A method for the determination of fullerenes in soil and sediment matrices using ultra-high performance liquid chromatography coupled with heated electrospray quadrupole time of flight mass spectrometry

Carboni, A.; Helmus, R.; Parsons, J.R.; Kalbitz, K.; de Voogt, P.

DOI
10.1016/j.chroma.2016.01.035

Publication date
2016

Document Version
Final published version

Published in
Journal of Chromatography A

Citation for published version (APA):
Carboni, A., Helmus, R., Parsons, J. R., Kalbitz, K., & de Voogt, P. (2016). A method for the determination of fullerenes in soil and sediment matrices using ultra-high performance liquid chromatography coupled with heated electrospray quadrupole time of flight mass spectrometry. *Journal of Chromatography A*, *1433*, 123-130. https://doi.org/10.1016/j.chroma.2016.01.035

Journal of Chromatography A, 1433 (2016) 123–130

Contents lists available at ScienceDirect

Journal of Chromatography A

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

A method for the determination of fullerenes in soil and sediment matrices using ultra-high performance liquid chromatography coupled with heated electrospray quadrupole time of flight mass spectrometry

Andrea Carboni [a,*], R. Helmus [a], John R. Parsons [a], Karsten Kalbitz [b], Pim de Voogt [a,c]

[a] University of Amsterdam, Institute for Biodiversity and Ecosystem Dynamics, Science Park 904, 1098 XH Amsterdam, The Netherlands
[b] Dresden University of Technology, Soil Resources and Land Use, Pienner Str. 19, 01737 Tharandt, Germany
[c] KWR, Watercycle Research Institute, P.O. Box 1072, 3433 PE Nieuwegein, The Netherlands

ARTICLE INFO

Article history:
Received 12 October 2015
Received in revised form 10 January 2016
Accepted 12 January 2016
Available online 16 January 2016

Keywords:
Fullerenes
Environmental analysis
Matrix effect
UHPLC
HRMS

ABSTRACT

The increasing production of fullerenes likely means a release of these chemicals in the environment. Since soils and sediments are expected to act as a sink, analytical tools are needed to assess the presence of fullerenes in these matrices. In the present work, a method was developed for the determination of fullerenes at environmental relevant levels employing Ultra High Performance Liquid Chromatograph coupled with High Resolution Mass Spectrometry (UHPLC-HRMS). Chromatographic separation was achieved with a core–shell biphenyl stationary phase that provided fast analysis with complete baseline separation. Ion Booster Electro Spray Ionization (IB-ESI) resulted in higher ionization efficiency and was much less susceptible to adduct formation in comparison with standard ESI, whereas Quadrupole Time of Flight (QTOF) MS granted high resolution mass spectra used for accurate identification. The Instrumental method limits of detection (ILoD) and quantification (ILoQ) were 6 and 20 fg, respectively, for C60 and 12 and 39 fg, respectively, for C70. Matrix effects related to co-extractants were systematically investigated in soil and sediments extracts through standard addition method (SAM) and monitoring the signal response during the chromatographic run of these samples. Consequently, minor chromatographic modifications were necessary for the analysis of matrices with high organic carbon content. The method limit of detection (MLoD)ranged from 84 pg/kg to 335 pg/kg, whereas limit of quantification (MLoQ) ranged from 279 pg/kg to 1.1 ng/kg. Furthermore, the method was successfully applied for the analysis of functionalized fullerenes (i.e. methanofullerenes). To the best of our knowledge, this is the first analytical method for the analysis of fullerenes in soils and sediments that employ core–shell biphenyl stationary phase as well as IB-ESI-QTOF MS hyphenated with UHPLC.

1. Introduction

Since their discovery [1], fullerenes and especially C60 have been subject of a large number of studies mostly focused on their innovative physical–chemical properties and possible applications. Fullerenes are already used in cosmetics (as antioxidants) and in photovoltaics (as electron acceptors) and possible fields of application include electronics and optics as well as biomedical engineering [2–6]. Furthermore, the possibility of derivatization of their structure e.g. with surface functionalization [7] as well as the encapsulation of other chemical species [8], is likely to increases the range of applications of these chemicals. Precise data regarding the manufactured amounts are missing but the worldwide production was estimated in tens of tons per year and is expected to increase in the near future [9,10]. This will inevitably mean a release into the environment and there are several concerns about their possible accumulation and toxic effects. Besides, fullerenes can also be naturally produced (e.g. in energetic events such as volcanic eruptions [11] or flame generation [12]) but data are limited and often contradictory [13]. Thus, the presence of fullerenes in the environment may be due to both natural and anthropogenic origins as well as resulting from transformation of engineered nanoparticles

---

[*] Corresponding author at: University of Amsterdam, Institute for Biodiversity and Ecosystem Dynamics, Earth Surface Science Research Group, Science Park 904, 1098 XH Amsterdam, The Netherlands.
E-mail address: A.carboni@uva.nl (A. Carboni).

http://dx.doi.org/10.1016/j.chroma.2016.01.035
0021-9673/

(e.g. fullerene derivatives). In this context, fullerenes are nowadays considered as emerging contaminants and analytical methods are needed for both environmental and toxicological assessment. However, only in the last few years have environmental monitoring and modeling studies been carried out and the knowledge is still limited. Data modeled based on the estimated production suggested that the concentrations (Predicted Environmental Concentration, PEC) of C60 in the environment should range between parts per billion and parts per trillion with higher concentrations in soil and sediments than water and air [14]. Thus, soil may act as a sink for the fullerenes whose source may be direct release and atmospheric deposition but also water transport (in the form of nC60 and/or associated with organic matter) and application of biosolids [15]. In the last few years, a number of studies have been published focused on the extraction of fullerenes and methods are now available that allow the extraction of these chemicals in a large number of matrices with acceptable if not excellent recoveries [e.g. 16–18]. High Performance Liquid Chromatography (HPLC) in combination with UV–vis and/or mass spectrometry (MS) is the most widely used technique for the determination of fullerenes and issues related to the chromatographic separation of the fullerenes have been recently reviewed elsewhere [19,20]. Briefly, mobile phases usually consisted of toluene as a main eluent in combination with a more polar solvent, such as acetonitrile or methanol, whereas stationary phases commonly employed include standard octadecyl silica (ODS) as well as functionalized silica. However, these methods are often time consuming and seldom optimized for the analysis of complex environmental samples [e.g. 16,18]. In this context, core–shell biphenyl is a novel stationary phase, never applied for the analysis of fullerenes, which may provide a retention mechanism similar to that of the pyrenylpropyl silica [21]. Regarding the detection, although UV–vis showed promising performance [22] for the determination of fullerenes in environmental matrices [18], its relatively low sensitivity and its lack of selectivity in comparison with mass spectrometric detection make it unsuitable for the analysis of the low concentrations expected in the environment. Thus, mass spectrometry is considered as the detection method of choice and low as well as high resolution methods have been described. Instruments commonly employed make use of triple quadrupole [23,24], LTQ Orbitrap [16,25–27], and FTICR [26] coupled with HPLC systems and Quadrupole time of flight (QTOF) MS with direct injection [28]. Atmospheric pressure ionization (API) methods were usually employed in negative mode. Specifically, the use of ESI in negative mode allowed the detection of fullerenes in soils and water at relatively low concentrations [16,23] but APPI was recently proposed as a better option due to the higher sensitivity and less complex mass spectra [24,26,27]. Heated interfaces such as H-ESI were seldom applied and showed efficiency of ionization generally higher than that of standard ESI, but lower in comparison with other systems such as atmospheric pressure photo (APPI) and chemical (APCI) ionizaton [24,26]. In this context, the Ion Booster-ESI (IB-ESI) consists of a modified ESI source that was recently employed in the screening of drugs in serum, showing better performance than standard ESI [29,30]. The additional soft voltage and vaporizer temperature are expected to enhance the ionization of the fullerenes in comparison with standard ESI, i.e. to improve the sensitivity. The analysis of fullerenes in the environment is challenging because of the low concentrations expected and only recently monitoring studies have highlighted the presence of these chemicals in environmental samples (e.g. soil [23,31], sediments [23,24], surface waters [24,26], wastewater [27,32] and air [33]). Furthermore, the complexity of some of the matrices that have to be analyzed (e.g. WWTPs, soil) is an issue during both the extraction and analysis of fullerenes [34]. The presence of co-extractants, with special regard to organic matter content, could hamper a correct quantification and it may have hindered the detection in other recent works focused on soil [17] and water [25]. To date a few studies addressed the challenges of a clean-up procedure [23] or accounted for the matrix effect during the detection and quantification of fullerenes. Although fortification (i.e. standard addition) of environmental samples under investigation was recently included in monitoring studies, this issue was never systematically approached and analytical methodologies such as matrix matched calibration were recommended [24,31]. Thus, in the present work we developed an analytical method that is specifically aimed for the analysis of fullerenes in complex environmental samples such as soil and sediment. The method included the use of a novel stationary phase, i.e. biphenyl functionalized silica, and of a heated interface (IB-ESI) in combination with high resolution mass spectrometry (QTOF-MS).

2. Materials and methods

2.1. Reagents and chemicals

Toluene, methanol, isopropanol and acetonitrile (Biosolve, Valkenswaard, The Netherlands) were all analytical grade (LC–MS). The mobile phase modifiers tested were acetic acid (Merck, Darmstadt, Germany), formic acid (Biosolve, Valkenswaard, The Netherlands) and ammonium acetate (Sigma–Aldrich, Zwijndrecht, The Netherlands) whereas sodium hydroxide was purchased by Merck KGaA (Amsterdam, The Netherlands). Fullerenes C60 (CAS: 99685-96-8) and C70 (CAS: 115383-22-7) were purchased from Sigma–Aldrich (Zwijndrecht, The Netherlands). Stock solutions of the individual fullerenes were prepared in toluene, at a concentration of 5 mg/L, and placed on an orbital shaker (Laboshake orbital shaker, Gerhardt, Königswinter, Germany) in the dark overnight. Further solutions needed for the experiments, including mixtures of the fullerenes, were obtained by dilution of the stock solutions, stored at 4 °C in the dark and sonicated for 2 min before use (Bransonic 12, Branson, Danbury CT, United states). The analyses were performed using an UHPLC system (Nexera, Shimadzu, Den Bosch, The Netherlands) equipped with a binary pump, autosampler and column oven. Retention of the chemicals was achieved with a core–shell Kinetex 2.6 μm biphenyl 100 Å chromatographic column (Phenomenex, Utrecht, The Netherlands) consisting of a biphenyl stationary phase. The UHPLC system was coupled to a high resolution Quadrupole-Time of Flight mass spectrometer (Q-TOF; maXis 4 G upgraded with HD collision cell, Bruker Daltonics, Wormer, The Netherlands) equipped with either an electrospray (ESI) or an Ion Booster electro spray (IB-ESI) ionization sources (Bruker Daltonics, Wormer, The Netherlands). High purity nitrogen was supplied by a N2 generator (Avilo, Dirksland, The Netherlands) and used for ionization and collision gas.

2.2. Analytical method settings

Internal mass calibration was performed automatically during each analysis in order to assure good mass accuracy for all the samples independently of the total analysis time. This was achieved at the beginning of the analysis (0.1–0.4 min) by infusing a 2 mM sodium acetate solution in a water isopropanol mixture (1:1, v:v), with a loop injection of 20 μl and a loop rinse of 20 μl. A temperature gradient was employed for the ionization source because of the different optimal temperatures needed for the analysis of the mass calibration solution and the analytes under investigation. Specifically, the temperature was set at 325 °C at the beginning of the run and was gradually increased after one minute in order to reach 450 °C at 2 min (Fig. S1, supplementary information). The sodium acetate cluster provided ten points of calibration ranging from m/z 387 to m/z 1207 (Table S2, Supplementary information) of which at

Table 1
Properties of the soil and sediment samples included in the present study.

Matrix	Substrate	N[%]	S[%]	Inorg C[%]	Org C[%]
1	Loess soil	0.11	0.02	n.d.	0.90
2	Urban park soil	0.18	0.03	n.d.	2.68
3	River clay sediments	0.14	0.03	n.d.	1.03
4	Sea clay sediments	0.21	0.06	0.64	1.69
5	Carbon-rich sandy soil	0.14	0.04	0.84	2.24
6	Dune sand	0.03	0.01	0.08	0.20

n.d. not detected.

least eight points (standard deviation ≤0.3 ppm) were taken for the mass calibration of each sample. The final chromatographic methods were optimized with regard to the methanol (eluent A) and toluene (eluent B) amounts employed and consisted of the following programs. Method A: flow rate of 400 μl, 40 s at 100% eluent A (focusing step), a linear gradient up to 50% eluent B in 0.3 min, an isocratic step of 3.50 min at 50% eluent B (elution step) and then a linear gradient of 20 s to reach 100% eluent B which was maintained for 2.5 min (cleaning step). Method B: flow rate of 600 μl starting with 40 s at 100% eluent A (focusing step), a linear gradient up to 35% eluent B in 20 s, an isocratic step of 4 min at 35% eluent B followed by a linear gradient of 1.5 min to reach 60% B (elution step), a linear gradient of 10 sec in order to reach 100% eluent B which was maintained for 2.5 min (cleaning step). The chromatograms were divided into four segments: segment 1 (from 0 min to 0.1 min) to assure the correct position of the loop, segment 2 (from 0.1 min to 0.4 min) dedicated to the mass calibration, segment 3 (from 0.4 min to 3.4 min in method A and 4.4 min in method B) for the SRM analysis of C60 (m/z 720.0005) and segment 4 (from 3.4 min to 7.6 min for method A and from 4.4 min to 9.6 min for method B) for the SRM analysis of C70 (m/z 840.0005). After the mass calibration was achieved, the LC flow was diverted to the waste (0.5 min) in order to avoid the exposure of the interface to the more polar co-extractants that eluted at the beginning of the chromatogram. The LC flow was redirected to the MS (1.5 min in method A and 2.5 min in method B) to allow the interface to equilibrate before the elution of the chemicals and then switched again to waste after the elution of the analytes (4.5 min in method A and 6.5 min in method B). Fullerenes ESI and IB-ESI spectra were obtained by infusing both individual and mixture standard solutions in toluene:methanol (1:1,v:v) at varying concentrations. The optimized working conditions for the ESI and IB-ESI in negative mode were: capillary voltage 1000 V, end plate offset −400 V, charging voltage 300 V, nebulizer gas 4.1, dry gas 3.0 l/min and dry heater 200 °C. Further settings optimized for the MS analysis were: funnel radio frequency (RF) at 325.0 Vpp (voltage point to point), Multipole RF at 300.0 Vpp, collision cell RF at 1600.0 Vpp, transfer time at 50 μs and prepulse storage time at 25 μs. The mass range analyzed in MS1 was 300–2000 m/z whereas the spectra rate frequency was set at 2 Hz during all the analysis performed. Collision energy for MS/MS was set at 100 V whereas the isolation width was 8.00 m/z. The present settings allowed a mass resolving power up to 80,000 (0.03 m/z FWHM). The $[M]^{-\bullet}$ and $[M+1]^{-\bullet}$ abundances relative to C60 and C70 were determined by analysing a standard solution at 500 ng/l repeatedly with both method A (n = 7) and method B (n = 7). More details about the molecular ions and isotopic clusters during the detection are provided in the discussion section. The Bruker Compass 1.7 software was employed in both the data collection and data processing.

2.3. Samples collection and treatment

In Table 1, the properties of the soil and sediment samples included in the present study are reported. The Loess soil (matrix 1) was collected from an agricultural field in South Limburg, The Netherlands (50°53′58″N, 5°53′16″E). Matrix 2 consisted of an urban park soil sample collected in Vondelpark, Amsterdam, The Netherlands (52.3580°N, 4.8680°E). River clay and sea clay (matrix 3 and matrix 4 respectively) were available from the collection of the Earth Surface Science research group at the University of Amsterdam. The sandy top soil rich in organic carbon (matrix 5) was collected in Flevopark, Amsterdam, The Netherlands (52.3611°N, 4.9492°E) whereas the dune sand (matrix 6) was sampled in a natural park near Castricum, The Netherlands (52.5500°N, 4.6667°E). The samples were placed in a freezer at −20 °C overnight and lyophilized with a Scanvac Coolsafe freeze–dryer (Labogene, Lynge, Denmark) in order to remove traces of water. The dried samples were finely ground with an agate mortar and sieved. The total carbon, sulfur and nitrogen contents of the dried soil samples were measured using a Vario EL Cube (Elementar, Hanau, Germany). The inorganic carbon content was determined as reported by Wesemael [35] as follows: 1 g of soil was weighed into a 250 ml Erlenmeyer provided with a silica gel lid and an excess of HCl 4 M was added. Control samples consisted of 0.250 g of $CaCO_3$. The samples were weighed again after 24 h of shaking at 60 rpm (Laboshake orbital shaker, Gerhardt, Königswinter, Germany) and the inorganic carbon content was calculated accordingly. The soil and sediment samples were extracted with the protocol already reported by our group [18] with minor modifications, i.e. the temperature was set at 20 ± 2 °C as recommended by Perez et al. [17]. Briefly, 10–15 g of each sample underwent two cycles of extraction with toluene with each cycle consisting of a combination of sonication for 20 min with a ultrasonic bath (Bransonic 12, Branson, Danbury CT, United states) and shaking for 2 h (Laboshake orbital shaker, Gerhardt, Königswinter, Germany). The extracts were concentrated evaporating the solvent under a gentle nitrogen flow (enrichment factor 20x) and stored in a refrigerator at 4 °C in the dark. A repeatability test for the extraction procedure was achieved by spiking and extracting the fullerenes into matrix 6 at the concentration of 100 ng/kg (n = 8) as reported by Carboni et al. [18].

2.4. Sample analysis

Methanol (25% in volume) was added to each sample and the analyses were performed with an injection volume of 10 μl. External calibration curves were obtained by analyzing standard solutions at concentrations ranging from 1 to 500 ng/l and quantification was based on chromatographic peak areas. Instrumental method limits of quantifications (ILOQs) were assumed as the lowest measured concentration in the linear range with a deviation less than 30% of the theoretical concentration injected [36]. The instrumental method limit of detection (ILoD) was calculated as 3/10 of the ILoQ observed. The method limits of detection (MLoDs) and quantification (MLoQs) in soil and sediment samples were extrapolated from the signal-to-noise ratio observed in the chromatograms assuming a 70% recovery of extraction [18]. In case of absence of matrix effect and noise, these values were assumed to be equal to the ILoD and ILoQ. The criteria used for the identification of the analytes were: 1 the expected chromatographic retention time (±0.2 min), 2 a mass accuracy threshold (≤5 ppm) and 3 an isotopic pattern fit threshold (≤50 mSigma), where mSigma represents the goodness of fit (the smaller the better) between the measured and theoretical isotopic pattern [37]. For the standard addition method (SAM) experiments, each matrix was spiked with both C60 and C70 fullerenes at increasing concentrations (25, 50, 100, 250 and 500 ng/l) in order to obtain concentration versus response curves (Supplementary information). Standard solutions at the same concentration were also analyzed to allow a comparison in terms of linearity, slope and intercept. In addition, the ion suppression/enhancement due to the matrix effect was evaluated in terms of response recovery by comparing the peak areas obtained for C60 and C70 spiked into the extracts with those obtained analyzing standard solutions at the same concentrations. The matrix effect (ME) was further investigated by continuously infusing a fullerenes standard (1 μg/l in toluene) into the MS source while injecting the matrices extracts. This allowed us to monitor the detector response during the chromatographic run, i.e. the matrix suppression/enhancement during the analysis. For this purpose a 5 ml glass chromatographic syringe and syringe pumps (KD Scientific, Holliston MA, United States) operating at 0.2 ml/h were employed.

3. Results and discussion

3.1. UHPLC-HRMS

The first aim of the present work was to assess the performance of the biphenyl stationary phase selected for the separation of the fullerenes. When method A was applied, C60 and C70 eluted at 3.1 min and 3.8 min respectively (Fig. 1) with fully resolved (Rs > 1.5) and highly symmetric chromatographic peaks. Similar to what is observed with columns that are specifically designed for the separation of fullerenes, e.g. the pyrenylpropyl functionalized silica (Buckyprep), with biphenyl groups the main interactions are expected to be the pi–pi-interactions and pi-stacking between the aromatic rings in the fullerenes and those of the stationary phase. However, as suggested by Núñez et al. [26] the size of the buckyballs may play a role in the retention mechanism. The biphenyl stationary phase was an optimal compromise for selectivity and rapidity when compared with other materials that were usually employed providing higher retention than the standard octadecyl silica and shorter analysis time in comparison with the pyrenylpropyl functionalized silica. It must be noticed that the analysis time may be shortened when using a shorter column with smaller particle size (i.e. 1.7 μm) that is currently available on the market. The "focusing step" i.e. the application of a 100% methanol at the beginning of the chromatographic run, resulted in a better peak shape and sensitivity and may be explained with the fact that the analytes accumulated at the start of the column (focused) until a stronger solvent was provided. The use of ESI-HRMS was already investigated by some of the authors of the present work [16]. In those studies, a consistent formation of adducts during the ionization process was reported, consisting of both oxidized products ($[M+O]^{-\bullet}$, $[M+OH]^{-\bullet}$) and methanol and/or toluene adducts (mostly $[M+OCH_3]^{-\bullet}$ and $[M+C_7H_7O_2]^{-\bullet}$). In contrast, IB-ESI-HRMS provided mass spectra that were dominated by the isotope cluster of the molecular ions with an only minor abundance of $[M+16]^{-\bullet}$ adducts as shown in Fig. 2. Similar to what has already been reported in other studies, the method was more sensitive for C60 than C70 (a factor of 2) at all the concentrations tested. Furthermore, in the present study the IB-ESI signal response was several orders of magnitudes higher than that of ESI stand-alone for both C60 and C70 (data not shown). Thus, ESI was not taken into consideration for further analysis. In the present work, sodium acetate was selected for internal mass calibration because it can stand higher temperatures in comparison with other commonly applied calibration solutions (e.g. sodium formate). This allowed the determination of the accurate masses of fullerenes with errors lower than 5 ppm. In comparison with APPI, where toluene was used as a dopant to improve the ionization efficiency, ESI analysis required the presence of methanol during ionization that can be added post-column as already described [16]. The post column addition was not needed in the present work since methanol was one of the constituents in the mobile phase during the elution of the chemicals. It must be noted that fragmentation of the fullerenes could not be obtained even at extremely high collision energies (>100 eV). Thus, although multiple reaction monitoring (MRM) mode was applied in the present work, the MS2 analysis corresponded to $[M]^{-\bullet}$à$[M]^{-\bullet}$ transitions (pseudo-MRM) and the MS2 spectra resembled the MS1 ones (Fig. 2b and e) in which adducts and background masses (e.g. m/z 731.4124 in Fig. 2a) were removed. Núñez et al., reported higher than expected relative abundances of the isotopic cluster ions when using APPI with special regard to the m/z $[M+1]^{-\bullet}$ and an enhanced effect on larger fullerenes [26]. In their work they demonstrated that the addition of hydrogenated products to the peaks corresponding to the ^{13}C natural abundance may account for the phenomenon. Furthermore, Emke et al., showed that the unexpected isotopic distribution was due to the presence of methanol during the ionization, a phenomenon not observed when toluene was the only solvent employed [27]. If highly resolving systems, such as FTICR MS, are not available, anomalous isotopic abundances may hinder a qualitative detection of fullerenes. Especially when analyzing non-functionalized structures, such as C60 and C70, the lack of MS/MS fragmentation implies that the main identification points must be provided by the accurate mass and cluster distribution. In the present work the C60 spectra presented molecular ions at m/z $[M]^{-\bullet}$, $[M+1]^{-\bullet}$, $[M+2]^{-\bullet}$ and $[M+3]^{-\bullet}$ consistent with the theoretical isotopic patterns (Fig. 1c) and those obtained by ESI, whereas C70 showed slightly higher than expected m/z $[M+1]^{-\bullet}$ abundance in MS1 Fig. (2a and f). In detail, C60 presented a $[M+1]^{-\bullet}/[M]^{-\bullet}$ ratio of 0.64 ± 0.05 (versus the expected value of 0.65) whereas C70 presented a ratio of 0.77 ± 0.03 (versus the 0.74 expected).

Identification points in the present work were provided by the chromatographic retention times and the accurate mass detection and isotopic clusters in both MS1 and MS2. Calibration curves, obtained analyzing standard solutions of the fullerenes, showed high linearity ($R^2 > 0.998$) and the ILoD and ILoQ were 0.6 and 2 ng/l, respectively, for C60 and 1.2 and 4 ng/l, respectively, for C70. These results indicate that the IB-ESI-HRMS system allowed the determination of fullerenes at concentrations lower than those of other ESI-MS and heated ESI methods applied [25] and similar to those of APPI-MS methods recently developed [24]. Finally, the method was also tested for the determination of fullerene derivatives to assess the possibility to include functionalized structures investigated in our previous works [16,18]. The method was suitable for the analysis of the methanofullerenes that were completely resolved (Rs > 1.5) from the related non functionalized structures at the beginning of the chromatogram (Fig. S5, Supplementary information).

3.2. Matrix effects

The method we developed was tested for the determination of fullerenes spiked into extracts of soils and sediments. Soil and sediment samples were chosen in order to represent a range of possible environmental matrices and textures (e.g. clay, sand) and the selection included samples with varying inorganic and organic carbon contents whereas nitrogen and sulfur concentrations were similar between the samples (Table 1). After the extraction, the sample extracts were spiked with fullerenes in order to investigate the analysis of the chemicals in presence of the co-extractants. The use of fullerene standards in organic solvent for the spiking of samples has often been the subject of debate since it is not expected to represent the real conditions at which the chemicals are present in the environment [18,38]. However, in the present work, standard solutions in toluene were directly added to the toluene extracts, thus the results hereby presented will help in understanding the behavior of fullerenes that are already present in the extracts with no effect of the extraction procedure applied to the original sample. According to a previous study, the texture of the soil samples should be of a minor concern when analyzing fullerenes [18]. On

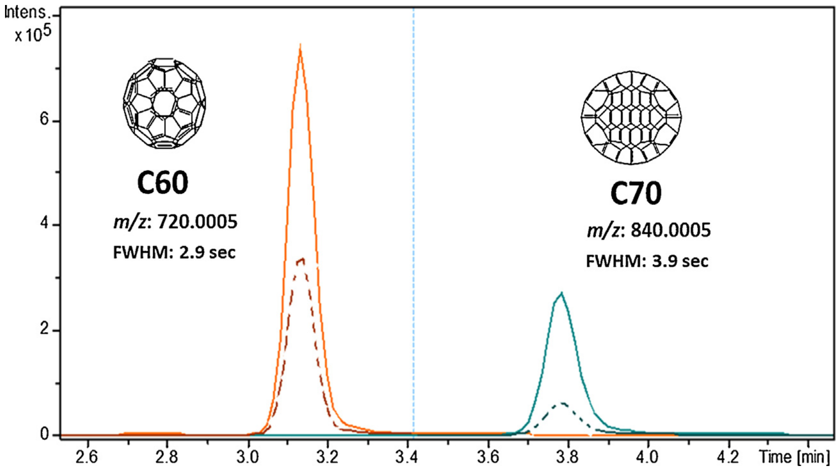

Fig. 1. Chromatographic separation of C60 (left) and C70 (right) standard at 500 ng/l. The continuous and dashed lines represent the MS1 and MS2 chromatograms respectively.

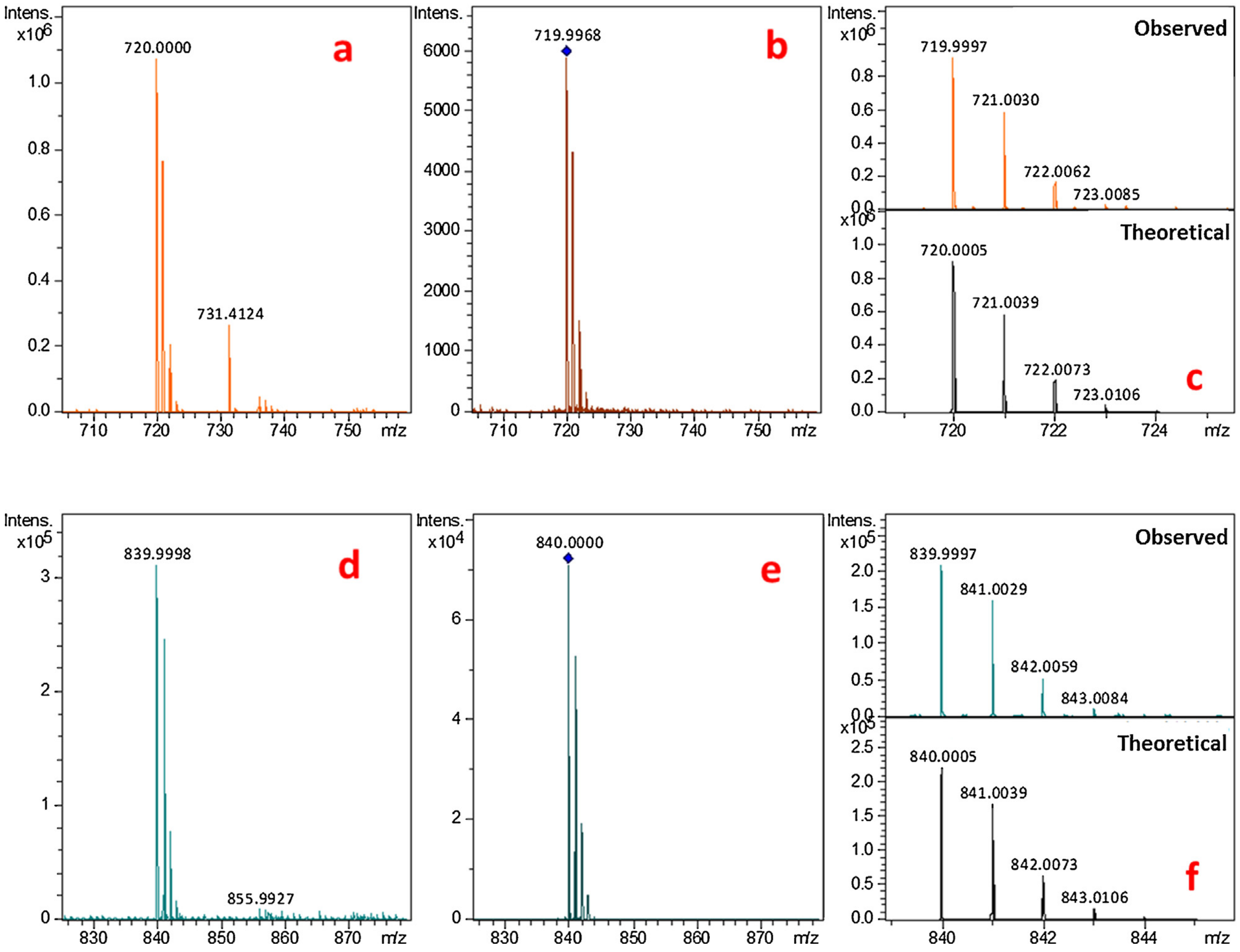

Fig. 2. (IB)ESI-QTOF mass spectra of C60 and C70 MS (a and d respectively) and MS2 (b and e respectively). In Fig. 2c (C60) and 2f (C70) a comparison between measured (top) and expected (bottom) *m/z* clusters.

the contrary, the presence of carbon and specifically organic matter in the samples is expected to play a major role, e.g. by hindering the detection and quantification of the chemicals (matrix effects, ME). The ME may either cause the enhancement or the decrease of the method's accuracy and sensitivity. It must be noted that during the sample preparation, an addition of methanol at percentages higher than 25% of the final volume caused precipitation in the extracts with the highest carbon content (matrix 2). This was due to the large content of non-polar co-extractants resulting from the extraction with 100% toluene. Therefore, although the initial optimization was achieved with a toluene:methanol ratio of 1:1 (v:v), the final composition of each sample injected into the UHPLC was a toluene:methanol ratio of 3:1 (v:v). The high collision energy values (100 V) applied during the analysis led to a "Background clean-up",

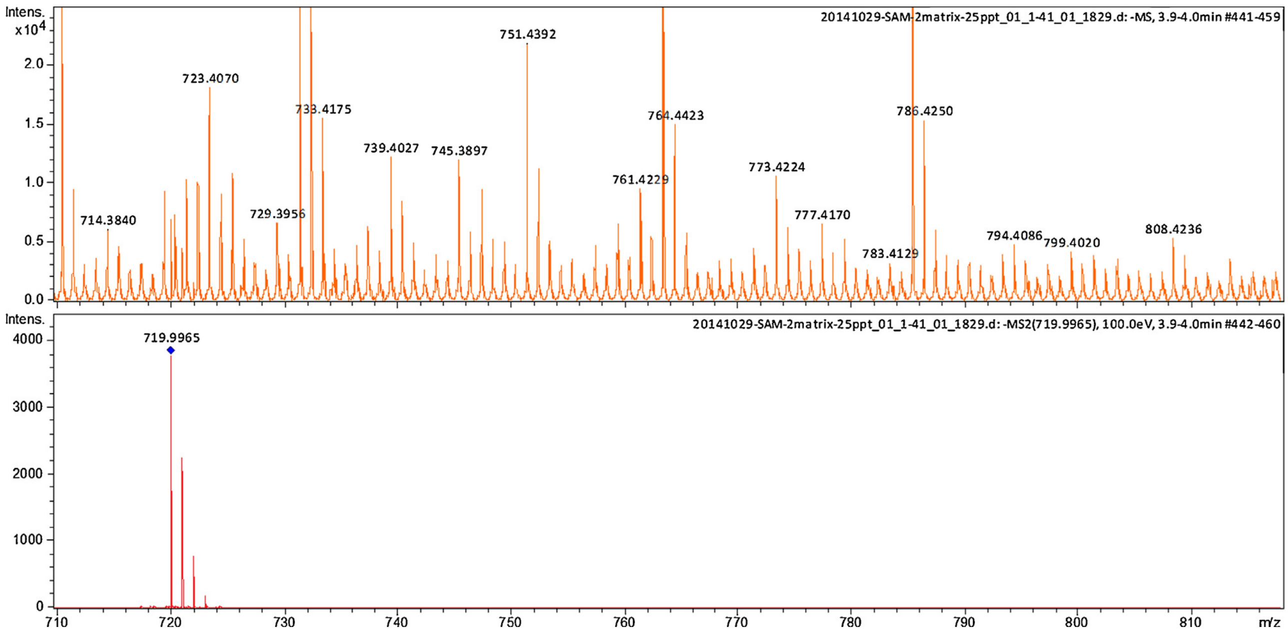

Fig. 3. MS1 (top) and MS2 (bottom) spectra of C60 spiked at the concentration of 25 ng/l in presence of high organic carbon content (matrix 2).

Table 2
Results of the standard addition experiment for C60 and C70 with the use of the method A (left) and method B (right)[a]. For matrix descriptions, see Section 2.3.

C60	Method A				Method B			
	MS1		MS2		MS1		MS2	
	r^2	%Rec	r^2	%Rec	r^2	%Rec	r^2	%Rec
Matrix 1	0.997	122 ± 15	0.993	123 ± 13	0.998	116 ± 14	0.999	120 ± 20
Matrix 2	0.998	6 ± 3	0.999	13 ± 10	1.000	67 ± 6	1.000	69 ± 9
Matrix 2 dil 10x	0.989	141 + 16	0.997	106 ± 5	0.998	123 ± 6	1.000	110 ± 6
Matrix 3	1.000	102 ± 6	0.997	105 ± 5	0.999	98 ± 6	1.000	108 ± 3
Matrix 4	0.999	112 ± 7	0.997	115 ± 7	1.000	110 ± 7	1.000	118 ± 9
Matrix 5	1.000	119 ± 6	0.997	108 ± 5	0.999	82 ± 8	0.981	101 ± 18
Matrix 6	0.999	113 ± 1	0.998	113 ± 3	0.999	107 ± 5	0.999	115 ± 8
C70								
Matrix 1	0.995	105 ± 8	0.998	101 ± 8	1.000	96 ± 5	0.999	107 ± 7
Matrix 2	0.997	29 ± 7	0.998	21 ± 1	0.998	45 ± 11	1.000	39 ± 3
Matrix 2 dil 10x	1.000	151 ± 5	1.000	133 ± 7	0.999	116 ± 11	1.000	108 ± 7
Matrix 3	0.999	100 ± 3	1.000	98 ± 6	1.000	88 ± 4	1.000	102 ± 4
Matrix 4	0.999	108 ± 9	0.999	105 ± 6	1.000	97 ± 3	1.000	106 ± 6
Matrix 5	1.000	113 ± 3	1.000	100 ± 4	1.000	77 ± 2	0.999	82 ± 4
Matrix 6	0.999	101 ± 4	1.000	101 ± 3	1.000	99 ± 3	1.000	104 ± 4

[a] r^2 = regression coefficient of the standard addition lines; Rec% = recovery; Matrix 2 dil 10x = matrix 2 diluted ten times; method A and method B differ in the mobile phase composition during the isocratic step (50:50,MeOH:Tol and 65:35,MeOH, respectively) and flow rate (400 and 600 ul/min, respectively).

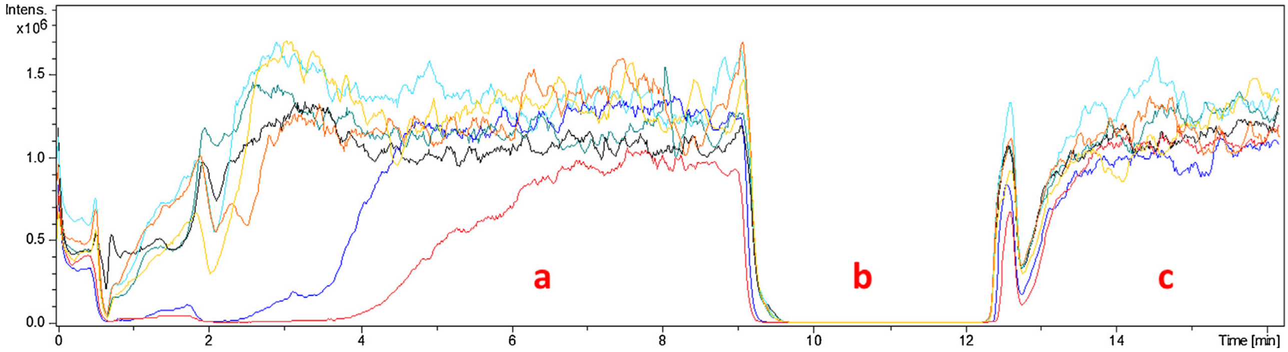

Fig. 4. Response of C60 post-column infused directly into the IB-ESI-MS during chromatographic runs of the soil or sediments extracts using a gradient elution of methanol:toluene (method A). The black line represents a pure solvent injection in comparison with matrix 1 (light blue), matrix 2 (red), matrix 3 (orange), matrix 4 (yellow), matrix 5 (blue) and matrix 6 (green). The start of section b corresponds to the moment the gradient reaches 100% toluene, at the start of c the gradient program returns to initial solvent composition (see Section 2.2). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

i.e. the degradation of the co-extractants, with resulting extremely clean MS2 spectra. This allowed the unambiguous determination of the fullerenes in all the samples, even at concentrations as low as 25 ng/l (Fig. 3). The results of the SAM experiments are reported in Table 2 and more comprehensively in Supplementary information (Table S6). The response was linear ($R^2 > 0.99$) for both C60 and C70 in all the matrices tested (with the only exception of C60 in matrix 5 when analyzed in MS2), and no significant difference ($P > 0.05$, F-test for covariances) was observed between MS1 and MS2 measurements in the same matrix. It should be noted that, although relative small intercepts were observed, these were not significantly different from zero ($P > 0.05$). However, a significant difference ($P < 0.05$) was present when comparing the response recovery of different samples. Indeed, although the majority of the matrices showed recoveries ranging from 100% to 122%, i.e. featuring either no ME or a slight enhancement of the response, a large signal suppression (specifically 6% recovery for C60 and 29% recovery for C70) was found in the matrix with the highest carbon content (matrix 2). This effect was not observed in other matrices with a high organic carbon content (matrix 4 and matrix 5) indicating that the percentage of organic matter in the samples was not the only property determining the ion suppression and that other feature such as its chemical composition may play a role. However, when the extract from matrix 2 was diluted ten times (matrix 2 dil 10x in Table 2), an enhancement of the response was found, which is consistent with that observed in matrices that have a lower organic carbon content. This suggests that although the chemical composition of the co-extractants played a role in the ion suppression, their concentration was likely the main reason for the response loss observed in the extract of matrix 2. The MLoDs were extrapolated assuming a 70% recovery of extraction from the matrices (see materials and methods) and ranged from 84 pg/kg for C60 to 168 pg/kg for C70 whereas MLoQs ranged from 279 pg/kg for C60 to 559 pg/kg for C70 (method A). However, in the case of matrix 2, which showed a strong matrix effect, these values were a factor of 30 higher for C60 and a factor of 5 higher for C70. The smaller suppression of the C70 signal in comparison with C60 may be explained by the fact that, being more retained during the analysis, C70 eluted in a position of the chromatogram that was less affected by the more polar co-extractants. The matrix effect due to the co-extractants in the samples was monitored by injecting the matrices while infusing the fullerenes in the MS system. The signal suppression was due to the more polar fraction of the co-extractants, eluting at the beginning of the chromatographic run where a higher percentage of methanol was present. In particular, as shown in Fig. 4, the matrix 2 (red line) suffered a large signal suppression at the beginning of the analysis (Fig. 4a) whereas signals from other matrices with a high organic carbon content were not suppressed (matrix 4 in yellow) or were affected to a lesser extent (matrix 5 blue line).

Flushing with toluene (100%) (Fig. 4b), in order to remove the more non-polar fraction that may remain in the column and affect the next injections, ultimately restored the response to the maximum levels as shown in Fig. 4c. During the flushing stage of the chromatogram (b), however, no signal was present because of the absence of any methanol and consequent lack of ionization of the fullerenes (Fig. 4b) in the ESI. Matrices 2 and 5 were selected for further experiments aimed to improve the analytical method for the determination of fullerenes in complex matrices with mitigation of the ion suppression. Three mobile phase modifiers: acetic acid, ammonium acetate and formic acid, were tested at varying concentrations with the purpose of attenuating the ion suppression. However, since the addition of formic acid resulted in a near or complete loss of the response whereas both acetic acid and ammonium acetate suppressed the signal to a large extent, these modifiers were not taken into account for further analysis. The large suppression may be due to the susceptibility of the ESI to buffer salts in comparison with APPI as mentioned by Núñez et al. [26]. Also the modification of the mobile-phase composition with increasing amounts of isopropanol and acetonitrile did not lead to any improvement in recovering the response and, in the case of acetonitrile, caused the formation of adducts at the m/z $[M+40]^{-\bullet}$ for both C60 and C70. Subsequently, the modification of the mobile phase composition was tested with regards to the methanol:toluene ratio. In detail, weakening the eluotropicity, i.e. increasing the amount of methanol, delayed the elution of the fullerenes and enhanced the separation of interfering compounds in the biphenyl column, with a general improvement of the signal response (Supplementary information, Fig. S7). Optimal conditions were found at 65:35 (methanol:toluene, v:v) whereas further addition of methanol (e.g. 70:30, v:v) increased the retention time of the fullerenes and broadened the peak, with no further enhancement of the response. Finally, the original method (method A) was modified accordingly by changing the methanol:toluene ratio in the isocratic step from 50:50 (method A) to 65:35 (method B). Further optimization of the methods consisted in the addition of a washing step (100% toluene) at the end of the analysis and to divert the LC flow to the waste in order to prevent the exposure of the source to the co-extractants at the beginning of the analysis and provide higher sensitivity and better peak shape of the fullerenes. In the resulting method B, the retention times of the fullerenes were delayed, with C60 and C70 eluting at 4 and 6 min respectively (Supplementary information, Fig. S8) and the ILOD and ILOQ were 12 and 39 fg, respectively, for C60 and 23 and 78 fg, respectively, for C70. When method B was applied, a considerable improvement in the response recovery of C60 was observed for the matrix 2 (from 6% to 67%) and minor but consistent improvements were obtained for C70 (from 29 to 45%), as reported in Table 2. This corresponded to a MLoD and MLoQ of 168 pg/kg and 559 ng/kg, respectively, for C60 and 335 pg/kg and 1.1 ng/kg, respectively, for C70. Furthermore, no significant difference ($P < 0.05$) was found when comparing the performances of the two methods for the analysis of the other matrices. Eventually, we recommend the use of method B (10 min) for monitoring studies, since it can provide a more reliable measurement of the fullerenes in a wider range of matrices. In particular, method B should be employed in the analysis of soils and sediments as well as wastewater samples that can consist of complex matrices possessing high organic carbon contents. However, method A can provide a faster (8 min) and more sensitive analysis for the determination of fullerenes in standard solutions and less complex samples such as sandy soils with low organic carbon contents.

4. Conclusions

In the present work we developed a sensitive and fast method for the determination of fullerenes in soils and sediments. The biphenyl-coated stationary LC phase provides an adequate retention and represents a valid alternative to other stationary phase materials currently applied with promising perspectives for the analysis of complex mixtures including functionalized fullerenes. We also showed how heated ESI, i.e. the IB-ESI employed in the present work, can grant an ionization efficiency higher than that of standard ESI and comparable to that of other recently developed APPI methods, with the advantage of producing isotopic patterns that resemble the theoretical ones. In general, the high resolution and the clarity of the mass spectra allowed an unambiguous determination of the fullerenes in all the samples under investigation, without the need of an additional clean up, and at low concentrations that are environmentally relevant. The matrix effect due to the presence of co-extractants was investigated and the method is robust and flexible with regard to the analysis of very complex matrices such as soils having high organic carbon contents. However, the matrix effect and specifically the ion suppression/enhancement during the analysis remains an issue and the use of internal standards (e.g. isotopically labeled), if available, as well as matrix matched calibration are recommended for a more precise determination. Due to its favorable qualitative and quantitative features, the method developed in the present study is a valid tool for the monitoring of fullerenes in soils and sediments and for the study of the fate of these novel contaminants at environmentally relevant concentrations.

Acknowledgements

This work is funded by NanoNextNL, a micro and nanotechnology consortium of the Government of The Netherlands and 130 partners, and by the Joint Research Program of the Dutch Water Utilities (BTO). The authors thank Joke Westerveld, Leo Hoitinga and Chiara Cerli for technical assistance and Manuel Jaramillo Garcia for the help in the sample collection and selection.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2016.01.035.

References

[1] H.J. Kroto, J.R. Heath, S.C. O'Brien, R.F. Curl, R.E. Smalley, C60: Buckminsterfullerene, Nature 318 (1985) 162–163.
[2] D.M. Guldi, N. Martin, Fullerenes: from synthesis to optoelectronic properties, Kluwer Academic Publishers, Norwell MA, 2002.
[3] Y. Kim, S. Cook, S.M. Tuladhar, S.A. Choulis, J. Nelson, J. Durrant, D.C. Bradley, M. Giles, I. McCulloch, C. Ha, M. Ree, A strong regioregularity effect in self-organizing conjugated polymer films and high-efficiency polythiophene: fullerene solar cells, Nat. Mater. 5 (2006) 197–203.
[4] N., Burangulov, A.P., Moravsky, Y.V., Kulikova, R.O., Loutfy, G.I., Dyachuk, Cosmetic compositions containing fullerene clusters, (2006) U.S. Patent Application 20050136079.
[5] N. Tagmatarchis, H. Shinohara, Fullerenes in medicinal chemistry and their biological applications, Mini-Rev. Med. Chem. 1 (4) (2001) 339–348.
[6] A. Maynard, E. Michelson, The Nanotechnology Consumer Products Inventory, Woodrow Wilson International Center for Scholars, 2006.
[7] J.C. Hummelen, B.W. Knight, F. Lepeq, F. Wudl, J. Yao, C.L. Wilkins, Preparation and characterization of fulleroid and methanofullerene derivatives, J. Org. Chem. 60 (1995) 532–538.
[8] Y. Chai, T. Guo, C. Jin, R.E. Haufler, L.P. Chibante Felipe, J. Fure, L. Wang, J.M. Alford, R.E. Smalley, Fullerenes with metals inside, J. Phys. Chem. 95 (20) (1991) 7564–7568.
[9] C.O. Hendren, X. Mesnard, J. Dröge, M.R. Wiesner, Estimating production data for five engineered nanomaterials as a basis for exposure assessment, Environ. Sci. Technol. 45 (2011) 2562–2569.
[10] F. Piccinno, F. Gottschalk, S. Seeger, B. Nowack, Industrial production quantities and uses of ten engineered nanomaterials in Europe and the world, J. Nanopart. Res. 14 (2012) 1109–1119.
[11] D. Heymann, L.P.F. Chibante, R.R. Brooks, W.S. Wolbach, R.E. Smalley, Fullerenes in the cretaceous-tertiary boundary-layer, Science 265 (1994) 645–647.
[12] J.B. Howard, J.T. Mc Kinnon, Y. Makarovsky, A.L. Lafleur, M.E. Johnson, Fullerenes C_{60} and C_{70} in flames, Nature 352 (1991) 139–141.
[13] J.E. Elsila, N.P. de Leon, F.L. Plows, P.R. Buseck, R.N. Zare, Extracts of impact breccia samples from Sudbury, Gardnos, and Ries impact craters and the effects of aggregation on C(60) detection, Geochim. Cosmochim. Acta 69 (11) (2005) 2891–2899.
[14] F. Gottschalk, T. Sonderer, R.W. Scholz, B. Nowack, Modeled environmental concentrations of engineered nanomaterials (TiO2, ZnO Ag. CNT, Fullerenes) for different regions, Environ. Sci. Technol. 43 (2009) 9216–9222.
[15] D.A. Navarro, R.S. Kookana, J.K. Kirby, S.M. Martin, A. Shareef, J. Du, M.J. Mc Laughlin, Behaviour of fullerenes (C60) in the terrestrial environment: potential release from biosolids-amended soils, J. Hazard. Mater. 262 (2013) 496–503.
[16] A. Kolkman, E. Emke, P.S. Bauerlein, A. Carboni, D.T. Tran, T.L. ter Laak, A.P. van Wezel, P. de Voogt, Analysis of (functionalized) fullerenes in water samples by liquid chromatography coupled to high-resolution mass spectrometry, Anal Chem. 85 (2013) 5867–5874.
[17] R.A. Perez, B. Albero, E. Miguel, J.L. Tadeo, C. Sanchez-Brunete, A rapid procedure for the determination of C60 and C70 fullerenes in soil and sediments by ultrasound-assisted extraction and HPLC-UV, Anal. Sci. 29 (2013) 533–538.
[18] A. Carboni, E. Emke, J.R. Parsons, K. Kalbitz, P. de Voogt, An analytical method for determination of fullerenes and fullerene derivatives in soil with high performance liquid chromatography and UV detection, Anal. Chim. Acta 807 (2013) 159–165.
[19] Y. Saito, H. Ohta, K. Jinno, Chromatographic separation of fullerenes, Anal. Chem. 76 (2004) 266A–272A.
[20] A. Astefanei, O. Núñez, M.T. Galceran, Fullerenes, Chemistry, Natural Sources and Technological Applications, in: S.B. Ellis (Ed.), Nova Science Publishers, New York, 2014, pp. 35–63.
[21] S. Lomas, Identifying the Increased Scope of Core-Shell Technology for HPLC and UHPLC Chromatographers, Chromatogr. Today (2015) 43–44 (Feb/Mar 2015).
[22] C. Wang, C. Shang, P. Westerhoff, Quantification of fullerene aggregate nC60 in wastewater by high-performance liquid chromatography with UV–vis spectroscopic and mass spectrometric detection, Chemosphere 80 (2010) 334–339.
[23] J. Sanchis, D. Bozovic, N.A. Al-Harbi, L.F. Silva, M. Farre, D. Barcelo, Quantitative trace analysis of fullerenes in river sediment from Spain and soils from Saudi Arabia, Anal. Bioanal. Chem. 405 (2013) 5915–5923.
[24] A. Astefanei, O. Núñez, M.T. Galceran, Analysis of C60-fullerene derivatives and pristine fullerenes in environmental samples by ultrahigh performance liquid chromatography–atmospheric pressure photoionization-mass spectrometry, J. Chromatogr. A 1365 (2014) 61–71.
[25] A.P. van Wezel, V. Morinière, E. Emke, T. ter Laak, A.C. Hogenboom, Quantifying summed fullerene nC60 and related transformation products in water using LC LTQ Orbitrap MS and application to environmental samples, Environ. Int. 37 (2011) 1063–1067.
[26] O. Núñez, H. Gallart-Ayala, C.P. Martins, E. Moyano, M.T. Galceran, Atmospheric pressure photoionization mass spectrometry of fullerenes, Anal. Chem. 84 (2012) 5316–5326.
[27] E. Emke, J. Sanchís, M. Farré, Determination of several fullerenes in sewage water by LCHR-MS using atmospheric pressure photoionisation, Environ. Sci. Nano (2015), http://dx.doi.org/10.1039/C4EN00133H (accepted).
[28] L. Song, A.D. Wellman, J. Huifang, Adcock, Electron capture atmospheric pressure photoionization mass spectrometry: analysis of fullerenes, perfluorinated compounds, and pentafluorobenzyl derivatives, Rapid Commun. mass Spectrom. 21 (2007) 1343–1351.
[29] J. Kempf, J. Traber, V. Auwarter, L.M. Huppertz, 'Psychotropics caught in a trap'—Adopting a screening approach to specific needs, Forensic Sci. Int. 243 (2014) 84–89.
[30] L.M. Huppertz, S. Kneisel, J. Auwarter, Kempf, A comprehensive library-based, automated screening procedure for 46 synthetic cannabinoids in serum employing liquid chromatography-quadrupole ion trap mass spectrometry with high-temperature electrospray ionizaton, J. Mass Spectrom. 49 (2014) 117–127.
[31] J. Sanchis, L.F. Oliviera, F.B. Leão, M. Farre, D. Barcelo, Liquid chromatography–atmospheric pressure photoionization–Orbitrap analysis of fullerene aggregates on surface soils and river sediments from Santa Catarina (Brazil), Sci. Total Eniron. 505 (2015) 172–179.
[32] M. Farre, S. Perez, K. Gajda-Schrantz, V. Osorio, L. Kantiani, A. Ginebreda, D. Barcelo, First determination of C60 and C70 fullerenes and *N*-methylfulleropyrrolidine C60 on the suspended material of wastewater effluents by liquid chromatography hybrid quadrupole linear ion trap tandem mass spectrometry, J. Hydrol. 383 (2010) 44–51.
[33] J. Sanchis, N. Berrojalbiz, G. Caballero, J. Dachs, M. Farre, D. Barcelo, Occurrence of aerosol-bound fullerenes in the mediterranean sea atmosphere, Environ. Sci. Technol. 46 (2012) 1335–1343.
[34] J. Jehlicka, O. Frank, V. Hamplova, Z. Pokorna, L. Juha, Z. Bohacek, Z. Weishauptova, Low extraction recovery of fullerene from carbonaceous geological materials spiked with C60, Carbon 43 (2005) 1909–1917.
[35] J.C. van Wesemael, De bepaling van het calciumcarbonaatgehalte van gronden, Chem. Weekbl 51 (1955) 35–36.
[36] Commission Directive 200/90/EC, laying down technical specifications for chemical analysis and monitoring of water status, Off. J. Eur. Union L201 (2009) 36–38.
[37] P. Gago-Ferrero, E.L. Schymansky, A.A. Bletsou, R. Aalizadeh, J. Hollender, N.S. Thomaidis, Extended suspects and non-target strategies to characterize emerging polar organic contaminants in raw watewater with LC-HRMS/MS, Environ. Sci. Technol. 49 (2015) 12333–12341.
[38] A. Shareef, G. Li, R.S. Kookana, Quantitative determination of fullerene (C60) in soils by high performance liquid chromatography and accelerated solvent extraction, Environ. Chem. 7 (2010) 292–297.