A liquid chromatography tandem mass spectrometry method for simultaneous analysis of 46 atmospheric particulate-phase persistent organic pollutants and comparison with gaschromatography/mass spectrometry

Francesca Buiarelli[a*], *Patrizia Di Filippo*[a,b], *Donatella Pomata*[a,b], *Carmela Riccardi*[a,b], *Martina Bartocci*[a]

[a]*Department of Chemistry, University of Rome "La Sapienza", P.le Aldo Moro, 5-00185 Rome, Italy*
[b]*INAIL-DIT, Via Roberto Ferruzzi 38 -00143 Rome, Italy;*

ABSTRACT

A novel multi-analyte method for the simultaneous determination of 46 compounds of environmental concern, most of them belonging to the persistent organic pollutants, was developed using high performance liquid chromatography and the results were compared to those obtained by gas chromatography. This study was performed in perspective of a cumulative exposure assessment of substances of health concern in environments where high levels, relatively to airborne particulate matter, can be found. The target compounds included polychlorinated biphenyls, brominated flame retardants and derivatives of polycyclic aromatic hydrocarbons.

The multi-analyte method was evaluated in air particulate matter in terms of reproducibility, linearity, recovery, limits of detection and quantification and matrix effect. The recovery was above 70% for all the analytes, whereas limits of quantification ranged between 23-390 pg/m^3 in Liquid Chromatography and less than ten times in Gas Chromatography-Mass Spectrometry.

Matrix effect was generally negligible for both the techniques, except the case of the detection of oxygenated derivatives of polycyclic aromatic hydrocarbons by Gas Chromatography.

In order to demonstrate the efficacy and to assess the method performances (accuracy and precision), both the techniques were applied to standard reference materials, and the results were compared, discussing the advantages and disadvantages of them.

The method was finally applied to a real sample of indoor airborne particulate matter with aerodynamic diameter ≤4μm (PM_4).

We demonstrated that the Liquid Chromatography was the only technique able to analyze the 46 compounds, including thermally degradable ones, with a single chromatographic run without derivatization steps. On the other hand, gas chromatography still presents higher sensitivity for the detection of some of the investigated compounds. This study can be considered only explorative and further improvements can be expected with new generation LC-MS instruments (10-100 times more sensitive).

Keywords: Persistent organic pollutants, Polychlorinated biphenyls, Brominated flame-retardants, Oxy-PAHs, atmospheric particulate matter, HPLC-MS-MS, GC-MS

1. Introduction

The persistent organic pollutants (POPs) are one of the major environmental concerns due to their persistence, long transportability, bio-accumulation and potentially adverse effects on living

[*]Corresponding author francesca.buiarelli@uniroma1.it (F. Buiarelli) tel. +39-06-49913645 fax +39-490631

organisms [1-3]. Polybrominated diphenyl ethers (PBDEs), Polychlorinated biphenyls (PCBs), Benzo[*a*]pyrene (BaP) belong to this class of compounds [4].

PCBs are a broad family of man-made organic chemicals. Due to their non-flammability, chemical stability, high boiling point and electrical insulating properties, chemical mixtures of chlorinated biphenyl congeners were used in hundreds of industrial and commercial applications [5, 6]. A few PCBs, referred to as "dioxin-like", have shown toxic responses similar to those observed for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). These include dermal toxicity, immunotoxicity, reproductive deficits, teratogenicity, endocrine toxicity and carcinogenicity/tumour promotion.

Brominated flame-retardant chemicals (BFR) are added to plastics and other products to make them difficult to burn [7, 8]. The most frequently used BFRs are polybrominated diphenyl ethers (PBDEs), tetrabromobisphenol A (TBBPA), and hexabromocyclododecane (HBCD). The PBDEs are persistent and bioaccumulative industrial chemicals, and structurally similar to PCBs [9]. Nowadays, both European Union (EU) and United States Environmental Protection Agency (US-EPA) banned the use of all PBDE formulations [10].

TBBPA and its derivatives are used as either reactive or additive intermediates in polymer manufacture [11]. HBCD is a ring-shaped brominated hydrocarbon molecule that presents three possible spatial arrangements of the bromine atoms: α, β, γ HBCD [12]. Because of its properties, HBCD has been identified as a "substance of very high concern" (SVHC) based on the criteria set by the European REACH Regulation [13].

Consumption of contaminated food is the main human exposure source to organo-halogen compounds [14]. However, the exposure to these multiple pollutants may be much more complex and massive [15] due to their widespread presence in the environment including water, soil, and air.

As regardsair, a relevant issue in risk assessment is the combined exposure to multiple chemicals. [16].

For example, samples collected from open burning areas where electricals and electronic equipments are disposed (e-waste) can contain numerous classes of organic chemicals, including many halogenated (chlorinated or brominated) chemicals and polycyclic aromatic hydrocarbons and their derivatives [17, 18]. In indoor environments (homes, workplaces and schools), where people spend a considerable amount of time, numerous sources of contamination may lead to exposure to flame-retardant chemicals [19]. Most of the published methods are focused on the analysis of a specific family of compounds and multi-class methods are rarely reported [20-22].

The determination of POPs belonging to different chemical classes, including Oxy-PAHs and Nitro-PAHs in airborne particulate matter , using a single procedure of sampling, extraction, clean-up and analysis has not yet been investigated.

The most commonly used technique for the identification and quantification of single classes of POP in different matrices, including air particulate matter, is gaschromatography-mass spectrometry (GC-MS) **[23-28]**.

On the other hand, high performance liquid chromatography-tandem mass spectrometry (HPLC-MS-MS) has been used mainly for the analysis of flame retardants [29-31] and, to our knowledge, no studies have been published about comprehensive analysis of PCBs with this technique.

An interesting paper has already compared [32] the chromatographic and mass spectrometric behaviors of 49 polybrominated diphenylether (PBDE) homologues with different liquid chromatographic separation systems and different gas chromatographic temperature programs .

Starting from the literature about the topic, the aim of this study was the development of an effective analytical method for the simultaneous determination of 46 priority and (re-)emerging POPs of environmental concern in airborne particulate matter.

The investigated classes are: PCBs, BFRs, BaP and 1-nitropyrene (1NP), as representative of polycyclic aromatic hydrocarbons (PAHs) and their nitrated derivatives, and the oxygenated derivatives of PAHs (Oxy-PAHs).

Despite not beingclassified as POPs, Nitro and Oxy-PAHs are included in this study for their chemical, physical and toxicological properties and because they frequently occur together with POPs in both complex industrial sites and residential areas [18].

Since ion suppression effects, observed primarily in electrospray ionization, may adversely affect the accuracy and precision of quantitative methods

(detecting analytes in complex matrices l) we performed an accelerated solvent extraction (ASE), and a solid phase purification (SPE) on cartridge, before the analysis by HPLC-MS-MS in multiple reaction monitoring (MRM). The performances of HPLC-MS-MS method were compared with those of GC-MS methods with two different ionization techniques (electronic interaction EI and chemical ionization CI) and were evaluated in terms of detection limits. Matrix effect was generally negligible for both the techniques, except for the detection ofoxygenated derivatives of polycyclic aromatic hydrocarbons by GC.

The techniques were applied to standard reference materials (SRM 1975, SRM 2975 and SRM 2585), and to a real sample of indoor airborne particulate matter with aerodynamic diameter ≤4μm (PM_4), in oder to compare the results and to discuss the main drawbacks of the two techniques. Although gas chromatography generally shows higher sensitivity for the detection of some of the investigated compounds, HPLC/MS-MS was the only technique that was able to analyze the 46 compounds in a single chromatographic run.

Furthermore the limits of detection of the HPLC were still compatible to those found in indoor air of e-waste recycling sites [17].

2. Material and Methods

2.1 Chemical, reagents and materials

All the standards and internal standards are reported in Supporting Information in table SI 1.

Individual stock standards were prepared at 0.2 mg mL^{-1} in methanol (MeOH), acetonitrile (AcN) or isooctane, and stored in the dark at −20 °C. Working standard solutions of each compound were prepared by diluting the stock standard kept at +4 °C, in amber vials.

Solvents: MeOH, Isooctane, AcN, Dichloromethane (DCM), n-Hexane, and Acetone were purchased from Sigma-Aldrich S. r. l. (Milan, Italy); Water HPLC Grade- was from ROMIL, Cambridge (GB).

Materials: Diatomaceous earth sorbent (hydromatrix) was obtained by Dionex (Thermo Fisher Scientific, Sunnyvale, CA); STRATA Silica and STRATA Florisil cartridges were purchased by Phenomenex (Torrance, CA, USA), polytetrafluoroethylene (PTFE) filters 37 mm x 2 μm, were from Pall Corporation (Michigan, USA); Standard Reference Materials (SRM) 2975, 1975 and 2585 were prepared by the U.S. National Institute of Standards and Technology (NIST) (Gaithersburg, MD, USA).

The SRM 2975, according to the certificate, is a diesel particulate material collected from a filtering system designed specifically for diesel-powered forklifts. A total of 13.7 kg of diesel particulate material was homogenized. 5.65 kg of material was extracted with DCM for the preparation of SRM 1975 (diesel particulate extract) and the remaining diesel particulate material (8.05 kg) was bottled for distribution as SRM 2975. A unit of SRM 1975 consists of four ampoules, each containing approximately 1.2 mL of a dichloromethane extract of diesel particulate matter SRM 2975.

The dust used for the preparation SRM 2585, as reported in the certificate, was taken from vacuum cleaner bags collected from homes, cleaning services, motels, and hotels in the states of North Carolina, Maryland, Ohio, New Jersey, Montana, and Wisconsin during 1993 and 1994.

2.2 Sampling

Sampling of atmospheric particulate matter in indoor environments was carried out by means of pumps SKC DeLuxe (Model 224-PCXR8), equipped with an aluminum cyclone, operating at a flow rate of 2.5 L min^{-1} to collect particulate matter with aerodynamic diameter $\leq 4\mu m$ (PM_4).

The filters were weighed before and after sampling on an analytical balance (Sartorius MC-5, $\Delta m = \pm$ 0.001mg) after conditioning for twenty-four hours in a chamber maintained at 50% relative humidity and 20 °C (Activa Climatic Cabinet, Aquaria MI) according to UNI EN 12341/2001; UNICHIM 285/2003 and D.M. 60/2002), then they were sealed, and stored in aluminum foils in refrigerator at -18°C. The particulate matter amounts on each filter ranged from 0.1 to 0.5 mg and the total sampled air volume was about 40 m^3,corresponding to 11 sampling days

2.3 Sample preparation

The sample preparation was carried out according to Figure 1.

5-100 mg of SRM and/or working solutions at different concentration levels were added on blank filters, previously conditioned and weighed. The SRM additions were carried out in order to obtain final concentrations of certificated analytes within the range of linearity found for each compound. The spiked samples were dried to evaporate the solvents and were aged for a week to establish the equilibration between the analytes and the matrix.

Blank filters BF (α), spiked filters (β), and sampled filters (γ) were extracted with two cycles of a mixture DCM/acetone 4:1 by accelerated solvent extraction (ASE 200–Dionex, Thermo Fisher Scientific, Sunnyvale, CA) at a temperature of 100°C and a pressure of 1500 psi (phase I). Due to the high complexity of the extracted samples, a subsequent purification by SPE cartridges was necessary to reduce ion suppression or to eliminate interferences (phase II) . The extracted volume (about 20 mL) was evaporated to dryness using a Glas-Col SE 500 automated evaporation system (Glas-Col, Terre Haute, IN) and redissolved in 200 μL of toluene before loading onto a florisil cartridge, conditioned by 3 mL of acetone, followed by 3 mL of DCM and 3 mL of n-hexane, using a vacuum manifold (Alltech12-Port model SPE Vacuum manifold) (Grace, Deerfield, IL). Compounds retained from the cartridge were eluted, by 6 mL of n-hexane, 6 mL of DCM/hexane

(2:1), followed by 6 mL of DCM and 3 mL of acetone (phase II). The three fractions were collected all together.

The extracts were evaporated under nitrogen stream before the GC-MS and HPLC-MS-MS analyses (Phase III and IV) and reconstituted by 50 µL of toluene or MeOH.

2.4 GC/MS equipment and conditions

An HP 6890 gas chromatograph fitted with a HP 7683B autosampler and connected to a HP 5973A single quadrupole mass-selective detector (Agilent Technologies, Palo Alto, CA) was used for GC/MS in Electronic Interaction (EI) and GC/MS in negative Chemical Ionization (NCI) analysis.

GC separations were achieved in 20 and 30 min respectively on a Column DB-5-5% phenyl-methylpolysiloxane (L=30m; I.D.=0.25 mm; film thickness = 0.25µm J&W Scientific) in GC-EI-MS and on a capillary column DB17-50% phenyl-methylpolysiloxane (L=30m; I.D.=0.25 mm; film thickness 0.25µm, J&W Scientific) in GC-NCI-MS, according to Di Filippo et al. **[25]**.

The temperature programme in GC-EI-MS (operating at 70 eV) was: 100 °C initial T, ramped at 25 °C min^{-1} to 310 °C, then held for 10 min and in NCI (using CH_4 as gas): 100 °C initial temperature, ramped at 15°Cmin-1 to 310 °C, then held for 10 min. Samples (1µL) were injected in splitless mode. The injector temperature was set at 280 °C. The helium carrier gas was set at a constant flow rate of 1.0 mL min^{-1}.

The quadrupole was set at 150°C, the ion source temperatures were set at 150°C for CI and 230°C for EI, whereas the transfer line at 300°C. Acquisitions were performed in selected ion monitoring (SIM) mode according to table SI 2 and SI 3 in Supporting Information section, using the Agilent MSD Chem Station D.01.00 software. In this case TBPA was not included, since, the derivatization is mandatory in the GC-MS analysis.

2.5 LC/MS/MS equipment and conditions

An HPLC pump system (Agilent 1100 Agilent Technologies, Santa Clara, CA, USA) with an autosampler (Agilent G1313A –Agilent Technologies, Santa Clara, CA, USA), was coupled to a triple quadrupole mass spectrometer (API 2000 AB SCIEX instrument S.r.l., Forster City, CA, USA) equipped with the Turbo Ion Spray (TIS) and Atmospheric Pressure Chemical Ionization (APCI) interfaces. To perform MS and MS/MS analyses in full scan (mass range m/z 50– 800) and in product ion mode, the acquisition parameters were optimized by flow injection analysis (FIA) at a mobile phase flow rate of 200 µL min^{-1}, injecting 5 µL of a solution of 10 ng $µL^{-1}$. The best results were obtained operating with APCI with no additives. Air was used as nebulizing gas, and

nitrogen as both curtain and collisional gas and the settings for the nebulizer, curtain and collision gas were 60 psi, 30 and 5 respectively. The source probe heater was set at 400°C and the nebulizer current (NC) of the APCI source at -4 μA. The declustering potential (DP), was optimized in order to maximize the parent ion intensities, and, operating in product scan mode, the collision energy (CE) was optimized (table SI 4 in Supporting Information). Finally, all the analyses were carried out by LC/MS/MS in multiple reaction monitoring (MRM) mode, acquiring two or more diagnostic product ions from the chosen precursors to obtain high specificity and sensitivity. The chromatographic column was a GEMINI C_{18} 150 mm x 2.00 mm i.d., 3 μm particle size (Phenomenex, Bo Italy). Table SI 4 shows retention times, precursors and fragments chosen for the definitive MRM analyses. In order to simplify the analyses and to increase sensitivity, seven different acquisition periods were created according to table SI 4. The elution was optimized both in terms of composition of mobile phase (MeOH-H_2O at a flow rate of 200 $\mu L min^{-1}$) and by modifying gradient elution.

A MeOH/water gradient starting at 90% MeOH and increasing to 100% by 1% min^{-1} and holding for 8 min was used to elute all the analytes . A single chromatographic run, switching APCI polarity from positive to negative simultaneously, allowed to analyze all the compounds together in less than 16 min.

Analyst software 1.6.2 was used for acquisition and analysis of data from the mass spectrometer.

2.6 Calibration curves for quantitative analysis and matrix effect

Different calibration curves ("A", "B") were built, both in HPLC-MS/MS and in GC-MS, in order to evaluate the instrumental linearity, the method linearity, to estimate any possible matrix effect and to determine analyte concentrations in SRM and in environmental samples. Curves "A" were built using seven standard solutions with increasing concentrations of the analytes, and constant concentrations of internal standards (IS), chosen in the middle of the calibration range.

The multi-standard solutions contained analytes at different concentrations, depending on the instrumental sensitivities. Thus, in LC-MS-MS, concentration ranged from 0.02 to 10 mgL^{-1}, whereas in GC-EI between 6 and 1000 μgL^{-1} and GC-NCI between 0.5 and 400 μgL^{-1}.

To reproduce the environment where the analytes are found and the interactions between the analytes and other compounds in the matrix (possibly altering the analytical response), we also prepared the matrix-matched calibration curves "B". One sampled filter was extracted and divided in seven aliquots. Each aliquot was spiked with the same standard solutions of curve "A", and

processed, according to the analytical procedure of the previous section 2.3, prior to the injection. The eventual endogenous contribution was subtracted from the analyte response.

A linear plot of the peak area ratios (analyte area on ISTD area) against the concentrations of standard added (abscissa) was drawn. Each solution was injected three times and the regression model was applied to the calibration data set.

In order to assess the matrix effects (ME) possibly altering the results, we determined the ratio between the slope of the method calibration curves (B) and the slope of the standard calibration curves (A), defining B/A x 100 as the matrix effect value. Thus, a value >100% corresponds to a signal enhancement, whereas a value <100% to a signal suppression **[33].**

2.7 Limit of detection and quantification

Limit of detection (LOD) and quantitation (LOQ) were determined for both–HPLC-MS/MS and GC-MS.

The limit of detection (LOD) of the method was determined by spiking the matrices with the analytes before the whole procedure. The concentration of injected analyte producing a peak with a signal-to-noise ratio (S/N) of 3 was chosen as LOD. The limit of quantification (LOQ) was estimated in the same way as for LOD, using the criterion of (S/N) of 10.

2.8 Reproducibility

Intra-day and inter-day reproducibility of the method were determined by analyzing the solution from a blank filter, spiked at LOQ level, ten times in the same day and in five non-consecutive days and were expressed as relative standard deviation (RSD).

The precision of the investigated methods were assessed via replicate analyses of the standard reference materials (SRMs).

2.9 Recovery

Analyte recovery was determined after each step of the procedure (ASE extraction, SPE purification, evaporation), using analyte standard solutions and adding the IS just before the injection.

Total recovery was instead determined on blank filters spiked, before the extraction, with different amounts of analyte, at LOQ level, once and a half, and twice more concentrated, adding the IS prior to chromatographic analysis. The solutions were analyzed in triplicate by both LC–MS/MS in MRM mode and GC-NCI-MS.

The accuracy of the methods were assessed via replicate analyses of the standard reference materials (SRMs).

3. RESULTS

3.1 Optimization of the analytical method

3.1.1 Optimization of HPLC-MS-MS

Firstly, the mass spectrometer parameters were optimized for all the analytes, to determine suitable source parameters for the best sensitivity and S/N ratio, as well as to determine the molecule-related ions. This allowed us to study the fragmentation, but also to provide qualitative and quantitative information.

Acquisition with an APCI source was the best compromise for all the compounds, since ESI was inefficient for some analytes (PBDE and some PCB), as already reported by other authors **[29]**.

The APCI source was operated in both positive and negative mode, depending on the different nature of the compounds, and the response of compounds was maximized without additives. In order to achieve the highest possible sensitivity and to find the specific MS-MS transitions, the instrument parameters and lens potentials were optimized, both in full and product ion scan, as described in section 2.2.4, by flow injection analysis of the investigated compounds. The MS-MS transitions used in the following MRM experiments and the ions used for quantitative and qualitative purposes are shown in table SI4. As noticed, within the same class of compounds, characteristic common fragmentations occur.

Analyses of PCBs are always carried out by GC **[16, 34]**, due to its sensitivity, nevertheless we have obtained interesting results by HPLC-MS-MS as regard the study of fragmentation and the possibility of application to environmental samples.

For the PCBs ionized by negative APCI, the precursor ion was neither the molecular ion $[M]^{\bullet-}$ nor the deprotonated molecule $[M-H]^-$, but $[M\text{-}Cl+OH]^-$. The product ion is due to the loss of a molecule of HCl. As example, following this pathway of fragmentation, figures 2a and 2b show negative Q1 and MS-MS spectra for CB 153. On the other hand, the precursor of the positively ionized PCBs was $[M]^{\bullet+}$ (considering the isotopic cluster) and the main product was the ion due to the simultaneous loss of two Cl atoms, as shown in figure 3a and 3b for CB 81. CB 52 was the only positively ionized CB having, as main product, an ion formed by the loss of one Cl atom.

As regard the investigated PBDEs, as already reported by other authors **[35],** neither the molecular ion $[M]^{\bullet-}$ nor the deprotonated molecule $[M-H]^-$ were formed. The phenoxide ion $[M-Br+O]^-$ was

the precursor, and a typical fragment is due to the loss of the Br_2. This pathway of fragmentation is also common to TBBP-A and HBCD, starting from deprotonated molecule $[M-H]^-$, as precursor ion **[29]**.

The oxy-derivatives of PAHs were all analysed by positive ionization and the precursor was the protonated molecule $[M+H]^+$. Product ions did not follow a predetermined pathway of fragmentation, as the other classes of compounds. Anyway, most of the fragment ions were characterized by frequent CO/CHO losses. Explanatory Q_1 and MS-MS spectra are shown in fig 4 a) and 4b) for BaF.

The HPLC-MRM experiments were then performed, following the transitions of the two most abundant ions (shown in table SI 4). The best mobile phase was water-MeOH in gradient elution. MeOH proved to be more selective than AcN, particularly in the case of the three isomers (α,β,γ) of HBC, allowing the perfect chromatographic separation of them, as shown in figure 5a and 5b and already reported by other authors **[12].**

The reproducibility of the retention time allowed to split the mass spectrometric acquisition into different periods (see table SI 4), thus increasing the S/N ratio. Despite being some compounds co-eluted , especially those belonging to the same class with similar chemical-physical characteristics, the m/z discrimination allowed their identification, as in the case of CB 28, 52 and 95.

3.1.2 Extraction efficiency and recovery

In order to optimize the extraction step, filters spiked with three different concentrations of each analyte were subjected to extraction by ASE. A solvent mixture of DCM/acetone 4:1 was used to extract all the analytes. Neat DCM was also tested, but the recovery drastically decreased. For the SPE purification, we tried two different cartridges: silica and florisil, and the last one showed a better performance. We also determined experimentally the loss due to the evaporation step, that ranged between 1%-5%.

The recovery was not dependent on the level of concentration and was determined by HPLC/APCI-MS/MS in MRM mode and in GC-NCI-MS in SIM mode. The recovery calculation was $R=C/Crefx100$,where C is the concentration found with the method and $Cref$ is the reference (added) concentration.The total recoveries of the whole procedure of figure 1, for each analyte, expressed as average of three different samples, were always above 70%, as in table 1, with a CV below 20%. Most of the analyte losses seems due to SPE purification, probably for the presence of interfering compound (data not shown).

3.1.3 Linearity, LOD/ LOQ, reproducibility and matrix effect

Good linearity was obtained in the investigated concentration range for each analyte, as demonstrated by R^2 values between 0.980 and 0.999 of the calibration curves (A) (data not shown). Matrix effect, measured according to section 2.6, occurred only in case of oxygenated derivates of PAHs analyzed in NCI-GC, whereas it was negligible in all the other cases (table 2).

Table 3a shows the LOD/LOQ values for each investigated compound obtained by HPLC-MS-MS and the range of the LOD/LOQ values in GC-MS (table 3b) in $\mu g\ L^{-1}$ or $pg\ m^{-3}$.

The intra-day reproducibility at the LOQ level ranged from 4% to 8%. The inter-day reproducibility varied from 4% to 14% for both the techniques.

3.2 Application to SRMs

3.2.1 Analysis of NIST SRM 1975

SRM 1975 extracts were analyzed, after dilution, by GC-MS and HPLC/MS-MS, in order to check the presence of oxy-PAHs. Three aliquots (50 µL) were prepared by dilution (1:7) and each of them was added by internal standards. The only certified value in the SRM 1975 was 1-Nitropyrene and both GC and HPLC were in good agreement, as shown in table 4. The relative error (E%) was 3.6% and 9.1 % respectively. Other not certified compounds were identified and quantified by both of the techniques, and the results for BaF and BaD were in good agreement, whereas FL, 9,10 AQ and BA, although of the same order of magnitude, showed bigger variability, similar to the values obtained by other authors **[36, 37]**. Each value is associated to its standard deviation in the bracket.

3.2.2 Analysis of NIST SRM 2975

The SRM 2975 was processed as well . Three aliquots of 5 mg of powder, were extracted by ASE and purified according to the procedure of fig 1. The reference value of 1NP was a bit different (25%) from our results (in good agreement by both the techniques). The values of the other compounds were compared to those measured by Nocun **[36]**as shown in table 5.

GC and HPLC values were usually in good agreement each other and higher than those reported by Nocun [36]. In particular in this study 9,10 AQ was in good accordance with the values obtained by Nocun [36]. BaD, FL and *BA* were in good agreement each other while BaF discordant values were observed .

The standard deviations were usually bigger than those found in SRM1975, due to the more complex matrix, requiring a sample purification step, that can introduce a factor of variability.

3.2.3 Analysis of NIST SRM 2585

In the case of the SRM 2585, supplied by NIST as standard material for the analysis of halogenated flame retardants and PCB, it was necessary to weight large amounts of dust, in order to obtain the analytes of interest in concentrations above the detection limits of the chosen technique. Three samples from 10 to 100 mg of powder were weighed according to the technique used (GC→HPLC). As regard GC, negative chemical ionization gave results in better agreement to the reference values of the NIST. GC-MS results are displayed in table 6. The 4th column shows the relative error (E%) with respect to the NIST value. Some compounds show E%>30%, that can still be considered acceptable for complex analytical samples, as airborne particulate matter.

Unfortunately, due to the low instrumental sensitivity , it was possible to identify and quantify, with a good confidence, only 5 compounds (table 6) in HPLC-MS-MS. The 6th column shows the relative error (E%) respect to the NIST value. BDE 209 was analyzed only by HPLC with a good accuracy, because thermal degradation occurs in GC. In addition, results for BaP are reported only by HPLC, since it is not detected in GC-NCI-MS, whereas it was determined with an E% of 2 in GC-EI-MS.

3.2.4 Analysis of POPs in a indoor atmospheric sample

For explorative purposes, between October and December 2015, four samples of particulate matter of aerodynamic diameter less than 4μm (PM_4) were collected in an office with electrical and electronic equipment (EEE) running continuously and ventilated from the outside. To reach the detection threshold of the analysis, a total sampling time (11 days), corresponding to approximately 40 m^3, was needed. After conditioning, filters were added with the IS at the same concentration of calibration curves and were processed, according to our procedure (see Fig.1).

Analyses were carried out by both the techniques, but only GC/NCI-MS was able to detect the analytes of interest in the samples. In figure 6, the sum of concentrations of PCBs, PBDEs and Oxy-PAHs (ng/m^3) are shown in the indoor samples.

The presence of other eventual coeluting interferences was examined by analyzing blank field filters, i.e. filters located in the sampler housing, during all the sampling period and handled as environmental samples.

Oxy-PAHs were the most abundant compounds while PCBs and PBDEs showed, as expected, very low concentrations, representative of a site free from massive contaminations. The concentrations of PBDEs (292.3 pg/m^3) were in agreement with literature values measured in indoor air such as 826.0

399 pg/m^3 [38], 538.5 pg/m^3 **[27]** and 166 pg/m^3 **[16]**; at the opposite, PCB concentrations (173.7
400 pg/m^3) were much lower than those measured in urban indoor air in other studies such as 18149
401 pg/m^3 **[16]** 1319 pg/m^3 **[19]**. As regards oxy-PAH concentrations (687.7 pg/m^3), these were
402 comparable with those already determined in our previous study (910 pg/m^3) **[25]** in outdoor
403 samples of particulate matter. The high presence of Oxy-PAHs has therefore been ascribed to
404 outdoor–indoor air exchange.
405

406 ## 4. DISCUSSION

407 ### *4.1 Comparison between GC and HPLC analysis*

408 The results show that only HPLC-MS-MS allows to analyze the 46 analytes in a single fast
409 chromatographic run (16 min). It provides a cost effective method (decreasing the use of the
410 instrumentation, solvents and time of analysis) for simultaneous detection of PCB, BFR, PAH,
411 Oxy-PAH and Nitro-PAH in complex environments characterized by multiple sources of pollution.
412 Specifically, high performance liquid chromatography allowed the analysis of the investigated
413 compounds, belonging to different classes, including PCB, so far analyzed mostly by GC.
414 In fact, only one author reports the analysis of PCB 126 in blood samples by HPLC-MS-MS (Q-
415 trap) **[39]**.
416 The overlap of the retention times and transitions for CB 123-126, CB 146-153, CB 156-157 and
417 CB180-190 allowed to determine only their sum. On the other hand, in this case gas
418 chromatography-mass spectrometry (with EI or CI) is able to analyze and individually quantify
419 these congeners., since the co-eluting compounds are different. CB 126, CB 156 and CB 157 belong
420 to "dioxin like" compounds and their correct determination is important, since their concentration is
421 used to calculate toxic equivalents in risk assessment applications.
422 As regards Oxy-PAHs analysis in GC-MS, only the negative chemical ionization permitted to
423 achieve the limits of detection suitable for analysis of atmospheric particulate matter samples, but a
424 not negligible matrix effect was observed (see table 2). Matrix effect can both reduce or enhance the
425 detector response when compared to response of the standards in neat solvent **[40]**. The matrix
426 effect may depend on the instrument, the type and amount of matrix (grams of matrix per milliliter
427 of extract), the sample pre-treatment procedure. In HPLC, matrix effects were always more
428 negligible than GC as proved by the ratio between the slope of the method calibration curves (B)
429 and the slope of the standard calibration curves (A) (within 90-110%) (see table 2), allowing to use
430 calibration curves (A) for direct quantitation of target compounds.

In addition HPLC avoids the derivatization step, mandatory in the case of analysis of TBBP-A by GC (in this case not analysed), providing a more rapid analytical method with low sample handling. Furthermore, HPLC allows to separate the three HBCD diastereoisomers and to analyze highly brominated congeners, as BDE 209, showing thermal degradation in GC.

The analysis by HPLC/MS-MS also overcomes the potential interferences occurring in GC/MS determination of PBDEs and PCBs, due to the co-presence of other chlorinated and brominated compounds. In fact, the two ionization modes, EI and NCI, are subjected to different type of interferences. In general, EI-MS is affected by chlorinated interferences, NCI-MS eliminates chlorinated interferences, but there are different brominated interferences, well resolved with EI-MS approach **[41]**.

The selected strategy demonstrated to be fit for purpose, by applying it to SRMs with the aim to verify the efficacy of the study, the precision of the method, and the accuracy. Both the techniques were also able to identify unambiguously the investigated analytes, when in adequate amount, thus confirming their validity in a complex matrix, such as that of atmospheric particulate matter.

In fact, although the HPLC LOQs were higher than GC ones, they resulted compatible to the BFR concentration values found in indoor air of Electronics Recycling Plants, as shown in table 7 **[17,42, 43]**. In the Shredding area the values range between 300-85000 pg/m^3 **[42,43]**, whereas in dismalting hall they range between 80-19900 pg/m^3 **[17]**.

In our opinion, the coupling of the proposed chromatographic separation to analyzers such as Q-TOF and the Orbitrap would doubtless provide better performances and strongly improve the overall applicability of the method.

5. CONCLUSION

To our knowledge, an analytical procedure (sampling, extraction, clean-up and chromatographic analysis) allowing the simultaneous analysis of POP belonging to different chemical classes, including Oxy-PAHs and Nitro-PAHs, has not yet been investigated in airborne particulate matter, since, , different analytical procedures, specific for each single class of compounds, are usually performed.

This paper describes a reliable methodology for the simultaneous determination of 46 pollutants of environmental concern, in atmospheric particulate matter, belonging to POPs by HPLC-MS-MS and the results were compared to GC-MS.

The main advantages of LC over GC are:

-the simultaneous determination of 46 analytes belonging to different chemical classes;

-the separation of the three diastereoisomers of HBCD;

-the analysis of very polar compounds (such as TBBP-A) without the need of derivatization and of thermally labile compounds;

-the absence of matrix effects.

GC-MS has still presented better sensitivity for some of the considered compounds, but we obtained a good compromise using LC-MS-MS with APCI source in MRM mode, even in the case of compounds that did not fragment. The optimized method could be suitable, for example, to analyze atmospheric particulate matter collected in sites of treatment, recycling and disposal of electrical and electronic waste, where the concentration of BFRs and PCBs can be quite significant.

Anyway, due to the dated LC-MS instrument available, our study can be considered only explorative and further improvements can be expected with new generation LC-MS instruments (10-100 times more sensitive). The method, in this present form, can be a support tool for epidemiological and risk assessments and for increasing the information about the simultaneous presence and the atmospheric concentrations of POPs in particularl polluted sites.

Acknowledgements

This work was supported by "Progetto di ricerca Ateneo Sapienza 2016" and by "INAIL/Ricerca Discrezionale /2016-2018/P3O3/UO2.

Supporting Information Available

Tables SI 1-4 give detailed information about standards purchasing, instrumental parameters by HPLC-MS-MS and GC-MS. This material is free of charge and available.

References

[1] W. Xu, X. Wang and Z. Cai, Anal. Chim. Acta **790**, 1 (2013).

[2] F. Wania and D. Mackay, Environ Sci & Technol/news **30** (9), 390 (1996).

[3] A. Beyer, D. Mackay, M. Matthies, F. Wania and E. Webster, Environ. Sci. Technol. **34**, 699 (2000).

[4] A.C. Ruiz-Fernández, J.F. Ontiveros-Cuadras, J.L. Sericano, J.A. Sanchez-Cabeza, L.L.W. Kwong, Sci. Total. Environ. **493**, 505 (2014).

[5] IARC Monogr. Eval. Carcinog. Risk Chem. Hum. Polychlorinated biphenyls and Polybrominated biphenyls, **7** (2015).

[6] M.D. Mullin and C.M. Pochini, Environ. Sci. Technol. **18**, 468 (1984).

[7] M.J. Dagani, H.J. Barda, T.J. Benya and D.C. Sanders, Encyclopedia of Bromine Compounds, Ullmann's Encyclopedia of Industrial Chemistry. Ed. Wiley-VCH Verlag GmbH & Co. (2000).
[8] A. Besis, A. Katsoyiannis, E. Botsaropoulou and C. Samara, Environ. Pollut. **188**, 64 (2014).
[9] C. De Wit, Chemosphere **46**, 583 (2002).
[10] P. Gramatica, S. Cassani and A. Sangion, J. Hazard Mater. **306**, 237 (2016).
[11] J. Alexander, D. Benford, A. Boobis and Å. Bergman, EFSA Journal, **9** (12), 2477 (2011).
[12] M.A.E. Abdallah and S. Harrad, Environ. Int. **35**, 870 (2009).
[13] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency. OJEC L396, 30.12.2006, pp. 1–849
[14] A. Polder, A. Tkachev, K.B. Løken and J.O. Odland, Sci. Total Environ. **408**, 5352 (2010).
[15] A. Sjodin, D.G. Patterson Jr and A.A. Bergman, Environ. Int. **29**, 829 (2003).
[16] S. Harrad, S. Hazrati and C. Ibarra, Environ. Sci. Technol. **40**, 4633 (2006).
[17] A. Sjodin, H. Carlsson, K. Thurreson, S. Sjolin, A. Bergman and C. Ostman, Environ. Sci. Technol. **35** (3), 448 (2001).
[18] M.H. Wong, S.C. Wu, W.J. Deng, X.Z. Yu and Q. Luo, Environ. Pollution **149**, 131 (2007).
[19] S. Hazrati and S. Harrad, Environ. Sci. Technol. **40**, 7584 (2006).
[20] L. Zhang, T. Zhang, L. Dong, S. Shi and L. Zhou, Sci. Total. Environ. **454-455**, 619 (2013).
[21] F.R. Brown, J. Winkler, P. Visita, J. Dhaliwal and M. Petreas, Chemosphere **64**, 276 (2006).
[22] Z. Shi, Y. Wangand, P. Niu, J. Wang and Z. Sun, J. Sep. Sci. **36**, 3402 (2013).
[23] F. Mercier, E. Gilles, G. Saramito, P. Glorennec and B. Le Bot, J. Chromatogr. A **1336**, 101 (2014).
[24] D. Geng, P. Kukucka and E.J. Ingrid, Talanta **162**, 648 (2017).
[25] P. Di Filippo, D. Pomata, C. Riccardi, F. Buiarelli, V. Gallo, J. Aerosol Sci. **87**, 126 (2015).
[26] A. Albinet, E. Leoz- Garziandia, H. Budzinski and E. Villenave, J. Chromatogr. A **1121** (1), 106 (2006).
[27] K. Thuresson, J.A. Björklund, C.A. de Wit, Sci. Total Environ. **414**, 713 (2012).
[28] N.M. Tue, A. Sudaryanto, T.B. Minh, T. Isobe and S. Takahashi, Sci. Total Environ. **408**, 2155 (2010).
[29] G. Mascolo, V. Locaputo and G. Mininni, J. Chromatogr. A **1217**, 4601 (2010).
[30] L. Debrauwer, A. Riu, M. Jouahri, E. Rathahao and I. Jouanin, J. Chromatogr. A **1082** (1), 98 (2005).
[31] M. Rani, W.J. Shim, G.M. Han, M. Jang and Y.K. Song, Chemosphere **110**, 111 (2014).

[32] R. Cariou, J.P. Antignac, L. Debrauwer, D. Maume and M. Fabrice, J. Chromatogr. Sci. **44**, 489 (2006).

[33] F.Buiarelli, P. Di Filippo, C. Riccardi, D. Pomata and E. Rumolo, Int. J. Environ. An. Ch. **95** (8), 713 (2015).

[34] A.M. Cicero, E. Pietrantonio, G. Romanelli and A.D. Muccio, Bull. Environ. Contam. Toxicol. **65**, 307 (2000).

[35] A. Riu, and D. Zalko and L. Debrauwer, Rapid Commun. Mass Spectrom. **20**, 2133 (2006).

[36] M.S. Nocun and M.M. Schantz, Anal. Bioanal. Chem. **405**, 5583 (2013).

[37] J.A. Layshock, G. Wilson and K.A. Anderson, Environ. Toxicol. Chem. **29** (11), 2450 (2010).

[38] S.A. Batterman, S. Chernyak, C. Jia, C. Godwin, S. Charles, Environ. Sci Technol. **43** (8), 2693 (2009).

[39] B.J. Newsome, M.C. Petriello, S.G. Han, M.O. Murphy and K.E. Eske, J. Nutritional Biochem. **25**, 126 (2014).

[40] D. Pomata, P. Di Filippo, C. Riccardi, F. Buiarelli and V. Gallo, Atmos. Environ. **84**, 332 (2014).

[41] E. Eljarrat, A. De la Cal and D. Barceló, J. Chromatogr. A. **1008**, 181 (2003).

[42] A. Julander, A. Westbergb, M. Engwalla and B. van Bavel, Sci. Total Environ. **350**, 151 (2005).

[43] T.M. Cahill, G. Danka, M.J. Charles, J.R. Sanborn, M.S. Denison and L. Baker, Environ. Sci. Technol. **41**, 6370 (2007).

Captions

Figure 1. Schematic diagram of the analytical procedure

Figure 2. a) Negative APCI-Q1 and b) MS-MS spectra for CB 153 (MW 358) Precursor ion m/z 340.6, parameters as in table SI 4. FIA conditions: injection 5 μL of a solution 10μg mL^{-1}, flow rate 200μL min^{-1}.

Figure 3. a) Positive APCI-Q1 and b) MS-MS spectra for CB 81 (MW 290) Precursor ion m/z 291.9, parameters as in tableSI4. FIA conditions: injection 5 μL of a solution 10 μg mL^{-1}, flow rate 200μL min^{-1}.

Figure 4. a) Positive APCI-Q1 and b) MS-MS spectra for BaF (MW230). Precursor ion m/z 231.1, parameters as in table SI 4. FIA conditions: injection 5 μL of a solution 10 μg mL^{-1}, flow rate 200μL min^{-1}.

Figure 5. a) HPLC-MS-MS analysis in MRM mode of the three isomers (α,β,γ) of HBCD using water-AcN (unresolved peaks) and b) water-MeOH (good resolution between the three isomers). Flow rate 200 µL min^{-1}, isocratic elution, column Gemini C_{18} (150 mm x 2.00 mm), conditions as in tableSI4.

Figure 6. Sum of PCBs, PBDEs and Oxy-PAHs(ng/m^3) in indoor samples.