DETERMINATION OF POLYBROMINATED DIPHENYL ETHERS IN ENVIRONMENTAL SAMPLES BY GAS CHROMATOGRAPHY COUPLED WITH INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

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DOLOČITEV POLIBROMIRANIH DIFENIL ETROV V OKOLJSKIH VZORCIH S PLINSKO KROMATOGRAFIJO IN MASNO SPEKTROMETRIJO Z INDUKTIVNO SKLOPLJENO PLAZMO

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Abstract

Polybrominated diphenyl ethers (PBDEs) are flame retardants. They are frequently present as pollutants in the environment due to their widespread use in the past. In the EU Water Framework Directive (WFD), six PBDE congeners (BDE 28, BDE47, BDE 99, BDE 100, BDE 153 and BDE 154) are listed as priority substances. The analytical method used for their determination in water samples at environmental quality standard (EQS) level (0.5 ng L^{-1} for the ΣPBDEs) should possess the uncertainty equal to or less than 50 %, while their limit of quantification (LOQ) for ΣPBDEs should be below 0.15 ng L^{-1}. To meet these requirements, an analytical procedure for the determination of six PBDEs in environmental water samples by GC-ICP-MS was developed. In this procedure, samples were acidified to pH 2 ensuring the stability of PBDEs for at least 20 days. The use of Tris – citrate buffer enabled efficient desorption of PBDEs from suspended particulate matter (SPM) or humic acids (HA) hence their quantitative extraction into iso-octane. The expanded uncertainty for determination of PBDEs at EQS level was found to be around 40 % (k = 2), and the LOQ for the ΣPBDEs 0.109 ng L^{-1}.

PBDEs are lipophilic organic contaminants, which enter the wastewater treatment plants (WWTPs) from urban, agricultural and industrial discharges. Up to 90 % of PBDEs are during the wastewater treatment accumulated in the sewage sludge. To estimate the possibilities of sludge re-use, reliable determination of PBDEs concentrations is of crucial importance. For this purpose, an analytical method with minimal sample preparation steps was developed for sensitive and reliable determination of the six PBDEs in sewage sludge by GC-ICP-MS. Mechanical shaking of sewage sludge with 0.1 mol L^{-1} HCl in MeOH and subsequent addition of Tris-citrate buffer and iso-octane, the most effectively extracted PBDEs from the complex sludge matrix. Spike recovery test and comparative analysis with the species-specific isotope-dilution (ID) GC-ICP-MS confirmed the accuracy of the newly developed analytical procedure. The procedure is sensitive (LODs between 0.2 to 0.3 ng g^{-1}), repeatable and reproducible.

The general human population is exposed to PBDEs mainly through diet and dust ingestion. Due to its bioaccumulative potential, fish is believed to be the main contributor to the dietary exposure to PBDEs. Thus, reliable and very sensitive analytical methods for the determination of PBDEs in fish and mussel are needed in order to evaluate PBDEs' pollution of the aquatic environment and consequently a risk for human health. For this purpose a new analytical procedure for the determination of the six PBDEs by GC-ICP-MS in fish and mussel was developed. The 25 % aqueous solution of TMAH as an extracting agent with applying the ultrasound-assisted extraction and followed by the addition of Tris-citrate buffer and iso-octane together with mechanical shaking provided the best

extraction efficiency. A fast and minor solvent consumption clean-up step, which includes passing the extract through the column filled with Florisil, was also established. The accuracy of the analytical procedure was confirmed by analyzing the NIST SRM 1946 (fish tissue) and the NIST SRM 2974a (freeze-dried mussel tissue) by a spike recovery test and by applying a species-specific ID GC-ICP-MS quantification.

To demonstrate the applicability and robustness of the newly developed GC-ICP-MS procedures, PBDEs were determined in river and seawater samples, sewage sludge samples from the municipal WWTP as well as in river and sea fish samples.

Povzetek

Polibromirani difenil etri (PBDE) so spojine, ki so se v preteklosti množično uporabljale kot zaviralci gorenja in jih zato kot onesnažila najdemo v okolju. Evropska vodna direktiva (EU WFD) je šest analogov PBDE (BDE 28, BDE 47, BDE 99, BDE 100, BDE 153 in BDE 154) uvrstila v skupino nevarnih onesnažil, katerih skupna vsebnost v površinskih vodah ne sme preseči mejne vrednosti 0,5 ng L^{-1}, ki jo dovoljuje okoljski standard (EQS). Da bi preprečili onesnaženje vodnega okolja, EU WFD predpisuje redne nadzorne meritve šestih analogov PBDE z uporabo analiznih metod, v katerih meja kvantifikacije (LOQ) za njihovo vsoto ne preseže 0,15 ng L^{-1}. V predstavljenem doktorskem delu smo razvili nov analizni postopek določitve šestih analogov PBDE v različnih vzorcih vode z GC-ICP-MS, ki izpolnjuje pogoje, določene v EU WFD. Odvzeti vzorec vode smo z dušikovo kislino nakisali na pH 2 ter ga tako stabilizirali za najmanj 20 dni. Učinkovito desorpcijo PBDE iz suspendiranih delcev in huminskih kislin ter njihovo kvantitativno ekstrakcijo v izooktan smo dosegli z dodatkom Tris-citratnega pufra in mehanskim stresanjem vzorca. Razširjena merilna negotovost določitve PBDE pri koncentraciji EQS je bila 40 % (k=2) in LOQ za ∑PBDE 0,109 ng L^{-1}.

PBDE so organska onesnažila lipofilne narave, ki pridejo na čistilno napravo iz mestnih, kmetijskih in industrijskih virov. Na čistilni napravi se jih kar 90 % nalaga v odpadnem blatu, ki se ga večinoma sežge ali odloži na tla. Za varno ponovno uporabo odpadnega blata je pomembno, da v njem niso presežene dovoljene vsebnosti PBDE. Za zanesljivo, dovolj občutljivo in hitro določitev PBDE v vzorcih odpadnega blata smo razvili analizni postopek, ki vključuje njihovo ekstrakcijo iz odpadnega blata v izooktan ter njihovo ločbo in detekcijo z GC-ICP-MS. Najboljši izkoristek ekstrakcije smo dobili s postopkom, v katerem smo vzorcem odpadnega blata najprej dodali 10 mL 0,1 mol L^{-1} HCl v MeOH in jih mehansko stresali 2 uri, temu pa je sledilo dodatno 2-urno stresanje po dodatku Tris-citratnega pufra izooktana. Točnost analiznega postopka smo dokazali z izračunom izkoristkov določitve vsebnosti posameznih PBDE v vzorcih, ki smo jim pred določitvijo z GC-ICP-MS dodali znane množine PBDE, in z metodo izotopskega redčenja. Eksperimentalni rezultati so potrdili, da je analizni postopek občutljiv (LOD med 0,2 in 0,3 ng g^{-1}), ponovljiv in obnovljiv.

Ljudje PBDE vnesemo v telo predvsem z uživanjem hrane in vdihavanjem prašnih delcev. PBDE se bioakumulirajo v maščobnih tkivih živih organizmov. Najbolj so jim izpostavljeni ljudje, ki pogosto uživajo ribe. Za določitev vsebnosti PBDE v tkivih rib in školjk smo razvili hitro in zanesljivo analizno metodo, ki omogoča določitve PBDE v izredno nizkih koncentracijah. Metoda vključuje ekstrakcijo PBDE v izooktan, čiščenje ekstrakta ter ločbo in kvantitativno določitev PBDE z GC-ICP-MS. Ekstrakcijo smo izvedli v dveh korakih.

V prvem smo uporabili ultrazvočno ekstrakcijo in 25 % vodno raztopino TMAH in v drugem, po dodatku Tris-citratnega pufra in izooktana, mehansko stresanje. Maščobe, ki so se ekstrahirale v organsko fazo, smo odstranili z uporabo kolon, polnjenih s Florisilom. Točnost analiznega postopka smo preverili z analizo standardnega referenčnega materiala NIST SRM 1946 za ribe in NIST SRM 2974a za školjke ter z izračunom izkoristkov določitve vsebnosti posameznih PBDE v vzorcih, ki smo jim pred določitvijo z GC-ICP-MS dodali znane množine PBDE, in z metodo izotopskega redčenja. Nove analizne metode smo uporabili za določitev PBDE v vzorcih rečnih in morskih vod, vzorcih odpadnega blata iz mestne čistilne naprave ter vzorcih rečnih in morskih rib.

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Abbreviations

ABS ... Acrylonitrile-butadiene-styrene
ASE ... Accelerated solvent extraction
Ar ... Argon
BFR ... Brominated flame retardant
BSEF ... The Bromine Science Environmental Forum
Br ... Bromine
CRM ... Certified reference material
DLLME ... Dispersive liquid-liquid microextraction
ECD ... Electron capture detector
ECNI ... Electron capture negative ionization
EDC ... Endocrine disrupting chemicals
EEE ... Electronic equipment
EFSA ... European Food Safety Authority
EI ... Electron impact
EQS ... Environmental quality standard
FPUF ... Flexible polyurethane foams
GC ... Gas chromatography
GPC ... Gel permeation chromatography
HA ... Humic acid
HCl ... Hydrochloric acid
Hg ... Mercury
HIPS ... High impact polystyrene
HPLC ... High pressure liquid chromatography
HR ... High resolution
I ... Iodine
ID ... Isotope dilution
IUPAC ... International union of pure and applied chemistry
K ... Potassium
Kow ... Octanol water partition coefficient
LC ... Liquid chromatography
LLE ... Liquid-liquid extraction
LOD ... Limit of detection
LOQ ... Limit of quantification
LR ... Low resolution
MAE ... Microwave-assisted extraction
MS ... Mass spectrometry
m/z ... Mass/charge
NIST ... National Institute for Standards and Technology
P ... Phosphorous
Pb ... Lead
PBB ... Polybrominated biphenyls

PBDD ... Polybrominated dibenzodioxin
PBDE ... Polybrominated diphenyl ether
PBDF ... Polybrominated dibenzofuran
PBT ... Polybutylene terephthalate
PCB ... Polychlorinated biphenyl
PE ... Polyethylene
PLE ... Pressurized-liquid extraction
POP ... Persistent organic pollutant
PP ... Polypropylene
PTV ... Programmable temperature vaporisation
QA ... Quality assurance
QC ... Quality control
S ... Sulphur
SFE ... Supercritical-fluid extraction
Sn ... Tin
SPE ... Solid phase extraction
SPM ... Solid phase material
TMAH ... Tetramethyl ammonium hydroxide
Tris ... Tris(hydroxymethyl)aminomethane
UAE ... Ultrasound-assisted extraction
UPE ... Unsaturated polyesters
Vp ... Vapour pressure
WFD ... Water framework directive
Ww ... Wet weight
WWTP ... Wastewater treatment plant

Chapter 1

Introduction

1.1 General Information About PBDEs

Polybrominated diphenyl ethers (PBDEs) are a group of organic chemicals introduced into the market in the 1970s as brominated flame retardants (BFRs), in response to the ban of previously used flame retardants, such as polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs). The general molecular structure of PBDEs is shown in Figure 1.1. There are 10 homologs based on the number of bromine atoms attached, i.e. mono, di, tri, tetra, penta, hexa, hepta, octa, nona, and deca BDEs, which have 3, 12, 24, 42, 46, 42, 24, 12, 3 and 1 different isomers, respectively. Therefore, theoretically there are 209 possible PBDE compounds or congeners. They vary in the number and positions of bromine atoms in the molecule and have a specific brominated diphenyl ether (BDE) number assigned by the International Union of Pure and Applied Chemistry (IUPAC) system (Wei, 2012). The molecular masses of BDE congeners range from 249.1 g/mole for mono-BDEs to 959.2 g/mol for deca-BDEs (Table 1.1).

PBDEs are non-ionizable compounds with low volatility (Vp $<$ 1 Pa at 25 °C), which is different between congeners. With an increasing number of bromine atoms the volatility of congeners decreases. Hence, congeners with more bromine atoms (e.g. decaBDE) are less volatile than congeners with a lower number of bromine atoms (e.g. triBDE) (Tittlemeier, et al., 2002). PBDEs are lipophilic and hydrophobic persistent organic pollutants (POPs) being tightly absorbed to organic matter and possessing a tendency to bioaccumulate (log Kow 5) (De Wit, 2002).

Vapour pressure (Vp_s), octanol water partition coefficient (log Kow) and nomenclature of different BDE congeners is presented in Table 1.2 and their corresponding structures in Figure 1.2.

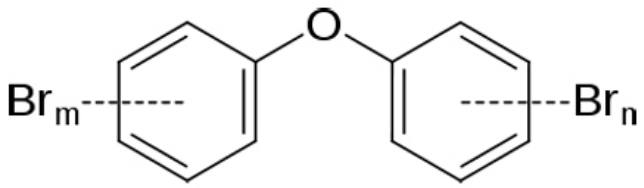


Figure 1.1: The general molecule structure of PBDEs.

Table 1.1: Homologues and number of isomers for each homologue group for PBDEs (Wei, 2012).

Number of Br atoms	**IUPAC**	**Formula**	**MW (g mol^{-1})**	**Isomers**	**Abbreviation**
1	1 - 3	$C_{12}H_9OBr$	249.1	3	mono
2	4 -15	$C_{12}H_8OBr_2$	328.0	12	di
3	16 - 39	$C_{12}H_7OBr_3$	406.9	24	tri
4	40 - 81	$C_{12}H_6OBr_4$	485.8	42	tetra
5	82 - 127	$C_{12}H_5OBr_5$	564.7	46	penta
6	128 - 169	$C_{12}H_4OBr_6$	643.6	42	hexa
7	170 - 193	$C_{12}H_3OBr_7$	722.5	24	hepta
8	194 - 205	$C_{12}H_2OBr_8$	801.4	12	octa
9	206 - 208	$C_{12}HOBr_9$	880.3	3	nona
10	209	$C_{12}OBr_{10}$	959.2	1	deca

Table 1.2: Vapour pressure (Vp_s), octanol water partition coefficient (log Kow) and nomenclature of different BDE congeners (Tittlemeier, et al., 2012).

PBDE congener	**Bromine substitution**	**Homologue series**	**Vapour pressure (Pa)**	**Log Kow**
BDE 28	2,2`,4-triBDE	triBDE	9.07×10^{-4}	5.98
BDE 47	2,2`,4,4`-tetraBDE	tetraBDE	5.52×10^{-5}	6.55
BDE 99	2,2`,4,4`,5-pentaBDE	pentaBDE	3.85×10^{-6}	7.13
BDE 100	2,2`,4,4`,6-pentaBDE	pentaBDE	5.50×10^{-6}	6.86
BDE 153	2,2`,4,4`,5,5`-heksaBDE	heksaBDE	5.80×10^{-6}	7.62
BDE 154	2,2`,4,4`,5,6`-heksaBDE	heksaBDE	2.67×10^{-7}	7.39
BDE 183	2,2`,3,4,4`,5,6`-heptaBDE	heptaBDE	1.85×10^{-8}	8.27
BDE 209	2,2`,3,3`,4,4`,5,5`,6,6`-decaBDE	decaBDE	9.28×10^{-9}	6.27

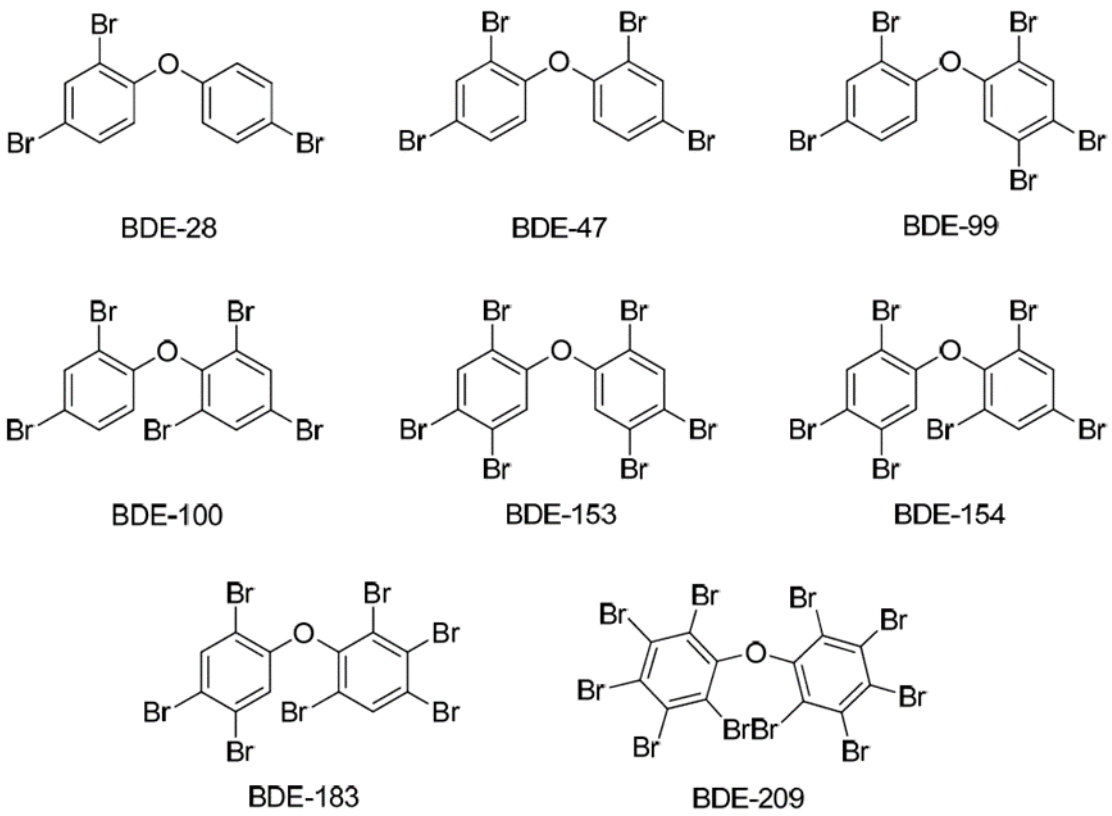


Figure 1.2: Structure of selected BDE congeners. Adopted from (EFSA, 2011).

PBDEs are applied as additives to numerous polymers, such as plastics, textiles, flexible polyurethane foams (FPUF), furniture, electrical or electronic devices and automobile parts to prevent or retard the spread of fire (de Boer, de Boer & Boon, 2000). PBDE additives interfere with the combustion process by forming hydrogen bromide gas, which, in turn, displaces the O_2 necessary to sustain the oxidation reactions. Hydrogen bromide formed then reacts with the high-energy H and OH radicals, releasing water vapor and the lower-energy Br radicals, which may remain available to repeat the cycle (Rahman, et al., 2001). Such dual processes severely retard and constrain thermal oxidation. In comparison with chlorinated flame retardants, the brominated compounds differ in their flame retardant properties. Bromine-hydrocarbon bonds are weaker than chlorine-hydrocarbon bonds, so bromine radicals are released more easily as the material is heated. These bromine radicals are also released over a narrower temperature range, so they tend to be more concentrated as the fire starts, thus, more effective at stopping the fire.

Formation of the high-energy OH and H radicals from O_2:
$H^{\cdot} + O_2 \dashrightarrow OH^{\cdot} + O^{\cdot\cdot}$
$O^{\cdot\cdot} + H_2 \dashrightarrow OH^{\cdot} + O^{\cdot\cdot}$

Release of lower energy bromine radical from BDE congener:
$R\text{-}Br \dashrightarrow R^{*} + Br^{*}$

Formation of hydrogen bromide and neutralization of energy-rich radicals:
$HBr + H^{*} \dashrightarrow H_2 + Br^{*}$
$HBr + OH^{*} \dashrightarrow H_2O + Br^{*}$

Combustion of PBDE treated materials can lead to the formation and emission of toxic polybrominated dibenzofurans (PBDFs) and polybrominated dibenzodioxins (PBDDs) as byproducts from PBDEs combustion (de Boer, de Boer & Boon, 2000; Bieniek, et al., 1989).

1.2 Production, Use and the Regulations on the Use of PBDEs

1.2.1 Production of PBDEs

Commercial production of PBDEs began in 1976 (WHO, 1994). Since then PBDEs have been marketed in three primary formulations: the PentaBDE formulation, commercially known as DE-71 and Bromkal 70-5DE, the OctaBDE formulation, DE-79 and the DecaBDE formulation, DE-83R or Saytex 102E. The formulations differ in their composition of BDE congeners. The Penta formulation is dominated (by weight) by penta congeners (50–62 %) with secondary contributions by tetra (24–38 %) and hexa congeners (4–12 %). The Octa formulation is dominated by hepta (45 %) and octa congeners (33 %), with secondary contributions by hexa (12 %) and nona (10 %) congeners. The Deca formulation is essentially composed of BDE 209 (97–99 %, with 1–3 % other, mainly nona congeners) (La Guardia, Hale & Harvey, 2006).

Worldwide, 40,000 – 67,000 metric tons of PBDE mixtures were produced annually between 1999 and 2003 (US EPA, 2010]. The Bromine Science Environmental Forum (BSEF) estimated the total market demand for the BDE commercial mixtures by region in 2001, the most recent year for which data are available (Table 1.3). This data shows the dominance of the DecaBDE formulation (DecaBDE, PentaBDE and OctaBDE accounted for 83 %, 11 % and 6 %, respectively) in the total PBDE production worldwide. In that

year, Europe accounted for about 14 %, 16 % and 2 % of the total DecaBDE, OctaBDE and PentaBDE mixture usage, respectively. The majority of the PBDEs produced globally were consumed in the Americas (Law, et al., 2006).

Table 1.3: The usage of PBDE commercial mixtures in different areas of the world in 2001 (in tonnes) (Law, et al., 2006).

Mixture	**Americas**	**Europe**	**Asia**	**Rest of the world**	**Total**
DecaBDE	24500	7600	23000	1050	56100
OctaBDE	1500	610	1500	180	3790
PentaBDE	7100	150	150	100	7500
Total	33100	8360	24650	1330	67390

1.2.2 Use of PBDEs

The main manufacturing sectors that have used PBDEs are as follows: organobromine industry, electrical and electronics industry, transport industry, construction industry, and furniture, textiles and carpet industry. Due to the phase out by the manufacturer, as well as the state-specific bans noted below, Penta and OctaBDE mixtures are no longer present in contemporary products to inhibit flammability. For the most part, DecaBDE mixture is not prohibited from production and continues to be used.

It is considered that approximately 95 % of the use of PentaBDE commercial mixture was for the treatment of flexible polyurethane foam materials (US EPA, 2010). These foams were mainly used in automotive and upholstery applications, including bedding matrices. Minor uses included textiles, printed circuit boards, insulation foam, cable sheets and conveyer belts.

The main former use of OctaBDE commercial mixture was in acrylonitrile-butadiene-styrene (ABS) polymers (plastics), accounting for about 95 %. The treated ABS was mainly used for housings/casings of electrical and electronic equipment (EEE). Other minor uses were high impact polystyrene (HIPS), polybutylene terephthalate (PBT) and polyamide polymers. OctaBDE mixture was typically added to ABS at a loading of 12−15 % weight of the final product (EU, 2003). Although the majority of these polymers were used in electronics, there was also some use in the transport sector (US EPA, 2010; Rahman, et al., 2001).

DecaBDE mixture is still used as a general-purpose additive flame retardant to a wide array of plastics having many product applications. High impact polystyrene (HIPS), polyethylene (PE), polypropylene (PP), polybutylene terephthalate (PBT) and unsaturated polyesters (UPEs) are common plastics treated with DecaBDE. HIPS-based plastics are used in the manufacture of housings and back panels to televisions, in casings of audio and video equipment, mobile phones, remote controls, PCs, and PC monitors, the PE-based plastics are used in the insulation of wire and cables of electrical equipment. The PP-based plastics are used in communication cables, capacitor films, building cables, pipes, stadium seats, lamp sockets and holders, and kitchen hoods, the PBT-based plastics are used as connectors in electrical and electronic equipment while the UPEs are used in building and construction materials as reinforced plastic panels. DecaBDE mixture is added to polymers in concentrations of 10−15 %, by weight of the final product (US EPA, 2010, Alaee, et al., 2003).

1.2.3 Restriction of production and usage of PBDEs

PBDEs were in widespread use and are now detected in all compartments of the environment. Concerns over their environmental persistence, bioaccumulation in food webs, and the occurrence in the environment have triggered regulatory and voluntary actions to reduce the use of PBDEs. Nowadays, PBDEs are considered to be an emerging class of contaminants, the usage and production of which should be minimized. Some adequate regulations in this direction have already been adopted by different World and European organizations. European Union (EU) banned the use of Penta and OctaBDE commercially available mixtures in 2004 (European Court of Justice, 2003), DecaBDE can no longer be used in electronics and electrical applications from 2008 (European Court of Justice, 2008), while, according to the EU directive, it is still allowed to use DecaBDE in plastics. In certain countries, the use of DecaBDE has been banned independently, for example in Sweden since January of 2007. Compared to EU, the United States of America (USA) and Asia are still in the phase out legislation for PBDEs, although the primary USA producer has voluntarily phased out production of the Penta and OctaBDE mixtures. Only 10 federal states of America have officially banned the use of PBDEs mixtures until 2008. However, the USA producers and the main USA importer of the DecaBDE decided to end production, import and sales of these chemical for all consumers, transportation, and military uses, by the end of 2013 (US EPA, 2010). In Canada, the manufacture of PBDEs (tetra to decaBDE congeners) is prohibited. Prohibited is also the use, sale and import of some PBDEs (tetraBDE, pentaBDE and hexaBDE congeners). Despite the bans there will be a continuous release of PBDEs from existing products for many years to come. Recently, Danon-Schaffer et al. (2013) reported that even with an immediate ban on adding all products treated with BFRs to landfill, concentrations of PBDEs would continue persisting within landfills beyond 2080, while PBDEs contained in existing landfilled waste (both e-waste and non-e-waste) would persist to nearly 2070.

The EU Water Framework Directive (WFD), which entered into force in December 2000, establishes a Community framework for water protection and management. Its main focus is the establishment of a strategy for dealing with chemical pollution of water. In 2008, WFD listed six BDE congeners (BDE 28, BDE 47, BDE 99, BDE 100, BDE 153 and BDE 154) into a group of priority hazardous substances (Directive 2008/105/EC, 2008). WFD recommends regular monitoring of the six PBDE congeners in surface waters and biota in order to prevent pollution of the aquatic environment. The current legislation specifies an environmental quality standard (EQS) for the sum concentration of the six PBDE congeners in surface water and biota (relating to fish) to equal or less than 0.5 ng L^{-1} and 8.5 ng kg^{-1} ww, respectively, (Directive 2009/90/EC, 2008). For other abiotic and biotic environments there is no such restriction yet.

The Stockholm Convention on POPs is a global treaty ratified by 176 parties (2013) to protect human health and the environment from chemicals that remain intact in the environment, animal and humans for long periods and have harmful impacts on human health and the environment. In 2009, tetra to hepta brominated BDE congeners were added to the POP list of the Stockholm Convention.

1.3 Toxicology of PBDEs

Increasing concentration levels of PBDEs in human tissues (blood, serum, breast milk) have caught worldwide health concern. There is a growing body of research showing that exposure to PBDEs can cause adverse health effects in animals, children and adults, including a disruption of endocrine functions, like thyroid hormone abnormalities, negative

impact on immune function, disruption of growth and causing neurodevelopmental delays. Exposures to PBDEs occur during vulnerable periods of human and wildlife development. From fertilization through fetal development and through nursing of young offspring, which raises particular concern. Children can be more exposed to PBDEs due to their hand-to-mouth activities and higher metabolic rate. Children and young adults are also more prone to developmental dysfunctions provoked by PBDEs exposure. Adverse neurodevelopmental effects associated with prenatal PBDE exposure can be detected in children at early ages (12–36 months) and as the children age (48 and 72 months) (Talsness, 2008; Bergman, et al., 2012).

Toxicological properties of PBDEs have been investigated mainly on laboratory experimental animals (mice, rats). Hence, the available evidence about the impact of PBDEs on human health is still very limited. A toxicological information is focused mainly on PBDEs mixtures, much less information is available on the individual congeners. There is some evidence suggesting that BDE 99 is more potent than BDE 47 and also that its toxic effects worsen and are more apparent with age (Viberg, et al., 2003). PBDEs share a structural similarity with thyroid hormones. This can help understand experimental and epidemiologic observations, which suggest that PBDEs can interfere with thyroid hormone pathways. Because thyroid hormones are critical for normal brain development, such interferences can cause the neurodevelopmental effects observed after neonatal PBDE exposure. This theory is further supported by toxicological evidence which includes potentially causal associations between neonatal exposure to BDE 47, BDE 99, or commercial BDE mixtures (DE-71 and Bromokal 70-5 DE) and reduced thyroxine (T4) concentrations in experimental murine mice (several genetic lines of mice that could help clarify the mechanisms by which chemical exposures can interfere with thyroid hormone action are now widely available) and rats models (Legler, 2008; Kuriyama, et al., 2007; Hallgren, et al., 2001; Fowles, et al., 1994).

PBDEs are endocrine disrupting chemicals (EDCs). Humans and animals are exposed to multiple EDCs (PCBs, pesticides, metals) at the same time, and there is justifiable concern that different EDCs can act together and result in an increased risk of adverse effects on human and animal health.

1.4 Occurrence of PBDEs in the Environment

PBDEs have contaminated the environment and were detected in its different compartments due to their large volume production and extensive use in the past. The first evidence of the presence of PBDEs in the environment was reported by Zweidingen in 1979 (Zweidingen, et al., 1979), who detected BDE 209 in air, followed by Anderson and Blomquist in 1981 (Andreson & Blomquist, 1981), who reported the presence of PBDEs in fish samples from Sweden.

Distribution of BDE congeners in the environment is governed by their respective physical and chemical properties. They have a high binding affinity to particles and organic matter (Fontala, et al., 2009) and have been found in soils, sediments, sewage sludge, surface waters, house dust, and in outdoor and indoor air (de Wit, 2002). For PBDEs, air and water are primary transport media, while soils and sediments are environmental sinks. In general, BDE congeners are hydrophobic, lipophilic persistent organic pollutants (POPs) that have low water solubility and low vapor pressures. PBDEs bio-accumulate into terrestrial and aquatic food webs and bio-magnify in living organisms. The body burdens of BDE congeners in a wide variety of avian species, fish, insects, and aquatic or terrestrial mammals indigenous to geographical areas ranging from the equator to the poles also

substantiate BDE's propensity for long-range transport. In mammals, PBDEs are found mainly in adipose tissue, blood serum and breast milk.

1.4.1 Sources and transport of PBDEs in the environment

Concentrations of PBDEs in air, water and soil have been found to vary across urban gradients with elevated concentrations near urban centers and decreasing concentrations moving outward from the population center. This indicates that sources of PBDEs are related to human activities. Within highly populated areas, elevated concentrations in air, water, sediments, and soil are associated closely to manufacturing, recycling, and waste disposal facilities, signifying that these may serve as point sources of PBDEs in abiotic media (Iqbal, et al., 2017; Hale, et al., 2006; Wang, et al., 2007). Additional significant source of PBDEs may be municipal sewage. In municipal wastewater treatment plants, up to 90 % of PBDEs from wastewater end up in sewage sludge. The majority of sewage sludge in Europe is disposed to landfills or applied to land. When sewage sludge is used as a fertilizer or regenerator for soil, such application can contribute to the release of PBDEs to the environment. Current major sources of PBDEs are waste processing and separation, and sewage sludge and landfill leachate releases. PBDEs that can be released into the atmosphere during trash burning, a common practice in the developing world and in rural areas of developed countries, could also contribute significantly to environmental PBDEs burdens (de Boer, et al., 2003; North, 2003).

As the distance from population centers increases, long-range environmental movements of PBDEs become more important (de Wit, Alaee & Muir, 2006). There is also evidence of various biota transporting contaminants from one place, which is known as "bio transport". Many studies have proved that salmon is involved in bio transport of POPs including PBDEs, and have contaminated lake food webs (O'Toole, et al., 2006; Morrisey, et al., 2011).

Figure 1.3 shows how PBDEs are transported in the freshwater environment. It demonstrates many mechanisms responsible for the transport and fate of PBDEs. Among them, the major processes involved in fresh water ecosystem are as follows: dry/wet deposition on fresh waters, air-water exchange, water-sediment exchange and sediment-biota or water-biota exchange.

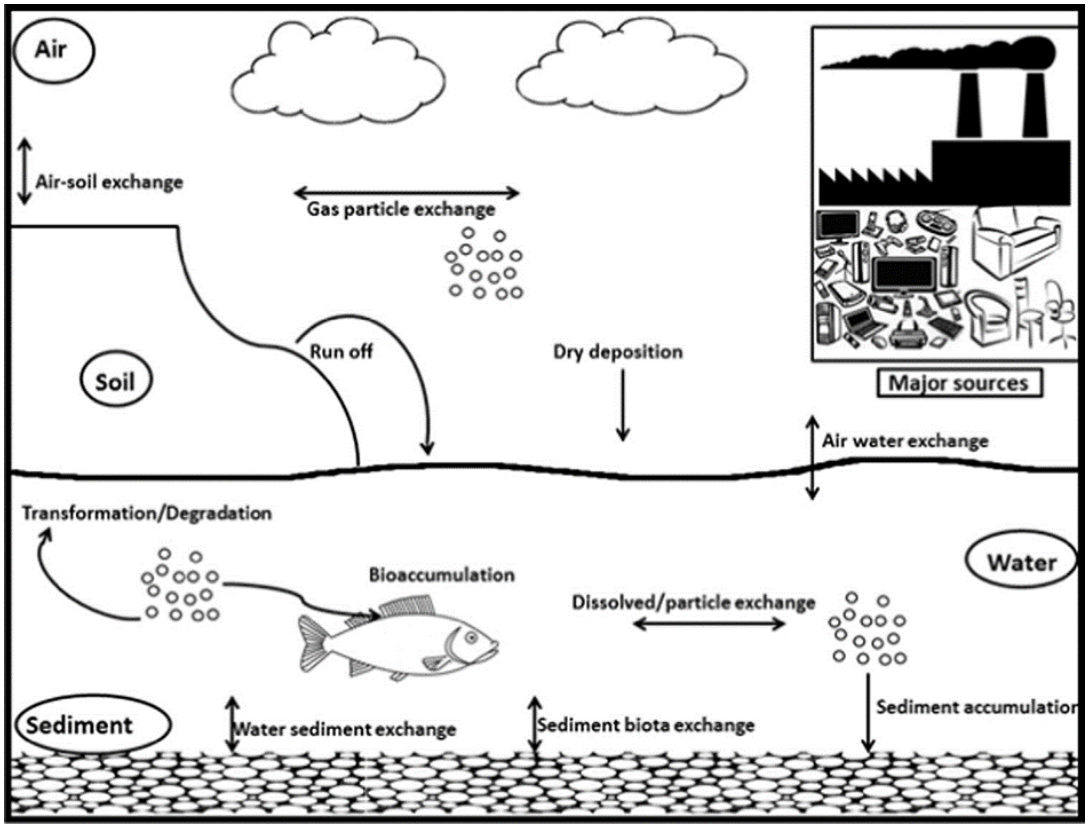


Figure 1.3: Mechanisms involved in transport and fate of PBDEs in the freshwater ecosystem. Adopted from (Iqual, et al., 2017).

1.4.2 Abiotic environment

PBDEs were found in various abiotic environmental compartments, including surface waters, soils, sediments, sewage sludge, dust, outdoor and indoor air. Their presence in different compartments has not been equally investigated. While many authors have reported on the presence of PBDEs in air and sediments, comparatively little is known about PBDEs in surface waters, sewage sludge and soils, despite the fact that sewage sludge and soils are predicted to be the dominant environmental sinks for PBDEs (Law, et al., 2006). Most reports on PBDEs concentrations in abiotic media are for locations in North America, Europe and in recent years also for Asia. Little information exists for parts of the Arctic, Australia, South America and Africa. Average concentrations of PBDEs in surface waters (ng L^{-1}), sediments (ng g^{-1}) and sewage sludge (ng g^{-1} dw) from locations found in the cited literature are presented in Table 1.4. In sediments and sewage sludge samples, PBDEs are present in concentrations that are much higher than those commonly found in natural waters. For that reason, only a few reports are found in the literature on the determination of trace concentrations of PBDEs in environmental water samples.

Table 1.4: Average concentrations of PBDEs in surface waters (ng L^{-1}), sediments (ng g^{-1} dw) and sewage sludge (ng g^{-1} dw) from different locations.

Location	**Sampling year**	**Sample type**	**PBDEs**	**References**
River Aire (UK)	2012	River water	295	(Christale, et al.,2013)
River Siene (France)	/	River water	2.1-2.5	(Labadie, et al., 2010
Lake Ontario (North America)	2011	Lake water	0.227	(Venier, et al., 2014)
Lake Michigan (North America)	2012	Lake water	0.038	(Venier, et al., 2014)
River Po (Italy)	2010	Sediment	3132	(Luigi, et al., 2015)
Ebro River (Spain)	2010	Sediment	186	(Barón, et al. 2014)
Llobregat River (Spain)	2010	Sediment	164	(Barón, et al. 2014)
Lake Maggiore (Italy)	2005	Sediment	27	(Mariani, et al., 2008)
Dongjiang River (South China)	2009	Sediment	186	(He, et al., 2012)
Lake Michigan (North America)	2004	Sediment	320	(Zhu & Hites, 2005)
Sweden	2000	Sewage sludge	130	(Law, et al., 2006)
Germany	2002-2003	Sewage sludge	105	(Knoth, et al., 2008)
Switzerland	2003	Sewage sludge	83	(Kupper, et al, 2008)
Spain	2005	Sewage sludge	74	(Eljarrat, et al., 2008)
Sweden	2010	Sewage sludge	43	(Olofsson, et al., 2012)
Italy	2009-2010	Sewage sludge	35	(Cincinelli, et al., 2012)
California (USA)	2003	Sewage sludge	2035	(North, 2004)
North Carolina (USA)	2010	Sewage sludge	1553	(Davis, et al., 2012)

1.4.3 Biotic environment

BDE congeners, which possess hydrophobic and lipophilic properties, can bio-accumulate in aquatic organisms through the exposure within their food web. Benthic invertebrates and mollusks living and feeding directly on contaminated sediments acquire a body burden of PBDEs that is then passed up the food chain from bottom-feeding fish to top piscivorous fish, raptors, and mammals. Mollusks internally absorb PBDEs from the water column by filtering the water. Fish acquire PBDEs through the dietary pathway and also from the water passing through their gills (Bragigand, et al., 2006). Factors like accumulation with time (the age of the fish), or trophic position in the food web as well as seasonal variations are likely to contribute to the PBDEs concentration in fish. PBDEs were detected in deep-water marine fish and mammals and in marine ecosystems ranging from the equator to the poles. This provides additional evidence for the long-range transport of PBDEs (EPA, 2010).

Field studies of the bioaccumulation of PBDEs in terrestrial environment have included fish-eating mammals, birds of prey and carnivorous mammals in the wild (Gavningen Sørmo, et al., 2006; Wolkers, et al., 2004). These animals are top predators in the food web and acquire a body burden of PBDEs from trophic level exposures in a similar manner as aquatic organisms. Birds of prey are ideal candidates to study the bioaccumulation of PBDEs because they are carnivores, typically secondary and tertiary consumers in terrestrial and aquatic food chains. Only a very limited number of terrestrial mammals have been studied for their body burden of PBDEs. Many mammals are top predators and, therefore, bioaccumulate and biomagnify PBDEs from the food web. Average concentrations of PBDEs in biota samples from the different locations are presented in Table 1.5.

Table 1.5: Average concentrations of PBDEs in various biota samples from different locations.

Location	**Sampling year**	**Sample type**	**PBDEs**	**References**
		Fish	**ng g^{-1} ww**	
Finland Sea	2007-2008	farmed fish	0.9 - 1.7	(Souminen, et al., 2011)
Baltic Sea	2004-2006	herring, salmon	1.2 - 2.5	(Szlinder-Richert et al. 2010)
Belgium Sea	2003	plaice, whiting	0.06 - 6.1	(Voorspoels, et al., 2003)
German rivers	2013	bream	0.14 – 18	(Lohmann, et al., 2015)
England rivers	2007-2011	roach	2 – 44	(Jürgens, et al., 2013)
Czech Rep. Elbe river	2002-2003	chub, bream, trout	2 – 18	(Pulkrabova, et al., 2007)
Norway lakes	1998-2004	brown trout	0.3 – 23	(Mariussen, et al., 2008)
Italy	2011-2012	halibut, blue fish, trout	0.03 - 0.5	(Martellini, et al., 2016)

		Mussels	**ng g^{-1} ww**	
Greece	2005-2007	mussel	0.07-1.51	(Dosis, et al., 2016)
Portugal	2002-2004	mussel	0.18-0.86	(Gama, et al., 2016)
Italy	2008	mussel	0.17-1.04	(Giandomenico, et al., 2013)
Belgium	2002	mussel	0.15-1.8	(Covaci, et al., 2005)
Norway	2002	mussel	0.2	(Knutsen, et al., 2008)
U.K.	2006	mussel	0.2-3	(Fernandes, et al., 2008)
		Birds	**ng g^{-1} lipid**	
Island	2002-2004	eider	44	(Jörundsdóttir, et al., 2013)
Island	2002-2004	great skua	2400	(Jörundsdóttir, et al., 2013)
Belgium	2001-2003	buzzard	17-4400	(Voorspoels, et al., 2005)
Belgium	2001-2003	sparrow hawk	79-18000	(Voorspoels, et al., 2005)
		Mammals	**ng g^{-1} lipid**	
Belgium	2004	red fox	1-44	(Voorspoels ,et al., 2006)
Norway	2001	polar bear	13-70	(Wolkers, et al., 2004)
Norway	1999	ringed seal	14.1-23.7	(Wolkers, et al., 2004)
Greenland	2006	dolphin	249-308	(Rotander, et al., 2012)
Island	2003-2006	Minke whale	64-111	(Rotander, et al., 2012)
Norway	2006	ringed seal	21-33	(Rotander, et al., 2012)

1.4.4 Human exposure to PBDEs

PBDEs are chemical additives that do not form a chemical bond with polymer resins and can be easily released into the environment by leaching and volatilization. When PBDEs enter different environmental compartments, they are persistent, with degradation half-lives in the order of years (Park, et al., 2014; de Wit, 2002). Being highly lipophilic and stable in the environment, with the potential for bioaccumulation in organisms and biomagnification through food chains, they can enter human bodies by various routes. The main routes by which humans are exposed to PBDEs are ingestion through diet or dust and inhalation of indoor and outdoor air (Domingo, et al. 2012; Linares, Bellés & Domingo, 2015).

1.4.4.1 Dietary intake

Today, it is hypothesized that the predominant route of human exposure to PBDEs could be a dietary intake. It is estimated that, except for occupationally exposed individuals, more than 90 % of total human exposure to organic pollutants such as PBDEs comes from food (Bocio, et al., 2007; Linares, Bellés & Domingo, 2015). The human body absorbs pollutants from food and accumulates them in the tissues. There is a lot of scientific studies confirming the presence of PBDEs in foodstuffs and the human dietary exposure to PBDEs in a number of European countries, USA, China, and Japan (Domingo, et al. 2012; Linares, Bellés & Domingo, 2015).

Differences in cultural, consumer product and life-style habits, as well as in policy regulations on the industrial production and the use of PBDEs across the Atlantic, are expected to influence the measured PBDEs exposure levels. Due to its bioaccumulative potential, fish is believed to be the main contributor of PBDEs in the diet (Bragigand, et al., 2006; Darnerud, et al., 2006) and thus it has been analyzed to a greater extent than other food groups. In other food products, PBDEs are most predominant in fatty products like butter, fats and oils. Solid milk products like cheese also contain relatively high amounts of PBDEs, while liquid milk products only contain very low concentrations of PBDEs. Their concentration levels in meat are usually significantly lower than in fish. In general, the difference is approximately a factor of ten although large variations are observed within the groups. The level of PBDEs in vegetables is expected to be considerably lower than in the other food categories due to the larger water and lower lipid content and their primary position in the ecosystem. The acceptable concentration levels of PBDEs in different foods are not yet set by the legislations. The occurrence values of the PBDEs that are used for the human exposure assessment are summarized in Table 1.6. These values are adapted from The Scientific Opinion on Polybrominated Diphenyl Ethers (PBDEs) in Food by European Food Safety Authority (EFSA) (EFSA, 2011) and were calculated on the basis of analyses of 3933 samples across seven broad food categories of the FoodEx food classification system. All the PBDEs levels (mean concentrations) reported are calculated on wet weight basic (ng g^{-1} ww).

Depending on the intake rates of these food categories, their relative importance varies. Dietary habits, including fish consumption, differ with the culture, thus there are great differences in the main food items that contribute to the dietary exposure between countries. In Scandinavia, fish is the main contributor to the dietary exposure to PBDEs. For example, in Sweden, fish is estimated to make up 47 % (Darnerud, et al., 2006) and in Finland 55 % of the total dietary PBDE-exposure (Kiviranta, et al., 2004), respectively. In North America, there is a tendency to eat less fish, which results in meat and fats being the two main contributing groups of food to the total dietary PBDE-exposure (Schecter, et al., 2006). Meat products, particularly poultry and processed meat, were found to

contribute about 60–70 % to the dietary PBDE-exposure for Americans with only 10–20 % arising from fish.

Table 1.6: Occurence values of PBDEs in different food categories reported by EFSA (EFSA, 2011).

Food categories	**Mean values of PBDEs (ng g^{-1} ww)**
Eggs and egg products	0.858 – 0.999
Milk and dairy products	0.042 – 0.276
Meat and meat products	0.060 – 0.254
Animal and vegetable fats and oils	0.357 – 2.288
Fish and other sea food	1.969 – 2.618
Food for infants and small children	0.429 – 0.456
Vegetables and vegetable products	0.127 – 0.173

1.4.4.2 Exposure through dust ingestion and inhalation

PBDEs are compounds with low vapor pressures, which means that, in indoor environments, they are preferentially found in dust. Hence, most studies of indoor contamination with PBDEs are focused on human exposure via ingestion of settled dust rather than via inhalation of indoor air. Inhalation exposure occurs as a result of release of PBDEs from household products into the air. Although dietary exposure is an important source of human exposure to PBDEs, some studies have indicated that ingestion of house dust can also be significant (Stapleton, et al., 2005; Ni, et al., 2013). This exposure route is particularly important for young children because of their tendency to play on the floors and frequent hand-to-mouth contact. Ingestion of contaminated dust can result in a 100 times greater exposure than average for a toddler living in a home where PBDEs are present (Jones-Otazo, et al., 2005). The PBDEs concentrations in outdoor air are between one and two orders of magnitude lower than in indoor air. This confirms that indoor air is not polluted from the outside but the sources are present inside the houses. Electrical appliances, especially computers and televisions, are the major PBDE sources in indoor environments. Other factors such as ventilation rate, proximity of the sampler to potential sources, and age of those sources can affect the levels of PBDEs in indoor air, although relatively few studies have focused on indoor air (Frederiksen, et al., 2009; Ni, et al., 2013).

1.4.4.3 Body burden

Unlike adults, infants are additionally exposed to PBDEs via placental transfer and by breast-feeding. Breast milk is an easy non-invasive medium for biomonitoring because any contamination of this medium is of great concern as the contamination is directly passed on to the next generation. Various studies have reported that lactation is a significant PBDEs exposure pathway to breast-fed infants (Darnerud, et al., 2011; Thomsen, et al., 2010; Frederiksen, et al., 2009). Assessment of the exposure to PBDEs for infants through breast-feeding has been performed in a number of countries. From them, it was indicated that PBDE concentrations in breast milk in North America are at least 10 times, can be 100 times, higher than those in Europe and Asia, most probably because of the much larger scale in production and wider usage of PBDEs in North America (Fängström, et al., 2008; Zhang, et al., 2011). Time-trend studies indicated that the PBDE levels in breast milk

increased rapidly from 1970s, but appeared to have stabilized or decreased since the late 1990s or early 2000s (Frederiksen, et al., 2009). This may be attributed to the restriction and ban on the production and usage of low brominated BDEs (technical Penta- and Octa-BDE products) in Europe and some countries in USA and Japan. There is limited information on PBDE levels in other human tissues such as adipose tissue, placenta, liver and hair. Due to its easiness of collection, transport, storage, and also the very low cost of acquisition, hair has been used widely for human biomonitoring of certain toxicants. PBDE levels have also been measured in human hair, suggesting that it can be used as a biomarker of PBDEs exposure in infants, children, and adults (Kucharska, et al., 2015; Tadeo, Sanchez-Brunete & Miguel, 2009; Ma, et al., 2011).

1.5 Degradation of PBDEs

The greatest source of uncertainty regarding the transport and fate of PBDEs in the environment is the extent of their degradation in abiotic media. Among PBDEs, the degradation of BDE 209 is of particular interest as this congener is still in use and its degradation may contribute to the environmental pools of lesser brominated congeners. Certain biotic and abiotic processes can transform PBDEs in the environment. The processes most important for the breakdown of PBDEs include biodegradation, biotransformation, and photolysis. Although it is suggested that in the environment, photochemical degradation and biodegradation of PBDEs are slow or negligible, it was demonstrated for both of them to take place in water, soil, and sediment under laboratory conditions (Vonderheide, et al., 2006; Stapleton & Dodder, 2008). BDE 209 has also been shown to be susceptible to abiotic degradation by metal oxides that occur naturally in soils and sediments. There is only limited scientific information supporting the action of these pathways in degrading PBDEs (Kim, et al., 2014). On the other hand, there is a growing body of scientific evidence to suggest that certain marine fish species and marine mammals are capable of metabolically debrominating PBDE congeners in vivo (biotransformation) (Stapleton, et al., 2006; Roberts, et al., 2011). The higher brominated BDE 209 and the DecaBDE commercial formulation have been successfully debrominated to lower brominated congeners through a metabolic pathway in fish. The exact mechanism involved in metabolic debromination remains unknown. It is also not clear whether all fish species, both marine and freshwater, have the innate propensity for biotransforming PBDEs. Debromination of PBDEs has been so far observed in juvenile rainbow trout, juvenile lake trout, and common carp (all of which are freshwater species).

1.6 Methods for the Determination of PBDEs in Environmental Samples

PBDEs have been found in terrestrial, aqueous, and atmospheric environment. This results in a significant increase of attention that is now paid to their analysis. In comparison to other persistent organic pollutants (POPs), PBDEs are not so well examined. It is, therefore, necessary to develop analytical methods that will enable the assessment of the degree of pollution of different environmental compartments with PBDEs. Analysis of PBDEs in environmental samples requires the specific analytical methods in which the main criteria are low detection limits and high selectivity. Analytical methods developed for the determination of PBDEs in environmental samples have to solve the problems, which arise from complex composition of sample matrices, that in practice means co-

extraction of interfering compounds and the need for their removal. Analysis of highly brominated PBDE compounds usually requires a different approach to be adopted in order to eliminate the risk of loss of analytes (e.g. degradation due to the high temperature) (Król, et al., 2012). Common to all the analytical procedures used for the determination of PBDEs in environmental and biological samples is that they comprised of sample collection, sample preparation (extraction, clean-up and pre-concentration) and final instrumental analysis by applying different coupled techniques.

1.6.1 Extraction methods

Extraction procedure applied in PBDEs analysis requires some additional attention because the wide range in molecular weights of the BDE congeners (between 250 and 960 g mol^{-1}) yields in their different physical behaviors, indicating that a given extraction method may not work equally well for all PBDE congeners alike (Vonderheide, 2009) and also because of the increasing environmental awareness and consequently the need for lower detection limits, which in practice usually means new adaptations of the existing extraction and clean-up procedures. PBDEs can be successfully extracted by Soxhlet or liquid–liquid extraction (LLE), but these modes of extraction are characterized by lengthy extraction times and high solvent consumption. More advanced extraction techniques, such as accelerated solvent extraction (ASE), pressurized liquid extraction (PLE), ultrasonic-assisted extraction (UAE), microwave-assisted extraction (MAE), solid phase extraction (SPE) and supercritical-fluid extraction (SFE), have recently been introduced to reduce the extraction time and solvent consumption, as well as to improve extraction efficiency. The typical extraction solvents used are toluene, dichloromethane, hexane, iso-octane, acetone and their combinations (Król, et al., 2012; Covaci, et al., 2011).
Advantages and disadvantages of the extraction techniques, which are commonly applied in PBDEs analysis of various environmental and biological samples, are compared in Table 1.7.

Table 1.7: Comparison of different extraction techniques commonly applied in PBDEs analysis of various environmental and biological samples (Król, et al., 2012, Wang, 2008).

Extraction technique	**Solvent consumption**	**Extraction temperature**	**Application**	**Advantages/Disadvantages**
Soxlet	50 – 300 mL	Boiling point temperature of solvent	Sewage sludge Fish, mussel Soil Dust Electronic equipment	+ Allows complicated matrices, high efficiency - Time and solvent consuming
ASE / PLE	15 – 75 mL	Up to 150 °C	Sediments Soil Fish Milk	+ Fully automated, reduces time and solvent consumption - Extensive clean-up of extracts is needed - Expensive instrumentation
UAE	50 – 150 mL	Up to 80 °C	Fish, mussel Soil Bird eggs	+ Short extraction time
MAE	Up to 30 mL	Up to 150 °C	Sewage sludge Fish, mussel Soil Electronic equipment	+ Short extraction time, small solvent consumption - Not for all extraction solvents, clean-up step must be done - Expensive instrumentation
SPE	Up to 100 mL	/	Water Human serum	+ Small solvent consumption, very efficient - Conditioning of sorbent bed must be done
SFE	10 – 50 mL	Up to 150 °C	Sediment Dust Fish	+ Small solvent consumption, environmentally friendly

1.6.1.1 Surface waters

The EU WFD requires the extraction of PBDEs from whole water, which contains also solid phase particulate matter (SPM) without prior filtration. Thus, the extraction method used should be able to extract PBDEs bound to humic acid as well as to other types of particles, which can be contained in surface waters. Prior to analysis, the SPM phase is often separated from the water with water filtration and analytes are then extracted separately (Fontana, et al., 2009; Labadie, et al., 2010). The most frequently used extraction procedures of PBDEs from whole water were LLE (Gonzáles-Gago, 2011) and SPE using SPE cartridges (Labadie, et al., 2010) or SPE discs (Erger, et al., 2012; Thurman & Snavely, 2000). In recent years, dispersive liquid-liquid microextractions (DLLME) have been investigated as promising extraction techniques for PBDEs, mainly from aqueous samples (Król, et al., 2012). It was concluded from experimental results that DLLME is

not acceptable for the extraction of PBDEs due to its poor selectivity and sensitivity and that, before usage, it needs to be further improved. Only a few reports are found in the literature about PBDEs determination in environmental water samples. Mainly because of the content of PBDEs, which is in these samples mostly at ultra-trace levels, requiring very sensitive detector systems for their determination. Analytical procedures found in the cited literature are sensitive, but still do not achieve the limit of quantification (LOQ) levels required by the WFD.

1.6.1.2 Sewage sludge

Samples with solid matrices, such as soils, sediments and sewage sludge are one of the most complicated among environmental samples. Several studies have been devoted to the determination of PBDE levels in such samples. From an analytical viewpoint, sewage sludge is a challenging matrix because it is not uniform in its composition. The concentrations of pollutants present in sewage sludge samples vary on the nature of the inputs to the wastewater treatment plant (WWTP). In addition to the analytes of interest (PBDEs), sewage sludge contains a number of other components that can potentially cause interferences during analysis. Some of the co-extracted interfering compounds are lipids and other naturally-occurring materials, as well as materials that may be added to the sewage during its processing (e.g. surfactants, ferric chloride, polymeric colloids). These components can manifest themselves as interferences at all stages of the analytical procedure, starting from sample preparation (Díaz-Cruz, et al., 2009). Most of the analytical methods developed for the determination of PBDEs in sewage sludge employ the time and solvent consuming Soxlet extraction method (Cincinelli, et al., 2012; Zennegg, et al., 2013; Ran, et al., 2013; Venkatesan & Halden, 2014). Recently, new extraction techniques based on ASE (Davis, et al., 2012; Gorga, et al., 2013), MAE (Shin, 2007) and UAE (Swarthout & Kucklich, 2008) have also been evaluated. Several advantages of these techniques have been demonstrated, including lower solvent consumption and reduced extraction times. However, all the developed analytical methods require an extensive clean-up step in the analytical procedure to remove the co-extracting components before the final instrumental determination of PBDEs.

1.6.1.3 Fish and mussel

Similar to the extraction of PBDEs from the sample matrix of various environmental samples, most analytical procedures for the determination of PBDEs in fish and mussel tissues developed in recent years apply the Soxhlet extraction method (Wolschke, et al., 2015; Jürgens, et al., 2013; Poma, et al., 2014). When used for this purpose, Soxhlet extraction was found to be a very robust and low-cost method that requires simple sample preparation, but, on the down side, large quantities or organic solvent and high extraction times (24 – 48h). There are published data on the use of ASE (Tapie, et al., 2008; Johansson, et al., 2006; Martellini et al., 2016), UAE (Pozo, et al., 2015) or MAE (Tapie, et al., 2008; Wang, et al., 2010) for the extraction of PBDE from fish and mussel samples. In these reports analytical methods were applied that possess relatively high limits of detection (LODs) and/or poor recoveries. Determination of the lipophilic contaminants in biological samples is directly related to the extraction of the fat content. Due to that fact, the elimination of lipids in the clean-up procedure should be carried out prior to the instrumental analysis of the PBDEs. This should be taken into account also for PBDEs analysis in mussels that in average contain 80 %, 15 %, 2 % and 1 % of water, proteins, fat and ash, respectively, and fish samples that are usually comprised of 66-81 %, 16-21 %,

0.2-25 % and 1.2-1.5 % of waterproteins, lipids and ash, respectively (Van Leeuwen & de Boer, 2008; Huss, 1995).

1.6.2 Clean-up procedures

Extraction techniques used for the extraction of PBDEs from different environmental and biological samples share their common disadvantage of poor selectivity. Since physicochemical properties of PBDEs are similar to those of some other organic compounds, e.g. polybrominated biphenyl (PBBs), polybrominated dibenzo-p-dioxins (PBDDs) and polybrominated dibenzofurans (PBDFs), they can all be extracted by similar or even identical extraction methods. The lack of appropriate purification of extracts may result in numerous interferences and analytical mistakes. For that reason, the clean-up step, which is very often described as a multistage procedure, is necessary in order to avoid co-extraction of pollutants, like those with similar physicochemical properties, polychlorinated biphenyls (PCBs), humic acids or lipids. As extracts from sediments, sewage sludge or soil samples often contain sulfur or water, first step of their purification usually involves treatment with copper powder (sulfur removal) and sodium sulphate (acts like a desiccant). In the case of biota extracts, they usually contain high concentrations of lipids, which have to be removed prior to chromatographic separation. The removal of lipids can be achieved by destructive or nondestructive methods. The addition of sulfuric acid or silica gel impregnated with potassium hydroxide into extracts are among those often-applied destructive methods that both may lead to the destruction of analyzed compounds. Alumina has been reported to offer less harsh lipid removal than sulfuric acid or potassium hydroxide. It is very often used for further clean-up of extracts before the instrumental analysis (Król, et al., 2012; Fulara & Czapliczka, 2012). Another common approach, which allows selective removal of lipids from biological extracts, is gel permeation chromatography (GPC). The separation of interferences is usually done by using a polystyrene–divinylbenzene column or by the combination of silica gel and Florisil (Covaci, et al., 2011). The clean-up procedures listed usually require large amounts of organic solvents, the retention capacity of the columns used is limited and they are prone to loss of analytes (Shin, et al., 2012). When GC-ICP-MS is used for the determination of PBDEs in organic extracts, there is no need to completely eliminate the co-extracted interferences, except lipids.

1.6.3 Instrumental determination of PBDEs

1.6.3.1 Separation of PBDEs

Instrumental analysis of PBDE starts with their separation. Principal separation technique employed for the analysis of PBDEs is gas chromatography (GC). During GC analysis special attention has to be paid towards a specific BDE congeners due to their physico-chemical properties (e.g. vapour pressure or polarity) and thermal instability. It was already demonstrated that thermal degradation of higher brominated BDE congeners may occur during GC analysis (especially BDE 209) (Dirtu, et al., 2008; Roosens, et al., 2008). Therefore, before the determination of PBDEs, the characteristics of the GC system used should carefully be optimized according to the properties of the analytes. These include the selection of the column brand, column stationary phase, length and internal diameter as well as the injection technique, all of them having a very strong influence on the accuracy of PBDEs analysis (Covaci, et al., 2011; Björklund, 2004). Additionally, due to the thermally labile nature of PBDEs the key issue in their separation by GC is to apply the

appropriate column temperature program, which needs to be kept below 300 °C. Adequate resolution to determine individual BDE congeners is offered by capillary GC columns. To achieve adequate separation between all the BDE congeners present in the sample and separate them from possible interferences, the capillary column has to be sufficiently long (30–50 m) with small internal diameters (< 0.25 mm). In the cases when extracts are clean, the use of short narrow-bore capillary columns (internal diameter < 0.1 mm) may also provide satisfactory resolution (Covaci, et al., 2002; Vonderheide, 2009).

The most commonly used injectors for the analysis of PBDEs are splitless, on-column and programmable temperature vaporisation (PTV) injection systems. Their advantages and disadvantages derive primarily from their availability, price, detection limits, extract constitution and discrimination of BDE congeners on the basis of molecular weight. Due to the relatively low levels of PBDEs in environmental samples, splitless injection is the preferred technique, but it is necessary to accurately determine the temperature of injection, which will not discriminate lower brominated BDEs and, at the same time, affect the degradation of higher brominated PBDEs. Thermally more labile PBDEs can breakdown if the temperature of injection is too high and will not be sufficiently vaporized for efficient transfer to the GC column if it is too low. (Björklund, 2004). An attractive way to reduce thermal degradation of PBDEs in injection system is to apply on-column injection. Its main advantage is that it shortcuts the injector interface between the extract and the column by delivering the extract directly to the capillary column. As a result, a higher precision for the analysis of BDE 209 was observed. One of the main disadvantages of the on-column injection is the need for very clean sample extracts so that potential matrix residues do not reach the capillary column. Sample matrix residues can increase signal noise and cause peak tailing, retention time shifts, more frequent column trimming and reduce column lifetime. Both, splitless and on-column injection methods are limited to sample volumes from 1 to 3 µL (Covaci, et al., 2011). In comparison, PTV injection may accommodate up to 125 µL of sample volume after its careful optimization. Injection in a properly used PTV system may have some advantages, such as minimal degradation of labile PBDEs and, due to higher injection volumes, lower detection limits (Stapleton, 2006; Toolback, 2003).

The risk of thermal degradation of less stable, highly brominated congeners, while operating GC, can be avoided also by applying liquid chromatography (LC) (Król, 2012; Ma, et al., 2011; Bacaloni, et al., 2009). Among LC columns that were employed for the separation of PBDEs, those with biphenyl and Ultra Aqueous C18 stationary phases have been reported to give adequate chromatographic separation and have been successfully used for chromatographic separation of PBDEs in fish samples (Zhou, et al., 2010). Despite this application, there is still a lack of information on the use of LC in the analysis of PBDEs.

1.6.3.2 Detection of separated PBDEs

Instrumental detection technique completes the steps in the analytical method for the determination of PBDEs in different samples. In this final step, the last set of parameters have to be optimized in order to assure the accuracy of measurements.

Due to the usually very low concentrations of PBDEs in the environment, their analysis in environmental samples requires specific analytical instrumental techniques in which the main criteria are low detection limits and high selectivity. For PBDEs determination, the most frequently applied and suitable detector systems, coupled to GC or LC, are mass spectrometry (MS) and electron capture (ECD) detectors. The MS instruments can operate either in electron impact (EI) or in electron capture negative ionization (ECNI) mode. Using EI-MS for PBDE analysis, the major ions formed are $[M]^+$ and $[M\text{-}2Br]^+$, which can

be used for their identification and quantification (Stapleton, 2006). The main potential interferences that may influence the accuracy of measurements originate from chlorinated compounds, such as PCBs (the nominal masses corresponding to ions monitored for di-BDEs (m/z = 326) or tetra-BDEs (m/z = 396) are the same as those of penta-chlorobiphenyls (penta-CBs) or hepta-CBs, respectively (Korytár, et al., 2005). EI-MS provides a higher selectivity in comparison to that of ECNI-MS. In the latter only $[Br]^-$ ions are monitored and measurement interferences in the PBDEs determination may additionally come from other bromoorganic contaminants such as methoxylated polybrominated diphenyl ethers (MeO-PBDEs) and PBBs (Eljarrat, et al., 2003; Hites, et al., 2008). When EI ionization is applied an increase in sensitivity can be achieved by using high resolution MS instruments, while for the ENCI ionization, the use of HRMS instead of low resolution MS showed no improvement in sensitivity.

ECD produces peaks due to compounds containing electronegative elements, but no distinction can be made between how many or what type of elements the compound contains. Use of such detection is advantageous because it is rather inexpensive and quite sensitive. Major disadvantage of ECD is its poor selectivity when real samples with complicated matrices are measured. Since identification is based solely on retention time, interferences can lead to erroneous results. Co-elutions of various PCBs and other classical organochlorine analytes with PBDEs are well-documented (Alaee, et al., 2001). Selectivity and sensitivity obtained while working with ECD cannot match those obtained by any of MS techniques, but it can be applied to the analysis of samples where PBDEs are present at high concentration levels (ng g^{-1}) (Król, 2012, et al.; Wang, 2008).

Besides MS detector operated in either EI or ECNI modes, other MS type detectors were used to analyse PBDEs, including the time of flight analyzer (TOF-MS) (Wang, 2008; Čajka, et al., 2005) and quadrupole ion storage MS (QISTMS) (Shangmuganathan, et al., 2011). In recent years, the gas chromatography coupled to inductively coupled plasma mass spectrometry (GC-ICP-MS) was also applied for this purpose (Vonderheide, 2002; Xiao, et al., 2007). Since only the bromide ions signal is measured by ICP-MS, interferences from compounds containing chlorine and sulphur that interfere in GC-MS and GC-ECD are eliminated. Beside effective removal of some common measurement interferences, this method is characterized also by excellent sensitivity, high selectivity and the possibility for precise isotope ratio measurements and with this isotope dilution (ID) quantification (Popp, et al., 2011). Despite these facts, the ICP-MS is not commonly used for the detection of separated PBDEs extracted from environmental, food or human samples.

1.6.3.3 GC - ICP - MS

1.6.3.3.1 ICP - MS basics

ICP-MS is a highly sensitive instrumental technique that enables simultaneous determination of over 90 % of the elements in the periodic table at concentrations below 0.01 µg L^{-1}. It is based on coupling together inductively coupled plasma with a mass spectrometer that detects ions as mass/charge (m/z) particles. Furthermore, it has the possibility to detect different isotopes of elements and can be used for isotope ratio measurements and isotope dilution quantifications (Nelms, 2005; Thomas, 2004).

The sample is typically introduced into the ICP as an aerosol, produced by passing the liquid sample through a simple pneumatic nebulizer. Larger aerosol droplets are removed from the gas stream by a spray chamber, and the remaining smaller droplets are swept into the central channel of the argon plasma. Because the plasma has a very high temperature, the droplets are rapidly dried, decomposed, vaporized, atomized and then

ionized, mostly as M^+ ions. The plasma is formed by the interaction of an intense magnetic field (produced by the radiofrequency time-varying electric current that is passed through the copper coil) on a rarefied gas (usually Ar) flowing tangentially through a concentrical quartz tube called torch. This has the effect of ionizing the gas and, when seeded with a source of electrons from a high voltage spark, forms a very high temperature plasma discharge (10,000 K maximum) at the open end of the tube. To ensure good repeatability of measurements the plasma needs to be very stable. This is done with power stability of the generator and its flexibility to cope with different plasma loadings. The torch, which is made of three concentric tubes, is used to generate positively charged ions. Plasma gas (Ar) is delivered through the middle tube, the inner tube is for sample aerosol and the outer tube is for cooling gas (also Ar). Once the ions are produced in the plasma, they are directed into the mass spectrometer via the interface region, which is maintained at a vacuum with a mechanical roughing pump. This interface region consists of two metallic cones, which allow the ions to pass through the ion optics, where they are guided into the mass separation device. The ion optics consists of a series of electrostatic lenses, which electrostatically focus the ion beam toward the mass separation device, while stopping photons, particulates and neutral species from reaching the detector. There are many different mass separation devices, including quadrupole, magnetic sector, time of flight and collision/reaction cell technology. They allow the analyte ions of a particulate mass-to-charge ratio through to the detector and filter out all the non-analyte, interfering and matrix ions. The final process is to convert the ions into an electrical signal with an ion detector. It contains a series of metal dynodes along the length of the detector. When the ions emerge from the mass filter, they impinge on the first dynode and are converted into electrons. As the electrons are attracted to the next dynode, electron multiplication takes place, which results in a very high stream of electrons emerging from the final dynode. The ICP-MS is very sensitive mainly because of the electron multiplier, which can transform a single ion into a measurable signal pulse. The detector has high sensitivity, a wide linear dynamic (up to 10^9) and low background noise. The background is affected by the plasma generator, lens configuration, analyzer vacuum and the quality of the detector electronics (Nelms, 2005; Thomas, 2004).

ICP-MS offers several important advantages, e.g. simultaneous multielement capability, rapid quantitative analysis, wide dynamic range, high sensitivity, high selectivity, low detection limits and simple hyphenation with chromatographic techniques for speciation analysis (Nelms, 2005; Thomas, 2004).

1.6.3.3.2 GC coupled to ICP – MS

Gas chromatography is a high resolving chromatographic separation technique, which is usually applied for the separation of volatile and semi-volatile compounds. Upon suitable derivatisation reactions, a great variety of higher boiling and more polar compounds become accessible to GC. In principle, GC instrumentation is fully compatible with ICP-MS and offers several advantages compared to HPLC-ICP-MS. GC-ICP-MS provides higher resolving power and 100 % introduction efficiency into the ICP. At the same time, as a consequence of the dry plasma conditions, the plasma is more stable and exhibits fewer spectral interferences. For the coupling of GC to ICP-MS several prerequisites have to be fulfilled. The analytes of interest usually elute as concentrated quasi-matrix-free peaks in gaseous form. In order to prevent condensation of the analytes of interest, a heated transfer line is required to maintain the temperature of the eluting gas flow and all cold spots between the GC compartment and the ICP have to be eliminated. To avoid decomposition or adsorption of the analytes, all surfaces in contact with them should be deactivated. As

typical effluent flow rates applied in GC separation are too low to obtain an annular plasma, the addition of a make-up/sheath gas is required to obtain typical sample gas flow rates for ICP-MS of approximately 1 L min^{-1}. Beside the make-up gas, the addition of oxygen to the nebulizer gas flow is recommended to prevent deposition of carbon arising from the sample solvent on the sampler cone, which can negatively affect the instrument performance. So far, several commercial transfer lines that permit the coupling of GC to ICP-MS have been introduced (Popp, et al., 2010; Nash, et al., 2004). Common to all of them is that the GC-eluent is directed through an inert capillary, which is surrounded by a heated tubing line. A constant flow of pre-heated make-up gas is added to the GC-eluent at the end of the transfer-line, close to the tip of the injector tube to transport the eluting gas into the plasma.

Traditional fields of application for GC-ICP-MS include speciation studies on the environmental occurrence of organometallic compounds of Pb, Hg and Sn. In recent years the use of GC-ICP-MS for organic compounds containing different hetero-atoms (e.g. P, S, Br, I) received rising attention (Popp, et al., 2010).

1.6.3.3.3 Quantification of separated species by ICP - MS and quality control

The assessment of quality control (QC) and quality assurance (QA) is considered a very important part of the analytical procedure. The QA/QC include the following measures: analysis of chemical and glassware blanks due to the possible contamination of solvents, control of instrumental blanks, identification based on retention time criteria as well as on internal standards, quantification based on the isotope dilution method with the use of internal standard, establishing of calibration curve with the use of matrix matched standards and internal standards, analysis of duplicate samples, check of method performance by analyzing control samples of known concentrations and analysis of certified reference material.

With the isotope dilution technique, samples are fortified prior to their preparation with analytes that are isotopically enriched. The element to be analyzed must have, therefore, at least two stable or long-lived radioactive isotopes able to be measured in a mass spectrometer and be free of spectral interferences. Knowledge of the isotope ratio of the enhanced addition (spike) allows the calculation of the sample concentration by measuring the new isotope ratio of the amended sample. It enables the elimination of measurement interferences that would still be visible with the conventional internal or external standard calibration and compensates for instrumental instabilities such as signal drift or matrix effects. The uncertainty in the concentration measurement depends only on the uncertainty in the measurement of the isotope ratios. For these reasons, the ID is the most accurate method of quantification. The concentration of the analyte in the sample is calculated using Equation [1], where $_S$ stands for sample and $_{Sp}$ for isotopically enriched spike. In this equation the concentration of the analyte in the sample c_S is determined just by measuring R_m as all other parameters in the equation are known. c = concentration of the analyte, A^a = abundance of the isotope a, A^b = abundance of the isotope b, Rm = isotope ratio a/b in the mixture, Rs = isotope ratio b/a in the sample and R_{Sp} = isotope ration a/b in the spike (Rodríguez-Gonzales, et al., 2005).

$$c_S = c_{Sp} \frac{m_{Sp}}{m_S} \frac{M_S}{M_{Sp}} \frac{A^b_{Sp}}{A^a_S} \left(\frac{R_m - R_{Sp}}{1 - R_m R_S}\right) \quad [1]$$

Internal standard calibration also uses a ratio, even though the internal standard is not always influenced to exactly the same degree as the analyte signal. Thus, it can be predicted that, in the case of analysis of PBDEs by GC-ICP-MS, using the isotopically enriched standard for each congener under consideration would be an excellent means to obtain highly accurate data. As the definition of internal standard refers to the compound that has similar properties and behaves in a similar way to studied analytes, the use of ^{13}C labeled PBB and PCB, or unlabeled BDE congeners that are not typically identified in samples (e. g. BDE 15, BDE 75, BDE 77) has been reported in literature (Hale, et al., 2001; Stapleton, 2006; Vonderheide, 2009).

In order to verify the trueness of the developed analytical procedure, the certified reference material (CRM) is often applied. To perform the analytical measurements with the greatest accuracy, it is beneficial to have reference materials with known concentrations of analytes of interest to validate results. The US National Institute for Standards and Technology (NIST) issues RMs certified for the concentrations of PBDEs in fish and mussel tissue, as well as in spiked and non-spiked human milk, human serum, house dust and marine sediment. No CRMs are available for PBDEs in food other than fish and for PBDEs in surface water. In case of lack of respective reference material, other approaches, such as the addition of standards, have to be used.

Chapter 2

Aims and Hypothesis

PBDEs have been found in all compartments of the environment (air, soil, sediment, natural waters, sewage sludge, dust, biota and human tissues). Despite their ubiquitous presence, various compartments have not been equally investigated. While many authors have reported on the presence of PBDEs in air and sediments, comparatively little is known about PBDEs in surface waters, sewage sludge, and biota tissues.
Various analytical methods have been developed for the determination of PBDEs in different environmental and biological samples. In the analysis, diversity of sample matrices represents a complex challenge. Composition and concentration of PBDEs in any specific sample depend on the sample type and the location where the sample has been collected. Concentrations of PBDEs in soils, sediments and house dust are much higher than those in natural waters. For that reason, only few reports are found in the literature on the determination of trace concentrations of PBDEs in natural water samples. The other problem in PBDEs determination is quantitative extraction from the sample matrix. While the extraction of PBDEs from water samples is relatively straightforward and the main problem is how to achieve low detection limits, problems in samples with complex matrix, like sewage sludge or mussel and fish tissue analyses include poor recovery rates and extraction efficiencies.
Most analytical procedures for the determination of PBDEs in environmental samples developed in recent years employ the time- and solvent-consuming Soxhlet extraction method and new extraction methods that would overcome these disadvantages should be developed. GC coupled to ICP-MS represents a superior analytical tool for the speciation analysis of PBDEs at low concentrations and enables analyses of samples with a complex matrix, such as sewage sludge or biota tissues without or with minimal cleaning of the extracts. Despite the fact that ICP-MS is a versatile and sensitive detector, it is not commonly used for the detection of the GC separated PBDEs.
The aims of the present doctoral thesis were to develop analytical procedures with minimal sample preparation for a sensitive and reliable determination of the six PBDEs in surface water, sewage sludge, mussel and fish samples by GC-ICP-MS.
For that purpose:

- The influence of different extracting agents and the subsequent simultaneous addition of the Tris-citrate buffer (co-extracting agent) and iso-octane on the

extraction efficiency were systematically investigated when applying different modes of extraction.

- The parameters influencing GC-ICP-MS detection were optimized.
- The procedure of acidification of the sample, which prevents adsorption of PBDEs on the surface of the glass bottles in case of storage of surface water samples, was investigated.
- The applicability and robustness of the developed analytical procedures was demonstrated by analyses of different environmental samples.

Chapter 3

Materials and Methods

3.1 Materials

All reagents used were of analytical-reagent grade. Milli-Q water (18.2 MΩ cm) (Milipore, Bedford, MA, USA) was used for the preparation of all aqueous solutions. Individual standards of seven PBDE congeners (28, 47, 77, 99, 100, 153, 154) at a concentration of 50 g mL^{-1} were purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). Standard stock solutions of PBDEs were prepared in iso-octane at concentration levels of 5 g mL^{-1} and stored in the dark at 4 °C. Further working standard solutions were prepared daily in methanol or in acetone.

^{81}Br isotopically enriched BDEs (BDE 28, BDE 47, BDE 99, BDE 100, BDE 153 and BDE 154) were obtained from ISC Science (Oviedo, Spain) and were used for the calculation of the BDEs' concentrations by ID-GC-ICP-MS. The compositions of the ^{81}Br enriched BDE 28, BDE 47, BDE 99 and BDE 153 determined for isotopes 81 and 79 were 99.53 % and 0.47 %, respectively. The compositions of the ^{81}Br enriched BDE 100 for isotopes 81 and 79 were 68.5 % and 31.5 %, respectively and of ^{81}Br enriched BDE 154, 76.3 % and 24.7 %, respectively.

Hydrochloric acid (HCl), nitric acid, acetone, acetic acid, sodium acetate, citric acid monohydrate, hydroxymethyl aminomethane (Tris) and tetramethyl ammonium hydroxide (TMAH) were obtained from Merck (Darmstadt, Germany). Hexane, methanol and iso-octane (2, 2, 4-trimethylpentane) were purchased from J. T. Baker (Deventer, Holland). The buffer solutions were prepared daily with Milli-Q water. For preparation of 1 mol L^{-1} acetate buffer (pH 4.8), sodium acetate and acetic acid were used. Tris-citrate buffer (pH 6.0) was prepared from a 0.2 mol L^{-1} solution of Tris with an appropriate addition of citric acid.

IHSS Suwannee River Humic Acid Standard II, which was used to study the influence of humic acids (HA) on the efficiency of extraction of PBDEs from water samples, was purchased from the International Humic Substances Society (Saint Paul, MN, USA).

The Standard reference material 2974a and the Standard reference material 1946 were purchased from the National Institute of Standards & Technology (NIST) (Gaitersburg, MD, USA).

The columns used to clean up the extract were Strata FL-PR Florisil (170um, 80A) 500mg/3mL obtained from Phenomenex, Inc. (Torrance, CA, USA).

3.2 Instrumentation

The determination of PBDEs was carried out on an Agilent 6890 gas chromatograph (GC) (Agilent Technologies, Santa Clara, CA, USA) equipped with an Agilent 6890 Series Autosampler Injector that was coupled to an Agilent 7700x ICP-MS (Agilent Technologies, Tokyo, Japan) via heated transfer line and fitted with a 15 m x 0.25 mm DB - 5MS capillary column (film thickness 0.25 µm) coated with 5 % phenylmethylpolysiloxane (Agilent J&W Scientific, Palo Alto, CA, USA). The control and operation of the coupled system was performed by using an Agilent MassHunter software.

Mechanical shaking of samples during the extraction procedure was performed on a Vibromix 40 orbital shaker (elliptical table shaker) (Tehtnica, Železniki, Slovenia). Ultrasound-assisted extraction of samples was performed with a 550D VWR International ultrasonic bath (West Chester, PA, USA). Microwave-assisted extraction was performed with a CEM MARS 5 microwave acceleration reaction system (CEM, Matthews, NC, USA). Centrifugation of sample extracts was performed on the Hettich Universal 320 Centrifuge (Hettich GmbH & Co., KG, Tuttlingen, Germany).

3.3 Cleaning Procedure

To avoid contamination, all glassware was rinsed three times with tap water, soaked in 10 % nitric acid for 48 hours, rinsed three times with tap water, three times with MilliQ water and heated at 400 °C for at least 4 hours. Just before use, all glassware was rinsed with hexane and acetone and dried at room temperature.

3.4 Methods

3.4.1 Sample collection and preparation

3.4.1.1 Water

For method development, river water samples were taken from the unpolluted river Gradaščica (close to Ljubljana city, Slovenia). To verify the applicability of the method, Slovenian river waters and seawater samples from the Northern Adriatic coast in Slovenia were taken at several sampling locations. Sampling was performed in July-August 2013. Samples were collected in 1 L amber glass bottles previously cleaned as described in *3.3.*, 1 mL of nitric acid was added and the acidified samples immediately transported to the laboratory in iceboxes. Until the analysis, samples were stored at 4 °C.

3.4.1.2 Sewage sludge

Sewage sludge sample from a previously performed proficiency testing scheme on trace elements in sewage sludge was chosen for method development. Data of its characterisation and homogeneity as well as the exact procedure followed for sample preparation are described by Van de Veen (Van de Veen, et al., 2001). Briefly, the material was collected at a WWTP, which receives wastewater of industrial (37 %) and municipal (63 %) origin. A representative sewage was dried at 40 °C to a target moisture content of 3-5 %. After drying, the material was ground and passed through a 0.108 mm sieve. Bottles containing 20 g of the material were then sterilized by irradiation to eliminate any bacterial activity.

On the same WWTP fresh sewage sludge samples were collected in year 1998 and 2014, dried at 40 °C in the dark for 3 days, homogenised and passed through a 0.108 mm sieve. The samples were then frozen at -20 °C until the analysis.

3.4.1.3 Fish and mussel

For the method development, the standard reference material NIST SRM 2974a (Freeze-Dried Mussel Tissue) was used. To confirm the accuracy of the developed analytical procedure, PBDEs were determined in two standard reference materials, NIST SRM 2974a (Freeze-Dried Mussel Tissue) and NIST SRM 1946 (Lake Superior Fish Tissue), which are certified for all the six PBDEs analysed. To verify the applicability of the method, fish samples from the Northern Adriatic Sea and from the Sava River were taken at several sampling locations. Sampling was performed in year 2015. Fish samples were filleted, homogenized in a blender, lyophilized and stored in darkness at -20 °C until the analysis. Soft mussel tissues were removed from the shells, homogenized in a blender and stored in the darkness at -20 °C until the analysis.

3.4.2 Optimized developed analytical procedures

3.4.2.1 Water samples

Liquid – liquid extraction of PBDEs from water samples was used prior to their determination by GC-ICP-MS. A 300 mL sample aliquot, acidified with nitric acid to pH 2 (1 mL HNO_3 per 1 L of water), was transferred to a 1 L glass reactor vessel. As internal standard BDE 77 was added, followed by the addition of 300 mL Tris – citrate buffer solution (pH 6). Then, 2 mL of iso-octane was added as an extracting agent. Samples were mechanically shaken for 6 h. After that the organic phase was collected and concentrated under a gentle nitrogen flow to a final volume of approximately 25 L. Finally, the organic phase was collected in a 2 mL amber vial by a Pasteur pipette and 2 L injected into the GC. A flow chart is presented in Figure 3.1.

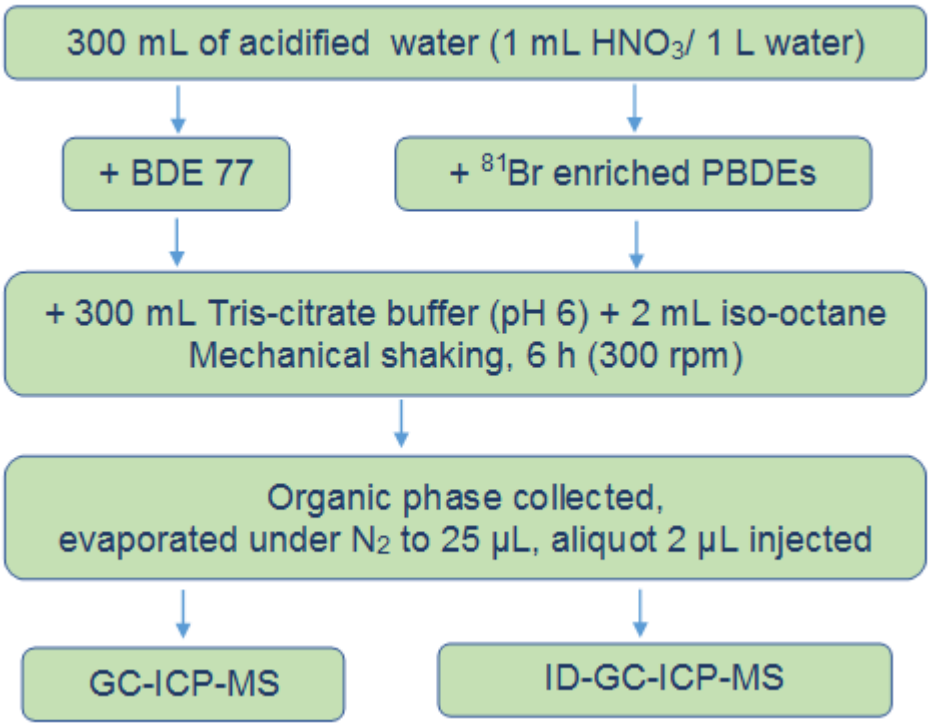


Figure 3.1: Flow chart of the analytical procedure for the determination of PBDEs in water samples.

3.4.2.2 Sewage sludge samples

0.5 g of dry sewage sludge sample was weighted into a glass reactor vessel to which BDE 77 (internal standard) or ^{81}Br enriched PBDEs spike, and 10 mL of 0.1 mol L^{-1} HCl in MeOH were added. Vessels were closed and mechanically shaken for the first 2 h (20 °C, 300 rpm). Then, 50 mL of Tris-citrate buffer solution (pH 6) and 2 mL of iso-octane were added and samples mechanically shaken again for another 2 h (20 °C, 300 rpm). Organic phase (iso-octane), which contained dispersed emulsion, was collected in a 10 mL glass vial by a Pasteur pipette. To separate the emulsion from the organic phase, 0.5 mL of 25 % KOH in MeOH was added and centrifuged for 5 min at 4200 × g. Clear organic phase was transferred into a 2 mL amber glass vial by a Pasteur pipette. An aliquot of 2 µL was injected into the GC. A flow chart is presented in Figure 3.2.

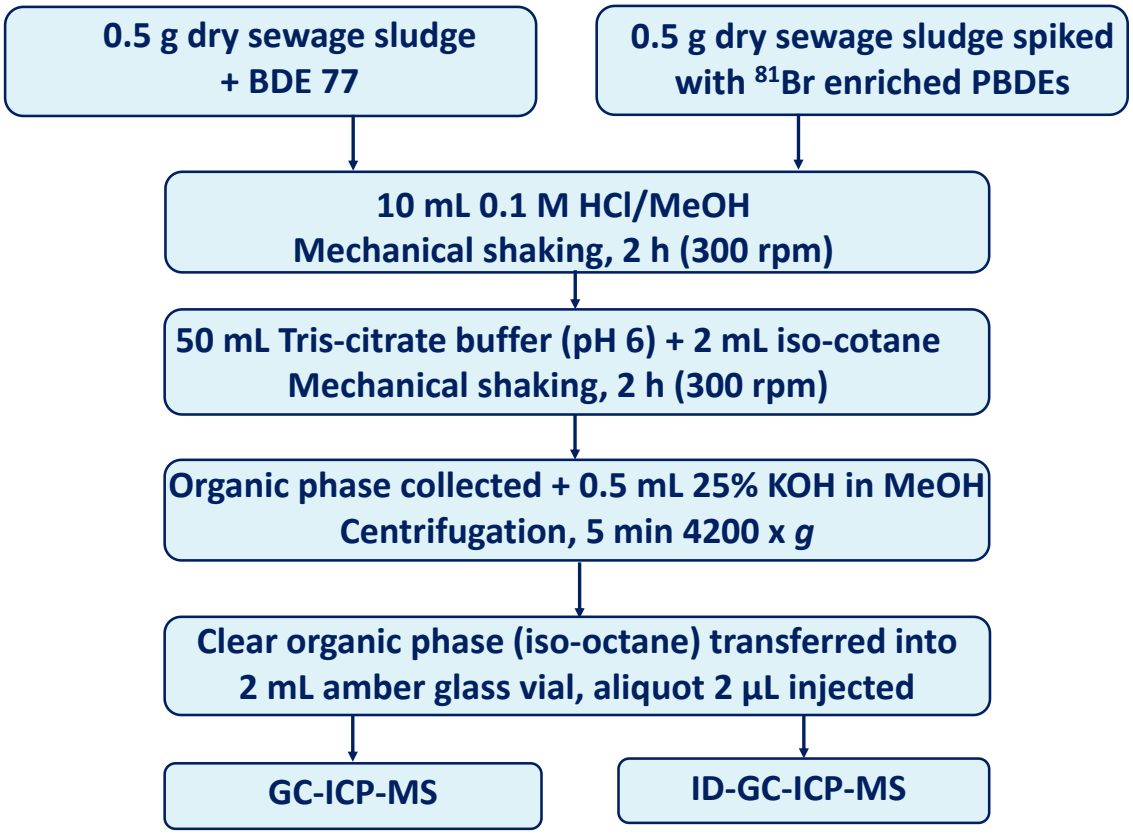


Figure 3.2: Flow chart of the analytical procedure for the determination of PBDEs in sewage sludge samples.

3.4.2.3 Fish and mussel samples

0.25 g of lyophilized or 1.5 g of fresh mussel or fish sample was weighed into a glass reactor vessel to which BDE 77 (internal standard) or ^{81}Br enriched PBDEs spike, and 10 mL of 25 % aqueous solution of TMAH were added. The vessels were closed and ultrasound-assisted extraction applied for 30 minutes. Then, 50 mL of Tris-citrate buffer solution (pH 6) and 2 mL of iso-octane were added and the samples mechanically shaken for 2 h (20 °C, 300 rpm). The organic phase (iso-octane), which contained the dispersed emulsion, was collected in a 10-mL glass vial with a Pasteur pipette. To separate the emulsion from the organic phase, 0.5 mL of 25 % KOH in MeOH was added and centrifuged for 5 min at 5000 × g. The clear organic phase was collected and passed through a 3 mL column packed with 500 mg of Florisil (preconditioned with 1 mL of iso-octane) to remove lipids from the matrix. The column was washed out with 2 mL of iso-octane and, afterward, the whole organic phase concentrated under a gentle nitrogen flow to a final volume to approximately 30 L. Finally, the concentrated extract was collected in a 2 mL amber vial by a Pasteur pipette and 2 L injected into the GC. A flow chart is presented in Figure 3.3.

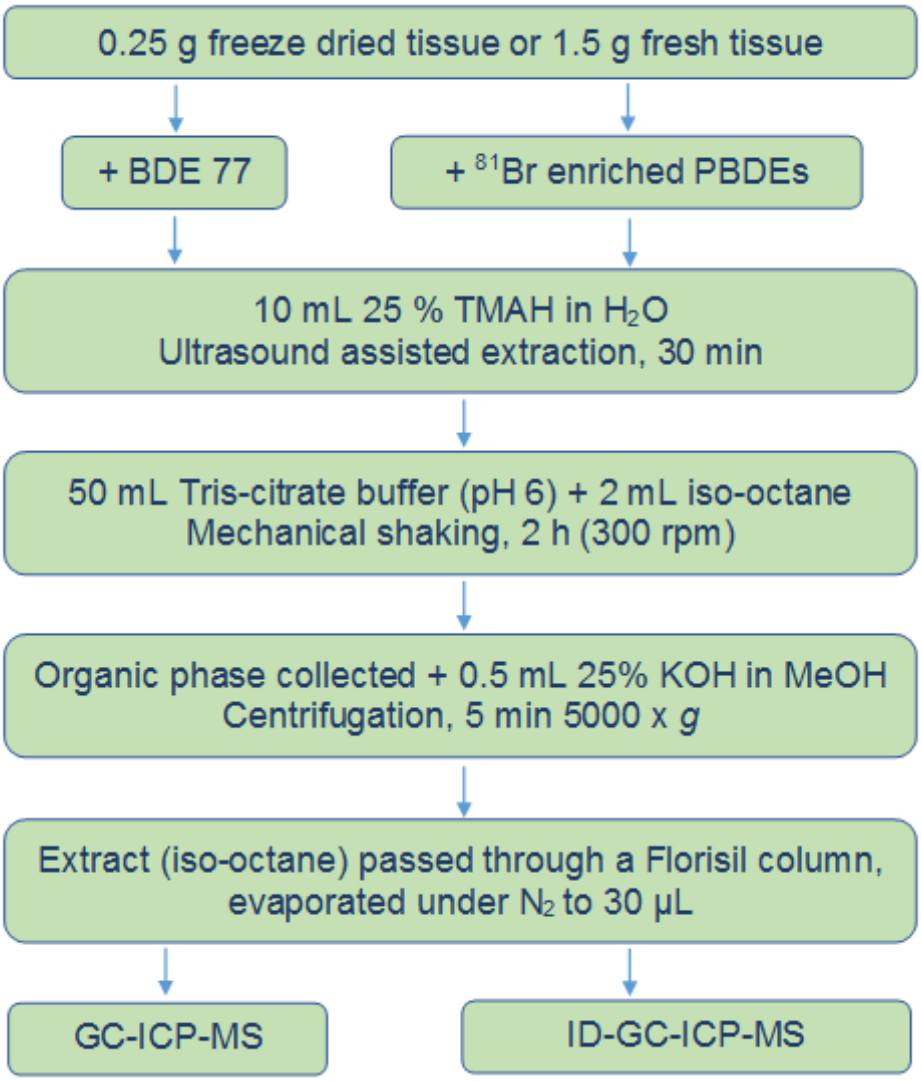


Figure 3.3: Flow chart of the analytical procedure for the determination of PBDEs in fish and mussel samples.

3.4.3 GC-ICP-MS parameters for separation and determination of PBDEs

For the separation of PBDEs on a 15 m column, the following GC temperature program was applied: first the column temperature was raised from 120 °C to 300 °C at a heating rate of 30 °C min^{-1} and held there for 5 min. The inlet temperature and transfer line was held at 280 °C. Helium at a flow rate of 1.5 mL min^{-1} was used as carrier gas, the injection mode was splitless and the injection volume 2 µL. The operating parameters of the GC-ICP-MS system are presented in Table 3.1.

Calculation of the PBDE concentrations was done by the standard addition calibration method and by the species-specific ID-GC-ICP-MS procedure, as described in detail by Rodríguez-Gonzales and coworkers (Rodríguez-Gonzales, et al., 2005). Blank samples were determined under the same analytical protocol as samples. All analyses were made in triplicate.

Table 3.1: GC and ICP operating parameters.

ICP-MS	**Unit**
RF power	1300 W
Sample depth	7.0 mm
Carrier gas (Ar)	1.50 L min^{-1}
Optional gas (20 % $^{v}/_{v}$ O_2 in Ar)	10 %
Integration time per isotope	0.1 s
Isotopes measured	^{79}Br, ^{81}Br
Tune gas	100 ppm Xe in Ar
Total acquisition time	666 s
GC	**Unit**
Injection volume	2 µL
Mode	Split-less
Carrier gas (He)	1.50 mL min^{-1}
Inlet temperature	280 °C
Transfer line temperature	280 °C

Chapter 4

Results and Discussion

In order to develop a very sensitive analytical procedure for reliable determination of six PBDE congeners in different environmental and biological samples by GC-ICP-MS, the instrumental parameters influencing GC-ICP-MS detection were first optimized, followed by optimization of the extraction procedures. The performances of the analytical procedures developed were evaluated. Finally, the applicability of the newly developed analytical procedures was demonstrated with the determination of PBDEs in marine and river water samples, sewage sludge samples from the municipal WWTP and in marine and river fish samples.

4.1 Optimization of the GC-ICP-MS Operating Parameters

For optimization of the GC-ICP-MS operating parameters a mixture of the six PBDE congeners and BDE 77 at a concentration of 400 ng L^{-1} for each particular congener was prepared in 2 mL of isooctane. By applying a 15 m chromatographic column, the GC temperature program and GC operating parameters described under *3.4.3*, all the PBDEs analysed were efficiently separated. In order to obtain the best signal intensities for the determination of the separated PBDEs, ICP-MS operating parameters were further optimized by modifying the ICP RF power, the flow rate of carrier gas and by the addition of oxygen as optional gas. The results are presented in Figure 4.1.

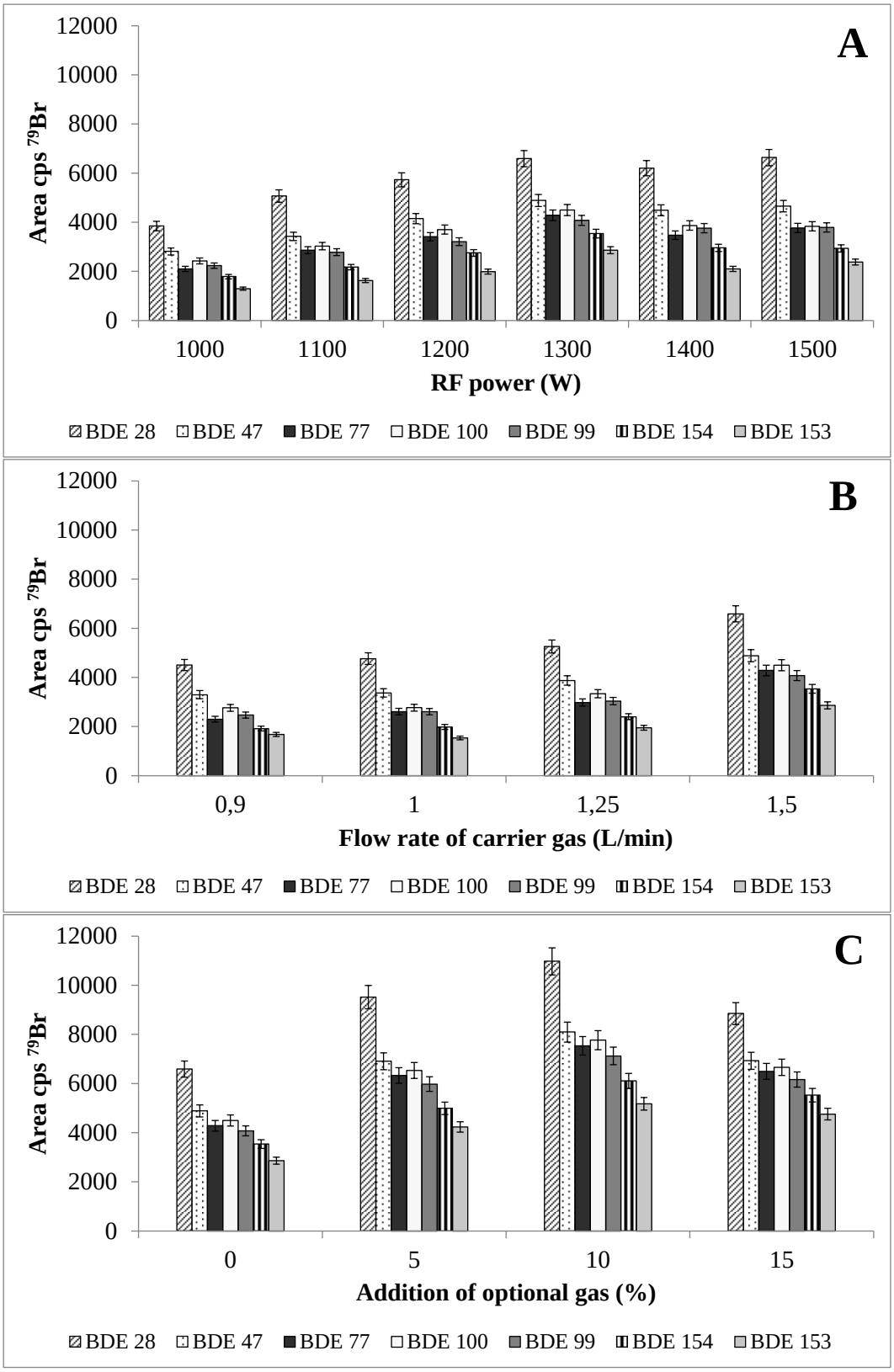


Figure 4.1: Influence of ICP RF power (A), the flow rate of carrier gas (B) and addition of optional gas (20 % $^{v}/_{v}$ O_2 in Ar) (C) on signal intensities of PBDEs (400 ng L^{-1}) applying the GC-ICP-MS procedure. Chromatograms were recorded at m/z 79. Results represent the average ± standard deviation of three replicates.

It is evident from Figure 4.1 that the highest signal intensities for all the PBDEs investigated were obtained at an ICP RF power of 1300 W (Figure 4.1A). At this RF power, the flow rate of carrier gas was then optimized. As can be further seen from Figure 4.1B the most intensive signals were obtained at a carrier gas flow rate of 1.5 L min^{-1}. Finally, the influence of the addition of optional gas was examined. The data in Figure 4.1C demonstrate that the addition of oxygen in optional gas substantially improved the signal sensitivity. This phenomenon is related to carbon removal, which was the most efficient on the addition of 10 % of optional gas containing 20 % v/v O2 in Ar.

4.2 Water Samples

To develop an analytical method for the determination of PBDEs in surface waters that would meet the requirements set by WFD, the parameters which influence the overall extraction efficiency in the liquid-liquid extraction step were critically evaluated. The goal was to achieve an LOQ of 0.15 ng L^{-1} for the ΣPBDEs in whole water sample and a relative expanded uncertainty of 50 % or less at the EQS level (0.5 ng L^{-1} of ΣPBDEs) (Directive 2008/105/EC, 2008). To improve the extraction efficiency of PBDEs from water into iso-octane by liquid-liquid extraction, the use of sodium acetate – acetic acid or Tris – citrate buffer was tested. The accuracy of the analytical procedure was checked by spike recovery tests and by applying species-specific isotope-dilution (ID) GC-ICP-MS quantification. The stability of PBDEs in acidified and non-acidified spiked surface water samples was checked over a time span of more than 30 days. To demonstrate the applicability of the GC-ICP-MS analytical procedure developed, PBDEs were determined in river and seawater samples.

4.2.1 Optimization of the extraction procedure

Experiments were carried out with spiked unpolluted river water samples, to which suspended particulate matter (SPM) as bottom sediment, wet sieved < 63 µm or SPM and humic acid (HA) was added at a concentration of 35 mg L^{-1} and 5 mg L^{-1}, respectively. 5 L of sample was prepared by homogenization on a mechanical shaker for 24 hours. 100 mL aliquots were then taken and spiked with the six BDE congeners (BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, BDE 154) and BDE 77 as internal standard at concentrations of 400 ng L^{-1} each. After spiking, they were shaken for another 24 hours. Before extraction, different sample treatments with or without the addition of nitric acid and acetate or citrate buffer were applied. 2 mL of iso-octane was then added and liquid-liquid extraction by mechanical shaking or ultrasound treatment performed for different time intervals. The organic phase containing PBDEs was collected in a 2 mL dark GC glass vial. Concentrations of PBDEs were determined by GC-ICP-MS under the optimized operating parameters (*3.4.3*).

4.2.1.1 Influence of different sample treatments on extraction efficiency

Different sample treatments were tested prior to the extraction of PBDEs from water samples spiked with 400 ng L^{-1} PBDEs, containing 35 mg L^{-1} SPM (a typical amount of SPM in river water during or after rain). Non-acidified and acidified samples without the addition of buffers or with the addition of acetate (pH 4.8) or Tris-citrate buffers (pH 6) were examined. For acidification 1 mL of HNO_3 was added to 1 L of sample (pH 2). The acid used and the choice of buffer was based on studies about the determination of organotin compounds in surface waters and landfill leachates (Vahčič, Milačič & Ščančar, 2011a; Vahčič, Milačič & Ščančar, 2011b). The influence of pH on the extraction efficiency

when applying acetate and Tris-citrate buffers was examined in the pH range from 3 to 7. The optimal pH for acetate or Tris-citrate buffer was found to be 4.8 or pH 6, respectively (data not shown). These experiments were performed by applying the following procedure: to 100 mL of spiked water sample, 100 mL of buffer and 2 mL of iso-octane were added. Then samples were mechanically shaken (20 °C, 300 rpm) for 1 hour to extract PBDEs into the organic phase where their concentrations were determined by GC-ICP-MS. Signal intensities of PBDEs for different sample treatments are presented in Figure 4.2.

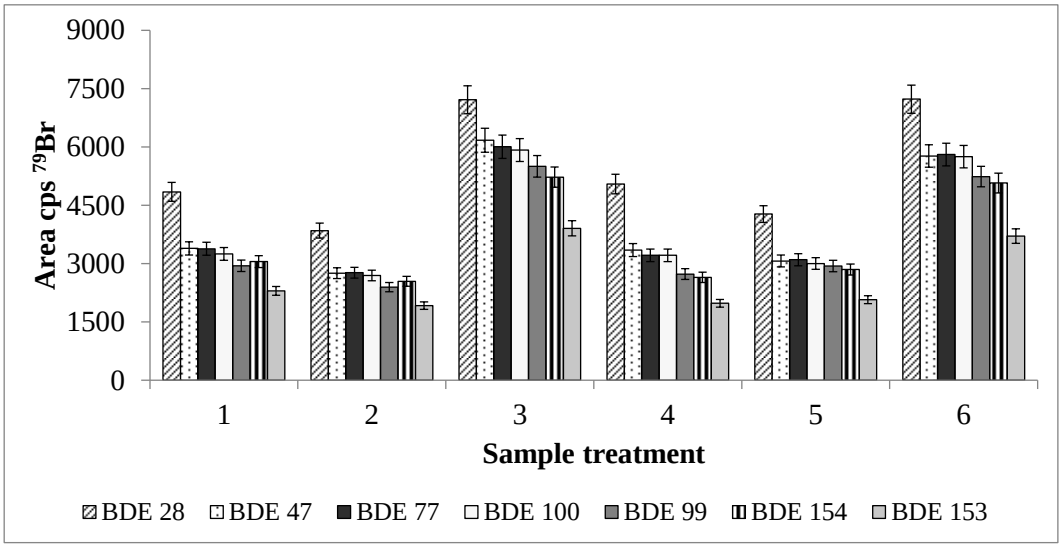


Figure 4.2: Influence of different sample treatments on signal intensities of spiked river water sample containing PBDEs (400 ng L^{-1}) when applying the GC-ICP-MS procedure (sample volume 100 mL). 1: Non-acidified sample. 2: Non-acidified sample followed by the addition of acetate buffer (pH 4.8). 3: Non-acidified sample followed by the addition of Tris-citrate buffer (pH 6). 4: Sample acidified with HNO_3 (pH 2). 5: Sample acidified with HNO_3 to pH 2, followed by the addition of acetate buffer (pH 4.8). 6: Sample acidified with HNO_3 to pH 2, followed by the addition of Tris-citrate buffer (pH 6). Results represent the average ± standard deviation of three replicates. Mode: Mechanical shaking (20 °C, 300 rpm, 1 h).

From the experimental results, it is evident that the use of Tris-citrate buffer facilitates the extraction of PBDEs from the matrix of the water sample analysed, as it almost doubles the signal intensities for both non-acidified and acidified samples in comparison to those samples where no Tris-citrate buffer or acetate buffer was used. Hence, in further experiments the stability of PBDEs over a time span of 30 days was followed in non-acidified and acidified water samples (Figure 4.3) using a Tris-citrate buffer in the liquid-liquid extraction stage.

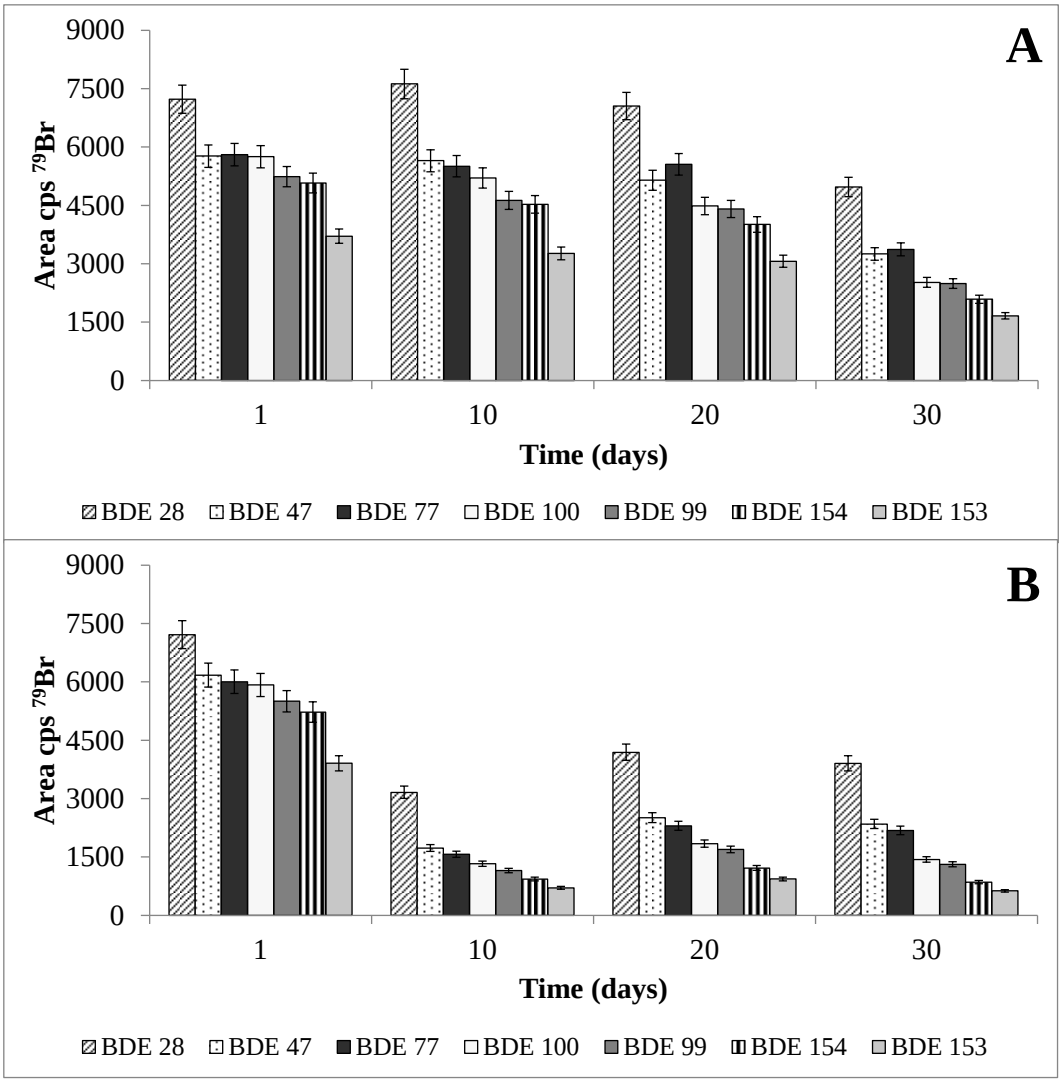


Figure 4.3: Stability of acidified and non-acidified river water samples spiked with PBDEs (400 ng L^{-1}) checked by applying the GC-ICP-MS procedure (sample volume 100 mL). Bars represent signal intensities of sample which was acidified with HNO_3 to pH 2, followed by the addition of Tris-citrate buffer (pH 6) (A) and non-acidified sample with the addition of Tris-citrate buffer (pH 6) (B). Results represent the average ± standard deviation of three replicates. Mode: Mechanical shaking (20 °C, 300 rpm, 1 h).

Data from Figure 4.3 clearly demonstrate that non-acidified samples are stable only for one day, while samples acidified prior to the Tris-citrate buffer addition are stable for at least 20 days. Acidification of the sample prevents adsorption of PBDEs on the surface of the glass bottles, while citric acid enables efficient desorption of PBDEs from SPM. For that reason, acidified samples, buffered to pH 6 with Tris-citrate buffer, were used in all the following experiments.

4.2.1.2 Influence of different modes of extraction on extraction efficiency

During optimization of the extraction procedure different modes of extraction were tested. Mechanical shaking (20 °C, 300 rpm) and ultrasound-assisted extraction (20 °C, 240 W) were applied for different time intervals. The results are presented in Figure 4.4.

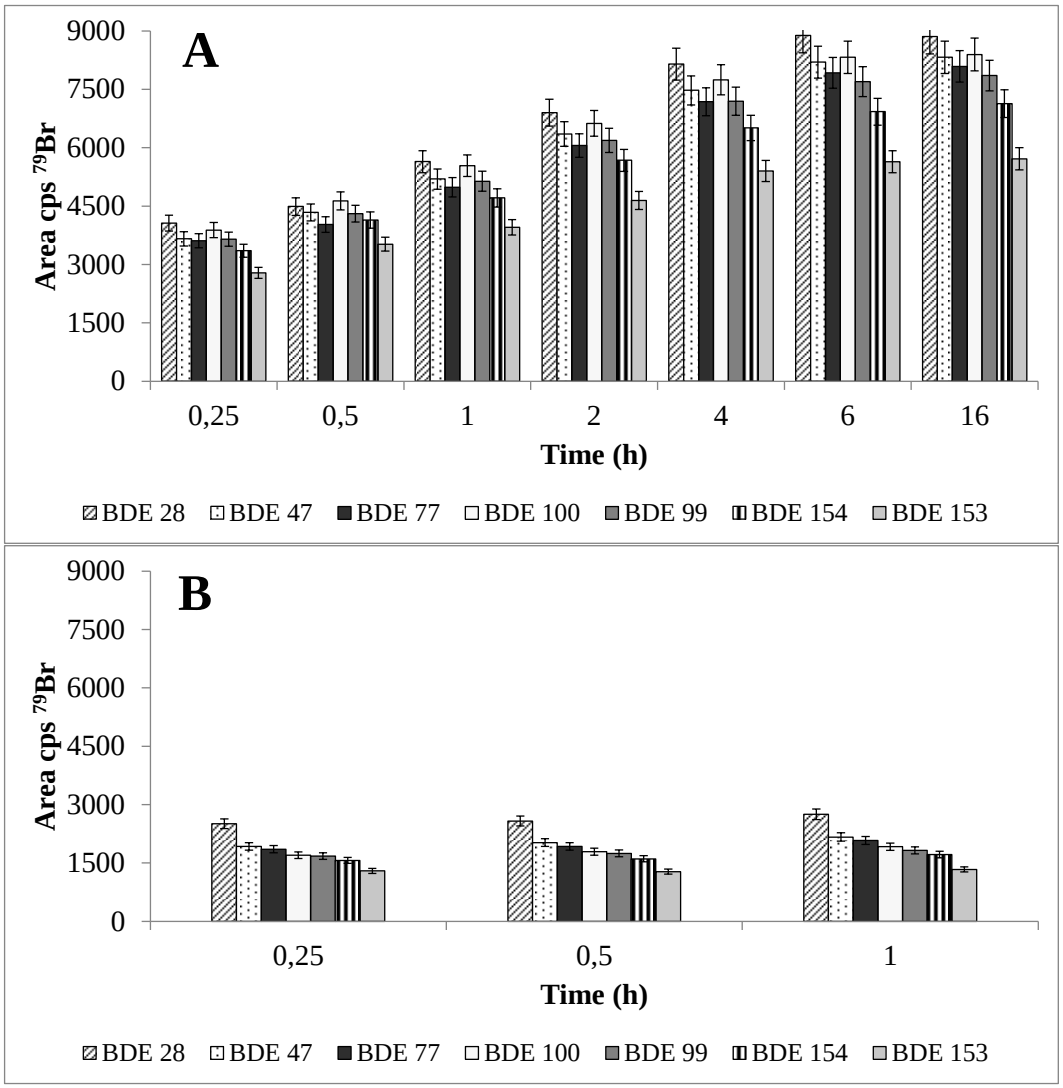


Figure 4.4: Influence of different modes and times of extraction on signal intensities of river water sample spiked with PBDEs (400 ng L^{-1}) on applying the GC-ICP-MS procedure (sample volume 100 mL). Samples were acidified with HNO_3 to pH 2, followed by the addition of the Tris-citrate buffer (pH 6). Results represent the average ± standard deviation of three replicates. Mode A: Mechanical shaking (20 °C, 300 rpm). Mode B: Ultrasound-assisted extraction (20 °C, 240 W).

From the signal intensities presented in Figure 4.4, it is evident that mechanical shaking enables much more effective extraction of PBDEs from spiked water samples containing 35 mg L^{-1} of SPM than ultrasound-assisted extraction, which was found to be rather inefficient. For mechanical shaking, the signal intensities gradually increased with time and reached their constant maximum values after 6 hours. For that reason, mechanical shaking for 6 hours was applied for liquid-liquid extraction of PBDEs from water samples.

4.2.1.3 Influence of humic acids on extraction efficiency

Environmental water samples may contain around 5 mg L^{-1} of humic substances, while in natural waters rich in humic substances, their content is appreciably higher and is

around 40 mg L^{-1}, of which about 4 mg L^{-1} represents HA (Malcolm, McKnight & Averett, 1994). In order to estimate the influence of HA on the extraction efficiency of PBDEs, river water samples containing 35 mg L^{-1} of SPM and 5 mg L^{-1} of HA were prepared and spiked with 400 ng L^{-1} of the six BDE congeners listed as priority substances in the WFD. Analyses were performed by the previously described optimized analytical procedure. A typical chromatogram obtained by GC-ICP-MS, recorded at *m/z* 79, is presented in Figure 4.5. As evident from this Figure, all six PBDE congeners and BDE 77 analyzed were completely separated.

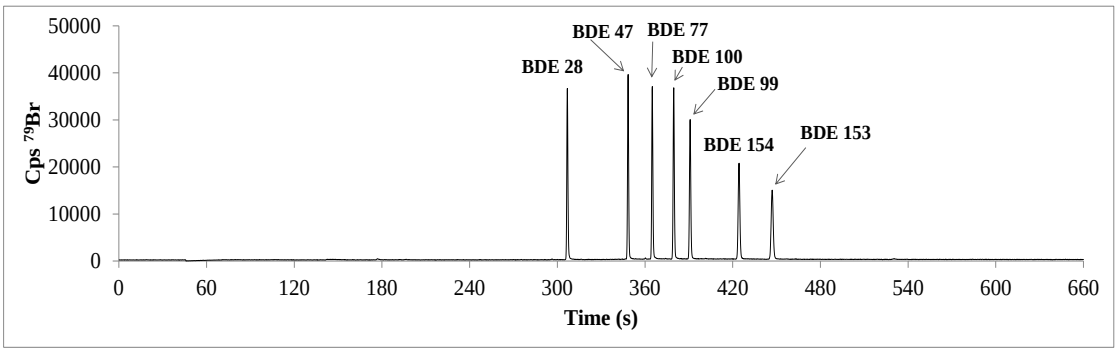


Figure 4.5: Chromatogram of river water sample containing 35 mg L^{-1} of SPM and 5 mg L^{-1} of HA, spiked with PBDEs (400 ng L^{-1}) by applying the GC-ICP-MS procedure and recorded at *m/z* 79 (sample volume 100 mL).

4.2.2 Figures of merit of the analytical procedure for the determination of PBDEs in environmental water samples by GC-ICP-MS

In order to improve the sensitivity of the developed analytical procedure, the organic phase (iso-octane) containing the PBDEs extracted was collected and concentrated under a gentle stream of nitrogen to approximately 25 µL. PBDEs were then determined by GC-ICP-MS.

4.2.2.1 Accuracy check

To evaluate the efficiency of extraction and the accuracy of the developed analytical procedure, 100 mL of river water sample containing 35 mg L^{-1} of SPM and a sample containing 35 mg L^{-1} of SPM and 5 mg L^{-1} of HA were spiked with 10 ng L^{-1} of PBDEs and the analysis performed by GC-ICP-MS using the standard addition calibration method. The recoveries calculated as the ratio between the added and found PBDE concentrations are presented in Table 4.1.

Table 4.1: Extraction recoveries for PBDEs added to 100 mL of river water containing 35 mg L^{-1} of SPM and river water containing 35 mg L^{-1} of SPM and 5 mg L^{-1} of HA. The organic phase was concentrated to 25 µL and concentrations of PBDEs determined by GC-ICP-MS using the standard addition calibration method. Results represent the mean value of three replicate determinations of a selected BDE with the standard deviation of the measurements.

Sample	**BDE congener**	**Concentration (ng L^{-1})**	**Added (ng L^{-1})**	**Found (ng L^{-1})**	**Recovery (%)**
River water containing 35 mg L^{-1} SPM	BDE 28	< 0.0054	10.0 ± 0.3	9.7 ± 0.3	97 ± 3
	BDE 47	< 0.0048	10.0 ± 0.3	10.3 ± 0.3	103 ± 3
	BDE 100	< 0.0052	10.0 ± 0.3	10.1 ± 0.3	101 ± 3
	BDE 99	< 0.0059	10.0 ± 0.3	9.8 ± 0.3	98 ± 3
	BDE 154	< 0.0051	10.0 ± 0.3	10.0 ± 0.4	100 ± 4
	BDE 153	< 0.0064	10.0 ± 0.3	9.9 ± 0.3	99 ± 3
River water containing 35 mg L^{-1} SPM and 5 mg L^{-1} HA	BDE 28	< 0.0054	10.0 ± 0.3	9.8 ± 0.3	98 ± 3
	BDE 47	< 0.0048	10.0 ± 0.3	10.1 ± 0.3	101 ± 3
	BDE 100	< 0.0052	10.0 ± 0.3	9.8 ± 0.3	98 ± 3
	BDE 99	< 0.0059	10.0 ± 0.3	9.7 ± 0.3	97 ± 3
	BDE 154	< 0.0051	10.0 ± 0.3	10.3 ± 0.4	103 ± 4
	BDE 153	< 0.0064	10.0 ± 0.3	10.1 ± 0.3	101 ± 3

As evident, the recoveries of spiked samples containing SPM or SPM and HA lay between 97 and 103 %, indicating quantitative extraction of PBDEs from both samples and confirming the accuracy of the analytical procedure developed. Since there is no standard reference material available for the determination of PBDEs in water samples, the accuracy of the analytical procedure was also verified by comparative species – specific ID-ICP-MS analysis. For this purpose, ^{81}Br enriched BDEs (BDE 28, BDE 47, BDE 99 and BDE 153) were used and the PBDE concentrations determined by GC-ID-ICP-MS using the equations published by Rodríguez-Gonzales et al. (Rodríguez-Gonzales, et al., 2005). The results of the comparative determination of PBDEs by GC-ID-ICP-MS and by the standard addition calibration method are presented in Table 4.2. It can be seen that the difference between the results determined by GC-ICP-MS and ID-GC-ICP-MS did not exceed 1 %, further confirming the accuracy of the analytical procedure.

Table 4.2: Extraction recoveries for PBDEs added to 100 mL of river water containing 35 mg L^{-1} SPM and 5 mg L^{-1} HA. The organic phase was concentrated to 25 µL and concentrations of PBDEs determined by GC-ICP-MS using standard addition calibration and GC-ID-ICP-MS methods. Results represent the mean value of three replicate determinations of a selected BDE with the standard deviation of the measurements.

Sample	**BDE congener**	**BDE (ng L^{-1})**	**BDE added (ng L^{-1})**	**BDE found standard addition (ng L^{-1})**	**BDE found GC-ID-ICP-MS (ng L^{-1})**
River water containing 35 mg L^{-1} SPM and 5 mg L^{-1} HA	BDE 28	< 0.0054	10.0 ± 0.3	9.8 ± 0.3	10.11 ± 0.15
	BDE 47	< 0.0048	10.0 ± 0.3	10.1 ± 0.3	9.88 ± 0.15
	BDE 99	< 0.0059	10.0 ± 0.3	9.7 ± 0.3	10.10 ± 0.15
	BDE 153	< 0.0064	10.0 ± 0.3	10.1 ± 0.3	9.90 ± 0.15

4.2.2.2 Limits of detection and quantification

Samples of Milli Q water and saline water (3.8 % NaCl) containing 35 mg L^{-1} of SPM and 5 mg L^{-1} of HA were prepared. The limits of detection (LODs) and of quantification (LOQs) for the determination of six BDE congeners (BDE 28, BDE 47, BDE 99, BDE 100, BDE 153 and BDE 154) were calculated on the basis of three times and ten times the standard deviation, respectively, of the signal (peak area), of the water samples analysed. 300 mL of samples were extracted and the organic phase concentrated to 25 µL. To calculate the LODs and LOQs, eight water and eight saline water samples were analysed by the GC-ICP-MS procedure developed. The corresponding LODs and LOQs are presented in Table 4.3.

Table 4.3: LODs and LOQs of analytical method for the determination of PBDEs in water and saline water (3.8 % NaCl) samples containing 35 mg $L^{-1}$of SPM and 5 mg L^{-1} of HA. 300 mL of samples were extracted, the organic phase concentrated to 25 µL and analyses performed by GC-ICP-MS.

BDE congener	**Water**		**Saline water**	
	LOD (ng L^{-1})	**LOQ (ng L^{-1})**	**LOD (ng L^{-1})**	**LOQ (ng L^{-1})**
BDE 28	0.0054	0.018	0.0052	0.017
BDE 47	0.0048	0.016	0.0054	0.018
BDE 100	0.0052	0.017	0.0047	0.016
BDE 99	0.0059	0.020	0.0051	0.017
BDE 154	0.0051	0.017	0.0054	0.018
BDE 153	0.0064	0.021	0.0051	0.017
ΣPBDEs	/	0.109	/	0.103
ΣPBDEs set by WFD	/	0.15	/	0.15

Under the optimized analytical procedure, low LODs and LOQs were obtained for the six BDE congeners in water and saline water samples. The LODs ranged between 0.0047 and 0.0064 ng L^{-1} and LOQs between 0.016 and 0.021 ng L^{-1}. The LOQs for the ΣPBDEs were 0.109 ng L^{-1} and 0.103 ng L^{-1} for water and saline water, respectively. They are below 0.15 ng L^{-1}, the LOQ for the ΣPBDEs required by the WFD.

4.2.2.3 Repeatability, reproducibility and linearity of measurement

To test the repeatability and reproducibility of measurement, river water samples containing 35 mg L^{-1} of SPM and 5 mg L^{-1} of HA were spiked with six BDE congeners at a concentration of 10 ng L^{-1} or 0.083 ng L^{-1}. For analysis, 100 mL or 300 mL of samples were taken. In order to measure the contaminants reliably at the EQS level (EQS for ΣPBDEs is 0.5 ng L^{-1}), analytical methods with a measurement uncertainty of 50 % or below (k = 2) are required by the WFD daughter directive 2009/90/EC (Directive 2009/90/EC, 2008). The sum of the six BDE congeners, each at a concentration of 0.083 ng L^{-1}, used to test the analytical performance was 0.498 ng L^{-1}, and was close to the EQS level (Directive 2008/105/EC, 2008).

For the evaluation of the repeatability of measurement six independent river water samples spiked with PBDEs at the two above-mentioned concentration levels were analysed on the same day. After seven days, the same experiment was repeated by a different analyst, applying the same analytical procedure to estimate the reproducibility of measurement. The concentrations of the analysed PBDEs were determined by the standard addition calibration method. For the sample spiked at 10 ng L^{-1}, standard solutions containing a mixture of PBDE from 1 to 20 ng L^{-1} were used for the calibration curve, while for the sample containing 0.083 ng L^{-1} of PBDEs, standard solutions from 0.02 to 1 ng L^{-1} were used. The correlation coefficients of the calibration curves, the repeatability and the reproducibility of measurement are presented in Table 4.4.

Table 4.4: Correlation coefficients, repeatability and reproducibility of measurement of analytical method for the determination of PBDEs in water samples. To 100 mL or 300 mL of river water samples containing 35 mg L^{-1} of SPM and 5 mg L^{-1} of HA (PBDEs concentrations below LODs), six BDE congeners at a concentration of 10 or 0.083 ng L^{-1} were added, respectively. The organic phase was concentrated to 25 µL and concentrations of PBDEs determined by GC-ICP-MS using the standard addition calibration method.

Sample volume	**Spike**	**BDE congener**	r^2	**Repeatability RSD (%)**	**Reproducibility RSD (%)**
100 mL	10 ng L^{-1}	BDE 28	0.996	2.9	4.4
		BDE 47	0.997	2.9	5.4
		BDE 100	0.995	3.3	5.3
		BDE 99	0.995	3.4	5.6
		BDE 154	0.999	3.7	4.8
		BDE 153	0.992	3.3	5.7
300 mL	0.083 ng L^{-1}	BDE 28	0.963	6.0	6.9
		BDE 47	0.976	21.3	23.1
		BDE 100	0.965	12.5	13.9
		BDE 99	0.972	19.1	17.1
		BDE 154	0.974	24.8	28.9
		BDE 153	0.953	14.0	15.3

As can be seen from Table 4.4, the correlation coefficients for calibration curves (r^2) for the PBDEs concentration ranges from 1 to 20 ng L^{-1} and from 0.02 to 1 ng L^{-1} are in general better than 0.992 and 0.963, respectively. Good repeatability of measurements, around 3 %, was obtained for a river water sample containing 35 mg L^{-1} of SPM and 5 mg L^{-1} of HA, spiked with the six BDE congeners at a concentration of 10 ng L^{-1}, while the reproducibility of measurement was slightly worse. For river water samples of the same matrix spiked with 0.083 ng L^{-1} of the six BDE congeners, whose sum of 0.498 ng L^{-1} is at EQS level, the repeatability of measurement for particular BDE congeners ranged between 6 and 25 %, whereas, when compared with repeatability, a slightly worse reproducibility of measurement was obtained. The expanded uncertainty at the EQS level was found to be around 40 % (k = 2), which is lower than the minimum performance criteria for water monitoring set by the WFD. To demonstrate that the performance of the developed analytical procedure complies with the requirements set by the WFD, uncontaminated river water to which 35 mg L^{-1} of SPM and 5 mg L^{-1} of HA were added (with PBDEs concentrations below the LODs) and a saline river water sample (3.8 % NaCl) of the same matrix were spiked with 0.025 ng L^{-1} of the six BDE congeners. The concentrations of the spikes were at the LOQ levels for individual PBDEs (see data of Table 4.3). The analytical chromatograms of these samples obtained by GC-ICP-MS are presented in Figure 4.6.

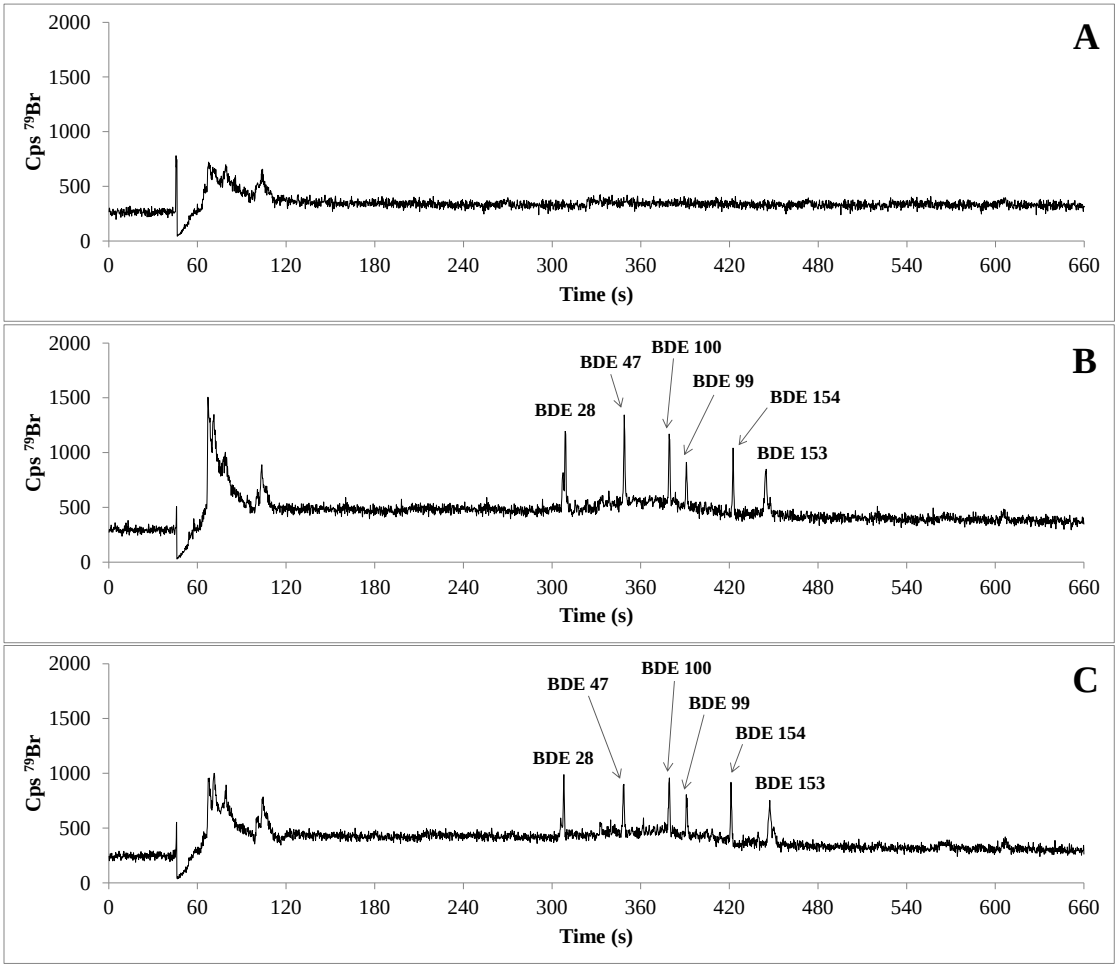


Figure 4.6: Chromatograms of six BDE congeners in the river water sample (containing 35 mg L^{-1} of SPM and 5 mg L^{-1} of HA) (A) and spiked samples of river water (B) and saline river water (C). Samples of river water (A) were spiked with six BDE congeners at 0.025 ng L^{-1} (sample volume 300 mL). The organic phase was concentrated to 25 µL and the GC-ICP-MS procedure applied. Chromatograms were recorded at m/z 79.

From this Figure, it can be seen that the peaks of all the PBDEs analysed at the LOQs concentration levels are well defined in both river water (Figure 4.6B) and saline river water (Figure 4.6C). They are clearly resolved from the baseline of the uncontaminated river water (Figure 4.6A), indicating the high sensitivity of the developed analytical procedure.

4.2.2.4 Analysis of PBDEs in environmental water samples

The newly developed GC-ICP-MS analytical procedure was applied for the determination of PBDEs in several Slovenian river water samples. For the water samples from the Drava river sampled at Brezno, the Krka river at Otočec, Gradaščica at Šujica and Hruševnik rivulet at Hruševo, the concentrations of the six BDE congeners were below the LODs. In addition, seawater samples from the Northern Adriatic were also analysed. A chromatogram of seawater from the Izola Marina is presented in Figure 4.7, while the results of the analyses of sea water samples are presented in Table 4.5.

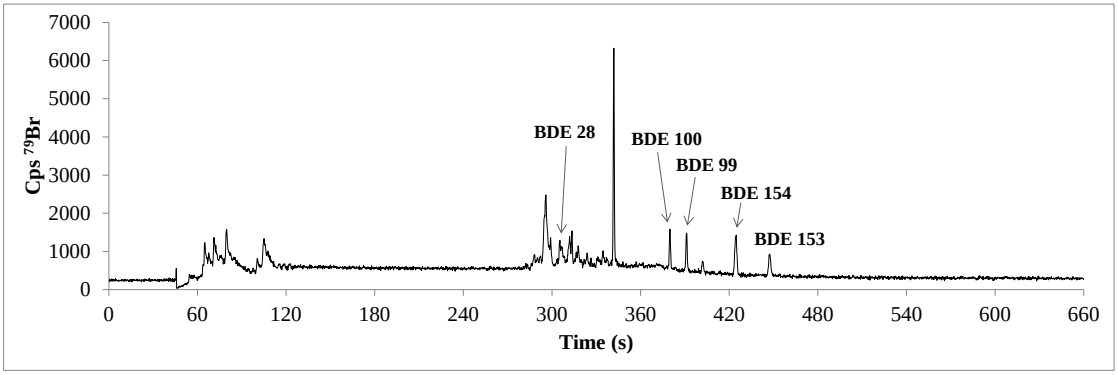


Figure 4.7: Chromatogram of PBDEs in the marine water sample from the Izola Marina (sample volume 300 mL). The organic phase was concentrated to 25 µL and the GC-ICP-MS procedure applied. The chromatogram was recorded at m/z 79.

Table 4.5: Concentrations of PBDEs found in seawater samples from the Northern Adriatic (Slovenia) determined by GC-ICP-MS, using 300 mL of sample. The organic phase was concentrated to 25 µL.

	PBDE congeners listed by WFD						
Sampling site	**BDE 28 (ng L^{-1})**	**BDE 47 (ng L^{-1})**	**BDE 99 (ng L^{-1})**	**BDE 100 (ng L^{-1})**	**BDE 153 (ng L^{-1})**	**BDE 154 (ng L^{-1})**	**ΣPBDE (ng L^{-1})**
Bele skale beach	1.14±0.03	< 0.0054	< 0.0051	0.160±0.005	< 0.0051	< 0.0054	1.3
Strunjan	< 0.0052	< 0.0054	< 0.0051	< 0.0047	0.213±0.007	0.153±0.006	0.366
Izola Dockyard	< 0.0052	< 0.0054	0.117±0.004	0.194±0.006	0.340±0.012	0.227±0.009	0.878
Izola Marina	0.053±0.004	< 0.0054	0.094±0.003	0.074±0.002	0.051±0.002	0.085±0.002	0.357
Port of Koper	0.540±0.016	< 0.0054	0.125±0.004	0.178±0.006	< 0.0051	0.108±0.004	0.951
Portorož Marina	0.169±0.005	< 0.0054	< 0.0051	< 0.0047	< 0.0051	< 0.0054	0.169

4.3 Sewage Sludge Samples

The aim of this part of the doctoral thesis was to develop a simple analytical procedure with minimal sample preparation for sensitive and reliable determination of the six PBDEs in sewage sludge samples by GC-ICP-MS. In order to check the accuracy of the developed analytical procedure, spike recovery test and the species-specific isotope-dilution (ID) GC-ICP-MS analysis were used. Finally, PBDEs were determined in sewage sludge samples from the municipal WWTP collected in years 1998, 2000 and 2014.

4.3.1 Optimization of the extraction procedure

Sewage sludge consists of a complex sample matrix. It is mostly composed of an organic matter (dead biomass). To release the hydrophobic PBDEs from organic and particulate matter, an appropriate digestion/extraction method must be applied. Hence, to develop a very sensitive and reliable analytical procedure for the determination of six PBDEs in sewage sludge by GC-ICP-MS, the parameters which influence the efficiency of their extraction were optimized. For this purpose the influence of different extracting agents (methanol (MeOH), acetic acid (AcOH)/MeOH mixture (3 : 1) and 0.1 mol L^{-1} HCl in MeOH) and subsequent addition of Tris-citrate buffer (co-extracting agent) and iso-octane on the extraction efficiency was studied applying different modes of extraction (mechanical shaking, microwave- and ultrasound-assisted extraction). For quantification of PBDEs a standard addition calibration method was applied. Optimization of the procedure was performed on the sewage sludge sample previously used in the proficiency testing scheme (sampling year 2000) (Van de Veen, et al., 2001).

4.3.1.1 Influence of different extraction agents on extraction efficiency

0.5 g of sludge samples were weighed into a glass reactor vessel and 10 mL of extracting agent (MeOH, AcOH/MeOH (3 : 1) or 0.1 mol L^{-1} HCl in MeOH) spiked with six BDE congeners (BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, and BDE 154) and BDE 77 as an internal standard, in concentration of 48 ng g^{-1} each, were added. Samples in closed glass reactors were mechanically shaken for 2 h. In order to further improve the extraction efficiency, Tris-citrate buffer was added as a co-extracting agent, followed by the addition of 2 mL of iso-octane, and samples mechanically shaken for another 16 h. It was experimentally found that the addition of Tris-citrate buffer also alleviated the separation of organic from the aqueous phase. Nevertheless, after mechanical shaking, dispersed emulsion was present in the organic phase. To separate the emulsion from iso-octane, 25 % KOH in MeOH was added as described in Paragraph *3.4.2.2* and concentrations of PBDEs in the clear organic phase determined by GC-ICP-MS. Data of these experiments are presented in Figure 4.8.

As can be seen, among the three tested extracting agents, analytical signal intensities were the highest for the 0.1 mol L^{-1} HCl in MeOH, indicating that this extracting agent most efficiently releases PBDEs from the sewage sludge sample matrix. Therefore, it was used in all further experiments.

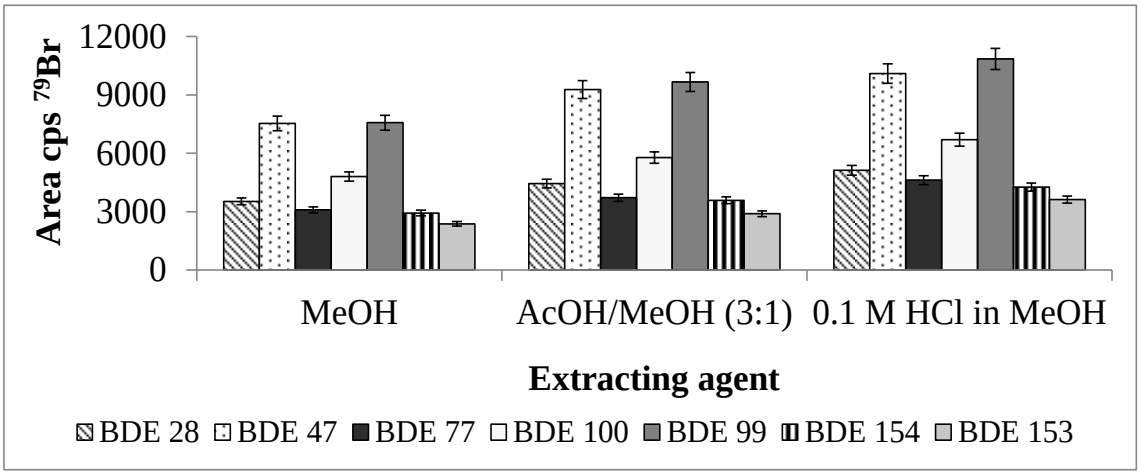


Figure 4.8: Influence of different extracting agents on signal intensities of sewage sludge sample spiked with PBDEs (48 ng g^{-1}) by applying mechanical shaking and speciation analysis with GC-ICP-MS. Results represent the average ± standard deviation of three replicates. Samples were shaken for 2 h. Tris-citrate buffer and iso-octane were added and samples shaken for another 16 h.

4.3.1.2 Influence of different modes of extraction on extraction efficiency

During optimization of the extraction procedure different modes of extraction were tested. Mechanical shaking (20 °C, 300 rpm), microwave-assisted extraction (50 °C or 90 °C, 1200 W) and ultrasound-assisted extraction (20 °C, 240 W) were applied for different time intervals. Microwave- and ultrasound-assisted extraction did not provoke the formation of dispersed emulsion in iso-octane, containing PBDEs. Hence, the clean-up step with 25 % KOH in MeOH was omitted.

4.3.1.2.1 Extraction applying mechanical shaking

To 0.5 g of the spiked sample, 10 mL of 0.1 mol L^{-1} HCl in MeOH was added. Samples were mechanically shaken for 16 h. After that, Tris-citrate buffer and iso-octane were added and sample mechanically shaken again for different time intervals from 0.25 to 6 h. The results are presented in Figure 4.9A.

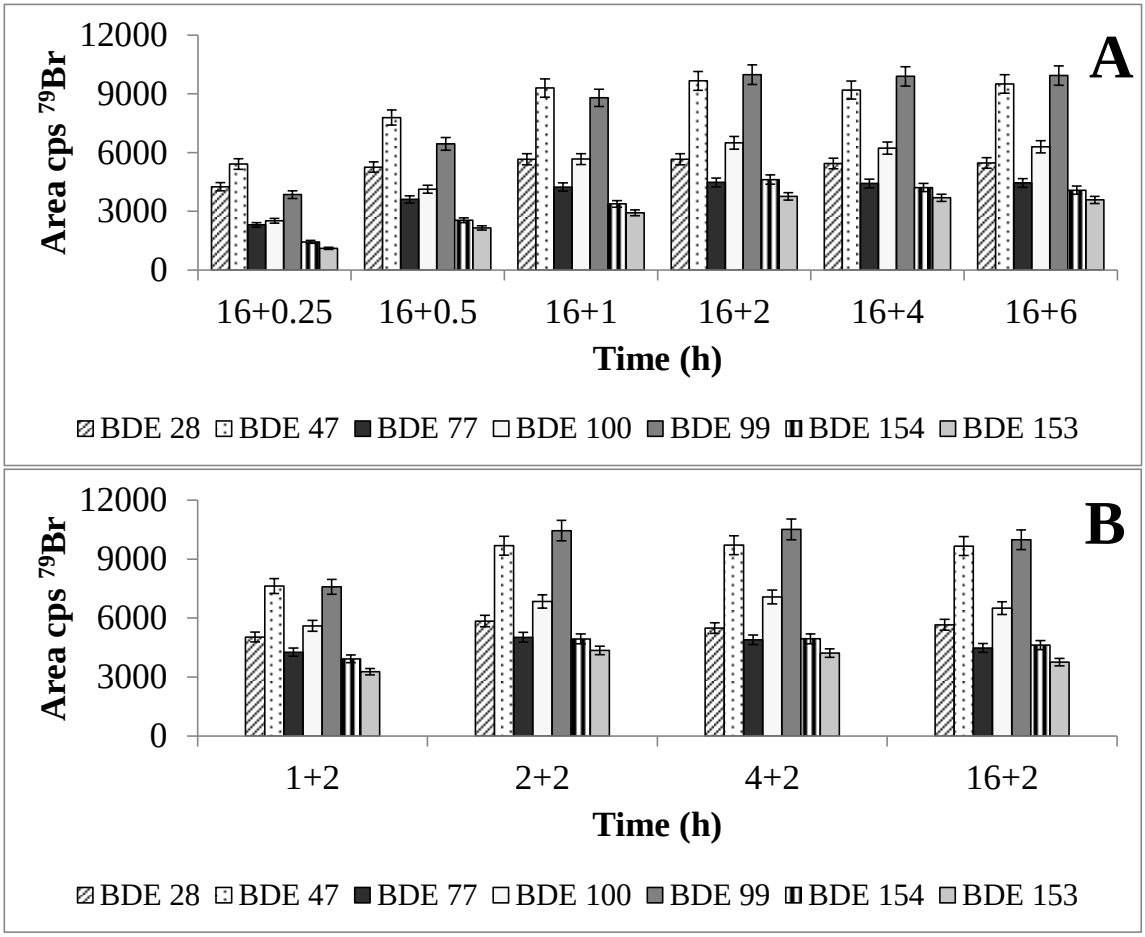


Figure 4.9: Influence of different modes of extraction on signal intensities of sewage sludge sample spiked with PBDEs (48 ng g^{-1}) by applying mechanical shaking and speciation analysis with GC-ICP-MS. Results represent the average ± standard deviation of three replicates.

(A) Samples were shaken with 0.1 mol L^{-1} HCl in MeOH for 16 h. Tris-citrate buffer and iso-octane were added and samples shaken again for different time intervals (0.25-6 h).

(B) Samples were shaken with 0.1 mol L^{-1} HCl in MeOH for different times (1-16 h). Tris-citrate buffer and iso-octane were added and samples shaken again for 2 h.

As can be seen, the signal intensities gradually increased with time and reached their constant maximum values when applying 16 h of shaking with 0.1 mol L^{-1} HCl in MeOH and subsequent shaking with Tris-citrate buffer and iso-octane for the next 2 h. In order to optimize the time of mechanical shaking after the addition of 0.1 mol L^{-1} HCl in MeOH, samples were shaken for different time intervals from 1 to16 h, while the time of subsequent shaking after the addition of the Tris-citrate buffer and iso-octane, was kept constant for 2 h. These results are presented in Figure 4.9B. They clearly demonstrate that by applying mechanical shaking, the optimal time for extraction of PBDEs from sewage sludge with 0.1 mol L^{-1} HCl in MeOH is 2 h, followed by the subsequent shaking with Tris-citrate buffer and iso-octane for the next 2 h.

4.3.1.2.2 Microwave-assisted extraction

0.5 g of spiked samples were supplemented with 10 mL of 0.1 mol L^{-1} HCl in methanol. The closed vessels were subjected to microwave-assisted extraction at 50 and 90 °C for 5 and 10 min. Microwave extraction conditions were as follows: 1200 W, 1 min ramp to 50 °C or 90 °C and hold at that temperature for the next 4 or 9 min. After that, the suspension was transferred into a glass reactor, Tris-citrate buffer and 2 mL of iso-octane were added and the sample mechanically shaken for 2 h. The results are presented in Figure 4.10A.

As evident, the extraction of PBDEs from sewage sludge is more efficient at 90 °C, while signal intensities for 5 min and 10 min extraction are comparable. Thus, 5 min extraction at 90 °C was chosen for the next experiment, where mechanical shaking was replaced with different times (5, 10 or 20 min) of microwave-assisted extraction at the same temperature (90 °C). The microwave extraction conditions for the first run were the same as in the first experiment and the conditions for the second run were as follows: 1200 W, 1 min ramp to 90 °C, held at that temperature for 4, 9 or 19 min. The results are presented in Figure 4.10B. It is evident that signal intensities gradually increased with time but are still much lower than signal intensities in Figure 4.10A. In the continuation of the optimization of the procedure, the next experiment was done in one step. 0.5 g of spiked samples were supplemented with 10 mL of 0.1 mol L^{-1} HCl in methanol, Tris-citrate buffer and iso-octane. The closed vessels were subjected to microwave-assisted extraction at 90 °C for 5, 10, or 20 min. Microwave extraction conditions were as follows: 1200 W, 1 min ramp to 90 °C, held at that temperature for 4, 9 or 19 min. These results are presented in Figure 4.10C. Data from Figure 4.10C clearly demonstrate that this extraction procedure was found to be rather inefficient.

4.3.1.2.3 Ultrasound-assisted extraction

0.5 g of spiked samples were supplemented with 10 mL of 0.1 mol L^{-1} HCl in methanol. The closed glass reactors were first subjected to ultrasound-assisted extraction for 15, 30 or 60 min. After that, Tris-citrate buffer and iso-octane were added. The suspension was again subjected to ultrasound-assisted extraction for 15, 30 or 60 min. Signal intensities were appreciably lower than those obtained by mechanical shaking, regardless of the time of extraction applied (signal intensities were too low to show the data).

From the signal intensities presented in Figures 4.9 and 4.10 it is evident that under optimized extraction procedures, mechanical shaking and microwave-assisted extraction provide comparable results. Due to simpler manipulation of samples, mechanical shaking was applied in further experiments.

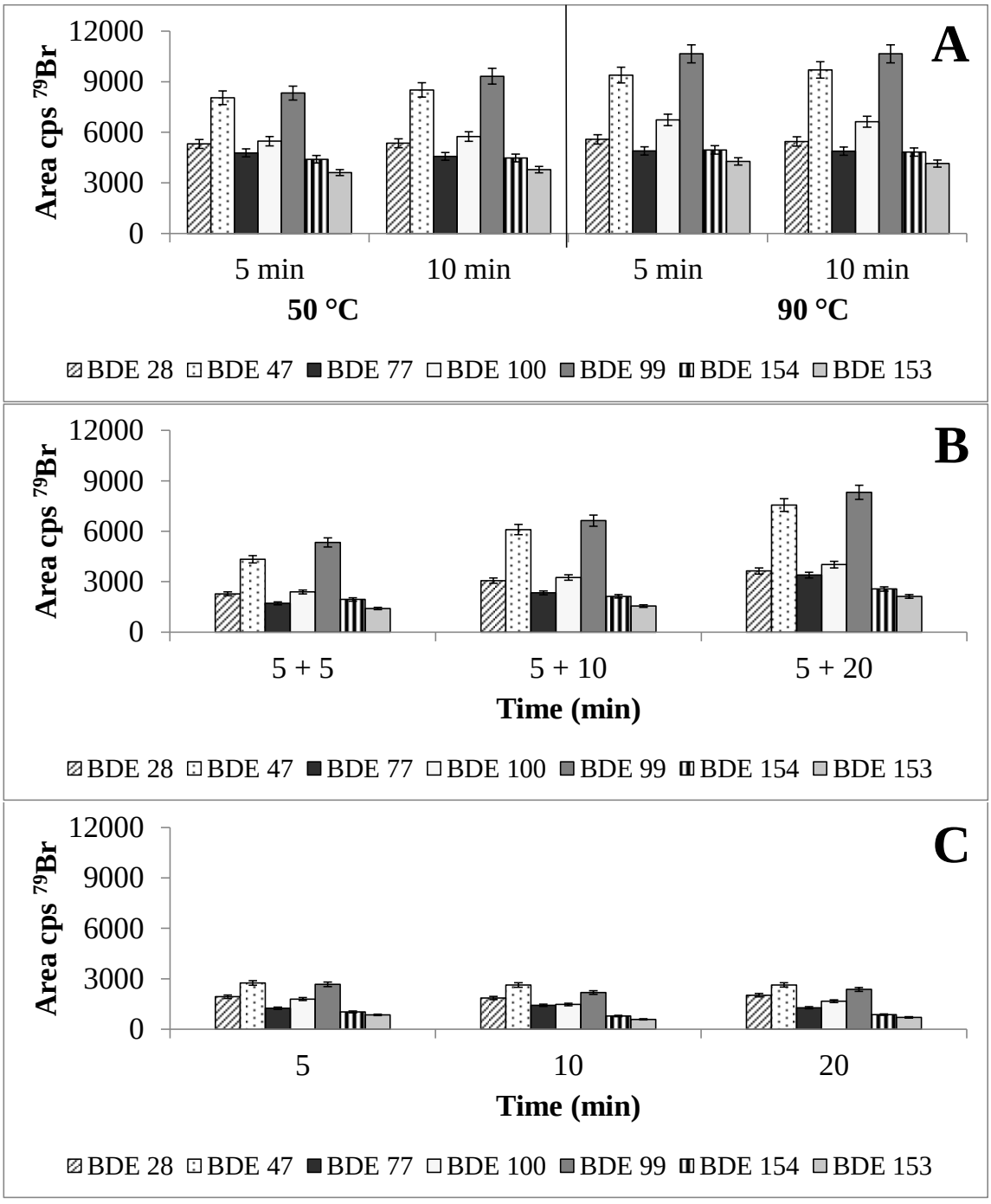


Figure 4.10: Influence of different modes of extraction on signal intensities of sewage sludge sample spiked with PBDEs (48 ng g^{-1}) by applying microwave-assisted extraction and speciation analysis with GC-ICP-MS. Results represent the average ± standard deviation of three replicates.

(A) Samples were supplemented with 10 mL of 0.1 mol L^{-1} HCl in methanol and subjected to microwave-assisted extraction at 50 °C and 90 °C for 5 and 10 min. Tris-citrate buffer and iso-octane were added and samples mechanically shaken for the next 2 h.

(B) Samples were supplemented with 10 mL of 0.1 mol L^{-1} HCl in methanol and subjected to microwave-assisted extraction at 90 °C for 5 min. Tris-citrate buffer and iso-octane were added and microwave-assisted extraction performed again at 90 °C for 5 to 20 min.

(C) Samples were supplemented with 10 mL of 0.1 mol L^{-1} HCl in methanol and 50 mL of Tris-citrate buffer and iso-octane, and subjected to microwave-assisted extraction at 90 °C for 5 to 20 min.

4.3.2 The performances of analytical procedure

The performances of the optimized analytical procedure for simultaneous speciation analysis of six PBDEs in sewage sludge by GC-ICP-MS were evaluated by applying mechanical shaking and extraction procedure as described in paragraph *3.4.2.2.* Typical chromatograms of aqueous standard solution of the mixture of PBDEs, sewage sludge and sewage sludge sample spiked with PBDEs are presented in Figure 4.11. Chromatogram of aqueous standard solution of the mixture of PBDEs is shown for comparison of the retention times.

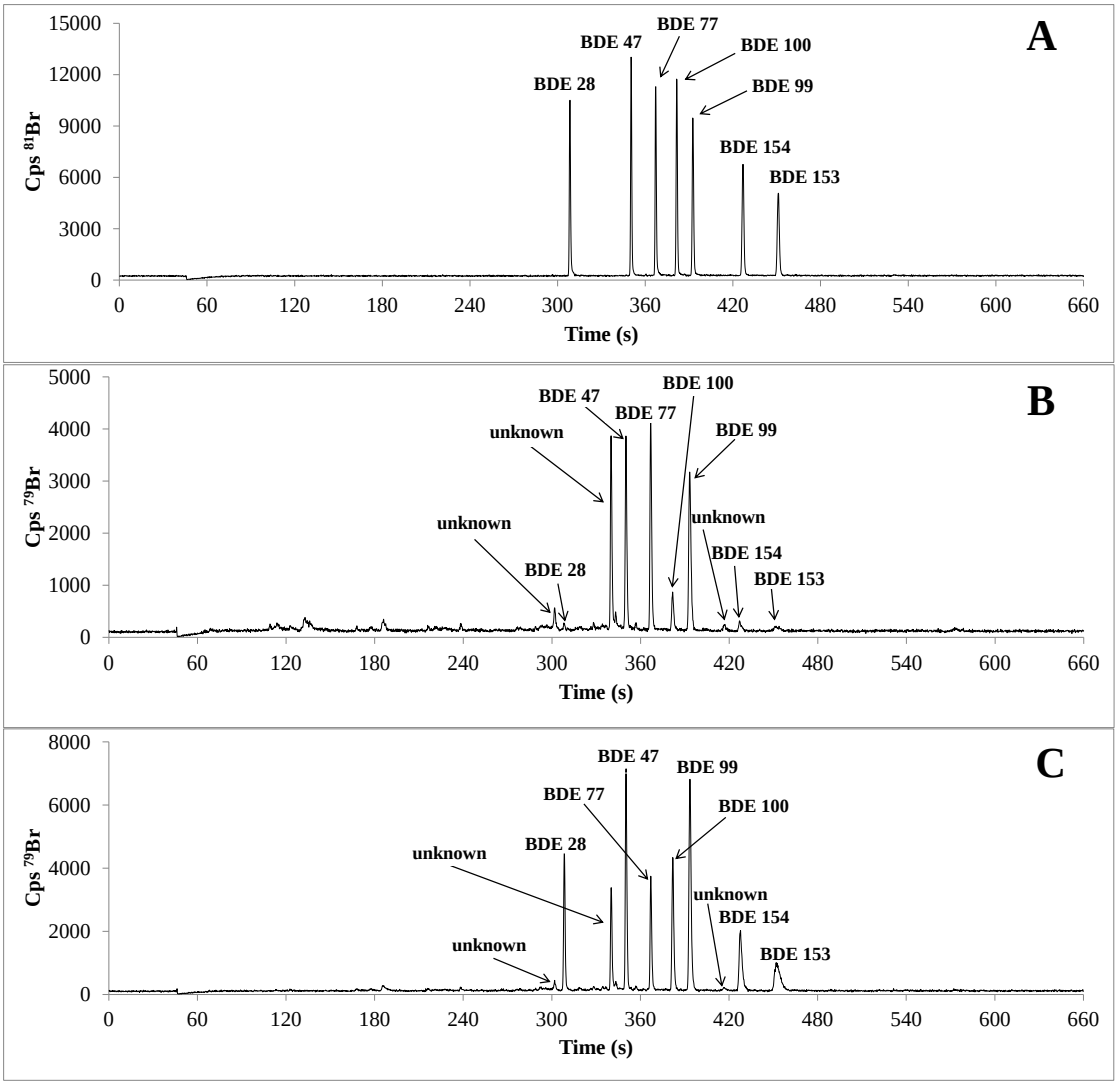


Figure 4.11: Chromatograms of (A) aqueous standard solution of the mixture of six BDE congeners and internal standard BDE 77 (20 ng mL^{-1} each), (B) sewage sludge sample (WTTP Domžale-Kamnik, sampling year 2000) spiked with internal standard BDE 77 (12 ng mL^{-1}) and (C) sewage sludge sample (WTTP Domžale-Kamnik, sampling year 2000) spiked with the mixture of six BDE congeners and internal standard BDE 77 (12 ng mL^{-1} each) by applying GC-ICP and recorded at m/z 79.

As evident from Figure 4.11, six BDE congeners and BDE 77 (internal standard) are well resolved. The same retention times for PBDEs are obtained from the aqueous standard, sewage sludge and spiked sludge sample. In the sewage sludge sample all six PBDEs were detected. In addition, three unidentified peaks were also observed.

4.3.2.1 Quantification of PBDEs and the accuracy check

For the evaluation of efficiency of extraction and accuracy of the developed analytical procedure, 0.5 g of sewage sludge was spiked with 12 ng g^{1} of BDE 28, BDE 100, BDE 153, and BDE 154 and 24 ng g^{-1} of BDE 47 and BDE 99, and the analysis performed by GC-ICP-MS using the standard addition calibration method. The recoveries calculated as the ratio between the added and found PBDE concentrations are presented in Table 4.6.

Table 4.6: Extraction recoveries for PBDEs added to 0.5 g of sewage sludge from WWTP Domžale-Kamnik (sampling year 2000) determined by GC-ICP-MS using the standard addition calibration method. Results represent the mean value of three replicate determinations of a selected BDE with the standard deviation of the measurements.

Sample	**BDE congener**	**Concentration (ng g^{-1})**	**Added (ng g^{-1})**	**Found (ng g^{-1})**	**Recovery (%)**
Sewage sludge sampling year 2000	BDE 28	1.12±0.03	12.0±0.2	12.5±0.4	95±3
	BDE 47	38.6±1.6	24.0±0.5	64±3	102±4
	BDE 99	29.3±0.7	24.0±0.5	54±1	101±2
	BDE 100	8.1±0.2	12.0±0.2	19.5±0.4	97±2
	BDE 153	7.3±0.4	12.0±0.2	20.1±0.8	104±4
	BDE 154	5.3±0.2	12.0±0.2	16.4±0.6	95±4

As evident, the recoveries of spiked samples lay between 95 and 104 %, indicating quantitative extraction of PBDEs from sewage sludge and confirming the accuracy of the developed analytical procedure. The recoveries obtained in the present study are similar to those reported for microwave-assisted extraction (Shin, Svoboda & Falleta, 2007) or better than obtained by accelerated solvent extraction (Gorga, et al., 2013) or Soxlet extraction (Ran, et al., 2013), which require tedious clean-up steps. Since there is no standard reference material available for the determination of PBDEs in sewage sludge samples, the accuracy of the analytical procedure was also verified by comparative analysis applying species-specific ID-ICP-MS. For this purpose ^{81}Br enriched BDEs were used and the PBDEs concentrations determined by ID-GC-ICP-MS using the equations reported by Rodríguez-Gonzales, et al. (2005). These results are presented in Table 4.7.

Table 4.7: Concentrations of PBDEs found in sewage sludge sample from WWTP Domžale-Kamnik, collected in year 2000, determined by GC-ICP-MS using the standard addition calibration and GC-ID-ICP-MS methods. Results represent the mean value of three replicate determinations of a selected BDE with the standard deviation of the measurements.

BDE congener	**BDE standard addition (ng g^{-1} dw)**	**BDE GC-ID-ICP-MS (ng g^{-1} dw)**
BDE 28	1.12 ± 0.03	1.010 ± 0.015
BDE 47	38.6 ± 1.6	38.8 ± 0.8
BDE 99	29.3 ± 0.7	29.4 ± 0.4
BDE 100	8.1 ± 0.2	8.0 ± 0.1
BDE 153	7.3 ± 0.4	7.6 ± 0.2
BDE 154	5.3 ± 0.2	5.5 ± 0.1

It can be seen that the difference between the two methods for BDE 28, BDE 47, BDE 99, and BDE 100 did not exceed 1 %, and was around 4 % for BDE 153 and BDE 154, which additionally confirmed the accuracy of the analytical procedure. It may be concluded that the developed analytical procedure is suitable for its intended use for the determination of six PBDEs in sewage sludge. Furthermore, in comparison to the regular coupling of GC to MS detection, the developed GC-ICP-MS procedure requires only minimal sample preparation and a very simple clean-up step.

4.3.2.2 Limits of detection and quantification, linearity, repeatability and reproducibility of measurements

Limits of detection (LODs) and quantification (LOQs) for the analytical procedure were calculated on the basis of the standard addition method calibration curves. To estimate the linearity of the calibration curves sewage sludge was spiked with six PBDEs in the concentration range from 1 to100 ng g^{-1} of each BDE. For the evaluation of the repeatability of measurements six independent sewage sludge samples were spiked with six PBDE congeners (48 ng g^{-1}) and were analysed on the same day. The concentrations of the PBDEs were determined by the standard addition calibration method. After seven days the same experiment was repeated by a different analyst, applying the same analytical procedure to estimate the reproducibility of measurement. The results for the repeatability and reproducibility were calculated as relative standard deviation (RSD). Data of the performances of the analytical procedure are presented in Table 4.8.

Table 4.8: LODs, LOQs, correlation coefficients, repeatability and reproducibility of measurements of the analytical method for the determination of six PBDEs in sewage sludge by GC-ICP-MS using the standard addition calibration method.

BDE congener	**LOD (ng g^{-1} dw)**	**LOQ (ng g^{-1} dw)**	r^2	**Repeatability RSD (%)**	**Reproducibility RSD (%)**
BDE 28	0.209	0.696	0.999	3.1	3.9
BDE 47	0.216	0.721	0.993	4.3	4.7
BDE 99	0.182	0.608	0.997	2.3	2.5
BDE 100	0.195	0.649	0.999	2.2	3.2
BDE 153	0.302	1.01	0.990	4.4	5.7
BDE 154	0.249	0.831	0.998	3.9	4.9

As evident, low LODs for particular BDE ranging from 0.2 to 0.3 ng g^{-1} and LOQs from 0.6 to 1.0 ng g^{-1}, enable sensitive determination of PBDEs in sewage sludge. Good linearity of measurement was obtained with correlation coefficients for calibration curves (r^2) for all PBDEs better than 0.990. It can be further seen that very good repeatability and reproducibility of measurements were also obtained (2.2 – 5.7 %).

4.3.3 Analysis of PBDEs in sewage sludge from wastewater treatment plant

The developed GC-ICP-MS analytical procedure was applied for the determination of PBDEs in sewage sludge samples from WWTP Domžale-Kamnik, Slovenia, collected in years 1998, 2000 and 2014. These results are presented in Table 4.9.

Table 4.9: Concentrations of PBDEs found in sewage sludge samples from WWTP Domžale-Kamnik determined by GC-ICP-MS.

Sampling year	**BDE congener (ng g^{-1} dw)**					
	BDE 28	BDE 47	BDE 99	BDE 100	BDE 153	BDE 154
1998	< 0.209	49.9±2.1	66.6±1.5	13.4±0.3	9.6±0.4	4.7±0.2
2000	1.12±0.03	38.6±1.6	29.3±0.7	8.1±0.2	7.3±0.4	5.3±0.2
2014	1.40±0.04	20.1±0.9	12.7±0.3	6.4±0.1	4.2±0.2	3.11±0.03

Concentrations of PBDEs calculated on a dry weight ranged between below LOD (BDE 28) and 49.9 ng g^{-1} (BDE 47). The most abundant BDE congeners in samples analysed were BDE 47 and BDE 99, which is in accordance with the typical profile usually found in sewage sludge samples. With the exception of BDE 28, concentrations of other BDE congeners decreased with time. The decreasing concentration changes are reasonable due to regulatory actions in 2004 and reduced usage of PBDEs in the society. The concentrations of PBDEs from the present study are of the same order of magnitude as those reported in the last 15 years for European countries, but lower than those determined in the USA. Concentration levels (ng g $^{-1}$ dw) of PBDEs in sewage sludge among countries in the last 15 years are presented in Table 1.4.

4.4 Fish and Mussel Samples

The goal of this part of the doctoral thesis was to develop a simple analytical procedure with minimal sample preparation for a sensitive and reliable determination of the six PBDEs in fish and mussel samples by GC-ICP-MS. The accuracy of the analytical procedure was checked by analyzing the NIST SRM 1946 (Lake Superior fish tissue) and the NIST SRM 2974a (freeze-dried mussel tissue) by spike recovery test and by applying species-specific isotope-dilution (ID) GC-ICP-MS quantification. To demonstrate the applicability of the GC-ICP-MS analytical procedure developed, PBDEs were determined in mussels and fish samples from the Northern Adriatic Sea and fish samples from the Sava River.

4.4.1 Optimization of the extraction procedure

The main difference between environmental (fresh or sea waters, soils or dust) and biological (fish or mussel) sample matrices is in the higher content of organic matter, proteins and lipids in the latter. Hence, to release the hydrophobic PBDEs from samples with such a complex matrix, an appropriate digestion/extraction method must be applied. For this purpose, the influence of different extracting agents (25 % aqueous solution of TMAH, 0.5 mol L^{-1} acetic acid in MeOH and 0.1 mol L^{-1} HCl in MeOH) and the subsequent addition of the Tris-citrate buffer (co-extracting agent) and iso-octane on the extraction efficiency was studied when applying different modes of extraction (mechanical shaking, microwave- and ultrasound-assisted extraction). A fast and minor solvent consumption clean-up step, which includes passing the extract through the column filled with Florisil, was also established in order to remove lipids, which can damage the GC column. Optimization of the analytical method was performed on freeze-dried mussel tissue reference material SRM 2974a with certified or referenced values for all six PBDEs. 0.25 g of SRM 2974a was weighed into a glass reactor vessel and spiked (200 ng g^{-1}, each) with BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, BDE 154 and BDE 77 as an internal standard. The spiking procedure consisted of the addition of a standard in acetone to a sample, homogenization of the resulting slurry and keeping samples in the hood until complete evaporation of the acetone.

4.4.1.1 Influence of different extracting agents on the extraction efficiency

The most efficient extracting agents were chosen for the extraction of PBDEs from mussels after a series of experiments in which 10 mL of a given extracting agent (25 % aqueous solution of TMAH, 0.5 mol L^{-1} acetic acid in MeOH or 0.1 mol L^{-1} HCl in MeOH) was added into glass reactor vessels containing 0.25 g of the spiked freeze-dried mussel tissue. The sample was mechanically shaken for 2 h. After that, 50 mL of Tris-citrate buffer, as the co-extracting agent, aiming to further improve the extraction efficiency, and 2 mL of iso-octane were simultaneously added and the sample mechanically shaken for another 2 h. It was experimentally proven that the addition of Tris-citrate buffer facilitates the extraction of PBDEs from the matrix and also substantially alleviates the separation of organic from the aqueous phase (Novak, Zuliani, Milačič & Ščančar, 2014). Nevertheless, after mechanical shaking, the dispersed emulsion was still partly present in the organic phase. To separate the emulsion from iso-octane, 25 % KOH in MeOH was added as described in Paragraph 3.4.2.3 and concentrations of PBDEs in the clear organic phase determined by GC-ICP-MS. Data of these experiments are presented in Figure 4.12.

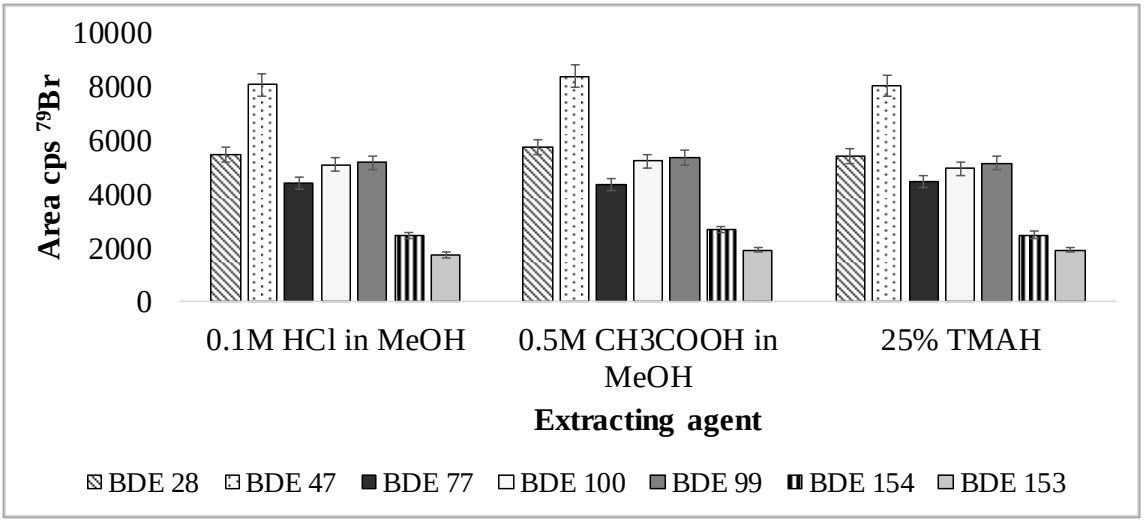


Figure 4.12: The influence of different extracting agents on signal intensities of mussel tissue sample (NIST SRM 2974a) spiked with PBDEs (200 ng g^{-1}) by applying mechanical shaking and speciation analysis with GC-ICP-MS. Results represent the average ± standard deviation of three replicates. The samples were shaken for 2 h. Tris-citrate buffer and iso-octane were added and samples shaken for another 2 h.

As can be seen from Figure 4.12, (analysis of spiked freeze-dried mussel tissue), similar analytical signal intensities, which indicate the extraction efficiency, for all the BDE congeners, including internal standard BDE 77 were obtained regardless the extracting agent applied. On the contrary, in the analysis of fresh fish samples, significant variations of analytical signal intensities were observed. These differences depend on a given extracting agent used and were mainly the consequence of the formation of thick slurry or less efficient separation between organic and aqueous phase that prohibited us to collect optimal quantity of organic phase in the case of 0.5 mol L^{-1} acetic acid in MeOH or 0.1 mol L^{-1} HCl in MeOH, respectively. Thus, on the basis of experimental observations and results, it was concluded that in order to apply the developed analytical procedure for both, freeze-dried and fresh samples, the 25 % aqueous solution of TMAH as an extracting agent is the most efficient.

4.4.1.2 Influence of different modes of extraction on extraction efficiency

Three modes of extraction, namely mechanical shaking (20 °C, 300 rpm), microwave-assisted extraction (40 °C, 60 °C or 90 °C, 15 % of 1600 W) and ultrasound-assisted extraction (20 °C, 240 W) were applied for different time intervals and their performances compared during optimization of the extraction of PBDEs from mussel and fish samples.

4.4.1.2.1 Extraction applying mechanical shaking

To 0.25 g of spiked SRM 2974a (freeze-dried mussel tissue sample) 10 mL of 25 % aqueous solution of TMAH was added. The sample was, in the first step, mechanically shaken (20 °C, 300 rpm) for 2 h. After that, in the second step, the Tris-citrate buffer (50 mL) and iso-octane (2 mL) were added and the sample shaken for different times in an interval from 0.25 to 16 h. Results of these experiments are presented in Figure 4.13A.

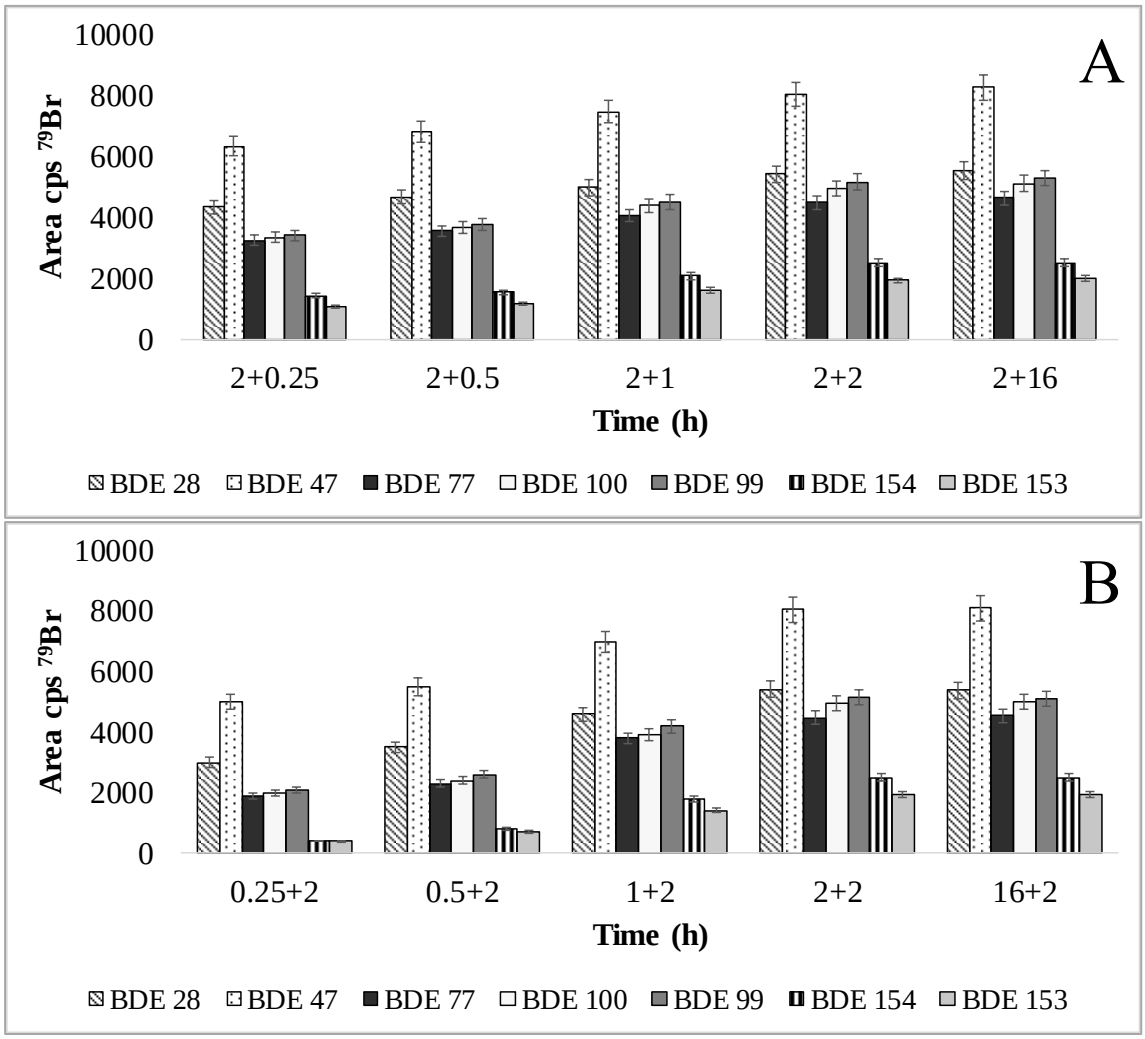


Figure 4.13: Influence of different modes of extraction on signal intensities of the mussel tissue sample (NIST SRM 2974a) spiked with PBDEs (200 ng g-1) by applying mechanical shaking and speciation analysis with GC-ICP-MS. Results represent the average ± standard deviation of three replicates.

(A) Samples were shaken with 10 mL of 25 % aqueous solution of TMAH for 2 h. Tris-citrate buffer and iso-octane were added and samples shaken again for different time intervals (0.25-6 h).

(B) Samples were shaken with 10 mL of 25 % aqueous solution of TMAH for different times (0.25-16 h). Tris-citrate buffer and iso-octane were added and samples shaken again for 2 h.

Results in Figure 4.13A show that constant maximum values of analytical signal intensities for all the PBDEs analysed were reached when the sample was first shaken with 25 % aqueous solution of TMAH for 2 h, followed by shaking in Tris citrate buffer and iso-octane for at least next 2 h. Similar, as for the second step, the time of extraction was optimized also for the first step of extraction, varying it in the time interval from 0.25 to 16 h in the first step, while keeping the time of extraction in the second step constant (2 h). These results are presented in Figure 4.13B, and show that the optimal time of the

mechanical shaking in the first step of extraction is 2 h. Thus, the optimal extraction time of the two-step mechanical shaking extraction method was 2 h (shaking with 25 % aqueous solution of TMAH) + 2 h (subsequent shaking after the addition of Tris-citrate buffer and iso-octane).

4.4.1.2.2 Ultrasound-assisted extraction

The closed glass reactors, which contained 0.25 g of spiked freeze-dried mussel tissue (SRM 2974a) sample and 10 mL of 25 % aqueous solution of TMAH were subjected to ultrasound-assisted extraction for 15, 30 or 60 min. Then, the Tris-citrate buffer and iso-octane were added and the sample mechanically shaken for 2 h in the second step. Corresponding results that are presented in Figure 4.14A demonstrate that the analytical signal intensities were increased with the time and become constant when 30 min of ultrasound-assisted extraction was applied. The maximum values of analytical signal intensities were slightly higher than those obtained after the extraction with mechanical shaking.

4.4.1.2.3 Microwave-assisted extraction

As in other two modes of extraction, microwave-assisted extraction was carried out in two steps. In the first step, closed vessels containing 0.25 g of spiked freeze-dried mussel tissue (SRM 2974a) sample and 10 mL of 25 % aqueous solution of TMAH were subjected to microwave-assisted extraction at different temperatures (40 °C, 60 °C or 90 °C). The program used was as follows: power 15 % of 1600 W, ramp to selected final temperature (40 °C, 60 °C or 90 °C) in 1 min and hold at that temperature for 3 min. After cooling, the suspensions were transferred into glass reactors, the Tris-citrate buffer and iso-octane added and samples mechanically shaken for 2 h. Compared to the analytical signal intensities presented in Figure 4.14B, it can be seen that at 40 °C the extraction of the PBDEs from the sample matrix is slightly less efficient than that at 60 °C or 90 °C.

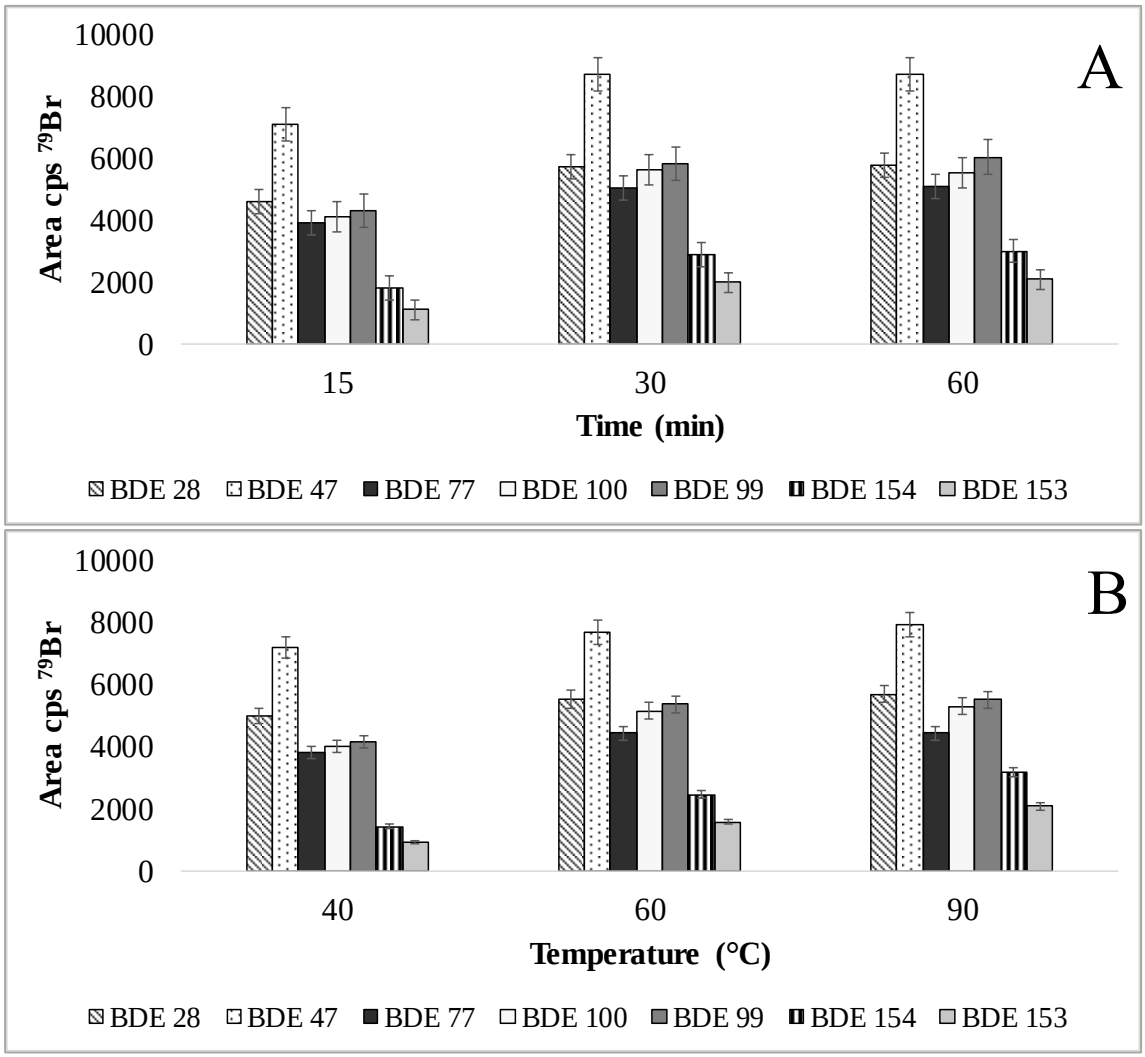


Figure 4.14: Influence of different modes of extraction on signal intensities of the mussel tissue sample (NIST SRM 2974a) spiked with PBDEs (200 ng g^{-1}) by applying ultrasound- and microwave-assisted extraction and speciation analysis with GC-ICP-MS. Results represent the average ± standard deviation of three replicates.

(A) Samples were supplemented with 10 mL of 25 % aqueous solution of TMAH and subjected to ultrasound-assisted extraction for 15, 30 and 60 min. Tris-citrate buffer and iso-octane were added and samples mechanically shaken for the next 2 h.

(B) Samples were supplemented with 10 mL of 25 % aqueous solution of TMAH and subjected to microwave-assisted extraction at 40, 60 and 90 °C for 4 min. Tris-citrate buffer and iso-octane were added and samples mechanically shaken for the next 2 h.

It can be deduced that among all the compared modes of extraction, microwave-assisted extraction is the least and the ultrasound-assisted the most efficient for the extraction of PBDEs from freeze-dried mussel sample matrix. This is consistent with the outcomes from optimization of the mode of extraction of PBDEs from sewage sludge (paragraph 4.3.1.2) where the efficiency of microwave extraction was found to be inferior to its compared alternatives (mechanical shaking, ultrasound-assisted extraction).

Once the extracting agents and relevant extracting conditions were optimized for mussels (SRM 2974a), it was experimentally proven that among the extracting agents tested for mussels, only 25 % aqueous solution of TMAH can be efficiently applied also for the extraction of PBDEs from the fresh fish sample matrix (NIST SRM 1946). When different modes of extraction were applied under their corresponding optimal parameters, experimental results showed that PBDEs can be extracted from this sample matrix by mechanical shaking or ultrasound-assisted extraction with almost identical efficacy, while microwave-assisted extraction was less efficient. Since ultrasound-assisted extraction applied in the first step exhibits several advantages, such as being the most robust, time efficient and the easiest for handling, the extraction method that consists of 30 min of ultrasound-assisted extraction with 25 % aqueous solution of TMAH in the first step, which is followed by 2 h of mechanical shaking with the Tris-citrate buffer and iso-octane in the second step, was chosen as the optimal for lyophilised mussel and fresh fish sample matrices.

4.4.2 Performance of the analytical procedure

The performance of the optimised analytical procedure (paragraph 3.4.2.3) for the simultaneous speciation analysis of six BDE congeners in freeze-dried mussel tissues (SRM 2974) and fresh fish tissue (SRM 1946a) was evaluated by the determination of its limits of detection and quantification, linearity, repeatability and reproducibility of measurement, and by the accuracy check.

4.4.2.1 Limits of detection and quantification, linearity, repeatability and reproducibility of the measurements

The limits of detection (LODs) and limits of quantification (LOQs) for the determination of six BDE congeners were calculated on the basis of three times (3s) and ten times the standard deviation (10s), respectively, of the signal intensities of the blank sample, measured in eight replicates. To estimate the linearity of the calibration curves SRM 1946a was spiked with six PBDEs in concentration range from 0.05 to 5 ng g^{-1} for each BDE. The repeatability of the determination was evaluated by the relative standard deviation (RSD) of six consecutive analyses of this SRM on the same day. The concentrations were determined by the standard addition calibration method. The reproducibility of the determination was calculated from a set of six analyses of the same sample over a period of 7 days. The data are presented in Table 4.10.

Table 4.10: LODs, LOQs, correlation coefficients, repeatability and reproducibility of measurements of the analytical method for the determination of six PBDEs in SRM 1946a (fresh fish tissue) by GC-ICP-MS using the standard addition calibration method.

BDE congener	**LOD (ng g^{-1} ww)**	**LOQ (ng g^{-1} ww)**	r^2	**Repeatability RSD (%)**	**Reproducibility RSD (%)**
BDE 28	0.003	0.011	0.999	3.2	8.3
BDE 47	0.006	0.019	0.999	3.2	5.7
BDE 99	0.008	0.027	0.997	3.0	8.3
BDE 100	0.004	0.013	0.999	3.3	7.8
BDE 153	0.005	0.015	0.997	5.0	8.1
BDE 154	0.009	0.030	0.998	3.4	7.3

Under the optimized analytical procedure, low LODs and LOQs were obtained for all the six BDE congeners ranging from 0.003 to 0.009 ng g^{-1} and from 0.011 to 0.030 ng g^{-1}, respectively. Correlation coefficient for calibration curves (r2) for all the PBDEs determined were better than 0.997. Considering the complex matrix of fish tissues, the good repeatability of measurements was obtained for PBDEs analyzed (RSDs from 3.0 to 5.0 %), while the reproducibility of measurements was slightly worse (RSDs from 5.7 to 8.3 %).

4.4.2.2 Accuracy check

The efficiency of the extraction and accuracy of the analytical procedure applied were evaluated by analysis of the standard reference materials NIST SRM 2974a, and NIST SRM 1946. The samples were spiked with the same concentration of each BDE congener as certified and the analysis performed by GC-ICP-MS using the standard addition method for quantification. The recoveries calculated as the ratio between the certified and determined PBDE concentrations are presented in Table 4.11.

Table 4.11: Extraction recoveries for PBDEs from SRM 2974a and SRM 1946 determined by GC-ICP-MS using the standard addition calibration method. Results represent the mean value of three replicate determinations of a selected BDE with the standard deviation of the measurements.

Sample	**BDE congener**	**Certified/Referenced**[a] **(ng g^{-1})**	**Found (ng g^{-1})**	**Relative Recovery (%)**
SRM 2974a (freeze-dried mussel tissue)	BDE 28	0.905±0.051	0.953±0.030	105±3
	BDE 47	14.3±2.8[a]	13.7±0.4	96±3
	BDE 99	4.78±0.24	4.81±0.16	101±3
	BDE 100	2.83±0.56[a]	2.79±0.08	99±3
	BDE 153	0.201±0.014	0.195±0.010	97±5
	BDE 154	0.297±0.014[a]	0.303±0.010	102±3
SRM 1946 (frozen fish tissue)	BDE 28	0.742±0.0272	0.769±0.025	104±3
	BDE 47	29.9±2.3	28.2±0.9	94±3
	BDE 99	18.5±2.1	18.0±0.6	97±3
	BDE 100	8.57±0.52	8.29±0.25	97±3
	BDE 153	2.81±0.41	2.74±0.14	98±5
	BDE 154	5.77±0.80	5.85±0.20	101±3

It can be seen from Table 4.11 that the recoveries lay between 94 and 105 %, indicating high extraction efficiency. It can be further seen that the determined concentrations of PBDEs are in good agreement with the certified values. The accuracy of the analytical procedure was also verified by the species-specific ID-GC-ICP-MS method applied for the quantification. For this purpose ^{81}Br enriched BDEs were used and the PBDEs concentrations determined by ID using the procedure and equations reported by Rodríguez-González, et al. (2005). These results are presented in Table 4.12. They indicate that the differences between the results determined by GC-ICP-MS and those determined by ID-GC-ICP-MS did not exceed 6 %, further confirming the accuracy of the analytical procedure.

Table 4.12: Extraction recoveries for PBDEs from SRM 2974a and SRM 1946 determined by the ID-GC-ICP-MS method. Results represent the mean value of three replicate determinations of a selected BDE with the standard deviation of the measurements.

Sample	**BDE congener**	**Certified/Reference[a] (ng g^{-1})**	**Found (ng g^{-1})**	**Relative Recovery (%)**
SRM 2974a (freeze-dried mussel tissue)	BDE 28	0.905±0.051	0.929±0.030	103±3
	BDE 47	14.3±2.8[a]	13.9±0.4	97±3
	BDE 99	4.78±0.24	4.85±0.15	102±3
	BDE 100	2.83±0.56[a]	2.76±0.08	98±3
	BDE 153	0.201±0.014	0.200±0.010	99±5
	BDE 154	0.297±0.014[a]	0.298±0.010	100±3
SRM 1946 (frozen fish tissue)	BDE 28	0.742±0.0272	0.727±0.023	98±3
	BDE 47	29.9±2.3	28.7±0.9	96±3
	BDE 99	18.5±2.1	17.6±0.6	95±3
	BDE 100	8.57±0.52	8.66±0.27	101±3
	BDE 153	2.81±0.41	2.92±0.14	104±5
	BDE 154	5.77±0.80	5.89±0.20	102±3

Typical chromatograms corresponding to the working standard solution (60 ng L^{-1}) of all the PBDEs analyzed, which was used to depict retention time of a given BDE, the NIST SRM 2974a, and the NIST SRM 1946 are presented in Figure 4.15 as Figure 4.15A, B and C, respectively. It can be seen that the retention times obtained for the PBDEs standard solution match those for both NIST standards. The chromatographic peaks are well resolved, indicating also the high selectivity of the analytical procedure developed.

On the basis of the results presented in this paragraph it can be concluded that the developed analytical procedure is suitable for its intended use (the determination of six PBDEs in mussel and fish samples, freeze-dried or fresh). When compared to the GC-MS procedures cited in the literature, it is characterized by faster sample preparation, simpler clean-up step, lower solvent consumption, very good recoveries and low LODs.

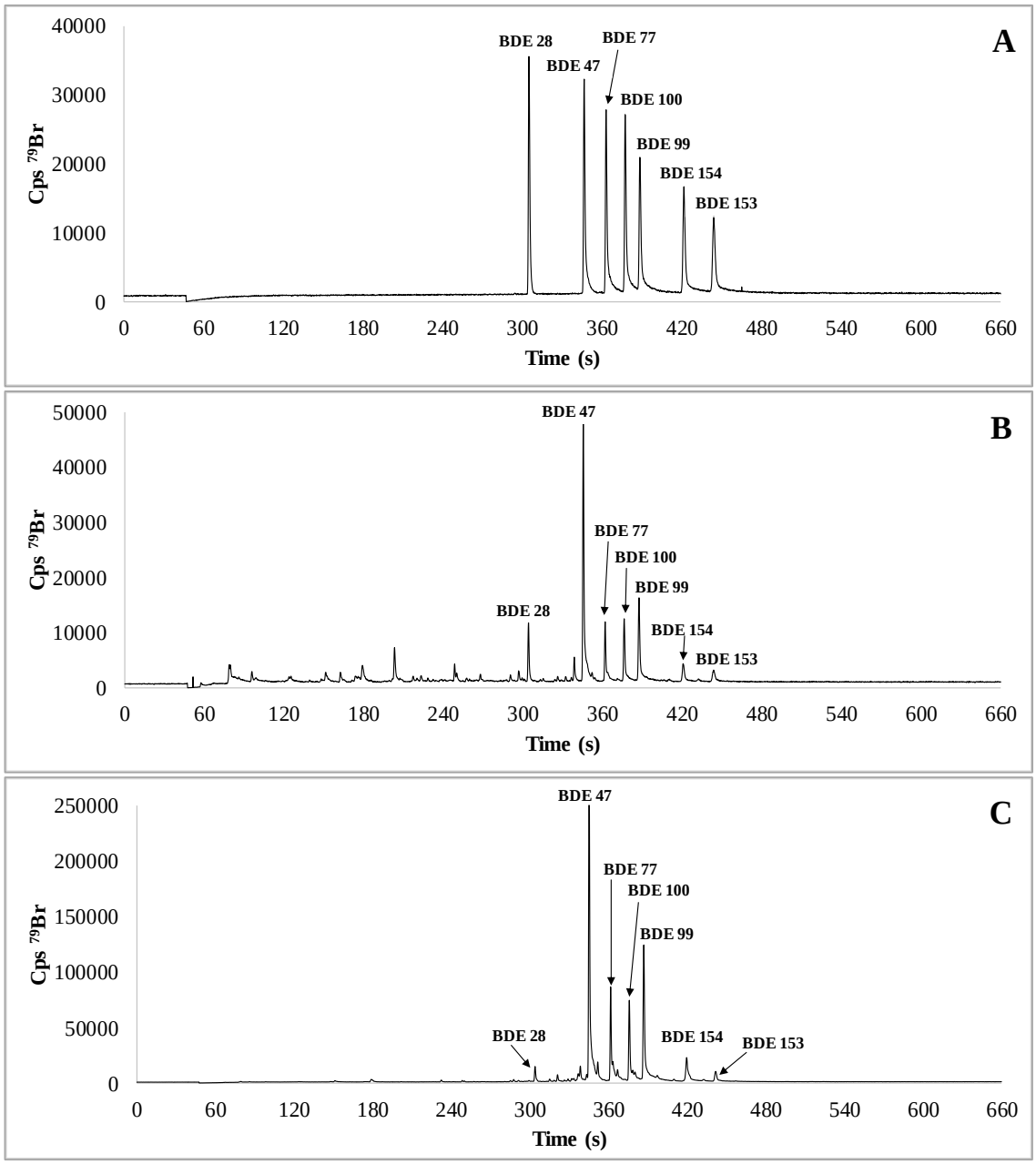


Figure 4.15: Chromatograms of (A) the mixture of six BDE congeners and internal standard BDE 77 in isooctane (60 ng mL^{-1} each), (B) the freeze-dried mussel tissue sample (NIST SRM 2974a) spiked with internal standard BDE 77 and (C) the frozen fish tissue homogenate sample (NIST SRM 1946) spiked with internal standard BDE 77. The organic phase of the samples was concentrated to 30 µL and the GC-ICP-MS procedure applied. Chromatograms were recorded at m/z 79.

4.4.3 Analysis of PBDEs in blue mussels and fish samples from the Northern Adriatic Sea and fish samples from the Sava River

In order to confirm the feasibility of the newly developed GC-ICP-MS analytical procedure for the determination of the content of PBDEs in fish and mussels, blue mussels and fish samples from the Northern Adriatic Sea (Slovenia) and chub river fish samples from the Sava River taken at different locations (Slovenia, Croatia, and Serbia) were analysed. These samples are commonly analysed in biomonitoring of particular toxic substances in the aquatic environment or to control the food safety. All the samples analysed were collected in 2015. The results are presented in Table 4.13.

Table 4.13: Concentrations of PBDEs in fish and mussel samples from the Northern Adriatic Sea and from the Sava River determined by GC-ICP-MS.

	Location	**Species**	**BDE congener (ng g^{-1} ww)**					
			BDE 28	**BDE 47**	**BDE 99**	**BDE 100**	**BDE 153**	**BDE 154**
The Sava River	Litija	chub	0.036	0.50	< lod	0.12	< lod	< lod
	Čatež	chub	< lod	0.19	< lod	0.09	< lod	< lod
	Zagreb	chub	0.036	0.54	0.047	0.12	< lod	< lod
	Beograd	chub	0.020	0.25	< lod	0.008	< lod	<lod
The Northern Adriatic Sea	Slovenia	sardine	0.11	0.53	0.11	0.080	< lod	< lod
		seabass	0.05	0.18	0.109	0.102	0.060	0.086
		whiting	0.07	0.14	0.069	0.068	0.034	0.065
Local market	Slovenia	blue mussel	0.03	0.09	0.05	0.07	< lod	< lod

The PBDEs concentrations in mussels and fish samples calculated on a wet weight (ww) ranged from below the LOD to 0.54 ng g^{-1} for a given BDE congener, and from 0.24 to 0.83 ng g^{-1} for the PBDE, respectively. All the six PBDE congeners listed in the WFD were detected, confirming their presence in the aquatic environment, from which samples were taken. This is the first evidence of the presence of PBDEs in fish from this part of Europe. The concentrations of the PBDEs from the present study were of comparable magnitude of order or lower than those reported in the literature for other European countries. Among the PBDEs determined, BDE 47 was the most abundant. Its concentrations ranged from 0.09 and 0.54 ng g^{-1} ww. Beside BDE 47, BDE 100 and BDE 99 were commonly detected. Such pattern of concentration of different BDE congeners was observed also by other authors whose results on the PBDEs concentration in fish (ng g^{-1} ww) from different sites in Europe are summarized in Table 4.14.

Our first results suggest that PBDEs are ubiquitous in the aquatic environment in this part of Europe. However, to evaluate the extent of pollution by PBDEs, more fresh and marine water biological samples, including fish and mussels from regular monitoring programs, should be analysed, especially since the PBDEs concentration in the fish samples analysed was above the environmental quality standard (EQS) for biological samples

(EQSbiota for the sum of BDE congeners 28, 47, 99, 100, 153 and 154 is set to be lower than 0.0085 ng g^{-1}).

Table 4.14: Comparison of 6PBDE (BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, BDE 154) concentrations (ng g^{-1} ww) in fish samples among European countries in last 18 years. All the compared concentrations were determined in fish muscle tissues.

Location	**Sampling year**	**Species**	**$\sum_6$PBDE (ng g^{-1} ww)**	**Reference**
Finland Sea	2007-2008	farmed fish	0.9 - 1.7	Souminen et al. (2011)
Baltic Sea	2004-2006	herring, salmon	1.2 - 2.5	Szlinder-Richert et al. (2010)
Belgium Sea	2003	plaice, whiting	0.06 - 6.1	Voorspoels et al. (2003)
German rivers	2013	bream	0.14 – 18	Lohmann et al. (2015)
English rivers	2007-2011	roach	2 – 44	Jürgens et al. (2013)
Czech Rep. Elbe River	2002-2003	chub, bream, trout	2 – 18	Pulkrabova et al. (2007)
Norwegian lakes	1998-2004	brown trout	0.3 – 23	Mariussen et al. (2008)
Italy	2011-2012	halibut, trout	0.03 - 0.5	Martellini et al. (2016)

Chapter 5

Conclusions

In the doctoral dissertation presented, new analytical procedures have been developed for the determination of six PBDEs in environmental and biological samples by GC-ICP-MS. A new analytical procedure was developed for the determination of PBDEs in environmental water samples by GC-ICP-MS. The instrumental parameters were optimized for efficient separation and sensitive detection of six PBDEs from the list of priority substances of the WFD, and the parameters that influence the extraction efficiency and sample stability investigated. Acidification of samples maintained the stability of PBDEs for at least 20 days, while the Tris-citrate buffer facilitated desorption of PBDEs from aqueous matrices containing SPM and HA. The optimized analytical procedure consists of acidification of the sample to pH 2 with nitric acid, simultaneous addition of Tris-citrate buffer solution to maintain a pH of 6 and facilitate desorption, followed by solvent extraction with 2 mL of iso-octane, mechanical shaking for 6 h, collection of the organic phase and its concentration to approximately 25 L and PBDE determination by GC-ICP-MS. Accuracy checks were performed by spike-recovery tests of water samples spiked with PBDEs and by the ID-GC-ICP-MS technique. The recoveries for six PBDEs (BDE 28, BDE 47, BDE 99, BDE 100, BDE 153 and BDE 154) in spiked river water samples containing 35 mg L^{-1} of SPM and 5 mg L^{-1} of HA ranged from 97 to 103 %, while the differences between the results determined by GC-ICP-MS and by ID-GC-ICP-MS did not exceed 1 %, confirming the accuracy of the analytical procedure. In river water samples spiked with the six PBDE congeners at concentrations of 10 ng L^{-1}, good repeatability of measurements for a particular PBDE of around 3 % was obtained, while for a sample spiked with six PBDE congeners whose sum of 0.498 ng L^{-1} was at the EQS level, the repeatability of measurement for a particular PBDE ranged between 6 and 25 %. The expanded uncertainty at the EQS level was found to be around 40 % ($k = 2$), which is better than the minimum performance criteria for monitoring the water status set by the WFD. When a 300 mL sample was analyzed, and the organic phase concentrated to 25 µL, the LOQ for ΣPBDEs in river water was 0.109 ng L^{-1} and was below the 0.15 ng L^{-1} limit value required by the WFD. The developed analytical procedure was successfully applied for reliable determination of the six PBDEs congeners in environmental river and seawater samples.

A new analytical procedure was also developed for sensitive and reliable determination of the six PBDEs (BDE 28, BDE 47, BDE 99, BDE 100, BDE 153 and BDE 154) in sewage sludge by GC-ICP-MS. For this purpose the extraction procedure was optimised. Different extracting agents (MeOH), acetic acid (AcOH)/MeOH mixture (3 : 1) and 0.1 mol L^{-1} HCl in MeOH) followed by the addition of Tris-citrate buffer (co-extracting agent) and iso-octane were applied under different modes of extraction (mechanical shaking, microwave- and ultrasound-assisted extraction). PBDEs were the most efficiently extracted from the sludge by applying mechanical shaking or microwave-assisted extraction with 0.1 mol L^{-1} HCl in MeOH and subsequent addition of Tris-citrate buffer and iso-octane. Since mechanical shaking is simpler for sample manipulation, it was applied in the extraction procedure. Quantification of PBDEs in iso-octane was performed by GC-ICP-MS using the standard addition calibration method. The procedure is simple and requires minimal sample preparation, which is the main advantage over the commonly applied procedures based on GC-MS analysis, which require tedious clean-up steps before MS detection. Accuracy checks were accomplished by the spike recovery test of sludge samples spiked with PBDEs and by the comparative analysis with the ID-GC-ICP-MS technique. The recoveries for six PBDEs ranged from 95 to 104 %, while the differences between the results determined by GC-ICP-MS and ID-GC-ICP-MS did not exceed 1 % (BDE 28, BDE 47, BDE 99, and BDE 100) or 4 % (BDE 153 and BDE 154), which confirmed the accuracy of the analytical procedure developed. Good repeatability and reproducibility of measurements RSDs (2.2 to 5.7 %) was obtained. The procedure is very sensitive with LODs for six PBDEs congeners ranging from 0.2 to 0.3 ng g^{-1}. In general, the RSDs are better and LODs lower than those reported in the literature. Analysis of sewage sludge samples from municipal WWTP Domžale-Kamnik, Slovenia in a time span from 1998 to 2014 demonstrated that the most abundant BDE congeners were BDE 47 and BDE 99. In this period a progressive decrease of PBDEs content was observed, while the PBDEs concentrations were comparable to those reported for most of the European countries. Due to its simplicity, reliability and sensitivity the developed method is appropriate to be used in routine laboratories for monitoring of PBDEs in sewage sludge.

In the last part, a new analytical procedure was developed for a sensitive and reliable determination of six PBDE congeners (BDE 28, BDE 47, BDE 99, BDE 100, BDE 153 and BDE 154) in mussels and fish samples by GC-ICP-MS. The extraction procedure was optimized. For this purpose, NIST SRM 2974a was used. It was found that 30 min of ultrasound-assisted extraction with 10 mL of 25 % aqueous solution of TMAH and afterward 2 h of mechanical shaking with 50 mL of Tris citrate buffer and 2 mL of iso-octane extracted the PBDEs from mussels and fish samples the most efficiently. This extraction approach has also the advantage of shortening the duration of extraction in comparison to mechanical shaking, and simplifies sample handling and manipulation in comparison to microwave-assisted extraction. The removal of lipids from the organic phase was performed by passing the extract through the column filled with Florisil. This clean-up step is simpler than those needed prior to detection of PBDEs by MS techniques. The quantification of PBDEs extracted in iso-octane was performed by GC-ICP-MS using a standard addition calibration method and by species-specific ID-ICP-MS. The results of analyses of the NIST SRM 2974a (freez-dried mussel tissue) and the NIST SRM 1946

(frozen fish tissue homogenate) reference materials were in agreement with the certified values confirming the accuracy of the analytical procedure. Good repeatability and reproducibility of measurements were obtained (RSDs from 3.0 to 8.3 %). The analytical method is sensitive with LODs for six PBDEs ranging from 0.003 to 0.009 ng g^{-1} ww. In general, RSDs are better and LODs lower or in the same range as those reported in the cited literature. The developed procedure was successfully applied for the determination of the six PBDEs in mussel and fish samples from the Slovenian part of the Northern Adriatic Sea and fish from the Sava River. The concentrations of the PBDEs and the observed PBDE profiles from the present study fall within the same magnitude of order or lower than those reported for other European countries. Due to its simplicity, sensitivity and reliability, the developed method can be used to assess the levels of PBDEs pollution in the biota samples in an attempt to enhance the ecosystem monitoring strategies.

References

Alaee, M., Backus, S. & Cannon, C. (2001). Potential interferences of PBDEs in the determination of PCBs and other organochlorine contaminants using electron capture detection. Journal of Separation Science 24, 465–469.

Anderson, O. & Blomkvist, G. (1981). Polybrominated aromatic pollutants found in fish in Sweden. Chemosphere 10, 1051-1060.

Bacaloni, A., Callipo, L., Corradini, E., Giansanti, P., Gubbiotti, R., Samperi, R. & Laganà, A. (2009). Liquid chromatography–negative ion atmospheric pressure photo ionization tandem mass spectrometry for the determination of brominated flame retardants in environmental water and industrial effluents. J. Chromatogr. A 1216, 6400–6409.

Barón, E., Santín, G., Eljarrat, E. & Barcel , D. (2014). Occurrence of classic and emerging halogenated flame retardants in sediment and sludge from Ebro and Llobregat river basins (Spain). J. Hazard. Mater. 265, 288–295.

Bergman, A. (ed.), Heindel, J. J., Jobling, S., Kidd, K. A. & Zoeller, T. (2012). State of the Science of Endocrine Disrupting Chemicals 2012, Summary for Decision-Makers.

Bieniek, D., Bahadir, M. & Kortte, F. (1989). Formation of heterocyclic hazardous compounds by thermal degradation of organic compounds. Heterocyles 1989 28, 719-722.

Björklund, J., Tollbäck, P., Hiarne, C., Dyremarck, E. & Östman, C. (2004). Influence of the injection technique and the column system on gas chromatographic determination of polybrominated diphenyl ethers. J Chromatogr A 1041, 201–210.

Bocio, A., Domingo, J. L., Falcó, G. & Llobet, J. M. (2007). Concentrations of PCDD/PCDFs and PCBs in fish and seafood from the Catalan (Spain) market: estimated human intake. Environ Int 33, 170–175.

Bragigand, V., Amiard-Triquet, C., Parlier, E, Boury, P., Marchand, P. & El Hourch, M. (2006). Influence of biological and ecological factors on the bioaccumulation of polybrominated diphenyl ethers in aquatic food webs from French estuaries. Sci Total Environ 368, 615−626.

Čajka, T., Hajšlová, J., Kazda, R. & Poustka, J. (2005). Challenges of gas chromatography-high-resolution time-of-flight mass spectrometry for simultaneous analysis of polybrominated diphenyl ethers and other halogenated persistent organic pollutants in environmental samples. J Sep Sci 28, 601–611.

Christensen, J. H. & Platz, J. (2001). Screening of polybrominated diphenyl ethers in bluemussels, marine and freshwater sediments in Denmark. J. Environ. Monit. 3, 543–547.

Cincinelli, A. Martellini, T. Misuri, L. Lanciotti, E. Sweetman, A. Laschi, S. & Palchetti, I. (2012). PBDEs in Italian sewage sludge and environmental risk of using sewage sludge for land application. Environ. Pollut. 161, 229-234.

Cincinelli, A., Martellini, T., Misuri, L., Lanciotti, E., Sweetman, A., Laschi, S. & Palchetti, I. (2012). PBDEs in Italian sewage sludge and environmental risk of using sewage sludge for land application. Environ. Pollut. 161, 229-234.

Commission directive 2009/90/EC of 31 July 2009 laying down, pursuant to Directive 2000/60/EC of the European Parliament and of the Council, technical specification for chemical analysis and monitoring of water status, (2009). Official Journal Of the European Union 201, 36-38.

Covaci, A., Bervoets, L., Hoff, P., Voorspoels, S., Voets, J., Van Campenhout, K., . . . Schepens, P. (2005). Polybrominated diphenyl ethers (PBDEs) in freshwater mussels and fish from Flanders, Belgium. J. Environ. Monit. 7, 132–136.

Covaci, A., de Boer, J., Ryan, J. J., Voorspoels, S. & Schepens, P. (2002). Determination of polybrominated diphenyl ethers and polychlorinated biphenyls in human adipose tissue bylarge-volume injection – narrow-bore capillary gas chromatography/electron impact low resolution mass spectrometry. Anal Chem 74, 790–798.

Covaci, A., Dirty, A. C., Voorspoels, S. & Roosens, L. (2011). Sample Preparation and Chromatographic Methods Applied to Congener-Specific Analysis of Polybrominated Diphenyl Ethers. In Ethel, E. & Damià, B. (Eds.), Brominated flame retardants. Springer.

Cristale, J., Katsoyiannis, A., Sweetman, A. J., Jones, K. C. & Lacorte, S. (2013). Occurrence and risk assessment of organophosphorus and brominated flame retardants in the River Aire (UK). Environ. Pollut. 179, 194–200.

Danon-Schaffer, M. N., Mahecha-Botero, A., Grace J. R. & Ikonomou, M. (2013). Mass balance evaluation of polybrominated diphenyl ethers in landfill leachate and potential for transfer from e-waste. Sci. Tot. Environ. 461–462, 290-301.

Darnerud, P. O., Atuma, S., Aune, M., Bjerselius, R., Glynn, A., Grawé, K. P. & Becker, W. (2006). Dietary intake estimations of organohalogen contaminants (dioxins, PCB, PBDE and chlorinated pesticides, e.g. DDT) based on Swedish market basket data. Food Chem Toxicol 44, 597–1606.

Darnerud, P. O., Aune, M., Larsson, L., Lignell, S., Mutshatshi, T., Okonkwo, J., . . . Agyei, N. (2011). Levels of brominated flame retardants and other pesistent organic pollutants in breast milk samples from Limpopo Province, South Africa. Sci Total Environ 409, 4048–4053.

Davis, E. F. Klosterhaus, S. L. & Stapleton, H. M. (2012). Measurement of flame retardants and triclosan in municipal sewage sludge and biosolids, Environ. Internat. 40, 1-7.

Davis, E. F., Klosterhaus, S. L. & Stapleton, H. M. (2012). Measurement of flame retardants and triclosan in municipal sewage sludge and biosolids. Environ. Internat. 40, 1-7.

De Boer, J., de Boer, K. & Boon, J. P. (2000). The Handbook of Environmental Chemistry Vol. 3 Part K: New Types of Persistent Compounds, Springer-Verlag.

De Boer, J., Wester, P. G., van der Horst, A. & Leonards, P. E. G. (2003). Polybrominated diphenyl ethers in influents, suspended particulate matter, sediments, sewage treatment plant and effluents and biota from the Netherlands, Environ. Pollut. 122, 63-74.

De Wit, C. A. (2002). An overview of brominated flame retardants in the environment. Chemosphere 46, 583–624.

De Wit, C. A. (2002). Chemosphere 46, 583-624.

De Wit, C. A., Alaee, M. & Muir, D. C. (2006). Levels and trends of brominated flame retardants in the Arctic. Chemosphere 64, 209–233.

Díaz-Cruz, M. S. García-Galán, M. J. Guerra, P., Jelic, A., Postigo, C., Eljarrat, E., . . ., Barceló D. (2009). Analysis of selected emerging contaminants in sewage sludge. TrAC Trends Anal. Chem. 28 (11), 1263-1275.

Directive 2003/11/EC of the European Parliament and of the council of 6 February 2003 amending for the 24th time council directive 76/769/EEC relating to restrictions on the marketing and the use of certain dangerous substances and preparations (pentabromodiphenyl ether and octabromodiphenyl ether) (2003). Official Journal L 042.

Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standard in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council, (2008). Official Journal Of the European Union 348, 84-97.

Dirtu, A. C., Ravindra, K., Roosens, L., van Grieken, R., Neels, H., Blust, R. & Covaci, A. (2008). Fast analysis of decabrominated diphenyl ether using low-pressure gas chromatography – electron capture negative ionization mass spectrometry. J Chromatogr A 1186, 295–301.

Domingo, J. L. (2012). Polybrominated diphenyl ethers in food and human dietary exposure: a review of the recent scientific literatura. Food Chem Toxicol 50, 238–249.

Dosis, I., Athanassiadis, I., & Karamanlis, X. (2016). Polybrominated diphenyl ethers (PBDEs) in mussels from cultures and natural population. Mar. Pollut. Bull. 107, 92–101.

EFSA (2011). Scientific opinion on polybrominated diphenyl ethers (PBDEs) in food. EFSA Journal 9(5), 2156-2427.

Eljarrat, E., de la Cal, A. & Barcelo, D. (2003). Potential chlorinated and brominated interferences on the polybrominated diphenyl ether determinations by gas chromatography-mass spectrometry. J Chromatogr A 1008, 181–192.

Erger, C., Balsaa, P., Werres, F. & Schmidt, T. C. (2012). Multi-component trace analysis of organic xenobiotics in surface water containing suspended particular matter by solid phase extraction/gas chromatography-mass spectrometry. J. Chromatogr. A 1249, 181–189.

European court of justice. Cases C-14/06 and C-295/06, Judgement of the court, 1 April 2008, Directive 2002/95/EC and Commission Decision 2005/717/EC (2008).

Fängström, B., Athanassiadis, I., Odsjö, T., Norén, K. & Bergman, A. (2008). Temporal trends of polybrominated diphenyl ethers and hexabromocyclododecane in milk from Stockholm mothers, 1980–2004. Mol Nutr Food Res 52, 187–193.

Fernandes, A., Dicks, P., Mortimer, D., Gem, M., Smith, F., Driffield, . . . Rose, M. (2008). Brominated and chlorinated dioxins, PCBs and brominated flame retardants in Scottish shellfish: methodology, occurrence and human dietary exposure. Mol. Nutr. Food Res. 52, 238–249.

Fontana, A. R., Silva, M. F., Martínez, L. D., Wuilloud, R. G. & Altamirano, J. C. (2009). J. Chromatogr. A 1216, 4339-4346.

Fontana, A. R., Silva, M. F., Martínez, L. D., Wuilloud, R. G. & Altamirano, J. C. (2009). Determination of polybrominated diphenyl ethers in water and soil samples by cloud point extraction-ultrasound-assisted back-extraction-gas chromatography–mass spectrometryJ. Chromatogr. A 1216, 4339-4346.

Fowles, J. R., Fairbrother, A., Baecher-Steppan, L. & Kerkvliet, N. I. (1994). Immunologic and endocrine effects of the flame-retardant pentabromodiphenyl ether (DE-71) in C57BL/6J mice. Toxicology 86, 49–61.

Frederiksen, M., Vorkamp, K., Thomsen, M. & Knudsen, L. E. (2009). Human internal and external exposure to PBDEs–a review of levels and sources. Int J Hyg Environ Health 212, 109–134.

Frederiksen, M., Vorkamp, K., Thomsen, M. & Knudsen, L. E. (2009). Human internal and external exposure to PBDEs – A review of levels and sources. Int. J. Hyg. Environ. Health 212, 109–134.

Fulara, I. & Czaplicka, M. (2012). Methods for determination of polybrominated diphenyl ethers in environmental samples – review. J. Sep. Sci. 35, 2075–2087.

Gama, A. C., Sanatcumar, P., Viana, P., Barceló, D., Bordado, J. C. (2006). The occurrence of polybrominated diphenyl ethers in river and coastal biota from Portugal. Chemosphere 64, 306–310.

Giandomenico, S., Spada, L., Annicchiarico, C., Assennato, G., Cardellicchio, N., Ungaro, N. & Di Leo, A. (2013). Chlorinated compounds and polybrominated diphenyl ethers (PBDEs) in mussels (Mytilus galloprovincialis) collected from Apulia Region coasts. Mar. Pollut. Bull. 73, 243–251.

Gonzáles- Gago, A., Brandsma, S. H., Leonards, P. E. G., de Boer, J. Marchante-Gayón, J. M. & Garcia Alonso, J. I. (2011). Anal. Bioanal. Chem. 401, 2639-2649.

Gorga, M., Martínez, E., Ginebreda, A., Eljarrat, E. & Barceló, D. (2013). Determination of PBDEs, HBB, PBEB, DBDPE, HBCD, TBBPA and related compounds in sewage sludge from Catalonia (Spain). Sci. Tot. Environ. 444, 51-59.

Gorga, M., Martínez, E., Ginebreda, A., Eljarrat, E. & Barceló, D. Determination of PBDEs, HBB, PBEB, DBDPE, HBCD, TBBPA and related compounds in sewage sludge from Catalonia (Spain). Sci. Tot. Environ. 444, 51-59.

Gravningen Sørmo, E., Pettersvik Salmer, M., Jenssen, B. M., Hop, H., Bæk, K., Kovacs, K., . . . Skaare, J. U. (2006). Biomagnification of polybrominated diphenyl ether and hexabromocyclododecane flame retardants in the polar bear food chain in Svalbard, Norway. Environ. Toxicol. Chem. 25, 2502-2511.

Hale, R. C., La Guardia, M. J., Harvey, E. P., Mainor, T. M., Duff, W. H. & Gaylor, M. O. (2001). Polybrominated Diphenyl Ether Flame Retardants in Virginia Freshwater Fishes (USA). Environ Sci Technol 35, 4585–4591.

Hale, R. C., La Guardia, M. J., Harvey, E., Gaylor, M. O. & Mainor, T. M. (2006). Brominated flame retardant concentrations and trends in abiotic media. Chemosphere 64, 181-186.

Hallgren, S., Sinjari, T., Hakansson, H. & Darnerud, P. O. (2001). Effects of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) on thyroid hormone and vitamin A levels in rats and mice. Arch Toxicol 75, 200-208.

He, M. J., Luo, X. J., Chen, M. J., Sun, Y. X., Chen, S. J. & Mai, B. X. (2012). Bioaccumulation of polybrominated diphenyl ethers and decabromodiphenyl ethane in fish from a river system in a highly industrialized area, South China. Sci. Total Environ. 419, 109–115.

Hites, R. A. (2008). Electron impact and electron capture negative ionization mass spectra of polybrominated diphenyl ethers and methoxylated polybrominated diphenyl ethers. Environ Sci Technol 42, 2243–2252.

Huss, H. H. (1995). Quality and quality changes in fresh fish. FAO Fisheries technical paper, 348.

Iqbal, M., Syed, J. H., Katsoyiannis, A., Malik, R. N., Farooqi, A., Butta, A., . . ., Jones, K. C. (2017). Legacy and emerging flame retardants (FRs) in the freshwater ecosystem: A review. Environmental Research 152, 26–42.

Johansson, I., Héas-Moisan, K., Guiot, N., Munschy, C., & Tronczyński, J. (2006). Polybrominated diphenyl ethers (PBDEs) in mussels from selected French coastal sites: 1981-2003. Chemosphere 64, 296-305.

Jones-Otazo, H. A., Clarke, J. P., Diamond, M. L., Archbold, J. A., Ferguson, G., Harner, T., . . . Wilford, B. (2005). Is house dust the missing exposure pathway for PBDEs?

An analysis of the urban fate and human exposure to PBDEs. Environ Sci Technol 39, 5121–5130.

Jörundsdóttir, H., Löfstrand, K., Svavarsson, J., Bignert, A. & Bergman, Å. (2013). Polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) in seven different marine bird species from Iceland. Chemosphere 93, 1526–1532.

Jürgens, M. D., Johnson, A. C., Jones, K. C., Hughes, D. & Lawlor, A. J. (2013). The presence of EU priority substances mercury, hexachlorobenzene, hexachlorobutadiene and PBDEs in wild fish from four English rivers. Sci. Tot. Environ. 461-462, 441-452.

Jürgens, M. D., Johnson, A. C., Jones, K. C., Hughes, D., & Lawlor, A. J. (2013). The presence of EU priority substances mercury, hexachlorobenzene, hexachlorobutadiene and PBDEs in wild fish from four English rivers. Science of the Total Environment, 461-462, 441-452.

Kim, E. J., Kim, J. H., Kim, J. H., Bokare, V. & Chang, Y. S. (2014). Predicting reductive debromination of polybrominated diphenyl ethers by nanoscale zerovalent iron and its implications for environmental risk assessment. Sci. Tot. Environ. 470-471, 1553 – 1557.

Kiviranta, H., Ovaskainen, M. L. & Vartiainen, T. (2004). Market basket study on dietary intake of PCDD/Fs, PCBs, and PBDEs in Finland. Environ Int 30, 923–932.

Knoth, W. Mann, W. Meyer, R. & Nebhuth, J. (2007). Polybrominated diphenyl ether in sewage sludge in Germany. Chemosphere 67, 1831-1837.

Knutsen, H. K., Kvalem, H. E., Thomsen, C., Froshaug, M., Haugen,M., Becher, G., . . . Meltzer, H. M. (2008). Dietary exposure to brominated flame retardants correlates with male blood levels in a selected group of Norwegians with a wide range of seafood consumption. Mol. Nutr. Food Res. 52, 217–227.

Korytár, P., Covaci, A., de Boer, J., Gelbin, A. & Brinkman, U. A. T. (2005). Retention-time database of 126 polybrominated diphenyl ether congeners and two bromkal technical mixtures on seven capillary gas chromatographic columns. J Chromatogr. A 1065, 239–249.

Król, S., Zabiegała, B. & Namieśnik, J. (2012). PBDEs in environmental samples: sampling and analysis. Talanta 93, 1-17.

Kucharska, A., Covaci, A., Vanermen, G. & Voorspoels, S (2015). Non-invasive biomonitoring for PFRs and PBDEs: New insights in analysis of human hair externally exposed to selected flame retardants. Sci. Tot. Environ. 505, 1062-1071.

Kupper, T., de Alencastro, L.F., Gatsigazi, R., Furrer, R., Grandjean, D. & Tarradellas, J. (2008). Concentrations and specific loads of brominated flame retardants in sewage sludge. Chemosphere 71, 1173-1180.

Kuriyama, S. N., Wanner, A, Figaldo-Neto, A. A., Talsness, C. E., Koerner, W. & Chahoud, I. (2007). Developmental exposure to low-dose PBDE-99: tissue distribution and thyroid hormone levels. Toxicology 242, 80–90.

La Guardia, M. J., Hale, R. C. & Harvey, E. (2006). Detailed polybrominated diphenyl ether (PBDE) congener composition of the widely used penta-, octa-, and deca-PBDE technical flame-retardant mixtures. Environ Sci Technol 40, 6247−6254.

Labadie, P., Tlili, K., Alliot, F., Bourges, C., Desportes, A. & Chevreuil, M. (2010). Development of analytical procedures for trace-level determination of polybrominated diphenyl ethers and tetrabromobisphenol A in river water and sediment. Anal. Bioanal. Chem. 396, 865–875.

Labadie, P., Tlili, K., Alliot, F., Bourges, C., Desportes, A. & Chevreuil, M. (2010). Development of analytical procedures for trace-level determination of polybrominated diphenyl ethers and tetrabromobisphenol A in river water and sediment. Anal. Bioanal. Chem. 396, 865-875.

Law, R. J., Allchin, C. R., de Boer, J., Covaci, A., Herzke, D., Lepom, P., . . ., de Wit, C.A. (2006). Levels and trends of brominated flame retardants in the European environment, Chemosphere 64, 187-208.

Legler, J. (2008). New insights into the endocrine disrupting effects of brominated flame retardants. Chemosphere 73, 216-222.

Linares, M., Bellés, M. & Domingo, J. L. (2015). Human exposure to PBDE and critical evaluation of health hazards. Arch Toxicol 89, 335–356.

Lohmann, N., Rolle, S., Paepke, O., Ruedel, H., Teuber, D., & Koschrreck, J. (2015). Polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCDD) in fish of German waters: a study in the context of the EU Water Framework Directive. Organohalogen Compounds, 77, 538-541.

Ma, J., Cheng, J. P., Wang, W. H., Kunisue, T., Wu, M. H. & Kannan, K. (2011). Elevated concentrations of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans and polybrominated diphenyl ethers in hair from workers at an electronic waste recycling facility in Eastern China. J Hazard Mater 186, 1966–1971.

Ma, Q., Wang, C., Bai, H., Xi, H. W., Xi, G. C. Ren, X. M., . . ., Guo, L. H. (2011). Comprehensive Two-Dimensional Separation of Hydroxylated Polybrominated Diphenyl Ethers by Ultra-Performance Liquid Chromatography Coupled with Ion Mobility-Mass Spectrometry. Am. Soc. Mass Spectrom. 22, 1851–1861.

Malcolm, R. L., McKnight, D. M. & Averett, R. C. (1994). History and Description of the Okefenokee Swamp Origin of the Suwannee River. In: Humic Substances in the Suwannee River, Georgia: Interactions, Properties, and Proposed Structures. United States Geological Survey Water-Supply Paper 2373. United States Government Printing Office, U.S. Geological Survey, Denver, CO.

Mariani, G., Canuti, E., Castro-Jimenéz, J., Christoph, E. H., Eisenreich S. J. Hanke G., . . . Umlauf, G. (2008). Atmospheric input of POPs into Lake Maggiore (Northern Italy): PBDE concentrations and profile in air, precipitation, settling material and sediments. Chemosphere 73, 114–121.

Mariussen, E., Fjeld, E., Breivik, K., Steinnes, E., Borgen, A., Kjellberg, G., & Schlabach, M. (2008). Elevated levels of polybrominated diphenyl ethers (PBDEs) in fish from Lake Mjøsa, Norway. Science of the Total Environment, 390, 132-141.

Martellini, T., Diletti, G., Scortichini, G., Lolini, M., Lanciotti, E., Katsoyiannis, A., & Cincinelli, A. (2016). Occurrence of polybrominated diphenyl ethers (PBDEs) in foodstuffs in Italy and implications for human exposure. Food and Chemical Toxicology, 89, 32-38.

Martellini, T., Diletti, G., Scortichini, G., Lolini, M., Lanciotti, E., Katsoyiannis, A., & Cincinelli, A. (2016). Occurrence of polybrominated diphenyl ethers (PBDEs) in foodstuffs in Italy and implications for human exposure. Food and Chemical Toxicology 89, 32-38.

Morrissey, C.A., Pollet, I. L., Ormerod, S. J. & Elliott, J. E. (2011). American dippers indicate contaminant biotransport by Pacific salmon. Environ. Sci. Technol. 46, 1153–1162.

Nash, M. & Krupp, E. (2004). Determination of Organotin Species in Sediment Extracts using GC Coupled with X-Series ICP-MS. Thermo Electron Application Note 40698.

Nelms, S. (2005). Inductively coupled plasma Mass Spectrometry Handbook. Blackwell, Oxford, England,.

Ni, K., Lua, Y., Wanga, T., Kannanc, K., Gosens, J., Xu, L., . . . Liu, S. (2013). A review of human exposure to polybrominated diphenyl ethers (PBDEs) in China. Int J Hyg Environ Health 216, 607–623.

North K.D. (2004). Tracking Polybrominated Diphenyl Ether Releases in a Wastewater Treatment Plant Effluent, Palo Alto, California. Environ. Sci. Technol. 38, 4484-4488.

North, K. D. (2004). Tracking Polybrominated Diphenyl Ether Releases in a Wastewater Treatment Plant Effluent, Palo Alto, California. Environ. Sci. Technol. 38, 4484-4488.

Olofsson, U. Bignert, A. & Haglund, P. (2012). Time-trends of metals and organic contaminants in sewage sludge, Water Res. 46, 4841-4851.

O'Toole, S., Metcalfe, C., Craine, I. & Gross, M. (2006). Release of persistent organic contaminants from carcasses of Lake Ontario Chinook salmon (Oncorhynchus tshawytscha). Environ. Pollut. 140, 102–113.

Park, J. E., Kang, Y. Y., Kim, W. I., Jeon, T.W., Shin, S. K., Jeong, M. J. & Kim, J. G. (2014). Emission of polybrominated diphenyl ethers (PBDEs) in use of electric/electronic equipment and recycling of e-waste in Korea. Sci Total Environ 470–471, 1414–1421.

Poma, G., Volta, P., Roscioli, C., Bettinetti, R. & Guzzella, L. (2014). Concentrations and trophic interactions of novel brominated flame retardants, HBCD, and PBDEs in zooplankton and fish from Lake Maggiore (Northern Italy). Sci. Tot. Environ. 481, 401-408.

Poppa, M., Hanna, S. & Koellenspergera, G. (2010). Environmental application of elemental speciation analysis based on liquid or gas chromatography hyphenated to inductively coupled plasma mass spectrometry—A review. Anal. Chim. Acta 668, 114–129.

Pozo, K., Kukučka, P., Vaňková, L., Přibylova, P., Klanova, J., Rudolph, A., … Ahumada, R. (2015). Polybrominated Diphenyl Ethers (PBDEs) in Concepcion Bay, central Chile after the 2010 Tsunami. Mar. Pollut. Bull. 95, 480–483.

Pulkrabova, J., Hajšlová, J., Poustka, J., & Kazda, R. (2007). Fish as Biomonitors of Polybrominated Diphenyl Ethers and Hexabromocyclododecane in Czech Aquatic Ecosystems: Pollution of the Elbe River Basin. Environmental Health Perspectives, 115 (1), 28-34.

Rahman, F., Langford, K. H., Scrimshaw, M. D. & Lester, J. N. (2001). Polybrominated diphenyl ether (PBDE) flame retardants. Sci. Tot. Environ. 275, 1-17.

Rahman, F., Langford, K. H., Scrimshaw, M. D. & Lester, J. N. (2001). Polybrominated diphenyl ether (PBDE) flame retardants. Sci Total Environ 275, 1-17.

Ran, Y., Yang, J., Liu, Y., Zeng, X., Gui, H. & Zeng, E. Y. (2013). Levels, compositions, and inventory of polybrominated diphenyl ethers in sewage sludge of Guangdong Province, South China. Environ. Sci. Pollut. Res. 20, 8780-8789.

Ran, Y., Yang, J., Liu, Y., Zeng, X., Gui, H. & Zeng, E.Y. (2013). Levels, compositions, and inventory of polybrominated diphenyl ethers in sewage sludge of Guangdong Province, South China. Environ. Sci. Pollut. Res. 20, 8780-8789.

Roberts, S. C., Noyes, P. D., Gallagher, E. P. & Stapleton, H. M. (2011). Species-Specific Differences and Structure-Activity Relationships in the Debromination of PBDE Congeners in Three Fish Species. Environ Sci Technol 45(5), 1999–2005.

Rodríguez-Gonzales, P., Marchante-Gayón, J. M., Garcia Alonso J. I. & Sanz-Medel, A. (2005). Isotope dilution analysis for elemental speciation: A tutorial review. Spectrochim. Acta B 60, 151-207.

Roosens, L., Dirtu, A. C., Goemans, G., Belpaire, C., Gheorghe, A., Neels, H., . . ., Covaci, A. (2008). Brominated flame retardants and organochlorine contaminants in fish from the Scheldt River, Belgium. Environ Int 34, 976–983.

Rotander, A., van Bavel, B., Polder, A., Rigét, F., Auðunsson, G. A., Gabrielsen, G. W., . . ., Dam, M. (2012). Polybrominated diphenyl ethers (PBDEs) in marine mammals

from Arctic and North Atlantic regions, 1986–2009. Environment International 40, 102–109.

Schecter, A., Papke, O., Harris, T. R., Tung, K. C., Musumba, A., Olson, J. & Birnbaum, L. (2006). Polybrominated diphenyl ether (PBDE) levels in an expanded market basket survey of U.S. food and estimated PBDE dietary intake by age and sex. Environ Health Perspect 114, 1515–1520.

Shanmuganathan, D., Megharaj, M., Chen, Z. & Naidu, R. (2011). Polybrominated diphenyl ethers (PBDEs) in marine foodstuffs in Australia: residue levels and contamination status of PBDEs. Mar. Pollut. Bull. 63, 154–159.

Shin, L. H., Boo, H. O., Bang, E., Gorinstein, S. & Seo, J. (2012). Development of a cleanup method for polybrominated diphenyl ether (PBDE) in fish by freezing-lipid filtration. Eur Food res Technol 235, 295-301.

Shin, M., Svoboda, L. & Falleta, P. (2007). Microwave-assisted extraction (MAE) for the determination of polybrominated diphenylethers (PBDEs) in sewage sludge. Anal. Bioanal. Chem. 387, 2923-2929.

Shin, M., Svoboda, L. & Falleta, P. (2007). Microwave-assisted extraction (MAE) for the determination of polybrominated diphenylethers (PBDEs) in sewage sludge. Anal. Bioanal. Chem. 387, 2923-2929.

Stapleton, H. (2006). Instrumental methods and challenges in quantifying polybrominated diphenyl ethers in environmental extracts: a review. Anal Bioanal Chem 1, 1–11.

Stapleton, H. M. & Dodder, N. G. (2008). Photodegradation of decabromodiphenyl ether in house dust by natural sunlight. Environ Toxicol Chem 27, 306-312.

Stapleton, H. M., Dodder, N. G., Offenberg, J. H., Schantz, M. M. & Wise, S. A. (2005). Polybrominated diphenyl ethers in house dust and clothes dryer lint. Environ Sci Technol 39, 925–931.

Stapleton, H. M., Harner, T., Shoeib, M., Keller, J. M., Schantz, M. M., Leigh, S. D. & Wise, S. A. (2006). Determination of polybrominated diphenyl ethers in indoor dust standard reference materials. Anal Bioanal Chem 384, 791–800.

Stapleton, H., Brazil, B., Holbrook, D. R., Mitchelmore, C. L., Benedict, R., Konstantinov, A. & Potter, D., (2006). In Vivo and In Vitro Debromination of Decabromodiphenyl Ether (BDE 209) by Juvenile Rainbow Trout and Common Carp. Environ. Sci. Technol. 40, 4653-4658.

Suominen, K., Hallikainen, A., Ruokojärvi, P., Airaksinen, R., Koponen, J., Rannikko, R., & Kiviranta, H. (2011). Occurrence of PCDD/F, PCB, PBDE, PFAS, and Organotin Compounds in Fish Meal, Fish Oil and Fish Feed. Chemosphere, 85, 300-306.

Swarthout Jr., R. F., Kucklick, J. R. & Davis, W. C. (2008). The determination of polybrominated diphenyl ether congeners by gas chromatography inductively coupled plasma mass spectrometry. J. Anal. At. Spectrom. 23, 1575-1580.

Szlinder-Richert, J., Barska, I., Usydus, Z., & Grabic, R. (2010). Polybrominated diphenyl ethers (PBDEs) in selected fish species from the southern Baltic Sea. Chemosphere, 78, 695-700.

Tadeo, J. L., Sanchez-Brunete, C. & Miguel, E. (2009). Determination of polybrominated diphenyl ethers in human hair by gas chromatography-mass spectrometry. Talanta 78, 138–143.

Talsness, C. E. (2008). Overview of toxicological aspects of polybrominated diphenyl ethers: A flame-retardant additive in several consumer products. Environ. Research 108, 158-167.

Tapie, N., Budzinski, H., & Le Ménach, K. (2008). Fast and efficient extraction methods for the analysis of polychlorinated biphenyls and polybrominated diphenyl ethers in biological matrices. Anal Bioanal Chem 391, 2169-2177.

Thomas, R. (2004). Practical guide to ICP-MS. Marcel Dekker, Inc., Newyork, Basel.

Thomsen, C., Stigum, H., Froshaug, M., Broadwell, S. L., Becher, G. & Eggesbo, M. (2010). Determinants of brominated flame retardants in breast milk from a large scale Norwegian study. Environ Int 36, 68–74.

Thurman, E. M. & Snavely, K. (2000). Advances in solid-phase extraction disks for environmental chemistry TrAC Trends Anal. Chem. 19, 18–26.

Tittlemier, S. A., Halldorson, T., Stern, G. A. & Tomy, G. T. (2002). Vapor pressures, aqueous solubility, and Henry's law constants of some brominated fire retardants. Environ Toxicol Chem 21(9), 1804−1810.

Tollback, P., Bjorklund, J. & Ostman, C. (2003). Large-volume programmed-temperature vaporiser injection for fast gas chromatography with electron capture and mass spectrometric detection of polybrominated dipheyl ethers, J. Chromatogr. A 991, 241–253.

US EPA (2010). An exposure assessment of polybrominated diphenyl ethers. EPA/600/R-08/086F. Washington DC: Office of Research and Development National Center for Environmental Assessment.

Vahčič, M., Milačič, R. & Ščančar, J. (2011a). Rapid and Sensitive Analytical Method for Monitoring of 12 Organotin Compounds in Natural Waters. Acta Chim. Sloven. 58, 185-194.

Vahčič, M., Milačič, R. & Ščančar, J. (2011b). Development of analytical procedure for the determination of methyltin, butyltin, phenyltin and octyltin compounds in landfill leachates by gas chromatography-inductively coupled plasma mass spectrometry. Anal. Chim. Acta 694, 21-30.

Van de Veen, A. M. H., Horvat, M., Milačič, R., Bučar, T., Repinc, U., Ščančar, J. & Jaćimović, R. (2001). Operation of a proficiency testing scheme of trace elements in sewage sludge with reference values. Accred. Qual. Assur. 6, 264-268.

Van de Veen, A. M. H., Horvat, M., Milačič, R., Bučar, T., Repinc, U., Ščančar, J. & Jaćimović, R. (2001). Operation of a proficiency testing scheme of trace elements in sewage sludge with reference values. Accred. Qual. Assur. 6, 264-268.

Van Leeuwen, S. P. J. & de Boer, J. (2008). Brominated flame retardants in fish and shellfish - levels and contribution of fish consumption to dietary exposure of Dutch citizens to HBCD. Mol. Nutr. Food Res. 52, 194-203.

Venkatesan, A. K. & Halden, R. U. (2014). Brominated flame retardants in U.S. biosolids from the EPA national sewage sludge survey and chemical persistence in outdoor soil mesocosms. Water Res. 55, 133-142.

Viberg, H., Fredriksson, A., Jakobsson, E., Orn, U. & Eriksson, P. (2003). Neurobehavioral derangements in adult mice receiving decabromodiphenyl ether (PBDE 209) during a defined period of neonatal brain development. Toxicol. Sci. 76, 112-120.

Viganò, L., Mascolo, G. & Roscioli, C. (2015). Emerging and priority contaminants with endocrine active potentials in sediments and fish from the River Po (Italy). Environ. Sci. Pollut. Res. 22, 1–17.

Vonderheide, A. P. (2009). A review of the challenges in the chemical analysis of the polybrominated diphenyl ethers. Microchem J 92, 49–57.

Vonderheide, A. P. (2009). A review of the challenges in the chemical analysis of the polybrominated diphenyl ethers. Microchemical Journal 92, 49–57.

Vonderheide, A. P., Montes-Bayon, M. & Caruso, J. A. (2002). Development and application of a method for the analysis of brominated flame retardants by fast gas chromatography with inductively coupled plasma mass spectrometric detection. J. Anal. At. Spectrom. 17, 1480–1485.

Vonderheide, A. P., Mueller-Spitz, S. R., Meija, J., Welsch, G. L., Mueller, K. E., Kinkle, B. K., . . . Caruso, J. A. (2006). Rapid breakdown of brominated flame retardants by soil microorganisms. J Anal At Spectrom 21, 1232-1239.

Voorspoels, S., Covaci, A., & Schepens, A. (2003). Polybrominated Diphenyl Ethers in Marine Species from the Belgian North Sea and the Western Scheldt Estuary: Levels, Profiles, and Distribution. Environmental Science & Technology, 37, 4348-4357.

Voorspoels, S., Covaci, A., Lepom, P., Cutenaire, S. & Schepens, P. (2006). Remarkable Findings Concerning PBDEs in the Terrestrial Top-Predator Red Fox (Vulpes vulpes). Environ. Sci. Technol. 40, 2937-2943.

Voorspoels, S., Covaci, A., Lepom, P., Jaspers, V. L. B. & Schepens, P. (2005). Levels and distribution of polybrominated diphenyl ethers in various tissues of birds of prey. Environmental Pollution 144, 218-227.

Wang, D., Atkinson, S., Hoover-Miller, A., Shelver, W. L. & Li, Q. X. (2008). Simultaneous use of gas chromatography/ion trap mass spectrometry-electron capture detection to improve the analysis of bromodiphenyl ethers in biological and environmental samples. Rapid Commun Mass Spectrom 22, 647–656.

Wang, P., Zhang, Q., Wang, Y., Wang, T., Li. X., Ding, L., & Jiang, G. (2010). Evaluation of Soxhlet extraction, accelerated solvent extraction and microwave-assisted extraction for the determination of polychlorinated biphenyls and polybrominated diphenyl ethers in soil and fish samples. Anal. Chim. Acta 663, 43-48.

Wang, Y., Jiang, G., Lam, P. K. S. & Li, A. (2007). Polybrominated diphenyl ether in the East Asian environment: a critical rewiev. Environment International 33, 963-973.

Wei, H. (2012). Polybrominated Diphenyl Ethers in Sediments from Arkansas, Illinois and Indiana of the United States.Chicago, Illinois: Public Health Sciences in the Graduate College of the University of Illinois at Chicago.

WHO (1994). Brominated diphenyl ethers. Environ Health Crit 1994 62.

Wolkers, H., van Bavel, B., Derocher, A., Wiig, Ø., Kovacs, K. M., Lydersen, C. & Lindström, G. (2004). Congener-Specific Accumulation and Food Chain Transfer of Polybrominated Diphenyl Ethers in Two Arctic Food Chains. Environ. Sci. Technol. 38, 1667-1674.

Wolschke, H., Meng, X., Xie, Z., Ebinghaus, R. & Cai, M. (2015). Novel flame retardants (N-FRs), polybrominated diphenyl ethers (PBDEs) and dioxin-like polychlorinated biphenyls (DL-PCBs) in fish, penguin, and skua from King George Island, Antarctica. Mar. Pollut. Bull. 96, 513-518.

Xiao, Q., Hu, B., Duan, J., He, M. & Zu, W. (2007). Analysis of PBDEs in soil, dust, spiked lake water, and human serum samples by hollow fiber-liquid phase microextraction combined with GC-ICP-MS. J. Am. Soc. Mass Spectrom. 18, 1740–1748.

Zennegg, M., Munoz, M., Schmid, P. & Gerecke, A. C. (2013). Temporal trends of persistent organic pollutants in digested sewage sludge (1993–2012). Environ. Internat. 60 202-208.

Zhang, L., Li, J. G., Zhao, Y. F., Li, X. W., Yang, X., Wen, S., . . . Wu, Y. N. (2011). A national survey of polybrominated diphenyl ethers (PBDEs) and indicator polychlorinated biphenyls (PCBs) in Chinese mothers' milk. Chemosphere 84, 625–633.

Zhou, S. N., Reiner, E. J., Marvin, C., Kolic, T., Riddell, N., Helm, P., . . ., Brindle, I. D. (2010). Liquid chromatography-atmospheric pressure photoionization tandem mass spectrometry for analysis of 36 halogenated flame retardants in fish. J. Chromatogr. A 1217, 633-641.

Zhu, L. Y. & Hites, R. A. (2005). Brominated flame retardants in sediment cores from Lakes Michigan and Erie. Environ. Sci. Technol. 39, 3488–3494.

Zweidinger, R. A., Cooper, S. D., Erickson, M. D., Michael, L. C. & Pellizzari, E. D. (1979). Sampling and analysis for semivolatile brominated organics in ambient air. ACS Symposium Series 94, 217-231.

Bibliography

Publications Related to the Thesis

Journal Articles

P. Novak, T. Zuliani, R. Milačič and J. Ščančar, "Development of an analytical procedure for the determination of polybrominated diphenyl ethers in environmental water samples by GC-ICP-MS," *Analytica chimica acta*, vol. 827, pp. 67-73, 2014.

P. Novak, T. Zuliani, R. Milačič and J. Ščančar, "Development of an analytical method for the determination of polybrominated diphenyl ethers in sewage sludge by the use of gas chromatography coupled to inductively coupled plasma mass spectrometry," *Analytica chimica acta*, vol. 915, pp. 27-35, 2016.

P. Novak, T. Zuliani, R. Milačič and J. Ščančar, "Newly develped analytical method for the determination of PBDEs in mussels and fish by GC-ICP-MS". Send for publications.

Conference Paper

P. Novak, T. Zuliani, R. Milačič and J. Ščančar, "Determination of PBDEs in environmental water samples," presented at *ICP-MS-Seminar,* Ljubljana, Slovenia, 2013.

P. Novak, T. Zuliani, R. Milačič and J. Ščančar, "Determination of PBDEs in environmental water samples by GC-ICP-MS," in *Proceedings : part 1 of the 6th Jožef Stefan International Postgraduate School Students' Conference*, Ljubljana, Slovenia, 2014.

P. Novak, T. Zuliani, R. Milačič and J. Ščančar, "Determination of polybrominated diphenyl ethers in environmental waters by GC-ICP-MS," *European Winter Conference on Plasma Spectrochemistry*, Münster, Germany, 2015.

J. Ščančar, P. Novak, T. Zuliani and R. Milačič, "Determination of polybrominated diphenyl ethers in sewage sludge by GC-ICP-MS," *in Book of abstracts of the 21st International Symposium on Separation Sciences*, Ljubljana, Slovenia, 2015.

P. Novak, T. Zuliani, R. Milačič and J. Ščančar, " Determination of polybrominated diphenyl ethers in sewage sludge by GC-ICP-MS," in *Proceedings : part 1 of the 8th Jožef Stefan International Postgraduate School Students' Conference*, Ljubljana, Slovenia, 2016.

P. Novak, T. Zuliani, R. Milačič and J. Ščančar, " Determination of polybrominated diphenyl ethers in sewage sludge by GC-ICP-MS," in *Program and abstract book of the 10th Young researchers' day*, Ljubljana, Slovenia, 2016.