Novel Analytical Methods for Flame Retardants and Plasticizers based on Gas Chromatography, Comprehensive Two-Dimensional Gas Chromatography and Direct Probe Coupled to Atmospheric Pressure Chemical Ionization-High Resolution Time-of-Flight-Mass Spectrometry

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1 **Abstract**

2   In this study, we assess the applicability of different analytical techniques, namely direct probe

3   (DP), gas chromatography (GC) and comprehensive two-dimensional gas chromatography

4   (GCxGC) coupled to atmospheric pressure chemical ionization (APCI) with a high resolution

5   (HR)-time-of-flight (TOF)-mass spectrometry (MS) for the analysis of flame retardants and

6   plasticizers in electronic waste and car interiors. APCI-HRTOFMS is a combination scarcely

7   exploited yet with GC or with a direct probe for screening purposes and to the best of our

8   knowledge, never with GCxGC to provide comprehensive information. Due to the increasing

9   number of flame retardants and questions about their environmental fate, there is a need for the

10   development of wider target and untargeted screening techniques to assess human exposure to

11   these compounds. With the use of the APCI source, we took the advantage of using a soft

12   ionization technique that provides mainly molecular ions, in addition to the accuracy of HRMS

13   for identification. The direct probe provided a very easy and inexpensive method for the

14   identification of flame retardants without any sample preparation. This technique seems

15   extremely useful for the screening of solid materials such as electrical devices, electronics and

16   other waste. GC-APCI-HRTOF-MS appeared to be more sensitive compared to liquid

17   chromatography (LC)-APCI/atmospheric pressure photoionization (APPI)-HRTOF-MS for a

18   wider range of flame retardants with absolute detection limits in the range of 0.5-25 pg. A variety

19   of tri- to decabromodiphenyl ethers, phosphorus flame retardants and new flame retardants were

20   found in the samples at levels from $\mu g\ g^{-1}$ to $mg\ g^{-1}$ levels.

21   KEYWORDS: Direct probe, atmospheric pressure chemical ionization, comprehensive two-

22   dimensional gas chromatography, flame retardants, electronic waste

INTRODUCTION

Halogenated flame retardants (FRs) [e.g. polybrominated diphenyl ethers (PBDEs) and tetrabromobisphenol A (TBBPA)] are widespread in the environment due to their use in a variety of electronics, clothes and furniture for enhancing fire safety. Due to their persistence, bioaccumulative and toxic properties [1,2] authorities have restricted the use of PBDEs. The European Union (EU) banned pentaBDE and octaBDE mixtures in 2004 [3] and decaBDE in electric and electronic products in 2009 [4], with limits of 0.1 % by mass being set for pentaBDE and octaBDE in products placed on the market. As a result of these regulations, the use of alternative flame retardants is increasing. Decabromodiphenyl ethane (DBDPE) is used as a substituent of decaBDE and the use of phosphorus flame retardants (PFRs), which are sometimes also used as plasticizers, is increasing. New flame retardants have been reported for the first time in the environment and in consumer products, such as tetrabromobisphenol-A-bis-(2, 3-dibromopropylether) (TBBPA-BDBPE) in dust [5] and sediments [6] or U-OPFR [2, 2-bis (chloromethyl) propane-1, 3-diyl tetrakis (1-chloropropane-2-yl) bis-(phosphate)] in baby products [7]. Due to the lack of data on the identity and levels of flame retardants currently in use, the development of proper, broad screening techniques for these compounds and possible unknowns is highly desirable. In addition, a fast screening technique for flame retardants in waste is needed.

Gas chromatography/electron capture negative ionization quadrupole MS (GC/ECNI qMS), GC/high resolution mass spectrometry (GC/HRMS) and GC ion trap MS (GC/ITMS) have been the most commonly used MS techniques for the determination of polybrominated diphenyl ethers (PBDEs) [8]. Recent articles have shown the suitability of liquid chromatography (LC)-atmospheric pressure chemical ionization (APCI) or LC-atmospheric pressure photoionization

46   (APPI) low resolution MS in the negative mode for the analysis of brominated flame retardants,

47   with [M−Br+O]- as the main ion due to in-source displacement reactions [9-11]. Owing to its

48   sensitivity in full-scan acquisition mode and high mass accuracy, HR full spectrum acquisition

49   techniques, such as HR-time-of-flight (TOF)-MS, have been increasingly used in the last decade

50   for both targeted and untargeted analysis [12]. The combination of a soft ionization technique

51   that generates spectral data rich in molecular ions with HRMS is especially suitable for

52   compound identification. In this study, APCI is assessed as ionization source for the analysis of

53   flame retardants coupled with a HR-TOF detector and for the first time in combination with

54   direct probe, GC or comprehensive two-dimensional GC (GCxGC). LC-electrospray

55   (ESI)/APCI/APPI-HRTOFMS methods were optimised and discussed for comparison with GC-

56   based methods.

57   Fast direct-probe or ambient ionization MS techniques without the need of chromatographic

58   separation and even sample treatment have arisen recently for the direct analysis of solid

59   materials [13] as a good alternative to matrix-assisted laser desorption/ionization (MALDI). In

60   desorption electrospray ionization (DESI), which was introduced in 2004 [14, 15], a sample

61   surface is ionized by directing charged droplets produced from a pneumatically-assisted

62   electrospray and generates a mass spectrum that is similar to ESI. The so-called atmospheric

63   pressure solids analysis probe (ASAP), first reported in 2005 [16,17] operates on the same basis

64   as the APCI-direct probe, being both assemblies quite similar. In both APCI-ASAP and APCI-

65   direct injection probe the sample ionization takes place under heating for gas phase generation

66   and with a corona discharge that initiates an ion/molecule reaction. Applications have been made

67   mainly in the bio-analytical fields (reviewed in [18]) and polymer material [19-20] and more

68   recently in the food [21, 22] and environmental area [23-24]. A recent article exploits also the

69   combination of ASAP with a HR Orbitrap for polymer additives, reporting the detection of one

70   flame retardant [25].The use of direct probe APCI-HRTOF-MS is here studied for the first time

71   for the fast screening of a wide variety of flame retardants and plasticizers without any sample

72   preparation.

73   APCI interfaces for use in combination with GC are recently commercially and are quite

74   promising for the analysis of non-polar volatile compounds, such as flame retardants, due the

75   higher resolution and less matrix ionization effects when compared to LC. First home-made

76   couplings were reported decades ago and more recently in 2005 [26-28]. GC-APCI couplings

77   have been scarcely exploited yet for real sample analysis and only some applications have been

78   reported, namely for the determination of pesticides using a GC-APCI-q-TOF-MS [29] or a triple

79   quadrupole analyzer (QqQ)-MS [30,31] as good alternatives to common electron ionization (EI)

80   sources, in which abundant fragmentation occurs.

81   The coupling of GCxGC with APCI-HRTOF-MS would provide with a comprehensive

82   information for screening. A number of articles have suggested the applicability of GCxGC with

83   low or medium resolution EI-TOF-MS (5000-7000 FWHM) [32-34] for screening of organic

84   contaminants, but to the best of our knowledge this is the first time that GCxGC is coupled to a

85   HRMS (>15000 FWHM) using a soft ionization source. GCxGC has been applied to the analysis

86   of PBDEs with EI-TOF low resolution MS using a combination of a non-polar and medium-

87   polar columns, the second dimension being mostly high temperature 8% phenyl-polycarborane-

88   siloxane [35], 50% phenyl-polysilphenylene-siloxane [36,37], or 65% phenyl-

89   methylpolysiloxane [38] with thermal degradation and/or band broadening of the highly

90   brominated PBDEs as the main drawback. The target compounds were mainly PBDEs, although

91   sometimes other compound classes such as polychlorinated biphenyls (PCBs) and polycyclic

92 aromatic hydrocarbons (PAHs) were included, while the most recent article also includes

93 methoxylated PBDEs and some BFRs [37]. The developed methodologies (based on direct

94 probe, GC and GCxGC) were applied to two electronic waste samples and two samples from car

95 interiors. Nowadays, electronic waste is one of the fastest growing types of waste with a global

96 production of about 50 million tons a year, which has been scarcely investigated yet for flame

97 retardants [39,40]. The fast screening provided by the direct probe could constitute a very useful

98 technique in the recycling of flame retarded polymers under compliance of legislation on the

99 high recycling quotas demanded by the European waste of electric and electronic equipment

100 (WEEE) directive [41]. On the other hand, GC and GCxGC coupled to APCI-HRTOF-MS all

101 provide quantitative results at a low sensitivity or comprehensive information useful for target

102 and untargeted screening, respectively. A total of 30 flame retardants including a variety of tri- to

103 decabrominated diphenyl ethers, new BFRs and PFRs were studied in order to assess the

104 applicability of the new methods for a screening of this type of compounds.

105 **EXPERIMENTAL SECTION**

106 Information about chemicals and suppliers and instrumentation and materials used for sample

107 extraction and analysis are provided in the Supporting Information of this article. The

108 abbreviations for flame retardants and plasticizers recently proposed by Bergman et al. were

109 employed in this article [42].

110 **Optimization of the analysis with direct probe, GC-APCI and GCxGC-APCI-HRTOF-**

111 **MS**

112 The chromatographic separation of flame retardants (including PBDEs and new BFRs

113 analyzed in negative mode) was optimized in different GC capillary columns (specified in

114 Supporting Information). A mix solution containing 24 PBDEs (tri- to decabrominated diphenyl

115 ethers) was tested to assure separations of congeners.   For GCxGC (flow modulator)

116 experiments, the flow in the first (0.3-0.8 mL/min) and second (15-55 mL/min) dimensions and

117 the modulation time (2-4 sec) were optimized.

118   Standard solutions of flame retardants (including PBDEs and new BFRs) at a level of 1 mg

119 mL^{-1} were infused in source and the main MS and source parameters influencing sensitivity

120 (capillary exit and skimmer1, hexapole RF, transfer time, pulse storage time and source

121 temperature) were optimized.

122 **Analysis of flame retardants in e-waste and car interiors samples with direct probe, GC,**

123 **GCxGC /APCI-HRTOF-MS**

124   Four samples, kindly supplied by the Flemish Institute for Technological Research (VITO,

125 Antwerp, Belgium), two of them coming from printed circuit boards and the other two from car

126 interiors from a recycling park were analyzed by GC and GCxGC-APCI-HR-TOF after solvent

127 extraction and directly without sample preparation with direct probe-APCI-HR-TOF. Samples

128 were supplied as shredder material with particle size < 1 mm.

129   For direct probe analysis no sample preparation was required at all. The glass probe was

130 introduced directly into the shredder material and large particles were removed with a lint-free

131 cotton cloth before introducing it into the MS source. For GC and GCxGC analysis samples were

132 extracted with a mixture of 50:50 v/v acetone:dichloromethane according to a method recently

133 reported for the extraction of plastic baby toys [43]. The extraction process consisted of two

134 consecutive steps of vortexing (1 min) and ultrasonication (15 min) with 5 mL of solvent for

135 aliquots of 200 mg. The samples were left in solvent overnight after the first extraction to assure

136   maximal recoveries. Aliquots of 250 μL were diluted in a ratio 1:3 v/v with toluene and filtered.

137   If necessary, further dilutions (10-250 times) were made to semi-quantify highly concentrated

138   compounds. Internal standards were added at the dilution step (due to the high concentration of

139   the target compounds), acting as injection IS for correction of matrix effects and fluctuations in

140   the signal of the instrument. No destructive clean-up step was applied to prevent losses of

141   analytes. Experiments were made in triplicate. Analytical conditions for analysis are specified in

142   Table 1.

143   Calibration solutions for quantification were made by appropriate dilution of the stock

144   solutions with toluene or methanol for GC and LC, respectively in the range 5-500 μg L^{-1} with a

145   concentration of ISs of 100 μg L^{-1}. The main ions used for quantification for each target

146   compound are given in Tables S-1 and S-2 and the sum of the main ion (M, as the most intense

147   isotope) plus M+2, M+4 and M-2, M-4 was employed for obtaining maximum sensitivity (see

148   Figure S-1 as an example of the spectra main ion of TBBPA).

149   **RESULTS AND DISCUSSION**

150   **HR-TOF-MS parameters optimization**
151
152   A total of 30 flame retardants and plasticizers were analyzed in negative (the new BFRs ATE,

153   BATE, DPTE, HCDBCO, EHTeBB, TBBPA, α,β,δ ,-HBCD, BEHTBP, BTBPE and tri- to deca-

154   BDE) and positive GC-APCI modes (the PFRs TiBP, TBP, TCEP, TCPP, DOPO, TEHP, EHDP,

155   TEHP, TBOEP, TMPP). Table S-1 and S-2 provide further information about the target

156   compounds. For most BFRs, $[M-Br+O]^-$ was the main ion except for TBBPA and HBCD ([M-

157   H]$^-$) and BTBPE (fragment $C_6Br_3H_2O^-$), which is in accordance with recent studies [9-11]. For

158   comparison with direct probe and GC-based methods, the main ions obtained in LC-APCI and

159   LC-APPI sources were also studied. HCDBCO behaved differently in LC-APCI/APPI ($[M+O_2]^-$)

160   than in GC/GCxGC-APCI ($[M-Cl+O]^-$). The mobile phase or carrier gas in this case influenced

161   the ionization (methanol:water for LC and He in GC and GCxGC). For BDE 209, the fragment

162    $C_6Br_5O^-$ was the major ion in LC, while the ion $[M-Br+O]^-$ was predominant in GC due to the

163   higher temperature of the source in LC (285°C and 200 °C in LC and GC, respectively). The

164   main secondary ions observed in direct probe, GC and GCxGC-APCI-HRTOF-MS were [M-

165    $HBr+O_2]^-$, $[M-Br+O_2]^-$ or $[M+O_2]^-$ and were used for confirmation purposes. The main ion of

166   PFRs in APCI positive mode was $[M+H]^+$, except for EHDP ($[M-C_8H_{17}+H_2]$), while secondary

167   ions with enough sensitivity for confirmation purposes were not observed (except for EHDP,

168    $[M+H]^+$). APCI in the negative mode was not sensitive enough for the analysis of the chlorinated

169   PFRs. Flame retardants (name, structure, molecular formula), main and secondary confirmation

170   ion in APCI/HRTOF-MS and retention times in GC and GCxGC are given in Tables S-1 and S-2

171   for negative and positive modes, respectively. The main ion *m/z* is the most intense isotope and

172   not the theoretical monoisotopic mass due to the complexity of the isotopic distribution of the

173   compounds.

174   The source temperature influenced the sensitivity of PBDEs. Temperatures higher than around

175   250 °C caused extra debromination of PBDEs and a decrease in the intensity of the main primary

176   and secondary ions cited above, with the higher masses less affected. The same behavior was

177   observed for DPTE. For direct probe experiments, higher source temperatures >300 °C caused a

178   faster desorption of flame retardants from the material and an increase in background noise. A

179   slow desorption is preferred to obtain a longer signal in time (several minutes). However, a high

180   temperature (around 400 °C) was employed for removal of residues in the source after each

181   measurement. The source temperature was set at 200 °C for GC and direct probe experiments.

182   This value is lower than that required for LC (285 °C) necessary to reach a compromise between

183   in-source degradation and an efficient mobile phase vaporization/ionization. The MS parameters

184   capillary exit and skimmer 1, hexapole RF, transfer time and pulse storage time had also a strong

185   influence on sensitivity, and were dependent of the *m/z* value. A compromise was selected as

186   optimal for the mass range of interest *m/z* 200-1000. (See Tables 1 and S-3).

187   For GCxGC data acquisition, a value of 25 Hz was selected as the minimal acquisition rate to

188   obtain reproducible signals (2-7% RSD for areas and 0.025-0.035% RSD for retention times;

189   peak width in second dimension 300-500 ms). The sensitivity decreased with a factor of 4.5 from

190   5 to 30Hz and with ca. 2.6 from 5 to 20 Hz. After conversion to a *cdf* file format, the size of the

191   file was reduced from ~1 GB to ~350 MB and could be handled by GC Image software. The use

192   of an interpolation tool (nearest neighbour) and an intensity threshold filter (set at 100) both

193   provided by the software was necessary for obtaining clear 2D or 3D plots.

194   **Gas chromatographic separation optimization**

195   For GC and GCxGC a number of columns were tested with the aim of obtaining the widest scope

196   of applicability for flame retardants. The main goal was to reduce the well-known degradation of

197   higher molecular weight PBDEs and to obtain a good separation between the target compounds

198   and matrix components in the case of GCxGC.

199   To obtain a good second dimension separation with GCxGC with a flow modulator, a slow

200   temperature gradient was required in the first dimension. Results for 1st dimension separation

201   experiments are given in Table S-4 and some examples of GC-ECD chromatograms are given in

202   Figure S-2. The trifluoropropyl polysiloxane phase gave better results in terms of peak intensity

203   and width of late eluting PBDEs (especially BDE 209) when compared to BDE 153 and also a

204   better separation of PBDEs and novel flame retardants (taking into account the ratio BDE

205   153/BTBPE as example) and good intensity for the more polar HBCD and TBBPA. The use of

206   fast gradients with high temperature columns did not improve results for the peak intensity and

207   peak width of BDE 209 in this type of columns. The use of ionic liquid columns was not possible

208   due to a strong degradation of compounds with more than five Br atoms.

209   The trifluoropropyl polysiloxane column (Rxi-200) was selected for both GC and GCxGC as

210   first dimension column in further experiments. For GC-HRTOF-MS a faster gradient (ramp of

211   10 ºC min^{-1} to 310 ºC) was used to elute all the compounds within about 20 min. Although

212   temperatures higher than the maximum temperature of the GC column program (>10-15 ºC) are

213   recommended to prevent peak broadening in the transfer line, the maximum is 300 ºC, according

214   to the supplier of the instrument. For this reason, peak broadening was observed for the last

215   eluting compounds (> 8 Br atoms), a behaviour that was not observed when coupling the column

216   to the ECD detector. For overcoming this disadvantage, an empty deactivated silica tube was

217   inserted in the transfer line instead of the column and the flow was increased up to 5 mL min^{-1} at

218   14 min for eluting the higher PBDEs (peak width of 0.3-0.5 min in the first dimension). The

219   silica tube for the transfer line was replaced within each batch of experiments (around 20

220   injections) to prevent an increase in the width of last eluting peaks, which is possibly due to

221   attached matrix components. A higher temperature in the transfer line temperature would be

222   anyway highly desirable. Figure 1 shows GC-APCI-HRTOF-MS chromatograms of a standard

223   solution (negative and positive mode), an e-waste sample (negative mode) and a car interior

224   sample (positive mode).

225   Regarding the optimisation of GCxGC with a flow modulator, best results were obtained with

226   a medium polar-apolar column combination that because this better prevented the degradation

227 and peak broadening of highly hydrophobic PBDEs and of TBBPA in comparison with the

228 commonly used apolar/medium polar or polar column combination. For example, with the use of

229 wax columns as second dimension, although better second dimension separation was obtained,

230 the peaks were in general very wide (500-1000 ms), even with the use of the recently developed

231 high temperature megaWAX columns (maximum temperature of 300 °C) and PBDEs with more

232 than 6 Br atoms, TBBPA and HBCD could not be analyzed. The final column configuration,

233 Rxi-200/DB-5 was selected as a compromise between the 2nd dimension separation and

234 applicability to a higher number of flame retardants (PBDEs, new flame retardants and PFRs

235 with exception of deca-BDE and HBCD). The length of the DB5 column was set at 5 m (after

236 optimization between 2 and 5 m). Figure S-3 shows the GCxGC-ECD chromatograms of

237 standard solutions of PBDEs and new flame retardants with the Rxi-200 column coupled to

238 megaWAX, ZB-35HT Inferno and DB5 columns as 2nd dimension. Figure 2 (above) shows a

239 GCxGC-APCI(-)-HRTOF-MS chromatogram of an e-waste sample.

240   For comparison purposes standards were also run with LC (for settings see Table S-3). For

241 separation of the HBCD isomers (which is not possible by GC or GCxGC) and the thermolabile

242 higher molecular weight PBDEs, LC-APCI/APPI-HRTOF offers a good alternative at a similar

243 level of sensitivity.

Analytical performance of direct probe, GC and GCxGC APCI-HRTOF-MS for

245 **analyzing flame retardants**

246   All techniques proposed offer identification of the flame retardants studied with a mass

247 accuracy below 5 ppm (value obtained for the target compounds with internal calibration in each

248 run and from the four samples analyzed with three replicates each in GC, GCxGC and direct

249 probe-APCI-HRTOFMS methods, see Table 3 for concentration). On top of that, the direct probe

250 method offers, an identification within a few minutes, without any sample preparation, a clear

251 advantage over GC methods for a fast screening at high concentration levels. The spectra

252 obtained by direct probe-APCI(-)-HRTOF-MS of an e-waste sample (Figure 2) show the

253 presence of PBDEs (159-1855 µg g^{-1}), TBBPA (16232 µg g^{-1}) and BTBPE (1689 µg g^{-1}).

254 The second dimension separation introduced by GCxGC provided additional information for

255 screening and better separation from co-eluting interferences and, consequently cleaner spectra.

256 As shown in Figure 2, in the second dimension the target compounds are clearly separated from

257 co-eluting interferences (an example is shown in Figure S-4 for a co-eluting interferent in the 1st

258 dimension that is separated from BDE153 in the 2nd dimension).

259 Direct probe sensitivity is related to the amount of sample and MS noise coming from the

260 matrix. Detection limits (LODs) for screening flame retardants and plasticizers with direct

261 probe-APCI-HRTOF-MS were about 0.025% w/w in samples, which is low enough for technical

262 and recycled materials and below the required level of PBDEs for compliance of legislation of

263 0.1 % w/w [European waste of electric and electronic equipment (WEEE) directive].

264 The sensitivity of GC and GCxGC with APCI in comparison with LC with APPI APCI and

265 ESI sources (already reported in literature with other MS detectors) was compared for a group of

266 flame retardants. Instrumental LODs were calculated from solvent blank determinations fortified

267 at low levels (prepared in triplicate and at concentrations around the expected LODs) by using a

268 signal-to-noise ratio of 3 on the basis of the sum of main ion (M, which does not correspond to

269 the theoretical monoisotopic mass but to the most intense isotope) and M+2, M+4 and M-2, M-4.

270   For GCxGC, the software data analysis 4.0 was used for calculation of LODs taking into account

271   the sum of the modulated peaks for each compound. Results are given in Table 2.

272   As reported before for LC-based methods, APPI and APCI are both suitable sources for the

273   screening of the selected flame retardants, while only TBBPA and HBCD could be also

274   efficiently ionized in the ESI source. When comparing with LC, GC-APCI-HRTOF-MS gave a

275   better range of sensitivity for the flame retardants studied (0.5-25 injected pg) in comparison

276   with LC (1-112 injected pg with APCI source and 2-250 injected pg in APPI source), for which

277   the first eluting compounds (BDE 28, BDE 47, ATE, BATE, DPTE and HCDBCO) showed a

278   significantly lower response (from 2 to 125 times lower). The lower sensitivity of the first eluting

279   compounds in LC could be related to the higher water content in the mobile phase in the first part

280   of the LC program suppressing somehow the ionization process at the relatively low vaporization

281   temperature (285 °C) set to restrict debromination of PBDEs. On the other hand, LC methods

282   showed better sensitivity for octa-, nona- and deca-BDEs, probably due to peak broadening for

283   these last eluting compounds in GC as a consequence of the relatively low temperature of the

284   transfer line. It is worth mentioning that LODs in Table 2 are expressed in absolute amounts

285   (pg) and multiplying factors have been included to calculate LODs in terms of concentration in

286   the samples. The injection volume of the LC method was five times higher. This disadvantage of

287   the GC method could be overcome with large injection volume (LVI) approaches for GC.

288   Anyway, even in concentration terms, the sensitivity would be higher for the GC method for the

289   most polar compounds (e.g. BDE 28, BDE 47, ATE).

290   The LODs in GC-APCI(+)-HRTOF-MS for PFRs were all within the same range and suitable

291   for screening purposes. The calculated values for instrumental LODs were the followings: TiBP

292   (30 pg), TNBP (15 pg) TCEP (2 pg), TCIPP isomers (5-10 pg), DOPO (2 pg), TBOEP (5 pg),

293   EHDP (5 pg), TEHP (10 pg), TPHP (2 pg), TMPP isomers (30-50 pg).

294   Limits of quantification (LOQs) were estimated for GC-APCI-HRTOF-MS taking into

295   account a sample amount of 200 mg and an extract of 10 mL (assuming a theoretical 100%

296   recovery "in a best case scenario", these values are just indicative since real extraction recoveries

297   were not calculated for samples). Estimated LOQs were in the range of 0.1-4 $\mu g\ g^{-1}$.

298   The recoveries of the internal standards (added after extraction) in the different matrices

299   expressed as mean±SD (range) were as follows: 90±13 (75-115%) for BDE58, 81±13 (65-104%)

300   for d15-TPHP and 82±6 (74-90%) for d27-TNBP in GC-APCI-HRTOF-MS. These values show

301   that matrix effects are, even without sample clean-up, were very low, which is a common

302   advantage of GC compared to LC.

303   The precision of the method for quantification was between 2-25% (expressed as relative

304   standard deviation, RSDs, n=6) in GC-APCI-HRTOF-MS for the different compounds mainly

305   due to the heterogeneous nature of the waste samples (even at 1 mm particle size), which

306   consisted of a mixture of very different components. A value of 200 mg was set as sample

307   aliquot to obtain an RSD below a maximum of 25%, suitable for screening purposes.

308   **Sample analysis**

309   Samples were extracted in triplicate and analyzed for screening purposes by direct probe

310   and GCxGC and for quantitative results (RSDs 2-25%) by GC coupled with APCI(+/-)-HRTOF-

311   MS. Retention times, mass accuracy (< 5ppm) and isotopic patterns and a S/N >5 were used for

312   positive identification. Results are shown in Table 3.

313   TBBPA and in to minor extent PBDEs are the main flame retardants present in electronic

314   equipment [44]. A recent article also reports TPHP and TPPO (triphenyl phosphine oxide) as

315   major PFRs in e-waste [45]. Regarding car interiors, PBDEs and some new flame retardants such

316   as DBDPE are the most detected compounds [46, 47].

317   TBBPA was found at a high concentration in one of the printed circuit board samples

318   (16200±3000 μg g^{-1} or 1.6 % w/w), which is within the expected concentration in a typical

319   circuit board (1-2% w/w) and at a lower level 71±15 μg g^{-1} in the other e-waste sample, which

320   contained in general the same flame retardants but at lower levels. Lower levels of TBBPA were

321   found in car interior samples (7±1 μg g^{-1} and 15±2 μg g^{-1}). Highly brominated PBDEs (> 6 Br

322   atoms) were found in printed circuit board sample 1 (0.02-0.2% w/w) and printed circuit board

323   sample 2 (0.001-0.01 % w/w). BTBPE was also present in these two samples (0.17-0.004 w/w).

324   Although not included as a target compound, DBDPE (decabromodiphenylethane) was also

325   detected when performing untargeted screening with GC-APCI(-)-HRTOF-MS in e-waste

326   sample 2 (1.6±1 ppm error). The retention time was confirmed by the injection of a standard

327   solution. Both DBDPE and BTBPE are used as substitutes for decaBDE and octaBDE mixtures,

328   respectively.

329   DOPO and EHDP were also detected in printed circuit board samples (<0.01 % w/w). It

330   is worth mentioning that there are not data available about the presence of DOPO in dust samples

331   yet and this is to the best of our knowledge the first time than DOPO is reported in e-waste.

332   DOPO has been proposed as an alternative non-halogenated flame retardant for TBBPA in

333   printed circuit boards since it can also be chemically bound and become part of the epoxy resin

334   backbone, although the higher costs has limited its applicability. TPHP and TMPP were the

335   PFRs at higher levels in printed circuit boards (0.4% and 0.2% w/w in printed circuit board 1 and

336   0.06 and 0.001% w/w in printed circuit board 2, respectively). Samples of car interiors contained

337   mainly PFRs, namely TCIPP (0.04-0.2% w/w) and TPHP (<0.001 % w/w). Levels of PFRs were

338   lower than required for meeting fire protective standards (~ 3% w/w [47]). The mixture of

339   different components in each sample may reflect the more complicated composition of modern

340   flame retardants, often mixtures of BFRs, PFRs and sometimes metal-based flame retardants.

341   In order to check the performance of the technique at lower levels of flame retardants,

342   three dust samples were also analyzed by GC and GC-GC-APCI/HRTOF-MS after a solvent

343   extraction with acetone and toluene and a concentration clean-up with a Na_2SO_4 column (20-80

344   mg sample to a final volume of 500 µL). Lower levels of flame retardants (~50-10.000 ng g^{-1})

345   were found, mainly PFRs (results are not shown). Finally, despite the suitability of the developed

346   techniques for screening of unknowns, this aspect was not investigated in this study and is the

347   focus of ongoing research in our lab. An example of an unknown peak with an halogenated mass

348   spectra in sample e-waste 1 is shown in Figure S-4.

349   **Conclusions**

350   New analytical methods were developed for flame retardants in e-waste and car interior

351   samples based on direct probe, GC and GCxGC coupled to APCI-HRTOF. Direct probe APCI-

352   HRTOF is a fast (few minutes), easy and inexpensive qualitative screening tool for the

353   identification of flame retardants under compliance of the European WEEE directive. In

354   addition, coupling of GCxGC to a HRTOF by using a soft ionization source was developed for

355   the first time to provide comprehensive information and cluster separation for screening of target

356   compounds and unknowns. As a quantitative technique, GC-APCI-HRTOF-MS generally

357   provides a good sensitivity for a wide range of flame retardants and higher resolution and lower

matrix effects than LC-APCI/APPI/ESI-HRTOF-MS. The latter is still required for the separation of HBCD isomers and offers a good alternative for the thermolabile higher molecular weight PBDEs. TBBPA, PBDEs, BTBPE, TPHP, TCIPP and TMPP were found at relatively high concentrations (at μg g^{-1} to mg g^{-1} levels) in the analyzed samples.

Acknowledgments

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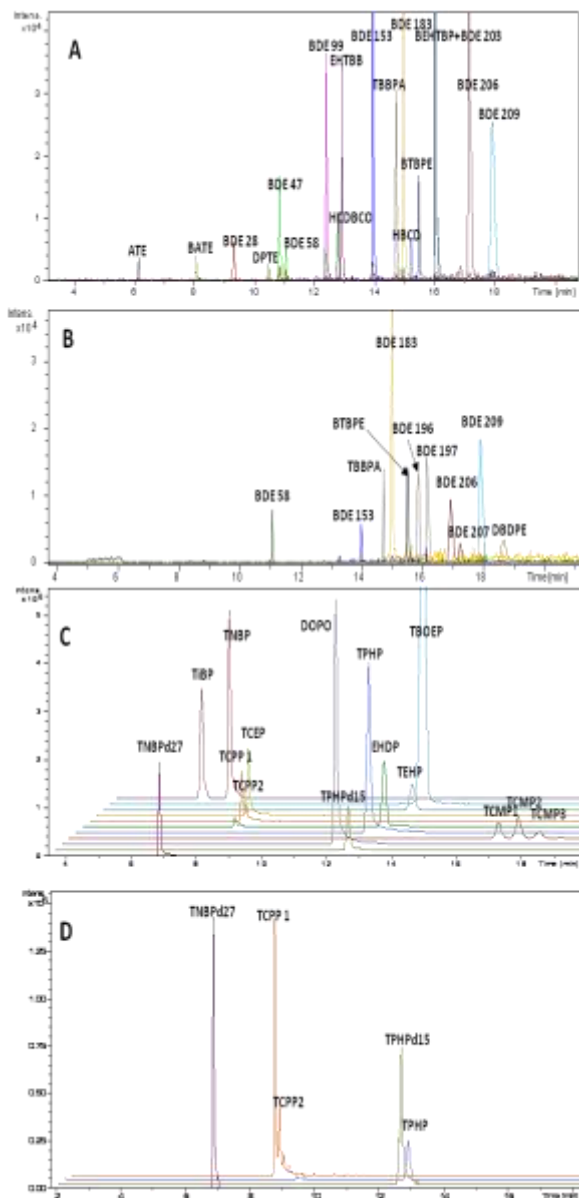
Figure 1. GC-APCI-HRTOF-MS extracted ions chromatograms of (A) a standard solution of 250 μg L^{-1} (100 μg L^{-1} of IS BDE 58) and (B) e-waste 2 both in APCI negative mode; (C) a standard solution of 250 μg L^{-1} (100 μg L^{-1} of IS TNBPd27 and TPHPd15) except for DOPO (2000 μg L^{-1}) and (D) car interior 2 both in APCI positive mode

Figure 2. GCxGC-APCI(-)-HRTOF-MS (above) and direct probe-APCI(-)-HRTOF-MS spectra (below) of e-waste 1 sample; (A) full mass range spectra; (B) 450-910 *m/z* spectra; (C) 250-350 *m/z* spectra

Table 1. GC, GCxGC and direct probe APCI-HR-TOF-MS parameters

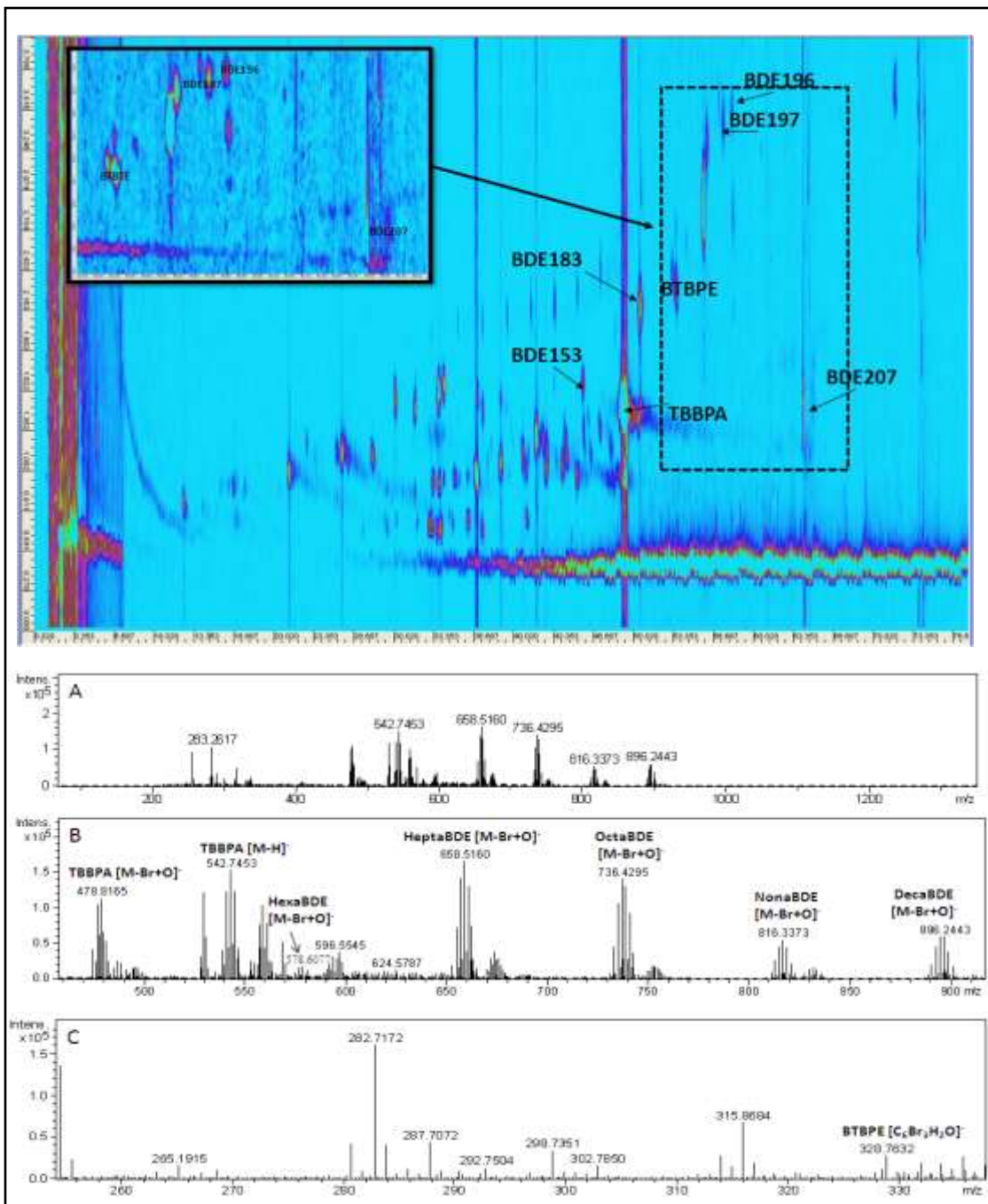| System	Chromatographic separation conditions	[1]TOF parameters
GC-APCI-HRTOF	- Capillary column *Rxi-200* (15m x 0.25 mm x 0.25 μm) - GC program negative mode: 1.5 mL min^{-1} He until 14 min and then up to 5 mL min^{-1} (100 °C for 3min, to 200 °C at a ramp of 20°C/min and to 310 °C at a ramp of 10°C/min); GC program positive mode: 1 mL min^{-1} He (100 °C for 3min, to 200 °C at a ramp of 20°C /min and to 225 °C at a ramp of 2°C /min) - Injection volume: 1 μL (pulsed splitless), injector temperature 285°C (negative mode) and 250 °C (positive mode)	Capillary (neg./pos.) -/+1000 V End plate offset (neg.) -1000 (pos.) -500 Corona (neg.) -10000 nA(pos.) +6000 nA Dry gas 2 L min^{-1} Nebulizer 4 bar Dry Heater 200 °C Vaporizer temperature 200°C
GCxGC-APCI-HRTOF	- First dimension: capillary column *Rxi-200* (15m x 0.25 mm x 0.25 μm); second dimension DB5 (5m x 0.25 mm x 0.1 μm) - Mobile phase: 0.5 mL/min He in first dimension (100 °C for 3min, to 200 C at a ramp of 15°C/min and to 310°C at a ramp of 2°C/min for positive mode and 3°C/min for negative mode and then hold 25 min) and 50 mL/min He in second dimension (130 °C for 3min, to 205 C at a ramp of 15°C/min and to 315 °C at a ramp of at a ramp of 3°C/min for negative mode and at 2°C/min for negative mode and then hold 25 min) - Injection volume: 1 μL (splitless), injector temperature 285°C (negative mode) and 250 (positive mode) - GCxGC flow modulator parameters: modulation delay 0.02s, modulation period 4s, sample injection: 3.9 s	Capillary (neg./pos.) -/+1000 V End plate offset (neg.) -1000 (pos.) -500 Corona (neg.) -10000 nA(pos.) +6000 nA Dry gas 2 L min^{-1} Nebulizer 4 bar Dry Heater 200 °C Vaporizer temperature 200 °C
Direct Probe-APCI-HRTOF	-	Capillary (neg./pos.) -/+1000 V End plate offset (neg.) -1000 (pos.) -500 Corona (neg.) -10000 nA(pos.) +6000 nA Dry gas 2 L/min bar Nebulizer 4 bar Dry Heater 200 °C Vaporizer temperature 200 °C

[1]TOF detection parameters: capillary exit: ±80 V; Skimmer1: ±26.7 V; Hexapole RF: 250 V (negative mode) and 200 V (positive mode); transfer time: 40; puls storage time: 10; focus on

**Table 2. Detection limits (LODs, pg injected) with different sources HRTOF-MS**

	LC-APCI	LC-APPI	LC-ESI	GC-APCI	GCxGC-APCI
BDE 28	112	250	-	5	10
BDE 47	22	50	-	1	5
BDE 99	3	5	-	0.5	2
BDE153	3	5	-	0.5	1
BDE183	2	4	-	0.5	5
BDE 203	1	4	-	10	25
BDE 206	1	4	-	10	100
BDE 209	3	3	-	10	-
ATE	94	250	-	25	500
BATE	27	75	-	10	500
DPTE	18	250	-	10	25
HCDBCO	38	250	-	2	10
EHTeBB	4	3	-	0.5	5
BTBPE	5	3	-	0.5	5
BEHTBP	2	2	-	2	10
TBBPA	19	4	20	10	250
α-HBCD	35	25	5		-
β-HBCD	25	10	5	[a]15	-
δ-HBCD	75	25	5		-

[a]LODs for HBCD in GC correspond to the sum of isomers (α-, β-,δ-HBCD); injection volume: 1 µL in GC- and 5 µL in LC-based methods; for calculation of instrumental LODs in terms of concentration (ng mL^{-1}) multiply by 0.2 in LC methods (according to injection volume) and for calculation of LODs in samples (µg g^{-1}) multiply values for a factor of 0.15 in GC methods and 0.03 in LC methods

Table 3. Flame retardants and plasticizers concentrations (x±SD µg g^{-1}, n=3) in waste samples and confirmation by GCxGC and direct probe experiments

	E-WASTE 1			E-WASTE 2			CAR INTERIOR 1			CAR INTERIOR 2		
	Quantification (ug/g) GC	Screening[a] GCxGC	DP	Quantification (ug/g) GC	Screening[a] GCxGC	DP	Quantification (ug/g) GC	Screening[a] GCxGC	DP	Quantification (ug/g) GC	Screening[a] GCxGC	DP
ATE	n.d.			n.d.			n.d.			n.d.		
BATE	n.d.			n.d.			n.d.			n.d.		
BDE 28	n.d.			n.d.			n.d.			n.d.		
DPTE	n.d.			n.d.			n.d.			n.d.		
BDE 47	1.5±0.2			n.d.			n.d.			n.d.		
BDE 99	n.d.			n.d.			n.d.			n.d.		
HCDBCO	n.d.			n.d.			n.d.			n.d.		
EHTeBB	n.d.			n.d.			n.d.			n.d.		
BDE 153	160±30	+	+	12.9±0.3	+	-	n.d.			n.d.		
BDE 183	1200±260	+	+	80±6	+	-	n.d.			n.d.		
TBBPA	16200±3200	+	+	71±15	+	+	7±1	-		15±2	-	
α,β,δ ,-HBCD	n.d.			n.d.			n.d.			n.d.		
BEHTBP	n.d.			n.d.			n.d.			n.d.		
BDE 197	290±50	+	+	11±1	+	-	n.d.			n.d.		
BDE 196	390±70	+	+	18±3	+	-	n.d.			n.d.		
BDE 203	n.d.			n.d.			n.d.			n.d.		
BTBPE	1700±250	+	+	41±3	+	-	n.d.			n.d.		
BDE 207	850±201	+	+	20±1	-	-	n.d.			n.d.		
BDE 206	40±6	-		10±0.5	-	-	n.d.			n.d.		
BDE 209	1860±200	-	+	71±10	-	-	n.d.			n.d.		
TiBP	n.d.			n.d.			n.d.			n.d.		
TBP	n.d.			n.d.			n.d.			n.d.		
TCEP	0.8±0.1	-	-	1.5±0.2	-	-	n.d.	-	-	n.d.		
TCPP[b]	n.d.			n.d.			200±13	+	+	1640±220	+	+
DOPO	90±20	-	-	35±3	-	-	n.d.			n.d.		
TPHP	3600±460	+	+	590±150	+	+	2.5±0.5	+	-	9±2	+	-
EHDP	10±1	+	-	9±2	+	-	n.d.			n.d.		
TEHP	n.d.			n.d.			n.d.			n.d.		
TBOEP	n.d.			n.d.			n.d.			n.d.		
TMPP[b]	1800±280	+	+	12±2	+	-	n.d.			n.d.		

[a]Presence of flame retardants confirmed by mass ppm error below 5), S/N above 5 and in case of GCxGC also by retention times in first and second dimension with a tolerance RSD level of ±0.1%; [b]Concentration calculated on the basis of the individual concentration of the first eluting isomer
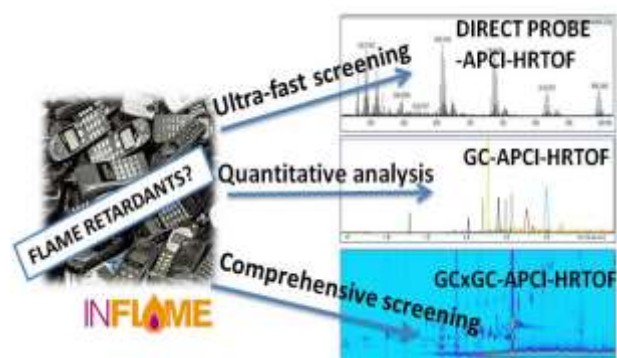
Abstract Graphic/'For Table of Contents Only"