Ion mobility quadrupole time-of-flight mass spectrometry for the identification of non-intentionally added substances in UV varnishes applied on food contact materials. A safety by design study.

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ABSTRACT:

Varnishes are normally applied on printed food packaging to protect it from smearing and scratching. Moreover, they may be applied on the food contact surface in order to improve resistance towards moisture and fat. Some of the compounds that make up the varnish formulation could migrate to the food. In this work, the ion mobility quadrupole time-of-flight mass spectrometry has been used to obtain drift time-aligned mass spectra in which accurate the mass of precursor ions and their fragments are used to identify both intentionally and non-intentionally added substances (NIAS). The compound 2-propenoic acid,1,1'-[2-[[3-[2,2-bis[[(1-oxo-2-propen-1-yl)oxy]methyl]butoxy]-1-oxopropoxy]methyl]-2-ethyl-1,3-propanediyl] ester was identified as a NIAS formed from the varnish monomer 2-propenoic acid, 1,1'-[2-ethyl-2-[[(1-oxo-2-propen-1-yl)oxy]methyl]-1,3-propanediyl] ester. The compound 5, 11-diethyl-7-oxo-4,6,10,12-tetraoxopentadecane-3,13-diyl diacrylate is a NIAS derived from the varnish monomer 2-propenoic acid, 1,1'-[oxybis(methyl-2,1-ethanediyl)] ester, and was found to migrate into the food simulant tested at a level of 0.03 mg kg−1. Finally, the NIAS, 2-{2-[2-(acryloyloxy)-1-methylethoxy]-1-methylethoxy}-1-methylethyl acrylate, an impurity of a photoinitiator used for UV curing of the varnish, was identified, and its migration of 0.14 mg kg−1 exceeded the threshold established as safe for human consumption.

*Keywords:*IM-HRMSE, NIAS, Food contact, UV varnish

1. Introduction

Varnishing is a finishing process used in food packaging where a thin transparent layer of varnish is applied on the printed material. In this way, the print is protected from smearing and scratching, and the surface is glossier and even. Varnishes are a kind of ink without colorant [1] and may be applied to the food contact surface in order to improve resistance towards moisture and fat [2]. UV varnishes contain reactive resins that polymerize and form a hard film under UV radiation. The main components are oligomers, monomers, prepolymers and photoinitiators [3]. Therefore, all these components are susceptible of migration from the packaging to the food in contact with it. When the varnish is applied over the printed surface, several migration mechanisms can occur, such as a) the direct migration, that is the transfer of matter from the printed side through the substrate onto the unprinted internal side, and b) the set off, where the compounds can be transferred to the internal food contact non printed layer due to its contact with the external printed surface, when the printed material is stored as reels. In the case of photoinitiators, there are many works that studied these migration phenomena [4–6]. Nevertheless, a lot of work has still to be done in the area of non-intentionally added substances (NIAS). NIAS are originated from impurities, oligomers reaction by-

products or degradation processes [7]. The phenomenon of NIAS were specifically mentioned in Article 19 of Regulation EU 10/2011 [8], then it has been studied in several areas of packaging such as paper and board [7], plastic materials [9], coatings [10,11], adhesives used for multilayer [12–15] and labels [16,17]. As it can be seen in these works, the study of NIAS is an analytical challenging work, where a wide series of unknowns can appear. Thus, high resolution mass spectrometry coupled to efficient separation techniques are required. Ion mobility mass spectrometry (IMS) is a gas-phase technique whose mechanism relies on the separation of ions with different mobility that pass through a drift cell in which a constant electric field is applied [18]. The ions are characterized by their collision cross-section value (CCS), a parameter related to the ion's rotationally averaged size, shape, total charge and charge distribution. Depending on the gas used, changes [19]. The ion mobility separations take only a few milliseconds to occur. This enables the ion mobility cell to be employed in line with a ultrahigh performance liquid chromatographer (UHPLC), where peaks typically present a few seconds width, and a Time of Flight (ToF) mass spectrometer, which operates on a microsecond time scale. As a result, a UHPLC-IM-QToF-MS system brings about an extra dimension of sample separation and definition, and three-dimensional spectra are obtained (mass-to-charge ratio, ion intensity, and drift time) [20]. This technique is particularly useful when dealing with complex matrices, since alignment of precursors and fragment ions on the basis on both retention time and drift time can be achieved. Ultimately, ion mobility filtered extracted ion chromatograms (XIC) are cleaner and present less interferences compared to those obtained from a non-ion mobility configuration [21]. This appears to be of great value for the identification of unknowns.
In this work the ion mobility technology has been used in order to obtain cleaner spectra which contribute to identify non-intentionally added substances that could migrate from UV varnishes used in food packaging materials to food simulants.

2. Materials and methods

2.1. Reagents

The standards benzophenone, 2-hydroxy-2-methylpropiophenone, 2-Hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone, ethanol, 2,2′,2″-nitrilotris- were purchased from Sigma-Aldrich Quimica S.A (Madrid, Spain). The compounds 1,1'-[2-ethyl-2-[[(1-oxo-2-propen-1- yl)oxy]methyl]-1,3-propanediyl] ester, 2-propenoic acid, 1,1'-[oxybis (methyl-2,1-ethanediyl)] ester, bis (2-methylbutan-2-yl) dicarbonate, [2-[2-(2-hydroxy-1-methyl-ethoxy)-1-methyl-ethoxy]-1-methyl-ethyl] 3-(diethylamino)propanoate, [1-methyl-2-[1-methyl-2-(1-methyl-2- prop-2-enoyloxy-ethoxy)ethoxy]ethyl]3-(diethylamino)propanoate, [(methylethylene)bis(oxy)]dipropanol, phenol, 4,4'-(1-methylethylidene) bis-, reaction products with 2-(chloromethyl)oxirane, 2-propenoate, reaction product of (4,4′-Isopropylidenediphenol, oligomeric reaction products with 1-chloro-2,3-epoxypropane) and 2-propenoic acid (molar ratio 1:3), reaction product of (4,4′-Isopropylidenediphenol, oligomeric reaction products with 1-chloro-2,3-epoxypropane) and 2-propenoic acid (molar ratio 1:1), reaction product of 2,2'-[propane-2,2-diylbis(4,1-phenyleneoxymethylene)] dioxirane and 2-propenoic acid (molar ratio 1:2), reaction product of 2,2'-[propane-2,2-diylbis(4,1-phenyleneoxymethylene)] dioxirane and 2-propenoic acid (molar ratio 1:3), reaction product of 3-(4-{1-methyl-1-[4-(oxiran-2-ylmethoxy) phenyl]ethyl} phenoxy) propane-1,2-diol and 2-propenoic acid (molar ratio 1:1), 2-methyl-2-propanyl 1-hydroxy-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-oate, poly[oxy(methyl-1,2-ethanediyl)], α,α′,α"-1,2,3-propanetriyltris[ω-[(1-oxo-2-propen-1-yl)oxy]- and oligo[2-hydroxy-2-methyl-1-[4-(1-

methylvynyl)phenyl] propanone were industrial standards with a purity up to 85%. Ethanol, water and methanol of HPLC grade were supplied by Scharlau Chemie S.A (Sentmenat, Spain).

2.2. Samples

Three different UV-curable varnishes were studied (named varnish 1, 2 and 3). All of them are intended for being used in food contact materials to cover the ink applied over Polypropylene (PP). The varnish is not in direct contact with food, as it is applied on the external side of the packaging. 5 g/m2□}0.1 of each varnish were applied over PP with 80 μm thickness using an atomatic coater K control Coater model 202 (RK PrintCoat Instruments Ltd, Litlington, Royston, Herts SG8 0QZ). An UV curing equiment HOK-2/1 ASP from BCB (Cerdanyola, Spain) was used for the curing. 100 mJ/cm2 were applied over the varnish applied in PP (varnished PP), a radiometer was used to control the UV exposure. Afterwards, several laminates of varnished PP were rolled over a glass rod simulating an industrial varnished polypropylene reel in order to study the set-off phenomenum. These rolled laminates were kept one month at room temperature, and after this period their migration was studied. Films of PP 80 μm were used as blanks.

2.3. Extraction of the cured varnish

A piece of 5 cm2 of cured varnish was extracted with 5 g of ethanol 95% (v/v) in an ultrasonic bath for 1 h at room temperature. PP was used as a blank since varnish was applied over this substrate.

2.4. Migration assays

Migration assays were done according the "EuPIA Guidance on Migration Test Methods for the evaluation of substances in printing inks and varnishes for food contact materials" [22]. Three replicates of PP blank, three replicates of the varnished PP side and three replicates of the unprinted PP side of the varnished PP rolled (set-off) were placed in contact with ethanol 95% (v/v). Migracell® migration cells were used to do the migration assays, 0.78 dm2 were placed in contact with 0.13 Kg of food simulant considering the ratio 6 dm2 to 1 Kg of food simulant established by the Regulation. Migration cells were kept at 60 °C for 10 days.

2.5. Gas chromatography-mass spectrometry single quadrupole (GC-MS/Q)

The equipment used was a CTC Analytics CombiPal autosampler coupled to an Agilent 6890 N gas chromatograph with a mass spectrometer MS 5975B detector. All of them from Agilent Technologies (Palo Alto, CA, USA). The capillary column used was a HP-5MS (30m×0.25 μm x250 μm) from Agilent Technologies (Madrid, Spain). The oven program was as follows: 40 °C for 2 min, with rate of 10 °C/min up to 300 °C, maintained for 2 min. The injection type was splitless, the injection volume was 1 μL and the helium flow was 1 mL/min. The acquisition was done in electron impact ionization (EI). The mass detector was set at SCAN mode (in the range *m/z* 45–350). NIST 08 mass spectral search program (v. 2.0) was used for library search and comparison of the acquired spectra with the reference ones.

2.6. Ultra-high-pressure-liquid chromatography coupled with ion mobilityquadrupole-time of flight analyzer (UHPLC- IMS/QTOF)

The analyses were carried out in a chromatograph Acquity UPLC™ system coupled to an electrospray interface (ESI) and VION® IMS/QTOF detector, supplied by Waters (Manchester, UK). An UPLC™ BEH C18 column of 1.7 μm particle size (2.1×100 mm)

was used with a flow rate of 0.3 mL/min and a column temperature of 35 °C. The mobile phase was water (phase A) and methanol (phase B), both with 0.1% formic acid. The gradient used was 95-5% of phase A-B finishing in 100% of phase B after 13 min. The volume of sample injected was 5 µL. The electrospray interface (ESI) was used in positive and negative ionization, sensitivity mode with a capillary voltage of 1 kV and a sampling cone at 30 V. The temperatures used were 120 °C and 500 °C for source and desolvation respectively, and the desolvation gas flow was 800 L h−1. The system was calibrated and acquired data in the range 50–1000 *m/z*. Leucine-Enkephalin [M+H]+ *m/z* 556.2765 was used as lock-mass for real time mass correction. Collision ramp applied was 20–40 V, argon was used as collision gas. The acquisition was set at high definition mass spectrometry (HDMSE), with 0.1 s scan time.

2.7. Software

NIST MS Search (v. 2.2., 2014) mass spectral search program was used on the GC-MS/Q system for library search and comparison of the acquired spectra with the reference ones [23]. Data from VION® IMS/QTOF were processed using UNIFI (v. 1.8.) software and MassLynx (v. 4.1.) software, both from Waters Corporation. Chemdraw Ultra (v. 12.0.2.1076., Cambridge Soft) was also used for generating the mol files when necessary. The software Toxtree® (v. 2.6.0.) was used for risk assessment. The classification depends on the molecular structure and maximum values of human exposure for each toxicity class: Class I, II and III, 1.8, 0.54 and 0.09 mg kg−1, respectively.

3. Results and discussion

In order to identify potential migrants from the varnishes, non-targeted analysis of the sample was performed.

3.1. GC-MS/Q identification

The identification of volatile compounds in the extracts from the varnishes was carried out by GC-MS/Q. NIST 2014 [23] returned a “hit list” of matched chemical compounds from the library. Then, standards of the compounds were analyzed for confirmatory purposes. Benzophenone and 2-hydroxy-2-methylpropiophenone were detected in the extract coming from varnish 1. Both are photoinitiators used for the UV curing. 2-Hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone was identified in the extract coming from varnishes 2, 3 and 4. This compound is also a photoinitiator for UV curing. These initiators start the cross-linking reaction in response to UV-radiation.

3.2. UHPLC- IMS/QTOF analysis

The identification of non-volatile compounds was carried out by UHPLC- IMS/QTOF. This technique provides mass accuracy of both precursor and fragmentations, as well as ion mobility separation. In this system, the ions produced in the ionization region enter the StepWave™ ion guide and reach the ion mobility chamber, where they are separated based on their mobility constant K. As a result, a collision cross section (CCS, units of A2) value is obtained for each compound. CCS values are a robust and precise physicochemical property of an ion, which is related to its chemical structure and three-dimensional conformation [24]. HDMSE acquisition mode combines the selectivity of ion mobility and the specificity of MS/MS. From the acquisition of a single analysis, UNIFI software processes the raw data of low and high collision energy, and generates spectra after the drift time-alignment of precursors with their product ions. The whole

process makes the identification of nontarget compounds more reliable compared to traditional high resolution mass spectrometry (HRMS).

Fig. 1 shows the spectrum of the compound 7, [2-[2-(2-hydroxy-1- methyl-ethoxy)-1-methyl-ethoxy]-1-methyl-ethyl] 3-(diethylamino) propanoate peak at low energy acquisition and high energy acquisition, with drift time alignment. Fig. 2 shows the spectrum of the same compound disabling the drift time alignment.

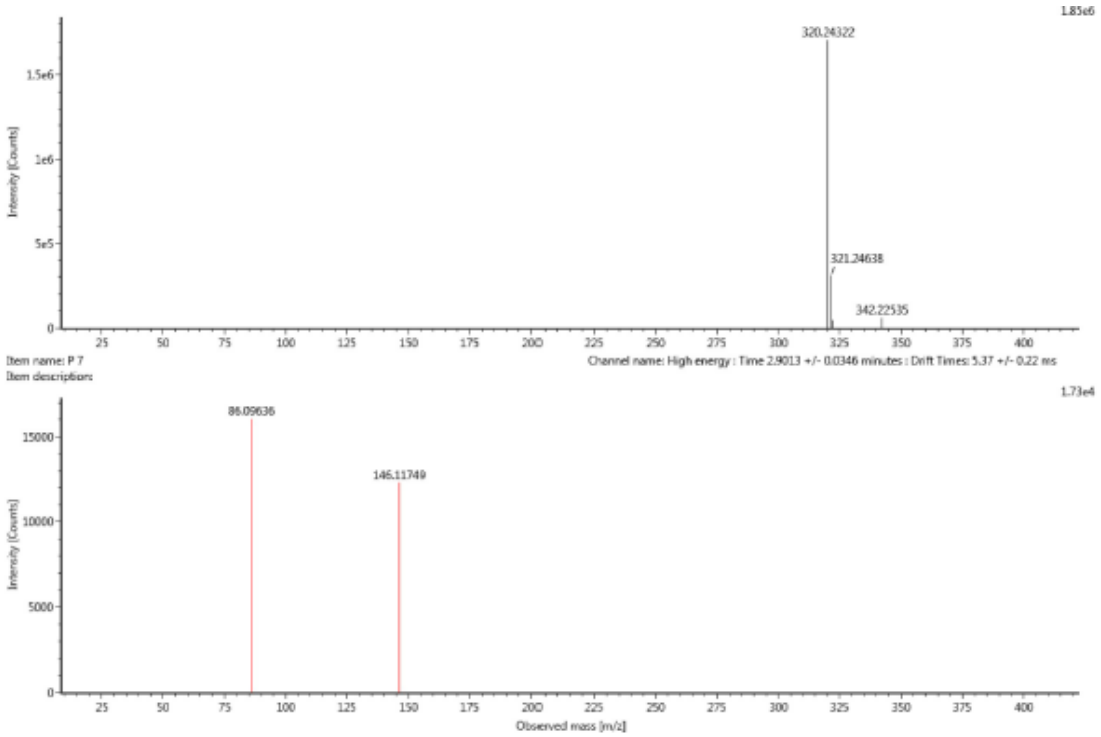

Fig. 1. Spectrum of the compound 7, [2-[2-(2-hydroxy-1-methyl-ethoxy)-1-methyl-ethoxy]-1-methyl-ethyl] 3-(diethylamino)propanoate peak at low energy acquisition and high energy acquisition, with drift time alignment.

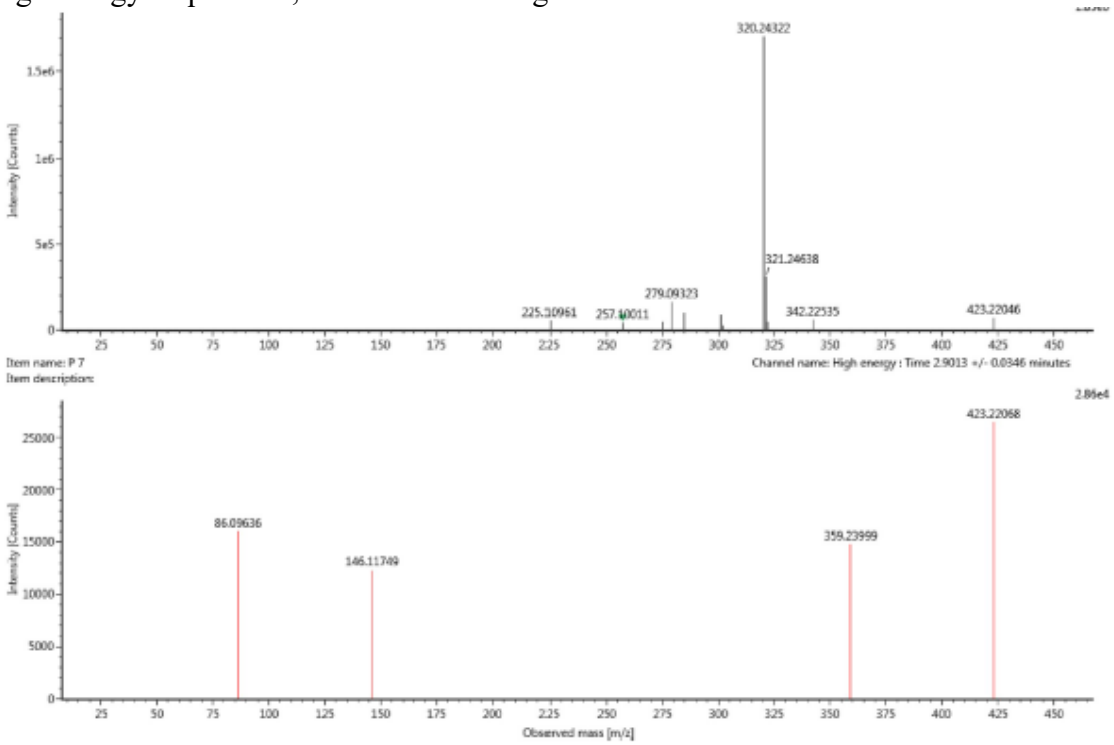

Fig. 2. Spectrum of the compound 7, [2-[2-(2-hydroxy-1-methyl-ethoxy)-1-methyl-ethoxy]-1-methyl-ethyl] 3-(diethylamino)propanoate peak at low energy acquisition and high energy acquisition, disabling drift time alignment.

It can be noted that the spectra in Fig. 1 exhibit a significant reduction of interfering ions as only components with same drift time of the precursor are displayed. This is a good example of how ion mobility helps reduce background noise, and remove many interferences coming from the matrix. The low energy spectrum was used to obtain the elemental composition of the unknown compounds, using a mass tolerance of 3 mDa. The elemental composition calculator was set to order the list of proposed elemental compositions according to the fit of the experimental data to the theoretical isotope distribution (i-FIT). The software applied an isotope predictive filtering to reduce the number of proposed elemental compositions, using algorithms to estimate the number of atoms in an unknown molecule, based on the mass of the molecular ion and the relative intensity of the first and second isotopes. Once a list of elemental compositions was obtained, the database Chemspider, that is embedded into UNIFI software [25], in combination with a bibliographic search about varnishes formulations, leads to a list of candidates for each mass detected. The fragments obtained in the high energy spectrum provided an additional confidence level to the identification process of the candidates. UNIFI® is also able to automatically match the most abundant experimental fragments obtained with the theoretical fragments of the proposed candidate, considering the accurate mass of each fragment, combined with a theoretical probability of bond-cleavage. Commercially available standards were analyzed for confirmation of the candidates. When no matches were found using the automatic procedure of UNIFI, the data were exported to MassLynx software. The MassFragment tool allowed to compare the theoretical fragments of any candidate, to the accurate masses of the fragments obtained in the high energy spectrum. The database Scifinder [26] was used in support of the identification process. Finally, when the candidates coming from this database didn't match the spectrum, Chemdraw was used to draw potential candidates and then the MassFragment tool compared the theoretical fragments with the actual ones.
Table 1 shows the list of compounds identified. The compound number 1, 2-propenoic acid 1,1'-[2-ethyl-2-[[(1-oxo-2-propen-1-yl)oxy] methyl]-1,3-propanediyl] ester (TMPTA) has been identified. It is a monomer used for the varnish polymer formation. Which means that free monomers can remain in the varnish after the curing. Moreover, the detected compound number 2, 2-propenoic acid,1,1'-[2-[[3-[2,2-bis [[(1-oxo-2-propen-1-yl)oxy]methyl]butoxy]-1-oxopropoxy]methyl]-2- ethyl-1,3-propanediyl] ester is considered a NIAS, as it is a reaction product coming from the monomer TMPTA. Several candidates were drawn based on the TMPTA structure and considering the elemental composition using ChemDraw. Then, MassFragment was used to compare the theoretical fragments with the spectrum. The compound number 2 was the compound that fit better with the spectrum.
Fig. 3 shows the high energy spectra of these two compounds.

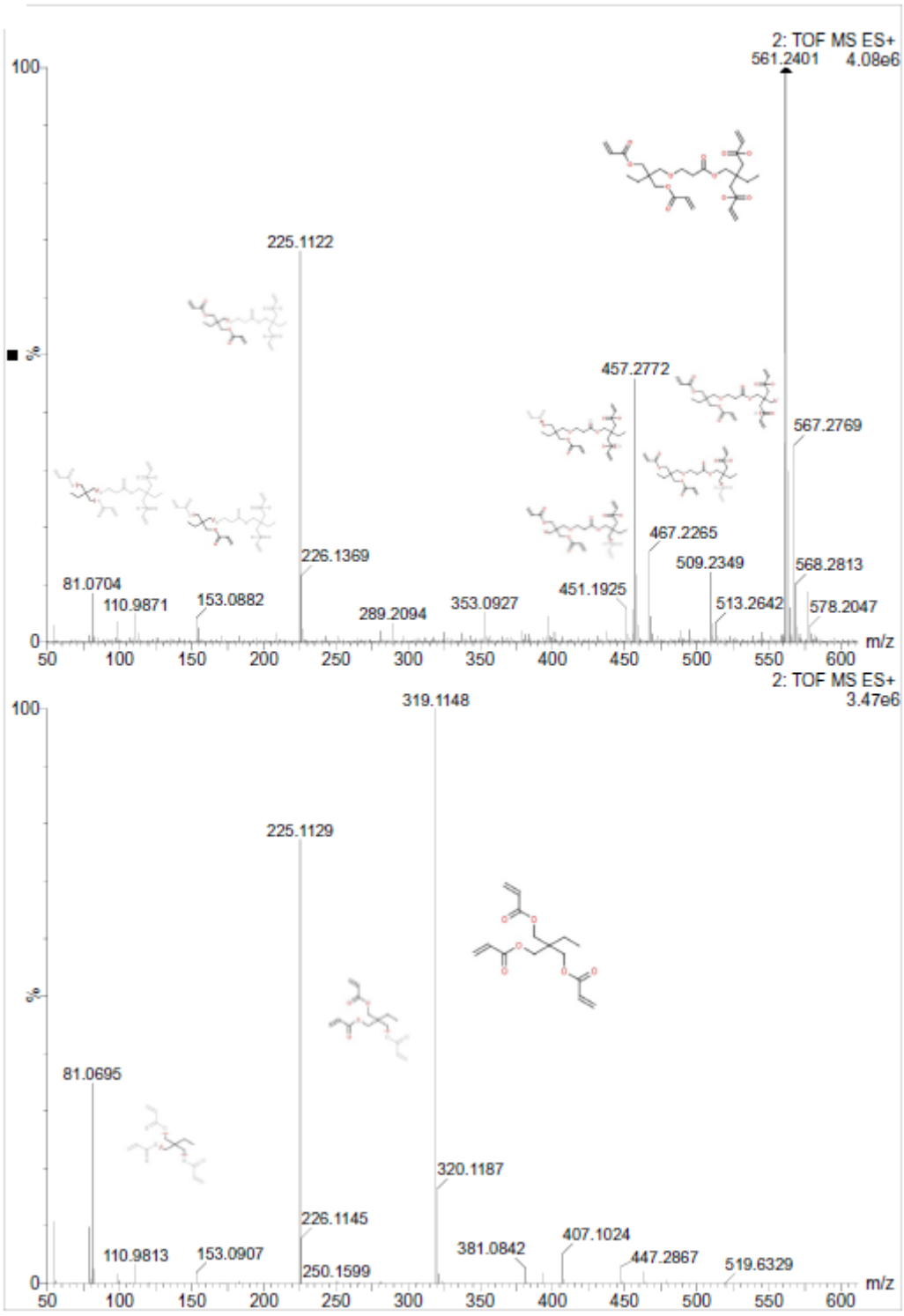

Fig. 3. Spectra of 2-propenoic acid 1,1'-[2-ethyl-2-[[(1-oxo-2-propen-1-yl)oxy]methyl]-1,3-propanediyl] ester (TMPTA) and 2-propenoic acid,1,1'-[2-[[3-[2,2-bis [[(1-oxo-2-propen-1-yl)oxy]methyl]butoxy]-1-oxopropoxy]methyl]-2-ethyl-1,3-propanediyl] ester. Molecular structure of the compounds and the fragments of the molecules in high energy spectra.

Fig. 3 shows the molecular structure of the compounds without taking into account the sodium adducts and the fragments of the molecules detected in the high energy spectra. As it can be seen, there are two fragments in common in both molecules (*m/z* 225.11 and

m/z 81.07), which is an indicator that the molecular structures can be correlated. The compound number 3, 2-propenoic acid, 1,1'-[oxybis(methyl- 2,1-ethanediyl)] ester (DPGDA) is a residual monomer as well, and it was identified in varnish 1. Following the same logic, compound 4, 11- diethyl-7-oxo-4,6,10,12-tetraoxopentadecane-3,13-diyl diacrylate can also be considered NIAS coming from compound 3. The compound number 5, ethanol 2,2′,2″-nitrilotris- is a photoinitiator for UV curing. Photoinitiators induce the photopolymerization or photocrosslinking process in which low molecular weight monomers and prepolymers are converted by the absorption of UV/visible light into highly crosslinked, solvent and chemically resistant films [27]. The compounds number 6, 7, 8 and 10 in Table 1 can be considered derivatives of the reaction between the photoinitiator 2-propenoic acid (1-methyl-1,2-ethanediyl) bis[oxy(methyl-2,1-ethanediyl)] ester and diethyl amine. These compounds were confirmed by analyzing an industrial sample of this photoiniciator. The compound number 9 was identified as 2-{2-[2-(acryloyloxy)-1-methylethoxy]-1-methylethoxy}.-1-methylethyl acrylate. It was found that this NIAS is an impurity of this photoiniciator [28]. The compounds 11–16 in Table 1 are part of a resin commonly used in varnishes [29], and the compound 17 is an antifoaming product [30]. A residual monomer poly[oxy(methyl-1,2-ethanediyl)], α,α′,α''-the polymer formation through the UV curing [31] was labelled as compound number 18, while compound number 19, oligo[2-hydroxy-2-methyl-1-[4-(1-methylvynyl)phenyl]propanone, is a polymeric photoinitiator. Considering the candidates identified using this technique, it is legitimate to think that a portion of these components can be susceptible to chemical migration from the polymeric material to food. In fact, free monomers typically remain in the varnish even after the curing. On these bases, chemical migration was assessed using food simulants.

Table 1: Number of varnish, number of compound, compound name, mass detected, elemental composition and collision cross section of each compound.

Varnish	N°	Compound name	Mass detected	Elemental composition	CCS
1	1	2-Propenoic acid, 1,1'-[2-ethyl-2-[[(1-oxo-2-propen-1-yl)oxy]methyl]-1,3-propanediyl] ester (TMPTA)	319.1168	C15H20O6 [Na+]	165.1
1	2	2-propenoic acid, 1,1'-[2-[[3-[2,2-bis[[(1-oxo-2-propen-1-yl)oxy]methyl]butoxy]-1-oxopropoxy]methyl]-2-ethyl-1,3-propanediyl] ester	561.2328	C27H38O11[Na+]	224.8
1	3	2-Propenoic acid, 1,1'-[oxybis(methyl-2,1-ethanediyl)] ester (DPGDA)	265.1057	C12H18O5 [Na+]	157.5
1	4	5,11-diethyl-7-oxo-4,6,10,12-tetraoxapentadecane-3,13-diyl diacrylate	453.2102	C21H34O9 [Na+]	196.1
1	5	Ethanol, 2,2',2''-nitrilotris-	150.113	C6H15NO3[H+]	132.2
1,2,3	6	Bis (2-methylbutan-2-yl) dicarbonate	269.1365	C12H22O5 [Na+]	159.3
1,2,3	7	[2-[2-(2-hydroxy-1-methyl-ethoxy)-1-methyl-ethyl] 3-(diethylamino)propanoate	320.244	C16H33NO5[H+]	174.8
1,2,3	8	[1-methyl-2-[1-methyl-2-prop-2-enoyloxy-ethoxy]ethoxy]ethyl] 3-(diethylamino)propanoate	374.255	C19H35NO6[H+]	187.1
1,2,3	9	2-(2-[2-(acryloyloxy)-1-methylethoxy]-1-methylethoxy)-1-methylethyl acrylate	323.147	C15H24O6[Na+]	170.6
1,2,3	10	[(methylethylene)bis(oxy)] dipropanol	215.126	C9H20O4[Na+]	146.5
1,2,3	11	Phenol, 4,4'-(1-methylethylidene)bis-, reaction products with 2-(chloromethyl)oxirane, 2-propenoate	769.35	C45H52O11[H+]	270.8
1,2,3	12	Reaction product of (4,4'-isopropylidenediphenol, oligomeric reaction products with 1-chloro-2,3-epoxypropane) and 2-propenoic acid (molar ratio 1:3)	822.9352	C48H53O12[H+]	219.3
1,2,3	13	Reaction product of (4,4'-isopropylidenediphenol, oligomeric reaction products with 1-chloro-2,3-epoxypropane) and 2-propenoic acid (molar ratio 1:1)	714.8404	C42H49O10[H+]	210.0
1,2,3	14	Reaction product of 2,2'-[propane-2,2-diylbis(4,1-phenyleneoxymethylene)] dioxirane and 2-propenoic acid (molar ratio 1:2)	484.5382	C27H31O4[H+]	190.5
1,2,3	15	Reaction product of 2,2'-[propane-2,2-diylbis(4,1-phenyleneoxymethylene)] dioxirane and 2-propenoic acid (molar ratio 1:3)	538.5856	C30H33O6[H+]	186.2
1,2,3	16	Reaction product of 3-(4-[1-methyl-1-[4-(oxiran-2-ylmethoxy) phenyl]ethyl]phenoxy) propane-1,2-diol and 2-propenoic acid (molar ratio 1:1)	430.4908	C24H29O7[H+]	166.2
1	17	2-Methyl-2-propanyl 1-hydroxy-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-oate	521.2938	C23H46O11[Na+]	201.0
2	18	Poly[oxy(methyl-1,2-ethanediyl)], α,α',α''-1,2,3-propanetriyltris[ω-[(1-oxo-2-propen-1-yl)oxy]- (GPTA)	393.152	(C3 H6 O)n (C3 H6 O)n (C3 H6 O)n C12H14 O6 [Na+]	156.4
			451.280		185.2
			509.239		211.0
			567.280		220.0
			625.319		237.1
2,3	19	oligo[2-hydroxy-2-methyl-1-[4-(1-methylvinyl)phenyl]propanone	431.221	$(C_{13}H_{16}O_2)_3[Na+]$	237.0
			635.784		256.0

3.3. Migration results

Migration assays were performed according to the "EuPIA Guidance on Migration Test Methods for the evaluation of substances in printing inks and varnishes for food contact materials" [22]. The guidelines state that migration tests should be done by using ethanol 95% (v/v) as the worst case scenario, applying the ratio of 6 dm2 of material surface to

1 kg of food simulant weight. Coatings, printing inks and adhesives are not yet covered by a specific EU legislation. Nevertheless, they must comply with the Regulation (EC) No 1935/2004 [32]. This Regulation establishes that materials and articles, including active and intelligent materials and articles, should be manufactured in compliance with good manufacturing practices (GMPs) so that, under normal or foreseeable conditions of use, they do not transfer their constituents to food in quantities which could: (a) endanger human health; or (b) bring about an unacceptable change in the composition of the food; or (c) bring about a deterioration in the organoleptic characteristics thereof. In order to establish a risk assessment procedure, the strategy described below was applied [33]. When the compound was listed in the plastics regulation 10/2011/EC [8] or in the Swiss ordinance 817.023.21 [34], and the migration value was below the specific migration limit (SML), it was considered that the compound complies with Regulation 1935/2004/EC. The problem arises when a compound is not present in this list. This is usually the case for NIAS. Therefore, a search for the relevant no-observed adverse effect level (NOAEL) obtained from repeat-dose toxicological studies was undertaken. To obtain the tolerable daily intake (TDI) from the NOAEL, a default assessment factor of 100 was applied: TDI (mg kg−1 body weight day−1) = NOAEL/assessment factor. This factor gives an additional margin to take into account the possibility that humans may be more sensitive than animals. Then, in order to obtain the self-derived SML, the convention of an average person body weight of 60 kg was adopted. Whilst it was considered an average consumption of 1 kg of packaged food per day. (self-derived SML = TDI*60). Finally, when the NOAEL did not exist, the TTC (threshold of toxicological concern) approach was used. This methodology is based on a decision-tree approach that treats information on the molecular structure of a target substance to assign the substance to one of the three Cramer classes. Each Cramer class is allocated to an exposure limit (mg/person/day) [35]. The same convention regarding the daily consumption of 1 kg packaged food per person was considered in this approach as well [33].
Table 2 shows the migration results from both volatile and nonvolatile compounds to ethanol 95%.

Table 2: Migration results from both volatile and non-volatile compounds to ethanol 95%. Varnish number, compound name, limit of detection (LOD), migration mg/kg, migration from set-off mg/kg, specific migration limit (SML) mg/kg and threehold according Cramer rules (mg/kg).

Varnish	Compound name	LOD	Migration mg/Kg	Migration set-off mg/Kg	SML mg/Kg	Threshold mg/Kg
1	2-Propenoic acid, 1,1'-[2-ethyl-2-[[(1-oxo-2-propen-1-yl)oxy]methyl]-1,3-propanediyl] ester (TMPTA)	0.01	< LOD	< LOD		0.09
1	2-propenoic acid, 1,1'-[2-[[3-[2,2-bis[[(1-oxo-2-propen-1-yl)oxy]methyl]butoxy]-1-oxopropoxy]methyl]-2-ethyl-1,3-propanediyl] ester	0.01	< LOD	< LOD		0.09
1	2-Propenoic acid, 1,1'-[oxybis(methyl-2,1-ethanediyl)] ester (DPGDA)	0.02	0.04	< LOD		0.09
1	5,11-diethyl-7-oxo-4,6,10,12-tetraoxopentadecane-3,13-diyl diacrylate	0.02	0.03	< LOD		0.09
1	Ethanol, 2,2′,2″-nitrilotris-	0.04	< LOD	< LOD	0.05	
1	Benzophenone	0.10	3.83	0.23	0.6	
1	2-Hydroxy-2-methylpropiophenone	0.1	0.70	< LOD		0.09
1	Bisphenol A	0.01	< LOD	< LOD	0.05	
2			< LOD	< LOD		
3			< LOD	< LOD		
2	2-Hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone	0.02	< LOD	< LOD	0.05	
3			< LOD	< LOD		
4			< LOD	< LOD		
1	Bis (2-methylbutan-2-yl) dicarbonate	0.02	0.73	< LOD		1.8
2			0.04	< LOD		
3			< LOD	< LOD		
4			< LOD	< LOD		
1	[2-[2-(2-hydroxy-1-methyl-ethoxy)-1-methyl-ethoxy]-1-methyl-ethyl] 3-(diethylamino) propanoate	0.02	0.42	< LOD		0.09
2			< LOD	< LOD		
3			< LOD	< LOD		
4			< LOD	< LOD		
1	[1-methyl-2-[1-methyl-2-(1-methyl-2-prop-2-enoyloxy-ethoxy)ethoxy]ethyl] 3-(diethylamino)propanoate	0.02	0.25	< LOD		0.09
2			< LOD	< LOD		
3			< LOD	< LOD		
4			< LOD	< LOD		
1	2-{2-[2-(acryloyloxy)-1-methylethoxy]-1-methylethoxy}-1-methylethyl acrylate	0.02	0.14	< LOD		0.09
2			< LOD	< LOD		
3			< LOD	< LOD		
4			< LOD	< LOD		
1	[(methylethylene)bis(oxy)]dipropanol	0.02	0.56	< LOD		0.09
2			0.05	< LOD		
3			< LOD	< LOD		
4			< LOD	< LOD		
1	Phenol, 4,4'-(1-methylethylidene)bis-, reaction products with 2-(chloromethyl)oxirane, 2-propenoate	0.02	< LOD	< LOD		0.09
2			< LOD	< LOD		
3			< LOD	< LOD		
1	Reaction product of (4,4′-Isopropylidenediphenol, oligomeric reaction products with 1-chloro-2,3-epoxypropane) and 2-propenoic acid (molar ratio 1:3)	0.02	< LOD	< LOD		0.09
2			< LOD	< LOD		
3			< LOD	< LOD		
1	Reaction product of (4,4′-Isopropylidenediphenol, oligomeric reaction products with 1-chloro-2,3-epoxypropane) and 2-propenoic acid (molar ratio 1:1)	0.02	< LOD	< LOD		0.09
2			< LOD	< LOD		
3			< LOD	< LOD		
1	Reaction product of 2,2'-[propane-2,2-diylbis(4,1-phenyleneoxymethylene)] dioxirane and 2-propenoic acid (molar ratio 1:2)	0.02	< LOD	< LOD		0.09
2			< LOD	< LOD		
3			< LOD	< LOD		
1	Reaction product of 2,2'-[propane-2,2-diylbis(4,1-phenyleneoxymethylene)] dioxirane and 2-propenoic acid (molar ratio 1:3)	0.02	< LOD	< LOD		0.09
2			< LOD	< LOD		
3			< LOD	< LOD		
1	Reaction product of 3-(4-{1-methyl-1-[4-(oxiran-2-ylmethoxy) phenyl]ethyl}phenoxy) propane-1,2-diol and 2-propenoic acid (molar ratio 1:1)	0.02	< LOD	< LOD		0.09
2			< LOD	< LOD		
3			< LOD	< LOD		
1	2-Methyl-2-propanyl 1-hydroxy-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-oate	0.03	< LOD	< LOD		0.09
2	Poly[oxy(methyl-1,2-ethanediyl)], α,α′,α″-1,2,3-propanetriyltris[ω-[(1-oxo-2-propen-1-yl)oxy]- (GPTA)	0.01	< LOD	< LOD		0.09
2	oligo[2-hydroxy-2-methyl-1-[4-(1-methylvynyl)phenyl]propanone	0.04	< LOD	< LOD	0.05	

The table shows the migration of compounds that diffuse through the varnished substrate (PP) and thereby come into contact with the food simulant, and the 'set-off' migration by which substances used in varnishes can transfer from the varnished (outer) surface to the inner, food-contact surface. It may happen during stacking or rolling of food contact materials that are varnished on the outside. DPGDA and the compound 4, 11-diethyl-7-oxo-4,6,10,12-tetraoxopentadecane-3,13-diyl diacrylate, as was described in the previous section, is a NIAS migrated to the food simulant. Moreover, several photoinitiators such as benzophenone, 2-hydroxy-2-methylpropiophenone and the compounds coming from the photoinitiator 2-propenoic acid (1-methyl-1,2-ethanediyl) bis[oxy(methyl-2,1-ethanediyl)] ester, and the reaction products with diethylamine, including the compound 2-{2-[2-(acryloyloxy)-1-methylethoxy]-1-methylethoxy}. -1-methylethyl acrylate, a NIAS, migrated to the food simulant, exceeding their SML established in the European Regulations [8,34] were found to migrate under the conditions applied. In the case of benzophenone, a common photoinitiator, the migration happened through both diffusion and the 'set-off' mechanism. These migration results justifiy the importance of investing in new technologies, such as an ion mobility/high resolution mass spectrometry system, in order to identify the NIAS in the food contact materials. This work demonstrates that the NIAS found in the cured varnish can migrate to the food simulants, in some cases at

a level that would exceed the European food contact regulation limits. Compounds that migrated over the SML were removed from the final varnish formulation. Then, it highlights the significance of studying the identification of NIAS and their migration to food, since it helps the companies to decide what compounds could be present in a formula that is going to be in contact with food.

4. Conclusions

The enhanced mass accuracy, combined with the drift time-aligned spectra obtained using the technique UHPLC-IMS/QTOF allowed to identify several NIAS in the cured varnishes used in food packaging. Two of the NIAS were neo-formed compounds coming from reactions between some of the ingredients in the formula, whereas others NIAS could be considered impurities of additives and ingredients intentionally added to obtain a suitable varnish formulation. Some of these compounds migrated to the food simulant over the established migration limits. This study also highlights the great benefits gained by the use of ion mobility mass spectrometry when it comes to spectral interpretation and structure elucidation of unknown compounds.

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