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Comparison of the migration of bisphenol A and bisphenol S from paper food contact materials into fatty foods with that of cold water extracts

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1 Introduction

Food contact materials (FCMs) made of paper, board and carton contain a variety of substances, which are able to migrate into foodstuffs, such as mineral oil hydrocarbons, polycyclic aromatic hydrocarbons, phtalates or bisphenols [1, 2]. Phtalates and bisphenol A for example may influence the organoleptic properties of food or might cause endocrine disrupting effects in humans, thus having an impact on human health [3]. Among raw materials, manufacturing aids and finishing fabrics, paper products made from primary fibers or recycling material may contain contaminants like by-products from the production of printing colors or color developers from thermal papers [1, 2, 4, 5]. When the product is intended to come into contact with food, those compounds may potentially migrate into foodstuffs.

To simulate the migration from FCMs into food, cold water extraction (CWE) according to DIN 645 is performed [2, 6]. CWE is a so-called conventional method to simulate migration processes. DIN 645 states that 10 g of sample should be used for extraction. In case that strongly absorptive paper products such as napkins and paper towels are used, most of the water is absorbed when 10 g of sample are used for extraction. This is not the case for paper or cardboard. The absorption of the solvent might have an impact on the extraction efficiency or migration rate of analyte into CWE.

In CWE from paper napkins, primary aromatic amines (PAA) have been found [4]. This is caused by the fact that FCMs made from paper, such as napkins, are often printed and printing inks may contain contaminations like PAAs. Another substance group that is often found in paper made from recycled fibers are bisphenols, especially bisphenol A (BPA) and bisphenol S (BPS). These substances originate from thermal paper, through which they are introduced in the recycling process of paper [1, 2, 7].

Besides the application of conventional methods like CWE, it is possible to perform migration tests with food. According to Regulation (EC) 10/2011 on plastic materials and articles intended to come into contact with food, the results of migration tests obtained with food prevail over those obtained with food simulant [8]. Migration tests with different kinds of food (wet, dry, acidic and fatty) were already performed for PAAs in printed napkins, but migration tests for bisphenols from paper were mainly conducted for dry foods up to this point [1, 4]. Wet and fatty foodstuffs that were tested with regard to the migration of bisphenols from paper FCMs were cucumber and sausage [9]. While those results were published, the author was informed about similar tests with cucumber and meatloaf conducted by the CVUA Stuttgart. These results have not been published yet. The data suggest that only small amounts of BPA and BPS are released into foodstuffs

from paper FCMs in a scale of ng per dm^2 of surface area. Since there are other foods that have a higher fat content and longer contact time with paper FCMs made from recycled fibers (e.g. pizza) determination of migration into those kind of foodstuffs seems necessary, too.

For the analysis of bisphenols, different methods for sample preparation and analysis were already in place. A very attractive method for sample preparation in general is the QuEChERS method (QuEChERS being an acronym for Quick, Easy, Cheap, Effective, Rugged, Safe) which was originally established for the determination of pesticide residues in fruits and vegetables [10]. The original method is comprised of solvent extraction with acetonitrile, followed by separation of organic solvent and water by addition of salt consisting of a mixture of sodium chloride and magnesium sulfate. Clean up and water removal are achieved by dispersive solid-phase extraction (dSPE). The dSPE material contains magnesium sulfate and primary secondary amines (PSA), but its composition can be altered with regard to the sample matrix. QuEChERS methods were established for BPA and BPS in different food and biological matrices like infant formula, sweetened condensed milk (can) and breast milk, tea, seafood (fish, seaweeds), canned vegetables and fruits, human urine and earthworms [11–17], but not for food that is likely to come into contact with paper FCMs and has a wet and/or fatty consistency.

1.1 Food contact materials

Food contact materials (FCMs) are defined by the European Food Safety Authority (EFSA) as materials that are created with the purpose to come in contact with foodstuffs. Examples for FCMs are dishes, napkins, packaging and kitchen utensils. FCMs are made from various raw or recycling materials such as paper, glass, metal, wood or plastics [18]. According to Regulation (EC) No. 1935/2004 on materials and articles intended to come into contact with food, FCMs should not release substances into foodstuffs in any amount that is suitable to induce a possible health risk, an unreasonable change in their composition or any alteration of their organoleptic properties. For some substances, a Specific Migration Limit (SML) was established, which determines the maximum concentration of that substance in food [8].

The production of FCMs from recycling materials is to be preferred with regard to the protection of the environment [19]. However, FCMs made from recycling materials can be problematic e. g. in the sense that during the recycling process hazardous chemicals from the original product can be accumulated [1]. Therefore it is necessary to examine the amount of harmful substances in recycling products and their actual transfer into food. Paper and board materials have the highest minimum targets for recycling by weight (see table 1). Thus it is very likely that hazardous substances are transferred into the recycled fibers. It is shown that Germany's regulation for recycling is stricter than the Directive of the European Parliament and Council [20, 21].

Table 1: Recycling rate according to Directive 94/62/EC and *Packaging Law in Germany [20, 21]. The given data represent the minimum targets in mass percentage.

material	until 2026 (EU)	until 2031 (EU)	from 2022*
glass	70	75	90
paper and board	75	85	90
ferrous metals	70	80	90
aluminum	50	60	90
plastics	50	55	70

1.1.1 Paper and board FCMs

Essentially, paper and board are made of fibrous materials such as cellulose (natural and synthetic origin), fibers of synthetic high polymers[1], wood pulp or recycled fibers of paper, board and carton. All of these are considered to be raw materials in the production of paper and board [2]. When paper is made from recycled fibers, the raw material is also called "secondary fibers" [22].

Amongst raw materials, recommendation XXXVI of the German Federal Institute for Risk Assessment (ger. Bundesinstitut für Risikobewertung, BfR) states manufacturing aids and finishing materials allowed for the production of FCMs made of paper and board. The recommendation is filling a legal loophole, while it is not legally binding itself. Paper and board FCMs are generally subject to Regulation (EC) No. 1935/2004, which contains a list of materials that may be covered by specific measures. Paper and board, printing inks, plastics, varnishes and coatings are among them, but no specific measure was enacted for neither paper and board materials nor printing inks. Thus, they lack further legal clarification [19, 23]. Members of the paper and board industry[2] published the "Food Contact Guidelines for the compliance of paper & board materials and articles". The guidelines do not provide a legal framework but aim at filling the void of harmonized standards in absence of a specific measure by the European Comission [24].

1.1.2 FCMs made from paper and board with recycled fibers

BfR Recommendation XXXVI states SMLs for different substances that may be transferred into food, such as primary aromatic amines (PAAs). FCMs made with secondary fibers have to meet additional criteria concerning contaminations from raw material, e. g. components of printing ink. Contamination can be limited by applying rules of Good Manifacturing Pratice (GMP) like thorough selection of raw material and appropriate cleaning techniques during the recycling process [2, 25]. It is advisable to consider the intended use of the final product (temperature, contact time etc.) when choosing the raw material,

[1] Those fibers have to fulfill the requirements of the corresponding food laws.

[2] Alliance For Beverage Cartons and the environment, Cepi ContainerBoard (CCB), European Carton Makers Association, European Tissue Symposium, International Confederation of Paper and Board Converters in Europe (CITPA), European Federation of Corrugated Board Manufacturers (FEFCO) [24].

since the selection of the starting material has a strong impact on the quality of secondary fibers [1, 24].

Due to the current level of knowledge certain phtalates, mineral oil hydrocarbons, benzophenone, BPA, diisopropylnaphthalene and 4,4'-bis(dimethylamino)-benzophenone are substances that are transferred and/or accumulated during the recycling process and have to be investigated concerning their potential for migration into food [26]. For 4,4'-bis(dimethylami-no)-benzophenone and BPA, it is only necessary to check whether the SML is exceeded, when the FCM is intended to come in contact with moist and/or fatty foodstuff [2]. This can be the case for a pizza box or paper plates, that are in contact with such food and are made with secondary fibers (see figure 1) [7].

Figure 1: Different moist and fatty foods in contact with FCMs made with paper from secondary fibers. In this case, the migration rate for certain analytes has to be determined to check whether their migration limits were exceeded [2, 27–29].

1.1.3 Legal regulations for migration tests of paper and board FCMs

Migration is defined by EFSA as the transfer of substances or particles from FCMs into food [30]. General requirements for the inertness of FCMs are given in Regulation (EC) No. 1935/2004. Additionally, Regulation (EC) No. 10/2011 functions as a specific measure and contains a list of permitted substances that are allowed for the production of plastics. The SML in this list might be applied to other FCMs as well. For example, PAAs should not be released from plastic FCMs into food (with a detection limit of 0.01 mg kg^{-1}). Since PAAs were also found in colored paper napkins, the SML is applicable for paper in this case, too [8, 31].

Food or food simulants are used to analyze the transfer of substances during migration tests. The results of migration tests with food have a higher priority than those performed with food simulants. Two examples for food simulants that are stated in Regulation (EC) No. 10/2011 are plant oil or 10 vol.-% solution of ethanol. Besides food simulants, Annex III of Regulation (EC) No. 10/2011 also defines contact times and temperature conditions for migration tests with FCMs made from plastics [8]. To ensure comparability of the results from migration tests of plastic kitchen untensils in contact with food, the European Union Reference Laboratory for Food Contact Materials (EURL-FCM) and the National Reference Laboratories (NRLs) of the network published a report with conditions for testing kitchenware articles in contact with foodstuffs, thus replacing the Joint Research Centre's (JRC) Guidelines on Testing Conditions for Articles In Contact With Foodstuffs (With a

Focus on Kitchenware) of 2009 [32].

Concerning the migration from paper FCMs, the BfR published a guideline in 2015. Simulation of the transfer into food is necessary to evaluate the migration. It is usually performed via conventional procedures that represent "worst-case scenarios" of the migration setting. Still, it is supposed to represent a realistic setting for the underlying conditions and the contact between food and FCM. The depiction of real migration processes is sometimes problematic because food simulants that are used according to different DIN norms may not show the same results for migration as real food. In that case, the test has to be performed according to latest scientific knowledge (contact conditions and test food). Otherwise testers should use a food that is a worst case representative for contact with that specific FCM. For simulation of the contact of a moist or fatty food with paper FCMs, either a migration test or a CWE (DIN EN 645) is prepared for analysis [33]. The conditions for migration tests determined in Regulation (EC) No. 10/2011 are applied with regard to temperature, food simulant and contact time [8]. For paper towels and napkins, the BfR recommendation specifically mentions that preparing CWE is the standard method for the analysis of migrating substances because the materials are not durable enough for a migration test[3]. Regardless of the raw material (primary or secondary fibers), the analytical methods for paper FCMs are the same and include chromatography, mass-spectrometry and spectroscopy [1, 33].

The CWE as a simulation for migration is being criticized by the industry, because it is assumed to be inadequate. For instance, there is a discussion whether paper FCMs like paper towels should be used at 10 g initial sample weight for CWE as DIN 645 states. Due to a variation in grammage from board materials, a bigger surface area is in contact with the water and therefore the comparability between lighter paper FCMs and board materials is poor. There is also a problem regarding paper FCMs that swell when they come in contact with water, since there is less water available for extraction because of the absorption into the paper.

1.2 Possible contaminants in paper and board

As the list of the BfR recommendation XXXVI defines, many substances may be used in the production of paper and board materials [2]. A lot of paper and board packaging is in contact with dry foods such as rice, noodles, flour, bread or sugar. In this case, the possible transfer of contaminants is taking place via the gas phase. Besides, paper and board FCMs are used for packing fast foods like pizza and hamburgers, which have a high-fat content [1, 7]. Also moist and/or fatty baked goods like cake or croissants may be wrapped in paper bags or put in cardboard boxes. Since not all of those FCMs are made from primary but (also) recycled fibers, the variety of substances that can migrate into the foodstuff increases. This is due to the raw material and its original use, because the composition of the raw material depends on its initial usage and it might not have been initially produced

[3]Materials are not durable for migration tests as long as they do not possess a functional barrier.

to fit the requirements of FCMs [1]. Regardless of the contaminants' origin, some of them present a risk to human health [7]. That risk depends on the contaminants' concentration in FCMs and the amount of hazardous substances that is released into food. That is why it is necessary to analyze the amount of substance in paper FCM as well as the foodstuff after the migration test to see whether the concentration of the substance of interest is within or above the SML.

1.2.1 Bisphenols

Bisphenols belong to the substances that may be recovered in paper FCMs made from recycled fibers. This is caused by the consumers' wrong disposal of thermal paper with paper waste instead of residual waste. Thermal paper contains BPA that was used as a color developer. Thus, paper products gained from recycling paper are contaminated with color developers from thermal paper that was introduced into the recycling process. The bulk of thermal paper is disposed correctly with residual waste, only 15 % of thermal paper are rediscovered in paper waste. Nevertheless, secondary fibers are contaminated with BPA in significant amounts [1]. Then, they are used in the production of FCMs, thus exposing foodstuff to the contaminant[4] [34]. The obligation to hand out receipts in Germany is in place since January 2020. In Austria the determination is valid since July 2016 [35, 36]. Due to these obligations, the amount of thermal paper in circulation does not decrease and even more thermal paper may be introduced into the recycling process. Thus, the amount of BPA and BPS in recycling paper FCMs and their release into foodstuff is of interest with regard to the public health since these compounds represent a harm to human health when they are consumed in certain amounts.

After an assessment of its potential health risk to cashiers and workers who often handle thermal paper, Regulation (EC) 2016/2235 amending Annex XVII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards bisphenol A, restricted the concentration of BPA in thermal paper to 0.02 wt%. The restriction took effect on the 2nd of January 2020. Since BPA has to be replaced in thermal paper, its structure analogue BPS seems to present an alternative as a color developer for the paper industry [37–40].

1.2.1.1 Bisphenol A

2,2-Bis(4-hydroxyphenyl)propane (BPA, CAS 80-05-7, see figure 2) occurs in coatings made from polycarbonate, since it is used as a starting material (monomer) in the production of plastics [8, 31, 41]. In epoxy resins, which are used as can coatings, BPA is used in both the formation of BPA diglycidyl ether (BADGE, CAS 1675-54-3, see figure 2) and in polymerization reactions [42]. BPA is known to act as an endocrine disruptor due to

[4] During flotation, a step of the recycling process, a little bit of BPA is deposited but the amount is so low that it is not quantifiable [1].

its spatial structure which is comparable to 17β-estradiol. Hence it is able to fit into the tertiary structure of estrogen receptors. BPA does not have strong estrogenic properties. Though the binding affinity to the estrogenic receptor is 10,000-100,000 weaker compared to estradiol, studies suggest that BPA may inhibit the activity of thyroid hormones and the synthesis of testosterone [43].

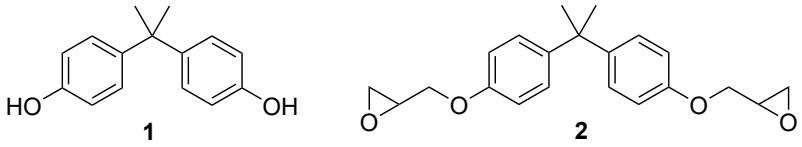


Figure 2: Structure of BPA (CAS 80-05-7, **1**) and BPA diglycidyl ether (BADGE, CAS 1675-54-3, **2**).

In 2015, EFSA published a new assessment on BPA with the intention to reevaluate its health risks. Recent data showed the need for a new evaluation concerning the exposition routes with focus on dietary exposition. A new temporary tolerable daily intake (t-TDI) with 4 µg per kg body weight per day was established and will be updated after data from an ongoing long time study in rodents are available [41]. There are different routes for the intake of BPA. For this work, the focus lies on the internal exposure through diet since it deals with the migration of BPA into food and resulting risks for the human health. It is also the main source of exposure. After absorption, BPA is conjugated to BPA-glucuronide by UDP-glucuronyl-transferase (UGT) which is its main metabolite in humans. Sulfation of BPA by sulfotransferases (ST) is possible as well (see figure 3). BPA and its conjugates are excreted via the kidneys and also via breast milk (rats, humans) [42].

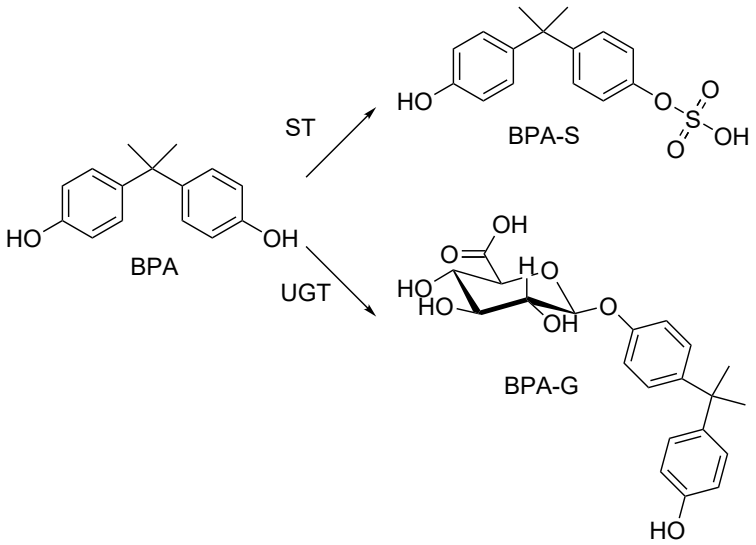


Figure 3: Human metabolism of BPA, according to the Scientific Opinion on the risks to public health related to the presence of bisphenol A, EFSA [42]. BPA is mainly glucuronidated via UDP-glucuronyl-transferase (UGT), but also metabolized by sulfation via sulfotransferases (ST).

Moreover, BPA is classified as probably reprotoxic (Repr. 1B) and STOT SE 3 (**S**pecific **T**arget **O**rgan **T**oxicity **s**ingle **e**xposure; irritating to the respiratory tract and/or has a narcotic effect). It can cause serious eye damage (Eye Dam. category 1 - irreversible effects) and skin allergies (Skin Sensitization, category 1) [44, 45]. Since BPA is defined as an endocrine disruptor of category 1B, this means that most data has been gained from animal studies. For reprotoxicity, the data has to present clear evidence of an effect on fertility and sexual funtion of men and women as well as being developmentally toxic to their descendants, while there is a lack of other toxic effects. In order to be classified as toxic to reproduction (category 1B), the impairment of reproduction may not be the secondary result of other toxic effects, if any occur. The term STOT SE describes the non-lethal, deleterious effect of a substance after single exposure. The classification as such is independent from the effect's reversibility and the elapsing time period before the appearance of symptoms. The classification does not only include the effect of the substance on the target organ, but can also include harm to other organs that are less severe. Substances with STOT of category 3 have a reversible effect on the human body, health is restored after a certain amount of time.

In 2016, the ECHA (European Chemicals Agency) received documents from different member states which identified BPA as a substance of very high concern (SVHC). As a result of the decision of the Member State Committee (MSC), BPA was put on the Candidate List for eventual inclusion in Annex XIV to REACH (Regulation (EC) 1907/2006) [46]. The recommendation was submitted once more in 2019 [5]. It was argumented that BPA is classified as a toxicant for reproduction (category 1B), it is produced in high volumes and widely spread [47].

In thermal paper, BPA is part of a reactive layer and serves as a color developer. It is a weak acid with a melting point between 80-120 °C. BPA serves as a kind of solvent for the reaction between the color developer and the leuco dye which is colorless before the reaction. The thermally-triggered reaction leads to a proton-transfer, so that the colored product is formed by the change of the pH [37, 48]. Thermal papers include receipts and self-adhesive labels (e. g. for shipping) as well as tickets for events or public transport (see figure 4). The amount of BPA in thermal papers is about 1-2 wt% of the paper product [37].

The handling of thermal paper is the second main source of exposure for the public to BPA [42]. BPA is not chemically bound to components of the paper matrix and therefore easily transferred to the skin [34]. The transfer rate increases significantly when the skin is moist, not only for BPA but for other color developers, too [37]. A market analysis in Germany between 2015-2017 performed by Simat et al. manifested that BPA was the most widely used color developer while other color developers were applied less often. Pergafast® 201 (N-p-Tolylsulfonyl-N'-3-(p-Tolylsulfonyloxy)Phenylurea, see figure 6), an alternatively used color developer, follows upon BPA in terms of usage [37]. From 2018 to 2019, the market analysis was repeated [5]. As a result, Pergafast 201 was present in

[5] This was the 9th recommendation of ECHA for putting BPA in in Annex XIV to REACH [47].

41.7 % of the samples with concentrations above 0.02 wt% and therefore represents the most common color developer on the German market. BPA occurred in 36.0 % of the samples, followed by BPS (13.3 %).

Figure 4: Thermal paper is a source for BPA and BPS. They are used as color developers. Since BPA is restricted to 0.02 wt% since January 2020, it is replaced by other color developers such as BPS [39, 40, 49].

When Regulation (EC) 2016/2235 came into place, BPA had to be replaced as a color developer. According to ECHA, BPS is the main replacement for BPA in thermal papers on the EU market, including imports. BPS-based thermal paper is estimated to make up 61 % of the EU market volume. The estimation was made by ECHA after the European Commission requested that the substitution of BPA in thermal papers would be monitored. The volume of BPA-containing thermal paper in Europe fell by 29 % from 2014-2019 and was nearly completely replaced by BPS-containing paper, which increased by 24 % (see figure 5) [40].

From a more global perspective, BPA was still the most used color developer in thermal paper. In samples from 39 countries, which were gathered by Frankowski et al. between May 2018 and May 2019, 69 % of the samples contained BPA [50]. BPS occurred in 20 % of all samples, but was the only color developer in samples from Japan and the USA. Non-bisphenol color developers were not analyzed.

Regulation (EC) 2018/213 on the use of bisphenol A in varnishes and coatings intended to come into contact with food and amending Regulation (EU) No 10/2011 as regards the use of that substance in plastic food contact materials states a specific migration limit (SML) of 0.05 mg kg^{-1} food/food simulant for BPA from plastic FCMs, which was adopted in the recommendation XXXVI of the BfR for paper and board FCMs[6] [2]. BPA was added to Annex XVII of REACH (Regulation (EC) 1907/2006 on **R**egistration, **E**valuation, **A**uthorisation and Restriction of **Ch**emicals) in 2016 due to its restriction in thermal papers [40, 51].

[6] An examination with regard to the BPA and BPS content of the paper FCM is only required, when the FCM is supposed to come in contact with moist or fatty foodstuffs.

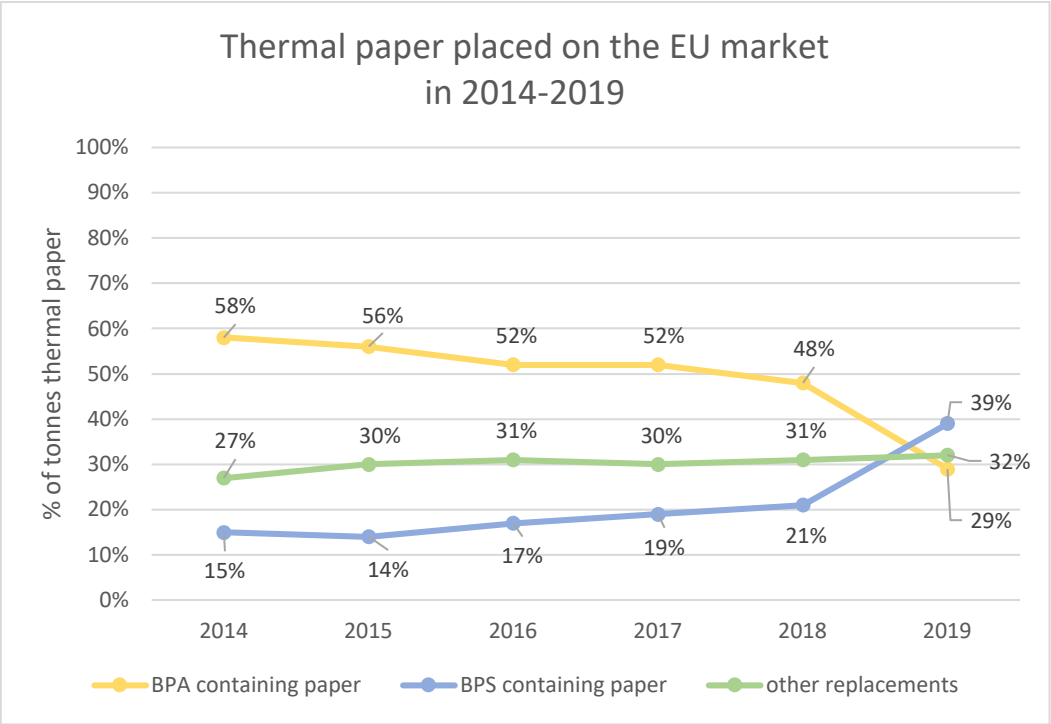


Figure 5: Monitoring of color developers in thermal paper on the EU market (2014-2019) by ECHA [40]. During this time period of four years, the amount of BPS in tonnes of thermal paper increased three times, while the amount of BPA decreased two times.

1.2.1.2 Bisphenol S

The compound 4,4´-dihydroxydiphenyl-sulphone (BPS, CAS 80-09-1) is found in plastic FCMs. The monomer is utilized for the synthesis of polyethersulfone [31]. In Germany, BPS is a substitute for BPA in thermal paper which was predicted by Regulation (EC) 2016/2355 on payment services in the internal market [38]. Because of their structural analogy, concerns have been raised about the toxicology and adverse health effects of BPS which might be similar to those of BPA [39]. BPS and other color developers such as D8 (4-hydroxyphenyl-4'-isopropoxyphenyl-sulfone, CAS 95235-30-6) and Pergafast 201 have been added to the Community Rolling Action Plan (CoRAP) because each of them is suspected to act as an endocrine disruptor. Evaluation of BPS is ongoing while evaluations for D8 and Pergafast 201 (see figure 6) have not started yet. All of the substances will be evaluated by Belgium [40, 52–54]. BPS is not only used as a replacement for BPA, but also appears in thermal paper as a contaminant of the color developer D8. Originally, BPA and BPS are purposefully added to thermal paper as color developers, making them intentionally added substances (IAS) in the first place. However, BPA and BPS are not intentionally added substances (NIAS) in FCMs made from recycling paper.

Besides, BPS is assumed to be CMR (carcinogenic, mutagenic and toxic to reproduction)[55]. BPS - as BPA - might inhibit spermatogenesis, depending on the time of exposure and concentration (human data). In both murine and human preadipocytes, BPS

was found to increase lipid accumulation as well as protein levels for several adipogenic markers. It was discovered that BPS is doing so by activating estrogenic receptors. BPS acts as a weaker estrogen receptor α (ERα) agonist than BPA. The exposure of human breast cancer cells (MFC-7) to different bisphenols (BPA and BPS among them) were found to stimulate hormone-dependent breast cancer, induce cell proliferation, decrease cell adhesion and therefore promote the ability of cells to migrate. All in all, bisphenols are involved in the promotion of cancer progression [56].

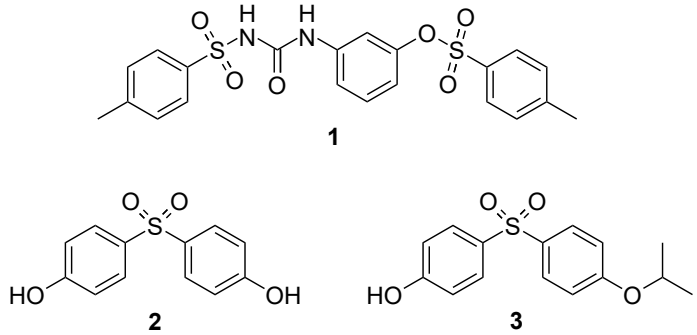


Figure 6: Structures of different color developers that are used in exchange for BPA in thermal paper: Pergafast 201 (CAS 232938-43-1, **1**), BPS (CAS 80-09-1, **2**), D8 (4-hydroxyphenyl-4'-isopropoxyphenyl-sulfone, CAS 95235-30-6, **3**).

The SML of BPA in plastic FCMs is applied for BPS, too (0.05 mg kg^{-1} food/food simulant). According to Regulation (EC) 2019/794 on a coordinated control plan with a view to establishing the prevalence of certain substances migrating from materials and articles intended to come into contact with food, there was no current information about the migration of BPS into food and information about the migration from lackered or coated FCMs was incomplete at the time. Therefore, further investigation of the matter was suggested [31]. A reevaluation of BPS by EFSA in 2020 did not have an impact on the SML of 0.05 mg kg^{-1} food/food simulant. EFSA recommends to gain further data concerning the migration and occurence of BPS in food and the use of BPS in plastic materials.

A study on toxicokinetics showed that BPS is efficiently absorbed ($\geq$ 90 %) by rats when administered orally. Excretion takes place within two days, the main path of elimination is through renal excretion. BPS-glucuronide (BPS-G) was recovered in urine and is also excreted via faeces and bile. In bile other metabolites - sulfated as well as sulfated and glucuronated BPS (BPS-S/BPS-GS) - were observed (see figure 7). The lowest NOAEL of 20 mg kg^{-1} bw/day does neither have an impact on the current SML nor on the general regulation of BPS (Regulation (EC) 10/2011) in FCM [55].

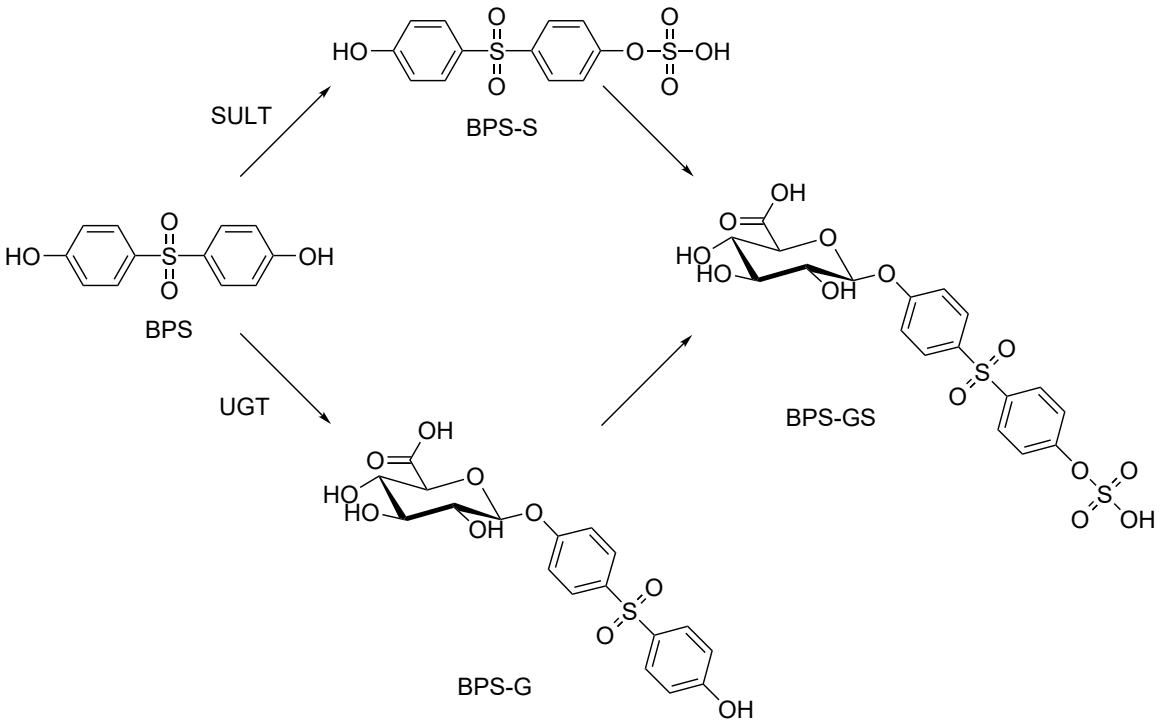


Figure 7: Metabolism of BPS in rats [55]. Involved enzymes are UDP-glucuronyltransferases (UGT) and sulfotransferases (ST) [57]. BPS is metabolized by sulfation, forming BPS-S, or glucuronidation, with BPS-G as the product. After the first conjugation reaction, the other functional group may additionally be introduced to the molecule, resulting in BPS-GS.

1.2.2 Aromatic amines

Aromatic amines form a large group of organic compounds. Two examples for their molecular structures are shown in figure 8.

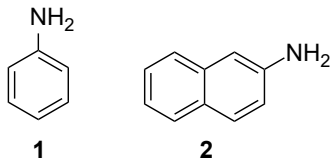


Figure 8: Structure of aniline (CAS 62-53-3, **1**) and 2-naphthylamine (CAS 91-59-8, **2**) [58]. Both compounds are primary aromatic amines.

The compounds differ concerning the number of aromatic rings and the kind and number of substituents attached to the rings. Through replacement of an aromatic hydrogen atom attached to an aromatic ring by an amino group (-NR_2(R = H, aryl, alkyl)), the common structural element is formed. When the polarity of the substituents of the aromatic structure increases, the toxicity of the compound decreases due to more efficient excretion [59]. Aromatic amines play an important role in the chemical industry. They serve as raw materials in the production of drugs, dyes, polyurethanes and cosmetics among other fields of application [60, 61].

1.2.2.1 Primary aromatic amines (PAA)

Primary aromatic amines (PAA) differ from secondary and tertiary aromatic amines in that regard that the substituents on the amino group are hydrogen atoms (R_1 = R_2 = H) while for secondary aromatic amines one of the side groups is an alkyl chain, an aryl or other functional group (R_1 or R_2 = aryl, alkyl). In case of a tertiary aromatic amine, both substituents are alkyl, aryl or other functional groups, though they do not necessarily have to be identical. The structures of aniline and 2-naphthylamine in figure 8 represent two PAAs. Some PAAs are hazardous to human health since they form covalent DNA-adducts after metabolic activation [62]. Thus DNA replication might be impaired which may cause cancer. Some PAAs are classified as carcinogenic (e. g. 2-naphthylamine, category 1A, human carcinogen) and possible carcinogens (e. g. o-anisidine, category 1B, probably carcinogenic for humans, data gained from animal studies), while others (e. g. aniline) have been suspected to act as carcinogens but have not been evaluated as such (yet) [4, 31, 63, 64]. The mechanism of the reaction of PAAs with DNA was described by Böge et al. [65].

PAAs are found in FCMs made of plastic or paper. Contamination of paper and board FCMs with PAAs is not restricted to the recycling process: in paper FCMs produced from primary fibers, they are present as either educts or impurities as well as by- or degradation products of azo dyes[7] (see figure 9). While azo dyes are used intentionally, this does not apply to the contamination of pigments with PAAs [24]. Therefore they are considered as NIAS.

A German state research laboratory recovered aniline (CAS 62-53-3), 3-hydroxy-2-naphthanilide (naphthol AS, CAS 92-77-3) and 3-hydroxy-2-naphthoic acid (HNA, CAS 92-70-6) along with two secondary aromatic amides in CWE from printed paper FCMs in quantitative amounts[8] [66]. Those three substances are substructures to different pigments that are named in a positive list in Annex 10 of the Swiss Consumer Goods Ordinance and in the draft of the German Federal Ministry of Food and Agriculture (ger. Bundesministerium für Ernährung und Landwirtschaft, BMEL) for the regulation of printing inks (see figure 10) [67, 68]. Since there is no specific measure in place, neither in Europe nor in Germany, the legal framework for printing inks remains incomplete.

Naphthol AS is an aromatic amide while HNA is a carboxylic acid and does not contain an amino group. That is why HNA and naphthol AS will be further examined in a separate paragraph.

[7] Azo dyes are formed by the coupling of one- or morefold diazodized arylamines [58].

[8] The secondary aromatic amines are not of further interest in the course of this work, since they were not detected in the analyzed samples. This was shown by a bachelor thesis that was performed by another student in the research group.

Figure 9: Printed paper napkins, paper straws and baking molds contain primary aromatic amines e. g. due to the contamination of pigments [69–71].

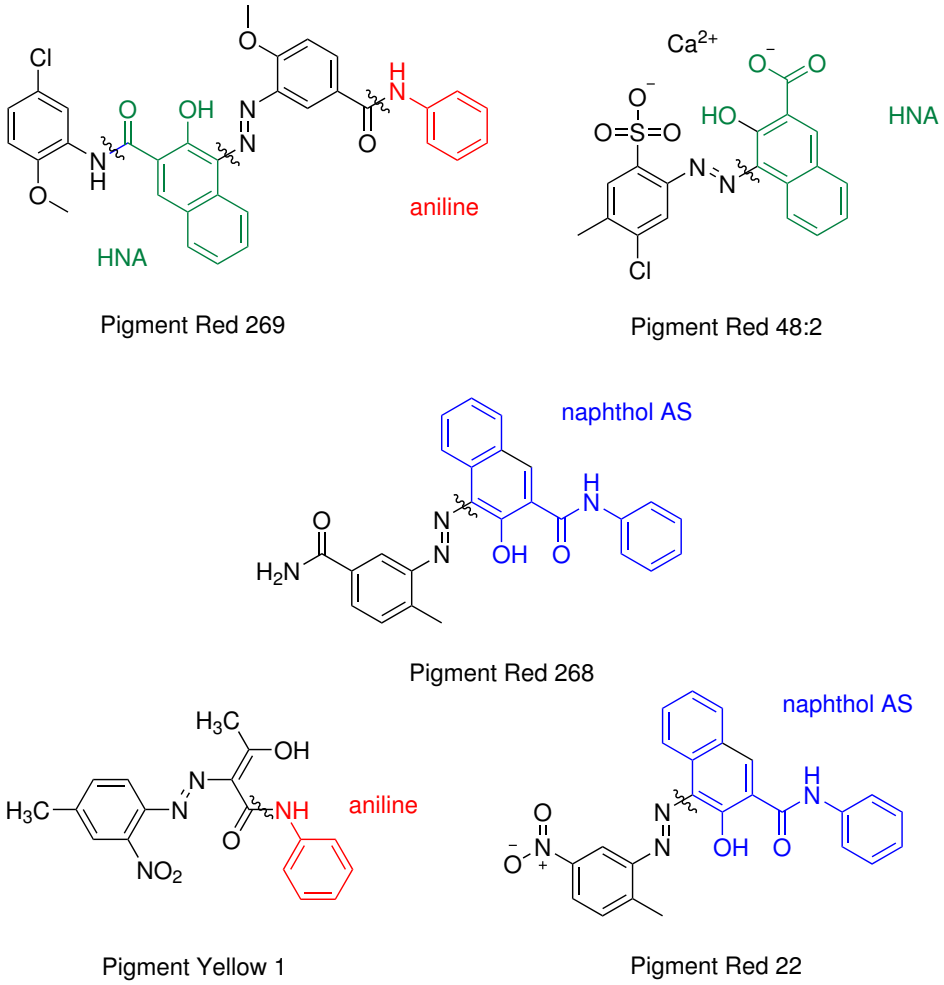


Figure 10: Pigments of azo dyes that contain HNA (3-hydroxy-2-naphthoic acid, green), naphthol AS (3-hydroxy-2-naphthanilide, blue) and aniline (red) as substructures: Pigment Red 269 (CAS 67990-05-0), Pigment Red 48:2 (CAS 7023-61-2), Pigment Red 268 (CAS 16403-84-2), Pigment Yellow 1 (CAS 2512-29-0), Pigment Red 22 (CAS 6448-95-9) [67, 68].

Aniline is the simplest PAA (see figure 8) and classified as carcinogenic (class 2), mutagenic (class 2) and acutely toxic (class 3, toxic via inhalation, dermal and oral exposure) [45]. The acute effect of the absorption of aniline is the formation of methemoglobin (Met-

Hb). Met-Hb is formed by oxidation of Fe^{2+} to Fe^{3+}, which is not able to bind oxygen and therefore its transport is inhibited [58]. The result is an oxygen deficit. Chronical intake leads to headaches, diarrhea, light cyanosis and fatigue. The main metabolite of aniline is N-acetylaniline which is hydroxylated and then sulfated or glucuronidated. Since phase II enzymes convert substances into more hydrophilic compounds, they are typically eliminated through renal excretion. This might be the reason for the association of the exposure to aniline with bladder cancer [58].

Because of their carcinogenic properties, some PAAs present a risk to human health e. g. when migrating into food [31, 66]. Regulation (EC) No. 10/2011 determines that PAAs should not be released from plastic FCMs into food in detectable amounts with a detection limit of 0.01 mg per kg food which is an aggregate limit [8]. For those PAAs that are classified as carcinogens of class 1A (known human carcinogens) and 1B (carcinogenic potential for humans), the BfR recommends to apply the ALARA-principle [4, 45]. Consumers should be able to avoid contact to those substances and have an exposition as low as reasonably achievable (ALARA). Additionally, the BfR opinion No. 021/2014 on the release of PAAs from printed FCMs like napkins and paper bags proposes that PAAs classified as carcinogens of category 1A and 1B should not be detectable with an analytical detection limit of 0.002 mg kg^{-1} food/food simulant. The migration limit of 0.01 mg kg^{-1} food/food simulant for the entirety of detected PAAs from plastic FCMs was confirmed for paper FCMs as well, with the exception of carcinogens of category 1A and 1B [63].

1.2.2.2 Aromatic amide (naphthol AS) and 3-hydroxy-2-naphthoic acid

In 2019, the BfR published an opinion about the adverse health effects of different aromatic amides including naphthol AS and HNA (see figure 11). Since no migration limit existed at the time, a hazard assessment was performed based on existing data and *in silico* data. The substances are orally absorbed after migration into food and well absorbed in the gastrointestinal tract according to the "Rule of Five"[9]. In accordance with *in silico* testing, hydrolysis of the amide bond of naphthol AS is not to be expected. Nevertheless, substructures of naphthol AS (aniline and HNA) are found in paper FCMs, which could indicate that naphthol AS is unstable and hydrolyzed during mechanical application. Thus, it is not out of scope that the same metabolic hydrolysis of the amide bond could occur in human metabolism, too [66].

[9] The "Rule of Five" was established by Lipinksi et al., stating that if molecules fit cetain criteria, poor absorption is to be expected. Those criteria are the number of H-bond donors, H-bond acceptors, the molecular weight and the calculated Log P (CLogP) [72]. Actually four parameters are crucial, though the name of this rule might suggest otherwise.

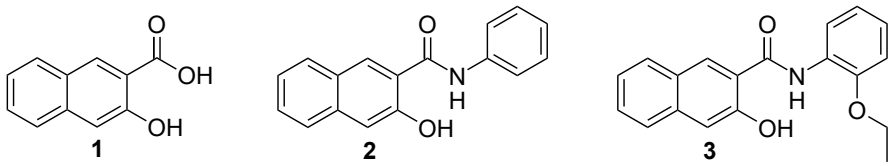


Figure 11: Structure of HNA (3-hydroxy-2-naphthoic acid, CAS 92-70-6**,** **1**), naphthol AS (3-hydroxy-2-naphthanilide, CAS 92-77-3, **2**) and 2'-ethoxy-3-hydroxy-2-naphthanilid (CAS 92-74-0, **3**) [66].

Both compounds neither have mutagenic properties nor do they act as carcinogens. HNA does not have genotoxic properties (*in vivo*). For naphthol AS it remains unclear whether the compound has genotoxic properties. 2'-ethoxy-3-hydroxy-2-naphthanilid (CAS 92-74-0), a substance with similar structure to naphthol AS is not genotoxic, although this analyte may release aniline as well (see figure 11). Therefore, and because of its potential to bind DNA and proteins, naphthol AS will be classified as possibly genotoxic (*in vivo*), until proven otherwise. The calculated tolerable daily intake and SML for HNA and naphthol AS are to be found in table 2 [66].

Table 2: Tolerable daily intake (TDI) and migration limits for naphthol AS and HNA into CWE and food [66].

limits	**naphthol AS**	**HNA**
Tolerable daily intake [µg/person/day]	0.42	360
Proposed migration limits [µg/L]	non detectable, with LOD < 10	360

2 Aims

PAAs and related compunds as well as BPA and BPS are byproducts in the production of paper FCMs or transferred into FCMs made from recycled fibers during the recycling process. They might migrate into the food that they come into contact with and depending on the transferred amount, they might present a risk to human health. Therefore, it is important to determine the quantity of such contaminants in food or food simulants like CWE. While a method for the quantitative analysis of PAAs and related compounds in food was already established, a method for the quantitative analysis of BPA and BPS in foodstuffs should be developed, validated and then applied for migration tests to compare CWEs and real migration into a food sample. Croissants should be used as food samples, meeting the mentioned conditions for a migration test under the worst foreseeable contact conditions. It is also important that this food matrix is fat-rich. With regard towards their octanol-water partition coefficients (log K_{ow}), BPA is likely to migrate into a fat-rich food. The same applies for BPS but due to its lower log K_{ow}, the level of migration would be expected to be lower than that of BPA (see table 3). The octanol-water partition coefficient is originally used to assess the solubility of contaminants and thus their distribution in the environment [73]. Since it is also applied for the assessment of a substance's gastrointestinal absorption and therefore applicable in different contexts, it is consulted here to gain a rough estimation of the substance's behavior concerning the migration into croissants [74]. It is also a realistic scenario that croissants, like other baked goods, come in contact with paper FCMs made with recycled fibers.

Table 3: Octanol-water partition coefficients (log K_{ow}) of BPA and BPS.

analyte	**log K_{ow}**	**reference**
BPA	3.32	Muhamad et al., 2016 [75]
	3.43	Wu et al., 2018 [73]
BPS	1.2	Björnsdotter et al., 2017 [76]
	1.65	Wu et al., 2018 [73]

The extraction efficiency of CWE for contaminants from absorptive paper FCMs may be influenced by the initial sample weight used for extraction. For this reason, CWE should be performed using different initial sample weights to determine its impact on extraction efficiency. As mentioned before, printed napkins may contain PAAs, thus napkins should be analyzed with regard to a correlation between PAA release into CWE and the sample weight. CWE should be analyzed with regard to the extraction efficiency of BPA and BPS

from paper towels made with recycled fibers, too. Thus, different samples and contaminants could be investigated to get a broader perspective on this problem. Since the homogeneity of BPA- and BPS-containing paper samples was already established by other members of the research group, but not the homogeneity of napkins with regard to their aniline, naphthol AS and HNA content, the homogeneity should be examined.

When the migration into CWE or food is analyzed, it is necessary to determine the total amount of analyte in the paper FCM to determine the percental amount of substance that is actually transferred into food or food simulant. For this reason, solvent extractions of the paper FCMs should be performed.

3 Theoretical background

3.1 High performance liquid chromatography

The purpose of chromatographic methods is to separate substances in a complex mixture for identification and quantification. Separation is achieved by molecular interaction of the molecules in the sample with a stationary phase: processes like repeated adsorption and desorption as well as distribution between two phases lead to separation after sufficient operation time. Substances in the sample solution are carried through a chromatographic column by a mobile phase. A crucial criterion concerning the choice of the mobile phase is the solvation of the analytes of interest in the mobile phase [77].

In liquid chromatography (LC), the stationary phase consists of small porous particles while liquid solvents are chosen as mobile phases. The smaller the particles of the stationary phase are, the better the separation and signal detection become. At the same time, the use of smaller particles results in more tightly packed columns, therefore higher pressures have to be applied to move the mobile phase through the stationary phase. Thus, the use of smaller particles in the stationary phase resulted in the advancement of the LC, the so called High Performance Liquid Chromatography (HPLC) [77]. Since molecules have different structural and therefore physico-chemical properties, they interact with the stationary phase to varying degrees. The higher the affinity of a substance towards the stationary phase is, the stronger the interactions and the higher the retention become. Depending on the interaction, molecules arrive at the detector at different times and have a characteristic retention time t_R by which they are identified. The detector then creates an electric signal from the chemical signal, resulting in a peak in the chromatogram. Quantitative analysis is performed by the injection of solutions that contain the analyte in known concentrations (calibration) into the LC apparatus, followed by the implementation of a regression analysis for the signal height or signal area and the analyte's concentration. The use of this correlation enables the calculation of the concentration of the analyte in the samples [78].

A scheme of an HPLC-apparatus is shown in figure 12. The mobile phase is degassed to avoid air pockets since they cause irregularities in the separation process and broadened peak shapes. The eluents are then mixed and transferred to the column by a high-pressure pump at pressures up to 20 MPa [78]. Injection volumes of typically 1-100 µL of sample solution are injected and transferred to the precolumn. The volume may vary depending on the chromatographic system that is used. The precolumn may serve as a protection for the analytical column and it consists of the same stationary phase as the analytical

column. The application of a guard column with a stationary phase that is different from that of the analytical column may still fulfill the purpose of a filter. Signals are recorded by a detector and processed by a computer [78]. Many different detectors can be applied in an HPLC-equipment, e. g. UV-Vis, fluorescence, conductivity or mass-spectrometrical detectors [77, 79].

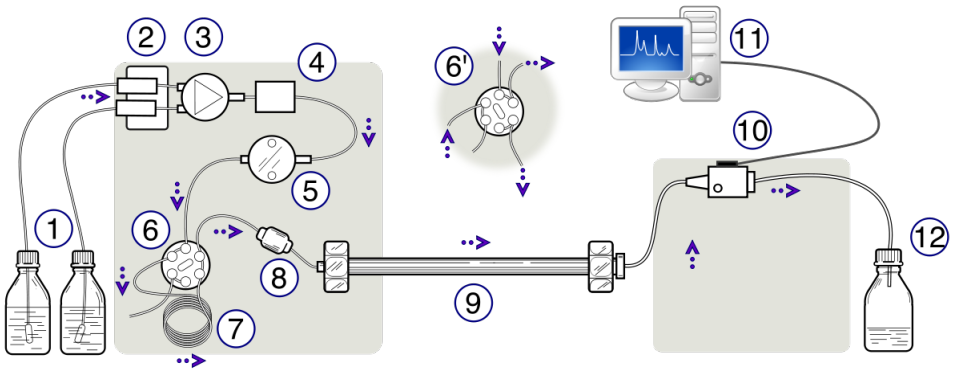


Figure 12: Exemplary construction of an HPLC-system: 1) eluents, 2) degasser, 3) mixing valve, 4) mixing chamber, 5) pump, 6) injection valve, 7) sample loop, 8) precolumn, 9) column, 10) detector, 11) computer for data analysis, 12) solvent waste [80].

Chromatographic systems are generally distinguished by the polarity of the mobile and the stationary phase. Systems with a polar stationary and an apolar mobile phase are called normal phase chromatography (NPC), while application of a more apolar stationary phase and a more polar mobile phase is defined as reversed phase chromatography (RPC) [77]. Stationary phases for NPC are silica gel or aluminum oxide, while silanol groups are chemically modified for RPC. Substituents for modification of the silanol groups are typically alkyl chains with and without different functional groups. Chromatographic separation can be performed with constant composition of eluents (isocratic elution) or changing composition of eluents (solvent gradient). A solvent gradient can proceed linearly, non-linearly or gradually. A solvent gradient is useful, when mixtures of substances with a wide range of retention times are analyzed [79].

3.2 Infra-Red Vortex Evaporator (IR-Dancer)

The Infra-Red Vortex Evaporator (IR-Dancer) is an equipment that is used for the concentration of solutions. In contrast to the rotary evaporator, the IR-Dancer applies infrared heating and a vortex function as well as a vacuum pump for the evaporation of the solvent, giving the IR-Dancer its name [81].

The construction of an IR-Dancer is shown in figure 13. The samples are put into the heated base plate, which is moved by coupling between the magnetic feet of the base plate and the magnetic drive mechanism. The latter is separated from the vacuum chamber [81]. The orbital motion of the base plate generates a larger surface area of the solution

inside the test tube. This way evaporation is accelerated. A side wall heating prevents the solvent from condensation on the interior walls of the IR-Dancer. Additionally, the base plate contains a heater that is activated by a temperature probe. The probe is set to the desired sample temperature. As soon as the solvent evaporates and the temperature drops, the ceramic infrared heaters in the lid are activated to maintain the sample's set temperature. A sample window allows the analyst to follow the evaporation process and to stop at any time. A cold trap is installed to protect the vacuum pump by condensation of the solvent on the cooling coils. After the evaporation process is finished, the solvents are defrosted and removed from the cold trap [81]. Since the evaporation process is taking place at low temperatures and in the absence of direct light, the equipment is well suited for the concentration of thermally unstable and photosensitive substances [82].

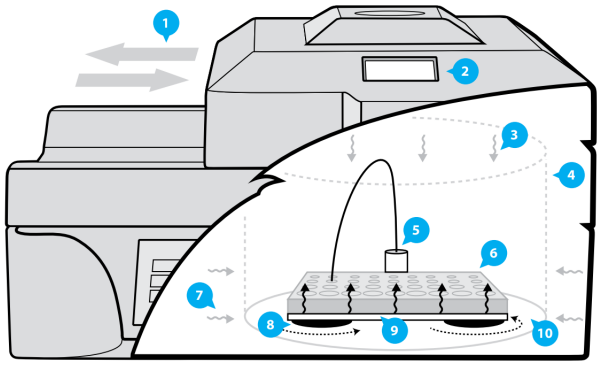


Figure 13: Construction of an IR-Dancer: 1) auto open & close lid, 2) sample window, 3) infrared heating from above, 4) stainless steel chamber, 5) temperature probe, 6) exchangeable sample racks, 7) side wall heating, 8) magnetic feet, 9) heated base plate, 10) vortex motion [81]. The model shown here is the Hettich Vortex Vacuum Concentrator (HVC), while the IR-Dancer from Zinsser, serial number 40061, was used in the course of this work. The working principle is the same for both models.

3.3 Mass spectrometry

Mass spectrometry (MS) is an analytical technique that is applied in a lot of different scientific disciplines like physics, pharmacy, medical professions and chemistry. The basic principle of this method is the formation of ions that are separated by their mass-to-charge ratio (m/z) [77]. In that way, substances can be qualitatively and quantitatively analyzed. First, analytes have to be transferred into the gas phase, to create single ions that are easily controlled by electric and/or magnetic fields. The creation of ions is crucial, because directed motion is necessary to transfer ions into the mass analyzer, where they are separated by their m/z-ratio. Otherwise, molecules would move unregulated into various directions [83].

The basic scheme of mass spectrometers is simple. The equipment contains an ion source,

a mass analyzer and a detector. It is run under high vacuum to maximize the mean free path since ions would otherwise be distracted from their trajectory [83].

3.3.1 Electrospray ionization

There are different methods for ionization, but charging the particle is basically achieved by addition or substraction of charge carriers like electrons, protons or other ions from the particle. Ionization methods are for example electron ionization (EI), chemical ionization (CI), fast atom bombardment and matrix-assisted laser desorption/ionization (MALDI). The ionization method used for the analyses presented in this work was electrospray ionization (ESI), therefore only this ionization method shall be described in detail [84].

ESI is the ionization method of choice when LC and mass spectrometry are coupled. It is a "soft ionization" under atmospheric pressure, i. e. the formed ions are not fragmented during the ionization process. The method is suited for non-volatile analytes, which are dissolved in volatile solvents in low concentrations (10^{-6}-10^{-4} mol L^{-1}). The sample solution is pumped into a needle or a capillary at a typical flow rate of 5–20 µL min^{-1} [83]. A potential of 3–4 kV is applied to the capillary. The arithmetic sign of the charge of the needle determines the kind of ions that are formed. If the potential is negative, anions are formed, if it is positive, cations are formed. Due to the same charge of the ions in the solution, they are repelled by each other. When the solution reaches the outlet of the capillary, the liquid forms a taylor cone (see figure 14). The ion source is heated to support evaporation of the solvent. In a next step a spray is formed, since solvent evaporation causes a decrease in the distance between ions which enables electrostatic repulsive forces to overcome surface tensions. This happens when the Rayleigh-limit is exceeded [83].

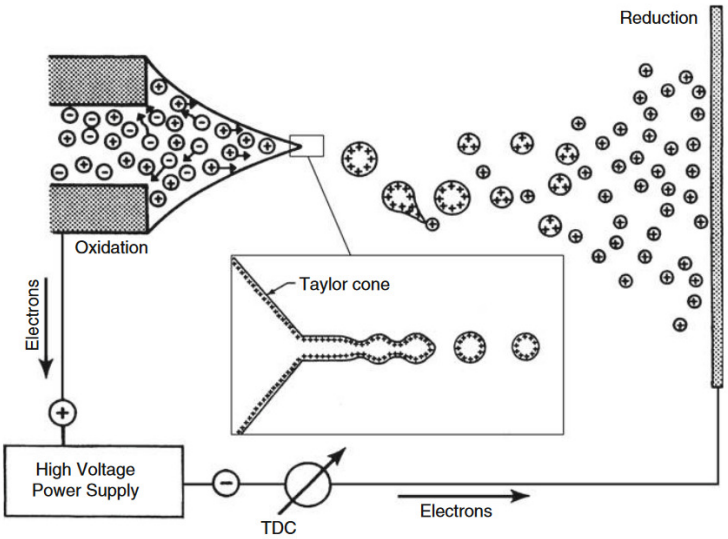


Figure 14: Schematic illustration of electrospray ionization (ESI) [85]. Positive ionization mode is depicted here, therefore cations are formed by oxidation on the inner wall of the capillary. The transferred electrons are transported to the high voltage source and towards the counter electrode [83].

Ions are accelerated towards the electrode with the opposite charge while the droplets of the spray are still shrinking in size due to a hot nitrogen stream that causes further evaporation of the solvent. In this process, the Rayleigh limit is reached repeatedly which leads to further decrease of the droplets' size. (Solvated) ions are brought into the high vacuum of the mass analyzer through a small aperture with gradually decreasing pressure or increasing vacuum, respectively [84].

3.3.2 Mass analyzer

For this work, a triple quadrupole was used as a mass analyzer (QqQ). A mass analyzer separates ions based on their m/z-ratio.

A quadrupole consists of four parallel electrodes. The electrodes opposing each other have the same charge, while the electrodes next to each other have opposite charges. The same DC voltage (U) is applied to the opposing electrodes (see figure 15) [77]. DC voltage is overlapped by high frequency AC voltage (Vcosωt) [79]. When ions move along the z-axis between the electrodes, they are attracted by the charge of one of the electrodes and move towards it. Due to the overlapping AC-voltage, the ions are repulsed and thus move in a sinusoidal trajectory. Ions with a fitting m/z ratio pass the mass analyzer and reach the detector. When the m/z ratio does not fit, the ion hits the electrode. Then it is neutralized and therefore no longer controlled by the electric field, thus it does not reach the detector [84].

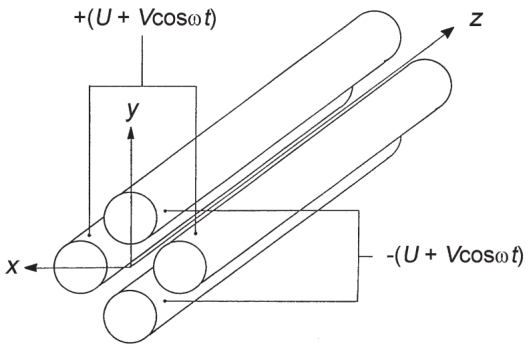


Figure 15: Schematic illustration of a quadrupole mass analyzer [85]. DC voltage (U) is applied to the opposite electrodes with the same charge which is overlapped by AC voltage with radio frequency (Vcosωt). Electrodes typically have a diameter of 1 cm and a length of 20 cm [84].

3.3.3 Triple quadrupole mass spectrometry

In general, when different mass analyzers are linked, it is referred to as tandem mass spectrometry (MS/MS). For quantitative analysis via quadrupole, a triple quadrupole mass analyzer is often used, because it can offer information about the structure of an unknown compound by fragmentation (product ion scan). This property is valuable in trace analysis, since biological and food samples contain a high amount of substances which are present in

low concentrations and thus it is even more significant to ensure that the present compound is identical with the analyte. It can also increase the specificity/selectivity of the analysis when used for precusor ion scan or neutral loss scan. The specifity is enhanced through the gain of information about the structure of the precursor ion as well as its fragment. Another advantage of MS/MS is the assignment of the precursor ion and the precursor fragment to one another [84]. For precusor ion scan, the first quadrupole Q_1 is set to scan mode, while the third quadrupole Q_3 is held at a constant value to identify possible precursor ions for a specific fragment. Neutral loss scan is carried out by setting Q_1 and Q_3 in scan mode with a constant mass difference, so e. g. loss of water is detected and ions can be assigned to a substance group such as alcohols [84].

Another operation mode for the triple quadrupole is the multiple reaction monitoring (MRM, see figure 16). This mode enables the selective generation of the spectrum of a specific ion fragment. MRM is based on the detection of selected fragments (daughter ions) of a target ion (parent ion). The daughter ions have to be known. Q_1 and Q_3 are set to certain m/z-ratios to specifically select a precursor ion and its corresponding fragment. If just one fragment of a charged molecule is analyzed in this fashion, the mode is called single reaction monitoring (SRM) [83].

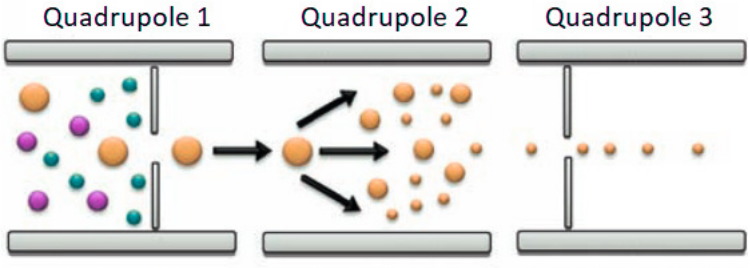


Figure 16: Schematic illustration of a triple quadrupole (QqQ) in MRM-mode (adapted from [86]). In Q_1, ions are separated according to their m/z-ratio. In q_2, which serves as a collision chamber, the selected ion is fragmented. Q_3 serves as a filter (m/z), so characteristic fragments of the molecule can be detected specifically [86].

Measurements for this work were performed in MRM mode. Since the SML for BPA and BPS from paper FCMs made with recycling fibers into food is at 50 µg kg^{-1} food/food simulant, analysis is performed at trace levels. Signals of croissant ingredients may overlap with the signals of BPA and BPS, if the retention time and the m/z ratio of another substance correlate with that of BPA or BPS. Quantification of fragments offers a more specific determination, because two compounds having the same retention time, m/z ratio and daughter ions is rather unlikely.

4 Material and methods

4.1 Samples

Two kinds of samples were analyzed: first, paper FCMs with regard to their bisphenol content and the migration of bisphenols/PAAs into CWE depending on the initial sample weight. Secondly, a food sample was used to validate a method for the quantitative analysis of BPA and BPS, and to perform migration tests with the paper samples. Paper FCMs that contained bisphenols were disposable paper plates (13 cm x 20 cm), pizza boxes (Ø 30 cm), and paper towels. All of those paper samples were made with recycled fibers. The grammage was determined by preparing three pieces of each paper sample of known measurement and weighing each of the paper samples (for results, see paragraph 5.3).

PAA containing samples were red paper napkins. The napkins were printed from one side and had a second, unprinted layer. Red colored napkins were chosen because they are likely to contain higher amounts of PAAs, HNA and naphthol AS compared to napkins of other colors, since red pigments contain most of the substructures shown in figure 10 and thus the probability for them to contain a significant amount of these contaminants is higher. Generally, paper towels and napkins were used for the examination of the extraction efficiency of CWE from absorbent paper FCMs depending on the amount of initial sample weight because the samples swell when they come in contact with water due to their absorptive properties. Homogeneity of the analytes in paper napkins was determined in the context of this thesis, while the homogeneity of BPA and BPS in the other paper samples (paper plates, pizza boxes, paper towels) was confirmed by other members of the research group before the work for this thesis started. The homogeneity of the paper napkins was determined by cold water extraction of the samples (for procedure, see paragraph 4.3.3). Twelve CWE, which were prepared from 10.0 g of sample, were analyzed (for results, see paragraph 5.1).

Croissants were chosen as food samples for the migration tests with FCMs made from recycling fibers. The sample is an industrial product, thus the variation of the matrix composition is minimized. Therefore it should have a minimal effect on the repeatability of the method. The package label claims that a croissant weighs about 60 g and that they contain 24 % butter. The list of nutrients is shown in table 4: the high content of carbohydrates originates from the main ingredient, which is wheat flour. Fat and protein stem from eggs and butter, other ingredients were water, sugar, table salt, yeast, ascorbic acid and xylanase.

Table 4: Nutritional composition of 100 g croissants that were used in the course of this work, according to the product label.

ingredient	mass per 100 g croissant
fat	20.5 g
saturated fatty acids	13.2 g
carbohydrates	33.8 g
sugar (included in carbohydrates)	5.1 g
protein	7.8 g
salt	0.71 g

4.2 Reagents

All solvents, standards and reagents were of analytical grade. Methanol (LC-MS grade, MeOH, CAS 67–56-1), acetonitrile (ACN, CAS 75-05-8), tetrahydrofuran (THF, CAS 109-99-9), acetone (CAS 67-64-1) and isopropanol (2-propanol, CAS 67-63-0) were purchased from Merck KGaA (Darmstadt, Germany). Hexane (CAS 110-54-3) was bought from Promochem (Hyderabad, Indiana), while formic acid (CAS 64-18-6) was purchased from Fisher Chemicals (Hampton, New Hampshire). 3-Hydroxy-2-naphthanilide (naphthol AS, CAS 92-77-3) and 3-hydroxy-2-naphthoic acid (HNA, CAS 92-70-6) were obtained from Tokyo Chemical Industry (TCI, Tokyo, Japan). Aniline (CAS 62-53-3) and aniline-d_5 (CAS 4165-61-1) were purchased from neochema GmbH (Bodenheim, Germany). 4-Aminobiphenyl-d_9 (CAS 344298-96-0) and bisphenol A (CAS 80–05-7) were obtained from Dr. Ehrenstorfer (Augsburg, Deutschland). $^{13}C_{12}$-ring-labeled bisphenol S (BPS-$^{13}C_{12}$, CAS 263261–65-0) and d_8-ring labeled bisphenol A (BPA-d_8, CAS 92739-58-7) were purchased from Cambridge Isotope Laboratories, Inc. (Tewksbury, Massachusetts). Bisphenol A-d_{16} (BPA-d_{16}, CAS 96210– 87-6) was bought from Isotec International (Canton, Georgia, USA). Bisphenol S (CAS 80-09-1), magnesium sulfate (CAS 7487-88-9), sodium sulfate (CAS 7757-82-6) and water (LC-MS grade, CAS 7732-18-5) were bought from Sigma Aldrich Chemie GmbH (Munich, Germany), while sodium chloride (CAS 7647-14-5) was purchased from Honeywell Fluka (Morristown, New Jersey)[10]. Different dSPE materials (a) 150 mg $MgSO_4$, 50 mg PSA, b) 150 mg $MgSO_4$, 25 mg PSA, 25 mg C18E) were bought from Phenomenex (Aschaffenburg, Germany). Other dSPE materials (C18ec and diamino materials) were purchased from Macherey-Nagel GmbH & Co. KG (Dueren, Deutschland). Sodium chloride-magnesium sulfate-mix (CAS 7647-14-5 /7487-88-9), dSPE material (unknown components) and magnesium sulfate (polish pouch) were obtained from Agilent (Waldbronn, Germany). Ultra pure water type I was obtained from a Mili-Q® Reference A+ System with a Q-POD® Disperser (Merck).

[10]Those three salts were used for method development only.

4.3 Experimental

4.3.1 Sample preparation

Paper-based FCMs that were selected for the determination of their bisphenol content and the transfer into foodstuff were stored wrapped in aluminum foil; napkins chosen for the determination of their PAA release into CWE were stored in aluminum cans, both at room temperature. The samples were always handled with gloves. Surfaces and scissors were cleaned with distilled water and isopropanol. All glassware was cleaned with methanol.

For method development, validation and migration tests, frozen croissants were baked in the preheated drying cabin according to the instructions provided on the packaging (170 °C, 19 min). The samples were torn into small pieces and homogenized for 1 min at stage 12 in a kitchen machine from Krups Prep&Cook. Afterwards, the container was cleaned with tap water and dish soap. The homogenized samples were stored at -18 °C. For migration tests, the samples were brought into contact with the FCMs for 1 h and 24 h at room temperature and homogenized afterwards.

4.3.2 Preparation of internal standard mixes, standard mixes and calibration solutions

Standard and internal standard mixes of PAAs were prepared at a concentration of 1 mg L^{-1} in MeOH and stored at 4 °C in brown glass headspace vials. Calibration levels ranged from 1-10 µg L^{-1} (1, 2, 4, 6, 8, 10 µg L^{-1} aniline, HNA, naphthol AS). During the preparation of the calibration solutions, the 10 mL volumetric flasks were filled to the mark with blank solution of a white paper napkin. Internal standard was added to all calibration solutions at a concentration of 5 µg L^{-1} (aniline-d_5 and 4-aminobiphenyl-d_9).

Bisphenol standard mixes and internal standard mixes were prepared from stock solutions at a concentration of 10 mg L^{-1} in MeOH (internal standards) or ACN (standards) and stored under the same conditions as PAA mixes, but not necessarily in brown glass headspace vials. Internal standard mixes contained either BPA-d_{16} and BPS-$^{13}C_{12}$ or BPA-d_8 and BPS-$^{13}C_{12}$. Standard mixes as well as internal standard mixes were diluted to different concentrations.

Different calibration solutions were prepared for the quantitative analysis of bisphenols. For analysis of the correlation of initial sample weight and extraction efficiency in CWE, calibration solutions were prepared in 50 % MeOH (MeOH: water, 1:1 (v/v)) with internal standard concentration (BPA-d_8 and BPS-$^{13}C_{12}$) at 25 µg L^{-1}. BPA/BPS concentrations ranged from 1-100 µg L^{-1} (1, 5, 10, 20, 30 40, 50, 60, 80, 100 µg L^{-1}).

For method development, calibration solutions were prepared in MeOH (MeOH: water, 1:1 (v/v)) with internal standard concentration (BPA-d_8 and BPS-$^{13}C_{12}$ or BPA-d_{16} and BPS-$^{13}C_{12}$) at 25 µg L^{-1}. BPA/BPS concentrations ranged from 0.5-100 µg L^{-1} (0.5, 1, 5, 10, 25, 50, 75, 100 µg L^{-1}). Another calibration was prepared in 50 % ACN (ACN:water, 1:1 (v/v)) with 50 µg L^{-1} internal standard (BPA-d_{16}, BPA-d_8 and BPS-$^{13}C_{12}$), BPA/BPS

concentrations ranged from 0.5 - 150 µg L^{-1} (0.5, 1, 5, 10, 25, 50, 75, 100, 125, 150 µg L^{-1}) and another calibration was prepared in 100 % ACN with the same internal standard concentration (BPA-d_{16}, BPA-d_8 and BPS-$^{13}C_{12}$) and the same calibration levels. A fourth calibration was prepared in 50% ACN with 25 µg L^{-1} of internal standard (BPA-d_8 and BPS-$^{13}C_{12}$), BPA/BPS concentrations ranged from 0.5-150 µg L^{-1} (0.5, 1, 5, 10, 25, 50, 75, 100, 125, 150 µg L^{-1}).

Solvents and internal standard concentrations were adjusted due to the sample preparation that was used. When 500 ng of internal standard are added to the food sample and dissolved in 10 mL ACN used for extraction, assuming that the internal standard is dissolved in the organic phase, the internal standard is present at a concentration of 50 µg L^{-1}. If the sample solution was diluted by factor 2, the concentration was at 25 µg L^{-1}, which is why those concentration levels of internal standard were used for calibration solutions. Concerning the solvent composition, it is known that its properties have an impact on the ionization process (ESI) [87]. Thus it is necessary to have the same solvent for calibration solutions and sample solutions, so that the quantification is not biased by different ionization conditions due to the usage of varying solvent compositions.

4.3.3 Cold water extracts

CWE preparation was performed according to DIN EN 645: paper samples were cut into pieces of 1-2 cm^2. The initial sample weight was varied from 1.0-10.0 g in double determination and in steps of 1.0 g. 200 mL of water were added to the sample in 500 mL Erlenmeyer flasks, the flasks were closed and put on a shaker at 90 mot min^{-1} and room temperature for 24 h[11]. After the extraction, the solutions were filtered through two overlapping filter papers (Whatman, Glass Microfiber Filters) in a Nutsch-type filter. The samples were washed twice with water and slightly pressed, so that the extract was removed from the sample under applied vacuum. The solutions were transferred to 250 mL volumetric flasks and filled up to the mark with water. The samples were stored at 4 °C. If an analyte's concentration (PAA) was above the operational range (10 µg L^{-1}), an aliquot of sample was diluted in 10 mL volumetric flasks with water. 50 µL internal standard (1 mg L^{-1} aniline-d_5 and 4-aminobyphenyl-d_9 in MeOH) were added before analysis. For the quantitative determination of bisphenols, a sample aliquot was diluted in 10 mL volumetric flasks with MeOH:water (1:1 (v/v)), if an analyte's concentration was above the operational range (100 µg L^{-1}). 25 µL internal standard (10 mg L^{-1} BPA-d_8 and BPS-$^{13}C_{12}$ in MeOH) were added before analysis.

For some double determinations, the deviation of results was greater than the comparative standard deviation σ_{pt} [µg L^{-1}] which was defined for the homogeneity tests. In this case, a third CWE for that specific initial sample weight was prepared, because the limit

[11] The use of a shaker to mix the CWE is not prescribed in DIN 645. It states that the samples should be shaken occasionally, so concerning this aspect the extraction deviates from DIN 645. An interlaboratory comparison study from 2015 came to the conclusion that a variation in the preparation of CWE according to DIN 645 (such as shaking procedure) did not have an impact on the results of the PAA-concentration in colored paper napkins [64].

for standard deviation under reproducibility conditions was exceeded. For aniline, the 22 % for relative standard deviation according to Horwitz were used as the comparative standard deviation σ_{pt} [%] instead of the σ_{pt} [µg L^{-1}] from the homogeneity tests (see table 11) [88]. The analyte's concentration is lower than that of the other analytes and therefore the percental scattering of the results is increased compared to naphthol AS and HNA. According to Horwitz, the relative standard deviation is also increased with decreased analyte concentration (see table 23), thus the application of a higher σ_{pt} [%] seems justified since 22 % are proposed for analyte concentrations below 100 ppb. The same approach was applied for BPA and BPS in paper towels since the mean analyte concentrations were below 100 ppb, too.

4.3.4 Solvent extraction of paper FCMs

The extraction of paper samples was performed in triplicate for each sample to determine the total amount of BPA and BPS in these paper FCMs. The samples were extracted with ACN:water (1:1 (v/v)) for the first step of extraction, while pure ACN was used for the following extraction steps.

2.0 g of sample were weighed in 50 mL centrifuge tubes. A reagent blank was carried along. 20 mL of 50 % ACN were added for the first extraction. The samples were then put into an ultrasonic bath for 1 h. During the different extraction steps, the temperature in the ultrasonic bath reached 70 °C. Samples were centrifuged for 10 min at 4,000 rpm and 20 °C. The supernatant was transferred into a 20 mL volumetric flask. If necessary, ACN was added to fill the flask to the mark after the first extraction step, since part of the solvent was absorbed by the paper sample. The extracts were then transferred into 20 mL headspace vials and stored in the refrigerator at 4 °C. 20 mL ACN were added to the samples into the centrifuge tubes and the extraction was repeated. If an analyte's concentration was above the operational range (100 µg L^{-1} BPA/BPS), an aliquot of sample was diluted in 2, 5 or 10 mL volumetric flasks with water. 20, 50 or 100 µL internal standard (2.5 mg L^{-1} BPA-d_8 and BPS-$^{13}C_{12}$ in MeOH, resulting in 25 µg L^{-1}) were added before analysis.

For quality control, a BPA-containing solution from an inter-laboratory comparison study was diluted and measured at any injection. According to the documents delivered with the FAPAS-solution[12] used in the inter-laboratory study, the concentration was 0.093 mg kg^{-1}. The solution's density was determined by participants of the study at 0.93 g mL^{-1}. From the mass concentration and the density, the solution's concentration was calculated and resulted in 86.49 µg L^{-1}. The calculation was performed to simplify comparability of the quality control and the sample solutions[13]. 100 µL of internal standard solution (5 mg L^{-1} BPA-d_8 and BPS-$^{13}C_{12}$ in MeOH) were added to 2.5 mL of solution in a 10 mL volumetric flask, so the concentration of internal standard was 50 µg L^{-1}. It was

[12] FAPAS is a proficiency testing provider.

[13] Recovery was calculated for the quality control and the factor was applied to the results for the measured BPA concentration in the sample solutions.

filled to the mark with 50 % ACN. Due to the application of the dilution factor 4, the BPA concentration was 21.62 µg L^{-1} for the quality control.

4.3.5 Method development

MEAL study method for analysis of plasticisers in oil or fat

1.0 g of sample was weighed in 50 mL centrifuge tubes. Reagent blank (containing 1 mL ACN) and sample blank were prepared as well as spiked samples with analyte concentration at 50 µg kg^{-1} and 100 µg kg^{-1}. The determinations were performed singlefold for each spike level. Internal standard was added, resulting in a concentration of 25 µg kg^{-1}. Samples were mixed on a vortex for 1 min, put in an ultrasonic bath for 10 min at 30 °C and left on the bench overnight.

The next day, 10 mL ACN (+ 1 % acetic acid (v/v)) were added to the samples. Extraction was performed for 15 min on a shaker. After extraction, samples were centrifuged for 10 min at 4,000 rpm and -9 °C and the supernatant was transferred into 30 mL centrifuge tubes, which contained 0.5 g dSPE-C18ec material (Macherey-Nagel GmbH & Co. KG). 10 mL fresh ACN (+ 1 % acetic acid (v/v)) were added to the samples and extraction was repeated. The supernatant of both extractions was combined with the 0.5 g dSPE C18ec material (Macherey-Nagel GmbH & Co. KG). Samples were mixed on a vortex and then frozen for 2 h at -20 °C. It was centrifuged for 10 min at 4,000 rpm and -9 °C. The supernatant was transferred into 50 mL centrifuge tubes that contained 1.0 g dSPE diamino material (Macherey-Nagel GmbH & Co. KG), and 300 µL LC-MS water were added. Samples were mixed on a vortex for 1 min, then frozen overnight at -20 °C.

On the next day, samples were centrifuged for 10 min at 4,000 rpm and -9 °C, the supernatant was transferrred into a 25 mL-screw glass containing Na_2SO_4 and samples were shaken. 10 mL of extract were pipetted into a Büchi flask and solutions were concentrated to a volume of $\leq$ 1 mL. It was filled up to the mark with ACN, resulting in 1 mL sample solution. The pressure of the rotavapory was set to 300 mbar in the beginning, coolant was set to -10 °C and shaker settings were adjusted to 30 °C and 280 rpm. Extracts were diluted in 50 % MeOH, which resulted in the formation of a precipitate. Thus, different approaches were used to remove the precipitate. First, 100 µL of diluted extract were transferred into an Eppendorf tube and centrifuged for 3 min at 12,500 rpm. The supernatant was transferred into vials. Another approach was to evaporate the solvent to dryness with a Pasteur pipette and the residue was dissolved in 100 µL 50 % MeOH, the supernatant was transferred into vials. Samples were stored at 4 °C.

QuEChERS method with Phenomenex dSPE materials

5.0 g and 10.0 g of sample were weighed in 50 mL centrifuge tubes to determine the appropriate amount of sample for analysis. 5 mL of water were added to the sample of 5 g. A reagent blank (10 mL of water) and sample blanks with 5.0 g and 10.0 g of sample were prepared as well as spiked samples with analyte concentration at 50 µg kg^{-1} and 100 µg kg^{-1}. The determinations were performed singlefold for each amount of sample and spike level.

Internal standard was added to the blanks and spiked samples, resulting in a concentration of 25 µg kg^{-1}. 10 mL ACN were added, samples were shaken for 1 min and centrifuged for 5 min at 3,500 rpm and 20 °C. Salt mix (1.0 g NaCl und 4.0 g $MgSO_4$) was added, the mix was shaken for 1 min and centrifuged for 5 min at 3,500 rpm and 20 °C. 1 mL of extract was transferred on each dSPE material Phenomenex, a) 150 mg $MgSO_4$, 50 mg PSA, b) 150 mg $MgSO_4$, 25 mg PSA, 25 mg C18ec (each in 1 mL tubes). For the sampling with 5.0 g of croissant, samples were cleaned up twice, once without dilution, another one with dilution factor 2. The same was planned for sampling with 10.0 g of croissant, but the matrix absorbed most of the solvent, so clean-up was performed only once. Tubes were shaken for 30-60 s, then put in the microcentrifuge for 3 min at 12,500 rpm. 100 µL of extract were diluted with 100 µL 50 % MeOH. A precipitate was formed, therefore the same approaches for its removal were used to as in the MEAL-study approach. Samples were stored at 4 °C.

For another extraction, 2.0 g of sample were weighed in 50 mL centrifuge tubes, therefore 2 mL ACN were used as the reagent blank. A sample blank was prepared as well as spiked sample with analyte concentration at 100 µg kg^{-1}. Each clean-up was performed singlefold for this spike level. Internal standard was added, resulting in a concentration of 250 µg kg^{-1}. 10 mL of water (LC-MS) were added, samples were shaken for 1 min and 10 mL ACN were added. Extraction was performed by shaking for 1 min, then samples were centrifuged for 5 min at 3,500 rpm and 20 °C. Salt mix (1 g NaCl und 4 g $MgSO_4$ in 50 mL tubes) was added, the mix was shaken for 1 min and centrifuged for 5 min at 3,500 rpm and 20 °C. Then, 1 mL of extract was transferred on both dSPE materials and samples were mixed by shaking for 30 s. The tubes were put into the microcentrifuge fo 3 min at 12,500 rpm, then 600 µL of supernatant were transferred into Eppendorf-tubes and diluted with 600 µL 50% MeOH. Two different syringe filters were used in comparison, a polyamide-filter (0.45 µm) and a polypropylene-Filter (0.45 µm). 100 µL of filtrated extract were transferred into vials with inserts. As another method for clean-up, samples were frozen at -18 °C for two days. Since no precipitate was formed, samples were brought to room temperature and 100 µL of extract were diluted with 100 µL 50 % MeOH in an Eppendorf-tube. Diluted solutions were centrifuged for 3 min at 12,500 rpm in a microcentrifuge, 100 µL of supernatant were pipetted into vials with inserts.

Comparison between freezing the samples and filtration with PA-filter was repeated. That time, the supernatant from the dSPE materials were frozen for 1.5 h instead of two days, then they were diluted, centrifuged and analyzed. Samples were stored at 4 °C. Analysis was also repeated with 2.0 g of sample and samples were spiked at 10 µg kg^{-1} and 50 µg kg^{-1} in triple determination. Moreover, addition of internal standard was performed before extraction and after extraction/before clean-up with dSPE material.

QuOil method with Phenomenex dSPE materials

First, 2.0 g of sample were weighed in 50 mL-centrifuge glasses. Then, 2 mL of LC-water were added to the reagent blank. Samples were spiked at concentrations of

100 µg kg^{-1} and internal standard was added at concentration of 50 µg kg^{-1}. 10 mL ACN were added and samples were shaken for 1 min. Centrifugation was performed for 5 min at 3,500 rpm and 20 °C. Then, 1 mL of supernatant were pipetted into dSPE C18ec material containing tubes (Phenomenex), samples were shaken for 30 s and centrifuged for 3 min at 12,500 rpm in the microcentrifuge. 600 µL of supernatant were transferred into an Eppendorf-tube and diluted with 600 µL 50 % MeOH (sample blank and spiked sample showed precipitation). The diluted samples were centrifuged (3 min, 12,500 rpm) and 100 µL of supernatant were pipetted into vials with insert. The second way to perform the QuOil-method was to freeze the samples. 4 mL of supernatant were transferred into 30 mL centrifuge glasses after extraction. They were put into the freezer for 1.5 h at -18 °C. Afterwards, it was centrifuged for 5 min at 20 °C and 3,500 rpm and 1 mL of supernatant was transferred to each Phenomenex dSPE material. Samples were shaken for 30 s and centrifuged for 3 min at 12,500 rpm. Then, 600 µL of supernatant were diluted with 600 µL 50 % MeOH in an Eppendorf-tube which again resulted in the formation of a precipitate. Samples were put into the microcentrifuge for 3 min at 12,500 rpm and 100 µL of supernatant were transferred into vials.

QuEChERS method with Agilent dSPE materials

The Agilent QuEChERS Kit followed with Agilent Bond Elut EMR-Lipid Cleanup was used, because it showed good results for quantitative determination of fipronil in eggs, which are considered to be high-fat food like croissants, too [89]. Since the sample preparation provided good recovery and reproducibility for fipronil and its metabolites in this complex matrix as well as efficient matrix removal, it presented a promising option for quantitative determination of BPA and BPS in croissants.

For testing the Agilent materials, 1.0 g and 2.0 g of sample were weighed in 50 mL tubes. Internal standard (BPA-d_{16} and BPS-$^{13}C_{12}$) was added, resulting in a concentration of 500 µg kg^{-1} or 250 µg kg^{-1}, respectively. Samples were spiked at concentrations of 10 µg kg^{-1} and 50 µg kg^{-1}. Each spike level was prepared in triple determination.

First, 10 mL of water were added and samples were shaken for 1 min. Then, 10 mL ACN were added and the mixture was shaken for 1 min. Bond Elut EN QuEChERS-salt was added, it was shaken for another minute and centrifuged for 5 min at 3,500 rpm and 4 °C. Phase separation is shown in figure 17.

After that, 5 mL water were pipetted to Bond Elut EMR-Lipid dSPE material, mixed on a vortex and 5 mL raw extract were added to the dSPE material. Samples were shaken for 5 min at 3,500 rpm and 4 °C. The supernatant was transferred into 50 mL-Tubes and Bond Elut EMR-Lipid (polish pouch, $MgSO_4$) was added. The samples were mixed by shaking, then samples were centrifuged (5 min, 3,500 rpm, 4 °C) and filtrated through a syringe filter (PA, 0.45 µm). Afterwards, 100 µL filtrate were pipetted into vials with inserts, samples were stored at 4 °C. Subsequent analyses were performed with 2.0 g of sample.

Different parameters were changed to optimize the extraction and recovery. The first

test of the Agilent Kit was performed with BPA-d_{16} and BPS-$^{13}C_{12}$ as internal standard. Afterwards, BPA-d_8 and BPS-$^{13}C_{12}$ were used during method development. Addition of internal standard was performed either in the beginning before the extraction was performed or after the extraction/before clean-up with dSPE material. The time frame for extraction was extended to 15 min instead of 1 min. Furthermore, the final step of filtration was compared to centrifugation of 600 µL of sample solution in the microcentrifuge for 3 min at 12,500 rpm.

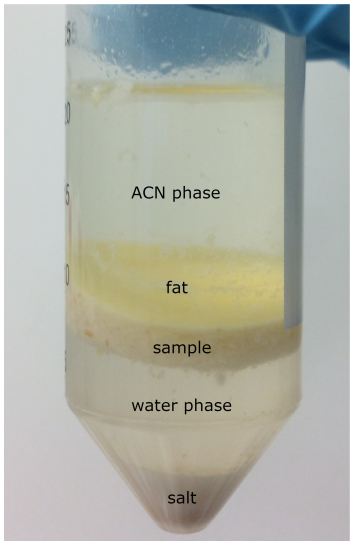


Figure 17: Phase separation of ACN and water after extraction of 2.0 g of croissant sample. The yellow layer was assumed to be fat, since the sample contains a high amount of fat and the layer was either solid or liquid, depending on temperature conditions before and after centrifugation (room temperature vs. centrifugation at 4 °C). Furthermore, the layer was always located towards the ACN phase not the water phase, thus it is assumed that this is a rather apolar substance.The layer was not further analyzed.

Samples were concentrated, too. The solvent evaporation was performed on a rotary evaporator and an IR-Dancer for comparison. In this case, the addition of internal standard was set to 50 µg kg^{-1}. After clean up with polish pouch, 2 mL of extract were pipetted into test tubes, put into the IR-Dancer and solvent was evaporated to dryness overnight (settings: 40 °C, p_{min} = 190 mbar). The residue was dissolved in 200 µL 50 % ACN. A phase separation occured, thus the concentration on the IR-Dancer was repeated. The 2 mL extract that were transferred into test tubes, were taken from the ACN-phase[14].

Before sample concentration on the rotary evaporator, samples were transferred to 15 mL tubes after clean up with polish pouch and centrifuged for 5 min at 4 °C and 3,500 rpm. 2 mL of the extract were taken from the ACN-phase and pipetted into 10 mL or 25 mL pear-shaped flasks.The solvent was evaporated to dryness and the residue was dissolved in

[14] After the use of the polish pouch, a phase separation occurred. Thus the salt that caused the phase separation after the absorption of the residue in 50 % ACN was introduced by part of the aqueous phase. Thus it was crucial to take extract from the ACN and not the water phase.

200 µL 50 % ACN. The sample solution was quantitatively transferred into an Eppendorf-tube, samples were put into a microcentrifuge for 5 min at 12,000 rpm and the supernatant was pipetted into vials with inserts.

Sample contamination

Reagent blanks and samples showed small signals at the same retention time as BPA. To find the source of contamination, MeOH, 50 % MeOH, water, ACN and internal standard were filled in vials. A reagent blank was extracted with the Agilent Kit that was used for sample preparation and after each step that required the usage of materials from the Kit, 100 µL of extract were filled in a vial with insert. The extracts were not only mixed with the $MgSO_4$ polish pouch, but also with heated Na_2SO_4 in comparison. To elucidate, whether contamination came from plastic tubes, a sample was prepared in glassware. Furthermore, the container in which the samples were homogenized was tested as a possible source of contamination. During migration tests, the containers were cleaned with soap and tap water. Afterwards, the containers were cleaned with MeOH two times and the solvent was analyzed for signs of contamination.

4.3.6 Method validation

New calibration solutions were prepared for method validation. The standard solution with the lowest BPA/BPS concentration amounted to 0.5 µg L^{-1}, upper limit was at 100 µg L^{-1} BPA/BPS (0.5, 10, 25, 40, 55, 70, 85, 100 µg L^{-1}). Volumetric flasks were filled to the mark with 50 % ACN (ACN:water (v/v)), internal standard concentration was 50 µg L^{-1} BPA-d_8 and BPS-$^{13}C_{12}$.

For determination of trueness/bias, recovery and precision, sixfold determination of spiked sample with 50 µg kg^{-1} was performed. Spike levels of 10 µg kg^{-1} and 100 µg kg^{-1} were analyzed in triple determination and blanks at each spike level were prepared as well as a reagent blank. 2 mL of water were added to each spiked blank and 2.0 g of sample were weighed, all in 50 mL-tubes. 200 µL internal standard solution (500 µg L^{-1} BPA-d_8 and BPS-$^{13}C_{12}$ in MeOH) were added, thus the internal standard concentration amounted to 50 µg kg^{-1}. Volumes of standard solution that were added to spiked samples, amounted to: 40 µL for 10 µg kg^{-1}, 200 µL for 50 µg kg^{-1} and 400 µL for 100 µg kg^{-1} (500 µg L^{-1} BPA/BPS in MeOH).

In the beginning, 10 mL water were added, samples were shaken for 1 min, then 10 mL ACN were added. Extraction was performed on a shaker for 15 min. Afterwards, QuEChERS salt mix ($NaCl/MgSO_4$) was added. Samples were shaken for 1 min and centrifuged for 5 min at 3,500 rpm and 4 °C. 5 mL water were pipetted on EMR-Lipid dSPE material, mixed on a vortex at maximum speed and 5 mL of raw extract were added. Samples were shaken for 1 min and centrifuged for 5 min at 3,500 rpm and 4 °C. The supernatant was decanted into 50 mL-tubes, Bond Elut EMR-Lipid (polish pouch, $MgSO_4$) was added. The mix was shaken for 1 min, samples were centrifuged for 5 min at 3,500 rpm and 4 °C, supernatant was decanted in 15 mL-tubes and centrifuged once more (5 min/3,500 rpm/4 °C).

Then, 2 mL of extract were pipetted into 25 mL pear shaped flasks and the solvent was evaporated to dryness on the rotary evaporator. Settings of the rotary evaporator were 10 °C for coolant, 40 °C for the water bath and rotation was set to 180 rpm. After the solvent was evaporated to dryness, 200 µL of 50 % ACN were pipetted on top of the residue and transferred into an Eppendorf-tube. Samples were put into a microcentrifuge for 5 min at 12,000 rpm and the supernatant was pipetted into a vial with insert.

Matrix extracts were prepared for the determination of LOD and LOQ. Sample preparation was performed according to determination of trueness and recovery, except that internal standard and standard solution were not added in the beginning, but were pipetted to 1.9 mL matrix extract in 25 mL pear shaped flasks before evaporation of solvent. The calibration for the determination of LOD and LOQ contained internal standard at 50 µg L^{-1} BPA-d_8 and BPS-$^{13}C_{12}$. The BPA/BPS concentration of calibration solutions (LOD/LOQ) ranged from 1-10 µg L^{-1} BPA/BPS. Solutions were pipetted twice into vial with inserts, so injection would be performed from different vials during analysis. Linearity, selectivity and calibration range were determined from the results.

4.3.7 Migration tests

Two migration tests were performed for different contact times: 1 h and 24 h. The contact time of 24 h was applied to enable a direct comparison with the CWE conditions. The other time frame was chosen, to gain another data point and because contact times of that dimension may be closer to realistic scenarios. Consumers buy baked goods in the morning and might prepare breakfast, while the baked goods remain in a paper bag.

From paper plates, pizza boxes and kitchen paper, six pieces à 4.9 cm x 10 cm (= 0.49 dm^2) were cut for each migration test. Four croissants were prepared per paper sample and contact time (see paragraph 4.3.1). One croissant served as a blank, three others were prepared for triple determination of the migration from any paper FCM with 1 h or 24 h of contact time. The blank croissant was wrapped in aluminum foil, while the others were sampled with paper FCM: one piece of paper was put on top of the croissant, another one was put below (resulting in a contact area of 0.98 dm^2) and this kind of "sandwich" was then wrapped in aluminum foil, too (see figure 18). To ensure contact between FCM and foodstuff, the croissants were wrapped tightly, then they were left on the bench for 1 h (24 h) at room temperature (ca. 25 °C). Room ventilation was turned off, so humidity might not be decreased. At the end of contact time, FCMs were removed and the croissants were weighed.

The ratio between initial sample weight and surface area was determined. All croissants were weighed after contact time and the mean was calculated out of 24 values resulting in 53.52 g. With the surface area of 0.98 dm^2, the ratio of initial sample weight and surface area was 54.61 g food per dm^2 FCM.

The samples were homogenized in accordance with paragraph 4.3.1. First, the blank croissant was homogenized in one jar, then the sampled croissants were homogenized. The

croissants that were brought into contact with one paper FCM were homogenized in the same jar. That way cross contamination was prevented. The jars were roughly wiped out between homogenization of each croissant. This was sufficient, since samples during method development showed small peaks for BPA, but the area was smaller than that of the lowest calibration standard (0.5 µg L^{-1})[15]. Thus, the samples were regarded to be free from BPA and it was concluded that contamination did not occur between the samples during the homogenization. Afterwards, containers were cleaned with distilled water and dish soap; after the first migration test (1 h), it was additionally cleaned with methanol.

For each croissant, including blanks, the determination was performed in triplicate to depict a certain amount of food regarding the migration. Since 2.0 g of sample were used for sample preparation, triplicates gave 6 g of croissant, while the mean weight of the croissants after baking and cooling to room temperature amounted to 53.52 g. On average 11 % of a whole croissant sample were analyzed. A reagent blank was prepared as well.

Extraction and sample preparation was performed according to the validated method (protocol see paragraph 4.3.6).

After the migration tests, two croissants were baked and their core temperature was measured. The temperature amounted to 87.9 °C for one croissant and 84.6 °C for the other croissant.

Figure 18: Setting for migration tests: a croissant is placed on top of a piece of paper FCM, another piece of paper is put on top of the croissant, resulting in a kind of sandwich. That "sandwich" is then wrapped in aluminum foil and left on the bench for a specific time frame (1 h or 24 h).

4.3.8 Instrumental conditions for the quantitative analysis of contaminants in red napkins

Chromatograms were recorded and processed with Analyst 1.7.0 (Sciex) while evaluations were performed with SCIEX OS 1.4.0 (Sciex) for all analyses including the quantitative determinations of bisphenols in different sample solutions. Retention times of all analytes are shown in table 5.

[15]The results of the validation showed that the LOD for BPA is 2 µg kg^{-1} and thus the concentration of BPA is below LOD. Therefore it is questionable whether the contamination occured from BPA or not.

Table 5: Retention times of the different analytes.

compound	retention time [min]
BPA	3.95
BPS	2.30
aniline	2.11
naphthol AS	10.36
HNA	9.75
4-aminobiphenyl-d_9	8.81

For quantification, the MS was operated in the multiple reaction monitoring (MRM) mode. MS/MS acquisition was operated in positive-ion mode, the source temperature was 500 °C. The mass transitions used for quantification and qualification are summarized in table 6, additional MS/MS settings are depicted in table 8. All equipment settings were already in place and were adopted by the author for the analyses. The same applies to the settings in paragraph 4.3.9.

Table 6: Mass transitions used for quantification and qualification. The incoherence of the quantifier and qualifier fragments of BPA and BPA-d_8 will be discussed in paragraph 5.4.

compound	precursor	quantifier		qualifier	
		Q1 mass [Da]	Q3 mass [Da]	Q1 mass [Da]	Q3 mass [Da]
BPA	$[M-H]^-$	227.0	212.0	227.0	133.0
BPA-d_8		235.1	137.0	235.1	220.1
BPA-d_{16}		241.1	223.0	241.1	141.9
BPS		248.9	107.9	248.9	92.0
BPS-$^{13}C_{12}$		261.4	113.9	261.4	98.0
aniline	$[M+H]^+$	94.1	77.0	94.1	51.1
aniline-d_5		98.9	82.1	98.9	54.1
HNA		189.0	170.9	189.0	114.9
naphthol AS		264.0	171.0	264.0	114.9
4-aminobiphenyl-d_9		179.1	160.2	179.1	162.2

LC-MS/MS with electrospray ionization was carried out on a Shimadzu LC-20AD prominence (Shimadzu, Duisburg, Germany) HPLC system coupled with an API 4000 Q TRAP mass spectrometer (Sciex, Darmstadt, Germany). The HPLC system contained two pumps (LC-20AD), a column oven (CTO-20AC HT), a degasser (DGU-20A5), a controller (CBM-20A) and a temperature-controlled autosampler (SIL-20AC). The injection volume of sample extract into the LC-MS/MS system was 10 µL. Chromatography was performed on a Kinetex 2.6 µm F5 column (100 mm × 2.1 mm, 2.6 µm particle size, 100 Å pore size, Phenomenex, Aschaffenburg, Germany) at 40 °C with a flow rate of 0.2 mL min^{-1}. The

mobile phase consisted of LC-MS grade methanol with 0.05 % formic acid and water with 0.05 % formic acid. The gradient conditions are given in table 7.

Table 7: Solvent gradient for PAA-analysis.

gradient time [min]	0	6	13	13.5	25
eluent A, water, 0.05 % formic acid [%]	95	5	5	95	95
eluent B, methanol, 0.05 % formic acid [%]	5	95	95	5	5

Table 8: Additional settings of MS/MS for the quantitative analysis of contaminants in red napkins.

MS/MS-parameters	
scans in period	625
relative start time	0.00 msec
scheduled ionization	off
experiments in period	1
scan type	MRM
scheduled MRM	no
polarity	positive
scan mode	none
ion source	ESI+
resolution Q1	unit
resolution Q3	unit
intensity thres.	0.00 cps
settling time	0.0000 msec
MR pause	5.0070 msec
MCA	no
detector parameters	
CEM	2500.0

4.3.9 Instrumental conditions for the quantitative analysis of bisphenols

For the quantitative analysis of bisphenols in solvent extracts from paper FCMs, the same equipment was used as for the quantitative analysis of contaminants in red napkins. Still, the parameters and column described in this paragraph were consistently applied to all bisphenol analyses. If differences occured, they are listed here.

ESI-LC-MS/MS analysis of CWE and croissant extracts during method development, validation and migration tests was carried out on an Agilent 1260 Infinity II LC System (Shimadzu, Kyoto, Japan) coupled with a MayLab MistraSwitch column oven and a 5500 Q TRAP mass spetrometer (Sciex, Darmstadt, Germany). The HPLC system consisted of two pumps (Agilent 1260 G1312B), a degasser (Agilent 1260 G1379B) and a temperature-controlled autosampler (Agilent 1260 G1367E). 2 µL of the sample extract were injected into the LC-MS/MS system. Chromatographic separation was conducted on

a Kinetex 5 µm Biphenyl column (150 mm x 3.0 mm, 5 µm particle size, 100 Å pore size, Phenomenex, Aschaffenburg, Germany) with a Phenomenex Kinetex 5 µm Biphenyl guard column (2.1 mm x 4.6 mm, 5 µm particle size) at 40 °C with a flow rate of 0.4 mL min^{-1}. The mobile phase consisted of LC-MS grade methanol and water**.** The gradient conditions are depicted in table 9.

Table 9: Solvent gradient for the separation of bisphenols during quantitative analysis.

gradient time [min]	0	4	11	11.5	16
eluent A, water	40	1	1	40	40
eluent B, methanol	60	99	99	60	60

The MS was operated in MRM-mode. MS/MS acquisition was operated in negative-ion mode, the source temperature was set to 450 °C (CWE: 47 °C, the different temperatures will be discussed in paragraph 5.2). Additional MS/MS settings are depicted in table 10. The mass transitions used for quantification and qualification are summarised in table 6, the corresponding fragments of BPA and BPS are shown in figures 19 and 20 [90].

Table 10: Additional settings of MS/MS for the quantitative analysis of bisphenols.

	CWE	**solvent extraction**	**method development, validation and migration tests**
scans in period	160	160	149
relative start time	1000.00 msec	0.00 msec	1000.00 msec
scheduled ionization	off		
experiments in period	1		
scan-type	MRM		
scheduled MRM	no		
polarity	negative		
scan mode	none		
ion source	ESI-		
resolution Q1	unit		
resolution Q3	unit		
intensity thres.	0.00 cps		
settling time	0.0000 msec		
MR pause	5.0000 msec	5.0070 msec	5.0000 msec
MCA	no		
detector parameters			
CEM	1850.0	2500	1850.0

With regard to table 10, the settings vary between the analysis of CWE, method development/validation/migration tests and the solvent extraction. The settings are different, because the samples were measured with different equipment as it was mentioned earlier.

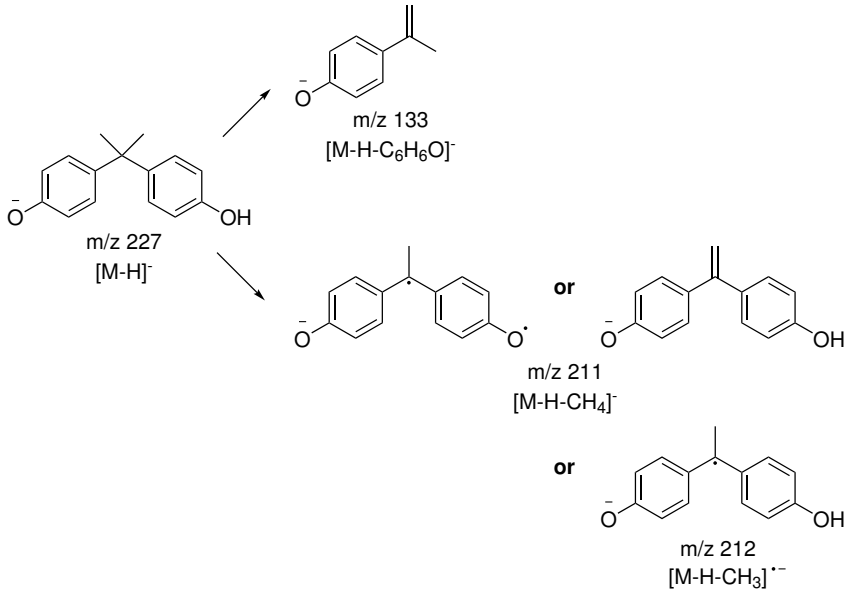


Figure 19: Fragmentation of BPA according to Zhao et al., 2016 [90]. The fragmentation of BPA leading to the fragment of m/z ratio 212 is a suggestion from the author.

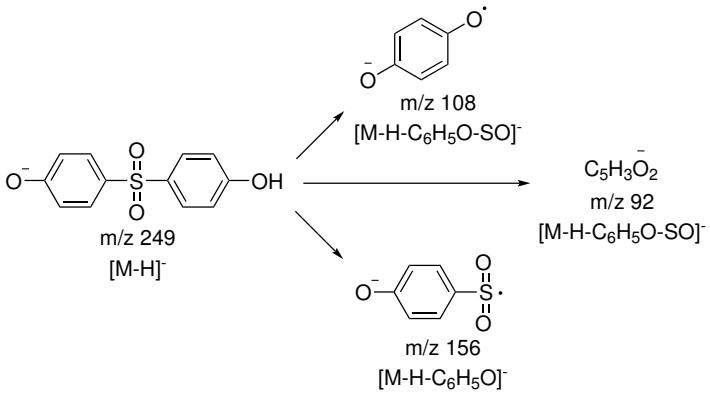


Figure 20: Fragmentation of BPS according to Zhao et al., 2016 [90].

5 Results and discussion

5.1 Homogeinity tests of colored paper napkins

The homogeneity of the paper napkins was determined by CWE of the samples. The results were evaluated in accordance with the International Union of Pure and Applied Chemistry (IUPAC) International Harmonized Protocol, International Organization for Standardization (ISO) 13528, and F-test check for significant heterogeneity [91, 92]. The results are shown in table 11.

Table 11: Results of the homogeneity test of the colored paper napkins and the defined comparative standard deviation σ_{pt} [µg L^{-1}] for each analyte. Twelve CWE were analyzed and evaluated. The results of the Cochran and Grubbs test showed an outlier for aniline. Therefore the outlier was removed, thus eleven out of twelve samples were taken into consideration for the evalution of the homogeneity of aniline in red printed napkins.

analyte	**mean x [µg L^{-1}]**	**comparative standard deviation σ_{pt} [µg L^{-1}]**	**homogeneity according to ISO 13528**	**homogeneity according to IUPAC**
aniline	5.21	1.15	yes	yes
naphthol AS	231.05	50.83	no	yes
HNA	166.22	36.57	no	yes
BPA	45.8	10.1	yes	yes
BPS	7.3	1.6	yes	yes

Neither the Cochran nor the Grubbs test showed any outliers for naphthol AS and HNA. Both tests showed an outlier for aniline. When the data point of the 12th sample was removed, no outlier was identifiable. Therefore eleven instead of twelve samples were included into the evaluation of the homogeneity of the paper napkins with regard to their aniline content. BPA, BPS and aniline are homogenous according to ISO 12528 and IUPAC, while naphthol AS and HNA are homogenous according to IUPAC but not according to ISO 13528.

5.2 Correlation between initial sample weight and analyte concentration in CWE according to DIN 645

CWEs (DIN EN 645) are used as alternatives for migration tests, especially for FCMs such as paper towels and napkins, since these materials are not durable enough for a migration test [33]. Another aspect that these FCMs have in common are their absorptive properties in contact with water. Less water is available for extraction due to the absorption into the FCM. The characteristic of those paper FCMs is illustrated in comparison to other paper FCMs like paper plates (see figure 21).

Figure 21: CWE of paper plates (**1**), paper towels and paper napkins (**2**) in comparison: while a supernatant is clearly visible for the CWE prepared from cardboard material (paper plates), the paper towels and napkins absorb most of the water. Thus, a supernatant is hardly perceivable.

The impact of the swelling properties on the efficiency of the extraction process and the related scalability of the results was the reason for preparing the CWEs with different initial sample weights, ranging from 1.0 g-10.0 g. The results of the initial sample weight depending concentrations of aniline, naphthol AS and HNA in CWE are shown in figure 22.

The data point for naphthol AS-concentration in CWE at 3.0 g initial sample weight was removed, because the concentration of the diluted extract was above the operational range (1-10 µg L^{-1}) due to insufficient dilution. A dilution factor of 20 was applied to CWEs with initial sample weight from 1.0 g-3.0 g with regard to their naphthol-AS concentration, while CWEs with initial sample weights $>$ 4.0 g were diluted by factor 50. Since this dilution factor proved to be adequate for CWEs with higher initial sample weight and factor 20 proved too low, it is recommendable for repetition of CWE with 3.0 g of sample.

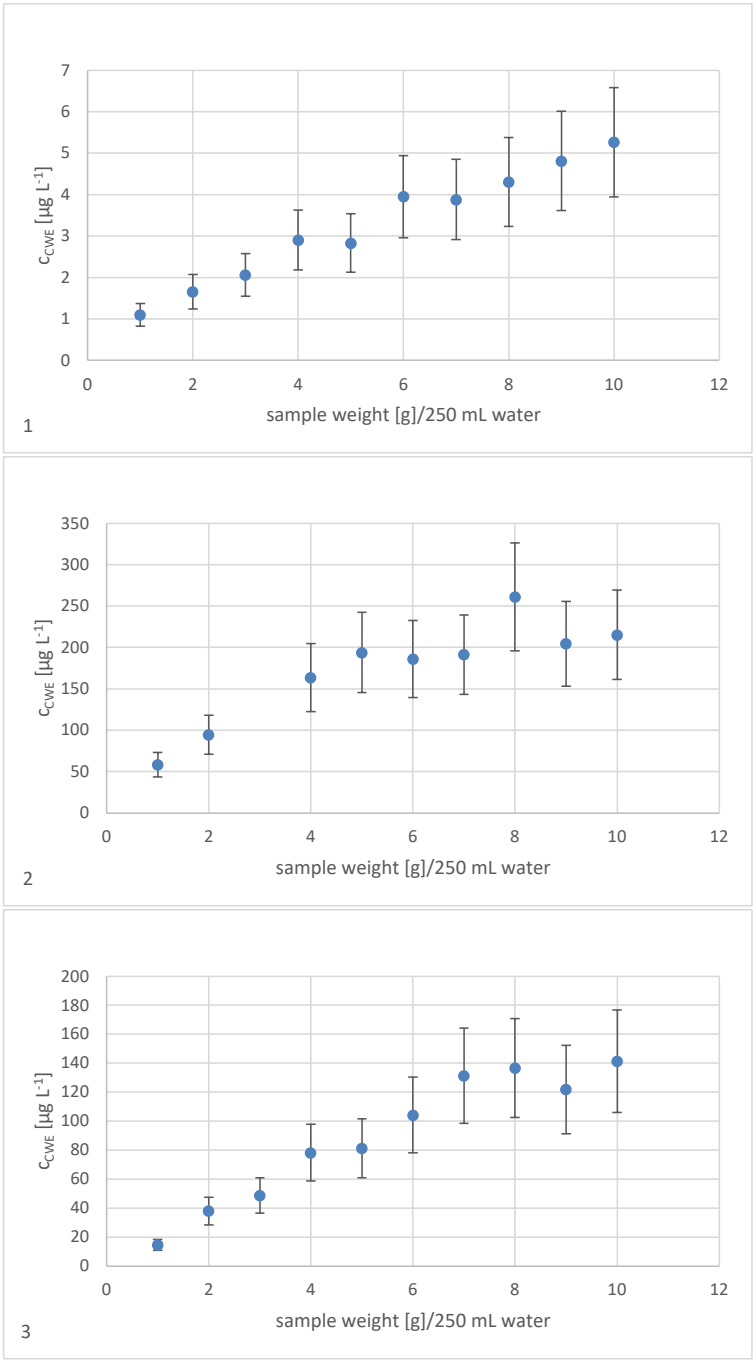


Figure 22: Concentrations of aniline (**1**), naphthol AS (**2**) and HNA (**3**) in CWE [µg L^{-1}]. The data point at 3.0 g initial sample weight was removed for naphthol AS, since its concentration exceeded the operational range due to insufficient dilution. Relative measurement uncertainty (MU) was set to 25 % based on expert judgement of the scientists of the research group and is shown for each data point.

The aniline-concentration of CWE increased with increased ratio of initial sample weight to the volume of CWE. The correlation appeared to be linear and no saturation occured, the same referred to HNA, see figure 22. Meanwhile, saturation seems to have occured

for naphthol AS: the solution appears to be saturated with naphthol AS at an initial sample weight of 5.0 g at 193.9 ± 48.5 µg L^{-1}. For naphthol AS, no reference for its water solubility could be found, so its methyl- and methoxy-derivative (3-hydroxy-2'-methyl-2-naphtanilide, CAS 135-61-5; 3-hydroxy-2'-methoxy-2-naphtanilide, CAS 135-62-6) were used for a rough estimation (see table 12) [93, 94]. Since the derivatives are soluble at concentrations of 2.1-6.6 mg L^{-1}, the water solubility of naphthol AS is expected to be in that dimension. The naphthol AS-concentration in CWE amounted to about 0.2 mg L^{-1} when the saturation effect occured (see table 13). Thus, it appears that naphthol AS did not exceed its solubility limits when the limits of its derivatives are applied.

With regard to the BfR recommendation 021/2014 that states that the aggregate detection limit for PAAs of 0.01 mg per kg food from Regulation (EC) No. 10/2011 applies to paper FCMs aswell, the release of aniline into the CWE prepared with 10.0 g of sample would be regarded as safe (see table 13)[16] [63]. Because of the density of water (1 g mL^{-1}), the c_{CWE}(aniline) [µg L^{-1}] is the same as β_{CWE}(aniline) [mg kg^{-1}] and thus directly comparable with the aggregate detection limit [mg kg^{-1}]. The amount of aniline in CWE is 0.00526 mg L^{-1}, thus not exceeding the aggregate limit for PAAs, at least not on its own. The migration limit for naphtol AS and HNA were given in the BfR opinion from 2019 as < 10 µg L^{-1} for naphthol AS and 360 µg L^{-1} for HNA (see table 13) [66]. The concentration of HNA in the CWE prepared with 10.0 g of sample is below the recommended limit (141.38 µg L^{-1}), while naphthol AS is clearly exceeding the limit (215.31 µg L^{-1}).

Table 12: Water solubility of the analytes that were quantified in CWE. No reference could be found for the water solubility of naphthol AS, therefore methyl- and methoxy-derivatives were used to roughly estimate the solubility of naphthol AS [93, 94].

substance	**solubility in water at certain temperature**
aniline	35 g L^{-1} at 20 °C (no pH given) [95]
naphthol AS	-
3-hydroxy-2'-methyl-2-naphtanilide	0.0066 g L^{-1} at 20 °C and pH of 7.2 [94]
3-hydroxy-2'-methoxy-2-naphtanilide	0.0021 g L^{-1}at 20 °C and pH of 6.7 [93]
HNA	0.474 g L^{-1} (no temperature or pH given) [96]
BPA	0.298 g L^{-1} at 25 °C (no pH given) [97]
BPS	1.1 g L^{-1} at 20 °C (no pH given) [73]

[16] According to the recommendation, the aggregate limit of 0.01 mg $kg^{-1}$for PAAs that migrate from paper FCMs into food does not apply to PAAs that are classified as carcinogens of category 1A and 1B. The ALARA principle should be applied to those compounds [63]. Since aniline is classified as carcinogenic (class 2), the aggregate limit is applicable in this case.

Table 13: Concentration of the analytes in paper napkins in CWE [µg L^{-1}]. The results are shown including MU which was set to 25 % based on expert judgement of the scientists in the research group.

initial sample weight	**c_{CWE}(aniline) [µg L^{-1}]**	**c_{CWE}(naphthol AS) [µg L^{-1}]**	**c_{CWE}(HNA) [µg L^{-1}]**
1.0 g	1.10 ± 0.28	58.52 ± 14.63	14.66 ± 3.66
2.0 g	1.66 ± 0.41	94.55 ± 23.64	38.00 ± 9.50
3.0 g	2.06 ± 0.51	-	48.76 ± 12.19
4.0 g	2.90 ± 0.73	163.64 ± 40.91	78.25 ± 19.56
5.0 g	2.83 ± 0.71	193.92 ± 48.48	81.38 ± 20.35
6.0 g	3.95 ± 0.99	186.15 ± 46.54	104.24 ± 26.06
7.0 g	3.88 ± 0.97	191.39 ± 47.85	131.36 ± 32.84
8.0 g	4.30 ± 1.08	261.05 ± 65.26	136.61 ± 34.15
9.0 g	4.81 ± 1.20	204.41 ± 51.10	121.83 ± 30.46
10.0 g	5.26 ± 1.32	215.31 ± 53.83	141.38 ± 35.35

Besides the preparation of CWE from paper napkins to determine potential saturation effects, CWEs were also prepared from paper towels made from recycling paper to examine whether saturation effects occured for BPA and BPS. Saturation effects seem to have occured for BPA (see figure 23), too. This was the case at an initial sample weight of $\geq$ 4.0 g. With a concentration of 136.8 ± 40.3 µg L^{-1}, the concentration of BPA did not exceed its water solubility limit in comparison to the literature (see table 12).

With regard to table 14, where the solvated amount of analyte is shown, it can be seen that the amount of BPA migrating into CWE is decreased by factor 2-3 with an increase of initial sample weight. The decrease is observable when the initial sample weight is increased from 4.0 g to more than 5.0 g. The same conclusion is drawn for naphthol AS in the tested paper napkins: there is a correlation between the saturation in CWE and the amount of sample used in its preparation for some analytes. BPA and naphthol AS have the lowest water solubility in comparison with the other analytes (see table 12) because even if the water solubility of naphthol AS was about factor 100 higher than that of its derivatives, it would still be lower than for the other analytes. At the same time, the highest concentrations in CWE were observed for BPA and naphthol AS. It is possible that for the other analytes no saturation was observed because their amount in the sample is too low and that saturation would occur, in case they were present in higher amounts, too. Since their water solubility is higher, it is expected that the saturation would occur at higher levels than for BPA and naphthol AS.

The concentrations of BPA and BPS in CWE were compared with the SML of 50 µg kg^{-1}. The result for BPA in CWE prepared from 10.0 g of sample was 159.6 µg L^{-1}, while the concentration for BPS amounted to 62.7 µg L^{-1}. The amount of BPS in CWE is close to the SML but when MU is considered, it is not unequivocally exceeding it. Meanwhile, the concentration of BPA is three times higher than the SML. Even when MU is considered

in its favor, it is still two times higher than the SML.

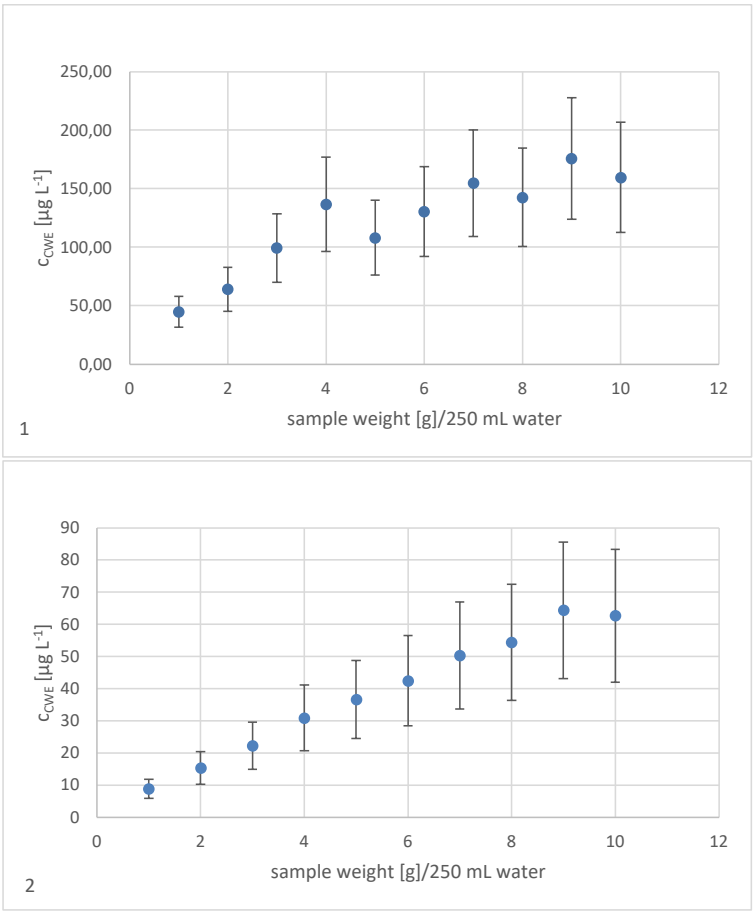


Figure 23: Concentrations of BPA (**1**) and BPS (**2**) in CWE [µg L^{-1}]. MU was established in an inter-laboratory study by members of the research group and amounted to 29.5 % for BPA and 33.0 % for BPS. MU is shown for each data point.

Table 14: Concentration of BPA and BPS from paper towles in CWE [µg L^{-1}]. The results are shown including MU which was determined in an inter-laboratory study and set to 29.5 % for BPA and 33 % for BPS.

initial sample weight	**c_{CWE}(BPA) [µg L^{-1}]**	**c_{CWE}(BPS) [µg L^{-1}]**
1.0 g	44.87 ± 13.24	8.96 ± 2.96
2.0 g	64.02 ± 18.89	15.41 ± 5.09
3.0 g	99.30 ± 29.29	22.27 ± 7.35
4.0 g	136.75 ± 40.34	30.93 ± 10.21
5.0 g	108.10 ± 31.89	36.69 ± 12.11
6.0 g	130.44 ± 38.48	42.48 ± 14.02
7.0 g	154.68 ± 45.63	50.32 ± 16.61
8.0 g	142.60 ± 42.07	54.44 ± 17.96
9.0 g	175.76 ± 51.85	64.38 ± 21.24
10.0 g	159.64 ± 47.09	62.68 ± 20.68

One reason for the occurring saturation effects is the partial absorption of the solvent by the paper samples. The absorption of water into the paper is motivated by capillary force. The classical liquid transport model of Lucas and Washburn in capillaries of porous media relates penetration x at time t with the viscosity μ, contact angle between liquid and capillary wall θ_{eq}, surface tension γ and the capillary radius R as follows [98, 99]:

$$x = \sqrt{\frac{\gamma R cos\theta_{eq}}{2\mu}}\sqrt{t}.$$

Songok et al. showed that the equation is inadequate for short contact times [s], which is caused by many factors, one of which is the change in material properties such as the swelling of the paper [100]. Because the CWE lasted for 24 h, contact time would not be categorized as short. Thus, the model can be applied on a general level for description of the varying absorption of water by different paper FCMs. When preparing CWE for different paper FCMs, such as paper napkins or paper plates according to DIN 645, the only parameter in the Lucas-Washburn-equation that varies for the samples concerning the penetration x is the capillary radius R. While R is different for various (paper) materials, the difference increases due to the absorptive capacities of the different papers. A paper towel will absorb more water than a cardboard plate, thus the capillary radius is increased and with it the penetration x of the paper towel compared to a paper plate.

Absorption of a liquid into a solid, which makes the solid grow in size, is the general definition of "swelling" [101]. When water is absorbed by the paper samples used for the preparation of CWE, the paper swells. This effect is caused by the main molecular component in paper which is cellulose. The polysaccharide consists of many glucose-subunits. Hence, lots of intermolecular hydrogen bonds are formed [102]. With increasing polarity of a solvent, the paper matrix is able to absorb an increased amount of solvent, because it is stabilized in the matrix due to said hydrogen bonds [103]. Thus, the absorbed water is stabilized in the paper matrix by two forces: the capillary force and intramolecular interactions. Consequently, cellulose is forming a hydrate shell, which - e. g. in paper chromatography - is responsible for dissolving hydrophilic substances [104].

According to the free-volume theory that describes diffusion in polymers by consideration of the probability that a substance migrates into a hole, pore or capillary because its size is bigger than the molecule itself, swelling of the pores and therefore their enlargement enhances diffusion [105]. By considering cellulose to be a biopolymer, the theory may be applied to the processes in the paper matrix. In this case, the swelling of the tight cellulose-matrix allows substances, which are originally too large to diffuse in the paper matrix more easily [24]. This may be a counteracting effect towards the deceleration of diffusion.

During the extraction the migrating substance diffuses into the solvent [106]. According to Fick's law, the solute flux J proceeds in opposition to the concentration gradient $\frac{dc}{dt}$ [107]:

$$J = -D\frac{dc}{dt},$$

with D as the diffusion coefficient for a solute in a specific solvent.

Thus, PAAs as well as BPA and BPS will diffuse from the tested FCMs into the water since the concentration in the paper is higher than in the solvent. A high amount of solvent or replacement of the solvent by fresh one increases the efficiency of the extraction [106]. According to Nernst's distribution law, the distribution of a solute between two phases achieves a constant value for the ratio of the concentrations in the different phases at constant temperature [77]:

$$K = \frac{c''}{c'}.$$

K is the equilibrium distribution coefficient, c'' and c' represent the solute's concentration in one of the different phases. When this constant value is achieved, the phases are in equilibrium. As soon as equilibrium is established, no more solute is extracted. The state could be rather described as an even exchange of solute between extract and sample. It is possible that equilibrium between the paper samples and water is established quicker, since the water is diffusing through the pores of the paper much slower since it is held in it by capillary force and stabilized by the hydrogen bonds of the paper matrix. In this case little to no fresh solvent would diffuse through the paper and thus the extraction process would not be promoted but instead hindered due to the presence of local equilibrium states. If only a small amount of solvent is present, equilibrium is established faster because less solute is necessary to achieve the equilibrium concentration. This also means that less solute can diffuse into the solvent since saturation occurs quickly due to the presence of a small amount of solvent. Therefore the literature value for the water solubility of BPA and naphthol AS might not be exceeded, but it might be achieved locally in certain parts of the paper matrix. Overall, the equilibrium in parts of the matrix appears to outweigh the possible enhanced diffusion by the swelling of the matrix since the saturation occured for BPA and naphthol AS even though their concentration in CWE were below their literature solubility limits.

As described in paragraph 4.3.9, the temperature setting of the ion source during the analysis of the CWE prepared from paper towels was 47 °C and thus much lower than during the analysis of samples from method development, method validation (450 °C) and CWEs prepared from paper napkins (500 °C). Since the CWEs from paper towels were prepared and analyzed before method development and validation, the setting was not especially changed for that purpose. Due to the lower sensitivity compared to earlier analysis, the settings were examined and it was noticed that the temperature was set too low. If the temperature of the ion source is set too low, the solvent does not evaporate efficiently and the release of ions from the solvent droplets is hindered [83]. Nevertheless, the samples were not analyzed for a second time, since the aim of the analysis was not to determine the exact concentration of BPA/BPS in the samples[17], but to characterize

[17]The concentration of BPA/BPS in the same paper towels was established in an inter-laboratory study by members of the research group.

whether a trend of saturation occurred with increasing initial sample weight. Therefore, the actual concentration of the CWEs are expected to be higher. However, the lower sensitivity of the signals should not affect the trend itself.

With regard to the saturation effects of certain analytes in the CWE, it was shown that the results are not scalable for the applied samples. Therefore, the concentration of one analyte in CWE, which was prepared from an absorptive material, is not necessarily calculable for another CWE, which was prepared from that same sample with a different initial sample weight . It is also possible that this effect depends on the analyte's concentration in the paper and if enough analyte is present, saturation might occur for any analyte.

5.3 Solvent extraction of paper and board FCMs

The aim was to determine the total amount of BPA and BPS in three paper FCMs via solvent extraction. Total extraction is achieved, when the last extract contains less than 1 % of the analyte concentration of the first extract. Extractions were performed with 50 % ACN (ACN:water, 1:1 (v/v)) for the first extraction step, the following steps were performed with pure ACN. In extractions performed earlier in the research group, it was shown that the addition of water in the first extraction step improved the extraction efficiency. BPA showed good solubility in ACN but extraction with pure ACN required more extraction steps without water than with an addition of water. The extractions also showed the best extraction efficiency for the procedure used in the course of this work (see paragraph 4.3.4), but after the calculation of the results obtained in the course of this work an error during the evaluation of the earlier extractions was revealed. Therefore, Soxhlet extraction actually shows better extraction efficiency and extracted more BPA from paper FCMs than the extraction in the ultrasonic bath. Thus, it is recommended for future extractions[18]. As a result, the amount of BPA and BPS determined in these samples cannot be considered to be the total amount in the tested paper FCMs. Nevertheless, the results will be used in comparison with CWE and migration tests to give the reader an idea of the quantity of analyte in the samples and the percental migration rate. Table 15 shows the results for each extraction step.

For the extraction of paper plates and pizza boxes, five extraction steps were performed. For paper towels, six steps were performed. The means were calculated from triple determinations. It is apparent that the amount of BPA in the FCMs is about tenfold higher than the amount of BPS. Total extraction after the mentioned definition was achieved for BPA and BPS in paper plates and BPA in pizza boxes. The amount of BPS in the last extract from pizza boxes amounted to 1.5 % of the first extract and thus it is not a total extraction. The same accounts for BPA (the last extract contains 5.8 % of the amount in the first extract) and BPS (the last extract contains 6.1 % of the amount in the first extract) in paper towels. LOD and LOQ of CWE from an inter-laboratory study

[18] Only BPA and not BPS was analyzed in these extract solutions. Therefore, no statement can be made concerning the results of BPS.

(LOD_{BPA}: 2 µg L^{-1}, LOD_{BPS}: 1 µg L^{-1}; LOQ_{BPA}: 8 µg L^{-1}, LOQ_{BPS}: 2 µg L^{-1}) were applied for the evaluation [19]. The MU for solvent extracts was set to 25 % based on expert judgement of the scientists in the research group while the MU for BPA and BPS in CWE was determined in the inter-laboratory study. The MU for BPA amounts to 29.5 %, for BPS it is 33 %.

Table 15: Amount of BPA and BPS [µg dm^{-2}] extracted from the tested paper FCMs per extraction step. The mean results were calculated from triple determinations and are shown including MU.

paper plates, extract no.	**BPA_{mean} [µg dm^{-2}]**	**BPS_{mean} [µg dm^{-2}]**
1	17.57 ± 4.39	1.08 ±0.27
2	5.42 ± 1.35	0.27 ±0.07
3	0.99 ± 0.25	< LOQ
4	< LOQ	< LOD
5	< LOD	< LOD
pizza boxes, extract no.	**BPA_{mean} [µg dm^{-2}]**	**BPS_{mean} [µg dm^{-2}]**
1	37.69 ± 9.42	3.97 ± 0.99
2	15.76 ± 3.94	1.05 ± 0.26
3	4.76 ± 1.19	0.67 ± 0.17
4	1.38 ± 0.34	0.17 ± 0.04
5	0.33 ± 0.08	< LOQ
paper towels, extract no.	**BPA_{mean} [µg dm^{-2}]**	**BPS_{mean} [µg dm^{-2}]**
1	5.03 ± 1.26	0.40 ± 0.10
2	3.17 ± 0.79	0.26 ± 0.06
3	1.71 ± 0.43	0.14 ± 0.03
4	0.79 ± 0.20	0.07 ± 0.02
5	0.51 ± 0.13	0.04 ± 0.01
6	0.29 ± 0.07	0.02 ± 0.01

The results of the CWE were compared with the release of BPA/BPS from paper FCMs according to solvent extraction. The results for CWE were obtained in an inter-laboratory comparison study by other members of the research group. For the preparation of CWE, 10.0 g of sample (paper plate, pizza box) or 1.0 g (paper towels) were used. Figure 24 shows that the pizza boxes released the highest amount of BPA per dm^2 with regard to the tested samples, followed by the tested paper plates and paper towels. Concerning the release of BPS per dm^2 sample, the pizza boxes released the highest amount, while paper plates and paper towels released about the same amount of BPS. Release per surface area is not to be confused with the amount of BPA and BPS per kg paper. As shown in table 16, paper towels have the lowest grammage and therefore the surface area in contact with solvent, food or food simulant at the same initial sample weight as for the other paper FCMs is always higher. Thus, the content of BPA and BPS per kg paper FCM is additionally shown

[19] The author did not take part in any inter-laboratory comparison study and therefore the results from those studies were always obtained by other members of the research group.

in table 17.

The grammage of the paper samples that were used for migration tests was determined by preparing three pieces of each paper sample of known measurement and weighing these samples. The determination of grammage was achieved through division of the mass by the measurements of the paper (see table 16).

Table 16: Grammage of paper FCMs used for determination of their bisphenol content and the migration of BPA and BPS into food.

sample	**mean grammage [g dm^{-2}]**	**standard deviation [g dm^{-2}]**
paper plate	3.0328	0.0267
pizza box	2.925	0.0255
paper towels	0.5483	0.0171

Table 17: Comparison of the content of BPA/BPS per kg paper FCM and the release of BPA/BPS per dm^2 for solvent extractions with 50 % ACN. The results are shown including MU.

paper FCM	**BPA**		**BPS**	
	[mg kg^{-1}]	**[µg dm^{-2}]**	**[mg kg^{-1}]**	**[µg dm^{-2}]**
paper plates	7.91 ± 1.98	23.98 ± 5.99	0.45 ± 0.11	1.36 ± 0.34
pizza boxes	20.48 ± 5.12	59.92 ± 14.98	2.00 ± 0.50	5.86 ± 1.47
paper towels	20.99 ± 5.25	11.51 ± 2.88	1.71 ± 0.43	0.94 ± 0.23

The results for the BPA content are in accordance with those of the final report of a scientific study organized by the BMEL concerning the migration of contaminants from FCMs made from recycled fibers into food [1]. The BPA content of board FCMs made from recycled fibers ranged from 2.5 to 24.4 mg kg^{-1}.

In figure 24 it is also shown that only 8 % of the BPA-content in the solvent extracts were found in CWE for paper plates and pizza boxes, while 61 % of the extracts BPA-content from paper towels migrated into the CWE. From the extracted BPS-content in paper plates, 68 % were found in CWE, 75 % were found in the CWEs of pizza boxes and 129 % of the result that was measured for paper towels after extraction with 50 % ACN were found in the CWE[20]. Of course, it is not possible to extract more than the

[20] This would support the Lucas Washburn approach: the highest amount of BPA from FCMs was extracted from paper towels by CWE (percental and absolute). The extracted amount of BPS was higher for pizza boxes than for paper towels but this FCM contains more BPS and the percental amount of the transferred BPS into CWE is higher for the extraction from paper towels.

total amount of BPS with CWE, but it has to be repeated that total extraction was not achieved for BPA/BPS in paper towels and the method for solvent extraction does not have the highest extraction efficiency. Therefore, it has to be assumed that the BPS-amount in the tested paper towels is higher than the result for extraction with 50 % ACN and that about 100 % are extracted by CWE. A much higher percental amount of BPS was extracted with CWE than of BPA. This might be caused by the circumstance that the absolute amount of BPS in paper is generally lower than that of BPA. The impairment of the extraction of BPA by CWE might be further increased by its lower water solubility.

The CWE is not a (total) extraction method and the results confirm this circumstance. It produces a leachate that should simulate the migration into food. The suitability of the CWE as a simulation for analytes like BPA and BPS will be compared later on (see paragraph 5.6).

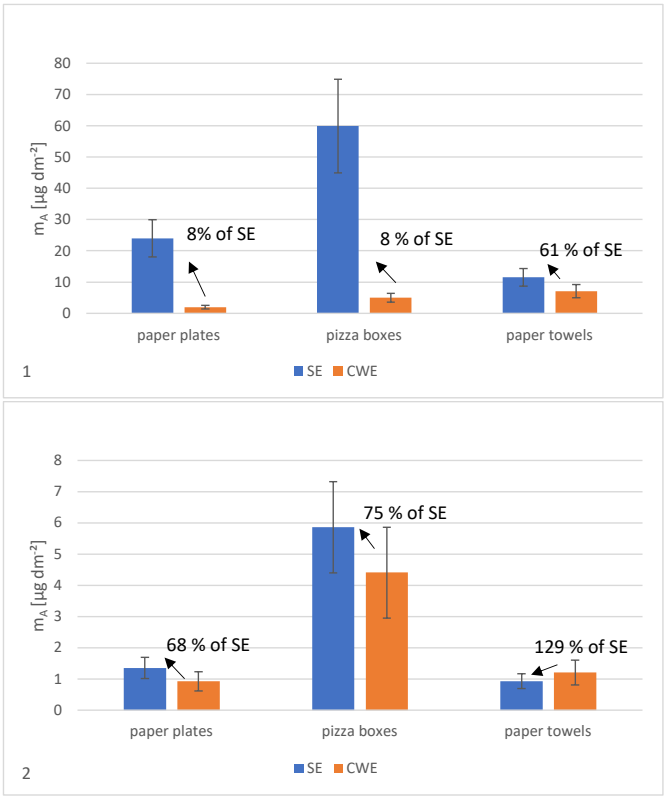


Figure 24: Amount of BPA (**1**) and BPS (**2**) in FCM according to solvent extraction (SE) compared to CWE [µg dm^{-2}]. The results for CWE were obtained in an inter-laboratory comparison study. The MU was set to 25 % based on expert judgement of scientists of the research group. The amount of BPA released into CWE relative to the amount that was released into the solvent extracts amounted to 8 % for both paper plates and pizza boxes and 61 % for paper towels. The amount of BPS released into CWE relative to the amount that was released into the solvent extracts amounted to 68 % for paper plates, 75 % pizza boxes and 129 % for paper towels.

A problem that occured during the analysis of the samples were the peak shapes (see

figure 25). As stated in paragraph 4.3.9, the solvent extracts were analyzed with different LC-MS/MS equipment than the samples that were analyzed during method development, method validation and the migration tests. When the LC-MS/MS equipment consisting of the Agilent 1260 Infinity II LC System and the 5500 Q TRAP mass spectrometer was applied the injection volume was set to 2 µL. In contrast, the injection volume on the Shimadzu LC-20AD prominence HPLC system coupled with an API 4000 Q TRAP mass spectrometer was set to 20 µL since the mass spectrometer was less sensitive **in comparison to the 5500 Q TRAP**. The column that was used for the separation of BPA and BPS was used for solvent extracts as well as the other analyses (Kinetex 5 µm Biphenyl column, 150 mm x 3.0 mm, 5 µm particle size, 100 Å pore size). Due to the application of different injection volumes it was suspected that the setting of 20 µL was responsible for the broadened and tooth shaped peaks and that the column was overloaded.

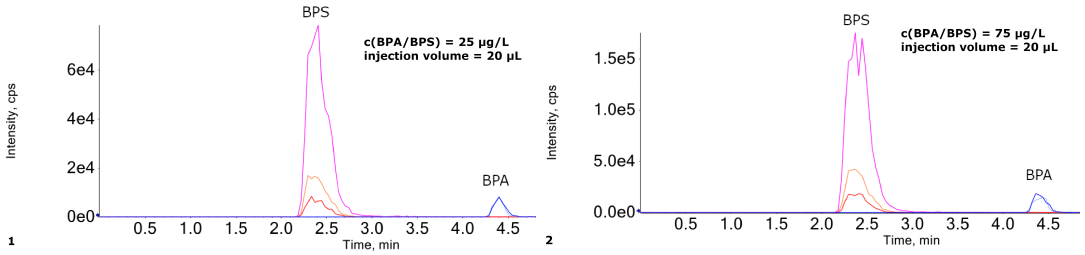


Figure 25: Calibration standards with 25 µg L^{-1} BPA/BPS (**1**) and 75 µg L^{-1} BPA/BPS (**2**). The transitions shown are the quantifier of BPA (dark blue, 227.0/212.0), qualifier of BPA (light blue, 227.1/133.0), quantifier of BPS (pink, 248.9/107.9), the first qualifier of BPS (orange, 248.9/92.0) and a second qualifier of BPS (red, 248.9/107.9). The injection volume was set to 20 µL.

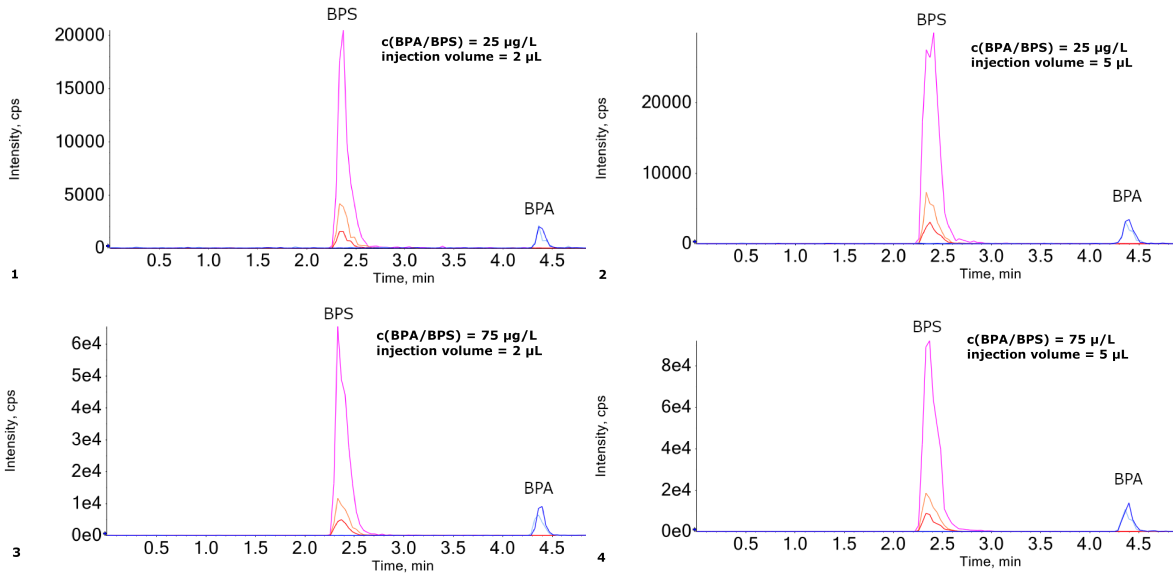


Figure 26: Calibration standards with 25 µg L^{-1} BPA/BPS (**1**, **2**) and 75 µg L^{-1} BPA/BPS (**3**, **4**) with injection volumes of 2 µL (**1**, **3**) and 5 µL (**2**, **4**).

Hence, injection volumes of 2 µL and 5 µL of the calibration standards were tested to find out which volume would not lead to an overload of the column and result in more Gaussian type peak shapes (see figure 26). As a result, 2 µL of injection volume were applied since the peaks were sharper than those that were obtained with an injection volume of 5 µL.

5.4 Method development

During method development, recovery of internal standard BPA-d_{16} proved to be problematic. This was a hinderance during evaluation of the recovery of the experiments. The problem with BPA-d_{16} is that the hydroxy groups are deuterated, too. In protic solvents such as water, ACN and MeOH, which were used for extraction and as solvents during analysis via LC-MS/MS, both acidic deuterium atoms are quickly exchanged for protons in a quantitative amount (see figure 27) [108].

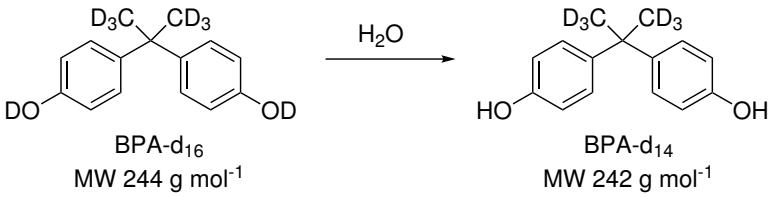


Figure 27: Exchange of the deuterium atoms of the hydroxy groups of the internal standard BPA-d_{16} for protons in protic solvents. Deuterium atoms belonging to the methyl groups might be exchanged, too [108].

The signal intensity of BPA-d_{16} varied widely under the present conditions even though the proton exchange on the hydroxy groups was taken into consideration. This may have been caused by the exchange of deuterium for protons that is also occuring on the deuterated methyl groups. The solution to this problem was to apply BPA-d_8, since only the aromatic structures are deuterated and therefore the standard is much more stable regarding the exchange of protons towards deuterium.

With regard to figure 28, it is apparent that the more sensitive fragmentation of BPA-d_8 was used as qualifier and not as quantifier. Additionally, the application of the fragments as qualifier and quantifier was not in accordance with that of the fragments of BPA (see table 6). During method development, the quantifer and qualifier of BPA were exchanged for each other, since it became apparent that the fragmentation of 227.0 m/z to the fragment of 212.0 m/z was more sensitive than the fragmentation to 133.0 m/z. It was assumed that the other fragment was originally applied as quantifier due to the interfering peaks that are shown in figure 29 and originate from tensides according to scientists of the research group. The equivalent fragment of BPA-d_8 (137.0 m/z) should have been established as quantifier, too. Since the evaluation of the calibration and the samples was performed in the same manner, this circumstance is expected not to have a significant impact on the final results of the method validation and the migration tests.

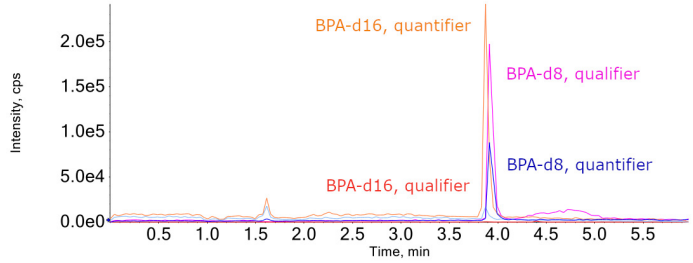


Figure 28: Comparison of the signal intensity of BPA-d_8 and BPA-d_{16}: the fragment with 141.9 m/z (red) was used as the qualifier Ion for BPA-d_{16} and could not be detected. The fragment with 223.0 has the highest intensity (orange) and is the quantifier for BPA-d_{16}. The m/z ratios are equivalent to the deuterated ions of the molecular structures that are shown in figure 19.

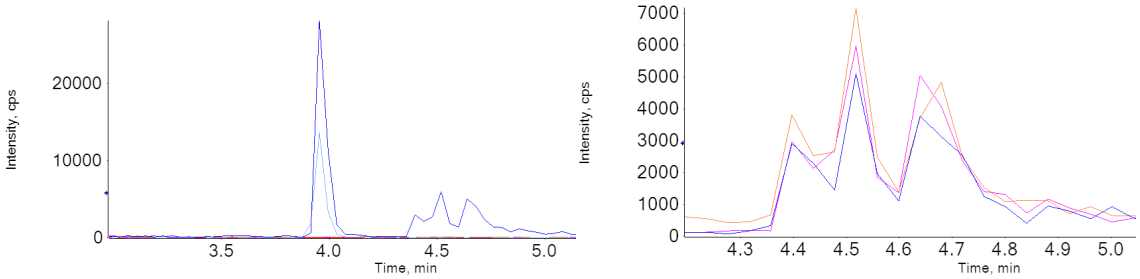


Figure 29: The left chromatogram shows the quantifier (dark blue, 212.0 m/z) and qualifier (light blue, 133.0 m/z) fragments of BPA. The interfering peaks only appear in the chromatogram of the quantifier. The right chromatogram shows the interfering peaks of the tensides in different calibration solutions that were analyzed during validation (blue: 0.5 µg L^{-1} BPA/BPS, pink: 10 µg L^{-1} BPA/BPS, orange: 85 µg L^{-1} BPA/BPS). The signal is virtually constant.

During method development, croissants were bought at self-service counters in different local supermarkets. The samples showed small peaks at the same retention time as BPA. That is why it was assumed that the samples were contaminated, so they were replaced by frozen goods from local supermarkets, which were baked in the drying oven of the laboratory. The signals still appeared, but the peak areas decreased compared to the fresh samples and therefore the frozen croissants were chosen for further analysis. The signals from the sample that was finally chosen, did not fit the area ratios of the quantifier and qualifier that were determined for BPA during validation (see paragraph 5.5), so the criteria for selectivity of BPA were not met. Signals were also detected from the reagent blank. Therefore, and because the peak areas of sample blanks were below those of the standard solution with the lowest concentration of BPA (0.5 µg L^{-1}), it was supposed that even if the detected substance was BPA, its concentration was < LOD according to the validation and thus the influence on the validation was negligible.

Solvents were checked for contamination as well and a reagent blank was prepared, using the materials from the Agilent Kit. After each clean-up step for which material from the kit

was applied, an aliquot of sample solution was transferred into a vial to find out whether a component of the QuEChERS-Kit might have introduced the contamination into the samples. Pure solvents showed no signals for BPA and were excluded as possible source of contamination. Only after the usage of Polish Pouch ($MgSO_4$) for clean up, a signal was detected. The area of the signal was still below that of the lowest calibration standard. Since the polish pouch comes in small packages which are coated with plastic on the insed, it could be the source of contamination.

Sample preparation was also performed in glass ware, to check whether the contamination could stem from the use of plastic tubes. However the detected signal area was even increased compared to that of the reagent and sample blank that were prepared in plastic tubes. Thus it was concluded, that the contamination did not stem from the plastic tubes used for sample preparation.

It was also suspected that a source of contamination might be the container in which the samples were homogenized. The container consists of steel and is not coated. It was cleaned between homogenization of samples with dish soap and tap water. During the migration tests, the container was rinsed with MeOH after the usual cleaning procedure, an aliqout of the solvent was analyzed via LC-MS/MS to determine whether a contamination occured. Then the container was cleaned with MeOH and afterwards it was rinsed with MeOH once more. Another aliqout of solvent was analyzed in comparison. That way it should be examined, whether an additional cleaning with MeOH could help to minimize a possible contamination. A signal occured at the same retention time as BPA, but it was below the standard solution with the lowest concentration of BPA (0.5 µg L^{-1}). There was also no difference between the cleaning procedures whether the container was additionally cleaned with or without MeOH, so the clean up step was not considered to be necessary.

Another possible source of contamination could be the container in which the croissants were stored. According to the manufacturer, the container (IKA Disperser DIS-300-S-M.10 Tube) and the cover consist of polyethylene (PE) [109]. Since BPA is used as an antioxidant in plastics, it can be released into a contact medium [110]. Fasano et al. showed that BPA migrated from various FCMs containing PE (low density PE film, bread bag, tetrapack, yogurt packaging) into different foodstuffs [3]. For the LDPE film and the tetrapack, the concentration was $<$ LOD (21 ng L^{-1}), while it could not be recovered as a migrant from the yogurt packaging. Only for the bread bag, a mean concentration of 189 $\pm$ 46 ng L^{-1} was recovered. Therefore, it is not unlikely that BPA might have migrated from the container into the croissant samples in small amounts that are even below the LOD. Since signals were also recovered in the solvent blanks it is probable that the source of contamination did not have an impact on the croissants. At this point it is rather likely that the signals are caused by a substance in the package of the Polish Pouch.

Different initial sample weights were applied to determine the amount of sample that would be suitable for sample preparation. Originally 10.0 g of sample were used for QuEChERS sample preparation [10], so this approach was used, too. However, samplings with 5.0 g and 10.0 g, respectively, contained too much of the dry croissant matrix, because most

of the solvent (ACN) was absorbed. Therefore it was decided to work with lower amounts of sample. Furthermore, the main component of fruits and vegetables, which QuEChERS sample preparation was established for originally, is water (see table 18), while the main components in croissant are carbohydrates and fat (see table 4). Therefore no water had to be added in the original method because the samples already contained a high amount of water. Additionally, the quantity of substances in the matrix that have to be removed is expected to be higher than in fruits or vegetables and it is reasonable to work with less sample when working with croissant. It was decided to use 2.0 g of the sample, to have as much sample as possible for analysis without losing solvent volume through absorption.

Table 18: Water content of different fruits and vegetables according to Souci, Fachmann and Kraut [111].

food	**water content per 100 g**
blueberry	84.6 g
carrot	88.2 g
cucumber	96.0 g
raspberry	84.5 g
tomato	94.2 g

When samples were concentrated on the rotary evaporator in comparison with the IR-Dancer, results were comparable concerning recovery. It was decided to use the rotary evaporator for different reasons: first, the shaker function of the IR-Dancer did not work properly, secondly the concentration of samples with BPA/BPS prepared from a different food matrix was already performed successfully on the rotary evaporator. Nevertheless, concentration of samples on an IR-Dancer is a good alternative, since this way > 20 samples can be concentrated in about 3 h. This would enable one person to prepare ≥ 20 samples for analysis on one day.

Different dSPE materials, different clean-up steps before analysis (filtration, centrifugation, dilution, freezing of the samples) and variation of parameters such as extraction time were evaluated to optimize and develop a method that is suitable for the quantitative analysis of BPA and BPS in croissants. The comparability of the different methods and therefore discussion of the results is problematic, since for all methods except the MEAL-study approach and the validated Agilent method, the analyte concentration was $<$ LOQ for BPA. Spiking samples to 50 µg kg^{-1} (100 ng BPA/BPS per 2.0 g sample) and dilution in 10 mL of ACN yields a concentration of 10 µg L^{-1} BPA/BPS. If samples are diluted at a factor 2 during the application of the dSPE material, the concentration is 5 µg L^{-1} and therefore below LOQ for BPA[21] - at least for the Agilent method, because for the other experiments the LOQ was not determined. The problem is rooted in data evaluation: calculations from µg L^{-1} to µg kg^{-1} were performed directly in the software SCIEX OS, instead of looking at concentrations of solutions first. The only approach where samples

[21] The LOQ for BPA is 6 µg L^{-1}.

were concentrated as a final step and thus the concentration of BPA was > LOQ apart from the validated method was the method from the MEAL study for analysis of fats and oils. The informational content of this analysis is limited in that regard that the experiment was performed in a single determination per spike level. The reason for single determination was that in the beginning of the method development, different approaches were tried and limited ressources (dSPE materials and time) were at hand. The results obtained with the method from the MEAL study had a recovery of 87 % (50 µg kg^{-1}) and 96 % (100 µg kg^{-1}) for BPA , for BPS it was 67 % (50 µg kg^{-1}) and 72 % (100 µg kg^{-1}). Results were evaluated without internal standard, because BPA-d_{16} could not be recovered. The results of the MEAL study method are promising for a start, but questionable due to the lack of internal standard and the fact that results were gained in single determination. Also, the sample preparation is quite more time consuming than the sample preparation of the validated method with the QuEChERS-Kit of Agilent.

5.5 Method validation

The validation of an analytical method is necessary to investigate the reliability and quality of that method. It confirms that the requirements for the intended use of the method are met and is performed for the purpose of quality control [112]. For this purpose, different parameters were determined via statistical evaluation and calibration like the precision, trueness, LOD, LOQ and selectivity. [77].

Calibration range and linearity

Linearity of a calibration is given when analyte concentration and measurement signal are directly proportional [112]. For FCMs and food in contact with FCMs, the target calibration range is supposed to contain at least five calibration points in the range from 0.2*SML up to 2*SML [88]. Since the SML of BPA and BPS amounts to 50 µg kg^{-1}, which is equal to 50 µg L^{-1} in the sample solution (conversion factor: 1, see paragraph 5.6), the lowest calibration level should be set to 10 µg L^{-1}. The calibration point with the highest BPA/BPS concentration should be set to 100 µg L^{-1}, according to literature.

Linearity was evaluated in the calibration range of 0.5 µg L^{-1} and 100 µg L^{-1} at eight calibration points (see paragraph 4.3.6). Each calibration solution was analyzed in triplicate and the mean of the resulting peak areas was calculated for the analyte and internal standard. From the calculation of the ratio of these results, the calibration curve was determined (see figure 30).

Linearity was verified via Mandel test and residual test [88]. The Mandel test creates linear and quadratic regression from the data of the calibration curve and determines whether the quadratic regression is a better fit for the calibration curve than the linear regression. The comparison is based on the regression coefficients: if the value F_{calc} that combines the effects of the residual variance of the linear (s_{y1}^2) and the quadratic (s_{y2}^2)

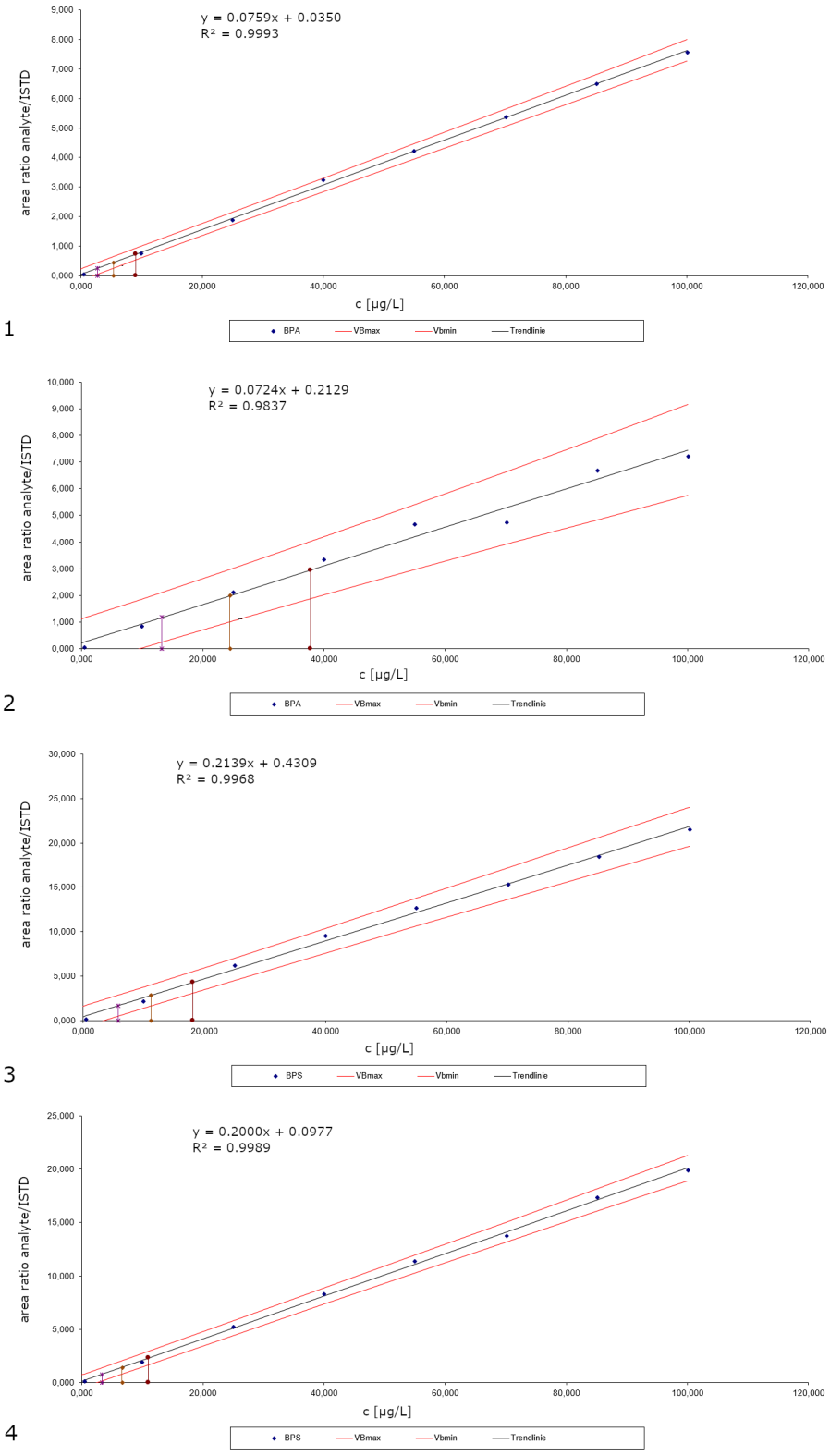


Figure 30: Calibration curves measured during the validation of linearity. The fit and regression coefficient are shown for each calibration curve (BPA day 1 (**1**), BPA day 2 (**2**), BPS day 1 (**3**), BPS day 2 (**4**)).

regression is compared with the tabulated F of Fisher and $F_{calc} < F$ applies, the quadratic regression does not fit the calibration curve better than the linear regression and thus linear regression is accepted. F_{calc} is calculated by the following equation:

$$F_{calc} = [\frac{(n-2)*s_{y1}^2-(n-3)*s_{y2}^2}{s_{y2}^2}]$$

with n as the number of calibration curves used for the verification of linearity.

The results for F, F_{calc} and regression coefficients R^2 are shown in table 19. Since $F_{calc} < F$ applies for all calibrations on two different days, the calibrations are considered to fulfill the criteria for linearity of the Mandel test.

Table 19: F_{calc}, F and regression coefficients R^2 from Mandel test for the calibration curves of BPA and BPS in the calibration range from 0.5-100 µg L^{-1}.

	day 1			**day 2**		
analyte	F_{calc}	F	R^2	F_{calc}	F	R^2
BPA	3.2348	16.26	0.999	1.1616	16.26	0.984
BPS	12.3302	16.26	0.997	1.6611	16.26	0.999

Residuals ε were plotted with the corresponding concentrations x to verify the linearity of the calibration. Linearity is confirmed when the plot shows randomly distributed points in comparison to a systematic pattern which indicates non-linearity. ε is the difference between the measured value y_i and the expected value y_i^{ex} [88]:

$$\varepsilon = y_i - y_i^{ex}.$$

Residual plots are shown in figure 31.

Since the scattering of the residuals was random for all calibrations, it was interpreted as another proof of the calibrations' linearity. Therefore, linearity is given in the range proposed by literature [88]. Linearity for the determination of BPA and BPS in breast milk via QuEChERS sample preparation and LC-MS/MS detection was obtained in the rage of 1-70 µg L^{-1}, while for BPA in tea the range was 5-500 µg L^{-1}[12, 13]. Thus, the range determined in this validation fulfills the proposed criteria and is in accordance with the results for breast milk. The maximum level was considerably lower than for the validated method for BPA-determination in tea but the calibration range is sufficient since the essential point is to determine whether the SML of 50 µg kg^{-1} is exceeded or not.

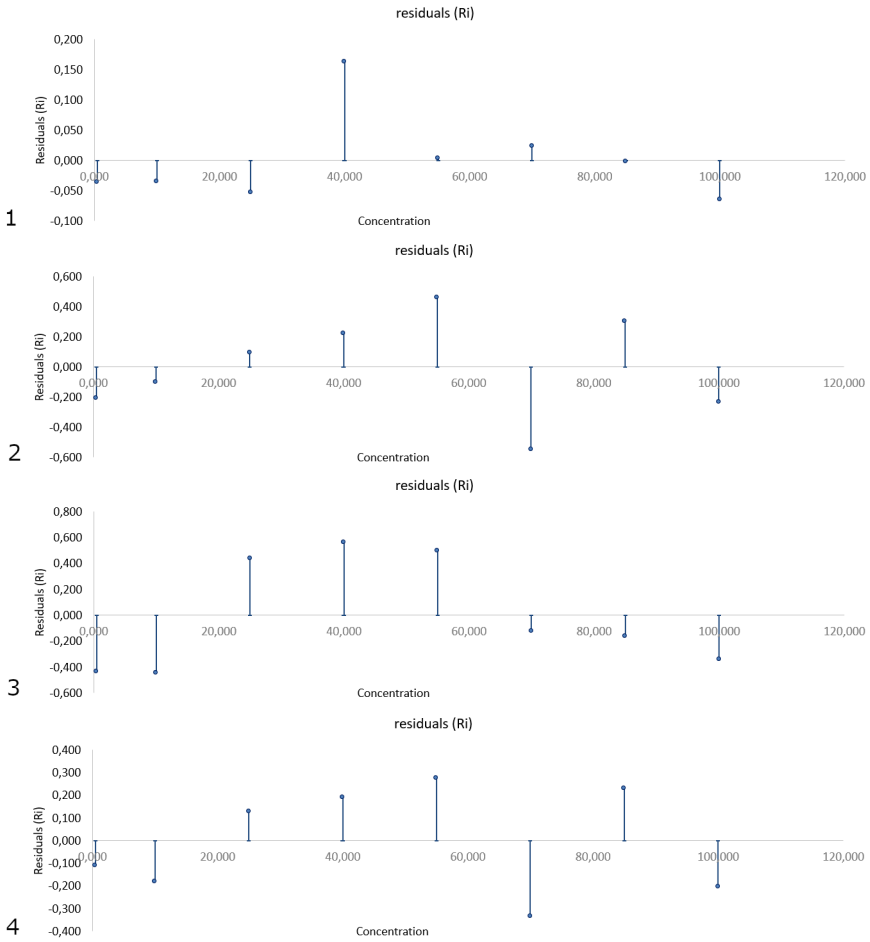


Figure 31: Residuals for the different calibration curves. Linearity is confirmed when the plot shows randomly distributed points in comparison to a systematic pattern which indicates non-linearity (BPA day 1 (**1**), BPA day 2 (**2**), BPS day 1 (**3**), BPS day 2 (**4**)).

Recovery

Recovery R is defined as the (percental) ratio of analyte that was recovered from a spiked sample to the actual concentration of the spiked sample [77]. It is calculated via the following equation:

$$R\ [\%] = \frac{x_{mean}}{x_{spike}} * 100,$$

with x_{mean} as the mean concentration determined in the spiked samples at a certain spike level while x_{spike} is the actual concentration of the spiked samples. The higher the recovery is, the less analyte is lost during sample preparation [112]. The maximum acceptable criteria for recovery of an analyte's concentration from 10-100 µg kg^{-1}(10-100 ppb) are listed in table 20.

Table 20: Maximum acceptable criteria for recovery according to CEN TS 15356 [88].

concentration	**mean recovery [%]**
$\leq$ 10 ppb	40-120
10-100 ppb	60-110
$>$ 100 ppb	80-110

In the validation plan, 80-110 % were established as maximum acceptable criteria for the mean recovery at all spike levels (10 µg kg^{-1}, 50 µg kg^{-1} and 100 µg kg^{-1}). The results were obtained by triple determination at spike levels of 10 µg kg^{-1} and 100 µg kg^{-1}. The results at spike level 50 µg kg^{-1} were obtained from sixfold determination. The mean results for the recovery of BPA and BPS both analyses (on different days) are shown in table 21.

Table 21: Mean results for the recovery R of BPA and BPS at different spike levels for analyses on two different days.

spike level [µg kg^{-1}]	**R_{mean}BPA [%], day 1+2**	**R_{mean}BPS [%], day 1+2**
10	99	85
50	95	93
100	103	101

The results for the recovery of BPA and BPS were in the range of the maximum acceptable criteria of 80-110 %. This applies for all spike levels. In literature, recovery of BPA and BPS was comparable to or lower than the results for the validation of this method (see table 22).

Table 22: Literature results for the recovery of BPA and BPS in food and biological matrices.

analyte	**sample**	**sample preparation**	**analytical technique**	**recovery**	**reference**
BPA	fish	QuEChERS	LC-MS/MS	67-82 %	[15]
	tea			88-109 %	[13]
	breast milk			70-92 %	[12]
BPS	breast milk			70-92 %	[12]

Precision

Precision is determined by the analysis of replicate samples and the calculation of their standard deviation (SD) to the mean. It may also be expressed as relative standard deviation (RSD) [88]. The standard deviation specifies the scattering of the measured values around the mean value [77]. Mean y_m and SD are calculated by the following equations:

$$y_m = \frac{1}{n} \sum_{i=1}^{n} y_i$$

n represents the number of replicates.

$$SD = \sqrt{\frac{\sum_{i=1}^{n}(y_i - y_m)^2}{n-1}}$$

RSD ist calculated by division of the standard deviation by the mean [77]:

$$RSD = \frac{SD}{y_m}$$

Since precision depends on the analyte concentration, it should be determined at different concentrations [88]. Acceptability criteria for precision are shown in table 23. The criteria in the validation plan were set to $\leq$ 10 %.

Table 23: The within laboratory standard deviation calculated according to Horwitz for different analyte concentrations. These levels should not be exceeded [88].

analyte concentration	**RSD [%] predicted**
100 ppm	8.0
10 ppm	11.3
1 ppm	16.0
100 ppb	22.6

RSD was determined for BPA and BPS at spike levels of 10 µg kg^{-1} and 100 µg kg^{-1} in sample solution, each in triple determination. For the spike level of 50 µg kg^{-1}, sample analysis was performed sixfold. The results for each spike level are shown in table 24, mean results are shown in table 30.

Table 24: Results of the relative standard deviation for all spike levels. The mean precision for all levels was calculated and is shown in table 30.

spike level	**RSD_{BPA} [%]**	**RSD_{BPS} [%]**
10 µg kg^{-1}	7.12	6.57
50 µg kg^{-1}	7.10	6.74
100 µg kg^{-1}	9.45	6.31

Deviations in the results for BPA are assumed to be higher than for BPS, because BPS is more sensitive and thus the peak areas are larger. Therefore, an alteration of the peak area of BPS causes a smaller scattering of results in comparison to an alteration of the peak areas of BPA in the same dimension. The results are below the limit set in the validation plan and in the range of the maximum acceptable criteria. The precision of this method is also comparable to or higher than for other methods that are used for quantification of BPA and BPS in food and biological matrices via QuEChERS sample preparation and LC-MS/MS detection. For breast milk, tea and fish, mean RSD was determined to be 14 %, 15 % or $\leq$ 10 % [12, 13, 15]

Trueness and bias

Trueness is represented in terms of bias which expresses the difference between the true value and the mean of the results [88]. The bias was calculated from the results for recovery of the spike level 50 µg kg^{-1} by the following equation:

$$bias\ [\%] = \sqrt{\frac{\sum(\frac{y_i - reference\ value}{reference\ value} * 100)^2}{n}}$$

Reference values may be obtained from certified reference material, reference materials, reference methods and reference laboratories. During the validation, none of these sources were available, therefore the results of the actual spike concentration of 50 µg kg^{-1} were used as reference value [88]. The maximum acceptable bias of quantitative methods is shown in table 25.

Table 25: Maximum acceptable bias of quantitative methods according to the commission decision concerning the performance of analytical methods and the interpretation of results [113].

concentration	**bias range [%]**
$\leq$ 1 ppb	-50 to +20
1-10 ppb	-30 to +10
$>$ 10 ppb	-20 to +10

Bratinova et al. stated that if the acception of significant bias is based on the maximum acceptable bias, the results should be considered in the calculation of the method's uncertainty [88]. The bias obtained in the course of the validation was 11.9 % for BPS and 12.4 % for BPA. They exceeded the maximum acceptable range for the bias at a spike level of 50 µg kg^{-1}. Since the range is exceeded by less than 3 %, the bias was still accepted and considered in the calculation of the uncertainty of the method.

Limit of detection (LOD) and Limit of quantification (LOQ)

The limit of detection (LOD) is the smallest amount of an analyte that can be detected in the sample solution with acceptable certainty. It is a qualitative parameter. In comparison to the LOD, the limit of quantification (LOQ) is the lowest concentration of an analyte that can be determined in the sample solution with acceptable certainty, thus it is a quantitative parameter [88]. Determination of the LOD and LOQ was performed according to DIN 32645 (indirect method): the calculation via a calibration function was based on a calibration range from 1-10 µg L^{-1} in matrix extract. Standard and internal standard solutions were added after the extraction (see paragraph 4.3.6). When the indirect method is applied, the calibration range is supposed to be close to the LOD, ranging from 0 to 10*LOD [114]. The parameters that were applied for the determination of LOD and LOQ via the indirect method are shown in table 26.

Table 26: Parameters that were applied for the determination of LOD and LOQ via the indirect method (calibration).

parameter	**type/value**
limit	LOD/LOQ
chemometric model	indirect mehod via calibration
number n of calibration solution	10
number m of measurements of the calibration solutions	2
significance level α [%]	5
reciprocal relative measurement uncertainty k	3

The calibration solutions were analyzed in duplicate on each day that measurements were performed. The mean of the peak areas was calculated from both injections for analyte and internal standard. From the results for analyte and internal standard, the peak area ratio was calculated. The LOD was calculated by the following equation [114]:

$$x_{LOD} = s_{xO} * t_{f;\alpha} \sqrt{\frac{1}{m} + \frac{1}{n} + \frac{x_{mean}^2}{Q_x}}.$$

s_{xO}...deviation for the method in case variances are homogen
$t_{f;\alpha}$...quantile of t-distribution for unilateral questions for errors of the first degree (f= n-2 degrees of freedom)
x_{mean}...mean value of the analyte concentration in all samples
Q_x...sum of mean squares of x

The LOQ was calculated according to the following equation [114]:

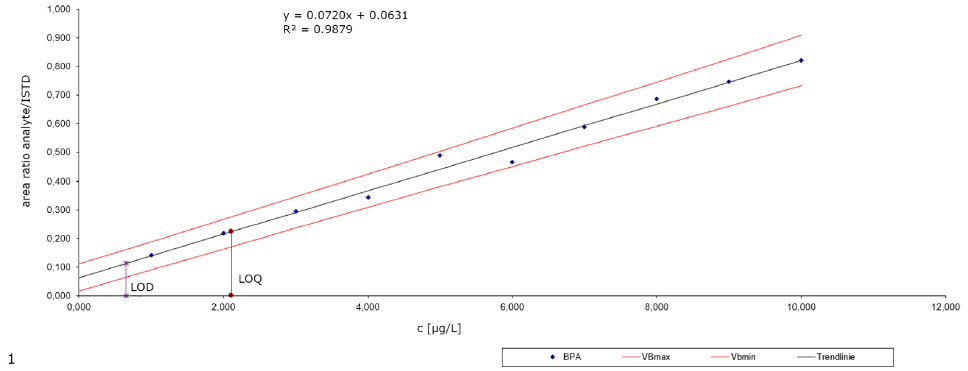
$$x_{LOQ} = k * s_{xO} * t_{f;\frac{\alpha}{2}} \sqrt{\frac{1}{m} + \frac{1}{n} + \frac{(k * x_{LOD} - x_{mean})^2}{Q_x}}$$

with $t_{f;\frac{\alpha}{2}}$ representing the quantile of t-distribution for bilateral questions for errors of the second degree (f= n-2 degrees of freedom). The results for LOD and LOQ are shown in table 27 and 30. The calibration curves for the determination of LOD and LOQ are shown in figure 32.

Table 27: The results of the determination of the LOD and LOQ for BPA and BPS.

limit	**BPA_{day1}**	**BPA_{day2}**	**BPS_{day1}**	**BPS_{day2}**
LOD [µg L^{-1}]	1	2	1	1
LOQ [µg L^{-1}]	3	6	3	2

From the two determinations, the higher result for LOD and LOQ was established as the final result. The maximum limit for the LOD in the validation plan was set to $<$ 5 µg L^{-1}, the LOQ was set to $<$ 10 µg L^{-1}.



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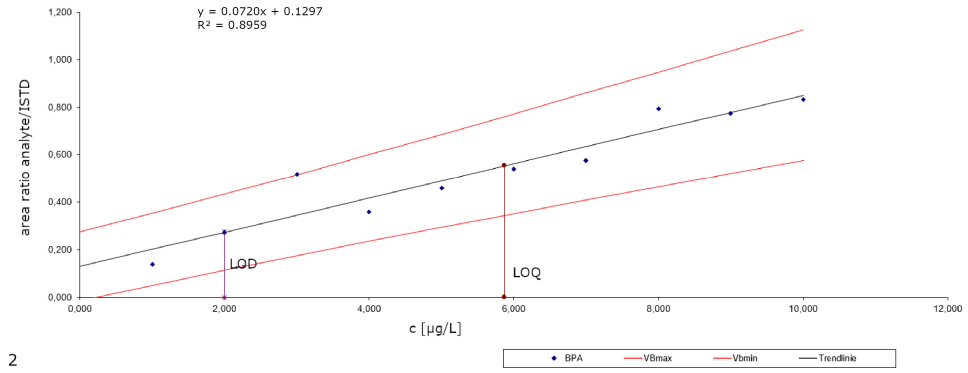

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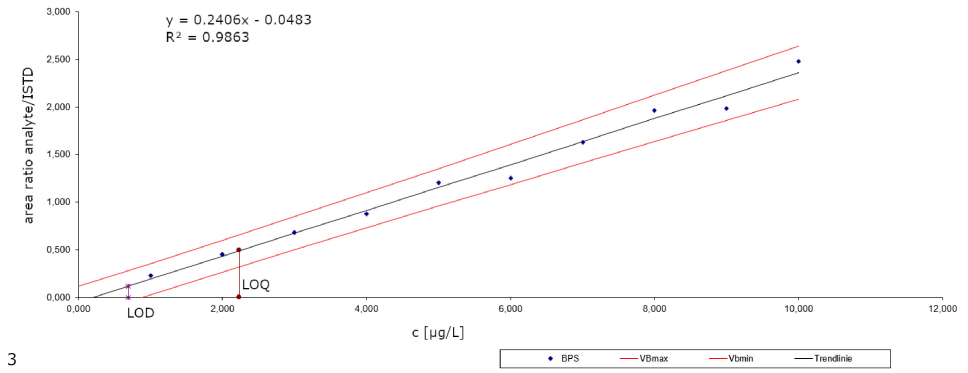

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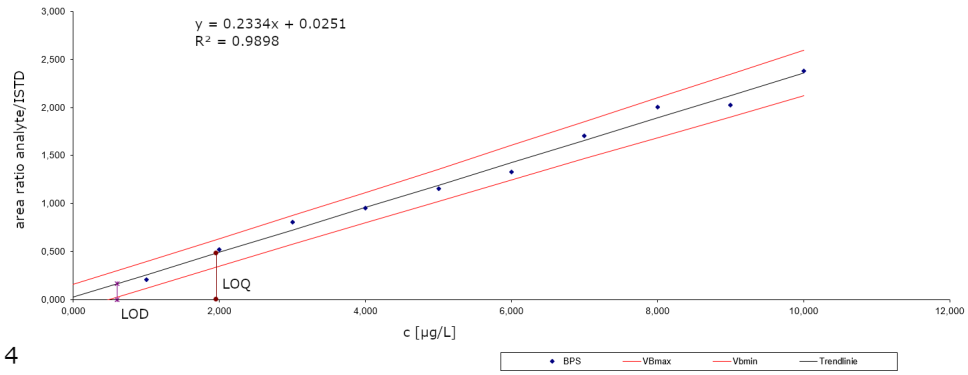

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Figure 32: The calibration for the determination of LOD and LOQ was performed in sample extract with analyte concentration ranging from 1-10 µg L^{-1}. Fit and regression coefficient are shown for each calibration curve (BPA day 1 (**1**), BPA day 2 (**2**), BPS day 1 (**3**), BPS day 2 (**4**)).

According to the expert opinion of scientists from the research group, the LOQ should be 10-fold lower than the SML, which applies for both analytes. The results were also below the limit that was set in the validation plan and therefore are considered to be adequate. Also, Tuzimski et al. determined simliar LOD and LOQ for BPA and BPS in breast milk (see table 28).

Table 28: Limit of detection (LOD) and limit of quantification (LOQ) for BPA and BPS in breast milk determined via QuEChERS sample preparation and LC-MS/MS detection [12].

analyte	**LOD [µg L^{-1}]**	**LOQ [µg L^{-1}]**
BPA	0.54	1.35
BPS	0.10	0.20

Selectivity

Selectivity describes the degree of quantification that the method can provide for an analyte in a complex sample which contains a variety of substances [88, 112]. Selectivity was determined by the ratio plot [112]. The peak area ratio of two daughter ions was calculated for the calibration solutions:

$$area\ ratio = \frac{peak\ area_{Qualifier}}{peak\ area_{Quantifier}}$$

The mean of these area ratios was calculated and the relative deviation of each area ratio from the mean value was calculated by division of the area ratio of a calibration solution by the mean value. The calculation of the relative deviation was repeated for the area ratios of each sample solution with the mean value of the calibration solutions. Additionally, the maximum deviation of the retention time was calculated for calibration solutions by the same procedure: first, the mean value was calculated for the retention time of the calibration solutions. Then the arithmetic difference of the retention time of each calibration solution was calculated with the mean value, giving the deviation of retention time. The procedure was repeated for each sample solution in combination with the mean retention time of the calibration solutions. The results were calculated for sample and calibration blanks, too, but were not considered for the determination of the selectivity criteria. The limit for selectivity criteria was set to a maximum deviation of the retention time $\leq$ 0.1 min and maximum deviation of the area ratio $\leq$ 20 % in the validation plan. The results for the spiked samples are shown in table 29. It can be concluded that the selectivity of the method fits the criteria set in the validation plan.

Table 29: The maximum deviation of the retention time and the area ratios of the spiked samples that were analyzed for the determination of recovery.

analyte	**BPA**	**BPS**
maximum deviation of retention time, t_R [min]	0.01	0.02
maximum deviation of area ratio	12 %	7 %

Relative measurement uncertainty (U_{rel})

The uncertainty of measurement (U) is generally characterizing the scattering of the measurement results and thus it is regarded as a parameter that expresses the accuracy of a method. Relative measurement uncertainty (U_{rel}) was determined by a procedure analogous to NORD-Test.

First, combined standard uncertainty (u_c) was calculated from the uncertainty of the spike (u_{spike}) - which was set to 2 % -, bias and standard deviation. The applied standard deviation is that of the sixfold determination of the spike level with 50 µg kg^{-1} BPA/BPS. The uncertainty of the spike is caused by the circumstance that the recovery of the analyte may differ from the recovery of the spike [115].

$$u_{bias} = \sqrt{(u_{spike} + bias)^2}$$

$$u_c = \sqrt{s^2 + u_{bias}^2}$$

Expanded relative uncertainty (U_{rel}) was calculated by multiplication of the combined standard uncertainty (u_c) with a factor k which associates a certain level of confidence to the uncertainty [88]. For this validation, this factor was set to k=2 with a level of confidence of 95 %.

$$U_{rel} = k * u_c$$

U_{rel} amounted to 32.8 % for BPA and 30.7 % for BPS.

All results from the validation are shown in table 30. Since maximal acceptance criteria from the *Guidelines for performance criteria and validation procedures of analytical methods used in controls of food contact materials* and limits set in the validation plan were met and comparable results were found in the literature, the method is well suited for the quantitative analysis of BPA and BPS in croissant matrix.

Table 30: Validation results of the QuEChERS method for the analysis of BPA and BPS in croissant matrix.

validation parameter	BPA	BPS
calibration range, linearity	5 µg L^{-1} - 100 µg L^{-1}	
recovery, 10 µg kg^{-1}	99 %	85 %
recovery, 50 µg kg^{-1}	95 %	93 %
recovery, 100 µg kg^{-1}	103 %	101 %
precision	7.9 %	6.5 %
trueness (bias)	12.4 %	11.9 %
limit of detection, LOD	2 µg L^{-1}	1 µg L^{-1}
limit of quantification, LOQ	6 µg L^{-1}	3 µg L^{-1}
selectivity retention time (max. deviation $\leq$ 0.1 min)	0.01 min	0.02 min
selectivity area ratio (max. deviation $\leq$ 20 %)	12 %	7 %
expanded relative measurement uncertainty, U_{rel} (k = 2)	32.8 %	30.7 %

5.6 Migration of bisphenols from paper and board materials into food

For the migration tests performed in the course of this work, food with a high-fat matrix was chosen to find out how much BPA/BPS would migrate into the food sample. Binderup et al. mentioned that when fruit or vegetables are packed in paper FCMs, an accumulation of moisture becomes recognizable [7]. When the croissants were sampled with paper FCM and wrapped in aluminum foil, the food released water after baking as well, which moistened the paper and may have increased the chance of transfer between food and FCM. This is in accordance with Regulation (EC) No. 10/2011 that states that a migration test should be performed under the worst foreseeable contact conditions.

First it has to be stressed, that only one migration test was performed and due to the limited number of data, the meaningfulness is restricted, too. Nevertheless, the results appear to be accurate, since similar unpublished results were achieved for migration tests concerning the release of BPA and BPS from paper FCMs into different foods[22]. For all FCMs, the BPA concentration in croissants was below LOD (2 µg kg^{-1}) after 1 h and 24 h of contact time. The results are similar to those for the migration of BPA and BPS into cucumber (1 h of contact time) that were achieved by the CVUA Stuttgart. Thus the results of the migration tests performed for this work can be considered more meaningful. A few samples showed signals with areas that would give concentrations above the LOD; in those cases the area ratios of quantifier and qualifier exceeded the 20 % limit for deviation, so selectivity criteria for BPA were not met. Also, areas for BPA were smaller than those for blank croissants, which did not come in contact with the paper FCMs, so even if any

[22] The results were achieved by the CVUA Stuttgart and disclosed to the author in personal communication by the head of the national reference laboratory for substances that are intended to come into contact with food, Dr. Torsten Bruhn.

BPA was present in the croissants, it is likely that it did not migrate from the paper. Concentrations of BPS were below LOD (1 µg kg^{-1}), too. The analyte concentrations are far below the SML with 50 µg kg^{-1} and thus do not present a risk to human health according to current knowledge.

Results were also calculated as µg BPA/BPS migrated per dm^2 of surface area, under the application of the LOD for the upper bound (UB) approach [116]. When the UB approach is applied to analytical results, the results that are below the LOD are substituted by the LOD. When the results are above the LOD but below the LOQ, results are substituted by the LOQ. Since the results were below LOD, the LOD of BPA and BPS (BPA: 2 µg L^{-1}, BPS: 1 µg L^{-1}) were put into place. To determine the uncertainty of the results, the LOD was multiplied with the concerning U_{rel} determined during the validation (see paragraph 5.5, U_{rel} BPA: 32.75 %, U_{rel} BPS: 30.69 %), results see table 31. The results in µg L^{-1} are equal to those in µg BPA/BPS per kg food sample, because during the conversion the different factors cancel each other out:

$$\beta\ [\frac{\mu g}{kg}] = c\ [\frac{\mu g}{L}] * \frac{V\ [L]}{m\ [g]} = 2\ \frac{\mu g}{L} * \frac{0.002\ L}{2\ g} * 1000 = 2\ \frac{\mu g}{kg}$$

The calculation was performed according to the method described in paragraph 4.3.6 for the method validation: 2 mL of extract solution were concentrated on the rotary evaporator and analyzed via LC-MS/MS, therefore the result applies to this volume of extract solution. The initial sample weight of croissant amounted to 2.0 g, and the factor of 1000 was applied to gain the result per kg sample.

Table 31: Results of the migration tests in µg L^{-1} (which is equal to the result in µg kg^{-1}) and the amount of µg BPA/BPS per dm^2 that migrated from the paper samples into the food samples. Since the results were ≤ LOD, the upper bound approach was applied. The MU was calculated by application of the U_{rel} determined during the validation (see paragraph 5.5).

FCM	**BPA [µg L^{-1}]**	**BPS [µg L^{-1}]**	**BPA [µg dm^{-2}]**	**BPS [µg dm^{-2}]**
paper plates	2 ± 0.66	1 ± 0.31	0.109 ± 0.036	0.055 ±0.017
pizza boxes				
paper towels				

The ratio of initial sample weight (whole croissant) and surface area was determined. As mentioned in paragraph 4.3.7, the mean mass of the croissants after baking and cooling to room temperature amounted to 53.52 g. The surface area of FCMs amounted to 0.98 dm^2, therefore the ratio of initial sample weight and surface area was 54.61 g food per dm^2 FCM. The commission for consumer goods recommended a ratio of 1 kg food per 13.3 dm^2 with regard to the migration of contaminants from board materials into food, which is equal to 75.19 g food per dm^2 FCM. The ratio of initial sample weight to surface area for the performed tests amounts to 73 % of the recommended ratio, meaning that more sample could have been used for the tests [117]. Since an increase of initial sample weight or a

decrease of surface area would have only resulted in lower BPA and BPS concentrations in croissants, the lack of accordance does not have an effect on the results in the sense that migration had been underestimated.

The results in µg dm^{-2} were calculated similarly to those in µg kg^{-1}, only the factor of 1000 was substituted by the amount of sample per surface area (54.61 g dm^{-2}):

$$m_A\ [\frac{\mu g}{dm^2}] = c\ [\frac{\mu g}{L}] * \frac{V\ [L]}{m\ [g]} * \frac{m\ [g]}{A\ [dm^2]} = 2\ \frac{\mu g}{L} * \frac{0.002\ L}{2\ g} * 54.61\ \frac{g}{dm^2} = 0.109\ \frac{\mu g}{dm^2}$$

The BPA concentration in croissants was < LOD after 24 h of contact time with all paper FCMs, too. When signals appeared, the area ratios for BPA deviated in a way that selectivity criteria were not met (> 20 %) and areas were comparable with reagent or sample blanks. The migration of BPA is considered not to have happened even after 24 h of contact time. For the migration from pizza boxes, no migration of BPS was detected (< LOD). Peak areas for BPS after migration from paper towels were lower than those of the standard solution with 1 µg L^{-1} and therefore < LOD for five out of nine samplings. No signal was detected for the other samples or it did not set apart from noise, respectively. Criteria for selectivity were met. Again, the sample blank showed a comparable/higher area for BPS than for sampled croissants, so the source of contamination/migration is still in question and is not likely to stem from paper FCMs. For the migration from paper plates, only one sample showed signals at both analyses, the selectivity criteria for area ratios were met and the signal area was bigger than the one of the sample blank. Nevertheless, the peak area was still below the one of the standard solution with 1 µg L^{-1} and therefore < LOD. All in all, it is concluded that the migration of BPS from all FCMs is < LOD. Signals might stem from BPS but the matter could not be established definitely. The source of contamination remains unclear.

As shown in figure 33, the migration of BPA and BPS into croissant matrix is much lower (if it occured at all) than into CWE. Upper bound approach was used for conservative assessment of the migration [116]. That way, the results for migration tests represent a "worst case-scenario". The results are shown as µg BPA/BPS per surface [dm^2], to enable better comparison of the results.

The results from CWE are highly overestimating the actual migration of BPA/BPS into croissant matrix, the concentration of BPA and BPS in CWE prepared from pizza boxes and BPA in CWE prepared from paper towels are even exceeding the SML of 50 µg kg^{-1} (equal to 2.73 µg per dm^2, see table 32). Even when assessed conservatively, the BPA and BPS concentration in food is far below the SML. The results of CWE suggest a risk to human health, while the performed migration tests show that there is actually no indication for this assumption.

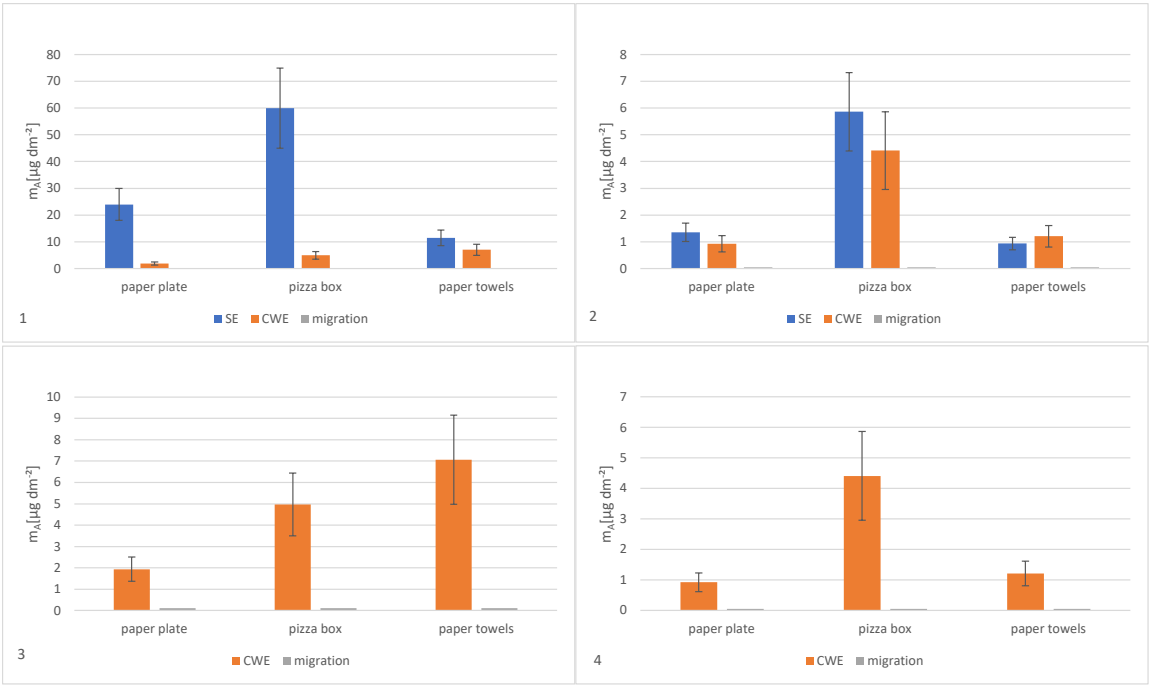

Figure 33: Amount of BPA (**1**) and BPS (**2**) that was released per dm^2 from paper FCM into solvent extract (SE), CWE and croissant (migration). Due to the scale of the y-axis in the diagrams, the amount of BPA and BPS that migrated into food is not perceivable, since it was < LOD. The results were assessed conservatively by UB approach (BPA: 2 ± 0.66 µg L^{-1}; 2.00 µg L^{-1} are equal to 0.11 ± 0.04 µg dm^{-2}; BPS: 1 ± 0.31 µg L^{-1}; 1.00 µg L^{-1} are equal to 0.06 ± 0.02 µg dm^{-2}). Therefore, the results for the transfer of BPA (**3**) and BPS (**4**) into CWE and croissants are additionally shown separately since the scale of the y-axis is decreased and the results for the migration tests are better recognized.

Table 32: Results for CWE and solvent extraction of paper FCMs. The results were calculated from the concentration in sample solutions to the migrated amount as µg per dm^2 surface area of paper FCM and are shown including MU.

FCM	**analyte**	**CWE [µg dm^{-2}]**	**solvent extraction [µg dm^{-2}]**
paper plates	BPA	1.94 ± 0.57	23.98 ± 5.99
	BPS	0.93 ± 0.31	1.36 ± 0.34
pizza boxes	BPA	4.97 ± 1.47	59.92 ± 14.98
	BPS	4.41 ± 1.46	5.86 ± 1.47
paper towels	BPA	7.07 ± 2.09	11.51 ± 2.88
	BPS	1.21 ± 0.40	0.94 ± 0.23

Exposure to contaminants from food packaging is typically low, ranging from below 10 µg kg^{-1} to 60 µg kg^{-1} in food [118]. In that regard, the obtained results for migration tests are neither surprising nor atypical. Migration rate depends on general parameters like size of the migrating molecule as well as number and size of pores in the packaging material. Solubility and polarity of the migrant have a strong impact on migration rates,

too. Two other impact factors, which are often used for the predicition of migrations are diffusion and partition coefficients. The diffusion coefficient D describes the migration rate and the partition coefficient K describes the ratio of analyte concentration in FCM to its concentration in food/food simulant at chemical equilibrium [105]. The more analyte migrates into food/food simulant, the smaller the partition coefficient becomes. Not many studies have been dealing with the prediction of partition coefficients for migration models. For the prediction of diffusion coefficients, different approaches exist but most of them were established for the prediction of diffusion coefficients from plastic FCMs. Therefore, the diffusion and partition coefficients cannot be easily determined for the migration from paper FCMs. This emphazises that migration processes and their predicitions by modeling are a complex endeavour and that migration tests are still important to evaluate the risk from migration from FCMs into food.

The ratio between surface area and initial sample weight (250 mL water/250 g food simulant) for CWE are listed here: paper plates - 13.19 dm^2 kg^{-1}, pizza boxes - 13.67 dm^2 kg^{-1} and paper towels - 7.30 dm^2 kg^{-1}. The results are obtained by division of initial sample weight by the grammage of the samples. By division of the resulting surface area by the amount of water used for CWE, the ratio of surface area to food simulant is calculated. The ratios for paper plates and pizza boxes are in accordance with the BfR recommendation for migration tests with paper FCMs (13.32 dm^2 kg^{-1})[23] which is only consequent since the recommendation refers to the migration from board materials [117]. Therefore, the weight per area of paper samples in the recommendation (300 g m^2) is comparable with the weight per area of the tested paper plates (303.3 g m^2) and pizza boxes (292.5 g m^2, see table 16). It has to be taken into account that the recommendation does not include paper materials such as the tested paper towels, hence the deviation of the grammage (54.8 g m^2) could be anticipated. For preparation of CWE just 1.0 g of paper towels was used in comparison to 10.0 g of the other samples and therefore the ratio of surface area and amount of food sample is decreased. If 10.0 g of paper towels were used, the ratio would amount to 72.95 dm^2 kg^{-1} and the surface area would be too high according to the BfR recommendation.

Furthermore, potential migrations of BPA and BPS from paper into food were calculated and migration tests with food simulants were performed in other studies. For both approaches, the migration of BPA and BPS was below the SML of 50 µg kg^{-1} (or at least the t-TDI 4 µg per kg body weight per day in the case of BPA) and therefore did not present a health risk [119, 120]. In an earlier migration test with a contact time of 9 months, no BPA could be recovered in different foods. The foodstuffs tested were chocolate biscuits, corn grits, rice, oatmehl, breadcrumbs and egg-based pasta - all foods with a dry non-greasy matrix and a big surface. The concentration of BPA in the folding box was 8.3 mg kg^{-1} and since no BPA could be detected in the foodstuffs, it is fair to assume that a transfer

[23] The average grammage of board FCMs is assumed to amount to 300 g m^{-2} [117]. When 10 g of sample are applied for CWE, the surface area in contact with the solvent is 0.0333 m^2 (= 3.33 dm^2). The application of 250 mL/250 g water results in a surface-to-volume ratio of 13.33 dm^2 kg^{-1} food/food simulant.

via gas phase does not take place either [1, 2]. In personal conversations, yet unpublished results of migration tests for other foodstuff in contact with paper FCM were disclosed and suggested that the migration of BPA and BPS from paper FCMs is below SML for other foodstuff, too.

6 Conclusions

A method for the quantitative analysis of BPA and BPS in dry high-fat matrix was successfully established and validated. The method was used for migration tests. In comparison with results of CWE according to DIN 645 from an inter-laboratory comparison study, the results of those tests show that little ($\leq$ LOD[24], LOD BPA: 2 µg kg^{-1} food, LOD BPS: 1 µg kg^{-1} food) if any amount of BPA and BPS is transferred into croissants from the tested paper FCMs made from recycled fibers. Since sample and solvent blanks showed similar signals, the contamination might not stem from BPA or BPS. Even if it is BPA or BPS, the contamination is probably not originating from the paper samples. In comparison with the CWE, it became recognizable that the CWE highly overestimated the migration into this food matrix. The SML at 50 µg kg^{-1} is even exceeded in CWE for two out of three paper samples, implying a risk to human health, while the recovery in food was below the LOD. This circumstance requires further investigation.

Concerning the variation of initial sample weight used for CWE of absorbent materials according to DIN 645, it was demonstrated that the solvation of analyte per kg paper decreases with increased initial sample weight for certain analytes such as BPA and naphthol AS. The alteration of sample weight does not have an impact on the extraction efficiency of other analytes which might be caused by their low concentrations in the paper FCMs in this case. With regard to the BPA release from the tested paper towels and the naphthol AS release from the examined red napkins into CWE, these substances exceeded their migration limits and thus would present a risk to human health.

[24]The UB approach was applied for data interpretation, see paragraph 5.6 [116].

Abstract

The aim of this thesis was the comparison of the migration of bisphenol A (BPA) and bisphenol S (BPS) from food contact materials (FCMs) made from paper with recycled fibers into cold water extracts (CWE) after DIN 645 with that into fatty foods (croissant). An HPLC-MS/MS method for quantification of BPA and BPS in croissants was developed, validated and applied for migration tests. The results of CWEs from three food contact materials (paper towels, paper plates and pizza boxes) were obtained before the work for this thesis started and used in comparison with solvent extractions and migration tests.

The following parameters were determined during the course of the validation of this method: linearity, recovery, precision, bias/trueness, relative measurement uncertainty U_{rel}, limit of detection, limit of quantification and selectivity. The mean recoveries are between 85-103 % for different spike levels (10, 50, 100 µg kg^{-1}). Precision amounts to 7.9 % for BPA and 6.5 % for BPS. Bias for BPA is 12.4 % and 11.9 % for BPS. Relative measurement uncertainty U_{rel} is 32.8 % for BPA and 30.7 % for BPS. The limit of detection (LOD) is 2 µg kg^{-1} for BPA and 1 µg kg^{-1} for BPS, while the limit of quantification (LOQ) is 6 µg kg^{-1} for BPA and 3 µg kg^{-1} for BPS. Therefore, quantification of BPA and BPS is working well below the Specific Migration Limit (SML) of 50 µg kg^{-1}.

Results of migration tests show that the transfer of BPA and BPS is below LOD (BPA: 2 µg kg^{-1}, BPS: 1 µg kg^{-1}) after contact times of 1 h and 24 h. CWE seems to overestimate the migration of BPA and BPS into croissants for all tested FCMs.

Additionally, CWE from swelling paper materials such as napkins and paper towels were prepared after DIN 645. The norm regulates that 10 g of sample should be used for the preparation of CWE, which is problematic in that regard that most of the water is absorbed by those FCMs when 10 g of sample are used. To determine whether the initial sample weight has an impact on the transfer rate into the CWE, sample weight was varied from 1 g-10 g. The amount of analyte that was transferred into CWE decreased for some analytes with increased initial sample weight.

Zusammenfassung

Ziel dieser Arbeit war es die Migration von Bisphenol A (BPA) und Bisphenol S (BPS) aus Lebensmittelkontaktmaterialien (engl. food contact materials, FCMs) hergestellt aus recyclierten Fasern in Kaltwasserextrakte nach DIN 645 mit der Migration in fettreiche Lebensmittelmatrix zu vergleichen. Als Lebensmittelproben wurden Croissants verwendet. Es wurde eine HPLC-MS/MS-Methode zur Quantifizierung von BPA und BPS in Croissant-Matrix erarbeitet, validiert und für die Migrationstests eingesetzt. Die Ergebnisse wurden mit Kaltwasserextrakten von drei verschiedenen Lebensmittelkontaktmaterialien (Küchenrolle, Pappteller und Pizzakarton), die vor Beginn dieser Arbeit analysiert wurden, verglichen. Diese Ergebnisse wurden außerdem mit jenen von Lösungsmittelextraktionen der genannten FCM verglichen.

Bei der Validierung wurden folgende Parameter der Methode bestimmt: Linearität, Wiederfindung, Präzision, Bias bzw. Richtigkeit, relative Messunsicherheit U_{rel}, Nachweis- und Bestimmungsgrenze sowie Selektivität. Die mittleren Wiederfindungsergebnisse liegen zwischen 85-103 % für verschiedene Spikelevel (10, 50, 100 µg kg^{-1}). Die Präzision beläuft sich auf 7,9 % für BPA und 6,5 % für BPS. Der Bias bzw. die Richtigkeit wurde mit 12,4 % für BPA und 11,9 % für BPS bestimmt. Die relative Messunsicherheit U_{rel} beträgt 32,8 % für BPA und 30,7 % für BPS. Die Nachweisgrenze für BPA beträgt 2 µg kg^{-1}, für BPS beträgt sie 1 µg kg^{-1}. Die Bestimmungsgrenze für BPA beträgt 6 µg kg^{-1}; für BPS liegt diese bei 3 µg kg^{-1}. Dementsprechend ist eine gute Quantifizierung auch deutlich unterhalb des Spezifischen Migrationsgrenzwertes von 50 µg kg^{-1} (engl. Specific Migration Limit, SML) möglich.

Die Ergebnisse der Migrationstests zeigten, dass der Übergang von BPA und BPS nach Kontaktzeiten von 1 h und 24 h unterhalb der Nachweisgrenzen (BPA: 2 µg kg^{-1}, BPS: 1 µg kg^{-1}) lag. Der Kaltwasserextrakt scheint die Migration von BPA und BPS für die hier untersuchten Lebensmittelkontaktmaterialien in Croissant zu überschätzen. Die hier durchgeführten Messungen bilden die Grundlagen für weitere Untersuchungen.

Darüber hinaus wurden Kaltwasserextrakte nach DIN 645 von stark quellenden Lebensmittelkontaktmaterialien aus Papier mit variierender Einwaage hergestellt. Die Norm schreibt vor 10 g Probeneinwaage zu verwenden, allerdings wird von derartigen Materialien bei entsprechender Einwaage der Großteil des Wassers absorbiert. Um zu prüfen, ob die Probeneinwaage einen Effekt auf den Übergang von Kontaminanten in den Kaltwasserextrakt bewirkt, wurden Einwaagen von 1 g-10 g Probe verwendet. Für einzelne Analyte sank die Menge an jeweiligem Analyt, die aus den Proben herausgelöst wurde, mit steigender Einwaage.

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