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Keywords

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Abstract

Polyester-polyurethane lacquer, used to cover the inner surface of metallic food contact materials, may transfer non-intentionally added substances (NIAS) to the food. The identification of such a diversity of compounds, considered as migrating substances, requires taking advantage of complementary analytical platforms. Therefore, four types of gas chromatography-mass spectrometry (GC-MS) couplings were investigated and compared for their abilities to identify migrating substances after acetonitrile extraction of two commercialised lacquers. In parallel, various ionisation sources, i.e. electron ionisation (EI) (70 eV and soft energies) and atmospheric-pressure chemical ionisation (APCI) as well as various mass analysers, i.e. quadrupole, time-of-flight (low and high resolution) and Orbitrap, were tested. Comparison of mass spectra with a commercial library for EI ionisation source led to the identification of two NIAS compounds, isophorone diisocyanate and 4,4'-diphenylmethane diisocyanate. Additionally, many cyclic oligoesters (four monomer units) were unambiguously identified according to supplier's declaration on starting materials used, primarily based on the molecular ion observed by APCI mode and characteristic fragment ions. High resolution mass analysers also enhanced confidence level in such NIAS identification. One- and two-dimensional GC were also investigated for separation assessment. Although GC×GC did not reveal additional NIAS, its use provided a valuable mapping of oligomers according to monomers composition. These results were compared to our previously published LC-MS study, carried out on the same lacquer samples. This study shows that LC and GC, along with their related ionisation techniques and their own selectivity, are complementary approaches, revealing different classes of compounds covering a wide range of volatility and polarity.

1. Introduction

Materials intended for direct food contact (FCMs) aim at protecting and preserving foodstuffs. In turn, FCMs may transfer some of their constituents to food, which may endanger human health, depending on the quantity and the toxicity of such substances. Thus, FCMs are subjected to dedicated frameworks [1], regulations [2–4] and directives [5, 6] to prevent and/or control this phenomenon. The study of FCMs has traditionally focused on intentionally added substances, which are voluntarily employed due to their technical properties, and which monitoring is not considered an analytical challenge. However, over the past few year, the investigation of migrating Non-Intentionally Added Substances (NIAS) has been raising interest in the scientific community. NIAS are for instance side-products or breakdown products appearing during the manufacture, impurities of start products or environmental or process contaminants. Moreover, depending on the details of formulations like the purity or diversity of chemicals used, the number of resulting NIAS may be huge with complex chemical structures and at trace level. Consequently, for dealing with such a range of possible chemical structures in a global risk assessment process, NIAS identification is required as a first step. However, as NIAS are generally not described substances with no corresponding commercially available analytical standards, their identification and quantification is a challenge. Regarding the particular case of polyester-polyurethane coatings, some related NIAS have already been tentatively identified and reported in the literature, mainly as cyclic or linear oligoesters [7–10]. Such substances were elucidated using liquid chromatography-high resolution mass spectrometry (LC-HRMS) technologies, whereas gas chromatography-mass spectrometry (GC-MS) fitted to electron ionisation source (EI) did not reveal any oligoester [9] and was consequently not recognised as the most relevant technique to achieve NIAS identification [8]. Still, GC-MS is widely used to identify potential migrants originating from a diverse range of packaging types, such as paper and board, adhesives, plastics, biodegradable and nano- or polypropylene films. Additionally, this technique already allowed characterisation of a large number of low molecular weight cyclic oligoesters from

69 food packaging lamination adhesives [11]. Therefore, the potential of the GC-MS, applied to
70 polyester-polyurethane coatings, deserves further investigation.
71 Previous studies related to NIAS identification have combined the use of LC-MS and GC-MS
72 approaches to allow the detection of substances ranging from non-volatile to semi-volatile ones [11–
73 15] in extracts obtained using either organic solvents or food simulant. However, when only GC-MS
74 technology is used to achieve identification, different ionisation modes, highly dissociative or softer,
75 are generally employed [13, 16–18]. EI at 70 eV generates spectra with high and standardised
76 fragmentation allowing identification based on comparison against commercial libraries, such as the
77 NIST library, which contain MS spectra of more than 200,000 substances. However, for the yet
78 undescribed migrating substances, identification is complicated since these compounds are absent
79 from databases. Moreover, strong ionisation induced at 70 eV leads to low abundance or even total
80 absence of the most relevant informative fragments required for identification. Therefore, the use of
81 soft ionisation techniques like chemical ionization (CI) or atmospheric-pressure chemical ionization
82 (APCI) is recommended to preserve the molecular ion. Another novel solution is EI at variable
83 ionisation energies, in order to simultaneously improve the molecular ion signal through the use of
84 softer ionisation energy and to facilitate identification of specific isomers [19].
85 More recently, comprehensive two-dimensional gas chromatography (GC × GC) was used for the
86 analysis of FCMs [20] to enhance the peak capacity and thus compound detectability in complex
87 mixtures [21–23]. Besides the choice of chromatographic separation, mass analyser types provide
88 different levels of confidence for identification [15], depending on achieved resolution.
89 The present work aims at more deeply exploring the potentialities of GC-MS based approaches to
90 identify novel NIAS extracted from two polyester-polyurethane lacquers with acetonitrile. Applying a
91 global, non-targeted strategy, full-scan MS spectra were acquired with various GC-MS instruments, in
92 order to investigate the semi-volatile fraction of extractible NIAS and to reveal the most
93 comprehensive method(s) of characterisation. We thus involved in the study four GC-MS platforms
94 including EI ionisation (qMS, 70 eV; time of flight-low resolution mass spectrometry: TOFLRMS, 70

and 14 eV; Orbitrap, 70, 12 and 8 eV), GC×GC-EI-TOFLRMS (70 eV) and GC-APCI-TOFHRMS. The different combinations were then compared in terms of (i) acquired profile, (ii) selectivity, i.e. ability to identify a substance by comparison with commercial library or by structural elucidation, (iii) resolving power, i.e. interest of the use of GC×GC compared to one-dimensional GC and (iv) complementarity with LC-ESI-Orbitrap technology based on information previously published [10]. Additionally, the interest of derivatisation was investigated.

2. Material and methods

2.1. Chemicals

N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) for GC derivatisation procedure (purity ≥98.5%) and standards of isophorone diisocyanate (IPDI) (CAS 4098-71-9) and 4,4'-diphenylmethane diisocyanate (DPMDI) (CAS 101-68-8) were purchased from Sigma-Aldrich (St-Louis, MO, USA). Acetonitrile was provided by Carlo Erba Réactifs (HPLC grade, Rodano, Italy). *n*-Nonane was obtained from Merck (Darmstadt, Germany).

2.2. Lacquer samples

Two different polyester-polyurethane lacquers (Lac1 and Lac2) were obtained from two major industrial suppliers. According to the supplier's information, both lacquers were based on a mixture of two phthalic acid isomers (PA), namely terephthalic (TPA) and isophthalic acid (IPA), as diacids. The diol monomers differed since Lac1 was based on neopentyl glycol (NPG) and 1,6-hexanediol (HD) while Lac2 was based on NPG, diethylene glycol (DEG) and ethylene glycol (EG). In addition, our previous study [10] revealed the presence of sebacic acid (SA) and triethylene glycol (TEG) for Lac2. Both lacquers were coated on one side of metal plates and cured according to the supplier technical recommendations [10].

2.3. Sample preparation

Sample extraction procedure was performed as previously described [10]. Briefly, pieces of 0.5 dm^2 of each coated plate was placed in glass tubes with 10 mL of acetonitrile at 40 °C during 24 h. Acetonitrile was selected as a strong extractive solvent to maximize extraction efficiency in order to facilitate the identification of substances possibly migrating in food or food simulants. Aliquots of 200 µL were evaporated at 40 °C under a gentle stream of nitrogen. Dried extracts were reconstituted in *n*-nonane or subjected to a derivatisation procedure occurring at 60 °C for 1 h after addition of 20 µL MSTFA. This derivative agent was used due to its ability to react by silylation with, among others, alcohol and carboxylic acid functions which are characteristic of monomers used for the polyester lacquer formulation. Dilution solvents (MSTFA or *n*-nonane), were systematically analysed as blanks.

2.4. Instrumental acquisition

Four technologies were compared (Table 1), including GC-(EI)qMS (Platf1) (7000 Triple Quad, Agilent Technologies, Inc., Santa Clara, CA, USA), GC-(EI)Orbitrap (Platf2) (Q-Exactive, Thermo Scientific, Bremen, Germany), GC-(APCI)TOFHRMS (Platf3) (Synapt G2-S HDMS, Waters, Manchester, UK), GC(×GC)-(EI)TOFLRMS (Platf4) (BenchTOF-Select, Markes International, Llantrisant, UK). GC conditions were kept as similar as possible across the four instruments under investigation. Injection volume was set at 1 µL in the splitless mode. Helium was used as carrier gas.

One-dimensional GC separation was achieved on a 30 m long × 0.25 mm internal diameter, 0.25 µm film thickness DB-5MS capillary column (Agilent Technology, Palo Alto, CA, USA). The oven temperature was programmed as follows: 120 °C (3 min), 10 $°C.min^{-1}$ to 270 °C (15 min), 5 $°C.min^{-1}$ to 310 °C (8 min) and held for 8 min. Injector temperature was set at 250 °C. Carrier gas flow rate was 1 $mL.min^{-1}$ for Platf1, Platf2 and Platf4 and 1.7 $mL.min^{-1}$ for Platf3.

GC×GC separation was achieved on Platf4 equipped with an INSIGHT flow modulator (SepSolve Analytical Ltd, Peterborough, UK), on a 20 m x 0.18 mm, 0.18 µm BPX5 capillary column (SGE,

Ringwood, Australia) followed by a 5 m x 0.25 mm, 0.25 µm BPX50 (SGE). Initial temperature at 120 °C for 4 min was ramped to 350 °C (5 °C.min^{-1}) and held for 3 min. Carrier gas flow rate was set at 0.5 mL.min^{-1} and 20 mL.min^{-1} for the first and second dimensions, respectively. The split ratio between TOF and FID was approximately 1:3.

Electron ionisation was performed at 70 eV with Platf1, at 70 and 14 eV simultaneously with a Tandem Ionisation source on Platf4 and at 70, 12 and 8 eV separately with Platf2. Regarding Platf3, the APCI ionisation process occurred with N_2 as auxiliary gas (500 L.h^{-1}), cone gas (50 L.h^{-1}) and make-up gas (400 mL.min^{-1}). Corona pin was set at 2 µA.

A solvent delay of 6 min was respected prior to data acquisition. Acquisition was operated in full scan mode over *m/z* range of [50–750] with Platf1, Platf2 and Platf4 and over *m/z* range of [50–1200] with Platf3. The resolving power (full width at half maximum) was set at 120 000@200 with Platf2 and 40 000 with Platf4.

2.5. Software

Data analysis was performed with MassHunter Qualitative Analysis B.05.00 (Agilent Technology, Palo Alto, CA, USA) (Platf1), TraceFinder 4.1 General Quan software (Thermo Fisher Scientific, San José, CA, USA) (Platf2), MassLynx version 4.1 (Waters, Manchester, UK) (Platf3) and ChromSpace 1.2. (Markes International, Llantrisant, UK) (Platf4). Library comparison was achieved with the NIST 2014 database, developed and supported by the National Institute for Standards and Technology.

3. Results and discussion

3.1. Lacquer profiles

The lacquer TICs obtained both with and without a derivatisation procedure (Figure 1a) were compared revealing significant differences as expected. In particular, the 6-23 min region of the chromatogram revealed early-eluting peaks in the derivatised samples. As they were not detected in the underivatised ones, this provides the first information regarding their chemical structures.

Indeed, these compounds probably contain polar functions (hydroxyl or acidic groups). Therefore, only analyses carried out after derivatisation were investigated further, in order to seek maximum characterisation of NIAS. Blank and both lacquer profiles were all clearly different, in particular for the second part of TICs (>25 min) which seems more complex for Lac2 (Figures 1 and S1). This description is supported by the results previously obtained using LC as chromatographic separation [10]. When comparing the profiles obtained by the four instruments, they appear slightly different for a given lacquer extract after MSTFA derivatisation. On the second part, between 24 and 32 min, the profile obtained by the EI-TOFLRMS for Lac2 is richer (Figure 1c) and more intense. Likewise, on the first part, profiles were enriched between 6 and 17 min for the APCI-TOFHRMS and EI-TOFLRMS (Figure 1b and 1c). This difference could be mainly explained by the high voltage used in the TOFLRMS mass analyser, which could allow improvement of the transmission rate of ions, increasing intensity detection of heavier fragment ions.

To be more specific regarding the comparison between lacquer profiles obtained by the different platforms, numbers of the integrated chromatographic peaks were reported for each platform in Table 2. For enumeration, threshold values were set at 1% of intensity of the largest peak for Platf1, 3,000 (absolute area) for Platf3 and S/N 20 for Platf2 and Platf4. Moreover, software related to each technology allowed integration of data, except for (EI)-TOFLRMS and (EI)Orbitrap technologies for which a deconvolution step prior to integration was performed.

The highest number of integrated peaks was reached using the GC-Orbitrap platform, with more peaks detected at low ionisation energies than at higher ones. Compared to this platform, GC-TOFLRMS highlighted a similar number of peaks for the 70 eV value and four times less at low ionisation energy. TOFHRMS and qMS display the lowest number of integrated chromatographic peaks, with about one hundred detected. Similar tendencies were observed with both lacquers and blank, Lac1 revealing less integrated chromatographic peaks compared to Lac2, and blank even less, as expected.

198 The use of a lower ionisation energy is theoretically expected to gain in selectivity even if a loss of
199 ionisation efficiency inevitably occurs, leading to a loss of sensitivity [19]. However, the lacquer
200 profiles appear not to be significantly affected when different energy values are employed for EI (8,
201 12 and 70 eV) according to the TIC (Figures S2 and S3). Regarding the comparison between low and
202 high ionisation energies for EI, the trend appeared opposite between TOFLRMS and Orbitrap
203 platforms. In contradiction with this theory, numbers of the integrated chromatographic peaks
204 obtained with the GC-Orbitrap at different ionisation energy values could be mainly explained by a
205 saturation phenomenon inside the C-trap, leading to an ion competition with the use of 70 eV.
206 Indeed, the GC-Orbitrap exhibited more detected chromatographic peaks at 8 eV than at 70 eV (see
207 TIC in Figure S4). Moreover, the higher number of chromatographic peaks detected with GC-
208 TOFLRMS and GC-Orbitrap technologies could be explained by respective software used, allowing for
209 a powerful deconvolution process leading to an efficient separation of co-eluted compounds based
210 on EIC shape and alignment.
211 A comparison between signals of blank and lacquer allows for the discrimination of signals
212 originating from external contamination. Indeed, it is undeniable that ubiquitous compounds come
213 from laboratory environment, glassware used or instrument (connection or gas purity).
214
215 *3.2. Compounds identification via library search*
216 *3.2.1. Library search methodology*
217 In GC-MS analysis, the use of EI at 70 eV is common practice to allow direct comparison against
218 commercial databases, such as the NIST library, which encompasses more than 200,000 EI mass
219 spectra. Therefore, as a first approach, the capacity to identify compounds from the raw data set
220 acquired was evaluated by comparing mass spectra of the detected compounds to those of the NIST.
221 Although such a strategy is theoretically not the most suitable to identify NIAS as they are not
222 expected to be present in databases, it is worth testing to investigate the chemical composition of
223 the lacquer extracts and eventually highlight possible structures of interest within the initial

224 composition of the lacquers. Any high match factor is to be further confirmed with genuine analytical
225 standard for unambiguous identification.
226 Whereas library matching was automatically processed with ChromSpace and Trace Finder softwares
227 for GC-TOFLRMS and GC-Orbitrap analysis, respectively, library searching was achieved manually
228 with the data acquired on the GC-qMS system. Among all matching compounds exhibiting a match
229 factor above 700, each suggestion was carefully verified in terms of mass spectra similarities and
230 absence in the reference blank. Additionally, for data acquired in GC-Orbitrap, mass deviation
231 between measured and theoretical fragments with a visualization of their overlapped extracted ion
232 chromatograms after deconvolution was performed.
233
234 *3.2.2. Library search results*
235 Several phthalic acid-derived compounds could be identified in both lacquers. However, it remains a
236 difficult challenge to assign them to a unique identification since mass spectra of such compounds
237 are extremely similar despite different alkyl chains, the fragment ion *m/z* 149 being for most of them
238 the base peak.
239 With this methodology, four relevant compounds were identified by library matching in both
240 lacquers using GC-(EI)TOFLRMS, GC-(EI)Orbitrap and possibly GC-(EI)qMS (Table S1). IPDI and DPMDI
241 were detected along with a match factor above 700 and were confirmed with standards. Two cyclic
242 oligoesters, namely 2EG+2TPA and 2NPG+2oPA, also seemed to be present in both lacquers.
243 Concerning IPDI and cyclic oligoesters, several isomers were detected (Table S1).
244
245 *3.2.3. Library search interpretation*
246 The four compounds considered relevant in the NIAS context were systematically detected in both
247 lacquer extracts, except when using the qMS platform, where some substances were not detected or
248 identified due to its lower sensitivity.

Regarding match factor values, slight differences were observed between platforms for a given compound. It can be explained by varying mass fragmentation profiles, probably due to the ability of the instrument to transfer ions depending to their *m/z*. Indeed, even if a fragmentation profile obtained at 70 eV is robust, differences were observed between the three technologies employed. In particular, different relative intensity values for some ions were illustrated in Figure S5. In this example, the *m/z* 149 appeared as the base peak for the three technologies. However, the second most intense fragment detected at *m/z* 193 was observed at 100%, 80% and 50% using GC-Orbitrap, GC-TOFLRMS and GC-qMS, respectively.

Unexpected IPDI and DPMDI have previously been identified in plastic packaging materials at low intensities [24]. They could be impurities or degradation products of IPDI trimer generally used as crosslinker for the polyurethane lacquer manufacture [25]. Two IPDI stereoisomers were detected in lacquer extracts with same proportion displayed for the standard, which is itself a mixture of *cis*-IPDI (75%) and *trans*-IPDI (25%) [26].

Regarding cyclic oligoesters, several of them have been identified using GC-MS and reported in a recent study related to polyester urethane-based adhesive materials [11]. In our can coating extracts, which are based on the same material chemistry, the detection of cyclic oligoesters is therefore not surprising.

The combination 2EG+2TPA detected in Lac2 extract was expected since EG and TPA were used as starting monomers. This combination matched at three retention times corresponding to three different compounds. It is likely that one of them is the correct compound and that the two others could correspond to isomers, such as 2EG+2IPA, and/or to a similar cyclic oligoester such as compounds with only one monomer of difference in the combination. In Lac1 extract, the combination 2EG+2TPA was not expected since EG was not stated in supplier information. Its presence at relatively low intensity is likely explained by the presence of EG monomer as impurity. The cyclic oligoester 2NPG+2oPA isomer should not be detected since only TPA and IPA were used for the Lac1 and Lac2. Therefore, matching compounds might rather correspond to isomers.

3.3. Identification of unknowns

Although a few NIAS could be identified thanks to the commercial library, most intense signals in the acquired fingerprints remained non elucidated. Therefore, structural elucidations were carried out and the potential of each technology, especially ionisation mode, was investigated.

For each platform, mass spectra were, as a first step, investigated for their abilities to highlight the chemical nature of compounds. First of all, *m/z* 149.02335 and 73.04679, two relevant *m/z* ions corresponding to well-known major fragments related to PA [27] and MSTFA [28], respectively, were investigated. In that respect, the detection of *m/z* 149 without *m/z* 73 reflected the presence of non-derivative substances like cyclic oligoesters containing PA monomer. With regard to ion exhibiting *m/z* 73, it signs reactive ending alcohol and/or carboxylic acid functions on the NIAS structure. This ion tracking strategy revealed the presence of intense cyclic oligoester for Lac2 (Figure 2b) and Lac1 (Figure S6b) between 23 and 34 min. This ions tracking was more obvious at the highest ionization energy (EI at 70 eV), logically allowing for stronger fragmentation leading to the detection of intense low *m/z* ions. Mass spectra related to predictive cyclic oligoesters were then further investigated in order to assign one combination, corroborated with fragmentation pattern consistency and retention time.

As expected, APCI ionisation readily revealed the quasi-molecular ions. Specifically, for the cyclic oligomer 2EG+2PA, APCI was the only ionisation mode allowing its detection (Figure 3). The precise mass of this specific ion could be directly compared to a homemade database to quickly identify the compound [10]. Interestingly, along with such quasi-molecular ions, nitrosyl adducts $[M + NO]^+$ were systematically produced. These characteristic adducts were actually already reported for ester studies using APCI ionisation mode [29].

EI mode at 70 eV seemed to provide richer information, in relation to a higher number of fragments. For cyclic tetramers, one of the relevant fragments corresponded to a loss of selective fragments belonging to the initial diol monomers (Figure S7). In the case of a cyclic tetramer composed by two

301 different diols, one, two or even three typical fragments could be detected. A number above the
302 theoretical maximum value possible (i.e. three) was observed when DEG diol was part of the
303 oligomer, as illustrated in Figure S7. Indeed, DEG is composed in some way of two EG and a rupture
304 can occur on each side of the central oxygen, leading to EG-like fragments. These relevant fragments
305 for identification were detected as well using APCI mode.
306 Low ionisation energy in EI mode was also applied to unknown identification. Fragmentation profiles
307 at low and high EI energy were compared for TOFLRMS between 14 and 70 eV (Figures 3b and 3c)
308 and for Orbitrap technology between 12 and 70 eV (Figures 3d and 3e). Mass spectra were obviously
309 different for 2EG+2PA. However, mass spectra at low and high energy for EG+NPG+PA+SA and
310 2NPG+2PA were quite similar. Low energy revealed slightly less fragments presenting low *m/z*, these
311 fragments being less intense. These differences are stronger for the TOFLRMS. Interestingly, profile
312 obtained at 14 eV with EI-TOFLRMS seemed closer to APCI-TOFHRMS profile, especially for
313 2NPG+2PA, and provided the molecular ion with higher relative intensity compared to profile
314 obtained at 12 eV with EI-Orbitrap. Therefore, independently from the ionisation energy values, the
315 geometry of the source and the ability to transfer ions influence the fragmentation profiles.
316 To discriminate cyclic oligoester isomers, the use of EI at variable ionisation energy appears to be a
317 promising strategy according to the literature [19]. TOFLRMS platform allowed for such assessment
318 since higher differences could be observed between mass spectra acquired at low and high energies
319 (Figure 3). Depending on the nature of oligomer, mass spectra of isomers displayed major differences
320 at 70 rather than 14 eV (Figure S8), a result that is probably related to their inner stability. This
321 observation is in disagreement with the literature relating to the discrimination of isomers [19].
322 Moreover, despite the information available at 70 eV, the chromatographic separation remains the
323 only solution to confirm the presence of several isomers.
324 In addition, HRMS techniques increase the confidence level related to identification, something
325 which is even more interesting without genuine analytical standards. For example, the cyclic
326 2NPG+2PA with the theoretical values of *m/z* 469.186 and 468.177 for $[M + H]^+$ and $[M]^{+\cdot}$,

327 respectively, were measured at *m/z* 469.184 using (APCI)TOFHRMS, *m/z* 468.177 using (EI)Orbitrap,
328 *m/z* 468.189 using (EI)TOFLRMS and *m/z* 467.7 using (EI)qMS.
329 Finally, the elucidated cyclic oligoesters were all tetramers. All intense signals for Lac1 (n=6) and Lac2
330 (up to 25) in the 24.5-34 min retention time range have been identified, corresponding to 2 and 11
331 combinations of oligomers (Figures S9 and S10), respectively. More specifically, for Lac1, isomers of
332 2NPG+2PA and NPG+HD+2PA were identified. Such combinations were in accordance with the
333 monomers declared by suppliers. Similarly, regarding Lac2, the combinations corresponded to
334 2EG+2PA, 2EG+PA+SA, EG+NPG+2PA, EG+DEG+2PA, EG+NPG+PA+SA, EG+DEG+PA+SA, 2NPG+2PA,
335 NPG+DEG+2PA, 2DEG+2PA, 2NPG+PA+SA and NPG+DEG+PA+SA, as expected with regards to the
336 supplier's declaration.
337
338 *3.4. GC×GC added value*
339 GC×GC was also investigated for its ability to enhance NIAS elucidation thanks to higher
340 chromatographic resolution provided compared to one-dimensional GC. Indeed, the 2D visualisation
341 is expected to reveal specific patterns of oligoesters depending on their physico-chemical properties.
342 In the first GC dimension where an apolar selectivity was chosen, cyclic oligoesters were eluted
343 according to the volatility of the diol monomers. Regarding Lac1 and Lac2, oligoesters involving EG
344 monomer (197 °C as boiling point (bp)) eluted first, followed by those based on NPG monomer
345 (207 °C as bp), DEG monomer (245 °C as bp) and at last HD monomer (250 °C as bp), as shown in the
346 horizontal axis on the Figure 4. In the second GC dimension, compounds eluted according to their
347 polarity on a 50% phenyl 50% dimethylsiloxane stationary phase. Thus, for oligoesters involving
348 similar diol monomers, those involving PA monomers (aromatic ring) were eluted prior to those
349 based on SA monomers (aliphatic acid), illustrating the increased confidence in compound
350 identification provided by the 2nd GC dimension.
351 Such mapping of oligoester families can be further used as a robust prediction tool for faster
352 identification of NIAS in unknown lacquer extracts. On Figure 4, highly reproducible retention times

between Lac1 and Lac2 can be observed regarding the 2NPG+2PA. A characteristic area related to this oligoester can also be determined as rectangular shape within the ranges 47.0 to 48.6 min and 2.5 to 3.8 sec for x-axis and y-axis, respectively. Similarly, the definition of specific areas corresponding to other oligoester families can be defined. Further, such mapping is also expected to enable highlighting undescribed NIAS families, other than cyclic oligoesters. Applying this tool to our datasets did not allow reveal any new cyclic oligoesters or additional undescribed NIAS in either lacquer. This observation is likely due to the efficiency of the deconvolution software used to perceive coeluting peaks in data acquired by one-dimensional GC.

3.5. Comparison of GC with LC

Since the same extract of both lacquers had previously been characterised using LC-HRMS platform describing a range of NIAS [10], we compared both approaches to assess their complementarities and respective added values.

Regarding oligoesters, LC separation combined with ESI revealed the presence of cyclic oligoesters ranging from tetra- to octamers [10]. In the present study, GC strategy was limited to cyclic tetramers detection, in line with results reported by Zhang et al. [11]. Oligoesters with more than 4 monomer units were likely not volatile enough due to their larger molecular weight. Zhang et al. also detected a few cyclic hexamers. Such substances were based on adipic acid as diacid monomer only, which is smaller compared to PA and SA.

Cyclic tetramers detected in Lac1 and Lac2, either by GC-MS or LC-MS, including isomers, are compiled in the Table 3. Overall, it appears that cyclic tetramers containing the most (EG) or the less (DEG) volatile diol monomers, present higher GC or LC responses, respectively. Interestingly, the most intense signal detected using GC technologies, namely 2EG+2PA, was not observed using LC system (Table 3). Moreover, during the isomers investigation, one-dimensional GC was able to discriminate as many or more compounds than LC, depending on the combination, due to higher chromatographic resolution.

With regard to the complementarity between both approaches, IPDI and DPMDI were only revealed by GC technologies, whatever the ionisation mode (EI at low and high energy or APCI) and, conversely, cyclic caprolactam oligomers could only be detected using LC-ESI-HRMS [10].

Regarding cyclic oligoesters detected by both LC and GC technologies, soft ionization modes like APCI or ESI coupled to GC or LC, respectively, displayed similar mass spectra profiles. However, molecular ion was more directly identified using APCI mode due to the larger number of adduct ions produced by ESI in positive mode. Similarly, mass spectra obtained after fragmentation by high-energy collisional dissociation mode using LC technology are similar to those obtained by EI mode. The characteristic ions formed by the loss of a selective diol monomer fragment was actually observed in both ionisation modes, as well as the *m/z* 149 related to the presence of PA (Figure S7).

Whereas no universal and exhaustive ESI mass spectra library is available so far, the NIST database registered at 70 eV in EI coupled to GC remains helpful for identification of some unexpected substances in an untargeted workflow.

4. Conclusions

The combined use of various GC platforms enabled the identification of 6 and 29 cyclic oligoester tetramers at best for Lac1 and Lac2, respectively. In addition, in both lacquer extracts, two suspected cross linker impurities, IPDI and DPMDI, were identified.

Derivatisation was not effective in the detection of cyclic oligoesters. However, it is expected to be helpful to reveal linear oligoesters and provide additional information relative to chemical functions, which is particularly interesting for identification of unknowns.

With regard to identification, the most relevant investigated technique was the EI source at 70 eV for substances present in the NIST library and APCI source for undescribed migrating substances. The use of variable ionisation, i.e. EI at low and high energies is considered a good compromise to allow NIST library search together with molecular ion detection, using only one system and avoiding ionisation

sources switching. In addition, the use of HRMS has been found to enhance the confidence in the identification of the compounds.

Even if TOFLRMS and Orbitrap appeared as the most informative, sensitivity of the detection did not appear as a bottleneck of NIAS investigation since in such studies, the sample size is generally not an issue and concentrated extracts may easily be obtained.

While GC×GC separation did not reveal additional compounds, it provided a valuable mapping which could highlight undescribed oligoesters. Further, the separation capacity of GC×GC is expected to be even more relevant when complex samples, like food items, are analysed.

Moreover, results obtained by the GC-MS and LC-MS studies confirm the need for a combination of complementary approaches to provide the most exhaustive fingerprint possible. Only one platform is therefore not able to answer risk assessment expectations for which all substances below the conventional limit of 1,000 Da have to be considered [30].

Finally, the responses obtained by GC-MS and LC-MS were different for several cyclic tetramers. Consequently, special attention must be paid to quantification issue. Therefore, the use of mimetic standards, some of them being recently synthesized [31], is undeniably necessary to quantitatively measure those compounds, after extraction by food simulants or directly in foodstuffs. This should be considered as the next step within risk assessment process.

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References

1. European Commission (2004) Regulation (EC) No 1935/2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC. Off J Eur Union L338:4–17

2. European Commission (2008) Regulation (EC) No 282/2008 on recycled plastic materials and articles intended to come into contact with foods and amending Regulation (EC) No 2023/2006. Off J Eur Union L86:9–18

3. European Commission (2009) Regulation (EC) No 450/2009 on active and intelligent materials and articles intended to come into contact with food. Off J Eur Union L135:3–11

4. European Commission (2011) Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food. Off J Eur Union L 12:1–89

5. European Commission (1984) Directive 84/500/EEC on the approximation of the laws of the Member States relating to ceramic articles intented to come into contact with foodstuffs. Off J Eur Communities 12–16

6. European Commission (2007) Directive 2007/42/EC relating to materials and articles made of regenerated cellulose film intended to come into contact with foodstuffs. Off. J. Eur. Union L172:71–82

7. Schaefer A, Ohm VA, Simat TJ (2004) Migration from can coatings: Part 2. Identification and quantification of migrating cyclic oligoesters below 1000 Da. Food Addit Contam 21:377–389 . doi: 10.1080/02652030310001637939

8. Bradley E, Driffield M, Guthrie J, Harmer N, Thomas Oldring PK, Castle L (2009) Analytical approaches to identify potential migrants in polyester–polyurethane can coatings. Food Addit Contam Part A 26:1602–1610 . doi: 10.1080/19440040903252256

9. Paseiro-Cerrato R, MacMahon S, Ridge CD, Noonan GO, Begley TH (2016) Identification of unknown compounds from polyester cans coatings that may potentially migrate into food or food simulants. J Chromatogr A 1444:106–113 . doi: 10.1016/j.chroma.2016.03.038

10. Omer E, Cariou R, Remaud G, Guitton Y, Germon H, Hill P, Dervilly-Pinel G, Le Bizec B (2018) Elucidation of non-intentionally added substances migrating from polyester-polyurethane lacquers using automated LC-HRMS data processing. Anal Bioanal Chem 1–13 . doi: 10.1007/s00216-018-0968-z

11. Zhang N, Kenion G, Bankmann D, Mezouari S, Hartman TG (2018) Migration studies and chemical characterization of low molecular weight cyclic polyester oligomers from food packaging lamination adhesives. Packag Technol Sci 31:197–211 . doi: 10.1002/pts.2367

12. Canellas E, Vera P, Nerín C (2015) UPLC-ESI-Q-TOF-MS(E) and GC-MS identification and quantification of non-intentionally added substances coming from biodegradable food packaging. Anal Bioanal Chem 407:6781–6790 . doi: 10.1007/s00216-015-8848-2

13. Onghena M, Van Hoeck E, Van Loco J, Ibáñez M, Cherta L, Portolés T, Pitarch E, Hernandéz F, Lemière F, Covaci A (2015) Identification of substances migrating from plastic baby bottles using a combination of low-resolution and high-resolution mass spectrometric analysers coupled to gas and liquid chromatography. J. Mass Spectrom. 50:1234–1244

14. Lago MA, Ackerman LK (2016) Identification of print-related contaminants in food packaging. Food Addit Contam Part A 33:518–529 . doi: 10.1080/19440049.2015.1136435

15. Martínez-Bueno MJ, Hernando MD, Uclés S, Rajski L, Cimmino S, Fernández-Alba AR (2017) Identification of non-intentionally added substances in food packaging nano films by gas and liquid chromatography coupled to orbitrap mass spectrometry. Talanta 172:68–77 . doi: 10.1016/j.talanta.2017.05.023

16. Canellas E, Vera P, Nerín C (2014) Atmospheric pressure gas chromatography coupled to quadrupole-time of flight mass spectrometry as a tool for identification of volatile migrants from autoadhesive labels used for direct food contact. J. Mass Spectrom. 49:1181–1190

17. Cherta L, Portolés T, Pitarch E, Beltran J, López FJ, Calatayud C, Company B, Hernández F (2015) 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. Food Chem 188:301–308 . doi: 10.1016/j.foodchem.2015.04.141

18. Humston-Fulmer E, Binkley J (2017) Detection and characterization of extractables in food packaging materials by GC-MS. Curr trends mass Spectrom 15:8–15

19. Alam MS, Stark C, Harrison RM (2016) Using Variable Ionization Energy Time-of-Flight Mass Spectrometry with Comprehensive GC×GC To Identify Isomeric Species. Anal Chem 88:4211–4220 . doi: 10.1021/acs.analchem.5b03122

20. Biedermann M, Grob K (2018) Advantages of comprehensive two-dimensional gas chromatography for comprehensive analysis of potential migrants from food contact materials. Anal Chim Acta. doi: 10.1016/J.ACA.2018.10.046

21. Biedermann M, Grob K (2006) Phenolic resins for can coatings: I. Phenol-based resole analysed by GC–MS, GC×GC, NPLC–GC and SEC. LWT - Food Sci Technol 39:633–646 . doi: 10.1016/J.LWT.2005.04.008

22. Biedermann M, Castillo R, Riquet A-M, Grob K (2014) Comprehensive two-dimensional gas chromatography for determining the effect of electron beam treatment of polypropylene used for food packaging. Polym Degrad Stab 99:262–273 . doi: 10.1016/J.POLYMDEGRADSTAB.2013.10.021

23. Biedermann M, Grob K (2015) Comprehensive two-dimensional gas chromatography for characterizing mineral oils in foods and distinguishing them from synthetic hydrocarbons. J Chromatogr A 1375:146–153 . doi: 10.1016/j.chroma.2014.11.064

24. García Ibarra V, Rodríguez Bernaldo de Quirós A, Paseiro Losada P, Sendón R (2018) Identification of intentionally and non-intentionally added substances in plastic packaging materials and their migration into food products. Anal Bioanal Chem 410:3789–3803 . doi: 10.1007/s00216-018-1058-y

25. Driffield M, Bradley EL, Castle L (2007) A method of test for residual isophorone diisocyanate trimer in new polyester-polyurethane coatings on light metal packaging using liquid chromatography with tandem mass spectrometric detection. J. Chromatogr. A 1141:61–66

26. Spyrou E, Metternich HJ, Franke R (2003) Isophorone diisocyanate in blocking agent free polyurethane powder coating hardeners: Analysis, selectivity, quantumchemical calculations. Prog Org Coatings 48:201–206 . doi: 10.1016/S0300-9440(03)00104-8

27. Friocourt MP, Picart D, Floch HH (1980) Mass spectral fragmentation of phthalic acid esters. Biol Mass Spectrom 7:193–200 . doi: 10.1002/bms.1200070504

28. Halket JM, Waterman D, Przyborowska AM, Patel RKP, Fraser PD, Bramley PM (2005)

Chemical derivatization and mass spectral libraries in metabolic profiling by GC/MS and LC/MS/MS. J Exp Bot 56:219–243 . doi: 10.1093/jxb/eri069

29. Halloum W, Cariou R, Dervilly-Pinel G, Jaber F, Le Bizec B (2016) APCI as an innovative ionization mode compared with EI and CI for the analysis of a large range of organophosphate esters using GC-MS/MS. J Mass Spectrom 52:54–61 . doi: 10.1002/jms.3899

30. European Food Safety Authority (2008) Food contact materials: Note for guidance for petitioners presenting an application for the safety assessment of a substance to be used in food contact materials prior to its authorisation. EFSA J 1–125

31. Pietropaolo E, Albenga R, Gosetti F, Valentina T, Koster S, Marin-Kuan M, Veyrand J, Patin A, Schilter B, Pistone A, Tei L (2018) Synthesis, identification and quantification of oligomers from polyester coatings for metal packaging. J Chromatogr A 1578:15–27 . doi: 10.1016/j.chroma.2018.10.002

Table 1. Details of the four different platforms used for comparison with type of GC, ionisation source and analyser.

	GC	Ionisation source	Analyser
Platf1	1D	EI (70 eV)	qMS
Platf2	1D	EI (8, 12 and 70 eV)	Orbitrap
Platf3	1D	APCI	TOFHRMS
Platf4	1D and 2D	EI (14 and 70 eV)	TOFLRMS

Table 2. Number of integrated chromatographic peaks for blank, Lac1 and Lac2 obtained by different GC-MS platforms.

	Ionisation	Blank	Lac1	Lac2
TOF (HRMS)	APCI	69	135	131
TOF (LRMS)	EI (14 eV)	54	139	199
	EI (70 eV)	94	404	537
Orbitrap	EI (8 eV)	380	608	824
	EI (12 eV)	260	632	796
	EI (70 eV)	134	334	616
qMS	EI (70 eV)	17	83	100

Table 3. List of cyclic tetraesters identified in Lac1 and Lac2 using GC-MS technologies and/or ESI-LC-Orbitrap [10] along with number of separated isomers. ND: not detected.

			GC		LC	
Lacquer	**Molecular mass**	**Oligomers combination**	**Number of separated isomers**	**Intensity**	**Number of separated isomers**	**Intensity**
1 and 2	384.08452	2EG+2PA	1	+++	ND	ND
2	420.17842	2EG+PA+SA	2	-	ND	ND
2	426.13147	EG+NPG+2PA	3	++	1	-
2	428.11074	EG+DEG+2PA	2 to 3	±	2	+
2	462.22537	EG+NPG+PA+SA	2	+	1	+
2	464.20464	EG+DEG+PA+SA	1 to 2	- - -	2	+
1 and 2	468.17842	2NPG+2PA	3	++	2	++
2	470.15769	NPG+DEG+2PA	3	+	3	+++
2	472.13695	2DEG+2PA	3	- - -	3	++
1	482.19407	NPG+HD+2PA	2	++	2	+++
1	496.20972	2HD+2PA	ND	ND	1	++
2	500.29854	EG+DEG+2SA	ND	ND	1	- - -
2	504.27232	2NPG+PA+SA	2	+	2	+
2	506.25159	NPG+DEG+PA+SA	2	- -	1	+
2	508.23085	2DEG+PA+SA	ND	ND	1	±
2	514.18390	NPG+TEG+2PA	ND	ND	1	- - -
2	516.16317	DEG+TEG+2PA	ND	ND	2	- - -

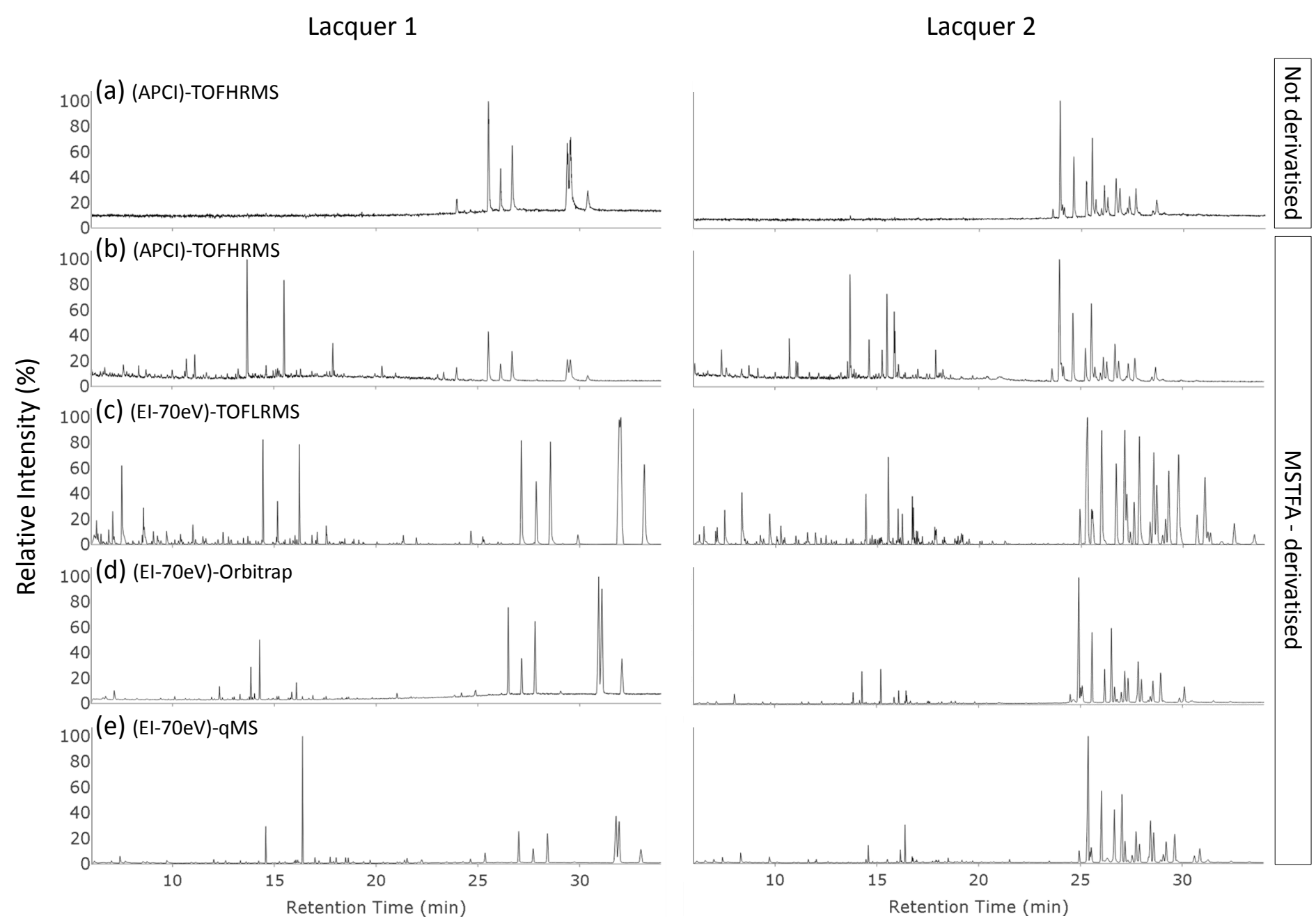
Figure captions

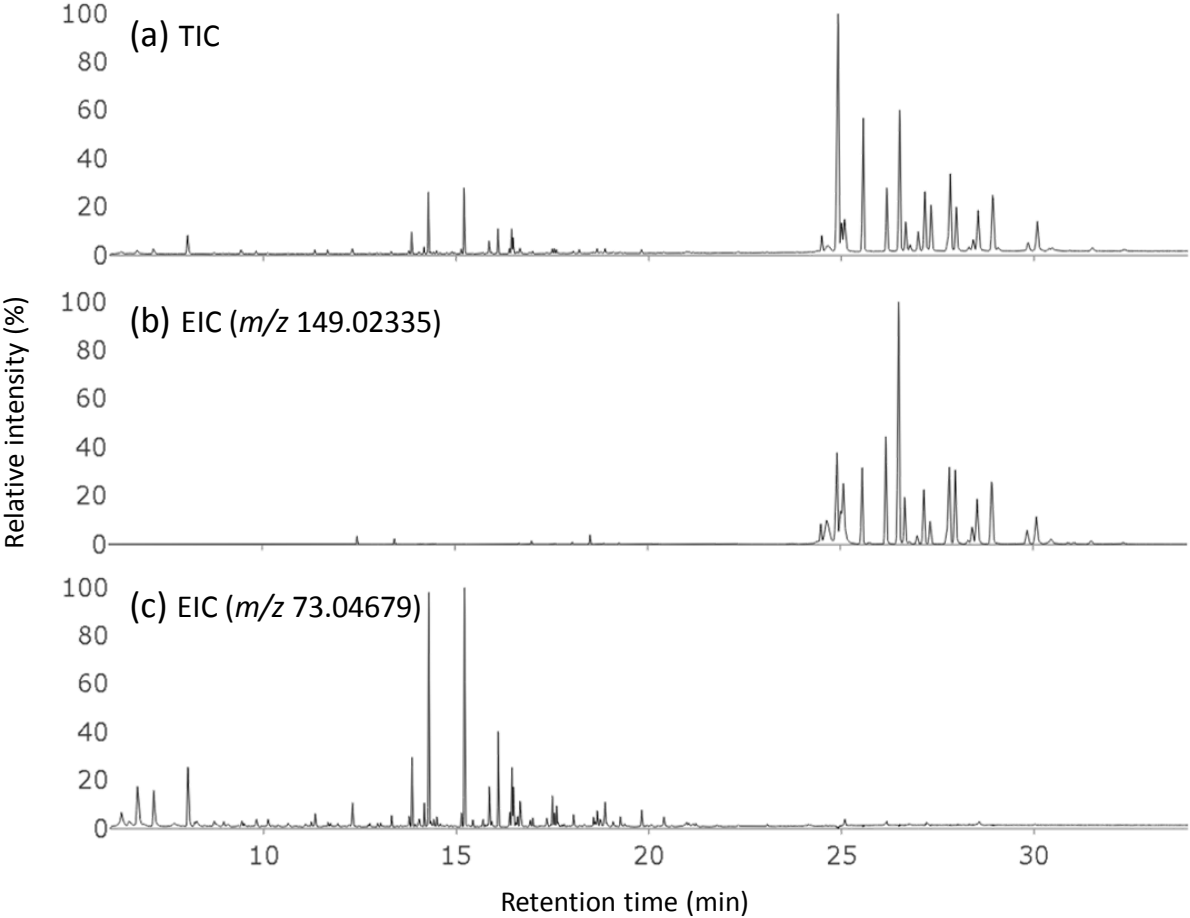
Figure 1. TICs of Lac1 extract on the left and Lac2 extract on the right obtained (a) without derivatisation procedure or (b, c, d and e) after derivatisation procedure. Platforms and sources used were GC coupled to (a and b) APCI-TOFHRMS, (c) EI-TOFLRMS at 70 eV, (d) EI-Orbitrap at 70 eV and (e) EI-qMS at 70 eV.

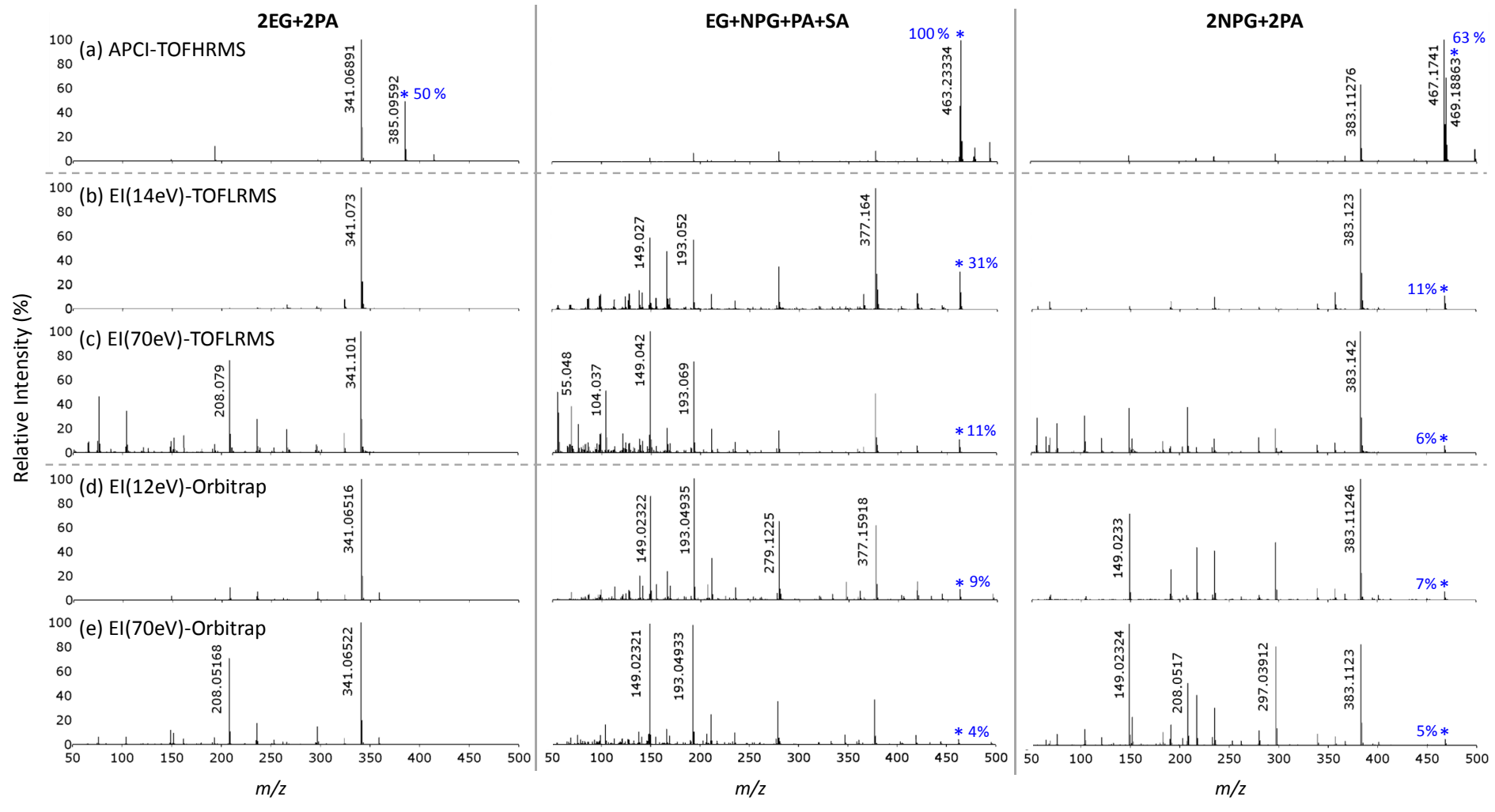
Figure 2. (a) TICs and EICs of *m/z* (b) 149.02335 and (c) 73.04679 from Lac2 extract obtained by EI-Orbitrap at 70 eV after MSTFA derivatisation.

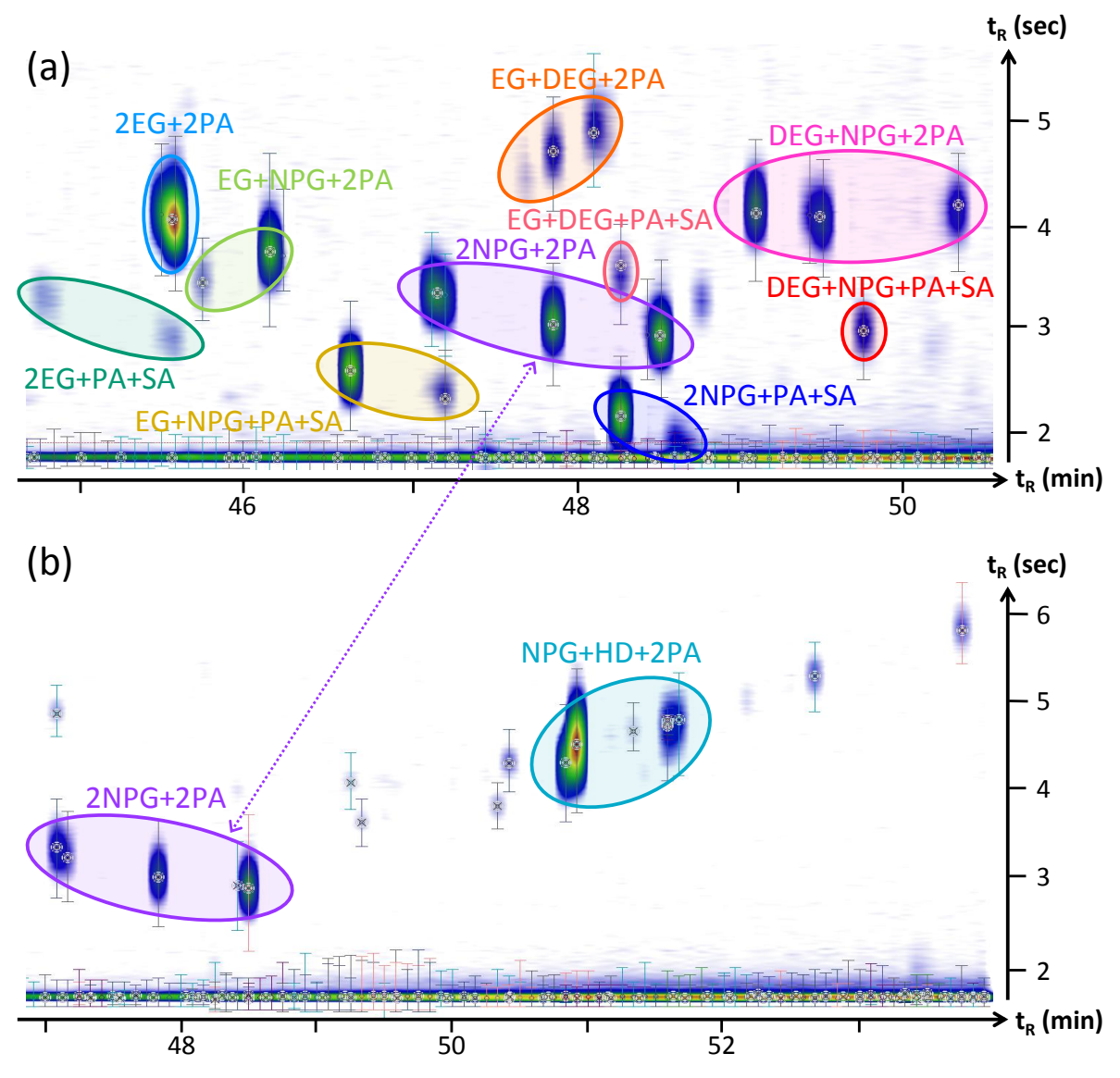
Figure 3. Mass spectra recorded for 2EG+2PA cyclic, EG+NPG+PA+SA cyclic and 2NPG+2PA cyclic with different GC-MS systems: (a) APCI-TOFHRMS, EI-TOFLRMS at (b) 14 eV and (c) 70 eV, EI-Orbitrap at (d) 12 eV and (e) 70 eV. *: (a) $[M+H]^+$ or (b, c, d and e) $[M]^{+\cdot}$

Figure 4. GC×GC-MS plots of Lac2 (a) and Lac1 (b) extracts with identified cyclic tetramers.


Lacquer 1
Lacquer 2
Not derivatised
MSTFA - derivatised
Relative Intensity (%)
(a) (APCI)-TOFHRMS
(b) (APCI)-TOFHRMS
(c) (EI-70eV)-TOFLRMS
(d) (EI-70eV)-Orbitrap
(e) (EI-70eV)-qMS
100
80
60
40
20
0
10
15
20
25
30
Retention Time (min)


(a) TIC
(b) EIC (m/z 149.02335)
(c) EIC (m/z 73.04679)
Relative intensity (%)
Retention time (min)
100
80
60
40
20
0
10
15
20
25
30


2EG+2PA
EG+NPG+PA+SA
2NPG+2PA
(a) APCI-TOFHRMS
(b) EI(14eV)-TOFLRMS
(c) EI(70eV)-TOFLRMS
(d) EI(12eV)-Orbitrap
(e) EI(70eV)-Orbitrap
Relative Intensity (%)
m/z
341.06891
385.09592
* 50 %
463.23334
100 % *
383.11276
467.1741
469.18863*
63 %
341.073
149.027
193.052
377.164
* 31%
383.123
11% *
208.079
341.101
55.048
104.037
149.042
193.069
* 11%
383.142
6% *
341.06516
149.02322
193.04935
279.1225
377.15918
* 9%
149.0233
383.11246
7% *
208.05168
341.06522
149.02321
193.04933
* 4%
149.02324
208.0517
297.03912
383.1123
5% *


(a)
2EG+2PA
EG+NPG+2PA
EG+DEG+2PA
DEG+NPG+2PA
EG+DEG+PA+SA
2NPG+2PA
DEG+NPG+PA+SA
2EG+PA+SA
EG+NPG+PA+SA
2NPG+PA+SA
t_R (sec)
5
4
3
2
t_R (min)
46
48
50
(b)
NPG+HD+2PA
2NPG+2PA
t_R (sec)
6
5
4
3
2
t_R (min)
48
50
52