The occurrence of Bisphenol A and Phthalates in Portuguese wines and the migration of selected substances from coatings in contact with a wine simulant

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Resumo

O Bisfenol A (BPA) e ésteres do ácido ftálico (ftalatos) são substâncias que podem ocorrer em diversos alimentos por contaminação proveniente de várias fontes, nomeadamente através da migração a partir de materiais e embalagens. Estas substâncias podem ser prejudiciais à saúde dado apresentarem propriedades de disrupção endócrina, por isso o seu controlo é muito importante. No presente trabalho foi realizado um estudo para a determinação da ocorrência de BPA e ftalatos em vinhos disponíveis no mercado e foi feita uma estimativa da exposição do consumidor a estes contaminantes a partir da ingestão de vinho. Dada a inter-relação entre estas substâncias e os materiais em contato com alimentos, incluindo vinhos, foi feito também um estudo de migração de dois revestimentos (epóxi-fenólico e poliéster-fenólico) usados em embalagens metálicas.

O BPA foi monitorizado em 16 amostras de vinho por cromatografia HPLC/FD. Este composto foi detetado em 13 amostras em níveis acima do limite de deteção (0,57 µg/l), sendo a concentração mais elevada de 3,52 µg/l, encontrada num vinho tinto. A exposição do consumidor ao BPA foi estimada usando valores médios de limite inferior e limite superior de BPA calculados a partir dos dados experimentais (LI e LS) para um consumo de vinho médio (0,116 l) e elevado (0,75 l). Os resultados para o consumo médio foram de 4,8% (LI) e 7,2% (LS) relativamente ao t-TDI atual (0,04 µg / kg de peso corporal dia) enquanto que para o consumo elevado foram de 31% (LI) e 46,5% (LS).

Foram determinados 11 ftalatos em 19 amostras de vinho, das quais 3 eram vinhos do Porto, por GC/MS. Pelo menos um ftalato foi detetado em todas as amostras analisadas. Os dois ftalatos mais comuns foram o dietil hexil ftalato (DEHP) e o dibutil ftalato (DBP) com concentrações médias variando nos intervalos 30,33 µg/l - 39,59 µg/l e 21,91 µg/l - 22,59 µg/l, respetivamente. A exposição do consumidor foi também estimada, obtendo-se resultados inferiores a 1% do TDI em vigor, para o consumo médio e elevado. Os três vinhos do Porto apresentaram valores significativamente elevados de DBP, a amostra mais contaminada atingiu 825 µg/l. Além disso, o di-isobutil ftlalato (DiBP), um composto não permitido em materiais destinados ao contato com alimentos, foi encontrado em níveis de 106 µg/l numa das amostras.

A migração a partir de revestimentos de resina epóxi e poliéster foi analisada segundo diferentes técnicas analíticas. Em primeiro lugar foram realizados ensaios para identificar as substâncias potencialmente migrantes. As amostras foram analisadas por GC/MS, acoplada a injeção após dessorção térmica direta com e sem extração prévia. Foram testados diversos solventes de extração (acetonitrilo, diclorometano e iso-octano). Os resultados permitiram selecionar três migrantes de cada revestimento para monitorização em condições de migração: BPA, ε-Caprolactona e óxido de fosfina-butil(difenil) do revestimento de epóxi, e, butil(difenil)-neopentilglicol, um oligómero cíclico e 1,3 dioxano-5-metanal-5-etil do revestimento de poliéster. Foi feito um estudo de migração destes compostos usando uma solução etanólica a 20%, a 60 ° C durante 3, 5 e 10 dias. O BPA, o óxido de fosfina-butil(difenil), o oligómero cíclico e o dioxano apresentaram um libertação mais rápida do que o ε-Caprolactona e o butil(difenil)-neopentilglicol.

Abstract

This work studied the occurrence of bisphenol A (BPA) and phthalates in commercially available wines along with the migration of compounds from coatings commonly used in food applications.

BPA was monitored in 16 wine samples using a HPLC/FD chromatographic methodology. Thirteen samples contained the contaminant at levels above the Limit of Detection (0.57 μg/l); the most contaminated sample was a red wine with 3.52 μg/l. An exposure assessment was performed using lower bound (LB) and higher bound (HB) averages calculated from the data, for an average wine consumer (0.116 l) and a high wine consumer (0.75 l). The results for an average consumer were 4.8% (LB) and 7.2% (HB) of the current t-TDI (0.04 μg/ kg bw day), while for high consumers the results were 31% (LB) and 46.5% (HB).

Eleven phthalate compounds were monitored in 19 wine samples, 3 of which were fortified wines, by means of a GC/MS detection method. At least one phthalate was detected in all the samples analyzed; the two most common contaminants were Diethyl Hexyl Phthalate (DEHP) and Dibutyl Phthalate (DBP) with averages ranging 30.33 μg/l -39.59 μg/l and 21.91 μg/l – 22.59 μg/l respectively. An exposure assessment was made; the estimated daily intakes from wine consumption were below 1% of the TDIs in vigor for both average and high consumers. The three Port wines were significantly contaminated by DBP, with the most contaminated sample reaching 825 μg/l. Additionally, Di-isobutyl phthalate (DiBP), which is non-permitted food contact material, was found at levels of 106 μg/l in one sample.

A migration study was made using epoxy and polyester coatings used in food contact materials. The samples were first screened using GC/MS, by means of a direct thermal desorption method and a liquid extraction using aggressive solvents (acetonitrile, dichloromethane and iso-octane).

The results allowed selecting three migrants from each coating: BPA, ε-Caprolactone and butyldiphenyl phosphine oxide from the epoxy coating and neopentyl glycol, a cyclic oligomer and 1,3 Dioxane-5-methanal-5 ethyl from the polyester coating. While BPA, butyldiphenyl phosphine oxide, the cyclic oligomer and the dioxane were rapidly released in the simulant; a slower migration rate was observerd for ε-Caprolactone and neopentyl glycol.

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Abbreviations

BBP – Benzyl butylphthalate

BPA – Bisphenol A

DBP – Di-butyl phthalate

DBP – Di-butylphthalate

DCHP – Di-cyclohexyl phthalate

DEHP – Di-ethylhexyl phthalate

DEP – Di-ethyl phthalate

DHP – Di-hexyl phthalate

DIBP – Di-isobutyl phthalate

DIDP – Di-isodecyl phthalate

DINP – Di-isononyl phthalate

DMP – Di-methyl phthalate

DNOP – Di-n-octyl-phthalate

ER – Epoxy Resin

GC-MS – Gas Chromatography with Mass Spectrometry detection

HPLC-FD – High Performance Liquid Chromatography with Fluorescence Detection

LOD – Limit of Detection

LOQ – Limit of Quantification

PC – Polycarbonate Plastic

PE – Polyester

PVC – Polyvinylchloride

t-TDI – Temporary Tolerable Daily Intake

TDI – Tolerable Daily Intake

WHO – World Health Organization

AC – Acetonitrile

HB – Higher Bound

LB – Lower Bound

EtOH – Ethanol

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1 Introduction

The use of some food contact materials, such as packaging, is unavoidable. It is important to ensure that dangerous chemicals do not migrate from these materials into food in quantities able to risk consumer health. Two commonly monitored contaminants, with endocrine disrupting properties, are Bisphenol A (BPA) and esters of phthalic acids, also known as phthalates.

The principal reason for the importance to control phthalates, on top of their potential toxicity, is how widespread they are: they have become ubiquitous environmental contaminants (Swan 2008). BPA, while also very widespread in the environment, has been at the centre of a lot of attention these past years as a result of new findings that may indicate a greater toxicity of the substance than was previously thought (Teeguarden & Hanson-Drury 2013). Properties of foods and conditions of contact influence the migration behaviour. For example the ethanol content may favour the migration of these contaminants.

Portugal is an important wine producer; it was estimated by OIV that it was the 11th greatest wine producer in the world, with a production surpassing 6 mhl in 2014 (OIV 2015). Wine may contain BPA and phthalates, as is evidenced by studies that will be described more in depth in the following sections. Therefore, it is important to monitor the presence of these foreign substances in Portuguese wines.

Phthalates may migrate into wine as a result of their use into several materials such as vats, pipes, pumps, hoses, gaskets and containers. The main source of contamination of BPA is, on the other hand, related to the internal coating of storage vats, which normally are epoxy-based.

The work performed had three specific objectives: (i) to monitor the occurrence of BPA and phthalates in Portuguese wines, (ii) to evaluate the potential migration from coatings to a wine simulant and (iii) to estimate the exposure of consumers to these contaminants while wine sourced.

Wine samples were collected from local producers and were analysed to detect, identify and quantify BPA and phthalates present (objective i). The concentration values found were used to estimate consumer exposure (objective iii). Two different coatings, one BPA based (epoxy-phenolic) and one BPA-free (polyester-phenolic) were tested to evaluate the potential migrants present (objective ii).

1.1 Bisphenol A and Phthalates

1.1.1 Bisphenol A

With a molecular formula $C_{15}H_{16}O_2$, BPA, shown in figure 1, has moderate solubility in water at room temperature, ranging from 120 to 300 mg/L, due to its predominantly hydrophobic structure (Staples et al. 1998). BPA is a well-known fluorophore, reason for which fluorescence detection is commonly used to detect and quantify it (Gomez et al. 2006).

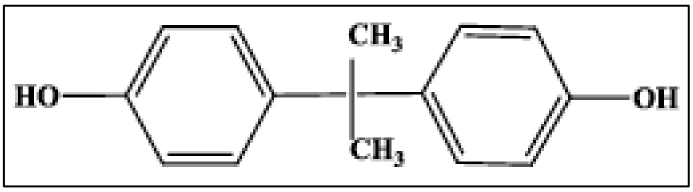


Figure 1: Structure of Bisphenol A

BPA was first commercialized in the 1950s, after its potential use as building block of for the production polycarbonate plastics (PCs) and epoxy resins (ERs) was discovered (Vogel 2009). Its production has thereafter become very significant, with a production capacity estimated being worth £10 billion in 2011 (vom Saal et al. 2012). Two grades of BPA are generally produced: one, with a maximum content of 0.2% of 2,4-isopropylidenediphenol is used for the synthesis of polycarbonate (PC) plastics, and another, with up to 7% of the unwanted isomer, is generally used in the manufacture of epoxy resins (ER) (Groshart et al. 2001). Other applications involve the use of BPA as an additive, examples include: polyvinylchloride plastics, methacrylate resins, polyetheramides, polyarylates, flame retardants, thermal and recycled paper (EFSA 2015).

BPA is a well-established endocrine disruptor, a result of its structural similarity with the estrogen receptor, as shown in figure 2 (Wang et al. 2012; Markey et al. 2001; Timms et al. 2005; Patricia A. Hunt et al. 2003). Because of this hormonal behavior and of its ubiquity, a lot of research has been performed to elucidate the toxicology of BPA. BPA has been associated with, among other things, obesity (Carwile & Michels 2011; Wang et al. 2012), cardiovascular disease (Melzer et al. 2010) and behavioral problems (Braun et al. 2011).

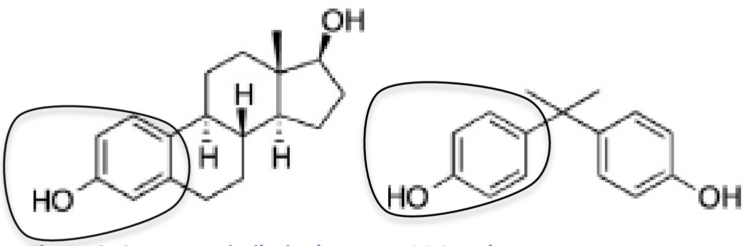


Figure 2: Structure similarity between BPA and estrogen receptor

Throughout the last decade several exposure assessments have been performed. Their aim was to determine whether exposure levels to BPA are high enough to cause the health issues evidenced by toxicological studies. In the following paragraphs, assessments made by EFSA will be described.

In 2006, EFSA set a Tolerable Daily Intake (TDI) of 50 µg/kg bw day, and calculated exposure levels well below this figure; the Specific Migration Limit (SML) of 0.6 mg BPA/kg food was therefore left unchanged. Given some studies, performed after the 2006 EFSA assessment, described potential toxicity of BPA at lower doses than what was previously thought, a new evaluation of BPA was made in 2015 (EFSA 2015). An interesting feature of the new assessment was that non-food exposure was also included into the exposure assessment.

The outcome was the introduction of a temporary TDI (t-TDI), set at 4 µg/kg bw day. This is more than 12 times lower than the previously set TDI. This change was explained by a more refined risk assessment methodology and by new data available for the assessment. This TDI is temporary because there is pending a long term toxicity study still under development. Exposure estimates were also found to be lower than the previous evaluation (4 to 15 times), as a result of less conservative assumptions and the use of higher quality data. The final assessment was that BPA did not pose a significant risk to the population, since the exposure level was calculated being 3-5 times lower than the t-TDI.

While these exposure assessments are reassuring, the use of BPA in baby bottles was restricted in the EU in 2011, as a result of the EC directive 20011/8/EU. The following year the FDA acted similarly and also banned BPA from these products in the USA. Some European national governments have adopted extra restrictions on the use of BPA. Examples are France, where BPA has been banned from all food contact materials in 2015, or Denmark, where the ban was applied to food contact materials of foods targeting children up to 3 years of age.

Exposure to BPA may come from a wide range of products such as thermal paper, sunglasses, building and medical materials (Seit & Cedex 2011; Biedermann et al. 2010). The most impactful exposure route is, however, the human diet (EFSA 2015). Given the great majority of BPA use is directed towards the production of polycarbonate plastics and epoxy resins (EFSA 2015), migration from these products into food may be significant.

1.1.2 Phthalates

Phthalates are anthropogenic, environmentally ubiquitous contaminants. They are generally synthesized reacting phthalic anhydride with an alcohol, which can contain up to 13 carbon atoms, with a catalytic amount of sulfuric acid. The size of the R chain is used to categorize phthalates into high (R = 6 or more carbon atoms) or low (R= less than 6 carbon atoms) molecular weight phthalates.

The phthalate that has historically been the most used as a plasticizer in the production of PVCs, is di-ethylhexyl phthalate DEHP (Fasano et al. 2015). DEHP, like most phthalates, is lipophilic and is scarcely soluble in water; it may be found in plastics at concentrations of up to 40% w/w (ATSDR 2002).

Phthalates are very widespread in the environment as result of their huge production volumes, only in Europe 1 billion tons were produced in 2010 (European Chemicals Agency 2013). Another reason, which relates to materials such as PVCs where they are used as plasticizers, is the absence of a covalent bond between the phthalates and the main polymer, which allows them to migrate very easily, especially when subject to high temperatures or in the presence of lipophilic substances (Fromme et al. 2002). The ubiquitous character of phthalates makes monitoring and analyzing them problematic due to potential cross contamination in laboratories even when special care measures are taken (Guo & Kannan 2012; Del Carlo et al. 2008).

Phthalates are mainly used as plasticizers in PVC and in inks, but they can be found in a wide range of products, from laboratory solvents (Guo & Kannan 2012) to personal care (Guo et al. 2014) and food products (Cao 2010). As was the case for BPA, the main route of exposure seems to be the human diet (Fromme et al. 2007). Since PVCs may come into contact with food throughout production and packaging, it may represent a risk factor regarding food contamination.

There is evidence for human exposure to these chemicals: metabolites of phthalates have been found in human urine (Silva et al. 2004), saliva (Silva et al. 2005) and even uteri (Latini et al. 2003). Given phthalates were shown to act as endocrine disruptors, they could represent a significant health hazard (Tran et al. 2016). Epidemiological studies have shown that phthalate exposure is correlated to, among other things, sperm damage (Rozati et al. 2002), early puberty in females (Wolff et al. 2010) and infertility (Tranfo et al. 2012).

The EU has set TDIs for, and restricted the use of, five phthalates. These are di-ethylhexyl phthalate (DEHP), benzyl butylphthalate (BBP), di-butylphthalate (DBP), di-isononyl phthalate (DINP) and di-isodecyl phthalate (DIBP) (EC 2011; Biedermann et al. 2010; EC 2005; EC 2005; EC 2006). These compounds should not be found at concentrations above 0.1% in toys that could be mouthed by children and, when in food contact materials, specific migration limits cannot be above those shown in table 1.

Table 1: Specific Migration Limits and TDIs set by the EU regarding DEHP, BBP, DBP, DINP and DIDP

Phthalate	CAS #	SML* (mg/l)	TDI (mg/kg bw day)
DEHP	117-81-7	1.5	0.5
BBP	85-68-7	30	5
DBP	84-74-2	0.3	0.1
DINP: substance mixture	28553-12-0, 68515-48-0	9	1.5
DIDP: substance mixture	26761-40-0, 68515-49-1	9	1.5

***: Specific Migration Limit from food contact material to food**

Some additional requirements related to the use of these 5 phthalates are (EC 2011):

DBP and DEHP

Only to be used as:

- “Plasticiser in repeated use materials and articles contacting non-fatty foods;”
- “Technical support agent in polyolefins in concentrations up to 0,05 % in the final product”.

DiNP and DiDP and BBP

Only to be used as:

- "Plasticiser in repeated use materials and articles";
- "Plasticiser in single-use materials and articles contacting non-fatty foods except for infant formulae and follow-on formulae as defined by Directive 2006/141/EC or processed cereal-based foods and baby foods for infants and young children as defined by Directive 2006/125/EC";
- "Technical support agent in concentrations up to 0,1 % in the final product".

1.2 Migration study from epoxy and polyester coatings

In the past, wines have been predominantly stored in cement tanks lined with an epoxy-phenolic resin, which may contain residual BPA once this is used as a monomer in the resin production. While modern wineries have largely adopted steel tanks that do not require coating with these resins, many producers still use the older cement tanks to some extent. As a result of the current concerns regarding the safety of BPA, it is important to assess potential alternatives to epoxy coatings. One potential candidate is polyester coating. Thus the work performed compared the migration from epoxy-phenolic and polyester-phenolic coatings.

Polyesters are very widespread polymers, produced reacting a dicarboxylic acid with a diol, as shown in figure 3. The ester thus produced having two available hydroxyl groups may react again and a polymeric chain builds up.

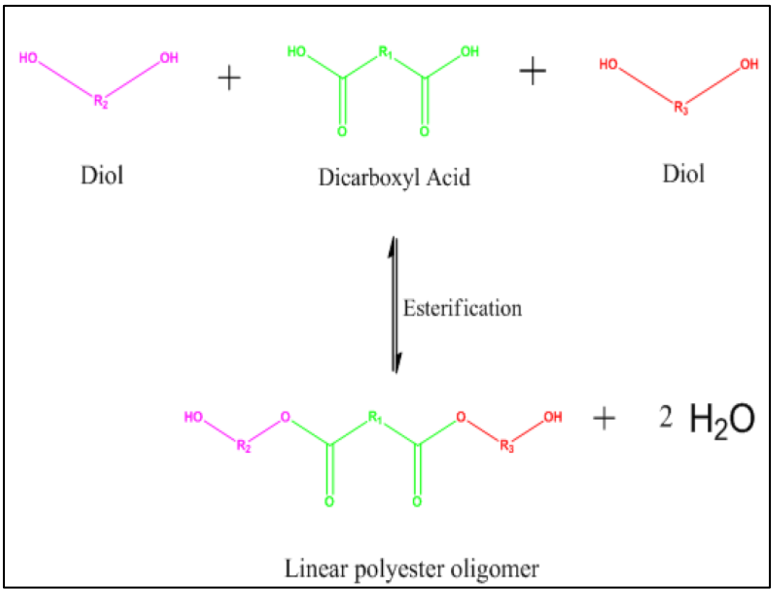


Figure 3: Synthesis of a polyester from a diol and a dicarboxylic acid (Aditi Shrikhande 2012)

Potential side products of these reactions are short-chained cyclic oligomers, shown in figure 4, which do not bind to the backbone of the polymer, making them prone to migration.

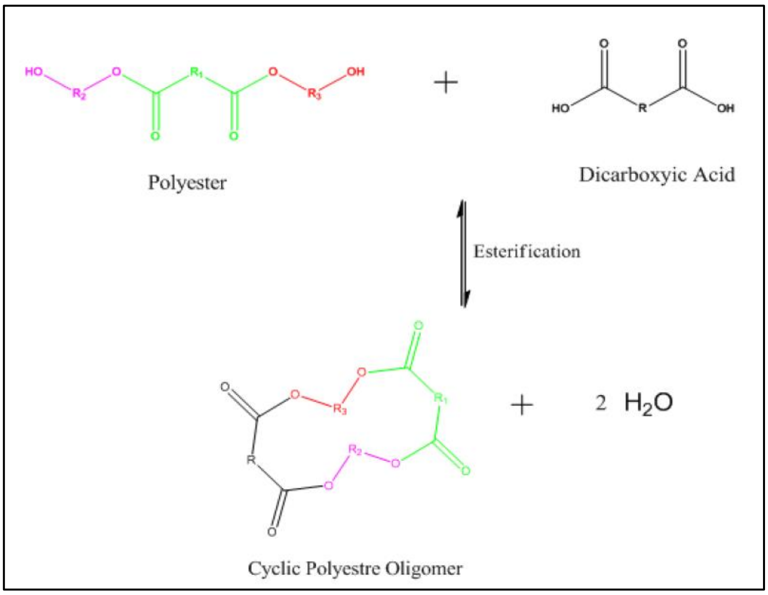


Figure 4: Formation of oligomeric compounds during the synthesis of polyesters (Aditi Shrikhande 2012)

The most widespread use of polyesters is the production of polyethylene terephthalate plastics (PET), which are used mainly in the production of water bottles. Their use as adhesives and coatings, however, relies on a wide range of potential starting materials, shown in table 2 (Schaefer 2004).

Table 2: Common starting materials used in the synthesis of polyesters for coating and adhesive applications

Polyols	Polyvalent carboxylic acid
1. Ethylene glycol	11. Trimellitic acid
2. 1,2-Propanediol	12. Phthalic acid
3. 1,3-Propanediol	13. Adipic acid
4. 1,3-Butanediol	14. Terephthalic acid
5. 2-Methyl-1,3-propanediol	15. Isophthalic acid
6. 2,2,-Dimethyl-1,3-propandiol (neopentyl glycol)	
7. 1,6-Hexanediol	
8. 2,2,4-Trimethylpentane-1,3-diol	
9. 1,1,1-Tris(hydroxymethyl)propane (trimethylolpropane)	
10. 1,4-*Bis*(hydroxy-methyl)-cyclohexane (cyclohexyldimethanol)	

Epoxy resins are a class of polymers synthesized reacting BPA with 3-chloro-1,2 epoxypropane, as shown in figure 5.

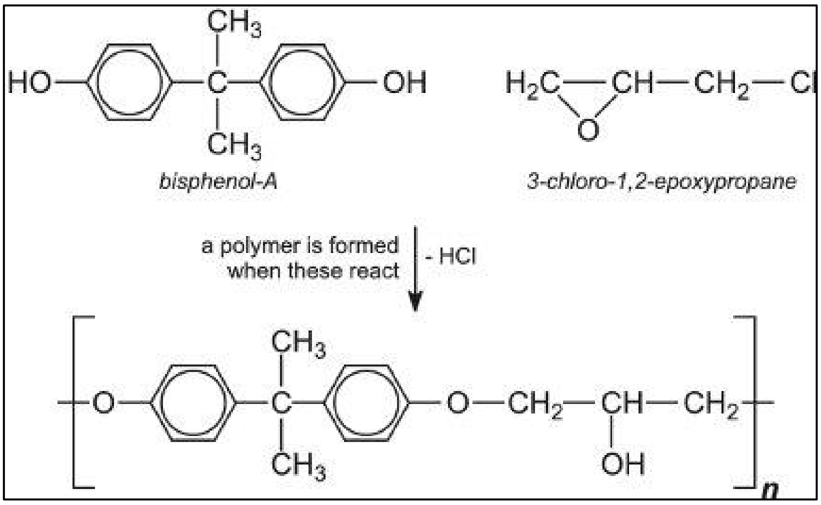


Figure 5: Reaction of BPA with 3-chloro-1,2 epoxypropane to synthesize epoxy resins

Once the BPA-containing backbone has been synthesized, cross-linking may be performed using several compounds, for example phenolic amino resins. ERs have very useful properties, such as resistance to high temperatures or chemicals and good adhesion. As a result of this these materials are used in several products, like the internal coatings of cans and food storage tanks.

Three important factors affect BPA migration from epoxy resins: temperature, pH and contact time (Goodson et al. 2004). Consumption of canned foods, containing an epoxy coating, has been shown to potentially increase dramatically BPA exposure. In a study made by Carwile et al (2011) daily consumption of canned soup over five days increased urine BPA levels of the participants by 1000%. Epoxy resins are also used to coat wine storage vats, which may enable BPA contamination of wine.

Typical factors that generally affect migration are time and temperature of contact, compatibility between the food and the material, mobility of the components of the material and the surface area of the material exposed to the food (Bradley et al. 2008). In order to assess the extent and the rate of migration from food contact materials into foods analyses are generally performed using food simulants (Muncke 2013). Food simulants are used instead of the actual foods in order to simplify the analysis (no matrix effects) and to improve the reproducibility of results (Muncke 2013). Usually food simulants are categorised, based on their chemical properties, as hydrophilic, lipophilic and amphiphilic. The most commonly used simulants are classified with letters from A to E (EC 2011):

- A: Ethanol 10%, simulates hydrophilic foods
- B: Acetic Acid 3%, simulates hydrophilic foods with pH<4.5
- C: Ethanol 20%, simulates hydrophilic foods with alcohol<20%
- D1: Ethanol 50%, simulates lipophilic materials, foods with alcoholic contents > 20%, oil and water in oil emulsions
- D2: Vegetable Oil, lipophilic foods
- E: Tenax, dry foods

1.3 Occurrence of Bisphenol A and Phthalates in wine

1.3.1 Bisphenol A

While the epoxy lining of wine storage vats is a potential risk factor for the contamination of wine, data regarding the occurrence of BPA in wine is, unfortunately, hard to find.

In 2002, when estimating the potential BPA intake arising from wine consumption, the EU had to use data on migration from epoxy products into wine simulants (no data on actual occurrence in wine was available) (EC 2002; Larroque, M., S. Brun 1989). It was estimated that 10 mg/kg epoxy-resin could migrate into a wine simulant (after 1-3 years). Taking into account this value and the 1.4 kg of the resin normally used to coat a 1,500 litres vat with thickness 100 µm, the migration estimate (based on total mass transfer assumption) was 9 µg (BPA)/kg of wine. As a result, a consumer (60 kg body weight) drinking a 0.75 l bottle in a single day would be exposed to 11 µg/kg bw day. This value is almost three times the current t-TDI of 0.04 µg/kg bw day. It is, however, a worse case scenario that made use of conservative and protective assumptions.

The first study that monitored the occurrence of BPA, through the use of a HPLC/FD method, in real wine was made in 2006 (Brenn-Struckhofova & Cichna-Markl 2006). 59 samples (46 white and 13 red), collected from Austrian markets, were analysed. The wines had been in contact with different materials, including steel, wood and plastic storage vats, glass bottles and tetra bricks. The LOQ of the method was 0.2 µg/l, and only 13 of these samples were below this level. The average BPA content of the 46 samples was 0.58 µg/l and the median value 0.40 µg/l. Using the maximum recorded BPA concentration (2.1 µg/l), if a 60 kg person was to drink 0.75 l of wine in a single day, he or she would be exposed to 0.026 µg/kg bw. This is a more reassuring exposure level when compared to the one based on the total mass transfer.

1.3.2 Phthalates

The most recent studies relevant to the occurrence of phthalates in wines are summarised in table 3.

Over 100 wine samples and 30 spirits were analysed by Chatonnetet al. (2014). The findings indicate that the most frequently detected phthalate was DBP, with 59 samples out of 100 contaminated. The average contamination levels were high, 273 µg/l for DBP, 8 µg/l for BBP and 134 µg/l for DEHP. The study also evaluated phthalate migration from materials that could come into contact with wine: the internal coating of wine storage vats, made of epoxy-resins, was considered the greatest risk factor.

Cinelli et al. (2013) made an investigation regarding phthalates in wines in 2013. While the main aim of the study was to compare two chromatographic techniques, measurements on commercial wine samples were also made. The samples were, on average, a lot more contaminated than the other studies. DBP was the most represented phthalate, reaching contents of up to 312.4 µg/l and 100% of the samples were found above the limit of detection.

Fasano et al. (2015), analysed several food types including wine. The contaminants investigated were BBP, DBP, DEHP, BPA and di-ethyl hexyl adipate. The most highly phthalate contaminated wine sample contained 9.72 µg/l of DBP (red wine).

Carillo et al. (2008), aimed to determine the impact of the use of deuterated phthalates as internal standards in the analyses of wine samples. The study also involved screening phthalates on 10 wine samples. The results were relatively moderate, with the most contaminated sample with 5.22 µg/l of DEHP. An interesting consideration came from the fact that wines containing different packages were analysed (Glass bottled with cork and synthetic stoppers, wines in cartons and wines bag in a box). However, no statistically significant differences were found for the results from different packages, which could indicate that contamination occurred prior to packaging.

Table 3: Occurrence studies on the presence of phthalates in wine (µg/l)

Country	Wine	Phthalate	Analytical Method	N	%+ive	LOD	LOQ	Average	Max	Source
Italy	White	DBP	GC-MS	2	100	2.08	NA	6.67	7.49	(Fasano et al. 2015)
	White	BBP		2	100	0.08	NA	3.08	3.7	
	White	DEHP		2	0	2.25	NA	-	-	
	Red	DBP		2	100	2.08	NA	8.72	9.72	
	Red	BBP		2	100	0.08	NA	1.69	1.7	
	Red	DEHP		2	0	2.25	NA	-	-	
Italy	Red	DBP	GC/ITMS	4	100	0.025	0.084	153.7975	312.4	(Cinelli et al. 2013)
	White	DBP		5	100			129.24	272.3	
	Red	BBP		4	50	0.1	0.335	45.475	135.7	
	White	BBP		5	60			50.58	130.8	
	Red	DEHP		4	100	0.073	0.246	18.425	26.6	
	White	DEHP		5	80			12.02	18.4	
Spain	Wine	DBP	HS-SPME-GCMS	10	100	0.0048	NA	1.607	5.37	(Carrillo et al. 2008)
	Wine	DEP		10	60	0.0055	NA	1.147	4.22	
	Wine	DMP		10	30	0.0164	NA	0.147	0.61	
	Wine	DEHP		10	60	0.055	NA	2.758	5.22	
	Wine	BBP		10	10	0.0288	NA	0.229	2.29	
France	Wine	DBP	GC-MS	100	59	0.004	0.01	273	2212	(Chatonnet, Boutou & A. Plana 2014)
	Wine	BBP		100	15			8	122	
	Wine	DEHP		100	15			134	1131	

2 Materials and Methods

2.1 Chemicals

2.1.1 BPA analysis

Bisphenol A (99%) from Sigma Aldrich and Methanol (99.9%), Acetonitrile (99.99%) from Fluka Chemicals. HPLC grade water was used. AFFINIMIP SPE Bisphenol A cartridges (AFFINISEP).

2.1.2 Phthalate analysis

n-Hexane (97%), Di-butyl phthalate-DBP (99%), Di-isodecyl phthalate-DIDP (99%), Di-cyclohexyl phthalate-DCHP (99%), Di-ethylhexyl phthalate DEHP (99.5%), Benzyl butylphthalate-BBP, (97.4%), Di-ethyl phthalate-DEP (99.5%), Di-ethylhexyl phthalate-d4 (98%), Di-butyl phthalate-d4 (98%), Di-n-octyl-phthalate-d4 (98%) from Sigma Aldrich. Di-isobutyl phthalate-DIBP (98%), Di-methyl phthalate-DMP (98%) Di-isononyl phthalate-DINP from Fluka Chemicals. Di-n-octyl-phthalate-DnOP was obtained thanks to the European Laboratory of Reference (JRC, Italy). Di-hexyl phthalate-DHP (98%) from Chem Service. Acetone from Merck.

2.1.3 Screening analysis

Dichloromethane (99.8%) from Sigma Aldrich, Acetonitrile (99.9%) from Fluka Chemicals and n-iso- Octane (98%) from Merck.

2.1.4 Migration analysis

Ethanol (99.8%) from Sigma Aldrich and water (de-ionized).

2.2 Samples

2.2.1 Wine

The 16 wines obtained from Portuguese producers, shown in Table 4, were analyzed for BPA and phthalates. According to the suppliers, these wines were stored in vats for periods of time ranging from 1 to 4 months. They consisted of 9 red, 5 white and 2 rosé. wines Additionally, 3 Porto wines, 2 red and one white were analyzed only for phthalates.

2.2.2 Epoxy and Polyester coatings

The epoxy-phenolic and polyester phenolic coatings, applied on 7.5 x 2.5 cm glass plates were kindly provided by a local coating producer. The polyester coatings contained an orange/yellow pigment while the epoxy coatings a red one. The company provided documentation regarding the formulations of the coatings. These can be consulted in annex 1 and 2. Due to confidential information these annexes are available only for the supervisors and thesis evaluator.

Table 4: Wine samples

Company	Sample	Type of wine	EtOH (%)	pH	Time of storage (months)
A	BB02	White	NA	NA	4
A	BB15	White	NA	NA	1
A	BB17	Rose'	NA	NA	2
A	BB18	Red	NA	NA	6
B	CC4	Red	12.61	3.86	2
B	CC135	Red	14.03	3.94	3
B	CC87	Red	12.37	3.83	2
B	CC35	Red	12.87	3.91	3
B	CC4x	Red	13.02	3.82	3.5
B	CC12	Red	12.79	3.84	3
B	CC14	Red	12.75	3.83	4
B	CC37	Red	13.05	3.69	2
C	A	White	10.15	3.03	2
C	B	Rose'	12.1	3.43	2
C	C	White	10.15	3.03	3
C	D	White	11.3	3.17	1

NA: not available

2.3 Wine Sample Preparation

2.3.1 Extraction for BPA analyses

BPA was extracted from the wines by means of Selective Solid Phase Extraction, using AFFINIM SPE columns. The procedure first involved conditioning the column with Methanol containing 2% formic acid (3 mL), then with acetonitrile (3mL) and finally with water (3mL, HPLC grade). 10 mL of the wine were loaded into the conditioned column, following washing with water (9 mL, HPLC grade) and acetonitrile/water 40:60 (6 mL, HPLC grade water). The column was then allowed to dry under vacuum (1 min) and elution was done with 3 mL of methanol. The extract was evaporated to dryness and dissolved in 0.5 mL of acetonitrile/water (40:60). The samples with detectable amounts of BPA were replicated.

2.3.2 Extraction for Phthalate analyses

To avoid cross-contamination of phthalates in the laboratory, all the relevant glassware was washed with acetone and hexane and heated to 160 ^{o}C overnight prior to the analyses.

An internal method (PE.E.PL70), based on OIV-MA-AS323-10 (OIV 2013) was followed. A solution containing 12.5 mL of the sample, 50 μL of the internal standards (DnBP-d4, DEHP-d4 and DnOP-d4, 0.5 g/L) and 10 mL of hexane was prepared and vortex mixed for 1 minute. The organic phase was separated by means of centrifugation (5 min, 2500 rpm); 8 mL of the organic phase were then collected. The extract was reduced to 1 mL by means of evaporation under nitrogen atmosphere. A blank (hexane), treated like all the samples was prepared every time analyses were performed. Every samples were analysed in replicate.

2.4 Calculation of Lower and Higher Bound averages

Calculating average contamination contents from an occurrence study is not as straight forward as it seems. A key factor is the treatment of non-detected (nd) and non-quantified (nq) samples. Unless there is a reason to believe that a contaminant is not present in a food, it should be assumed that the nd and nq samples may still contain the contaminant (WHO 2009).

While there are various strategies to approach this, the one used here involved calculating Lower Bound (LB) and Higher Bound (HB) averages (WHO 2009). The LB average was calculated by substituting values below the Limit of Detection (LOD) by 0 and values below the Limit of Quantification (LOQ) by the LOD. The HB average was calculated by substituting values below the LOD by the LOD and values below the LOQ by the LOQ, yielding a more conservative estimate.

2.5 Exposure estimates

Exposure estimates are usually calculated using the following equation (FAO/WHO 2010):

$$Dietary\ Exposure = \frac{\Sigma(Concentration\ of\ chemical\ in\ food\ x\ daily\ food\ consumption)}{Body\ weight} \quad (1)$$

Where:

- Dietary exposure (is µg/kg bw day) is the daily exposure of an average person to a chemical.
- Concentration of chemical in food (µg/kg) is normally obtained from occurrence data, using for example LB and HB averages.
- Daily consumption (kg/day) is normally derived from surveys or using assumed default values.
- Body weight normally used is by the EU is 70 Kg (EFSA 2012). Please note that the default value used to be 60 Kg, which is more a restrictive and protective value. It was considered, however by EFSA that 70 Kg is a more accurate representation of the average weight for adults in Europe

2.6 Determination of potential migrants

2.6.1 Studies on the composition of the coatings

In order to identify the potential migrants present in the coatings, extracts using acetonitrile, dichloromethane and iso-octane (10 mL, 24h) were analyzed. The performance between the 3 extraction solvents was compared. The extracts were evaporated to 0.5 mL under nitrogen flow and analyzed by GC/MS.

The coatings also were analyzed directly (without extraction), by treating approximately 10 µg of the coatings using a thermal-desorption GC/MS technique (ChromatoProbe). The coatings were removed physically from the glass slides before introduction in the system.

2.6.2 Migration studies

Migration of selected substances into wine simulant C (20% EtOH), at 60 ºC, was monitored. The coated sample (fragmented into 3 parts) was brought into contact with 10 mL of the simulant. After 3, 5 and 10 days the simulants were evaporated to dryness under vacuum, using a rotary evaporator and were re-dissolved into 1mL of 99% ethanol. 10 µL were then extracted and analyzed. A blank of the wine simulant, treated exactly like the samples was also analyzed.

2.7 Chromatographic techniques

2.7.1 HPLC-FD – Analysis of BPA in wine

The samples were analyzed using a Beckman System Gold 126 Solvent Module chromatography system, with a C18 column (150mm x 4.6mm). The separation was performed using a gradient and the flow rate was 1 ml/min. A Jasca FP-2020 Plus fluorescence detector was used and set at excitation/emission of 230 nm and 315 nm. The injection volume was 50 µL. The mobile phase was acetonitrile (40%) and HPLC grade water (60%).

2.7.2 GC-MS – Analysis of Phthalates in wine

A Brucker Scion 456-GC chromatography system was used, with VF-5MS column (30m * 0.25 mm I.D.; Df: 0.25 µm). Injector temperature was 320 °C. Oven temperature was 80 °C during the first minute, after which it was gradually increased to 320 °C at 10 C/min and left at 320 °C during 8 minutes. Injection volume was 1 µL (split less time 0.5 min). The system was coupled to a quadruple mass spectrometer, with transfer line temperature set as 320 °C and source temperature as 220 °C. The ionization was performed by means of electron impact at 70 eV. SIM mode was applied (33-700 m/z). The qualification and quantification ions used are shown in table 5.

Table 5: Quantification and qualification ions of the phthalates analysed

	Ion Quantification m/z	Ions qualification m/z	
DMP	163	77	194
DEP	149	177	222
DiBP	149	167	223
DBP	149	205	223
BBP	149	91	206
DCHP	149	167	249
DEHP	149	167	249
DnOP	149	167	279
DINP	149	293	
DIDP	149	397	
DBP - d4	153	94	210
DnOP - d4	153	171	283
DEHP - d4	153	171	283

2.7.3 GC-MS – operational conditions

Three protocols were used: one for the determinations on the coating (dry) without prior extraction, one for the determination in the extraction solvents of the coatings, and one for the determination of the migrated substances into the wine simulant.

Compositional analysis using the dry coating:

A ChromatoProbe was used with a Programmable Temperature Vaporizer (PTV).

The temperature injection program was the following: the initial temperature was 10 ºC (for 0.5 min), it was then increased (50 ºC/min) until it reached 320 ºC until the end of the run. The split ratio was off during the first 7 minutes of the run, it was thereafter opened to 30 µL/min.

Compositional analysis using liquid extracts of the coatings:

A Brucker Scion 456-GC chromatography system was used, with VF-5MS column (30m * 0.25 mm I.D.; Df: 0.25 µm). Injector temperature was 320 °C. Oven temperature was 40 °C during the first 5 minutes, after which it was gradually increased to 320 °C at 10 C/min and left at 320 °C during 8 minutes. Injection volume was 1 µL (split less time 0.5 min). The system was coupled to a quadruple mass spectrometer, with transfer line temperature set as 320 °C and source temperature as 220 °C. The ionization was performed by means of electron impact at 70 eV. Full scan mode was applied (45-700 m/z).

Migration study of substances from the coating into a wine simulant:

The same equipment was used; the temperature injection program was, however, modified in order to flush the solvent prior to the measurement. The initial temperature, 10 ºC, was increased to 90 ºC (100 ºC/min) for the first 4 minutes of the run; it was then increased to 320 ºC (200 ºC/min) until the end of the run. The split ratio was 50 µL/min for the first 5 minutes (to allow the ethanol to flush), it was then closed and opened again at 40 minutes to 30 µL/min.

The mass spectra peaks were analysed with the same settings described in 8.4.2 and the mass spectra were qualified using the NIST library, Version 2.2 (June 2014).

2.8 Performance of the quantification methods

2.8.1 Performance of the BPA analytical method

A calibration curve was performed and is shown in figure 6. Solutions containing increasing contents of BPA were used (1.86 µg/l, 3.72 µg/l, 9.3 µg/l, 18.6 µg/l). The R^2 value was 0.99938. The LOD of the method, calculated from the standard deviation of the intercept of the curve, was 0.57 µg/l and the LOQ 1.91 µg/l.

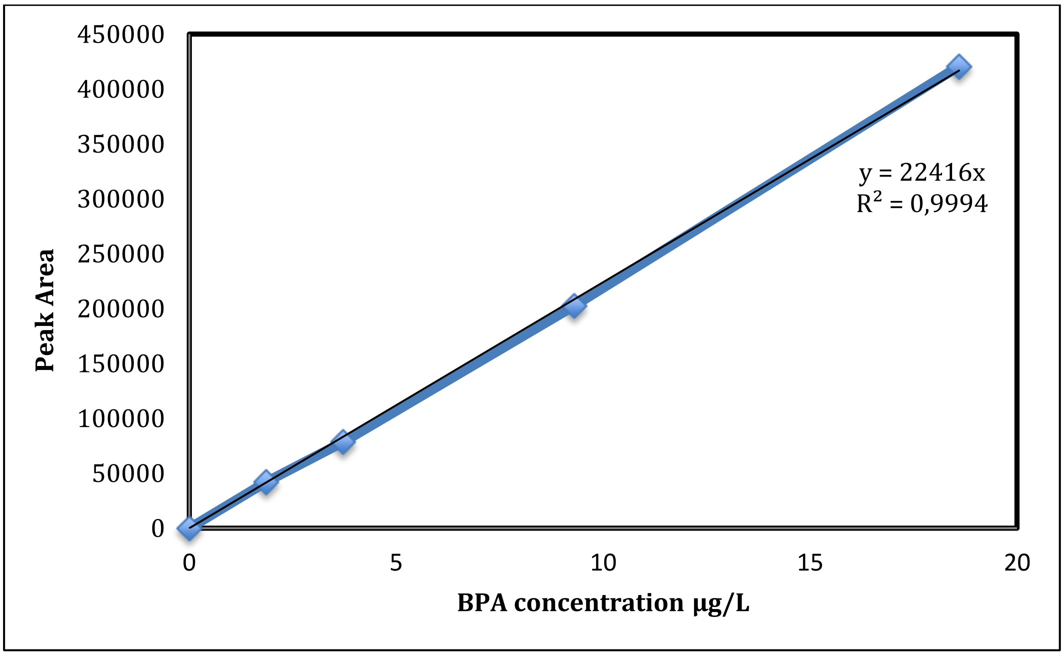


Figure 6: Calibration curve of BPA

Five samples were spiked with 9.73 µg/l to assess the recovery of the method. The results are shown in Table 6. The average recovery of the spiked wines was 54.14%.

Table 6: Amount of BPA measured in spiked wine samples (9.73 µg/l)

WINE	µg/l	Recovery (%)
Sample 1	6.7	68.65
Sample 2	5.2	53.43
Sample 3	5.4	55.55
Sample 4	4.3	44.23
Sample 5	4.7	48.81

2.8.2 Performance of the Phthalate analytical method

A work solution, prepared dissolving the required phthalates in hexane was prepared: the concentrations used for each phthalate are shown in Table 7.

Table 7: Work solution for phthalates

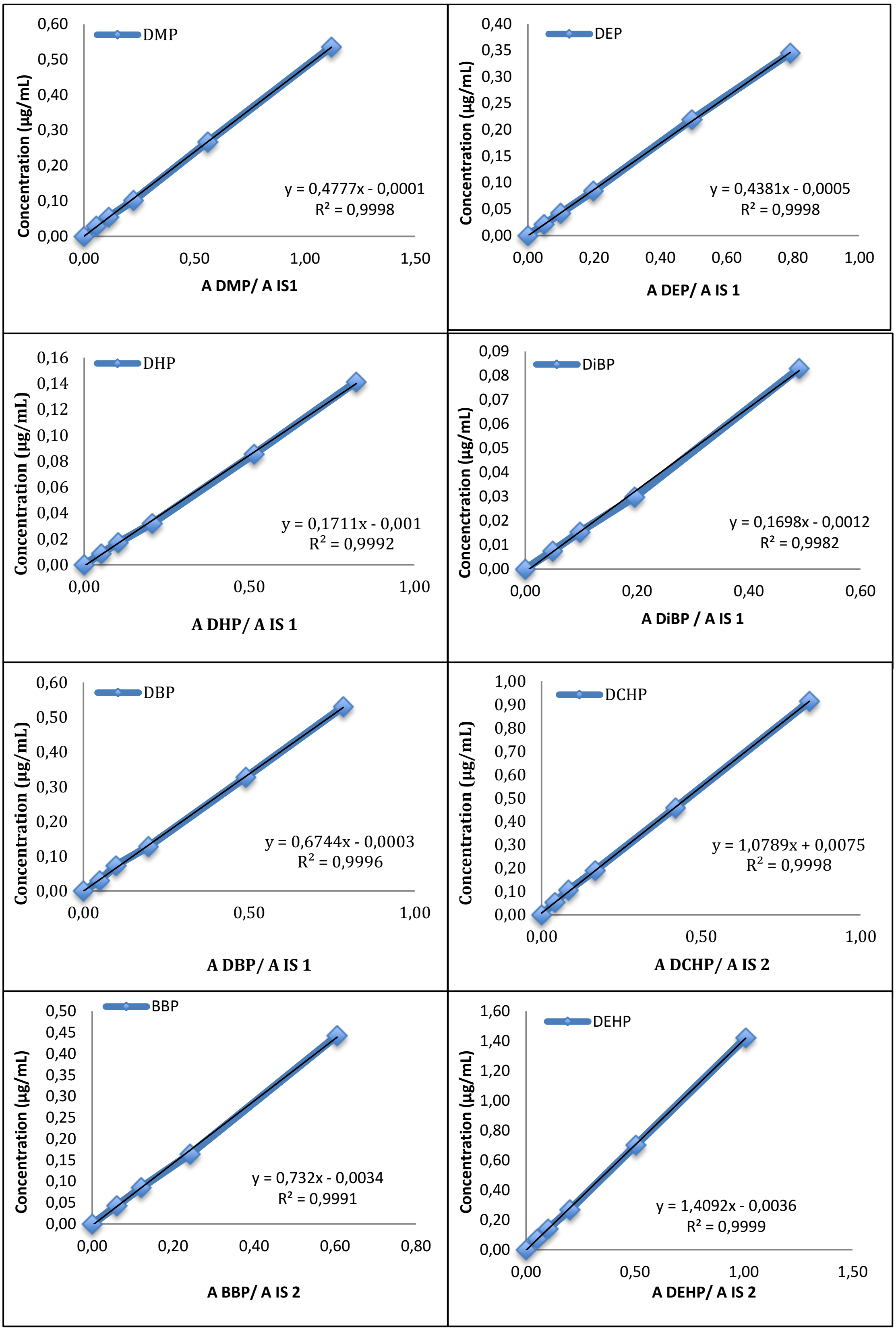
Phthalate	mg/L
DMP	5.6
DEP	4.95
DHP	5.15
DiBP	4.9
DBP	4.9
DCHP	4.2
BBP	6.05
DEHP	5.05
DnOP	5.4
DINP	25.1
DIDP	23

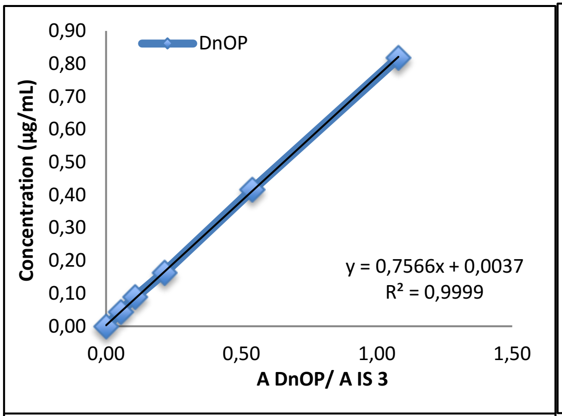
The work solution was used to prepare six standards with increasing amounts of phthalate contents (Table 8).

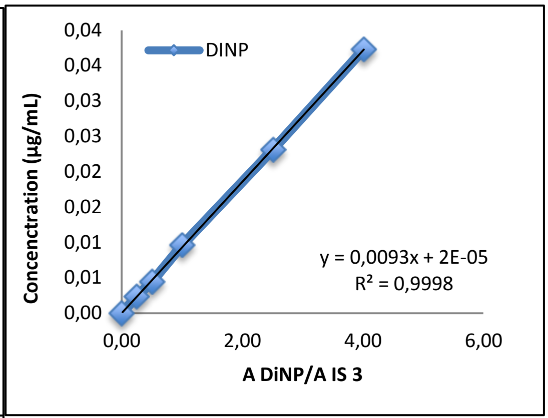
Table 8: Standards used for the phthalate analysis

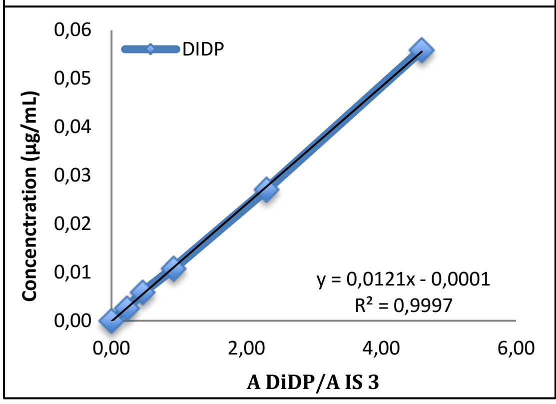
Standard	Hexane (µL)	Spiking from work solution (µl)	Internal Standards (µl)
S0	950	0	50
S1	940	10	50
S2	930	20	50
S3	910	40	50
S4	850	100	50
S5	790	160	50
S6	750	200	50

The standards were analyzed and the peaks were identified with MS using table 4. In order to account for variability in injected volume, each peak area was divided by its corresponding deuterated internal standards (IS). Please note that IS 1 (DBP – d4) was used for phthalates with lower molecular weights, namely DMP, DEP, DHP, DiBP and DBP. IS 2 (DEHP – d4) was used for phthalates with intermediate molecular weights and IS 3 (DnOP – d4) was used for phthalates with higher molecular weights (DnOP, DiNP, DiDP). The resultant calibration curves are shown below.


DMP
y = 0,4777x - 0,0001
R² = 0,9998
Concentration (µg/mL)
A DMP/ A IS1
DEP
y = 0,4381x - 0,0005
R² = 0,9998
Concentration (µg/mL)
A DEP/ A IS 1
DHP
y = 0,1711x - 0,001
R² = 0,9992
Concentration (µg/mL)
A DHP/ A IS 1
DiBP
y = 0,1698x - 0,0012
R² = 0,9982
Concentration (µg/mL)
A DiBP / A IS 1
DBP
y = 0,6744x - 0,0003
R² = 0,9996
Concentration (µg/mL)
A DBP/ A IS 1
DCHP
y = 1,0789x + 0,0075
R² = 0,9998
Concentration (µg/mL)
A DCHP/ A IS 2
BBP
y = 0,732x - 0,0034
R² = 0,9991
Concentration (µg/mL)
A BBP/ A IS 2
DEHP
y = 1,4092x - 0,0036
R² = 0,9999
Concentration (µg/mL)
A DEHP/ A IS 2







The R^2 value, Limits of Detection (LODs) and Limits of Quantification (LOQs) for the 11 phthalates are shown below and their recoveries are shown in table 9.

Table 9: R^2 values, LODs and LOQs of the method for the analysed phthalates

	R^2	LOD (µg/l)	LOQ (µg/l)
DMP	0.99984	1.437	4.790
DEP	0.99984	1.058	3.528
DHP	0.99925	2.370	7.900
DiBP	0.99982	2.265	7.550
DBP	0.99956	2.328	7.760
DCHP	0.99981	1.192	3.974
BBP	0.99985	2.025	6.749
DEHP	0.99985	1.256	4.186
DnOP	0.99986	1.309	4.364
DINP	0.99982	5.623	18.742
DIDP	0.99966	6.017	20.058

The recovery of the samples was assessed by spiking a wine sample containing low amounts of phthalates with increasing amounts of the stock solution, as shown in table 10. The average recoveries ranged from 135.4% to 49%.

Table 10: Spiking levels of wine samples used in the recovery study

Phthalate	0 µl/ L	40 µl/ L	80 µl/ L	400 µl/ L	Average Recovery (%)
DMP	-----	0.0044	0.0088	0.044	70.8
DEP	-----	0.0036	0.0072	0.0216	135.4
DiBP	-----	0.00384	0.00768	0.0384	119.4
DBP	-----	0.00452	0.00904	0.0452	103.1
DHP	-----	0.0046	0.0092	0.046	107.6
BBP	-----	0.00512	0.01024	0.05632	110.8
DCHP	-----	0.00472	0.00944	0.0472	110.4
DEHP	-----	0.00464	0.00928	0.0464	83.3
DnOP	-----	0.00448	0.00896	0.0448	78.4
DINP	-----	0.01856	0.03712	0.1856	65.2
DIDP	-----	0.01804	0.03608	0.1804	49

3 Results and Discussion

3.1 Occurrence of BPA in wine

Of the 16 samples analyzed, 13 contained detectable amounts of BPA, 6 of which being higher than the LOQ (Table 11). The most contaminated sample had 3.52 µg/l of BPA, which is higher than the highest value found by Brenn-Struckhofova & Cichna-Markl (2.1 µg/l).

Table 11: BPA content of the samples (µg/L)

Sample Name	Type of wine	Time of storage (months)	Contamination level (µg/l)
BB02	White	4	<LOQ
BB15	White	1	<LOQ
BB17	Rosé	2	<LOQ
BB18	Red	6	1.96
4	Red	2	3.03
135	Red	3	<LOQ
87	Red	2	3.52
35	Red	3	<LOQ
4x	Red	3.5	<LOD
12	Red	3	<LOD
14	Red	4	<LOQ
37	Red	2	<LOD
A	Verde	2	<LOQ
B	Rosé	2	2.06
C	Verde	3	2.35
D	White	1	2.25

The mean BPA content of the samples was analyzed using the methodology described in section 2.4. The following values were found:

- LB average: 1.16 µg/l
- HB average: 1.74 µg/l

If the censored data had been set to 0 µg/l, the overall average would have been 0.95 µg/l, slightly lower than the LB estimate.

The LB and HB values are harder to compare when averages have been calculated differently. In the case of the work by Brenn-Struckhofova & Cichna-Markl (YEAR), no information has been provided regarding how the average, 0.54 µg/l, was calculated. Nevertheless, both LB and HB found in the present study are well above 0.54 µg/l.

White, red and rosé wines are produced in different manners and as a result of this they could have been exposed to different amounts of BPA. Other factors, such as pH, which is generally lower in Verde wines, or ethanol content, which is generally higher in red wines (see table 4, section 2.2.1) may have played a role in the potential migration of BPA.

The two most contaminated samples were red wines (3.03 and 3.52 µg/l), it could be that their higher ethanol content favored the migration of BPA during storage. The small sample size does not allow, however, to produce statistically strong considerations. Storage time, which usually occurs in epoxy-coated vats, could theoretically be correlated to the BPA content of the wines. The data presented here did not, however, indicate correlation between the two. The size of the storage tanks, which ultimately affects the surface area of the wine exposed to BPA, the thickness and composition of the epoxy lining used in the vats, and the storage temperature may all be important factors regarding BPA migration. More information would be needed for the establishment of relationships between the various factors that may play a role.

3.2 Occurrence of Phthalates in wine

3.2.1 Table wines

The results of the analyses made on table wines are shown in tables 12 and 13. DEHP and DBP were the most common phthalates, with averages (LB-HB) of 30.33 µg/l - 30.59 µg/l and 21.91 µg/l - 22.59 µg/l respectively. The sample found to be the most contaminated was BB02, with 158.5 µg/l of DEHP. On the other hand, no samples above limits of quantification were found for the phthalates DMP, DHP, DiBP, DCHP, DnOP, DiNP and DiDP.

During the measurements some cross-contamination issues were experienced, i.e. some blanks were found contaminated with DEHP. The source of contamination was identified (a rubber pompette) and the measurements affected were repeated.

The average DEHP contents (30.33 µg/l -30.59 µg/l) were higher than those found in the literature (Section 1.3.2), with the closest results coming from the study made by Cinelli et al. (2013), with an average content of 18.43 µg/l in red wines. In the case of BBP (3.97 µg/l -7.53 µg/l) results seem to be similar to what was found by Fasano et al. (2015) (3.08 µg/l), while the other studies all measured significantly lower BBP contents.

The current study measured levels of DBP (21.91 µg/l - 22.59 µg/l) a lot lower than was found by Chatonnet et al. (2014) (273 µg/l) and Cinelli et al. (2013) (312.4 µg/l). These are very high values. It seems legitimate to question whether they may have been calculated using only the positive samples (i.e. results above LOQ).

Given the relatively small sample size, it is hard to correlate phthalate contamination with the type of wine.

Table 12: Phthalates in table wine (μg/l)

Sample	Wine Type	Storage (months)	DMP	DEP	DHP	DiBP	DBP	DCHP	BBP	DEHP	DnOP	DINP	DIDP
135	White	4	<LOD	<LOD	<LOD	<LOQ	**12.77**	<LOD	55.49	**158.54**	<LOQ	<LOD	<LOD
35	White	1	<LOD	<LOD	<LOD	<LOQ	**8.83**	<LOD	<LOQ	**26.29**	<LOD	<LOD	<LOD
4	Rosé	2	<LOQ	<LOD	<LOD	<LOQ	**60.66**	<LOD	<LOQ	**15.91**	<LOD	<LOD	<LOD
87	Red	6	<LOD	<LOD	<LOD	<LOQ	**57.87**	<LOD	<LOD	**20.38**	<LOD	<LOD	<LOD
4 BR	Red	2	<LOD	**3.72**	<LOD	<LOD	**11.57**	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD
12	Red	3	<LOD	<LOD	<LOD	<LOD	**61.41**	<LOD	<LOQ	**28.52**	<LOD	<LOD	<LOD
14	Red	2	<LOD	<LOD	<LOD	<LOD	**17.58**	<LOD	<LOQ	**39.75**	<LOD	<LOD	<LOD
37	Red	3	<LOD	<LOQ	<LOD	<LOD	**5.05**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
BB02	Red	3.5	<LOD	<LOD	<LOD	<LOD	**20.79**	<LOD	<LOD	**30.7**	<LOD	<LOD	<LOD
BB15	Red	3	<LOD	<LOD	<LOD	<LOD	**17.15**	<LOD	<LOD	**23.17**	<LOD	<LOD	<LOD
BB17	Red	4	<LOD	<LOD	<LOD	<LOD	**12.1**	<LOD	<LOD	**51.45**	<LOD	<LOD	<LOD
BB18	Red	2	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	**19.21**	<LOD	<LOD	<LOD
A	Verde	2	<LOQ	<LOD	<LOD	<LOQ	**7.55**	<LOD	<LOD	**19.97**	<LOD	<LOD	<LOD
B	Rosé	2	<LOQ	<LOQ	<LOD	<LOQ	**20.76**	<LOD	<LOD	**15.76**	<LOD	<LOD	<LOD
C	Verde	3	<LOQ	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	**25.5**	<LOD	<LOD	<LOD
D	White	1	<LOQ	<LOD	<LOD	<LOQ	**31.88**	<LOD	<LOD	**8.87**	<LOD	<LOD	<LOD

Table 13: LB and HB average contamination levels of phthalates in the table wines (µg/l)

	LB average	HB average
DMP	0.36	1.65
DEP	0.36	1.53
DHP	0	2.37
DiBP	1.76	4.58
DBP	21.91	22.59
DCHP	0	1.19
BBP	3.97	7.53
DEHP	30.33	30.59
DnOP	0.08	1.31
DINP	0	5.62
DIDP	0	6.02

3.2.2 Port wines

The Port wines showed results significantly higher for DBP, as shown in table 14. While Port Wine 2 was below legislative limits (300 µg/l), with 215 µg/l, the other two samples were well above the EU permitted levels, with 440 and 825 µg/l respectively. Port wine 2 was also found to contain significant amounts of DiBP (106 µg/l), which is not listed in the European regulation, and therefore is not permitted as food contact substance. Therefore its origin should be investigated.

Table 14: Content of phthalates in three Porto wines (µg/l)

	Porto Wine 1 (Red)	Porto Wine 2 (Red)	Porto Wine3 (White)
DEP	2.5	1	4
DiBP	3	3	3
DBP	440	215	825
BBP	5	1	7
DEHP	12	62	44
DiDP	Nd	106	nd
DCHP	Nd	Nd	nd
DiPP	Nd	Nd	nd
DnOP	Nd	Nd	nd
DINP	Nd	Nd	nd
DHP	Nd	Nd	nd

In comparison with the table wines, that have a different winemaking process, Port wines have additional risk factors:

- Contact with storage materials: Port wines may potentially be in contact with sources of phthalates for longer times during transfers and handling.
- Ethanol Content: Phthalates are generally poorly soluble in water and tend to solubilize better with the ethanol fraction; higher ethanol contents can therefore be seen as an additional risk factor for the migration of phthalates.
- Addition of spirit: The fermentation of Port wines is stopped through the addition of "Aguardente", a spirit with very high ethanol contents which may be a major source of phthalates in these wines.

The increased susceptibility of beverages with higher ethanol contents towards phthalate presence had already been observed by Chatonnet et al. (2014), in a study where 19% of 30 spirit samples did not comply with legislative limits. Furthermore, an increase of DiDP concentration proportional to time of aging was observed. Given that only three samples were analyzed averages and exposure estimates were not calculated for these samples.

3.3 Exposure Assessment

3.3.1 Exposure assessment of BPA from the consumption of wine

The lower and higher bound exposure estimates, shown below, were calculated using the concentration data from 3.1 and equation 1, in section 2.5. The average daily wine consumption used was 0.116 l, derived from the Portuguese wine consumption statistics from 2014 (Bettini 2015). The following estimates were found.

- LB exposure: 0.00192 μg/ kg bw day
- HB exposure: 0.00288 μg/ kg bw day

The values indicate controlled exposure levels, accounting for 4.8% and 7.2% of the t-TDI currently in force. In order to take into account an high wine consumer, a daily intake of 0.75 l of wine was also used, similarly to what was done by Brenn-Struckhofova & Cichna-Markl (2006). The following results were found:

- LB exposure: 0.0124 μg/ kg bw day
- HB exposure: 0.0186 μg/ kg bw day

Both the LB (31% t-TDI) and HB (46.5% t-TDI) exposure estimates would be reasonably below the t-TDI, even accounting for the high daily wine consumption. These levels of exposure could, however represent a potential issue if the other sources of BPA were to be significant. In particular knowing that, according to the last EFSA BPA assessment, canned food is the major source of BPA exposure in Europe, these relative contributions from wine consumption in high consumers may be relevant. The biggest limitation of these calculations is the reduced sample size of the survey.

3.3.2 Exposure Assessment of Phthalates in Wine

Similarly to what was done for BPA, exposure were estimated using average (0.116 l/day) and high (0.75 l/day) wine consumptions, and an average body-weight of 70 Kg. Table 15 describes the results obtained for the average wine consumers. It can be seen that all the exposure estimates seem to be low and, for the phthalates with a TDI, the results are all below 0.1% of the TDI.

Table 15: Exposure assessment of phthalates in wines (LB,HB, average consumer), µg/ kg bw day

	LB Exposure	% TDI	HB Exposure	% TDI
DMP	0.00059	-	0.00273	-
DEP	0.00060	-	0.00254	-
DHP	0	-	0.00393	-
DiBP	0.00291	-	0.00759	-
DBP	0.03631	0.036	0.03744	0.037440
DCHP	0	-	0.00197	-
BBP	0.00658	0.00013	0.01247	0.000249
DEHP	0.05026	0.01005	0.05069	0.010138
DnOP	0.00013	-	0.00216	-
DINP	0	0	0.00932	0.000621
DIDP	0	0	0.00997	0.000664

Table 16 shows the exposure estimates made for the high wine consumers (0.75 l). While the magnitude of the results increased, exposure estimates remained well under control, with the highest % TDI coming from DBP with 0.24%.

Table 16: Exposure assessment of phthalates in wines (LB,HB, high consumer), µg/ kg bw day

	LB Exposure	% TDI	HB Exposure	% TDI
DMP	0.00385	-	0.0176	-
DEP	0.00391	-	0.0164	-
DHP	0	-	0.0254	-
DiBP	0.01888	-	0.0490	-
DBP	0.2348	0.2348	0.2420	0.24207
DCHP	0	-	0.0128	-
BBP	0.04258	0.00085	0.0806	0.001613
DEHP	0.3249	0.06600	0.32776	0.065552
DnOP	0.00088	-	0.01403	-
DINP	0	0	0.06025	0.004016
DIDP	0	0	0.06447	0.004298

3.4 Screening of epoxy-coating

The following section will describe the results obtained from the compositional analyses of the epoxy coating, made to select potential migrants to be monitored into the wine simulant.

3.4.1 Dry coating

The chromatograph obtained from the direct analysis of the epoxy coating is shown below in figure 7.

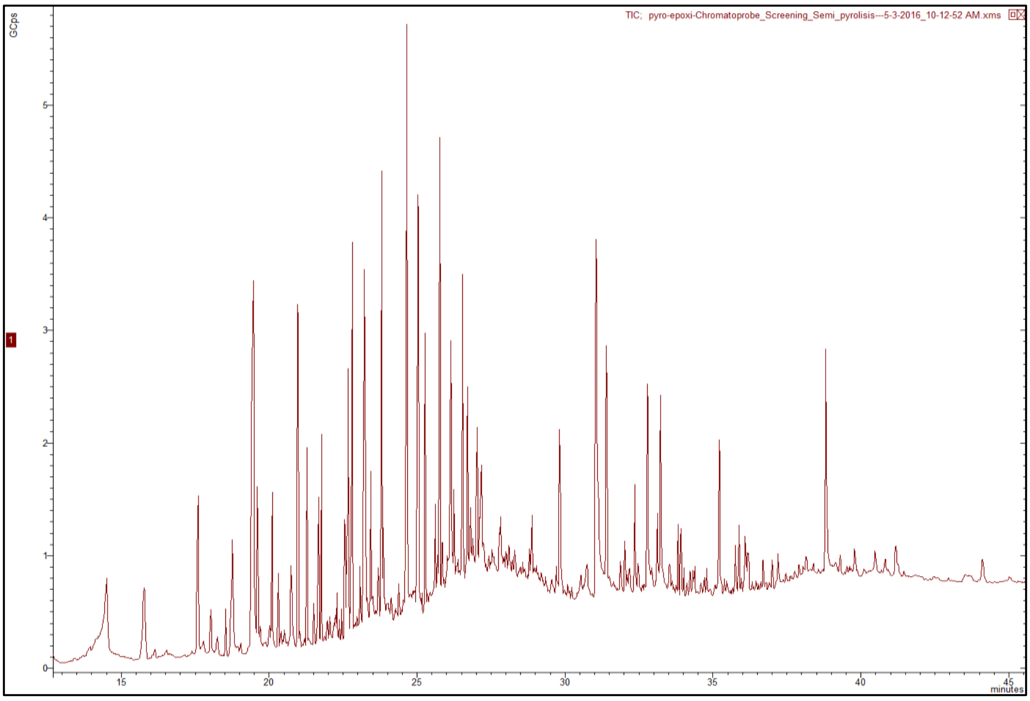


Figure 7: Chromatograph of the direct analysis of the epoxy-phenolic coating

The signal was very intense, which is a result of the sensitivity of the ChromatoProbe technique and of the purity of the sample (i.e. no solvents were used). The qualitative analysis of these peaks will be made in section 3.6.

3.4.2 Liquid extracts (acetonitrile, dichloromethane and iso-octane)

The coatings were also analysed using three solvents, one relatively polar (acetonitrile), one apolar (iso-octane) and one very aggressive (dichloromethane). The resultant chromatograms are shown in figure 8, 9 and 10.

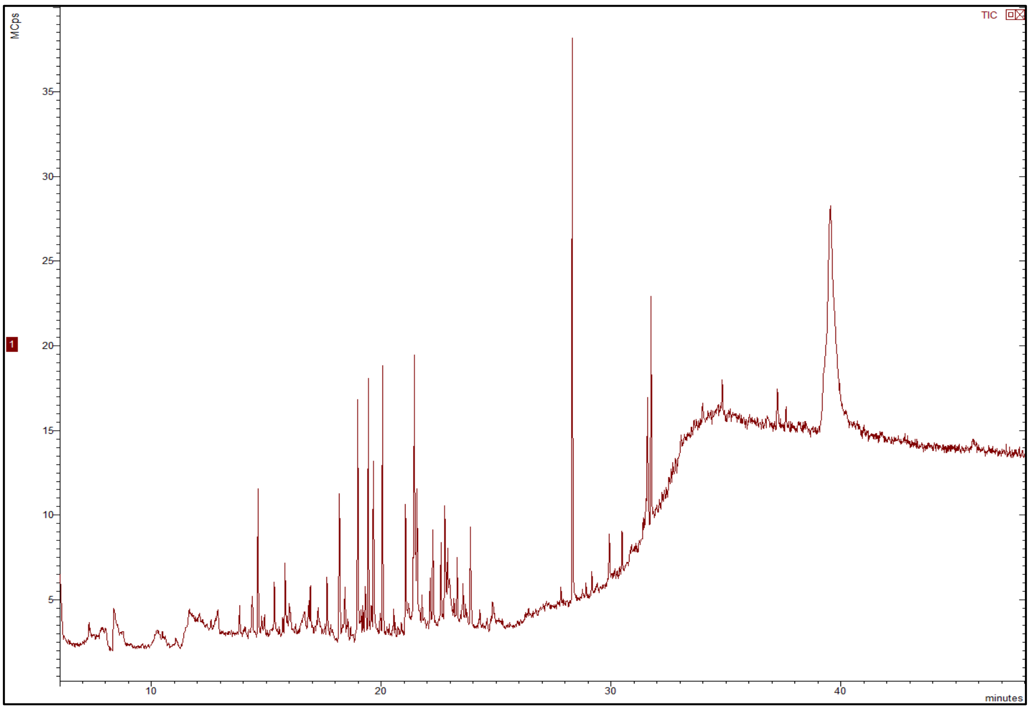


Figure 8: Extract from epoxy sample after 24h in Acetonitrile

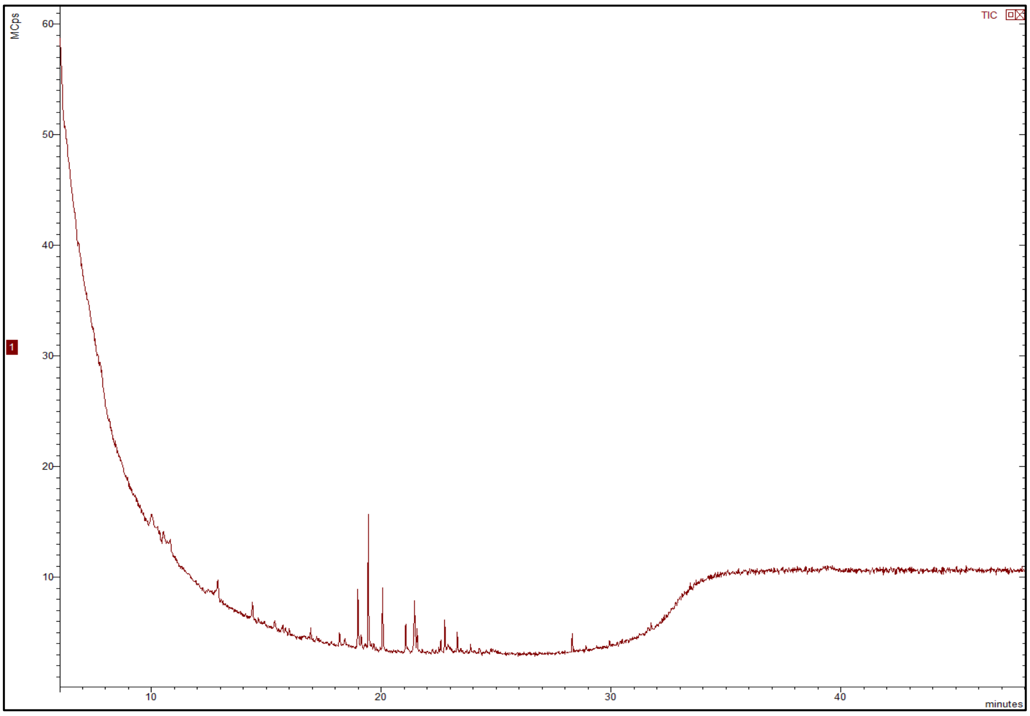


Figure 9: Extract from epoxy sample after 24h in Dichloromethan

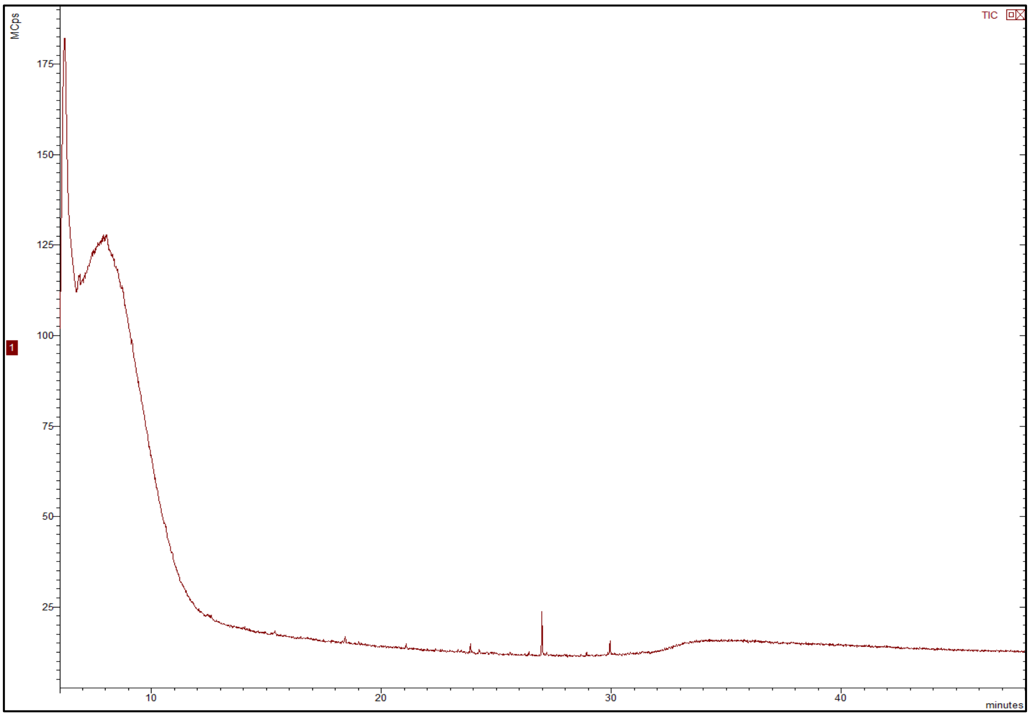


Figure 10: Extract from epoxy sample after 24h in iso-octane

The solvent with better extraction performance in terms of the number of substances detected in the extracts as evaluated by the richness of the chromatogram was acetonitrile. Iso-octane extract showed very poor extraction power and dichloromethane was intermediate. Therefore, the results from acetonitrile (AC) extract were considered in the present study. They yielded comparable peaks to the DCM extract but with better mass spectra matches.

Overall the detection signal was about 100 times weaker than the one with the ChromatoProbe dry measurement, and the reliability of the identified peaks was also weaker as evaluated by the probability of the match provided by the data analyses software.

3.4.3 Migration to Wine simulant

The samples were brought into contact with the wine simulant (20% EtOH) and left at 60 ºC for 3, 5 and 10 days. Below, in figure 11, the chromatogram of the simulant analysis after 10 days is shown.

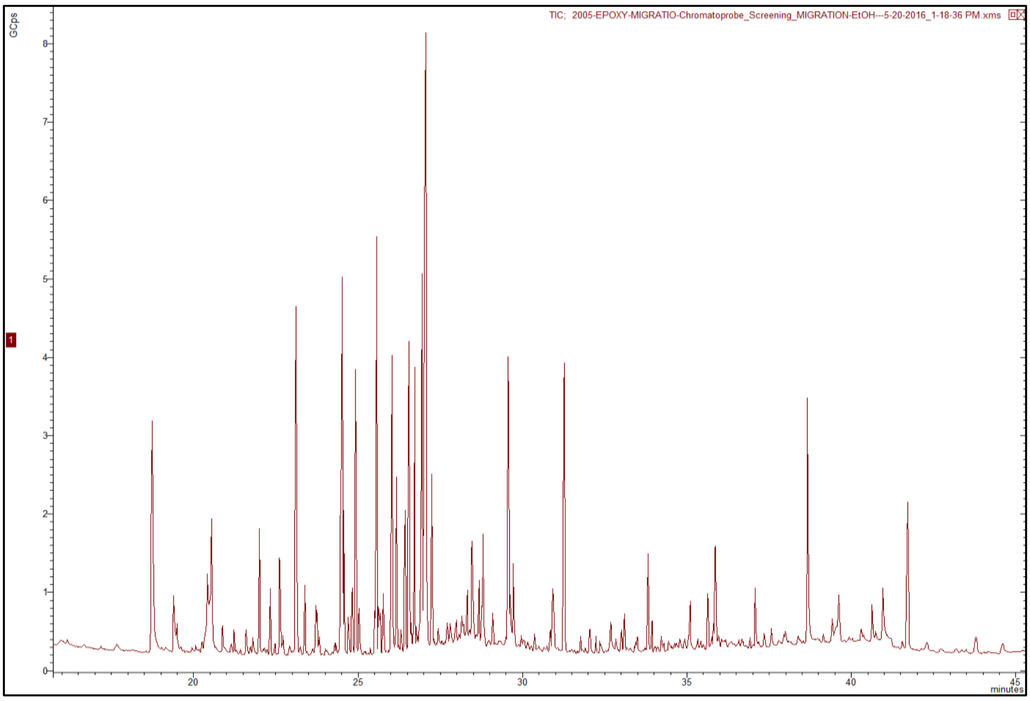


Figure 11: Chromatograph of epoxy sample into a wine simulant for 10 days at 60 C

The chromatographs after 3 and 5 days can be consulted in annex 3. Some background noise was experienced in some of the measurements, probably as a result of the presence of impurities in the samples. The blank analyses enabled verifying which peaks were originated from the samples and disregard those peaks due to blanks.

The overall amount of peaks and their intensity was a lot greater than observed in the AC extract, probably a result of the more sensitive technique and of the more extreme conditions used in the study of this sample (10 days, 60 ºC instead of 24 hours at room temperature).

3.5 Screening of polyester coating

The following section will describe the results obtained from the compositional analyses of the polyester coating, made to select potential migrants to be monitored into the wine simulant.

3.5.1 Dry coating

The same procedure applied to the epoxy resin coating was used for the polyester coating. The chromatogram of the sample is shown in figure 12. The most significant peaks, with signals greater than 1 GPcs, had their mass spectra compared to the NIST database.

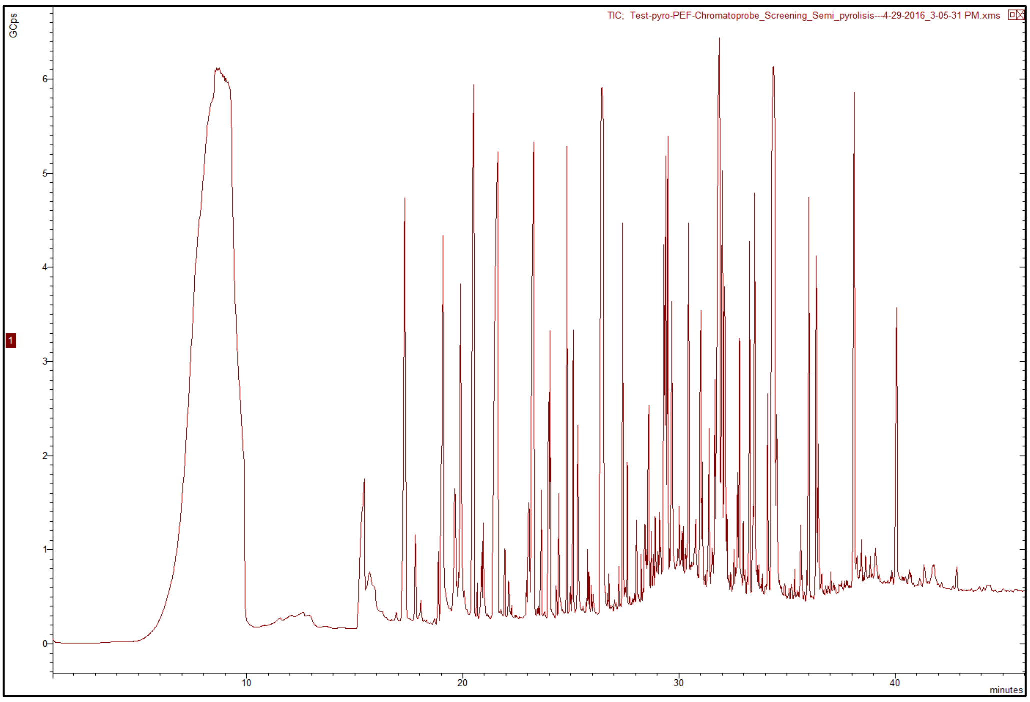


Figure 12: Chromatograph of the direct analysis on the dry polyester-phenolic coating

3.5.2 Liquid extracts (acetonitrile, dichloromethane and iso-octane)

The polyester coating was also analysed using three extraction solvents: acetonitrile, iso-octane and dichloromethane. The resultant chromatograms are shown in figure 13, 14 and 15.

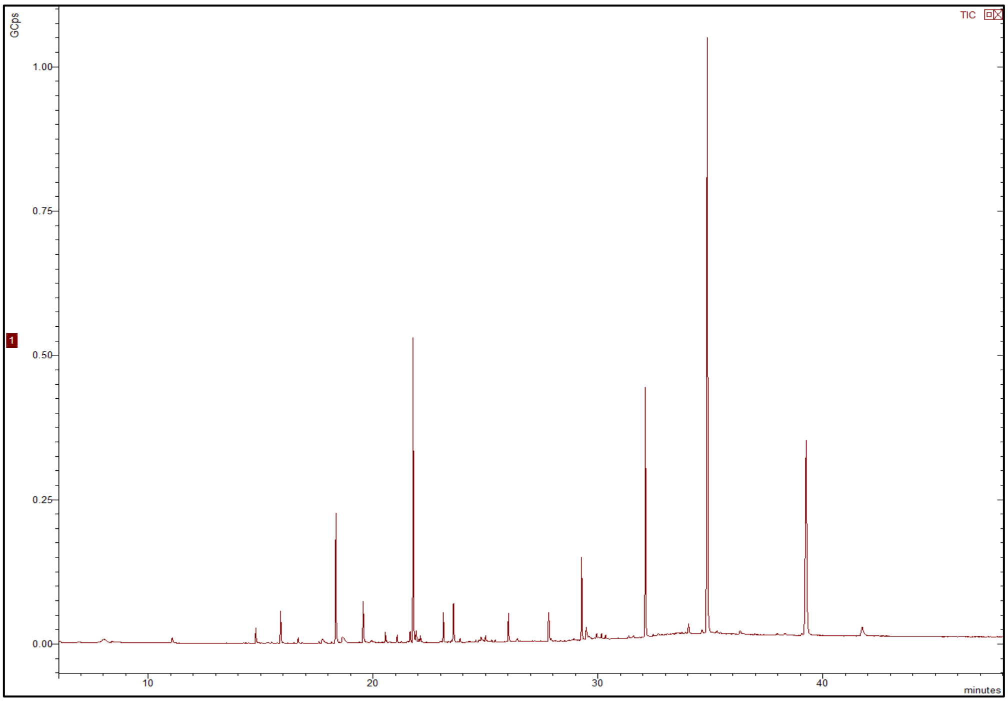


Figure 13: Extract from polyester coating after 24h in Acetonitrile

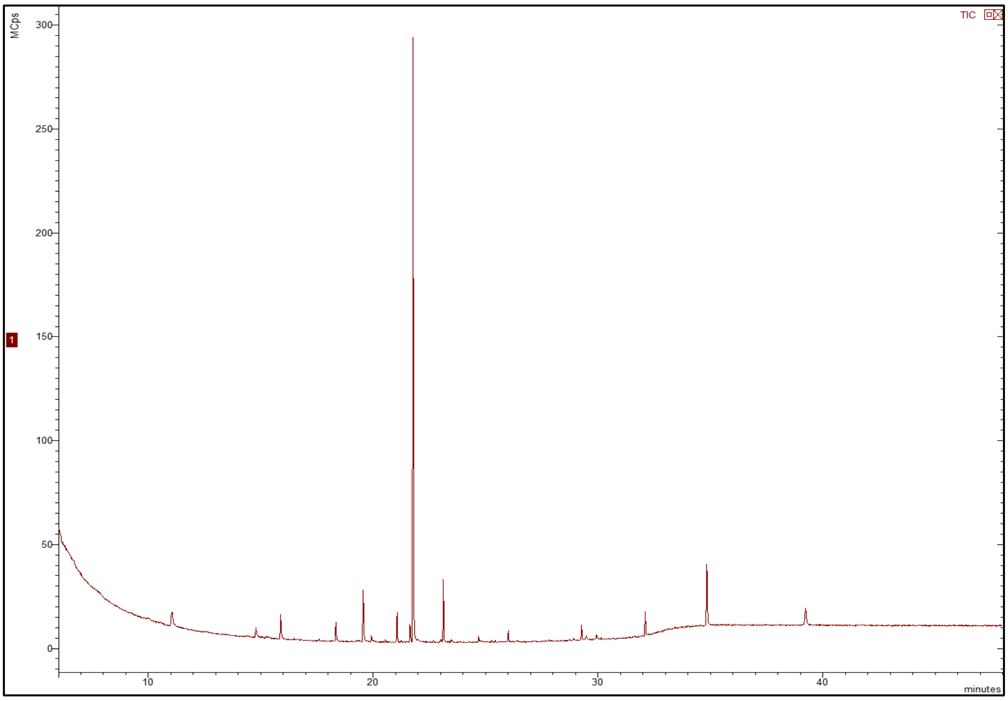


Figure 14: Extract from polyester coating after 24h in dichloromethane

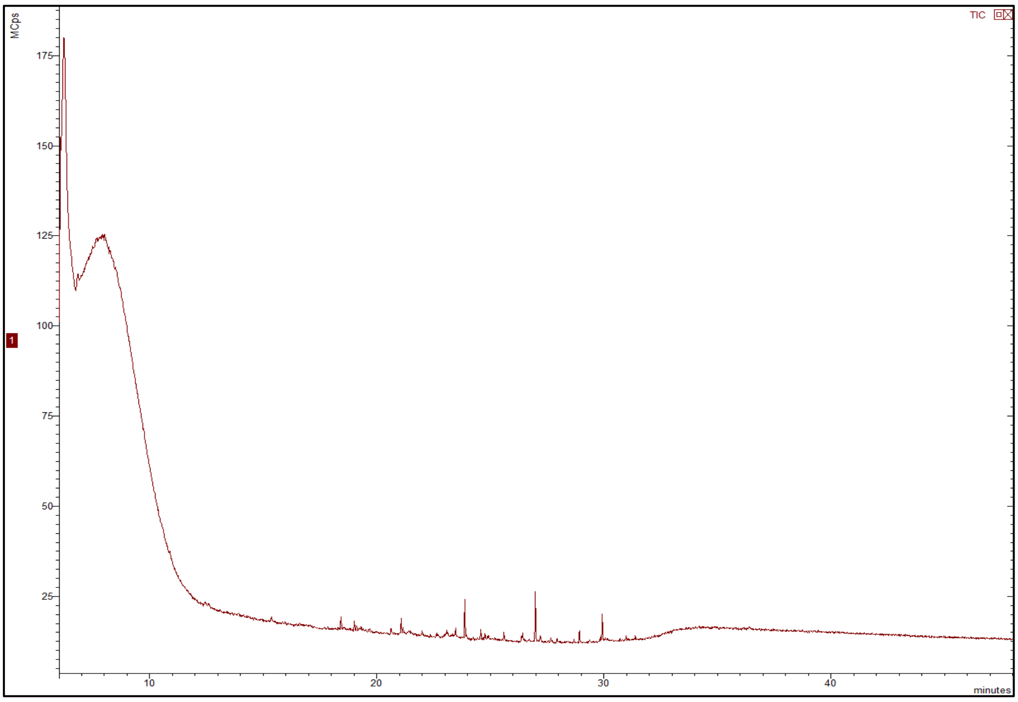


Figure 15: Extract from polyester coating after 24h in iso-octane

Similarly to the epoxy coating, the solvent more efficient was acetonitrile. Fewer peaks were found in the dichloromethane extract and even fewer in iso-octane.

While the magnitude of the signal remained well below the direct measurement with the ChromatoProble technique, the intensity of these peaks was approximately 10 times higher than the one recorded for the epoxy coating AC extract (figure 8). Therefore, it seems that the polyester coating is more susceptible to chemical extraction from acetonitrile than epoxy resin.

3.5.3 Migration to Wine simulant

The samples were brought into contact with the wine simulant (20% EtOH) and left at 60 ºC for 3, 5 and 10 days. Below, in figure 16, the chromatogram of the simulant after 10 days is shown. To consult the chromatograms after 3 and 5 days please check annex 4.

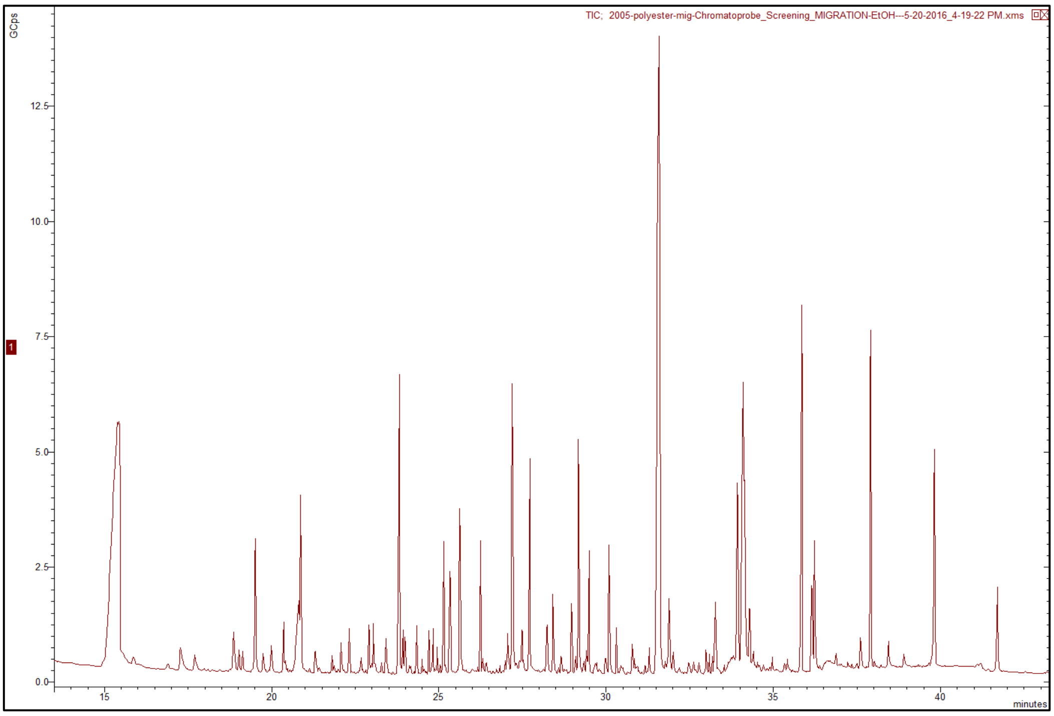


Figure 16: Polyester coating extract (20% EtOH) after 10 days at 60 C

Similarly to the epoxy coating, the overall amount of peaks and their intensity is higher than what observed in the AC extract, probably a result of the more extreme conditions used in the study of this sample.

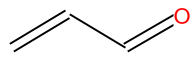
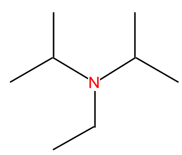
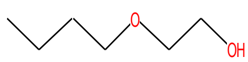
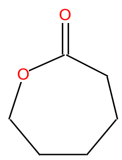
3.6 Identification of the peaks

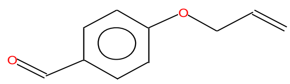
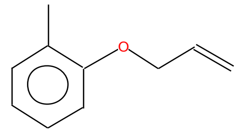
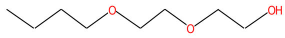
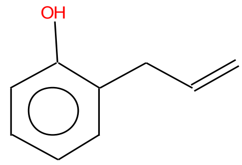
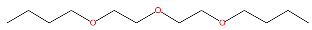
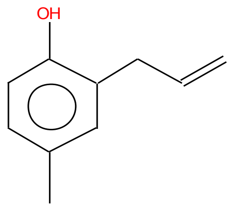
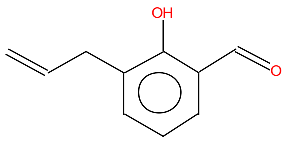
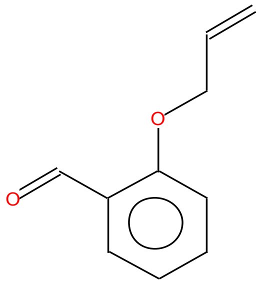
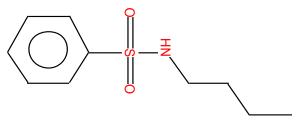
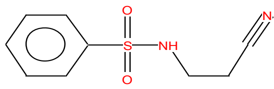
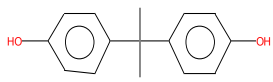
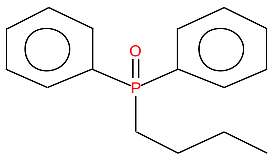
The most significant peaks had their mass spectra was compared to the NIST database (Version 2.2, January 2014). The match between the spectra and the library was used to identify the samples. The software assigned probabilities based on the likelihood of the identification and it is a common practice, in this area, to consider a good match results above 60% (Vladimir & Sparkman 2004). For all the peaks with a signal greater than 1 GPcs (for the samples measured with the ChromatoProbe) and 5 MPcs (for the AC extracts), the overall retention times, structures and probabilities can be found in annexes 5-10.

Table 17 present the substances identified with at least a 45 % probability for the measurements performed on the epoxy samples. Similarly, table 18 contains the results obtained for the polyester coating.

Because the scope of this work was to elucidate the potential migration of substances into the wine simulant, every single identified molecule will not be discussed in this thesis. Besides comparison between the different methodologies, results allowed for the selection of the migrants to monitor in the wine simulant.

Table 17: Peaks with at least a 45% probability found in the measurement of the dry epoxy-phenolic sample and of its acetonitrile (AC) and wine simulant (20% EtOH) extracts.

Found in	RT (min.)	Prob (%)	NAME	CAS n	Structure
Dry sample	8.5	86.8	2-propenal	107-02-8	
Dry sample	12.2	72.8	Diisopropyl-ethylamine	7087-68-5	
Dry sample	14.5	70.8	Ethanol, 2-butoxy	111-76-2	
Dry Sample AC Extract Wine Similant	18.8 15.8 18.72	62.5 50.9 68.7	ε-Caprolactone	502-44-3	

AC extract	18.9	46.3	Benzaldehyde, 4-(2-propenyloxy)-	40663-68-1	
Dry Sample	19.48	58	Allyl-o-tolyl-ether	936-72-1	
AC extact	14.6	81.7			
Dry Sample	19.5	47.9	Butyl Diglycol	112-34-5	
Dry Sample	19.61	66.7	2-Allylphenol	1745-81-9	
Dry Sample	20.103	50	Dibutyl Carbitol	112-73-2	
Dry Sample	20.92	55	2-Allyl-p-cresol	6628-06-4	 77
Dry Sample	21.28	65.2	Benzaldehyde, 2-hydroxy-3- (2-propenyl)	24019-66-7	
Dry Sample	22.81	53.2	Benzaldehyde, 2-(2-propenyloxy)	28752-82-1	
AC Extract	23.9	47.5	Benzenesulfonamide, N-butyl-	3622-84-2	
		58.6	N-(2-Cyano-ethyl)-benzenesulfonamide	2619-21-8	
Dry Sample	31.06	85.3	BPA	80-05-7	
Wine Simulant	30.92	85.4			
Dry Sample	31	64.5	Phosphine oxide, butyldiphenyl	4233-13-0	
AC extract	28.3	65.6			
Wine Simulant	31.2	60.6			

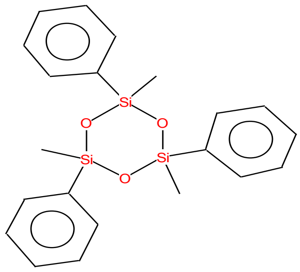
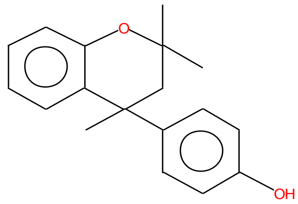
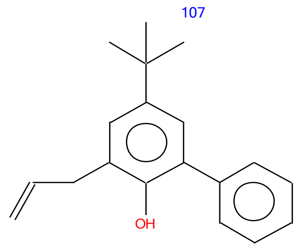
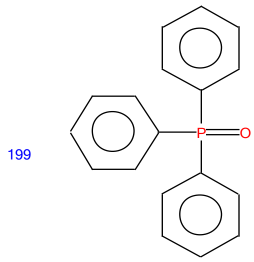
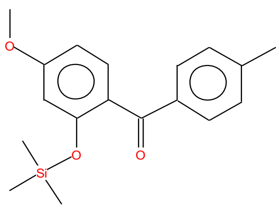
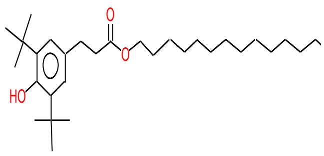
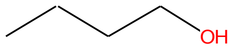
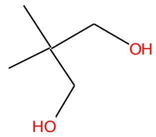
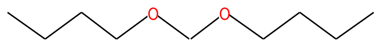
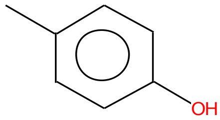
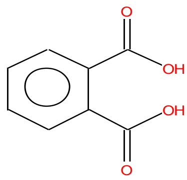
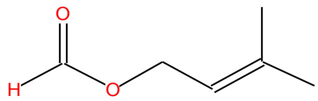
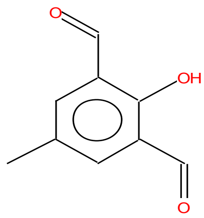
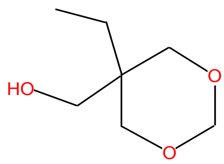
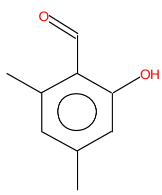
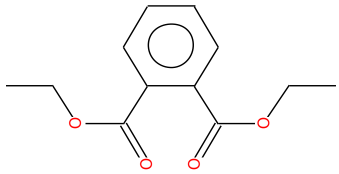
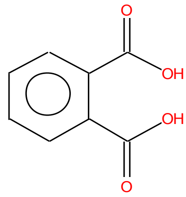
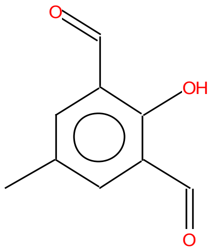
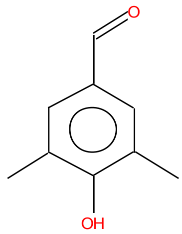
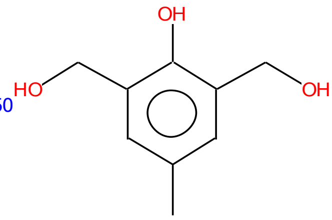
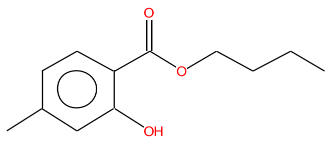
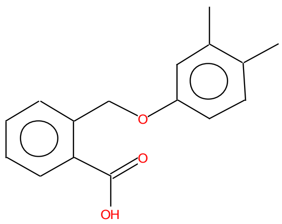
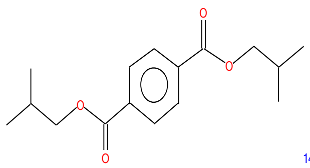
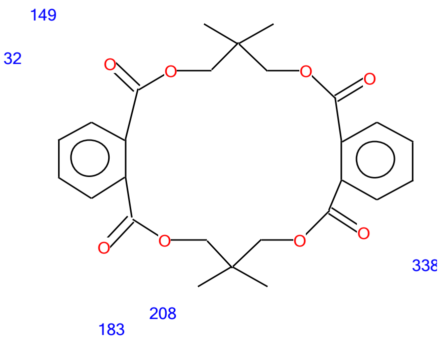
AC extract	31.5	53.8	Cyclotrilosiloxane, 2,4,6 – trimethyl-2,4,6-triphenyl	546-45-2	
Dry Sample	32.4	48	Phenol, p-(2,2,4-trimethyl-4-chromanyl)-	472-41-3	
Dry Sample	32.8	50.9	2-Hydroxy-3-allyl-5-t-butylbiphenyl	23079-51-8	
Dry Sample	33.9	88.1			
AC extract	31.7	94.6	Triphenyl-phospine	791-28-6	
Wine Simulant	33.81	95.9			
Dry Sample	35.208	51,5	Related to BADGE		
Wine Simulant	41.693	94.3	acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, octadecyl ester	2082-79-3	

Table 18: Peaks with at least a 45% probability found in the measurement of the dry polyester-phenolic sample and of its acetonitrile (AC) and wine simulant (20% EtOH) extracts

Found in	RT (min.)	Prob (%).	NAME	CAS n	Structure
Dry sample	8.3	59.8	1-butanol	71-36-3	
Dry sample Wine Simulant	15.3 15.4	81.6 80.5	Neopentyl glycol	126-30-7	
Dry sample AC extract	17.2 11.05	69.9 66	Dibutoxy methane	2568-90-3	
Dry Sample	17.78	46	p-cresol	106-44-5	
AC extract	18.3	71.5	Phthalic Acid	88-99-3	
Dry sample	18.87	56	Formic acid, 3-methyl-2-butenyl ester	68480-28-4	
AC extract	19.6	89.2	2-Hydroxy-5-methylisophthalaldehyde	7310-95-4	
Dry sample Wine Simulant	19.63 19.5	83 88.9	1,3 Dioxane-5-methanal-5ethyl	5187-23-5	
Dry Sample	20.46	52.7	2-Hydroxy-4,6-dimethylbenzaldehyde	1666-02-0	

AC Extract	21.1	78.7	Diethyl Phthalate	84-66-2	
Dry Sample	21.502	46.9	Phthalic Acid	88-99-3	
Dry Sample	23.26	88.3	2-Hydroxy-5-methylisopthaldehyde	7310-95-4	 77
Dry Sample	23.61	65	3,5-Dimethyl-4-hydroxybenzaldehyde	2233-18-3	
Dry Sample	25.35	48.1	1,3-Benzenedimethanol, 2-hydroxy-5-methyl	91-04-3	150 
Dry Sample	28	73	butyl-2-didioxy-4-methyl benzoate	NA	
Dry Sample	31.38	70	Benzoic acid, 2 (3,4-dimethylphenoxymethyl)	NA	
Dry Sample	31.66	44.9	Diisobutyl terephthalate	18699-48-4	50  149

Dry Sample	40.06	83.5	7H,18H-dibenzo[g,p][1,5,10,14]tetraoxacyclooctadecin-5,11,16,22-tetrone, 8,9,19,0-	NA	
Wine Simulant	39.81	80.6			

3.7 Comparing results from different methodologies

The present section will only discuss the findings shown in tables 17 and 18, where the most likely identifications (45% probability or more) are shown. Only three substances were found in all of the measurements performed for the epoxy-phenolic coating: ε – Caprolactone, butyldiphenyl phosphine oxide and triphenyl phosphine.

In the case of the polyester coating no substance was found reliably in all the three studies made. However Neopentyl Glycol, 1,3 Dioxane-5-methanal-5ethyl and the oligomer 7H,18H-dibenzo[g,p][1,5,10,14]tetraoxacyclooctadecin-5,11,16,22-tetrone, 8,9,19,0-tetrahydro-8,8,19 (will be referred to as "oligomeric compound" from now on), were found with good reliability and intensity in both the dry coating/ChromatoProbe analysis and the wine simulant extract.

The reasons for the differences in the results obtained may be related to several factors, such as the different chromatographic conditions used and the different treatments of the samples.

The biggest difference between the three methods is that one did not involve using solvents. The dry measurement/ChromatoProbe technique yielded more peaks compared to the two measurements that made use of liquid extracts; this could be a result of the greater concentration of substances found on the actual coating; furthermore, some chemicals may not have been extracted into the solvents. Additionally, besides the efficiency this technique is much faster and less work loaded, but requires manual injection. The results obtained using the extracts may also be subject to issues resulting from solvent interactions with the migrants.

Prior to the ChromatoProbe measurements, the wine simulants had to be evaporated using a rotary evaporator (and heated to 60 ºC), before being reconstituted into ethanol (1mL). Some potential migrants may have been lost during the evaporation and the reconstitution step (i.e. some residues may have remained on the surface of the flask).

The two liquid extracts were treated very differently, which could have affected the amount of migrants in the samples. While the wine simulants were stored at 60 ºC for up to 10 days, the acetonitrile extracts were kept at room temperature for 24 hours. The harsher conditions experienced by the wine simulant, together with the more sensitive technique used (the Chromatoprobe was not used for the AC extract), may have contributed to the greater amount of peaks found.

While the three experiments yielded relatively different results, there also were common findings, which allowed selecting specific migrants that will be described in the following sections.

3.8 Migrants from the epoxy coating

ε-Caprolactone and butyldiphenyl phosphine oxide were found with in quite intensity and reliability in all the three analyses, while BPA was only detected with good intensity and reliability in the wine simulant and on the dry coating. Because it is a key substance for the investigation its migration was also be monitored. The migration behaviour of these substances was monitored by integration of their peaks at the 3 time intervals (3, 5 and 10 days).

The migration behaviour of ε-Caprolactone is shown in figure 17. After 10 days at 60 °C the migration is still increasing; the experiment should therefore have been conducted for a longer time (even though the European legislation indicates that 10 days is the time required for compliance). It seems that the kinetics of migration was slow enough and that the rate of migration remained constant throughout the experiment.

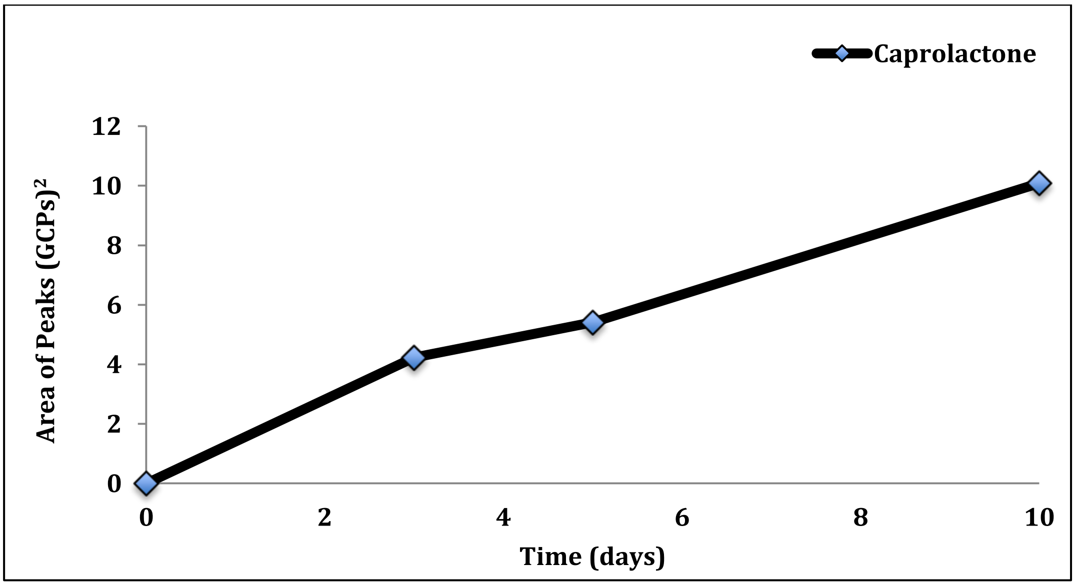


Figure 17: Migration of Caprolactone after 3, 5 and 10 days (20% EtOH, 60 C)

The migration behaviour of BPA, however, was significantly different (Figure 18). A first look at the graph shows that the amount of migrant decreased between day 5 and day 10. A possible explanation is that the migrant reached a plateau after day 3, and that the subsequent variations are simply a result of the experimental error and correspond to fluctuations around this plateau value. Even small changes in the injected volume would be able to affect significantly the results because internal standard was not used.

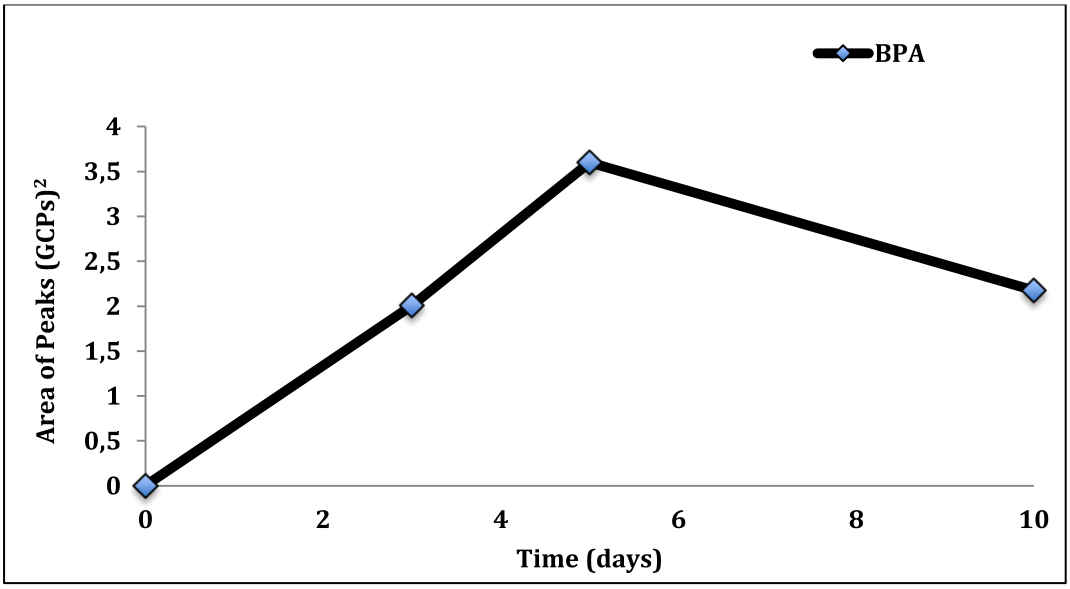


Figure 18: Migration of BPA after 3, 5 and 10 days (EtOH 20%, 60 C)

Thus, it seems that the migration rate was faster for BPA compared to ε-Caprolactone's. A molecular factor generally thought to have an impact on migration rate is molar weight (Poças et al. 2011). The molar weight of Caprolactone is 114.14 g/mol while BPA's 228.29 g/mol; given usually the rate of migration is thought to be inversely proportional to molecular weight, some other factors must have come into play.

The relative slower migration of ε-Caprolactone could be due to its potential partial binding, by means of H-bonds, to the resin's backbone. ε-Caprolactone has been shown to be able to form such bonds when blended into epoxy-resins (Ni & Zheng 2005). On the other hand, the faster BPA migration may have been related to the absence of chemical bonds between the molecule and resins (i.e. the unbound, residual BPA migrated).

The migration of butyl diphenyl phosphine oxide is shown in figure 19. The sample seemed to reach a plateau after the third day; which indicates that this material is probably present as an unbound additive or residue of the material.

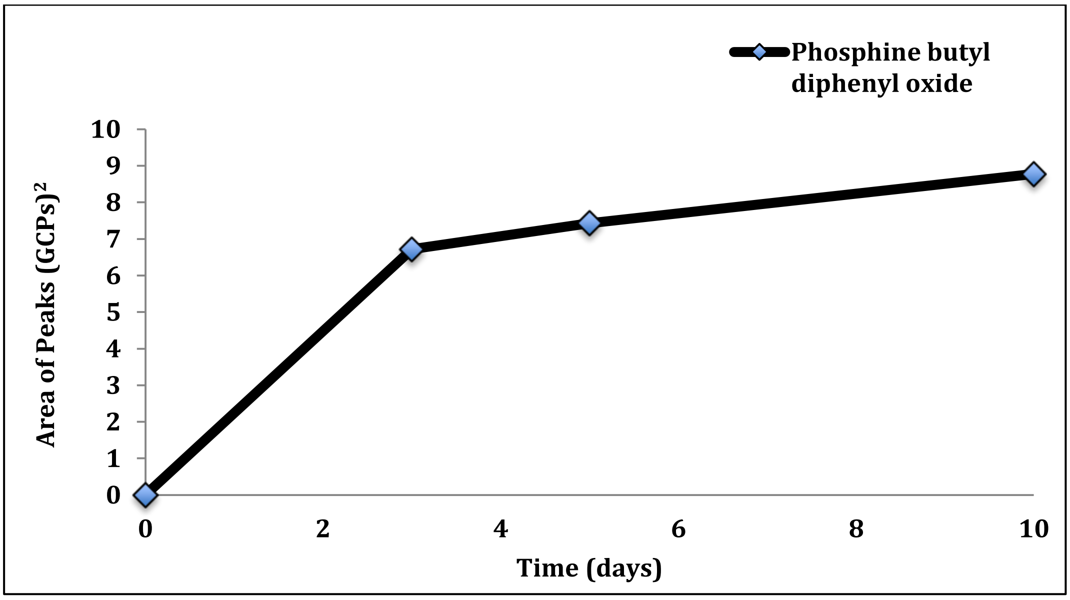


Figure 19: Migration of Phosphine butyl diphenyl oxide after 3, 5 and 10 days (EtOH 20%, 60 C)

3.9 Migrants from the polyester coating

Neopentyl glycol, the cyclic oligomeric compound and 1,3 Dioxane-5-methanal-5 ethyl were found with good intensity and reliability in the dry coating and the wine simulant. Their migration behaviour was monitored by integration of their peaks at the 3 time intervals (3, 5 and 10 days).

The migration behavior of the glycol is shown in figure 20. It can be observed that the substance did not seem to have reached a plateau by the end of the experiment, as was observed for ε-Caprolactone and again, a longer migration study may have been more adequate.

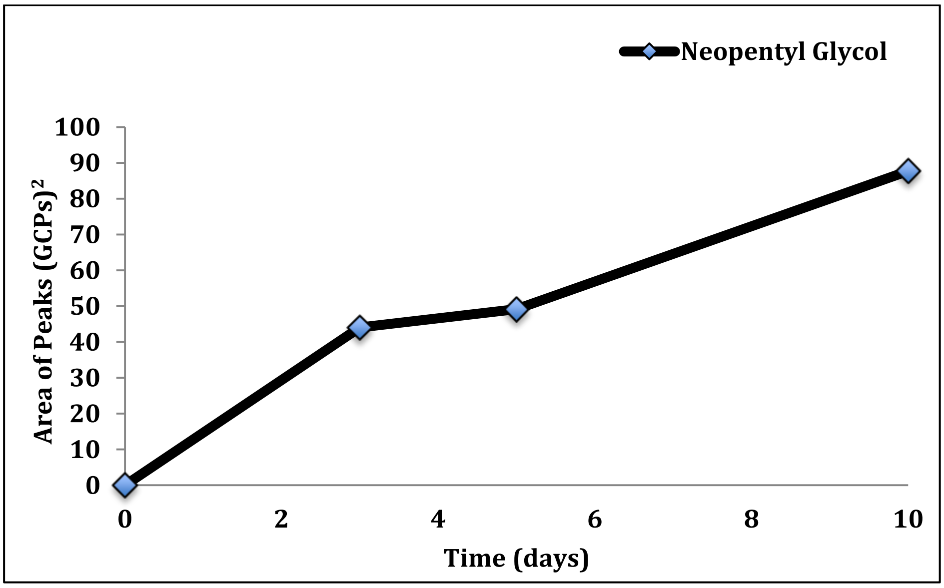


Figure 20: Migration of Neopentyl Glycol after 3, 5 and 10 days (EtOH 20%, 60 C)

A possible explanation for this slow release may be that this substance is bonded with the polymeric backbone of the material. Actually, neopentyl glycol is often used as a starting reagent in the synthesis of polyesters (Schaefer 2004), in combination with a dicarboxylic acid, as is described in section 1.3. Another possible explanation may rely on that this material may be a building block of the material in the cyclic oligomer that was also detected with good reliability, shown in figure 21.

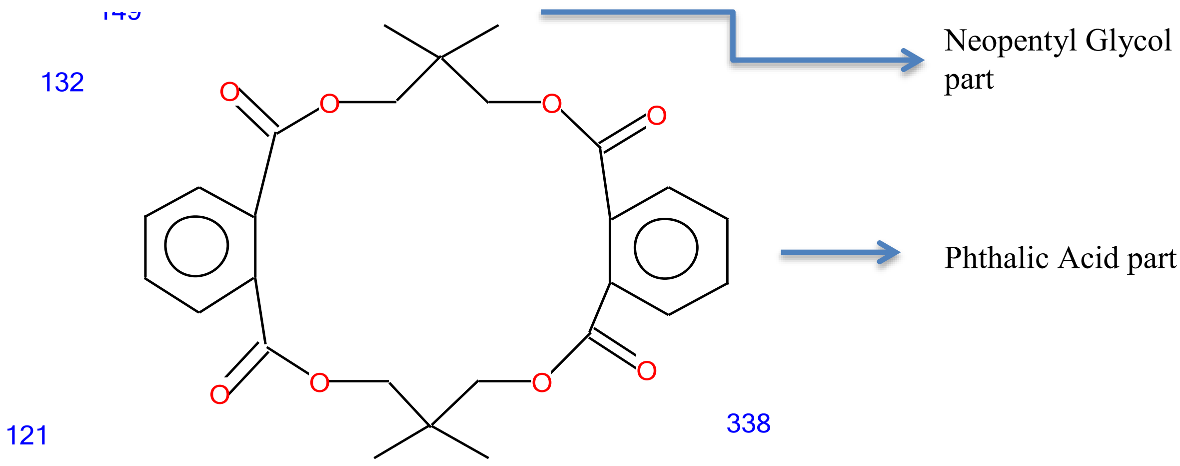


Figure 21: Cyclic oligomer

It could be hypothesised, from the structure of the oligomer, that it was formed as a side product of the polymerisation reaction between neopentyl glycol and phtalic acid.

The oligomer, on the other hand, had a much faster initial rate of migration and seemed to have reached a plateau after day 3 (Figure 22). This is in line with a material that is not chemically bonded to the backbone of the sample and gets released rapidly, as would be expected with such a substance in a polyester coating (Aditi Shrikhande 2012).

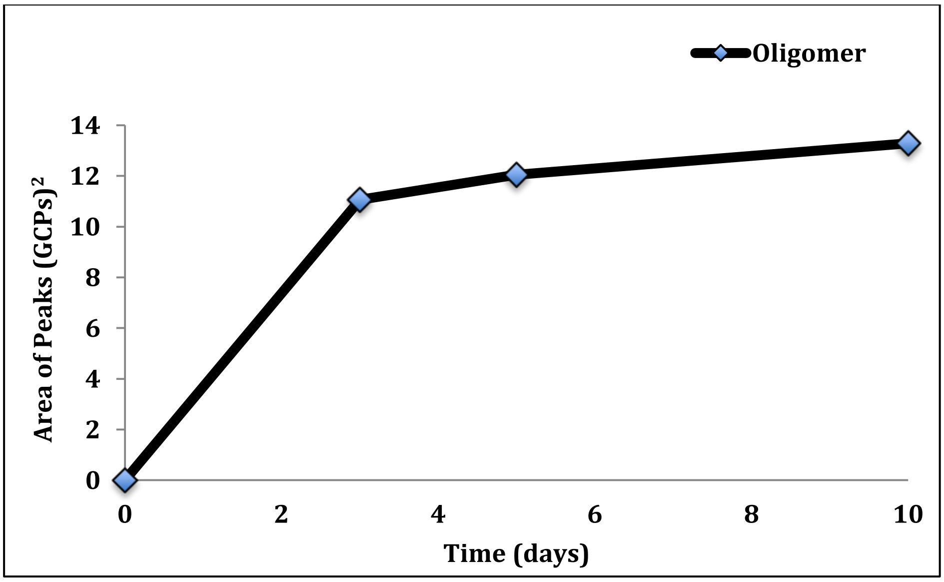


Figure 22: Migration of cyclic oligomer after 3, 5 and 10 days (EtOH 20%, 60 C)

Potential uses of 1,3 Dioxane-5-methanal-5 ethyl in the polyester are not known. Its use was not foreseen according to the information provided by the producer and relevant information could not be found in the literature. However, for the sake of completeness, its migration pattern into the wine simulant is shown in Figure 23.

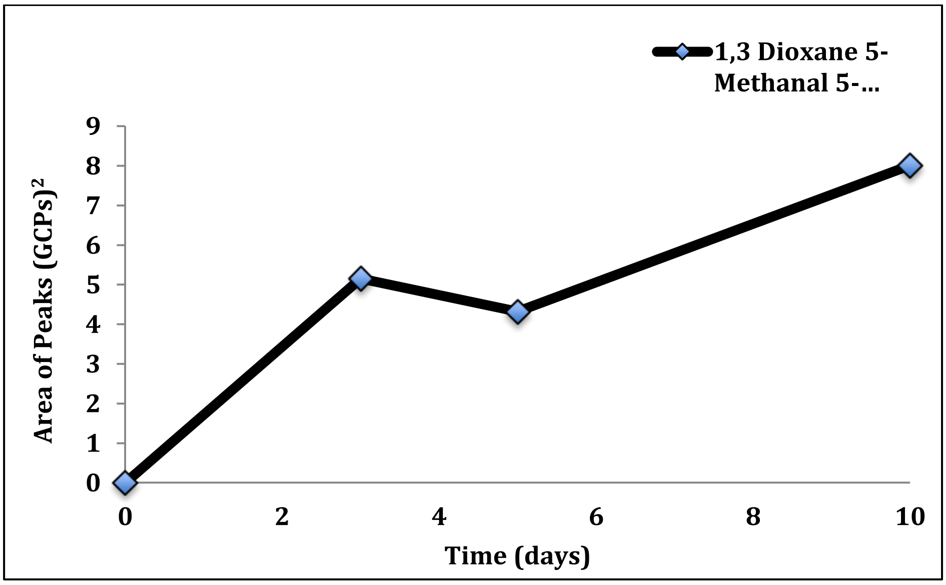


Figure 23: Migration of 1,3 Dioxane-5-methanal after 3, 5 and 10 days (EtOH 20%, 60 C)

The substance, after a significant initial increase, seemed to migrate more slowly after day 3. Some of the variation found thereafter may be related to the experimental error of the method, as was the case for BPA.

4 Future Work

The occurrence and exposure estimates that were made could be improved by measuring a larger sample size. Especially Port wines, which were found contaminated with concerning levels of DBP.

Given some phthalate cross-contamination was experienced, it may prove to be beneficial to adopt extra decontamination practices. Anja Fankhauser-Noti and Koni Grob (2007) have shown, for example, that adding Al_2O_3 to solvents can prior to their use could drastically reduce the contents of DEHP. Another step to limit this issue could be to use separated rooms for the preparation of standards from other sample handling procedures.

This experimental design of the migration study could also be improved: including an internal standard able to account for changes in volume and repeating the measurements at least twice, for example, could help accounting for experimental error. Another possible improvement could be to decrease the temperature during the evaporation of the extracts, in order to limit the loss and degradation of the migrants.

Given the study was purely qualitative, trying to plot a calibration curve using standards could also be interesting, since the method proved to be sensitive enough to detect a wide range of contaminants, such as BPA without any need of sample treatment.

5 Conclusions

BPA levels in wine were found to be moderate, with averages ranging between 1.16 µg/l (LB) and 1.76 µg/l (HB). The exposure estimates for an average consumer were low compared to the t-TDI (4.8% LB, 7.2% HB); as could be expected, they were higher for high consumers (31%LB, 46.5%HB).

Of the eleven phthalates monitored in wine, DEHP and DBP were the most commonly found, with averages ranging 30.33 µg/l -39.59 µg/l and 21.91 µg/l – 22.59 µg/l. The estimated exposure levels of the phthalates subject to regulations always were at least 100 times lower than the TDI. The Port wines analyzed, however, were significantly contaminated by DBP, with the most contaminated sample reaching 825 µg/l. DiBP, a non-permitted food contact material, was also found at levels of 106 µg/l in one sample. The higher susceptibility of Port wines towards migration of phthalates could be related to their higher ethanol contents and to a possible exposure to sources of phthalates during fortification or aging.

The qualitative chromatographic method used showed that it was possible to detect compounds such as BPA or ε-Caprolactone in the epoxy coating sample analyzed. The fast migration behaviour of BPA seems to indicate that it was released as a result of the residual free BPA of the sample. The slower release of ε-Caprolactone could be related to a stronger degree of binding to the polymeric backbone, which could be related to the formation of hydrogen bonds.

The analysis of the polyester extracts highlighted the probable use of neopentyl glycol and phthalic acid as building block of the material. Unbound oligomeric side-products were detected; the release profile of which was very rapid, in line with their presence as unbound side-products.

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7 Annexes

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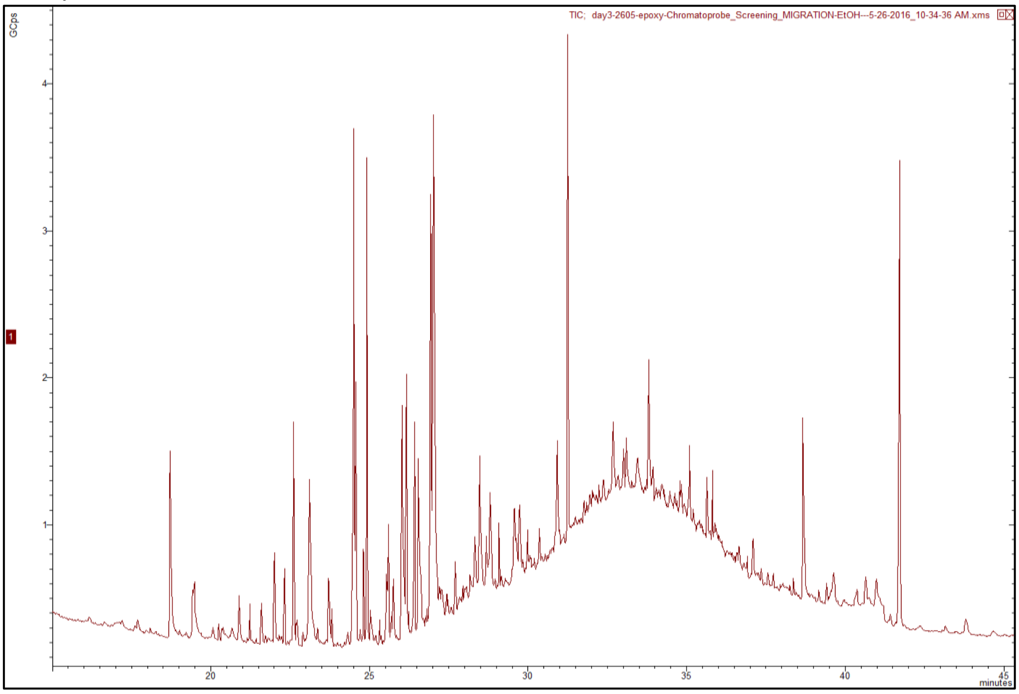
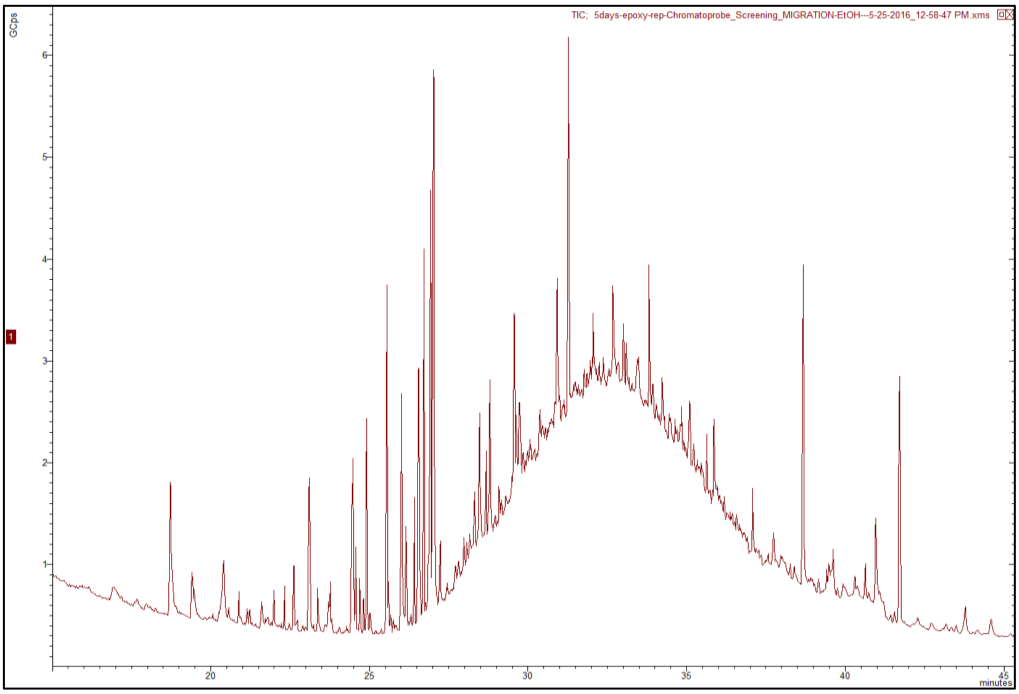
Annex 3: Chromatogram of extract of the epoxy phenolic coating after 3 and 5 days (20% EtOH, 60 ºC)

3 Days

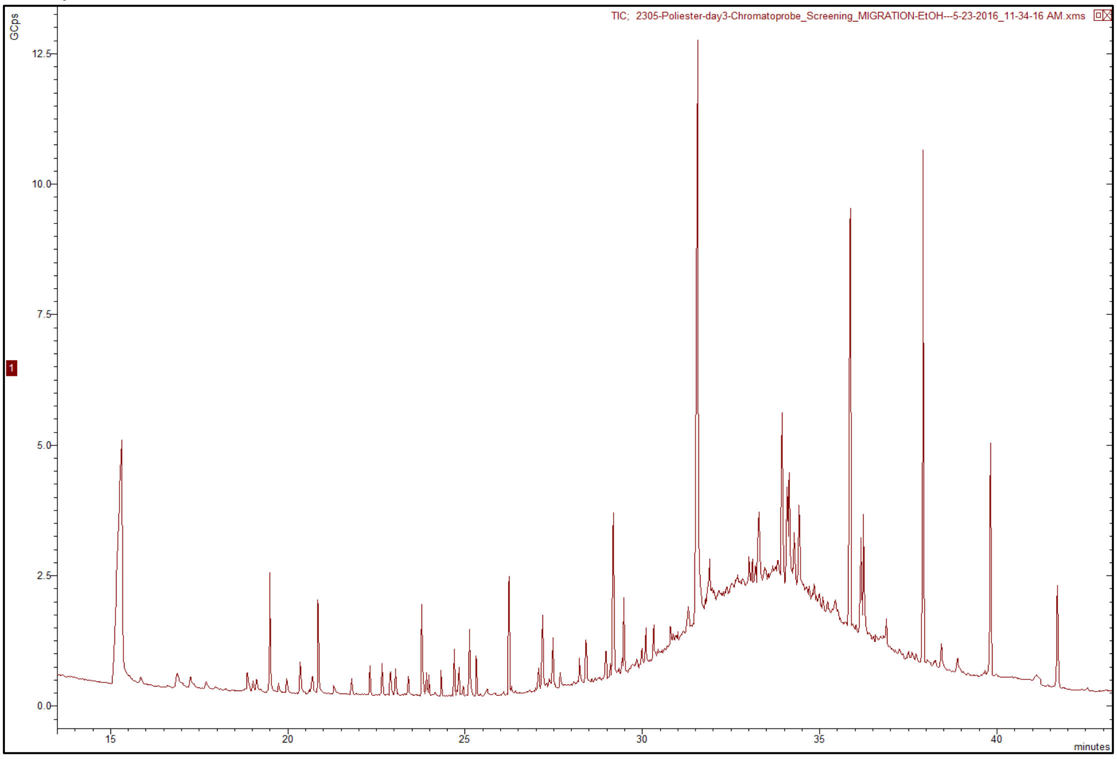


5 Days

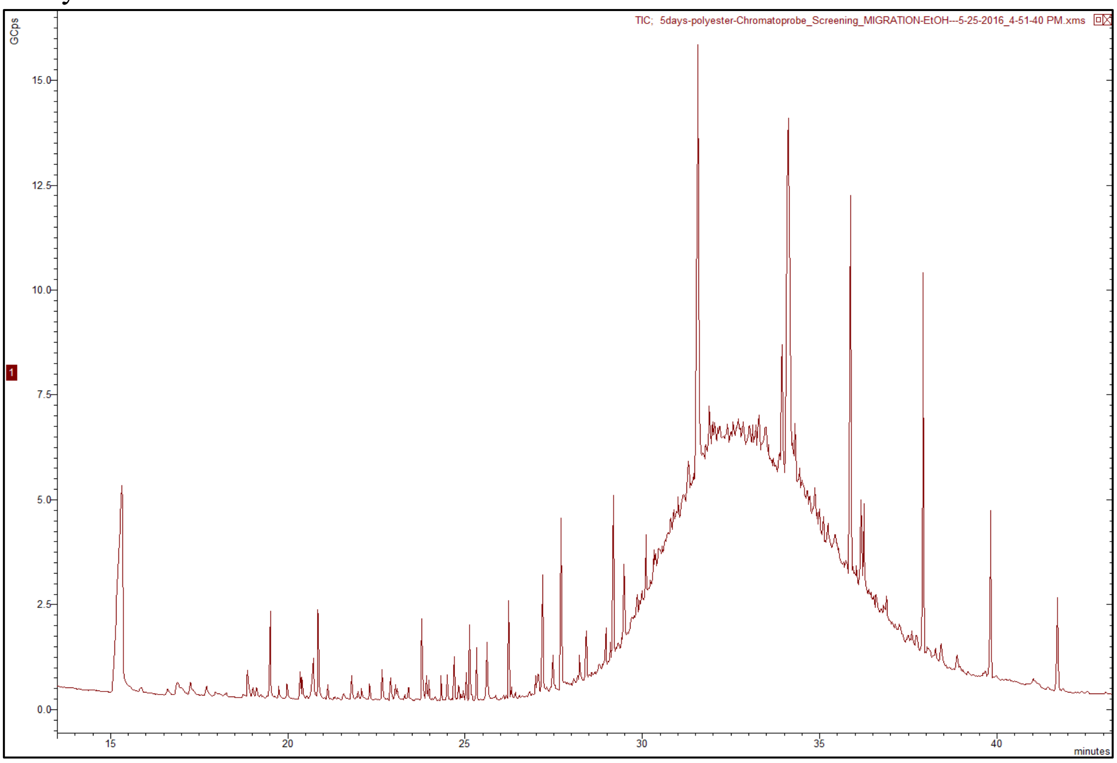


Annex 4: Chromatogram of extract of the polyester coating after 3 and 5 days (20% EtOH, 60 ºC)

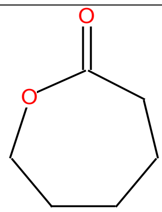
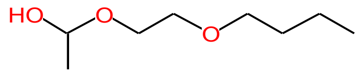
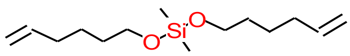
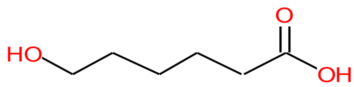
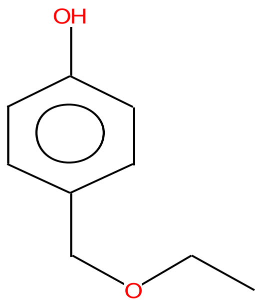
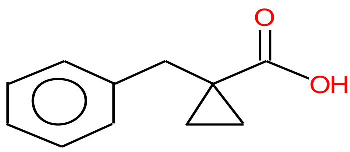
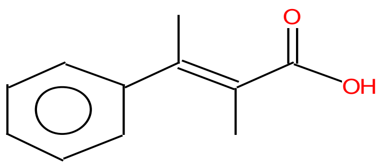
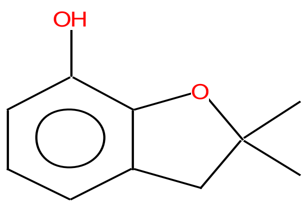
3 Days

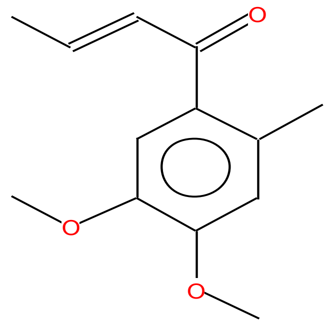
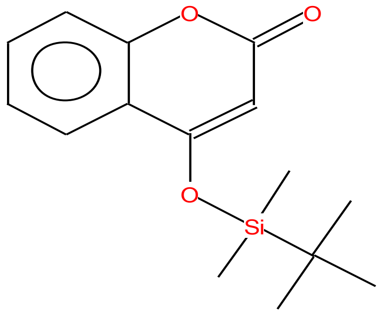
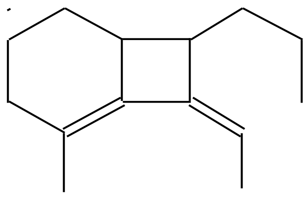
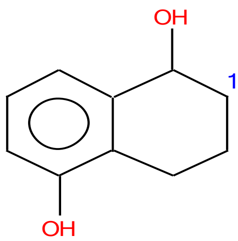
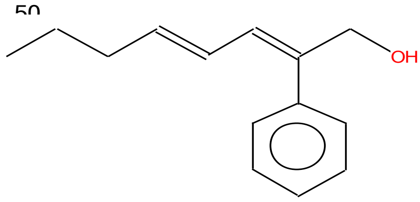
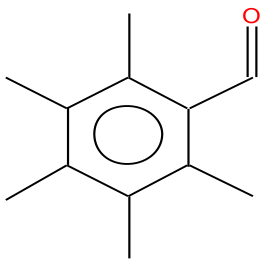


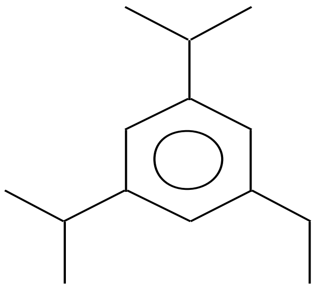
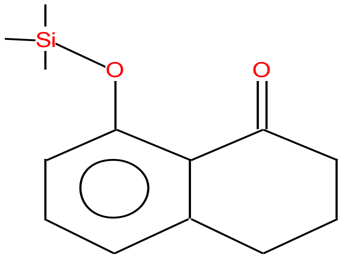
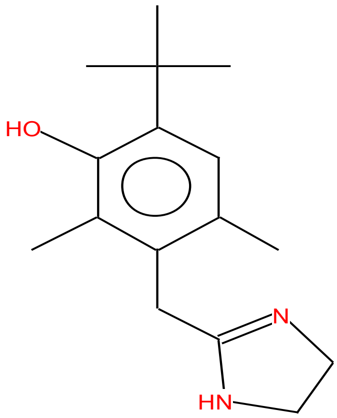
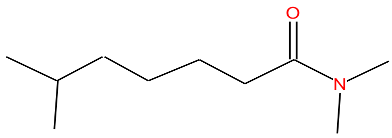
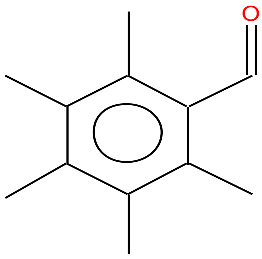
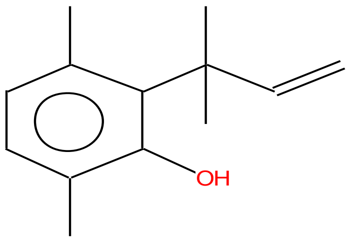
5 Days

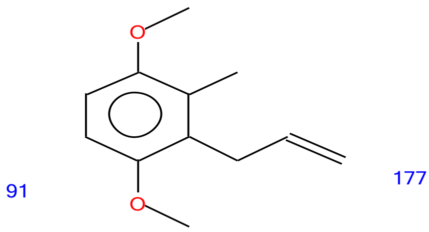
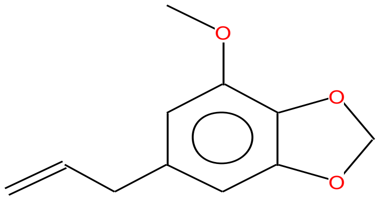
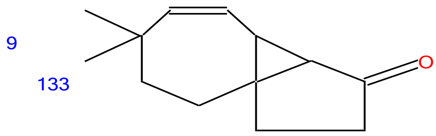
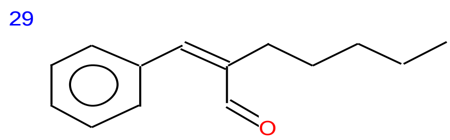
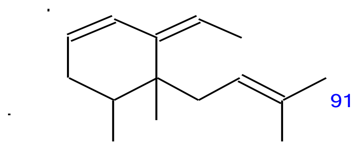
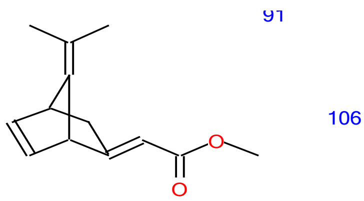
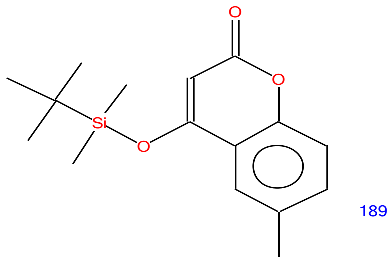
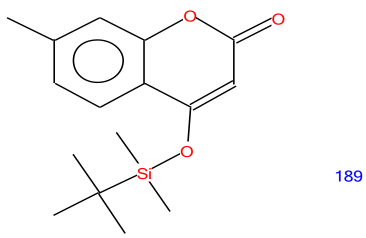


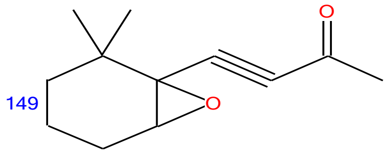
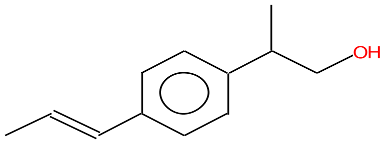
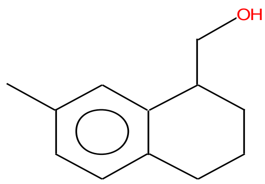
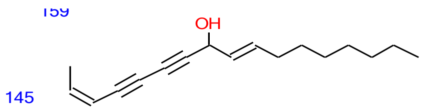
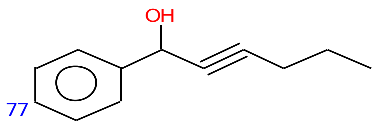
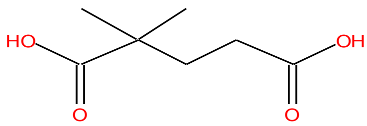
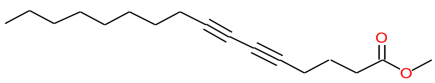
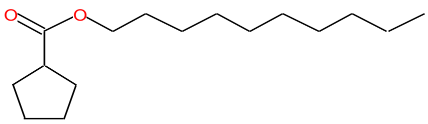
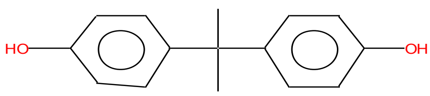
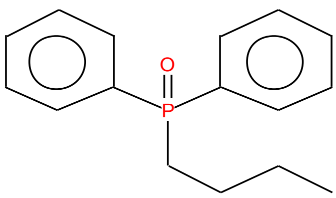
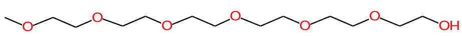
Annex 5: Probable structure of peaks (1 GPcs or more) of the epoxy coating (10 days, 20% EtOH, 60 ºC)

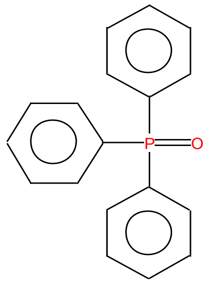
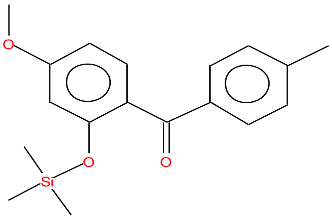
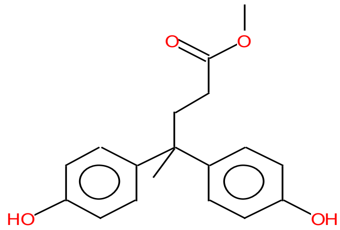
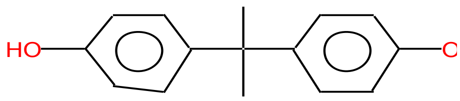
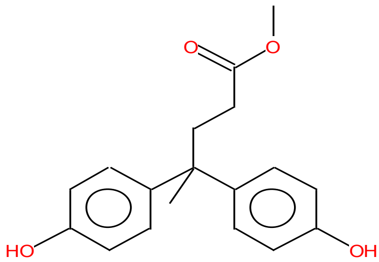
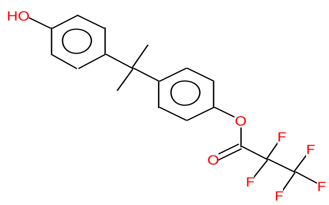
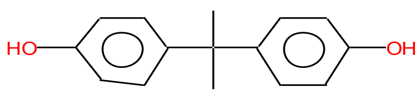
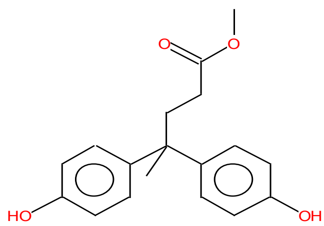
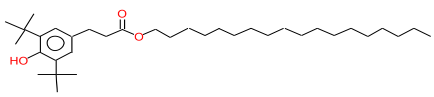
RT (min)	NAME	CAS n	Prob (%)	Structure
18.725	Caprolactone	502-44-3	68.7	
19.397	Ethanol, 1-(2-butoxyethoxy)-	54446-78-5	42.3	
20.424	bis(Hex-5-en-1-yloxy)(dimethyl)silane	NA	6.66	
20.536	Hexanoic Acid	1191-25-9	43.9	
21.975	4-Ethoxymethylphenol	57726-26-8	94.7	
22.611	Cyclopropanecarboxylic acid, 1-(phenylmethyl)-	27356-91-8	20	
	Cinnamic acid, α,β-dimethyl	NA	11.5	
23.115	7-Benzofuranol, 2,3-dihydro-2,2-dimethyl-	1563-38-8	34.4	

23.376	2-Methyl-4,5-dimethoxycrotonophenone	207233-94-1	16.6	39  179
23.731	4-Hydroxycoumarin, TBDMS derivative	NA	16.6	 175
	Biphenylene, 1,2,3,6,7,8,8a,8b-octahydro-4,5-dimethyl-	106988-87-8	15.4	
24.478	1,5-Dihydroxy-1,2,3,4-tetrahydronaphthalene	40771-26-4	10.5	 107 136
24.571	2-Phenyl-2,4-octadienol	NA	17.2	50 
24.926	Pentamethylbenzaldehyde	17432-38-1	13.2	 13

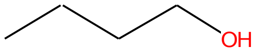
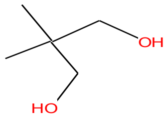
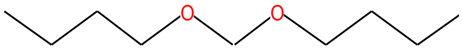
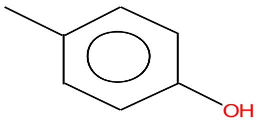
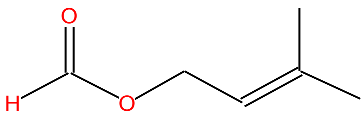
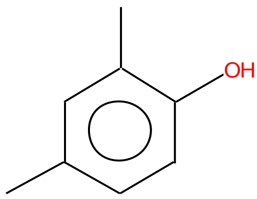
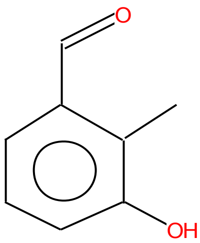
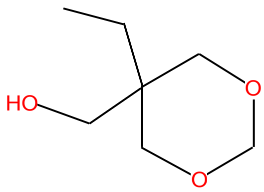
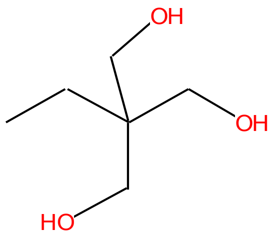
	Benzene, 1-ethyl-3,5-diisopropyl-	15181-13-2	9.05	
	8-Hydroxy-1-tetralone, TMS derivative	NA	26.6	
25.579	Oxymetazoline	1491-59-4	20.4	
25.616	Isooctanamide, N,N-dimethyl-	NA	14.7	
25.766	Pentamethylbenzaldehyde	17432-38-1	9.43	
26.027	Phenol, 2-(1,1-dimethyl-2-propenyl)-3,6-dimethyl-	92617-73-7	7.6	

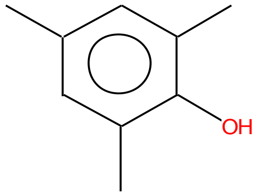
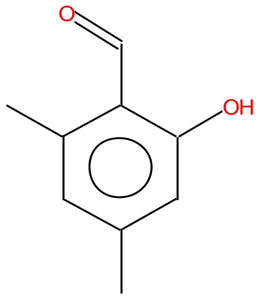
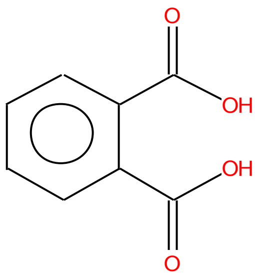
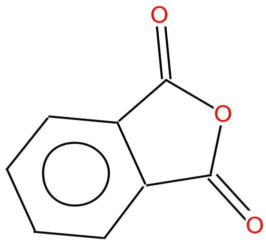
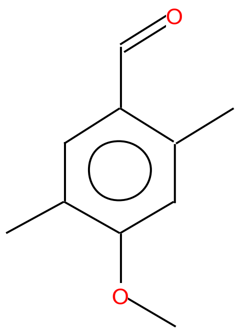
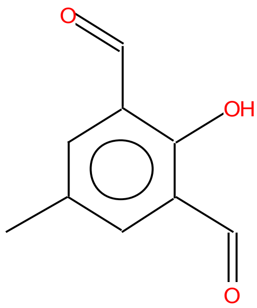
	2-Allyl-1,4-dimethoxy-3-methyl-benzene	NA	19.1	
26.158	1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)-	607-91-0	16.2	
	Cyclopenta[1,3]cyclopropa[1,2]cyclohepten-3(3aH)-one, 1,2,3b,6,7,8-hexahydro-6,6-dimethyl-	91531-58-7	18.8	
26.419	Cinnamaldehyde, α-pentyl-	122-40-7	10.5	
	β-Vatirenene	NA	8.05	
26.531	(7-Isopropylidenebicyclo[2.2.1]hept-5-en-2-ylidene)acetic acid, methyl ester	NA	10.6	
26.699	4-(tert.-Butyldimethylsilyl)oxy-6-methylcoumarin	NA	34	0 
	4-(Tert.-butyldimethylsilyl)oxy-7-methylcoumarin	NA	26.7	0 

26.923	1-Butyn-3-one, 1-(6,6-dimethyl-1,2-epoxycyclohexyl)-	NA	19.7	
27.24	Benzeneethanol, β-methyl-4-(1-propenyl)-	NA	27.7	
28.304	1-Naphthalenemethanol, 1,2,3,4-tetrahydro-8-methyl-	NA	16.8	
	2,9-Heptadecadiene-4,6-diyn-8-ol, (Z,E)-	NA	16.2	
28.473	Benzenemethanol, α-1-pentynyl-	108946-34-5	19.1	
28.659	?	NA		
28.791	?	NA		
29.556	2,2-Dimethylglutaric acid	681-57-2	36.8	
29.631	Methyl 5,7-hexadecadiynoate	NA	31.1	
29.725	Cyclopentanecarboxylic acid, decyl ester	NA	19.8	
30.919	**BPA**	**80-05-7**	**85.4**	
31.256	Phosphine oxide, butyldiphenyl-	4233-13-0	60.6	
32.04	2,5,8,11,14,17-Hexaoxanonadecan-19-ol	23601-40-3	26.4	

33.813				
	Triphenylphosphine oxide	791-28-6	95.9	
	{4-Methoxy-2-[(trimethylsilyl)oxy]phenyl}(4-methylphenyl)methanone	NA	41.3	
35.102				
	4-Hydroxy-γ-(4-hydroxyphenyl)-γ-methylbenzenebutanoic acid, methyl ester	7297-85-0	23.8	
	BPA	80-05-7	25.8	
35.643				
	4-Hydroxy-γ-(4-hydroxyphenyl)-γ-methylbenzenebutanoic acid, methyl ester	7297-85-0	19.5	
37.08	Isopropylidenediphenol, 4-hydroxy-4'-pentafluoropropionyloxy-	NA	33	
	BPA	80-05-7	42.5	
38.667				
	4-Hydroxy-γ-(4-hydroxyphenyl)-γ-methylbenzenebutanoic acid, methyl ester	7297-85-0	18.1	
41.693	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-	2082-79-3	94.3	

Annex 6: Probable structure of peaks (1 GPcs or more) from the polyester coating (dry measurement)

RT (min)	NAME	CAS n	Prob (%)	Structure
8.3	1-butanol	71-36-3	59.8	
15.3	Neopentyl glycol	126-30-7	81.6	
17.2	Dibutoxymethane	2568-90-3	69.9	
17.78	p-cresol	106-44-5	46	
18.87	Formic acid, 3-methyl-2-butenyl ester	68480-28-4	56	
18.96	Phenol 2,4 dimethyl	105-67-9	22.8	
19.04	5-Hydroxy-2-methylbenzaldehyde	23942-00-9	27.1	
19.63	1,3 Dioxane-5-methanal-5ethyl	5187-23-5	83	
	trimethylol prapane	77-99-6	9.59	

19.9	Phenol, 2,3,6-trimethyl	2416-94-6	36.9	
20.46	2-Hydroxy-4,6-dimethylbenzaldehyde	1666-02-0	52.7	
21.502	Phthalic Acid	88-99-3	46.9	
	Phthalic Anhydride	85-44-9	37.8	
23.05	Benzaldehyde, 4-methoxy-2,5-dimethyl-	6745-75-1	35	
23.26	2-Hydroxy-5-methylisopthaldehyde	7310-95-4	88.3	

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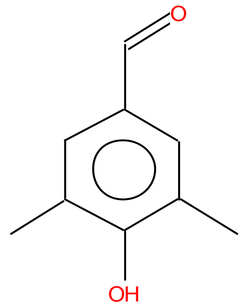
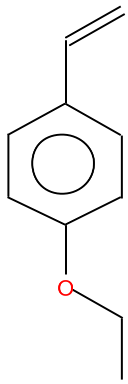
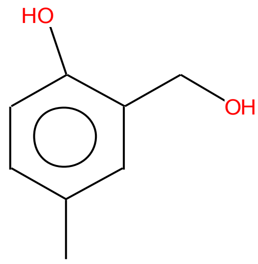
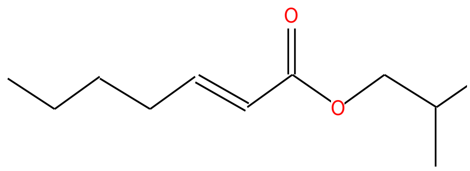
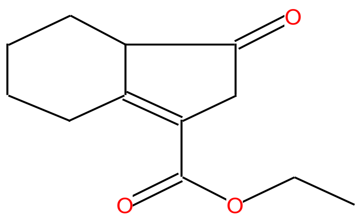
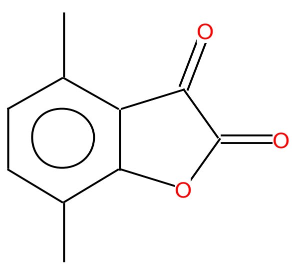
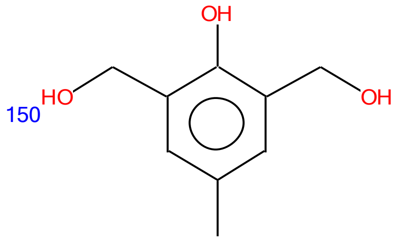
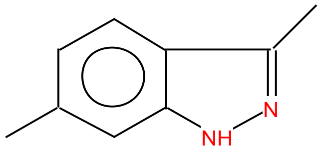
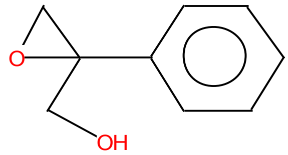
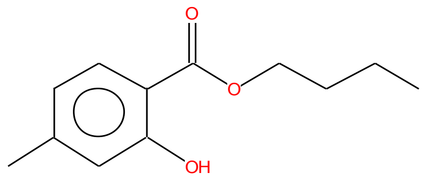
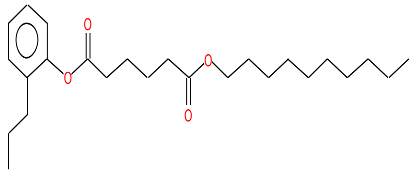
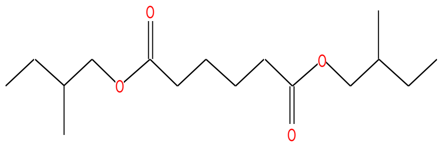
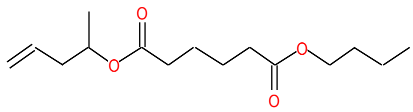
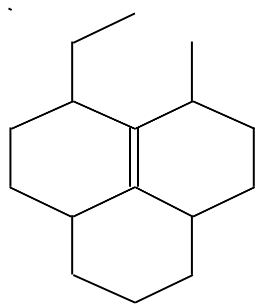
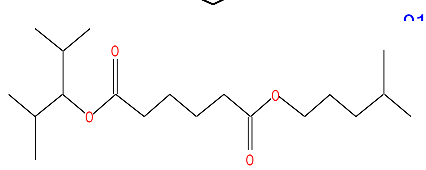
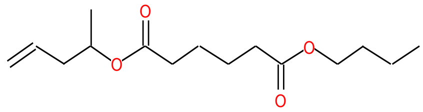
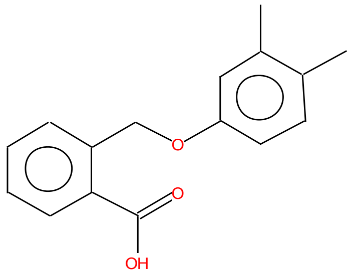
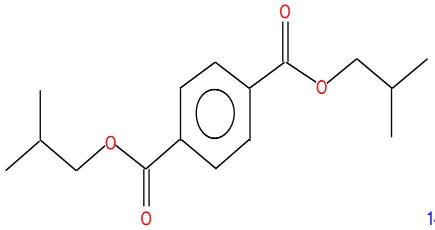
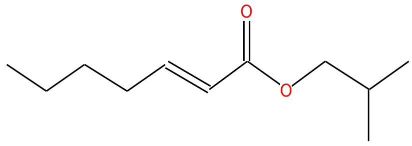
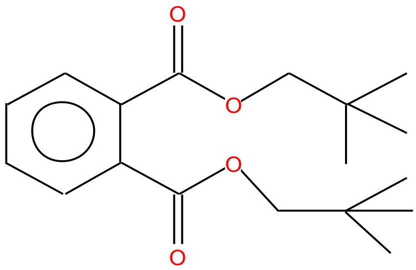
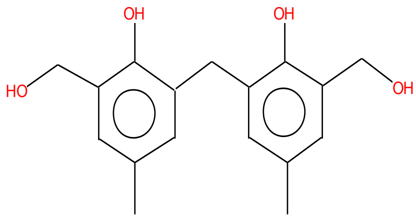
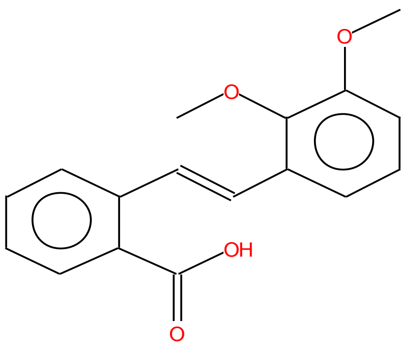
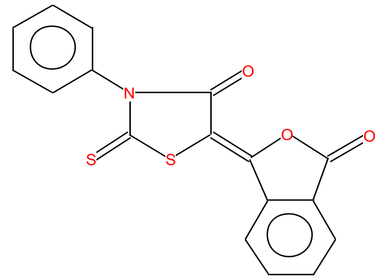
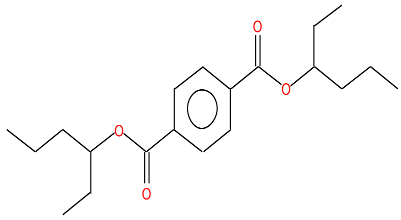
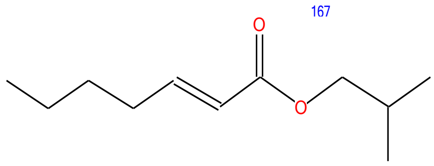
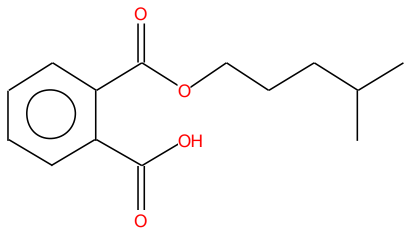
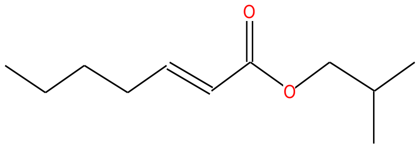
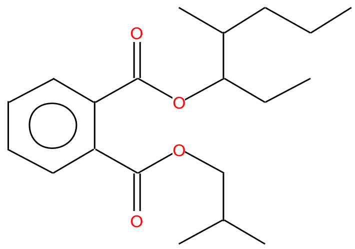
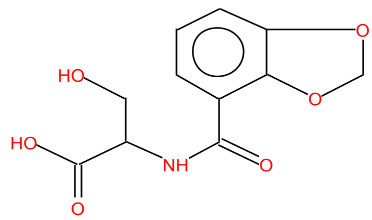
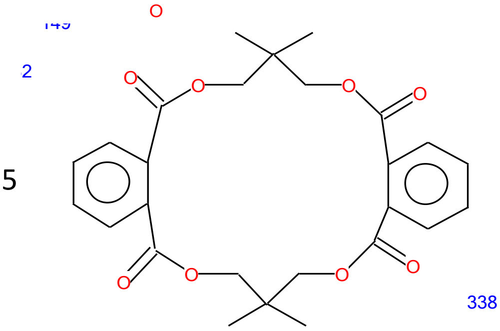
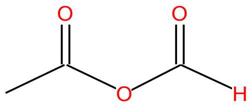
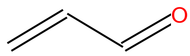
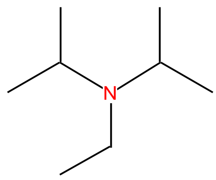
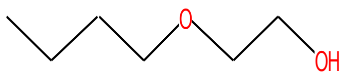
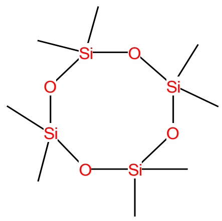
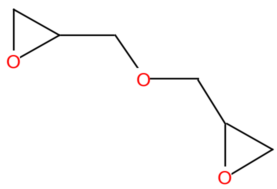
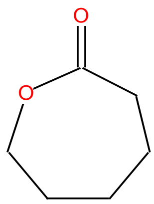
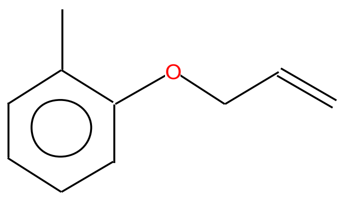
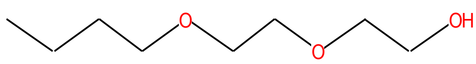
23.61	3,5-Dimethyl-4-hydroxybenzaldehyde	2233-18-3	65	
23.97	4-Ethoxystyrene	5459-40-5	26	148 
24.04	2-Hydroxy-5-methylbenzyl alcohol	4383-07-7	7.51	
24.4	Ester adipic acid	NA	5	
24.85	3-oxo-3,3A-4,5,6,7-Hexahydro 2H indene-1-carboxilic acid ethyl ester	NA	23	
25.09	Benzofuran-2,3-dione, 4,7-dimethyl-2,3-dihydro-	31297-30-0	30	

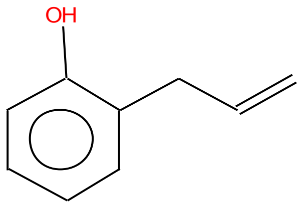
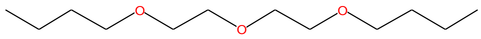
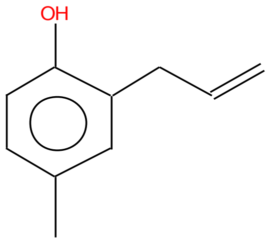
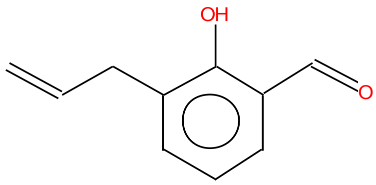
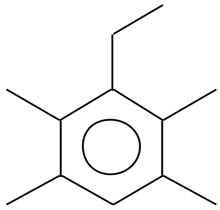
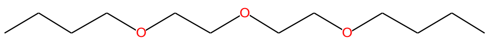
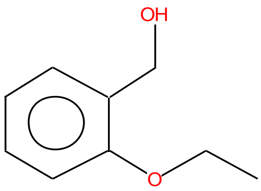
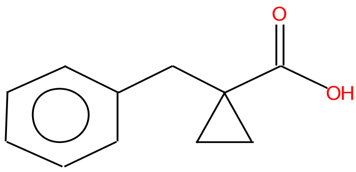
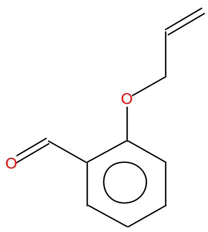
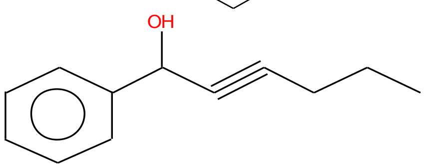
25.35	1,3-Benzenedimethanol, 2-hydroxy-5-methyl	91-04-3	48.1	
25.8	1H-Indazole-3,6-Dimethyl	7746-28-3	36	
26.417	Oxiranemethanol, 2-phenyl	141248-89-7	20.6	
27.4	Unknown 1			
27.6	Unknown 2			
28	butyl-2-didioxy-4-methyl benzoate	NA	73	
28.6	Ester adipic acid	NA	5	
29.347	Hexanedioic acid, bis (2-methylpropyl) ester	141-04-8	16.6	
29.4	adipic acid ester	NA	4.6	
29.5	Unknown 3			
29.67	1(3H)-Isobenzofuranone, 3-(2-hydroxy-oxo-1-cyclohexenyl)	NA	14.2	
30.449	Adipic acid like compound	NA	9.04	

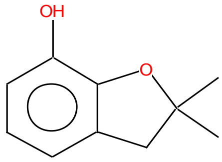
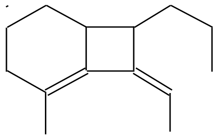
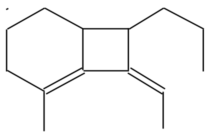
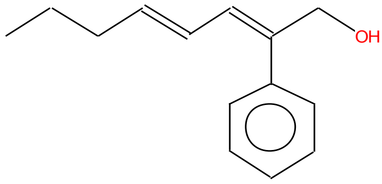
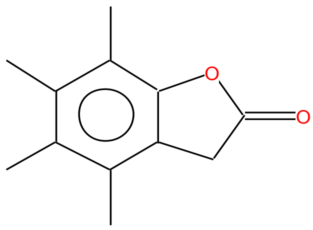
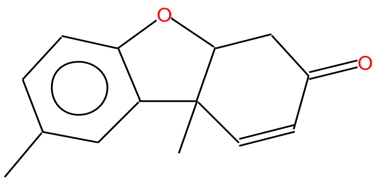
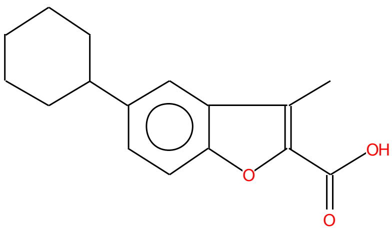
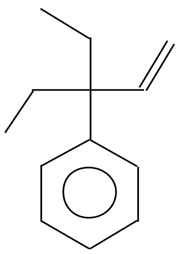
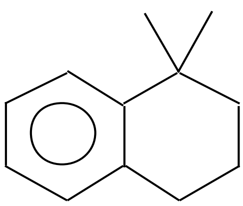
31.03	Adipic acid ester	NA	9.18	
31.38	Benzoic acid, 2 (3,4-dimethylphenoxymethyl)	NA	70	
31.662	Diisobutyl terephthalate	18699-48-4	44.9	 149
31.849	Adipic acid ester	NA	7.84	
31.961	Dineopentyl phthalate	2553-24-4	17.7	
32.110	Benzenemethanol, 3,3'-methylenebis(2-hydroxy-5-methyl)	22247-58-1	22.2	 145
32.801	Benzoic acid, 2-(2-(2,3-dimethoxyphenyl)ethenyl	NA	24.1	

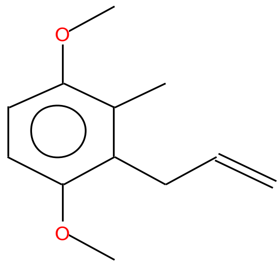
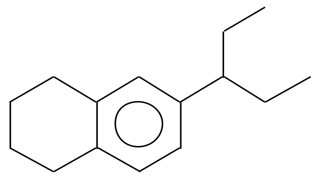
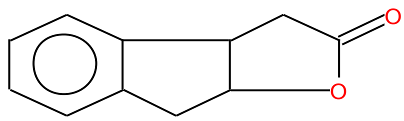
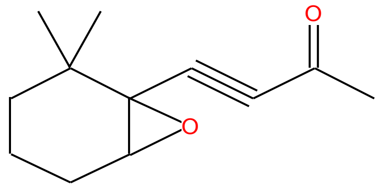
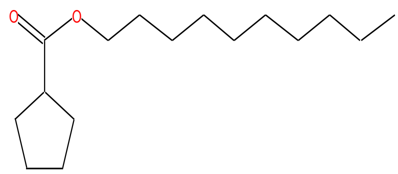
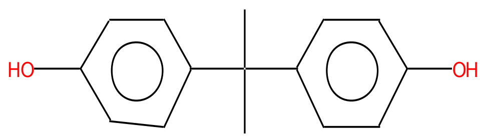
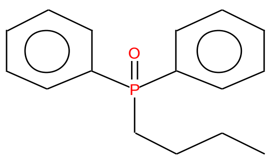
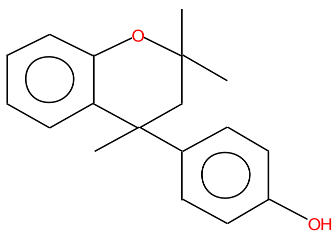
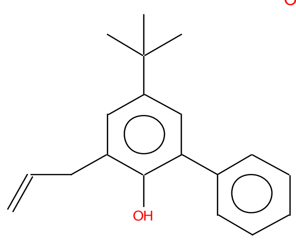
33.268	Phthalic Acid ester	NA	13.8	
33.512	Phthalic Acid ester	NA	7.32	
34.100	Adipic acid ester	NA		
34.389	Phthalic Acid ester like compound	84131-14-6	12.3	
36.014	Ester Adipic acid compound	NA	8.29	
36.350	Mono -3,5,5 trimethylhexyl-phthalate	84131-14-6	5.2	
38.100	N-(1,3-Benzodioxol-4-ylcarbonyl) serine	NA	6.55	
40.065	7H,18H-dibenzo[g,p][1,5,10,14]tetraoxacyclooctadecin-5,11,16,22-tetrone, 8,9,19,20-tetrahydro-8,8,19	NA	83.5	

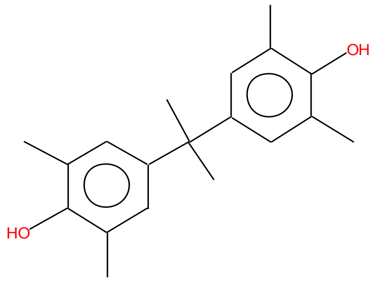
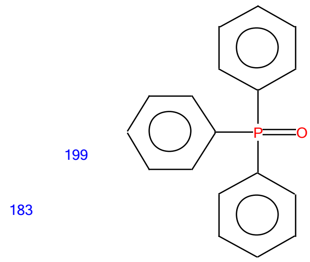
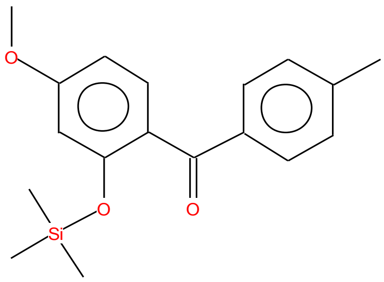
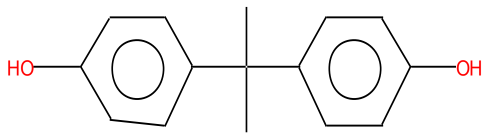
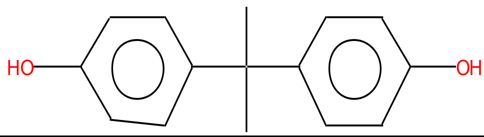
Annex 7: Probable structures of peaks with at least 1 GPcs intensity from the epoxy phenolic coating (dry measurement)

RT (min.)	NAME	CAS n	Prob (%)	Structure
8	Acetic Acid, anhydride with formic acid	2258-42-6	41.2	
8.5	2-propenal	107-02-8	86.8	
12.2	Diisopropylethylamine	7087-68-5	72.8	
14.5	Ethanol, 2-butoxy	111-76-2	70.8	
15.8	Cyclotetrasiloxane, octamethyl	556-67-2	49.3	
17.6	diglycidil ether	2238-07-5	12.9	
18.8	ε-Caprolactone	502-44-3	62.5	
19.48	Allyl-o-tolyl-ether	936-72-1	58	
19.5	Butyl Diglycol	112-34-5	47.9	

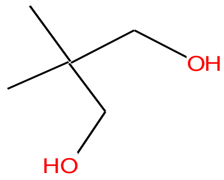
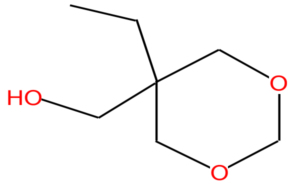
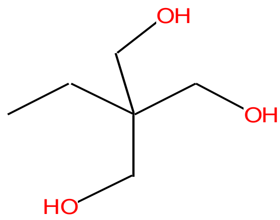
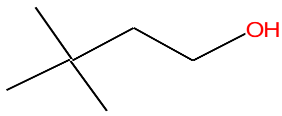
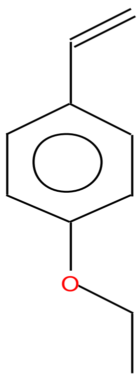
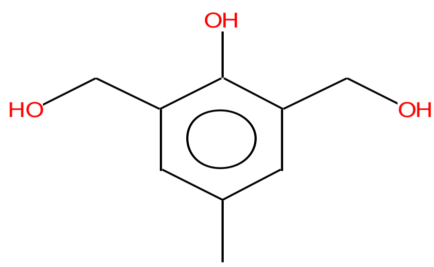
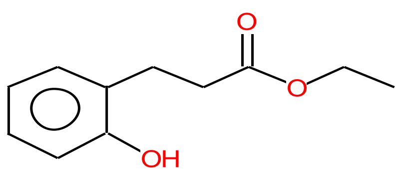
19.61	2-Allylphenol	1745-81-9	66.7	
20.103	Dibutyl Carbitol	112-73-2	50	
20.92	2-Allyl-p-cresol	6628-06-4	55	 77
21.28	Benzaldehyde, 2-hydroxy-3-(2-propenyl)	24019-66-7	65.2	
21.6	Benzene, 3-ethyl-1,2,4,5-tetramethyl	31365-98-7	11.4	
21.76	Dibutyl Carbitol	112-73-2	23	
22.57	2-Ethoxybenzyl Alcohol	71672-75-8	39	50 
22.68	Cyclopropane carboxylic acid,1-(phenulmethyl)	27356-91-8	23	
22.81	Benzaldehyde, 2-(2-propenyloxy)	28752-82-1	53.2	
23.239	Benzenemethanol, alpha-1-pentynyl	108946-34-5	20.3	

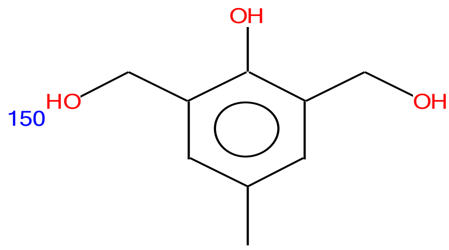
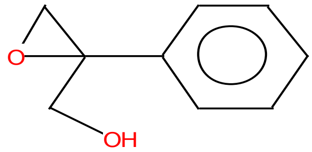
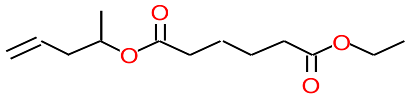
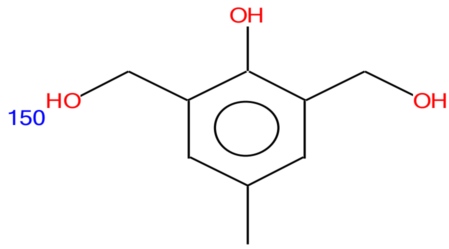
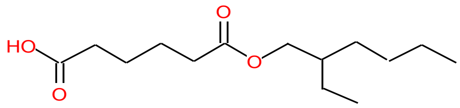
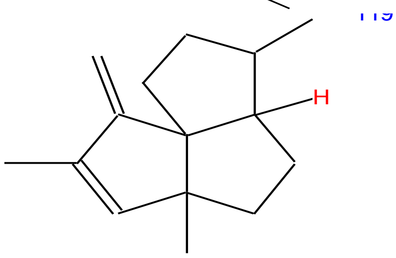
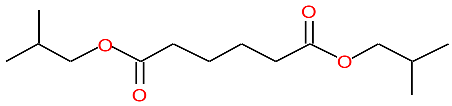
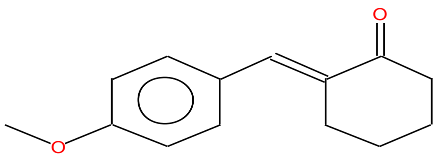
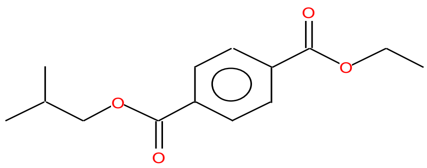
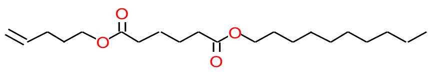
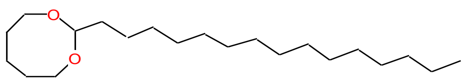
	2,3-Dihydro-2,2-dimethyl-7-benzofuranol	1563-38-8	16.3		14
23.43	Biphenylene, 1,2,3,6,7,8a,8b-octahydro-4,5-dimethyl	106988-87-8	33		91 50
23.81	Biphenylene, 1,2,3,6,7,8a,8b-octahydro-4,5-dimethyl	106988-87-8	21		91 50
24.64	2-Phenyl-2,4-Octanedienal	NA	14		
25	4,5,6,7 -Tetramethylphthalide	29002-54-8	46.4		
25.27	3(4H)-Dibenzofuranone, 4a,9b-dihydro-8,9b-dimethyl	546-24-7	15.4		115
	Benzofurane-2-carboxylic-acid,5-cyclohexyl-3-methyl	NA	12.1		1.
25.76	3-Ethyl-3-phenyl-1-pentene	19781-34-1	11.8		
	1,1-Dimethyltetralin	1985-59-7	10.9		

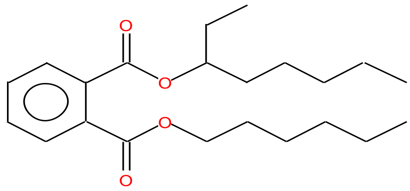
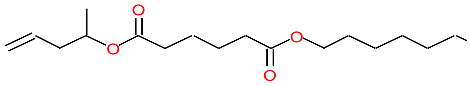
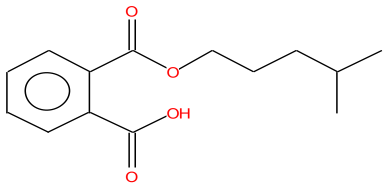
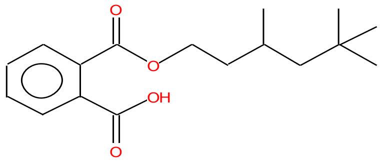
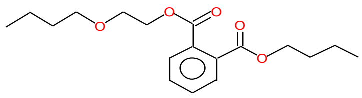
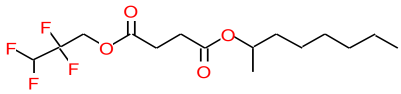
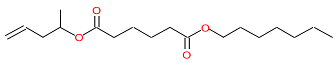
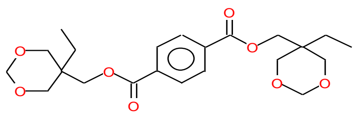
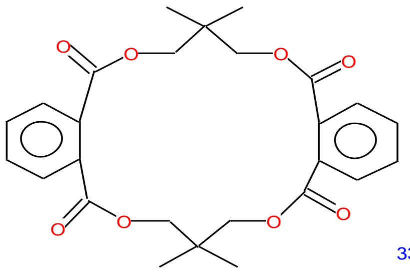
26.133	2-Allyl-1,4-dimethoxy, 3-methylbenzene	NA	19	
26.5	Naphtalene, 6-(1-ethylpropyl)-1,2,3,4 -tetrahydro	54889-56-4	11.7	
26.712	2H-Indeno(2,1b)furan-2-one, 3,3a,8,8a-tetrahydro	80343-98-2	29.6	
27.02	1-Butyn-3-one, 1-(6,6-dimethyl-1,2-epoxycyclohexyl)	NA	22	
29.83	Cyclopentane carboxylic acid, decyl ester	NA	39.6	
31.06	**BPA**	**80-05-7**	**85.3**	
31	Phosphine oxide, butyldiphenyl	4233-13-0	64.5	
32.4	Phenol, p-(2,2,4-trimethyl-4-chromanyl)-	472-41-3	48	
32.8	2-Hydroxy-3-allyl-5-t-butylbiphenyl	23079-51-8	50.9	

33.23	bisphenol a, tetramethyl	5613-46-7	13	
33.9	Triphenylphosphine	791-28-6	88.1	
35.208	Related to BADGE		51,5	
35.8	BPA-Based	NA	25	
38.83	BPA-Based	NA	27	

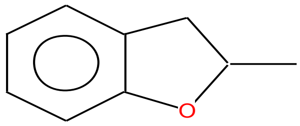
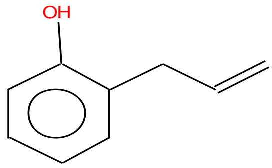
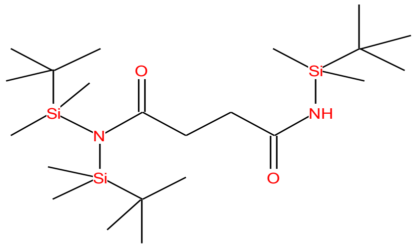
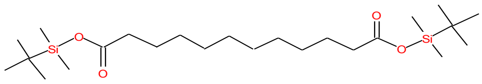
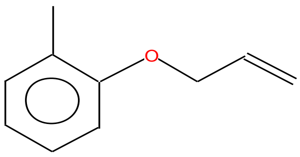
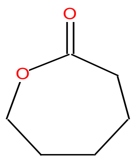
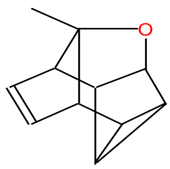
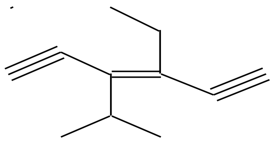
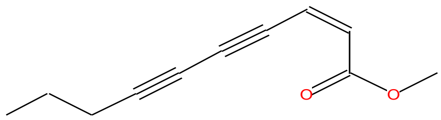
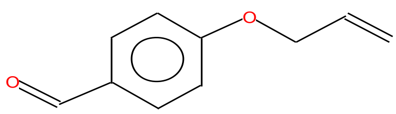
Annex 8: Probable structure of peaks (with 1 GPcs or more) from the polyester coating after 10 days (20% EtOH, 60 °C)

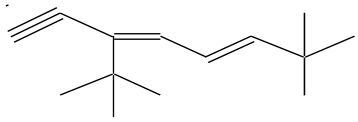
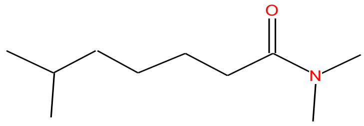
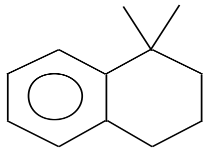
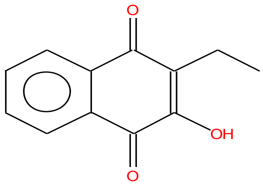
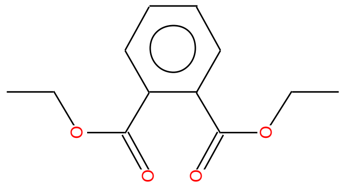
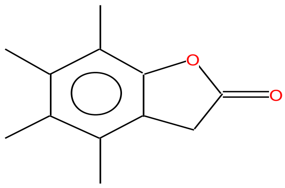
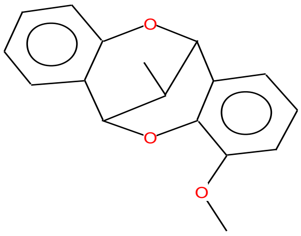
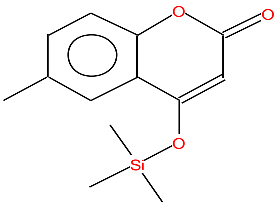
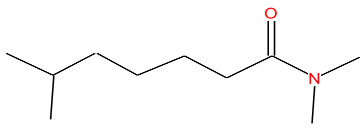
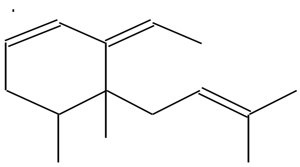
RT(min)	NAME	CAS n	Prob (%)	Structure
15.409	Neopentyl Glycol	126-30-7	80.5	73 
19.518	1,3 Dioxane-5-methanal-5ethyl	5187-23-5	88.9	
20.807	1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)-	77-99-6	88.3	
20.882	1-Butanol, 3,3-dimethyl-	624-95-3	9.8	
23.794	4-Ethoxystyrene	5459-40-5	19.2	148 
25.139	1,3-Benzenedimethanol, 2-hydroxy-5-methyl	91-04-3	64.5	
25.344	Ethyl 3-(2-hydroxyphenyl)propanoate	20921-04-4	21.9	

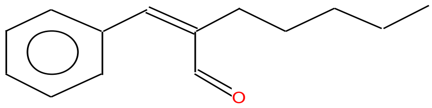
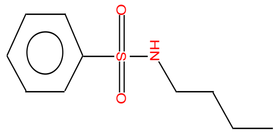
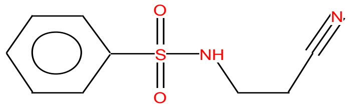
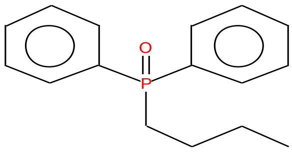
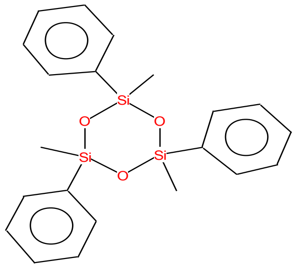
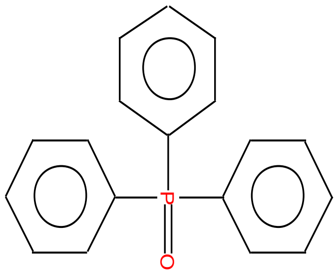
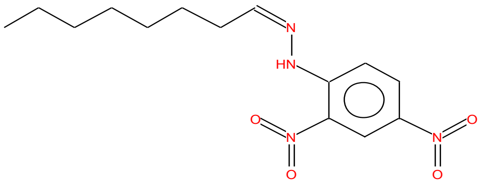
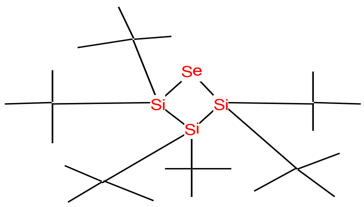
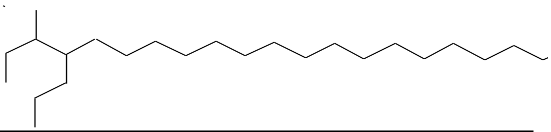
25.624	1,3-Benzenedimethanol, 2-hydroxy-5-methyl	91-04-3	50	
26.24	Oxiranemethanol, 2-phenyl-	141248-89-7	11.5	
27.212	Adipic acid, ethyl pent-4-en-2-yl ester	NA	31	
27.679	1,3-Benzenedimethanol, 2-hydroxy-5-methyl	91-04-3	46.4	
28.41	Hexanedioic acid, mono(2-ethylhexyl)ester	4337-65-9	17.4	
28.897	Silphiperfola-4,7(14)-diene	210637-49-3	24.3	
29.173	Hexanedioic acid, bis(2-methylpropyl) ester	141-04-8	23.3	
29.473	Cyclohexanone, 2-[(4-methoxyphenyl)methylene]-	5765-29-7	9.86	
30.108	Terephthalic acid, ethyl isobutyl ester	NA	14.1	
31.546	Adipic acid, decyl pent-4-enyl ester	NA	6.54	
31.883	1,3-Dioxocane, 2-pentadecyl-	41583-11-3	4.95	

33.267	Phthalic acid, hexyl oct-3-yl ester	NA	11.7	
33.92	Adipic acid, heptyl pent-4-en-2-yl ester	NA	54.1	
34.108	2-((4-Methylpentyloxy)carbonyl)benzoic acid	848131-14-6	8.79	
	2-((3,5,5-Trimethylhexyloxy)carbonyl)benzoic acid	297182-83-3	8.79	
34.276	1,2-Benzenedicarboxylic acid, 2-butoxyethyl butyl ester	33374-28-6	50.4	
35.845	Succinic acid, 2-octyl 2,2,3,3-tetrafluoropropyl ester	NA	20.6	
36.107	Adipic acid, heptyl pent-4-en-2-yl ester	NA	27.7	 295
36.218	Terephthalic acid, di((5-ethyl-1,3-dioxan-5-yl)methyl) ester	NA	40	
39.809	7H,18H-dibenzo[g,p][1,5,10,14]tetraoxacyclooctadecin-5,11,16,22-tetrone, 8,9,19,20-tetrahydro-8,8,19	NA	80.6	 338

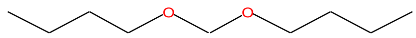
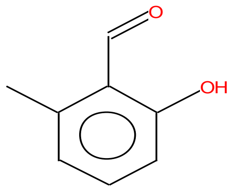
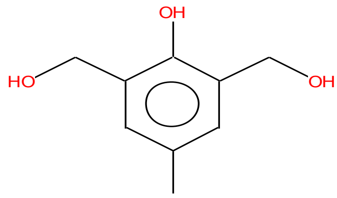
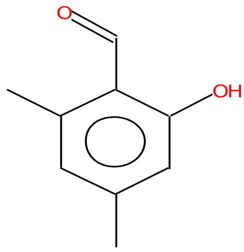
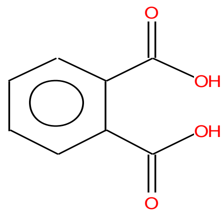
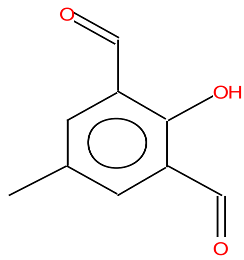
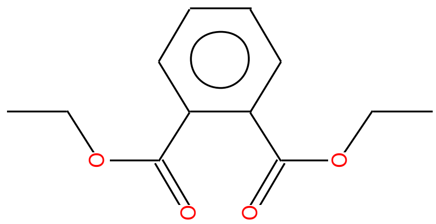
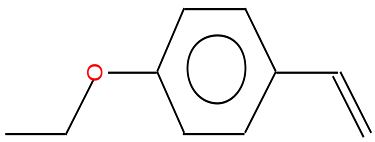
Annex 9: Probable structure of peaks identified in the epoxy coating AC extract

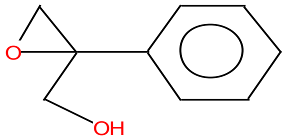
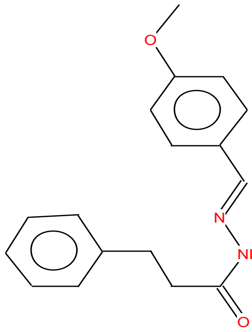
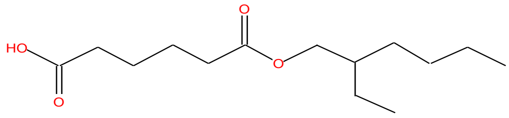
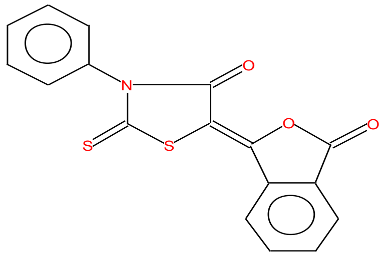
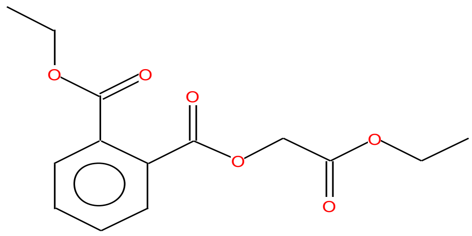
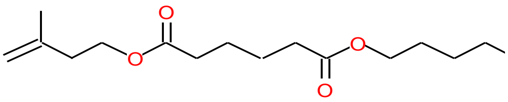
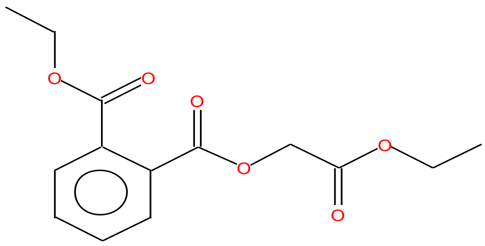
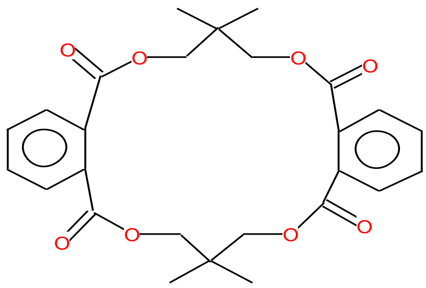
RT (min.)	NAME	CAS n	Prob (%)	Structure
	Benzofuran, 2,3-dihydro-2-methyl-	1746-11-8	26.1	
13.9	2-Allylphenol	1745-81-9	23.1	
14.4	N1,N1,N4-Tris(tert-butyldimethylsilyl) succinamide		38.2	
	Dodecanedioic acid, 2TBDMS derivative	104255-99-4	28.5	
14.6	Allyl o-tolyl ether	936-72-1	81.7	148 
15.8	ε-Caprolactone	502-44-3	50.9	
17.7	1,2,4-Metheno-1H-cyclobuta[b]cyclopenta[d]furan, 2,2a,3a,4,6a,6b-hexahydro-3a-methyl-	78323-74-7	18.8	
	Hex-3-ene-1,5-diyne, 3,4-diisopropyl-		12.5	
18.1	Methyl (Z)-dec-2-en-4,6-diynoate	505-01-1	9.6	
18.9	Benzaldehyde, 4-(2-propenyloxy)-	40663-68-1	46.3	

19.3	Oct-3-ene-1,5-diyne, 3-t-butyl-7,7-dimethyl-	-	40.1	
19.4	Isooctanamide, N,N-dimethyl-	-	14.9	
19.7	Naphthalene, 1,2,3,4-tetrahydro-1,1-dimethyl-	1985-59-7	11.4	
20.1	2-Phenyl-2,4-octadienol	-	17.4	
21.1	Diethyl Phthalate	84-66-2	52.2	
21.5	4,5,6,7-Tetramethylphthalide	29002-54-8	37	
21.6	Dibenzo[b,f]1,5-dioxacyclooctane, 4-methoxy-6,12-(ethylideno)-	205692-58-6	13.9	
22.2	4 –(Trimethylsilyl)oxy-6-methylcoumarin		18.1	
22.6	Isooctanamide, N,N-dimethyl-		10.5	
22.7	β-Vatirenene		10.1	

	Cinnamaldehyde, α-pentyl-	122-40-7	9.72	
23.9	Benzenesulfonamide, N-butyl-	3622-84-2	47.5	
	N-(2-Cyano-ethyl)-benzenesulfonamide	2619-21-8	58.6	
28.3	Phosphine oxide, butyldiphenyl-	4233-13-0	65.6	
31.5	Cyclotrilosiloxane, 2,4,6 –trimethyl-2,4,6-triphenyl	546-45-2	53.8	
31.7	Triphenylphosphine oxide	791-28-6	94.6	
	Octanal, (2,4-dinitrophenyl)hydrazone	1726-77-8	4.50	
39.5	Hexa-t-butylselenatrisiletane	93194-15-1	19.7	
	Cyclohexane, 1,4-dimethyl-2-octadecyl	55282-02-5	5.88	

Annex 10: Probable structure of peaks identified in the polyester coating AC extract

RT (min.)	NAME	CAS n	Prob (%)	Structure
11.05	Di-n-butoxymethane	2568-90-3	66	
14.8	Benzaldehyde, 2-hydroxy-6-methyl-	18362-36-2	43	
15.9	1,3-Benzenedimethanol, 2-hydroxy-5-methyl-	91-04-3	20.7	
	2-Hydroxy-4,6-dimethylbenzaldehyde	1666-02-0	18.3	
18.3	Phthalic Acid	88-99-3	71.5	
19.6	2-Hydroxy-5-methylisophthalaldehyde	7310-95-4	89.2	
21.1	Diethyl Phthalate	84-66-2	78.7	
21.6	4-Ethoxystyrene	5459-40-5	14.2	

21.8	Oxiranemethanol, 2-phenyl-	141248-89-7	29.8	
23.1	3-Phenyl-propionic acid (4-methoxy-benzylidene) hydrazide	-	8.4	149 
26	Hexanedioic acid, mono(2-ethylhexyl)ester	4337-65-9	30.3	
	3-Phenyl-5-phthalidylidenerhodanine	30162-50-6	13.8	
29.3				
	1,2-Benzenedicarboxylic acid, 2-ethoxy-2-oxoethyl ethyl ester	84-72-0	12.7	
32.1	Adipic acid, 3-methylbut-3-enyl tetradecyl ester	-	6.18	
34.8	Phthalic acid, hexyl oct-3-yl ester		5.11	
39.2	7H,18H-dibenzo[g,p][1,5,10,14]tetraoxacyclooctadecin-5,11,16,22-tetrone, 8,9,19,20-tetrahydro-8,8,19,19-tetramethyl-		29.1	49  338