The use of ion mobility time-of-flight mass spectrometry to assess the migration of polyamide 6 and polyamide 66 oligomers from kitchenware utensils to food

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ABSTRACT

Oligomers, are, in general, unknown components of the polymer. These oligomers can migrate from the polymer into the food and become a non-intentionally added substance to the food. In this work, ion mobility time-of-flight mass spectrometry has been used to identify oligomers migrating from kitchenware. The structure elucidation of oligomers from polyamide 6 and polyamide 66 was achieved through the analysis of accurate *m*/*z* values of adducts and collision cross section values of precursor ions together with high-energy fragmentation patterns. Additionally, a method to extract oligomers from sunflower oil, cooked beans, soup and whole milk has been developed. Extraction recoveries ranged from 87 to 102% and limits of detection were from 0.03 to 0.11 mg/kg. It was observed that the migration from kitchenware to real food was below the specified migration limit of 5 mg/kg. However, this limit was exceeded for food simulants, which therefore overestimated the oligomer migration.

Keywords: Polyamide kitchenware Oligomers migration UPLC-IM-Q/TOF Migration to foodstuff

1. Introduction

A food contact material is any material or article that is intended to come into contact with food as defined in regulation 1935/2004 (Commision, 2011). The safety of food contact materials must be evaluated since compounds can migrate from the materials into food. The materials must be manufactured in compliance with European regulations so that any potential transfer to foods does not raise safety concerns, change the composition of the food in an unacceptable way or have adverse effects on the taste or odour of food (EU, 2004). Kitchenware has been broadly studied in the context of intentionally added substances proven to migrate to food or food simulants (Gelbke, Buist, Eisert, Leibold, & Sherman, 2019; Ibarra, de Quiros, & Sendon, 2016; Shiozawa, Haneishi, Suzuki, Ogimoto, Takanashi, Tomioka, et al., 2017). According to the European Commission though, the migration of non-intentionally added substances (NIAS) should also be investigated (Commision, 2011). The study of NIAS is complex due to the fact that they can occur as impurities, reaction products, breakdown products, degradation products or oligomers (Nerin, Alfaro, Aznar, & Domeno, 2013). As such, accurate mass spectrometry-based techniques are required to detect and identify potential NIAS, especially in the case of oligomers, (Canellas, Vera, & Nerin, 2014; Hoppe, Pim, & Roland, 2018; Kappenstein, Ebner, Forster, Richter, Weyer, Pfaff, et al., 2018; Maria,

Pim, & Roland, 2018; Rusko, Perkons, Rasinger, & Bartkevics, 2020; Tsochatzis, Lopes, Kappenstein, Tietz, & Hoekstra, 2020; Ubeda, Aznar, & Nerin, 2018; Wrona & Nerin, 2020). However, even using such high-resolution techniques, it is not always possible to identify every NIAS present in a sample due to the complexity of the mass spectral data and the interpretation thereof. To address this, ion-mobility quadrupole time-of-flight mass spectrometry, coupled to the ultra-high performance liquid chromatography (UPLC-IM-Q/TOF) has been used for this work. This technique produces cleaner, less complex spectra, since the alignment of precursors and fragment ions is based on both retention time and drift time. Moreover, in addition to accurate *m*/*z* values of precursor and fragmentation ions, ion-mobility mass spectrometry enables the determination of a Collision Cross Section (CCS) value, which reflects the size and shapes of each molecule. Therefore, the use of this technique can provide more information than traditional accurate mass spectrometry such as traditional time-of-flight or Orbitrap techniques. UPLC-IM-Q/TOF analysis has previously been used to successfully identify NIAS migrating from several different food contact materials (Canellas, Vera, & Nerin, 2019; Canellas, Vera, Nerin, Dreolin, & Goshawk, 2020; Vera, Canellas, Barknowitz, Goshawk, & Nerin, 2019). Once the NIAS have been identified, a risk assessment must be performed to ensure that there is no risk to human health due to their migration into food. Food simulants are used as substitutes for food due to the simplification of the chemical analysis they provide. Food simulants vary in terms of their chemical properties in order to simulate different food types,for example hydrophilic, lipophilic or amphiphillic. In general, the use of food simulants provides a good approximation to the actual migration into foods. However, for some compounds, the migration to simulants can overestimate or underestimate the extent of migration to food due to the fact that temperatures, time and the matrix affect the partition and diffusion coefficients responsible for migration mechanism (Elizalde, Aparicio, & Rincon, 2020; Martinez-Lopez, Gontard, & Peyron, 2018; Starker & Welle, 2019). Although migration studies from food contact material to foodstuff require further analytical developments, they can still be used to confirm the compliance of a product for intended use, and determine the suitability of a given simulant for a migrant or a group of migrants. In the present study, identification and quantification of oligomers migrating from kitchenware is studied using ion mobility-quadrupole time of flight mass spectrometry coupled to the ultra-high pressure liquid chromatography. The individual oligomers were isolated and purified to be used as pure standards to confirm the identification and to quantify the migration. Migration to food simulants and to several foods has been studied, and the risk associated with the migration to both food simulants and foodstuffs has been assessed.

2. Materials and methods

2.1. Reagents

Ethanol and methanol of high performance liquid chromatography (HPLC) grade were supplied by Scharlau Chemie S.A (Sentmenat, Spain). Glacial acetic acid was supplied by Scharlau Chemie S.A (Sentmenat, Spain). Caprolactam was purchased from Sigma-Aldrich Química S.A (Madrid, Spain). Oligomers from polyamide 6 (PA6) and polyamide 66 (PA 66) were isolated from PA6 pellets and PA 66 pellets, respectively, which were purchased from Sigma-Aldrich Química S.A (Madrid, Spain). 6 g and 200 mg OASIS HBL cartridges were supplied by Waters (Spain).

2.2. Oligomer isolation

The oligomers were isolated following the method developed by Abe et al. (Abe, Mutsuga, Ohno, Kawamura, & Akiyama, 2016) with some minor modifications. 10 g of pure PA 6 and PA66 were extracted with methanol (50 mL) at 40 ◦C overnight, and the residue was subsequently removed by filtration. The filtrates and washes were evaporated using a rotary evaporator and the residue was dissolved in 10% methanol (v/v). The solution was passed through 6 g OASIS HLB solid phase extraction cartridges. 50 mL of 10, 20, 30, 40, and 50% methanol (v/v) were then passed through the cartridge and each collected separately. The monomers and oligomers in each fraction were determined using ultra high performance liquid chromatography and triple quadrupole mass spectrometry (UPLC-TQ-MS) and the solvent was evaporated. The residue remaining from each fraction was re-dissolved in ethanol 10% and passed through a 200 mg OASIS HLB cartridge. Subsequently, 50 mL of 10, 20, 30, 40, and 50% methanol (v/v) was passed through a 200 mg OASIS HLB cartridges and 10 mL of each sample was collected. Isolated monomers and oligomers in each fraction were determined by UPLC-TQ-MS. These steps were repeated for each fraction until pure PA cyclic monomers and oligomers were obtained. The concentration of each oligomer was then calculated using a gravimetric method. To separate caprolactam and the PA6 dimer, caprolactam was hydrolyzed following the method developed by Abe. et al (Abe, Mutsuga, Ohno, Kawamura, & Akiyama, 2016).

2.3. Migration samples

Four kitchenware utensils, each belonging to a different brand, were purchased from a local market; two spatulas, which were labelled as spatula 1 and spatula 2 and two ladles, labelled as ladle 1 and ladle 2 were tested.

2.4. Food used for migration studies

Cans of beans, “Fabada Litoral”, (8.3% fat content), chicken soup, “Caldo casero de pollo Gallina Blanca”, (0.3% w/w of fat content), whole milk, “Leche Pascual”, (3% w/w of fat content) and sunflower oil, “Koipesol”, (100% w/w of fat content) were purchased from a local market.

2.5. Oligomer extraction from food matrices

Two methods were followed for the extraction of oligomers from sunflower oil and food. The first method was a slight variant of that developed by Heimrich 2015 (Heimrich, Nickl, Bonsch, & Simat, 2015). The method consisted of taking an aliquot of 50 g of homogenized food, transferring it into a separation funnel and adding 50 mL of hexane to dissolve the fat content. Cyclic oligomers were extracted three times using 25 mL acetonitrile/water 1:1 (v/v). The combined acetonitrile fractions were washed with another fraction of 50 mL hexane and, together with the extract, evaporated in a rotary evaporator and subsequently re-dissolved in 50 mL of 10% methanol (v/v). This solution was then passed through 200 mg OASIS HLB solid phase extraction (SPE) cartridges which had been activated with methanol and water. 30 mL of methanol was required to ensure the elution of all oligomers. Recovery was determined by spiking the oil and food with the oligomers previously isolated. For the second procedure, an aliquot of 5 g of homogenized food was collected in a separation funnel. 50 mL of hexane was added to

dissolve the fat and subsequently 50 mL of methanol was added. The resulting solution was shaken until it separated into two phases. The methanol phase was collected, evaporated in a rotary evaporator and then dissolved in 50 mL of 10% methanol (v/v). The solution was passed through the 200 mg OASIS HLB SPE cartridges that had previously been activated with methanol and water. 30 mL of methanol was required to ensure the elution of all oligomers. Recovery was determined by spiking the oil and the food with the mixture of oligomers previously isolated. Comparing the results from the two extraction methods, the second method was selected for the extraction of oligomers from beans, chicken soup and whole milk samples.

2.6. Migration assays

Migration assays were performed following the guidelines set out in the BfR document (036/2019/BfR, 2019). In the document the migration value is determined to be 5 mg/kg of food/day (group migration value). This is based on the available toxicological data for the group of PA 6 oligomers with n = 2 to 8 (dimer to octamer) and PA 6,6 oligomers with n = 1 to 4 (monomer to tetramer). . Based on the finding in the BfR report, the 5 mg/kg value is considered as the SML. The test conditions used were 30 min or 2 h at 100 ◦C which agree with those published by Jakubowska, Beldi, Robouch, and Hoekstra (2020). Therefore, to compare the migration results presented here with the SML established in the BfR report, the samples were analysed as follows. The samples were dispensed into DURAN® glass beakers together with 1L of simulant solution at a temperature of 100 ◦C for 0.5 h. An area of 12 dm2 of the spatulas and 9 dm2 of ladles were immersed in the simulants. The same sample size was used for the migration assays of beans, soup, milk and sunflower oil. The temperature was monitored in water and food experiments using two thermometers inside the Duran beaker. In the analysis of other simulants, the solution was preheated to the temperature used in the test and dispensed into beaker with the sample inside a preheated oven. A laboratory oven with an internal thermometer and accurate temperature control was used. When using simulants containing ethanol, evaporation occurs during the migration test and if the test is performed in a glass beaker. Therefore, for the simulants containing ethanol 10%, ethanol 50% and ethanol 95% (v/v), the samples were cut into 1 × 5 cm pieces and placed into pressure resistant tubes filled with 41.6 g of simulant, thereby ensuring the same ratio of spatula to simulant (area/w) to that in the beakers. Preheated ethanol 10% (v/v) was placed into the pressure resistant tubes, the spatula pieces were introduced and the tube was closed and placed in the oven at a temperature of 100 ◦C for 0.5 h. The same procedure was followed in ethanol 50% and ethanol 95% (v/v) with the exception that the oven temperature was held at 60 ◦C for 2.5 h. Three replicates of each test were carried out and blanks were also prepared for both the simulants and the food.

2.7. Ultra-high-pressure-liquid chromatography coupled to an ion mobility-quadrupole time of flight analyzer (UPLC-IM-Q/TOF)

Screening analyses were carried out using an AcquityTM UPLC chromatography system coupled to an electrospray interface (ESI) and VION® ion mobility-quadrupole time of flight (IMS/Q/TOF) mass spectrometer, from Waters (Manchester, UK). A 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 phases were water (phase A)

and methanol (phase B), both with 0.1% formic acid. The gradient used was 95% A to 100% B after 13 min, with 2 min of re-equilibration to initial conditions. The volume of sample injected was 5 μL. The electrospray interface (ESI) was used in positive ionization, sensitivity mode with a capillary voltage of 3 kV and a sampling cone of 30 V. The temperatures used were 120 ◦C and 500 ◦C for source block and desolvation gas, respectively, and the desolvation gas flow rate was 800 L h□ 1. The system was calibrated and data were acquired in the range 50–1000 *m*/*z*. Leucine-Enkephalin [M + H]+, *m*/*z* 556.2765, was used as the lock-mass compound for real time mass correction. A collision energy ramp of 20 to 40 V was applied with argon used as the collision gas. Nitrogen was used as the mobility gas. The acquisition was set to high definition mass spectrometry (HDMSE) mode, with a 0.1 s scan time. The ion mobility resolving power was ~ 20 Ω/ΔΩ FWHM. Data acquisition and processing were carried out using UNIFI v.1.8 software.

2.8. Ultra-high-pressure-liquid chromatography coupled to tandem quadrupole mass spectrometer (UPLC-TQ-MS)

Quantitation analyses were carried out using a AcquityTM UPLC chromatography system coupled to an electrospray interface (ESI) and TQ detector, supplied by Waters (Manchester, UK). Same UPLC method/ conditions were used as in 2.7. The electrospray interface (ESI) was used in positive ionization mode with a capillary voltage of 3 kV and an optimized sampling cone voltage for each single compound (table 1). The temperatures used were 120 ◦C and 450 ◦C for source and desolvation gas, respectively, and the desolvation gas flow was 600 L h□ 1. Selected Ion Recording (SIR) mode was used for monitoring the precursor ions of the target analytes. MassLynx v.4.1 software was used for data acquisition and processing.

3. Results and discussion

3.1. Identification of migrants

The kitchenware samples were purchased in a supermarket, therefore the additives used in the production of the polymer of which the kitchenware was composed were unknown. UPLC-IM-Q/TOF was selected to identify the compounds migrating from the kitchenware to the food simulants. In addition to mass accuracy and fragment ion information, this technique also provides separation by means of a traveling wave ion mobility cell (TWIMS). The ions, which are subjected to a constant electric field while traveling through a buffer gas, are separated based on their shape and size. The time ions take to traverse the drift cell is called the ion-mobility “drift time”. By applying a calibration, a collision cross section (CCS) value can be derived from the drift time of each compound. The CCS value is related to the three-dimensional conformation of the of the chemical structure compound. Since, HDMSE was used for this analysis, cleaner spectra were obtained as the alignment of precursors and fragment ions is based on both retention time and drift time. The spectra are drift time-aligned, fragments can be distinguished from background ions and can be easily assigned to the precursor ion, thus bringing additional confidence in the identification of unknowns. The workflow for the identification of the compounds begins with molecular ion of the unknown compound in the measured low energy spectrum. The elements considered for the derivation of the elemental formula were: carbon, oxygen, hydrogen, nitrogen, chlorine, bromine,

fluorine, sulfur, phosphorous, silicon and sodium. Since they are the most common elements in the molecular formulae of plastic additives. Sodium was included specifically because the compounds have a high tendency to form sodium adducts with the mobile phase. Two criteria were used to establish the elemental formula for an unknown: (1) the i- Fit, which is a measure of the goodness-of-fit of the theoretical isotope pattern of a particular elemental composition to the peaks in the measured spectrum, and (2) the mass tolerance, which was set at 3 mDa. Once the molecular formulae of each accurate mass had been determined, it was necessary to use a database of chemical compounds (ChemSpider (RSC, 2020)) was searched to obtain a list of candidate compounds for the unknowns. An *in-silico* fragmentation algorithm was applied to the structure of each candidate compound to produce theoretical fragments. The mass of each theoretical fragment was then compared to masses of the ions in the measured high-energy spectrum for the corresponding unknown compound. Therefore, assignment of a compound to an unknown migrant was made on the basis of the accurate mass of the precursor and fragment ions and additionally on the accuracy of the measured isotope pattern as compared to the theoretical isotope pattern. In all cases, the mass error between measured and theoretical values for the compounds identified by UPLC-IM-Q/TOF was<3 mDa. This workflow was applied to identify migrants from spatula 1 to the food simulants used in the migration studies. Table 1 shows the compounds identified, their retention times, CCS values and measured *m*/*z* values, together with the theoretical *m*/*z* and elemental composition of the proposed candidate molecule. Fig. 1 and Fig. 2 show the fragment assignment for the candidates proposed for the accurate mass *m*/*z* 453.344 and an elemental composition of C24H45N4O4. In the absence of fragmentation data, it is not possible to distinguish between the candidate compounds since they have the same elemental composition and similar CCS values (table 1). CCS has been useful to find out that not only the *m*/*z* of the pairs candidates was equal but also to know that the shape was equal too. Therefore, the molecules must be similar in shape and it could lead to discard other candidates in the identification process. CCS is a parameter related with the molecular shape and size, therefore it can be complementary to accurate mass and fragmentation patterns. The candidate compounds are PA 6 tetramer and the PA 66 dimer. The fragments measured in the high energy function for each compound are clearly different, though, and thereby enables the identification through the agreement between the experimental fragments and the predicted fragments for each candidate compound. The measured fragments 79.054, 114.091, 226.191 *m*/*z* correspond to theoretical fragments of the PA 6 tetramer molecule and the measured fragments 100.112, 182.154 and 209.165 *m*/*z* correspond to the theoretical fragments of the PA 66 dimer molecule. The accurate mass fragment assignment was performed for all compounds observed in the sample, leading to the identification of the oligomers shown in Table 1. Some fragments were found on several PA 6 and PA 66 oligomers since part of their molecular structure is common in all of them. Table 1 shows, that two candidate compounds, with the same elemental composition, were found for three of the unknown migrants. As such a definitive assignment could not be made for these migrants based on the *m*/*z* of the precursor ion alone. In all cases though, a definitive assignment was made by comparing the theoretical fragmentation patterns for each candidate compound with ions in the high energy data. The ability to differentiate the compounds using their fragmentation pattern was the key to the structural elucidation in all cases and was simplified by having cleaner high energy spectra afforded by ion mobility. Moreover, in

order to confirm the identification, the self-generated standards (2.2. section) were injected. Retention time and mass fragmentation of the standards match with the substances found in the migration solutions. All unknown migrants were identified as oligomers of PA 6 and PA 66 and therefore, the material of which the spatula is made is a mixture of PA6 and PA 66. Since, oligomers are produced as by-products of the polymerization processes of PA and remain in the PA-based materials, they can be considered as NIAS. According to Article 19 of Regulation (EU) No 10/2011 (Commision, 2011) no migration limits for these compounds have been established. Nevertheless, a specific migration limit (SML) of 15 mg/kg in food is specified for the PA 6 monomer (caprolactam). Additionally, the BfR document on PA oligomers (BfR, 2019) states that a group migration value of<5 mg/kg in food is considered toxicologically acceptable. Therefore, migration studies were carried out to establish whether the migration from these kitchenware utensils exceeds this limit.

3.2. Oligomer extraction from food matrices

Upon identifying the migrants from the spatula, a quantitation method was developed to determine the extent of the migration. Commercial standards of the oligomers are not available and therefore they must be isolated from the polymer. The purity of each isolated monomer and oligomer was determined from their mass chromatogram peak area on the total ion chromatograms (TIC) obtained by UPLC-IM-Q/TOF (SCAN) mode ranging from *m*/*z* 50–1,200. The peak purities were calculated by dividing the peak area of each isolated monomer or oligomer by the total peak area on the TIC. Purities were determined as follows; PA 6 monomer 95%, PA 6 dimer 90%, PA 6 trimer 82%, PA 6 tetramer 87%, PA 6 heptamer 84%, P 6 hexamer 86%, PA 66 monomer 91%, PA 66 dimer 83% and PA 66 trimer 82%. The purity of each standard was considered in all calculations. Once the oligomers were obtained, they were used to confirm the identity of the migrants by their analysis by UPLC-IM-Q/TOF. They were used for identification confirmation and quantification purposes. Moreover, CCS values were determined with the standard solutions of theses pure oligomers obtained. CCS values were constant in the range ± 5%. It is remarkable, that a library of compounds for UPLC-MS-IMS/ Q-TOF was being built by the injection of standards of these NIAS and collection of their CCS data. The migrants found in this study have been included and will be used to facilitate future screening analysis. CCS values were determined with the standard solutions of the pure oligomers previously obtained and described in this paper. CCS values were constant in the range ± 5%. A UPLC-TQ-MS method was developed to perform the quantitation of the oligomers. Each single oligomer was infused directly through the ESI the at a concentration of 10 mg/kg in order to determine the capillary voltage, desolvation nitrogen flow and cone voltages optimal for that oligomer. An efficient separation of all the PA oligomers was achieved in a single run using the UPLC BEH C18 column of 1.7 μm particle size column. Table 1 shows the optimum cone voltage obtained for each compound. Calibration curves were generated using the isolated oligomers and a commercial standard of caprolactam. Regression coefficients were at least 0.999 for all of the calibration curves. Limits of detection were calculated as follows: (LOD) = 3SD of blank/slope of calibrators, and are shown in Table 2. Similar LOD were obtained for the molecules with the same mass but different structure. Moreover, the method was less sensitive for the larger oligomers than for the smaller ones. Good recoveries were found for sunflower oil ranging from 78 to 102%, chicken

soup ranging from 83 to 106% and for milk ranging from 88 to 100%. The recoveries found for the extraction from the beans were lower due to the fact that it is the most complex matrix; a heterogeneous mixture of solid and liquid content, with beans and sauce (73 to 95%). The RSD % for the food matrices ranged from 1.6% to 15% depending on the food matrix and the oligomer. The worst RSD % was found for beans ranging (5.5 to 15%). This again could be due to the fact that this is the most complex matrix of those studied. Although, PA oligomer analytical methods with high sensitivity have been presented recently (Kappenstein, et al., 2018; Song, Chang, Lyu, Yon, Lee, Park, et al., 2018), no methods for the extraction of the oligomers from very complex food matrices have been developed. In this work a methodology for the extraction of the oligomers migrating from kitchenware has been optimized. A mixture of the standards at a concentration of 1 mg/L was spiked into the sunflower oil in order to calculate recoveries from both methods previously described. Recoveries were calculated as follows: Recovery (%) = (Concentration found on the sunflower oil/ concentration spiked) *100%. Two methods were investigated for the extraction of the compounds from the food matrices. After the samples had been extracted from the food SPE was applied, using SPE cartridges as that for the isolation of the oligomers, to clean the matrix for the extraction and concentration of the oligomers. The methanol phase resulting from the extraction was evaporated in a rotary evaporator and dissolved in 50 mL of 10% methanol (v/v). Ten fractions, each of 5 mL, of the methanol solution were eluted through the SPE cartridge. Each fraction was collected and analyzed by UPLC-TQ/MS in order to determine the quantity of methanol required to elute the oligomers. Six fractions of 5 mL (30 mL) were needed to completely elute the oligomers. For the first method, described in section 2.5, analysis by UPLC-TQ-MS revealed recoveries ranging from 78 to 103% for sunflower oil. The table 1 also indicates recoveries ranging from 78 to 102% for the second extraction methodology which is also described in section 2.5. The latter extraction method was deemed to be the most desirable due to the fact that the extraction process is cleaner and only one extraction is required. Therefore, it was more efficient than the first extraction method for this class of compounds. Subsequently, the food products selected for the study were spiked, extracted, analyzed by UPLC-TQ-MS and recoveries were obtained. Recoveries ranged from 78 to 98% for beans, 83–106% for chicken soup and 88–100% for whole milk (Table 2). Employing a two steps sample preparation, with a liquid–liquid extraction followed by a SPE extraction, good recoveries were obtained. The limits of detection obtained using this technique ranged from 0.031 to 0.110 mg/kg. This was considered to be sufficiently sensitive since the SML of the sum of the oligomers is 5 mg/kg.

3.3. Migration study

Table 2 shows the migration results from spatula 1 to each of the simulants and food products studied. Results for the migration to the food simulants show higher values were obtained for ethanol 95%, with the sum of oligomers (without caprolactam) close to 16 mg/kg. The main contributor to the total migration of the oligomers was PA 66 monomer in ethanol 95% (migration concentration of 12 ± 1 mg/kg). It was also observed that the migration of low molecular weight PA 66 oligomers was higher than that for heavier PA 66 oligomers. Similar findings were obtained by Kappenstein et al. (Kappenstein, et al., 2018) and Soto-Valdez et al. (Soto-Valdez, 1997). Ethanol 95% is the food simulant usually used as substitute for the fat in sunflower oil. Therefore, the migration values

obtained for ethanol 95% were compared to those for sunflower oil. Fig. 3 shows the UPLC-TQ-MS base peak ion (BPI) chromatograms which combines the nine SIR channels monitored for the extract obtained from the migration to sunflower oil and ethanol 95%, together with the BPI chromatogram of a standard mix of PA 6 and PA 66 oligomers. The values obtained for the ethanol 95% simulant and the sunflower oil were very different. The migration of PA 6 dimer, PA 6 trimer, PA 6 tetramer, PA 6 pentamer, PA 6 hexamer and PA 66 trimer was below the limit of detection in sunflower oil. Additionally, the migration of PA66 monomer and PA 66 dimer was much lower in sunflower oil than in ethanol 95% (12 ± 1 and 1.1 ± 0.1 mg/kg respectively for ethanol 95%; and 0.95 ± 0.31 and 0.42 ± 0.02 mg/kg respectively for sunflower oil). The total concentration of the oligomers migrating to ethanol 95% was over the established limit, whereas the migration concentration was below this limit for sunflower oil. Therefore, in general, ethanol 95% overestimates the migration of PA6 and PA 66 oligomers to sunflower oil. This agrees with the work published by Heimrich et al. in which ethanol 95% was found to overestimate the migration when compared to the migration in oil (Heimrich, Nickl, Bonsch, & Simat, 2015), In this work, the main contribution to the migration to sunflower oil was caprolactam, which at 3.2 ± 0.3 mg/kg, and below the SML of 15 mg/kg, exceeded the migration of the compound in ethanol 95% (0.73 ± 0.21 mg/kg). This is in contrast twith Abe et al. and Heimrich et al. ((Abe, Mutsuga, Ohno, Kawamura, & Akiyama, 2016; Heimrich, Nickl, Bonsch, & Simat, 2015), who found that the concentration in the simulant was higher than that in the oil. The sum of the migration values obtained for the oligomers in each of the ethanol 10%, ethanol 50%, ethanol 95% and acetic acid 3% (v/v) simulants exceeded the established migration limit. The compound that contributes most to this migration is the PA 66 monomer (4.8 ± 0.4, 7.0 ± 0.2, 12 ± 1, and 5.5 ± 1.2 mg/kg respectively). In contrast to this, the migration of the PA 6 monomer, also known as caprolactam, was below the SML (15 mg/kg) for all simulants studied. Since the results of migration to ethanol 95% were inconsistent with those for the migration to sunflower oil, the migration of oligomers to the three food products was performed in order to establish migration values of the oligomers to real food. Cooked beans, milk and chicken soup were selected for the study since they are common foods (high consumption rate) and usually transferred with a spatula or a spoon when heated. The sum of the oligomers in the three foods studied was below the established limit of 5 mg/kg (in the absence of caprolactam which has a SML 15 mg/kg). The simulants representative of beans were ethanol 10% and sunflower oil. The measured migration of the oligomers to the beans was closed to 3 mg/kg. Whilst the total oligomer migration to ethanol 10% was found to be 7 mg/kg, therefore overestimating the migration to the beans. Consequently, the oligomer migration from the spatula to the ethanol 10% as simulant exceeded the SML, whereas the migration to the real food was below the SML. In this work, it has been observed that the PA 66 monomer migrates to ethanol far more readily than it does to food with no ethanol but a high of water content. It is important to remark that, no swelling effect was observed, the spatulas maintained their structure after the migration process. Therefore, the ethanol effect on migration it is not due to swelling. This behaviour for ethanol has been shown previously, for example by Abe et al., for the migration of PA oligomers from ladles in which the migration of PA 66 monomer was an order of magnitude higher in ethanol 20% than in water (Abe, Mutsuga, Ohno, Kawamura, & Akiyama, 2016). The simulants used for chicken soup were ethanol 10%, acetic acid 3% and sunflower oil (with a correction factor of 1/3

according to Regulation (EU) No 10/2011 (Commision, 2011)). The total oligomer migration to ethanol 10% (7 mg/kg), and to acetic acid 3% (8 mg/kg) both exceeded the SML and overestimated the migration to the chicken soup (4.3 mg/kg). Migration of caprolactam to chicken soup (0.15 mg/ kg) was below migration of this monomer to sunflower oil (3.2 mg/kg), and this again may be attributed to the lower amount of fat in the soup. Additionally, the migration of caprolactam to the soup was less than that found for the beans which have a higher level of fat (8.3% fat). Based on these findings, it is suggested that fat content is the most likely factor contributing to the migration of caprolactam. Moreover, the sum of oligomer migration of soup and oil is nearly the same but the migration of the single oligomers varies. The migration of PA66 monomer was much lower in sunflower oil than in the soup. As it was explained before, PA 66 had a very low tendency to migrate to sunflower oil when compared with food with a high content of water or ethanol based simulants. Finally, whole milk was studied, with the simulant for the whole milk being ethanol 50%. The simulant overestimated the oligomer migration (9.8 mg/kg) to that for the whole milk (3% fat content) which was found to be 3.3 mg/kg and below the SML. As previously demonstrated, ethanol tends to extract PA oligomers more readily than the food it is used to simulate. Therefore, its utility as a simulant for food for these compounds should be considered carefully. In all cases, the oligomer migration from the spatula to the simulants were above the SML, however, after studying the migration to real foods, the migration level was found to be below the SML. To extend the sample set, three additional kitchenware utensils were purchased and the oligomer migration from each utensil to beans was measured (table 3). The aim of this study was to compare the migration from different kitchenware utensils to real food, thus improving the coverage of items that are available to consumers. It was found that the monomer and oligomers of PA 6 did not migrate from spatula 2 or either of the ladles. It is possible that this is because they may only be made of PA 66. PA 66 monomer was the main migrant from the four utensils studied (2.2 mg/kg, 1.7 mg/kg, 2.1 mg/kg and 3.2 mg/kg for spatula 1, spatula 2, ladle 1 and ladle 2 respectively). Migration of PA 66 dimer was also observed and found to be higher from spatula 2 and ladles 1 and 2 (2.1, 1.9 and 2.5 mg/kg, respectively) than that from spatula 1 (0.31 mg/kg). The total migration of oligomers from ladle 2 was the only value found to exceed the recommended limit of 5 mg/kg.

4. Conclusions

The use of hyphenated traveling wave ion mobility-high resolution mass spectrometry system has been successfully used to identify oligomers in migration extracts. The technique was able to differentiate oligomers with the same elemental composition and similar CCS values through unique fragmentation patterns. Migration of the identified compounds from different kitchenware utensils to a range of food products and simulants was performed. It was observed that the migration of PA6 and PA 66 oligomers to ethanol 95%, commonly used as substitute for sunflower oil, overestimated the migration when compared to migration to sunflower oil itself. Moreover, an extraction method of oligomers from cooked beans, soup and whole milk was developed, and the migration to simulants and real foods was studied. Migration values of the sum of oligomers obtained in the simulants were higher than those obtained for real food. Finally, ethanol generally overestimated the migration and therefore careful consideration should be given to using ethanol as a food simulant for this family of compounds.

CRediT authorship contribution statement Elena Canellas: Conceptualization, Investigation. **Paula Vera:** Investigation. **Xue-Chao Song:** Investigation. **Cristina Nerin:** Supervision, Funding acquisition. **Jeff Goshawk:** Investigation, Supervision. **Nicola Dreolin:** Investigation, Supervision.

Declaration of Competing Interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Tables and Figures:

Table 1: Compounds detected via UPLC-IM-Q/TOF in simulant 95% ethanol after exposure, retention time, measured *m*/*z*, theoretical *m*/*z*, mass error, elemental composition and CCS detected. UPLC-TQ cone voltages (V), limits of detection and recoveries of the polyamide oligomers (percentage Relative Standard Deviation in brackets)

Compound	RetentionTime (min)	Experimental m/z	Theoretical m/z	Mass error (mDa)	Elemental composition	CCS (Å²)	Cone voltage (V)	LOD (mg/kg)	Percentage Recovery (RSD%) Sunflower oil	Percentage Recovery (RSD%) Beans	Percentage Recovery (RSD%) Chicken soup	Percentage Recovery (RSD%) Whole milk
PA 6 monomer	2.50	113.0841	113.0841	0.0	C6H12NO	127.88	30	0.031	98 (3.0)	78 (5.5)	96 (3.5)	88 (5.6)
PA 6 dimer	2.29	227.1753	227.1760	-0.7	C12H23N2O2	155.44	30	0.062	100 (5.6)	95 (7.6)	106 (2.6)	90 (5.2)
PA 6 trimer	3.10	340.2593	340.2600	-0.7	C18H34N3O3	182.05	30	0.110	99 (7.1)	85 (8.1)	102 (5.1)	85 (4.8)
PA 6 tetramer	3.66	453.3436	453.3441	-0.5	C24H45N4O4	209.73	30	0.082	87 (2.6)	80 (9.2)	98 (1.6)	81 (8.6)
PA 6 pentamer	4.03	566.4269	566.4281	-1.2	C30H56N5O5	237.61	40	0.054	80 (5.0)	79 (10.3)	91 (2.2)	93 (4.8)
PA 6 hexamer	4.33	701.4928	701.4942	-1.4	C36H66N6O6Na	265.41	40	0.110	78 (11.0)	73 (15.0)	83 (5.0)	87 (2.6)
PA 66 monomer	2.61	227.1753	227.1760	-0.7	C12H23N2O2	153.61	30	0.054	97 (5.3)	98 (7.9)	89 (6.9)	91 (8.1)
PA 66 dimer	3.88	453.3437	453.3441	-0.4	C24H45N4O4	214.07	30	0.071	102 (5.8)	95 (8.7)	100 (8.0)	100 (6.3)
PA 66 trimer	4.55	701.4935	701.4942	-0.7	C36H66N6O6Na	270.69	40	0.120	89 (8.0)	81 (10.)	99 (7.0)	98 (1.9)

Table 2 Concentrations of PA oligomers found after the migration assays from spatula 1 in the food and food simulant tested (standard deviation in brackets).

Concentrations of PA oligomers found after the migration assays from spatula 1 in the food and food simulant tested (standard deviation in brackets).

Compound	Ethanol 10% (mg/kg)	Acetic acid 3% (mg/kg)	Ethanol 50% (mg/kg)	Ethanol 95% (mg/kg)	Sunflower oil (mg/kg)	Beans (mg/kg)	Chicken soup (mg/kg)	Whole milk (mg/kg)
PA 6 monomer	0.28 ± 0.02	0.37 ± 0.10	0.61 ± 0.13	0.73 ± 0.21	3.2 ± 0.3	0.32 ± 0.02	0.15 ± 0.01	0.42 ± 0.01
PA 6 dimer	0.081 ± 0.001	0.11 ± 0.04	0.098 ± 0.001	0.16 ± 0.05	<0.062	<0.062	0.082 ± 0.013	<0.062
PA 6 trimer	0.160 ± 0.001	0.19 ± 0.03	0.230 ± 0.003	0.31 ± 0.03	<0.110	0.24 ± 0.02	0.24 ± 0.02	0.54 ± 0.01
PA 6 tetramer	<0.082	<0.082	<0.082	0.28 ± 0.07	<0.082	<0.082	<0.082	<0.082
PA 6 pentamer	<0.054	0.071 ± 0.001	<0.054	0.08 ± 0.02	<0.054	<0.054	1.2 ± 0.3	<0.054
PA 6 hexamer	<0.110	<0.110	<0.110	0.16 ± 0.03	<0.110	<0.110	<0.110	<0.110
PA 66 monomer	4.8 ± 0.4	5.5 ± 1.2	7.0 ± 0.2	12 ± 1	0.95 ± 0.31	2.2 ± 0.2	2.6 ± 0.4	2.3 ± 0.4
PA 66 dimer	0.74 ± 0.03	0.78 ± 0.18	0.87 ± 0.02	1.1 ± 0.1	0.42 ± 0.02	0.31 ± 0.01	0.11 ± 0.01	0.32 ± 0.02
PA 66 trimer	1.1 ± 0.1	1.1 ± 0.4	1.5 ± 0.1	2.2 ± 0.2	<0.120	<0.120	<0.120	<0.120
Sum of oligomers	7.2	8.1	10.3	16.9	4.6	3.1	4.4	3.6

Table 3 Concentrations of PA oligomers (mg/Kg) found after the migration assays from spatula 1, 2 and ladle 1 and 2

Compound	Beans Spatula 1 mg/Kg	Beans Spatula 2 mg/Kg	Beans Ladle 1 mg/Kg	Beans Ladle 2 mg/Kg
PA 6 monomer	0.32 ± 0.01	<0.031	<0.031	<0.031
PA 6 dimer	0.041 ± 0.001	<0.062	<0.062	<0.062
PA 6 trimer	0.24 ± 0.02	<0.110	<0.110	<0.110
PA 6 tetramer	<0.082	<0.082	<0.082	<0.082
PA 6 pentamer	<0.054	<0.054	<0.054	<0.054
PA 6 hexamer	<0.110	<0.110	<0.110	<0.110
PA 66 monomer	2.2 ± 0.2	1.7 ± 0.4	2.1 ± 0.5	3.2 ± 0.3
PA 66 dimer	0.31 ± 0.01	2.1 ± 0.3	1.9 ± 0.3	2.5 ± 0.4
PA 66 trimer	<LOD	0.11 ± 0.25	0.091 ± 0.030	0.31 ± 0.02

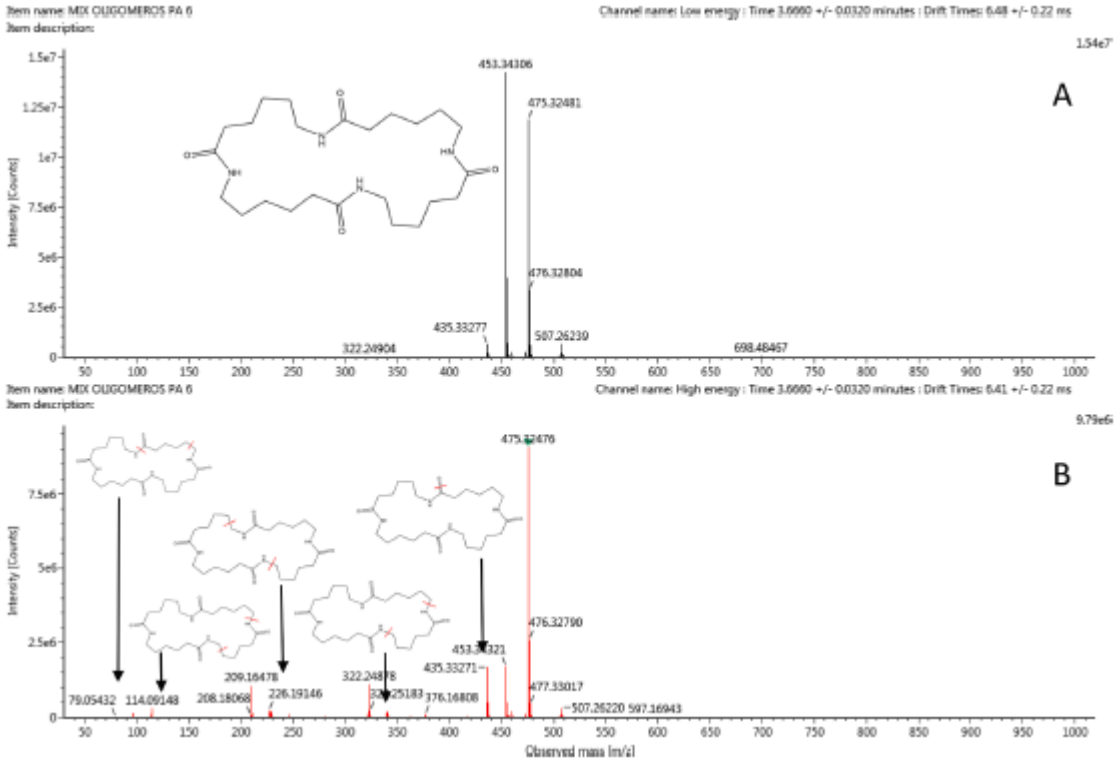

Fig. 1. Low-energy (A) and high energy drift time-aligned spectrum (B) of the PA 6 tetramer obtained by UPLC-IM-Q/TOF.

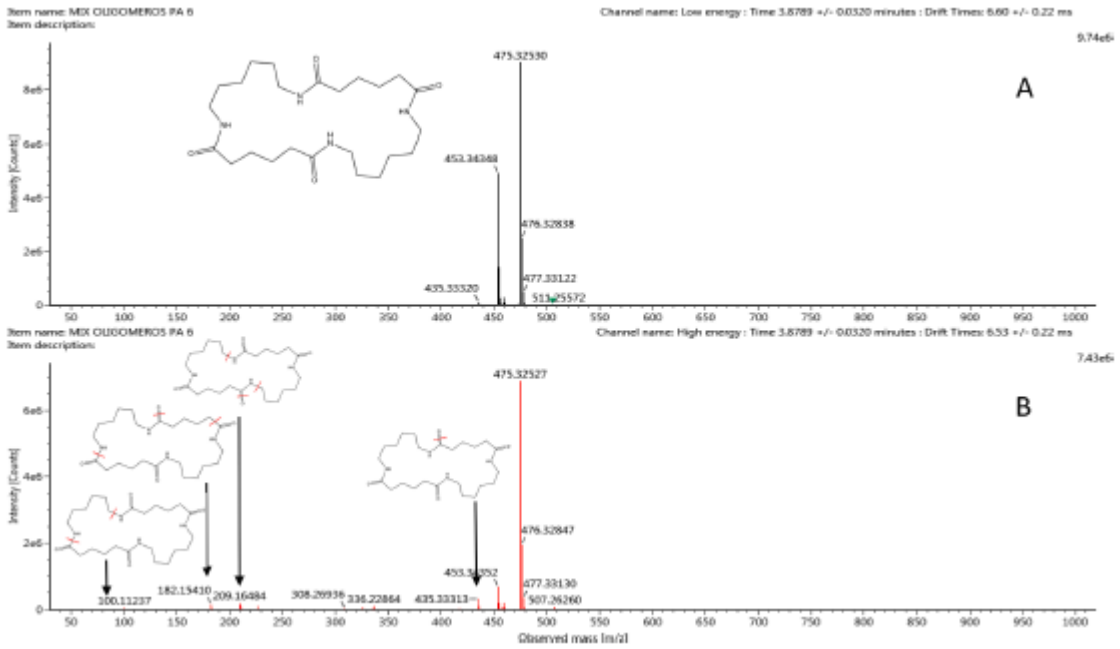

Fig. 2. Low-energy (A) and high energy drift time-aligned spectrum (B) of the PA 66 dimer obtained by UPLC-IM-Q/TOF.

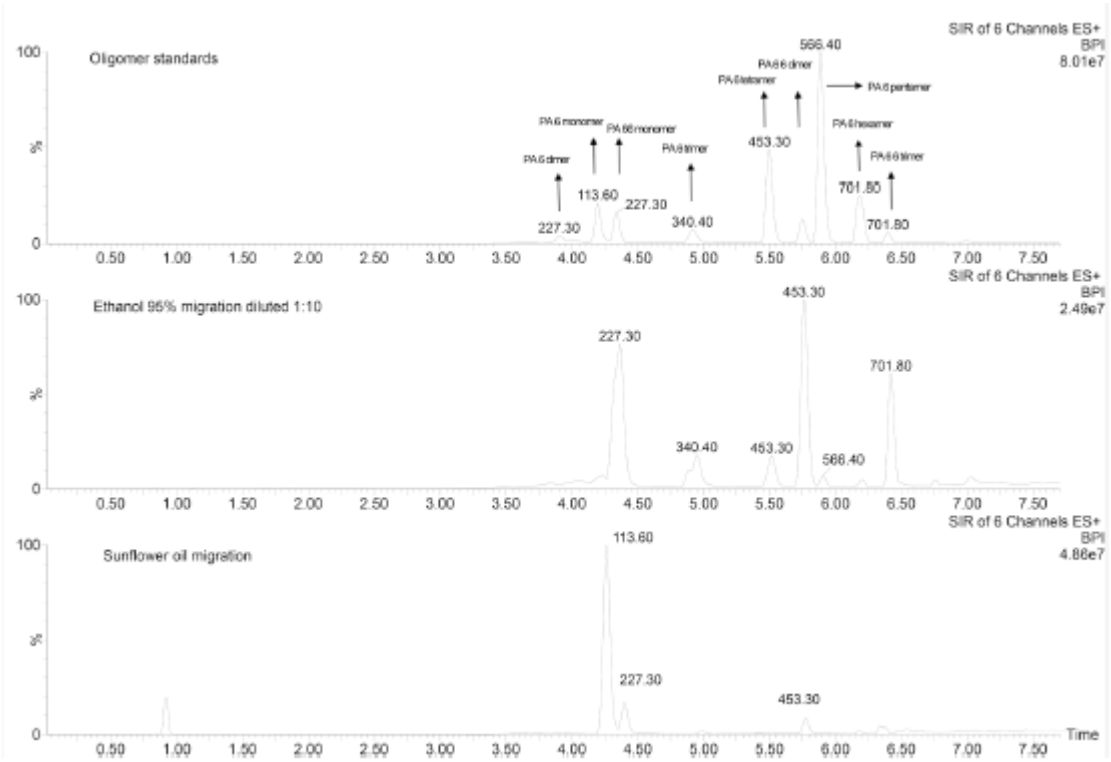

Fig.3. Combined SIR chromatograms of the PA oligomers. From top to bottom: standard mix, migration to ethanol 95%, and migration to sunflower oil.