TARGETED AND NON-TARGETED ANALYSIS OF PLASTIC-RELATED CHEMICALS IN FOOD

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Abstract

Plastic-related chemicals (PRCs) are substances related to plastics including the initial components of the plastics (e.g. monomers, antioxidants, additives) and the degradation products of plastics. The occurrence of PRCs in food and their potential adverse health effects have raised concerns about the health of consumers. To date, the surveillance of PRCs in food has mostly focused on the targeted screening and quantification of specific residues using tools such as high performance liquid chromatography (HPLC) or gas chromatography (GC) coupled with mass spectrometry (MS). For example, bisphenol A (BPA) and several phthalates have been detected in different types of food. To ensure food safety though, it is now acknowledged there is a need for analytical tools able to screen and identify not only "known" PRCs in food, but also the new "unknown" compounds. The main objective of my research is to develop and optimize a non-targeted method to investigate PRCs in food with an emphasis on the investigation of the influence of data processing parameters on the identification of trace residues in food. In Chapter 3, a non-targeted workflow was optimized based on the HPLC hyphenated to quadruple time-of-flight MS (HPLC-QTOF-MS) analysis to investigate leachable residues from reusable bottles. Low method detection limits (MDL) and high mass accuracy were achieved for 11 bisphenol analogues. Results indicated that all tested bottles are free of BPA, and the bisphenol analogues were not applied as BPA replacement in these bottle manufacture. The effect of data post-processing parameters on the feature extraction in non-targeted analysis was also systematically investigated, and results confirmed that these parameters need to be carefully optimized to extract all the features and

identify them accurately. The optimized method was effectively applied to identify monomethyl terephthalate at trace levels in food simulants in contact with Tritan™ bottles. In Chapter 4, the non-targeted workflow was developed and optimized for the analysis of PRCs as well as other environmental contaminants in a complex food matrix (pike fish fillets). None of the bisphenol analogues used for targeted method validation were detected in pike samples suggesting that these chemicals do not accumulate at detectable concentrations in muscle of pike naturally-exposed in the St. Lawrence River at two sampling sites. Peak height related parameters show high importance in chromatographic data filtering for fish samples and need for optimization before the non-targeted analysis. The non-targeted workflow was shown to accurately identify chemicals of high environmental and health concern (i.e., diethylhexyl phthalate and perfluorooctanesulfonic acid) in pike muscle extracts. In Chapter 5, the optimized non-targeted workflow was applied to screen PRCs in different types of food (namely fish, chicken, canned tuna, leafy vegetables, bread and butter). The MDLs (below 3.6 ng g^{-1}) and recoveries for the targeted bisphenols among the different food matrices (76% to 122%) were satisfactory. A range of contaminants in different food matrices were detected and identified, including BPA, bisphenol S (BPS), bis(2-ethylhexyl) adipate, dibutyl adipate, hexadecyl methacrylate and Irganox®1076. BPS was first reported in Canadian fresh fish and chicken breast samples. In Chapter 6, the optimized non-targeted workflow was applied to study the thermal degradation of BPA and BPS in water (model matrix) and fish muscles (real food). BPA and BPS did not degrade in water (less than 0.1% degradation) but degraded in fish matrix (about 35% degradation in fish for both BPA and BPS). The

degradation products in spiked fish samples are different from those in incurred group. Overall, this research demonstrated that non-targeted analysis is crucial in understanding the occurrence and the fate of PRCs in food, and the results of the present research will contribute to refining current food safety risk assessments.

Résumé

Les substances chimiques associées aux plastiques (abrév. PRCs) sont définies comme un groupe incluant les monomères, les antioxydants, les additifs, mais aussi tout produit de dégradation des plastiques ou de ces composés. La présence des PRCs dans les aliments et les effets potentiels sur la santé humaine ont créé des préoccupations pour la santé des consommateurs. A date, la surveillance des PRCs dans les aliments s'est concentrée sur l'analyse ciblée et la quantification de résidus spécifiques, basées sur des outils comme la chromatographie en phase (HPLC) ou gazeuse (GC) couplée a la spectrométrie de masse (MS). Ainsi, le bisphénol A (BPA) et plusieurs phthalates ont été détectés dans divers types. Des outils analytiques sont désormais nécessaires pour protéger la santé des consommateurs afin de passer au crible et identifier les PRCs dans les aliments, en incluant non seulement les composés « connus » mais aussi ceux « inconnus ». Récemment seulement, des approches non-ciblées sont apparus pour la recherche des résidus chimiques et restent à être validés pour le domaine des PRCs. L'objectif principal de cette recherche est de développer et d'optimiser des approches non-ciblées pour l'étude des PRCs dans les aliments, avec comme emphase l'étude de l'influence du traitement post-analyse des données sur l'identification des résidus traces. Dans le Chapitre 3 de cette thèse, une méthode non-ciblée, base sur la spectrométrie de masse en tandem quadripolaire/a temps de vol (HPLC-QTOF-MS) a été optimisée pour évaluer la migration chimique de résidus depuis les bouteilles d'eau en plastique réutilisables. Une limite de détection de la méthode (MDL) et une justesse de la masse a été obtenue pour onze analogues du BPA utilisés comme composes modèles. Les résultats indiquent

que toutes les bouteilles d'eau testées ne contiennent pas de BPA, ou d'analogues du BPA. L'effet du traitement post-analyse des données sur la détection des composes a été étudié de manière systématique, et les résultats ont confirmés que les paramètres doivent être optimisés pour l'extraction et l'identification des composés. La méthode optimisée a été appliquée efficacement pour identifier le téréphtalate mono méthyl dans des simulants alimentaires en contact avec les bouteilles en Tritan^{TM}. Dans le Chapitre 4, une méthode non-ciblée a été optimisée pour évaluer les PRCs et d'autres contaminants environnementaux dans une matrice biologique alimentaire complexe (des filets de brochet). Par une méthode ciblée, aucun des analogues du BPA n'ont été détectés dans des échantillons de brochets de deux sites du Saint-Laurent dans la région de Montréal, suggérant que peu ces résidus ne s'accumulent pas dans les muscles des poissons pêchés a ces deux sites. La hauteur des pics chromatographiques et des paramètres associes sont tres importants pour l'étape de traitement des signaux pour l'approche non-ciblée. La méthode non-ciblée a permis d'identifier dans les extraits de brochet des substances d'importants pour l'environnement et la santé des consommateurs (par exemple le phtalate de diéthylhéxyle et l'acide perfluorooctanesulfonique). Dans le Chapitre 5, une méthode non-ciblée a été optimisée pour le criblage des PRCs dans différents types d'aliments (poisson, poulet, thon en conserve, légumes, pain et beurre). Les MDLs (< 3.6 ng g^{-1}) et les taux de recouvrement sont acceptables pour l'analyse ciblée des bisphénols parmi ces matrices alimentaires (76% - 122%). Divers contaminants ont été détectés, notamment le BPA, le bisphénol S (BPS), l'adipate de bis(2-éthylhéxyle), l'adipate de dibutyle, le méthacrylate d'hexadécyle et l'Irganox$^{®}$1076. Ces résultats

montrent pour la première fois le BPS dans les échantillons de poisson et de poulets au Canada. Enfin, dans le Chapitre 6, la méthode non-ciblée a été appliquée à la dégradation thermique du BPA et du BPS dans l'eau (matrice modèle) et des muscles de poisson (matrice réelle). Le BPA et le BPS ne se dégradent dans l'eau (<0.1% de dégradation) mais se dégradent dans le poisson (environ 35% pour le BPA et le BPS). Les produits de dégradation dans les matrices de poisson dopées, sont différents de ceux observés dans les matrices contaminées naturellement. En conclusion, cette recherche montre que l'approche non-ciblée est critique pour comprendre la présence et le devenir des PRCs dans les aliments, et les résultats contribuent à raffiner l'évaluation des risques pour la santé pour les consommateurs.

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Contribution of Authors

This thesis is presented in a manuscript form and consists of seven chapters. In Chapter 1, a general introduction of plastic-related chemicals (PRCs) in food and the research objectives of the thesis are presented. In Chapter 2, a literature review is presented which summarizes the analytical methods, the challenges and knowledge gaps of analyzing PRCs in food. Then, Chapter 3 to Chapter 6 are prepared in a manuscript form and arranged sequentially through connecting text. Out of these four manuscripts, Chapter 3 has been published in the journal *Talanta*, and Chapter 4 has been published in the journal *Environmental Pollution*. Chapter 5 was submitted for publication in the journal *Food Chemistry*, and Chapter 6 was submitted for publication in the journal *Food Chemistry*. Finally, Chapter 7 presents an overall conclusion of the thesis as well as some recommendations for future research.

The present author was responsible for the concepts, design of experiments, experimental work, data acquisition, data treatment and manuscript preparation in all the manuscripts. Dr. Stéphane Bayen, the thesis supervisor and the co-authors for all the manuscripts, had direct advisory for all the experimental design and the manuscripts. In Chapter 3 "Optimization of the post-acquisition data processing for the non-targeted screening of trace leachable residues from reusable plastic bottles by high performance liquid chromatography coupled to hybrid quadrupole time of flight mass spectrometry", Lan Lin collected the bottle samples and conducted the migration test, is the co-author of Chapter 3. In Chapter 4, "Suspect screening of plastic-related chemicals in northern pike (*Esox lucius*) from the St. Lawrence River, Canada", Dr. JonathanVerreault and Dr. Magali Houde managed the sampling of the pike fish tissues and they also edited the manuscript before

submission. Mr. Jingyun Zheng and Dr. Cindy Gate Goodyer are the co-authors for both Chapter 5 “Targeted and non-targeted analysis of PRCs in food collected in Montreal, Canada” and Chapter 6 “Thermal degradation of bisphenol A and bisphenol S in water and fish (cod and basa) fillets”. Mr. Zheng supported this work during food sample collection and sample preparation. Dr. Goodyer edited both manuscripts before the submissions.

Publications

- Tian, L., Lin, L., & Bayen, S. (2019). Optimization of the post-acquisition data processing for the non-targeted screening of trace leachable residues from reusable plastic bottles by high performance liquid chromatography coupled to hybrid quadrupole time of flight mass spectrometry. *Talanta*, *193*, 70-76.

- Tian, L., Verreault, J., Houde, M., & Bayen, S. (2019). Suspect screening of plastic-related chemicals in northern pike (*Esox lucius*) from the St. Lawrence River, Canada. *Environmental Pollution*, *255*, 113223.

- Tian, L., Zheng, J., Goodyer, C. G., & Bayen, S. (2019). Non-targeted screening of plastic-related chemicals in food collected in Montreal, Canada. *Revised manuscript submitted*.

- Tian, L., Zheng, J., Goodyer, C. G., & Bayen, S. (2019). Thermal degradation of bisphenol A and bisphenol S in water and fish (cod and basa) fillets. *Revised manuscript submitted*.

Conference Presentations

- Tian, L., Zheng, J., Goodyer, C. G., Bayen, S. Non-targeted analysis of the degradation of four plastic-related contaminants in food during thermal treatment. 9th International Symposium on Recent Advances in Food Analysis (RAFA 2019), November 5-8, 2019. Prague, Czech Republic.

- Tian, L., Goodyer, C. G., Zheng, J., Bayen, S. Determination of bisphenol S and ten other bisphenol analogues in fresh fish from Canada. 9th International Symposium on Recent Advances in Food Analysis (RAFA 2019), November 5-8, 2019. Prague, Czech Republic.

- Tian, L., Zheng, J., Goodyer, C. G., Bayen, S. Targeted and non-targeted analysis of plastic-related contaminants in food from Montreal using HPLC-QTOF-MS. 63rd International Conference on Analytical Sciences and Spectroscopy, June 25-28, 2019. Montreal, QC, Canada.

- Tian, L., Zheng, J., Bayen, S. Determination of 11 bisphenols and other plastic-related contaminants in food using high performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (HPLC-QTOF-MS). 102nd Canadian Chemistry Conference and Exhibition (CSC 2019), June 3-7, 2019. Quebec City, QC, Canada.

- Tian, L., Bayen, S. Non-targeted analysis of leachable residues from food contact materials (FCMs) using HPLC-QTOF-MS. ILSI North America 2019 Food Packaging Conference:

Scientific Advances and Challenges in Safety Evaluation of Food Packaging Materials, April 2-3, 2019. Washington DC, USA.

- Tian, L., Reinling, J., Bayen, S. Development of a LC-MS/MS-based method for screening of non-targeted chemicals of potential concern in Northern Pike. SETAC 28th Europe Annual Meeting, May 13, 2018, Rome, Italy.

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- Tian, L., Bayen, S. Targeted and non-targeted analysis of organic migrants from reusable plastic bottles using high performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (HPLC-QTOF-MS) 100th Canadian Chemistry Conference and Exhibition (CSC 2017). 2017, Toronto, ON, Canada.

- Tian, L., Lin, L., Bayen, S. Non-targeted identification of organic migrants from reusable plastic bottles using HPLC-QTOF-MS: influence of data processing. 61st ICASS (International conference on analytical science and spectrometry), June 19, 2017, Quebec City, QC, Canada.

- Bayen, S., Tian, L., von Eyken, A., Lin, L. From targeted to non-targeted analysis: applications to food safety. 29th Annual Tandem Mass Spectrometry Workshop, Nov 30-Dec 3, 2017, Lake Louise, AB, Canada.

Abbreviations

ALARA	as low as reasonably achievable
ANOVA	a nested analysis of variance
APCI	atmospheric pressure chemical ionization
BADGE	BPA diglycidyl ether
BPA	bisphenol A
BPAF	bisphenol AF
BPAP	bisphenol AP
BPB	bisphenol B
BPBP	bisphenol BP
BPC	bisphenol C
BPE	bisphenol E
BPF	bisphenol F
BPG	bisphenol G
BPM	bisphenol M
BPP	bisphenol P
BPPH	bisphenol PH
BPS	bisphenol S
BPZ	bisphenol Z
CPU	central processing unit

DBA	dibutyl adipate
DBP	dibutyl phthalate
DEHA	bis(2-ethylhexyl) adipate
DEP	diethyl phthalate
EC	European Commission
ED	electrochemical detection
EFSA	European Food Safety Authority
ESI	electrospray ionization
EU	European Union
FAO	Food and Agricultural Organiization
FCMs	food contact materials
FD	fluorescence detection
GC	gas chromatography
HPLC	high performance liquid chromatography
HPMA	N-(2-Hydroxypropyl) methacrylamide
HRMS	high resolution mass spectrometry
IAS	intentionally added substances
LDA	liner discrimination analysis
LE	liquid extraction
LOD	limit of detection

MDL	method detection limit
MLE	microwave-assisted liquid extraction
MMME	mean mass measurement error
MPP	MassHunter Profiler Professional
MS	mass spectrometry
MS/MS	tandem mass spectrometry
N/A	not available
NC	not conducted
ND	not detected
NIAS	non-intentionally added substances
PC	polycarbonate
PCA	principal component analysis
PFOS	perfluorooctanesulfonic acid
PLE	pressure liquid extraction
PLS-DA	partial least squares discriminant analysis
PRCs	plastic-related chemicals
PVC	polyvinyl chloride
QA	quality assurance
QC	quality control
QqQ	triple quadrupole

QTOF	quadrupole time-of-flight
RSD	relative standard deviation
RT	retention time
SLE	sonication-assisted liquid extraction
SML	specific migration limit
SPE	solid phase extraction
SUPRASE	supramolecular solvent extraction
TOF	time-of-flight

Chapter 1. Introduction

1.1 General introduction

Plastic materials are ubiquitous in our modern life. Through food contact materials (FCMs) (e.g. packaging) or environmental pollution, some chemicals can leach out from plastic materials and enter the human food chain. The occurrence of such residues (plastic-related chemicals, PRCs) in food and their potential adverse health effects have raised serious public health concerns (Muncke, 2009). For example, bisphenols and phthalates, two key chemical families in plastics manufacturing, are now ubiquitous food and environmental contaminants, and some of them have been identified as potential endocrine disrupting compounds (Muncke, 2009; Rosenmai et al., 2014; Schierow & Lee, 2008).

Due to the potential toxicity of some of these plasticizers or monomers, a range of regulations have been established to control their usage. In this way, bisphenol A (BPA) was banned from baby bottle materials in Canada and EU for example (Government of Canada, 2010; European commission, 2011). However, a ban may just result in its replacement with another equally hazardous chemical. For example, bisphenol S (BPS), which is reported as a replacement of BPA in different materials, was shown to exert similar toxicity as BPA (Mathew et al., 2014). Surprisingly though, for most applications, little information is publicly available on the replacement(s) of a chemical being phased out. To address potential food safety issues in this field, there is a need to develop analytical tools able to identify and screen for the presence of contaminants coming from plastics in food, including “known” and any new “unknown” ones.

To date, the study of chemicals leaching or migrating from plastic materials, or present in environment as pollutants, has mostly focused on the targeted screening and quantification of specific residues using tools such as high performance liquid chromatography (HPLC) or gas chromatography (GC) coupled with mass spectrometry (MS) (Lorber et al., 2015). The detection and the identification of "unknown" chemicals, without *a priori* knowledge on their structures, relies however on a non-routine approach called non-targeted analysis. Non-targeted analysis requires specific considerations in terms of sample preparation, instrumental analysis and data treatment (Vuckovic, 2012). Only recently, non-targeted workflows have been applied to food analysis, in a field now named as "foodomics" (Knolhoff and Croley, 2016). Bignardi et al. (2014), for example, developed a non-targeted method to investigate chemicals migrating from specific plastic cooking ware. To date though, this approach has been limited to the suspected screening of compounds from packaging materials and packaged food, notably bottled water and canned food (Skjevrak et al., 2005; Bignardi et al., 2014; Gosetti et al., 2016). While some have optimized the sample preparation and instrumental analysis steps, other important steps of the non-targeted workflows have not yet been optimized to improve the identification of "unknown" trace residues in food, notably post-acquisition data processing (Nerin et al., 2013).

Furthermore, the fate of any contaminants in food, including PRCs, during food processing and human digestion should be better understood to refine food safety risk assessments. As most foods, especially those of animal origin, are consumed after thermal processing, it is important to

understand the thermal degradation of PRCs in food as well as to identify the thermal degradation products.

1.2 Research objectives

The main objective of my research is to develop and optimize a non-targeted method to investigate the occurrence of PRCs in food. In addition to optimizing the sample preparation steps, this research proposes an in-depth investigation of the influence of data processing parameters on the identification of trace residues in food. Bisphenol analogues were used as model chemicals for method validation, and also as a case study to understand the fate of PRCs in food under thermal treatment. The specific objectives of this research are:

(i) to develop and optimize a non-targeted workflow to identify unknown PRCs in food simulants;

(ii) to optimize a non-targeted workflow to identify unknown PRCs and other contaminants in a complex food matrix (pike fish as a case study);

(iii) to screen the bisphenol analogues in multiple food matrices (targeted screening) as well as to apply a non-targeted workflow to detect and identify "unknown" PRCs in multiple food matrices (non-targeted screening);

(iv) to investigate the fate of PRCs in food under thermal treatment by a non-targeted approach using bisphenol analogues as a case study.

Chapter 2. Literature Review

2.1 Introduction

According to the *Codex Alimentarius* (FAO), food contaminants are substances that have not been intentionally added into food, including the substances from processing, as well as those come from the environment. To ensure food safety and to protect consumers' health, the presence of food contaminants must be monitored carefully. Chemical contaminants include agricultural chemicals, environmental and industrial contaminants, and natural toxins (Jackson, 2009). The presence of chemical contaminants in food has been recognized as one of the most prominent issues that affect food safety, which is highly concerned by consumers (Fu et al., 2017; Nerin et al., 2016) For examples, it was reported that in 2010, about 339,000 illnesses and 20,000 deaths were related to just four chemicals, namely aflatoxin, cyanide in cassava, dioxin, peanut allergens (World Health Organization, 2015).

Plastic is defined as a group of synthetic resinous or other substances that can be molded into any form (Robertson, 2005). Plastic materials are ubiquitous in our modern life, and the use of plastic packaging has increased continuously in recent decades (Groh et al., 2019). About 60% of plastic packagings are reported to be used for food and beverages (Groh et al., 2019). Some chemicals can migrate into food from plastic materials used as food contact materials (FCMs, e.g. packaging) or enter the human food chain because of environmental pollution. Such chemicals are defined as plastic-related chemicals (PRCs), and include the initial components of the plastics (e.g., monomers and polymer fragments, and additives which are intentionally added substances (IAS)) and the impurities of initials components and plastic degradation products (which are non-

intentionally added substances (NIAS)). Many PRCs are hazardous and can induce adverse health effects on both human and animals (Ng et al., 2015; Rochester, 2013). For example, bisphenol A (BPA) as well as some phthalates (e.g., dibutyl phthalate and diethyl phthalate) have been shown to exhibit endocrine disruptive properties in humans and aquatic organisms (Tiwari et al., 2016; Wei et al., 2011).

Some PRCs are not authorized or have been banned for application in FCMs because of their toxicity (e.g. BPA). When a plastic additive is banned though, the actual chemical replacement of the substance in the polymer is often unknown to the public as the composition of a materials is often proprietary and confidential (Nerin et al., 2016). Worryingly, some molecules, discussed as possible replacements have been reported to be as toxic as the original chemical they may replace. For example, bisphenol S (BPS), a compound structurally analogous to BPA, is presently used as an alternative of BPA in thermal printing papers and has been detected in different foods (Eladak et al., 2015). Recent studies suggest that BPS toxicity is comparable to BPA (Rosenmai et al., 2014; Eladak et al., 2015).

The surveillance of PRCs in food has focused on specific compounds or a class of chemicals to date, which does not appear to be sufficient to ensure food safety as some "unknown" or "unexpected" toxic PRCs may be present in food. In this context, non-targeted approaches, able to operate without any prior knowledge about the chemical, are necessary for the detection and the identification of PRCs in food. Non-targeted strategies, first developed in the field of metabolomics, are now increasingly applied in food analysis (Knolhoff & Croley, 2016).

In the present Chapter, available methods for the analysis of PRCs in food are first reviewed. The challenges and knowledge gaps in PRCs analysis are identified. Bisphenol analogues in food are then presented as a case study to understand the occurrence, level, and potential risk of PRCs in food.

2.2 Analytical methods for the detection, identification and quantification of PRCs in food

The general workflow for the analysis of PRCs in food can be described by the following steps: sample pretreatment, extraction, clean-up, background removal, concentration, derivation and instrumental analysis (qualitative and quantitative), and data processing (Caballero-Casero et al., 2016). Sample pretreatment includes homogenization, filtration, centrifugation, precipitation, and a clean-up step can be applied to achieve more effective results, especially for the high-fat content samples (Ballesteros-Gómez et al., 2009; Caballero-Casero et al., 2016). Sometimes, a deconjugation step is required to assess the free and total concentrations of the contaminants. In this case, ß-glucuronidase and sulphatase are commonly used to release conjugated compounds (Yan et al., 2009). Each individual step is critical for the analysis, although only the optimization of extraction and instrumental analysis steps are generally reported in literature (Knolhoff & Croley, 2016).

2.2.1 Extraction methods

A range of extraction methods have been successfully applied to the analysis of PRCs in food (detailed information in Table 2.1). Among them, liquid extraction (LE) (or solvent extraction) and solid phase extraction (SPE) are the most widely used techniques for the extraction PRCs from solid and liquid food, respectively, mainly because the two methods are simple and versatile. QuEChERS is another extraction technique reported for food samples which show some advantages like saving solvent and satisfactory recovery (Luo et al., 2017; Aparicio et al., 2018). Sometimes, a clean-up step is necessary to improve the selectivity of extraction where SPE is generally applied. On specific occasions, the combination of different techniques may be used to improve the extraction efficiency (Lorber et al., 2015). Specific approaches are described in the following sections.

2.2.1.1 Liquid extraction

Liquid extraction (LE) is probably the most common method for solid food samples, and is sometimes also applied to liquid samples (Ballesteros-Gómez et al., 2009). Soxhlet extraction was for example reported for the extraction of BPA, 4-nonylphenol, and octylphenol in fish (Mortazavi et al., 2013). Other methods based on liquid extraction, including pressure liquid extraction (PLE), sonication-assisted liquid extraction (SLE) and microwave-assisted liquid extraction (MLE), have also been applied to food samples to either increase the extraction speed or to limit solvent consumption (Ballesteros-Gómez et al., 2009).

The amount of food sample used for the extraction for most of study is about 5 g (ranging from 0.5 to 30 g) for solid food (Schecter et al., 2010) and 10–50 mL for liquid food, although in some cases, very large sample size have been reported (e.g. 120 g for canned jalapeño peppers (Munguia-Lopez, Peralt et al., 2002) or 500 mL for mineral water (Toyo'oka & Oshige, 2000) (Ballesteros-Gómez et al., 2009). Generally, acetonitrile, hexane and methanol are effective solvents for liquid extraction (Caballero-Casero et al., 2016). Supramolecular solvent extraction (SUPRASE) were also reported to be effective (Alabi et al., 2014). The benefit of supramolecular solvent is the capacity to extract food samples that widely range in polarity, and it was reported to be time-saving as the isolation and clean-up can be conducted at the same time thanks to the properties of some supramolecular solvents (Alabi et al., 2014). The overall solvent consumption per sample usually ranged from 15 to 300 mL (Ballesteros-Gómez et al., 2009). Extraction times range from 10 min to 120 min depends on the equipment applied (Table 2.1).

2.2.1.2 Solid phase extraction (SPE)

SPE is commonly reported for the extraction of liquid food samples, but it is also frequently used as a clean-up step for many types of food extracts. Sorbents including C18, magnesium silicate and many others have been reported depending on the physico-chemical properties of the analytes (Caballero-Casero et al., 2016; Grumetto et al., 2008). The advantage of SPE compared to solvent extraction is often a higher selectivity and a lower solvent consumption. However, the recovery

for SPE is sometimes low (57-89%, Caballero-Casero et al., 2016), and SPE may sometimes be more time-consuming compared to PLE, SLE and MLE (Cao et al., 2011; Liao & Kanna, 2013).

2.2.1.3 QuEChERS extraction

The QuEChERS (for Quick, Easy, Cheap, Effective, Rugged and Safe) method was first developed for the extraction of pesticides in fruit and vegetables. Recently it has been widely applied in other types of contaminants and food, such as milk, honey and fish tissues (Anastassiades et al., 2003; Liu et al., 2016; Luo et al., 2017). The advantage of QuEChERS is that it is easy to operate, little equipment is required, fast and it does not consume large amounts of solvent. QuEChERS has been reported for the extraction of bisphenols in seafood and canned food with satisfactory recoveries (68-104%) (Table 2.1).

Table 2.1 Common methods for the extraction of PRCs in food matrices

Extraction Method	Food type	Recovery range	Reference
LE	Fish	73-106%	Liao and Kannan (2013); Schecter et al. (2013)
	Meat	73-106%	Liao and Kannan (2013); Schecter et al. (2013)
	Crop (flour)	73-106%	Liao and Kannan (2013); Schecter et al. (2013)
	Bread	73-106%	Liao and Kannan (2013); Schecter et al. (2013)
	Beverage	93%	Geens et al. (2010)

	Canned fish	73-106%	Liao and Kannan (2013); Schecter et al. (2013)
	Canned soup	71%	Thomson* and Grounds (2005)
PLE	Fish	94-100%	Carabias-Martínez et al. (2005)
	Meat	92-99%	Shao et al. (2007)
	Crop (flour)	81-104%	Carabias-Martínez et al. (2005)
SLE	Fish	65-112%	Schecter et al. (2010)
	Meat	65-112%	Schecter et al. (2010)
	Canned fish	65-112%	Schecter et al. (2010)
	Canned soup	65-112%	Schecter et al. (2010)
Soxhelt extraction	Fish	85-100%	Mortazavi et al. (2013)
QuEChERS	Fish	74-113%	Luo et al. (2017)
	Canned fish	68-104%	Cunha et al. (2012)
	Vegetables	81-126%	Aparicio et al. (2018)
SPE	Beverage	95%	Geens et al. (2010)
	Milk	97-104%	Maragou et al. (2006)
	Infant formula	85−94%	Cao et al. (2008)

2.2.2 Detection and quantification of known PRCs in food (targeted analysis)

Targeted analysis consists of confirming the occurrence of specific known compounds in food and quantifying their levels. The targeted compounds are generally identified through comparison with pure analytical standards, based on properties such as chromatographic retention times (RT) and mass spectra (e.g. ion ratios for the qualifier and quantifier ions) or UV-Vis absorbance spectra. When quantifying the known compound, internal standard, “matrix match calibration” or

"standard addition" methods can be applied to correct improve the quantification, especially for complex matrices (Luo et al., 2017).

As PRCs are generally present at trace level in food samples and given the complexity of food matrices, highly selective and sensitive instruments are required for the detection and the quantification of the target compounds. In this context, techniques based on high performance liquid chromatography (HPLC) and gas chromatography (GC) have generally been applied, although other approaches such as immunochemical techniques, have also been successful in detecting some PRCs (e.g. bisphenols) in food (Ballesteros-Gómez et al., 2009). HPLC based methods including HPLC-fluorescence detection (HPLC-FD), HPLC-electrochemical detection (HPLC-ED) and HPLC-MS have been successfully applied to detect and quantify some PRCs (Ballesteros-Gómez et al., 2009). HPLC-MS shows the advantage of low detection limits over the other two detectors (Table S2.1).

GC based methods also play an important role in PRCs analysis especially for nonpolar semi-volatile compounds, for example, phthalate plasticizers (Fierens et al., 2012; Schecter et al., 2013). For some applications, GC-MS often requires a derivatization step, which can further lower the detection limit and improve the separation (Ballesteros-Gómez et al., 2009). It is for example reported that a derivatization step provides sharper peaks for BPA and a better separation from other analytes and coextracted matrix components (Wingender et al., 1998). After derivatization, the LOD for BPA by GC-MS decreased about 200 times (from 0.8 ng to 0.004 ng) compared to a method without derivatization (Stuart et al., 2005). Silylation and acetylation are the most

frequently used derivatization procedures (Ballesteros-Gómez et al., 2009). In addition to the derivatization step, extensive clean-up is often required before GC analysis, especially for fatty foods, as lipids can significantly reduce the analytical performances of GC (Dodo & Knight, 1999). Some examples for the application of LC or GC based methods for the quantification of PRCs are summarized in Supplementary Table S2.1.

2.2.3 Identification of unknown PRCs in food (non-targeted analysis)

To date, many studies have focused on the analysis of specific "known" contaminants which is insufficient to ensure the food safety (Fu et al., 2017). In recent decades, the detection and the identification of unknown or unexpected contaminants using the non-targeted strategy have been more and more popular (Knolhoff & Croley, 2016). The non-targeted analysis includes both suspect screening and unknown screening, both of which aim to investigate compounds without analytical standards of the pure compound and without prior knowledge on the occurrence of the substance in food (Krauss et al., 2010).

A general workflow for non-targeted food analysis was described by Fu et al. (2017) (Figure 2.1). In this approach, food samples are extracted and then analyzed by the HPLC or GC coupled with HRMS followed by data treatments and database searching. While this workflow may identify a compound, the confirmation of the identity of an unknown still requires a comparison with a chemical standard (Fu et al., 2017).

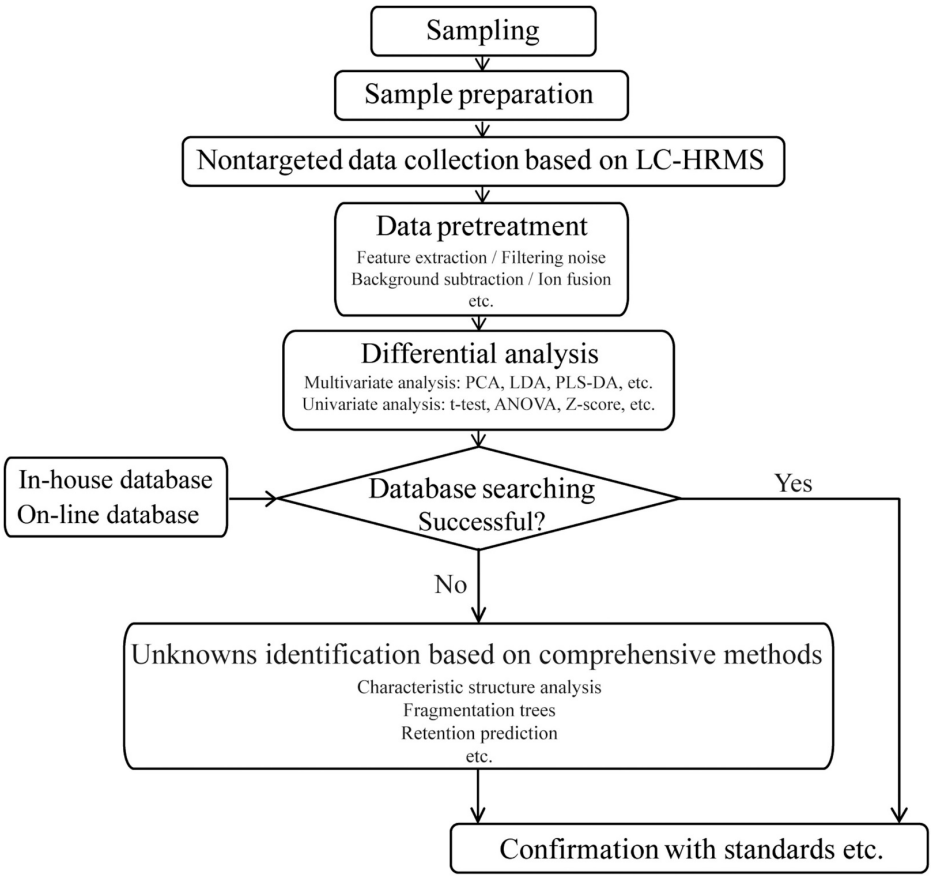


Figure 2.1 General workflow for non-targeted screening of contaminants in food (Fu et al., 2017)

2.2.3.1 Sample preparation for MS based non-targeted analysis

The quality of chromatogram is important for non-targeted identification, and the sample preparation is a crucial step in improving the quality of chromatogram (Plaßmann et al., 2014). For example, ion suppression can be reduced by sample dilution, resulting in better detection of compounds of interest (Stahnke et al., 2012). Common extraction methods for PRCs in food have been summarized in 2.2.1. Critical specific aspects of non-targeted analysis are reviewed below.

Unselective extraction. The extraction approach for non-targeted analysis should not be specific to a particular contaminant, as the intent is to screen the widest range of contaminant classes in food, possibly with different physical and chemical properties (Fu et al., 2017). In this case, "dilute-and-shoot", LE, SPE and QuEChERS are the most common methods applied for food extraction in non-targeted analysis, although these methods still need to be examined for many food matrices. So far, QuEChERS has been validated for various classes of contaminants (e.g., pesticides, veterinary drugs and toxins) in different food matrices, and is recognized to be sufficient and easily adapted for the non-targeted analysis of food contaminants (Knolhoff & Croley, 2016).

Minimum sample processing. Excessive clean-up steps should be avoided in non-targeted extraction as they can remove some compounds of interest (Knolhoff & Croley, 2016). Frequently, a mere sample extracts filtration is only applied before injection (Knolhoff & Croley, 2016).

Sample replication and pooling. Sample replication is important in non-targeted analysis for the quality assurance purposes (reproducibility), as well as for statistical analysis of the data (Knolhoff & Croley, 2016). According to the European Commission regulations on dioxin analysis in food (European Commission, 2014a), duplication of analysis should be at least performed, while in literature, the replication numbers of samples for food contaminants analysis vary in studies (Aparicio et al., 2018; Newton et al., 2018; Wood, Du Preez, Steenkamp, Duvenage, & Rohwer, 2017). To reduce analytical variation and to quantitatively determine analytical precision in non-

targeted analysis, quality control samples (QCs), which are pooled samples containing an aliquot of each sample, are generally applied.

2.2.3.2 Instrumental analysis

HPLC or GC coupled with MS detectors have become increasingly popular in food safety analysis. MS detectors commonly reported for the analysis of PRCs include triple quadrupole (QqQ) MS, and hybrid quadrupole-time-of-flight (QTOF) MS, and quadrupole-Obitrap MS. In the case of non-targeted analysis, HRMS with accurate mass is preferred as it can offer high mass accuracy and resolving power (Fu et al., 2017). Therefore, HPLC or GC coupled to quadrupole Orbitrap MS or TOF MS have generally been applied for non-targeted analysis in food. QqQ MS is often applied for targeted screening and quantification due to its low sensitivity in full-scan mode compared with TOF MS (Herrmann et al., 2012; Soler et al., 2007). The criteria for TOF MS and Obitrap MS in non-targeted analysis will be illustrated in this section.

Several criteria are proposed for unknown identification including RT, molecular weight, precursor ion, mass tolerance of ±20% to ±50% with the reference MS/MS spectra etc. (European Commission, 2002). The determination of the elemental composition is a critical step for compound identification and "Seven Golden Rules" were proposed by Kind and Fiehn (2007) to generate formula from thousands of possible compounds (endogenous compounds and xenobiotics). The accuracy of instrument measurement was also included among these rules, and a mass accuracy lower than 3 ppm and the relative isotopic ratio accuracies lower than 5% are

generally required to yield formulas with high accuracy (e.g. based on the 6000 tested compounds, the correct formulas were retrieved as top hit at 80–99% probability with these criteria) (Kind & Fiehn, 2007). Both TOF MS and Orbitrap MS can achieve the mass accuracies below 5 ppm, and QTOF show more advantages than Obitrap in overcoming peak coelution and ion suppression (Croley et al., 2012).

Another important criterion for non-targeted instrument analysis is high resolution. Higher the resolving power are more effective at isolating the target from interferences in complex matrices, notably when low concentration of unsuspected compounds is expected. For example, Kellmann et al. (2009) reported that for analytes in intermediate complexity samples (e.g. honey) (at levels near 25 ng g^{-1}), a resolving power of 7,000–10,000 may be sufficient, while for analytes in complex matrices such as food, a resolving power up to 70,000 would be desirable (Kellmann et al., 2009). Both TOF and Orbitrap analyzers can reach this criterion (Kellmann et al., 2009).

In addition to the mass accuracy and resolution power, ionization mode is another important factor that impact the instrument analysis. Electrospray ionization (ESI) and an atmospheric pressure chemical ionization (APCI) are frequently applied as ionization sources for non-targeted analysis. In many cases, ESI can provide high signals and less isobaric interferences compared to APCI, and thus, ESI is preferred for PRCs analysis (Gallart-Ayala et al., 2010; Vaclavikova et al., 2016). Once using ESI, it is recommended that both positive and negative ionization mode should be used for a non-targeted analysis as some compounds only ionize in positive or negative mode (Knolhoff & Croley, 2016).

2.2.3.3 Method validation and data analysis

In non-targeted analysis, the robustness of the analytical method can be assessed by selecting a number of representative compounds (e.g. in the same class as the suspected contaminants or cover a large polarity range) and analyzing them as targets. For example, this strategy has been applied for the validation of non-targeted method for multiclass contaminants identification in milk and water (Kunzelmann et al., 2018; Wood et al., 2017).

A general framework for data analysis workflows in non-targeted analyses was proposed by Knolhoff and Croley (2016) (Figure 2.2). Data are first extracted and filtered then followed by the database searching and chemical standard confirmation. The structural identification mainly relies on the mass spectrum, thus peak extraction and features filtration are very important steps (Knolhoff & Croley, 2016). Statistical analysis such as principal component analysis (PCA), linear discrimination analysis (LDA), a nested analysis of variance (ANOVA) or partial least squares discriminant analysis (PLS-DA) can distinguish sample groups, and is often helpful in removing chemical background for future analysis (Knolhoff & Croley, 2016). To date, there are no guidelines available for the peak extraction and database searching steps in non-targeted analysis.

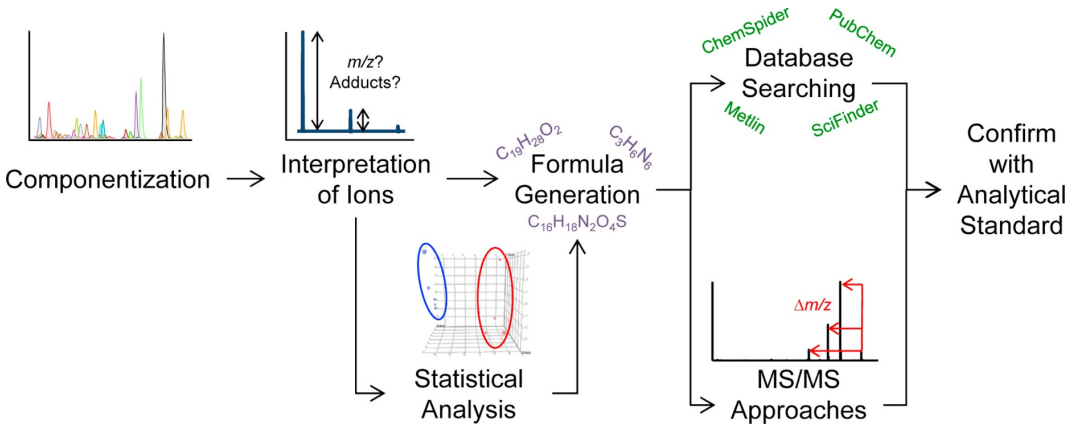


Figure 2.2 Data analysis workflow for non-targeted screening (Knolhoff & Croley, 2016)

2.3 The occurrence of PRCs in food: bisphenols as a case study

Bisphenols are a group of chemicals with two hydroxyphenyl functional groups. The most representative compound in this group is BPA. Many studies have reported the occurrence of bisphenols in food and environmental matrices such as dust, soil and water (Chen et al., 2016; Schecter et al., 2010). According to a review paper, food is recognized to be one of the major source of bisphenols exposure to human (Caballero-Casero et al., 2016).

Increasing concerns have emerged about the occurrence of bisphenols in food due to their potential toxicity. Starting from 2010, BPA has been banned for applications in baby bottles in Canada and in the European Union (Government of Canada, 2010; European Commission, 2011a). Since then, a wide range of food containers and packaging have been marketed as “BPA-free”. Although this label is often highlighted, the actual chemical replacement occurring in these materials is rarely specified. Some studies have reported that structural analogues, such as bisphenol B (BPB), bisphenol E (BPE), bisphenol S (BPS) and bisphenol F (BPF) may have replaced BPA for some

applications (Ng et al., 2015). Most of these structural analogues of BPA have been reported to have negative impact on estrogen receptor and androgen receptor activity comparable to BPA (Rosenmai et al., 2014). In this section, bisphenols were used as a case study to understand the fate of PRCs in food including the occurrence, level, source, and impact factors for migration etc.

2.3.1 BPA and its analogues

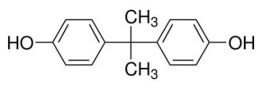
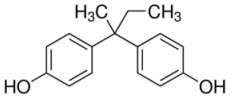
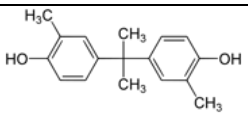
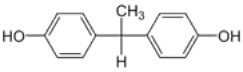
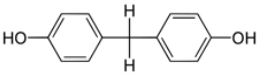
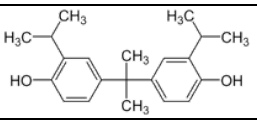
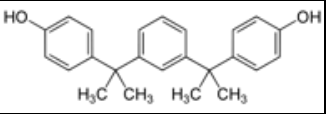
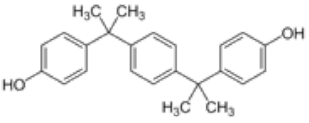
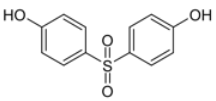
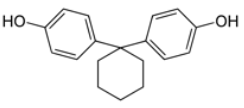
2.3.1.1 Physico-chemical properties and applications of BPA

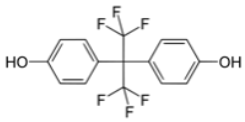
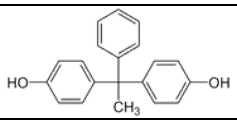
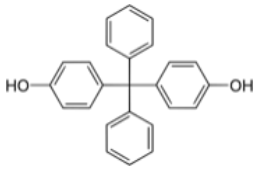
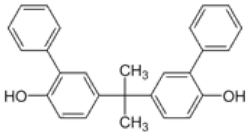
BPA is a white solid organic chemical which is synthesized by acetone and phenols with the acid catalyst. BPA is relatively hydrophobic with the Octanol−Water Partition Coefficient (log K_{ow}) of 3.3 and slightly polar owing to its two hydroxyl groups (Table 2.2). It is soluble in most of the organic solvent and alkaline solutions, but shows relatively low solubility in water (120-300 mg L^{-1}) (EFSA, 2015).

BPA was first synthesized in 1890s by a Russian chemist Aleksandr Dianin, and it was applied for commercial use in the 1950s because of its ability to make plastic durable and shatterproof (EFSA, 2015). BPA is mainly used as the monomer or additive to manufacture polycarbonate (PC) plastics and applied to produce epoxy resins, polyvinyl chloride (PVC) materials, thermal paper products and so on. Both PC plastics and epoxy resins are used as FCMs, and PC plastics are for example widely used as reusable food and drink containers, tableware, and water pipes, while epoxy resins are used as inner coatings of cans and lids of glass jars and bottles for food and beverages (Caballero-Casero et al., 2016). BPA is also used to produce BPA methacrylate, polyetherimides,

polysulphone resins and polyarylates materials, which have been used in manufacture of medical materials, microwave ware, electronical devices and vehicles in human daily life (EFSA, 2015).

Table 2.2 Physiochemical property of bisphenol compounds

Bisphenols	CAS No.	Molecular Mass (g/mol)	log K_{ow}	p*K*a	Melting point (°C)	Structure
Bisphenol A (BPA)	80-05-7	228.291	3.32	9.6	153	
Bisphenol B (BPB)	77-40-7	242.318	4.13	10.1	120.5	
Bisphenol C (BPC)	79-97-0	256.339	4.74	N/A	152.1	
Bisphenol E (BPE)	2081-08-5	214.264	3.19	N/A	123-127	
Bisphenol F (BPF)	620-92-8	200.237	2.91	7.55	162-164	
Bisphenol G (BPG)	127-54-8	312.446	6.55	N/A	127.3	
Bisphenol M (BPM)	13595-25-0	346.47	6.25	N/A	199	
Bisphenol P (BPP)	2167-51-3	346.47	6.25	N/A	165.5	
Bisphenol S (BPS)	80-09-1	250.268	1.65	8.2	245-250	
Bisphenol Z (BPZ)	843-55-0	268.356	5.0	N/A	165.5	

Bisphenol AF (BPAF)	1478-61-1	336.233	4.47	9.2	159-162	
Bisphenol AP (BPAP)	1571-75-1	290.362	4.86	N/A	182	
Bisphenol BP (BPBP)	1844-01-5	352.425	6.08	N/A	216.6	
Bisphenol PH (BPPH)	24038-68-4	380.478	7.17	N/A	240.6	

Note: information is derived from "chemspider.com"

2.3.1.2 Physico-chemical properties and applications of BPA analogues

Bisphenol analogues have similar structure to BPA and some of them are commonly used as the substitutes of BPA (Table 2.2). BPE and BPF both have very similar structure to BPA, and show slightly higher polarity and solubility than BPA. BPE is commercially used in the manufacturing of resins and plastics (Pozdnyakov et al., 2011), while the application of BPE in FCMs is not clear. BPF has a broad application including the production of plastics, epoxy resins, liners, coating of food cans as well as dental materials (Rochester & Bolden, 2015). BPS is the most polar compound (log K_{ow}=1.65) among all the bisphenol analogues. It was first made in 1869 as a dye and is commonly used to produce polycarbonate plastic, epoxy glues and thermal paper products as it is lighter and more heat-stable than BPA (Kuruto-Niwa et al., 2005). It is suggested BPS is used as an alternative of BPA in thermal printing papers (Eladak et al., 2015).

Bisphenol B (BPB), bisphenol C (BPC), bisphenol Z (BPZ), bisphenol AF (BPAF) and bisphenol AP (BPAP) are slightly less polar than BPA with a log K_{ow} values ranging from 4.13 to 5.0. BPB is a BPA derivative (synthesized by butanone and phenol) used in the manufacture of phenolic resins and has been reported with a similar estrogen-like activity as BPA (Cunha et al., 2012). BPZ is synthesized by phenol and cyclohexanone under acid conditions, and is commercially used as a precursor to make polycarbonate plastics and epoxy resins (Gregor, 2012). BPAF and BPAP are synthesized by phenol with acetophenone and hexafluoroacetone, respectively. BPAF and BPAP are used as alternatives of BPA in manufacturing paper products (EPA, 2012), while whether they have been applied in FCMs is not illustrated in the literature. BPAF is also used to produce hoses used in food processing equipment and polymers for electronic devices (LaFleur & Schug, 2011). The other bisphenol analogues (namely BPG, BPM, BPP, BPBP and BPPH) have relatively larger molecular weight and are more hydrophobic than BPA (Table 2.2), and they all show low solubility in water. The commercial applications of these compounds are not very clear.

2.3.2 Bisphenols in food

Diet is the predominant source of human bisphenol contaminants exposure, especially for BPA (Caballero-Casero et al., 2016). Indeed, exposure to BPA from food source is generally greater than that from non-food sources by at least one order of magnitude for most studied subgroups (Geens et al., 2012). As other bisphenols such as BPF, BPS and BPAF have been detected in food, monitoring bisphenol residues in food is highly important for public's health.

2.3.2.1 Levels of bisphenol residues in food

In this section, a total of 49 papers were selected from the literature and reviewed. Caballero-Casero et al. (2016) reviewed studies with at least 10 samples, while in the present review, any sampling size larger than three was included. Food samples were grouped into 17 categories including water, milk, infant formula and so on. All detailed information is presented in supplementary materials (Table S2.1).

Based on the reviewed studies, BPA is the most abundant bisphenol contaminant and is detected in almost all the food categories, followed by BPS and BPF in terms of detection frequency. BPA is often detected at relatively higher concentration in food than other bisphenols, although in some case BPF and BPS concentrations in food may exceed that of BPA (Figure 2.3). The highest BPA concentrations were reported in water in China (568 ng g^{-1}) then followed by other beverages in China (267 ng g^{-1}) and Japan (213 ng g^{-1}) (Kawamura et al., 1999; Li et al., 2006; Niu et al., 2015). Relatively high BPA levels have been also recorded in cereals, canned seafood and meat products in countries such as China, Japan and Belgium (Table S2.1).

BPA concentrations have been assessed in many food types, however studies that explored the multi bisphenol analogues in food are scarce. Generally, the levels of BPF and BPS residues in food are lower than 5 ng g^{-1}. However, in some exception cases, BPF and BPS have been detected in food at relatively greater levels. For example, BPF was detected in mustard from Switzerland at an average level of 1850 ng g^{-1} (detection frequency of 78.7%) (Zoller et al., 2016). Liao and Kannan (2014) reported that an average concentration 15.4 ng g^{-1} for BPF in vegetables from nine

cities of China was, which was about 5 times higher than the concentrations of BPA detected in the same samples (2.88 ng g^{-1}). Among all the reviewed studies, the highest BPS residue level (36.1 ng g^{-1}) were reported in canned bean products from Spain (Viñas et al., 2010). Other analogues like BPB, BPP, BPZ, BPAF and BPAP were also detected in food, and their levels are generally lower than BPA. In some cases though, the BPAP concentration detected in vegetables in China was higher than the BPA concentration in the same study, but the detection frequency of BPAP was three times lower than that of BPA (Liao & Kannan, 2014). When comparing the bisphenol residue level in food from different countries, relatively higher contamination levels have been reported in food from markets in Canada, China and Spain followed by Japan and other countries. As only papers published in English were reviewed, the information for some countries may be missing and create a bias in this assessment.

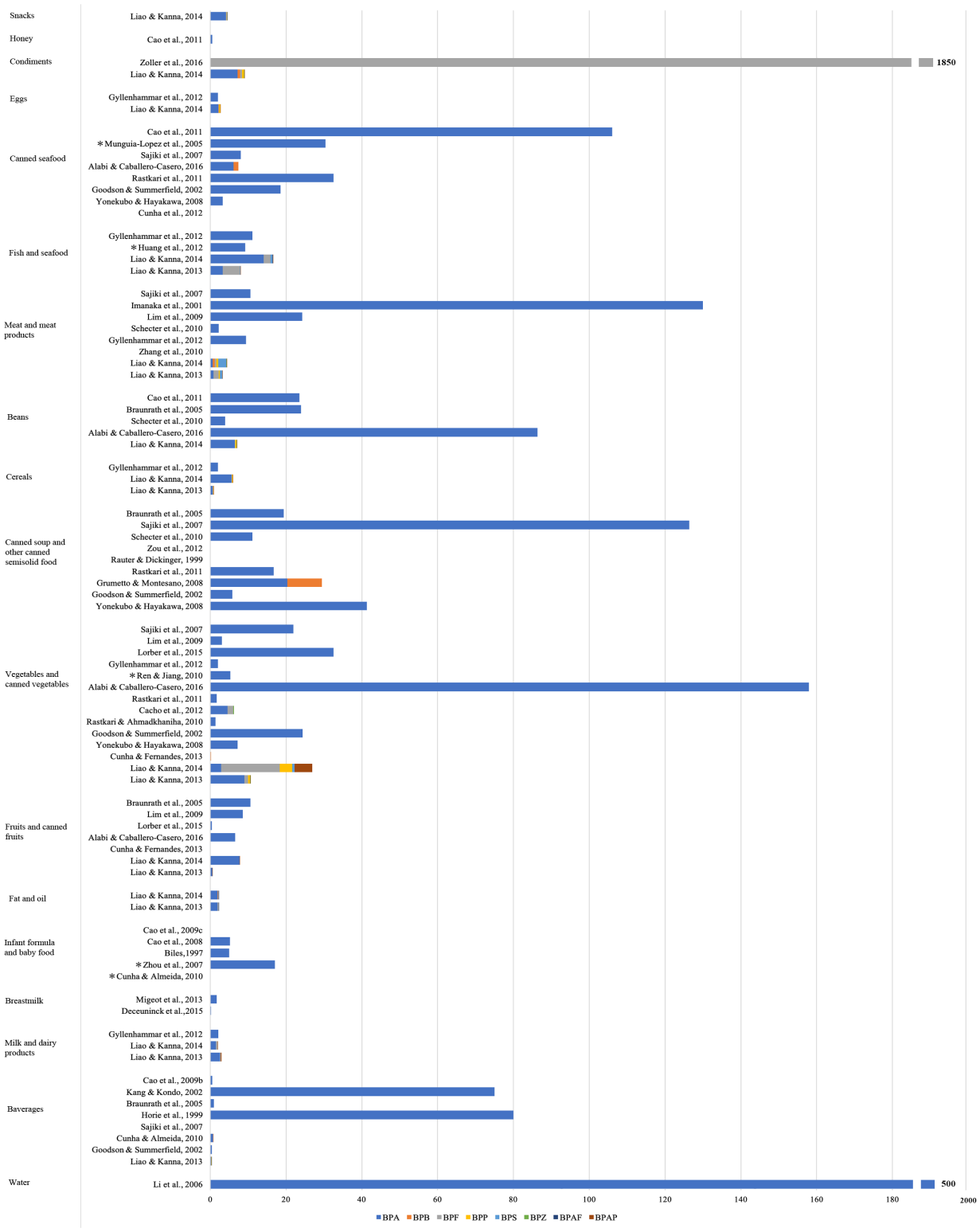


Figure 2.3 Total bisphenol levels in different food categories (generated from Table S2.1)

* data expressed on the dry weight basis.

2.3.2.2 Pathways of bisphenol residues in food

The origin of bisphenol residues in food may be traced back to the packaging materials or specific food containers, accumulation in crops and food producing animals from contaminated water and soil, or formation during food processing.

Migration from packaging materials. Migration from packaging is one of the most important sources of bisphenol residues in food. Monomers and plasticizers with low or medium molecular weights can migrate from plastic packaging materials into the food (Bui et al., 2016). As a result, canned food and packaged beverages show higher bisphenol residue level than the non-packaged food. Among all migrants originated from plastic food contact materials, BPA is the most prevalent (and the most studied).

Polyethylene (PE) is commonly used as packaging film, bags, and yogurt packaging. In a migration study, BPA was also shown to migrate from PE materials into food simulants ranging from lower than the limit of detection (LOD) to 234 ng L^{-1} (high BPA residues were detected in bread bags made of low density PE) (Fasano et al., 2012). Many epoxy resins are produced by the reaction of BPA with BPA diglycidyl ether (commonly abbreviated to BADGE, made from BPA and epichlorohydrin), which represents the second largest usage of BPA (EFSA, 2015). As a result, BPA has been frequently detected in food can containers coated with epoxy resins. BPA in infant food sold in cans ranged from 8.3 to 13.2 ng g^{-1} (Biles et al., 1997). During storage, BPA could reach 105.4 ng g^{-1} in oily food such as canned tuna (Munguia-Lopez et al., 2005). BPA increased

to about 4.2 ng g^{-1} in canned jalapeno peppers (during storage at 25°C for 70 days) (Munguia-Lopez & Soto-Valdez, 2001), while in the study of Alabi et al. (2014), BPA concentration is about 241 ng g^{-1} in canned red paper.

Other bisphenol analogues such as BPS and BPAF have also been reported to migrate from packaging materials into food. For example, BPS was reported to migrate from epoxy can coatings in canned vegetables at levels up to 175 ng g^{-1} (Viñas et al., 2010). BPAF was detected in food simulants (10% ethanol in water) in contact with the coated metal lid of glass jar (Česen et al., 2016). BPA had been used in the past as an additive for PVC, but this usage was discontinued in Europe from December 2001 (EFSA, 2015). Nonetheless, a study on stretchable PVC film used for food packaging in Spain revealed BPA migration up to 61.2 μg dm^{-2} (Lopez-Cervantes & Paseiro-Losada, 2003).

Migration of bisphenol from reused utensils. PC materials are commonly used in the manufacturing of food containers, such as jars and water bottles. Since BPA is a monomer to produce PC, and BPA is reported to leach out from PC materials. BPA migration from new baby bottles into water (at 100°C, 0.23±0.12 ng g^{-1}) was reported by a study in Norway prior to the ban of BPA-based materials in baby bottles in European Union (Brede et al., 2003; European Commission, 2011a). In a similar study in Republic of Korea, the mean level of BPA migration from new baby bottles into water was 0.03 ng g^{-1} at 40°C and 0.13 ng g^{-1} at 95 °C (Nam et al., 2010).

Accumulation in crops and food-producing animals from the environment. Many bisphenols are ubiquitous contaminants in the environment, they are frequently detected in the environment like water, air and soil (Corrales et al., 2015). Plants can rapidly absorb bisphenols, especially BPA, in water via their roots (Kang et al., 2006). BPA was also reported that accumulated in different fish species from lake and rivers in Hunan Province, China (Luo et al., 2017).

Food processing. Bisphenol compounds can also form during food processing. In a study by Zoller et al. (2016), while raw materials were BPF-free, BPF was detected only in mustard containing white mustard seed but not in other mustard products under the same processing conditions. The result indicated that BPF was formed during the processing. The authors proposed a mechanism for BPF formation involving the transformation of glucosinalbin into 4-hydroxybenzyl alcohol, a compound found in many plants, then further converted into BPF under acidic condition (Zoller et al., 2016).

2.3.2.3 Factors that affect the migration of bisphenols from FCMs into food

According to Fick's Law, in chemical systems, the diffusion of a chemical species is related to the temperature, concentration and the chemical potential. Similarly, these factors also impact the migration of contaminants to food. In this section, factors influencing bisphenols migration were summarized. Based on the reviewed studies, most of the investigations were performed for BPA.

Temperature. The higher heating temperature, the higher level of BPA migrated from can coating to food (Kawamura et al., 1999; Sajiki et al., 2007). Kang et al. (2003) reported that heating at

121°C for 30 min induced more than twice of BPA migration from can coating to food than heating at 105°C for 30 min. However, Goodson et al. (2004) reported that reheating the canned food in boiling water for 15 to 35 min did not induce significant change of BPA level in different kinds of canned food compared with unheated ones.

Fat content of food. As many of the bisphenols are lipophilic, it is not surprising to detect relatively higher bisphenol concentrations in packaged food with high-fat-content than those with low-fat-content. In reviewed literatures, canned fish and meat products contained relatively higher BPA residue levels than any other packaged food including milk and fruits (Figure 2.3). Kang et al. (2003) compared the migration of BPA from can materials into water or oil, and results indicated that BPA level were higher in oil than in water (after heating at 121°C for 30 min).

Storage time. Storage time shows mild impact on the migration of BPA from packaging materials to food. Goodson et al. (2004) reported that BPA started to migrate when contact with the packaging material but when it reached a particular level, the migration will be stable (no more migration during the extended storage).

Other factors. In addition to the factors above, other factors such as the salt content or the food type can also impact the migration of bisphenols from packaging materials. Kang et al., 2003 reported that BPA migration into a food simulant containing 5% of NaCl was about twice higher than in the simulant without NaCl. The presence of glucose in the food simulant also increased BPA migration (Kang et al., 2003). Furthermore, it has been reported that caffeine can also affect

BPA migration, as a correlation has been observed between caffeine and BPA levels in beverages (Kang & Kondo, 2002). The reason why caffeine can affect the BPA migration level is still unclear.

2.3.3 Regulations for bisphenol residue concentrations in food

According to the European Commission, the overall migration to a food of all the substances together should not exceed the limit of 60mg kg^{-1} food or 10 mg dm^{-2} of FCM (European Commission, 2014b). For BPA, the specific migration limit (SML) was fixed to 0.6 mg kg^{-1} food in 2004 and has not been changed, except for baby bottles, for which BPA is banned in EU since 2011. BPS has a SML of 0.05 mg kg^{-1} food (European Commission, 2011b), while BPF is not included in the authorized substances list for plastic materials intended to come in contact with food by European regulation (for plastic materials in contact with food, SMLs have been fixed assuming that 1 kg of food is consumed daily by a person of 60 kg for a lifetime exposure) (European Commission, 2011b).

In Canada, Food Directorate of Health Canada conducted a risk assessment on BPA in 2008 and concluded that the current dietary exposure to BPA through food packaging cannot pose any health risk to the general population, including infants and newborns (Health Canada, 2008). Health Canada recommended the general principle of ALARA (as low as reasonably achievable) to limit the exposure to BPA, because BPA does induce adverse health effects at low dose in certain animal studies (Health Canada, 2008). Similar conclusions can also be found in the document of Food and

Drug Administration in US that the application of BPA for the currently approved uses in food containers and packaging is safe (Food and Drug Administration, 2013).

2.4 Conclusions

Although the occurrence of bisphenol analogues in food has been widely investigated, there are still a number of knowledge gaps which need to be filled comprehensively. First, investigations are needed to characterize the occurrence of bisphenols other than BPA in food and the environment. As the exact composition of FCMs is not available, knowledge about the role of other bisphenols as a replacement of BPA is limited. Chemical migration processes for other bisphenols should be better described. Secondly, the fate of PRCs in food during food processing and in human digestive tract should be described to support food safety risk assessments. As most foods, especially for those of animal origin, are consumed after thermal processing (cooking), it is important to understand the fate of PRC residues in food during thermal treatment and to identify the potential thermal degradation products.

The non-targeted analysis based on the MS strategies is recognized to be powerful in identifying new contaminants as well as dealing with the analytical interferences in complex food matrices (Herrero et al., 2012). However, due to the trace level of contaminants in food and the dynamic concentration of food components, non-targeted screening remains challenging for the analysis of contaminants in food (Herrero et al., 2012). Thus, extraction methods for food in non-targeted analysis should be further developed to overcome these challenges. A major part in non-targeted

analysis is data analysis including the data processing for peak picking (or peak extraction), the statistical analysis and the compound identification. However, the influence of data processing parameters on trace residues identification has not been well illustrated so far (Krauss et al., 2010). In recent decades, a suite of commercial and open-access software has been developed to deal with chromatographic data for peak deconvolution, isotope ratio calculation, retention time alignment, statistical analysis and spectral library search. As peak picking is crucial in non-targeted analysis (Knolhoff & Croley, 2016), data processing parameters thus need to be optimized, but there is no standard protocol for this step to date. Furthermore, the suspected screening is data-dependent (which is highly relied on the library capacity and the MS-structure correlation tools), which is a big challenge for non-targeted identification. In future studies, efforts should be given to the development of comprehensive database for the various classes of contaminants and MS-structure correlation tools to improve the identification rates. The identification of all the "unknowns" in food is not feasible (Schymanski et al., 2014), and how to take the advantage of data from non-targeted analysis should be also considered in future studies.

2.5 Supplementary materials

Table S2.1 Bisphenol residues in different food (ng g^{-1})

Food categories	**References**	**Analytical method**	**BPA**	**BPB**	**BPF**	**BPP**	**BPS**	**BPZ**	**BPAF**	**BPAP**
Water	Fan et al., 2013	LC-MS/MS	0.13	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Water	Li et al., 2006	N/A[#]	568.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Beverages	Liao & Kanna, 2013	LE, HPLC-MS/MS	0.24	N/A	N/A	0.03	0.01	N/A	0.01	N/A
Beverages	Gallart-Ayala et al., 2010	SPE, HPLC-MS/MS	0.607	N/A	0.22	N/A	N/A	N/A	N/A	N/A
Beverages	Goodson & Summerfield, 2002	LE (dispersive), GC-MS	0.44	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Beverages	Cacho et al., 2012	SBSE, GC-MS	0.30	N/A	0.04	N/A	N/A	0.01	N/A	N/A
Beverages	Cunha & Almeida, 2010	LE (dispersive), GC-MS	0.74	0.04	N/A	N/A	N/A	N/A	N/A	N/A

Beverages	Sakhi et al., 2014	LE, GC-MS	0.37	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Beverages	Yumin Niu et al., 2015	LE+SPE, HPLC-MS/MS	267.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Beverages	Schecter et al., 2010	SLE, GC-MS	0.71	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Beverages	Geens et al., 2010	SPE, GC-MS	4.79	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Beverages	Lim et al., 2009	SLE, HPLC	45.51	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Beverages	Sajiki et al., 2007	LE+SPE, HPLC-MS/MS	0.13	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Beverages	Horie et al., 1999	SPE, HPLC	80.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Beverages	Braunrath et al., 2005	SLE, HPLC	0.93	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Beverages	Kim et al.,2001	GC-MS	57.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Beverages	Kawamura et al., 1999	SPE, GC-MS	213.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Beverages	Kang & Kondo, 2002	SPE, HPLC	75.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Beverages	Cao et al., 2011	SPE, GC-MS	0.74	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Beverages	Cao et al., 2009b	SPE, GC-MS	0.55	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Milk and dairy products	Liao & Kanna, 2013	LE, HPLC-MS/MS	2.55	0.01	0.134	N/A	0.04	N/A	0.03	0.19
Milk and dairy products	Liao & Kanna, 2014	LE, HPLC-MS/MS	1.47	N/A	0.38	0.04	0.01	N/A	N/A	0.06
Milk and dairy products	Gyllenhammar et al., 2012	LE, GC-MS	2.10	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Milk and dairy products	Sakhi et al., 2014	LE, GC-MS	0.72	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Milk and dairy products	Sajiki et al., 2007	LE+SPE, HPLC-MS/MS	43.50	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Milk and dairy products	Maragou et al., 2006	SPE, HPLC-MS	15.20	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Milk and dairy products	Cao et al., 2011	SPE, GC-MS	15.30	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Breast milk	Deceuninck et al.,2015	SPE, GC-MS/MS	0.23	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Breast milk	Migeot et al., 2013	SPE, UPLC-MS/MS	1.69	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Infant formula and baby food	Cunha & Almeida, 2010	LE (dispersive), GC-MS	0.09*	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Infant formula and baby food	Zhou et al., 2007	HPLC	17.00*	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Infant formula and baby food	Schecter et al., 2010	SLE, GC-MS	1.24	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Infant formula and baby food	Ackerman et al., 2010	SPE, HPLC-MS/MS	9.60	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Infant formula and baby food	Kuo & Ding, 2004	SPE, GC-MS	63.20	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Infant formula and baby food	Biles,1997	SPE, HPLC-fluorescence detection	5.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Infant formula and baby food	Cao et al., 2008	SPE, GC-MS	5.12	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Infant formula and baby food	Cao et al., 2009	SPE, GC-MS	7.20	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Fat and oil	Liao & Kanna, 2013	LE, HPLC-MS/MS	1.90	N/A	0.26	N/A	N/A	N/A	0.01	N/A
Fat and oil	Liao & Kanna, 2014	LE, HPLC-MS/MS	1.92	0.05	0.19	N/A	0.01	N/A	0.02	N/A
Fruits and canned fruits	Liao & Kanna, 2013	LE, HPLC-MS/MS	0.53	N/A	N/A	N/A	0.01	N/A	0.02	0.06
Fruits and canned fruits	Liao & Kanna, 2014	LE, HPLC-MS/MS	7.76	N/A	N/A	N/A	N/A	N/A	0.01	0.01
Fruits and canned fruits	Cunha & Fernandes, 2013	QuEChERS+ LE (dispersive), GC-MS	0.01	0.003	N/A	N/A	N/A	N/A	N/A	N/A
Fruits and canned fruits	Alabi & Caballero-Casero, 2014	LE (dispersive)+ SUPRASE, HPLC-fluorescence detection	6.50	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Fruits and canned fruits	Gyllenhammar et al., 2012	LE, GC-MS	2.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Fruits and canned fruits	Lorber et al., 2015	SLE+SPE, GC-MS	0.40	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Fruits and canned fruits	Geens et al., 2010	SLE, GC-MS	20.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Fruits and canned fruits	Lim et al., 2009	SLE, HPLC	8.60	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Fruits and canned fruits	Braunrath et al., 2005	LE (dispersive), HPLC	10.55	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Fruits and canned fruits	Cao et al., 2011	SPE, GC-MS	3.24	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Vegetables and canned vegetables	Liao & Kanna, 2013	LE, HPLC-MS/MS	8.99	N/A	1.00	0.47	0.02	0.08	0.01	0.12
Vegetables and canned vegetables	Liao & Kanna, 2014	LE, HPLC-MS/MS	2.88	N/A	15.40	3.27	0.64	0.04	0.03	4.68

Vegetables and canned vegetables	Cunha & Fernandes, 2013	QuEChERS+ LE (dispersive), GC-MS	0.07	0.003	N/A	N/A	N/A	N/A	N/A	N/A
Vegetables and canned vegetables	Yonekubo & Hayakawa, 2008	LE, HPLC-MS+HPLC-MS/MS	7.20	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Vegetables and canned vegetables	Goodson & Summerfield, 2002	LE (despersive), GC-MS	24.30	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Vegetables and canned vegetables	Rastkari & Ahmadkhaniha, 2010	SPE, GC-MS	1.33	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Vegetables and canned vegetables	Cacho et al., 2012	SBSE, GC-MS	4.56	N/A	1.50	N/A	N/A	0.17	N/A	N/A
Vegetables and canned vegetables	Rastkari et al., 2011	SPE, GC-MS	1.67	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Vegetables and canned vegetables	Alabi & Caballero-Casero, 2014	LE (dispersive)+ SUPRASE, HPLC-fluorescence detection	158.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Vegetables and canned vegetables	Ren & Jiang, 2010	GC-MS	5.31*	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Vegetables and canned vegetables	Gyllenhammar et al., 2012	LE, GC-MS	2.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Vegetables and canned vegetables	Lorber et al., 2015	SLE+SPE, GC-MS	32.50	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Vegetables and canned vegetables	Yumin Niu et al., 2015	LE+SPE, HPLC-MS/MS	33.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Vegetables and canned vegetables	Thomson and Grounds, 2005	LE, GC-MS	24.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Vegetables and canned vegetables	Schecter et al., 2010	SLE, GC-MS	2.30	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Vegetables and canned vegetables	Geens et al., 2010	SLE, GC-MS	116.30	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Vegetables and canned vegetables	Lim et al., 2009	SLE, HPLC	3.10	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Vegetables and canned vegetables	Vinas & Campillo, 2010	SPE, GC-MS	18.80	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Vegetables and canned vegetables	Sajiki et al., 2007	LE+SPE, HPLC-MS/MS	21.90	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Vegetables and canned vegetables	Cao et al., 2011	SPE, GC-MS	83.70	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Canned soup and other semisolid food	Yonekubo & Hayakawa, 2008	LE, HPLC-MS+HPLC-MS/MS	41.30	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Canned soup and other semisolid food	Goodson & Summerfield, 2002	LE (despersive), GC-MS	5.80	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Canned soup and other semisolid food	Grumetto & Montesano, 2008	SPE, HPLC	20.30	9.10	N/A	N/A	N/A	N/A	N/A	N/A
Canned soup and other semisolid food	Rastkari et al., 2011	SPE, GC-MS	16.750	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Canned soup and other semisolid food	Thomson and Grounds, 2005	LE, GC-MS	21.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Canned soup and other semisolid food	Schecter et al., 2010	SLE, GC-MS	11.10	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Canned soup and other semisolid food	Geens et al., 2010	SLE, GC-MS	29.30	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Canned soup and other semisolid food	Sajiki et al., 2007	LE+SPE, HPLC-MS/MS	126.40	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Canned soup and other semisolid food	Braunrath et al., 2005	LE (dispersive), HPLC	19.40	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Canned soup and other semisolid food	Cao et al., 2011	SPE, GC-MS	44.40	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Cereals	Liao & Kanna, 2013	LE, HPLC-MS/MS	0.61	0.02	0.08	0.13	0.01	N/A	0.01	0.01
Cereals	Liao & Kanna, 2014	LE, HPLC-MS/MS	5.60	0.01	0.13	0.13	0.04	N/A	0.01	0.03
Cereals	Gyllenhammar et al., 2012	LE, GC-MS	2.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Cereals	Sakhi et al., 2014	LE, GC-MS	0.24	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Cereals	Yumin Niu et al., 2015	LE+SPE, HPLC-MS/MS	187.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Cereals	Niu et al., 2012	SPE, HPLC-MS/MS	0.60	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Cereals	Cao et al., 2011	SPE, GC-MS	1.73	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Bean	Liao & Kanna, 2014	LE, HPLC-MS/MS	6.48	0.01	0.05	0.32	0.05	0.08	0.01	0.03
Bean	Alabi & Caballero-Casero, 2014	LE (dispersive)+ SUPRASE, HPLC-fluorescence detection	86.30	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Bean	Thomson and Grounds, 2005	LE, GC-MS	17.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Bean	Schecter et al., 2010	SLE, GC-MS	3.90	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Bean	Vinas & Campillo, 2010	SPE, GC-MS	77.70	N/A	N/A	N/A	36.10	N/A	N/A	N/A
Bean	Braunrath et al., 2005	LE (dispersive), HPLC	23.90	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Bean	Cao et al., 2011	SPE, GC-MS	23.50	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Meat and meat products	Liao & Kanna, 2013	LE, HPLC-MS/MS	0.85	N/A	1.34	0.35	0.61	0.03	0.01	0.05

Meat and meat products	Liao & Kanna, 2014	LE, HPLC-MS/MS	0.58	0.52	0.43	0.52	2.16	0.07	0.01	0.10
Meat and meat products	Gyllenhammar et al., 2012	LE-Gel permeation chromatography, GC-MS	9.40	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Meat and meat products	Sakhi et al., 2014	LE, GC-MS	3.20	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Meat and meat products	Lorber et al., 2015	SLE+SPE, GC-MS	1.50	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Meat and meat products	Yumin Niu et al., 2015	LE+SPE, HPLC-MS/MS	57.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Meat and meat products	Thomson and Grounds, 2005	LE, GC-MS	98.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Meat and meat products	Schecter et al., 2010	SLE, GC-MS	2.20	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Meat and meat products	Lim et al., 2009	LE (centrifuge), HPLC	24.29	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Meat and meat products	Imanaka et al., 2001	LE+SPE, GC-MS	130.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Meat and meat products	Sajiki et al., 2007	LE+SPE, HPLC-MS/MS	10.60	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Meat and meat products	Cao et al., 2011	SPE, GC-MS	10.50	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Fish and seafood	Liao & Kanna, 2013	LE, HPLC-MS/MS	3.23	N/A	4.63	N/A	0.02	N/A	0.01	0.01
Fish and seafood	Liao & Kanna, 2014	LE, HPLC-MS/MS	14.10	N/A	1.74	N/A	0.56	N/A	0.09	0.01
Fish and seafood	Huang et al., 2012	N/A[#]	9.18[*]	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Fish and seafood	Gyllenhammar et al., 2012	LE, GC-MS	11.10	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Fish and seafood	Sakhi et al., 2014	LE, GC-MS	7.30	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Fish and seafood	Yumin Niu et al., 2015	LE+SPE, HPLC-MS/MS	11.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Fish and seafood	Niu et al., 2012	SPE, HPLC-MS/MS	109.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Fish and seafood	Cao et al., 2011	SPE, GC-MS	0.89	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Canned seafood	Cunha et al., 2012	QuEChERS, GC-MS	0.02	0.02	N/A	N/A	N/A	N/A	N/A	N/A
Canned seafood	Yonekubo & Hayakawa, 2008	LE, HPLC-MS+HPLC-MS/MS	3.30	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Canned seafood	Goodson & Summerfield, 2002	LE (despersive), GC-MS	18.50	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Canned seafood	Rastkari et al., 2011	SPE, GC-MS	32.55	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Canned seafood	Alabi & Caballero-Casero, 2014	LE (dispersive)+ SUPRASE, HPLC-fluorescence detection	61.00	13.30	N/A	N/A	N/A	N/A	N/A	N/A

Canned seafood	Schecter et al., 2010	SLE, GC-MS	3.77	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Canned seafood	Geens et al., 2010	SLE, GC-MS	169.30	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Canned seafood	Lim et al., 2009	LE (centrifuge), HPLC	125.25	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Canned seafood	Sajiki et al., 2007	LE+SPE, HPLC-MS/MS	8.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Canned seafood	Munguia-Lopez et al., 2005	LE, HPLC fluorescence detection	30.40*	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Canned seafood	Cao et al., 2011	SPE, GC-MS	106.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Eggs	Liao & Kanna, 2014	LE, HPLC-MS/MS	2.15	0.01	0.12	0.34	N/A	N/A	0.02	0.01
Eggs	Gyllenhammar et al., 2012	LE, GC-MS	2.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Eggs	Yumin Niu et al., 2015	LE+SPE, HPLC-MS/MS	2.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Condiments	Liao & Kanna, 2014	LE, HPLC-MS/MS	7.19	0.67	0.45	0.60	0.02	0.03	0.02	0.07
Condiments	Zoller et al., 2016	LE (centrifuge), HPLC-MS/MS	N/A	N/A	1850.00	N/A	N/A	N/A	N/A	N/A
Honey	Inoue, et al., 2003	SPE, HPLC-MS	33.30	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Honey	Cao et al., 2011	SPE, GC-MS	0.50	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Snacks	Liao & Kanna, 2014	LE, HPLC-MS/MS	4.16	N/A	0.14	0.09	0.07	N/A	0.01	N/A

Note: * data was reported in dry weight.

** "+" indicates a combination of different methods.

N/A= not available in reviewed literature.

[#] Information not available, as data was derived from review articles and the original article is not accessible.

Connecting Text

Chapter 2 provided a summary of literature on the methods for analyzing plastic-related chemicals (PRCs) in food with the emphasis on the application of non-targeted strategies for the analysis of unknown contaminants in food as well as an overview of the bisphenol residues in food. After identifying the multiple knowledge gaps in this field, Chapter 3 presents the development and optimization of a non-targeted workflow in identifying unknown PRCs in food simulant with an emphasis on the optimization of the post-acquisition data processing. Chapter 3 has been published in *Talanta:* Tian, L., Lin, L., & Bayen, S. (2019). Optimization of the post-acquisition data processing for the non-targeted screening of trace leachable residues from reusable plastic bottles by high performance liquid chromatography coupled to hybrid quadrupole time of flight mass spectrometry. *Talanta*, *193*, 70-76.

Chapter 3. Optimization of the Post-Acquisition Data Processing for the Non-Targeted Screening of Trace Leachable Residues from Reusable Plastic Bottles by High Performance Liquid Chromatography Coupled to Hybrid Quadrupole Time of Flight Mass Spectrometry

3.1 Abstract

Food safety regulations for food contact materials (FCM) usually rely on the assessment of chemical migration in order to reduce human exposure to chemical residues that could leach from the FCM into the food. In this field, there is a need for non-targeted analytical tools which can identify unknown or unexpected leachable residues, and therefore avoid unwanted human exposure. In this study, a method based on high performance liquid chromatography coupled to hybrid quadrupole time of flight mass spectrometry (HPLC-QTOF-MS) was developed and optimized to investigate leachable residues from 30 reusable plastic bottles. Firstly, a method was validated for the targeted analysis of the 11 bisphenol analogues. None of the bisphenols were detected in the food simulants (ethanol/water; 50:50 v/v), indicating that all tested bottles are free of BPA, and that bisphenol analogues were not applied as BPA replacement in bottle manufacture. The effect of post-acquisition data processing parameters on the feature extraction in non-targeted analysis was then systematically investigated. Several parameters significantly reduced the number of correct identifications of some target trace residues, which confirms that data post-processing has to be carefully optimized to decrease the risk of false negatives. The optimized method was effectively applied to the 30 bottle samples, and monomethyl terephthalate was identified at trace level in food simulants in contact with Tritan^{TM} bottles (migration rate of 0.054±0.002 to 0.53±0.021 μg cm^{-2} per 10 days at 40°C). This method can therefore be applied to study the leachable residues from other FCMs and offer some novel information for human risk assessments.

3.2 Introduction

Chemical residues including unreacted monomers, plasticizers or other additives can migrate from polymeric food contact materials (FCM) into the food they are in contact with (Bui et al., 2016). As a result, packaging materials have been identified as a major dietary source of leachable

chemicals for humans (Baner et al., 1996). Chemical migration from FCMs has become a major health concern, notably since key monomers and plasticizers residues (e.g. phthalates or bisphenol A (BPA)) have been associated with adverse health effects (Fasano et al., 2012). Although each of these residues is frequently detected at the trace level, there are growing concerns about their combined effects on health (Nerin et al., 2013).

Among polymeric materials, polycarbonate (PC) is commonly used to produce food containers such as jars and reusable water bottles. BPA is a monomer used in the fabrication of PC materials, and it can migrate into food or water in the case of incomplete polymerization or hydrolysis of the polymer (Brede et al., 2003). BPA has been banned for applications in baby bottles in Canada and in the European Union (Rosenmai et al., 2014). Since then, a wide range of containers have been marketed as "BPA-free". Although this label is often highlighted, the actual chemical replacement occurring in the polymer remains unidentified on most containers. Some studies have reported that structural analogues, bisphenol B (BPB), bisphenol E (BPE), bisphenol S (BPS) and bisphenol F (BPF) may have replaced BPA for some applications (Ng et al., 2015). However, whether BPA-free products may contain one or more bisphenol analogues remains unclear.

The conventional approach to assess the safety of FCMs consists in measuring the amount of leachables migrating from the material to the food (García-Córcoles et al., 2018). High-end analytical tools such as HPLC and gas chromatography (GC) coupled with mass spectrometry (MS) are commonly reported to perform the screening and the quantification of target leachable residues in food (Gallart-Ayala et al., 2011; Munguia-Lopez & Soto-Valdez, 2001; Lorber et al., 2015; Oliveira et al., 2014). This type of analysis can be relatively time-consuming when it comes to track trace levels of multiple substances in complex food matrices (Baner et al., 1996). Therefore, food simulants (e.g. diluted acetic acid, ethanol) are often used as a surrogate for actual foodstuffs to assess chemical migration from FCMs (European Commission, 2011). The targeted analysis with tools such as LC-MS enables the detection of trace chemical residues in such food simulants, and concentrations of BPA as low as 2.4 ng mL^{-1} could be measured for example in simulants in

contact with PC baby bottles (Maragou et al., 2008). However, this targeted approach does not allow the investigation of other "unexpected" or unknown leachables (e.g. impurities, degradation products of the polymer…), hence calling for the need for non-targeted tools in this field.

Non-targeted analysis explores the occurrence of unexpected or unknown compounds, in particular when no reference information is available about the analytes, and relies on a thorough data treatment of chromatograms and mass spectra (Plaßmann et al., 2014). Non-targeted approaches follow specific analytical workflows in terms of sample preparation, instrumental analysis and data treatment (Vuckovic, 2012; Madsen et al., 2010). Non-targeted workflows, initially developed in the field of human metabolomics, are now being developed and applied in the field of food safety and food quality (Knolhoff & Croley, 2016). This approach benefits from the rapid technological developments in the fields of nuclear magnetic resonance, GC-MS and HPLC-MS (Kaufmann, 2012; Bradley & Coulier, 2007).

High resolution mass spectrometry (HRMS), for example using quadrupole time-of-flight (QTOF), allows for the collection of full scan mass spectra with a high mass accuracy. As a result, HRMS has become a popular tool to hyphenate with HPLC for the non-targeted analysis of food contaminants (Kaufmann, 2012). HRMS data (e.g. accurate mass, isotope patterns) are then explored using algorithms to generate a molecular formula and eventually suggest a structure. While HRMS provides some advantages over low resolution MS in terms of identification, there are still some challenges with the approach. First of all, peak extraction parameters (match tolerance: mass, isotope pattern and retention time) may be challenging to set because proper peak resolution (mass resolution and peak separation) is not easily achieved when chemicals are present as trace residues or as a suite of isomers (Nerin et al., 2013). The identification of "unknown" chemicals occurring at trace level is also quite challenging and requires advanced data treatment software and comprehensive database (Nerin et al., 2013). To cope with these issues, Knolhoff and Croley focused on optimizing the sample extraction and instrumental performances, as these steps significantly affect data quality and the subsequent identification of unknowns (Knolhoff &

Croley, 2016). To date, the influence of chromatographic data processing parameters on the detection of trace leachables has not been studied, although this step may be equally important (Nerin et al., 2013).

In the present study, a non-targeted workflow was developed and optimized to study leachables from polymeric reusable water bottles. More specifically, the objectives of this study were (i) to validate the analytical performances of a HPLC-QTOF-MS based method for the detection of trace model substances (eleven bisphenol analogues) in simulants in contact with the plastic bottles; (ii) to optimize the data treatment parameters in order to detect and identify trace substances using a non-targeted workflow, using the suite of bisphenols spiked at trace level as challenge samples and (iii) to apply the optimized non-targeted workflow to identify "unknown" leachable migrants in food simulants in contact with the plastic bottles.

3.3 Materials and method

3.3.1 Reagent, material and standards

Formic acid and ammonium acetate (both MS grade), and HPLC grade solvents (water, methanol and ethanol) were purchased from Fisher Scientific (Hampton, USA). Standards of BPA (IUPAC: 4,4'-(propane-2,2-diyl)diphenol, purity ≥ 99%), BPF (IUPAC: 4,4'-methylenediphenol, purity ≥ 98%), BPS (IUPAC: 4,4'-sulfonyldiphenol, purity ≥ 98%) were purchased from Sigma-Aldrich (St. Louis, USA). BPB (IUPAC: 4,4'-(2,2-butanediyl)diphenol, purity ≥ 98%) was purchased from Toronto Research Chemicals (Toronto, CA). Bisphenol C (BPC) (IUPAC: 4,4'-(2,2-propanediyl)bis(2-methylphenol), purity ≥ 99%), BPE (IUPAC: 4,4'-(1,1-ethanediyl)diphenol, purity ≥ 98%), bisphenol P (BPP) (IUPAC: 4,4'-(1,4-phenylenedi-2,2-propanediyl)diphenol, purity ≥ 99%), bisphenol Z (BPZ) (IUPAC: 4,4'-(1,1-cyclohexanediyl)diphenol, purity ≥ 99%), bisphenol AF (BPAF) (IUPAC: 4,4'-(1,1,1,3,3,3-hexafluoro-2,2-propanediyl)diphenol, purity ≥ 99%), bisphenol AP (BPAP) (IUPAC: 4,4'-(1-phenyl-1,1-ethanediyl)diphenol, purity ≥ 99%),

bisphenol BP (BPBP) (IUPAC: 4,4'-(diphenylmethylene)diphenol, purity ≥ 98%), monomethyl phthalate (≥97%), monomethyl isophthalate (≥ 97%), monomethyl terephthalate (≥ 97%) were purchased from Sigma-Fluka (St. Louis, USA). Stock solution of each individual bisphenol was prepared in methanol (100 mg L^{-1}). A working standard mixture solution of the 11 bisphenol analogs was prepared weekly at a concentration of 1 mg L^{-1} in methanol, stored in amber glass vials at -20°C in the dark. To reduce the background contamination, glass materials were used in place of plastic materials. All glassware was baked at 320°C for 4 hours and rinsed with methanol prior to use. A range of reusable bottles (n=30) were purchased in local markets in the region of Montreal (Canada). Detailed information about the bottles is presented in Table 3.1.

Table 3.1. Information on bottle samples collected from the Canadian market

Sample code	Materials	Color	Contact area (cm^2)	Country of origin
UT1	Unknown	Transparent	189.9	China
UT2	Unknown	Transparent	194.9	China
UT3	Unknown	Transparent	205.6	China
UT4	Unknown	Transparent	183.4	China
UG1	Unknown	Green	191.5	China
UG2	Unknown	Green	175.2	China
UG3	Unknown	Green	194.9	China
UG4	Unknown	Green	181.3	China
UG5	Unknown	Green	191.5	Malaysia
UR1	Unknown	Red	175.2	China
UR2	Unknown	Red	174.7	China
UR3	Unknown	Red	194.9	China
PT1	Polypropylene	Transparent	188.4	China
PT2	Polypropylene	Transparent	238.8	Malaysia
PT3	Polypropylene	Transparent	194.9	China
PG1	Polypropylene	Green	238.8	Malaysia
PG2	Polypropylene	Green	194.9	China
PG3	Polypropylene	Green	194.9	Thailand
PR1	Polypropylene	Red	238.8	Malaysia
PR2	Polypropylene	Red	162.1	China
PR3	Polypropylene	Red	219.6	China
TT1	Tritan ™	Transparent	179.6	China

TT2	Tritan ™	Transparent	187.0	China
TT3	Tritan ™	Transparent	179.6	China
TG1	Tritan ™	Green	181.3	USA
TG2	Tritan ™	Green	181.3	China
TG3	Tritan ™	Green	187.0	China
TR1	Tritan ™	Red	187.0	China
TR2	Tritan ™	Red	181.3	China
TR3	Tritan ™	Red	181.3	China

Note: the contact area was calculated from the diameter of the bottle and the height of solvent (all the bottles contained the same volume (250 mL) of simulant).

3.3.2 Migration test

All the plastic wraps, label stickers and straws were removed from bottles prior to the test. Twenty mL of HPLC water was used to rinse the inner surface of each bottle by shaking the bottle manually during at least 15 seconds in order to rinse off dusts and other residues. Each bottle was filled with 250 mL of ethanol/water (50:50, v/v) used as a food simulant for the migration test, as reported by others and following the recommendations of the Europe Commission Regulation 10/2011/EU (Fasano et al., 2012; Kubwabo et al., 2009). While the thirty bottles had different geometric shapes and dimensions (capacity ranging from 250 to 1000 mL), they were filled with the same volume (see below for normalization of the leaching quantities). The bottle openings were then covered with aluminum foil to prevent any migration from the lid (often made of a different plastic material). The bottles were then placed in preheated oven at 40°C for 10 days. Five glass amber vials filled with about 20 mL of ethanol/water were used as procedural blanks, and stored in the oven under the same conditions. Five quality control (QC) samples were prepared as identical pooled samples in which 20 μL of each sample in the study were mixed together (Dervilly-Pinel et al., 2015).

3.3.3 Instrument condition

Samples were then analyzed with an Agilent 1290 Infinity II liquid chromatogram (LC) system (Agilent technologies, Santa Clara, USA) coupled to a 6545 QTOF mass spectrometer (Agilent Technologies, Santa Clara, USA) operating in the negative electrospray ionization mode. The LC separation was conducted on a Poroshell 120 Phenyl Hexyl (Agilent Technologies; 2.7 µm × 3.0 mm × 100 mm) fitted with a Poroshell 120 EC-C18 (2.7 µm × 3.0 mm ×10 mm) guard column. The mobile phase (0.2 $mL.min^{-1}$) consisted in a mixture (gradient mode) of A= water and B = methanol (both containing 0.1% formic acid). The percentage of organic mobile phase B increased linearly as follows: 1min, 5% 15-20 min 100% and 20.10 min 5%. The injection volume was set to 10 µL and the column temperature was maintained at 20°C. Nitrogen was used as the drying gas (325°C). The gas flow was 5 L min^{-1}. Samples were run in the All Ions mode (collision energy: 0; 10; 20 40 V) with a fragmenter energy of 175 V. MS data was acquired in the 50-1700 *m/z* range.

3.3.4 Data treatment

For the targeted analysis of bisphenols, data were analyzed using Agilent MassHunter Quantitative analysis (B07.00) software. For non-targeted analyses, the extraction and the identification of the compounds were performed with the MassHunter Profiling software series, which is recognized as a robust computer tool for the treatment of LC-MS data (Nerin et al., 2013). Data were first aligned using Agilent MassHunter Profinder (B.06.00) based on the optimized different data processing parameters obtained from section 3.3.6 (see Results section 3.4).

The statistical comparison of the chemical profiles among the samples was completed using MassHunter Profiler Professional (MPP, version B14.0). Samples were grouped according to their type (“blank” and “sample”) and material (“unknown”, “polypropylene” and “Tritan”). Principal components analysis (PCA) was applied to identify common or unique features among sample groups. PCA data treatment is recognized as an effective method for data grouping (Tengstrand et al., 2013; Cotton et al., 2014). A “fold change” analysis in MPP was applied to identify features

whose intensity is significantly in one group compared to the others. Molecular formulas were generated from the exact mass and the isotopic patterns. Finally, formula with the lowest mass errors and with the most similar relative ion abundance ratios were selected by software as the top candidate and compared with the library database. The Agilent Extractables & Leachables LC/QTOF PCDL containing 1006 compounds (360 with MS/MS spectra) was used as a database in this work.

3.3.5 Method performances for the detection of the eleven bisphenol analogues in simulants

The first objective was to validate the overall method performances for the eleven bisphenol analogues. For this purpose, calibration standards were prepared at six different concentrations (ranging from 5 to 100 µg L^{-1}). The linearity of the instrument response was assessed from these standards for each analyte. The method detection limit (MDL) was calculated as three times of standard deviation of the blank signals in the food simulant matrix. The precision of method was assessed based triplicate analysis. Matrix effect was estimated by comparing the slopes of standard curves in pure solvent and matrix-matched calibration curves (containing the same level of bisphenols in the food simulants). The matrix effect percentage was calculated based on Equation (1) (Cheng et al., 2017).

$$\text{Matrix effect (\%)} = \left(1 - \frac{S_m}{S_0}\right) \times 100\% \qquad \text{Equation (1)}$$

, where S_m is the slope for matrix-matched calibration curve, and the S_0 is the slope for calibration curve in pure solvent.

3.3.6 Optimization and validation of the non-targeted workflow

The second objective of this study was to optimize and validate the data treatment parameters for the detection and the identification of substances present at trace level in food simulants using a non-targeted workflow (Supplementary materials - Figure S3.1). This task consisted in challenging the data treatment in detecting “blindly” individual bisphenols spiked at trace levels in the

simulants (20 µg L^{-1}). This level was in the range of those reported in other studies on BPA migration (Maragou et al., 2008; Kang & Kondo, 2002). The eleven bisphenol analogues cover a broad range of polarity (log K_{ow} ranging from 1.65 to 6.08) and compounds spread neatly in the chromatogram (Figure 3.1). Different combinations of data file batches (with a different set of blank, non-spiked and spiked samples; see below) were analyzed with Agilent Profinder (B06.00) with the goal to identify the conditions leading to (i) the maximum number of features (compounds or compound adducts), and (ii) the complete identification of all 11 bisphenols. Under real conditions, a leachable may be detected only in a few samples of a batch (Munguia-Lopez & Soto-Valdez, 2001; Kubwabo et al., 2009). Therefore, the influence of the frequency of samples containing the unknown chemical in a batch was also studied. Different batches, all containing 30 samples and 4 blanks, were selected as follows: "Batch A" contained only samples (30) spiked with bisphenols; Batch B1, B2 and B3 contained 5 different spiked samples (each batch was made of a different set of spiked samples); Batch C1, C2 and C3 contained only one spiked sample (all different).

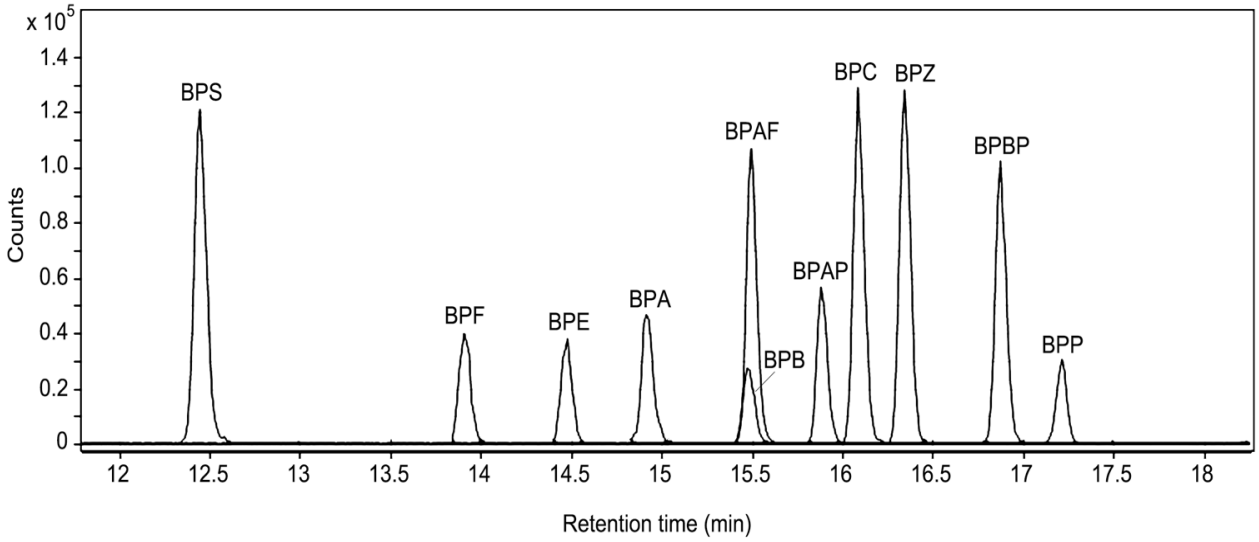


Figure 3.1 Chromatogram for bisphenol standard

Data treatment was conducted using the "*Targeted Feature Extraction*" mode, which extracts all the features in the samples and compares the resulting mass and mass spectra information with a

library (database). Data were first extracted using the initial set of parameters (Table 3.2), which are mostly the default values in the software. The number of extracted features and the number of the accurate detections of the bisphenols were then compared for different sets of data processing conditions. For Isotope peak spacing tolerance range, values from 1 to 50 ppm were tested. For *Expansion values for chromatogram extraction*, the tested range was 10-50 ppm (the default value is 35 ppm). Although HRMS was used to record the data, wide *Expansion values for chromatogram extraction* value are recommended to adequately describe the peak shape for substances with low abundance (e.g. trace contaminants). Similar value have been also reported by other algorithms (Tautenhahn et al., 2008).

Table 3.2. Initial set of parameters applied for feature extraction

Parameter	**Initial Value**
Isotope peak spacing tolerance range	7 ppm
Expansion values for chromatogram extraction (*m/z*) (+/-)	35 ppm
Limit EIC extraction range (expected RT +/-)	1.5 min
Peak filter (absolute height)	≥200 counts
Limit to the largest 2000 features	Not selected
Score filter: "don't match when < 70 " and "do not match if the unobserved second ion's abundance is expected to be > 200"	Not selected
Integrator method	Agile 2
Peak spectra: spectra to include how much percent of average scan	>10%
TOF spectra: exclude if above how much saturation	20%
Post processing: Find by formula peak filter (absolute height)	≥200 counts

3.4 Results and discussion

3.4.1 Method validation (targeted analysis of bisphenols)

Instrument response for calibration standards was linear ($r^2 > 0.98$) for all the analytes in the 5 to 500 μg L^{-1}concentration range (Table 3.3). Matrix effects below 20% are generally treated as mild effects (Kmellár et al., 2008), and in this study, matrix effects were <2% (enhancement of signal).

The estimated MDLs ranged from 0.15 to 0.95 μg L^{-1}, which is comparable with the detection limit for bisphenols in literature using similar tools (Carabias-Martinez et al., 2006). Intra-day and inter-day precisions were assessed (n=5 replicates) and the relative standard deviations (RSD) were below 4% in both cases, reflecting an overall satisfactory precision for the analysis.

Table 3.3. Retention times (RT) and method performances for bisphenols

Compounds	RT (min)	Linearity (r^2)	Intra-day precision (RSD, %)	Inter-day precision (RSD, %)	MDL (μg L^{-1})
BPS	12.5	0.9992	1.6	2.6	0.88
BPF	13.9	0.9910	3.9	2.2	0.71
BPE	14.5	0.9829	1.9	2.9	0.90
BPA	14.9	0.9913	1.8	1.9	0.81
BPB	15.5	0.9868	2.9	2.9	0.98
BPAF	15.5	0.9931	2.0	2.6	0.20
BPC	15.9	0.9889	0.6	2.1	0.41
BPAP	16.1	0.9944	1.9	0.9	0.89
BPZ	16.4	0.9860	0.9	1.8	0.46
BPBP	16.9	0.9904	3.2	1.8	0.69
BPP	17.2	0.9983	1.0	1.2	0.55

3.4.2 Occurrence of bisphenol analogues in real samples

None of the 11 bisphenol analogues was detected in any of the food simulants that had been in contact with the plastic bottles for 10 days. Based on individual MDLs, it can be concluded that specific migration for each of the bisphenols was therefore below 0.00015 mg dm^{-2} per 10 days. As the LODs of the method were satisfactory, this result suggests that neither BPA nor the bisphenol analogs were used in the manufacturing of the plastic bottles, regardless they were labeled “BPA-free” or not.

3.4.3 Optimization of the post-acquisition data processing parameters

The non-targeted workflow was then optimized and the results of the feature extraction and targeted compound identification are presented in Table 3.4. In this table, the number between brackets is the total number of features. Using the initial conditions, all the target bisphenols were detected in the spiked samples regardless of how many spiked samples were present in the sample batch (Table 3.4). The present workflow was able to identify all the 11 bisphenols even for a batch (34 samples) containing just a single sample with traces of the bisphenols, supporting the potential of non-targeted workflows to identify "unknown" or "unexpected" leachables.

The influence of the various parameters on the total number of extracted features and identification of the target bisphenols was then assessed (Table 3.4). Parameters such as "*Peak filter (absolute height)*", "*Post processing: find by formula peak filter (absolute height)*" and "*Limit to the largest 2000 features*" had a significant influence on the number of features ($p < 0.01$, t-test) and the correct identification of the target bisphenols ($p < 0.01$, t-test). For example, the number of bisphenols detected decreased from 11/11 to 3/11 when the "*Post processing: Find by formula peak filter (absolute height)*" value increased from 200 to 2500. This finding highlights the risk of false negatives for trace contaminants if this parameter is not properly optimized. However, roughly four times more features were obtained when the "*Post processing: Find by formula peak filter (absolute height)*" value decreased from 2500 to 200. While a higher number of features may seem attractive, a lot of these "additional" features may correspond to noise. Their treatment may result in possible false positives and a waste of time at a later stage to remove them. An increasing number of features was also obtained when the "*Expansion values for chromatogram extraction*" value was increased from 10 to 50 ppm. Parameters such as "*Expansion values for chromatogram extraction*" could be expected to impact the number of bisphenols correctly detected because of co-eluting interfering compounds, but this was not the case in the present study. A careful examination of the extracted chromatograms confirmed the absence of co-eluting isobaric

interferences in the real samples for the bisphenols, probably explaining the lack of impact of the "*Expansion values for chromatogram extraction*" for this type of food simulants.

The present results illustrate that post-acquisition data processing parameters need to be carefully optimized for the study of trace chemicals. As the main objective of this study was to identify "unknown" compounds at trace levels, parameters were set to conditions corresponding to all the target bisphenols could be identified. This corresponded overall to the initial conditions (Table 3.2), except that "*Isotope peak spacing tolerance range*" was set to 1 ppm and "*Peak spectra: spectra to include average scan*" to >30%.

To the best of our knowledge, this is the first paper reporting the optimization of the data processing parameters for non-targeted database-screening analysis on leachable residues. Moschet et al. used the Agilent series software for the non-targeted screening of micropollutants in water, and reported the parameters for database matching by "*Find by Formula*" analysis in Agilent Qualitative analysis (B07.0) in their study (Moschet et al., 2017). These parameters were similar as the ones used for database searching in the present study, though Moschet et al. did not report how these parameters were selected. A possible reason for the research gap in the field of data processing optimization could be that studying each parameter is time-intensive. The central processing unit (CPU) capacity is a key factor controlling data processing time, which has been reported in a database-screening study (Wood et al., 2017). In this study, a 3.6GHz CPU with 32GB RAM computer was used, which offered two to three times faster than the computer with 16GB RAM in study of Wood et al. (2017). With the abovementioned computer in the present study, each set of conditions in Table 3.4 required approximately 1-3 hours of computer time, i.e. 14 days for the entire optimization of the data treatment.

Table 3.4 Influence of the different data treatment parameters on the output of the feature extraction

Parameter		**Batch A**	**Batch B1**	**Batch B2**	**Batch B3**	**Batch C1**	**Batch C2**	**Batch C3**
Initial conditions		11/11 (17323)	11/11 (21754)	11/11 (21456)	11/11 (20424)	11/11 (19140)	11/11 (21410)	11/11 (19570)
Isotope peak spacing tolerance range +/-	1ppm	11/11 (15068)	11/11 (20189)	11/11 (19478)	11/11 (20175)	11/11 (19518)	11/11 (21523)	11/11 (18524)
Isotope peak spacing tolerance range +/- (7 ppm→initial)	7ppm	11/11 (17323)	11/11 (21754)	11/11 (21456)	11/11 (20424)	11/11 (19140)	11/11 (21410)	11/11 (19570)
Isotope peak spacing tolerance range +/-	50ppm	11/11 (17257)	11/11 (20883)	11/11 (20465)	11/11 (19268)	11/11 (19931)	11/11 (18749)	11/11 (17675)
Expansion values for chromatogram extraction	10 ppm	11/11 (17323)	11/11 (21754)	11/11 (21456)	11/11 (20424)	11/11 (19140)	11/11 (21410)	11/11 (19570)
Expansion values for chromatogram extraction (35 ppm→initial)	35 ppm	11/11 (19284)	11/11 (23247)	11/11 (23175)	11/11 (23558)	11/11 (29160)	11/11 (23080)	11/11 (22463)
Expansion values for chromatogram extraction	50 ppm	11/11 (21900)	11/11 (28163)	11/11 (28931)	11/11 (27547)	11/11 (29260)	11/11 (28483)	11/11 (28301)
Limit EIC extraction range for RT +/-	0.15 min	11/11 (17323)	11/11 (21754)	11/11 (21456)	11/11 (20424)	11/11 (21338)	11/11 (21410)	11/11 (21560)
Limit EIC extraction range for RT +/-	0.5 min	11/11 (17323)	11/11 (21754)	11/11 (21456)	11/11 (20424)	11/11 (21338)	11/11 (21410)	11/11 (21560)
Limit EIC extraction range for RT (1.5 min→initial)	1.5 min	11/11 (17323)	11/11 (21754)	11/11 (21456)	11/11 (20424)	11/11 (21338)	11/11 (21410)	11/11 (21560)
Limit EIC extraction range for RT (un take)	No limit	11/11 (17323)	11/11 (21754)	11/11 (21456)	11/11 (20424)	11/11 (21338)	11/11 (21410)	11/11 (21560)
“do not match when < 70” and “do not match if the	Selected	11/11 (17717)	11/11 (17843)	11/11 (18469)	11/11 (17209)	11/11 (18220)	11/11 (16504)	11/11 (16528)

unobserved second ion's abundance is expected to be > 200"								
Isotope abundance score is 60→100	100	11/11 (18430)	11/11 (19864)	11/11 (19280)	11/11 (17370)	11/11 (17506)	11/11 (17447)	11/11 (18430)
Peak filter: absolute height ≥ 100 counts *	100	11/11 (17323)	11/11 (21754)	11/11 (21456)	11/11 (20424)	11/11 (21338)	11/11 (21001)	11/11 (20685)
Peak filter: absolute height ≥ 200→initial	200	11/11 (17323)	11/11 (21754)	11/11 (21456)	11/11 (20424)	11/11 (19140)	11/11 (21410)	11/11 (19570)
Peak filter: absolute height ≥ 1000 counts	1000	9/11 (13457)	9/11 (19094)	9/11 (14482)	9/11 (11937)	9/11 (19457)	9/11 (11755)	9/11 (11535)
Peak spectra: spectra to include average scan > 1%	1%	11/11 (17681)	11/11 (19058)	11/11 (21302)	11/11 (20215)	11/11 (21890)	11/11 (19544)	11/11 (18566)
Peak spectra: spectra to include average scan > 10%→initial	10%	11/11 (17323)	11/11 (21754)	11/11 (21456)	11/11 (20424)	11/11 (19140)	11/11 (21410)	11/11 (19570)
Peak spectra: spectra to include average scan > 30%	30%	11/11 (16969)	11/11 (19001)	11/11 (20962)	11/11 (20033)	11/11 (21381)	11/11 (19698)	11/11 (19479)
Peak spectra: spectra to include at apex of peak	Selected	11/11 (17038)	11/11 (20842)	11/11 (22308)	11/11 (20978)	11/11 (20838)	11/11 (19462)	11/11 (19658)
TOF spectra: exclude if above saturation (20%→initial)	20%	11/11 (17323)	11/11 (21754)	11/11 (21456)	11/11 (20424)	11/11 (19140)	11/11 (21410)	11/11 (19570)
TOF spectra: exclude if above saturation	40%	11/11 (17323)	11/11 (21754)	11/11 (21456)	11/11 (20424)	11/11 (19140)	11/11 (21410)	11/11 (19570)
TOF spectra: exclude if above saturation (un take)	--	11/11 (17323)	11/11 (21754)	11/11 (21456)	11/11 (20424)	11/11 (19140)	11/11 (21410)	11/11 (19570)

Post processing (find by formula peak filter: ≥ 200→counts initial	200	11/11 (17323)	11/11 (21754)	11/11 (21456)	11/11 (20424)	11/11 (19140)	11/11 (21410)	11/11 (19570)
Post processing (find by formula peak filter: ≥ 1000 counts	1000	10/11 (7771)	7/11 (10857)	7/11 (10842)	7/11 (11376)	5/11 (10902)	5/11 (10757)	5/11 (10933)
Post processing (find by formula peak filter: ≥ 2500 counts	2500	3/11 (3565)	3/11 (4953)	3/11 (4950)	3/11 (5214)	4/11 (4913)	4/11 (4785)	4/11 (5121)
Post processing: limit to the largest 2000 features	Selected	3/11 (2000)	3/11 (2000)	3/11 (2000)	3/11 (2000)	3/11 (2000)	3/11 (2000)	3/11 (2000)

Note: * When the peak height filter is selected to be 100, the Post processing “Find by formula” peak filter was not selected.

3.4.4 Non-targeted analysis of leachable residues

Based on the optimized parameters obtained in section 3.4.3, non-spiked samples and blanks were aligned and extracted by Agilent Profinder (B06.00) under "*Targeted Feature Extraction*" mode. Features were extracted, and then the data were analyzed using Agilent Mass Profiler Professional 14.0. PCA discriminated QCs, blank and samples (see supplementary Figure S3.2-A), confirming some actual differences between samples and procedure blanks. In this study, the features detected in both the bottle extract samples and the blanks were not considered for further data treatment. Then, samples for different materials (unknown, polypropylene and Tritan^{TM}) were compared using the "*fold change*" analysis (p value cut-off: 0.05, multiple testing correction: Benjamini-Hochberg). There was no significant difference amongst the features in the "unknown" group and the polypropylene group ($p > 0.1$). However, six features were exclusively found in the Tritan^{TM} group (Figure S3.2-B). The feature (*m/z* of 179.0344) with the highest abundance and the highest matching score (91.2%) was identified as monomethyl phthalate using the PCDL database (Figure 3.2), and was selected for further identification (see below).

As a comparison, PCA results were also assessed for data obtained using non-optimized conditions for the data post-processing (detailed parameters described in the supplementary Table S3.1). In that case, QCs, blank and samples did not group satisfactorily (see supplementary Figure S3.2-C), and there were no distinct differences among the 3 types of polymers (supplementary Figure S3.2-D). This clearly illustrates further the importance of optimizing data post-processing parameters as inconclusive data would be obtained otherwise.

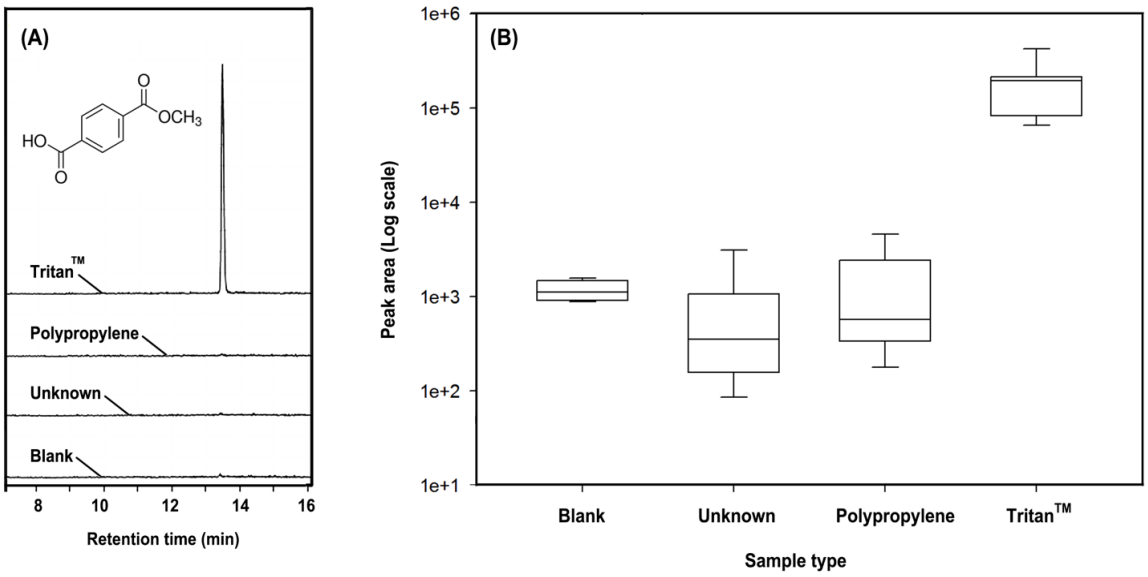


Figure 3.2 (A) Comparative chromatograms extracted at *m/z* 179.0344 in various samples and structure of mono-methyl terephthalate; (B) Boxplot of the peak area of feature *m/z* 179.0344 (retention time = 13.5 min) among the various groups of samples.

3.4.5 Identification and quantification of non-targeted compound

The identity of the suspected compound was finally confirmed using analytical standards. As the identity of monomethyl phthalate was predicted based on the exact mass and isotope signature, monomethyl isophthalate and monomethyl terephthalate, two isomers of monomethyl phthalate, were also considered as potential candidates. Individual standards (1 mg L^{-1}) of the three phthalates were prepared in methanol and were analyzed using HPLC-QTOF-MS. Ammonium acetate (0.1 mM) was added into the mobile phase A instead of formic acid and mobile phase B was changed to pure methanol to improve the chromatographic separation of the 3 isomers. Results confirmed that the feature with *m/z* 179.0344 is monomethyl terephthalate (retention time difference <0.1 min, exact mass difference <10 ppm and main fragment ions matched with standard). The reason why monomethyl terephthalate leached out of Tritan™ bottles is not determined in this study, and this is the first time that monomethyl terephthalate is reported as leachable residue from Tritan™

bottles. Dimethyl terephthalate has been reported as a monomer in the production of Tritan^{TM} materials (Osimitz et al., 2012), and monomethyl terephthalate could be a hydrolytic product of unreacted dimethyl terephthalate.

A five-point calibration curve was built for monomethyl terephthalate in the 5 to 500 µg.L^{-1} range to quantify the concentration of monomethyl terephthalate in the extracts from the Tritan^{TM} bottles. Monomethyl terephthalate ranged from 10 to 99 µg L^{-1} in the extracts, which corresponds to a migration of 0.054±0.002 to 0.53±0.021 µg cm^{-2} of monomethyl terephthalate from bottles to food simulant over 10 days.

3.5 Conclusions

In this study, a non-targeted workflow was optimized based on HPLC-QTOF-MS analysis to investigate leachable residues from reusable bottles. First, a method based on HPLC-QTOF-MS was first validated for the targeted analysis of the 11 bisphenol analogues (low MDL, high accuracy). None of the bisphenols were detected in food simulants indicating that all tested bottles are free of BPA and bisphenol analogues were not applied as BPA replacement in bottle manufacture. The effect of data post-processing parameters on the feature extraction in non-targeted analysis was then systematically investigated, and results confirmed these parameters need to be carefully optimized to extract all the features and identify them accurately. The optimized method was effectively applied to identify monomethyl terephthalate at trace level in food simulants in contact with Tritan^{TM} bottles. This method can therefore be applied to study the leachable residues from other FCMs and offer some novel information for human risk assessments. Future studies will focus on optimizing non-targeted workflows for more complex food matrices instead of food simulants.

3.6 Acknowledgements

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3.8 Supplementary materials

Table S3.1. Data post-processing parameters under different conditions

Parameter	**Optimized condition**	**Non-optimized condition**
Isotope peak spacing tolerance range	1 ppm	50 ppm
Expansion values for chromatogram extraction (m/z) (+/-)	35 ppm	50 ppm
Limit EIC extraction range (expected RT +/-)	1.5 min	No limit
Peak filter (absolute height)	≥200 counts	≥1000 counts
Limit to the largest 2000 features	Not selected	Not selected
Score filter: "don't match when < 70 " and "do not match if the unobserved second ion's abundance is expected to be > 200"	Not selected	Not selected
Integrator method	Agile 2	Agile 2
Peak spectra: spectra to include how much percent of average scan	>30%	>10%
TOF spectra: exclude if above how much saturation	20%	Not selected
Post processing: Find by formula peak filter (absolute height)	≥200 counts	≥1000 counts

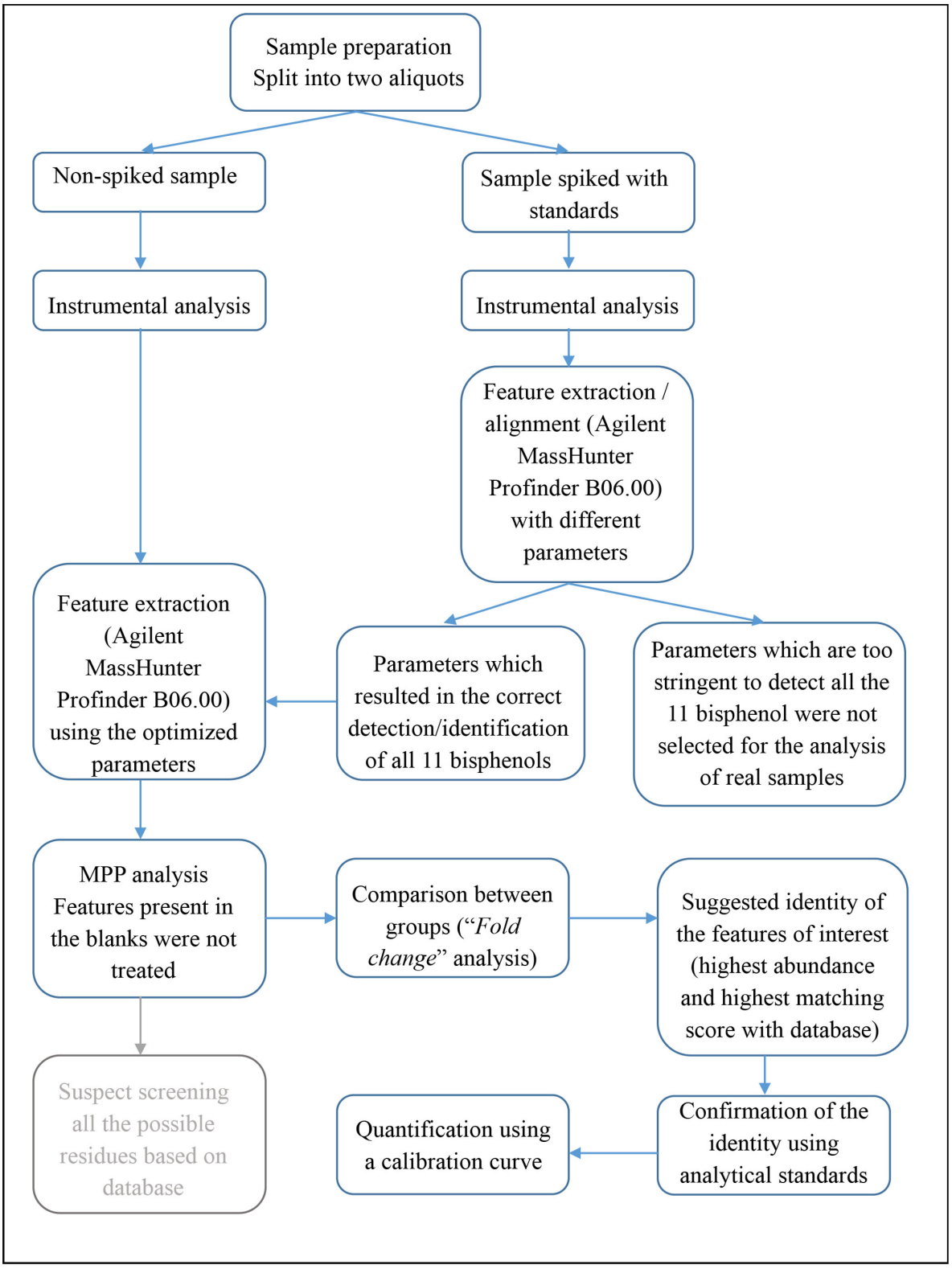

Figure S3.1 Non-targeted workflow with post-acquisition data processing optimization

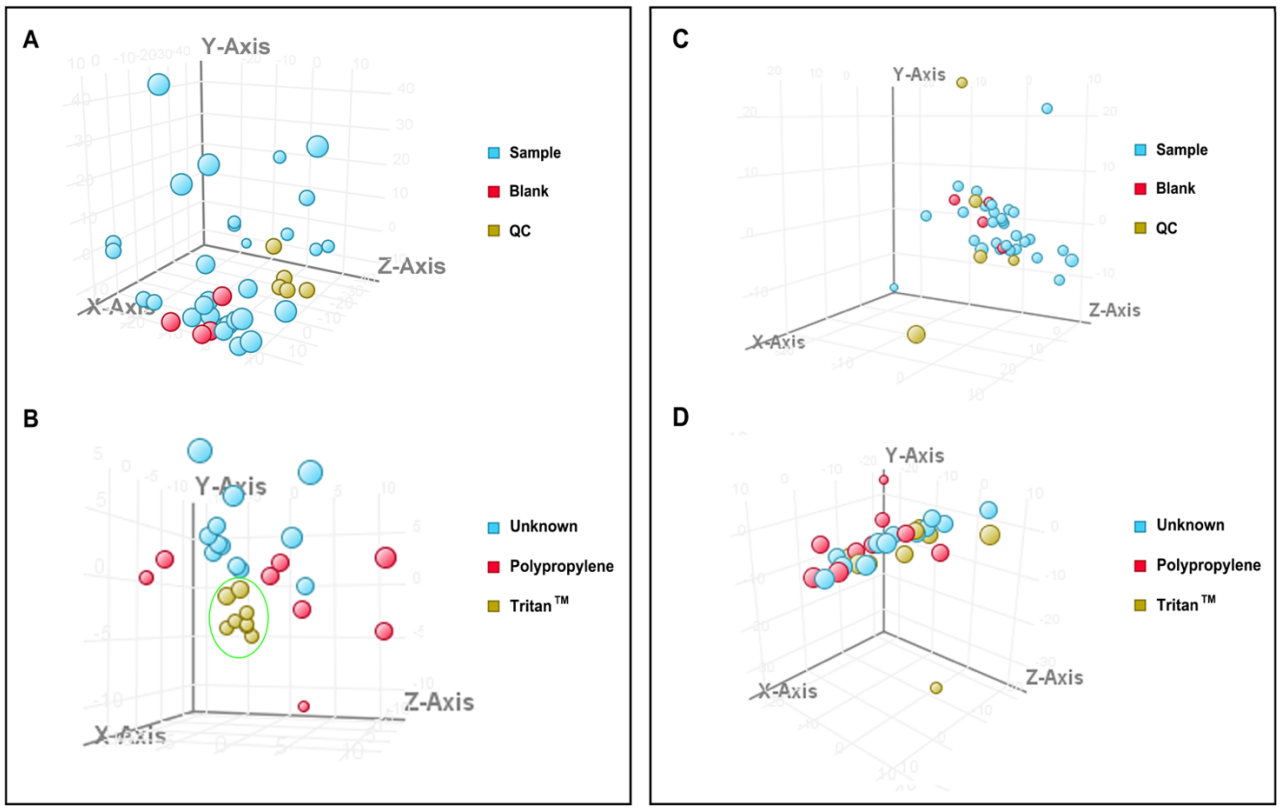

Figure S3.2. Principal Component Analyses conducted on datasets obtained for (i) the samples, blanks and QCs with optimized (Fig. A) and non-optimized data post-processing parameters (Fig. C); and (ii) samples from the 3 groups of polymers: "unknown", polypropylene and Tritan ™ with optimized parameters (Fig. B) and non-optimized data post-processing parameters (Fig. D).

Connecting Text

Chapter 3 reported the effect of data post-processing parameters on the feature extraction in non-targeted analysis, and results confirmed the importance of data post-processing parameters optimization in non-targeted analysis. In Chapter 4, the optimization of a non-targeted workflow will be applied for a more complex food matrix instead of food simulants. Pike fish fillets is used as a case study. Chapter 4 has been published in the Journal *Environmental Pollution*: Tian, L., Verreault, J., Houde, M., & Bayen, S. (2019). Suspect screening of plastic-related chemicals in northern pike (Esox lucius) from the St. Lawrence River, Canada. *Environmental Pollution*, *255*, 113223.

Chapter 4. Suspect Screening of Plastic-related Chemicals in Northern Pike (*Esox lucius*) from the St. Lawrence River, Canada

4.1 Abstract

Environmental contaminant monitoring traditionally relies on targeted analysis, and very few tools are currently available to monitor "unexpected" or "unknown" compounds. In the present study, a non-targeted workflow (suspect screening) was developed to investigate plastic-related chemicals and other environmental contaminants in a top predator freshwater fish species, the northern pike, from the St. Lawrence River, Canada. Samples were extracted using sonication-assisted liquid extraction and analyzed by high performance liquid chromatography coupled with quadrupole time of flight mass spectrometry (HPLC-QTOF-MS). Ten bisphenol compounds were used to test the analytical performances of the method, and satisfactory results were obtained in terms of instrumental linearity (r^2>0.97), recoveries, (86.53-119.32%), inter-day precision and method detection limits. The non-targeted workflow data processing parameters were studied, and the peak height filters (peak filtering step) were found to influence significantly the capacity to detect and identify trace chemicals in pike muscle extracts. None of the ten bisphenol analogues were detected in pike extracts suggesting the absence of accumulation for these chemicals in pike muscle. However, the non-targeted workflow enabled the identification of diethyl phthalate (DEP) and perfluorooctanesulfonic acid (PFOS) in pike extracts. This approach thus can be also applied to various contaminants in other biological matrices and environmental samples.

4.2 Introduction

As a result of the widespread use of plastic materials in our societies, a range of chemicals that we are defining as plastic-related chemicals (PRCs) may be disseminated into the environment, contaminate food or accumulate in human and animal tissues (Alimba & Faggio, 2019; Guzzetti et al., 2018). PRCs include the initial components of the plastics (e.g., monomers and polymer fragments, and additives) and their degradation products. PRCs such as bisphenol A (BPA), dibutyl phthalate (DBP) and diethyl phthalate (DEP) have been detected in water, soil and biota

(Mohapatra et al., 2010; Salgueiro-González et al., 2015; Selvaraj et al., 2015). PRCs including BPA and nonylphenol have also been detected in raw or treated wastewater in France (Dupuis et al., 2012), and may be transferred to vegetables when contaminated wastewater is used for irrigation (Lu et al., 2015). At polluted sites, aquatic organisms and notably fish have also been shown to accumulate BPA and nonylphenol (Lee et al., 2015). The exposure-related effects of these compounds have not been comprehensively documented, but BPA as well as some phthalates (e.g., DBP and DEP) have been shown to exhibit endocrine disruptive properties in humans and aquatic organisms (Tiwari, Sahu, & Pandit, 2016; Wei et al., 2011). For example, it was reported that BPA can induce estrogenic response in japanese medaka (*Oryzia latipes*) (Kang et al., 2002; Lee et al., 2015).

Conventional environmental monitoring relies on targeted analysis. In this approach, analytical methods are developed to detect and quantify a suite of target contaminants using authentic analytical standards. Analytical tools such as high performance liquid chromatography (HPLC) or gas chromatography coupled to various detectors (e.g., mass spectrometer (MS) and diode array) are commonly used for this purpose (Luo et al., 2017; Petrović et al., 2001; Sun et al., 2014). MS detectors have become the dominant tools for the trace level analysis of contaminants in environmental and biological samples as they are not only highly sensitive, but also allow for effective multi-residue analyses (Sosa-Ferrera, Mahugo-Santana, & Santana-Rodríguez, 2013). Although targeted analysis is the current standard in environmental monitoring programs (Petrovic et al., 2004), it is mostly limited to a finite list of known contaminants.

Recently, a new approach called non-targeted analysis (which includes suspect screening) has emerged with the objective to identify "unknown" contaminants. This approach has the potential to provide a more comprehensive overview of chemical pollution and will therefore improve ecological and human health risk assessments. Non-targeted analysis involves the automated detection of compounds in the complex chromatograms and a comparison of the mass spectra with compound libraries, and therefore often relies on high-quality data acquisition and libraries (Díaz

et al., 2012; Krauss, Singer, & Hollender, 2010). The identification of "unknown" also relies on the application of advanced data processing methods which can filter chromatograms and mass spectra, and thus minimize interferences (Bletsou at al., 2015; Krauss et al., 2010). Chromatography coupled to high resolution MS (HRMS) has become popular in non-targeted analysis of emerging contaminants, and has been applied to matrices such as water, wastewater, soil and aquatic organisms (Blum et al., 2017; Hollender at al., 2017; Kong et al., 2018). Accurate mass measurement using HRMS including time-of-flight MS (TOF-MS) allows for structural predictions with a high degree of confidence (Sosa-Ferrera et al., 2013). However, there are still some challenges with a HRMS-based approach related to both the sample preparation (clean-up) and data processing (von Eyken & Bayen, 2019). Several methods have been applied for the extraction of environmental organic contaminants in fish tissues including Soxhlet-based extraction (Mortazavi et al., 2013), pressurized liquid extraction (Carabias-Martínez et al., 2005), microwave-assisted extraction (Basheer, Obbard, & Lee, 2005; Bayen, Lee, & Obbard, 2004), sonication-assisted extraction (Zhang, Bayen, & Kelly, 2015), and QuEChERS (quick, easy, cheap, effective, rugged and safe) (Anastassiades et al., 2003; Luo et al., 2017). These methods generally yield satisfactory recoveries (about 80 to 110%) for targeted contaminants (e.g., BPA and 4-nonylphenol). In the context of non-targeted analysis, a strategy may be to extract chemicals with a wide range of polarity and decrease the number of cleanup steps to capture a maximum of "unknown" contaminants, but this remains to be tested for many environmental matrices. Unlike the sample preparation, the influence of data processing parameters have not been well examined so far (Krauss et al., 2010). In recent decades, a suite of commercial and open-access software has been developed to deal with chromatographic data for peak deconvolution, isotope ratio calculation, retention time alignment, statistical analysis and spectral library search. For example, Agilent MassHunter and Waters ChromaLynx (within MassLynx) have been reported for the identification of contaminants in environmental samples (Ibáñez et al., 2008; Wood et al., 2017).

Data processing parameters need to be optimized, but there is no standard protocol for this step to date.

The overall objective of the present study was to develop and apply a non-targeted analytical approach to determine PRCs in muscle of wild-caught northern pikes. In addition to the validation of sample preparation steps, this research made an in-depth investigation of the influence of data processing parameters on the identification of trace residues in fish tissues. Specific objectives were to: (i) validate the analytical performance of a targeted analysis method of ten bisphenols in pike muscle; (ii) optimize the data processing parameters of the peak filtering step to detect and identify trace substances in pike muscle extracts using a non-targeted workflow; (iii) to apply the optimized non-targeted workflow to identify "unknown" PRCs and potentially other environmental contaminants in pike muscle and (iv) to perform a multivariate analysis of the non-targeted to compare muscle of pike collected upstream and downstream of a major wastewater treatment plant in Montreal in the St. Lawrence River (Canada). The northern pike (*Esox lucius*) was selected for this study as it is a top predator fish, which can impact the fish communities by shaping the composition, abundance and distribution of their prey (Forsman et al., 2015). Pike is important for recreational and commercial fishing (Forsman et al., 2015). It is consumed by many populations in Canada (Blanchet et al., 2013), and has been shown to accumulate a wide range of organic contaminants (Binelli & Provini, 2003; Kierkegaard et al., 2004; Reinling, Houde, & Verreault, 2017). Specifically, occasionally elevated tissue concentrations of polychlorinated biphenyls, polybrominated diphenyl ethers and perfluoroalkyl substances have been reported in pike (Houde et al., 2013; Kierkegaard et al., 2004; Reinling et al., 2017). The abundance of certain of these contaminants has led to the development of food safety guidelines for the consumption of pike, for example in Canada (information retrieved from Ontario government official website: https://www.ontario.ca/page/eating-ontario-fish-2017-18).

To date, there has been no application of non-targeted analysis of PRCs in muscle tissues of predatory fish species.

4.3. Materials and methods

4.3.1 Chemicals

To avoid the plastic-related contamination during experimental procedure, the use of plastic labware was limited as much as possible, and only polypropylene centrifuge tubes and filter syringes, and polytetrafluoroethylene HPLC sample vial caps were used. All the glass vials used for standard preparation (Thermo Fisher Scientific, Waltham, US) and the other glassware were baked at 320°C for 4 h before use.

Formic acid (LC-MS grade) and HPLC-grade solvents (water and methanol) were purchased from Fisher Scientific (Hampton, USA). Analytical standards of BPA (purity ≥ 99%), bisphenol F (BPF; purity ≥ 98%), bisphenol S (BPS; purity ≥ 98%), perfluorooctanesulfonic acid (PFOS; purity ≥88%), diethyl phthalate (DEP; purity ≥ 99%), bisphenol E (BPE; purity ≥ 98%), bisphenol P (BPP; purity ≥ 99%), bisphenol Z (BPZ; purity ≥ 99%), bisphenol AF (BPAF; purity ≥ 99%), bisphenol AP (BPAP; purity ≥ 99%), bisphenol BP (BPBP; purity ≥ 98%), paxilline (purity ≥ 98%) and chloramphenicol (purity ≥ 99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Bisphenol B (BPB, purity ≥ 98%) was purchased from Toronto Research Chemicals (Toronto, ON, Canada). Stock solutions of the individual bisphenols were prepared in methanol (100 mg L^{-1}). Working standard mixture solutions of the ten bisphenol analogues were prepared weekly at a concentration of 1 mg L^{-1} in methanol. PFOS, DEP and paxilline stock solutions were also prepared in methanol at 1 mg L^{-1} before use. All the standard stocks were prepared less than 24 hours before the experiment and stored in amber glass vials in the freezer (-20°C) prior to analysis.

4.3.2 Fish sampling and preparation

Pike samples were collected in late May to early June 2014 and 2015 using a beach seine in the St. Lawrence River (QC, Canada), 4 km upstream (Iles de Boucherville (IB), n=12) and 4 km downstream (Îlet Vert (IV), n=14) of the point of discharge of a major primary wastewater

treatment plant. Pikes were euthanized using a clove oil solution (250 mg L^{-1}), and then pike filets were prepared (boneless and skinless) and stored in the freezer (-80°C) before sample extraction. Detailed information on pike sample collection and preparation can be found in Reinling et al. (2017).

4.3.3 HPLC-MS sample preparation

Pike muscle samples were first thawed at room temperature and then homogenized using a Waring stainless steel blender (Torrington, USA). About 2 g (fresh weight) of fish filet was weighed and transferred into a 50-mL polypropylene centrifuge tube. Ten mL of methanol was added into the tube. Tubes were sonicated using a Branson 3510 sonication bath (40 KHz) for 10 min and centrifuged at 8000 rpm for 10 min. The supernatant was collected and filtered through 0.22 μm filter (Norm-Ject, Tuttlingen, Germany) into HPLC amber glass vials. The extracts were kept at -20°C freezer until HPLC analysis.

4.3.4 Instrument condition

Samples were analyzed using an Agilent 1290 Infinity II LC system (Agilent technologies, Santa Clara, USA) coupled to a 6545 quadrupole TOF-MS (Agilent Technologies, Santa Clara, USA) operating in both the positive (ESI+) and negative (ESI-) electrospray ionization modes. The LC separation was conducted on a Poroshell 120 Phenyl Hexyl column (Agilent Technologies; 2.7 μm × 3.0 mm × 100 mm) fitted with a Poroshell 120 EC-C18 (2.7 μm × 3.0 mm ×10 mm) guard column. The mobile phase (0.2 mL min^{-1}) consisted of a mixture (gradient mode) of water (solvent A) and methanol (solvent B), both containing 0.1% formic acid. The percentage of organic mobile phase B increased linearly and the gradient was as follows: started at 5% for 1 min, then increased to 100% at 1-15 min, 15-20 min kept 100% and at 20 min, the eluent was restored to the default conditions for 5 min to re-equilibrate the column for the next injection. The injection volume was set to 10 μL and the column temperature was maintained at 20°C. Nitrogen was used as the drying

gas (325°C). The gas flow was 5 L min^{-1}. Samples were run in the All Ions MS/MS mode at four collision energies (0; 10; 20; 40 V) with a fragmenter energy of 200 V. MS data was acquired in the *m/z* 50-1700 range.

4.3.5 Quality assurance / quality control

Solvent blanks (methanol) and procedural blanks were injected with each batch of six samples. Procedural blanks were prepared the same way as the pike muscle samples (section 4.3.3). Features present in solvent blanks and procedural blanks were treated as "background features" and were not considered in the non-targeted analysis. Pooled quality control (QC) samples are critical when performing non-targeted analysis using liquid chromatography–mass spectrometry (Gika et al., 2014). Five pooled QC samples were prepared by mixing equal aliquot (10 µL) of each individual fish muscle sample together. QC samples were analyzed randomly in the HPLC-MS batch among the pike muscle samples to control for RT drifts, mass measurement reproducibility as well as the instrumental background noise (for detailed information, see section 4.4.4).

4.3.6 Chromatographic data processing

4.3.6.1 Analytical performance validation by targeted screening

HPLC-MS data was analyzed using Agilent MassHunter Quantitative analysis (B.07.01) software to confirm whether 10 targeted bisphenol analogues were present in fish samples and procedural blanks (targeted screening). The most abundant isotopes of the $[M-H]^-$ ion were used as quantifier for the ten bisphenols (Table 4.1). The chromatogram extraction window was ± 10 ppm for mass and ± 0.5 min for retention time (RT).

Table 4.1 Targeted screening mass and RT for bisphenol analogues

Compound	**RT (min)**	***m/z* ***
BPS	12.5	249.0222
BPF	13.9	199.0759

BPE	14.5	213.0916
BPA	14.9	227.1072
BPB	15.5	241.1229
BPAF	15.5	335.0507
BPAP	16.1	289.1229
BPZ	16.4	267.1385
BPBP	16.9	351.1385
BPP	17.2	345.1855

* *m/z* for the most abundant isotope of $[M-H]^-$ for each compound was calculated using Exact Mass Calculator (https://www.sisweb.com).

4.3.6.2 Non-targeted identification

Non-targeted data processing was conducted using the Agilent MassHunter Profiling software series following a workflow developed in a previous study (Tian, Lin, & Bayen, 2019) with an updated version of software (Figure 4.1). Agilent MassHunter Profinder (B.08.00) was used for data alignment and molecular feature extraction with the data processing parameters obtained from section 4.3.6 (see Results section 4.4.3). A personalized library with the 10 bisphenols (including MS/MS spectra) was built using Agilent PCDL Manager B07.00. to understand the effect of data processing parameters on compound identification with the suspect screening approach. Comparisons of the chemical profiles among the samples were conducted using MassHunter Profiler Professional (MPP, version B14.0). Samples files after alignment (CEF file) were grouped according to their type ("blank", "sample" and QC). A "principal components analysis" (PCA) was first produced to check common and unique components among sample groups as well as QCs and blanks. A "fold change" analysis in MPP was applied on the feature abundance to identify molecular features that may be significantly more concentrated in a sample group compared to blanks or other groups.

Formulae were generated based on the exact mass and the isotopic patterns for selected molecular features. Formulae with the lowest mass errors and with the most similar relative ion abundance ratios were selected by software as the top candidate and compared with the library for

identification. The Agilent Extractables & Leachables LC/QTOF PCDL containing 1006 compounds and Agilent Water LC/QTOF PCDL containing 1000 compounds were used as databases. Several features were reported with a library matching score >80% based on a default matching score distribution as follows: mass score contributes to 50%, isotope abundance score contributes to 25% and isotope spacing score contributes to 25% of the total 100% matching score, then those features and suggested identity were checked manually using Agilent Qualitative analysis (B07.00) by "*Find by Formula*" option. Only those features with an isotope signature matching score above 85% were selected for predicted RT checking. A relatively lower isotope signature matching score threshold (60%) was reported in von Eyken & Bayen (2019). However, based on the observations obtained for selected pike samples in the present study, relatively large mass differences between the features and the suggested formula (>5 ppm) were obtained when selecting an isotope signature matching score below 85%. For each candidate, the log octanol-water partition coefficient (log K_{ow}) was obtained from ChemSpider and PubChem website (Table S4.3), and used to predict the RT of the compound during the LC separation. The RT prediction is a simple and effective way to reduce the rate of false positive candidates in non-targeted analysis using LC/MS (Bade et al., 2015). In the present study, a simple linear relationship ($r^2 > 0.91$) between log K_{ow} value and RT was built based on the eleven known analytes (ten bisphenols and chloramphenicol standard), selected to cover a broad log K_{ow} value range (1.00 - 6.25; see Table S4.3) (Bade et al., 2015). The relationship between log K_{ow} value and RT in HPLC analysis is not always linear (Creek et al., 2011), thus a wide RT window (± 5 min) was applied in this study in order to reduce false discovery rate (Bade et al., 2015; Ji et al., 2009). For example, log K_{ow} 5.25 has been reported for PFOS, which positions this compound between BPA (log K_{ow} 3.32) and BPBP (log K_{ow} 6.08). In that case, the RT for a molecular feature potentially identified as PFOS should fall in between (± 5 min) the actual RTs of BPA and BPBP (Table S4.3). Suspect candidates failing to match this criterion were excluded from further identification. When the RT matched the criterion, MS/MS information for the candidates was searched in the literature to support the

identification of tentative structures. Finally, selected analytical standards were purchased to validate the identity of a molecular feature as a proof of concept (Figure 4.1).

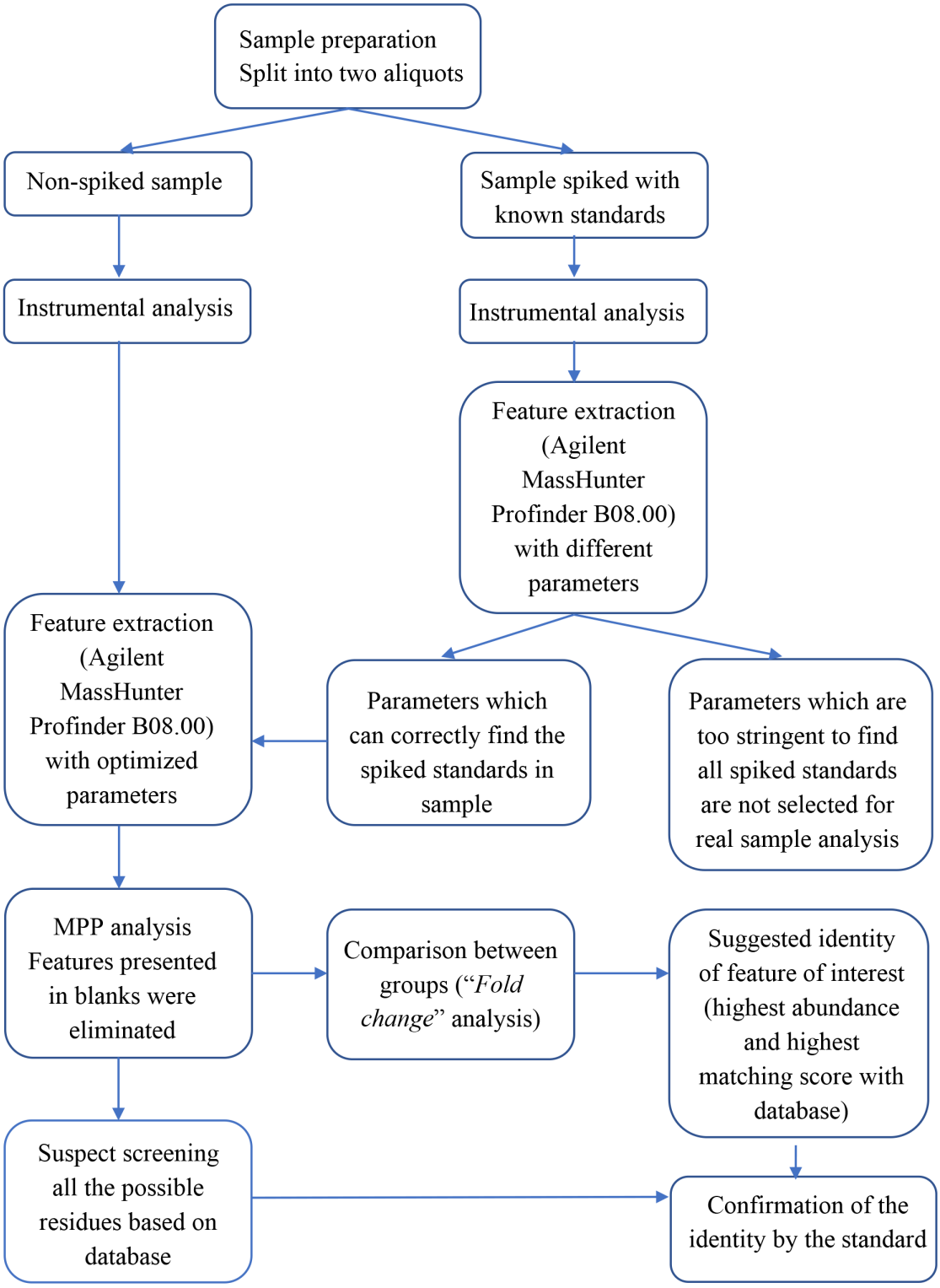

Figure 4.1 Non-targeted workflow

4.3.7 Method validation

4.3.7.1 Method performance for the targeted analysis of bisphenol analogues in fish muscle

The first objective was to validate the method performance for the ten bisphenol analogues in pike muscle tissues. Quality assurance (QA) included procedural blanks (n=3) as well as solvent and matrix-matched calibrations (six levels ranging from 5 to 500 µg L^{-1}). The linearity of the instrument response was assessed using the analysis of standards in methanol. The method detection limit (MDL) was calculated as three times of standard deviation of blanks divided by the slope of the calibration curve (Bayen et al., 2013). The relative standard deviation (RSD) for the inter-day precision was calculated based on the analysis of three replicates of spiked fish muscle extracts at 20 µg L^{-1} on different days (n=3). An inter-day precision (RSD) lower than 15% was judged acceptable (Rezk et al., 2015). Matrix effects were studied through the comparison of the slope of matrix-matched calibration curve and the slope of calibration curve in pure solvent. The matrix effect percentage was calculated based on Equation (1) (Cheng et al., 2017).

$$\text{Matrix effect (\%)} = \left(1 - \frac{S_m}{S_0}\right) \times 100\% \qquad \text{Equation (1)}$$

where S_m is the slope for matrix-matched calibration curve, and the S_0 is the slope for calibration curve in pure solvent.

4.3.7.2 Optimization and validation of the non-targeted workflow

A "challenge test" was conducted to assess appropriate data processing conditions in terms of data alignment and molecular feature extraction with Agilent Profinder (B08.00). This test consisted in challenging the data processing in detecting individual bisphenols spiked at trace level in the fish extracts. The use of known standard mixtures is recognized as a useful tool to develop a non-targeted workflow for complex matrices prior to the testing of actual samples (Knolhoff & Croley, 2016). Therefore, pike muscle extracts that were confirmed to be free for bisphenols were spiked

with the 10 bisphenol analogues at a level (20 μg L^{-1}) corresponding to those reported for BPA in catfish (*Arius maculatus*) (Lee et al., 2015).

The resulting HPLC-QTOF-MS data files were grouped into batches with different combinations of blanks, non-spiked and spiked samples. The frequency of some contaminants in environmental samples can be quite low. As an example, a detection frequency (4.35%) was reported for BPAF in aquatic food (Liao & Kannan, 2013). The number of spiked samples in a batch was then assessed as a parameter possibly influencing the correct identification. Therefore, different batches of data files were prepared as follow: Batch A contained the files of 4 blanks and 26 samples all spiked with bisphenols; Batch B contained the files of 4 blanks and 26 samples including 5 spiked and 21 non-spiked samples (Batch B1, B2 and B3 were prepared and the spiked samples in each batch were different from the other two sub-batches); Batch C contained the files of 4 blanks and 26 extraction samples with only one spiked sample (Batch C1, C2 and C3 were prepared and the spiked sample in each batch was different from the other two sub-batches).

Data processing was conducted in the "*Targeted Feature Extraction*" mode with the personalized library with 10 bisphenols (section 4.3.5). Data was initially processed using the default parameters (Table S4.1) reported in a previous study (Tian et al., 2019).

4.4 Results and discussion

4.4.1 Method validation

Mean mass measurement error (MMME) was calculated based on the method reported by Brenton and Godfrey (2010) to evaluated the accuracy of QTOF-MS in the present study. The MMME ranged from -1.2 to 3.6 ppm for 10 bisphenols in pure methanol, and from 0.4 to 5.9 ppm in spiked pike muscle extracts (Table S4.2). Dasenaki et al. (2015) have reported the similar mass accuracy for multi-contaminants in sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) muscle extracts analyzed by LC-QTOF-MS.

A chromatogram depicting the signals for the ten targeted bisphenols in spiked pike muscle extracts is presented in Figure 4.2. Instrument response for calibration standards was linear (r^2>0.97) in the 5 to 500 μg L^{-1} concentration range in pike extract. Matrix effects were between 2% and 11% (suppression of signal) for BPP, BPBP and BPAF (Figure S4.1), while for other compounds, the matrix effects were below 1%, which are generally treated as mild matrix effects (Kmellár et al., 2008). Except for BPB, the estimated MDLs were lower than 2.53 ng g^{-1} for all the bisphenols, which is comparable to what Lee et al. (2015) reported for the freshwater fish (*Tilapia zillii* and *Chanos chanos*). The inter-day precision (RSD) in the present study was below 6%, which is similar to the value reported in the literature for contaminants analysis in tilapia and carp tissue extracts (Zhang et al. 2011) and can reflect an overall satisfactory precision for the analysis (Rezk et al., 2015). The mean extraction recovery was corrected by the matrix matched calibration curve (Table 4.2).

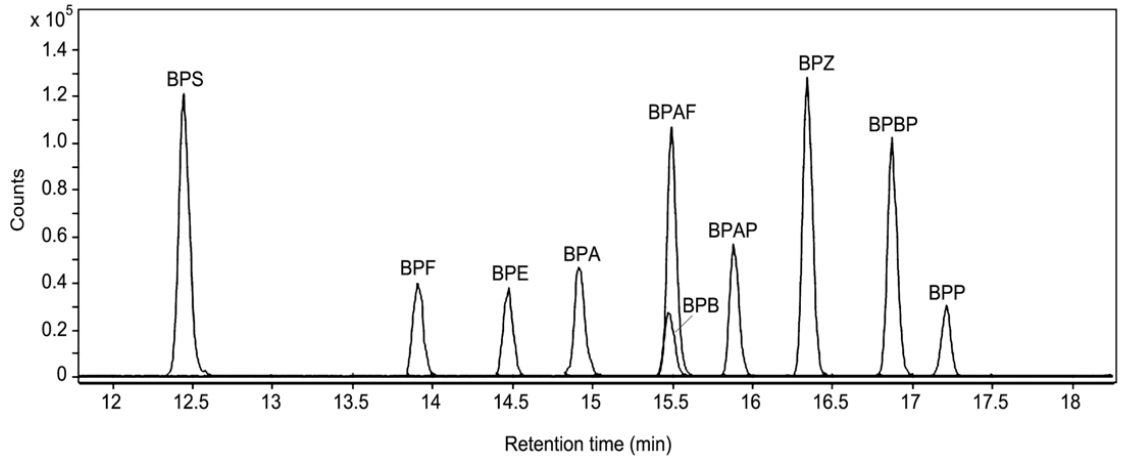

Figure 4.2 HPLC-QTOF-MS chromatogram for ten bisphenols in spiked northern pike muscle extracts

Table 4.2 Instrument performance and recoveries for targeted bisphenols in pike extracts

Bisphenol	Linearity (r^2)	RSD (%)	MDL (ng g^{-1})	Recovery(n=3)
BPS	1.00	1.41	0.10	100.91 ±0.57%
BPF	0.99	2.40	1.40	119.32 ±4.67%

BPE	0.97	2.74	2.53	118.04 ±5.64%
BPA	0.98	2.98	0.90	92.20 ±4.60%
BPB	0.98	2.24	3.56	98.13 ±0.57%
BPAF	1.00	1.66	0.05	95.38 ±0.59%
BPAP	0.99	6.04	1.60	95.28 ±2.05%
BPZ	0.99	1.29	1.00	105.16 ±0.14%
BPBP	0.98	6.02	0.10	104.80 ±10.79%
BPP	0.99	3.74	0.90	86.53 ±2.33%

4.4.2 Occurrence of bisphenol analogues in fish samples

The detection of BPA in several fresh water fish species has been reported in the literature (Lee et al., 2015; Wei et al., 2011). However, in the present study, none of the ten bisphenol analogues were detected in the muscle tissues of the 26 northern pikes collected in the St. Lawrence River. As the sensitivity of the method was satisfactory (see section 4.4.1), these results suggest that neither BPA nor the other bisphenol analogues were accumulated at detectable levels in pike muscle in this study.

4.4.3 Validation of the non-targeted workflow and the optimization of data processing parameters

The non-targeted workflow was validated and the results of the molecular feature extraction and targeted compound identification are presented in Table 4.3. Using the default data processing conditions, all the target bisphenols were detected in the spiked pike samples regardless of the number of spiked samples in a sample batch (Table 4.3). The present workflow was able to identify all 10 bisphenols even for a batch (26 samples and 4 blanks) containing a single sample with traces of the bisphenols, supporting the potential of non-targeted workflows to identify "unknown" or "unexpected" residues in complex matrix (pike muscle extracts). Similarly to a previous study on food simulants (Tian et al., 2019), parameters like "*Peak filter (absolute height)*", "*Post processing: find by formula peak filter (absolute height)*" and "*Limit to the largest 2000 features*", which all related to peak height, showed a significant influence on the number of model compound identification (all $p<0.01$, t-test). For example, the number of bisphenols detected decreased from

10/10 to 2/10 when the "*Post processing: Find by formula peak filter (absolute height)*" value increased from 200 to 2500 counts. Similarly, the "*peak filter (absolute height)*" also impacted the identification of model contaminants in all batches (Table 4.3). As the main objective of this study was to identify "unknown" compounds at trace levels in pike muscle tissues, parameters corresponding to complete identification of the target bisphenols were selected for the rest of the study, which essentially corresponded to the default conditions described in Table S4.1.

This is the first study validating the impact of data processing parameters on the non-targeted screening of contaminants in a fish matrix. Similar parameters were also reported in the literature for environmental samples though the rationale for selected these parameters was no available (Moschet et al., 2017; Wood et al., 2017). A possible explanation for the absence of studies in the field of data processing optimization could be because the study of each parameter is time-intensive (Tian et al., 2019). Using a 3.6GHz CPU with 32GB RAM computer, each set of conditions in Table 4.2 required approximately 1-3 hours of computer time, and therefore a total of 15 days for the present study.

Table 4.3 Impact of different parameters on model compounds identification

Parameter		Batch A	Batch B			Batch C		
			B1	B2	B3	C1	C2	C3
Default conditions		10/10	10/10	10/10	10/10	10/10	10/10	10/10
Expansion values for chromatogram extraction	**10 ppm**	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	35 ppm	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	50 ppm	10/10	10/10	10/10	10/10	10/10	10/10	10/10
Limit EIC extraction range for RT +/-	0.15 min	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	0.5 min	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	1.5 min	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	No limit	10/10	10/10	10/10	10/10	10/10	10/10	10/10
"do not match when < 70" and "do not match if the unobserved second ion's abundance is expected to be > 200"	**Yes**	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	No	10/10	10/10	10/10	10/10	10/10	10/10	10/10
Isotope abundance score is 60	**60**	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	80	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	100	10/10	10/10	10/10	10/10	10/10	10/10	10/10
Peak filter: absolute height ≥___ counts	100	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	200	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	1000	6/10	8/10	8/10	8/10	6/10	6/10	6/10
Peak spectra: spectra to include average scan larger than	1%	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	10%	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	30%	10/10	10/10	10/10	10/10	10/10	10/10	10/10
Peak spectra: spectra to include at apex of peak	**Yes**	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	Not selected	10/10	10/10	10/10	10/10	10/10	10/10	10/10
TOF spectra: exclude if above ___ saturation	**20%**	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	40%	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	Not selected	10/10	10/10	10/10	10/10	10/10	10/10	10/10

Post processing (find by formula peak filter: ≥ ___counts)	**200**	10/10	10/10	10/10	10/10	10/10	10/10	10/10
	1000	6/10	8/10	8/10	8/10	6/10	6/10	6/10
	2500	2/10	2/10	2/10	2/10	2/10	2/10	2/10
Post processing: limit to the largest 2000 features	Yes	3/10	3/10	3/10	3/10	3/10	3/10	3/10
	No limit	10/10	10/10	10/10	10/10	10/10	10/10	10/10

Note: each time only one parameter was changed; 2/10 indicates only 2 bisphenols were identified correctly out 10 bisphenols and 10/10 indicates all the ten bisphenols were correctly identified. Parameters in bold correspond to the default condition.

4.4.4 Non-targeted analysis of plastic-related contaminants and other environmental contaminants

Based on the optimized parameters (section 4.4.3), raw data for non-spiked samples, QCs and blanks were aligned and extracted by Agilent Profinder (B08.00) with the "*Batch Molecular Feature Extraction*" mode. In this step, QC samples were included to control the RT drifts, mass measurement reproducibility as well as the instrumental background noise (if a molecular feature is present in one group but not in any QC, this feature was eliminated for analysis) (Gika et al., 2014). In total, 15,234 and 15,977 molecular features were extracted in positive and negative ionization modes, respectively. These molecular features were then further analyzed using Agilent MPP (B14.0). Pike muscle extracts were first compared with blanks, and features presented in both the pike muscle extracts and the blank groups were eliminated from further identification (von Eyken & Bayen, 2019).

Recently, database for different contaminant groups were made commercially available, however, the MS/MS information is not always included in these databases for all compounds (Moschet et al., 2017). In that case, the validation of the identification requires the analysis of an authentic standard. In the present study, identifying every single feature was not feasible, and the focus was placed on features with relatively high abundances and matching scores relative to the compound libraries. In total, nine features passed the predicted RT checking criteria (Table 4.4). Features with *m/z* 223.0970 (ESI+) and *m/z* 498.9297 (ESI-) presented in all the fish extracts were identified using the library as DEP ($[M+H]^+$) and PFOS ($[M-H]^-$), and this identification was later confirmed using an authentic standard (RT match < 0.1 min and MS/MS main fragments match). DEP is a phthalic ester used as plasticizer and has been detected in wastewater in China and in tissues of various fish species (*Cyprinidae* and *Percidae*) in France (Gao et al., 2014; Teil et al., 2012). However, data on DEP in aquatic organisms from the St. Lawrence River has so far not been reported. Laboratory and field studies tend to indicate that phthalate esters such as DEP do not

biomagnify in aquatic food webs (Gobas et al., 2003). PFOS is a synthetic anionic surfactant which has been applied for decades before being listed on the Stockholm Convention on Persistent Organic Pollutants in 2009 and regulated in many countries (Zhang et al., 2012). PFOS was reported in fish and aquatic organisms (Kwadijk et al., 2010; Labadie & Chevreuil, 2011) and in surface water worldwide including the St. Lawrence river in Canada (Kwadijk et al., 2010; Scott et al., 2009). It is reported that PFOS can induce expression change in genes related to energy metabolism, reproduction and stress response in carp liver as well as induce oxidative damage in rainbow trout (Hagenaars et al., 2008; Oakes et al., 2005). Results from the present study indicates the ubiquity of PFOS in predator fish from the St. Lawrence River. PFOS has been shown to biomagnify in aquatic food chain (Bossi et al., 2005), which highlights the importance of screening this compound and other related compounds in fish.

The feature *m/z* 241.1303 ($[M+H]^+$) was first tentatively identified as dimetilan, an insecticide (isotope signature matching score = 86.9% and within the predicted RT range). However, the fragment ion of feature *m/z* 241.1303 did not match the MS/MS information of dimetilan reported in the literature, thus only the formula of this feature can be confirmed at the present stage. Similarly, the features *m/z* 239.1487 and *m/z* 286.2012 ($[M+H]^+$) in ESI positive mode and *m/z* 239.1149, *m/z* 301.2178, and *m/z* 329.2440 ($[M-H]^-$) in ESI negative mode were only confirmed for their formula. Feature *m/z* 434.2331 ($[M-H]^-$) was suggested to be paxilline according to the Agilent Extractables & Leachables LC/QTOF PCDL database. Most strikingly, the RT of feature *m/z* 434.2331 ($[M-H]^-$) matched the one for pure paxilline standard. However, when compared the most abundant daughter fragments, the one for paxilline standard (*m/z* 376.1897 ($[M-H]^-$)) was different from the one in the fish extracts (*m/z* 378.1551 ($[M-H]^-$)), which indicated a false identification for feature *m/z* 434.2331($[M-H]^-$). This result further highlights that identification of unknowns based on a formula predicted from accurate mass measurement is insufficient, even if predicted RT match observations.

Table 4.4 Identification of selected features

Features (m/z)	**ESI mode**	**Neutral mass**	**RT (min)**	**Suggested identity based on database**	**Isotope signature matching[1]**	**RT criterion matching[2]**	**MS/MS matching in literature[3]**	**Authentic standard confirmation[4]**
241.1303	Positive	240.1222	2.37	$C_{10}H_{16}N_4O_3$ (Dimetilan)	+	+	-	NC
239.1487	Positive	238.1429	8.12	$C_{11}H_{18}N4O_2$ (Pirimicarb)	+	+	-	NC
286.2012	Positive	285.1940	12.76	$C_{15}H_{27}NO_4$ (Lindelofine)	+	+	NA	NC
223.0970	Positive	222.0892	15.42	$C_{12}H_{14}O_4$ (DEP)	+	+	+	+
239.1149	Negative	240.1222	2.37	$C_{10}H_{16}N_4O_3$ (Dimetilan)	+	+	-	NC
498.9297	Negative	499.9374	15.15	$C_8HF_{17}O_3S$ (PFOS)	+	+	+	+
434.2331	Negative	435.2409	17.55	$C_{27}H_{33}NO_4$ (Paxilline)	+	+	NA	-
301.2178	Negative	302.2245	18.04	$C_{20}H_{30}O_2$ (Pimaric acid)	+	+	-	NC
329.2440	Negative	330.2558	18.39	$C_{22}H_{34}O_2$ (Ethyl abietate)	+	+	-	NC

Notes:

[1.] "+" means the matching score > 85%;

[2.] "+" means that the RT criterion was met based on the log K_{ow} value of suspect chemical;

[3.] "+" means the two main daughter ions matched with literature; "-" means the two main daughter ions did not match with literature.

[4.] "+" means both the RT and two main daughter ions of suspect chemical matched with reference standard, "-" means the RT or the daughter ions did not match with the reference standard;

NA means not available, NC means: not conducted.

4.4.5 Comparison between sampling sites by non-targeted analysis

Samples from the two sampling sites: upstream and downstream of Montreal's point of discharge of the wastewater treatment plant effluent in the St. Lawrence River were compared by "*fold change*" analysis in Agilent MPP (B14.0) (p value cut-off: 0.05, multiple testing correction: Benjamini-Hochberg). Features present only at one site or that showed significantly high abundance (p<0.05) in one site were of interest, as they could be used later as a possible marker of exposure to wastewater in future studies. For example, the molecular feature (*m/z* 239.1487 ($[M+H]^+$)) was significantly more abundant (p<0.05) in pike from the downstream group (Table 4.4, Figure 4.3A (in red color) and 4.3B) and was not present in procedural blanks. This feature is potentially a bioaccumulative chemical in pike tissues that could be used a marker of exposure to wastewater in the downstream site (Figure S4.2). Further identification was performed following the procedure described in section 4.4.4, and this feature was suggested to be the insecticide pirimicarb, with a high isotope signature matching score (96 %) and a RT range match. However, when compared to the MS/MS data for pirimicarb in the literature, the main daughter ion of feature *m/z* 239.1487 ($[M+H]^+$) did not agree with the literature (Figure S4.3) (Bobeldijk et al., 2001). Although the feature *m/z* 239.1487 ($[M+H]^+$) can only be temporarily identified for its chemical formula, the results indicated that non-targeted workflows may allow for the comparison of fish samples. With the development of correlation tools of MS/MS and chemical structures and the increase of database capacity, the structure of feature *m/z* 239.1487 ($[M+H]^+$) will be determined in future study.

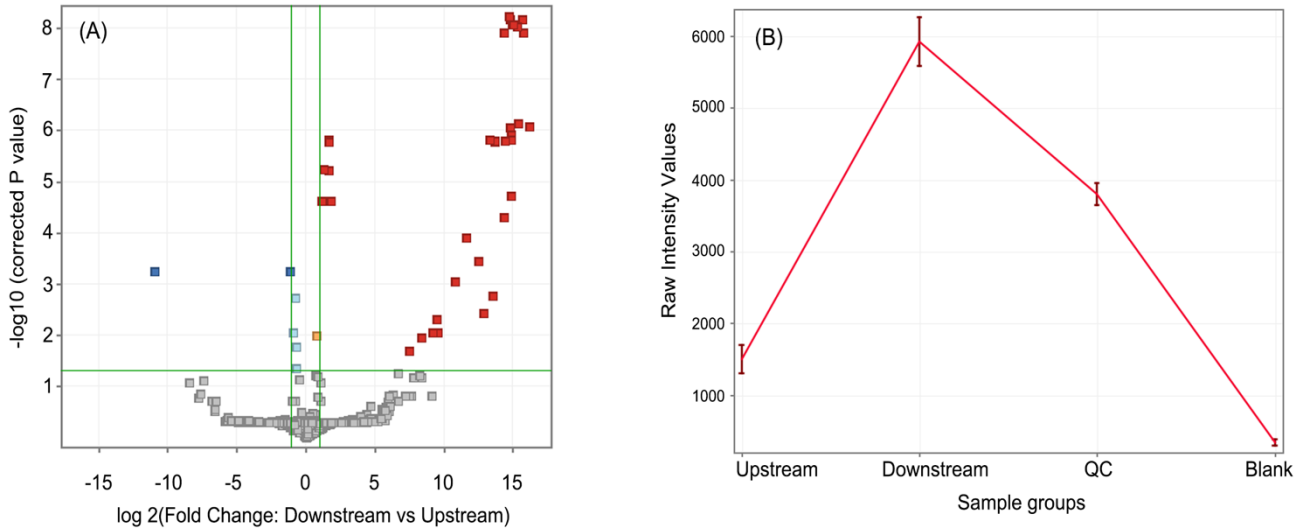

Figure 4.3 "*Fold Change*" analysis of pike from downstream and upstream groups (A. Volcano plot of downstream vs upstream, molecule features with high abundance in downstream group are in red; B. Average abundance for feature *m/z* 239.1487 ($[M+H]^+$) in different sites)

4.5 Conclusions

In this study, we present the application and validation of a non-targeted workflow to investigate unknown contaminants in a complex biological matrix (northern pike muscle). Sonication-assisted liquid extraction followed by HPLC-QTOF-MS analysis yielded satisfactory recovery and low LOD for the ten target compounds (bisphenol analogues). None of the 10 bisphenol analogues used for targeted method validation were detected in pike samples suggesting that these chemicals do not accumulate at detectable concentrations in muscle of pike naturally-exposed in the St. Lawrence River at two sites including one located downstream of Montreal's wastewater treatment plant effluent. Peak height related parameters show high importance in chromatographic data filtering for fish samples and need be optimized before the non-targeted analysis.

Suspected screening and non-targeted workflows can be used as an early warning system for environmental and food contaminant surveillance, and offers new perspective in the context of regulatory framework. For example, Brüggen & Schmitz (2018) described how an approach

combining target, suspected target and non-target screening could improve current water monitoring and assessment. The present non-targeted workflow was shown to accurately identify chemicals of high environmental and health concern (i.e., DEP and PFOS) in pike muscle extracts. As suspected screening is limited by the compound library capacity, efforts should focus on developing comprehensive libraries for the various classes of contaminants, including in particular MS/MS information to increase identification rates. Some other steps of the non-targeted workflows such as the chromatographic acquisition or the data deconvolution method, also need to be systematically studied to improve the rate of identification, with a particular focus on decreasing false identification rates.

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4.8 Supplementary materials

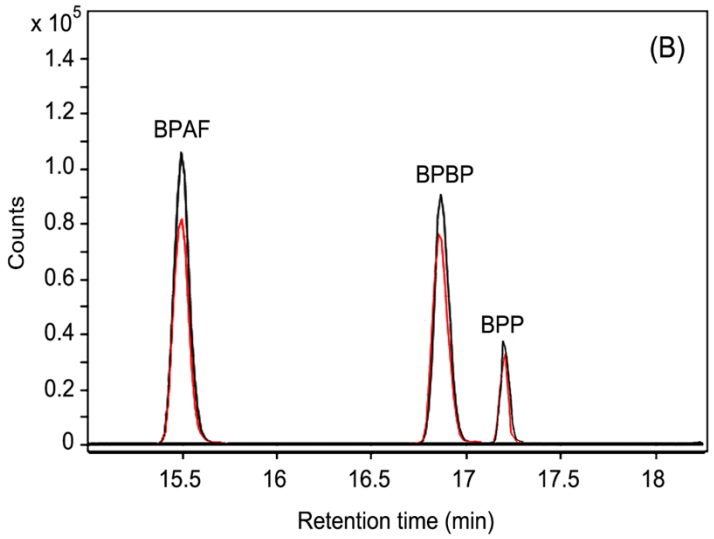

Figure S4.1 Matrix effects for BPAF, BPBP and BPP with a comparison of the chromatogram in pure solvent (methanol, black) and in the matrix (fish muscle extracts, red)

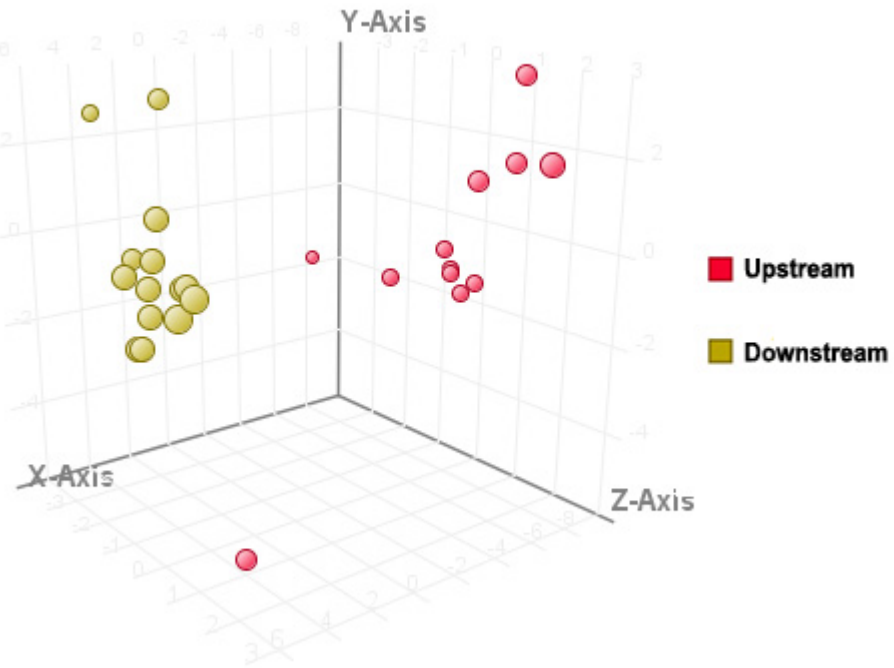

Figure S4.2 The PCA result with the feature list generated from Volcano plot in Figure 4.2

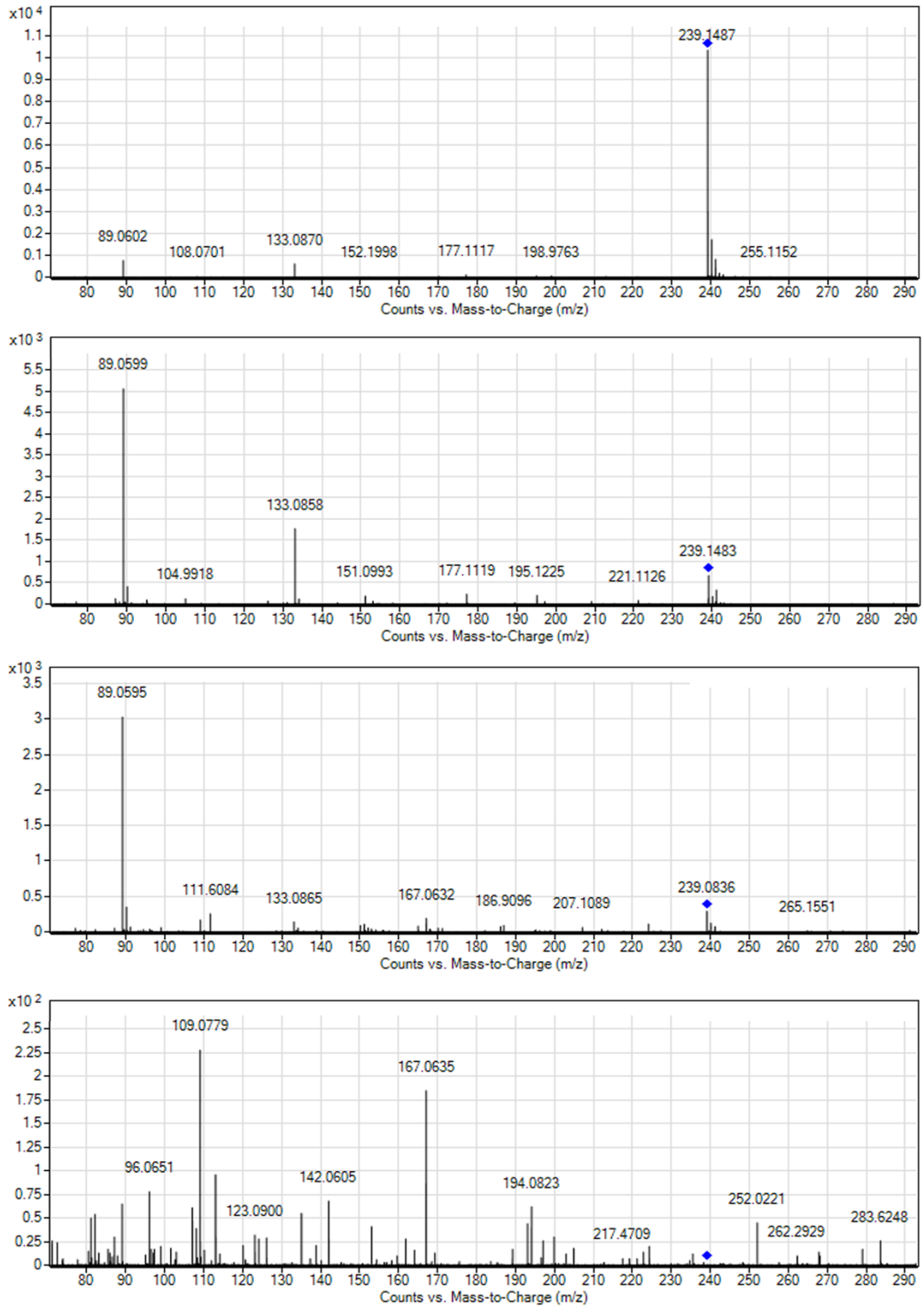

Figure S4.3 Product ion spectra of feature *m/z* 239.1487 ($[M+H]^+$) obtained at different collision energies: 0, 10, 20 and 40 eV (from top to bottom)

Table S4.1 Initial parameter for feature extraction

Parameter	Initial Value
Isotope peak spacing tolerance range	7 ppm
Expansion values for chromatogram extraction (m/z) (+/-)	10ppm
Limit EIC extraction range (expected RT +/-)	1.5 min
Peak filter (absolute height)	≥200 counts
Limit to the largest 2000 features	Not selected
Score filter: "don't match when < 70 " and "do not match if the unobserved second ion's abundance is expected to be > 200"	Not selected
Integrator method	Agile 2
Peak spectra: spectra to include how much percent of average scan	>10%
TOF spectra: exclude if above how much saturation	20%
Post processing: Find by formula peak filter (absolute height)	≥200 counts

Table S4.2 Mean mass measurement error (MMME) (ppm) for the 10 bishenols in solvent and in the fish muscle extracts

Compound	MMME in pure solvent (methanol)	MMME in fish muscle extracts	Significance of difference*
BPS	3.61±0.57	4.42±1.98	No
BPF	2.01±0.36	1.51±0.71	No
BPE	0.94±0.33	0.47±1.98	No
BPA	1.76±1.56	3.96±0.31	Yes
BPB	-1.24±1.17	0.41±1.1	No
BPAF	1.19±1.48	3.28±2.74	No
BPAP	1.73±3.67	5.92±2.92	Yes
BPZ	-0.75±2.12	2.25±1.32	Yes
BPBP	1.42±1.61	3.70±0.01	Yes
BPP	-0.29±3.07	4.06±2.25	Yes

Note: * "Yes" means the MMME difference is significant (p<0.05) between pure solvent and fish muscle extracts; "No" means the MMME difference is not significant (p>0.05) between pure solvent and fish muscle extracts.

Table S4.3 RT and log K_{ow} for chloramphenicol and bisphenol analogues

Compounds	Log K_{ow}*	Measured RT (min)
Chemical standards		
Chloramphenicol	1.00	9.8
BPS	1.65	12.5
BPF	2.91	13.9
BPE	3.19	14.5
BPA	3.32	14.9
BPB	4.13	15.5
BPAF	4.47	15.5
BPAP	4.86	16.1
BPZ	5.00	16.4
BPBP	6.08	16.9
BPP	6.25	17.2
Molecular features (Suspected identity)		
$C_{10}H_{16}N_4O_3$ (Dimetilan)	0.27	2.4
$C_{11}H_{18}N4O_2$ (Pirimicarb)	1.40	8.1
$C_{15}H_{27}NO_4$ (Lindelofine)	2.54	12.8
$C_{12}H_{14}O_4$ (DEP)	2.70	15.4
$C_8HF_{17}O_3S$ (PFOS)	5.00	15.2
$C_{27}H_{33}NO_4$ (Paxilline)	4.13	17.6
$C_{20}H_{30}O_2$ (Pimaric acid)	6.45	18.0
$C_{22}H_{34}O_2$ (Ethyl abietate)	7.24	18.4

Note: * log K_{ow} values were obtained from *chemspider.com* and *pubchem.ncbi.nlm.nih.gov*

Connecting Text

Chapter 4 further proved the importance of optimizing data processing parameters in non-targeted analysis of contaminants in pike fish fillet samples. The results of Chapter 4 indicate the robustness of the non-targeted workflow developed in previous studies (Chapter 3 and Chapter 4), which could be applied to study the other food categories. In Chapter 5, the non-targeted workflow will be applied to investigate unknown PRCs in different types of food. The targeted screening of 11 bisphenol analogues will be simultaneously conducted on the same food samples. Chapter 5 has been submitted to the journal "*Food Chemistry*": Tian, L., Zheng, J., Goodyer, C. G., & Bayen, S. Non-targeted screening of plastic-related chemicals in food collected in Montreal, Canada.

Chapter 5. Non-Targeted Screening of Plastic-Related Chemicals in Food Collected in Montreal, Canada

5.1 Abstract

A non-targeted screening method based on ultrasound-assisted extraction followed by high performance liquid chromatography coupled with quadrupole time of flight mass spectrometry (HPLC-QTOF-MS) was developed to screen for the presence of plastic-related chemicals (PRCs) in different types of food (fish, chicken, canned tuna, leafy vegetables, bread and butter). Eleven bisphenols were used as targeted compounds to validate the method. Instrument linearity ($r^2 \geq 0.98$), inter-day precision (RSD ≤ 9.0%) as well as method detection limits (MDLs below 3.6 ng g^{-1}) were satisfactory. Recoveries of the eleven bisphenols ranged from 76% to 122% among the different food matrices. The method was applied to food collected from Montreal, Canada in 2017-2018. The non-targeted screening approach identified a range of contaminants in different food matrices, including BPA, BPS, bis(2-ethylhexyl) adipate, dibutyl adipate, hexadecyl methacrylate and Irganox®1076. Further research is suggested to investigate the concentration of these PRCs, the consumption habits of average and specific populations and the potential routes of contamination.

5.2 Introduction

Plastic-related chemicals (PRCs) are substances related to plastics, including residual monomers, antioxidants, additives or the degradation products of plastics (Tian, Verreault et al., 2019; von Eyken et al., 2019). PRCs such as bisphenol analogues (e.g. bisphenol A (BPA), bisphenol F (BPF), bisphenol S (BPS) and bisphenol AF (BPAF)), 4-nonylphenol and some phthalates have been detected in bottle water, water, seafood, vegetables and many different types of packaged food (Careghini et al., 2015; Li et al., 2016; Liao & Kannan, 2013, 2014). Many of these chemicals can cause adverse health effects in humans including endocrine disruption, changes in neurobehavioral development, and metabolic diseases such as diabetes and obesity (Rochester, 2013; Rosenmai et al., 2014), raising concerns of consumer safety.

PRCs can enter the environment and food through pathways such as the discharge of industrial wastes to the environment, irrigation with reclaimed water or through the application of polymers in agriculture and food or as food contact material (Lu et al., 2015; Nerín et al., 2003) (Figure S5.1). A wide range of PRCs have been detected in food due to migration from plastic packaging. As a result, food has been identified as a major route of human exposure to various PRCs (Careghini et al., 2015; Fasano et al., 2012). Many studies have been conducted to determine the levels of target PRCs in food and to estimate human exposure (Rudel et al., 2011; Sakhi et al., 2014). However, food monitoring strategies are mostly limited to a finite list of "known" PRCs (Fu et al., 2017). Investigating "unknown" or "unexpected" contaminants (including the non-intentional added substances, NIAS) in food has only recently emerged as necessary to provide more comprehensive information for food safety risk assessments (Fu et al., 2017; Knolhoff and Croley, 2016).

In this context, non-targeted workflows, based on chromatography coupled to mass spectrometry (MS), have been developed in recent years to identify contaminants in various matrices (Knolhoff et al., 2016; Tian, Lin, & Bayen, 2019). For example, non-targeted workflows have been developed to investigate environmental pollution and, more recently, food matrices (Knolhoff and Croley, 2016; Krauss et al., 2010; Liu et al., 2019). However, due to the complexity and heterogeneity of food matrices, non-targeted screening in food analysis remains challenging from the sample preparation to the data analysis. This is because the success of contaminant identification based on non-targeted analysis relies on the concentration and purity of contaminants in food as well as the resolution achieved in the analysis (Nerin et al., 2013). Some contaminants in complex food matrices may co-elute resulting in difficulties in contaminant identification. Thus, sample treatments (including the extraction as well as the removal of interferences like lipid and protein in food) and data treatment software (including the chromatographic deconvolution tools and statistical tools) play a very important role for non-targeted identification (Nerin et al., 2013). In addition, according to Knolhoff and Croley (2016), the extraction methods examined for non-

targeted analysis are limited to a few biological sample types which cannot cover the diversity of food. Thus, it is important to validate sample treatment methods based on a variety of food matrices. Our previous studies have investigated the importance of data treatments in the non-targeted analysis of PRCs (Tian, Lin, et al., 2019; Tian, Verreault, et al., 2019). They have also highlighted the validity of the non-targeted workflow based on ultrasound-assisted liquid extraction followed by high performance liquid chromatography coupled with MS (HPLC-MS) in food analysis (Tian, Verreault et al., 2019). Ultrasound-assisted liquid extraction and HPLC-MS analysis are also preferred by many researchers to investigate multi-classes of chemicals including PRCs in different types of food (Pico, 2013). Using HPLC-MS, multiple classes of compounds can be screened within a single run which is helpful when analyzing with a large number of samples. Furthermore, a derivatization step is not necessary which makes the LC-MS more popular than gas chromatography-MS (GC-MS) (Knolhoff and Croley, 2016). High resolution MS (HRMS; e.g. quadrupole time-of-flight (QTOF)), allows the collection of full scan mass spectra with a high mass accuracy for formula generation and high confidence in structure prediction in non-targeted analysis (Knolhoff et al., 2016). As a result, HPLC coupled with QTOF-MS (HPLC-QTOF-MS) has proved to be a powerful tool in the non-targeted analysis of contaminants in food contact materials (FCMs) (Tian, Lin, et al., 2019) and in pike fish tissues (Tian, Verreault et al., 2019).

In the present study, a non-targeted screening method based on ultrasound-assisted liquid extraction and HPLC-QTOF-MS analysis was applied to determine the unknown PRCs in different types of food sampled from markets in Montreal, Canada. Eleven bisphenols were used as targeted compounds for target screening as well as to validate the non-targeted workflow. This study aims to develop and validate a simple and effective analytical method for non-targeted analysis of different types of food as well as to identify unknown PRCs in food. This approach can offer useful information for food safety monitoring and risk assessment.

5.3 Materials and methods

5.3.1 Reagents and standard preparation

Ammonium acetate (LC-MS grade), sodium sulfate anhydrous (purity ⩾ 99%) and HPLC-grade solvents (water, acetonitrile and methanol) were purchased from Fisher Scientific (Hampton, USA). Analytical standards of BPA (purity ⩾ 99%), BPF (purity ⩾ 98%), BPS (purity ⩾ 98%), BPAF (purity ⩾ 99%), bisphenol E (BPE; purity ⩾ 98%), bisphenol P (BPP; purity ⩾ 99%), bisphenol Z (BPZ; purity ⩾ 99%), bisphenol AP (BPAP; purity ⩾ 99%), bisphenol BP (BPBP; purity ⩾ 98%), octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate (Irganox®1076, purity ⩾ 99%) and dibutyl adipate (purity ⩾ 96%) were purchased from Sigma-Aldrich (St. Louis, USA). Hexadecyl methacrylate (purity ⩾ 98%), bisphenol C (BPC, purity ⩾ 98%) and BPB (purity ⩾ 98%), BPA-$^{13}C_{12}$ (purity ⩾ 98%), BPF-$^{13}C_{12}$ (purity ⩾ 98%), BPS-$^{13}C_{12}$ (purity ⩾ 98%) and BPAF-d_4 (purity ⩾ 98%) were purchased from Toronto Research Chemicals (Toronto, Canada). Stock solutions of the individual bisphenols were prepared in methanol (100 mg L^{-1}). Working standard mixture solutions of the eleven native bisphenols and four labeled bisphenols were prepared weekly at a concentration of 1 mg L^{-1} in methanol, respectively. Irganox®1076, dibutyl adipate and hexadecyl methacrylate stock solutions (1 mg L^{-1}) were also prepared in methanol prior to use. All the standard stocks were prepared less than 24 hrs before the experiment and stored in amber glass vials in the freezer (-20 °C) prior to analysis.

5.3.2 Background contaminants control

To avoid plastic-related contamination during experimental procedures, the use of plastic labware was limited as much as possible, and only polypropylene centrifuge tubes, filter syringes and polytetrafluoroethylene HPLC sample vial caps were used. Amber glass vials used for standard preparations (Thermo Fisher Scientific, Waltham, US), mortars and pestles were baked at 320 °C

for 4 hrs prior to use. Stainless-steel knives and meat grinders were washed with detergent, rinsed with MilliQ water and HPLC grade methanol, and then air dried prior to use. Anhydrous sodium sulfate was used as blank material and was processed following the same procedure as food composites (see section 5.3.3) to prepare procedural blanks.

5.3.3 Food sampling and pretreatment

5.3.3.1 Food sample collections

Six types of packaged food, namely fish fillets, chicken, canned tuna, leafy vegetables, bread and butter, and three types of non-packaged food including fish fillets, vegetables and bread were purchased from six different local markets in Montreal, Canada in November 2017 and May 2018 (detailed information see Supplementary Table S5.1). Packaged foods were directly purchased from the markets and transported by a cooler while the "non-packaged" fish and vegetables (which were not in a packaged form in the retail market, but how these foods were collected and transported is unknown) were entirely wrapped in aluminum foil before weighing and transport by cooler. The core (middle of the bread, not in direct contact with packaging) and several samples of the outer layer (including the part not directly in contact with packaging and the crust that was in contact) of packaged bread loafs were cut from each other using a stainless-steel knife, and then processed into composites as described below) to investigate for differences between the core and the outer layer of bread.

5.3.3.2 Food composite preparation

All the food samples were first stored in the refrigerator (4 °C) as soon as they arrived in the lab and then the food composites were prepared within 24 hrs. Fish and chicken were ground in a stainless-steel manual meat grinder. Other types of food (vegetables, canned tuna, bread and butter) were cut by a stainless-steel knife on aluminum foil. Each food composite was prepared by transferring about 30 g of the individual food sample into an amber glass jar (250 mL) followed

by freeze drying (Martin Christ Gamma 1-16 LSC freeze-dryer, Osterode am Harz, Germany). Additional aliquots of each individual food homogenate were wrapped in aluminum foil, vacuum sealed by a polypropylene bag and stored in a freezer (-80 °C). Freeze-dried food composites were further homogenized using mortar and pestle and were stored in amber glass jars at -80 °C. Detailed information for each composite is presented in Table S5.1 and Figure S5.2 (in Supplementary materials).

5.3.4 Sample extraction

About 0.5 g (±0.05 g) of each freeze-dried food composite (except for vegetables) was weighed and transferred into a 15-mL polypropylene centrifuge tube. Samples were spiked with 60 μL bisphenol labeled standard mixture solutions (1 mg L^{-1}). Six mL of methanol was added into the tube. Tubes were vortexed for 1 min using a Vortex Mixer (Fisher Scientific, Hampton, USA), sonicated using a Branson 3510 sonication bath (40 kHz) for 30 min and, finally, centrifuged at 4500 rpm for 10 min at room temperature (23±2 °C). The supernatant was collected and filtered through a 0.22 μm filter (Norm-Ject, Tuttlingen, Germany) into HPLC amber glass vials. The extracts were kept at -20 °C until HPLC analysis.

Vegetable composites were extracted following the same procedure as above, except that acetonitrile was used for extraction instead of methanol; acetonitrile yields higher recoveries for PRCs in leafy vegetables than methanol (Aparicio et al., 2018). After filtration into the HPLC amber glass vials, samples were dried under nitrogen gas and reconstituted in water/methanol (v/v=1:1) (Aparicio et al., 2018).

5.3.5 Instrumental analysis

Samples were analyzed using an Agilent 1290 Infinity II LC system (Agilent Technologies, Santa Clara, USA) coupled to a 6545 quadrupole TOF-MS (Agilent Technologies, Santa Clara, USA) operating in both the positive (ESI+) and negative (ESI-) electrospray ionization modes. The LC

separation was conducted on a Poroshell 120 Phenyl Hexyl column (Agilent Technologies; 2.7 μm × 3.0 mm × 100 mm) fitted with a Poroshell 120 Phenyl Hexyl guard column (Agilent Technologies; 2.7 μm × 3.0 mm ×10 mm). The mobile phase (0.2 mL min^{-1}) consisted of a mixture (gradient mode) of water (solvent A) and methanol (solvent B), both containing 10 mM ammonium acetate. The percentage of organic mobile phase B increased linearly and the gradient times were as follows: initially at 5% for 1 min, then increased to 100% over 1-15 min, and maintained at 100% during 15-20 min; at 20 min, the eluent was restored to the initial conditions for 5 min to re-equilibrate the column for the next injection. The injection volume was set at 10 μL and the column temperature was maintained at 20 °C. Nitrogen was used as the drying gas (325 °C). The gas flow was 5 L min^{-1}. Samples were run in the All Ions MS/MS mode at four collision energies (0; 10; 20; 40 V) with a fragmenter energy of 150 V. MS data was acquired in the m/z 50-1700 range.

5.3.6 Method validation and quality assurance

Mass accuracy of the TOF was continuously maintained by the API-TOF reference mass solution containing hexakis(1H, 1H, 3H-tetrafluoropropoxy)phosphazine, purine and trifluoroacetic acid ammonium salt (Agilent Technologies, Santa Clara, USA) via an ESI nebulizer. To validate the instrumental performance for the eleven bisphenol analogues in the various matrices, quality assurance (QA) included procedural blanks (n=6), solvent blanks (n=3) as well as solvent and matrix-matched calibrations (six levels ranging from 10 to 200 μg L^{-1} with labeled standards inside at 60 μg L^{-1}) were assessed. The linearity of the instrument response was assessed using the analysis of standards in methanol. The method detection limit (MDL) was calculated as three times the standard deviation of procedural blanks divided by the slope of the matrix-matched calibration curve (Bayen et al., 2013). Samples used for recovery testing were first screened to confirm the absence of the four bisphenols (spiked with labeled standard mixture). The relative standard deviation (RSD) for the inter-day precision was calculated based on the analysis of three replicates of spiked food extracts (about 30 μg kg^{-1} fresh weight for each compound) on different days (n=3).

An inter-day precision (RSD) lower than 15% was judged acceptable (Rezk et al., 2015). The data obtained for spiked food extracts were also processed following the non-targeted workflow to validate whether the non-targeted workflow can correctly find all eleven bisphenols in different food matrices. Five pooled quality control samples (QCs) were prepared by mixing equal aliquots (10 µL) of each individual food composite sample together and analysis by HPLC of every tenth sample to control the drift of RT, the reproducibility of mass measurement as well as the instrumental background noise (Gika et al., 2014; Tian, Lin, et al., 2019).

5.3.7 Chromatographic data treatment

5.3.7.1Targeted screening

LC-MS data were analyzed using Agilent MassHunter Quantitative analysis (B07.01) software to confirm whether four bisphenol analogues were present in food samples and procedural blanks. The most abundant isotopes of $[M-H]^-$ were used as quantifier for the eleven bisphenols (Table 5.1). The chromatogram extraction window was ± 10 ppm for mass and ± 0.5 min for retention time (RT) (Tian, Verreault et al., 2019). The matrix-matched calibration curves were applied for the quantification once the presence of bisphenols was confirmed by targeted screening.

5.3.7.2 Non-targeted screening and identification

Non-targeted data treatment was conducted using the Agilent MassHunter Profiling software series following a workflow adapted from our previous study identifying unknown chemicals in pike fish muscle tissues (Tian, Verreault et al., 2019) (Figure 5.1). Briefly, chromatographic data were first aligned and features were extracted with the data processing parameters obtained from Tian, Verreault et al. (2019). Then features were analyzed by MassHunter Profiler Professional (MPP, version B14.0). A principal component analysis (PCA) was first produced to check common and unique components among samples, QCs and blanks. A “fold change” analysis in MPP was applied on the feature abundance to identify molecular features that were more concentrated in

sample groups compared to blanks (fold change cut-off: 2.0, *p*-value cut off: 0.05). Fold change analysis has been reported to be useful in distinguishing features from different groups in non-targeted analysis (Knolhoff et al., 2016). Formulas for molecular features higher in sample groups than in the blanks were generated based on the exact mass and the isotopic patterns. Formulas with the lowest mass errors and with the most similar relative ion abundance ratios were selected by software as the top candidates and compared with the library for identification. The Agilent Extractables & Leachables LC/QTOF PCDL containing 1006 compounds (notably the present eleven bisphenol analogs of interest) was used in this study.

Features with suspected identity were checked manually using Agilent Qualitative analysis (B07.00) by "Find by Formula" option. Only those features showing a coincident formula with a suspected identity (identified by MPP) were selected for the next step. For each candidate, the log octanol-water partition coefficient (log K_{ow}) was obtained from ChemSpider and PubChem websites and used to predict the RT range of the compound during the LC separation (the model was developed and validated in a previous study) (Tian, Verreault et al., 2019). Suspected candidates failing to match this criterion were excluded from further identification. When the RT matched the criterion, MS/MS information for the candidates was searched for in the literature if available to support the identification of tentative structures. In the end, selected analytical standards were purchased to confirm the identity of the molecular features.

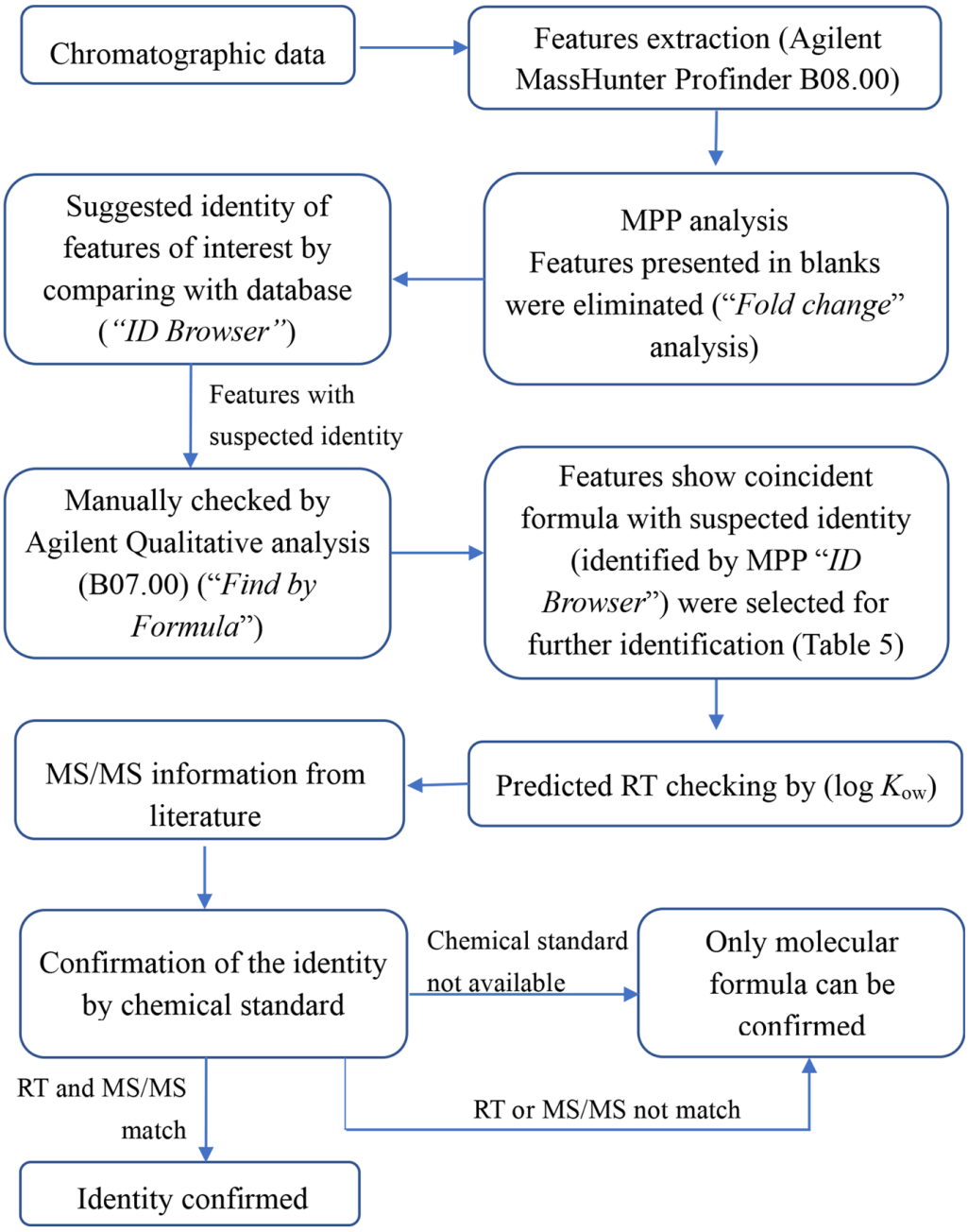

Figure 5.1 Non-targeted identification workflow

5.4 Results and discussions

5.4.1 Method validation

Instrument response for calibration standards was linear ($r^2 > 0.98$) for all the analytes (Table 5.1), the mass error was less than 1.4 ppm, and the RT drift was less than 0.02 min. The mean extraction recoveries for four bisphenols in different food ranged from 76% to 122% (Supplementary Table S5.2) and the recoveries are comparable to the values reported in literature for chemical contaminants in food (Cladière, Delaporte, Le Roux & Camel, 2018). MDLs were lower than 3.6 ng g^{-1} (dry weight) for all the bisphenols in all the matrices (Table S5.3), which is comparable with the literature for bisphenols in food (Aparicio et al., 2018; Lee et al., 2015). The inter-day precision (RSD) in the present study was below 9.0% (Table S5.3), which is similar to values reported in the literature for contaminants in food extracts (Niu et al., 2015) and is satisfactory for this type of analysis (Rezk et al., 2015).

Table 5.1 Mass to charge ratio and RT of four targeted bisphenols used in chromatogram extraction

Compound	RT (min)	m/z ($[M-H]^-$)*	Mass error (ppm)	Linearity (r^2)
BPA	14.9	227.1072	0.4	0.99
BPA-$^{13}C_{12}$	14.9	239.1477	1.4	
BPF	13.9	199.0759	0.5	0.99
BPF-$^{13}C_{12}$	13.9	211.1168	0.8	
BPS	12.2	249.0222	0.1	0.98
BPS-$^{13}C_{12}$	12.2	261.0630	0.1	
BPAF	15.5	335.0507	0.6	0.99
BPAF-d_4	15.5	339.0819	0.8	
BPE	14.4	213.0916	0.5	0.99
BPB	15.3	241.1229	0.3	0.99
BPC	15.8	255.1385	0.7	0.99
BPAP	16.0	289.1229	0.1	0.99
BPZ	16.3	267.1385	0.2	0.99

BPBP	16.8	351.1385	0.6	0.99
BPP	17.1	345.1855	0.3	0.99

* m/z was calculated for the most abundant isotope of $[M-H]^-$ for each compound.

Table 5.2 Detection frequency of BPS in different food composites

Food category	**Composite sum**	**Frequency**	**Detected in individual food composite**	**Concentration (ng g^{-1}, dw)**
Packaged fish	17	64.7%	Tilapia[1,2], basa[1,2], cod[1,2], sole[1,2], hake[2] haddock[2], salmon[2] fillets	ND-408.0
Non-packaged fish	14	21.4%	Cod[1], basa[2], salmon[2] fillets	ND-367.1
Packaged vegetables	8	12.5%	Watercress[1]	ND-below 4.5*
Non-packaged vegetables	7	ND	ND	ND
Chicken	2	100%	Chicken breast[1,2]	ND-291.4
Packaged bread	4	ND	ND	ND
Bread core	4	ND	ND	ND
Canned tuna	4	ND	ND	ND
Butter	4	ND	ND	ND

Note: ND=Not detected. 1 and 2 indicates the composite was sampled in 2017 or 2018, respectively. * 4.5 ng g^{-1} is the limit of quantification for BPS in vegetables.

5.4.2 Targeted screening

Except for BPA and BPS, none of the other bisphenols was detected in any of the food samples in the present study. BPA was only detected in one packaged cod composite (91.4 ng g^{-1}, dry weight (dw), sampled in 2017) while BPS was detected in several food composites; the detection frequency for BPS in different food composites is shown in Table 5.2. To confirm the presence of

bisphenols in food, the analysis of all the positive food composites (where BPA and BPS were detected) were replicated three times (from sample extraction to HPLC analysis).

BPS was more frequently detected in fish and chicken, with the highest frequency in packaged fish. BPS was expected to be used as the alternative where BPA was initially in use (Rosenmai et al., 2014), and this could be the reason for the higher BPS detection frequency in 2017 and 2018 food samples than BPA and other analogues. However, there are multiple studies more recently suggesting that BPS is not a safe alternative to BPA (Rosenmai et al., 2014).

According to the literature, BPA and BPS have been detected in different types of food including fish, vegetables, meat products, dairy products, etc. in the United States, China and Spain (Liao and Kannan, 2013, 2014; Viñas et al., 2010). Detection of BPA and BPS in Canadian food was limited to the study of Cao et al. (Cao et al., 2019; Cao et al., 2011). BPA was found in different food composites including marine fish (0.48 ng g^{-1}, ww) (Cao et al., 2011) while BPS was only detected in fresh meat and sausage (no chicken tested) from Canada (ND to 35 ng g^{-1}, ww) (Cao et al., 2019). Our study is the first to report BPS in fresh chicken and fish sampled from Canada. The level of BPS in chicken is comparable with the result for other meats reported in the study of Cao et al. (2019). The mean concentration of BPS in fish composite samples in the present study is much higher than the level of BPS reported in fish from the US (mean level: 0.02 ng g^{-1}, ww) (Liao and Kannan, 2013), but comparable with the results from Europe (Staniszewska et al., 2014).

In the literature, BPF and BPAF were detected in different types of food from the US, including fish, meat products, vegetables, cereals and dairy products (Liao and Kannan, 2013), and the detection frequency of BPF and BPAF in fish and seafood was 17.4% and 4.4% (n=23), respectively, while the detection frequency of both bisphenols in meat and meat products was 7.84% (n=51). Except for fish (4.6 ng g^{-1}, wet weight (ww)) and meat (1.3 ng g^{-1}, ww), the average level of BPF in food from the US is generally low (< 1 ng g^{-1}, ww) while the level of BPAF is lower than 0.021 ng g^{-1} (ww) (Liao and Kannan, 2013). Similar results were also reported in food from China and Spain (Cacho et al., 2012; Liao and Kannan, 2014). However, neither BPF nor BPAF

was detected in any food composites in the present study. The reason for this absence could be related to, but not limited to, the differences in sampling times and sampling locations. The investigation about relationship between packaging types and food contaminants is an on-going work which will provide some clues for this point.

5.4.3 Non-targeted screening

Chromatographic data, including samples, pooled QCs and procedure blanks were first aligned and extracted by Agilent Profinder (B08.00) under the "Batch Molecular Feature Extraction" mode. Pooled QC samples were included in alignment to control the RT drifts and mass measurement reproducibility (Gika et al., 2014; Tian, Verreault et al., 2019). Food extract samples were then compared with procedure blanks, and a tentative identity was studied for the selected features (MPP B.14.0).

In the present non-targeted workflow (for data-dependent screening), confirming the identity of every single feature was not feasible due to the limitation of library capacity and the availability of chemical standards. Thus, the identification priority was given to features with relatively high abundance and matching scores relative to the compound libraries.

A "blind" screening of spiked model bisphenols was used to assess the suspect screening capacity of the proposed workflow for the various matrices (section 5.3.6). The non-targeted workflow correctly identified all of the eleven bisphenols in all the spiked food matrices (n=3), which supports the validity of the non-targeted workflow in the present study. For real samples, after the confirmation of a molecular formula with "*find by formula*" (Agilent Qualitative analysis B07.00), eight features under ESI+ and nine features under ESI- (Table 5.3) were selected for further identification following the workflow in Figure 5.1.

Features at *m/z* 227.1068 ($[M-H]^-$, 14.9 min) and *m/z* 249.0223 ($[M-H]^-$, 12.2 min) were identified by the library as BPA and BPS, respectively, and these identities were directly confirmed by the chemical standards. The non-targeted screening result matches the result of targeted screening for

BPA and BPS in different foods, which further proves the effectiveness of non-targeted workflow in the present study.

Features at *m/z* 145.0864 ($[M+H]^+$, 17.6 min), *m/z* 101.0598 ($[M+H]^+$, 18.5 min) and *m/z* 111.0443 ($[M+H]^+$, 18.5 min) did not pass the RT criterion, and they were therefore not considered for further attempts at identification.

Features at *m/z* 371.3161 ($[M+H]^+$, 18.5 min) and *m/z* 259.1904 ($[M+H]^+$, 15.3 min) were identified by library as bis(2-ethylhexyl) adipate (DEHA) and dibutyl adipate (DBA), respectively. This identification was further confirmed by authentic standards (with RT match < 0.1 min and MS/MS main fragments match). DEHA and DBA have been applied in manufacturing FCMs, and DEHA has been reported to be present in packaged food, including fish, chicken, beverages and curry paste (Page and Lacroix, 1995). Data for DBA in food are limited to the controlled migration study of Wei et al. (2009) illustrating that DBA can migrate from food packaging to ham. In the present study, DBA was found in a "non-packaged" haddock composite, which indicates that DBA may have accumulated in the fish itself or have contaminated the fish during earlier handling steps before sale in the retail market. Both DEHA and DBA are reported to be toxic from animal tests, but the data for their toxicity in humans as well as the bioaccumulation in organisms are scanty in the published literature (LCSP, 2011).

Features at *m/z* 309.2796 ($[M-H]^-$, 17.7 min) and *m/z* 529.4619 ($[M-H]^-$, 20.3 min) were identified by the library as hexadecyl methacrylate and Irganox®1076, respectively. While some long-chain methacrylates have been used to produce FCMs (Franz and Brandsch, 2013), there is little specific information in the literature about the use of hexadecyl methacrylate in FCMs. So far, there is little information on the toxicity of hexadecyl methacrylate through diet in humans (European Chemicals Agency, retrieved from: https://echa.europa.eu/substance-information/-/substanceinfo/100.017.885). Irganox®1076 is an antioxidant applied in low density polyethylene (LDPE) plastic manufacturing, and LDPE has been reported to be used for making bread bags (Arvanitoyannis and Bosnea, 2004; Fasano et al., 2012). Studies as early as 1985 have reported

the migration of Irganox®1076 from packaging to different foods and food simulants (Arvanitoyannis & Bosnea, 2004; Bieber et al., 1985). This is the first time, however, that Irganox®1076 is reported in bread. Interestingly, this compound was present in the composite sample of the outer layer of the packaged brown bread but not in the composite of cores of the same bread sample, which suggests that the occurrence of Irganox®1076 in the outer layer of the bread may be as a result of bread processing, handling or migration from packaging. Similarly, there are only limited data about the toxicity of Irganox®1076 in humans but, according to the dashboard database of the US Environmental Protection Agency (EPA), Irganox®1076 can cause subchronic disease in dogs (https://comptox.epa.gov/dashboard/dsstoxdb/results?search=2082-79-3).

The MS/MS patterns of features *m/z* 245.1290 ($[M+H]^+$, 12.3 min), *m/z* 142.0660 ($[M-H]^-$, 12.4 min), 229.1446 m/z ($[M-H]^-$, 13.1 min) and 221.1541 m/z ($[M-H]^-$, 15.6 min) did not match the MS/MS information of their suspected identities in the literature and, thus, the identification of these features was limited to their molecular formulas.

The identity of features at *m/z* 341.2691 ($[M+H]^+$, 17.3 min) and *m/z* 313.2387 m/z ($[M-H]^-$, 14.9 min) were confirmed by chemical standards that did not match the suggested identities from the library. Both the MS/MS information and chemical standards were not available at the time the experiment was conducted; the identification of features at *m/z* 339.2171 ($[M+H]^+$, 17.1 min) and *m/z* 239.2015 ($[M-H]^-$, 16.7 min) is, thus, limited to their molecular formulas.

The results from the present study show the capacity of the non-targeted screening approach to identify chemicals of health concern (i.e., DEHA, DBA and Irganox®1076) in complex food matrices. Compared with the traditional method (targeted screening) applied in food safety monitoring, MS-based non-targeted analysis offers the powerful ability to identify new contaminants as well as deal with the analytical interferences in complex food matrices (Herrero et al., 2012). However, due to the trace level of contaminants in food and the dynamic concentration of food components, non-targeted screening remains a challenge for the analysis of

contaminants in food (Herrero et al., 2012). With optimization of sample preparation, chromatographic acquisition, data deconvolution methods and improvement in library capacity, the non-targeted screening will be more robust in identifying food contaminants and provide important information for regulatory frameworks in the future.

Table 5.3 Non-targeted identification of selected features from food composites

ESI+	Features ($[M+H]^+$, m/z)	RT (min)	Food composite*	Molecular formula	Suspected identity from library	RT criterion matching	MS/MS matching literature	Authentic standard confirmation
	245.1290	12.3	Canned Tuna in oil[1]	$C_{14}H_{16}N_2O_2$	3,3-Dimethoxybenzidine	Yes	No	NC
	259.1904	15.3	Haddock[1, np]	$C_{14}H_{26}O_4$	Dibutyl adipate (DBA)	Yes	Yes	Yes
	339.2171	17.1	Butter[2, p]	$C_{19}H_{30}O_5$	Dodecyl gallate	Yes	NA	NC
	341.2691	17.3	Brown bread[2, p]	$C_{20}H_{36}O_4$	2-Ethylhexyl fumarate	Yes	NA	No
	145.0864	17.6	Butter[1, p]	$C_7H_{13}NO_2$	N-(2-Hydroxypropyl) methacrylamide (HPMA)	No	NC	NC
	371.3161	18.5	Basa[1, p] & Chicken breast[1]	$C_{22}H_{42}O_4$	Bis(2-ethylhexyl) adipate (DEHA)	Yes	Yes	Yes
	101.0598	18.5	Sole[1, np]	$C_5H_8O_2$	Glutaral	No	NC	NC
	111.0443	18.5	Sole[1, np] & Basa[1, p]	$C_6H_6O_2$	Hydroquinone	No	NC	NC
ESI-	**($[M-H]^-$, m/z)**	**RT (min)**	**Food composite**	**Molecular formula**	**Suspected identity from library**	**RT criterion matching**	**MS/MS matching literature**	**Authentic standard confirmation**
	249.0223	12.2	Tilapia[1, p; 2, p], Basa[1, p; 2, p; 2, np], Cod[1, p; 2, p; 1, np], Sole[1, p; 2, p], Hake[2, p], Haddock[2, p], Salmon[2, p; 2, np],	$C_{12}H_{10}O_4S$	BPS	Yes	Yes	Yes

			Watercress[1, p], & Chicken breast[1, p; 2, p]					
	142.0660	12.4	Brown bread[2, p]	$C_{10}H_9N$	2-Naphthylamine	Yes	No	NC
	229.1446	13.1	White bread[1, p]	$C_{12}H_{22}O_4$	Di-isopropyl adipate	Yes	No	NC
	227.1068	14.9	Cod[1,p]	$C_{15}H_{16}O_2$	BPA	Yes	Yes	Yes
	313.2387	14.9	Brown bread[2, p]	$C_{18}H_{34}O_4$	Dibutyl sebacate	Yes	NA	No
	221.1541	15.6	Halibut[1, np]	$C_{14}H_{22}O_2$	2,5-Di-tert-butylhydroquinone	Yes	No	NC
	239.2015	16.7	Butter[2, p]	$C_{15}H_{28}O_2$	Lauryl acrylate	Yes	NA	NC
	309.2796	17.7	Chicken breast[1]	$C_{20}H_{38}O_2$	Hexadecyl methacrylate	Yes	NA	Yes
	529.4619	20.3	Brown bread[2, p]	$C_{35}H_{62}O_3$	Irganox®1076	Yes	Yes	Yes

Note: NA=Not available; NC=Not conducted; * 1 and 2 indicate the sampling time (1=November, 2017; 2=May, 2018), p=packaged, (for bread, p=outer layer of packaged bread); np=non packaged.

5.5 Conclusions

Although numerous studies have reported the detection of bisphenols in food, to the best of our knowledge, this is the first study to report the occurrence of BPS in fresh fish filets and chicken breasts from Canada. Notably, the highest detection frequency of BPS was observed in packaged fish compared to other food categories, and this should be further investigated. Although the non-targeted method was validated for the analysis of bisphenols, it was also successful in identifying compounds from other chemical families (e.g. DBA, hexadecyl methacrylate and Irganox®1076). In addition, this is also the first study reporting DBA in fish, hexadecyl methacrylate in chicken and Irganox®1076 in bread from the market. Few data were available in the literature on the occurrence of these "unexpected" PRCs in food, and future research should focus on better characterizing dietary exposure to PRCs in food among both general and specific populations in the context of food risk assessment. Overall, the non-targeted screening method developed in this study was effective as it correctly identified all the spiked bisphenols in all food matrices, and also allowed for the identification of several unexpected PRCs in different food types. Confirmation of the structures of unknown contaminants is currently the main bottleneck of the approach, as the non-targeted identification step relies on library capacity and the MS-structure correlation tools. Future efforts should focus on the development of a comprehensive database for the various classes of food contaminants as well as MS-structure correlation tools to improve the identification rates. Furthermore, future work should investigate the relationship between packaging materials and the various contaminants identified in food using non-targeted analysis.

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5.7 References

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5.8 Supplementary materials

Table S5.1 Food composites information

Food category	**Composite name ***	**Numbers of individual samples ****
Fish	Tilapia 1	6
	Rainbow trout 1	6
	Basa 1	5
	Haddock 1	2
	Cod 1	6
	Salmon 1	6
	Halibut 1	1
	Sole 1	3
	Tilapia (non-packaged) 1	4
	Rainbow trout (non-packaged) 1	4
	Haddock (non-packaged) 1	3
	Cod (non-packaged) 1	4
	Salmon (non-packaged) 1	5
	Sole (non-packaged) 1	5
	Tilapia 2	6
	Rainbow trout 2	6
	Basa 2	6
	Hake 2	5
	Haddock 2	6
	Cod 2	6
	Salmon 2	6
	Halibut 2	1

	Sole 2	6
	Tilapia (non-packaged) 2	6
	Rainbow trout (non-packaged) 2	6
	Basa (non-packaged) 2	6
	Haddock (non-packaged) 2	6
	Cod (non-packaged) 2	6
	Salmon (non-packaged) 2	6
	Sole (non-packaged) 2	6
Leafy vegetables	Spinach 1	6
	Arugula 1	4
	Romaine lettuce 1	6
	Watercress 1	1
	Spinach (non-packaged) 1	6
	Arugula (non-packaged) 1	6
	Romaine Lettuce (non-packaged) 1	6
	Watercress (non-packaged) 1	6
	Spinach 2	6
	Arugula 2	6
	Romaine lettuce 2	6
	watercress 2	6
	spinach (non-packaged) 2	6
	Romaine lettuce (non-packaged) 2	6
	watercress (non-packaged) 2	6
Chicken	Chicken breast 1	6
	Chicken breast 2	6
Bread	White bread 1	6
	Brown bread 1	6
	White bread (core) 1	6
	Brown bread (core) 1	6
	White bread 2	6
	Brown bread 2	6
	White bread (core) 2	6
	Brown bread (core) 2	6
Canned tuna	Canned tuna in water 1	6
	Canned tuna in oil 1	6
	Canned tuna in water 2	6
	Canned tuna in oil 2	6
Butter	Non-salted butter 1	6

	Salted butter 1	6
	Non-salted butter 2	6
	Salted butter 2	6

Note: * 1 indicates the food sampled in November 2017; 2 indicates the food sampled in May 2018;

** Some food was not available in all the six markets at the sampling time, thus the number may be lower than six.

Table S5.2. Average recoveries of bisphenols in different matrices (n=3)

Food	Salmon*	Cod*	Romaine lettuce**	Chicken	Brown bread	White bread	Canned tuna in water	Canned tuna in oil	Butter
BPS	100±13%	82±5%	90 ±12%	91±10%	90±16%	90±6%	83±8%	97±1%	98±2%
BPF	107±17%	87±19%	93 ±12%	114±5%	76±11%	77±14%	97±13%	98±18%	104±12%
BPE	96±6%	105±5%	78±8%	101±3%	87±3%	90±7%	95±9%	97±6%	99±3%
BPA	88±11%	120±14%	90 ±16%	99±16%	97±14%	81±20%	98±10%	81±8%	105±15%
BPB	121±3%	122±4%	102±2%	122±9%	89±5%	88±4%	117±3%	108±5%	92±2%
BPAF	102±4%	97±3%	88 ±1%	101±2%	94±4%	100±5%	100±3%	98±1%	100±3%
BPC	90±3%	106±7%	82±4%	103±4%	109±3%	105±9%	107±8%	101±9%	85±4%
BPAP	80±8%	80±9%	76±5%	101±1%	81±7%	88±4%	109±6%	116±5%	100±2%
BPZ	86±6%	122±2%	77±3%	83±6%	76±1%	79±8%	116±5%	108±7%	80±7%
BPBP	81±9%	99±4%	116±5%	83±7%	76±5%	77±2%	109±6%	110±11%	87±2%
BPP	103±7%	104±1%	80±2%	73±3%	84±2%	80±7%	81±5%	86±10%	74±1%

Note: * Salmon and cod represent the high-fat fish (fat content ≥ 3%, wet weight) and low-fat fish (fat content < 1%, wet weight) in this study, respectively. The fat content was measured by Soxhlet extraction in this study (data not shown here).

** Romaine lettuce represents the four types of vegetables in this study, as they have similar moisture (≥ 92%) and fat content (< 0.5%, wet weight) (data not shown here).

Table S5.3. Method detection limit (MDL, ng g^{-1}, dw) and inter-day precision (RSD, %)

Food	Salmon		Cod		Romaine lettuce		Chicken		Brown bread		White bread		Canned tuna in water		Canned tuna in oil		Butter	
Bisphenol	MDL	RSD	MDL	RSD	MDL	RSD	MDL	RSD	MDL	RSD	MDL	RSD	MDL	RSD	MDL	RSD	MDL	RSD
BPS	1.2	1.5	1.7	3.0	1.5	3.0	1.1	2.2	1.5	3.7	1.5	3.3	1.5	2.9	1.5	3.0	2.0	4.3
BPF	2.1	3.3	2.5	2.2	2.4	2.6	2.2	2.7	2.9	3.0	3.6	3.5	2.0	3.3	1.9	3.3	2.1	3.9
BPE	2.3	6.0	2.2	5.5	2.7	5.9	2.6	4.7	2.1	5.3	2.0	5.0	1.9	4.3	1.9	5.1	2.2	5.5
BPA	1.9	1.4	2.4	2.7	2.0	3.5	1.7	2.6	3.0	3.9	3.4	4.4	2.3	3.5	2.1	3.2	2.2	4.0
BPB	3.1	3.7	2.9	4.0	3.1	5.3	3.0	5.0	3.1	4.9	3.0	4.1	2.9	3.8	3.0	3.7	3.1	5.0
BPAF	1.4	1.4	1.3	2.4	1.6	2.2	1.2	1.3	1.8	1.7	1.9	1.9	1.9	1.5	1.8	1.6	1.9	1.9
BPC	0.9	8.1	1.3	4.9	1.7	5.8	0.7	4.5	0.9	5.1	1.0	5.7	0.8	3.9	0.9	6.2	0.5	4.0
BPAP	1.2	4.1	1.5	3.3	1.3	2.7	1.1	4.8	1.7	3.0	1.7	3.8	1.2	3.1	1.3	3.9	1.8	3.9
BPZ	1.3	2.9	1.3	2.9	1.4	3.3	1.5	4.1	1.1	4.4	1.3	4.6	1.3	4.0	1.7	4.9	3.4	5.1
BPBP	1.1	3.1	1.0	3.9	1.6	4.4	1.4	4.3	1.7	5.0	1.8	4.8	1.2	3.9	1.5	3.9	1.7	4.7
BPP	1.1	4.6	1.3	4.1	1.5	3.9	1.5	4.3	1.6	4.6	1.5	5.0	1.4	4.4	1.6	6.2	0.7	9.0

Note: MDL was calculated based on dry weight.

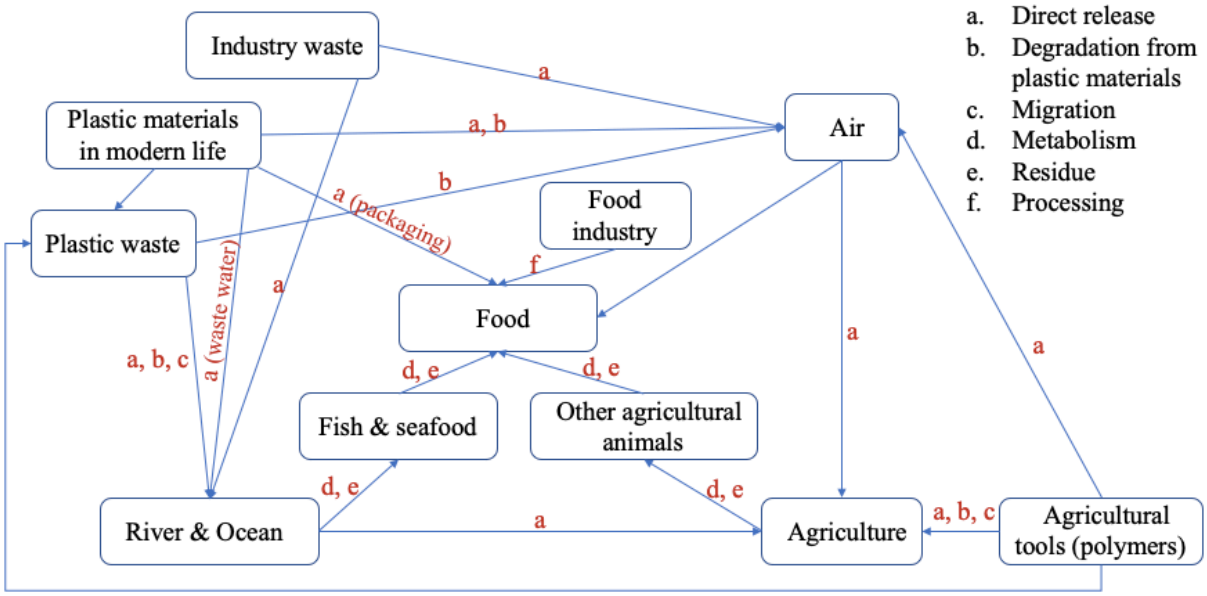

Figure S5.1 Possible pathways for PRCs in food

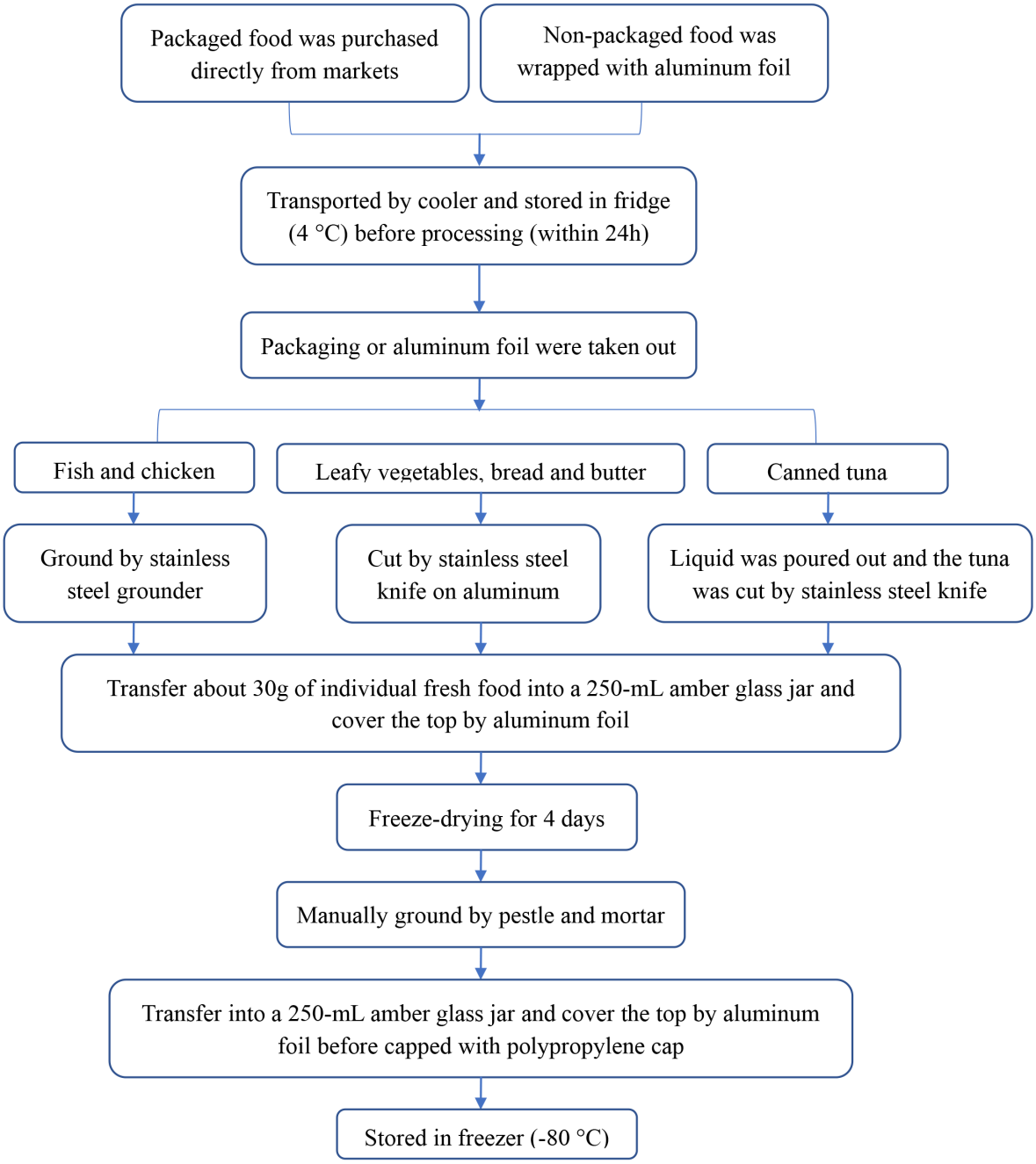

Figure S5.2 Food composite process procedure

Connecting Text

The non-targeted screening method for the analysis of PRCs has been developed and optimized in previous chapters (Chapter 3 and Chapter 4) and proved to be effective as it could identify several unexpected PRCs in different types of food (Chapter 5). In Chapter 6, the optimized non-targeted method is applied to investigate the thermal degradation of bisphenol A and bisphenol S in different matrices (water, spiked fish and incurred fish) as well as to identify their degradation products. Chapter 6 has been submitted for publication in the journal "*Food Chemistry*": Tian, L., Zheng, J., Goodyer, C. G., & Bayen, S. Thermal degradation of bisphenol A and bisphenol S in water and fish (cod and basa) fillets.

Chapter 6. Thermal degradation of bisphenol A and bisphenol S in Water and Fish (cod and basa) Fillets

6.1 Abstract

The thermal degradation of bisphenol A (BPA) and bisphenol S (BPS) was investigated in water and fish (cod, basa) fillets. Ultrasound assisted solvent extraction followed by high performance liquid chromatography coupled with quadrupole time of flight mass spectrometry (HPLC-QTOF-MS) was used to analyze residues in fish. Good instrumental linearity (r^2>0.99) and recoveries (83.3-128.4%) were achieved. BPA and BPS did not degrade (1 hour; 100 °C) in water (< 0.1% degradation) but degraded in fish matrices. The degradation percentage of BPA was 33.0±1.5% and 35.4±1.2% in incurred and spiked cod, respectively; and the degradation percentage of BPS was 34.7±1.7% and 37.5±1.4% degradation in incurred and spiked basa, respectively. The degradation products in spiked samples were different from those in the incurred group under the same conditions. This first study on the thermal degradation of plastic-related chemicals in food using a non-targeted approach will contribute to the refining of food safety risk assessments.

6.2 Introduction

Bisphenol A (BPA) (Figure 6.1) is a plastic-related chemical (PRC) which is used widely in applications such as food contact materials (FCMs), thermal papers and electronical devices. The main use of BPA in FMCs is to manufacture epoxy resins, polycarbonate plastics and polyvinyl chloride films (Cao et al., 2011). BPA can migrate from the FCMs into the food, and diet has been identified as a major source of BPA exposure for humans (Cao et al., 2011). BPA has been found to display some endocrine disruption activity, and the application of BPA in manufacturing baby bottles and FCMs for infant formula has been banned in the United States, in Canada and in the European Union (Government of Canada, 2010; European Commission, 2011; US FDA, 2014). Bisphenol S (BPS) (Figure 6.1), a compound structurally analogous to BPA, is presently used as an alternative to BPA in thermal printing papers and has been detected in different foods (Eladak et al., 2015). Recent studies suggest that BPS toxicity is comparable to BPA (Eladak et al., 2015).

BPA is often reported in food, and has been detected for example in wild-caught fish from Poland (Staniszewska et al., 2014) as well as fresh fish from the Canadian market (Cao et al., 2015), at levels up to several hundred nanograms per gram in fish muscles (Staniszewska et al., 2014). Data for BPS in fresh fish are scarcer in the literature. Liao and Kannan (Liao & Kannan, 2013, 2014) reported average levels of 0.02 ng g^{-1} and 0.56 ng g^{-1} for fish samples from markets in the US and China, respectively. The occurrence of BPA and BPS in fish can be a human health concern and the monitoring program in Canada has been focusing on the level of these compounds in food (Cao et al., 2011).

Current guidelines for assessing contaminants in processed food is limited to pesticides (OECD 508, 2008), and there are no standards for PRCs or other non-intentionally added substances (NIAS). For the purpose of exposure assessment as well as food safety monitoring, the fate of particular chemicals under thermal processing should be considered, especially for food of animal origin (Tian & Bayen, 2018). Chemical contaminants in food can undergo different reactions under thermal treatments, including the breakdown of chemical bonds which results in breakdown products with a smaller molecular weight compared to the original parent compound (Göckener, Kotthoff, Kling & Bücking, 2019), and reactions with food components which results in new chemicals (e.g. bisphenol A diglycidyl ether (BADGE) can react with protein in food during sterilization) (Petersen, Biereichel, Burseg, Simat & Steinhart, 2008). To the best of our knowledge, there is no systematic study on the thermal degradation of BPA and BPS under cooking conditions (e.g. boiling) in food or in water. Most of the studies on BPA and BPS degradation are limited to their biodegradation (enzyme or bacteria), photodegradation or oxidation in the presence of chemical catalysts (Eio, Kawai, Tsuchiya, Yamamoto & Toda, 2014; Wang et al., 2017; Watanabe, Horikoshi, Kawabe, Sugie, Zhao & Hidaka, 2003). The identity of some degradation products has been confirmed or proposed based on their fragmentation patterns in high resolution mass spectrometry (HRMS) (Eio et al., 2014; Wang et al., 2017; Watanabe et al., 2003). HRMS is commonly applied for the identification of unknown chemicals as the relatively higher resolving

power can provide additional structural information for complex sample matrices, including food (Tian, Verreault, Houde & Bayen, 2019). Furthermore, accurate mass measurements with HRMS can also help to determine the formula of unknown compounds (Picó and Barceló, 2008).

The water model is most frequently applied when studying the degradation of food contaminants (Goeckener, Kotthoff, Kling & Buecking, 2019; Tian, Khalil & Bayen, 2017). However, it has been reported that the thermal degradation of a chemical in water model may be different from those in food matrices (Tian & Bayen, 2018). In addition, investigation of the fate of emerging contaminants in incurred samples is more important than the spiked model as it can reflect the real fate of contaminants in food (Tian & Bayen, 2018). BPA was detected in one cod fillet sample (92.1 ng g^{-1}, wet weight), and BPA was detected in one basa fillet sample containing (71.4 ng g^{-1}, wet weight) in our previous screening study (Tian, Zheng, Goodyer & Bayen, 2019), and these two incurred sample matrices were therefore selected to study the thermal degradation of bisphenols.

The present study was conducted with the hypothesis that BPA and BPS can degrade in water and fish matrices under thermal treatment. In other words, the present study has aimed to 1) investigate the fate of BPA and BPS in water, spiked fish muscles and incurred fish muscles under thermal processing; 2) identify the thermal degradation products of BPA and BPS in different heating models; and 3) compare the degradation products of BPA and BPS from cooking with those from other degradation pathways (e.g. photodegradation and biodegradation).

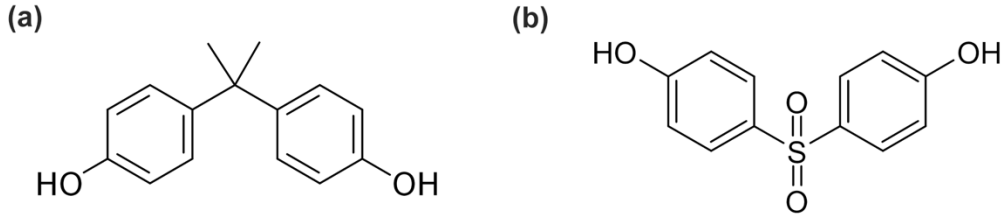

Figure 6.1 Chemical structures of BPA (a) and BPS (b)

6.3 Materials and methods

6.3.1 Chemicals

Ammonium acetate (LC-MS grade) and HPLC-grade solvents (water, acetonitrile, ethyl acetate and methanol) were purchased from Fisher Scientific (Hampton, USA). Analytical standards of BPA (purity ≥ 99%), BPS (purity ≥ 98%) and β-glucuronidase (type HP2, ≥100,000 units/g) were purchased from Sigma-Aldrich (St. Louis, USA). BPA-$^{13}C_{12}$ (purity ≥ 98%), BPS-$^{13}C_{12}$ (purity ≥ 98%) and 4,4-dihydroxystilbene (purity ≥ 98%) were purchased from Toronto Research Chemicals (Toronto, Canada). Stock solutions of the individual bisphenols were prepared in methanol (100 mg L^{-1}). Stock solutions of the two native bisphenols and two labeled bisphenols were prepared weekly at a concentration of 1 mg L^{-1} in methanol. All the stock solutions were stored in amber glass vials in the freezer (-20 °C) prior to analysis.

6.3.2 Fish sample collection and incurred sample selection

The workflow for fish sample collection and analysis as well as incurred sample selection is presented in Figure S6.1. In brief, fish samples, cod (*Gadus morhua*) and basa (*Pangasius bocourti*), were collected in six local markets in Montreal, Canada in November 2017 and May 2018. Fish composites were prepared following the methods described in a previous study (Tian, Zheng et al., 2019). In brief, fish samples were ground in a stainless-steel manual meat grinder. Each food composite was prepared by transferring about 30 g of the individual food sample into an amber glass jar (250 mL) followed by freeze drying (Martin Christ Gamma 1-16 LSC freeze-dryer, Osterode am Harz, Germany). Additional aliquots of each individual food homogenate were wrapped in aluminum foil, vacuum sealed by a polypropylene bag and stored in a freezer (-80 °C). Freeze-dried food composites were further homogenized using a mortar and pestle and were stored in amber glass jars at -80 °C.

All the composites were extracted and analyzed by high performance liquid chromatography coupled with quadruple time-of-flight mass spectrometry (HPLC-QTOF-MS) as described in the study of Tian, Zheng et al. (2019) to screen for BPA and BPS in fish. Based on these results, several fish samples were selected for the present study. One cod fillet sample containing BPA (92.1 ng g^{-1}, wet weight), and one basa fillet sample containing BPS (71.4 ng g^{-1}, wet weight) were selected as incurred samples for thermal treatment. Two other cod and basa samples, measured to be free of the two bisphenols (below 30 pg g^{-1}) were used as controls and spiked matrices as described below (section 6.3.3).

6.3.3 Thermal treatment

About 2 g (± 0.05 g) of fresh incurred cod (named as "*IC cod*"), fresh incurred basa ("*IC basa"*), fresh control cod ("*BL cod*") and basa ("*BL basa*") were weighed in 20 mL glass amber vials. 100 µL BPA stock solution (1 mg L^{-1}) and 100 µL BPS stock solution (1 mg L^{-1}) were spiked into the *BL cod* and *BL basa*, to produce "*SP cod*" and "*SP basa*" samples, respectively. "*IC cod-L*" and "*IC basa-L*" samples were prepared by spiking with 100 µL $^{13}C_{12}$-labeled BPA stock solution (1 mg L^{-1}) and 100 µL $^{13}C_{12}$-labeled BPS stock solution (1 mg L^{-1}) in "*IC cod*" and "*IC basa*", respectively. For the water model, two mL of HPLC grade water were added to two 20 mL glass vials and then spiked with 100 µL BPA stock solution (1 mg L^{-1}) or 100 µL BPS stock solution (1 mg L^{-1}), respectively. The spiking levels of the bisphenols in the water model were much lower than the solubility for both BPA and BPS (300 and 1100 mg L^{-1}, respectively).

All of the sample vials were capped with polypropylene caps and then heated in a water bath at 100°C for 1 hour. After cooking, samples were taken out from the water bath and allowed to cool down to room temperature. Repeated experiments (n=5) were performed for each treatment. According to the OECD guideline, heating for 50 min is acceptable for a boiling condition (OECD 507, 2007); in the present study, 1 hour was applied to mimic extreme cooking conditions as well as to yield enough degradation for compound identification (Tian & Bayen, 2018).

6.3.4 HPLC sample extraction

After cooling, 60 μL of $^{13}C_{12}$-BPA stock solution (1 mg L^{-1}) was spiked into IC cod, SP cod and water (containing BPA) samples. Sixty μL of $^{13}C_{12}$-BPS stock solution (1 mg L^{-1}) was spiked into IC basa, SP basa and water (containing BPS) samples. Mass labeled surrogates were used to quantify BPA or BPS in these samples. *IC cod-L* and *IC basa-L* samples were not used for quantification purpose and, thus, bisphenol-labeled stock solutions were not added to these samples before extraction.

All of the samples, except water, were then transferred into individual 15-mL polypropylene centrifuge tubes using 6 mL of methanol. Tubes were vortexed for 1 min using a Vortex Mixer (Fisher Scientific, Hampton, USA), sonicated using a Branson 3510 sonication bath (40 KHz) for 30 min and, finally, centrifuged at 4500 rpm for 10 min. The supernatant was collected and filtered through a 0.22 μm polytetrafluoroethylene (PTFE) filter (Norm-Ject, Tuttlingen, Germany) into HPLC amber glass vials. Unheated samples (n=5 for each group) were extracted the same way as heated ones. All extracts were stored at -20 °C in the dark until HPLC-MS analysis. Water model samples were directly injected without extraction steps.

6.3.5 BPA and BPS deconjugation tests

As BPA can be metabolized to BPA glucuronide in fish (e.g. carp (*Cyprinus carpino*)) (Kang, Katayama & Kondo, 2006; Yokota, Miyashita & Yuasa, 2002), a deconjugation test was conducted to compare the levels of free/total bisphenols in fish muscles before and after thermal treatment. The method in the present study was adapted from Tan et al. (2019). β-Glucuronidase from *Helix pomatia* is commonly used to deconjugate the glucuronidated BPA and BPS as well as sulfated forms of BPA (Tan et al., 2019). In our work, four fresh *IC cod* samples (1 g each) were weighed in 20 mL glass tubes. Two of them were heated at 100°C for 1 h in water bath (one used as a control sample and one used as a test sample in the deconjugation test described below) while the other two were not heated (one used as a control sample and one used as a test sample in the

deconjugation test discussed below). Ten ng of $^{13}C_{12}$-BPA standard was added to each sample. Then 2 mL of 1 M ammonium acetate buffer (pH 4.7) containing 2000 units of β-glucuronidase were added. The mixture was gently mixed and incubated for 18 hrs at 37 °C. After incubation, 1 mL of water was added to the solution. The mixture was extracted three times with 3 mL of ethyl acetate by vortexing for 5 minutes followed by centrifugation (4000 rpm, 10 min). The organic phase was taken out of the tube, evaporated to dryness under a gentle stream of nitrogen and then reconstituted with 1 mL of methanol. This liquid was filtered through a 0.22 μm PTFE filter (Norm-Ject, Tuttlingen, Germany) into HPLC amber glass vials and kept in the -20 °C freezer until HPLC analysis. Control samples were treated the same way as above but without β-Glucuronidase in the buffer. This test was performed twice.

To study the possible conjugation of BPS in fish fillets, *IC basa* samples were treated the same way as above except that 10 ng of $^{13}C_{12}$-BPS were spiked prior to the deconjugation.

6.3.6 HPLC-MS analysis

Samples were analyzed using an Agilent 1290 Infinity II LC system (Agilent Technologies, Santa Clara, USA) coupled to a 6545 quadrupole TOF-MS (Agilent Technologies, Santa Clara, USA) operating in both positive (ESI+) and negative (ESI-) electrospray ionization modes. The LC separation was conducted on a Poroshell 120 Phenyl Hexyl column (Agilent Technologies; 2.7 μm × 3.0 mm × 100 mm) fitted with a Poroshell 120 Phenyl Hexyl (2.7 μm × 3.0 mm ×10 mm) guard column. The mobile phase (0.2 mL min^{-1}) consisted of a mixture (gradient mode) of water (solvent A) and methanol (solvent B), both containing 10mM ammonium acetate. The percentage of organic mobile phase B increased linearly and the gradient was as follows: 5% solvent B for 1min, from 1-5min the solvent B was increased to 100%, from 15-20 min the solvent B was kept at 100%, and at 20 min the eluent was restored to the initial conditions for 5 min to re-equilibrate the column for the next injection. The injection volume was set at 10 μL and the column temperature was maintained at 20 °C. Nitrogen was used as the drying gas (325 °C). The gas flow was 5 L min^{-1}.

Samples were run in the All Ions MS/MS mode at four collision energies (0 V; 10 V; 20 V; 40 V) with a fragmenter energy of 150 V. MS data were acquired in the *m/z* 50-1700 range.

6.3.7 Data treatment

6.3.7.1 Quantification for degradation percentage

Chromatographic data were analyzed using Agilent MassHunter Quantitative analysis (B.07.01) software to quantify the concentrations of BPA and BPS in fish before and after thermal treatment. The most abundant isotopes of $[M-H]^-$ were used as quantifier for BPA (227.1072 *m/z*) and BPS (249.0222 *m/z*). The chromatogram extraction window was ± 10 ppm for mass and ± 0.5 min for retention time (RT). The recovery difference between raw and cooked matrix was corrected by the internal standards ($^{13}C_{12}$-BPA and $^{13}C_{12}$-BPS) for quantification. The degradation percentage was calculated based on the mass of bisphenols in food before and after cooking. As the whole sample after cooking (cooked fish muscle as well as the juice around) were extracted (section 6.3.4), correction for the moisture loss was not required.

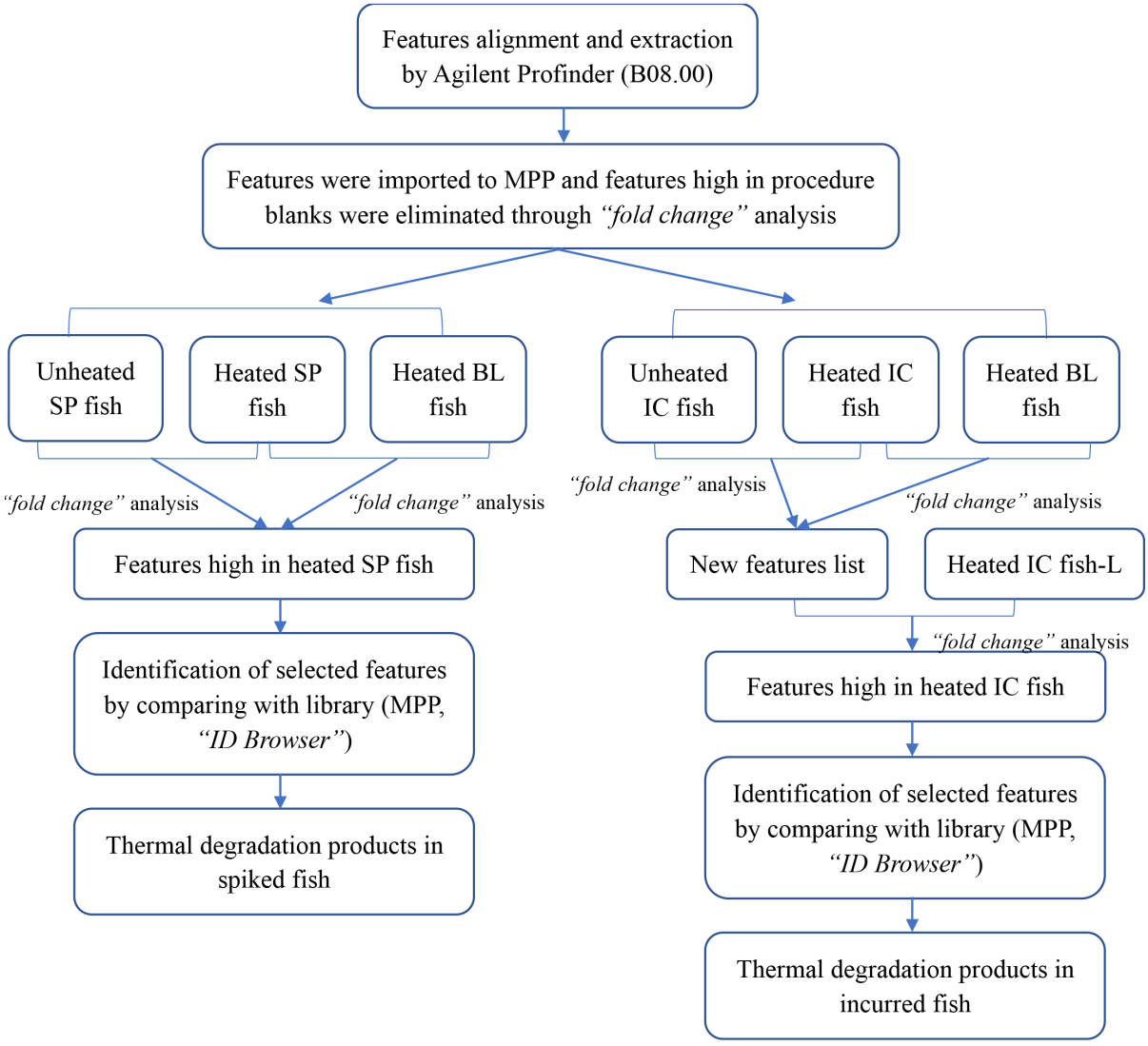

Figure 6.2 The workflow for thermal degradation product identification

6.3.7.2 Thermal degradation product identification

The thermal degradation products of BPA and BPS were identified using the Agilent MassHunter Profiling software series following a workflow (Figure 6.2) adapted from a previous study (Tian, Verreault, Houde & Bayen, 2019). Briefly, chromatographic data were aligned and features were extracted using Agilent Profinder (B08.00) with optimized parameters from Tian, Verreault et al. (2019). Molecular features were then compared among the samples using MassHunter Profiler

Professional (MPP, version B14.0). A "*fold change*" analysis in MPP was applied on the feature abundance to identify relatively more abundant molecular features in heated sample groups as compared to unheated ones (Figure 6.2). Fold change analysis has been reported to be a useful tool to distinguish features from different groups when applying non-targeted analysis (Knolhoff, Zweigenbaum, & Croley, 2016). Formulas for specific molecular features were generated using exact mass (mass accuracy < 5 ppm) and the isotopic pattern information (ion abundance ratio difference < 5%). Formulas with the lowest mass errors and with the most similar relative ion abundance ratios were selected by software as the top candidates and compared with the library for identification. The Agilent Extractables & Leachables LC/QTOF Personal Compound Database and Library (PCDL) containing 1,006 compounds and the METLIN Metabolite PCDL containing 24,000 compounds were used in this study. The mass of degradation products and metabolites of BPA and BPS reported in literature were also included in the screening (see Table 6.1 and Table 6.2).

Table 6.1 Metabolites and degradation products of BPA proposed or reported in the literature

Molecular formula	Molecular weight*	Reaction type	References
$C_3H_6O_3$	90.0316	Metabolite	Zhang et al., 2013
$C_4H_6O_6$	150.0164	Metabolite	Daâssi et al., 2016
$C_4H_8O_3$	104.0473	Metabolite	Daâssi et al., 2016
C_6H_6O	94.0418	Degradation	Lee, Kim, & Kim, 2004
$C_6H_6O_2$	110.0367	Metabolite	Eio et al., 2014
$C_6H_6O_4$	142.0266	Degradation	da Silva et al., 2014)
C_6H_7O	95.0496	Degradation	Watanabe et al., 2003
C_7H_6O	106.0418	Metabolite	Zhang, Yin, & Chen, 2013
$C_7H_6O_2$	122.0367	Degradation/ Metabolite	Watanabe et al., 2003; Zhang et al., 2013; Ike et al., 2002; Eio et al., 2014
$C_7H_6O_3$	138.0316	Metabolite	Ike et al., 2002; Eio et al., 2014
$C_7H_8O_4$	156.0423	Degradation	da Silva et al., 2014
C_8H_8	104.0626	Degradation	Li et al., 2008
$C_8H_{10}O$	122.0731	Degradation/ Metabolite	Watanabe et al., 2003; Zhang et al., 2013
$C_8H_{11}O$	123.0809	Metabolite	Zhang et al., 2013
C_8H_{12}	108.0939	Degradation	Lee, Kim, & Kim, 2004
$C_8H_8O_2$	136.0524	Metabolite	Zhang et al., 2013; Ike et al., 2002; Eio et al., 2014
$C_8H_8O_3$	152.0473	Metabolite	Ike et al., 2002; Eio et al., 2014
$C_9H_{10}O$	134.0731	Degradation	Barbieri et al., 2008; Huang & Weber, 2005; Li et al., 2008
$C_9H_{10}O_4$	182.0579	Degradation	da Silva et al., 2014
$C_9H_{12}O$	136.0888	Degradation	Watanabe et al., 2003; Li et al., 2008
$C_9H_{12}O_2$	152.0837	Degradation/ Metabolite	Watanabe et al., 2003; Huang & Weber, 2005; Zhang et al., 2013
$C_9H_{12}O_3$	168.0786	Degradation	da Silva et al., 2014
$C_{11}H_{16}O$	164.1201	Degradation	Lee, Kim, & Kim, 2004

$C_{14}H_{14}O_2$	214.0993	Metabolite	Zhang et al., 2007
$C_{15}H_{14}O_2$	226.0993	Metabolite	Zhang et al., 2013; Ike et al., 2002
$C_{15}H_{14}O_3$	242.0943	Degradation	da Silva et al., 2014; Barbieri et al., 2008; Deborde et al., 2008
$C_{15}H_{14}O_4$	258.0892	Metabolite	Zhang et al., 2013; Ike et al., 2002; Eio et al., 2014
$C_{15}H_{15}O_3$	243.1021	Metabolite	Zhang et al., 2013
$C_{15}H_{16}O_3$	244.1099	Degradation/ Metabolite	Choi & Lee, 2017; da Silva et al., 2014; Barbieri et al., 2008; Deborde et al., 2008; Zhang et al., 2013; Ike et al., 2002; Eio et al., 2014
$C_{15}H_{16}O_4$	260.1049	Degradation/ Metabolite	Choi & Lee, 2017; da Silva et al., 2014; Zhang et al., 2013; Ike et al., 2002
$C_{15}H_{16}O_5$	276.0998	Degradation/ Metabolite	Choi & Lee, 2017; Deborde et al., 2008
$C_{15}H_{18}O_6$	294.1103	Metabolite	Choi & Lee, 2017
$C_{15}H_{20}O_3$	248.1412	Metabolite	Zhang et al., 2013
$C_{15}H_{20}O_5$	280.131	Degradation	Watanabe et al., 2003
$C_{16}H_{16}$	208.1252	Degradation	Li et al., 2008
$C_{16}H_{26}O$	234.1983	Degradation	Lee, Kim, & Kim, 2004
$C_{18}H_{20}O_2$	268.1463	Degradation	Huang & Weber, 2005
$C_{21}H_{20}O_3$	320.1412	Degradation	Huang & Weber, 2005
$C_{27}H_{30}O_3$	402.2194	Degradation	Huang & Weber, 2005
$C_{30}H_{30}O_4$	454.2144	Degradation	Huang & Weber, 2005

Note: Molecular formulas in bold were confirmed with chemical standards.

* Molecular weight was calculated using Exact Mass Calculator (https://www.sisweb.com).

Table 6.2 Metabolites and degradation products of BPS proposed or reported in the literature

Molecular formula	**Molecular weight***	**Reaction type**	**References**
$C_2H_4O_4S_2$	195.9551	Degradation	Wang et al., 2017
$C_2HO_3S_2$	136.9367	Degradation	Wang et al., 2017
$C_6H_{14}O_5S$	198.0561	Degradation	Shao, Ren, et al., 2017

C_6H_6O	94.0418	Degradation	Shao, Ren, et al., 2017
$C_6H_6O_2$	110.0367	Degradation	Cao et al., 2013
$C_6H_6O_2S$	142.0088	Degradation	Shao, Duan, et al., 2017
$C_6H_6O_4S$	173.9986	Degradation	Cao et al., 2013; Shao, Ren, et al., 2017; Shao, Duan, et al., 2017
$C_6H_6O_5S$	189.9935	Degradation	Shao, Ren, et al., 2017
$C_7H_{11}O_6S$	223.0276	Degradation	Wang et al., 2017
C_7H_{14}	98.1095	Degradation	Shao, Duan, et al., 2017
$C_7H_{14}O_4S$	194.0612	Degradation	Shao, Ren, et al., 2017
$C_7H_{14}S$	130.0816	Degradation	Shao, Duan, et al., 2017
$C_8H_4O_6S$	227.9728	Degradation	Wang et al., 2017
$C_8H_7O_2$	135.0446	Degradation	Wang et al., 2017
$C_8H_8O_4S$	200.0143	Metabolite	Choi & Lee, 2017
$C_{10}H_{14}S$	166.0816	Degradation	Shao, Duan, et al., 2017
$C_{11}H_9O_4S$	237.0221	Degradation	Wang et al., 2017
$C_{12}H_{10}O_2S$	218.0401	Degradation	Shao, Duan, et al., 2017
$C_{12}H_{10}O_3S$	234.035	Degradation	Shao, Duan, et al., 2017
$C_{12}H_{10}O_7S$	298.0147	Metabolite	Choi & Lee, 2017
$C_{12}H_{10}O_8S$	314.0096	Metabolite	Choi & Lee, 2017
$C_{12}H_{12}S$	188.0659	Degradation	Shao, Duan, et al., 2017
$C_{24}H_{18}O_8S_2$	498.0443	Degradation	Wang et al., 2017

Note: * Molecular weight was calculated using Exact Mass Calculator (https://www.sisweb.com).

6.3.8 Method performances and quality assurance

6.3.8.1 Method validation

To validate the method performance for the two bisphenols in each matrix, solvent and matrix-matched calibrations (six levels ranging from 10 to 200 μg L^{-1} with labeled standard inside at 60 μg L^{-1}) were built. The linearity of the instrument response was assessed through the analysis of standards prepared in methanol. The method detection limit (MDL) was calculated as three times the standard deviation of procedure blanks divided by the slope of the matrix-matched calibration curve (Tian, Verreault et al., 2019). Control fish fillets were used for the recovery tests. Extraction recoveries for BPA and BPS were assessed for both raw and cooked fish muscles (n = 3 for each matrix) by standard addition method (Diana Di Mavungu et al., 2009).

6.3.8.2 Quality assurance

Quality assurance (QA) in the present study was conducted by analyzing different QA samples including procedural blanks (n=6), solvent blanks (n=3) as well as quality control samples (QC samples) (n=6) in between the sample batches, using reference solutions during the LC-MS analysis and controlling for background contaminants (e.g. avoiding using plastic lab ware as much as possible and baking all glassware at 320 °C for 5 hrs before use).

Retention time drifts of the HPLC-MS analysis were detected using "pooled QC samples" (Gika, Theodoridis, Plumb, & Wilson, 2014). In this study, pooled QC samples were prepared by mixing 10 μL aliquots from each experimental vial (including procedure blanks, heated and unheated samples) and analyzed every ten samples. Mass accuracy of the TOF was continuously maintained by an API-TOF reference mass solution (contains hexakis(1H, 1H, 3H-tetrafluoropropoxy)phosphazine, purine and trifluoroacetic acid ammonium salt) (Agilent Technologies, Santa Clara, USA) via an ESI nebulizer.

The relative standard deviation (RSD) for the inter-day precision was calculated based on the analysis of three replicates of the same sample (contains 10 μg L^{-1} of BPA or BPS in fish extracts) on different days. An inter-day precision (RSD) lower than 15% was judged acceptable.

6.4 Results and discussion

6.4.1 Method validation

The mean mass measurement error (MMME) was calculated based on the method reported by Brenton and Godfrey (2010) to evaluate the accuracy of mass measurement in the present study. The MMME ranged from 1.7–3.6 ppm for BPA and BPS in pure methanol and water, and from 3.9 to 4.5 ppm in fish muscle extracts. The instrumental response linearity for HPLC-QTOF-MS was satisfactory ($R^2 > 0.99$), and the MDLs were lower than 91.2 pg g^{-1} for both raw and cooked muscles (Table S6.1). The inter-day precision was estimated to be below 6.1% for both BPA and BPS. The average extraction efficiency of BPA and BPS in raw and cooked fish muscles shown in Table S1 confirmed the efficiency of the present method for bisphenol analyses. The extraction efficiency for incurred samples was considered to be the same as for spiked samples.

6.4.2 Degradation percentages of BPA and BPS in water, incurred samples and spiked samples

Both BPA and BPS did not degrade (< 0.1% degradation) in water at 100°C after 1 hour heating. The thermal degradation percentage of BPA was 33.0±1.5% and 35.4±1.2% in incurred and spiked cod, respectively; the thermal degradation percentage of BPS was 34.7±1.7% and 37.5±1.4% degradation in incurred and spiked basa, respectively (Table S6.2). The percentages were not significantly different (t-test, p>0.05) when comparing incurred and spiked models for both BPA and BPS. The peak area for $^{13}C_{12}$-labeled BPA and $^{13}C_{12}$-labeled BPS also decreased (about 35%) in *IC cod-L* and *IC basa-L* after thermal treatment, respectively, which provides further evidence for the degradation of bisphenols in the fish matrix. The concentrations of BPA and BPS in fish

muscles (both cooked and raw) were not changed (p>0.05) before and after the deconjugation test (n=2 for each condition). These results indicate that (i) BPA and BPS in fish muscles were mostly present in their free form, and (ii) the thermal treatment applied in the present study did not yield any conjugated BPA or conjugated BPS.

6.4.3 Identification of thermal degradation products of BPA

The thermal degradation products of BPA were identified following the workflow in Figure 6.2. Features showing a high abundance in "heated *SP cod*" and "heated *IC cod*" but not high in corresponding unheated groups were exported for further analysis (features from *IC cod-L* was used to ensure that the features exported from the IC cod group were related to BPA degradation but not only to the fish matrix). Molecular formulas were generated (Table S6.3) based on the isotopic patterns and exact masses following the criteria reported in a previous study (Tian, Verreault et al., 2019). The "*Seven Golden Rules*" for formula generation by mass spectrometry (Kind & Fiehn, 2007) were applied in the present study. Notably, the N/C ratio should be lower than 1.3, and for molecules with a molecular weight <1000, the N number should not exceed 25. Under ESI- mode, the differences between features from heated *SP cod* and heated *BL cod* were investigated using the results of the *principal component analysis* (PCA) and *fold change* (Figure 6.3). In the end, 12 and 13 features detected in ESI- mode, were confirmed for their formulas in spiked and incurred cod muscles, respectively. However, for the ESI+ mode, only two features were suggested based on the workflow in incurred fish muscles and no features could be found that were related to BPA in heated spiked cod (Table S6.3). Most notably, none of the degradation products reported in the literature (Table 6.1) matched the neutral masses of the features in the heated *SP cod* or *IC cod* samples.

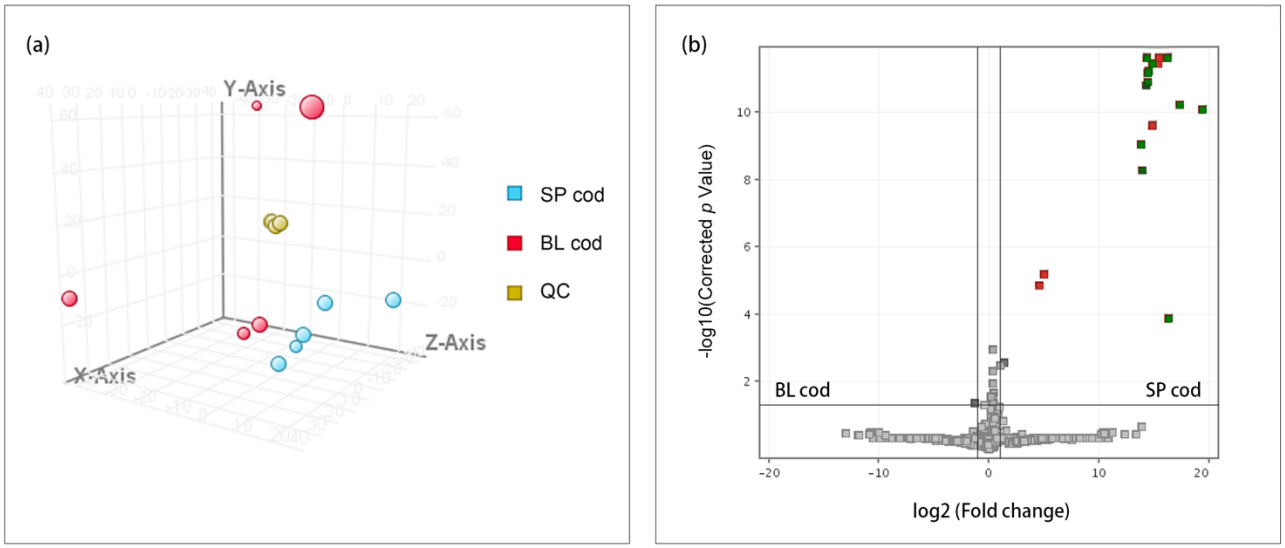

Figure 6.3 Differences between features from heated *SP cod* and heated *BL cod* (a. PCA result; b. Fold change result. In Figure 6.3b, features with confirmed formulas from heated *SP cod* in Table S6.3 are highlighted in green; red squares represent features in heated *SP cod* that are significant different from those in BL cod but cannot be identified for their formula due to the big mass difference (>10ppm); grey squares represent features without a significant difference between heated BL cod and heated SP cod (p>0.05)).

Most of the features in Table S6.3 have a higher mass and additional atoms compared to BPA, which tends to suggest complex degradation/recombination mechanisms of BPA happened under the thermal treatment in cod muscles. Feature 212.0837 (*m/z* 211.0763, $[M-H]^-$) was tentatively identified as 4,4-dihydroxystilbene (Figure S6.2a) using the library. A feature *m/z* 223.0763 ($[M-H]^-$) was also detected in heated *IC cod-L* samples (i.e. spiked with $^{13}C_{12}$-BPA) with the same RT, providing support that feature 212.0837 (*m/z* 211.0763, $[M-H]^-$) is related to BPA. This structure would suggest that a recombination occurred, leading to the formation of a C=C double bound during heating. A similar structure (4,4'-dihydroxy-α-methylstilbene) has been reported by Ike et al. (2002) during the bio-degradation of BPA (Figure S6.2b). An analytical standard of 4,4-dihydroxystilbene was purchased to confirm the identity of feature 212.0837. However, the RT of

4,4-dihydroxystilbene did not match with the RT for feature 212.0837 (RT difference of 0.7 min), invalidating the tentative identity of feature 212.0837 suggested by library.

6.4.4 Identification of thermal degradation products of BPS

The literature on the degradation of BPS is much scarcer than for BPA. Some proposed degradation products of BPS from biodegradation and photodegradation are summarized in Table 6.2. In the present study, the identification of thermal degradation products of BPS in the basa fillet matrix was conducted the same way as for BPA. Under ESI-, only one feature in heated *SP basa* was filtered as a potential thermal degradation product of BPS using the "*fold change*" analysis (Table S6.4), and four features were recorded for *IC basa*. Under ESI+, the PCA analysis did not reveal any clear differences between heated *SP basa* and heated *BL basa* (Figure S6.3 in supplementary material), and no feature could be related to BPS degradation in heated SP basa. Four features possibly related to BPS were detected in the heated *IC basa*. However, no tentative structure was suggested for these features from the library or from the literature. Indeed, none of the mass or formula for the features detected in the heated *SP basa* or *IC basa* samples matched those reported in Table 6.2.

6.4.5 Comparison of thermal degradation products in different heating models

OECD has suggested the unsuitability of using spiked samples to study the stability of pesticides during processing (OECD 508, 2008). Also, the differences among water, incurred and spiked samples have been suggested previously in the literature (Tian & Bayen, 2018). To date, there are no specific guidelines for testing the stability of contaminants in food matrices during processing besides pesticides. In this study, the fate of BPA and BPS in water and fish matrices was investigated for the first time. Results indicated that BPA and BPS did not degrade in water when exposed to 100°C for 1 hour. However, under the same conditions, both BPA and BPS degraded in spiked and incurred fish fillet models.

Except for feature 212.0837 (neutral mass), all the degradation products of BPA and BPS were different between spiked samples and incurred samples. These results indicate that food matrices can influence the degradation of BPA and BPS. In other words, the "water model" cannot reflect the real thermal degradation of bisphenol residues in food samples (e.g. fish). The unsuitability of using the water model in studying the fate of contaminants in food was also reported for antibiotics (Tian & Bayen, 2018; Tian, Khalil, & Bayen, 2017). Instead, incurred samples (e.g. IR-cod and IC basa in this study) appear to be essential to study contaminant degradation and provide credible information for risk assessment.

6.4.6 Relationship to risk assessment

Although the application of BPA has decreased (Baluka & Rumbeiha, 2016), it can still be detected in fish from the present study. BPS, suggested to be the BPA alternative in some applications (Eladak et al., 2015), was also detected in fish. Only a small fraction of BPA (33±1.5%) and BPS (34.7±1.7%) degraded in incurred fish muscles after heating for 1 hour (at 100 °C). These results tend to indicate that, under normal cooking conditions (internal temperature < 100 °C, time <1 hour), most of BPA and BPS residues in a fish fillet portion would remain as the parent compound. Nonetheless, since the toxicity of any of the bisphenol degradation products remains unknown, it is not currently possible to conclude if cooking would alter the risks associated with these residues. Based on the present results, it seems that monitoring only the levels of the parent contaminants, such as BPA and BPS, is insufficient to fully assess the risks of such compounds in cooked food, as complex reactions may occur between the contaminants and the food matrices. More efforts should be placed on identifying and assessing the degradation products of particular contaminants in food produced during processing to avoid any unwanted impact on consumers. This task is quite intricate because of the diversity of food matrices, contaminants and processes (cooking) applied to food.

6.5 Conclusions

In the present study, thermal degradation of BPA and BPS in water and fish models was investigated using sonication-assisted liquid extraction followed by HPLC-QTOF-MS analysis. Satisfactory linearity, LOD and recoveries were achieved. The degradation percentages were similar for bisphenols in the SP and IC groups, as well as for their $^{13}C_{12}$-labeled analogs spiked to the IC group. In addition, in the spiked model, unique features were observed after heating as compared to the control blank matrix. In particular, one unique feature (*neutral mass* 212.0837) was observed in the heated fish spiked with BPA, and was matched by its equivalent (*neutral mass* 224.0836) in the heated fish spiked with $^{13}C_{12}$-BPA. The results of the present study provide evidences that both BPA and BPS degraded in spiked and incurred fish muscle models, but not in water models under the same conditions. Furthermore, the degradation products in spiked samples were found to be different from those in incurred groups. These results indicate that the degradation of BPA and BPS is matrix-dependent and highlight the limitation of relying only on the "water model" or "spiking model" to investigate the thermal degradation of food contaminants.

Non-targeted analysis based on LC-MS has been become a popular tool to identify unknown chemicals in food (Picó & Barceló, 2008). However, applying a non-targeted approach to study the degradation products of food contaminants still has many challenges, including the high price of the HRMS instrument and the limitations of library capacity and the deconvolution and MS-structure correlation software (Picó & Barceló, 2008). Future studies should focus on overcoming these issues to increase the identification rate. Furthermore, the toxicity of thermal degradation products of food contaminants should also be investigated in future studies to ensure food safety.

6.6 Acknowledgement

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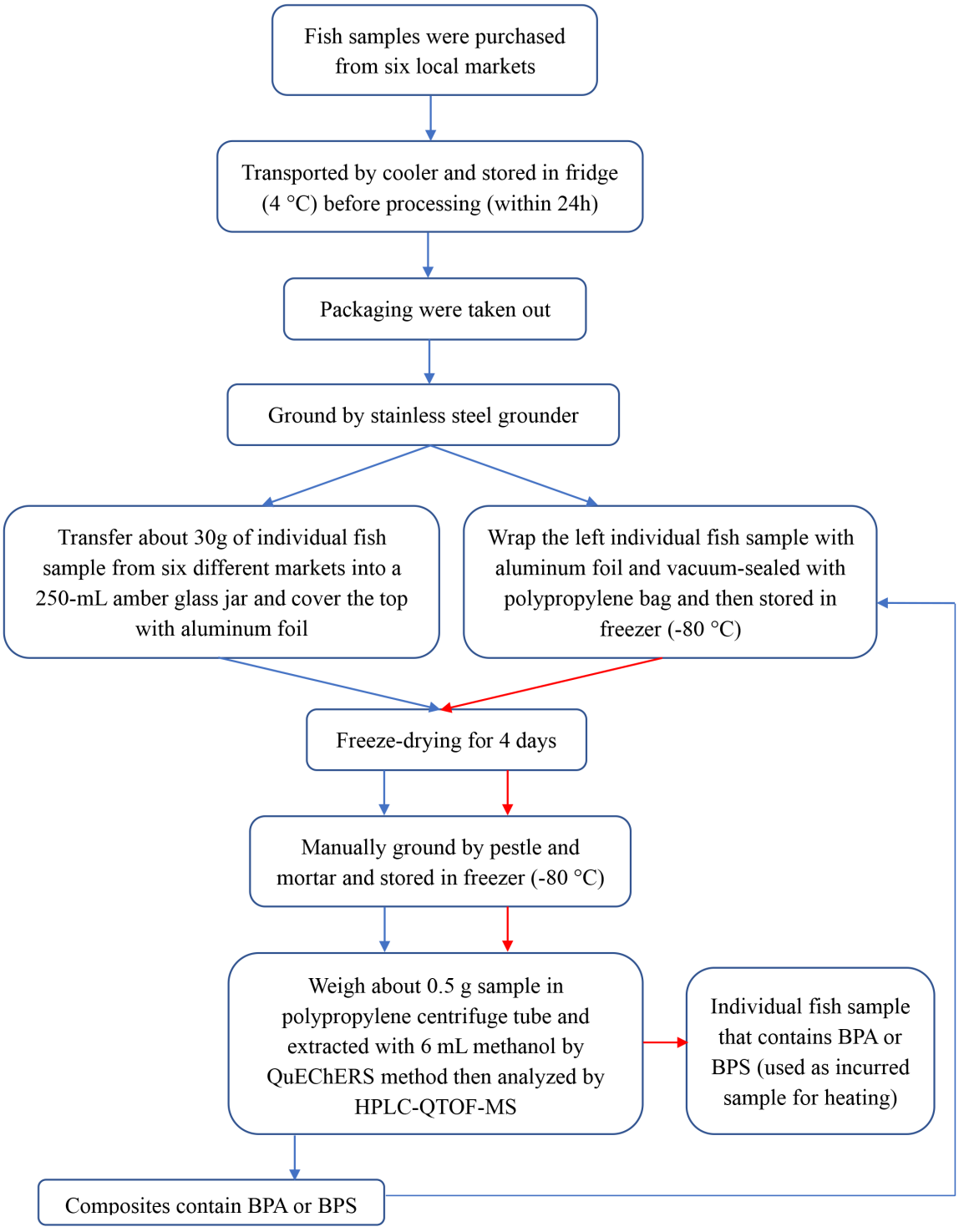

Figure S6.1 Workflow for fish sample collection and incurred sample selection

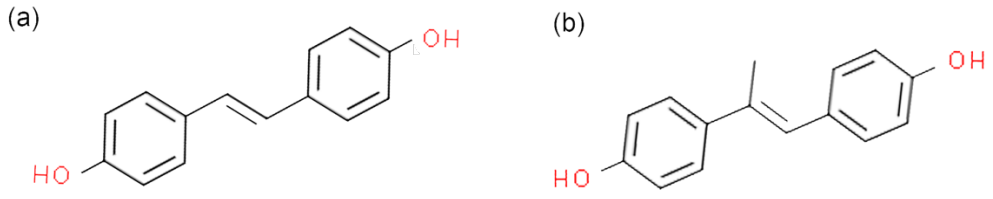

Figure S6.2 Chemical structures of 4,4'-dihydroxystilbene (a) and 4,4'-dihydroxy-α-methylstilbene (b)

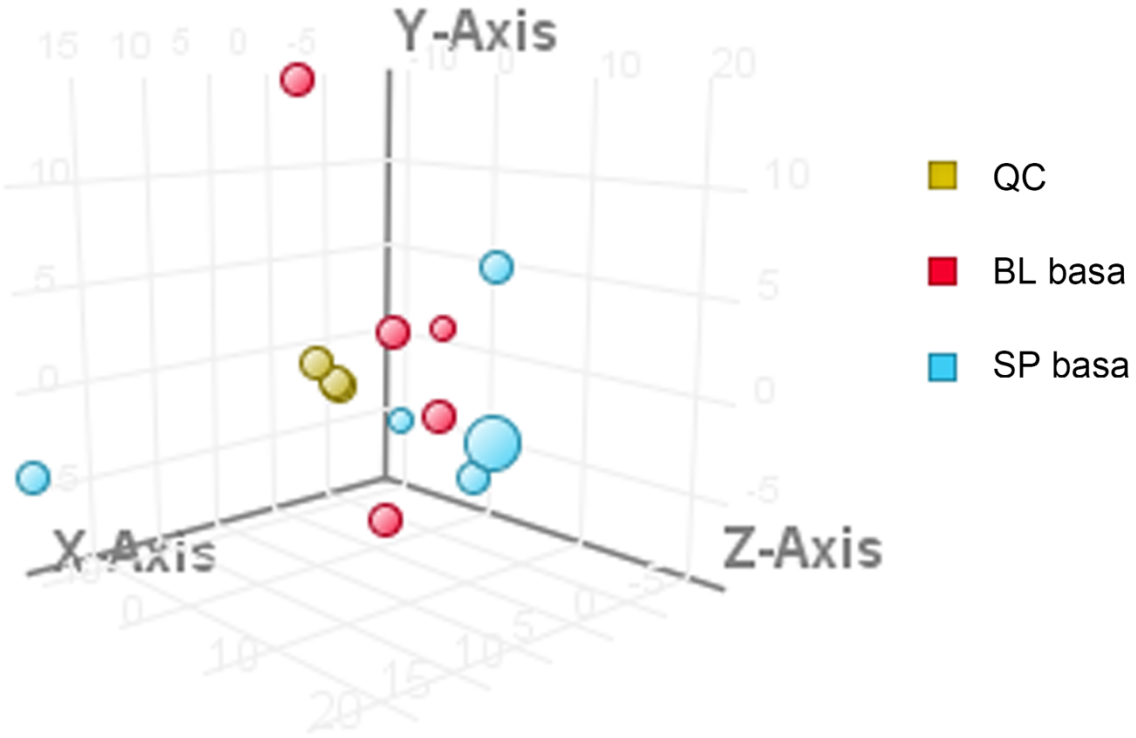

Figure S6.3 PCA results for cooked *SP basa* and cooked *BL basa*

Table S6.1 Method validation for BPA and BPS in fish muscles

Bisphenols	**MDL (pg g^{-1}, wet weight, n=6)**		**Recovery (%, n=3)**		**RSD (%, n=3)**	
	Raw	Cooked	Raw	Cooked	Raw	Cooked
BPA in cod	77.8	91.2	124.2±11.3	118.1±9.2	5.4	6.1

BPS in basa	31.1	39.5	83.3±12.0	128.4±5.1	3.3	5.9

Table S6.2 Thermal degradation percentage (%) for BPA and BPS in incurred and spiked samples (n=5)

Bisphenols	Incurred samples	Spiked samples
BPA in cod	33.0±1.5	35.4±1.2
BPS in basa	34.7±1.7	37.5±1.4

Table S6.3 Degradation products of BPA in *SP cod* and *IC cod*

Ionization mode	Features in SP cod (neutral mass)	RT (min)	Suggested molecular formula
ESI-	212.0837	14.9	$C_{14}H_{12}O_2$
	392.2527	15.2	$C_{17}H_{30}N_9O_2$
	495.3307	17.8	$C_{26}H_{45}N_3O_6$
	646.3671	15.3	$C_{29}H_{42}N_{16}O_2$
	686.5451	21.3	$C_{40}H_{66}N_{10}$
	687.3783	13.5	$C_{26}H_{39}N_{24}$
	718.4291	14.1	$C_{40}H_{52}N_{11}O_2$
	829.4486	13.5	$C_{29}H_{51}N_{25}O_5$
	834.4107	12.8	$C_{33}H_{44}N_{25}O_2$
	875.4363	13.2	$C_{44}H_{61}N_9O_8S$
	889.4879	13.8	$C_{36}H_{51}N_{29}$
	932.4594	15.0	$C_{40}H_{52}N_{24}O_4$
Ionization mode	**Features in IC cod (neutral mass)**	**RT (min)**	**Suggested molecular formula**
ESI-	204.0251	14.4	$C_8H_4N_4O_3$
	212.0837	14.9	$C_{14}H_{12}O_2$
	218.0391	14.5	$C_{10}H_8N_3OS$
	299.1264	12.4	$C_{16}H_{17}N_3O_3$
	299.1381	6.3	$C_{13}H_{15}N_8O$
	319.0731	8.0	$C_{18}H_{11}N_2O_4$
	322.0328	10.2	$C_{15}H_{14}O_4S_2$
	379.1848	8.2	$C_{16}H_{19}N_{12}$
	398.337	18.0	$C_{24}H_{46}O_4$
	468.1694	8.1	$C_{21}H_{24}N_8O_3S$
	503.1981	3.3	$C_{20}H_{21}N_{15}O_2$
	684.3783	10.3	$C_{40}H_{46}N_9O_2$

	902.5188	14.0	$C_{44}H_{62}N_{20}S$
ESI+	303.2179	16.7	$C_{16}H_{31}O_5$
	509.3597	17.4	$C_{27}H_{43}N_9O$

Table S6.4 Degradation products of BPS in *SP basa* and *IC basa*

Ionization mode	**Features in SP basa (neutral mass)**	**RT (min)**	**Suggested molecular formula**
ESI-	188.1408	13.9	$C_{10}H_{20}O_3$
Ionization mode	**Features in IC basa (neutral mass)**	**RT (min)**	**Suggested molecular formula**
ESI-	245.0511	13.1	$C_{13}H_{11}NO_2S$
	288.1225	10.8	$C_{14}H_{16}N_4O_3$
	324.2657	16.5	$C_{20}H_{36}O_3$
	332.1486	10.8	$C_{16}H_{20}N_4O_4$
ESI+	322.2484	16.4	$C_{18}H_{32}N_3O_2$
	376.2535	17.9	$C_{21}H_{34}N_3O_3$
	424.2609	18.0	$C_{23}H_{32}N_6O_2$
	433.2269	17.3	$C_{12}H_{27}N_{13}O_5$

Chapter 7. General Conclusions

7.1 Conclusions

In the present research, non-targeted methods were developed and optimized to analyze multiple PRCs in different types of food. An emphasis was given to the optimization of data post-processing parameters in non-targeted workflow as this had not been studied systematically before.

First, a method based on HPLC-QTOF-MS was successfully validated for the targeted analysis of the 11 bisphenol analogues (low MDL and high accuracy). None of the bisphenols were detected in food simulants indicating that all tested bottles are free of BPA and bisphenol analogues were not applied as BPA replacement in bottle manufacture. The effect of data post-processing parameters on the feature extraction in non-targeted analysis was then systematically investigated, and results confirmed these parameters need to be carefully optimized to extract all the features and identify trace contaminants accurately. The optimized method was effectively applied to identify monomethyl terephthalate at trace level in food simulants in contact with Tritan™ bottles. To the best of our knowledge, this is the first paper reporting the optimization of the data processing parameters for non-targeted database-screening analysis on leachable residues.

Secondly, the non-targeted workflow was applied and optimized to investigate unknown contaminants in a complex biological matrix (northern pike muscle). Sonication-assisted liquid extraction followed by HPLC-QTOF-MS analysis yielded satisfactory recovery and low LOD for the target bisphenol analogues. None of the bisphenol analogues used for targeted method validation were detected in pike samples suggesting that these chemicals do not accumulate at detectable concentrations in muscle of pike naturally-exposed in the St. Lawrence River at two

sites. Peak height related parameters show high importance in chromatographic data filtering for fish samples and further demonstrated the importance of data post-processing parameters optimization in non-targeted analysis. The present non-targeted workflow was shown to accurately identify chemicals of high environmental and health concern (i.e., DEP and PFOS) in pike muscle extracts.

Then, the optimized non-targeted workflow was applied in identifying PRCs in different kinds of food collected in Canada. The non-targeted screening method developed in this study was effective as it could identify several PRCs in different food including BPA, BPS, DBA, hexadecyl methacrylate and Irganox®1076. Although numerous studies have reported the detection of bisphenols in food, to the best of our knowledge, this study is the first to report the occurrence of BPS in fresh fish filets and chicken breasts from Canada. Also, this is the first study reporting DBA in fish, hexadecyl methacrylate in chicken and Irganox®1076 in bread from the market.

Finally, the optimized non-targeted workflow was used to investigate the thermal degradation of BPA and BPS in water and fish models. Results indicate the thermal degradation of BPA and BPS in both spiked fish muscles and incurred fish muscles but not in water models under the same condition. Furthermore, the degradation products in spiked samples are different from those in incurred group. These results indicate that the degradation mechanisms of BPA and BPS are matrix dependent, and the results also highlights the limitation of relying only on the "water model" or "spiking model" to investigate the thermal degradation products of food contaminants.

Overall, the non-targeted method developed in the present research was effective as it could identify several PRCs in different types of samples including simple food simulant, various complex food matrices and cooked food. The non-targeted workflow can also be used as an early warning system for environmental and food contaminant surveillance, and offers new perspective in the context of regulatory framework. Thus, the methodology of the present thesis can be applied to study the other types of contaminants in food and environmental samples and offer some novel information for human risk assessments.

7.2 Scientific contributions

The work presented in this thesis contribute to several research novelties as follow:

- The systematic assessment of the impact of each data processing parameter (in total 9 parameters) on trace residue identification in food simulant and complex food matrix (fish) using a non-targeted approach.
- The development of targeted methods for the simultaneous analysis of multiple contaminants (bisphenols) in various foods.
- The application of the optimized non-targeted workflow to study PRCs in various food.
- Several PRCs and other contaminants were first time detected in particular matrices including: monomethyl terephthalate in food simulants in contact with Tritan™ bottles, DBA in fresh fish, hexadecyl methacrylate in chicken breast and Irganox®1076 in bread from the local markets of Montreal, and BPS in fresh fish and chicken breast from Canada.

- Determination of the fate of BPA and BPS in different matrices under thermal treatment, and the identification of thermal degradation products by a non-targeted method.

7.3 Recommendations for future research

Based on the findings in this thesis, several recommendations for future research were identified as follow:

- Application and optimization of a non-targeted method to study other emerging trace contaminants in food.
- As suspected screening is limited by the compound library capacity, efforts should focus on developing comprehensive libraries for the various classes of contaminants, including in particular MS/MS information to increase identification rates.
- The confirmation of the structure of unknown contaminants without library is currently the main bottleneck of the non-targeted workflow as it highly relies on the MS-structure correlation tools. Some other steps of the non-targeted workflow such as the chromatographic acquisition or the data deconvolution method, also need to be systematically studied to improve structural identification.
- The highest detection frequency of BPS was observed in packaged fish compared to other food categories, and this should be further investigated. Few data were available in the literature on the occurrence of these “unexpected” PRCs in food, and future research should

focus on better characterizing dietary exposure to PRCs in food among the general and specific populations in the context of food risk assessment.

- The relationship between packaging materials and the various contaminants identified in food should be investigated to understand the source of PRCs in food, especially for those first-time detected (e.g. hexadecyl methacrylate in chicken breast).
- The thermal degradation of other PRCs in food should be investigated to generate key data for food safety risk assessments.

General Reference List

Note: In accordance with the Guidelines for Thesis Preparation, each of the manuscript chapters (i.e. Chapters 3-6) contain their own reference list. Hence, the following reference list corresponds to the references included in the remaining chapters of the thesis (i.e. Chapters 1 and 2).

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