# SPME – GC - MS analysis of selected photoinitiators in paper and board food packaging materials

**Skef, Lara**

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Lara Skef

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# SPME – GC - MS ANALYSIS OF SELECTED PHOTOINITIATORS IN PAPER AND BOARD FOOD PACKAGING MATERIALS

The work was done under mentorship of Mario Ščetar, PhD, Associate professor (Faculty of Food Technology and Biotechnology, University of Zagreb). The experimental part is done at the Institute of Analytical Chemistry and Food Chemistry at Graz University of Technology under the guidance of Erich Leitner, PhD, Full professor and with the assistance of Lisa Hoffellner, PhD.

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# SPME – GC - MS ANALYSIS OF SELECTED PHOTOINITIATORS IN PAPER AND BOARD FOOD PACKAGING MATERIALS

*Lara Skef*, univ. bacc. ing. techn. aliment.,
*058211286*

**Abstract:** Most food products are packaged. Paper is mainly used for this purpose, especially due to the increased awareness of the plastics impact on the environment. Due to its good printability, it is usually printed with UV inks. These contain photoinitiators, which have a low molecular weight and can be prone to migration. However, in order to comply with the framework regulation (EC) 1935/2004, printing inks must not release substances to the packaged food in quantities that could lead to undesirable changes. Therefore, this work focused on the development of a highly sensitive, selective and accurate SPME-GC-MS method for the determination of selected photoinitiators in paper and board food packaging materials. An internal standard method was used for quantification, so method is optimised to be less labour intensive and require little sample preparation time. Selected packaging materials from supermarkets were analysed and photoinitiators were found in some of them.

**Keywords:** *photoinitiators, UV-inks, paper food packaging materials, board food packaging materials, SPME-GC-MS method*
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**Mentor:** Mario Ščetar, PhD, Associate professor
**Co-mentor:** Erich Leitner, PhD, Full professor, University of Technology Graz, Austria
**Technical support and assistance**: Lisa Hoffellner, PhD

**Reviewers:**
1. Mia Kurek, PhD, Associate professor (president)
2. Mario Ščetar, PhD, Associate professor (mentor)
3. Antonela Ninčević Grassino, PhD, Associate professor (member)
4. Marina Krpan, PhD, Associate professor (substitute)

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# SPME-GC-MS ANALIZA ODABRANIH FOTOINICIJATORA U PAPIRNATIM I KARTONSKIM MATERIJALIMA ZA PAKIRANJE HRANE

*Lara Skef*, univ. bacc. ing. techn. aliment.,
*058211286*

**Sažetak:** Većina prehrambenih proizvoda je pakirana. U tu se svrhu uglavnom koristi papir, posebice zbog povećane svijesti o utjecaju plastike na okoliš. Zbog dobre mogućnosti ispisa većina ih se tiska UV-tintama. One sadrže fotoinicijatore koji su niske molekularne mase te su skloni migraciji. Kako bi bile usklađene s Uredbom (EZ) 1935/2004, tiskarske tinte ne smiju ispuštati tvari u pakiranu hranu u količinama koje bi mogle dovesti do neželjenih promjena. Stoga je cilj ovog rada razvoj vrlo osjetljive, selektivne i točne SPME-GC-MS metode za određivanje odabranih fotoinicijatora u papirnim i kartonskim materijalima za pakiranje hrane. Za kvantifikaciju je korištena interna standardna metoda, tako da je metoda optimizirana da bude manje radno intenzivna i zahtijeva malo vremena za pripremu uzorka. Analizirani su odabrani materijali za pakiranje iz supermarketa te su u nekima od njih pronađeni fotoinicijatori.

**Ključne riječi:** *fotoinicijatori, UV-tinte, papirnati pakirni materijal, kartonski pakirni materijal, SPME-GC-MS metoda*
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**Mentor:** izv. prof. dr. sc. Mario Ščetar
**Komentor:** prof. dr. sc. Erich Leitner, Tehnološki fakultet Graz, Austrija
**Pomoć pri izradi**: dr. sc. Lisa Hoffellner

**Stručno povjerenstvo za ocjenu i obranu:**
1. izv. prof. dr. sc. Mia Kurek (predsjednik)
2. izv. prof. dr. sc. Mario Ščetar (mentor)
3. izv. prof. dr. sc. Antonela Ninčević Grassino (član)
4. izv. prof. dr. sc. Marina Krpan (zamjena)

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# 1. INTRODUCTION

Any material that is intended to be used as a food contact material (FCM) needs to fulfil certain legal requirements. According to the regulation (EC) 1935/2004, FCMs must be sufficiently inert to preclude substances from being transferred to food in quantities large enough to endanger human health, to bring an unacceptable change in the composition of the food and a deterioration of the organoleptic properties under normal and foreseeable conditions of use (European Parliament and European Council, 2004). With the production and design of new products i.e., food packaging materials, the safety of those products needs to be evaluated. Therefore, analytical methods based on different sample preparation techniques combined with chromatographic methods have to be developed.

Besides the main function of food packaging which is to protect the content from loss or damage, it also has the function to promote the product and can serve as a place for information storage (Himanshu and Goswami, 2019). As paper and board materials feature good printability, it is not surprising that most of the packaging is nowadays printed (Robertson, 2019). For that purpose, printing inks are employed, mostly UV-curing printing inks. The legislation on printing inks is diverse and, until today, no European-specific community legislation concerning printing inks for food packaging exists. However, the framework regulation (EC) 1935/2004 is also applicable to inks. Following that fact, inks should also not release substances to the packed food in quantities that could result in unwanted changes (European Parliament and European Council, 2004). In UV-inks, the focus is on photoinitiators that are usually of low-molecular weight and might tend to migrate, either through the packaging or via reverse-side migration (set-off). Therefore, the goal of this work was to develop a screening method for the targeted and untargeted analysis of the inks with the focus on photoinitiators, like benzophenone derivatives, thioxanthone derivatives, and aminobenzoate derivatives.

# 2. FOOD PACKAGING MATERIALS

Nowadays, packaging has become an indispensable part of the food manufacturing process. By far the most important functions of food packaging are preserving, protecting and distributing food to customers in a safe form without risk to human health (Raheem, 2013). Another important contribution is its communication function which, in addition to the appearance of encouraging the customer to purchase, serves as a place of information storage. It includes the submission of all necessary regulatory information, such as providing the identity of the product, the net quantity of the content, name/address of the manufacturer, packer, or distributor (Shin and Selke, 2014). It also contains information about special handling or storage instructions, together with instructions for opening and using the content (Marsh and Bugusu, 2007). The communication function is not limited only to textual information but also includes elements such as packaging shape, colour and symbols as it plays the important role of a silent seller (Shin and Selke, 2014).

As already mentioned, food packaging provides protection of food from external environmental factors and potential physical, chemical and microbiological hazards which plays an important role in predicted food shelf-life (Avinash and Mittal, 2019). Therefore, the process of selecting an optimal packaging material plays a significant role in fulfilling its functions. The major factors that should be taken into consideration during selection are costs, quality of the product and the capability of preserving product freshness, quality and safety.

Commonly used materials in food industries employed as food packaging are plastics, paper, glass and metal (Alamri et al., 2021). Among them, paper can be highlighted as the most used, especially because of the increasing pressure on raising awareness of the effects plastic materials have on the environment (Avinash and Mittal, 2019).

## 2.1. PAPER AND BOARD MATERIAL

Paper is by far the oldest discovered material. The history of paper material starts with the ancient Egyptians who used reedy plant papyrus to produce the world’s first writing material. Paper can be defined as sheet material made from an interlaced network of cellulose plant fibres. About 97 % of plant fibres are derived from wood, however, remaining sources such as wheat, rye, barley or rice straws, sugar cane bagasse, flax, corn husks, bamboo and others can be used as well. The papermaking process starts with the pulping, where the fibre mass, the so-called pulp, is produced in a mechanical, chemical, or a combination process (Robertson, 2019). This initial papermaking step greatly affects the final paper's quality. Paper can be

bleached or treated with chemicals such as slimicides and strengthening agents as well. The main advantages of paper materials are mechanical strength, biodegradability, and relatively low process costs to be printed. However, poor barrier properties to oxygen, carbon dioxide and water vapour, as well as opaquest, porosity and thermolability limit the range of their usage (Raheem, 2013).

The many different types of paper materials used in food packaging industries can be categorized as kraft, sulphite, greaseproof, glassine paper, vegetable parchment and waxed paper (Robertson, 2012).

Due to the good printability of paper and board packaging materials, most of the packaging is nowadays printed.

# 3. CONTAMINATION OF FOOD FROM FOOD PACKAGING MATERIAL

Since the last few decades, the rising number of food contaminations from the migration of substances from food packaging has brought big concerns about food safety to the limelight. When the contamination originating from pesticide residues or environmental pollution is took as a reference, as main perspective of food contamination, it is estimated that nowadays the contamination from the migration is up to 100 times higher (Van Bossuyt et al., 2016). Migration, together with sorption and permeation processes are three classical categorization levels of interactions between food and the packaging material. All types of interaction processes that may happen during the manufacturing process or further steps of a supply chain are crucial to be understood when direct or indirect contact of food with packaging material is present. The mentioned interactions have consequently the intrusion of gases and volatiles, moisture, microorganisms, and other low-weight compounds. Even though they may also result in desirable effects, the worst outcome is causing alterations in the quality and safety of the packed foods, as well as unwanted changes in food flavours. Thus, since the preservation of both quality and safety is considered critical control point in the packaging, storage, transportation, and retail processes, it is crucial to ensure prescribed food safety standards (Alamri et al., 2021; Lago et al., 2019).

The focus of this work is on compounds that participate mainly in migration processes, thus other types of interactions will not be explained in further detail.

## 3.1. MIGRATION

Migration is a term which describes the transfer of chemical compounds between the food and the package. The migration process can result in unwanted changes in two directions, from packaging material to foodstuff or via reverse-side migration. One case represents the diffusion of low molecular weight substances into the food, while the latter-mentioned scenario includes the mass transfer of food colour, aroma, flavour, and nutrients from food products which impacts the organoleptic properties of foods. The migration of substances can come from two possible sources (a) from packaging materials, for example paper, and (b) from the packaging components, like printing inks, adhesives, and coatings (Alamri et al., 2021).

### 3.1.1. Migration mechanism

The migration phenomenon is a mass transfer process from higher to lower concentration gradient until the equilibrium is reached. The mentioned mass transfer is mainly based on the diffusion process, which in most cases obeys Fick's laws of diffusion.

The first Fick's law relates the already mentioned concentration gradient with diffusion flux, and can be described as follows (Mairinger, 2012):

$$F = -Dp\ (\delta Cp/\delta x) \quad [1]$$

The second Fick's law represents a mathematically expressed diffusion rate and can be described with the following equation:

$$dCp/dt = D\ (d^2Cp/dx^2) \quad [2]$$

in which Cp represents the concentration (mg/g) of the migrant in the packaging material, D is the coefficient of diffusion ($cm^2/s$), T stands for the time in which the diffusion takes place and x is the distance between the food and the packaging material in cm (Aparicio and Elizalde, 2015; Arvanitoyannis and Kotsanopoulos, 2014).

Both equations represent the base for the development of suitable mathematical models to describe the migration process that would be able to predict the concentration of a migrant, as well as its dependence on time. However, mathematical models are still in the stage of continuous development since robust simulations or models for accurate evaluation of migration are still not made (Alamri et al., 2021).

### 3.1.2. Types of migration

When talking about types of migration, two unconnected categorizations can be made. The first categorization can be based on the migration nature and divided into three groups: migration according to migrant number, migration related to food nature and migration based on the coefficient of diffusion.

Migration according to migrant number includes two terms that should be distinguished - overall migration (OM) and specific migration (SM). While OM is related to the sum of all releasing substances, SM is related to the mass transfer of specific compounds.

Migration related to food nature includes defining a food system as non-migrating, volatile or leaching. Non-migrating systems imply very low mass transfer of pigments or inorganic compounds. Volatile systems take into consideration small aroma compounds that can transfer

without direct contact. Lastly, leaching systems are systems where migrant mass transfer starts with diffusion, goes to dissolution, and ends up with dispersion into the foodstuff.

The second division is more based on how the migration process occurs and in which conditions:

(a) Contact migration as the name says, occurs only if direct contact is present.

(b) Gas-phase migration describes mass transfer carried with the gas phase from the outer layer to the inner one.

(c) Permeation migration describes the transfer of a substance through the packaging material from the outer layer that can be whether coated or printed towards the inner layer or contact side of the packaging material. The substance upon reaching the inner side of the package can then migrate to the packed food by direct contact or by migration through gas phase.

(d) Set-off migration is a term mostly related to inks, varnishes and coatings, where the mass transfer occurs from the printed side when it gets in contact with the internal content. The contact can occur by stacking or reeling when the printed packages are kept nested in each other.

(e) Condensation migration where substance transfer occurs during the boiling or sterilization processes of the pouched food or food in trays or cartons (Alamri et al., 2021).

### 3.1.3. Factors influencing the migration

Even though a lot of factors can affect the migration process, several of them should be highlighted as general and main ones. Triantafyllon et al. (2007) reported the significant influence of food nature on migration levels when discovered that high-fat content food is proved to be related to high migration levels. As with the nature of food, the nature of packaging material also affects migration levels. For example, thinner packaging materials are brought into correlation with higher migration levels. Furthermore, factors related to contact of packaging material and packed food, such as time, temperature and type can also be explained affecting the level of migration. Studies so far confirmed significantly increased migration extent when direct contact is present, during a longer time of contact and if the temperature of contact is increasing or is increased. The last two factors can be categorized as dependencies on the migrant, such as the amount of it in the packaging material and migrant characteristics. As can be assumed, higher concentrations of migrants present are in direct correlation with higher migration levels. On the other hand, migrant characteristics are more complex to be

explained. The low molecular weight substances have a bigger tendency to migrate in comparison to ones with higher weight (Alamri et al., 2021). Also, the low molecular weight substances (below 1000 Daltons) are found to be a safety issue because of their possible physiological activity. From the same perspective the ones above 1000 Da, which are classified as high molecular weight substances, are not found to be of great concern as they are not absorbed in the gastrointestinal tract (Mairinger, 2012). Furthermore, the dependent characteristics, such as the complexity of molecular configuration, stability and volatility should also be taken into consideration (Aparicio and Elizalde, 2015).

### 3.2. REGULATIONS RELATED TO PACKAGING MATERIALS

„Food contact materials are all the materials and articles intended to come into contact with food, such as kitchen equipment, cutlery and dishes. They also include materials used in processing equipment, such as coffee makers or production machinery, as well as the containers used to transport foodstuffs“ (EFSA, 2023). Depending on the composition of the food contact materials (FCM) and its properties, when put in contact with food, different materials can transfer chemical substances to the food. Those chemical substances might endanger human health or change the food properties.

Food contact materials and articles are EU-regulated with Regulation (EC) 1935/2004. The purpose of regulation 1935/2004 is to ensure the effective functioning of the internal market together with ensuring FCM safety by providing the base for securing a high level of protection of human health and customer interests (European Parliament and European Council, 2004).

The current FCM legal framework lays down 2 general safety requirements. First is that materials and articles, including active and intelligent materials and articles, should be manufactured in compliance with good manufacturing practices. This means that under normal and foreseeable conditions of use, they do not transfer their constituents to food in quantities that could endanger human health, bring an unacceptable change in the composition of the food, or cause a deterioration in the organoleptic characteristics. The second one relates to labelling, advertising, and other ways of presentation of products that should not mislead the customers (European Parliament and European Council, 2004).

Additionally, in Annex I. of Regulation (EC) 1935/2004 seventeen groups of substances are alphabetically listed, for which specific measures should be covered at some future time. They are as follows: 1. Active and intelligent materials and articles 2. Adhesives 3. Ceramics 4. Cork

5. Rubbers 6. Glass 7. Ion-exchange resins 8. Metals and alloys 9. Paper and board 10. Plastics 11. Printing inks 12. Regenerated cellulose 13. Silicones 14. Textiles 15. Varnishes and coatings 16. Waxes 17. Wood. The mentioned specific measures, so far, have been put in place for four out of seventeen material types, namely: plastics and recycled plastics, active and intelligent food contact materials, regenerated cellulose, and ceramics. Specific measures have also been put in place for FCMs containing certain epoxy substances and for teats and sooths made of rubber or elastomers. These FCMs need to comply not only with the Framework and the GMP Regulations but also with their specific measures, which can contain detailed restrictions on the manufacture and use of FCMs (European Parliament and European Council, 2004).

Further regulations regarding good manufacturing practice are defined within Regulation (EC) 2023/2006 which applies to all FCMs through all manufacture steps, including processing and distribution of materials and articles, with exclusion of the production of starting substances (European Commission, 2006).

Since this work is mainly oriented to paper and broad materials as FCMs of interest, specific regulations were reviewed.

### 3.2.1. Specific regulations for paper and board materials

Paper and board materials are one out of seventeen groups of food contact materials. However, there is no uniform legal regulation within the EU. Nevertheless, food businesses should ensure that all paper products that may get in contact with food during whether food production or packaging process (for example paper towels that can be used to dry food or on which food is placed during production) meet the requirements of food contact materials legislation, including the composition of any dyes that may have been used in its manufacture.

However, Council of Europe Policy Statement XXXVI. (The German Federal Institute for Risk Assessment, 2022) concerning paper and board materials represents a valid recommendation for single and multi-layered commodities (articles, materials) made of paper or paperboard and cast fibres that may come into contact with packed food and/or have an effect on it. It refers to paper or paperboard intended to be used at temperatures up to 90 °C (for keeping food warm or reheating it).

# 4. PHOTOINITIATORS

Due to low cast operations and good printability of paper, 90 % of packed food is printed (Van Bossuyt et al., 2016). The often-used types of inks for packaging material printing are UV light-cured printing inks, which are considered safer than the classic inks that were used previously. The reason for that is that they do not contain solvents and do not absorb into porous materials but stay on the surface. UV inks consist of different substances. Then it is worth mentioning for this study are photoinitiators which catalyse the UV-initiated polymerisation process and consequently cure the ink on the printed surface (Fouassier and Lalevée, 2021a).

## 4.1. CHEMISTRY OF PHOTOINITIATORS

In general, the polymerization reaction represents the process of adding many monomer units to create a macromolecule. It consists of decomposition of the initiator to initiating specie, which can attach monomer units, and other units add further to form the macromolecule. The polymerization process can be activated through a thermal process, light, electron beam, X-rays, γ- rays, plasma, microwaves or even pressure. When exposure to light is used for the initiation step, the reaction is called photopolymerization. Since monomers and oligomers are usually not light-sensitive due to their absorption properties, the addition of a photoinitiator or photoinitiating system is necessary. Under light exposure, a photoinitiator converts absorbed light energy, UV or visible, into a chemical to form an initiating species whose nature depends on the used starting molecule. They can be radical, cationic, or anionic (Fouassier and Lalevée, 2021a). The most important properties that photoinitiators must fulfil are high absorption, high molar extinction coefficient, the high quantum yield of formation of initiating species and high reactivity towards the monomer (Zheng et al., 2020).

The photopolymerization process is applied in the so-called UV curing process or „radiation curing “. Light is used to transform a liquid photosensitive formulation into an insoluble, dried, and solid film. The film thickness, depending on the application, varies within a range from a few micrometres to a few hundred μm (newly developed methods also allow thicker films if needed) (Fouassier and Lalevée, 2021a).

Two types of polymerization processes are worth mentioning when talking about printing inks - free radical forming photopolymerization which is by far the main one and cationic with completely different photoinitiators chemistry (Metin et al., 2020).

Free radical forming photopolymerization consists of three steps: initiation, in which the formation of free radical species occurs, propagation step of adding a radical to a low molecular weight monomer (or prepolymer), including chain growth polymerization, and eventually termination step. The two main types of free radical UV photoinitiators can be distinguished into type I and type II. Type I photoinitiators absorb the UV energy, i.e., photons, and divide them into two primary radicals which are then able to initiate a rapid addition reaction with unsaturated groups, such as acrylic, often present in the prepolymers and monomers (Fouassier and Lalevée, 2021b). Most of them contain a benzoyl (phenyl-CO-) functionality group. It includes benzoin derivates, benzilketals and acetophenone derivates from the aromatic carbonyl compounds group. On the other hand, type II does not undergo a fragmentation process for the formation of secondary free radicals but rather interacts with co-initiators or photosensitizers for extraction of hydrogen, usually an amine known as an amine synergist, for example 2-Ethylhexyl-4-dimethylaminobenzoate (EHDAB) and Ethyl-4-(Dimethylamino) benzoate (EDAB) (Zheng et al., 2020; Mairinger, 2012). The far most popular type II photoinitiator is benzophenone (BP) due to its low costs and characteristics (Mueller et al., 2022). This type includes aromatic ketones like benzophenone and its derivates and thioxanthones (Mairinger, 2012). Mostly used UV-light photoinitiators are Type II (Aldrich, 2013). Cationic photoinitiators include sulfonium salts, iodonium salts and iron complexes. These salts can produce either Brönsted or Lewis acids, depending on their chemical structure. They were mainly developed for epoxy- and vinyl ether-based monomers where they present very active initiators for the ring-opening polymerization through the oxonium ion (Zheng et al., 2020; Schwalm, 2001).

### 4.2. FOOD SCARES

The topic of migration of photoinitiators from food packaging material is well known for almost two decades now. It was especially brought to the limelight by two big people's outcries, among a lot of them recorded within the last 15 years through the Rapid Alert System for Food and Feed (RASFF). It was made in 1979 as an effective tool for the exchange of information about measures taken regarding the insurance of food safety (Aparacio et al., 2015).

Aparicio and Elizalde (2015) investigated RASFF notifications in the period from 2000 to 2011, concluding that 143 notifications were issued for the migration of photoinitiators. 2-Isopropylthioxanthone (2-ITX) was involved in 119 of them, with the biggest values reached in 2005 and 2006 presenting 30 % of total notifications received for food contact material in the aforementioned period. 2-ITX was found in infant milk from Spain in amounts ranging

from 120 to 300 µg/L, which led to withdrawals in Italy, France, Spain and Portugal. In total, approximately 30 million litres of milk were withdrawn from markets at the time (Aparicio and Elizalde, 2015). Due to the high public pressure, this situation enforced EFSAs opinion published soon after, in December 2005. „Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to 2-Isopropyl thioxanthone (ITX) and 2-ethylhexyl-4-dimethylaminobenzoate (EHDAB) in food contact materials contained safety evaluation regarding the mentioned case and for 2-ITX as a UV ink component. In conclusion, due to 2-ITX testing negatively in two adequate limited *in vivo* genotoxicity studies, no doubts about its genotoxic potential were raised. Therefore, the specific migration limit for 2-ITX has been set to 50 µg/kg of food. (EFSA, 2005).

The second big crisis occurred in 2009 when large amounts of BP and 4-methyl benzophenone (4-MBP) were found in breakfast cereals and bars. To be exact, German authorities reported 4-MBP found in a concentration of 798 µg/kg of food in cereals. Later on, the Belgian Scientific Committee reported amounts of up to 3729 µg/kg of food of 4-MBP and 4210 µg/kg of food of BP (Van Bossuyt et al., 2016). This situation once again raised interest in EFSAs opinion, which was then published in May 2009. BP was already defined as not genotoxic, based on negative in vitro and in vivo tests. The same opinion followed for 4-MBP. Also, EFSA concluded short term consumption of breakfast cereals with large quantities of 4-MBP and BP do not pose a risk to people. Both now have established SMLs of 0.6 mg/kg of food (EFSA, 2009).

Because of situations arisen both in 2005 and 2009, 2-ITX and BP are found to be the most widely studied photoinitiators from an analytical point of view.

### 4.3. LEGAL BACKGROUND

The legal background for printing inks is diverse and until today, no European-specific community legislation concerning printing inks for food packaging exists. However, printing inks are one of seventeen groups listed within the framework regulation (EC) 1935/2004 and therefore must fulfil the general requirements from Article 3. Moreover, they must agree with Commission Regulation (EC) 2023/2006 on good manufacturing practices for materials and articles intended to come into contact with food (European Commission, 2006; European Parliament and European Council, 2004).

In addition, the regulation (EU) 10/2011 European legislation for plastic materials and articles intended to come into contact with foodstuffs also makes specific reference to printing

inks and is sometimes used as a framework for paper-based packaging materials due to a lack of specific regulations for paper packaging materials. So far, SML limits for BP and its derivates have been set, but that is an exception because of its possible usage as a UV blocker (European Commission, 2011).

Due to the lack of specific regulations for printing inks, some countries implemented national legislation for the regulation of printing inks intended to be used in food packaging. The first of them was Switzerland with the implementation of the Swiss Ordinance of the FDHA on materials and articles intended to come into contact with foodstuffs with the latest edition 2.1 published in December 2020. The Ordinance contains several Annexes, presenting lists of permitted substances, including plastics, cellulose films, ceramics and glass, silicone and printing inks. Provisions related to printing inks applied on the non-food contact surface of food contact materials are set in section 12, while permitted substances are those listed in Annex 2 and Annex 10. Currently, Annex 10 contains 5343 substances listed. They are divided into A and B statuses. While status A represents evaluated substances i.e., for which toxicological data have been evaluated so far, status B substances remain unevaluated due to a lack of data considered by EFSA or other regulatory bodies or generally insufficient data available at the moment (Federal Department of Home Affairs, 2020). The list was established together with the support of EuPIA (European Printing Ink Association). EuPIA, due to the absence of specific EU legislation, issued a document for Good Manufacturing Practice (GMP) for Printing Inks for Food Contact Materials. In the 4$^{th}$ completely revised version issued in March 2016, the mechanism for raw materials selection for FCM inks is revised (EuPIA, 2016). Also, Guideline on Printing Inks applied to Food Contact Materials from April 2020 and Guidance on specific ink technologies: UV for FCM, the „Suitability List of Photo-Initiators and Photosynergists for FCM“, issued in October 2020 are established (EuPIA, 2020a and 2020b).

# 5. ANALYSIS OF PHOTOINITIATORS

## 5.1. GAS CHROMATOGRAPHY

IUPAC defines chromatography as „A physical method of separation in which the components to be separated, are distributed between two phases, one of which is stationary (stationary phase) while the other (the mobile phase) moves in a definite direction“(IUPAC, 1997). The mobile phase is responsible for the transport of the analytes along the stationary phase.

Methods can be classified using different factors; however, a fundamental criterion is the aggregation state of the mobile phase which may be a gas, liquid or supercritical fluid. The mobile phase aggregation state is the main factor for distinguishing chromatography types. Gas chromatography (GC) is a type of chromatography in which gas is applied as a mobile phase. The other two known types based on mobile phase aggregation are liquid chromatography (LC) with a liquid mobile phase and supercritical fluid chromatography (SFC) with supercritical fluid employed as the mobile phase (Engewald and Dettmer-Wilde, 2014).

Another criterion is the separation mechanism which can be based on adsorption, solubility, ion exchange, size exclusion or selective interaction. When talking about GC, only solubility and adsorption are applicable. Further on, if the stationary phase employed is solid, then we are talking about gas-solid chromatography (GSC) and the adsorption mechanism takes place. If the stationary phase is on the other side liquid, a solution process takes place and it is called gas-liquid chromatography (GLC) (Geiger and McElmurry, 2020).

Based on the property of gases, GC must always be performed in closed systems. The stationary phase is located in the chromatographic column, the so-called heart of the GC system, which is purged by the gas mobile phase provided by the carrier gas supply. Columns can be distinguished into two groups, packed and capillary columns. Packed columns are completely filled with a fine-grained solid stationary phase or with a thin film of a highly viscous liquid, whether used for the aforementioned GSC or GLC. On the other side, capillary columns nowadays find much wider application ranges, feature an inner diameter of less than 1 mm and the stationary phase is a thin layer on the inner wall. It can be a thin layer of highly viscous liquid presenting wall-coated open-tubular columns (WCOT) or a thin layer of an adsorbent when talking about porous layer open-tubular columns (PLOT). The column is a fundamental part of the GC system because there the chromatographic separation takes place. When talking about simply defining a chromatographic process, laying on the plate theory, we

are talking about column segments, so-called plates, whose number depends on column length. In each plate, a series of discontinuous equilibrium steps take place between the partition of solute in the mobile phase and in the stationary phase. That partition is defined by the solute-specific distribution constant, K. When equilibrium is reached in one column plate, the portion of the remained solute in the mobile phase is then transported to the next column segment, where again equilibrium is established (Engewald and Dettmer-Wilde, 2014).

When the analytes are eluted from the column, the detector produces an electric signal. As the endpoint, the data system registers, stores, and analyses the produced data. The detector signal is in the data system presented as a gas chromatogram, providing peaks. It is basically defined as the detector signal plotted over time (Wagner et al., 2021). In the gas chromatogram the x-axis represents the retention time, while the y-axis shows the abundance i.e., signal intensity. If only a carrier gas reaches the detector, a baseline will be recorded. A baseline is a flat line with common slight fluctuations - so-called baseline or background noise. When the analyte reaches the detector, an increasing intensity will be visible ideally following a normal distribution and having a Gaussian shape. Peaks are characterised by position on the chromatogram, expressed as retention time which delivers information about analyte identity, peak height, width and shape. While its height represents a measure for relative concentration or amount of analyte, peak width and shape are indicators of column performance and working conditions (Engewald and Dettmer-Wilde, 2014).

GC is in general a powerful and accurate analytical technique that separates different mixture complexities. Its domain is the separation, identification and quantitation of volatile, non-polar or weakly polar compounds with less than 60 carbon atoms, molecular mass below 500 Da and boiling points above 500 °C (Moldoveanu and David, 2019). Before starting the analysis, it is very important to take into consideration whether the solid or liquid form of the sample will be used for analysis, which column should be used, as well as the carrier gas and oven temperature. The improperly selected column will generate inaccurate and unreliable separation. While selecting carrier gas, which can be either helium, nitrogen or hydrogen, inertness, absence of oxygen, dryness, safety, cost and availability should be taken into consideration (Teonata et al., 2021). Regarding sample introduction, the most common systems are split and splitless. The split injection mode includes introducing only a small amount of sample onto the column (ratio varies from 10:1 up to 100:1) and it is suitable for targeted analysis of compounds with boiling points lower than the product or a matrix. On the other hand, the splitless mode allows compounds with high boiling points to condense and

concentrate at the head of the column (Geiger and McElmurry, 2020). It is necessary that analytes are stable volatiles and that their boiling points are not too low to avoid sample decomposition. Non-volatile compounds can be analysed with GC as well, but in that case derivatization to a less polar, as a sample preparation step, or pyrolysis is obligatory (Teonata et al., 2021). The end goal is to combine GC instrumentation with appropriate sample preparation techniques to achieve increased precision, a reduced number of preparation steps and reduced relative standard deviation (RSD) (Engewald and Dettmer-Wilde, 2014).

GC finds usage in various fields such as pharmaceuticals and drugs, environmental studies, petroleum industries and the food industry. Applications in food analysis are various such as the determination of food composition like additives, flavours and aromas, and the detection of contaminants like toxins, pesticides, fumigants, pollutants, drugs and compounds from packaging materials (Teonata et al., 2021).

### 5.1.1. Solid Phase Microextraction (SPME)

Sample preparation is a very important step in GC and it usually takes two-thirds of the total time spent for GC analysis. Its significance can be taken into the limelight whether talking about reducing errors or uncertainties due to the more significant number of steps or transferring compounds into a more suitable format for analysis (Falaki, 2019). With the right sample preparation steps, smaller initial sample and solvent amounts can be used, and higher specificity and selectivity of the analysed compounds can be achieved. There are several options to make non-suitable analytes like polar, less volatile, or thermally fragile compounds amenable to GC analysis. Usually, it includes derivatization by chemical reactions, such as silylation, alkylation or acylation or the introduction of „detector-specific groups“ (Munir et Badri, 2020). For non-volatile compounds degradation by controlled thermal break-down, GC pyrolysis, into analysable smaller molecules, is also possible (Engewald and Dettmer-Wilde, 2014).

In food monitoring, for extraction and analysis of volatile analytes, the SPME technique as modern and solvent-free sample preparation technology is often used (Badawy et al., 2022). The advantages of this technique are easy automation, compatibility with both GC and HPLC instruments, reusability, low-cost process and fastness with no destructive nature towards samples (Lancioni et al., 2022).

Generally, a sorbent-coated fibre or rod is placed into the vial containing a sample of interest. In the SPME process the fibre coating extracts the compounds from the sample until

equilibrium of the analytes between the sample and fibre is reached (Geiger and McElmurry, 2020). The SPME concentrates analytes on the fibre after which thermal desorption and analysis follow. The SPME injection technique can be conducted in three ways, depending on the sample nature (Merkle et al., 2015). Direct extraction implies immersion of coated fibre into the aqueous sample, headspace configuration is used for sampling of volatile analytes from the headspace above the sample, while membrane protection configuration includes protecting of coating fibre with a membrane for uses such as dirty samples. Also, based on the different polarities of the sample, different fibres are used, which differ in the polarities of applied coatings (Engewald and Dettmer-Wilde, 2014).

Coatings can be polymeric films for absorption or embedded particles in a polymeric film. So far on the market, nonpolar, bipolar, and polar coatings are available. The commonly used are nonpolar fibre coatings from polydimethylsiloxane (PDMS) since most of the volatile analytes are found to be nonpolar or slightly polar. The polar coating can be whether polyacrylate (PA) or CARBOWAX$^{TM}$-Polyethylene Glycol (PEG) coatings. Bipolar coatings represent a combination of PDMS with embedded particles like Carboxen adsorbent (CAR), divinylbenzene (DVB) or both. Since the volatility of analytes can be put in a close relationship with their molecular weight, for each fibre different analyte molecular weight ranges are applicable. The second important parameter is fibre coating thickness which is also brought in direct correlation with the volatility of compounds, i.e., thicker fibre coatings are used for highly volatile compounds, or in cases when a larger organic matrix volume is used. Fibre core type can be fused silica, StableFlex$^{TM}$ (SF), metal alloy, and nitinol, of which fused silica is originally used due to high inertness. The biggest disadvantage of its usage is high fragility; however, the thin coat of polymer can be added on the fused silica, reducing its shortcomings by making it more flexible (Sigma Aldrich, Supelco manual).

### 5.1.2. Gas chromatography – Mass spectrometry (GC-MS)

Coupling GC with an advantageous detector such as a mass spectrometer spawned one of the most sensitive and selective techniques for the separation, identification and quantification of volatile and semi-volatile complex organic mixtures (Pastor et al., 2019). The reason for that is the two-dimensional identification consisted of retention time by separation in the GC and a specific mass spectrum for each component in a mixture (Harvey, 2005). The instrumentation essentially consists of the gas chromatograph, the mass spectrometer, and a data system. However, it can be as well provided with features like an autosampler, or other detector types like an infrared spectrometer or a flame-ionization detector (FID).

In general, MS detection includes three steps: ionisation, separation and detection. Ionisation is the first step and includes generating ions from inorganic or organic compounds. There are two types of ionisation sources used when coupled to GC, electron impact (EI), which is more commonly used, and chemical ionization (CI). In EI molecules are being bombarded with a high energy of 70 eV beam of electrons and molecules are being ionised due to the removal of an electron. Further on, the aforementioned ions are separated according to their mass-to-charge ratio (m/z) which leads to changes in the field in the quadrupole. Consequently, it allows only a particular m/z ratio through the detector (Moeder, 2014).

MS instruments can operate in full scan mode which allows identification of unknown compounds, or in selected ion monitoring (SIM) mode when a more sensitive analysis of target compounds is wanted (Harvey, 2005).

# 6. EXPERIMENTAL SECTION

## 6.1. MATERIALS

To test the applicability of the developed method and to obtain some information on the contamination of certain paper food packaging materials with photoinitiators, in total, twelve samples of paper food packaging were bought. All samples of interest were purchased in local supermarkets. The sample range includes cereals, coffee, pasta and confectionery products. Samples names and brands will not be represented within this work, therefore codes P01-P12 will be used to represent the samples.

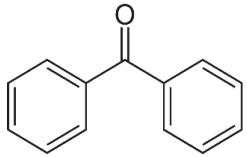
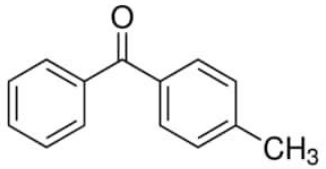
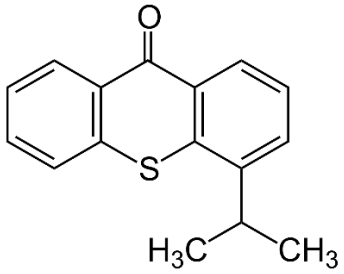
Since food packaging materials without printing inks were not available, unbleached paper with 75 $g/m^2$ grammage has been used for the development of measuring method. The fundamental criteria for the selection of control paper was to be as much matrix-matched to the real test samples.

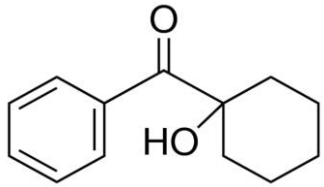
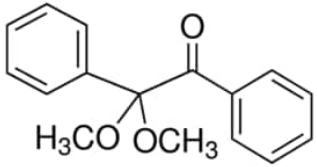
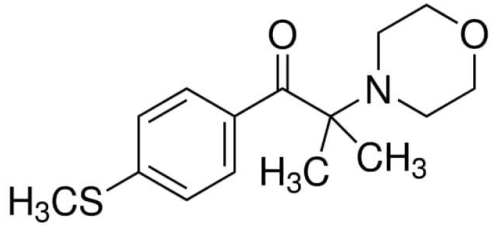
### 6.1.1. Analytes

Six photoinitiators were chosen. The selected photoinitiators and their important physicochemical properties are listed in Table 1. In the table, the analytes are grouped according to their chemical structures in: benzophenone derivatives, morpholino derivatives, aminobenzoate derivates and xanthone derivatives.

The photoinitiators were primarily chosen according to the recent research data, and due to the highest frequency of identification in food packaging materials. Also, three of them – BP, 4-MBP and 2-ITX, caused the two biggest outcries related to the migration of photoinitiators so far, in the years 2005 and 2009. Since in this work GC-MS was used, the chosen photoinitiators had to fulfil the requirements in the way of physicochemical properties, especially molecular weight (MW) and boiling points. Ionic photoinitiators are characterized by very high boiling points, thus only radical-forming photoinitiators were suitable in this study. All chosen photoinitiators are listed within EuPIA's list and the Nestle Guidance.

**Table 1.** Selected photoinitiators

| ABBREVIATION | CHEMICAL NAME | CAS# | LOGP-VALUE | $B_P$ (°C) | MW | CHEMICAL STRUCTURE |
|---|---|---|---|---|---|---|
| **BENZOPHENONE DERIVATES** | | | | | | |
| **BP** | Benzophenone | 119-61-9 | 3.18 | 305.4 | 182.22 |  |
| **4-MBP** | 4-Methylbenzophenone | 134-84-9 | 3.64 | 328.1 | 196.24 |  |
| **XANTHONE DERIVATES** | | | | | | |
| **2-ITX** | 2-Isopropythioxanthon | 5495-84-1 | 5.33 | 398.9 | 254.35 |  |

| AMINOBENZOATE DERIVATES | | | | | | |
|---|---|---|---|---|---|---|
| **HCPK** | 1-Hydroxycyclohexylphenyl ketone | 947-19-3 | 2.344 | 339 | 204.26 |  |
| **DMPA** | 2,2-Dimetoxy-2-phenylacetophenone | 24650-42-8 | 4.75 | 371.1 | 256.3 |  |
| **MORPHOLINO DERIVATES** | | | | | | |
| **MMTPMP** | 2-methyl-4'-(methylthio)-2-morpholinopropiophenone | 71868-10-5 | 2.997 | 420.1 | 279.40 |  |

### 6.1.2. Chemicals

All chemicals used in this study are listed in Table 2.

All used chemicals were of analytical grade. Methanol and hexane were stored at room temperature, light protected. Analytes were supplied in solid form, they were stored in the fridge at +6 °C, light protected.

**Table 2.** List of chemicals, suppliers and CAS numbers used in this study

| ***Chemicals*** | **Supplier** | **CAS#** |
|---|---|---|
| *Methanol* | Thermo Fisher Scientific, USA | 67-56-1 |
| *Hexane* | Thermo Fisher Scientific, USA | 110-54-3 |
| *Benzophenone* | Sigma Aldrich, USA | 119-61-9 |
| *1-Hydroxycyclohexylphenyl ketone* | TCI, Japan | 947-19-3 |
| *4-Methylbenzophenone* | Sigma Aldrich, USA | 134-84-9 |
| *2-Isopropythioxanthon* | TCI, Japan | 5495-84-1 |
| *2,2-Dimetoxy-2-phenyl acetophenone* | Sigma Aldrich, USA | 24650-42-8 |
| *2-methyl-4'-(methylthio)-2-morpholinopropiophenone* | TCI, Japan | 71868-10-55 |
| *Deuterated benzophenone* | Sigma Aldrich, USA | 22583-75-1 |

### 6.1.3. Labware and instruments

All labware used in this study is listed in Table 3.

**Table 3.** List of labware used in this study

| ***Labware*** | ***Volume (if applicable)*** |
|---|---|
| *Dropper* | / |
| *Volumetric flask* | 10 mL |
| *Microman pipette with Gilson tips* | 10-100 µL |
| *Disposable capillary pipets* | 10-100 µL |
| *Glass screw vials with Teflon-coated septum* | 40 mL |
| *Screw vials with caps* | 2 mL |
| *Headspace crim vials with magnetic crimp caps* | 20 mL |
| *Elliptic magnetic stirring bars with Teflon* | / |
| *Beaker glass* | 100 mL |
| *Scissors* | / |
| *Crimping pliers for closing the vials* | / |

All laboratory instruments used in this study, including model and manufacturer, are listed in Table 4.

**Table 4.** All laboratory instruments used in this study

| ***Laboratory instruments*** | **Model** | **Manufacturer** |
|---|---|---|
| *Transferpettor 1000 µL* | / | Brand Gmbh + Co. Kg, Germany |
| *Gas chromatograph* | 7890A | Agilent Technologies, USA |
| *Mass spectrometer* | 5975C | Agilent Technologies, USA |
| *SPME fibre* | PDMS/DVBS, 65 µm | Supelco, USA |

6.1.4. Software

Data analysis was done with 91701DA GC/MSD ChemStation Data Analysis provided by Agilent Technologies, USA. It controls data flow from its acquisition to its export into desired selected reports via methods or sequences. Therefore, it performs review of data, provides library searches and quantitation and generates.

## 6.2. METHODS

6.2.1. Stock and standard solutions preparation

*6.2.1.1. Analyte stock solutions*

Stock solutions of the six analytes listed in Table 1 were prepared at a concentration of 1 g/L in both methanol (MeOH) and hexane.

Approximately 10 mg were weighed into a clean 10 ml volumetric flask. A respective amount of methanol or hexane was added into the volumetric flask to the graduation mark positioned on the long neck, obeying the rule of positioning the meniscus for the accurate and precise preparation of stock solutions. The flask was caped and shaken properly until the whole amount of solid was dissolved without any visible particles. The stock solutions were transferred in glass screw vials with Teflon-coated septa and stored light protected at -22 °C.

*6.2.1.2. Analyte solutions*

A 10 mg/L solution of each analyte (Table 1) was prepared in 2 mL screw vials with caps. It was prepared by mixing 10 µL of analyte stock solution and filling up to 1 mL with 990 µL of solvent. Two sets of analyte solutions were prepared, in hexane for liquid injection (only to confirm suitability of analytes for determination with GC) and in MeOH for SPME injection.

*6.2.1.3. Analyte mixture solution*

An mixture solution of all six analytes was prepared in MeOH in a concentration of 10 mg/L. It was prepared by mixing 10 µL of each analyte stock solution in 2 mL screw vials with caps and filling up to 1 mL with 940 µL of MeOH. The analyte mixture solution was stored light-protected at +6 °C.

*6.2.1.4. Internal standard solution*

Deuterated benzophenone (d-10), listed in Table 1., was used as an internal standard (IS). The IS stock solution was initially purchased in 1 g/L concentration and diluted in MeOH. For this study, it was diluted to 10 mg/L by adding 10 µL of stock solution and 990 µL of MeOH. The prepared solution was placed in a 2 mL screw vial, stored light protected at +6 °C.

*6.2.1.5. Analyte mixture solutions prepared for calibration*

Five calibration levels containing the IS and, representing mixes of all six analytes were prepared in methanol. Concentrations used for calibration levels, together with the preparation steps are shown in Table 5.

**Table 5.** Analytes mixture solutions for calibration

| Calibration level | Concentration (mg/L) | Preparation of standard solution |
|---|---|---|
| **1** | 5 | 100 µL of 50 mg/L* analyte mixture solution + 900 µL of MeOH |
| **2** | 10 | 10 µL of each PI stock solution in concentration 1 g/L + 10 µL of internal standard solution in concentration 10 mg/L + 940 µL of MeOH |
| **3** | 20 | 20 µL of each PI stock solution in concentration 1 g/L + 10 µL of IS solution in concentration 10 mg/L + 890 µL of MeOH |
| **4** | 30 | 30 µL of each PI stock solution in concentration 1 g/L + 10 µL of IS solution in concentration 10 mg/L + 840 µL of MeOH |
| **5** | 40 | 40 µL of each PI stock solution in concentration 1 g/L + 10 µL of IS solution in concentration 10 mg/L + 790 µL of MeOH |

*50 mg/L analyte mixture solution was prepared as mid-step to obey the rule for not taking less than 10 µL of solution. Preparation of 50 mg/L analytes mixture solutions included adding 50 µL of each PI stock solution in concentration 1 g/L, 10 µL of IS solution in concentration 10 mg/L and filling up to 1 mL volume with 740 µL of MeOH

6.2.2. Sample preparation of packaging material

Firstly, the content was removed from the food packaging. Since neither of the selected food packaging were in direct contact with liquid food, there was no need for cleaning and/or drying of packaging material. The food packaging was cut into smaller, representative pieces. A 100 mg were weighed and transferred into 20 mL headspace crimp vials. Food packaging pieces were spiked with 10 µL of IS solution in concentration 10 mg/L using a Microman pipette 10-100 µL with 10-100 µL Gilson tips. Disposable 10 µL capillary pipets could also be used.

### 6.2.3. GC-MS analysis

The analysis was carried out on a GC-MSD system consisting of an Agilent 7890A GC coupled to an Agilent MS 5975C detector. Separation was performed on a Restek Rxi-5ms column (30 m, 0.25 mm i.d., 0.25 µm thickness). Helium was used as a carrier gas, with a constant flow rate of 1 mL/min.

Analysis was performed with two injection modes – liquid and SPME injection. The method should have been developed for SPME injection, however liquid injection was used only to confirm the analytes suitability for analysis with GC and to determine the retention times of each analyte.

The GC-MS system was controlled by 91701DA GC/MSD ChemStation Data Analysis software.

#### *6.2.3.1. Analysis with liquid injection*

*GC parameters*

The injector temperature was 240 °C. The samples were injected splitless with an initial pressure of 51 kPa. A single taper splitless ultra inert liner with glass wool was employed. The injection volume was 1 µl. The oven temperature after optimization is showed on Figure 1. First stage of the temperature programme was an initial isothermal period of 1 min at 60 °C followed with the 10 °C/minute ramp up to final temperature of 300 °C, with 1 minute hold.

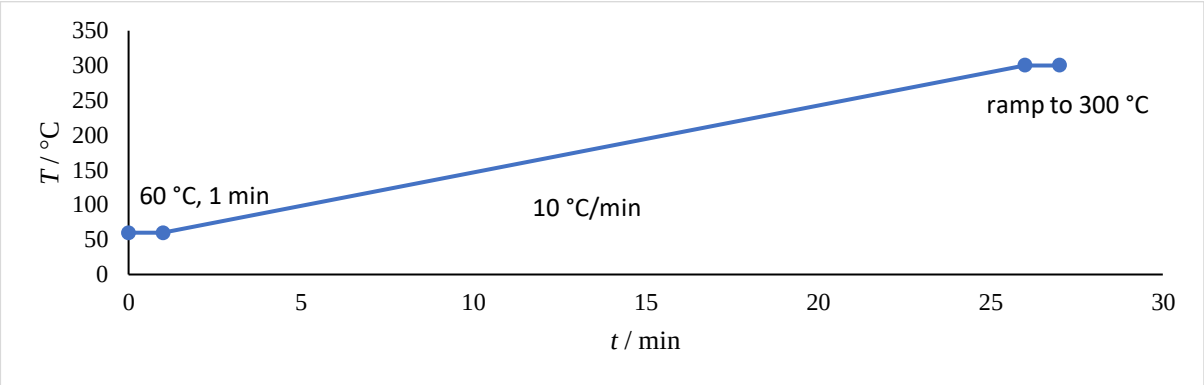



**Figure 1.** GC temperature programme

*MS parameters*

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 290 °C, the ion source at 230 °C and the quadrupole at 150 °C. The solvent delay was set to 5.5 minutes.

Data was acquired in full scan mode. Within scan parameters lowest mass was set to 35 Da while the highest mass to 350 Da, with the threshold set at 50.

*6.2.3.2. Analysis with SPME injection*

*GC parameters*

The injector temperature was 270 °C. The samples were injected splitless with an initial pressure of 78.9 kPA. A splitless straight Ultra Inert Liner without glass wool was employed. The injection volume was 1 µL. After optimization the oven temperature programme was the same as shown in Figure 1. (Chapter 6.3.2.1. GC parameters): an initial isothermal period of 1 min at 60 °C, ramp to 300 °C at 10 °C/minute with 1 minute hold.

*MS parameters*

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 290 °C, the ion source at 230 °C and the quadrupole at 150 °C. The solvent delay was set to 12 minutes. Data was acquired in select ion monitoring (SIM) mode. In SIM mode, at least two and maximum three fragment ions were acquired for each substance. Depending on the number of fragment ions selected from the SIM group a dwell time was set from 40 to 80 ms, resulting in 6 cycles/s. Retention times and ion ratios were used for unambiguous identification of the individual analytes. Ion ratios were calculated using the peak areas.

*SPME parameters*

For the SPME injection a Supelco Stablex PDMS/DVB, 65 µm, pink fibre was used. After optimization of the SPME parameters, an extraction time of 20 min and an extraction temperature of 120 °C were employed. Preincubation time was 5 minutes at 120 °C. Desorption time was set to 10 minutes.

6.2.4. Quantification

Depending on the requirement, the quantification of photoinitiators was performed using an IS or standard addition with 5-point calibration from 5 to 40 mg/L (Table 5). Paper samples were cut into smaller, representative pieces. 100 mg of paper was weighed and spiked with the desired concentration of standard, depending on the calibration level. Since paper represents a complex matrix, peak areas received for 100 mg of paper containing 10 µL of MeOH were employed as values with 0 mg/L of spiked solution for standard addition calibration. For IS calibration with benzophenone-d10, respective values were subtracted from peak area values

of all five calibration levels, therefore 0 mg/L value is taken as the peak area for calibration level 0 mg/L.

# 7. RESULTS AND DISCUSSION

Since paper and board packaging materials feature a good printability in combination with low costs most of the packaging material is printed with UV-curing inks. UV curing inks are among others, consisted of photoinitiators that due to their low-molecular weight have tend to migrate. The aim of this graduate thesis was therefore to develop an accurate, highly sensitive and selective method for determination of photoinitiators in paper and board food packaging materials.

In that purpose GC coupled with MS and SPME injection were used for development of method being able to determine and quantify six selected photoinitiators in different food packaging materials.

Accordingly, to achieve that, following issues had to be taken into account:

(a) careful selection and optimization of gas chromatography parameters;
(b) careful selection and optimization of mass spectrometry parameters:
(c) a simple sample preparation to be suitable for performing an analysis without using solvents:
(d) both IS and standard addition method had to be tested to select the most suitable quantification method having in mind complexity of sample matrix and usual quantity of samples if applied in laboratory and/or industries
(e) to achieve that limit of detection (LOD) and limit of quantification (LOQ) are as lower as possible, and to be in the SML range of selected photoinitiators;
(f) the analysis of different packaging materials had to be performed to confirm if developed method was accurate enough for testing of packaging materials.

Even though the method was not validated through this study, it was aspired to be as least labour-intensive as possible but highly accurate.

## 7.1. METHOD DEVELOPMENT

### 7.1.1. Selection and optimization of gas chromatography parameters

#### *7.1.1.1. Selection and optimization of column parameters*

As already stated, the column is the so-called heart of GC where chromatographic separation takes place. For the purpose of this study, a WCOT capillary column was selected. The rule of choosing a non-polar column due to the higher thermal and chemical stability recommendation for using a non-polar stationary phase as possible was obeyed. In fact, columns with nonpolar and weakly polar siloxane phases, containing methyl or 5 % phenyl are found to be the commonly used ones. Despite a longer lifetime, being more inert and resistant to oxidation, hydrolysis, and aggressive sample components, they are found to have better efficiency, a wider range of operating temperatures and bleed less at higher temperatures which are fundamental advantages for selecting them for this method development due to the boiling points of the chosen analytes. Capillary column dimensions selection included length (L), inner diameter (ID) and film thickness. Out of the few most frequently used dimensions, 30 m length, 25 mm inner diameter and 0.25 μm film thickness were selected - the so-called standard or general-purpose columns. A length of 30 m was chosen due to its universal application. While shorter columns would be suitable only for simple separations, longer columns would demand very complex mixtures. Thus, selecting longer columns would only result in longer analysis time and consequently higher costs. Due to the medium to high boiling points of the analytes, a thickness of 0.25 μm was consequently selected. Since MS detection was used in combination with GC, the recommendation of choosing an inner diameter of 0.25 mm was obeyed. In conclusion, due to the selection of a column with standard dimensions having a broad range of applications, its suitability did not need any further optimization or selection steps.

#### *7.1.1.2. Selection of carrier gas*

The carrier gas must be inert and must not undergo chemical reactions with the sample components. Also, it must be compatible with the chosen detector. Helium was selected for MS study.

It’s velocity was set to 35.17 cm/s, which fits to the mean range suitable for the inner column diameter of 0.25 mm (15-50 cm/s).

*7.1.1.3. Selection and optimization of column oven temperature programme*

The first rule that should be obeyed when a liquid stationary phase is used rather than a solid is that the column temperature should be set below the boiling point of the analytes. Out of the six analytes used in this study, benzophenone had the lowest boiling point of 305.4 °C. Therefore, the maximum possible final oven temperature was selected and set to 300 °C. The initial temperature was chosen based on the preferred solvent, following the fact that it should be set from 5 °C to 10 °C below the boiling point of the solvent. Since MeOH was used as solvent with a boiling point of 64.7 °C the initial temperature set was to 60 °C with an isothermal period of 1-minute hold. Further on, because the boiling range of analytes is much higher than 100 °C, an isothermal mode over the whole analysis was not suited, so a temperature program had to be set. For the first test measurement, a linear temperature program was used, where the column oven temperature was raised at a linear rate of 10 °C per minute until the final temperature was reached. Since a satisfactory separation, resolution and analysis run time of 26 minutes was achieved, the column oven temperature programme was not further optimized.

7.1.2. Optimization of SPME conditions

*7.1.2.1. Selection and optimization of SPME fibre*

Due to the analytes´ wide range of polarity, both polar and non-polar fibres were found not to be suitable. So, a bipolar fibre had to be selected, due to the fact that these fibres have an adsorbent extraction mechanism and extract primarily by size so both polar and non-polar analytes can be extracted. Two fibres commercially available from Supelco were tested - Polydimethylsiloxane/ Divinylbenzene (PDMS/DVB), 65 µm, pink and Divinylbenzene/ Carboxen/ Polydimethylsiloxane (DVB/CAR/PDMS), 50 µm/30 µm, grey. The PDMS/DVB fibre, 65 µm, pink was selected based on better sensitivity for the analytes i.e., bigger peak areas were obtained for all components (Figure 2).

*7.1.2.2. Selection and optimization of extraction temperature and time*

Parallel to testing different types of bipolar SPME fibres, different extraction temperatures were set: 60 °C, 80 °C, 100 °C and 120 °C for both fibres, while the extraction time was set to 20 minutes and was not further optimized. From Figure 2 it can be concluded that the best peak intensity was received when combining the PDMS/DVB, 65 µm, pink fibre with 120 °C extraction temperature for all selected analytes.

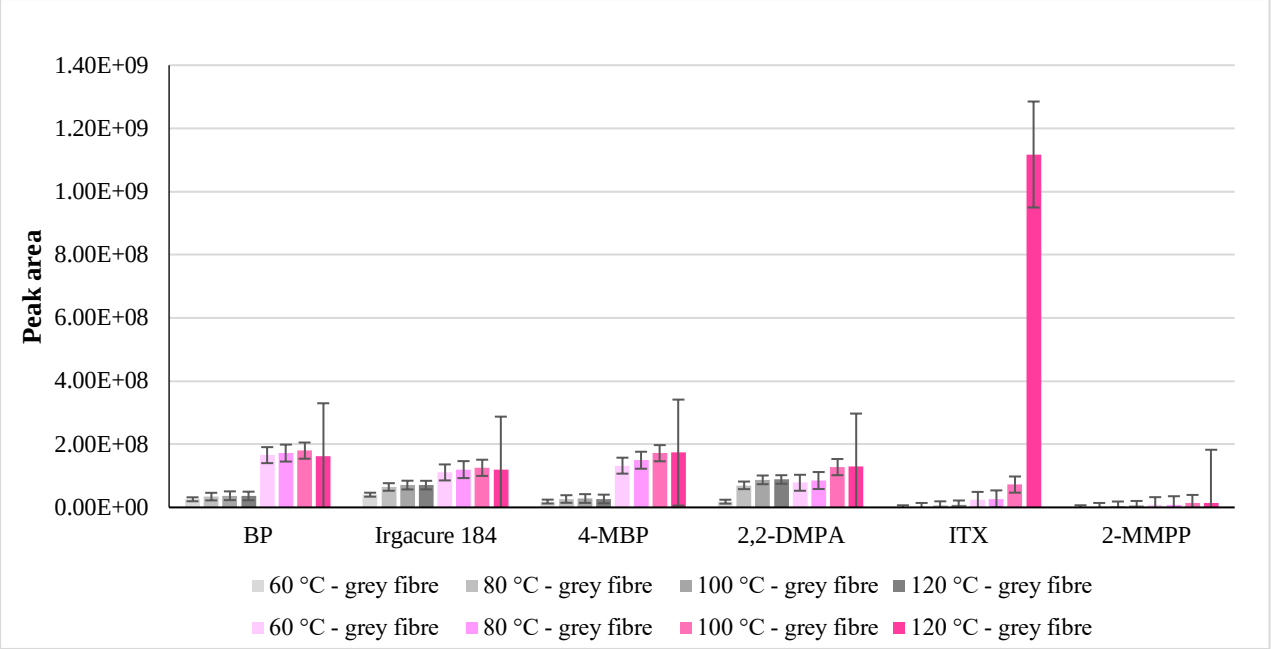



**Figure 2.** Comparison of peak areas for each of the six analytes using two different fibres in combination with four different extraction temperatures

### 7.1.3. Optimization of mass spectrometry parameters

After the successful optimization of the gas chromatography parameters, confirmed by measurements in the full scan mode, a SIM acquisition method was developed to increase detection sensitivity and selectivity. The six SIM groups were set, one for each of the selected analyte, according to their retention times. When possible, three selective fragment ions were set presenting one quantifier ion and two qualifier ions. However, for most of them, it was not possible to select three of them because they either had too low of a mass which made them not unique enough and not easily separated from interferences, or because the two compounds had the same selective ion, or both. The selective ions for each group were gathered from an analysis of 100 ng of each analyte with liquid injection and SPME injection. Their suitability was also confirmed with the NIST Library Database.

Once the fragment ions were chosen, the dwell time, which represents the amount of time spent sampling a specific ion, had to be set. The goal was to set the dwell time for each group to get at least 12 data points over each peak. The dwell time was set to at least 40 ms up to 80 ms when the group contained less selective fragment ions. The SIM groups and the corresponding analytes are listed in Table 6, together with dwell times set for each group.

**Table 6.** Overview of selected SIM groups with the corresponding analytes, retention times and dwell times

| ***SIM Group*** | **SIM group start time (min)** | **Analytes** | **Fragment ions** | **MW** | **$B_p$ [°C]** | **RT** | **Dwell time (ms)** | **Cycles/sec** |
|---|---|---|---|---|---|---|---|---|
| *Group 1* | 12.00 | Deuterated benzophenone (IS) | **110**, 192 | 84.1488 | 79 | 14.45 | 40 | 5.46 |
| | | Benzophenone | **105**, 182 | 182.22 | 305.4 | 14.50 | | 5.46 |
| *Group 2* | 14.91 | 1-Hydroxycyclohexylphenyl ketone | 81, **99**, 105 | 204.26 | 339 | 15.20 | 50 | 5.82 |
| *Group 3* | 15.75 | 4-Methylbenzophenone | **119**, 196 | 196.24 | 328.1 | 15.93 | 80 | 5.51 |
| *Group 4* | 16.50 | 2,2-Dimetoxy-2-phenyl acetophenone | 105, **151** | 256.3 | 371.1 | 17.25 | 80 | 5.52 |
| *Group 5* | 20 | 2-methyl-4'-(methylthio)-2-morpholinopropiophenone | **128**, 129 | 279.40 | 420.1 | 20.97 | 80 | 5.53 |
| *Group 6* | 21.50 | 2-Isopropythioxanthon | **239**, 254 | 254.35 | 398.9 | 21.90 | 80 | 5.53 |

*Bolded fragment ions represent the quantifier ion chosen for each analyte

## 7.2. QUANTIFICATION OF SELECTED PHOTOINITIATORS

For the quantification of the six selected photoinitiators, two independent quantification methods were used. The standard addition method was selected to be tested as the reference method to account for potential matrix effects. The results obtained from standard addition method were compared to results obtained while using the IS method to ensure the accuracy of the latter method.

It should be noted that quantification of all six analytes was achieved by using the quantifier ion extracted for each compound, in both IS and standard addition methods. The quantifier ion represented the fragment ion with the highest abundance. The selected quantifier ions are the fragment ions bolded in Table 6. Since these ions were unique to each of these compounds, accurate qualification and quantification could be achieved.

For both methods of quantification i.e., calibration methods, spiking of samples was used. Before starting the measurements, two spiking methods were tested – 1) direct spiking of paper samples, and 2) spiking solutions onto the glass of the vial, instead of direct spiking of paper. Received abundances for both methods were compared and no significant difference in abundances were noticed. Therefore, direct spiking of 100 mg of paper was selected for further measurements. Selection was arbitrary, however direct spiking was selected as it better describes real conditions. Since the packaging material of real samples without printing inks and consequently, photoinitiators, could not be obtained, a similar possible complex matrix tried to be employed. For that purpose, an unbleached paper, with 75 $g/m^2$ grammage was selected.

Specific migration limits (SML) for each of the selected photoinitiators, regulated in the Swiss Ordinance of the FDHA on materials and articles intended to come into contact with foodstuffs, edition 2.1 (Federal Department of Home Affairs, 2020) are shown in Table 7. They were used as framework values in calculating worst case scenario and accordingly for setting up of the calibration levels.

**Table 7.** Regulated SML values for each selected PI and calculated worst-case scenario values for 100 mg of paper

| ***Analytes*** | **SML values (mg/kg of food)** | **Calculated worst-case scenario for spiking 100 mg of unbleached paper with grammage 75 g/m$^2$ (µg/100 mg of paper)** |
|---|---|---|
| *Benzophenone* | 0.6 | 13.33 |
| *1-Hydroxycyclohexylphenyl ketone* | 0.01 | 0.22 |
| *4-Methylbenzophenone* | 0.6 | 13.33 |
| *2,2-Dimetoxy-2-phenyl acetophenone* | 0.01 | 0.22 |
| *2-methyl-4'-(methylthio)-2-morpholinopropiophenone* | 0.01 | 0.22 |
| *2-Isopropythioxanthon* | 0.05 | 1.11 |

The SML values are usually expressed in mg per kilogram of food. However, if needed they can be easily recalculated and expressed in different units. For example, the EU cube rule assumes that a person with an average weight of 70 kg, consuming 1 kilogram of food per day, packed in a packaging material of 6 dm$^2$ area. Consequently, the SML can be expressed as mg/dm$^2$. That correlation was used to determine worst-case evaluation for selected photoinitiators when measuring 100 mg of unbleached paper with 75 g/m$^2$ grammage. For the calculation of worst-case scenario firstly it was calculated how much food can be packed in 100 mg of paper (75 g/m$^2$). A 100 mg of that paper represents area of 0.1333 dm$^2$ calculated from paper grammage. When compared to EU cube assumption it is equivalent to 0.0222 kg of food. If the SML is then, for example, 0.6 mg/kg of food, the value 13.33 µg represents the equivalent to 100 mg of paper. The calculated values are also shown in Table 7.

Analyte concentration selection for the calibration levels was calculated according to the calculated worst-case scenarios for 100 mg of selected paper. The calibration levels were set in a way to effectively cover the range of calculated values for selected photoinitiators. Since the fourth calibration level expressed as SML was 13.5 µg/kg of food and the highest SML of

all employed analytes was 0.6 mg/kg of food, which when recalculated as explained before was 13.33 ug/kg of food, last calibration point was set to 18 μg/kg of food. Also, in that way a minimum of 5 calibration levels required was obeyed.

The solution concentrations used for spiking ranged from 5 mg/L to 40 mg/L. When correlated with a spiking volume of 10 μL that means that a range from 50 ng to 400 ng of analytes was measured. If expressing it as corrected SML, a range from 2.25-18 μg/kg of food was used.

### 7.2.1. Internal standard method

For fully effective use of the IS method, two fundamental criteria should be fulfilled. Namely, an IS should not be present in the sample that will be analysed, and the compound or compounds used as ISs should have a chemical structure as similar as possible to the compound of interest to be calibrated.

The calibration curves when using the IS method are constructed by plotting the response for each analyte to be calibrated against a known concentration of the photoinitiator. The response represents the ratio of analyte area to IS area. Both peak areas of each compound and IS peak areas were calculated as averages from 3 measurements. To determine the concentration of an analyte of interest, the peak area received was divided by the IS peak area and plotted onto the obtained calibration curve.

In the ideal case, the addition of ISs to the sample should be as soon as possible, so the IS was added during the stock solution dilution. That way inaccuracies that might result from the sample preparation are accounted for i.e., all the possible preparation mistakes will reflect on the IS as well. Also, when used as calibration method, injection processes are accounted for as well.

Deuterated benzophenone (d10-BP) was used as an IS for all analytes. It was present in all measurements in the same quantity of 100 ng.

Typical calibration curves obtained for each photoinitiator are shown in Figures 3 – 8.

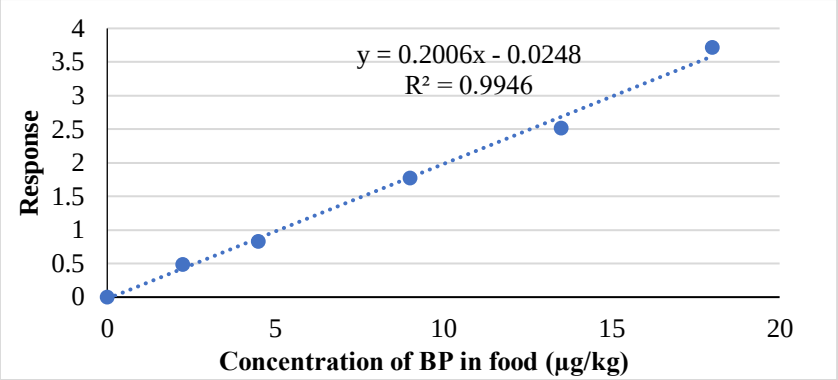



**Figure 3.** Internal standard calibration curve obtained for Benzophenone (BP)

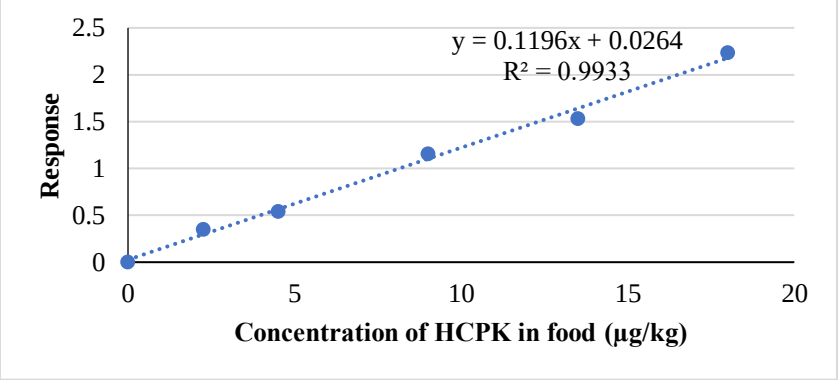



**Figure 4.** Internal standard calibration curve obtained for 1-Hydroxycyclohexylphenyl ketone (HCPK)

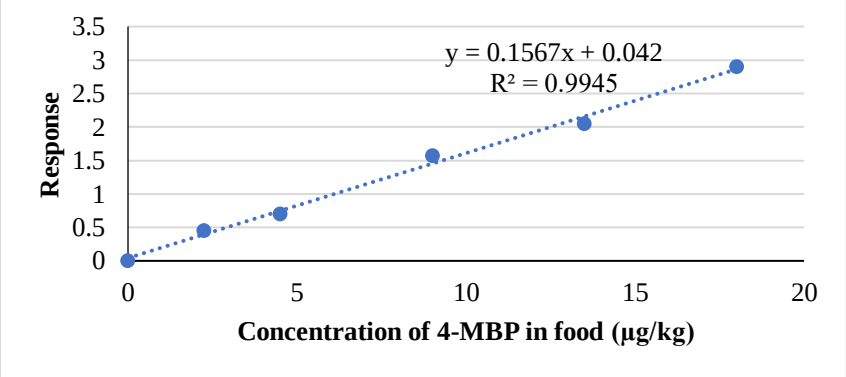



**Figure 5.** Internal standard calibration curve obtained for 4-Methylbenzophenone (4-MBP)

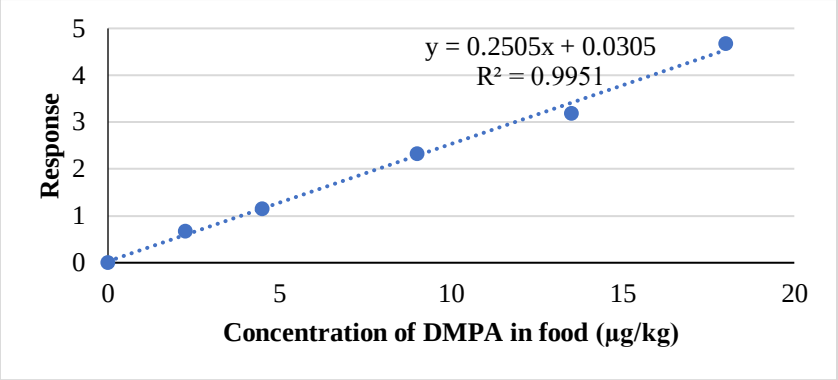



**Figure 6.** Internal standard calibration curve obtained for 2,2-Dimetoxy-2-phenyl acetophenone (DMPA)

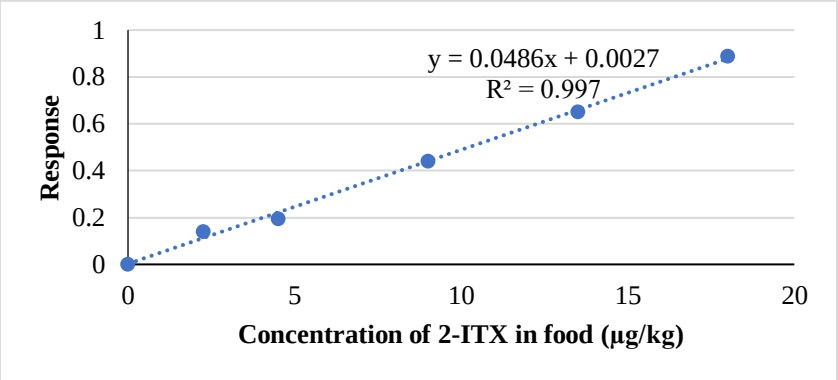



**Figure 7.** Internal standard calibration curve obtained for 2-Isopropythioxanthone (2-ITX)

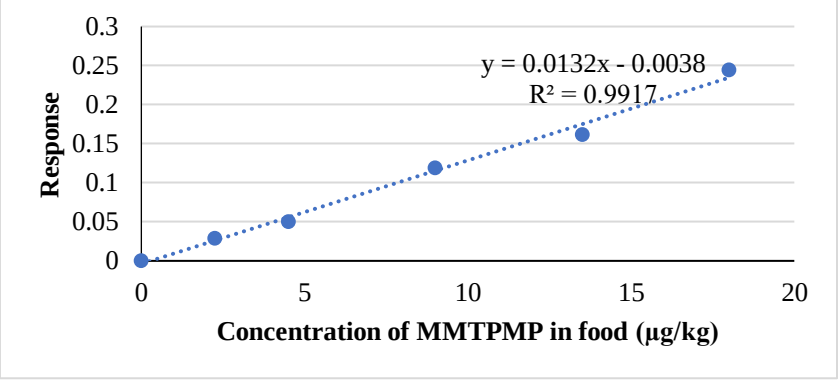



**Figure 8.** Internal standard calibration curve obtained for 2-Methyl-4'-(methylthio)-2-morpholinopropiophenone (MMTPMP)

For all curves, it was focused on coefficients of determination ($R^2$) having values bigger than 0.99 which is considered good accuracy. The highest value of $R^2$ was obtained for 2-ITX, while the lowest value of 0.9917 was obtained for MMTPMP. Due to the fact that MMTPMP is an analyte with the highest MW (279.40) and highest boiling point (420.1 °C) of all six

analytes, it was concluded that to obtain the bigger value of $R^2$ for MMTPMP a longer extraction time should be employed, for example, 30 minutes. However, it is concluded that improvement of all $R^2$ trying to reach even higher values, could be set as a goal if the method will be further optimized and validated, which is out of the scope of this study.

### 7.2.2. Standard addition method

Even though the standard addition method is considered to be more accurate because possible matrix effects are accounted for, it is found as more labour-intensive. Peak areas in the graph provided to entail calibration curves present the average of peak areas received from 3 parallel measurements.

Typical calibration curves obtained for each analyte using the standard addition method are shown in Figures 9 – 14.

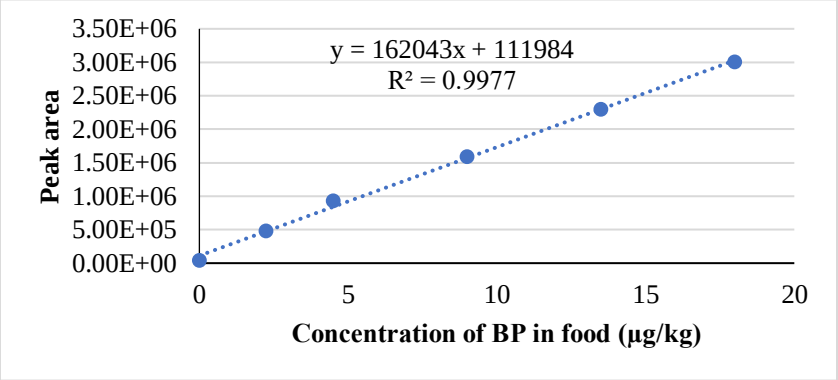



**Figure 9.** Standard addition calibration curve obtained for Benzophenone (BP)

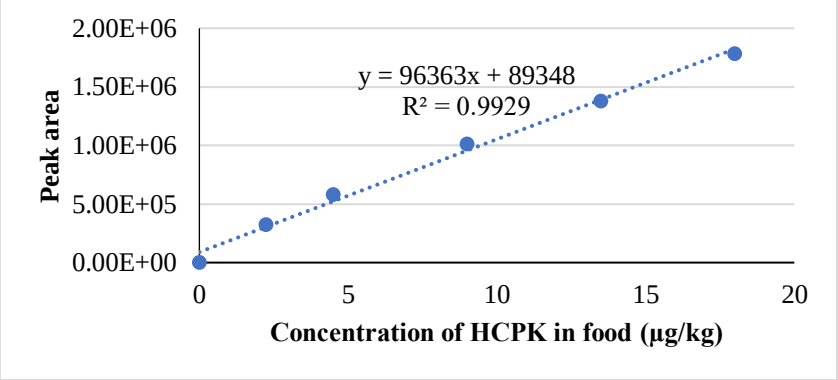



**Figure 10.** Standard addition calibration curve obtained for 1-Hydroxycyclohexyphenyl ketone (HCPK)

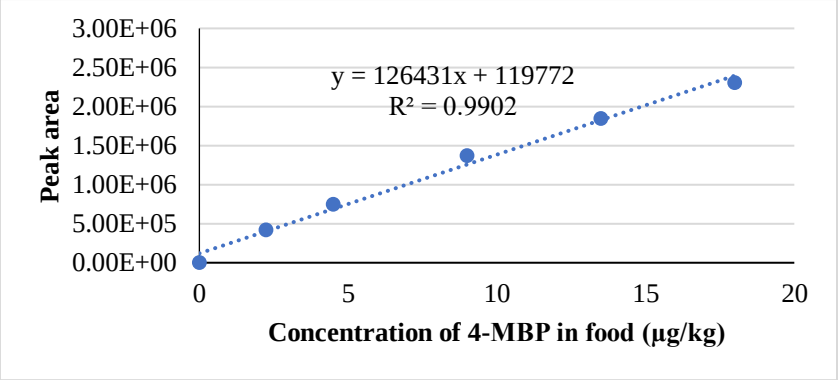



**Figure 11.** Standard addition calibration curve obtained for 4-Methylbenzophenone (4-MBP)

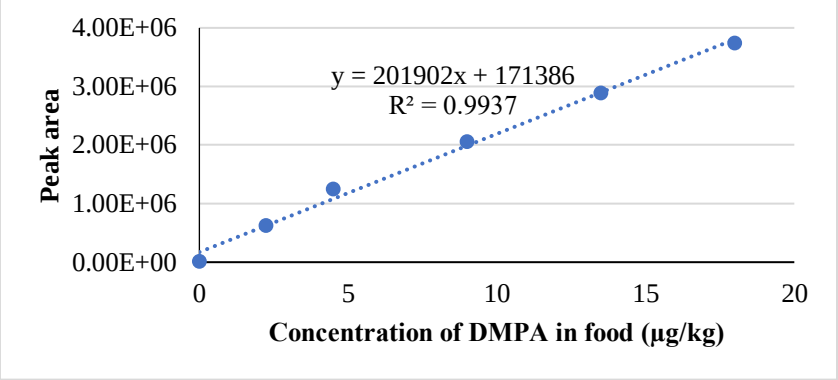



**Figure 12.** Standard addition calibration curve obtained for 2,2-Dimetoxy-2-phenyl acetophenone (DMPA)

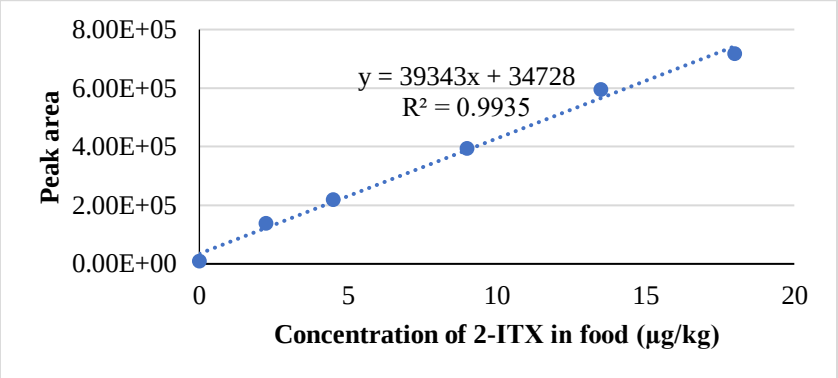



**Figure 13.** Standard addition calibration curve obtained for 2-Isopropythioxanthone (2-ITX)

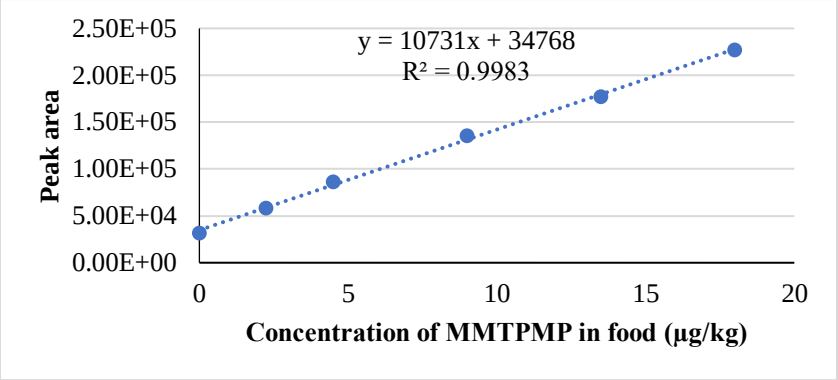



**Figure 14.** Standard addition calibration curve obtained for 2-Methyl-4'-(methylthio)-2-morpholinopropiophenone (MMTPMP)

For all curves, the $R^2$ obtained were bigger than 0.99 which is considered a good accuracy.

Based on all aforementioned, it can be concluded that the results obtained from both quantitative methods were found to be in good agreement. However, the IS method is selected for further analysis of food packaging materials because the IS method was deemed to fit more for routine analysis since it is less labour-intensive.

### 7.2.3. Limits of detection and limits of quantification

A validation of the analytical method is necessary for further usage in laboratory conditions with great certainty in received results. The validation includes the assessment of the linearity of the calibration curves, the determination of recovery and precision as well as of the LOD and the LOQ. However, since validation of the method was out of the scope of this study, it was not performed.

However, the LODs and LOQs were calculated (Table 8). The LOD presents the minimum concentration of an analyte that can be detected with acceptable certainty, in that case the analyte is only qualifiable. On the other hand, the LOQ refers to the minimum concentration of an analyte that can be quantified, with acceptable precision.

The LODs and LOQs were determined with the signal-to- noise ratio. The signal was manually measured value of the analyte peak height, while noise was calculated as peak-to-peak noise of the baseline around the target analyte. For the calculation the lowest calibration point, 5 mg/L, n = 3 was used. The signal-to-noise ratio was then used to calculate the concentrations that would give signal-to-noise ratios of 3:1 and 10:1.

**Table 8.** Limits of Detection and Quantification for the analytes in solvent standards

| Analyte | Ion fragment used to calculate LOD and LOQ | LOD (µg/kg) | LOQ (µg/kg) |
|---|---|---|---|
| Benzophenone | 105 | 0.1689 | 0.5628 |
| 1-Hydroxycyclohexylphenyl ketone | 99 | 0.4804 | 1.6014 |
| 4-Methylbenzophenone | 119 | 0.1661 | 0.5536 |
| 2,2-Dimetoxy-2-phenyl acetophenone | 151 | 0.1077 | 0,3589 |
| 2-methyl-4'-(methylthio)-2-morpholinopropiophenone | 128 | 0.3835 | 1.2784 |
| 2-Isopropythioxanthon | 239 | 0.1788 | 0.5961 |

The great sensitivity of the developed method can be confirmed by obtained LOD and LOQ values. All obtained values are bellow 1 µg/kg, with exceptions on LOQ values of HCPK and MMTPMP. The similiar results were also reported in Mairinger (2012) with similiary high LOQ values for the MMTPMP of 1.56 µg/kg. However, it should be noted that differencies in obtained values are visible since compared work did not used SPME as injection method.

### 7.3. ANALYSIS OF THE RESPECTIVE FOOD PACKAGING MATERIAL

The selected food packaging materials were analysed by the developed SPME GC-MS method in the context of this master thesis. A 100 mg of each packaging material were cut into smaller pieces and spiked with 100 ng of deuterated benzophenone as an IS.

The results of the analysis of the 12 foodstuff respective packaging materials are summarised in Table 9 and Figure 15. BP was found to be present in every analysed packaging material, while MMTPMT was not found in any of it. The Figure 15 shows the percentage of packaging materials in which selected photoinitiator analytes were found.

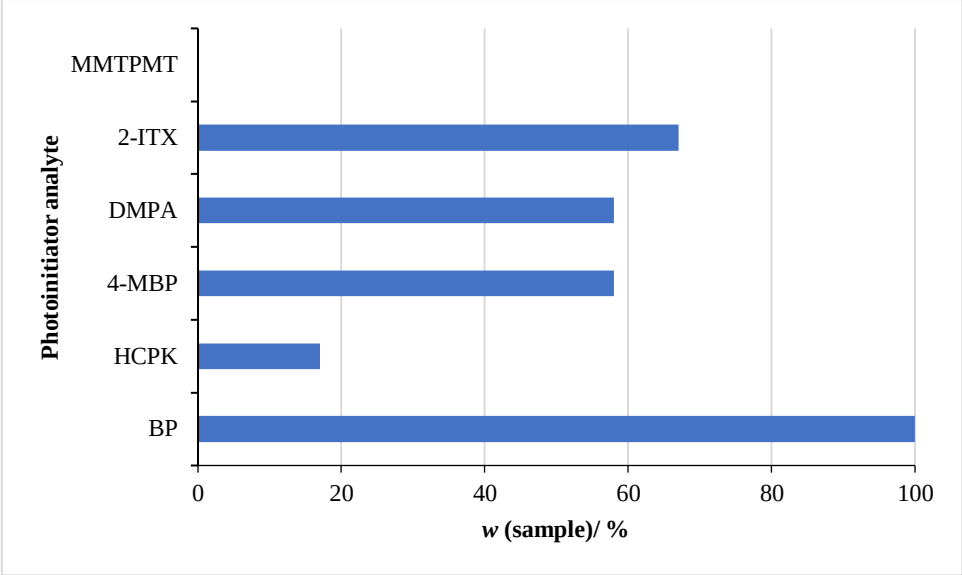



**Figure 15.** Percentages of analysed packaging materials containing the respective photoinitiator analytes

An example of SIM gas chromatogram received for the analysis of P01 spiked with 100 ng of IS is shown on Figure 16. Also, peaks of each analyte found in the respective packaging is marked.

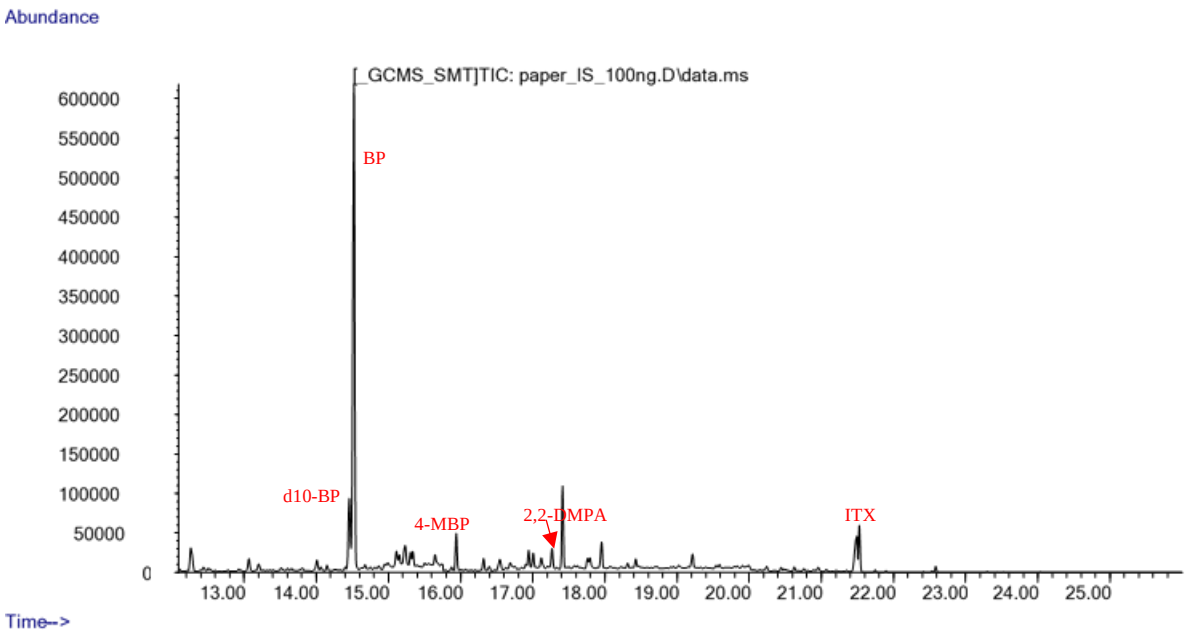



**Figure 16.** Gas chromatogram of the packaging material P01

After determination of photoinitiators present in each packaging material, the quantification was performed. For quantification, calibration curves obtained from selected IS method of quantification (Figures 9-14) were used. For each of the analyte (if present) the selected unique

fragment ion was extracted and integrated presenting the peak area of the analyte. Since all the measurements were done in three parallels, the average of peak areas was taken for further measurements. The received peak area was then divided with average internal peak from same three measurements. Ratio of analyte peak area and IS area was plotted onto the calibration curve to calculate the quantity of analyte present in respective packaging material. Table 9 shows the values received after plotting peak areas onto the obtained calibration curves.

**Table 9.** Photoinitiators found in analysed packaging materials

| Food packaging material sample | Quantity of photoinitiators in food (μg/kg)* | | | | | |
|---|---|---|---|---|---|---|
| | BP | HCPK | 4-MBP | DMPA | 2-ITX | MMTPMT |
| P01 | 39.23 | / | 3.36 | 1.32 | 0.53 | / |
| P02 | 4.41 | / | 3.01 | 0.69 | 0.44 | / |
| P03 | 4.97 | / | 5.02 | / | 0.08 | / |
| P04 | 4.18 | / | 9.74 | 0.74 | 0.08 | / |
| P05 | 10.87 | / | 4.71 | 0.46 | 0.31 | / |
| P06 | 12.82 | / | / | / | 0.35 | / |
| P07 | 10.20 | / | / | / | 0.17 | / |
| P08 | 9.14 | 1.81 | / | / | / | / |
| P09 | 5.76 | / | / | / | 0.30 | / |
| P10 | 19.42 | / | 18.51 | 0.76 | / | / |
| P11 | 7.38 | 3.61 | / | 0.94 | / | / |
| P12 | 6.19 | / | 6.06 | 0.66 | / | / |

*The values are expressed as μg/kg of food, converted obtaining the EU cube rule, already afore explained in the Chapter 7.2. Quantification of selected photoinitiators

As can be seen, in all analysed food packaging material samples at least two out of the 6 analysed photoinitiators were detected. Out of the six selected photoinitiators the most prominent ones in the anaylsed samples were BP and 4-MBP. BP was found in all analysed sampled in quantities ranging from 4 up to 40 μg/kg of food. Only one more sample contained higher quantities of photoinitiators; 4-MBP detected in P10 in total quantity of 19 μg/kg of food, while in the other samples it ranged from 3 to 10 μg/kg of food. However, both were

bellow regulated SML of 0.6 mg/kg. All other photoinitiators were detected in samples below 10 μg/kg of food and bellow regulated SMLs.

The BP and 4-MBP found in the majority of samples can be explained with the fact that both photoinitiators have highest SML values, which is probably the reason for higher frequency of their usage. The same was also confirmed within the results from Mairinger (2012) and Anderson and Castle (2010) founding BP and 4-MBP in almost all the samples in highest concentration. Even though, the numerous works were published regarding method development for determination of different photoinitiators, most of them were based on migration studies and analysis of food samples packed in printed packaging material. Also, so far there were no published GS-MS methods developed with SPME injection found, which is one of the reasons why it was chosen as injection method in this work.

# 8. CONCLUSION

1. Due to the good printability of paper and board materials, most of the packaging materials are nowadays printed. For that purpose, often UV inks are employed. They contain, among other compounds, photoinitiators that are characterized by low-molecular-weight and therefore might tend to migrate from the packaging into the food.

2. Even though the legislation on printing inks is diverse and, until today no European-specific community legislation concerning printing inks for food packaging exists, inks should not release substances to the packed food in quantities that could result in unwanted changes.

3. Compounds that might migrate to food and endanger human health or change the characteristics of food itself have regulated specific migration limits. Also, if there are more migrating substances present, the overall migration limit should also be obeyed. Those limits are usually very low. Therefore, accurate, highly selective and sensitive method in combination with a simple sample preparation procedure should be developed for the determination of respective photoinitiators.

4. A GC-MS method in combination with SPME injection was successfully developed for the determination of 6 selected photoinitiators in various packaging material samples.

5. The analysis of different packaging materials showed the presence of various photoinitiators. All results were below the specific migration levels. BP is found in all samples in concentrations from 4 up to 40 μg/kg of food, while MMTPMP was not found in any of the analysed samples. 4-MBP is detected in highest quantity of 19 μg/kg of food in one sample, while all other photoinitiators were detected in samples below 10 μg/kg of food.

6. The developed method represents a worst-case evaluation and in case of exceeding the SML alternative migration studies are necessary.

7. If the method would be submitted for routine laboratory analysis, it should be validated.

# 9. REFERENCES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DECLARATION OF ORIGINALITY

I, LARA SKEF declare that this master's thesis is an original result of my own work and it has been generated by me using no other resources than the ones listed in it.

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Signature