Title

Identification of unknown compounds from polyester cans coatings that may potentially migrate into food or food simulants

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1. Abstract

Cross-linked polyester resins are being introduced into the market as alternatives to epoxy resins as coatings for metal food cans. Identification of potential migrants, from these coatings into food, is a significant analytical challenge due to the diversity of substances employed in the manufacture of the coatings. However, such identification is required to assess migration from the can coating into the food and quantify dietary exposure. Polyester can coatings were extracted with acetonitrile at 40 °C for 24 h and the extracts were analyzed by a variety of analytical techniques, including GC-MS, HPLC-DAD/MS, HPLC-DAD/CAD and UHPLC-HRMS. Twenty nine non-volatile oligomers were tentatively identified using retention times, UV spectra, and accurate mass measurements. Identified oligomers suggest the coating in use for food cans is a polyester coating and is mainly based on the monomers isophthalic acid, terephthalic acid and nadic acid. To give confidence in the identification, one of the tentatively identified oligomer was synthesized and analyzed by ^{13}C and ^1H NMR and UHPLC-HRMS. The NMR and HRMS results, confirmed the presence of this compound in the can extracts. Finally, to determine if rapid, direct detection of the oligomers was practical, the coatings were analyzed by DART-HRMS. Twenty three out of the 29 oligomers were identified in the coating by direct measurement with DART-HRMS in few minutes.

Keywords: Food packaging, polyesters can coatings, identification, direct analysis in real time (DART), non-intentionally added substances (NIAS), migration.

2. Introduction

Polymeric coatings have been traditionally employed as functional barriers between food and metal cans. Cans with polymeric coatings protect food from external agents such as light, oxygen, microorganisms, and facilitate transport and storage of the canned food. They preserve the quality of food in terms of flavor, odor, color and extend self-life. Traditionally, one of the most commonly used coatings are epoxy-resins based in part on bisphenol A (BPA). BPA is a monomer that has been receiving much attention because of its possible effect as endocrine disruptor at lower doses [1, 2]. However, recently, two independent agencies, such as Food and Drug Administration (FDA) and European Food Safety Authority (EFSA) independently reached the same conclusion, that BPA poses no health risk to consumers at the exposure concentrations in food[3][4].In spite of these conclusions, consumers remain wary about the use of BPA in food contact materials. In an effort to satisfy consumer demand, can manufactures and food producers have been developing new coatings to replace epoxy-resins.

One of the new alternatives for food packaging are polyester can liners [5, 6].Polyester coatings are usually manufactured from the reaction of carboxylic acids and alcohols [7], starting materials which are approved for use in food packaging. During the polymerization process, intermediate compounds known as oligomers can remain incompletely reacted in the polymeric chain and could potentially migrate into the food. These side products are considered as non-intentionally added substances (NIAS). Most of these compounds have not been evaluated because of lack of standards and may pose food safety issues. The diversity of starting compounds (i.e. multiple monomers, crosslinkers, additives, solvents) and the high temperatures employed during processing, results in a very diverse variety of oligomers in the final polymer and makes identification difficult [8]. There are several studies focused on oligomer

identification, but most characterize epoxy-resins based on bisphenol A diglycidyl ether (BADGE) and BPA [8-12]. Only a limited number of studies have characterized polyester resins [5, 13] and as a consequence there is very little information about potential oligomers that can migrate from packaging into food. Due to the high diversity of monomers that can be used for manufacture of polyesters resins as well as variability from one producer to another in coating manufacturing process, the oligomers that are present could vary between polyester resins. Thus, it is advantageous to continue characterizing oligomers in the polyester coatings.

Polyethylene terephthalate (PET) is a polyester polymer commonly used for food contact applications, such as trays for packaging food and beverage bottles [14]. Main monomers have been already analyzed as well as oligomers that may potentially migrate from PET. Phthalates used as monomers in polyester such as terephthalic and isophthalic acid have been determined by high performance liquid chromatography (HPLC) coupled to different types of detector such as diode array detector (DAD) [15-18] and mass spectrometers (MS) [19]. Oligomers such as Bis-(2-hydroxyethyl) terephthalate (BHET) and PET cyclic trimer have been analyzed by using HPLC coupled to DAD [20, 21], MS [19, 22] and evaporative light scattering detector (ELSD) [5]. Additionally, techniques that do not require sample preparation such as direct analysis in real time (DART) were used to determine compounds in food contact materials [23, 24]. The aim of this work was to characterize and identify substances, mainly oligomers, which are present in polyester can coatings and may migrate into foods. Briefly, the procedures includes FTIR identification of the coating, extraction of coating with appropriate solvent and identification of volatiles and non-volatiles compounds by using GC and LC techniques coupled to DAD, charged aerosol detector (CAD) and MS. A small subset of the tentatively identified the oligomers were synthesized and their identification determined by NMR spectroscopy. Finally, to determine if

rapid, direct detection of the oligomers was practical, the coatings were analyzed by DART-HRMS. The identified compounds will be useful for migration studies from polyesters can coatings to food or food simulants.

3. Materials and methods

3.1. Chemicals and analytical standards for synthesis

Hexanes, dichloromethane (DCM) (HPLC grade), ethyl acetate (HPLC grade), acetonitrile (ACN) (Optima® for LC-MS) and water (Optima® for LC-MS) were obtained from Fisher Scientific (Fair Lawn, New Jersey). Ethyl alcohol absolute was purchased from Acros (New Jersey, USA). Ammonium Hydroxide (NH₄OH) 20% ultrapure reagent was purchased from J.T. Baker (Phillipsburg, New Jersey). Bicyclo [2.2.1] hept-5-ene-2,3-dicarboxylic anhydride (NAD) $\geq 95\%$, cis-5-norbornene-endo-2,3-dicarboxylic acid (NA) 98%, tert-butyldimethylsilyl chloride (TBCl) 97 %, 1,4-cyclohexanedimethanol (COH) mixture cis and tran 99%, isophthalic acid (IPA) 99%, terephthalic acid (TA) 98%, 4-tert-butylphenol (tBuPh) 99%, 2-methyl-1,3-propanediol (MBO) 99%, vanillin 99%, and sulphuric acid 95-98% were purchased from Aldrich (St. Louis, MO). Pyridine (PIR) 99+%, sodium chloride solution $\sim 26\%$ (saturated in water at 20 °C, AT), sodium sulphate $\geq 99\%$ anhydrous granular, trimethylamine (TMA) $\geq 99\%$, 4-(dimethylamino)pyridine (DMAP) $\geq 99\%$, N-N-dicyclohexylcarbodiimide (DDC) 99%, thionyl chloride $\geq 99\%$, and 2,2,5,7,8-pentamethyl-6-chromanol 97% (CRM) were obtained from Sigma-Aldrich (St. Louis, MO). Bis-(2-hydroxyethyl) terephthalate (BHET) was purchased from Polysciences Inc (Warrington, PA). PET cyclic trimer was purchased from Eastman Chemical Products Inc. (Kingsport, TN). Test mixture 2 for apolar capillary columns according to Grob, silica gel TLC plates, silica gel 70-230 mesh, ammonium chloride $\geq 99.9995\%$, and formic acid

for LC/MS ~ 98% were purchased from Fluka (St. Louis, MO). Chloroform-d (CDCl_3) 99.96%, was purchased from Cambridge Isotopes Laboratories, Inc. (Andover, MA).

3.2 Sample preparation

Empty cans with lids coated with polyester resins were supplied by an industrial partner. Cans were filled with ACN, sealed and placed into an oven at 40 °C for 24 hours. After extraction, one aliquot was reserved for analysis and a second was concentrated (25 to 1 ml) under nitrogen stream at 40 °C without going to dryness. Polyester cans were also extracted with ethyl acetate 24h at room temperature (20 °C). Concentrated and non-concentrated acetonitrile samples were analysed by gas chromatography-mass spectrometry (GC-MS), high performance liquid chromatography in series with a diode array detector and a mass spectrometer (HPLC-DAD/MS), high performance liquid chromatography coupled to a diode array detector and a charge aerosol detector (HPLC-DAD/CAD), ultra-high performance liquid chromatography tandem high resolution mass spectrometry (UHPLC-HRMS) and direct analysis in real time-mass spectrometry (DART-HRMS). All sample extracts were stored in glass vials with polypropylene lid and polytetrafluoroethylene septa.

3.3. Instrumentation

3.3.1. FTIR

Infrared spectra of the can coatings were collected using a Nicolet 6700 FTIR spectrometer (Madison, WI), equipped with an attenuated total reflectance (ATR) (Thermo Scientific, Madison, WI) attachment and a diamond ATR crystal. The diamond crystal was cleaned with ethanol after each can coating sample and a background was collected and subtracted from the sample spectra. Pieces of the can coatings including top, bottom and body were analyzed by IR

analysis. The FTIR spectrometer was operated in the range from 4000 to 650 cm^{-1} using OMNIC 8.3 software (Thermo Electron Corporation). For coating identification, obtained spectra were compared with several polymer IR libraries. These libraries indicated the coating was a terephthalate/ isophthalate based coating with multiple diol type linkages.

3.3.2. GC-MS analysis

GC-MS analysis was performed using an Agilent Technologies 5975C inert XL MSD with triple-axis detector equipped with Gerstel multipurpose sampler MPS 2. An HP-5MS 30 m x 0.32 mm ID, 0.25 μm column with a flow rate of 1 mL/min of helium was used for chromatographic separation. A single taper glass liner was used. Injection volume was 1 μL . The inlet temperature started at 70 $^{\circ}\text{C}$, increased at a rate of 12 $^{\circ}\text{C}/\text{sec}$ until a temperature of 350 $^{\circ}\text{C}$ was reached. The source temperature and, quad temperature were set at 230 and 150 $^{\circ}\text{C}$, respectively. Full scan spectra from m/z 50 – 500 were collected throughout the analysis. The initial oven temperature was 30 $^{\circ}\text{C}$, held for 1.5 min and then increased at a rate of 10 $^{\circ}\text{C}/\text{min}$ until a temperature of 300 $^{\circ}\text{C}$ was achieved, which was held for 5 min. Total run was 33.5 min. Data analysis was performed using Agilent MSD Chemstation and NIST database for detection and identification.

3.3.3. HPLC-DAD/MS

A HPLC Agilent 1290 Infinity system that consisted of a quaternary pump, degasser, autosampler, thermostatted column compartment, and DAD, was coupled to a triple quadrupole mass spectrometer (Agilent Technologies 6410). The DAD acquired spectra from 200 to 400 nm and single wavelength at 225, 254, 280, 288 and 330 nm. The MS was equipped with electrospray ionization source, was operated in the positive and negative mode collecting full

scan spectra from 100 to a 1000 m/z. The following conditions: Scan time 500, gas temperature 350 °C, gas flow 10, nebulizer 25 and capillary voltage of 4000 V. The chromatographic separation was performed on an Agilent Polaris 3 (C18) (150 x 2.0 mm) thermostatted at 25 °C. The injection volume was 10 µL. Flow rate was 0.3 mL/min. The mobile phases consisted of water 0.1% formic acid (A) and acetonitrile 0.1% formic acid (B). The gradient, starting at 80 % of A, was held for 2 min. then, was reduced to 50 % over 3 min. Afterwards, A was decreasingly reduce to a 0% in 25 min and held for 10 min. A post-time of 5 min was used after each analysis. The total run was 45 min. MassHunter workstation software was employed for data analysis.

3.3.4. HPLC-DAD/CAD

A HPLC Agilent 1100 system that consisted of a binary pump, a degasser, autosampler, thermostatted column compartment, and DAD was coupled to a Dionex Corona CAD (Thermo Scientific ultra RS charged aerosol detector). An Agilent Zorbax Extend C18, 80 Å (2.1 x 150 mm, 3 µm) was used as chromatographic column. Chromatographic conditions and DAD setting were the same as used for HPLC-DAD/MS with the exception that the mobile phase did not contain formic acid. The Corona CAD was run under normal conditions. Full scan range 100 pA, filter was set to 4, power function was 1.00. Nitrogen was used as nebulizer gas, gas pressure was 35 psi, ion trap volt was 20.5 V and temperature set point was 25 °C for the nebulizer thermal control. An Agilent Chem-Station software was employed for data analysis.

3.3.5. UHPLC-HRMS

An UHPLC-ESI-MS system comprised of Accela 1250 pump fitted with a degasser, and an autosampler coupled to a Q Exactive mass spectrometer controlled by Xcalibur (Thermo Fisher

Scientific, San José, CA, USA) was employed for the collection of high resolution mass spectra. An Acquity UPLC BEH C18 1.7 μ m 2.1 x 150 mm column with a flow rate of 0.2 mL/min and injection volume of 1 μ L was used for chromatographic separation. The mobile phases consisted of 5% acetonitrile in water 0.1% formic acid (A) and 5% water in acetonitrile 0.1% formic acid (B). The gradient started at 100 % of A and held for 1 min. then A was reduced to 0 % over 11 min and held for 11 min. Post time was 6 min. Detector conditions were as follows: For positive mode, detector was operated in the full scan from 100-800 m/z, sheath gas flow rate was 35, aux gas flow rate 10, sweep gas flow rate 8, spray voltage 3.50 KV, capillary temp 350 °C, s-lens RF level 50 and heater temp was 350 °C. For the negative mode detector was operated in the full scan from 100-1000 m/z, sheath gas flow rate was 35, aux gas flow rate 10, sweep gas flow rate 8, spray voltage 3.50 KV, capillary temp 350 °C, s-lens RF level 50 and heater temperature was 350 °C. For dd-MS² a similar chromatographic method was used and settings were as follows: Resolution was 17,500, AGC Target was $1e^5$, maximum IT 50 ms, isolation window 4.0 m/z, dynamic inclusion 4.0 s, loop count was 140, intensity threshold $2e^4$ and NCE % were from 20 to 50.

3.3.6. ¹³C and ¹H NMR spectroscopy

The synthesized compound was dissolved in CDCl₃ and analysed using a Varian MR600 with a ¹³C/¹H optimized Oneprobe. The ¹H NMR analysis was performed at 599.76 MHz at 25 °C. A 45 degree excitation pulse was used with a 4 sec acquisition time. The relaxation delay was 2 sec with 32 scans and a spectral width of 9615.4 Hz (16 ppm.) The ¹³C experiments were performed at 150.8 MHz at 25 °C. The relax delay was 1 sec and the excitation pulse was 45 degrees. Acquisition time was set to 0.865 sec with a spectral width of 37878.8 Hz (250 ppm.) All ¹³C experiments used 5000 scans.

3.3.7. DART-HRMS

DART-HRMS was operated in positive and negative ionization mode. The DART source from Ion Sense (Saugus, MA) was coupled to a Thermo Q exactive. The instrument conditions were as follows: Heater temperature of 450 °C. Spray voltage of 1.5KV, S-lens level 50, and capillary temperature of 250 °C. The mass spectra acquisition was from 100 to 1000 m/z. The source was set at 10.3 cm from the cone. Helium was used as the ionization gas. A solution of NH₄OH 20% was placed in two HPLC vials on each side of the cone detector. For sample preparation, the coating was swabbed with a glass tip and the tip was manually placed between the source and the MS inlet and maintained for approximately three seconds. Acetonitrile can extracts and concentrated acetonitrile can extracts were analysed by placing a drop onto a glass tip and holding the tip for 3 sec in the ionization region. A blank of acetonitrile was analyzed before and after each analysis. A new glass capillary was used for every sample. DART data was processed by using TOX IDTM (Thermo Scientific) with an internal polyester oligomers data base using a mass tolerance of 5 ppm.

3.4. Synthesis of compounds

3.4.1. Synthesis of (3-(((tert-butyldimethylsilyl)oxy)methyl)cyclohexyl)methanol (monoCOH)

A total of 10 grams (5 eq) of COH were added to a 100 mL round bottom flask with 0.2 eq of DMAP, 1 eq of TMA and 35 mL of dry DCM. The solution was stirred and a solution with 1eq of TBCL in 10 mL of DCM was added drop wise. The reaction was stirred until completion. The DCM solution was washed twice with water and with saturated ammonium chloride solution respectively. The organic phase was separated from the aqueous solution. Remaining water was removed from DCM by the addition of sodium sulphate. To achieve separation of the compound,

a silica column was used. The solution was evaporated to dryness and re-dissolve in mobile phase (30% ethyl acetate and 70 % hexanes), then, poured into a silica column. Mobile phase was added and aliquots were collected in glass tubes and spotted on TLC silica plates. Separation was performed in the TLC using the same mobile phase as in silica column. To determine in which glass tubes the target compound was present, TLC silica plate was immersed in a solution containing 1.0% vanillin and 1.5% sulphuric acid in ethanol followed by heating the plate. Tubes with target analyte were collected and concentrated to dryness under nitrogen until obtaining transparent syrup which would correspond with the monoCOH.

3.4.2. Synthesis of 3-(((4-(hydroxymethyl)cyclohexyl)methoxy)carbonyl)bicyclo[2.2.1]hept-5-ene-2-carboxylic acid (NA+COH)

Twenty mL of thionyl chloride was added to 1 gram of cis-5-norbornene-endo-2,3-dicarboxylic acid or nadic acid (NA) and heated to 55 °C for 45 minutes with stirring and then evaporated to dryness and dissolved in DCM. One eq of monoCOH, 1.5 eq of PIR, 3 eq of DCC and 1 eq of DMAP dissolved in DCM were added to solution and stirred overnight. Target compound was separated on a silica column with similar condition as used for monoCOH. Deprotection of the compound was achieved by using a 5:1 solution of DCM/Ethanol 0.05 N HCl with stirring overnight. Afterwards, the target compound was purified in silica gel column with mobile phase (75% ethylacetate and 25 % hexanes). The fractions containing the product were evaporated to dryness under inert conditions with nitrogen until a white solid powder was obtained.

4. Results and discussion

4.1. Can coating extraction

After polymer identification by FTIR and based on previous experience and cited literature [13], acetonitrile at 40 °C for 24 h was selected to extract migrants from the polyester can coatings.

Moreover, another appropriate solvent (ethyl acetate) at 20 °C for one day was used for extraction. Samples were analysed by GC and LC, in triplicate, and compared with a blank solvent used as a control. Based on the number of compounds and the intensity of responses, acetonitrile was a more efficient extraction solvent.

4.2 Identification of unknowns compounds

4.2.1. Analysis of polyester can coatings extract by GC-MS

GC-MS was used to tentatively identify volatile and semi-volatile compounds that could potentially migrate from polyester coatings. A GC-MS method that covered a wide mass range (from 50 to 500 Da) with a suitable gradient of temperatures for inlet and oven was used. Samples were injected in split 1:10 and splitless modes. To verify that the proposed method would detect compounds with different polarities and different mass range, test mixture 2 for apolar capillary columns according to Grob was used as a positive control. All of the compounds present in the mixture were successfully detected. Additionally, several standard solutions, corresponding to monomers that may be used in manufacture of polyester polymers, including carboxylic acids (IPA, TA and NA), alcohols (tBuPh, MBO, COH) and the PET cyclic trimer, were analysed by the method. Only the alcohols were detected as it was expected, since the selected carboxylic acids and the PET cyclic trimer would be consider non-volatiles substances and the suitable technique to determine these compounds would be LC. These pre-test analyses confirmed that the GC-MS method was appropriate to detect potential volatiles and semi-volatiles compounds from polyesters coatings.

Acetonitrile and ethyl acetate can extracts, concentrated and non-concentrated, were analysed and several low intensity signals were detected in the concentrated samples, some corresponding to polyesters derivatives. However, poor matches for the proposed substances were achieved.

Just one substance had a fragmentation pattern that matched a potential starting material (NAD) was detected. A standard of the compound was purchased, prepared in acetonitrile and injected. The retention time (t_R) did not match the t_R of the unknown, therefore none of the signals were selected as potential migrants.

4.2.2. Analysis of polyester coating extract by HPLC-DAD/MS, HPLC-DAD/CAD and UHPLC-HRMS

The first step of the characterization and identification process of non-volatiles was the analysis of the acetonitrile can extracts by HPLC-DAD/MS. Only the concentrated extracts had significant responses in these detectors. To enhance the range of the compounds detected, a long gradient of 40 minutes starting from 20% to a 100% of organic solvent was used. The DAD was employed to obtain the spectra and the chromatographic profile of the polyester extracts. To avoid interferences because of formic acid at lower wavelengths, the same chromatographic conditions but without the acid were also used. Additionally, to enhance chromatographic separation of highly polar compounds that would elute at the beginning of the chromatogram, a set of ACN extracts were diluted with water to obtain a solution of 20% ACN. UV maximal absorption for most of peaks was at 214, 236 and 288 nm (Figure 1A). The spectra are similar to those obtained for the BHEI by Schaefer [5] which suggests that the oligomers are primarily based on IPA. A few peaks have maximal absorption at 242 and 288nm (Figure 1B). This spectrum is similar to BHET and PET cyclic trimer, agreement with these two spectra is most likely due to TA being used in the manufacture of the coating. Appropriate chromatograms, showing significant responses and good separation between peaks were achieved for the sample extracts (Figure 2).

Samples were also analysed by HPLC-DAD/CAD. Corona CAD is a universal detector that can be useful for the detection non-volatile substances that do not have strong UV-Vis absorption [25-27]. No significant differences were observed by using the CAD. This is consistent with previous reports that UV-Vis and MS analysis are appropriate for the detection of the starting materials used for polyester coatings and the unreacted oligomers.

Mass spectra were processed by extracting ions from the TIC chromatogram in mass-to-charge intervals of 100 (ie: from 100 to 200 m/z, from 200 to 300 m/z, etc.) By using this mass selection, several peaks were obtained, and each peak supplied nominal masses. The nominal masses obtained were introduced into an in-laboratory database containing most probable monomers employed in the coating. All of the possible combinations of the potential monomers (≤ 1000 Da), including linear and cyclic structures, were included in the database as were adducts commonly formed during electrospray ionization (e.g H^+ , NH_4^+ , Na^+ , K^+ and $H_4C_2N^+H^-$). The mass range was set up to 1000 Da since it is generally assumed that hydrocarbons above this molecular weight are not absorbed in the gastrointestinal track and they do not represent a risk from the food safety perspective provided they do not hydrolyse to smaller molecules and become absorbed. If the nominal mass matched compounds in the database, the exact mass of the proposed structure was calculated using Chem Bio office software. The exact mass was then used to search the mass chromatogram obtained using HRMS. If selected compound has an exact mass with good ppm agreement (< 5 ppm), good peak shape and appropriate t_R , the compound was consider as tentatively identified. In addition to using the low resolution mass chromatograms as a starting point, any high intensity peaks in the high resolution mass chromatograms were compared to the database. A total of 29 oligomers were identified (table 1) within a 5 ppm match to an exact mass. Structures are displayed in table S1. All correspond to

the reaction of carboxylic acids and diols (except for tBuPh which is a mono-alcohol). Of the 29 identified compounds, 28 were detected in the positive mode. Most of them have high intensity with adducts Na^+ , H^+ and NH_4^+ . Adducts with K^+ were detected but with low intensity. Acetonitrile adducts were not found. Twelve compounds were detected in the negative mode, of which 6 have higher intensity than positive mode. PA+ tBuPh+ COH has a ppm agreement of - 4.9, is the only compound that has tBuPh in the structure, a substance that may be used as chain stopper in resins [9]. The fact that the ppm agreement is close to the limit of the ppm acceptable range in addition to is the only oligomers that contain tBuPh in the structure, makes questionable the identification. However, it is one of the compounds that have a higher response in the MS detectors. Isobaric compounds with several peaks were detected. This is attributed to the presence of isomers or substances with the same composition but different structure or oligomers with different identities [13] as well as oligomers high MW fragmenting in the source to give same fragment compound but at different retention time. Most of the oligomers correspond to linear structures, but cyclic structures were also identified, which is consistent with previous literature in can coatings [5, 13] and also in certain adhesives employed for packaging [28, 29]. The cyclic compounds elute later than the linear compounds, although some have the same monomer composition, such as 3PA+2MBO+COH and 3PA+2MBO+COH cyclic, where the t_R is 14.8 and 16.4 respectively. This is due to cyclic oligomer react more with stationary phase because they most likely are structures with lower polarity (for example, estimated log P for one of the possible isomers of 3PA+2MBO+COH is 6.63, whilst is 7.17 for the cyclic structure). In other studies for characterization of oligomers from polyester coatings, most of proposed structures are cyclic oligomers [5, 13], while in this study are linear compounds, this might be because of variability from one producer to another in the manufacture process of the coating.

316 The retention times of identified compounds are consistent with the molecular weight as well as
317 the size and structure of the proposed molecule.

318 Five peaks detected in the negative mode were not consider as identified since no overwhelming
319 evidence of their structure was obtained nor with the reacting compounds, but because of their
320 high response in the MS detector it was necessary to mention their detection (Table 2). The
321 proposed molecular formula for these compounds suggests that they are formed from the
322 reaction of a carboxylic acid and an aliphatic chain end-capping reagent. For the two compounds
323 that have the lower molecular weight, it might be possible that they are fragments from other
324 oligomers or that they reacted with ethanol or butanol during the manufacturing process.

325 However, taking into account the proposed molecular formula, the other three compounds with a
326 higher molecular weight could correspond with the reaction of a carboxylic acid and a large
327 unsaturated aliphatic chain; it might be possible that they are products of decomposition or
328 products formed in the end-capping curing process. This is a good example of the complexity of
329 the characterization and identification process.

330 A dd-MS² analysis of the tentatively identified peaks was also performed. Main detected
331 fragments are presented in the Table 1. Majority of fragments were obtained by using a NCE %
332 at 40. Compounds 1, 6 and 13 did not generate fragments probably because of the low detector
333 response. The most repeated fragments were 121.0296, 109.1015 and 149.0.235 which were
334 expected because oligomers have similar molecular structure and consequently, may have similar
335 fragments, which gives confidence in the oligomers identification.

336 Once the oligomers were tentatively identified in the coating, identities of the peaks obtained by
337 DAD were assigned (Figure 2). Given this, it was observed that to retain oligomers such as
338 PA+COH and PA+MBO, the use of formic acid 0.1% in mobile phase is required to avoid

elution of these compounds in the dead volume, but under these conditions a more selective wavelength such as 288 nm had to be used to obtain an appropriate profile of the extract. Additionally, measured peaks were quite consistent with the order of t_R obtained in the UHPLC-HRMS. Derivatives that only have NA as the di-carboxylic acid in their structure will not have a significant UV absorption because of the lack of aromatic ring, so the NA derivatives marked in Figure 2 are probably co-eluting with other derivatives with phthalates in their structure. HRMS data was also used to tentatively identify unreacted monomers and additives employed in the manufacture of the coating. For this purpose two internal data bases were used. One contained all substances approved through the food contact notification system through 2013. The other list, were prepared with all monomers and additives listed in the positive list of the European Union. To process the HRMS data with the identified databases, TOX IDTM software was used. This software was set to identify the exact mass of the compound listed, including adducts, in database within 5 ppm. Several monomers and additives were detected including the expected carboxylic (IPA, TA and NA) as well as the COH. For the rest of the proposed compounds, only one, the CRM was selected as potential migrant because it was the only compound that had an appropriate retention time, an acceptable peak shape and a significant response in the detector. A standard solution (10 µg/ml) of CRM was injected using the same conditions as the acetonitrile polyester extraction, but a different retention time was obtained, thus it was not considered identified.

4.2.3. Synthesis of NA+COH and NMR analysis

To give confidence in the identification of some polyester derivatives in food can coatings, one of identified polyester oligomer was selected for synthesis, the NA+COH (Figure 3). The organic synthesis of NA+COH was performed in three steps: Syntheses of monoCOH, syntheses of the

dichloride of the NA and then reaction between both. After synthesis of the compound very little sample was obtained and corresponds to a solid white powder. This compound was analyzed by ^{13}C NMR and ^1H NMR. The ^{13}C NMR analysis exhibited signals at 37.08, 37.76, 39.67, 40.33, 46.22 to 48.74, 68.52, 70.10, 134.45, 135.42, 172.37 and 176.17 ppm correspond with C^{15} and C^{19} , C^1 to C^3 and C^7 , C^{20} , C^{22} , C^4/C^5 , C^3 and C^6 carbons respectively corresponding with NA+COH structure. Signals for ^1H NMR were as follows: Signals shifts at 1.2, 1.4, 1.5, 3.2, 3.3, 3.5, 4.1, 6.2 and 6.3 ppm correspond to protons 14 to 19, 7, 17, 1/2, 20/3, 6, 22, 4/13 and 21 respectively. NMR analyses suggested that the synthesized compound was not pure because other signal interferences were obtained, and signal corresponding to atom 22 was weak. Even though all the expected signals were obtained by NMR analysis, more data was required to verify the presence of the oligomers detected. Consequently, the obtained product from the synthesis, was dissolved in acetonitrile and injected through HRMS, the exact mass corresponding to the NA+COH was obtained as well as same retention time as in polyester extract (Figure 4). These results from HRMS were compared with a blank as well as the concentrated polyester acetonitrile extracts.

Additionally, we attempted to synthesize other of the identified oligomers (PA+COH) by using isophthalic acid as the main monomer. Synthesis of this compound was similar to NA+COH. However, NMR signals were weaker and contained interferences; thus, the synthesis was not included in this study. Analysis of IPA+COH by HRMS showed the exact mass and same t_R as the polyester extract for this compound. HRMS data along with data obtained from NMR, gives confidence in the identification of the polyester oligomers. These results prove that identification of polyester oligomers was appropriate. Because the synthesized compounds were not pure, they will not be used as standards in future migration studies. The impurity of the compounds is most

likely due to the nature of the monomers that are used to manufacture polymers. Because of the monomer high reactivity, they probably react in a high extend to form polymeric chains with higher molecular weight than NA+COH and IPA+COH, making very complicated the synthesis of these particular low molecular weight polyester derivatives by the chosen synthetic pathway.

4.2.4. DART-HRMS

DART-MS is a powerful technique for qualitative analysis of target compounds, and it was used in this study for direct identification of the previous identified potential migrants. DART settings were optimized regarding to the source temperature, distance of the cone to the source and sample preparation. Improvement of sample preparation was achieved by scratching the can coatings with previously cleaned scissors and then swabbing with a glass capillary. The glass tip with swabbed sample was introduced between the DART source and the detector. The employed sample preparation was compared to the used by Paseiro-Cerrato [24] and it was observed that swabbing the material showed a considerable increase in sensitivity. Moreover, a significant increase in sensitivity in positive and negative modes was observed by using NH₄OH 20% as a dopant. The can top, body and adhesive were analyzed separately in triplicate. In addition, concentrated and not concentrated acetonitrile extract were also analyzed. The total time of the analysis was less than 30 min. Data was processed with an internal laboratory database which included the identified polyesters oligomers. Only adducts with NH₄⁺, H⁺ and H⁻ were searched in the list.

From the 29 identified compounds by UHPLC-HRMS, 23 (79%) were detected by DART directly in the can coating, 24 (83%) in the concentrated acetonitrile extract and 17 (59%) in acetonitrile. Most of compounds were detected in the positive ionization mode, 22 were detected in the coating and in the concentrated acetonitrile extract, while only 13 were detected in the

non-concentrated acetonitrile in the negative ionization mode, 17, 16 and 15 compounds were detected in the coating, concentrated acetonitrile and acetonitrile respectively. These results are also consistent with those results obtained by UHPLC-HRMS, where most of the potential migrants were detected in the positive ionization mode. Only 3 false positives were obtained in the worst case scenario, which correspond to the analyzed coating sample using the detector in the negative mode. Three oligomers, compounds number 20, 21 and 25 (Table 1) were not detected in any test sample or extract using DART. The non-detection of these compounds is most likely because these substances with high molecular weight would need a higher temperature to be thermo-desorbed from the material and subsequently evaporated as was suggested by other authors [30, 31]. The fact that identification in the coating and in the concentrated extract is very similar, shows that DART is a powerful tool to determine in few minutes polyester oligomers directly in the coating, avoiding sample preparation and the development of long and expensive analytical methods.

5. Conclusions

During last few years, new coating formulations have been introduced into the market place with the aim of replacing existing coatings that contain BPA. One disadvantage is that composition of new coatings is not well known and identification of the potential migrants is necessary to assess migration into food. A protocol that employs several analytical techniques (GC-MS, HPLC-DAD/MS, HPLC-DAD/CAD and UHPLC-HRMS) was successfully applied for identification of potential migrants from polyester coatings. Twenty nine oligomers were tentatively characterized and identified. The synthesis of one of the oligomers (NA+COH) proved that the proposed protocol to identified unknowns in polyester can coatings is robust. The synthesis of polyester low molecular weight oligomers based on multiple monomers is not a simple task, one

431 compound was synthesized and was not pure and consequently, other synthetic pathways should
432 be used to obtain pure analytical standards that may be used for tracking migration test. Besides,
433 DART-HRMS gives very similar results to those obtained by conventional techniques for
434 detection of unknowns in minutes, which involves a considerable progress in development of
435 potential migrant identification strategies. Identified substances in this work are currently being
436 used to monitor the migration process from polyester can coatings into food simulants [32] and
437 foodstuff.

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Figure captions

Figure 1A: UV Spectra of and isophthalic acid derivative.

Figure 1B: UV Spectra of a terephthalic acid derivative.

Figure 2: HPLC-DAD chromatogram of acetonitrile polyester extraction concentrated 25 times detected at 288 nm. See table 1 for the numbers of the proposed compounds.

Figure 3. Numbered structure of NA+COH.

Figure 4. A) NA+COH in polyester can coating. B) Synthesized NA+COH.

Figure 1A

Figure 1B

Figure 4.

Figure 2

Figure 3.

Tables

Table 1. Tentatively identified oligomers in the UHPLC-HRMS. PA are phthalic acids (IPA and/or TA) reacted with alcohols. The obtained exact masses correspond with adducts of highest intensity in the detector (Bold letters). ^aNumber. ^bNot detected. ^cRetention times corresponding with the peak with highest response. ^dNumber of peaks detected per proposed oligomer.

Table 2. Unknowns with high response in MS detectors in the negative mode. Phthalic acids (PA) are either IPA and/or TA most likely reacted with carbons (C) and alcoholic groups (OH).^a Retention times obtained using the UHPLC-HRMS.

Supplementary Material.

Table S1. Structures of the tentatively identified oligomers. Note that in most of the structures isophthalic acid was included as main monomers because most of the peaks obtained in the HPLC-DAD had a UV spectra consistent with isophthalic acid derivatives. However, oligomers with terephthalic acid could also be present in the structure. In addition, isomers or oligomers with the same composition but different structure could also occur.

No ^a	Proposed identified oligomers	Exact mass	ppm	Pos. mode(Adducts)	Neg. mode(H ⁻)	Predicted formula	t _R ^c	Peaks ^d	Fragments	Structure
1	NA+2EG	293.0999	1.1	H ⁺ , NH ₄ ⁺ , Na ⁺ , K ⁺	x ^b	C ₁₃ H ₁₈ O ₆	6.9	1	x	Linear
2	PA+MBO	237.0771	1	H ⁺ , Na ⁺	H ⁻	C ₁₂ H ₁₄ O ₅	8.7	2	121.0296/165.0192	Linear
3	NA+COH	331.1519	0.9	H ⁺ , NH ₄ ⁺ , Na ⁺ , K ⁺	x	C ₁₇ H ₂₄ O ₅	9.4	1	205.0473/299.1263	Linear
4	PA+2MBO	333.1212	1	H ⁺ , NH ₄ ⁺ , Na ⁺ , K ⁺	x	C ₁₆ H ₂₂ O ₆	9.7	1	149.0229/210.1239	Linear
5	PA+COH	291.1242	1.2	x	H ⁻	C ₁₆ H ₂₀ O ₅	10.1	1	121.0292/223.0897	Linear
6	2PA+MBO	385.0935	1.6	H ⁺ , NH ₄ ⁺ , Na ⁺	H ⁻	C ₂₀ H ₁₈ O ₈	10.8	1	x	Linear
7	NA+COH+MBO	403.2095	0.9	H ⁺ , Na ⁺ , K ⁺	x	C ₂₁ H ₃₂ O ₆	11	2	337.1622/211.0578	Linear
8	PA+MBO+COH	387.1781	0.8	H ⁺ , NH ₄ ⁺ , Na ⁺ , K ⁺	x	C ₂₀ H ₂₈ O ₆	11	2	204.9858/191.1068	Linear
9	NA+PA+2MBO	497.1790	1.6	H ⁺ , NH ₄ ⁺ , Na ⁺ , K ⁺	H ⁻	C ₂₅ H ₃₀ O ₉	11.5	2	293.1383/221.0809	Linear
10	2PA+2MBO	457.1511	1.5	H ⁺ , NH ₄ ⁺ , Na ⁺ , K ⁺	H ⁻	C ₂₄ H ₂₆ O ₉	11.6	1	121.0296/225.0556	Linear
11	NA+PA+COH	474.2126	0.9	H ⁺ , NH ₄ ⁺ , Na ⁺ , K ⁺	H ⁻	C ₂₅ H ₂₈ O ₈	12	5	183.0636/109.1015	Linear
12	NA+2COH	457.2566	1.1	H ⁺ , NH ₄ ⁺ , Na ⁺ , K ⁺	x	C ₂₅ H ₃₈ O ₆	12.2	2	391.2092/239.0916	Linear
13	2PA+COH	463.1363	1.3	H ⁺ , NH ₄ ⁺ , Na ⁺ , K ⁺	H ⁻	C ₂₄ H ₂₄ O ₈	12.3	1	x	Linear
14	NA+PA+3MBO	569.2364	1.3	H ⁺ , NH ₄ ⁺ , Na ⁺ , K ⁺	x	C ₂₉ H ₃₈ O ₁₀	12.5	2	503.1891/293.1386	Linear
15	PA+NA+COH+MBO	551.2259	1.3	H ⁺ , NH ₄ ⁺ , Na ⁺ , K ⁺	H ⁻	C ₂₉ H ₃₆ O ₉	12.8	3	205.0473/313.1411	Linear
16	PA+tBuPh+COH	425.2302	-4.9	H ⁺	x	C ₂₆ H ₃₂ O ₅	12.9	1	99.0078/179.9489	Linear
17	2PA+MBO+COH	511.1979	1.1	H ⁺ , NH ₄ ⁺ , Na ⁺ , K ⁺	H ⁻	C ₂₈ H ₃₂ O ₉	13.2	2	121.0296/225.0556	Linear
18	2PA+2MBO+COH	607.2521	1.2	H ⁺ , NH ₄ ⁺ , Na ⁺ , K ⁺	x	C ₃₂ H ₄₀ O ₁₀	13.9	2	109.1014/205.0477	Linear
19	2PA+2COH	565.2450	1.2	H ⁺ , NH ₄ ⁺ , Na ⁺ , K ⁺	H ⁻	C ₃₂ H ₃₈ O ₉	14.2	1	121.0296/395.1503	Linear
20	3PA+2COH+MBO	787.3306	-2.4	H ⁺	H ⁻	C ₄₄ H ₅₀ O ₁₃	14.8	3	721.2827/375.1049	Linear
21	3PA+2MBO+COH	750.3124	0.6	H ⁺ , NH ₄ ⁺ , Na ⁺	H ⁻	C ₄₀ H ₄₄ O ₁₃	14.8	2	149.0235/221.0809	Linear
22	2PA+2COH+MBO	639.3170	1	H ⁺ , NH ₄ ⁺ , Na ⁺ , K ⁺	x	C ₃₆ H ₄₆ O ₁₀	14.9	2	149.0234/109.1012	Linear
23	3PA+3MBOc	678.2500	0.7	H ⁺ , NH ₄ ⁺ , Na ⁺ , K ⁺	x	C ₃₆ H ₃₆ O ₁₂	15.8	2	149.0235/221.0809	cyclic
24	2NA+MBO+COHc	544.2913	1.4	H ⁺ , NH ₄ ⁺ , Na ⁺ , K ⁺	x	C ₃₀ H ₃₈ O ₈	16.2	5	109.1013/171.0652	cyclic
25	3PA+2MBO+COHc	715.2726	-3.2	H ⁺ , NH ₄ ⁺ , Na ⁺ , K ⁺	x	C ₄₀ H ₄₂ O ₁₂	16.4	3	583.1782/109.1013	cyclic
26	2PA+COH+MBOc	517.1840	1.4	H ⁺ , NH ₄ ⁺ , Na ⁺ , K ⁺	x	C ₂₈ H ₃₀ O ₈	16.4	3	109.1013/67.0544	cyclic
27	2NA+PA+3MBOc	710.3172	0.1	H ⁺ , NH ₄ ⁺ , Na ⁺ , K ⁺	x	C ₃₈ H ₄₄ O ₁₂	16.6	3	293.1383/221.0809	cyclic
28	PA+NA+COH+MBOc	511.2331	0.9	H ⁺ , NH ₄ ⁺ , Na ⁺ , K ⁺	x	C ₂₉ H ₃₄ O ₈	16.6	1	109.1013/149.0235	cyclic
29	NA+PA+2COHc	565.2804	1.4	H ⁺ , NH ₄ ⁺ , Na ⁺ , K ⁺	x	C ₃₃ H ₄₀ O ₈	18.6	4	109.1015/67.0544	cyclic

Table 1.

Proposed structure	Exact mass	Predicted formula	t _R ^a
NA+2C	209.0822	C ₁₁ H ₁₄ O ₄	10.4
PA+4C	221.0819	C ₁₂ H ₁₄ O ₄	11.3
PA+25C	501.3010	C ₃₃ H ₄₂ O ₄	18.8
PA+26C	515.3167	C ₃₄ H ₄₄ O ₄	19.9
PA+26C+OH	529.2959	C ₃₄ H ₄₂ O ₅	20.5

Table 2.