Master's thesis

NTNU
Norwegian University of Science and Technology
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Department of Chemistry

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Parabens and triclocarban in baby foodstuffs from Norway

Master's thesis in Natural Science with Teacher Education
Supervisor: Alexandros Asimakopoulos
June 2020

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Kunnskap for en bedre verden

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Acknowledgement

In the work with this thesis I have gained a deeper understanding about trace level analysis, and the modern techniques one can apply to get valid results. As a future chemistry teacher, insight into modern method and analysis techniques are advantageous to bring along in teaching. Understanding chemical analysis and interpretation of the obtained results are a part of the curriculum in the chemistry subject in Norwegian high schools (Utdanningsdirektoratet, 2020). This makes this master thesis relevant for the teacher profession.

This master thesis could never have been completed without good supporters. First of all I have to thank my supervisor, Alexandros Asimakopoulos, for always being supportive, professional and positive. Even though we have not been able to meet in these Covid-19 times, you have been a fast email responder and a great help. Thanks to Kristine Vike for being patient, responsive and a great mentor. I have to thank Vishwesh Venkatraman for the help with PCA and correlation analysis. Susanna Gonzales deserves a thank for easy, descriptive and thorough review of the instrumental part, and help with the analysis. A special thanks to Ragnhild and Tonje, my fellow nerve wrecks and overthinkers, for good advice and wise words in times of crisis. Maren, thank you for regular long and cheerful conversations. Thanks, Einar, for keeping up with my roller coaster emotions and mood. I am forever grateful for my long-time cohabitant, Malin, for making the first five years in Trondheim magic, and to Guri, my parter-in-crime, for making the years at Gløshaugen fun. Thanks to Ida, for regular dinners, and laughter and singing moments.

Abstract

Antimicrobial chemicals are added to consumer products to prevent microbial growth. For consumer safety and quality assurance, addition of antimicrobial chemicals are regulated, in terms of labelling and quantites permitted. Alkyl esters of para-hydrobenzoic acid, namely parabens, and triclocarban are chemicals that posess antimicrobial properties. Due to their potential negative health effects, detection of these chemicals is important. The main goal of this study was to estimate and quantify the occurrence of these chemical, as well as paraben derivates, in foodstuffs intended for children. The foodstuffs (n=49) were purchased on a local supermarket in Norway, and included grain products, dairy products, snack products, vegetable products and fruit products.

The target analytes were extracted by a solid supported liquid liquid extraction, and analysed by UHPLC-MS/MS. Due to variable detection values of the target analytes in the samples used for quality assurance, the concentrations were semiquantified. Parabens were detected in 35 of 49 samples. The highest estimated concentrations was obtained by the parabens with short chain lenghts (Methyl- and Ethyl paraben). Contrary to previous studies, this study obtained the highest detection rate of the long chained paraben, benzyl paraben (BezP). The finding is worrying due to BezPs possible endocrine disruptive properties. All samples except one sample contained paraben derivates, and their estimated concentrations were much higher than for the parabens. TCC was found in one sample in low concentration. A PCA-analysis was perfomed to detect the possible variation and correlation in the obtained dataset. Positive correlation was obtained by the paraben derivates Vanillic and 4-HB, and for Vanillic and the sum of parabens and derivates. This study reinfoces a need to detect, understand and investigate the origin of parabens in foodstuffs.

Sammendrag

For forbrukersikkerhet og kvalitetssikring av forbrukerartikler, er tilsetning av bakteriebekjempende kjemikalier strengt regulert, både med hensyn på merking og mengde. Alkylestere av parahydroksibenzosyre, kalt parabener, og triclocarban er kjemikalier som har bakteriebekjempende egenskaper. Grunnet potensielle negative helseeffekter ved eksponering av disse kjemikaliene, er deteksjon og kvantifisering viktig. Målet for denne studien var å kartlegge kostholdseksponering av triclocarban og parabener, samt parabenderivater, i matvarer som er ment for barn. Matvarene (n=49) ble kjøpt på en lokal matvarebutikk i Norge, og inkluderte korn-, meieri-, snacks-, frukt- og grønnsaksprodukter.

Analyttene ble ekstrahert ved hjelp av en fast-fase-støttet væske-væske-ekstraksjon, og analysert med UHPLC-MS/MS. Grunnet variable deteksjoner av analyttene i kvalitetskontrollprøvene, ble konsentrasjonene semikvantifisert. Parabener ble detektert i 35 av 49 prøver, og de høyest estimerte konsentrasjonene ble funnet for parabener med kortest sidekjeder (metyl- og etylparaben). I motsetning til funnene i andre studier, fant denne studien høyest forekomst av det langkjedede parabenet benzylparaben. Funnet er urovekkende med hensyn på dets mulige hormonforstyrrende egenskaper. Samtlige prøver, utenom én, inneholdt parabenderivater. Triclocarban ble funnet i én prøve i en lav konsentrasjon. En PCA-analyse ble gjennomført for å detektere variasjonen og potensielle korrelasjoner i datasettet. Positiv korrelasjon ble funnet for parabenderivatene Vanillic og 4-HB, samt Vanillic og summen av parabener og derivater. Videre forsterker denne studien behovet for å detektere, forstå og undersøke opprinnelsen til parabener i mat.

Innhold

Figures

Tables

Abbreviations

MeP	Methyl paraben
EtP	Ethyl paraben
PrP	Propyl paraben
BuP	Butyl paraben
HeP	Heptyl paraben
4-HB	4-/ para-hydroxybenzoic acid
3,4-DHB	3,4-dihydroxybenzoic acid
OH-EtP	Ethyl-protocatechuate
Vanillic	3-hydroxy-3-methoxybenzoic acid
TCC	Triclocarban
IS	Internal standard
TA	Target analyte
LOD	Lowest limit of detection
LOQ	Lowest limit of quantification
EDC	Endocrine disruptor chemical
HPLC	High pressure/performance liquid chromatography
UHPLC	Ultrafast high pressure/ performance liquid chromatography
EFSA	European Food Safety Autority
QC	Quality control
QA	Quality assurance

1 Introduction

In the recent years there has been a greater understanding and focus on the effects and fates that anthropogenic chemicals might have on consumers and the environment (Miljøstatus, 2020, Darbre and Harvey, 2008, Boberg et al., 2010). Chemicals are added to electronics as flame retardants, as pesticides to protect our food crops, and in consumer products to increase shelf life and prevent microbial growth (Miljøstatus, 2020, ECHA, 2020). Whenever there is a suspicion that chemicals might have adverse health effect, the request for studies that map the extent of exposure are being strengthened. Parabens and triclocarban are examples of chemicals that works as antimicrobial chemicals (Asimakopoulos et al., 2014a). Parabens are found in pharmaceuticals, cosmetics and foodstuffs, while triclocarban is found in personal care products. Some studies have suggested that exposure to parabens and triclocarban, might have adverse health effects. In that regard their detection rate and concentration in consumer products is important for consumer awareness and safety, as well as quality assurance (Saad et al., 2005).

The supply of baby foods is extensive, and includes everything from breast milk substituents to ready-to-eat dinner products. In Norwegian regulations, children foodstuffs should not contain substances endanger the child´s health (Forskrift om barnemat, 2002). As children are vulnerable, addition of preservatives, artificial sweeteners and colorants are prohibited in children foodstuffs (Mattilsynet, 2017). In this study the aim was to analyse baby foods to chart whether children are exposed to triclocarban, and parabens and its derivates, by ingestion of baby food products. Part of the aim was also to compare different food categories, to chart the potential concentration differences of target analytes. This was done by applying a liquid extraction protocol for extraction tailored to Liquid-chromatography-tandem mass spectrometry for analysis. A PCA and correlation analysis was performed to look at any correlations among some of the target analytes in the different categories.

2 Background

Chemicals might be added to foodstuffs to change the color, the consistency, the taste, to increase the shelf life and to prevent bacterial growth (Mattilsynet, 2019). The chemicals found in food can either be added intentionally or non-intentionally. They can occur through processing or be present in ingredients of which a foodstuff is composed from (EFSA, 2020). In this study, baby foodstuffs were analyzed for parabens and its derivates, and triclocarban. In the following, background material are presented. Firstly the target analytes (TA) are introduced, their exposure sources, the effects, and some of the recent studies regarding their presence in foodstuffs. Secondly the extraction and detection methods are presented, followed by an introduction of quality control and quality assurance parameters applied in the validation of the method and quantification protocol.

2.1 Target analytes

Parabens are alkyl esters of p-hydroxybenzoic acid (Liao et al., 2013a). The hydroxybenzoic acid consists of a benzyl ring with a hydroxyl group at the para position and a carboxyl group in the ortho position. The alkyl ester (OR) is linked to the carboxyl group. A general illustration of parabens is found in Figure 2.1.

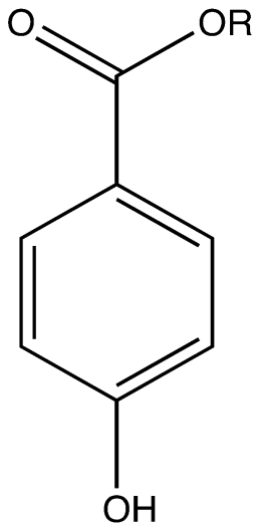

Figure 2.1: The general structure of parabens. The OR symbolizes alkyl esters that are attached.

Parabens are suited to act as antimicrobial preservatives due to their chemical and thermal stability, low cost, and wide application area (Piao et al., 2014). Their antimicrobial activity is found to increase, and the water solubility to decrease, with the chain length of the ester group (Soni et al., 2005). The parabens included the present study were methylparaben (MeP), ethyl paraben (EtP), propyl paraben (PrP), butyl paraben (BuP), benzyl paraben (BezP) and heptyl paraben (HeP). BezP somewhat stands out as it has an aromatic ring instead of an alkyl chain. MeP, EtP and PrP, which are referred to as parabens with short chain lengths, are illustrated in Figure 2.2, respectively. BuP, BezP and HeP is illustrated in Figure 2.3, respectively.

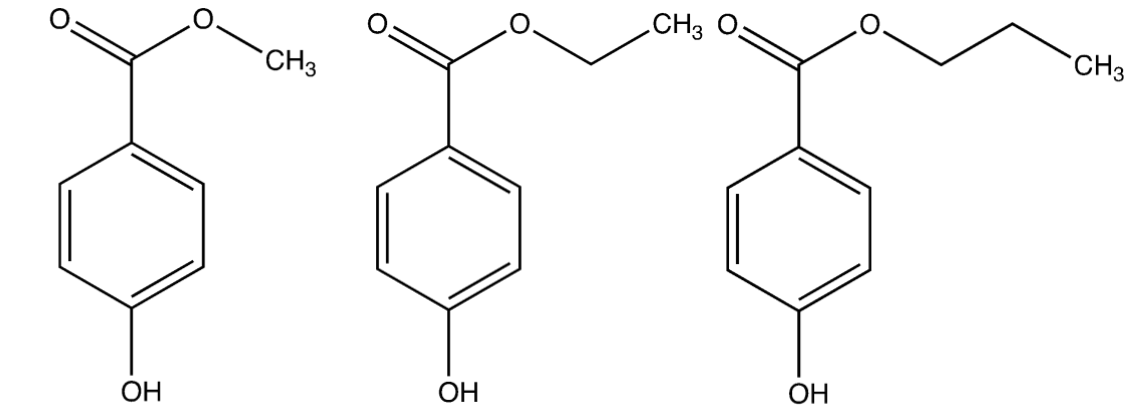

Figure 2.2 The chemical structure of methyl-, ethyl- and propyl paraben.

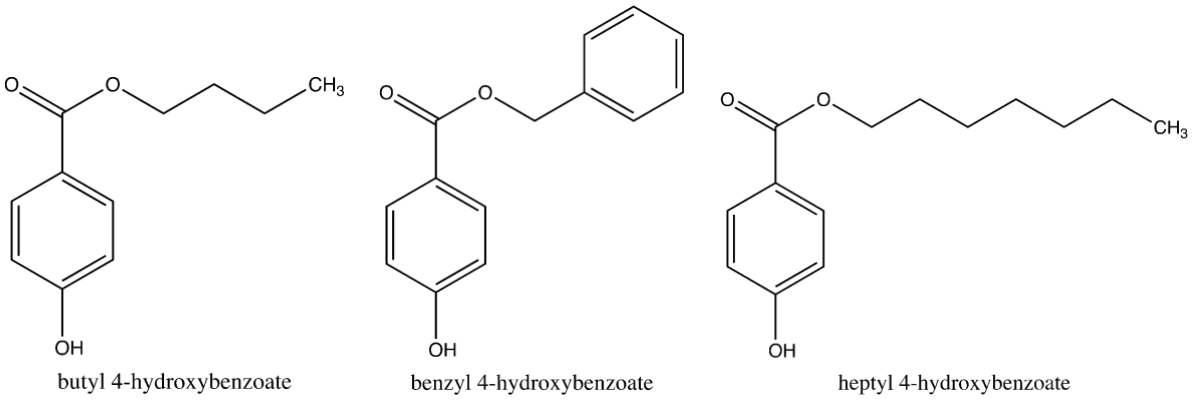

Figure 2.3 The chemical structure of butyl-, benzyl- and heptyl paraben.

The paraben derivates, parahydroxybenzoic acid (4-HB), 3-hydroxy-3-methoxybenzoic acid (Vanillic acid/ Vanillic) and 3,4-dihydroxybenzoic acid (3,4-DHB), were also investigated in the present study. Hydrolysis of the ester linkage of the paraben gives 4-HB, that is the main metabolite of parabens in the human body (Darbre and Harvey, 2008, Boberg et al., 2010). Methoxylation and hydroxylation of the aromatic ring in 4-HB, can lead to the formation of Vanillic and 3,4-DHB, respectively (Tomás-Barberán et al., 2000). Vanillic can be added to food for the intention of flavouring purposes (Noubigh and Abderrabba, 2016). Ethyl-protocatechuate (OH-EtP) is a novel metabolite of EtP, and is found to be correlated with exposure to EtP when detected in urine (Asimakopoulos et al., 2014a, Wang and Kannan, 2013). OH-EtP was along with 4-HB, 3,4-DHB and Vanillic acid included in this study. Their structures are illustrated in Figure 2.4, respectively**.**

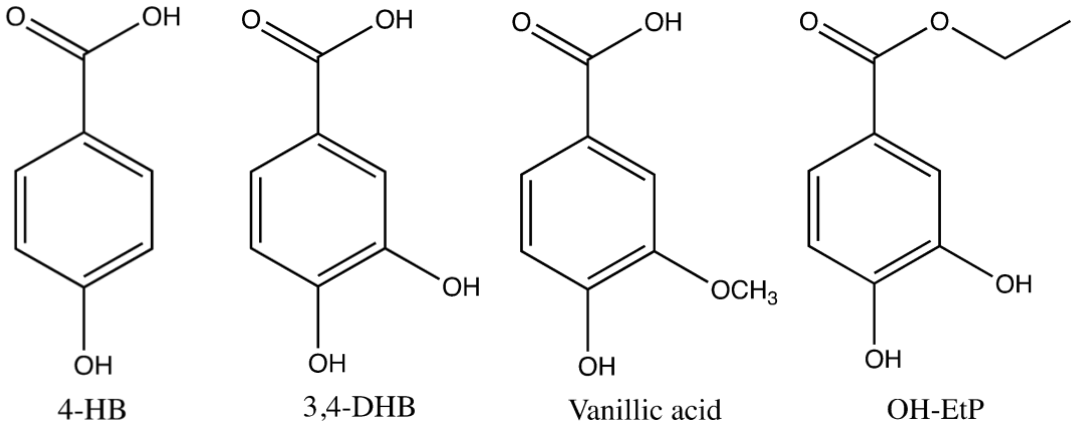

Figure 2.4 The chemical structure of 4-HB, 3,4-DHB, Vanillic acid and OH-EtP

Triclocarban (TCC) is a polychlorinated diphenylurea (SCCP, 2005) . It consists of two phenyl rings attached on both sides of an urea group. The chloro groups are attached in the 3´and 4´position, and in the 3´of the phenyl rings, respectively. Triclocarban is used as antimicrobial agent in personal care products (Snyder and O'Connor, 2013). Figure 2.5 illustrates the chemical structure of TCC.

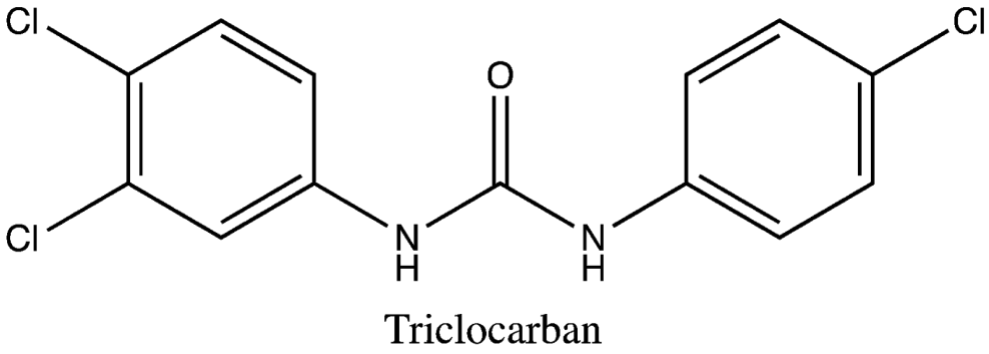

Figure 2.5 The chemical structure of Triclocarban

2.2 Exposure

Humans can be exposed to parabens both by dietary and non-dietary consumption, in that regard the accurate exposure rate of parabens can be hard to estimate (Asimakopoulos et al., 2014a). Yet, evidences that humans are exposed to parabens and their metabolites are found in serum, urine, placenta and wastewater (Van Overmeire et al., 2019, Asimakopoulos et al., 2014a, Asimakopoulos et al., 2016b, Carrasco-Correa et al., 2015, Ocaña-González et al., 2015, Li et al., 2020, Wang and Kannan, 2013, Wang et al., 2013). Parabens with the shorter chain lengths are usually found in the greatest concentrations. Studies have shown the presence of parabens both as intact parabens and as the metabolite 4-HB in the human body, suggesting that the humans are capable to metabolize parabens (Boberg et al., 2010, Asimakopoulos et al., 2016a). However, when analyzing consumer products it is important to keep in mind that quantification of metabolites is not only an indication of the parabens. 4-HB and its conjugates can occur naturally in some vegetables, plants and cereal species (Tomás-Barberán et al., 2000, Zhang et al., 2019, Boberg et al., 2010). Inter- and intraspecies variation have been reported in fruits and vegetables (Tomás-Barberán et al., 2000). Methoxylation and hydroxylation of 4-HB can also occur during processing. As mentioned above, Vanillic acid can be added for the intention of vanilla flavor. It is also an intermediate in the production of vanillin, commonly used for vanillic flavor in food (Noubigh and Abderrabba, 2016). OH-EtP have been reported as a natural compound in wine an peanut seed coat (Wang and Kannan, 2013). The transformation of EtP to OH-EtP have been reported in the human body, but the potential transformation process in consumer products, have not been established yet.

TCC is used as an antimicrobial agent in personal care products such as shampoos, cosmetics and soaps to prevent bacterial growth (Asimakopoulos et al., 2016a, Clarke and Smith, 2011,

SCCP, 2005). TCC have been detected in different environmental medias, like wastewater, river water, in soil, and in the human body (Clarke and Smith, 2011, Sapkota et al., 2007, Zhou et al., 2012). The use of biosolids have raised concern of the release of TCC to the environment and its possibility to biomagnify up the food chain. TCC seems to be able to sorb to soil and sediment, and can be taken up by plant roots and thereby biotransfer to animals (Clarke and Smith, 2011, Snyder and O'Connor, 2013). TCC has been detected in some species, suggesting that the release from personal care products can end up in living organisms, and thereby into food (Yao et al., 2019, Ramaswamy et al., 2011). As a lot of environmental contaminants, it can be hard to calculate the accurate exposure of TCC (Clarke and Smith, 2011).

2.3 Effects

The health effects of parabens have been studied in vivo and in vitro. The potential of paraben to interfere with the estrogen receptor (ER), the androgen receptor (AR), the possible genotoxicity and to increase the risk of cancer have been studied (Darbre and Harvey, 2008, EFSA, 2004b, Golden et al., 2005). One of the major concerns, is that they might act as a endocrine disruptor. An endocrine disruptor chemical (EDC) is an exogenous chemical that might interact with the natural hormone receptors (Klaassen and Casarett, 2019). It is suggested that the potential for parabens to act as an EDC is increasing with the chain length of the ester group (Boberg et al., 2010). This is why use of parabens with longer chain lengths are limited compared with parabens with shorter chain lengths. In the studies concerning parabens with short chain lengths (MeP, EtP and PrP) there are conflicting results. Some studies demonstrate that these parabens might mimic natural hormones or block hormone receptors. Other studies state they are so weak endocrine disruptors, that it requires an unrealistic magnitude to work similar to natural hormones, or to block receptors (Golden et al., 2005, Libei et al., 2016).

Chen et al. (2008) has tried to predict the endocrine disruption effects of TCC (Chen et al., 2008). Although there is no evidence that TCC will act as a EDC alone, there is a possibility that it might work synergistic with natural testosterone and enhance the natural signal. This can disrupt the natural hormone homeostasis. The European Commission of Health and Consumer protection Directorate-General have considered TCC to be at low risk for humans

exposed through personal care products (SCCP, 2005). Thus, they concluded that there is a lack of studies on the potential adverse effects of TCC when it comes from other sources, like environmental contamination.

2.3.1 Acceptable daily intake

For determining how much of an additive that is a tolerable consumption, an acceptable daily intake (ADI) is estimated (Klaassen and Casarett, 2019). Prior to the elaboration of an ADI, there are studies to determine the highest dose possible where there is no observed adverse effects. This limit is called NOAEL (no observed adverse effect level), and the ADI is based on this. Due to conflicting results concerning the potential of parabens to have adverse health effects, the European Food Safety Autority (EFSA) allows addition of methyl and ethyl paraben to food, but only to certain categories; dried meat products, jelly coatings of meat products, liquid dietary food supplements, and in confectionary (EFSA, 2004b). EFSA have, based on several studies, set an ADI for these parabens, accordingly. The ADI of methyl- and ethyl-paraben have been set to 0-10 mg/ kg body weight (EFSA, 2004b). No ADI have been set for the parabens with longer chain lengths (Boberg et al., 2010). Nevertheless these parabens should be limited in use due to their higher potential to act as an EDC. Concerning TCC no ADI have been set for the purpose of food additive.

2.4 Detection of parabens, paraben derivates and triclocarban in food

2.4.1 Parabens and paraben derivates

The paraben concentration in foodstuffs have been reported in several studies from different countries. Table 2.1 displays some of the recent studies and the results. If the studies have reported concentrations in a single product, the average of the reported concentrations in a given category have been calculated by the undersigned and listed up. The studies in question have been marked with a star (*). Some of the articles listed up have mainly focused on developing a solid method to detect parabens, and this is why the number of samples are scare. Different exposure to parabens between countries have been suggested by obtained detection rates and concentrations by comparing urine in USA and China (Wang et al., 2013). Accordingly, it is important to keep in mind that there are different regulations across countries concerning the use of preservatives in food, which might be an explanation of concentration variations in the products or categories (Yang et al., 2014). In Norway, the addition of preservatives are prohibited in foodstuffs meant for children

Table 2.1: Reported median/ mean concentration of parabens, 4-HB 3,4-DHB Vanillic and OH-EtP in foodstuffs and beverages from previous studies

Unit (mean/ median)	Analysis method	Food/ beverage	n	MeP	EtP	PrP	BuP	BezP	HeP	4-HB	3,4-DHB	V.A	EtP-OH	(Reference) Country
ng/g fw (mean)	*HPLC-MS/MS*													(Liao et al., 2013a) China
		Cereal	39	16.6	5.39	1.94	1.24	0.011	0.005					
		Meat	19	2.27	1.87	1.11	0.371	0.027	0.006	-	-	-	-	
		Fish and seafood	10	1.45	0.692	0.377	0.185	0.020	0.005	-	-	-	-	
		Egg	11	1.17	0.275	0.173	0.155	0.011	0.005	-	-	-	-	
		Dairy product	16	17.7	0.715	1.57	0.288	0.005	0.005	-	-	-	-	
		Bean product	27	11.0	4.36	0.685	0.486	0.028	0.005	-	-	-	-	
		Fruit	20	9.68	6.89	3.36	0.384	0.074	0.005	-	-	-	-	
		Vegetables	39	81.1	10.9	14.7	1.75	0.107	0.006	-	-	-	-	
		Cookies/ snacks	26	12.9	3.70	4.41	0.394	0.028	0.005	-	-	-	-	
		Beverages	4	0.524	0.283	0.007	0.009	0.011	0.005	-	-	-	-	
		Cooking oils	11	6.32	4.22	0.250	0.016	0.103	0.005	-	-	-	-	
		Conduments	47	20	42.8	12.1	0.168	0.309	0.006	-	-	-	-	
		Others	13	24	0.037	0.017	0.005	0.014	0.005	-	-	-	-	
µg/g fw (mean)*	*HPLC-PDA*													(Maher et al., 2020) Saudi-Arabia
		Cereals	21	75.77	0.13	0.01	0.57	-	-	-	-	-	-	

		Meat product	7	28.95	0.55	0.01	ND	-	-	-	-	-	-	
		Fish	4	0.51	ND	ND	0.02	-	-	-	-	-	-	
		Dairy product	42	26.28	13.16	ND	ND	-	-	-	-	-	-	
		Bean product	9	0	0.98	ND	0.06	-	-	-	-	-	-	
		Fruits	20	0.15	0.96	0.96	ND	-	-	-	-	-	-	
		Vegetables	10	0.05	0.07	0.02	ND	-	-	-	-	-	-	
		Cookies and snacks	41	0.17	0.28	0.01	ND	-	-	-	-	-	-	
		Beverages	18	23.60	0.26	ND	ND	-	-	-	-	-	-	
		Condiments	16	495.7	0.69	0.01	0.11	-	-	-	-	-	-	
		Others (μg/g)	27	13.99	3.35	0.12	19.46	-	-	-	-	-	-	
ng/g dw (not stated)	*GC-MS*													(Djatmika et al., 2016)
		Shrimp	10	10.8	8.0	5.5	7.4	-	-	-	-	-	-	
		Cod	1	11.5	5.6	6.8	5.6	-	-	-	-	-	-	
		Tilapia	1	6.2	5.5	ND	5.0	-	-	-	-	-	-	
		Striped bass	1	18.5	15.1	4.9	6.2	-	-	-	-	-	-	
ng/g dw	*HPLC-UV*													(Yang et al., 2011)
		Pancakes	3	ND	ND	ND	ND	-	-	-	-	-	-	
ng/g fw (median)	*HPLC-UV*	*Dog food*												(Karthikraj et al., 2018) **
		Dry food	7	8.2	1.6	1.4	<LOQ	<LOQ	<LOQ	1250	600	<LOQ	<LOQ	
		Wet food	3	1.8	<LOQ	1.5	<LOQ	<LOQ	<LOQ	310	136	0.8	<LOQ	
		Broths	13	0.9	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	220	28.8	0.5	<LOQ	
ng/g fw (median)	*HPLC-UV*	*Cat food*												(Karthikraj et al., 2018)

		Dry food	5	20.8	7.5	4.2	<LOQ	<LOQ	0.19	1760	840	1.6	<LOQ	
		Solid	8	9	0.8	1.4	<LOQ	<LOQ	<LOQ	1085	560	<LOQ	<LOQ	
		Wet food	8	0.9	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	130	10.5	<LOQ	<LOQ	
		Broths	14	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	140	21.4	<LOQ	<LOQ	
*µg/ g fw**	*UPLC-MS/MS*													(Zhou et al., 2015)
		Radish	1	5.0	2.0	0.2	ND	-	-	-	-	-	-	
		Tomato	1	1.5	0.5	0.2	ND	-	-	-	-	-	-	
		Cabbage	1	1.3	1.6	1.2	ND	-	-	-	-	-	-	
		Cowpea	1	0.9	ND	0.5	0.1	-	-	-	-	-	-	
		Cucumber	1	1	0.1	0.8	ND	-	-	-	-	-	-	
mg/ kg fw (mean)*	*HPLC-UV*													(Yang et al., 2014)*
		Candy	3	ND	ND	56.86	ND	-	-	-	-	-	-	
		Pastry	4	63.425	63.125	ND	ND	-	-	-	-	-	-	
		Jam	3	18.966	85.733	23.93	ND	-	-	-	-	-	-	
		Pickle	5	17.72	123.66	17.98	ND	-	-	-	-	-	-	
		Sausage	4	30.5	27.425	13.075	ND	-	-	-	-	-	-	
ng/g fw (median)	*HPLC-MS/MS*													(Liao et al., 2013b) USA
		Beverages	33	0.095	0.005	0.005	0.005	0.005	-	-	-	-	-	
		Dairy products	31	0.344	0.010	0.014	0.005	0.005	-	-	-	-	-	
		Fats and Oils	5	0.005	0.005	0.005	0.005	0.011	-	-	-	-	-	
		Fish and shellfish	23	0.336	0.009	0.042	0.005	0.005	-	-	-	-	-	
		Grains	54	2.25	0.048	0.104	0.005	0.005	-	-	-	-	-	
		Meat	52	1.10	0.018	0.070	0.005	0.005	-	-	-	-	-	
		Fruits	20	0.328	0.012	0.005	0.005	0.005	-	-	-	-	-	

		Vegetables	49	1.06	0.051	0.023	0.005	0.005	-	-	-	-	-	
g/kg ww (mean)	*UHPLC-PDA*													(Xiu-Qin et al., 2008)* **
		Cola	6	ND	ND	ND	ND	-	-	-	-	-	-	
		Fruit-flavored carbonate	6	ND	ND	ND	ND	-	-	-	-	-	-	
		Fruit juice	6	ND	ND	ND	ND	-	-	-	-	-	-	
ng/g ww (Median)	*UHPLC-MS/MS*													(Kimura et al., 2019)
		Grain	102	0.75	0.92	0.08	0.18	0.15	0.07	42.3	12.7	61.8	0.5	
		Gelatin	10	8.13	4.46	0.19	0.11	0.07	0.05	21.5	1.89	17.3	0.15	
		Seafood	9	0.61	1.68	0.05	1.33	0.04	-	126	47.9	624	0.32	
		Meat	4	0.48	0.11	0.05	0.30	0.19	0.03	60.2	33.3	79.6	0.45	
		Chocolate	39	3.29	10.3	0.18	0.53	0.22	0.05	45.5	334	42.2	4.39	
		Fruit	11	0.16	0.13	0.03	0.06	0.06	-	106	303	102	0.16	
		Vegetable	4	0.09	0.19	-	-	0.12	-	57.7	46.4	58.4	-	
		Sugar	2	-	0.12	-	-	-	-	7.18	0.13	8.14	-	
ng/g fw (Median)*	*LC-HRMS*	*Baby food*												(Nobile et al., 2020)* ** Italy
		∑	112	4.14	-	1.33	ND	ND	-	176.6	10.1	-	7.3	
ng/g lw (Median)	*UHPLC-MS/MS*	Fish												(Ramaswamy et al., 2011) Phillippines
		∑	58	470	12	42	4.2	-	-	-	-	-	-	
ng/kg fw (do not say)*	*SPE-GC-MS*	*Grain products*												(Azzouz et al., 2020) **Spain
		Wheat	3	120	ND	ND	ND	ND	-	-	-	-	-	

Rice	3	450	61	ND	ND	ND	-	-	-	-	-
Spagetthi	3	22	ND	ND	ND	ND	-	-	-	-	-
Tortellini with cheese	3	ND	ND	ND	ND	ND	-	-	-	-	-
Macaroni	3	71	82	ND	ND	ND	-	-	-	-	-
Noodles	3	89	73	ND	ND	ND	-	-	-	-	-
Sesame reganas	3	ND	ND	ND	ND	ND	-	-	-	-	-
Wheat tortillas	3	18	ND	ND	ND	ND	-	-	-	-	-
Corn flakes	3	ND	180	ND	ND	ND	-	-	-	-	-
Crunchy muesli with fruits	3	ND	94	ND	ND	ND	-	-	-	-	-
Cookies	3	39	70	ND	ND	ND	-	-	-	-	-

V.A: Vanillic acid

*The average concentrations were calculated by the undersigned manually, because the concentration was listed for the individual samples.

**Some of the analytes that was detected in this study, was not included in this table

"-": The chemicals were not analyzed

$\sum$: The sum concentrations of the samples that were analyzed

ND: Not detected

dw = dry weight

fw = fresh weight

*fw** = no freeze drying process was reported, so it was assumed that the reported concentration were in fresh weight

lw = lipid weight

ww = wet weight

Some of the studies listed in Table 2.1 have detected parabens in fish bought on local food markets (Ramaswamy et al., 2011, Djatmika et al., 2016). In the latest years, studies have detected amounts of parabens in marine plants, invertebrates, fishes and in marine mammals (Xue et al., 2017, Xue et al., 2015, Zhao et al., 2019). Unlike most chemicals found to bioaccumulate, parabens have a lower octanol/water partition coefficient (K_{ow}), ranging from 1.66 to 3.56 (Xue et al., 2017, Golden et al., 2005). Parabens have not been found to bioaccumulate in the human body (Boberg et al., 2010). The potential of parabens and the metabolites to bioaccumulate in the marine environment have been investigated, with conflicting results. Xue et al. (2017) reported increasing concentration of MeP and decreasing 4-HB in higher trophic levels in the marine environment, suggesting that bioaccumulation is not only dependent on the K_{ow}, but also on the biotransformation potential of an organism. Zhao et al. (2019) found the bioaccumulation potential of parabens to be low, but significant for both 4-HB and OH-EtP. A lot of research still remains, but the presence of intact parabens in species living in remote areas, gives an understanding of the scope and widespread distribution of the chemicals. Another potential source of paraben contamination in food have been the package material. Thus none of the investigated literature have revealed a correlation between the paraben content and the package material (Maher et al., 2020, Liao et al., 2013a, Liao et al., 2013b).

2.4.2 Triclocarban

There are few studies determining the concentration of TCC in foodstuffs. The reason might be that these chemicals are not intentionally added in foodstuffs. Table 2.2 lists up three of the studies done on TCC in food. In the study presented by Yao et al. (2019), the mean concentration was calculated by the undersigned manually, since the obtained detection values was reported for the single samples and not as a sum. For the chicken and egg category, the presence of TCC was found in 1 and 2 out of 6 samples, respectively. Yao et al. (2019) opened up to the possibility that this could be due to sponges or food service wipes that was used to clean up contacts in contact with the foodstuff.

Table 2.2: Recent studies of TCC detection in foodstuffs

Unit (Median/ mean)	*Analytical method*	*Food*	*Number of samples*	*TCC*	*(Reference) Country*
µg/ kg fw[a] (Mean)	*UHPLC-MS/MS*				(Yao et al., 2019)* China
		Beer	6	ND	
		Sodas	5	ND	
		Chicken	4	0.025	
		Cherry	4	ND	
		Egg	5	0.18	
ng/g ww (Median)	*UHPLC-MS/MS*				(Kimura et al., 2019) Norway
		Grain	102	0.17	
		Gelatin	10	0.11	
		Seafood	9	0.12	
		Meat	4	0.76	
		Chocolate	39	0.16	
		Fruit	11	0.07	
		Vegetable	4	-	
		Sugar	2	-	
ng/g (Mean)	*UHPLC-MS/MS*				(Ramaswamy et al., 2011) Phillipines
		Fish	58	10	

*The average was calculated by the undersigned manually, as concentration of the different categories were calculated for each of the samples.

fw[a] = no freeze drying process was reported, so it was assumed that the reported concentration were in fresh weight

2.4.3 Estimated daily intake

Based on the calculation of the content of a compound in foodstuffs, an estimated daily intake (EDI) can be calculated (Liao et al., 2013a, Liao et al., 2013b, Ramaswamy et al., 2011). The EDI is calculated by taking the average value of the paraben content times the average estimated daily food consumption, and divide this by the body weight of the consumer, as illustrated in equation 2.1.

$$EDI = \frac{\Sigma C_i \times DC_i}{BW} \quad (2.1)$$

The Norwegian Health Authorities guidelines concerning food consumption for children is different for the different age groups (Helsenorge, 2018). For children under 6 months, it is recommended to have 2-3 meals, and that each meal consist of 1-1.5 dl food. For the age group >8 months, the amount increases to 2 dl, and it is recommended to have 3-4 meals. 1-2 snack meals can be included for the children over 8 months. Liao et al. (2013b) calculated the EDI of infants (<1 year) and toddlers (1-<6 years), to be 940 and 879 ng/kg bw/ day, respectively, for foodstuffs obtained from the United States. The average food consumption can be hard to predict due to differences in terms of age, gender etc. (Liao et al., 2013b). In addition food might not be the only exposure route for infants and children. Evidences are present that children can be exposed to parabens and triclocarban even before birth as they have been found in the serum of pregnant woman (Li et al., 2020). Parabens have also been reported in the human placenta (Van Overmeire et al., 2019). Parabens and TCC have also been reported in baby teethers (Asimakopoulos et al., 2016a).

2.5 Sample preperation

A sample preparation step to isolate the chemicals of interest might be required before an analysis procedure (Bedson, 1996). This is necessary if the sample needs to be converted from one phase to another (e.g. solid to liquid), or to remove impurities from the sample matrix. If the sample is in solid state, it can also be beneficial to freeze dry the sample prior to the extraction procedure. The moist content in a sample might influence the penetration of the solvent that is used in the extraction.

2.5.1 Liquid liquid extraction (LLE)

To extract target analytes, liquid-liquid extraction (LLE) can be applied (Snyder et al., 2010). LLE is based on Nernst distribution law. By introducing analytes to an aqueous and an organic phase, the analytes will distribute between these phases until equilibrium is reached. Hydrophobic compounds will have greatest affinity to the organic phase, while hydrophilic compounds will have greatest affinity to the aqueous phase. The equilibrium between the two phases can be described by the partitioning coefficient constant (K_{LLE}) as illustrated in equation 2.2 (Lundanes et al., 2014).

$$K_{LLE} = \frac{[Analyte]_{Organic}}{[Analyte]_{Aqueous}} \qquad (2.2)$$

$[Analyte]_{organic}$ is the concentration of analyte in the organic phase, $[analyte]_{aqueous}$ is the concentration of the analyte in the aqueous phase. If the target analyte is supposed to be extracted into the organic phase, it is desirable that K_{LLE} is greater than one. Addition of salt can enlarge K_{LLE} by decreasing the concentration of the target analyte in the aqueous phase. This is called “the salting out effect”. By removing the phase with no target analytes, the target analytes are isolated. Opposite to the aqueous phase, the organic phase often require treatment before injection to a HPLC instrument. If the analytes is in the organic phase, then evaporation of the solvent might be necessary prior to injection (Snyder et al., 2010).

2.5.2 Solid samples

As mentioned above, analysis and quantification of chemicals often requires the samples to be in a liquid phase (Snyder et al., 2010). When analyzing solid samples, like food, the sample

itself might be insoluble, but the analytes of interest might be soluble. By introducing the sample to a solvent, the analytes can be extracted to the solvents. Hence the analytes can be isolated with filtration or centrifugation. Extraction from solid samples might be supported by ultrasonication to increase the resolution. When extraction TA from foods, the various content of fat, protein, water, salts and fiber might influence the extraction rate (Bedson, 1996)

2.6 Instrumental

To be able to qualify or quantify the analytes in a sample, the analytes needs to be separated and detected. In this section the separation technique (U)HPLC and the detection technique MS/MS are introduced.

2.6.1 HPLC and UHPLC

High Performance Liquid Chromatography (HPLC) is a practical separation technique due to its versatility. It can separate a variety of compounds with different properties, provide reproducibility, and have strong sensitivity (McMaster, 2007, Niessen, 2006). The separation principle of HPLC is based on the principle that same solves equal (McMaster, 2007). It is operating with two different phases; a stationary phase and a mobile phase. Different chemicals will have various affinity to the two phases, dependent on the chemical´s properties. The HPLC system consist of a reservoir with solvent, a pump, an injector, a column, a detector and a recorder as shown in Figure 2.6 (McMaster, 2007, Niessen, 2006). The mobile phase, often consisting of one or two solvents, is pumped to the column. Before the column inlet, the sample is injected to the mobile phase that will transport it to the column where the stationary phase is. The compounds with the greatest affinity to the mobile phase, will be the first ones eluate through the column. While the compounds with the greatest affinity to the stationary phase will use longer time through the column. When the analyte passes the column, it will enter a detector flow cell which produces chromatograms (McMaster, 2007). A chromatogram show separated peaks in a coordinate system, where the y-axis represent the peak intensity and the x-axis show the Retention Time (RT). The RT is defined as the time it takes from a sample is injected to the peak is detected in a chromatogram. Ideally, each peak represents a single compound.

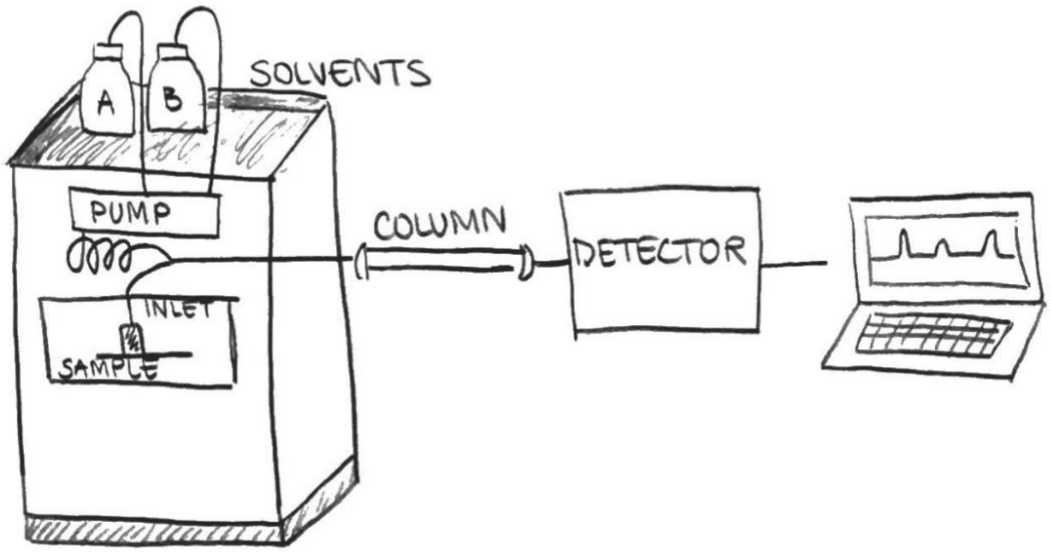

Figure 2.6: A general illustration of a HPLC system: solvents, pump, sample inlet, column, detector and processor. Reproduced from Lundanes (2014) and Snyder (2010).

Some problems can occur in the separation of analytes when applying HPLC (Snyder et al., 2010). If the separation of peaks is not optimal, two compounds can eluate at the same time, and show up as one peak in the chromatogram. This can be due to the composition of the mobile phase. The simplest way of liquid chromatography is isocratic elution where the mobile phase is consistent through the entire analysis. It can be hard to find a satisfying composition of the mobile phase that will give tolerable separation both for the peaks eluting first and the peaks eluting last. Gradient elution, where there is a continuous change in the mobile phase during the separation, can be the solution.

By using the principle of HPLC, but with an increased pressure, the system are able to deliver effluent fast to the detection instrument (McMaster, 2007). This is made possible by using columns with small diameter that are packed with small particle sized materials. The technique is called Ultrafast high performance liquid chromatography (UHPLC/ UPLC). UHPLC allows the system to run with high mobile phase flow rates with little resolution loss. This is a common method used in the pharmaceutical market. Due to the small diameter of the column in UHPLC, the column have a greater risk of plugging by the sample, or mobile phase contamination.

2.6.2 Detection

After separation by the UHPLC it is desirable to qualify or quantify the target analytes (Gross, 2017). An instrument that is widely used is the mass spectrometer that consist of an ion source, a mass analyzer and a detector. Detection by a mass spectrometer requires the analytes to be in ionized form. This is done in the interface between the (U)HPLC and the MS.

2.6.2.1 ESI – ionization source

A method for ionizing the sample prior to the detection by MS is electrospray ionization (Hoffmann and Stroobant, 2007). The mobile phase, containing the analytes, is introduced to a capillary tube that is exposed to an electric field (3-5 kV) under atmospheric pressure. The electric field will produce charged ions, and at the outlet of the capillary tube the ions are introduced to a heated gas (e.g. N_2) (Snyder et al., 2010, Lundanes et al., 2014). This will lead to the formation of droplets and the mobile phase will evaporate. As the droplets leave the capillary tube, the repulsive forces inside them will exceed the surface tension. This will lead to the droplets exploding and decrease in size as they move towards the detection unit. The ESI can be performed in a positive or negative mode. In the positive mode, the ions are protonated (oxidation), and in the negative mode the ions are deprotonated (reduction) (Lundanes et al., 2014). Figure 2.7 illustrates the ESI in the negative ionization mode.

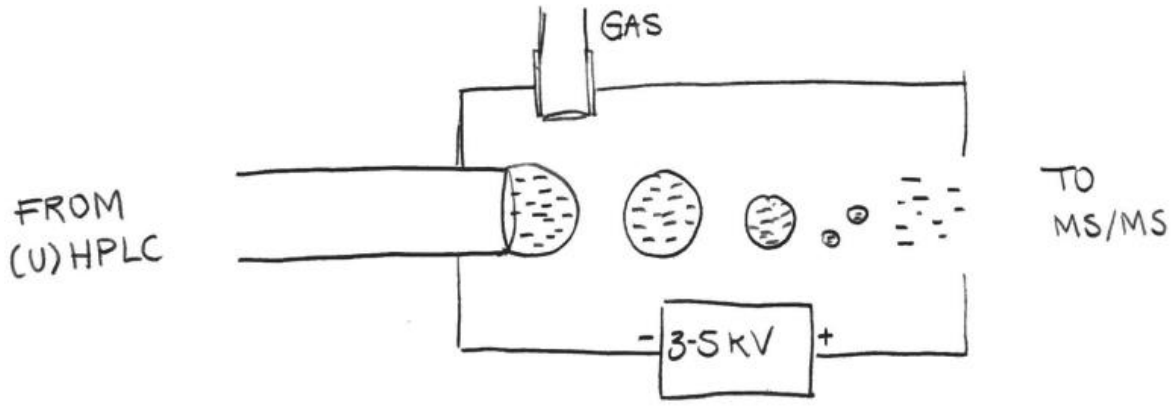

Figure 2.7: General illustration of an electron spray ionization source in negative ionization mode. Reproduced from Snyder et al. (2010) and Hoffmann and Stroobant (2007)

2.6.2.2 MS/ MS

A mass spectrometric detector is an instrument designed to separate, select and detect ions according to their mass to charge ratio (m/z) (Hoffmann and Stroobant, 2007). The ions detected can give information about the initial compound. The triple-quadrupole /tandem MS-detector have great sensitivity and selectivity as it involves two stages of mass analysis. First it detects a parent ion, then fragments this ion, and further confirms the parent ion by monitoring the daughter ions. A quadrupole consist of four parallel rods that are in contact with an electric field (Snyder et al., 2010, Hoffmann and Stroobant, 2007). The ions generated in the interface between the LC and the MS, with a specific m/z-ratio, enter the first quadrupole. Here the ions are isolated, and sent to the second quadrupole. In the second quadrupole, the collision cell, the ions are fragmented further by an inert gas, and these new fragments are send to the next quadrupole. The third quadrupole isolates fragments with a specific m/z ratio, before they are being sent to an electron multiplier for detection and quantification. The MS/MS can be run in different scan modes (Hoffmann and Stroobant, 2007). The "product ion scan" will determine all the daughter ions generated from a parent ion. "Parent ion scan" will determine the parent ion based on the daughter ions. "Natural loss scan" will determine generated fragments by choosing a neutral fragment. In "Selected reaction monitoring" (SRM) there is no scan mechanism, thus the analyzers are focused on selected masses, concerning the parent and daughter ion. The mass analyzers will only recognize daughter fragments if they are a result of the parent ion. SRM is also called multiple reaction monitoring (MRM) (Kinter and Kinter, 2013).

2.7 Quantification

2.7.1 Internal standard

Whenever there is a sample preparation step prior to the analysis, there is a higher chance of both sample loss and sample contamination (Snyder et al., 2010). By adding an internal standard (IS) prior to the sample preparation this type of error can be accounted for. An internal standard (IS) is a compound that, preferably, have a similar retention time as the target analytes (TA). The IS should not have the exact same m/z-ratio as the target analyte, and should therefore not exist in the sample (Lundanes et al., 2014). In LC-MS a isotope of the TA is often used as IS (Snyder et al., 2010). Application of the IS method makes it

possible to have a reference value for the target analytes both when considering the peak height and the concentration. When several TA are supposed to be detected, it is preferred to have a number of IS.

2.7.2 Internal standard calibration curve

An internal standard calibration curve can be made to determine unknown concentration of a target analyte (Snyder et al., 2010). The calibration curve is made by making samples with known concentrations of the target analytes, together with a constant concentration of the IS. The ratio between the peak height/ area of the target analytes and the IS is plotted against the different concentrations of the target analytes. Linear regression gives an equation that is applicable to use for determination of unknown concentrations of target analytes.

2.8 Quality control and Quality assurance

Quality control (QC) and quality assurance (QA) refers to validation of the process and product quality, respectively (Snyder et al., 2010, Lundanes et al., 2014). This is important to make sure the process give similar results independently on the time of analysis and the type of matrix. There are several parameters to take into consideration when evaluating QC and QA. In the following some of them are listed.

2.8.1 Limit of detection and Limit of quantification

To limit the possibility of errors in the quantification due to noise, a limit of detection (LOD) should be set. The LOD expresses the limit where a compound can be qualified, but not quantified (Snyder et al., 2010). The LOD can be determined in different ways. A way of establishing a LOD is consider the signal-to noise ratio. The LOD can be set to three times the noise signal (Lundanes et al., 2014). To ensure a reliable quantification of the target analytes, a limit of quantification (LOQ) should be established. This is the limit where a compound can be quantified. LOQ can be set to the peak height 9 or 10 times the height of the noise level. (Lundanes et al., 2014, Snyder et al., 2010).

2.8.2 Matrix effect

When comparing LC-MS/MS-signals obtained from a clean sample solution with a matrix sample with the same concentration of a target analyte, variations in signal might be detected

(Choi et al., 2001). This can be due to co-eluting compounds from the matrix that suppress or enhance the analyte signal. This is called the matrix effect. When the sample enters the ionization source in the interface between the LC and MS/MS, there can be a competition between the analyte ions and the matrix components with similar retention time. The components may also slow down the evaporation of the solvent by changing the surface tension of the droplets, or enclose them (Stahnke et al., 2009). Matrix effect can be accounted for by comparing the signal in a spiked matrix sample post-extraction by a pure standard solvent solution as shown in Equation 2.3 (Asimakopoulos et al., 2014b):

$$Matrix\ effects = \frac{(Peak\ of\ spiked\ matrix\ (post\ extraction)) - (Peak\ reagent\ blank)}{(Peak\ area\ of\ standard\ solvent\ solution) - 1}\ x\ 100 \qquad (2.3)$$

2.8.3 Recovery

The sample recovery includes both losses and gains due to sample preparation (Bedson, 1996). The absolute recovery can be evaluated by comparing matrixes spiked with standard solutions pre- and post-extraction (Asimakopoulos et al., 2014b). This is done by comparing samples where target analytes are added to the matrixes pre-extraction, with samples added TA post-extraction. The peak area of the TA minus a blank sample of the pre-extracted and the post-extracted are divided and multiplied by a 100%, shown in equation 2.4

$$Absolute\ recovery = \frac{[Peak\ area\ of\ TA\ (pre-extraction)] - [Peak\ of\ blank\ sample]}{[Peak\ are\ of\ TA\ (post-extraction] - [Peak\ of\ blank\ sample]}\ x\ 100\% \qquad (2.4)$$

To calculate the relative recovery, the concentration of an IS is taken into consideration when comparing pre- and post-extraction. The peak are of the TA is divided by the peak area of a specific IS for both pre-and post-extraction matrixes, and consequently these numbers are divided and multiplied by 100%, as shown in equation 2.5:

$$Relative\ recovery\ = \frac{\frac{[Peak\ area\ of\ TA\ (Pre-extraction] - [Peak\ of\ blank\ sample]}{[Peak\ area\ of\ IS]}}{\frac{[Peak\ are\ of\ TA\ (Post-extraction] - [Peak\ of\ blank\ sample]}{[Peak\ are\ of\ IS]}}\ x\ 100\% \qquad (2.5)$$

2.8.4 Retention time and relative retention time

The retention time (RT) is most often measured in decimal minutes (Snyder et al., 2010). The RT should be constant (<0.05 minutes) when the chromatographic conditions are kept constant, and can be used as a qualitative assessment of a compound. The relative retention time (RRT) is the retention time of a compound relative to the retention time of a reference compound, often an IS, as shown in equation 2.6. The reference compound should be a compound that is unlikely to have overlapping peaks with other analytes. The retention time can be influenced by the type of matrix, so the retention time in a matrix sample might vary from the retention time in a blank matrix.

$$RRT = \frac{Retention\ time\ of\ target\ analyte}{Retention\ time\ of\ reference\ compound} \quad (2.6)$$

2.8.5 Relative response

The relative response (RR) is the response of the target analyte divided by the response of a reference sample, like an IS (Kimura et al., 2019). If the IS is added as early as possible in the extraction process, it can compensate for potential sample losses. In that regard concentration determination would be more accurate using RR than evaluating the response of the analyte alone. RR can be calculated as shown in equation 2.7.

$$RR = \frac{Response\ of\ target\ analyte}{Response\ of\ reference\ compound} \quad (2.7)$$

2.9 Precision

Repetitive measurements should be done to ensure the precision of a method (Bedson, 1996). Evaluation of the precision can clarify for variations in different factors that can occur in the analysis, e.g. temperature, shaking times, extraction conditions and flow rates. By analyzing

replicates of samples under the same conditions, it is possible to evaluate a methods repeatability (Snyder et al., 2010). The repeatability indicates if the method are able to produce the same results within a short time interval under identical conditions. It should be determined by analyzing three replicates and cover a specified range of the procedure. The reproducibility of a method refers to the properties of the methods to give similar results despite different conditions, for instance in different laboratories. A relative standard deviation (RSD) can be calculated for both reproducibility and repeatability. RSD is calculated by taking the standard deviation and dividing it by the average value of a sample set, as shown in equation 2.7.

$$Relative\ standard\ deviation = \frac{STD}{Average} \tag{2.7}$$

2.10 Correlation analysis

A correlation analysis can be performed to evaluate the degree of association between two variables (Asuero et al., 2006). It can be evaluated by their linear relationship, and in quantitative analysis, the parameter to evaluate is the correlation coefficient, r. The r-value ranges between -1 and 1, and the greater the correlation, the greater the r-value. Negative value indicates a negative correlation. A r-value > 0.7 is considered high correlation, and r-value between 0.5 and 0.7 is considered a moderate correlation.A r-value < 0.5 is considered low correlation. The larger the number of samples, n, the lower the acceptable r-value becomes.

2.11 Principal component analysis (PCA)

After an experiment or an analysis, the obtained results can be provided as quantitative data (Abdi and Williams, 2010). These data can be placed in a table and be sorted into sets of inter-correlated variables, and these variables can be interpreted individually. A Principle Component Analysis (PCA) tries to unite these variables and extract the main findings. In this way the variables might propose a pattern of similarities as the quantitative data are added to a map. The aim is to extract the most important information, and still show the variation in the

obtained data, but reduce the size of the original data tables (Naik, 2018, Abdi and Williams, 2010). In this way the obtained information gets structured and simplified.

3 Materials and method

3.1 Sample collection

Baby foodstuffs samples were purchased at a grocery store in Norway. The liquid and semi-solid samples were stored in the freezer at -20$_{o}$C, while the solid samples were stored in room temperature until analysis. Before extraction the samples were freeze dried. The samples where categorized by type of food and the brand. The categories where inspired by the article published by Liao et al. (2013a), where foodstuffs in China where analyzed. These categories where: grain (e.g. whole grain products, n=7), dairy (infant formula (powder and ready-to-drink), n=3), cookies and snacks (e.g. maize puffs and snack bars, n=12) , fruit (e.g. smoothies and fruit purées, n= 18) and vegetables (e.g. dinner products, vegetable purées, n= 9). A lot of the baby food samples were full meals with a variety of ingredients. For these samples, they were placed in the category that fit their main ingredient. Due to the lab lockdown, obtained information about the ingredients in the samples, was obtained from the brand´s webpages, online grocery shops and pictures taken of the samples (Nestlé, 2020, Semper, 2020, Organix, 2020, Ella´s kitchen, 2020, Coop, 2020, Norges online, 2020). The samples are listed in Appendix A.

3.2 Chemicals

3.2.1 Internal standards, target analytes and solvents

Analytical isotope standard mix solution, containing $^{13}C_6$-MeP, $^{13}C_6$-EtP, $^{13}C_6$-PrP and $^{13}C_6$-BuP, were obtained from Sigma Aldrich. The analytes standards, MeP (99%), EtP (99%), PrP (≥ 99%), BuP (≥ 99%), BezP (≥ 98%), HeP (99%), 4-HB (99%), 3,4-DHB (≥97%), Vanillic acid (97%), OH-EtP (97%) and TCC (99%), were purchased from Sigma Aldrich.

Ammonium acetate, ethyl acetate and methanol was purchaced from Sigma Aldrich. The Milli-Q water was purified by Millipore water distribution system.

3.2.2 Calibration curve

A mix of the target analytes were used to make a calibration curve. The concentration in the standard mixed used for the calibration curve is found in Appendix F. Known concentrations of the analytes were spiked with a constant volume of internal standard mix solution with four internal standards (10 ppb). The concentrations of the target analytes were 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50 ppb, respectively.

3.3 Extraction

The samples were prepared and extracted according to the procedure used by Kimura (2019). 0.2-1.3 gram (dry weight) of each sample was weighed out and added to a polypropylene (PP) tube (15 mL). Accordingly they were spiked Internal Standard (IS) (10 µL) and ammonium acetate (1M, 2 mL) and stored in the fridge overnight. The next day ethyl acetate (5 mL) was added to the tubes. The tubes were then sonicated (10 minutes) before being mechanically shaken (45 min). After shaking, the tubes were centrifuged (3500 rpm in 5 minutes), and the supernatant was transferred to new test tubes. Addition of ethyl acetate (5 mL), mechanic shaking and centrifugation was repeated two more times, and the respective supernatants were combined. Thus, the combined supernatant were added Milli-Q water to remove salts, and thereby centrifuged (3500 rpm, 5 minutes). The organic phase where transferred to new PP tubes, and concentrated to near-dryness by a nitrogen steam using a TurboVap LV (Biotage). After evaporation, the elutes were added to UHPLC-vials with additional methanol (~1 mL).

3.4 Analysis by UHPLC-MS/MS

The analysis parameters was set as described in Asimakopoulos et al. (2016b). The UHPLC used was delivered from Waters Acquility. It was combined with Waters Acquility Column Manager, sample manager and 1 UHPLC class binary solvent manager. The target analytes was separated using Kinetex C18 seperation column (2.1 mm #50 mm, 1.3 mm; Phenomenex Inc, Torrance, CA, U.S) serially connected to a SecurityGuard ULTRA C18 guard column (2.1 mm, sub-2 mm core-shell column;Phenomenex Inc.). The mobile phase consisted of water with 0.1% formic acid and methanol at flow rate 200 µL/ min. The injection volume was 3 µL.

The mobile phase consisted an organic phase (A) with water added 0.1% formic acid and a water phase (B) with methanol. The flow rate was set to 200 µL/ min. Analysis was preformed with gradient elution program, starting with 1% A and 99% B, and increased to 75% A and 25% B after 0.4 minutes. It continued to increase, and after 0.8 minutes it consisted of 95% A and 5% B. This composition where kept until 2.5 minutes, then A was increased and B decreased further, and after 2.55 minutes the composition was 99% A and 1% B. This composition where kept until 3.3 minutes, before the phases was set to start composition The ESI spray was run in a negative ionization mode, with the capillary voltage of 1.8 kV. The flow rate of the cone gas (N_2) was set to 0.15 mL/ min. The source temperature and the desolvation temperature was set to 150$_o$C and 350$_o$C, respectively. The MS/MS was set in MRM mode. Table 3.1 lists up the primary and secondary transition for the target analytes and the internal standards. The retention time values are the average retention time based on the highest concentrations in the calibration curve. The highlighted transitions are the once used for quantitative ions.

Table 3.1: Target analytes and internal standard with their retention time, primary and secondary transition, respectively. Quantifying ions are highlighted

Primary and secondary transition of the target analytes			
Compound	*Retention time (RT)*	*Primary transition*	*Secondary transition*
MeP	1.63	151>136	**151>92**
$^{13}C_6$-MeP	1.63	157>142	**157>98**
EtP	1.83	165>137	**165>92**
$^{13}C_6$-EtP	1.83	171>142	**171>98**
PrP	2.02	179>136	**179>92**
$^{13}C_6$-PrP	2.02	185>142	**185>98**
BuP	2.20	193>137	**193>98**
$^{13}C_6$-BuP	2.20	199>142	**199>98**
BezP	2.20	227>136	**227>92**
HeP	2.67	235>136	**227>92**
4-HB	1.25	**137>93**	-
3,4-DHB	1.05	**153>109**	-

Vanillic	1.30	**167>152**	157>108
OH-EtP	1.64	181>153	**181>108**
TCC	2.66	**313>160**	313>126

The assessment of which samples that had acceptable RRT was based on the European Unions criteria regarding the interpretation of results (The Commission of the European Communities, 2002). Acceptable RRT was considered ±2.5% of the average RRT in the calibration curve. For 4-HB and 3,4-DHB it was determined, in collaboration with the supervisor, that due to their polarity, the lower limits were exceeded. The lower acceptable limits for RRT was determined from the spike samples RRT, which was lower than for the calibration curve. The respective internal standard reference for the different target analytes in calculation of RRT is listed in Table E.2 in Appendix E.

3.5 QC/ QA

For about every 15th sample, a procedure blank was made to make sure that any contaminations were taken into account. Any presence of the TA in the procedure blanks was subtracted from the samples in the sample set. Initially the samples supposed to be used for QC and QA should have consisted of several types of baby food to make sure that several matrix types were accounted for. Due to the Covid-19 situation, the lab was locked down, and these samples could not be prepared. Samples analyzed in January, which was intended to be prepared, extracted and analyzed so that the undersigned and two other master students were acquainted with the method, had to be used. These samples consisted of one type of baby food only (Sample 2, Appendix A), and was not freeze dried prior to the analysis. The samples used for QA is referred to as samples from experiment 1, while the actual samples and the calibration curve are referred to as samples from experiment 2.

3.6 Data treatment

For analyzing the chromatograms obtained from the UHPLC-MS/MS analysis the software programs MassLynx and TargetLynx (version V4.1, Waters Corporation Milford MA, USA) were used. The data obtained was prosessed in a spreadcheet (Excel, 2019). For the drawing was used.

4 Results and discussion

4.1 Quality assurance

As mentioned in chapter 3.5, the samples used for QC and QA obtained from experiment 1 did only contain one type of baby food. This might have influenced some of the parameters in the QC. Liao et al. (2013a) reported that the recoveries were slightly different among the food categories, that is, the different food matrixes. The samples used in the present study was in the category "grain" witch only accounts for about 14% of the samples. If several types of food matrices were accounted for, than the parameters in QC and QA might have been more accurate and representative for all of the different samples. Some food are inhomogeneous and complex matrixes with various content of proteins, salts, fat, water and fiber (Bedson, 1996). The fat and water content could for instance influence the extraction rate. Due to this it would be preferred to include the samples containing various amount of fat, and to use the freeze dried samples for QA purposes. Accordingly results presented in the QA section needs to be interpreted with caution.

From experiment 2, standard calibration curves of 0.1, 0.2, 0.5, 1, 2, 5, 10, 20 and 50 ppb were obtained for the different target analytes, with good correlation coefficients (R^2=0.99 for all, except 3,4-DHB: R^2>0.98), presented in Appendix C.

4.1.1 LOD and LOQ

The LOD varied from <0.03 ng/mL to 11.72 ng/mL based on the signal-to-noise values times 3. The LOD was determined by the average noise values of the lowest concentrations, 0.1, 0.2 and 0.5 ppb, of the calibration curve from experiment 2. The LOD was consequently estimated from instrumental, and not from matrix samples. Due to possible co-eluting components in the matrix, it might be assumed that the LOD would be different if it was based on spiked matrix samples.

Since some of the signal-to-noise values were much lower than the lowest calibration curve point, the LOQ value was determine to be 0.1 ng/mL (lowest calibration curve point). The LOD was consequently set to LOQ/ 3. Higher LOD values were obtained of the derivates compared to the parabens, and this corresponds good to the studies performed by Kimura (2019) and in the study were the method of Nobile et al. (2020) was developed (Chiesa et al., 2018). The LOQ was ranging from 0.1 ng/mL to 35.15 ng/mL. The LOD and the LOQ values for the target analytes are presented in Table 4.1. Concentrations below LOD was removed from the data set.

Table 4.1: The target analytes and their LOD and LOQ (ng/mL), respectively

LOD and LOQ		
Compound	*LOD (ng/mL)*	*LOQ (ng/ mL)*
MeP	0.19	0.58
EtP	0.03	0.1
PrP	0.03	0.1
BuP	0.048	0.14
BezP	0.03	0.056
HeP	0.03	0.1
4-HB	9.86	29.56
3,4-DHB	5.76	17.27
Vanillic acid	11.72	35.16
OH-EtP	0.03	0.1
TCC	0.03	0.1

4.1.2 Matrix effect

Table 4.2 illustrates the calculated matrix effect of the target analytes. What can be clearly seen in the table is the general pattern of decreasing matrix effect as the chain length of the parabens increase. For MeP and EtP the matrix effects indicate that the signal is being enhanced due to possible interfering compounds in the matrix. For PrP, BuP, BezP and HeP the signals are being suppressed due to negative matrix effects. For BezP and HeP with matrix effects of -77.84% and -91.98%, respectively, suggests that over half of the concentration of the compounds is being suppressed due to the matrix.

Table 4.2: The calculated matrix effect of the target analytes obtained by spiked samples (10ppb)

Matrix effect	
Compound	*ME (%)*
MeP	52.50
EtP	8.79
PrP	-33.94
BuP	-60.48
BezP	-77.84
HeP	-91.98
4-HB	-248.62
3,4-DHB	76.36
Vanillic	-73.28
OH-EtP	45.62
TCC	-95.11

In studies were parabens and metabolites have been quantified, the concentrations of the derivates are usually higher than the paraben concentration (Nobile et al., 2020, Karthikraj et al., 2018, Kimura et al., 2019). Due to their natural presence in food, it is natural that the concentrations in food matrix samples are high. Regarding 4-HB the concentration found in the non-spike samples were 1.3 and 1.2 times greater than the pre- and post-spiked samples,

respectively. This led to negative corrected areas for both pre- and post-spiked samples. The matrix effect for 4-HB is in that regard somewhat misleading. Negative values indicates that the ions are being suppressed, but in this case the matrix effect calculations gave negative values because the post-spiked samples had negative corrected area. The non-spike samples, on the other hand, suggests that the matrix contributes greatly to amplified signals compared to the "clean" reference sample spiked with 10 ppb of 4-HB. The matrix effect of Vanillic acid and TCC suggests that the ions are being suppressed, while for OH-EtP it is being enhanced.

4.1.3 Precision

Table 4.3: Calculated precision of four replicates of pre-spiked samples (10 ppb) with absolute and relative values, using the areas and the relative areas, respectively

	Absolute				**Relative**			
	Median	*Mean*	*STD*	*RSD(%)*	*Median*	*Mean*	*STD*	*RSD(%)*
MeP	2878.59	2919.35	348.47	11.94	0.44	0.46	0.07	15.09
EtP	5296.63	5469.17	1138.27	20.81	0.64	0.69	0.14	20.34
PrP	10073.90	9698.89	2833.99	29.22	0.84	0.83	0.25	30.11
BuP	8228.13	7561.00	2034.56	26.91	0.92	0.93	0.30	31.80
BezP	6625.55	6252.21	2117.97	33.88	0.76	0.70	0.21	29.78
HeP	2208.66	2453.07	1588.97	64.77	0.29	0.29	0.17	58.88
4-HB	23376.77	24176.36	2919.61	12.08	3.04	3.51	1.02	28.96
3,4-DHB	20557.01	21913.00	3549.23	16.20	3.51	3.33	0.41	12.26
Vanillic	16628.62	15525.34	2388.77	15.39	2.48	2.40	0.58	24.02
OH-EtP	11973.14	11812.00	2169.50	18.37	1.45	1.49	0.25	17.00
TCC	3994.08	5459.47	4967.89	91.00	0.52	0.64	0.53	81.84

As for the matrix effect, Table 4.3 show a pattern for the parabens regarding the relative standard deviation for both the absolute and relative values; The longer the chain length of the ester group, the higher the RSD. The relative values are comparable to the absolute values,

indicating that the IS did not vary in signals as much as the target analytes. The RSD values indicates that the replicates of a sample gave various peak areas with the same concentration of spiked target analytes. The highest RSD obtained from HeP and TCC, suggest that the repeatability of the method was unsatisfactory. Kimura (2019) applied the same method and reported lower RSD values for all of the target analytes. Based on the precision parameters in the present study compared to the precision results obtained by Kimura (2019), this suggests that the reproducibility of the method is unsatisfactory (Snyder et al., 2010). Thus, the high RSD-values in the present study might be due to the water content in the samples as they were not freeze-dried prior to analysis. The moisture content of a sample can influence the penetration of the solvent in an extraction process, and thereby lead to different extractions of the target analytes (Bedson, 1996). In addition it would be preferred to determine the RSD for several spike concentrations, to see if there was a differences with higher spike concentration (Snyder et al., 2010). As mentioned above, this was not possible due to the Covid-19 situation.

4.1.4 Absolute and relative recoveries

Table 4.4 Calculated absolute and relative recovery for the target analytes (n=4). Spiked samples contained 10 ppb TA-mix and 10 ppb IS.

Absolute and relative recovery		
Compound	*Absolute recovery*	*Relative recovery*
MeP	50.63	99.36
EtP	48.08	98.16
PrP	41.98	91.41
BuP	36.29	100.00
BezP	36.24	89.12
HeP	36.58	100.00
4-HB	169.12	53.25
3,4-DHB	11.66	-70.41
Vanillic	-767.13	-16.11
OH-EtP	45.62	92.45
TCC	38.18	90.08

Table 4.4 illustrates the obtained absolute and relative recoveries of the target analytes. The absolute recovery values of the parabens suggests sample losses. Only half of the concentrations in the samples was recovered after the sample preparation procedure, as the recoveries range from 36.14-50.63%. The relative recoveries, on the other hand, show that the IS compensated for these losses in that these recoveries are high, ranging from 89.12-100.00%. Taken together, these results supports that determining the sample concentrations would be more accurate by using the relative ratio instead of the areas of the samples alone. The relative recoveries are similar to that reported by Liao et al. (2013a) (MeP, EtP, PrP, BuP, BezP, HeP) and Maher et al. (2020) (MeP, EtP, PrP, BuP).

For the metabolites, the high absolute recovery of 4-HB is most likely due to the fact that the non-spiked samples had a greater peak area than the pre- and post-spiked samples. After correcting the areas by subtracting the non-spiked, the areas of the pre-spiked had a greater negative value than the post-spiked. This made the absolute recovery high, although it would be smaller if the areas had not been corrected by the non-spike samples. Unsatisfactory recoveries was calculated for 3,4-DHB as well, with a low absolute recovery, and a negative relative recovery. Regarding the negative values of Vanillic acid, this result may be explained by the fact that the areas of the non-spiked samples were higher than the pre-spiked samples. This influenced both the absolute and the relative recovery calculations. TCC and OH-EtP showed similar recoveries as the parabens, suggesting that the internal standards is representative and compensates for potential sample losses.

4.1.5 Quantification

It was determined that the samples used for QA and QC failed to meet the standards for use in the quantification process. Very high and low recoveries were observed for the derivates, Vanillic, 4-HB and 3,4-DHB. In addition it was not observed a closeness of agreement of the replicates in the precision evaluation of the parabens, OH-EtP and TCC as the RSD% was high (except for MeP) (Snyder et al., 2010). Due to this all the TA were semi-quantified, meaning that the values presented in the following are estimates of the analyte concentration (Bedson, 1996). Thus, it can be argued that the quantification of parabens were slightly more accurate compared to the derivates due to more satisfying recovery values.

The calculation of concentrations, should initially be based on the slope of the spiked samples. Thus, due to the unsatisfactory results, and that the samples only accounted for one matrix type, other slopes was used. For the determination of concentration for MeP, EtP, PrP and BuP, that all had internal standards with similar chemical structure, the concentrations were determined by using the slope of the calibration curve from experiment 2 (Appendix C). For the remaining chemicals, the slope used to determine the concentrations were obtained from previous work done by the supervisor (Kimura, 2019). The slopes for quantifications are added in Table E.1 in Appendix E. For one sample, addition of IS was forgotten, and the concentrations for the different target analytes was determined by external calibration (Appendix E).

The calculated concentrations of 4-HB, 3,4-DHB and Vanillic listed in the following, was much higher than the highest calibration point in the standard calibration curve (Kimura et al., 2019). The linearity of the curve is only proved to be from 0.1-50 ppb, so concentrations over 50 ppb might not follow the same linear relationship between concentration and response (Snyder et al., 2010). This gave an even greater reason to semi-quantify the derivates.

4.2 Occurrence and semi-quantification of parabens, paraben derivates and triclocarban in baby food

In the following the results from the semi-quantification of the target analytes in the present study are introduced. The results have been compared with the studies listed in Table. 2.1. The research on the paraben content in baby foodstuffs is scare, and the only obtained study was the one reported by Nobile et al. (2020). Thus, comparing the obtained concentration estimates in the present study with obtained results from other studies might gain an understanding of how the concentrations in baby food could differ or be similar to other types of food. It could also contribute to get an overview of the exposure rate of the target analytes from food as this is found to be hard to estimate when analyzing parabens and triclocarban in biological matrices (Asimakopoulos et al., 2014a). However, it is important to keep in mind that the regulations concerning additives in food might be different in other countries (Yang et al., 2014). This might lead to variations in detection and quantification as a natural consequence of this. Most of the studies listed in Table 2.1, reported concentrations in fresh weight, while the present study reported dry weight. Also, the concentration obtained by this study are estimates as the target analytes are semi-quantified. The results must in that regard be interpreted with caution.

4.2.1 Occurence overall

Table 4.5 show the semi-quantified median, mean, maximum and minimum dry weight concentrations (ng/g) and the detection rate of the target analytes detected in the present study. The concentrations varied from 0.05 ng/g (HeP) to 71.27 ng/g (MeP). The estimated median concentration of parabens was 2.17 ng/g. The derivates 4-HB, 3,4-DHB and Vanillic were found in high concentrations and with high detection rates (95.92%, 53.06% and 87.76%, respectively). OH-EtP was found in 30.61% of the samples, while TCC was found in 1 of the samples. The calculations were based on the samples showing concentrations above LOD. The detection rate was determine by taking the number of samples over LOD and dividing it by the number of samples in the entire sample set.

Table 4.5 The semi-quantified median, mean, max and minimum concentration (ng/g), and the detection rate (DR) of the target analytes, ∑parabens, ∑derivates and ∑derivates and parabens. Concentrations are listed in dry weight (dw)

Concentration of target analytes					
Compounds	*Median (ng/g, dw)*	*Mean (ng/g, dw)*	*Max (ng/g, dw)*	*Min (ng/g dw)*	*DR (%)*
MeP	11.87	21.17	71.27	1.14	24.49
EtP	1.05	3.81	29.70	0.31	30.61
PrP	0.72	0.72	1.00	0.44	4.08
BuP	0.14	0.15	0.31	0.05	12.24
BezP	0.26	1.08	7.70	0.05	51.02
HeP	0.06	0.06	0.06	0.05	4.08
∑parabens	2.17	9.73	72.90	0.05	71.43
4-HB	1097.94	3383.16	19731.91	16.45	95.92
3,4-DHB	4926.41	20516.23	152764.77	25.49	53.06
Vanillic	413.22	1136.29	7238.34	39.73	87.76
OH-EtP	1.03	2.72	14.50	0.08	30.61
∑derivates	2923.65	15450.05	161301.44	16.45	97.96
TCC	0.04	0.04	0.04	0.04	2.04
∑all	2924.41	15451.51	161315.93	16.45	97.96

∑parabens: Sum MeP, EtP, PrP, BuP, BezP, HeP

∑derivates: Sum 4-HB, 3,4-DHB, Vanillic

∑parabens and derivates: All TA

4.2.2 Paraben detection

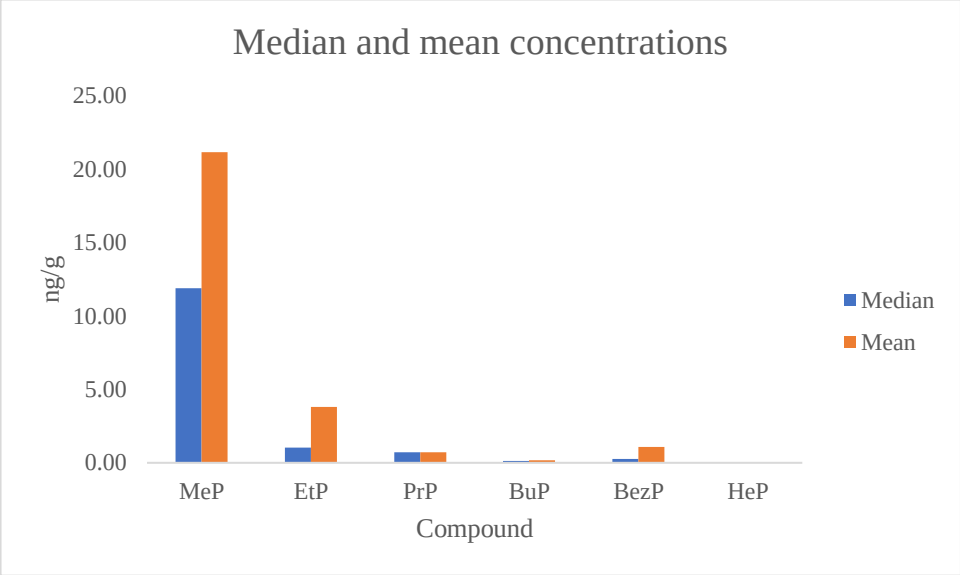

Figure 4.1 A column chart illustrating the estimated median and mean concentrations of the parabens detected in baby foodstuffs

Figure 4.1 provides an illustration of the estimated median and mean concentrations of the parabens in the present study. The greatest median and mean concentration was obtained by MeP, which correlates well with the findings from foodstuff listed up in Table 2.1 (Liao et al., 2013b, Liao et al., 2013a, Maher et al., 2020, Zhou et al., 2015, Yang et al., 2014, Djatmika et al., 2016). MeP was also the paraben reported in highest concentrations in human placenta, urine, serum and house dust (Van Overmeire et al., 2019, Asimakopoulos et al., 2014a, Li et al., 2020, Ramirez et al., 2011, Wang and Kannan, 2013). This might indicate that MeP is the paraben that humans are exposed to in the highest concentrations. Relatively speaking these findings are reassuring in that MeP is the paraben considered to be least harmful (Darbre and Harvey, 2008, Boberg et al., 2010, EFSA, 2004b). In the samples containing MeP along with other parabens, MeP accounted for 83.79% of the total paraben content on average. The estimated median concentration of parabens was decreasing with increasing chain length of the ester, despite BezP who obtained higher estimated median concentration than BuP and

HeP (BezP = 0.26 ng/g, BuP =0.14 ng/g, HeP = 0.06 ng/g). Parabens with shortest chain lengths (MeP, EtP, PrP) were found in greatest concentrations, with highest median and mean values in several of the studies listed in Table 2.1 (Liao et al., 2013b, Liao et al., 2013a, Azzouz et al., 2020, Djatmika et al., 2016, Maher et al., 2020). Greater concentrations of the parabens with shorter chain length correlates well with results from biological samples as well, suggesting greatest exposure of these parabens (Asimakopoulos et al., 2014a, Li et al., 2020). The estimated mean value for the sum of parabens (9.73 ng/g) is comparable to those observed by Liao et al. (2013b) (9.67 ng/g), but lower than the mean value obtained from Liao et al. (2013a) (39.3 ng/g) and Maher et al. (2020). Nobile et al. (2020) reported lower concentrations of parabens in their study on baby food.

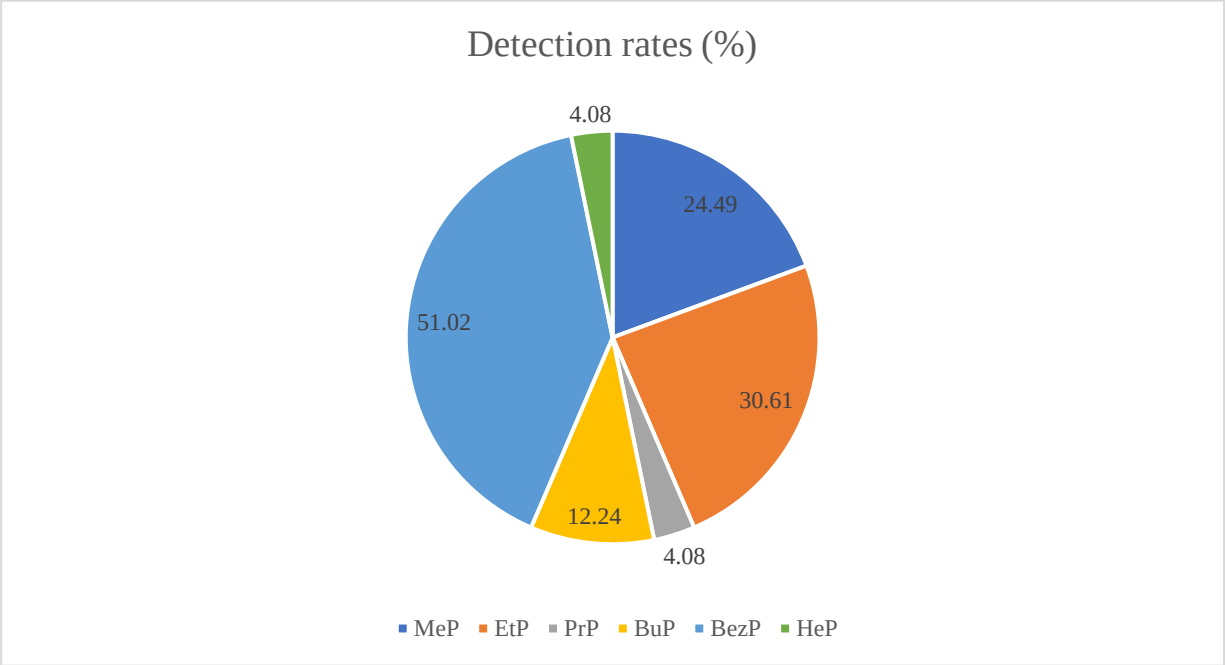

Figure 4.2: Pie chart illustrating the detection rates of the different parabens

The detection rates obtained in the present study are illustrated in Figure 4.2.MeP was present in 12 of the 49 samples. Contrary to expectations, the detection rate of MeP was not the highest among the parabens in the present study. Both EtP and BezP showed higher detection rates, as they were found in 15 and 25 samples, respectively. The detection rate of BezP was greater than reported in other studies, and none of the studies reported higher detection rates of BezP than MeP (Liao et al., 2013b, Liao et al., 2013a, Nobile et al., 2020, Kimura et al.,

2019). Detection rates of BezP in biological samples, have also been reported to be low, and less frequent than for parabens with shorter chain lengths (Asimakopoulos et al., 2014a, Wang and Kannan, 2013, Li et al., 2020, Wang et al., 2013). BezP is not allowed to use as food additive, and is one of the parabens that is of highest concern when it comes to endocrine disruption (EFSA, 2004b). It is therefore unclear how BezP could be detected in this extent in the present study. How parabens could end up in baby food is further discussed in chapter 4.6. Regarding the other parabens, PrP and HeP was only found in 2 samples, and BuP was found in 6 samples. Low incidence of PrP (2.7%) was also reported in the study published by Nobile et al. (2020). Other studies have reported PrP to have high and comparable detection rates as MeP and EtP (Liao et al., 2013a, Liao et al., 2013b). For the sum of parabens, MeP, EtP, PrP, BuP, BezP and HeP, the detection frequency were 71.43%. This was a rather unexpected detection rate, as the paraben content in baby food stuff are expected to be scare. In summary these results suggests that children are exposed to parabens through the consumption of baby foodstuffs.

4.2.3 Derivate detection

4-HB and Vanillic showed the highest detection rates overall, as they were found in 47 and 43 of the samples, respectively. 3,4-DHB was detected in 26 of the samples. The three derivates possessed the greatest concentrations estimates overall in the present study. The findings of the current study support some of the previous research in that higher concentrations was reported for derivates than for parabens (Karthikraj et al., 2018, Kimura et al., 2019, Nobile et al., 2020, Xue et al., 2017, Xue et al., 2015). The obtained results might further support the idea the intake of 4-HB as a natural component in food to be greater than the contribution from parabens (Boberg et al., 2010). The derivates 4-HB and 3,4-DHB were reported in high concentrations in snacks samples and baby food in previous studies (Kimura, 2019, Nobile, 2020). They have also been found in excessive amounts in mammals, marine plants and fish, proposing a widespread occurrence of these chemicals(Xue et al., 2015, Xue et al., 2017). The metabolite OH-EtP had a detection rate of 30.61%, higher than the detection rate reported by Kimura (2019) and Nobile (2020). However, the estimated median concentration of OH-EtP, was lower than both Nobile (2020) and Kimura (2019) reported (7.3 and 3.75 ng/g, respectively). The detection rate of OH-EtP and EtP was the same (30.61%). Their common presence was found in 60% of the samples they were detected. Their relationship in the

human body is known, as OH-EtP is the novel metabolite of EtP, but the relationship in consumer products is still not fully understood (Wang and Kannan, 2013). A simple correlation test was performed in Excel, but no correlation was obtained ($r<0.5$). This might suggest that OH-EtP have other origins than from EtP in foodstuffs. The natural presence of OH-EtP have been found in peanut seed coating and wine, indicating that the presence of OH-EtP can have a natural origin in some plant species (Wang and Kannan, 2013).

4.2.4 TCC detection

TCC showed the lowest detection frequency of all the analytes of interest. It was only found in 1 out of 49 samples. Since TCC is an antimicrobial in hygiene products and not in food, this agrees well with the low detection rate. In some of the studies were TCC have been detected in foods, it has been found in unprocessed foods. This might suggest that the detection of TCC was due to contamination of the organism, rather than addition from processing (Ramaswamy et al., 2011, Yao et al., 2019). On the other hand, Kimura (2019) reported the detection rate of TCC to be 0-100% in different processed foodstuffs, suggesting that contamination or addition can arise in processing. In studies were urine, fish and serum have been analyzed, were parabens and TCC have been detected, parabens have been found in much higher concentrations and detection rates (Asimakopoulos et al., 2016a, Ramaswamy et al., 2011, Li et al., 2020). This might implicate a greater widespread occurrence and exposure of parabens than TCC. This can be a result of the wider application area for parabens then for TCC. Nevertheless the reported detections of TCC in foods provides an understanding of how humans can be exposed to unintentionally added chemicals in foodstuffs. Unintentionally added chemicals detected in foods also reinforce a need for mapping of chemicals in food for consumer safety and quality assurance (Saad, 2005).

4.3 Occurrence of target analytes based by category

The different baby food samples were categorized as described in section 3.1. Table 4.6 show the obtained results regarding the target analyte concentration in the grain, dairy and cookies and snacks (C&S) category. Table 4.7 show the obtained results in the fruit and vegetable category. The variations within the different food categories in regard to the content of 4-HB, Vanillic, ∑parabens (Sum_6_Parabens) and ∑parabens and derivates (Sum_parabens) was also investigated. Sum_6_Parabens include MeP, EtP, PrP, BuP, BezP, HeP, while Sum_parabens include Sum_6_Parabens, OH-EtP, 4-HB and 3,4-DHB. The obtained results are presented in the box-plot in Figure G.1 in Appendix G. The category "grain" and "Grain/cereal" should be looked upon as one category, namely "grain".

Table 4.6: Semi-quantified median, mean, maximum and minimum concentration (ng/g dw) and the detection rate (DR) of the target analytes in the grain, dairy and cookies and snack (C&S) category

	Grain (n=7)					**Dairy (n=3)**					**C&S (n=12)**				
	Median (ng/g)	*Mean (ng/g)*	*Max (ng/g)*	*Min (ng/g)*	*DR (%)*	*Median (ng/g)*	*Mean (ng/g)*	*Max (ng/g)*	*Min (ng/g)*	*DR (%)*	*Median (ng/g)*	*Mean (ng/g)*	*Max (ng/g)*	*Min (ng/g)*	*DR (%)*
MeP	10.84	15.89	35.70	1.14	42.86	ND	ND	ND	ND	ND	6.22	21.67	71.27	2.95	33.33
EtP	15.23	15.23	29.70	0.75	28.57	ND	ND	ND	ND	ND	0.61	0.65	1.05	0.31	33.33
PrP	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.44	0.44	0.44	0.44	8.33
BuP	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.31	0.31	0.31	0.31	8.33
BezP	0.22	0.91	2.48	0.06	71.43	ND	ND	ND	ND	ND	0.18	0.21	0.45	0.08	58.33
HeP	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.05	0.05	0.05	0.05	8.33
∑parabens	1.46	13.78	43.06	0.22	85.71	ND	ND	ND	ND	ND	0.77	8.32	72.90	0.08	91.67
4-HB	293.92	384.39	1004.13	71.83	100.00	18.25	17.75	18.55	16.45	100.00	830.24	1928.12	10147.04	213.85	100.00
3,4-DHB	855.52	1550.72	3576.85	219.80	42.86	ND	ND	ND	ND	ND	2295.00	7304.60	25629.72	25.49	66.67
Vanillic	284.05	365.14	947.42	39.73	100.00	ND	ND	ND	ND	ND	214.20	458.92	2606.75	107.18	100.00
OH-EtP	0.08	0.08	0.08	0.08	14.29	ND	ND	ND	ND	ND	0.30	0.79	1.76	0.09	41.67
∑derivates	761.23	1414.12	4865.03	111.55	100.00	18.25	17.75	18.55	16.45	100.00	2441.47	7256.78	27558.00	346.52	100.00
TCC	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.04	0.04	0.04	0.04	8.33
∑all	796.99	1425.95	4908.05	112.30	100.00	18.25	17.75	18.55	16.45	100.00	2441.70	7264.73	27559.85	354.16	100.00

∑parabens: MeP, EtP, PrP, BuP, BezP, HeP, ∑ derivates: 4-HB, 3,4-DHB, Vanillic, *DR*: Detection rate, ND: Not detected

Table 4.7 Semi-quantification, mean, maximum and minimum concentration (ng/g dw) and the detection rate (DR) of the target analytes in the fruit and vegetable category

	Fruit (n=18)					**Vegetables (n=9)**				
	Median (ng/g)	*Mean (ng/g)*	*Max (ng/g)*	*Min (ng/g)*	*DR (%)*	*Median (ng/g)*	*Mean (ng/g)*	*Max (ng/g)*	*Min (ng/g)*	*DR (%)*
MeP	15.73	14.65	24.74	2.41	22.22	61.10	61.10	61.10	61.10	11.11
EtP	2.44	2.91	8.98	0.62	44.44	0.92	0.92	0.92	0.92	11.11
PrP	ND	ND	ND	ND	ND	1.00	1.00	1.00	1.00	11.11
BuP	0.14	0.13	0.20	0.05	22.22	0.08	0.08	0.08	0.08	11.11
BezP	0.15	0.13	0.18	0.05	22.22	1.47	2.26	7.70	0.47	100.00
HeP	ND	ND	ND	ND	ND	0.06	0.06	0.06	0.06	11.11
Sum parabens	3.51	9.21	26.42	0.05	50.00	2.47	9.28	61.98	0.47	100.00
4-HB	4053.86	5806.15	18477.18	477.37	88.89	1097.94	4469.87	19731.91	186.25	100.00
3,4-DHB	35982.26	55243.24	152764.77	6634.92	44.44	2109.99	4055.29	10321.13	272.72	77.78
Vanillic	1021.12	1484.63	6463.71	57.64	88.89	856.34	2130.44	7238.34	89.08	88.89
OH-EtP	3.31	4.08	14.50	0.09	50.00	ND	ND	ND	ND	ND
Sum derivates	11631.76	32858.73	161299.27	134.39	94.44	3153.40	9517.71	34480.90	276.29	100.00
TCC	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sum all	11655.02	32865.76	161315.93	134.39	94.44	3154.69	9526.99	34483.39	276.76	100.00

∑parabens: MeP, EtP, PrP, BuP, BezP, HeP, ∑ derivates: 4-HB, 3,4-DHB, Vanillic, *DR*: Detection rate, ND: Not detected

4.3.1 Occurrence of target analytes in grain

In the grain category parabens were detected in 6 out of 7 samples, and the estimated median concentration was 1.46 ng/g. The maximum single concentration was obtained by MeP (35.70 ng/g) and the minimum concentration by BezP (0.06 ng/g). The highest paraben concentration (43.06 ng/g) was obtained by a sample containing MeP, EtP, and BezP. Liao et al. (2013a) and Liao et al. (2013b) reported parabens to be present in all of the samples in the grain category, with MeP accounting for the greatest concentration and greatest detection rate. In the present study BezP had the highest detection rate, while EtP showed the highest median concentration. The estimated median and mean concentration of EtP (15.23 ng/g) was higher than the median and mean values reported by Liao et al. (2013a), Liao et al. (2013b), Azzous et al. (2020) and Kimura (2019) in this category, while a higher mean value was reported by Maher et al. (2020). However, EtP was only found in 2 samples, making the detection rate lower than the reported detection rate of Liao et al. (2013a) and to Liao et al. (2013b) studies (85% and 66.7%, respectively). 4-HB and Vanillic showed 100% detection rate in the grain category. The highest concentration of the derivates was obtained by 3,4-DHB, that was found in 3 of the samples. Natural presence of these paraben derivates have been reported in some grain products like wheat and oats (Tomás-Barberán et al., 2000). OH-EtP was only found in one of the samples with an estimated concentration of 0.08 ng/g.

In the study performed by Azzouz et al. (2020), pure grain/ cereal products were analyzed. None of the products exceeded paraben concentrations over 0.45 ng/g (450 µg/g). In the present study the grain category included samples in the range from porridge with fruit flavor to dinner products with grain (e.g. whole grain pasta), meat and vegetables. It is therefore conceivable that the paraben concentrations were not fully correlated with the grain content in these products. It is interestingly to note that the sample that contained the highest sum of parabens was a multi-component dinner product, while the sample that did not contain any parabens was a porridge sample. This might suggest that the origin of parabens detected in the grain category did not derive from the grain alone in all of the samples.

4.3.2 Occurrence of target analytes in Dairy

The samples in the dairy category consisted of infant formula, both powder and ready-to-drink products. No parabens were found in this category, and the only concentrations obtained was of 4-HB. 4-HB was found in all of the samples, but the concentrations were the lowest reported concentrations in the entire data set. The dairy category was the only category that did not contain products with ingredients derived from grain, fruit or vegetables, where the natural presence of Vanillic, 3,4-DHB and 4-HB have been reported (Tomás-Barberán et al., 2000). This might explain the absence of Vanillic and 3,4-DHB and the low concentration of 4-HB, compared to the other categories.

All though infant formula is included in the dairy category in the studies of Liao et al. (2013a), Liao et al. (2013b) and Maher et al. (2020), their dairy category also included yoghurt, milk, cheese and other dairy products. The reported median and mean values show the total content of the category, and not the infant formulas alone. However, all of the above studies reported paraben content in their dairy category, with the highest average concentration of MeP (Liao et al., 2013a, Liao et al., 2013b, Maher et al., 2020). These findings might indicate that the estimated concentrations obtained in infant formula are not representative for the entire dairy category.

4.3.3 Occurrence of target analytes in cookies and snacks

All of the parabens were detected in the cookie and snack category. Only 1 out of 12 samples did not contain any parabens. The detection rates were ranging from BezP>MeP=EtP>BuP=PrP=HeP. The maximum single concentration was of MeP (71.27 ng/g), and this was the highest concentration reported in the entire data set. The concentration exceeded the highest point in the calibration curve, which causes greater uncertainty. The minimum single concentration was of HeP (0.05 ng/g). This was one out of two categories where PrP was reported. For the sum of parabens, the median concentration in this category was the lowest obtained (0.77 ng/g) (despite dairy that did not detect any parabens). The estimated mean value (8.23 ng/g) in the present study were lower than found in other studies in the cookie category (Liao et al., 2013a, Maher et al., 2020).

Vanillic and 4-HB were found in all of the samples. 3,4-DHB obtained the highest median concentration, and was detected in 8 of the 12 samples. OH-EtP was found in 5 samples, with a median concentration of 0.3 ng/g. The cookies and snack category was the only category where one of the samples showed concentration of TCC.

4.3.4 Occurrence of target analytes in fruits

Parabens were found in half of the samples in the fruit category, with a median concentration of 3.51 ng/g. The maximum single concentration was of MeP (24.74 ng/g), and the minimum single concentration was of BezP and BuP (0.05 ng/g). The highest obtained concentration for the sum of parabens was 26.42 ng/g in a sample were MeP, EtP and BezP was detected. EtP, and its novel metabolite OH-EtP, showed the highest detection rate in the fruit category, 44.44% and 50%, respectively. A correlation between their presence have been proven in detection in urine, but their correlation in consumer products, has not been established yet (Wang and Kannan, 2013). A simple correlation analysis in Excel was also performed to see if there was a positive correlation in the fruit category, but no correlation was observed. OH-EtP had higher median, mean and max concentration than EtP. This might implicate that the presence of OH-EtP has a natural origin in food, or that it can arise from other compounds.

4-HB, 3,4-DHB and Vanillic obtained the highest concentration in the fruit category, with detection rates of 88.89%, 44.44% and 88.89%, respectively. Their estimated median and mean concentrations was the highest obtained values in the entire data set. The variation of these derivates was also the highest obtained, as illustrated in Figure G.1 (Appendix G). These results might be related to the natural presence of these compounds in various types of fruits (Tomás-Barberán et al., 2000, Boberg et al., 2010). Accordingly, natural variations of these compounds have been reported among different fruit species and within the same species (Tomás-Barberán et al., 2000). High obtained concentration of 4-HB and 3,4-DHB in the fruit category are in agreement of those obtained by Nobile et al. (2020) and Kimura (2019). Thus, neither of them reported median concentrations as high as the estimated median concentrations reported in the present study.

4.3.5 Occurrence of target analytes in vegetables.

For the sum of parabens the median concentration was 2.47 ng/g, and parabens were detected in all of the samples. The single maximum value was obtained by MeP (61.10 ng/g), while HeP obtained the single minimum concentration (0.06 ng/g). Along with the cookies and snack category, the vegetable category also obtained an estimated concentration of PrP. Higher concentrations of MeP, EtP and PrP (0.1-5 µg/g) were obtained by Zhou et al. (2015) in the analysis of different vegetables. For the sum of parabens, the maximum concentration (61.98 ng/g) was obtained by a sample containing MeP and BezP. All of the samples in the vegetable category showed the presence of BezP, and the highest median and mean values were obtained for BezP in this category. There was similarities between the high detection rates in this study and those described by other studies listed in Table 2.1 (Liao et al., 2013b, Liao et al., 2013a, Kimura et al., 2019). Among the different categories, the highest detection rate of BezP was found to be the 2nd greatest in the vegetable category in the study performed by Liao et al. (2013a) (51%), the 3rd greatest in the study performed by Liao et al. (2013b) (46.9%), and the 2nd greatest in the study performed by Kimura (2019) (75%). Thus, none of them reported a detection frequency of 100% as in the present study. The estimated mean and median values in the present study were higher than the reported by all of the above studies. It is difficult to explain these results, due to the fact that BezP should not be used as a preservative in food (EFSA, 2004a). However, caution must be applied both due to the fact that the sample size in the vegetable category is scare and due to the semi-quantification.

The derivates 4-HB, 3,4-DHB and Vanillic showed high detection frequencies in the vegetable category (100%, 77.78% and 88.89%, respectively). The highest concentration was obtained by 3,4-DHB. The vegetable category was the only one where OH-EtP was not detected. As EtP was found in one of the samples in the vegetable samples, this might support that a correlation in food products is not as described in biological samples.

4.4 Correlation and PCA

Due to high detection rates of the derivates 4-HB and Vanillic, these compounds was investigated further. Variables evaluated in the correlation and PCA analysis was the content of Vanillic, 4-HB, ∑parabens (Sum_6_Parabens) and ∑parabens and derivates (Sum_parabens). Sum_6_Parabens and Sum_6_Parabens includes the same compounds as

described in section 4.3.. A positive correlation was obtained by 4-HB and Sum_6_Parabens, and between 4-HB and Sum_parabens, thus, neither of them were significant (p=0.05) This might confirm that the content of 4-HB in food is due to a natural origin rather than from the contribution from parabens alone (Boberg et al., 2010). A significant positive correlation, on the other hand, was obtained between Vanillic and 4-HB ($r=0.61$, $p \leq 0.05$). These findings might confirm the conversion from 4-HB to Vanillic by methoxylation of in the 3´position in the aromatic ring, that is known to occur through processing (Tomás-Barberán et al., 2000). Still, the addition of Vanillic for flavouring purposes, and the natural presence of 4-HB and Vanillic, makes it hard to explain the exact origin of the correlation. Accordingly neither of 4-HB or Vanillic can evidently be related to the exposure of parabens due to their natural presence in some foods (Tomás-Barberán et al., 2000, Boberg et al., 2010). A positive, significant correlation between Vanillic and Sum_parabens was also obtained ($r=0.56$, $p \leq 0.05$). This might suggest that Vanillic concentrations have come from parabens or derivates (4-HB, 3,4-DHB, OH-EtP). Figure 4.3 illustrates the Correlation plot (corrplot) obtained in the correlation analysis.

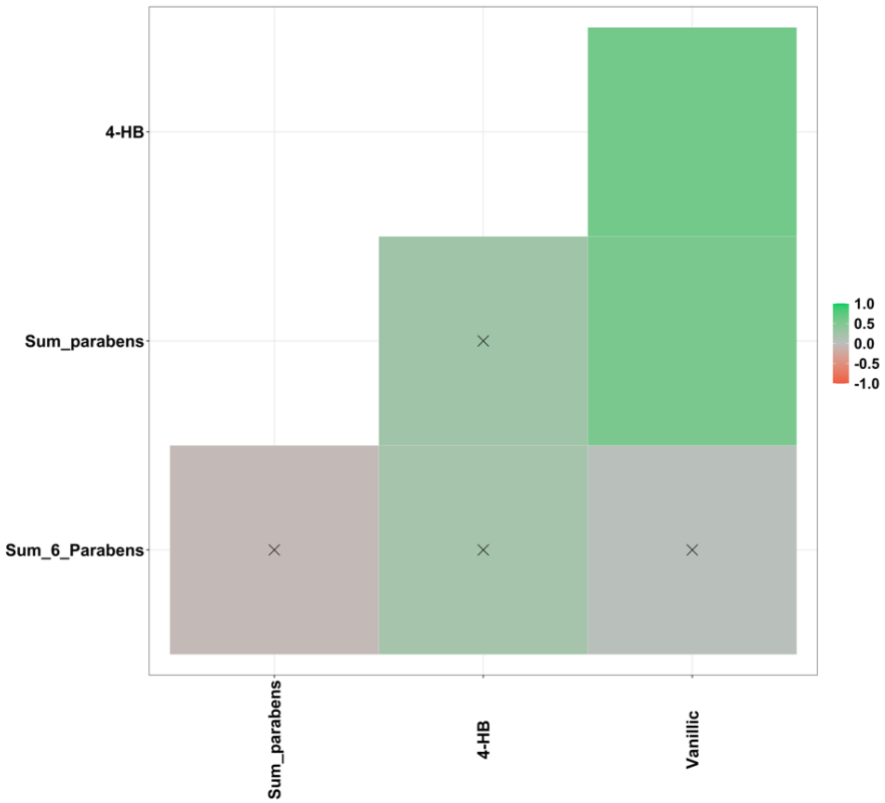

Figure 4.3: Corrplot of the ∑parabens, ∑parabens and derivates, 4-HB and Vanillic. Crosses in the boxes implicate that no significant correlation was obtained.

A PCA was performed to illustrate the correlation and variations between the different food categories and the Sum_parabens, Sum_6_parabens, Vanillic and 4-HB. The obtained biplot is found in Figure 4.4. The category “grain” and “Grain/cereal” represents the category “grain”. The highest sum of parabens are obtained in the cookies and snacks category. Also the correlation between 4-HB and Vanillic, and Vanillic and the Sum All are illustrated in this biplot, as their presence in the biplot are close.

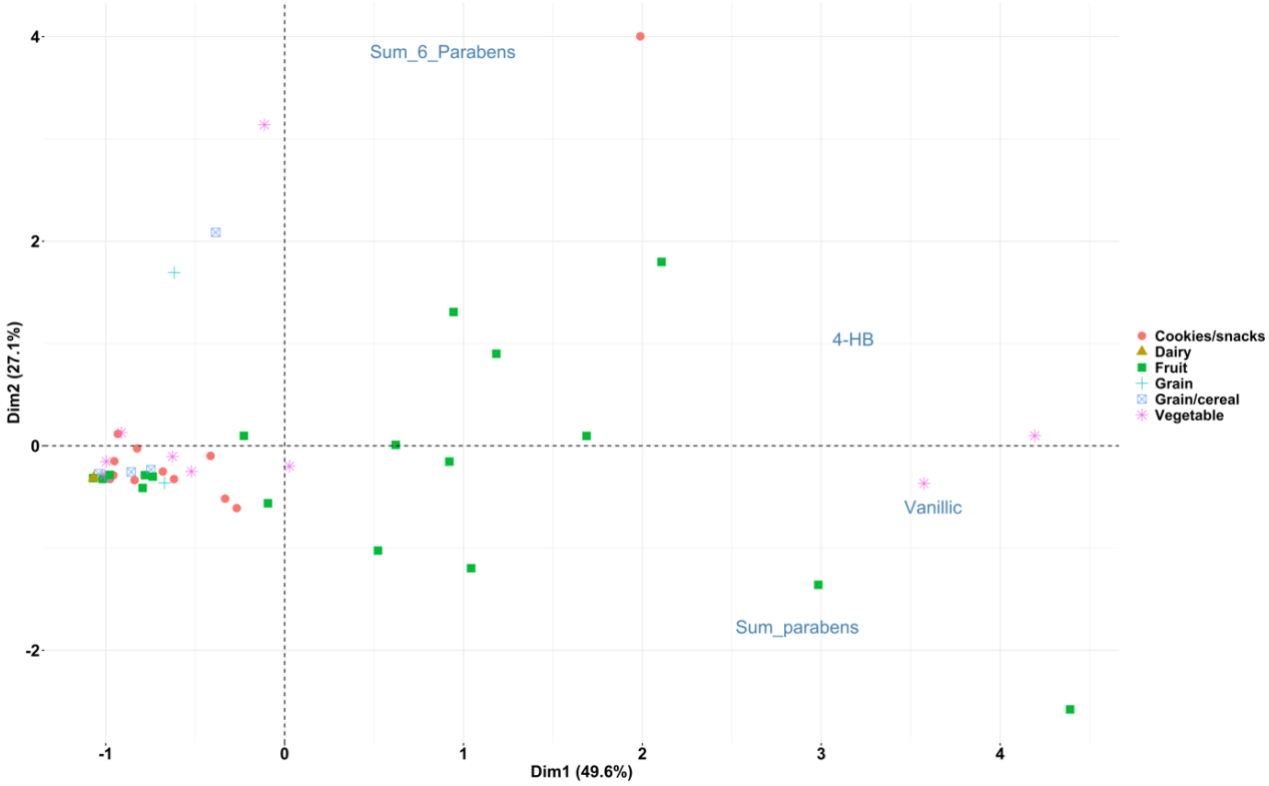

Figure 4.4: Biplot obtained from the correlation between the ∑ parabens, ∑ parabens and derivates, 4-HB and Vanillic.

4.5 Estimated daily intake

An estimation of the daily intake (EDI) of parabens from baby foodstuff was performed. This was based on the Norwegian Health Authorities guidelines for the transition from liquid to solid food for infants (>6 months), and the portion size information obtained from some of the samples (Helsenorge, 2018). The calculation was based on equation 2.1. The EDI was

based on an assumption of babies eating 4 meals, and calculated by taking the mean values of the baby food times the estimated food ingestion.

The estimation was set to be 3892 ng/g/kg bw. The calculated estimation is higher than the calculated estimation for infants and toddlers reported by Liao et al. (2013b). Thus, this is only an estimation, and there are several errors that can be taken into consideration. First of all, this study did not include a variety of the food categories that children can be exposed to in a varied diet, e.g. eggs, fat, bean and fish products (Liao et al., 2013b). Secondly, the obtained concentration was estimated in dry weight. After freeze drying with excessive water loss, the samples lose weight. In that regard, the parabens per g fresh weight estimation could be lower. A major uncertainty also follows as the estimation is based on semi-quantified concentrations.

A calculation to evaluate the amount of food a child would have to ingest to exceed the ADI of MeP and EtP was performed (Appendix H). This was estimated to be several folds higher than the food consumption for an adult, for all the investigated food categories. Thus, children are more vulnerable than adults because they are in their growth stages, and might be more vulnerable for EDCs. In addition, the findings in this study suggests exposure to the parabens with longer chain lengths, where there is no set limits of intake. As the endocrine disruptive potential is estimated to be higher for parabens with longer chain lengths, it is worrying that BezP was detected in over half of the samples. Food might not be the only route of exposure of parabens for children, as it is found in baby teethers, personal care products and house dust (Asimakopoulos et al., 2016a, Ramirez et al., 2011). Children might be exposed to parabens from various sources. It thereby reinforce further investigation to map the exposure, and to understand the possible negative health effects to a greater extent.

4.6 Occurrence of parabens in foodstuffs

So far, little attention have been paid to the potential for parabens to end up in food unintentionally. Search in literature revealed few studies in witch discussed the source of parabens to be other than intentionally addition. The results in this study, did not explain the occurrence of parabens in foodstuffs. Nevertheless the potential for parabens to end up in food unintentionally, must be considered due to the fact that the addition of preservatives in

foodstuff for small children is prohibited in Norway (Mattilsynet, 2017). A source that have been considered to be the contamination source is the package material. Thus, Maher et al. (2020), Liao et al (2013b) and Liao et al. (2013a) investigated the correlation between the paraben content and the package material, but neither of them found an association between them. Due to the lock-down of the lab, and unavailable samples, there was not performed a correlation analysis between package material and paraben content in the present study. Further research should be done to investigate paraben content in food samples to derive from external sources such as the package material. Despite the fact that the present study did not analyze any fish containing products, the studies performed by Ramaswamy et al. (2011) and Djatmika et al. (2016), where detectable amount of parabens were present in wild caught fish bought on fish markets, provide an understanding that parabens in foodstuffs might have other sources than intentionally addition. Parabens have been found in measurable concentrations in several trophic levels in the marine biota (Xue et al., 2015, Xue et al., 2017). The detection of parabens in the range from house dust to mammals living in remote areas, provides an understanding of the scope of exposure and the widespread occurrence of parabens (Xue et al., 2015, Ramirez et al., 2011). The bioaccumulation potential needs further research, not only for marine organisms, but as a potential source of parabens in food.

5 Conclusions

The aim of the present study was to investigate the presence of parabens, their derivates and triclocarban in baby foodstuffs in Norway. The target analytes were semi-quantified due to unsatisfactory reproducibility of the quality assurance samples, and some poor recoveries. However, the obtained results suggested that small children are exposed to parabens through consumption of baby foodstuffs, as parabens were detected over LOD in 35 of the 49 samples. All of the different target analytes were detected in greater or less extent. The highest estimated median and mean values was obtained by methyl paraben (MeP) and ethyl paraben (EtP). The highest detection rate was obtained by benzyl paraben (BezP), that were found in 51.02% of the samples. The other parabens (PrP, BuP, HeP) were detected with low detection rates and in low concentrations. Part of the aim was to look at differences and similarities among the different food categories. Parabens were detected in all of the categories, except the dairy category that consisted of infant formula samples. The highest detection rate of parabens was found in the vegetable category, followed by cookies and snacks, grain and fruit, respectively. The dairy category only obtained detectable concentrations of the derivate 4-HB, and the concentration estimates was the lowest in the entire data set. The grain, cookies and snacks, fruit and vegetable category obtained high concentration estimates of the paraben derivates 4-HB, 3,4-DHB and Vanillic acid. The derivate and novel metabolite of EtP, OH-EtP, was detected in 30.61% of the samples, with the highest detection rate obtained in the fruit category. A correlation between the content of OH-EtP and EtP was not obtained in the present study, as in studies concerning these compounds in biological matrices. A correlation analysis revealed a significant correlation between Vanillic and 4-HB, and between Vanillic and the Sum of parabens and derivates (MeP, EtP, PrP, BuP, BezP, HeP, OH-EtP, 3,4-DHB and 4-HB). TCC was found in only 1 out of 49 samples.

The findings in the present study enhances the need to map the concentration of parabens in foodstuffs. The use of parabens is limited to certain types of food, and prohibited in food meant for children in Norway. There is, therefore, a need for studies investigating the

quantities of parabens in different foodstuffs, as well as investigations concerning the possible origin of how parabens can to end up in foodstuffs.

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Appendices

Appendix A - sample list

Table A. 1: The baby food samples included in the analysis, brand, type of food, flavor, category and the dry weight. The highlighted text in the category is the main ingredient.

Sample number	Brand	Type of food	Flavor	Category	Weight (g dw)
1	Semper	Whole grain porridge	With fruit and yoghurt	Grain	0.5413
2	Nestlé	Oatmeal porridge	Mango and banana	Grain	1.0154
3	Änglemark	Whole grain porridge	Natural, ecologic	Grain	0.9669
4	NAN	Brest milk replacement/ formula feed		Dairy product/	1.0377
5	HIPP (?)	Crackers	Whole grain crackers	Cookies and snacks	1.1614
6	Änglemark	Corn snacks	Ecologic	Cookies and snacks	1.0812
7	Mini MI	Snacks	Corn and millet (hirse)	Cookies and snacks	0.9233
8	Ella´s kitchen	Maize puffs	Strawberry and banana, ecologic	Cookies and snacks	1.0636
9	Änglemark	Corn snacks	Carrot and apple	Cookies and snacks	1.0442
10	Organix	Corn puffs	Carrot, 7+ months	Cookies and snacks	1.0720
11	Organix	“Goodies”	Raspberry and apple fruits	Cookies and snacks	0.8530

12	Kiddylicious	Snacks	“fruktsnøre” strawberry taste	Cookies and snacks	1.109
13	Kiddylicious	Smoothie bites	Strawberry and banana	Cookies and snacks	1.006
14	Nestlé	Fruit bar	Fruit and whole grain (oatmeal, blueberry and blackcurrants)	Cookies and snacks	0.9726
15	Nestlé	Fruit bar	Grape, oatmeal and apple	Cookies and snacks	1.3632
16	Ella´s kitchen	Fruit and oatbar	Strawberry and apple	Cookies and snacks	1.3821
17	Nestlé	Porrige/ “välling”	Corn	Grain	0.9450
18	Nestlé	Porrige/ “välling”	Mild oat “good night”	Grain	0.9739
19	Nestlé	My fruit	Apple and pear	Fruit	1.0030
20	Nestlé	My fruit	Apple, blueberry and banana	Fruit	0.9919
21	Nestlé	My fruit	Apple and apricot	Fruit	0.9683
22	Nestlé	My fruit	Apple, pear, raspberry and blueberry	Fruit	0.9962
23	Nestlé	My fruit	Apple and mango	Fruit	0.9822

24	Nestlé	Naturnes babys organic	Apple, and rosehip	Fruit	1.0831
25	Nestlé	Naturnes baby´s organic	Pear	Fruit	1.0687
26	Nestlé	Naturnes baby´s organic	Apple, banana, blackberry and blueberry	Fruit	1.0275
27	Nestlé	Junior fruit smoothie	Banana and apple	Fruit	1.0209
28	Ella´s kitchen	Organic	Blueberry, apple, banana and vanilla	Fruit	0.9867
29	Ella´s kitchen	Ecologic	Red berries	Fruit	1.0939
30	Änglamark	Ecologic smoothie	Pear, banana and strawberry	Fruit	1.0118
31	Änglamark	Ecologic smoothie	Prune	Fruit	0.9600
32	Änglamark	Ecologic smoothie	Banana, blackberry and raspberry	Fruit	1.0773
33	Änglamark	“Kräms” ecologic smoothie	Banana, strawberry and yoghurt	**Fruit**/ dairy product	1.1064
34	Hipp	Øko “Hippis”	Apple, banana and strawberry	Fruit	0.9822
35	Semper	Dinner	Pasta Bolognese	**Vegetable**/ meat	1.0071
36	Semper	Dinner	Pasta and ham	**Vegetable**/ grain	0.4724

37	Semper	Dinner	Rice, chicken and vegetables	**Vegetable**/ meat	1.0236
38	Änglamark	“Matis” dinner	Sweet potato, chicken and vegetables	**Vegetable**/ meat	1.0163
39	Änglamark	“Matis” dinner	Couscous and vegetables	**Vegetables** > grain	0.9724
40	Nan	Organic	Milk	Dairy product	1.0369
41	Nan	Pro	Milk	Dairy product	1.0678
42	Nestlé	Junior	Spaghetti Bolognese whole grain	**Grain** > vegetables	1.0020
43	Nestlé	Eco	Whole grain pasta with vegetables	**Vegetables**/ grain	1.0301
44	Nestlé		Moms chicken balls with pasta	**Grain/** meat	1.0222
45	Nestlé	Naturnes	Lasagna whole grain	**Vegetables /** Grain	0.9733
46	Semper		Meat balls in brown gravy	**Vegetables**/ meat	0.9891
47	Hipp	Eco (dessert)	Apple	Fruit	0.5238
48	Nestlé		Carrot and potato	Vegetables	0.4964
49	Nestlé		Fruit salad	Fruit	1.2293

Appendix B – QA and QC samples

Table B. 1: Sample weight and content samples used for QA and QC obtained from experiment 1.

Samples used for QA and QC		
Sample	**Weight (g)**	**Content**
Non-spike sample 1	0.9670	10 ppb IS
Non-spike sample 2	1.0472	10 ppb IS
Non-spike sample 3	0.9190	10 ppb IS
Pre-spike sample 1	0.9517	10 ppb TA and IS
Pre-spike sample 2	1.0351	10 ppb TA and IS
Pre-spike sample 3	1.0494	10 ppb TA and IS
Pre-spike sample 4	1.1820	10 ppb TA and IS
Post-spike sample 1	1.0157	10 ppb TA and IS
Post-spike sample 2	1.1301	10 ppb TA and IS

Appendix C – calibration curves

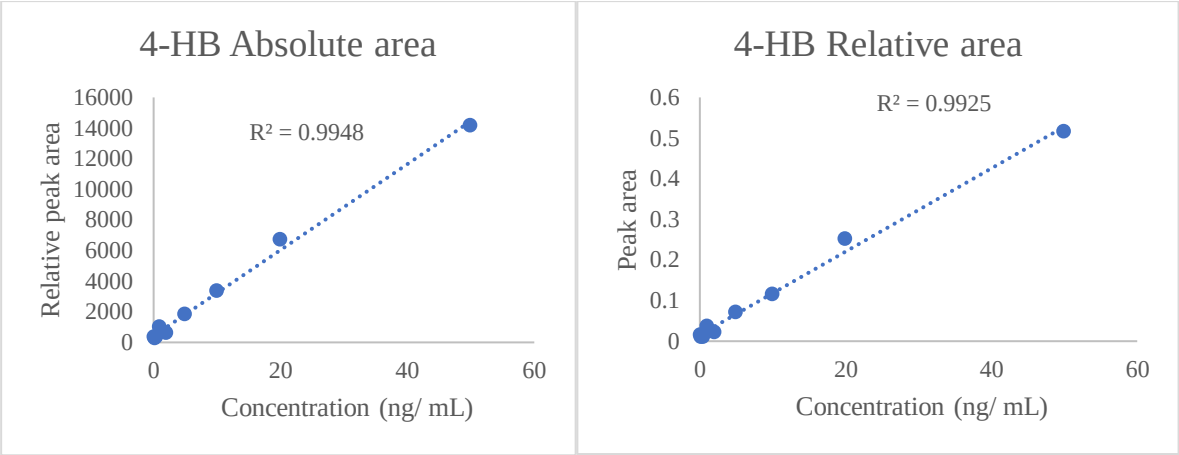


Figure C. 1: The obtained calibration curves based on absolute and relative area for 4-HB with spiked concentrations of TA and TA and IS, respectively

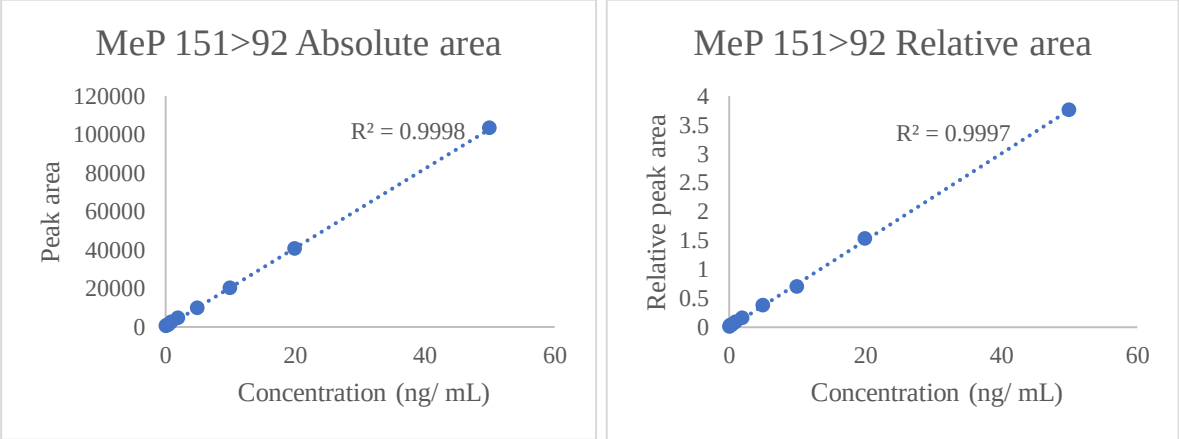


Figure C. 2 The obtained calibration curves based on absolute and relative area for MeP with spiked concentrations of TA and TA and IS, respectively

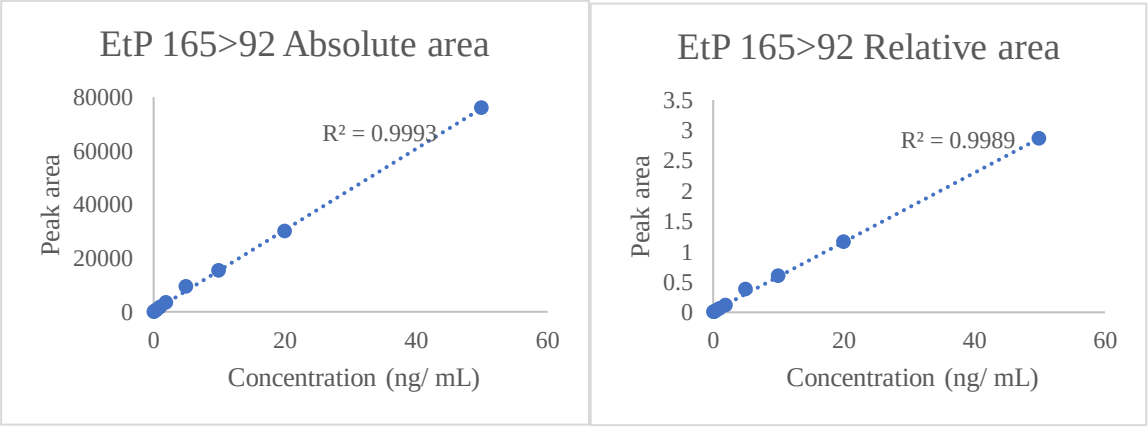


Figure C. 3 The obtained calibration curves based on absolute and relative area for EtP with spiked concentrations of TA and TA and IS, respectively

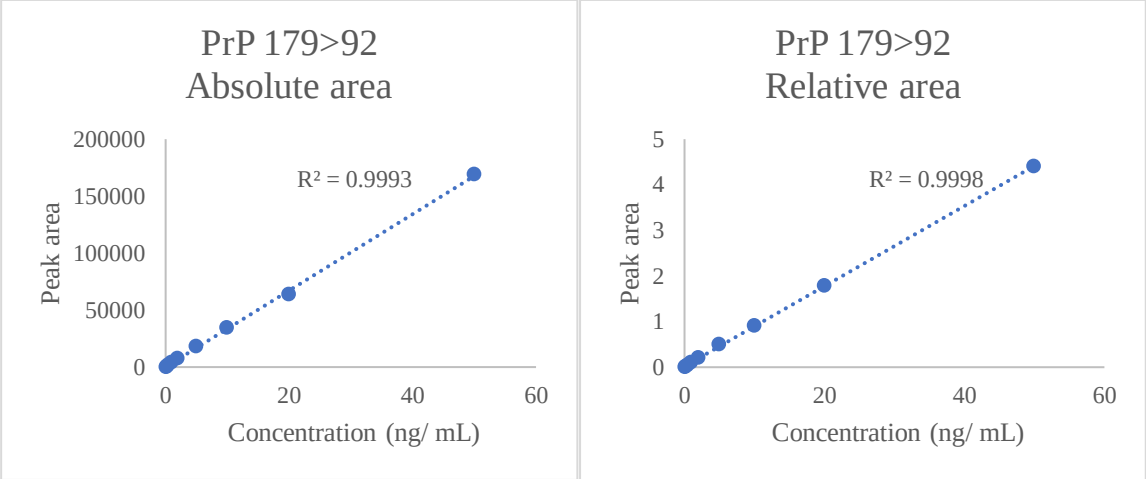


Figure C.4 The obtained calibration curves based on absolute and relative area for PrP with spiked concentrations of TA and TA and IS, respectively

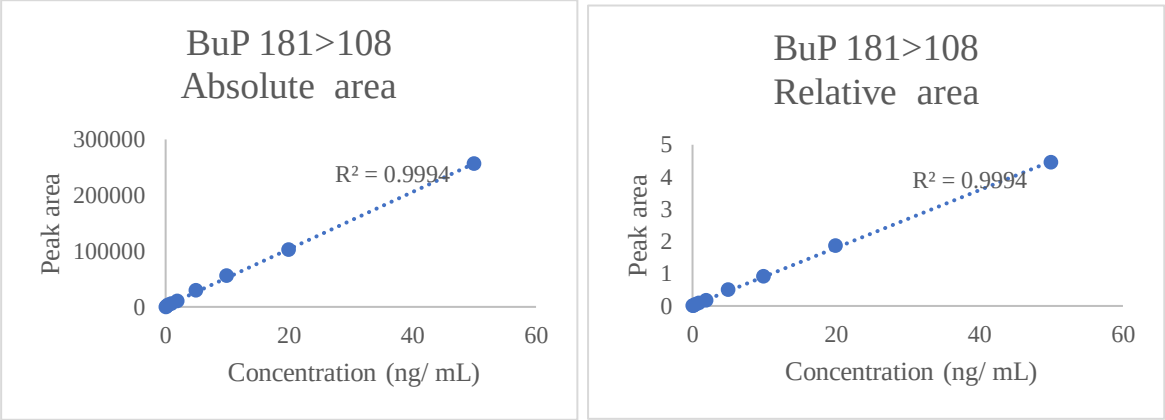


Figure C. 5 The obtained calibration curves based on absolute and relative area for BuP with spiked concentrations of TA and TA and IS, respectively

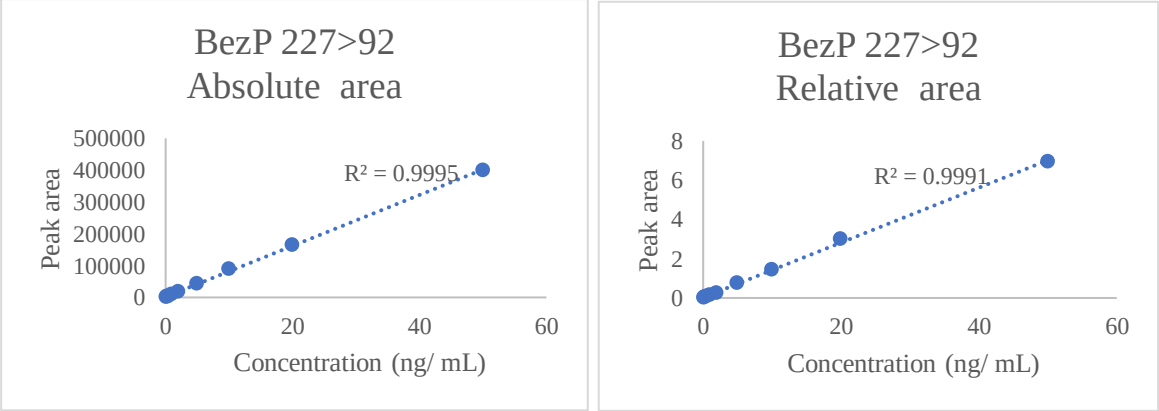
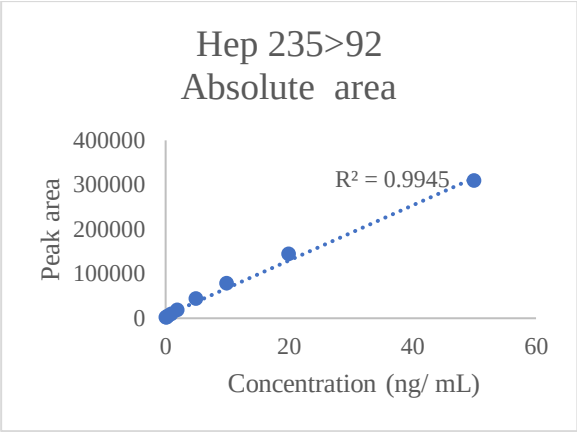


Figure C. 6 The obtained calibration curves based on absolute and relative area for BezP with spiked concentrations of TA and TA and IS, respectively



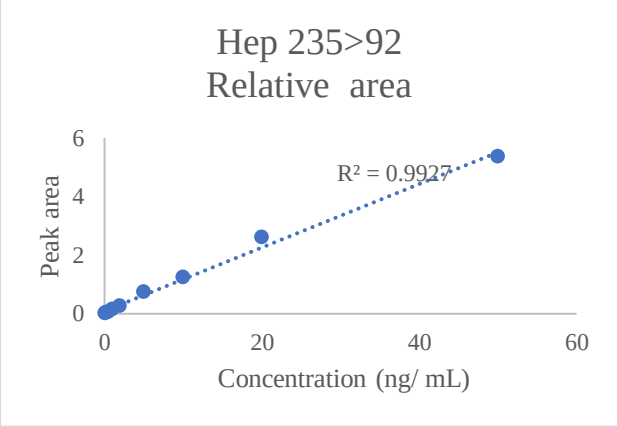
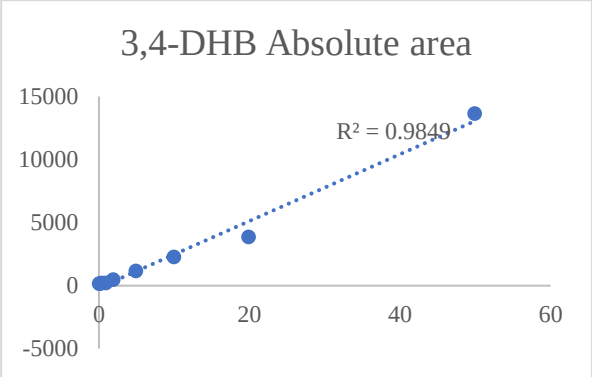


Figure C. 7 The obtained calibration curves based on absolute and relative area for HeP with spiked concentrations of TA and TA and IS, respectively



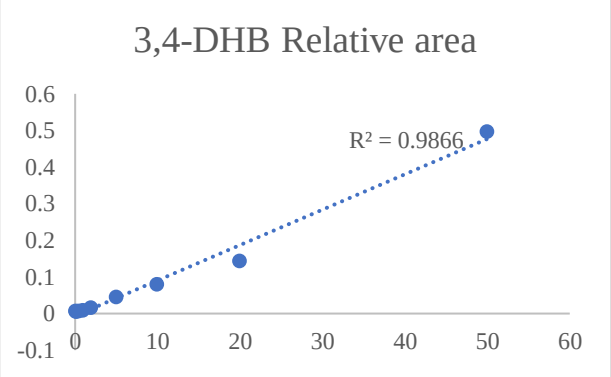
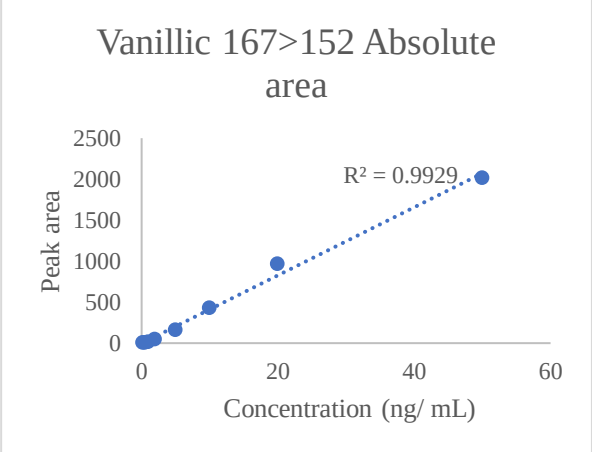


Figure C. 8 The obtained calibration curves based on absolute and relative area for 3,4-DHB with spiked concentrations of TA and TA and IS, respectively



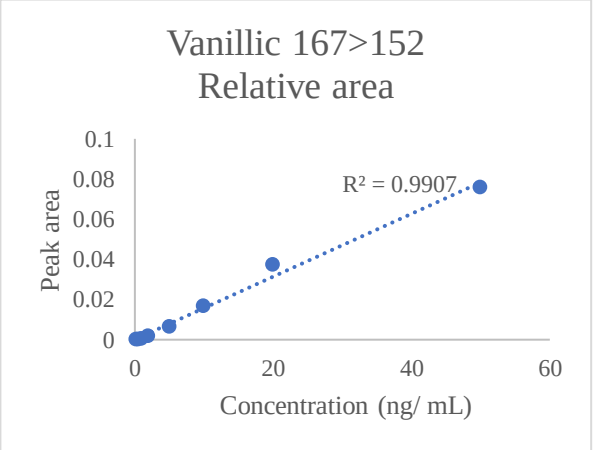
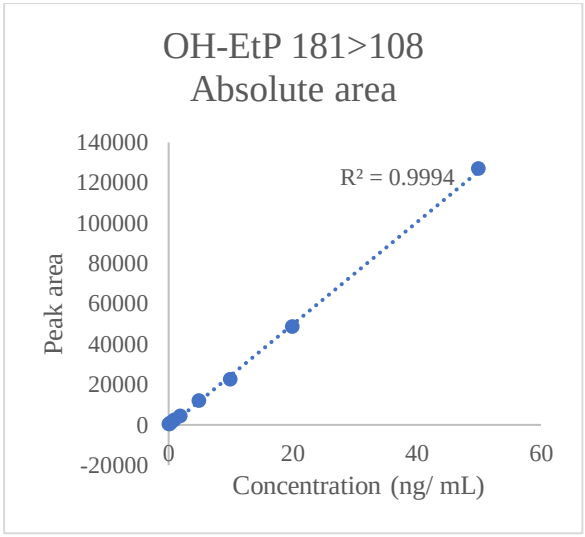


Figure C. 9 The obtained calibration curves based on absolute and relative area for Vanillic with spiked concentrations of TA and TA and IS, respectively



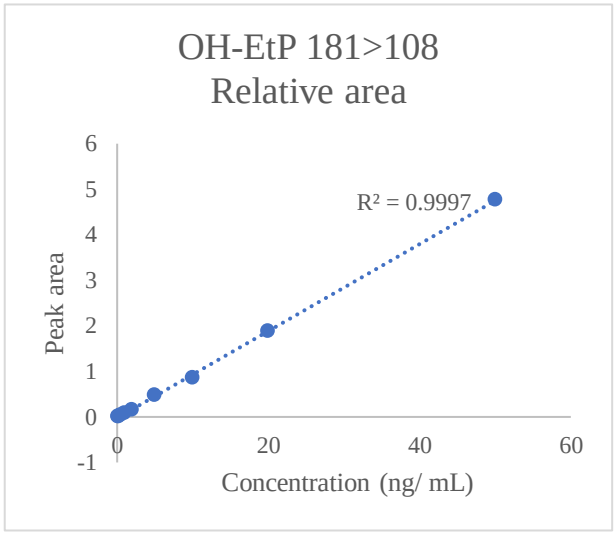
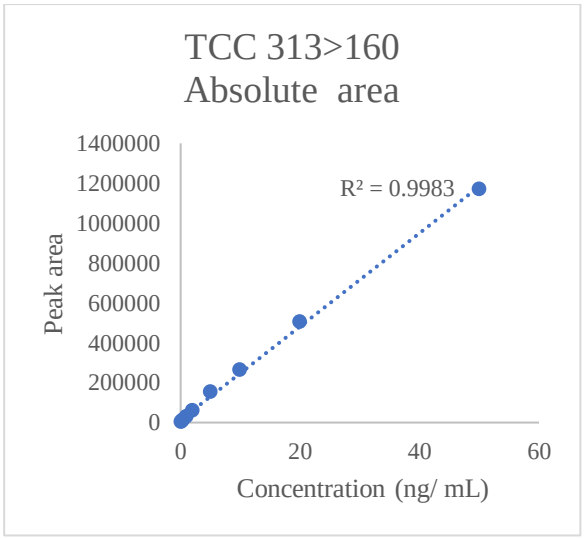


Figure C. 10 The obtained calibration curves based on absolute and relative area for OH-EtP with spiked concentrations of TA and TA and IS, respectively



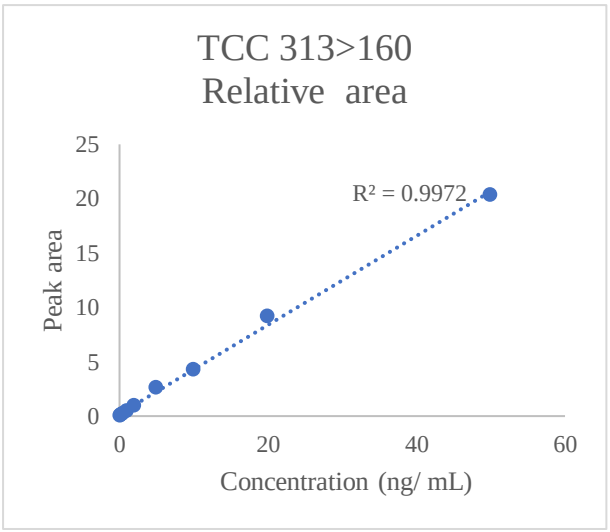


Figure C. 11 The obtained calibration curves based on absolute and relative area for TCC with spiked concentrations of TA and TA and IS, respectively

Appendix D – gradient elution program and chromatograms

Gradient elution program is presented in table D.1. Chromatograms obtained from target analytes (20 ppb) and the internal standards (10ppb) are illustrated in Figures D.1-D.11.

Table D. 1: Gradient elution program used in the analysis of the target analytes. Flow rate was 0.2 µL throughout the entire analysis.

Gradient elution program: Mobile phase composition		
Time	A(%)	B (%)
Initial	1	99
0.4	75	25
0.8	95	5
2.5	95	5
2.55	99	1
3.3	99	1
3.5	1	99
4.0	1	99

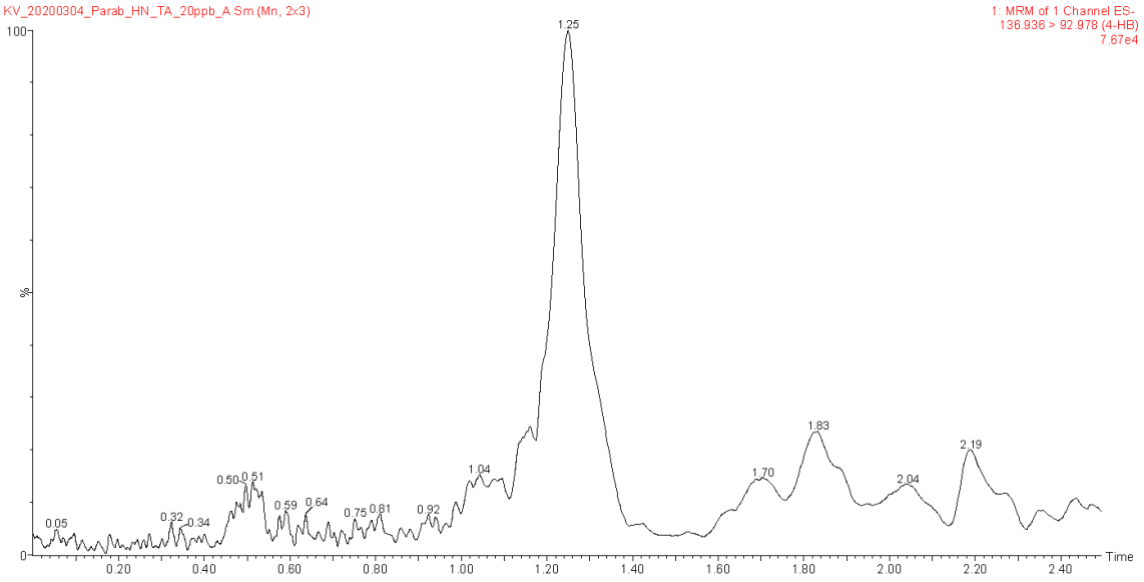


Figure D. 1: MRM chromatogram of 4-HB primary transition

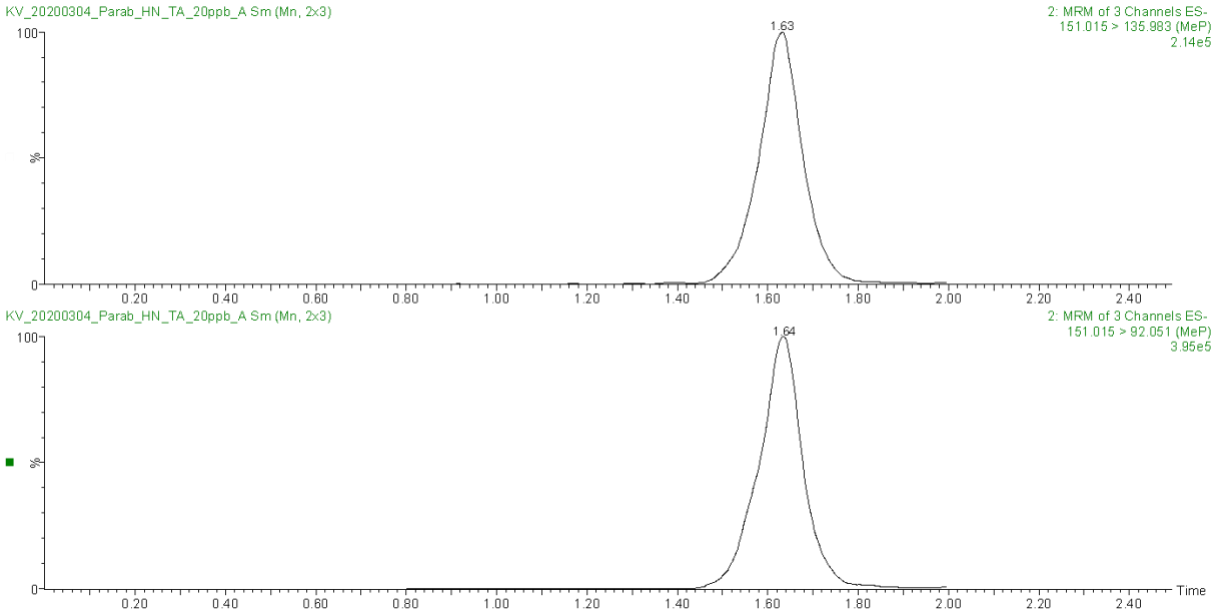


Figure D. 2: MRM chromatogram of MeP primary and secondary transition (151>136 and 151>92)

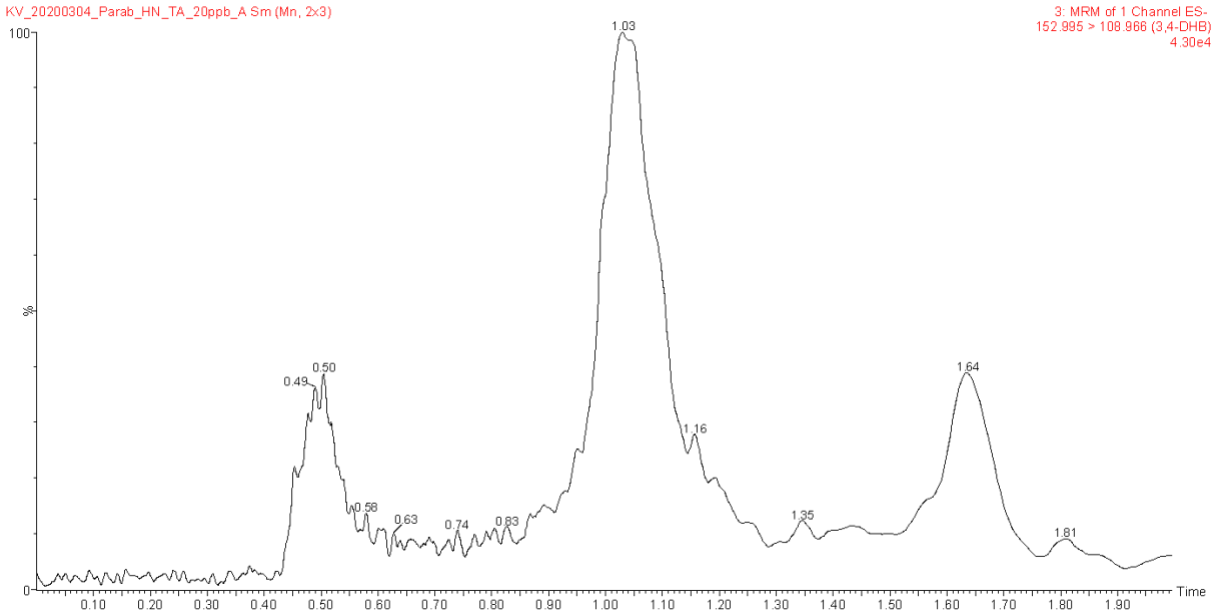


Figure D. 3: MRM chromatogram of 3,4-DHB, primary transition (153>109)

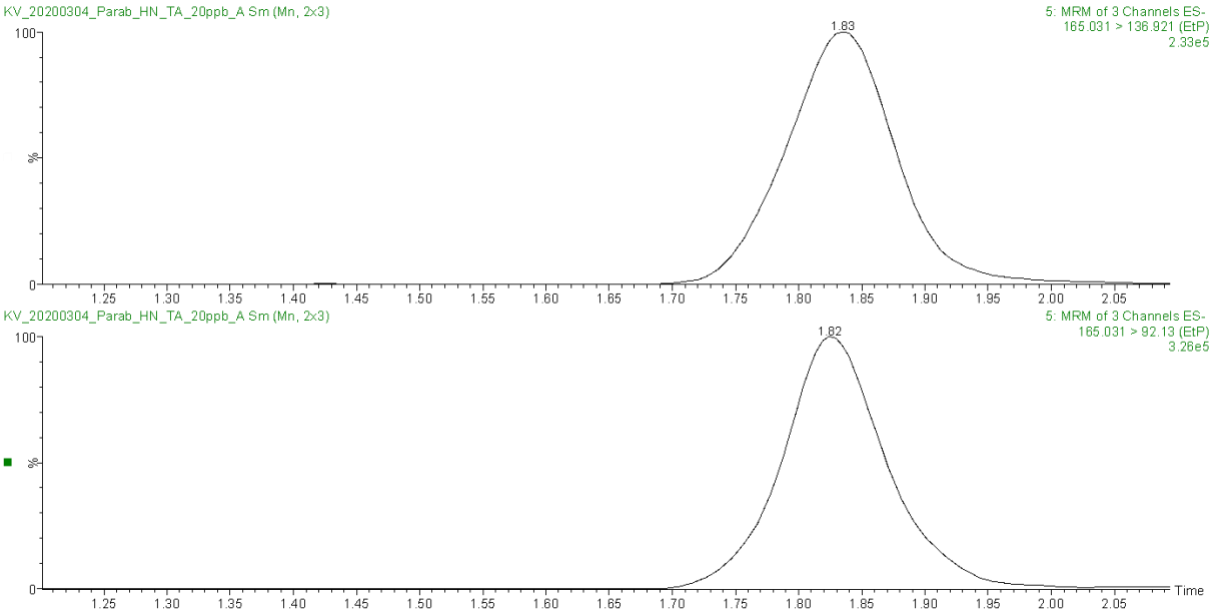


Figure D. 4: MRM chromatogram of EtP primary and secondary transition (165>136 and 165>92)

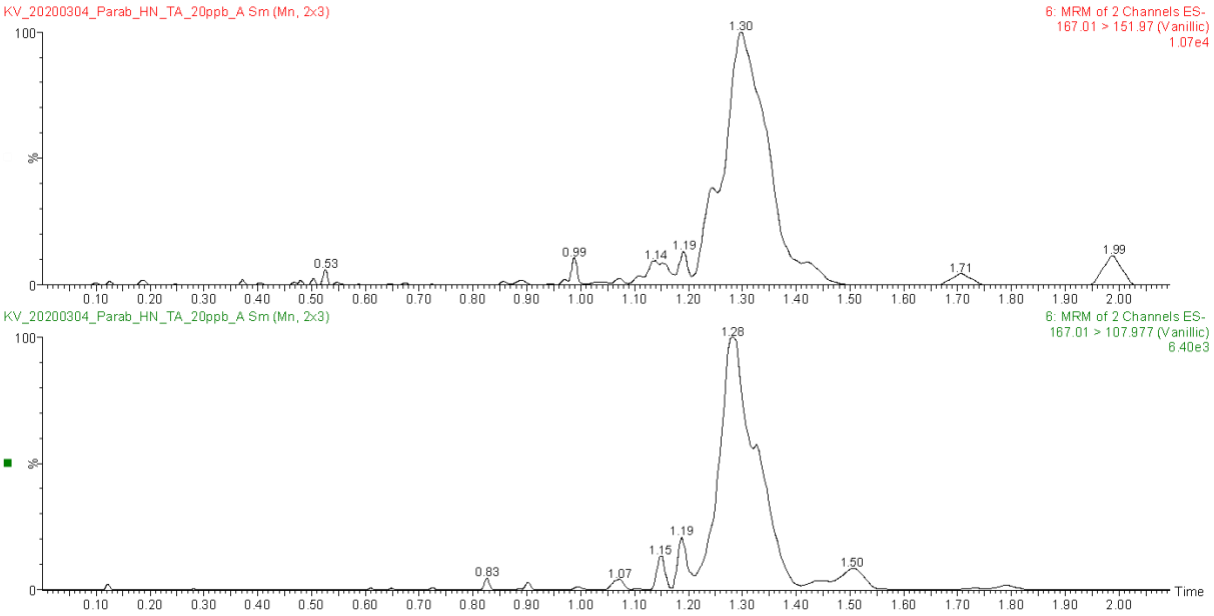


Figure D. 5: MRM chromatogram of Vanillic, primary and secondary transition (167>152 and 167>108)

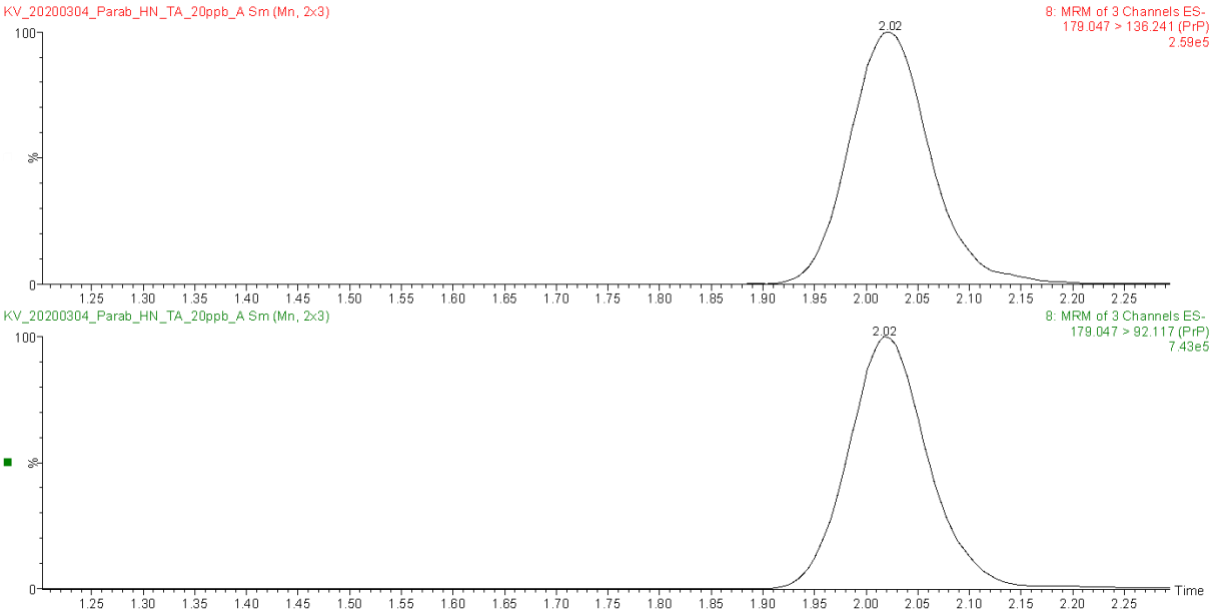


Figure D. 6:MRM Chromatogram of PrP primary and secondary transition (179>136 and 179>92)

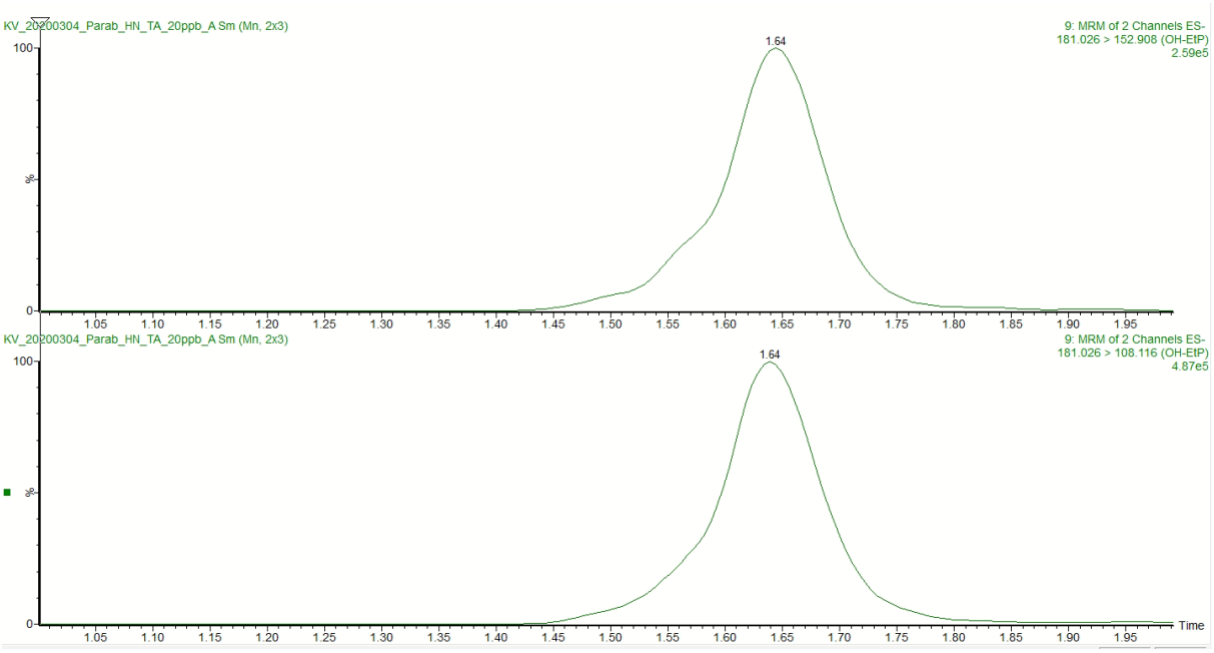


Figure D. 7: MRM Chromatogram of OH-EtP primary and secondary transition (181>152 and 181>108)

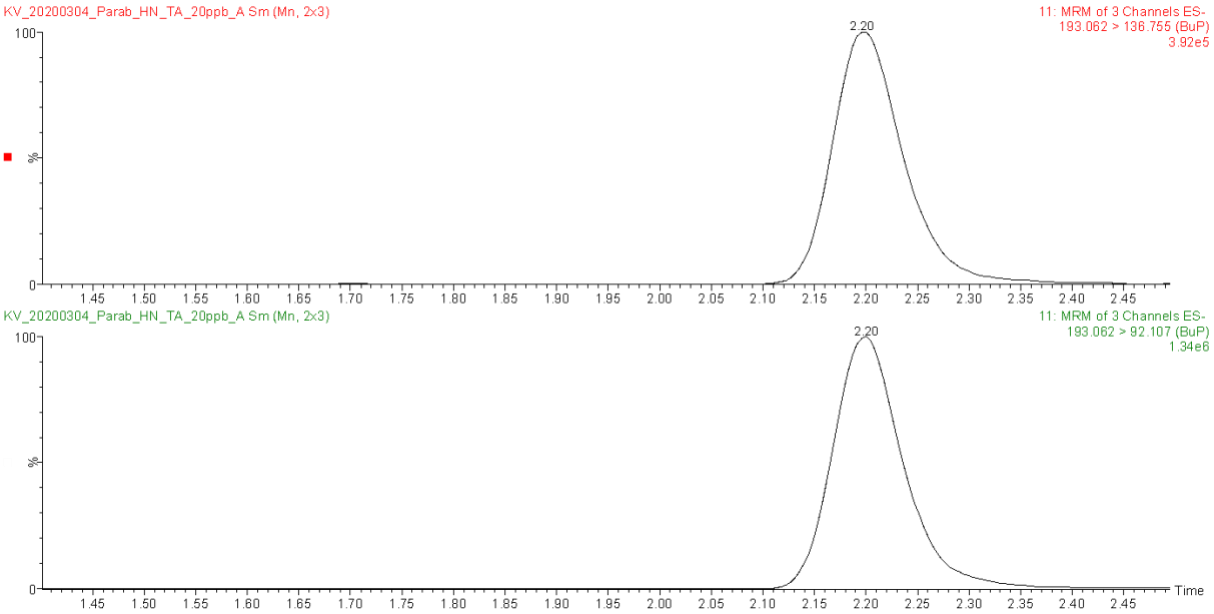


Figure D. 8: MRM Chromatogram of BuP primary and secondary transition (193>136 and 193>92)

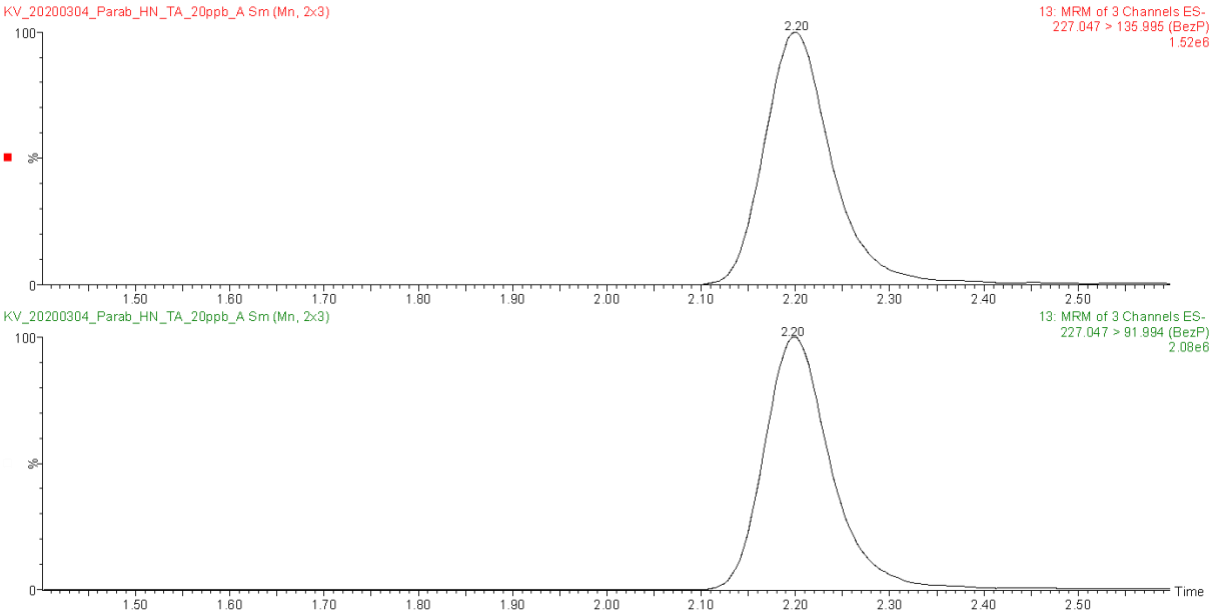


Figure D. 9: MRM chromatogram of BezP, primary and secondary transition (227>136 and 227>92)

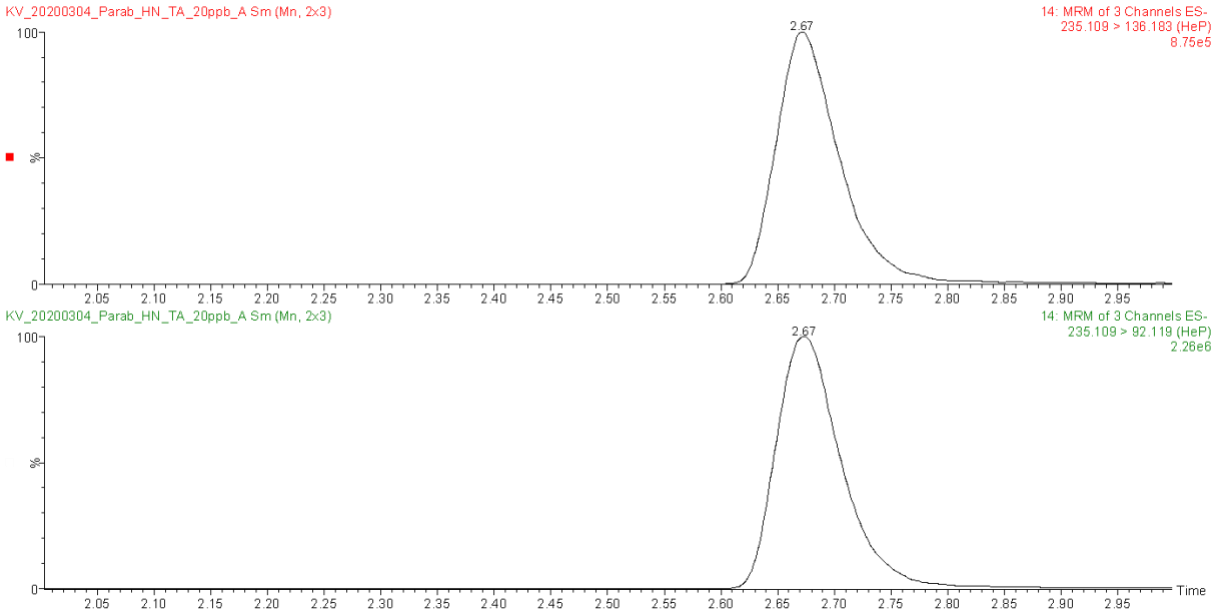


Figure D. 10: MRM chromatogram from HeP, primary and secondary transition (235>136 and 235>92)

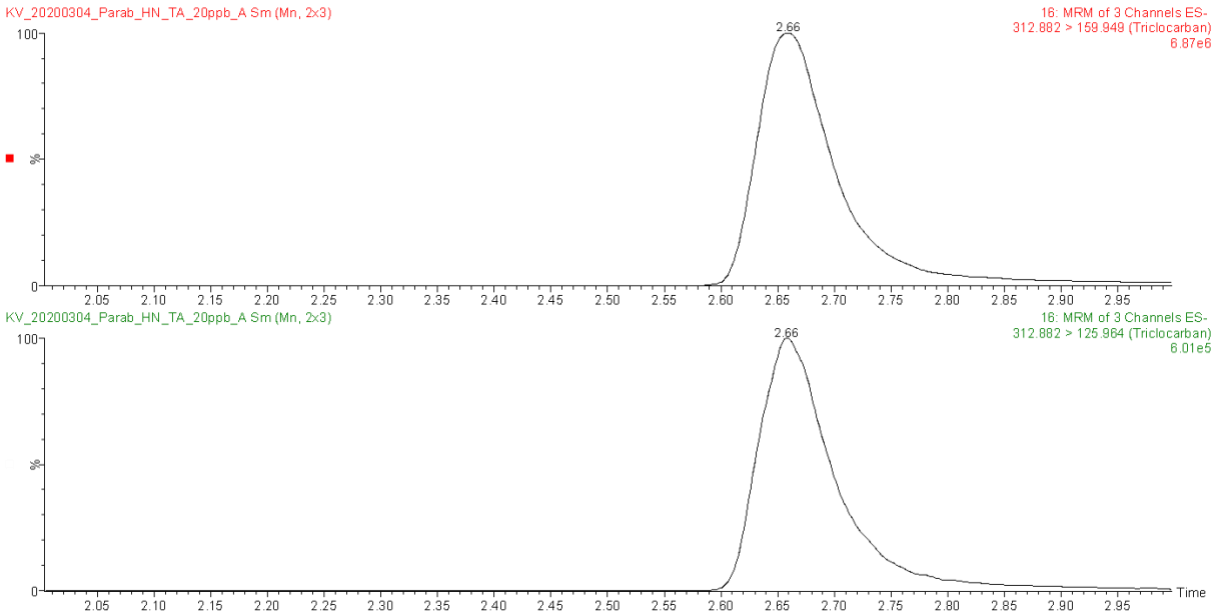


Figure D. 11: MRM Chromatogram from TCC, primary and secondary transition (313>160 and 313>126)

Appendix E – Slopes, TA and associated IS and external standardization

Table E. 1: Slopes used for the determination of concentrations obtained by Kimura (2019)

Slopes for quantification	
Compound	*Slope*
BezP	0.068
HeP	0.13
4-HB	0.012
3,4-DHB	0.0067
Vanillic	0.002
OH-EtP	0.087
TCC	0.33

Table E. 2: Target analyte and associated internal standard used for reference in calculation of RRT and RR

Internal standard used for quantification	
Target analyte (Quantification transition)	*Internal standard (Quantification transition)*
MeP (151>92)	$^{13}C_6$-MeP (157>98)
EtP (165>92)	$^{13}C_6$-EtP (171>98)
PrP (179>92)	$^{13}C_6$-PrP (185>98)
BuP (193>92)	$^{13}C_6$-BuP (199>98)
BezP (227>92)	$^{13}C_6$-BuP (199>98)
HeP (235>92)	$^{13}C_6$-BuP (199>98)
4-HB (137<93)	$^{13}C_6$-MeP (157>98)
3,4-DHB (153>109)	$^{13}C_6$-MeP (157>98)
Vanillic (167>152)	$^{13}C_6$-EtP (171>98)
OH-EtP (181>108)	$^{13}C_6$-EtP (171>98)
TCC (313>160)	$^{13}C_6$-BuP (199>98)

External standardization

For one of the samples, Internal standard addition was forgotten. The concentration in the sample was based on the external standardization (Snyder et al., 2010). It was determined by taking the area of the sample and dividing it by the slope of the calibration curve with absolute area for the respective target analyte (Appendix C).

Appendix F – Accurate concentration standard TA mix

Table F. 1: The accurate concentration in the target analyte mix used for making the calibration curve.

Compound	ppm	TA (µL)	MeOH (µL)	Concentration
MeP	990	101	899	99.99
EtP	1000	100	900	100
PrP	980	102	989	99.96
BuP	990	101	999	99.99
BezP	1100	91	909	100.1
HeP	990	101	899	99.99
4-HB	1030	97	903	99.91
3,4-DHB	1060	94	906	99.64
Vanillic	100	100	900	100
TCC	101	99	901	99.99

Appendix G – Box plot

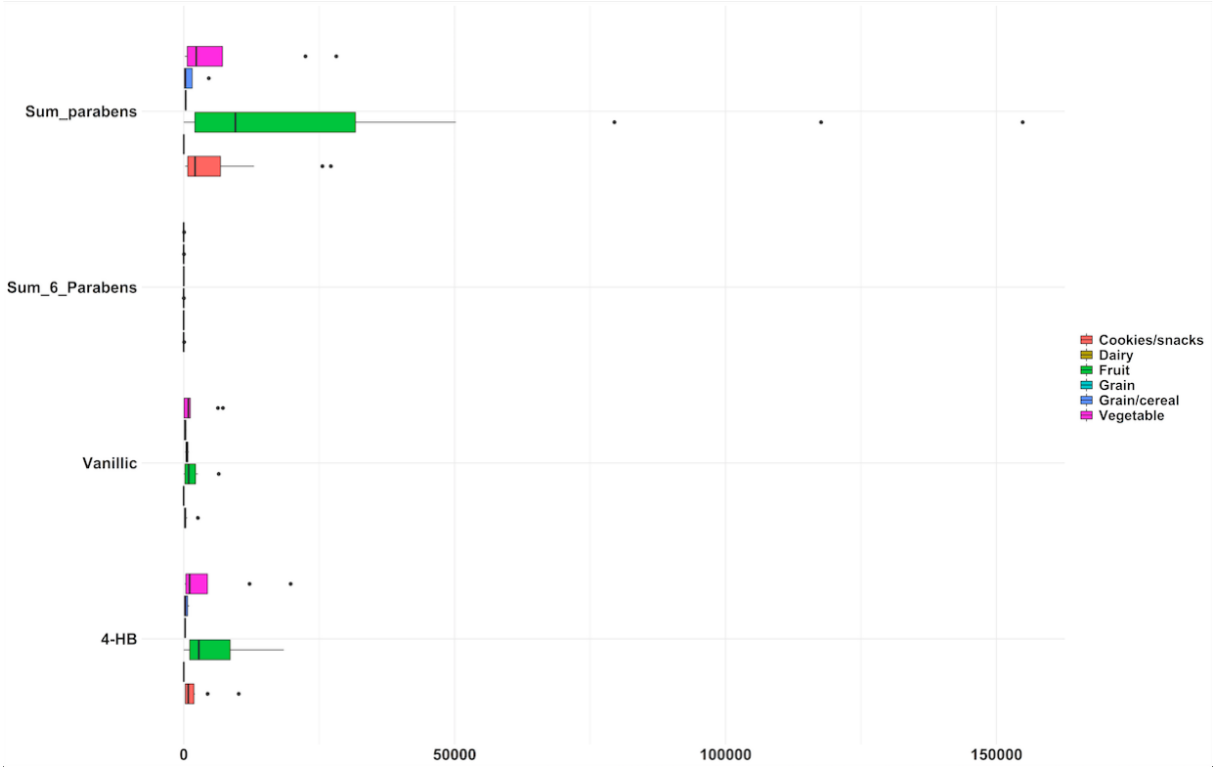


Figure G. 1: Box-plot illustrating the concentrations of ∑parbens and derivates (Sum_parabens), ∑parabens (Sum_6_Parabens), Vanillic and 4-HB. The horizontal line in the boxes represents the median value.

Appendix H – ADI and EDI calculations

Table H. 1: Estimated mean, median and detection rate of MeP and EtP for the different food categories

	∑MeP+EtP		
	Mean (ng/g)	*Median (ng/g)*	*DR (%)*
Grain	19,53	18,42	57,14
C&S	12,75	2,95	58,33
Fruit	10,23	4,41	44,44
Vegetables	31,01	31,01	22,22

ADI: 10 mg/kg bw = 10,000,000 ng/kg bw

Mean ∑MePEtP: 14,82 ng/g

- Grain: $\frac{10{,}000{,}000\frac{ng}{kg\ bw}}{19.53\frac{ng}{g}}$ = 512032.77 g/kg bw = 512.03 kg/ kg bw
- C&S: $\frac{10{,}000{,}000\frac{ng}{kg\ bw}}{12.75\frac{ng}{g}}$=784313.73 g/kg bw = 784.31 kg/ kg bw
- Fruit: $\frac{10{,}000{,}000\frac{ng}{kg\ bw}}{10.23\frac{ng}{g}}$=977517.11 g/kg bw = 977.52 kg/kg bw
- Vegetables; $\frac{10{,}000{,}000\frac{ng}{kg\ bw}}{31.01\frac{ng}{g}}$ = 322476.62 g/kg bw = 322.48 kg/kg bw

EDI-estimate

The conversion from dl to grams was difficult to evaluate when the labs were locked down. Instead the portion sizes for products in the different food categories was used in the EDI calculations. The example calculation was based on the assumption that children eat 4 meals (breakfast, lunch, dinner and supper). A porridge sample portion size is about 30 g, while a dinner portion can be about 190 g, based on the information gained in some of the samples, as illustrated in Figure H.1-H4. If the average intake of a meal is thereby somewhere in between 30-200g per day. 4 meals was estimated to be about 400 g of food per day.

$$EDI = \frac{9.73\frac{ng}{g} \times 400\frac{g}{day}}{kgBW} = \frac{3892\frac{ng}{day}}{kgBW}$$

Portion size information

Figure H. 1: Illustrated picture of a dinner product with portion size 120 g

Figure H. 2: Illustrating picture of a dinner product with portion size 190 g

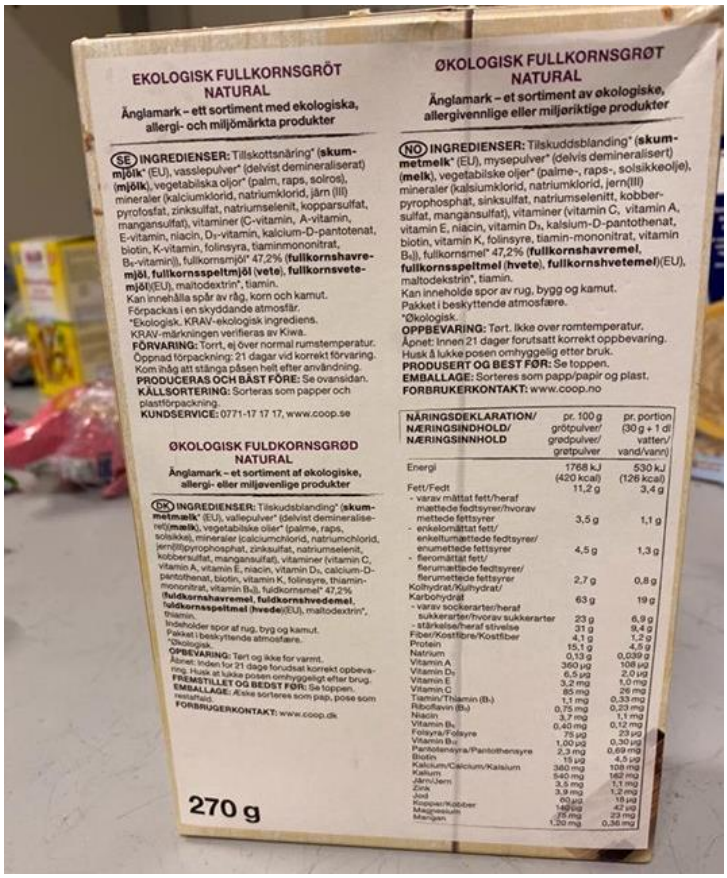


Figure H. 3: Illustrating picture of a grain product (porridge) with portion size 30 g

Figure H. 4: Illustrating fruit smoothie with portion size 110 g

Figure H. 5: Illustrating picture of snack bar with portion size 25 g.

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