UPLC-ESI-Q-TOF-MS^E and GC-MS identification and quantification of non-intentionally added substances coming from biodegradable food packagings

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Abstract

Biodegradable packagings are made by combination of several materials creating a multilayer with the needed properties. Each material, including the adhesive, could contain substances that could migrate to the food. In this work, gas chromatography coupled to mass spectrometry (GC-MS) and ultra-high pressure liquid chromatography coupled to quadrupole time of flight mass spectrometry (UPLC–ESI-Q-TOF-MS^E) have been used to identify the biodegradable adhesive compounds. Five of the thirteen compounds identified were non- intentionally added substances (NIAS); they were neoformed compounds created by the reaction of added compounds in the adhesive. Moreover, the migration of the compounds was studied through four different biodegradable materials, paper, polylactide, Ecovio® and polyvinyl alcohol for the first time. Three of the thirteen compounds identified in the adhesive migrated from the adhesive to Tenax that was used as solid food simulant. One of them, 2,4,7,9-tetramethyl-5-decyne-4,7-diol, was an intentionally added substance and the other two were 1,6-dioxacyclododecane-7,12-dione and 1,6,13,18-tetraoxacyclotetracosane-7,12,19,24-tetraone that were NIAS identified in this work. Higher migration values (ranging from 0.81 to 2.07 mg/Kg) were observed through Ecovio® than through the multilayer made by combination of Ecovio®-polyvinyl alcohol (0.07 to 0.39 mg/Kg) due to the barrier effect provided by polyvinyl alcohol. Migration values through paper and polylactide were below the limits of detection.

Keywords

Ultra high pressure liquid chromatography coupled to quadrupole time of flight mass spectrometry, biodegradable packaging, non intentionally added substances, migration

1. Introduction

Food packaging industry is becoming increasingly interested in the usage of bioplastics for packaging due to the growing environmental awareness. According to the European Bioplastics organization, bioplastics can be defined as plastics based on renewable resources (biobased) or as plastics which are biodegradable and/or compostable [1]. Biopolymers are polymers produced by living organisms, they are polymeric biomolecules but are not necessarily biodegradable . It is the type of chemical bond which defines the biodegradability. In fact, there are several synthetic polymers that are biodegradable and compostable [2,3].

Most of the food packages are formed by combination of several materials creating a multilayer. It is due to the packaging requirements such as surface characteristics, thickness/body of the package, gas or aroma barrier, chemical resistance, sealability, formability or shrink properties, which are not found in a single material. In order to have the desired mechanical or physical properties it is necessary to combine the materials that provide specific characteristics. Depending on the materials used in the multilayer, they are created by coextrusion or lamination process using adhesives [4]. Biodegradable plastics are also combined in order to obtain multilayers. In fact, biodegradable adhesives have been recently designed in order to get totally compostable multilayers [5-14]. In this work the multilayers studied are built by the combination of several compostable materials bonded by a compostable adhesive.

Each material, including the adhesive that joins the different layers, is made of many components such as plasticizers , antioxidants, thermal stabilizers, tackifiers, thickeners, fillers, surfactants, emulsifiers, waxes, slip agents, light stabilizers, biocides, solvents, monomers and oligomers carrier or plasticizers that provide the properties needed [15,4].

Moreover, not only the intentionally added substances (IAS) mentioned before could appear in the material but also non intentionally added substances (NIAS) can be present. These NIAS could appear as impurities from the raw materials used, decomposition of the initial components or because of chemical interactions between them (neoformed compounds) or even with the food in contact with them [16-19].

When multilayers come in contact with foods all these chemicals, including NIAS, can migrate to the food and contaminate it. Consequently, the identification of the migrants from a food packaging material is an essential step. Migration from plastics, paper and adhesives have been broadly studied [20-23]. Nevertheless, no attention was paid yet to the migration from compostable multilayers. This work deals with the migration of compounds coming from a new biodegradable adhesive used to build several compostable multilayers. The different behavior of these migrants on the different compostable materials used to build the multilayers is also studied and discussed.

2. Experimental

2.1. Materials

The adhesive selected for the study was a water-base biodegradable adhesive. This adhesive is compostable according to EN 13432 [24]. It is normally used for the production of multilayer films based on a broad variety of compostable films and papers. The adhesive was supplied by Samtack (Barcelona, Spain).

The following compostable multilayer complexes made with the biodegradable adhesive were provided by Samtack for the migration study:

-Multilayer 1: Polilactide (PLA) film of a thickness of 20 µm -adhesive 4 g/m^2- offset paper of a grammage of 90 g/m^2-Multilayer 2 Ecovio® EXP 0.5 SL® film of a thickness of 40 µm- adhesive 4 g/m^2- Ecovio® EXP 0.5 SL® film of a thickness of 40 µm - Multilayer 3: Ecovio® EXP 0.5 SL® film of a thickness of 40 µm - adhesive 4 g/m^2- polyvinyl alcohol (PVOH) film of a thickness of 40 µm - adhesive 4 g/m^2- Ecovio® EXP 0.5 SL® film of a thickness of 40 µm

The following reagents: 1,4-butandiole, 2,4,7,9-tetramethyl-5-decyne-4,7-diol, butylated hydroxytoluene, bis (2-ethylhexylmaleate), adipic acid , 2-methyl-2H-isothiazol-3-one, 1,2-benzisothiazol-3(2H)-one and 1,4,7-trioxacyclotridecane-8,13-dione were purchased from Sigma–Aldrich Química S.A (Madrid, Spain).

HPLC-MS quality methanol and ultrapurified water were supplied by J. T. Baker (Deventer, The Netherlands).

Tenax® TA 80/100 mesh was supplied by Supelco (Bellefonte, USA).

2.2. Sample preparation and migration tests

In order to identify the compounds in the adhesive, solutions of the adhesive were prepared. 1 g of adhesive was dissolved in 100 g of metanol. The solution was filtered. Then 1 µL of this solution was analyzed by GC-MS/Q and 5 µL were analyzed by UPLC-MS-Q-TOF.

The migration study was done with poly(2,6-diphenyl-p-phenylene oxide) (Tenax) following the procedure optimized in previous works [25-27]. The cutouts of each multilayer, 5 x 5 cm in size, were placed in Petri dishes and covered with 1 gram of Tenax ® forming a uniform layer (4 g Tenax per dm^2 laminate in accordance with UNE-EN 14338 [28]). This system was kept in the oven at 40 ºC for 10 days. After that, it was extracted two consecutive times with 3.4 mL of methanol. The total solution containing the two extracts was concentrated under a stream of N_2 to 200 µL and analyzed by GC-MS/Q and UPLC-MS/QTOF. Three replicates of each laminate and substrate were studied.

2.3 Instrumentation

Gas chromatography-mass spectrometry_single quadrupole (GC-MS/Q)

The equipment used was a CTC Analytics CombiPal autosampler coupled to an Agilent 6890N gas chromatograph with a mass spectrometer MS 5975B detector. All of them from Agilent Technologies (Palo Alto, CA, USA).

The capillary column used was a HP-5MS (30 m x 0,25 µm x 250 µm) from Agilent Technologies (Madrid, Spain). The oven program was as follows: 40ºC for 2 min, with rate of 10°C/min up to 300 º C, maintained for 2 min. The injection type was splitless, the injection volume was 1 µL and the helium flow was 1ml/min. The acquisition was done in electron impact ionization (EI). The mass detector was set at SCAN mode (in the range m/z 45-350).

NIST 08 mass spectral search program version 2.0 was used for the identification of the compounds.

Ultra high pressure liquid chromatography-mass spectrometry_quadrupole_time of flight (UPLC-MS/Q-TOF)

Chromatography was carried out in an Acquity^{TM} system using an Acquity UPLC BEH C18 column of 17 µm particle size (2.1 mm x 100 mm), both from Waters (Milford, MA, USA)

Chromatography was carried out at 0.3 mL/min column flow and the column temperature was 35ºC. The gradient used here was 5-95% methanol, (0-10min) and the volume of sample injected was 5 µL.

The detector consisted of an API source (atmospheric pressure ionization) with an electrospray interface (ESI) coupled to mass spectrometer consisting of a hexapole, a cuadrupole, a collision cell and a time of flight analyzer (QTOF) Xevo G2 from Waters (Milford, MA, USA).

The electrospray probe was set in both positive (ESI+) and negative (ESI-) modes. The corona voltage was 2.5 kV for (ESI+) and 0.5 kV for (ESI-), the sampling cone voltage was optimized between 20 and 50V, finally 30 V was selected for the screening because more peaks were detected. Nitrogen was used as a desolvation gas, the flow was 500 L h^{-1} at a temperature of 400 °C, the cone gas flow was 20 L h^{-1}.

MS^E mode was selected for the acquisition; collision ramp energy from 5 to 30 V was used. The mass range considered was 10-1200 Da. Data were collected in centroid mode and the sensitivity analyzer mode was selected. The accuracy and reproducibility of all the analyses was guaranteed by using a lockspray. Leucine-enkephalin was employed as the lock mass at a concentration of 2 ng mL^{-1} in water/acetonitrile with 0.1% formic acid and a flow rate of 5 µL min^{-1}. MassLynx v.4.1 software (Waters, Milford MA, USA) was used to analyze the samples.

3. Results and discussion

3.1. Identification

In this work, non-targeted analysis of the sample was done in order to identify all the components that are included in the adhesive before studying their migration.

In the first place, the identification of the volatile compounds in the adhesive was carried out by GC-MS/Q. NIST 08 returned a "hit list" of matched chemical compounds from the library. Then, standards of the compounds were analyzed for confirmatory purposes. Figure 1 shows the chromatogram obtained by GC-MS/Q. Six peaks were observed in the chromatogram and they were identified following the procedure explained. Five out of the six compounds were normal constituents of an adhesive formulation, 1,4-butandiole (RT 7.7 min) that is a common monomer used in adhesives [29], 2,4,7,9-tetramethyl-5-decyne-4,7-diol (RT 14.1 min) that was the non- ethoxylated derivative of 2,2'-((2,4,7,9-tetramethyldec-5-yne-4,7-diyl)bis(oxy))diethanol (RT 16.7 min), butylated hydroxytoluene (RT 15.4 min) that is a common antioxidant [30], 2,2'-((2,4,7,9-tetramethyldec-5-yne-4,7-diyl)bis(oxy))diethanol (RT 16.7 min) that is a surfactant commonly used in water based adhesives [31] and bis(2-ethylhexylmaleate) (RT 22.0 min) that is a common plasticizer (dioctyl maleate) [30]. The compound with the dominat peak in the chromatogram was 1,6-dioxacyclododecane-7,12-dione (RT 16.3 min). This compound wasn't found as a normal constituent of an adhesive formulation. This compound is not commercially available but Watanabe et al. [32] reported the identification of this substance as a degradation product in resins, so it could be a NIAS. However, Watanabe et al. did not deep in the exact reaction that lead to this compound.

The identification of non-volatile compounds was carried out by UPLC-MS/Q-TOF. This technique provides molecular fragmentation combined with mass accuracy in order to elucidate the molecular structure that could lead to the identification of the compounds. Conditions used for the acquisition are explained before. The mass range acquired was limited up to 1200 Da because it was considered unlikely that compounds heavier than 1000 Da would migrate from the adhesive to the food. The chromatogram was acquired in MS^E mode. MS^E mode is a method of data acquisition that involves the fast alternation between two energy conditions, thus providing the accurate mass of the precursor ion, in addition to fragment ions, for further confirmatory purposes. As a result, two functions are obtained in a single run. Function 1 that is the low-energy function that provides the precursor ion spectra that can lead to a molecular formula of the compound. Function 2, the high energy function, which is the result of applying a ramp of collision voltage in the collision cell. Mass fragments that can lead to a structure elucidation are obtained in this function.

Figure 2 shows the chromatogram obtained by UPLC-MS/Q-TOF ESI+ mode Spectra obtained with function 1 contained the accurate mass for the molecular ion. Two criteria were used to establish its elemental formula: a) the i-Fit, that is the probability that the isotope pattern of a particular elemental composition in the list of results matches the peaks in the measured spectrum and b) the mass tolerance, that was set at 3 mDa. Once the molecular formula of each accurate mass were known, it was necessary to use a database of chemical compounds and to know the typical composition of an adhesive in order to elucidate the possible compounds that could be present in the sample. For this work the databases [www.chemspider.com] [33] and [www.scifinder.org] [34] were used in order to obtain a list of candidates for the identification. A bibliographic search was done about if these compounds are commonly used to manufacture adhesives. Then, using the high energy function (function 2) the fragmentation spectra were obtained. The accurate masses of the fragments were considered in order to find out if they could be generated from the candidates obtained in the databases and then confirm their identification.

Following these procedure, three non volatile compounds were identified. Adipic acid was identified using ESI- mode. It is a common monomer used in adhesives [29]. On the other hand, using ESI+ mode 2-methyl-2H-isothiazol-3-one (RT 1.7 min) and 1,2-benzisothiazol-3(2H)-one (RT 3.8 min) were identified. These compounds are two common biocides used in water-base adhesives [30]., However, the compounds with the high areas in the chromatogram (RT 5.7, 6.3, 6.6 and 6.8 min) still remained as unknown compounds as the fragments of the candidates proposed by the databases did not match with the fragments obtained experimentally by the Q-TOF.

We hypothesized that both the volatile compound 1,6-dioxacyclododecane-7.12-dione and the four unknown compounds could be neoformed compounds coming from the reaction between some volatile and non-volatile compounds identified.

Figure 3 shows the reaction of the two compounds identified in the sample, butane-1,4-diol and adipic acid, proposed for the formation of the compound 1,6-dioxacyclododecane-7,12-dione.

The first attempt was to assume that these two compounds could react to form bigger lactones with the same structure (figure 3). Taking into account this hypothesis, the molecular mass of these neoformed compounds was calculated. It matches with the m/z of the unknowns compounds minus the sodium mass since these compounds formed sodium adducts by the electrospray ionization (figure 2: 423.2022, 623.3059, 823.4108 and 1023.5125). Then, the accurate masses of the fragments of these compounds were considered in order to find out if they could be generated from these candidates obtained by the reaction between butane-1,4-diol and adipic acid.

Figure 4 shows the high energy spectrum (function 2) of the compound 1,6,13,18-tetraoxacyclotetracosane-7,12,19,24-tetraone (RT 5.7 min). As it can be seen, all the fragments match with the fragments of the proposed candidate. The same happened with the other three compounds showed in table 2, 1,6,13,18,25,30-Hexaoxacyclohexatriacontane-7,12,19,24,31,36-hexone (RT 6.3 min); 1,6,13,18,25,30,37,42-octaoxacyclooctatetracontane-7,12,19,24,31,36,43,48-octaone (RT 6.6 min) and 1,6,13,18,25,30,37,42,49,54-decaoxacyclohexacontane-7,12,19,24,31,36,43,48,55,60-decaone (RT 6.8 min). There are not standards commercially available for these compounds for the confirmation step. Nevertheless, the identification of the compounds 1,6-dioxacyclododecane-7,12-dione, butane-1,4-diol and adipic acid, followed by the match with accurate masses of the experimentally obtained fragments by UPLC-MS/Q-TOF with these lactones lead to consider that the identification was reliable.

3.2. Migration study

Once the composition of the compostable adhesive was known the migration of these compounds was studied when the adhesive takes part of a multilayer. This adhesive is a new compostable adhesive so it should be used to form multilayer complexes with compostable materials in order to form compostable packaging.

The multilayer complexes studied were based on several compostable films found in the market, PLA (polilactic acid), Ecovio® (that consist of the biodegradable Ecoflex® (fossil basis) and polylactic acid (PLA)), compostable PVOH and paper. The description of the samples under study is described in the experimental section.

None of these multilayer complexes are barrier to liquids. They are intended to be used for dry food. Because of that, it was decided to do the migration assays with Tenax following the indications described in both Regulation EC/10/2011 and UNE-EN 14338.

Tables 1 and 2 show limits of detection, migration values to Tenax® and the estimated daily intake (EDI) for both sides of the multilayer 1, multilayer 2 and multilayer 3. The estimated daily intake (EDI) was calculated according the equation established by the FDA (Food and Drug Administration of United States) (FDA, 1995. Recommendations for Chemistry Data for Indirect Food Additive Petitions):

EDI(mg/personxday)=migration (mg/Kg)x3Kg(food intake per person and day)xC_F

Where C_F is the fraction of the daily diet expected to be in contact with a specific packaging material (for adhesives this is 0.14).

Migration of all the compounds previously identified was below the limits of detection in multilayer 1. However, three compounds migrated from multilayers 2 and 3, 2,4,7,9-tetramethyl-5-decyne-4,7-diol, 1,6-dioxacyclododecane-7,12-dione and 1,6,13,18-tetraoxacyclotetracosane-7,12,19,24-tetraone (migration values were 0.21, 2.07 and 0.81 mg/Kg respectively for multilayer 2 and 0.04, 0.39 and 0.07 respectively for multilayer 3) . Two of these compounds were neoformed substances, NIAS, coming from the adhesive. It highlights the importance of this study since without the previous identification the pattern recognition of these NIAS would not be possible.

Multilayer 1 was built from paper and PLA, and these two materials seem to be acting as barrier for these compounds. Nevertheless, in the case of multilayers 2 and 3, the compounds were able to pass through the materials they were made of. This is the first time that migration of these kinds of multilayers is studied. Ecovio® is a material that consists of Ecoflex® (fossil basis) and polylactic acid (PLA). Ecoflex® provides better mechanical properties to polylactic acid (PLA), but this work evidences that it provides worst barrier properties to some compounds than PLA itself.

On the other hand, the effect on the migration through the multilayer provided by the biodegradable PVOH was studied. The migration through multilayer 2, that was built with Ecovio® and adhesive, was compared with the migration through multilayer 3, that also contained PVOH with the objective to minimize the water and oxygen permeability through the multilayer [4]. Higher migration values were observed in multilayer 2 than in multilayer 3. Therefore, the presence of PVOH decreases the migration compared with the multilayer 2. Then it can be concluded that PVOH not only decreases the permeability of water and oxygen but also the migration of these compounds coming from the adhesive.

Adhesives are not yet covered by a specific European legislation but as any food contact material they have to comply with the frame Regulation 1935/2004/CEE. Then, for adhesives to be used in plastic materials and articles adequate information should be provided to the manufacturer of the final plastic article that would enable him to ensure the compliance to both Regulation 1935/2004/CEE and 10/2011/EU. It means that the migration of the compounds should be below the limit established in this Regulation. Table 3 shows the specific migration limit for the substances found that are included in the positive list of Regulation EU/10/2011. To evaluate if the rest of the compounds found could endanger the human health a bibliographic search about their LOAEL was done. However, the LOAEL or the toxicity of these neoformed compounds has not been studied yet. Then, the evaluation of the safety of these materials poses a problem because toxicity of the compounds is difficult to predict. Therefore, only a theoretical evaluation could be done. The theoretical evaluation based on the Cramer rules and TTC (Threshold of Toxicological Concern) was selected. This classification assigns the toxic effect of a substance according to its molecular structure [35]. The neoformed compounds found here were classified in the lowest toxicity class (table 3). The estimated daily intake (EDI) of these migrants (table 1 and 2) was below the threshold established by the International Life Sciences Institute (ILSI) for this class of toxicity [36]. Then it can be concluded that, taking into account this theoretical toxicity data, the adhesive used in these multilayers will be safe for food contact applications.

4. Conclusions

This work has established that the identification of the compounds present in a sample intended to be in contact with food is an essential step, since the compounds can react between them producing neoformed substances that could be present in the final sample even at higher concentrations than the intentionally added substances.

This work has studied for the first time the migration of compounds coming from a compostable adhesive through different industrial biodegradable materials. It has been proved that non-intentionally added substances (NIAS) that have been identified in the adhesive studied here, are able to diffuse through some of the biodegradable materials migrating to the food simulant used here for the migration assays.

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Table 1. Compounds identified by GC-MS, limits of detection expressed as mg/Kg, migration values to Tenax® (mg/Kg) from both sides of the multilayer 1 (PLA -adhesive 4 g/m^2-paper), multilayer 2: ecovio®-adhesive 4 g/m^2-ecovio® and multilayer 3: ecovio®- adhesive 4 g/m^2-PVOH- adhesive 4 g/m^2-ecovio® and estimated daily intake (mg/Kg/day).

RT (min)	Compound	LOD	Multilayer 1 (PLA side)		Multilayer 1 (paper side)		Multilayer 2		Multilayer 3	
		mg/Kg	mg/Kg	mg/Kg/d	mg/Kg	mg/Kg/d	mg/Kg	mg/Kg/d	mg/Kg	mg/Kg/d
7,7	1,4-Butandiole	0.1	<LOD		<LOD		<LOD		<LOD	
14.1	2,4,7,9-tetramethyl-5-decyne-4,7-diol	0.03	<LOD		<LOD		0.21	0.08	0.04	0.02
15.4	Butylated hydroxytoluene	0.3	<LOD		<LOD		<LOD		<LOD	
16.3	1,6-dioxacyclododecane-7,12-dione[1]	0.6[1]	<LOD		<LOD		2.07	0.86	0.39	0.16
16.7	2,2'-((2,4,7,9-tetramethyldec-5-yne-4,7-diyl)bis(oxy))diethanol[2]	0.03	<LOD		<LOD		<LOD		<LOD	
22.0	Bis (2-ethylhexylmaleate)	3	<LOD		<LOD		<LOD		<LOD	

[1] Compound quantified with 1,4,7-trioxacyclotridecane-8,13-dione

[2] Compound quantified with 2,4,7,9-tetramethyl-5-decyne-4,7-diol

Table 2. Retention times of the compounds identified by UPLC-MS/Q-TOF, number of the compound in the chromatogram, measured mass, type of ion found, ΔmDa (measured mass- calculated mass from the formula), formula, compound name, limits of detection expressed as mg/Kg, migration values to Tenax® (mg/Kg) coming from both sides of the multilayer 1 (PLA -adhesive 4 g/m^2-paper), multilayer 2: ecovio®-adhesive 4 g/m^2-ecovio® and multilayer 3: ecovio®- adhesive 4 g/m^2-PVOH- adhesive 4 g/m^2-ecovio® and estimated daily intake (mg/Kg/day).

RT)ESI ESI le	Nº	Measured mass [ion found]	ΔmDa	Formula	Compound	LOD	Multilayer 1 (PLA side)		Multilayer 1 (paper side)		Multilayer 2		Multilayer 3	
							mg/Kg	mg/Kg/d	mg/Kg	mg/Kg/d	mg/Kg	mg/Kg/d	mg/Kg	mg/Kg/d
1.9 ESI -		145.0505 [-H]	0.4	$C_6H_{10}O_4$	Adipic acid	0.05	<LOD		<LOD		<LOD		<LOD	
1.7 ESI +	1	116.0178 [+H]	0.8	C_4H_5NOS	2-methyl-2H-isothiazol-3-one	0.002	<LOD		<LOD		<LOD		<LOD	
3.8 ESI+	2	152.0180 [+H]	1.0	C_7H_5NOS	1,2-Benzisothiazol-3(2H)-one	0.004	<LOD		<LOD		<LOD		<LOD	
5.7 ESI+	3	423.2022 [+Na]	2.7	$C_{20}H_{32}O_8$	1,6,13,18-tetraoxacyclotetracosane-7,12,19,24-tetraone	0.005[1]	<LOD		<LOD		0.81	0.34	0.07	0.03
6.3 ESI+	4	623.3059 [+Na]	1.6	$C_{30}H_{48}O_{12}$	1,6,13,18,25,30-Hexaoxacyclohexatriacontane-7,12,19,24,31,36-hexone	0.005[1]	<LOD		<LOD		<LOD		<LOD	
6.6 ESI+	5	823.4108 [+Na]	1.6	$C_{40}H_{64}O_{16}$	1,6,13,18,25,30,37,42-octaoxacyclooctatetracontane-7,12,19,24,31,36,43,48-octaone	0.005[1]	<LOD		<LOD		<LOD		<LOD	
6.8 ESI+	6	1023.5125 [+Na]	1.6	$C_{50}H_{80}O_{20}$	1,6,13,18,25,30,37,42,49,54-decaoxacyclohexacontane-7,12,19,24,31,36,43,48,55,60-decaone	0.005[1]	<LOD		<LOD		<LOD		<LOD	

[1] Compounds quantified with 1,4,7-trioxacyclotridecane-8,13-dione

Table 3. Specific migration limits (mg/Kg), lowest observed adverse effect level (LOAEL) (mg/Kg/day) and human exposure threshold (mg/Kg/day) of the compounds studied.

Compound	SML (mg/Kg)	LOAEL (mg/Kg/day)	HET (mg/Kg/day)
1,4-Butandiole	5 (Regulation EU/ 10/2011)		
2,4,7,9-tetramethyl-5-decyne-4,7-diol		200	
Butylated hydroxytoluene	3 (Regulation EU/ 10/2011)		
1.6-dioxacyclododecane-7,12-dione[1]			1.8 (class I)
2,2'-((2,4,7,9-tetramethyldec-5-yne-4,7-diyl)bis(oxy))diethanol[2]		200	
Bis (2-ethylhexylmaleate)		30	
Adipic acid	No limit (Regulation EU/ 10/2011)		
2-methyl-2H-isothiazol-3-one	0.5 (Regulation EU/ 10/2011)		
1,2-Benzisothiazol-3(2H)-one	0.5 (EFSA[1])		
1,6,13,18-tetraoxacyclotetracosane-7,12,19,24-tetraone			1.8 (class I)
1,6,13,18,25,30-Hexaoxacyclohexatriacontane-7,12,19,24,31,36-hexone			1.8 (class I)
1,6,13,18,25,30,37,42-octaoxacyclooctatetracontane-7,12,19,24,31,36,43,48-octaone			1.8 (class I)
1,6,13,18,25,30,37,42,49,54-decaoxacyclohexacontane-7,12,19,24,31,36,43,48,55,60-decaone			1.8 (class I)

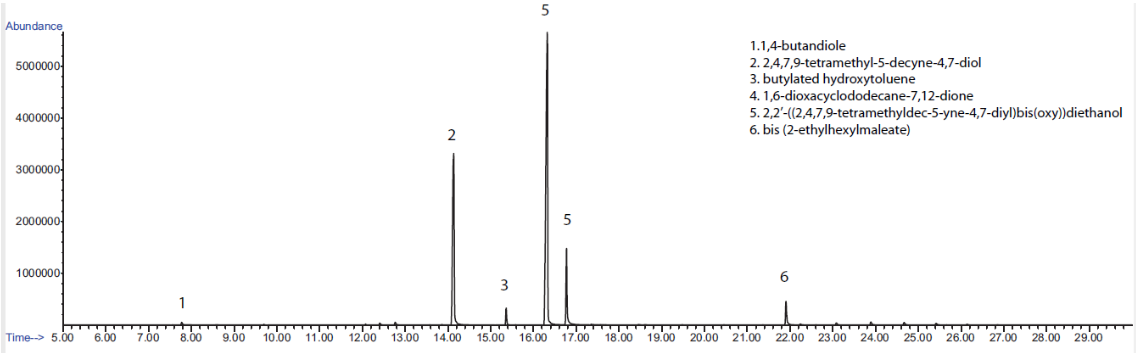

Fig 1 Chromatogram of the biodegradable adhesive obtained by GC-MS, number of the peaks and compound names.

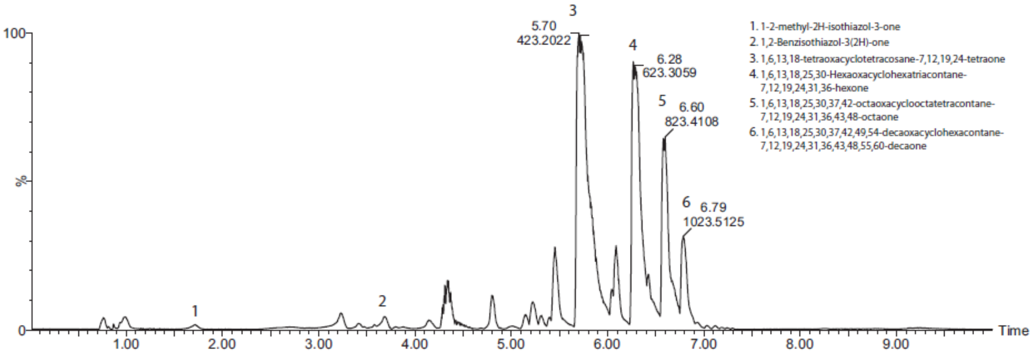

Fig 2 Chromatogram of the biodegradable adhesive obtained by UPLC-MS/Q-TOF number of the peaks and compound names.

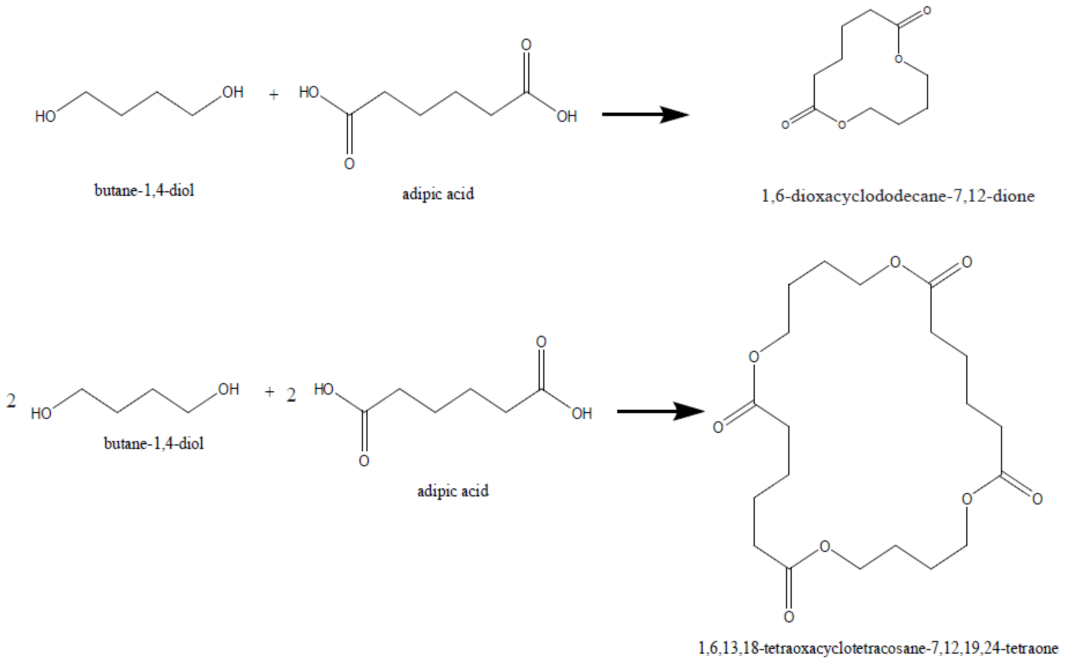

Fig 3 Reaction of adipic acid with butane-1,4-diol to form several NIAS identified by UPLC-MS/Q-TOF

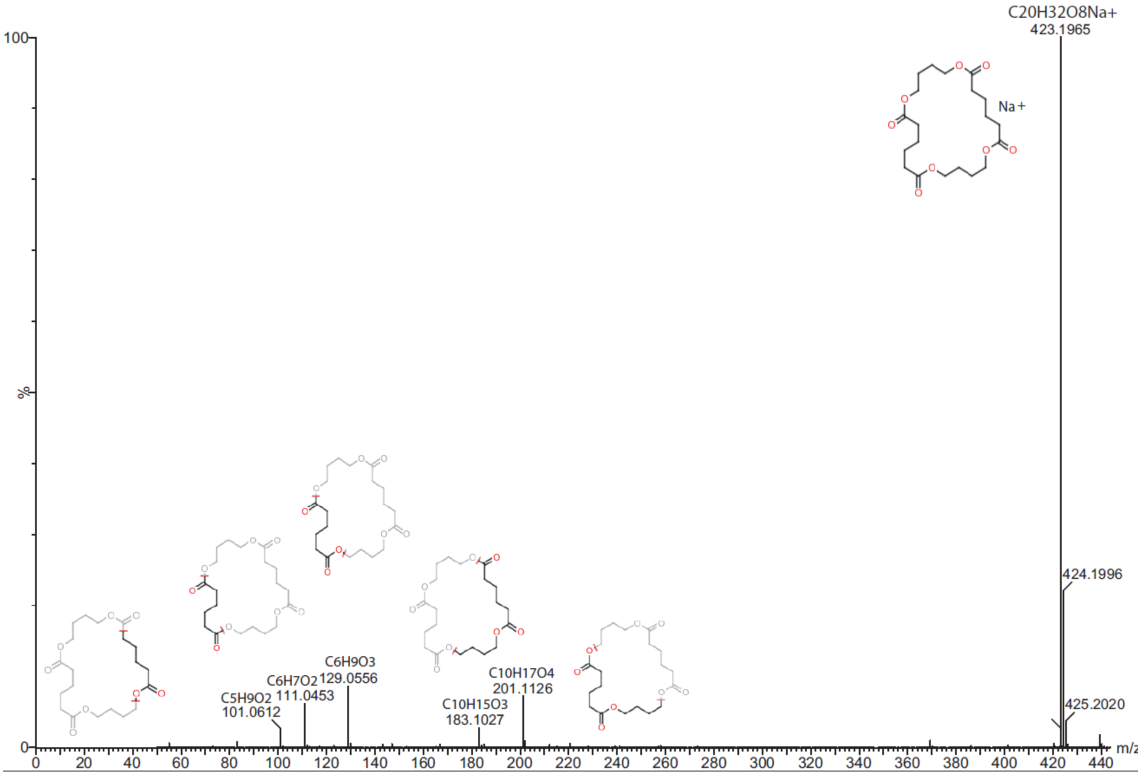

Fig 4 High energy spectrum of the compound 1,6,13,18-tetraoxacyclotetracosane-7,12,19,24-tetraone obtained by UPLC-MS/Q-TOF.