Talanta 101 (2012) 428–434

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

An evaluation of uncertainty associated to analytical measurements of selected polycyclic aromatic compounds in ambient air

Ana Isabel Barrado-Olmedo, Rosa María Pérez-Pastor, Susana García-Alonso*

Chromatography unity, Department of Technology, CIEMAT. Avda. Complutense 40, 28040 Madrid, Spain

ARTICLE INFO

Article history:
Received 11 June 2012
Received in revised form
11 September 2012
Accepted 22 September 2012
Available online 29 September 2012

Keywords:
PAHs
Hydroxy-PAHs
Nitro-PAHs
HPLC
Fluorescence
Ambient air

ABSTRACT

This paper presents an evaluation of uncertainty associated to analytical measurement of 18 polycyclic aromatic compounds (PACs) in ambient air by liquid chromatography with fluorescence detection (HPLC/FD). The study was focused on analyses of PM_{10}, $PM_{2.5}$ and gas phase fractions. Main analytical uncertainty was estimated for 11 polycyclic aromatic hydrocarbons (PAHs), four nitro-polycyclic aromatic hydrocarbons (nitro-PAHs) and two hydroxy-polycyclic aromatic hydrocarbons (OH-PAHs) based on the analytical determination, reference material analysis and extraction step. Main contributions reached 15–30% and came from extraction process of real ambient samples, being those for nitro-PAHs the highest (20–30%). Range and mean concentration of selected PACs measured in gas phase and $PM_{10}/PM_{2.5}$ particle fractions during a full year are also presented. Concentrations of OH-PAHs were about 2–4 orders of magnitude lower than their parent PAHs and comparable to those sparsely reported in literature.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Regarding polycyclic aromatic compounds (PACs), aromatic hydrocarbons have been extensively investigated in ambient air, meanwhile other derivatives such as nitro- and especially hydroxyl-derivatives have received less attention in spite of their toxicity, even higher than their parent PAHs [1]. These compounds have been reported to show estrogenic activity when present in ambient particulate matter [2]. As a result, the growing concern over concentration levels of PACs in ambient air made it necessary to optimise analytical methods on the basis of simplicity and efficiency, in order to process large batches of samples.

Among analytical tools for measurement of organic compounds, high performance liquid chromatography with fluorescence detector (HPLC/FD) provides enough sensitivity and selectivity to determine a lot of them in diverse interdisciplinary applications. HPLC methods usually are simple and rapid, with low cost associated if compared with other analytical techniques. These advantages make HPLC/FD an interesting alternative for analysis of PACs, which can exhibit direct fluorescence properties or after derivatisation reactions. Thus, HPLC/FD is rather used for PAH analyses, although not usually employed for nitro-PAHs [3]. This technique has been rarely applied to OH-PAHs determination on airborne particulates [4] despite involving advantages such as no derivatisation requirements and high sensitivity.

Considerable attention has to be paid to quality in order to obtain reliable analytical measurements. From a practical point of view, uncertainty estimations are usually hard tasks in which the evaluation of dominant contributions such as precision and recovery studies [5] is necessary to minimise complex mathematical approaches. For these purposes, the analyses of standard reference materials leads to recovery estimates and associated uncertainty, whereas precision studies are based on variability of data obtained from analyses of representative matrices and levels of concentration. Analytical measurement has also to be considered as an important uncertainty source, mainly affected by preparation of calibration standards and calibration graphs [6].

The aim of this study was to evaluate the major uncertainty contributions associated with the analytical chain for selected PAC measurements in ambient air by HPLC/FD. In particular, 12 PAHs, 4 nitro-PAHs and 2 OH-PAHs were investigated. Uncertainty contributions were grouped from chromatographic determination, reference material analyses and field ambient air samples. Contribution from analyses of real samples was included to give a measure of the confidence in results obtained under field conditions. Glass fibre filters and polyurethane foam plug (PUF) were used for particulate fraction and gas phase collection, respectively. An intensive field campaign was performed weekly from January 2008 to February 2009. The purpose of these measurements was to evaluate the effectiveness of the analytical methods by analysing about 50 each type of ambient air samples. Taking into account the lack of this kind

---

* Corresponding author. Tel.: +34 134 66 539; fax: +34 134 660 05.
E-mail address: susana.garcia@ciemat.es (S. García-Alonso).

0039-9140/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2012.09.053

of studies and the need to assess reliability of analytical results for environmental evaluations, the work here presented can be considered as an interesting contribution to achieve knowledge on analytical variability associated to results.

2. Material and methods

2.1. Reagents and standards

Hexane, dichloromethane and methanol for organic trace analysis and HPLC grade acetonitrile were obtained from SDS, Carlo Erba (Val de Reuil, Cedex). Sodium borohydride (99%) and copper (II) chloride (97%) were obtained from Sigma Aldrich (Steinheim, Germany).

The PAH standards were PAH-Mix 9 from Dr. Ehrenstörfer GMbH containing 10 ng/μL of fluorene (F), phenanthrene (Ph), anthracene (An), fluoranthene (Fl), pyrene (Pyr), benz[*a*]anthracene (BaAn), chrysene (Chry), benz[*b*]fluoranthene (BbFl), benz[*k*]fluoranthene (BkFl), benzo[*a*]pyrene (BaPyr), dibenzo(*a*,*h*)anthracene (DBA) and benzo[*g*,*h*,*i*]perylene (BghiP) in acetonitrile. Following standard reference materials from the National Institute of Standards and Technology (NIST, USA) were used for method validation: SRM 1649, 1975 and 2975, an urban dust, a dichloromethane extract of diesel particulate and diesel particulate matter (also used to prepare SRM1975).

The OH-PAH standards included 1-hydroxypyrene (1-OHPyr) and 2-hydroxyphenantrene (2-OHPh), also from Dr. Ehrenstörfer GMbH, 10 μg mL^{-1} in acetonitrile.

Individual NPAH calibration check solutions (methanol, 10 μg mL^{-1}) were supplied by Dr. Ehrenstörfer Reference Materials (Augsburg, Germany). Selected Nitro-PAHs were 3-nitro phenanthrene (3-NPh), 9-nitrophenanthrene (9-NPh), 1-nitro pyrene (1-NPyr) and 3-nitro fluoranthene (3-NFl). Stock solutions prepared in methanol were used for identification and quantification.

2.2. Sample collection

The sampling site selected was the CIEMAT research centre, 7 km northwest of Madrid city. The site can be considered as an open urban area of busy traffic at rush hour without any direct influence from other sources.

A high volume MCV CAV-A/mb sampler loaded with Whatman glass fibre filters (102 mm diameter) and polyurethane foam plug (PUF) of 5.5 cm diameter by 7.5 cm long were used for the sampling of the particulate and the vapour phase, respectively. Particulate ($PM_{2.5}$ and PM_{10}) air pollution samples were collected on previously heat-backed (400 °C for 24 h) filters. Regarding PUFs, they were previously cleaned up by washing with detergent and rinsing with tap water, and then by Soxhlet extraction with dichloromethane for 24 h.

The high-volumen sampler was used to collect 24 h ambient air samples and the flow rate was 22.5 m^3 h^{-1}. Coarse particulate (PM_{10}) and gas phase (PUF) samples were taken simultaneously, while fine particulate samples ($PM_{2.5}$) were taken separately. Filter and PUF samples were wrapped separately in aluminium foil. After collection, the filters were stored in a freezer at -20 °C until analysis within 2 weeks of collection, while PUFs were stored in a dessicator at room temperature within 3 days of collection. The risk of sample contamination, mainly for PUF samples, was the reason for no delay respective analysis.

2.3. Sample preparation

Filters were half cut as sub-samples for PAH/nitro-PAH and OH-PAH analyses. Each filter half was then weighted and treated as follows.

PAHs and Nitro-PAHs. The filter sub-samples were extracted by microwave at 900 w (120 °C), during 40 min. Dichloromethane was used as extraction solvent (20 mL). The samples were then filtered (0.22 μm-pore size PTFE) and concentrated in a rotary evaporator. To avoid losses, the last solvent fraction was eliminated under nitrogen flow. Once the solvent had evaporated, the extracts were re-dissolved in 1.0 mL acetonitrile and injected in the chromatograph for PAH analyses. Then, acetonitrile extracts were slowly concentrated under nitrogen flow to re-dissolve it in methanol and proceeding for derivatisation reaction.

The reduction to fluorescent amino PAHs was done by treatment with $NaBH_4$–$CuCl_2$ as indicate Gibson [7]. The procedure was based on addition of 0.5 mL of 0.05% aqueous solution of $CuCl_2$ and 0.050 g of $NABH_4$ to 1 mL of methanolic aliquot sample [8]. This mixture was sonicated for 15 min and then maintained at room temperature for 1 h. After this, 1 mL of water and 1 mL of dichloromethane were added and then hand shacked for extraction of amino-PAHs. Dichloromethane phase was collected with a syringe from the bottom of the amber conical vial used. A re-extraction was performed to assure recovery and both organic layers were combined and concentrated under a nitrogen stream. Amino-PAHs were recovered in acetonitrile and analysed by HPLC/FD.

OH-PAHs. Two sonication cycles (15 min) using a Selecta Ultrasound-H, 40 kHz/400 W were performed in closed tubes containing 10 mL of methanol. The combined extracts were then left to stand for 15 min in a freezer. After this, the extracts were filtered (PTFE, 0.22 μm) and the solvent was evaporated under a slow nitrogen flow (1 mL).

PUF samples were Soxhlet extracted with 90 mL of dichloromethane (8 h) only for NPAH and PAH analyses and the samples were then concentrated by rotary evaporator/nitrogen flow until 1.0 mL of acetonitrile. Regarding OH-PAHs, previous studies showed null recovery efficiencies for hot extractions, such as Soxhlet and microwave extractions [9]. Taking into account this limitation and those associated to larger PUF size, OH-PAHs were only investigated in particulate fraction. Moreover, these compounds tend to be more particle associated [10].

2.4. Chromatographic conditions

An Agilent series 1200 liquid chromatograph equipped with C18 thermostated column, (36.7 °C) and an Agilent 1100 fluorescence detector were used. Particular conditions to determine analytes of interest were established as follows:

PAHs. A Supelcosil C18 column (LC-PAH 250 × 4.6 mm, particle size 5 μm) was used. The mobile phase was a mixture of HPLC acetonitrile/water (45:55) programmed up to 100% of acetonitrile in 23 min and keeping it there for 10 min; the mobile phase flow rate was 1.5 mL min^{-1} and injection volume was 25 μL. Analysis time was 37 min and an equilibration delay of 6 min was applied to the next injection. Optimised operation conditions of fluorescence detector were well established in previous work [11].

OH-PAHs were determined isocratically with an Eclipse XDB C-18 (Agilent) column (150 × 4.6 mm, 5 μm) [9]. The mobile phase was a mixture of acetonitrile/water (60/40) (including an acetic-acetate buffer-1%) and the flow rate was 1.0 mL min^{-1}. Injection volume was 25 μL. Fluorescence was detected at wavelengths of 259/386 nm for 2-OHPH and 242/388 nm for 1-OHPYR and the analysis time was 7 min.

Nitro-PAHs. An Eclipse XDB C18 thermostated column (150 × 4.6 mm) was also used for nitro-PAH analysis. The mobile phase was a mixture of HPLC grade acetonitrile/water (acetic acid, 1%) (50:50) programmed up to 100% of acetonitrile in 12 min and keeping it there for 3 min; the mobile phase flow rate was 1.0 mL min^{-1}. Analysis time was 20 min, including an equilibration delay of 6 min to the next injection. The volume injected was 25 μL. The selected wavelengths were 254/410, 254/444, 244/438 and 244/528 nm for analyses of 3-amino Ph, 9-amino Ph, 1-amino Pyr and 3-amino Fl, respectively. 1-amino Pyr and 3-amino Fl could not be separated under these conditions, although both could be quantified separately using its own optimal wavelengths and performing a second analysis for 3-amino fluoranthene (at 528 nm of emission wavelength). Analytical signal of co-eluting amino derivatives was not registered for each target compounds.

3. Results

3.1. Analytical methods

Studied analytical parameters were linear dynamic range, regression coefficients and detection limits. Linear response was established from 5 to 1000 ng mL^{-1} and from 5 to 500 ng mL^{-1} for selected PAHs/Nitro-PAHs and OH-PAHs, respectively. Regarding correlation coefficients, they were higher than 0.995 for the target PAHs and OH-PAHs. In the case of Nitro-PAHs, coefficient values higher than 0.991 were obtained from 10 to 100 ng mL^{-1} and when calibration solutions were prepared in the same day.

Instrumental detection limits were calculated analysing solutions containing 5 ng mL^{-1} of selected PAHs/OH-PAHs and 10 ng mL^{-1} for Nitro-PAHs and they were considered as twice the standard deviation on average of five replicate analyses. Experimental values were about 1 and 3 ng mL^{-1} for selected PAHs/OH-PAHs and Nitro-PAHs, respectively.

Blanks of filters and PUFs were analysed for estimation of method detection limits as twice standard deviation associated with blank content average. Detection limits of filter blanks were about 1, 5 and 5.0–10 ng mL^{-1} for PAHs, OH-PAHs and nitro-PAHs, respectively, while PUF blank values led to 5 and 10–15 ng mL^{-1} for PAHs and nitro-PAHs, respectively.

Finally, the method validation was performed by choosing a reference material with similar matrix and representative levels of concentration. In particular, the uncertainty contribution of the analysis of reference material was estimated using SRM 1649 (urban particulate matter) from the National Institute of Standards and Technology (NIST, USA). This reference material provides typical urban dust matrix for evaluating the reliability of analytical methods. Different aliquots were subjected to analytical protocols and results are shown in Table 1. PAH concentration values were in the range of those certified, whereas clear differences between certified values and experimental mean data were obtained for some of the selected nitro-PAHs. Thus, 3-NPh and 9-NPh results agreed with those of the certified, although 1NPyr and 3NFl values resulted in important differences. In this respect, insufficient selectivity of the optimised method would lead to co-elution among isomers of amino-pyrene and amino-fluoranthene, respectively [12].

Table 1
Operational conditions of fluorescence detector for PAH analysis.

Time (min)	λ excitation (nm)	λ emission (nm)	Analysed PAH
0′	280	320	Na, Ace, F
13.4′	250	360	Ph
14.4′	250	400	An
15.6′	240	460	Fl
16.4′	280	390	Pyr
18.1′	290	420	BaA
19.7′	290	400	Chry
21.5′	300	430	BbF, BkF, BaP, DBA, BghiP

Regarding OH-PAHs, there were no suitable reference materials available, so method validation of selected compounds was carried out comparing the emission and excitation spectra obtained from analyses of airborne particulate extracts and standard solutions, as proposed by Kisikawa et al. [4]. The excitation and emission spectra of OH-Ph and OH-Pyr in airborne particulates were in good agreement with those obtained from the standard solutions [9]. For the target nitro-PAHs, a comparative study between emission/excitation spectra of extracts and standard solutions was also performed to confirm each chromatographic peak.

3.2. Uncertainty estimation

In this study, the evaluation of uncertainty contributions was accomplished after combining them into the following groups:

- Analytical determination.
- Reference material analysis.
- Extraction step.

3.2.1. Analytical determination

These contributions were mainly affected by:

1. Preparation of calibration standards (u_{Cstd}).
2. Chromatographic calibrate ($u_{\mathrm{C}x}$) from linear least squares calibration.

Uncertainty associated with preparation of calibration solutions includes the uncertainty of the stock solution according to certificates and dilution chain. While uncertainty associated to stock solution was supplied by the manufacturer as ±1%, the main contributions of dilution chain during preparation of calibration solutions included repeatability, temperature and specification limits of used syringes. All of them were no significant, being the main contribution from the initial concentration of stock solution [11]. As a result, the relative uncertainty associated with each concentration of calibration standard ($u_{\mathrm{Cstd}}/C_{\mathrm{std}}$) was estimated as 0.02 (1% supplied by Dr. Ehrenstörfer, 10 μg mL^{-1}) and was used for all standards in the studied concentration range.

Uncertainty from linear least squares calibration is mainly due to variability in responses showed by the instrument. Chromatographic determination has therefore uncertainty due to regression coefficients, which was evaluated from standard deviations of slope and intercept in calibration line. For this, a set of four concentration levels were independently prepared and analysed for a period of 3 months (five points for each concentration level). Repeatability of the instrumental analytical response is the second contribution source to chromatographic determination and it was estimated from analyses of six aliquots of a standard solution. In definitive, the relative uncertainty of the predicted analyte concentration ($u_{\mathrm{C}x}/C_x$) from linear least squares calibration was calculated applying the following equation:

$$\frac{u_{\mathrm{C}x}^2}{\mathrm{C}x^2}=\frac{u_y^2}{(y-b)^2}+\frac{u_b^2}{(y-b)^2}+\frac{u_m^2}{m^2}$$

where "y" is the mean value of the analytical response of six standard solutions (25, 10 and 50 ng mL^{-1} of selected PAHs, OH-PAHs and nitro-PAHs, respectively), "u_y" its uncertainty ($ws_y/\sqrt{n}$) and applying the expression for the lineal regression of least squares, "b" is the y-intercept of the calibration graph, "u_b" is the uncertainty deduced from standard deviation of i-intercept, "m" is the slope of calibration graph and "u_m" is the uncertainty of slope.

The final combination of uncertainties (u_x^2/x^2) associated with preparation of standard solutions and calibration graph was in general below 5% for selected PACs, being uncertainty from the standards higher than those of calibration graphs (Table 2). Only the measurements of Pyr, 9-NPh and NFl showed higher contributions from calibration graph, especially for NFl which raised 22%. These results are consequence of the low analytical sensitivity associated due to the weaker instrumental responses (“y”) to a concentration changes (“x”).

3.2.2. *Uncertainty contribution of the recovery*

The standard relative deviation of reference material analyses for PAHs and nitro-PAHs of interest was calculated to evaluate the uncertainty contribution of recovery step. It was not possible to obtain this uncertainty contribution for studied OH-PAHs due to the lack of reference materials. In this case, recovery estimates was obtained from spiking studies, being below 5% which is consistent with analytical measurement component as the main uncertainty contribution. However, it must be mentioned that spiking studies can be unrepresentative for estimating recovery uncertainty.

Estimation of the uncertainty derived from the recovery was performed as indicate Yenisoy-Karakas [13]. In particular, a significance test was used to determine when the mean recovery (R_m) was significantly different from 1.0, calculating “t” as follows:

$$t=\frac{|1-Rm|}{u(Rm)}$$

where

$$u(Rm)=Rm\sqrt{\left(\frac{u_C}{C}\right)^2+\left(\frac{u_{C_{RM}}}{C_{RM}}\right)^2}$$

Being u_C the standard deviation of replicate recovery divided by $n^{1/2}$, C is the measured concentration, u_{CRM} is the uncertainty of reference material and C_{RM} is the spiked concentration of reference material. Two-tailed critical t_{crit} for $(n-1)$ degrees of freedom was considered as 2.776 and 4.303 for studied PAHs and nitro-PAHs, respectively (95% confidence and 2-tail). When calculated value was greater than t_{crit} a correction factor was applied (R_m). In particular, it was used for Pyr, BbFl, BkFl, BaPyr, DBA and NPyr. As it was pointed previously, NPyr and NFl showed co-elution among isomers, so SRM 1649b leads to R_m substantially greater (above 1.7) and it was inappropriate for consideration of uncertainty contribution from recovery of NPyr and NFl.

Uncertainty results deduced from reference material analyses are compiled in Table 1. In general, the standard uncertainty was 5–10% for selected PAHs, which were lightly above than those corresponded from analytical determination.

In order to verify the traceability of the results obtained, the routine samples must be similar to the used reference material according to the levels of concentration and type of matrix. As a result, the reference material that best matched in this study was the SRM1649, from a typical urban environment. However, the study of more complex matrices such as SRM2975 and SRM1975, which proceed from diesel matrix has been considered of interest to proceed with greater difficulty analytical extraction of these compounds [14]. Sample matrix could affect the final result and it would be interesting to consider its influence on the measurement uncertainty. Table 3 compiles the estimated uncertainty contribution by analyses of reference materials SRM1975 and SRM2975, which correspond to an extract of diesel particulate and the used diesel particulate matter to prepare it, respectively. As it can be seen, higher dispersions of Pyr, BaAn and BkFl were obtained for diesel materials, which is coherent with their reduced concentration levels. Results obtained from diesel particulate matter were the highest, about 10–24%, which might be due to the difficulty for extraction of analytes from the more complex matrix.

3.2.3. *Analysis of real ambient air samples*

The uncertainty component associated with the extraction step of real samples was based on a series of four analyses of each type of sample. Therefore the evaluation of uncertainty was considered as a type A contribution and deduced from standard deviation ($w^*s/\sqrt{n}$, being $n=4$).

$$u^2_{\text{air sample}}=u^2_{\text{extr}}+u^2_{\text{rec}}+u^2_{\text{anal}}$$

In particular, an ambient air sample of each sampling device was cut into four similar pieces which were previously weighted and then considered as independent sub-samples for extraction

Table 2
Concentrations (μg g^{-1}) obtained from the analyses of the reference materials: 1649a for PAH and 1649b for NPAH, respectively. Uncertainty contributions from reference material analyses are also included.

	SRM 1649					
	Mean value (μg g^{-1})	Certified (μg g^{-1})	*R*%	*t*	*u* (*R*)	*u* (%)
PAHs						
Ph	4.5 ± 0.3	4.1 ± 0.4	1.1	1.0	5.49E−02	5.5
An	0.51 ± 0.05	0.43 ± 0.08	1.2	0.80	1.08E−01	11
Fl	6.2 ± 0.1	6.45 ± 0.2	0.96	1.3	1.70E−02	1.7
Pyr	6.6 ± 0.4	5.3 ± 0.25	1.25	3.5	4.85E−02	4.8
BaAn	2.07 ± 0.09	2.21 ± 0.07	0.94	1.7	2.70E−02	2.7
Chry	3.2 ± 0.3	3.05 ± 0.06	1.0	0.75	4.51E−02	4.5
BbFl	5.0 ± 0.1	6.45 ± 0.6	0.78	2.8	3.95E−02	3.9
BkFl	1.63 ± 0.03	1.91 ± 0.03	0.85	9.3	1.09E−02	1.1
BaPyr	1.8 ± 0.3	2.51 ± 0.09	0.72	5.0	5.28E−02	5.3
DBA	0.150 ± 0.007	0.29 ± 0.02	0.53	11	2.48E−02	2.5
BghiP	4.0 ± 0.9					
Nitro – PAHs						
3 – NPh	28 ± 4	22.1 ± 0.3	1.3	2.9	1.05E−01	11
9 – NPh	1.4 ± 0.2	1.8 ± 0.1	*0.78*	3.7	6.77E−02	6.8
1 – NPyr	120 ± 10	71.8 ± 1.3	*1.7*	*10*	8.19E−02	8.8
3 – NFl	120 ± 16	4.6 ± 0.1		*14*		

Table 3
Uncertainty contributions deduced from analytical determination.

	$(u_{\text{Cpat}})^2/C_{\text{pat}}^2$	$(u_{Cx})^2/C_x^2$	u_x^2/x^2	u_x/x (%)
PAHs				
F	4.00E−04	3.47E−04	7.47E−04	2.7
Ph	4.00E−04	7.30E−05	4.73E−04	2.2
An	4.00E−04	3.66E−04	7.66E−04	2.8
Fl	4.00E−04	6.14E−04	1.01E−03	3.2
Pyr	4.00E−04	1.88E−03	2.28E−03	4.8
BaAn	4.00E−04	2.18E−04	6.18E−04	2.5
Chry	4.00E−04	6.60E−05	4.66E−04	2.2
BbFl	4.00E−04	3.77E−04	7.77E−04	2.8
BkFl	4.00E−04	1.10E−05	4.11E−04	2.0
BaPyr	4.00E−04	1.84E−04	5.84E−04	2.4
DBA	4.00E−04	1.04E−04	5.04E−04	2.2
BghiP	4.00E−04	3.47E−04	7.47E−04	2.7
OH – PAHs				
OH – Ph	4.00E−04	3.33E−04	7.33E−04	2.7
OH – Pyr	4.00E−04	8.15E−05	4.82E−04	2.2
Nitro – PAHs				
3 – NPh	4.00E−04	3.22E−04	7.22E−04	2.7
9 – NPh	4.00E−04	6.36E−04	1.04E−03	3.2
1 – NPyr	4.00E−04	8.83E−03	9.23E−03	9.6
3 – NFl	4.00E−04	2.31E−04	6.31E−04	2.5

and later analysis. Hence, four sub-samples for PM_{10}, $PM_{2.5}$ and PUF were treated and analysed separately for evaluating extraction process contribution. Some PAC analyses, such as DBA/BghiP (particulate fractions) and BghiP/3-NFl (PUF samples), were below detection limit due to the significant reduction of analytical signal (1/4). Therefore, corresponding standard deviations could not be deduced.

The main contributions of uncertainty as coefficient of variation in the determination of the selected PACs in air ranged from 15 to 30% (Table 4).

However, uncertainty results of OHPAH derivatives were similar to those provided by analytical determination ($<5\%$). Therefore, the most important contribution of uncertainty arises from the analytical determination, without great influence from extraction process of sample.

Regarding the influence of the type of collected sample in analytical variability, higher dispersions were obtained when polyurethane foam samples were evaluated. In particular, values for NPAH uncertainties corresponded approximately with 30%, while results of particulate were about 20%. Heavier PAHs (from chrysene) in PUF samples further reached 20% versus 15% obtained from filter samples. Measurements of the more volatile compounds presented uncertainties of about 20% in both types of substrates, which can be justified based on the lower concentration and higher volatility.

3.3. Ambient air measurements

The proposed methods were applied to determine the selected PACs in filters and PUF samples collected over a full year. The range and mean concentration of results obtained from the vapour and particulate phases and measured weekly from January 2008 to February 2009 are given in Table 5.

In general, results indicated a lower concentration in the particulate fraction and abundance of volatile PAHs in the gaseous phase, according to the frequent pattern on PAH determinations in ambient air [15,16]. The average total concentrations of PAHs obtained in the particulate fractions were about 1 ng m^{-3} for PM_{10} and $PM_{2.5}$, while for gas phase concentration values were 16 ng m^{-3}. F, Ph, Fl and Pyr were found to be the most abundant PAHs in vapour fraction, which reached levels of 8.7, 42, 6.7, 19 and 22%, respectively, of this gas phase. Ph, Fl, Pyr and Cry predominated in the particulate phase, a finding which is in agreement with other authors [1,17–21] Table 6.

Regarding NPAH results, the average relative composition was similar in both kind of samples for most compounds; only volatile 3-NPh and 9-NPh values were slightly higher in PUF samples. Nitro-pyrene results obtained from particulate fraction reached the highest values. In general, they were also comparable to those found in literature [22].

Finally, hydroxy-phenanthrene was predominant (OH-Ph/OH-Pyr ratio of about 3). Due to the lack of studies on measurements of OH-PAHs in ambient air, it is difficult to compare among data from literature, although particulate concentrations presented in this paper were in agreement to those scarce found [1,4,23]. Mean concentrations of OH-Ph and OH-Pyr were around 60 and 20 pg m^{-3}, respectively, and 2–4 orders of magnitude less than their parent PAHs. In this sense, hydroxylated PAHs can be more easily degraded than their parent PAHs under atmospheric conditions.

4. Conclusions

HPLC/FD was applied to analyse selected PACs in aerosol and gas phase atmospheric samples. They showed satisfactory validation parameters such as precision, recovery and selectivity. The optimised methodologies involved low cost and could be considered as an attractive alternative for laboratories with reduced instrumental resources.

The main uncertainty contributions were estimated based on analytical determination, reference material analysis and extraction step. The highest uncertainty contributions were provided by extraction process of real ambient air samples which were about 15–20% for most PAHs and about 20–30% for the nitro-PAHs. Higher uncertainty component for nitro-PAHs was associated to derivatisation reaction involved in chromatographic method.

Table 4
Concentrations obtained from the analyses of the reference materials: SRM1975 extract (A) and SRM 2975 particulate matter (B). Uncertainty contributions from reference material analyses are also included.

1975	**Mean value (μg mL^{-1})**	**Certified (μg mL^{-1})**	***t***	***R*%**	***u* (*R*)**	***u* (%)**
(A)						
PAHs						
F	0.14 ± 0.005	0.110 ± 0.003	9.92	1.28	3.23E−02	2.9
Ph	8.6 ± 0.3	8.0 ± 0.2	3.45	1.08	3.35E−02	2.3
Fl	13.7 ± 0.5	13.5 ± 0.6	0.40	1.01	3.60E−02	2.9
Pyr	0.33 ± 0.01	0.4 ± 0.1	1.84	0.78	2.96E−02	12
BaAn	0.060 ± 0.002	$0.09 \pm 0.01_5$	7.15	0.63	2.79E−02	5.2
Chry	2.17 ± 0.08	1.95 ± 0.07	3.91	1.11	3.50E−02	2.8
BbFl	3.2 ± 0.1	3.2 ± 0.1	0.19	0.99	3.15E−02	2.7
BkFl	0.140 ± 0.006	0.17 ± 0.05	1.55	0.82	3.26E−02	12
DBA	0.086 ± 0.003	0.08 ± 0.01	0.94	1.09		9.1

2975	**Mean value (μg g^{-1})**	**Certified (μg g^{-1})**	***t***	***R*%**	***u* (*R*)**	***u* (%)**
(B)						
PAHs						
Ph	16.5 ± 0.3	17 ± 3	0.39	0.97	8.11E−02	8.1
Fl	26.2 ± 0.6	27 ± 5	0.17	0.98	9.55E−02	9.6
Pyr	1.1 ± 0.2	0.9 ± 0.2	1.00	1.23	2.33E−01	23
BaAn	0.45 ± 0.08	0.32 ± 0.07	1.73	1.41	2.37E−01	24
Chry	5.2 ± 0.2	4.6 ± 0.2	3.89	1.15	3.75E−02	3.8
BkFl	0.6 ± 0.1	0.68 ± 0.08	1.73	0.81	1.10E−01	11
BghiP	0.3 ± 0.1	0.50 ± 0.04	2.30	0.64	1.56E−01	16
Nitro−PAHs						
1NPyr	34 ± 9	36		0.93		

Table 5
Uncertainty contributions deduced from analyses of real ambient air samples. No data corresponds to levels of concentration below detection limit.

	PM2.5						PM10						PUF					
					u^2	u (%)					u^2	u (%)					u^2	u (%)
PAHs		pg m^{-3}						pg m^{-3}						pg m^{-3}				
F	9.2	8.0	9.2	9.2	3.28E−03	5.7	5.7	5.0	5.6	5.9	3.28E−03	5.7	2684	2230	2517	2574	4.32E−03	6.6
Ph	45	29	47	55	4.35E−02	21	28	18	29	33	3.99E−02	20	1781	1089	1368	1240	3.40E−02	18
An													520	423	393	665	4.32E−02	21
Fl	76	51	82	89	3.57E−02	19	47	32	50	56	3.52E−02	19	651	536	473	791	3.77E−02	19
Pyr	92	61	91	104	3.20E−02	18	57	38	56	65	3.16E−02	18	425	291	353	430	2.25E−02	15
BaAn	12	8.3	10	13	2.63E−02	16	7.5	5.2	6.2	7.9	2.33E−02	15	14	9.5	14	20	6.62E−02	26
Chry	85	57	81	95	2.92E−02	17	52	36	50	57	2.46E−02	16	46	41	28	44	3.10E−02	18
BbFl	64	44	56	68	2.41E−02	16	39	28	34	42	2.13E−02	15	6.8	4.7	6.3	8.3	3.75E−02	19
BkFl	25	17	22	27	2.48E−02	16	15	11	13	16	2.19E−02	15						
BaPyr	22	11	12	25	1.17E−01	34	14	10	8.5	20	1.06E−01	33						
OH−PAHs																		
OHPh	39	41	41	38	1.23E−03	3.5	29	29	28	28	4.19E−04	2.0						
OHPyr	13	12	13	12	1.42E−03	3.8	13	14	12	12	1.30E−03	3.6						
Nitro−PAHs																		
NNa	13	6.9	8.0	15	9.11E−02	30							8.2	9.0	9.8	16	7.29E−02	27
3NPh	33	25	27	34	1.45E−02	12	9.2	5.7	8.2	5.8	4.30E−02	21	8.4	7.0	3.0	5.7	1.05E−01	32
9NPh	12	8.5	6.4	13	7.24E−02	27	18	17	22	14	2.25E−02	15	20	18	32	36	7.86E−02	28
NPyr	29	18	28	34	4.04E−02	20	12	9.2	6.9	15	7.60E−02	28	2.2	2.2	3.8	4.8	1.11E−01	33
NFl	9.1	6.5	7.7	10	2.15E−02	15	6.5	14	8.4	9.0	7.61E−02	28						

Table 6
Mean values of concentration measured of PACs of interest from January 2008 to February 2009.

	PM$_{10}$ n=55 **Mean (min-max)**	**PM$_{2.5}$** n=49 **Mean (min-max)**	**PUF** n=55 **Mean (min-max)**
PAHs (pg m^{-3})			
F	20 (< 2.2–64)	22 (< 2.2–55)	1400 (170–5300)
Ph	160 (10–610)	145 (7.6–435)	5600 (1200–12000)
An	18 (< 2.4–56)	14 (< 2.4–70)	1100 (18–4600)
Fl	255 (25-820)	220 (< 4.2–690)	2400 (460–4900)
Pyr	250 (< 4.4–820)	290 (< 6.1–685)	2500 (920–4900)
BaAn	90 (< 4.7–500)	76 (< 4.7–320)	110 (10–410)
Chry	175 (< 5.0–920)	150 (18–525)	95 (26–300)
BbFl	140 (< 3.8–690)	115 (< 3.8–505)	28 (< 10–62)
BkFl	60 (< 3.1–295)	45 (< 3.1–210)	18 (< 8.7-53)
BaPyr	70 (< 2.9–410)	57 (< 2.9–300)	21 (< 11–61)
BghiP			31 (< 6.4–89)
***Nitro-PAHs* (pg m^{-3})**			
3-NPh	18 (7.4–47)	13 (< 6.4–35)	23 (8.3–49)
9-NPh	20 (12–64)	22 (9.1–72)	33 (11–65)
NPyr	41 (19–159)	32 (16–84)	11 (8.8–15)
NFl	21 (9.2–64)	15 (7.1–29)	7.7 (< 5.7–12)
***OH-PAHs* (pg m^{-3})**			
OHPh	63 (10–250)	70 (12–355)	
OHPyr	21 (< 7.8–120)	25 (< 7.8–210)	

However, contribution from analytical determination was found the main source of uncertainty for selected OHPAH measurements (5%). Regarding the type of sampling device, PUF measurements implied higher dispersions:

- For nitro-PAHs, approximately 30% were reached (about 20% in filter samples).
- For heavier PAHs, approximately 20% were obtained (15% in filter samples).

Finally, the effectiveness of the proposed methods was evaluated by analysing PM$_{10}$, PM$_{2.5}$ and PUF ambient air samples collected during a full year, providing their applicability for routine analysis. Concentration results were in agreement to literature.

References

[1] G. Wang, K. Kawamura, X. Zhao, Q. Li, Z. Dai, H. Niu, Atmos. Environ. 41 (2007) 407–416.
[2] D. Wenger, A.C. Gerecke, N.V. Heeb, O. Schmid, C. Hueglin, H. Naegeli, R. Zenobi, J. Appl. Toxicol. 29 (2008) 223–232.
[3] O. Delhomme, P. Herckes, M. Millet, Anal. Bioanal. Chem. 389 (2007) 1953–1959.
[4] N. Kishikawa, S. Morita, M. Wada, Y. Ohba, K. Nakashima, N. Kuroda, Anal. Sci. 20 (2004) 129–132.
[5] V.J. Barwick, S.L.R. Ellison, Anal. Commun. 35 (1998) 377–383.
[6] EURACHEM/CITAC Guide CG 4, Quantifying uncertainty in analytical measurement, 2nd ed., 2000.
[7] T.L. Gibson, Atmos. Environ. 16 (8) (1982) 2037–2040.
[8] X. Jinhui, Anal. Chim. Acta 316 (2000) 111–115.
[9] A.I. Barrado, S. García, Y. Castrillejo, R.M. Pérez, Atmos. Poll. Res. 3 (2012) 81–87.
[10] D. Vione, S. Barra, G. De Gennaro, M. De Rienzo, S. Gilardoni, M.G. Perrone, L. Pozzoli, Ann. Chim. 94 (2004) 257–268.
[11] S. García-Alonso, R.M. Pérez Pastor, M.L. Sevillano-Castaño, O. Escolano, F.J. García-Frutos, Polycyclic Aromat. Compd. 28 (2008) 67–83.
[12] H.A. Bamford, D.Z. Bezabeh, M.M. Schantz, S.A. Wise, J.E. Baker, Chemosphere 50 (2003) 575–587.
[13] S. Yenisoy-Karakas, Anal. Chim. Acta 571 (2006) 298–307.
[14] M. Piñeiro-Iglesias, P. López-Mahía, E. Vázquez-Blanco, S. Muniategui-Lorenzo, D. Prada-Rodríguez, Polycyclic Aromat. Compd. 22 (2002) 129–146.
[15] K. Ravindra, L. Bencs, E. Wauters, J. Hoog, F. Deutsch, E. Roekens, N. Bleux, P. Berghamns, R.V. Grieken, Atmos. Environ. 40 (2006) 771–785.
[16] I.A. Leal-Granadillo, J.I. García-Alonso, A. Sanz-Medel, J. Environ. Monit. 2 (3) (2000) 218–222.

[17] M.S. Callén, J.M. Lopez, A.M. Mastral, J. Environ. Monitor. 13 (2010) 319–327.
[18] M.S. Callén, M.T. de la Cruz, J.M. López, A.M. Mastral, Fuel Process. Technol. 92 (2011) (2011) 176–182.
[19] Z. Li, A. Sjodin, E.N. Porter, D.G. Patterson, L.L. Needham, S. Lee, A.G. Russell, J.A. Mulholland, Atmos. Environ. 43 (2009) 1043–1050.
[20] K. Saarnio, M. Sillanpää, R. Hillamo, E. Sandell, A.S. Pennanen, R.O. Salonen, Atmos. Environ. 42 (2008) 9087–9097.
[21] Y.W.F. Tham, K. Takeda, H. Sakugawa, Atmos. Res. 88 (2008) 224–233.
[22] P. Ciccioli, A. Cecinado, E. Brancaleoni, M. Frattoni, P. Zacchei, A.H. Miguel, P.D. Vasconcellos, J. Geophys. Res. (Atmos.) 101 (1996) 19567–19581.
[23] M.T. Galcerán, E. Moyano, J. Chromatogr. A 715 (1995) 41–48.