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).

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

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

Different mass analyzers have been used for the determination of potential migrants in food packaging materials, usually applying target methodologies (Alin, & Hakkarainen, 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-

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

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

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

2.2. Samples

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

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

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

2.5. Data processing

2.5.1. GC-(EI)TOF MS

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

The deconvolution package ChromaLynx Application Manager, a module of MassLynx software, was used to automatically process the data. Parameters such as scan width, 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.

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

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

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

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

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

Acknowledgements

The authors acknowledge the financial support of Generalitat Valenciana (research group of excellence PROMETEO/2009/054; ISIC 2012/016) and are very grateful to the Serveis Centrals d'Instrumentació Científica (SCIC) of the University Jaume I for the use of GC-TOF MS (GCT) and to MS Technologies Center (Waters Corporation, Manchester, UK) for using the GC-APGC-Xevo QTOF, as well as to the Ainia technological center for providing the sample extracts. L. Cherta is grateful to University Jaume I for her pre-doctoral grant and T. Portolés is very pleased to Conselleria de Educación, Formación y Empleo for her post-doctoral grant.

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Figure Captions

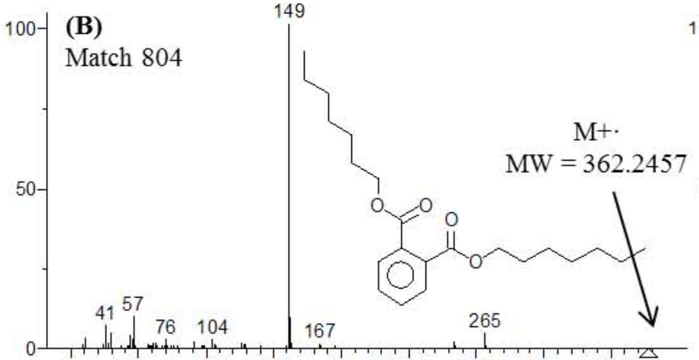
Figure 1. Theoretical mass spectra of the different candidates (B-I) that fit with the experimental spectrum (shown in the center, A) for a chromatographic peak obtained by GC-(EI)TOF MS.

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

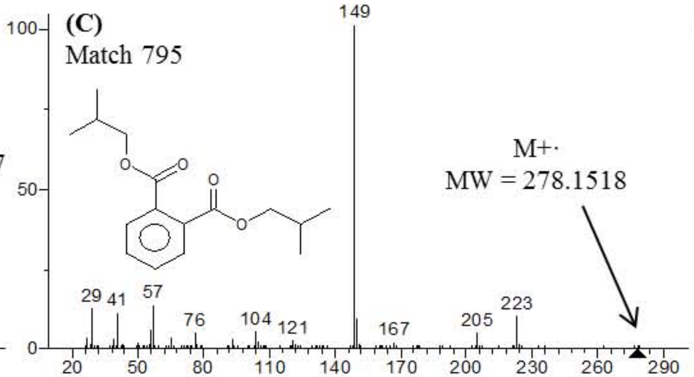
Figure 3. a) Experimental spectrum (A) obtained by GC-(EI)TOF MS for the peak at 32.22 min. Theoretical mass spectra (B-C) of the two candidates proposed for the unknown compound given in (A). b) Low and high energy spectra from the chromatographic peak obtained by GC-(APCI)QTOF MS for the unknown compound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

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

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



CAS 3648-21-3, Diheptyl phthalate, $C_{22}H_{34}O_4$



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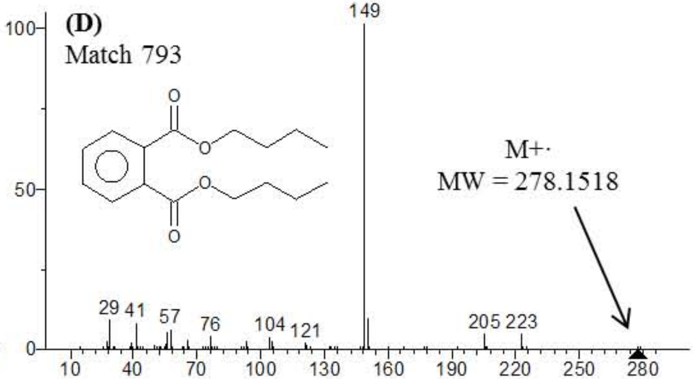

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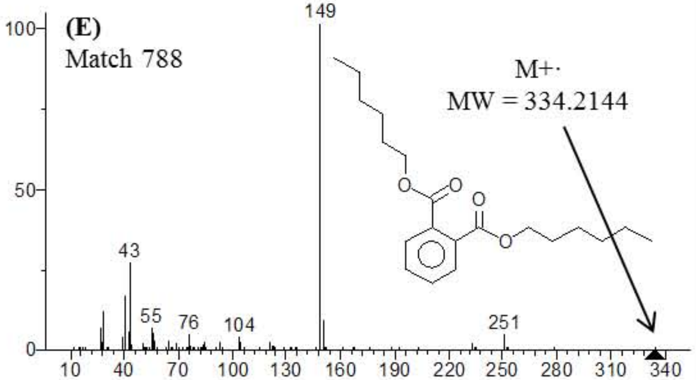
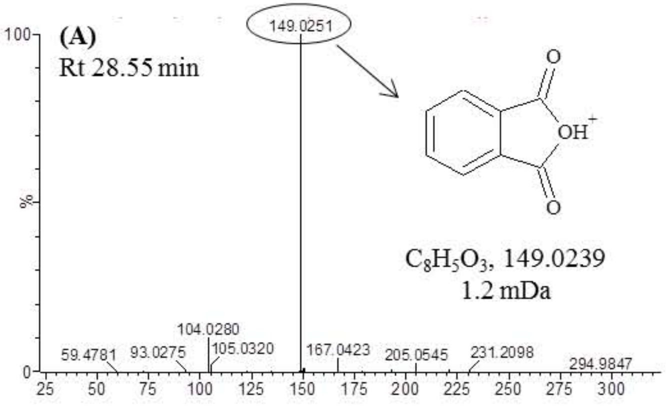
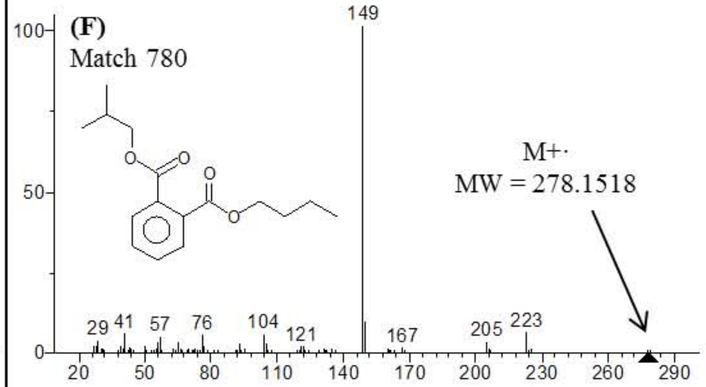


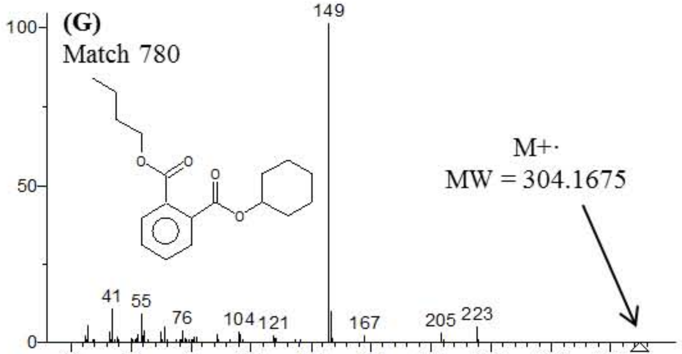
CAS 84-75-3, Dihexyl phthalate, $C_{20}H_{30}O_4$



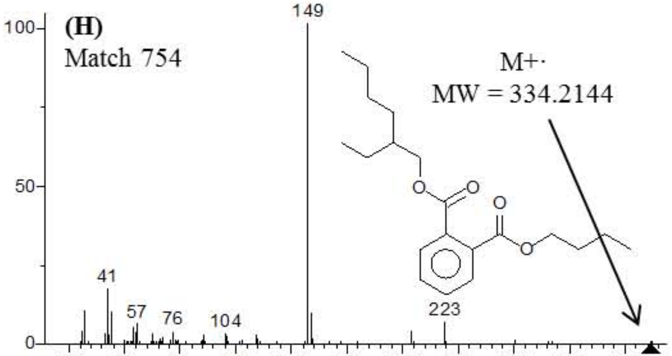
Experimental spectrum



CAS 17851-53-5, 1-Butyl, 2-isobutyl phthalate, $C_{16}H_{22}O_4$



CAS 84-64-0, Butyl cyclohexyl phthalate, $C_{18}H_{24}O_4$



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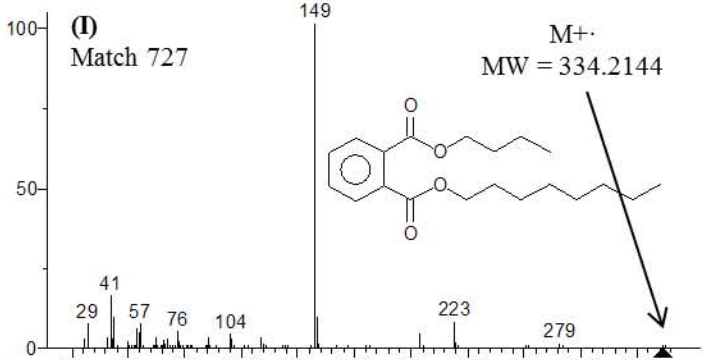

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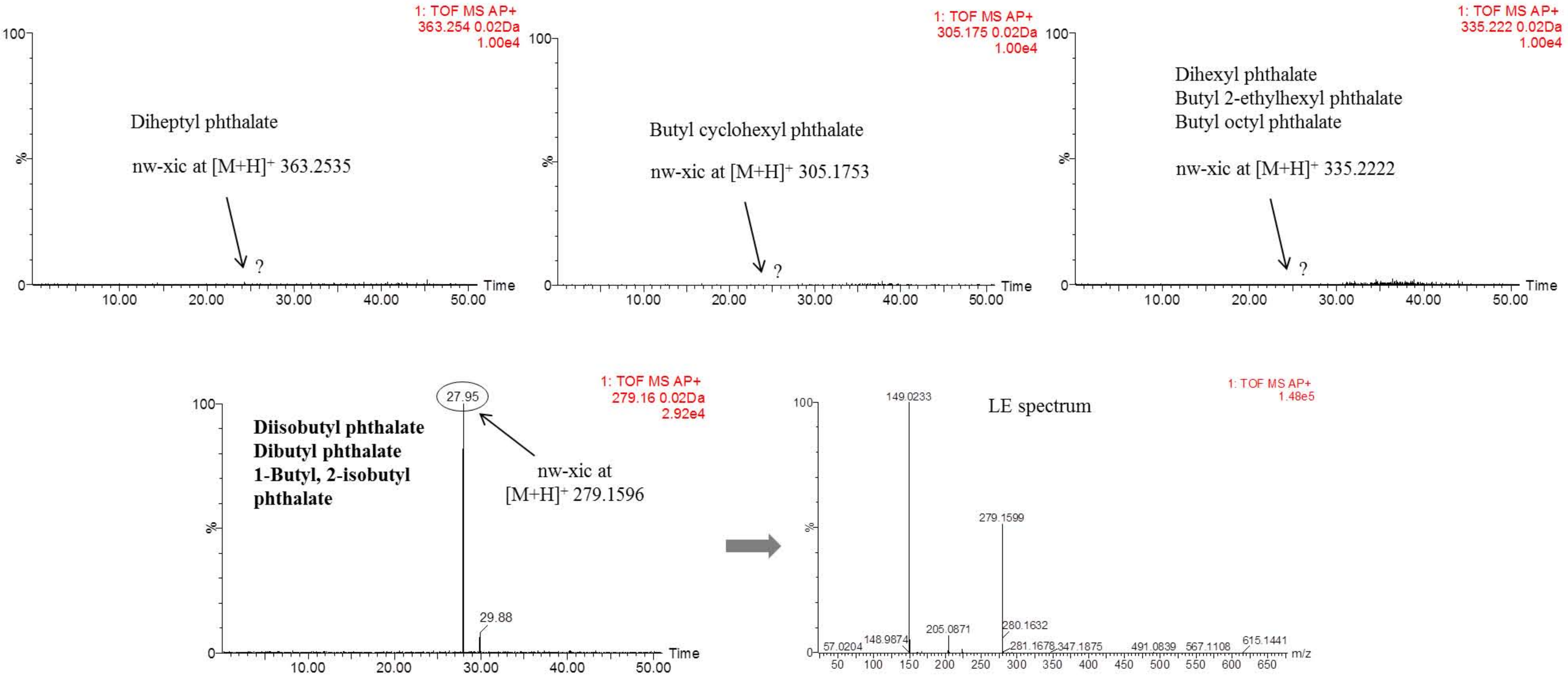
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363.254 0.02Da
1.00e4
100
%
0
Time
10.00
20.00
30.00
40.00
50.00
Diheptyl phthalate
nw-xic at [M+H]+ 363.2535
?
1: TOF MS AP+
305.175 0.02Da
1.00e4
Butyl cyclohexyl phthalate
nw-xic at [M+H]+ 305.1753
?
1: TOF MS AP+
335.222 0.02Da
1.00e4
Dihexyl phthalate
Butyl 2-ethylhexyl phthalate
Butyl octyl phthalate
nw-xic at [M+H]+ 335.2222
?
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279.16 0.02Da
2.92e4
Diisobutyl phthalate
Dibutyl phthalate
1-Butyl, 2-isobutyl
phthalate
27.95
29.88
nw-xic at
[M+H]+ 279.1596
LE spectrum
1: TOF MS AP+
1.48e5
149.0233
279.1599
57.0204
148.9874
205.0871
280.1632
281.1678
347.1875
491.0839
567.1108
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m/z
50
100
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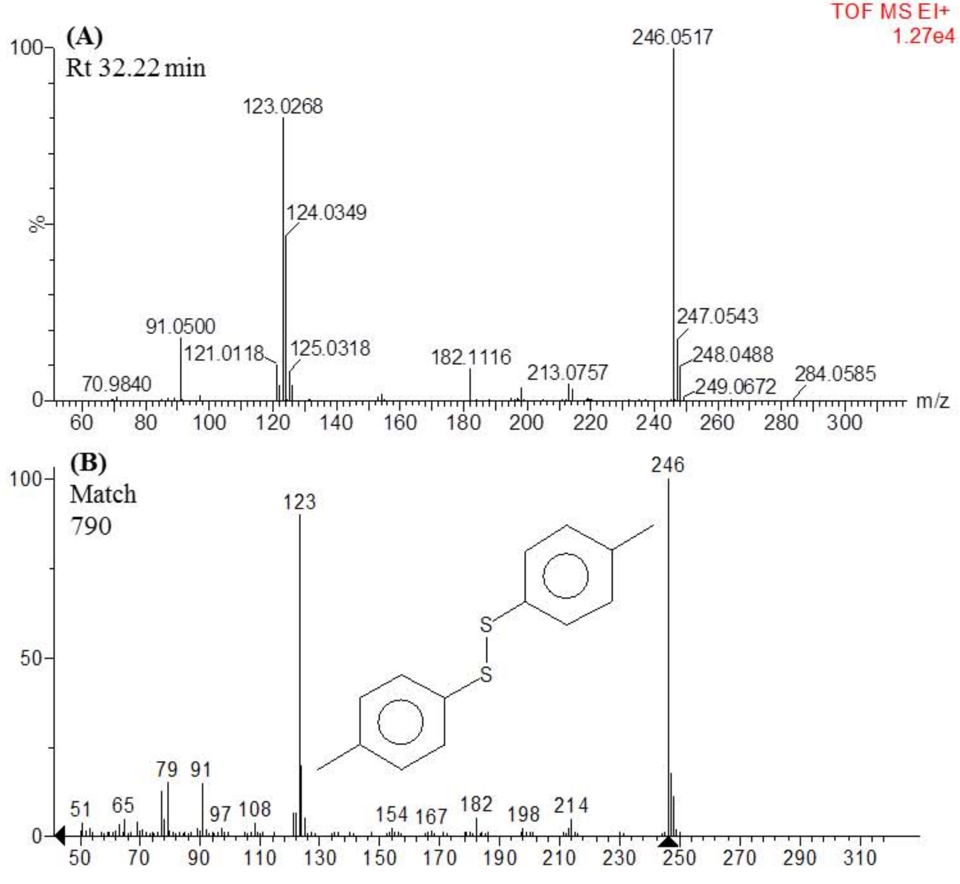
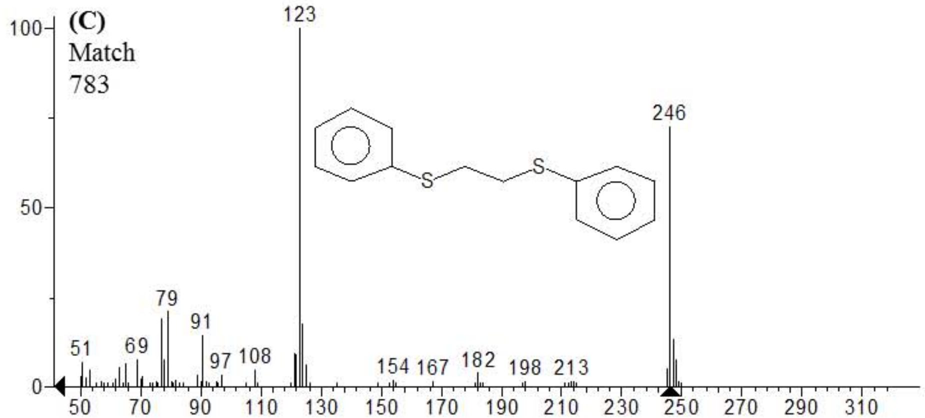
200
250
300
350
400
450
500
550
600
650

GC-(EI)TOF MS



CAS 103-19-5, p-Tolyldisulfide, $C_{14}H_{14}S_2$, MW = 246.0537



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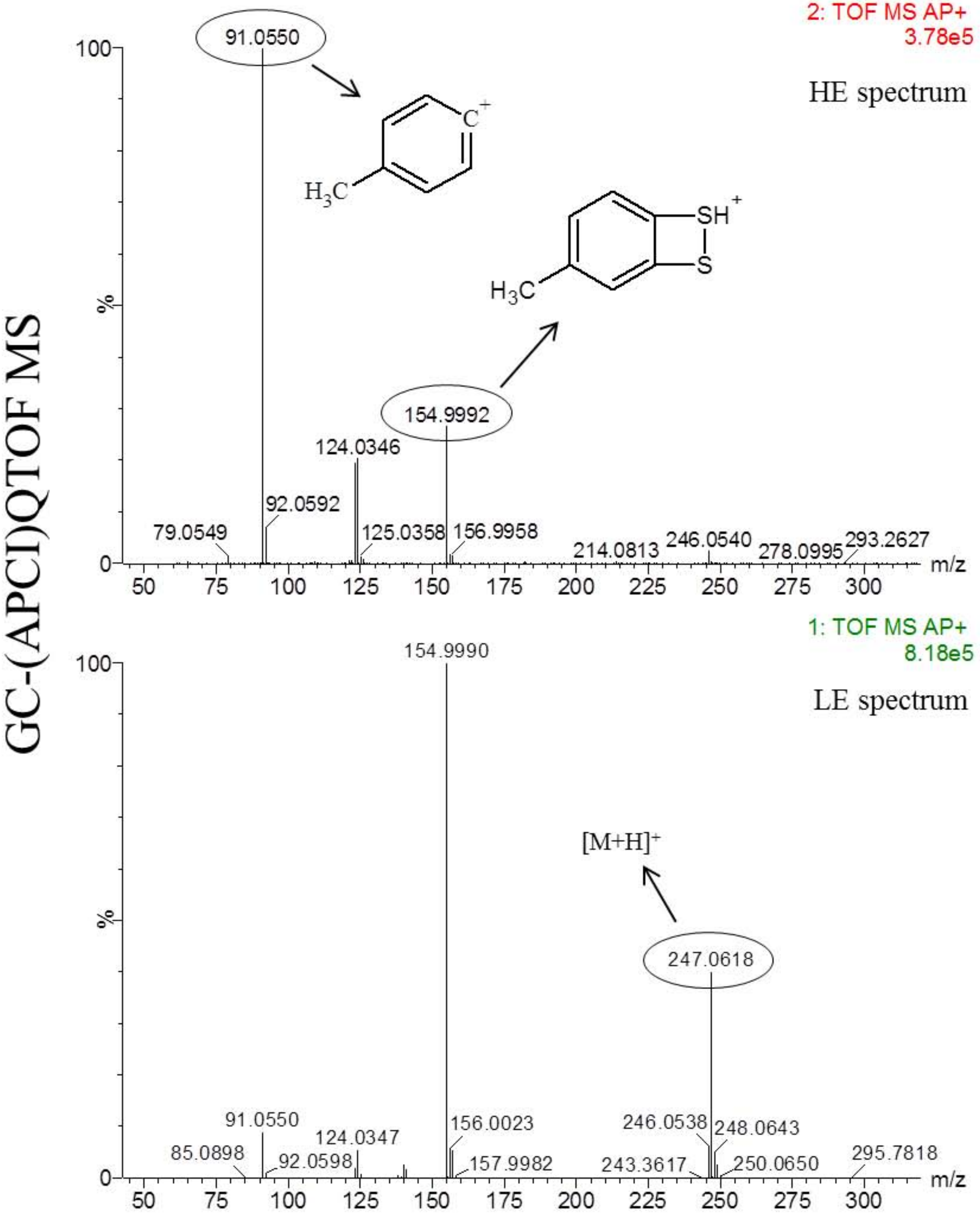
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HE spectrum
91.0550
H3C
C+
SH+
S
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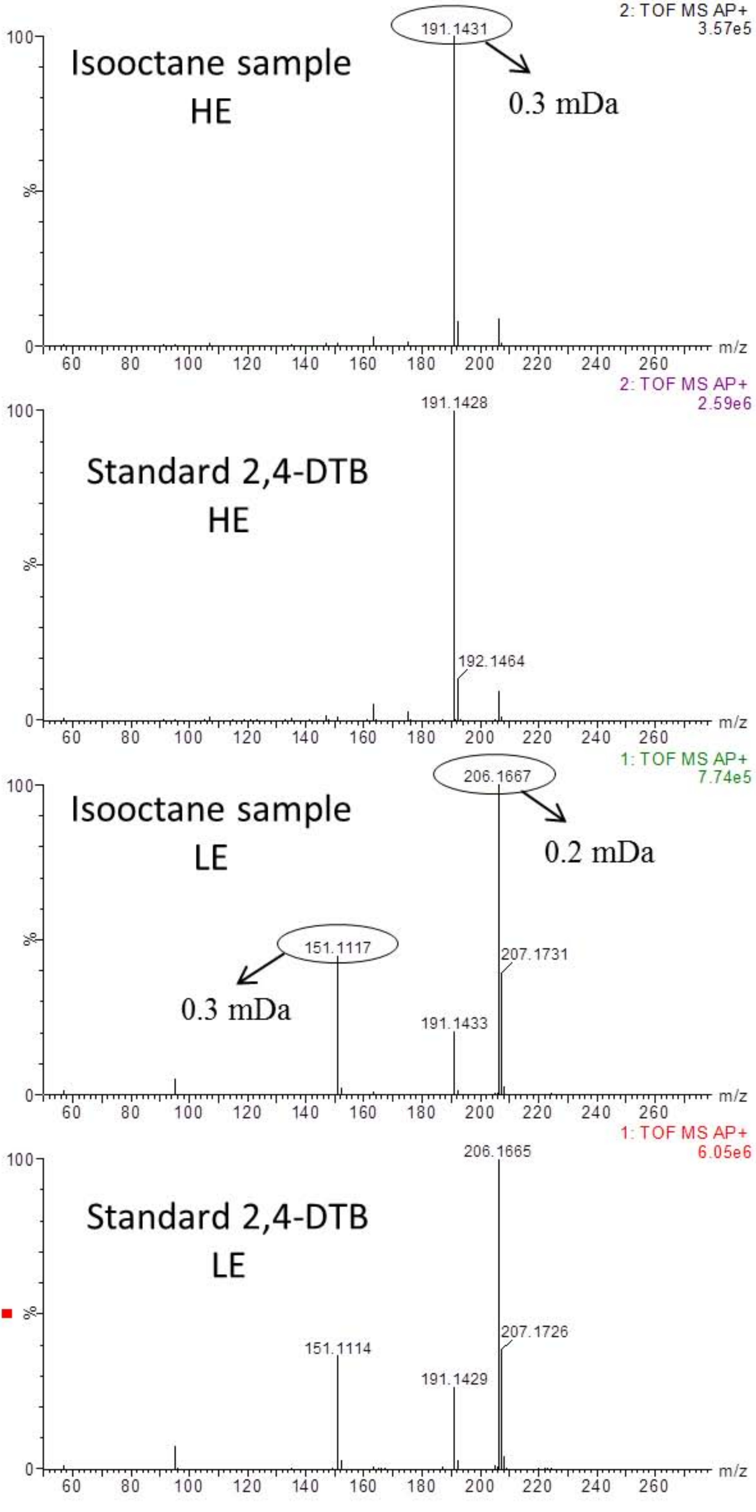
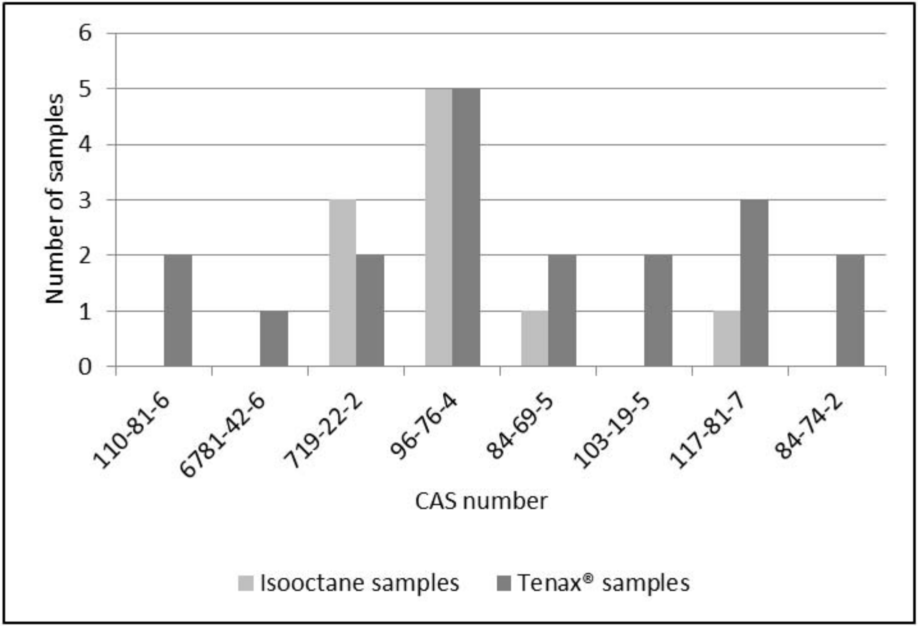
92.0592
79.0549
125.0358
156.9958
214.0813
246.0540
278.0995
293.2627
1: TOF MS AP+
8.18e5
LE spectrum
154.9990
[M+H]+
247.0618
91.0550
124.0347
156.0023
85.0898
92.0598
157.9982
246.0538
248.0643
243.3617
250.0650
295.7818
100
%
0
m/z
50
75
100
125
150
175
200
225
250
275
300


2: TOF MS AP+
3.57e5
191.1431
0.3 mDa
Isooctane sample
HE
100
%
0
m/z
60
80
100
120
140
160
180
200
220
240
260
2: TOF MS AP+
2.59e6
191.1428
192.1464
Standard 2,4-DTB
HE
1: TOF MS AP+
7.74e5
206.1667
0.2 mDa
Isooctane sample
LE
151.1117
0.3 mDa
207.1731
191.1433
1: TOF MS AP+
6.05e6
206.1665
Standard 2,4-DTB
LE
207.1726
151.1114
191.1429



Number of samples
0
1
2
3
4
5
6
110-81-6
6781-42-6
719-22-2
96-76-4
84-69-5
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
18.8	**17.93**	**6781-42-6**	5	**m-Acethyl acetophenone**	**C10H10O2**	available	**Confirmed**
		1009-61-6		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**
20.68	**19.9**	**96-76-4**	6	**2,4-di-tert-butyl-phenol (2,4-DTB)**	**C14H22O**	available	**Confirmed**
		1138-52-9		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
28.55	**27.93**	**84-69-5**	8	**Diisobutyl phthalate**	**C16H22O4**	available	**Confirmed**
		84-74-2		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**
40.42	**40.34**	**117-81-7**	4	**Bis(2-ethylhexyl) phthalate (DEHP)**	**C24H38O4**	available	**Confirmed**
		27554-26-3		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