



Identification of substances migrating from plastic baby bottles using a combination of low and high resolution mass spectrometric analyzers coupled to gas and liquid chromatography



Journal:	<i>Journal of Mass Spectrometry</i>
Manuscript ID:	JMS-15-0051.R3
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	n/a
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Keywords:	Baby bottles, Migration, GC-(Q)TOF-MS, UHPLC-QTOF-MS, food contact materials

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6 2 **combination of low and high resolution mass spectrometric analyzers**
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3 20 **Abstract**

4 21 This work presents a strategy for elucidation of unknown migrants from plastic food contact
5 22 materials (baby bottles) using a combination of analytical techniques in an untargeted
6 23 approach. First, gas chromatography (GC) coupled to mass spectrometry (MS) in electron
7 24 ionization (EI) mode was used to identify migrants through spectral library matching. When
8 25 no acceptable match was obtained, a second analysis by GC-(EI) high resolution mass
9 26 spectrometry (HRMS) time-of-flight (TOF) was applied to obtain accurate mass
10 27 fragmentation spectra and isotopic patterns. Databases were then searched to find a possible
11 28 elemental composition for the unknown compounds. Finally, a GC hybrid quadrupole QTOF-
12 29 MS with an atmospheric pressure chemical ionization (APCI) source was used to obtain the
13 30 molecular ion or the protonated molecule. Accurate mass data also provided additional
14 31 information on the fragmentation behaviour as two acquisition functions with different
15 32 collision energies were available (MS^E approach). In the low energy (LE) function, limited
16 33 fragmentation took place, whereas for the high energy (HE) function, fragmentation was
17 34 enhanced. For less volatile unknowns, ultra-high pressure liquid chromatography (UHPLC)-
18 35 QTOF-MS was additionally applied. Using a home-made database containing common
19 36 migrating compounds and plastic additives, tentative identification was made for several
20 37 positive findings based on accurate mass of the (de)protonated molecule, product ion
21 38 fragments and characteristic isotopic ions. Six illustrative examples are shown to demonstrate
22 39 the modus operandi and the difficulties encountered during identification. The combination of
23 40 these techniques was proven to be a powerful tool for the elucidation of unknown migrating
24 41 compounds from plastic baby bottles.

25 42
26 43 **Keywords:** Baby bottles; migration; GC-(Q)TOF-MS; UHPLC-QTOF-MS; food contact
27 44 materials
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46 Introduction

47 Nowadays, there is an increasing concern over the presence of hazardous chemicals in
48 food contact materials (FCMs) [1,2]. Many of these FCMs are made of plastics, which, next
49 to the polymer, contain complex mixtures of compounds, such as monomers, additives,
50 catalysts or degradation products. Consequently, migration of these chemicals from the plastic
51 FCMs into the food could arise, resulting in off-flavours and taints in the food or even
52 harmful effects to human health. For plastic FCMs, all authorized starting substances have
53 been assembled in a Union List in EU Regulation 10/2011 together with their migration limit
54 and/or restricted use. [3]. Furthermore, the use of Bisphenol-A was banned for the
55 manufacture of polycarbonate (PC) infant feeding bottles and their placement on the
56 European market. [4]. As a consequence, baby bottles made of other polymer types, e.g.
57 polypropylene (PP) or polyamide (PA), are now present on the market.

58 The migration phenomenon in the alternative materials for baby bottles has been
59 understudied up to now and little is known about the possible migrants from these polymer
60 alternatives. GC quadrupole-MS (GC-Q-MS) with electron impact (EI) ionization source has
61 been used to investigate the presence of unknown compounds in food simulant that has been
62 in contact with the alternative baby bottle plastics [5,6]. The drawback of this approach is that
63 a conclusive library match cannot always be obtained when comparing experimental and
64 library EI spectra, as many migrating compounds can be new, unregulated, or even non-
65 intentionally added substances (NIAS); e.g. degradation products of polymerisation reaction,
66 and are thus not included in commercially available libraries.

67 Using high-resolution time-of-flight mass spectrometry (TOF-MS), the identification
68 process improves as accurate masses of the ions are obtained. Moreover, the sensitivity is
69 notably higher than of the quadrupole MS when working in full-spectrum acquisition. The
70 compounds tentatively identified by library matching can be confirmed by checking the
71 accurate-masses of the product ions and the molecular ion (if present in the EI spectrum) and
72 ambiguous results in the library search can be partly resolved [7]. Only recently, such
73 accurate-mass instruments have also been coupled to alternative (softer) ionization sources for
74 GC, e.g. atmospheric pressure chemical ionization (APCI), facilitating the detection of the
75 molecular ion (or protonated molecule) which in turn eases the derivation of possible
76 molecular formulae. The potential of GC-(APCI)TOF-MS has recently been demonstrated in
77 other fields, such as pesticide residue or water analysis [8–10]. To our knowledge, its
78 application to the analysis of migrants from plastic FCMs has been rather limited. This
79 technique has been explored for the analysis of adhesives and non-intentionally added

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3 80 substances [11–13], though no work applying the APCI source was yet conducted on plastic
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5 81 baby bottles.

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7 82 To study the migration of non-volatile compounds from FCMs, LC-MS with
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9 83 electrospray ionization (ESI) is the most suitable approach to be applied [14]. Only for few
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11 84 classes of compounds, such as pharmaceuticals or pesticides, LC mass spectral libraries are
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13 85 available due to the prominent spectral differences induced by the use of different ionization
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15 86 sources. Therefore, until now, most of the analysis of non-volatile plastic migrants has been
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17 87 limited to targeted approaches by monitoring pre-selected families of compounds, such as
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19 88 phthalates, UV-ink photoinitiators or antioxidants [14]. On the other hand, the use of HRMS
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21 89 is mandatory for screening purposes. LC-TOF-MS has already shown its efficiency for
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23 90 screening and confirmation in the analysis of forensic (illicit drugs) and environmental
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25 91 samples (pesticides, flame retardants, etc.) [15–20]. Furthermore, few non-targeted studies
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27 92 have been published on possible contaminants migrating from FCMs [21–26],

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29 93 The aim of this work was to develop and apply a methodology for the identification of
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31 94 unknowns observed during non-targeted screening of plastic migrants from baby bottles,
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33 95 based on the use of low and high resolution MS. GC and LC hyphenated to a variety of mass
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35 96 analyzers were used for this purpose. To our knowledge, this is the first time that a
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37 97 combination of these techniques has been applied in a non-targeted approach to elucidate
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39 98 unknown migrants from plastic baby bottles. While it was not the goal of this work to give a
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41 99 complete overview of all detected compounds in the tested baby bottles [6], some particular
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43 100 examples have been selected to demonstrate the potential of the applied methodology for the
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45 101 elucidation of unknown plastic migrants.

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48 103 **Materials and methods**

49 104 *Materials*

50 105 *Samples and sample treatment*

51 106 Ten polypropylene (PP) baby bottles and one polyamide (PA) baby bottle from the Belgian
52 107 market [6], consisting the majority of the market share, were selected for the application of
53 108 the developed methodology. The use of simulants is prescribed in the EU Regulation 10/2011
54 109 to mimic the migration testing towards real foods, leading to the selection of simulant D1
55 110 (water:EtOH (50:50)) as a simulant for milk [3]. After sterilisation of the bottles during ten
56 111 minutes with boiling water, three consecutive migrations for 2h at 70°C were performed with
57 112 the water-EtOH simulant. Afterwards, a non-targeted liquid-liquid extraction with ethyl
58 113 acetate:n-hexane (1:1) was performed on the simulant samples as previously described [6].
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3 114 The obtained organic extracts were then further concentrated to $\pm 75 \mu\text{L}$ under a gentle N_2
4 115 stream for analysis by GC or evaporated until dryness and dissolved in $75 \mu\text{L}$ MeOH for LC
5 116 injection. All bottles were tested in duplicate. Deuterated 2,6-di-*tert*-butyl-4-methylphenol-
6 117 D24 (Campro Scientific GmbH, Berlin, Germany) was added as an internal standard (IS) for
7 118 GC analysis to the simulant prior to LLE to correct for potential variations in the extraction
8 119 method or instrumental response. For LC, $^{13}\text{C}_{12}$ -Bisphenol-A was selected (Cambridge
9 120 Isotope Laboratories, Inc. Andover, Massachusetts, USA).
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16 122 *Chemicals*

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18 123 Methanol (gradient grade for liquid chromatography LiChrosolv) and ethyl acetate (for liquid
19 124 chromatography LiChrosolv) were purchased from Merck (Darmstadt, Germany). N-hexane
20 125 (for residue analysis and pesticides, 95%) was purchased from Acros Organics (Geel,
21 126 Belgium). Ultrapure water was prepared by means of an Elga Purelab Prima (Tienen,
22 127 Belgium). Helium (99.999%) and nitrogen (99.99%) were purchased from Air Liquide (Liège,
23 128 Belgium). For GC-(Q)TOF-MS analysis hexane for ultra-trace analysis grade was purchased
24 129 from Scharlab (Barcelona, Spain). For UHPLC-QTOF-MS analysis HPLC-grade methanol
25 130 (MeOH), acetonitrile (ACN) and sodium hydroxide (>99%) were purchased from ScharLab
26 131 (Barcelona, Spain). Formic acid (HCOOH) (>98% w/w) was obtained from Fluka. HPLC-
27 132 grade water was obtained from deionized water passed through a Milli-Q water purification
28 133 system (Millipore, Bedford, MA, USA). Dicyclopentyl-dimethoxysilane (>98%) was
29 134 purchased from TCI chemicals (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan).
30 135 Pentaerythritol tetrakis(3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionate) (98%) was
31 136 purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany).
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43 138 *Methods*

44 139 *GC-(EI)MS*

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46 140 Initial non-target analyses of simulant extracts were performed with an Agilent 6890 gas
47 141 chromatograph coupled to an Agilent 5973 mass selective detector (MSD) equipped with an
48 142 electron impact (EI) ionization source and operated in full scan mode from m/z 40 to 700. The
49 143 GC column was a 30 m x 0.25 mm x 0.25 μm DB-5ms column (Agilent JW Scientific,
50 144 Diegem, Belgium). The temperature of the oven was set at 60°C for 3 min, and was then
51 145 increased to 300°C at a rate of $10^\circ\text{C min}^{-1}$ where it was held for 15 min. The total run-time
52 146 was 42 min. Helium was used as a carrier gas, with a constant flow rate of 1.0 mL min^{-1} . A
53 147 volume of $2 \mu\text{L}$ extract was injected so that a sufficiently detectable amount of analyte was
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3 148 brought on the column. The MS spectra obtained for the migrating chemicals extracted by the
4 149 simulant were compared with commercially available WILEY and NIST mass spectra
5 150 libraries by use of the Agilent MSD Chemstation® for peak identification.
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10 152 *GC-(EI)TOF-MS*

11 153 An Agilent 6890N GC system (Palo Alto, CA) equipped with an Agilent 7683 autosampler,
12 154 was coupled to a GCT time-of-flight (TOF) mass spectrometer (Waters Corporation,
13 155 Manchester, U.K.), operating in EI mode (70 eV). The GC separation was performed using
14 156 the same column type and oven program as for the GC-(EI)MS. The interface and source
15 157 temperatures were both set to 250°C and a solvent delay of 3 min was selected. The TOF-MS
16 158 was operated at 1 spectrum/s acquisition rate over the mass range m/z 50-700, using a
17 159 multichannel plate voltage of 2800 V. TOF-MS resolution was approximately 8500 at full
18 160 width half maximum (FWHM) at m/z 614. Heptacosafuorotributylamine (Sigma Aldrich,
19 161 Madrid, Spain), used for the daily mass calibration and as lock mass, was injected via syringe
20 162 in the reference reservoir at 30°C to monitor the m/z ion 218.9856. The application manager
21 163 ChromaLynx, also a module of MassLynx software, was used to investigate the presence of
22 164 unknown compounds in samples. Library search was performed using the commercial NIST
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34 167 *GC-(APCI)QTOF-MS*

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36 168 An Agilent 7890A GC system (Palo Alto, CA, USA) coupled to a quadrupole TOF mass
37 169 spectrometer XevoG2 QTOF (Waters Corporation, Manchester, UK) with an APCI source
38 170 was used. The instrument was operated under MassLynx version 4.1 (Waters Corporation).
39 171 Sample injections were made using an Agilent 7693 autosampler. The GC separation was
40 172 performed using the same conditions as described in the previous 2 GC techniques. 1 μ L was
41 173 injected at 280°C under splitless mode. Helium was used as carrier gas at 1.2 mL min^{-1} . The
42 174 interface temperature was set to 310°C using N_2 as auxiliary gas at 150 L h^{-1} , make up gas at
43 175 300 mL min^{-1} and cone gas at 16 L h^{-1} . The APCI corona pin was operated at 1.6 μ A with a
44 176 cone voltage of 20 V. The ionization process occurred within an enclosed ion volume, which
45 177 enabled control over the protonation/charge transfer processes. Xevo QTOF-MS was operated
46 178 at 2.5 spectra/s acquiring a mass range m/z 50-1200. TOF-MS resolution was approximately
47 179 18 000 (FWHM) at m/z 614. For MS^E measurements, two alternating acquisition functions
48 180 were used applying different collision energies: a low-energy function (LE), selecting 4 eV,
49 181 and a high-energy function (HE). In the latter case, a collision energy ramp (25-40 eV) rather
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3 182 than a fixed higher collision energy was used. Heptacosfluorotributylamine (Sigma Aldrich,
4 183 Madrid, Spain) was used for the daily mass calibration. Internal calibration was performed
5 184 using a background ion coming from the GC-column bleed as lock mass (protonated molecule
6 185 of octamethyl-cyclotetrasiloxane, m/z 297.0830). MassFragment software (Waters) was used
7 186 to explain the fragmentation behavior of the detected compounds. This software applies a
8 187 bond disconnection approach to suggest possible structures for the product ions from a given
9 188 molecule.
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15 190 *LC-QTOF-MS*

16 191 A Waters Acquity UPLC system (Waters, Milford, MA, USA) was interfaced to a hybrid
17 192 quadrupole-orthogonal acceleration-TOF mass spectrometer (XEVO G2 QTOF, Waters
18 193 Micromass, Manchester, UK), using an orthogonal Z-spray-ESI interface operating in positive
19 194 and negative ionization modes. The UPLC separation was performed using an Acquity UPLC
20 195 BEH C18 1.7 μm particle size analytical column 100 mm L \times 2.1 mm I.D. (Waters) at a flow
21 196 rate of 300 $\mu\text{L min}^{-1}$. The mobile phases used were A= H_2O with 0.01% HCOOH and
22 197 B=MeOH with 0.01% HCOOH. The percentage of organic modifier (B) was changed linearly
23 198 as follows: 0 min, 10%; 14 min, 90%; 16 min, 90%; 16.01 min, 10%; 18 min, 10%. Nitrogen
24 199 (from a nitrogen generator) was used as the drying and nebulizing gas. The gas flow was set
25 200 at 1000 L h^{-1} . The injection volume was 20 μL . The resolution of the TOF mass spectrometer
26 201 was approximately 20,000 at full width half maximum (FWHM) at m/z 556. MS data were
27 202 acquired over an m/z range of 50–1200. A capillary voltage of 0.7 and 2.5 kV was used in
28 203 positive and negative ion modes, respectively. A cone voltage of 20 V was used. Collision gas
29 204 was argon 99.995% (Praxair, Valencia, Spain). The interface temperature was set to 600°C
30 205 and the source temperature to 130°C. The column temperature was set to 40°C.

31 206 For MS^E experiments, two acquisition functions with different collision energies were
32 207 created. The first one, the low energy function (LE), selecting a collision energy of 4 eV, and
33 208 the second one, the high energy (HE) function, with a collision energy ramp ranging from
34 209 25 eV to 40 eV in order to obtain a greater range of product ions. The LE and HE functions
35 210 settings were for both a scan time of 0.4 s.

36 211 Calibrations were conducted from m/z 50 to 1200 with a 1:1 mixture of 0.05 M NaOH:5%
37 212 HCOOH diluted (1:25) with acetonitrile:water (80:20). For automated accurate mass
38 213 measurement, the lock-spray probe was used, using as lockmass a solution of leucine
39 214 enkephalin (10 $\mu\text{g mL}^{-1}$) in acetonitrile:water (50:50) at 0.1% HCOOH pumped at 20 μL
40 215 min^{-1} through the lock-spray needle. The leucine enkephalin $[\text{M}+\text{H}]^+$ ion (m/z 556.2771) for
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3 216 positive ionization mode and $[M-H]^-$ ion (m/z 554.2615) for negative ionization were used for
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5 217 recalibrating the mass axis and to ensure a robust accurate mass measurement over time. It
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7 218 should be noted that all the exact masses shown in this work have a deviation of 0.55 mDa
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9 219 from the “true” value, as the calculation performed by the MassLynx software uses the mass
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11 220 of hydrogen instead of a proton when calculating $[M+H]^+$ exact mass. However, because this
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13 221 deviation is also applied during mass axis calibration, there is no negative impact on the mass
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15 222 errors presented in this article. MS data were acquired in centroid mode and were processed
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17 223 by the ChromaLynx XS application manager (within MassLynx v 4.1; Waters Corporation).
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225 *Data processing*

226 *GC data processing*

227 A schematic overview of the GC approach is given in Figure 1a. The analytical
228 strategy to perform a non-target analysis with GC-MS techniques started from the results
229 obtained in our previous work [6]. In a first screening based on GC-(EI)MS data using
230 commercially available WILEY and NIST libraries with Agilent MSD Chemstation®
231 software, peaks with an area of at least 10% of the area of the internal standard were selected
232 for identification. Only compounds with library matches above 90% were accepted as
233 tentative candidates. When the returned match was below 90%, peaks were defined as
234 “unidentified” as they were most probably not included in the commercial libraries and
235 further research was conducted with GC-(EI)TOF-MS based on accurate mass data.

236 By means of the ChromaLynx Application Manager, a module of Masslynx software,
237 the remaining unidentified peaks were deconvoluted and searched again in the commercial
238 nominal mass NIST02 library. A hit list with five positive matches > 700 was generated.
239 Next, an elemental composition calculator (maximum deviation 5 mDa) was applied to
240 determine the five most likely formulae of the five most intense ions acquired in the accurate
241 mass spectrum. The proposed formulae of these five fragments were then compared with the
242 proposed molecular formulae of the top-five library hits using criteria like mass error and
243 isotopic fit. When a possible molecular formula could be derived in this way, candidates with
244 this particular empirical formula were searched in the Chemspider database. By using the
245 ChromaLynx MassFragment, which is a tool for fragmentation prediction, the obtained
246 accurate mass EI spectrum could be compared with the predicted fragments of a selected
247 possible structure and scorings were given. In this way, a differentiation could also be made
248 between different structures with same empirical formula and those which generate fragments
249 which are not in accordance with the obtained experimental spectrum, could be rejected.

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3 250 When no conclusive match could be obtained (e.g. more than one identity fit of
4 251 possible molecular formulae with the experimental GC-(EI)TOF spectrum), the samples could
5 252 be re-injected into the GC-(APCI)QTOF system to confirm or exclude preceding tentative
6 253 GC-(EI)TOF identifications. Due to the reduced fragmentation generally occurring in the
7 254 APCI source, a search was conducted for the accurate mass molecular ion and the protonated
8 255 molecule of the suggested molecular formulae candidates from the (EI)TOF. If one of the two
9 256 was present, a narrow window-extracted ion chromatogram (nw-XIC, ± 0.02 Da) resulted in a
10 257 chromatographic peak eluting approximately 2 minutes earlier than the values obtained in the
11 258 GC-(EI)TOF-MS. If no chromatographic peak appeared performing the nw-XIC for the
12 259 selected masses, the obtained spectrum at the expected retention time was manually examined
13 260 for other possible ions that could be the M^{++} or $[M+H]^+$. In this case, by comparing the
14 261 (EI)TOF and the (APCI)QTOF spectra, generally M^{++} or $[M+H]^+$ could be retrieved as often
15 262 the (EI)TOF spectrum still contains minor amounts of M^{++} (or $[M+H]^+$) which are more
16 263 abundant in the (APCI)QTOF. Again, the elemental composition software (± 5 mDa) was used
17 264 to determine the molecular formula of the unknown compound. Then, the fragmentation
18 265 pattern in the (APCI)QTOF of the unknown compound was studied by examining the MS^E
19 266 data, which provide useful further information about the fragmentation. Normally, the HE
20 267 mode offers most information about how the compound fragments as the presence of M^{++} or
21 268 $[M+H]^+$ diminishes and fragmentation increases. For some compounds, quite severe
22 269 fragmentation occurs already in the LE mode. Experimentally recorded fragmentation patterns
23 270 can also here be compared with software generated ones for possible candidates by the use of
24 271 MassFragment. When commercially available, standards were bought to confirm the actual
25 272 presence of the suggested compounds.

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42 274 *LC data processing*

43 275 A graphical overview of the LC-workflow was given in Figure 1b. No commercial MS
44 276 libraries of common plastic migrants are available for LC-MS, and a genuine non-target
45 277 approach of the raw data would result in a far too laborious data processing. Therefore, we
46 278 constructed a home-made database to facilitate a wide-scope suspect screening. By including
47 279 the empirical formula of a compound in the database, the ChromaLynx software processes
48 280 this against the obtained accurate mass spectra and positive matches are returned if the mass
49 281 error (± 0.002 Da) is appropriate. First, approximately 50 migrants that were previously
50 282 detected in the alternative plastics to PC baby bottles were included in this list [5,6]. Because
51 283 all analytical standards of these compounds were available to us, their experimental data

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3 284 (retention time and product ions) were also included in the database. Second, the empirical
4 285 formulae of around 190 common plastic additives were added, since these compounds could
5 286 also migrate from the alternative plastics. Last, more than 800 compounds authorised for
6 287 plastic FCMs by the European Union Regulation No. 10/2011 [3] were included in the
7 288 database.

8 289 For most compounds in this database, the only criterion to obtain a positive match was
9 290 to search by the exact mass of the empirical formula. This commonly led to several false
10 291 positive hits. Therefore, every positive hit (a peak detected, commonly corresponding to the
11 292 exact mass of the (de)protonated molecule) was checked manually evaluating the product ions
12 293 and characteristic isotopic ions, leading to the tentative identification of the candidate, based
13 294 on structure compatibility and comparison with available literature data. Adducts, such as
14 295 $[M+Na]^+$ or $[M+K]^+$, were also included to facilitate the detection of some compounds in
15 296 those cases where information existed on their possible formation. Also here, the analytical
16 297 standards were purchased for confirmation when commercially available.
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299 **Results and Discussion**

300 *Selection of techniques*

301 Until now, most analytical methods employed for the determination of plastic migrants
302 have been focused on the targeted analysis of a restricted number of a priori selected
303 compounds [27–29]. However, potential migrating compounds other than the target analytes
304 cannot be detected using this approach. Electron impact (EI) ionization used in GC produces
305 highly reproducible fragmentation spectra which makes the identification of unknown
306 compounds possible by comparison with commercially available mass spectral libraries (e.g.
307 Wiley, NIST). Due to its ability to obtain sensitive full scan data and accurate mass
308 measurements [7,30,31], GC-TOF-MS and hybrid quadrupole-TOF-MS (QTOF-MS) are
309 powerful mass analyzers for a wide variety of non-target applications for semi-volatiles
310 [7,32]. Due to a high degree of fragmentation in EI ionization, the molecular ion has often a
311 low abundance. This is an important limitation for structural elucidation, as the presence of
312 the molecular ion in a mass spectrum, especially if measured at accurate mass, provides
313 crucial information. In APCI ionization, a stable (quasi)molecular ion is formed by means of
314 charge transfer ($M^{+\bullet}$) and/or by protonation ($[M+H]^+$). The APCI interface used in GC can be
315 coupled with a wide range of high resolution mass analyzers (TOF, QTOF).

316 For LC analysis, the accurate-mass product ion spectra obtained in MS/MS mode on
317 the QTOF-MS provide relevant structural information. However, since the pre-selection of

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3 318 analyte precursor ions has to be done in the quadrupole, this results in the usual loss of
4 319 isotopic pattern information. This drawback can be overcome by MS^E data-acquisition, in
5 320 which both accurate-mass (de)protonated molecule (LE function) and product ions (HE
6 321 function) are obtained in the same injection without the need of selecting any precursor ion.
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8 322 The sequential collection of LE and HE data during sample analysis is a significant advantage
9 323 towards the structural elucidation of unknown compounds in a non-targeted screening
10 324 approach [33].

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14 325 In this manuscript, we have included a selection of examples to demonstrate the
15 326 developed strategy for the elucidation of unknown migrants from plastic baby bottles. The
16 327 selection of the cases was based on their ability to illustrate the contribution of each ionization
17 328 technique and mass analyzer towards the final identification. A detailed overview of all
18 329 identified compounds and the used techniques can be found in Table 1. Since most migrating
19 330 compounds are small molecules (molecular weight < 1200 Da), the parameters to calculate
20 331 the possible molecular formulae with the Elemental Composition software were generally set
21 332 as follows: C: 0-50, H: 0-100, O: 0-10, N: 0-10 and P: 0-5. Other atoms were included in the
22 333 search if after manual inspection of the spectrum the isotope pattern indicated the presence of
23 334 other elements. A maximum deviation of 2 mDa from the measured mass was applied. When
24 335 searching for the M⁺ (if existing), the option 'odd-electron ions only' was added. For
25 336 [M+H]⁺, this option was 'even-electron ions only'. For fragments, both odd and even options
26 337 were selected. Within the workflows proposed in Figure 1a and 1b, the criteria introduced by
27 338 Schymanski et al. [34] were used towards the acceptance of an unambiguous identification of
28 339 a compound. Here, five different levels of identification were defined, each with their
29 340 corresponding requirements varying from a level 5 mass of interest identification to an
30 341 unequivocal molecular formula (level 4), tentative candidate (level 3), probable structure
31 342 (level 2) and confirmed structure (level 1). Due to the lack of commercial availability or
32 343 sometimes relatively high prices of some products, not all analytical standards of tentatively
33 344 identified migrants were obtained. Here, identification was only done until level 2 of these
34 345 criteria.

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52 347 *Case study 1*

53 348 In the GC-(EI)MS, an unknown chromatographic peak with a retention time of 14.30
54 349 min was detected in most PP samples tested. No firm library match was obtained and scores
55 350 were very poor (<70%). Due to its detection frequency and because the intensity was
56 351 comparable to that of the internal standard ($\pm 10 \mu\text{g kg}^{-1}$ assuming an equal response factor,
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3 352 which is a considerable amount for plastic migrants), this compound was of major interest.
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5 353 Therefore, the compound was analysed further with GC-(EI)TOF-MS (Fig 1a). When
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7 354 performing a database search using the accurate mass fragmentation data obtained, no
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9 355 improvement in the match factors was perceived. Regarding the (EI)TOF spectrum (Figure 2),
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11 356 the ion m/z 159.0843 would be assumed to be the possible M^{+} . A clear isotope pattern at $M+1$
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13 357 and $M+2$ was seen and therefore both S and Si were included for the Elemental Composition
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15 358 search. This resulted in five possible molecular formulae, though only two of them ($C_6H_{13}N_3S$
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17 359 and $C_5H_{13}N_3OSi$) could possibly explain the isotope pattern seen.

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19 360 Looking at the LE APCI spectrum (Figure 2), m/z 229.1626 is the highest mass
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21 361 acquired, suggesting that this would be the M^{+} or $[M+H]^+$ of the unknown compound and
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23 362 that 159.0843 is a major fragment ion. Indeed, a very small and hardly visible peak was
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25 363 perceived at m/z 228.1531 in the (EI)TOF spectrum, suggesting that m/z 229.1626 was
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27 364 $[M+H]^+$. A large number of molecular formulae (>20) were calculated, but after considering
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29 365 the mass errors, only three formulae remained. Of these three, already one could be discarded,
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31 366 as $C_5H_{21}N_6O_4$ is not an existing chemical structure. This reduced the possible empirical
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33 367 formulae to $C_{13}H_{24}OS$ or $C_{12}H_{24}O_2Si$. Investigating the isotope ratios and the elemental
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35 368 compositions of the fragments starting from these two formulae, the option implying a Si
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37 369 atom clearly fitted best to the obtained spectra. A number of 116 positive hits were returned
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39 370 when searched in the Chemspider database. At this point, a literature search using the term
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41 371 ' $C_{12}H_{24}O_2Si$ + polypropylene' quickly returned the suggestion of dicyclopentyl-
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43 372 dimethoxysilane (structure 3, Figure 2). This alkyl silane is used in combination with Ziegler-
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45 373 Natta catalysts to increase the isotactic index of PP [35]. This structure was also suggested by
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47 374 Chemspider as the third most cited one. The first two structures (Figure 2) were considered as
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49 375 well, but already when checking the APCI spectrum with the MassFragment prediction
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51 376 software, the ions m/z 197.1363 (loss of CH_4O), 159.0844 (loss of C_5H_{10}) or 129.0736 (loss of
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53 377 $C_6H_{12}O$) could only be explained by structure 3. The respective masses m/z 215.1469,
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55 378 177.0947 and 147.0844 could be explained as the adduction of a water molecule to these
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57 379 fragments. The inclusion of a small amount of water in the APCI source to promote the
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59 380 formation of the $[M+H]^+$ could explain this phenomenon as already described by Wachsmuth
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381 et al [36]. Therefore, dicyclopentyl-dimethoxysilane was retained as the probably identified
382 migrant. The presence of this compound (level 1 identification) was afterwards
383 unambiguously confirmed by injection of the purchased commercial standard (Figure SI-1).
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385 *Case study 2*

Two peaks with an EI spectrum that exhibited similarities to those of the previously identified [6], respectively hexa- (22.54 min) and octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (24.22 min), were found in a PP sample at high intensities (more than 6 times the area of the IS). Library matching gave poor results (<70%) and did not suggest any structures with realistic possibilities either. The abundant presence of ion m/z 343.3209 in the LE function of the (APCI)QTOF suggested that for the compound related to the octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester this had to be the $[M+H]^+$. The low abundant presence of ion m/z 342.3108 in the (EI)TOF spectrum indeed confirmed that ion m/z 343.3209 was the protonated molecule, resulting in a molecular formula of $C_{21}H_{42}O_3$. Chemspider returned 59 possible structures for this empirical formula. The presence of ions m/z 284.2723 and 285.2791 in the (EI)TOF and the LE (APCI)QTOF spectrum, respectively, indicated the presence of an integral stearic acid moiety ($C_{18}H_{36}O_2$) in the structure, which made us discard all other possible molecular structures and thus, only five possibilities remained (see Figure 3B). The detection of this m/z also revealed that, for the remaining C_3H_6O moiety, the position of the third O-atom of this molecule had to be at the ultimate or the penultimate C-atom, whether or not incorporated as an ether (structures 1 and 2) or as an alcohol group (structures 3-5) (Figure 3B). Indeed, to explain the presence of fragment m/z 284.2723, the rules of the McLafferty rearrangement had to be applied, stating that the sixth atom starting from the carbonyl-O has to be a hydrogen atom. In this way, structure 2 (Figure 3B) could already be rejected as a possibility. The presence of m/z 325.3109 in the LE (APCI)QTOF spectrum, explained by the loss of a water molecule, suggests, on the other hand, the presence of a free alcohol group instead of an ether, because the loss of water is easier and more probable in this case, which eliminates structure 1 as well. Within the available MS spectra, it was not possible though to differentiate between the remaining structural isomers of structures 3-5 to determine which the actual unknown migrant was and only a probable identification could be reached (level 2). Injection of the different analytical standards is the only way to bring a decisive answer here. For the hexadecanoic acid based unknown migrant, the same conclusions could be drawn.

Case study 3

In this case, an unknown compound with a double intensity of the IS peak was seen in the first migration step of the PA bottle, though it completely disappeared in the next migration steps. Both GC-(EI)MS and GC-(EI)TOF-MS database searches gave poor matches (<40%), indicating that the structure of the unknown migrant was very different from the

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3 420 structures present in the database. The abundant ion m/z 394.3612 in the GC-(EI)TOF-MS
4 421 (RT 31.79 min) seemed to be the M^{+} , which was indeed confirmed by the highly abundant
5 422 presence of m/z 395.3638 (protonated molecule) in the LE GC-(APCI)QTOF-MS spectrum.
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7 423 Since no significant isotope patterns were noticed, an elemental composition search including
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9 424 only elements C, O, H and N resulted in a molecular formula of $C_{24}H_{46}N_2O_2$ (mass error of -
10 425 0.2 mDa) for which Chemspider returned 32 hits. For this molecular formula, all fragment
11 426 ions of both GC-(EI)TOF-MS and the HE of the GC-(APCI)QTOF-MS could be explained
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13 427 with very low mass errors (generally <2 mDa for the TOF and <0.2 mDa for the QTOF),
14 428 differentiating clearly the realistic possible fragments. It was noticeable that the most
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16 429 abundant (EI)TOF-MS ion (m/z 198.1868, $C_{12}H_{24}NO$) and the second most abundant
17 430 (APCI)QTOF-MS fragment ion (m/z 197.2014, $C_{12}H_{25}N_2$) exhibited a mass difference of only
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19 431 one amu with different though very similar empirical formulae, suggesting a common origin.
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23 432 This observation, together with the presence in this sample of a large amount of
24 433 lauro lactam, a polyamide monomer with m/z 197.1780 and a molecular formula of $C_{12}H_{23}NO$,
25 434 (GC-(EI)TOF-MS RT 17.08 min) suggested that this unknown might be a dimer of
26 435 lauro lactam, since its molecular formula is exactly the double of this compound and the ion
27 436 m/z 395.3638 is two times the mass of the protonated form of lauro lactam. Another evidence
28 437 is the disappearance of this unknown compound after the first migration step. Because this
29 438 dimer is a side-product of the polymerisation reaction, it is probably unbound in the polymer
30 439 skeleton. Therefore, it can easily be transferred to the migration solution and disappear in the
31 440 second migration step. Although data were rather conclusive, LC-QTOF-MS was also used to
32 441 confirm the presence of this dimer, since no commercial standard was available. Indeed, the
33 442 protonated monomer (m/z 198.1861, $C_{12}H_{23}NO$, RT: 7.41 min), the dimer (m/z 395.3626,
34 443 $C_{24}H_{46}N_2O_2$, RT: 7.74 min) and even the trimer (m/z 592.5419, $C_{36}H_{70}N_3O_3$, RT: 8.39 min,
35 444 most probably not eluted on GC) were seen in the LC-QTOF-MS (Figure 4B). The MS
36 445 spectra of these oligomers were undeniably confirmed by Stoffers et al. [37]. Regarding the
37 446 identification criteria proposed by Schymanski et al. [34], this leads us only to a level 2a
38 447 identification: probable structure, unambiguous literature spectrum-structure match, but not
39 448 confirmed by a reference standard. It has to be noticed though that, in this particular case, the
40 449 degree of confirmation could already be considered as high, because three different ionization
41 450 techniques (EI, APCI and ESI) have been applied. Yet, this is not always possible, since some
42 451 compounds are not suited for both GC and LC.
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453 *Case study 4*

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3 454 This was based on a positive accurate mass match of a peak eluted in the LC with RT
4 455 of 7.85 min having the accurate mass of bis(3,4-dimethylbenzylidene)sorbitol ($C_{24}H_{30}O_6$,
5 456 Millad 3988, a nuclear clarifying agent for PP) [38], with the processed LC data in ESI+
6 457 mode. For nine out of ten PP bottles, the protonated mass of m/z 415.2118 was matched with
7 458 an error < 2 mDa and with good isotope fittings. To confirm its presence, a literature search
8 459 was conducted to compare the obtained MS spectra with available literature. McDonald et al.
9 460 [38] provided characteristic MS data for this compound which indeed matched with our data
10 461 (Figure 5). The protonated molecule m/z 415.2121 was in the LE mode also the most
11 462 abundant ion. Furthermore, the $[M+Na]^+$ and $[M+K]^+$ adducts were also identified with
12 463 masses m/z 437.1941 and 453.1682, respectively. The m/z 119.0862 (C_9H_{11}), which originates
13 464 from the loss of one of the two dimethylbenzene moieties, was already seen in the LE
14 465 function, and this ion was the most significant in the HE spectrum. Ions m/z 397.2010 (loss of
15 466 H_2O), 295.1187 ($C_{15}H_{19}O_6$) and 277.1802 ($C_{15}H_{17}O_5$) were also retrieved in the HE function,
16 467 though in relatively small abundances. The Elemental Composition calculator confirmed that
17 468 all these fragments were indeed present, calculating their empirical formulas with low mass
18 469 errors ($< \pm 0.8$ mDa). It was noteworthy that 3,4-dimethylbenzaldehyde, a degradation product
19 470 of Millad 3988, was retrieved in the GC-MS injections of all PP samples which contained this
20 471 compound, confirming indirectly its presence. Therefore, we conclude the identification with
21 472 a high confidence (level 2) of Millad 3988 as migrant from most PP baby bottles.
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36 474 *Case study 5*

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38 475 The accurate mass of the protonated molecule $C_{26}H_{27}N_2O_2S$, m/z 431.1789 (LC RT
39 476 11.9 min), corresponding to 2,5-bis(5'-*tert*-butyl-2-benzoxaolyl)thiophene, an optical
40 477 brightening agent for polymers, was returned as a possible positive hit when comparing a PP
41 478 sample acquired in ESI+ mode to the LC database part containing plastic additives (mass
42 479 error 0.4 mDa) (Figure SI-2). Literature search [39] supported this finding, as besides the
43 480 protonated molecule, it also explained the fragments m/z 415.1467 and 401.1303 which were
44 481 seen in the HE mode and which were matched by the Elemental Composition calculator as
45 482 $C_{25}H_{23}N_2O_2S$ (1 mDa error) and $C_{24}H_{21}N_2O_2S$ (2.6 mDa error), respectively. No further
46 483 fragments could be seen due to the complexity of this structure. To obtain a higher confidence
47 484 degree in the identification of the compound, more fragments are necessary to be obtained by
48 485 applying higher collision energies.
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56 486 57 58 487 *Case study 6*

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3 488 The last example involves the compound Pentaerythritol tetrakis(3-(3,5-di-*tert*-butyl-
4 489 4-hydroxyphenyl)propionate), an anti-oxidant better known under its commercial name
5 490 Irganox 1010. An accurate mass matching for mass m/z 1175.7821 ($C_{73}H_{107}O_{12}$) was obtained
6 491 for this compound in all PP samples injected under ESI(-) mode in LC-QTOF-MS. Although
7 492 the protonated molecule was not present in the positive mode, its deprotonated molecule was
8 493 seen in the ESI- mode. Comparison of our experimental spectra with literature data only could
9 494 confirm the deprotonated molecule [40]. However, the injection of an available reference
10 495 standard of Irganox 1010 matched perfectly in retention time and fragmentation pattern
11 496 confirming in this way the unequivocal identification of this compound (Figure SI-3).

12 497 The presence of Irganox 1010 was already suggested in our previous work because
13 498 several potential degradation products of this compound were found by GC-(EI)MS analysis
14 499 [6]. The compound methyl-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl) propionate ($C_{18}H_{28}O_3$),
15 500 originating from a loss of one of the four “arms” of the original anti-oxidant (Figure SI-4),
16 501 was detected in all PP samples tested before, though until now, no concrete link with its origin
17 502 from Irganox 1010 could be established. This example demonstrates again the power of the
18 503 simultaneous use of these complementary techniques for the analysis of unknown migrants
19 504 from plastic products.

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21 506 **Critical considerations**

22 507 An efficient analytical strategy based on the combination of several mass analyzers
23 508 coupled to both gas and liquid chromatography has been applied for non-target analysis of
24 509 migrating components from plastic baby bottles. The complementary use of GC-(EI)MS, GC-
25 510 (EI)TOF-MS, GC-(APCI)QTOF-MS and UHPLC-QTOF-MS allowed an efficient and wide-
26 511 scope target and non-target screening on samples coming from a food simulant, in this case
27 512 H_2O -EtOH (50/50; v/v), that had been previously into contact with plastic baby bottles. The
28 513 methodology was applied to six case studies to illustrate the analytical challenges when the
29 514 mass spectra of the unknown compounds did not match with commercially available GC-
30 515 (EI)MS libraries. Furthermore, the use of a home-made database including a large number of
31 516 compounds of interest for detection of compounds via LC-QTOF was discussed into detail.
32 517 The strategy applied in this work has been proven to be successful for the elucidation of
33 518 several unknown plastic migrants, from non-polar volatile compounds to semi-polar non-
34 519 volatiles. Despite the success of the (tentative) identification of some relevant compounds, the
35 520 successful elucidation of unknowns is not only a matter of easily following a standardized
36 521 procedure, but it also requires next to the use of several analytical techniques, experience and

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3 522 creative insight of the analyst, which still makes it a challenging and quite tedious labour.
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6 524 **Acknowledgements**

7
8 525 Matthias Onghena wishes to thank the Federal Government Service for Public Health of

9
10 526 Belgium for funding his PhD scholarship through the project ALTPOLYCARB (RT 12/10).

11 527 The Research Institute for Pesticides and Water acknowledges the financial support from

12 528 Generalitat Valenciana (Group of Excellence Prometeo II/2014/023; ISIC/2012/016 Envi-

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For Review Only

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3 674 **Figure Captions:**
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7 676 **Figure 1:** Schematic overview of GC- (A) and LC (B)-methodology for the non-target
8 677 screening and elucidation of unknown plastic migrants.
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13 679 **Figure 2:** (A) (EI)TOF (top), (APCI)QTOF low energy (middle) and high energy (bottom)
14 680 spectra of unknown 1 with indicated fragments originating from structure number 3. (B)
15 681 Possible elemental compositions for m/z 159.0843 and 229.1626. (C) Top 3 Chempider
16 682 possible structures for $C_{12}H_{24}O_2Si$.
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21 684 **Figure 3:** (A) (EI)TOF (top) and (APCI)QTOF low energy spectra of unknown 2 with
22 685 structures of the most abundant fragments (B) Possible molecular structures for unknown 2
23 686 with molecular formula $C_{21}H_{42}O_3$.
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27 688 **Figure 4:** (A) GC-(EI)TOF (top), GC-(APCI)QTOF low energy (middle) and high energy
28 689 (bottom) spectra of unknown 3 with empirical formulae and fragments of the most abundant
29 690 peaks. (B) LC-QTOF spectra of lauro lactam monomer (top), dimer (middle), trimer (bottom).
30 691 (Source structures Stoffers et al., 2003)
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35 693 **Figure 5:** Literature ([38] +LC-MS spectrum (upper left corner) compared to the spectra
36 694 obtained by us on ESI+ LC-QTOF MS (upper right LE mode, lower right HE mode) for
37 695 suggested compound bis(3,4-dimethylbenzylidene)sorbitol.
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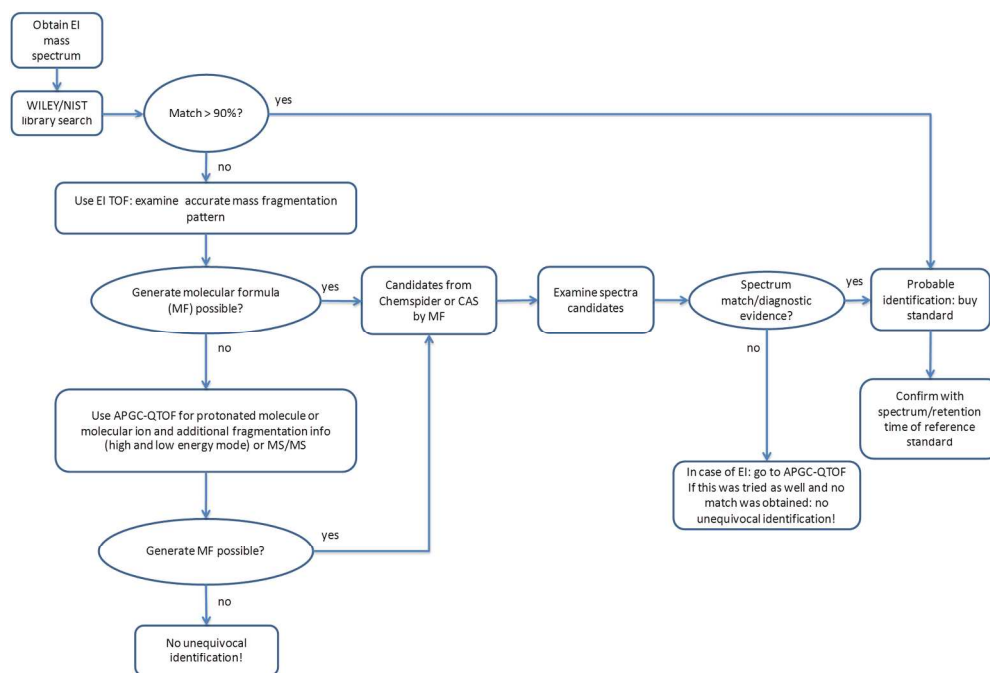
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698 **Table Captions:**

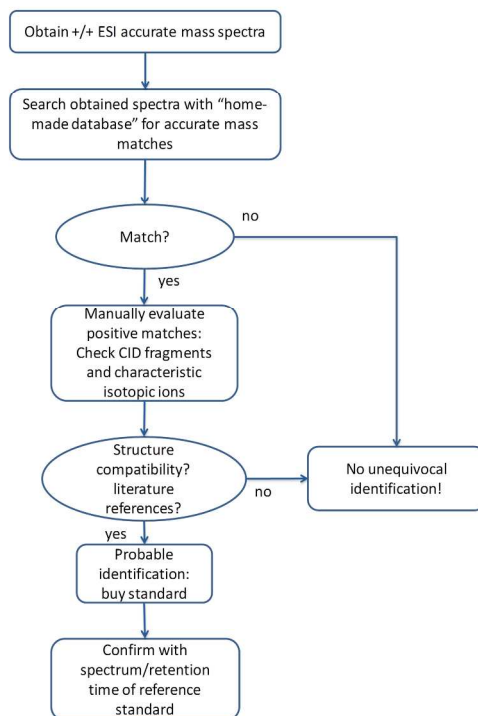
699 **Table 1:** Summary of detected compounds, techniques used and related errors

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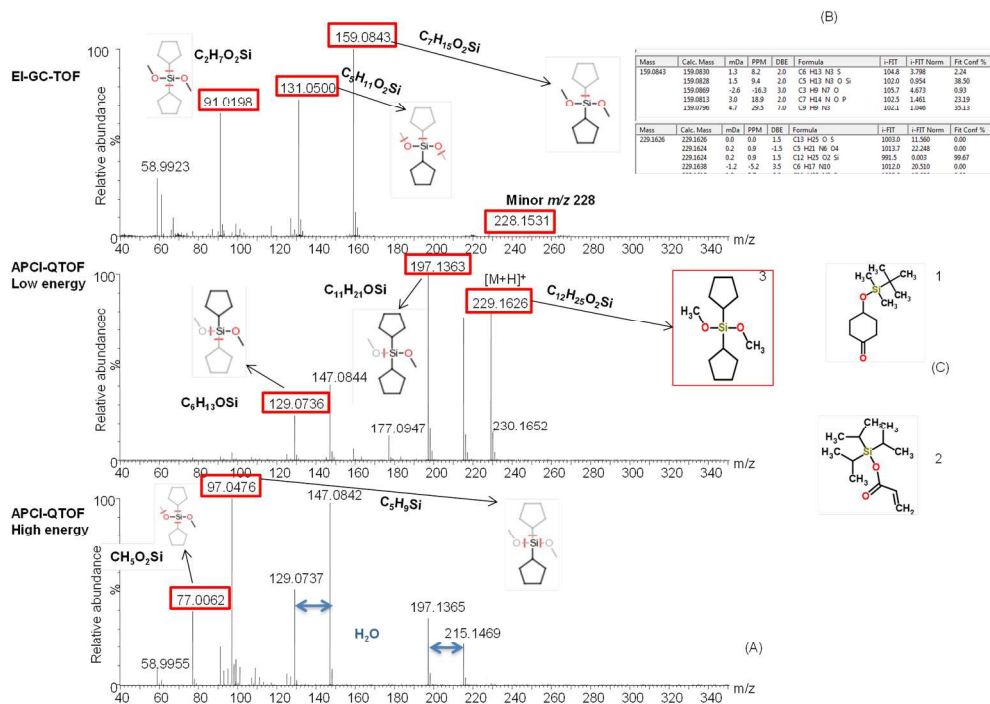
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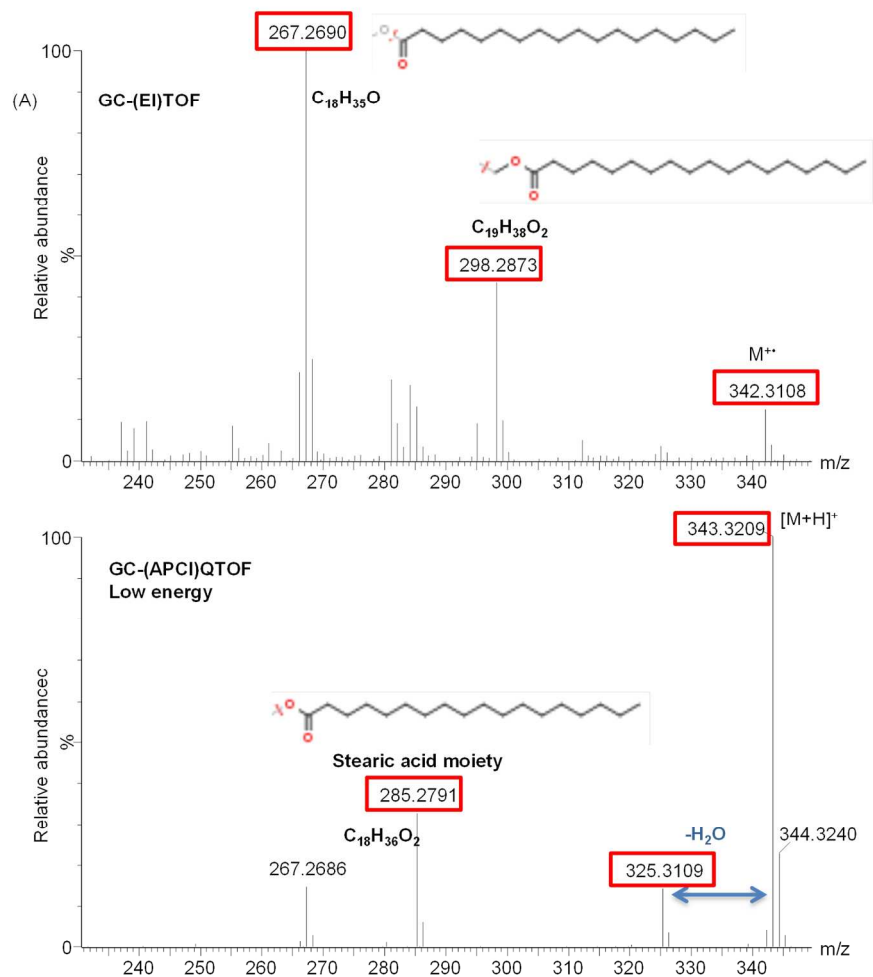
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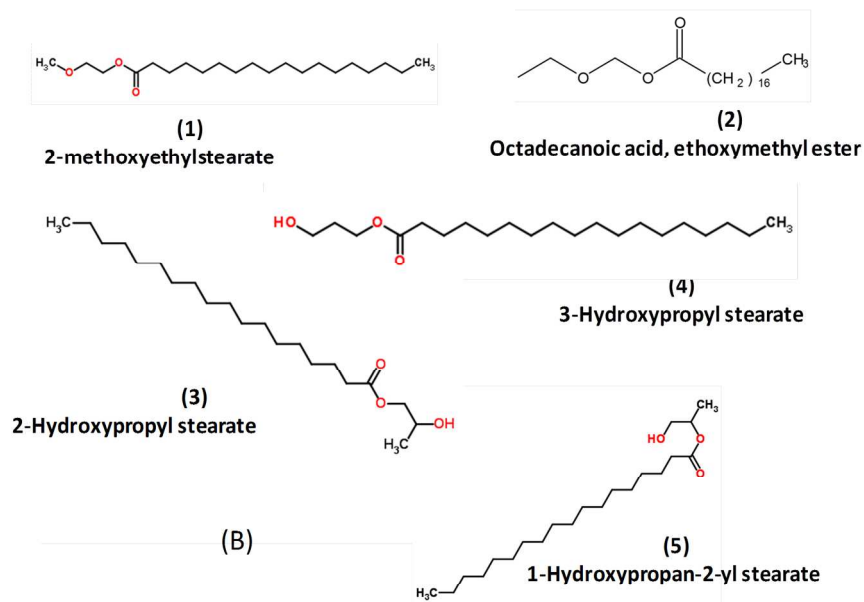
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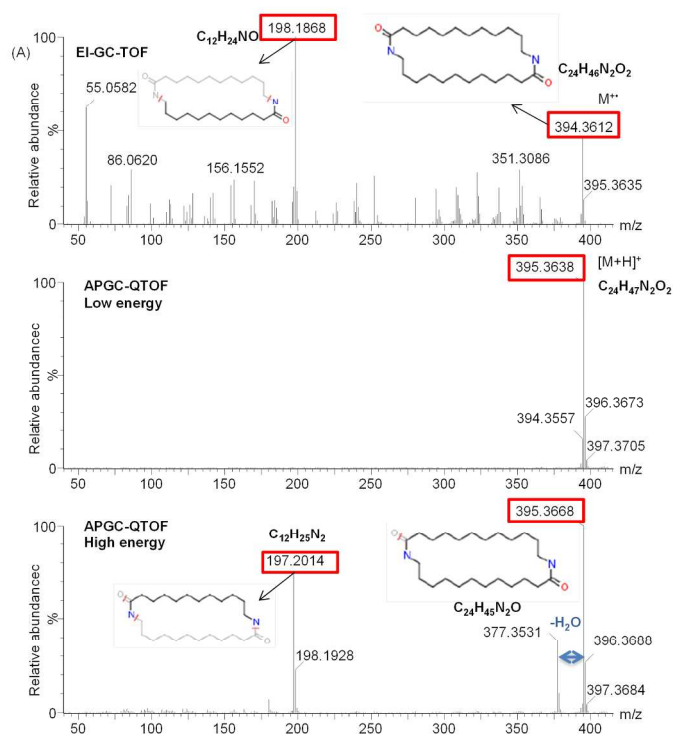
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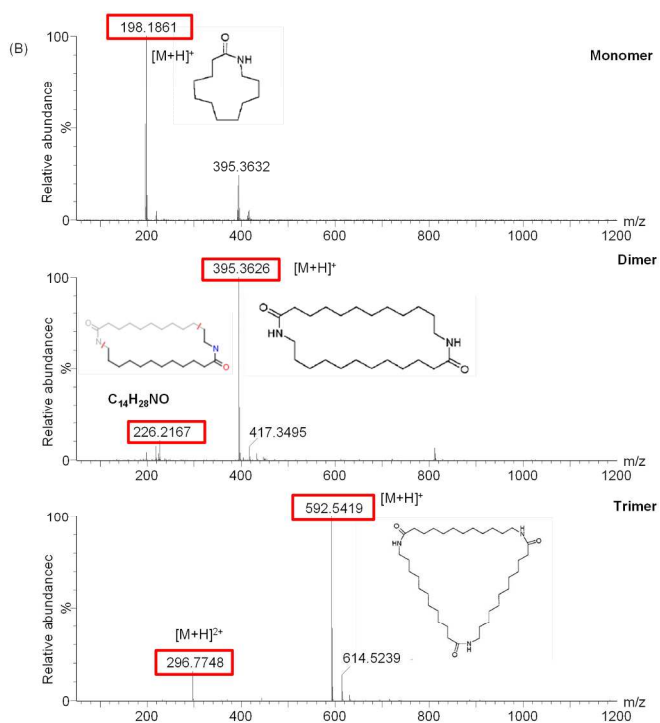
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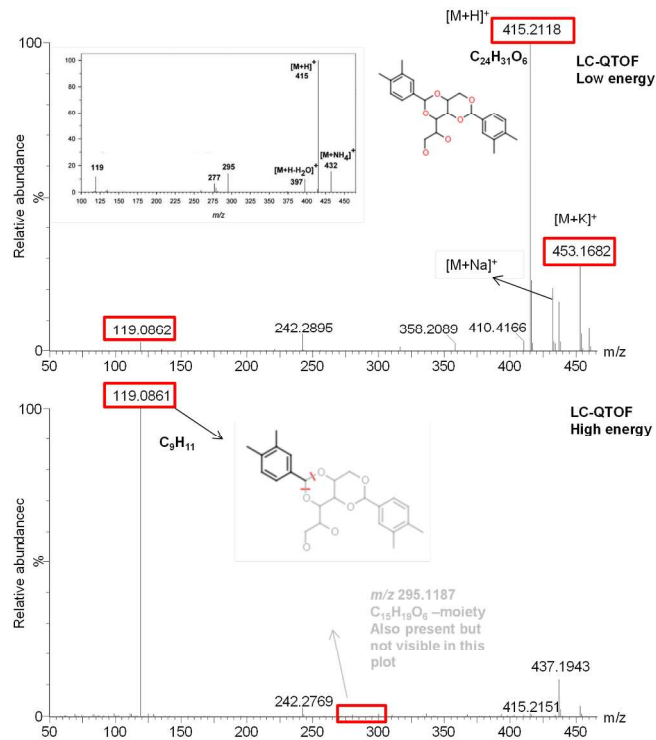
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Table 1: Summary of detected compounds, techniques used and related errors

Compound name	Identification level	Techniques used for identification	GC-(EI)TOF-MS error (ppm)	GC-(APCI)QTOF-MS error (ppm)	LC-QTOF-MS error (ppm)
Dicyclopentyl-dimethoxysilane	1	GC-(EI)MS / GC-(EI)TOF-MS / GC-(APCI)QTOF-MS	-6.6	0.9	/
(2)-hydroxypropylstearate / (3)-hydroxypropylstearate / 1-hydroxypropan-2-yl-stearate	2	GC-(EI)MS / GC-(EI)TOF-MS / GC-(APCI)QTOF-MS	-7.6	-0.9	/
Lauro lactam monomer / dimer / trimer	2	GC-(EI)MS / GC-(EI)TOF-MS / GC-(APCI)QTOF-MS / LC-QTOF-MS	-13.4	0.0	-3.0
bis(3,4-dimethylbenzylidene)sorbitol	2	LC-QTOF-MS	/	/	-0.7
2,5-bis(5'- <i>tert</i> -butyl-2-benzoxaolyl)thiophene	2	LC-QTOF-MS	/	/	-0.9
Irganox 1010	1	LC-QTOF-MS	/	/	4.9
p-t-octylphenol	1	GC-(EI)-MS / GC-(EI)TOF-MS	3.4	/	/
Diisopropylxanthate	2	GC-(EI)MS / GC-(EI)TOF-MS / GC-(APCI)QTOF-MS	6.7	0.6	/
Dibutylphthalate	1	GC-(EI)-MS / LC-QTOF-MS	/	/	1.4
Diisobutylphthalate	1	GC-(EI)-MS / LC-QTOF-MS	/	/	-0.4
Benzoic acid, 4-ethoxy-, ethyl ester	2	LC-QTOF-MS	/	/	1.5

Figure SI-1: GC-(EI)TOF-MS spectrum of dicyclopentyl-dimethoxysilane standard.

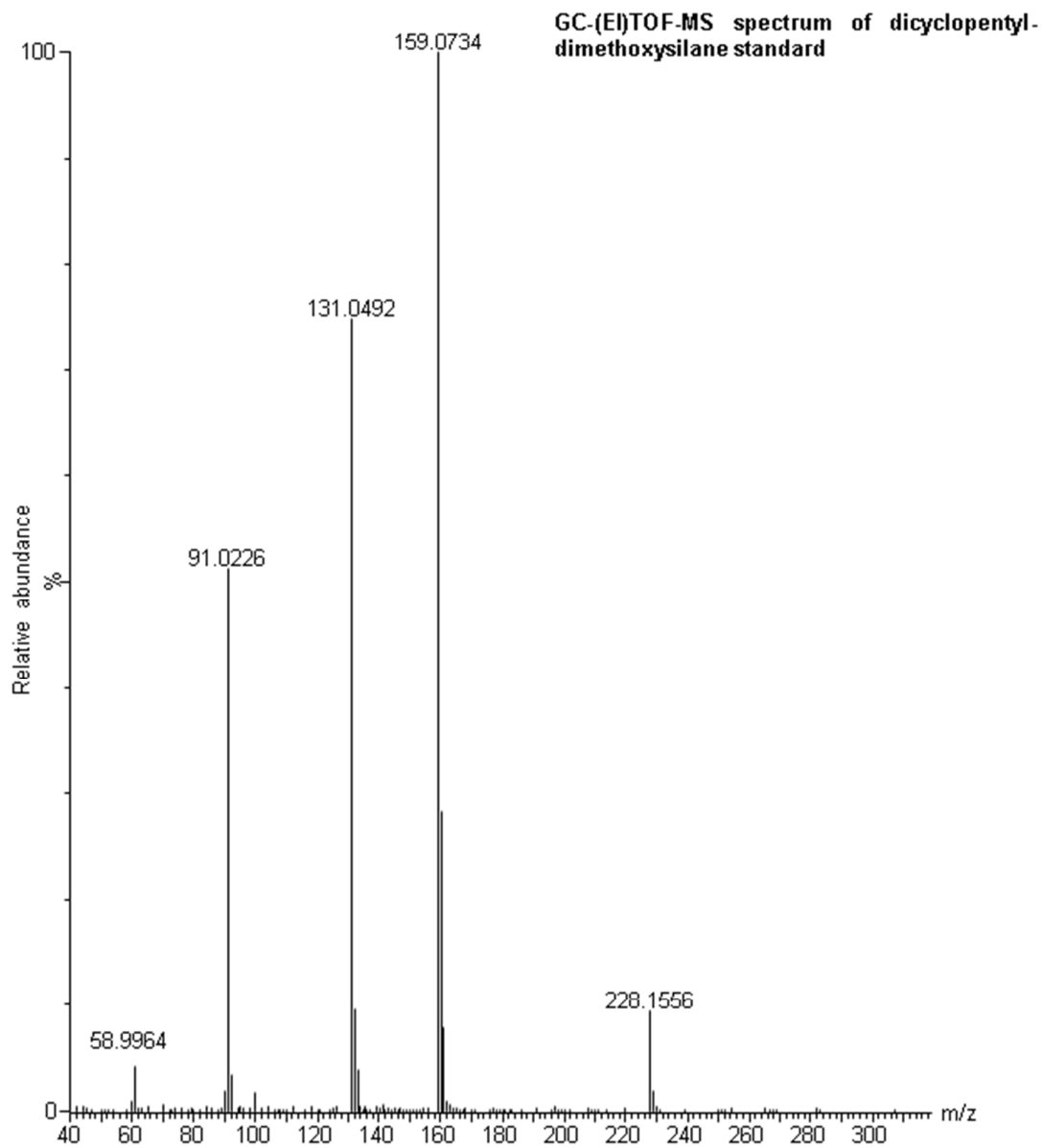


Figure SI-2: Literature (Guo et al. 2013) +LC-MS spectrum (left) compared to the spectra obtained by us on ESI+ LC-QTOF MS (upper right LE mode, lower right HE mode) for suggested compound 2,5-bis(5'-*tert*-butyl-2-benzoxaolyl)thiophene.

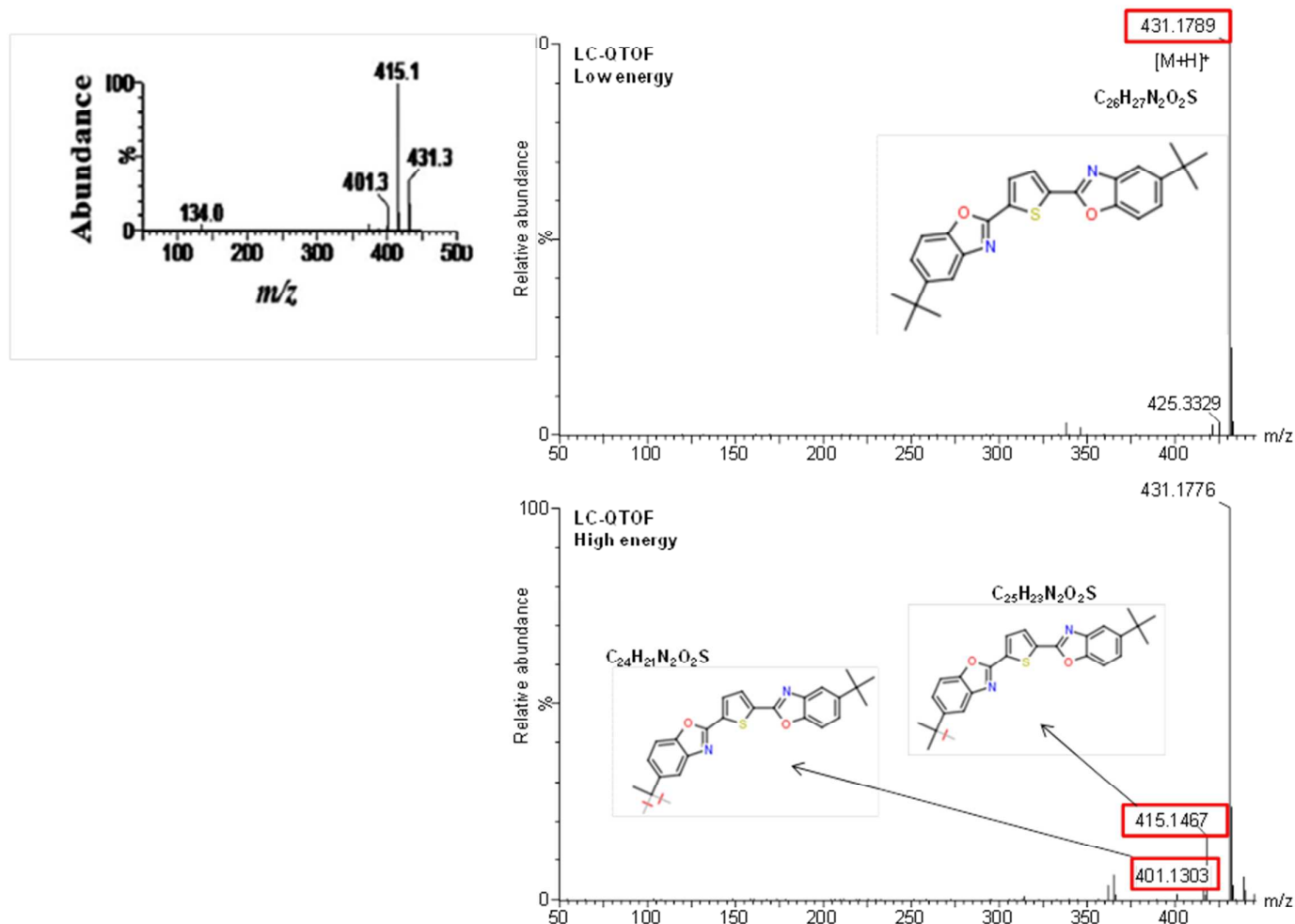


Figure SI-3: ESI(-) LC-QTOF-MS spectrum of Irganox 1010 standard.

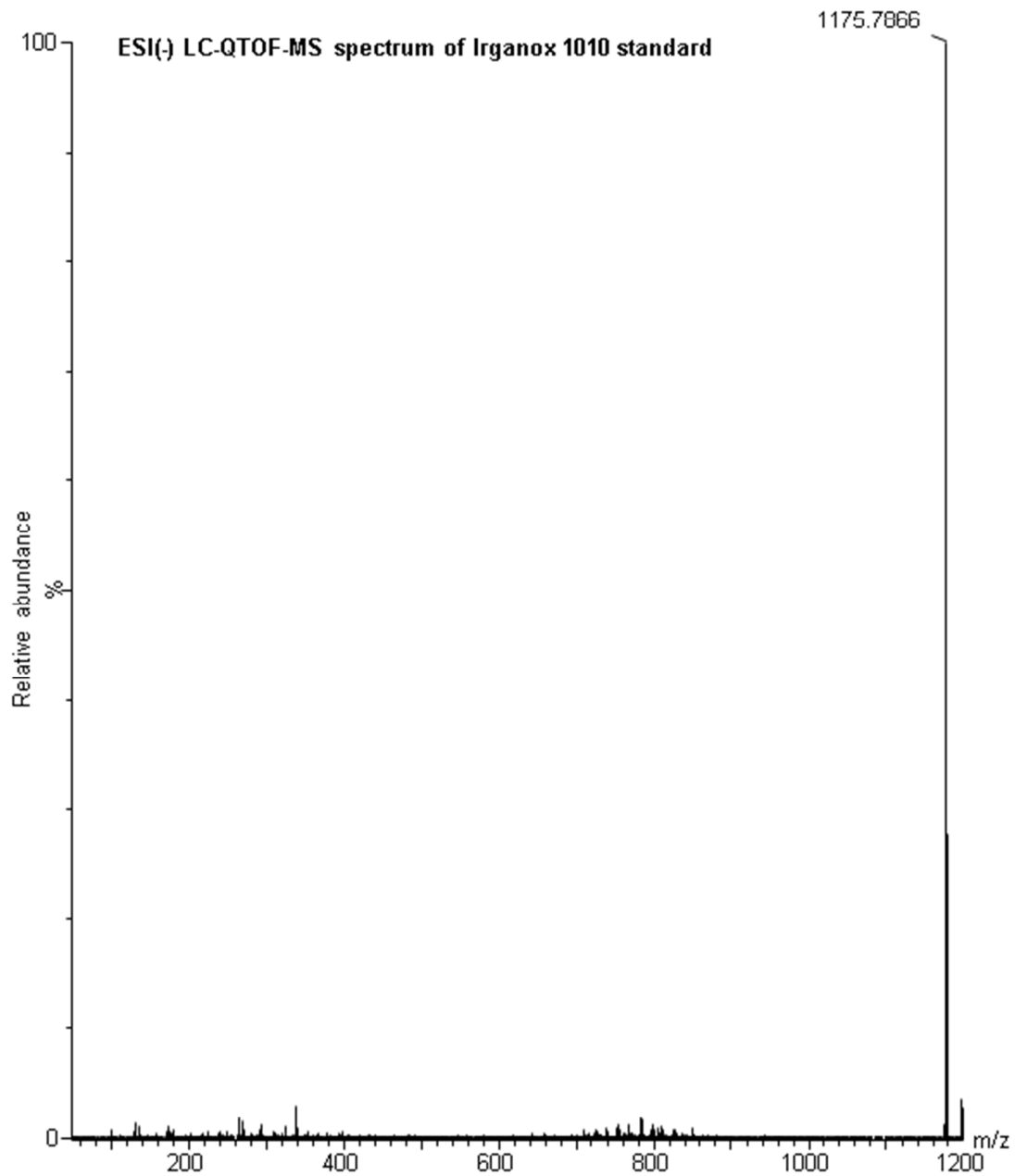
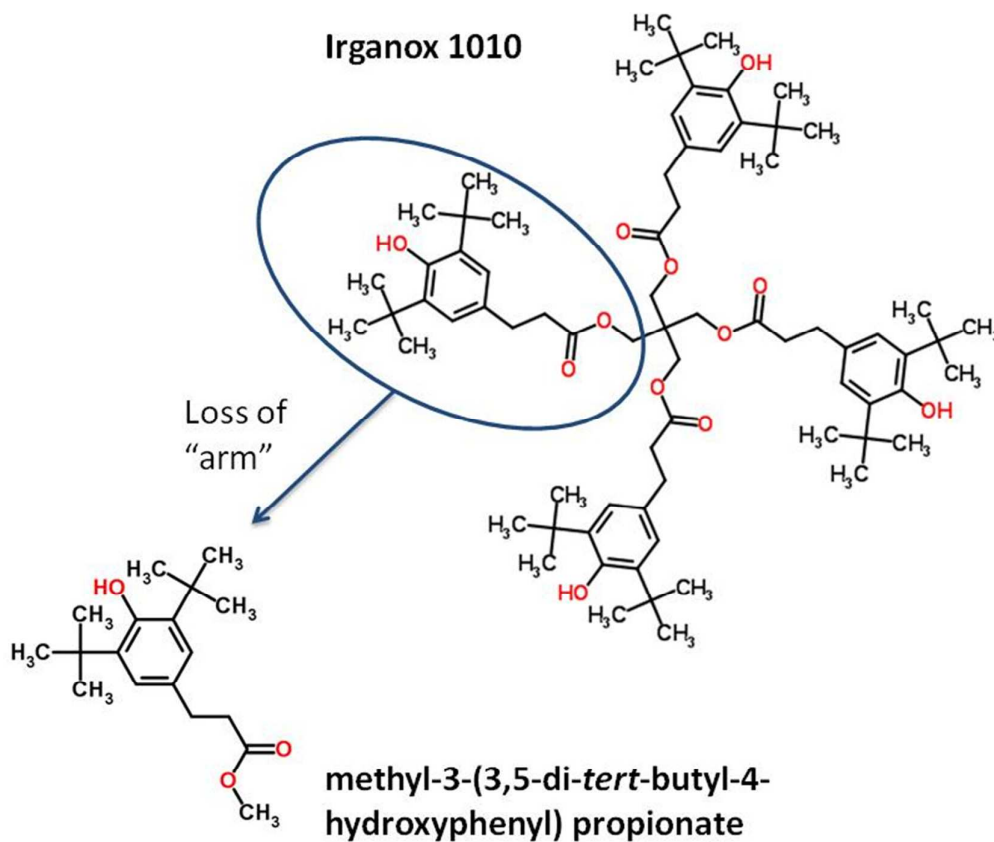


Figure SI-4: Indication of the loss of methyl-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl) propionate (found in GC-MS analysis) from the original structure of Irganox 1010 (found by LC-MS).



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8 **1 Identification of substances migrating from plastic baby bottles using a**
9 **2 combination of low and high resolution mass spectrometric analyzers**
10 **3 coupled to gas and liquid chromatography**
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18 6 Portolés³, Elena Pitarch³, Félix Hernández³, Filip Lemièrè⁴, Adrian Covaci¹
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7 **Abstract**

8 21 This work presents a strategy for elucidation of unknown migrants from plastic food contact
9 22 materials (baby bottles) using a combination of analytical techniques in an untargeted
10 23 approach. First, gas chromatography (GC) coupled to mass spectrometry (MS) in electron
11 24 ionization (EI) mode was used to identify migrants through spectral library matching. When
12 25 no acceptable match was obtained, a second analysis by GC-(EI) high resolution mass
13 26 spectrometry (HRMS) time-of-flight (TOF) was applied to obtain accurate mass
14 27 fragmentation spectra and isotopic patterns. Databases were then searched to find a possible
15 28 elemental composition for the unknown compounds. Finally, a GC hybrid quadrupole QTOF-
16 29 MS with [an](#) atmospheric pressure chemical ionization (APCI) source was used to obtain the
17 30 molecular ion or the protonated molecule. Accurate mass data also provided additional
18 31 information on the fragmentation behaviour as two acquisition functions with different
19 32 collision energies were available (MS^E approach). In the low energy (LE) function, limited
20 33 fragmentation took place, whereas for the high energy (HE) function, fragmentation was
21 34 enhanced. For less volatile unknowns, ultra-high pressure liquid chromatography (UHPLC)-
22 35 QTOF-MS was additionally applied. Using a home-made database containing common
23 36 migrating compounds and plastic additives, tentative identification was made for several
24 37 positive findings based on accurate mass of the (de)protonated molecule, product ion
25 38 fragments and characteristic isotopic ions. Six illustrative examples are shown to demonstrate
26 39 the modus operandi and the difficulties encountered during identification. The combination of
27 40 these techniques was proven to be a powerful tool for the elucidation of unknown migrating
28 41 compounds from plastic baby bottles.
29 42

30 43 **Keywords:** Baby bottles; migration; GC-(Q)TOF-MS; UHPLC-QTOF-MS; food contact
31 44 materials
32 45

46 Introduction

47 Nowadays, there is an increasing concern over the presence of hazardous chemicals in
48 food contact materials (FCMs) [1,2]. Many of these FCMs are made of plastics, which, next
49 to the polymer, contain complex mixtures of compounds, such as monomers, additives,
50 catalysts or degradation products. Consequently, migration of these chemicals from the plastic
51 FCMs into the food could arise, resulting in off-flavours and taints in the food or even
52 harmful effects to human health. For plastic FCMs, all authorized starting substances have
53 been assembled in a Union List in EU Regulation 10/2011 together with their migration limit
54 and/or restricted use. [3]. Furthermore, the use of Bisphenol-A was banned for the
55 manufacture of polycarbonate (PC) infant feeding bottles and their ~~placement~~placing on the
56 European market. [4]. As a consequence, baby bottles made of other polymer types, e.g.
57 polypropylene (PP) or polyamide (PA), are now present on the market.

58 The migration phenomenon in the alternative materials for baby bottles has been
59 understudied up to now and little is known about the possible migrants from these polymer
60 alternatives. GC quadrupole-MS (GC-Q-MS) with electron impact (EI) ionization source has
61 been used to investigate the presence of unknown compounds in food simulant that has been
62 in contact with the alternative baby bottle plastics [5,6]. The drawback of this approach is that
63 a conclusive library match cannot always be obtained when comparing experimental and
64 library EI spectra, as many migrating compounds can be new, unregulated, or even non-
65 intentionally added substances (NIAS); e.g. degradation products of polymerisation reaction,
66 and are thus not included in commercially available libraries.

67 Using high-resolution time-of-flight mass spectrometry (TOF-MS), the identification
68 process improves as accurate masses of the ions are obtained. Moreover, the sensitivity is
69 notably higher than of the quadrupole MS when working in full-spectrum acquisition. The
70 compounds tentatively identified by library matching can be confirmed by checking the
71 accurate-masses of the product ions and the molecular ion (if present in the EI spectrum) and
72 ambiguous results in the library search can be partly resolved [7]. Only recently, such
73 accurate-mass instruments have also been coupled to alternative (softer) ionization sources for
74 GC, e.g. atmospheric pressure chemical ionization (APCI), facilitating the detection of the
75 molecular ion (or protonated molecule) which in turn eases the derivation of possible
76 molecular formulae. The potential of GC-(APCI)TOF-MS has recently been demonstrated in
77 other fields, such as pesticide residue or water analysis [8–10]. To our knowledge, its
78 application to the analysis of migrants from plastic FCMs has been rather limited. This
79 technique has been explored for the analysis of adhesives and non-intentionally added

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7 80 substances [11–13], though no work applying the APCI source was yet conducted on plastic
8 81 baby bottles.

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10 82 To study the migration of non-volatile compounds from FCMs, LC-MS with
11 83 electrospray ionization (ESI) is the most suitable approach to be applied [14]. Only for few
12 84 classes of compounds, such as pharmaceuticals or pesticides, LC mass spectral libraries are
13 85 available due to the prominent spectral differences induced by the use of different ionization
14 86 sources. Therefore, until now, most of the analysis of non-volatile plastic migrants has been
15 86 limited to targeted approaches by monitoring pre-selected families of compounds, such as ~~e.g.~~
16 87 phthalates, UV-ink photoinitiators or antioxidants [14]. On the other hand, the use of HRMS
17 88 is mandatory for screening purposes. LC-TOF-MS has already shown its efficiency for
18 89 screening and confirmation in the analysis of forensic (illicit drugs) and environmental
19 90 samples (pesticides, flame retardants, etc.) [15–20]. Furthermore, few non-targeted studies
20 91 have been published on possible contaminants migrating from FCMs [21–26],
21 92

22 93 The aim of this work was to develop and apply a methodology for the identification of
23 94 unknowns observed during non-targeted screening of plastic migrants from baby bottles,
24 95 based on the use of low and high resolution MS. GC and LC hyphenated to a variety of mass
25 96 analyzers were used for this purpose. To our knowledge, this is the first time that a
26 97 combination of these techniques has been applied in a non-targeted approach to elucidate
27 98 unknown migrants from plastic baby bottles. While it was not the goal of this work to give a
28 99 complete overview of all detected compounds in the tested baby bottles [6], some particular
29 100 examples have been selected to demonstrate the potential of the applied methodology for the
30 101 elucidation of unknown plastic migrants.
31 102

32 103 **Materials and methods**

33 104 *Materials*

34 105 *Samples and sample treatment*

35 106 Ten polypropylene (PP) baby bottles and one polyamide (PA) baby bottle from the Belgian
36 107 market [6], consisting the majority of the market share, were selected for the application of
37 108 the developed methodology. The use of simulants is prescribed in the EU Regulation 10/2011
38 109 to mimic the migration testing towards real foods, leading to the selection of simulant D1
39 110 (water:EtOH (50:50)) as a simulant for milk [3]. After sterilisation of the bottles during ten
40 111 minutes with boiling water, three consecutive migrations for 2h at 70°C were performed with
41 112 the water-EtOH simulant. Afterwards, a non-targeted liquid-liquid extraction with ethyl
42 113 acetate:n-hexane (1:1) was performed on the simulant samples as previously described [6].
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7 114 The obtained organic extracts were then further concentrated to $\pm 75 \mu\text{L}$ under a gentle N_2
8 115 stream for analysis by GC or evaporated until dryness and dissolved in $75 \mu\text{L}$ MeOH for LC
9 116 injection. All bottles were tested in duplicate. Deuterated 2,6-di-*tert*-butyl-4-methylphenol-
10 117 D24 (Campro Scientific GmbH, Berlin, Germany) was added as [an](#) internal standard (IS) for
11 118 GC analysis to the simulant prior to LLE to correct for potential variations in the extraction
12 119 method or instrumental response. For LC, $^{13}\text{C}_{12}$ -Bisphenol-A was selected (Cambridge
13 120 Isotope Laboratories, Inc. Andover, Massachusetts, USA).
14 121
15 122

18 122 *Chemicals*

19 123 Methanol (gradient grade for liquid chromatography LiChrosolv) and ethyl acetate (for liquid
20 124 chromatography LiChrosolv) were purchased from Merck (Darmstadt, Germany). N-hexane
21 125 (for residue analysis and pesticides, 95%) was purchased from Acros Organics (Geel,
22 126 Belgium). Ultrapure water was prepared by means of an Elga Purelab Prima (Tienen,
23 127 Belgium). Helium (99.999%) and nitrogen (99.99%) were purchased from Air Liquide (Liège,
24 128 Belgium). For GC-(Q)TOF-MS analysis hexane for ultra-trace analysis grade was purchased
25 129 from Scharlab (Barcelona, Spain). For UHPLC-QTOF-MS analysis HPLC-grade methanol
26 130 (MeOH), acetonitrile (ACN) and sodium hydroxide (>99%) were purchased from ScharLab
27 131 (Barcelona, Spain). Formic acid (HCOOH) (>98% w/w) was obtained from Fluka. HPLC-
28 132 grade water was obtained from deionized water passed through a Milli-Q water purification
29 133 system (Millipore, Bedford, MA, USA). Dicyclopentyl-dimethoxysilane (>98%) was
30 134 purchased from TCI chemicals (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan).
31 135 Pentaerythritol tetrakis(3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionate) (98%) was
32 136 purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany).
33 137

41 138 *Methods*

42 139 *GC-(EI)MS*

43 140 Initial non-target analyses of simulant extracts were performed with an Agilent 6890 gas
44 141 chromatograph coupled to an Agilent 5973 mass selective detector (MSD) equipped with an
45 142 electron impact (EI) ionization source and operated in full scan mode from m/z 40 to 700. The
46 143 GC column was a $30 \text{ m} \times 0.25 \text{ mm} \times 0.25 \mu\text{m}$ DB-5ms column (Agilent JW Scientific,
47 144 Diegem, Belgium). The temperature of the oven was set at 60°C for 3 min, and was then
48 145 increased to 300°C at a rate of $10^\circ\text{C min}^{-1}$ where it was held for 15 min. The total run-time
49 146 was 42 min. Helium was used as a carrier gas, ~~this~~ with a constant flow rate of 1.0 mL min^{-1} .
50 147 A volume of $2 \mu\text{L}$ extract was injected so that a sufficiently detectable amount of analyte was
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7 148 brought on the column. The MS spectra obtained for the migrating chemicals extracted by the
8 149 simulant were compared with commercially available WILEY and NIST mass spectra
9 150 libraries by use of the Agilent MSD Chemstation® for peak identification.
10 151

12 152 *GC-(EI)TOF-MS*

13
14 153 An Agilent 6890N GC system (Palo Alto, CA) equipped with an Agilent 7683 autosampler,
15 154 was coupled to a GCT time-of-flight (TOF) mass spectrometer (Waters Corporation,
16 155 Manchester, U.K.), operating in EI mode (70 eV). The GC separation was performed using
17 156 the same column type and oven program as for the GC-(EI)MS. The interface and source
18 157 temperatures were both set to 250°C and a solvent delay of 3 min was selected. The TOF-MS
19 158 was operated at 1 spectrum/s acquisition rate over the mass range m/z 50-700, using a
20 159 multichannel plate voltage of 2800 V. TOF-MS resolution was approximately 8500 at full
21 160 width half maximum (FWHM) at m/z 614. Heptacosafuorotributylamine (Sigma Aldrich,
22 161 Madrid, Spain), used for the daily mass calibration and as lock mass, was injected via syringe
23 162 in the reference reservoir at 30°C to monitor the m/z ion 218.9856. The application manager
24 163 ChromaLynx, also a module of MassLynx software, was used to investigate the presence of
25 164 unknown compounds in samples. Library search was performed using the commercial NIST
26 165 library.
27 166

34 167 *GC-(APCI)QTOF-MS*

35 168 An Agilent 7890A GC system (Palo Alto, CA, USA) coupled to a quadrupole TOF mass
36 169 spectrometer XevoG2 QTOF (Waters Corporation, Manchester, UK) with an APCI source
37 170 was used. The instrument was operated under MassLynx version 4.1 (Waters Corporation).
38 171 Sample injections were made using an Agilent 7693 autosampler. The GC separation was
39 172 performed using the same conditions as described in the previous 2 GC techniques. 1 μL was
40 173 injected at 280°C under splitless mode. Helium was used as carrier gas at 1.2 mL min^{-1} . The
41 174 interface temperature was set to 310°C using N_2 as auxiliary gas at 150 L h^{-1} , make up gas at
42 175 300 mL min^{-1} and cone gas at 16 L h^{-1} . The APCI corona pin was operated at 1.6 μA with a
43 176 cone voltage of 20 V. The ionization process occurred within an enclosed ion volume, which
44 177 enabled control over the protonation/charge transfer processes. Xevo QTOF-MS was operated
45 178 at 2.5 spectra/s acquiring a mass range m/z 50–1200. TOF-MS resolution was approximately
46 179 18 000 (FWHM) at m/z 614. For MS^E measurements, two alternating acquisition functions
47 180 were used applying different collision energies: a low-energy function (LE), selecting 4 eV,
48 181 and a high-energy function (HE). In the latter case, a collision energy ramp (25-40 eV) rather
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7 182 than a fixed higher collision energy was used. Heptacosfluorotributylamine (Sigma Aldrich,
8 183 Madrid, Spain) was used for the daily mass calibration. Internal calibration was performed
9 184 using a background ion coming from the GC-column bleed as lock mass (protonated molecule
10 185 of octamethyl-cyclotetrasiloxane, m/z 297.0830). MassFragment software (Waters) was used
11 186 to explain the fragmentation behavior of the detected compounds. This software applies a
12 187 bond disconnection approach to suggest possible structures for the product ions from a given
13 188 molecule.
14 189

18 190 *LC-QTOF-MS*

19 191 A Waters Acquity UPLC system (Waters, Milford, MA, USA) was interfaced to a hybrid
20 192 quadrupole-orthogonal acceleration-TOF mass spectrometer (XEVO G2 QTOF, Waters
21 193 Micromass, Manchester, UK), using an orthogonal Z-spray-ESI interface operating in positive
22 194 and negative ionization modes. The UPLC separation was performed using an Acquity UPLC
23 195 BEH C18 1.7 μm particle size analytical column 100 mm L \times 2.1 mm I.D. (Waters) at a flow
24 196 rate of 300 $\mu\text{L min}^{-1}$. The mobile phases used were A= H_2O with 0.01% HCOOH and
25 197 B=MeOH with 0.01% HCOOH. The percentage of organic modifier (B) was changed linearly
26 198 as follows: 0 min, 10%; 14 min, 90%; 16 min, 90%; 16.01 min, 10%; 18 min, 10%. Nitrogen
27 199 (from a nitrogen generator) was used as the drying and nebulizing gas. The gas flow was set
28 200 at 1000 L h^{-1} . The injection volume was 20 μL . The resolution of the TOF mass spectrometer
29 201 was approximately 20,000 at full width half maximum (FWHM) at m/z 556. MS data were
30 202 acquired over an m/z range of 50–1200. A capillary voltage of 0.7 and 2.5 kV was used in
31 203 positive and negative ion modes, respectively. A cone voltage of 20 V was used. Collision gas
32 204 was argon 99.995% (Praxair, Valencia, Spain). The interface temperature was set to 600°C
33 205 and the source temperature to 130°C. The column temperature was set to 40°C.

34 206 For MS^E experiments, two acquisition functions with different collision energies were
35 207 created. The first one, the low energy function (LE), selecting a collision energy of 4 eV, and
36 208 the second one, the high energy (HE) function, with a collision energy ramp ranging from
37 209 25 eV to 40 eV in order to obtain a greater range of product ions. The LE and HE functions
38 210 settings were for both a scan time of 0.4 s.

39 211 Calibrations were conducted from m/z 50 to 1200 with a 1:1 mixture of 0.05 M NaOH:5%
40 212 HCOOH diluted (1:25) with acetonitrile:water (80:20). For automated accurate mass
41 213 measurement, the lock-spray probe was used, using as lockmass a solution of leucine
42 214 enkephalin (10 $\mu\text{g mL}^{-1}$) in acetonitrile:water (50:50) at 0.1% HCOOH pumped at 20 μL
43 215 min^{-1} through the lock-spray needle. The leucine enkephalin $[\text{M}+\text{H}]^+$ ion (m/z 556.2771) for

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7 216 | positive ionization mode; and $[M-H]^-$ ion (m/z 554.2615) for negative ionization; were used
8 217 | for recalibrating the mass axis and to ensure a robust accurate mass measurement over time. It
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10 218 | should be noted that all the exact masses shown in this work have a deviation of 0.55 mDa
11 219 | from the “true” value, as the calculation performed by the MassLynx software uses the mass
12 220 | of hydrogen instead of a proton when calculating $[M+H]^+$ exact mass. However, because this
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14 221 | deviation is also applied during mass axis calibration, there is no negative impact on the mass
15 222 | errors presented in this article. MS data were acquired in centroid mode and were processed
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17 223 | by the ChromaLynx XS application manager (within MassLynx v 4.1; Waters Corporation).
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19 225 | *Data processing*

20 226 | *GC data processing*

21 227 | A schematic overview of the GC approach is given in Figure 1a. The analytical
22 228 | strategy to perform a non-target analysis with GC-MS techniques started from the results
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24 229 | obtained in our previous work [6]. In a first screening based on GC-(EI)MS data using
25 230 | commercially available WILEY and NIST libraries with Agilent MSD Chemstation®
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27 231 | software, peaks with an area of at least 10% of the area of the internal standard were selected
28 232 | for identification. Only compounds with library matches above 90% were accepted as
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30 233 | tentative candidates. When the returned match was below 90%, peaks were defined as
31 234 | “unidentified” as they were most probably not included in the commercial libraries and
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33 235 | further research was conducted with GC-(EI)TOF-MS based on accurate mass data.

34 236 | By means of the ChromaLynx Application Manager, a module of Masslynx software,
35 237 | the remaining unidentified peaks were deconvoluted and searched again in the commercial
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37 238 | nominal mass NIST02 library. A hit list with five positive matches > 700 was generated.
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39 239 | Next, an elemental composition calculator (maximum deviation 5 mDa) was applied to
40 240 | determine the five most likely formulae of the five most intense ions acquired in the accurate
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42 241 | mass spectrum. The proposed formulae of these five fragments were then compared with the
43 242 | proposed molecular formulae of the top-five library hits using criteria like mass error and
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45 243 | isotopic fit. When a possible molecular formula could be derived in this way, candidates with
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47 244 | this particular empirical formula were searched in the Chemspider ~~internet~~ database. By using
48 245 | the ChromaLynx MassFragment, which is a tool for fragmentation prediction, the obtained
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50 246 | accurate mass EI spectrum could be compared with the predicted fragments of a selected
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52 247 | possible structure and scorings were given. In this way, a differentiation could also be made
53 248 | between different structures with same empirical formula and those which generate fragments
54 249 | which are not in accordance with the obtained experimental spectrum, could be rejected.

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7 250 When no conclusive match could be obtained (e.g. more than one identity fit of
8 251 possible molecular formulae with the experimental GC-(EI)TOF spectrum), the samples could
9 252 be re-injected into the GC-(APCI)QTOF system to confirm or exclude preceding tentative
10 253 GC-(EI)TOF identifications. Due to the reduced fragmentation generally occurring in the
11 254 APCI source, a search was conducted for the accurate mass molecular ion and the protonated
12 255 molecule of the suggested molecular formulae candidates from the (EI)TOF. If one of the two
13 256 was present, a narrow window-extracted ion chromatogram (nw-XIC, ± 0.02 Da) resulted in a
14 257 chromatographic peak eluting approximately 2 minutes earlier than the values obtained in the
15 258 GC-(EI)TOF-MS. If no chromatographic peak appeared performing the nw-XIC for the
16 259 selected masses, the obtained spectrum at the expected retention time was manually examined
17 260 for other possible ions that could be the M^{++} or $[M+H]^+$. In this case, by comparing the
18 261 (EI)TOF and the (APCI)QTOF spectra, generally M^{++} or $[M+H]^+$ could be retrieved as often
19 262 the (EI)TOF spectrum still contains minor amounts of M^{++} (or $[M+H]^+$) which are more
20 263 abundant in the (APCI)QTOF. Again, the elemental composition software (± 5 mDa) was used
21 264 to determine the molecular formula of the unknown compound. Then, the fragmentation
22 265 pattern in the (APCI)QTOF of the unknown compound was studied by examining the MS^E
23 266 data, which provide useful further information about the fragmentation. Normally, the HE
24 267 mode offers most information about how the compound fragments as the presence of M^{++} or
25 268 $[M+H]^+$ diminishes and fragmentation increases. For some compounds, quite severe
26 269 fragmentation occurs already in the LE mode. Experimentally recorded fragmentation patterns
27 270 can also here be compared with software generated ones for possible candidates by the use of
28 271 MassFragment. When commercially available, standards were bought to confirm the actual
29 272 presence of the suggested compounds.
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41 274 *LC data processing*

42 275 A graphical overview of the LC-workflow was given in Figure 1b. No commercial MS
43 276 libraries of common plastic migrants are available for LC-MS, and a genuine non-target
44 277 approach of the raw data would result in a far too laborious data processing. Therefore, we
45 278 constructed a home-made database to facilitate a wide-scope suspect screening. By including
46 279 the empirical formula of a compound in the database, the ChromaLynx software processes
47 280 this against the obtained accurate mass spectra and positive matches are returned if the mass
48 281 error (± 0.002 Da) is appropriate. First, approximately 50 migrants that were previously
49 282 detected in the alternative plastics to PC baby bottles were included in this list [5,6]. Because
50 283 all analytical standards of these compounds were available to us, their experimental data
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7 284 (retention time and product ions) were also included in the database. Second, the empirical
8 285 formulae of around 190 common plastic additives were added, since these compounds could
9 286 also migrate from the alternative plastics. Last, more than 800 compounds authorised for
10 287 plastic FCMs by the European Union Regulation No. 10/2011 [3] were included in the
11 288 database.

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14 289 For most compounds in this database, the only criterion to obtain a positive match was
15 290 to search by the exact mass of the empirical formula. This commonly led to several false
16 291 positive hits. Therefore, every positive hit (a peak detected, commonly corresponding to the
17 292 exact mass of the (de)protonated molecule) was checked manually evaluating the product ions
18 293 and characteristic isotopic ions, leading to the tentative identification of the candidate, based
19 294 on structure compatibility and comparison with available literature data. Adducts, such as
20 295 $[M+Na]^+$ or $[M+K]^+$, were also included to facilitate the detection of some compounds in
21 296 those cases where information existed on their possible formation. Also here, the analytical
22 297 standards were purchased for confirmation when commercially available.
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28 299 **Results and Discussion**

29 300 *Selection of techniques*

30 301 Until now, most analytical methods employed for the determination of plastic migrants
31 302 have been focused on the targeted analysis of a restricted number of a priori selected
32 303 compounds [27–29]. However, potential migrating compounds other than the target analytes
33 304 cannot be detected using this approach. Electron impact (EI) ionization used in GC produces
34 305 highly reproducible fragmentation spectra which makes the identification of unknown
35 306 compounds possible by comparison with commercially available mass spectral libraries (e.g.
36 307 Wiley, NIST). Due to its ability to obtain sensitive full scan data and accurate mass
37 308 measurements [7,30,31], GC-TOF-MS and hybrid quadrupole-TOF-MS (QTOF-MS) are
38 309 powerful mass analyzers for a wide variety of non-target applications for semi-volatiles
39 310 [7,32]. Due to a high degree of fragmentation in EI ionization, the molecular ion has often a
40 311 low abundance. This is an important limitation for structural elucidation, as the presence of
41 312 the molecular ion in a mass spectrum, especially if measured at accurate mass, provides
42 313 crucial information. In APCI ionization, a stable (quasi)molecular ion is formed by means of
43 314 charge transfer ($M^{+•}$) and/or by protonation ($[M+H]^+$). The APCI interface used in GC can be
44 315 coupled with a wide range of high resolution mass analyzers (TOF, QTOF).
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53 316 For LC analysis, the accurate-mass product ion spectra obtained in MS/MS mode on
54 317 the QTOF-MS provide relevant structural information. However, since the pre-selection of
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7 318 analyte precursor ions has to be done in the quadrupole, this results in the usual loss of
8 319 isotopic pattern information. This drawback can be overcome by MS^E data-acquisition, in
9 320 which both accurate-mass (de)protonated molecule (LE function) and product ions (HE
10 321 function) are obtained in the same injection without the need of selecting any precursor ion.
11 322 The sequential collection of LE and HE data during sample analysis is a significant advantage
12 323 towards the structural elucidation of unknown compounds in a non-targeted screening
13 324 approach [33].

14 325 In this manuscript, we have included a selection of examples to demonstrate the
15 326 developed strategy for the elucidation of unknown migrants from plastic baby bottles. The
16 327 selection of the cases was based on their ability to illustrate the contribution of each ionization
17 328 technique and mass analyzer towards the final identification. A detailed overview of all
18 329 identified compounds and the used techniques can be found in Table 1 ~~of the Supplemental~~
19 330 ~~Information (SI)~~. Since most migrating compounds are small molecules (molecular weight <
20 331 1200 Da), the parameters to calculate the possible molecular formulae with the Elemental
21 332 Composition software were generally set as follows: C: 0-50, H: 0-100, O: 0-10, N: 0-10 and
22 333 P: 0-5. Other atoms were included in the search if after manual inspection of the spectrum the
23 334 isotope pattern indicated the presence of other elements. A maximum deviation of 2 mDa
24 335 from the measured mass was applied. When searching for the M⁺ (if existing), the option
25 336 'odd-electron ions only' was added. For [M+H]⁺, this option was 'even-electron ions only'.
26 337 For fragments, both odd and even options were selected. Within the workflows proposed in
27 338 Figure 1a and 1b, the criteria introduced by Schymanski et al. [34] were used towards the
28 339 acceptance of an unambiguous identification of a compound. Here, five different levels of
29 340 identification were defined, each with their corresponding requirements varying from a level 5
30 341 mass of interest identification to an unequivocal molecular formula (level 4), tentative
31 342 candidate (level 3), probable structure (level 2) and confirmed structure (level 1). Due to the
32 343 lack of commercial availability or sometimes relatively high prices of some products, not all
33 344 analytical standards of tentatively identified migrants were obtained. Here, identification was
34 345 only done until level 2 of these criteria.

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347 *Case study 1*

348 In the GC-(EI)MS, an unknown chromatographic peak with a retention time of 14.30
349 min was detected in most PP samples tested. No firm library match was obtained and scores
350 were very poor (<70%). Due to its detection frequency and because the intensity was
351 comparable to that of the internal standard ($\pm 10 \mu\text{g kg}^{-1}$ assuming an equal response factor,

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7 352 which is a considerable amount for plastic migrants), this compound was of major interest.
8 353 Therefore, the compound was analysed further with GC-(EI)TOF-MS (Fig 1a). When
9 354 performing a database search using the accurate mass fragmentation data obtained, no
10 355 improvement in the match factors was perceived. Regarding the (EI)TOF spectrum (Figure 2),
11 356 the ion m/z 159.0843 would be assumed to be the possible M^{++} . A clear isotope pattern at $M+1$
12 357 and $M+2$ was seen and therefore both S and Si were included for the Elemental Composition
13 358 search. This resulted in five possible molecular formulae, though only two of them ($C_6H_{13}N_3S$
14 359 and $C_5H_{13}N_3OSi$) could possibly explain the isotope pattern seen.

15 360 Looking at the LE APCI spectrum (Figure 2), m/z 229.1626 is the highest mass
16 361 acquired, suggesting that this would be the M^{++} or $[M+H]^+$ of the unknown compound and
17 362 that 159.0843 is a major fragment ion. Indeed, a very small and hardly visible peak was
18 363 perceived at m/z 228.1531 in the (EI)TOF spectrum, suggesting that m/z 229.1626 was
19 364 $[M+H]^+$. A large number of molecular formulae (>20) were calculated, but after considering
20 365 the mass errors, only three formulae remained. Of these three, already one could be discarded,
21 366 as $C_5H_{21}N_6O_4$ is not an existing chemical structure. This reduced the possible empirical
22 367 formulae to $C_{13}H_{24}OS$ or $C_{12}H_{24}O_2Si$. Investigating the isotope ratios and the elemental
23 368 compositions of the fragments starting from these two formulae, the option implying a Si
24 369 atom clearly fitted best to the obtained spectra. A number of 116 positive hits were returned
25 370 when searched in the Chemspider database. At this point, an [internet literature](#) search using
26 371 the term ' $C_{12}H_{24}O_2Si$ + polypropylene' quickly returned the suggestion of dicyclopentyl-
27 372 dimethoxysilane (structure 3, Figure 2). This alkyl silane is used in combination with Ziegler-
28 373 Natta catalysts to increase the isotactic index of PP [35]. This structure was also suggested by
29 374 Chemspider as the third most cited one. The first two structures (Figure 2) were considered as
30 375 well, but already when checking the APCI spectrum with the MassFragment prediction
31 376 software, the ions m/z 197.1363 (loss of CH_4O), 159.0844 (loss of C_5H_{10}) or 129.0736 (loss of
32 377 $C_6H_{12}O$) could only be explained by structure 3. The respective masses m/z 215.1469,
33 378 177.0947 and 147.0844 could be explained as the adduction of a water molecule to these
34 379 fragments. The inclusion of a small amount of water in the APCI source to promote the
35 380 formation of the $[M+H]^+$ could explain this phenomenon as already described by Wachsmuth
36 381 et al [36]. Therefore, dicyclopentyl-dimethoxysilane was retained as the probably identified
37 382 migrant. The presence of this compound (level 1 identification) was afterwards
38 383 unambiguously confirmed by injection of the purchased commercial standard (Figure SI-1).

39 384
40 385 *Case study 2*

Two peaks with an EI spectrum that exhibited similarities to those of the previously identified [6], respectively hexa- (22.54 min) and octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (24.22 min), were found in a PP sample at high intensities (more than 6 times the area of the IS). Library matching gave poor results (<70%) and did not suggest any structures with realistic possibilities either. The abundant presence of ion m/z 343.3209 in the LE function of the (APCI)QTOF suggested that for the compound related to the octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester this had to be the M^{++} or $[M+H]^+$. The low abundant presence of ion m/z 342.3108 in the (EI)TOF spectrum indeed confirmed that ion m/z 343.3209 was the protonated molecule, resulting in a molecular formula of $C_{21}H_{42}O_3$. Chemspider returned 59 possible structures for this empirical formula. The presence of ions m/z 284.2723 and 285.2791 in the (EI)TOF and the LE (APCI)QTOF spectrum, respectively, indicated the presence of an integral stearic acid moiety ($C_{18}H_{36}O_2$) in the structure, which made us discard all other possible molecular structures and thus, only five possibilities remained (see Figure 3B). The detection of this m/z also revealed that, for the remaining C_3H_6O moiety, the position of the third O-atom of this molecule had to be at the ultimate or the penultimate C-atom, whether or not incorporated as an ether (structures 1 and 2) or as an alcohol group (structures 3-5) (Figure 3B). Indeed, to explain the presence of fragment m/z 284.2723, the rules of the McLafferty rearrangement had to be applied, stating that the sixth atom starting from the carbonyl-O has to be a hydrogen atom. In this way, structure 2 (Figure 3B) could already be rejected as a possibility. The presence of m/z 325.3109 in the LE (APCI)QTOF spectrum, explained by the loss of a water molecule, suggests, on the other hand, the presence of a free alcohol group instead of an ether, because the loss of water is easier and more probable in this case, which eliminates structure 1 as well. Within the available MS spectra, it was not possible though to differentiate between the remaining structural isomers of structures 3-5 to determine which the actual unknown migrant was and only a probable identification could be reached (level 2). Injection of the different analytical standards is the only way to bring a decisive answer here. For the hexadecanoic acid based unknown migrant, the same conclusions could be drawn.

Case study 3

In this case, an unknown compound with a double intensity of the IS peak was seen in the first migration step of the PA bottle, though it completely disappeared in the next migration steps. Both GC-(EI)MS and GC-(EI)TOF-MS database searches gave poor matches (<40%), indicating that the structure of the unknown migrant was very different from the

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7 420 structures present in the database. The abundant ion m/z 394.3612 in the GC-(EI)TOF-MS
8 421 (RT 31.79 min) seemed to be the M^+ , which was indeed confirmed by the highly abundant
9 422 presence of m/z 395.3638 (protonated molecule) in the LE GC-(APCI)QTOF-MS spectrum.
10 423 Since no significant isotope patterns were noticed, an elemental composition search including
11 424 only elements C, O, H and N resulted in a molecular formula of $C_{24}H_{46}N_2O_2$ (mass error of -
12 425 0.2 mDa) for which Chempider returned 32 hits. For this molecular formula, all fragment
13 426 ions of both GC-(EI)TOF-MS and the HE of the GC-(APCI)QTOF-MS could be explained
14 427 with very low mass errors (generally <2 mDa for the TOF and <0.2 mDa for the QTOF),
15 428 differentiating clearly the realistic possible fragments. It was noticeable that the most
16 429 abundant (EI)TOF-MS ion (m/z 198.1868, $C_{12}H_{24}NO$) and the second most abundant
17 430 (APCI)QTOF-MS fragment ion (m/z 197.2014, $C_{12}H_{25}N_2$) exhibited a mass difference of only
18 431 one amu with different though very similar empirical formulae, suggesting a common origin.

19 432 This observation, together with the presence in this sample of a large amount of
20 433 lauro lactam, a polyamide monomer with m/z 197.1780 and a molecular formula of $C_{12}H_{23}NO$,
21 434 (GC-(EI)TOF-MS RT 17.08 min) suggested that this unknown might be a dimer of
22 435 lauro lactam, since its molecular formula is exactly the double of this compound and the ion
23 436 m/z 395.3638 is two times the mass of the protonated form of lauro lactam. Another evidence
24 437 is the disappearance of this unknown compound after the first migration step. Because this
25 438 dimer is a side-product of the polymerisation reaction, it is probably unbound in the polymer
26 439 skeleton. Therefore, it can easily be transferred to the migration solution and disappear in the
27 440 second migration step. Although data were rather conclusive, LC-QTOF-MS was also used to
28 441 confirm the presence of this dimer, since no commercial standard was available. Indeed, the
29 442 protonated monomer (m/z 198.1861, $C_{12}H_{23}NO$, RT: 7.41 min), the dimer (m/z 395.3626,
30 443 $C_{24}H_{46}N_2O_2$, RT: 7.74 min) and even the trimer (m/z 592.5419, $C_{36}H_{70}N_3O_3$, RT: 8.39 min,
31 444 most probably not eluted on GC) were seen in the LC-QTOF-MS (Figure 4B). The MS
32 445 spectra of these oligomers were undeniably confirmed by Stoffers et al. [37]. Regarding the
33 446 identification criteria proposed by Schymanski et al. [34], this leads us only to a level 2a
34 447 identification: probable structure, unambiguous literature spectrum-structure match, but not
35 448 confirmed by a reference standard. It has to be noticed though that, in this particular case, the
36 449 degree of confirmation could already be considered as high, because three different ionization
37 450 techniques (EI, APCI and ESI) have been applied. Yet, this is not always possible, since some
38 451 compounds are not suited for both GC and LC.

39 452
40 453 *Case study 4*

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7 454 | This was based on a positive [accurate mass](#) match of a peak eluted in the LC with RT
8 455 | of 7.85 min having the accurate mass of bis(3,4-dimethylbenzylidene)sorbitol (C₂₄H₃₀O₆,
9 456 | Millad 3988, a nuclear clarifying agent for PP) [38], with the processed LC data in ESI+
10 457 | mode. For nine out of ten PP bottles, the protonated mass of m/z 415.2118 was matched with
11 458 | an error < 2 mDa and with good isotope fittings. To confirm its presence, [an internet](#)
12 459 | [literature](#) search was conducted to compare the obtained MS spectra with available literature.
13 460 | McDonald et al. [38] provided characteristic MS data for this compound which indeed
14 461 | matched with our data (Figure 5). The protonated molecule m/z 415.2121 was in the LE mode
15 462 | also the most abundant ion. Furthermore, the [M+Na]⁺ and [M+K]⁺ adducts were also
16 463 | identified with masses m/z 437.1941 and 453.1682, respectively. The m/z 119.0862 (C₉H₁₁),
17 464 | which originates from the loss of one of the two dimethylbenzene moieties, was already seen
18 465 | in the LE function, and this ion was the most significant in the HE spectrum. Ions m/z
19 466 | 397.2010 (loss of H₂O), 295.1187 (C₁₅H₁₉O₆) and 277.1802 (C₁₅H₁₇O₅) were also retrieved in
20 467 | the HE function, though in relatively small abundances. The Elemental Composition
21 468 | calculator confirmed that all these fragments were indeed present, calculating their empirical
22 469 | formulas with low mass errors (<±0.8 mDa). It was noteworthy that 3,4-
23 470 | dimethylbenzaldehyde, a degradation product of Millad 3988, was retrieved in the GC-MS
24 471 | injections of all PP samples which contained this compound, confirming indirectly its
25 472 | presence. Therefore, we conclude the identification with a high confidence (level 2) of Millad
26 473 | 3988 as migrant from most PP baby bottles.

27 474 28 475 *Case study 5*

29 476 | The accurate mass of the protonated molecule C₂₆H₂₇N₂O₂S, m/z 431.1789 (LC RT
30 477 | 11.9 min), corresponding to 2,5-bis(5'-*tert*-butyl-2-benzoxaolyl)thiophene, an optical
31 478 | brightening agent for polymers, was returned as a possible positive hit when comparing a PP
32 479 | sample acquired in ESI+ mode to the LC database part containing plastic additives (mass
33 480 | error 0.4 mDa) (Figure SI-2). Literature search [39] supported this finding, as besides the
34 481 | protonated molecule, it also explained the fragments m/z 415.1467 and 401.1303 which were
35 482 | seen in the HE mode and which were matched by the Elemental Composition calculator as
36 483 | C₂₅H₂₃N₂O₂S (1 mDa error) and C₂₄H₂₁N₂O₂S (2.6 mDa error), respectively. No further
37 484 | fragments could be seen due to the complexity of this structure. To obtain a higher confidence
38 485 | degree in the identification of the compound, more fragments are necessary to be obtained by
39 486 | applying higher collision energies.

40 487

488 *Case study 6*

489 The last example involves the compound Pentaerythritol tetrakis(3-(3,5-di-*tert*-butyl-
490 4-hydroxyphenyl)propionate), an anti-oxidant better known under its commercial name
491 Irganox 1010. An accurate mass matching for mass m/z 1175.7821 ($C_{73}H_{107}O_{12}$) was obtained
492 for this compound in all PP samples injected under ESI(-) mode in LC-QTOF-MS. Although
493 the protonated molecule was not present in the positive mode, its deprotonated molecule was
494 seen in the ESI- mode. Comparison of our experimental spectra with literature data only could
495 confirm the deprotonated molecule [40]. However, the injection of an available reference
496 standard of Irganox 1010 matched perfectly in retention time and fragmentation pattern
497 confirming in this way the unequivocal identification of this compound (Figure SI-3).

498 The presence of Irganox 1010 was already suggested in our previous work because
499 several potential degradation products of this compound were found by GC-(EI)MS analysis
500 [6]. The compound methyl-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl) propionate ($C_{18}H_{28}O_3$),
501 originating from a loss of one of the four “arms” of the original anti-oxidant (Figure SI-4),
502 was detected in all PP samples tested before, though until now, no concrete link with its origin
503 from Irganox 1010 could be established. This example demonstrates again the power of the
504 simultaneous use of these complementary techniques for the analysis of unknown migrants
505 from plastic products.

507 **Critical considerations**

508 An efficient analytical strategy based on the combination of several mass analyzers
509 coupled to both gas and liquid chromatography has been applied for non-target analysis of
510 migrating components from plastic baby bottles. The complementary use of GC-(EI)MS, GC-
511 (EI)TOF-MS, GC-(APCI)QTOF-MS and UHPLC-QTOF-MS allowed an efficient and wide-
512 scope target and non-target screening on samples coming from a food simulant, in this case
513 H_2O -EtOH (50/50; v/v), that had been previously into contact with plastic baby bottles. The
514 methodology was applied to six case studies to illustrate the analytical challenges when the
515 mass spectra of the unknown compounds did not match with commercially available GC-
516 (EI)MS libraries. Furthermore, the use of a home-made database including a large number of
517 compounds of interest for detection of compounds via LC-QTOF was discussed into detail.
518 The strategy applied in this work has been proven to be successful for the elucidation of
519 several unknown plastic migrants, from non-polar volatile compounds to semi-polar non-
520 volatiles. Despite the success of the (tentative) identification of some relevant compounds, the
521 successful elucidation of unknowns is not only a matter of easily following a standardized

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7 522 procedure, but it also requires next to the use of several analytical techniques, experience and
8 523 creative insight of the analyst, which still makes it a challenging and quite tedious labour.

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11 525 **Acknowledgements**

12 526 Matthias Onghena wishes to thank the Federal Government Service for Public Health of
13
14 527 Belgium for funding his PhD scholarship through the project ALTPOLYCARB (RT 12/10).

15 528 The Research Institute for Pesticides and Water acknowledges the financial support from
16
17 529 Generalitat Valenciana (Group of Excellence Prometeo II/2014/023; ISIC/2012/016 Envi-
18 530 Food).

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For Review Only

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7 675 **Figure Captions:**

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10 677 **Figure 1:** Schematic overview of GC- (A) and LC (B)-methodology for the non-target
11 678 screening and elucidation of unknown plastic migrants.

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15 680 **Figure 2:** (A) (EI)TOF (top), (APCI)QTOF low energy (middle) and high energy (bottom)
16 681 spectra of unknown 1 with indicated fragments originating from structure number 3. (B)
17 682 Possible elemental compositions for m/z 159.0843 and 229.1626. (C) Top 3 Chemspider
18 683 possible structures for $C_{12}H_{24}O_2Si$.

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22 685 **Figure 3:** (A) (EI)TOF (top) and (APCI)QTOF low energy spectra of unknown 2 with
23 686 structures of the most abundant fragments (B) Possible molecular structures for unknown 2
24 687 with molecular formula $C_{21}H_{42}O_3$.

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28 689 **Figure 4:** (A) GC-(EI)TOF (top), GC-(APCI)QTOF low energy (middle) and high energy
29 690 (bottom) spectra of unknown 3 with empirical formulae and fragments of the most abundant
30 691 peaks. (B) LC-QTOF spectra of lauro lactam monomer (top), dimer (middle), trimer (bottom).
31 692 (Source structures Stoffers et al., 2003)

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35 694 **Figure 5:** Literature ([38] +LC-MS spectrum (upper left corner) compared to the spectra
36 695 obtained by us on ESI+ LC-QTOF MS (upper right LE mode, lower right HE mode) for
37 696 suggested compound bis(3,4-dimethylbenzylidene)sorbitol.

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7 699 | **Table Captions:**

8 | **Table 1:** Summary of detected compounds, techniques used and related errors

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3 **Ms. No.: JMS-15-0051-R2**

4 **Title:** Identification of substances migrating from plastic baby bottles using a combination of
5 low and high resolution mass spectrometric analyzers coupled to gas and liquid
6 chromatography
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8 **Corresponding Author:** Matthias Onghena
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11 We thank the reviewers for their thorough reading of our manuscript and for their
12 suggestions and remarks. We are pleased to see that the reviewers have discussed
13 important points of the manuscript and suggested essential improvements. In line with the
14 reviewer's suggestions, we have reviewed the manuscript and we strongly believe that these
15 revisions have further improved our manuscript.
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17
18 Therefore, we have taken into account all objections and suggestions (marked with track
19 changes in the text in the revised version of the manuscript). Please find here below a point-
20 by-point reply to the reviewers' comments. **(A – authors' comments)**
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23 **A: The figure quality should be improved as we tried to optimize the resolution. If there**
24 **still would be problems we are happy to discuss with the support team how to provide the**
25 **adequate format.**
26
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29 **Reviewing: 1**

30 Comments to the Author

31 I have revised this corrected manuscript as well as the previous versions. The manuscript
32 now addresses all the questions performed by the reviewers.
33

34 **A: Thank you.**
35
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37 **Reviewing: 2**

38 Comments to the Author

39 The paper claims to have developed a strategy for the analysis of non-target compounds
40 using mass spectrometry (MS), specifically looking at chemicals leaching from plastic baby
41 bottles. With six case studies, it demonstrates the complementary use of various MS
42 separation and ionization modes for identification of unknown compounds.
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46 Broad Comments

47 I found Case Study 2 to be somewhat difficult to follow, especially in the last third of the
48 section. Case Study 4 could be organized for better comprehension.
49

50 **A: The authors have gone through the mentioned case studies and found that they read**
51 **good and can be rather easily followed. It is possible that it appears difficult, just because**
52 **the subject is rather complex.**
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56 I will strongly suggest to move Table 1 of the supplemental material to the main manuscript,
57 it will make things easier for the readers. MS Spectra are hard to read.
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3 **A: Table 1 has been included in the main manuscript as suggested by the reviewer.**
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6 Specific Comments

7 Line 29: add "an" before atmospheric

8 Line 55: placement instead of placing

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10 Line 64: insert comma after unregulated

11 Line 65: add comma after reaction)

12 Line 87: remove e.g.

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14 Line 117: add "an" before internal

15 Line 146: remove ", this"

16
17 Line 216: remove both commas

18 Line 257: change min with minutes

19 **A: The specific line comments have been adapted adequately following the reviewer's**
20 **instructions.**
21

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23 Line 247: What were the scorings based on? What was the threshold score for being
24 accepted?

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26 **A: Mass fragment does not return actual scoring values though it calculates the mass error**
27 **between the predicted fragments of a possible structure and the experimentally measured**
28 **ions. The only threshold applied here was an absolute mass error between the**
29 **experimental and theoretical value < 5mDa. Additionally there was checked if all the**
30 **experimentally observed fragments could be explained by a suggested structure. At this**
31 **point, the analyst had to take a decision based on logic and common sense to accept a**
32 **certain structure or not. Therefore "scoring" should not be seen as an actual numerical**
33 **value here but more as how a possible candidate performs taking into account the above**
34 **described criteria.**
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40 Line 276: comma needed after LC-MS (run-on sentence)

41 **A: changed accordingly.**
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44 Line 365: What was the cut-off used for eliminating candidates by their mass error?

45 **A: A cut-off value of 5 mDa was used.**

46
47 Line 370: An "internet search" does not seem fit for a reproducible scientific method.

48 **A: changed accordingly to "literature search".**
49

50
51 Line 392: based on the proposed structures it cannot be a molecular ion since there is no
52 nitrogen atom to be an odd mass (343.3209) please review nitrogen rule.

53 **A: The reviewer is indeed right here and the text was changed adequately.**
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56 Line 403: McLafferty is spell wrong

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58 Line 451: LC...not clear what the authors want to say with this last sentence
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Line 454: What was based on the positive peak match? Start the case study more properly.

Line 458: Tell the readers you searched the literature, not the internet.

A: The specific line comments have been adapted adequately following the reviewer's instructions.

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