

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27

**Analysis of C<sub>60</sub>-Fullerene Derivatives and Pristine Fullerenes in Environmental samples by Ultrahigh Performance Liquid Chromatography-Atmospheric Pressure Photoionization-Mass Spectrometry**

Alina Astefanei, Oscar Núñez \*, Maria Teresa Galceran

Department of Analytical Chemistry, University of Barcelona. Martí i Franquès 1-11, E08028 Barcelona, Spain

\* Corresponding author: Oscar Núñez

Department of Analytical Chemistry, University of Barcelona  
Martí i Franquès 1-11, E-08028, Barcelona, Spain.  
Phone: 34-93-403-3706  
Fax: 34-93-402-1233  
e-mail: oscar.nunez@ub.edu

**KEYWORDS:** C<sub>60</sub>-fullerene derivatives; Pristine fullerene; APPI; Ultrahigh performance liquid chromatography; Mass spectrometry; Environmental samples

28 **Abstract**

29 In this work, a method is proposed for the simultaneous analysis of several pristine  
30 fullerenes ( $C_{60}$ ,  $C_{70}$ ,  $C_{76}$ ,  $C_{78}$ , and  $C_{84}$ ) and three  $C_{60}$ -fullerene derivatives (N-methyl  
31 fulleropyrrolidine, [6,6]-Phenyl  $C_{61}$  butyric acid methyl ester and [6,6]-Phenyl  
32  $C_{61}$  butyric acid buthyl ester) in environmental samples. The method involves the use of  
33 ultrahigh performance liquid chromatography coupled to atmospheric pressure  
34 photoionization mass spectrometry (UHPLC-APPI-MS) and allowed the  
35 chromatographic separation in less than 4.5 minutes. The product ions from tandem  
36 mass spectrometry studies of fullerene derivatives, were characterized and the most  
37 abundant one ( $m/z$  720), corresponding to  $[C_{60}]^+$ , was selected for quantitation. Selected  
38 reaction monitoring (SRM at 0.7  $m/z$  FWHM) by acquiring two transitions using both  
39 isotopic cluster ions  $[M]^+$  and  $[M+1]^+$  as precursor ions was proposed for quantitation  
40 and confirmation purposes. For pristine fullerenes, highly-selective selected ion  
41 monitoring (H-SIM) acquisition mode by monitoring the isotopic cluster ions  $[M]^+$  and  
42  $[M+1]^+$  was used. Pressurized solvent extraction conditions were optimized in order to  
43 improve recoveries of the studied fullerene compounds from sediment samples. Values  
44 up to 87-92% for  $C_{60}$ -fullerene derivatives and lower but still acceptable, 70-80%, for  
45 pristine fullerenes were obtained. Method limits of quantitation (MLOQs) ranging from  
46 1.5  $\text{pg L}^{-1}$  to 5.5  $\text{ng L}^{-1}$  in water samples and from 0.1  $\text{ng Kg}^{-1}$  to 523  $\text{ng Kg}^{-1}$  in  
47 sediments were obtained with good precision (relative standard deviations always lower  
48 than 13%). The applicability of the developed method was evaluated by analyzing  
49 several environmental samples such as sediments and pond water and the detected  
50 levels for  $C_{60}$ -fullerene derivatives were of 0.1-2.7  $\text{ng Kg}^{-1}$  and 1.5-8.5  $\text{pg L}^{-1}$ ,  
51 respectively.  $C_{60}$  and  $C_{70}$  were the only pristine fullerenes detected in the analyzed  
52 samples (0.1-7.2  $\text{ng Kg}^{-1}$  in sediments and 9-330  $\text{pg L}^{-1}$  in water pond samples).

53

54

55

56

57

58

59

60

61

62

63

64

## 65 **1. Introduction**

66 Since their discovery [1], fullerenes have gained a prime role on the scientific  
67 scene because of their exceptional properties and versatility. Fullerenes are used in a  
68 wide range of applications such as in optical and electronic devices (polymer additives,  
69 solar cells, photovoltaic and electro-optical devices) [2], in commercial cosmetic  
70 products [3], as well as in biomedicine (antiviral, anticancer, and antioxidant agents, in  
71 drug delivery systems, or as gene carriers) [4]. Nevertheless, nowadays surface-  
72 functionalized fullerenes are produced in larger quantities than empty fullerenes in an  
73 attempt to create more biologically compatible forms [5,6]. The most common  
74 modification of fullerenes consist of the addition of esters of the butyric acid as in [6,6]-  
75 Phenyl C<sub>61</sub> butyric acid methyl ester (PCBM) and [6,6]-Phenyl C<sub>61</sub> butyric acid butyl  
76 ester (PCBB) and also the addition of azomethineylide groups to form N-  
77 methylfulleropyrrolidines. N-methyl fulleropyrrolidine (C<sub>60</sub>-pyrr) is being used as an  
78 intermediate for the synthesis of other fullerene compounds with medical and biological  
79 applications [7] and PCBM and PCBB are commonly used as electron acceptors in solar  
80 cells [8,9]. Pristine fullerenes, mostly C<sub>60</sub> and C<sub>70</sub>, are used in cosmetic products due to  
81 their antioxidant behavior [10] as well as in organic photovoltaic cells [11]. The  
82 industrial scale production (currently exceeding several tones/year) and extensive use of  
83 fullerenes and fullerene derivatives would increase human and environmental exposure  
84 although it has been reported that they also occur in a variety of natural materials [13].  
85 The potential toxicity of fullerene compounds is an intensely debated issue. Several  
86 reports showed that pristine fullerenes are non-toxic and that they have a wide spectrum  
87 of positive and unique biological activity while other works are suggesting the  
88 possibility of some adversary effects [3,14].

89 Nowadays, these compounds are being considered emerging contaminants  
90 hence, sensitive and reliable methods for their analysis in environmental samples are  
91 needed. Chromatographic separation of fullerenes has been studied since the 1990s  
92 [15,16] but their detection in environmental samples occurred only recently [17-20].  
93 This partly due to the lack of methodology for the detection and characterization of  
94 fullerene compounds, especially functionalized fullerenes, in complex matrices, *i.e.*  
95 water, soil, etc. Among the analytical methods reported in the literature for fullerene  
96 determination in environmental samples, liquid chromatography-mass spectrometry  
97 (LC-MS) is the method of choice for the quantification of low concentrations of  
98 fullerenes, and the use of MS analyzers of both low [21] and high resolution [22,23],

99 has been described. Regarding ionization sources, electrospray ionization (ESI) and  
100 atmospheric pressure chemical ionization (APCI) are the most frequently used [21,24-  
101 27] although lately, atmospheric pressure photoionization (APPI) has been proposed for  
102 the analysis of fullerenes in water samples [27-29]. Most of the reported studies focus  
103 on the analysis of pristine fullerenes, especially C<sub>60</sub> and C<sub>70</sub> [18,21,22,28-30] and only  
104 few studies describe analytical methodologies for some functionalized fullerenes  
105 [21,23,31]. Most of the works dealing with the analysis of environmental samples are  
106 devoted to the determination of pristine fullerenes, especially C<sub>60</sub> and C<sub>70</sub> in industrial  
107 effluents and surface waters [29], although recently their presence has also been  
108 reported in airborne samples from the Mediterranean Sea as well as in some soil and  
109 sediment samples [32]. Only one paper in the literature [21] reported the presence of a  
110 C<sub>60</sub>-fullerene derivative, C<sub>60</sub>-pyrr, in wastewater treatment plant effluents.

111 For the extraction of fullerenes from environmental waters, liquid-liquid  
112 extraction (LLE) [18,24,25,30] and solid phase extraction (SPE) [19,22-24] are most  
113 commonly used although recently, Chen *et al.* [29] reported ultrasound assisted  
114 dispersive liquid-liquid micro-extraction for the extraction of C<sub>60</sub>, C<sub>70</sub> and PCBM. In  
115 general LLE provides higher recoveries [20,24,30] and the addition of salts is  
116 recommended to destabilize the aqueous nC<sub>60</sub> aggregates and facilitate its transfer into  
117 the organic solvent [30,33]. Ultrasound extraction has been also proposed for the  
118 extraction of fullerenes from the water suspended solids [21,28]. Very few reports have  
119 been published regarding sediments and soil sample treatment. In general, Soxhlet and  
120 ultrasound assisted extraction with toluene [32,34-38] are the most frequently used,  
121 although pressurized liquid extraction (PLE) [32,39] has also been proposed. However,  
122 most of the works evaluate the extraction of C<sub>60</sub> or C<sub>70</sub> fullerene and very few are  
123 devoted to functionalized fullerenes [21,23,37] and generally they do not report method  
124 performance characteristics.

125 In the present work, a method based on LLE for water and PLE for sediments  
126 and analysis by UHPLC-MS(/MS) of several pristine fullerenes (C<sub>60</sub>, C<sub>70</sub>, C<sub>76</sub>, C<sub>78</sub>, and  
127 C<sub>84</sub>) and three C<sub>60</sub>-fullerene derivatives (C<sub>60</sub>-pyrr, PCBM and PCBB), which present  
128 increasing industrial applications, was developed. The behavior of the compounds in  
129 different reversed phase columns and mobile phases was studied. Mass spectrometry  
130 atmospheric pressure ionization sources (ESI, APCI and APPI) were evaluated for the  
131 ionization of C<sub>60</sub>-fullerene derivatives and the obtained spectra were discussed. Tandem  
132 mass spectrometry behavior of the derivatives was studied to characterize the product

133 ions and to select the most abundant and selective ones for quantitation and  
134 confirmation. Moreover, PLE conditions for the extraction of the compounds from  
135 sediment samples were optimized. Finally the proposed method was applied for the  
136 determination of the studied compounds in environmental samples.

137

## 138 **2. Experimental**

### 139 **2.1. Chemicals and standard solutions**

140  $C_{60}$  (CAS: 99685-96-8),  $C_{70}$  (CAS: 115383-22-7),  $C_{76}$  (CAS: 142136-39-8),  $C_{78}$   
141 (CAS: 136316-32-0),  $C_{84}$  (CAS: 135113-16-5) fullerenes and  $C_{60}$  fullerene derivatives:  
142 N-methyl fulleropyrrolidine ( $C_{60}$ -pyrr) (CAS: 151872-44-5), [6,6]-Phenyl  $C_{61}$  butyric  
143 acid methyl ester (PCBM) (CAS: 160848-22-6), and [6,6]-Phenyl  $C_{61}$  butyric acid  
144 butyl ester (PCBB) (CAS: 571177-66-7) were purchased from Sigma-Aldrich  
145 (Steinheim, Germany). The chemical structures and abbreviations of these compounds  
146 are given in Figure 1. Methanol, acetonitrile and toluene, all of them LC-MS grade,  
147 were also supplied by Sigma-Aldrich. Sodium chloride (NaCl) was purchased from  
148 Merck (Darmstadt, Germany) and diatomaceous earth sorbent (Hydromatrix) from  
149 Varian (California, USA). Stock standard solutions of fullerenes ( $10 \text{ mg kg}^{-1}$ ) were  
150 individually prepared by weight in toluene and stored at  $4^\circ\text{C}$ . Working solutions were  
151 prepared weekly by appropriate dilution of the stock standard solution in  
152 toluene/methanol (30:70 v/v).

153 Accucore C18 ( $150 \times 2.1 \text{ mm}$ ,  $2.6 \mu\text{m}$  particle size) and Hypersil GOLD C18  
154 ( $150 \times 2.1 \text{ mm}$ ,  $1.9 \mu\text{m}$  particle size) chromatographic columns were both purchased  
155 from Thermo Fisher Scientific (San José, CA, USA).

156 Nitrogen (99.98% pure) supplied by Claind Nitrogen Generator N2 FLO (Lenno,  
157 Italy) was used for the API sources, and high purity Argon (Ar1), purchased from Air  
158 Liquid (Madrid, Spain), was used as a collision-induced dissociation gas (CID gas) in  
159 the triple quadruple instrument.

160

161

### 162 **2.2. Instrumentation**

163 The chromatographic separation was carried-out using an ultrahigh performance  
164 liquid chromatography (UHPLC) system (Accela system; Thermo Fisher Scientific),

165 equipped with a quaternary pump, autosampler, and column oven. The UHPLC final  
166 separation of the studied compounds was performed with a Hypersil GOLD C18  
167 column (150 mm x 2.1 mm, 1.9  $\mu\text{m}$  particle size, Thermo Scientific). Toluene (mobile  
168 phase A) and methanol (mobile phase B) at a flow rate of 500  $\mu\text{L}/\text{min}$  (column back  
169 pressure~600 bar) was used. Gradient elution was as follows: 1 min at 30 % of solvent  
170 A, a linear gradient from 30 to 55% in 1 min, then an isocratic step of 3 min at 55 %,  
171 and returning to initial conditions in 1 min which were maintained for 5 min in order to  
172 prepare the column for the next analysis.

173 Mass spectrometric analysis was performed using a TSQ Quantum Ultra AM  
174 (Thermo Fischer Scientific) triple quadrupole instrument, equipped with hyperbolic  
175 rods that permit operation in enhanced mass resolution (isolation window: 0.1–0.04  $m/z$ ,  
176 FWHM) and with an Ion Max API source housing device (Thermo Fisher Scientific)  
177 equipped with ESI and APCI probes. The working conditions for ESI and heated-  
178 electrospray ionization (H-ESI) (Thermo Fisher Scientific) in negative mode were:  
179 electrospray voltage -3 kV, capillary temperature 350  $^{\circ}\text{C}$ , vaporizing temperature for H-  
180 ESI probe 300  $^{\circ}\text{C}$ . For negative APCI, the discharge current was 10  $\mu\text{A}$  and the  
181 vaporizing temperature 300  $^{\circ}\text{C}$ . When operating with negative APPI, the Ion Max  
182 source housing was equipped with a Syagen Photo Mate VUV light source (krypton  
183 discharge lamp, 10.0 eV) (Syagen Technology Inc., Tustin, CA, USA), and the APCI  
184 probe was used as a nebulizer-desolvation device (no corona discharge was applied).  
185 For all studies, nitrogen (purity >99.98%) was employed as sheath gas and auxiliary gas  
186 at a flow rate of 30 and 25 a.u. (arbitrary units), respectively. Tube lens offset was 180  
187 V and the ion sweep gas was kept at 0 a.u. Ion transfer tube and the vaporizer  
188 temperatures were set at 300  $^{\circ}\text{C}$ . To optimize both API source parameters and mass  
189 spectrometry conditions, infusions of fullerene standard solutions prepared in  
190 toluene/methanol (40:60,  $v/v$ ) of 1  $\text{mg L}^{-1}$ , at a flow rate of 10  $\mu\text{L min}^{-1}$ , were used.  
191 Additionally, fullerene solutions of 100  $\mu\text{g L}^{-1}$  were chromatographically analyzed by  
192 isocratic elution with toluene-methanol (40:60,  $v/v$ ), at a flow rate of 500  $\mu\text{L min}^{-1}$ , in  
193 order to evaluate the purity of the standards used. Both,  $\text{C}_{60}$ -pyrr and PCBB standards  
194 showed a 10% contamination with pristine  $\text{C}_{60}$  fullerene. This contamination has been  
195 taken into account when working with mixed standard solutions for calibration. For  
196 instrument control and data processing, Xcalibur v2.0 (Thermo FisherScientific)  
197 software was used.

198 For the acquisition, the chromatogram was divided in 2 segments, with different  
199 acquisition modes for each one of them. For C<sub>60</sub>-fullerene derivatives (segment 1: 0-2.8  
200 min), selected reaction monitoring (SRM, Q1 and Q3 at 0.7 *m/z* FWHM) by monitoring  
201 two SRM transitions (using the isotopic cluster ions [M]<sup>+</sup> and [M+1]<sup>+</sup> as precursor ions)  
202 was used (Table 1). For the analysis of pristine fullerenes (segment 2: 2.8-5 min)  
203 highly-selective selected ion monitoring (H-SIM) mode working at enhanced resolution  
204 in Q3 (0.06 *m/z* FWHM, with a mass resolving power higher than 12,500), and  
205 monitoring the isotopic cluster ions [M]<sup>+</sup> and [M+1]<sup>+</sup>, was used as previously described  
206 [28]. For H-SIM mode, the scan width was set at 0.1 *m/z* and a 10 ms scan time (1  
207 μscan) and for SRM mode 0.7 and 10 ms, respectively, were used.

208

### 209 **2.3. Sampling and sample treatment**

210 Water and sediment samples were collected from several ponds located around  
211 Barcelona's Airport (El Prat de Llobregat, Spain) and were stored at 4°C and room  
212 temperature, respectively, and in the dark for a maximum of 2 weeks.

213 For method validation, blank water and sediment samples collected from a zone  
214 located away from industrialized urban areas were used. Chromatograms of these blank  
215 samples are included in supplementary data (Figure 1S).

216

#### 217 *Water samples*

218 Liquid-liquid extraction (LLE) with toluene following a previously described  
219 procedure [30] with some modifications was used. Samples (200 mL) were processed in  
220 triplicate, without filtration, as follows: each sample was placed in a separatory funnel  
221 and 20 % (w/v) of NaCl was added prior to the addition of 20 mL of toluene. After  
222 shaking the funnel on a shaker (Selecta, Barcelona, Spain) for 10 minutes, the toluene  
223 phase was collected. This procedure was repeated 3 times for each sample. The final  
224 toluene extract (60 mL) was then evaporated to approx. 5 mL using nitrogen in a  
225 Turbovap II Concentration Workstation (Zymark Corporation, Hopkinton,  
226 Massachusetts, USA), and finally evaporated to almost dryness (0.1 mL) with a Visidry  
227 vacuum manifold (Supelco, Bellefonte PA, USA). The extract was reconstituted in  
228 toluene-methanol (30:70, v/v) solution to a final volume of 0.5 mL and transferred to an  
229 amber-glass injection vial. Finally, 10 μL of this extract was injected into the UHPLC-  
230 APPI-MS(/MS) system.

231

## 232 *Sediment samples*

233 Prior to extraction, the samples were air dried for 48 h, and then homogenized  
234 using a mortar and pestle and then sieved using a 2 mm sieve. Two different extraction  
235 methods were evaluated: ultrasound extraction and pressurized solvent extraction  
236 (PLE). For ultrasound extraction, 15g of homogenized sediment sample were dispersed  
237 in 50 mL toluene and sonicated for 1 hour in an ultrasound bath. The solution was then  
238 centrifuged at 4500 RPM for 10 min using a Selecta Centronic Centrifuge (Barcelona,  
239 Spain). The extraction was repeated three times collecting a total toluene extract of 150  
240 mL. PLE extractions were performed on an ASE 100 Accelerated Solvent Extractor  
241 system (Dionex, San Jose, CA, USA). Sediment samples were extracted as follows: 15g  
242 of homogenized sample were placed in 34 mL steel cell, filling the remaining cell  
243 volume with Hydromatrix (Varian, Harbor City, CA, USA). The cell was sealed at each  
244 end by cellulose glass fiber paper circles. Samples were extracted at 150 °C with  
245 toluene and working at a pressure of 1300 psi applying one static cycle of 10 minutes  
246 and a flush volume of 100% (100 s of purge with N<sub>2</sub>). The solvent was flushed out of  
247 the cell by N<sub>2</sub>, obtaining a final toluene extract volume of approx. 34mL.

248 Finally, toluene extracts obtained by both ultrasound extraction and PLE were  
249 evaporated and treated as previously described for the pond water samples.

250

## 251 **3. Results and discussion**

### 252 **3.1 Mass spectrometry studies**

253 In a previous study carried out in our research group [28], high ionization  
254 efficiency for pristine fullerenes was observed when using solvent-mediated negative-  
255 ion APPI, in comparison to other API sources. One of the aims of this work was to  
256 analyze three C<sub>60</sub> fullerene derivatives, whose presence in the environment is expected  
257 to rise due to their increasing use, together with the five pristine fullerenes previously  
258 studied. For this purpose, different API sources (APPI, ESI, H-ESI, and APCI) were  
259 evaluated by infusing individual standard solutions of the C<sub>60</sub> fullerene derivatives (1  
260 mg L<sup>-1</sup>) in toluene/methanol (40:60, v/v) in both, positive and negative modes. For all  
261 the evaluated API sources, higher ionization efficiency was observed in negative mode  
262 and the MS spectra was always dominated by the isotope cluster of the molecular ion  
263 [M]<sup>+</sup>, with *m/z* ions at M, M+1, M+2, and M+3, in agreement with previous reports  
264 [23,29]. A comparison of the ionization efficiency of the tested API sources (APPI, ESI,  
265 H-ESI, and APCI) is shown in Figure 2 where the signals obtained with each source for



266 the molecular ion  $[M]^\bullet$  of the three  $C_{60}$ -fullerene derivatives (PCBM, PCBB and  $C_{60}$ -  
267 pyr), normalized to the signal observed in APPI, is given. As can be seen, the  
268 sensitivity of  $C_{60}$ -fullerene derivatives is considerably higher when using solvent-  
269 mediated (toluene) APPI as previously reported for pristine fullerenes [28] and also for  
270 PCBM [29]. Among the other sources, APCI provided better results than electrospray in  
271 both, ESI and H-ESI modes, which can be explained because electron capture is favored  
272 in the APCI plasma.

273 In the full scan MS spectra of some of the fullerene derivatives obtained by  
274 infusion of the standard solutions, in addition to the isotope cluster of the molecular ion  
275  $[M]^\bullet$ , there is another cluster at  $m/z$  720 corresponding to the isotopic pattern of the  
276 molecular ion  $[M]^\bullet$  of the  $C_{60}$  buckyball structure. Figure 3a shows, as an example, the  
277 full scan MS spectrum of  $C_{60}$ -pyrr in negative APPI mode where both the molecular  
278 ions  $[M]^\bullet$  ( $m/z$  777) and the cluster at  $m/z$  720 appeared. This could be due to the  
279 presence of  $C_{60}$  fullerene residues in the commercial  $C_{60}$ -pyrr standard or to the loss of  
280 the pyrrolidine functional group by in-source fragmentation. In the LC chromatogram of  
281 the  $C_{60}$ -pyrr standard two peaks appeared, one at the retention time of  $C_{60}$ -pyrr and  
282 another one at the retention time of  $C_{60}$  fullerene indicating a contamination of  $C_{60}$ -pyrr  
283 by  $C_{60}$  (~10%). PCBB standard also contained a 10% contamination by  $C_{60}$  fullerene.  
284 Additionally, a minor contribution (5%) of an ion at  $m/z$  910 was present in the  
285 spectrum of this compound, resulting from the loss of propene ( $CH_2=CH-CH_3$ ). This ion  
286 was caused by thermal degradation and its intensity increased with the vaporizer  
287 temperature. In contrast, no contamination by  $C_{60}$  was observed for PCBM standard.

288 As previously reported [28], pristine fullerenes presented isotopic patterns with  
289 relative abundances of  $m/z$  M+1, M+2, and M+3 higher than the theoretical ones,  
290 because of the addition of hydrogen to a double bond of the fullerene structure. In  
291 contrast to this behaviour, for the  $C_{60}$ -fullerene derivatives studied in this work, the  
292 isotope patterns obtained using APPI matched with those obtained by H-ESI, APCI, and  
293 also with the theoretical ones (Figure 3a). A possible explanation for this different  
294 behavior can be the presence of the functional groups which reduces the tendency of  
295 hydrogen addition to the double bonds. In addition, it must be mentioned that when  
296 analyzing low concentration levels of  $C_{60}$  the isotope cluster matched with the  
297 theoretical pattern, in contrast to the previously reported behavior of pristine fullerenes.  
298 This fact seems to indicate that addition of hydrogen to the fullerene double bond in the  
299 ion source could be related to the fullerene concentration. In order to confirm this

300 assertion, the LC-MS spectra of C<sub>60</sub> and C<sub>70</sub> (isocratic elution with toluene-methanol,  
301 40:60, v/v) were obtained by injecting standard solutions at several concentrations (0.1-  
302 1000 µg L<sup>-1</sup>), and the variation of the ion ratio (M/M+1) with the concentration was  
303 evaluated. The results showed that the isotope pattern matched with the theoretical one  
304 at low concentration levels (<100 µg L<sup>-1</sup>) while at higher concentrations, the ion ratio  
305 M/M+1 decreased, showing abundances of M+1, M+2 and M+3 higher than the  
306 theoretical ones. A similar behavior with a decrease in the signal of the [M+1]<sup>•</sup> ion of  
307 the isotopic cluster at low concentrations (<10 µg L<sup>-1</sup>) was also observed for the  
308 fullerenes of higher size.

309 With the objective of charactering the product ions obtained in tandem mass  
310 spectrometry and selecting the most abundant and selective ones for quantitation and  
311 confirmation purposes, negative APPI MS/MS spectra of C<sub>60</sub>-fullerene derivatives were  
312 obtained by analyzing individual standard solutions (100 µg L<sup>-1</sup>) under chromatographic  
313 isocratic conditions (toluene/methanol, 40:60, v/v). In contrast to the behavior observed  
314 for pristine fullerenes which cannot be fragmented [28], C<sub>60</sub>-fullerene derivatives did  
315 show fragmentation. The product ion scan spectra of each compound at different  
316 collision energies were acquired. Table 1 shows the product ions obtained for the [M]<sup>•</sup>  
317 of each compound in the triple quadrupole MS/MS spectrum (relative abundance ≥5%),  
318 the collision energies (CE) and the corresponding ion assignment. All the compounds,  
319 showed a product ion at *m/z* 720 corresponding to the radical [C<sub>60</sub>]<sup>•</sup> which was always  
320 the most intense product ion of the spectra and whose intensity increased with the  
321 applied collision energy. No additional fragmentation occurred for C<sub>60</sub>-pyrr at any of the  
322 evaluated collision energies (10 to 100 eV). In contrast, for PCBM and PCBB, with  
323 larger functional groups, the loss of both, the propyl acid alkyl ester group,  
324 (CH<sub>2</sub>)<sub>2</sub>COO(CH)<sub>x</sub>H, and the butyl acid alkyl ester group, (CH<sub>2</sub>)<sub>3</sub>COO(CH)<sub>x</sub>H, was  
325 observed in the product ion spectra at collision energies lower than 80 eV (*m/z* 823). For  
326 PCBB, an additional product ion was observed at *m/z* 896 corresponding to the loss of  
327 the butyl group of the carboxylate ester. Moreover, the loss of propene (CH<sub>2</sub>=CH-CH<sub>3</sub>)  
328 was also observed at collision energies lower than 30 eV (*m/z* 910). The cleavage of the  
329 carboxylate ester for fullerenes with large alkyl moieties and the loss of the propyl acid  
330 alkyl ester group in the MS/MS spectra of PCBM and PCBB have been reported  
331 [23,29]. However, no information has been found in the literature about the fragment  
332 ion at *m/z* 809 corresponding to the loss of the butyl acid alkyl ester group observed in  
333 the MS/MS spectra of PCBM and PCBB, at CE < 80eV with a relative abundance from

334 5 to 10%. At high collision energies ( $[C_{60}\text{-pyrr}]^+$ : 80 eV,  $[\text{PCME}]^+$ : 100 eV,  $[\text{PCBE}]^+$ :  
335 150 eV) only the loss of the functional groups,  $(\text{CH}_2)_2\text{N-CH}_3$  for  $C_{60}\text{-pyrr}$ ,  
336  $\text{C}_6\text{H}_5(\text{CH}_2)_3\text{COOCH}_3$  for PCBM and  $\text{C}_6\text{H}_5(\text{CH}_2)_3\text{COO}(\text{CH}_2)_3\text{CH}_3$  for PCBB, occurred  
337 providing for all the compounds a product ion at  $m/z$  720 corresponding to the  $[C_{60}]^+$   
338 and this fragment ion was selected for quantitation. Hence, for  $C_{60}$ -fullerene derivatives  
339 two SRM (Q1 and Q3 at 0.7  $m/z$  FWHF) transitions selecting as precursors the ions  
340  $[M]^+$  and  $[M+1]^+$  of their isotopic cluster, and as product ions  $[M]^+$  and  $[M+1]^+$   
341 corresponding to the buckyball ( $C_{60}$ ) (Table 1) is proposed. As an example, Figure 3b  
342 shows the MS/MS spectra with the two SRM transitions selected for  $C_{60}\text{-pyrr}$ . For  
343 pristine fullerenes, H-SIM (Q3 at 0.06  $m/z$  FWHM, mass resolving power >12,500  
344 FWHM) acquisition mode by monitoring the two most intense ions of the fullerene  
345 isotope clusters ( $[M]^+$  and  $[M+1]^+$ ) was used [28].

346

### 347 **3.2. Chromatographic separation**

348 For the chromatographic separation of fullerenes C18 reversed-phase  
349 chromatography with conventional LC columns (5  $\mu\text{m}$  particle size) [18,21,24,40] and  
350 mainly with toluene/methanol or toluene/acetonitrile mixtures as mobile phase are  
351 usually used. In a previous work, the separation of pristine fullerenes was achieved  
352 using a Hypersil GOLD C18 column (150 x 2.1 mm, 1.9  $\mu\text{m}$  particle size, 175 Å) and  
353 toluene/methanol as mobile phase under isocratic elution conditions [28]. In the present  
354 work, in order to optimize the separation of pristine fullerenes and the  $C_{60}$ -fullerene  
355 derivatives, two columns, the previously used Hypersil GOLD C18 column and a core-  
356 shell Accucore C18 column (150 x 2.1 mm, 2.6  $\mu\text{m}$  particle size, 80Å) with  
357 superficially porous particles, were evaluated. As expected,  $C_{60}$ -fullerene derivatives  
358 eluted first in both C18 columns because of their higher polarity, followed by  $C_{60}$  and  
359 the other pristine fullerenes, their retention time increasing with the number of carbon  
360 atoms and, consequently with their hydrophobicity. The analysis time (retention time of  
361  $C_{84}$ ) was significantly shorter when Hypersil GOLD column was used, 4.3 min instead  
362 of 11.2 min in the Accucore C18 column, under isocratic conditions (toluene/methanol,  
363 60:40, v/v). This can be due to higher interactions between fullerenes with big size and  
364 symmetric shape and the column with the smaller porous size packaging, as previously  
365 reported [51]. Among the studied  $C_{60}$ -fullerene derivatives, only  $C_{60}\text{-pyrr}$  presented a  
366 slightly higher retention in the Accucore C18 column (1.9 min) than in the Hypersil  
367 GOLD one (1.7 min). This could be related to the size of the functional groups of the

368 fullerene derivatives, and therefore, their symmetry. C<sub>60</sub>-pyrr has the smallest functional  
369 group, and therefore its symmetry and retention behavior is more similar to that of  
370 pristine fullerenes. The other two C<sub>60</sub>-fullerene derivatives showed lower retention  
371 times in the column with superficially porous particles as currently happens when core-  
372 shell columns and columns with totally porous particles (sub-2 μm) are compared [41-  
373 43]. Since shorter analysis time and better asymmetry factors were obtained with the  
374 Hypersil GOLD column, it was selected for the separation of the studied compounds.

375 Two mobile phases, toluene/methanol and toluene/acetonitrile were evaluated  
376 using the Hypersil GOLD column under isocratic elution conditions (40% toluene and  
377 60 % methanol or 60% acetonitrile). Figure 4 shows the chromatograms obtained in  
378 both cases. Although the eluotropic strength of the mixture toluene/acetonitrile is higher  
379 than that of toluene/methanol, fullerene compounds presented higher retention times  
380 when acetonitrile mixtures were used, behavior that was also observed by Jinno *et al.*  
381 [44]. For instance, the retention time of C<sub>84</sub> increased from 4.3 min in toluene/methanol  
382 to 7.1 min in toluene/acetonitrile. This curious behavior of fullerenes can be related to  
383 their ability to form aggregates in polar solvents that explains for instance, their  
384 solubility in water [45,46]. It has been reported that stable dispersed colloidal particles  
385 are formed when C<sub>60</sub> and C<sub>70</sub> dissolved in non-polar solvents such as toluene, are added  
386 into polar solvents, like MeOH, ACN, ethanol or THF and that the hydrodynamic  
387 diameter of these small particles depends on the properties of both solvents, mainly  
388 their miscibility and the dielectric constant of the polar solvent [47,48]. Moreover, it has  
389 been suggested that increasing the polarity of the solvent leads to the formation of  
390 smaller aggregates [48]. On the bases of this assumption, fullerene clusters in  
391 toluene/ACN would be bigger than in toluene/MeOH and as a result, fullerenes will be  
392 more retained when toluene/ACN is used compensating the higher eluotropic strength  
393 of this mobile phase.

394 Furthermore, higher responses were observed with LC-APPI-MS when  
395 toluene/methanol solutions were used in agreement with the results previously reported  
396 by Chen *et al.* [29] for C<sub>60</sub>, C<sub>70</sub>, and PCME. Since lower retention times and better  
397 sensitivity were obtained, toluene/methanol was selected as the optimum mobile phase.  
398 Finally, gradient elution was optimized to improve the separation of the first two eluting  
399 compounds, PCBM and PCBB. Under gradient elution (see Experimental Section) the  
400 resolution between PCBM and PCBB increased from 1.2 (isocratic conditions) to 2.0 .

401

### 402 3.3. Sample treatment

403 In this work, pond water samples were extracted by LLE with toluene after the  
404 addition of NaCl to facilitate the transfer to the toluene phase, following a procedure  
405 based on the one described by Chen *et al.* [30] for the analysis of C<sub>60</sub> in water (see  
406 *Experimental Section*). Recoveries were evaluated by spiking blank pond water samples  
407 (200 mL) with fullerene and fullerene derivatives standard solutions in toluene at 5  
408 concentration levels between 1 and 10 ng L<sup>-1</sup> for PCBM, PCBB, C<sub>60</sub>-pyrr, C<sub>60</sub>, and C<sub>70</sub>,  
409 and between 10 and 100 ng L<sup>-1</sup> for C<sub>76</sub>, C<sub>78</sub> and C<sub>84</sub> fullerenes. Recoveries were  
410 calculated from the slope of the plot of the calculated amount versus the added  
411 concentration and the values obtained were always higher than 83% and slightly better  
412 for C<sub>60</sub>-fullerene derivatives (93-96 %) than for pristine fullerenes (83-89 %) (Table 2).

413 Regarding the extraction of fullerene compounds from sediments, both  
414 ultrasound extraction and pressurized liquid extraction (PLE) using toluene as  
415 extraction solvent were evaluated. For this purpose, blank sediment samples were  
416 spiked with standards prepared in toluene at levels of 50 ng Kg<sup>-1</sup> for PCBM, PCBB,  
417 C<sub>60</sub>-pyrr, C<sub>60</sub> and C<sub>70</sub>, and at 50 µg Kg<sup>-1</sup> for the higher fullerenes and kept at room  
418 temperature (25 °C) for 24 h before extraction. The extracts obtained in both, ultrasound  
419 and PLE extractions were collected, evaporated and analyzed as described in the  
420 *Experimental Section*.

421 For ultrasound extraction, the recoveries obtained were very low, ranging from  
422 <1 % to 33 % and decreased for the pristine fullerenes of high molecular weight. These  
423 values are lower than those reported by Sanchis *et al.* [32] (35-108 %) for the extraction  
424 of C<sub>60</sub>, C<sub>70</sub> and some fullerene derivatives from sediments. However these authors used  
425 a very long extraction time, 2 cycles of 4h each and the shorter extraction time (1h) used  
426 in our work can explain the lower recoveries obtained.

427 PLE extractions were initially carried out following a procedure proposed by  
428 Shareef *et al.* [39] for the extraction of C<sub>60</sub> from soils. In our work, beside C<sub>60</sub> fullerene,  
429 the extraction of C<sub>70</sub> and higher pristine fullerenes together with three C<sub>60</sub>-fullerene  
430 derivatives (C<sub>60</sub>-pyrr, PCBM and PCBB) was studied. Sediment samples were extracted  
431 with 100% toluene, at 50 °C and applying 5 static cycles of 10 minutes each. At these  
432 conditions, recoveries between 15 and 45% were obtained which were lower than those  
433 reported by Shareef *et al.* [39] (84 -107 % for C<sub>60</sub>). However, it is worth mentioning that  
434 in the present work the recoveries were calculated in sediments spiked at much lower  
435 concentration levels (ng Kg<sup>-1</sup>- µg Kg<sup>-1</sup>) than the soils reported in the Shareef work (mg

436  $\text{Kg}^{-1}$ ][39].  $\text{C}_{60}$ -fullerene derivatives showed higher recoveries (40-45%) than pristine  
437 fullerenes (15-34%) and the extraction efficiency decreased with the molecular weight  
438 as previously observed by ultrasound extraction.

439 In order to improve PLE recoveries, several parameters such as the extraction  
440 temperature and the number of extraction cycles were optimized. The effect of the  
441 extraction temperature, 50 °C to 200 °C, was evaluated by maintaining all the other  
442 conditions unchanged (5 cycle number of 10 minutes each) and the recoveries obtained  
443 are shown in Figure 5. As can be seen, an important improvement on recoveries was  
444 obtained up to 150 °C. Since no significant increase was observed at higher  
445 temperatures, 150 °C was chosen as optimum temperature for further studies. The effect  
446 of the number of extraction cycles was also evaluated and it was observed that the  
447 recoveries decreased with the number of cycles mainly for the pristine fullerenes of  
448 higher molecular weight, probably due to the co-extraction of interfering compounds  
449 from the matrix as the cycle number increased. The highest recoveries were obtained by  
450 using only 1 extraction cycle. Thus, one static extraction cycle of 10 minutes at 150 °C  
451 was chosen as optimum conditions for the extraction of the studied fullerenes from  
452 sediment samples.

453 Recovery values were determined by the standard addition method fortifying  
454 blank sediment samples at 5 different concentration levels, between 10 and 100  $\text{ng Kg}^{-1}$   
455 for  $\text{C}_{60}$ -fullerene derivatives,  $\text{C}_{60}$  and  $\text{C}_{70}$  and from 1 to 50  $\mu\text{g Kg}^{-1}$  for larger fullerenes.  
456 The recoveries were then calculated from the slope obtained by plotting the found  
457 concentration versus the added one, and values between 70 and 92 % were obtained  
458 (Table 2). These recoveries are higher than the ones reported by Sanchis *et al.* [32]  
459 (around 60 %) for the extraction of  $\text{C}_{60}$ ,  $\text{C}_{70}$ , PCBM and  $\text{C}_{60}$ -pyrr from sediments by  
460 using PLE.

461

### 462 **3.4. Method performance and application**

463 Both instrumental and method quality parameters were determined, and are  
464 given in Table 2. Instrumental limits of detection (ILODs), based on a signal-to-noise  
465 ratio of 3:1, calculated using the confirmation ion (for pristine fullerenes) or the  
466 confirmation SRM transition (for  $\text{C}_{60}$ -fullerene derivatives), were obtained by analyzing  
467 solutions of the studied compounds at very low concentration levels. ILODs down to  
468 0.001 pg injected were obtained for the  $\text{C}_{60}$ -fullerene derivatives, while values between  
469 0.007 and 5.5 pg injected were found for pristine fullerenes. Calibration curves based on

470 the peak area of the studied fullerenes at concentrations between ILOQ and 100  $\mu\text{g}$   
471  $\text{L}^{-1}$  were obtained, showing good linearity ( $r^2 > 0.998$ ) for all the compounds. To evaluate  
472 method detection limits (MLODs), blank pond water and blank sediment samples were  
473 spiked at low concentration levels and were subjected to the sample treatments  
474 previously described. For water samples, MLODs values between 1.4 and 3.4  $\text{pg L}^{-1}$   
475 (PCBM, PCBB,  $\text{C}_{60}$ -pyrr,  $\text{C}_{60}$  and  $\text{C}_{70}$ ) and between 0.6 and 1.6  $\text{ng L}^{-1}$  (higher  
476 fullerenes) were obtained. These values are in agreement with those reported previously  
477 for pristine fullerenes [28] and lower than the ones reported by Farre *et al.* [21] for  $\text{C}_{60}$ ,  
478  $\text{C}_{70}$  and  $\text{C}_{60}$ -pyrr. MLOD values for sediment samples, ranged between 45 and 250  $\text{pg}$   
479  $\text{Kg}^{-1}$  for PCBM, PCBB,  $\text{C}_{60}$ -pyrr,  $\text{C}_{60}$  and  $\text{C}_{70}$  and between 31 and 158  $\text{ng Kg}^{-1}$  for  
480 higher fullerenes and were also lower than those reported by Sanchis *et al.* [32] for  $\text{C}_{60}$ ,  
481  $\text{C}_{70}$ ,  $\text{C}_{60}$ -pyrr and PCBM (9-21  $\text{ng Kg}^{-1}$ ) in sediment and soil samples. MLOQs, based  
482 on a signal-to-noise ratio of 10, were also calculated following the same procedure, and  
483 the values are indicated in Table 2.

484 The matrix effect was evaluated by comparing the peak areas obtained by  
485 analyzing a standard solution of target fullerenes (500  $\text{ng L}^{-1}$  for PCBM, PCBB,  $\text{C}_{60}$ -  
486 pyrr,  $\text{C}_{60}$  and  $\text{C}_{70}$  and 50  $\mu\text{g L}^{-1}$  for  $\text{C}_{76}$ ,  $\text{C}_{78}$ , and  $\text{C}_{84}$ ) with those of the extracts spiked  
487 post extraction treatment at the same concentration level. A reduction of the peak areas,  
488 ranging from less than 10% for water samples to 20% in sediments, was observed for  
489 large pristine fullerenes ( $\text{C}_{76}$ - $\text{C}_{84}$ ). The higher matrix effect observed for sediments  
490 compared to water samples which is more pronounced for larger fullerenes is due to the  
491 higher content of organic matter. To prevent this effect, matrix matched calibration is  
492 proposed for quantitation.

493 Instrumental run-to-run precision was evaluated by analyzing a total of five replicates of  
494 a standard at 100  $\text{ng L}^{-1}$  for PCBM, PCBB,  $\text{C}_{60}$ -pyrr,  $\text{C}_{60}$ ,  $\text{C}_{70}$ , and 10  $\mu\text{g L}^{-1}$  for higher  
495 fullerenes on the same day. The relative standard deviations (RSDs) based on  
496 concentration ranged from 5 to 7 %. Method day-to-day precision was evaluated by  
497 performing 15 replicate determinations on 3 nonconsecutive days (five replicates per  
498 day) of spiked blank water and sediment samples at two concentration levels, a low  
499 level (MLOQ values) and a high level (10  $\text{ng L}^{-1}$  for PCBM, PCBB,  $\text{C}_{60}$ -pyrr,  $\text{C}_{60}$ , and  
500  $\text{C}_{70}$ , and 100  $\text{ng L}^{-1}$  for higher fullerenes in water samples, and 100  $\text{ng Kg}^{-1}$  for PCBM,  
501 PCBB,  $\text{C}_{60}$ -pyrr,  $\text{C}_{60}$  and  $\text{C}_{70}$ , and 50  $\mu\text{g Kg}^{-1}$  for  $\text{C}_{76}$ ,  $\text{C}_{78}$  and  $\text{C}_{84}$  in sediment samples).  
502 Relative standard deviations (RSDs) based on concentration ranged from 10 to 13 %

503 (high level) and from 14 to 21 % (low level) showing an acceptable performance (Table  
504 2).

505 In order to evaluate the applicability of the developed method for the analysis of  
506 C<sub>60</sub>-fullerene derivatives and pristine fullerenes in complex matrices, 6 pond water  
507 samples and 7 sediment samples collected from different ponds located close to  
508 Barcelona's Airport (El Prat de Llobregat, Spain) were analyzed and the results are  
509 given in Table 3. Fullerene derivatives were detected in all the analyzed sediment  
510 samples at low ng Kg<sup>-1</sup> concentration levels. Figure 6 shows as an example, the  
511 chromatogram obtained for one of the samples. PCBM and C<sub>60</sub>-pyrr (Table 3) were  
512 quantified at levels between 0.14-2.7 ng Kg<sup>-1</sup>, while PCBB was detected at slightly  
513 higher concentration levels, up to 5.1 ng Kg<sup>-1</sup>. To the best of our knowledge this is the  
514 first report of the presence of PCBM, PCBB and C<sub>60</sub>-pyrr, compounds in sediment  
515 samples. Among pristine fullerenes, C<sub>60</sub> and C<sub>70</sub> were detected in most of the samples,  
516 but they could only be quantified in some of them, at levels up to 1.1 ng Kg<sup>-1</sup> and 2.1 ng  
517 Kg<sup>-1</sup>, respectively, while higher fullerenes were not detected. C<sub>60</sub> and C<sub>70</sub> fullerenes  
518 have been recently found in sediments and superficial soils [32], also at low ng L<sup>-1</sup>  
519 concentration levels and in samples from highly industrial and urban areas and near  
520 international airports. Thus, their presence in the samples analyzed in this work could be  
521 related to the location of the sampling points, close to the landing strips of Barcelona's  
522 airport (El Prat de Llobregat, Spain). The C<sub>60</sub>-fullerene derivatives were also detected in  
523 almost all the analyzed water samples at low pg L<sup>-1</sup> concentration levels (Table 3), and  
524 the highest concentration detected was ~8 pg L<sup>-1</sup> (PCBM and C<sub>60</sub>-pyrr). There are no  
525 previous reports of the presence of these compounds in water samples, except for C<sub>60</sub>-  
526 pyrr that has been found in wastewater effluents [21]. Regarding pristine fullerenes, our  
527 results are in agreement with previous reports about the presence of these compounds in  
528 environmental water samples, which are detected usually at low ng L<sup>-1</sup> levels [21,28,32]  
529 except in one paper [21] where C<sub>60</sub> was found at µg L<sup>-1</sup> levels. As regards larger  
530 fullerenes which have not been found in the analyzed samples, the presence of C<sub>84</sub> in  
531 water has been recently reported [28], although C<sub>76</sub> and C<sub>78</sub> have not yet been found in  
532 environmental samples.

533

#### 534 **4. Conclusions**

535 A fast and sensitive UHPLC-APPI-MS/(MS) method was developed for the analysis  
536 three C<sub>60</sub>-fullerene derivatives (PCBM, PCBB and C<sub>60</sub>-pyrr) with increasing industrial



537 applications together with five pristine fullerenes. So far there are no other works  
538 describing a comparable analytical method suitable for the simultaneous analysis of all  
539 these fullerene compounds, and at the very low detection levels ( $\text{ng L}^{-1}$ ) relevant for  
540 environmental analysis. Among the different MS API sources tested, APPI was selected  
541 because it showed the best sensitivity. In contrast to pristine fullerenes, the molecular  
542 ion  $[\text{M}]^+$  isotope pattern of the studied  $\text{C}_{60}$ -fullerene derivatives match with the  
543 theoretical ones probably due to a lower tendency of hydrogen addition to the double  
544 bonds caused by the presence of their functional groups reducing the carbon cage  
545 insaturation. Unlike pristine fullerenes, these compounds did present fragmentation,  
546 mainly losing the functional groups and providing the buckyball structure  $[\text{C}_{60}]^+$ . The  
547 specific characteristics of fullerene compounds regarding their size, their peculiar  
548 solubility behavior and tendency to form aggregates allowed us to explain their  
549 retention behavior in different chromatographic columns (totally porous vs core-shell)  
550 and mobile phase mixtures (toluene-MeOH and toluene-ACN).  $\text{C}_{60}$ -fullerene derivatives  
551 were detected at low  $\text{ng Kg}^{-1}$  and  $\text{pg L}^{-1}$  levels, in sediments and water samples  
552 collected from highly industrialized areas (near Barcelona's airport). This work is the  
553 first report of their presence in these matrices. Among pristine fullerenes,  $\text{C}_{60}$  and  $\text{C}_{70}$   
554 were detected in most of the samples, while higher fullerenes were not detected. The  
555 results presented in this work demonstrate that the developed method can be proposed  
556 for the analysis of fullerene and  $\text{C}_{60}$ -fullerene derivatives in environmental samples.

557

## 558 **Acknowledgements**

559

560 The authors gratefully acknowledge the financial support received from Spanish  
561 Ministry of Economy and Competitiveness under the project CTQ2012-30836. Alina  
562 Astefanei thanks the Spanish Ministry of Economy and Competitiveness for a Ph.D.  
563 grant (FPI).

564

565

566

## 567 **References**

568

- 569 [1] H.W. Kroto, J.R. Heath, S.C. O'Brien, R.F. Curl, R.E. Smalley,  $\text{C}_{60}$ :  
570 buckminsterfullerene, Nature 318 (1985) 162-163.

- 571 [2] D. Kronholm, J.C. Hummelen, Fullerene-based n-type semiconductors in  
572 organic electronics, *Mater. Matters (Milwaukee, WI, U. S. )* 2 (2007) 16-19.
- 573 [3] L. Xiao, H. Takada, K. Maeda, M. Haramoto, N. Miwa, Antioxidant effects of  
574 water-soluble fullerene derivatives against ultraviolet ray or peroxy lipid through  
575 their action of scavenging the reactive oxygen species in human skin  
576 keratinocytes, *Biomed. Pharmacother.* 59 (2005) 351-358.
- 577 [4] N. Tagmatarchis, H. Shinohara, Fullerenes in medicinal chemistry and their  
578 biological applications, *Mini-Rev. Med. Chem.* 1 (2001) 339-348.
- 579 [5] T. Da Ros, M. Prato, Medicinal chemistry with fullerenes and fullerene  
580 derivatives, *Chem. Commun. (Cambridge)* (1999) 663-669.
- 581 [6] B.H. Chen, J.P. Huang, L.Y. Wang, J. Shiea, T.L. Chen, L.Y. Chiang, Synthesis  
582 of octadecahydroxylated C70, *Synth. Commun.* 28 (1998) 3515-3525.
- 583 [7] M. Lens, L. Medenica, U. Citernesi, Antioxidative capacity of C(60)  
584 (buckminsterfullerene) and newly synthesized fulleropyrrolidine derivatives  
585 encapsulated in liposomes, *Biotechnol. Appl. Biochem.* 51 (2008) 135-140.
- 586 [8] H. Hoppe, M. Drees, W. Schwinger, F. Schaffler, N.S. Sariciftcia, Nano-  
587 Crystalline Fullerene Phases in Polymer/Fullerene Bulk-Heterojunction Solar  
588 Cells: A Transmission Electron Microscopy Study, *Synth. Met.* 152 (2005) 117-  
589 120.
- 590 [9] H. Zhu, J. Wei, K. Wang, D. Wu, Applications of carbon materials in  
591 photovoltaic solar cells, *Sol. Energy Mater. Sol. Cells* 93 (2009) 1461-1470.
- 592 [10] T.M. Benn, P. Westerhoff, P. Herckes, Detection of fullerenes (C60 and C70) in  
593 commercial cosmetics, *Environ. Pollut.* 159 (2011) 1334-1342.
- 594 [11] S. Erkoç, Perspectives of Fullerene Nanotechnology Edited by Eiji Osawa,  
595 *Mater. Manuf. Processes* 17 (2002) 881-883.
- 596 [12] C.O. Hendren, X. Mesnard, J. Droge, M.R. Wiesner, Estimating Production  
597 Data for Five Engineered Nanomaterials As a Basis for Exposure Assessment,  
598 *Environ. Sci. Technol.* 45 (2011) 4190.
- 599 [13] L. Becker, R.J. Poreda, A.G. Hunt, T.E. Bunch, M. Rampino, Impact event at  
600 the Permian-Triassic boundary: evidence from extraterrestrial noble gases in  
601 fullerenes, *Science (Washington, DC, U. S. )* 291 (2001) 1530-1533.
- 602 [14] K. Aschberger, H.J. Johnston, V. Stone, R.J. Aitken, C.L. Tran, S.M. Hankin,  
603 S.A.K. Peters, F.M. Christensen, Review of fullerene toxicity and exposure -  
604 Appraisal of a human health risk assessment, based on open literature, *Regul.*  
605 *Toxicol. Pharmacol.* 58 (2010) 455-473.
- 606 [15] Y. Saito, H. Ohta, K. Jinno, Chromatographic separation of fullerenes, *Anal*  
607 *Chem* 76 (2004) 266A-272A.

- 608 [16] K. Jinno, J. Wu, Technical advances in the liquid chromatographic separation of  
609 fullerenes, *Sep. Fullerenes Liq. Chromatogr.* (1999) 1-24.
- 610 [17] M. Farre, J. Sanchis, D. Barcelo, Analysis and assessment of the occurrence, the  
611 fate and the behavior of nanomaterials in the environment, *TrAC, Trends Anal.*  
612 *Chem.* 30 (2011) 517-527.
- 613 [18] C.W. Isaacson, M. Kleber, J.A. Field, Quantitative analysis of fullerene  
614 nanomaterials in environmental systems: a critical review, *Environ. Sci.*  
615 *Technol.* 43 (2009) 6463-6474.
- 616 [19] B.F.G. Pycke, T.M. Benn, P. Herckes, P. Westerhoff, R.U. Halden, Strategies  
617 for quantifying C60 fullerenes in environmental and biological samples and  
618 implications for studies in environmental health and ecotoxicology, *TrAC,*  
619 *Trends Anal. Chem.* 30 (2011) 44-57.
- 620 [20] C. Wang, C. Shang, P. Westerhoff, Quantification of fullerene aggregate nC60  
621 in wastewater by high-performance liquid chromatography with UV-vis  
622 spectroscopic and mass spectrometric detection, *Chemosphere* 80 (2010) 334-  
623 339.
- 624 [21] M. Farre, S. Perez, K. Gajda-Schranz, V. Osorio, L. Kantiani, A. Ginebreda, D.  
625 Barcelo, First determination of C60 and C70 fullerenes and N-  
626 methylfulleropyrrolidine C60 on the suspended material of wastewater effluents  
627 by liquid chromatography hybrid quadrupole linear ion trap tandem mass  
628 spectrometry, *J. Hydrol.* 383 (2010) 44-51.
- 629 [22] A.P. van Wezel, V. Moriniere, E. Emke, T. ter Laak, A.C. Hogenboom,  
630 Quantifying summed fullerene nC60 and related transformation products in  
631 water using LC LTQ Orbitrap MS and application to environmental samples,  
632 *Environ. Int.* 37 (2011) 1063-1067.
- 633 [23] A. Kolkman, E. Emke, P.S. Baeuerlein, A. Carboni, D.T. Tran, T.L. ter Laak,  
634 A.P. van Wezel, P. de Voogt, Analysis of (Functionalized) Fullerenes in Water  
635 Samples by Liquid Chromatography Coupled to High-Resolution Mass  
636 Spectrometry., *Anal. Chem.* 85 (2013) 5867-5874.
- 637 [24] D. Bouchard, X. Ma, Extraction and high-performance liquid chromatographic  
638 analysis of C60, C70, and [6,6]-phenyl C61-butyrac acid methyl ester in  
639 synthetic and natural waters, *J. Chromatogr. A* 1203 (2008) 153-159.
- 640 [25] C.W. Isaacson, C.Y. Usenko, R.L. Tanguay, J.A. Field, Quantification of  
641 Fullerenes by LC/ESI-MS and Its Application to in Vivo Toxicity Assays, *Anal.*  
642 *Chem.* 79 (2007) 9091-9097.
- 643 [26] S.Y. Xie, S.L. Deng, L.J. Yu, R.B. Huang, L.S. Zheng, Separation and  
644 identification of perchlorinated polycyclic aromatic hydrocarbons and fullerenes  
645 (C60, C70) by coupling high-performance liquid chromatography with  
646 ultraviolet absorption spectroscopy and atmospheric pressure chemical  
647 ionization mass spectrometry, *J. Chromatogr. A* 932 (2001) 43-53.

- 648 [27] S.i. Kawano, H. Murata, H. Mikami, K. Mukaibatake, H. Waki, Method  
649 optimization for analysis of fullerenes by liquid chromatography/atmospheric  
650 pressure photoionization mass spectrometry, *Rapid Commun. Mass Spectrom.*  
651 20 (2006) 2783-2785.
- 652 [28] O. Núñez, H. Gallart-Ayala, C.P.B. Martins, E. Moyano, M.T. Galceran,  
653 Atmospheric Pressure Photoionization Mass Spectrometry of Fullerenes, *Anal.*  
654 *Chem.* 84 (2012) 5316-5326.
- 655 [29] H.C. Chen, W.H. Ding, Determination of aqueous fullerene aggregates in water  
656 by ultrasound-assisted dispersive liquid-liquid microextraction with liquid  
657 chromatography-atmospheric pressure photoionization-tandem mass  
658 spectrometry, *J. Chromatogr. A* 1223 (2012) 15-23.
- 659 [30] Z. Chen, P. Westerhoff, P. Herckes, Quantification of C60 fullerene  
660 concentrations in water, *Environ. Toxicol. Chem.* 27 (2008) 1852-1859.
- 661 [31] J. Sanchis, N. Berrojalbiz, G. Caballero, J. Dachs, M. Farre, D. Barcelo,  
662 Occurrence of Aerosol-Bound Fullerenes in the Mediterranean Sea Atmosphere,  
663 *Environ. Sci. Technol.* 46 (2012) 1335-1343.
- 664 [32] J. Sanchis, D. Bozovic, N.A. Al-Harbi, L.F. Silva, M. Farre, D. Barcelo,  
665 Quantitative trace analysis of fullerenes in river sediment from Spain and soils  
666 from Saudi Arabia, *Anal. Bioanal. Chem.* 405 (2013) 5915-5923.
- 667 [33] J. Brant, H. Lecoanet, M.R. Wiesner, Aggregation and deposition characteristics  
668 of fullerene nanoparticles in aqueous systems, *J. Nanopart. Res.* 7 (2005) 545-  
669 553.
- 670 [34] D. Heymann, L.P.F. Chibante, R.R. Brooks, W.S. Wolbach, R.E. Smalley,  
671 Fullerenes in the Cretaceous-Tertiary boundary layer, *Science* 265 (1994) 645-  
672 647.
- 673 [35] L. Becker, J.L. Bada, R.E. Winans, J.E. Hunt, T.E. Bunch, B.M. French,  
674 Fullerenes in the 1.85-billion-year-old Sudbury impact structure, *Science* 265  
675 (1994) 642-645.
- 676 [36] J. Jehlicka, O. Frank, V. Hamplova, Z. Pokorna, L. Juha, Z. Bohacek, Z.  
677 Weishauptova, Low extraction recovery of fullerene from carbonaceous  
678 geological materials spiked with C60, *Carbon* 43 (2005) 1909-1917.
- 679 [37] A. Carboni, E. Emke, J.R. Parsons, K. Kalbitz, P. de Voogt, An analytical  
680 method for determination of fullerenes and functionalized fullerenes in soils  
681 with high performance liquid chromatography and UV detection, *Anal. Chim.*  
682 *Acta* 807 (2014) 159-165.
- 683 [38] R.A. Perez, B. Albero, E. Miguel, J.L. Tadeo, C. Sanchez-Brunete, A rapid  
684 procedure for the determination of C60 and C70 fullerenes in soil and sediments  
685 by ultrasound-assisted extraction and HPLC-UV, *Anal. Sci.* 29 (2013) 533-538.

- 686 [39] A. Shareef, G. Li, R.S. Kookana, Quantitative determination of fullerene (C60)  
687 in soils by high performance liquid chromatography and accelerated solvent  
688 extraction technique, *Environ. Chem.* 7 (2010) 292-297.
- 689 [40] F. Moussa, M. Pressac, E. Genin, S. Roux, F. Trivin, A. Rassat, R. Ceolin, H.  
690 Szwarc, Quantitative analysis of C60 fullerene in blood and tissues by high-  
691 performance liquid chromatography with photodiode-array and mass  
692 spectrometric detection, *J. Chromatogr. B* 696 (1997) 153-159.
- 693 [41] H. Gallart-Ayala, E. Moyano, M.T. Galceran, Fast liquid chromatography-  
694 tandem mass spectrometry for the analysis of bisphenol A-diglycidyl ether,  
695 bisphenol F-diglycidyl ether and their derivatives in canned food and beverages,  
696 *J. Chromatogr. A* 1218 (2011) 1603-1610.
- 697 [42] A. Staub, D. Zurlino, S. Rudaz, J.L. Veuthey, D. Guillarme, Analysis of peptides  
698 and proteins using sub-2 $\mu$ m fully porous and sub 3- $\mu$ m shell particles., *J.*  
699 *Chromatogr. A* 1218 (2011) 8903-8914.
- 700 [43] P. Yang, T. McCabe, M. Pursch, Practical comparison of LC columns packed  
701 with different superficially porous particles for the separation of small molecules  
702 and medium size natural products, *J. Sep. Sci.* 34 (2011) 2975-2982.
- 703 [44] K. Jinno, T. Uemura, H. Ohta, H. Nagashima, K. Itoh, Separation and  
704 identification of higher molecular weight fullerenes by high-performance liquid  
705 chromatography with monomeric and polymeric octadecylsilica bonded phases,  
706 *Anal. Chem.* 65 (1993) 2650-2654.
- 707 [45] C.T. Jafvert, P.P. Kulkarni, Buckminsterfullerene's (C60) Octanol-Water  
708 Partition Coefficient (Kow) and Aqueous Solubility, *Environ. Sci. Technol.* 42  
709 (2008) 5945-5950.
- 710 [46] S. Deguchi, R.G. Alargova, K. Tsujii, Stable Dispersions of Fullerenes, C60 and  
711 C70, in Water. Preparation and Characterization, *Langmuir* 17 (2001) 6013-  
712 6017.
- 713 [47] A. Mrzel, A. Mertelj, A. Omerzu, M. Copic, D. Mihailovic, Investigation of  
714 Encapsulation and Solvatochromism of Fullerenes in Binary Solvent Mixtures,  
715 *J. Phys. Chem. B* 103 (1999) 11256-11260.
- 716 [48] R.G. Alargova, S. Deguchi, K. Tsujii, Stable colloidal dispersions of fullerenes  
717 in polar organic solvents, *J. Am. Chem. Soc.* 123(43) (2001) 10460-10467.
- 718

719 **Figure Captions**

720

721 Figure 1. Pristine fullerenes and C<sub>60</sub>-fullerene derivatives chemical structures

722 Figure 2. Signal of the [M]<sup>-</sup> ion of C<sub>60</sub>-fullerene derivatives with different API sources.

723 Figure 3. LC-APPI-MS spectra of C<sub>60</sub>-pyrr. (a) Full scan MS spectrum, (b) MS/MS  
724 spectrum of the precursor ion at *m/z* 777 (CE: 80 eV), and (c) MS/MS spectrum of the  
725 precursor ion at *m/z* 778 (CE: 80 eV).

726 Figure 4. UHPLC-APPI-MS(/MS) separation of fullerenes in a Hypersil GOLD C18  
727 column (150 x 2.1 mm, 1.9 μm) using (a) toluene:methanol (40:60, v/v) and (b)  
728 toluene:acetonitrile (40:60, v/v) as mobile phase, Flow rate: 500 μL min<sup>-1</sup>.

729 Figure 5. Effect of the extraction temperature on PLE. Blank sediment samples spiked  
730 at 50 ng Kg<sup>-1</sup> for C<sub>60</sub>, C<sub>70</sub> and C<sub>60</sub>-fullerene derivatives, and at 50 μg Kg<sup>-1</sup> for the higher  
731 fullerenes (5 extraction cycles of 10 min each).

732 Figure 6. UHPLC-APPI-MS(/MS) chromatogram of (a) standard mixture (100 ng L<sup>-1</sup>)  
733 and (b) sediment sample.

734

735

736

737

738

739

740

741

742

743

744

745

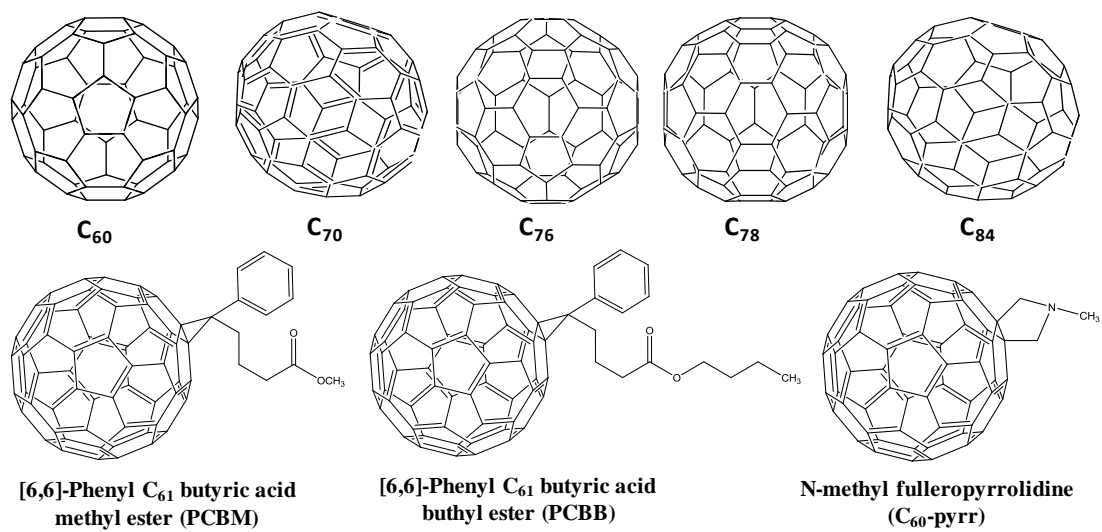
746

747

748

749 Figure 1

750



751

752

753

754

755

756

757

758

759

760

761

762

763

764

765

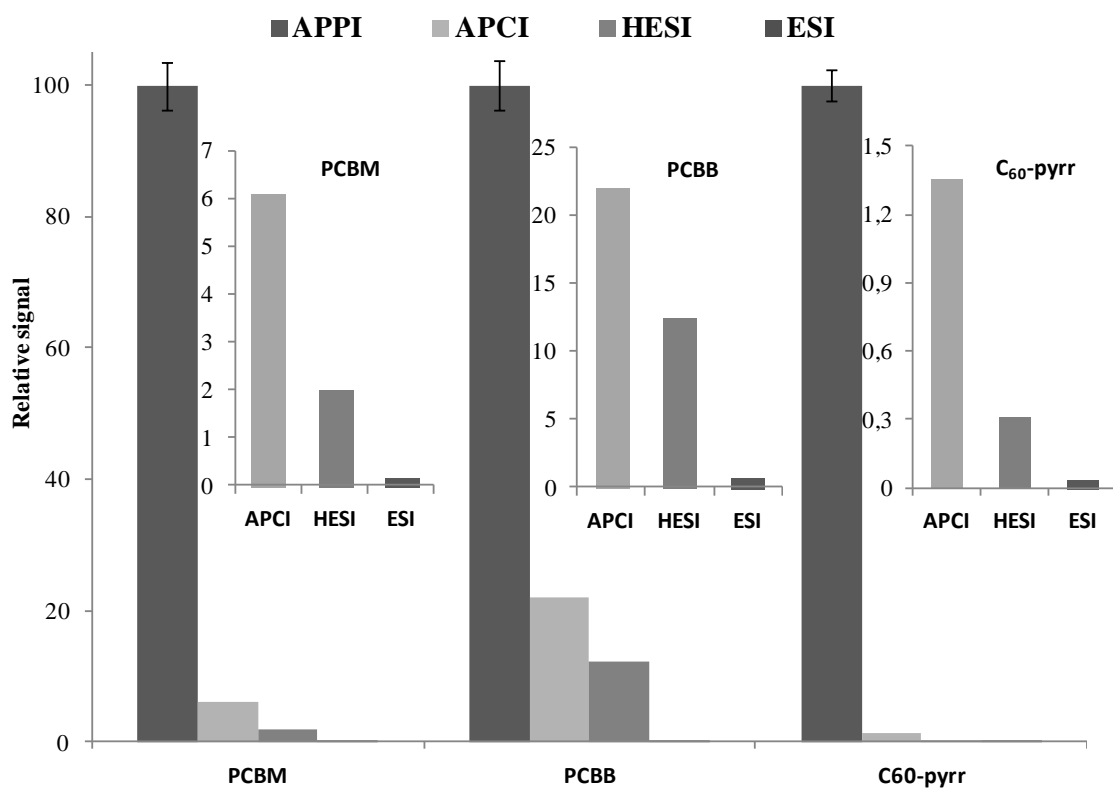
766

767

768

769 Figure 2

770



771

772

773

774

775

776

777

778

779

780

781

782

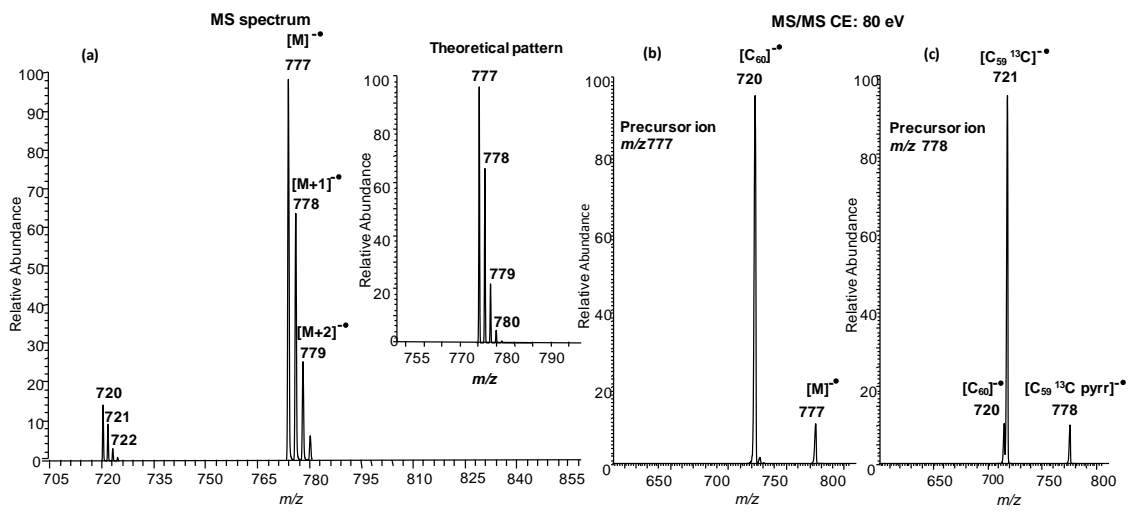
783

784



785 Figure 3

786



787

788

789

790

791

792

793

794

795

796

797

798

799

800

801

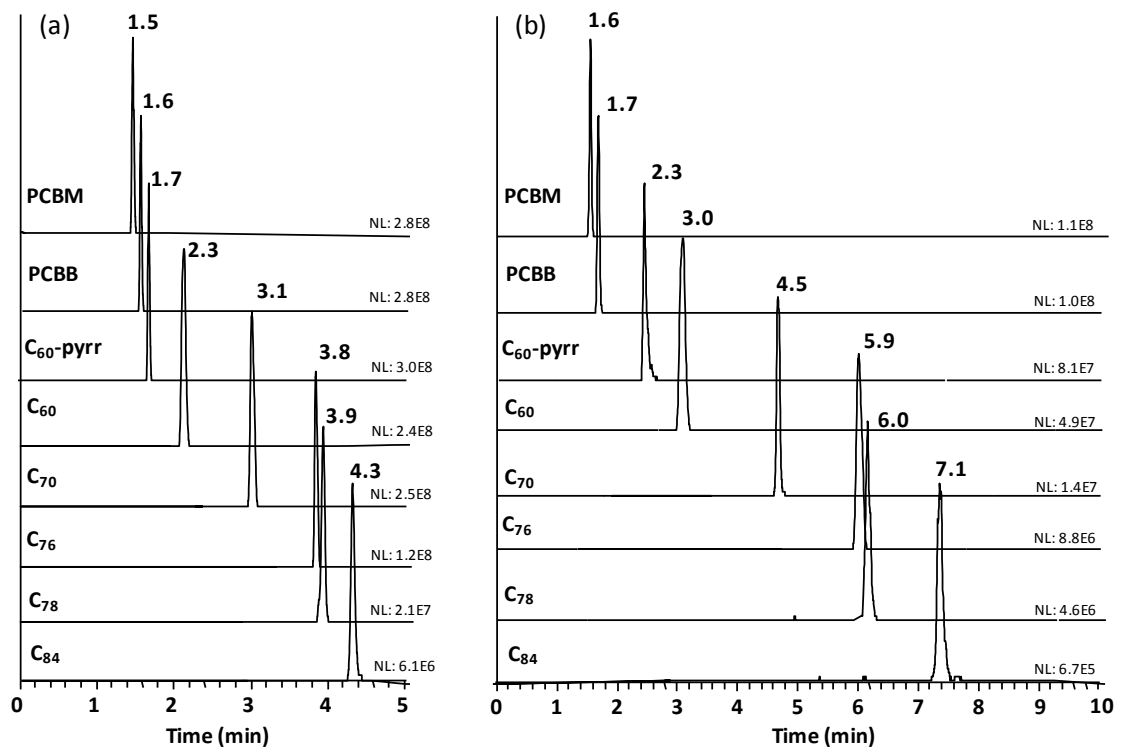
802

803

804

805 Figure 4

806



807

808

809

810

811

812

813

814

815

816

817

818

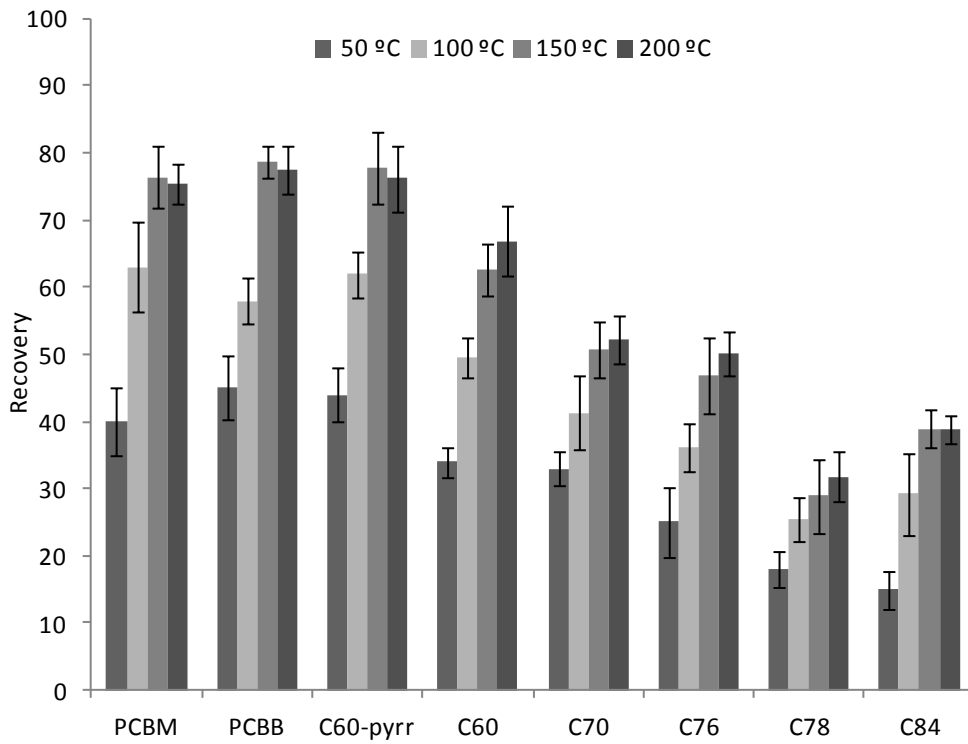
819

820

821

822 Figure 5

823



824

825

826

827

828

829

830

831

832

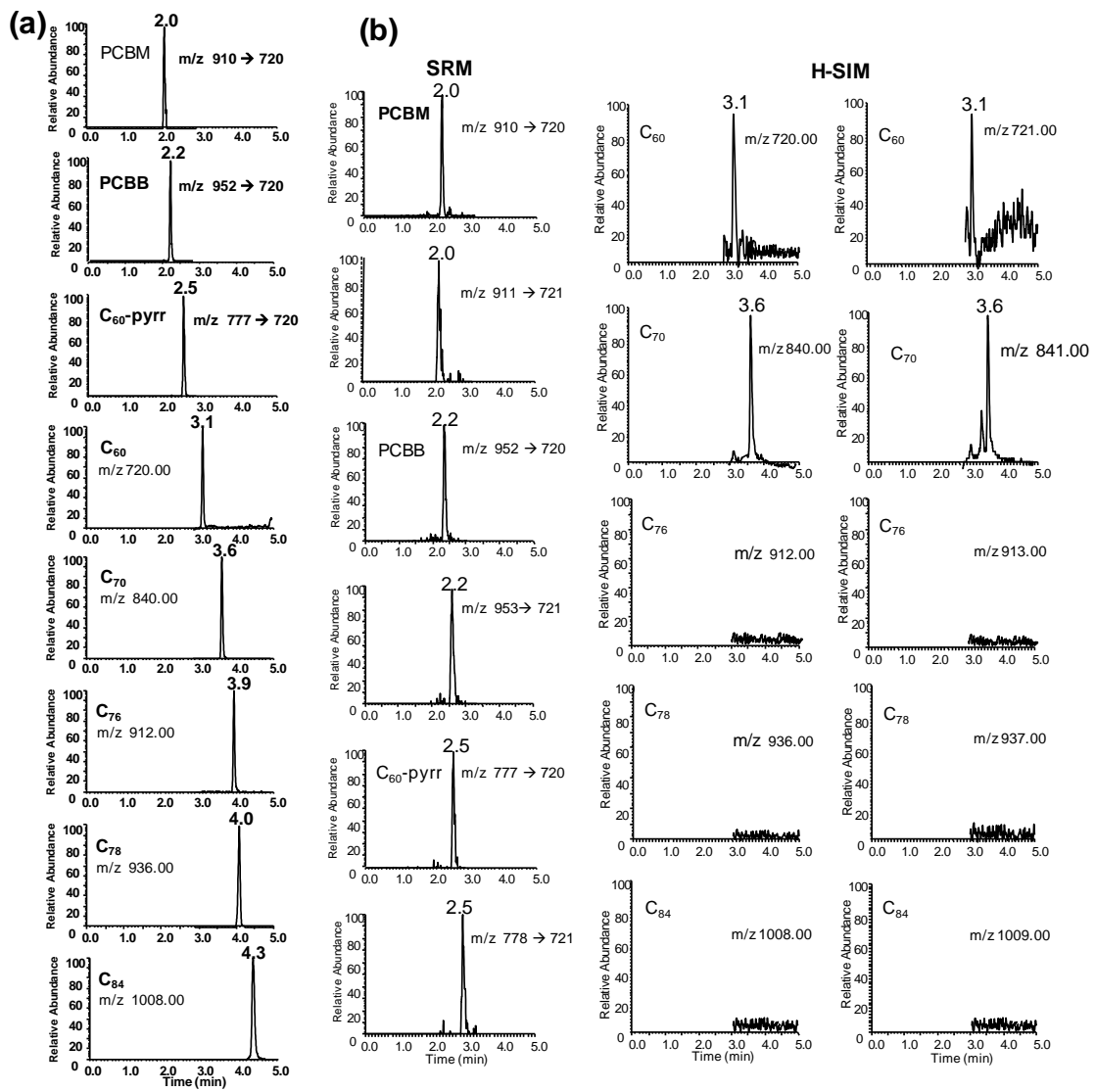
833

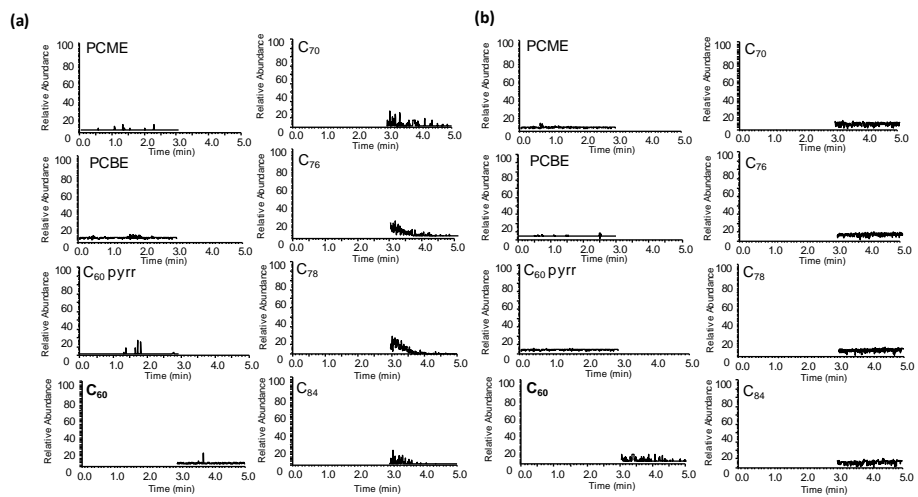
834

835

836

837





849

Figure 1S. UHPLC-APPI-MS/(MS) chromatogram of (a) blank sediment sample and (b) blank water sample

Table 1. Product ion spectra of C<sub>60</sub>-fullerene derivatives and selected SRM transitions

Compound	Precursor ion [M] <sup>-•</sup> ( <i>m/z</i> )	Product ion spectra		Selected reaction monitoring Transitions (CE, eV)
		<i>m/z</i> (%Rel.Ab.)	Ion assignment (CE, eV)	
PCBM	910	910 (40%)	[M] <sup>-•</sup> , 80	910→720 (100) <sup>a</sup>
		823(5%)	[M-(CH <sub>2</sub> ) <sub>2</sub> COOCH <sub>3</sub> ] <sup>-•</sup>	911→721 (100) <sup>b</sup>
		809(10%)	[M-(CH <sub>2</sub> ) <sub>3</sub> COOCH <sub>3</sub> ] <sup>-•</sup>	
		720 (100%)	[M-C <sub>6</sub> H <sub>5</sub> C(CH <sub>2</sub> ) <sub>3</sub> COOCH <sub>3</sub> ] <sup>-•</sup>	
PCBB	952	952 (30%)	[M] <sup>-•</sup> , 80	952→720 (150) <sup>a</sup>
		896(5%)	[M-(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub> ] <sup>-•</sup>	953→721 (150) <sup>b</sup>
		823 (5%)	[M-(CH <sub>2</sub> ) <sub>2</sub> COO(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub> ] <sup>-•</sup>	
		809(5%)	[M-(CH <sub>2</sub> ) <sub>3</sub> COO(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub> ] <sup>-•</sup>	
720 (100%)	[M-C <sub>6</sub> H <sub>5</sub> C(CH <sub>2</sub> ) <sub>3</sub> COO(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub> ] <sup>-•</sup>			
C <sub>60</sub> -pyrr	777	777 (40%)	[M] <sup>-•</sup> , 50	777→720 (80) <sup>a</sup>
		720 (100%)	[M-(CH <sub>2</sub> ) <sub>2</sub> NCH <sub>3</sub> ] <sup>-•</sup>	778→721 (80) <sup>b</sup>

<sup>a</sup>Quantitation <sup>b</sup>Confirmation

Table2. Quality parameters

Compound	Instrument quality parameters				Method quality parameters									
	ILODs Standard		Ion ratio <sup>a</sup>	Run to run precision <sup>b</sup>	Water				Sediment					
	pg injected	ng L <sup>-1</sup>			MLODs (pg L <sup>-1</sup> )	MLOQs (pg L <sup>-1</sup> )	Recovery (%)	Day to day precision <sup>c</sup>		MLODs (ng Kg <sup>-1</sup> )	MLOQs (ng Kg <sup>-1</sup> )	Recovery (%)	Day to day precision <sup>c</sup>	
							Low level <sup>d</sup>	High level <sup>e</sup>				Low level <sup>d</sup>	High level <sup>e</sup>	
PCBM	0.004	0.4	1.40±0.07	7	1.4	4.5	93	16	12	0.045	0.15	87	21	13
PCBB	0.002	0.2	1.42±0.07	6	0.9	3.0	94	14	10	0.036	0.12	92	20	11
C <sub>60</sub> -pyrr	0.001	0.1	1.35±0.06	7	0.4	1.5	96	15	12	0.030	0.10	87	19	12
C <sub>60</sub>	0.007	0.7	1.28±0.10	5	2.3	7.5	85	14	10	0.23	0.78	80	16	10
C <sub>70</sub>	0.01	1.0	1.10±0.10	6	3.4	11.2	89	15	11	0.25	0.82	78	18	12
C <sub>76</sub>	2.0	200	1.07±0.09	7	1200	4090	87	16	12	46	154	76	15	11
C <sub>78</sub>	1.0	97	0.95±0.07	5	600	1870	83	14	10	31	103	70	17	12
C <sub>84</sub>	5.5	550	0.98±0.05	6	1600	5460	85	16	12	158	523	73	18	13

<sup>a</sup>(M/M+1)±S.D; <sup>b</sup>%RSD (n=5); <sup>c</sup>%RSD (n= 3 x 5); <sup>d</sup>MLOQs, <sup>e</sup>10 ng L<sup>-1</sup> (PCBM, PCBB, C<sub>60</sub>-pyrr, C<sub>60</sub>, and C<sub>70</sub>), 100 ng L<sup>-1</sup> (C<sub>76</sub>-C<sub>84</sub>)

Table 3. Analysis of Fullerenes in Water and Sediment Samples by UHPLC-APPI-MS/(MS)

Sample	PCBM		PCBB		C <sub>60</sub> -pyrr		C <sub>60</sub>		C <sub>70</sub>		C <sub>76</sub>	C <sub>78</sub>	C <sub>84</sub>
	Concentration <sup>a</sup>	Ion ratio	Concentration <sup>a</sup>	Ion ratio	Concentration <sup>a</sup>	Ion ratio	Concentration <sup>a</sup>	Ion ratio	Concentration <sup>a</sup>	Ion ratio			
<b>Pond water</b>													
1	5.2 ± 0.5	1.38 ± 0.04	<MLOQ		8.5 ± 0.5	1.32 ± 0.03	15 ± 1	1.30 ± 0.06	330 ± 20	1.18 ± 0.02	n.d.	n.d.	n.d.
2	8.0 ± 0.9	1.38 ± 0.04	5.0 ± 0.5	1.40 ± 0.04	2.0 ± 0.1	1.34 ± 0.05	25 ± 2	1.20 ± 0.02	31 ± 2	1.11 ± 0.06	n.d.	n.d.	n.d.
3	n.d.		n.d.		MLOD		<MLOQ		22 ± 1	1.15 ± 0.04	n.d.	n.d.	n.d.
4	MLOD		MLOD		5.1 ± 0.6	1.34 ± 0.03	11 ± 1	1.24 ± 0.04	<MLOQ		n.d.	n.d.	n.d.
5	MLOD		<MLOQ		n.d.		9 ± 1	1.27 ± 0.06	n.d.		n.d.	n.d.	n.d.
6	6.1 ± 0.7	1.39 ± 0.05	n.d.		n.d.		22 ± 1	1.25 ± 0.03	<MLOQ		n.d.	n.d.	n.d.
<b>Sediment</b>													
1	0.15 ± 0.01	1.36 ± 0.02	2.45 ± 0.04	1.40 ± 0.05	0.36 ± 0.01	1.36 ± 0.03	<MLOQ		<MLOQ		n.d.	n.d.	n.d.
2	MLOQ		0.18 ± 0.01	1.43 ± 0.05	0.140 ± 0.001	1.32 ± 0.01	<MLOQ		MLOD		n.d.	n.d.	n.d.
3	0.16 ± 0.01	1.41 ± 0.04	0.23 ± 0.01	1.43 ± 0.07	0.562 ± 0.003	1.35 ± 0.02	1.0 ± 0.01	1.26 ± 0.08	MLOD		n.d.	n.d.	n.d.
4	<MLOQ		0.91 ± 0.03	1.41 ± 0.06	0.220 ± 0.002	1.32 ± 0.02	n.d.		n.d.		n.d.	n.d.	n.d.
5	2.6 ± 0.3	1.37 ± 0.02	5.1 ± 0.3	1.40 ± 0.04	2.7 ± 0.3	1.30 ± 0.01	1.1 ± 0.1	1.21 ± 0.02	2.1 ± 0.1	1.17 ± 0.02	n.d.	n.d.	n.d.
6	<MLOQ		0.18 ± 0.02	1.38 ± 0.02	0.52 ± 0.02		0.82 ± 0.04	1.20 ± 0.02	7.2 ± 0.2	1.13 ± 0.03	n.d.	n.d.	n.d.
7	MLOD		<MLOQ		<MLOQ		MLOD		n.d.		n.d.	n.d.	n.d.

<sup>a</sup>pg L<sup>-1</sup> <sup>b</sup>ng Kg<sup>-1</sup> Concentrations given as  $\bar{X} \pm S.D$ ; n.d. = not detected  
 Ion ratio values given as (M/M+1)±S.D.