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MASTERARBEIT

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Development of a multimethod for the analysis of photoinitiators migrating into foodstuffs

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ADI	acceptable daily intake
AP	analyte protectant
APPI	atmospheric pressure photoionisation
ASE	accelerated solvent extraction
BADGE	bisphenol A diglycidyl ether
BDMAM	2-benzyl-2-4(dimethylamino)-4′-morpholinobutyrophenone
BP	benzophenone
BPA	bisphenol A
BP-d10	benzophenone-d10
DAD	diode array detection
DART	direct analysis in real time
DEAB	4,4'-bis(diethylamino)benzophenone
DETX	2,4-diethyl-9H-thioxanthen-9-one
DMAB	4-(dimethylamino)benzophenone
DMPA	2,2-dimethoxy-2-phenylacetophenone
DPTMBPO	diphenyl-(2,4,6-trimethylbenzoyl)phosphine oxide
EA	2-ethylanthraquinone
EBF	ethyl-benzoylformate
ECD	electron capture detector
EDMAB	ethyl-4-dimethylaminobenzoate
EFSA	European Food Safety Authority
EHDAB	2-ethylhexyl-4-dimethylaminobenzoate
EI	electron impact ionisation
ESBO	epoxidized soy bean oil
ESI	electrospray ionisation
FCM	food contact material
FHBP	4-fluoro-4′-hydroxybenzophenone

FID	flame ionisation detection
FLD	fluorescence detector
GC	gas chromatography
GMP	good manufacturing practice
4HBP	4-hydroxy-benzophenone
HCPK	1-hydroxycyclohexyl-1-phenylketone
HHEMPP	2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone
HMBP	2-hydroxy-4-methoxybenzophenone
HMPP	2-hydroxy-2-methylpropiophenone
HOBP	2-hydroxy-4-(octyloxy)benzophenone
HPLC	high performance liquid chromatography
HPTLC	high performance thin-layer chromatography
ISTD	internal standard
2-ITX	2-isopropylthioxanthone
4-ITX	4-isopropylthioxanthone
ITX-d3	isopropylthioxanthone-d3
ITX-d7	isopropylthioxanthone-d7
LC	liquid chromatography
LLE	liquid-liquid extraction
LOD	limit of detection
LOQ	limit of quantification
MOAH	mineral oil aromatic hydrocarbons
MBB	methyl-2-benzoylbenzoate
MBF	methyl-benzoylformate
4MBP	4-methylbenzophenone
MK	Michler’s Ketone
MMTPMP	2-methyl-1-(4-(methylthio)phenyl)-2-morpholinopropan-1-one
MOSH	mineral oil saturated hydrocarbons
MRL	maximum residue level
MRM	multiple reaction monitoring

MS	mass spectrometry
OML	overall migration limit
PBZ	phenylbenzophenone
PE	polyethylene
PET	polyethylene terephthalate
PLE	pressurized liquid extraction
PSA	primary secondary amine
PTMBP	phenyl-bis(2,4,6-trimethylbenzoyl)phosphine
PVC	polyvinylchloride
QuEChERS	quick easy cheap effective rugged safe
RASFF	rapid alert system for food and feed
SIM	selected ion monitoring
SML	specific migration limit
S/N	signal to noise ratio
SPE	solid phase extraction
SPME	solid phase micro extraction
TDI	tolerable daily intake
TIC	total ion chromatogram

1. INTRODUCTION

The unintentional contamination of food by (potentially) toxic substances is highly undesirable and of great concern to consumers. A wide array of chemical compounds may contaminate foodstuffs. Well known environmental contaminants, which have been continuously monitored over many years, include e.g. mycotoxins, dioxins and heavy metals. However, food processing and packaging may also constitute an important source of food contaminants. Since the middle of the last century the amount of packaged food steadily increased. Nowadays over 90% of all manufactured foodstuffs are enclosed in printed packaging material. (Leks-Stepien 2011)

Food packaging materials are often printed with so-called UV-curable inks, which consist of a mixture of acrylates and photoinitiators. The photoinitiators catalyse the UV initiated polymerisation process that fixes the ink to the printed surface. (Papilloud and Baudrauz 2002a)

The issue of printing ink components migrating into foodstuffs was highlighted especially by the detection of photoinitiators in milk products and fruit juices in 2005 and in cereal products in 2009. (European Food Safety Authority 2005b, 2009a, 2009b) Currently photoinitiators are not regulated by a specific EU legislation and maximum residue levels in food are not established yet, although according to the EFSA the presence of certain substances could be considered undesirable. (European Food Safety Authority 2005b)

A wide variety of chemical compounds may be used as photoinitiators; however, so far only a limited number of substances have been targeted in analytical methods reported for determining photoinitiators in foodstuffs. Moreover, only a few methods for the simultaneous analysis of several photoinitiators have been described in the literature so far.

Therefore the main objective of this master thesis was the development of a selective and sensitive analytical method for the simultaneous identification and quantification of a wide range of photoinitiators in foodstuffs.

The subsequent analysis of various cereal products and beverages as well as their respective packaging materials for the presence of these photoinitiators should provide information regarding the extent of contamination of two important groups of packaged foodstuffs with photoinitiators and assess which photoinitiators are presently used in the respective packaging materials.

2. CONTAMINATION OF FOOD UPON MIGRATION OF SUBSTANCES FROM PACKAGING MATERIALS

Food packaging has the purpose of containing the food product, protecting the foodstuff from external influences and damage, and providing ingredient and nutritional information. (Coles 2003) Food packaging should contain food in a cost-effective way that fulfils both industry requirements as well as consumer desires. (Marsh and Bugusu 2007)

Since the 1950s the proportion of packaged food steadily increased so that the majority of food products are nowadays sold in packaged form. (Chatwin and Katan 1989) Alongside the many advantages food packaging provides to both the consumer and the industry (e.g. prolongation of shelf life, easier handling during transport, use as advertisement space), one must, however, also consider and evaluate the risks that may arise from the materials used for packaging food. These risks can concern several areas including environmental issues upon disposal but most importantly undesired interactions with the foodstuff.

2.1. MIGRATION FROM PACKAGING MATERIALS INTO FOODSTUFFS

Frequently undesired interactions between foodstuffs and their packaging may take place. Interactions can be classified into the following categories:

- Migration
  is the transfer of components from the packaging material into the foodstuff.
- Absorption
  is the transfer of components from the foodstuff into the packaging material.

The transfer of chemical substances from the food packaging material into the food contaminates the foodstuff and may result in a toxic hazard or the formation of off-flavours. The interaction processes are influenced by the characteristics of the packaging material and the foodstuff as well as by different parameters such as temperature, storage time and other environmental factors. (Mannheim and Passy 1990)
The following discussion will focus only on migration being the process resulting potentially in a hazardous food contamination. (Sanches-Silva et al. 2008a)

Migration is a diffusion process, which is described in most cases by Fick's laws of diffusion. Fick's first law relates the diffusive flux to a concentration gradient, in the present case between the foodstuff and the packaging material. Fick's second law relates the change of concentration over time to the concentration gradient.

Fick's first law: $F = -D_p (\delta C_p/\delta x)$

Fick's second law: $(\delta C_p/\delta t) = D_p (\delta^2 C_p/ \delta x^2)$

F is the rate of transport per unit area of the packaging material

D_p is the diffusion coefficient of the migrant in the packaging material, which can be measured by kinetic experiments [cm^2/s]

C_p is the migrant concentration in the packaging material [mg/g]

x is the space coordinate, orthogonal to the contact surface between the foodstuff and the packaging material [cm]

t is the elapsed time [s]

These two equations are the basis for mathematical models to describe the migration process which should allow the prediction of the concentration of a certain migrant in a foodstuff and its dependence on time. (Chatwin and Katan 1989) Different models that take into account additional parameters such as the density of the packaging material, the molecular weight of the migrant, temperature, mass transfer coefficient or partition coefficient have been

developed. (Arvanitoyannis and Bosnea 2004, Vitrac et al. 2007) However, as no robust mathematical models or simulations to evaluate migration exist at present, chemical analysis is still irreplaceable for studying the migration process. (Arvanitoyannis and Bosnea 2004) Nevertheless, mathematical models are regarded as a valuable tool to identify the factors that affect migration most. (Simoneau and Hannaert 1999)

Migrating substances with a molecular weight below 1000 Dalton are considered as possibly physiologically active and are therefore a potential safety issue. (Grob 2002) High molecular weight substances (≥ 1000 Da) are not considered to be of concern, as they are not absorbed by the gastrointestinal tract, except for fragmented macromolecules occurring during digestion or being resorbed by specific transport mechanisms. (Grob 2002, Sanches-Silva et al. 2008a) Especially in foods packed in plasticized PVC cling films, recycled paper and cardboard or cans with an inner coating, significant concentrations of migrated substances with potential health concerns have been identified in the past. (Vitrac et al. 2007, Sanches-Silva et al. 2008e)

2.2. LEGAL BACKGROUND

At least parts of a food packaging have direct contact with the enclosed foodstuff and thus most packaging materials are to be regarded as **f**ood **c**ontact **m**aterials (FCM). The safety of food contact materials relies on ensuring that during contact with the foodstuff no migration of unsafe levels of chemical substances from the material to the food takes place. (Arvanitoyannis and Bosnea 2004)

In the European Union food contact materials and articles are regulated by the Framework Regulation EC 1935/2004, which sets up general requirements for all food contact materials. In addition there are various legislations on specific materials stated in the Framework Regulation as well as directives on individual substances or groups of substances, which are employed in the manufacture of

food contact materials. Additionally national legislations cover groups of materials and articles for which EU legislation is not yet in place. (European Commission-Health and Consumers Directorate 2011)

Regarding migration from food contact materials article 3 of Regulation EC 1935/2004 is of particular importance. According to this article materials and articles in contact with food “shall be manufactured in compliance with good manufacturing practice, so that under normal and foreseeable conditions of use, they do not transfer their constituents to food in quantities which could endanger human health or bring about an unacceptable change in the composition of the food or bring about a deterioration in the organoleptic characteristics thereof“. (European Parliament and European Council 2004) Rules on **g**ood **m**anufacturing **p**ractice (GMP) relating to groups of materials and articles intended to come into contact with food are laid down in regulation EC 2023/2006. (European Commission 2006)

For plastic materials and articles intended to come into contact with foodstuffs (including plastic layers in multilayer materials) a specific legislation exists in form of Regulation 10/2011 which lays down an **o**verall **m**igration **l**imit (OML) and **s**pecific **m**igration **l**imits (SML) or maximum contents in the material or article for individual substances. (European Commission 2011)

The overall migration limit is set to control the total amount of substances migrating from the packaging material into the foodstuff, regardless of the toxicological significance of these substances. The overall migration limit is set at 10 mg/dm^2 (corresponding to 60 mg/kg foodstuff) for chemical migration from plastic materials and articles intended to come into contact with food. However, an overall migration below this threshold does not mean that the package is safe, as the chemical natures and toxicities of the migrants are not identified and taken into account, respectively. (European Commission 2011)

For individual authorized substances, which are toxicologically evaluated, specific migration limits are set according to the **t**olerable **d**aily **i**ntake (TDI) or the **a**cceptable **d**aily **i**ntake (ADI) values. The limit is based on the assumption

that every day throughout lifetime a person weighing 60 kg eats 1 kg of food packed in plastics containing the substance in the maximum permitted quantity. (European Commission-Health and Consumer Directorate 2011)

Regulation 10/2011 also contains a list of monomers, additives and other substances authorised for use in the manufacture of plastics for food contact applications. (European Commission 2011) This positive list is restricted to starting materials and assumes that the main migrants from plastic packaging materials come from starting materials such as monomers and additives. (European Commission 2011) However, this is not true for more complex systems, where beside of the desired product many other different compounds are formed for which the origin from the authorised starting materials is no assurance of safety. (Grob 2002)

With regards to the analytical determination of migration detailed regulations for performing this task have been established in EU legislation. According to Article 18 of Regulation 10/2011, the specific migration of plastic food contact materials that are already in contact with a foodstuff shall be determined in the foodstuff itself. Where the food contact material is not yet in contact with the food, food simulants are employed. (European Commission 2011) The simulants to be used for mimicking a certain class of foodstuffs and the migration conditions (time, temperature) are laid down in Directives 82/771/EEC and 85/572/EEC. (European Council 1982, European Council 1985)

In order to minimize the number of experimental migration tests, Regulation 10/2011 allows the use of mathematical models based on diffusion and mass balance equations as a screening tool for checking the compliance of plastic food contact materials with the SMLs. (European Commission 2011)

Besides the regulation on plastics discussed above, specific legislations exist for ceramics, regenerated cellulose films, recycled plastic materials as well as for, active and intelligent materials and articles. (European Commission-Health and Consumers Directorate 2011)

In practice checking for compliance of packaging materials with the respective EU regulations is difficult and tedious, due to a lack of information on the potential migrants from the manufacturer and a lack of standardized analytical methods. (Arvanitoyannis and Bosnea 2004)

2.3. CONTAMINANTS IN FOOD FROM PACKAGING MATERIALS

A wide variety of materials are used for packaging food and many of them consist of or contain numerous chemical substances from primary production, recycling processes and finishing steps (e.g. coating, printing). In this section a short overview of some selected food contaminants originating from packaging material is given.

2.3.1. Contaminants from Plastic Packaging Materials

Due to their many advantageous properties (e.g. light weight, robustness) plastics are widely used as food packaging materials. More than 30 different plastic polymers are currently being employed for food packaging materials. This variety is additionally enlarged by different types of additives, which are used. (Lau and Wong, 2000)

Plastic materials consist of a vast number of different chemical species, with macromolecules constituting the basis. Additionally, oligomers, additives and manufacturing residues may be present. Macromolecular components are regarded as an immobile and inert matrix through which the lower molecular weight components can migrate. (Chatwin and Katan 1989)

Plastic additives such as plasticisers, stabilizers, antioxidants, lubricants, anti-blocking and anti-static agents are used to improve the performance either during processing or in use of these packaging materials. Plastic additives are generally present in a small amount only, ranging from 0.1-1 %, and are dispersed in the polymer matrix. Due to their low molecular weights they have a

certain potential to migrate from the food packaging materials into the enclosed foodstuffs. (Lau and Wong, 2000)

Probably the most intensely studied group of plastic additives are plasticisers, which are used to increase the flexibility of the polymeric material. Their migration from food contact materials into food has raised many concerns since the early 1980s, as some of them showed carcinogenic effects in rodents and potential estrogenic effects in humans in toxicological studies. (Lau and Wong 2000) Nowadays butyl stearate, acetyltributyl citrate, alkyl sebacates and adipates are commonly used types of plasticisers, as they have a low toxicity. (Lau and Wong 2000) The use of phthalate plasticisers on the other hand has been restricted due to the potential carcinogenic and estrogenic effects and possible impairment on human fertility of some members of this compound group. (European Food Safety Authority 2005a, Lau and Wong 2000) A frequently used alternative **e**poxidized **s**oy **b**ean **o**il (ESBO), was also evaluated by the **E**uropean **F**ood **S**afety **A**uthority (EFSA) due to its potential migration and a SML was set for this compound as well. (European Food Safety Authority 2006) Both phthalates and ESBO are widely used as plasticisers in sealing gaskets of metal lids for glass jars and therefore can frequently be found in foodstuffs like e.g. sauces, condiments and products in oil. (European Food Safety Authority 2005a, 2006)

During the polymerisation process in the production of plastics the monomeric building blocks are generally not converted into polymeric structures to 100% so that unreacted monomers and shorter chain oligomers are also present in the final material. In contrast to the polymer, these monomers and oligomers are reactive substances, which may possess a certain toxicity. For example, vinyl chloride monomer has a high toxicity and thus monomer levels in **p**oly**v**inyl**c**hloride (PVC) food packaging material need to be strictly controlled. (Lau and Wong 2000) The residual monomer content of the polymer as well as the migration into food are regulated. (European Commission 2011)

Poly**e**thylene **t**erephthalate (PET) is a copolymer of ethylene glycol with either terephthalic acid or dimethyl terephthalate and is used for packaging materials

for beverages and edible oils. As no thermal deformation takes place below 220 °C, PET is also used for trays and dishes for microwave and conventional cooking. PET contains small amounts of low molecular weight oligomers ranging from dimer to pentamer, which may migrate into in the foodstuff. (Lau and Wong 2000)

Bis**p**henol **A** (BPA) is a plastic monomer, which is extensively used in many different types of food packaging. It is known to be an endocrine disruptor. (Muncke 2009, Ozaki et al. 2004) Epoxy resins of the BPA type, like **B**isphenol **A** **d**i**g**lycidyl **e**ther (BADGE), are starting materials for cold-cured epoxy polymers. (Lau and Wong 2000) These epoxy polymers are employed for example as coatings in food cans. (Lau and Wong 2000) Unreacted epoxy groups have cytotoxic effects in tissues with high rates of cell division. (Lau and Wong 2000) Due to their toxicity it is necessary to control the unreacted epoxy resins to limit their migration into food. (Lau and Wong 2000)

2.3.2. Contaminants from Paper and Cardboard Packaging Materials

Besides plastic paper and cardboard constitute a second major material group used for food packaging. Paper and cardboard are either used as primary packaging in direct contact with dry foodstuffs, as a layer in multilayer materials or as secondary packaging (e.g. corrugated board boxes). (Jickells et al 2005, Nerin and Asensio 2007) In the past several years the use of paper and board containing recycled fibres for food contact applications has increased. (Ozaki et al. 2004) Despite various processing steps, recycled paper and board has become an area of particular concern, since it was found that the content of heavy metals, formaldehyde, halogenated aromatic hydrocarbons, bleaching agents, volatiles, slimicides and residues of e.g. printing inks may be higher in recycled fibre than in fresh fibre. (Triantafyllou et al. 2002, Castle et al. 1997)

Regarding (recycled) paper and cardboard packaging special attention has lately been paid to the migration of mineral oil components. Mineral oils consist of a saturated hydrocarbon fraction (MOSH: **m**ineral **o**il **s**aturated

hydrocarbons) and a fraction of mainly alkylated polycyclic aromatic hydrocarbons (MOAH: **m**ineral **o**il **a**romatic **h**ydrocarbons). (Biedermann et al. 2009) Though the toxicological data is not yet complete (Bundesinstitut für Risikobewertung 2010), a contamination of foodstuff with mineral oil components is not desirable, especially with regard to the well-investigated accumulation of these compounds in fat tissue and certain organs. (Biedermann et al. 2009) The main source of contamination by paperboard packaging materials is printing ink, where mineral oils are used as solvents. (Droz and Grob 1997) Especially printing inks from newspapers, which enter the paper recycling process, but also inks used for packaging printing, are responsible for this contamination. (Moret et al. 2011)

2.3.3. Contamination from printing inks

Over 90% of all processed foodstuffs are sold in printed packaging materials. (Leks-Stepien 2011) Thus, printing inks constitute a wide-spread source for the potential contamination of foodstuffs. There are two possible ways how printing ink substances can become contaminants in foodstuffs. They can either migrate through the packaging material as mentioned in section 2.1 or the so called set-off effect can occur, which takes place when the printed material is rolled on spools or stacked and therefore the inner surface, which later is in direct contact with the foodstuff, comes into contact with the outer printed surface. (Bradley et al. 2005, Gallart-Ayala et al. 2011a)

In the course of the printing process the liquid ink needs to be fixed to the printed surface. Conventional inks typically can be fixed either by means of oxidation (e.g. inks based on unsaturated oils like linseed oil) or by evaporative drying (e.g. solvent-based inks). However, both of these fixation processes have disadvantages: For oxidation inks fixation takes a long time to be fully completed. For solvent-based inks evaporation needs only a few minutes, but it may take a couple of days or even weeks for residual solvents to be minimised to levels that are organoleptically tolerable. (Anderson and Castle 2003)

A possibility of avoiding these drawbacks is the use of polymeric inks, in which the dye is mixed with monomers and photoinitiators. Fixation is effected by radiation. Thereby, the photoinitiators start a polymerisation process (“curing”) that leads to the formation of a macromolecular network with embedded colour particles. For this curing process UV and electron beam radiation are employed, although UV radiation is more frequently used. The UV-curable monomers have to contain an unsaturated chemical group that is able to react with other unsaturated molecules to produce a solid coherent film. Generally, acrylate, epoxy or vinylether derivatives are used nowadays. (Allen 1996)

The radiation curing process is very fast, with a touch-dry cure realised in a matter of seconds. (Anderson and Castle 2003) Additionally, there is no longer a potential problem with residues of organic solvents from the printing process contaminating the food through the printed packaging material. (Anderson and Castle 2003) However, polymeric inks fixed by curing may bring with them new possible contaminants, such as acrylates and photoinitiators. (Papilloud and Baudraz 2002a) Indeed, it was shown that photoinitiators can contaminate foodstuffs either by migration or the set-off effect. (Rothenbach et al. 2007)

The following chapter will discuss photoinitiators and contamination of foodstuffs by them in detail, as the objective of this master thesis was to develop a multimethod for the analysis of these substances in foodstuffs and the respective packaging materials.

3. PHOTOINITIATORS IN FOOD AND THEIR ANALYSIS

As discussed in the previous chapter photoinitiators constitute an integral part of polymeric inks, which are fixed to the printed surface by a radiation curing process. In the course of this curing process the photoinitiator, a photoactivator system, absorbs the radiation energy and transforms it into active species, which then induce a photochemical polymerisation or crosslinking of a monomer, oligomer or prepolymer formulation. (Allen 1996)

3.1. CHEMISTRY OF PHOTOINITIATORS

For radiation-cured printing inks two types of photoinitiators are available to induce the photopolymerisation process. They differ in the type of reaction, which can either be a radical addition that starts the polymerisation or an ionic, mostly cationic mechanism. (Allen 1996)

3.1.1. Radical-forming Photoinitiators

In the case of photoinitiators inducing a free radical chain process, the first reaction step is the formation of free radical species through the absorption of radiation. In the following step the radical adds to a low molecular weight monomer or prepolymer, inducing a chain growth polymerisation/crosslinking reaction and eventually termination. To control the reactivity, certain photochemical and photophysical properties are essential. Among others absorptivity, quantum yield, solubility and stability play an important role. Additionally the suppression of the quenching effect of oxygen constitutes a challenge. Photoinitiators inducing a radical addition can be classified into two basic categories. (Allen 1996)

In the case of the so called type I photoinitiators radiation with UV or visible light wavelength leads to a unimolecular bond cleavage generating free radicals. (Sigma Aldrich 2011) Type I photoinitiators cover different chemical structures,

but except for the azo compounds, they all have a benzoyl functionality in common. (Allen 1996) Examples of type I photoinitiators are shown in Figure 1.

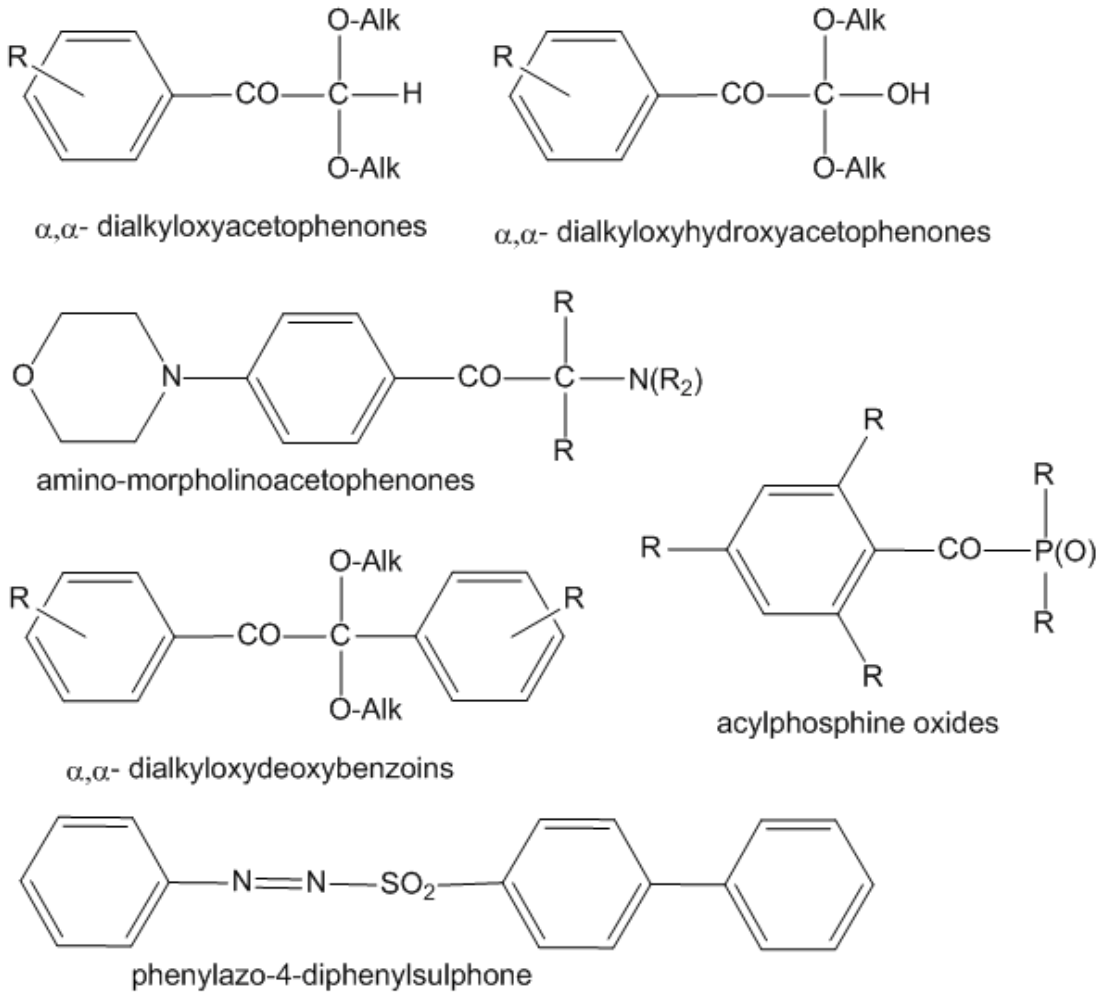


Figure 1: Examples for type I photoinitiators
(modified from Allen 1996)

In contrast, type II photoinitiators undergo a bimolecular reaction, where the free radical is formed by the interaction of the type II photoinitiator in the excited state with a second molecule (synergist). (Sigma Aldrich 2011) Type II photoinitiators have various chemical structures, including camphorquinone, benzils, α-ketocoumarines, xanthones, benzophenones and anthraquinones (Figure 2). (Allen 1996) Different substituents enhance the chromophore reactivity and provide oil or water solubility. (Allen 1996) For an enhanced efficiency tertiary amine co-synergists are used in conjunction with the aromatic

ketones. (Allen 1996) These co-synergists are also believed to inhibit the above mentioned oxygen quenching effect. (Allen 1996, Sigma Aldrich 2011)

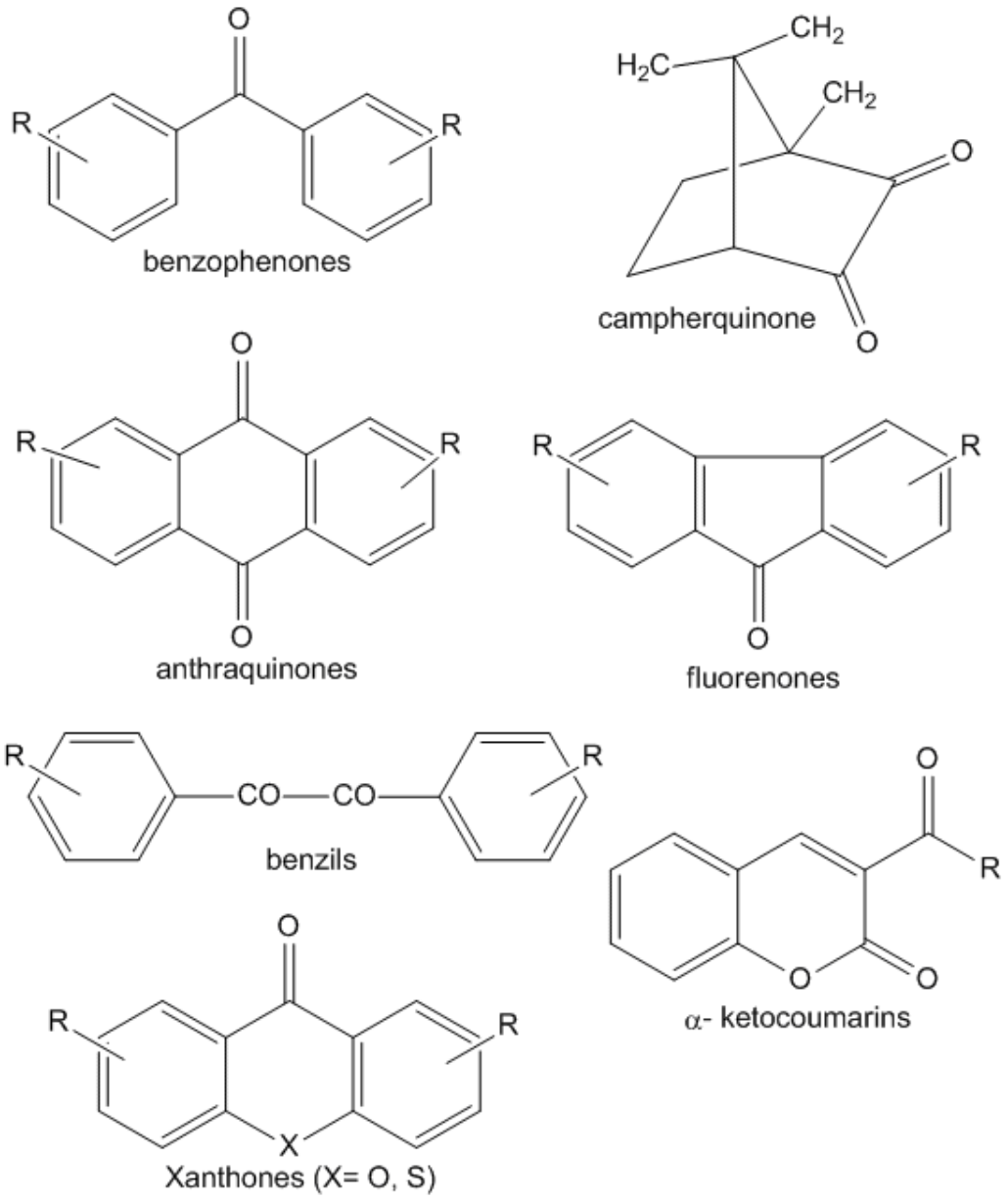


Figure 2: Examples for type II photoinitiators
(modified from Allen 1996)

Benzophenone is one of the most important type II photoinitiators as the use of benzophenone combined with type I photoinitiators and tertiary amines enhances reactivity and thus provides a way for a cost-effective formulation of UV-cured printing inks. (Allen et al. 1999, Anderson and Castle 2003)

3.1.2. Cationic Photoinitiators

Cationic photoinitiators were developed for the polymerisation of expoxy- and vinylether-based monomers. Depending on the chemical structure, cationic photoinitiators are able to produce either Brönsted acids or Lewis acids. Figure 3 shows some examples for cationic photoinitiators. (Allen 1996)

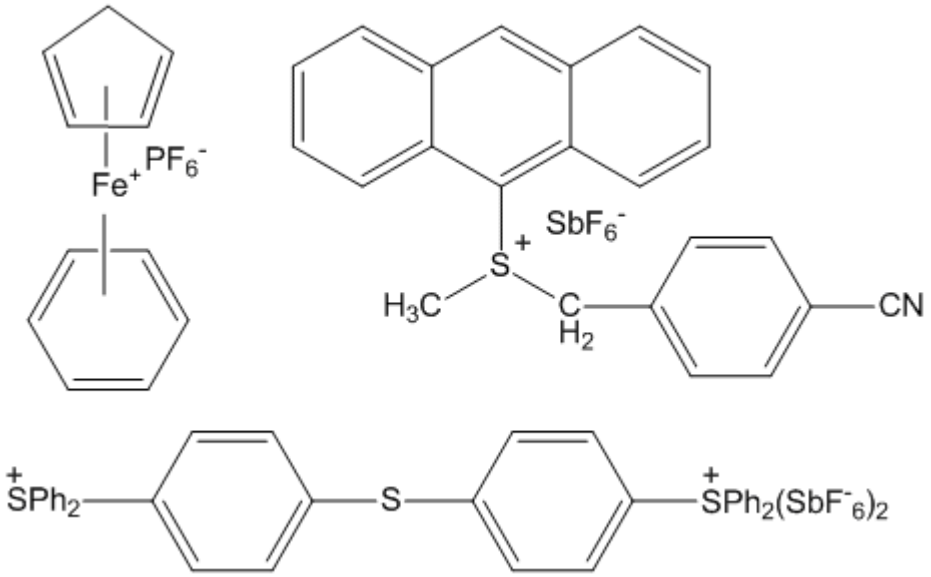


Figure 3: Examples for cationic photoinitiators
(modified from Allen 1996)

Other cationic photoinitiators are complex metal halides or diaryliodonium, triarylsulphonium and -selenonium salts. Upon photolysis substances of the latter type produce strong Brönsted acids. In order to induce polymerisation, the acid reacts with a monomer. Generally termination of the polymerisation process occurs through impurities. (Allen 1996)

Cationic photoinitiators have the main advantage of being insensitive to oxygen, but many of them are expensive and toxic, which makes them unsuitable for printing inks formulations for food packaging materials. (Allen 1996)

3.2. PHOTOINITIATORS AS FOOD CONTAMINANTS

In recent years the migration of ink components, especially photoinitiators, from paper, cardboard or plastic food packaging into foodstuffs has attracted considerable interest. This topic was especially brought to the limelight by two food scares.

3.2.1. Food scares

In 2005 **i**sopropyl**t**hio**x**anthone (ITX) and 2-**e**thyl**h**exyl-4-**d**imethyl**a**mino**b**enzoate (EHDAB) were the content of a food alert, as they had been found in baby milk, milk products and cloudy juices packaged in beverage cartons. (European Food Safety Authority 2005b) As milk is a fatty food, non-polymer-bonded ITX is easily soluble in this matrix due to its high lipophilicity. In the case of fruit juice, fibre could incorporate the photoinitiators and hence effect a contamination of the juice. (Sargatini et al. 2006)

The Italian Food Control Authority detected that the photoinitiator ITX migrated into baby milk at concentrations ranging from 120 to 300 µg/L. (European Food Safety Authority 2005b, Gil-Vergara et al. 2007) As a result over 30 million litres of ready-to-feed infant formula were re-called by producers in Italy, France, Portugal and Spain. (Sun et al. 2007) As a consequence of the alert, scientific investigations of ITX and EHDAB, the latter being used as a co-synergist, were conducted by EFSA. (European Food Safety Authority 2005b) The food industry reported results of analysis for ITX and EHDAB in several food products packaged in cartons printed with UV-cured inks containing these two photoinitiators. (European Food Safety Authority 2005b) Both ITX and EHDAB were found in milk products, soy-based products and fruit juices at varying levels up to over 100 µg/L. (European Food Safety Authority 2005b) On the basis of this information a risk assessment was carried out by EFSA. The existing genotoxicity studies did not indicate a genotoxic potential for ITX. However, the toxicological data of 2-ITX was insufficient and the presence of ITX in food could be considered as undesirable. A maximum migration level of

50 µg/kg foodstuff was set as this contamination was deemed to be unlikely to pose a health risk.

In 2009 the migration of **b**enzo**p**henone (BP) and 4-**m**ethyl**b**enzo**p**henone (4-MBP) from printed carton packages into cereals was detected, once again heightening the interest towards migration of printing ink compounds. (European Food Safety Authority 2009a, Rodriguez-Bernaldo de Quiros 2009) Upon the notification on the migration of non-evaluated 4-methylbenzophenone from cardboard, the European Commission asked EFSA to evaluate the possible risk of the presence of 4-MBP in cereals and whether the existing group TDI of BP and 4-**h**ydroxy**b**enzo**p**henone (4-HBP) of 0.01 mg/kg bodyweight could be also applied to 4-MBP. (European Food Safety Authority 2009a) The Scientific Panel on food contact materials, enzymes, flavourings, and processing aids (CEF) concluded that a short-term consumption of cereals contaminated with 4-MBP should not constitute a risk to human health, but that if 4-MBP was to be used continuously, more data would be needed to carry out a full risk assessment. (European Food Safety Authority 2009a) The panel also stated that the TDI of BP could not be applied to 4-MBP and that HBP should not be included in the TDI of BP due to the lack of data. In a renewed toxicological evaluation of BP the panel raised the TDI to 0.03 mg/kg bodyweight. (European Food Safety Authority 2009b)

3.2.2. Regulation of Photoinitiators as Food Contaminants

Regulation 2023/2006 related to GMP refers specifically to printing inks applied to the non-food contact side, prescribing that printing inks components shall not be transferred into foodstuffs by set-off effect or transfer through the substrate at concentrations that do not conform with the requirements of article 3 of Regulation (EC) 1935/2004. (European Commission 2006) A resolution of the Council of Europe on this topic can be regarded as a reference text, until the EU establishes a specific regulation relating to printing inks. (Council of Europe 2005)

Legal regulations specifically dedicated to printing inks compounds do not exist at present in the EU. However, when food packaging materials are printed the printing ink and its components become a part of a food contact material and thus general legal rules regarding FCMs apply to them. Consequently, printing ink components have to comply with Article 3 of Framework Regulation 1935/2004, which lays down the general requirements for all food contact materials (see chapter 2.2).

Thus up to now **m**aximum **r**esidue **l**evels (MRL) for photoinitiators in foods have not been established in the European Union, although according to EFSA the presence of some of them could be considered undesirable. (European Food Safety Authority 2005b) Only for BP for its use as an additive in plastics a SML was set at 0.6 mg/kg foodstuff. (European Commission 2011)

Recently, Swiss regulations have set two lists of authorized ink photoinitiators. One list contains those compounds that have been toxicologically evaluated and therefore a specific migration limit has been established. On the other list the non-evaluated substances can be found. For these substances a migration limit of 10 µg/kg food applies. (Federal Department of Home Affairs 2011)

As manufacturers of packaging materials are in charge of ensuring that their products comply with Regulation 1935/2004, the **Eu**ropean **P**rinting **I**nks **A**ssociation (EuPIA) defined a guideline on printing inks applied to the non-food contact surface of food packaging materials and articles. (European Printing Ink Association 2011) According to this guideline a printing ink substance is seen to be acceptable if its specific migration does not exceed:

- 10 µg/kg in the case of insufficient toxicological data
- 50 µg/kg for non-genotoxic compounds, consistent with EFSA Guidance
- ≥ 50 µg/kg, if supported by toxicological data and/or an evaluation performed according to EFSA Guidance

(European Printing Ink Association 2011)

3.2.3. Migration behaviour of Photoinitiators

Contamination of packaged foodstuffs by photoinitiators can occur by three different mass transfer ways. It can take place by migration, set-off or permeation. (Jung et al. 2010) A transfer by migration is only possible in cases where no functional barrier, such as aluminium foil is present between the printed surface and the foodstuff, e.g. yoghurt cups. (Jung et al. 2010) In the case of composite materials with a functional barrier, e.g. beverage cartons, contamination with photoinitiators can only take place by set-off. (Semi)volatile compounds like BP or 4-MBP can also undergo permeation and be transferred via the gas phase into foodstuffs packaged in cartons with inner plastic bags. (Jung et al. 2010, Rothenbacher et al. 2007, Pastorelli et al. 2008, Sanches-Silva, et al. 2008a, European Food Safety Authority 2009b)

Apart from the construction of the packaging material, contamination by photoinitiators is also influenced by other factors. It was shown by Triantafyllou et al. that the migration from paper and board depends on the chemical nature of the photoinitiators as well as time and temperature conditions. (Triantafyllou et al. 2007) Jung et al. conducted a study on the transfer of ITX, 2-**m**ethyl-1(4-**m**ethyl**t**hio**p**henyl)-2-**m**orpholino**p**ropan-1-one (MMTPMP) and the amine synergist **e**thyl-4-(**d**i**m**ethyl**a**mino)-**b**enzoate (EDMAB) into yoghurt cups under different conditions with yoghurt and 50% ethanol as food simulant. (Jung et al. 2010) Under certain conditions contamination with ITX and EDMAB took place by set-off and not via permeation. In contrast, contamination with MMTMP could not be detected. For ITX it could be shown that the age of the UV-lamp used for curing and the storage time had an influence on the extent of set-off. The amount of ITX migrating into the yoghurt increased with the age of the UV-lamp and the storage time.

It was observed that for benzophenone in cardboard packaging material there is a reduction of migration into foodstuffs depending on the type of contact (lower migration for indirect contact than for direct contact) and on the storage temperature (migration decreased with lower temperatures). (Anderson and Castle 2003) Nevertheless, Johns et al. could observe that even under low

temperature conditions (-20 °C) and with no direct contact between packaging material and foodstuff, migration of benzophenone still takes place. (Johns et al. 2000) Pastorelli et al. showed that BP can even migrate from secondary packaging material through the packaging layer in direct contact with the foodstuff and into the food itself. (Pastorelli et al 2007)

Sanches-Silva et al. studied the migration of six photoinitiators (BP, ITX, EHDAB, 2,2-**d**i**m**ethoxy-2-**p**henyl**a**cetophenone (DMPA), 1-**h**ydroxy**c**yclohexyl-1-**p**henyl**k**etone (HCPK) and MMTPMP) into food simulants. (Sanches-Silva et al. 2009) With the experimental data a mathematical model based on Fick's second law and the key parameters were calculated. It was observed that a relationship exists between R, which is the ratio between log $K_{o/w}$ and molecular weight and total migration if log $K_{o/w}$ < 5. This condition is fulfilled for HCPK, DMPA, BP and MMTPMP. For these photoinitiators the following relationship was formed: the higher R is, the lower is migration. In general migration tends to increase with an increase of ethanol percentage in the food simulant, except for HCPK. (Sanches-Silva et al. 2009)

Rodriguez-Bernaldo de Quiros et al. studied the permeation of seven photoinitiators (4-HBP, **m**ethyl-2-**b**enzoyl**b**enzoate (MBB), BP, 2-HBP, 4-MBP, **p**henyl**b**en**z**ophenone (PBZ) and 4,4'-bis(**die**thyl**a**mino)**b**enzophenone (DEAB)). (Rodriguez-Bernaldo de Quiros et al. 2009) Migration of 2-HBP, BP and 4-MBP was observed to a higher extent compared with the other selected photoinitiators. The reason for this phenomenon is the higher vapour pressures of these compounds. The porosity of the foodstuffs also seems to influence the permeation process. Compared to the other matrices (bread, cereal, pasta and rice), the highest concentrations of photoinitiators were found in cake, which is not surprising, as the substances are all quite lipophilic. These results agree with those reported by Anderson and Castle and Triantafyllou et al.. (Anderson and Castle 2003, Triantafyllou et al. 2007)

3.3. ANALYSIS OF PHOTOINITIATORS

In order to assess the exposure of consumers towards photoinitiators contaminants in food, to check for compliance with legal or toxicological limits, to ensure food safety and to study the transfer of photoinitiators from packaging material into foodstuffs accurate and sensitive methods of analysis are required. Several methods have been developed to analyse photoinitiators qualitatively and quantitatively in foodstuffs and packaging materials.

As food matrices are complex and usually contain relatively low concentrations of photoinitiators, efficient pre-concentration and clean-up procedures are necessary. Most commonly **l**iquid-**l**iquid **e**xtraction (LLE), **p**ressurized **l**iquid **e**xtraction (PLE) and **s**olid **p**hase **e**xtraction (SPE) are employed for these purposes. Food packaging material is mostly extracted by different organic solvents, such as acetonitrile or dichloromethane overnight. Techniques commonly used to determine photoinitiators either in foodstuffs or in packaging materials are gas chromatography (GC) coupled to mass spectrometry (MS) or flame ionisation detector (FID) and liquid chromatography (LC) with mass spectrometric or UV detection.

In an early migration study on paper and board food packaging materials by Castle et al., which was conducted with no preconception of what compounds might be present, the finding of **M**ichlers's **k**etone (MK, 4,4'-bis(dimethylamino)-benzophenone) in unbleached Kraft paper was unexpected (Castle et al. 1997a), especially because Michler's ketone is reported to be a potential carcinogen. (NCI 1979, Ozaki et al. 2004) Moreover the UK printing ink industry had voluntarily placed it on a list of compounds that it did not recommend for use in printing inks for cardboard food packaging. (Castle et al. 1997b) In a follow-up work of Castle et al. Michler's Ketone and its analogue 4,4'-bis(diethylamino)-benzophenone (DEAB) as well as 4-(**d**i**m**ethyl**a**mino)-**b**enzophenone (DMAB) were investigated in cardboard and paper packaging materials and various foodstuffs, including cereal products, dried fruit, vegetables, dairy products, fats, meat and fish. (Castle et al. 1997b) In the

packaging materials the concentrations of MK, DEAB and DMAB were low and in foods these photoinitiators were not detectable (LOD 2 µg/kg). According to Castle et al. recycled paper was the most likely source of the contamination. (Castle et al. 1997b)

As BP is one of the most frequently used photoinitiators and is additionally used in the production of polyethylene coating films (Song et al. 2000), it is often part of analyses of migrants from food packaging materials. Song and his co-workers published an analytical procedure, employing GC-FID or GC-ECD, for the determination of five different contaminants, benzophenone, dimethyl phthalate, anthracene, methyl stearate, and pentachlorophenol, in recycled paper and board used for food packaging. (Song et al. 2000) In all recycled paperboards BP was present. In another study on the migration of different organic pollutants from recycled paper and cardboard packaging material into foodstuffs with fat contents ranging from 2% to 28%, BP was also in the scope of the analysis. (Triantafyllou et al. 2007) In the foodstuffs with the highest fat content, the highest levels of migration of the selected organic pollutants were observed. (Triantafyllou et al. 2007) Benzophenone has also been determined in PET flakes (Nerin et al. 2003) and in a multilayer plastic-paper food packaging by GC-MS. (Nerin and Asensio 2007) Pastorelli et al. reported a rapid and specific reversed-phase liquid chromatographic method with UV-detection to quantify benzophenone in packaging materials and cake samples. (Pastorelli et al. 2008)
Though positive findings of BP in foodstuffs are often reported, one should bear in mind that the presence of benzophenone may also be due to its application in the production of polyethylene coating films, and consequently not necessarily due to its use as a UV ink photoinitiator. (Di Gianni et al. 2004)

In the wake of the detection of ITX in baby milk in 2005, a large variety of methods to analyse for this photoinitiator in foodstuffs was developed. An overview of the different methodologies is given in Table 1.

Table 1: Overview of methods for the analysis of isopropylthioxanthone

reference	analyte	matrices	sample preparation	measurement	LOQ
Morlock and Schwack 2006	ITX	milk based products, margarine, soybean oil	accelerated solvent extraction⇨ liquid-liquid extraction	HPTLC-FLD HPTLC-MS	milk based products: 32 µg/L margarine and soybean oil: 1 µg/kg
Sagratini et al. 2006	ITX	fruit juices	pressurised liquid extraction⇨ liquid-liquid extraction	LC-MS (different mass analysers tested)	0.05-10 µg/L (depending on analysers)
Bagnati et al. 2007	2-ITX, 4-ITX	milk	liquid-liquid extraction	HPLC-MS/MS (different columns tested)	2.5 - 7.2 µg/L (depending on column)
Gil-Vergara et al. 2007	ITX, EHDAB	milk and milk based beverages	pressurised liquid extraction⇨ liquid-liquid extraction	GC-MS or LC-MS	GC-MS: 0.5- 1 µg/L LC-MS: 0.02-0.1 µg/L
Sun et al. 2007	ITX	milk, yoghurt drink, tea and juice packaging material	liquid-liquid extraction⇨ solid phase extraction	LC-MS/MS	0.5 µg/kg
Allegrone et al. 2008	ITX	milk with different fat content	solid phase extraction	GC-MS/MS	0.5 µg/L
Benetti et al. 2008	ITX	dairy products	liquid-liquid extraction	LC-MS	
Gallart-Ayala et al. 2008	2-ITX, 4-ITX	baby food, fruit juices, milk beverages, broth	liquid-liquid extraction⇨ solid phase extraction	LC-MS/MS	milk: 12- 13 ng/kg fruit puree: 2- 3.6 ng/kg

While the separation of the 2-isomer and 4-isomer of ITX generally presents no problem in gas chromatography based methods, coelution occurred in LC methods employing reversed phase (C8 or C18) columns. However, using a zirconium column (Bagnati et al. 2007) or a pentafluorophenylpropyl column (Gallart-Ayala et al. 2008) the two ITX isomers were also separated in LC methods.

A related photoinitiator that is employed either instead of or together with BP is 4-MBP. (European Food Safety Authority 2009a) Shortly after the detection of 4-MBP in breakfast cereals in 2009, Van Hoeck et al. published a method for the determination of BP and 4-MBP in breakfast cereals using GC-MS/MS. (Van Hoeck et al. 2010) After extraction the samples were cleaned up using SPE; BP-d10 was used as internal standard.

As a multitude of photoinitiators has been developed to date and it is generally unknown which substance(s) has/have been employed in the printing process of a certain food packaging, analytical methods focussing on only one or two photoinitiators can be considered of being of limited value for investigating foodstuffs and their packaging with regard to overall photoinitiator contamination and presence, respectively. However, only a limited number of multimethods targeting several to many photoinitiators has been reported so far. The studies that have been published on this analytical challenge to date are summarised in Table 2. Some interesting aspects of these studies are discussed in the following.

In the course of a study on the migration of printing ink components into food simulants Papilloud and Baudraz developed a multimethod for the determination of six photoinitiators (BP, EHDAB, MMTPMP, ITX, HCPK and DMPA) but also 9 acrylates using gas chromatography coupled to mass spectrometry. (Papilloud and Baudraz 2002a) Eight acrylates were also screened alongside a multitude of photoinitiators in a very recent report. (Bion et al. 2011)

Table 2: Overview of multimethods for the analysis of various photoinitiators

reference	analytes	matrices	sample preparation	measurement
Papilloud and Baudraz 2002a	BP, DMPA, EHDAB, HCPK, ITX, MMTPMP	food simulants	solid phase extraction	HPLC-DAD GC-MS
Sagratini et al. 2008	BP, EDMAB, EHDAB, HCPK, 2-ITX	milk, fruit juice, wine packaging material	liquid-liquid extraction⇨ solid phase extraction	GC-MS or LC-MS
Sanches-Silva et al. 2008c	BP, DMPA EHDAB, HCPK ITX, MMTPMP	milk powder	solvent extraction	HPLC-DAD
Sanches-Silva et al. 2008b	BP, DMPA. EHDAB, HCPK, ITX, MMTPMP	infant formula packaging material	liquid-liquid extraction	HPLC-UV HPLC-MS
Sanches-Silva et al. 2008d	BP, DMPA. EHDAB, HCPK, ITX, MMTPMP	orange juice packaging material	liquid-liquid extraction	HPLC-UV GC-MS
Koivikko et al. 2010	BP, DEAB, EHDAB, 2-HBP, 4-HBP, MBB, MBP, PBZ	cake, chocolate, pasty, nuts, dried fruits, packaging material	solvent extraction	HPLC-DAD
Negreira et al. 2010	BP, DMPA, EDMAB, EHDAB, ITX, MBP, MMTPMP	milk packaging material	solid phase micro extraction solvent extraction	GC-MS
Gallart-Ayala et al. 2011a	BP, DEAB, DETX, DMPA, EDMAB, EHDAB, HCPK, HMPP, 2-ITX, 4-ITX, PBZ	baby food, fruit juice, gazpacho, white wine, sangria, water	QuEChERS-method	LC-MS/MS
Bugey et al. 2011	35 photoinitiators	flour, cakes, pasta, cornmeal	accelerated solvent extraction ⇨ liquid-liquid extraction	GC-MS/MS
Bion et al. 2011	37 photoinitiators	meat, dairy and cereal products	QuEChERS-method	LC-MS/MS

As a consequence of the 4-MBP food scare, Koivikko et al. developed a multimethod for 4-MBP and BP as well as for 7 other photoinitiators (PBZ, 4-HBP, 2-HBP, MBB, DEAB and EHDAB) using HPLC-DAD and GC-MS. The cardboard material from the food packaging and in some cases also the foodstuff itself were analysed. Koivikko and his coworkers found out that BP and 4-MBP were not usually present in the same samples. (Koivikko et al. 2010)

Regarding the extraction of cardboard packaging material studies reported over the last few years all seem to use very similar procedures based on an extraction with acetonitrile for 24 hours at 70 °C by shaking. (Sun et al. 2007, Pastorelli et al. 2008, Negreira et al. 2010, Koivikko et al. 2010)

The challenging nature of the analysis of photoinitiators in foodstuffs was highlighted by the difficulties reported by Gallart-Ayala et al. in unambiguously confirming the presence of BP in an LC-MS/MS method, as ion ratio errors higher than 20% were obtained. (Gallart-Ayala et al. 2011a) In order to overcome this analytical problem the authors switched to high-resolution mass spectrometry for detection. (Gallart-Ayala et al. 2011b)

Only in the past year methods targeting more than ten photoinitiators simultaneously have been reported. Gallart-Ayala et al. developed a method for eleven photoinitiators using LC-MS/MS. The importance of addressing a wide range of analytes can be derived from the finding that 4 to 8 photoinitiators were present in the analysed packaging materials. (Gallart-Ayala et al. 2011a)
At the 5th International Symposium on Recent Advances in Food Analysis two multimethods with the largest scope so far were presented. One contribution described a method for screening and quantifying 35 photoinitiators in various foodstuffs and packagings. (Bugey et al. 2011) Samples were extracted by accelerated solvent extraction (ASE) and analysed by GC-MS/MS in the multiple reaction monitoring (MRM) mode. The second presentation reported a method for the analysis of UV ink ingredients, in which 37 photoinitiators, including five polymeric ones and additionally eight acrylates were screened in seven different food matrices using LC-MS/MS. (Bion et al. 2011)

4. STUDY DESIGN AND EXPERIMENTAL

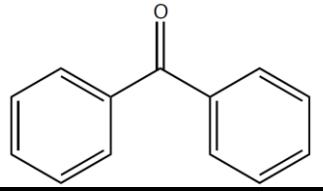
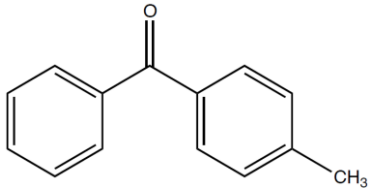
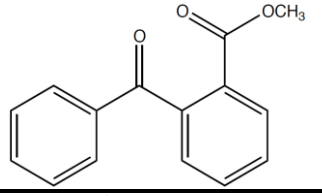
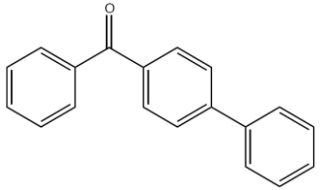
4.1. STUDY DESIGN

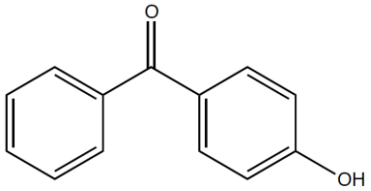
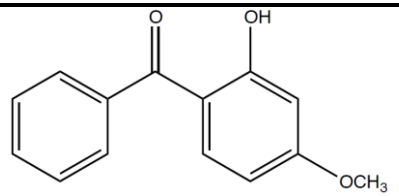
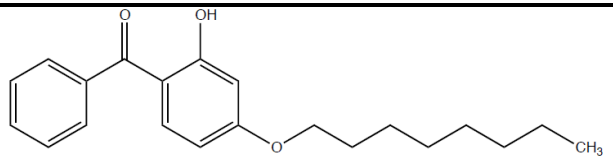
As multimethods for the determination of photoinitiators in foodstuff that target a substantial proportion of potential analytes are scarce (as described in section 3.3), the aim of this master thesis was to develop a method which can be applied for a broad screening of photoinitiators in foodstuffs as well as in packaging materials. The objective of this master thesis was the development of a multimethod for UV-ink photoinitiators in various cereal products and fruit juices as well as in their respective packagings by GC-MS.

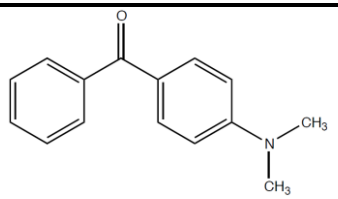
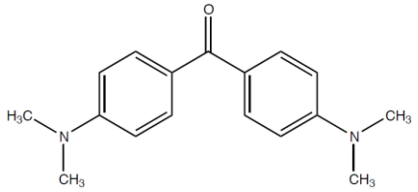
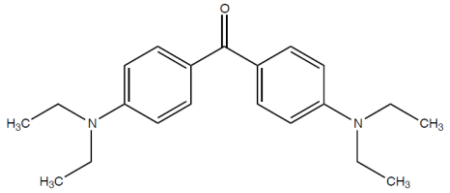
4.1.1. Analytes

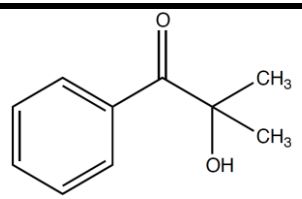
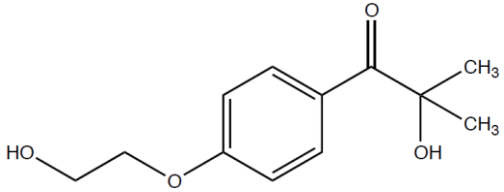
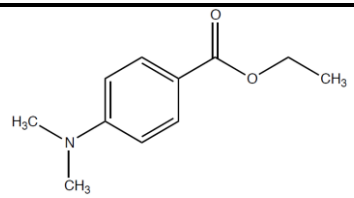
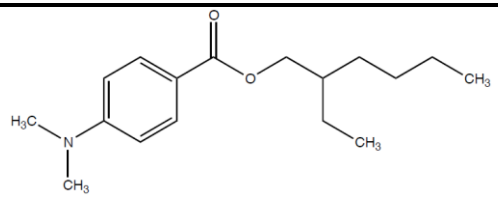
The selection of the photoinitiators studied was mainly governed by the physico-chemical properties of the potential substances, especially molecular weight and boiling point, as gas chromatography was the selected technique. Hence only radical forming photoinitiators were within the scope, as ionic photoinitiators have very high boiling points and are therefore not GC-amenable. Additionally the selection of the photoinitiators was restricted by their commercial availability, as not all photoinitiators enlisted in the EuPIA public inventory list (European Printing Ink Association 2008) were purchasable. The 26 selected photoinitiators together with important physico-chemical properties are listed in Table 3. In the table the analytes are grouped according their chemical structures in benzophenone derivatives, benzoate derivatives, morpholino derivatives, xanthone derivatives and phosphine oxide derivatives.

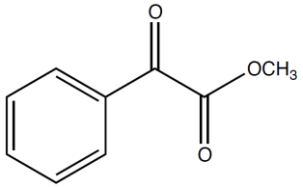
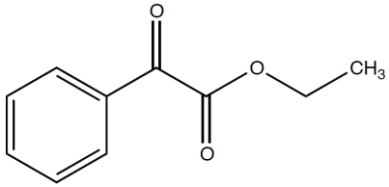
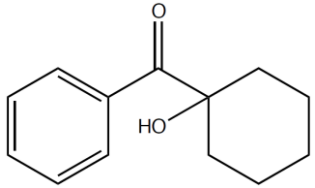
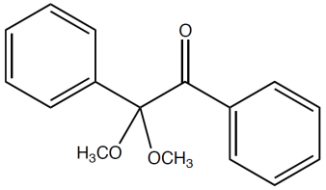
Table 3: Selected photoinitiators

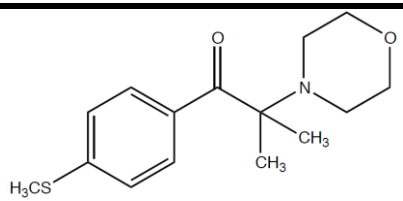
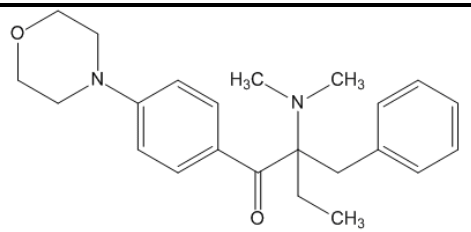
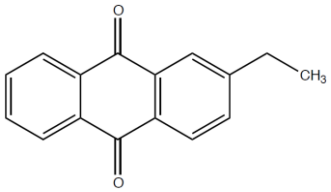
acronym	chemical name	CAS#	logP-value [a]	B_p [°C] [a]	MW	chemical structure
benzophenone derivatives						
BP	**b**enzo**p**henone	119-61-9	3.18	305.4	182.22	
4MBP	4-**m**ethyl-**b**enzo**p**henone	134-84-9	3.64	328.1	196.24	
MBB	**m**ethyl-2-**b**enzoyl**b**enzoate	606-28-0	2.828 ±0.315	351.5 ±15.0	240.25	
PBZ	**p**henyl**b**en**z**ophenone	2128-93-0	5.142 ±0.343	419.1 ±24.0	258.31	

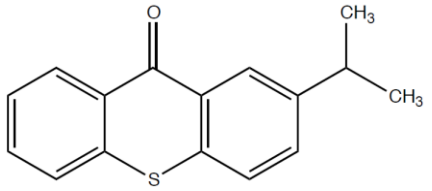
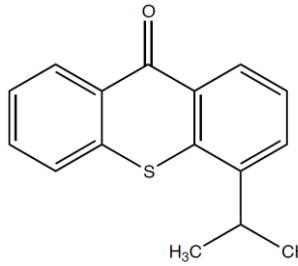
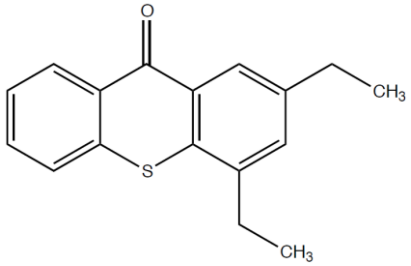
acronym	chemical name	CAS#	logP-value [a]	B_p [°C] [a]	MW	chemical structure
4HBP	4-**h**ydroxy**b**enzo**p**henone	1137-42-4	2.924 ±0.310	367.3 ±25.0	198.22	
HMBP	2-**h**ydroxy-4-**m**ethoxy-**b**enzo**p**henone	131-57-7	3.995 ±0.366	370.3 ±27.0	228.24	
HOBP	2-**h**ydroxy-4-(**o**ctyloxy)-**b**enzo**p**henone	1843-05-6	7.562 ±0.366	457.9 ±30.0	326.43	

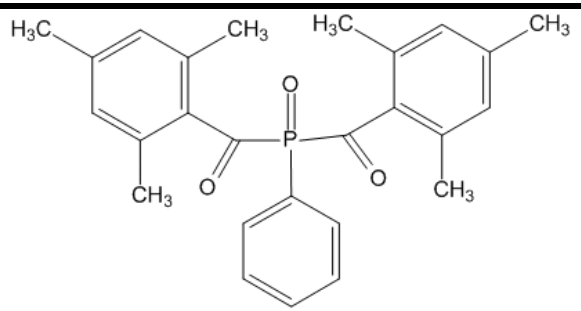
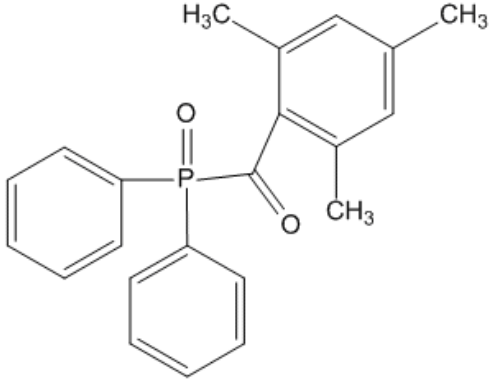
acronym	chemical name	CAS#	logP-value [a]	B_p [°C] [a]	MW	chemical structure
DMAB	4-(**dim**ethyl**a**mino)-**b**enzophenone	530-44-9	3.542 ±0.324	369.0 ±25.0	225.29	
MK	4,4'-bis(dimethylamino)-benzophenone **M**ichler's **K**etone	90-94-8	3.87 ±0.354	427.7 ±30.0	268.35	
DEAB	4,4'-bis(diethylamino)-benzophenone	90-93-7	5.994	475.7	324.46	

acronym	chemical name	CAS#	logP-value [a]	B_p [°C] [a]	MW	chemical structure
benzoate derivatives						
HMPP	2-**h**ydroxy-2-**m**ethyl**p**ropio**p**henone	7473-98-5	1.485 ±0.380	260.8 ±13.0	164.20	
HHEMPP	2-**h**ydroxy-4′-(2-**h**ydroxy**e**thoxy)-2-**m**ethyl**p**ropio**p**henone	106797-53-9	0.738 ±0.415	405.0 ±25.0	224.25	
EDMAB	**e**thyl-4-**d**i**m**ethyl**a**mino-**b**enzoate	10287-53-3	3.14	296.5	193.24	
EHDAB	2-**e**thyl**h**exyl-4-**d**imethyl**a**mino**b**enzoate	21245-02-3	6.15	325	277.40	

acronym	chemical name	CAS#	logP-value [a]	B_p [°C] [a]	MW	chemical structure
MBF	**m**ethyl-**b**enzoyl**f**ormate	15206-55-0	1.464 ±0.423	247.0	164.16	
EBF	**e**thyl-**b**enzoyl**f**ormate	1603-79-8	1.974 ±0.423	256.5	178.18	
HCPK	1-**h**ydroxy**c**yclohexyl-1-**p**henyl**k**etone	947-19-3	2.344	339	204.26	
DMPA	2,2-**dim**ethoxy-2-**p**henyl**a**cetophenone	24650-42-8	4.75	371.1	256.3	

acronym	chemical name	CAS#	logP-value [a]	B_p [°C] [a]	MW	chemical structure
morpholino derivatives						
MMTPMP	2-**m**ethyl-1-(4-**m**ethyl**t**hio**p**henyl)-2-**m**orpholino**p**ropan-1-one	71868-10-5	2.997	420.1	279.40	
BDMAM	2-**b**enzyl-2-(**dim**ethyl**a**mino)-4′-**m**orpholinobutyrophenone	119313-12-1	4.023 ±0.535	528.8 ±50.0	366.50	
xanthone derivatives						
EA	2-**e**thyl**a**nthraquinone	84-51-5	4.325 ±0.291	415.4 ±35.0	236.27	

acronym	chemical name	CAS#	logP-value [a]	B_p [°C] [a]	MW	chemical structure
2-ITX	2- **i**sopropyl**t**hio**x**anthone	5495-84-1	5.33	398.9	254.35	
4-ITX	4- **i**sopropyl**t**hio**x**anthone	83846-86-0	5.048 ±0.214	391.0 ±32.0	254.35	
DETX	2,4-**di****e**thyl-9H-**t**hio**x**anthen-9-one	82799-44-8	5.673 ±0.213	427.9 ±45.0	268.37	

acronym	chemical name	CAS#	logP-value [a]	B_p [°C] [a]	MW	chemical structure
phosphine oxide derivatives						
PTMBPO	**p**henylbis (2,4,6-**tri**ethyl**b**enzoyl) **p**hosphine **o**xide	162881-26-7	5.488 ±0.627	590.0 ±60.0	418.46	
DPTMBPO	**di****p**henyl (2,4,6-**tri**ethyl**b**enzoyl) **p**hosphine **o**xide	75980-60-8	4.620 ±0.420	519.6 ±60.0	348.43	

[a] Chemical Abstract Service, SciFinder 2008 ®

4.1.2. Sample preparation

The analysis of low-level photoinitiator contaminants in highly complex matrices as are foodstuffs requires suitable sample preparation to extract the analytes from the bulk matrix and to remove potentially interfering co-extracted matrix components as much as possible. Moreover a concentration step may be necessary to achieve the required low limits of quantification. At the same time, however, the sample preparation process should be rapid, easy to perform and should not require excessive amounts of chemicals.

In recent years the so-called QuEChERS method (**qu**ick, **e**asy, **ch**eap, **e**ffective, **r**ugged and **s**afe), which was originally developed for the determination of pesticide residues in various food matrices with low fat content, has become a frequently employed sample preparation approach in food analysis, not only for pesticides, but also for various food contaminants, including photoinitiators. Due to the simplicity of this sample preparation method, it is also suitable for routine laboratories, as 25 samples can easily be prepared for analysis each day. (Anastassiades et al. 2003a, Anastassiades 2005)

As food packaging materials constitute a far less complex matrix than foodstuffs a less sophisticated sample preparation procedure usually suffices. Thus, in the present work a simple solvent extraction was employed for the sample preparation of the food packaging material.

4.1.3. GC-MS

Despite efficient sample preparation the finally obtained solution still constitutes a complex mixture of compounds in which the analytes need to be identified and accurately quantified with high confidence and reliability. For this purpose a separation system coupled to a sensitive and selective detector is necessary. Highly efficient separations can be obtained by gas chromatography. The requirement for the analysis by gas chromatography is that the compounds are sufficiently volatile and can be vaporized without decomposition. In order to

unambiguously identify an analyte the information on the retention time provided by GC is not sufficient, as other compounds may coelute. Therefore a specific detector is required. For this purpose mass spectrometry is a very suitable technique as analytes with similar retention times can be unambiguously discriminated upon their mass spectrometric behaviour.
Thus for the simultaneous determination of the 26 photoinitiators in foodstuffs and packaging materials gas chromatography in combination with mass spectrometry was chosen.

4.1.4. Samples

In order to test the applicability of the developed method in routine analysis and to obtain some information of the contamination of certain foodstuffs with photoinitiators, several dozen food samples were purchased in different local supermarkets. The sample range included cereal products, packaged in printed cardboard boxes with inner plastic bags or a paper bag with printed surface, and fruit juice samples in printed multi-layer cartonboard packagings (e.g. Tetra Pak®).

4.2. EXPERIMENTAL

4.2.1. Chemicals

The photoinitiator analytes listed in Table 3 and the internal standard compounds **b**enzo**p**henone-**d10** (BP-d10) and 4-**f**luoro-4′-**h**ydroxy-**b**enzo**p**henone (FHBP) were obtained from Sigma-Aldrich and were all of analytical grade. They were stored at room temperature protected from light.
The chemicals used for sample preparation and GC-MS analysis are listed in Table 4.

Table 4: Chemicals employed for sample preparation and GC - MS analysis

Chemical	**Supplier**
Acetonitrile	VWR, Austria
Methanol	VWR, Austria
Hexane	VWR, Austria
Deionized water	Mili-Q purification system
Formic acid	Riedel de Haën, Germany
Magnesium sulphate anhydrous, coarsely grained	Fluka, Germany
Sodium chloride	Merck, Germany
PSA 40 µm	Supelco, USA
C18 bonded silica	Supelco, USA
Sorbitol	Sigma Aldrich, Germany
D-(+)-gluconic acid-δ-lactone	Sigma Aldrich, Germany

4.2.2. Standard Solutions

Analyte Stock Solutions

Stock solutions of the analytes listed in Table 3 and the internal standard compounds were prepared at a concentration of 1 g/L in acetonitrile (in the case of 4,4'bis(dimethylamino)benzophenone in methanol). Approximately 10 mg

were weighed into a screw-cap glass tube and the appropriate amount of acetonitrile was added gravimetrically taking the purity of the standard substances into account. The stock solutions were stored light protected at -18 °C.

Analyte Mixture Solutions

A 10 mg/L analyte mixture solution was prepared by mixing 200 µL of each analyte stock solution in a volumetric flask and filling up to 20 mL with acetonitrile. By 1:10 dilution of this 10 mg/L analyte mixture solution with acetonitrile, a further analyte mixture solution with a concentration of 1 mg/L was prepared. The analyte mixture solutions were stored light protected at +6 °C.

Internal Standard Solutions

A 500 mg/L internal standard solution of BP-d10 and FHBP was prepared by mixing the stock solutions of BP-d10 and FHBP in a 1:1 ratio. Additionally a 100 mg/L internal standard solution was prepared by mixing 1 mL of each internal standard stock solution in a volumetric flask and filling up to 10 mL with acetonitrile. The internal standard solutions were stored light protected at +6 °C.

Analyte Protectant Stock Solutions

A stock solution of sorbitol with a concentration of 50 mg/mL was prepared by dissolving 500 mg of sorbitol in 10 mL of acetonitrile:water (1:1 v/v). A stock solution of D-(+)-gluconic acid-δ-lactone with a concentration of 50 mg/mL was prepared by dissolving 500 mg of the lactone in 10 mL of acetonitrile:water (6:4 v/v). The analyte protectant stock solutions were stored at +6 °C.

Analyte Protectant Working Solution

The analyte protectant working solution (AP MIX) with a concentration of 5 mg/mL sorbitol and 10 mg/mL D-(+)-gluconic acid-δ-lactone was prepared by mixing 2 mL of D-(+)-gluconic acid-δ-lactone stock solution and 1 mL sorbitol stock solution in a volumetric flask and filling up to 10 mL with acetonitrile:water (7:3 v/v). The AP MIX was stored at +6 °C.

Solvent Standards

Solvent standards with concentrations of 12.5, 25, 50, 125, 250, 500, 2500 µg/L were prepared in acetonitrile by appropriate dilution of the 10 mg/L analyte mixture solution and addition of the 100 mg/L internal standard solution so that all standards contained the internal standard substances at a level of 5000 µg/L. Prior to analysis by GC-MS 1 mL of each solvent standard level was filled in a brown glass vial and 30 µL of analyte protectant working solution were added. The solvent standards were stored light protected at +6 °C.

Matrix-matched Standards

A freshly squeezed orange juice and a muesli packed in a plastic bag purchased at a local supermarket were used as blank matrices. Ten portions of the blank matrices were worked up according to the QuEChERS sample preparation method (see section 4.2.3), whereby no internal standard solution was added at the beginning. The blank matrix samples were pooled after the reconstitution of the evaporated sample in acetonitrile. To obtain the various matrix-matched standard solutions 500 µL of pooled blank matrix were evaporated under a stream of nitrogen at 45 °C and reconstituted in 500 µL of the respective solvent standard solution. Matrix-matched standards were stored light protected at +6 °C.

Due to the different sample preparations of the two matrices concentration factors of 5 for the cereal matrix and 10 for the fruit juice matrix were obtained. Thus the matrix-matched standards had concentration levels of 2.5, 5, 10, 25, 50, 100 and 500 µg/kg for the cereal matrix and 1.25, 2.5, 5, 12.5, 25, 50 and 250 µg/kg for the fruit juice matrix, respectively. The internal standard concentration was 1000 µg/kg for the cereal matrix and 500 µg/kg for the fruit juice matrix, respectively.

4.2.3. Sample Preparation of Foodstuffs

The methods used to work up the foodstuff samples were based on the QuEChERS methodology. (Anastassiades et al. 2003a, Anastassiades 2005)

Three different variants of the QuEChERS methodology, which only differed in the clean-up step, were employed. The clean-up consisted of a dispersive SPE with PSA, a dispersive SPE with PSA and C18 bonded silica or a dispersive SPE with PSA followed by liquid-liquid extraction with n-hexane. An overview of the sample preparation procedure, including the three variants is given in Figure 4. In detail it was performed as follows:

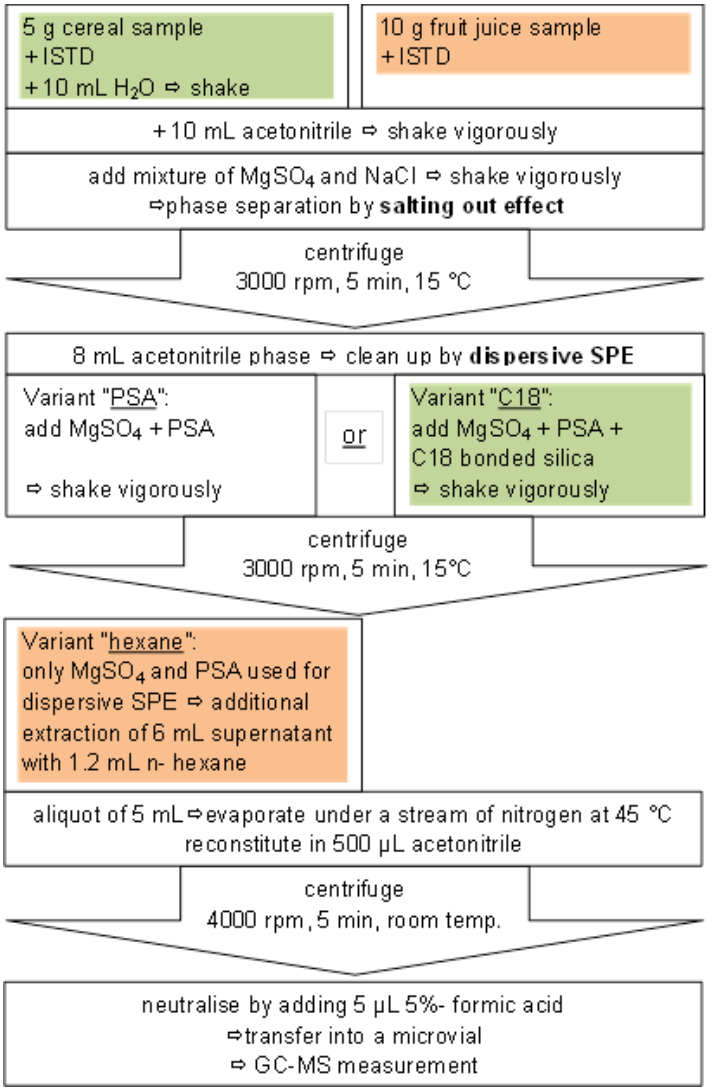


Figure 4: Flow chart of the sample preparation variants
The finally adopted variants for the two matrices are highlighted in green for the cereal matrix and in orange for the fruit juice matrix

In a preparatory step the following salt mixtures were weighed into screw-cap pyrex tubes:

salting out mixture:

- 4 g magnesium sulphate anhydrous
- 1 g sodium chloride

dispersive SPE mixture “PSA”

- 1.2 g magnesium sulphate anhydrous
- 200 mg PSA

dispersive SPE mixture “PSA + C18”

- 1.2 g magnesium sulphate anhydrous
- 200 mg PSA
- 200 mg C18 bonded silica

In order to obtain homogenous samples, the cereal samples were ground with dry ice, for the fruit juice samples, the juice was shaken well.
In the first step 5 g of homogenous cereal sample or 10 g fruit juice, respectively, were weighed into a 50 mL centrifuge tube. The 100 mg/L internal standard solution was added to the samples (50 µL for cereal samples and 100 µL for fruit juice samples) and was allowed to diffuse for approximately 10 minutes. To the cereal samples 10 mL of water were added. Subsequently 10 mL of acetonitrile were added to the sample and the mixture was shaken vigorously by hand for at least one minute. The salting out mixture was added to the centrifuge tube, which was then shaken vigorously for one minute. Afterwards the mixture was centrifuged at 3000 rpm for 5 minutes at 15 °C. An aliquot of 8 mL of the upper acetonitrile phase was transferred into a screw cap pyrex tube, containing one of the two dispersive SPE mixtures depending on the variant used. The tube was shaken vigorously by hand for 30 seconds and centrifuged at 3000 rpm for 5 minutes at 15 °C.
In the case of the variant containing an additional liquid-liquid-extraction step 6 mL of the supernatant were extracted with 1.2 mL n-hexane.

5 mL of the acetonitrile phase/supernatant were transferred to a glass test tube and the solvent was evaporated using a stream of nitrogen at 45 °C.
The residue was reconstituted in 500 µL acetonitrile and centrifuged at 4000 rpm for 5 minutes at room temperature. After adding 5 µL of a 5 % solution of formic acid in acetonitrile, the solution was transferred into a brown glass microvial for GC- MS measurement.

4.2.4. Sample Preparation of Packaging Material

The food packaging was opened and the content was removed. In the case of fruit juice cartons, the inner side of the packaging material was gently washed with distilled water and then wiped off. Fifty 1x1 cm pieces were cut out of different parts of the packaging material and were then extracted with 20 mL acetonitrile in a glass centrifuge tube on a shaker overnight. Due to the photosensitivity of the analytes the glass centrifuge tube was covered with aluminium foil. After that an aliquot of 15 mL was reduced to 3 mL by evaporation using a stream of nitrogen at 45 °C. Subsequently the solution was centrifuged at 4000 rpm for 5 minutes at room temperature. To an aliquot of 1 mL, 50 µL of internal standard solution (100 mg/L) and 30 µL of analyte protectant mix were added before being transferred to a brown glass vial for GC-MS analysis. The conversion factor from µg/L measured by GC-MS to µg/dm^2 packaging material is 1/125.

4.2.5. Analysis by GC-MS

The analyses were carried out on a GC-MSD system consisting of an Agilent 6890 GC coupled to an Agilent 5975 Mass Spectrometer. Separation was performed on a HP-5-MS column (60 m, 250 µm i.d., 0.25 µm thickness) from Agilent. A 2 m guard column of the same column type was used. Helium was used as carrier gas, with a constant flow rate of 2 mL/min. The injector temperature was 270 °C. The samples were injected pulsed splitless with an

initial pressure of 60 psi. A single taper splitless Ultra Inert Liner with glass wool was employed. The injection volume was 3 µL.

After optimization the oven temperature programme was as follows: initial isothermal period of 3.9 min at 60 °C, to 100 °C at 25 °C min^{-1}, 1 min hold, ramp to 250 °C at 8 °C min^{-1}, 4 min hold, ramp to 310 °C at 25 °C min^{-1}, hold for 15 min (see Figure 5).

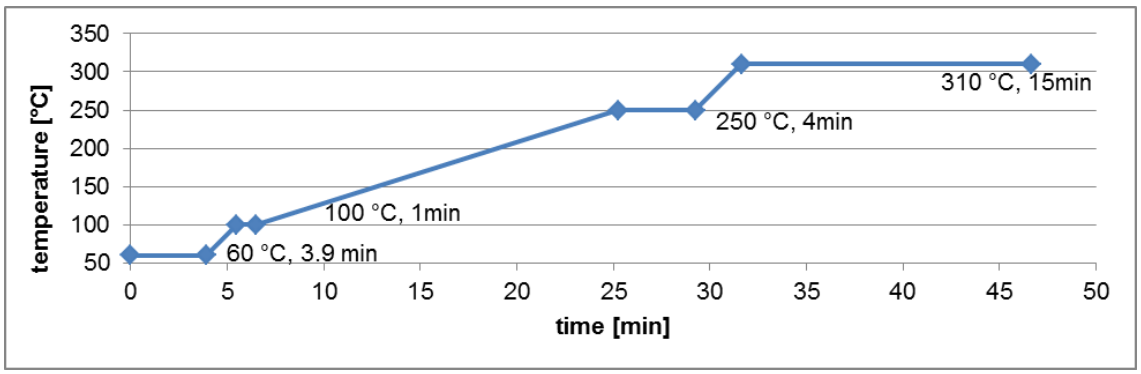


Figure 5: GC-temperature programme

The mass spectrometer was equipped with an electron impact (EI) ion source and was operated in positive mode at a voltage of 70 eV. The MSD transfer line was kept at 280 °C, the ion source at 220 °C and the quadrupole at 150 °C. Data were acquired in **s**elected **i**on **m**onitoring (SIM) mode. For each substance at least 3 ions were acquired and each ion had a dwell time of 25 ms, resulting in 6-10 cycles/s depending on the SIM group. The GC-MS system was controlled by MSD ChemStation E 02.02. software.

Retention times and ion ratios were used for unambiguous identification of the individual analytes. Ion ratios were calculated using the peak areas. The maximum deviations of the ion ratios in a sample from the average ones of the standards were as follows: For ion ratios below 10 %, between 10 and 20 %, between 20 and 50 % and over 50 % they were ± 50 %, ± 30 %, ± 25 % and ± 20 %, respectively. (adapted from European Commission DG SANCO 2009)

For the food samples quantification was performed by using 4 to 7-point calibrations of matrix-matched standards ranging from 25-500 µg/kg, 10-500 µg/kg, 5-500 µg/kg or 2.5-500 µg/kg for cereal matrix and from 12.5-250 µg/kg, 5-250 µg/kg, 2.5-250 µg/kg or 1.25-250 µg/kg for fruit juice

matrix, respectively. For the packaging material samples quantification was performed by using a 4 to 7-point calibration of solvent standards ranging from 125 to 2500 µg/L, 50 to 2500 µg/L, 25 to 2500 µg/L or 12.5 to 2500 µg/L. Calibration curves were obtained by linear regression using 1/x weighting. Depending on the retention time of the analyte, the internal standard benzophenone-d10 or 4-fluoro-4’hydroxybenzophenone was employed.

5. Results and Discussion

5.1. Method Development

5.1.1. GC-MS

In the first step the chromatographic and mass spectrometric behaviours of the analytes (retention times and mass spectra, respectively) were assessed by measuring dilutions of the stock solutions of the analytes (10 mg/L) by GC-MS in full scan mode.

The oven temperature programme of the GC was based on an existing method for the analysis of benzophenone in foodstuffs (Heidrun Unterweger, personal communication) and was as follows: isothermal segment at 50 °C for 2.1 minutes, ramp to 280 °C at 20 °C/min hold for 40 minutes. At least three ions for each analyte were selected for the following SIM method. If present the molecule ion was among the chosen ions, as it possesses a high selectivity in most cases. In addition ions with high m/z ratios or characteristic fragment ions were selected.

In this step the phosphine oxides (phenylbis(2,4,6 trimethylbenzoyl)phosphine oxide and (2,4,6-trimethylbenzoyl) phosphine oxide) turned out to be not volatile enough for the analysis by GC. Besides it was observed that the response for BDMAM was very low and thus this compound was also not suitable for the analysis by GC.

In the next step the GC separation was optimised with the objective of a baseline separation of all analytes to avoid potential interferences between co-eluting analytes and to be able to differentiate between the two ITX isomers. Besides this issue the separation of DMAB and EHDAB was challenging. Thus, different oven temperature programmes were examined, which are depicted in Figure 6.

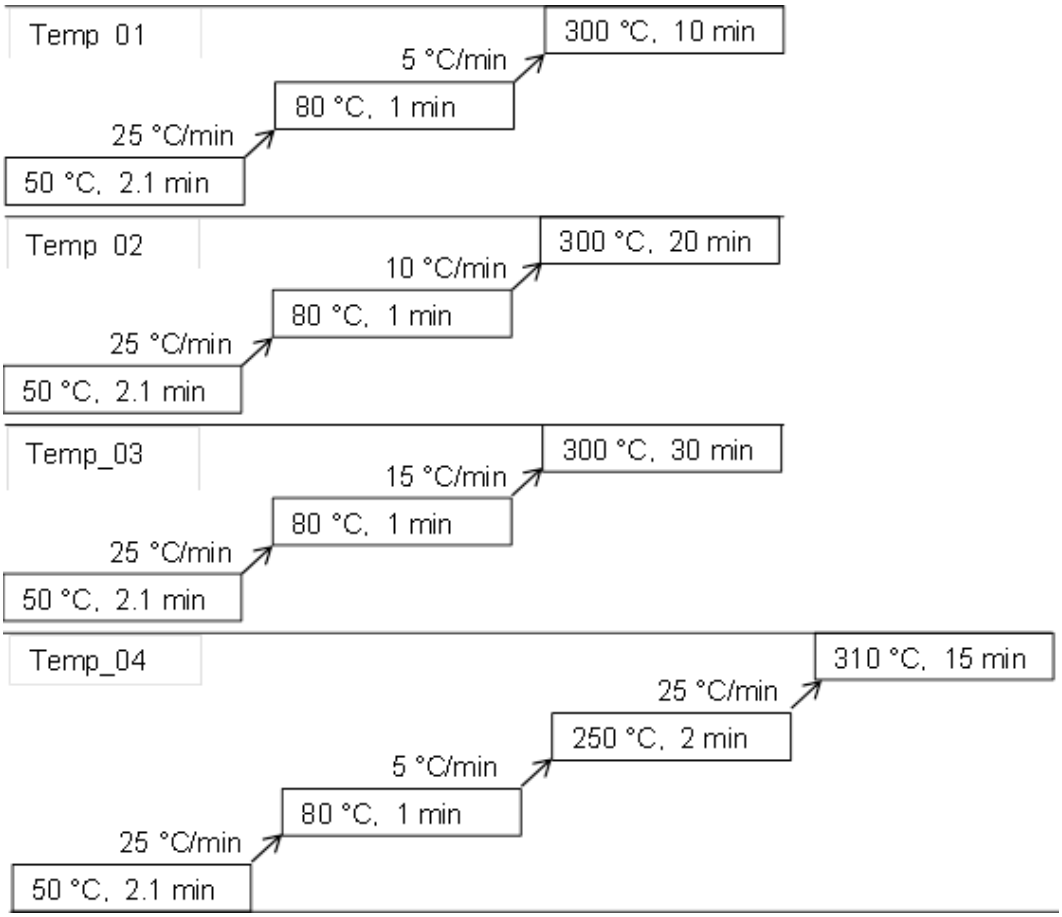


Figure 6: Optimisation of the oven temperature programme regarding baseline separation of all analytes

Aiming at the baseline separation of the ITX isomers and DMAB and EHDAB, in the first three oven temperature programmes (Temp_01- Temp_03) different heating rates to the third isothermal section and different holding times of the third isothermal section were investigated. However, these approaches were unsuccessful. By introducing a fourth isothermal segment in the oven temperature programme Temp_04, a separation of DMAB and EHDAB as well as of the two ITX isomers was achieved.

As the temperature programme had a total length of approximately 58 minutes, the next step was to optimise it with regards to the time of analysis. The different temperature programmes evaluated for achieving this goal are shown in Figure 7.

In programmes Temp_05 and Temp_06 the heating rate of the second temperature ramp was increased to 8 °C/min and 7 °C/min, respectively. However, in these temperature programmes, the hold time of 2 minutes after the second temperature ramp was too short to obtain baseline separation of the ITX isomers. By increasing the hold time to 4 minutes (oven temperature programme Temp_07) it was possible to baseline separate all analytes, while at the same time compared to temperature programme Temp_04, an improvement in run time of 11 minutes was achieved.

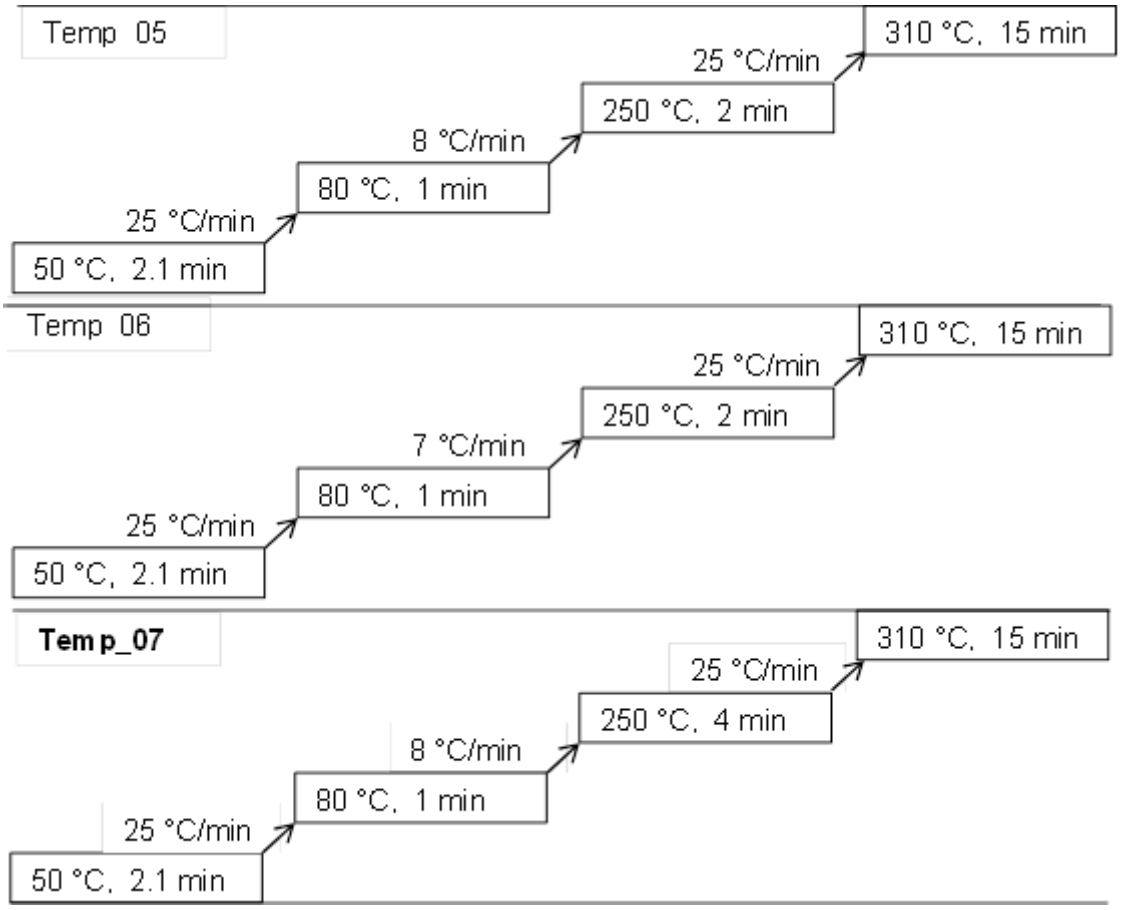


Figure 7: Optimisation of the oven temperature programme regarding overall run time

The first isothermal hold of the oven temperature programme forms the basis of solvent trapping. Slow transfer of the analytes into the column leads to a broadening of the initial chromatographic bands. To overcome this negative effect solvent trapping is employed. At the beginning the column temperature is set about 5-25 °C below the boiling point of the solvent, so that the solvent

condensates at the head of the column and forms a kind of stationary phase. The vaporized analytes are resolved into this condensated phase and as a consequence are reconcentrated. Afterwards the column temperature is rapidly increased to about 25 °C above the boiling point of the solvent in order to start the chromatographic separation. Narrow peaks are obtained as a result.

Acetonitrile, which has a boiling point of 82 °C, was used as solvent. Thus the effect of solvent trapping was not performed well for the oven temperature programmes Temp_01 to Temp07, as the first rapid temperature ramp did not reach a high enough temperature. Therefore, the upper point of the first temperature ramp was changed to 100 °C, instead of 80 °C (final GC oven temperature programme depicted in Figure 8).

After developing an oven temperature programme that successfully separated all analytes at baseline, a SIM acquisition method was developed to increase detection sensitivity. The analytes were grouped according to their retention times and at least three selective ions for each analyte, one quantifier ion as well as two qualifier ions, were monitored in the respective group. The use of the ion ratios quantifier/qualifier and quantifier/qualifier 2 ensured the correct identification of the analytes. The dwell time, which represents the amount of time spent sampling a specific ion, was set to 25 ms for each ion in every group. The SIM groups and the corresponding analytes are listed in Table 5.

Table 5: SIM-Groups, Ions and Retention times

SIM-Group	Analytes	*MW*	*B_P [°C]*	RT	ISTD
Group 1	**Ions: 59, 77, 105, 106, 136**				
13-15 min	2-**h**ydroxy-2-**m**ethyl**p**ropio**p**henone	*164.2*	*260.8 ± 13.0*	**14.04**	BP d10
	methyl **b**enzoyl**f**ormate	*164.2*	*247*	**14.35**	BP d10
Group 2	**Ions: 51, 77, 105, 106, 150**				
15-19.2 min	**e**thyl **b**enzoyl**f**ormate	*178.18*	*256.5*	**15.53**	BP d10
Group 3	**Ions: 105, 110, 152, 160, 182, 192**				
19.2-20.3 min	**b**enzo**p**henone-**d10**	*192.28*	*305.4*	**19.57**	
	benzo**p**henone	*182.22*	*305.4*	**19.64**	BP d10
Group 4	**Ions: 77, 81, 99, 105, 148, 164, 193**				
20.3-21.2 min	1-**h**ydroxy**c**yclohexyl-1-**p**henyl**k**etone	*204.26*	*339*	**20.53**	BP d10
	ethyl-4-**dim**ethyl**a**mino**b**enzoate	*193.24*	*296.5*	**20.71**	BP d10
Group 5	**Ions: 119, 181, 196**				
21.2-22.8 min	**m**ethyl**b**enzo**p**henone	*196.24*	*328.1*	**21.42**	BP d10
Group 6	**Ions: 91, 105, 121, 151, 163, 165, 166, 197, 209, 225, 240**				
22.8-24.2 min	2,2-**dim**ethoxy-2-**p**henyl**a**cetophenone	*256.3*	*371.1*	**23.04**	FHBP
	2-**h**ydroxy-4'-(2-**h**ydroxy**e**thoxy)- 2-**m**ethyl**p**ropiophenone	*224.25*	*405 ± 25.0*	**23.32**	FHBP
	methyl-2-**b**enzoyl**b**enzoate	*240.25*	*351.5 ± 15.0*	**23.73**	FHBP
Group 7	**Ions: 77, 105, 121, 141, 151, 159, 198, 216, 227, 228**				
24.2-27 min	4-**f**luoro-4'-**h**ydroxy**b**enzo**p**henone	*216.21*	*374.3*	**24.42**	
	4- **h**ydroxy**b**enzo**p**henone	*198.22*	*367.3 ± 25.0*	**24.72**	FHBP
	2-**h**ydroxy-4-**m**ethoxy**b**enzo**p**henone	*228.24*	*370.3 ± 27.0*	**24.93**	FHBP
Group 8	**Ions: 84, 108, 128, 148, 151, 164, 165, 181, 193, 221, 224, 225, 226, 236, 277**				
27-29.7 min	2-**e**thyl**a**nthraquinone	*236.27*	*415.4 ± 35.0*	**27.56**	FHBP
	4-(**dim**ethyl**a**mino)**b**enzophenone	*225.29*	*369.0 ± 25.0*	**27.87**	FHBP
	2-**e**thyl**h**exyl-4-**d**imethyl**a**mino**b**enzoate	*277.4*	*325*	**28.08**	FHBP
	2-**m**ethyl-1-(4-**m**ethyl**th**io**p**henyl)-2-**m**orpholino**p**ropan-1-one	*279.4*	*420.1*	**28.45**	FHBP
Group 9	**Ions: 184, 239, 254**				
29.7-30.6 min	**4-i**sopropyl**th**io**x**anthone	*254.35*	*391.0 ± 32.0*	**29.93**	FHBP
	2-isopropyl**th**io**x**anthone	*254.35*	*398.9*	**30.05**	FHBP
Group 10	**Ions: 152, 181, 258**				
30.6-31.2 min	**p**henyl-**b**en**z**ophenone	*258.31*	*419.1 ± 24.0*	**30.76**	FHBP
Group 11	**Ions: 84, 128, 152, 239, 253, 268**				
31.2-33 min	2,4-**die**thyl-9H-**th**io**x**anthen-9-one	*268.37*	*427.9 ± 45.0*	**31.39**	FHBP
Group 12	**Ions: 137, 213, 326**				
33-34 min	2-**h**ydroxy-4-(**o**ctyloxy)**b**enzo**p**henone	*326.43*	*457.9 ± 30.0*	**33.31**	FHBP
Group 13	**Ions: 148, 224, 251, 268**				
34-36.5 min	**M**ichler's **k**etone	*268.35*	*427.7 ± 30.0*	**34.36**	FHBP
Group 14	**Ions: 265, 309, 324**				
36.5-46 min	4,4'-bis(**die**thyl**a**mino)**b**enzophenone	*324.46*	*475.7*	**36.91**	FHBP

Matrix components with no or low volatility in the sample lead to a degradation of the column performance over time. Since the matrix compounds accumulate in the first meter(s) of the column, cutting off this first section would restore the separation capacity of the analytical column. However, this shortening of the column changes retention times and requires re-optimisation of the SIM time windows. A possible solution is the use of a guard column, which protects the analytical column from contamination, as the non-volatile residues stay in the guard column and do not interact with the stationary phase of the analytical column. (De Zeeuw, 2011) Thus, it is not necessary anymore to trim the analytical column, as the guard column can be easily changed. However, the connection between guard column and analytical column constitutes a potential problem area, as it can create dead volume and is a possible source of leaks and reactivity. (De Zeeuw, 2011) To overcome this effect a special connector has to be used.
Thus, in the present method a 2 m guard column with the same stationary phase as the analytical column (5 % phenyl-methylsiloxane) was employed. The guard column was replaced with a new one, when contamination affected peak shape or response.

In order to improve sensitivity the injection volume was increased by employing pulsed splitless injection instead of splitless injection. The injection volume depends on the volume of the vaporisation chamber (i.e. the liner), on the solvent as it affects the expansion volume as well as on the inlet temperature and pressure. The pulsed splitless injection is based on an increase of inlet pressure for a short period of time during sample injection (generally for 1 or 2 minutes). Due to the increased initial inlet pressure higher volumes, up to 5 µL, can be injected by making use of Boyle's law ($p_1V_1=p_2V_2$), which describes the inversely proportional relationship between the absolute pressure and the volume of a gas for a closed system with constant temperature. (Godula et al. 1999, Doherty 2011)
Hence, the following rule of thumb can be applied: a doubling of the inlet pressure leads to twice the injection volume. Based on this rule the initial inlet pressure was increased from 30 psi to 60 psi. The injection volume was only

raised from 2 µL to 3 µL. During method development injection volumes of 4 µL and 5 µL were also tested, but it was calculated that these volumes led to an overload of the vaporization chamber and as a consequence to irreproducible results.

In order to employ pulsed splitless injection effectively, the oven temperature programme was additionally optimised. The hold time of the initial temperature was prolonged to 3.9 min, which was obtained empirically. This time results of the 1 min hold time of the increased inlet pressure and additionally the time required to decrease the pressure back to 30 psi. Consequently only after 3.9 min the purge valve was opened and the first ramp of the oven temperature programme started. The final GC oven temperature programme, being optimised in terms of analyte separation, solvent trapping effect and pulsed splitless injection is shown in Figure 8.

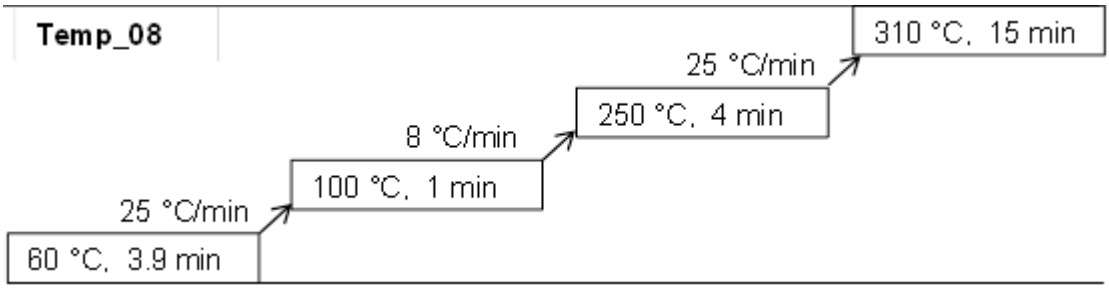


Figure 8: Final GC-oven temperature programme

Figure 9 shows the GC-MS chromatogram for 1 mg/L analyte mixture of 23 the photoinitiator analytes using the optimised conditions described above.

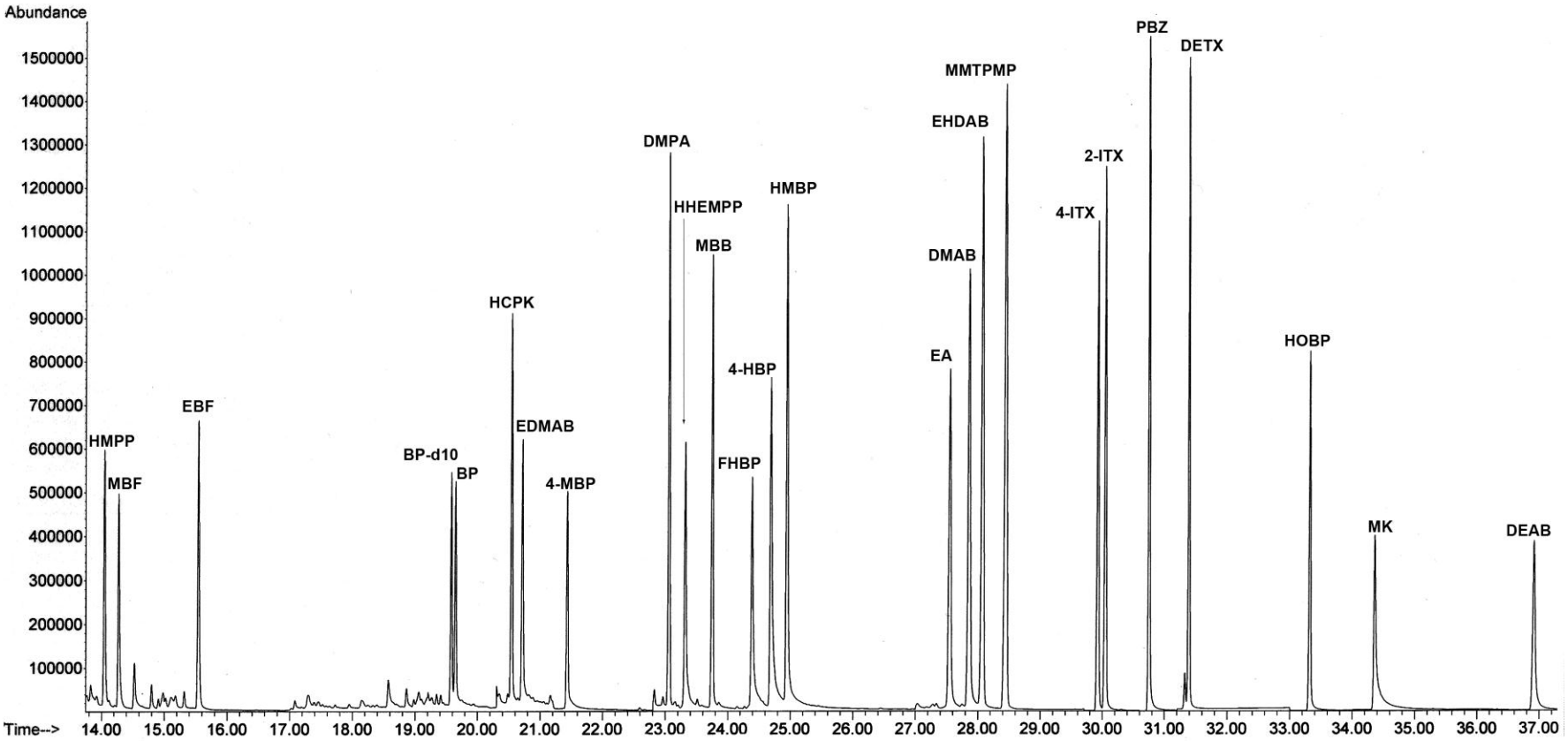


Figure 9: GC-MS chromatogram of 1 mg/L analyte mixture solution of 23 photoinitiators employing optimised conditions (BP-d10 and FHBP are internal standards)

Upon the first injections of matrix samples in combination with solvent standards, problems regarding GC-MS sensitivity and stability were encountered. The problems concerning sensitivity were manifested by a significant decrease in response and peak broadening. These phenomena were observed especially for solvent standards at the end of a sequence after only a few injections of samples containing matrix. The extent of the decrease in response and of the peak broadening was not equal for all analytes and thus could not be corrected by the internal standards. To overcome these problems, different approaches were tested: A higher injector temperature of 310 °C instead of the previously used temperature of 270 °C was evaluated. Since some analytes have high boiling points, an increase of vaporization by a higher injector temperature was expected. As no significant increase in response was observed, this approach was not pursued any further. Up to this point a single taper splitless inert liner with glass wool was employed. From the troubles mentioned above, it was suspected that there were too many active sites in the liner. These active sites can interact with the analytes, leading to degradation or adsorption of the analytes, resulting in poor linearity for calibration curves and loss of sensitivity. (Zhao et al. 2011) Therefore a specially deactivated single taper splitless Ultra Inert Liner with glass wool was employed, which resulted in a better chromatography with regard to peak broadening and loss of sensitivity. In order to condition the liner and saturate any still remaining active sites, two injections of a high level matrix-matched standard were performed at the beginning of each sequence.

In addition it was necessary to clean the instrument intensively after every sequence in order to get the described problems under control. After every sequence the inlet was cleaned with isooctane, the septum and the liner were changed and the ion source was cleaned as well. Periodically the gold seal in the inlet was cleaned or changed and the guard column was replaced.

5.1.2. Standard Solutions and Calibration

Due to the photoactivity of the analytes, a storage experiment was conducted at the beginning of method development. Vials containing the analyte mixture solution with a concentration of 10 mg/L were stored at three different places over three days. The selected places were a windowsill with high sun exposure, the tray of the autosampler in the air conditioned analysis room and the fridge. The solution stored light protected in the fridge at +6 °C was used as a reference. As expected the highest losses of analytes were observed for the vial exposed to the sun, some analytes (EDMAB, FHBP, HPB, MK, DEAB) were not even detectable anymore. To a far lesser extent, losses occurred in the vial stored on the autosampler tray. As a result of this storage experiment, all volumetric flasks containing analyte solutions were wrapped in aluminium foil and brown glass vials were used for the standard solutions and samples. In addition a sun blind with UV protection was used in the laboratory and the analysis room.

Among the 23 analytes, five compounds possess a hydroxyl group (HMPP, HHEMMPP, HBP, HMBP and HOBP), which leads to a tailing in the GC-MS chromatogram as the hydroxyl group interacts with active sites of the inlet and the column. This tailing complicates integration.

It was observed that in spiked cereal samples these analytes showed a significant improvement regarding peak shape and intensity in comparison to solvent standard solutions. This phenomenon is called “matrix-induced chromatographic response enhancement” as matrix components saturate active sites in the GC system and thus reduce (undesired) analyte interactions. (Anastassiades et al. 2003b) It is practically impossible to make a GC completely inert, as even the most stable column phase polymers will degrade over time at hot temperatures generating active surfaces. (Anastassiades et al. 2003b)

A possible solution for the problem that active sites pose for solvent standards are so-called analyte protectants, which mask the active sites and effect a response enhancement. (Anastassiades et al. 2003b) Anastassiades et al. tested several different compounds with strong hydrogen bonding capabilities, as hydrogen bonds play an important role in the interaction between analytes and active sites. (Anastassiades et al. 2003b) Substances with multiple hydroxyl groups, such as sugars and sugar derivatives, turned out to be the most effective protectants.

To obtain equivalent peak shapes and intensities in solvent standards and in almost matrix-free samples, analyte protectants were therefore introduced. For this purpose, an analyte protectant mix was prepared. It consisted of sorbitol, which protects late eluting substances and D-(+)-gluconic acid-δ-lactone, which protects early and middle eluting substances. The analyte protectant mix was added to the solvent standards as well as to the sample extracts before analysis by GC-MS. The addition of the analyte protectant mix indeed led to a chromatographic response enhancement and although tailing still occurred to a certain extent, it also led to an improvement of the peak shape. Nevertheless the application of analyte protectant was not sufficient to overcome the differences with regard to peak shape and sensitivity between solvent standards and spiked matrix samples. As a consequence matrix-matched standards were prepared for each sample type (cereals and fruit juices).

Upon changing from solvent to matrix-matched standards, the ions monitored for each analyte were checked for possible interferences from matrix components and ions were changed if necessary. The resulting ions monitored for the solvent standards as well as for the two types of matrix-matched standards are listed in Table 6. A consequence of the introduction of matrix-matched standards was that MBF had to be excluded from the method, as it was not possible to obtain ions selective enough for unequivocal identification within the matrices.

Table 6: Ions monitored depending on standard type[a]

	cereal matrix			fruit juice matrix			solvent standards		
Analytes	**Quan**	**Qual 1**	**Qual 2**	**Quan**	**Qual 1**	**Qual 2**	**Quan**	**Qual 1**	**Qual 2**
HMPP	105	106	77	59	77	105	59	106	77
EBF	105	77	106	105	77	51	105	77	106
BP-d10	110	192		110	192		110	192	
BP	105	182	152	182	105	152	105	182	152
HCPK	81	99	77	99	81	77	99	81	77
EDMAB	193	148	164	193	148	164	193	148	164
MBP	196	119	181	196	119	181	119	196	181
DMPA	151	105	225	151	105	225	151	105	225
HHEMPP	166	121	165	166	165	121	166	165	121
MBB	240	163	209	240	209	163	163	240	209
FHBP	216	121		216	121		216	121	
HBP	198	121	105	198	121	105	198	121	105
HMBP	151	227	228	151	227	228	227	151	228
EA	236	221	193	236	221	193	236	221	193
DMAB	225	148	224	225	226	148	225	148	226
EHDAB	277	165	164	165	277	164	165	164	277
MMTPMP	128	84	151	128	84	151	128	84	151
4-ITX	254	239	184	239	254	184	239	254	184
2-ITX	254	239	184	239	254	184	239	254	184
PBZ	258	181	152	181	258	152	181	258	152
DETX	268	253	239	268	253	239	253	268	239
HOBP	213	326	137	326	213	137	213	326	137
MK	148	268	224	268	148	224	268	224	148
DEAB	309	324	265	309	324	265	309	324	265

[a]Quan…quantifier ion

Qual …qualifier ion

As the packaging material samples contained only low amounts of matrix, solvent standards could be used. To the solvent standards and the packaging sample extracts the analyte protectant mix was added leading to satisfactory peak shapes and responses.

Calibration curves were obtained by linear regression, using 1/x weighting.
In an unweighted least-squares linear regression, equal emphasis is given to the variability of data points throughout the calibration curve. In general the absolute variation of responses, however, increases for higher concentrations and data at the high end of the curve tend to dominate the calculation of the linear regression. As a result there is an excessive error at the lower end of the calibration curve. One solution to reduce this error and to obtain a better fit of the experimental data to the calibration curve is to weight the data inversely with the concentration. The weighting will often minimise the overall error of the method and improve the quality of the analytical results. (Dolan 2009)

Two internal standards, BP-d10 and FHBP, were employed in the quantification of the analytes. The idea was that BP-d10 should be a suitable internal standard for the analytes without a hydroxyl group, while FHBP should suitably compensate for sample preparation losses and variations in the GC-MS measurement for the five analytes containing a hydroxyl group (HMPP, HHEMMPP, HBP, HMBP and HOBP). However, the differences in the chemical structures of the analytes preclude the ideal fulfilment of the compensatory role of the internal standard for every analyte. An overlay of a standard injected several times throughout a sequence seemed to indicate a better functioning of the internal standards if they were assigned to the analytes according to retention times (HMPP, EBF, BP, HCPK, EDMAB and MBP assigned to BP-d10, the other 16 analytes to FHBP). As the GC separation is mainly determined by temperature/analyte boiling point due to the non-polar column used, this assignment is also eligible. All further analyses were performed with the internal standard assignment by retention time (see Table 5).

5.1.3. Sample Preparation

As starting point for sample preparation the QuEChERS-method was employed in a variant where the dispersive SPE clean-up included C18 bonded silica, as cereals contain a certain amount of fat and this can be effectively removed by the C18 material. (Anastassiades 2005)

In order to optimise sample preparation with regards to remaining matrix, in the next step four different variants of the QuEChERS-method were investigated. Three variants differed only in the clean-up step performed by dispersive SPE as shown in Figure 4, the forth type completely excluded the dispersive SPE step. Cereal and fruit juice samples spiked at 10 µg/kg, 25 µg/kg and 100 µg/kg were analysed with each of the variants.
An overlay of the chromatograms of a cereal sample worked up by the four different variants is depicted in Figure 10.

As the extracts obtained from the variant excluding the dispersive SPE clean-up showed a much higher matrix load than those prepared using dispersive SPE this variant was considered to be unsuitable.

Regarding the different dispersive SPE variants the one where only PSA was used, was excluded as it seemed to yield too “dirty” extracts with regards to the amount of remaining matrix for both cereal and fruit juice samples. The difference between the “C18-variant” and “hexane-variant” in terms of remaining matrix was not significant. The “hexane-variant” was chosen for both matrices due to the perceived possibility that the C18 bonded silica could bind the analytes and therefore would lead to lower recovery rates.

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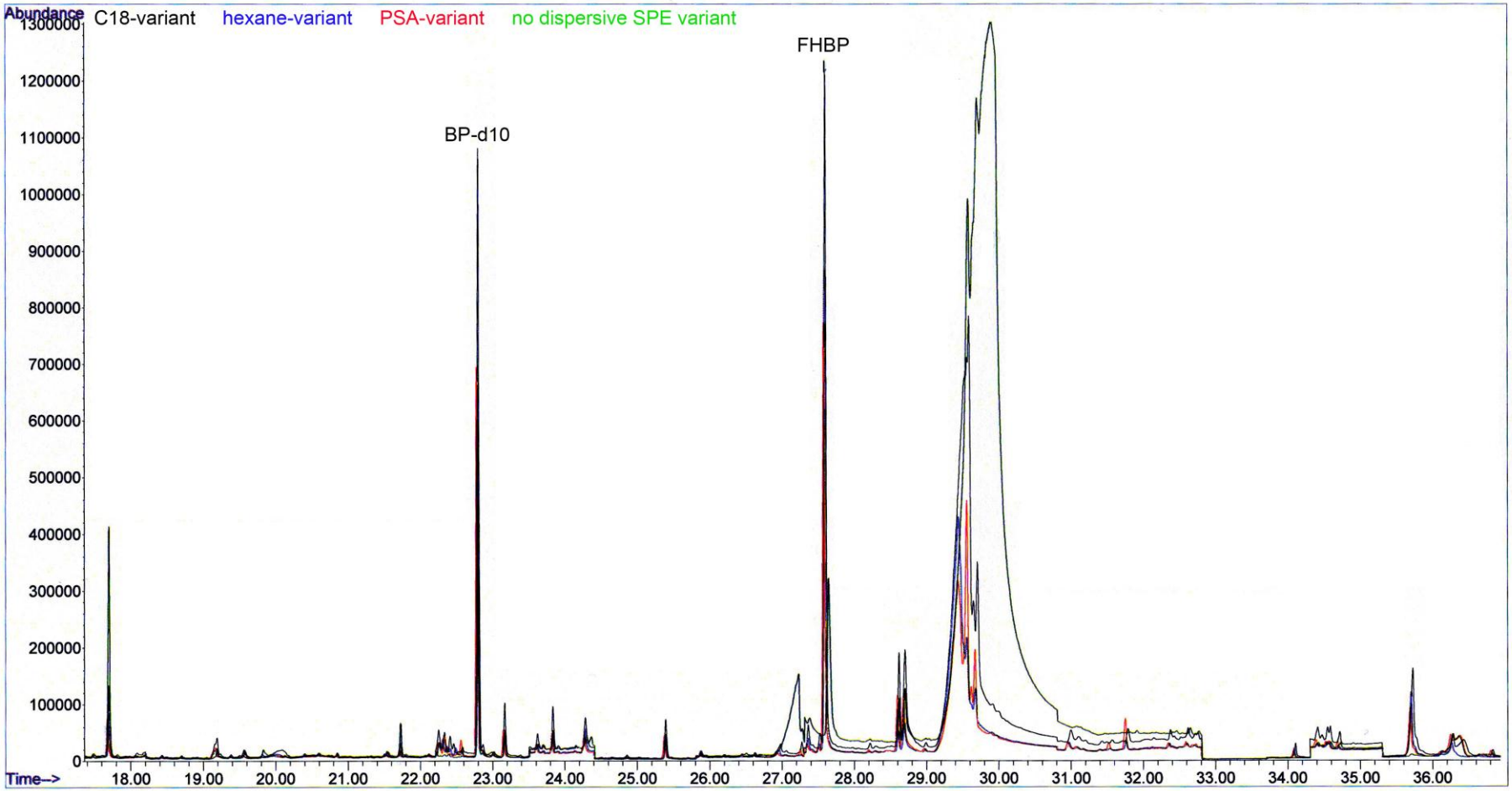

Figure 10: Overlay of GC-MS chromatograms of cereal samples worked up by the four different variants

In the next step the ”hexane-variant” was used to prepare the two matrices sixfold at three fortification levels in order to obtain information regarding the recoveries of the analytes.

In this evaluation, the “hexane-variant” provided good recovery results for the fruit juice matrix (recoveries ranged from 68 to 123%). Thus this variant was a suitable sample preparation and was subsequently validated.

For the cereal matrix, the “hexane-variant” turned out to be not as suitable as expected. Only poor recoveries were obtained for EHDAB, ITX, DETX and HOBP (recoveries ranged between 38% and 71%), the hexane phase was analysed and showed the presence of these analytes. A repetition of the recovery study with the “C18-variant” showed that better recoveries were achieved for these problematic analytes (recoveries ranged from 57 to 92%), while at the same time no negative influence on the other analytes was noted. Consequently the “C18-variant” became the method of choice for cereal matrix and was validated in the following.

5.2. Method Validation for Food Samples

In order to demonstrate the suitability of a developed procedure for the intended analytical application, a validation of the analytical method is necessary. The validation includes the assessment of the linearity of the calibration curves, the determination of recovery and precision as well as of the limit of detection and the limit of quantification. Currently no official rules or requirements regarding validation exist for contaminants from packaging materials. In the present work the SANCO document for method validation and quality control procedures for pesticide residues analysis in food and feed was used as guideline. (European Commission DG SANCO 2009)

As already discussed in chapter 5.1, of the 26 initially targeted photoinitiators three analytes (BDMAM and the two phosphine oxides) turned out to be not volatile enough for GC and for one analyte, MBF, the acquired ions were too unselective for the matrix samples. Thus, 22 photoinitiator analytes were validated in two different matrices (cereal and fruit juice samples).

5.2.1. Limits of Detection and Limits of Quantification

The limit of detection (LOD) is defined as the minimum concentration of an analyte that can be detected with acceptable certainty, however not being quantifiable with acceptable precision, whereas the limit of quantification (LOQ) refers to the minimum concentration of an analyte that can be quantified with acceptable certainty and precision. (European Commission DG SANCO 2009) As no legal regulations specifically dedicated to photoinitiators exist at present in the EU, the migration limit of the list for non-authorised substances of the Swiss Ordinance was taken as a basis. According to Swiss Regulation non-authorised substances must not be detectable in the foodstuff (LOD ≤10 µg/kg) (Federal Department of Home Affairs 2011), therefore one of the targets for the development of the present multimethod was to achieve a maximum LOQ of 10 µg/kg for all analytes.

For the determination of the LOD a signal-to-noise ratio (S/N ratio) of 3:1 was used, whereas for the LOQ a S/N ratio of 10:1 was employed. In order to determine the LODs and LOQs of the developed method, the S/N ratios were calculated using the analytical data of matrix-matched standards. The S/N ratios of all acquired ions of an analyte were compared with each other. The three ions, which were the best in terms of low matrix background noise, were chosen for the validation (see section 5.1.2, Table 6). The ion with the smallest S/N ratio was employed for the determination of the respective LOD and LOQ.

The LODs and LOQs for the cereal and fruit juice matrices are given in Table 7. Due to different matrix backgrounds, the ions used for determination of the LODs and LOQs sometimes differ between the cereal and the fruit juice matrix.

Table 7: Limits of detection and quantification for cereal and fruit juice matrices

	cereal matrix			***fruit juice matrix***		
Analyte	***Ion*[a]**	**LOD (µg/kg)**	**LOQ (µg/kg)**	***Ion*[a]**	**LOD (µg/kg)**	**LOQ (µg/kg)**
HMPP	*77*	1.0	3.3	*105*	1.3	4.3
EBF	*106*	0.4	1.3	*51*	1.6	5.4
BP	*152*	2.2	7.3	*152*	2.0	6.8
HCPK	*77*	1.0	3.3	*77*	1.4	4.7
EDMAB	*164*	0.3	1.1	*164*	0.2	0.6
MBP	*181*	1.6	5.3	*181*	1.4	4.8
DMPA	*105*	0.4	1.3	*225*	0.5	1.5
HHEMPP	*166*	0.7	2.3	*121*	0.4	1.3
MBB	*209*	0.2	0.7	*209*	0.2	0.5
HBP	*105*	4.1	13.5	*105*	0.5	1.6
HMBP	*151*	0.5	1.6	*151*	0.03	0.1
EA	*193*	1.3	4.3	*193*	0.4	1.2
DMAB	*224*	1.0	3.4	*226*	0.2	0.5
EHDAB	*164*	0.7	2.3	*164*	0.2	0.5
MMTPMP	*151*	14.5	48.3	*151*	1.0	3.3
4-ITX	*184*	0.3	1.0	*184*	0.1	0.4
2-ITX	*184*	0.6	1.9	*184*	0.1	0.3
PBZ	*152*	0.4	1.3	*152*	0.1	0.2
DETX	*239*	4.0	13.3	*239*	0.4	1.2
HOBP	*137*	0.8	2.8	*137*	0.3	1.0
MK	*224*	0.6	2.1	*224*	0.2	0.6
DEAB	*265*	1.7	5.8	*265*	0.7	2.2

[a]m/z of ion used to calculate LOD and LOQ

As can be seen in Table 7 the LOQs in cereal matrix differ significantly from the LOQs in fruit juice matrix. For the majority of analytes lower LOQs were achieved for the fruit juice matrix than for the cereal matrix, even taking the higher concentration factor from sample preparation for fruit juice into account. This can be explained by a higher load of matrix constituents present for the cereal matrix in the GC-MS measurement solution, which results in an increased signal background. For all analytes, with the exception of HBP, MMTPMP and DETX in cereal matrix, LOQs below 10 µg/kg were achieved. For HBP and MMTPMP the selectivity of the ions was rather low resulting in a high background noise level and consequently only rather high LOQ could be achieved. In addition for HBP the free hydroxyl group resulted in some peak tailing, further negatively affecting sensitivity. DETX eluted late together with fat compounds, resulting in a high noise level leading to a higher LOQ. Nevertheless, also in the cereal matrix LOQs below 10 µg/kg were achieved for most analytes, indicating that the method is highly sensitive and allows the detection and quantification of photoinitiators in cereal matrix as well as in fruit juice matrix at very low concentrations.

The achieved LOQs compare favourably to those of other recently published wide-scope multimethods for the analysis of photoinitiators. A GC-MS/MS method developed by Bugey et al. generally yielded higher LOQs for cereal samples than the present method (with the exception of MMTPMP and DETX) (Bugey et al. 2011), while similar or slightly lower LOQs were achieved by Bion et al. for yoghurt (ranging between 0.5 and 5 µg/kg) employing a LC-MS/MS method. (Bion et al. 2011)

5.2.2. Linearity

Matrix-matched standards were used for the acquisition of the calibration curves and were obtained by linear regression, using 1/x weighting. Calibration was performed using 7 levels ranging from 2.5 to 500 µg/kg for cereals and from 1.25- 250 µg/kg for fruit juice. Due to the quite high matrix backgrounds for

some ions and resulting elevated limits of detection (see section 5.2.1) it was not possible to perform a 7-point calibration for some analytes.

Thus, in the case of the cereal matrix a 6-point calibration was obtained for HBP and DETX, and a 4-point calibration for MMTPMP. In the case of fruit juice matrix a 6-point calibration was obtained for HMPP, EBF, BP, HCPK and MBP.

As shown in Table 8, the regression coefficients of the calibration curves were ≥ 0.998 for all analytes in both matrices, indicating excellent linearity. An examplary calibration curve is depicted in Figure 11.

Table 8: Regression coefficients of the calibration curves for cereal and fruit juice matrices

Analyte	cereal matrix	fruit juice matrix
HMPP	0.9996	0.9985
EBF	0.9990	0.9979
BP	0.9998	0.9996
HCPK	0.9997	0.9989
EDMAB	0.9999	0.9997
MBP	0.9997	0.9994
DMPA	0.9992	0.9994
HHEMPP	0.9999	0.9996
MBB	0.9997	0.9998
HBP	0.9999	0.9989
HMBP	0.9999	0.9997
EA	0.9998	0.9998
DMAB	0.9996	0.9998
EHDAB	0.9992	0.9995
MMTPMP	0.9991	0.9993
4-ITX	0.9992	0.9993
2-ITX	0.9994	0.9995
PBZ	0.9997	0.9993
DETX	0.9993	0.9994
HOBP	0.9999	0.9998
MK	0.9999	0.9993
DEAB	0.9999	0.9990

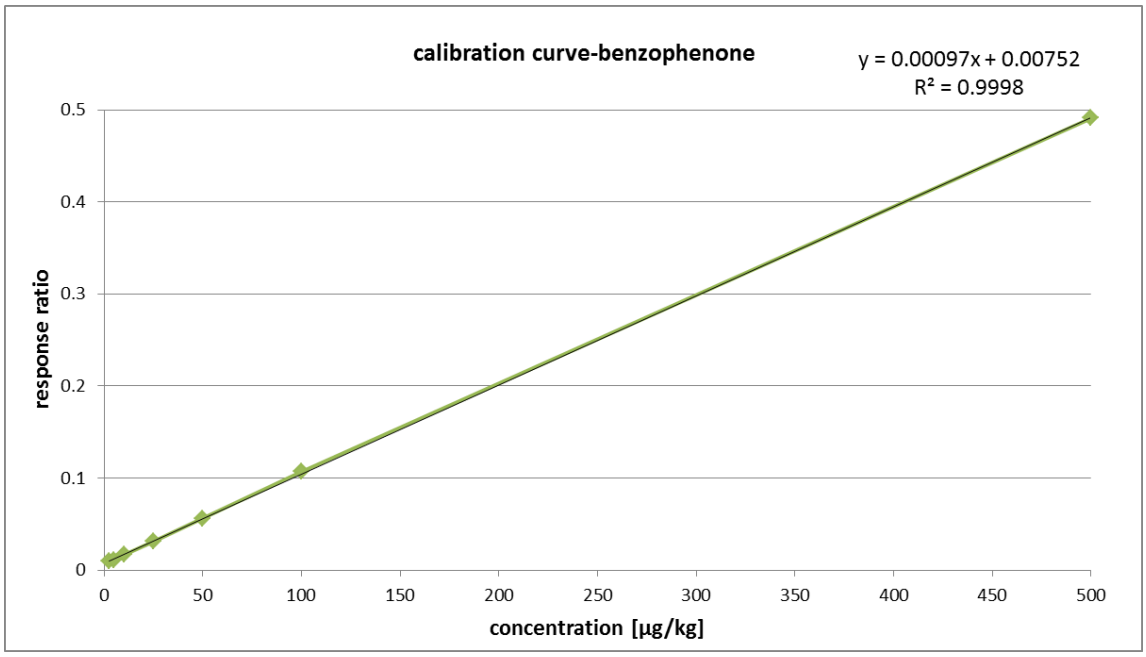

Figure 11: Calibration curve of benzophenone in cereal matrix

5.2.3. Recovery Rates and Precision

According to the SANCO guideline recoveries for all analytes included in a method should range between 70 and 120 % for all fortification levels with relative standard deviations ≤ 20 %. (European Commission DG SANCO 2009) For the determination of recovery and precision data blank samples were spiked at three concentration levels (10 µg/kg, 25 µg/kg and 100 µg/kg) and each was analysed six times. The obtained recovery and precision data are shown in Table 9 and Figure 12 for the cereal matrix and in Table 10 and Figure 13 for the fruit juice matrix.

Table 9: Recovery and precision data for cereal matrix

	10 µg/kg		25 µg/kg		100 µg/kg	
Analyte	RR[%][a]	RSD[%][b]	RR[%][a]	RSD[%][b]	RR[%][a]	RSD[%][b]
HMPP	91.3	11.7	97.3	7.8	81.7	14.3
EBF	94.4	9.8	90.7	12.1	83.3	13.6
BP	153.0	14.1	132.7	5.7	98.4	4.3
HCPK	129.8	5.9	122.6	4.0	107.3	2.4
EDMAB	106.1	8.3	105.3	2.1	101.7	3.6
MBP	105.5	5.3	105.3	3.9	99.3	2.0
DMPA	118.8	5.4	112.8	2.7	98.5	5.2
HHEMPP	103.1	6.9	104.2	3.5	98.3	6.2
MBB	104.2	2.6	112.1	2.8	101.0	1.8
HBP	*	*	104.7	1.7	95.4	6.2
HMBP	102.4	6.3	103.2	5.1	95.3	4.8
EA	94.1	2.4	95.4	2.9	91.0	4.8
DMAB	98.6	5.3	102.4	3.2	101.0	4.7
EHDAB	85.1	3.9	88.1	2.6	82.3	6.6
MMTPMP	*	*	*	*	100.3	5.0
4-ITX	87.4	3.8	91.6	2.4	80.6	5.7
2-ITX	87.7	2.9	91.6	2.0	80.8	6.3
PBZ	94.0	3.8	97.4	2.6	90.2	5.9
DETX	*	*	81.4	4.2	76.1	5.0
HOBP	62.3	4.1	66.4	4.2	56.4	12.1
MK	102.4	3.5	95.8	1.4	90.8	5.0
DEAB	90.2	1.6	94.6	2.9	94.3	3.0

* spiking level below the LOQ

[a] recovery rate

[b] relative standard deviation (n=6)

For the cereal matrix the recovery rates were within the range of 70-120 % for all analytes for all levels with the exception of BP, HCPK and HOBP. HOBP might experience some losses during sample preparation as the recoveries were below 66 % at all concentrations. This compound might partially bind to the C18 silica due to its long non-polar octyl chain. All relative standard deviations were smaller than 15 %, thus indicating a high precision of the method.

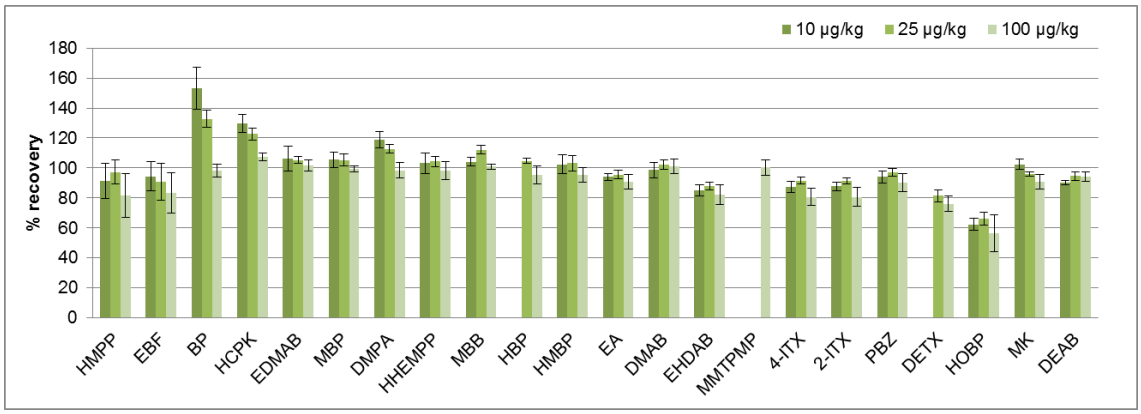

Figure 12: Average recovery rates in cereal matrix
Error bars denote standard deviations

For the fruit juice matrix the recovery rates were within the range of 70- 120 % for all analytes for all levels, with the exceptions of MMTPMP and EBF. EBF might experience some losses during sample preparation, as its recoveries were below 70 %. All relative standard deviations were below 10 %, thus indicating an excellent precision.

A comparison of the recoveries obtained for the two matrices shows that more accurate and precise results were obtained for the fruit juice matrix. This may be explained by the more complex nature of the cereal matrix compared to the fruit juice matrix. Those recoveries that deviated significantly from 100 % might be explained by the fact that the internal standards did not fit perfectly for all analytes concerning their chemical structure.

Table 10: Recovery and precision data for fruit juice matrix

	10 µg/kg		25 µg/kg		100 µg/kg	
Analyte	RR[%][a]	RSD[%][b]	RR[%][a]	RSD[%][b]	RR[%][a]	RSD[%][b]
HMPP	83.2	3.7	98.7	6.2	114.0	5.4
EBF	86.4	2.6	77.9	3.3	67.9	3.5
BP	98.0	3.0	97.4	2.9	96.7	1.1
HCPK	74.7	5.5	93.8	8.1	108.1	4.1
EDMAB	94.6	5.5	100.4	5.0	100.2	2.5
MBP	105.0	2.1	100.3	5.6	95.6	3.1
DMPA	101.1	2.6	114.5	3.1	112.0	2.2
HHEMPP	103.3	1.8	102.8	4.1	113.3	1.4
MBB	104.9	1.8	111.9	4.4	108.0	2.2
HBP	101.1	2.1	100.4	5.0	98.0	5.1
HMBP	97.8	3.3	102.2	1.9	105.6	1.7
EA	105.7	1.9	101.0	4.5	100.8	3.3
DMAB	113.2	0.9	110.2	3.2	108.1	1.7
EHDAB	99.6	3.1	101.0	6.6	94.3	2.4
MMTPMP	116.7	2.1	122.9	3.4	118.5	1.9
4-ITX	103.5	2.6	102.0	6.3	95.6	2.1
2-ITX	103.3	2.4	101.9	6.0	95.1	2.0
PBZ	113.6	2.0	112.0	3.9	107.1	1.1
DETX	99.2	3.0	97.1	7.6	91.3	2.7
HOBP	86.7	4.6	79.9	6.4	76.4	3.2
MK	106.4	3.1	92.4	5.6	102.2	3.3
DEAB	104.9	1.4	99.0	6.4	105.7	2.1

[a] recovery rate
[b] relative standard deviation (n=6)

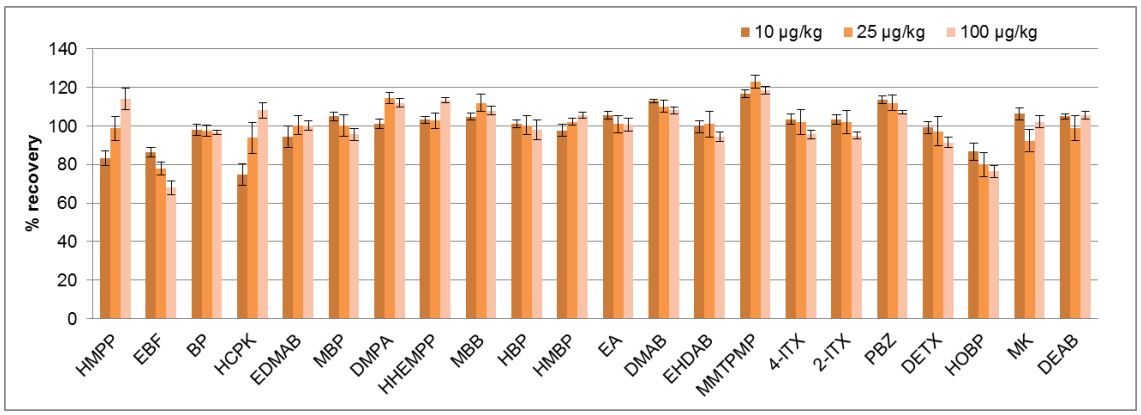

Figure 13: Average recovery rates in fruit juice matrix
Error bars denote standard deviations

5.3. METHOD CHARACTERISTICS FOR PACKAGING MATERIAL SAMPLES

The main focus of the analyses of the packaging materials of the cereal and fruit juice samples was placed on obtaining qualitative information. Due to this aim and because spiking of packaging material would not have been easily achievable, the method for analysing packaging materials was not validated. However, in the course of the solvent standards calibration LODs and LOQs as well as linearity were obtained.

5.3.1. Limits of Detection and Limits of Quantification

As described in section 5.2.1, the LOD was determined by a S/N ratio of 3:1, whereas for the LOQ a S/N ratio of 10:1 was used. The obtained LODs and LOQs are shown in Table 11.

Table 11: Limits of Detection and Quantification for the analytes in solvent standards

	solvent		
Analyte	***Ion*** [a]	**LOD ($\mu g/dm^2$)**	**LOQ ($\mu g/dm^2$)**
HMPP	*77*	0.04	0.13
EBF	*106*	0.04	0.14
BP	*152*	0.16	0.55
HCPK	*77*	0.02	0.07
EDMAB	*164*	0.02	0.05
MBP	*181*	0.05	0.16
DMPA	*225*	0.03	0.11
HHEMPP	*121*	0.02	0.06
MBB	*209*	0.02	0.06
HBP	*105*	0.08	0.28
HMBP	*151*	0.007	0.02
EA	*193*	0.03	0.10
DMAB	*226*	0.02	0.06
EHDAB	*164*	0.005	0.02
MMTPMP	*151*	0.08	0.26
4-ITX	*184*	0.014	0.05
2-ITX	*184*	0.014	0.05
PBZ	*152*	0.008	0.03
DETX	*239*	0.04	0.13
HOBP	*137*	0.02	0.08
MK	*224*	0.13	0.42
DEAB	*265*	0.20	0.66

[a] m/z of ion used to calculate LOD and LOQ

5.3.2. Linearity

The calibration curves for the solvent standards were obtained by linear regression using 1/x weighting. Calibration was performed using 7 levels ranging from 12.5 to 2500 µg/L. For BP, MK and DEAB it was not possible to obtain a 7 point calibration, as their LOD were above the lowest calibration level. A 6 point calibration was performed for these 3 analytes.

As shown in Table 12, the regression coefficients of the calibration curves used for quantification were ≥0.999 for all analytes with the exception of MK, indicating excellent linearity.

Table 12: Regression coefficients for solvent standards

Analyte	regression coefficient
HMPP	0.9997
EBF	0.9999
BP	0.9999
HCPK	0.9999
EDMAB	0.9999
MBP	0.9999
DMPA	0.9999
HHEMPP	0.9999
MBB	1.0000
HBP	0.9999
HMBP	0.9999
EA	0.9999
DMAB	0.9999
EHDAB	0.9999
MMTPMP	0.9999
4-ITX	0.9999
2-ITX	0.9999
PBZ	0.9999
DETX	0.9999
HOBP	0.9999
MK	0.9898
DEAB	0.9997

5.4. Analysis of Food and respective Packaging Materials

A total of 45 foodstuff samples and their respective packaging materials were analysed in the context of this master thesis. 21 cereal samples packaged in printed cardboard boxes with inner plastic bags or just a paper bag, and 24 fruit juice samples packaged in beverage cartons were analysed by the developed GC-MS multimethod.

5.4.1. Analysis of Cereal Samples and their respective Packaging Materials

The results of the analysis of the 21 cereal samples and their respective packaging materials are summarised in Table 13 and Figure 14. BP was found to be present in every cereal sample and its cardboard box packaging. The concentrations of BP in the foodstuff ranged from 25- 50 µg/kg product and were therefore all below the SML of 0.6 mg/kg. In 8 cereal samples at least one other photoinitiator was also found, though all findings were below 10 µg/kg or below the LOQ, respectively.

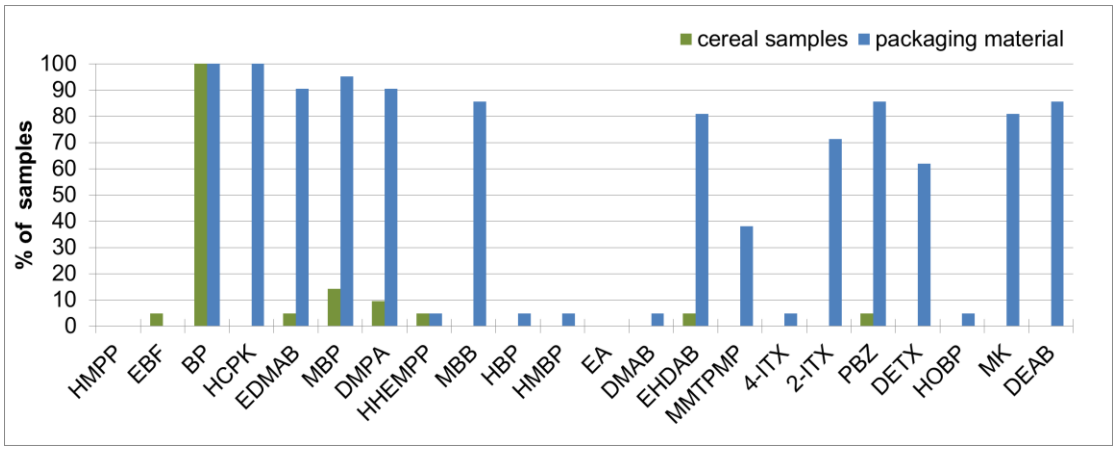

Figure 14: Percentages of cereal samples and their respective packaging materials containing the photoinitiator analytes

Besides BP the photoinitiators PBZ, DMPA, HHEMPP and EBF were determined in concentrations above the respective LOQ. Additionally the analytes MBP, EHDAB and EDMAB were detected (<LOQ).

With regards to the food scare in 2009, it was interesting that MBP was found in three samples in detectable amounts. In the respective packaging materials this photoinitiator was also present.

Interestingly, the photoinitiators found in addition to BP in the cereal samples C17, C18 and C21 were not detected in their respective packaging materials (see Table 13). The found analytes EBF, DMPA and HHEMPP have no other known source of contamination besides of UV-cured printing inks. However, a migration from a secondary packaging material into the foodstuff could be a possible source. Jickells et al. showed that a migration of BP and DMPA can take place from secondary packaging material into foodstuffs by transfer via the gas phase. (Jickells et al. 2005) They showed that paper is a poor barrier against the transfer of substances volatile enough to enter the gas phase.

The packaging of all cereal samples, with the exception of C21, which was only packed in a paper bag, consisted of an inner plastic bag in addition to the cardboard box. However, this additional packaging component could obviously not prevent the migration of the photoinitiators into the foodstuffs. This finding is in good agreement with the literature. The possible contamination through a functional barrier, such as plastic bags, was described for example also by Pastorelli and her coworkers. (Pastorelli et al. 2007) They reported the migration of BP from printed paperboard packages into cakes through different plastic films.

Figure 15 and Figure 16 show chromatograms of the cereal sample C07 and its packaging material. For each analyte found in the sample one characteristic ion was extracted and is shown.

Table 13: Photoinitiators found in cereal samples and the respective packaging materials

sample number	Analyte		Analyte		Analyte		Analytes present in packaging material, *in descending concentrations*[a]
	abbrev.	conc. [µg/kg]	abbrev.	conc. [µg/kg]	abbrev.	conc. [µg/kg]	
C01	BP	27.8					**BP**, MK, MBP, DEAB, HCPK, PBZ, MBB, EDMAB, DMPA, DETX, EHDAB, *MMTPMP*
C02	BP	51.2					**BP**, MK, MBP, DEAB, PBZ, HCPK, MBB, DMPA, EDMAB, EHDAB
C03	BP	29.7	MBP	<LOQ			**BP**, **MBP**, MBB, MK, HCPK, DMPA, DEAB, PBZ, EDMAB, EHDAB, DMAB, HOBP
C04	BP	31.3					**BP**, MBB, MBP, MK, HCPK, DMPA, PBZ, DEAB, 2-ITX, EDMAB, EHDAB, DETX, *MMTPMP*
C05	BP	33.0					**BP**, MMTPMP, MBP, HCPK
C06	BP	29.2					**BP**, MMTPMP, HCPK, MBP, EDMAB, *DMPA*
C07	BP	48.4	PBZ	2.0	MBP, EHDAB	<LOQ	**BP**, **PBZ**, MBB, EDMAB, MK, **EHDAB**, **MBP**, HCPK, 2-ITX, DMPA, DETX, *DEAB*
C08	BP	36.8	DMPA	2.7			**BP**, PBZ, MK, MBB, HCPK, MBP, HCPK, MBP, EDMAB, **DMPA,** EHDAB, 2-ITX, *DEAB*
C09	BP	25.0					**BP**, MBB, MK, MBP, HCPK, 2-ITX, EDMAB, DETX, PBZ, DEAB, DMPA, EHDAB
C10	BP	36.5					**BP**, PBZ, MK, EHDAB, MBB, MBP, DMPA, EDMAB, HCPK, 2-ITX, HHEMPP, *DEAB*
C11	BP	34.7					**BP**, MK, MBP, MBB, PBZ, HCPK, 2-ITX, EDMAB, EHDAB, DMPA, DETX, *DEAB*
C12	BP	50.9	MBP	<LOQ			**BP**, **MBP**, PBZ, MK, MBB, DMPA, HCPK, EDMAB, 2-ITX, EHDAB, *DEAB*
C13	BP	35.5					**BP**, PBZ, EHDAB, MK, MBP DMPA, MBB, DETX, DEAB, HCPK, EDMAB, 2-ITX, HMBP
C14	BP	42.7					**BP**, MK, MBP, PBZ, MBB, DMPA, DEAB, 2-ITX, EDMAB, HCPK, EHDAB, DETX, *MMTPMP*
C15	BP	35.0					**BP**, MK, MBP, DMPA, MBB, HCPK, PBZ, EDMAB, 2-ITX, DETX, EHDAB, *DEAB*
C16	BP	46.3	EDMAB	<LOQ			**BP**, MK, MBP, DEAB, HCPK, 2-ITX, MBB, PBZ, **EDMAB**, DMPA, DETX, EHDAB
C17	BP	39.4	HHEMPP	8.1			**BP**, HCPK, MBP, PBZ, 2-ITX, MBB, EDMAB, DMPA, DEAB, DETX, EHDAB, MMTPMP
C18	BP	40.4	EBF	2.6			**BP**, MK, MBP, DEAB, PBZ, MBB, 2-ITX, EDMAB, DMPA, EHDAB, HCPK, DETX, *MMTPMP*
C19	BP	36.2					**BP**, MBP, MK, MBB, PBZ, HCPK, 2-ITX, EDMAB, DMPA, DETX, *DEAB*
C20	BP	28.5					**BP**, MK, PBZ, MBP, MBB, HCPK, EDMAB, DMPA, 2-ITX, HBP, EHDAB, MMTPMP, *DEAB*
C21	BP	48.3	DMPA	1.5			HCPK, ***BP***

[a]Analytes underlined and written in bold indicate compounds that were found in both the packaging material and the foodstuff. Analytes written in cursive were detected at a concentration < LOQ.

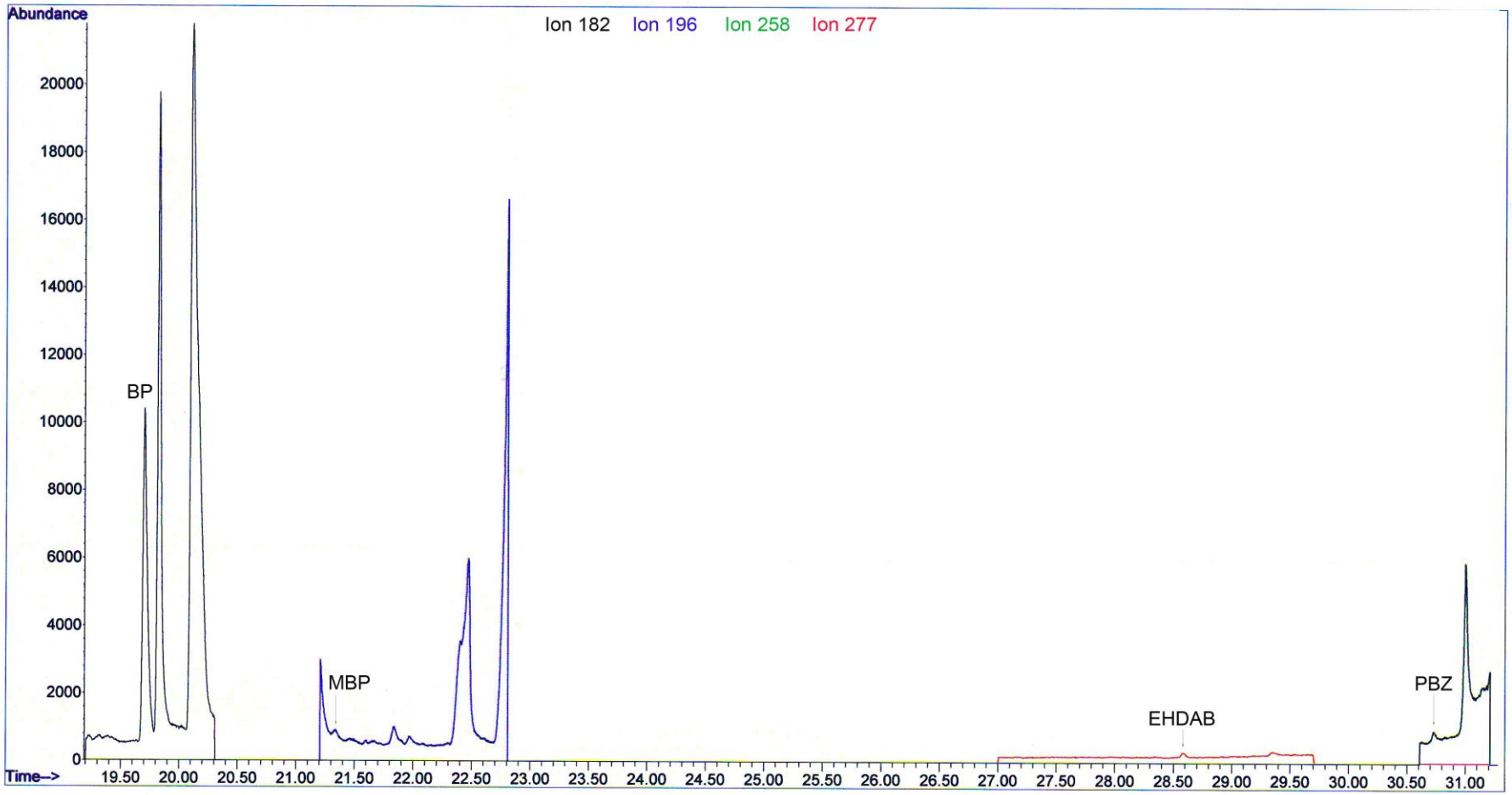

Figure 15: GC-MS chromatogram of cereal sample number C07

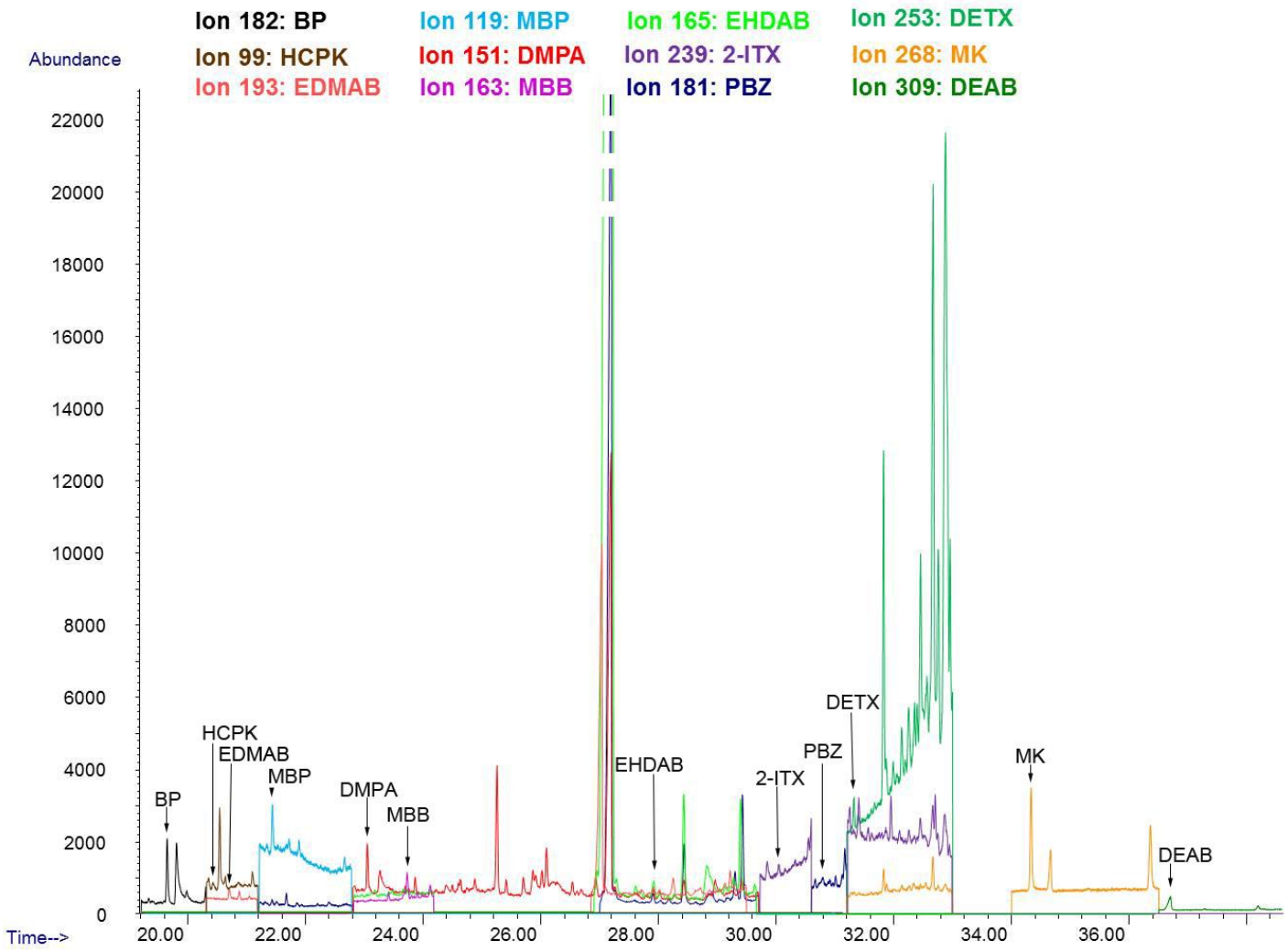

Figure 16: GC-MS chromatogram of the respective packaging material of cereal sample number C07

The quantitative results of the analyses of the packaging materials are shown in Table 14. On average in the cereal packaging materials 11 different photoinitiators were found. Thereby BP and HCPK were found in every packaging material sample. The concentrations of BP ranged from 0.7-50.8 µg/dm^2. EDMAB, MBP, DMPA, MBB, EHDAB, PBZ, MK and DEAB were found in ≥ 80 % of the packaging materials.

These results deviate from the findings of Koivikko et al. as they observed that BP and MBP were usually not present in the same packaging material sample. (Koivikko et al. 2010)

Table 14: Concentrations and Numbers of analytes found in cereal packaging materials

analyte [µg/dm^2]	sample number																				
	C01	C02	C03	C04	C05	C06	C07	C08	C09	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21
BP	6.0	10.0	19.8	16.9	0.7	0.9	12.9	49.1	10.4	17.0	5.2	26.6	50.8	9.7	8.5	11.9	12.0	9.1	6.8	6.0	<LOQ
HCPK	0.7	0.9	2.6	2.0	0.1	0.3	0.9	2.4	1.9	0.6	0.7	0.7	1.0	0.5	0.8	2.1	3.6	0.3	0.8	0.8	0.2
EDMAB	0.4	0.5	0.7	0.7		0.1	1.8	1.1	1.3	0.7	0.5	0.3	0.9	0.5	0.7	1.1	1.0	0.5	0.6	0.6	
MBP	1.8	2.4	18.2	4.1	0.2	0.3	1.3	2.3	2.8	1.0	1.2	5.1	3.2	1.6	2.3	4.2	3.1	2.0	2.4	1.2	
DMPA	0.3	0.5	2.5	1.3		<LOQ	0.6	1.1	0.7	0.9	0.3	0.8	3.2	1.0	1.7	0.8	0.8	0.4	0.5	0.5	
HHEMPP										0.2											
MBB	0.5	0.8	3.0	5.3			2.2	2.6	4.7	1.1	0.9	0.9	2.3	1.2	0.9	1.4	1.2	1.0	1.2	1.0	
HBP																				0.4	
HMBP													0.2								
DMAB																					
EHDAB	0.2	0.2	0.3	0.3			1.3	0.7	0.3	1.4	0.3	0.2	4.1	0.4	0.2	0.4	0.4	0.3		0.4	
MMTPMP	0.2			<LOQ	0.3	0.4								<LOQ			0.4	<LOQ		0.3	
2-ITX				0.8			0.9	0.4	1.8	0.3	0.7	0.3	0.8	0.6	0.6	1.6	1.2	0.6	0.8	0.5	
PBZ	<LOQ	1.1	1.0	1.2			6.5	4.5	0.9	4.1	0.8	3.2	10.6	1.3	0.7	1.2	1.9	1.1	0.8	1.5	
DETX	0.2			0.2			0.2		0.9		0.2		1.4	0.2	0.4	0.4	0.6	0.2	0.3	0.2	
HOBP			0.2																		
MK	2.1	2.9	2.9	2.5			1.7	3.1	3.0	1.9	1.8	2.7	3.4	2.4	2.4	4.3		2.5	1.7	2.0	
DEAB	1.6	1.4	1.6	0.9			<LOQ	<LOQ	0.8	<LOQ	<LOQ	<LOQ	1.4	0.9	<LOQ	2.1	0.7	1.3	0.4	<LOQ	
number of analytes	12	10	12	13	4	6	12	11	12	12	12	11	13	13	12	12	12	13	11	14	2

The frequent finding of MK is of special interest as several years ago the UK printing ink industry placed MK on a list of substances not recommended for the use for cardboard food packaging due to its potential cancerogenicity. (Castle 1997b)

As several factors influence the migration from photoinitiators into foodstuffs (see chapter 3.2.), no relationship between the concentrations of an analyte in the foodstuff and in the respective packaging material can be established.

5.4.2. Analysis of Fruit Juice Samples and their respective Packaging Materials

Various fruit juices including orange juice, orange nectar, red orange juice, grapefruit juice, apple juice, carrot juice and multivitaminic fruit juice were analysed. The results are shown in Table 15 and Figure 17. In 92% of the analysed fruit juice samples BP was found in a range from 7.9-28.5 µg/kg. In one sample BP was detected below the LOQ. In addition to BP of the other analytes only MBB was present in 3 samples.

As fruit juices contain only low amounts of fats, photoinitiators mainly contaminate the juice by being adsorbed to the fibres. (Sagratini et al. 2006) However, no correlation between the determined amounts of photoinitiators and the type of juice/fibre content was found.

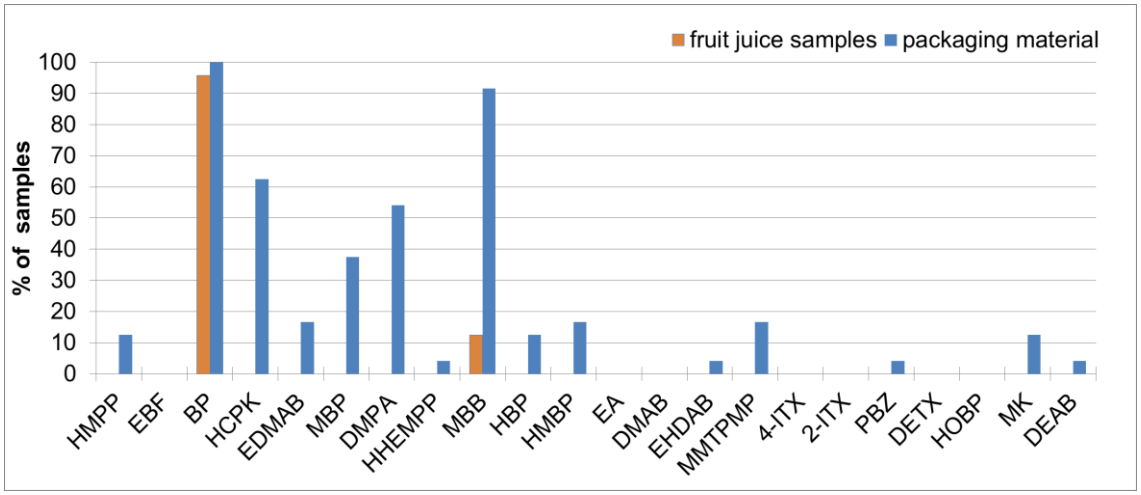

Figure 17: Percentages of fruit juice samples and their respective packaging materials containing the photoinitiator analytes

The findings of BP in nearly every fruit juice sample are in good agreement with the recently reported results of Gallart Ayala et al.. (Gallart Ayala et al. 2011a) Apart from BP they also reported only very low concentrations of other photoinitiators (PBZ, DEAB, 2-ITX, 4-ITX, DETX, EHDAB, DMPA and EDMAB)

Interestingly, the ITX isomers were not found in any of the fruit juice samples and also in none of the packaging materials. In several publications findings of ITX in fruit juice have been reported. (Rothenbacher et al. 2007, Bagnati et al. 2007) As these studies were published some years ago in the wake of the ITX

scare in 2005, one may speculate that this photoinitiator has been replaced by others in the meantime or that the issue of set-off is under control nowadays. However, other published studies also described only low findings of ITX and other photoinitiators in fruit juices. (Sanches-Silva et al. 2008d, Sagratini et al. 2008, Gallart Ayala et al. 2008) Sagratini and coworkers studied various fruit juices, where contents of ITX ranged from 0.05 to 0.78 µg/L. (Sagratini et al. 2006)

Figure 18 and Figure 19 show chromatograms of fruit juice sample F05 and its packaging material. For the fruit juice sample the quantifier ion and the two qualifier ions of BP were extracted and are shown. For each analyte found in the respective packaging material one characteristic ion was extracted and is shown.

Table 15: Photoinitiators found in fruit juice samples and the respective packaging materials

sample number	BP conc. [µg/kg]	MBB conc. [µg/kg]	Analytes present in packaging material, *in descending concentrations*[a]
F01	10.0		MBB, **BP**, HCPK, HMBP, DMPA, PBZ, *DEAB*
F02	7.9	4.5	**MBB**, **BP**, *HMPP*
F03	8.0		**BP**, MBB, HCPK, DMPA
F04	< LOD	2.19	BP, MK, **MBB**
F05	15.1		MK, **BP**, MBB, HMBP, DMPA, HCPK, EDMAB, *MMTPMP*
F06	15.6		**BP**, MBB, MBP, HCPK, DMPA, HMBP
F07	9.4		MK, MBB, **BP**, MBP, HHEMPP, HCPK, HMBP
F08	<LOQ		**BP**, MBP, MBB, DMPA, HCPK
F09	16.5		**BP**, MBB, HCPK
F10	17.0		**BP**, *MMTPMP*
F11	17.7		MBP, ***BP***, *DMPA* , *MBB*
F12	17.1		**BP**, MBB, DMPA
F13	18.6		**BP**, DMPA, HMPP, HCPK, *MMTPMP*
F14	28.5		**BP**, DMPA, MBB, EDMAB, HCPK,
F15	27.3		**BP**, MBP, MBB, HCPK, DMPA
F16	23.3		**BP**, MBP, MBB, HCPK, DMPA, *MMTPMP*
F17	20.2		**BP**, HCPK, MBB, DMPA
F18	16.8		MBB, **BP**, MBP, HCPK
F19	21.3		**BP**, MBB, EHDAB
F20	15.7		**BP**, MBB, MBP, HCPK
F21	14.0		**BP**, MBB, DMPA
F22	16.7		HBP, **BP**, MBB, EDMAB, HMPP
F23	13.5	0.65	HBP, **MBB**, **BP**, MBP, HCPK, EDMAB
F24	23.5		HBP, MBB, **BP**

[a]Analytes underlined and written in bold indicate compounds that were found in both the packaging material and the foodstuff. Analytes written in cursive were detected at a concentration <LOQ.

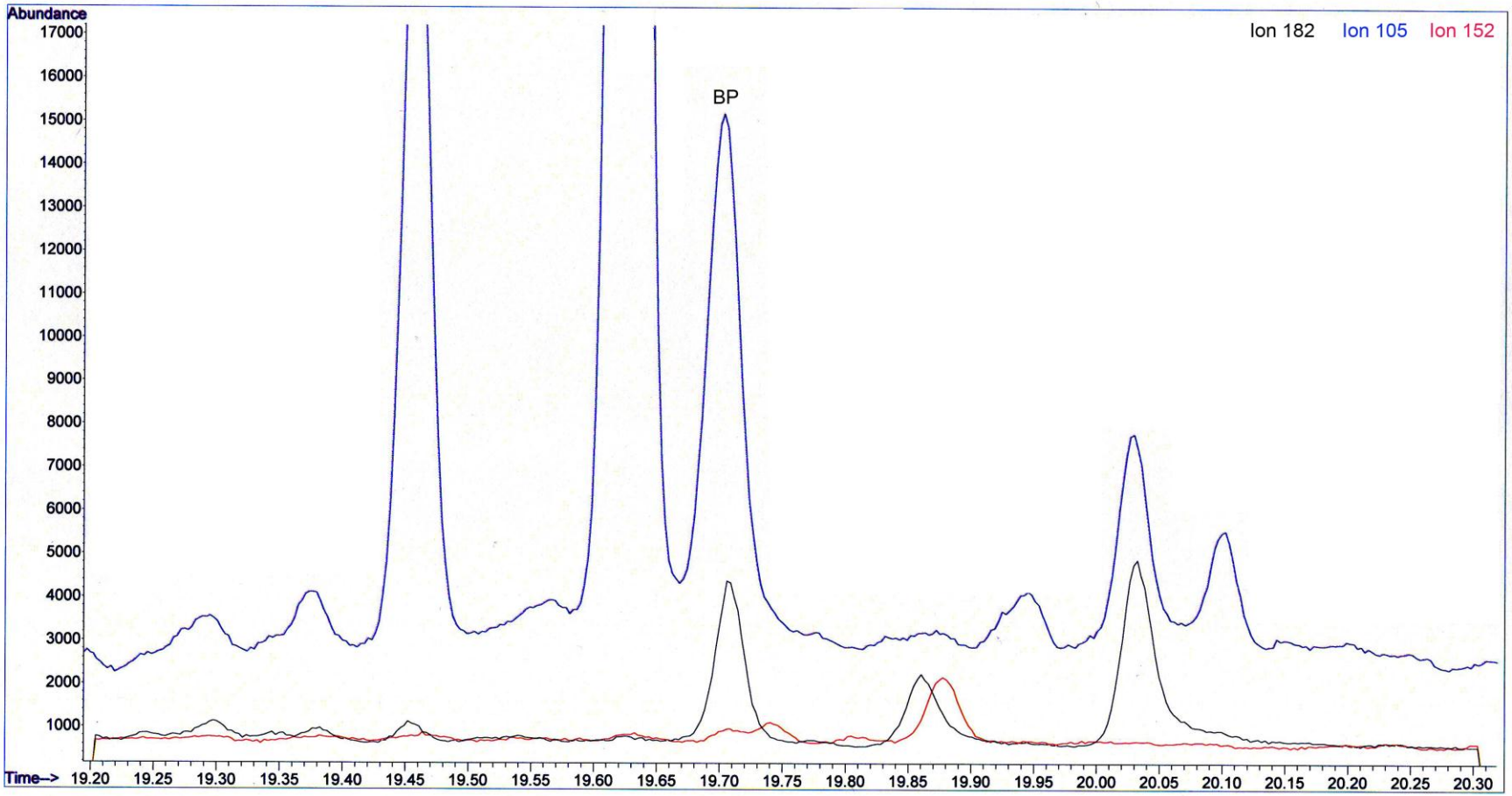

Figure 18: GC-MS chromatogram of fruit juice sample number F05

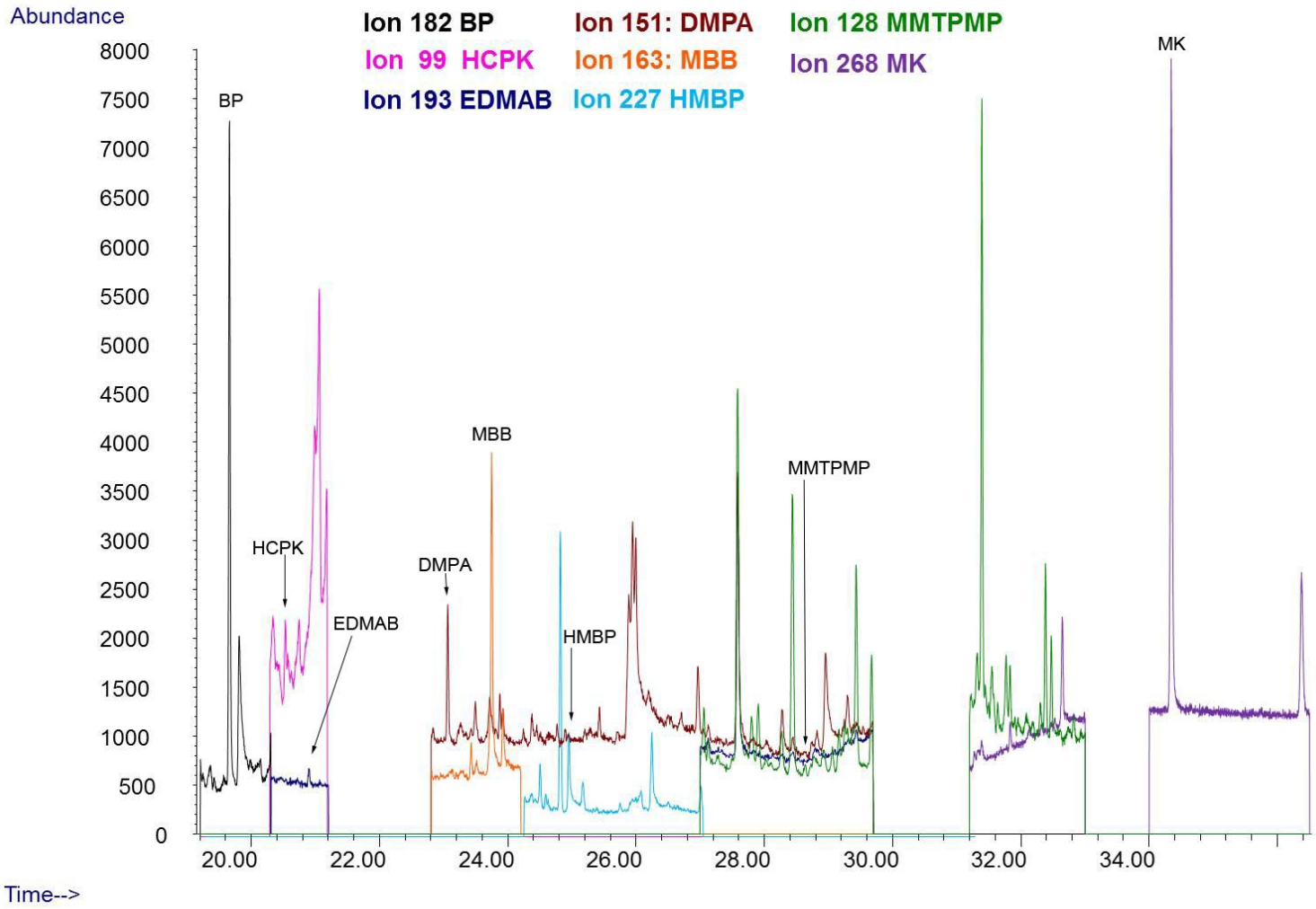

Figure 19: GC-MS chromatogram of the respective packaging material of fruit juice sample number F05

In all cases where BP and MBB were found in the foodstuff, these analytes were present in the packaging material as well.

Table 16 shows the concentrations and numbers of analytes present in the fruit juice packaging materials. An average of five different photoinitiators was found. BP was determined in all samples and concentrations ranged from 0.6-3.8 µg/dm^2. Apart from BP MBB was also present in more than 90 % of the packaging materials. HCPK, MBP and DMPA were also frequently found.

Table 16: Concentrations and Numbers of analytes found in fruit juice packaging material

analyte [µg/dm^2]	sample number																							
	F01	F02	F03	F04	F05	F06	F07	F08	F09	F10	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20	F21	F22	F23	F24
HMPP		<LOQ											0.2									0.2		
BP	0.8	1.6	3.8	2.9	2.8	2.6	1.8	2.6	2.9	1.1	<LOQ	0.9	1.5	1.1	1.5	2.1	1.4	1.0	2.0	1.9	0.9	0.9	0.6	0.9
HCPK	0.2		0.2		0.1	0.2	0.2	0.2	0.2				0.2	0.2	0.4	0.3	0.3	0.2		0.2			0.2	
EDMAB					0.1									0.3								0.4	0.1	
MBP						0.3	0.5	0.7			0.19				0.9	0.49		0.3		0.4			0.4	
DMPA	0.1		0.1		0.2	0.2		0.2			<LOQ	0.2	0.3	0.6	0.2	0.2	0.2				0.2			
HHEMPP							0.3																	
MBB	2.3	4.8	1.4	2.7	0.7	0.5	2.5	0.6	0.3		<LOQ	0.3		0.3	0.9	0.4	0.2	2.1	0.6	0.6	0.4	0.5	1.5	1.1
HBP																						10	6.5	4.4
HMBP	0.2				0.2	0.1	0.1																	
EHDAB																			0.1					
MMTPMP					<LOQ					<LOQ			<LOQ			<LOQ								
PBZ	0.1																							
MK				2.8	4.2		2.8																	
DEAB	<LOQ																							
number of analytes	7	3	4	3	8	6	7	5	3	2	4	3	5	5	5	6	4	4	3	4	3	5	6	3

6. CONCLUSIONS

A GC-MS multimethod was successfully developed for the determination of 22 photoinitiators in two different types of foodstuff (cereals and fruit juices) and their respective packaging material. Sample preparation was performed by the QuEChERS method, which is based on an acetonitrile extraction and a subsequent clean-up step by dispersive solid-phase extraction. Validation results showed that the developed multimethod was highly selective and sensitive.

The analysis of different cereal and fruit juice samples showed the presence of BP in 43 out of 45 samples. All results were below the specific migration level of 0.6 mg/kg product. Other photoinitiators (PBZ, DMPA, HHEMPP, EBF, EHDAB, EDMAB and MBB) were found in only a few samples at low concentrations, all below 10 µg/kg. In contrast, numerous photoinitiators were found in the packaging materials.

As the migration of substances from packaging materials is influenced by various factors, it was not possible to link the analytical findings for the foodstuffs and the packaging materials.

Despite the presence of barriers between the printed packaging surface and the foodstuff (multilayer beverage carton and inner plastic bag, respectively) migration still took place and photoinitiators contaminated the samples.

Although in none of the analysed samples concentrations above the SML were found, the presence of photoinitiators is not desirable and further monitoring of foodstuffs on the market seems to be necessary. Moreover further investigations with regards to migration and packaging (technology) should be worthwhile to tackle this issue and reduce contamination of foodstuffs with photoinitiators in the future.

7. SUMMARY

Photoinitiators are constituents of UV-cured printing inks, which are widely used in the printing of food packaging materials. The photoinitiators can migrate from the packaging material into the foodstuff, leading to an undesirable contamination.

The aim of this master thesis was the development of an analytical method for the simultaneous determination of a wide range of photoinitiators in foodstuffs and their respective packaging materials. A total of 22 photoinitiators (various substituted benzophenones, xanthones, benzoates, etc.) were included in this multimethod. The developed method comprised a sample preparation procedure consisting of an acetonitrile extraction and a subsequent clean-up step by dispersive solid-phase extraction followed by a measurement using gas chromatography-mass spectrometry. The method was successfully validated for cereal and fruit juice matrix and provided limits of quantification below 10 µg/kg food for almost all analytes in both matrices.

The method was employed for the analysis of various cereal products and fruit juices from the Austrian market as well as their respective packaging materials. The analysis of the different cereal samples showed the presence of benzophenone in all analysed samples and their respective packaging materials. Benzophenone was also found in most fruit juice samples. All findings of benzophenone were below the specific migration level of 0.6 mg/kg product. Although the packaging materials contained numerous different photoinitiators, other photoinitiators apart from benzophenone were found in only a few food samples at very low concentrations. It was observed that the number as well as the concentrations of photoinitiators present in the cereal samples and their respective packaging materials was higher than in the fruit juice samples and the beverage cartons.

8. ZUSAMMENFASSUNG

Photoinitiatoren sind Bestandteile von UV-getrockneten Druckertinten, welche oft beim Bedrucken von Lebensmittelverpackungsmaterialien zum Einsatz kommen. Photoinitiatoren können aus Verpackungsmaterialien in das Lebensmittel migrieren, wodurch es zu einer unerwünschten Kontamination kommt.

Das Ziel dieser Masterarbeit war die Entwicklung einer analytischen Methode für die simultane Bestimmung mehrerer Photoinitiatoren in Lebensmitteln und deren Verpackungsmaterialien. Diese Multimethode umfasste insgesamt 22 Photoinitiatoren (unterschiedlich substituierte Benzophenone, Xanthone, Benzoate, etc). Die entwickelte Methode umfasste eine Probenaufarbeitung bestehend aus einer Extraktion mit Acetonitril und anschließender Aufreinigung mittels dispersiver Festphasenextraktion gefolgt von einer Vermessung mittels Gaschromatographie-Massenspektrometrie. Die Methode wurde erfolgreich für Cerealien- und Fruchtsaftmatrix validiert. Für beide Matrices lagen die Bestimmungsgrenzen in den meisten Fällen unter 10 µg/kg Produkt.
Mit Hilfe der entwickelten Multimethode wurden unterschiedliche Cerealien und Fruchtsäfte, sowie deren Verpackungsmaterialen, aus österreichischen Supermärkten untersucht. Benzophenon wurde in allen Cerealienproben und deren Verpackungsmaterialien sowie in fast allen Fruchtsaftproben gefunden. In allen Lebensmitteln lagen die Werte unterhalb des spezifischen Migrationslevels von 0.6 mg/kg Lebensmittel. Obwohl in den Verpackungsmaterialien zahlreiche weitere Photoinitiatoren gefunden wurden, wurden in den Lebensmitteln selbst, nur wenige andere Photoinitiatoren in geringen Konzentrationen gefunden. Es wurde beobachtet, dass sowohl die Anzahl als auch die Konzentrationen der Photoinitiatoren in Cerealienproben und deren Verpackungsmaterialien höher waren als in den Fruchtsaftproben und deren Getränkekartons.

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10. PUBLICATIONS

A poster entitled “GC-MS multimethod for the analysis of photoinitiators migrating from packaging material into foodstuff” was presented at the 5th International Symposium on Recent Advances in Food Analysis in Prague, in November 2011.

Abstract:

GC-MS MULTIMETHOD FOR THE ANALYSIS OF PHOTOINITIATORS MIGRATING FROM PACKAGING MATERIALS INTO FOODSTUFF

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Competence Centre for Residue Analysis, Austrian Agency for Health and Food Safety, Spargelfeldstrasse 191, 1220 Vienna, Austria

Photoinitiators are used in UV-cured printing inks to start a polymerisation process that leads to the formation of a macromolecular network with embedded colour particles. Due to their technological advantages UV-cured inks are also widely used in the printing process of food packaging materials. Subsequently, the photoinitiators can migrate from the packaging material into the enclosed foodstuff. A wide variety of chemical compounds may be used as photoinitiators; however, so far only a limited number of substances (mostly ITX and benzophenones) have been targeted in analytical methods reported for determining photoinitiators in foodstuffs. We have developed a GC-MS multimethod that allows the screening for more than twenty different compounds used as photoinitiators (various substituted benzophenones, thioxanthones, benzoates, etc.). The various steps of method optimisation will be discussed and the characteristics of the final method will be described. The developed GC-MS method allows the sensitive and selective analysis of photoinitiators in both foodstuffs as well as packaging materials. Limits of quantification are mostly at or below 10 µg/kg food. The photoinitiator

multimethod was employed for the screening of various cereal products and beverages as well as their respective packagings for the presence of photoinitiators. The observed substances and their concentrations in the foodstuffs and packaging materials will be presented.

11. CURRICULUM VITAE

Teresa Mairinger

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Personal Information

- date of birth: 09-26-1988
- nationality: Austrian

Education

February 2011 - date **Master thesis in Nutritional Sciences**

- "Development of a multimethod for the analysis of photoinitiators migrating from packaging materials into foodstuff"

Competence Centre for Residue Analysis, Austrian Agency for Health and Food Safety, Vienna

2009 - date **Master degree programmes**

- master programme Nutritional Sciences, with focus on Molecular Nutrition
- master programme Chemistry

University of Vienna, Austria

2006 - 2009 **Bachelor degree programme**

- Nutritional Sciences
- bachelor thesis "Flavonoide in der Ernährung- Sekundäre Pflanzeninhaltsstoffe in der Ernährung am Beispiel der Flavonoide" supervised by Prof. Dr. Robert Ebermann, Department of Nutritional Sciences, University of Vienna

University of Vienna, Austria

1998 - 2006 **"Gymnasium"**

- specialized subjects for general qualification for university entrance: English, French, History
- final exam passed with distinction

AHS GRG Hagenmüllergasse, Vienna, Austria

Work Experience

November 2010 **Internship**

- cell culture, production of cell lysates, Western Blot and Immunodetection

work group of Prof. Dr. Hildegard Laggner, Department of Medical Chemistry, Medical University of Vienna, Austria

July 2010 **Internship**

- determination of food contaminants (GC-MS, HPLC-MS, LC-MS/MS)

Competence Centre for Residue Analysis, Austrian Agency for Health and Food Safety, Vienna

February 2010 **Internship**

- immunoassays of human blood samples (ELISA)

Department of Nutritional Sciences, University of Vienna, Austria

March - June 2009 **voluntary collaboration**

- taxidermy of guinea pig, intracellular microelectrode technique

work group of Prof. Dr. Christian Studenik, Department of Pharmacology and Toxicology, University of Vienna, Austria

July 2008 **Internship**

- microbiological quality control in food production, water analysis, food routine analysis, product development

Mautner Markhof Feinkost GmbH, Austria

Personal Skills

Languages:	German, mother tongue
	English, very good spoken and written
	French, good spoken and written
Computer skills:	very good command: Microsoft Office™, MSD Chemstation
	experience with: SPSS 12.0, Derive 6.0,
	programming language PERL

Vienna 3rd January 2012