TECHNISCHE UNIVERSITÄT MÜNCHEN
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Development of analytical screening methods for migration estimation of adhesives related substances in food packaging materials

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Contents

Abbreviations

ARF	Absolute Response Factor
BfR	Bundesinstitute für Risikobewertung
BgVV	Bundesinstitute für gesundheitlichen Verbraucherschutz und Veterinärmedizin
CAD	Charged Aerosol Detector
CEN	Committee Européen de Normalisation (European Standardisation Organisation)
EC	European Commission
ELSD	Evaporated Light Scattering Detector
EVA	Ethylene Vinyl Acetate
FID	Flame Ionisation Detector
FL	Fluorescence
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
IVK	Industrieverband Klebstoffe
LC	Liquid Chromatography
LFGB	Lebensmittel- und Futtermittelgesetzbuch
LMBG	Lebensmittel- und Bedarfgegentändegesetz
LOD	Limit of Detection
MS	Mass Spectrometry
MPPO	Modified Polyphenylene Oxide
NP	Normal Phase
PSA	Pressure Sensitive Adhesive
PU	Polyurethane
PVAc	Poly(vinyl acetate)
PVOH	Polyvinyl alcohol
QM	Quantity in Material (limits for residual amounts)
RI	Reflection Index Detector
RP	Reverse Phase
RRF	Relative Response Factor
SEC	Size Exclusion Chromatography
SML	Specific Migration Limit
UV	Ultraviolet

VAE	Vinyl Acetate-Ethylene
VOC	Volatile Organic Compounds

1. Introduction

1.1. Adhesives in food packaging

1.1.1. Application areas

The use of adhesives for manufacture of food packaging materials is inevitable to create the multi-layer composite films, to make the shape of the packaging and to attach the labels. For these applications, adhesives are used in various ways for adhesion, sealing or seaming between different two or more-layers of plastic and paper or their combined forms, for instance flexible film-to-film lamination, paper-film/cardboard-film combination, rigid multi-layer plastic packaging systems, sacks and pouches, and labels. Besides these applications, there are special applications like refrigerators, microwaves, kitchen furniture and corks for alcoholic beverage bottles.

The demand and production of adhesives is increasing year after year in Germany. In 2002 and 2007, 640.000 and 798.000 tons of adhesives were produced in Germany respectively. In comparison with the outturn of 2006, 67.000 tons were increased [Handbuch Klebtechnik 2008/2009].

The paper & packaging industry is the major market segment for the consumption of adhesives occupying 35 % of the entire German adhesive consumption (Figure 1-1). The total adhesive consumption of EU market is almost 3 million tons [FEIKA 2004]. 22 % of the adhesives and sealants that were manufactured in 27 European Union member states in 2007 were used for packaging (Figure 1-2) [BASA 2007].

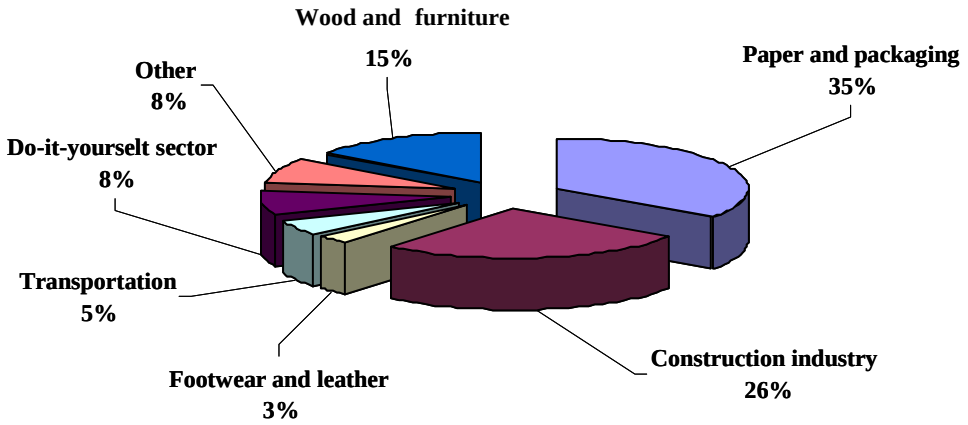


Figure 1-1. German adhesive consumption by market segments [Peters et al. 2002]

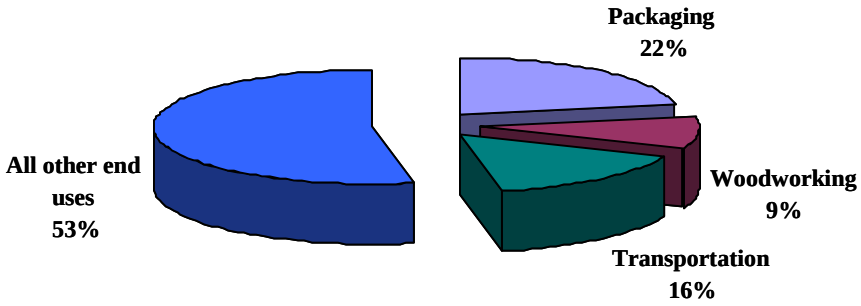


Figure 1-2. End-Use Markets segments of adhesive and sealant in 27 European Union member states [BASA 2007].

Various adhesive systems such as water/solvent based, hot-melt, cold/heat seal and pressure sensitive systems are mainly used for food packaging materials. Table 1-1 shows the use of adhesives to manufacture food packaging materials.

Table 1-1. Use of adhesives for food contact materials [Bradley 2006].

Application	Type of food contact	Adhesive system
Flexible packaging laminates	Indirect	- Reactive polyurethane adhesives - Reactive epoxide systems - Water based adhesives - Hot-melt adhesives
Box closure	Indirect	- Water based adhesives - Hot-melt adhesives
Sealable lidding	Direct / indirect	- Cold seal / heat seal coatings - Solvent based adhesives - Water based adhesives - Hot-melt adhesives
Labelling	Direct / indirect	- Pressure sensitive adhesives - Solvent based adhesives - Water based adhesives - Hot-melt adhesives

Since the volatile organic compounds (VOC) emissions are tightly restricted by government, the flexible film lamination and paper laminating industry is gradually and rapidly changing from organic solvent-based adhesives to water-based adhesives or solvent-free adhesives like hot-melts types. The consumption of organic solvents for the manufacture of adhesives had been gradually decreased about 70 % for 15 years (1989 ~ 2005) in

Germany [Handbuch Klebtechnik 2006/2007]. The water-based or organic solvent free systems in the adhesive types produced in Germany (1999) were more than 90 % [Peters et al. 2002].

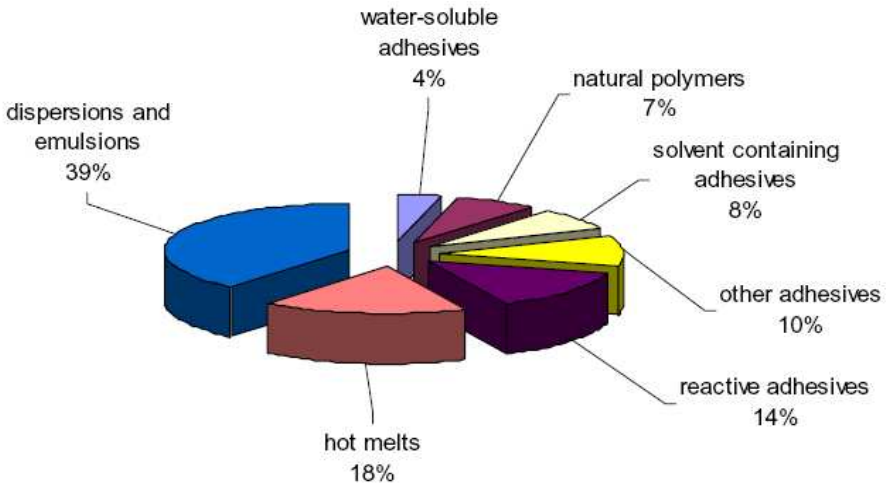


Figure 1-3. Proportions of the total amount of the adhesives produced in Germany [Peters et al. 2002]

1.1.2. Composition and classification of adhesives for food packaging

An adhesive is composed of basic raw materials, which are called binders and which determine its adhesiveness (adhesion) and its internal strength (cohesion), and additives, which determine particular end-use and processing characteristics [Gierenz and Karmann 2001]. Polymers are generally used as the binders. In addition to these polymers, adhesives consist of various additives such as tackifier resins, plasticizers, fillers, thickeners, solvents, wetting agents (surfactants), stabilizers (antioxidants and heat- and UV-stabilizers) and biocides and more according to the intention of its end-use. Table 1-2 shows the raw materials to manufacture adhesives using for food packaging materials.

These adhesives can be classified according to setting-mechanism with two types of reactions, reactive adhesives with a chemical reaction and non-reactive adhesives without a chemical reaction. These adhesives can be subdivided into 100 %-systems, water based and solvent based systems according to their compositions again (Table 1-3). The detailed information on the classification of adhesives will be presented in chapter 9.5.

Table 1-2. Raw materials of adhesives used for manufacture of food packaging materials.

Classification	Raw materials	Examples	Technical function
Binder	Polymers	EVA (Ethylene-vinyl acetate copolymer) PVAc (Polyvinylacetate) VAE (Vinylacetate-ethylene copolymer) Acrylics PU (Polyurethane) Polyolefins PVOH (Polyvinylalcohol) Rubbers (natural and synthetic) Starch and Dextrin Casein Cellulose Others	- Binder - To give cohesion to adhesive system
Additives	Tackifier resins	Natural Resins, e.g. rosin, oleoresin and fossil resin	To improve the cohesive strength of the adhesive film before solidification
	Plasticizers	Phthalates, Adipates, Dibenzoates	To improve flexibility and wet-out of the final adhesive system without sacrificing adhesion performance
	Stabilizers	Antioxidants, UV- and Heat stabilizers, e.g. hindered phenols, phosphates and hydroxy phenyl benzotriazole classes	To protect from heat, UV and oxidation
	Fillers	Pyrogenic and precipitated silicas, chalks, light and heavy apar	To increase solids contents
	Thickeners	Polyacrylate, Urethane based	To control viscosity
	Wetting agent	Surfactants, e.g. Alcohol ethoxylates, Modified silicates	To improve wetting
	Biocides or antimicrobials	2-octyl-2H-isothiazol-3-one, 2-bromo-2-nitropropane-1,3-diol	For natural product adhesive, paper and board adhesives
Solvents		Water, Toluene, Xylene, Ethyl acetate, Acetone	Carrier medium

Table 1-3. Classification of adhesives used for manufacture of food packaging materials.

Application method	Setting-mechanism			
	Adhesion with Chemical reaction (Reactive)		Adhesion without Chemical reaction (Non-reactive)	
	Raw material (main polymer)	Application system	Raw material (main polymer)	Application system
100 % system (carrier-free adhesives)	Acrylic	PSA labels, film/film	Acrylic	PSA
	PU	film/film, clear boxes, cork	PU	Film/paper
			EVA	Boxes, labelling, PSA
			Polyolefine	Boxes, hygiene article, PSA, labelling
			Synthethic rubber	Boxes, hygiene article, PSA, labelling
Water based	PU	film/film, film/paper	PU	Paper/paper
			Acrylic	Film / paper
			Casein	Labelling, laminating
			Cellulose derivatives	Paper sacks
			Dextrin	Labelling, most packaging, mainly with paper
			EVA	All types of packaging
			VAE	Boxes, paper/paper
			Natural rubber	Cold seals
			PVOH	Paper/paper, tissue laminating
			Starch	Labelling, paper/paper, corrugated board
			Synthetic rubber	Film/paper, alu/paper
Solvent based	PU	film/film	Acrylic	PSA, film/film
			Natural rubber	PSA tapes, labels, cork
			Synthetic rubber	PSA

PSA : Pressure sensitive adhesive, EVA : Ethylene-vinylacetate, VAE : Vinylacetate-ethylene, PVOH : Polyvinylalcohol, PU : Polyurethane

1.2. Overview of German and EU legislation related to adhesives used for manufacture of food packaging materials

The safety of food contact materials in EU is currently accomplished by several regulations and directives as follows [European Commission 2009].

- The Framework Regulation (EC) 1935/2004 on the general principles for food packaging materials and articles intended to come in contact with food.

- GMP Regulation (EC) 2023/2006 on good manufacturing practice for materials and articles intended to come into contact with food.

- Directives and regulations on specific materials or articles which contact directly with food.

- Directives and regulations on single chemical substances or groups of substances.

The Framework Regulation (EC) 1935/2004 was published in the Official Journal of the European Union (OJEU) at October 27, 2004 and replaced the Directive 89/109/EEC and Directive 80/590/EEC. This Framework Regulation was not promulgated as a directive, but as a regulation. Therefore, it needed not to be transferred to the national legislation of the EU member states, but came into force directly in all EU member states.

The general requirements for food contact materials and articles are defined in Article 3 of Regluation (EC) 1935/2004 [2004] as follows.

> 1. Materials and articles, including active and intelligent materials and articles, shall be manufactured in compliance with good manufacturing practice so that, under normal or foreseeable conditions of use, they do not transfer their constituents to food in quantities which could:
>
> (a) endanger human health;
>
> (b) bring about an unacceptable change in the composition of the food;
>
> (c) bring about a deterioration in the organoleptic characteristics thereof.

In the Annex I of the EU Framework Regulation (EC) 1935/2004, several groups of materials and articles are listed for which specific measures may be adopted. Figure 1-4

shows the groups of materials and articles listed in the Annex I of the EU Framework Regulation (EC) 1935/2004 with the corresponding specific directives and regulations, which have been established on EU level so far.

Among the materials and articles listed in Annex 1 of the EU Framework Regulation (EC) 1935/2004, currently, plastic materials, regenerated cellulose films, ceramics and elastomers and rubbers are regulated by individual specific directives and regulations.

Plastic materials for food contact purposes are regulated by the EU Plastics Directive 2002/72/EC [2002], which was amended by the following Directives 2004/1/EC, 2004/19/EC, 2005/79/EC, 2007/19/EC and 2008/39/EC and by Regulation (EC) 975/2009. The EU Plastics Directive represents a positive list (PL) for potential migrants such as monomers, other starting substances and additives. It also lays down general restrictions, e.g. an overall migration limit as well as specific restriction like specific migration limits (SML) and limits for residual amounts (QM) in the end-articles. The determination of these potential migrants is achieved by individual analytical methods.

The EU Plastics Directive 2002/72/EC provides a complete positive list for monomers and other starting substances and ,since January 2010, also for additives.

The materials and articles made of regenerated cellulose film are regulated by Directive 2007/42/EEC [2007a]. Ceramic articles intended to come into contact with foodstuffs are currently regulated by Directive 2005/31/EC [2005a] amending Directive 84/500/EEC including the performance criteria of the analytical method.

In addition to the EU Plastics Directive, further regulations are established for plastic materials intended for food contact applications. According to the Directive 78/142/EEC, the maximum vinyl chloride monomer level may not exceed 1 mg/kg in the final product. Further details on the analysis of vinyl chloride in food and polyvinylchloride (PVC) are established by the Directives 80/766/EEC [1980] and 81/432/EEC [1981]. For plasticizers used in gaskets of lids transitional migration limits are laid down by Regulation (EC) 372/2007 [2007b] and Regulation (EC) 597/2008 [2008].

The use of the epoxy derivatives BADGE, BFDGE and NOGE is regulated by Regulation (EC) 1895/2005 [2005b]. Furthermore N-nitrosamines and N-nitrostable substances used in teats and soothers made of elastomer or rubber are regulated by Directive 93/11/EEC [1993].

The basic rules for migration testing regarding time and temperature conditions are laid down in Directive 82/711/EEC [1982]. This Directive was amended two times up to now by Directive 93/8/EC and by Directive 97/48/EC. In addition, Directive 85/572/EC [1985] “List

of simulants" states which simulant(s) shall be used for a particular foodstuff or group of foodstuffs.

Adhesives used for food contact materials are currently not specifically regulated on EU level. However, adhesives are covered by the Framework Regulation (EC) 1935/2004 and therefore must comply with the general requirements stated in Article 3 of the Framework Regulation. Verification of this requirement is in the responsibility of packaging producers. The fact that there are no clear or standardized verification procedures was one of the driving forces for this thesis within the EU project No COLL-CT-030309 'Migresives'.

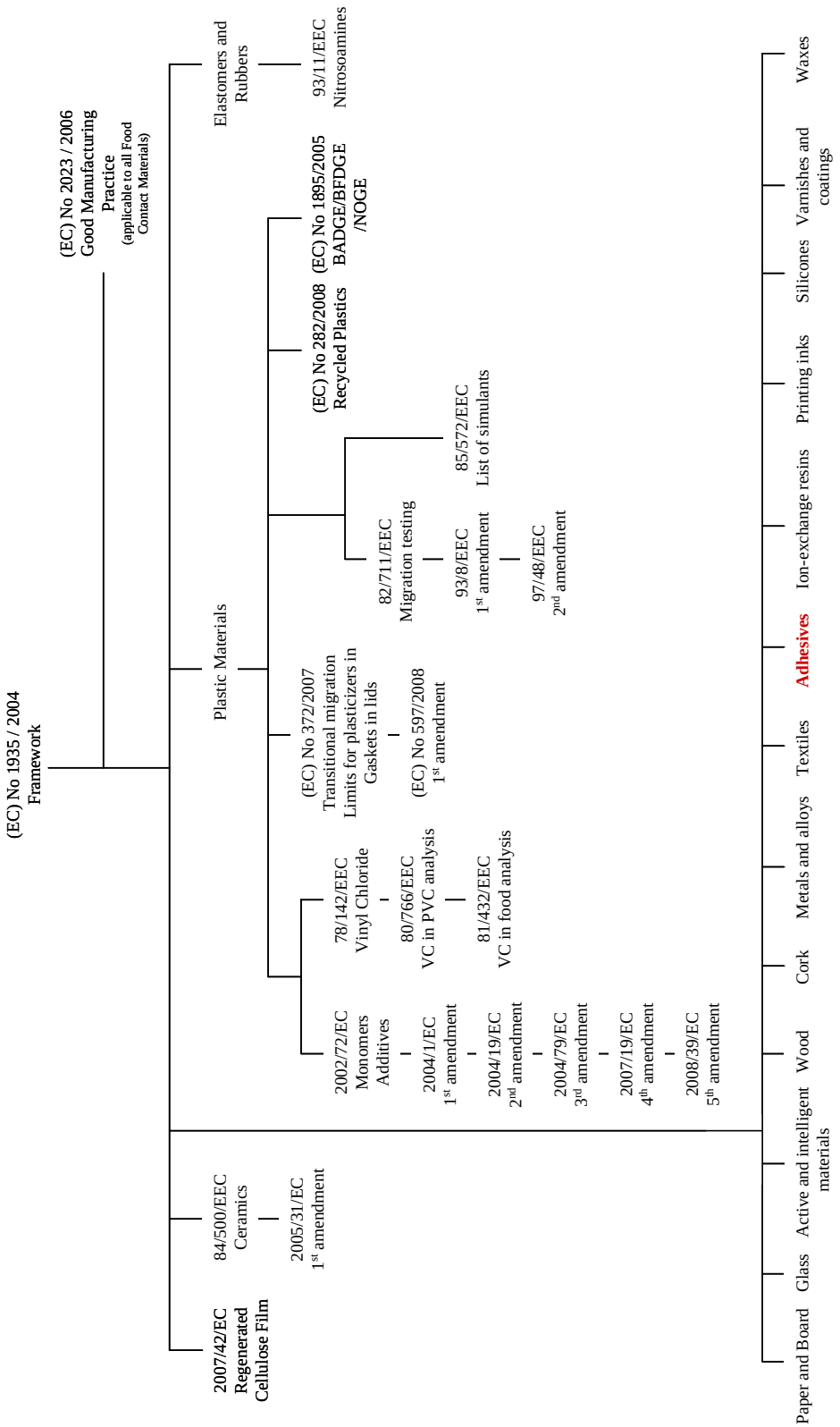


Figure 1-4. Overview of community legislation [European Commission 2008].

In Germany, all food contact materials must comply with the requirements of § 30 and § 31 of the Foods, Consumer Goods and Feedstuffs Code (Lebensmittel und Futtermittelgesetzbuch, LFGB) [LFGB 2005], the Article 3 of the EU Framework Regulation (EC) 1935/2004 and the Consumer Goods Ordinance (Bedarfsgegenständeverordnung, BedGgstV) [BedGgstV 2008] that is the German implementation of EU Directive 2002/72/EC and their consecutive amendments relating to plastic materials and articles intended to come into contact with foodstuffs. The European directives regarding regenerated celluose film, ceramics and vinylchloride monomers are also comprised in this Consumer Goods Ordinance.

In addition to these legislations, food contact materials and articles are regulated by the recommendations of Federal Institute for Risk Assessment (Bundesinstitut für Risikobwertung, BfR) [BfR Recommendation 2008]. These recommendations define specific positive list of monomers, starting substances and additives used for the manufacture of food contact materials. They represent the current level of science and technology for the conditions under which consumer goods made of high polymer substances meet the requirements of § 31, para 1, LFGB and the Article 3, para 1 of the EU Framework Regulation (EC) 1935/2004 [BfR Online 2008].

The plastics recommendations are generally and/or legally recognized in Germany and EU member states, but are not compulsory executed. However, if consumer goods are produced in a manner that deviates from the provisions in these recommendations, responsibility for any complaints based on food law provisions (§§ 30, 31 para 1 LFGB) lies solely with the manufacturer and user [BfR Online 2008]. The recommendations are continuously deliberated and amended by the Plastics Committee of the BfR which consists of experts from surveillance, research and industry and shall be coexisted with EU directives in Germany until EU directives are completed.

As mentioned above, there are no specific directives or regulations for adhesives on EU level. However, some monomers, starting substances and additives used for the manufacture of adhesives are regulated by other directives and regulations, primarily by the EU Plastics Directive 2002/72/EC. Additionally, adhesive related substances not mentioned in EU Directive 2002/72/EC or in the Consumer Goods Ordinance and its amendments may be regulated by some of the BfR recommendations. There are in total 44 BfR recommendations (I - LIII) for plastics (and other food contact materials such as paper and rubber) intended to come into contact with foodstuffs. Adhesives may partially be covered by following eight recommendations [BfR Recommendation 2008].

- VII. Polypropylene
- X. Polyamides
- XIV. Plastics Disperisions
- XVI. Polyvinyl Ethers
- XX. Polyisobutylene, Isobutylene Copolymers and Mixtures of Polyisobutylene with other Polymers
- XXII. Polymers Based on Esters of Acrylic and Methacrylic Acids, their Copolymers, and Mixtures of these with other Polymers
- XXV. Hard Paraffins, Microcrystalline Waxes and Mixtures of these with Waxes, Resins and Plastics
- XXVIII. Cross-Linked Polyurethanes as Adhesive Layers for Food Packaging Materials

For polyurethane based adhesives special focus must be addressed to primary aromatic amines. Primary aromatic amines (PAA) are derived from the residues of aromatic isocyanate monomers in polyurethane based adhesives used for multi-layered plastic materials as the non-intended reaction by-products. Primary aromatic amines (PAA) are tightly regulated by Directive 2002/72/EC because of the potentially high exposure and the carcinogenic properties. According to Annex V of Directive 2002/72/EC [2002], primary aromatic amines (expressed as aniline) migrated from the material and articles manufactured by using aromatic isocyanates should not be detectable using an analytical method with a detection limit of 0.01 mg/kg of food or food simulants.

1.3. Principles of interaction between adhesive layers in food packaging materials and packed food

Interactions between packed foods and the packaging materials can be classified into three categories [Mannheim 1990, Piringer 2008 a].

- Migration : The transfer of components from the packaging materials into foodstuffs.
- Permeation : The transfer of gases or organic vapours and water vapour through the packaging materials.
- Absorption : The transfer of components from the foodstuffs into the packaging materials.

Figure 1-5 shows the interactions that may occur between a food packaging material consisting of two substrates combined by one adhesive layer and foodstuffs.

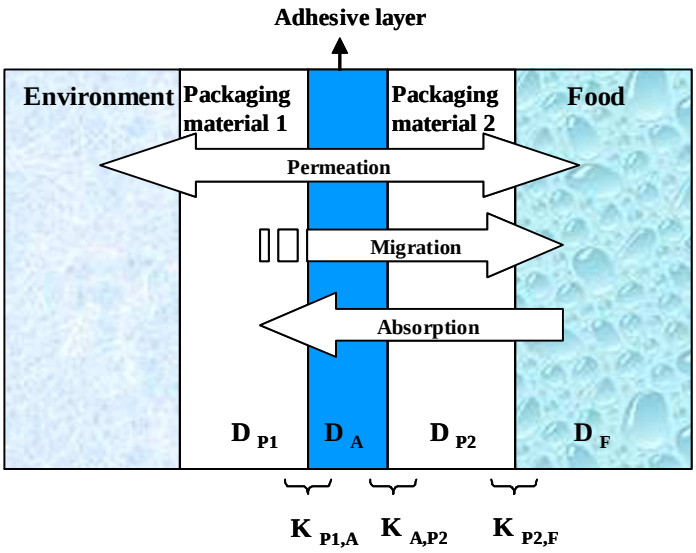


Figure 1-5. Illustration of interaction between food packaging materials and foodstuffs

Where D_{P1}, D_A and D_{P2} are the diffusion coefficients in adhesive layer, packaging material 1 and 2. $K_{P1,A}$, $K_{A,P2}$ and $K_{P2,F}$ are the partition coefficients between two different phases.

Among these interactions migration is a major consideration factor to ensure the safety and quality of packaged food and to impose restrictions on the transfer levels of undesirable

constituents. The migration of adhesive related substances from the adhesive layer depends on the following key factors.

- Initial concentration of the migrants in the adhesive layer, $C_{A,0}$
- Mobility of the migrant, i.e. diffusion coefficients in packaging material 1 and 2 and in the adhesive layer (D_{P1}, D_{P2} and D_A) as well as in the foodstuff or food simulant (D_F)
- Partition coefficients ($K_{P1,A}$, $K_{A,P2}$ and $K_{P2,F}$) are defined by the ratio of the migrant concentration at equilibrium between two layers, for instance in adhesive layer and packaging material 2 or in packaging material 2 and foodstuff, repectively.
- Molecular size (molecular weight) of the migrating substance
- Surface area (contact area) and thickness of the packaging material layers
- Contact temperature and time

Especially, diffusion coefficients (D_P) in packaging materials and partition coeffcients ($K_{P,F}$) between the packaging material and foodstuffs or food simulants of migrants are the most relevant physical parameters in the migration process.

The diffusion process in the packaging materials can be explained in one dimension by Fick's 2 nd law of diffusion (equation 1). Equation 2 describes the partitioning. It is equivalent to Henry's law.

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial X^2}$$ --------- Equation 1

C : Concentration of a migrant in packaging material
t : Contact time
X : Distance from the origin of the x-axis
D : Diffusion coefficient in packaging material

$$K_{1/2} = \frac{C_{1,e}}{C_{2,e}}$$ --------- Equation 2

$C_{1,e}$: Concentration at equilibrium of a migrant in material 1
$C_{2,e}$: Concentration at equilibrium of a migrant in material 2

Consequently, the mass transfer from the area of contact between packaging materials and foodstuffs or food simulants is a function of these migration influencing factors. The amounts of migrants transferred from a homogeneous layer of a mono material can be mathematically

predicted by using the following equation [Begley et al. 2005] which is based on related mathematics established by Crank [Crank 1975].

$$\frac{m_{L,t}}{A} = C_{P,0}\ \rho_P\ d_P\left(\frac{\alpha}{1+\alpha}\right)\left[1-\sum_{n-1}^{\infty}\frac{2\alpha(1+\alpha)}{1+\alpha+\alpha^2 q_n^2}exp\left(-D_P t\frac{q_n^2}{d_P^2}\right)\right],\ \alpha = \frac{V_F / V_P}{K_{P,F}}$$ -- Equation 3

$m_{L.t}/A$	(µg/cm^2)	: Amount of the migrated migrant after the contact time
t	second (s)	: Contact time
$C_{P,0}$	(mg/kg)	: Initial concentration of migrant in packaging material
ρ_P	(g/cm^3)	: Density of packaging material
d_P	(cm)	: Thickness of packaging material
V_P *and* V_F	(cm^3)	: Volumes of packaging material ($\boldsymbol{V_P}$) and foodstuff ($\boldsymbol{V_F}$)
D_P	(cm^2/s)	: Diffusion coefficient of migrant in packaging material
$K_{P,F}$		: Partition coefficient (the ratio of the migrant concentrations (w/v) in packaging material and foodstuff at equilibrium)
q_n		: The positive roots of the trigonometric identity tan q_n = -α q_n

In most cases, adhesive layers in food packaging materials are not in direct contact with foods. They are usually separated from the foods by a plastic layer or another materials. Therefore, the type and morphology of the packaging materials (in most cases paper and plastic film) are important factors in the transfer of migrants from an adhesive layer through a barrier layer into a food. The exact calculation of the amount of a migrated substance from such multilayer cases is only possible by numerical solution of the diffusion equation (Equation 1) [Brandsch 2000].

1.4. Analysis aspects for qualitative and quantitative determination of adhesive related substances

The traditional approach for qualitative and quantitative analysis of the potential migrants in food contact materials is accomplished by the process of solvent extraction. Overall and specific migration are assessed by using food simulants, chromatographic, colorimetric and gravimetric analysis. For specific migration testing, the typical chromatographic instruments and techniques are used like GC-FID, HPLC-UV, GC-MS, LC-MS and more, which are sensitive, accurate and reliable techniques. For the semi-quantitation determining the approximate amount of substances migrated from food packaging materials, the overall

migration test by using gravimetric analysis can be performed, but it cannot provide the specific physico- and chemical informations on the potential migrants.

On the other hand, scientific research on the systematic analysis of adhesive related substances is lacking. Only some results have been reported for the screening analysis and for semi- or specific migration tests of adhesive related substances in multi-layer composite films bonded with the adhesives. Brede et al. [2001] determined aromatic amines in flexible food packaging materials by using spectrophotometry and identified them by GC-MS. Lawson et al. [2000] determined volatile and non-volatile migrants in adhesive samples using a combination of techniques including GC-MS, LC-MS, MALDI-TOF-MS, HPLC and colorimetric determination. For semi-quantification, Petersen [2001] conducted overall migration testing from stretch films and flexible films according to CEN_EN(V) 1186 standard method. Begley et al. [1991] analyzed the diglycidyl ether of Bisphenol A (BADGE) in an epoxy adhesive by using HPLC-UV technique. Gruner and Piringer [1999] examined the amounts of the potential migrants from food packaging materials bonded with various adhesives based on ethylne-vinylacetate (EVA) copolymer, dextrin, starch, polyvinylacetate (PVAc) homopolymer and vinylacetate-ethylene (VAE) copolymer by overall migration test and semi-quantitation test using GC-FID. This semi-quantitation approach was to quantify unknown volatile to semi-volatile substances by universal internal standard substances. However, this method was not fully validated by a statistical determination of the response factors using a set of appropriate chemical compounds. Further, the results did not give a specific identification of potential migrants.

In conclusion, so far the qualitative and quantitative methods for the determination of adhesive related substances have focused on the specific migration test of a certain substance or a substance group like aromatic amines and the overall migration test.

1.5. Potential migrants in adhesive layers of food packaging materials

As mentioned in chapter 1.1.2, many types of adhesives are used to manufacture food packaging materials. It is difficult to predict potential migrants contained in adhesive layers, since thousands of substances are used for the formulation of different adhesives. Migration studies only for adhesive related substances from food packaging materials have not been carried out extensively. Some studies have been published up to now. Bonell and Lawson [1999] examined the amounts of the migratable substances from cold seal adhesives based typically on natural rubber and from acrylic polymer and polyurethane adhesives. Brede et al.

[2001] determined the concentration of 4,4'-Methylenedianiline (4,4-MDA) as a reaction by-product orginating from polyurethane adhesives. Davies [2003] identified diethylene glycol dibenzoate, dipropylene glycol dibenzoate, polyethylene glycol (PEG) and an ethylene oxide based polyol as the main potential migrants in water based adhesives. He also found phenanthene carboxylic acid derivatives, styrene, butylated hydroxyl toluene (BHT), long chain hydrocarbons and cyclic hydrocarbons as the main compounds in hotmelt adhesives.

There are some representative adhesive systems that are frequently used for multi-layer food packaging materials. Therefore, the representative adhesive related substances for multi-layer food packaging materials could be estimated through a survey of the related literature. The surveyed potential migrants are presented in table 1-4 below.

For flexible multi-layer films, solvent based adhesive and 100 % solids adhesives are used in most cases. Ethylene-vinylacetate (EVA) copolymer, acrylate and methacrylate polymers and polyurethanes are used as main binders for these adhesives. In addition, these adhesive mixtures contain additives and solvents. Polyurethanes are the best known polymers in the manufacture of adhesives.

Polyurethanes consist of two base components as starting substances, which are diisocyanates and polyols. Residual free monomeric isocyanates in polyurethanes will rapidly be hydrolysed with moisture in foodstuffs forming the corresponding aromatic amines. Primary aromatic amines are known or suspected carcinogenic substances. For the investigation of isocyanate compounds in aqueous solutions, the corresponding amines are usually determined because of their rapid reaction with moisture. For the hot-melt adhesives, EVA copolymer is used almost exclusively in a mixture with additional polymers and additives [Brede et al. 2001]. Acrylate and methacrylate polymers are important raw materials for pressure-sensitive adhesives (PSA) [Gierenz and Karmann 2001].

Water based adhesives are commonly formulated from natural occurring materials such as dextrins, starches, caseins and natural rubbers, as well as from synthetic polymers based on acrylates, polyurethane, synthetic rubber, vinylacetate -ethylene (VAE) copolymer and polyvinyl alcohol (PVOH).

Plasticizers are important to increase the plasticity of the adhesives. Phthalate plasticizers are most widely used. Other plasticizers, such as benzoates, citric esters and glycerol triacetate are also used [Gierenz and Karmann 2001]. Stabilizers are used in adhesive formulations to protect against degradation by reaction with oxyen, UV light and high processing temperature. Expecially, the use of antioxidants for adhesive formulations is

inevitable, since the oxidation can occur at all stages from synthesis to final end-use of an adhesive [Petrie 2004].

Table 1-4. Potential migrants in adhesive layers of food packaging materials.

Classification	Raw materials		Potential migrants	Main application for food packaging
Binders	Acrylate and methacrylate copolymer		**Acrylate and methacrylate monomers :** Ethyl acrylate, Methyl acrylate, Butyl acrylate, Ethyl methacrylate, Methyl methacrylate, Butyl methacrylate	Solvent based and 100 % solids adhesives
	Ethylene-vinylacetate copolymer (EVA)		Vinylacetate monomer	100 % solids adhsives
	Polyurethane (PU)	Diisocyanates	**Aromatic diisocyanates :** Toluene diisocyanate (TDI), Diphenylmethane diisocyanate (MDI) **Aliphatic diisocyanates :** Hexamethylene diisocyanate (HDI), Isophoron diisocyanate (IPDI)	Solvent based and 100 % solids adhsives
		Polyols	**Polyether polyols :** Ethylene glycol, Diethylene glycol, 1,2-propanediol, 1,4 butanediol, Dipropylene glycol, Glycerol	
		By-products : Amines	**Aromatic amines :** Toluene 2,4-diamine (2,4-TDA), Toluene 2,4-diamine (2,6-TDA), 4,4'-Methylenedianiline (4,4-MDA) **Aliphatic amines :** Isophorone diamine (IPDA), Hexamethylene diamine (HMDA)	
	Vinylacetate-ethylene (VAE)		Vinylacetate monomer	Water based adhesives
	Rubber (natural and synthetic)		Isoprene, Butadiene, Styrene, Chloroprene, Acrylonitrile	Water based adhesives
	Polyvinyl alcohol (PVOH)		Vinylacetate monomer	Water based adhesives
	Dextrin, starch, casein			Water based adhesives
Additives	Plasticizers		**Phthalate and Adipate :** Diisobutyl phthalate (DIBP), Dibutyl phthalate (DBP), Bis(2-ethylhexyl) phthalate (DEHP), Bis(2-ethylhexyl) adipate (DEHA), **Benzoate :** Diethylene glycol dibenzoate (DEGDB), Triethylene glycol dibenzoate (TEGDB), Dipropylene glycol dibenzoate (DPGDB)	All types of adhesives
	Stabilizers	Antioxidants	**Primary antioxidants :** - 2,6-Di-tert-butyl-4-methylphenol (BHT) - Octadecyl 3,5-bis (1,1-dimethylethyl)-4-hydroxybenzene propanoate (Irganox 1076), - Tetrakis [methylene-3 (3´,5´-di-t-butyl-4-hydroxyphenyl) propionate] methane (Irganox 1010) **Secondary antioxidants :** - 2,4-Bis (1,1 dimethylethyl) phenyl-phosphite (Irgafos 168) - Bis(2,4-di-tert-butylphenyl) pentaerythritoldiphosphite (Ultranox 626) - Dodecyl 3-(3-dodecoxy-3-oxo-propyl)sulfanylpropanoate (Irganox PS 800) - Octadecyl 3-(3-octadecoxy-3-oxo-propyl)sulfanylpropanoate (Irganox PS 802)	
		Light stabilizers	- (2-hydroxy-4-octoxy-phenyl)-phenyl-methanone (Chimasorb 81) - 2-(2'-Hydroxy-3,5'-di-tert-butylphenyl)-5-chlorobenzotriazole (Tinuvin 327)	
Solvents			Toluene, xylene, ethyl acetate, acetone, methanol, MEK	Solvent based adhesives

2. Objectives of this work

To investigate the total amount of migrating substances from a food packaging into foodstuff, the overall migration test is usually performed according to the EN 1186 series of European Committee for Standardization (CEN), CEN TC 194/SC1/WG1 [CEN 2000]. However, the overall migration test does not give any information on the identity of migrated substances and not very sensitive. It would be a very problematic and an unrealistic attempt to analyze the migration from adhesives based on this overall migration method.

In conclusion, a more suitable quantitative approach with screening character or a new concept for the determination of adhesive related substances in food packaging materials is necessary. This new quantitative approach should be quick, of multi-analyte character and sufficiently accurate to cover broad ranges of physico-chemical properties like polarity and volatility and to minimise the number of the analysis techniques and chromatographic conditions.

Therefore, multi methods for the simultaneous analysis of a broad range of substances with low detection limit for sensitive analysis are needed. Furthermore, often the exact composition of raw materials as well as of by-products or reaction products are not known. For this, screening methods and the possibility to estimate the concentrations of such unknown or non-intentionally added substances would be important tools for the proper assessment of food law conformity and food safety related to substances migrating from adhesive layers of food packaging materials.

The adhesive related substances in food packaging materials have various physico-chemical properties from volatile to non-volatile, from polar to non-polar and the potential number of these substances has been estimated to be more than several thousands [Bonell and Lawson 1999]. Therefore, it is impossible that all adhesive related substances can be analysed with individual analytical methods for quantification. For this reason, new evaluation procedures based on analogical and predictive conclusions extrapolated from measurable parameters to other, non-measurable ones will be needed.

- The first objective of this work was to develop analytical multi methods for the screening covering various chemical classes of adhesive related substances up to a molecular weight of 1000 g/mol. For this, an universal detection system, which can detect most compounds with sensitive and analogous response, should be selected as

well as efficient separation systems, that ensure sufficient peak capacity and resolution on the separation column, should be developed.

- The second objective which has a more quantitative dimension is the development of analytical approaches for the semi-quantification of the potential migration of known and unknown constituents of adhesives by using a statistically averaged detection response of known and representative adhesive related substances.

- The third objective is to confirm and validate the applicability of the developed screening and semi-quantification methods in practical migration and extraction tests.

3. Theoretical considerations

3.1. General conceptual design of the analytical approach for simultaneous determination of multiple analytes

For the traditional quantitative analysis, it is essential to calibrate the analytical system using standard substances corresponding to each target substance. However, this method is not practical or even not applicable for the quantification of a larger number of known or unknown substances due to the unavailability of all standard substances and because the unknown substances would first need to be identified. A semi-quantitative approach using a statistical response factor obtained from a large number of suitable standard substances could therefore be a powerful alternative tool.

For this approach, first of all, more than 50 adhesive related substances should be defined as representative analytes. These substances need to represent the physico-chemical properties of the adhesive related substances by covering the range of different physico-chemical properties from polar to non-polar, from volatile to non-volatile and from low to high molecular weights.

Secondly, the relative response factors (RRF) of the selected substances should be measured and determined by suitable detection systems coupled with a chromatographic technique such as gas chromatography (GC) and high performance liquid chromatography (HPLC) system.

Finally, from the determined RRF values, a distribution range of RRF values of all adhesive related substances can be established at a 95 % coverage level. From this distribution, a statistical RRF can then be derived, which can be used for the semi-quantification of larger amounts of known and unknown substances.

For facilitation and practical performance of this concept, a few suitable internal standards should be selected for which the relationship of the statistical RRF and their own RRF has to be established. Through this relationship, these internal standards can be used as so-called ‘universal internal standards’, since they allow then the intended semi-quantification of any other substance.

3.2. Considerations on detector response factors

The detector response factor which expresses the sensitivity of a detector relative to a standard substance can be described in two ways. For a concentration sensitive detector, the substance specific absolute response factor (ARF) of a detector is defined as the signal amount (like peak area) divided by the concentration of the analyte; for a mass sensitive detector, the signal amount divided by the known mass of the analyte (Equation 4).

$$\text{Absolute Response Factor (ARF)} = \frac{A_s}{C_s} \text{ or } \frac{A_s}{M_s} \quad \text{---------- Equation 4}$$

A_s : peak area of analyte

C_s : concentration of analyte

M_s : mass of analyte introduced in column

Ideally, all chromatographic detectors produce a peak as a signal and the area of this peak is proportional to the concentration or mass of the compound represented by this peak. However, practically the response factor of each individual substance may change for different concentrations. Therefore, it needs to make a calibration curve. If the calibration curve is linear and the calibration line passes through the origin of the x and y axis, then the response factor is constant. The average of the constant response factors within the calibration range can be used to calculate the correct concentration of a known analyte (Equation 5).

$$C_s^{known} \text{ or } M_s^{known} = \frac{A_s^{known}}{ARF^{average}} \quad \text{---------- Equation 5}$$

C_s^{known} : concentration of known analyte

M_s^{known}: mass of known analyte introduced in column

A_s^{known} : peak area of known analyte in sample

$ARF^{average}$: average absolute response factor of known analyte in defined calibration range

This quantitative method for a known analyte using ARF value of the corresponding standard can be also applied for semi-quantitative approach of unknown analytes in complex sample (Equation 6). In this thesis, for the semi-quantitative approach the statistical response factor ($ARF^{statistic}$) is used. This can be derived from the distribution of ARF values of the

representative adhesive standard substances. It is assumed that these represent the physico-chemical properties of all adhesive related substances.

$$C_s^{\,unknown} \;\; or \;\; M_s^{\,unknown} = \frac{A_s^{\,unknown}}{ARF^{\,statistic}} \quad \text{---------- Equation 6}$$

$C_s^{\,unknown}$: concentration of unknown analyte

$M_s^{\,unknown}$: mass of unknown analyte introduced in column

$A_s^{\,unknown}$: peak area of the unknown analyte

$ARF^{\,statistic}$: statistical absolute response factor obtained from the response factor distribution of more than 50 substances

However, the absolute response factor (ARF) value could scatter in the range of a few percents, since gas or liquid flow rates and injection volume sizes could vary from run to run. To minimise or even eliminate these scatterings effects usually an internal standard is applied. The relative response calculated via an internal standard is not dependent on injection volume and operation conditions, since an internal standard is included in each sample analysed.

The relative response factor (RRF) is defined as signal/concentration ratio between analyte and the internal standard (Equation 7). The RRF is calculated as mass related concentration (mg/l, RRF w/w) and as molar concentration (mol/l, RRF mol/mol).

$$\text{Relative Response Factor (RRF)} = \frac{Area_s \times C_{is}}{C_s \times Area_{is}} \quad \text{---------- Equation 7}$$

$Area_s$: Peak area of analyte

$Area_{is}$: Peak area of the Internal standard

C_s : Concentration of analyte

C_{is} : Concentration of the Internal standard

The concentration of the known analyte can be calculated by using the RRF of the corresponding standard as follows.

$$C_s^{\,known} = \frac{C_{is} \times Area_s^{\,known}}{Area_{is} \times RRF^{\,average}} \quad \text{---------- Equation 8}$$

$Area_s^{\,known}$: peak area of known analyte

$Area_{is}$: peak area of the Internal standard

$C_s^{\,known}$: concentration of known analyte

C_{is} : concentration of the Internal standard

$RRF^{average}$: average relative response factor of known analyte in defined calibration range

The statistical relative response factor ($RRF^{statistic}$) can be likewise derived from the 95% distribution of RRF values of the representative adhesive standard substances. As shown in Equation 6, the concentration of unknown analyte in complex sample can be estimated by using the statistical relative response factor ($RRF^{statistic}$) as follows.

$$C_s^{unknown} = \frac{C_{is}^{universal} \times Area_s^{unknown}}{Area_{is}^{universal} \times RRF^{statistic}} \quad \text{---------- Equation 9}$$

$Area_s^{unknown}$: peak area of unknown analyte

$Area_{is}^{universal}$: peak area of the universal internal standard

$C_s^{unknown}$: concentration of unknown analyte

$C_{is}^{universal}$: concentration of the universal internal standard

$RRF^{statistic}$: statistical relative response factor obtained from the response factor distribution of more than 50 substances

3.3. More specific considerations on analytical systems

In a chromatographical analysis, detection and separation of the complex mixture are another question. The universality, high sensitivity and response constancy of the detector are the main requirements to develop a multi-screening method for the semi-quantitative approach of unknown substances in adhesive samples by using HPLC and GC systems. On the other hand, for the separation of the complex mixtures, the selection of a suitable column and mobile phase composition are the main consideration parameters in HPLC systems. On the contrary, the separation behavior in the GC system is almost independent from carrier gas as a mobile phase. Therefore, the choice of an appropriate column is the most important consideration parameter in a GC system.

3.3.1. Universal detection system for volatile and semi-volatile substances

The flame ionization detector (FID) is a universal detector and the most common detector in GC analysis. Its popularity can be explained by its universal response which is proportional to the mass of organic substances and by the ease of use. The FID responds to the mass of carbon per time unit passing the detector. Therefore, it produces a signal for all carbon containing compounds that elute from the GC column and therefore the signal is largely independent from the chemical structure. The detector has a linear response over more than 7 orders of magnitude of intensity. From the above mentioned reasons, the GC-FID system is expected to show appropriate analysis capability for the quantification of volatile and semi-volatile substances than other detection systems resulting for a wide application range of target substances.

3.3.2. Universal detection system for non-volatile substances

HPLC systems suffer from the limitation that there is no universal detector with high sensitivity and universality like the flame ionization detector used in GC system. Some of the frequently used detectors for the analysis of the non-volatile substances used in food packaging materials, are ultraviolet (UV) -, fluorescence (FL) -, refractive index (RI) - and evaporative light scattering (ELSD) - detectors as well as mass spectrometer (MS). However the applicability of an UV detector is limited, since some analytes show no or low absorption

of UV light at a wavelength range higher than 200 nm [Dreux et al. 1996, Young and Dolan 2004].

The FL detector is in general more sensitive than UV detector, and it has a very high selectivity but for the FL detection a derivatization procedures is often required. The pretreatment procedures of samples are complex and therefore the application range is restricted.

The refractive index (RI) detector and the evaporative light scattering detector (ELSD) are being used as universal detectors. However, the RI detector suffers from the compatibility problem with gradients elution and from low sensitivity. The ELSD is more sensitive than the RI detector and can use a gradient elution. When the target substances eluting from a HPLC column are less volatile than the used mobile phase, a high enough number of dry-particles can be generated for detection of the ELSD. However, the ELSD has some limitations that include non-linear calibration and lower sensitivity than UV, FL and mass spectrometry (MS) [Lucena et al 2007]. According to Górecki et al. [2006], the day-to-day reproducibility and the precision of the results obtained from ELSD are unstable, which leads to the need for regular recalibration. Therefore, since both universal detectors may not give satisfying analytical results, another detector has to be considered for further development of the multi-screening method.

The charged aerosol detector (CAD) is a new system with a potential for universal detection, since it combines sensitivity with independence from UV- or fluorescence active structures. The principle of the CAD is that the mobile phase is nebulized with nitrogen and then evaporated in a drying tube. The resultant non-volatile particles are charged by ionized nitrogen and finally detected by a sensitive electrometer (Figure 4-3). The response of CAD is not affected by physico-chemical properties of the analytes and the sensitivity is high enough to achieve detection limits in the ppb range [ESA Inc online]. According to McCarthy et al. [2005], the CAD showed consistent response factors (peak height/mass injected), wide dynamic range, high sensitivity in the nanogram level and excellent reproducibility (relative standard deviation in the 1 – 10 % range) in the analysis of non-volatile substances with various physico-chemical properties. Pistorino and Pfeifer [2008] reported that the LOD (limit of detection) value of CAD surpassed that of evaporative light scattering detector (ELSD) as well as mass spectroscopy in the analysis of polyketides. Takahashi et al. [2008] reported that the LOD value determined by CAD was 10 times lower than that by ELSD in the comparison test using polyethylene glycol (MW 1000 g/mol) as a target analyte.

The CAD may also work for the purpose of this thesis because of its working principles. However, until now, no attempts to verify this or to establish general detector response factors have been carried out and made available in the published literature. With this technique the molecular weight fraction below 1000 g/mol can be cut out of the HPLC chromatogram and quantified by universal standards. This technique would be useful for extracts from adhesive samples and have the advantage to give direct information on molecular weight of migrants.

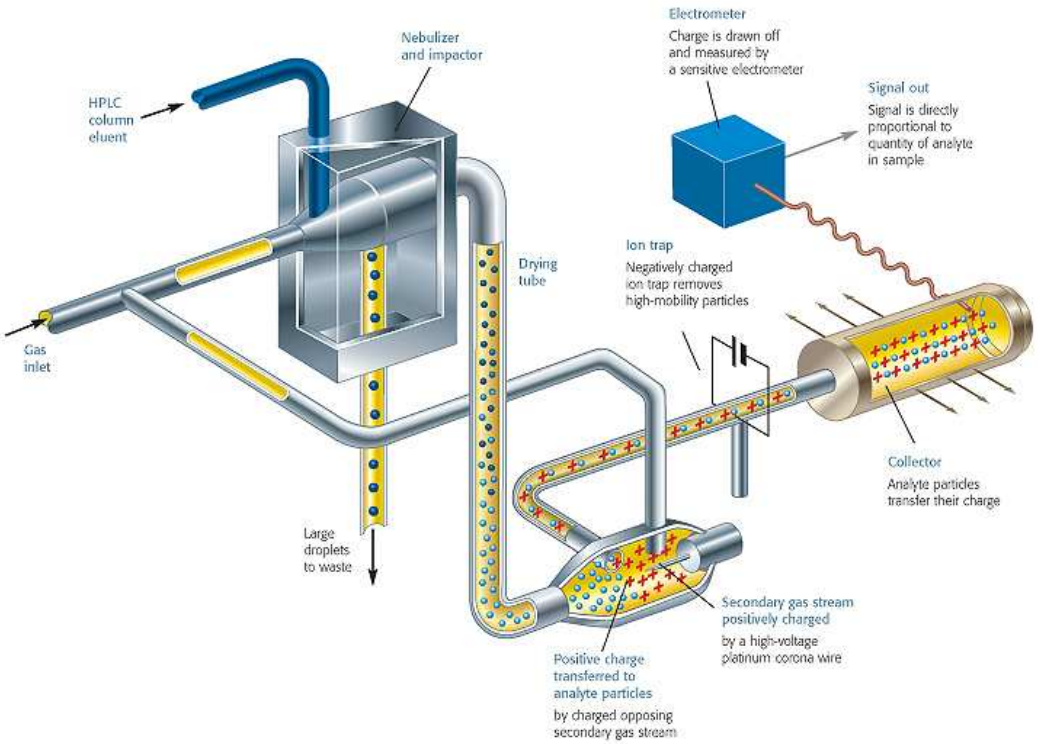


Figure 4-3. Schema of the operation principle of the charged aerosol detector (CAD) [ESA Inc online].

3.3.3. Separation systems for volatile and semi-volatile substances

Since the capillary columns provide much a higher theoretical plate number and therefore peak resolution than packed columns and reduce the separating time, these column types are used in most cases. The most common stationary phases for capillary columns are polysiloxanes and polyethylene glycols.

Fully non-polar columns coated with dimethylpolysiloxanes which attained by substituting 100 % with methyl groups generally separate substances according to their boiling point which is more or less according to the molecular weight. Therefore, it is expected that unknown target substances with a broad range of molecular weights and various physico-

chemical properties show a good relationship of their retention time with their molecular weights. However, poorer response is expected in the analysis of high polar substance groups on such non-polar columns. On the other hand, when the polyethylene glycols are used as coating materials for GC columns, the intention is mainly to analyse high-polar analytes like alcohols, phenols and carboxylic acids. The separation of this polar substances occurs mainly because of interaction between solutes and the stationary phase of the column and not due to boiling point differences.

3.3.4. Separation systems for non-volatile substances

The performance of HPLC separation columns is usually limited and will not allow complete separation of complex mixtures into all individual substances. For this reason, there is no HPLC multi-screening method available so far with broad applicability to various chemical substance groups. Among various separation column techniques based on liquid chromatography, RP-HPLC (reversed phase – high performance liquid chromatography) is a very powerful separation technique and widely used. Reverse phase C18 columns using gradient mobile phase are often used for screening tests of non-volatile migrants. Kawamura et al. [1996], Dopico-García et al. [2003], Mansouri et al. [1998] and Block et al. [2006] separated various additives such as antioxidants and ultraviolet stabilizers in polyethylene by using HPLC system equipped with a C18 column and a mobile phase composition based on organic solvent and water without pH adjustment.

Methanol and acetonitrile are unquestionably the most important and preferable organic solvents as mobile phase in reverse phase liquid chromatography. The use of methanol in mobile phase composition gives rise to better selectivity because of the higher polar/ionic interaction than acetonitrile. However, methanol cannot avoid the broadening of peaks and the retention prolongation of non-polar/neutral compounds. On the contrary, more symmetrical peak shapes can be obtained from the use of acetonitrile in mobile phase because of its lower viscosity. In addition, acetonitrile generally provides sufficient selectivity for non- or semi-polar substances.

The gradient elution chromatography is a powerful tool for chemical analysis due to its broad range of retentivity, high peak capacity and short operation cycle [Anita and Horvath 1989]. In comparison with isocratic elution, gradient elution has great advantages in separating compounds which differ widely in retention on a chromatographic column.

Consequently, gradient elution chromatography provides fast and highly resolved separations, which also implies high loading capacity [Truei et al. 1992].

3.3.5. Online two-dimensional chromatography

Complex mixtures require analytical methods of extremely high resolving power in order to provide reliable analysis of the sample components [Bushey and Jorgenson 1990]. However typical one-dimensional chromatography using one separation column and isocratic or gradient elution does not always offer sufficient peak capacity and resolution for the separation of complex mixtures. A two-dimensional separation technique based on two independent separation mechanisms is in principle a useful tool to increase the peak capacity and resolving power. It has been used and explored for several years to separate and characterize synthetic polymers, biomolecules and complex mixtures [Murphy et al. 1998].

The peak capacity, one of the parameters to show the efficiency of a chromatography system, describes the maximum number of resolvable peaks that can be separated in a chromatogram and this peak capacity can be an indicator to represent the column resolution [Giddings 1967]. In the one-dimensional separation, the peak capacity (P_{1D}) under isocratic condition can be defined as follows [Giddings 1967]:

$$P_{1D} = 1 + \frac{\sqrt{N}}{4} \cdot 1n\left(\frac{V_p}{V_o}\right) \text{ ---------- Equation 10}$$

where N is the theoretical plate number and V_p and V_o are the retention time of the first and last peak.

On the contrary, the peak capacity (P_{2D}) for two-dimensional chromatography is calculated by multiplication of peak capacities of the two separation dimensions as shown in equation 11 [Chang 2003]. The maximum peak capacity is achieved in so-called “orthogonal” systems with non-correlated retention mechanisms in both dimensions [Dugo et al. 2008]. Figure 3-1 shows an example of the typical chromatogram and the orthogonal separation in a two-dimensional system. For this, the separation columns used in the first and second dimension should be quite different in view of their separation mechanisms [Jandera et al. 2005].

$$P_{2D} = P1 \times P2 = \left[1 + \frac{\sqrt{N_1}}{4} \cdot 1n \frac{V_{1p}}{V_{1o}}\right] \times \left[1 + \frac{\sqrt{N_2}}{4} \cdot 1n \frac{V_{2p}}{V_{2o}}\right] \text{---- Equation 11}$$

P_{2D} : the peak capacity for two-dimensional chromatography

$P1$: the peak capacity of first dimension

$P2$: the peak capacity of second dimension

N_1 : the number of theoretical plate of first dimension

N_2 : the number of theoretical plate of second dimension

V_{1o} and V_{1p}: the retention time of first and last peak in first dimension

V_{2o} and V_{2p}: the retention time of first and last peak in second dimension

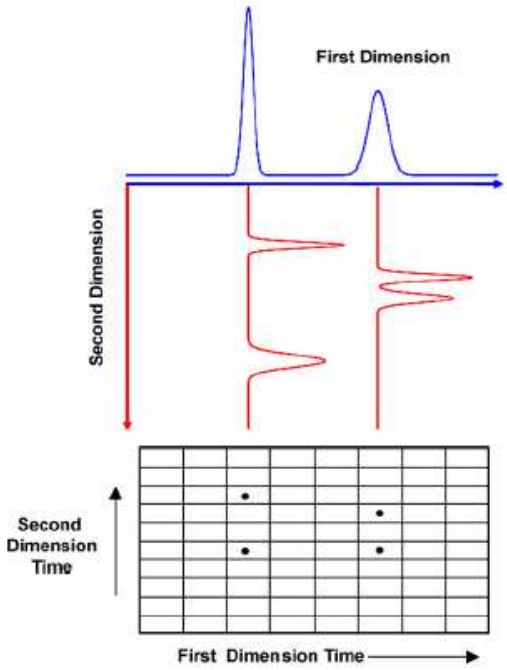


Figure 3-1. Illustration of the multiplicative relationship between the peak capacities of the independent first and second dimensions in the multi-dimensional separation system [Stoll et al. 2007].

The two-dimensional separation can be achieved either offline or in an online system. In an offline approach, effluent from the first separation LC (liquid chromatography) system are collected in vials and reinjected into the second LC separation system.

On the contary, in an online two-dimensional chromatography setup, the different two dimensions are connected through a switching valve equipped with two identical-volume sampling loops. The effluent eluted from the first dimension is automatically transferred via the switching valve into the second dimension. Although the operation of the offline is simpler than the online system, the offline system has many disadvantages such as time

inefficiency, diffculty of automation and reproduction, sample loss and contamination as well as formation of artefacts [Dugo et al. 2008]. These disadvantages can often be overcome by the online approach. However, the operation of the online system is not too easy, because various parameters for the online system should be necessarily considered in the practical aspect such as compatibility of mobile phase and stationary phase [Jandera 2006] as well as the setup and programming of the modulator (switching valve) to transfer into the second dimension [Dugo et al. 2008].

For the two-dimensional separations, various liquid chromatography (LC) separation systems can be connected to each other depending on the target substances. There are RP (reverse phase)×RP, RP×NP (normal phase), NP×RP, SEC (size exclusion chromatography)×RP or NP, NP or RP×SEC and more combinations.

The combination of NP×RP or RP×NP two-dimensional systems is very difficult to accomplish because of poor compatibility of the mobile phases used in the NP and in the RP systems [Blahová et al. 2006]. The combinations of the same separation mechanisms such as RP×RP and NP×NP two-dimensional systems show very poor orthogonality between two RP or NP dimensions [Stoll et al. 2007].

SEC×NP or SEC×RP are mainly applied to the separation and characterization of synthetic polymers, copolymers or polymer blends that soluble in organic solvents [Jandera 2007]. The combination of SEC×NP or SEC×RP provides good orthogonality, since the separation mechanism of SEC is based solely on size (to be exact hydrodynamic volume), and RP and NP separate by hydrophobicity or polarity [Stoll et al. 2007]. Since many polymers are well soluble in organic solvents, NP is used more often than RP, while RP is mainly used for water-soluble polymers [Jandera 2007, Stoll et al. 2007]. In this study, the relevant molecular weight range of adhesive related substances is up to 1000 g/mol, since substances with a molecular weight above 1000 g/mol are considered to be not adsorbed in the gastrointestinal tract [SCF 2002] and are not expected to migrate through polymer films. Therefore, when SEC is used as the first dimension, the substances with molecular weights above 1000 g/mol can be comfortably excluded from the sample mixture. Therefore, SEC systems used as the first dimension represent a separation technique as well as a purification process of the sample mixtures. Consequently, the combination of SEC (as a first dimension) and NP (as a second dimension) will be therefore the most attractive selection for this study. In addition, the combination of the two-dimensional separation technique and a universal charged aerosol detector (CAD) as a detection technique permits the sensitive and universal detection for the non-volatile adhesive related substances.

For the SEC×NP system, the compatibility of the mobile phase is the most important consideration. Tetrahydrofuran-cyclohexane, dichloromethane-heptane, dichloromethane-acetonitrile, dichloromethane-methanol, trichloromethane-cyclohexane, etc. are usually used as mixed mobile phases, either with isocratic or with gradient elution in the NP dimension [Jandera 2006]. Since the charged aerosol detector (CAD) is very sensitive for changes of the mobile phase composition, the mobile phase composition of the first and second dimensions should be identical. However, the packing material in SEC column has a different swelling characteristic depending on the organic solvents. Therefore, when organic solvents with different swell volumes are mixed, the swelling volume of the packing material in the SEC column should be considered as well as the optimized mobile phase composition that allows the increase of peak height and the decrease of peak width in the NP dimension. As a first attempt for SEC×NP-HPLC×CAD, the mobile phase combinations using dichloromethane-heptane and dichloromethane -hexane were tested in this work.

In recent studies for polymer analysis, NP or RP×SEC combinations are mainly applied to improve the overall separating power by using gradient elution in the first dimension [Van der Horst and Schoenmakers 2003].

3.4. Method validation

The validation of an analytical test method is the process of demonstrating that the analytical procedures are suitable for the intended use. Therefore, for the validation of the multi analytical methods for screening of adhesive related substances, the following validation parameters are important.

- Selectivity (Specificity) : The ability to discriminate between the target analyte and other substances in the test samples. This can be confirmed by some retention parameters such as retention time and/or retention index.
- Linearity : The calibration curve is a graphic representation of the detection system’s response as a function of the quantity of analyte. The linearity is evaluated by a graphical presentation and linear regression analysis.
- Limit of detection : The smallst amount and concentration of analyte in a sample that can be reliably distinguished, with stated significance, from the background or blank level.

- Practicability : The ease of operation, in terms of sample throughput and costs, to achieve the required performance criteria and thereby meet the specified purpose.

The developed analytical method through these validation procedures can be described by a complete report according to the CEN (European Committee for Standardization) standard format. This format is obligatory for method descriptions in EU petitions for the approval of new substances for food-contact materials and also described in the Note for Guidance [EFSA 2006]. For instance, the EN 13130 series [CEN 2004] and the BCR project 'Monomer' report [Franz and Rijk 1997] that are describing standard analysis methods for the quantification of migratable monomers used for food-contact plastic materials were prepared according to CEN standard format. The final standard methods for semi-quantitation will be prepared according to the CEN standard format in this thesis.

3.5. Identification of volatile and semi-volatile substances

Various retention parameters such as retention time, relative retention time and linear retention index can be applied for the identification of unknown peaks eluting from a GC column. The retention time of an analyte on GC column varies according to operational conditions such as phase ratio and column length, gas flow rate and temperature. [Zellner et al. 2008]. For this reason, it is useful only to compare the retention times determined by one GC instrument and the same operational conditions. The retention time in GC analysis can only provide the base information to identify unknown substances in a sample through comparison with the retention time of known reference substances. On the other hand, relative retention time is the ratio between the retention time of an analyte and that of an internal standard. This parameter is useful to compare the retention data from one GC instrument to another. However, for comparing the relative retention times between different GC systems, the operational conditions of GC systems should be just the same, since this relative retention time is also influenced by the phase ratio and different temperature programs.

In order to overcome these limitations, the retention index (*RI*) system has often been used for the identification of unknown peaks in GC analysis. This retention index (see Equation 12) is independent from phase ratio, column length, gas flow rate and column temperature. It is only influenced by the kind of the stationary phase [Goodner 2008]. Therefore, the retention index system is a reliable and reproducible parameter for the identification of unknown peaks eluting from a GC column. The retention index system which are used a

homologue series of *n*-alkanes as reference substances was first introduced by Kováts [1958], but the equation given by Kováts for calculation of the retention indices is only for isothermal gas chromatographic condition. The use of isothermal condition in GC analysis is not practical for the separation of a complex mixture containing many compounds with a wide range of boiling points. For this reason, Van den Dool and Kratz [1963] first proposed an equation that was transformed to a more general form to include also the programmed temperature condition as shown in equation 12.

$$RI = 100 \times \frac{tR_{(X)} - tR_{(n)}}{tR_{(n+1)} - tR_{(n)}} + 100\,n \text{ -------- Equation 12}$$

RI : retention index

X : target compound

$tR_{(X)}$: retention time of target compound

n : number of carbon atoms in the n-alkanes

$tR_{(n)}$: retention time of n-alkane with *n* carbon atoms eluting before target compound *(X)*

$tR_{(n+1)}$: retention time of n-alkane with (*n*+1) carbon atoms eluting after target compound *(X)*

3.6. Practical applications of screening methods

In order to confirm and validate the applicability of the developed screening and semi-quantification methods, migration and extraction tests using the real adhesive samples were performed.

3.6.1. Migration test

There are no specific directives for paper and board on EU level and therefore no standardized methods are available for the migration testing of paper and board. Paper and board for food packaging are mainly intended to contact with dry and non-fatty food. Also, the packaging materials listed in table 4-9 do not usually come into contact with liquid foodstuffs. The selection of solvents as food simulant was therfore not considered. Tenax (modified polyphenylene oxide_MPPO) has been used in this thesis, since it is recognized as a suitable food simulant for dry and fatty foods [Ottenio et al. 2004].

3.6.2. Extraction test

In general, the solvents for extraction test are selected according to the polarity of food contact plastics. For example, ethanol is used for polar polymers such as polyamide, rigid polyvinyl chloride (PVC) and polyethylene terephthalate (PET), whereas isooctane is used for nonpolar polymers such as polyolefines (PO). The recommended extraction condition is 24 hours at 40 °C [Franz and Störmer 2008, Gruner and Piringer 1999, Berghammer et al. 1994]. However, the identification of the suitable extraction solvent for other polymers with medium polarity such as polystyrene (PS) and plasticized PVC is not obvious [Franz and Störmer 2008]. On the other hand, dichloromethane (DCM) was recommended as a suitable extraction solvent for PO, PET and polystyrene (PS) by EU DG XII Research programme [1994-1997] and the extraction test was conducted at 40 °C for over 18 hours. This means that DCM shows efficient extraction power independent of the polarity of the polymers. The adhesive samples selected for this study consist of two different basic raw materials, vinyl acetate ethylene (VAE) copolymer and polyvinyl acetate (PVAc) (Table 4-8). Therefore, the adhesive samples in this thesis will be extracted by DCM under the condition of 24 hour at 40 °C.

Polymers with high molecular weight (MW > 1000 g/mol) may be also extracted from adhesive samples. Although the extracts are filtered by micro syringe filter (0.45 µm), the nebulizer in CAD and separation column could be clogged with the sticky polymers used as a binder of the adhesive. Therefore, the polymers with a molecular weight higher than about 1000 g/mol in the extracts should be excluded through an appropriate clean-up procedure. For this, size exclusion chromatography (SEC) has been carried out prior to analysis.

4. Experimental

4.1. Chemicals

4.1.1. Representative adhesive related substances

A wide range of substances has been selected for the development of analytical methods in this thesis. They are shown in Table 4-1. The purity of the representative adhesive related substances was greater than 95 % except for dipropylene glycol dibenzoate. 3-tert.-butyl-4-hydroxy-anisole (BHA) and 2-(2H-benzotriazol-2-yl)-4,6-bis(1-methyl-1 phenylethyl) -phenol (Tinuvin 234) were used as internal standards for calibration. Details about the source and purity of the standard substances as well as solvents used to prepare stock solutions are also given in table 4-1.

Table 4-1. List of representative adhesive related substances for the development of the screening methods.

Nr.	Name	Synonyms	MW (g/mol)	CAS-No.	Montfort list	Manufacturer	Purity (%)	Solvents for Stock solution	
								GC analysis	HPLC analysis
Group A Acrylate									
1	Acrylic acid methyl ester	Methyl acrylate	86.09	96-33-3		Aldrich	> 99 %	DCM	MeOH
2	Acrylic acid ethyl ester	Ethyl acrylate	100.11	140-88-5	Y	Aldrich	> 99 %	DCM	MeOH
3	2-Methylacrylic acid methyl ester	Methyl methacrylate	100.11	80-62-6	Y	Aldrich	99 %	DCM	MeOH
4	2-Methyl-2-propenoic acid ethyl ester	Ethyl methacrylate	114.14	97-63-2	Y	Aldrich	99 %	DCM	MeOH
5	Acrylic acid butyl ester	Butyl acrylate	128.18	141-32-2	Y	Aldrich	> 99 %	DCM	MeOH
6	Methacrylic acid, butyl ester	Butyl methacrylate	142.19	97-88-1	Y	Aldrich	99 %	DCM	MeOH
7	2-ethylhexyl prop-2-enoate	Ethylhexyl acrylate	184.28	1322-13-0		Fluka	> 98 %	DCM	MeOH
Group B Plasticizers									
8	Diisobutyl phthalate	DIBP	278.35	84-69-5	Y	Merck	> 98 %	DCM	MeOH
9	Dibutyl phthalate	DBP	278.35	84-74-2	Y	Merck	> 98 %	DCM	MeOH
10	Bis(2-ethylhexyl) phthalate	DEHP or DOP	390.56	117-81-7		Merck	> 98 %	DCM	MeOH
11	Diethylhexyl adipate	DEHA	370.57	103-23-1		Merck	> 98 %	DCM	MeOH
12	Glycerol triacetate	Triacetin	218.20	102-76-1	Y	Sigma	≥ 99 %	DCM	MeOH
13	2-Ethylhexyl diphenyl phosphate	Phosflex 362	362.44	1241-94-7		Riedel-de-Haën	99 %	DCM	MeOH
14	Diethylene glycol dibenzoate	DEGDB	314.34	120-55-8	Y	Aldrich	96 %	DCM	MeOH
15	Triethylene glycol dibenzoate	TEGDB	358.40	120-56-9		Aldrich	99 %	DCM	MeOH
16	Dipropylene glycol dibenzoate	DPGDB	342.42	27138-31-4	Y	Aldrich	80 %	DCM	MeOH
17	Propylene glycol dibenzoate	Bezoflex 284	284.3	19224-26-1		Aldrich	> 96 %	DCM	MeOH
18	2,2,4-Trimethyl-1,3-pentanediol dibenzoate	Benzoflex 354	354.45	68052-23-3		Aldrich	> 99.9 %	DCM	MeOH
Group C Carboxylic acid									
19	2-Propenoic acid	Acrylic acid	72.06	079-10-7	Y	Aldrich	> 99 %	DCM	MeOH
20	trans-Butenedioic acid	Fumaric acid	116.07	110-17-8		Aldrich	> 99 %	DCM	MeOH
21	cis-Butenedioic acid	Maleic acid	116.07	110-16-7		Aldrich	> 99 %	DCM	MeOH
22	Hexanedioic acid	Adipic acid	146.14	124-04-9	Y	Aldrich	99 %	DCM	MeOH
23	1,4-Benzene-dicarboxylic acid	Terephthalic acid	166.13	100-21-0		Fluka	> 99 %	DCM	MeOH
24	1,3-Benzene-dicarboxylic acid	Isophthalic acid	166.13	121-91-5	Y	Fluka	99 %	DCM	MeOH
Group D Alcohol									
25	1,2-Dihydroxyethane	Ethylene glycol	62.06	107-21-1	Y	Fluka	> 99.5 %	DCM	MeOH
26	1,2-Dihydroxypropane	Propylene glycol	76.1	57-55-6	Y	Fluka	> 99.5 %	DCM	MeOH
27	1,4-Butanediol	1,4-Butylene glycol	90.12	110-63-4	Y	Fluka	99 %	DCM	MeOH
28	2,2'-Dihydroxydiethyl ether	Diethylene glycol	106.12	111-46-6	Y	Fluka	> 99 %	DCM	MeOH
29	1,3-Benzenediol	Resorcinol	110.11	108-46-3		Fluka	> 99 %	DCM	MeOH
30	1,2,3-Propanetriol	Glycerol	92.09	56-81-5	Y	Sigma-aldrich	≥ 99.5 %	DCM	MeOH
31	2-Bromo-2-nitropropane-1,3-diol	Bronopol	200.01	52-51-7		Riedel-de-Haën	99.9 %	DCM	MeOH

Continued table 4-1.

Nr.	Name	Synonyms	MW (g/mol)	CAS-Nr.	Montfort list	Manufacturer	Purity (%)	Solvents for Stock solution	
								GC analysis	HPLC analysis
Group E Amine									
32	Hexamethylenediamine	HMDA	116.21	124-09-4		Fluka	> 99 %	DCM	MeOH
33	Toluene 2,4-diamine	2,4-TDA	122.17	95-80-7		Aldrich	98 %	DCM	MeOH
34	1,3,5-Triazine-2,4,6-triamine	Melamine	126.12	108-78-1		Fluka	> 99 %	DCM	MeOH
35	Isophorone diamine	IPDA	170.3	2855-13-2		Fluka	> 99 %	DCM	MeOH
36	4,4'-Methylenedianiline	4,4 MDA	198.26	101-77-9		Fluka	> 97 %	DCM	MeOH
Group F Antioxidants									
37	2,6-Di-tert-butyl-4-methylphenol	BHT	220.35	128-37-0	Y	Merck	> 99 %	DCM	MeOH
38	Octadecyl 3,5-bis (1,1-dimethylethyl)-4-hydroxybenzene propanoate	Irganox 1076	531	2082-79-3	Y	Ciba	-	Acetone	Acetone
39	2,4-Bis (1,1 dimethylethyl) phenyl-phosphite	Irgafos 168	646.93	31570-04-4		Ciba	-	Acetone	Acetone
40	1,3,5-Trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl) benzene	Irganox 1330	775.21	1709-70-2	Y	Ciba	-	Acetone	Acetone
41	Tetrakis [methylene-3 (3´,5´-di-t-butyl-4-hydroxyphenyl) propionate] methane	Irganox 1010	1177.7	6683-19-8	Y	Ciba	-	Acetone	Acetone
Group G Others									
42	Propanoic acid, ethenyl ester	Vinyl propionate	100.12	105-38-4	Y	Fluka	> 98 %	DCM	MeOH
43	Ethenylbenzene	Styrene	104.15	100-42-5	Y	Aldrich	99 %	DCM	MeOH
44	Dimethylbenzene	p-Xylene	107.17	106-42-3	Y	Riedel-de-Haën	99.9 %	DCM	MeOH
45	1,6-Hexalactam	Caprolactam	113.16	105-60-2	Y	Fluka	> 99 %	DCM	MeOH
46	N-Vinyl-2-pyrrolidinone	1-Vinyl-2-pyrrolidinone	114.14	88-12-0	Y	Acros	99 %	DCM	MeOH
47	2-Phenylpropene	α-Methylstyrene	118.18	98-83-9	Y	Aldrich	99 %	DCM	MeOH
48	Diphenyl keton	Benzophenone	182.23	119-61-9		Aldrich	≥ 99 %	DCM	MeOH
49	2-(2-Butoxyethoxy)ethanol acetate	Butyl diglycol acetate	204.27	124-17-4	Y	Aldrich	> 99.2 %	DCM	MeOH
50	2-Octyl-2H-isothiazol-3-one	Octhilinone	213.34	26530-20-1		Riedel-de-Haën	99.9 %	DCM	MeOH
51	4,4'-Dihydroxy-2,2-diphenylpropane,	Bisphenol A	228.29	80-05-7		Fluka	97 %	DCM	MeOH
52	Methanone, bis(4-(diethylamino)phenyl)-	4,4'-Bis(diethylamino) benzophenone, BDBP	324.46	90-93-7		Aldrich	> 99 %	DCM	MeOH
53	Bisphenol A diglycidyl ether	BADGE	340.42	1675-54-3	Y	Fluka	97 %	DCM	MeOH
54	5-tert-butyl-2-[5-(5-tert-butyl-1,3-benzoxazol-2-yl)-2-thienyl]-1 ,3-benzoxazole	Uvitex OB	430.06	7128-64-5		Ciba	-	DCM	MeOH
55	Sodium dioctyl sulfosuccinate	Docusate sodium	445.63	577-11-7	Y	Sigma	≥ 99 %	DCM	MeOH
Additional additives for HPLC-CAD analysis									
56	(2-hydroxy-4-octoxy-phenyl)-phenyl-methanone	Chimassorb 81	326.19	1843-05-6		Ciba	-	-	Acetone
57	2-(2'-Hydroxy-3,5'-di-tert-butylphenyl)-5-chlorobenzotriazole	Tinuvin 327	357.16	3864-99-1		Ciba	-	-	Acetone
58	2,2'-Thiobis(4-methyl-6-tert-butylphenol)	Irganox 1081	358.54	90-66-4		Ciba	-	-	Acetone
59	Acrylic acid, 2-tert-butyl-6-(3-tert-butyl-2-hydroxy-5-methylbenzyl)-4-methylphenyl ester	Irganox 3052	394.25	61167-58-6		Ciba	-	-	Acetone
60	Dodecyl 3-(3-dodecoxy-3-oxo-propyl)sulfanylpropanoate	Irganox PS 800	514.41	123-28-4		Ciba	-	-	Acetone
61	N,N'-Bis(3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionyl)hydrazide	Irganox MD 1024	552.39	32687-78-8		Ciba	-	-	Acetone
62	Triethyleneglycol bis[3-(3-tert-butyl-4-hydroxy -5-methylphenyl) propionate]	Irganox 245	586.37	36443-68-2		Ciba	-	-	Acetone
63	2,4-Bis(octylmercapto)-6-(4-hydroxy-3,5-ditert-butylanilino)-1,3,5-triazine	Irganox 565	588.39	991-84-4		Ciba	-	-	Acetone
64	3-(3,5-ditert-butyl-4-hydroxy-phenyl)-N-[6-[3-(3,5-ditert-butyl-4-hydroxy-phenyl)propanoylamino]hexyl]propanamide	Irganox 1098	636.49	23128-74-7		Ciba	-	-	Acetone
65	Bis(2,4-di-tert-butylphenyl) pentaerythritoldiphosphite	Ultranox 626	640.33	26741-53-7		Ciba	-	-	Acetone
66	2-[2-[3-(3,5-ditert-butyl-4-hydroxy-phenyl)propanoyloxy]ethylsulfanyl]ethyl 3-(3,5-ditert-butyl-4-hydroxy-phenyl)propanoate	Irganox 1035	642.40	41484-35-9		Ciba	-	-	Acetone
67	1,3,5-tris[(3,5-ditert-butyl-4-hydroxy-phenyl)methyl]-1,3,5-triazinane-2,4,6-trione	Irganox 3114	783.52	27676-62-6		Ciba	-	-	Acetone
Universal Internal Standard									
IS	3-tert-butyl-4-hydroxy-anisole	BHA	180.24	25013-16-5		Fluka	> 98 %	DCM	-
IS	2-(2H-benzotriazol-2-yl)-4,6-bis(1-methyl-1 phenylethyl)–phenol	Tinuvin 234	448	70321-86-7		Ciba	-	DCM	MeOH

DCM : Dichloromethane, MeOH : Methanol

4.1.2. Test substances used for GC column selection

Four different columns were tested to find a suitable column for the development of the multi screening method. For this, 26 adhesive related substances which have various representative physico-chemical properties were selected (Table 4-2).

Table 4-2. List and details of the substances selected for GC column selection.

Nr.	Substances	MW (g/mol)	CAS-No.	Manufacturer	Purity (%)
1	Acrylonitrile	53.06	107-13-1	Aldrich	> 99 %
2	1,3 Butadiene	54.09	106-99-0	Aldrich	> 99 %
3	Ethylene glycol	62.06	107-21-1	Fluka	> 99 %
4	Vinyl chloride	62.49	75-01-4	Aldrich	> 99 %
5	Propylene glycol	76.11	57-55-6	Fluka	> 99 %
6	Vinyl acetate	86.09	108-05-4	Aldrich	> 99 %
7	Methyl acrylate	86.09	96-33-3	Aldrich	> 99 %
8	Ethyl acetate	88.11	141-78-6	Aldrich	> 99 %
9	1,4-Butanediol	90.12	110-63-4	Fluka	99 %
10	Vinylidene chloride	96.94	75-35-4	Aldrich	> 99 %
11	Ethyl acrylate	100.11	140-88-5	Aldrich	> 99 %
12	Methyl methacrylate	100.11	80-62-6	Aldrich	> 99 %
13	Styrene	104.15	100-42-5	Aldrich	99 %
14	Diethylene glycol	106.12	111-46-6	Fluka	> 99 %
15	m-Xylene	106.17	108-38-3	Riedel-de-Haën	99.9 %
16	Caprolactam	113.16	105-60-2	Fluka	> 99 %
17	Ethyl methacrylate	114.14	97-63-2	Aldrich	> 99 %
18	N-Vinyl-2-Pyrrolidinone	114.14	88-12-0	Aldrich	99 %
19	α –Methylstyrene	118.18	9011-11-04	Aldrich	99 %
20	Butyl acrylate	128.18	141-32-1	Aldrich	> 99 %
21	Butyl methacrylate	142.19	97-88-1	Aldrich	> 99 %
22	2,6-Di-tert-butyl-4-methylphenol	220.35	128-37-0	Fluka	> 99 %
23	Diisobutyl phthalate	278.35	84-69-5	Merck	> 98 %
24	Dibutyl phthalate	278.35	84-74-2	Merck	> 98 %
25	Bis(2-ethylhexyl) adipate	370.57	103-23-1	Merck	> 98 %
26	Bis(2-ethylhexyl) phthalate	390.56	117-81-7	Merck	> 98 %
IS	3-tert.-butyl-4-hydroxy-anisole, BHA	180.24	25013-16-5	Fluka	> 98 %

4.1.3. Test substances used for online two-dimensional chromatogaphic analysis

For the studies by online two-dimensional chromatography (SEC×NP-HPLC×CAD), 11 additives related to adhesives (Table 4-3) with molecular weights from 326 to 1177 g/mol were selected.

Table 4-3. List of the substances selected for online two-dimensional HPLC analysis.

Chemical name	Trade name	MW (g/mol)	CAS-No.
Phenolic antioxidants			
Pentaerythritoltetrakis[3-(3,5-di-tert.butyl-4-hydroxyphenyl) propionate]	Irganox 1010	1177	6683-19-8
1,3,5-tris[(3,5-ditert-butyl-4-hydroxy-phenyl)methyl]-1,3,5-triazinane-2,4,6-trione	Irganox 3114	784	27676-62-6
1,3,5-trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl) benzene	Irganox 1330	775	1709-70-2
2-[2-[3-(3,5-ditert-butyl-4-hydroxy-phenyl)propanoyloxy]ethylsulfanyl]ethyl 3-(3,5-ditert-butyl-4-hydroxy-phenyl)propanoate	Irganox 1035	642	41484-35-9
n-Octadecyl-3-(4-hydroxy-3,5-di.tert.butylphenyl)propionate	Irganox 1076	531	2028-79-3
Phosphorus antioxidants			
Tris(p-tert.butylphenyl) phosphate	Irgafos 168	647	31570-4-4
Thioester stabilizers			
Dodecyl 3-(3-dodecoxy-3-oxo-propyl)sulfanylpropanoate	Irganox PS800	515	123-28-4
Octadecyl 3-(3-octadecoxy-3-oxo-propyl)sulfanylpropanoate	Irganox PS802	683	693-36-7
UV Absorbers			
2-(benzotriazol-2-yl)-4,6-bis(1-methyl-1-phenyl-ethyl)phenol	Tinuvin 234	447	70321-86-7
(2-hydroxy-4-octoxy-phenyl)-phenyl-methanone	Chimassorb 81	326	1843-05-6
Fluorescent whitening agent			
5-tert-butyl-2-[5-(5-tert-butyl-1,3-benzoxazol-2-yl)-2-thienyl]-1,3-benzoxazole	Uvitex OB	431	7128-64-5

4.1.4. Standard solution of n-Alkanes for determination of retention indices

For the determination of retention indices, *n*-C8 to *n*-20 and *n*-C21 to *n*-40 alkanes mixtures were obtained from Fluka Germany.

4.1.5. Solvents and Reagents

Methanol and acetonitrile of HPLC grade were obtained from VWR (Darmstadt, Germany), and acetone, analytical grade, was obtained from Chem^{solute} (Renningen, Germany). Water, HPLC grade and analytical grade tetrahydrofuran, dichloromethane, n-heptane, n-hexane and diethyl ether were obtained from Merck (Darmstadt, Germany).

Formic acid (purity > 96 %) and ammonium acetate (purity > 98 %) were obtained from Sigma-Aldrich.

4.2. Preparation of standard solutions

4.2.1. Representative adhesive related substances and internal standards

Stock solution

Standard stock solutions for GC analysis were prepared for each substance including internal standards BHA and Tinuvin 234 in dichloromethane and acetone at a concentration of 1000 ± 50 µg/ml.

For HPLC-CAD analysis, the standard stock solutions including the internal standard Tinuvin 234 were prepared in methanol and acetone at a concentration of 1000 ± 50 µg/ml. In each case the correct concentrations of the stock solutions were calculated. The solvents used for the preparation of stock solutions of each substance are listed in table 4-1.

Calibration solutions

1, 5, 10, 50, 100, 250 and 500 µl of the standard stock solutions of each substance at a concentration of 1000 ± 50 µg/ml were filled into a series of 10 ml volumetric flasks. For GC-FID analysis, 500 µl of the internal standard stock solutions of BHA and Tinuvin 234 were added to each of the flasks. For HPLC-CAD analysis, 500 µl of Tinuvin 234 was spiked. The flasks were filled up to the marks with dichloromethane for GC analysis and with methanol for HPLC analysis. These standard solutions contained approximately 0.1, 0.5, 1, 5, 10, 25 and 50 µg/ml of each substance.

4.2.2. Test substances and internal standard for GC column selection

Stock solution

Standard stock solutions of test substances and internal standard (BHA) were prepared by dissolving 1000 ± 50 µg/ml of each substance in 10 ml of methanol. The correct concentrations of each substance were calculated.

Calibration solutions

Standard calibration solutions of 1, 5, 10, 25 and 50 µg/ml were prepared from the stock solutions by dilution with methanol. The calibration solutions were spiked with 25 µg/ml of BHA as an internal standard.

4.2.3. Test substances for online two-dimensional chromatogaphic analysis

Stock solutions

The standard stock solutions were prepared in dichloromethane (DCM) at a concentration of 1000 ± 50 µg/ml. The correct concentrations of each standard stock solution were calculated.

Standard solutions

1 ml of the stock solution was filled into 10 ml volumetric flask. The flask was filled up to the marks with the mobile phase solution composed of 30 % DCM and 70 % n-heptane. These standard solutions contained approximately 100 µg/ml.

4.2.4. n-Alkanes for the determination of retention indices

n-Alkanes standard solutions with a concentration of 40 mg/l were prepared in *n*-hexane and toluene respectively.

4.3. Instrumental analysis for non-volatile substances

4.3.1. HPLC-CAD analysis

Apparatus

The HPLC system from DIONEX consisted of Pump P680 A HPG, Autosampler ASI-100 and Column oven TCC-100. A charged aerosol detector (CAD) from ESA Biosciences was used to detect non-volatile substances.

HPLC-CAD conditions

A C18 column (HyperClone 250 × 4.60 mm, 5 μm particle size) was used for the separation of the selected substances. The CAD was set to a gas pressure of 35 psi, none filter mode and a range of 100 pA. The detailed analytical parameters of the HPLC-CAD are summarized in Table 4-4.

Table 4-4. HPLC-CAD conditions used for non-volatile substances

<table>
<tr><th>Analyses</th><th>HPLC conditions</th><th>Mobile phase conditions</th></tr>
<tr><td rowspan="2">Representative adhesive related substances</td><td rowspan="2">- Column temperature : 40 °C
- Flow rate : 1.0 ml/min.
- Injection volume : 20 μl</td><td>Gradient condition A
<table>
<tr><td>Time (min)</td><td>0</td><td>3</td><td>27</td><td>42</td><td>47</td><td>57</td></tr>
<tr><td>A(%)</td><td>10</td><td>10</td><td>0</td><td>0</td><td>10</td><td>Stop</td></tr>
<tr><td>B(%)</td><td>90</td><td>90</td><td>100</td><td>100</td><td>90</td><td></td></tr>
</table>
A : Water B : Acetonitrile</td></tr>
<tr><td>Gradient condition B
<table>
<tr><td>Time (min)</td><td>0</td><td>1</td><td>25</td><td>45</td><td>50</td><td>60</td></tr>
<tr><td>A(%)</td><td>40</td><td>40</td><td>0</td><td>0</td><td>40</td><td>Stop</td></tr>
<tr><td>B(%)</td><td>60</td><td>60</td><td>100</td><td>100</td><td>60</td><td></td></tr>
</table>
A : Water B : Acetonitrile</td></tr>
<tr><td>Isophthalic acid and Terephthalic acid</td><td>- Column temperature : 35 °C
- Flow rate : 1.0 ml/min.
- Injection volume : 25 μl</td><td>
<table>
<tr><td>Time (min)</td><td>0</td><td>3</td><td>13</td><td>15</td><td>25</td><td>25</td></tr>
<tr><td>A(%)</td><td>80</td><td>80</td><td>0</td><td>80</td><td>80</td><td>Stop</td></tr>
<tr><td>B(%)</td><td>20</td><td>20</td><td>100</td><td>20</td><td>20</td><td></td></tr>
</table>
A: Formic acid buffer (pH 2.5) B: Acetonitrile</td></tr>
</table>

4.3.2. SEC×NP-HPLC×CAD analysis

Apparatus

The HPLC system from DIONEX consisted of Pump P680 A HPG, Autosampler ASI-100 and Column oven TCC-100. A diode array detector (PDA-100) from DIONEX and charged aerosol detector (CAD) from ESA Biosciences were used to detect the 11 test substances in the first and second dimensions, respectively. Transfer of effluent fractions from the first to the second dimension was done with a 10 port switching valve (I-valve ®, Techlab GmbH Germany).

System set-up

Figure 4-1 shows the schematic of the on-line two-dimensional separation system used in this thesis. The flow of the SEC (size exclusion chromatography) dimension should be regulated through a restrictor to keep a consistant pump pressure. The injected sample is separated in the first dimension (SEC column) according to the molecular size (hydrodynamic volume) of each compound. Then the UV detector monitored the absorbance of the separated substances at 240 nm. The effluents from the first dimension are tranferred into the second dimension via the switching valve equipped with two identical-volume sampling loops (200 µl volume).

The effluents separated from the first dimension are alternately collected in two sampling loops. While the effluents are filled in one loop, the effluents collected in the other loop are separated in the second dimension NP-HPLC (normal phase - high performance liquid chromatography) according to the chemical composition of samples. Therefore, the analysis time in the second dimension should be at least equal or less than the duration that the effluents separated in the first dimension are being filled in a loop. The loop sampling time was 1 min and therefore the valve was switched every 1 min. The samples separated in the second dimension were detected by using the charged aerosol detector (CAD) from ESA Biosciences. The CAD settings were the same as shown above.

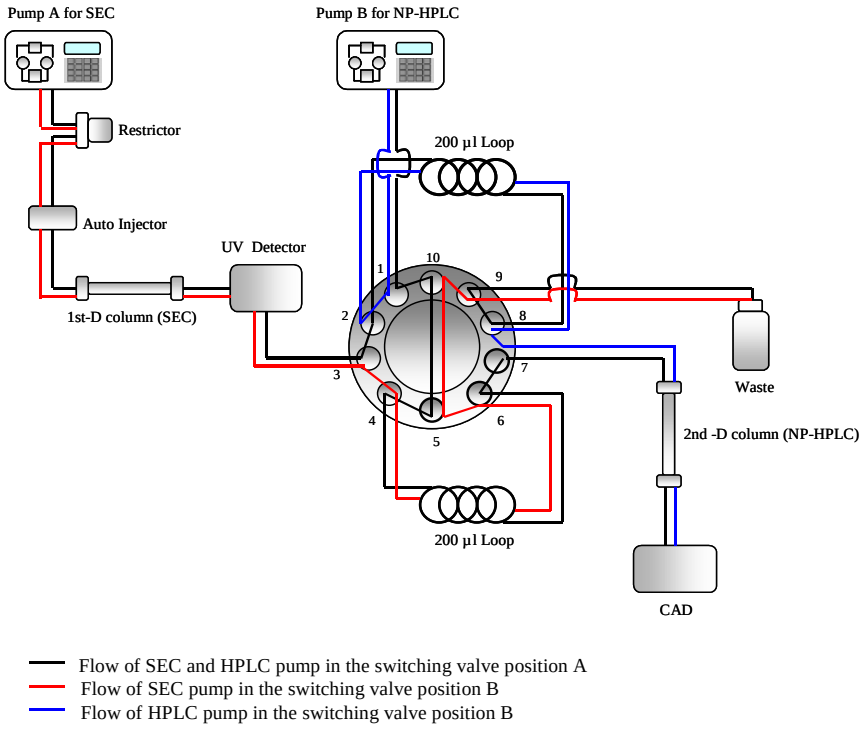


Figure 4-1. Schematic of the on-line SEC×NP-HPLC×CAD system using 10 way switching valve.

SEC×NP-HPLC×CAD conditions

Dichloromethane (DCM):n-heptane and DCM:n-hexane compositions were tested as the candidate mobile phase for both dimensions. The first dimension used KF-401 HQ (250 × 4.6 mm, 3 μm) semi-micro SEC column (Shodex) based on styrene-divinylbenzene (S-DVB) copolymer packing material. The mass exclusion limit of this column, estimated for linear molecules by using polystyrene (PS), is up to 1500 g/mol. In order to enhance the column efficiency, two identical SEC columns in series were connected.

For the second dimension, Diol phase column (LiChrospher Diol 250 × 4.0 mm, 5 μm) was tested. The detailed operating conditions are summarized in Table 4-5.

Table 4-5. SEC×NP-HPLC×CAD conditions

Analyses	Dimensions	Conditions
11 additives related to adhesive	SEC dimension	Mobile phase : DCM (30 %) : *n*-Heptane (70 %), Isocratic Oven temperature : 35 °C Flow rate : 0.2 ml/min. Injection volume : 10 μl Detection : UV 240 nm
	NP-HPLC dimension	Mobile phase : DCM (30 %) : *n*-Heptane (70 %), Isocratic Oven temperature : 35 °C Flow rate : 2.0 ml/min. Detection : CAD
Optimization test for SEC dimension Irganox 1010		Mobile phase : DCM (50 %) : *n*-Heptane (50 %), Isocratic DCM (50 %) : *n*-Hexane (50 %), Isocratic Oven temperature : 35 °C Flow rate : 0.2 ml/min. Injection volume : 10 μl Detection : UV 240 nm
Optimization test for NP-HPLC dimension Irganox 1076, Irganox 1330, Irgafos 168, Uvitex OB		Mobile phase : DCM (50 %) : *n*-Heptane (50 %), Isocratic DCM (30 %) : *n*-Heptane (70 %), Isocratic Oven temperature : 35 °C Flow rate : 2.0 ml/min. Detection : CAD

4.3.3. LC-MS analysis of dipropylene glycol dibenzoate

Apparatus

Waters Alliance 2695 HPLC system was used. Mass spectrometric analysis was carried out with Waters Micromass Quattro LC equipped with an electrospray ionization (ESI) source.

LC-MS conditions

The analytical parameters of the LC separation and mass spectrometer are summarized as follows.

Table 4-6. LC-MS conditions for the identification of dipropylene glycol dibenzoate.

LC conditions	
Column	HyperClone C18 column (250 × 4.60 mm, 5 µm particle size; Phenomenex)
Mobile phase	75 % acetonitrile and 25 % Ammonium acetate buffer (5 mM, pH=3.5)
Flow rate	0.6 ml/min
Injection volumn	10 µl
Oven Temperature	40 °C
MS conditions	
Ion source	Electron Ionization (ESI)
Polarity	Positive
Mass range	150 ~ 1000 g/mol
Scan rate	0.5 seconds / mass range
Desolvation temperature	400 °C

Sample preparation

The standard stock solution of dipropylene glycol dibenzoate was prepared in methanol at a concentration of approximately 1000 µg/ml. The standard stock solution was diluted with methanol to 10 µg/ml.

4.4. Instrumental analysis for volatile and semi-volatile substances

Gas chromatography (GC) equipped with flame ionization detector (FID) was used for analysis of volatile and semi-volatile substances. Samples were injected into the GC system by an automatic liquid sampler. Two different GC-FID instruments were used (HP Agilent 6890N GC and HP 6890 GC). The instruments and columns are summarized in Table 4-7. The detailed GC-FID conditions are shown in Appendix (Chapter 9.6).

Table 4-7. Instrumentation used for GC-FID analysis.

Instrument	Column	Analyses	Remarks
HP Agilent 6890N GC	**DB-1**, 30 m × 0.32 mm i.d. × 0.25 µm film thickness	**Column selection test** : 26 volatile and semi-volatile adhesive related substances	Chapter 9.6.1 and 5.4.1
	DB-FFAP, 30 m × 0.32 mm i.d. × 0.25 µm film thickness		
	DB-Wax, 30 m × 0.32 mm i.d. × 0.25 µm film thickness		
	Zebron (ZB) 624 , 60 m × 0,25 mm i.d. × 1.4 µm film thickness		
HP Agilent 6890N GC	**DB-1**, 30 m × 0.32 mm i.d. × 0.25 µm film thickness	**Multi-screening test and the determination of relative response factors (RRF)** : 55 representative adhesive related substances	Chapter 9.6.2, 5.4.2 and 5.4.3
	DB-FFAP, 30 m × 0.32 mm i.d. × 0.25 µm film thickness		
HP Agilent 6890N GC	**DB-1**, 30 m × 0.32 mm i.d. × 0.25 µm film thickness	**Linear retention indices_ Condition A and B** : 55 representative adhesive related substances	Chapter 9.6.3 and 5.4.6
HP 6890 GC		**Linear retention indices_ Condition C** : 55 representative adhesive related substances	
HP Agilent 6890N GC	**DB-FFAP**, 30 m × 0.32 mm i.d. × 0.25 µm film thickness	**Linear retention indices_ Condition D** : 55 representative adhesive related substances	

4.5. Statistical data analysis of relative response factor (RRF) values

The RRF value for a given substance will depend on the nature of the particular molecule and the specific physical or chemical response to it of the used detector. Consequently, for the whole set of representative substances a statistical pattern of RRF values can be expected which can be described by a distribution curve.

4.5.1. Normality test

Estimation of normality of RRF values was carried out using Minitab 15 [Minitab version 15 2007] Ryan-Joiner correlation test for normality.

Many statistical tests and intervals are based on the assumption of normality. However, in many real cases, the data do not follow a normal distribution and therefore it is not possible to estimate the distribution of RRF values at a certain confidence level. For this reason, an appropriate transformation was needed for non-normal data. The Box-Cox transformation proposed by Box and Cox [1964] is useful for non-normal data. It is defined by equations 13 and 14 :

$$\text{Response variable (T)} = \frac{(Y^{\lambda}-1)}{\lambda} \qquad \text{if } \lambda \neq 0 \text{ ------ Equation 13}$$

$$\text{Response variable (T)} = log_e(Y+\lambda) \qquad \text{if } \lambda = 0 \text{ ------ Equation 14}$$

Where Y is the variable and λ is the Box-Cox parameter indicating a number that represents the optimal transformation for correcting non-normality. The optimal value of λ can be determined by the Box-Cox plot that gives a correlation between the pooled standard deviations (SD) versus the λ values. At this time, SD is the Y axis and Lambda is the X axis. All these procedures for Box-Cox transformation were also carried out using Minitab 15 [Minitab version 15 2007].

4.5.2. Estimation of the distribution range of RRF values

The distribution range of RRF values at a 95 % coverage level is shown as follows in figure 4-2 and can be defined by equations 15 and 16 [Gottwald 1999], depending on the number of substances.

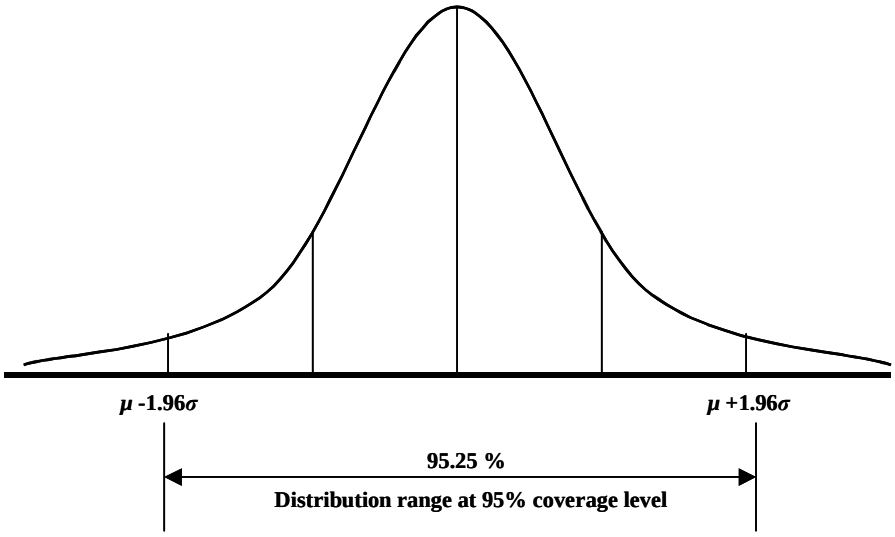


Figure 4-2. Schematic of the distribution range characteristics of RRF values

Distribution range of response factors = $\mu \pm 1.96\ \sigma$ $n > 30$ ---- Equation 15

Distribution range of response factors = $\mu \pm t\ \sigma$ $n < 30$ ---- Equation 16

where μ is mean of RRF values, σ is standard deviation, t is a t-variable of Student's t-distribution and n is number of sample.

In the normal distribution, 95.25% of the data are located in the range of $\mu \pm 1.96\,\sigma$. This is a distribution range of RRF values. When the sample size is high enough ($n > 30$), the normal distribution is usually applied for the estimation of the distribution range. However, if the sample size is too small ($n < 30$), the t-distribution (student distribution) should be applied to estimate the distribution range of the population from the experiment samples. This t-distribution depends on the degree of freedom ($f = n - 1$). When the t-variable is $f = \infty$, P = 95% (t-table), the t-distribution overlaps with normal distribution.

4.6. Linearity and Limit of detection (LOD)

Linearty of calibration curve was determined between the ratios of peak area of the analytes to that of internal standard and the corresponding concentrations.

The limit of detection (LOD) is usually defined as a signal/noise ratio of 3:1 and shows the sensitivity of the analysis method. The LOD values were determined from the calibration curves according to the guidelines of the German standard DIN 32645 [DIN 32645 1994].

4.7. Practical applications

4.7.1. Sample generation

Six pure water-based adhesives (VAE 1 ~ 5 and PVAc) and the corresponding composite samples (VAE 1-C ~ 5-C and PVAc-C) bonded with each pure water-based adhesive, details of which are given in table 4-8 and 4-9, were obtained from the manufacturers of each adhesive.

Table 4-8. List of the pure water-based adhesive samples.

Sample name	Application Method	Setting-Mechanism
VAE 1[1)]	Water based	Non-reactive
VAE 2	Water based	Non-reactive
VAE 3	Water based	Non-reactive
VAE 4	Water based	Non-reactive
VAE 5	Water based	Non-reactive
PVAc	Water based	Non-reactive

[1)] VAE 1 ~ 5 : Pure adhesives based on Vinyl acetate ethylene, PVAc : Pure adhesive based on Polyvinyl acetate

Table 4-9. List of composite samples bonded with water-based adhesives.

Sample name	Composites	End Application	Food Types	Food Contact Conditions
VAE 1-C [1)]	Cardoard / Cardboard	Folding box	Dry food	Room temperature
VAE 2-C	Cardboard / plastic window	Folding box	Dry food	Room temperature
VAE 3-C	Cardoard / Cardboard	Folding box	Hamburger, pommes frites	Hot fill
VAE 4-C	Paper / Paper, Coated paper	Paper bags : side seam	Bread, pastry ; all kinds of fast food	Deep frozen up to -60 °C
VAE 5-C	Paper / Paper, Coated paper	Paper bags : side seam	Bread, pastry ; all kinds of fast food	Deep frozen up to -60 °C
PVAc-C	Board / Board	Folding box side seam	-	-

[1)] VAE 1-C ~ 5-C and PVAc-C : Composites bonded with the pure adhesives VAE 1 ~ 5 and PVAc (e.g. VAE 1-C composite sample was bonded with VAE 1 pure adhesive)

4.7.2. Sample preparation

Extraction of pure water based adhesives

0.5 g of the pure adhesive was extracted with 10 ml dichloromethane (DCM) for 24 hours at 40 °C. The extracts were filtered using a teflon filter (PTFE) of 0.45 µm.

For the quantification, the extracts were spiked with the internal standards solution consisting of BHA (3-tert-butyl-4-hydroxy-anisole) and 2-(2H-benzotriazol-2-yl)-4,6-bis(1-methyl-1-phenylethyl)phenol (Tinuvin 234) prior to injection for GC-FID.

A clean-up procedure was performed for the HPLC-CAD analysis. Figure 4-3 shows the schematic of SEC clean up procedures. The HPLC system from DIONEX consisted of Pump P680 A HPG, Autosampler ASI-100 and Column oven TCC-100. Refractive Index (RI) Detector from Gynkotek RI was used to monitor the effluent fractions. Fraction collector 201 from Gilson-Abimed was used to collect the effluent fraction of interest.

A KF-401 HQ (250 × 4.6 mm, 3 µm) semi-micro SEC column (Shodex) was used for the separation of the extracts. The column temperature and mobile phase flow rate were kept at 40 °C and constant at 0.3 ml/min. The injection volume was 50 µl. Tetrahydrofurane (THF) was used as the mobile phase for isocratic eluent. RI detection was conducted at ambient temperature.

The fraction of interest (from 8 to 13 min, 1.2 mL) was collected with a fraction collector. The final dilution ratio of DCM extracts was 1 : 4 and for the correct calculation of the concentration, it should be multiplied by factor 4.

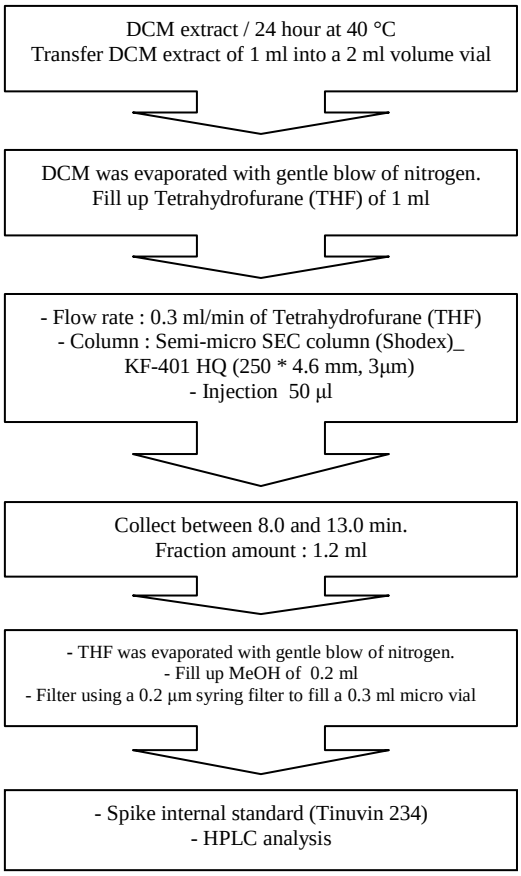


Figure 4-3. Schematic of SEC clean-up procedure

Migration testing using Tenax

Three sample sheets of each 0.5 dm^2 were placed into petri dishes and 2 g of Tenax was poured onto the surface area of each sample sheet. For the blank determination, Tenax of the same mass was placed in an empty Petri dish without a sample sheet. The prepared test samples were placed inside an air-heated oven at 40 °C for 10 days. After this time, Tenax was transferred into the Erlenmeyer flask for extraction, at the same time the internal standards (BHA and Tinuvin 234) were spiked for semi-quantification and then the Tenax was extracted three times using in total 90 ml of diethyl ether. The extracts were transferred into 100 ml vials and diethyl ether was completely evaporated with the aid of a gentle nitrogen flow at room temperature.

5 ml dichloromethane for GC-FID analysis and 5 ml methanol for HPLC-CAD analysis were added into the fully evaporated vials respectively. The extracts were filtered by a teflon filter of 0.2 μm and analyzed by GC-FID and HPLC-CAD respectively.

4.7.3. Analysis of extracts and migrants using the developed screening methods

The prepared extracts and migration solutions were analyzed by using the developed screening methods. The HPLC-CAD analysis was performed by gradient condition B as described in chapter 4.3.1. The GC-FID analysis was performed by the DB-1 column condition as described in the chapter 4.4.

5. Results

5.1. Selection of representative adhesive related substances

The multi-method and the semi-quantitative approach were developed using a selection of representative substances. As the essential procedure for the quantitative determination of unknown substances migrating from adhesives, the adhesive related substances were investigated through the literature search [Bonell and Lawson 1999, Gierenz and Karmann 2001, Ullmann's Encyclopedia of Industrial Chemistry 2008] and the opinions obtained from the industry partners of the EU project No COLL-CT-030309 ‘Migresives’. Subsequently, among these substances, 55 representative adhesive related substances were selected according to the following considerations.

The selected adhesive related substances should be typically used for adhesives formulations and represent different chemical structures, polarities and molecular weights.

The maximum molecular weight was about 1000 g/mol. According to the Guidelines of the Scientific Committee on Food (SCF) [SCF 2002], substances with molecular weight below 1000 g/mol are regarded as toxicologically relevant, since the substances with a molecular weight above 1000 g/mol will not be adsorbed in the gastrointestinal tract and are not expected to migrate through polymer films. This opinion was only presented for the polymeric additives in the SCF guideline. However, the extended application for any substances that may migrate from packaging materials into foodstuff is also reasonable.

A prerequisite was that reference standards of the single compounds needed to be available for calibration purposes. Therefore low molecular weight oligomers or other mixtures of substances could not be included in the list. The selection of substances contains monomers, additives and some solvents.

The selected substances were classified according to application intention or to functional group of the substances (Group A ~ G). More detailed information on these substances can be found in Appendix 9.2. Table 4-1 shows the list of the representative adhesive related substances. The list contains 7 acrylates, 11 plasticizers, 6 carboxylic acids, 7 alcohols and phenols, 5 amines, 5 antioxidants and 14 other substances. Organic solvents, monomers and various additives (UV absorber, thermal stabilizers, fluorescent whitening agent, surfactant and biocide) were included in the category “other substances”. The molecular weights of the substances ranged from 62 g/mol to 1177 g/mol. Figure 5-1 shows the distribution of molecular weight of the selected adhesive related substances.

Our source for adhesive related substances is the Montfort list complied in the MAFF (Ministry of Agriculture, Fisheries and Food) project FS 2223 [Bonell and Lawson 1999]. It is a representative list on raw materials of adhesives for food packaging. In this list, more than 360 substances are registered. Non-intentionally added substances such as reaction by-products were not considered in the Montfort list. For instance, primary aromatic amines originating from isocyanates which are starting substances for polyurethane adhesives are representative reactive by-products. In case of thermal decomposition from non-reactive systems further chemical compounds are of interest and need to be considered. The selected 55 substances (see Table 4-1) were compared with those listed in the Monfort list. It was found that 31 of the substances were contained in the Montfort list.

In the selection there were only few substances with molecular weight greater than 500 g/mol. For the development of the HPLC methods 12 plastic additives (antioxidants and UV-stabilizers) with a range of molecular weights from 300 to 700 g/mol were additionally included (Table 4-1).

The physico-chemical properties, especially the functional groups in the molecular structure, of an internal standard should be similar with those of targeted analyte. For screening purposes and semi-quantitative estimates, more generally applicable internal standards were needed. 3-tert-butyl-4-hydroxy-anisole (BHA) and 2-(2H-benzotriazol-2-yl)-4,6-bis(1-methyl-1 phenylethyl)–phenol (Tinuvin 234) were selected as the universal internal standards, because BHA and Tinuvin 234 have been established as internal standards for GC screening analyses for a long time at Fraunhofer IVV. BHA and Tinuvin 234 are used as an antioxidant and an ultraviolet light absorber respectively. But they are seldomly used so that most packaging materials are free of there both substances. BHA and Tinuvin 234 are containing hetero atoms and some polar functional groups that are considered to give a more conservative estimation for a semi-quantitative estimate. For GC-FID analysis, BHA and Tinuvin 234 are used as the internal standards. For HPLC-CAD analysis, only Tinuvin 234 was used, since BHA cannot be detected by CAD because of its relatively high volatility.

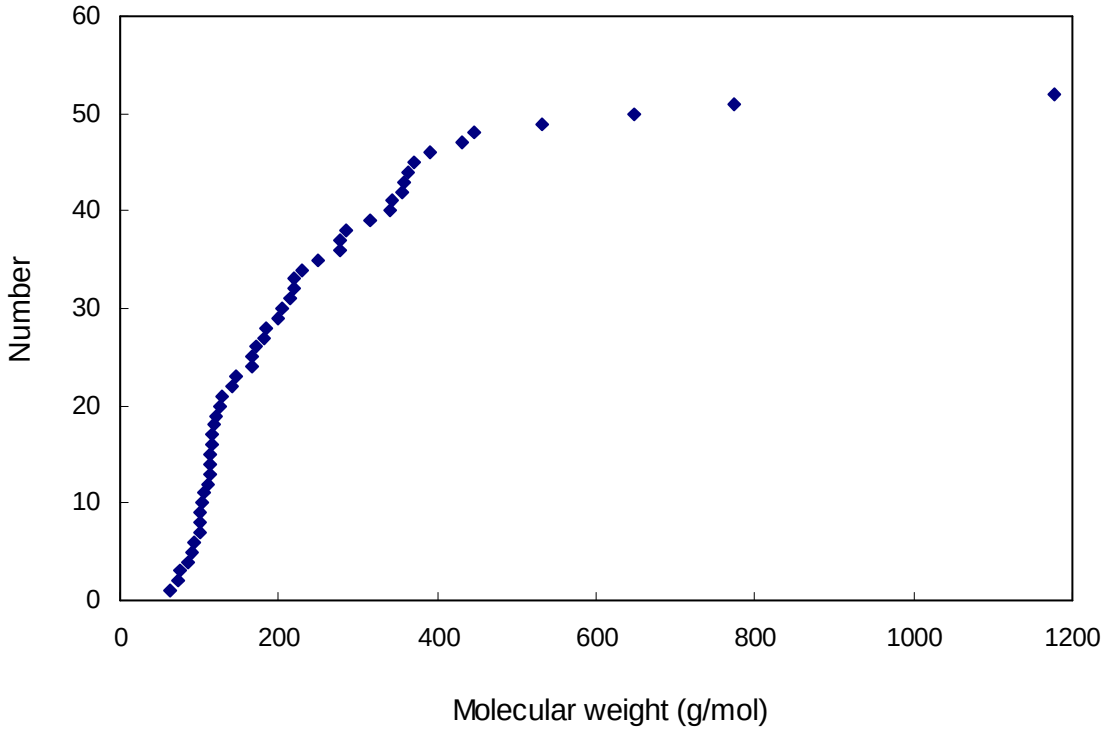


Figure 5-1. Molecular weight distribution of 55 adhesive related substances.

5.2. Development of screening method by HPLC-CAD

5.2.1. HPLC-CAD analysis data

For the development of the HPLC method, standards of all 55 plus 12 additional substances were analyzed using the charged aerosol detector (CAD). An internal standard (Tinuvin 234) was used.

Table 5-1 shows the screening results of all substances that were detectable by CAD. The analysis data of all detected substances and the comparison results for two different mobile phase compositions are summarized in this table. Linear regression was obtained between the ratios of peak area of the analytes to that of the internal standard and the corresponding concentrations. The limit of detection (LOD) according to the German standard DIN 32645 [1994] was studied with calibration curves.

As discussed in chapter 3.3.4, C18 column and gradient elution using acetonitrile (ACN) and water would be the most useful conditions to separate the selected adhesive related substances. The detection sensitivity of the CAD is influenced by the content of organic solvents in the mobile phase composition [Garmache et al. 2005, Brunelli et al. 2007 and Górecki et al. 2006]. Therefore, the influence of the content of organic solvents in the mobile phase composition was also characterized.

In order to obtain improved resolution, a good sensitivity of CAD and a suitable chromatographic run-time, two different mobile phase compositions of ACN and water (Gradient condition A and B, see chapter 4.3.1) were tested for gradient elution in this thesis. Total 28 substances of 67 test substances were detected under the gradient condition A and B. Figure 5-2 and 5-3 show the related chromatograms.

The use of gradient condition A does not permit sufficient resolution of 28 substances detected by CAD. The substances that have relatively high polarity such as BADGE, triethylene glycol dibenzoate (TEGDB), diethylene glycol dibenzoate (DEGDB), Irganox 245, Benzoflex 284, dipropylene glycol dibenzoate (DPGDB) and Irganox MD 1024 were all eluted at the retention time range between 3 and 4 minutes in gradient condition A.

On the contrary, besides some substances that overlapped each other (DEGDB with TEGDB and diethylhexyl phthalate with diethylhexyl adipate), most substances were separated with improved peak resolution under the gradient condition B (Figure 5-3). Especially, Bisphenol A was eluted at column dead-volume in gradient condition A, but it

was retarded after the column dead-volume in gradient condition B. Also, besides Chimasorb 81 and dipropylene glycol dibenzoate, most substances were detected with a good peak shape.

Chimasorb 81 showed a tailing peak in both gradient conditions and therefore the response was poor. The tailing may be caused by additional chemical interaction between Chimasorb 81 and the residual silanol groups of the stationary phase. The peak tailing of Chimasorb 81 could be overcome by pH modification of the mobile phase composition by using acetic acid [Quinto-Fernandez et al. 2003, Specific migration].

Docusate sodium, an ionic compound, was not retarded on the C18 column with acetonitrile/water mobile phase compositions, but the peak was found in the column dead-volume. Besides docusate sodium, some carboxylic acids (isophthalic and terephthalic acid) in the Group C were detected by CAD (Figure 5-4), but it was necessary to adjust the pH of the mobile phase using an appropriate buffer to prevent the ionization and to obtain a sufficient retention.

As shown in figure 5-3, dipropylene glycol dibenzoate standard was eluted with two separated peaks (peak No. 6 and 6-1). In order to identify of these peaks, dipropylene glycol dibenzoate was analyzed by LC-MS. As the result, dipropylene glycol dibenzoate was eluted with four separated peaks (peak 1 ~ 4, see Figure 5-5) in the HPLC analysis using a C18 column and ammonium acetate buffer (5 mM, pH=3.5) mobile phase (Table 4-6). The sodium adduct of dipropylene glycol dibenzoate (m/z 365) was only confirmed from the mass spectra of four separated peaks (Figure 5-5). This means that the peak No. 6-1 was identified as isomer peak of dipropylene glycol dibenzoate. Therefore, for the calculation of the relative response factors of dipropylene glycol dibenzoate, the sum of areas of both peaks (peak No. 6 and 6-1) was taken.

The minimum molecular weight of the detected substances by CAD was 228.29 g/mol (Bisphenol A, vapor pressure 5.21E-05 Pa). The molecular weights of most substances which could not be detected by CAD, were below 300 g/mol.

The maximum vapor pressure of representative adhesive related substances for detection by CAD was 8.39E-03 Pa (2-ethylhexyl diphenyl phosphate, MW 362.44 g/mol). The more volatile substances with high vapor pressure were not detected in the calibration range as well as at high concentrations (about 1000 µg/ml). Vapor pressures of all substances detected by CAD except diethylhexyl adipate and 2-ethylhexyl diphenyl phosphate were below 10^{-5} Pa.

The response of CAD is not affected by physico-chemical properties of the analytes [ESA Inc online]. Therefore, it provides a universal detection for non-volatile substances. Irganox PS 800 has no UV chromophores in the molecular structure and therefore it cannot be

detected by an UV detector. However, Irganox PS 800 could be detected with good sensitivity in this study (Figure 5-3).

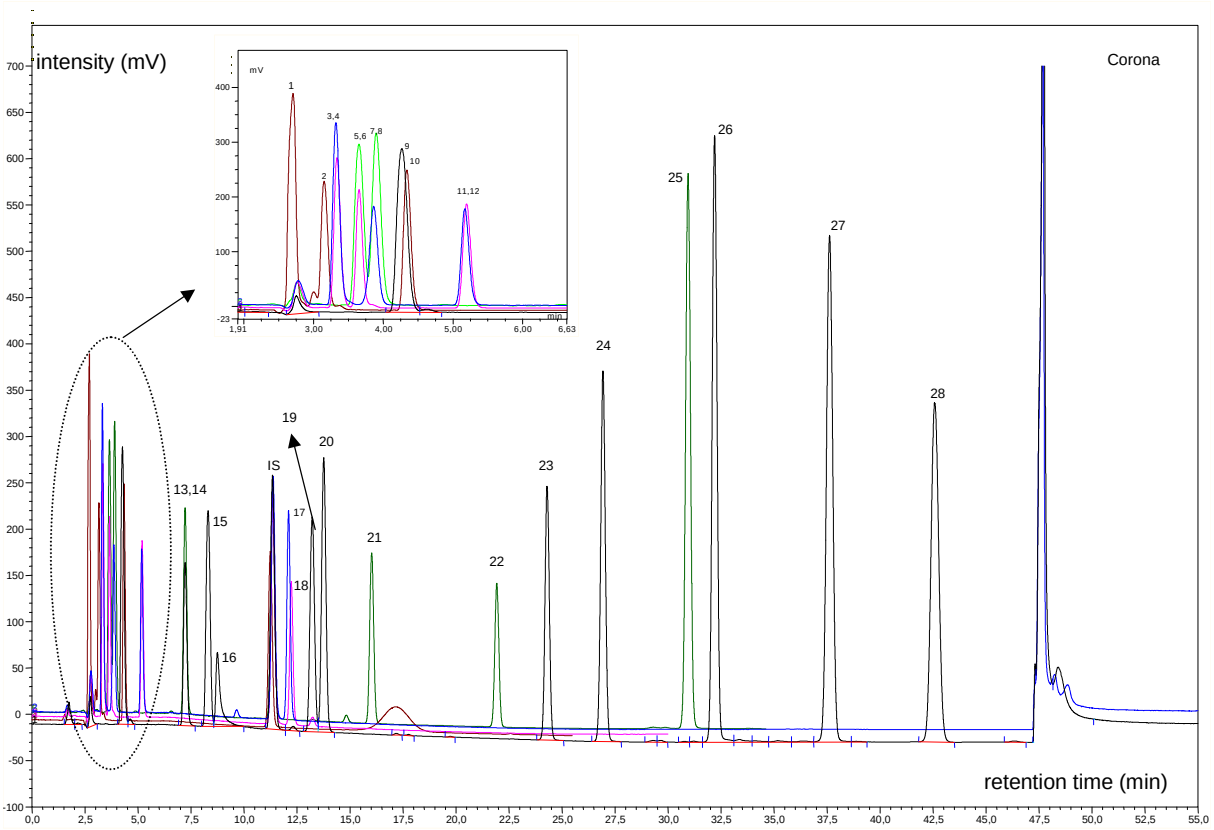
Response plots of the analyte concentration versus the peak area ratio between analyte and internal standard were observed to have good linearity in both mobile phase compositions (see chapter 9.1.1). For all substances in the concentration range from 0.1 to 50 µg/ml, the correlation coefficients varied between 0.9960 and 1.000 in the gradient condition A, and between 0.9952 and 0.9999 in the gradient condition B, respectively. All calibration curves via Tinuvin 234 are presented in chapter 9.1.1 separately. Diethylhexyl phthalate, diethylhexyl adipate, dipropylene glycol dibenzoate and propylene glycol dibenzoate showed a non-linear slope.

In order to evaluate the sensitivity of the CAD for the substances detected under two different mobile phase compositions, the limit of detection (LOD) values were determined. The LOD values for the individual substances ranged from 0.30 to 3.28 µg/ml in the gradient condition A, and from 0.45 to 3.53 µg/ml in the gradient condition B, respectively. However, the LOD values of 17 substances measured under the gradient condition A were lower than those obtained from the gradient condition B. On the whole, it is considered that the CAD sensitivity of the non-volatile substances was somewhat influenced by organic solvent content in mobile phase composition.

Table 5-1. LOD, correlation coefficient R values and retention data of representative adhesive related substances analyzed with HPLC-CAD under two different gradient conditions.

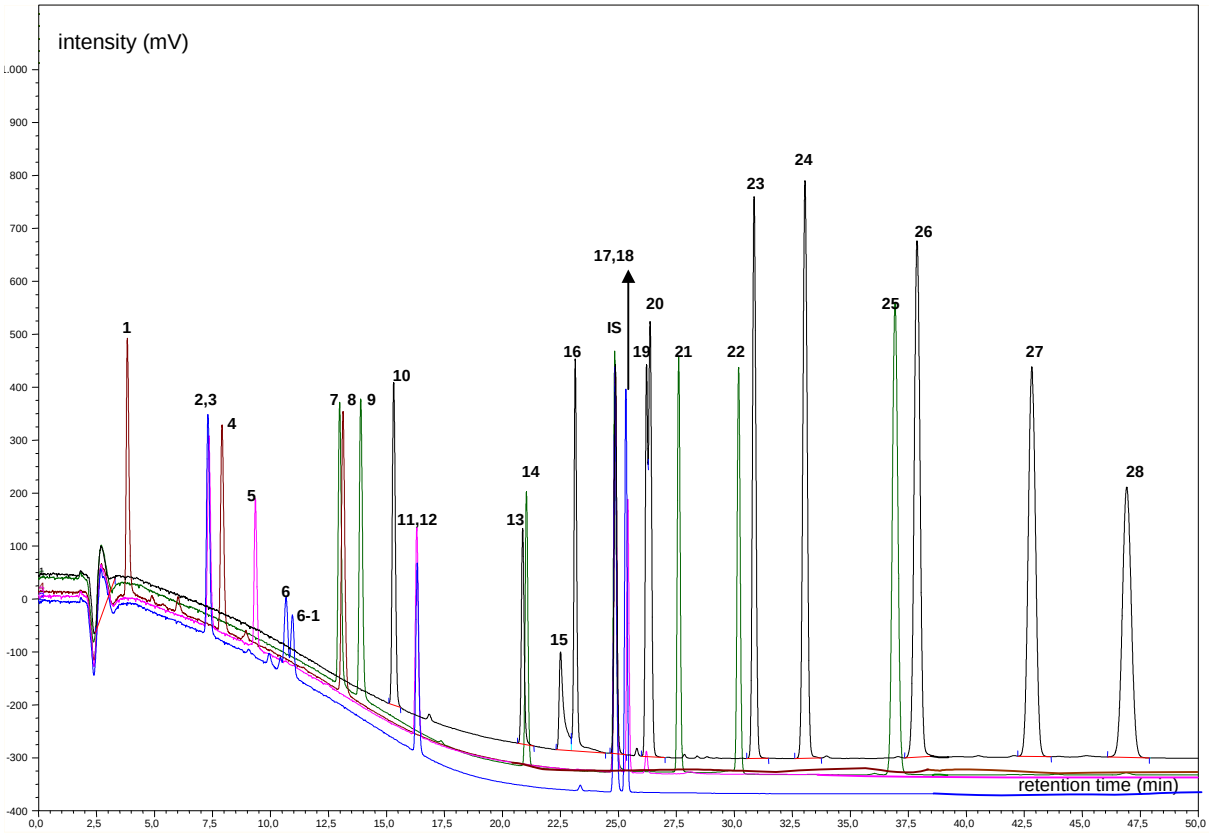
Classification	Nr.	Substances	MW (g/mol)	Vapor pressure (Pa)	LOD (µg/ml)		Correlation coefficient R	
					Gradient condition A	Gradient dondition B	Gradient condition A	Gradient dondition B
Group B Plasticizers	1	Diehylhexyl phthalate	390.56	1.89E-05	2.12	2.49	0.9984	0.9978
	2	Diethylhexyl adipate	370.57	1.13E-04	2.47	3.53	0.9973	0.9952
	3	2-ethylhexyl diphenyl phosphate	362.44	8.39E-03	1.95	0.79	0.9985	0.9998
	4	Diethylene glycol dibenzoate	314.34	9.63E-05	1.40	0.73	0.9992	0.9998
	5	Triethylene glycol dibenzoate	358.40	6.33E-06	0.54	0.93	0.9999	0.9997
	6	Dipropylene glycol dibenzoate	342.42	6.13E-05	2.25	ND < 5 ppm	0.9982	ND < 5 ppm
	7	Propylene glycol dibenzoate	284.3	2.53E-05	3.28	ND < 5 ppm	0.9960	ND < 5 ppm
	8	2,2,4-trimethyl-1,3-pentanediol dibezoate	354.45	1.16E-06	2.09	1.03	0.9986	0.9996
Group F Antioxidants	9	Irganox 1076	531	4.51E-11	0.59	0.90	0.9999	0.9997
	10	Irgafos 168	646.93	2.45E-11	0.52	0.45	0.9999	0.9999
	11	Irganox 1330	775.21	4.19E-20	1.31	1.54	0.9992	0.9989
	12	Irganox 1010	1177.7	1.55E-31	0.36	1.39	1.0000	0.9993
Group G Others	13	Bisphenol A	228.29	5.21E-05	NR	0.93	NR	0.9996
	14	4,4'-bis (diethylamino)benzophenone	324.46	4.33E-07	0.77	1.52	0.9998	0.9990
	15	BADGE	340.42	1.44E-05	0.94	0.45	0.9996	0.9999
	16	Uvitex OB	430.06	2.29E-10	0.36	0.78	0.9999	0.9997
	17	Docusate sodium	445.63	2.89E-09	NR	NR	NR	NR
Group H Additional Substances	18	Chimasorb 81	326	7.00E-07	ND < 10 ppm	ND < 10 ppm	ND < 10 ppm	ND < 10 ppm
	19	Tinuvin 327	357.16	2.67E-07	0.66	0.92	0.9999	0.9996
	20	Irganox 1081	358.5	6.53E-06	0.41	1.22	0.9999	0.9993
	21	Irganox 3052	394.25	3.65E-08	0.84	0.68	0.9997	0.9998
	22	Irganox PS 800	515	2.35E-11	0.96	1.13	0.9997	0.9995
	23	Irganox MD 1024	552.39	1.65E-15	0.87	2.44	0.9997	0.9974
	24	Irganox 245	586.37	1.09E-16	0.77	2.46	0.9998	0.9975
	25	Irganox 565	588.4	1.77E-16	0.51	1.39	0.9999	0.9992
	26	Irganox 1098	637	1.59E-20	0.30	1.39	1.0000	0.9997
	27	Ultranox 626	640.3	1.07E-09	0.55	0.76	0.9999	0.9998
	28	Irganox 1035	642	7.17E-16	0.43	0.90	0.9999	0.9997
	29	Irganox 3114	784	1.19E-21	1.01	1.00	0.9996	0.9996

ND : not detected, NR : not retarded,
Source of vapor pressure : SRC (http://www.syrres.com/what-we-do/databaseforms.aspx?id=386)



1. Bisphenol A	2. BADGE	3. Triethylene glycol dibenzoate	4. Diethylene glycol dibenzoate
5. Irganox 245	6. Propylene glycol dibenzoate	7. Dipropylene glycol dibenzoate	7. Irganox MD 1024
9. Irganox 1098	10. 4,4-Bis(diethylamino) benzophenone	11. 2-Ethylhexyl diphenyl phophate	12. 2,2,4-Trimethyl-1,3-pentanediol dibenzoate
13. Irganox 3052	14. Irganox 1081	15. Irganox 1035	16. Chimasorb 81
17. Diethylhexyl phthalate	18. Diethylhexyl adipate	19. Irganox 3114	20. Uvitex OB
21. Tinuvin 327	22. Ultranox 626	23. Irganox 1010	24. Irganox 1330
25. Irganox 565	26. Irganox PS 800	27. Irganox 1076	28. Irgafos 168

Figure 5-2. Representative chromatograms of the non-volatile substances that were analysed by HPLC-CAD equipped with C18 separation column after injection of 20.0 µl of a standard solution containing 50 µg/ml _Gradient condition A.



1. Bisphenol A	2. Diethylene glycol dibenzoate	3. Triethylene glycol dibenzoate	4. BADGE
5. Propylene glycol dibenzoate	6. Dipropylene glycol dibenzoate	6-1. Isomer of Nr.6 peak	7. Irganox 245
8. 4,4-Bis(diethylamino) benzophenone	9. Irganox MD 1024	10. Irganox 1098	11. 2-Ethylhexyl diphenyl phophate
12. 2,2,4-Trimethyl-1,3-pentanediol dibenzoate	13. Irganox 1081	14. Irganox 3052	15. Chimasorb 81
16. Irganox 1035	17. Diethylhexyl adipate	18. Diethylhexyl phthalate	19. Irganox 3114
20. Uvitex OB	21. Tinuvin 327	22. Ultranox 626	23. Irganox 1010
24. Irganox 1330	25. Irganox 565	26. Irganox PS 800	27. Irganox 1076
28. Irgafos 168			

Figure 5-3. Representative chromatograms of the non-volatile substances that were analysed by HPLC-CAD equipped with C18 separation column after injection of 20.0 µl of a standard solution containing 50 µg/ml_Gradient condition B.

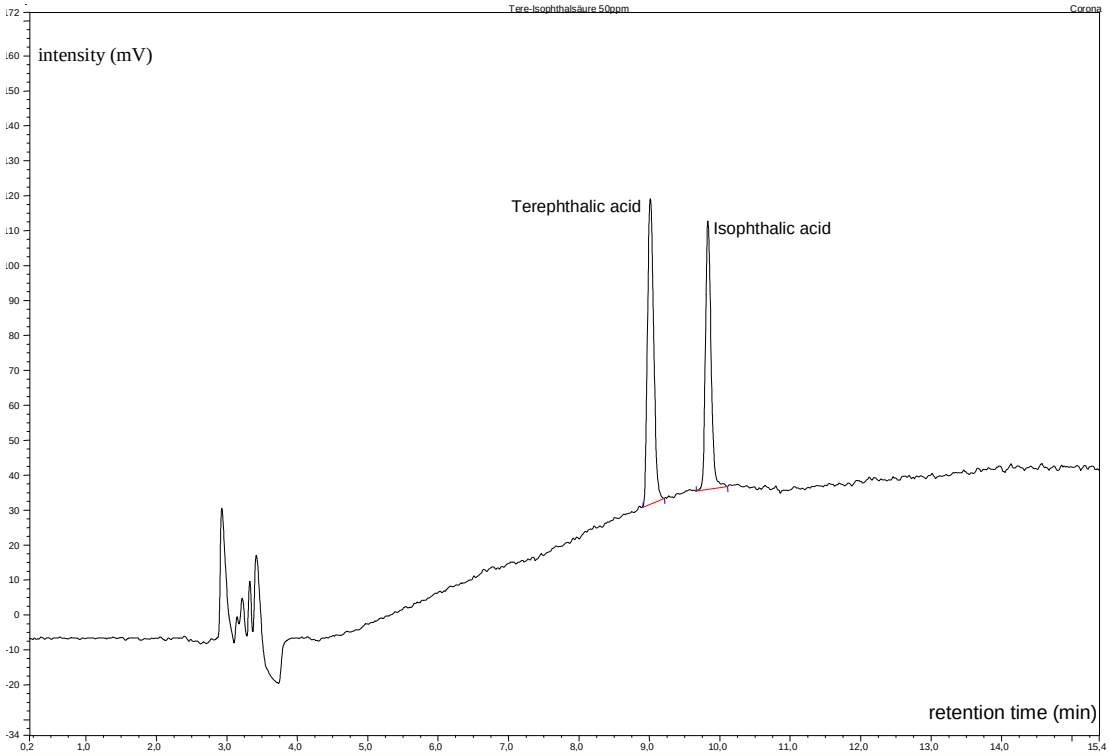


Figure 5-4. HPLC-CAD Chromatogram of isophthalic and terephthalic acid (MW: 166.13 g/mol) at the concentration 50 mg/l.

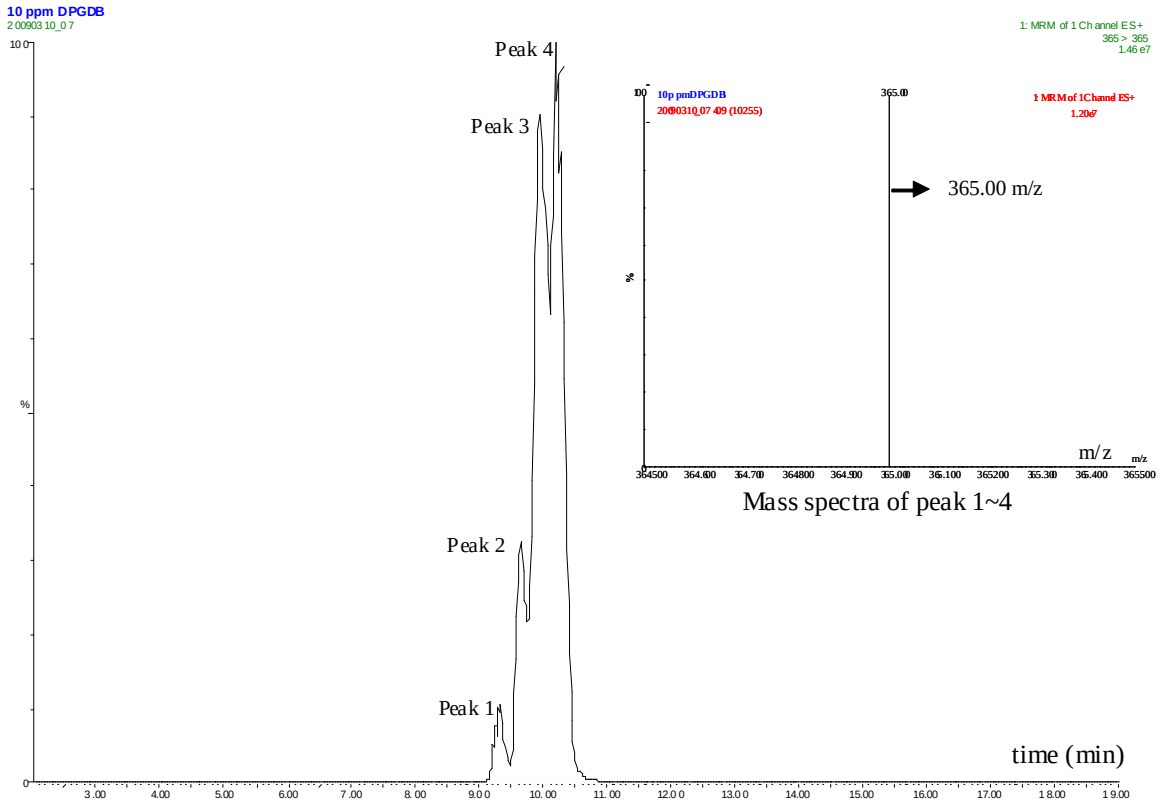


Figure 5-5. LC-MS chromatogram and spectrum of dipropylene glycol dibenzoate.

5.2.2. HPLC-CAD relative response factors

The applicability of a semi-quantitative approach using universal standard substances was investigated via the relative responses of the substances in relation to the internal standard.

The RRF values of total 27 substances in gradient condition A and 28 substances in gradient condition B were calculated for mass related concentration (mg/l, RRF w/w) and molar concentration (mol/l, RRF mol/mol).

The mass and molar related response factors (RRF w/w and mol/mol) relative to an internal standard (Tinuvin 234) are summarized in table 5-2. The RRF values (w/w) are highly variable from 0.29 to 2.61 in the gradient condition A and from 0.25 to 2.44 in the gradient condition B. Especially, Irganox 1330, Irganox 565, Irganox PS 800, Irganox 1076 and Irgafos 168 showed much higher responses than other substances. The molarity related relative responses (RRF mol/mol) are also highly scattered in the range from 0.17 to 3.43 in the gradient condition A and from 0.14 to 4.22 in the gradient condition B. Also, these values showed not a linear correlation increasing with the molecular weight in both gradient conditions (Figure 5-7 and 5-9). The relationship between the molecular weights and CAD responses (w/w) of the test substances are depicted in figure 5-6 for the gradient condition A and in figure 5-8 for the gradient condition B. The ideal range of the RRF values is usually recognized for 0.8 to 1.2. [Kazakevich and LoBrutto 2007, Burgard and Kuznicki 1990]. Among the substances listed in table 5-2, the substances with molecular weight higher than 400 g/mol or with a vapor pressure below 10^{-9} Pa, respectively, showed a response (RRF w/w) higher than 0.8 both for gradient condtion A and B (partition B in Figure 5-6 and partition B-1 in Figure 5-8). On the contrary, the relative response (RRF w/w) of substances with a molecular weight below 400 g/mol or with a vapor pressure above 10^{-9} Pa was less than 0.8 for both mobile phase compositions (partition A in Figure 5-6 and partition A-1 in Figure 5-8). As an exception, in spite of small molecular weight and high vapor pressure, 4,4'-bis(diethylamino) benzophenone (BDBP) showed good response by CAD with the RRF value (RRF w/w) 0.83 and 0.93 in both gradient conditions, respectively. In conclusion, analytes for CAD detection need to fulfil the two criterions, higher than 400 g/mol molecular weight and lower than 10^{-9} Pa vapor pressure.

The change of organic solvent content in mobile phase composition during the chromatographic run versus the CAD response are depicted in figure 5-10 and 5-11, respectively. When comparing the responses under the two different gradient conditions, no dependency from the organic solvent content in the mobile phase was visible. For instance,

Irganox 3114, Irganox 1098, Irganox 245, Irganox 1035 and more with greater molecular weight and/or lower vapor pressure were eluted with lower organic solvent content, nonetheless, these substances were detected with similar responses in both mobile phase compositions.

Table 5-2. Relative response factors of representative adhesive related substances retarded under two different separation conditions.

Classification	No.	Substances	MW (g/mol)	Vapor pressure (Pa)	RRF (w/w)		RRF (mol/mol)	
					Gradient condition A	Gradient condition B	Gradient condition A	Gradient condition B
Group B Plasticizers	1	Diehylhexyl phthalate	390.56	1.89E-05	0.54	0.52	0.48	0.46
	2	Diethylhexyl adipate	370.57	1.13E-04	0.46	0.41	0.38	0.34
	3	2-ethylhexyl diphenyl phosphate	362.44	8.39E-03	0.34	0.38	0.27	0.40
	4	Diethylene glycol dibenzoate	314.34	9.63E-05	0.42	0.47	0.29	0.28
	5	Triethylene glycol dibenzoate	358.40	6.33E-06	0.57	0.58	0.46	0.46
	6	Dipropylene glycol dibenzoate	342.42	6.13E-05	0.29	0.32	0.22	0.14
	7	Propylene glycol dibenzoate	284.3	2.53E-05	0.30	0.25	0.19	0.16
	8	2,2,4-trimethyl-1,3-pentanediol dibezoate	354.45	1.16E-06	0.34	0.38	0.27	0.30
Group F Antioxidants	9	Irganox 1076	531	4.51E-11	2.21	2.26	2.78	2.68
	10	Irgafos 168	646.93	2.45E-11	1.95	2.04	2.81	2.96
	11	Irganox 1330	775.21	4.19E-20	1.82	2.44	3.10	4.22
	12	Irganox 1010	1177.7	1.55E-31	1.09	1.51	2.50	4.05
Group G Others	13	Bisphenol A	228.29	5.21E-05	NR	0.64	NR	0.33
	14	4,4'-bis (diethylamino)benzophenone	324.46	4.33E-07	0.83	0.93	0.53	0.67
	15	BADGE	340.42	1.44E-05	0.70	0.59	0.53	0.45
	16	Uvitex OB	430.06	2.29E-10	0.99	0.96	0.96	0.92
	17	Docusate sodium	445.63	2.89E-09	NR	NR	NR	NR
Group H Additional Substances	18	Chimasorb 81	326	7.00E-07	0.28	0.26	0.17	0.19
	19	Tinuvin 327	357.16	2.67E-07	0.61	0.74	0.54	0.47
	20	Irganox 1081	358	6.53E-06	0.45	0.34	0.47	0.29
	21	Irganox 3052	394.25	3.65E-08	0.68	0.57	1.06	0.50
	22	Irganox PS 800	515	2.35E-11	2.10	2.15	2.22	2.44
	23	Irganox MD 1024	552.39	1.65E-15	0.95	0.99	1.17	1.22
	24	Irganox 245	586.37	1.09E-16	0.81	0.85	1.06	1.09
	25	Irganox 565	588.4	1.77E-16	2.61	2.44	3.43	3.05
	26	Irganox 1098	637	1.59E-20	0.92	0.91	1.20	1.30
	27	Ultranox 626	640.3	1.07E-09	0.59	0.92	0.84	1.31
	28	Irganox 1035	642	7.17E-16	0.84	0.83	1.20	1.20
	29	Irganox 3114	784	1.19E-21	0.94	1.05	1.62	1.84
Mean					0.91 a [1)]	0.96 a	1.14 b [2)]	1.20 b

NR : Not retarded,

[1) and 2)] Means with the same letter are not significantly different ($p > 0.05$).

Source of vapor pressure : SRC (http://www.syrres.com/what-we-do/databaseforms.aspx?id=386)

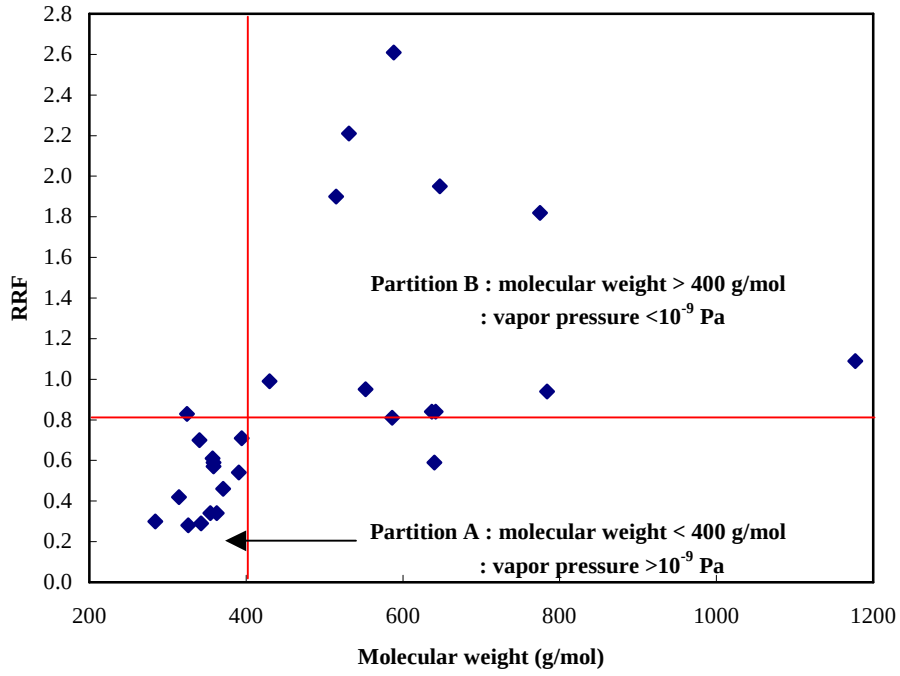


Figure 5-6. Correlation of the relative response factors (w/w) with molecular weight on CAD coupled with reverse phase HPLC system by using gradient condition A.

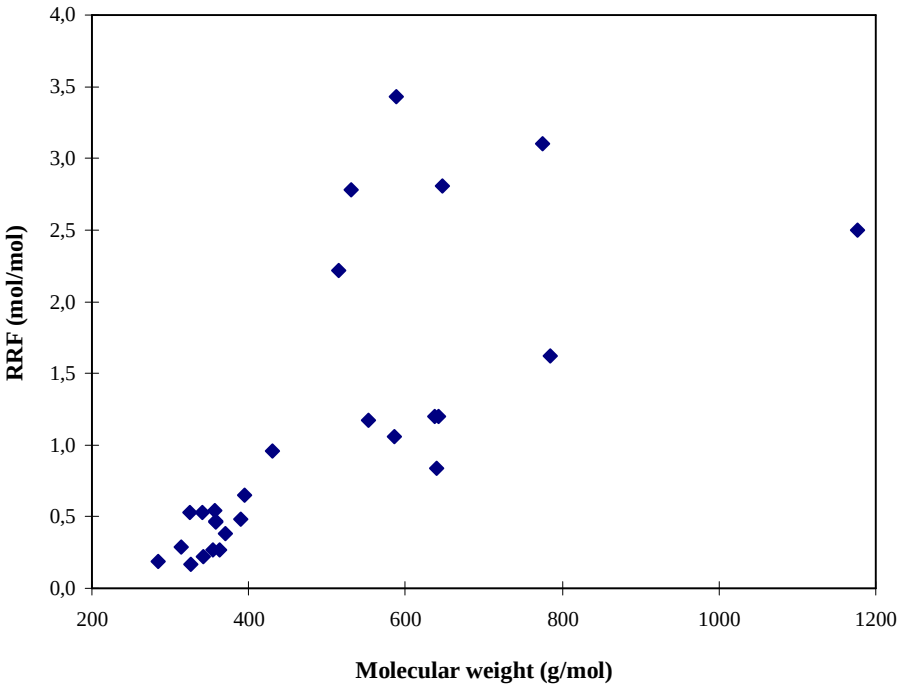


Figure 5-7. Correlation of the relative response factors (mol/mol) with molecular weight on CAD coupled with reverse phase HPLC system by using gradient condition A.

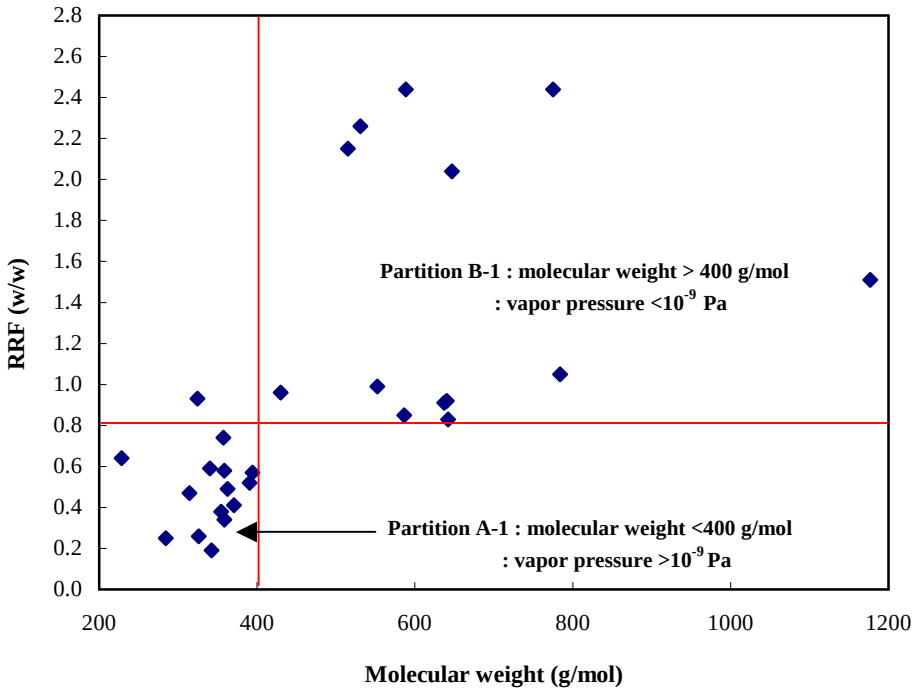


Figure 5-8. Correlation of the relative response factors (w/w) with molecular weight on CAD coupled with reverse phase HPLC system by using gradient condition B.

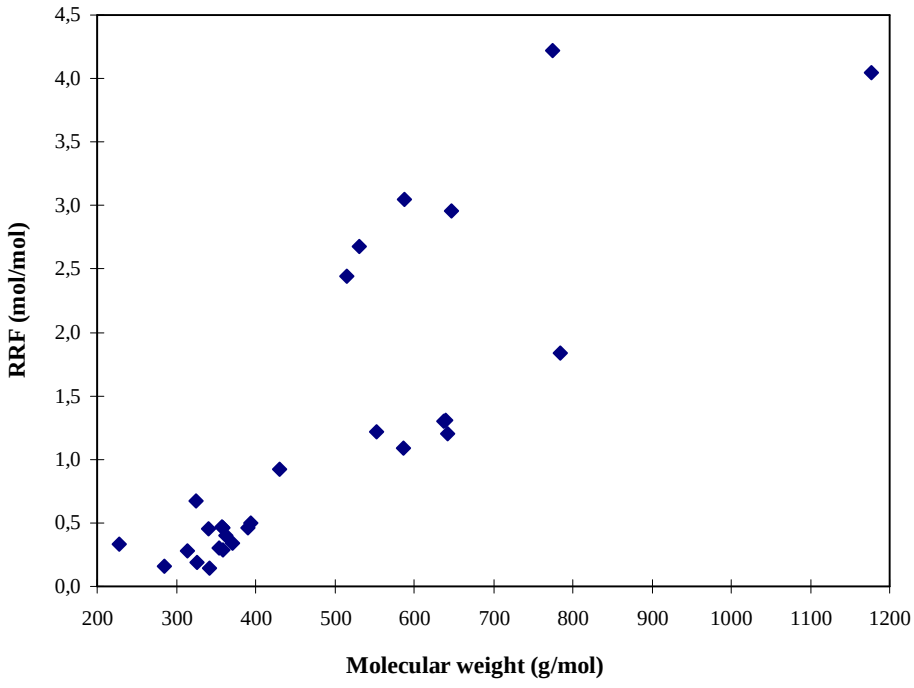


Figure 5-9. Correlation of the relative response factors (mol/mol) with molecular weight on CAD coupled with reverse phase HPLC system by using gradient condition B.

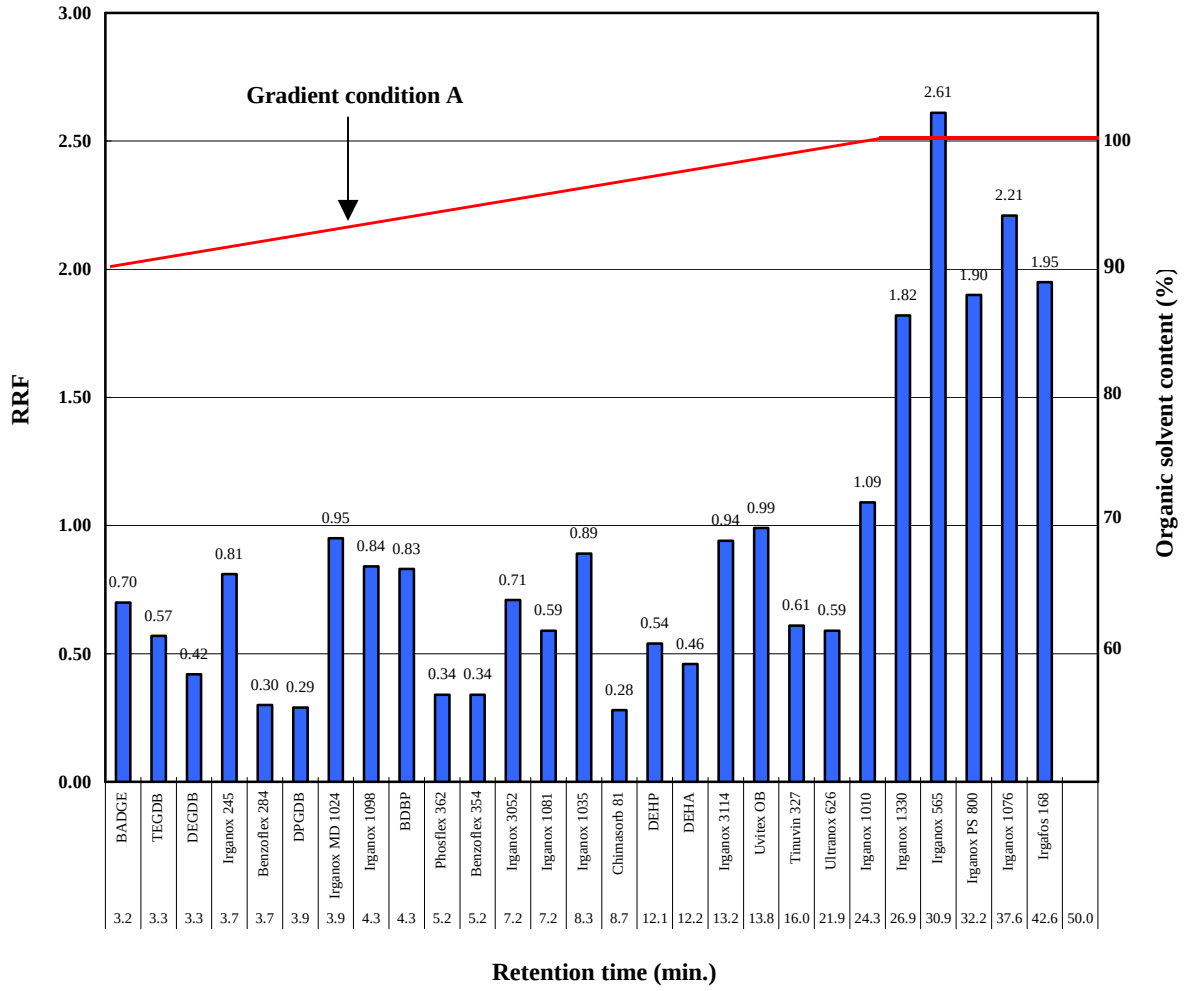


Figure 5-10. Change of relative response factors depending on the organic content in mobile phase Gradient A.

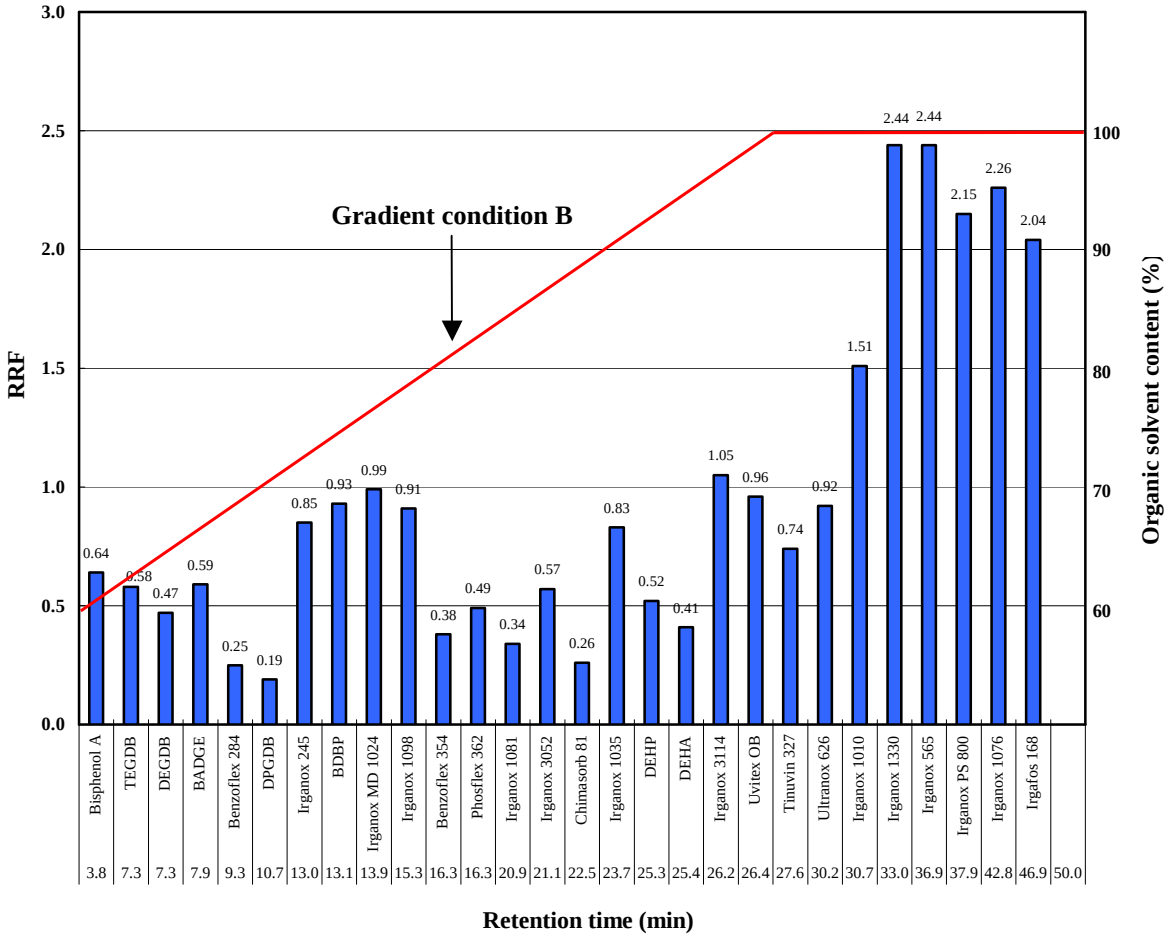


Figure 5-11. Change of relative response factors depending on the organic content in mobile phase Gradient B.

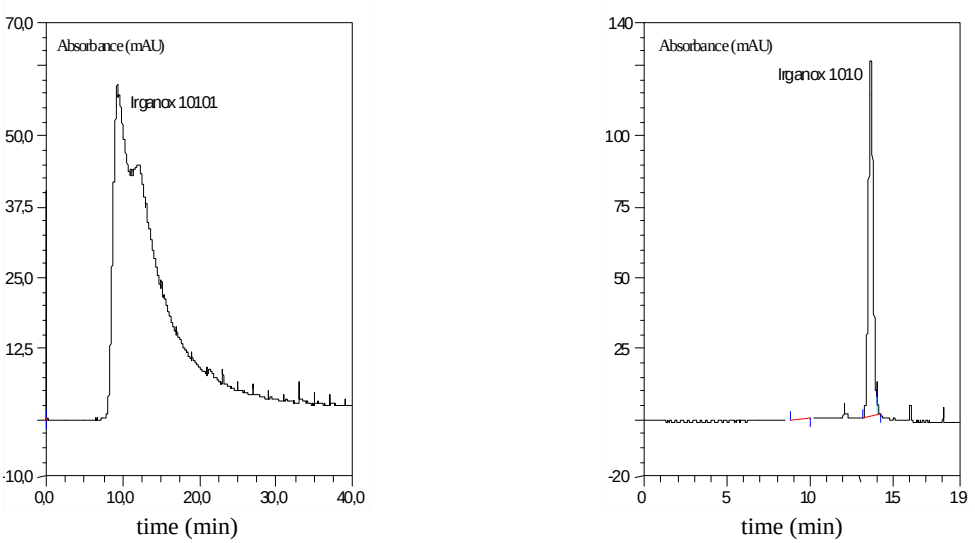
5.3. SEC×NP-HPLC×CAD two-dimensional chromatography

5.3.1. Optimization of the SEC×NP-HPLC conditions

Two-dimensional separation techniques with two different stationary phases shall improve peak capacity and resolving power compared to one-dimensional ones in this thesis. For the optimization of the online two-dimensional separation system, the combination of stationary phase for the first and second dimension, the compatability of mobile phase and the sample transfer modulation using switching valve are most important considerations.

Size exclusion chromatography (SEC) and normal phase high performance liquid chromatography (NP-HPLC) were choosen for the first and second dimension respectively. For the first dimension a nonaqueous semi-micro SEC column (KF- 401 HQ 250 × 4.6 mm, 3 µm, Shodex) based on styrene-divinylbenzene (S-DVB) copolymer was selected and a diol colunm (LiChrospher Diol 250 × 4.0 mm, 5 µm) was selected for the second dimension.

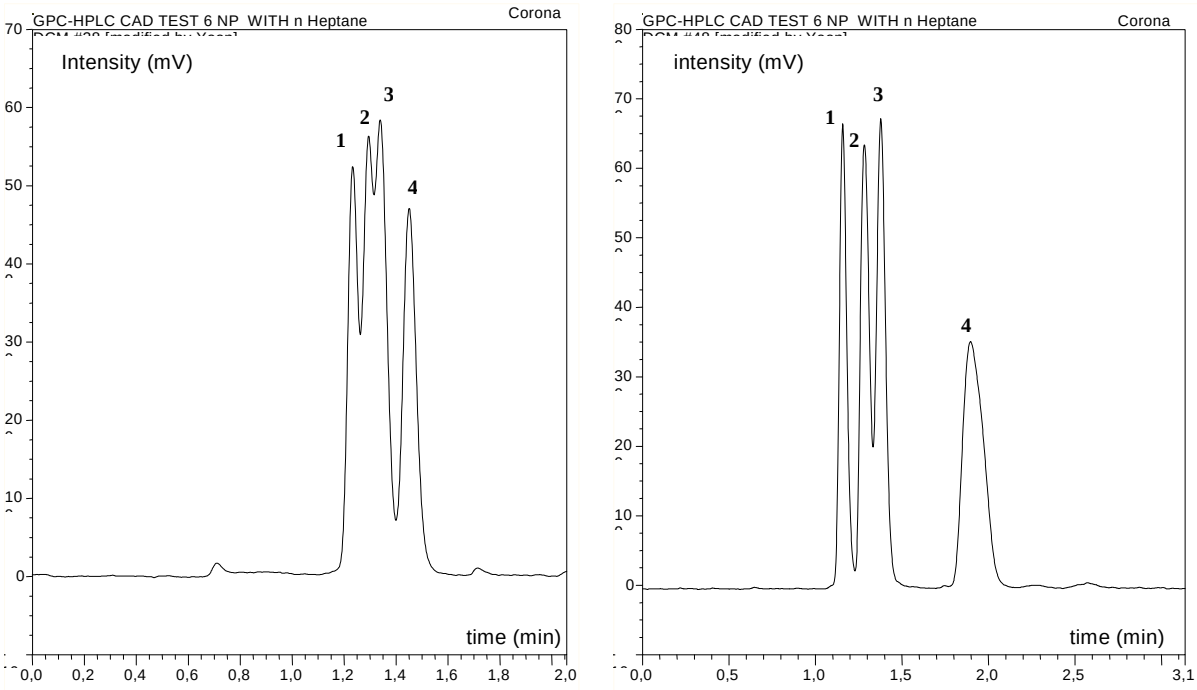
The compatibility of the mobile phases is very important for the two-dimensional separation. In this study, the mobile phase composition of first and second dimension should be identical because the charged aerosol detector (CAD) is very sensitive for changes of the mobile phase composition. In order to optimize the mobile phase composition, an antioxidant (Irganox 1010) was analyzed with two different mobile phase compositions (dichoromethane:n-hexane and dichloromethane:n-heptane) on the first dimension (SEC). Using dichoromethane:n-hexane, the peak eluted from the SEC dimension showed a broad tailing peak, whereas dichloromethane:n-heptane showed a sharp and symetrical peak (Figure 5-12).



50:50 Dichloromethane/n-Hexane 50:50 Dichloromethane /n-Heptane

Figure 5-12. SEC chromatograms of Irganox 1010 separated with two different mobile pahse compositions.

In order to find a suitable separation condition for the second dimension, four additives with different polarities (Irganox 1076, Irganox 1330, Irgafos 168, Uvitex OB) were analyzed by using two different dichloromethane : n-heptane ratios as mobile phase compositions (NP-composition A and B). As stated above, a diol phase column (LiChrospher Diol 250 × 4.0 mm, 5 µm) was used and the flow rate was 2.0 ml/min. As shown in figure 5-13, the four additives were separated within 1 minute in both mobile phase compositions but NP-composition B with a ratio of 30:70 (Dichloromethane : n-heptane) showed better resolution than NP-composition A.



50:50 DCM/n-Heptane (NP-composition A) 30:70 DCM/n-Heptane (NP-composition B)

Figure 6-13. Second dimension chromatograms

1 : Irganox 1076, 2 : Irganox 1330, 3 : Irgafos 168, 4 : Uvitex OB

5.3.2. SEC×NP-HPLC×CAD two-dimensional separation

As the first step in order to reach maximum peak separation when analysing complex adhesive samples, 11 additives related to adhesives (Table 4-3 in chapter 4.1.3) were selected and analyzed by the online two-dimensional system.

For the first dimension, two 250 mm SEC columns were connected in series, since a single SEC column had too limited peak capacity and resolution. This effect is already described in literature [Opiteck and Jorgenson 1997, Gilar et al. 2005].

Based on the results described above (chapter 5.3.1), a dichloromethane:n-heptane mobile phase mixture was selected for the development of the online two-dimensional screening method. Isocratic analysis with the same mobile phase was performed on both SEC and NP-HPLC dimension.

Figure 5-14 shows the raw chromatograms of a mixture of 11 additives in the first and second dimension. The 11 additives were eluted in the first dimension (SEC) according to their hydrodynamic volume and were monitored by UV-detection at 240 nm. In the second dimension each effluent fraction was separated according to hydrophobicity or polarity of the substances and monitored by CAD. The two raw chromatograms need to be combined and the sampling fractions in both dimensions need to be considered in order to achieve a two-dimensional chromatogram. This was done by hand in a graphic software programm OriginPro 8 [Originlab, Northampton, MA, USA]. The result is shown in figure 5-15.

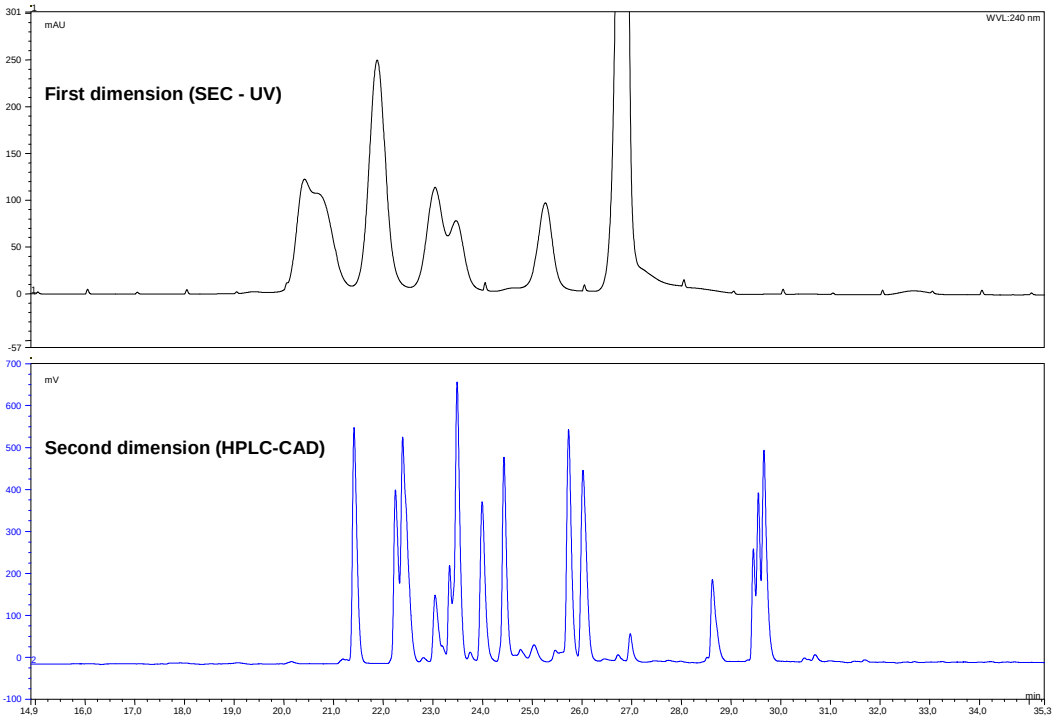


Figure 5-14. SEC-HPLC-CAD two-dimensional chromatograms of 11 additives analyzed by Dichloromethane : n-Heptane (30 : 70) isocratic elutions.

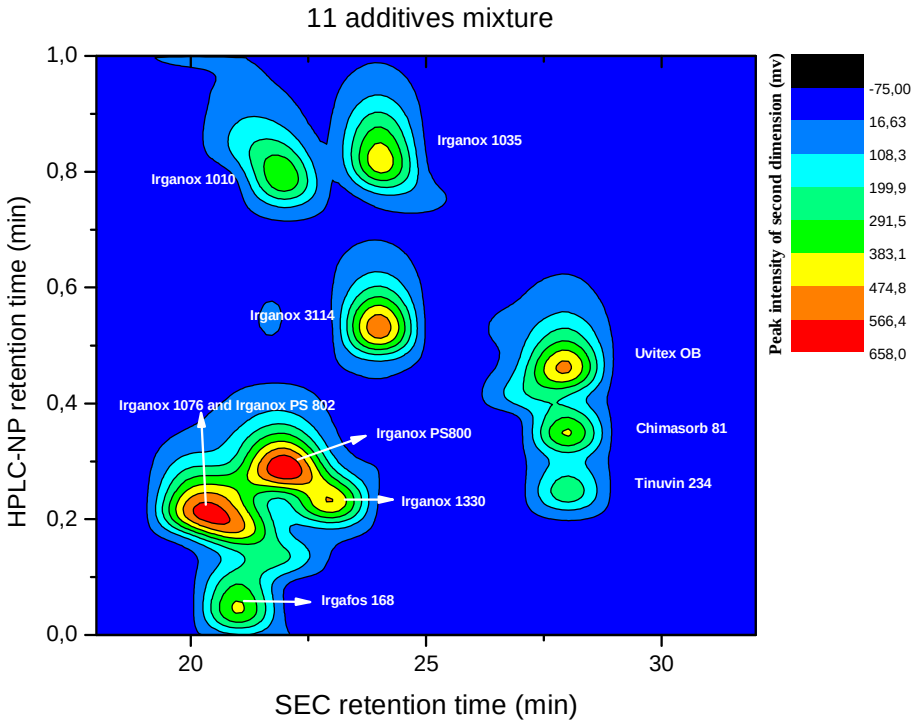


Figure 5-15. The two-dimensional SEC-HPLC contour plot of 11 additives.

5.4. Development of a screening method by GC-FID

5.4.1. Pre-selection of GC columns

Four different columns of different polarity were tested to select the best suitable column(s) for the development of a semi-quantitative screening method. 26 substances from table 4-2 in chapter 4.1.2 were analyzed by the GC-FID systems equipped with four different candidate columns (DB-1, DB-FFAP, ZB-624, ZB-Wax). The results are summarized in table 5-3. Figure 5-16 to 5-19 show the chromatograms.

The ZB-624 column is specifically designed for the separation of volatile organic compounds and coated with a low-polar stationary phase. 22 substances were eluted from the ZB-624 column. The main advantage of thick film columns is the strongly increased retention, allowing analysis of volatile compounds at normal capillary GC temperatures [Steenackers and Sandra 1995]. For above mentioned reasons, most volatile substanes among 26 substances showed enough response and good sensitivity on the ZB-624 column. Especially, the hydrocarbons such as styrene, p-xylene and alpha-methylstyrene showed a higher response than other substances, because the FID response is proportional to the number of carbon atoms in hydrocarbon molecules. Calibrations using the internal standard BHA (3-tert.-butyl-4-hydroxy-anisole) showed good linearity in the concentration range from 1 to 50 µg/ml at a correlation coefficient of more than 0.9956. The limits of detection (LOD) values determined according to DIN 32645 ranged from 0.42 to 2.53 µg/ml. Polar substances such as alcohols and caprolactam showed a tailed peak shape and therefore low sensitivity. The plasticizers such as phthalates and adipates which have higher boiling points were not eluted on the ZB-624 column, since the upper temperature limit of the stationary phase is 260 °C.

21 substances were eluted from the DB-1 column. High polar substances like the alcohols did not show enough response or were not detected on the DB-1 column because of the polarity difference between the alcohols and stationary phase of the DB-1 column. Acrylonitrile, 1,3 butadiene, vinyl chloride and vinylidene chloride were not retarded on the DB-1 column because of their extreme high volatility. On the contrary, non or semi-polar substances such as hydrocarbons, acrylates, plasticizers (phthalates and adipate) and the antioxidant BHT were eluted with good response. Calibrations of these substances using the internal standard (BHA) showed good linearity in the concentration range from 1 to 50 µg/ml

at a correlation coefficient of minimum 0.9967. The LOD values ranged from 1.18 to 3.25 µg/ml.

As polar stationary phases, DB-FFAP column and ZB-Wax were tested. Methyl acrylate and ethyl acetate overlapped with the solvent peak. Other substances were detected by using the DB-FFAP and ZB-Wax.

The DB-FFAP column was specially developed to analyze carboxylic acids like fatty acids [Hewlett Packard Application Note 1998]. The DB-FFAP provided the more symmetric peak shapes and enough response from polar to non-polar substances compared to the other columns (Figure 5-16).

ZB-Wax column is specified for separation of polar complex mixtures such as alcohols, phenols and carboxylic acids. The maximum applicable temperature of the DB-FFAP and the ZB-Wax columns is 260 °C. Nevertheless, the non-volatile plasticizers with relatively high boiling point could be detected on both columns.

Response plots of the analyte concentration versus peak area showed good linearity. The correlation coefficients of all detected substances in the calibration range from 1 to 50 µg/ml varied between 0.9920 and 0.9997 for the DB-FFAP column, and between 0.9922 and 0.9998 for the ZB-Wax column respectively. The LOD values for the individual substances ranged from 0.91 to 9.12 µg/ml in the analysis using DB-FFAP, and from 0.71 to 12.8 µg/ml in the analysis using ZB-Wax, respectively.

In conclusion, the high boiling point plasticizers like DIBP, DBP, DEHA and DEHP were not eluted from the ZB-624 column because of its temperature limit. On the contrary, the plasticizers were detected with enough responses on DB-1 and DB-FFAP.

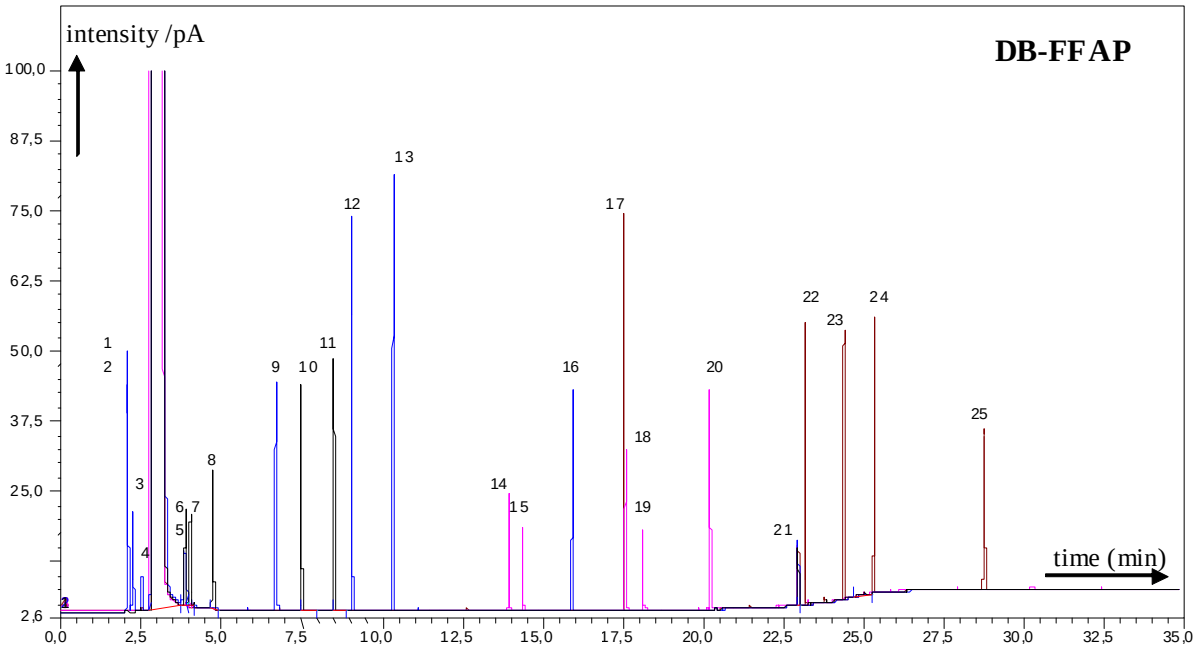
Alcohols, high polar substance groups, showed very poor responses on non- or semi-polar stationary phases (DB-1 and ZB-624). However, the alcohols showed better peak shape on the DB-FFAP and ZB-Wax coated with polar stationary phases and therefore higher sensitivity than on DB-1 and ZB-624 was achieved. In addition, all test substances were detected on DB-FFAP and ZB-Wax.

Based on the above stated results, DB-1, DB-FFAP and ZB-Wax could be used to develop the multi-screening method. However, the ZB-Wax column exibited nearly similar separation properties to DB-FFAP. Therefore, DB-1 and DB-FFAP were finally used and tested further for the development of the multi-screening method by using the representative adhesive related substances.

Table 5-3. Comparision of the relative response factors (RRF), limits of detection (LOD) and correlation coefficients, R values of 26 test substances on 4 columns of different polarity (GC-FID).

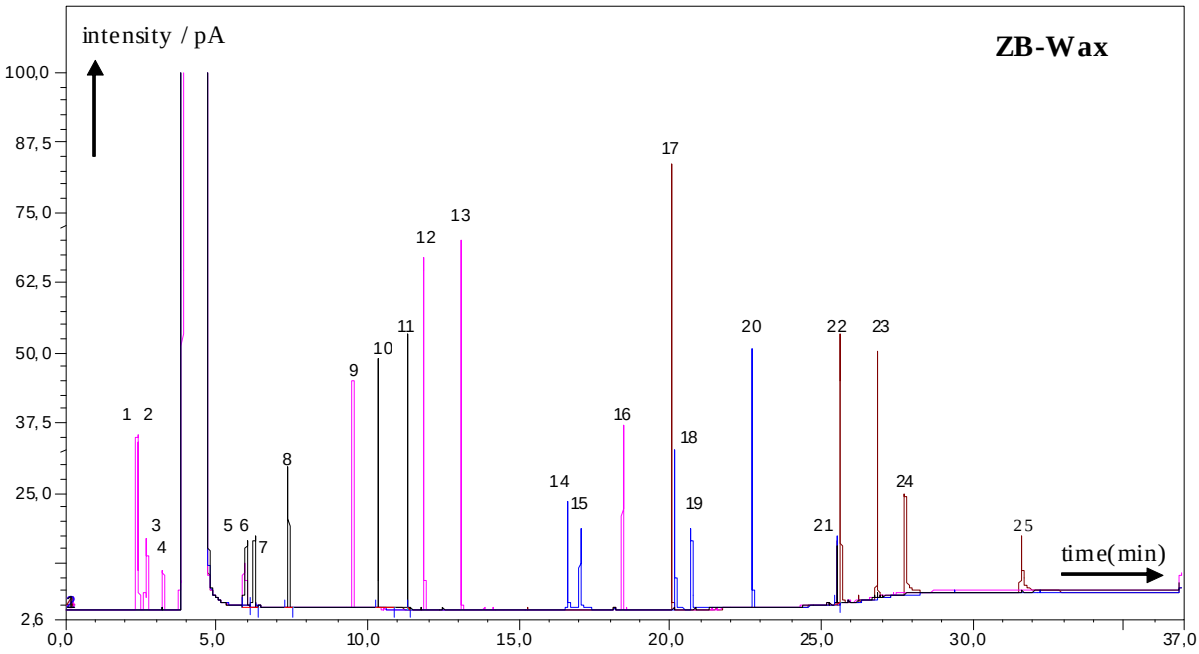
No.	Substances	MW (g/mol)	CAS Nr.	ZB-624			DB-1			DB-FFAP			ZB-Wax		
				RRF	Correlation coefficient R	LOD (µg/ml)	RRF	Correlation coefficient R	LOD (µg/ml)	RRF	Correlation coefficient R	LOD (µg/ml)	RRF	Correlation coefficient R	LOD (µg/ml)
1	Acrylonitrile	53.06	107-13-1	1.36	0.9996	1.31	ND	ND	ND	0.93	0.9965	3.05	1.25	0.9971	2.74
2	1,3 Butadiene	54.09	106-99-0	1.00	0.9994	1.88	ND	ND	ND	0.85	0.9920	5.07	0.80	0.9932	4.97
3	Ethylene glycol	62.06	107-21-1	0.19 [1)]	-	-	0.22 [1)]	-	-	0.34	0.9957	4.64	0.37	0.9982	3.02
4	Vinyl chloride	62.49	75-01-4	0.48	0.9997	1.05	ND	-	-	0.49	0.9960	9.12	0.32	0.9922	12.76
5	Propylene glycol	76.11	57-55-6	0.29 [1)]	-	-	0.37 [1)]	-	-	0.52	0.9941	4.61	0.50	0.9978	2.81
6	Vinyl acetate	86.09	108-05-4	1.13	0.9997	1.17	0.16 [2)]	-	-	0.32	0.9972	3.09	0.38	0.9935	4.74
7	Methyl acrylate	86.09	96-33-3	0.69	0.9993	2.53	0.72	0.9995	1.27	ND	ND	ND	ND	ND	ND
8	Ethyl acetate	88.11	141-78-6	0.96	0.9999	0.60	0.74	0.9967	3.25	ND	ND	ND	0.29	0.9958	3.64
9	1,4 Butanediol	88.53	110-63-4	0.48 [2)]	-	-	0.39 [1)]	-	-	0.62	0.9941	5.24	0.64	0.9952	4.73
10	Vinylidene chloride	96.94	75-35-4	0.41	1.0000	0.42	ND	ND	ND	0.44	0.9985	2.90	0.45	0.9957	5.01
11	Ethyl acrylate	100.11	140-88-5	0.93	0.9999	0.56	0.78	0.9996	1.18	0.86	0.9988	1.99	0.75	0.9998	0.86
12	Methyl methacrylate	100.11	80-62-6	1.03	0.9998	0.87	0.86	0.9996	1.19	0.89	0.9988	2.06	0.98	0.9993	1.58
13	Styrene	104.15	100-42-5	1.98	0.9998	0.97	1.91	0.9993	1.54	1.87	0.9992	1.64	1.76	0.9989	1.86
14	Diethylene glycol	106.12	111-46-6	0.22 [1)]	-	-	ND	ND	ND	0.35	0.9947	4.33	0.35	0.9946	4.39
15	m-Xylene	106.17	108-38-3	1.73	0.9999	0.68	1.88	0.9991	1.61	1.75	0.9968	3.06	1.65	0.9958	3.54
16	ε -Caprolactam	113.16	105-60-2	0.72 [2)]	-	-	0.72 [2)]	-	-	0.91	0.9926	5.89	0.91	0.9927	5.88
17	Ethyl methacrylate	114.14	97-63-2	1.07	0.9999	0.65	0.91	0.9996	1.19	0.90	0.9996	1.14	0.88	0.9997	0.99
18	N-Vinyl-2-Pyrrolidinone	114.14	88-12-0	0.60	0.9999	0.75	0.68 [2)]	0.9987	2.24	0.87	0.9969	3.44	0.84	0.9968	3.50
19	alpha -Methylstyrene	118.18	98-83-9	1.97	0.9998	0.81	1.65	0.9994	1.58	1.69	0.9966	3.85	1.53	0.9961	4.15
20	Butyl acrylate	128.18	141-32-2	1.18	0.9999	0.50	1.06	0.9995	1.35	1.10	0.9997	0.91	1.07	0.9998	0.74
21	Butyl methacrylate	142.19	97-88-1	1.19	0.9999	0.78	1.11	0.9995	1.24	1.15	0.9997	0.91	1.22	0.9998	0.71
22	2,6-Di-*tert*-butyl-4-methylphenol (BHT)	220.35	128-37-0	1.72	0.9996	1.54	1.52	0.9994	1.63	1.45	0.9989	2.11	1.46	0.9992	1.80
23	Diisobutyl phthlate (DIBP)	278.35	84-69-5	-	-	-	1.02	0.9990	2.19	0.99	0.9982	2.87	0.99	0.9989	2.22
24	Dibutyl phthalate (DBP)	278.35	84-74-2	-	-	-	0.97	0.9993	1.77	0.95	0.9986	2.58	0.97	0.9993	1.82
25	Diethylhexyl adipate (DEHA)	370.57	103-23-1	-	-	-	0.99	0.9993	1.83	1.05	0.9985	2.58	0.87	0.9979	3.10
26	Diethylhexyl phthalate (DEHP)	390.56	117-81-7	-	-	-	1.15	0.9992	1.88	1.17	0.9986	2.55	0.63	0.9981	2.96

ND : not detected or overlap with solvent (MeOH) peak
[1)] : not detected less than 25 ppm
[2)] : not detected less than 5 ~ 10 ppm



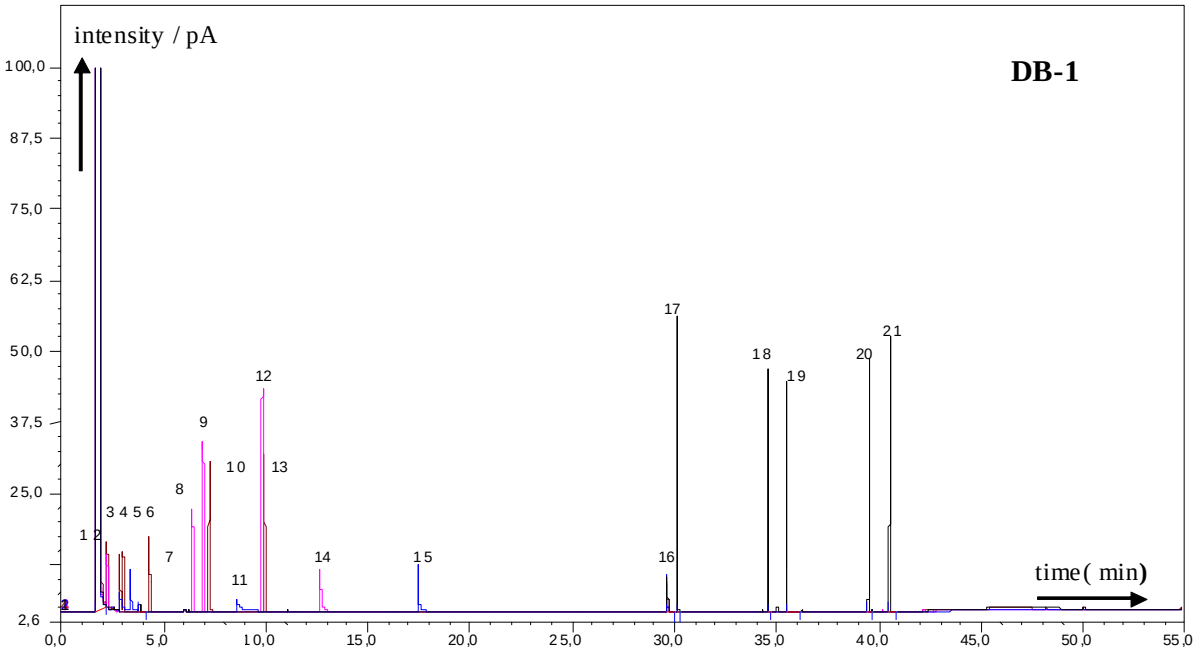
1. 1,3 Butadiene 2. Vinyl chloride 3. Vinylidene chloride 4. Vinyl acetate
5. Acrylonitrile 6. Ethyl acrylate 7. Methyl methacrylate 8. Ethyl methacrylate
9. m-Xylene 10. Butyl acrylate 11. Butyl methacrylate 12. Styrene
13. α-Methylstyrene 14. Propylene glycol 15. Ethylene glycol 16. N-vinyl-2-pyrrolidone
17. BHT 18. 1,4-Butanediol 19. Diethylene glycol 20. Caprolactam
21. BHA : IS 22. DIBP 23. DBP 24. DEHA 25. DEHP

Figure 5-16. Comparison of chromatograms that were analysed by GC-FID equipped with DB-FFAP column after injection of 1.0 µl of a standard solution containing 50 µg/ml.



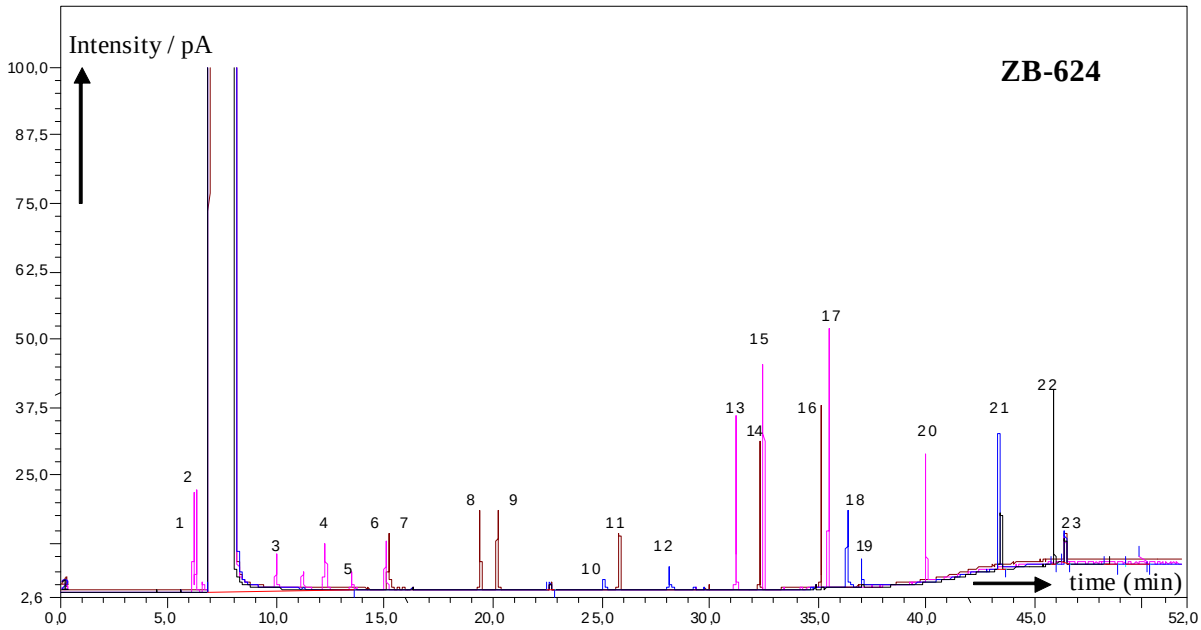
1. Vinyl chloride 2. 1,3 Butadiene 3. Vinylidene chloride 4. Vinyl acetate
5. Acrylonitrile 6. Ethyl acrylate 7. Methyl methacrylate 8. Ethyl methacrylate
9. m-Xylene 10. Butyl acrylate 11. Butyl methacrylate 12. Styrene
13. α-Methylstyrene 14. Propylene glycol 15. Ethylene glycol 16. N-vinyl-2-pyrrolidone
17. BHT 18. 1,4-Butanediol 19. Diethylene glycol 20. Caprolactam
21. BHA : IS 22. DIBP 23. DBP 24. DEHA 25. DEHP

Figure 5-17. Comparison of chromatograms that were analysed by GC-FID equipped with ZB-Wax column after injection of 1.0 µl of a standard solution containing 50 µg/ml.



1. Vinyl acetate
2. Ethyl acetate
3. Ethyl acrylate
4. Ethylene glycol
5. Methyl methacrylate
6. Propylene glycol
7. Ethyl methacrylate
8. m-Xylene
9. Styrene
10. Butyl acrylate
11. 1,4-Butanediol
12. α-Methylstyrene
13. Butyl methacrylate
14. N-vinyl-2-pyrrolidone
15. Caprolactam
16. BHA : IS
17. BHT
18. DIBP
19. DBP
20. DEHA
21. DEHP

Figure 5-18. Comparison of chromatograms that were analysed by GC-FID equipped with DB-1 column after injection of 1.0 µl of a standard solution containing 50 µg/ml.



1. Vinyl chloride
2. 1,3 Butadiene
3. Vinylidene chloride
4. Acrylonitrile
5. Vinyl acetate
6. Ethyl acetate
7. Methyl acrylate
8. Ethyl acrylate
9. Methyl methacrylate
10. Ethylene glycol
11. Ethyl methacrylate
12. Propylene glycol
13. m-Xylene
14. Butyl acrylate
15. Styrene
16. Butyl methacrylate
17. α-Methylstyrene
18. 1,4-Butanediol
19. Diethylene glycol
20. N-vinyl-2-pyrrolidone
21. Caprolactam
22. BHT
23. BHA : IS

Figure 5-19. Comparison of chromatograms that were analysed by GC-FID equipped with ZB-624 column after injection of 1.0 µl of a standard solution containing 50 µg/ml.

5.4.2. GC-FID separation on non-polar DB-1 column

In order to develop the multi-screening methods for semi-quantification of volatile to semi-volatile unknown substances, 55 of representative universal standards were analyzed by GC-FID equipped with a DB-1 column. The linearity of the calibration curves and the limit of detection (LOD) according to the German standard DIN 32645 [DIN 32645 1994] were studied.

The results are summarized in table 5-4 and figure 5-20 shows the chromatogram. The LOD values did not show marked difference between the methods using 3-tert.-butyl-4-hydroxy-anisole (BHA) and 2-(2H-benzotriazol-2-yl)-4,6-bis(1-methyl-1 phenylethyl) -phenol (Tinuvin 234) as the internal standards. The LOD values of all detected substances ranged from approximately 0.2 to 2.6 µg/ml using BHA and from 0.4 to 2.7 µg/ml using Tinuvin 234. Linear calibration curves were determined by linear regression analysis using the peak area ratios between the detected substances and internal standards and a seven point calibration in the concentration range of 0.1 ~ 50 µg/ml. Calibration using BHA and Tinuvin 234 showed good linearity in the range from 0.1 to 50 µg/ml at correlation coefficients of minimum 0.9967 and 0.9959 respectively. All calibration curves using BHA are presented in chapter 9.1.2.

Most substances in the groups of acrylates, plasticizers, antioxidants and other substances were detected in the whole calibration range. Irganox 1010 among the antioxidants (Group F) was not detectable even at the highest concentration of about 1000 µg/ml because of its low volatility and the temperature limit of the DB-1 column. The phenol type (BHT, Irganox 1076 and Irganox 1330) and phosphite type (Irgafos 168) antioxidants were detected with enough response. The peaks of docusate sodium and carprolactam in Group G were not detected under the calibration standard concentration of 5.0 and 1.0 µg/ml, respectively.

The substances containing a polar functional group in molecular structure such as carboxyl, alcohol and amine were not detected with enough response or did not show response and symmetric peak shape on the DB-1 column (Figure 5-20 and 5-21).

Alcohols except resorcinol (1,3-Benzenediol) were not detected at calibration standard concentrations lower than 5.0 µg/ml. Bronopol (2-Bromo-2-nitropropane-1,3-diol) containing alcohol functional groups, a halogen element (Br) and a heteroatom N showed a bad response by FID regardless of the types of separation columns. Figure 5-22 shows the chromatograms of a bronopol standard that were analyzed with two different columns (DB-1 and DB-FFAP). Several unknown peaks were found only in the chromatogram of DB-1 column. Carboxylic

acids are not volatile enough and were not detectable by GC system [Waksmundzka-Hajnos 1998].

The amines showed a better sensitivity on DB-1 than alcohols. However, amines are likely to be adsorbed and decomposed on GC column and the adsorption and decomposition of amines on GC column gives rise to tailing peaks, ghosting phenomena and poor sensitivity [Terashi et al. 1990, Ábalos et al. 2001, Pfundstein et al. 1991]. As shown in figure 5-20, melamine in Group E was not detected at the concentration of 1000 µg/ml. Isophoron diamine and 4,4-methylenedianiline were eluted as two peaks respectively. However, the decomposition peak of 4,4-methylenedianiline was not found for calibration concentrations below 50 mg/l. Isophoron diamine is usually produced as the mixture of cis and trans isomers (cis/trans ca. 3:1) [Berkessel et al. 2006]. Two peaks of *cis-trans* isomers were found in GC-MS chromatogram and the peak areas of two isomers were combined for the quantification [Dalene et al. 1994]. In this study, two isomeric peaks of isophoron diamine were combined to determine the relative response factors (RRF).

Table 5-4. LOD and correlation coefficient R values of representative universal standards analyzed with GC-FID equipped with DB-1 column.

Classi-fication	No.	Substances	MW	Retention time (min.)	Correlation coefficient R		LOD (μg/ml)		Remark
					via BHA	via Tinuvin 234	via BHA	via Tinuvin 234	
Group A Acrylate	1	Methyl acrylate	86.09	1.559	0.9999	0.9999	0.51	0.49	ND < 0.5 ppm
	2	Ethyl acrylate	100.11	2.068	0.9999	0.9999	0.44	0.42	
	3	Methyl methacrylate	100.11	2.217	0.9998	0.9987	0.69	0.71	
	4	Ethyl methacrylate	114.14	3.317	0.9999	0.9998	0.63	0.49	ND < 0.5 ppm
	5	Butyl acrylate	128.18	6.308	0.9998	0.9997	0.60	0.63	
	6	Butyl methacrylate	142.19	9.093	0.9996	0.9996	0.82	0.72	
	7	Ethylhexyl acrylate	184.28	16.677	0.9997	0.9997	0.68	0.60	
Group B Plasticizers	8	Di-*iso*-butyl phthalate	278.35	30.849	0.9999	0.9997	0.41	0.83	
	9	Dibutyl phthalate	278.35	32.611	0.9999	0.9997	0.42	0.77	
	10	Diethylhexyl phthalate	390.56	42.567	0.9998	0.9998	0.76	0.76	
	11	Diethylhexyl adipate	370.57	40.577	0.9977	0.9975	2.06	2.15	
	12	Triacetin	218.20	19.325	1.0000	0.9996	0.27	0.78	
	13	Phosflex 362	362.44	40.530	0.9967	0.9968	2.51	2.46	
	14	Diethylene glycol dibenzoate	314.34	18.208	0.9999	0.9998	0.53	0.60	
	15	Triethylene glycol dibenzoate	358.40	44.904	0.9997	0.9993	0.84	1.28	
	16	Dipropylene glycol dibenzoate	342.42	41.040	0.9998	0.9998	0.72	0.75	ND < 0.5 ppm
	17	Propylene glycol, dibenzoate	284.3	35.829	0.9999	0.9998	0.48	0.63	
	18	2,2,4-Trimethyl-1,3-pentanediol dibenzoate	354.45	41.485	0.9997	0.9993	0.74	1.12	
Group C Carboxylic acid	19	Acrylic acid	72.06						ND in Calibration range
	20	Fumaric acid	116.07						
	21	Maleic acid	116.07						
	22	Adipic acid	146.14						
	23	Terephthalic acid	166.13						
	24	Isophthalic acid	166.13						
Group D Alcohol	25	Ethylene glycol	62.06	2.083					ND < 10 ppm
	26	Propylene glycol	76.1	2.512	0.9991	0.9992	2.07	1.97	ND < 5 ppm
	27	1,4-Butanediol	90.12	7.599					ND < 10 ppm
	28	Diethylene glycol	106.12	8.140					ND < 10 ppm
	29	Resorcinol	110.11	17.834	0.9999	0.9997	0.52	0.83	ND < 0.5 ppm
	30	Glycerol	92.09	10.047					ND < 50 ppm
	31	Bronopol	200.01						ND in Calibration range
Group E Amine	32	Hexamethylene diamine	116.21	12.136					ND < 25 ppm
	33	Toluene -2,4 -diamine	122.17	19.546	0.9995	0.9998	1.06	0.71	ND < 0.5 ppm
	34	Melamine	126.12						ND
	35	Isophorone diamine	170.3	19.135/ 18.737					Two peaks ND < 5 ppm
	36	4,4-Methylenedianiline	250.25	34.756/ 33.570					Two peaks ND < 0.5 ppm
Group F Antioxidants	37	2,6-Di-*tert*-butyl-4-methylphenol	220.35	23.666	0.9999	0.9998	0.41	0.61	
	38	Irganox 1076	531	54.016	0.9993	0.9997	1.28	0.91	
	39	Irgafos 168	646.93	54.170	0.9994	0.9996	1.11	0.87	
	40	Irganox 1330	775.21	67.944	0.9992	0.9989	1.40	1.62	
	41	Irganox 1010	1177.7						ND
Group G Others	42	Vinyl propionate	100.12	1.934	0.9996	0.9996	0.88	0.88	
	43	Styrene	104.15	5.985	0.9997	0.9997	0.68	0.74	
	44	*para*-Xylene	107.17	5.496	0.9997	0.9996	0.71	0.79	
	45	Caprolactam	113.16	15.923	0.9998	0.9998	0.65	0.76	ND < 1.0 ppm
	46	N-Vinyl-2-pyrrolidinone	114.14	11.848	0.9997	0.9996	0.84	1.00	ND < 0.5 ppm
	47	Alpha-Methylstyrene	118.18	8.958	0.9991	0.9990	1.28	1.34	
	48	Benzophenone	182.23	25.605	0.9999	0.9997	0.48	0.81	
	49	Butyl diglycol acetate	204.27	19.947	1.0000	0.9997	0.22	0.71	
	50	2-Octyl-2H-isothiasol-3-one	213.34	29.992	0.9999	0.9998	0.33	0.62	
	51	Bisphenol A	228.29	35.983	0.9975	0.9979	2.33	2.16	
	52	4,4'-Bis(diethylamino) benzophenone	324.46	49.609	0.9996	0.9999	0.87	0.50	
	53	BADGE	340.42	46.749	0.9990	0.9980	1.28	1.83	
	54	Uvitex OB	430.06	58.034	0.9998	0.9999	0.72	0.57	
	55	Docusate sodium	445.63	37.980	0.9983	0.9987	2.66	2.35	ND < 5 ppm

ND < xx ppm : not detected below the concentration of xx ppm
ND : not detected

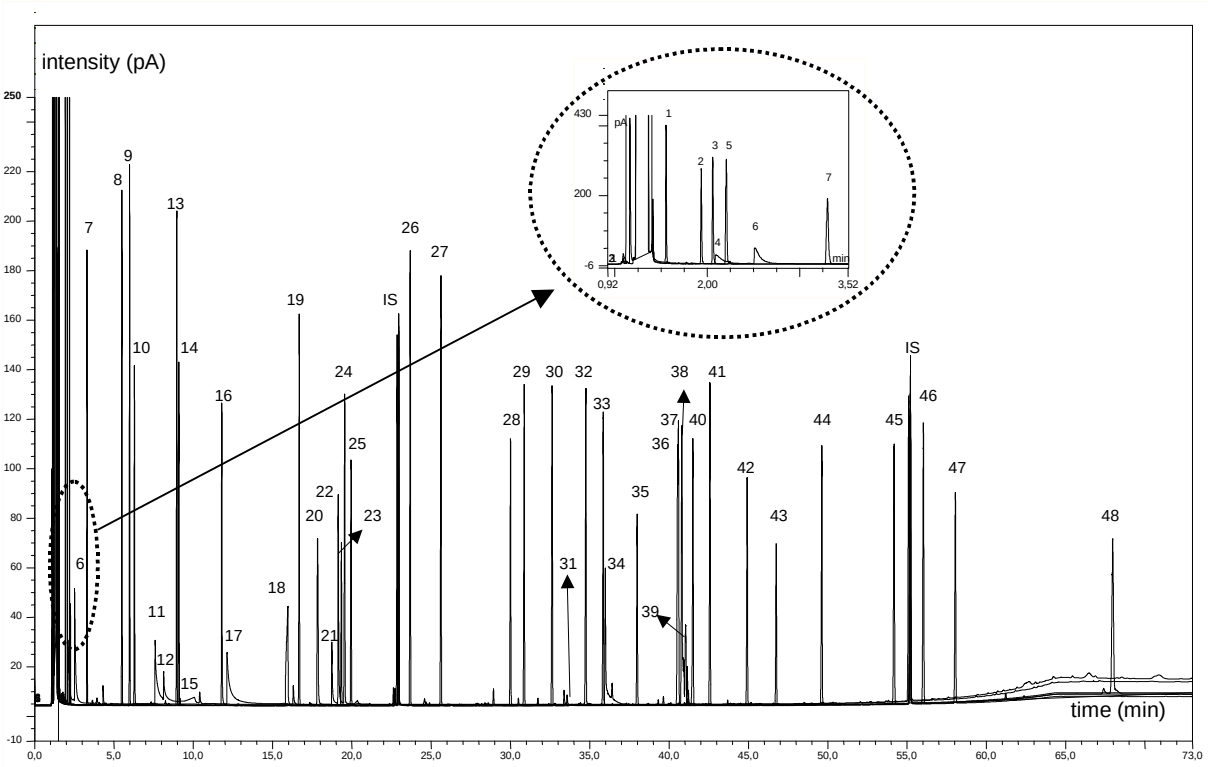


Figure 5-20. Representative chromatograms of the adhesive related substances detected by using GC-FID equipped with DB-1 separation column after injection of 5.0 µl of a standard solution containing 50 µg/ml.

1, Methyl acrylate	2, Vinyl propionate	3, Ethyl acrylate	4, Etylene glycol	5, Methyl methacrylate	6, Propylene glycol
7, Ethyl methacrylate	8, p-Xylene	9, Styrene	10, Butyl acrylate	11, 1,4-butanediol	12, Diethylene glycol
13, Alpha-methylstyrene	14, Butyl methacrylate	15, Glycerol	16, N-vinyl-2-pyrrolidone	17, Hexamethylene diamine	18, Carprolactam
19, Ethylhexyl acrylate	20, 1,3-Benzenediol (Resorcinol)	21, Isophoron diamine (*trans*)_2nd peak	22, Isophoron diamine (*cis*)_main peak	23, Glycerol triacetate	24, Toluene 2,4-diamine
25, Butyl diglycol acetate	26, 2,6-di-tert-butyl-4-methylphenol	27, Benzophenone	28, 2-octyl-2H-isothiasol-3-one	29, Diisobutyl phthalate	30, Dibutyl phthalate
31, 4,4-methyllenedianiline_2nd peak	32, 4,4-methyllenedianiline_main peak	33, Propylene glycol dibenzoate (Bezoflex 284)	34, Bisphenol A	35, Docusate sodium	36, 2-ethylhexyl diphenyl phosphate
37, Diethylhexyl adipate	38, Diethylene glycol dibenzoate	39, Dipropylene glycol dibenzoate	40, 2,2,4-Trimethyl-1,3-pentanediol dibenzoate (Benzoflex 354)	41, Diethylhexyl phthalate	42, Triethylene glycol dibenzoate
43, BADGE	44, 4,4-bis(4-glycidyloxyphenyl)propane	45, Irgafos 168	46, Irganox 1076	47, Benzoxazole, 2,2'-(2,5-thiophenediyl) *bis*(5-(1,1-dimethylethyl)	48, Irganox 1330

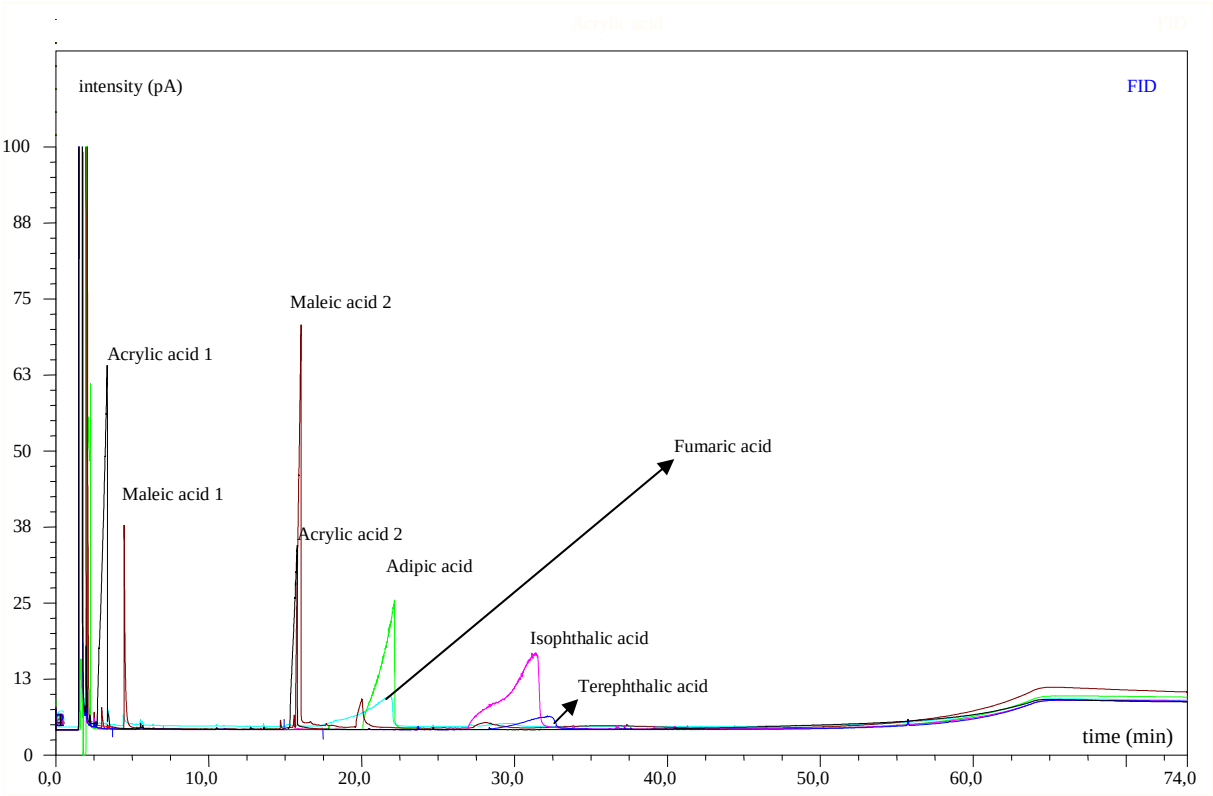


Figure 5-21. Chromatogram of group C in the RARS analyzed GC-FID equipped with DB-1 column at the concentration 1000 µg/ml.

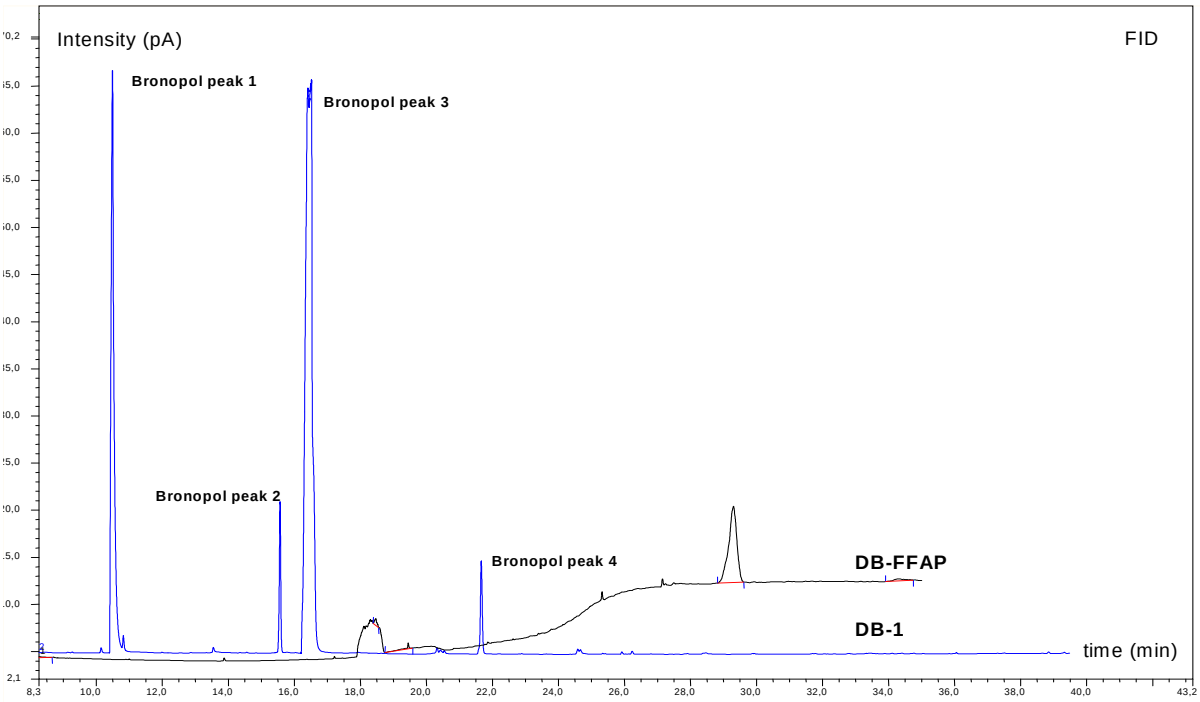


Figure 5-22. Chromatograms of Bronopol analyzed GC-FID equipped with DB-1 and DB-FFAP column at the concentration 1000 µg/ml.

5.4.3. GC-FID separation on polar DB-FFAP column

For the simultaneous analysis including the high polar substances, the DB-FFAP column coated with ‘nitroterephthalic acid modified polyethylene glycol’ was tested. The obtained data are shown in table 5-5.

On the DB-FFAP, the alcohols showed a symetric peak shape and the sensitivity was improved related to the DB-1 column. The peaks of the alcohols except bronopol and glycerol were detected up to a calibration standard concentration of 0.1 µg/ml (propylene glycol, 1,4 butanediol and diethylene glycol) and 0.5 µg/ml (ethylene glycol and resorcinol) respectively. Alcohols on the DB-FFAP column showed also good linearity with a correlation coefficient R values of 0.9985 ~ 0.9995 and the LOD values were between 1.22 and 1.89 µg/ml. Amines were not retained on the FFAP phase (Table 5-5 and Figure 5-23).

The carboxylic acids listed in table 5-5 also could not be detected with enough response or did not show any responses on FFAP phase. In the analysis of the carboxylic acids, the non-volatile acids with low vapor-pressure such as terephthalic acid, isophthalic acid, fumaric acid and maleic acid were not detected at the concentration of 1000 µg/ml. Acrylic acid and Adipic acid could be analyzed at concentrations higher than 50 µg/ml, but was not detectable in the calibration range of 0.1 to 50 µg/ml.

Some plasticizers which are important for the manufacture of adhesives such as diethylene glycol dibenzoate and triethylene glycol dibenzoate were not detected by the method on the FFAP column. The antioxidants which have been detected by the method on DB-1 could not be analyzed by the FFAP column.

Table 5-5. Relative response factor (RRF), LOD and correlation coefficient R values of all of the RARS analyzed with GC-FID equipped with DB-FFAP column.

Classification	No.	Substances	MW	Correlation coefficient R	LOD (µg/ml)	Remark
Group A Acrylate	1	Methyl acrylate	86.09			overlap with solvent peak
	2	Ethyl acrylate	100.11	0.9981	1.96	
	3	Methyl methacrylate	100.11	0.9913	4.04	
	4	Ethyl methacrylate	114.14	0.9992	1.19	
	5	Butyl acrylate	128.18	0.9990	1.31	
	6	Butyl methacrylate	142.19	0.9991	1.21	
	7	Ethylhexyl acrylate	184.28	0.9996	0.79	
Group B Plasticizers	8	Di-*iso*-butyl phthalate	278.35	0.9992	1.37	
	9	Dibutyl phthalate	278.35	0.9992	1.40	
	10	Diethylhexyl phthalate	390.56	0.9986	1.84	
	11	Diethylhexyl adipate	370.57	0.9990	1.40	
	12	Triacetin	218.20	0.9989	1.51	
	13	Phosflex 362	362.44	0.9994	1.10	
	14	Diethylene glycol dibenzoate	314.34	-	-	
	15	Triethylene glycol dibenzoate	358.40	-	-	
	16	Dipropylene glycol dibenzoate	342.42	0.9976	2.03	
	17	Propylene glycol, dibenzoate	284.3	0.9988	1.53	
	18	2,2,4-Trimethyl-1,3-pentanediol dibenzoate	354.45	0.9987	1.59	
Group C Carboxylic acid	19	Acrylic acid	72.06	-	-	Two peaks
	20	Fumaric acid	116.07	-	-	
	21	Maleic acid	116.07	-	-	
	22	Adipic acid	146.14	-	-	ND < 50 ppm
	23	Terephthalic acid	166.13	-	-	
	24	Isophthalic acid	166.13	-	-	
Group D Alcohol	25	Ethylene glycol	62.06	0.9995	1.33	
	26	Propylene glycol	76.1	0.9993	1.52	
	27	1,4-Butanediol	90.12	0.9991	1.52	
	28	Diethylene glycol	106.12	0.9992	1.22	
	29	Resorcinol	110.11	0.9985	1.89	
	30	Glycerol	92.09	-	-	ND < 5 ppm
	31	Bronopol	200.01	-	-	
Group E Amine	32	Hexamethylene diamine	116.21	-	-	
	33	Toluene -2,4 -diamine	122.17	-	-	
	34	Melamine	126.12	-	-	Not detected in calibration range
	35	Isophorone diamine	170.3	-	-	
	36	4,4-Methylenedianiline	250.25	-	-	
Group F Antioxidants	37	2,6-Di-*tert*-butyl-4-methylphenol	220.35	0.9999	0.49	
	38	Irganox 1076	531	-	-	
	39	Irgafos 168	646.93	-	-	
	40	Irganox 1330	775.21	-	-	
	41	Irganox 1010	1177.7	-	-	
Group G Others	42	Vinyl propionate	100.12	0.9994	1.00	
	43	Styrene	104.15	0.9999	0.40	
	44	*para*-Xylene	107.17	0.9999	0.40	
	45	Caprolactam	113.16	0.9999	0.33	
	46	N-Vinyl-2-pyrrolidinone	114.14	0.9998	0.67	
	47	Alpha-Methylstyrene	118.18	0.9994	1.01	
	48	Benzophenone	182.23	0.9999	0.52	
	49	Butyl diglycol acetate	204.27	0.9999	0.34	
	50	2-Octyl-2H-isothiasol-3-one	213.34	0.9991	1.25	
	51	Bisphenol A	228.29	-	-	
	52	4,4'-Bis(diethylamino) benzophenone	324.46	-	-	
	53	BADGE	340.42	-	-	
	54	Uvitex OB	430.06	-	-	
	55	Docusate sodium	445.63	0.9992	1.57	

ND < xx ppm : not detected below the concentration of xx ppm
ND : not detected

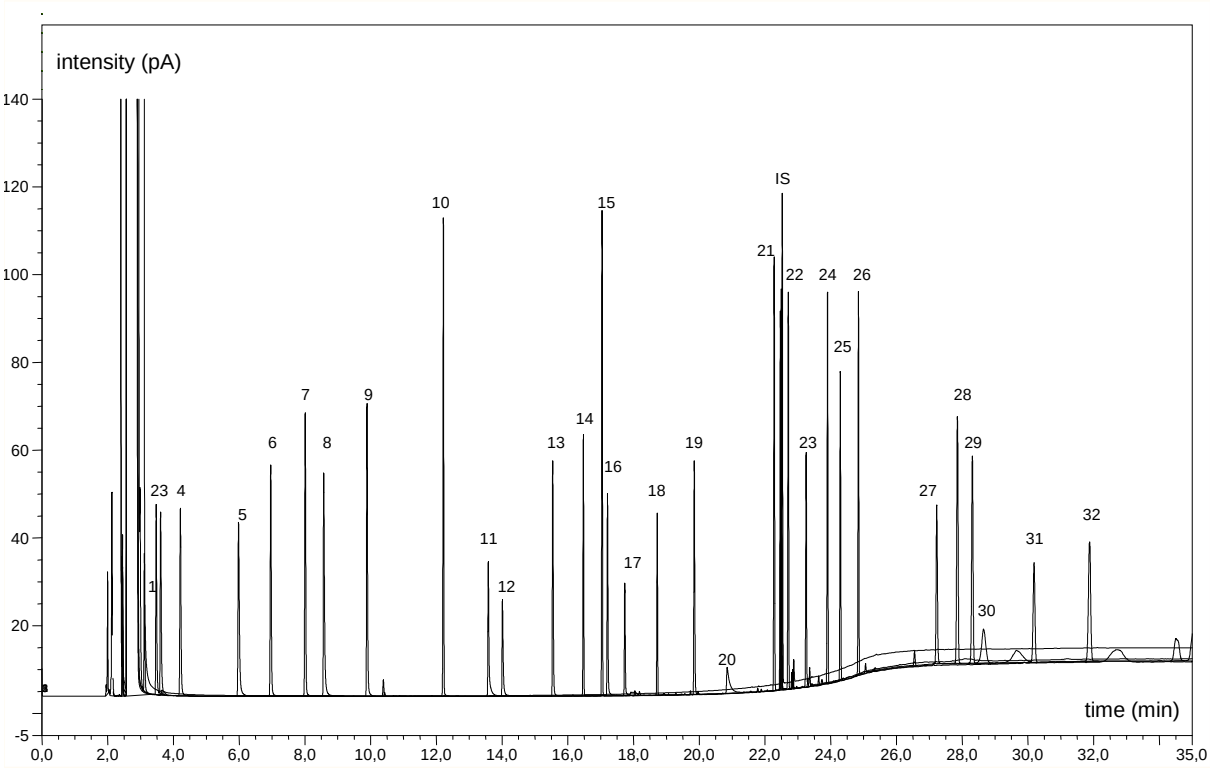


Figure 5-23. Representative chromatograms of the adhesive related substances detected using GC-FID equipped with DB-FFAP separation column after injection of 2.0 µl of a standard solution containing 50 µg/ml.

1, Vinyl propionate	2, Ethyl acrylate	3, Methyl methacrylate	4, Ethyl methacrylate	5, p-Xylene	6, Butyl acrylate
7, Butyl methacrylate	8, Styrene	9, Alpha-methylstyrene	10, Ethylhexyl acrylate	11, Propylene glycol	12, Ethylene glycol
13, N-vinyl-2-pyrrolidone	14, Butyl diglycol acetate	15, 2,6-di-tert-butyl-4-methylphenol	16, 1,4-butanediol	17, Diethylene glycol	18, Glycerol triacetate
19, Carprolactam	20, Glycerol	21, Benzophenone	22, Diisobutyl phthalate	23, Docusate sodium	24, Dibutyl phthalate
25, 2-octyl-2H-isothiasol-3-one	26, Diethylhexyl adipate	27, Resorcinol	28, Diethylhexyl phthalate	29, Bezoflex 284	30, Dipropylene glycol dibenzoate
31, 2-ethylhexyl diphenyl phosphate	32, Benzoflex 354				

5.4.4. GC-FID relative response factors

In order to validate the semi-quantitative approach by using an universal internal standard, the relative response factors of the 55 adhesive related substances were determined by calibrating in seven different concentrations from 0.1 to 50 µg/ml. BHA (3-tert.-butyl-4-hydroxy-anisole) and Tinuvin 234 (2-(2H-benzotriazol-2-yl)-4,6-bis(1-methyl-1 phenylethyl)-phenol) were used as the universal internal standards. The test substances were analysed by GC-FID equipped with a DB-1 column.

Table 5-6 gives the RRF values of the 55 representative adhesvie related substances compared to BHA and Tinuvin 234. The ideal range of the RRF values related to an internal standard is usually recognized from 0.8 to 1.2 [Kazakevich and LoBrutto 2007, Burgard and Kuznicki 1990]. Among the 55 substances, the RRF values of 25 substances via BHA and 13 substances via Tinuvin 234 were included within this range, respectively. All RRF values via Tinuvin 234 are markedly lower than those via BHA, since Tinuvin 234 was detected with higher response than BHA using the flame ionization detector (FID). This means that if Tinuvin 234 will be used for the semi-quantitative approach without correction factor, substances are more underestimated than if BHA is used as a internal standard. In other words, BHA as internal calibration standard permits a more conservative estimation than the use of Tinuvin 234. Blanco et al. [1992] reported that the RRF values vary related to the chosen internal standards.

The RRF values via BHA and Tinuvin 234 of acrylate compounds (Group A) ranged from 0.71 to 1.25 and increased with increasing the molecular weight. The responses of triacetin and dipropylene glycol dibenzoate in plasticizers (Group B) showed lower response versus BHA (0.46 and 0.51). The RRF values of other plasticizers were higher (0.75 ~ 1.25). Especially, the phthalate and adipate type plasticizers (0.99 ~ 1.17) showed a good response related to BHA on the DB-1 column. Except for dipropylene glycol dibenzoate (RRF = 0.51), the RRF values for benzoate type plasticizers were 0.75 ~ 1.12.

The phenol type (BHT, Irganox 1076 and Irganox 1330) and phosphite type (Irgafos 168) antioxidants were detected with sufficient response. The RRF values of these substances varied between 0.89 and 1.34.

As expected, the hydrocarbons (styrene, p-xylene and alpha-methylstyrene) and ketones [benzophenone and 4,4'-Bis(diethylamino) benzophenone] showed good response on the DB-1 column and their RRF values via BHA ranged from 0.93 to 1.36.

The RRF values of vinyl propionate, BADGE, docusate sodium, carprolactam, 1-vinyl-2-pyrrolidone, butyl diglycol acetate and Uvitex OB varied between 0.43 and 0.94. Especially, vinyl propionate, BADGE, docusate sodium did not show high responses and the RRF values varied 0.43 ~ 0.52. Except resorcinol, the alcohols in Group D showed the tailed peak shapes and therefore lower response or did not show any response in calibration range (bronopol). The RRF values of alcohols ranged from 0.14 to 0.45.

Since the substances containing carboxylic functional groups were not detected in the calibration range, the RRF values could not be calculated.

Table 5-6. Relative response factors obtained from GC-FID equipped with DB-1 column.

Classification	No.	Substances	Formula	MW (g/mol)	RRF	
					BHA	Tinuvin 234
Group A Acrylate	1	Methyl acrylate	$C_4H_6O_2$	86.09	0.71	0.59
	2	Ethyl acrylate	$C_5H_8O_2$	100.11	0.64	0.51
	3	Methyl methacrylate	$C_5H_8O_2$	100.11	0.67	0.55
	4	Ethyl methacrylate	$C_6H_{10}O_2$	114.14	0.82	0.66
	5	Butyl acrylate	$C_7H_{12}O_2$	128.18	0.90	0.73
	6	Butyl methacrylate	$C_8H_{14}O_2$	142.19	0.96	0.78
	7	Ethylhexyl acrylate	$C_{11}H_{20}O_2$	184.28	1.25	1.02
Group B Plasticizers	8	Diisobutyl phthalate	$C_{16}H_{22}O_4$	278.35	0.99	0.81
	9	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.35	1.00	0.82
	10	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.56	1.15	0.95
	11	Diethylhexyl adipate	$C_{22}H_{42}O_4$	370.57	1.17	0.96
	12	Glycerol triacetate	$C_9H_{14}O_6$	218.20	0.46	0.38
	13	2-Ethylhexyl diphenyl phophate	$C_{20}H_{27}O_4P$	362.44	0.81	0.67
	14	Diethylene glycol dibenzoate	$C_{18}H_{18}O_5$	314.34	0.87	0.72
	15	Triethylene glycol dibenzoate	$C_{20}H_{22}O_6$	358.40	0.75	0.62
	16	Dipropylene glycol dibenzoate	$C_{20}H_{22}O_5$	342.42	0.51	0.42
	17	Propylene glycol, dibenzoate	$C_{17}H_{16}O_4$	284.3	0.99	0.82
	18	2,2,4-Trimethyl-1,3-pentanediol dibenzoate	$C_{22}H_{26}O_4$	354.45	1.12	0.92
Group D Alcohol	25	Ethylene glycol	$C_2H_6O_2$	62.06	0.20	0.15
	26	Propylene glycol	$C_3H_8O_2$	76.1	0.30	0.22
	27	1,4-Butylene glycol	$C_4H_{10}O_2$	90.12	0.45	0.33
	28	Diethylene glycol	$C_4H_{10}O_3$	106.12	0.15	0.11
	29	Resorcinol	$C_6H_6O_2$	110.11	0.67	0.51
	30	Glycerol	$C_3H_8O_3$	92.09	0.14	0.10
	31	2-Bromo-2-nitropropane-1,3-diol	$C_3H_6BrNO_4$	200.01	ND	ND
Group E Amine	32	Hexamethylenediamine	$C_6H_{16}N_2$	116.21	0.81	0.52
	33	Toluene 2,4-diamine	$C_7H_{10}N_2$	122.17	0.65	0.64
	34	1,3,5-Triazine-2,4,6-triamine	$C_3H_6N_6$	126.12	ND	ND
	35	Isophorone diamine	$C_{10}H_{22}N_2$	170.3	0.54	0.43
	36	4,4'-Methylenedianiline	$C_{13}H_{14}N_2$	250.25	0.94	0.75
Group F Antioxidants	37	2,6-Di-tert-butyl-4-methylphenol	$C_{15}H_{24}O$	220.35	1.34	1.11
	38	Irganox 1076	$C_{35}H_{62}O_3$	531	1.28	1.05
	39	Irgafos 168	$C_{42}H_{63}O_3P$	646.93	1.16	0.60
	40	Irganox 1330	$C_{54}H_{78}O_3$	775.21	0.89	0.74
Group G Others	42	Vinyl propionate	$C_5H_8O_2$	100.12	0.52	0.40
	43	Styrene	C_8H_8	104.15	1.31	0.99
	44	para-Xylene	C_8H_{10}	107.17	1.36	1.03
	45	Caprolactam	$C_6H_{11}NO$	113.16	0.69	0.55
	46	1-Vinyl-2-pyrrolidinone	C_6H_9NO	114.14	0.72	0.56
	47	α-Methylstyrene	C_9H_{10}	118.18	1.35	1.02
	48	Benzophenone	$C_{13}H_{10}O$	182.23	1.25	0.95
	49	Butyl diglycol acetate	$C_{10}H_{20}O_4$	204.27	0.70	0.53
	50	2-Octyl-2H-isothiazol-3-one	$C_{11}H_{19}NOS$	213.34	0.82	0.68
	51	Bisphenol A	$C_{15}H_{16}O_2$	228.29	1.23	0.93
	52	4,4'-Bis(diethylamino) benzophenone	$C_{21}H_{28}N_2O$	324.46	0.93	0.71
	53	BADGE	$C_{21}H_{24}O_4$	340.42	0.43	0.33
	54	Uvitex OB	$C_{26}H_{26}N_2O_2S$	430.06	0.94	0.71
	55	Docusate sodium	$C_{20}H_{37}NaO_7S$	445.63	0.44	0.36
Group C Carboxylic acid	19 ~ 24	All substances were not detected in calibration range				
Mean					**0.83 a** [1)]	**0.65 b**

ND : Not detected, 1) Means with the same letter are significantly different ($p<0.05$).

5.4.5. Estimation of molecular weight by retention time

Figure 5-24 and 5-25 shows the correlation between molecular weight and retention times analyzed by GC-FID equipped with a DB-1 and a DB-FFAP column, respectively.

The correlation between the molecular weight versus the retention times shows a relatively good linearity (R^2 = 0.8451, n=44) on the DB-1 column (Figure 5-24). As expected, the retention time did not correlate with molecular weight on the FFAP (Figure 5-25).

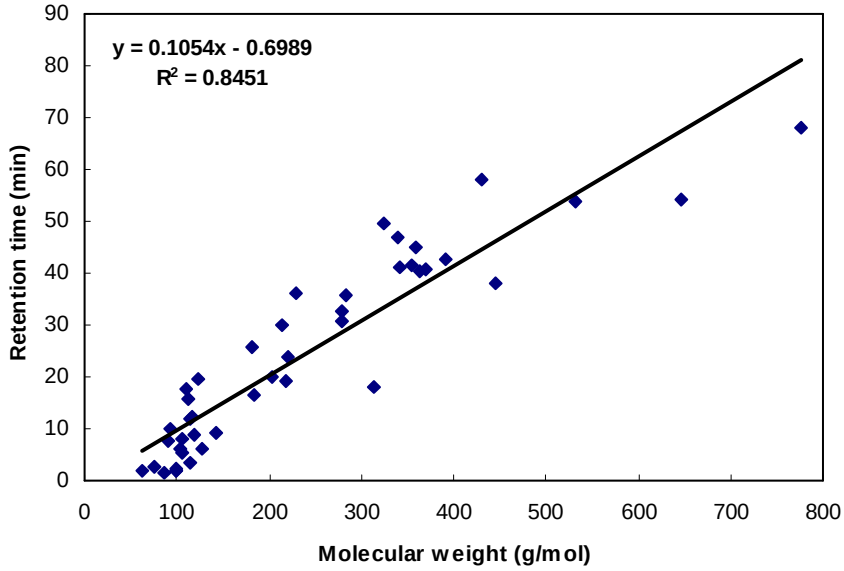


Figure 5-24. Correlation of the retention time with the molecular weight on GC-FID equipped with DB-1 column.

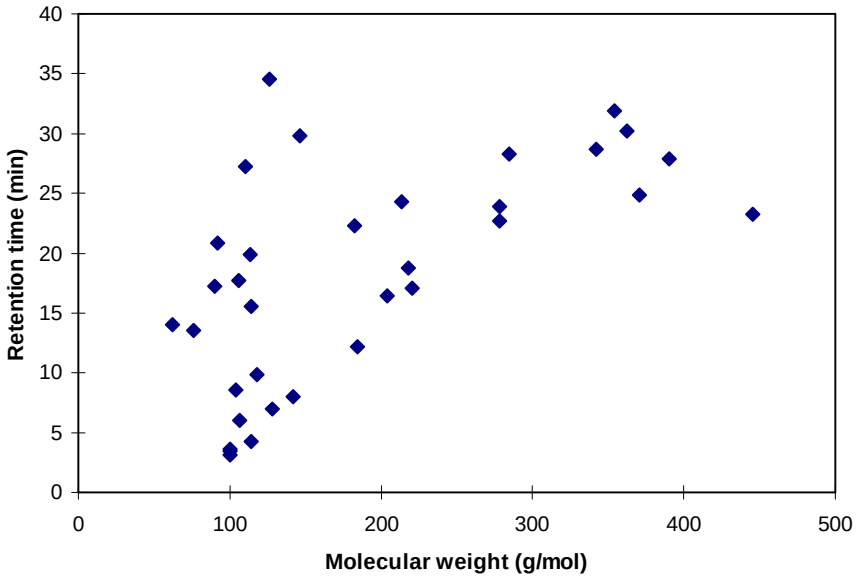


Figure 5-25. Correlation of the retention time with the molecular weight on GC-FID equipped with DB-FFAP column.

5.4.6. Characterisation of unknown substances by linear retention indices

In order to compare the linear retention indices varying depending on the operating conditions like carrier gases (He and H_2), temperature programs and different GC instruments, three different operating conditions (condition A, B and C, see Appendix 9.6.3) were applied for the DB-1 column. The linear retention indices obtained were compared in order to evaluate the influence of GC operational conditions on retention indices variation. In addition, in order to evaluate the retention indices variation according to column stationary phase, the retention indices determined by the condition D using a DB-FFAP column were compared with those determined by the condition A, B and C using DB-1 column.

A mixture of n-alkanes (C8 - C20 and C21 – C40) obtained from a chemical supplier was used as standards to cover the total analysis time. The 55 representative adhesive related substances were injected into the constituted GC-FID systems. Many ghost peaks were eluted from the mixture of C21 – C40 n-alkanes in the elution time for C8 – C20 n-alkane mixture. Therefore, two representative chromatograms (Figure 5-26 A and 5-26 B) of the homologous series of n-alkanes were presented.

The linear retention indices of each substance did not show a marked variation between different instruments (HP Agilent 6890N GC and HP 6890 GC) and operational conditions (condition A ~ C) (Table 5-7). The relative standard deviations (% RSD) of the retention indices calculated from three different analytical conditions for the DB-1 column ranged from 0.0 to 2.4 %. The retention indices of the GC-FID condition C measured by using a different instrument (HP 6890 GC) and carrier gas (H_2) from the condition A and B (see Table 4-7 in chapter 4.4), were not significantly different from the retention indices determined at the condition A and B using HP Agilent 6890N GC system and Helium carrier gas. This indicates that the precision of the retention indices is, in most substances, quite good independently from the GC instruments and analysis conditions.

Figure 5-27 and 5-28 show the correlation between the retention indices and molecular weights or carbon numbers. The correlation between the molecular weight versus the retention indices show a good linearity.

The retention indices on DB-FFAP column are also presented in table 5-7. Figure 5-29 shows a correlation between the molecular weights and retention indices of the substances detected on DB-FFAP column. The stationary phases influence the retention indices [Peng 2000, Goodner 2008, Ruther 2000]. The retention indices on the DB-FFAP column were

different from those on the DB-1 column and did not show a linear relation with molecular weights corresponding to the results shown in chapter 5.4.5.

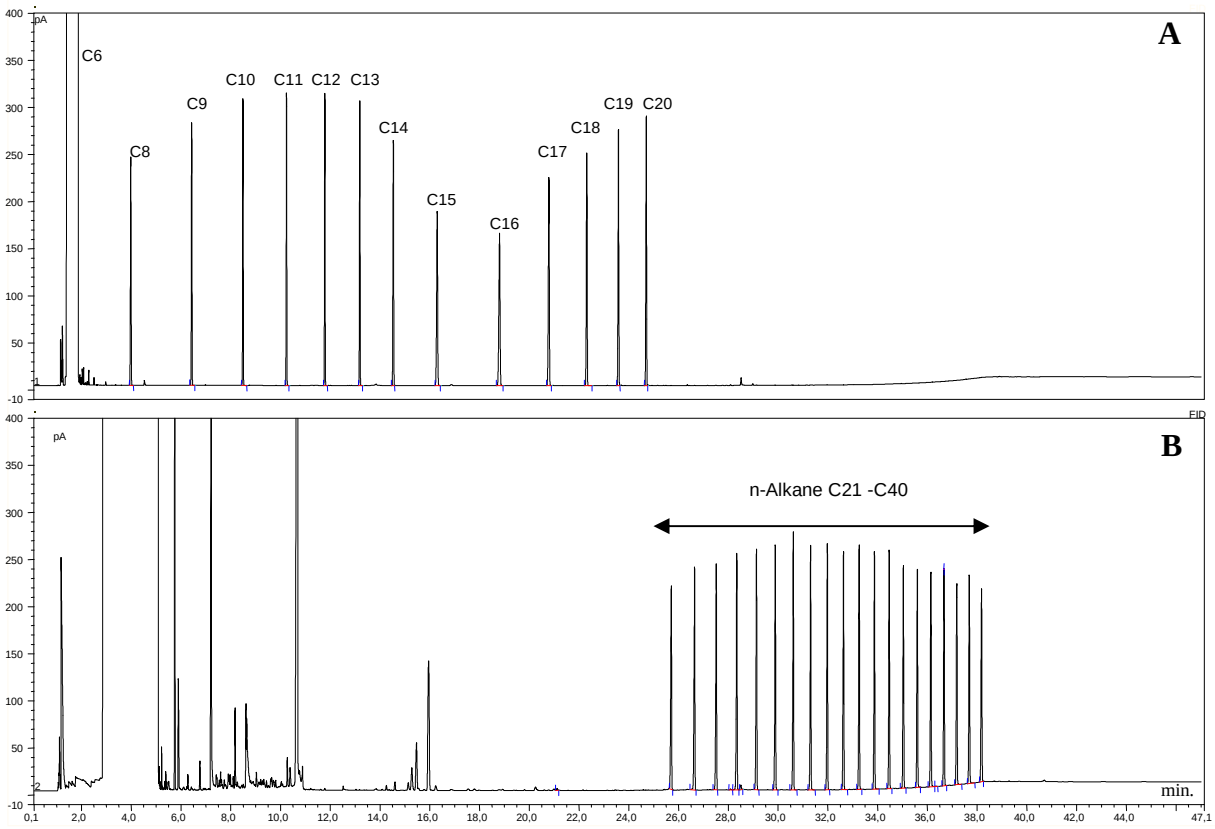


Figure 5-26. GC-FID chromatograms of n-Alkanes (A : C8 – C20, B : C21–C40).

Table 5-7.

1. Comparison of retention indices of representative adhesive related substances on DB-1 with different temperature programms and instruments.

2. Comparison of retention indices of representative adhesive related substances on DB-1 and DB-FFAP.

Classification	No	Substances	Molecular weight (g/mol)	Con. A [1] / DB -1	Con. B [1] / DB -1	Con. C [1] / DB -1	SD [2] Con. 1 ~3	RSD (%) [3] Con. 1 ~3	Con. D [1] / FFAP
Group A acylate	1	Methyl acrylate	86.09	-	-	-	-	-	-
	2	Ethyl acrylate	100.11	620	620	624	2	0.4	998
	3	Methyl methacrylate	100.11	626	626	631	3	0.5	1007
	4	Ethyl methacrylate	114.14	671	671	677	3	0.5	1041
	5	Butyl acrylate	128.18	876	878	876	1	0.1	1177
	6	Butyl methacrylate	142.19	964	965	963	1	0.1	1227
	7	Ethylhexyl acrylate	184.28	1213	1212	1212	1	0.0	1485
Group B Plasticizers	8	Glycerol triacetate	218.2	1311	1307	1305	3	0.2	2084
	9	Diisobutyl phthalate	278.35	1818	1820	1826	4	0.2	2552
	10	Dibutyl phthalate	278.35	1908	1910	1916	4	0.2	2708
	11	Propylene glycol dibenzoate	284.3	2084	2090	2100	8	0.4	3216
	12	Diethylene glycol dibenzoate	314.34	2381	2388	2402	11	0.4	-
	13	Dipropylene glycol dibenzoate	342.42	2397	2406	2416	10	0.4	3242
	14	2,2,4-Trimethyl-1,3-pentanediol dibenzoate	354.45	2427	2439	2450	12	0.5	3441
	15	Triethylene glycol dibenzoate	358.4	2658	2672	2682	12	0.5	-
	16	Phosflex 362	362.44	2366	2366	2371	3	0.1	3347
	17	Diehtylhexyl adipate	370.57	2368	2366	2379	7	0.3	2838
	18	Diethylhexyl phthalate	390.56	2498	2502	2506	4	0.2	3178
Group C Alcohol	19	Ethylene glycol	62.06	621	621	620	1	0.1	1629
	20	Propylene glycol	76.1	639	638	641	2	0.2	1593
	21	1,4-Butanediol	90.12	918	918	911	4	0.4	1925
	22	Glycerol	92.09	993	992	951	24	2.4	2324
	23	Diethylene glycol	106.12	935	935	930	3	0.3	1979
	24	Resorcinol	110.11	1255	1248	1245	5	0.4	3120
Group D Amines	25	Hexamethylene diamine	116.21	1061	1064	1066	3	0.2	-
	26	Toluene -2,4 -diamine	122.17	1329	1336	1334	4	0.3	-
	27	4,4-Methylenedianiline	250.25	2037	2048	2054	9	0.4	-
Group E Antioxidants	28	2,6-Di-*tert*-butyl-4-methylphenol	220.35	1486	1485	1493	4	0.3	1908
	29	Irganox 1076	531	3550	3567	3576	13	0.4	-
	30	Irgafos 168	646.93	3366	3396	3404	20	0.6	-
	31	Irganox 1330	775.21	-	-	-	-	-	-
Group F Others	32	Vinyl propionate	100.12	615	615	616	1	0.1	954
	33	Styrene	104.15	866	869	871	3	0.3	1257
	34	*para*-Xylene	106.17	850	854	855	3	0.3	1133
	35	Caprolactam	113.16	1188	1192	1193	3	0.2	2207
	36	1-Vinyl-2-pyrrolidinone	114.14	1049	1052	1056	4	0.3	1764
	37	α-Methylstyrene	118.18	960	962	964	2	0.2	1329
	38	Benzophenone	182.23	1571	1574	1587	9	0.5	2496
	39	2-Octyl-2H-isothiasol-3-one	213.34	1775	1778	1789	7	0.4	2761
	40	Butyl diglycol acetate	204.27	1335	1332	1332	2	0.1	1853
	41	Bisphenol A	228.29	2092	2097	2107	8	0.4	-
	42	4,4'-Bis(diethylamino) benzophenone	324.46	3012	3029	3041	15	0.5	-
	43	BADGE	340.42	2791	2805	2816	13	0.4	-
	44	Uvitex OB	430.06	3758	3796	3818	30	0.8	-
	45	Docusate sodium	445.63	2208	2207	2209	1	0.0	2622

1) GC-FID condition / column (see Annex 9.6.3)

2) Standard deviation of the retention indices determined by GC-FID condition A, B and C

3) Relative standard deviation of the retention indices determined by GC-FID condition A, B and C

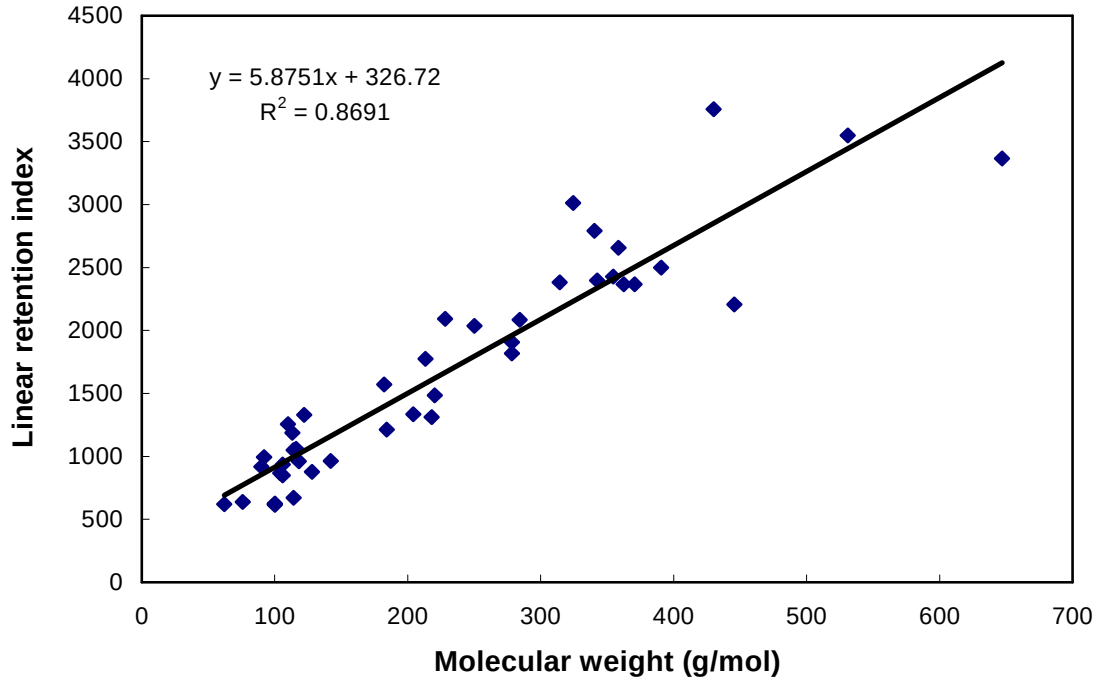


Figure 5-27. Correlation of the retention indices with the molecular weight on GC-FID equipped with DB-1 column.

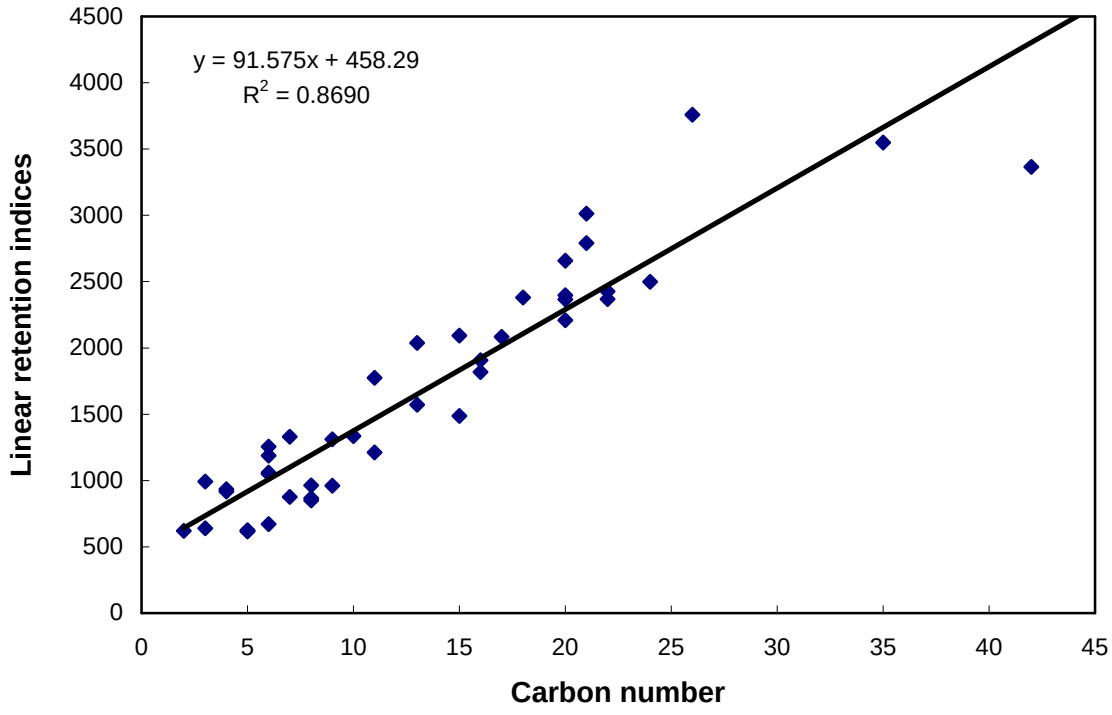


Figure 5-28. Correlation of the retention indices with carbon numbers on GC-FID equipped with DB-1 column.

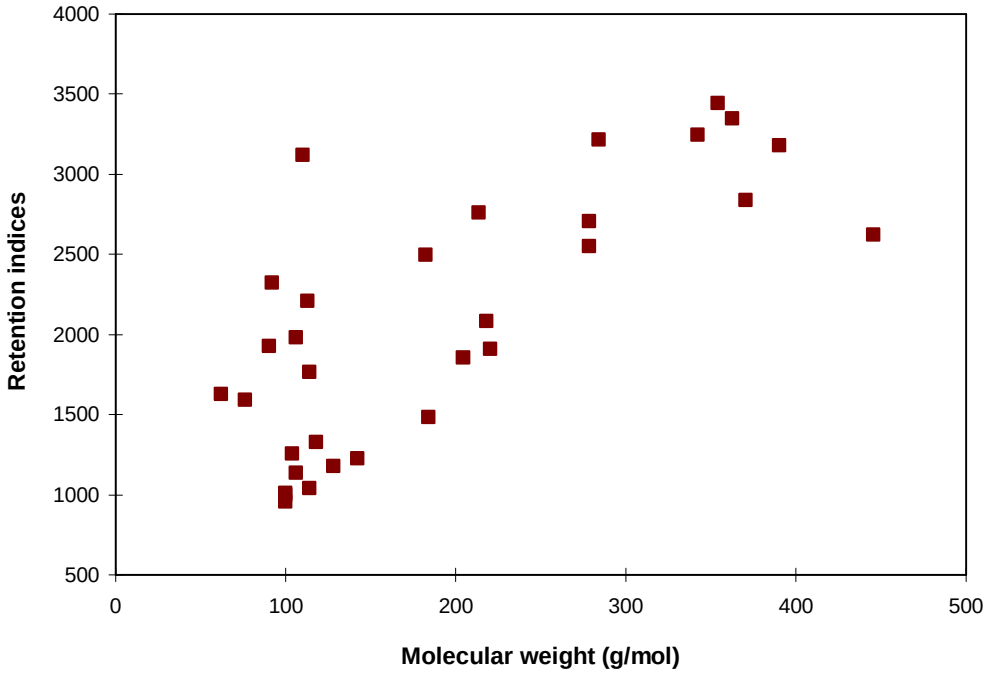


Figure 5-29. Correlation between molecular weights and the retention indices of the substances obtained by DB-FFAP column

5.5. Statistical data analysis of relative response factor (RRF) values

5.5.1. Normality test for HPLC-CAD analysis data

There was no significant difference between the CAD response factors determined by the two different mobile phase compositions (Table 5-2). Since the Gradient condition B showed better peak resolution than gradient condition A, the RRF data determined by Gradient condition B was used to establish the distribution range of RRF values at 95% coverage level for the semi-quantitative approach. Diethylhexyl phthalate, diethylhexyl adipate, dipropylene glycol dibenzoate and propylene glycol dibenzoate were excluded, because the relative response factors (RRF) of these substances were not constant in the calibration range. Thus, total 24 substances from 67 substances were taken for the statistical analysis. The RRF values of the 24 substances were in fact not approximately normal in mobile phase composition B (Figure 5-30). However, normality of the data is a prerequisite to estimate the distribution range and therefore the RRF values of 24 substances should show a normal distribution. Therefore, the data were transformed into an approximately normal distribution by using Box-Cox transformation [Box and Cox 1964]. The optimal Box-Cox parameter λ value was zero, therefore the natural log (Equation 14 in chapter 4.5.1) was taken for transforming the RRF values. The log-transformed response variables of the RRF values showed a normal distribution with a mean value of -0.14 and standard deviation of 0.64 (Figure 5-31). These values can be back-calculated to estimate the mean values and standard deviations of the original variables (chapter 5.5.2). The frequency distribution of the tranformed response variable of RRF values showed a normal distribution after Box-Cox transformation.

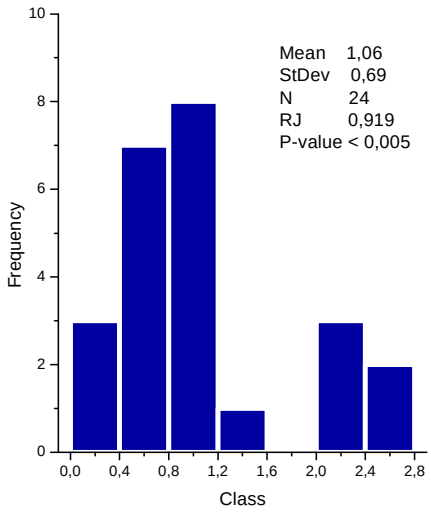


Figure 5-30. Normality test of RRF values **before** Box-Cox transformation (Gradient B).

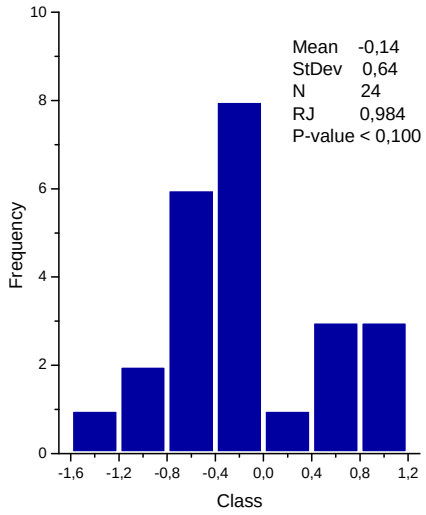


Figure 5-31. Normality test of RRF values **after** Box-Cox transformation (Gradient B).

5.5.2. Distribution range estimation of HPLC-CAD analysis data

Since the sample size (N) is relatively small (N < 30), the distribution range at 95 % coverage level was estimated by Student's t-distribution. The t-variables of Student's t-distribution with degree of freedom according to sample size and P=95% were obtained from the t-Table [Gottwald 1999].

The transformed response variables of the RRF values were in the range from –0.45 (Bisphenol A) to 0.89 (Irganox 1330 and Irganox 565) at a mean (± standard deviation) of –0.14 ± 0.64. The distribution range at 95 % coverage level was between –1.46 and 1.18. Thus backcalculated distribution range of RRF values of all adhesive related substances (population) was estimated between 0.23 and 3.25. For a conservative estimation of the concentration when using Tinuvin 234 as universal internal calibration standard for HPLC-CAD a RRF of 0.23 should be used. According to the definition of RRF in chapter 3.2, the concentration of unknown substances can be simply calculated as follows.

$$C_s^{known} = \frac{C_{is} \times Area_s^{known}}{Area_{is} \times RRF} \quad : \text{Calculation using RRF}$$

$Area_s^{known}$: peak area of known analyte
$Area_{is}$: peak area of the Internal standard
C_s^{known} : concentration of known analyte
C_{is} : concentration of the Internal standard
RRF : average relative response factor of known analyte in difined calibration range

$$C_s^{unknown} = \frac{C_{is}^{universal} \times Area_s^{unknown}}{Area_{is}^{universal} \times RRF^{statistic}(0.23)} : \text{Estimation using factor 0.23}$$

$Area_s^{unknown}$: peak area of unknown analyte
$Area_{is}^{universal}$: peak area of the Universal internal standard
$C_s^{unknown}$: concentration of unknown analyte
$C_{is}^{universal}$: concentration of the Universal internal standard
$RRF^{statistic}$: statistical relative response factor obtained from the response factor distribution of 24 substances

5.5.3. Normality test for GC-FID analysis data

The relative response factors (RRF) related to BHA as internal standard could be calculated for total 46 substances from the 55 representative adhesive related substances. For the statistical evaluation, the RRF values were divided in classes of 0.2 units in order to obtain relative frequency distributions. According to the Ryan-Joiner test for normality in Minitab 15 [Minitab version 15 2007], the frequency distribution of RRF values of 46 substances showed a normal distribution (Figure 5-32) with a mean value of 0.83 and a standard deviation of 0.33.

As stated above, four alcohols (ethylene glycol, 1,4-butanediol, diethylene glycol and glycerol) showed tailed peak shapes on the DB-1 column and therefore very low sensitivity. So that the RRF values were not constant in the calibration range. This indicates that the DB-1 column was not appropriate for the quantification of some alcohols using RRF values, same for carboxylic acids. Therefore, the RRF values of four alcohols should be excluded to establish the distribution range.

The frequency distribution of 42 substances without the four alcohols showed also a normal distribution with a mean value of 0.88 and a standard deviation of 0.29 (Figure 5-33).

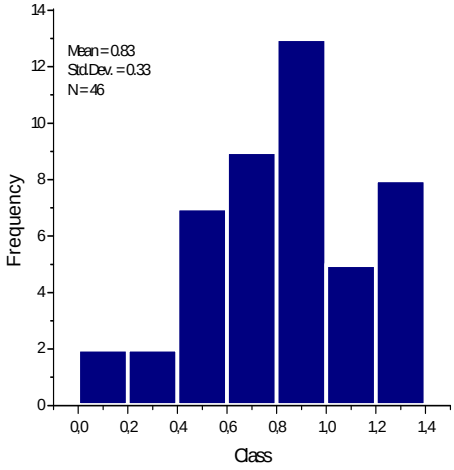


Figure 5-32. Frequency distribution of relative response factors (RRF) of 46 substances.

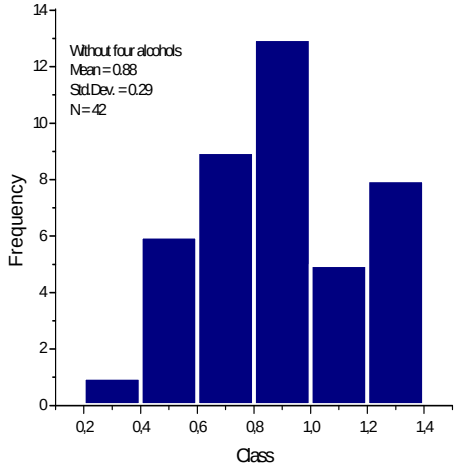


Figure 5-33. Frequency distribution of relative response factors of 42 substances without alcohols.

5.5.4. Distribution range estimation of GC-FID analysis data

The RRF values related to the response of BHA ranged between 0.14 (glycerol) and 1.36 (para-Xylene) at a mean (± standard deviation) of 0.83 ± 0.33. This means a concentration of 1 µg/ml corresponds to BHA-equivalent concentration between 0.14 µg/ml and 1.36 µg/ml. Since the number of the calculated RRF values was more than 30, the distribution range of the RRF values at 95% coverage level was calculated according to the equation 15 in chapter 4.5.2, The distribution of RRF values ranged from 0.18 to 1.48.

The RRF values without the four alcohols (ethylene glycol, 1,4-butanediol, diethylene glycol and glycerol) were between 0.30 (propylene glycol) and 1.36 (para-Xylene) at a mean 0.88 ± 0.29. the distribution range of the RRF values was between 0.31 and 1.44. Thus for a conservative estimation of the concentration of substances by using BHA as universal internal calibration standard a RRF of 0.31 should be used.

$$C_s^{\,unknown} = \frac{C_{is}^{\,universal} \times Area_s^{\,unknown}}{Area_{is}^{\,universal} \times RRF^{\,statistic}(0.31)} \quad : \text{ Estimation using factor 0.31}$$

$Area_s^{\,unknown}$: peak area of unknown analyte
$Area_{is}^{\,universal}$: peak area of the Universal internal standard
$C_s^{\,unknown}$: concentration of unknown analyte
$C_{is}^{\,universal}$: concentration of the Universal internal standard
$RRF^{\,statistic}$: statistical relative response factor obtained from the response factor distribution of 46 substances

5.6. Practical application of the multi-screening methods

5.6.1. HPLC-CAD analysis data of extracts from adhesives and glued samples and of migration solutions

Six different pure water-based adhesive samples (VAE 1 ~ 5 and PVAc) listed in table 4-8 in chapter 4.7.1 were extracted with dichloromethane (DCM) and the extracts were analyzed by the developed multi-screening method using the HPLC-CAD system equipped with a C18 column. A clean-up procedure using size exclusion chromatography (SEC) was carried out prior to injection, since the nebulizer in CAD and the separation column could be clogged with the sticky polymers used as a binder in the adhesives. Finally, for HPLC analysis, the extracts were prepared in methanol. The non-volatile compounds greater than 1000 g/mol in the extracts were excluded through SEC clean-up. The compounds smaller than 300 g/mol could not be detected by CAD (see chapter 5.2.1). Thus the detectable range of the compounds by CAD was 300 to 1000 g/mol.

Benzoate type plasticizers (Diethylene glycol dibenzoate, Dipropylene glycol dibenzoate and Triethylene glycol dibenzoate) were identified in 2 out of the 6 water-based adhesives (Table 5-8). Figure 5-34 and 5-35 show the chromatograms obtained from these sample extracts. Other non-volatiles were not found using the HPLC-CAD screening method. Diethylene glycol dibenzoate and triethylene glycol dibenzoate were observed in VAE 3 pure adhesive by GC-FID analysis (Figure 5-42 and Figure 5-48). However, these substances were eluted at the same retention time in HPLC-CAD analysis (Figure 5-34).

Migration experiments were performed using Tenax in contact with six different composite samples (VAE 1-C ~ 5-C and PVAc-C) bonded with the corresponding water-based adhesives (VAE 1 ~ 5 and PVAc) (Table 4-9 in chapter 4.7.1). In this case a clean-up procedure for HPLC-CAD analysis was not necessary. The chromatograms are presented in figure 5-36 ~ 5-39. The three dibenzoate type plasticizers were identified as main substances in the Tenax migration solutions of VAE 3-C and 5-C composite samples. In addition, some unknown peaks were found in the samples of VAE 1-C (RT at 29 and 37.5 min), 3-C (RT at 37.5 min) and 4-C (RT at 37.5 min) (Table 5-8). The unknown peaks can be allocated to compounds migrated from paper and cardboard used as substrates for the composite samples.

Table 5-8. List of the main components in six pure adhesive and composite samples identified by HPLC-CAD screening test.

Pure adhesives [1)]	**Identified main compounds**	**Composites** [2)]	**Identified main compounds**
VAE 1	-	VAE 1-C	Unknown substance
VAE 2	-	VAE 2-C	-
VAE 3	Diethylene glycol dibenzoate	VAE 3-C	Diethylene glycol dibenzoate
	Dipropylene glycol dibenzoate		Dipropylene glycol dibenzoate
	Triethylene glycol dibenzoate		Triethylene glycol dibenzoate
			Unknown substance
VAE 4	-	VAE 4-C	Unknown substance
VAE 5	Diethylene glycol dibenzoate	VAE 5-C	Diethylene glycol dibenzoate
	Dipropylene glycol dibenzoate		Dipropylene glycol dibenzoate
PVAc	-	PVAc-C	-

1) VAE 1 ~ 5 : Pure adhesives based on Vinyl acetate ethylene, PVAc : Pure adhesives based on Polyvinyl acetate
2) VAE 1-C ~ 5-C and PVAc-C : Composites bonded with the pure adhesives VAE 1 ~ 5 and PVAc
(e.g. VAE 1-C composite sample was bonded with VAE 1 pure adhesive)

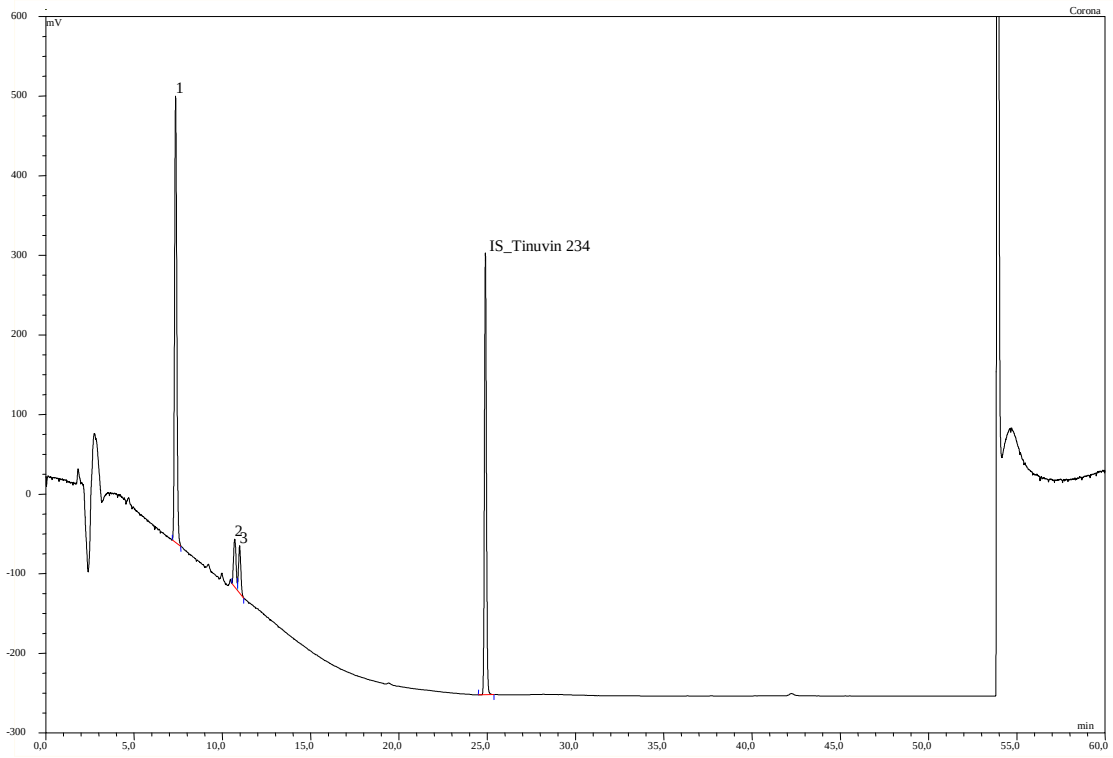


Figure 5-34. HPLC-CAD chromatogram of DCM extracts of **VAE 3** adhesive.
1 : Diethylene glycol dibenzoate and Triethylene glycol dibaenzoate
2 : Dipropylene glycol dibenzoate, 3 : Isomer of second Peak

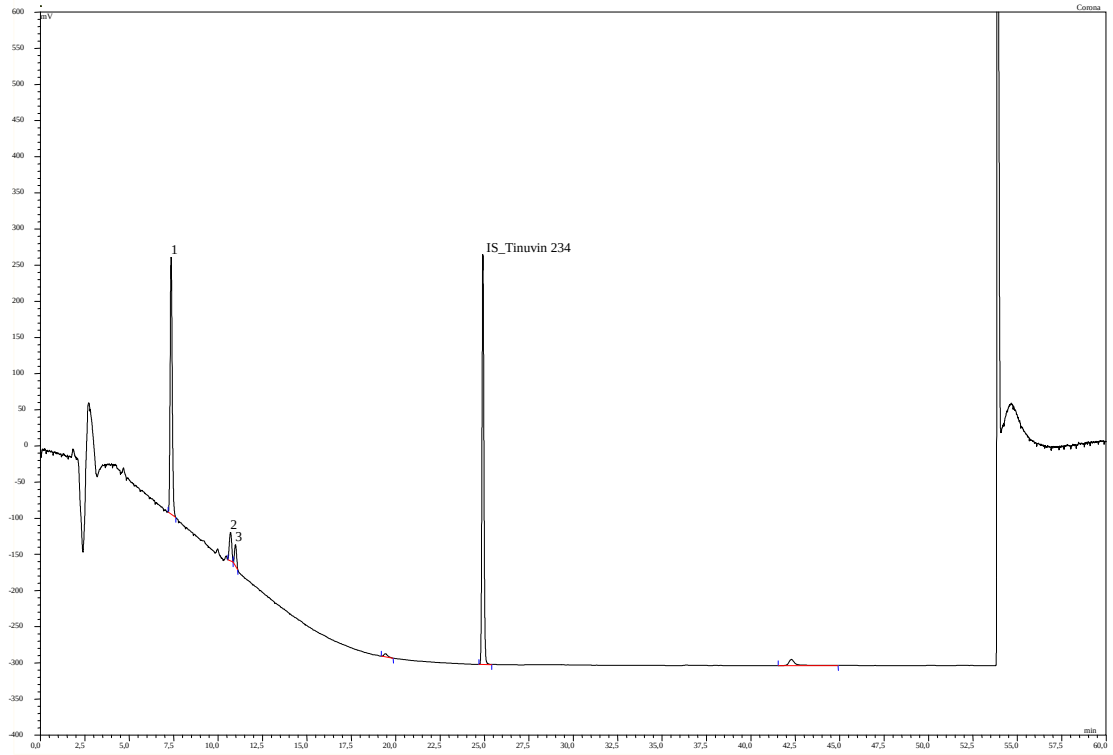


Figure 5-35. HPLC-CAD chromatogram of DCM extracts of **VAE 5** adhesive.
1 : Diethylene glycol dibenzoate 2 : Dipropylene glycol dibenzoate, 3 : Isomer of second Peak

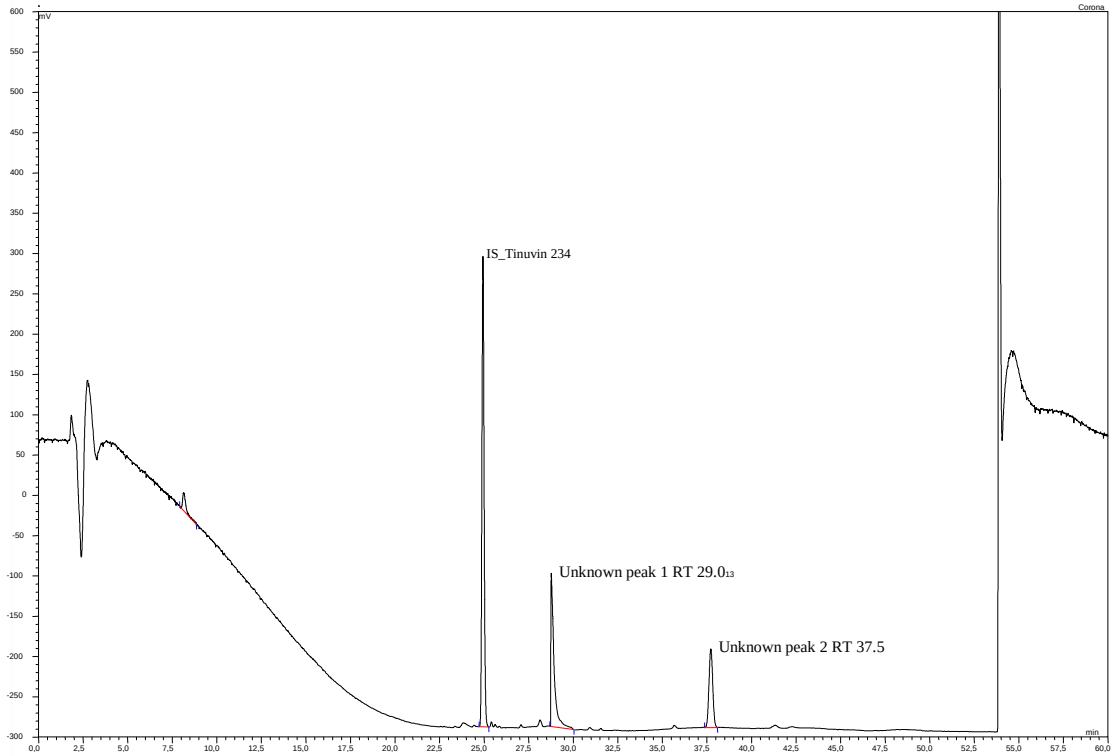


Figure 5-36. HPLC-CAD chromatogram of Tenax migrants of **VAE 1-C** sample.

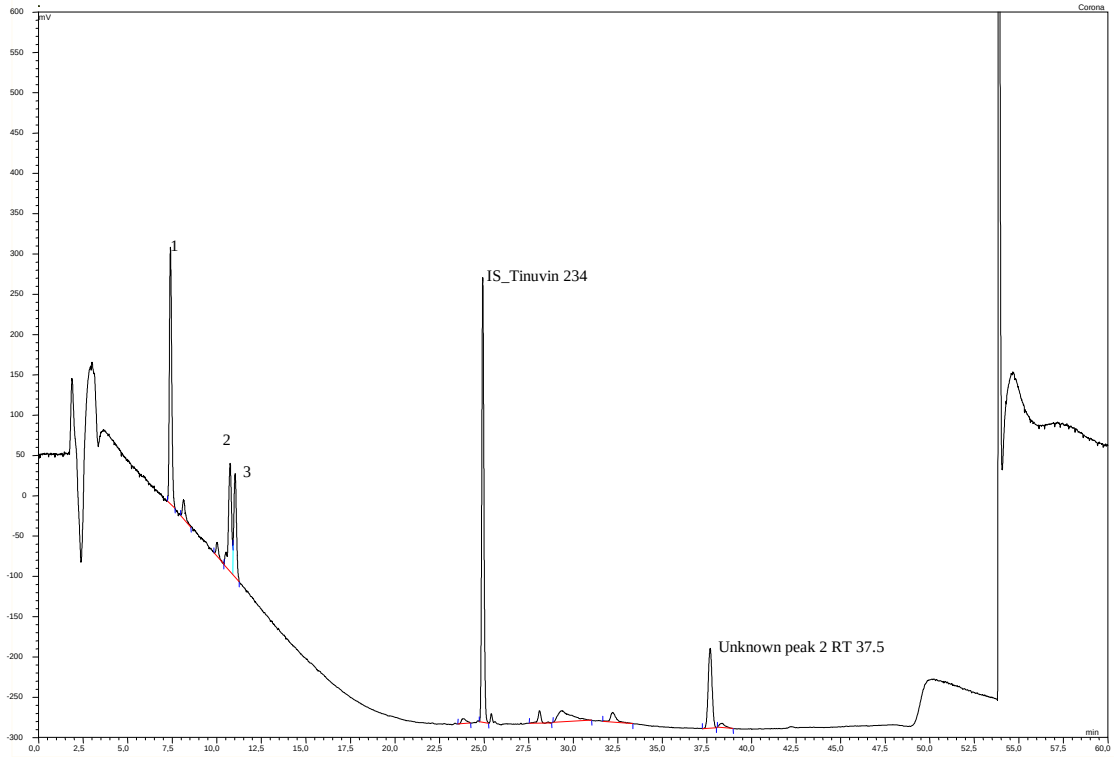


Figure 5-37. HPLC-CAD chromatogram of Tenax migrants of **VAE 3-C** sample.
1 : Diethylene glycol dibenzoate and Triethylene glycol dibaenzoate
2 : Dipropylene glycol dibenzoate, 3 : Isomer of second Peak

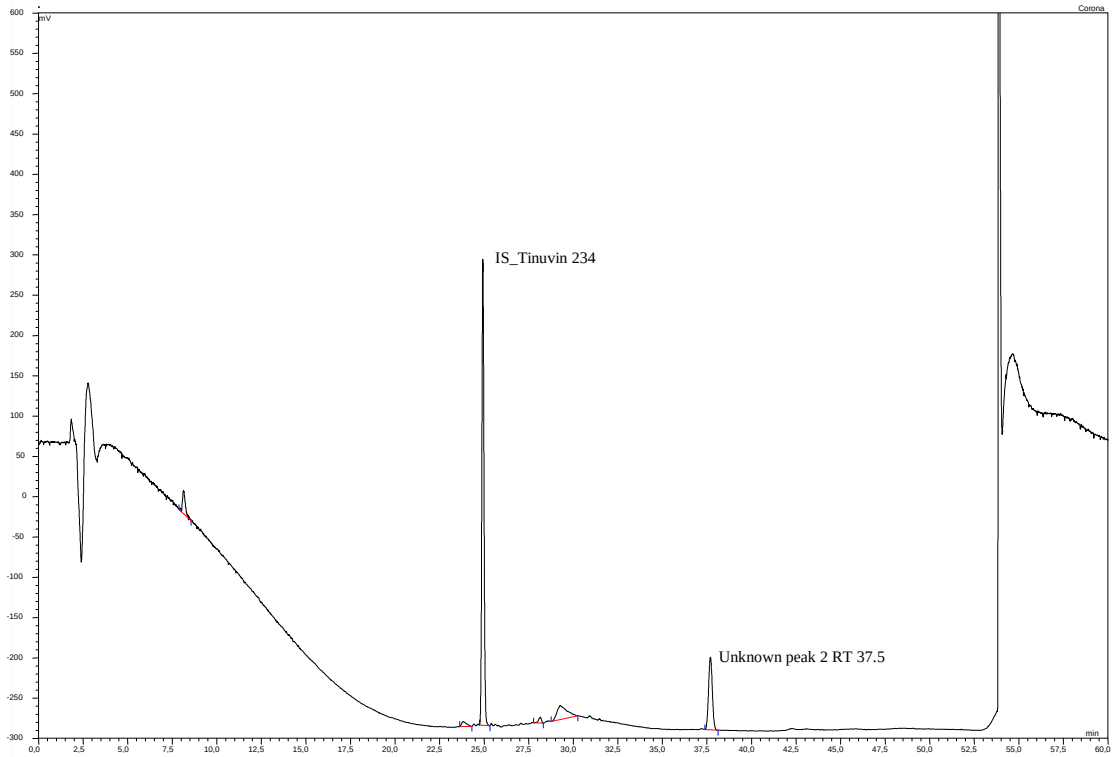


Figure 5-38. HPLC-CAD chromatogram of Tenax migrants of **VAE 4-C** sample.

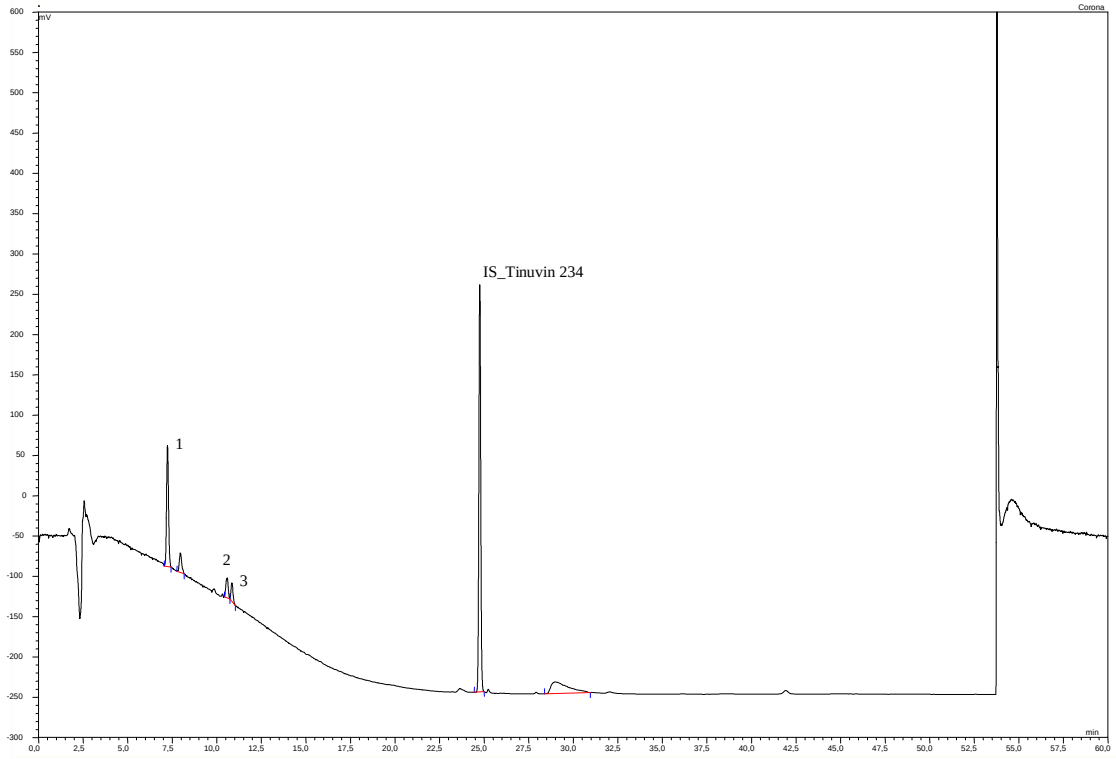


Figure 5-39. HPLC-CAD chromatogram of Tenax migrants of **VAE 5-C** sample.
1 : Diethylene glycol dibenzoate 2 : Dipropylene glycol dibenzoate, 3 : Isomer of second Peak

5.6.2. GC-FID analysis data of extracts and migrantion solutions

The same adhesive samples (VAE 1 ~ 5 and PVAc) were analyzed by GC-FID equipped with the DB-1 non-polar column. Figure 5-40 ~ 5-45 show the examples of chromatograms obtained from the GC-FID screening test of the dichloromethane extractions. The identified substances are summarized in table 5-9.

The migration experiments using Tenax as a simulants were performed with the composite samples (VAE 1-C ~ 5-C and PVAc-C) each bonded with one of the adhesives. Figure 5-46 ~ 5-51 show the GC-FID chromatograms. The main components in the extraction test were found likewise in this migration test. However, Benzoic acid ethylester and Benzoic acid ethylmethylester were not detected in the migration solution of VAE 5-C composite sample.

Table 5-9. List of the main components in six adhesive and composite samples identified by GC-FID screening test.

Pure adhesives [1)]	**Identified main compounds**	**Composites** [2)]	**Identified main compounds**
VAE 1	Triacetin	VAE 1-C	Triacetin
VAE 2	Butyl diglycol acetate	VAE 2-C	Butyl diglycol acetate
VAE 3	Diethylene glycol dibenzoate	VAE 3-C	Diethylene glycol dibenzoate
	Dipropylene glycol dibenzoate		Dipropylene glycol dibenzoate
	Triethylene glycol dibenzoate		Triethylene glycol dibenzoate
VAE 4	Triacetin	VAE 4-C	Triacetin
VAE 5	Diethylene glycol dibenzoate	VAE 5-C	Diethylene glycol dibenzoate
	Dipropylene glycol dibenzoate		Dipropylene glycol dibenzoate
	Benzoic acid ethylester		-
	Benzoic acid ethylmethylester		-
PVAc	Triacetin	PVAc-C	Triacetin

1) VAE 1 ~ 5 : Pure adhesives based on Vinyl acetate ethylene, PVAc : Pure adhesives based on Polyvinyl acetate
2) VAE 1-C ~ 5-C and PVAc-C : Composites bonded with the pure adhesives VAE 1 ~ 5 and PVAc
(e.g. VAE 1-C composite sample was bonded with VAE 1 pure adhesive)

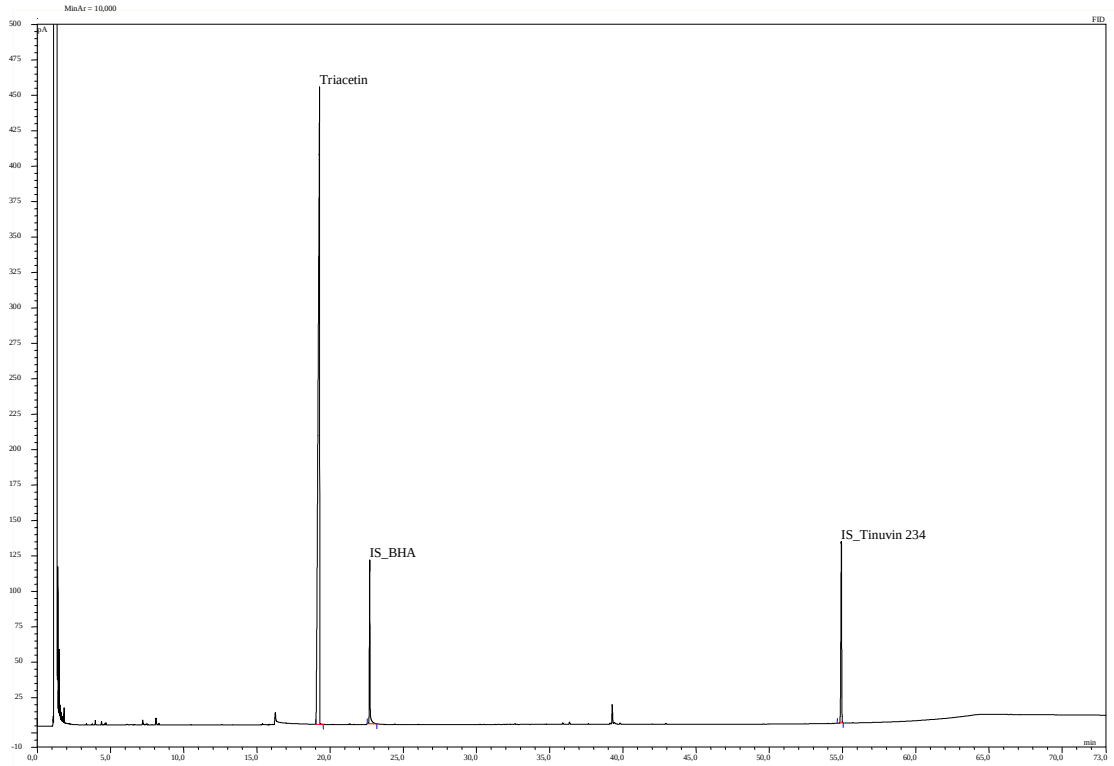


Figure 5-40. GC-FID chromatogram of DCM extracts of **VAE 1** adhesive.

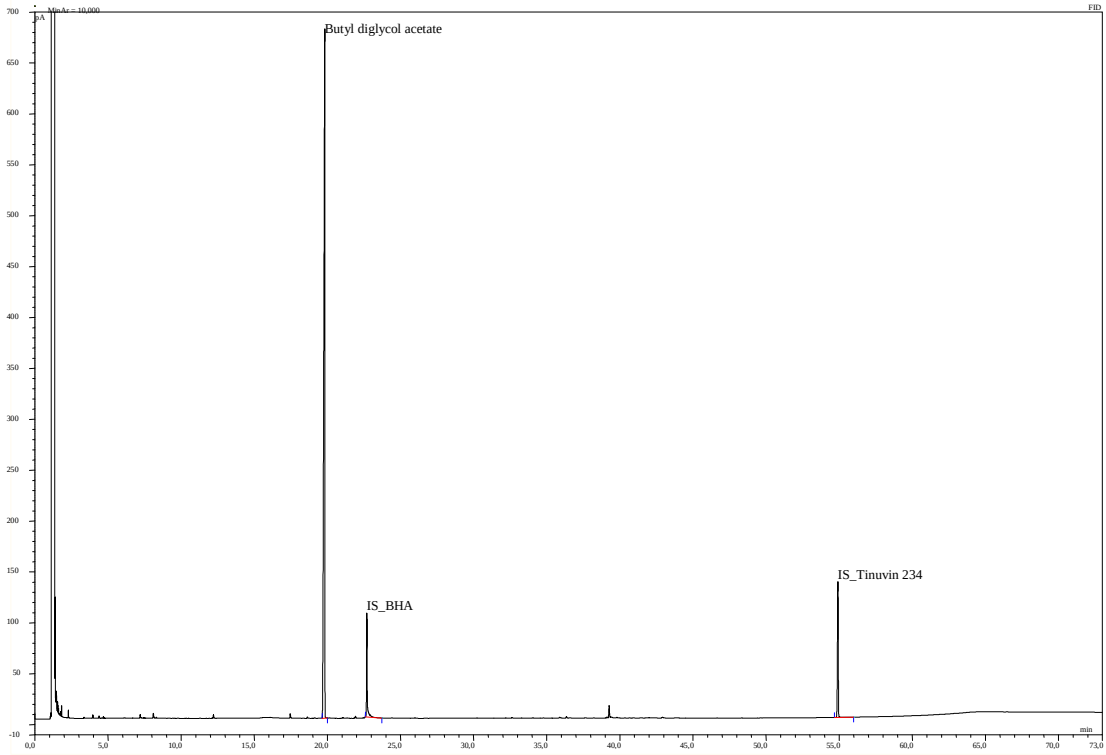


Figure 5-41. GC-FID chromatogram of DCM extracts of **VAE 2** adhesive.

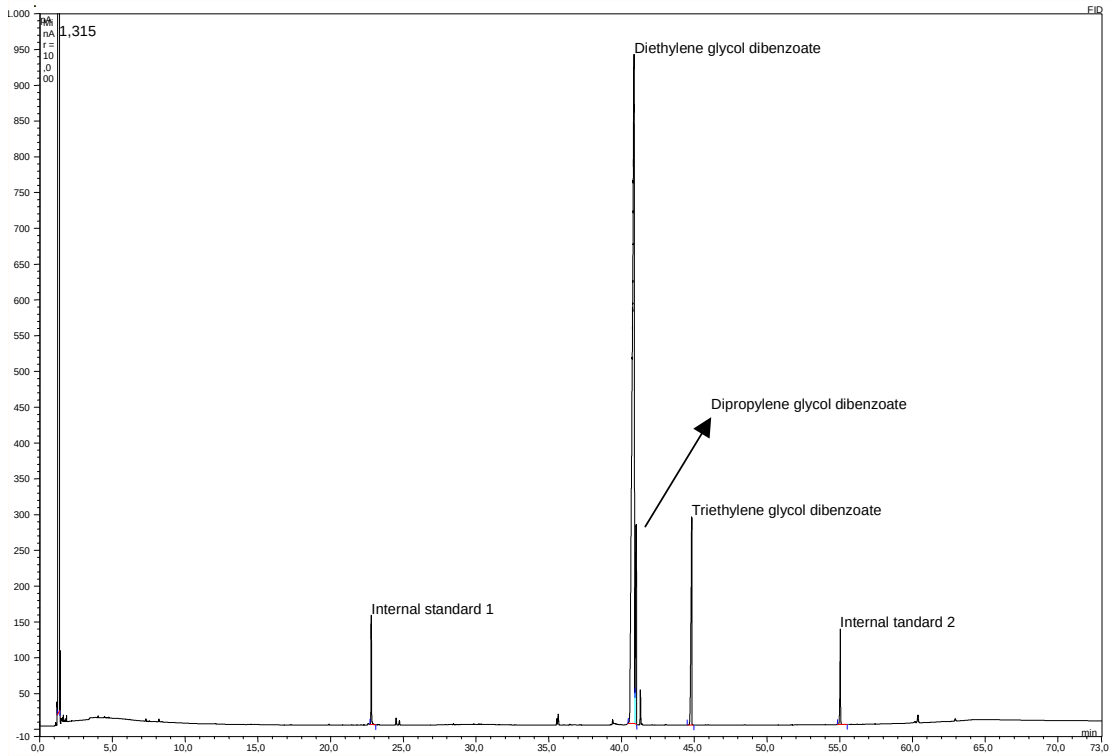


Figure 5-42. GC-FID chromatogram of DCM extracts of **VAE 3** adhesive.

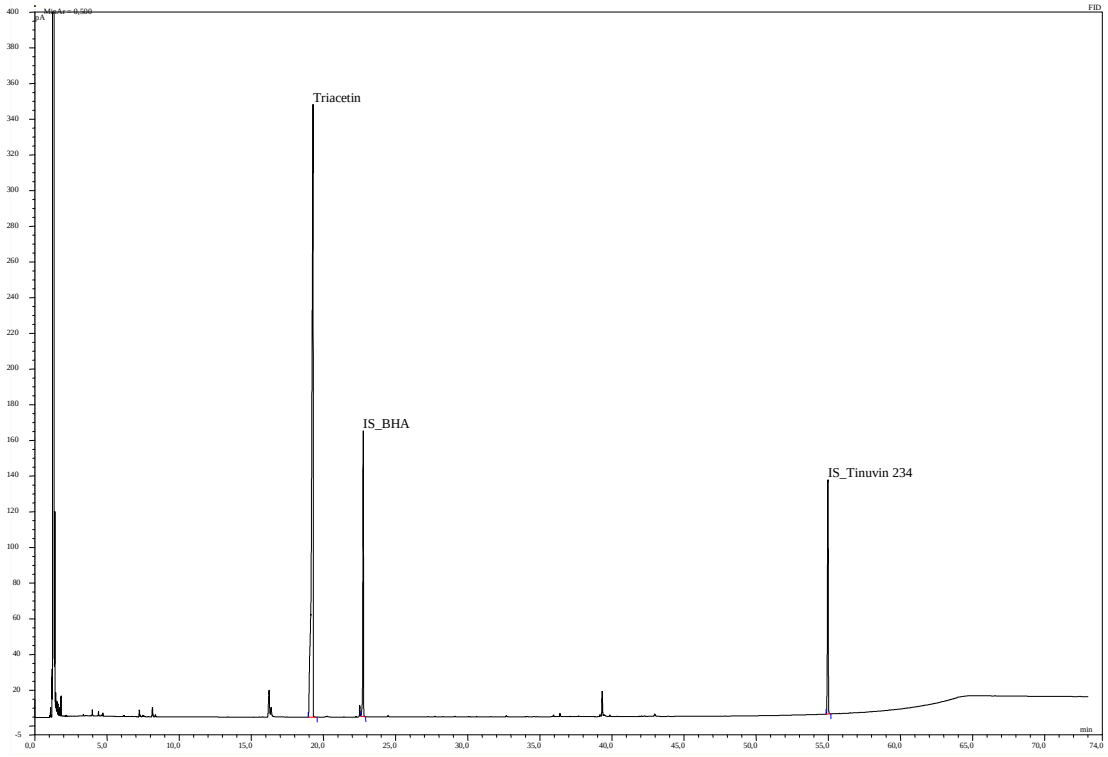


Figure 5-43. GC-FID chromatogram of DCM extracts of **VAE 4** adhesive.

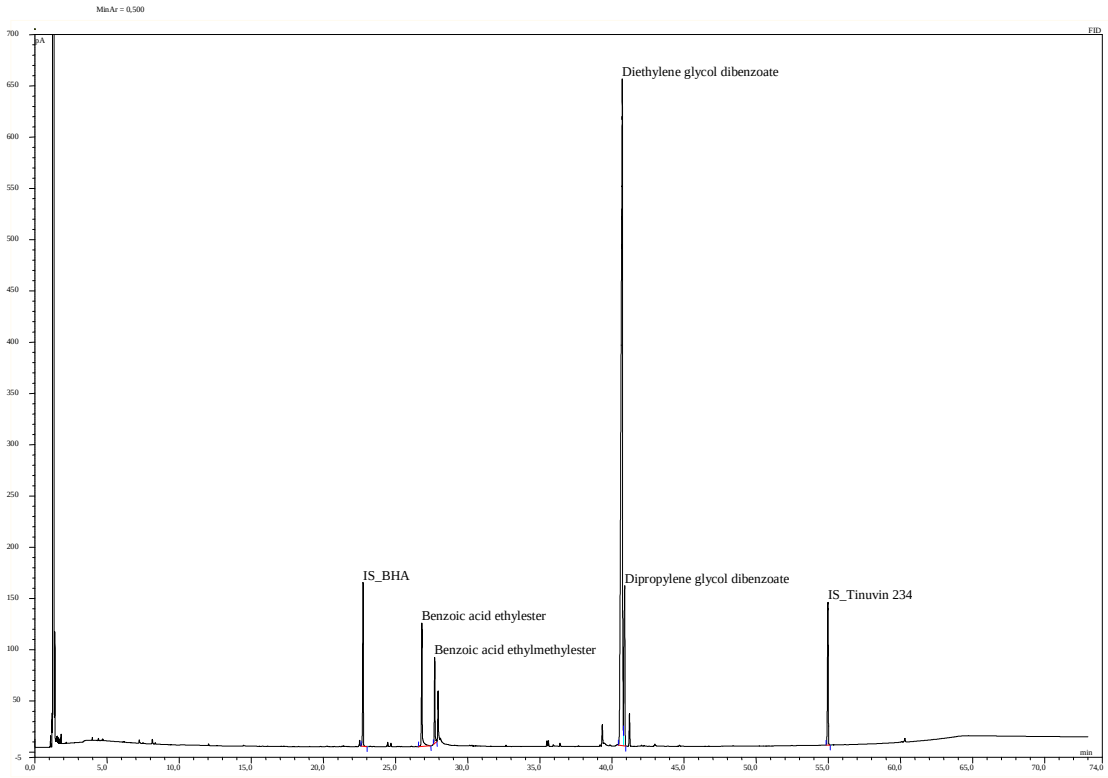


Figure 5-44. GC-FID chromatogram of DCM extracts of **VAE 5** adhesive.

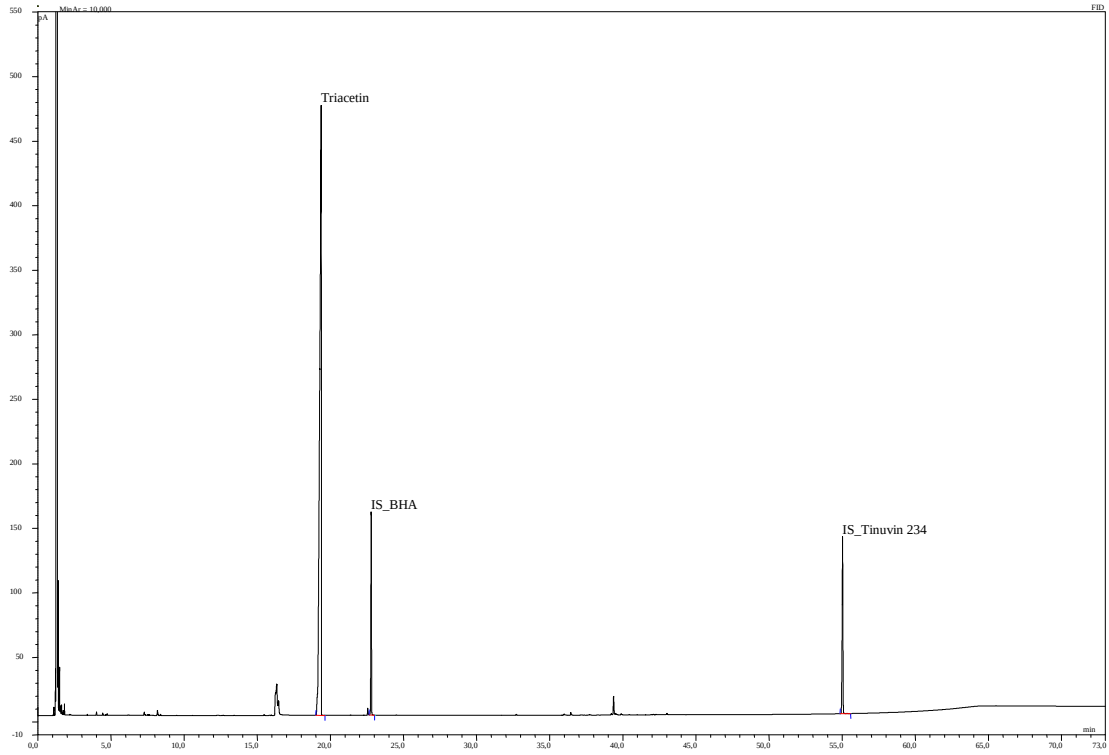


Figure 5-45. GC-FID chromatogram of DCM extracts of **PVAc 1** adhesive.

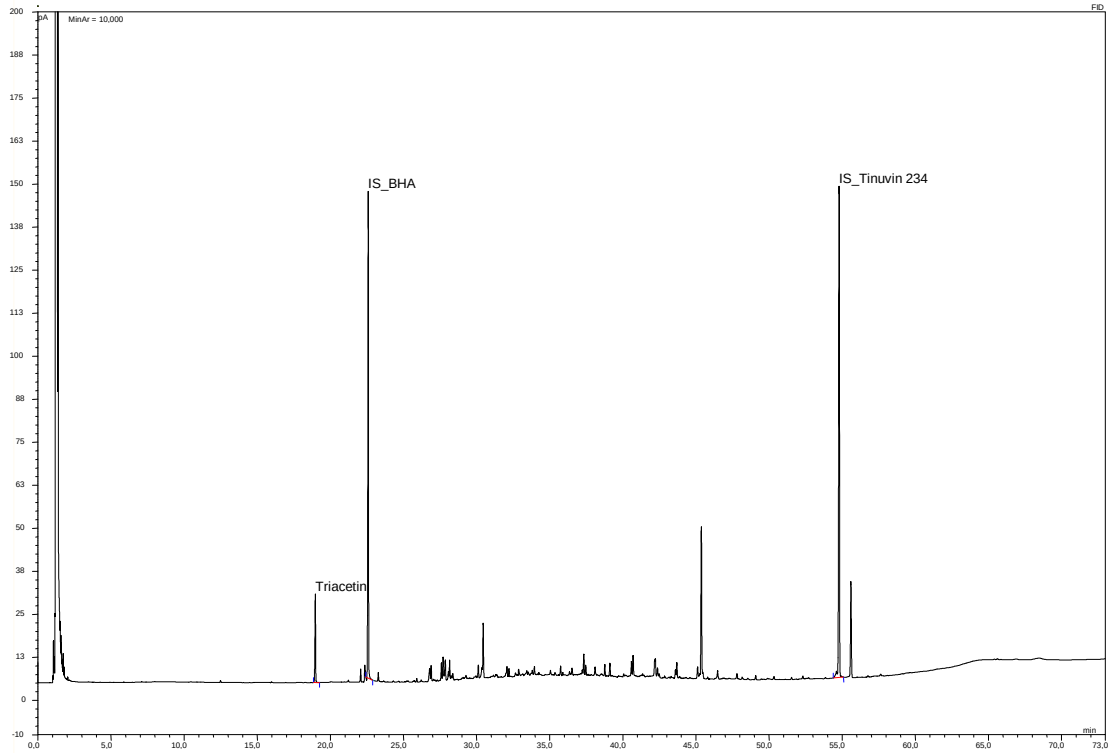


Figure 5-46. GC-FID chromatogram of the migrants transferred from the composite samples bonded with **VAE 1** adhesive into Tenax.

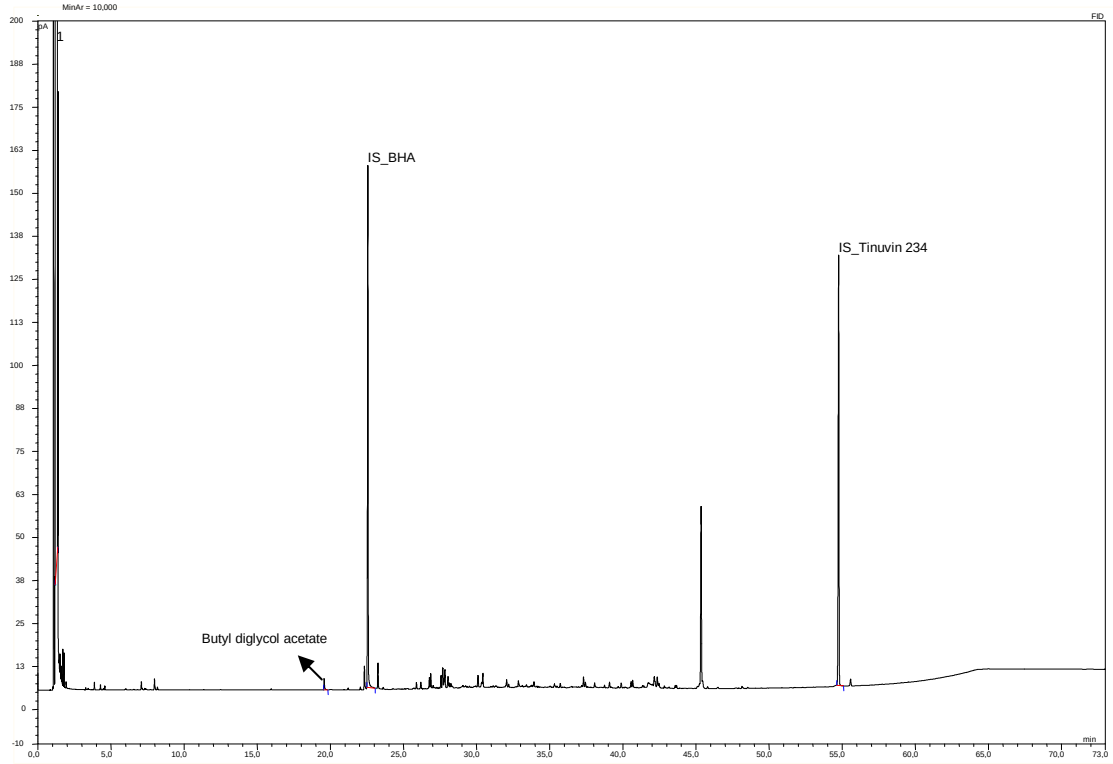


Figure 5-47. GC-FID chromatogram of the migrants transferred from the composite samples bonded with **VAE 2** adhesive into Tenax.

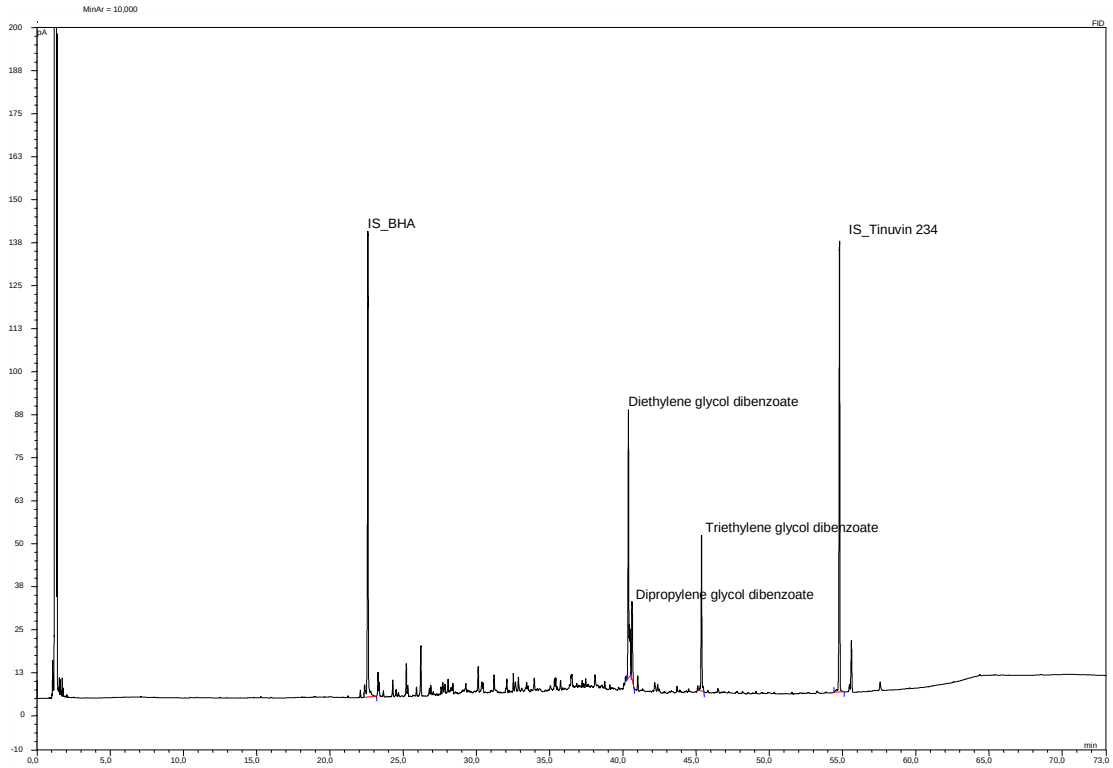


Figure 5-48. GC-FID chromatogram of the migrants transferred from the composite samples bonded with **VAE 3** adhesive into Tenax.

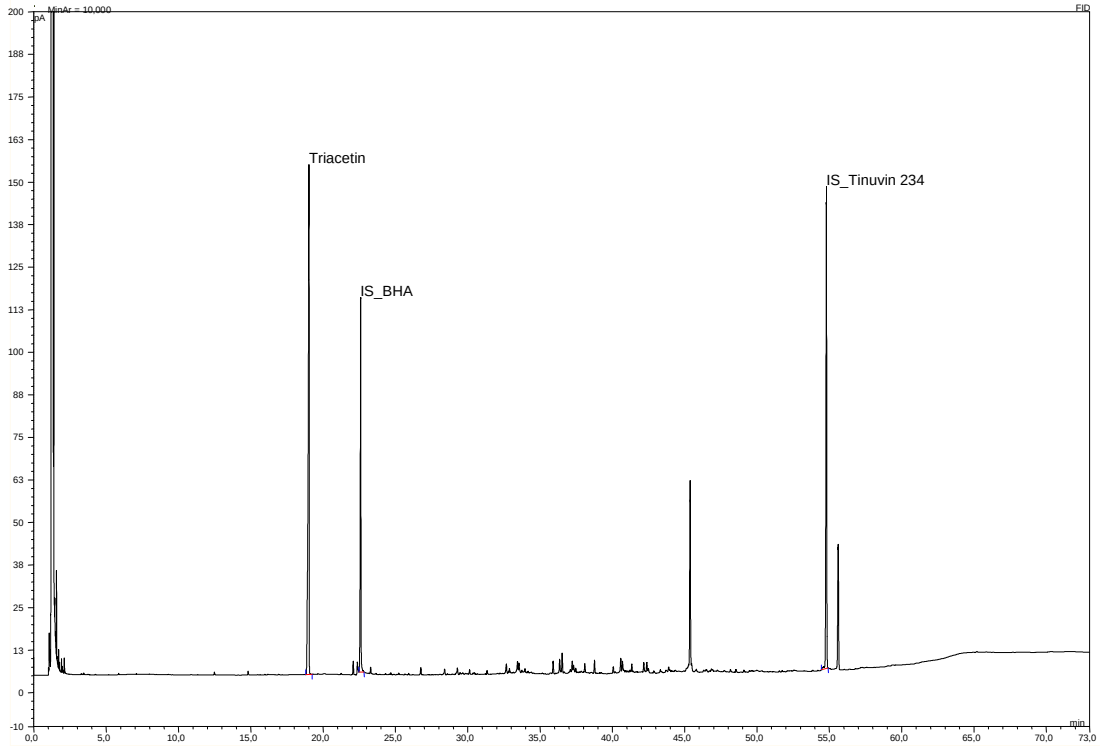


Figure 5-49. GC-FID chromatogram of the migrants transferred from the composite samples bonded with **VAE 4** adhesive into Tenax.

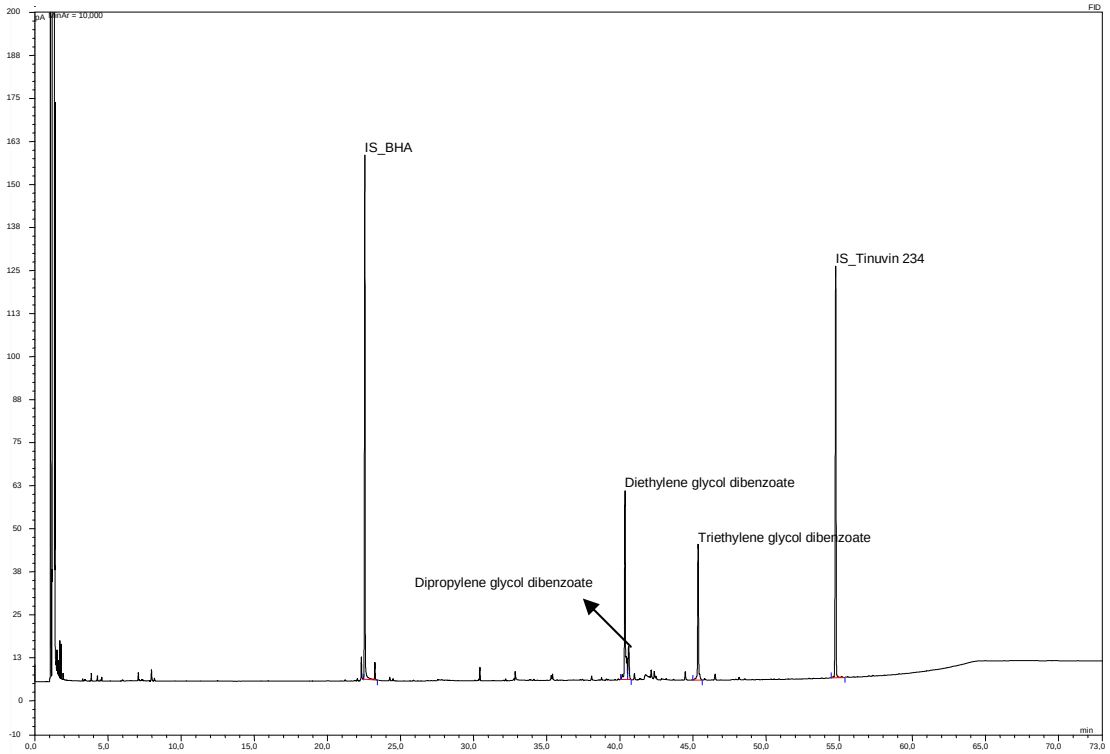


Figure 5-50. GC-FID chromatogram of the migrants transferred from the composite samples bonded with **VAE 5** adhesive into Tenax.

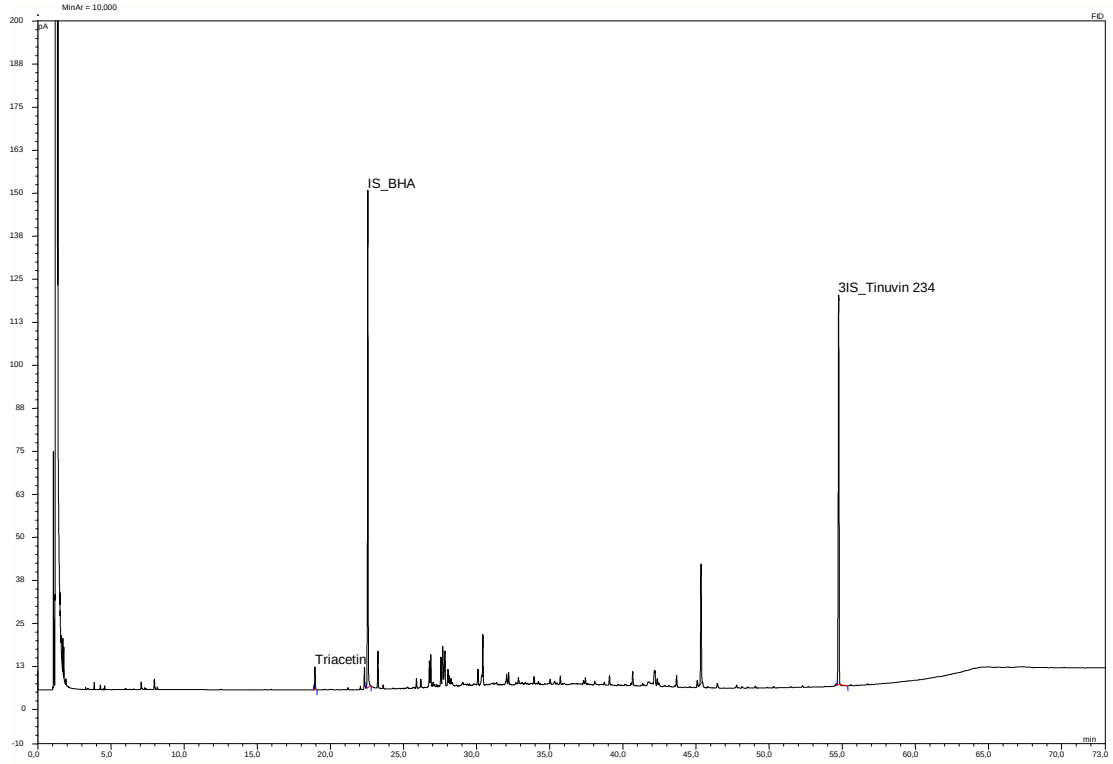


Figure 5-51. GC-FID chromatogram of the migrants transferred from the composite samples bonded with **PVAc** adhesive into Tenax.

5.6.3. Semi-quantification of adhesive related susbtances

Using the extracts and migration solutions of the 6 real samples, traditional quantification using calibration curves of standard substances was compared to the semi-quantitative estimates (conservative estimations) using the statistical RRF value. Table 5-10 and 5-11 show the comparison results between the conservative estimation and traditional quantification of the identified substances in the extracts and migration solutions of the pure adhesive samples and their composites. Additionally the concentrations of the identified substances were calculated by using the real RRF obtained from the calibration experiments of each identified substance and compared with other quantitative results.

The distribution range of the RRF values was between 0.31 and 1.44 in the GC-FID analysis. For a conservative estimation of the concentration the RRF of 0.31 was applied. These conservative estimations using the lower limit of the distribution range overestimate the quantitative results in a range of 129 to 305 % in the extraction test and of 130 to 305 % in the migration test.

Without correction factor (RRF = 1) the concentration would be underestimated. The traditional quantification results were also compared with the estimation results using a RRF

value of specific substance (substance specific RRF estimation in Table 5-10 ~ 5-12). The values obtained from migration and extraction test corresponded well with those of the traditional quantifition in the range of 79 to 109 % in both extraction and migration tests.

Table 5-10. Comparison of the GC-FID quantitative results of the identified substances in the adhesive extracts.

Sample [1)]	Substances	RRF	Traditional quantitation (µg/ml)	Semi-quantitative estimation (µg/ml)	Approximation ratio A [2)] (%)	Substance specific RRF estimation (µg/ml)	Approximation ratio B [3)] (%)
VAE 1	Triacetin	0.46	943	1341	142	904	96
VAE 2	Butyl diglycol acetate	0.70	670	1567	234	694	104
VAE 3	Diethylene glycol dibenzoate	0.87	1208	3648	302	1300	108
	Dipropylene glycol dibenzoate	0.51	381	492	129	299	79
	Triethylene glycol dibenzoate	0.75	237	572	242	237	100
VAE 4	Triacetin	0.46	600	847	141	570	95
VAE 5	Diethylene glycol dibenzoate	0.87	522	1589	305	566	109
	Dipropylene glycol dibenzoate	0.51	157	206	131	125	80
PVAc	Triacetin	0.46	1070	1507	141	1061	95

1) VAE 1 ~ 5 : Pure adhesives based on Vinyl acetate ethylene, PVAc : Pure adhesives based on Polyvinyl acetate
2) Approximation ratio of the semi-quantitative estimation to the traditional quantitation.
3) Approximation ratio of the substance specific RRF estimation to the traditional quantitation.

Table 5-11. Comparison of the GC-FID quantitative results of the identified substances in the migration solutions.

Sample [1)]	Substances	RRF	Traditional quantitation (µg/ml)	Semi-quantitative estimation (µg/ml)	Approximation ratio A [2)] (%)	Substance specific RRF estimation (µg/ml)	Approximation ratio B [3)] (%)
VAE 1-C	Triacetin	0.46	20.8	29.6	143	20.0	96
VAE 2-C	Butyl diglycol acetate	0.70	1.5	3.5	238	1.5	105
VAE 3-C	Diethylene glycol dibenzoate	0.87	38.5	117.2	305	41.8	109
	Dipropylene glycol dibenzoate	0.51	41.5	53.7	130	32.6	79
	Triethylene glycol dibenzoate	0.75	24.6	59.4	242	24.5	100
VAE 4-C	Triacetin	0.46	201	282.4	140	190.3	95
VAE 5-C	Diethylene glycol dibenzoate	0.87	21.8	66.4	305	23.7	109
	Dipropylene glycol dibenzoate	0.51	14.7	19.2	131	11.7	80
PVAc-C	Triacetin	0.46	4.8	7.0	145	4.7	98

1) VAE 1-C ~ 5-C and PVAc-C : Composites bonded with the pure adhesives VAE 1 ~ 5 and PVAc
(e.g. VAE 1-C composite sample was bonded with VAE 1 pure adhesive)
2) Approximation ratio of the semi-quantitative estimation to the traditional quantitation.
3) Approximation ratio of the substance specific RRF estimation to the traditional quantitation.

Semi- and traditional quantitative results determined by HPLC-CAD are given in table 5-12. Diethylene glycol dibenzoate and triethylene glycol dibenzoate were eluted with same retention time. Therefore a quantitative analysis was not possible.

The distribution range of the RRF values was between 0.23 and 3.25 in the HPLC-CAD analysis. Thus the lower limit is 0.23 for a conservative estimation of the concentration of the identified substances.

The conservative estimation values of the identified substances were approximated to traditional values in the range from 102 to 219 % in the extraction test of the pure adhesive samples and from 86 to 216 % in the migration test of the composite samples, respectively. The traditional quantitation results were compared with those of the estimation using the analytically obtained RRF values. The estimation values corresponded well with those of the traditional quantitation in the range of 73 to 107 % in the extraction test and of 62 to 105 % in the migration test.

Table 5-12. Comparison of the quantitative results by HPLC-CAD.

Sample [1]	**Substances**	**RRF**	**Traditional quantitation (µg/ml)**	**Semi-quantitative estimation (µg/ml)**	**Approximation ratio A** [2] **(%)**	**Substance specific RRF estimation (µg/ml)**	**Approximation ratio B** [3] **(%)**
Extraction Test							
VAE 3	Diethylene glycol dibenzoate	0.47	-	-	-	-	-
	Dipropylene glycol dibenzoate	0.32	494.8	516.0	104	370.8	75
	Triethylene glycol dibenzoate	0.58	-	-	-	-	-
VAE 5	Diethylene glycol dibenzoate	0.47	327.6	717.0	219	350.8	107
	Dipropylene glycol dibenzoate	0.25	278.6	284.6	102	204.6	73
Migration Test							
VAE 3-C	Diethylene glycol dibenzoate	0.47	-	-	-	-	-
	Dipropylene glycol dibenzoate	0.32	119.0	120.5	101	86.6	73
	Triethylene glycol dibenzoate	0.58	-	-	-	-	-
VAE 5-C	Diethylene glycol dibenzoate	0.47	31.9	68.8	216	33.7	105
	Dipropylene glycol dibenzoate	0.32	29.3	28.0	86	20.1	62

[1] VAE 1 ~ 5 : Pure adhesives based on Vinyl acetate ethylene, PVAc : Pure adhesives based on Polyvinyl acetate
VAE 1-C ~ 5-C and PVAc-C : Composites bonded with the pure adhesives VAE 1 ~ 5 and PVAc
(e.g. VAE 1-C composite sample was bonded with VAE 1 pure adhesive)

[2] Approximation ratio of the semi-quantitative estimation to the traditional quantitation.

[3] Approximation ratio of the substance specific RRF estimation to the traditional quantitation.

6. Discussion

6.1. Development of screening methods by using HPLC-CAD system

6.1.1. HPLC-CAD analysis data

Many analytical separations for the non-volatile compounds in food packaging materials have been carried out by means of reverse phase HPLC system (RP-HPLC). In order to optimize the chromatographic separation condition, the selection of suitable mobile phase composition and stationary phase is very important.

The retention of non-polar/non-ionic compounds on the C18 columns increases with increasing the hydrophobicity (or increasing alkyl chain length) of the column stationary phase. In addition, the retention of high polar compounds can be achieved by adjusting the pH value of mobile phase, since the high polar compounds do not retain on C18 columns. For these reason, C18 phase among the RP columns is widely used for analytical separations of most compounds including polar ones. In this study, the non-polar/non-ionic compounds showed suitable retention on the C18 column without the pH adjustment of the mobile phase. However, the high polarity substance groups such as carboxylic acids, amines and alcohols did not show retention. As described above, for the retention of these substance groups the mobile phase composition with adjusted pH will be needed. However, this adjustment of mobile phase composition brings the contradictory problem that the retention of non-polar substances may be prolonged too much [Neue et al. 2006].

Organic modifiers in water or aqueous buffer solutions are usually used as the mobile phases for RP-HPLC system. Acetonitrile and methanol are most preferable organic solvents as organic modifiers. The use of acetonitrile in the mobile phase composition provides more symmetrical peak shapes and lower column back-pressure in comparison with methanol because of its lower viscosity [Kromidas 2005]. In addition, acetonitrile gives rise to lower baseline-noise on CAD than other organic modifiers for RP-HPLC analysis [Moreau 2006] and thus the CAD sensitivity would be improved by decreasing the baseline-noise level. Acetonitrile cannot give sufficient selectivity for the separation of non-polar/non-ionic compounds. For this reason, a gradient elution using adequate aqueous proposition in mobile phase composition can provide more improved selectivity. Since isocratic elution is impossible for the separation of the test substances with various physico-chemical properties in one chromatographic run, gradient elutions composed with acetonitrile and water were

tested in this study. The gradient elution chromatography is a powerful tool for chemical analysis due to its broad range of retentivity, high peak capacity, high resolving power and short operation cycle [Anita and Horvath 1989, Truei et al. 1992]. In this study, the higher organic content in mobile phase (gradient condition A) resulted in a decrease of resolution of the substances that have a relatively high polarity (Figure 5-2). On the contrary, the use of higher aqueous mobile phase composition (gradient condition B) was suitable and useful for separating most of the test substances except high polar and ionic compounds such as carboxylic acids and docusate sodium (Figure 5-3).

Volatile substances cannot be detected by a charged aerosol detector (CAD). In this study volatile or semi-volatile substances with the molecular weight below 300 g/mol and the vapor pressure above 10^{-5} Pa were not detected by the CAD. This is in agreement with the finding of McCarthy et al. [2005], who reported that the responses of the volatile and semi-volatile compounds were poor or there was no response at all on CAD. They explained the reason of this result by the evaporation step in the CAD. In this step a significant portion of the compound is evaporated prior to detection. Although this is a limitation of the CAD, the volatile or semi-volatile substances can be detected and quantified with complementary techniques, such as GC-FID or GC-MS.

In order to evaluate the sensitivity of the CAD between two gradient conditions with different water contents (gradient condition A and B), LOD (signal to noise ratio of 3:1) values were determined according to the German standard DIN 32645 [DIN 32645 1994]. The response of CAD depends on the organic content in mobile phase composition, with higher response observed at higher organic content [Garmache et al. 2005]. As discussed above, the use of acetonitrile in mobile phase composition can reduce the baseline-noise on CAD. However, the CAD baseline-noise would be increased with increasing water content in the mobile phase composition, since water gives rise to higher baseline-noise on CAD [Moreau 2006]. According to Górecki et al. [2006], the peak areas at 90% acetonitrile were nearly five times greater than at 10% acetonitrile. Comparing the sensitivity (or LOD) of the CAD under two mobile phase conditions it was confirmed that the sensitivity of the CAD is somewhat influenced by water content in mobile phase compositions. However, the LOD values of the individual substances under two mobile phase compositions were distributed within similar range. The CAD under the gradient condition B (40 – 0 % water in 50 min) was able to attain LOD values in the range from 9 to 71 ng injected on the column. This is in good accord with the minimum LOD value range reported by the manufacturer of CAD instrument, with low ng limits of detection [ESA Inc online].

The correlation coefficient (r) is commonly used to evaluate the linear correlation between two variables and a calibration curve with $r \geq 0.995$ is usually considered to be linear [Van Loco et al. 2002]. As discussed in chapter 3.2, the linearity of the calibration curve is very important for the semi-quantitative approach of an unknown analyte. The linear calibration curve means the constancy of response factors in a calibration range and the average of response factors in a calibration range can be used to calculate the correct concentration of a known analyte. Some researchers have been reported the nonlinearity of calibration curve plotted by CAD response [Nair and Werling 2009, Vervoort et al. 2008]. In this study, most calibration curves using an internal standard (Tinuvin 234) under the gradient condition B showed linear correlation with $r \geq 0.995$. However, some plasticizers (Diethylhexyl phthalate, diethylhexyl adipate, dipropylene glycol dibenzoate and propylene glycol dibenzoate) showed a quadratic slope (see chapter 9.1.1). This means that these substances did not show a consistent response within the calibration range.

Many commonly used additives, e.g. Irganox PS 800 and PS 802, do not have a chromophorous group in their molecular structures and therefore cannot be detected by an UV detector [Arpino et al. 1990]. The universality of detection independent of physico-chemical properties of the analytes is the most distinctive advantage of CAD and it could be verified by the analysis of Irganox PS 800. The universality of CAD has been already confirmed by many researchers. Although poly(ethylene glycol) [Takahashi et al. 2008] and free fatty acids (Linolenic, linoleic, palmitic, oleic, stearic) [Nair and Werling 2009] have no UV- active structures, the substances could be detected by CAD with high sensitivity.

In conclusion, the reversed phase HPLC separation combined with CAD is powerful tool for the screening of the non-volatile adhesive related migrants, with sufficient separation efficiency, sensitivity and linearty.

6.1.2. HPLC-CAD relative response factors

The relative response factor (RRF) of 1 defines that an unknown substance and internal standard at identical concentrations have the same analytical responses. Therefore, the RRF values between 0.8 and 1.2 can be generally recognized as an ideal range [Kazakevich and LoBrutto 2007, Burgard and Kuznicki 1990]. If an unknown substance has an RRF value out of this range, the concentration of the unknown substance would be overestimated or underestimated. The response of CAD can not be simply interpreted. That is, it could be

multiply affected by some parameters such as the content of organic solvent in the mobile phase and the molecular weight and volatility (vapor pressure) of substances.

According to the general theory of the charged aerosol detector (CAD), a lower sensitivity would be expected, when a substance is eluted with lower organic solvent content, since a decrease of the organic content in the mobile phase leads to a decrease in the transport efficiency of the nebulizer [Górecki et al. 2006]. Several studies comparing CAD with other detectors such as UV, MS and ELSD and practical applications of CAD have been reported [Brunelli et al. 2007, Gamache et al. 2005, Forsatz and Snow 2007, Cascone et al. 2006, Sun et al. 2008, Lísa et al. 2007, Schönherr et al. 2009, Takahashi et al. 2008, Pistorino and Pfeifer 2008, McCarthy et al, 2005]. The majority of these publications reported that the CAD response increases with increasing the organic solvent content in the mobile phase. However, in these studies, the molecular weights and vapor pressures of most test substances used for the experiments were smaller than 400 g/mol and greater than 10^{-8} Pa level respectively. Therefore, the CAD response properties for the substances with a molecular weight higher than 400 g/mol were not broadely interpreted in these studies.

CAD provided nearly consistent and universal response to most non-volatile and/or semi-volatile compounds irrespective of chemical structure in an isocratic analysis according to Sun et al. [2008], but in a gradient analysis the response was dependent on the mobile phase compositions [Brunelli et al. 2007, Gamache et al. 2005, Górecki et al. 2006]. That is, the response increases with increasing the ratio of organic solvent in the mobile phase. As mentioned in chapter 5.2.1, although the limit of detection (LOD) values of the substances detected were somewhat influenced by the content of organic solvent in mobile phase, on the whole, there was statistically no significant difference between the CAD relative responses of the test substances obtained from two different mobile phase compositions (Table 5-2).

The RRF (w/w) values of the substances with a molecular weight higher than 400 g/mol were extremely variable from 0.83 to 2.44 in the gradient condition B. Especially, the substances which eluted with 100 % organic mobile phase during separation run-time such as Irganox 1330, Irganox 565, Irganox PS 800, Irganox 1076 and Irgafos 168 showed very higher responses than other substances with molecular weight above 400 g/mol and/or low vapor pressure less than 10^{-9} Pa. The RRF (w/w) values of these substances were from 1.82 to 2.61 in the gradient condition A and from 2.04 to 2.44 in the gradient condition B, respectively. This could not be clearly interpreted by the difference of the organic content in the mobile phase composition, because Tinuvin 327, Ultranox 626 and Irganox 1010 were likewise eluted with 100 % organic solvent in the gradient condition B and showed lower

RRF (w/w) values in the gradient condition A than in gradient condition B. In spite of high molecular weight and low vapor pressure, Irganox 1330, Irganox 1010 and Ultranox 626 showed poorer responses in gradient condition A than in gradient condition B with lower organic content and the difference between the two was significant. A reason could not be rationalized by the general detection principle of CAD. According to the principle of CAD, non-volatile particles formed in drying tube are charged by ionized nitrogen and finally detected by a sensitive electrometer. Here, the magnitude of CAD response depends on the size of the analyte particles charged by ionized nitrogen and the maximum response per particle mass could be achieved at particle diameter range of 10 ~ 32 nm [Dixon and Peterson 2002]. For the reason, the response factors of the substances detected on CAD would be mainly influenced by the size of the formed particles in this process. However, the particle formation process according to the physico-chemical properties of the substances has not been clarified yet. Therefore, we could not find the reason why the RRF values of the substances detected on CAD were highly variable. And also, the RRF values did not show a linear correlation with molecular weights of the detected substances in this study (Figure 5-6 and 5-8). Gamache et al. [2005] have determined the response factors of 735 pharmaceutical compounds with the molecular range from 168 to 684 g/mol and with a wide range of chemical structures using CAD. As the results of their study, the response factors were highly variable with more than 50 % RSD (relative standard deviation).

In order to correct the differences in CAD response to the substances, relative molar response factors (RRF mol/mol) can be also used as a correction factor. For reasons mentioned above, the relative molar response factors (RRF mol/mol) of the detected substances did not show a linear correlation with molecular weights (Figure 5-7 and 5-9).

When plotting the ratio of areas of some test substances to internal standard (Tinuvin 234) versus the concentrations of the substances (DEHP, DEHA, DPGDB and propylene glycol dibenzoate) with molecular weights smaller than 400 g/mol, the calibration curves did not show a linear correlation (see chapter 9.1.1) and the calibration line did not pass through the origin of the x and y-axis. This means that the RRF values of the substances are not consistent at the calibration concentration range from 0.1 µg/ml to 50 µg/ml. Therefore, the RRF values (w/w) of four substances in the calibration range cannot be used to establish the distribution range. Additionally, the sensitivities of these substances were lower than other substances with a molecular weights of more than 400 g/mol. It can be therefore concluded that the responses of the test substances on CAD were definitely influenced by the molecular weights and/or volatilities of the substances.

In conclusion, the RRF values of the test substances were highly variable. However, the reason could not be clearly demonstrated, since the particle formation process in CAD depending on the physico-chemical properties of the substances is not clear yet. Nevertheless, HPLC-CAD combination showed sufficient sensitivity and consistent response in a defined calibration range for the substances with molecular weight of more than 400 g/mol or vapor pressure smaller than 10^{-9} Pa independent of mobile phase composition. Therefore, a HPLC-CAD system equipped with a C18 column can be a powerful analytical tool and the RRF values determined by this technique can be used for the semi-quantitative approach of non-volatile unknown substances migrated from food packaging materials containing adhesive layers.

6.1.3. SEC×HPLC-CAD two-dimensional separation

As discussed in chapter 3.3.5, the conventional one-dimensional chromatography using one separation column and isocratic or gradient elution does not provide sufficient separation efficiency for the complex mixtures. Online two-dimensional separation technique is very useful to improve the separation efficiency. In this study, a SEC×NP-HPLC system combined with an universal detector, charged aerosol detector (CAD), was investigated to improve the peak capacity and resolution.

According to the supplier [Phenomenex technical notes 2000], the swelling volume of the packing materials (S-DVB gels) in dichloromethane is 60 %, but 30 % in n-hexane. In contrary, the dichloromethane : n-heptane mobile phase mixtures showed symmetrical peak shape with narrow peak width (Figure 5-12). It might be concluded that the packing material in SEC column has similar swelling volume in n-heptane as in dichloromethane. The swelling of packing materials (S-DVB gels) influences the peak shape. Some authors [Stuurman and Köhler 1987, Bowers and Pedigo 1986] suggested that the peak shape could be improved due to increasing of the swelling volume of packing material (S-DVB gels) in columns by the presence of THF (70 – 80 % swelling volume). Ells et al. [1999] have demonstrated that the swelling of packing material by THF contributes to reduction of sample peak width and tailing. Table 6-1 shows the swelling characteristics of S-DVB gels packed in nonaqueous SEC column for several organic solvents.

Table 6-1. Swell volume of the packing materials in SEC column depending on the organic solvents. [Phenomenex technical notes 2000].

Swell volume (%)	Organic solvents
30	Acetonitrile, Cyclohexane, *n*-Hexane, *Iso*-Propanol, *n*-Butyl Alcohol, Methanol
50	Acetone, m-Cresol, *o*-Chrolophenol, Dimethyl Formamide, Dimethyl Acetamide, n-methyl pyrrolidone, Dioxane
60	Ethyl Ether, Methylene Chroride, Methyl ethyl Ketone, Cycloheptane, Ethyl Acetate
70-80	Toluene, THF, *p*-Xylene, Chloroform, Cyclopentane, Benzene, Pyridine, *o*-dichlorobenzene

N-heptane is known as strong solvent in normal phase chromatography compared to water in reversed phase (RP) chromatography and therefore better peak resolution was obtained by the mobile phase NP-composition B.

The two-dimensional separation system has a maximum peak capacity when the selectivity of the separation systems is fully independent (or orthogonal) [Stoll et al. 2007]. Figure 6-1-A shows that the area of the bins covered by the normalized data points is 10 %. This represents 0 % orthogonality of a two-dimensional separation system. On the contrary, Figure 6-1-C shows an ideal orthogonal separation. Here, the coverage area by the normalized data points is 63 % representing 100 % orthogonality.

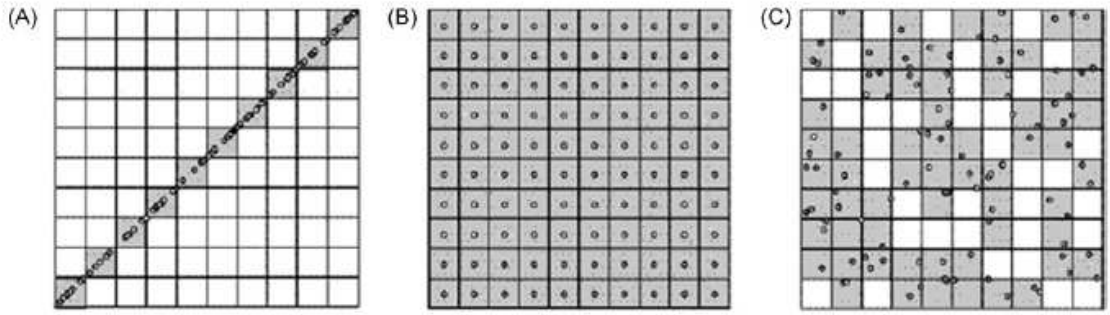


Figure 6-1. Geometric orthogonality concept. Hypothetical separation of 100 analytes in 10 × 10 normalized space. (A) Nonorthogonal system, 10 % area coverage represents 0 % orthogonality. (B) Hypothetical ordered system, full area coverage. (C) Random, ideally orthogonal system, area coverage is 63 % representing the 100 % orthogonality.

The orthogonality of a two-dimentional separation system is estimated by the coverage area (%) of the rectangular bins that contain peaks detected. The degree of orthogonality (coverage area) can be calculated according to equation 17 [Gilar et al. 2005].

$$O\,(\%) = \frac{\sum bins - \sqrt{P_{max}}}{0.63 P_{max}} \times 100 \text{ ---------- Equation 17}$$

O : orthogonality percent (%)

$\sum$bins : number of bins containing the detected peaks

P_{max} : total peak capacity obtained as a sum of all bins

Theoretically, the combination of SEC×NP-HPLC would provide good orthogonality, since the separation mechanisms are highly different. The plots of the normalized retention times of all peaks eluted from the 11 additives in both dimensions were constructed in figure 6-2. The 15 peaks originated from the 11 additives were evenly distributed onto 3×5 normalized separation space. The degree of orthogonality was calculated according to equation 17. The number of bins used for separation was 10 out of 15 bins. Thus the degree of the orthogonality for SEC×NP-HPLC was 65 %. However, the number of test compounds was very small for the estimation of the orthogonality. Thus the orthogonality between both dimensions could not be practically and accurately estimated by such geometric approach. According to Liu et al. [2008], the more complex of the sample, the more orthogonality of two-dimensional chromatography system would be improved in such geometric orthogonality estimation, since normalized retention points of all peaks detected on a two-dimensional separation system would be filled into the bins. That is, the number of bins for the partition of the two-dimensional separation space would be increased with increasing the number of the detected peaks.

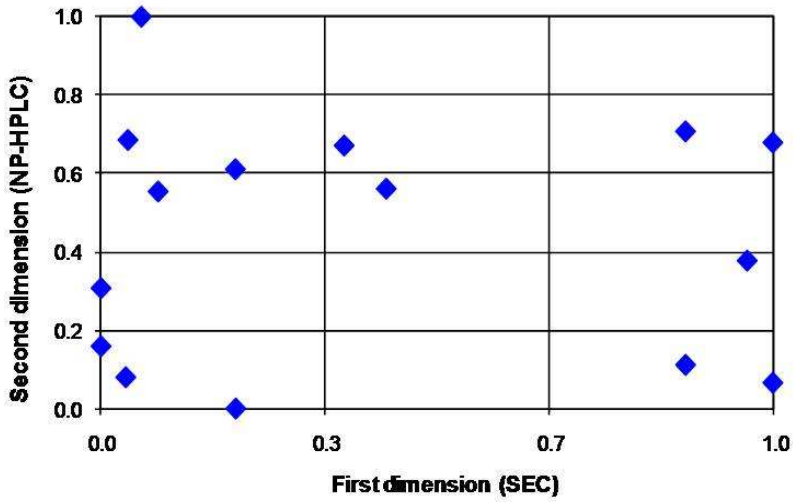


Figure 6-2. Normalized retention time plot for SEC×NP-HPLC two-dimensional separation system.

According to the theoretical assumption, the combination of SEC×NP-HPLC can provide good orthogonality. Thus the total peak capacity for the first and second dimension can be calculated according to equation 11 in chapter 3.3.5. The number of peaks for the first dimension, which was connected two columns in series, was 22 and that of the Diol column for the second dimension was 182. Thus the total peak capacity of the SEC×NP-HPLC separation was 4004.

$$P_{2D} = P1 \times P2 = 22 \times 182 = 4004$$

P_{2D} : the peak capacity for two-dimensional chromatography
P1 : the peak capacity of first dimension
P2 : the peak capacity of second dimension

As described above, the total peak capacity in the SEC×NP-HPLC two-dimensional separation will be markedly improved compared to the conventional one-dimensional ones. However, the 100 % orthogonality in two-dimensional chromatography is extremely rare. For the reason, Gilar et al. [2005] have calculated a practical peak capacity as the theoretical peak capacity according to equation 18. In this study, the practical peak capacity of the SEC×NP-HPLC system was 2683.

$$N_p = P_{2D} \frac{\sum bins}{P_{max}} = 4004 \times 0.67 = 2683 \text{ ---------- Equation 18}$$

P_{2D} : the peak capacity for two-dimensional chromatography
$\sum$bins : number of bins containing the detected peaks
P_{max} : total peak capacity obtained as a sum of all bins

The theoretically calculated peak capacity is only the estimation of the separation efficiency and they often tend to overestimate the separation power of the system in real analyses [Kivilompolo and Hyötyläinen 2007]. However, it was obvious that clearly higher separation efficiency can be obtained by the SEC×NP-HPLC two-dimensional system than by a conventional one-dimensional system.

The separation efficiency of the SEC column for the first dimension is limited. Several identical SEC columns in series are connected to improve the peak capacity [Gilar et al. 2005]. In this study, two identical SEC columns in series were connected. However, the efficiency of

the SEC dimension was not improved. The total analysis time in the first dimension (SEC) for the 11 additives was 7 min. That is, the number of fractions collected from the first dimension was limited. As a result, the overall peak capacity was reduced. Therefore, it needs to improve the overall separating power. For this, other combinations like NP×SEC, other SEC columns and the connection of three or more SEC columns can be considered for the further study. In the recent studies for polymer analysis, NP×SEC combination is mainly applied to improve the overall separating power by using gradient elution in the first dimension [Van der Horst and Schoenmakers 2003] and to characterize the fractions transferred from the first dimension [Berek 2010].

The main disadvantage of LC×LC (liquid chromatography × liquid chromatography) is that there is currently no a commercial data processing software for qualification and quantification available. The peaks obtained from two-dimensional chromatographic analysis are integrated as contour plot or peak volumes and the quantification can be usually performed by the calculation of peak volume. Data acquisition and handling for the quantitative GC×GC (gas chromatography) analysis has been performed by some developed commercial software, for instance HyperChrom GC×GC Data Interpretation Software (Thermo Scientific). However, the quantification tools in LC×LC are limited [Kallio et al. 2009]. Many researchers have therefore used in-house written software [Murphy et al. 1998]. From the above mentioned reason, the quantification procedures could not be performed in this study and would need further research activities.

6.2. Development of screening methods by GC-FID

6.2.1. GC-FID analysis

The non- or semi-polar substances were successfully analyzed with sufficient response by a GC-FID system equipped with a DB-1 non-polar column.

The polar substances like alcohols (glycol, polyol), amines and carboxylic acids showed a bad peak shape and therefore low sensitivity. The essential reason of these results is that the DB-1 column is coated with a non-polar stationary phase (dimethylpolysiloxane). For analysis of polar compounds, the polar stationary phase columns are often chosen because of their column deactivating properties and miscibility with polar solutes. Peaks that tailed or that are irreversibly adsorbed on non-polar columns are often eluted with good peak shape when a polar stationary phase is used [Klee 1985].

The peaks of all substances containing carboxylic functional groups were not detected in the calibration range. The analysis of carboxylic acids using GC is generally difficult because of their low volatility, strong adsoption on stationary phase and/or dimerisation of acid molecules [Waksmundzka-Hajnos 1998]. Therefore, for the analysis of carboxylic acids using GC system, polarity should be lower and volatility should be improved through a derivatisation procedure like esterification by using diazomethane and/or the reaction of silver salts of carboxylic acids with methyl iodide. Pyrolysis of carboxylic acids can also be used to obtain more volatile fragments of the analytes. However, for a sensitive analysis of carboxylic acids, the most general method is analysis by liquid chromatography (LC) such as anion-exchange and ion-exclusion chromatography [Waksmundzka-Hajnos 1998, Liebich et al. 1980, Destandau et al. 2005].

The DB-FFAP column which were selected for the simultaneous analysis including the high polar substances is a specialized separation column to analyse the volatile free acids [Hewlett Packard Application Note 1998]. The FFAP (free fatty acid phase) is generally applicable for the analysis of acidic compounds such as organic acids, phenols, alcohols, amides, N-acrylamino acids, all type of esters, ketones, lactones and more, but it is not suitable for alkaline compounds and aldehydes, since these compounds may be adsorbed on the FFAP or may react with the terminal groups of the stationary phase [Rotzsche 1991, Sandara 2002]. This explains that the amines were not detected on the FFAP phase. The DB-FFAP column has a lower usable temperature range (up to 260 ˚C) compared to DB-1. The substances with high boling points in the list of representative adhesive related subtances could not be

measured on this column. Compared with DB-1, the only advantage of DB-FFAP column is that the sensitivity to the alcohol compounds was significantly improved. This advantage was considered to the too small in relation to the effort of using a second method for the screening.

Consequently, the screening method using the combination of GC-FID and DB-1 column is applicable to a broad range of substances except highly polar substances such as amines, alcohols and carboxylic acids and the method covers unknown susbtances migrating from packaging materials and adhesives in a molecular range of approximately 100 to 800 g/mol.

6.2.2. GC-FID relative response factors

Theoretically, the response of the FID shall be proportional to the mass of carbon per time unit passing the detector [Jorgensen et al. 1990]. It produces a signal for all carbon atoms present in compounds that elute from the GC column. However, substantially, the FID response is not exactly proportional to the carbon number and therefore FID is not equally sensitive to the different compounds. Several studies have been reported on the FID response and its influence parameters. The response of FID is influenced by the heteroatoms present in various functional groups such as oxygen, nitrogen and halogens [Tong and Karasek 1984, Jorgensen et al. 1990, Kállai and Balla 2002], furthermore by the operating conditions of the GC-FID systems, for instances column stationary phase, detector temperature, injection mode (split or splitless) and injector temperature [Dressler and Cigánek 1994, Cicchetti et al. 2008]. In addition to these influence factors, the FID response is also influenced by the choice of an internal standard [Blanco et al. 1992].

Consequently, as described in chapter 5.4.4, the FID showed lower responses for the compounds with heteroatoms. Also the polarity of the compounds containing a heteroatom is relatively higher than that of pure hydrocarbon compounds. Tong and Karasek [1984] reported that the substitution of a heteroatom on a hydrocarbon lowers the FID response of the parent compounds but with increasing molecular weight the influence of the heteroatom on the FID response becomes less significant, and the response factor approaches that of the parent compound. This tendency is in good accord with the experimental results given in table 6-2. The RRF values of the heteroatom compounds in the list of which a part in molecular structures was substituted with heteroatoms (N, S, or P) including oxygen (O), relatively increased with increasing molecular weights or carbon number. However, in spite of its high molecular weight (MW : 445 g/mol, RRF : 0.44), the docusate sodium on FID showed lower response than other substances with similar molecular weight or carbon number. Since the

docusate sodium is an ionic compound, it could be strongly absorbed on the DB-1 stationary phase, with the reponse decrease in consequence of the peak tailing. However, peak tailing was not observed (Figure 5-20). Therefore, the response of the docusate sodium on FID was more influenced by heteroatoms in the molecular structure, because it was substituted with much more heteroatoms than other substances (Table 6-2).

Table 6-2. Change of RRF values according to number of heteroatom and of carbon in molecular structure.

Substances	Heteroatom / Number	Carbon Number / MW (g/mol)	RRF via BHA
Carprolactam	N / 1, O / 1	6 / 113.16	0.69
1-Vinyl-2-pyrrolidone	N / 1, O / 1	6 / 114.14	0.72
2-Octyl-2H-isothiazol-3-one	N / 1, S / 1, O / 1	11 / 213.34	0.82
2-Ethylhexyl diphenyl phosphate	P / 1, O / 4	20 / 362.44	0.81
Docusate sodium	Na / 1, S / 1, O / 7	20 / 445.63	0.44
4,4'-Bis(diethylamino)benzophenone	N / 2, O / 1	21 / 324.46	0.93
Uvitex OB	N / 2, S / 1, O / 2	26 / 430.06	0.94
Irgafos 168	P / 1, O / 3	42 / 646.93	1.16

All acrylic ester compounds shown in Table 6-3 have two heteroatoms (oxygen) in molecular structure and the RRF values were increased with increasing the carbon number. According to Jorgensen [1990], if the substances have heteroatoms of same number in molecular structure, the increase of carbon number is accompanied with the increase of RRF values.

Table 6-3. Change of RRF values of the 7 acrylic esters according to number of heteroatom and of carbon in molecular structure.

Substances	Heteroatom / Number	Carbon Number / MW (g/mol)	RRF via BHA
Methyl acrylate	O / 2	4 / 86.09	0.71
Ethyl acrylate	O / 2	5 / 100.11	0.64
Methyl methacrylate	O / 2	5 / 100.11	0.67
Ethyl methacrylate	O / 2	6 / 114.14	0.82
Butyl acrylate	O / 2	7 / 128.18	0.90
Butyl methacrylate	O / 2	8 / 142.19	0.96
Ethylhexyl acrylate	O / 2	11 / 184.28	1.25

Generally, compounds with -OH groups show a good response on FID using an appropriate polar stationary phase column. The DB-1 column has a non-polar phase

(dimethylpolysiloxane). Polar substances like alcohols (glycol, polyol) or amines showed on DB-1 a bad peak shape (Figure 5-20), low sensitivity and low RRF values.

Bronopol is widely used as a biocide in adhesives. Bronopol is easily transformed to formaldehyde in aqueous solution [Wang et al. 2002, Lian et al. 1997]. Rapid hydrolysis may occur at increased temperature and/or higher pH [US EPA 2006a]. Bronopol contains an alcohol functional group, a halogen element (Br) and a heteroatom N. It showed a bad response on FID regardless of selection of various columns, because FID is insensitive to halogens such as F, Cl, Br and I. Furthermore, Bronopol may be decomposed in the injector because of the increased temperature. Figure 5-22 shows two chromatograms for Bronopol that were analyzed with two different columns (DB-1 and DB-FFAP). Several peaks were eluted from both chromatograms.

Melamine did not show a FID response in the calibration range, because of its high polarity caused from polar functional group and many heteroatoms (six nitrogen atoms) in the molecular structure. Especially, the high polarity was attributed to the strong interaction between amines and DB-1 stationary phase, and therfore this interaction prohibited the amines from reaching the FID. Additionally, the lack of carbon number in the molecular structure caused furthermore lower sensitivity on FID. The FID response of carboxylic acids could be also rationalized by the same reasons.

Therefore, the multi-screening method using the DB-1 column combined with FID was not suitable to obtain an adequate retention, enough response and symmetric peak shape for the high polar substances such as amines, multifunctional alcohols and carboxylic acids. Furthermore, the presence of “heteroatom” in a molecular structure decreased the FID response.

The RRF values of alcohols varied between 0.14 and 0.45 except resorcinol (0.67). The bi- and trifunctional aliphatic alcohols among the alcoholic substances were not detected with sufficient response on the GC-FID system equipped with a DB-1 column because of the presence of low carbon numbers (low molecular weight) and more than two -OH groups. Resorcinol (Benzene-1,3-diol) is an aromatic alcohol and has a larger carbon number (6 carbon atoms) than the other investigated alcohols (2 ~ 4 carbon atoms). These should enhance the response. Especially, glycerol (RRF : 0.14) with three –OH groups and three carbon atoms in molecular structure showed the lowst response among the alcoholic substances and did not show the response under the calibration standard concentration of 50 µg/ml.

If all RRF values including that of alcohols are used for the establishment of a distribution range at 95% coverage level, it would be a worst case for the conservative concentration estimation, since the response of alcohols are insufficient on the DB-1 column. Therefore, the RRF values of the alcoholic substances should be excluded for the estimation of distribution range.

In conclusion, the GC-FID system equipped with a DB-1 non-polar column showed sufficient sensitivity and consistent response for most representative adhesive related substances except some high polar substances. Therefore, the RRF values obtained from this analytical tool could be used for the semi-quantitative approach of volatile or semi-volatile unknwon substances migrated from adhesive layers in food packaging materials.

6.2.3. Estimation of molecular weight by retention time

The DB-1 column coated with dimethylpolysilixane separates according to the boiling point (volatility) differences of the analytes [Golby 1999]. The boiling point correlates to the molecular weight and therefore the retention time and molecular weight of the representative adhesive related substances showed a good linear relation on DB-1 column. Franz et al. [2004] reported that the retention times of various substances from polar to non-polar were correlated with the molecular weight on DB-1 column using volatiles in Headspace-GC analysis.

On the contrary, the separation of the DB-FFAP column coated with 100 % polyethyleneglycol esterified by nitroterephthalic acid occurs according to direct interaction between the analyte and stationary phase. That is, the analytes can be mainly separated by polarity differences between stationary phase and analyte. According to Golby [1999], the compounds on a polar stationary phase are also separated by the volatility of analyte, but more emphasis is that it depends on the molecular interaction between analyte and stationay phase including the forces such as dipole-dipole, induced dipole-dipole interactions, Van der Waals and hydrogen bonding. Consequently in this study the test substances on DB-FFAP did not show correlation between retention time and molecular weight.

As mentioned in chapter 1.3, the molecular weight for theoretical migration estimation is one of the most important parameters, since the migration quantity decreases with the increase of molecular weight of the migrating substances [Figge 1996, Piringer and Baner 2008 b]. The representative adhesive related substances have a wide molecular weight range from 62 to 1177 g/mol and various physico-chemical characteristics. Therefore, the correlation of the

retention time with the molecular weight on the DB-1 column can be used for the estimation of the molecular weight of a not identified substance. Together with a semiquantitative estimate of the concentration, mathematical modelling of the migration of not identified substances gets possible.

6.2.4. Characterisation of unknown substances by linear retention indices

Retention time, relative retention time and retention indices are important parameters in qualitative analysis. However, the relative retention time is only reproducible within a single GC system and the retention time in GC analysis is strictly related to the measurement parameters, because it is influenced by the operational conditions such as phase ratio and column length, gas flow rate and temperature programm [Peng 1994, Zellner et al. 2008].

The linear retention indices depend on the stationary phase of the column [Goodner 2008, Peng 2000]. In this study, the retention indices determined on the DB-FFAP column were significantly different from those on the DB-1 column (Table 5-7). It is therefore clear that the linear retention indices are influenced by the type of stationary phase and the structure of analyte.

The different GC systems and operating conditions besides column stationary phase did not influence the retention indices [Peng 1994, Goodner 2008]. A comparison of the retention indices from literature [Peng 1988, Deutsche Forschungsgemeinschaft (DFG) 1992, Kondjoyan and Berdagué 1996, Gramshaw et al. 1995] and the mean values of the retention indices calculated from experimental data are given in table 6-4 and figure 6-3. The linear retention indices of 85 compounds including 45 adhesive related substances from this study were obtained from the published literatures and compared with the experimental retention indices. The additional 40 substances for the comparison with the experimental retention indices derive from various chemical classes such as hydrocarbons, alcohols, amines, esters and more that cover the whole range of physico-chemical properties from volatile to non-volatile, from polar to non-polar. All of these retention indices were determined on DB-1 and equivalent stationary phase (100 % dimethylpolysiloxane). The experimental retention indices of total seven substances including three acrylates, triacetin, diethylene glycol dibenzoate, 2-ethylhexyl diphenyl phosphate (phosflex 362) and propylene glycol were markedly different from the literature data with ± 20 retention index units. However, on the whole, the correlation between retention indices and molecular weights of the experiment data was in

good accord with those of the literature data (Figure 6-3). Especially, the hydrocarbons such styrene, *para*-xylene and alpha-methylstyrene showed same retention index units.

The comparison of the retention indices and molecular weights or carbon numbers revealed a linear relation between them and the retention indices were not influenced by the different GC system and the operating conditions. Therefore, the characterization such as molecular weight or carbon number of unknown substances in migration solutions could be estimated by using the linear retention indices. Because of their independence from the analytical parameters they are better suitable than the retention time.

Table 6-4. Comparison of retention indices of the representative adhesive related substances on same apolar columns with reference surveys.

Classification	No.	Substances	Molecular weight (g/mol)	Linear Retention Indices		
				Experiment	Literature	
					Retention indices	Literature Nr.
Group A acylate	1	Methyl acrylate	86.09	-	591	1
	2	Ethyl acrylate	100.11	621	683	1
	3	Methyl methacrylate	100.11	628	694	4
	4	Ethyl methacrylate	114.14	673	773	1
	5	Butyl acrylate	128.18	877	883	1
	6	Butyl methacrylate	142.19	964	967	1
	7	Ethylhexyl acrylate	184.28	1212	-	-
Group B Plasticizers	8	Glycerol triacetate	218.2	1308	1282	2
	9	Diisobutyl phthalate	278.35	1821	1835	2
	10	Dibutyl phthalate	278.35	1911	1913	2
	11	Propylene glycol dibenzoate	284.3	2091	-	
	12	Diethylene glycol dibenzoate	314.34	2390	2445	2
	13	Dipropylene glycol dibenzoate	342.42	2406	-	-
	14	2,2,4-Trimethyl-1,3-pentanediol dibenzoate	354.45	2439	-	-
	15	Triethylene glycol dibenzoate	358.4	2671	-	-
	16	Phosflex 362	362.44	2368	2450	2
	17	Diehtylhexyl adipate	370.57	2371	2381	2
	18	Diethylhexyl phthalate	390.56	2502	2507	2
Group C Alcohol	19	Ethylene glycol	62.06	621	< 1000	2
	20	Propylene glycol	76.1	639	753 / < 1000 / 726	1 / 2 / 4
	21	1,4-Butanediol	90.12	916	< 1000 / 922	2 / 5
	22	Glycerol	92.09	979	-	-
	23	Diethylene glycol	106.12	933	-	-
	24	Resorcinol	110.11	1249	1258	2
Group D Amines	25	Hexamethylene diamine	116.21	1064	-	-
	26	Toluene –2,4 –diamine	122.17	1333	-	-
	27	4,4-Methylenedianiline	250.25	2046	-	-
Group E Antioxidants	28	2,6-Di-*tert*-butyl-4-methylphenol	220.35	1488	1488 / 1505 / 1439 / 1491	1 / 2 / 3 / 4
	29	Irganox 1076	531	3564	-	-
	30	Irgafos 168	646.93	3389	-	-
	31	Irganox 1330	775.21	-	-	-
Group F Others	32	Vinyl propionate	100.12	615	-	-
	33	Styrene	104.15	869	< 1000 / 868	2 / 4
	34	*para*-Xylene	106.17	853	875 / 853 / 850	1 / 3 / 4
	35	Caprolactam	113.16	1191	-	-
	36	1-Vinyl-2-pyrrolidinone	114.14	1052	-	-
	37	α-Methylstyrene	118.18	962	963	4
	38	Benzophenone	182.23	1577	1611 / 1610	1 / 2
	39	2-Octyl-2H-isothiasol-3-one	213.34	1781	-	
	40	Butyl diglycol acetate	204.27	1333	-	
	41	Bisphenol A	228.29	2099	2155	2 /
	42	4,4'-Bis(diethylamino) benzophenone	324.46	3027	-	-
	43	BADGE	340.42	2804	-	-
	44	Uvitex OB	430.06	3791	3750	2 /
	45	Docusate sodium	445.63	2208	-	-
Additional substances for the comparison of retention indices	46	Ethyl amine	45.09	-	413 /	1
	47	1,3 Butadiene	54.09	-	395	2
	48	1,3-Propanediol	76.09	-	814	5
	49	Benzene	78.11	-	642 / 640	3 / 4
	50	Acetic acid, ethyl ester	88.11	-	599	3
	51	Methyl propionate	88.11	-	613 / 618	1 / 3
	52	Toluene	92.14	-	764 / 752 / 741	1 / 3 / 4
	53	Aniline	93.12	-	955	1
	54	Diethanolamine	105.14	-	1075	2
	55	Ethyl benzene	106.17	-	846	5
	56	m-xylene	106.17	-	871 / 851 / 850	1 / 3 / 4
	57	o-Xylene	106.17	-	895	1
	58	Naphthalene	128.17	-	1190 / 1156 / 1152	2 / 3 / 4
	59	2-Ethylhexanol	130.23	-	1015 / 1014	2 / 3
	60	Dipropylene glycol	134.17	-	1008	4
	61	Limonene	136.24	-	1053 / 1020	2 / 3
	62	Trimethtyl phosphate	140.07	-	1000 / 854	2 / 6
	63	o-phthalic anhydride	148.12	-	1322	2
	64	Triethylene glycol	150.18	-	1230	2
	65	4-hydroxy-3-methoxybenzaldehyde	152.15	-	1630	2
	66	Dipheylamine	169.22	-	1537 / 1595	1 / 2
	67	2-Phenylphenol	170.21	-	1550	2

Continued table 6-4

Additional substances for the comparison of retention indices	68	Dimethyl adipate	174.19	-	1213 / 1205	2 / 6
	69	Triethylphosphate	182.16	-	1109 / 1091	2 / 6
	70	Dimethyl isophthalate	194.18	-	1488	2
	71	Dimethyl phthalate	194.19	-	1406 / 1425	2 / 6
	72	Diethyl phthalate	222.24	-	1564 / 1558	2 / 6
	73	Diallyl phthalate	246.25	-	1712 / 1708	2 / 6
	74	Dipropyl phthalate	250.30	-	1746	2
	75	Dicyclohexyl phthalate	266.29	-	2461 / 2472	2 / 6
	76	Tributyl phosphate	266.32	-	1690 / 1690	2 / 6
	77	Dibutyl terephthalate	278.34	-	2066	2
	78	1,4-butanediol dibenzoate	298.33	-	2400	2
	79	Dibenzyl phthalate	346.38	-	2690	2
	80	2-(4-tert-butylphenyl)-5-(4-phenylphenyl)-1,3,4-oxadiazole	354.44	-	3342	2
	81	Diisooctyl adipate	370.58	-	2444	2
	82	1,4-bis(5-phenyl-2-oxazolyl)benzene), POPOP	392.44	-	3525	2
	83	Diisodecyl adipate	426.67	-	2745	2
	84	Dioctyl sebacate	426.67	-	2787 / 2778	2 / 6
	85	2,5-bis(5-tert-butyl-2-benzoxazolyl)thiophene, BBOT	430.56	-	2745	2

Literature 1 : Peng et al. 1988
Literature 2 : Deutsche Forschungsgemeinschaft (DFG) and The International Association of Forensic Toxicologists (TIAFT) 1992
Literature 3 : Kondjoyan and Berdagué 1996
Literature 4 : Gramshaw et al. 1995
Literature 5 : Peng 2000
Literature 6 : Messadi and Vergnaud 1979

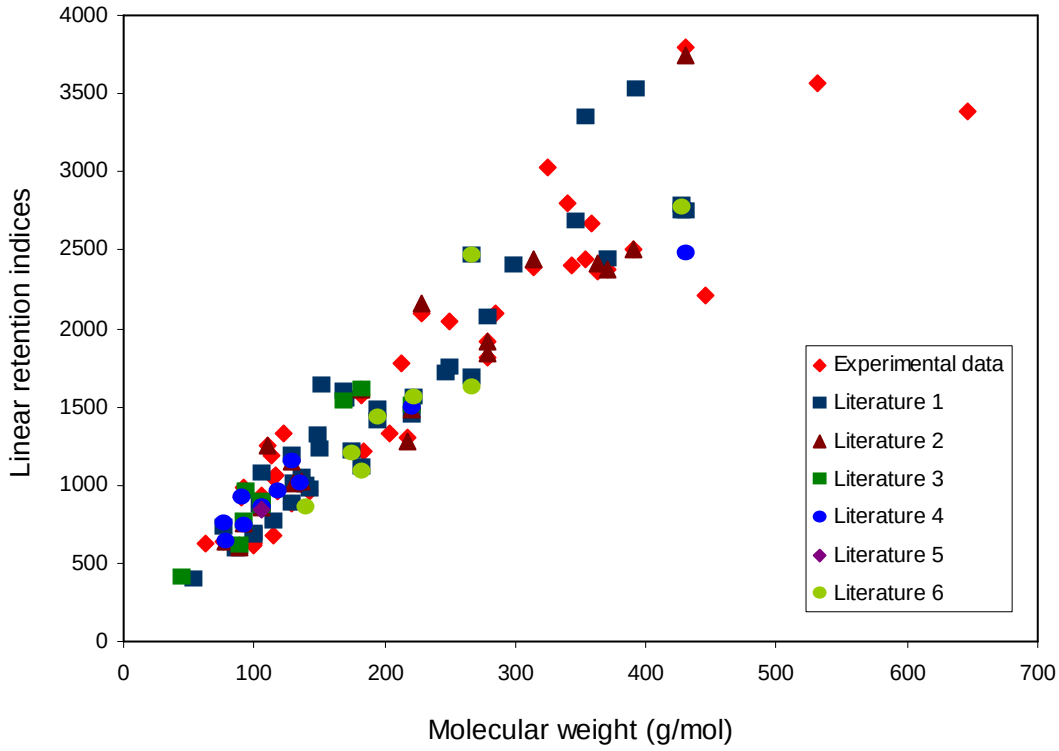


Figure 6-3. Comparison of the correlations between retention indices and molecular weight obtained from experimental data and the literature data (Table 6-1).

Literature 1 : Peng et al. 1988
Literature 2 : Deutsche Forschungsgemeinschaft (DFG) and The International Association of Forensic Toxicologists (TIAFT) 1992
Literature 3 : Kondjoyan and Berdagué 1996
Literature 4 : Gramshaw et al. 1995
Literature 5 : Peng 2000
Literature 6 : Messadi and Vergnaud 1979

6.3. Practical application of the developed screening methods

6.3.1. HPLC-CAD analysis data of extracts and migrants

As discussed in chapter 6.1.1, the responses of the volatile and semi-volatile compounds were poor or there was no response on a charged aerosol detector (CAD) because of the evaporation step in the CAD [McCarthy et al. 2005]. In this thesis, the detection properties of CAD were demonstrated by the screening test of 67 substances. Most substances with a molecular weight lower than 300 g/mol and a vapor pressure higher than 10^{-4} Pa were not detected in the CAD. These results are in good agreement with those obtained by the extraction and migration tests of the real adhesive samples. The molecular weights of the substances identified ranged from 314 g/mol to 358 g/mol (diethylene glycol dibenzoate, dipropylene glycol dibenzoate, triethylene glycol dibenzoate). The semi-volatile substances such as triacetin (MW 218.2 g/mol) and butyl diglycol acetate (MW 204.3 g/mol) detected by the GC-FID screening test could not be detected by CAD because of their higher volatilities than mobile phase composed of acetonitrile and water.

Some unknown peaks were detected in the migration solution of VAE 1-C, VAE 3-C and VAE 4-C samples bonded with pure adhesives based on vinyl acetate ethylene copolymers (VAE). These peaks can be allocated to substance that migrated from the substrates such as paper and card board.

In conclusion, the HPLC-CAD screening method developed for non-volatile adhesive related substances was applicable to the real migration and extraction test.

6.3.2. GC-FID analysis data of extracts and migrants

The GC-FID multi-screening method using DB-1 column was demonstrated to be useful and reliable for the semi-quantitation of semi-volatile and some non-volatile substances in adhesive and composite samples. Detection and separation of the substances with various physico-chemical properties on DB-1 column and flame ionization detector (FID) were correctly interpreted by the screening test of 55 representative adhesive related substances in this thesis.

With this method, some plasticizers as the main components in adhesive samples were identified by extraction and migration test. The molecular weights of the plasticizers were

between 200 and 400 g/mol. Some unknown peaks originated from the substrates were detected in the migration solution of VAE1-C ~ VAE 4-C and PVAc-C samples.

6.3.3. Semi-quantification of adhesive related substances

The distribution range of the RRF values determined by GC-FID was established from 0.31 to 1.44. In this study, the conservative estimation using an overall RRF value of 0.31 in GC-FID analysis overestimated the concentration of the relevant substances in a range of 129 ~ 305 % in the extraction and migration test. On the other hand, a quantification using real RRF values determined by the calibration curves of the substances detected were close to those obtained from traditional quantification data (Table 5-10 and 5-11), except for dipropylene glycol dibenzoate (DPGDB) which was not in good accord with the traditional estimation. The reason is that it could not be calibrated with a high purity standard as DPGDB of purity 80 % (technical grade) had to be inevitably used in this study.

In the quantitative analysis by using the real RRF value, the inconsistency of the RRF values in the calibration range leads to a quantitative error. In HPLC-CAD analysis, diethylene glycol dibenzoate (DEGDB) quantified by the real RRF value was very close to the traditional quantitation data (Table 5-12), but the concentration of DPGDB in the pure adhesives and the composite samples was markedly underestimated, since the RRF values of DPGDB in the calibration range are not consistent. In other words, the calibration curve of DPGDB was not linear and the calibration line did not pass through the origin of the x and y axis. For this reason, DPGDB was excluded to establish the distribution range in this study. The conservative estimation in the HPLC-CAD analysis should also overestimate in the same way as in GC-FID analysis, but DPGDB in the migration test of VAE 5-C was underestimated with the approximation ratio of 86 % because of the inconsistency of the RRF value of DPGDB.

In this thesis, a statistical design using universal substances was applied for the semi-quantitative approach of unknown substances related to adhesives in food packaging materials. The results of conservative estimation overestimated the real substance concentrations at maximum by 305 % in GC-FID analysis and by 219 % in HPLC-CAD analysis compared to those of the traditional quantification. In a conservative concept which systematically overestimates the concentrations of potentially harmful substances, however, these values are acceptable for the semi-quantitation of unknown substances migrated from multi-layer films for food packaging.

7. Summary

The core objective of this thesis was to develop an analytical method and analytical approach for semi-quantification of potential migrants from adhesive samples. For this, multi-analytical screening methods covering various physico-chemical classes of adhesive related substances up to a molecular weight of 1000 g/mol were developed. Furthermore, the analytical uncertainty of the semi-quantitative approach using universal substances was estimated.

For this, 55 substances were selected which cover different chemical structure, polarities and molecular weights and represent the physico-chemical properties of potential migrants in adhesives. They were used for the development of screening methods and of a semi-quantitative approach for unknown compounds. The list contains 7 acrylates, 11 plasticizers, 6 carboxylic acids, 7 alcohols and phenols, 5 amines, 5 antioxidants and 14 other substances. 12 additives (antioxidants and UV-stabilizers) with a wide range of molecular weights from 300 to 700 g/mol were additionally selected for HPLC-CAD analysis

Gas chromatography with flame ionization detector (GC-FID) and high performance liquid chromatography with charged aerosol detector (HPLC-CAD) were used for separation and detection. In total, 46 out of 55 substances were detected by GC-FID analysis equipped with a DB-1 column. Multifunctional alcohols and amines showed a bad peak shape and therefore low sensitivity. Since carboxylic acids are not volatile enough, they were not detectable by GC-FID. The GC-FID system equipped with a DB-FFAP column showed a more sensitive response for alcohols. Amines and carboxylic acids and some plasticizers were still not detected on the FFAP column. In conclusion, the multi-screening method on a DB-1 column is applicable to a broad range of substances except for highly polar substances.

For the identification of volatile unknown substances, retention time and retention indices were determined. Both retention parameters showed a linear relation with molecular weights or carbon numbers of the representative adhesive substances on the DB-1 column. It was shown that the linear retention indices were not influenced by the GC systems and operating conditions except the stationary phase of the column. Therefore, the molecular weight or carbon number of unknown substances could be reliably estimated from the retention indices. This could be confirmed by comparison with retention indices from the literature.

The non-volatile substances were analyzed by reverse phase HPLC coupled with a charged aerosol detector (CAD). Two mobile phase compositions were tested. CAD showed good relative responses for substances with a molecular weight higher than 400 g/mol or vapor pressure lower than 10^{-9} Pa. The mobile phase water-acetonitrile gradients on a C 18 column were suitable for separating most test substances. Highly polar and ionic compounds need specific mobile phases with adjusted pH. The response of CAD was not influenced by the organic content in water-acetonitrile mobile phase compositions. Therefore, the developed multi-screening method using CAD coupled with a reverse-phase HPLC system is capable for screening of unknown non-volatile migrants except for highly polar substances.

Online two-dimensional separation technique (SEC×NP-HPLC×CAD) was proposed to improve the peak capacity and resolving power. Higher separation efficiency could be obtained by this two-dimensional system in comparison to the one-dimensional system. However, the main disadvantage of the two-dimensional separation is the sophisticated automated data processing for qualification and quantification. Literally, there is no commercial software available. Therefore, quantification was not possible in this thesis.

Relative response factors (RRF) related to 3-tert-butyl-4-hydroxy-anisole (BHA) as internal standard of total 46 substances among 55 representative adhesive related substances could be determined by using the developed GC-FID multi-screening method for semi-volatile substances. The distribution range of the RRF values without alcohols was between 0.31 and 1.44. For a conservative estimation, a RRF value of factor 0.31 should be used for semi-quantitative estimation. On the other hand, relative response factors of total 28 substances out of 67 representative adhesive related substances could be determined by the HPLC-CAD multi-screening method. In order to establish the distribution range, the RRF values which were inconsistent in the calibration range were excluded. The distribution range was between 0.23 and 3.25. For a conservative estimation, a statistically derived RRF value of 0.23 should be used with this detection method.

Some substances were detected by the HPLC-CAD and the GC-FID multi-screening methods in the extraction and migration test. The results of the conservative estimation and the real RRF estimation using the RRF of the identified substances were compared with that of traditional quantification. In GC-FID analysis, the conservative estimations of the identified substances using an RRF value of 0.31 overestimated substance concentrations

compared to traditional quantification in a range from 129 to 305 % in the extraction test and from 130 to 305 % in the migration test, respectively. In HPLC-CAD analysis for non-volatile substances, an RRF value of 0.23 was used for the conservative estimation. The conservative estimation led to overestimations of the identified substances in relation to traditional values in the range from 102 to 219 % in the extraction test and from 86 to 216 % in the migration test, respectively. However, the conservative estimation of dipropylene glycol dibenzoate was not reliable because of its inconsistency of RRF value in the calibration range.

Consequently, the multi-screening methods covered a broad range of physico-chemical properties of compounds and the semi-quantitative approach was very reliable with an acceptable range of uncertainty. It can be proposed as an useful semi-quantitative approach for the potential migrants related to adhesives. This semi-quantitative multi method can be applied not only for the adhesives compliance testing area, but also for migratable substances from other material categories, for instance laquers, coatings, printing inks and more.

8. References

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9. Appendix

9.1. Calibration curves

9.1.1. Calibration curves for HPLC-CAD analysis

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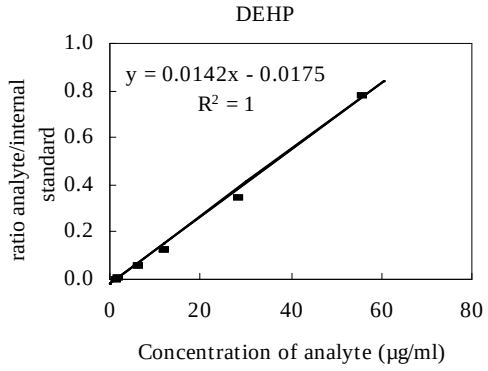
<td> 
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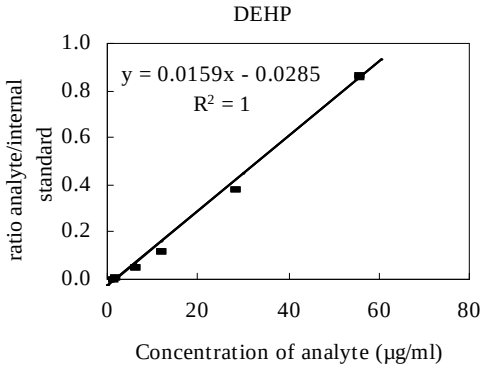
<td> 
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Limit of detection (LOD) : 2.12 µg/ml</td>
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Limit of detection (LOD) : 2.49 µg/ml</td>
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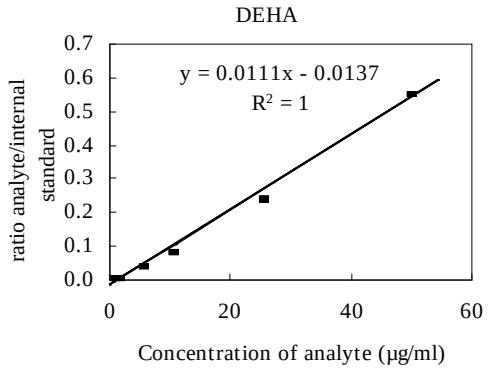
<td> 
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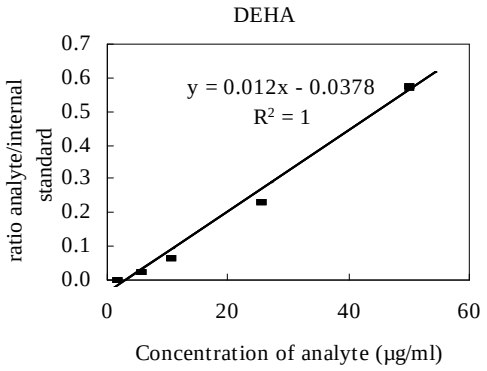
<td> 
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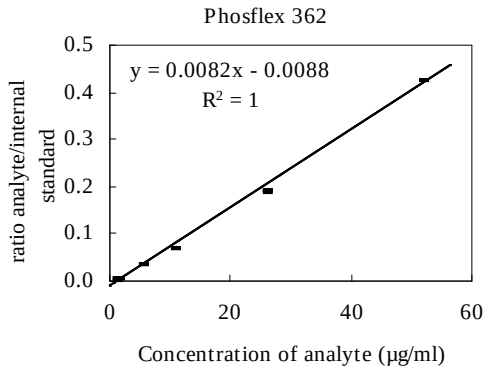
<td> 
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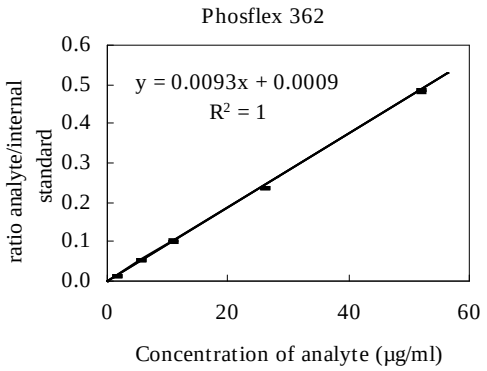
<td> 
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Limit of detection (LOD) : 0.79 µg/ml</td>
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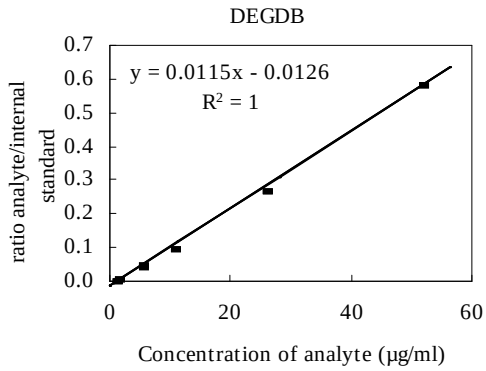

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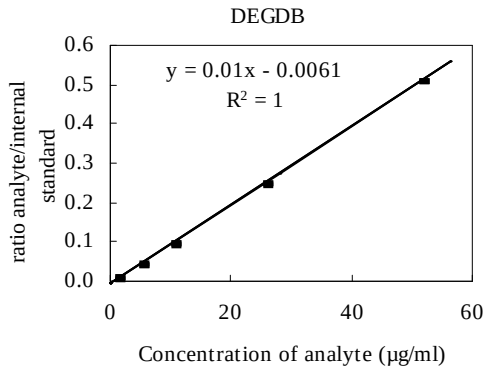

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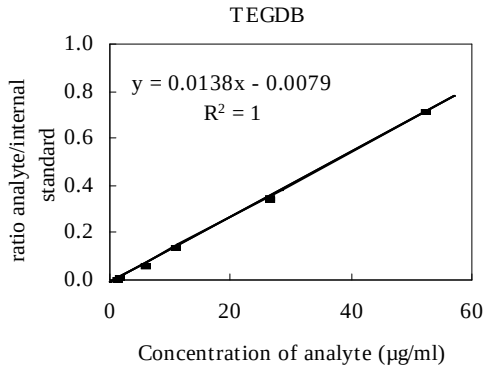
Limit of detection (LOD) : 0.73 µg/ml



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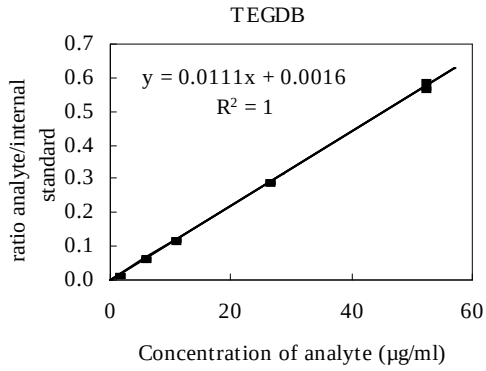
Limit of detection (LOD) : 0.54 µg/ml



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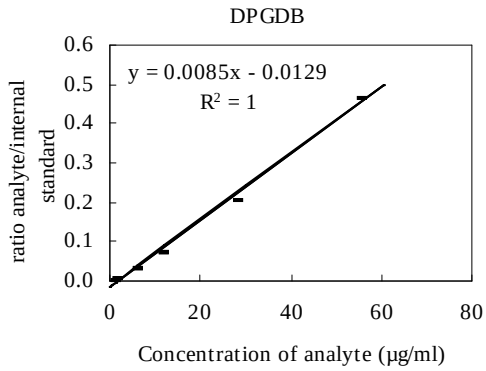
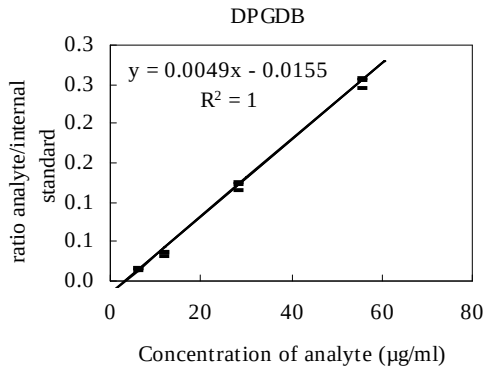
Limit of detection (LOD) : 0.93 µg/ml

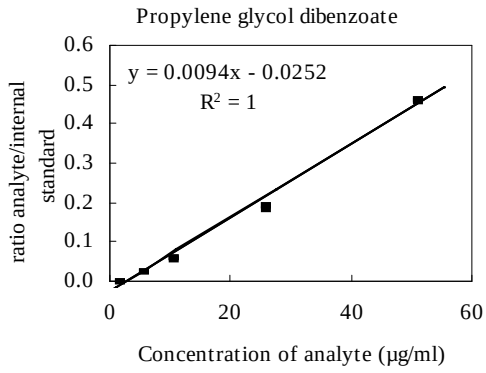


Gradient A Correlation coefficient R : 0.9982

Limit of detection (LOD) : 2.25 µg/ml

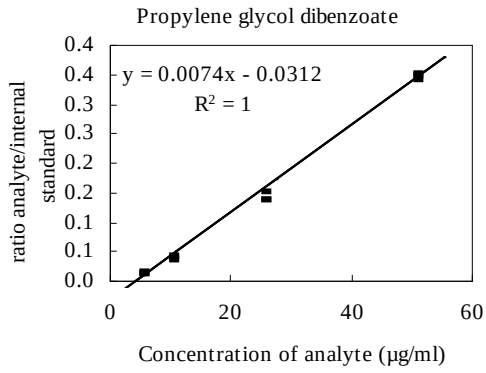


Gradient B not detected < 5 µg/ml

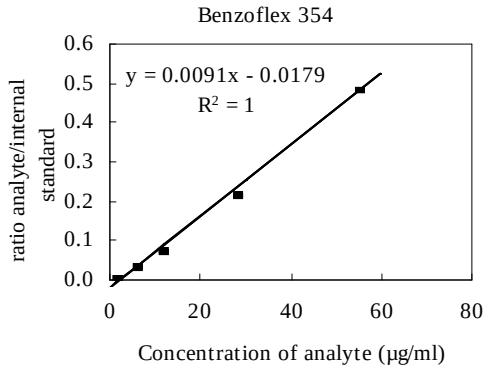


Gradient A Correlation coefficient R : 0.9960

Limit of detection (LOD) : 3.28 µg/ml

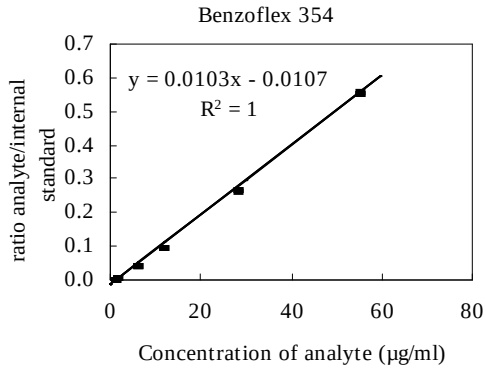


Gradient B not detected < 5 µg/ml



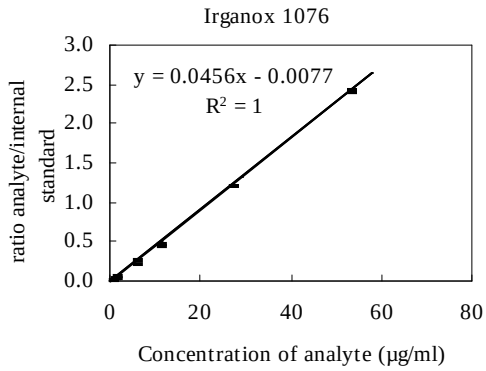
Gradient A Correlation coefficient R : 0.9986

Limit of detection (LOD) : 2.09 µg/ml



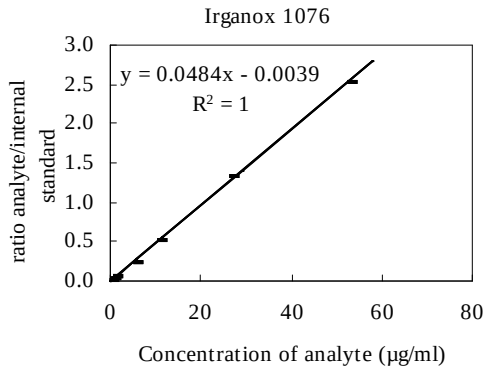
Gradient B Correlation coefficient R : 0.9996

Limit of detection (LOD) : 1.03 µg/ml



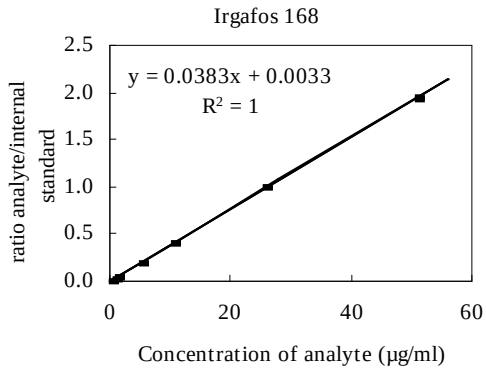
Gradient A Correlation coefficient R : 0.9999

Limit of detection (LOD) : 0.59 µg/ml



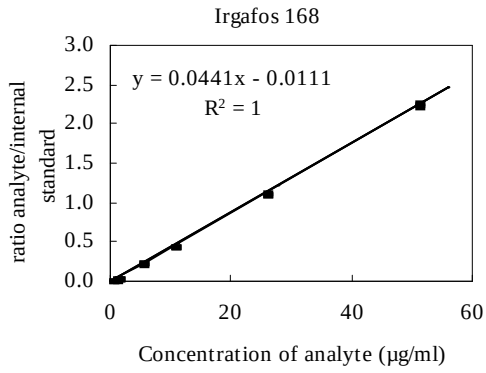
Gradient B Correlation coefficient R : 0.9997

Limit of detection (LOD) : 0.90 µg/ml



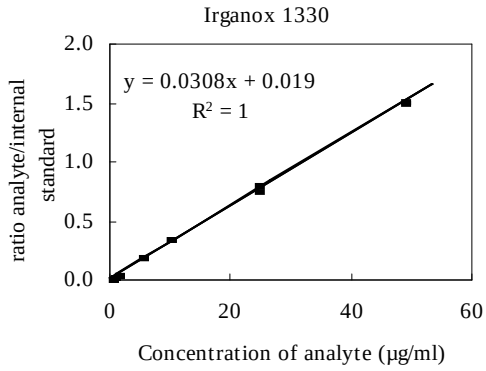
Gradient A Correlation coefficient R : 0.9999

Limit of detection (LOD) : 0.52 µg/ml



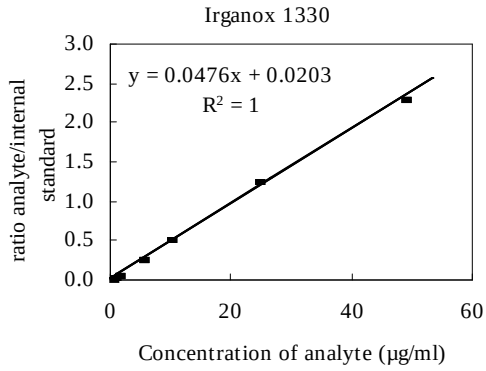
Gradient B Correlation coefficient R : 0.9999

Limit of detection (LOD) : 0.45 µg/ml



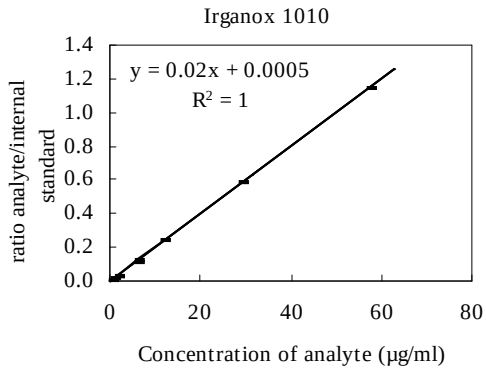
Gradient A Correlation coefficient R : 0.9992

Limit of detection (LOD) : 1.31 µg/ml



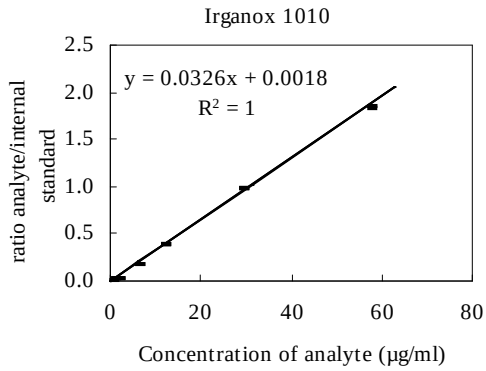
Gradient B Correlation coefficient R : 0.9989

Limit of detection (LOD) : 1.54µg/ml



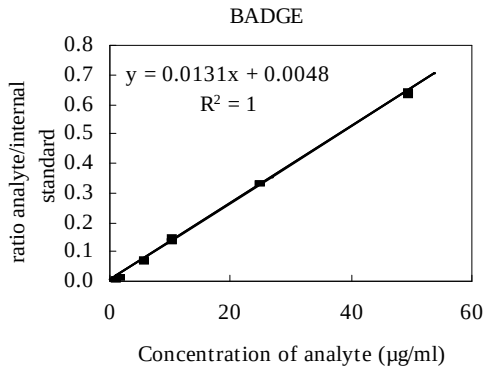
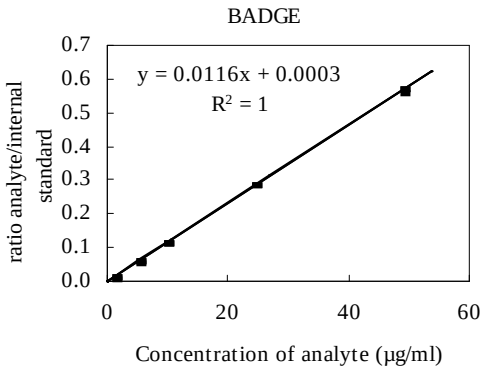
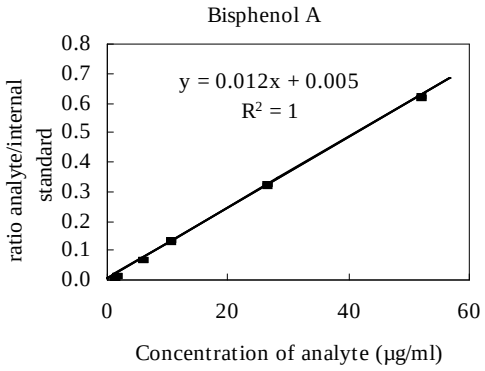
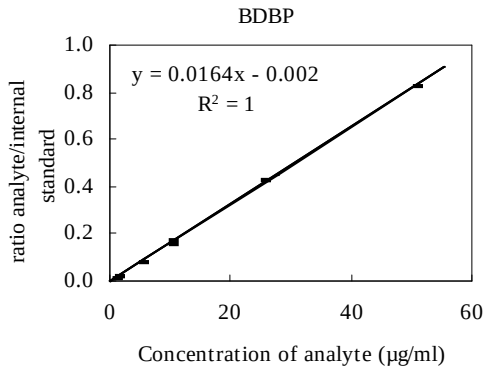
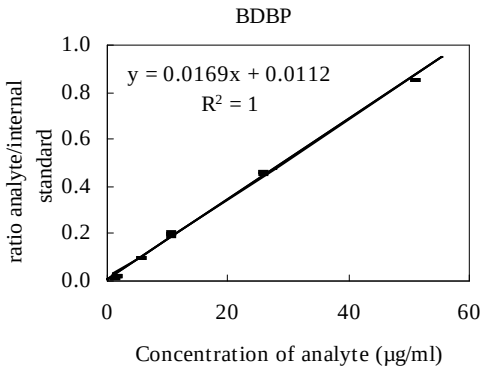
Gradient A Correlation coefficient R : 1.000

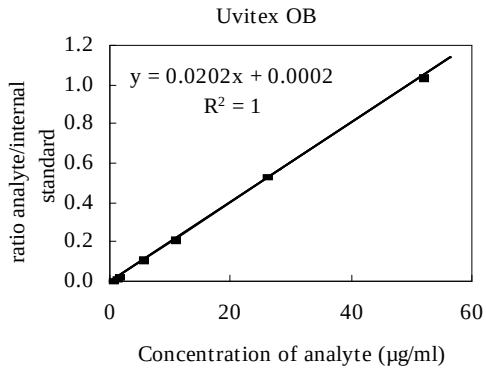
Limit of detection (LOD) : 0.36 µg/ml



Gradient B Correlation coefficient R : 0.9993

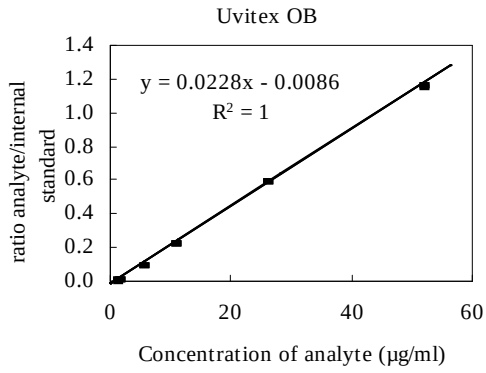
Limit of detection (LOD) : 1.39µg/ml

	
Gradient A Correlation coefficient R : 0.9996 Limit of detection (LOD) : 0.94 μg/ml	**Gradient B** Correlation coefficient R : 0.9999 Limit of detection (LOD) : 0.45 μg/ml
Bisphenol A was not retarded in this gradient condition.	
-	**Gradient B** Correlation coefficient R : 0.9996 Limit of detection (LOD) : 0.93 μg/ml
	
Gradient A Correlation coefficient R : 0.9998 Limit of detection (LOD) : 0.77 μg/ml	**Gradient B** Correlation coefficient R : 0.9990 Limit of detection (LOD) : 1.52 μg/ml



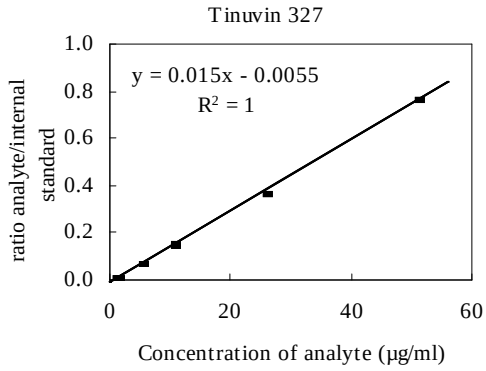
Gradient A Correlation coefficient R : 0.9999

Limit of detection (LOD) : 0.36 µg/ml



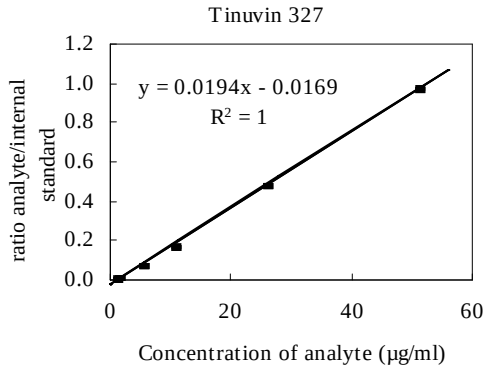
Gradient B Correlation coefficient R : 0.9997

Limit of detection (LOD) : 0.78 µg/ml



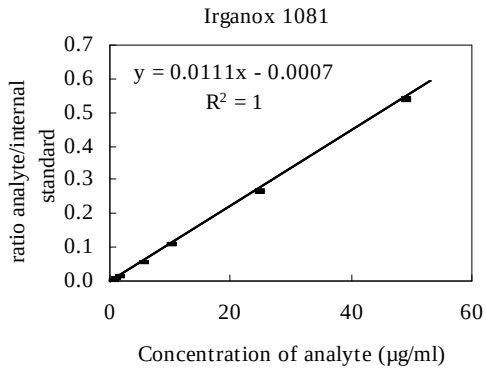
Gradient A Correlation coefficient R : 0.9999

Limit of detection (LOD) : 0.66 µg/ml



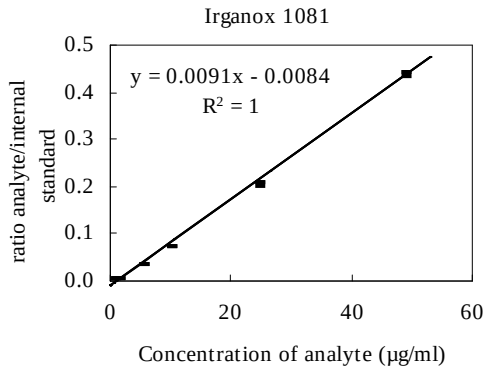
Gradient B Correlation coefficient R : 0.9996

Limit of detection (LOD) : 0.92 µg/ml



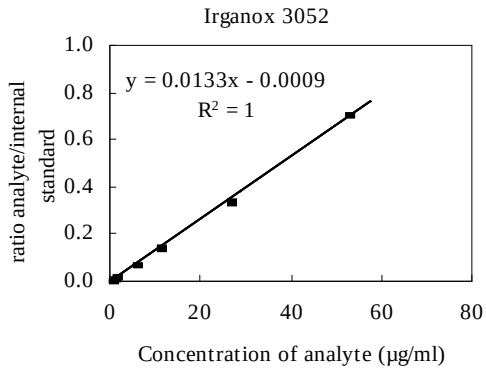
Gradient A Correlation coefficient R : 0.9999

Limit of detection (LOD) : 0.41 µg/ml



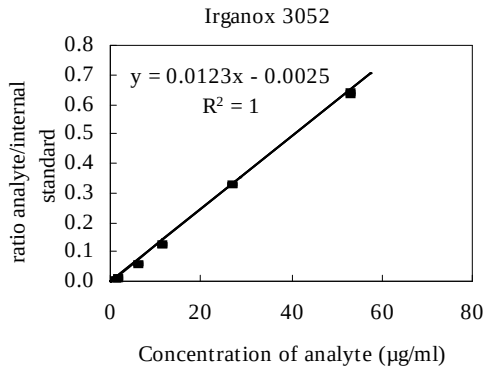
Gradient B Correlation coefficient R : 0.9993

Limit of detection (LOD) : 1.22 µg/ml



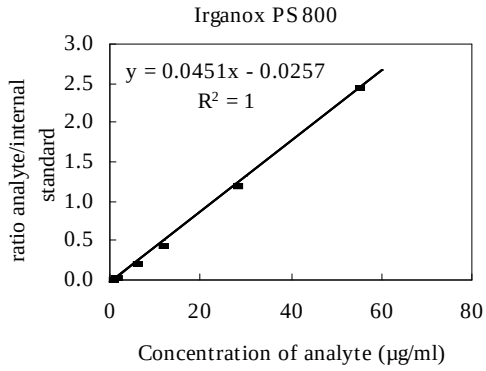
Gradient A Correlation coefficient R : 0.9997

Limit of detection (LOD) : 0.84 µg/ml



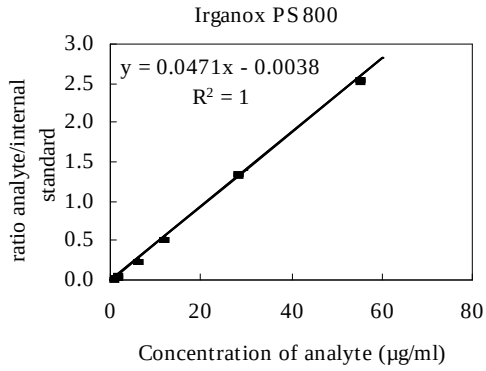
Gradient B Correlation coefficient R : 0.9998

Limit of detection (LOD) : 0.68 µg/ml



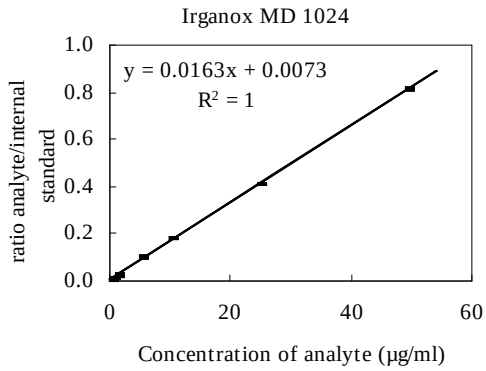
Gradient A Correlation coefficient R : 0.9997

Limit of detection (LOD) : 0.96 µg/ml



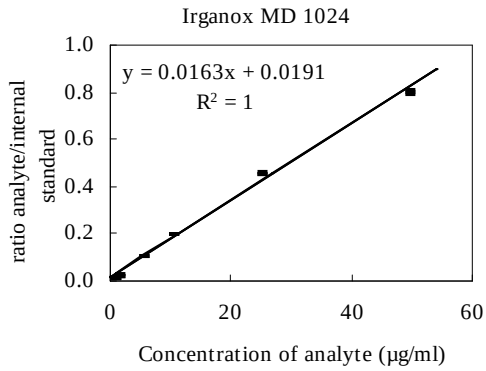
Gradient B Correlation coefficient R : 0.9995

Limit of detection (LOD) : 1.13 µg/ml



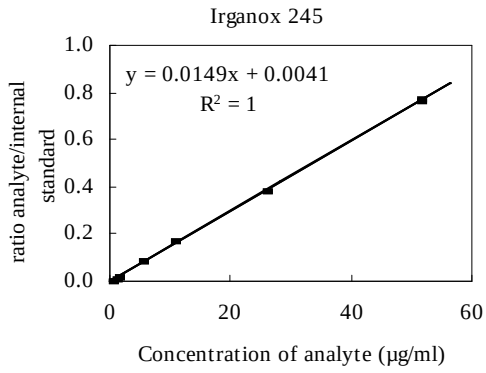
Gradient A Correlation coefficient R : 0.9997

Limit of detection (LOD) : 0.87 µg/ml



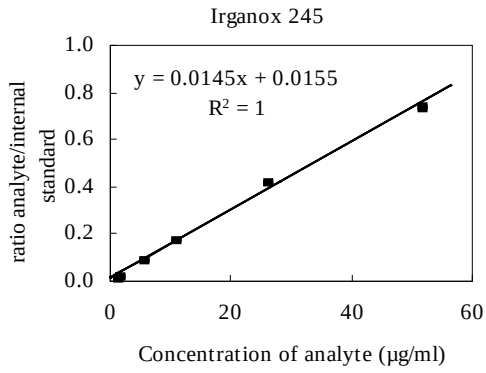
Gradient B Correlation coefficient R : 0.9974

Limit of detection (LOD) : 2.44µg/ml



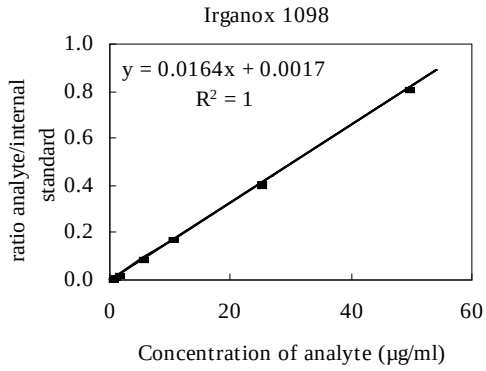
Gradient A Correlation coefficient R : 0.9998

Limit of detection (LOD) : 0.77 µg/ml



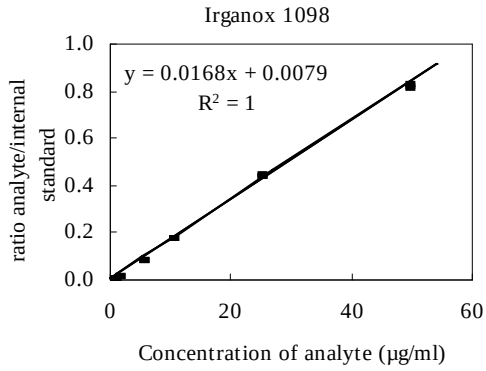
Gradient B Correlation coefficient R : 0.9975

Limit of detection (LOD) : 2.46 µg/ml



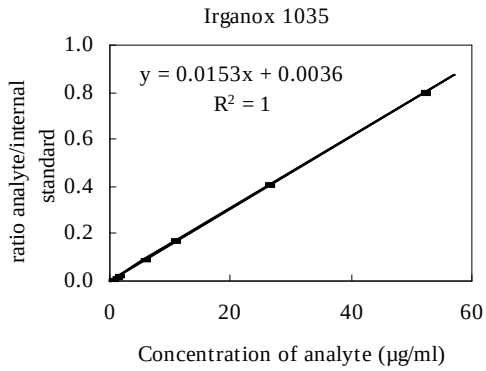
Gradient A Correlation coefficient R : 1.000

Limit of detection (LOD) : 0.30 µg/ml



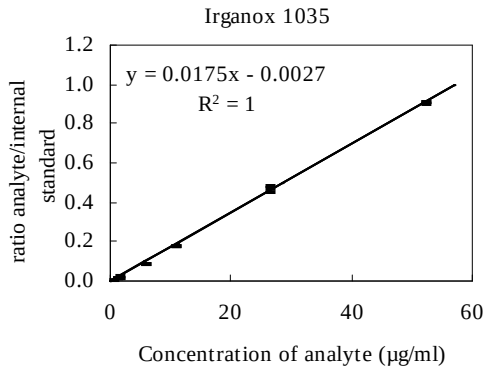
Gradient B Correlation coefficient R : 0.9991

Limit of detection (LOD) : 1.39 µg/ml



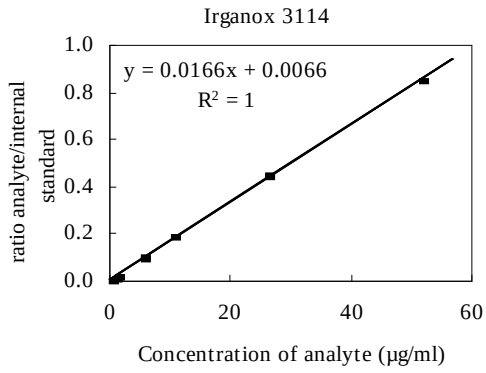
Gradient A Correlation coefficient R : 0.9999

Limit of detection (LOD) : 0.43 µg/ml



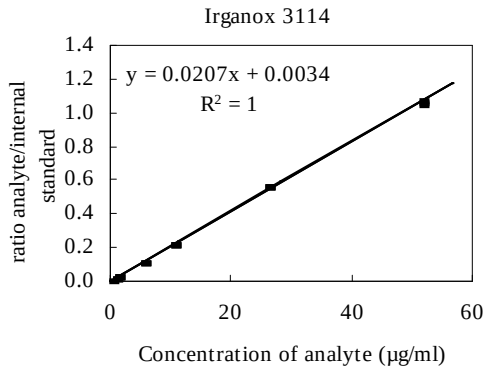
Gradient B Correlation coefficient R : 0.9997

Limit of detection (LOD) : 0.90 µg/ml



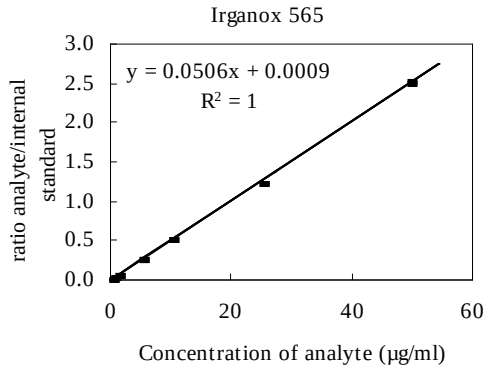
Gradient A Correlation coefficient R : 0.9996

Limit of detection (LOD) : 1.01 µg/ml



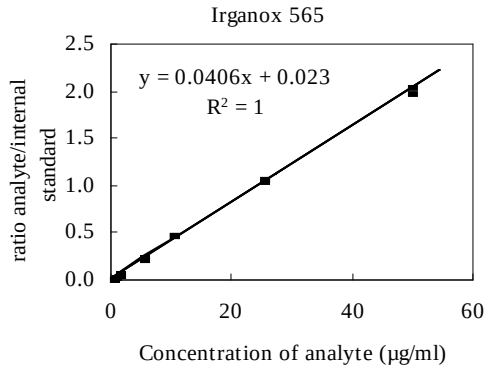
Gradient B Correlation coefficient R : 0.9996

Limit of detection (LOD) : 1.00 µg/ml



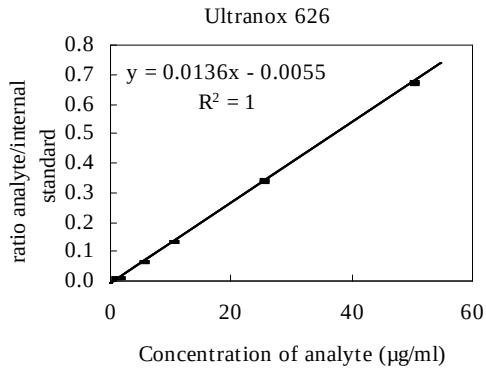
Gradient A Correlation coefficient R : 0.9999

Limit of detection (LOD) : 0.51 µg/ml



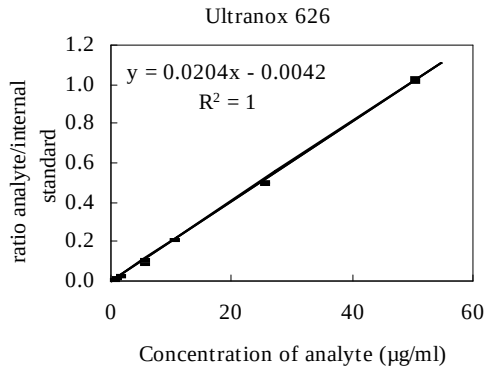
Gradient B Correlation coefficient R : 0.9992

Limit of detection (LOD) : 1.39 µg/ml



Gradient A Correlation coefficient R : 0.9999

Limit of detection (LOD) : 0.55 µg/ml



Gradient B Correlation coefficient R : 0.9998

Limit of detection (LOD) : 0.76 µg/ml

9.1.2. Calibration curves for GC-FID analysis

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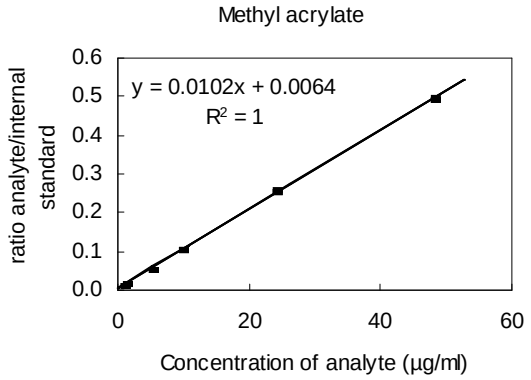
<td> 
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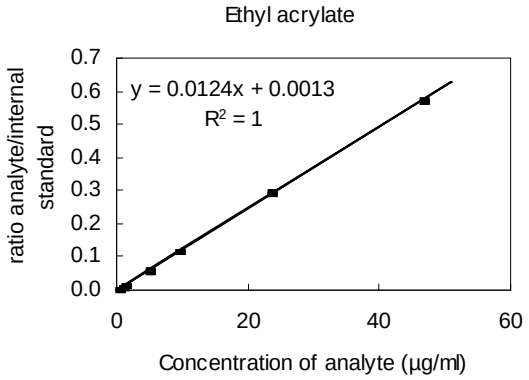
<td> 
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Lmit of detection (LOD) : 0.51 μg/ml</td>
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Lmit of detection (LOD) : 0.44 μg/ml</td>
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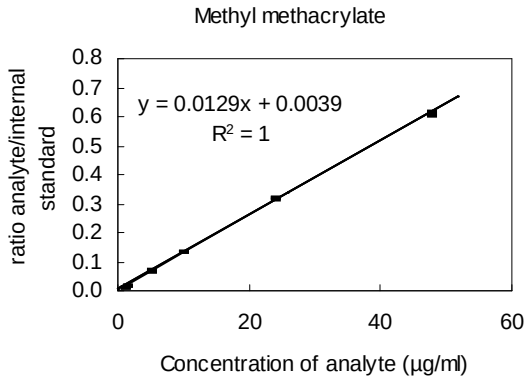
<td> 
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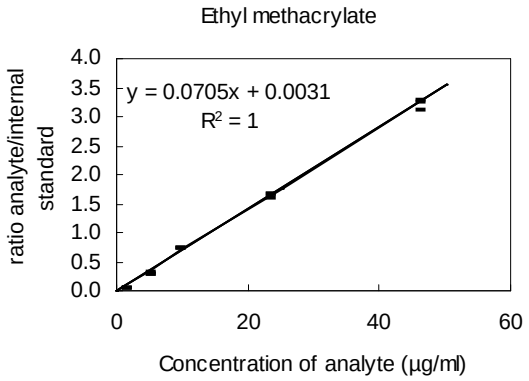
<td> 
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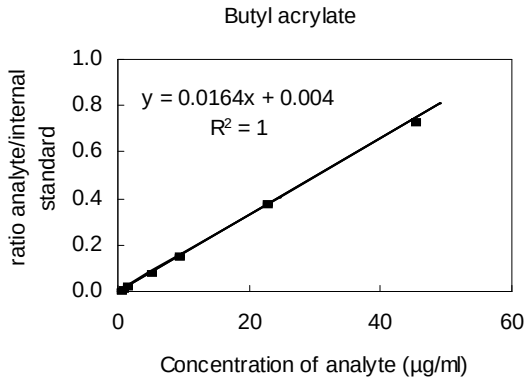
<td> 
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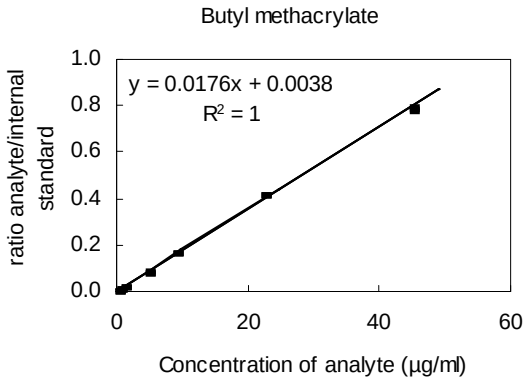
<td> 
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Lmit of detection (LOD) : 0.82 μg/ml</td>
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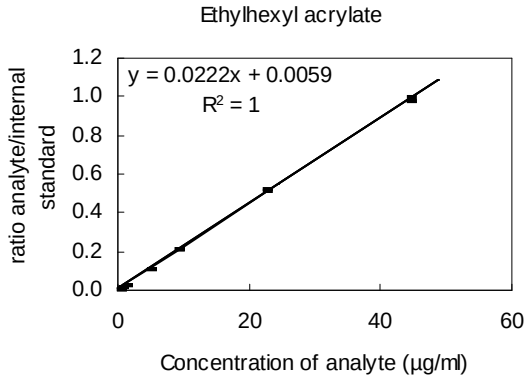
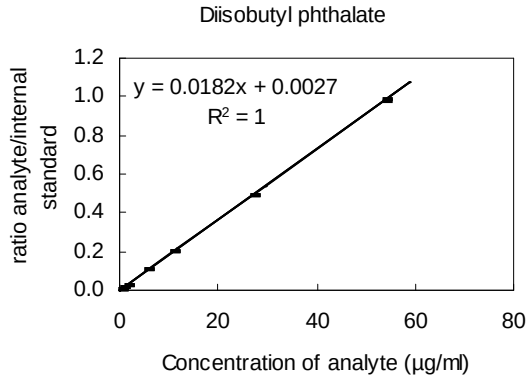


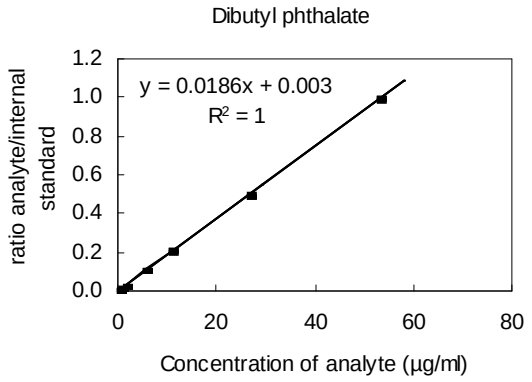
Correlation coefficient R : 0.9997

Lmit of detection (LOD) : 0.68 μg/ml



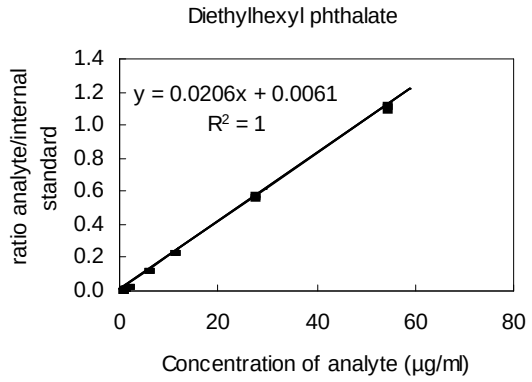
Correlation coefficient R : 0.9999

Lmit of detection (LOD) : 0.41 μg/ml



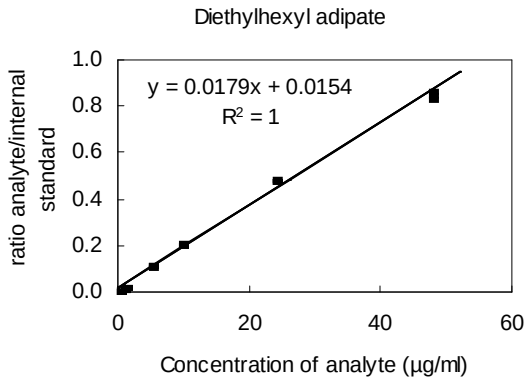
Correlation coefficient R : 0.9999

Lmit of detection (LOD) : 0.42 μg/ml



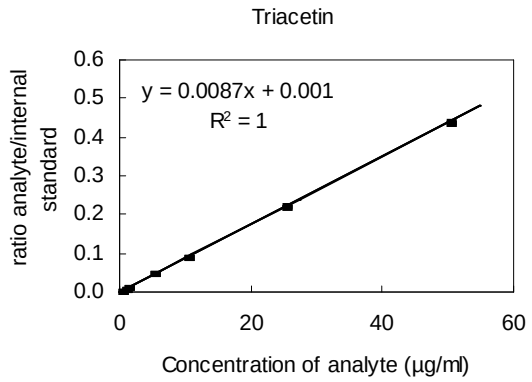
Correlation coefficient R : 0.9998

Lmit of detection (LOD) : 0.76 μg/ml



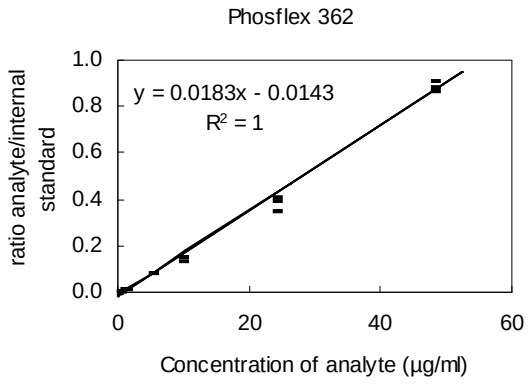
Correlation coefficient R : 0.9977

Lmit of detection (LOD) : 2.06 μg/ml



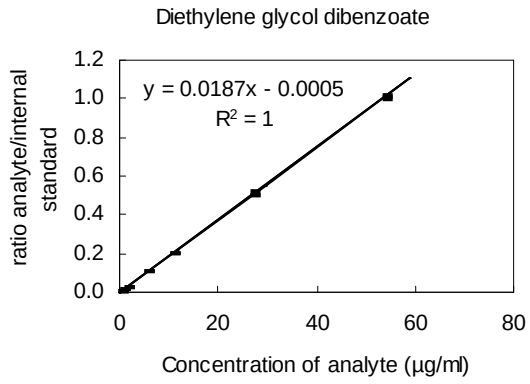
Correlation coefficient R : 1.0000

Lmit of detection (LOD) : 0.27 μg/ml



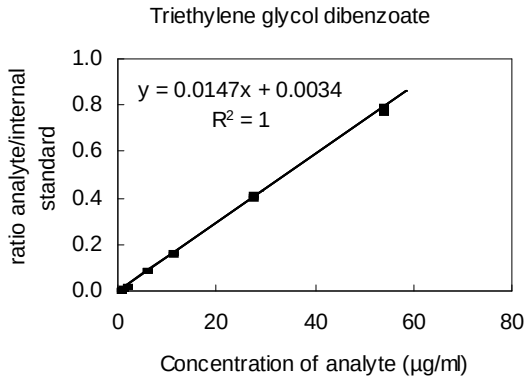
Correlation coefficient R : 0.9967

Lmit of detection (LOD) : 2.51 µg/ml



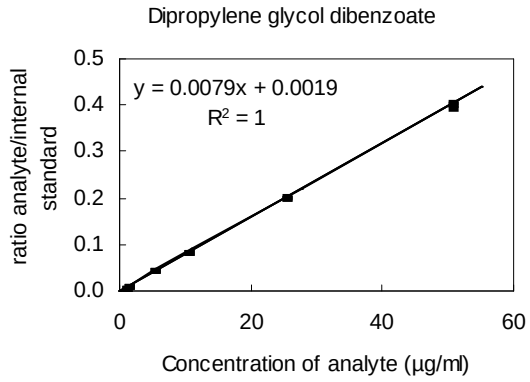
Correlation coefficient R : 0.9999

Lmit of detection (LOD) : 0.53 µg/ml



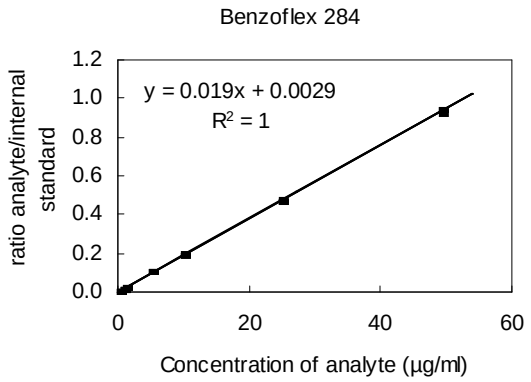
Correlation coefficient R : 0.9997

Lmit of detection (LOD) : 0.84 µg/ml



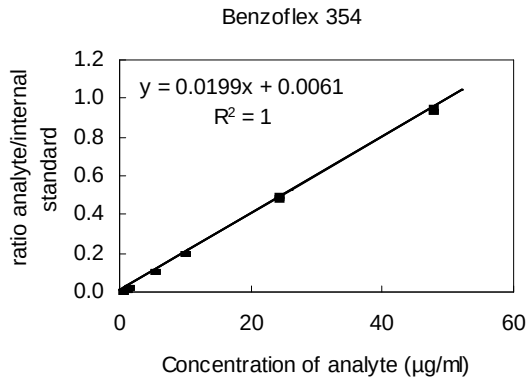
Correlation coefficient R : 0.9998

Lmit of detection (LOD) : 0.72 µg/ml



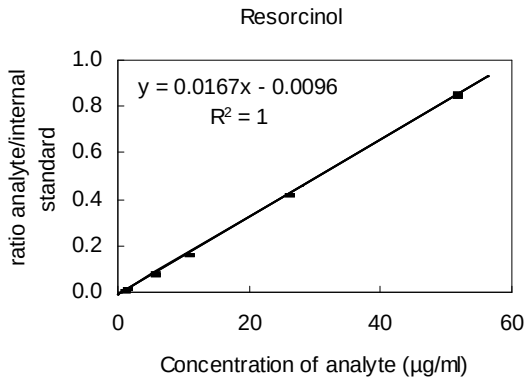
Correlation coefficient R : 0.9999

Lmit of detection (LOD) : 0.48 µg/ml



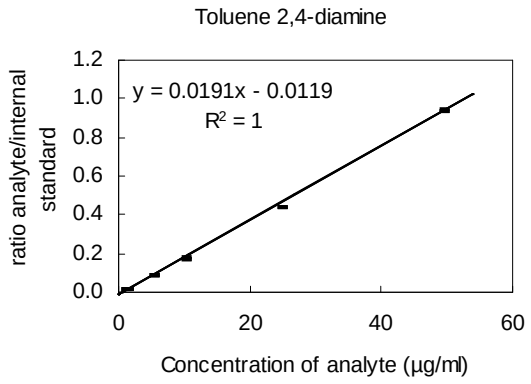
Correlation coefficient R : 0.9997

Lmit of detection (LOD) : 0.74 µg/ml



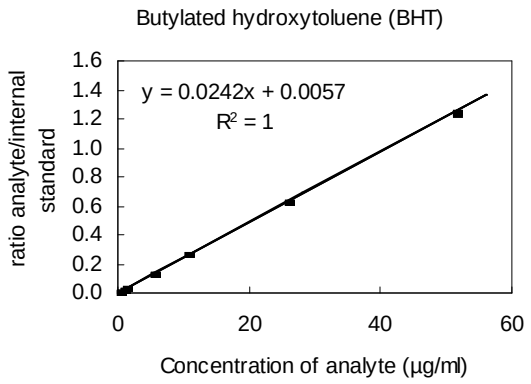
Correlation coefficient R : 0.9999

Lmit of detection (LOD) : 0.52 µg/ml



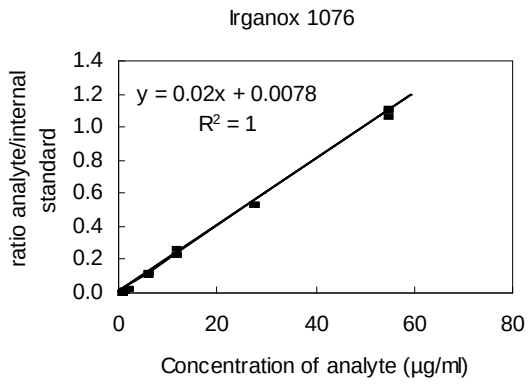
Correlation coefficient R : 0.9995

Lmit of detection (LOD) : 1.06 µg/ml



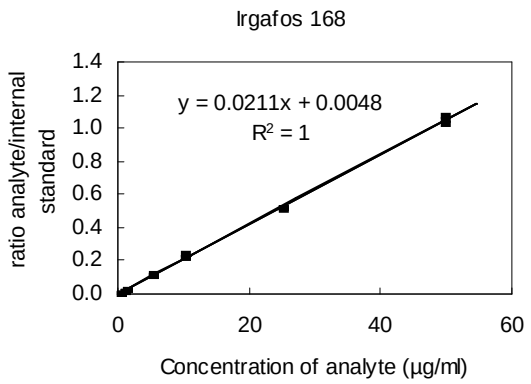
Correlation coefficient R : 0.9999

Lmit of detection (LOD) : 0.41 µg/ml



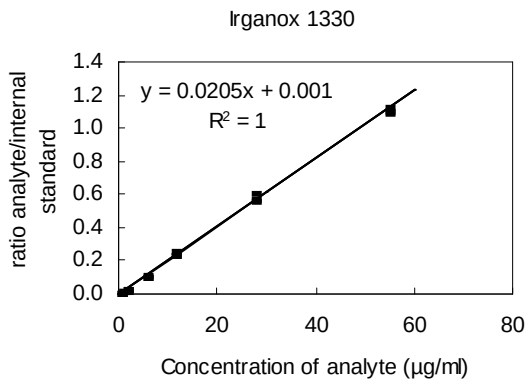
Correlation coefficient R : 0.9993

Lmit of detection (LOD) : 1.28 µg/ml



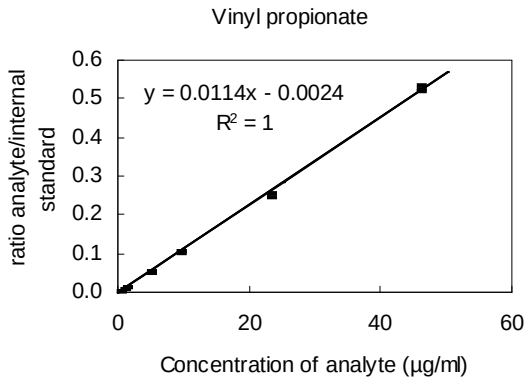
Correlation coefficient R : 0.9994

Lmit of detection (LOD) : 1.11 µg/ml



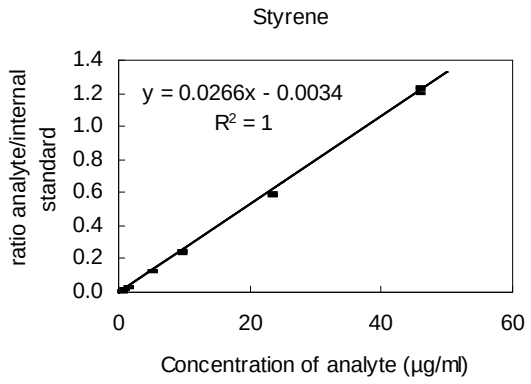
Correlation coefficient R : 0.9992

Lmit of detection (LOD) : 1.40 µg/ml



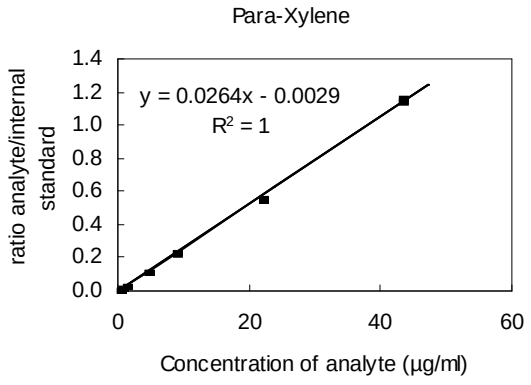
Correlation coefficient R : 0.9996

Lmit of detection (LOD) : 0.88 μg/ml



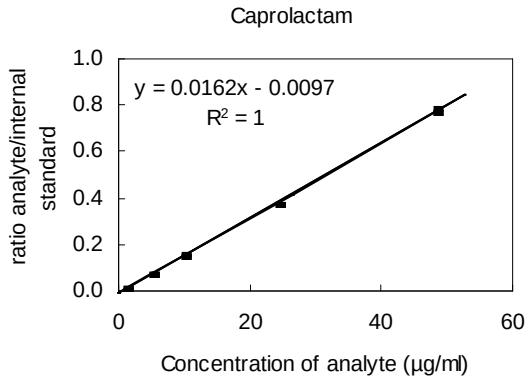
Correlation coefficient R : 0.9997

Lmit of detection (LOD) : 0.68 μg/ml



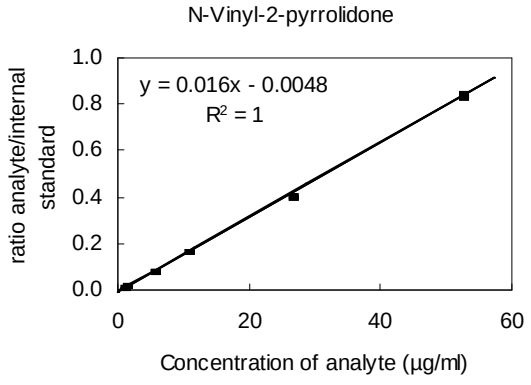
Correlation coefficient R : 0.9997

Lmit of detection (LOD) : 0.71 μg/ml



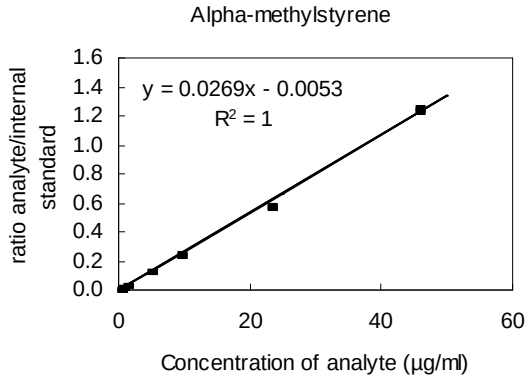
Correlation coefficient R : 0.9998

Lmit of detection (LOD) : 0.65 μg/ml



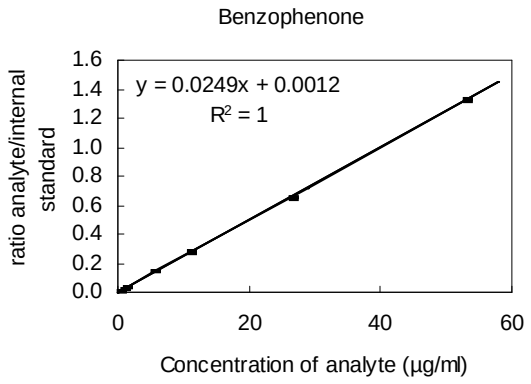
Correlation coefficient R : 0.9997

Lmit of detection (LOD) : 0.84 μg/ml



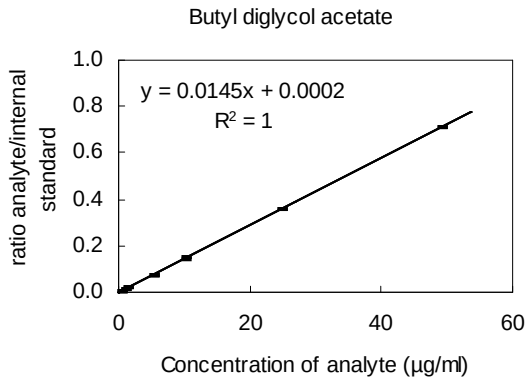
Correlation coefficient R : 0.9991

Lmit of detection (LOD) : 1.28 μg/ml



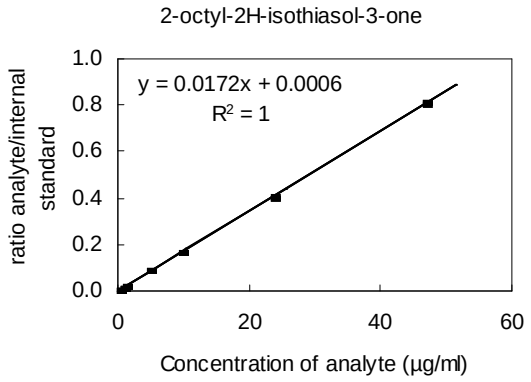
Correlation coefficient R : 0.9999

Lmit of detection (LOD) : 0.48 µg/ml



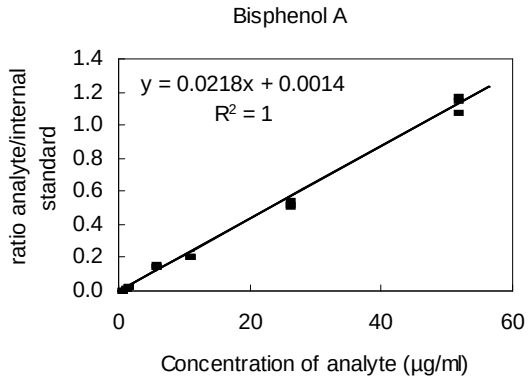
Correlation coefficient R : 1.0000

Lmit of detection (LOD) : 0.22 µg/ml



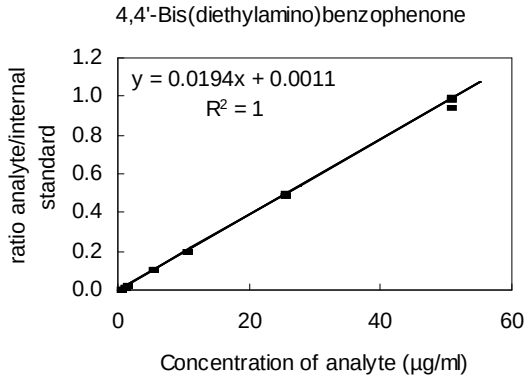
Correlation coefficient R : 0.9999

Lmit of detection (LOD) : 0.33 µg/ml



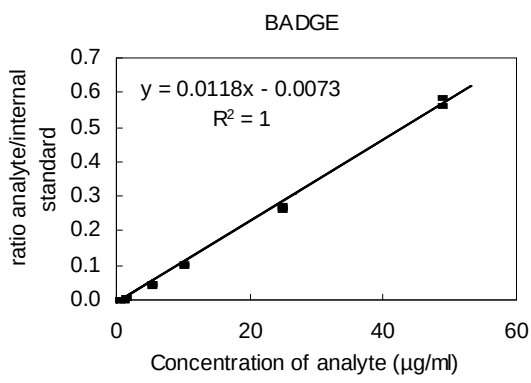
Correlation coefficient R : 0.9975

Lmit of detection (LOD) : 2.33 µg/ml



Correlation coefficient R : 0.9996

Lmit of detection (LOD) : 0.87 µg/ml



Correlation coefficient R : 0.9990

Lmit of detection (LOD) : 1.28 µg/ml

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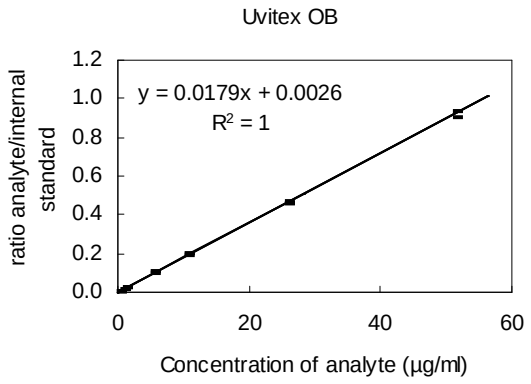
<td> 
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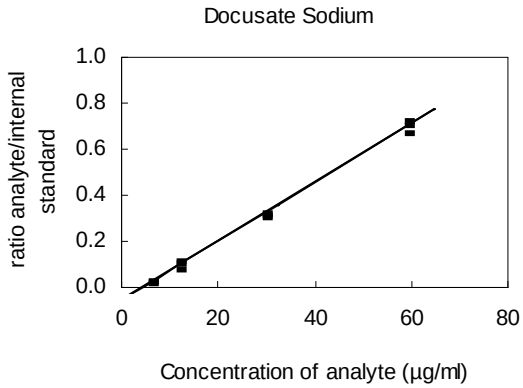
<td> 
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Lmit of detection (LOD) : 0.72 µg/ml</td>
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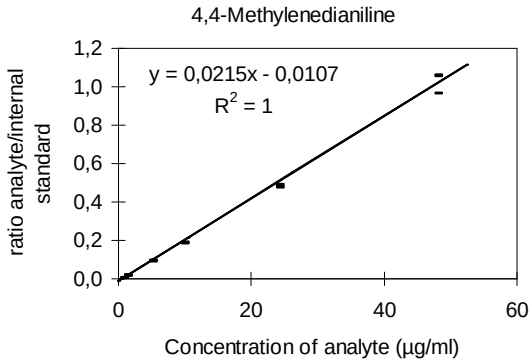
<td> 
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<td>Correlation coefficient R : 0.9983
Lmit of detection (LOD) : 1.87 µg/ml</td>
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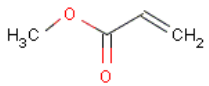
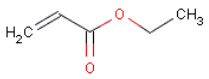
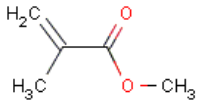
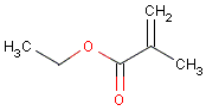
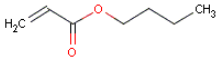
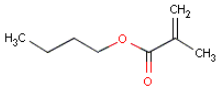
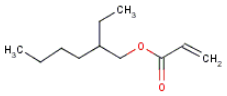
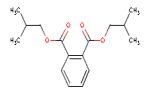
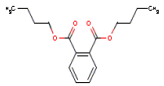
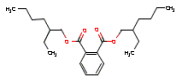
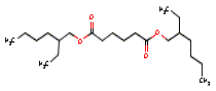
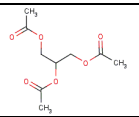
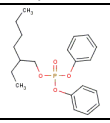
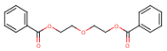
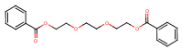
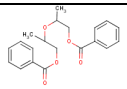
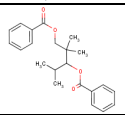
9.2. List of the representative adhesive related substances

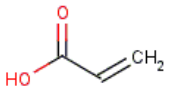
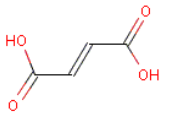
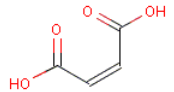
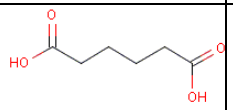
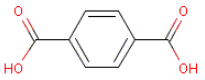
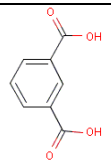
1: List of adhesive related, representative substances for establishing multi-screening methods and semi-quantitative estimates : Acrylates

Substances		MW (g/mol)	CAS RN./ PM Ref.	Formula	Chemical structure	Chemical classification	mp / bp (℃)	Water Solubility mg/L (25 deg C)	Log PO/W	Solubility	Vapour Pressure mm Hg (25 deg C)	Uses	EU Restriction	Way to analyse via
Name	**Synonyms**													
Methyl acrylate	Acrylic acid, methyl ester	86.09	96-33-3 / 11710	$C_4H_6O_2$		Ester	-76.5/ 80.2	4.94E+04	0.8	-	86.6	Monomer	SML(T) = 6 mg/kg	GC-FID, OV-101 Horna et al.,1985 GC-FID, DB-35 NIOSH Manual
Ethyl acrylate	Acrylic acid, ethyl ester	100.11	140-88-5 / 11470	$C_5H_8O_2$		Ester	-71.2/ 99.4	15000	1.32	Soluble in Alcohol, Ether and Oxigenated Solvents	38.6	Monomer	SML(T) = 6 mg/kg	GC-FID, DB-Wax NIOSH Manual
Methyl methacrylate	2-Methylacrylic acid, methyl ester	100.11	80-62-6 / 21130	$C_5H_8O_2$		Ester	-48/ 100.5	15000	1.38	Solubile in MEK, THF, Esters, Aromatic and Chlorinated hydrocarbon	38.5	Monomer	SML(T) = 6 mg/kg	GC-FID, DB-35 NIOSH Manual
Ethyl methacrylate	2-Methyl-2-Propenoic Acid, Ethyl Ester	114.14	97-63-2 / 20890	$C_6H_{10}O_2$		Ester	/ 117	5400	1.94	Solubile in oxigenated solvents	20.6	Monomer	SML(T) = 6 mg/kg	GC-FID, DB-35 NIOSH Manual
Butyl acrylate	Acrylic acid, butyl ester	128.18	141-32-2/ 10780	$C_7H_{12}O_2$		Ester	-64.6/ 145	2000	2.36	-	5.45	Monomer	SML(T) = 6 mg/kg	GC-FID, OV-101 Horna et al.,1985
Butyl methacrylate	Methacrylic acid, butyl ester	142.19	97-88-1 / 20110	$C_8H_{14}O_2$		Ester	-75.0/ 160	800	2.88	-	2.12	Monomer	SML(T) = 6 mg/kg	GC-FID, OV-101 Horna et al.,1985
Ethylhexyl acrylate	2-ethylhexyl prop-2-enoate; Acrylic acid, 2-ethylhexyl ester	184.28	1322-13-0	$C_{11}H_{20}O_2$		Ester			3.9			Monomer	SML= 0.05 mg/kg	

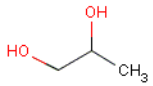
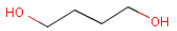
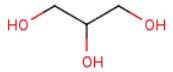
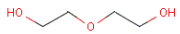
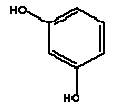
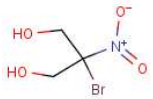
2: List of adhesive related, representative substances for establishing multi-methods and semi-quantitative estimates : Plasticizers

Substances		MW (g/mol)	CAS RN./ PM Ref.	Formula	Chemical structure	Chemical classification	mp / bp (℃)	Water Solubility mg/L (25 deg C)	Log PO/W	Solubility	Vapour Pressure mm Hg (25 deg C)	Uses	EU Restriction	Way to analyse via
Name	**Synonyms**													
Diisobutyl phthalate	Phthalic acid diisobutyl ester, DIBP	278.35	84-69-5 / 75280	$C_{16}H_{22}O_4$		Ester	< 25/ 296	6.2	4.11	Miscible with common organic solvents	0.0067	Plasticizer (non-food applications)	SML= 3.0 mg/kg	GC-MS,DB-5MS PA_M 1.605 (Fraunhofer IVV method)
Dibutyl phthalate	1,2-Benzenedicarboxylic acid, dibutyl ester, DBP	278.35	84-74-2 / 74880	$C_{16}H_{22}O_4$		Ester	-35/ 340	11.2	4.5	Miscible with common organic solvents	2.01 E-05	Plasticizer	SML = 3.0 mg/kg	GC-MS, DB-5MS PA_M 1.605 (Fraunhofer IVV method)
Bis(2-Ethylhexyl) Phthalate	DEHP or DOP	390.56	117-81-7 / 74640	$C_{24}H_{38}O_4$		Ester	-55/ 384	0.27	7.6	-	1.42E-07	Plasticizer	SML = 3.0 mg/kg	GC-MS, DB-5MS PA_M 1.605 (Fraunhofer IVV method)
Diethylhexyl adipate	Adipic acid bis (2-ethylhexyl) ester, DOA or DEHA	370.57	103-23-1 / 31920	$C_{22}H_{42}O_4$		Ester	-67.8/ 417	0.78	6.11	-	8.50E-07	Plasticizer	SML = 3.0 mg/kg	GC-MS, DB-5MS PA_M 1.605 (Fraunhofer IVV method)
Glycerol triacetate	Triacetin, 1,2,3-Propanetriol triacetate	218.20	10 2-76-1 /	$C_9H_{14}O_6$		Ester	78 / 259	5.80E+04	0.25	Soluble in Alcohol, Ether, other organic solvents	0.00248	Plasticizer	-	
2-Ethylhexyl diphenyl phophate	DPOF, Diphenyl octyl phosphate, Octicizer	362.44	1241-94-7	$C_{20}H_{27}O_4P$		Phosphate ester	-80 / 239	1.9	5.76	-	6.29E-05	Plasticizer		
Diethylene glycol dibenzoate	Benzoflex 2-45, DEGDB	314.34	120-55-8 / 47720	$C_{18}H_{18}O_5$		Ester	33.5 / 225	193	3.04	-	0.096	Plasticizer		
Triethylene glycol dibenzoate	Benzoflex T 150, TEGDB	358.4	120-56-9	$C_{20}H_{22}O_6$								Plasticizer		
Dipropylene glycol dibenzoate	Benzoflex TPU 405	342.42	27138-31-4 / 51840	$C_{20}H_{22}O_5$				15	3.88		4.6E-07	Plasticizer		
Propylene glycol, dibenzoate	Benzoflex 284	284.3	19224-26-1	$C_{17}H_{16}O_4$			103.83 / 443.1	23.93	2.77	Soluble in Aliphatic- and Aromatic Hydrocarbon	3.84E-07	Plasticizer		
2,2,4-trimethyl-1,3-pentanediol dibenzoate	Benzoflex 354	354.45	68052-23-3	$C_{22}H_{26}O_4$								Plasticizer		

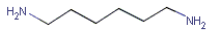
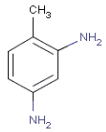
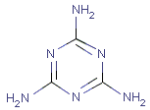
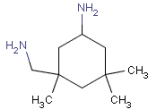
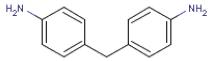
3: List of adhesive related, representative substances for establishing multi-methods and semi-quantitative estimates: Carboxylic acids

Substances		MW (g/mol)	CAS RN./ PM Ref.	Formula	Chemical structure	Chemical classification	mp / bp (℃)	Water Solubility mg/L (25 deg C)	Log PO/W	Solubility	Vapour Pressure mm Hg (25 deg C)	Uses	EU Restriction	Way to analyse via
Name	**Synonyms**													
Acrylic acid	2-Propenoic acid	72.06	79-10-7 / 10690	$C_3H_4O_2$		Unsaturated Carboxylic acid	13.5 / 141.2	1.0E+06	0.35		3.97	Monomer	SML(T) = 6 mg/kg	
Fumaric acid	2-Butenedioic acid	116.07	110-17-8 / 17290	HOOCCH=CHCOOH		Carboxylic acid	287dec/ 522	7000	0.46	Soluble in Ethanol and Acetone	0.000154	Monomer	-	HPLC-UV, 214 nm ODS column, H. S. Lee, 1993
Maleic acid	2-butenedioic acid, Cis-butenedioic acid	116.07	110-16-7 / 19540	HOOCCH=CHCOOH		Carboxylic acid	130.5/	4.41E+05	-0.48	Soluble in Alcohol and Acetone	3.59E-05	Monomer	SML(T) = 30 mg/kg	HPLC-UV, 220 nm ODS, EN 13130-24
Adipic acid	Hexanedioic acid	146.14	124-04-9 / 12130	$HOOC(CH_2)_4COOH$		Carboxylic acid	153.2/ 337.5	3.08E+04	0.08	Soluble in Alcohol and Acetone	3.18E-07	Monomer	-	HPLC
Terephthalic acid	1,4-Benzene-dicarboxylic acid	166.13	100-21-0 / 24910	$HOOCC_6H_4COOH$		Carboxylic acid	>300/	15	2	g/100g Methanol : 0.1 Acetic acid : 0.013	9.2E-06	Monomer	SML = 7.5 mg/kg	HPLC-UV, 242 nm ODS column EN 13130-2
Isophthalic acid	1,3-Benzene-dicarboxylic acid	166.13	121-91-5 / 19150	$HOOCC_6H_4COOH$		Carboxylic acid	347/	130	1.66	g/100g Methanol : 1.06 Acetic acid : 0.23	2.6E-08	Monomer	SML = 5 mg/kg	HPLC-UV, 242 nm ODS column EN 13130-2

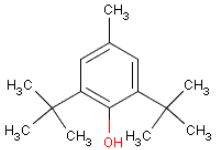
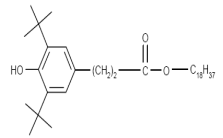
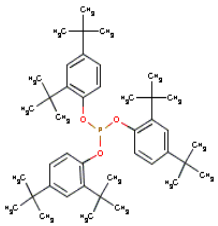
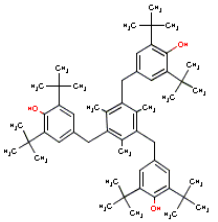
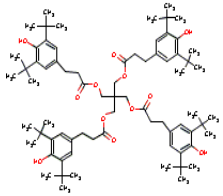
4: List of adhesive related, representative substances for establishing multi-methods and semi-quantitative estimates : Alcohols and Phenols

Substances		MW (g/mol)	CAS RN./ PM Ref.	Formula	Chemical structure	Chemical classification	mp / bp (℃)	Water Solubility mg/L (25 deg C)	Log PO/W	Solubility	Vapour Pressure mm Hg (25 deg C)	Uses	EU Restriction	Way to analyse via
Name	**Synonyms**													
Ethylene glycol	1,2-Dihydroxyethane	62.06	107-21-1 / 16990	$C_2H_6O_2$		Aliphatic dihydric alcohol	- 13 / 197.3	1.0E+06	-1.36	Soluble in most organic solvents, Poor soluble in Toluene, Benzene and Chloroform	0.092	Monomer for polyester, PU	SML(T) = 30 mg/kg	GC-FID, DB-FFAP, EN 13130-7 GC-FID, SPB-1, Flanagan, Streete, et al., 1997
Propylene glycol	1,2-Dihydroxypropane	76.10	57-55-6 / 23740	$C_3H_8O_2$		Aliphatic dihydric alcohol	-60 / 187.6	1.0E+06	-0.92	Soluble in Acetone, Chloroform, Ether and Ethanol. Miscible with oxygenated solvents	0.129	Monomer for polyester, PU	-	GC-FID, DB-1, Peng, 2000
1,4-Butanediol	1,4-Butylene glycol	90.12	110-63-4 / 13720	$C_4H_{10}O_2$		Aliphatic dihydric alcohol	20.1 / 235	1.0E+06	-0.83	Soluble in Alcohol Miscible with Acetone	0.0105	Monomer	SML(T) = 0.05 mg/kg	GC-FID, DB-1 Peng, 2000
Glycerol	1,2,3-Propanetriol, Glycerin	92.09	56-81-5 / 18100	$C_3H_8O_3$		Polyhydric alcohol	18.2 / 290	1.0E+06	-1.76	Soluble in Water and Alcohol	1.68E-04	Monomer, additive: humectand (prolongs open time)	-	GC-FID, Rtx-35 NIOSH
Diethylene glycol	2,2'-Dihydroxydiethyl ether, DEG	106.12	111-46-6/ 15760	$C_4H_{10}O_3$		Aliphatic diol	-10.4/ 245.8	1.0E+06	-1.47	Miscible with Ethanol, Acetone and Ether	0.0057	Polyester	SML(T) = 30 mg/kg	GC-FID, DB-FFAP, EN 13130-7
Resorcinol	1,3-Dihydroxybenzene, 1,3-Benzenediol	110.11	108-46-3 / 15910	$C_6H_6O_2$		Phenol	111/ 280	7.17E+05	0.8	g/100g (40 deg C) Benzene : 0.85 Acetone : 243.3 Chroloform : 0.78 Very soluble in Alcohol and Ether	0.0005	Resorcinol resins	SML = 2.4mg/kg	GC-FID, Mtx-1™ NIOSH Manual HPLC-UV 270 nm, ODS column, OSHA Manual
Bronopol	2-Bromo-2-nitropropane-1,3-diol	200.01	52-51-7 / 40460	$C_3H_6BrNO_4$			131.5/	2.5E+05	-0.64		1.26E-05	Antibacterial preservative, biocide		

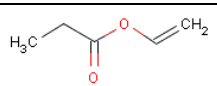
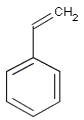
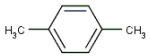
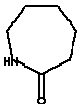
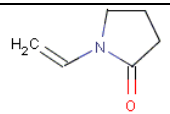
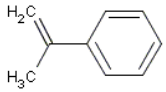
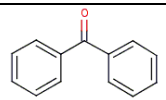
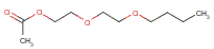
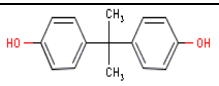
5: List of adhesive related, representative substances for establishing multi-methods and semi-quantitative estimates : Amines

Substances		MW (g/mol)	CAS RN./ PM Ref.	Formula	Chemical structure	Chemical classification	mp / bp (℃)	Water Solubility mg/L (25 deg C)	Log PO/W	Solubility	Vapour Pressure mm Hg (25 deg C)	Uses	EU Restriction	Way to analyse via
Name	**Synonyms**													
Hexamethylene diamine	1,6-Diaminohexane HMDA	116.21	124-09-4 / 18460	$C_6H_{16}N_2$		Amine	41.5/ 205	2.46E+06	0.35	Soluble in alcohols and aromatic solvents	1.1	Monomer, hydrolysis product of isocyanate	SML = 2.4 mg/kg	GC-FID, DB-1 or 100% methylsilicone coated column DIN CEN/TS 13130-21, 2005
Toluene 2,4-diamine	2,4-TDA	122.17	95-80-7 /	$C_7H_{10}N_2$		Amine	99/ 292	7.48E+04	0.14	Soluble in Alcohol, Ether and Oxigenated Solvents	0.00017	Hydrolysis product of isocyanate	-	LC-MS, APCI+ SRM : 123, 108 Fraunhofer IVV-PA LC-MS/MS, ESI+ MRM : 123.1, 108.3 S.K. Mortensen etc. 2005
Melamine	1,3,5-Triazine-2,4,6-triamine	126.12	108-78-1/ 25420	$C_3N_3(NH_2)_3$		Amine	345dec/	3240	-1.37	g/100 mL (30 deg C) Ethanol : 0.06 Acetone : 0.03 Dimethylformamide : 0.01 Ethyl cellosolve : 1.12	3.59-010 (20 deg C)	Monomer for amino resins	SML = 30 mg/kg	HPLC-UV, 230 nm Amino column, EN 13130-27
Isophorone diamine	IPDA, 1-Amino-3-aminomethyl-3,5,5-trimethyl cyclohexane	170.3	2855-13-2 / 19145, 12670	$C_{10}H_{82}N_2$		Amine	10/ 247	25200	1.9	-	0.015	Monomer, hydrolysis product of isocyanate	SML = 6 mg/kg	HPLC-FL, ODS EX. : 394, EM. : 480 Fraunhofer IVV PA
4,4'-Methylenedianiline	4,4 MDA, 4,4'-diaminodiphenzlmethan	198.26	101-77-9 / 16630	$C_{13}H_{14}N_2$		Amine	92.5/ 398	1000	1.59	Very soluble in Alcohol, Benzene and Ketones	2.97	Hydrolysis product of isocyanate	-	LC-MS, APCI+ SRM : 199, 106 Fraunhofer IVV-PA LC-MS/MS, ESI+ MRM : 199.1, 105.2 S.K. Mortensen etc. 2005

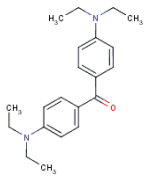
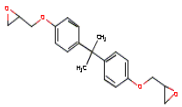
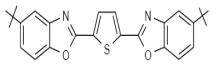
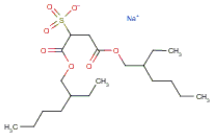
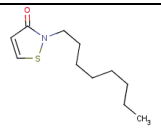
6: List of adhesive related, representative substances for establishing multi-methods and semi-quantitative estimates : Antioxidants

Substances		MW (g/mol)	CAS RN./ PM Ref.	Formula	Chemical structure	Chemical classification	mp / bp (℃)	Water Solubility mg/L (25 deg C)	Log PO/W	Solubility	Vapour Pressure mm Hg (25 deg C)	Uses	EU Restriction	Way to analyse via
Name	**Synonyms**													
Butylated hydroxytoluene	2,6-Di-tert-Butyl-4-Methylphenol, BHT	220.35	128-37-0 / 46640	$C_{15}H_{24}O$		Substituted toluene	71/ 265	0.6	5.1	Soluble in Toluene, Alcohos, Acetone, Chloroform, Benzene and Most Hydrocarbon solvents	0.0052	Antioxidants	SML = 3.0 mg/kg	GC-FID, DB-1 Fraunhofer IVV PA
n-Octadecyl-3-(4-hydroxy-3,5-di.tert.butylphenyl)propionate	Irganox 1076	531	2082-79-3 / 68320	$C_{35}H_{62}O_3$		Hindered phenol	50-55/	6.09E-09	13.41	% w/w Aceton : 19 Benzene : 57 Chloroform : 57 Cyclohexane : 40 Ethanol : 1.5 Ethylacetate : 38 n-Hexane : 32 Methanol : 0.6 Toluene : 50	3.38 E-13	Antioxidants	SML = 6 mg/kg	HPLC-UV 230 nm, ODS 2 column PA_K_1.340 (Fraunhofer IVV method)
Tris(p-tert.butylphenyl) phosphate	Irgafos 168	646.93	31570-04-4 / 74240	$C_{42}H_{63}O_3P$		Aryl phosphite	183-186/	< 0.01 (g/100g solution)	17.56	g/100g solution Aceton : 1 Chloroform : 36 Cyclohexane : 16 Ethanol : 0.1 Ethyl acetate : 4 n-Hexane : 11 Methanol : <0.01 Methylene chloride : 36 Toluene : 30	-	Antioxidants	-	HPLC-UV 230 nm, ODS 2 column PA_K_1.340 (Fraunhofer IVV method)
1,3,5-Trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl) benzene	Irganox 1330, Ionox 330, Irganox 330	775.21	1709-70-2 / 95200	$C_{54}H_{78}O_3$		Hindered phenol	244/	1.2	17.17	g/100g solution Aceton : 18 Chloroform : 28 Ethyl acetate : 27 n-Hexane : 10 Methanol : 3 Methylene chloride : 34	3.14E-22	Antioxidants	-	HPLC-UV 230 nm, ODS 2 column PA_K_1.340 (Fraunhofer IVV method)
Pentaerythritoltetrakis [3-(3,5-di-tert.butyl-4-hydroxyphenyl] propionate	Irganox 1010	1177.7	6683-19-8 / 71680	$C_{73}H_{108}O_{12}$		Sterically hindered phenol	110-125/	1.37E-18	1.36	g/100g solution Aceton : 47 Chloroform : 71 Ethanol : 1.5 Ethyl acetate : 47 n-Hexane : 0.3 Methanol : 0.9 Methylene chloride : 63 Toluene : 60	1.16E-33	Antioxidants	-	HPLC-UV 230 nm, ODS 2 column PA_K_1.340 (Fraunhofer IVV method)

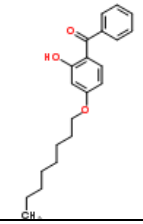
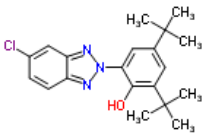
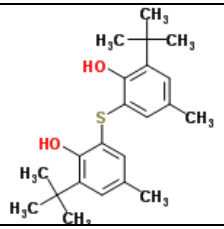
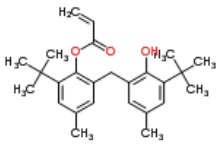
7: List of adhesive related, representative substances for establishing multi-methods and semi-quantitative estimates : Other

Substances		MW (g/mol)	CAS RN./ PM Ref.	Formula	Chemical structure	Chemical classification	mp / bp (℃)	Water Solubility mg/L (25 deg C)	Log PO/W	Solubility	Vapour Pressure mm Hg (25 deg C)	Uses	EU Restriction	Way to analyse via
Name	**Synonyms**													
Vinyl propionate	Propanoic acid, ethenyl ester	100.12	105-38-4 / 23920	$C_5H_8O_2$		Ester	-80/ 91.2	10600	1.22	-	36.6	Monomer Poly(vinyl esters)	SML(T) = 6 mg/kg	GC
Styrene	Ethenylbenzene, Vinyl bezene	104.15	100-42-5 / 24610	C_8H_8		Aromatic hydrocarbon; alkenylbenzene	-31/ 145	310	2.95	Soluble in Alcohol, Ether, Methanol and Acetone	6.4	Monomer for Synthetic rubber, PS	SML = 0.6 mg/kg	GC-FID, DB-Wax or DB-35 NIOSH Manual GC-FID, DB-1 S. Kent Hoekman, 1993 GC-FID, DB 1, PA_M_ 1_334
p-Xylene	Dimethylbenzene, Methyl toluene	106.17	106-42-3/	$C_6H_4(CH_3)_2$		Aromatic Hydrocarbon	13.2 / 138.5	162	3.15	Solubile in Alcohol,Ethanol, Diethyl ether and many organic solvents	8.84	Solvent	-	GC-FID, DB-Wax or DB-35 NIOSH Manual GC-FID, DB-1 S. Kent Hoekman, 1993
Caprolactam	1,6-Hexalactam	113.16	105-60-2 / 14200	$C_6H_{11}NO$		Amine and carboxylic acid	69.3/ 270	7.72E+05 at 10 deg C	0.66	wt % (20 deg C) Toluene : 24.2 Ethyl acetate : 24.2 MEK : 34.6 Cyclohexane : 2	0.0016	Monomer for polyamide	SML(T) = 15 mg/kg	GC-FID, CP-Sil 19 CB or DB 1701 EN 13130-16
N-Vinyl-2-Pyrrolidinone	1-Vinyl-2-pyrrolidinone	114.14	88-12-0 / 26230	C_6H_9NO		-	13.5/	52100	0.37	-	0.114	Monomer for poly(vinyl pyrrolidone)	SML = 0.05 mg/kg	GC-FID, DB-Wax, OSHA Manual
α-Methylstyrene	2-Phenylpropene, α-Methylstyrene	118.18	98-83-9 / 22210	C_9H_{10}		Aromatic hydrocarbon	-23.2/ 164.5	116	3.48	Miscible with Alcohol and Ether	1.9	Monomer: Styrene co-polymer	-	GC-FID, DB-Wax or DB-35 NIOSH Manual
Benzophenone	Diphenyl keton	182.23	119-61-9	$C_{13}H_{10}O$		Keton	49 / 305	137	3.18		0.00193	Photoinitiator		
2-(2-Butoxyethoxy)ethanol acetate	Butyl diglycol acetate	204.27	124-17-4 /	$C_{10}H_{20}O_4$		-	-32 / 245	3.10E+04	1.30	Miscible most organic solvents	0.04	solvent, rheolog. agent, brightness enhancer	-	
Bisphenol A	4,4'-Dihydroxy-2,2-diphenylpropane, BPA	228.29	80-05-7 / 13480	$C_{15}H_{16}O_2$		Phenol	153/	120	3.32	Soluble in Alcohol, Benzene and Ketones	3.91E-07	Monomer for epoxy resins	SML = 3 mg/kg	HPLC-FL, ODS, EX. : 235, EM. : 317 DIN CEN/TS 13130-13; 2005

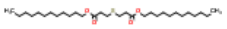
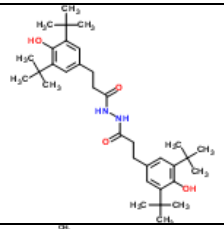
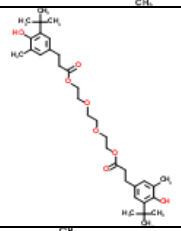
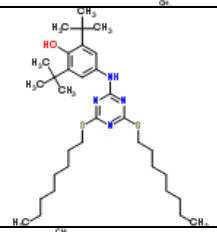
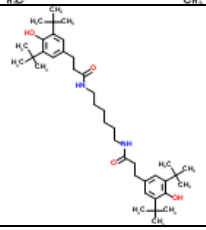
7: List of adhesive related, representative substances for establishing multi-methods and semi-quantitative estimates : Other (continuing)

4,4'-Bis(diethylamino)benzophenone	DEAB, bis(4-diethyl-aminophenyl) methanone, Michler's ketone	324.46	90-93-7	$C_{21}H_{28}N_2O$								Intermediate in pigment production, photoinitiator, crosslinker (not food contact applications), possible contaminant in recycling paper		
2,2-Bis(4-glycidyloxyphenyl)propane	BADGE, Bisphenol A diglycidyl ether	340.42	1675-54-3 / 13510	$C_{21}H_{24}O_4$		-	8-12/	3.69	3.84	-	1.08E-07	Monomer for epoxy resins	SML(T) = 1 mg/kg in FP or SML ND (DL = 0.020 mg/kg)	HPLC-Fl EX. : 275, EM. : 305 or UV 225 nm ODS2 column PA_M_1_338 (Fraunhofer IVV method)
Benzoxazole, 2,2'-(2,5-thiophenediyl)bis(5-(1,1-dimethylethyl)-	Uvitex OB	430.06	7128-64-5 /	$C_{26}H_{26}N_2O_2S$		Bis (benzoxazolyl) deriv.	196-202/ >350	6.79E-06	8.61	% w/w Aceton 0.5 Chloroform : 14 Ethyl acetate : 1 n-Hexane : 0.2 Methol : < 0.1	1.72E-12	Fluorescent whitening agent	-	HPLC-UV, ODS, 200 nm (Fraunhofer IVV method)
Sodium dioctyl sulfosuccinate	Docusate sodium	445.63	577-11-7 /	$C_{20}H_{38}O_7SNa$			176 /	7.10E+04	6.10	Soluble in Alcohol, Glycerol, CCl4, Acetone, Xylene, Hexane	2.17E-11	Wetting agent for water based emulsion (adhesives)	-	
2-Octyl-2H-isothiazol-3-one	Octhilinone	213.34	26530-20-1	C11H19NOS			< 25	500	2.45		3.68E-05	biocide		

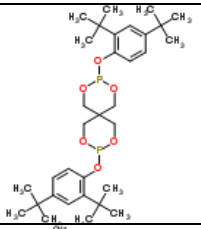
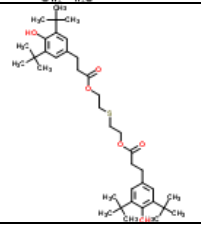
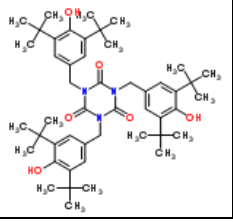
8: List of adhesive related, representative substances for establishing multi-methods and semi-quantitative estimates : Additional additives

Substances		MW (g/mol)	CAS RN./ PM Ref.	Formula	Chemical structure	Chemical classification	mp / bp (℃)	Water Solubility mg/L (25 deg C)	Log PO/W	Solubility	Vapour Pressure mm Hg (25 deg C)	Uses	EU Restriction	Way to analyse via
Name	**Synonyms**													
(2-hydroxy-4-octoxy-phenyl)-phenyl-methanone	Chimassorb 81	326.19	1843-05-6 / 61600	$C_{21}H_{26}O_3$		Organic benzophenone deriv.	45 / 458	0.56	6.96	Soluble in Acetone, Benzene	5.25E-09	UV absorber for PE, PP, PVC EVA, Adhesive	SML(T) = 6 mg/kg (15)	HPLC-UV
2-(2'-Hydroxy-3,5'-di-tert-butylphenyl)-5-chlorobenzotriazole	Tinuvin 327	357.16	3864-99-1 / 60480	$C_{20}H_{24}ClN_3O$		Phenol	/ 469	1.03	6.91	Soluble in Acetone, Benzene	2E-09	UV absorber	SML(T) = 30 mg/kg (19)	HPLC
2,2'-Thiobis(4-methyl-6-tert-butylphenol)	Irganox 1081	358.54	90-66-4 /	$C_{22}H_{30}O_2S$		Phenol	/ 431	-	6.83	sol. (g/100 ml): 123 g in toluene, 87 g in acetone, 55 g in hexane, 52 g in IPA, 30 g in ethanol insoluble in water	4.9E-08	Antioxidant	-	HPLC
Acrylic acid, 2-tert-butyl-6-(3-tert-butyl-2-hydroxy-5-methylbenzyl)-4-methylphenyl ester	Irganox 3052	394.25	61167-58-6 / 31520	$C_{26}H_{34}O_3$		Ester	198 / 470	0.0008	8.40	Soluble in Acetone	2.74E-10	Antioxidant	SML = 6 mg/kg	HPLC

8: List of adhesive related, representative substances for establishing multi-methods and semi-quantitative estimates : Additional additives (continuing)

dodecyl 3-(3-dodecoxy-3-oxo-propyl)sulfanylpropanoate	Irganox PS 800	514.41	123-28-4 / 93120	$C_{30}H_{58}O_4S$		Phenol	40 / 240	-	12.88	sol. (g/100 g): 65 g toluene, 60 g ethyl acetate, 55 g acetone, 52 g heptane; insol. in water	1.76E-13	Antioxidants for adhesive, PE, Styrene homo and copolymer	SML(T) = 5 mg/kg (21)	-
N,N'-Bis(3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionyl)hydrazide	Irganox MD 1024	552.39	32687-78- 8 / 38800	$C_{34}H_{52}N_2O_4$		-	/ 653	-	8.37	Soluble in Acetone	1.24E-17	Antioxidant	SML = 15 mg/kg	HPLC
Triethyleneglycol bis[3-(3-tert-butyl-4-hydroxy -5-methylphenyl) propionate]	Irganox 245	586.37	36443-68-2 / 94400	$C_{34}H_{50}O_8$		Sterically hindered phenol	79 / 674	-	6.55	Soluble in Acetone, Chloroform, Dichloromethane, Benzene, Methanol	8.21E-19	Antioxidant	SML = 9 mg/kg	HPLC
2,4-Bis(octylmercapto)-6-(4-hydroxy-3,5-ditert-butylanilino)-1,3,5-triazine	Irganox 565	588.39	991-84-4 / 40000	$C_{33}H_{56}N_4OS_2$		Phenol	/ 670	-	12.68	Soluble in Acetone	1.33E-18	Antioxidant	SML = 30 mg/kg	HPLC
3-(3,5-ditert-butyl-4-hydroxy-phenyl)-N-[6-[3-(3,5-ditert-butyl-4-hydroxy-phenyl)propanoylamino]hexyl]propanamide	Irganox 1098	636.49	23128-74-7 / 59120	$C_{40}H_{64}N_2O_4$		Substituted benzenepropanamide	159 / 740	-	8.80	Soluble in chloroform, methanol, acetone, ethyl acetate, water, benzene, and hexane	1.19E-22	Antioxidant	SML = 45 mg/kg	HPLC

8: List of adhesive related, representative substances for establishing multi-methods and semi-quantitative estimates : Additional additives (continuing)

Bis(2,4-di-tert-butylphenyl) pentaerythritoldiphosphite	Ultranox 626	640.33	26741-53-7 / 38820	$C_{33}H_{50}O_6P_2$		-	175 / 556	-	11.76	Soluble (g/100 ml): 41 g toluene, 35.7 g THF, 34.2 g dichloromethane, 10.7 g acetone, 7.3 g hexane; Insoluble in water	8.01E-12	Antioxidant	SML = 0.6 mg/kg	HPLC
2-[2-[3-(3,5-ditert-butyl-4-hydroxy-phenyl)propanoyloxy]ethylsulfanyl]ethyl 3-(3,5-ditert-butyl-4-hydroxy-phenyl)propanoate	Irganox 1035	642.40	41484-35-9 / 92880	$C_{38}H_{58}O_6S$		-	73 / 659	-	10.64	Soluble in Acetone, Chloroform, Ethanol, Ethyl acetate	5.38E-18	Antioxidant	SML = 2.4 mg/kg	HPLC
1,3,5-tris[(3,5-ditert-butyl-4-hydroxy-phenyl)methyl]-1,3,5-triazinane-2,4,6-trione	Irganox 3114	783.52	27676-62-6 / 95360	$C_{48}H_{69}N_3O_6$		-	233 / 757	-	10.34	Soluble in Acetone, Toluene, Dichloromethane, Methanol	8.89E-24	Antioxidant	SML = 5 mg/kg	HPLC

9.3. Methods (CEN_ European Committee for Standardization Format)

Migration estimation of adhesives related substances in food packaging materials by using analytical multi-method.

Method A for non-volatile substances

Contents

Foreword

This analytical method has been prepared within the collective reaearch project 030309 WP2b Migresives “Research programme on migration from adhesives in food packaging materials in support of european legislation and standardisation”. This method is prepared according to a CEN standard format.

1 Introduction

The adhesive related substances used for the manufacture of the food packaging materials can remain in the finished products and may migrate into foodstuffs. They have different physico-chemical properties concerning volatility and polarity as well as functional groups. Analysing all adhesive related substances with individual analytical methods for quantification is not manageable due to the multitude of methods which would have to be applied.

Therefore a multi-screening method which covers a broad range of physico-chemical properties and a semi-quantitative method using universal standard substances were developed for the investigation of the volatile and semi-volatile adhesive related substances.

2 Scope

This document describes a multi-screening method for the non-volatile adhesive related substances in food packaging materials and a semi-quantitative method of unknown substances by universal standard substances.

The method should be also applicable not only for the adhesives compliance testing area but also for other migration potentials from other material categories for instance laquers, coatings, printing inks and more.

3 Principle

For the semi-quantitative approach of the adhesive related substances a multilayer material is extracted by a solvent which is then analysed by high performance liquid chromatography (HPLC) combined with a universal detector (Charged Aerosol Detector). Unknowns are semi-quantified using the universal internal standard Tinuvin 234. To enhance the accurancy of the semi-quantification the relative response factors (RRF) of many adhesive representative compounds are determined by HPLC-CAD analysis in relation to Tinuvin 234.

By using these RRF values, a distribution range at 95 % coverage level could be established. The calculation factor that should be used for the semi-quantification of unknown substances could then be derived from this distribution range.

NOTE: For this semi-quantitative approach the adhesive representatives should be selected according to the following considerations.

- The selected adhesive related substances should be typically used for adhesives formulations and represent different chemical structures, polarities and molecular weights.
- The maximum molecular weights of the adhesive representatives are about 1000 g/mol. According to the Guidelines of the Scientific Committee on Food (SCF), substances with molecular weight below 1000 g/mol are regarded as toxicologically relevant, since the substances with a molecular weight above 1000 g/mol will not be adsorbed in the gastrointestinal tract.
- The adhesive representatives should be available for calibration purposes. Therefore low molecular weight oligomers or other mixtures of substances could not be included in the list. The selected substances contain monomers, additives and some solvents.

4 Reagents

NOTE: All reagents should be of recognised analytical quality unless otherwise stated.

4.1 Analytes

4.1.1 Representative adhesive related substances, Purity $\geq$ 95 %

See the list presented in Annex A.

NOTE: There is no pure Dipropylene glycol dibenzoate (DPGDB) standard more than purity 95 % from reagent dealers. As the next best way, DPGDB of purity 80 % (technical grade) was purchased from sigma-aldrich.

4.1.2 Internal standards_2-(2H-benzotriazol-2-yl)-4,6-bis(1-methyl-1-phenylethyl)phenol (Tinuvin 234)

4.2 Chemicals

4.2.1 Acetone

4.2.2 Acetonitrile (HPLC grade)

4.2.3 Methanol (HPLC grade)

4.2.4. Water (HPLC grade)

4.3 Solutions

4.3.1 Stock solution of the representative adhesive related substances (1 mg/ml)

Weigh to the nearest 0,1 mg approximately 10 mg of into a 10 ml volumetric flask, which contains approximately 10 ml of Acetone or Methanol (Annex A). Make up to the mark with Methanol and mix carefully.

NOTE : The representative adhesive related substances classfy according to application intention or functional group of substances for convenience of analysis groups (Group A ~ G) and the classified substances divide into eight groups (Group A ~ G) and the detail list presented in separated list.

Calculate the correct concentration of each representative adhesive related substance.

4.3.2 Internal standard stock solution of 2-(2H-benzotriazol-2-yl)-4,6-bis(1-methyl-1-phenylethyl)phenol (Tinuvin 234) (1 mg/ml)

Weigh to the nearest 0.1 mg, approximately 10 mg of Tinuvin 234 into a 10 ml volumetric flask. Make up to the mark with Acetone. Calculate the correct concentration of Tinuvin 234.

4.3.3 Standard solutions of the representative adhesive related substances

1, 5, 10, 50, 100, 250 and 500 µl of the standard stock solutions (4.3.1) of each analysis group (Group A ~ G) at a concentration of approximately 1000 µg/ml were filled into a series of 10 ml volumetric flasks. The flasks were filled up to the marks with Methanol. These standard solutions contain approximately 0.1, 0.5, 1, 5, 10, 25 and 50 µg/ml of each substance.

The standard solutions were spiked with the internal standard mixture consisting of Tinuvin 234 prior to injection for HPLC-CAD.

Calculate the correct concentration of each representative adhesive related substance.

5 Apparatus

NOTE : An instrument or item of apparatus is listed only where it is special or made to a particular specification, the usual laboratory glassware and equipment being assumed to be available.

5.1 High performance liquid chromatography equipped with a charged aerosol detector (CAD) produced by ESA Biosciences Inc.

5.2 High performance liquid chromatographic parameters

NOTE: HPLC apparatus should be optimised according to manufacturer's instruction.

High performance liquid chromatograph analytical column packed with a bonded reverse phase C18 25 cm x 4.6 mm I.D 5 µm particle size silica based packing maintained at a constant temperature of 40 °C ± 1 °C. Allow the analytical column to equilibrate at the correct flow rate for an hour.

Appropriate operating conditions have to be established for the specific equipment used for the determination.

The following column and chromatographic conditions have been found to be suitable.

Column: HyperClone C18 25 cm x 4,6 mm internal diameter 5 µm particle size maintained at a constant 40 °C ± 1 °C.

Mobile phase: Acetonitrile and Water gradient

Gradient condition

Time (min)	0	1	25	45	50	60
A(%)	40	40	0	0	40	Stop
B(%)	60	60	100	100	60	

A: Water B: Acetonitrile

Flow rate: 1.0 ml/min

Injector: 20 µl loop

Detection: The CAD was set to a gas pressure of 35 psi, none filter mode and a range of 100 pA

NOTE : 27 substances in 67 representative adhesive related substances have been observed with corresponding retention times as shown in Annex B. The minimum molecular weight of the observed substances on CAD was 228.29 g/mol (Bisphenol A). However, the molecular weights of most observed substances which could not be detected on CAD, are below 300 g/mol.

Dipropylene glycol dibenzoate (DPGDB) among the observed substances is detected with two separated peaks. The second peak is isomer peak of DPGDB and therfore the sum of areas of both peaks should be taken to calculate the relative response factor.

6 Samples

The samples of food simulants to be analysed are obtained as described in Directive 85/572/EEC "List of simulants" and Directive 97/48/EC for the basic rules of migration testing regarding time and temperature conditions. Samples are to be kept refrigerated with the exclusion of light.

6.1 Test sample preparation

Filter the migration solutions using a teflon filter of 0.2 μm. Transfer 0.95 ml of the solution into a vial suitable for HPLC injection and spike 0.05 ml of the internal standard (4.3.2) into the vial.

6.2. Blank sample preparation

Treat food simulants which have not been in contact with packaging material in the same way as described in clause 6.1.

7 Procedure

7.1 HPLC-CAD analysis

Examine the baseline stability and response linearity of the detector before starting measurements.

Maintain the same operating conditions throughout the measurements of all calibration solutions prepared in 4.3.3.

7.2 Calibration

Inject the calibration solutions prepared in 4.3.3.

The calibration samples prepared in 4.3.3 are analysed as they are without further sample treatment.

Identfy of the observed adhesive related substances peaks on the basis of the retention times and measure the total peak areas.

Obtain the integrated peak areas of the calibration solutions of each known adhesive related substance. Construct the calibration curves by plotting the peak area ratios (known adhesive related substance/internal standard) against the concentrations of each known adhesive related substance.

The calibration curves of the universal standard substances should be rectilinear and the correlation coefficient should be 0,996 or better and also the calibration line pass through the origin of the x and y axis. The universal standard substances which are not met these prerequisites should be excluded from the data evaluation.

7.3 Evaluation of data

7.3.1 HPLC interferences

Following the method described above no interferences have been detected in standard solutions.

7.3.2 Calculation of relative response factor (RRF) values

The relative response factor (RRF) is defined as signal/concentration ratio between analyte and the internal standard and can be calculated as following equation.

$$\text{Relative Response Factor (RRF)} = \frac{Area_s \times C_{is}}{C_s \times Area_{is}}$$

$Area_s$: Peak area of analyte
C_s : Concentration of analyte
$Area_{is}$: Peak area of the Internal standard
C_{is} : Concentration of the Internal standard

7.3.3. Statistical data analysis of relative response factor (RRF) values

Normality test

The normality of the data is a prerequisite to estimate the distribution range. The normality test can be performed by like Wilks Shapiro test, Ryan-Joiner test and other methods. For this, various statistical software packages can be used as follows.

a) MINITAB : Minitab Inc., Pennsylvania, USA
b) SAS : SAS institute, North Carolina, USA

NOTE: Many statistical tests and intervals are based on the assumption of normality. However, in many real cases, the data do not follow a normal distribution and therefore it is not possible to estimate the distribution range. For these non-normal data, an appropriate transformation is needed. The Box-Cox transformation proposed by Box and Cox is useful. It is defined as follows.

$$\text{Response variable (T)} = \frac{(Y^{\lambda} - 1)}{\lambda} \quad \text{if } \lambda \neq 0$$

$$\text{Response variable (T)} = log_e(Y + \lambda) \quad \text{if } \lambda = 0$$

Where Y is the variable and λ is the Box-Cox parameter indicating a number that represents the optimal transformation for correcting non-normality. The optimal value of λ can be determined by the Box-Cox plot that gives a correlation between the pooled standard deviations (SD) versus the λ values. At this time, SD is the Y axis and Lambda is the X axis. All these procedures for Box-Cox transformation can be accomplished by using 'Minitab' statistical package.

Distribution range estimation

The distribution range can be calculated as follow equations.

Distribution range of response factors = $\mu \pm 1.96\,\sigma$ $n > 30$

Distribution range of response factors = $\mu \pm t\,\sigma$ $n < 30$

where μ is mean of RRF values, σ is standard deviation, t is a t-variable of t-distribution and n is number of sample.

7.3.4 Calculation of unknown adhesive related substances

The RRF values of the universal adhesive related substances are calculated from the chromatographic results analyzed with HPLC-CAD screening method and established in a distribution range (7.3.3). For the semi-quantitative (or conservative) estimation, a statistical RRF value can be derived from the established distribution range of RRF values. The concentration of unknown substances can be simply calculated as follows.

Typical estimation using RRF

$$C_s^{known} = \frac{C_{is} \times Area_s^{known}}{Area_{is} \times RRF}$$

$Area_s^{known}$: peak area of known analyte
$Area_{is}$: peak area of the Internal standard
C_s^{known} : concentration of known analyte
C_{is} : concentration of the Internal standard
RRF : average relative response factor of known analyte in defined calibration range

Semi-quantitative estimation using statistical RRF

$$C_s^{unknown} = \frac{C_{is}^{universal} \times Area_s^{unknown}}{Area_{is}^{universal} \times RRF^{statistic}}$$

$Area_s^{unknown}$: peak area of unknown analyte
$Area_{is}^{universal}$: peak area of the Universal internal standard (Tinuvin 234)
$C_s^{unknown}$: concentration of unknown analyte
$C_{is}^{universal}$: concentration of the Universal internal standard (Tinuvin 234)
$RRF^{statistic}$: statistical relative response factor for semi-quantitative determination

8. Confirmation

8.1 Requirement for confirmation

In cases that a substance in the universal standards are detected with two or more separated peaks, the peaks shall be confirmed and all separated peaks originated from the substance, the sum of areas of all peaks should be taken for the calculation of the relative response factor.

Dipropylene glycol dibenzoate standard in the list was detected with two separated peaks. Therefore these peaks shall be confirmed by the method described in 8.2.

8.2 Confirmation by liquid chromatography (LC) using mass spectrometry (MS)

The calibration solutions prepared in section 4.3 shall be reanalysed using LC-MS.

LC conditions

Column	HyperClone C18 column (250 × 4.60 mm, 5 μm particle size)
Mobile phase	75 % acetonitrile and 25 % Ammonium acetate buffer (5 mM, pH=3.5)

Flow rate	0.6 ml/min
Injection volumn	10 µl
Oven Temperature	40 °C

MS conditions

Ion source	Electron Ionization (ESI)
Polarity	Positive
Mass range	150 ~ 1000 g/mol
Scan rate	0.5 seconds / mass range
Desolvation temperature	400 °C

NOTE : Sodium adducts of dipropylene glycol dibenzoate (m/z 365) was only confirmed from the mass spectra. Therefore, the second peak was identified as isomer peak of dipropylene glycol dibenzoate. For the calculation of the relative response factors, the sum of areas of both peaks was taken.

9 Precision

The within-laboratory detection limits (WDL) of the calibrated, based on the calibration curve method according to DIN 32645, were found to be in the range of 0.2 – 2.6 µg/ml.

10 Test report

The test report shall contain as a minimum, the following :

- date of analysis and reporting ;
- clear identification of the test laboratory and the responsible analyst;
- universal standard substances (representative adhesive related substances) and test method;
- sample details like origin and specification, type of food/simulant/material/article, reception date, and storage condition;
- results expressed in milligram unknown substances per kilogram food simulant or packaging material.

Annex A
(informative)

List of known adhesive related substances as candidates for universal standards for development of screening methods.

Classification	Nr.	Name	Synonyms	MW (g/mol)	CAS-Nr.	Manufacturer	Purity (%)	Stock Solution in
Group A Acrylate	1	Acrylic acid methyl ester	Methyl acrylate	86.09	96-33-3	Aldrich	> 99 %	MeOH
	2	Acrylic acid ethyl ester	Ethyl acrylate	100.11	140-88-5	Aldrich	> 99 %	MeOH
	3	2-Methylacrylic acid methyl ester	Methyl methacrylate	100.11	80-62-6	Aldrich	99 %	MeOH
	4	2-Methyl-2-propenoic acid ethyl ester	Ethyl methacrylate	114.14	97-63-2	Aldrich	99 %	MeOH
	5	Acrylic acid butyl ester	Butyl acrylate	128.18	141-32-2	Aldrich	> 99 %	MeOH
	6	Methacrylic acid, butyl ester	Butyl methacrylate	142.19	97-88-1	Aldrich	99 %	MeOH
	7	2-ethylhexyl prop-2-enoate	Ethylhexyl acrylate	184.28	1322-13-0	Fluka	> 98 %	MeOH
Group B Plasticizers	8	Diisobutyl phthalate	DIBP	278.35	84-69-5	Merck	> 98 %	MeOH
	9	Dibutyl phthalate	DBP	278.35	84-74-2	Merck	> 98 %	MeOH
	10	Bis(2-ethylhexyl) phthalate	DEHP or DOP	390.56	117-81-7	Merck	> 98 %	MeOH
	11	Diethylhexyl adipate	DEHA	370.57	103-23-1	Merck	> 98 %	MeOH
	12	Glycerol triacetate	Triacetin	218.20	102-76-1	Sigma	≥ 99 %	MeOH
	13	2-Ethylhexyl diphenyl phophate	Phosflex 362	362.44	1241-94-7	Riedel-de-Haën	99 %	MeOH
	14	Diethylene glycol dibenzoate	DEGDB	314.34	120-55-8	Aldrich	96 %	MeOH
	15	Triethylene glycol dibenzoate	TEGDB	358.40	120-56-9	Aldrich	99 %	MeOH
	16	Dipropylene glycol dibenzoate	DPGDB	342.42	27138-31-4	Aldrich	80 %	MeOH
	17	Propylene glycol, dibenzoate	Bezoflex 284	284.3	19224-26-1	Aldrich	> 96 %	MeOH
	18	2,2,4-Trimethyl-1,3-pentanediol dibenzoate	Benzoflex 354	354.45	68052-23-3	Aldrich	> 99.9 %	MeOH
Group C Carboxylic acid	19	2-Propenoic acid	Acrylic acid	72.06	079-10-7	Aldrich	> 99 %	MeOH
	20	trans-Butenedioic acid	Fumaric acid	116.07	110-17-8	Aldrich	> 99 %	MeOH
	21	cis-Butenedioic acid	Maleic acid	116.07	110-16-7	Aldrich	> 99 %	MeOH
	22	Hexanedioic acid	Adipic acid	146.14	124-04-9	Aldrich	99 %	MeOH
	23	1,4-Benzene-dicarboxylic acid	Terephthalic acid	166.13	100-21-0	Fluka	> 99 %	MeOH
	24	1,3-Benzene-dicarboxylic acid	Isophthalic acid	166.13	121-91-5	Fluka	99 %	MeOH
Group D Alcohol	25	1,2-Dihydroxyethane	Ethylene glycol	62.06	107-21-1	Fluka	> 99.5 %	MeOH
	26	1,2-Dihydroxypropane	Propylene glycol	76.1	57-55-6	Fluka	> 99.5 %	MeOH
	27	1,4-Butanediol	1,4-Butylene glycol	90.12	110-63-4	Fluka	99 %	MeOH
	28	2,2'-Dihydroxydiethyl ether	Diethylene glycol	106.12	111-46-6	Fluka	> 99 %	MeOH
	29	1,3-Benzenediol	Resorcinol	110.11	108-46-3	Fluka	> 99 %	MeOH
	30	1,2,3-Propanetriol	Glycerol	92.09	56-81-5	Sigma-aldrich	≥ 99.5 %	MeOH
	31	2-Bromo-2-nitropropane-1,3-diol	Bronopol	200.01	52-51-7	Riedel-de-Haën	99.9 %	MeOH
Group E Amine	32	Hexamethylenediamine	HMDA	116.21	124-09-4	Fluka	> 99 %	MeOH
	33	Toluene 2,4-diamine	2,4-TDA	122.17	95-80-7	Aldrich	98 %	MeOH
	34	1,3,5-Triazine-2,4,6-triamine	Melamine	126.12	108-78-1	Fluka	> 99 %	MeOH
	35	Isophorone diamine	IPDA	170.3	2855-13-2	Fluka	> 99 %	MeOH
	36	4,4'-Methylenedianiline	4,4 MDA	198.26	101-77-9	Fluka	> 97 %	MeOH
Group F Antioxidants	37	2,6-Di-tert-butyl-4-methylphenol	BHT	220.35	128-37-0	Merck	> 99 %	MeOH
	38	Octadecyl 3,5-bis (1,1-dimethylethyl)-4-hydroxybenzene propanoate	Irganox 1076	531	2082-79-3	Ciba	-	Acetone
	39	2,4-Bis (1,1 dimethylethyl) phenyl-phosphite	Irgafos 168	646.93	31570-04-4	Ciba	-	Acetone
	40	1,3,5-Trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl) benzene	Irganox 1330	775.21	1709-70-2	Ciba	-	Acetone
	41	Tetrakis [methylene-3 (3´,5´-di-t-butyl-4-hydroxyphenyl) propionate] methane	Irganox 1010	1177.7	6683-19-8	Ciba	-	Acetone
	56	(2-hydroxy-4-octoxy-phenyl)-phenyl-methanone	Chimassorb 81	326.19	1843-05-6	Ciba	-	Aceton
	57	2-(2'-Hydroxy-3,5'-di-tert-butylphenyl)-5-chlorobenzotriazole	Tinuvin 327	357.16	3864-99-1	Ciba	-	Aceton
	58	Acrylic acid, 2-tert-butyl-6-(3-tert-butyl-2-hydroxy-5-methylbenzyl)-4-methylphenyl ester	Irganox 3052	394.25	61167-58-6	Ciba	-	Aceton
	59	2,2'-Thiobis(4-methyl-6-tert-butylphenol)	Irganox 1081	358.54	1709-70-2	Ciba	-	Aceton
	60	dodecyl 3-(3-dodecoxy-3-oxo-propyl)sulfanylpropanoate	Irganox PS 800	514.41	123-28-4	Ciba	-	Aceton
	61	N,N'-Bis(3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionyl)hydrazide	Irganox MD 1024	552.39	32687-78-8	Ciba	-	Aceton
	62	Triethyleneglycol bis[3-(3-tert-butyl-4-hydroxy -5-methylphenyl) propionate]	Irganox 245	586.37	36443-68-2	Ciba	-	Aceton
	63	2,4-Bis(octylmercapto)-6-(4-hydroxy-3,5-ditert-butylanilino)-1,3,5-triazine	Irganox 565	588.39	991-84-4	Ciba	-	Aceton
	64	3-(3,5-ditert-butyl-4-hydroxy-phenyl)-N-[6-[3-(3,5-ditert-butyl-4-hydroxy-phenyl)propanoylamino]hexyl]propanamide	Irganox 1098	636.49	23128-74-7	Ciba	-	Aceton
	65	Bis(2,4-di-tert-butylphenyl) pentaerythritoldiphosphite	Ultranox 626	640.33	26741-53-7	Ciba	-	Aceton
	66	2-[2-[3-(3,5-ditert-butyl-4-hydroxy-phenyl) propanoyloxy]ethylsulfanyl]ethyl 3-(3,5-ditert-butyl-4-hydroxy-phenyl)propanoate	Irganox 1035	642.40	41484-35-9	Ciba	-	Aceton
	67	1,3,5-tris[(3,5-ditert-butyl-4-hydroxy-phenyl)methyl]-1,3,5-triazinane-2,4,6-trione	Irganox 3114	783.52	27676-62-6	Ciba	-	Aceton

Continued Annex A

Group G **Others**	42	Propanoic acid, ethenyl ester	Vinyl propionate	100.12	105-38-4	Fluka	> 98 %	MeOH
	43	Ethenylbenzene	Styrene	104.15	100-42-5	Aldrich	99 %	MeOH
	44	Dimethylbenzene	p-Xylene	107.17	106-42-3	Riedel-de-Haën	99.9 %	MeOH
	45	1,6-Hexalactam	Caprolactam	113.16	105-60-2	Fluka	> 99 %	MeOH
	46	N-Vinyl-2-pyrrolidinone	1-Vinyl-2-pyrrolidinone	114.14	88-12-0	Acros	99 %	MeOH
	47	2-Phenylpropene	α-Methylstyrene	118.18	98-83-9	Aldrich	99 %	MeOH
	48	Diphenyl keton	Benzophenone	182.23	119-61-9	Aldrich	≥ 99 %	MeOH
	49	2-(2-Butoxyethoxy)ethanol acetate	Butyl diglycol acetate	204.27	124-17-4	Aldrich	> 99.2 %	MeOH
	50	2-Octyl-2H-isothiazol-3-one	Octhilinone	213.34	26530-20-1	Riedel-de-Haën	99 %	MeOH
	51	4,4'-Dihydroxy-2,2-diphenylpropane,	Bisphenol A	228.29	80-05-7	Fluka	97 %	MeOH
	52	Methanone, bis(4-(diethylamino)phenyl)-	4,4'-Bis(diethylamino) benzophenone, BDBP	324.46	90-93-7	Aldrich	> 99 %	MeOH
	53	Bisphenol A diglycidyl ether	BADGE	340.42	1675-54-3	Fluka	97 %	MeOH
	54	Benzoxazole, 2,2'-(2,5-thiophenediyl) bis(5-(1,1-dimethylethyl)-	Uvitex OB	430.06	7128-64-5	Ciba	-	MeOH
	55	Sodium dioctyl sulfosuccinate	Docusate sodium	445.63	577-11-7	Sigma	≥ 99 %	MeOH

Annex B
(informative)

Retention time of representative adhesive related substances detected by HPLC-CAD.

Classification	Nr.	Substances	MW (g/mol)	Retention time (min)
Group B Plasticizers	1	Diethylhexyl phthalate	390.56	25.3
	2	Diethylhexyl adipate	370.57	25.4
	3	2-ethylhexyl diphenyl phosphate	362.44	16.3
	4	Diethylene glycol dibenzoate	314.34	7.3
	5	Triethylene glycol dibenzoate	358.40	7.3
	6	Dipropylene glycol dibenzoate	342.42	10.7 / 10.9
	7	Propylene glycol dibenzoate	284.3	9.3
	8	2,2,4-trimethyl-1,3-pentanediol dibezoate	354.45	16.3
Group F Antioxidants	9	Irganox 1076	531	42.8
	10	Irgafos 168	646.93	46.9
	11	Irganox 1330	775.21	33.0
	12	Irganox 1010	1177.7	30.7
	13	Chimasorb 81	326	22.5
	14	Tinuvin 327	357.16	27.6
	15	Irganox 1081	358.5	20.9
	16	Irganox 3052	394.25	21.1
	17	Irganox PS 800	515	37.9
	18	Irganox MD 1024	552.39	13.9
	19	Irganox 245	586.37	13.0
	20	Irganox 565	588.4	36.9
	21	Irganox 1098	637	15.3
	22	Ultranox 626	640.3	30.2
	23	Irganox 1035	642	23.7
	24	Irganox 3114	784	26.2
Group G Others	25	Bisphenol A	228.29	3.8
	26	4,4’-bis (diethylamino)benzophenone	324.46	13.1
	27	BADGE	340.42	7.9
	28	Uvitex OB	430.06	26.4
	29	Docusate sodium	445.63	-

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9.4. Methods (CEN_ European Committee for Standardization Format)

Migration estimation of adhesives related substances in food packaging materials by using analytical multi-method.

Method B for volatile and semi-volatile substances

Contents

Foreword

This analytical method has been prepared within the collective reaearch project 030309 WP2b Migresives “Research programme on migration from adhesives in food packaging materials in support of european legislation and standardisation”. This method is prepared according to a CEN standard format.

1 Introduction

The adhesive related substances used for the manufacture of the food packaging materials can remain in the finished products and may migrate into foodstuffs. They have different physico-chemical properties concerning volatility and polarity as well as functional groups. Analysing all adhesive related substances with individual analytical methods for quantification is not manageable due to the multitude of methods which would have to be applied.

Therefore a multi-screening method which covers a broad range of physico-chemical properties and a semi-quantitative method using universal standard substances were developed for the investigation of the volatile and semi-volatile adhesive related substances.

2 Scope

This document describes a multi-screening method for the volatile and semi-volatile adhesive related substances in food packaging materials and a semi-quantitative method for unknown substances by using universal standard substances.

The methods are applicable not only for the adhesives compliance testing area but also for the determination of migration potentials from other material categories like laquers, coatings, printing inks and more.

3 Principle

For the semi-quantitative approach of the adhesive related substances a multilayer material is extracted by a solvent which is then analysed by gas chromatography (GC) equipped with a universal flame ionisation detector (FID). Unknowns are semi-quantified using the universal internal standard BHA. To enhance the accurancy of the semi-quantification the relative response factors (RRF) of many adhesive representative compounds are determined by GC-FID analysis in relation to BHA.
By using these RRF values, a distribution range at 95 % coverage level could be established. The calculation factor that should be used for the semi-quantification of unknown substances could then be derived from this distribution range.

NOTE: For this semi-quantitative approach the adhesive representatives should be selected according to the following considerations.

- The selected adhesive related substances should be typically used for adhesives formulations and represent different chemical structures, polarities and molecular weights.

- The maximum molecular weights of the adhesive representatives are about 1000 g/mol. According to the Guidelines of the Scientific Committee on Food (SCF), substances with molecular weight below 1000 g/mol are regarded as toxicologically relevant, since the substances with a molecular weight above 1000 g/mol will not be adsorbed in the gastrointestinal tract.

- The adhesive representatives should be available for calibration purposes. Therefore low molecular weight oligomers or other mixtures of substances could not be included in the list. The selected substances contain monomers, additives and some solvents.

4 Reagents

NOTE: All reagents should be of recognised analytical quality unless otherwise stated.

4.1 Analytes

4.1.1 55 Representative adhesive related substances, Purity $\geq$ 95 %

The list presented in Annex A.

NOTE: There is no pure Dipropylene glycol dibenzoate (DPGDB) standard more than purity 95 % from reagent dealers. As the next best way, DPGDB of purity 80 % (technical grade) was purchased from sigma-aldrich.

4.1.2 Internal standards_3-tert.-butyl-4-hydroxy-anisole (BHA), Purity $\geq$ 98%

4.2 Chemicals

4.2.1 Acetone

4.2.2 Dichloromethane

4.3 Solutions

4.3.1 Stock solution of 55 representative adhesive related substances (1 mg/ml)

Weigh to the nearest 0,1 mg approximately 10 mg of substance into a 10 ml volumetric flask, which contains approximately 10 ml of Acetone or Dichloromethane (Annex A). Make up to the mark with Dichloromethane and mix carefully.

NOTE : The representative adhesive related substances are classfied according to application intention and functional group of substances or convenience of analysis groups (Group A ~ G, see Annex A)

Calculate the correct concentration of each representative adhesive related substance.

4.3.2 Internal standard stock solution of 3-tert.-butyl-4-hydroxy-anisole (BHA) (1 mg/ml)

Weigh to the nearest 0.1 mg, approximately 10 mg of BHA into a 10 ml volumetric flask. Make up to the mark with dichloromethane. Calculate the correct concentration of BHA.

4.3.3 Standard solutions of 55 representative adhesive related substances

1, 5, 10, 50, 100, 250 and 500 µl of the standard stock solutions (4.3.1) of each analysis group (Group A ~ G) at a concentration of approximately 1000 µg/ml were filled into a series of 10 ml volumetric flasks. The flasks were filled up to the marks with dichloromethane. These standard solutions contain approximately 0.1, 0.5, 1, 5, 10, 25 and 50 µg/ml of each substance.

The standard solutions were spiked with the internal standard mixture consisting of BHA prior to injection for GC-FID.

Calculate the correct concentration of each representative adhesive related substance.

5 Apparatus

NOTE: An instrument or item of apparatus is listed only where it is special or made to a particular specification, the usual laboratory glassware and equipment being assumed to be available.

5.1 Gas chromatograph, equipped with a flame ionisation detector (FID)

5.2 Gas chromatographic parameters

Gas chromatographic columns coated with 100 % dimethylpolysilioxane are available, for example DB-1, Rtx-1, SPB-1, HP-1, AT-1, BP-1, CP-sil 5 CB and OV-1.

Appropriate operating conditions have to be established for the specific equipment used for the determination.

The following chromatographic conditions have been found to be suitable.

Column	A wall coated open tubular (WCOT) column coated with 100 %-dimethylpolysilioxane 30 m × 0.32 mm i.d. × 0.25 µm film thickness
Carrier gas & flow rate	He, 1.5 ml/min (constant flow)
Injector temperature & mode	300°C with split ratio 20:1
Injection volume	5 µl
Detector temperature	320 °C
Oven temperature	40 °C (4 min), rate 5 °C / min, 340 °C (10 min)

NOTE : 46 substances in 55 representative adhesive related substances have been observed with corresponding retention times as shown in Annex B. Irganox 1010, Melamine, Bronopol and 6 carbolylic acids among the 55 substances have not been detected by the chromatographic condition in calibration range.

6 Samples

The samples of food simulants to be analysed are obtained as described in Directive 85/572/EEC “List of simulants” and Directive 97/48/EC for the basic rules of migration testing regarding time and temperature conditions. Samples are to be kept refrigerated with the exclusion of light.

6.1 Test sample preparation

Filter the migration solutions using a teflon filter of 0.2 µm. Transfer 0.95 ml of the solution into a vial suitable for GC injection and spike 0.05 ml of the internal standard (4.3.2) into the vial.

6.2. Blank sample preparation

Treat food simulants which have not been in contact with packaging material in the same way as described in clause 6.1.

7 Procedure

7.1 GC-FID analysis

Examine the baseline stability and response linearity of the detector before starting measurements.

Maintain the same operating conditions throughout the measurements of all calibration solutions prepared in 4.3.3.

7.2 Calibration

Inject the calibration solutions prepared in 4.3.3.

The calibration samples prepared in 4.3.3 are analysed as they are without further sample treatment.

Identfy 55 adhesive related substances peaks on the basis of the retention times and measure the total peak areas.

Obtain the integrated peak areas of the calibration solutions of each known adhesive related substance. Construct the calibration curves by plotting the peak area ratios (known adhesive related substance/internal standard) against the concentrations of each known adhesive related substance.

The calibration curves of the universal standard substances should be rectilinear and the correlation coefficient should be 0,996 or better and also the calibration line pass through the origin of the x and y axis. The universal standard substances which do not meet these prerequisites should be excluded from the data evaluation.

7.3 Evaluation of data

7.3.1 GC interferences

Following the method described above no interferences have been detected in standard solutions.

7.3.2 Calculation of relative response factor (RRF) values

The relative response factor (RRF) is defined as signal/concentration ratio between analyte and the internal standard and can be calculated as following equation.

$$\text{Relative Response Factor (RRF)} = \frac{Area_s \times C_{is}}{C_s \times Area_{is}}$$

$Area_s$: Peak area of analyte
C_s : Concentration of analyte
$Area_{is}$: Peak area of the Internal standard
C_{is} : Concentration of the Internal standard

7.3.3. Statistical data analysis of relative response factor (RRF) values

<u>Normality test</u>

The normality of the data is a prerequisite to estimate the distribution range. The normality test can be performed by like Wilks Shapiro test, Ryan-Joiner test and other methods. For this, various statistical software packages can be used as follows.

a) MINITAB : Minitab Inc., Pennsylvania, USA
b) SAS : SAS institute, North Carolina, USA

NOTE: Many statistical tests and intervals are based on the assumption of normality. However, in many real cases, the data do not follow a normal distribution and therefore it is not possible to

estimate the distribution range. For these non-normal data, an appropriate transformation is needed. The Box-Cox transformation proposed by Box and Cox is useful. It is defined as follows.

$$\text{Response variable (T)} = \frac{(Y^{\lambda} - 1)}{\lambda} \qquad \text{if } \lambda \neq 0$$

$$\text{Response variable (T)} = log_e(Y + \lambda) \qquad \text{if } \lambda = 0$$

Where Y is the variable and λ is the Box-Cox parameter indicating a number that represents the optimal transformation for correcting non-normality. The optimal value of λ can be determined by the Box-Cox plot that gives a correlation between the pooled standard deviations (SD) versus the λ values. At this time, SD is the Y axis and Lambda is the X axis. All these procedures for Box-Cox transformation can be accomplished by using 'Minitab' statistical package.

Distribution range estimation

The distribution range can be calculated as follow equations.

Distribution range of response factors = $\mu \pm 1.96\,\sigma \quad n > 30$

Distribution range of response factors = $\mu \pm t\,\sigma \quad n < 30$

where μ is mean of RRF values, σ is standard deviation, t is a t-variable of t-distribution and n is number of sample.

7.3.4 Calculation of unknown adhesive related substances

The RRF values of the universal adhesive related substances are calculated from the chromatographic results analyzed with GC-FID screening method and established in a distribution range (7.3.3). For the semi-quantitative (or conservative) estimation, a statistical RRF value can be derived from the established distribution range of RRF values. The concentration of unknown substances can be simply calculated as follows.

Typical estimation using RRF

$$C_s^{known} = \frac{C_{is} \times Area_s^{known}}{Area_{is} \times RRF}$$

$Area_s^{known}$: peak area of known analyte
$Area_{is}$: peak area of the Internal standard
C_s^{known} : concentration of known analyte
C_{is} : concentration of the Internal standard
RRF : average relative response factor of known analyte in defined calibration range

Semi-quantitative estimation using statistical RRF

$$C_s^{unknown} = \frac{C_{is}^{universal} \times Area_s^{unknown}}{Area_{is}^{universal} \times RRF^{statistic}}$$

$Area_s^{unknown}$: peak area of unknown analyte
$Area_{is}^{universal}$: peak area of the Universal internal standard (BHA)
$C_s^{unknown}$: concentration of unknown analyte
$C_{is}^{universal}$: concentration of the Universal internal standard (BHA)
$RRF^{statistic}$: statistical relative response factor for semi-quantitative determination

8. Confirmation

8.1 Requirement for confirmation

In order to derive information on molecular weight of an unknown peak observed by the GC-FID screening method in real samples, the unknown peak shall be confirmed by the method described in 8.2.

8.2 Confirmation using retention indices

The information on molecular weight of an unknown substance can be derived through comparison between the retention indices of an unknown substance in a sample mixture and that of known substance. The retention indices are only influenced by the kind of the column stationary phase and therefore gas chromatographic columns coated with 100 % dimethylpolysilioxane should be used for the determination of retention indices. The calibration solutions prepared in section 4.3 and C6 – C40 n-alkane mixture shall be reanalysed according to 5.2.

The retention indices can be calculated as following equation.

$$RI = 100 * \frac{X - M_{(n)}}{M_{(n+1)} - M_{(n)}} + 100n$$

RI : retention index

X : retention time of analyte

n : number of carbon atoms in the n-alkanes

$M_{(n)}$: retention time of n-alkane with n carbon atoms eluting before X

$M_{(n+1)}$: retention time of n-alkane with (n+1) carbon atoms eluting after X

NOTE : The retention indices of the universal standards were calculated by using the homologous series of n-alkane C_nH_{2n+2} as reference substances. The calculated retention indices are given in Annex B.

9. Precision

The within-laboratory detection limits (WDL) of the calibrated substances, based on the calibration curve method according to DIN 32645, were found to be in the range of 0.2 – 2.6 µg/ml.

10 Test report

The test report shall contain as a minimum, the following :

- date of analysis and reporting ;
- clear identification of the test laboratory and the responsible analyst;
- universal standard substances (representative adhesive related substances) and test method;
- sample details like origin and specification, type of food/simulant/material/article, reception date, and storage condition;
- results expressed in milligram unknown substances per kilogram food simulant or packaging material.

Annex A
(informative)

List of known adhesive related substances as candidates for universal standards for development of screening methods.

Classification	Nr.	Name	Synonyms	MW (g/mol)	CAS-Nr.	Manufacturer	Purity (%)	Stock Solution in
Group A Acrylate	1	Acrylic acid methyl ester	Methyl acrylate	86.09	96-33-3	Aldrich	> 99 %	DCM [1)]
	2	Acrylic acid ethyl ester	Ethyl acrylate	100.11	140-88-5	Aldrich	> 99 %	DCM
	3	2-Methylacrylic acid methyl ester	Methyl methacrylate	100.11	80-62-6	Aldrich	99 %	DCM
	4	2-Methyl-2-propenoic acid ethyl ester	Ethyl methacrylate	114.14	97-63-2	Aldrich	99 %	DCM
	5	Acrylic acid butyl ester	Butyl acrylate	128.18	141-32-2	Aldrich	> 99 %	DCM
	6	Methacrylic acid, butyl ester	Butyl methacrylate	142.19	97-88-1	Aldrich	99 %	DCM
	7	2-ethylhexyl prop-2-enoate	Ethylhexyl acrylate	184.28	1322-13-0	Fluka	> 98 %	DCM
Group B Plasticizers	8	Diisobutyl phthalate	DIBP	278.35	84-69-5	Merck	> 98 %	DCM
	9	Dibutyl phthalate	DBP	278.35	84-74-2	Merck	> 98 %	DCM
	10	Bis(2-ethylhexyl) phthalate	DEHP or DOP	390.56	117-81-7	Merck	> 98 %	DCM
	11	Diethylhexyl adipate	DEHA	370.57	103-23-1	Merck	> 98 %	DCM
	12	Glycerol triacetate	Triacetin	218.20	102-76-1	Sigma	≥ 99 %	DCM
	13	2-Ethylhexyl diphenyl phophate	Phosflex 362	362.44	1241-94-7	Riedel-de-Haën	99 %	DCM
	14	Diethylene glycol dibenzoate	DEGDB	314.34	120-55-8	Aldrich	96 %	DCM
	15	Triethylene glycol dibenzoate	TEGDB	358.40	120-56-9	Aldrich	99 %	DCM
	16	Dipropylene glycol dibenzoate	DPGDB	342.42	27138-31-4	Aldrich	80 %	DCM
	17	Propylene glycol, dibenzoate	Bezoflex 284	284.3	19224-26-1	Aldrich	> 96 %	DCM
	18	2,2,4-Trimethyl-1,3-pentanediol dibenzoate	Benzoflex 354	354.45	68052-23-3	Aldrich	> 99.9 %	DCM
Group C Carboxylic acid	19	2-Propenoic acid	Acrylic acid	72.06	079-10-7	Aldrich	> 99 %	DCM
	20	trans-Butenedioic acid	Fumaric acid	116.07	110-17-8	Aldrich	> 99 %	DCM
	21	cis-Butenedioic acid	Maleic acid	116.07	110-16-7	Aldrich	> 99 %	DCM
	22	Hexanedioic acid	Adipic acid	146.14	124-04-9	Aldrich	99 %	DCM
	23	1,4-Benzene-dicarboxylic acid	Terephthalic acid	166.13	100-21-0	Fluka	> 99 %	DCM
	24	1,3-Benzene-dicarboxylic acid	Isophthalic acid	166.13	121-91-5	Fluka	99 %	DCM
Group D Alcohol	25	1,2-Dihydroxyethane	Ethylene glycol	62.06	107-21-1	Fluka	> 99.5 %	DCM
	26	1,2-Dihydroxypropane	Propylene glycol	76.1	57-55-6	Fluka	> 99.5 %	DCM
	27	1,4-Butanediol	1,4-Butylene glycol	90.12	110-63-4	Fluka	99 %	DCM
	28	2,2'-Dihydroxydiethyl ether	Diethylene glycol	106.12	111-46-6	Fluka	> 99 %	DCM
	29	1,3-Benzenediol	Resorcinol	110.11	108-46-3	Fluka	> 99 %	DCM
	30	1,2,3-Propanetriol	Glycerol	92.09	56-81-5	Sigma-aldrich	≥ 99.5 %	DCM
	31	2-Bromo-2-nitropropane-1,3-diol	Bronopol	200.01	52-51-7	Riedel-de-Haën	99.9 %	DCM
Group E Amine	32	Hexamethylenediamine	HMDA	116.21	124-09-4	Fluka	> 99 %	DCM
	33	Toluene 2,4-diamine	2,4-TDA	122.17	95-80-7	Aldrich	98 %	DCM
	34	1,3,5-Triazine-2,4,6-triamine	Melamine	126.12	108-78-1	Fluka	> 99 %	DCM
	35	Isophorone diamine	IPDA	170.3	2855-13-2	Fluka	> 99 %	DCM
	36	4,4'-Methylenedianiline	4,4 MDA	198.26	101-77-9	Fluka	> 97 %	DCM
Group F Antioxidants	37	2,6-Di-tert-butyl-4-methylphenol	BHT	220.35	128-37-0	Merck	> 99 %	DCM
	38	Octadecyl 3,5-bis (1,1-dimethylethyl)-4-hydroxybenzene propanoate	Irganox 1076	531	2082-79-3	Ciba	-	Acetone
	39	2,4-Bis (1,1 dimethylethyl) phenyl-phosphite	Irgafos 168	646.93	31570-04-4	Ciba	-	Acetone
	40	1,3,5-Trimethyl-2,4,6-tris(3,5-di-t-butyl-4-hydroxybenzyl) benzene	Irganox 1330	775.21	1709-70-2	Ciba	-	Acetone
	41	Tetrakis [methylene-3 (3´,5´-di-t-butyl-4-hydroxyphenyl) propionate] methane	Irganox 1010	1177.7	6683-19-8	Ciba	-	Acetone
Group G Others	42	Propanoic acid, ethenyl ester	Vinyl propionate	100.12	105-38-4	Fluka	> 98 %	DCM
	43	Ethenylbenzene	Styrene	104.15	100-42-5	Aldrich	99 %	DCM
	44	Dimethylbenzene	p-Xylene	107.17	106-42-3	Riedel-de-Haën	99.9 %	DCM
	45	1,6-Hexalactam	Caprolactam	113.16	105-60-2	Fluka	> 99 %	DCM
	46	N-Vinyl-2-pyrrolidinone	1-Vinyl-2-pyrrolidinone	114.14	88-12-0	Acros	99 %	DCM
	47	2-Phenylpropene	α-Methylstyrene	118.18	98-83-9	Aldrich	99 %	DCM
	48	Diphenyl keton	Benzophenone	182.23	119-61-9	Aldrich	≥ 99 %	DCM
	49	2-(2-Butoxyethoxy)ethanol acetate	Butyl diglycol acetate	204.27	124-17-4	Aldrich	> 99.2 %	DCM
	50	2-Octyl-2H-isothiazol-3-one	Octhilinone	213.34	26530-20-1	Riedel-de-Haën	99 %	DCM
	51	4,4'-Dihydroxy-2,2-diphenylpropane,	Bisphenol A	228.29	80-05-7	Fluka	97 %	DCM
	52	Methanone, bis(4-(diethylamino)phenyl)-	4,4'-Bis(diethylamino) benzophenone, BDBP	324.46	90-93-7	Aldrich	> 99 %	DCM
	53	Bisphenol A diglycidyl ether	BADGE	340.42	1675-54-3	Fluka	97 %	DCM
	54	Benzoxazole, 2,2'-(2,5-thiophenediyl) bis(5-(1,1-dimethylethyl)-	Uvitex OB	430.06	7128-64-5	Ciba	-	DCM
	55	Sodium dioctyl sulfosuccinate	Docusate sodium	445.63	577-11-7	Sigma	≥ 99 %	DCM

[1)] DCM : Dichloromethane

Annex B
(informative)

Retention data of representative adhesive related substances detected by GC-FID.

Classification	Nr	Substances	Molecular weight (g/mol)	Retention indices	Retention time (min)
Group A acylate	1	Methyl acrylate	86.09	-	1.56
	2	Ethyl acrylate	100.11	621	2.06
	3	Methyl methacrylate	100.11	628	2.21
	4	Ethyl methacrylate	114.14	673	3.30
	5	Butyl acrylate	128.18	877	6.29
	6	Butyl methacrylate	142.19	964	9.10
	7	Ethylhexyl acrylate	184.28	1212	16.69
Group B Plasticizers	8	Glycerol triacetate	218.2	1308	19.34
	9	Diisobutyl phthalate	278.35	1821	30.87
	10	Dibutyl phthalate	278.35	1911	32.63
	11	Propylene glycol dibenzoate	284.3	2091	35.85
	12	Diethylene glycol dibenzoate	314.34	2390	40.80
	13	Dipropylene glycol dibenzoate	342.42	2406	41.04
	14	2,2,4-Trimethyl-1,3-pentanediol dibenzoate	354.45	2439	41.50
	15	Triethylene glycol dibenzoate	358.4	2671	44.92
	16	Phosflex 362	362.44	2368	40.54
	17	Diehtylhexyl adipate	370.57	2371	40.58
	18	Diethylhexyl phthalate	390.56	2502	42.58
Group C Alcohol	19	Ethylene glycol	62.06	621	2.09
	20	Propylene glycol	76.1	639	2.51
	21	1,4-Butanediol	90.12	916	7.60
	22	Glycerol	92.09	979	10.05
	23	Diethylene glycol	106.12	933	8.14
	24	Resorcinol	110.11	1249	17.84
Group D Amines	25	Hexamethylene diamine	116.21	1064	12.14
	26	Toluene -2,4 -diamine	122.17	1334	19.55
	27	4,4-Methylenedianiline	250.25	2046	34.74
	28	Isophorondimamine	170.3	-	18.74/19.14
Group E Antioxidants	29	2,6-Di-*tert*-butyl-4-methylphenol	220.35	1488	23.67
	30	Irganox 1076	531	3594	56.03
	31	Irgafos 168	646.93	3389	54.18
	32	Irganox 1330	775.21	-	67.97
Group F Others	33	Vinyl propionate	100.12	615	1.94
	34	Styrene	104.15	869	5.99
	35	*para*-Xylene	106.17	853	5.50
	36	Caprolactam	113.16	1191	15.97
	37	1-Vinyl-2-pyrrolidinone	114.14	1052	11.80
	38	α-Methylstyrene	118.18	962	8.96
	39	Benzophenone	182.23	1577	25.62
	40	2-Octyl-2H-isothiasol-3-one	213.34	1781	30.00
	41	Butyl diglycol acetate	204.27	1333	19.95
	42	Bisphenol A	228.29	2099	35.98
	43	4,4’-Bis(diethylamino) benzophenone	324.46	3027	49.62
	44	BADGE	340.42	2804	46.75
	45	Uvitex OB	430.06	3791	58.05
	46	Docusate sodium	445.63	2208	37.98

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9.5. Classification of adhesives

Classification of Adhesives by the Setting-mechanism without chemical reaction [Gierenz and Karmann 2001].

Classification			Raw Materials	Uses
Application without Volatile Solvent	Hot-melt adhesives		- EVA, PP, PA, Polyesters, PVA, PE - Thermoplstic elastomers	paper, fiberboard, plastics, textiles, leather
	Plastisol Adhesives		- PVC or poly(methyl methacrylate)	Metals(scheet-metal constructions), silicate-containing materials
Application of Solutions, Solvent Escapes before Bonding	Heat-Sealing Adhesives		- Vinyl polymers Copolymers of vinyl chloride or vinylidene chloride) Copolymers of vinyl acetate and poly-methacrylates - PU, polyesters etc.	Paper, plastics, lamination of plastic films to metal foils(packaging, metal-foil lamination)
	High-Frequency-sensitive heat seal coats		- Vinyl chloride, Polyacrylates, vinyl acetate, resins and plasticizers	
	Contact Cements		- Synthetic rubbers, Resins (phenolic resins, rosins and hydrocarbon resins) Solution of PU elastomer	Wood, plastics, rubber, metals
	Pressure-Sensitive Adhesives		- Natural and synthetic rubbers, Rosins, phenol-formaldehyde resins, hydro-carbon resins - Polyacrylates, poly(vinyl ethers), poly-methacrylates, polyisobutenes	Paper, film tapes, adhesive labels, self-adhesive decorative sheeting
Application of Solutions, Solvent Evaporates during Bonding	Solvent-containing adhesives	Adhesion Adhesives	- Nitrocellulose, Poly(vinyl acetate) - Natural and Synthetic rubbers, PU rubbers, EVA etc. - Solvent mixtures : Consist of esters and ketones or alcohol	Paper, plastics, silicate-containing materials, wood
		Solvent Adhesives	- PVC, Acetone, cyclohexanone, THF or mixtures thereof	
	Uses in Water		- Starch, dextrins, casein, cellulose, ethers, water-soluble derivatives of poly(acrylic acid), poly(vinyl alcohol), poly(vinyl pyrrolidone)	Paper, fiberboard, moistenable adhesive tapes, wood
Emulsion of Water-Insoluble Substances in Water that Escapes during Bonding	Aqueous emulsion	Emulsion-based adhesives	- Vinyl acetate homo- and copolymers Comonomers : maleic esters, acrylates, ethylene, vinyl chloride, vinyl laurate and unssturated carboxyl acids - Polyacrylate homo- and copolymers, styrene copolymers - Plasticizers, solvents and resins	Paper, wood, plastics
		Latex adhesives	- Natural and synthetic rubbers, synthetic resins and solvents	

Classification of reactive adhesives (setting by chemical reaction) [Gierenz and Karmann 2001].

Classification			Raw Materials	Uses
Polymerization adhesives	One-component	Cyanoacrylate adhesive	- Methyl, ethyl, butyl, and methoxy-ethyl esters of cyanoacrylic acid - Soluble polymers, plasticizers	Paper, fiberboard, plastics, textiles, leather
		Anaerobic adhesives	- Methacrylate	Metal
	Two-component		- Unsaturated polyesters, vinyl compounds (styrene or methyl methacrylate) - Hardeners : peroxides, accelerators : amines or heavy-metal salts	Metals(scheet-metal constructions), silicate-containing materials, plastics
Polyaddition adhesives	Epoxy resin adhesives - Epoxy phenol adhesives - Nylon epoxy adhesives - Elastomer epoxies adhesives		- Diglycidyl ether of bisphenol A, polyamide, nitrile rubber - Hardeners 1) hot-setting formulation : dicarboxylic acid anhydrides, dicyandiamide, and certain aromatic amines. 2) cold-setting system : aliphatic and cycloaliphatic amines and polyamines etc. - Other additives : accelerators, reactive diluents, plasticizers, resin modifiers, fillers	Paper, plastics, lamination of plastic films to metal foils(packaging, metal-foil lamination)
	Reactive Plyurethane adhesives		- polyisocyanates(aromatic and aliphatic), - polyols(polyesters or polyether)	Plastics, metals, silicate-containing materials
Polycondensation adhesives	Polyhydroxymethyl compounds		- Phenol-formaldehyde resins, urea-and melamine-formaldehyde resins, poly(vinyl formal)resins, nitrile rubber, epoxy resins, resorcinol-formaldehyde resins - Hardeners	Wood, metal
	Silicone adhesives		- Silicon	Metal, glass, paper, plastics
	MS polymers		- Poly(propylene glycol)	Many surfaces
	Reactive polyurethane hot-melt adhesives		- Diisocyanates, - Polyols(polyesters or polyether)	Bookbinding, wood gluing, shoe manufacturing
	Polyimides and poly- benzimidazoles		- Polyimides, - Polybezimidazoles	Metal
Vulcanizing Adhesives			-	Rubber-to-Metal bonding agents (Natural and synthetic rubber to metals)
Ultraviolet / Electron Beam (UV/EB) curing adhesives			- Acrylic esters - Epoxy resin, urethanes, polyesters, polyethers	Laminating, Pressure-sensitive products
Conductive Adhesives			- Epoxies, polyurethanes, silicones, polyimides - Fillers : alumina, silver	

9.6. Detailed GC-FID conditions

9.6.1. GC-FID analysis for the column selection test

Column	**DB-1**, 30 m × 0.32 mm i.d. × 0.25 µm film thickness (100 %-dimethylpolysiloxane)
Carrier gas & flow rate	He, 1.5 ml/min (constant flow)
Injector temperature & mode	300 °C with split ratio 10:1
Injection volume	2 µl
Detector temperature	320 °C
Oven temperature	40 °C (4 min), rate 5 °C / min, 340 °C (10 min)

Column	**DB-FFAP**, 30 m × 0.32 mm i.d. × 0.25 µm film thickness (Nitroterephthalic acid modified polyethylene glycol)
Carrier gas & flow rate	He, 1.5 ml/min (constant flow)
Injector temperature & mode	230 °C with split ratio 10:1
Injection volume	2 µl
Detector temperature	250 °C
Oven temperature	40 °C (5 min), rate 10 °C / min, 250 °C (10 min)

Column	**DB-Wax**, 30 m × 0.32 mm i.d. × 0.25 µm film thickness (Nitroterephthalic acid modified polyethylene glycol)
Carrier gas & flow rate	He, 1.5 ml/min (constant flow)
Injector temperature & mode	240 °C with split ratio 10:1
Injection volume	2 µl
Detector temperature	260 °C
Oven temperature	40 °C (6 min), rate 10 °C / min, 250 °C (10 min)

Column	**Zebron (ZB) 624** , 60 m × 0,25 mm i.d. × 1.4 µm film thickness (6 %-cyanopropylphenyl-94%-methylpolysilioxane)
Carrier gas & flow rate	He, 1.2 ml/min (constant flow)
Injector temperature & mode	240 °C with split ratio 10:1
Injection volume	2 µl
Detector temperature	260 °C
Oven temperature	40 °C (6 min), rate 5 °C / min, 90 °C (10 min), rate 10 °C / min, 250 °C / 10 min.

9.6.2. GC-FID analysis for the multi-screening test and the determination of relative response factors (RRF)

Column	**DB-1**, 30 m × 0.32 mm i.d. × 0.25 µm film thickness (100 %-dimethylpolysilioxane)
Carrier gas & flow rate	He, 1.5 ml/min (constant flow)
Injector temperature & mode	300°C with split ratio 20:1
Injection volume	2 µl
Detector temperature	320 °C
Oven temperature	40 °C (4 min), rate 5 °C / min, 340 °C (10 min)

Column	**DB-FFAP**, 30 m × 0.32 mm i.d. × 0.25 µm film thickness (Nitroterephthalic acid modified polyethylene glycol)
Carrier gas & flow rate	He, 1.5 ml/min (constant flow)
Injector temperature & mode	230°C with split ratio 10:1
Injection volume	2 µl
Detector temperature	250 °C
Oven temperature	50 °C (5 min), rate 10 °C / min, 250 °C (10 min)

9.6.3. GC-FID analysis for the determination of linear retention indices

Column	**DB-1**, 30 m × 0.32 mm i.d. × 0.25 µm film thickness (100 %-dimethylpolysilioxane)
Carrier gas & flow rate	He, 1.5 ml/min (constant flow)_for condition 1 and 2 H_2, 1.5 ml/min (constant flow)_for condition 3
Injector temperature & mode	300°C with split ratio 20:1
Injection volume	2 µl
Detector temperature	320 °C
Oven temperature	40 °C (4 min), rate 5 °C / min, 340 °C (10 min)_**condition A** 40 °C (4 min), rate 10 °C / min, 140 °C (4 min), rate 10 °C / min 340 °C (10 min)_**condition B** 50 °C (2 min), rate 10 °C / min,340 °C (10 min)_**conditon C**

Column	**DB-FFAP**, 30 m × 0.32 mm i.d. × 0.25 µm film thickness (Nitroterephthalic acid modified polyethylene glycol)
Carrier gas & flow rate	He, 1.5 ml/min (constant flow)
Injector temperature & mode	230°C with split ratio 20:1
Injection volume	2 µl
Detector temperature	250 °C
Oven temperature	50 °C (5 min), rate 10 °C / min, 250 °C (10 min) _ **condition D**

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