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>
Naphthalene <sup>a</sup>	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Acenaphthylene	0	0	0	22	47	33	53	30	0	8	0	8
Acenaphthene	0	0	0	0	51	56	49	44	0	0	0	0
Fluorene	0	0	0	0	0	33	100	67	0	0	0	0
Phenanthrene	0	0	0	0	0	5	98	92	1	2	1	1
Anthracene	0	0	0	0	0	31	100	69	0	0	0	0
Fluoranthene	0	0	0	0	0	0	99	99	1	1	0	0
Pyrene	0	0	0	0	0	0	100	100	0	0	0	0
Benz(a)Anthracene	0	0	0	0	0	0	100	100	0	0	0	0
Chrysene	0	0	0	0	0	0	100	100	0	0	0	0
Benzo(b)fluoranthene	0	0	0	0	0	0	100	100	0	0	0	0
Benzo(k)fluoranthene	0	0	0	0	0	0	100	100	0	0	0	0
Benzo(a)pyrene	0	0	0	0	0	0	100	100	0	0	0	0
Indeno (1,2,3-cd) pyrene	0	0	0	0	0	0	100	100	0	0	0	0
Dibenz(a,h)anthracene	0	0	0	0	0	0	100	100	0	0	0	0
Benzo(ghi)perylene	0	0	0	0	0	0	100	100	0	0	0	0

<sup>a</sup> Compound was not detected using other clean-ups as well

**Table 3.** Percent recovery (%) of PAHs in all eluted fractions for activated alumina SPE column

Compound	Alumina active (%)					
	1-3 mL <i>n</i> -hexane	4-6 mL <i>n</i> -hexane	7-9 mL <i>n</i> -hexane	10 mL <i>n</i> -hex/DCM	11-20 mL <i>n</i> -hex/DCM	21-25 mL <i>n</i> -hex/DCM
Naphthalene	27	24	22	27	0	0
Acenaphthylene	24	20	13	31	8	4
Acenaphthene	45	34	21	0	0	0
Fluorene	0	0	0	88	9	3
Phenanthrene	9	5	3	83	0	0
Anthracene	0	0	0	100	0	0
Fluoranthene	5	3	1	91	1	0
Pyrene	19	13	9	59	0	0
Benz(a)Anthracene	0	0	0	94	6	0
Chrysene	0	0	0	96	4	0
Benzo(b)fluoranthene	0	0	0	81	17	2
Benzo(k)fluoranthene	0	0	0	79	18	3
Benzo(a)pyrene	1	0	0	94	5	0
Indeno (1,2,3-cd)pyrene	0	0	0	76	20	4
Dibenz(a,h)anthracene	0	0	0	97	0	3
Benzo(ghi)perylene	0	0	0	97	3	0

**Table 4.** Percent recovery (%) of PAHs in all eluted fractions for activated silica gel SPE column

Compound	Silica active (%)																	
	1-3 mL <i>n</i> -hexane			4-6 mL <i>n</i> -hexane			7-9 mL <i>n</i> -hexane			10 mL <i>n</i> -hex/DCM			11-20 mL <i>n</i> -hex/DCM			21-25 mL <i>n</i> -hex/DCM		
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>
Naphthalene <sup>a</sup>	0	N.D.	N.D.	0	N.D.	N.D.	36	N.D.	N.D.	41	N.D.	N.D.	12	N.D.	N.D.	11	N.D.	N.D.
Acenaphthylene	0	0	0	0	0	0	0	0	0	90	100	77	10	0	12	0	0	10
Acenaphthene	0	0	0	0	0	0	0	0	0	74	100	100	26	0	0	0	0	0
Fluorene	0	0	0	0	0	0	0	0	0	100	100	100	0	0	0	0	0	0
Phenanthrene	0	0	0	0	0	0	0	0	0	100	98	100	0	1	0	0	1	0
Anthracene	0	0	0	0	0	0	0	0	0	100	100	100	0	0	0	0	0	0
Fluoranthene	0	0	0	0	0	0	0	0	0	100	100	100	0	0	0	0	0	0
Pyrene	0	0	0	0	0	0	0	0	0	100	100	100	0	0	0	0	0	0
Benz(a)Anthracene	0	0	0	0	0	0	0	0	0	100	100	100	0	0	0	0	0	0
Chrysene	0	0	0	0	0	0	0	0	0	100	100	100	0	0	0	0	0	0
Benzo(b)fluoranthene	0	0	0	0	0	0	0	0	0	100	100	100	0	0	0	0	0	0
Benzo(k)fluoranthene	0	0	0	0	0	0	0	0	0	100	100	100	0	0	0	0	0	0
Benzo(a)pyrene	0	0	0	0	0	0	0	0	0	100	100	100	0	0	0	0	0	0
Indeno (1,2,3-cd) pyrene	0	0	0	0	0	0	0	0	0	100	100	100	0	0	0	0	0	0
Dibenz(a,h)anthracene	0	0	0	0	0	0	0	0	0	100	100	100	0	0	0	0	0	0
Benzo(ghi)perylene	0	0	0	0	0	0	0	0	0	100	100	100	0	0	0	0	0	0

<sup>a</sup> Compound was not detected using other clean-ups as well

**Table 5.** Percent recovery (%) of PAHs in all eluted fractions for activated florisil SPE column

Compound	Florisil active (%)																	
	1-3 mL			4-6 mL			7-9 mL			10 mL			11-20 mL <i>n</i> -hex/DCM			21-25 mL <i>n</i> -hex/DCM		
	<i>n</i> -hexane			<i>n</i> -hexane			<i>n</i> -hexane			<i>n</i> -hex/DCM			<i>n</i> -hex/DCM			<i>n</i> -hex/DCM		
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>
Naphthalene <sup>a</sup>	0	N.D.	N.D.	0	N.D.	N.D.	0	N.D.	N.D.	68	N.D.	N.D.	17	N.D.	N.D.	15	N.D.	N.D.
Acenaphthylene	0	0	0	0	0	0	0	0	0	89	79	86	11	21	14	0	0	0
Acenaphthene	0	0	0	0	0	0	0	0	0	100	100	100	0	0	0	0	0	0
Fluorene	0	0	0	0	0	0	0	0	0	98	100	78	2	0	11	0	0	11
Phenanthrene	0	0	0	0	0	0	0	0	0	95	97	95	4	2	3	1	1	2
Anthracene	0	0	0	0	0	0	0	0	0	87	80	77	9	20	12	4	0	11
Fluoranthene	0	0	0	0	0	0	0	0	0	97	98	98	3	1	1	0	1	1
Pyrene	0	0	0	0	0	0	0	0	0	98	97	98	2	2	1	0	1	1
Benz(a)Anthracene	0	0	0	0	0	0	0	0	0	94	91	96	6	9	3	1	0	1
Chrysene	0	0	0	0	0	0	0	0	0	96	95	97	4	5	2	0	0	1
Benzo(b)fluoranthene	0	0	0	0	0	0	0	0	0	95	92	97	5	8	2	0	0	1
Benzo(k)fluoranthene	0	0	0	0	0	0	0	0	0	94	90	96	6	10	3	0	0	1
Benzo(a)pyrene <sup>b</sup>	0	N.D.	0	0	N.D.	0	0	N.D.	0	53	N.D.	71	38	N.D.	24	9	N.D.	5
Indeno (1,2,3-cd) pyrene	0	0	0	0	0	0	0	0	0	72	0	86	27	62	12	1	38	2
Dibenz(a,h)anthracene	0	0	0	0	0	0	0	0	0	82	71	90	18	29	10	0	0	0
Benzo(ghi)perylene <sup>b</sup>	0	N.D.	0	0	N.D.	0	0	N.D.	0	69	N.D.	87	29	N.D.	11	2	N.D.	1

<sup>a</sup> Compound was not detected using other clean-ups as well<sup>b</sup> Compounds were found in sample extracts if other SPE columns were used

Clean-ups in which non-active columns were used (silica, florisil) resulted in less reproducible results and worse separation of fractions containing aliphatics and UCM from PAHs. This was true also for alumina column, despite the fact that it was activated before use. When PAHs concentrations were summed up and compared between different columns, similar results were obtained for PAHs with MW higher than fluorene. The RSD's of the mean concentration were generally below 10%. The reproducibility between different SPE columns was worse for more volatile compounds (RSD close to 30%) and was also higher on some of the days for benzo(k)fluoranthene, benzo(a)pyrene, and indeno(1,2,3-cd)pyrene.

Following these findings activated silica gel SPE columns were used since the best separation of PAHs and aliphatic fraction and high reproducibility was obtained with their use.

### 3.4 Method validation with standard reference material (ERM-CZ100)

The method was validated using the European Reference Material (ERM-CZ100, fine dust, PM10-like). This material was certified for concentration of seven PAHs (Table 6), the concentrations of seven other PAHs are given as additional material information. Spiking of a sample with the standard mix and subsequent extraction of filters for the method validation is not considered a completely accurate approach [19] as it has been shown that the extraction rates from solid matrices may vary significantly for native and spiked compounds [19 and references therein]. Hence, we resorted to the use of an established PM reference material ERM-CZ100. Four samples (100-150 mg) of reference material were extracted and cleaned using the above optimized protocol. The recoveries of PAHs were between 55 % (phenanthrene) and 115 % (benz(a,h)anthracene) (Table 6). The average recovery for PAHs was 88 % (94 % for certified values and 81% for non-certified). Similarly, Fang et al. 2004 [20] reports the average of 86% recovery efficiency for 16 EPA PAH extracted from air particulate matter by Soxhlet. Ding et al. 2007 [21] found recoveries of PAHs from NIST reference material 1941 ranging between 80-120 %. Bi et al. 2003 [12], between 39 % (naphthalene) and 101 % (benzo(g,h,i)perylene), the experiments were conducted on matrix spikes. Caricchia et al. 1999 [22] found average recovery of 75 % for PAHs with similar boiling range. The relative standard deviations of four replicates in our checks were between 4-12%. The RSDs reported, for example, for Accelerated Solvent Extraction (ASE) [23] were between 2-6.7 % for PAHs extracted from Urban Dust (SRM 1649, NIST) with the average of 5.2 %. The average RSD for the same compounds using our method was 8.9 %, thus somewhat higher. In another study [3] recoveries ranging from not detected to 157% in a contaminated soil were found; the study compared Soxhlet and ASE extraction combined with either silica or alumina open chromatography clean-ups.

**Table 6.** The concentration of PAHs in the reference material (ERM-CZ100) in ng/mg.

Compound	1st	2nd	3rd	4th	Average	SD	RSD (%)	Certified value (ng/mg)	Recovery (%)
Certified values									
Benz(a)anthracene	0.79	0.85	0.68	0.79	0.78	0.07	9.3	0.91	<b>85.5</b>
Benzo(b)fluoranthene	1.50	1.55	1.38	1.67	1.53	0.12	7.9	1.42	<b>107.4</b>
Benzo(k+j)fluoranthene	1.09	1.14	0.87	1.06	1.04	0.12	11.2	1.42	<b>73.3</b>
Benzo(a)pyrene	0.57	0.67	0.49	0.59	0.58	0.07	12.4	0.72	<b>80.7</b>
Indeno (1,2,3-cd) pyrene	1.11	1.11	1.00	1.09	1.08	0.05	5.0	1.07	<b>100.6</b>
Dibenz(a,h)anthracene	0.21	-	-	-	-	-	-	0.18	<b>114.6</b>
Benzo(ghi)perylene	1.41	1.56	1.22	1.41	1.40	0.14	9.7	1.76	<b>79.5</b>
Non-certified values									
Phenanthrene	1.23	1.30	1.08	1.27	1.22	0.10	7.9	2.23*	<b>54.8</b>
Anthracene	0.21	0.23	0.21	0.22	0.22	0.01	4.2	0.28*	<b>78.3</b>
Fluoranthene	3.34	3.62	2.94	3.53	3.36	0.30	8.9	4.67*	<b>71.9</b>
Pyrene	3.58	3.77	3.24	3.75	3.59	0.24	6.8	4.59*	<b>78.1</b>
Chrysene	1.53	1.64	1.30	1.56	1.51	0.14	9.6	1.61*	<b>93.7</b>
Other PAHs									
Naphthalene	-	-	-	-	-	-	-	-	-
Acenaphthylene	0.09	0.11	0.10	0.10	0.10	0.01	5.5	-	-
Acenaphthene	0.08	0.09	0.09	0.07	0.08	0.01	9.1	-	-
Fluorene	0.12	0.14	0.14	0.13	0.13	0.01	6.2	-	-

\*additional value (not certified)

The reason of dibenz(a,h)anthracene being recovered only in one of the four replicates (Table 6) is unclear. It had the lowest concentration of all certified PAHs, Marr et al. [24] as well reported losses of higher molecular weight PAHs during SPE when their concentration in the analysed fuel was below a certain level when compared to unprocessed samples. Perhaps, similar was the reason in our case.

The concentration of PAHs in particulate matter samples were corrected for the recovery efficiency.

### 3.5 Validation of the analytical procedure for isotopic ratio measurement

The optimized method was finally checked for isotopic fractionation artefacts. PAH standard was subjected to the ultrasound extraction and both steps of the clean-up procedure.

The isotope ratios were then measured and compared to the unprocessed solution. Figure 3 gives the  $\delta^{13}\text{C}$  values of PAHs obtained during this experiment.

**Figure 3.** Comparison of carbon isotope ratio values in two standard solutions that has been subjected to sample extraction and purification procedure (1<sup>st</sup> and 2<sup>nd</sup> clean-up step) with unprocessed solution. The error bars correspond to 1 SD of the mean of four injections.

The differences in  $\delta^{13}\text{C}$  values were statistically insignificant (Student's t-test) between standards subjected to clean-up and the unprocessed solution, with the exception of naphthalene. The recovery of naphthalene was much lower than that of other PAHs, thus we contribute this difference rather to a very low peak of naphthalene, superimposed on relatively higher background that occurred in this part of chromatogram for proceeded samples. Further concentration of the solution in order to increase the amount injected on column was not done as naphthalene is predominantly present in the gas phase and the focus of this study was rather the air particulate matter.

### 3.6 Application to air particulate matter samples

#### 3.6.1 Concentration of PAHs

The optimized procedure was tested on a set of samples collected in three VMM air monitoring stations in Belgium: Gent, Zelzate and Borgerhout from 28<sup>th</sup> till 30<sup>th</sup> November 2011. The concentrations of 12 PAH compounds are listed in Table 7. The highest concentrations of all PAHs amongst these locations were registered in Borgerhout, consistent with previous findings [25] as this site is influenced by a heavy traffic. The concentrations found in Gent and Zelzate were lower and comparable to each other.

**Table 7.** Concentration of PAH compounds ( $\text{ng m}^{-3}$ ) at Gent, Zelzate and Borgerhout on 28, 29 and 30 November 2011

Location	Phe	Ant	Flu	Pyr	B(a)A	Chry	B(b)F	B(jk)F	BeP	BaP	Ind	D(ah)	B(ghi)P	Sum PAHs
GE1	0.08	0.01	0.18	0.17	0.14	0.29	0.37	0.41	0.37	0.28	0.31	0.05	0.37	3.02
GE2	0.03	0.01	0.05	0.05	0.04	0.07	0.10	0.13	0.10	0.07	0.09	0.02	0.10	0.86
GE3	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.03	0.02	0.03	0.02	0.02	0.18
ZL1	0.12	0.01	0.22	0.19	0.24	0.31	0.48	0.35	0.38	0.35	0.31	0.07	0.40	3.43
ZL2	0.07	0.01	0.12	0.10	0.11	0.15	0.24	0.22	0.22	0.16	0.17	0.04	0.23	1.83
ZL3	0.01	0.01	0.02	0.02	0.03	0.04	0.06	0.07	0.06	0.06	0.06	0.02	0.07	0.52
BO1	0.58	0.12	1.15	0.89	1.34	1.48	1.78	0.69	0.97	1.10	0.61	0.14	0.64	11.49
BO2	1.30	0.29	2.48	1.80	1.61	1.59	1.57	0.68	0.98	1.27	0.77	0.18	0.71	15.24
BO3	0.46	0.09	0.97	0.73	0.56	0.67	0.58	0.39	0.42	0.46	0.29	0.07	0.34	6.03

Figure 4 shows the relative contribution of PAHs at each location. The PAH profiles in Gent and Zelzate are similar, that is to say, 5- and 6-rings PAHs predominate over lower



molecular weight PAHs in both locations. Whilst in Borgerhout, it is the 4-ring PAHs that have the highest percentages amongst all PAHs. Similar was found by Vercuteren et al. [26], who concluded that lighter PAHs are more typical for Borgerhout than for other locations (Zelzate and Aarschot).

**Figure 4.** Comparison of carbon isotope ratio delta values of air particulate samples collected in three consecutive days in three different locations in Belgium (GE- Gent, ZE-Zelzate, BO-Borgerhout).

The authors sampled both the gas and particulate phases thus broader picture, especially of more volatile PAHs was obtained, nevertheless the results agree with ours. On the other hand, when factor analyses were done on the results from all the sites together, in the winter samples, first factor, which explained 60% of the total variance, contained high loadings of PAHs from naphthlene up to benzo(a)pyrene, and was interpreted as pointing out to domestic heating as a common source. This is consistent with the VMM report of evolution of pollutants emissions over Belgium in the years 2000-2012 [27], which assesses the contributions of various sources to the emissions of four PAHs (benzo(a)pyrene, benzo(b)- and benzo(k)fluornathenes and indeno(1,2,3-cd)pyrene) and lists households heating as the main contributor (90%). The second factor in the study of Verkouteren et al., [26] contained higher loadings of heavier PAHs, indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene, benzo(g,h,i)perylene whilst the third factor contained only high loadings of two more volatile PAHs (acenaphthene and fluorene). In 2006, Ravindra et al. [25, 28] has conducted a study of PAHs in six different locations in Belgium (Borgerhout and Zelzate among them) and concluded that vehicular emissions was the major source of PAHs in Flanders followed by minor contributions from incineration, petroleum/oil burning, coke production and wood/coal combustion.

Subsequently we calculated some of the molecular diagnostic ratios that are used across the literature [1,9, 25, 29-32] for identification of sources of these pollutants (Table 8). Significant differences ( $p < 0.05$ ) in most of these ratios (except for  $\text{Ind}/(\text{Ind}+\text{BghiP})$ ) were detected between Borgehout and other two locations. The differences in ratios of PAHs between in Zelzate and Gent were on the other hand statistically insignificant.

**Table 8.** The selected diagnostic ratios of PAHs at Gent, Zelzate and Borgerhout on 28, 29 and 30 November 2011.

Location	Flu/(Flu+Pyr)	BaA/(BaA+Chry)	Ind/(Ind+BghiP)	BaP/BghiP	BaA/Chry	Flu/BeP
GE1	0.52	0.33	0.46	0.76	0.49	0.49
GE2	0.54	0.35	0.45	0.70	0.54	0.51
GE3	0.52	0.56	0.54	1.07	1.30	0.17
ZL1	0.53	0.43	0.44	0.89	0.75	0.58
ZL2	0.54	0.42	0.43	0.69	0.72	0.53
ZL3	0.54	0.43	0.47	0.86	0.77	0.37
BO1	0.56	0.47	0.49	1.73	0.90	1.18
BO2	0.58	0.50	0.52	1.78	1.02	2.54
BO3	0.57	0.46	0.46	1.36	0.84	2.31

The values of the ratios in all locations fall within the ranges for vehicular emissions (Flu/(Flu+Pyr), BaA/(BaA+Chry), Ind/(Ind+BghiP)) but also other emissions, such as coal or wood combustion (e.g. Flu/(Flu+Pyr) equal to 0.4-0.5 was cited in the literature for vehicular emissions, whilst >0.5 for coal or wood combustion). In Belgium, oil and gas [33], is generally used for households heating thus coal can be excluded as a source of PAHs. Flu/BeP is reaching 3.5 for automobiles [31 and references therein], only at Borgerhout the ratios were close to this value, in other locations they were much lower (around or lower than 0.5). On the other hand the ratio of BaP/BghiP for vehicular emissions fits to the literature values only at Zelzate and Gent, in Borgerhout it is higher, rather within the range found previously for coal combustion [29 and references therein], what is unexpected as Borgerhout has the highest contribution of traffic amongst all the locations.

### 3.6.2 $\delta^{13}\text{C}$ measurements of PAHs

The samples were purified using the two step clean-up procedure and  $\delta^{13}\text{C}$  of PAHs was measured (Figure 4). Low amounts of PAHs were seen in samples which originated from Gent and Zelzate; these samples (three filters from each location) were combined. Samples from Borgerhout exhibited high levels of PAHs and single 24h filters were used for carbon stable isotope ratio analysis. These samples showed the most positive  $\delta^{13}\text{C}$  values amongst the three locations. The standard deviations for Borgerhout samples ranged from 0.23 to 2.3 ‰ (0.52 ‰ on the average) for BO1, 0.08 to 0.67 ‰ (0.46 ‰ on average) for BO2 and 0.18 to 1.9 ‰ (0.72 ‰ on average) for BO3. The highest SD were registered for dibenz(a,h)anthracene and anthracene. Both compounds were present in low concentrations (amplitude m/z=44 of about 300 mV). Moreover, dibenz(a,h)anthracene is co-eluting with indeno(1,2,3-cd)pyrene. Co-eluting PAHs: benz(a)anthracene and chrysene, benzo(b,j,k)fluoranthenes and above mentioned indeno(1,2,3-cd)pyrene with dibenz(a,h)anthracene were subsequently integrated together. No statistically significant differences (Student's t-test) were observed between  $\delta^{13}\text{C}$  of PAHs in BO samples on different days, except for benzo(e)- and benzo(a)pyrene between BO1 or BO2 and BO3, phenanthrene between BO2 and BO3 and the sum of indeno(1,2,3-cd)pyrene+dibenz(a,h)anthracene between BO1 and BO2.

**Figure 5.** Comparison of carbon isotope ratio delta values of air particulate samples collected in three consecutive days in three different locations in Belgium (GE- Gent, ZE-Zelzate, BO-Borgerhout).

The average  $\delta^{13}\text{C}$  values for fluoranthene up to benzo(g,h,i)perylene were -24.7 ‰ (range -25.3 to -24.2 ‰), -24.2 ‰ (range -24.8 to -23.9 ‰) and -25.2 ‰ (range -25.7 to -24.0 ‰), for samples BO1, BO2 and BO3, respectively. These values are in agreement with values reported for vehicle emission PAH  $\delta^{13}\text{C}$  studies [34- 35]. The range was thus very narrow and no trend was seen between delta value and MW of PAHs. Borgerhout location has the highest

contribution of traffic amongst VMM sampling stations as was determined by the measurements of EC [26, 33], which is thought to predominantly originate from traffic and often considered a marker for traffic emissions and especially diesel engine [30 and the references therein]. Samples collected on the first and the second day of sampling in Borgerhout, exhibited the same pattern and the values were within 1 SD away from each other. The sample collected on the third day exhibited slightly lower delta values for most of PAHs.

The combined samples collected in Gent are characterized by the most negative delta values. Standard deviations of the mean  $\delta^{13}\text{C}$  value obtained for three injections were the largest for this sample (due to the relatively low amplitudes of PAHs), they were in the range of 0.65 – 1.5 ‰ with an average of 1 ‰. The sample of Zelzate exhibited intermediate delta values. For these two locations an increase in delta values with increasing molecular weight can be noticed. The  $\delta^{13}\text{C}$  of benzo(g,h,i)perylene is similar for all three sampling places, thus indicating a common source of this PAH, this is not unexpected as BghiP is generally thought to originate from automotive exhaust [36 and references therein] and has been used as a marker of mobile emissions [30]. The most negative values for PAHs collected in Gent (Pyr-BaP) might be associated with a contribution of wood burning PAHs or, more generally, domestic heating. PAHs originating from wood burning exhibit relatively negative  $\delta^{13}\text{C}$  ranges as well, for example for both hardwood and softwood combustion a range between -28.8 and -28 ‰ for PAHs (naphthalene through pyrene) was measured [37]. In another study [38], range of -30 to -36.6 ‰ was obtained for all 16 EPA PAHs from different wood samples. Whilst some of the coal originating PAHs showed depletion with increasing molecular weight [38, 39], the wood combustion products showed enrichment [38]. The range of -28.9 to -30.6 ‰ was seen for some of the 3-5 ring PAHs that originated from soot from a chimney in which both coal and wood were burned, whilst more enriched delta values, -24.2 to -26.1 ‰, were seen for the same PAHs in soot originating from solely coal combustion [40]. These results are in line with our range for Gent samples. In Gent, the contribution of wood burning in autumn/winter period to the total organic carbon (OC) determined in PM10 samples during wintertime may be as high as 40% [33]. Although the contribution of wood burning to OC was almost the same in Borgerhout [33], it seems as though high amount of PAHs that originated from heavy traffic dominated the  $\delta^{13}\text{C}$  of PAHs. Subsequently statistical significance was tested between different locations, Gent PAHs were significantly different from Borgerhout PAHs with the exception of fluoranthene and benzo(ghi)pyrene during all three days, pyrene in BO1 and BO2, sum of indeno(1,2,3,-cd)pyrene and dibenz(a,h)anthracene in BO1 and BO3 and BaA+Chry in BO3. Zelzate PAHs  $\delta^{13}\text{C}$  were significantly different from PAHs in BO1 and BO2, except for phenanthrene, sum of Ind+D(ah)A and BghiP, whilst generally no differences were seen between BO3, with two exceptions (BbjkF and BeP). No statistically significant differences were detected (Student's t test) between Zelzate and Gent PAHs with the exception of BeP and BaP. These differences are generally in agreement with the differences in distribution analyses of PAHs described previously.

## 4 Conclusions

Based on the results of the conducted SPE column comparisons, best performance for the clean-up of 24-h air particulate matter samples was obtained with activated silica-gel columns in terms of selectivity and reproducibility. Generally for interferences removal and PAH recovery, all SPE columns performed similarly. However, in alumina columns lighter MW PAHs tended to elute already in n-hexane fraction, whilst florisil (2g) tended to irreversibly adsorb some of the higher MW PAHs. One step clean-up was optimized for concentration determination and in case of co-elutions present, second step was additionally used for carbon isotope ratio analysis. Subsequently the standard reference material, fine dust, PM10-like was used to determine the recoveries of PAHs, which were comparable to other literature studies. The sample preparation method was also checked for carbon isotope fractionation artefacts. No significant differences in  $\delta^{13}\text{C}$  values were found for unprocessed solutions of PAHs and the same compounds subjected to extraction and purification procedure. The procedure was tested on air particulate matter samples collected in three different locations in Belgium. Statistically significant differences in carbon isotope ratio of PAHs between Borgerhout location and Zelzate or Gent were noticed, confirming the differences in distribution and diagnostic ratios found during the concentration analyses and different PAH sources in these locations. The results, therefore, seem very promising for the use of  $\delta^{13}\text{C}$  of PAHs as an additional information helpful in source identification of these pollutants. The analyses were done on a limited data set (9 samples) and only 3 days were considered, thus for a more accurate assessment of the use of PAHs  $\delta^{13}\text{C}$  in the source identification and apportionment a more extensive sampling campaign and longer term isotopic analyses are needed.

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## 5 References

[1] G.L. Ströherr, N. Ré Poppi, J.L. Rapso Jr., J.B. Gomes de Souza, Determination of polycyclic aromatic hydrocarbons by gas chromatography-ion trap tandem mass spectrometry and source identifications by methods of diagnostic ratio in the ambient air of Campo Grande, Brazil, *Microchem J* 86 (2007) 112-118.

- [2] M. Akyüz, H. Çabuk, Particle-associated polycyclic aromatic hydrocarbons in the atmospheric environment of Zonguldak, Turkey, *Sci Total Environ* 405 (2008) 62-70.
- [3] M.P. Ricci, D.A. Merrit, K.H. Freeman, J.M. Hayes, Acquisition and processing of data for isotope-ratio-monitoring mass spectrometry, *Org Geochem* 21 (1994) 561-571.
- [4] J.S Fritz, M. Macka, Solid-phase trapping of solutes for further chromatographic or electrophoretic analysis, *J Chromatogr A* 902 (2000) 137-166.
- [5] A.J. Buczynska, B. Geypens, R. Van Grieken, K. De Wael, Large volume injection combined with gas chromatography-isotope ratio mass spectrometry for analysis of polycyclic aromatic hydrocarbons, *Rapid Commun Mass Sp* 28 (2013) 200-208.
- [6] T. Guerin, The extraction of aged polycyclic aromatic hydrocarbons (PAH) residues from a clay soil using sonication and Soxhlet procedure: a comparative study, *J Environ Monitor* 1 (1999) 63-67.
- [7] M.C. Graham, R. Allan, A.E. Fallick, J.G. Farmer, Investigation of extraction and clean-up procedures used in the quantification and stable isotopic characterisation of PAHs in contaminated urban soils, *Sci Total Environ* 360 (2006) 81-89.
- [8] F. Sun, D. Littlejohn, M.D. Gibson, Ultrasonication extraction and solid phase extraction clean-up for determination of US EPA 16 priority pollutant polycyclic aromatic hydrocarbons in soils by reversed-phase liquid chromatography with ultraviolet absorption detection, *Anal Chim Acta* 364 (1998) 1-11.
- [9] D.R. Banjoo, P.K. Nelson, Improved ultrasonic extraction procedure for the determination of polycyclic aromatic hydrocarbons in sediments, *J Chromatogr A* 1066 (2005) 9-18.
- [10] E. Menichini, F. Monfredini, F. Merli, The temporal variability of the profile of carcinogenic polycyclic aromatic hydrocarbons in urban air : a study in a medium traffic area in Rome , 1993 – 1998, *Atmos Environ* 33, (1999) 3739-3750.
- [11] M. Zheng, M. Fang, F. Wang, K.L.To, Characterization of the solvent extractable organic compounds in PM<sub>2.5</sub> aerosols in Hong Kong, *Atmos Environ* 34 (2000) 2691-2702.
- [12] X. Bi, G. Sheng, P. Peng, Y. Chen, Z. Zhang, J. Fu, Distribution of particulate- and vapor-phase n-alkane and polycyclic aromatic hydrocarbons in urban atmosphere of Guangzhou, China, *Atmos Environ* 37 (2003) 289-298.
- [13] M.A. Bari, G. Baumbach, B. Kuch, G. Scheffknecht, Wood smoke as a source of particle-phase organic compounds in residential areas, *Atmos Environ* 43 (2009) 4722-4732.
- [14] A. Mikołajczuk, B. Geypens, M. Berglund, P. Taylor, Use of a temperature-programmable injector coupled to gas chromatography-combustion-isotope ratio mass spectrometry for compound-specific carbon isotopic analysis of polycyclic aromatic hydrocarbons, *Rapid Commun Mass Sp* 23 (2009) 2421-2427.
- [15] J.-K. Jang, A. Li, Separation of PCBs and PAHs in sediment samples using silica gel fractionation chromatography, *Chemosphere* 44 (2001) 1439-1445.
- [16] V. Yusa, G. Quintas, O. Pardo, A. Pastor, M. de la Guardia, Determination of PAHs in airborne particles by accelerated solvent extraction and large-volume injection-gas-chromatography-mass spectrometry, *Talanta* 69 (2006) 807-815.
- [17] U. EPA, Compendium Method TO-13A. Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Ambient Air Using Gas Chromatography/Mass Spectrometry

- (GC/MS), Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air, EPA/625/R-96/010b. Cincinnati, OH, USA
- [18] N. Itoh, M. Numata, Y. Aoyagi, T. Yarita, Comparison of low-level polycyclic aromatic hydrocarbons in sediment revealed by Soxhlet extraction, microwave-assisted extraction and pressurized liquid extraction, *Anal Chim Acta* 612 (2008) 44-52.
- [19] N. Alexandrou, M. Smith, R. Park, K. Lumb, K. Brice, The extraction of polycyclic aromatic hydrocarbons from atmospheric particulate matter samples by accelerated solvent extraction (ASE), *Int J Environ An Ch*, 81 (2001) 257-280.
- [20] G.-C. Fang, C.-N. Chang, Y.-S. Wu, P. P.-C. Fu, I.-L. Yang, M.-H. Chen, Characterization, identification of ambient air and road dust polycyclic aromatic hydrocarbons in central Taiwan, Taichung, *Sci Total Environ* 327 (2004) 135-146.
- [21] X. Ding, X.-M. Wang, Z.-Q Xie, C.-H. Xiang, B.-X. Mai, L.-G. Sun, M. Zheng, G.-Y. Sheng, J.-M. Fu, U. Pöschl, Atmospheric polycyclic aromatic hydrocarbons observed over the North Pacific Ocean and the Arctic Sea: Spatial distribution and source investigation, *Atmos Environ* 41 (2007) 2061-2072.
- [22] A.M. Caricchia, S. Chiavarini, M. Pezza, Polycyclic aromatic hydrocarbons in the urban atmospheric particulate matter in the city of Naples (Italy), *Atmos Environ* 33 (1999) 3731-3738.
- [23] B.E. Richter, B.A. Jones, J.L. Ezzel, N.L. Porter, Accelerated solvent extraction: A technique for sample preparation., *Anal Chem* 68 (1996) 1033-1039.
- [24] L.C. Marr, T.W. Kirchstetter, R.A. Harley, Characterization of polycyclic aromatic hydrocarbons in motor vehicle fuels and exhaust emissions, *Environ Sci and Technol* 33 (1999) 3091-3099.
- [25] K. Ravindra, Polycyclic Aromatic Hydrocarbons in the Atmosphere: Fast Determination, Concentrations, Sources and Health Risks. PhD dissertation, University of Antwerp, Antwerp, Belgium, 2006.
- [26] J. Vercauteren, C. Matheussen, E. Wauters, E. Roekens, R. Van Grieken, A. Krata, Y. Makarovska, W. Maenhaut, X. Chi, B. Geypens, Chemkar PM10: An extensive look at the local differences in chemical composition of PM10 in Flanders, Belgium, *Atmos Environ* 45 (2011) 108-116.
- [27] Vlaamse Milieumaatschappij (VMM) report. Lozingen in de lucht 2000-2012. <http://www.vmm.be/pub/lozingen-in-de-lucht-2000-2012/>
- [28] K. Ravindra, L. Bencs, E. Wauters, J. de Hoog, F. Deutsch, E. Roekens, N. Bleux, P. Bergmans, R. Van Grieken, Seasonal and site-specific variation in vapour and aerosol phase PAHs over Flanders (Belgium) and their relation with anthropogenic activities, *Atmos Environ* 40 (2006) 771-785.
- [29] M. Akyüz, H. Çabuk, Gas-particle partitioning and seasonal variation of polycyclic aromatic hydrocarbons in the atmosphere of Zonguldak, Turkey, *Sci Total Environ*, 408, (2010) 5550-5558.
- [30] M.F. Simcik, S.J. Eisenreich, P.J. Lioy, Source apportionment and source/sink relationships of PAHs in the coastal atmosphere of Chicago and Lake Michigan, *Atmos Environ* 33 (1999) 5071-5079.

- [31] C. Oliveira, N. Martins, J. Taveres, C. Pio, M. Cerqueira, M. Matos, H. Silva, C. Oliveira, F. Camões, Size distribution of polycyclic aromatic hydrocarbons in a roadway tunnel in Lisbon, Portugal, *Chemosphere* 83 (2011) 1588-1596.
- [32] M.S. Callén, M.T. de la Cruz, J.M. López, A.M. Mastral, PAH in airborne particulate matter. Carcinogenic character of PM10 samples and assessment of the energy generation impact, *Fuel Process Technol* 92 (2011) 176-182.
- [33] W. Maenhaut, R. Vermeylen, M. Claeys, J. Vercauteren, C. Matheussen, E. Roekens, Assessment of the contribution from wood burning to the PM10 aerosol in Flanders, Belgium, *Sci Total Environ* 437 (2012) 226-236.
- [34] V.P. O'Malley, T.A. Abrajano Jr., J. Hellou, Determination of the  $^{13}\text{C}/^{12}\text{C}$  ratios of individual PAH from environmental samples: can PAH sources be apportioned? *Org Geochem* 26 (1994) 809-822.
- [35] T. Okuda, H. Takada, H. Naraoka, Thermodynamic behavior of stable carbon isotopic compositions of individual polycyclic aromatic hydrocarbons derived from automobiles, *Polycycl Aromat Comp* 23 (2003) 219-236.
- [36] E. Lichtfouse, Ancient polycyclic aromatic hydrocarbons in modern soils:  $^{13}\text{C}$ ,  $^{14}\text{C}$  and biomarker evidence, *Org Geochem* 26 (1997) 353-359.
- [37] V.P. O'Malley, R.A. Burke, W.S. Schlotzhauer, Using GC-MS/Combustion/IRMS to determine the  $^{13}\text{C}/^{12}\text{C}$  ratios of individual hydrocarbons produced from the combustion of biomass materials-application to biomass burning, *Org Geochem*, 27 (1997) 567-581.
- [38] C. McRae, G.D. Love, I.P. Murray, C.E. Snape, A.E. Fallick, Potential of gas chromatography isotope ratio mass spectrometry to source polycyclic aromatic hydrocarbon emissions, *Anal Commun* 33 (1996) 331-333.
- [39] C. Sun, M. Cooper, C.E. Snape, Use of compound-specific delta  $^{13}\text{C}$  and delta D stable isotope measurements as an aid in the source apportionment of polyaromatic hydrocarbons, *Rapid Commun Mass Sp* 17 (2003) 2611-2613.
- [40] C. McRae, C. Sun, C.F. McMillan, C.E. Snape, A.E. Fallick, Sourcing of Fossil Fuel-Derived PAH in the Environment, *Polycycl Aromat Comp* 20 (2000) 97-109.

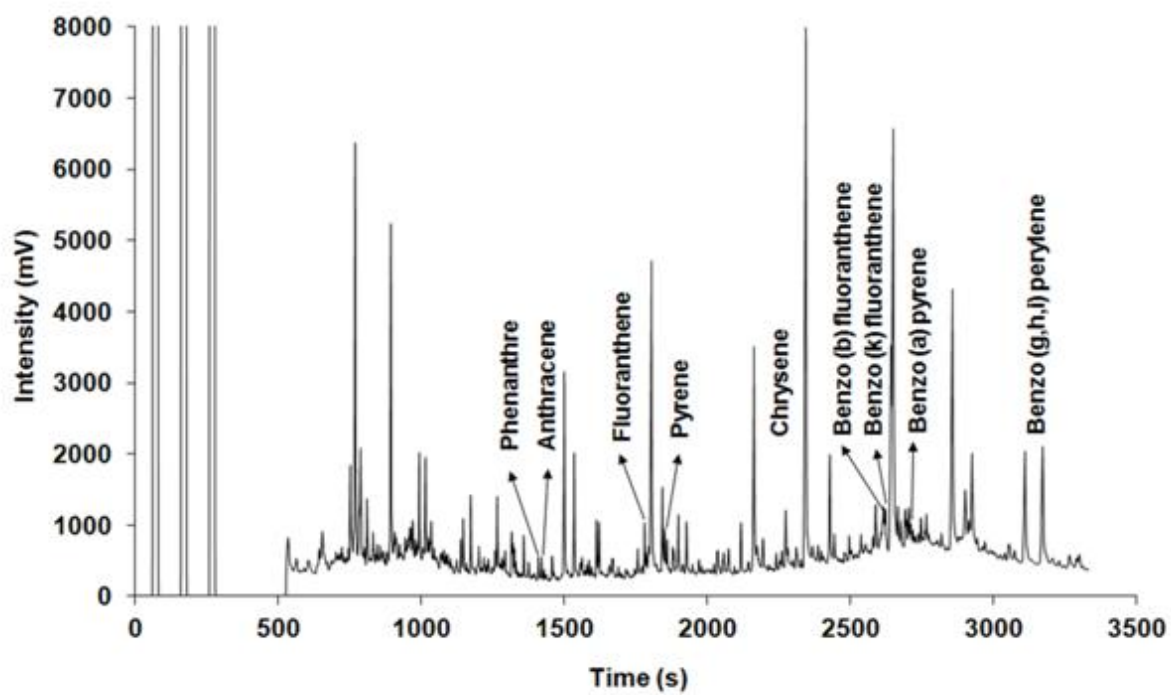


Fig 1

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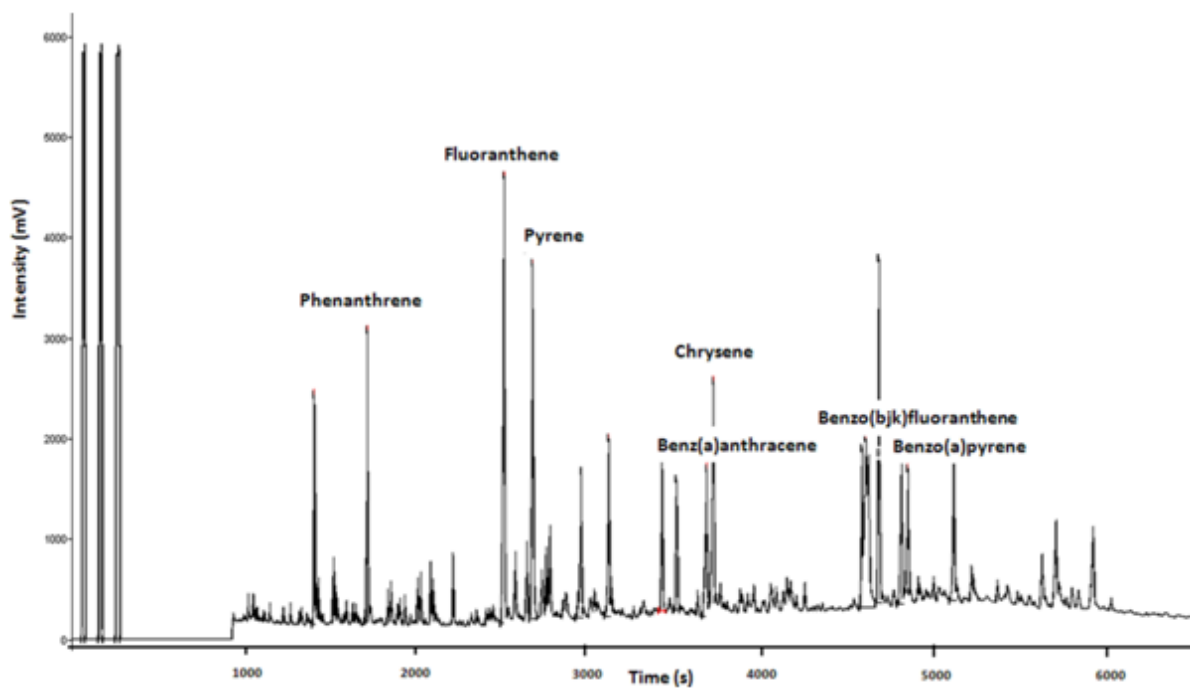


Fig 2

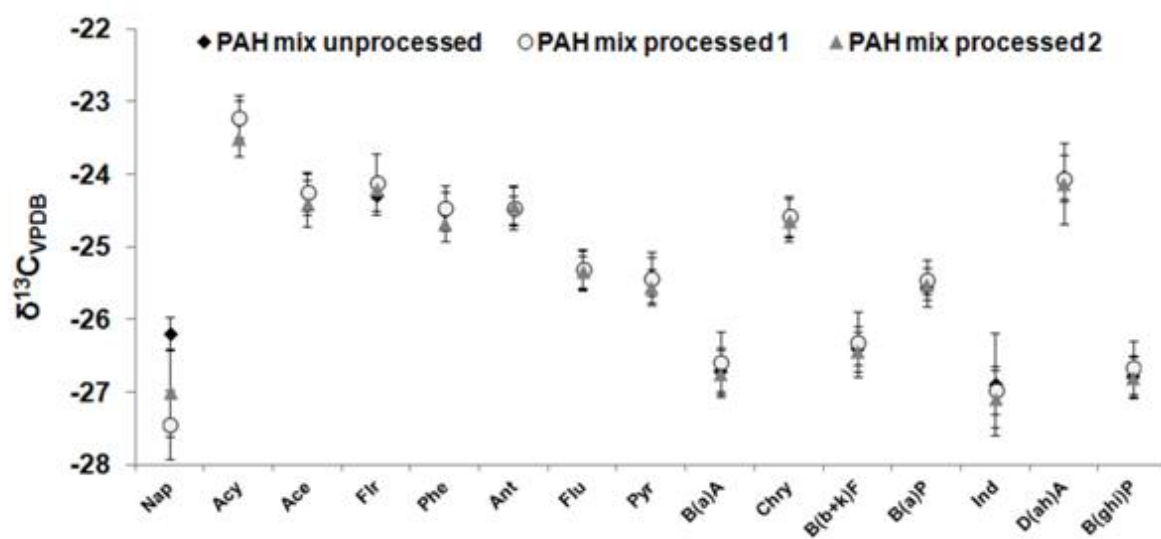


Fig 3

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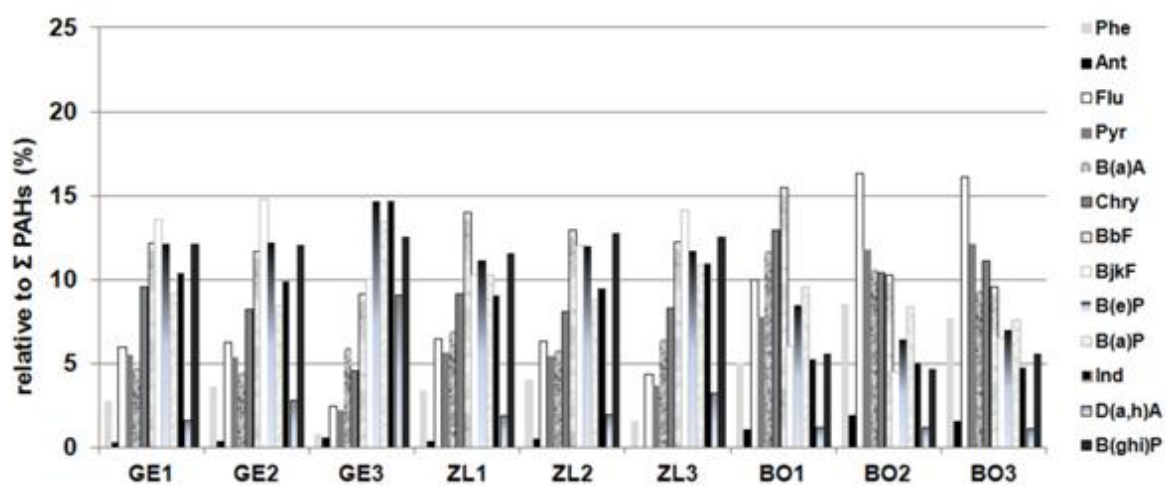


Fig 4

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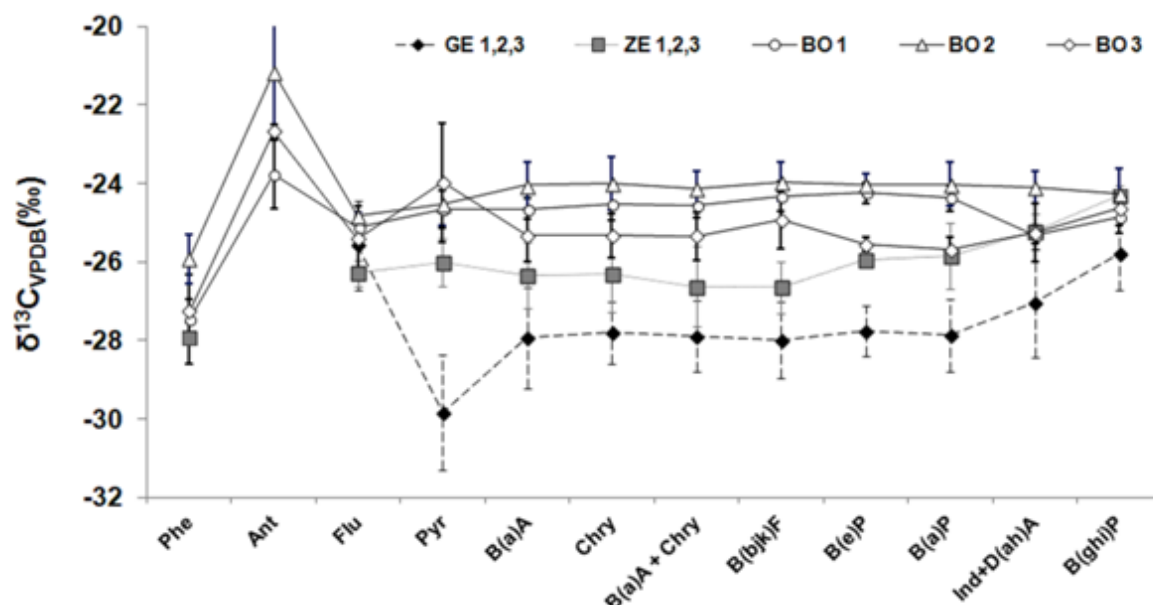


Fig 5

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### Highlights

- Sample clean-up for the analysis of stable carbon isotope ratio of PAHs is optimized
- Best performance was obtained with activated silica-gel cartridges
- The method was tested on air particulate matter from different locations in Belgium

Statistically significant differences between some of the locations were seen

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