Ion-mobility QTOF mass spectrometry: a novel technique applied to migration of non-intentionally added substances (NIAS) from polyethylene films intended for use as food packaging

Paula Vera [1], Elena Canellas [2], Gitte Barknowitz [3], Jeff Goshawk [3] and Cristina Nerín[1]*

[1] Analytical Chemistry Department, GUIA Group, I3A, University of Zaragoza, Mª de Luna 3, 50018 Zaragoza, Spain

[2] Samtack Adhesivos Industriales, C/ Cerámica, nº3, Pol. Magarola, 08292, Esparreguera, Barcelona (Spain)

[3] Waters Corporation, Manchester, UK

ABSTRACT: Non-target analysis of non-volatile substances in complex samples is a very challenging task that requires powerful analytical techniques and experience of analyzing such samples. An extensive study was conducted in order to identify non-intentionally added substances (NIAS) migrating from eighteen polyethylene (PE) samples intended to be in contact with food. The migration assays were performed in five simulants and analyzed by ultra-high-performance liquid chromatography (UPLC) coupled to an ion-mobility separation (IMS) quadrupole-time of flight (QTOF) mass spectrometer. This experimental set-up is a novel and powerful tool for this type of non-volatile and non-targeted analysis. Thirty-five compounds were identified, seventeen of which were NIAS. Methyl and ethyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propanoate were found to be degradation products of either irganox 1010 or 1076. Additionally, break-down products including hexa - Heptadecanamide, N,N'-1,2-ethanediylbis- and 11-Eicosenamide were identified together with impurity reaction products .g. dibutyl amine or compounds of unknown origin like phosphine oxide, tributyl-. Forty-five percent of the detected compounds were part of in the positive list contained in Regulation 10/2011/EU and their migration values were below their specific migration limits. The risk assessment for the rest of the compounds was carried out by comparing their migration values to the maximum concentration recommended by Cramer. E.g. ethyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propanoate and benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, 1,1'-[2,2-bis(hydroxymethyl)-1,3-propanediyl] ester (both class II toxicity), Heptadecanamide, N,N'-1,2-ethanediylbis- and phosphine oxide, tributyl- (both class III toxicity) were above the maximum concentration values in three samples migrated to ethanol 95 % , and therefore these samples are not suitable for food contact. The analytical tools and procedures used in this study are presented and discussed in detail.

Over the past decade, there has been a growing demand for the development of materials for food packaging. At least thirty different types of plastics are found in the market. The most common packaging materials are polyolefins, copolymers of ethylene, substituted olefins, polyesters, polyamides and polycarbonates [1]. Within the polyolefin group, polypropylene and polyethylene (low density LDPE and high density HDPE) are probably the most abundant materials. Therefore, PE was chosen as the subject of this study.

One important function of packaging is the chemical protection. Therefore, a potential migration of compounds present in packaging into the food must be considered [2,3]. In fact, plastic packaging contains many additives like lubricants, antioxidants, stabilizers and plasticizers which could migrate into the product. These substances, intentionally added to the polymer, are included in Regulation 10/2011/EU [4] where a positive list of compounds as well as the conditions of migration tests (temperature, time and type of simulants) are stated.

Many studies have tested the migration of intentionally added substances (IAS) from PE materials by different analytical techniques. For example, volatile compounds are analyzed by GC-MS and include phenolic antioxidants (BHA and BHT), plasticizers (BP, DiBP, DEHA, ATBC)[5,6], photoinitiators (benzophenone), diphenylbutadiene which is intended as a broad-spectrum antimicrobial agent [7]. Recently the importance of MOSH; POSH and MOAH analysis [8]has been emphasized. Non volatile compounds typically analyzed by LC-MS include stabilisers (tinuvins or chimassorbs [9]), antioxidants (irgafos 168, irganox 1010, irganox 1076 [9] and irganox 3114 [10]), plasticizers (phthalates [11], linear alkanamides and bisphenol A) and surfactants nonylphenols [12,13].

In addition, the use of Raman technology to detect Uvitex OB [14,15] has been described for this application area as well. It is worth mentioning that a lot of studies have been published relating to the determination of inorganic elements and nanocopper or nanosilver using ICP-MS [16-20]. In addition, mathematical models have been developed to estimate the migration of benzophenone, Irgafos 168 or diphenylbutadiene, for example, from PE plastics[21,22].

There are growing concerns by consumers and plastic manufacturers regarding the presence of non-intentionally added substances (NIAS) in food contact materials [23,24]. The identification of these compounds is a complex task that presents a great analytical challenge. UPLC coupled to a QTOF mass spectrometer is probably the most useful technique for the non-targeted analysis of non-volatile NIAS [2,25-27]. However, almost no information relating to NIAS in PE samples was found in literature [28]. Some information is available for the non-targeted analysis of volatile compounds by GC-MS [29,30], where the identification is facilitated by the availability of libraries like WILEY and NIST.

The novel technique of ion-mobility QTOF mass spectrometry (IMS QTOF) can be used for the confirmation of NIAS [31]. This technique, besides acquiring full scan mass spectra with high sensitivity and accurate masses for high and low collision energy, provides an additional dimension of separation by inclusion of ion-mobility. The technique, high definition mass

spectrometry (HDMSE), enables cleaner spectra to be generated and coeluting compounds to be separated.

A new parameter, the collisional cross section (CCS) value is determined automatically during data processing and depends on the size, shape and charge of the molecule. The CCS value is a characteristic measurement of the compound and does not depend on the chromatographic conditions or the column used; this represents an additional advantage in a laboratory environment. The analysis of standards enables CCS libraries to be created under different experimental conditions. The inclusion of CCS values within a library can provide a higher confidence level for identification of compounds. For some substances differentiation between isomers is possible due to their distinct CCS values.

The aim of this work was to identify non-volatile NIAS that migrate from PE samples, intended as food contact materials, and to expand the knowledge of unexpected compounds that may appear from degradation processes, impurities or reaction products from this type of plastic. The study was carried out by IMS QTOF and a detailed description of the procedures followed for non-targeted analysis and the identified NIAS are provided. Quantification and risk assessments were made.

Materials and methods

Reagents: The standards triacetine (102-76-1), ethanol,2-(2-butoxyethoxy), acetate (124-17-4), acetyl butyl citrate (77-90-7), octylphenol ethoxylate (9036-19-5), dodecyl alcohol ethoxylated (9002-92-0), 2-ethylhexyl sebacate (122-62-3)), oleamide (301-02-0), palmitic acid (57-10-3), bis(2-ethylhexyl) adipate (103-23-1), dinonyl phthalate (84-76-4), stearic acid (57-11-4), irganox 1010 (6683-19-8) , bis(2-ethylhexyl) phthalate (117-81-7), erucamide (112-84-5), irganox 1076 (2082-79-3), octadecanamide, N,N'-1,2-ethanediylbis- (110-30-5), irgafos 168 (31570-04-4), dibutyl amine (111-92-2), N,N-Bis(2-hydroxyethyl) dodecylamine (1541-67-9), phosphine oxide, tributyl- (814-29-9), tridodecylamine (102-87-4), methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propanoate (6386-38-5), 11-eicosenamide, (11Z)- (10436-08-5), formic acid and acetic acid were purchased from Sigma-Aldrich Química S.A (Madrid, Spain). All standards were of analytical quality. Ethanol, water and methanol of HPLC grade were supplied by Scharlau Chemie S.A (Spain). Tenax was supplied by Supelco (Bellefonte, USA).

Commercial samples: Eighteen different PE samples used as food contact materials were studied. Two types of PE; LDPE and HDPE supplied by different European companies were tested. These samples are used in contact with different types of foods like milk, sugar, rice, pasta, biscuits, nuts, chocolate, pizza, meat, fish, cheese and different sauces and creams.

The simulants used for each sample were chosen according to the intended use of the material and are stated in detail in table 1. No information about the sample composition was provided by the manufacturers.

Table 1: Plastic type, code and food simulants tested.

Plastic	Code	Food simulants				
		ET95	Tenax	ET10	AC3	ET50
HDPE	PE1					X
HDPE	PE2		X			
HDPE	PE3		X			
LDPE	PE4		X			
LDPE	PE5	X	X	X	X	
LDPE	PE6	X	X	X	X	
HDPE	PE7	X		X	X	
LDPE	PE8	X		X	X	
LDPE	PE9	X		X	X	
LDPE	PE10	X		X	X	
LDPE	PE11	X		X	X	
LDPE	PE12	X		X	X	
LDPE	PE13	X		X	X	
LDPE	PE14	X		X	X	
HDPE	PE15	X		X	X	
LDPE	PE16	X		X	X	
LDPE	PE17	X				
LDPE	PE18	X				

Migration assays: Five different simulants were used for the migration assays according to Regulation 10/2011/EU [4]. The tested simulants were ethanol 95 % (as simulant D2) when the materials were intended to be in contact with fatty foods (like cheese, meat or fish), ethanol 50% (simulant D1) for lipophilic foods (for example milk or liquids with alcohol >20%), Tenax ® (simulant E) for dry foods (like chocolate, biscuits, pasta, nuts) and ethanol 10% (simulant A) and acetic acid 3 % (simulant B) for hydrophilic and acidic foods (like creams, sauces and pizza) respectively.

For the migration test, the samples were immersed in liquid simulants (A, B, D1 or/and D2), according to the proportions stated in Regulation 10/2011/EU (6 dm2/1 Kg simulant). Afterwards, the samples were stored for 10 days at 60ºC.

When Tenax ® was used as a simulant, the films were cut into pieces of 2x4 cm and covered with 0.32 grams of Tenax® according to Regulation UNE-EN 14338[32] (4 g Tenax/dm2). The samples were subsequently placed on an aluminum foil inside a Petri dish and stored for 10 days at 60ºC. Tenax® was then extracted two consecutive times with 3 mL of ethanol. Before use, Tenax was cleaned up with acetone and ethanol for 6 hours by Soxhlet extraction.

The D2 and the Tenax extracts were concentrated six times under a stream of N2 and analyzed by UPLC-IMS QTOF. Simulants A, B and D1 were directly analyzed.

UPLC IMS QTOF analysis: The analyses were carried out on a ACQUITY UPLC® I-Class system coupled to a Vion IMS QTOF with electrospray interface (ESI) (Waters, Milford, MA, USA). A UPLC BEH C18 column (1.7 µm particle size, 2.1 mm x 100 mm) was used with a flow rate of 0.3 mL/min and kept at 35ºC to separate the samples. The mobile phases were water (phase A) and methanol (phase B) with 0.1 % formic acid. The gradient used was 5% phase B to 100% phase B in 13 min. The injected sample volume was 5 µL.

Data were acquired in positive and negative ionization mode. Vion IMS QTOF was operated in sensitivity mode (capillary voltage 1 kV, cone voltage 30V). The source and desolvation temperature was set to 120 ºC and 500 ºC respectively and the desolvation gas flow was 800 Lh-1. The instrument was calibrated for a mass range of 50 to 1200 Da.

The acquisition was carried out in high definition mass spectrometry (HDMSE) mode described above. Data were processed using UNIFI v1.8 software (Waters, Milford, MA,

USA). The acceptance criteria were set to 2% CCS variation between library entry and sample.

Identification of migrant compounds: For the identification, ethanol 95% and Tenax migration samples were analyzed using the Binary Compare function within the UNIFI software. This function allows a comparison between reference and sample which can be displayed as peak intensity chromatograms (BPI) which can be mirrored or it can be displayed as a separate table of candidate masses. For this work, only the compounds unique in the samples and absent in the references (blanks) were taken into count as markers.

Once the markers were chosen for each sample, the UNIFI Discovery Tool which is linked to Chemspider database search was used to identify each marker. The tool combines analytical information like accurate mass, isotope pattern and fragment ion information from the high collision energy mode obtaining possible molecular formulas and fragments for each ion which are then searched against the database.

The assignment for each candidate from the Chemspider database was made according to chemical criteria, background knowledge, candidates with higher values of fragment matches and/or the number of citations given as output in UNIFI.

Afterwards, the analytical standards of the candidates were analyzed under the same conditions as the samples and their identifications were confirmed by the retention time, CCS values and fragment matches. When the identification status of the candidate changed, it was being assigned as an identified compound and was added to the UNIFI Scientific Library. A typical compound entry in the library includes compound name, molecular formula, structure, fragment ions, CCS value and detected adducts. The data in this library will provide a significant benefit for the future identification of NIAS.

Quantification of migrant compounds and risk assessment: After identifying all candidates and creating the library, a quantification of the compounds was performed. The extracts of all of samples were analyzed by IMS QTOF as described earlier. The results were processed against a target list in UNIFI which was created from library entries to quantify the migrated compounds that were already identified. For this purpose, calibration curves of standard solutions in ethanol were prepared. When the standard was not commercially available, the compound was quantified using another standard with a similar molecular structure. Three replicates of each concentration level were analyzed for confirmation.

The concentrations were expressed as mg of compound per kg of simulant. For the simulant Tenax®, firstly, calculate the absolute mass of the compound that migrated (taking into account the volume of the extract after the concentration process), which was then divided by the surface area used for the migration assay (0.08 dm2) and finally, the ratio of 6 dm2/1Kg of simulant (Regulation 10/2011/EU) was applied [4].

To conduct the risk assessment, when the compounds were not in the positive list of Regulation 10/2011/EU [4], Cramer's classification with the software Toxtree® was applied. The classification depending on molecular structure and the estimated maximum values of human exposure for each toxicity class were:Class I, II and III with 1.80,0.54 and 0.09 mg/Kg [33].

Results and discussion: Eighteen different PE samples (HDPE and LDPE) intended to be used as food packaging for different types of food were analyzed by UPLC-IMS QTOF to determine non-volatile compounds migrated.

The most important aim of this work was not only to detect additives, but also new compounds (NIAS). This challenging task required a powerful technique, a significant amount of manual assessment and experience for evaluation. In order to evaluate if these materials fulfill the legislation, the migrated compounds were quantified and risk assessments were conducted according to Regulation 10/2011/EU [4] and Cramer [33].

Identification of migrant compounds from PE films: The migrant compounds found are shown in table 2, ordered according to their retention times. They are classified into two groups: common additives and unknown compounds/NIAS. Additionally, their detected mass-to-charge ratios (m/z), their type of ionization (H^+ or H^- or Na^+), matched fragments and CCS values are highlighted.

Amongst these additives several plasticizers like triacetine, acetyl butyl citrate (ATBC), dibutyl sebacate (DBS), bis(2-ethylhexyl) adipate (DEHA), dinonyl phthalate (DNP) and bis(2-ethylhexyl) phthalate (DEHP) [11] were found. These compounds are usually added to increase the flexibility improving performance of the packaging material. Antioxidants such as irganox 1010, irganox 1076 [34] and irgafos 168 [9,35]or slip agents like oleamide and erucamide, lubricants as palmitic, stearic acid [35] and octadecanamide, N,N'-1,2-ethanediylbis- were found as well. Ethanol,2-[2-(2-butoxyethoxy)ethoxy]- and Ethanol,2-(2-butoxyethoxy) acetate were found and are usually used as surfactants or solvents.

As table 2 shows, the most common additives in this type of samples were erucamide, Bis(2-ethylhexyl) phthalate and Irganox 1010, present in several samples. It should be highlighted that sample PE4 no migrant compound was detected.

The compounds number 5 and 6, in sample PE6, had a peculiar pattern of fragmentation and the identification was a very difficult task. A lot of masses appeared in both spectra which made it impossible to draw conclusions about the molecular mass to obtain their elemental compositions.

After studying the spectra it was observed that the mass difference between consecutive fragments was 44.0261 m/z, which corresponded to the gain of (C_2H_4O). It meant the loss of an ethoxylated group for each fragment. To identify these compounds, an exhaustive search was carried out of ethoxylated surfactants that could be used in the industry [36,37]. A list of all compounds with their respective molecular masses including sodiated adducts was created. For each of these, a sequence of fragment masses losing $(C_2H_4O)_n$ n=1,2,3… was also calculated. Finally, it was checked if any one of these sequences of fragment masses matched with the spectra obtained of both compounds in this study.

Table 2: Migrant compounds found in PE samples, retention time (RT) and mass detected (m/z), elemental composition (EC), fragments obtained, CCS values, candidate name, remarks and samples where these compounds were found.

Nº	RT and m/z	EC	Fragments	CCS	Candidate name	Remarks	Samples
Aditives							
1	3.50_241.0686	C9H14O6Na	-	147.3±1.4	Triacetine	Plasticizer	PE14, PE16
2	4.49_229.1416	C10H22O4Na	-	150.4±1.9	Ethanol,2-[2-(2-butoxyethoxy)ethoxy]-	Surfactant	PE14 , PE16
3	5.03_227.1261	C10H20O4Na	-	152.3±0.7	Ethanol,2-(2-butoxyethoxy), acetate	Surfactant	PE14, PE16
4	7.12_425.2146	C20H34O8Na	331.1751/359.206	205.3±4.1	Acetyl butyl citrate	Plasticizer	PE15
5	7.14_537.3427	(C2H4O)nC14H220Na	-	226.9±1.5	Octylphenol ethoxylate	Surfactant	PE6
6	7.42_473.3448	(C2H4O)nC12H260Na	89.0602	219.4±0.7	Dodecyl alcohol, ethoxylated	Surfactant	PE6
7	7.67_337.2351	C18H34O4Na	-	190.9±3.2	Dibutyl sebacate	Plasticizer	PE15
8	7.79_304.2617	C18H35NONa	-	186.4±2.6	Oleamide	Slip agent	PE12
9	7.98_255.2336	C16H33O2	-	196.6±3.5	Palmitic acid	Lubricant	PE1, PE5
10	8.13_393.2983	C22H42O4Na	281.1747/167.070	219.1±4.4	Bis(2-ethylhexyl) adipate	Plasticizer	PE2
11	8.43_441.2985	C22H42O4Na	-	227.6±0.2	Dinonyl phthalate	Plasticizer	PE3
12	8.43_283.2637	C18H37O2		184.7±1.0	Stearic acid	Lubricant	PE1, PE5
13	8.86_1199.7827	C73H108O12Na	-	372.1±5.5	Irganox 1010	Antioxidant	PE5, PE9, PE10, PE12, PE16
14	8.10_413.2676	C24H38O4Na	135.0441	217.5±4.3	Bis(2-ethylhexyl) phthalate	Plasticizer	PE7, PE10, PE14, PE16
15	8.29_360.3250	C22H43NONa	67.0542	201.8±4.3	Erucamide	Slip agent	PE7, PE8, PE9, PE10, PE11, PE12, PE13, PE16, PE17 and PE18
16	10.22_553.4596	C35H62O3Na	283.2632	248.6±3.7	Irganox 1076	Antioxidant	PE17, PE18
17	11.55_615.5823	C38H76N2O2Na	310.3095/237.3360/ 282.2779	278.1±4.1	Octadecanamide, N,N'-1,2-ethanediylbis-,	Lubricant	PE2
18	11.56_647.4586	C42H64O3P	591.3963	283.4±4.5	Irgafos 168	Antioxidant	PE5, PE12, PE15
	NIAS						
19	0.84_130.1604	C8H20N	-	136.6±2.8	Dibutyl amine	Inhibitor	PE6, PE7, PE10
20	5.82_246.2480	C14H32NO2	228.2318/102.0911/ 88.0753/202.2155	175.7±1.7	N,N-Bis(2-hydroxyethyl) decylamine		PE11
21	5.87_274.2828	C16H36NO2	256.2634/228.2318/ 102.0911/88.0753	187.7±1.8	N,N-Bis(2-hydroxyethyl) dodecylamine CAS 1541-67-9	Standard	PE8, PE11, PE13
22	6.41_302.3140	C18H40NO2	284.2949/102.0911/ 88.0753/258.2791	198.9±2.0	N,N-Bis(2-hydroxyethyl) tetradecylamine		PE11
23	6.82_330.3433	C20H44NO2	*	209.3±2.1	N,N-Bis(2-hydroxyethyl) hexadecylamine		PE11
24	7.15_358.3715	C22H48NO2	*	218.5±2.3	N,N-Bis(2-hydroxyethyl) octadecylamine		PE11
25	6.09_ 219.1889	C12H28OP	163.1246/191.1559/ 175.1246	158.5±2.2	Phosphine oxide, tributyl-	Standard	PE15
26	7.04_315.1936	C18H28O3Na	219.1730/203.143	178.5±1.6	Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propanoate, Ralox 35	Standard	PE10, PE17, PE18
27	7.22_329.2103	C19H30O3Na	*	184.3±5.2	Ethyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propanoate		PE10, PE17, PE18
28	7.51_679.4181	C39H60O8Na	*	257.7±3.4	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, 1,1'-[2,2-bis(hydroxymethyl)-1,3-propanediyl] ester		PE10, PE12, PE16
29	8.09_939.5989	C56H84O10Na	*	322.2±3.9	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, 1,1'-[2-[[3-[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl] -1-oxopropoxy]methyl]-2-(hydroxymethyl)-1,3-propanediyl] ester		PE10, PE12, PE16
30	7.79_522.5992	C36H76N	354.4090	276.9±5.2	Tridodecylamine	Standard	PE13
31	8.14_531.4916	C34H68N2O2Na	*	258.0±3.8	Pentadecanamide, N,N'-1,2-ethanediylbis-		PE2
32	9.76_559.5181	C34H68N2O2Na	282.2787/254.2466/ 299.3060/271.2755	263.3±3.4	Hexadecanamide, N,N'-1,2-ethanediylbis-		PE2
33	11.77_587.5502	C36H72N2O2Na	*	270.2±3.8	Heptadecanamide, N,N'-1,2-ethanediylbis-		PE2
34	9.42_685.4371	C42H63O4PNa	495.2657/551.3289/ 439.2037	290.5±2.9	Irgafos 168 OXO	Standard	PE5, PE12, PE15
35	10.25_332.2935	C20H39NONa	-	194.2±1.9	11-Eicosenamide, (11*Z*)-	Standard	PE12

- - Not fit fragments were shown *the compound was not found in the chemspider library.

-

Table 2 shows the identified compounds; Octylphenol ethoxylate and Dodecyl alcohol-ethoxylated, where the mass fragmentation matched with the database search.

Figure 1 shows a spectrum of ethoxylated octyl phenol, highlighting the fragments by a gain of C_2H_4O, where the molecular formula corresponded to $(C_2H_4O)n\ C_{14}H_{22}O$, and all fragments from n=1 (m/z= 273.1841 without and with Na^+ adduct) to n=22 (m/z= 1197.7305 without and with Na^+ adduct) appeared.

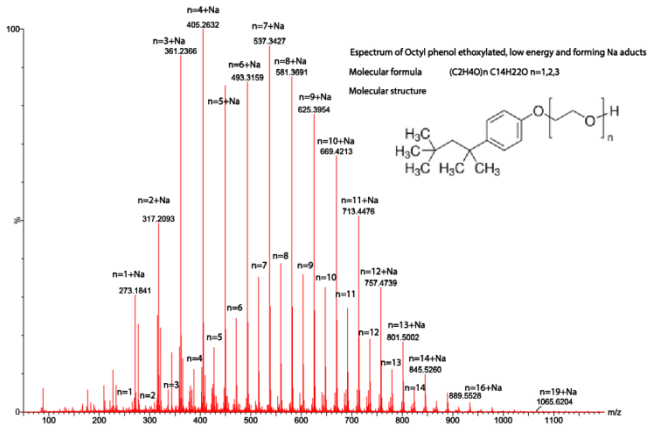

Figure 1. Spectrum of Octyl phenol, ethoxylated with their mass fragments by low energy collision.

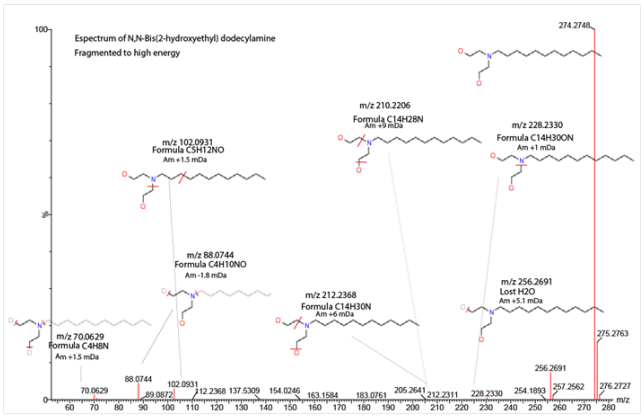

Figure 2 Spectrum of N,N-Bis(2-hydroxyethyl) dodecylamine with their ions mass and fragments according to UNIFI.

The second part of table 2 shows NIAS or unknown compounds. The first compound identified was dibutyl amine. It was detected in three samples. No information about the relationship between this compound and polyethylene was found in literature, only that this is a flotation agent used as a corrosion inhibitor in the manufacture of emulsifiers [38].

The compounds 20 to 24 belong to the group of N,N-Bis(2-hydroxyethyl)alkylamines, whose presence may be associated with an impurity-reaction or breakdown product [39,40]. Its molecular formula corresponded to $C_xH_{2x+3}NO_2$, and the mass difference between the consecutive compounds was the gain of 28.0348 m/z, meanings, C_2H_4 is contained in its alkyl chain. These compounds were detected in sample PE11, where the smallest compound had an alkyl chain of ten carbons and the biggest compound had eighteen carbons. Only, the compounds with pair carbons chain were detected.

Checking their spectra, all these compounds had the same fragmentation pattern but only the compound N,N-Bis(2-hydroxyethyl) dodecylamine was found and identified searching against the Chemspider database. The rest of the compounds did not correspond to any entries in Chemspider

Figure 2 shows the spectrum of N,N-Bis(2-hydroxyethyl) dodecylamine at high energy, where the ions are shown with their possible fragmented structures, as shown in UNIFI.

As this figure shows, the spectrum showed little fragmentation, and in all cases the common mass-to-charge ratios of 88.0757 and 102.5931 appeared as well as the mass related with the loss of a H_2O molecule (18.0105 m/z) from the molecular ion (274.2748) that corresponded to 256.2691 m/z. Additionally, this compound was a commercially available standard. It was also detected in two other samples (PE8 and PE11).

The compounds tributylphosphine and tridodecylamine (compounds 25 and 30 respectively) were identified and confirmed with their standards. They were only found in the samples PE15 and PE13 respectively. In literature, it was found that tributylphosphine is industrially used as a catalyst modifier in the cobalt-catalyzed hydroformylation of alkenes, where, the double bond of an alkene is broken forming an aldehyde compound [35]

Methyl (Ralox 35) and ethyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propanoate (compound 26 and 27) were detected in three samples. Ralox 35 could be identified with a commercial standard. And both compounds can be a break-down product of Irganox 1010 or Irganox 1076 [41]. Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, 1,1'-[2,2-bis(hydroxymethyl)-1,3-propanediyl] ester and Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, 1,1'-[2-[[3-[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]-1-oxopropoxy]methyl]-2-(hydroxymethyl)-1,3-propanediyl] ester (28 and 29, respectively) were considered as NIAS, coming from the degradation of Irganox 1010 in one or two positions of the molecule [42].

Other compounds, such as 31, 32 and 33 belong to the family of alkylamides, N,N'-1,2-ethanediylbis- that could be considered as breakdown or impurity products of Octadecanamide, N,N'-1,2-ethanediylbis, above named [43].

Irgafos 168 OXO (34) was an oxo-derivative from irgafos 168. It was corroborated by oxidizing the irgafos 168 standard with tetrahydrofuran for 1 day at 40ºC in an oven[42].

Finally, the compound 11-Eicosenamide, (11Z) (compound 35) was detected in sample PE12 and was confirmed by retention time and spectrum match. By its similar structure, it is reasonable to think that it may be derived of oleamide [12].

CCS evaluations: CCS values, shown in table 2, were calculated as averages of different replicates at different concentrations. Figure 3 shows the plot of these CCS experimental values versus molecular masses of each compound identified (from ~150 to 1180 m/z).

Our results demonstrated a linear correlation with a slope of ~ 0.23 and an R2 value of ~ 0.93. However, when the CCS values were grouped for N,N-Bis(2-hydroxyethyl)alkylamines or alkylamide, N,N'-1,2-ethanediylbis- families these R^2 values increased up to ~ 0.99.

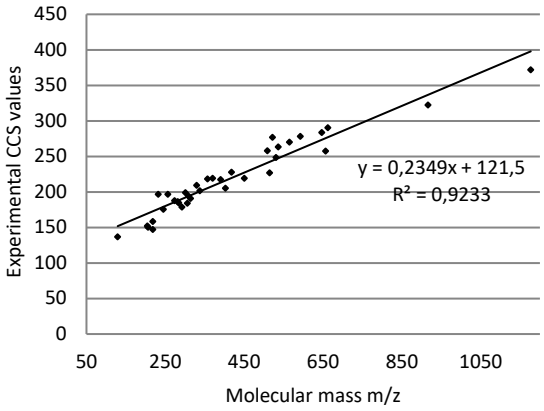

Figure 3.CCS experimental values vs molecular masses

Quantification of migrant compounds and risk assessment: Migrant concentrations were calculated for each simulant (Table 3). When the compound was not commercially available, it was quantified using a similar standard.

Limits of detection were between 7 and 50 µg/Kg for triacetine and Octadecanamide, N,N'-1,2-ethanediylbis-. The highest concentrations were found for the compounds irganox 1010 (PE5), erucamide (PE9), irgafos 168 (PE15) and phosphine oxide, tributyl- (PE15) in all cases in ethanol 95%.

The migration values were generally higher for the simulant ethanol 95% than for the rest of the simulants. It should also be emphasized that for ethanol 10% and acetic 3%, most of the migrant compounds were below their limits of detection, except for the group of N,N-Bis(2-hydroxyethyl) alkylamine in PE11, where the migration was above the quantification limit.

Sixteen out of thirty five compounds detected were included in the positive list of Regulation 10/2011/EU [4], some compounds were authorized without migration limits and others had specific migration limits, like acetyl butyl citrate, bis(2-ethylhexyl) adipate, bis(2-ethylhexyl) phthalate, irganox 1076 and N,N-Bis(2-hydroxyethyl) alkylamine but their values of migration were far below the corresponding SML (Table 3).

For the rest of the compounds, where no toxicity values existed, TTC and Cramer classification were applied. Six compounds were classified as class III of toxicity due to the presence of nitrogen in their structures, besides ethoxylate compounds and phosphine oxide, tributyl- by the presence of phosphorous. For this last compound, its migrant concentration was well above the maximum value recommended by Cramer for sample PE15 in the simulant ethanol 95%.

On the other hand, the PE15 and PE10 did not comply with the EU legislation either because the compounds Ethyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propanoate and Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, 1,1'-[2,2-bis(hydroxymethyl)-1,3-propanediyl] ester gave migration values of 0.89 ± 0.18 and 0.56 ± 0.06 mg/Kg (respectively), above 0.54 mg/Kg (Cramer for class II).

Finally, in sample PE2, heptadecanamide, N,N'-1,2-ethanediylbis concentration was above the recommended value of 0.09 mg/Kg for class III. Here, it is important to highlight that the compounds of the same family as hexa or octa-decanamide, N,N'-1,2-ethanediylbis were found in the Regulation [4] and they were authorized and without SML. However, this compound (with only a CH2 less in its alkyl chain) was not found in the legislation and applying Cramer, it must have a maximum limit of migration of 0.09 mg/Kg for class III. This fact is not conclusive, as compounds of the same family with similar structure should differ in their migration limits.

Conclusions: A non-volatile migrant compounds originating from migration of PE commercial samples has been carried out by UPLC-IMS QTOF, in order to identify most of the migrant compounds including NIAS. UNIFI software allows an automatic and integrated workflow to obtain in one step: elemental composition, structural database search and fragmentation assignment for each compound, reducing the amount of time required for review of this type of analysis. It has also provided the creation of a library and target list with CCS values, reducing the number of false positives during the quantification and increasing the confidence level of identification. In contrast, for the identification of some compounds a further search outside of this software was required, due to the fact that the Chemspider search did not provide results. Many NIAS are not contained in Chemspider, making the identification time consuming.

A total of thirty five compounds were identified working with high sensitivity. Eighteen identified compounds were additives like surfactants, plasticizers and lubricants like stearic acid, irganox 1010, bis(2-ethylhexyl) phthalate and erucamide being the most common compounds. 50% of identified compounds were NIAS coming from the rupture of these additives, such as for example, methyl and ethyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propanoate from irganox 1010 or 1076, or Hexa-heptadecanamide, N,N'-1,2-ethanediylbis from octadecanamide, N,N'-1,2-ethanediylbis, or also impurities, such as N,N-Bis(2-hydroxyethyl) amines, or compounds of unknown origin like phosphine oxide, tributyl-. This emphasizes the importance of analyzing the samples in depth and the methodology described in this article is a powerful way to obtain identification for the majority of migrant compounds.

For all compounds, the migration values were higher for the simulant ethanol 95%. This was expected, as most of the compounds are better soluble in ethanol than in acidic medium or aqueous simulants. Three samples (PE2, PE10 and PE15) were not suitable for food contact because of the migrant concentration of NIAS: Phosphine oxide, tributyl-, Ethyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propanoate, heptadecanamide, N,N'-1,2-ethanediylbis- and benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, 1,1'-[2,2-bis(hydroxymethyl)-1,3-propanediyl] ester were above the maximum values recommended by Cramer. This statement could change if exposure would be estimated. However, in this work the exposure factors have not been considered and only the migration values and the established limits either by the Regulation 10/2011/EU or by Cramer have been taken into account.

This work provides a general overview of compounds that can be present in PE plastics, not only the additives but also new NIAS. These results can be very helpful for food packaging manufacturers to know more about their products, what allows them to reformulate and avoid the presence of these NIAS, also referred to as “safety by design”.

AUTHOR INFORMATION

Corresponding Author

* E-mail address: cnerin@unizar.es Tel.: +34 976761873

Table 3: Migrant concentrations at simulants (et95%, Tenax, et10%, acetic 3% and et50%) expressed in mg/Kg of simulant, detection and quantification limits (expressed in µg/Kg) and specific migrantion limits or class Cramer classification (CC).

Migrant	LOD (µg/Kg) LOQ (µg/Kg)	PE	Migration to different simulants (mg/Kg)					SML or CC
			ET95%	Tenax	ET10%	AC3%	ET50%	
Triacetine	LOD=51/LOQ=170	PE14	<LOQ		<LOD	<LOD		Authorized
		PE16	<LOQ		<LOD	<LOD		
Ethanol,2-[2-(2-butoxyethoxy)ethoxy]	*	PE14	<LOQ		<LOD	<LOD		I
		PE16	<LOQ		<LOD	<LOD		I
Ethanol,2-(2-butoxyethoxy), acetate	LOD=7.7/LOQ=25	PE14	<LOQ		<LOD	<LOD		
		PE16	<LOQ		<LOD	<LOD		
Acetyl butyl citrate	LOD=25/LOQ=83	PE15	0.29±0.07		<LOD	<LOD		SML=60
Octylphenol ethoxylate	LOD=5.2/LOQ=17	PE6	<LOD	0.03±0.004	<LOD	<LOD		III
Dodecyl alcohol, ethoxylated	LOD=3.1/ LOQ=10.3	PE6	<LOD	0.03±0.003	<LOD	<LOD		III
Dibutyl sebacate	LOD=11/LOQ=37	PE15	0.75±0.15		<LOD	<LOD		I
Oleamide	LOD=33/LOQ=110	PE12	<LOQ		<LOD	<LOD		Autorized
Palmitic acid	LOD =30/LOQ=100	PE1					3.66±0.42	Autorized
		PE5	0.81±0.09	0.73±0.08	<LOD	<LOD		
Bis(2-ethylhexyl) adipate	LOD=9.9/LOQ=33	PE2		0.20±0.02				SML=18
Dinonyl phthalate	LOD=13/ LOQ=43	PE3		0.05±0.01				I
Stearic acid	LOD=30/LOQ=100	PE1					3.07±0.25	Autorized
		PE5	1.10±0.05	0.62±0.09	<LOD	<LOD		
Irganox 1010	LOD=16/ LOQ=153	PE5	3.28±0.40	0.03±0.005	<LOD	<LOD		Autorized
		PE9	<LOQ		<LOD	<LOD		
		PE10	0.21±0.03		<LOD	<LOD		
		PE12	0.77±0.08		<LOD	<LOD		
		PE16	0.11±0.03		<LOD	<LOD		
Bis(2-ethylhexyl) phthalate	LOD=22/ LOQ=73	PE7	0.09±0.01		<LOD	<LOD		SML=1.5
		PE10	0.08±0.01		<LOD	<LOD		
		PE14	<LOQ		<LOD	<LOD		
		PE16	<LOQ		<LOD	<LOD		
Erucamide	LOD=12/ LOQ=40	PE7	0.12±0.01		<LOD	<LOD		Autorized
		PE8	0.18±0.04		<LOD	<LOD		
		PE9	9.98±0.12		<LOD	<LOD		
		PE10	0.39±0.02		<LOD	<LOD		
		PE11	0.13±0.03		<LOD	<LOD		
		PE12	1.15±0.11		<LOD	<LOD		
		PE13	0.05±0.02		<LOD	<LOD		
		PE16	0.08±0.03		<LOD	<LOD		
		PE17	0.81±0.06					
		PE18	1.63±0.07					
Irganox 1076	LOD=11/ LOQ=37	PE17	0.42±0.03					SML=6
		PE18	1.21±0.05					
Octadec, N,N'-1,2-ethanediylbis-	LOD=50/LOQ=166	PE2		0.49±0.08				Autorized
Irgafos 168	LOD=5.5/ LOQ=18	PE5	1.73±0.21	0.22±0.02	<LOD	<LOD		Autorized
		PE12	0.77±0.08		<LOD	<LOD		
		PE15	3.20±0.55		<LOD	<LOD		
Dibutyl amine	LOD=9.8/ LOQ=32	PE6	<LOQ	<LOD	<LOD	<LOD		III
		PE7	<LOQ		<LOD	<LOD		
		PE10	0.06±0.02		<LOD	<LOD		
N,N-Bis(2-hydroxyethyl)decylamine	**	PE11	<LOQ		<LOQ	<LOQ		Limit group [N,N-Bis(2-hydroxyethyl) alquil (C8-C18) amine] 1.2
N,N-Bis(2-hydroxyethyl)dodecylamine	LOD=3.3/LOQ=11	PE11	0.17±0.02		0.15±0.02	0.19±0.01		
		PE8	<LOQ		<LOD	<LOD		
		PE13	<LOQ		<LOD	<LOD		
N,N-Bis(2-hydroxyethyl) tetradecylamine	**	PE11	0.12±0.02		0.04±0.01	0.11±0.01		
N,N-Bis(2-hydroxyethyl) hexadecylamine	**	PE11	0.07±0.01		<LOQ	0.02±0.005		
N,N-Bis(2-hydroxyethyl) octadecylamine	**	PE11	0.02±0.003		<LOD	<LOD		
Phosphine oxide, tributyl-	LOD=12/LOQ=40	PE15	2.33±0.30		<LOD	<LOD		III
Methyl 3-(3,5-di-tert-butyl-4-hydroxy-phenyl) propanoate	LOD=7.8/LOQ=40	PE14	0.05±0.01		<LOD	<LOD		II
		PE15	0.19±0.01		<LOD	<LOD		
Ethyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propanoate	LOD=7.8/LOQ=40	PE1					).03±0.005	II
		PE14	<LOQ		<LOD	<LOD		
		PE15	0.89±0.18		<LOD	<LOD		
Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-,1,1'-[2,2-bis(hydroxymethyl)-1,3-propanediyl]ester	***	PE10	0.56±0.06		<LOD	<LOD		II
		PE12	0.19±0.04		<LOD	<LOD		
		PE16	0.16±0.03		<LOD	<LOD		
Benzenepropanoicacid3,5-bis(1,1dimethylethyl)-4-hydroxy-,1,1'-[2-[[3-[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]-oxopropoxy]methyl]-2-(hydroxymethyl)1,3ester	***	PE10	<LOQ		<LOD	<LOD		II
		PE12	<LOD		<LOD	<LOD		
		PE16	<LOD		<LOD	<LOD		
Tridodecylamine	LOD=9.7/LOQ=32	PE13	<LOD		<LOD	<LOD		III
Pentadecanamide, N,N'-1,2-ethanediylbis-	****	PE2		<LOQ				III
Hexadecanamide, N,N'-1,2-ethanediylbis-	****	PE2		0.10±0.03				Autorized

Heptadecanamid, N,N'-1,2-ethanediylbis-	****	PE2		0.21±0.08			III
Irgafos 168 OXO	LOD=15/ LOQ=50	PE5	0.09±0.01	0.07±0.01	<LOD	<LOD	
		PE12	0.10±0.01		<LOD	<LOD	Autorized
		PE15	1.08±0.18		<LOD	<LOD	
11-Eicosenamide, (11*Z*)-	LOD=7.8/LOQ=40	PE12	0.05±0.01		<LOD	<LOD	Autorized

Quantified with *Ethanol2-(2-butoxyethoxy),acetate,**N,N-Bis(2-hydroxyethyl)dodecylamine,***Irganox1010,***Octadecanamide,N,N'-1,2-ethanediylbis

ACKNOWLEDGEMENTS

The authors acknowledge the financial help given by Gobierno de Aragón and European Social Funds to GUIA T53_17R and the Project FOODYPLAST "Ecofriendly and healthy Food plastic packaging" EFA099/15 (POCTEFA 2014-2020). Thank for Waters Corporation for providing VION IMS QTOF instrumentation.

REFERENCES

(1) Bhunia, K.; Sablani, S. S.; Tang, J.; Rasco, B. *Comprehensive Reviews in Food Science and Food Safety* **2013**, *12*, 523-545.

(2) Canellas, E.; Vera, P.; Nerin, C. *Food Chemistry* **2016**, *197*, 24-29.

(3) Nerin, C.; Gaspar, J.; Vera, P.; Canellas, E.; Aznar, M.; Mercea, P. *International Journal of Adhesion and Adhesives* **2013**, *40*, 56-63.

(4) *COMMISSION REGULATION (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food*.

(5) Boragno, L.; Stagnaro, P.; Losio, S.; Sacchi, M. C.; Menichetti, S.; Viglianisi, C.; Piergiovanni, L.; Limbo, S. *Journal of Applied Polymer Science* **2012**, *124*, 3912-3920.

(6) Jakubowska, N.; Beld, G.; Bach, A. P.; Simoneau, C. *Food Additives and Contaminants Part a-Chemistry Analysis Control Exposure & Risk Assessment* **2014**, *31*, 546-555.

(7) Silva, A. S.; Freire, J. M. C.; Franz, R.; Losada, P. P. *Food Research International* **2008**, *41*, 138-144.

(8) Biedermann-Brem, S.; Kasprick, N.; Simat, T.; Grob, K. *Food Additives and Contaminants Part a-Chemistry Analysis Control Exposure & Risk Assessment* **2012**, *29*, 449-460.

(9) Coulier, L.; Orbons, H. G. M.; Rijk, R. *Polymer Degradation and Stability* **2007**, *92*, 2016-2025.

(10) Bodai, Z.; Kirchkeszner, C.; Novak, M.; Nyiri, Z.; Kovacs, J.; Magyar, N.; Ivan, B.; Rikker, T.; Eke, Z. *Food Additives and Contaminants Part a-Chemistry Analysis Control Exposure & Risk Assessment* **2015**, *32*, 1358-1366.

(11) Guart, A.; Bono-Blay, F.; Borrell, A.; Lacorte, S. *Food Additives and Contaminants Part a-Chemistry Analysis Control Exposure & Risk Assessment* **2011**, *28*, 676-685.

(12) Silano, V.; Bolognesi, C.; Cravedi, J. P.; Engel, K. H.; Fowler, P.; Franz, R.; Grob, K.; Gurtler, R.; Husoy, T.; Karenlampi, S.; Mennes, W.; Milana, M. R.; Penninks, A.; Smith, A.; Pocas, M. D. T.; Tlustos, C.; Wolfle, D.; Zorn, H.; Zugravu, C. A.; Kolf-Clauw, M., et al. *Efsa Journal* **2017**, *15*.

(13) Hamlin, H. J.; Marciano, K.; Downs, C. A. *Chemosphere* **2015**, *139*, 223-228.

(14) Mauricio-Iglesias, M.; Guillard, V.; Gontard, N.; Peyron, S. *Food Additives and Contaminants Part a-Chemistry Analysis Control Exposure & Risk Assessment* **2009**, *26*, 1515-1523.

(15) Martinez-Lopez, B.; Peyron, S.; Gontard, N.; Mauricio-Iglesias, M. *Industrial & Engineering Chemistry Research* **2015**, *54*, 4725-4736.

(16) Ozaki, A.; Kishi, E.; Ooshima, T.; Hase, A.; Kawamura, Y. *Food Additives and Contaminants Part a-Chemistry Analysis Control Exposure & Risk Assessment* **2016**, *33*, 1490-1498.

(17) Banadda, N.; Lule, F.; Sempala, C.; Kigozi, J. *International Journal of Agricultural and Biological Engineering* **2016**, *9*, 194-200.

(18) Kiyataka, P. H. M.; Dantas, S. T.; Pallone, J. A. L. *Food Analytical Methods* **2015**, *8*, 2331-2338.

(19) Hannon, J. C.; Kerry, J. P.; Cruz-Romero, M.; Azlin-Hasim, S.; Morris, M.; Cummins, E. *Food Additives and Contaminants Part a-Chemistry Analysis Control Exposure & Risk Assessment* **2016**, *33*, 167-178.

(20) Echegoyen, Y.; Rodriguez, S.; Nerin, C. *Food Additives and Contaminants Part a-Chemistry Analysis Control Exposure & Risk Assessment* **2016**, *33*, 530-539.

(21) Pocas, M. F.; Oliveira, J. C.; Oliveira, F. A. R.; Hogg, T. *Critical Reviews in Food Science and Nutrition* **2008**, *48*, 913-928.

(22) Palkopoulou, S.; Joly, C.; Feigenbaum, A.; Papaspyrides, C. D.; Dole, P. *Trends in Food Science & Technology* **2016**, *49*, 110-120.

(23) Leeman, W.; Krul, L. *Current Opinion in Food Science* **2015**, *6*, 33-37.

(24) Canellas, E.; Vera, P.; Nerin, C. *Journal of Mass Spectrometry* **2014**, *49*, 1181-1190.

(25) Canellas, E.; Vera, P.; Nerin, C. *Analytical and Bioanalytical Chemistry* **2015**, *407*, 6781-6790.

(26) Canellas, E.; Vera, P.; Nerin, C. *Food and Chemical Toxicology* **2015**, *75*, 79-87.

(27) Canellas, E.; Vera, P.; Nerin, C. *Food Additives and Contaminants Part a-Chemistry Analysis Control Exposure & Risk Assessment* **2017**, *34*, 1721-1729.

(28) Dutra, C.; Freire, M. T. D.; Nerin, C.; Bentayeb, K.; Rodriguez-Lafuente, A.; Aznar, M.; Reyes, F. G. R. *Journal of the Brazilian Chemical Society* **2014**, *25*, 686-696.

(29) Kontominas, M. G.; Goulas, A. E.; Badeka, A. V.; Nerantzaki, A. *Food Additives and Contaminants Part a-Chemistry Analysis Control Exposure & Risk Assessment* **2006**, *23*, 634-641.

(30) Welle, F. *Food Additives and Contaminants* **2005**, *22*, 999-1011.

(31) Lapthorn, C.; Pullen, F. S.; Chowdhry, B. Z.; Wright, P.; Perkins, G. L.; Heredia, Y. *Analyst* **2015**, *14*, 6814-6823.

(32) *UNE-EN 14338:2004. Papel y cartón para contacto alimentario. Condiciones para la determinación de la migración en papel y cartón utilizando óxido de polifenileno modificado (MPPO) como simulante*.

(33) *Threshold of toxicological concern (TTC). ILSI Europe concise monograph series (2005)*

(34) Coltro, L.; Machado, M. P. *Polimeros-Ciencia E Tecnologia* **2011**, *21*, 390-397.

(35) Gillet, G.; Vitrac, O.; Desobry, S. *Journal of Applied Polymer Science* **2011**, *119*, 1492-1515.

(36) Zhu, Y. K.; Free, M. L.; Woollam, R.; Durnie, W. *Progress in Materials Science* **2017**, *90*, 159-223.

(37) Thurman, E. M.; Ferrer, I.; Rosenblum, J.; Linden, K.; Ryan, J. N. *Journal of Hazardous Materials* **2017**, *323*, 11-17.

(38) Lide, D. R. *Handbook of Chemistry and Physics* CRC Press, 1998, p 3–160.

(39) Lahimer, M.; Ayed, N.; Horriche, J.; Belgaied, S. *Arabian Journal of chemistry* **2013**.

(40) Bradley, E.; Coulier, L. *FD07/01 report: An investigation into the reaction and breakdown products from starting substances used to produce food contact plastics* **2007**.

(41) Onghena, M.; van Hoeck, E.; Vervliet, P.; Scippo, M. L.; Simon, C.; van Loco, J.; Covaci, A. *Food Additives and Contaminants Part a-Chemistry Analysis Control Exposure & Risk Assessment* **2014**, *31*, 2090-2102.

(42) Vera, P.; Canellas, E.; Nerin, C. *Talanta* **2018**, *188*, 750-762.

(43) Fernandes, F. C.; Gadioli, R.; Yassitepe, E.; De Paoli, M. A. *Polymer Composites* **2017**, *38*, 299-308.