

Method development for the LC-MS/MS determination of C₆₀ and C₇₀ fullerenes and their functionalized derivatives in airborne particulate matter, settled dust and soot

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ABSTRACT

Fullerenes are organic contaminants of growing concern due to their increasing use for industrial purposes, in addition to their emission by natural and anthropogenic combustion sources.

Their analysis is made tricky by their unique chemical structure and their acute hydrophobicity. Thus, analytical developments are required for further environmental evaluation of their occurrence. This article presents such a development for the analysis of C₆₀ and C₇₀ fullerenes together with three of their functionalized derivatives in airborne particulate matter, settled dust and soot from diesel exhaust.

Pressurized Liquid Extraction was performed with toluene and acetonitrile as extraction solvents, and gave mean extraction recoveries from 75% to 95% for the five fullerenes investigated.

Liquid Chromatography was optimized in terms of stationary phase and mobile phase to obtain a good separation of the compounds. Optimal separation of the compounds by liquid chromatography were obtained using a C18 stationary phase and a mixture of toluene - acetonitrile as mobile phase in gradient mode.

The mass spectrometry detection method was also optimized with a comparison of three ionization sources: Electrospray ionization (ESI), Atmospheric Pressure Chemical Ionization (APCI) and Atmospheric Pressure Photoionization (APPI). APPI gave the most sensitive and repeatable detection with instrumental limits of quantification (LOQ) down to 10 pg injected. MS/MS was used in pseudo-MRM mode to enhance signal intensity.

The optimized LC-APPI-MS/MS method was then validated and applied to indoor PM₁₀ and household dust samples, and to a Diesel Particulate Matter Reference Material. None of these samples showed concentrations of fullerenes above the method quantification limits.

1. Introduction

Fullerenes are a class of compounds that can either occur in the environment from natural sources (volcanoes, wildfires, meteoritic impacts, etc.) or from anthropogenic combustion processes, particularly industrial and vehicular sources [1–5].

These compounds are also increasingly used for various industrial applications such as photovoltaic cells, microelectronics, cosmetics,

water treatment or medicine among others [6–9]. For such applications, fullerenes are categorized as engineered nanomaterials and thus belong to the family of organic engineered nanoparticles, which have been increasingly produced in recent years, around tens of tons annually [6, 10–12]. This growing production is therefore of an order of magnitude similar to their release via combustion [5] and combination of both sources raises questions about the release of these carbon-based nanoparticles in the environment [13].

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Indeed, the occurrence of fullerenes in a nanoparticulate form raises questions about their potential adverse effects for human health and environment [12,14–16]. In 2010, Johnston et al. concluded that the hazards of fullerenes exposure to human health were uncertain [17]. For instance, DNA damage, increases in oxidative stress and decreases in aerobic respiration rates were reported as negative influences from C₆₀ fullerene on living organisms [18–20]. Even though recent studies tend to show that the toxicity of pure fullerenes is quite low under realistic environmental conditions [21], fullerenes can act as strong adsorbents for other organic pollutants, and therefore transport these pollutants in the environment and induce human exposure, as it was shown by Freixa et al. for riverine ecosystems [22]. This characteristic could also be particularly meaningful for airborne fullerenes in the particulate phase.

While the presence of fullerenes has already been investigated in environmental matrices such as natural water, wastewater, soils and sediments [10,23–25], only three previous analytical studies reported their occurrence in atmospheric samples, specifically in the particulate phase [2,26,27]. As fullerenes are not volatile compounds, they will sorb on the particulate matter (PM) when released in the atmosphere. This particulate matter is also called “aerosol” and can be made up of a wide variety of inorganic, organic and biological components.

One of these three studies resulted in the positive identification of C₆₀ with a level below the lowest calibration level, estimated in the low pg m⁻³ range, but only in parking garage samples [2]. Similar conclusions were drawn by Encinas and Gómez-de-Balugera who reported C₆₀ levels from 0.08 to 2.27 pg m⁻³ outdoors and up to a dozen of pg m⁻³ in a garage [26]. Sanchís et al. found higher concentrations of C₆₀ and C₇₀ in airborne particulate matter samples from the Mediterranean Sea, but also very wide distributions among all their samples, from 33 pg m⁻³ up to 234 ng m⁻³. On the other hand, they did not detect any functionalized fullerene [27].

However, such functionalized derivatives of fullerenes can still be considered as emerging contaminants. They present peculiar interests in nanotechnology for some of the industrial applications listed above [7, 28,29], but they also raise toxicological issues [17]. Among those functionalized derivatives are *N*-methylfulleropyrrolidine C₆₀ (NMFP), [6,6]-phenyl C₆₁ butyric acid methyl ester (C₆₁-PCBM), and [6,6]-phenyl C₇₁ butyric acid methyl ester (C₇₁-PCBM). This kind of compounds have been detected in matrices other than aerosols, such as in pond waters at the pg L⁻¹ concentration and in sediments at the ng kg⁻¹ level [28] or in wastewater effluents at concentrations up to the µg L⁻¹ level [23]. These findings confirm the necessity of monitoring these molecules in various environments for further risk assessment purposes.

Therefore, this paper presents a method development for the trace-level determination of the two main pristine fullerenes (C₆₀ and C₇₀), together with three of their functionalized derivatives (NMFP, C₆₁-PCBM, C₇₁-PCBM), in airborne PM, settled dust and soot. This work is thus the first determination of fullerenes and derivatives in the atmospheric compartment of indoor environments.

In the previously published fullerene determination methods applied to aerosols, Ultrasound-Assisted Extraction (UAE) was systematically used [2,26,27], though Pressurized Liquid Extraction (PLE) has proved itself very efficient and robust for the extraction of non-polar compounds from airborne PM [30]. Prior to this work, only a very limited amount of research papers reported the use of PLE for fullerene extraction, and none of them applied this method to airborne particulates, but rather to soils, sediments or carbonaceous geological materials [24,28,31,32]. In this work, the application of PLE is presented for the first time for an optimal extraction of fullerenes from airborne particles.

Liquid Chromatography – Mass Spectrometry (LC-MS) is almost universally applied for the determination of fullerenes in environmental samples. Octadecyl (C₁₈) stationary phases are largely predominant in the previously presented methods for fullerene environmental determination [2,25,28,33]. Here, a C₁₈ phase was compared with a biphenyl stationary phase. To the best of our knowledge, the use of a biphenyl phase for fullerenes separation was only presented once in the literature,

with a sole focus on C₆₀ and C₇₀, and not on their functionalized derivatives [10].

The most critical aspect of fullerene instrumental analysis resides in the optimization of the Mass Spectrometry (MS) detection method. For this purpose, we compared three different ionization sources (Electrospray ionization (ESI), Atmospheric Pressure Chemical Ionization (APCI) and Atmospheric Pressure Photoionization (APPI)) to select the most sensitive one.

In this paper, we also critically discuss quality assurance and performance of the Liquid Chromatography – Tandem Mass Spectrometry (LC-MS/MS) method. These are very delicate aspects to tackle in the case of method development for fullerenes trace-level analysis, since this family of compounds is very tricky. Acute hydrophobicity, sorption capacity on organic matter and on glassware, absence of commercial isotope-labeled standards, etc. are major analytical challenges which could hinder an accurate determination of fullerenes in environmental samples [5,10,13,23,31,34–37]. Pinpointing and tackling them was another important objective of this study.

2. Material and methods

2.1. Chemicals

Pure standards of C₆₀, C₇₀, C₆₁-PCBM, C₇₁-PCBM and NMFP were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France). LC-MS grade solvents (acetonitrile, methanol and toluene) were purchased from VWR Chemicals (Fontenay-sous-Bois, France) and Merck Millipore (Molsheim, France).

The Standard Reference Material® 2975 (SRM 2975) was purchased from the National Institute of Standards & Technology (NIST, Gaithersburg, MD, USA). It is made up of Diesel Particulate Matter from an Industrial Forklift and has been certified for its content in Polycyclic Aromatic Compounds [38].

2.2. Sample collection

Airborne PM samples were collected in February 2022 in the parking lot of a residential building. The building was located in the urban area of Strasbourg (Alsace, France, 48°35'26" N, 7°43'34" E).

The sampling was done using a Sven Leckel LVS6-RV medium-volume air sampler (Sven Leckel Ingenieurbüro GmbH, Berlin, Germany) operated at 2.3 m³ h⁻¹ during one week, and equipped with a PM₁₀ sampling head. A first set of three samples was obtained by collecting PM₁₀ during daytime only. The corresponding sampled volumes were from 209 to 213 m³. A second set of three samples was corresponded to PM₁₀ sampling during daytime and night-time, for the same total duration of one week. This resulted in sampled volumes were from 384 to 386 m³.

PM₁₀ was collected on glass fiber filters (GFFs) (Ø 47 mm, GF6, Whatman, GE Healthcare, Little Chalfont, United Kingdom).

Indoor dust samples were collected in February 2021 in urban households from the same residential building as the parking lot PM samples employing a household vacuum cleaner and Kudzu Science kits designed for settled dust sampling (Kudzu Science, Strasbourg, France). Depending on the dust load of the sampled floor, the mass of dust collected varied between 0.17 and 0.99 g.

After sampling, the filters and the dust samples were stored in aluminium foil at –20 °C until extraction.

2.3. Sample preparation

After sampling, PLE was applied to GFFs and dust samples at 104 bar and 110 °C thanks to an ASE 300 device (Dionex, Sunnyvale, CA, USA). Heating time was 6 min, then 2 static cycles of 10 min were performed.

Irrespective of the nature of the matrix and of the extraction method chosen, toluene is the preferred and almost only extraction solvent for

fullerenes in the literature [4,23,28,39]. We also observed the benefits of toluene for the solubilization of fullerenes and toluene was selected as extraction solvent. However, acetonitrile was added in equal proportion so that the extraction with a toluene/acetonitrile mixture (1:1, v/v) could be applicable for multi-class analysis purposes, including Polycyclic Aromatic Compounds. The volume of PLE extracts was around 90 mL. These extracts were further concentrated to 0.5 mL under a gentle nitrogen stream on a TurboVap II (Biotage AB, Uppsala, Sweden) and stored at $-20\text{ }^{\circ}\text{C}$ before analysis.

2.4. Instrumental analysis

2.4.1. LC-MS instruments

LC-ESI-MS/MS analyses were performed on a TSQ Quantum Access Max triple quadrupole mass spectrometer coupled to an Accela 1250 LC pump (Thermo Fisher Scientific Inc., Waltham, MA, USA).

LC-APCI-MS/MS and LC-APPI-MS/MS analyses were performed on an Esquire 3000^{plus} ion trap mass spectrometer (Bruker Scientific Instruments, Billerica, MA, USA) coupled to an Agilent 1100 HPLC (Agilent Technologies, Inc., Santa Clara, CA, USA).

Comparative direct infusion analyses to assess the electrospray ionization of fullerenes were performed on three other instruments: two Ion Traps (IT), a LCQ Fleet and a LTQ XL (Thermo Fisher Scientific Inc., Waltham, MA, USA), and an Agilent 6120 Single Quadrupole (SQ) (Agilent Technologies, Inc., Santa Clara, CA, USA).

2.4.2. Liquid chromatography

Two types of columns were compared for fullerene separation: a Nucleodur C₁₈ Pyramid (150 × 3 mm, 3 μm; Macherey-Nagel GmbH&Co., Düren, Germany) and a Raptor® Biphenyl (150 × 3 mm, 2.7 μm; Restek Corp., Bellefonte, PA, USA).

The comparison of the performance of these two LC columns was performed on the Accela 1250 – TSQ Quantum Access Max instrument (Thermo Fisher Scientific).

Compound elution with the optimized LC method was carried out on the C₁₈ column using toluene (solvent A) and acetonitrile (solvent B) in gradient mode. Elution was programmed as follows: 40% A maintained for 1 min, then ramped to 50% A in 7 min, then from 50% to 60% A in 4 min. The total duration of the LC run was 12 min before driving the solvent composition back to 40% A for the next analysis. The eluent flow rate was 0.7 mL min⁻¹ and the injection volume was set at 20 μL.

2.4.3. Mass spectrometry

Electrospray ionization was performed in negative mode on a heated ESI source (HESI-II, Thermo Fisher Scientific) with the following parameters: spray voltage -4000 V , sheath gas 35 (arb.), auxiliary gas 5 (arb.), capillary temperature $300\text{ }^{\circ}\text{C}$, vaporizer temperature $300\text{ }^{\circ}\text{C}$.

Both APCI and APPI analyses were performed in positive and negative ionization mode (see Section 3.1.2.) using the following source parameters: capillary voltage $\pm 3500\text{ V}$, nebulizer gas 43.5 psi, dry gas 6 L min⁻¹, capillary temperature $350\text{ }^{\circ}\text{C}$, vaporizer temperature $420\text{ }^{\circ}\text{C}$. For APCI analysis, the Corona discharge was $\pm 4\text{ }\mu\text{A}$.

Direct infusion experiments for the optimization of the MS response of fullerenes and the identification of the most abundant ions generated (see Section 3.1.) were performed with neat solutions in a toluene/acetonitrile (1:1, v/v) mixture, at an infusion flow rate of 50 μL min⁻¹, combined with the same composition of mobile phase at 200 μL min⁻¹.

For all ionization modes, pseudo-MRM was the detection mode implemented to perform LC-MS/MS analyses. It relies on Multiple Reaction Monitoring (MRM) with no mass loss transitions, as described previously by our group [40]. When possible, other MRM transitions were used only as a confirmation.

The optimized MS/MS transitions finally selected for the developed LC-MS/MS method relying on APPI are given in Table 1.

Table 1

Optimized tandem Mass Spectrometry quantitation (Q) and confirmation (q) transitions of the LC-APPI-MS/MS method developed on the Esquire 3000^{plus} instrument. Collision energies are reported under brackets after the *m/z* values of the parent and fragment ions.

LC-APPI-MS/MS transitions	Q	q
C ₆₁ -PCBM (–)	910 → 910 (0.3)	910 → 720 (1.5)
C ₇₁ -PCBM (–)	1030 → 1030 (0.5)	1030 → 840 (2.0)
NMFP (+)	778 → 778 (0.4)	777 → 777 (0.4)
C ₆₀ (–)	720 → 720 (0.5)	721 → 721 (0.5)
C ₇₀ (–)	840 → 840 (0.5)	841 → 841 (0.5)

2.5. Method validation

The LC-MS/MS method was validated against linearity of the calibration, precision, intra-day and inter-day repeatability, recovery and limit of quantification.

To obtain the best possible fit of the calibration curve, it was built in quadratic mode with 1/X weighting. These parameters are typical of tandem mass spectrometry detection. External calibration was performed given the absence of an available commercial internal standard (see Section 3.5.). Precision of the calibration was validated by verifying that the coefficient of determination (*R*²) was higher than 0.99 and by calculating deviations between theoretical concentrations and recalculated concentrations of calibration solutions.

To evaluate recoveries, GFFs which had been previously used for PM₁₀ sampling (380 m³) were washed once with the PLE method described in Section 2.3 to eliminate natural traces of analytes. Then, the pre-washed GFFs were fortified with five different levels of a standard mixture containing the five fullerenes (20 ng, 50 ng, 100 ng, 200 ng and 500 ng). Three replicates were performed for each level, and the whole analytical protocol was applied to these samples. Recoveries were defined as the measured concentrations of the concentrated extracts divided by their theoretical concentrations following the spike.

The instrumental limits of quantification (LOQ) had to meet all of the following criteria to be validated: 1) signal-to-noise ratio (S/N) > 10 for the corresponding standard in toluene, 2) superior to the measured “mean + standard deviation” concentration of fullerenes in at least five solvent blanks, 3) validated as a standard concentration of the calibration range according to the precision criteria.

The LOQ was confirmed for each target compound at each series of injection by analyzing the extract of an unfortified pre-washed GFF serving as matrix-matched blank sample. All these samples were below the LOQ for each fullerene.

The method LOQs for our environmental samples were derived from the instrumental LOQs taking into account the sampled mass or volume and the extraction recovery of the analytes at the lowest concentration levels (see Section 3.4.).

3. Results and discussion

3.1. Mass spectrometry of fullerenes

In the following section, results of fullerene neat standard direct infusion using various MS instruments and different ionization sources are presented. The purpose of these experiments was to identify the MS instruments, ionization sources and conditions which could enable the optimal LC-MS detection of fullerenes in various matrices.

It was noticed that toluene was required to obtain a satisfying response for any of the following experiments. In the absence of toluene, the use of other solvents resulted in the severe decrease of fullerene solubility.

3.1.1. ESI-MS

ESI-MS experiments were performed on a Triple Quadrupole (QqQ), TSQ Quantum Access Max, equipped with an Ion Max API heated

electrospray (H-ESI) ion source.

Due to the highly unusual chemical properties of fullerenes, i.e. their composition exclusively made up of carbon atoms, classical electrospray ionization pathways such as protonation and deprotonation in aqueous phase are not possible. With ESI, fullerenes can only be ionized by electron capture in negative mode to form $M^{\cdot-}$, due to their electron affinity (around 2.7 eV) [41].

By performing direct infusion of fullerene standard solutions in a toluene/acetonitrile mixture on the TSQ Quantum Access Max instrument, a high prevalence of the $M^{\cdot-}$ ion was observed, with additional epoxide adducts ($[M+O]^{\cdot-}$ and $[M+O_2]^{\cdot-}$) for all compounds. Mass spectra obtained by ESI-MS direct infusion of the C_{60} pristine fullerene and its functionalized derivative C_{61} -PCBM are represented in Figs. 1a and 2a, respectively. The corresponding mass spectra of other fullerenes are reported in Figure S1a, S2a and S3a of the Supplement.

The major finding of the ESI(-)MS experiments deals with the unreliability of electron-capture ESI for fullerenes on different instruments. Indeed, parallel studies were carried out on two IT mass spectrometers (LCQ Fleet and LTQ XL) equipped with an Ion Max API ESI source. Although the source design was the same for these instruments and for the above-mentioned TSQ, no signal could ever be obtained on these two ITs. A first hypothesis envisaged to explain this observation was the absence of vaporizer heating on these ITs in contrast to the H-ESI of the TSQ, but it was quickly discarded by checking that a satisfying signal was obtained on the TSQ instrument even when the vaporizer was set at

ambient temperature.

Obtaining any response was also not possible using ESI in hyphenation with the Esquire 3000^{plus} Ion Trap from Bruker Scientific Instruments, whereas the latter was successfully used with an APCI and APPI source (see Section 3.1.2. and 3.1.3.).

Furthermore, other ESI infusion tests were performed on a Single Quadrupole (Agilent 6120, Santa Clara, CA, USA), and an intense response was observed for the five fullerenes investigated on this instrument.

Notably, the previous works reporting successful electrospray ionization used TQ [23,27,28,42], Q-TOF [10] or Orbitrap instruments [25].

To conclude, the combination of these observations could suggest an instability of the electrospray-generated fullerene radical anions in ion trap instruments (confirmed on three different ITs) which would hinder their detection, while the same radical anions are observable on single quadrupole and triple quadrupole instruments.

3.1.2. APCI-MS

Unlike ESI, APCI can lead to fullerene ionization both in positive and negative mode. In both modes, charge transfer is the responsible ionization process, either O_2 -mediated electron transfer in APCI(-) or N_2 -mediated proton transfer in APCI(+).

Similarly to the mass spectra obtained using ESI, APCI spectra of fullerenes were dominated by their molecular ions $M^{+\cdot}$ and $M^{\cdot-}$. In the case of the APCI(+) spectra, only the molecular ion and related isotopic

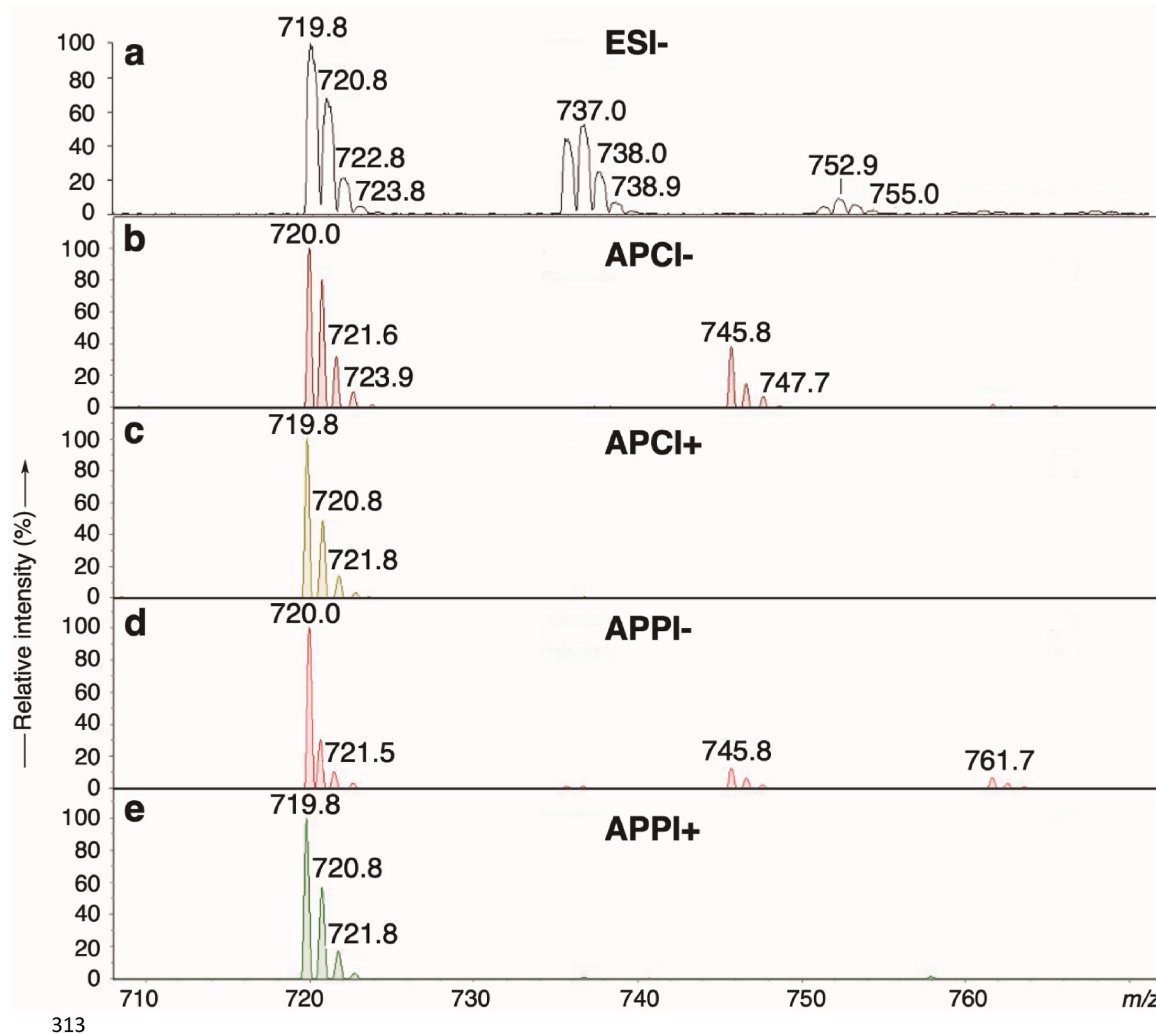


Fig. 1. Mass Spectrum obtained by direct infusion of C_{60} fullerene with a) negative ElectroSpray Ionization, b) negative Atmospheric Pressure Chemical Ionization, c) positive Atmospheric Pressure Chemical Ionization, d) negative Atmospheric Pressure PhotoIonization, e) positive Atmospheric Pressure PhotoIonization.

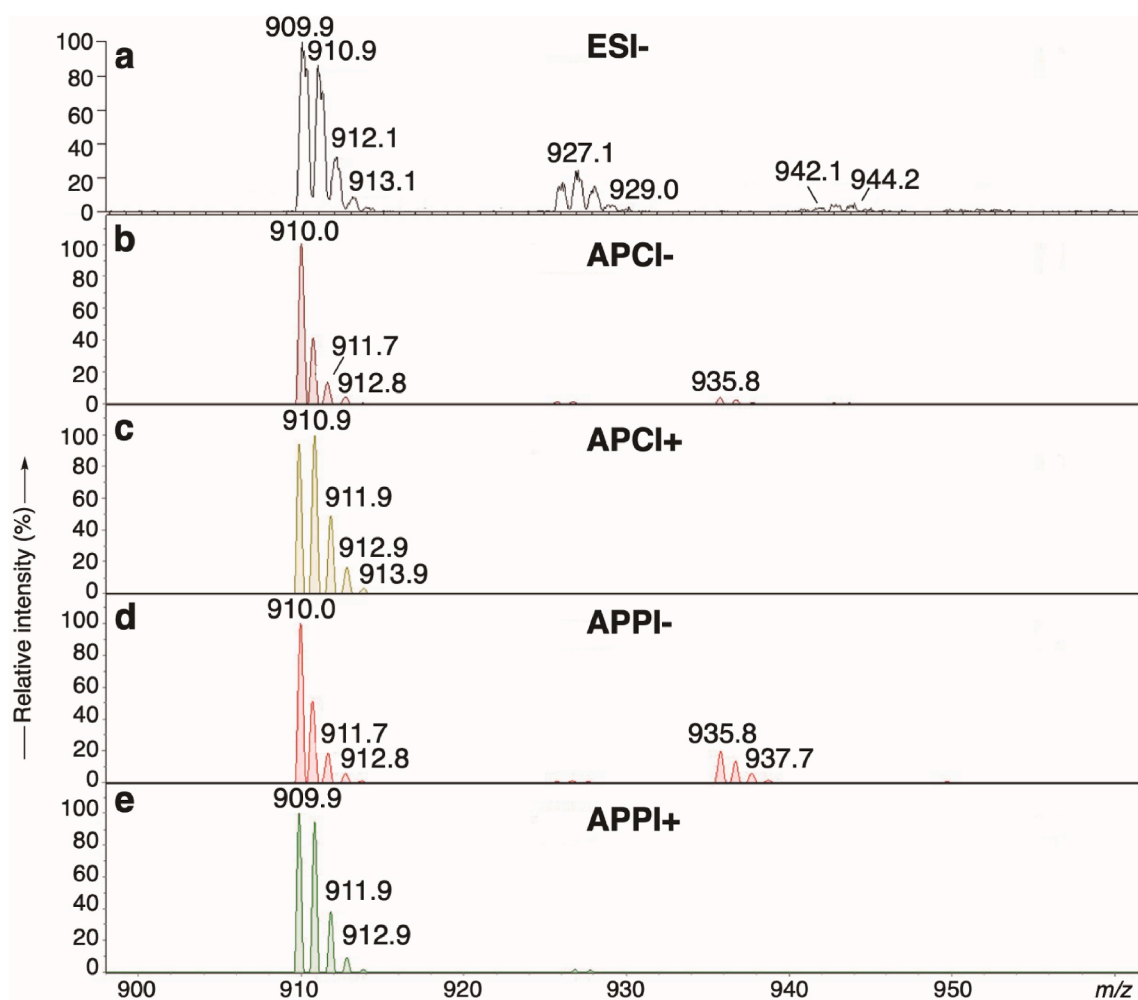


Fig. 2. Mass Spectrum obtained by direct infusion of C_{61} -PCBM fullerene with a) negative ElectroSpray Ionization, b) negative Atmospheric Pressure Chemical Ionization, c) positive Atmospheric Pressure Chemical Ionization, d) negative Atmospheric Pressure Photoionization, e) positive Atmospheric Pressure Photoionization.

ions were observed, whereas with APCI(–), M+26 and M+42 adducts were formed. They correspond to CN addition due to the presence of acetonitrile in the solvent ($[M + CN]^-$) and its corresponding epoxide adduct ($[M + CN + O]^-$). Interestingly, the epoxide M+16 adduct described in section 3.1.1 was either not detected, or only present at very low abundance with APCI(–). APCI mass spectra of studied analytes are presented in windows b and c of Figs. 1 and 2, Fig. S1, Fig. S2 and Fig. S3.

The mass spectra of functionalized fullerenes obtained in the positive mode show a variation in the relative proportion of M versus M+1 ions (see Fig. 2, Fig. S2 and Fig. S3). It can be assumed that in the positive mode, the ionization by protonation resulting in $[M+H]^+$ formation competes with the charge transfer ionization, and the M+1 peak would then correspond to a combination of this $[M+H]^+$ ion with the M^+ ion resulting from a ^{13}C isotope. The addition of these two ionization processes caused an increase of the M+1 peak intensity relative to the M peak.

While both polarities are applicable to fullerenes using APCI, they are far from being equivalent in terms of signal intensity. Indeed, for most fullerenes, negative APCI is much more efficient than positive APCI (see Fig. S4). For instance, the LC-MS/MS peak of C_{60} in pseudo-MRM mode is not visible at 1 mg L^{-1} in the positive mode, while it has a peak intensity in the 10^5 order of magnitude in the negative mode. For NMFP however, the signal is much more intense using positive ionization (ca. $6 \cdot 10^4$ vs. $1.5 \cdot 10^4$ intensity; cf. Fig. S4).

3.1.3. APPI-MS

APPI has proved to be very efficient for fullerenes ionization, considering their very low polarity and the use of toluene, which is universally used in LC separation of fullerenes, and serves as a solvent-mediated ionization agent [34,35,41,43,44].

APPI mass spectra of fullerenes were found to be very similar to APCI spectra (see Figs. 1 and 2, Fig. S1 and Fig. S2). In the negative mode, the $[M + CN]^-$ and $[M + CN + O]^-$ adducts were observed (Figs. 1d and 2d, Fig. S1d and Fig. S2d), whereas in the positive mode, only the M^+ ion occurred.

In general, it seems that observed adducts are dependent of various experimental parameters, including the nature of the solvent. Indeed, fullerene adducts other than those detected in the present study were observed when methanol was used instead of acetonitrile [7,34,35,43].

Comparison of APPI negative versus positive modes gives about the same results as with APCI (see Section 3.1.2.). Indeed, negative ionization is highly favored for all the fullerenes, except NMFP which is better ionized in the positive mode. The corresponding overlaid chromatograms are presented in Fig. S5.

3.1.4. Tandem mass spectrometry (MS/MS)

Fullerenes are stable “aromatic-like” structures. Their big carbon cage results in a very high resistance to collision-induced dissociation (CID). Therefore, previous studies presented the MS detection of fullerenes in either Single Ion Monitoring (SIM) mode or in pseudo-MRM

mode [7,10,23,27,35], but it was reported that the use of pseudo-MRM highly increases method sensitivity [23]. The principle and benefits of pseudo-MRM were reported in detail previously [40]. Briefly, it consists in monitoring MS/MS transitions with no mass loss between the parent and fragment ions, but still applying non-zero collision energies to preserve the selectivity of the MRM approach [40].

The MS/MS behavior of fullerene ions obtained using negative ESI with a triple quadrupole was investigated through MS/MS breakdown curves obtained by direct infusion of fullerenes neat standards in toluene on the TSQ Quantum Access Max. The most intense ions observed in MS¹ were used as precursor ions in MS², and the obtained fragment ions were monitored at various collision energies (CE) applied in the collision cell. The corresponding results are presented in Fig. 3.

As expected, no fragmentation of pristine fullerenes C₆₀ and C₇₀ was observed, and only the intact precursor ion was observed in Q3 after CID in Q2 (i.e. 720 for C₆₀ and 840 for C₇₀), irrespective of the CE (see Fig. 3a). The observation of the same phenomenon for functionalized fullerenes was however less expected. Indeed, the chemical structures of functionalized derivatives such as PCBM and NMFP suggest a possible loss of the functionalized group by CID, and therefore an intense MS/MS transition from the molecular ion M⁻ to a fragment corresponding to the bare carbon buckyball was expected. Surprisingly, according to our results presented in Fig. 3b for C₆₁-PCBM, these fragment ions only occur quantitatively at collision voltages above 45 V, and the maximal intensity of the corresponding transition only accounts for about 20% of the intensity of the pseudo-MRM transition.

Finally, one can notice an increase of the pseudo-MRM signal intensity from 0 to about 15 V applied, while one could expect a constant signal decrease already from very low collision voltages. This phenomenon was already described and was attributed to the geometry of the source from the mass spectrometer [40]. The TSQ Quantum Access Max is three-dimensional L-shaped, therefore the ions path is not direct from

Q1 towards Q3, and the application of collision voltages in Q2 could help facilitating the parent ion transmission in the TQ, thus enhancing the signal.

This pattern is actually an advantage for environmental analysis of fullerenes, because it maintains the specificity of MRM: the application of slight collision energies could result in the fragmentation of possible isobaric interferences which would be less resistant to CID than fullerenes. Therefore, with TQ instruments, pseudo-MRM mode highly increases the sensitivity for fullerene detection without affecting its specificity.

The pseudo-MRM pattern was also observed on the ion trap with APCI and APPI sources. C₆₀ and C₇₀ are not subject to fragmentation, whether positively or negatively charged, even at high fragmentation amplitudes (CE > 1.5) (Fig. S6).

However, in the case of functionalized fullerenes, it is worth noting that positively charged ions obtained with APCI and APPI sources were more subject to fragmentation than negatively charged ions. Already at moderate collision energies, the intensity of the positively charged molecular ion decreased, in favor of a range of fragments corresponding to the breaking of covalent bonds from the functional group, down to the fragment made up of the carbon buckyball skeleton only (see Fig. S7). On the other hand, negatively charged molecular ions of functionalized fullerenes only yielded their corresponding pristine fullerene, at fairly low intensities, and only when high collision energies were applied (see Fig. S7).

In general, tandem mass spectra obtained were very similar using APCI and APPI sources. The only differences concerned the relative intensities of fragments which could vary between both sources at the same collision energy.

3.2. Liquid chromatography

To separate the five fullerenes investigated in our study, a C₁₈ stationary phase was used: the Nucleodur C₁₈ Pyramid (150 × 3 mm, 3 μm particle size; Macherey-Nagel GmbH&Co., Düren, Germany). The performance of this column was compared to that of another kind of column having a different stationary phase: the Raptor® Biphenyl (150 × 3 mm, 2.7 μm particle size; Restek Corp., Bellefonte, PA, USA).

The biphenyl phase, even after careful optimization of the various LC parameters (see caption of Fig. 4), did not enable a satisfying separation of our compounds. The LC-ESI(-)-MS chromatogram obtained for the fullerene mixture with the Raptor® Biphenyl column in the optimized conditions is represented in Fig. 4a. The comparative LC-ESI(-)-MS chromatogram obtained with the Nucleodur C₁₈ Pyramid column on the same instrument is displayed in Fig. 4b.

To support the visual impression of a better fullerene separation on the Nucleodur C₁₈ Pyramid column, the number of theoretical plates (N) of each column for each studied fullerene, and the resolution (R) between consecutive peaks obtained with each column, are reported in Table 2. It can be concluded that for all the peaks, the value of N was always the highest with the C₁₈ column (from 2 to 8 times higher than with the biphenyl column). The higher efficiency of the C₁₈ column was confirmed by the resolution values, from 1.9 to 6.3, while those obtained using the biphenyl column ranged from 0.4 to 3.5, including three of the four resolutions below the critical value of 1.5.

It could be hypothesized that the lack of selectivity of the biphenyl phase was due to the interference of toluene used in the mobile phase, which could saturate the π-π interaction sites of the biphenyl phase, making them unavailable for our analytes. Therefore, the C₁₈ phase was selected for our analytical method.

Two polar solvents were also compared in association with toluene: acetonitrile and methanol. It was reported in the literature that no significant differences were observed between these two solvents in terms of peak shape and resolution using a C₁₈ stationary phase. However, compounds are eluted faster when methanol is used instead of acetonitrile [28,43]. After optimization of the LC method, we reached the

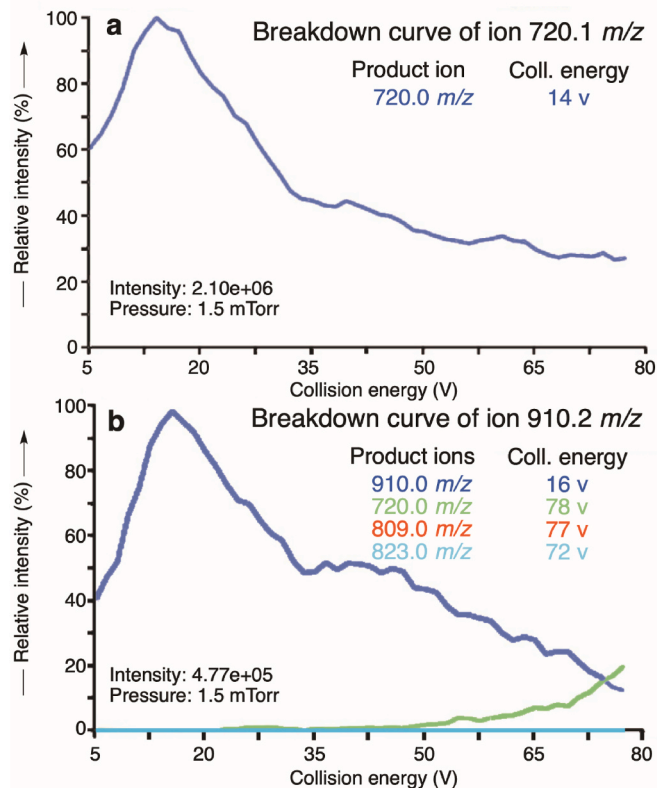


Fig. 3. MS/MS breakdown curve obtained by negative electrospray (ESI⁻) direct infusion and Collision-Induced Dissociation from 5 to 80 V of a) C₆₀ and b) C₆₁-PCBM on a Triple Quadrupole instrument.

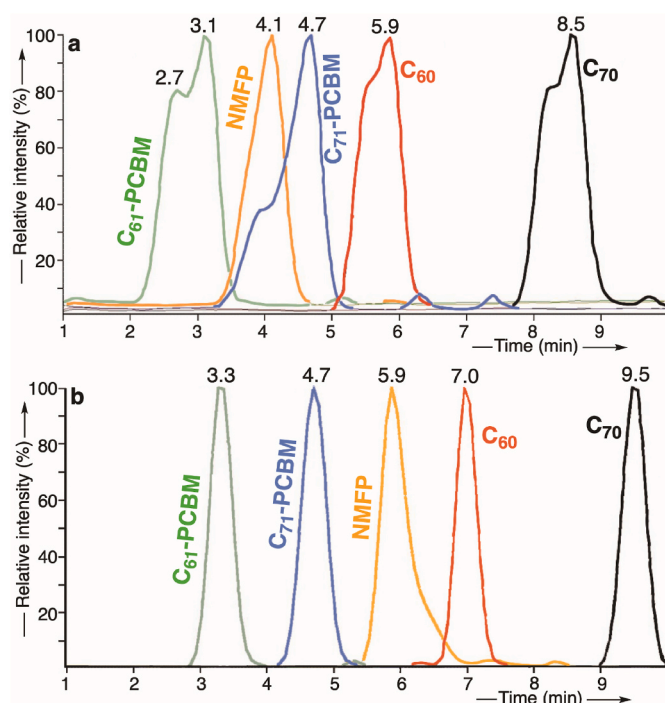


Fig. 4. LC-ESI(-)-MS Single Ion Monitoring Chromatogram of a C₆₀, C₇₀, C₆₁-PCBM, C₇₁-PCBM and NMFP mixture obtained a) on the biphenyl column (150 × 3 mm, 2.7 μm; Restek Corp., Bellefonte, PA, USA) and b) on the C₁₈ column (150 × 3 mm, 3 μm; Macherey-Nagel GmbH&Co., Düren, Germany) (relative intensities for each compound). For the biphenyl column (a), the LC gradient was 30% toluene/70% acetonitrile initial to 45% toluene/55% acetonitrile final in 14 min. For the C₁₈ column (b), the LC gradient was 40% toluene/60% acetonitrile initial (maintained for 1 min) to final 60% toluene/40% acetonitrile in 10 min. The LC flow rate was set at 0.7 mL min⁻¹.

Table 2

Number of theoretical plates (N) for each fullerene peak and in-between peak resolution (R) measured by LC-ESI(-)-MS with the biphenyl and C₁₈ columns.

N (LC-ESI(-)-MS)	Biphenyl	C ₁₈	C ₁₈ /Biphenyl Ratio
C ₆₁ -PCBM	157	832	5.3
C ₇₁ -PCBM	204	1724	8.5
NMFP	402	838	2.1
C ₆₀	870	5122	5.9
C ₇₀	1611	8190	5.1
R (LC-ESI(-)-MS)	Biphenyl	C ₁₈	C ₁₈ /Biphenyl ratio
Peaks # 1–2	1.4	3.0	2.2
Peaks # 2–3	0.4	1.9	5.1
Peaks # 3–4	1.2	2.0	1.6
Peaks # 4–5	3.5	6.3	1.8

same conclusion, as illustrated in Figs. S8 and S9. Broadly, the peak of C₇₀ was more intense and more focused with methanol in the eluent, while the shape of the other peaks was generally better with acetonitrile. The elution order of NMFP and C₇₁-PCBM was inverted when methanol was used instead of acetonitrile in the eluent.

Acetonitrile in combination with toluene was finally chosen as the optimal mobile phase. The same LC method was used for LC-ESI-MS/MS, LC-APCI-MS/MS and LC-APPI-MS/MS analyses (reconstituted Total Ion Chromatogram in Fig. S8). The five fullerenes studied in this work elute in the following order: first C₆₁-PCBM, then C₇₁-PCBM, NMFP, C₆₀ and last C₇₀.

3.3. Comparison between LC-ESI-MS/MS, LC-APCI-MS/MS and LC-APPI-MS/MS

The results obtained from direct infusion of fullerene standard solutions (see Section 3.1.) were used to select the most intense MS/MS transitions, which were then employed for the LC-MS/MS methods. Those transitions are summarized for each LC-MS/MS analysis in Tables S1–S3. Apart from variations of optimal collision energies, all transitions were shown to be identical in ESI, APCI and APPI modes in terms of precursor and product ions.

The main method parameter which we wanted to compare in this section was the LOQ. The goal was to obtain the most sensitive detection, given that fullerenes are generally found at very low concentrations. Instrumental LOQ obtained with each ionization source are reported in Table S4.

It appears clearly that ESI is less sensitive than APCI and APPI from two to ten times, with all instrumental LOQ equal or higher to 100 pg.

LOQ obtained with APCI and APPI are much closer for most compounds, even equal for the two pristine fullerenes C₆₀ and C₇₀ (100 and 40 pg, respectively). The only major difference observed concerns NMFP, for which the instrumental LOQ is ten times lower with APPI (10 vs. 100 pg), due to an enhanced response of NMFP with APPI in the positive ionization mode, as stated in Section 3.1.3 (Fig. S5). These results are in good agreement with those of Li et al. who only studied pristine fullerenes, and of Astefanei et al. who only presented results about the functionalized derivatives [28,34]. Both works resulted in a broad rating like APPI > APCI > ESI. However, the study of Astefanei et al. was based on negative ionization of NMFP [28], while we rather used positive ionization which we found more efficient for this compound.

In addition to its higher LOQs, the LC-ESI-MS/MS method was not selected because of some additional issues. First, the linearity of its calibration range (from the LOQ to 500 μg L⁻¹) was poorer than the linearity obtained using APCI and APPI sources. With the use of a quadratic fit and 1/X weighting, correlation coefficients (R²) were frequently lower than 0.99. The same conclusion was previously drawn by Li et al. [34].

Moreover, the intra-day repeatability was also lower, with relative standard deviations (RSD) often exceeding 15% (comparative values for the LC-APPI-MS/MS method are given in Section 3.5.). This suggests that the ionization processes involved in APCI and APPI are more robust for fullerenes than electrospray electron capture ionization which would be more sensitive to parameters such as ambient moisture or traces of water in the solvent for example.

LC-APCI-MS/MS and LC-APPI-MS/MS methods exhibited similar performances, but APPI was finally selected as the most appropriate source due to its low LOQs and its known specificity towards non-polar compounds [35].

3.4. Extraction recoveries

In this section are reported the recoveries of the method, including Pressurized Liquid Extraction yields and other steps of the sample preparation such as concentration of the extract.

The whole experiment designed for the determination of method recoveries was presented in Section 2.5. Those recoveries are represented for each of the five analytes at each of the five spiking levels in Fig. 5.

Preliminary tests showed that using toluene was necessary to dissolve and extract fullerenes from solid samples such as airborne PM collected on GFFs. In the present PLE method, acetonitrile was added (1:1, v/v) so that this extraction method could be applicable for multi-class extraction and analysis. This method was particularly optimized for the analysis of Polycyclic Aromatic Compounds [45].

The mean recoveries over all the spiking levels ranged from 75% for NMFP to 95% for C₆₀. Compared to studies determining fullerenes in

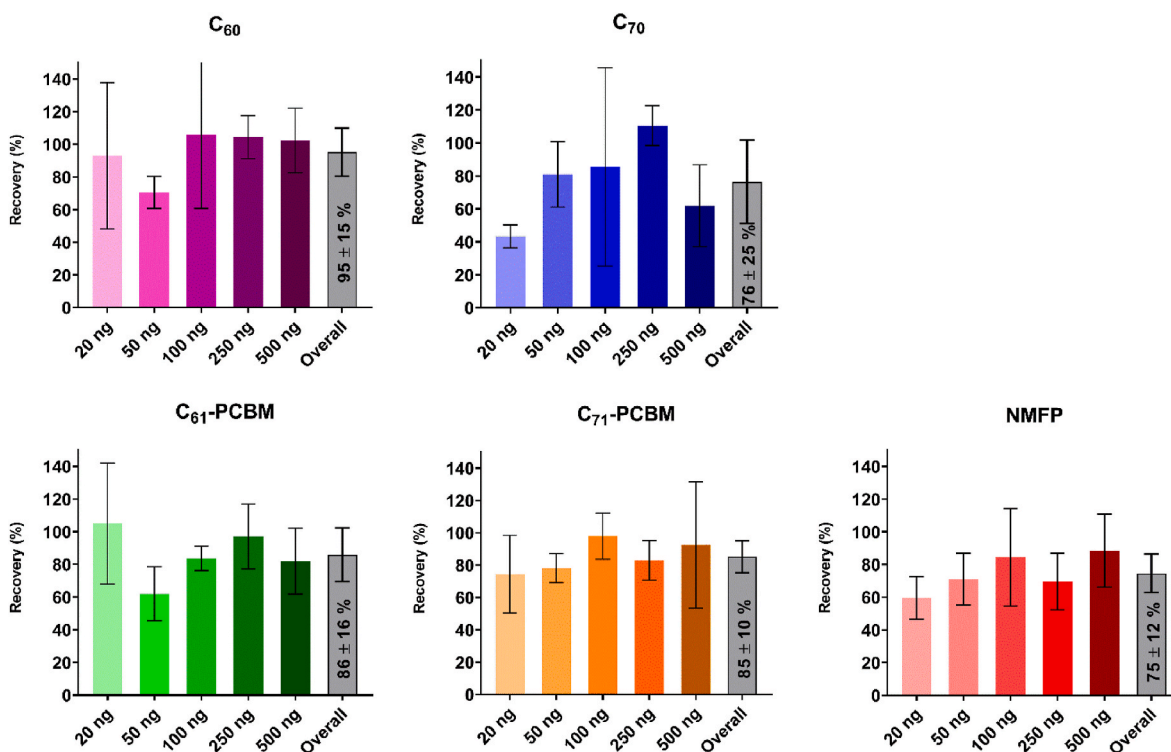


Fig. 5. Recovery (in %) of the five fullerenes spiked at five different levels on GFFs using Pressurized Liquid Extraction.

aerosol samples, these recoveries are higher than those previously published using ultrasonication of filters in toluene: from 24 to 53% in the study of Benn et al., from 50 to 56% in that of Encinas and Gómez, and from 60 to 71% in the study of Sanchís et al. [2,26,27]. This demonstrates the higher performance of our method, and more generally the interest of using PLE for the extraction of fullerenes from aerosol, as was already shown by Shareef et al. for soil samples and by Astefanei et al. for sediment samples [28,32].

It is likely that the extraction yields could have been even higher with the use of 100% toluene as PLE solvent, but in this case the extraction method would have been less flexible towards other classes of organic contaminants, and the enhancement of extraction recovery thanks to the PLE method enables to compensate for this limited recovery loss due to the addition of another solvent (i.e. acetonitrile in this work).

RSD over the five spiking levels ranged from $\pm 12\%$ for C₇₁-PCBM to $\pm 33\%$ for C₇₀. For the five studied fullerenes, a general trend is a lower recovery and/or a higher variability at the lowest spiking levels (see Fig. 5). It is generally expected that method reproducibility is lower at low concentrations. Indeed, small losses of analytes can lead to high relative variations of the recovery when the spiked amount is low.

In the case of fullerenes, glassware sorption seems to be the major pathway for analyte loss during sample preparation. This issue was already raised multiple times in the literature. Núñez et al. used plastic vials to avoid sorption on glass materials [35], whereas Sanchís et al. advised for the use of silica glassware to avoid sorption on plastic materials [9,37]. In our method, the extraction was performed in ASE stainless steel cells, which is an efficient material to reduce sorption [32]. However, the extract was then recovered in glass bottles, and the concentration of the extract was performed in glass tubes. To minimize losses, glassware was rinsed with toluene at each transfer from a container to another, following the advice of Farré et al. [23]. Still, it could be insufficient to ensure a total recovery of fullerenes, and other issues such as photodegradation during the concentration of the extract should be kept in mind as possible sources of analyte loss [9].

Hence, even though some of our matrix recoveries exhibit substantial

standard deviations (SD) inside triplicates (e.g. $93 \pm 45\%$ for C₆₀ fortified at 20 ng), the overall recoveries and RSD are in a satisfying range for the validation of the analytical method, considering the great analytical complexity related to these analytes [13].

3.5. LC-APPI-MS/MS method validation

The LC-APPI-MS/MS method was selected as the ideal method compared to LC-APCI-MS/MS and LC-ESI-MS/MS methods (see Section 3.3).

The first step of the method validation consisted in confirming the accuracy of the calibration. To this purpose, eight calibration curves were built from eight different sets of ten fullerene standard solutions in toluene, with concentrations ranging from 0.5 to 500 $\mu\text{g L}^{-1}$. At most, there could be ten validated calibration levels in a series, but for each series, six was considered as the minimal acceptable number of validated calibration levels. All quadratic calibration curves showed very good fit with all $R^2 > 0.99$, most of them being even > 0.999 . The calibration curves for each targeted fullerene are shown in Figs. S10–S14 of the Supplement.

For a more complete validation of the calibration range, the latter was evaluated in terms of precision. Deviations between theoretical concentrations and recalculated concentrations of standard solutions obtained from the calibration curve were determined. The average and SD of this deviation for each compound at each calibration point over the eight series were reported (see Fig. S15). The acceptability criterion was set at $\pm 30\%$ for all levels, except for the LOQ at which $\pm 60\%$ was allowed due to commonly higher deviations at the lowest calibration levels [46,47]. For C₇₀, C₆₁-PCBM and NMFP, deviations are indeed below the $\pm 30\%$ tolerance for all levels above the LOQ, and below the $\pm 60\%$ tolerance at the LOQ. In the case of C₆₀ and C₇₁-PCBM, the “mean - SD” value at the 10 $\mu\text{g L}^{-1}$ level is below the -30% tolerance (Fig. S15).

For quality assurance purposes, all calibration curves used for the analysis of real-world samples or recovery validation extracts were checked against these precision criteria and re-injected or even re-prepared if exceeding the maximal acceptable deviations.

External calibration was performed because we could not purchase any commercial isotope-labeled fullerene such as the $^{13}\text{C}_{60}$ used in previous studies. The supplier of $^{13}\text{C}_{60}$ reported in those studies is no longer in business [5,24,33]. We found no other chemical capable of acting as internal standard for our method because of the uniqueness of fullerene behavior. It would have to match, even approximately, the hydrophobicity of fullerenes for the sample preparation steps, their reversed-phase retention in non-aqueous conditions, and their ionization properties. We argue in favor of more availability of ^{13}C -labeled fullerenes, either pristine or functionalized. This would enhance method performance and facilitate method validation.

Intra-day repeatability was validated at a low-range level ($10\ \mu\text{g L}^{-1}$) and at a high-range level ($200\ \mu\text{g L}^{-1}$) over five replicate injections performed during the same day. For all five fullerenes, RSD were below the maximal accepted value of 15%. They ranged from 3.1% for C_{60} at $10\ \mu\text{g L}^{-1}$ to 10.6% for C_{70} at $10\ \mu\text{g L}^{-1}$ (see Table 3).

Inter-day repeatability was validated similarly to intra-day repeatability, at the same low-range and high-range levels, with five replicates injected over five consecutive days. For all five fullerenes, RSD were below the maximal accepted value of 15%. They ranged from 5.4% for C_{61} -PCBM at $10\ \mu\text{g L}^{-1}$ to 12.6% for C_{71} -PCBM at $10\ \mu\text{g L}^{-1}$ (see Table 3).

Together with repeatability, accuracy of the method was evaluated at $10\ \mu\text{g L}^{-1}$ and $200\ \mu\text{g L}^{-1}$. The intra-day accuracy of the developed LC-MS/MS method was between 83.5% for C_{60} at $10\ \mu\text{g L}^{-1}$ and 98.6% for NMFP at $200\ \mu\text{g L}^{-1}$. The inter-day accuracy varied between 81.0% for NMFP at $10\ \mu\text{g L}^{-1}$ and 103.4% for C_{61} -PCBM at $200\ \mu\text{g L}^{-1}$ (see Table 3).

Limits of quantification (LOQ) were defined according to several criteria reported in Section 2.5. In addition to the common “S/N > 10” rule, we specified that the LOQ level should be validated against precision with a deviation within the $\pm 60\%$ range defined previously. Moreover, the validated LOQ should be below the mean +SD of concentrations measured when integrating the background noise signal of solvent blanks.

For C_{60} , C_{61} -PCBM, C_{71} -PCBM and NMFP, all criteria were met at the same calibration level which could then be validated as LOQ. For C_{70} however, while $1\ \mu\text{g L}^{-1}$ could have been considered as LOQ regarding S/N and precision of the calibration, the measured mean +SD of blank injections often exceeded $1\ \mu\text{g L}^{-1}$, therefore only $2\ \mu\text{g L}^{-1}$ could be the successfully validated LOQ.

All validated instrumental LOQs are reported in Table S4. In Table S5, these instrumental LOQs are associated with their corresponding method LOQ for each environmental sample of this work.

Generally, our instrumental LOQs were quite higher than those

Table 3

Intra-day and inter-day repeatability calculated as relative standard deviations on the measured concentration, and accuracy calculated as ratio of measured concentration over theoretical concentration of Quality Control solutions, for each fullerene over five replicates (%).

	Level	C_{60}	C_{70}	C_{61} -PCBM	C_{71} -PCBM	NMFP
Intra-day accuracy (%)	$10\ \mu\text{g L}^{-1}$	83.5%	92.0%	90.5%	97.5%	86.1%
	$200\ \mu\text{g L}^{-1}$	85.2%	86.9%	98.3%	87.2%	98.6%
Intra-day repeatability (%)	$10\ \mu\text{g L}^{-1}$	3.1%	10.6%	6.0%	12.6%	5.4%
	$200\ \mu\text{g L}^{-1}$	6.6%	7.4%	7.7%	9.0%	4.8%
Inter-day accuracy (%)	$10\ \mu\text{g L}^{-1}$	85.8%	87.7%	94.0%	101.7%	81.0%
	$200\ \mu\text{g L}^{-1}$	90.4%	88.0%	103.4%	91.4%	91.9%
Inter-day repeatability (%)	$10\ \mu\text{g L}^{-1}$	6.6%	8.2%	5.4%	12.6%	8.9%
	$200\ \mu\text{g L}^{-1}$	5.7%	7.9%	8.4%	5.7%	11.6%

obtained in studies involving more recent and powerful MS instrumentation [10,37,44], but were still lower than those from several other studies [23,26,32].

Finally, this analytical method was validated in terms of recovery. All results regarding method recoveries were already presented in Section 3.4. and in Fig. 5.

The common standards in environmental analytical chemistry state that recoveries should be between 70% and 120% of the spiked amount, with less than 20% SD [46,47].

In our case, not all experiments could meet this standard. Indeed, as discussed previously, no internal standard was available. This complicated severely our method validation, because the sample preparation issues mentioned in Section 3.4. could not be corrected using an internal standard. However, on average, over the five concentration levels tested, all compounds showed mean recoveries between 75% and 95%, and four of them had SD < 20%. This was a satisfying outcome given the results previously published in the literature and the present analytical challenge (see Section 3.4.).

3.6. Application to environmental samples

A first series of three weekly medium-volume PM_{10} samples was collected during daytime only. Therefore, the method quantification limits for these samples were from $1.2\ \text{pg m}^{-3}$ for NMFP to pg m^{-3} for C_{60} (see Table S5).

A second series of three weekly medium-volume PM_{10} samples was collected during a whole week, including day and night. The resulting sampled volume was thus higher, and the associated method LOQs were from $0.65\ \text{pg m}^{-3}$ for NMFP to $6.5\ \text{pg m}^{-3}$ for C_{60} (see Table S5).

Among all these samples, none exhibited any fullerene concentration above the quantification limit.

This means that in the parking lot aerosol samples, C_{60} and C_{70} occur at far lower levels than those reported by Sanchis et al. in aerosols from the Mediterranean Sea (from 33 to $233\ 800\ \text{pg m}^{-3}$, with median values of $60\ \text{pg m}^{-3}$ for C_{60} and $480\ \text{pg m}^{-3}$ for C_{70}) [27]. In this same work, no functionalized fullerenes were detected in the environmental samples, like in the case of our samples [27]. C_{60} is also present at lower concentrations than the highest detected C_{60} concentrations in the study of Encinas and Gómez (about $10\ \text{pg m}^{-3}$). However, in this latter study, 26 samples out of 36 showed no trace of C_{60} above the method LOQ which was $2\ \text{ng}$ per filter, i.e. around $2\ \text{pg m}^{-3}$ according to the disclosed information about the sampling [26]. The high prevalence of the parking lot PM samples below $2\ \text{pg m}^{-3}$ is in line with our results. Finally, Benn et al. reported C_{60} concentrations in the range of $2\text{--}3\ \text{pg m}^{-3}$ below their lowest calibration point, which are also inferior to our LOQ for this compound.

The absence of any fullerene detection in the airborne PM samples from the present study is therefore consistent with the previously disclosed fullerene levels in PM, below the LOQ of this method.

The amount of collected settled dust varied highly depending on the household, from $0.17\ \text{g}$ to $1.02\ \text{g}$. Hence, the method LOQ varied from 0.24 to $1.5\ \text{ng g}^{-1}$ for NMFP and from 2.4 to $15\ \text{ng g}^{-1}$ for C_{60} (see Table S5).

To our knowledge, this study is the first ever dealing with the determination of fullerenes in household settled dust. All analyzed samples did not show the presence of detectable fullerenes, suggesting that household dust is not a major pathway leading to substantial human exposure to fullerenes. However, other similar studies would still be relevant and very useful for a more comprehensive assessment of fullerenes occurrence in similar indoor environments.

A Diesel Particulate Matter Standard Reference Material (SRM 2975) was also analyzed with the view of studying the presence of fullerenes in such a combustion exhaust sample. From the Diesel PM studied in this work, two distinct sample preparations were performed, where 29.5 and $37.1\ \text{mg}$ of this SRM were deposited on a GFF. The lowest resulting method LOQ was from $6.8\ \text{ng g}^{-1}$ for NMFP to $68\ \text{ng g}^{-1}$ for C_{60} (see

Table S5).

In this sample as well, no fullerene concentration above the LOQ could be measured.

The most comprehensive previous investigation of fullerenes in combustion exhausts was performed by Tiwari et al. [5]. These authors only targeted C₆₀, which was measured at 130 ng g⁻¹ and 41 µg g⁻¹ in two exhaust particulates samples of a diesel-powered generator operated at 90% load, but they did not detect it in the exhaust particulates of a diesel-powered generator operated at 0% load. They also quantified C₆₀ at 44 ng g⁻¹ and 120 ng g⁻¹ in two samples of exhaust plume particles from a gasoline-powered lawn mower [5]. As we did not detect this compound in SRM 2975, the C₆₀ concentration in this material should therefore be inferior to the values measured by Tiwari et al. in exhaust plumes particles.

More generally, Tiwari et al. underlined the possibility of a high variability in combustion conditions which could affect the release of fullerenes and also their extractability from the particles. Moreover, when C₆₀ was investigated in four ash samples, it could not be detected, and a ¹³C₆₀ surrogate spiked onto these samples could not be recovered [5]. Similarly, in the study of Benn et al., no C₆₀ could be detected in the analyzed soot samples [2]. This could mean that soot and ash are very sorptive matrices from which fullerenes could not be extracted, explaining why we were not able to quantify fullerenes in SRM 2975.

As stated by Tiwari et al. [5], combustion processes can still be considered as major incidental sources of fullerenes, hence their monitoring in such kind of samples, as well as in environmental samples (outdoor aerosols, indoor dust, soil, sediment, water, etc.), should be further undertaken in future studies.

A more comprehensive and reliable environmental assessment of fullerenes would then require available internal standards such as isotope-labeled fullerenes and even certified reference materials to enable inter-laboratory studies.

The use of new generation mass spectrometers with higher sensitivities could also help to reach lower limits of quantification, so that very low levels of fullerenes could be detected to gather more data about their environmental occurrence.

4. Conclusion

In this article, a method development for the environmental assessment of C₆₀ and C₇₀ fullerenes and some of their functionalized derivatives was described. Several parameters of the analytical method were optimized and comparison of two liquid chromatography stationary phases for a more efficient separation of fullerenes, as well as three ionization sources for the enhancement of the detection sensitivity, were reported. In the end, a C₁₈ stationary phase was preferred to a biphenyl stationary phase, and APPI was selected as the optimal source as compared to ESI and APCI sources. Furthermore, MS detection was enhanced with the implementation of pseudo-MRM.

A major improvement about fullerenes determination in aerosols was the higher extraction recoveries reached (from 75% to 95%) as compared to the recoveries reported in the literature, thanks to Pressurized Liquid Extraction which was used instead of Ultrasound Assisted Extraction.

Even though we managed to validate our analytical method according to linearity, precision, repeatability and LOQ, some RSD on recoveries were fairly high. This issue is likely due, in part at least, to the absence of commercially available isotope-labeled standard, and we argue for a better availability of such internal standards and surrogates in order to improve method performance for target analysis of fullerenes in the environment.

No quantification of fullerenes could be obtained from the environmental samples investigated here, due to their absence or too low concentration. Therefore, our results do not raise a particular health or environmental risk associated to fullerenes in such matrices. However, in view of the literature on this topic, we would like our analytical

development to serve as a step forward towards more environmental assessment of these compounds in a wide range of matrices (water, sediment, soil, atmosphere, indoor dust, biomonitoring in living organisms, etc.) and additional compounds among polar derivatives of fullerenes.

CRedit authorship contribution statement

Mathieu Galmiche: Conceptualization, Methodology, Validation, Investigation, Writing – original draft. **Eden Esslinger:** Validation, Investigation. **Olivier Delhomme:** Conceptualization, Writing – review & editing. **Philippe Schaeffer:** Resources, Visualization, Writing – review & editing. **Estelle Motsch:** Investigation. **Emmanuelle Leize-Wagner:** Resources, Writing – review & editing, Supervision. **Yannis-Nicolas François:** Conceptualization, Resources, Writing – review & editing, Project administration. **Maurice Millet:** Conceptualization, Resources, Writing – review & editing, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijms.2023.117072>.

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