Analytical strategy based on the combination of gas chromatography coupled to time-of-flight and hybrid quadrupole time-of-flight mass analyzers for non-target analysis in food packaging

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Abstract

The potential of an advanced analytical strategy based on the use of gas chromatography (GC) coupled to high resolution mass spectrometry (HRMS) with two different analyzers and ionization sources has been investigated and applied to the non-target analysis of food packaging contaminants. Initially, the approach based on GC-time-of-flight (TOF) MS with electron ionization (EI) source allowed performing a library search and mass accurate measurements of selected ions. Then, a second analysis was performed using hybrid quadrupole (Q) TOF MS with an atmospheric pressure chemical ionization (APCI) source in order to search for the molecular ion or the protonated molecule and study the fragmentation behavior.

This analytical strategy was applied to the analysis of four polypropylene/ethylene vinyl alcohol/polypropylene (PP/EVOH/PP) multilayer trays and one PP/Al foil/PP film, each

one subjected to migration assays with the food simulants isooctane and Tenax®, in order to investigate its potential on the determination of migrant substances.

Keywords

Gas chromatography; high resolution mass spectrometry; atmospheric pressure chemical ionization; electron ionization; food packaging; PP/EVOH/PP; PP/Al foil/PP; potential migrants.

1. Introduction

Time-of-flight (TOF) is considered the mass analyzer of choice for non-target analysis due to its well-known capability of performing accurate mass measurements, which increases the identification efficiency, together with its good sensitivity in full scan acquisition (Cervera, Portolés, Pitarch, Beltrán, & Hernández, 2012; Hernández, Portolés, Pitarch, & López, 2011). It provides a notable amount of chemical information that, after mass spectrometry (MS) acquisition, allows searching for a high number of compounds, even without any previous information or analyte selection. Moreover, the availability of hybrid mass analyzers as the quadrupole TOF (QTOF) MS enhances the identification reliability owing to the possibility of performing fragmentation experiments. Previous separation of the non-polar, volatile and thermostable substances is usually carried out by gas chromatography (GC). Recent progress in analytical instrumentation has increased the use of TOF mass analyzers coupled to GC in different fields as environmental analysis, food safety and toxicology (Hernández, Portolés, Pitarch, & López, 2007; Hajšlová, Pulkrabová, Poustka, Čajka, & Randák, 2007; Meyer & Maurer, 2012).

Electron ionization (EI) is by far the most widely used in GC-MS based methods (including GC-TOF MS) because of its capability of ionizing virtually any organic compound in a robust and reproducible way (Koesukwiwat, Lehotay, Miao, & Leepipatpiboon, 2010; Lehotay, Koesukwiwat, Van Der Kamp, Mol, & Leepipatpiboon, 2011). Commercial standardized libraries including more than 200000 MS spectra under EI are available; so, as a first approach, the identification of unknown compounds can be performed by a simple search matching. However, the high fragmentation occurred under EI may complicate the finding of a conclusive library match, especially due to the spectral similarity between many substances and the absence/low abundance of the molecular ion ($M^{+\bullet}$) in most cases. Another limitation is that the use of nominal mass spectra from the databases may not be powerful enough for confirmation, so accurate mass confirmation has to be done in a subsequent step by specific software tools. Softer ionization sources, as chemical ionization (CI), can be used as a complement for the identification using GC-TOF MS (Portolés, Pitarch, López, Hernández, & Niessen, 2011), although it is quite restricted to specific chemical classes.

The new commercially available atmospheric pressure chemical ionization (APCI) (commonly used in liquid chromatography-mass spectrometry) coupled to GC produces a soft and universal ionization, so the favorable presence of the molecular or quasi molecular ion notably facilitates a rapid and sensitive screening, as it has been already demonstrated in pesticide residue analysis using a GC-QTOF MS system (Portolés, Sancho, Hernández, Newton, & Hancock, 2010; Portolés, Mol, Sancho, & Hernández, 2014; Nácher-Mestre, Serrano, Portolés, Berntssen, Pérez-Sánchez, & Hernández, 2014).

67 The potential of this APCI source in GC-MS is becoming an attractive tool for food
68 safety concerning food-contact materials (Domeño, Canellas, Alfaro, Rodriguez-
69 Lafuente, & Nerin, 2012; Canellas, Vera, Domeño, Alfaro, & Nerín, 2012), especially
70 regarding non-target approaches.

71 Plastic food contact materials, widely used in the manufacture of food packaging, are
72 typically a mixture of polymers of high molecular mass and other starting substances, as
73 monomers and additives, which are susceptible to migrate from the package food due to
74 their low molecular mass (European Regulation No 10/2011). The migration of these
75 substances into food in contact with the packaging is considered as a potential source of
76 pollution because the migrants could alter the food composition, deteriorate the
77 organoleptic properties and, even, incur a human health risk. The European Regulation
78 No 1935/2004 about materials and articles intended to come into contact with food
79 appeals for the Good Manufacturing Practice (Rg 2023/2006) and establishes the
80 authorization process of substances. Specific measures for food-contact plastic materials
81 are contemplated in the European Regulation No 10/2011 that establishes the specific
82 migration limits (SML) in order to prevent the transfer of plastic constituents at harmful
83 levels. Demonstration of compliance must be tested using food simulants, which are
84 assigned to simulate certain foodstuff according to their chemical properties. The
85 literature shows examples of studies that follow the procedures for migration tests given
86 in the Directive 82/711/EEC and evaluate the main factors affecting migration to food
87 (Canellas, Aznar, Nerín, & Mercea, 2010; Vera, Aznar, Mercea, & Nerín, 2011).

88 Different mass analyzers have been used for the determination of potential migrants in
89 food packaging materials, usually applying target methodologies (Alin, & Hakkarainen,
90 2011; Burman, Albertsson, & Höglund, 2005; Fasano, Bono-Blay, Cirillo, Montuori, &

Lacorte, 2012; Simoneau, Van den Eede, & Valzacchi, 2012). However, special attention requires the non-regulated compounds that can be present in packaged food: the non-intentionally added substances (NIAS), which consist of impurities generated from manufacturing and/or degradation processes. The lack of information about the real composition of the final packaging complicates the identification of these compounds (Nerin, Alfaro, Aznar, & Domeño, 2013; Skjevrak et al., 2005). The identification of NIAS and unknown compounds, usually expected at low concentration levels, requires considerable time and effort. Up to now, very few applications using GC-TOF MS for the determination of migrants from food packaging materials have been reported based on a non-target approach (Nerín, Canellas, Aznar, & Silcock, 2009), as sensitive advanced analytical techniques are needed in this case.

Thus, in this work, the potential of a strategy based on the combination of GC-(EI)TOF MS and GC-(APCI)QTOF MS has been investigated for non-target analysis and applied for the identification of unknown substances capable to migrate from plastic materials to food simulants (isooctane and Tenax®).

2. Experimental

2.1. Reagents

A total of 21 commercial analytical standards were used for confirmation purposes. Diethyl sulphide (CAS No 110-81-6), tetramethylurea (632-22-4), octamethylcyclotetrasiloxane (556-67-2), m-acethyl acetophenone (6781-42-6), p-acethylacetophenone (1009-61-6), 3-(methylthio)phenyl isothiocyanate (51333-80-3), guaiazulene (489-84-9) and cinchophen (132-60-5) were purchased from ABCR GmbH & Co. KG (Karlsruhe, Germany). Sigma-Aldrich (Madrid, Spain) provided the standards: ethyl p-tolylsulfide (622-63-9), butylated hydroxytoluene (97123-41-6), 5,6-

115 dimethyl-2-aminobenzothiazole (29927-08-0), p-tolyldisulfide (103-19-5), di-n-octyl
116 phthalate (117-84-0) and bis(2-ethylhexyl) phthalate (117-81-7). 2,4-di-tert-butyl-
117 phenol (2,4-DTB) (CAS No 96-76-4), 2,4-di-tert-butyl-6-methylphenol (616-55-7),
118 diisobutyl phthalate (84-69-5), dibutyl phthalate (84-74-2) and diisooctyl phthalate
119 (27554-26-3) were acquired from Dr. Ehrenstorfer (Augsburg, Germany). 2,6-di-tert-
120 butyl-p-benzoquinone (2,6-DTBQ) (719-22-2) was purchased from Chempur Co.
121 (Karlsruhe, Germany) and 2-(methylthio)phenyl isothiocyanate (51333-75-6) was
122 acquired from Fluorochem Co. (Glossop, United Kingdom).

123 Individual stock solutions (around 500 mg/L) were prepared by dissolving each solid
124 reference standard in acetone and stored in a freezer at -20ºC. Each standard solution
125 was volume diluted in hexane (to around 1 mg/L) for the individual injection into the
126 chromatographic system.

127 Hexane and acetone, both for ultra-trace analysis grade, were purchased from Scharlab
128 (Barcelona, Spain). Diethyl ether for residue analysis and Tenax® adsorbent (60-80
129 mesh) were acquired from Sigma-Aldrich. Trimethylpentane (isooctane) (HPLC grade)
130 was purchased from VWR Chemicals.

131 **2.2. Samples**

132 A total of five samples were analyzed. Four samples were multilayer trays (of different
133 providers and two different size) made of polypropylene/ethylene vinyl
134 alcohol/polypropylene (PP/EVOH/PP) with different colour and without any printed
135 material. One sample was a film made of PP/Al foil/PP and used for closing food
136 containers.

137 **2.3. Migration experiments**

Migration experiments were carried out by AINIA (Paterna, Spain) within a collaborative study with our laboratory. The main objective of the work was to test the applicability of the non-target analytical approach in the analysis of those samples subjecting to migration assays.

In order to broaden the range of food packaging contaminants, both food stimulants, isooctane and polyoxide 2,6- diphenyl-p-phenylene (Tenax®), were used to perform the migration experiences in the selected samples. Isooctane was selected as oily food simulant while Tenax® was used as dry food simulant. Although isooctane is not mentioned as simulant in the plastic regulation (Directive 82/711), it was adopted as substitute simulant in order to obtain an extract that could be injected directly into GC system and avoiding any additional sample extraction. The migration procedures were mainly carried out based on Reg No 10/2011 (Appendix V, Chapter 2).

Different relations of sample surface to simulant volume were applied depending on the size of food containers tested: 20 dm^2/Kg for PP/Al foil/PP film and 10 and 6 dm^2/Kg for smaller and larger PP/EVOH/PP trays, respectively.

Test specimens were filled with pre-warmed isooctane and placed in the thermostatically controlled oven. The materials were subjected to two successive time temperature conditions (1.5 hours at 60 ºC followed by 10 days at 20 ºC) to simulate a thermal treatment and a subsequent storage at room temperature. The combination of these conditions was not specifically included in Regulation 10/2011 or related directives, but it has been used considering the worst predictable conditions of use. After exposure to the simulant, the test specimen was emptied and 1 mL of the food simulant was transferred to a vial for the GC injection. The followed protocol is described in the regulation UNE-EN 13130-1.

Before the use of Tenax® as simulant, this chemical was cleaned with diethyl ether in a Soxhlet extractor for 6 h and dried in an oven for other 6 h. Then the migration test was performed by keeping the Tenax® in contact with the test specimens in a Petri dish and incubating it for 30 minutes at 121 ºC followed by 10 days at 60 ºC (combined conditions extracted from the Regulation 10/2011 considering the worst predictable conditions of use). Finally, the analytes were extracted from the simulant with diethyl ether at room temperature and 1mL was transferred to a vial for the GC injection.

For each food simulant assayed, a blank of simulant was placed in the oven at the same conditions of test specimens.

2.4. Instrumentation

GC-(EI)TOF MS

An Agilent 6890N GC system (Palo Alto, CA, USA) coupled to a TOF mass spectrometer (GCT, Waters Corporation, Manchester, UK) with an EI source (70 eV) was used. The instrument was operated under MassLynx version 4.1 (Waters Corporation). Sample injections were made using an Agilent 7683 autosampler.

The GC separation was performed using a fused-silica HP-5MS capillary column with a lenght of 30 m x 0.25 mm i.d. and a film thickness of 0.25 μm (J&W Scientific, Folson, CA, USA). Injector was operated in splitless mode, injecting 1 μL at 280 ºC. The oven temperature was programmed as follows: 60 ºC (1 min); 5 ºC/min to 300 ºC (2 min); total chromatographic time of 51 min. Helium was used as a carrier gas at constant flow of 1 mL/min.

The interface and ion source temperatures were both set to 250 ºC and a solvent delay of 3 min was selected. TOF MS was operated at 1 spectrum/s acquiring a mass range *m/z*

50-650 using a multi-channel plate voltage of 2800 V. TOF MS resolution was about 8500 (FWHM) at *m/z* 614. Perfluorotributylamine (PFTBA) (Sigma Aldrich, Madrid, Spain), used for the daily mass calibration, was injected via syringe into the reference reservoir at 30 ºC for this purpose. Additionally, PFTBA was used as a lock mass correction for EI experiments (monitoring the ion with *m/z* 218.9856).

The application manager Chromalynx, a module of Masslynx 4.1 software, was used to investigate the presence of non-target (unknown) compounds in sample extracts. Library search was performed using the commercial NIST library (LIB2NIST v1.0.0.12).

GC-(APCI)QTOF MS

An Agilent 7890A GC system (Palo Alto, CA, USA) coupled to a quadrupole TOF mass spectrometer XevoG2 QTOF (Waters Corporation, Manchester, UK) with an APCI source was used. The instrument was operated under MassLynx version 4.1 (Waters Corporation). Sample injections were made using an Agilent 7683 autosampler.

The GC separation was performed using a fused silica HP-5 MS capillary column with a length of 30 m × 0.25 mm i.d. and a film thickness of 0.25 μm (J&W Scientific). The oven temperature was programmed as follows: 60 ºC (1 min); 5 ºC/min to 300 ºC (2 min). 1 μL was injected at 280 ºC under splitless mode. Helium was used as carrier gas at 1.2 mL/min.

The interface temperature was set to 310 ºC using N_2 as auxiliary gas at 150 L/h, make-up gas at 300 mL/min and cone gas at 16 L/h. The APCI corona pin was operated at 1.6 μA with a cone voltage of 20 V. The ionization process occurred within an enclosed ion volume, which enabled control over the protonation/charge transfer processes. The

208 water, used as modifier when working under proton-transfer conditions, was placed in
209 an uncapped vial, which was located within a specially designed holder placed in the
210 source door.

211 Xevo QTOF MS was operated at 2.5 spectra/s acquiring a mass range *m/z* 50–650. TOF
212 MS resolution was approximately 18000 (FWHM) at *m/z* 614. For MS^E measurements,
213 two alternating acquisition functions were used applying different collision energies: a
214 low-energy function (LE), selecting 4 eV, and a high-energy function (HE). In the latter
215 case a collision energy ramp (10-40 eV) rather than a fixed higher collision energy was
216 used. PFTBA (Sigma Aldrich, Madrid, Spain) was used for the daily mass calibration.
217 Internal calibration was performed using a background ion coming from the GC-column
218 bleed as lock mass (protonated molecule of octamethylcyclotetrasiloxane, *m/z*
219 297.0830). MassFragment software (Waters) was used to justify the fragmentation
220 behavior of the compounds detected. This software applies a bond disconnection
221 approach to suggest possible structures for the fragment ions from a given molecule.

222 **2.5. Data processing**

223 ***2.5.1. GC-(EI)TOF MS***

224 Analytical strategy to perform the non-target analysis from the accurate mass GC-
225 (EI)TOF MS data was based on our previous work based on the screening and
226 confirmation of organic pollutants in water (Hernández, Portolés, Pitarch, & López,
227 2007; Portolés, Pitarch, López, Sancho, & Hernández, 2007).

228 The deconvolution package ChromaLynx Application Manager, a module of MassLynx
229 software, was used to automatically process the data. Parameters such as scan width,
230 spectra rejection factor or peak width at 5% height were previously defined. For every

sample, this software detected all peaks that satisfied the established conditions and displayed their deconvoluted mass spectra. A library search was subsequently executed (NIST02 library) and a hit list with positive matches (library match >700) was generated. The formulae from these candidates were submitted to an Elemental Composition Calculator and the accurate mass measurements of the five most intense ions were evaluated for the confirmation/rejection of the finding. More than one identity fit with the experimental spectrum was expected (in terms of library match and accurate mass of main fragment ions –and molecular ion if this existed–).

In those cases where a component was found in both blank and samples, only those with a signal 10 times higher than that observed in the blank samples were considered as tentative candidates for further research.

2.5.2. GC-(APCI)QTOF MS

In order to confirm/reject previous tentative identifications performed by GC-(EI)TOF MS, samples were re-injected in the GC-(APCI)QTOF MS following the basis of our previous developed procedure (Portolés, Sancho, Hernández, Newton, & Hancock, 2010).

Owing to the lack of mass spectra libraries under APCI, in this case the search was done by taking profit of the soft ionization occurred in the APCI source. Thus, both the molecular ion and the protonated molecule ($[M+H]^+$) of the candidates proposed from the (EI)TOF MS data were searched by performing a narrow window-extracted ion chromatogram (nw-XIC, ±0.01 Da) in the (APCI)QTOF MS data. A chromatographic peak was expected at very similar retention time (approximately 1 min less than the value obtained in (EI)TOF MS).

The absence of a chromatographic peak when performing a nw-XIC at $M^{+\bullet}$ and/or $[M+H]^+$ did not involve the rejection although decreased the probability, since the APCI fragmentation degree depends on the compound nature and, although not as the common trend, the molecular ion can be lost in some cases under APCI conditions.

Further investigation on the fragmentation was performed by evaluating the MS^E acquisition, which provides two functions at low and high energy in the same injection. The low-energy function was used to investigate the presence of the molecular ion and/or protonated molecule, while the high-energy function was used to evaluate fragment ion information. Taking profit of the hybrid analyzer, tandem MS (MS/MS) experiments at different collision energies were also performed, in some cases, in order to improve the understanding of the fragmentation of the molecular ion or the protonated molecule, increasing reliability.

3. Results and discussion

The analytical non-target methodology proposed based on the combination of GC-(EI)TOF MS and GC-(APCI)QTOF MS was applied to 10 samples obtained from migration tests using isooctane and Tenax® as food simulants, and their corresponding blank samples.

In a first step, sample extracts were analyzed by using GC-(EI)TOF MS. In order to obtain spectra as pure as possible, a GC temperature program with a single soft temperature ramp was used to get a good chromatographic separation and reduce coelutions. Both library searching and accurate mass measurement of the five most intense ions were applied and tentative candidates were obtained. In order to confirm or reject those identifications, samples were re-analyzed by using GC-(APCI)QTOF MS.

277 Searching for the molecular ion and the protonated molecule in the APCI mass spectra
278 revealed essential information about the candidates proposed by (EI)TOF MS. Thus, in
279 those cases where the absence of the molecular ion in the EI spectra made difficult the
280 correct identification, molecular ion information obtained from the soft ionization
281 occurred in the APCI source was useful.

282 After sample analysis, 18 detected peaks accomplished the established requirements of
283 proposed strategy by (EI)TOF MS and (APCI)QTOF MS (**Table 1**). The number of the
284 candidates obtained by (EI)TOF MS were reduced by approximately half after applying
285 (APCI)QTOF MS (from a total of 63 candidates proposed by (EI)TOF for these 18
286 detected peaks, 36 were tentatively identified by (APCI)QTOF MS). However, in many
287 cases, still more than one structure could justify the identity of a chromatographic peak
288 due to the isomerism. As it can be seen in **Table 1**, discarding among those structures
289 was not always feasible in spite of performing MS/MS experiments. Only the
290 acquisition of commercial standards would ensure the unequivocal identity. After the
291 injection of 21 available standards by GC-(APCI)QTOF MS, 8 compounds could be
292 confirmed as positives and 3 identifications were rejected based on retention time and
293 ionization and fragmentation behavior. The remaining detected peaks could not be
294 finally confirmed due to the lack of their corresponding commercial standards and they
295 were considered as tentatively identified.

296 Next, some examples are shown to better illustrate the performed methodology for the
297 investigation of potential migrants in the samples studied.

298 *Example 1*

Figure 1 shows a GC-(EI)TOF MS experimental accurate mass spectrum (A) of a detected peak found in an isooctane and two Tenax® samples at 28.55 min, which presented a library match >700 for eight different candidate compounds (B-I). These spectra are all characterized by the absence of the $M^{+\bullet}$ and the abundant presence of the *m/z* ion 149, whose structure can derive from any of the eight candidates with an accurate mass in accordance with the experimental value. Although some of the matched compounds have different molecular masses (see **Figure 1**), the high fragmentation degree observed in the experimental EI spectrum of the unknown compound did not allow assuring its molecular mass. Thus, none of the eight possible compounds could be discarded with this first approach using (EI)TOF MS.

The soft ionization provided by GC-(APCI)QTOF MS resulted crucial in order to investigate the mentioned example. When nw-XICs (±0.01 Da) were obtained for the different four *m/z* values corresponding to the eight protonated molecules proposed in **Figure 1** using their exact masses, only a chromatographic peak at $[M+H]^+$ 279.1596 was observed at the expected retention time 27.95 min (**Figure 2**). So, after evaluating the corresponding LE spectrum, the previous list of eight candidates was reduced to three compounds with molecular formula $C_{16}H_{22}O_4$ (MW=278.1518). The information derived from the HE did not reveal additional information about the fragmentation; neither MS/MS experiments could be helpful to find distinguishing fragments due to the isomerism between the three candidates. In order to guarantee the unequivocal confirmation, the available commercial standards were acquired and their injection under GC-(APCI)QTOF MS confirmed the peak identity as diisobutyl phthalate due to ionization, fragmentation and retention time accordance.

Moreover, the aforementioned example gave more relevant information as an additional chromatographic peak at 29.88 min was observed in the nw-XIC at *m/z* ion 279.1596 by (APCI)QTOF MS (see **Figure 2**) and unnoticed by (EI)TOF MS. The LE and HE functions of this peak were identical to that at 27.95 min, probably corresponding to an isomer of the identified positive. Luckily, the injection of the commercial standards acquired confirmed the peak identity as dibutyl phthalate.

Example 2

Figure 3 shows another singular example which proved the potential of the analytical strategy proposed. The experimental spectrum obtained from a chromatographic peak detected by GC-(EI)TOF MS in two Tenax® samples (see **Figure 3 a**) presented a library match >750 with the theoretical spectra of two isomeric compounds, but EI fragmentation did not reveal significant information to distinguish between them. Then, the samples were analyzed by GC-(APCI)QTOF MS and the fragmentation under these conditions, and using water as modifier, provided the fragments 154.9992 and 91.0550 (see **Figure 3 b**). The structure proposed for those fragments only could be originated from the candidate p-tolyldisulfide. The injection of the commercial standard confirmed its identity.

Example 3

As an example of the confirmation with the commercial standards, **Figure 4** shows a positive finding of an isomer of the di-tert-butyl-phenol (DTB) in an isooctane sample. In this case, after performing the methodology developed based on GC-(EI)TOF MS and GC-(APCI)QTOF MS, four isomers were possible candidates for the chromatographic peak at the retention time 20.68 min (see **Table 1**). The commercial

345 standards could not be acquired for three of them. Only 2,4-DTB was available and its
346 confirmation could be expected as it is a common finding in plastic related studies.
347 After sample and standard solution injections, the same retention time was obtained for
348 both chromatographic peaks and, as can be observed in **Figure 4,** the mass accuracy
349 deviations calculated lower than 0.5 mDa confirmed its identity.

350

351 As a summary of the results obtained, **Figure 5** shows the detection frequency of the
352 potential migrants confirmed in the 10 samples analyzed coming from both simulants
353 isooctane and Tenax®. Among positive findings, only DEHP [117-81-7] and dibutyl
354 phthalate [84-74-2] are compounds regulated in the European Regulation No 10/2011,
355 with their corresponding SML. They are common plasticizers which can be only used in
356 articles containing non-fatty foods, according to the mentioned directive. Residues of
357 these migrants are usually found in plastic bottled waters (Schmid, Kohler, Meierhofer,
358 Luzi, & Wegelin, 2008; Bach, Dauchy, Chagnon, & Etienne, 2012; Al-Saleh, Shinwari,
359 & Alsabbaheen, 2011; Lee, Lai, Dou, Lin, & Chung, 2011) and can be also detected in
360 food packaging materials (Fromme et al., 2011; Aznar, Vera, Canellas, Nerín, Mercea,
361 & Störmer, 2011). The rest of the identified compounds were non-regulated substances.
362 The migrants more frequently detected were 2,4-DTB [96-76-4], present in all samples
363 analyzed, and 2,6-DTBQ [719-22-2], identified in three and two samples coming from
364 isooctane and Tenax®, respectively. Both compounds are common degradation products
365 from the antioxidants Irgafos 168 and Irganox 1010 (Denberg, Mosbæk, Hassager, &
366 Arvin, 2009) and they are frequently detected as NIAS in migration studies (Félix,
367 Isella, Bosetti, & Nerín, 2012; Nerin, Alfaro, Aznar, & Domeño, 2013; Vera, Aznar,
368 Mercea, & Nerín, 2011; Skjevrak et al., 2005). Diisobutyl phthalate [84-69-5], found in

three samples, is a plasticizer commonly associated with printing inks and it has been also reported as NIAS in plastic films (Skjevrak et al., 2005; Félix, Isella, Bosetti, & Nerín, 2012). p-Tolyldisulphide [103-19-5], a rubber accelerator, was present in two Tenax® samples, as well as diethyl disulphide [110-81-6], which is a by-product of the commercial production of ethanethiol, an intermediate and starting material in manufacture of plastics. m-Acethyl acetophenone [6781-42-6] was identified in one sample coming from Tenax® but the lack of awareness in the literature makes difficult to know about their properties and migration from plastic materials.

4. Conclusions

The use of two different and complementary ionization sources (EI and APCI) in GC-(Q)TOF MS has notably enhanced the identification potential of food packaging contaminants by performing a non-target analysis. The analysis by GC-(APCI)QTOF MS allowed reducing considerably the number of candidates previously proposed by GC-(EI)TOF MS, thus obtaining a reliable approach to the compounds identity. In some cases, around half of candidates from a detected peak by (EI)TOF could be rejected after searching for the molecular ion/protonated molecule in (APCI)QTOF and/or studying the fragmentation under these conditions. In order to get an unequivocal confirmation, the injection of available reference standards was performed, which allowed the confirmation of the identity of 8 migrants.

In most cases, the difficulty of arriving to conclusive results was evident in this kind of samples due to the extensive list of possible structures that are compatible with the data acquired, especially due to the isomeric nature of most candidates. Identification of unknowns is a challenge and, in addition, when standards are available their acquisition involves a considerable expense without ensuring conclusions, but the powerful

393 combination of techniques applied in this work allowed a rapid screening that simplified
394 and facilitated the identification process.

395

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532

533 **Figure Captions**

534 **Figure 1**. Theoretical mass spectra of the different candidates (B-I) that fit with the
535 experimental spectrum (shown in the center, A) for a chromatographic peak obtained by
536 GC-(EI)TOF MS.

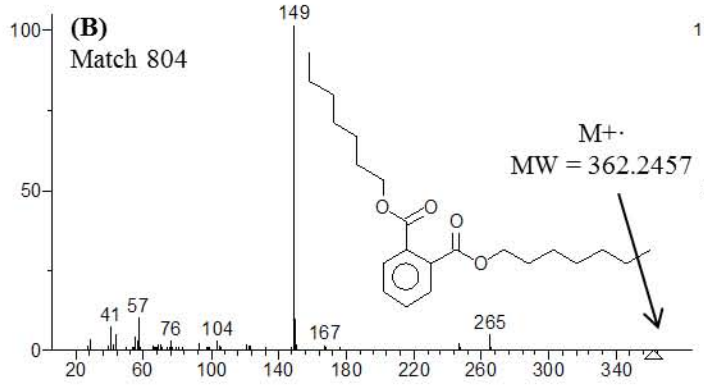
537 **Figure 2**. nw-XIC from the (APCI)QTOF MS data for the corresponding protonated
538 molecule of the candidates in Figure 1. LE spectrum of the detected peak at 27.95 min.

539 **Figure 3.** a) Experimental spectrum (A) obtained by GC-(EI)TOF MS for the peak at
540 32.22 min. Theoretical mass spectra (B-C) of the two candidates proposed for the
541 unknown compound given in (A). b) Low and high energy spectra from the
542 chromatographic peak obtained by GC-(APCI)QTOF MS for the unknown compound
543 detected by GC-(EI)TOF MS.

544 **Figure 4**. Mass spectra at high and low energy functions for 2,4-DTB in an isooctane
545 sample and the standard solution.

546 **Figure 5**. Frequency distribution of migrants confirmed in 10 samples analyzed by GC-
547 (EI)TOF MS and GC-(APCI)QTOF MS after performing a migration study using the
548 simulants isooctane and Tenax®.

549



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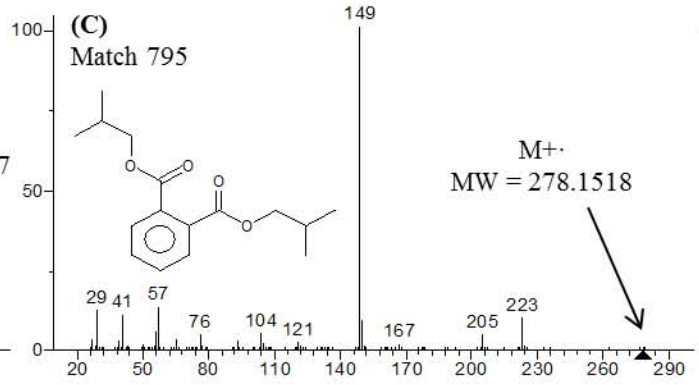

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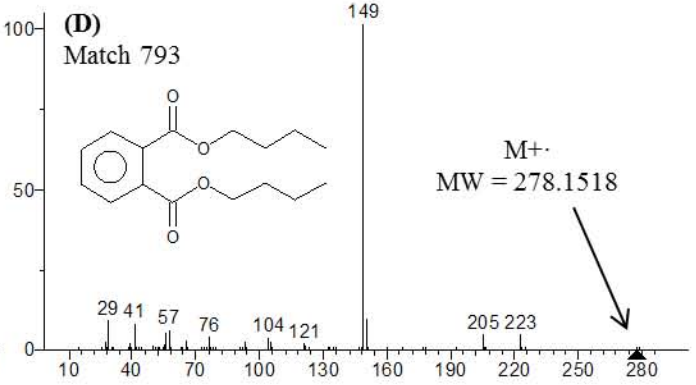

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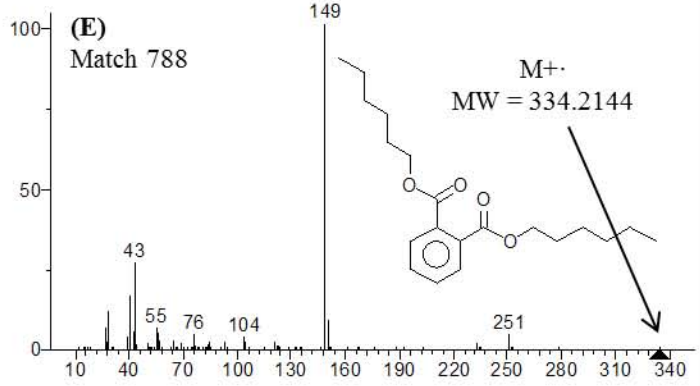

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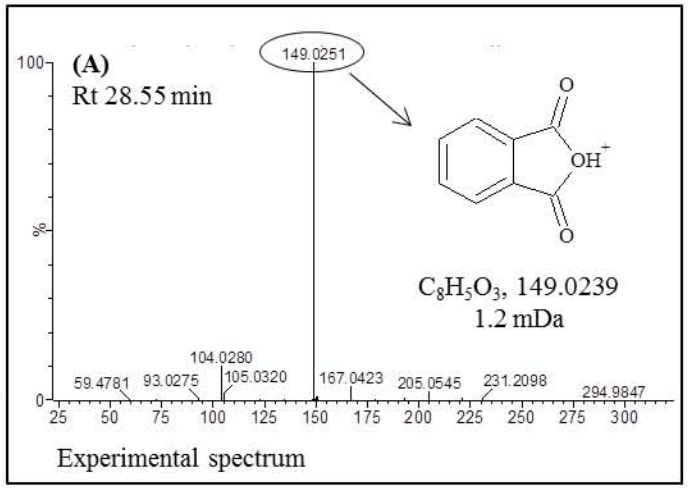

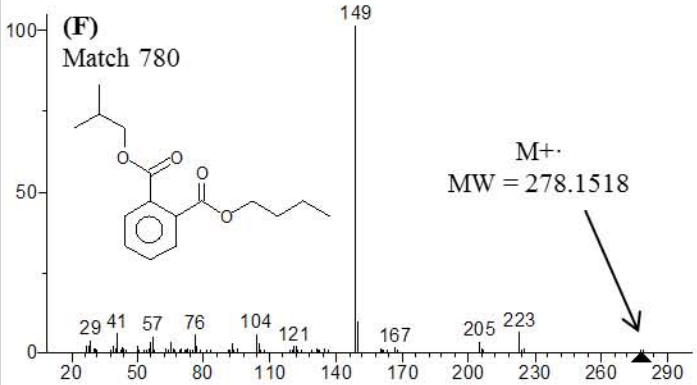

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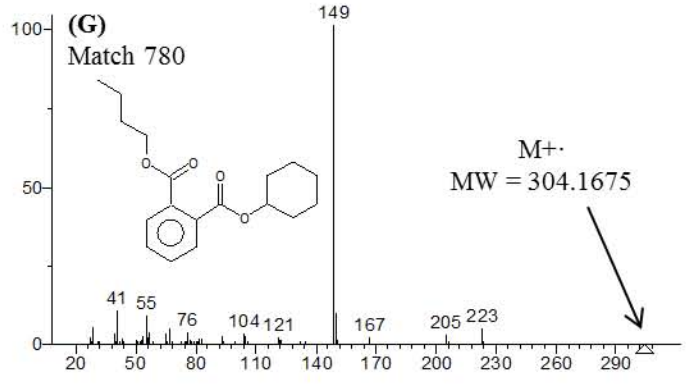

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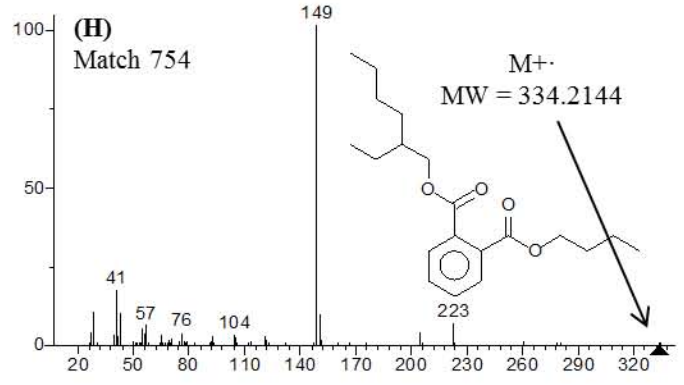

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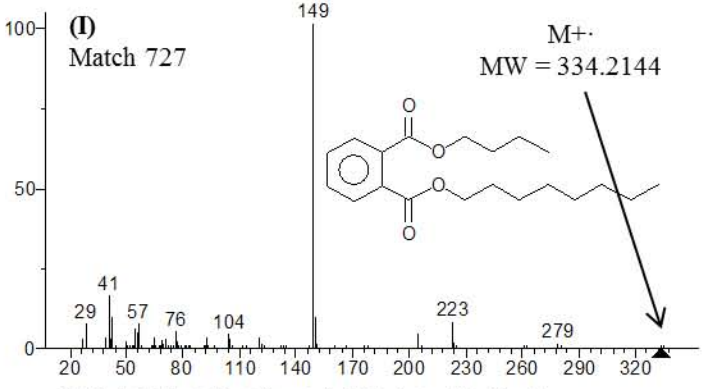

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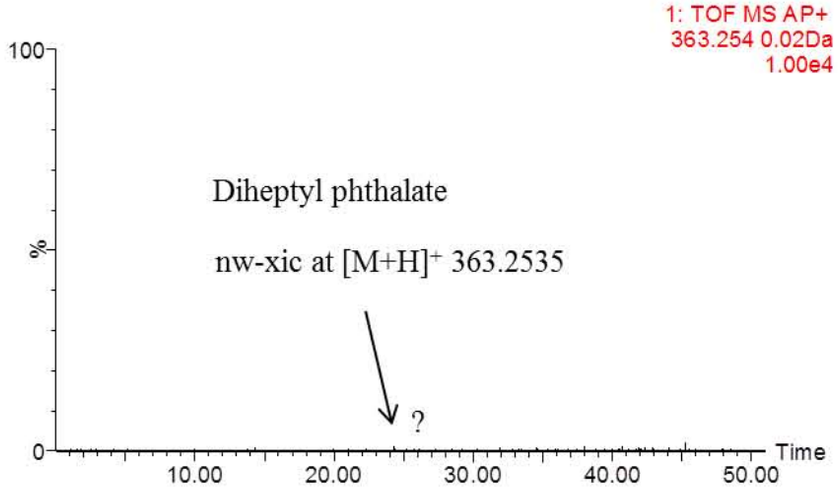
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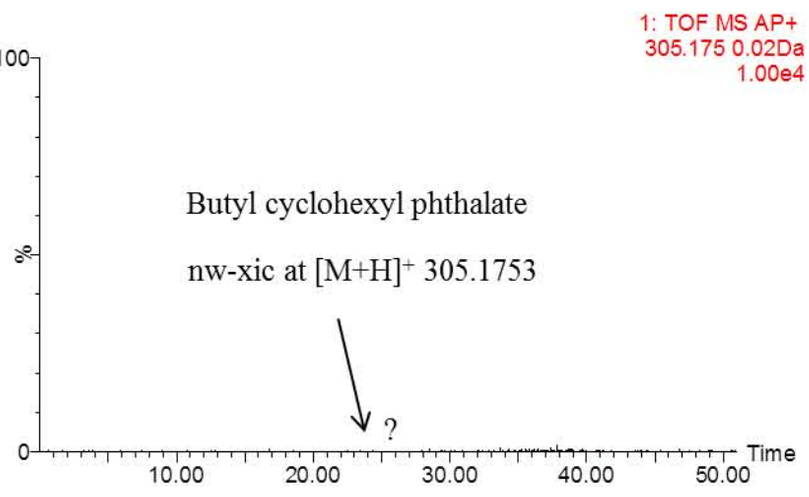
1.00e4
Diheptyl phthalate
nw-xic at [M+H]+ 363.2535
?
Time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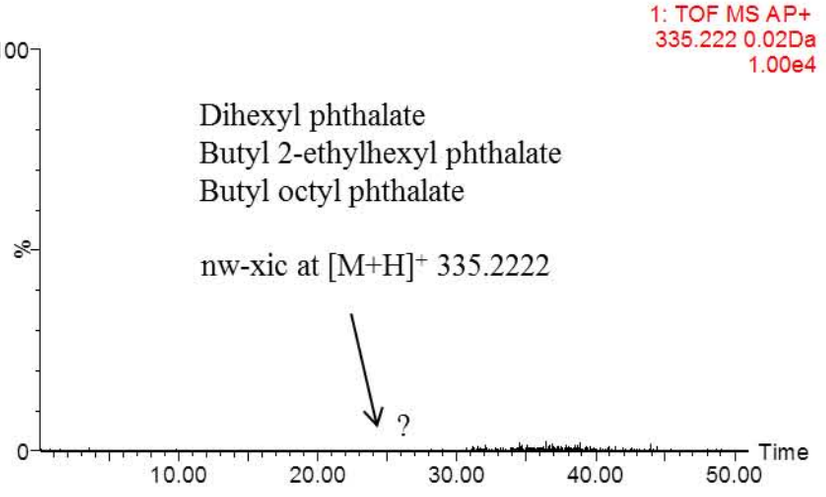
1.00e4
Butyl cyclohexyl phthalate
nw-xic at [M+H]+ 305.1753
?
Time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1.00e4
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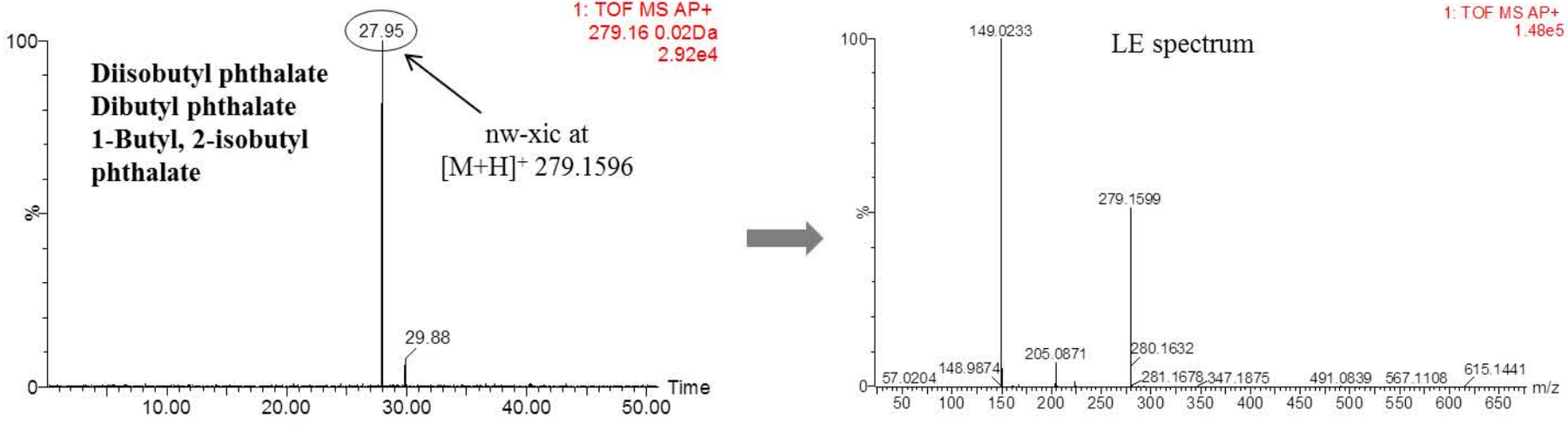
Butyl 2-ethylhexyl phthalate
Butyl octyl phthalate
nw-xic at [M+H]+ 335.2222
?
Time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2.92e4
27.95
Diisobutyl phthalate
Dibutyl phthalate
1-Butyl, 2-isobutyl
phthalate
nw-xic at
[M+H]+ 279.1596
29.88
Time
1: TOF MS AP+
1.48e5
LE spectrum
149.0233
279.1599
205.0871
280.1632
57.0204
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281.1678
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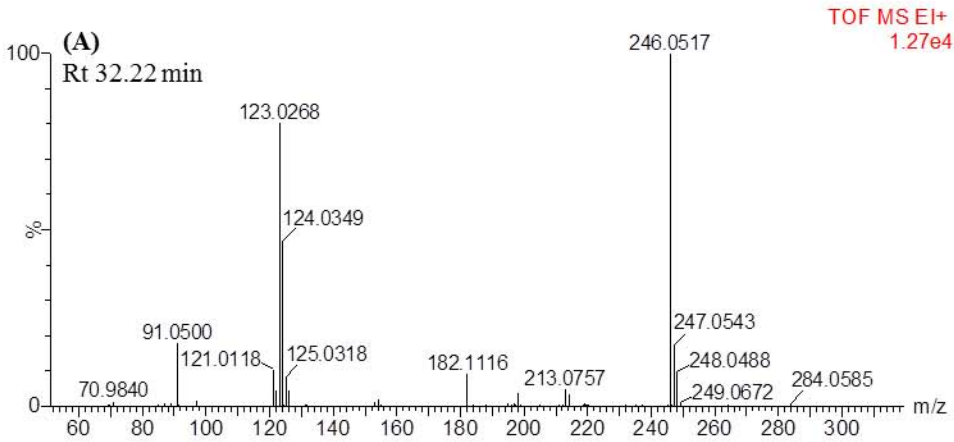
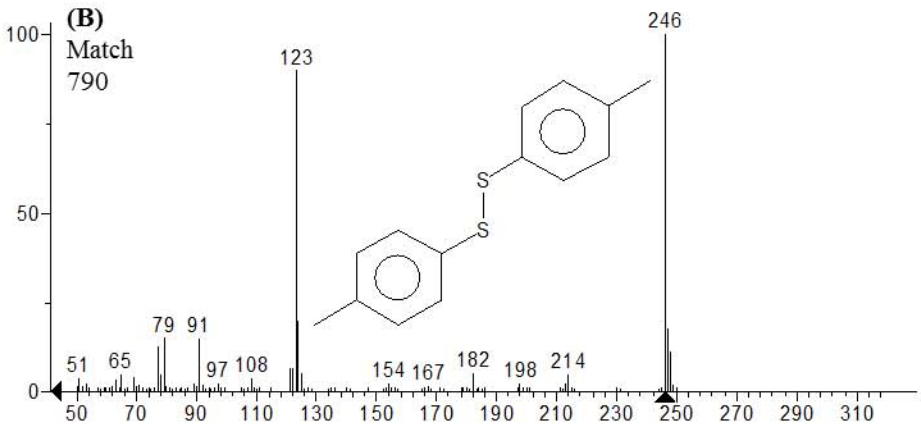
491.0839
567.1108
615.1441
m/z

GC-(EI)TOF MS





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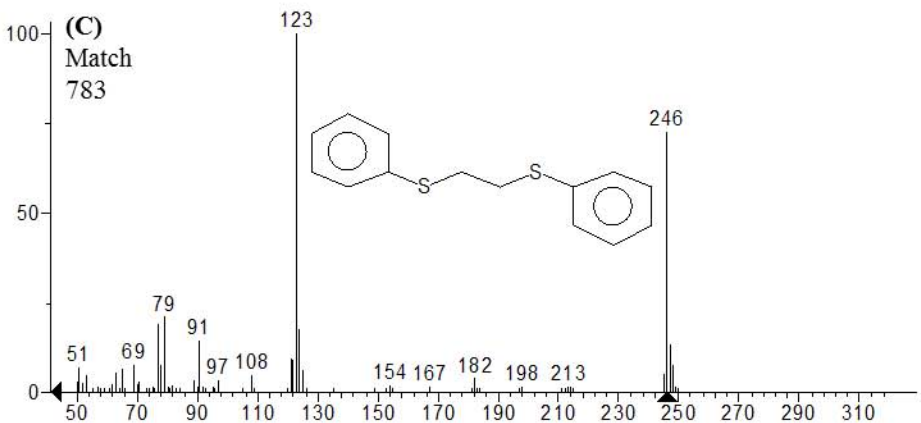

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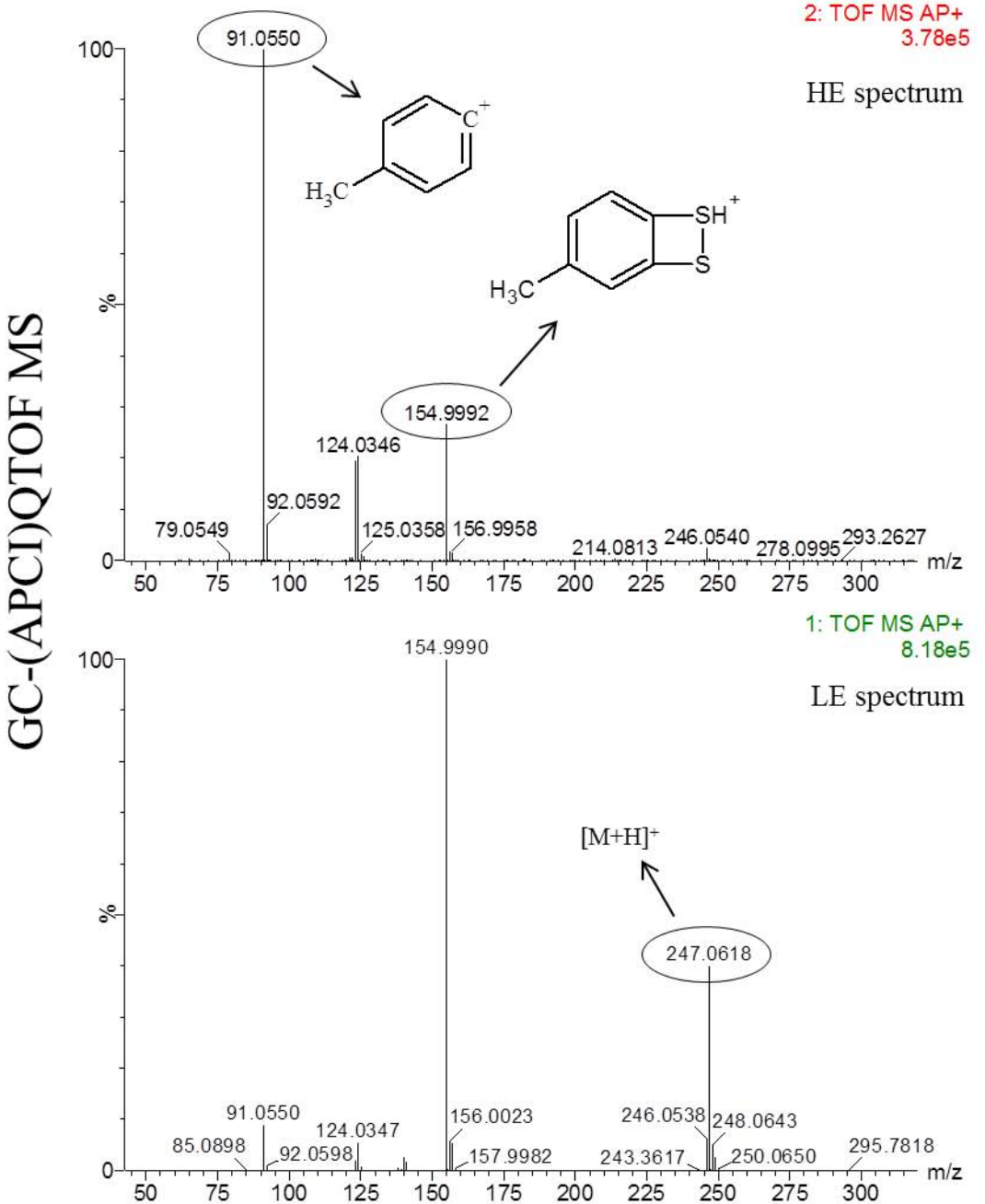
GC-(APCI)QTOF MS
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HE spectrum
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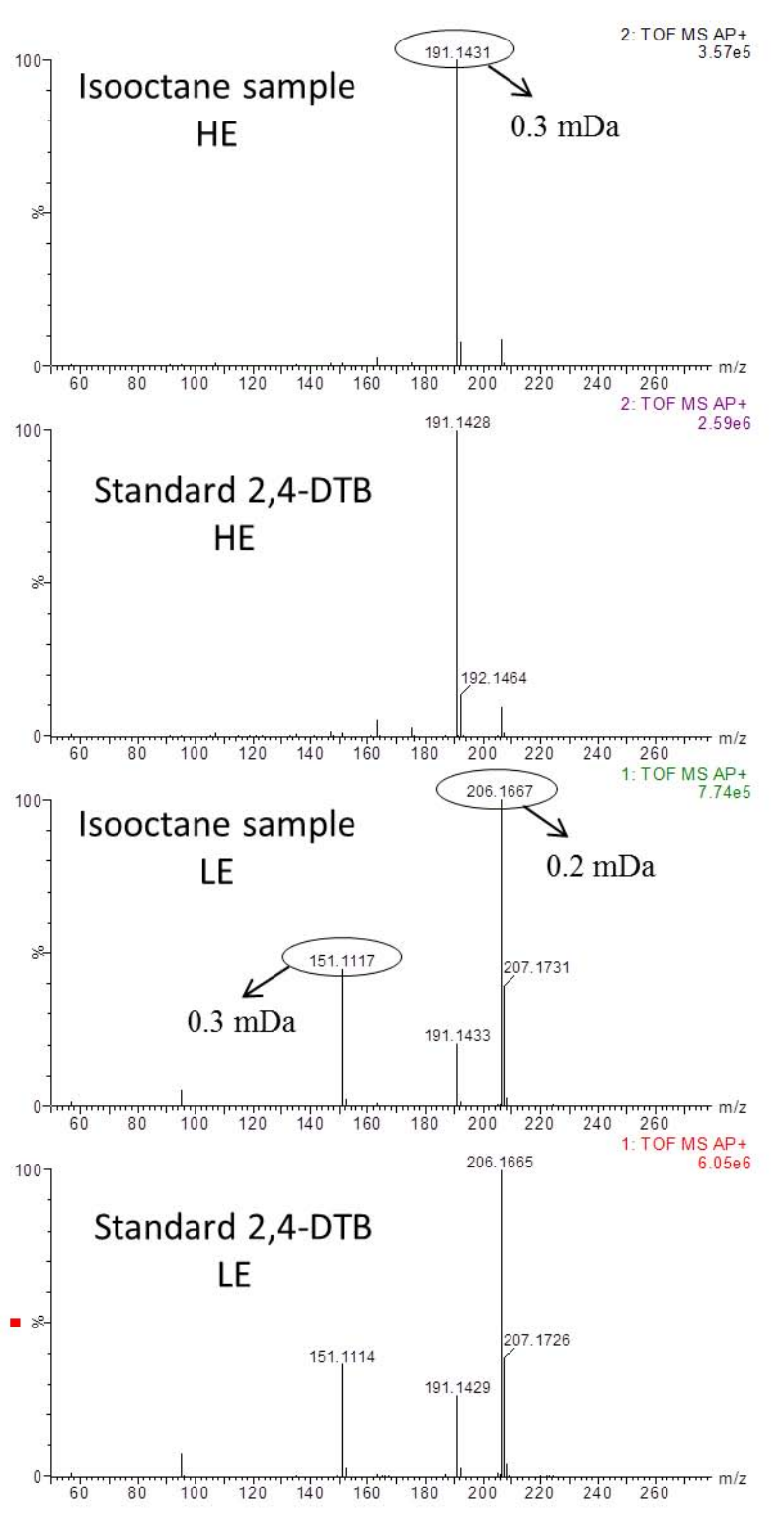
154.9992
124.0346
92.0592
79.0549
125.0358
156.9958
214.0813
246.0540
278.0995
293.2627
1: TOF MS AP+
8.18e5
LE spectrum
154.9990
[M+H]+
247.0618
91.0550
124.0347
85.0898
92.0598
156.0023
157.9982
246.0538
248.0643
243.3617
250.0650
295.7818
%
m/z


2: TOF MS AP+
3.57e5
Isooctane sample
HE
191.1431
0.3 mDa
2: TOF MS AP+
2.59e6
Standard 2,4-DTB
HE
191.1428
192.1464
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7.74e5
Isooctane sample
LE
206.1667
0.2 mDa
151.1117
0.3 mDa
207.1731
191.1433
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Standard 2,4-DTB
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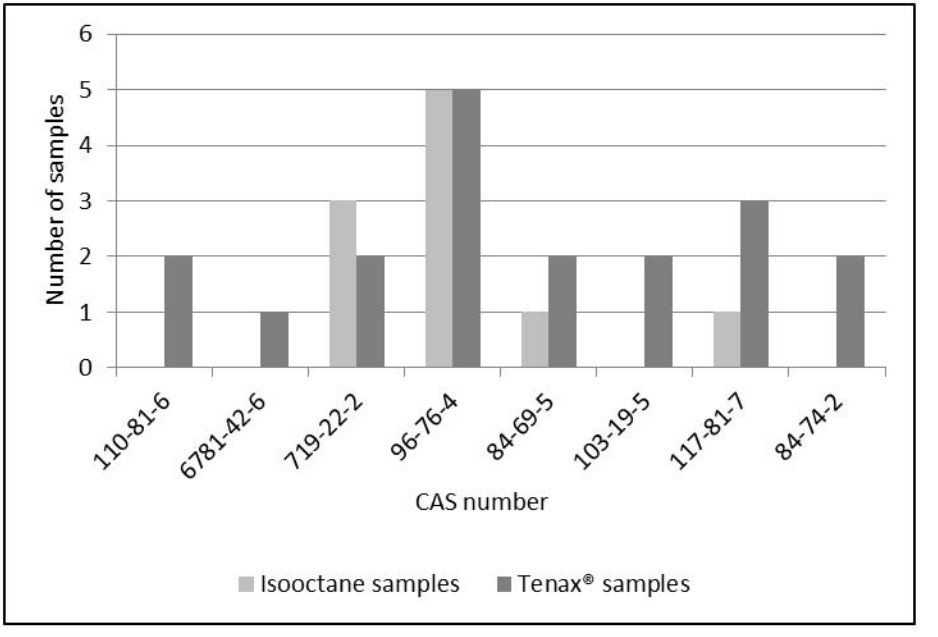
151.1114
207.1726
191.1429
%
m/z


6
5
4
3
2
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Number of samples
110-81-6
6781-42-6
719-22-2
96-76-4
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103-19-5
117-81-7
84-74-2
CAS number
Isooctane samples
Tenax® samples

Table 1. Migrants detected in samples coming from the simulants isooctane and Tenax® after applying the combination of GC-(EI)TOF MS and GC-(APCI)QTOF MS. Confirmed compounds are shown in bold.

Rt (TOF) (min)	Rt (QTOF) (min)	CAS No	Candidates number by (EI)TOF MS	Candidates by (APCI)QTOF MS	Formula	Commercial standards	Status
5.55	**4.28**	**110-81-6**	2	**Diethyl disulfide**	**C4H10S2**	available	**Confirmed**
7.04	5.96	632-22-4	1	Tetramethylurea	C5H12N2O	available	Non-confirmed
7.26	6.3	556-67-2	3	Octamethylcyclotetrasiloxane	C8H24O4Si4	available	Non-confirmed
13.96	12.98	622-63-9	6	Ethyl p-tolylsulfide	C9H12S	available	Non-confirmed
		-		Benzene, 1-(ethylthio)-3-methyl-	C9H12S	n.a.	Tentative
18.7	17.84	115754-89-7	5	2-(1-Hydroxycycloheptyl)-furan	C11H16O2	n.a.	Tentative
		6781-42-6		**m-Acethyl acetophenone**	**C10H10O2**	available	**Confirmed**
18.8	**17.93**	1009-61-6	5	p-Acethyl acetophenone	C10H10O2	available	Non-confirmed
		1689-09-4		3,3-Dimethyl-2-benzofuran-1(3H)-one	C10H10O2	n.a.	Non-confirmed
19.65	**18.81**	**719-22-2**	3	**2,6-di-tert-butyl-p-benzoquinone (2,6-DTBQ)**	**C14H20O2**	available	**Confirmed**
		96-76-4		**2,4-di-tert-butyl-phenol (2,4-DTB)**	**C14H22O**	available	**Confirmed**
20.68	**19.9**	1138-52-9	6	3,5-di-tert-butyl-phenol	C14H22O	n.a.	Non-confirmed
		5875-45-6		2,5-di-tert-butyl-phenol	C14H22O	n.a.	Non-confirmed
		50356-17-7		2,6-di-tert-butyl-phenol	C14H22O	n.a.	Non-confirmed
20.75	19.98	97123-41-6	5	Butylated Hydroxytoluene	C15H24O	available	Non-confirmed
		2934-07-8		2,4,6-Triisopropylphenol	C15H24O	n.a.	Tentative
		616-55-7		2,4-Di-tert-butyl-6-methylphenol	C15H24O	available	Non-confirmed
22.84	22.03	2254-94-6	4	2-Benzothiazolinethione, 3-methyl-	C8H7NS2	n.a.	Tentative
		51333-80-3		3-(Methylthio)phenyl isothiocyanate	C8H7NS2	available	Non-confirmed
		51333-75-6		2-(methylthio)phenyl isothiocyanate	C8H7NS2	available	Non-confirmed
		64036-43-7		Benzothiazolethiol, 2-methyl-	C8H7NS2	n.a.	Tentative
23.9	23.1	28291-69-2	2	2-(Ethylamino)-1,3-benzothiazole	C9H10N2S	n.a.	Tentative
		29927-08-0		5,6-Dimethyl-2-aminobenzothiazole	C9H10N2S	available	Non-confirmed
24.59	23.84	489-84-9	5	Guaiazulene	C15H18	available	Non-confirmed
		483-78-3		Naphthalene, 1,6-dimethyl-4-(1-methylethyl)-	C15H18	n.a.	Tentative
		489-77-0		6-Isopropyl-1,4-dimethylnaphthalene	C15H18	n.a.	Tentative
		84-69-5		**Diisobutyl phthalate**	**C16H22O4**	available	**Confirmed**
28.55	**27.93**	84-74-2	8	Dibutyl phthalate	C16H22O4	available	Non-confirmed
		17851-53-5		1-Butyl 2-isobutyl phthalate	C16H22O4	n.a.	Non-confirmed
30.46	**29.88**	**84-74-2**	-	**Dibutyl phthalate**	C16H22O4	available	**Confirmed**
31.82	31.13	115725-44-5	1	Cyclic octaatomic sulfur	S8	n.a.	Tentative
32.22	**31.67**	**103-19-5**	2	**p-Tolyldisulfide**	**C14H14S2**	available	**Confirmed**
		117-81-7		**Bis(2-ethylhexyl) phthalate (DEHP)**	**C24H38O4**	available	**Confirmed**
40.42	**40.34**	27554-26-3	4	Diisoctyl phthalate	C24H38O4	available	Non-confirmed
		117-84-0		Di-n-octyl phthalate	C24H38O4	available	Non-confirmed
42.32	42.32	132-60-5	1	Cinchophen	C16H11NO2	available	Non-confirmed

n.a. not available