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The application of ion mobility time of flight mass spectrometry to elucidate neo-formed compounds derived from polyurethane adhesives used in champagne cork stoppers

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ABSTRACT

Polyurethane adhesives are used to bond agglomerated cork and natural disk cork to produce cork stoppers that are used in champagne bottles. These adhesives are manufactured by reacting polyols with an excess of diisocyanates. Isocyanates are highly reactive compounds that have a propensity to form non-intentionally added substances (NIAS) in the end product. In this work, ion mobility-time of flight-mass spectrometry was used to elucidate such NIAS, through the comparison of accurate mass spectra with the fragmentation patterns of proposed candidates. Twelve neo-formed compounds, including amines, amides and urethanes, resulting from the reaction of isocyanates with acetic acid and ethanol used as food simulants, were identified. Additionally, markers from champagne vs. champagne after its exposure to the adhesive were investigated using the supervised multivariate analysis method of Orthogonal Projection to Latent Structures – Discriminant Analysis. Four neo-formed compounds, resulting from the reaction of diisocyanates with malic acid or tartaric acid contained in the champagne, were identified for the first time in this work. All of the compounds identified were subsequently quantified using ultra-high pressure liquid chromatography coupled to a triple quadrupole mass spectrometer. Limits of detection were below 5 μg/kg in the food simulants and below 30 μg/kg in champagne samples. Migration levels ranged from 70 to 721 μg/kg, with most of them exceeding the specific migration limit established for Cramer class III compound (90 μg/kg).

1. Introduction

Polyurethane (PU) polymers are commonly produced from the reaction between polyols and an excess of low molecular weight isocyanates [1]. The aromatic isocyanates, diphenylmethane diisocyanate (MDI) or toluene diisocyanate (TDI) and the aliphatic hexamethylene diisocyanate (HDI) or isophorone diisocyanate (IPDI) are the most common diisocyanates used for polyurethane formation [2].

Polyurethane adhesives are broadly used in food contact materials to bond several layers of film into multilayers structures [3–6] or to bond either the agglomerated cork or the natural cork disks of cork stoppers used for wine, cava or champagne bottles [7,8].

The normal reaction products of PU, polymers with a molecular weight well above 1000 g/mol, are not expected to raise any public health issue in terms of their migration to food [7]. However, residual monomers, together with reaction and degradation products from these monomers must also be considered. Regulation 10/2011/EC [9] establishes that any potential health risk in the final material or article arising from reaction and degradation products should be assessed by the manufacturer in accordance with internationally recognised scientific principles on risk assessment [9]. Therefore, the non-intentionally added substances (NIAS) derived from reactions or degradation must be identified, the migration of these compounds has to be quantified, and a risk assessment must be performed [10]. It has been demonstrated that NIAS may endanger the human health [11–13], therefore it is crucial to ensure that there is no risk to human health resulting from migration into food.

The raw materials used in the production of PU adhesives are very reactive and have complex chemistries. Consequently, it is possible for unexpected neo-formed compounds to be produced, which may interact with both simulants and food. In this work, the interaction of PU adhesives, used in the production of corks, with beverages in contact with

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the corks, are studied.

Primary aromatic amines (PAAs) coming from polyurethane adhesives are well known NIAS that have been reported by several authors [14–17]. Nevertheless, there are many other NIAS that could be formed by the interaction between polyurethane adhesives and food or food simulants. The identification of unexpected NIAS requires accurate mass spectrometry techniques. Among the available techniques, ion-mobility quadrupole time-of-flight mass spectrometry, coupled to ultra-high performance liquid chromatography (UPLC-IM-Q/TOF) that produces less complex spectra, has demonstrated to be and effective tool for the identification of NIAS [18–20].

In this context, the aim of our study is to use this technique combined with a multivariate analysis method of Orthogonal Projection to Latent Structures – Discriminant Analysis to try to identify new NIAS in both the food simulants recommended by the European legislation and in champagne, that is the food in contact with the cork stoppers. Then, a comparison of the NIAS detected in food and in food simulants is done. The neoformed compounds found from the interaction between the corks and the champagne can be highlighted as important scientific improvement, as it is the first time that these compounds are identified. This fact emphasizes the importance of migration studies with real food in addition to those usually applied to food simulants. Finally, a quantification method to assess the migration levels of the identified neoformed compounds is developed and a risk assessment of the migration levels of the identified and quantified NIAS is performed.

2. Materials and methods

2.1. Reagents

Ethanol and methanol, of high-performance liquid chromatography (HPLC) grade, and glacial acetic acid were supplied by Scharlau Chemie S.A (Sentmenat, Spain). 1,6-hexadiamine and isophorone diamine were purchased from Sigma-Aldrich Química S.A (Madrid, Spain). Ultrapure water was used for the chromatography. It was obtained using an Ultramatic GR from Wasserlab (Spain). It is system for purification of Type I Ultrapure Water (Reagent Grade).

Cabré and Sabaté Spanish cava (Spanish champagne) with an 11.5% vol. Of ethanol were purchased from a supermarket. It was selected since it is a medium quality champagne where the cork with an agglomerated cork (body) assembled with a cork disk using the PU adhesive are used.

PU adhesive was supplied by a company under a confidentiality agreement. The PU adhesive was a one component PU, based on a polymer obtained by the reaction of a polyol with an excess of alkyl diisocyanates.

Cork stoppers, for which an agglomerated cork (body) was assembled with a cork disk at the top and bottom using the PU adhesive, were supplied by the same company. The corks had 42 cm^2 agglomerated body, made of granules of 2–8 mm pressed together and bound with the adhesive, glued to a 5 cm^2 disk of natural cork also with adhesive.

2.2. Sample extraction

Sections of aluminum foil were coated with the PU adhesive and left to dry. Subsequently, 0.1 g of the dry adhesive was introduced into a glass vial with either 20 mL of simulant B (3% v/v acetic acid in water), 20 mL of simulant C (20% v/v ethanol in water) or 20 mL of champagne. The vials were maintained at a temperature of 60 °C for 10 days in order to model the migration conditions that would occur during a shelf-life of over six months.

2.3. Quantification method

A targeted method was developed in order to quantify the compounds identified.

Samples of the champagne were spiked with an aqueous solution of 1,6-hexadiamine and isophorone diamine, at concentrations of 100 ng/g and 200 ng/g respectively. The solutions were diluted with ultrapure water to minimize the effect of the matrix champagne and three concentration prepared: 1:1, 1:5 and 1:10 (v/v). The spiked dilutions were analyzed using both UPLC-IM-Q/TOF and UPLC-TQ-MS and the dilution prepared at 1:10 (v/v) was found to exhibit the least matrix effect.

External calibration curves were prepared and analyzed for both amines. NIAS were semi-quantified using these calibration curves since no commercial standards area available.

2.4. Migration studies

Migration tests from corks were performed following the guidelines set out in Regulation 10/2011/EC [9]. Simulant B and simulant C were selected for the study in line with the Regulation. The results obtained suggested that migration studies using food material were necessary, in addition to those performed with the simulants. Therefore, migration assays with champagne were also done.

The bottle with the lowest volume in which the cork stoppers are used holds 20 cL of champagne. Therefore, bottles containing 20 cL of simulant or champagne were sealed with the cork stoppers replicating the use of the corks in champagne bottles. The disk of pure cork was placed in the interior of the bottle. Three replicates of each simulant and champagne were studied and the bottles were laid horizontally 10 days and maintained at a temperature of 60 °C.

After exposure to the cork stoppers, the simulants were analyzed using UPLC-IM-Q/TOF for identification and UPLC-TQ-MS for quantification. The champagne used for the migration study was diluted 1/10 (v/v) with ultrapure water prior to injection.

2.5. 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 Acquity 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 100% A to 95% B after 13 min. The volume of sample injected was 10 μL.

The electrospray interface (ESI) was used in positive ionization, sensitivity mode with a capillary voltage of 3 kV and a sampling cone voltage of 30 V. The temperatures used were 120 °C and 500 °C for the source and desolvation gas, respectively, and the desolvation gas flow rate was 800 L h^{-1}. The system was calibrated using Major Mix test solution (Waters Corp.) and data were acquired in the range 50–1000 *m/z*. Leucine-Enkephalin $[M+H]^+$, *m/z* 556.2766, was used as the lock-mass compound for real-time mass correction at a concentration of 100 ng/mL and infusion rate of 15 μL/min was used. IMS gas flow rate was 25 mL/min, wave velocity 250 m/s, and IMS pulse height 45 V. A collision energy ramp of 20–40 V was applied with argon used as the collision gas in the high energy function. Nitrogen was used as the mobility gas.

Data independent analysis was set to high definition mass spectrometry ($HDMS^E$) mode, with a 0.1 s acquisition time. Data acquisition and processing were carried out using UNIFI v.1.8 software. In $HDMS^E$ mode two data channels are acquired simultaneously in a single run; a low energy channel for which the fragmentation of precursor ions is minimized, and a high energy channel for which a collision energy ramp is applied to induce fragmentation of precursor ions. On detecting an unknown component of interest, the first step in the elucidation process is to propose an elemental composition that corresponds to the *m/z* of the precursor ion. In this work, all unknowns were assumed to consist of the following chemical elements; carbon, oxygen, hydrogen, nitrogen,

chlorine, bromine, fluorine, sulfur, phosphorous and silicon. Sodium was also included in the elemental composition search since some compounds have a 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 a maximum deviation of 3 mDa. Following the determination of a molecular formula for each component of interest, a database search was performed for each elemental formula using the integrated link to ChemSpider [22] within the UNIFI software. The database search returns proposed compounds for each elemental composition, with each proposal consisting of the compound name and a list of synonyms together with the mol file, which represents the structure of the compound. Fragment Match, an in-silico fragmentation tool also integrated in the UNIFI software, is then applied to each structure. Fragment Match produces a series of substructures using a systematic bond disconnection algorithm from the mol file of each of the compounds proposed, and automatically compares the *m/z* values of the substructures to the *m/z* values of the fragment ions in the high energy data. For the NIAS, where the mol files were not available through ChemSpider, they were generated using Chemdraw Ultra 12.0, by taking into account the likely structures that would be derived from the reaction of intentionally added substances (IAS) present in the sample and IAS in the adhesive, cork and the compounds in the simulant or food.

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

Quantitation analyses were carried out using an Acquity UPLC chromatography system coupled to an electrospray interface (ESI) and Xevo TQ-S micro detector, supplied by 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 5 min. The volume of sample injected was 5 μL.

The electrospray interface (ESI) was used in positive ionization mode with a capillary voltage of 3 kV and the sampling cone voltage was optimized for each compound. 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}. Multiple reaction monitoring (MRM) mode was used as MS acquisition function. Optimization of the cone voltages and collision energies for 1,6-hexanediamine and isophorone diamine was performed by combined infusion. Argon was used as collision gas. Table 2 shows the cone voltage, mass transition of interest and collision energy used for each of the analytes. MassLynx v.4.1 software was used for data acquisition and processing.

2.6. Software

All sample data coming from UPLC-IM-Q/TOF were processed using the multivariate analysis tools available in UNIFI v1.8. UNIFI can generate marker matrices based upon user-defined criteria that can be automatically transferred to EZInfo software for multivariate analysis.

Data coming from UPLC-TQ-MS were processed by MassLynx v.4.1.

Molecular structures of neo-formed compounds were drawn with the software ChemDraw Ultra 12.0.

Toxicity assessment based on the Threshold of Toxicological Concern (TTC) was done using the software Toxtree v 3.1.0.1851.

3. Results and discussion

3.1. Identification

UPLC-IM-Q/TOF was the technique selected to detect and identify the compounds migrating from the PU adhesive to the food simulants and champagne (section 2.2). This technique provides separation by means of an ion mobility, mass accuracy, fragment ions and retention time. The time the ions take to traverse the drift cell is called the ion-mobility "drift time". A collision cross section (CCS) value can be derived from the drift time of each compound and is related to the three-dimensional conformation of the chemical structure of the compound. Ion-mobility can also provide simplified spectra since the fragment ions of a detected compound are aligned to the precursor ion for that compound using both retention time and drift time. This reduces the number of interfering ions aiding the elucidation of unknowns and providing additional confidence in the identification [18–21].

Fig. 1 shows the high energy spectrum of 2-hydroxy-*N*-(6-isocyanatohexyl)propanamide. It can be seen that the mass spectrum has few interferences due to the use of ion mobility. As a result of this it is straightforward to distinguish between background ions and fragments ions, aiding the complex task of structural elucidation. The fragments of 2-hydroxy-*N*-(6-isocyanatohexyl)propanamide are annotated on Fig. 1 and the fragmentation chemistry, summarized in Table 1, is described in the following paragraphs.

The cork stoppers studied here are manufactured for champagne bottles and as such will be in contact with champagne. Regulation 10/2011/EC [9] states that migration studies for alcoholic beverages of a strength of between 6% vol. and 20% vol. must be performed using food simulant C (20% ethanol v/v). Additionally, food simulant B (3% acetic acid v/v) should be used for foods which have a pH below 4.5. Since the typical pH of champagne is 2.9 [22], simulant B was also selected for the study.

In section 2.2, it was stated that the PU adhesive was placed in contact with food simulants B and C for 10 days at a temperature of 60 °C in order to determine whether NIAS were formed under the experimental conditions. Table 1 shows the neo-formed compounds identified in the simulants following the migration study, together with their accurate mass (mDa), CCS value, molecular structure and fragments. All of the neo-formed compounds found were added to a scientific library in UNIFI software to enable rapid and confident identification of these compounds in future sample analyses.

The two diamines, 1,6-hexanediamine and isophorone diamine, were identified through the workflow previously described and Fragment Match was used for identification of product ion fragments with the mol files used downloaded from Chemspider. com [23] as mentioned above. It is shown in Table 1, that alpha cleavage is the dominant reaction for amines with the loss of the largest alkyl groups [24]. Only one fragment was observed for 1,6-hexadiamine under the high energy conditions applied, and five fragments were observed for isophorone diamine. The fragment with a *m/z* value of 154.1596 was the most abundant of the five and corresponds to a single cleavage of a primary amine, with two different cleavages from the primary amine being possible. The other fragments are generated via at least two separate cleavages, with the most abundant these, with a *m/z* values of 137.1332, demonstrating the two possible cleavages from the primary amine. For the other fragments a cycloalkane cleavage is implied, in which the skeletal ring decompositions involve the cleavage of at least two bonds [25]. The identification of these two amines was confirmed with commercial standards. Retention time, fragment ion and CCS information generated using the standards, matched the values measured for the compounds identified in samples.

The two diamines identified are the result from the reaction (Reaction 1) between residual diisocyanates, isophorone diisocyanate and 1,6-hexanediisocyanate used as monomers, and water:

$$RNCO + H_2O \rightarrow RNHCOOH \rightarrow RNH_2 + CO_2 \quad (1)$$

The PU used for the cork stoppers under study is based on isophorone diisocyanate (IPDI) and hexamethylene diisocyanate (HDI). The main advantage of these diisocyanates from the point of view of food safety is that as aliphatic isocyanates they generate aliphatic amines which are

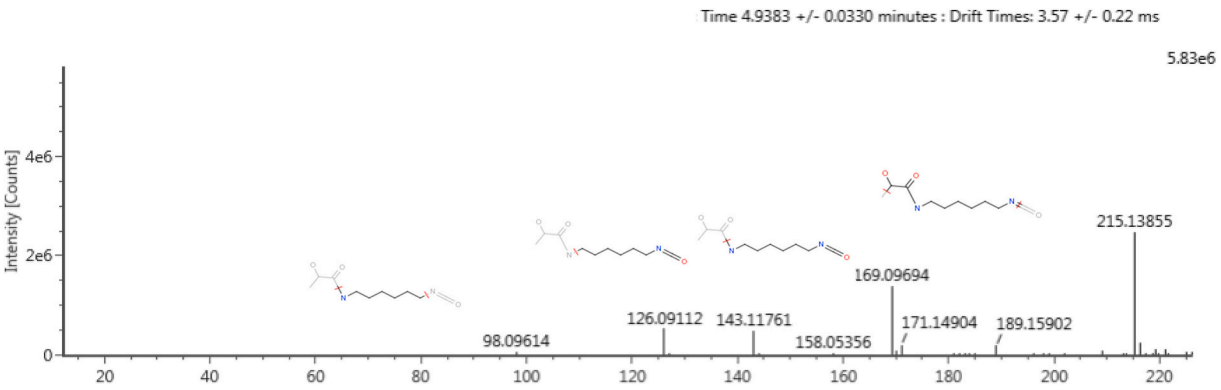


Fig. 1. UPLC-IM-Q/TOF high-energy mass spectrum for the compound 2-hydroxy-*N*-(6-isocyanatohexyl)propanamide identified in champagne exposed to polyurethane adhesive.

not carcinogenic. This is in contrast to aromatic isocyanates, that react with water and to yield PAAs which are carcinogenic [26].

Moreover, the compounds 6-isocyanatohexan-1-amine and (5-isocyanato-1,3,3-trimethylcyclohexyl)methanamine were identified in both simulants. These compounds result from Reaction 1 following the reaction of one of the isocyanates, from the two present on the diisocyanate molecule, with water.

The alpha cleavage of amines was also observed here for both diamines. Additionally, isocyanate fragmentation was observed, implying the loss of a $(NO)^+$, in-line with the fragmentation of isocyanates [27]. In the case of (5-isocyanato-1,3,3-trimethylcyclohexyl)methanamine these fragmentation patterns were also found in conjunction with fragmentation that leading to a cycloalkane cleavage (Table 1).

Furthermore, reaction 2 describes the reaction of IPDI and HDI with ethanol in the 20% ethanol simulant. The compounds are the result of the reaction between both isocyanates in the molecules of IPDI and HDI. The compounds ethyl (6-aminohexyl)carbamate, ethyl (3-(aminomethyl)-3,5,5-trimethylcyclohexyl)carbamate and diethyl hexane-1,6-diyldicarbamate result from the reaction between one of the isocyanates from the molecules of IPDI or HDI, with ethanol (Reaction 2).

$$RNCO + R'OH \rightarrow RNHCOOR' \quad (2)$$

Table 1 shows the fragmentation of these molecules and Fragment Match was used for the assignment of substructures to the product ion fragments. The mol files used in the analysis drawn using the Chemdraw software, as above described. A number of possible molecular structures were investigated, each being derived using the proposed elemental composition together with knowledge of compounds previously identified, in addition to the intentionally added compounds present in the sample. Fragment Match was then used to determine the most likely structure by matching substructures from each proposed compound to the peaks in the high energy spectrum. The compounds listed in Table 1 were those for which the theoretical fragmentation best matched the measured data to within a mass accuracy of 3 mDa. The cleavage implying the NHCO–O bond was observed, this cleavage was also observed by Gies et al. [28]. Moreover, the RNH-COOR' cleavage observed here was also reported by Harris et al. [29].

Additionally, the compound 1-(6-aminohexyl)-3-(6-isocyanatohexyl)urea was also identified in the 20% ethanol simulant. This compound results from the reaction of ethanol with two 1,6-hexanediisocyanates (Reaction 3):

$$\begin{aligned} &RNCO + H_2O \rightarrow RNHCOOH \rightarrow RNH_2 + CO_2 \\ &RNH_2 + RNCO \rightarrow RNHCONHR \end{aligned} \quad (3)$$

1-(6-aminohexyl)-3-(6-isocyanatohexyl)urea was detected as a sodium adduct. Since, sodium adducts have a low tendency to fragment under the collision energy conditions applied in mass spectrometry, only one fragment, corresponding to *N*–O cleavage, was found.

Table 1 also shows the following neo-formed compounds that were only identified in simulant B, (3% acetic acid (v/v)): *N*-(6-aminohexyl) acetamide, *N*,*N*'-(hexane-1,6-diyl)diacetamide, *N*-(3-(aminomethyl)-3,5,5-trimethylcyclohexyl)acetamide and *N*-((5-acetamido-1,3,3-trimethylcyclohexyl) methyl)acetamide. These compounds are amides that results from the reaction between a carboxylic acid (acetic acid) and diisocyanates [30]. The reaction occurs as follows, and might be promoted by storing the samples at a temperature of 60 °C for 10 days.

$$RNCO + R'COOH \rightarrow RNHCOOCOR' \rightarrow RNHCOR' + CO_2 \quad (4)$$

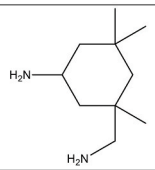
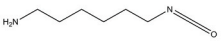
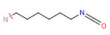
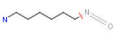
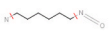
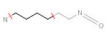
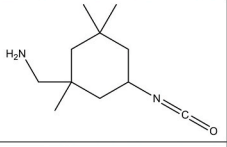
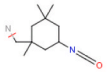
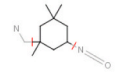
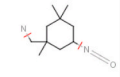
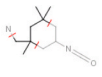
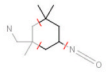
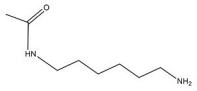
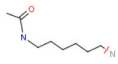
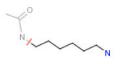
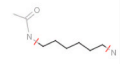
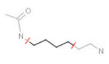
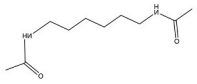
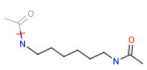
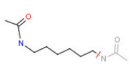
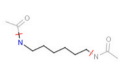
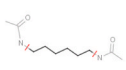
Table 1 shows the fragments detected for these compounds in the high energy data. The strategy for substructure assignment was the same as that described previously. The mol files were created using Chemdraw and Fragment Match was used for the substructure assignment to the measured fragment ions. An N–CO α-cleavage, a characteristic fragmentation pattern of amides, was observed [31], together with a β-cleavage at the *N*-R bond [32].

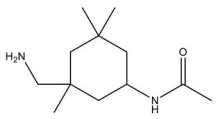
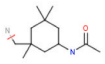
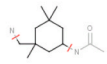
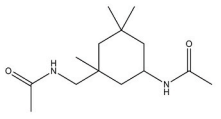
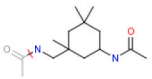
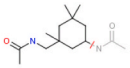
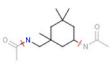
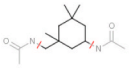
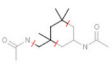
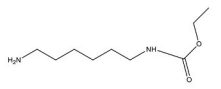
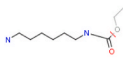
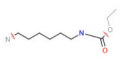
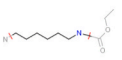
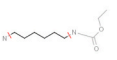
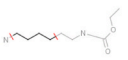
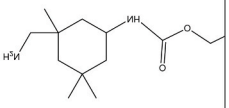
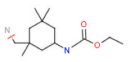
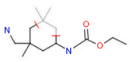
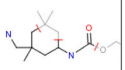
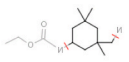
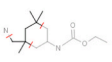
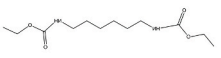
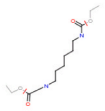
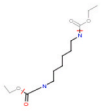
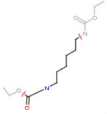
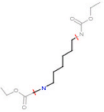
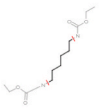
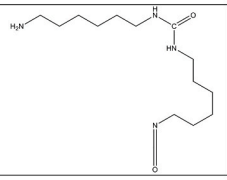
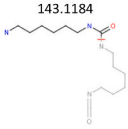
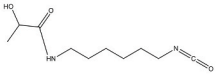
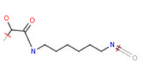
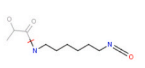
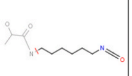
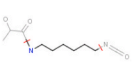
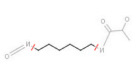
Following the results obtained for simulant B (3% acetic acid), in which neo-formed compounds were created through the reaction between IPDI and HDI, and acetic acid, it was decided to determine whether neo-formed compounds would result from the contact between the diisocyanates and champagne, that is the beverage to be in contact with the cork stoppers. The complexity of a food matrix sample increases the difficulty of the detection of non-intentionally added substances. Therefore, multivariate analysis (MVA) was used to compare the blank (pure champagne) and the extract obtained using the method described in section 2.2.

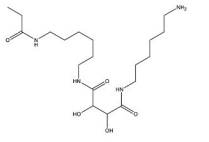
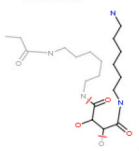
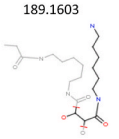
Following the peak detection for a set of samples, user-defined tolerances can be set in UNIFI to align the detected components across the sample set and generate a marker matrix in which each marker is uniquely defined by a combination of a *m/z* value, a retention time and a drift time. The marker matrix can be automatically transferred from UNIFI into the MVA software EZInfo. The two groups of samples (blanks and extracts) were investigated using the supervised MVA method of Orthogonal Projection to Latent Structures – Discriminant Analysis (OPLS-DA). OPLS-DA provides the ability to highlight even subtle differences between two sample groups. This additional level of detail is required since potentially harmful markers resulting from NIAS in the extract samples, may be masked by dominant markers in the complex champagne matrix.

OPLS-DA yields an S-plot which is a plot of the covariance of the markers – the magnitude of change (x-axis), against the correlation of the markers – the consistency of the change (y-axis) values. Fig. 2 shows the S-plot. The upper right quadrant of the S-plot shows markers which are elevated in the extract (champagne with polyurethane), while the lower left quadrant shows components elevated in the blank (champagne). The further along the x-axis the marker is located, the greater its contribution to the variance between the groups, while markers further

Table 1
Neo-formed compounds identified, retention time, accurate mass detected (mDa), CCS value, molecular structure of the compounds and their fragments.

Compound identified/simulant or food	Retention time (min) and accurate mass detected (mDa)	CCS ($Å^2$)	Molecular structure	Fragment 1	Fragment 2	Fragment 3	Fragment 4	Fragment 5
1,6-Hexanediamine (B, C, CAVA)	117.1392	148.9		100.1126 				
Isophorone diamine (B, C, CAVA)	171.1861	207.01		154.1596 	137.1332 	100.1126 	95.0863 	81.0712 
6-isocyanatohexan-1-amine (B, C, CAVA)	143.118	189.71		126.0919 	100.1126 	83.0844 	55.0560 	
(5-isocyanato-1,3,3-trimethylcyclohexyl)methanamine (B, C, CAVA)	197.1647	152.57		180.1388 	123.1174 	137.1330 	95.0861 	81.0701 
N-(6-aminohexyl)acetamide B	159.1497	255.1		142.1232 	100.1133 	83.0853 	55.0550 	
N,N'-(hexane-1,6-diyl)diacetamide B	201.160	144.66		159.1494 	142.1232 	100.1126 	83.0886 	

N-(3-(aminomethyl)-3,5,5-trimethylcyclohexyl)acetamide B	213.1972	157.3		196.1706 	137.1329 	95.0865 	81.0704 	
N-((5-acetamido-1,3,3-trimethylcyclohexyl)methyl)acetamide B	255.207	160.3		213.1933 	196.1701 	154.1596 	137.1331 	95.0865 
ethyl (6-aminohexyl)carbamate (C, CAVA)	189.1589	152.46		143.1187 	126.0915 	100.1126 	83.0859 	55.0548 
ethyl (3-(aminomethyl)-3,5,5-trimethylcyclohexyl)carbamate (C, CAVA)	243.2083	267.04		226.1807 	189.1579 	143.1187 	137.1338 	95.0894 
diethyl hexane-1,6-diyldicarbamate (C, CAVA)	261.1822	164.2		169.0971 	143.1186 	126.0925 	98.0982 	83.0863 
1-(6-aminohexyl)-3-(6-isocyanatohexyl)urea (C, CAVA)	285.2307	177.11		143.1184 				
2-hydroxy-N-(6-isocyanatohexyl)propanamide (CAVA)	215.1396	267.6		169.0981 	143.1194 	126.0926 	98.0977 	83.0848 

N^1-(6-aminohexyl)-2,3-dihydroxy-N^4-(6-propionamidohexyl)succinanamide (CAVA)	403.2932	213.93		215.1412 	189.1603 	169.0982	143.1184	126.0925
2-hydroxy-N-(5-isocyanato-1,3,3-trimethylcyclohexyl)methyl)propanamidel (CAVA)	269.1885	152.98		137.1337	102.0563	95.0873		
N-(6-(3-(3-(aminomethyl)-3,5,5-trimethylcyclohexyl)ureido)hexyl)-2-hydroxypropanamide (CAVA)	385.3166	206.85		189.1606	154.1596	143.1183	137.1304	102.0556

along the y-axis represent a higher reliability of the analytical result. The differences between the groups can result from markers which are only present in one group, or from markers with a significant change in intensity (concentration) between the groups.

The *m/z* values of the markers that were found to be elevated in the extract samples were elucidated following the procedure described previously, with mol files created using the Chemdraw and Fragment Match used assign substructures to fragment ions in the high energy data. Champagne contains various organic acids that could react with diisocyanates [33] with malic acid, lactic acid and tartaric acid being the main contributors. The presence of lactic acid depends on the malolactic fermentation process which is not the same in all champagne

production.

Table 1 shows the compounds 2-hydroxy-*N*-(6-isocyanatohexyl) propanamide, 2-hydroxy-*N*-(5-isocyanato-1,3,3-trimethylcyclohexyl) methyl)propanamidel and *N*-(6-(3-(3-(aminomethyl)-3,5,5-trimethylcyclohexyl)ureido)hexyl)-2-hydroxypropanamide which are formed from by the reaction between the diisocyanates and malic acid (reaction 4). Additionally, N1-(6-aminohexyl)-2,3-dihydroxy-N4-(6-propionamidohexyl)succinanamide was identified and is the results of the reaction between two molecules of 1,6-hexanediisocyanate and tartaric acid. These neo-formed compounds have not been published before and their presence highlights the importance of research into NIAS from substances that are to be food contact materials. The discovery of these compounds in the champagne extracts also highlights that simulants are not always able to model food composition. In summary, the NIAS found using the champagne matrix would not have been identified if the study had been performed only using the simulants recommended by Regulation 10/2011/EC [9].

None of the neo-formed compounds identified here have a specific migration limit that has been established in legislation. Nevertheless, Regulation 10/2011/EC [9] states that any potential health risk in the final material or article arising from reaction and degradation products should be assessed by the manufacturer in accordance with internationally recognised scientific principles on risk assessment. Such an assessment uses the Threshold of Toxicological Concern (TTC), a concept that establishes of a level of exposure for three Cramer classes described by Kroes et al., 2004 [34]. Compounds are classified into Cramer classes I, II and III which reflect a presumption of low, moderate or serious toxicity, respectively [35]. The NIAS identified here were classified as Cramer class III for which the TTC is 90 μg/person/day and a quantification study was performed to establish the level of risk to human health they pose corresponding to an SML of 90 ng/g.

3.2. Quantification

The quantification of the migration of the two diamines to simulants B and C was performed by external calibration from the calibration plots of certified standards of the two amines under study, using UPLC-IM-Q/TOF. The limit of detection of the compound 1,6-hexanediamine in simulant B was 51 ng/g and in simulant C was 59 ng/g. The limit of detection of the compound isophorone diamine was 32 ng/g for both simulants.

Since the NIAS are neo-formed compounds, no commercial standards were available, so a semi-quantification method was employed using the amines as standards.

An optimization procedure described in section 2.3. was undertaken to determine the influence of the champagne matrix on the determination of the compounds. Champagne was spiked with an aqueous solution of 1,6-hexadiamine and isophorone diamine at concentrations of 100 ng/g and 200 ng/g. The two spiked champagne samples were then diluted with ultrapure water at the following ratios: 1:1, 1:5 and 1:10 v/v before the analysis using UPLC-IM-Q/TOF in order to study the influence of the dilution on the matrix effect. Then, each dilution was analyzed by UPLC-IM-Q/TOF.

Finally, dilution 1:10 was selected and the limits of detection in the spiked champagne solution were determined as 554 ng/g for 1,6-hexanediamine and 412 ng/g for isophorone diamine, after accounting for the dilution.

These limits of detection obtained in champagne were higher than the estimated SML based on TTC and the Cramer classification for the neo-formed compounds (90 ng/g) therefore it was not sensitive enough for the purpose of the study done.

As a result, an appropriate experimental method was developed using the UPLC-TQ-MS to be able to quantify lower levels. Multiple-reaction-monitoring (MRM) mode was chosen as it provides ultimate selectivity and increased sensitivity for targeted analysis. Table 2 shows the MRM transitions, together with the cone voltages and the optimized collision energies for each compound. The limits of detection were 2.1 ng/g in 3% acetic acid, 3.1 ng/g in 20% ethanol and 25.3 ng/g in champagne for 1,6-hexanediamine and 2.4 ng/g in 3% acetic acid, 3.1 ng/g in 20% ethanol and 28.3 ng/g in champagne (after accounting for the dilution) for isophorone diamine.

The MRM method developed resulted in limits of detection an order of magnitude lower than the quantification method developed using UPLC-IM-Q/TOF and, crucially, lower than the SML of the neo-formed compounds. Therefore, this technique was selected to quantify the migration of the neoformed compounds.

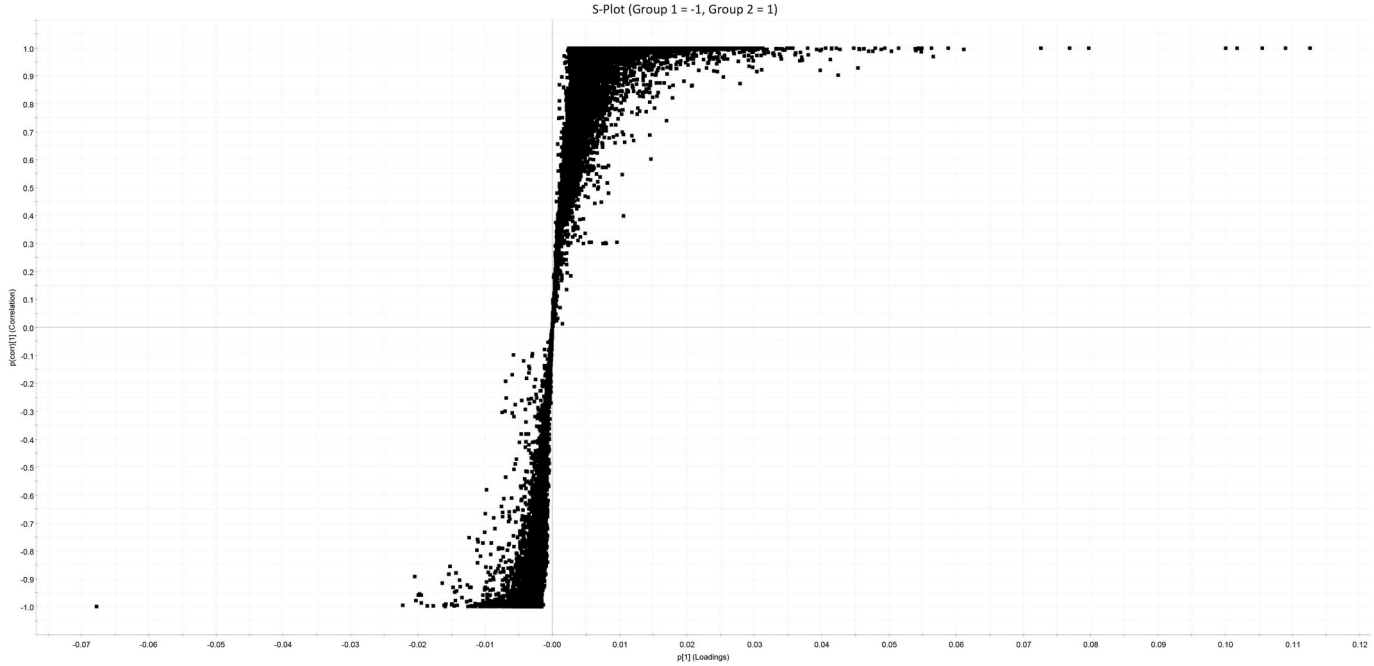


Fig. 2. S-plot, the upper right quadrant of the S-plot shows markers which are elevated in the extract (champagne with polyurethane), while the lower left quadrant elevated in the blank (champagne).

Table 2
Compounds found to migrate from PU adhesives in corks to simulants and champagne; UPLC-MS MRM transitions used, cone voltages, collision energies and migration values (ng/g).

Compound identified	Retention time (min)	MRM (*m/z*)	Cone voltage (V)	Collision energy (V)	SML mg/kg	Migration simulant B (ng/g)	Migration simulant C (ng/g)	Migration champagne (ng/g)
1,6-Hexanediamine	0.78	117.1 > 100.1	10	10	2.4	542.2 ± 12.3	358.3 ± 21.2	257.1 ± 31.2
Isophorone diamine	1.48	171.2 > 154.1	40	20	6	689.3 ± 25.6	357.5 ± 15.6	240.0 ± 25.3
6-isocyanatohexan-1-amine	2.13	143.1 > 126.1	30	20	0.09	258 ± 21	225 ± 17	148 ± 24
(5-isocyanato-1,3,3-trimethylcyclohexyl) methanamine	2.51	197.2 > 137.1	30	20	0.09	126 ± 8	89 ± 2	<LOD
N-(6-aminohexyl)acetamide	1.91	159.1 > 100.1	30	20	0.09	587 ± 28		
N,N'-(hexane-1,6-diyl)diacetamide	3.02	201.2 > 100.1	30	20	0.09	478 ± 46		
N-(3-(aminomethyl)-3,5,5-trimethylcyclohexyl)acetamide	3.33	213.2 > 137.1	30	20	0.09	478 ± 24		
N-((5-acetamido-1,3,3-trimethylcyclohexyl) methyl)acetamide	3.41	255.2 > 100.1	30	20	0.09	525 ± 47		
ethyl (6-aminohexyl)carbamate	2.13	189.2 > 143.1	30	20	0.09		721 ± 41	258 ± 14
ethyl (3-(aminomethyl)-3,5,5-trimethylcyclohexyl)carbamate	3.02	243.2 > 143.1	30	20	0.09		70 ± 10	<LOD
diethyl hexane-1,6-diyldicarbamate	3.13	261.2 > 143.1	30	20	0.09		88 ± 11	<LOD
1-(6-aminohexyl)-3-(6-isocyanatohexyl) urea	2.68	285.2 > 143.1	30	20	0.09		289 ± 9	200 ± 8
2-hydroxy-*N*-(6-isocyanatohexyl) propanamide	3.14	215.1 > 143.1	30	20	0.09			265 ± 15
N^1-(6-aminohexyl)-2,3-dihydroxy-N^4-(6-propionamidohexyl)succinanamide	3.30	403.3 > 143.1	30	20	0.09			289 ± 29
2-hydroxy-*N*-(5-isocyanato-1,3,3-trimethylcyclohexyl)methyl) propanamidel	3.51	269.2 > 137.1	30	20	0.09			254 ± 29
N-(6-(3-(3-(aminomethyl)-3,5,5-trimethylcyclohexyl)ureido)hexyl)-2-hydroxypropanamide	3.02	385.3 > 143.1	30	20	0.09			221 ± 12

3.3. Migration results

The results of the migration study of the polyurethane adhesive used in corks, detailed in section 2.4, are shown in Table 2. The migration tests were performed in accordance with Regulation 10/2011/EC in terms of temperature of exposure [9].

Most of the neo-formed compounds identified in the extract of the pure adhesive, described in section 2.2, were found to migrate from the corks to the simulants and champagne. Table 2 shows that migration of compounds ethyl (6-aminohexyl)carbamate, ethyl (3-(aminomethyl)-3,5,5-trimethylcyclohexyl)carbamate, diethyl hexane-1,6-diyldicarbamate and 1-(6-aminohexyl)-3-(6-isocyanatohexyl)urea), resulting from reaction 2 was lower in champagne than in 20% ethanol. This is most likely due to the lower proportion of ethanol in champagne, 11.5%, compared to that in 20% ethanol.

The level of migration of both diamines was below the SML established by Regulation 10/2011/EC [9]. However, the migration levels of most of the neo-formed compounds identified here, including those formed exclusively by the reaction between champagne and the monomers, exceeded their respective SML. This highlights the importance of performing the migration studies using real food in addition to using simulants. This is particularly true when there is the possibility that chemical interactions with real food can lead to the production of NIAS that would not be observed with the simulants.

4. Conclusions

Hyphenated traveling wave ion mobility-high resolution mass spectrometry has been successfully used to elucidate neo-formed compounds resulting from the reaction between a polyurethane adhesive used in cork stoppers and either food simulants or champagne. NIAS, not previously identified, have been observed in the simulants and were formed by the reaction between acetic acid or ethanol with the residual monomers from the PU adhesives (diisocyanates). In addition, other neo-formed compounds, that have not been reported previously, were identified in champagne and result from the reaction between the organic acids contained in the champagne and the residual monomers. A quantification method, based UPLC-TQ-MS in MRM mode to reach low limits of detection for the diamines studied, has also been developed. It has been shown that the combination of UPLC-IM-Q/TOF for identification of NIAS, and UPLC-TQ-MS for their quantification enables the complete migration process to be studied efficiently. The migration levels of all neo-formed compounds identified here, were found to exceed the SML. Some of the neo-formed compounds were found exclusively in the champagne extracts which highlights the importance of studying migration in real food samples in order to identify and quantify all potential NIAS.

Credit author statement

Elena Canellas: Conceptualization, Investigation, Supervision, writing; Paula Vera: Investigation, Supervision, writing; 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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