



NATIONAL AND KAPODISTRIAN UNIVERSITY OF ATHENS

SCHOOL OF SCIENCES

DEPARTMENT OF CHEMISTRY

**MASTER OF SCIENCE IN “ANALYTICAL CHEMISTRY-QUALITY
ASSURANCE”**

MASTER THESIS

**“Development and validation of an analytical protocol for the
simultaneous determination of organic micropollutants in
biota specimens by GC-APCI-QToF MS”**

ANASTASIA ORFANIOTI

CHEMIST

ATHENS

OCTOBER 2021

MASTER THESIS

Development and validation of an analytical protocol for the simultaneous determination of organic micropollutants in biota specimens by GC-APCI-QToF MS.

ANASTASIA ORFANIOTI

Registration Number:192214

SUPERVISING PROFESSOR:

Nikolaos S. Thomaidis, Professor NKUA

THREE-MEMBER EXAMINATION COMITEE:

Anastasios Economou, Professor, NKUA

Evangelos Gikas, Professor, NKUA

Nikolaos S. Thomaidis, Professor, NKUA

DEFENDING DATE: 21/10/2021

ΕΡΕΥΝΗΤΙΚΗ ΕΡΓΑΣΙΑ ΔΙΠΛΩΜΑΤΟΣ ΕΙΔΙΚΕΥΣΗΣ

Ανάπτυξη και επικύρωση ενός αναλυτικού πρωτοκόλλου για τον ταυτόχρονο προσδιορισμό οργανικών ρύπων σε ζωικούς οργανισμούς με GC-APCI-QToFMS.

ΑΝΑΣΤΑΣΙΑ ΟΡΦΑΝΙΩΤΗ

ΑΜ:192214

ΕΠΙΒΛΕΠΩΝ ΚΑΘΗΓΗΤΗΣ:

Νικόλαος Σ. Θωμαΐδης, Καθηγητής, ΕΚΠΑ

ΤΡΙΜΕΛΗΣ ΕΞΕΤΑΣΤΙΚΗ ΕΠΙΤΡΟΠΗ:

Αναστάσιος Οικονόμου, Καθηγητής, ΕΚΠΑ

Ευάγγελος Γκίκας, Καθηγητής, ΕΚΠΑ

Νικόλαος Σ. Θωμαΐδης, Καθηγητής, ΕΚΠΑ

ΗΜΕΡΟΜΗΝΙΑ ΕΞΕΤΑΣΗΣ: 21/10/2021

ABSTRACT

Over the last decade, the development of high resolving power mass analyzers (HRMS) has contributed significantly to the expansion of the scientific interest from the restricted determination of Persistent Organic Pollutants (POPs) to the wide-scope screening of both POPs and Emerging Contaminants (ECs). In light of their potential risk to human and environmental health, action is urgently required for their monitoring. In the ECs' analysis field, there is a clear trend toward Liquid Chromatography (LC)-HRMS as the majority of ECs are more polar and less volatile. However, a wide range of highly used ECs, as well as POPs, are volatile and thermostable, therefore Gas Chromatography (GC)-HRMS strategies should be approached. So far, less polar and more volatile compounds remain unexploited, pinpointing the need for the establishment of efficient GC-HRMS workflows in order to extend the chemical domain of the applied screening approaches in monitoring studies.

This thesis reports the development of a generic analytical protocol for the simultaneous determination of non-polar organic micropollutants in fish tissues. Since the chemical domain of the analysis includes compounds with a wide variety of physicochemical properties, a generic sample preparation protocol should be followed. For sample treatment, an Accelerated Solvent Extraction protocol was used for the extraction of the analytes of interest followed by an in-cell clean-up step with three different sorbent materials. The resulting extracts were analyzed using Gas Chromatography coupled to an Atmospheric Pressure Chemical Ionization (APCI) interface, followed by a hybrid Quadrupole Time of Flight mass analyzer (GC-QToF MS).

The characteristics of the developed method, were evaluated with spiked samples of a representative group of compounds, for which reference standards were available. A smart validation dataset, including chemicals from different chemical classes of GC-amenable compounds, like Polycyclic Aromatic Hydrocarbons (PAHs), Polychlorinated Biphenyls (PCBs), Organochlorine Pesticides (OCPs), Polychlorinated Naphthalenes (PCNs) and Plant Protection Products (PPPs), was used for the validation experiments.

Following the aforementioned protocol, fish tissues from different regions of the Black Sea were analyzed for the determination of xenobiotics. The results indicate the overall contamination degree of this environmental compartment due to the presence of several non-volatile pollutants.

SUBJECT AREA: Environmental Analytical Chemistry

KEYWORDS: Method Development, Organic Micropollutants, Biota Specimens, GC-APCI-QToF MS

ΠΕΡΙΛΗΨΗ

Τα τελευταία χρόνια, η ανάπτυξη αναλυτών μάζας υψηλής διακριτικής ικανότητας (HRMS) συνέβαλε σημαντικά στην επέκταση του ενδιαφέροντος της επιστημονικής κοινότητας από τον περιορισμό στον προσδιορισμό των έμμονων οργανικών ρύπων (POPs) στον εκτεταμένο έλεγχο των αναδυόμενων ρύπων (ECs). Εξαιτίας της πιθανότητας να αποτελούν κίνδυνο για την ανθρώπινη υγεία και το υδάτινο περιβάλλον, απαιτείται επείγοντως δράση για την παρακολούθησή τους. Στην ανάλυση των αναδυόμενων ρύπων υπάρχει μια σαφής τάση προς την χρήση υγροχρωματογραφίας συζευγμένης με αναλυτές μαζών υψηλής διακριτικής ικανότητας LC-HRMS, καθώς η πλειονότητα αυτών των ουσιών είναι πιο πολικές και θερμικά ασταθείς. Ωστόσο πολλοί αναδυόμενοι ρύποι είναι πτητικοί και θερμοσταθεροί και επομένως απαιτείται η ανάπτυξη μεθόδων αεριοχρωματογραφίας συζευγμένης με αναλυτές μαζών υψηλής διακριτικής ικανότητας GC-HRMS. Μέχρι σήμερα, οι λιγότερο πολικές και πιο πτητικές ενώσεις της κατηγορίας των αναδυόμενων ρύπων δεν έχουν μελετηθεί επαρκώς, επισημαίνοντας την ανάγκη ανάπτυξης αποδοτικών μεθόδων GC-HRMS.

Σκοπός της παρούσας μελέτης ήταν η ανάπτυξη μίας νέας μεθοδολογίας για τον ταυτόχρονο προσδιορισμό μη πολικών οργανικών μικρο-ρύπων σε ιστούς ψαριών. Εφόσον το πεδίο της ανάλυσης περιλαμβάνει ενώσεις με διαφορετικές φυσικοχημικές ιδιότητες, μια γενικευμένη προκατεργασία δείγματος θα πρέπει να ακολουθείται. Για την προκατεργασία, χρησιμοποιήθηκε ένα πρωτόκολλο που περιλάμβανε την χρήση της τεχνικής Accelerated Solvent Extraction για την εκχύλιση των αναλυτών στόχων της μελέτης, ακολουθούμενης από ένα βήμα καθαρισμού εντός των κελιών της συσκευής, με τρία διαφορετικά προσροφητικά υλικά. Τα παραγόμενα εκχυλίσματα αναλύθηκαν χρησιμοποιώντας αέρια χρωματογραφία σε συνδυασμό με πηγή χημικού ιονισμού ατμοσφαιρικής πίεσης (APCI) συζευγμένης με υβριδικό Τετράπολο-Αναλυτή Χρόνου Πτήσης ανιχνευτή μαζών (GC-QToF-MS).

Τα χαρακτηριστικά της αναπτυγμένης μεθόδου αξιολογήθηκαν με την χρήση εμβολιασμένων δειγμάτων με αντιπροσωπευτικούς αναλύτες από διάφορες

κατηγορίες χημικών ενώσεων, για τους οποίους υπήρχαν διαθέσιμα πρότυπα αναφοράς στο εργαστήριό μας. Η επικύρωση της μεθόδου πραγματοποιήθηκε με πτητικούς αναλύτες προερχόμενους από διάφορες κατηγορίες, όπως Πολυκυκλικοί Αρωματικοί Υδρογονάνθρακες (PAHs), Οργανοχλωριωμένα Φυτοφάρμακα (OCPs), Πολυχλωριωμένα Διφαινύλια (PCBs), Πολυχλωριωμένα Ναφθαλένια (PCNs) και Φυτοπροστατευτικά Προϊόντα (PPPs).

Ακολουθώντας το προαναφερθέν πρωτόκολλο, ιστοί ψαριών από διαφορετικές περιοχές της Μαύρης Θάλασσας αναλύθηκαν για τον προσδιορισμό ξενοβιοτικών ενώσεων. Τα αποτελέσματα υποδεικνύουν τον συνολικό βαθμό ρύπανσης αυτού του περιβαλλοντικού διαμερίσματος, λόγω της παρουσίας αρκετών μη πτητικών ρύπων.

ΘΕΜΑΤΙΚΗ ΠΕΡΙΟΧΗ: Περιβαλλοντική Αναλυτική Χημεία

ΛΕΞΕΙΣ ΚΛΕΙΔΙΑ: Ανάπτυξη Μεθόδου, Οργανικοί Ρύποι, Ζωικοί Οργανισμοί, GC-APCI-QToF MS

CONTENTS

ABSTRACT.....	7
ΠΕΡΙΛΗΨΗ.....	9
INDEX OF TABLES	13
INDEX OF FIGURES.....	14
<i>Theoretical Part</i>	17
CHAPTER 1	18
Introduction.....	18
1.1 Persistent Organic Pollutants (POPs)	18
1.1.1. Polycyclic Aromatic Hydrocarbons (PAHs).....	20
1.1.2. Polychlorinated Dibenzo-p-Dioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs).....	21
1.1.3. Polychlorinated Biphenyls (PCBs)	21
1.1.4. Pesticides.....	22
1.1.4.1. Organochlorine Pesticides (OCPs).....	22
1.1.5 Legislation on POPs	23
1.2 Emerging Contaminants (ECs).....	25
1.2.1 Flame Retardants (FRs)	27
1.2.2 Chlorinated Paraffins (CPs)	28
1.2.3 Polychlorinated Naphthalenes (PCNs).....	28
1.2.4 Per- and Polyfluoroalkyl Substances (PFASs)	29
PFASs.....	29
1.2.5 Polybrominated diphenyl ethers (PBDEs)	30
1.3 Biota specimens as indications of environmental contamination.....	31
1.3.1 Bioaccumulation.....	31
1.3.2 Biomagnification	32
Chapter 2.....	33
Determination of POPs and ECs in biota matrices-Literature Review	33
2.1. Sample treatment.....	33
2.1.1 Sample pre-treatment	33
2.1.2 Sample extraction techniques	34
2.1.2.1. Pressurized Liquid Extraction (PLE)	34
2.1.2.1.1. Selective ASE.....	36
Temperature.....	37
Solvent	37
In-cell clean-up	37
2.2. Sample Analysis	38

2.2.1. Gas Chromatography	38
2.2.2. Ionization Technique of Atmospheric Pressure Chemical Ionization (APCI)	41
2.2.3. Mass Spectrometry.....	43
2.2.3.1. Low Resolution Mass Spectrometry (LRMS)	43
2.2.3.1.1. Quadrupole (Q).....	43
2.2.3.1.2. Triple Quadrupole (QqQ)	44
2.2.3.2. High Resolution Mass Spectrometry (HRMS)	45
2.2.3.2.1. Time-of-Flight (TOF).....	45
2.2.3.3. Hybrid Instruments	47
2.3. Acquisition Modes in HRMS	47
2.3.1. Data Dependent Acquisition (DDA)	47
2.3.2. Data Independent Acquisition (DIA)	48
2.4. Data Treatment-Approaches in HRMS screening	48
2.5. Literature Review.....	51
Chapter 3.....	63
Scope.....	63
<i>Experimental Part</i>	65
CHAPTER 4	67
Materials and Methods	67
4.1. Chemicals and Reagents.....	67
4.2. Biota Samples	69
4.3. Sample Pretreatment.....	69
4.4. Sample Preparation	70
4.5. Instrumental Analysis.....	72
4.6. Method Development.....	73
4.7 Method Validation	76
4.8 Target screening of fish samples	82
4.9 Identification criteria.....	82
4.7. Application to real samples	83
CHAPTER 5	84
Results and Discussion	84
5.1 General Observations.....	84
5.1 Method Development Results.....	84
5.2 Method Validation	87
5.2.1 Linearity.....	87
5.2.2 Limits of Detection and Limits of Quantification	93
5.2.3 Trueness.....	97

5.2.4 Matrix effect.....	100
5.2.5 Precision	103
5.3 Application of target screening in fish tissue samples.....	106
CHAPTER 6	109
Conclusions and Future Perspectives	109
ABBREVIATIONS AND ACRONYMS.....	111
References	113

INDEX OF TABLES

Table 1: Biota Matrices, Analytes, Clean-Up Techniques for Gas Chromatography Analysis.....	53
Table 2: Biota Matrices, Analytes, Clean-Up Techniques for Gas Chromatography Analysis.....	57
Table 3: Sorbents for in-cell clean-up using the ASE apparatus.	61
Table 4: Conditions which were used in Accelerated Solvent Extraction.	72
Table 5: Four examined subtests.	75
Table 6: Validation dataset.	79
Table 7: Number of detected compounds and %Recoveries in different temperatures.	86
Table 8: Calibration curves for the method using GC-APCI-QTOFMS.	89
Table 9: Total results of LODs and LOQs.....	94
Table 10: % Recoveries at four concentration levels.	97
Table 11: % Factor of Matrix Effect (FME%) at four concentration levels.	100
Table 12: Total results for Precision at two concentration levels, ($n_{\text{replicates}}=6$).	104
Table 13: Total results of the determination of organic compounds in fish samples.	107
Table 15: Abbreviations and acronyms.	111

INDEX OF FIGURES

Figure 1: Categories of POPs	19
Figure 2: Molecular formulas of 16 PAHs.	20
Figure 3: General formula of PCDDs and PCDFs	21
Figure 4: General formula of PCBs.....	22
Figure 5: Structures of OCPs.....	23
Figure 6: Distribution of synthetic chemicals and main transformations in the environment and the technosphere	27
Figure 7: PCNs structure.....	29
Figure 8: Molecular structures of PFOS and PFOA.....	30
Figure 9: Occurrence of PFASs in the environment	30
Figure 10: Molecular structure of PBDEs.....	31
Figure 11: Bioaccumulation and Biomagnification of ECs through the food chain.....	32
Figure 12: Stainless-steel cell of PLE.....	35
Figure 13: The steps involved in PLE of analytes, together with interferences, from a solid sample particle	35
Figure 14: ASE process.....	36
Figure 15: Typical ASE cell assembly	38
Figure 16: GC Instrumentation	39
Figure 17: Types of capillary columns	40
Figure 18: Schematic diagram of APCI in GC-MS.....	42
Figure 19: Reactions in APCI	42
Figure 20: Quadrupole mechanism	44
Figure 21: Linear QqQ instrument parts	45
Figure 22: Scheme of TOF instrument (Maxis Impact, Bruker)	46
Figure 23: Scheme of QqToF instrument (Maxis Impact, Bruker)	47
Figure 24: Proposed identification confidence levels in high resolution mass spectrometric analysis. Note: MS ² is intended to also represent any form of MS fragmentation (e.g., MS ^e , MS ⁿ)	49
Figure 25: Systematic workflow for target, suspect and non-target screening ..	50
Figure 26: Final Protocol.....	70
Figure 27: Dionex™ ASE™ 350 Accelerated Solvent Extractor.. ..	71
Figure 28: The GC-APCI-QToF-MS system	73
Figure 29: Process of Method Development.....	76
Figure 30: Evaluation of identification criteria for Penconazole	83
Figure 31: Diagram (% Recoveries, comparison of protocols with SPE and In-Cell Clean-Up)	85
Figure 32: Box-Plot (% Recoveries, comparison of 4 subtests).....	86
Figure 33: Linearity of the instrument for 4,4-DDE.....	87
Figure 34: LOD (µg/Kg w.w.) in fish tissues in the different chemical classes ..	96
Figure 35: Total results of % Recovery.	99
Figure 36: %FME at four concentration levels.....	103
Figure 37: Repeatability (RSDr %) for the method.	106
Figure 38: Chromatogram of PAH Phenanthrene.....	114
Figure 39: Chromatogram of PAH Fluorene.....	114
Figure 40: Chromatogram of 4,4-DDE.....	115

PREFACE

This master thesis was performed at the Laboratory of Analytical Chemistry (Department of Chemistry, National and Kapodistrian University of Athens, Greece), under the supervision of Professor Nikolaos S.Thomaidis.

First of all, I would like to thank my supervisor, Prof. N. S. Thomaidis, for his guidance and support during my master and for giving me the opportunity to become a member of his research group. Also, I would like to thank the members of the examination committee, Professor Anastasios Economou and Professor Evangelos Gikas for their comments and remarks regarding my master thesis.

Particularly, I would like to thank PhD candidate George Gkotsis for his constant assistance, guidance and encouragement. His advices and knowledge were valuable and had a significant impact in my personal improvement as an analytical chemist. Moreover, I would like to thank post-doctoral researcher Maria-Christina Nika for her help and support during the experimental process of this master thesis, as well as for the constant guidance whenever I needed it.

A special thanks to all of my colleagues and friends in TrAMS group, for providing a great work environment and an enjoyable atmosphere in the laboratory.

Last but not least, I would like to deeply thank my family and friends for their huge support and encouragement during all these years and their constant support through my life.

Theoretical Part

CHAPTER 1

Introduction

Over the last few decades, urbanization, higher consumer demands and increase in living standards have led to the uncontrolled production of innovative technologies and products, including chemicals, for the maintenance of this achieved quality of life, at any cost. The main victim of these practices is the wider ecosystem. The pollution of various environmental compartments has been amplified, due to the distribution of greenhouse gases, chemicals, hazardous wastes and non-biodegradable materials among others, posing a potential risk to human and wildlife health [1]. The environmental movement, probably, began with the publication of Rachel Carson's book "Silent Spring", in 1962, which described the detrimental effects of pesticides on the ecosystem, making a call to consider unintended consequences of the release of man-made compounds [2]. Since then, the scientific community has mobilized and has focused its interest on the control and monitoring of these life-threatening xenobiotics for their reduction and future elimination.

1.1 Persistent Organic Pollutants (POPs)

POPs are organic compounds of both natural (volcanic activity) and anthropogenic origin (industrial, household and agricultural activity) that possess a particular combination of physicochemical properties, such that, once released into the environment, they remain intact for exceptionally long periods of time, as they resist photolytic, chemical and biological degradation [3]. They are divided into two categories: **(i)** intentionally and **(ii)** unintentionally produced ones, as shown in **Figure 1**. In the first category, belong organochlorine compounds produced as products intended for usage. Representative examples are Organochlorine Pesticides (OCPs) and industrial chemicals. The second category consists of compounds considered to be unwanted by-products. They are divided into three subcategories: Polycyclic Aromatic hydrocarbons (PAHs), dioxins and furans [4].

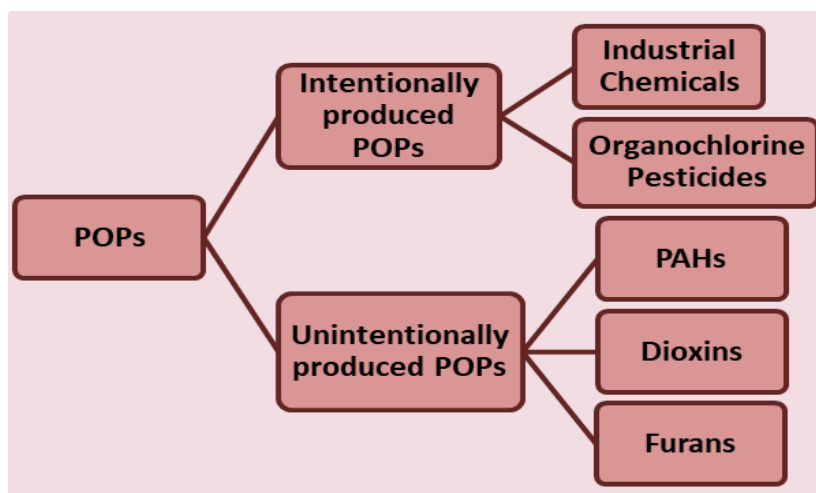


Figure 1: Categories of POPs [4].

POPs are a matter of concern owing to their toxicity and tendency to accumulate in biota tissues, ending up in the food chains. They are characterized as hormone disrupters, that can alter normal function of endocrine and reproductive systems. Additionally, they are accused of causing cancer, birth defects and learning, immunological, behavioral and neurological disabilities [4].

In Stockholm in 2004, has been signed the Stockholm Convention on POPs to reduce and/or eliminate the release of 12 original POP substances, named “the dirty dozen” [3]. This list includes nine Organochlorine Pesticides (OCPs), Polychlorinated Biphenyls (PCBs), Polychlorinated Dibenzo-dioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs). An addition of nine pesticides, flame retardants and surfactants occurred five years later. Moreover, Polychlorinated Naphthalenes (PCNs) were added in 2015 [5].

In 2014, the U.S Environmental Protection Agency (EPA) published a list of POPs for which the agency intends to promulgate discharge control standards. This publication refers to a list of 126 specific pollutants that includes heavy metals, organotins, chlorobenzenes, Volatile Organic Compounds (VOCs), alkylphenols, Polychlorinated Biphenyls (PCBs), pesticides, Polycyclic Aromatic Hydrocarbons (PAHs), and Polybrominated Diphenyl Ethers (PBDEs) [6].

1.1.1. Polycyclic Aromatic Hydrocarbons (PAHs)

PAHs are widespread environmental pollutants, produced by forest fires, volcanic activity and incomplete combustion of fossil fuels [7]. USEPA has listed 16 PAHs -naphthalene, acenaphthylene, acenaphthene, fluorine, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene, and indeno(1,2,3c,d)pyrene- as most priority ones to be analyzed in various environmental matrices [8,9]. Chemically, PAHs consist of two or more benzene rings bonded in linear, cluster, or angular arrangements [10]. Their molecular structures are presented in **Figure 2**. Because of their high hydrophobicity, they are accumulated in the fat tissue of aquatic organisms. As a result of their widespread presence and inclusion in European Union (EU) Directives, they are generally introduced in monitoring programs [8]. Finally, they are among the most notorious semi-volatile pollutants and are considered as hazardous air pollutants in the group of the non-halogenated organic compounds along with benzene, phenols and aldehydes, according to International Agency for Research on Cancer (IARC) [9].

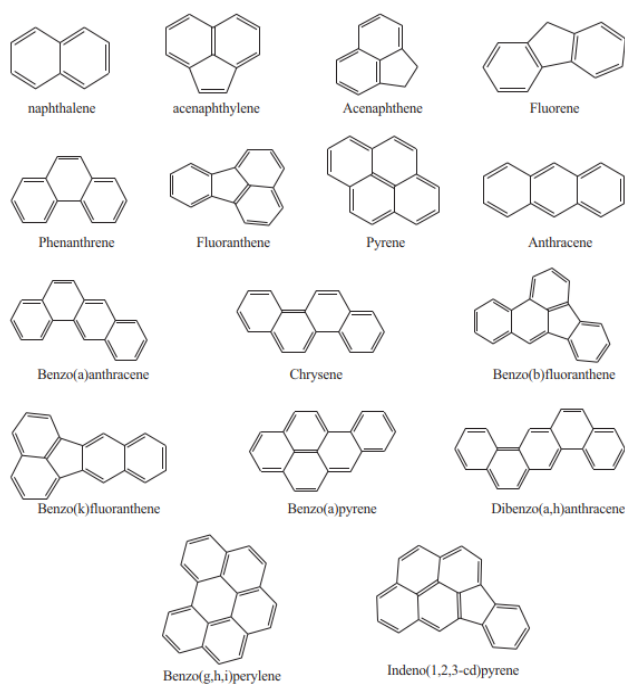


Figure 2: Molecular formulas of 16 PAHs [9].

1.1.2. Polychlorinated Dibenzo-p-Dioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs)

Polychlorinated Dibenzo-p-Dioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) constitute a class of ubiquitous pollutants with high chemical stability, extremely poor water solubility and tricyclic, chlorine-substituted benzene structure. The number of chlorine substituents on the benzene rings ranges from one to eight, resulting in 75 theoretical possible PCDD congeners and 135 possible PCDF congeners. Their structures are presented in **Figure 3** [11]. Both classes are formed unintentionally during industrial processes in the presence of chlorine and during incomplete combustion processes [12]. Dioxins have been reported as “the most toxic man-made compounds”. IARC named 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) as a known human carcinogen [11,13].

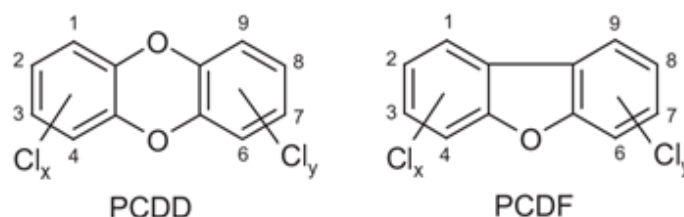


Figure 3: General formula of PCDDs and PCDFs [13].

1.1.3. Polychlorinated Biphenyls (PCBs)

PCBs comprise a class of 209 compounds produced from biphenyls by the replacement of hydrogens with chlorine atoms (**Figure 4**). Due to their chemical stability, insulating properties and non-flammability, they have been utilized in commercial and chemical industries for a variety of applications, such as in heat transfer, rubber and plastic products, hydraulic machines, dyes, pigments and carbonless copy paper [14]. In the 1970s and 1980s, production and export of PCBs was terminated in most countries, accused of environmental pollution. In the technical product and in environmental compartments, PCBs occur as mixtures [15]. The major route of human exposure to these harmful compounds is through diet, especially from meat, fish and dairy products. Besides their toxicity, several transformation products (TPs) of PCBs are potentially more

dangerous, by causing alterations of the cellular operations in lungs, interfering with estrogen receptors and influencing the reproductive system [14].

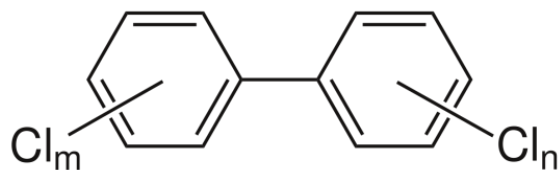


Figure 4: General formula of PCBs [16].

1.1.4. Pesticides

Pesticides constitute a broad group of compounds that includes any substance or mixture used to prevent, destroy, or control pests including insects, fungus, rodents or, unwanted species of plants causing harm during production and storage of crops. These compounds are classified according to the sources of origin as either being chemical pesticides or biopesticides. Despite their recognized importance, chemical pesticides are among the most important environmental pollutants, existing in huge quantities. They are further subdivided into pyrethroids, organochlorine, organophosphate and carbamate pesticides [17]. Although they are applied mostly on soils, they have a high potential to reach the aquatic environment, via runoff, agricultural storm-water discharges, and return flows from irrigated fields [2].

1.1.4.1. Organochlorine Pesticides (OCPs): encompass hexachlorocyclohexane (HCH), dichlorodiphenyltrichloroethane (DDT), Hexachlorobenzene (HCB), chlordanes, aldrin, dieldrin, toxaphene and endosulfans (**Figure 5**) [18]. Although most OCPs were banned about three decades ago, their presence and effects still linger on, due to their resistance to environmental degradation [19,20]. They result in a variety of toxic and adverse health effects, including carcinogenesis, immunological and reproductive disorders in living organisms including humans and wildlife [21].

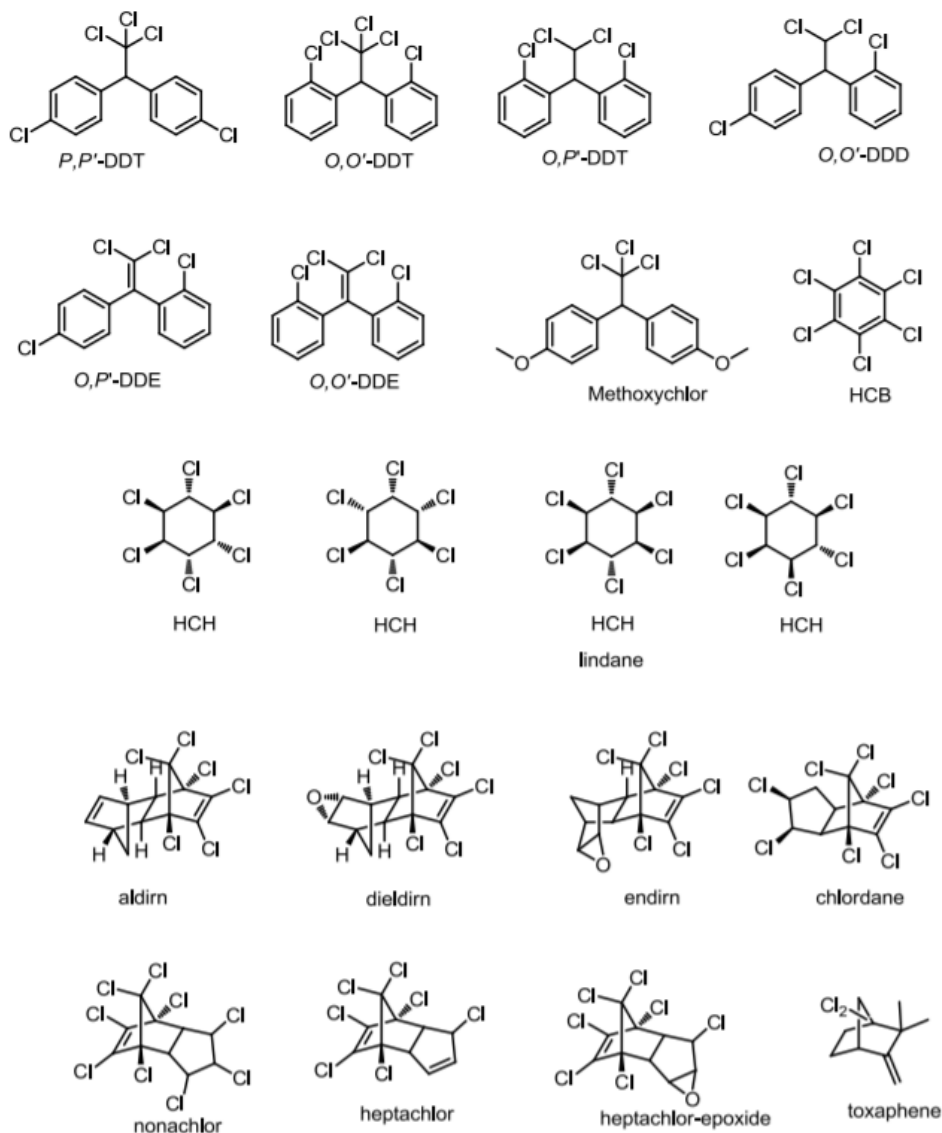


Figure 5: Structures of OCPs [22].

1.1.5 Legislation on POPs

The strong presence of POPs in the environment and their negative impacts on the quality of the ecosystem has caused worldwide interest. At European Union level, significant progress towards their elimination has been achieved. Several agreements, monitoring programs and regulations are in force for the control of these xenobiotics.

POPs are regulated worldwide by the Stockholm Convention, as mentioned earlier, and the UNECE (Aarhus) Protocol. The Executive Body to the UNECE Convention on Long-Range Trans-boundary Air Pollution (CLRTAP) adopted the UNECE Protocol on POPs on 24 June 1998 in Aarhus, Denmark. The UNECE Protocol on POPs, focuses currently on a list of 16 substances encompassing eleven pesticides, two industrial chemicals and three unintentional by-products. The ultimate objective of both agreements is to control, reduce or eliminate any discharges, emissions and losses of these POPs into the environment. The main legal instrument for implementing the Stockholm Convention and the UNECE Protocol on POPs is Regulation (EC) No 850/2004 on POPs (hereafter called the “POP Regulation”). This Regulation entered into force on 20 May 2004 and as a regulation it is directly applicable in all Member States, including those which are not yet parties to the Stockholm Convention or the UNECE POP Protocol. The POP Regulation contains provisions regarding production, placing on the market and use of chemicals, management of stockpiles and wastes and measures to reduce releases of unintentionally produced POPs. The POP Regulation has been amended several times. At the moment, Regulation (EU) 2019/1021, a recast of Regulation (EC) No 850/2004 is in force [23].

Under the POPs Regulation, ECHA helps to identify and propose new POPs from the EU to the Stockholm Convention. ECHA also supports in identifying the necessary future actions of the EU for the POPs Union Implementation Plan [24].

Since its publication in 2000, the Water Framework Directive(2000/60/EC) (WFD) and relative daughter Directives became the most important legal acts for water protection within the EU, establishing a framework for community action in the field of water policy. Within its strategy, the WFD identifies POPs that present a significant risk to the aquatic environment. The goal is to reduce the presence of all POPs at least below substance-specific Environmental Quality Standards (EQS). EQS are expressed as the concentration in water and mass fraction in biota of a particular pollutant (or group of pollutants) which

should not be exceeded in order to protect human health and the environment [25,26].

REACH Regulation (EC) No 1907/2006 provides provisions to ensure that industrial chemicals having POP characteristics are identified and prevented from being produced or imported in the EU. This regulation requires EU companies which manufacture or import chemical substances in quantities of one tone or more per year to register these substances and ensure that they can be used safely.

They exist also some EU regulations about some specific POPs, such as Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market and PCB Directive (Directive 96/59/EC) aiming for the approximation of the laws of the Member States on the controlled disposal of PCBs, the decontamination or disposal of equipment containing PCBs and/or the disposal of used PCBs in order to eliminate them completely [23].

1.2 Emerging Contaminants (ECs)

The development of new and more sensitive analytical techniques has evidenced the presence of a much larger number of potentially dangerous xenobiotic substances, globally known as Emerging Contaminants (ECs) [3]. According to Sauvé et al. there are three scenarios where the word “emergent” can be used: **i)** new or previously unknown compounds just recently reported, **ii)** known compounds for which the environmental contamination issues were not fully understood, and **iii)** “emerging issues” about contaminants where new information is jostling our understanding of their environmental and human health risk [27]. According to the NORMAN database, there are more than 700 compounds grouped in 20 classes of emerging pollutants that have been identified in the European aquatic environment [28]. They encompass a diverse group of compounds, including: Pharmaceuticals and Personal Care Products (PPCPs), illicit drugs and drugs of abuse, hormones and steroids, benzothiazoles, benzotriazoles, Polychlorinated Naphthalenes (PCNs),

Perfluorochemicals (PFCs), Polychlorinated Alkanes (PCAs), Polydimethylsiloxanes (PDMSs), synthetic musks, Quaternary Ammonium Compounds (QACs), Bisphenol A (BPA), triclosan, triclocarban, surfactants, Flame Retardants (FRs), plasticizers, as well as polar pesticides, veterinary products, industrial compounds, food additives, engineered nanomaterials and Transformation Products (TPs) [29,30].

Most ECs are produced and consumed in high amounts and, consequently, their introduction in the environment is continuous. Therefore, since they do not need to be persistent- to cause potentially negative effects- they are considered as pseudo-persistent contaminants [31,32]. The main sources are untreated urban wastewaters and wastewater treatment plants (WWTPs) (**Figure 6**), which normally are released into surface waters and then they end up into sediment, soil, groundwater and sea [33]. Once released into the environment, ECs are subject to degradation processes that contribute to their elimination. Depending both on the environmental compartment in which they are present [30] and on their physicochemical properties [34], different transformations can take place, sometimes producing products that can differ in their environmental behavior and ecotoxicological profile from their precursors [30].

ECs are currently not included in routine monitoring programmes and their fate, behavior and ecotoxicological effects are often not well understood. Moreover, for them, there is no appropriate legislation in comparison with POPs [33]. Detection, identification and quantification of ECs and their TPs in the various environmental compartments is essential for gaining knowledge on their occurrence and fate [34]. Therefore, one of the major trends in analytical chemistry is to develop fast and efficient procedures for the analysis of target and non-target ECs.

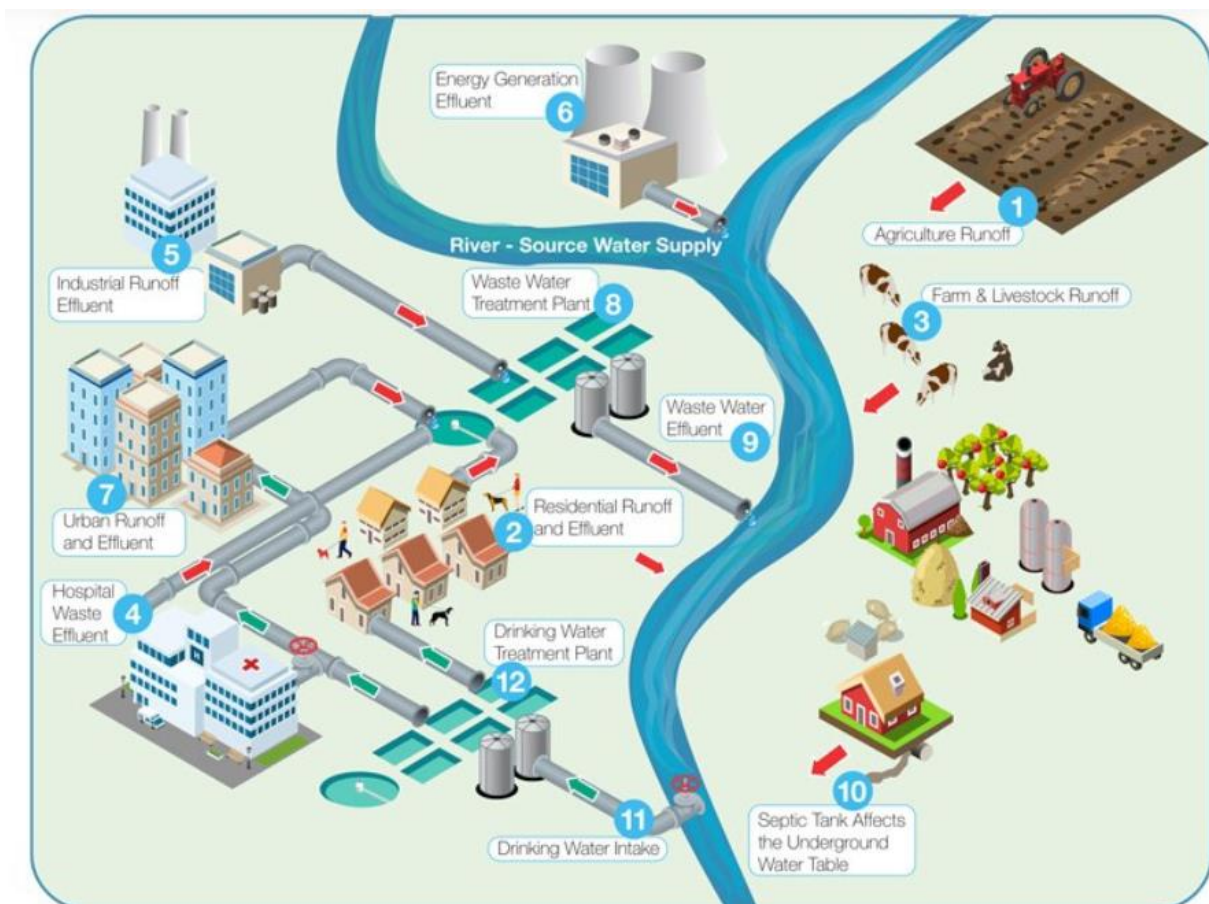


Figure 6: Distribution of synthetic chemicals and main transformations in the environment and the technosphere [35].

1.2.1 Flame Retardants (FRs)

FRs are chemical additives incorporated into polymers, in order to prevent combustion and to delay the spread of fire after ignition. With the gradual discontinuation of the use of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), due to their confirmed negative impacts, an urgent demand for alternative FRs has been increasing [36]. The alternative FRs can be divided into two major groups based on their structure: the halogenated FRs (HFRs), containing bromine or chlorine (or both), and the Organophosphorous FRs (OPFRs), containing one or more phosphate groups. The alternative FRs, 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), pentabromotoluene (PBT), 1,4-dimethyltetrabromobenzene (TBX) and hexabromobenzene (HBB) often have similar physicochemical properties as the restricted ones [37].

1.2.2 Chlorinated Paraffins (CPs)

CPs, also known as polychlorinated alkanes, are a complex mixture of different chlorinated hydrocarbons with variable composition, first introduced on the global market in the 1930s. This group contains many congeners with carbon chain length ranging from C10 to C30, diverse chlorination degree from 40% to 70% by weight, as well as structural and spatial isomers. The classification of CPs in general is based on chain length and is divided into three general groups: **(i)** short-chain CPs (SCCPs – chain length ranged from C10 to C13); **(ii)** medium-chain CPs (MCCPs – chain length from C14 to C17); **(iii)** long-chain CPs (LCCPs – chain length above C17). They are additives with high resistance and they are used in metalworking fluids, paints, and extreme-pressure lubricants, and as secondary plasticizers and flame retardants in plastics, sealants, and leather [38]. In 2017, members of the Persistent Organic Pollutants Review Committee (POPRC) of the Stockholm Convention classified SCCPs as POPs [39]. Moreover, they have also been qualified by members of the IARC as compounds belonging to group 2B – potentially possessing carcinogenic properties for humans [40]. They are also included in several regulatory lists, such as the European Water Framework Directive, in order to monitor them regularly in the European aquatic environment [41]. Considering the possible combinations of technical mixtures and the fact that more than one Cl can be attached to a carbon, the actual number of CP congeners in an environmental sample can be many tens of thousands, making CPs one of the most challenging groups of compounds to analyze [42]

1.2.3 Polychlorinated Naphthalenes (PCNs)

PCNs were first synthesized in 1833 as one of the first groups of industrial chemicals. Technical mixtures of PCNs have been used since the early 1900s as dielectric fluids, engine oil additives, electroplating masking compounds, wood, fabric, and paper preservatives preservatives, lubricants, and for dye production. There are 75 PCN congeners, substituted with one to eight chlorine atoms per naphthalene molecule (**Figure 7**). They are planar molecules composed of two six- membered connected aromatic rings with the chemical formula $C_{10}H_nCl_{(8-n)}$. Because of reports of a variety of serious health effects including chloracne, anorexia, nausea, headaches, weight loss, abdominal

pains, insomnia, alopecia, impotence, and mortality, and because of the development and increased production of supposedly less toxic PCBs and CPs, the use of PCNs began to decline in the mid '30s [42–44].

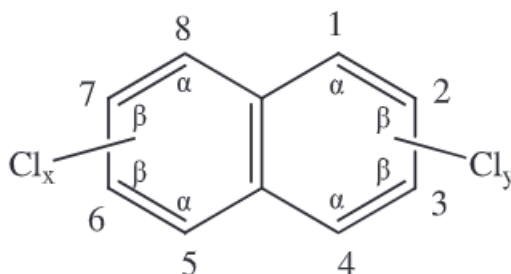


Figure 7: PCNs structure [44].

1.2.4 Per- and Polyfluoroalkyl Substances (PFASs)

PFASs are a class of synthetic fluorinated organic chemicals with unique properties, such as high surface activity, resistance to thermal and chemical degradation, and strong water and oil repellency [45]. They are chemically unusual compounds, as they are both hydrophobic and lipophobic, and they contain one of the strongest chemical bonds (C-F) known. The two most common groups of PFCs that are measured and detected in environmental matrices are Perfluoroalkyl Sulphonates (PFASs) with main representative compound Perfluorooctane Sulphonate (PFOS) and Perfluoroalkyl Carboxylates (PFACs), with main representative compound Perfluorooctanoic Acid (PFOA) [43]. Their structures are presented in **Figure 8**. Based on their persistence, toxicity, bioaccumulation, and capacity for long-range transport, PFOS and related substances were listed under Annex B (restriction of production and use) of the Stockholm Convention in 2009 [45]. Studies have shown that PFOA and PFOS, are a subject of concern, as described in Figure 9, due to their potential to cause liver cancer, affect the lipid metabolism, disturb the immune system, and reduce human fertility [2].

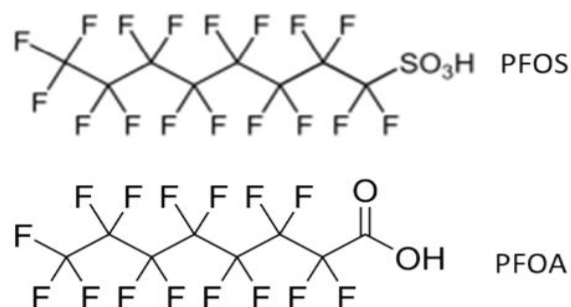


Figure 8: Molecular structures of PFOS and PFOA [46].

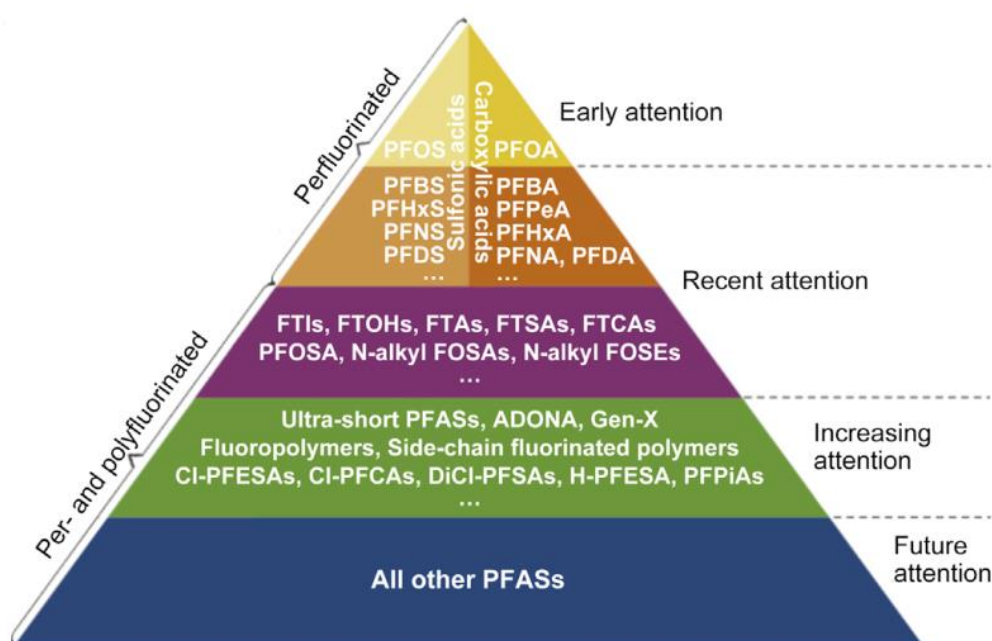


Figure 9: Occurrence of PFASs in the environment. [45].

1.2.5 Polybrominated diphenyl ethers (PBDEs)

PBDEs are a class of brominated flame retardants (BFRs) that were used in plastics, textiles, electronic circuitry, and other materials to reduce flammability of products. There are 209 PBDE congeners with a main molecular structure, shown in **Figure 10**. There is concern about their use, because of their widespread presence in the environment and in human and wildlife samples, as well as their presence in locations far from where they were produced or used [43,47].

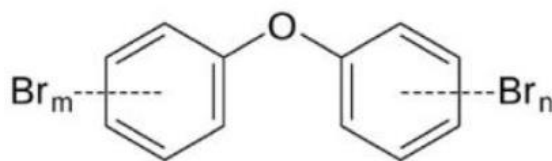


Figure 10: Molecular structure of PBDEs [48].

1.3 Biota specimens as indications of environmental contamination

An understanding of the spatial extent, magnitude, and source of contaminant exposure in biota is necessary to formulate appropriate conservation measures to reduce or remediate contaminant exposure [49]. Raptors (e.g. birds, fish, seals, otters, dolphins) are especially suitable for monitoring substances that are persistent and bioaccumulate in the environment, because **(a)** they are typically relatively long-lived, apex predators, **(b)** they effectively integrate contaminant exposure over time and over relatively large spatial areas, **(c)** they are easily captured which facilitates sampling (of blood, feather, muscle, liver, eggs) and **(d)** populations can be relatively easily monitored and quantified [50,51].

There are two terms which are mainly used for the description of the distribution of ECs in the environment; bioaccumulation and biodegradation.

1.3.1 Bioaccumulation: it is the simple uptake of substances from the environment or an accumulation over time or retention of the substance. Bioaccumulation factors are ordinarily calculated as the ratio of the compound of interest concentration in the biota sample to that in the surrounding media (e.g. soil or water). One criterion for bioaccumulation is the use of octanole water partition coefficient (K_{ow}) being > 5 as listed in Annex D of the Stockholm Convention [52].

1.3.2 Biomagnification: is defined as the transfer of xenobiotic substances from food, which can be also an organism or a part of it, to an organism in which a higher concentration, compared to the source concentration, will be found. It may be defined as an increase in the (fat-adjusted) internal concentration of a substance in organisms at higher trophic levels in a food chain. Longevity and size of the studied organism are examples for mechanisms provoking an increased concentration of contaminants [52].

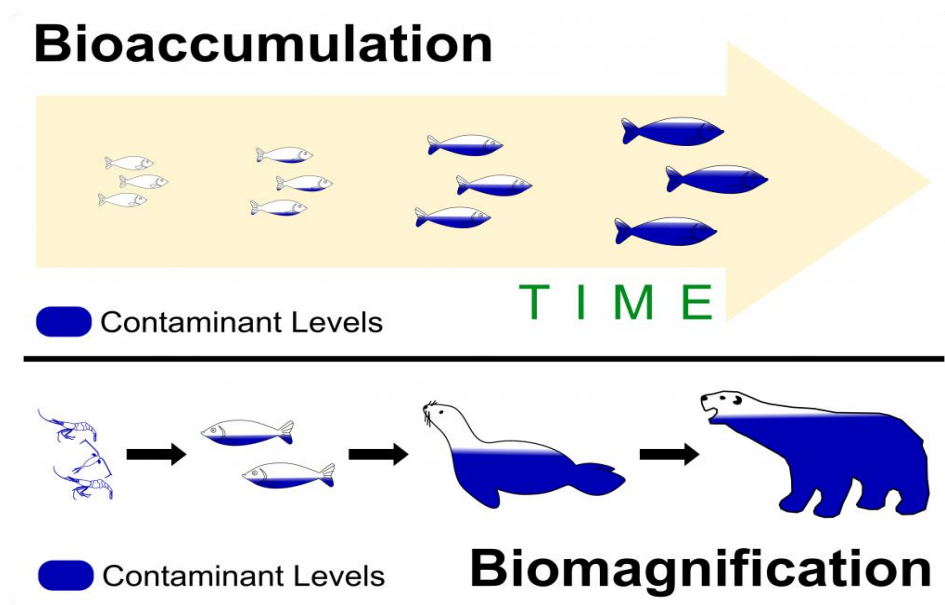


Figure 11: Bioaccumulation and Biomagnification of ECs through the food chain [53].

Chapter 2

Determination of POPs and ECs in biota matrices-Literature Review

The analysis of a wide range of organic contaminants in biota matrices is always a challenging task, not only due to the complexity of the samples, but also, due to the fact that these target analytes are present at trace levels. For this reason, generic and multi-residue methodologies must be developed and applied in monitoring programs in order to provide a broad and realistic knowledge about the overall pollution in a rapid, sensitive and selective way.

2.1. Sample treatment

Sample preparation is a crucial step in any analytical protocol designed for the trace analysis of organic contaminants. Usually, includes several steps; sample pre-treatment, extraction of analytes, and extract clean-up. Due to its numerous stages, sample preparation is the most error-prone, labor-intensive and time-consuming step in every analytical procedure. Therefore, automation can help eliminate or reduce possible inconsistencies, improve analytical throughput and reduce analytes' loss [54].

2.1.1 Sample pre-treatment

Pre-treatment, typically, involves homogenization followed by sample drying. The homogenization increases the surface area of the sample and enhances the accessibility of entrapped or bound analytes in sample matrices to the solvent(s). Sample drying is usually achieved with lyophilization. Moisture removal from homogenized samples eliminates the extraction variability among samples with different moisture contents [54].

2.1.2 Sample extraction techniques

Extraction step is of high importance, especially when a very complex matrix, like biota specimens, which contain fat matter, proteins, sugars etc., is analyzed [55]. As general considerations, the extraction is necessary, in order to guarantee the removal of interferences that could affect the determination of the analytes, the enrichment of target compounds to detectable concentration levels and the solvent switching, in order to meet the characteristics of the available instrumental detection [55,56].

As has been mentioned in several publications, the most common extraction techniques applied to solid and semisolid matrices include Soxhlet extraction, Supercritical Fluid Extraction (SFE), Microwave Assisted Extraction (MAE), Pressurized Liquid Extraction (PLE) and Matrix Solid-Phase Dispersion (MSPD) [7,57–59].

2.1.2.1. Pressurized Liquid Extraction (PLE)

One of the most powerful and relatively new techniques used for the extraction of micropollutants from biota specimens is Pressurized Liquid Extraction (PLE), or Accelerated Solvent Extraction (ASE), as it was originally launched by Dionex Inc. in 1995 [57]. It uses elevated temperature and pressure to achieve extraction from solid and semi-solid matrices in very short periods of time [59,60]. The extraction process is conducted in a tightly closed stainless-steel cell, as presented in **Figure 12** [56]. High temperature increases the solubility of the analytes, promotes a higher diffusion rate and aids the breaking of matrix-analyte interactions. Furthermore, high pressure keeps the solvent below its boiling point, maintaining its liquid phase. These conditions, also reduce the viscosity and the surface tension of the solvent. Thus, the solvent can penetrate more efficiently in the matrix structure, enhancing the extraction of target analytes, as shown in **Figure 13** [57,61,62].



Figure 12: Stainless-steel cell of PLE [63].

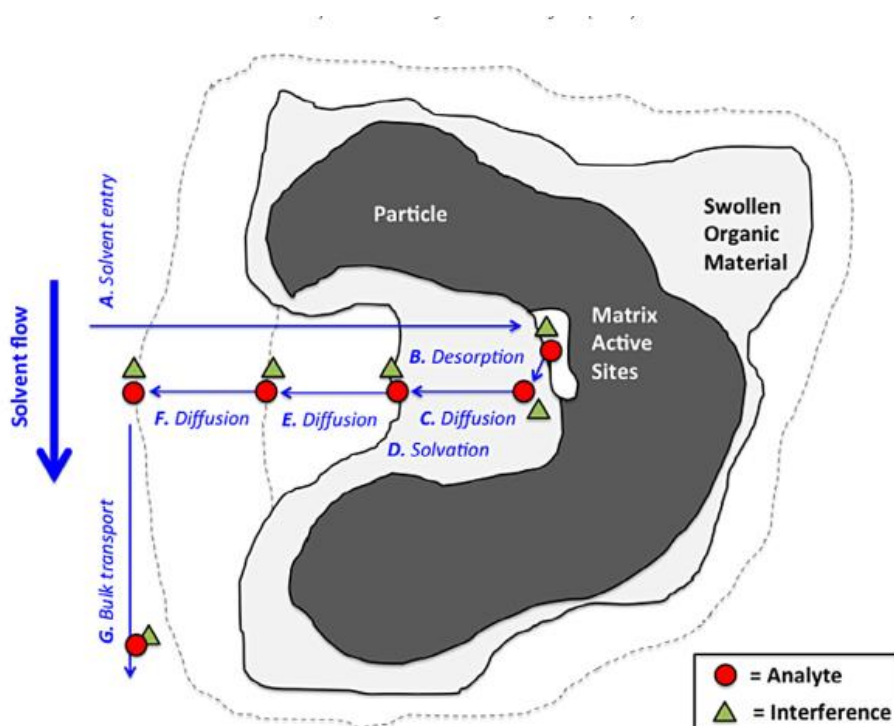


Figure 13: The steps involved in PLE of analytes, together with interferences, from a solid sample particle [54].

Figure 14 provides an overview of the general steps involved in the extraction of an analyte, by the ASE apparatus.

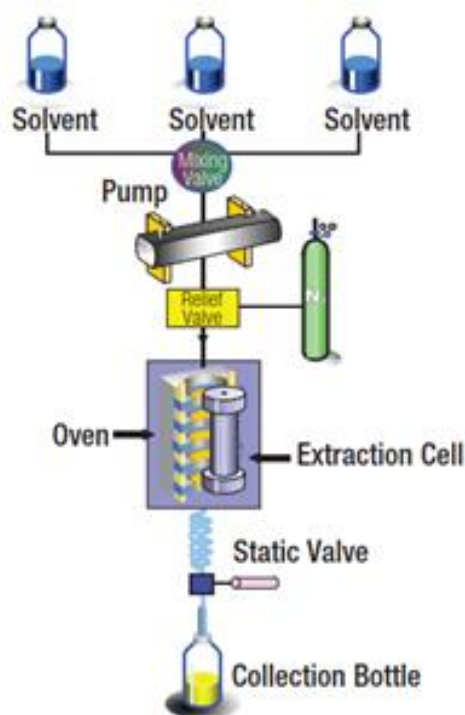


Figure 14: ASE process [59].

ASE is an attractive technique, due to its unique characteristics. It is fast (e.g. extraction time is ~15 min/sample), uses less solvent volume (~20 mL), no filtration is needed after extraction, the instrumentation allows extraction in unattended operation (at least 24 samples can be processed sequentially) and different sample sizes can be accommodated (e.g. 11, 22 and 33 mL vessels are available). However, its main two disadvantages are the restricted selectivity, which means that a further clean-up step is required, and the high purchasing cost in comparison with other extraction techniques [59].

2.1.2.1.1. Selective ASE

There are three parameters to optimize, so as to obtain a more selective extraction of the target compounds in ASE systems; temperature, solvents, and

use of adsorbent materials for the clean-up of extracts. Of course, the most powerful strategy is to use an appropriate combination of all three [59].

Temperature: Temperature is one of the most important parameters to be optimized, because it modifies the physicochemical properties of the solvents, which influences the efficiency of all steps described earlier. It should be considered that, although an increase in temperature may increase the solubility of the analyte, it may also increase the solubility of other undesired matrix compounds. However, a lower temperature would increase the extraction time. Furthermore, the use of high temperatures must be carefully evaluated when the target compounds are thermolabile since degradation of the analyte can occur during the extraction. In a common ASE system, temperature elevates up to 200°C [56,61].

Solvent: The type of solvent, or mixture of solvents used is crucial to achieve an efficient recovery. As in traditional extractions, solvent polarity has to be similar to the analytes; “like dissolves like”. However, it is also significant to obtain an “equilibrium” so that the solvent not only extracts the maximum amount of analyte possible, but also the minimum of other interfering compounds [60]. Several solvents such as methanol, water, toluene, dichloromethane, ethyl acetate and acetonitrile have been used [56,62].

In-cell clean-up: Advances in ASE systems have demonstrated the potential to selective removal of interferences during the sample extraction, i.e., combination of the extraction and clean-up into a single step. To use ASE as a method for extraction and clean-up combined in a single or in multiple steps, solid phases and/or filters should be added to the PLE cell. This process is referred to as “in-cell clean-up”. Typically, the adsorbent is loaded into the sample cell first and the sample is loaded on top of the adsorbent. This way, the flow of solvent during the extraction is such that unwanted compounds are retained in the cell by the adsorbent. Some adsorbents that have been used include copper, alumina, C₁₈ resin, florisil, silica gel, acid-impregnated silica gel, and ion-exchange resins [56,59].

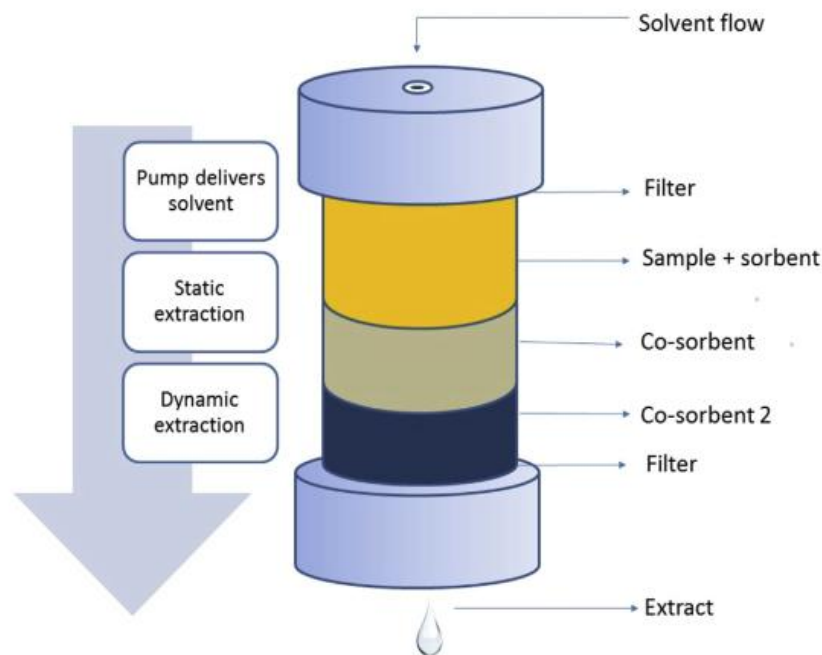


Figure 15: Typical ASE cell assembly [56].

2.2. Sample Analysis

2.2.1. Gas Chromatography

Gas chromatography (GC) is one of most widely used techniques for qualitative and quantitative analysis, based on the separation of a mixture in its components, due to their different boiling points [64]. During a GC separation, the sample is vaporized and injected right into the head of a chromatographic column. Elution is brought about by the flow of an inert gaseous mobile phase, which does not interact with analyte's molecules; its only function is to transport the analyte through the column. Column temperature is an important parameter that must be controlled, during a GC separation. Thus, the column is housed in a thermostated oven. The optimum column temperature depends on the boiling point of the target compounds and the degree of separation required. Roughly, a temperature equal to or slightly above the average boiling point of a sample results in a reasonable elution time. For samples with a broad boiling range of their analytes, it is often desirable to use a temperature program, whereby the column temperature is increased either continuously or gradually as the separation proceeds.

There are two types of gas chromatography:

- ✓ Gas-Solid Chromatography (GSC): the stationary phase is solid. The retention of analytes takes place as a result of their adsorption/desorption from the solid phase.
- ✓ Gas-Liquid Chromatography (GLC): the stationary phase is liquid and holds on a finely divided inert solid support. The retention of analytes is based on solute partitioning between mobile (gas) and liquid phase. GLC is most common used in sciences and simply is mentioned as GC.

A GC instrument (**Figure 16**) consists of simple components. Usually helium, hydrogen or nitrogen gas compressed in cylinders is used as the carrier gas. A GC column is attached to the injection port and samples are introduced into the carrier gas stream at a temperature sufficient to ensure vaporization of all analytes. Typically, the sample is introduced with a microliter syringe which is forced through a rubber septum at the injection port. An attached detector right after the column exit monitors each individual compound that elutes from the column. The detector must be insensitive to the carrier gas, while detecting the eluted analytes. A recording of its response to the analytes with time forms a chromatogram. The chromatogram contains the analytical data for the components of a mixture. Qualitative information appears in the characteristic retention time of each compound. Quantitative information is contained in its peak area [65].

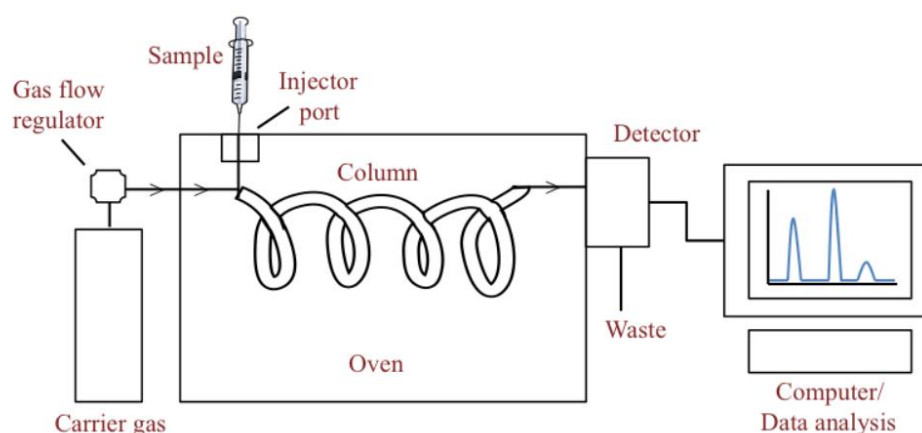


Figure 16: GC Instrumentation [66].

2.2.1.1 GLC column

As was mentioned earlier, the column is where the chromatographic separation occurs. The liquid stationary phase is coated on the inside wall of the capillary column and the inert mobile phase flows through the hollow tube. There are two general types of columns; packed and capillary ones. Packed columns contain a finely divided, inert, solid support material coated with liquid stationary phase. Most packed columns are 1.5 - 10m in length and have an internal diameter of 2 - 4mm. Typical dimensions for capillary columns range from 15 to 60 meters in length and from 0.20 to 0.53 mm in internal diameter. Capillary columns can be one of two types (**Figure 17**):

- ✓ wall-coated open tubular (WCOT)
- ✓ support-coated open tubular (SCOT)

WCOT columns consist of a capillary tube whose walls are coated with liquid stationary phase. In SCOT columns, the inner wall of the capillary is lined with a thin layer of support material such as diatomaceous earth, onto which the stationary phase has been adsorbed. SCOT columns are generally less efficient than WCOT columns. Both types of capillary column are more efficient than packed columns.

The chemical nature of the stationary phase has a great impact on the quality of the separation. The stationary phase usually has a thickness ranging from 0.1 to 1 μm [67].

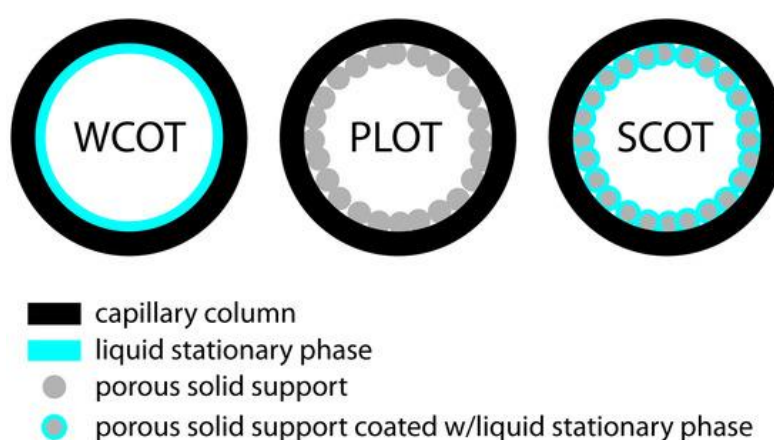


Figure 17: Types of capillary columns [68].

2.2.2. Ionization Technique of Atmospheric Pressure Chemical Ionization (APCI)

APCI was developed by Horning in the 1970s. Since then, the technique has been an invaluable tool in the development of interfaces for separation procedures [69]. The difference between APCI and other ionization techniques lies in the fact that the ionization process occurs at atmospheric pressure conditions and outside the vacuum system. A schematic illustration of the APCI source is presented in **Figure 18** [70]. This ionization technique involves three primary steps: **i)** conversion of the analyte to a gas-phase species (nebulization and desolvation), **ii)** ionization of the analyte through gas-phase ion–molecule reactions at atmospheric pressure and **iii)** extraction of the ions into the mass spectrometer with simultaneous exclusion of most of the nonionized species [71]. It can work in two ionization modes; positive and negative.

Ionization in positive mode happens by reaction of the analyte with protonated solvent molecules, generally giving rise to an abundant protonated analyte molecule $[M+H]^+$ or adduct ions like $[M+NH_4]^+$, $[M+Na]^+$, $[M+K]^+$ if ammonium, sodium and potassium salts are added to the solvent. In the negative mode, ions are generated by proton abstraction by oxygen ions O_2^* or by the formation of adducts with anions such as acetate or chloride present in the sample or solvent.

When the reagent gas is nitrogen, electrons produced from the corona discharge needle could ionize nitrogen plasma, creating N_2^{*+} and N_4^{*+} [Eqs. (1) and (2)]. These primary ions collide with the vaporized solvent molecules to form secondary reactant gas ions - e.g. H_3O^+ and $(H_2O)_nH^+$ [Eq. (3)]. These reactant gas ions then undergo repeated collisions with the analyte resulting in the formation of analyte ions. With the presence of water, H_2O^{*+} is formed by the charge transfer, and then reacts with another molecule of water to produce H_3O^+ [Eqs. (4) and (5)]. The formed H_3O^{*+} would further react with more water molecules to give water clusters $H_3O^+(H_2O)_n$, which ionizes the analyte molecule by the proton transfer. The high frequency of collisions results in a high ionization efficiency and thermalization of the analyte ions. This results in spectral data typically rich in predominantly molecular species and adduct ions with very little fragmentation, categorizing the APCI in low energy (soft) ionization sources [70].

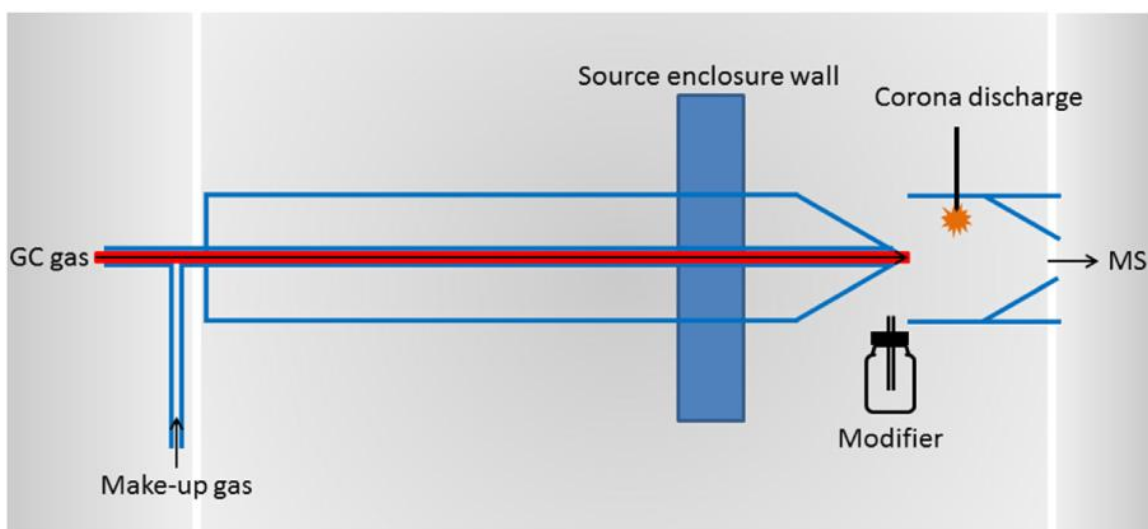


Figure 18: Schematic diagram of APCI in GC-MS [70].

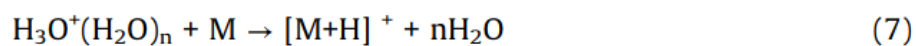


Figure 19: Reactions in APCI [70].

APCI has primarily been used to interface an MS with LC. However, GC/APCI was never fully commercialized, probably because of the high costs of the specialized instrumentation needed for these analyses at that time. Nowadays, new APCI sources are commercially available and capable to be interfaced with both GC and LC instruments. This fact adds versatility and extends analytical

capabilities providing flexibility to determine volatile and semi-volatile compounds of low and intermediate polarity, traditionally analyzed by dedicated vacuum GC-MS instruments [72].

2.2.3. Mass Spectrometry

Mass Spectrometry is based on the vacuum separation of ions, in the gas phase, according to their mass-to-charge (m/z) ratio. It can be divided into two groups, depending on the mass measurement: Low Resolution Mass Spectrometry (LRMS) and High Resolution Mass Spectrometry (HRMS) [73].

2.2.3.1. Low Resolution Mass Spectrometry (LRMS):

LRMS measurements provide information about nominal mass of the analyte i.e., the m/z for each ion is measured to single-digit mass units. The most common LRMS instruments are Quadrupole (Q), Triple Quadrupole (QqQ), and Ion Trap (IT).

2.2.3.1.1. Quadrupole (Q)

A quadrupole analyzer is typically limited to unit mass resolution measurements, but its excellent linear dynamic range, sensitivity, ease of use and low operating costs make it a popular choice for initial screening using targeted compound analysis and full scan applications [64]. However, in some cases it suffers from limitations in terms of selectivity and sensitivity, mainly because of interfering matrix components [74]. A quadrupole analyzer (**Figure 20**) uses a combination of radio frequency alternating current (AC) and direct current (DC) voltages as a mass filter, for separating ions. The quadrupole consists of four parallel rods. The positive DC voltage is applied on two opposite rods, and the same value of the negative DC voltage is applied on the remaining two rods. The AC is connected to all four rods. Combined DC and RF potentials on the quadrupole rods can be set to pass only a selected m/z ratio. All other ions do not have a stable trajectory through the quadrupole mass analyzer and

will collide with the quadrupole rods, never reaching the detector [75]. One of the major reasons why low resolution GC-MS methods dominate in environmental applications is due to the successful use of extensive mass spectral libraries obtained under standardized electron ionization conditions and nominal resolution [76].

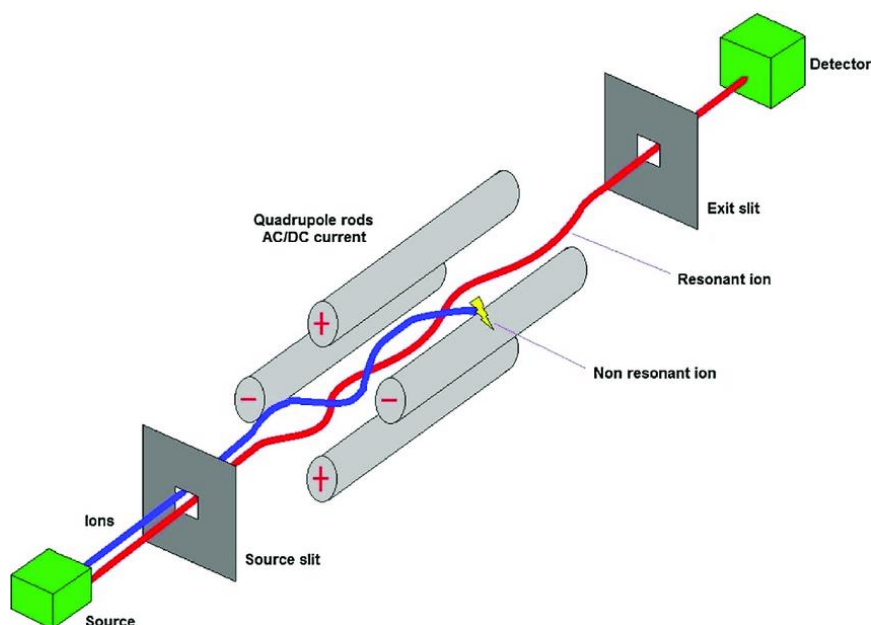


Figure 20: Quadrupole mechanism [77].

2.2.3.1.2. Triple Quadrupole (QqQ): A QqQ mass spectrometer (**Figure 21**) offers MS/MS in which the first and third Qs act as mass filters, while the second Q is used for fragmentation of the precursor ion through interaction with a collision gas (usually nitrogen or argon). The main MS/MS scan modes are product ion, precursor ion, neutral loss, single reaction monitoring (SRM), multiple reaction monitoring (MRM), and MSⁿ scans. The main benefits of analysis in MS/MS mode are minimum matrix component interferences, and at the same time, thanks to the possibility of selecting suitable precursor and product ions, possible identification and quantification of the above-mentioned contaminants even at (ultra)trace concentrations [74]. The unit mass resolution achieved by the typical Q instruments corresponds to 0.7Da (full width at half maximum (FWHM)). However, with the advanced QqQ instruments, a resolution up to 0.1Da (ultraselective) could be obtained [78].

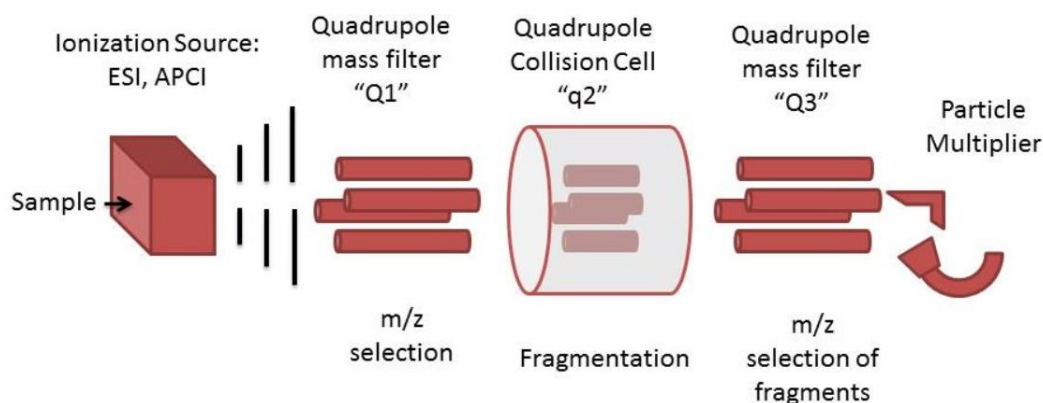


Figure 21: Linear QqQ instrument parts [79].

2.2.3.2. High Resolution Mass Spectrometry (HRMS)

HRMS provides information about the exact mass of the analyte, i.e., the m/z of each ion is measured from four to six decimal points. As a result, these instruments offer high sensitivity, fewer interferences and thus improved accuracy of the measurement but at much higher cost [74]. The HRMS instruments that have been used mostly are Time-of-Flight (TOF) and Orbitrap mass analyzers.

2.2.3.2.1. Time-of-Flight (TOF)

TOF (**Figure 22**) is based on the fact that ions with the same energy, but different mass, travel with different velocities. Basically, ions formed by a short ionization event are accelerated by an electrostatic field to a common energy and travel over a drift path to the detector. The lighter ones arrive before the heavier ones, and a mass spectrum is recorded. Measuring the flight time for each ion allows the determination of its mass. This cycle is repeated with a repetition rate that depends on the flight time of the highest mass to be recorded. The enhancement in the mass resolution is obtained by using reflectron (ion mirror). The reflectron is a series of ring electrodes with increasing voltage that creates retarding fields. The higher-energy ions reaching the reflectron area penetrate more deeply inside, and this results in extension of the time until they are reflected. Due to this phenomenon, the ions of the same m/z value with different initial energies hit the detector at almost the same time. The flight times of the ions separated

in a field-free region are proportional to the square root of the respective m/z value [80]. The inherent characteristics of TOF/MS are its high sensitivity in scan mode (all ions are detected), theoretically unlimited mass range as well as high acquisition speed (the duty cycle of modern instruments can attain 100 Hz). In addition, high-end TOF instruments afford resolving power of 40,000-60,000 and mass accuracies below 2 ppm [81]. Furthermore, the full spectrum dataset remains and offers the analyst the possibility of performing a retrospective analysis, i.e. to make a careful examination of old raw data looking for the presence of any compound that becomes interesting later [72]. The ability of the TOF to collect data for an entire mass range gives them the excellent potential as a tool for non-targeted screening and for the simultaneous determination of multiple POPs [64]. The only drawback to TOF analyzers is its limited dynamic range and quantitative performance [81].

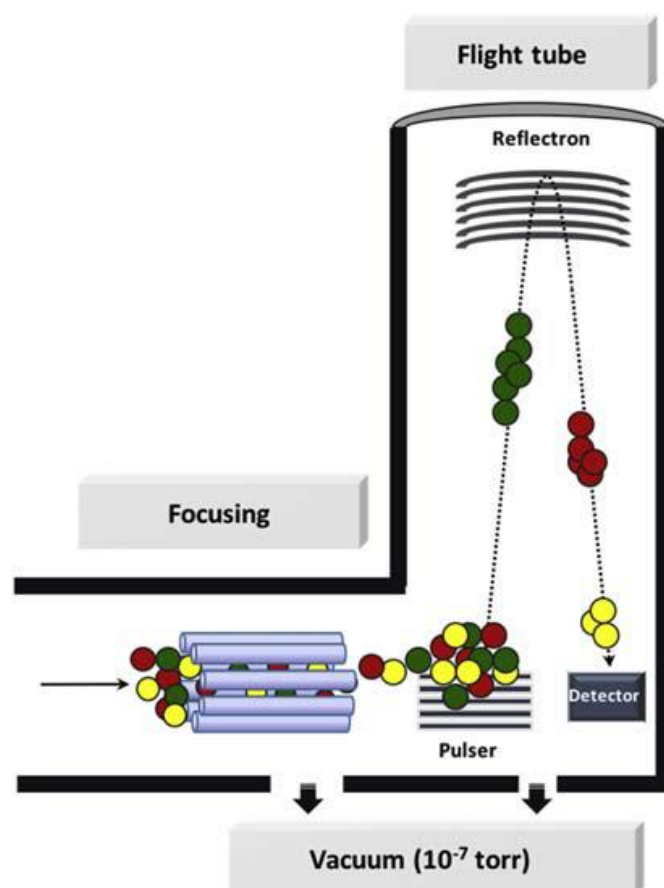


Figure 22: Scheme of TOF instrument (Maxis Impact, Bruker) [82] .

2.2.3.3. Hybrid Instruments

The coupling of two different analyzers is known as hybrid instrument. Tandem instruments usually consist of two quadrupole mass analyzers, sandwiching an ion guide collision cell. An example is QqTOF (**Figure 23**), where the first Q is used for initial mass selection and focusing, the second serves as a collision cell filled with a collision gas (helium, nitrogen, oxygen or argon) to produce specific fragments that are subsequently mass separated by the third mass analyzer, TOF [64]. An advantage is the high resolving power of TOF, typically in the range 20,000-40,000. As a result, interfering peaks from ions having the same nominal mass can be resolved, thus improving the signal to noise ratio [75].

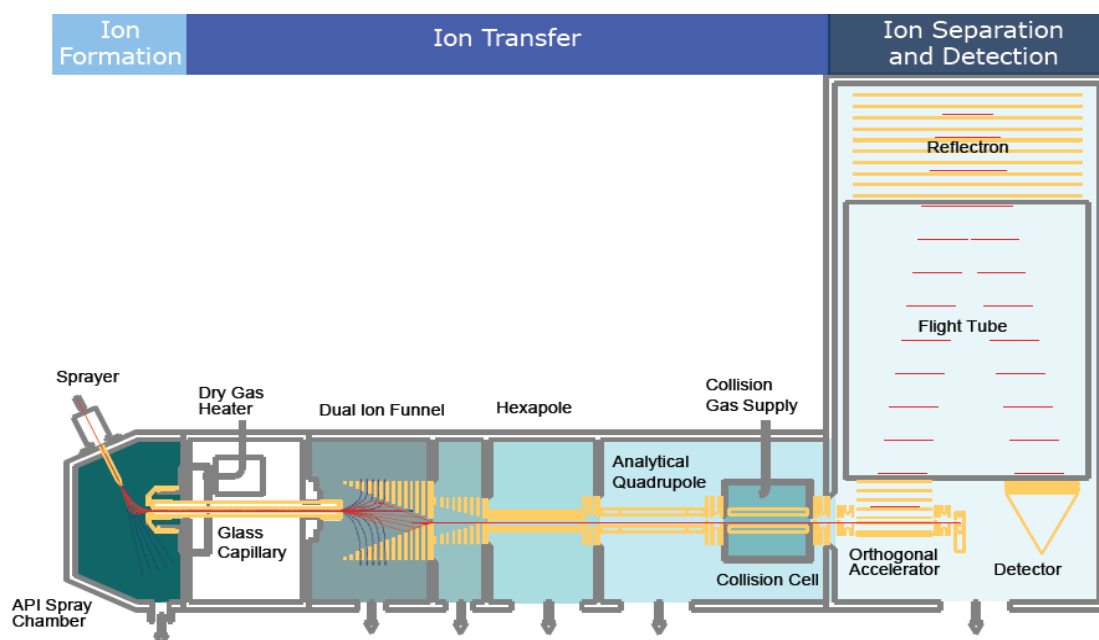


Figure 23: Scheme of QqTOF instrument (Maxis Impact, Bruker) [83].

2.3. Acquisition Modes in HRMS

2.3.1. Data Dependent Acquisition (DDA): In this acquisition, there is firstly a full scan which is defined as the survey scan and data are processed “on-the-fly” to determine the candidates of interest based on predefined selection criteria, such as intensity threshold or suspect inclusion list. If the selection

criteria are met, MS/MS analysis is then triggered and MS/MS scans (data-dependent) are performed. With this acquisition, 'clean' spectra with structural information are obtained in one injection. However, if the number of candidates of interest is big, the number of scans is decreased, so there are less data points that affect the detectability of the chromatographic peak [84].

2.3.2. Data Independent Acquisition (DIA): With this acquisition, there is no need to pre-select the precursor ion. Full-scan spectra at different collision energies are obtained in one injection. This acquisition provides simultaneously accurate mass data of parent compounds and fragment ions in a single run using two scans, one at low and one at high collision energy. By applying low energy in the collision cell, no fragmentation is performed. A full-scan spectrum is obtained that provides information for the parent ion (the (de)-protonated molecule) and, in some cases, the adduct ions and the in-source fragments. By applying high energy in the collision cell, fragmentation is performed and a spectrum similar to MS/MS experiments is obtained. This approach is called all-ions MS/MS, MSE or bbCID, according to the QTOF manufacturer [84].

2.4. Data Treatment-Approaches in HRMS screening

The HRMS based identifications of emerging contaminants and their transformation products vary among analytes and scientific projects, because, in many cases, it is impossible to synthesize and verify each compound. Schymanski et.al. 2014, suggested a system for the identification of emerging contaminants and their transformation products, in order to facilitate the communication between the various scientific groups. This system is consisted of the following 5 levels (**Figure 24**):

- ❖ **Level 1:** corresponds to the confirmed structure by the use of a reference standard with MS, MS/MS and retention time matching.
- ❖ **Level 2:** refers to a probable structure using literature or diagnostic data.

- ❖ **Level 3:** refers to tentative candidate(s) with possible, not exact, structures as the experimental information is insufficient to the exact proposal.
- ❖ **Level 4:** describes the case of an unambiguous formula which is assigned by the spectral information but there is no sufficient evidence to propose possible structures.
- ❖ **Level 5:** refers to the exact mass (m/z), which is detected in the sample, but no experimental information exists, in order to propose even a formula [85].

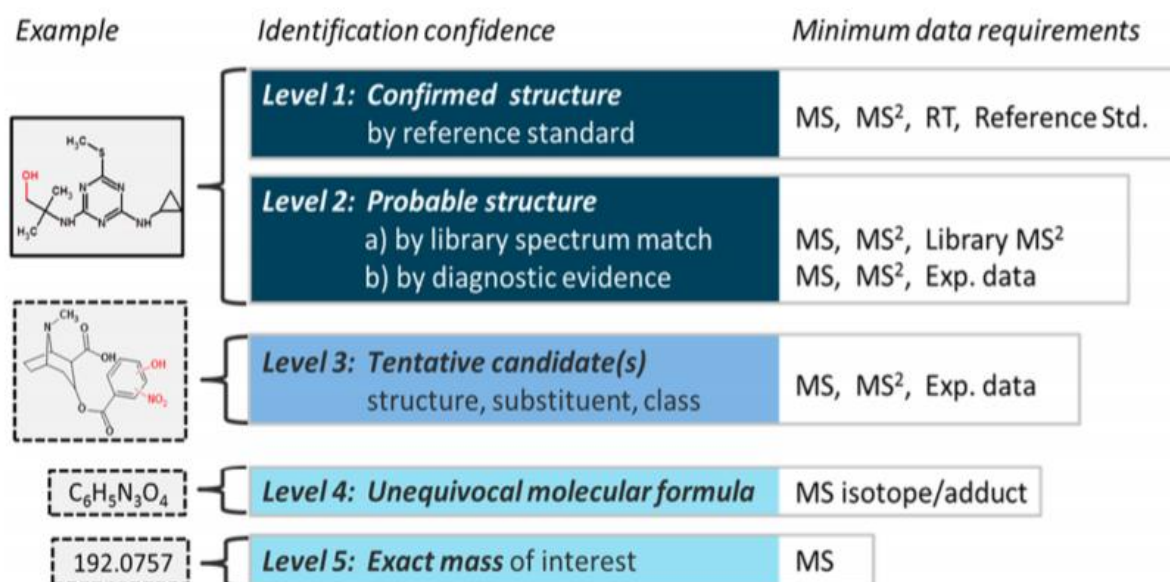


Figure 24: Proposed identification confidence levels in high resolution mass spectrometric analysis. Note: MS² is intended to also represent any form of MS fragmentation (e.g., MS_e, MS_n) [85].

In the literature, there are three approaches normally used for the analysis of compounds after the sample preparation; target analysis, suspect and non-target screening. The strategy for each workflow is depending on the instrumentation and the software, which is available in the laboratory. A systematic workflow for all three approaches is depicted in **Figure 25**.

✓ **target analysis**, in which an in-house developed database is used for the screening of a large number of compounds [84–86].

✓ **suspect screening**, in which a list of suspect compounds that are possible to be found in specific samples is built, according to literature and prediction models. The screening is based only on the exact m/z of the expected ions, which are usually the pseudo-molecular ions $[M+H]^+$ [84–86].

✓ **non-target screening**, in which samples are searched for contaminants and their transformation products without any previous information on them. These unknown compounds are actually new, unexpected or not searched ones in specific samples. Identification is a challenge in this approach, as more than one elemental formula and several plausible structures are obtained for a given unknown compound detected in a sample [84–86].

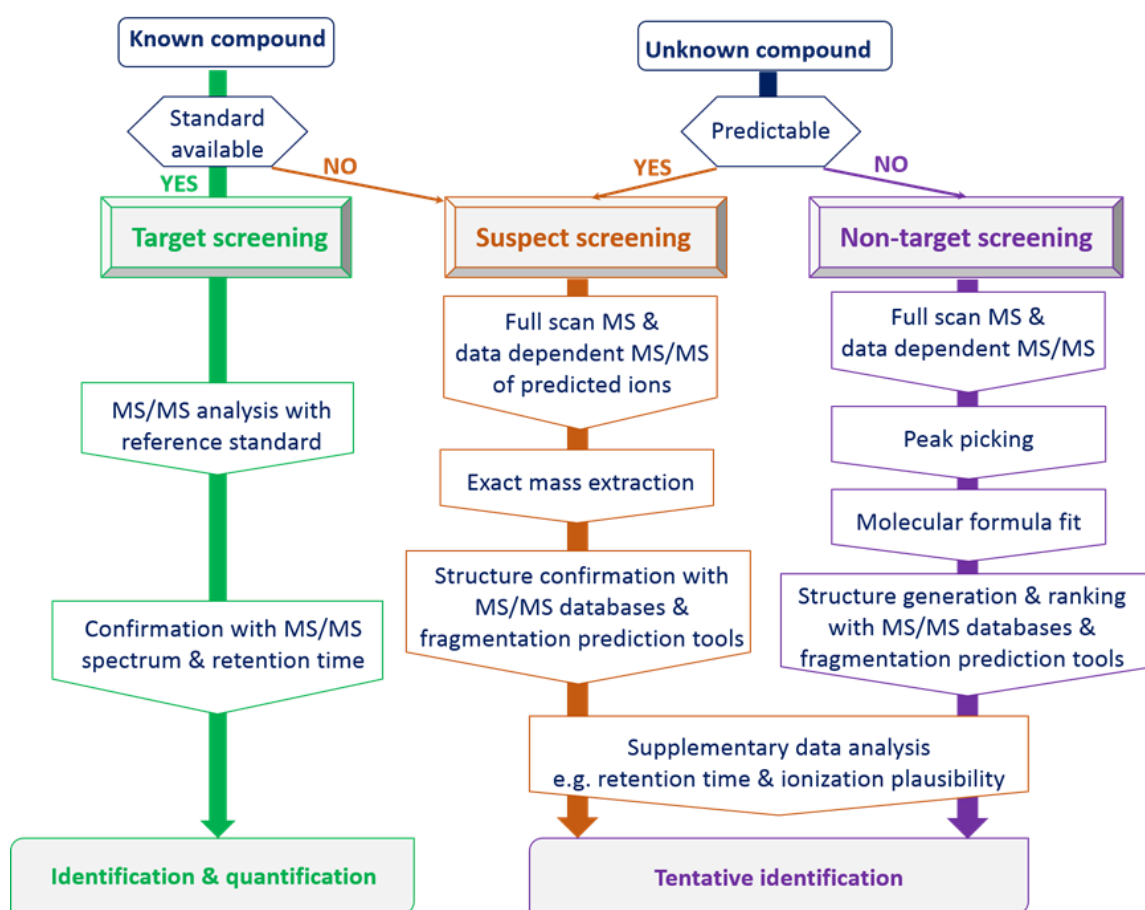


Figure 25: Systematic workflow for target, suspect and non-target screening [85].

2.5. Literature Review

So far, there are many studies focusing on the determination of specific classes of organic micropollutants in organisms from the lower and the upper trophic levels. An overview of the analytical procedures and methods that have been applied for the detection of ECs and POPs in biota is performed afterwards. The extraction techniques, used in such biomonitoring studies, are, among others, Soxhlet Extraction [5,58,87–98], MAE [25,99], MSPD [100–102], UAE [103–108] and ASE. The acceptance of PLE as an official EPA method (method 3545) for the determination of POPs in a variety of environmental solid samples has convinced many authors to use this technique for sample preparation in environmental analysis [1,30,109]. Therefore, numerous applications of PLE for the extraction of POPs from biota samples (vegetal and animal) have been reported [5,110].

For the scope of the current thesis, the research in scientific resources was conducted focusing on ASE, as an extraction technique of interest due to its unique benefits, on GC as analytes' separation technique and on mass spectrometry as detector of the produced precursor and fragment ions. In **Table 1** are presented the biota matrices, the clean-up (solvents, extraction time and cycles) and the followed analytical techniques for the determination of target analytes. Additionally, in **Table 2** are presented the aforementioned parameters in monitoring surveys for the simultaneous detection of micropollutants, belonging to different chemical classes. In **Table 3**, are presented ASE parameters for the in-cell clean-up. Many scientific groups have used ASE, in order to extract a specific group of contaminants such as, PAHs [25,99,111–116], PCBs [117–122], OCPs [123–127], FRs [109,128–134], SCCPs [135–138], Furans and Dioxins [110,139–141] and PCNs [91,142–144]. For the extraction of PCBs the solvents mainly used were hexane:acetone (4:1, v/v) or hexane:dichloromethane (1:1, v/v), whereas temperature ranged between 80 °C and 120 °C, with 100 °C being the most popular choice. For the efficient extraction of PAHs a mixture of hexane:dichloromethane (1:1, v/v) was used and the ideal temperature of instrumentation of ASE was 100 °C. OCPs were effectively extracted with hexane:acetone or hexane:dichloromethane in various

ratios with the temperature ranging between 60 °C and 120 °C. As far as FRs are concerned, the optimal extraction solvent is proved to be a mixture of hexane:dichloromethane in ratio 1:1, v/v and the temperature, under which the extraction process takes place, is ranging similarly to OCPs, from 60 °C to 125 °C. According to the relative bibliography, SCCPs were extracted with the common mix of hexane: dichloromethane (1:1, v/v) at 100°C. For the extraction of furans and dioxins many solvents have been used, such as hexane:acetone (1:1, v/v), toluene:acetone (70:30, v/v), cyclohexane:ethyl acetate (1:1, v/v) and dimethyl sulfoxide (DMSO):acetonitrile (1:9, v/v) with the temperature ranging at higher degrees compared to the previously mentioned classes of organic micropollutants. Finally, for PCNs, the extraction was conducted using dichloromethane:hexane, in the majority of the respective studies, keeping the temperature of 100 °C. The presence of co-extracted interfering materials requires clean-up step(s) prior to chromatographic analysis. ASE incorporates various clean-up sorbents with the sample, in order to eliminate the clean-up step(s) and simplify the sample preparation procedure [92]. Depending on the complexity of the sample matrix, different clean-up techniques can be applied. Classical liquid adsorption chromatography is still the dominant technique, involving alumina, silica gel and florisil. For extracts containing considerable amounts of lipid, sulphuric acid can be used prior to adsorption chromatography in order to destroy fatty acids [93]. Only few scientific groups chose to include an in-cell clean up step to their analytical procedure, in order to eliminate the clean-up step(s) and simplify the sample preparation procedure. The sorbents of choice were those suggested by the EPA 1613 b method [94], silica gel, or acidic/basic/ neutral silica [95], alumina, florisil and carbon as layers in the stainless steel vessel of ASE [96,97]. This strategy is considered to be less time consuming, avoiding the excessive sample manipulation needed and environmentally friendly, using less quantities of toxic solvents [98].

Table 1: Biota Matrices, Analytes, Clean-Up Techniques for Gas Chromatography Analysis.

<i>Analytes</i>	<i>Matrices</i>	<i>Extraction Technique</i>	<i>Temperature (°C)</i>	<i>Cycles</i>	<i>Static Time (min)</i>	<i>Solvents</i>	<i>Clean -Up Technique</i>	<i>Analytical Technique</i>	<i>Ref.</i>
OCPs	swine, bovine,sheep	ASE	60	3	2	HEX, MeOH	SPE	GC-MS, GC-ECD	[87]
OCPs	fish	ASE				HEX:ACE (1:1,v/v)	sulphuric acid	GC-MS/MS	[88]
OCPs	oyster	ASE	100			HEX:ACE (1:1, v/v)		GC-MS	[89]
OCPs	fish	ASE				DCM: HEX (1:1,v/v)	florisil column	GC-ECD	[90]
OCPs	fish	PLE	100	1 or 2	5	HEX:DCM (7:3, v/v)		GC-MS	[125]
OCPs	fish	MSPD-ASE	60	2	5	HEX:DCM (7:3, v/v)	acid alumina-Na ₂ SO ₄ column	GC-ECD	[126]
OCPs	fish	PLE	100	2 or 3	5	HEX:DCM (7:3, v/v)		GC-MS/MS	[126]
OCPs	chicken, pork, lamb	ASE	120	2	5	EtoAc	GPC	GC-QqQ-MS	[127]
PAHs	fish	ASE	100	2	7		GPC	GC-MS	[145]
PAHs	canned fish	ASE	120	2	5-6	HEX:DCM	SPE	GC-FID	[100]
PAHs	fish	ASE	100			DCM		GC-MS (SIM)	[117]
PAHs	oyster	ASE				DCM	silica-alumina column	GC-MS	[143]
PAHs	marine organisms	ASE	110		5	HEX:DCM (1:1, v/v)	alumina column	GC-MS (SIM)	[101]

<i>Analytes</i>	<i>Matrices</i>	<i>Extraction Technique</i>	<i>Temperature (°C)</i>	<i>Cycles</i>	<i>Static Time (min)</i>	<i>Solvents</i>	<i>Clean -Up Technique</i>	<i>Analytical Technique</i>	<i>Ref.</i>
PAHs	seafood	ASE	100		2	DCM:ACE (6:4, v/v)		GC-MS/MS	[146]
FRs	fish	ASE				HEX:DCM (1:1, v/v)	silica gel column	GC-MS/MS	[120]
FRs	fish	ASE	100	2	10	HEX:DCM (1:1, v/v)	sulfuric acid, SPE	GC–NCI–MS/MS	[102]
HFRs	fish	ASE	125	3	3	HEX:DCM (1:1, v/v)	silica column, GPC, florisil column	GC-NCI-MS, GC-HRMS, GC-MS/MS, GC-API-MS/MS	[132]
FRs	fish	ASE, SPME	150	1	5	H ₂ O:ACN (9:1, v/v)		GC-FPD	[109]
HFRs	mussel	ASE		2		HEX:DCM	Teflon silica column, carbon column	HRGC–HRMS	[103]
PBDEs	dab	ASE	100	2	8	DCM	GPC, silica gel column	GC–MS	[128]
PBDEs	fish	ASE					GPC, SPE	GC-MS	[129]
PBDEs	fish	ASE	80	2	10	HEX:DCM (1:1, v/v)		GC-QqQ-MS	[130]
PBDEs	marine amphipod	ASE	100	2	5	DCM	Na ₂ SO ₄ -florisil column	GC-ECD	[131]
PBDEs	shiner surfperch, white croaker, cormorant eggs, harbor seal blubber	ASE	100	2	5	HEX:DCM (1:1, v/v)	Na ₂ SO ₄ column, SEC, alumina chromatography	GC-HRMS	[133]

<i>Analytes</i>	<i>Matrices</i>	<i>Extraction Technique</i>	<i>Temperature (°C)</i>	<i>Cycles</i>	<i>Static Time (min)</i>	<i>Solvents</i>	<i>Clean -Up Technique</i>	<i>Analytical Technique</i>	<i>Ref.</i>
PBDEs	fish	ASE	100			HEX:DCM (95:5, v/v)		GC-ITMS-MS	[134]
PCBs	oyster	ASE	100		5	HEX:ACE (1:1, v/v)	silica gel/ alumina columns	GC-ECD	[104]
PCBs	catfish	ASE				DCM	SEC HPLC	GC-MS	[105]
PCBs	fish	ASE	100	1	5	HEX:ACE (1:1, v/v)	SPE	GC-MS	[106]
PCBs	fish	ASE				MeCl	silica/aluminum columns, SEC HPLC	GC-MS (SIM)	[107]
PCBs	eggs of spoonbill	ASE	100	2	10	DCM:PE (15:85, v/v)		GC-MS	[121]
PCBs	fish	ASE	125			HEX	filtration with Na ₂ SO ₄	GC-MS	[93]
PCNs	fish	PLE	80 °C~10 min, 100 °C~20 min			HEX:DCM (9:1,v/v)	FMS Automated Power Prep System	GC-HRMS	[142]
PCNs	fish	PLE	100			HEX:DCM (9:1,v/v)	FMS Automated Power Prep System	GC-HRMS	[143]
PCNs	fish	ASE	120	3	10	HEX:DCM (1:1,v/v)	acid silica/silica gel/basic alumina columns	GC-HRMS	[144]

<i>Analytes</i>	<i>Matrices</i>	<i>Extraction Technique</i>	<i>Temperature (°C)</i>	<i>Cycles</i>	<i>Static Time (min)</i>	<i>Solvents</i>	<i>Clean -Up Technique</i>	<i>Analytical Technique</i>	<i>Ref.</i>
PCNs	mussel, clam	PLE	120	3	7	HEX:DCM (1:1,v/v)	GPC, neutral silica column	GC-MS	[91]
SCCPs	zooplankton, invertebrates, fish	ASE				ACE:HEX (1:1,v/v)	acid/neutral/basic alumina/Na ₂ SO ₄ columns	GC-ECNI-MS	[136]
SCCPs	biota	ASE	100	3	10	HEX:DCM (1:1,v/v)	silica-florisil column	GC-ECNI-MS	[137]
SCCPs	fish	ASE				HEX:DCM (1:1,v/v)	acid silica-florisil column	HRGC-ECNI-LRMS	[138]

Table 2: Biota Matrices, Analytes, Clean-Up Techniques for Gas Chromatography Analysis.

<i>Analytes</i>	<i>Matrices</i>	<i>Extraction Technique</i>	<i>Temperature (°C)</i>	<i>Cycles</i>	<i>Static Time (min)</i>	<i>Solvents</i>	<i>Clean -Up Technique</i>	<i>Analytical Technique</i>	<i>Ref.</i>
OCPs, PCBs	fish	ASE	80	3	10	HEX:ACE (4:1, v/v)		GC– MS/MS	[119]
dl-PCBs, PCDD/Fs	macroalgae, macrophyta, bivalve, shrimp, fish	ASE				TOL:ACE (7:30,v/v)	silica/florisil/celite-carbon columns	GC-MS	[141]
HFRs, PBDEs	fish, herring gull eggs	ASE	125	3	3	HEX:DCM (1:1,v/v)	silica/florisil columns, GPC	GC-API-MS/MS (HFRs), GC-EI-MS (PBDEs)	[132]
OCPs, PCBs	fish	ASE	80	3	10	HEX:ACE (4:1, v/v)	in-line	GC-MS/MS	[108]
OCPs, PCBs, PAHs	fish, squid, bivalve shells, octopuses, shrimp	ASE	120			HEX:DCM (9:1,v/v)	GPC	GC-NCI (OCPs,PCBs) GC-EI-MS (PAHs)	[147]
OCPs, PCBs, PBDEs	fish	ASE	100			HEX:DCM	ACN,freezing ,centrifuging, alumina-N column	GC-ECD	[148]
PAHs	mussel	ASE	100			DCM	SEC, SPE	GC-MS	[112]
OCPs	fish	ASE	100	3	10	Cy:EtoAc (1:1,v/v)	GPC, SPE C18, SPE PSA	GC-MS	[25]
PAHs, PBDEs, OCPs	fish	ASE	150	2	10	HEX:DCM (1:1,v/v)	centrifugation (5x)	GC-MS	[99]

<i>Analytes</i>	<i>Matrices</i>	<i>Extraction Technique</i>	<i>Temperature (°C)</i>	<i>Cycles</i>	<i>Static Time (min)</i>	<i>Solvents</i>	<i>Clean -Up Technique</i>	<i>Analytical Technique</i>	<i>Ref.</i>
PAHs, PCBs	mussel	ASE	100,125	5,4	4,6	DCM	in-cell (alumina)	GC-MS, GC-ECD	[59]
PAHs, PCBs	mussel	ASE	100,125	5,4	4,6	DCM		GC-MS, GC-ECD	[113]
PAHs, PCBs	fish	ASE	150	3	8	HEX:ACE (4:1, v/v)	multilayer columns	HRGC-HRMS	[114]
PBDEs, PCBs, PAHs	mussel	ASE	60	2	5	HEX	silica column	GC-ECNIMS	[115]
PCBs, OCPs, PBDEs	fish	ASE	90	5		HEX:DCM (1:1,v/v)	glass wool/ Na ₂ SO ₄ /deactivated florisil/acid silica columns	GC-HRMS	[116]
PCBs, OCPs, PBDEs	fish	ASE	80		3	HEX:ACE (4:1, v/v)	Na ₂ SO ₄	GC/MS-MS	[149]
PCBs, PBDEs, DDT, OCPs	fish	ASE				MeCl		GC-MS	[150]
PCBs, PBDEs, OCPs	oyster	ASE	100		8	DCM	acid silica gel column	GC-ECD	[118]
PCBs, PBDEs, OCPs, PAHs	mussel	ASE	100		8	DCM	alumina/silica gel micro-column	GC-MS (PAHs, GC-ECD (OCs, PBDEs)	[151]
PCBs, PBDEs, PAHs, BFRs	fish	PLE	100	3	5	HEX:DCM (1:1,v/v)		GC×GC ToFMS	[152]
PCBs, PCNs, PAHs	mussels, clams	ASE	100	3	7	HEX:DCM (1:1,v/v)	GPC, neutral silica column	GC-MS	[91]

<i>Analytes</i>	<i>Matrices</i>	<i>Extraction Technique</i>	<i>Temperature (°C)</i>	<i>Cycles</i>	<i>Static Time (min)</i>	<i>Solvents</i>	<i>Clean -Up Technique</i>	<i>Analytical Technique</i>	<i>Ref.</i>
PCBs,PBDEs	dolphin, whale, harbour porpoise, striped dolphin, bottlenose dolphin	ASE	100	2	5	HEX	silica column	GC–EI-MS (PCBs), GC–ECNIMS(PBDEs)	[153]
PCBs,PBDEs,DDTs	harbor seal pups	ASE	100			DCM	column of silica gel and alumina (for polar compounds), SEC with HPLC (for POPs)	GC-MS	[154]
PCBs,PBDEs,OCPS	fish	ASE	80		3	HEX:ACE (4:1, v/v)	Na ₂ SO ₄	GC-MS/MS	[155]
PCBs,PBDEs,OCPS	fish	ASE	125	3	5	DCM	GPC, SPE PSA	GCxGC-HR-ToF MS	[156]
PCBs,PBDEs,OCPS,PAHs	canned smoked sprats	PLE	100	2	5	HEX:DCM(1:1,v/v)	GPC	GC-ToF MS	[157]
PCBs,PCDD, Fs	fish, fish oil	In-cell carbon fractionation PLE	100		5	HP, HP:ACE (1:2.5, v/v), TOL	silica columns	GC-HRMS	[158]
PCDD, PCBs, PCDF	anchovy, squid,mussel, norway lobster,mackerel, red mullet, clam	ASE				HEX:ACE (1:1, v/v)		HRGC-HRMS (SIM)	[139]
PCDD/Fs	mussel	ASE				DCM	GPC, silica/alumina columns, sulphuric acid, 2D-HPLC	HRGC-HRMS	[140]

<i>Analytes</i>	<i>Matrices</i>	<i>Extraction Technique</i>	<i>Temperature (°C)</i>	<i>Cycles</i>	<i>Static Time (min)</i>	<i>Solvents</i>	<i>Clean -Up Technique</i>	<i>Analytical Technique</i>	<i>Ref.</i>
<i>PCDDs, PCDFs, PCBs</i>	clam, crab	ASE	100	1	5	DCM:HEX (1:1,v/v) (PCBs), TOL (PCDD/F)		HRGC-HRMS	[159]
<i>PCDDs/Fs, polychlorinated dibenzo-p-dioxins/dibenzo furans, PCBs</i>	beef, pork, chicken, fish	ASE	180		15	DMSO: ACE (1:9, v/v)	silica gel-carbon column, DMSO/ACN/HEX partitioning	HRGC-HRMS	[110]
<i>PAHs, PCBs, PBDEs, OCPs, pyrethroids, DEET</i>	fish	ASE	100	3	5	HEX: DCM (1:1,v/v), HEX:ACE (1:1,v/v)	florisil cartridges	GC-MS, LC-MS/MS	[160]

Table 3: Sorbents for in-cell clean-up using the ASE apparatus.

Analytes	Matrices	Adsorbents (quantity,g)	Activation	Analytical Technique	Ref.
PCDDs, PCDFs, PCBs	fish	Na ₂ SO ₄ (ratio 1 (sample) :4)	Na₂SO₄ baked at 500°C for 5h, silica gel —rinsed with DCM, baked at 180°C for more than an hour, activated silica gel —bake at 180 °C for more than 1h, acid silica gel —mix 44g of concentrated H ₂ SO ₄ , basic silica gel —mix 30g of 1N NaOH, acid alumina—heating to 130°C for more than 12h, basic alumina heating to 600°C for more than 24h, mix of Carbopak C and Celite 545 - heating at 130°C for at least 6h	HRGC–HRMS	[96]
		alumina (10g)			
		mix of celite (5g) & carbopack (0.5g)			
		florisil (10g)			
		silica gel (5g)			
PCDD/Fs, PCBs	fish, pork, oil	Na ₂ SO ₄ , sulfuric acid impregnated silica	Na₂SO₄, silica gel -baked at 400°C for 10h, impregnated silica gel - heating for 10 h to 40°C and addition of 400g of H ₂ SO ₄	GC–HRMS	[95]
musk compounds	fish	alumina (5g)	heating at 500 °C for 4h	GC-MS	[161]
PCBs	pork, chicken	silica gel	silica gel/neutral alumina -heating at 400 °C, florisil -heating at 650°C, acid silica gel -mix of 50g sulfuric acid with 50g silica gel	GC-ECD	[162]
		acid silica gel			
		florisil			
		neutral alumina			
PCBs, OCPs, PAHs		silica gel	heating for at least 16h at 130°C		[163]
PCBs, PAHs	compost	deactivated silica gel, KOH impregnated silica gel, H ₂ SO ₄ impregnated silica gel	Na₂SO₄, silica gel -baked at 500°C overnight. acid silica gel -mix with 40g H ₂ SO ₄ , neutral silica gel -mix with 10g Milli-Q water, basic silica gel -mix with 168g KOH and dissolve in 700mL MeOH	GC-MS	[164]
PCBs, OCPs, PBDEs		alumina	heating overnight at 400-450°C		[165]
		florisil	heating at 675°C		[166]
		florisil	heating at 650°C		
		silica gel	rinsed with methylene chloride, baked at 180°C for less than an hour		
		acid silica gel (30% w/w)	mix with 44g of concentrated H ₂ SO ₄		

Analytes	Matrices	Adsorbents (quantity,g)	Activation	Analytical Technique	Ref.
		basic silica gel	mix with 30g of 1N NaOH		[89]
pyrethroid pesticides	dust, feed for poultry	acid silica/silica	impegrated silica gel - mix with 40% (w/w) H ₂ SO ₄ , neutral silica gel -heating for 24h at 190°C	GC/MS	
PCBs, OCPs, PBDEs	whale earwax	basic alumina (5g), silica gel (15g), florisil (10g)		GC-MS	[97]
α-HCH, HCB, HC, ALD, 4,4-DDE, 4,4-DDT	chicken	florisil (2.5g)	heating at 130°C for at least 48h	GC-MS	[167]
SCCPs	mollusks	florisil (3g), silica gel (2g), acid silica gel(5g), Na ₂ SO ₄ (4g)	silica gel -heating at 550°C for 12 h, florisil -heating at 140°C overnight, Na₂SO₄ -heating at 550°C for 10h	GC-MS	[168]
SCCPs	soil	column 1: 2g of neutral silica gel, 5g of concentrated H ₂ SO ₄ -treated silica gel, column 2: 5g of basic alumina and 2g of Na ₂ SO ₄ , column 3: 16g of florisil, column 4: 3g of florisil, 2g of neutral silica gel, 5g of 30% acid silica gel and 2g of Na ₂ SO ₄	neutral silica gel -baked at 130°C, acid silica -baked at 130°C, basic alumina -baked at 130°C, florisil -baked at 600°C, neutral silica gel -baked at 550°C	GC-ECNI-MS	[169]
PAHs	corals	neutral silica (2g), florisil (1.75g), acidic alumina (2g)	heating at 250°C for 12h (acidic alumina)	GC-MS	[170]
HFRs	mollusks	florisil (1.75g), acid silica (1.75g), silica (0.5g)	silica, florisil -heating at 130°C overnight	GC-NCI-MS	[170]

Chapter 3

Scope

As mentioned above, chemical monitoring data from biota specimens are of particular value, providing an overall image of the revelation of harmful (persistent, bio accumulative, toxic – PBT) substances, in environment. Under these circumstances, the present research is focused on the development and validation of a generic analytical method for the determination of volatile, thermally stable and non-polar organic ECs and POPs in biota matrices. The samples that will be analyzed, in the framework of this work, are freeze-dried fish samples from low contaminated areas and from three different regions of the Black Sea, in order that overall chemical pollution can be estimated. An innovated extraction technique will be used for the extraction of these contaminants; Accelerated Solvent Extraction (ASE). As the biota samples of choice are complex matrices, a clean-up step will also be demanded, in order to diminish the possible matrix effect on the subsequent procedure. In this point, one of our targets is the development of an in-cell clean-up step, which will take place in the vessel of ASE, right after the extraction process. Few solvents, sorbents and different temperatures, according to the existing literature, will be applied and the whole extraction and clean-up development will be evaluated in terms of %Recovery, %FME and detection of compounds belonging to different categories. After the optimization of the examined parameters, the validation of the method will take place. Quality parameters, such as linearity, precision, trueness, accuracy, repeatability, recovery, LODs, LOQs etc. are going to be determined. Data obtained from method validation can be used afterwards to judge the quality, reliability and consistency of analytical results. For the method's validation, a representative mix of GC target list compounds will be used. In National and Kapodistrian University of Athens (NKUA), a GC-APCI-HRMS database with more than 300 organic micropollutants [NKUA GC target list (DOI: 10.5281/zenodo.3753372) is available in the following link: <https://zenodo.org/record/3753372>] has been developed over the last years. Following the validation, an application of the developed method will occur in real biota samples, for the assessment of its suitability for its intended use (fit for purpose).

Experimental Part

CHAPTER 4

Materials and Methods

4.1. Chemicals and Reagents

A total of 101 organic non- and semi-volatile micropollutants and 18 internal standards (IS) were included in this smart validation dataset. The compounds of interest were selected based on environmental relevance and laboratory availability. Together they represent a wide range of chemical classes, including PPPs, PAHs, PCBs, PCNs and OCPs.

Standards of 4,4'-DDT, 4,4'-DDD, 4,4'-DDE, 2,4'-DDT, Acetochlor, Alachlor, Aldrin, Chlorpyrifos Ethyl, Chlorthal-dimethyl, Dichlorvos, Dicofol, Endosulfan alpha, Endosulfan sulphate, Hexachlorobenzene, Isodrin, Propachlor and Propazine and the internal standard Triphenyl phosphate (TPP), (>99%purity) were ordered from Fluka-Sigma-Aldrich (Steinheim, Germany). Standard stock solutions of the aforementioned compounds were prepared by dissolving 0.01g of the crystalline standards with hexane in 10 mL volumetric flask, so the concentration was 1000 mg L⁻¹ and stored at -20°C, to avoid possible decay. Standards of PCBs, 16-PAHs and PCNs from Fluka-Sigma-Aldrich (Steinheim, Germany) were used. Also, a standard solution of 16 PAHs certificated as CRM (Naphthalene-d8, Acenaphthylene-d8, Acenaphthene-d10, Fluorene-d10, Phenanthrene-d10, Anthracene-d10, Fluoranthene-d10, Pyrene-d10, Benzo (a) anthracene-d12, Chrysene-d12, Benzo (b) Fluoranthene-d12, Benzo (k) fluoranthene-d12, Benzo (a) Pyrene-d12, Indeno (1,2,3-c,d) Pyrene-d12, Dibenzo (a,h) anthracene-d14, Benzo (g,h,i) perylene-d12) from CPACChem was used as internal standard. Acrinathrin, Atrazine, Beflubutamid, Benalaxyl, Benfluralin, Bifenthrin, Bupirimate, Cadusafos, Carfentrazone Ethyl, Chlozolate, Clomazone, Cyanazine, Cyhalothrin-lambda major (mix 2), Diazinon, Dimethenamid, Dimethoate, Dimoxystrobin, Diuron, Ethalfluralin, Ethion, Ethoprophos, Etoxazole, Fenamiphos-sulfone, Fenitrothion, Fenoxycarb, Fenpropathrin, Fenthion, Fludioxonil, Fluopicolide, Flusilazole, Fosthiazate, Iprovalicarb, Malaoxon, Malathion, Mepanipyrim, Metazachlor, Methacrifos, Methidathion, Methoprotetryne, Metolachlor, Metribuzin, Mevinphos,

Myclobutanil, Napropamide, Oxadiazon, Oxadixyl, Oxyfluorfen, Paclobutrazol, Parathion-Ethyl, Parathion-Methyl, Penconazole, Pirimicarb, Procymidone, Proquinazid, Prothiophos, Pyraflufen Ethyl, Pyrazophos, Pyrimiphos Me, Quinalphos, Quinoxifen, Tebuconazole, Tebufenpyrad, Tefluthrin, Terbutylazine, Tolclofos Methyl, Triadimefon, Triallate and Trifluralin were all obtained from Thermo Scientific (Waltham, Massachusetts, USA).

Multi-compound standard working solutions containing the target analytes were prepared in hexane at 10 ppm, by combining appropriate aliquot of the individual stock solutions. This mixture was used in recovery experiments as spiking solution for the level of 300ppb. A surrogate cocktail mixture of deuterium-labeled PAHs was prepared at 10 ppm and used as analytical standard for quality control for GC-APCI-QToF MS analysis at 200 ppb, by being spiked into samples before extraction. The solutions were stored at -20°C.

Solvents used for sample treatment were of HPLC grade, including acetonitrile, isooctane, hexane, acetone and 2-propanol were acquired from Fischer Scientific (Loughborough, UK) and dichloromethane from Sigma Aldrich (Steinheim, Germany).

Solvents used for sample reconstitution (hexane) and GC-MS instrumentation (hexane, acetone, isopropanol) were of Pesticides and LC-MS grade and were provided by Honeywell (New Jersey, USA) and Carlo Erba (Barcelona, Spain). Deionized water was purified using a Milli-Q purification apparatus (Millipore Direct-Q UV, Bedford, MA, USA) with a resistance of 18.2 MΩ/cm.

Regarding the extraction using ASE apparatus, cellulose ASE Extraction Filters and Diatomaceous Earth for ASE were obtained from Thermo Scientific (Waltham, Massachusetts, USA).

For dispersion and clean-up purposes, different solid sorbents were used. Alumina (50-200 μm, 60Å) and silica gel (0.060-0.200mm, 60Å) were purchased from Acros Organics (Waltham, Massachusetts, USA) and florisil (< 200 mesh) was obtained from Sigma Aldrich (Steinheim, Germany).

Furthermore, for the sample preparation Na₂SO₄ was purchased by Fluka Analytical (Buchs, Switzerland).

Regenerated cellulose (RC) syringe filters (diameter 15 mm, pore size 0.2 µm), used for the filtering of the final extracts before transporting them to the vials, were obtained from MACHEREY-NAGEL GmbH & Co. KG (Düren, Germany).

To avoid contamination at low levels, gloves were worn during all the sampling preparation and glassware was cleaned with abundant tap water using a commercial detergent to discard the lipid matrices, following rinse with pure water (mili-pore) and methanol or acetone of HPLC grade.

4.2. Biota Samples

Muscle tissues from breams (*Abramis Brama*) were obtained from naturally low contaminated areas and were used for both method development and method validation procedures. Thereafter, the feasibility of the optimized method for the intended analytes' determination was evaluated by the treatment and analysis of three muscle tissues from different fish species (*Mullus barbatus*, *Merlangius merlangus*, *Scophthalmus*), collected from three different regions of the Black Sea, and more specifically, from Georgia, Ukraine and Russian Federation, respectively.

4.3. Sample Pretreatment

All samples used in the present study, were lyophilized in petri dishes, before any further treatment, using the Telstar's Freeze-Dryer LyoQuest (temperature: -55°C, vacuum: 5×10^{-2} mbar), in order to enhance extraction efficiency, improve the precision and achieve lower detection limits. After lyophilization, the freeze-dried samples were homogenized, using pestle and mortar. After homogenization, the samples were stored in amber glass vials and conserved in the freezer (-80°C) prior to analysis.

4.4. Sample Preparation

A schematic illustration showing the various steps of processing of fish samples is presented below (**Figure 26**). The described analytical strategy was followed in the experiments of validation of the developed method and its application to real samples. Under optimal conditions, one gram of lyophilized and homogenized fish tissue was inserted into the 22 mL cell of the ASE apparatus (**Figure 27**), right above a mix of silica:alumina:florisil in ratio 2:2:4 g, which was used for the purification step of the produced extracts. Diatomaceous earth was placed at the top of the sample, so as to fill the remaining empty part of cell.

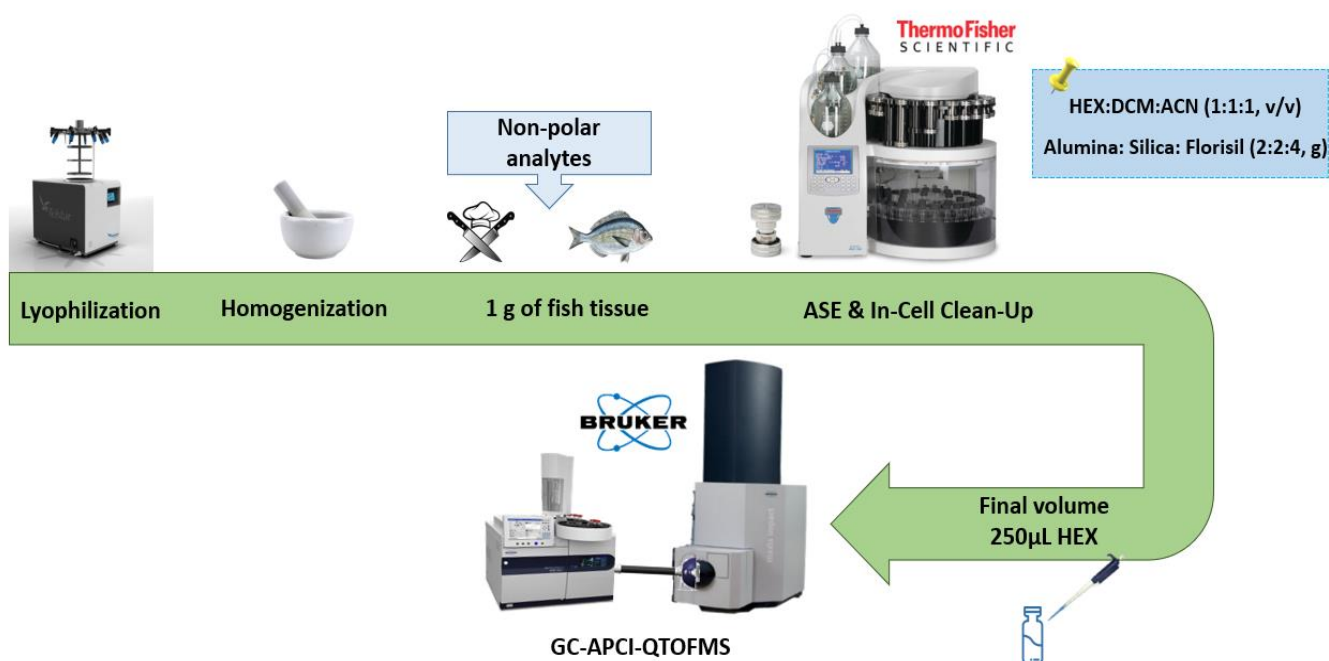


Figure 26: Final Protocol.



Figure 27: Dionex™ ASE™ 350 Accelerated Solvent Extractor [171].

For the spiking experiments, a 75µL solution containing a mixture of native standards was added in the ASE cell, at the top of the sample. Non-fortified samples were also prepared to generate blank matrices for matrix-matched calibration standards. For a reagent blank sample, Na₂SO₄ was used in the place of biological sample, following the samples' preparation procedure. Extraction was carried out with a mix of HEX:DCM:ACN in ratio 1:1:1, v/v. The extraction conditions are summarized in the following Table. Around 30 minutes were required for each extracting cell. Extracts were collected in ASE bottles and were condensed at room temperature, using a rotary evaporator, following an evaporation till dryness, under a gentle N₂ stream. Worth mentioning is the fact that before the condensation with rotary evaporator and nitrogen, 50 µL isooctane were added as keeper in each test tube. Keepers are substances (usually solvents) which are added to the samples to prevent analyte losses during solvent removal by evaporation. Once evaporated, the collected extracts were reconstituted with n-hexane for Pesticides' analysis at a final volume of 250µL. The final extract was filtered directly in an analysis vial with a 0.2 µm RC membrane filter to eliminate lipids and solid particles, which could obstruct GC-MS, and was ready for the instrumental analysis.

Table 4: Conditions which were used in Accelerated Solvent Extraction.

<i>Temperature (°C)</i>	100
<i>Pressure (psi)</i>	1500
<i>Heating Time (s)</i>	300
<i>Static Time (s)</i>	420
<i>#Static Cycles</i>	3
<i>Flush Volume (%)</i>	60
<i>Purge Time (s)</i>	180
<i>Extraction Solvent</i>	HEX:DCM:ACN

4.5. Instrumental Analysis

The instrumental analysis was carried out using a Bruker 450 gas chromatograph, equipped with a CP-8400 AutoSampler, coupled to a hybrid quadrupole time of flight mass spectrometer (QToF-MS) (Maxis Impact, Bruker Daltonics), using as interface, an atmospheric pressure chemical ionization (APCI) source for the ionization of the analytes (**Figure 28**).

In brief, for chromatographic separation in positive ionization mode, 1 μ L of extract or standard was injected in the splitless mode (Restek Split liner w/Glass Frit (4mm x 6.3 x 78.5)) and the splitless purge valve was activated 1 min after injection. A Restek Rxi-5Sil MS capillary column system of 30 m (0.25 mm i.d. x 0.25 μ m film thickness) was used with helium (>99.999%) as carrier gas with a constant flow rate of 1.5 mL min⁻¹. The oven temperature program used for the separation of the target compounds was as follows: 55°C initial hold for 3 min, increase at a rate of 15°C min⁻¹ to 180°C, then increase with a step of 6.5°C min⁻¹ to 280°C and hold for 5 min followed by an increase of 10°C min⁻¹ to 300°C and hold for 5.28 min. The temperature of splitless injector port, GC-MS transfer

line and MS source were set at 280, 290 and 250°C, respectively. All data were collected in data-dependent acquisition mode. Perfluorotributylamine (FC43) was infused prior to each injection for lock mass correction and calibration of the QToF mass spectrometer. Nitrogen gas (>99.999%) was used as collision gas. Bruker's software that was used for raw data analysis was DataAnalysis 5.1 and TASQ Client 2.1.



Figure 28: The GC-APCI-QToF-MS system.

4.6. Method Development

The aim of this study was the development of a novel, generic protocol for the determination of non-polar, semi-polar and volatile organic micropollutants. The extension of the chemical domain succeeded from the samples' preparation protocol is of high importance, due to the fact that the usage of HRMS permits not only the application of wide-scope target screening strategies, but also suspect and non-target methodologies and retrospective analysis. The first step of the method development was the planning of the protocol. In accordance with the bibliographic review, for the extraction of the analytes from the biota matrices, the usage of the ASE was considered as the best choice, due to its unique previously mentioned advantages. The focus was on the parameters that

affect the extraction efficiency and the overall recoveries of the target compounds. The crucial parameters, which needed to be tested, were the oven temperature and the extraction solvents. During the sample preparation is important to avoid or at least minimize the co-extraction of matrix interferences in the final extract and for this reason, a clean-up step was also examined, by testing Solid Phase Extraction (SPE) and in-cell clean-up inside the cell-in parallel with the extraction- for their ability to purify the produced extracts.

GC-MS sensitivity and recovery results during method development were assessed by spiking the samples with a representative mix of native and labeled analytes belonging to different chemical classes (OCPS, PCNS, PAHs, PCBs and PPPs) at a final concentration level of 300ppb. These 101 analytes are covering almost the 40% of the current total laboratory GC database.

% Recovery of each one of the spiked compounds, which was assessed by the equation: $\% \text{Recovery} = [(\text{Spiked Sample} - \text{Blank Sample}) / (\text{Matrix Matched} - \text{Blank Sample})] \times 100$, % Factor of Matrix Effect, which was calculated by the equation $\% \text{FME} = [(\text{Matrix Matched} - \text{Sample}) / (\text{Standard} - \text{Solvent})] \times 100$, and the number of detected compounds were considered, in order to conclude to the final optimal protocol. As blank sample, unfortified fish tissue was used, obtained from naturally low contaminated areas.

1st Test: The focus of the first test was on the clean-up step. Two different approaches were assayed; SPE and in-cell clean-up by ASE apparatus. The extraction protocol followed in this phase of the research was based on the isolation of the target analytes using ASE apparatus with a mixture of solvents of HEX:DCM:ACN (1:1:1, v/v), at 100°C. Cells were prepared as mentioned in section 4.4. After the extraction the two previously mentioned clean-up routes were tested.

-Combination of ASE extraction with separate clean-up step (SPE)

The cleaning procedure followed in this phase was based on a previously in-house developed and validated method. After the extraction, extracts were purified by SPE GC-Florisil cartridges [Strata® FL-PR Florisil [(170 µm, 80 Å), 5 g /20 mL, Giga Tubes, Phenomenex] previously conditioned with 20mL 10%

isopropanol (IPA) in DCM, followed by 30mL of HEX. After conditioning, the samples were loaded in the SPE cartridges. The elutes were collected during the sample's loading. The elution of the analytes from the adsorbent material was performed using 20mL of HEX:DCM (50: 50, v/v), followed by 20 mL of HEX.

-Combination of ASE extraction with on-line clean-up

In this test, cells for the ASE in-cell clean-up step were prepared by adding different types and amounts of sorbents at the space between the cellulose micro-filter and the sample. At this stage, two different sorbents were examined for the in-cell clean up. Florisil (5g) and a mix of silica:alumina:florisil (2:2:2, g). Extracts in HEX:DCM:ACN (1:1:1,v/v) with no further clean-up were handled as mentioned earlier in section 4.4.

2nd Test: After the first test, in which the in-cell clean-up was chosen for the purification of the produced extract, this second test followed. This experiment was divided into 4 subtests. For all of them the preparation protocol described in section 4.4 was followed, examining two different mixtures of extraction solvents and two different mixtures of sorbents for the in-cell clean-up. The oven temperature of the ASE apparatus was kept at 100°C for all of the tested cases. The conditions applied at these 4 approaches are presented in the following Table.

Table 5: Four examined subtests.

Test A	HEX:DCM:ACE	Alumina : Silica : Florisil (2:2:4, g)
Test B	HEX:DCM:ACN	
Test C	HEX:DCM:ACE	Florisil (5, g)
Test D	HEX:DCM:ACN	

3rd Test: Having already concluded to the in-cell clean-up and the usage of specific sorbents and extraction solvents, the last crucial parameter needed to be tested was the oven temperature of the ASE apparatus. For this reason, in

this third test, three different temperatures at 80, 100 and 150°C were assayed. The whole sample preparation was conducted as described in section 4.4, by following in-cell clean-up of the extracts by a mix of silica:alumina:florisil (2:2:4, g) and ASE with a mix of HEX:DXM:ACN (1:1:1, v/v). The rest of the parameters were remained as described in section.

Figure 29 presents a schematic illustration of the whole method development procedure.

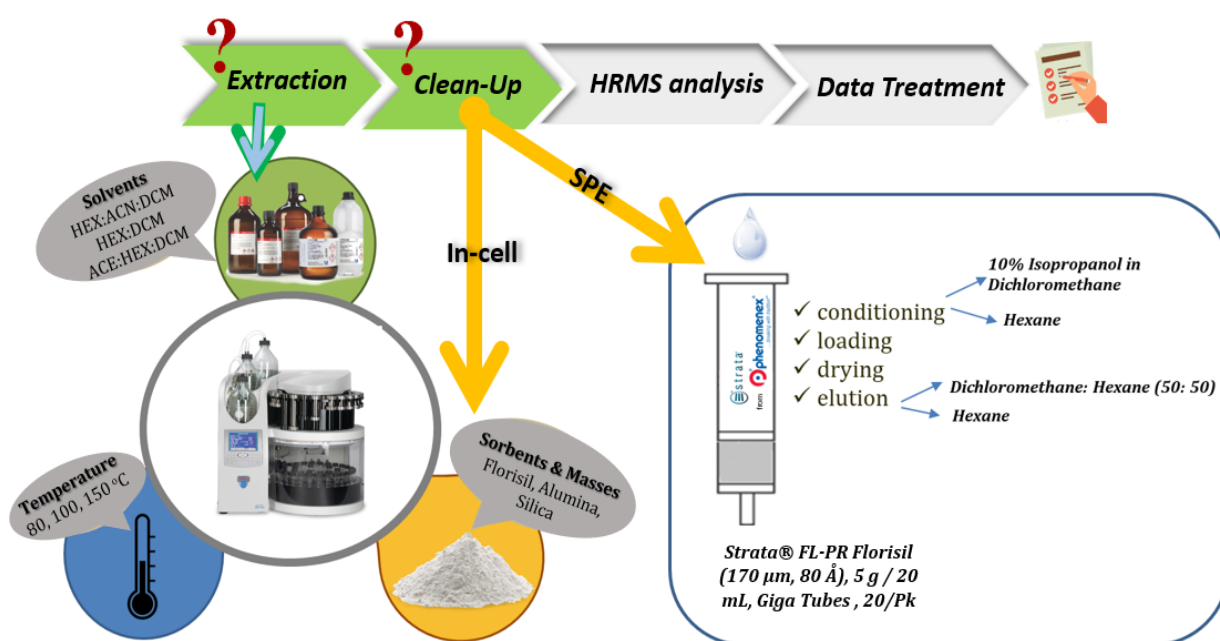


Figure 29: Process of Method Development.

4.7 Method Validation

Analytical method validation is an essential requirement to perform the chemical evaluation. It is a procedure of performing numerous assessments designed to verify that an analytical test system is suitable for its intended reason and is capable of providing beneficial and legitimate analytical data.

A number of experiments with spiked samples were performed, in order to assess the performance characteristics of the final method proposed in terms of recovery, linearity, precision, matrix effect and detectability of the method for a representative validation dataset of 101 compounds from different chemical classes and with different physicochemical properties, so that are eluted all over the chromatogram. The compounds of the validation dataset are shown in **Table 6**. All the validation studies were performed using fish tissue samples.

Validated parameters were:

- Linearity

Linearity of the instrument was studied for each compound by analyzing standard solutions at seven different concentration levels (**2, 5, 10, 25, 50, 100, 200 $\mu\text{g L}^{-1}$**). Moreover, linearity of the method was assessed for each compound by analyzing spiked samples at four different levels (**6.2, 12.4, 24.8, 49.6 $\mu\text{g/Kg w.w.}$**). The calibration curves were constructed using a least-squares linear regression. A coefficient of determination (r^2) of >0.995 was acceptable proof of calibration.

- Limits of Detection and Limits of Quantification

Concerning the evaluation of the sensitivity, Limits Of Detection (LOD) and Quantitation (LOQ) for both the instrument and the method were determined, by using data from regression analysis and calculating the standard deviation of intercept from calibration curves and spiked curves, respectively.

- Trueness

Accuracy of the method was determined comparing the recoveries of three spiked samples at four different concentration levels (**6.2, 12.4, 24.8, 49.6 $\mu\text{g/Kg w.w.}$**), as quality controls (QCs). The extracts were injected and analyzed for each QC. Recoveries were determined by comparing the obtained concentrations between the spiked extracted sample and the blank matrix samples spiked at the same concentration after the extraction.

- Matrix Effect

Matrix effect was determined by comparing the response of the analytes in matrix matched samples and their response in standard solutions at four different concentration levels (**6.2, 12.4, 24.8, 49.6 µg/Kg w.w.**).

- Precision

Precision was expressed in terms of repeatability (% RSDr). Repeatability of the method was determined by comparing RSDr from the analysis of six replicates of different spiked samples at two different concentration levels (**12.4, 49.6 µg/Kg w.w.**)

Table 6: Validation dataset.

Analyte	Group	Analyte	Group
2.4-DDT	<i>Organochlorine pesticides (OCPs)</i>	PCB 52	<i>Polychlorinated Biphenyls (PCBs)</i>
4.4-DDD	<i>Organochlorine pesticides (OCPs)</i>	PCB 101	<i>Polychlorinated Biphenyls (PCBs)</i>
4.4-DDE	<i>Organochlorine pesticides (OCPs)</i>	PCB 138	<i>Polychlorinated Biphenyls (PCBs)</i>
4.4-DDT	<i>Organochlorine pesticides (OCPs)</i>	PCB 180	<i>Polychlorinated Biphenyls (PCBs)</i>
Acetochlor	<i>Organochlorine pesticides (OCPs)</i>	Hexachloronaphthalene (PCN 67)	<i>Polychlorinated Naphthalenes (PCNs)</i>
Alachlor	<i>Organochlorine pesticides (OCPs)</i>	Pentachloronaphthalene (PCN 52)	<i>Polychlorinated Naphthalenes (PCNs)</i>
Aldrin	<i>Organochlorine pesticides (OCPs)</i>	Pentachloronaphthalene (PCN 54)	<i>Polychlorinated Naphthalenes (PCNs)</i>
Chlorpyrifos Ethyl	<i>Organochlorine pesticides (OCPs)</i>	Benz(a)anthracene	<i>Polycyclic Aromatic Hydrocarbons (PAHs)</i>
Chlorthal-dimethyl	<i>Organochlorine pesticides (OCPs)</i>	Benzo(a)pyrene	<i>Polycyclic Aromatic Hydrocarbons (PAHs)</i>
Dichlorvos	<i>Organochlorine pesticides (OCPs)</i>	Benzo(b)fluoranthene	<i>Polycyclic Aromatic Hydrocarbons (PAHs)</i>
Dicofol	<i>Organochlorine pesticides (OCPs)</i>	Chrysene	<i>Polycyclic Aromatic Hydrocarbons (PAHs)</i>
Endosulfan alpha	<i>Organochlorine pesticides (OCPs)</i>	Fluorene	<i>Polycyclic Aromatic Hydrocarbons (PAHs)</i>
Endosulfan sulphate	<i>Organochlorine pesticides (OCPs)</i>	Phenanthrene	<i>Polycyclic Aromatic Hydrocarbons (PAHs)</i>
Hexachlorobenzene	<i>Organochlorine pesticides (OCPs)</i>	Pyrene	<i>Polycyclic Aromatic Hydrocarbons (PAHs)</i>
Isodrin	<i>Organochlorine pesticides (OCPs)</i>	Acrinathrin	<i>Plant Protection Products (PPPs)</i>
Propachlor	<i>Organochlorine pesticides (OCPs)</i>	Atrazine	<i>Plant Protection Products (PPPs)</i>
Propazine	<i>Organochlorine pesticides (OCPs)</i>	Beflubutamid	<i>Plant Protection Products (PPPs)</i>

Analyte	Group	Analyte	Group
Benalaxyl	<i>Plant Protection Products (PPPs)</i>	Fenitrothion	<i>Plant Protection Products (PPPs)</i>
Benfluralin	<i>Plant Protection Products (PPPs)</i>	Fenoxycarb	<i>Plant Protection Products (PPPs)</i>
Bifenthrin	<i>Plant Protection Products (PPPs)</i>	Fenpropathrin	<i>Plant Protection Products (PPPs)</i>
Bupirimate	<i>Plant Protection Products (PPPs)</i>	Fenthion	<i>Plant Protection Products (PPPs)</i>
Cadusafos	<i>Plant Protection Products (PPPs)</i>	Fludioxonil	<i>Plant Protection Products (PPPs)</i>
Carfentrazone Ethyl	<i>Plant Protection Products (PPPs)</i>	Fluopicolide	<i>Plant Protection Products (PPPs)</i>
Chlozolinate	<i>Plant Protection Products (PPPs)</i>	Flusilazole	<i>Plant Protection Products (PPPs)</i>
Clomazone	<i>Plant Protection Products (PPPs)</i>	Fosthiazate	<i>Plant Protection Products (PPPs)</i>
Cyanazine	<i>Plant Protection Products (PPPs)</i>	Iprovalicarb	<i>Plant Protection Products (PPPs)</i>
Cyhalothrin-lambda major	<i>Plant Protection Products (PPPs)</i>	Malaoxon	<i>Plant Protection Products (PPPs)</i>
Diazinon	<i>Plant Protection Products (PPPs)</i>	Malathion	<i>Plant Protection Products (PPPs)</i>
Dimethenamid	<i>Plant Protection Products (PPPs)</i>	Mepanipyrim	<i>Plant Protection Products (PPPs)</i>
Dimethoate	<i>Plant Protection Products (PPPs)</i>	Metazachlor	<i>Plant Protection Products (PPPs)</i>
Dimoxystrobin	<i>Plant Protection Products (PPPs)</i>	Methacrifos	<i>Plant Protection Products (PPPs)</i>
Diuron	<i>Plant Protection Products (PPPs)</i>	Methidathion	<i>Plant Protection Products (PPPs)</i>
Ethalfuralin	<i>Plant Protection Products (PPPs)</i>	Methoprotryne	<i>Plant Protection Products (PPPs)</i>
Ethion	<i>Plant Protection Products (PPPs)</i>	Metolachlor	<i>Plant Protection Products (PPPs)</i>
Ethoprophos	<i>Plant Protection Products (PPPs)</i>	Metribuzin	<i>Plant Protection Products (PPPs)</i>
Etoxazole	<i>Plant Protection Products (PPPs)</i>	Mevinphos (Phosdrin)	<i>Plant Protection Products (PPPs)</i>
Fenamiphos-sulfone	<i>Plant Protection Products (PPPs)</i>	Myclobutanil	<i>Plant Protection Products (PPPs)</i>
Napropamide	<i>Plant Protection Products (PPPs)</i>	Pyrazophos	<i>Plant Protection Products (PPPs)</i>
Oxadiazon	<i>Plant Protection Products (PPPs)</i>	Pyrimiphos Me	<i>Plant Protection Products (PPPs)</i>

Analyte	Group	Analyte	Group
Oxadixyl	<i>Plant Protection Products (PPPs)</i>	Quinalphos	<i>Plant Protection Products (PPPs)</i>
Oxyfluorfen	<i>Plant Protection Products (PPPs)</i>	Quinoxifen	<i>Plant Protection Products (PPPs)</i>
Paclobutrazol	<i>Plant Protection Products (PPPs)</i>	Tebuconazole	<i>Plant Protection Products (PPPs)</i>
Parathion-Ethyl	<i>Plant Protection Products (PPPs)</i>	Tebufenpyrad	<i>Plant Protection Products (PPPs)</i>
Parathion-Methyl	<i>Plant Protection Products (PPPs)</i>	Tefluthrin	<i>Plant Protection Products (PPPs)</i>
Penconazole	<i>Plant Protection Products (PPPs)</i>	Terbuthylazine	<i>Plant Protection Products (PPPs)</i>
Pirimicarb	<i>Plant Protection Products (PPPs)</i>	Tolclofos Methyl	<i>Plant Protection Products (PPPs)</i>
Procymidone	<i>Plant Protection Products (PPPs)</i>	Triadimefon	<i>Plant Protection Products (PPPs)</i>
Proquinazid	<i>Plant Protection Products (PPPs)</i>	Tri-allate	<i>Plant Protection Products (PPPs)</i>
Prothiophos	<i>Plant Protection Products (PPPs)</i>	Trifluralin	<i>Plant Protection Products (PPPs)</i>
Pyraflufen Ethyl	<i>Plant Protection Products (PPPs)</i>		

4.8 Target screening of fish samples

An in-house build database of more than 300 organic compounds was used for the target screening of both validation set and real samples, using GC-APCI-QToF. This database contains different groups of organic compounds, mainly PPPs, OCPs, PAHs, PCNs, PCBs and it is continuously being updated. The database contains information about retention time, m/z and formula for precursor, qualifiers and adducts and in source fragments, as well as some chemical identifiers (e.g. CAS numbers). This information was acquired from the analysis of the standard solutions of these compounds, which were available in the laboratory, with the bbCID method, or was part of the manufacturer's database, Bruker's ToxScreener 2.1, which was built with the same bbCID method.

After the analysis the raw data were processed by Bruker TASQ Client 2.1 and DataAnalysis 5.1. In TASQ method, the Extracted Ion Chromatogram for each analyte that belongs to database was created with a mass error window of $\pm 0.005\text{Da}$.

4.9 Identification criteria

The identification of each detected peak was based on the evaluation of a set of identification criteria and on manual inspection. The first criterion was the retention time shift, which refers to the difference between the experimental retention time and the theoretical (one that is recorded in the database) retention time (ΔRT : $<0.2\text{ min}$). The second one was the mass accuracy, which refers to the difference between the experimental accurate mass and the theoretical mass in mDa or ppm. In our case a mass error less than 2 mDa was considered as acceptable.

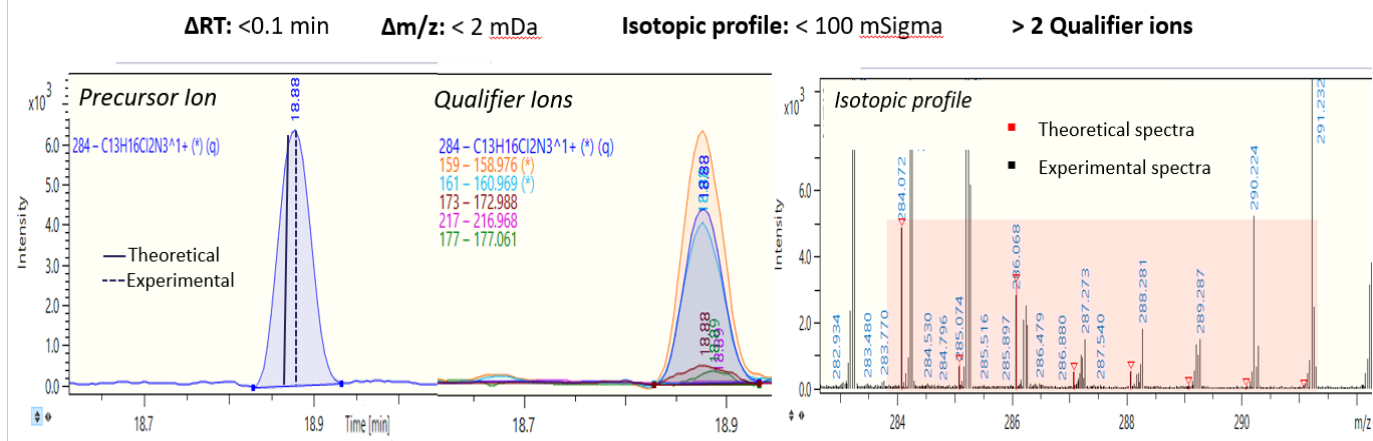


Figure 30: Evaluation of identification criteria for Penconazole.

Moreover, the isotopic pattern fidelity was evaluated, which refers to the correlation between the theoretical and the experimental isotopic pattern. Its calculation is based on the standard deviation of the masses and the intensities for all isotopic peaks and is expressed in mSigma value (Isotopic profile: < 100 mSigma). Lower mSigma value indicates better isotopic fitting. The presence of additional characteristic ions for each compound (qualifier ions) apart from the precursor ion, was considered as an additional identification criterion. It is evident that this evaluation process, provides us high identification confidence for our final results in biota samples.

4.7. Application to real samples

To demonstrate the applicability and suitability of the validated method, 3 fish samples obtained from three different regions of the Black Sea, were prepared and analyzed following the developed protocol. A procedural blank, a replicate sample and a matrix spike sample were included in each batch of samples for QA/QC purposes.

CHAPTER 5

Results and Discussion

5.1 General Observations

As mentioned in the chapter 4, a generic method for the determination of GC-amenable POPs and ECs has been developed in the Laboratory of Analytical Chemistry in the National and Kapodistrian University of Athens. After the analysis of the tested samples using GC-APCI (+)-QToF, it was proved that in-cell clean-up with a mix of silica:alumina:florisil (2:2:4, g) provides better results concerning the clean-up of the fish samples. Recoveries, %factor of matrix effect and the number of the recovered analytes were determined, in order to conclude to the optimal sample preparation protocol. As regards the extraction of the analytes, a mixture of HEX:DCM:ACN (1:1:1, v/v) was selected, as the extraction solvent. The optimal oven temperature was set at 100°C. Moreover, the final method was validated for its performance characteristics (linearity, LODs, LOQs, trueness, matrix effect and precision) for a set of 101 analytes. Finally, real fish samples from different species and regions were analysed using GC-APCI(+)-QToFMS.

5.1 Method Development Results

In the following diagram (**Figure 31**), is presented a comparison of the analytes' %Recoveries obtained conducting the 1st test, in which SPE and in-cell clean-up (mix) were compared. Apparently, for both clean-up steps there were not observed any significant differences between them in terms of the number of extracted analytes and their recoveries. However, even though SPE provided a little bit more satisfying recoveries of the spiked compounds at the level of 75-125 %, the fact that with the in-cell clean-up were recovered analytes from more chemical classes, combined with the previously mentioned advantages of ASE made the in-cell clean-up predominant for the rest of the method development. Also, the % Factor of Matrix Effect (%FME) was at an acceptable level for both of the two tested clean-up steps, indicating the efficiency of the developed

method to reduce the impact of the matrix on the analytes. It was also observed that the in-cell clean-up, performed by florisil sorbent, provided high recoveries, too. However, according to the literature review, the most used sorbents for this type of cleaning, is a mixture of silica:alumina:florisil. For this reason, it was decided to increase the amount of florisil to 4g, instead of 2g, in the cases where the mix would be used, for the rest of the method development.

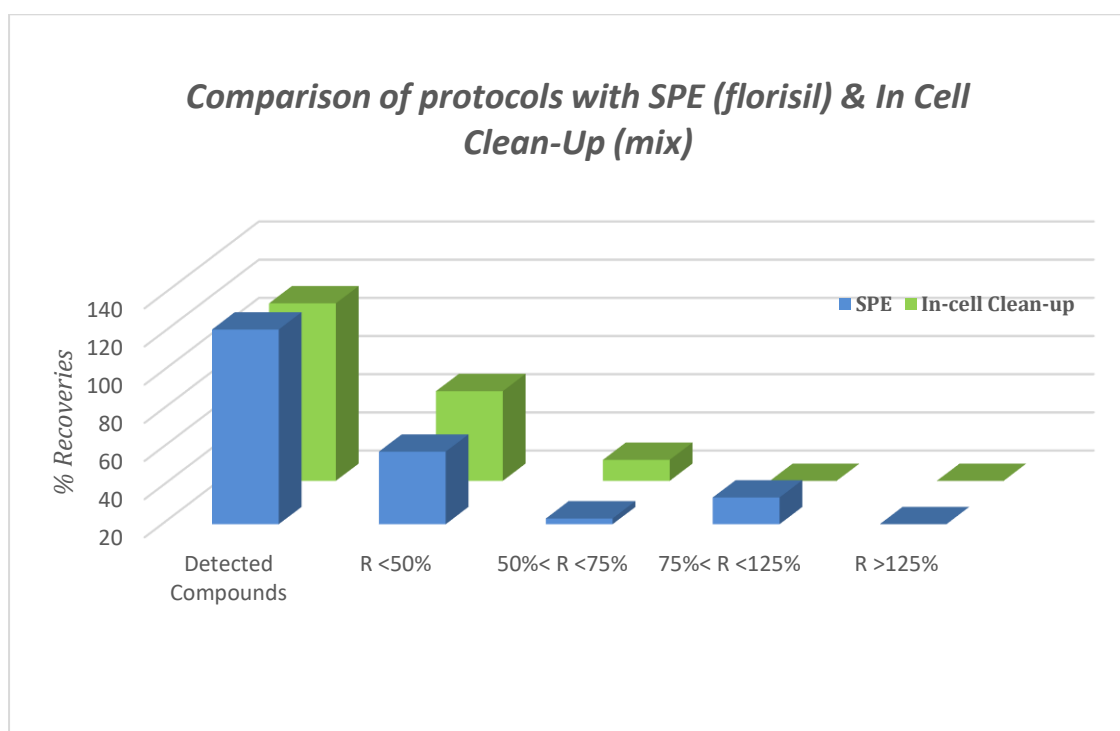


Figure 31: Diagram (% Recoveries, comparison of protocols with SPE and In-Cell Clean-Up).

With respect to the 2nd conducted test, 4 different subtests were compared, using two different mixtures of solvents; HEX: DCM: ACN (1:1:1, v/v) (test B & D) and HEX: DCM: ACE (1:1:1, v/v) (test A & C) and two different sorbents for the in-cell clean-up; a mix of silica:alumina:florisil (2:2:4, g) (test A & B) and florisil (5g) (test C & D).

In the Box-Plot diagram (**Figure 32**), a comparison of the %Recoveries protocols among the 4 assays is presented. Among these tests, test B provided

the highest number of recovered analytes from spiked samples, with their recoveries reaching satisfying levels.

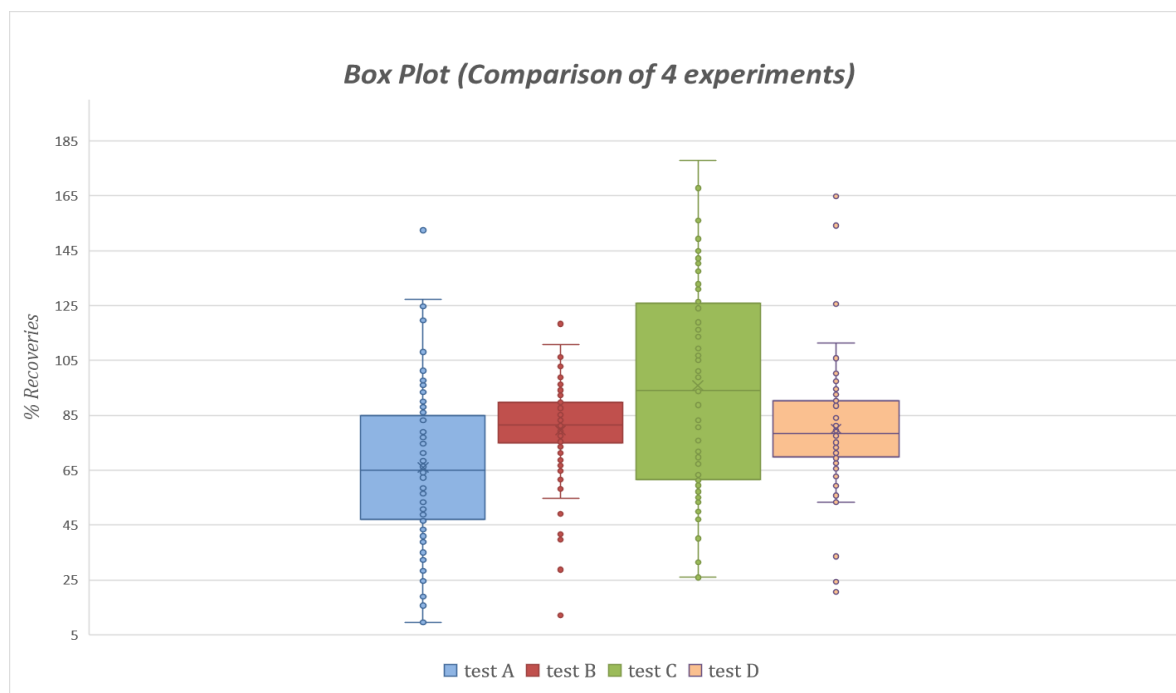


Figure 32: Box-Plot (% Recoveries, comparison of 4 subtests).

In the different temperatures, which were compared during the extraction procedure of the 3rd test, the evaluation of their efficiency took place using the number of the total recovered compounds and the %FME. The number of the detected compounds, in total, as well as the number of the %FME on them are presented respectively for the three tested temperatures at the following **Table 7**.

Table 7: Number of detected compounds and %Recoveries in different temperatures.

<i>Oven Temperatures</i>	<i>Extracted Analytes</i>	<i>%FME <20</i>
<i>80°C</i>	103	54
<i>100°C</i>	106	63
<i>150°C</i>	79	40

Although the differentiation in the number of total compounds is almost the same in the tests, in the test with extraction temperature 100°C higher recoveries were observed and a smallest %FME for more compounds.

5.2 Method Validation

As mentioned in chapter 4.7, for the evaluation of the parameters concerning the method's performance, a representative validation dataset of 101 compounds with different physicochemical properties from different chemical classes was used.

5.2.1 Linearity

Linearity of the instrument was studied for each compound by analyzing standard solutions at seven different concentrations levels (**2, 5, 10, 25, 50, 100, 200 µg/L**). These standards were prepared in solvent (Hexane). In **Figure 33** is an example of instrument linearity of the analyte 4.4-DDE.

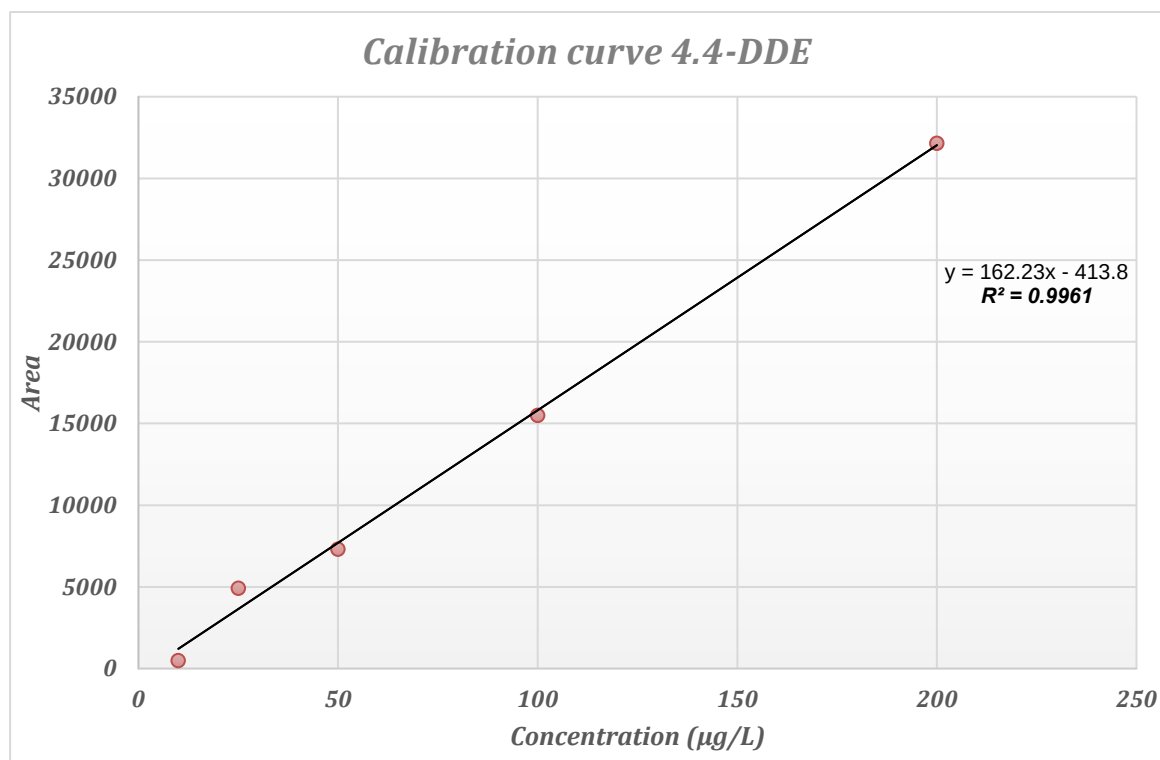


Figure 33: Linearity of the instrument for 4.4-DDE.

Moreover, linearity of the method was studied for each compound by analyzing spiked samples at four different concentration levels (**6.2, 12.4, 24.8, 49.6 µg/Kg w.w.**).

The regression lines were determined by the least squares method, and were of the form:

$$y = (b \pm S_b) * C + (a \pm S_a)$$

in which,

y: Relative peak area of each analyte

b: the slope

a: the intercept

C: concentration of analyte

S_b: Standard deviation of slope

S_a: Standard deviation of intercept

Regarding linearity, the slope, the intercept and the correlation coefficient (R²) of the standard addition curve for each compound are listed in **Table 8**.

The correlation coefficients (R²) were above 0.86 for all of the tested compounds in spiked samples. Moreover, the correlation coefficients (R²) were above 0.997 for most of the target analytes in standard solutions.

Table 8: Calibration curves for the method using GC-APCI-QToFMS.

Classification	Compound	I.S.	Spiked Samples				
			R^2	a	S_a	b	S_b
PAHs	Chrysene	Chrysene D12	0.92	1.1E-03	3.5E-04	-4.6E-02	4.0E-02
	Phenanthrene	Phenanthrene D10	0.98	-3.2E-03	2.0E-03	1.1E+00	1.9E-01
	Pyrene	Pyrene D10	0.993	1.8E-03	4.6E-04	-2.8E-02	4.4E-02
PCBs	PCB 101	PCB 209	0.91	3.7E-04	1.0E-04	-1.1E-02	1.1E-02
	PCB 138	PCB 209	0.88	4.2E-04	1.1E-04	-7.0E-03	1.1E-02
PCNs	Pentachloronapthalene (PCN 54)	PCB 209	0.89	4.2E-04	1.5E-04	-1.8E-02	1.7E-02
OCPs	2,4-DDT	Triphenyl phosphate	0.994	1.4E-03	3.6E-04	-2.9E-02	3.4E-02
	4,4-DDD	Triphenyl phosphate	0.991	1.4E-03	4.1E-04	-3.1E-02	3.8E-02
	4,4-DDE	Triphenyl phosphate	0.96	7.7E-03	2.4E-03	-5.1E-02	2.3E-01
	Acetochlor	Triphenyl phosphate	0.98	5.6E-03	1.7E-03	-2.6E-01	2.0E-01
	Chlorpyrifos Ethyl	Triphenyl phosphate	0.96	2.2E-03	6.1E-04	-7.6E-02	6.3E-02
	Chlorthal-dimethyl	Triphenyl phosphate	0.97	6.3E-04	2.2E-04	-2.9E-02	2.5E-02
	Dicofol	Triphenyl phosphate	0.99	5.5E-04	1.4E-04	-1.3E-02	1.3E-02
	Hexachlorobenzene	Triphenyl phosphate	0.98	6.2E-04	1.5E-04	-2.3E-02	1.7E-02
	Isodrin	Triphenyl phosphate	0.991	2.8E-04	1.0E-04	-1.3E-02	1.2E-02
	Propachlor	Triphenyl phosphate	0.97	1.9E-02	3.5E-03	-4.6E-01	3.3E-01
	Propazine	Triphenyl phosphate	0.96	6.6E-02	1.3E-02	-1.6E+00	1.2E+00

Classification	Compound	I.S.	Spiked Samples				
			R^2	a	Sa	b	Sb
Plant Protection Products (PPPs)	Atrazine	Triphenyl phosphate	0.993	9.0E-03	1.8E-03	-3.5E-01	2.1E-01
	Benfluralin	Triphenyl phosphate	0.97	1.0E-03	1.9E-04	-3.8E-02	2.2E-02
	Bifenthrin	Triphenyl phosphate	0.93	2.7E-03	8.6E-04	-1.2E-01	9.9E-02
	Bupirimate	Triphenyl phosphate	0.87	5.3E-04	2.3E-04	-1.9E-02	2.7E-02
	Cadusafos	Triphenyl phosphate	0.991	1.0E-02	2.0E-03	-3.2E-01	2.1E-01
	Carfentrazone Ethyl	Triphenyl phosphate	0.92	1.3E-03	4.4E-04	-5.9E-02	5.1E-02
	Chlozolate	Triphenyl phosphate	0.94	4.8E-04	1.5E-04	-2.2E-02	1.7E-02
	Cyanazine	Triphenyl phosphate	0.95	3.5E-03	1.0E-03	-1.6E-01	1.2E-01
	Diazinon	Triphenyl phosphate	0.98	1.1E-02	1.8E-03	-2.5E-01	1.7E-01
	Dimethenamid	Triphenyl phosphate	0.95	3.7E-03	1.1E-03	-1.7E-01	1.3E-01
	Dimethoate	Triphenyl phosphate	0.98	6.6E-03	1.4E-03	-2.6E-01	1.6E-01
	Dimoxystrobin	Triphenyl phosphate	0.93	1.5E-03	4.7E-04	-6.4E-02	5.4E-02
	Ethalfuralin	Triphenyl phosphate	0.98	1.0E-03	2.6E-04	-4.0E-02	3.0E-02
	Ethion	Triphenyl phosphate	0.94	8.4E-04	2.6E-04	-3.8E-02	3.0E-02
	Ethoprophos	Triphenyl phosphate	0.98	1.5E-02	2.8E-03	-3.8E-01	2.7E-01
	Etoxazole	Triphenyl phosphate	0.94	8.3E-04	2.6E-04	-3.5E-02	3.0E-02
	Fenitrothion	Triphenyl phosphate	0.92	4.5E-04	1.5E-04	-1.5E-02	1.7E-02
	Fenoxycarb	Triphenyl phosphate	0.93	1.5E-03	4.8E-04	-6.7E-02	5.6E-02

	Compound	I.S.	Spiked Smples				
			R^2	a	Sa	b	Sb
	Fludioxonil	Triphenyl phosphate	0.91	1.6E-03	5.5E-04	-6.8E-02	6.3E-02
	Fluopicolide	Triphenyl phosphate	0.86	6.5E-04	2.2E-04	-2.9E-02	2.5E-02
	Flusilazole	Triphenyl phosphate	0.93	2.8E-03	9.1E-04	-1.3E-01	1.0E-01
	Malaoxon	Triphenyl phosphate	0.95	1.9E-03	5.8E-04	-9.6E-02	6.7E-02
	Malathion	Triphenyl phosphate	0.94	1.9E-03	6.0E-04	-9.0E-02	6.9E-02
	Metazachlor	Triphenyl phosphate	0.94	2.3E-03	7.2E-04	-1.0E-01	8.3E-02
	Methacrifos	Triphenyl phosphate	0.996	4.4E-03	1.1E-03	-1.4E-01	1.1E-01
	Methidathion	Triphenyl phosphate	0.93	2.7E-03	8.5E-04	-1.1E-01	9.8E-02
	Methoprottryne	Triphenyl phosphate	0.93	2.7E-03	8.7E-04	-1.2E-01	1.0E-01
	Metolachlor	Triphenyl phosphate	0.94	7.4E-03	2.3E-03	-3.4E-01	2.6E-01
	Metribuzin	Triphenyl phosphate	0.95	3.7E-03	1.1E-03	-1.6E-01	1.3E-01
	Mevinphos (Phosdrin)	Triphenyl phosphate	0.94	1.2E-03	2.6E-04	-3.3E-02	2.5E-02
	Myclobutanil	Triphenyl phosphate	0.93	2.9E-03	9.4E-04	-1.3E-01	1.1E-01
	Napropamide	Triphenyl phosphate	0.93	4.6E-03	1.5E-03	-2.1E-01	1.7E-01
	Oxadiazon	Triphenyl phosphate	0.996	1.7E-03	1.6E-04	-3.0E-03	1.7E-02
	Oxyfluorfen	Triphenyl phosphate	0.85	1.1E-03	3.3E-04	-3.6E-02	3.4E-02
	Paclobutrazol	Triphenyl phosphate	0.93	1.6E-03	5.2E-04	-7.6E-02	6.0E-02
	Parathion-Ethyl	Triphenyl phosphate	0.93	2.5E-03	8.0E-04	-1.1E-01	9.2E-02

	Penconazole	Triphenyl phosphate	0.92	2.4E-03	8.0E-04	-1.1E-01	9.2E-02
	Procymidone	Triphenyl phosphate	0.89	8.5E-04	3.1E-04	-3.8E-02	3.5E-02
	Proquinazid	Triphenyl phosphate	0.95	1.2E-03	2.9E-04	-2.7E-02	2.8E-02
	Prothiophos	Triphenyl phosphate	0.93	1.4E-03	4.5E-04	-6.4E-02	5.2E-02
	Pyraflufen Ethyl	Triphenyl phosphate	0.88	1.9E-03	5.9E-04	-6.6E-02	6.0E-02
	Pyrimiphos Me	Triphenyl phosphate	0.88	3.9E-02	1.0E-02	-	9.9E-01
	Quinoxifen	Triphenyl phosphate	0.91	8.0E-04	2.7E-04	-3.5E-02	3.1E-02
	Tefluthrin	Triphenyl phosphate	0.97	2.7E-03	5.8E-04	-1.1E-01	6.7E-02
	Terbuthylazine	Triphenyl phosphate	0.98	9.6E-03	2.1E-03	-3.7E-01	2.4E-01
	Tolclofos Methyl	Triphenyl phosphate	0.93	2.4E-03	7.7E-04	-1.2E-01	8.9E-02
	Triadimefon	Triphenyl phosphate	0.95	6.5E-03	2.1E-03	-4.3E-01	2.8E-01
	Tri-allate	Triphenyl phosphate	0.995	3.0E-03	5.3E-04	-7.1E-02	5.0E-02
	Trifluralin	Triphenyl phosphate	0.96	4.4E-04	1.0E-04	-1.8E-02	1.2E-02

The rest of compounds comprising the validation dataset are not presented in the Table, due to the fact that they were recovered to some of the concentration levels, making it impossible to construct a calibration curve.

5.2.2 Limits of Detection and Limits of Quantification

Limits of detection (LOD) is the lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value) with a stated confidence level. Limit of Quantification (LOQ) is the lowest concentration of the analyte that has been validated with acceptable accuracy by applying the complete analytical method. For their estimation the following equations were used:

$$LOD = 3.3 \times \frac{S_a}{b}$$

$$LOQ = 10 \times \frac{S_a}{b}$$

In which,

S_a: Standard deviation of intercept

b: the slope of curve

The results of MLODs and MLOQs are presented in **Table 9**.

Table 9: Total results of LODs and LOQs.

Classification	Compound	Fish	
		MLOD ($\mu\text{g/Kg w.w.}$)	MLOQ ($\mu\text{g/Kg w.w.}$)
PAHs	<i>Benz(a)anthracene</i>	4.80	16.00
	<i>Benzo(a)pyrene</i>	6.76	22.55
	<i>Benzo(b)fluoranthene</i>	4.38	14.59
	<i>Chrysene</i>	3.23	10.78
	<i>Fluorene</i>	0.06	0.20
	<i>Phenanthrene</i>	0.31	1.04
	<i>Pyrene</i>	1.00	3.33
PCBs	<i>PCB 52</i>	2.10	6.99
	<i>PCB 101</i>	1.42	4.72
	<i>PCB 138</i>	1.10	3.67
	<i>PCB 180</i>	3.17	10.55
PCNs	<i>Pentachloronapthalene (PCN 52)</i>	1.09	3.65
	<i>Pentachloronapthalene (PCN 54)</i>	2.71	9.02
	<i>Hexachloronapthalene (PCN 67)</i>	2.66	8.86
OCPs	<i>2,4-DDT</i>	0.58	1.93
	<i>4,4-DDD</i>	0.58	1.94
	<i>4,4-DDE</i>	0.07	0.22
	<i>4,4-DDT</i>	2.71	9.02
	<i>Acetochlor</i>	0.26	0.85
	<i>Alachlor</i>	1.75	5.84
	<i>Aldrin</i>	3.04	10.12
	<i>Chlorpyrifos Ethyl</i>	0.08	0.25
	<i>Chlorthal-dimethyl</i>	0.78	2.58
	<i>Dichlorvos</i>	2.29	7.63
	<i>Dicofol</i>	1.49	4.96
	<i>Endosulfan alpha</i>	1.84	6.12
	<i>Endosulfan sulphate</i>	3.38	11.27
	<i>Hexachlorobenzene</i>	0.74	2.46
	<i>Isodrin</i>	1.13	3.76
	<i>Propachlor</i>	0.05	0.16
	<i>Propazine</i>	0.03	0.08
PPPs	<i>Acrinathrin</i>	4.65	15.50
	<i>Atrazine</i>	0.18	0.60
	<i>Beflubutamid</i>	0.43	1.43
	<i>Benalaxyl</i>	0.63	2.10
	<i>Benfluralin</i>	0.70	2.33
	<i>Bifenthrin</i>	0.38	1.26
	<i>Bupirimate</i>	2.25	7.52
	<i>Cadusafos</i>	0.09	0.31
	<i>Carfentrazone Ethyl</i>	0.50	1.68
	<i>Chlozolate</i>	2.71	9.02
	<i>Clomazone</i>	0.38	1.27

	Cyanazine	0.28	0.92
	Cyhalothrin-lambda major	4.96	16.53
	Diazinon	0.03	0.09
	Dimethenamid	0.14	0.48
	Dimethoate	0.11	0.38
	Dimoxystrobin	1.14	3.82
	Diuron	2.81	9.36
	Ethalfuralin	0.45	1.49
	Ethion	0.31	1.04
	Ethoprophos	0.05	0.16
	Etoxazole	0.83	2.77
	Fenamiphos-sulfone	1.55	5.17
	Fenitrothion	1.21	4.03
	Fenoxycarb	0.95	3.18
	Fenpropathrin	1.58	5.28
	Fenthion	1.93	6.44
	Fludioxonil	0.92	3.06
	Fluopicolide	1.17	3.91
	Flusilazole	0.24	0.79
	Fosthiazate Isomer 1	2.81	9.36
	Fosthiazate Isomer 2	2.44	8.13
	Iprovalicarb Isomer 1	1.08	3.59
	Iprovalicarb Isomer 2	1.10	3.67
	Malaoxon	0.42	1.40
	Malathion	0.17	0.55
	Mepanipyrim	1.19	3.97
	Metazachlor	0.77	2.57
	Methacrifos	0.13	0.42
	Methidathion	0.33	1.10
	Methoprotryne	1.08	3.59
	Metolachlor	0.27	0.90
	Metribuzin	1.01	3.37
	Mevinphos (Phosdrin)	0.81	2.71
	Myclobutanil	0.47	1.56
	Napropamide	0.57	1.89
	Oxadiazon	0.27	0.90
	Oxadixyl	1.58	5.28
	Oxyfluorfen	0.71	2.37
	Paclobutrazol	1.19	3.97
	Parathion-Ethyl	0.16	0.16
	Parathion-Methyl	1.49	4.96
	Penconazole	0.42	1.39
	Pirimicarb	0.44	1.45
	Procymidone	0.90	2.99
	Proquinazid	0.39	1.31
	Prothiophos	0.24	0.78
	Pyraflufen Ethyl	0.28	0.93

5.2.3 Trueness

The trueness was assessed by measuring the recovery rate for each analyte after the sample preparation of the spiked samples at four concentration levels (6.2, 12.4, 24.8 and 49.6 µg/Kg w.w.). In this work the recovery is given by the following equation:

$$\%R = \frac{\text{Relative area}_{(\text{spiked samples})} - \text{Relative area}_{(\text{blank sample})}}{\text{Relative area}_{(\text{M.M. samples})} - \text{Relative area}_{(\text{blank sample})}} \times 100$$

The results of % Recoveries for all concentration levels are shown in **Table 10**.

Table 10: % Recoveries at four concentration levels.

Classification	Compounds	%Recovery			
		C= 6.2 µg/Kg	C= 12.4 µg/Kg	C= 24.8 µg/Kg	C= 49.6 µg/Kg
PAHs	<i>Benz(a)anthracene</i>	ND	ND	60	76
	<i>Benzo(a)pyrene</i>	ND	ND	51	55
	<i>Benzo(b)fluoranthene</i>	ND	ND	50	41
	<i>Chrysene</i>	ND	74	79	92
	<i>Fluorene</i>	50	45	50	61
	<i>Phenanthrene</i>	62	71	69	75
	<i>Pyrene</i>	66	70	73	75
PCBs	<i>PCB 52</i>	ND	66	79	77
	<i>PCB 138</i>	39	63	76	87
	<i>PCB 101</i>	66	75	81	84
	<i>PCB 180</i>	ND	92	77	88
PCNs	<i>Pentachloronapthalene (PCN 52)</i>	78	66	76	84
	<i>Pentachloronapthalene (PCN 54)</i>	ND	60	74	77
	<i>Hexachloronapthalene (PCN 67)</i>	ND	56	73	89
OCPs	<i>2,4-DDT</i>	56	69	76	91
	<i>4,4-DDD</i>	59	77	87	96
	<i>4,4-DDE</i>	57	78	86	95
	<i>4,4-DDT</i>	ND	67	73	76
	<i>Acetochlor</i>	48	63	80	87
	<i>Alachlor</i>	45	52	49	61
	<i>Aldrin</i>	ND	66	62	75
	<i>Chlorpyrifos Ethyl</i>	56	69	80	102
	<i>Chlorthal-dimethyl</i>	47	64	78	81
	<i>Dichlorvos</i>	ND	56	58	64

	<i>Dicofol</i>	53	65	87	89
	<i>Endosulfan alpha</i>	61	78	81	82
	<i>Endosulfan sulphate</i>	ND	54	59	68
	<i>Hexachlorobenzene</i>	54	62	89	96
	<i>Isodrin</i>	30	55	68	152
	<i>Propachlor</i>	42	54	75	81
	<i>Propazine</i>	44	63	75	89
PPPs	<i>Acrinathrin</i>	ND	ND	48	56
	<i>Atrazine</i>	37	55	67	71
	<i>Beflubutamid</i>	17	52	89	79
	<i>Benalaxyl</i>	24	47	42	58
	<i>Benfluralin</i>	28	53	79	93
	<i>Bifenthrin</i>	46	45	68	75
	<i>Bupirimate</i>	ND	78	86	124
	<i>Cadusafos</i>	40	42	65	75
	<i>Carfentrazone Ethyl</i>	52	66	82	86
	<i>Chlozolate</i>	ND	38	41	60
	<i>Clomazone</i>	58	64	76	85
	<i>Cyanazine</i>	40	38	73	65
	<i>Cyhalothrin-lambda major</i>	ND	ND	79	83
	<i>Diazinon</i>	49	76	89	120
	<i>Dimethenamid</i>	52	71	84	93
	<i>Dimethoate</i>	48	51	79	111
	<i>Dimoxystrobin</i>	43	38	61	73
	<i>Diuron</i>	ND	76	83	108
	<i>Ethalfluralin</i>	38	65	86	93
	<i>Ethion</i>	29	51	74	87
	<i>Ethoprophos</i>	32	48	67	73
	<i>Etoazole</i>	56	75	115	122
	<i>Fenamiphos-sulfone</i>	29	36	42	38
	<i>Fenitrothion</i>	49	52	78	73
	<i>Fenoxycarb</i>	72	93	63	102
	<i>Fenpropathrin</i>	77	104	81	95
	<i>Fenthion</i>	ND	79	64	56
	<i>Fludioxonil</i>	67	56	91	103
	<i>Fluopicolide</i>	26	42	69	72
	<i>Flusilazole</i>	40	60	97	101
	<i>Fosthiazate Isomer 1</i>	ND	40	35	46
	<i>Fosthiazate Isomer 2</i>	ND	37	41	43
	<i>Iprovalicarb Isomer 1</i>	44	39	53	64
	<i>Iprovalicarb Isomer 2</i>	42	37	67	79
	<i>Malaoxon</i>	47	73	83	92
	<i>Malathion</i>	41	48	76	80
	<i>Mepanipyrim</i>	72	85	109	89
	<i>Metazachlor</i>	56	47	65	84
	<i>Methacrifos</i>	55	64	79	86
	<i>Methidathion</i>	35	65	69	73
	<i>Methoprottryne</i>	47	52	65	71
	<i>Metolachlor</i>	37	56	63	77
	<i>Metribuzin</i>	46	57	69	89
	<i>Mevinphos (Phosdrin)</i>	40	55	81	76
	<i>Myclobutanil</i>	63	70	93	107
	<i>Napropamide</i>	53	61	64	87
	<i>Oxadiazon</i>	38	51	79	90
	<i>Oxadixyl</i>	62	76	91	106
	<i>Oxyfluorfen</i>	38	75	70	86
	<i>Paclobutrazol</i>	48	73	84	87

	<i>Parathion-Ethyl</i>	74	82	89	117
	<i>Parathion-Methyl</i>	46	87	83	118
	<i>Penconazole</i>	32	60	57	104
	<i>Pirimicarb</i>	37	48	56	67
	<i>Procymidone</i>	48	76	84	97
	<i>Proquinazid</i>	36	56	68	75
	<i>Prothiophos</i>	62	78	80	120
	<i>Pyraflufen Ethyl</i>	33	52	62	79
	<i>Pyrazophos</i>	ND	42	58	70
	<i>Pyrimiphos Me</i>	48	66	79	84
	<i>Quinalphos</i>	38	34	48	46
	<i>Quinoxifen</i>	68	76	83	80
	<i>Tebuconazole</i>	ND	28	34	46
	<i>Tebufenpyrad</i>	ND	79	83	87
	<i>Tefluthrin</i>	45	67	82	106
	<i>Terbutylazine</i>	44	79	76	82
	<i>Tolclofos Methyl</i>	21	33	46	51
	<i>Triadimefon</i>	61	77	87	83
	<i>Tri-allate</i>	42	64	78	86
	<i>Trifluralin</i>	77	79	94	129

Furthermore, the average %Recovery of four different concentration levels was calculated and the overall results are shown in **Figure 35**.

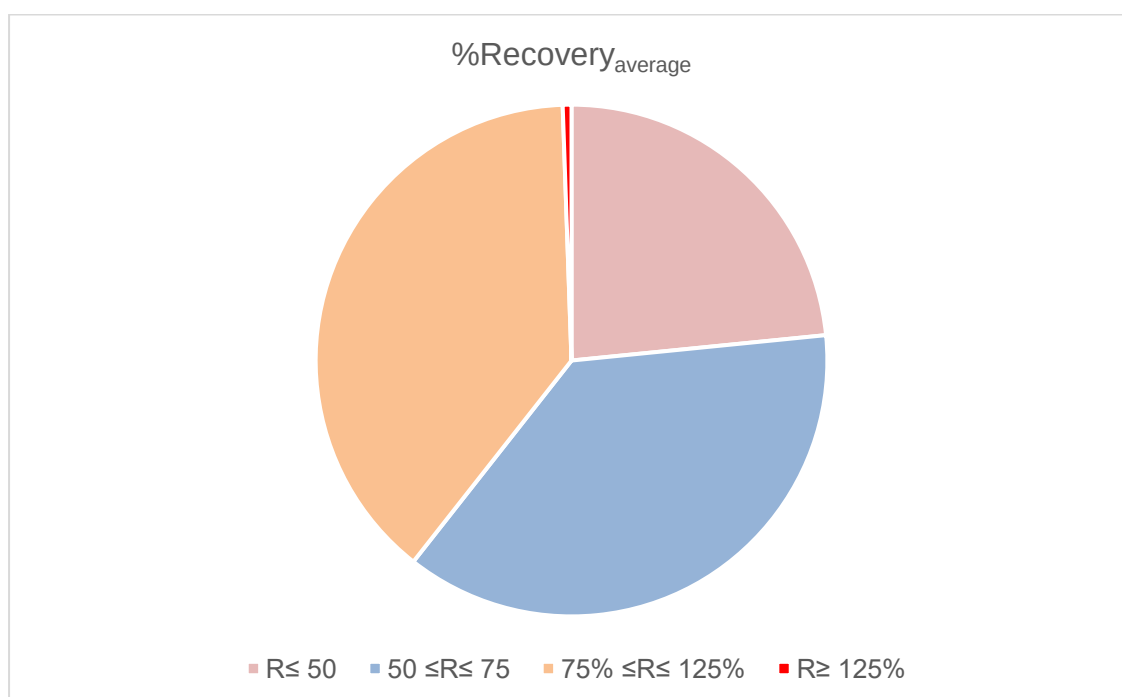


Figure 35: Total results of % Recovery.

5.2.4 Matrix effect

The determination of ME is important, so as to assess the selectivity of a proposed method. Ion suppression or enhancement may be caused by sample matrix and/or interferences. The mechanism and the origin of the matrix effect is not fully understood, but it may originate from the competition between an analyte and a co-eluting compound or undetected matrix components reacting with primary ions formed in the interface. Depending on the environment in which the ionization and ion evaporation processes take place, this competition may effectively decrease (ion suppression) or increase (ion enhancement) the efficiency of formation of the desired analyte ions, which are present at the same concentrations in the interface. This effect may reduce or increase the intensity of analyte ions and affect the reproducibility and accuracy of the assay.

Matrix effect was determined in terms of %Factor of Matrix Effect (%FME) by comparing the response of analyte between matrix matched samples and standard solution at four different concentration levels (**6.2, 12.4, 24.8 and 49.6 µg/Kg w.w.**). Results of %FME are shown in **Table 11**.

Table 11: % Factor of Matrix Effect (FME%) at four concentration levels.

Classification	Compound	%FME			
		C= 6.2 µg/Kg	C= 12.4 µg/Kg	C= 24.8 µg/Kg	C= 49.6 µg/Kg
PAHs	<i>Benz(a)anthracene</i>			15.0	16.4
	<i>Benzo(a)pyrene</i>			9.6	8.8
	<i>Benzo(b)fluoranthene</i>			13.2	12.8
	<i>Chrysene</i>		18.1	13.0	4.6
	<i>Fluorene</i>	11.5	10.2	14.1	13.8
	<i>Phenanthrene</i>	7.4	8.9	6.4	5.2
	<i>Pyrene</i>	13.9	7.8	5.0	7.6
PCBs	<i>PCB 52</i>		8.7	7.5	6.1
	<i>PCB 101</i>	14.9	16.0	9.8	9.7
	<i>PCB 138</i>	18.5	13.7	10.2	9.5
	<i>PCB 180</i>		9.6	8.9	8.3
PCNs	<i>Pentachloronapthalene (PCN 52)</i>	18.4	17.3	19.7	23.6
	<i>Pentachloronapthalene (PCN 54)</i>		21.1	18.2	16.5
	<i>Hexachloronapthalene (PCN 67)</i>		9.6	11.7	10.4
OCPs	<i>2,4-DDT</i>	24.9	19.3	12.5	12.1
	<i>4,4-DDD</i>	19.3	15.2	12.5	12.1
	<i>4,4-DDE</i>	15.4	10.5	12.7	9.3

Classification	Compound	%FME			
		C= 6.2 µg/Kg	C= 12.4 µg/Kg	C= 24.8 µg/Kg	C= 49.6 µg/Kg
	4,4-DDT		19.2	22.9	21.8
	Acetochlor	20.2	20.3	16.4	16.0
	Alachlor	14.5	15.3	17.8	19.1
	Aldrin		12.6	10.2	14.8
	Chlorpyrifos Ethyl	12.6	18.2	15.5	19.3
	Chlorthal-dimethyl	11.0	11.3	10.3	13.9
	Dichlorvos		9.8	7.8	12.4
	Dicofol	24.1	15.4	18.4	20.2
	Endosulfan alpha	9.3	8.7	8.1	5.5
	Endosulfan sulphate		8.0	7.5	9.8
	Hexachlorobenzene	16.4	16.8	17.0	20.3
	Isodrin	15.4	7.6	9.8	8.5
	Propachlor	6.2	17.5	18.2	19.3
	Propazine	16.6	16.4	14.2	19.8
PPPs	Acrinathrin			6.5	7.3
	Atrazine	3.2	4.8	4.7	6.2
	Beflubutamid	3.0	4.7	3.5	4.8
	Benalaxyl	7.4	11.7	10.6	9.8
	Benfluralin	1.9	2.0	1.8	2.3
	Bifenthrin	18.9	16.2	19.3	21.7
	Bupirimate		8.8	9.0	6.9
	Cadusafos	1.6	7.5	5.7	9.9
	Carfentrazone Ethyl	7.7	10.1	9.9	11.2
	Chlozolate		6.7	5.6	6.5
	Clomazone	19.3	18.9	20.6	19.7
	Cyanazine	10.5	9.9	14.4	23.4
	Cyhalothrin-lambda major			9.1	7.2
	Diazinon	20.8	20.3	20.4	26.7
	Dimethenamid	13.1	17.9	16.0	22.4
	Dimethoate	3.8	5.3	5.9	7.5
	Dimoxystrobin	17.7	18.9	14.2	20.3
	Diuron		9.2	6.7	5.3
	Ethalfuralin	7.9	8.5	3.7	5.1
	Ethion	5.7	8.9	6.2	10.0
	Ethoprophos	5.4	7.8	6.9	11.7
	Etoxazole	6.9	4.7	5.0	5.1
	Fenamiphos-sulfone	10.2	8.8	9.6	6.6
	Fenitrothion	10.8	5.6	3.6	4.2
	Fenoxycarb	13.8	10.7	9.0	11.6
	Fenpropathrin	12.6	14.5	8.8	5.0
	Fenthion		4.6	2.6	3.4
	Fludioxonil	20.1	17.8	19.4	14.4
	Fluopicolide	14.2	12.3	9.7	13.5
	Flusilazole	10.8	12.8	14.9	16.5
	Fosthiazate Isomer 1		22.8	18.4	12.3
	Fosthiazate Isomer 2		15.3	9.4	6.7
	Iprovalicarb Isomer 1	16.4	13.6	14.6	18.1
	Iprovalicarb Isomer 2	17.1	13.7	12.7	17.6
	Malaoxon	3.2	4.1	8.8	5.3
	Malathion	15.5	23.2	18.1	20.7
	Mepanipyrim	17.9	19.1	20.3	21.4
	Metazachlor	15.3	14.7	14.3	17.0
	Methacrifos	5.2	6.3	7.2	8.5
	Methidathion	5.9	7.8	5.5	7.8

Classification	Compound	%FME			
		C= 6.2 µg/Kg	C= 12.4 µg/Kg	C= 24.8 µg/Kg	C= 49.6 µg/Kg
	<i>Methoprottryne</i>	14.5	13.5	10.8	16.3
	<i>Metolachlor</i>	13.1	11.5	12.4	16.7
	<i>Metribuzin</i>	15.6	19.9	14.3	14.4
	<i>Mevinphos (Phosdrin)</i>	5.3	7.7	7.2	9.6
	<i>Myclobutanil</i>	13.4	11.3	11.4	12.2
	<i>Napropamide</i>	16.2	21.5	15.7	22.6
	<i>Oxadiazon</i>	19.6	24.6	16.7	21.6
	<i>Oxadixyl</i>	7.2	7.5	8.7	9.7
	<i>Oxyfluorfen</i>	8.1	8.2	5.8	7.0
	<i>Paclobutrazol</i>	16.7	12.9	11.0	11.4
	<i>Parathion-Ethyl</i>	4.5	5.8	5.0	6.2
	<i>Parathion-Methyl</i>	5.1	5.7	7.5	9.4
	<i>Penconazole</i>	13.6	16.1	15.1	15.7
	<i>Pirimicarb</i>	6.8	4.3	3.5	5.8
	<i>Procymidone</i>	1.6	2.8	2.2	2.6
	<i>Proquinazid</i>	14.4	16.5	13.1	17.1
	<i>Prothiophos</i>	8.4	12.9	11.1	15.9
	<i>Pyraflufen Ethyl</i>	14.9	18.9	14.8	17.4
	<i>Pyrazophos</i>		8.6	7.9	9.4
	<i>Pyrimiphos Me</i>	5.2	6.4	5.8	5.5
	<i>Quinalphos</i>	13.9	14.5	7.3	12.1
	<i>Quinoxifen</i>	12.8	12.3	9.6	11.8
	<i>Tebuconazole</i>		13.5	11.6	10.2
	<i>Tebuftenpyrad</i>		6.7	7.2	5.4
	<i>Tefluthrin</i>	14.0	13.7	6.3	9.4
	<i>Terbuthylazine</i>	8.6	4.9	4.3	6.8
	<i>Tolclofos Methyl</i>	17.0	14.0	7.4	7.9
	<i>Triadimefon</i>	16.6	18.7	18.5	21.7
	<i>Tri-allate</i>	26.9	35.7	31.6	48.8
	<i>Trifluralin</i>	9.2	4.6	3.4	3.2

The results of FME% are summarized in the **Figure 36** for 6.2, 12.4, 24.8 and 49.6 µg/Kg w.w. concentration levels.

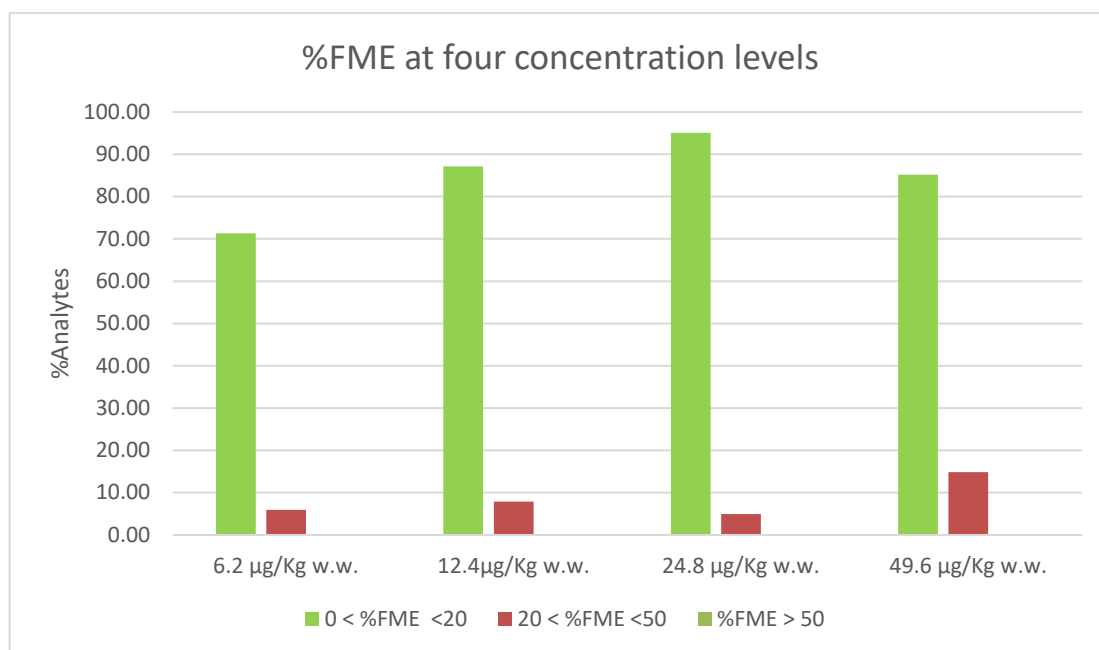


Figure 36: %FME at four concentration levels.

Ideally, the ME % is equal to zero meaning that neither ion suppression nor enhancement takes place. Total results for four different concentration levels shown that this tested parameter was ranging at levels lower than 50% and had insignificant effects on the signal for the majority of the tested compounds.

5.2.5 Precision

Precision of the developed method was assessed by the evaluation of repeatability (RSDr%) of three different spiked samples at two different concentration levels (**12.4 µg/Kg w.w.** and **49.6 µg/Kg w.w.**) in the same day. Total results for the estimation of precision are presented in **Table 12**.

Table 12: Total results for Precision at two concentration levels, ($n_{\text{replicates}}=6$).

Classification	Compound	%Repeatability	
		C= 12.4 $\mu\text{g/Kg}$	C= 49.6 $\mu\text{g/Kg}$
PAHs	<i>Benz(a)anthracene</i>		14
	<i>Benzo(a)pyrene</i>		12
	<i>Benzo(b)fluoranthene</i>		14
	<i>Chrysene</i>	10	14
	<i>Fluorene</i>	27	11
	<i>Phenanthrene</i>	15	11
	<i>Pyrene</i>	13	11
PCBs	<i>PCB 52</i>	4	2
	<i>PCB 101</i>	12	8
	<i>PCB 138</i>	5	6
	<i>PCB 180</i>	12	8
PCNs	<i>Pentachloronapthalene (PCN 52)</i>	9	8
	<i>Pentachloronapthalene (PCN 54)</i>	7	8
	<i>Hexachloronapthalene (PCN 67)</i>	12	9
OCPs	<i>2,4-DDT</i>	19	15
	<i>4,4-DDD</i>	10	9
	<i>4,4-DDE</i>	16	15
	<i>4,4-DDT</i>	12	10
	<i>Acetochlor</i>	47	60
	<i>Alachlor</i>	21	18
	<i>Aldrin</i>	19	38
	<i>Chlorpyrifos Ethyl</i>	21	19
	<i>Chlorthal-dimethyl</i>	18	10
	<i>Dichlorvos</i>	41	35
	<i>Dicofol</i>	14	17
	<i>Endosulfan alpha</i>	47	50
	<i>Endosulfan sulphate</i>	13	19
	<i>Hexachlorobenzene</i>	29	19
	<i>Isodrin</i>	7	15
	<i>Propachlor</i>	34	21
	<i>Propazine</i>	18	21
PPPs	<i>Acrinathrin</i>		34
	<i>Atrazine</i>	23	19
	<i>Beflubutamid</i>	29	31
	<i>Benalaxyl</i>	20	17
	<i>Benfluralin</i>	25	27
	<i>Bifenthrin</i>	25	28
	<i>Bupirimate</i>	16	11
	<i>Cadusafos</i>	14	15
	<i>Carfentrazone Ethyl</i>	27	37
	<i>Chlozolinat</i>	26	39
	<i>Clomazone</i>	66	72
	<i>Cyanazine</i>	32	39
	<i>Cyhalothrin-lambda major</i>		28
	<i>Diazinon</i>	24	19
	<i>Dimethenamid</i>	19	29
	<i>Dimethoate</i>	5	17

	Dimoxystrobin	22	26
	Diuron	26	27
	Ethalfluralin	21	26
	Ethion	32	38
	Ethoprophos	36	25
	Etoxazole	21	31
	Fenamiphos-sulfone	22	19
	Fenitrothion	36	38
	Fenoxycarb	18	26
	Fenpropathrin	17	46
	Fenthion	24	20
	Fludioxonil	16	13
	Fluopicolide	16	17
	Flusilazole	23	31
	Fosthiazate Isomer 1	21	20
	Fosthiazate Isomer 2	20	16
	Iprovalicarb Isomer 1	19	17
	Iprovalicarb Isomer 2	20	18
	Malaoxon	24	20
	Malathion	22	30
	Mepanipyrim	31	27
	Metazachlor	27	20
	Methacrifos	14	9
	Methidathion	5	70
	Methoprotetryne	13	28
	Metolachlor	3	1
	Metribuzin	27	31
	Mevinphos (Phosdrin)	27	28
	Myclobutanil	17	27
	Napropamide	25	26
	Oxadiazon	27	23
	Oxadixyl	26	21
	Oxyfluorfen	22	10
	Paclobutrazol	27	23
	Parathion-Ethyl	6	18
	Parathion-Methyl	9	13
	Penconazole	24	27
	Pirimicarb	8	6
	Procymidone	21	26
	Proquinazid	26	31
	Prothiophos	36	35
	Pyraflufen Ethyl	10	20
	Pyrazophos	12	10
	Pyrimiphos Me	21	38
	Quinalphos	16	15
	Quinoxifen	25	29
	Tebuconazole	26	24
	Tebufenpyrad	32	20
	Tefluthrin	23	26
	Terbutylazine	31	30
	Tolclofos Methyl	26	27
	Triadimefon	21	37
	Tri-allate	19	21
	Trifluralin	14	19

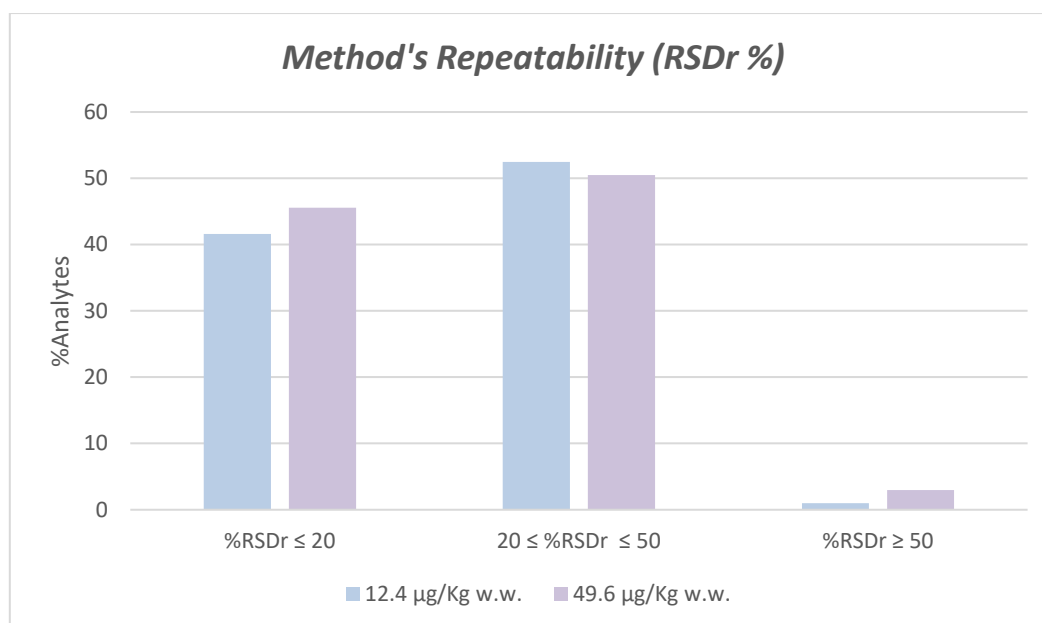


Figure 37: Repeatability (RSDr %) for the method.

As presented on the diagram, the repeatability for the method for most of the tested compounds was ranging between 20% and 50% (**Figure 37**).

5.3 Application of target screening in fish tissue samples

Fish tissue samples from the Black Sea and more specifically from Georgia, Ukraine and Russian Federation were sent to our laboratory, so as to estimate the overall contamination of these regions. These samples were prepared following the developed and validated protocol and they were analyzed using GC-APCI-QToFMS. Afterwards, the samples were screened using the software TASQ CLIENT 2.1 (Bruker Daltonic). A method was composed using the already mentioned in-house database and the screening of samples was based on the identification criteria which were mentioned earlier. In total, twelve analytes from different chemical classes were detected, as shown in **Table 13**. OCPs and PAHs were furtherly confirmed by analysis conducted by GC-EI MS/MS in SRM mode.

For the quantification of the samples the following procedure was followed.

Firstly, the ideal internal standard was selected for each analyte. Then, the quantification of the analytes which were detected in procedural blank samples was performed. The quantification of target analytes was performed using standard addition calibration curves. The concentration of each analyte was determined using the corresponding equation with the relative area which has been corrected with the appropriate internal standard.

From the concentration of the analytes that were detected in the samples, the concentration of the respective analytes in the procedural blank samples was subtracted. The final concentration came up after the calculation of the preconcentration of the samples during the sample treatment.

Table 13: Total results of the determination of organic compounds in fish samples.

<i>Classification</i>	<i>Analytes</i>	<i>fish_A</i>	<i>fish_B</i>	<i>fish_C</i>	<i>LOD (µg/Kg w.w.)</i>	<i>LOQ (µg/Kg w.w.)</i>
PAHS	<i>Fluorene</i>	<LOD	<LOD	<LOD	0.06	0.20
	<i>Phenanthrene</i>	1.15	3.62	1.56	0.31	1.04
PCBs	<i>PCB 101</i>	<LOD	2.05	<LOD	1.42	4.72
	<i>PCB 138</i>	<LOD	<LOD	<LOD	1.10	3.67
	<i>PCB 52</i>	<LOD	2.15	<LOD	2.10	6.99
OCPs	<i>2,4-DDT</i>	<LOD	1.11	<LOD	0.58	1.93
	<i>4,4-DDD</i>	<LOD	1.05	<LOD	0.58	1.94
	<i>4,4-DDE</i>	5.38	4.32	3.25	0.07	0.22
	<i>Chlorpyrifos Ethyl</i>	<LOD	<LOD	<LOQ	0.08	0.25
PPPs	<i>Benfluralin</i>	<LOD	<LOD	<LOQ	0.70	2.33
	<i>Diazinon</i>	<LOQ	<LOD	<LOD	0.03	0.09
	<i>Quinalphos</i>	<LOD	<LOD	9.91	1.25	4.17

The overall results indicate that the tested fish samples were contaminated, with OCPs having the highest contribution. This points the need for efficient workflows for the estimation of the environment contamination. As it is shown in **Table 13** most of the compounds were below of the LOD of the method

All three tested samples were found to be positive in PAHs (Phenanthrene and Fluorene). This arises the need for prioritization of these organic micropollutants for routine analysis of biota specimens in the future. Their extracted ion chromatograms are presented in **Figure 38** and **Figure 39**, respectively.

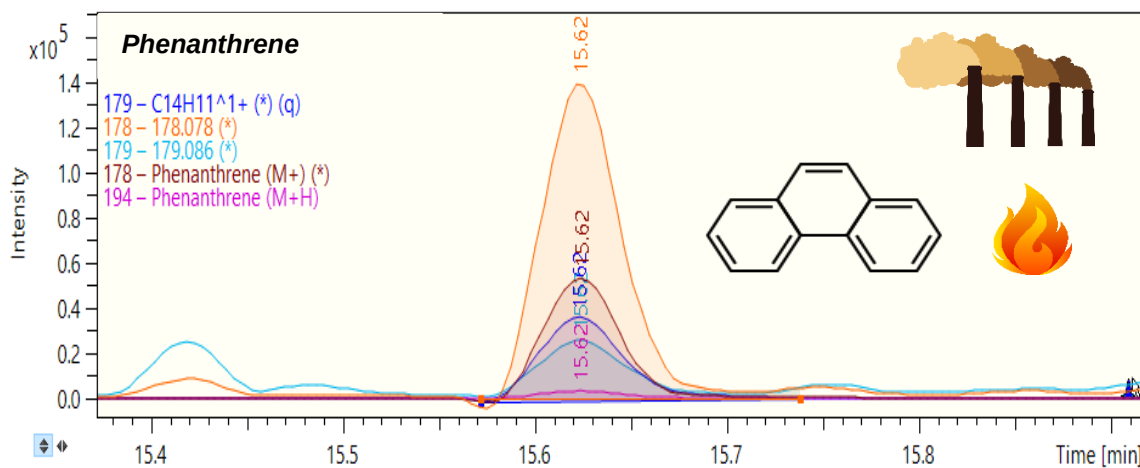


Figure 38: Chromatogram of PAH Phenanthrene.

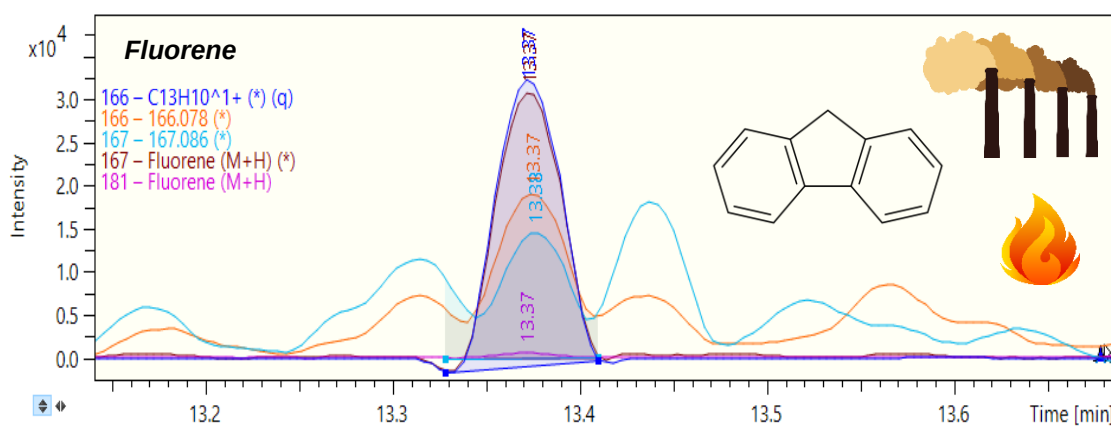


Figure 39: Chromatogram of PAH Fluorene.

Furthermore, the breakdown product of DDT, the 4,4-DDE chemical (**Figure 40**) was detected in all of the tested samples, indicating the persistency of DDT and its transformation products in the environment even after many years of banning its agricultural use.

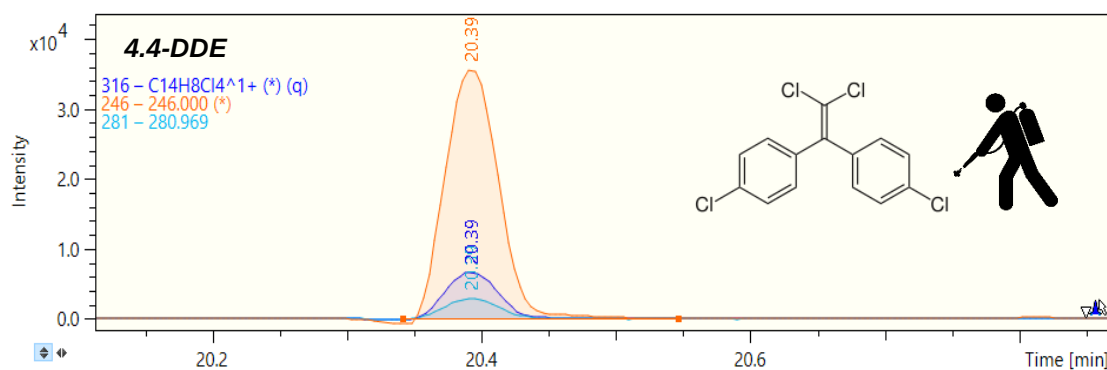


Figure 40: Chromatogram of 4,4-DDE.

CHAPTER 6

Conclusions and Future Perspectives

Emerging Contaminants and Persistent Organic Pollutants end up to the aquatic ecosystem through various ways and afterwards, they enter into the food webs, where they are bioaccumulated through the food chain. Consumers belonging to different trophic levels, due to their unique biology, habits and physiology, are integrated indicators of the overall environmental pollution caused by them. For this reason, the analysis of biota specimen is of great importance.

The evaluation of the results of this thesis demonstrates the need for the development of GC-HRMS workflows in order to extend the chemical domain of the applied screening approaches in monitoring studies. Wide-scope screening of POPs and ECs will provide the complete view of the contamination of the aquatic environment and the necessary measures will be taken.

In this master a generic method for the determination of GC-amenable organic xenobiotics had been developed. ASE apparatus was used for the extraction of the target analytes and the clean-up of the samples' extracts. The validation of the developed protocol and the evaluation of the linearity, MLODs, MLOQs, trueness, matrix effect, precision, and the detectability, using a representative validation dataset of 101 compounds with different physicochemical properties from a wide variety of classes of xenobiotics had followed.

Fish muscle samples were collected from three different sampling points at the Black Sea and they were prepared and analyzed following the developed method. Wide-scope target screening using a database with more than 300 organic contaminants was applied based on some performance criteria; mass accuracy, retention time, isotopic pattern and MS/MS information, were attributed in order to facilitate confidence.

The results of our research assured that few organic micropollutants from wide variety of classes are existed in these species. The same extracts can be used the suspect, non-target screening and retrospective analysis, due to the generic protocol, which was used in the sample preparation.

Last but not least, the developed method is intended to be applied to different matrices, coming from different regions, in order to prove its generic properties on an extended chemical field.

ABBREVIATIONS AND ACRONYMS

Table 14: Abbreviations and acronyms.

POPs	Persistent Organic Pollutants
OCPs	Organochlorine Pesticides
PCBs	Polychlorinated Biphenyls
PAHs	Polycyclic Aromatic Hydrocarbons
PCDDs	Polychlorinated Dibenzo-dioxins
PCDFs	Polychlorinated Dibenzofurans
PCNs	Polychlorinated Naphthalenes
PPPs	Plant Protection Products
EPA	Environmental Protection Agency
VOCs	Volatile Organic Compounds
PBDEs	Polybrominated Diphenyl Ethers
EU	European Union
EC	European Commission
EQs	Environmental Quality Standards
IARC	International Agency for Research on Cancer
TPs	Transformation Products
ECs	Emerging Contaminants
FRs	Flame Retardants
CPs	Chlorinated Paraffins
PFASs	Per- and Polyfluoroalkyl Substances
PLE	Pressurized Liquid Extraction
ASE	Accelerated Solvent Extraction
GC	Gas chromatography
GSC	Gas-Solid Chromatography
GLC	Gas-Liquid Chromatography
WCOT	Wall-Coated Open Tubular
SCOT	Support-Coated Open Tubular
APCI	Atmospheric Pressure Chemical Ionization
LC	Liquid Chromatography
MS	Mass Spectrometry
LRMS	Low Resolution Mass Spectrometry
HRMS	High Resolution Mass Spectrometry
Q	Quadrupole
QqQ	Triple Quadrupole
IT	Ion trap
ToF	Time of Flight
QToF	Quadrupole Time of Flight mass spectrometer
DDA	Data Dependent Acquisition
DIA	Data Independent Acquisition
SFE	Supercritical Fluid Extraction

MAE	Microwave Assisted Extraction
MSPD	Matrix Solid-Phase Dispersion
UAE	Ultrasound Assisted Extraction
GC-ECD	GC with Electron Capture Detector
GC-FID	GC with Flame Ionization Detector
HEX	Hexane
DCM	Dichloromethane
MeOH	Methanol
ACE	Acetone
ACN	Acetonitrile
EtoAC	Ethyl Acetate
TOL	Toluene
GPC	Gel Permeation Chromatography
SPE	Solid phase extraction
IPA	Isopropanol/2-propanol
RC	Regenerated Cellulose
HPLC	High Performance Liquid Chromatography
FC43	Perfluorotributylamine
ME	Matrix Effect
MF	Matrix factor
CRM	Certified Reference Material
LOD	Limit of Detection
LOQ	Limit of Quantification
R	Recovery
RSDr	Repeatability

References

- [1] M. Gavrilesu, K. Demnerová, J. Aamand, S. Agathos, F. Fava, Emerging pollutants in the environment: Present and future challenges in biomonitoring, ecological risks and bioremediation, *New Biotechnology*, vol. 32, 2015, pp.147–156.
- [2] P. Mira, S. Sabater, A. Elosegi, D. Barceló, *Emerging Contaminants in River Ecosystems*, 46th ed., Springer, Spain, 2016.
- [3] M. Lorenzo, J. Campo, Y. Picó, Analytical challenges to determine emerging persistent organic pollutants in aquatic ecosystems, *TrAC - Trends in Analytical Chemistry*, vol. 103, 2018, pp.137–155.
- [4] M.S. El-shahawi, A. Hamza, A.S. Bashammakh, W.T. Al-saggaf, Talanta An overview on the accumulation , distribution , transformations , toxicity and analytical methods for the monitoring of persistent organic pollutants, *Talanta*, vol. 80, 2010, pp.1587–1597.
- [5] X. Zhao, T. Cui, R. Guo, Y. Liu, X. Wang, Y. xia An, X. Qiao, B. Zheng, A clean-up method for determination of multi-classes of persistent organic pollutants in sediment and biota samples with an aliquot sample, *Analytica Chimica Acta*, vol. 1047, 2019, pp.71–80.
- [6] Priority Pollutant List-EPA, n.d.,. <https://www.epa.gov/sites/production/files/2015-09/documents/priority-pollutant-list-epa.pdf> (accessed September 25, 2021).
- [7] W. Wang, B. Meng, X. Lu, Y. Liu, S. Tao, Extraction of polycyclic aromatic hydrocarbons and organochlorine pesticides from soils: A comparison between Soxhlet extraction, microwave-assisted extraction and accelerated solvent extraction techniques, *Analytica Chimica Acta*, vol. 602, 2007, pp.211–222.
- [8] E. Martinez, M. Gros, S. Lacorte, D. Barceló, Simplified procedures for the analysis of polycyclic aromatic hydrocarbons in water, sediments and mussels, *Journal of Chromatography A*, vol. 1047, 2004, pp.181–188.
- [9] K. Hussain, R.R. Hoque, S. Balachandran, *Monitoring and Risk Analysis of PAHs in the Environment*, 2020.
- [10] H.I. Abdel-Shafy, M.S.M. Mansour, A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation, *Egyptian Journal of Petroleum*, vol. 25, 2016, pp.107–123.
- [11] OSPAR Commission 2007, *OSPAR Background Document on Dioxins*, 2007.
- [12] J.C. Larsen, P. Norhede, Polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs), *Environmental Project*, vol. No. 1521, 2013, pp.50.
- [13] E. Eljarrat, D. Barceló, Congener-specific determination of dioxins and related compounds by gas chromatography coupled to LRMS, HRMS, MS/MS and TOFMS, *Journal of Mass Spectrometry*, vol. 37, 2002, pp.1105–1117.
- [14] A.V.B. Reddy, M. Moniruzzaman, T.M. Aminabhavi, Polychlorinated biphenyls (PCBs) in the environment: Recent updates on sampling, pretreatment, cleanup technologies and their analysis, *Chemical Engineering Journal*, vol. 358, 2019, pp.1186–1207.
- [15] S. Harrad, *Persistent Organic Pollutants*, *Persistent Organic Pollutants*, vol. 2000, 2009, pp.1–277.
- [16] PCBs-general formula, n.d.,.

https://commons.wikimedia.org/wiki/File:PCB_allgemeine_Strukturformel.svg
(accessed September 25, 2021).

- [17] Y. Abubakar, H. Tijjani, C. Egbuna, C.O. Adetunji, S. Kala, T.L. Kryeziu, K.C. Patrick-Iwuanyanwu, Pesticides, history, and classification, *Natural Remedies for Pest, Disease and Weed Control*, 2019, pp.29–42.
- [18] W. Chen, M. Jing, J. Bu, J. Ellis Burnet, S. Qi, Q. Song, Y. Ke, J. Miao, M. Liu, C. Yang, Organochlorine pesticides in the surface water and sediments from the Peacock River Drainage Basin in Xinjiang, China: A study of an arid zone in Central Asia, *Environmental Monitoring and Assessment*, vol. 177, 2011, pp.1–21.
- [19] G.O. Duodu, A. Goonetilleke, G.A. Ayoko, Optimization of in-cell accelerated solvent extraction technique for the determination of organochlorine pesticides in river sediments, *Talanta*, vol. 150, 2016, pp.278–285.
- [20] L. Lupi, F. Bedmar, D.A. Wunderlin, K.S.B. Miglioranza, Organochlorine pesticides in agricultural soils and associated biota, *Environmental Earth Sciences*, vol. 75, 2016, pp.1–11.
- [21] U. Ali, J.H. Syed, R.N. Malik, A. Katsoyiannis, J. Li, G. Zhang, K.C. Jones, Organochlorine pesticides (OCPs) in South Asian region: A review, *Science of the Total Environment*, vol. 476–477, 2014, pp.705–717.
- [22] G.P. Satish, D.M. Ashokrao, S.K. Arun, Microbial degradation of pesticide: A review, *African Journal of Microbiology Research*, vol. 11, 2017, pp.992–1012.
- [23] European Union Implementation Plan for the Stockholm Convention on Persistent Organic Pollutants, vol. 8, 2014,.
- [24] Understanding POPs-ECHA, n.d.,. <https://echa.europa.eu/understanding-pops> (accessed September 25, 2021).
- [25] C. Lourencetti, M. Ricci, Determination of organochlorine priority substances in fish tissue: Optimisation of the clean-up step balancing removal of lipids with analytes' recovery, *Journal of Chromatography A*, vol. 1619, 2020, pp.460944.
- [26] J. Gasperi, S. Garnaud, V. Rocher, R. Moilleron, Priority pollutants in surface waters and settleable particles within a densely urbanised area: Case study of Paris (France), *Science of the Total Environment*, vol. 407, 2009, pp.2900–2908.
- [27] S. Sauvé, M. Desrosiers, A review of what is an emerging contaminant, *Chemistry Central Journal*, vol. 8, 2014,.
- [28] Norman Network, n.d.,. www.norman-network.net (accessed September 25, 2021).
- [29] T. Rasheed, M. Bilal, F. Nabeel, M. Adeel, M.N. Iqbal, Environmentally-related contaminants of high concern : Potential sources and analytical modalities for detection , quanti fi cation , and treatment, vol. 122, 2019, pp.52–66.
- [30] M. la Farré, S. Pérez, L. Kantiani, D. Barceló, Fate and toxicity of emerging pollutants, their metabolites and transformation products in the aquatic environment, *TrAC - Trends in Analytical Chemistry*, vol. 27, 2008, pp.991–1007.
- [31] N. Mastroianni, M.L. De Alda, D. Barceló, Emerging organic contaminants in aquatic environments: state-of-the-art and recent scientific contributions, *Contributions to Science*, vol. 6, 2010, pp.193–197.

- [32] M. Taheran, M. Naghdi, S.K. Brar, M. Verma, R.Y. Surampalli, Emerging contaminants: Here today, there tomorrow!, *Environmental Nanotechnology, Monitoring and Management*, vol. 10, 2018, pp.122–126.
- [33] M. Petrović, S. Gonzalez, D. Barceló, Analysis and removal of emerging contaminants in wastewater and drinking water, *TrAC - Trends in Analytical Chemistry*, vol. 22, 2003, pp.685–696.
- [34] V. Geissen, H. Mol, E. Klumpp, G. Umlauf, M. Nadal, M. van der Ploeg, S.E.A.T.M. van de Zee, C.J. Ritsema, Emerging pollutants in the environment: A challenge for water resource management, *International Soil and Water Conservation Research*, vol. 3, 2015, pp.57–65.
- [35] Thermo Fisher SCIENTIFIC, n.d.,
<https://www.thermofisher.com/gr/en/home/industrial/environmental/environmental-learning-center/contaminant-analysis-information/emerging-contaminants-analysis.html%0A%0A> (accessed September 17, 2020).
- [36] M. Giulivo, E. Capri, E. Eljarrat, D. Barceló, Analysis of organophosphorus flame retardants in environmental and biotic matrices using on-line turbulent flow chromatography-liquid chromatography-tandem mass spectrometry, *Journal of Chromatography A*, vol. 1474, 2016, pp.71–78.
- [37] J. Gustavsson, L. Ahrens, M.A. Nguyen, S. Josefsson, K. Wiberg, Development and comparison of gas chromatography–mass spectrometry techniques for analysis of flame retardants, *Journal of Chromatography A*, vol. 1481, 2017, pp.116–126.
- [38] R. Sun, X. Luo, B. Tang, Z. Li, L. Huang, T. Wang, B. Mai, Short-chain chlorinated paraffins in marine organisms from the Pearl River Estuary in South China: Residue levels and interspecies differences, *Science of the Total Environment*, vol. 553, 2016, pp.196–203.
- [39] X.J. Luo, Y.X. Sun, J.P. Wu, S.J. Chen, B.X. Mai, Short-chain chlorinated paraffins in terrestrial bird species inhabiting an e-waste recycling site in South China, *Environmental Pollution*, vol. 198, 2015, pp.41–46.
- [40] K. Kalinowska, P. Lenartowicz, J. Namieśnik, M. Marć, Analytical procedures for short chain chlorinated paraffins determination - How to make them greener?, *Science of the Total Environment*, vol. 671, 2019, pp.309–323.
- [41] L.M. van Mourik, I. van der Veen, S. Crum, J. de Boer, Developments and interlaboratory study of the analysis of short-chain chlorinated paraffins, *TrAC - Trends in Analytical Chemistry*, vol. 102, 2018, pp.32–40.
- [42] R.E. Clement, E.J. Reiner, S.P. Bhavsar, Organohalogen contaminants of emerging concern in Great Lakes fish: A review, *Analytical and Bioanalytical Chemistry*, vol. 404, 2012, pp.2639–2658.
- [43] N.S. Thomaidis, A.G. Asimakopoulos, A.A. Bletsou, Emerging contaminants: A tutorial mini-review, *Global Nest Journal*, vol. 14, 2012, pp.72–79.
- [44] G. Liu, Z. Cai, M. Zheng, Sources of unintentionally produced polychlorinated naphthalenes, *Chemosphere*, vol. 94, 2014, pp.1–12.
- [45] H. Feng, Y. Ruan, K. Zhang, P.K.S. Lam, Current analytical methodologies and gaps for per- and polyfluoroalkyl substances determination in the marine environment, *TrAC - Trends in Analytical Chemistry*, vol. 121, 2019, pp.115372.

- [46] E.E.L. Tr-- , V.F. Medina, S.A. Waisner, J. Mattei-sosa, E. Martinez-guerra, C.S. Griggs, J.M. Lalley, D.L. Henderson, L.C. Moores, B.H. Prager, Simulated Field Evaluation of the Decontamination Effluent Treatment System (DETS) for Wash Water from Mass Personnel Decontamination (MPD), Road Test , and Evaluation Treating Perfluorinated Alkyl Substances (PFAS), 2019,.
- [47] P. Wardrop, P.D. Morrison, J.G. Hughes, B.O. Clarke, Comparison of in-cell lipid removal efficiency of adsorbent mixtures for extraction of polybrominated diphenyl ethers in fish, *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, vol. 990, 2015, pp.1–6.
- [48] PBDEs-Molecular Structure, n.d.,. https://www.wikiwand.com/en/Polybrominated_diphenyl_ethers (accessed September 25, 2021).
- [49] S.M. O'Neill, A.J. Carey, L.B. Harding, J.E. West, G.M. Ylitalo, J.W. Chamberlin, Chemical tracers guide identification of the location and source of persistent organic pollutants in juvenile Chinook salmon (*Oncorhynchus tshawytscha*), migrating seaward through an estuary with multiple contaminant inputs, *Science of the Total Environment*, vol. 712, 2020, pp.135516.
- [50] P. Movalli, R. Dekker, J. Koschorreck, G. Treu, Bringing together raptor collections in Europe for contaminant research and monitoring in relation to chemicals regulations, *Environmental Science and Pollution Research*, vol. 24, 2017, pp.24057–24060.
- [51] F. Ebruary, H.L. Rees, Ices T Echniques in M Arine E Nvironmental S Ciences G Uidelines for the Study of the Epibenthos of Subtidal Environments, 2009,.
- [52] A. Zenker, M.R. Cicero, F. Prestinaci, P. Bottoni, M. Carere, Bioaccumulation and biomagnification potential of pharmaceuticals with a focus to the aquatic environment, *Journal of Environmental Management*, vol. 133, 2014, pp.378–387.
- [53] Bioaccumulation-Biomagnification of ECs, n.d.,. https://in.pinterest.com/pin/295056213068568304/?nic_v2=1a7b77vcr (accessed September 25, 2021).
- [54] B. Subedi, L. Aguilar, E.M. Robinson, K.J. Hageman, E. Björklund, R.J. Sheesley, S. Usenko, Selective pressurized liquid extraction as a sample-preparation technique for persistent organic pollutants and contaminants of emerging concern, *TrAC - Trends in Analytical Chemistry*, vol. 68, 2015, pp.119–132.
- [55] C. Nerín, C. Domeño, J. Salafranca, Advances in sample preparation of environmental solid matrices, *Comprehensive Sampling and Sample Preparation*, vol. 3, 2012, pp.783–796.
- [56] R.B. Hoff, T.M. Pizzolato, Combining extraction and purification steps in sample preparation for environmental matrices: A review of matrix solid phase dispersion (MSPD) and pressurized liquid extraction (PLE) applications, *TrAC - Trends in Analytical Chemistry*, vol. 109, 2018, pp.83–96.
- [57] N. Fidalgo-Used, E. Blanco-González, A. Sanz-Medel, Sample handling strategies for the determination of persistent trace organic contaminants from biota samples, *Analytica Chimica Acta*, vol. 590, 2007, pp.1–16.
- [58] H. Zhang, S. Bayen, B.C. Kelly, Co-extraction and simultaneous determination of multi-class hydrophobic organic contaminants in marine sediments and biota using GC-El-

MS/MS and LC-ESI-MS/MS, *Talanta*, vol. 143, 2015, pp.7–18.

- [59] A. Kettle, Use of Accelerated Solvent Extraction with In-Cell Cleanup to Eliminate Sample Cleanup During Sample Preparation, White Paper 70632 - Thermo Scientific, 2013, pp.1–7.
- [60] V. Andreu, Y. Picó, Pressurized liquid extraction of organic contaminants in environmental and food samples, *TrAC - Trends in Analytical Chemistry*, vol. 118, 2019, pp.709–721.
- [61] G. Alvarez-Rivera, M. Bueno, D. Ballesteros-Vivas, J.A. Mendiola, E. Ibañez, Pressurized liquid extraction, *Liquid-Phase Extraction*, 2019, pp.375–398.
- [62] H. Sun, X. Ge, Y. Lv, A. Wang, Application of accelerated solvent extraction in the analysis of organic contaminants, bioactive and nutritional compounds in food and feed, *Journal of Chromatography A*, vol. 1237, 2012, pp.1–23.
- [63] ASE stainless steel cell, n.d., <https://www.thermofisher.com/order/catalog/product/068085#/068085> (accessed September 25, 2021).
- [64] D. Megson, E.J. Reiner, K.J. Jobst, F.L. Dorman, M. Robson, J.F. Focant, A review of the determination of persistent organic pollutants for environmental forensics investigations, *Analytica Chimica Acta*, vol. 941, 2016, pp.10–25.
- [65] W. Skoog, *Analytical Chemistry*, 2013.
- [66] GC Instrumentation, n.d., <https://bitesizebio.com/28687/carrying-gas-chromatography/> (accessed September 25, 2021).
- [67] M.S. Tswett, Mass Spectrometry and Gas Chromatography, *Journal of the Mass Spectrometry Society of Japan*, vol. 1959, 2012, pp.3–3.
- [68] Types of capillary columns, n.d., <http://delloyd.50megs.com/moreinfo/gaschrom.html> (accessed September 25, 2021).
- [69] F.J. Andrade, J.T. Shelley, W.C. Wetze, M.R. Webb, G. Gamez, S.J. Ray, G.M. Hieftje, Atmospheric pressure chemical ionization source. 1. Ionization of compounds in the gas phase, *Analytical Chemistry*, vol. 80, 2008, pp.2646–2653.
- [70] J. Fang, H. Zhao, Y. Zhang, M. Lu, Z. Cai, Atmospheric pressure chemical ionization in gas chromatography-mass spectrometry for the analysis of persistent organic pollutants, *Trends in Environmental Analytical Chemistry*, vol. 25, 2020, pp.e00076.
- [71] M.A. Tarr, J. Zhu, R.B. Cole, *Atmospheric Pressure Ionization Mass Spectrometry*, 2000.
- [72] T. Portolés, J. V. Sancho, F. Hernández, A. Newton, P. Hancock, Potential of atmospheric pressure chemical ionization source in GC-QTOF MS for pesticide residue analysis, *Journal of Mass Spectrometry*, vol. 45, 2010, pp.926–936.
- [73] V.Y. J.R. Jocelyn Paré, Chapter 7 Mass spectrometry: Principles and applications, 1997.
- [74] K. Kalachova, J. Pulkrabova, T. Cajka, L. Drabova, M. Stupak, J. Hajslova, Gas chromatography-triple quadrupole tandem mass spectrometry: A powerful tool for the (ultra)trace analysis of multiclass environmental contaminants in fish and fish feed Rapid Detection in Food and Feed, *Analytical and Bioanalytical Chemistry*, vol. 405, 2013, pp.7803–7815.

- [75] D.G. V. Desfontaine, J.L. Veuthey, Hyphenated Detectors: Mass Spectrometry, 2017,.
- [76] A.T. Lebedev, O. V. Polyakova, D.M. Mazur, V.B. Artaev, The benefits of high resolution mass spectrometry in environmental analysis, *Analyst*, vol. 138, 2013, pp.6946–6953.
- [77] G. Santoiemma, Recent methodologies for studying the soil organic matter, *Applied Soil Ecology*, vol. 123, 2018, pp.546–550.
- [78] A.N.H. A.L. Rockwood, M.M. Kushnir, N.J. Clarke, K. Crockett, U. Eigner, S.A. Hofstadler, *Principles and Applications of Clinical Mass Spectrometry*, Elsevier Inc., 2018.
- [79] Linear QqQ instrument parts, n.d.,. https://www.wikiwand.com/en/Triple_quadrupole_mass_spectrometer (accessed September 25, 2021).
- [80] Principles of Instrumental Analysis, n.d.,. https://books.google.gr/books?id=D13EDQAAQBAJ&printsec=frontcover&dq=W.+Skoog,+Analytical+Chemistry,+2013&hl=en&sa=X&ved=2ahUKEwj49N_G--frAhXRpYsKHRGiCGsQ6AEwBnoECACQAg#v=onepage&q&f=false (accessed September 17, 2020).
- [81] I. Ferrer, E.M. Thurman, Liquid chromatography/time-of-flight/mass spectrometry (LC/TOF/MS) for the analysis of emerging contaminants, *TrAC - Trends in Analytical Chemistry*, vol. 22, 2003, pp.750–756.
- [82] Scheme of TOF instrument, n.d.,. [https://www.mcponline.org/article/S1535-9476\(20\)32864-4/fulltext](https://www.mcponline.org/article/S1535-9476(20)32864-4/fulltext) (accessed September 25, 2021).
- [83] S. Beck, A. Michalski, O. Raether, M. Lubeck, S. Kaspar, N. Goedecke, C. Baessmann, D. Hornburg, F. Meier, I. Paron, N.A. Kulak, J. Cox, M. Mann, The impact II, a very high-resolution quadrupole time-of-flight instrument (QTOF) for deep shotgun proteomics, *Molecular and Cellular Proteomics*, vol. 14, 2015, pp.2014–2029.
- [84] M. Krauss, H. Singer, J. Hollender, LC – high resolution MS in environmental analysis : from target screening to the identification of unknowns, 2010, pp.943–951.
- [85] E.L. Schymanski, J. Jeon, R. Gulde, K. Fenner, M. Ru, H.P. Singer, J. Hollender, Identifying Small Molecules via High Resolution Mass Spectrometry: Communicating Confidence, 2014, pp.2097–2098.
- [86] B.P. Gumbi, B. Moodley, G. Birungi, P.G. Ndungu, Target , Suspect and Non-Target Screening of Silylated Derivatives of Polar Compounds Based on Single Ion Monitoring GC-MS, 2019,.
- [87] H. Yu, Y. Tao, T. Le, D. Chen, A. Ishsan, Y. Liu, Y. Wang, Z. Yuan, Simultaneous determination of amitraz and its metabolite residue in food animal tissues by gas chromatography-electron capture detector and gas chromatography-mass spectrometry with accelerated solvent extraction, *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, vol. 878, 2010, pp.1746–1752.
- [88] C. Miège, A. Peretti, P. Labadie, H. Budzinski, B. Le Bizec, K. Vorkamp, J. Tronczyński, H. Persat, M. Coquery, M. Babut, Occurrence of priority and emerging organic compounds in fishes from the Rhone River (France), *Analytical and Bioanalytical Chemistry*, vol. 404, 2012, pp.2721–2735.
- [89] P. Perati, S.M.R. Ullah, Extraction of Organochlorine Pesticides from Oyster Tissue Using

Accelerated Solvent Extraction, n.d.,.

- [90] R. Yang, Y. Wang, A. Li, Q. Zhang, C. Jing, T. Wang, P. Wang, Y. Li, G. Jiang, Organochlorine pesticides and PCBs in fish from lakes of the Tibetan Plateau and the implications, *Environmental Pollution*, vol. 158, 2010, pp.2310–2316.
- [91] S. Pizzini, R. Piazza, G. Cozzi, C. Barbante, Simultaneous determination of halogenated contaminants and polycyclic aromatic hydrocarbons: a multi-analyte method applied to filter-feeding edible organisms, *Analytical and Bioanalytical Chemistry*, vol. 408, 2016, pp.7991–7999.
- [92] J.M. Van Emon, J.C. Chuang, *Analytica Chimica Acta* Development of a simultaneous extraction and cleanup method for pyrethroid pesticides from indoor house dust samples, *Analytica Chimica Acta*, vol. 745, 2012, pp.38–44.
- [93] E. Cocco, C. Guignard, L. Hoffmann, T. Bohn, Rapid analysis of polychlorinated biphenyls in fish by pressurised liquid extraction with in-cell cleanup and GC-MS, vol. 7319, 2011,.
- [94] Method 1613 Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, n.d.,. https://well-labs.com/docs/epa_method_1613b_1994.pdf (accessed October 7, 2020).
- [95] K. Wiberg, S. Sporning, P. Haglund, E. Björklund, Selective pressurized liquid extraction of polychlorinated dibenzo-p-dioxins, dibenzofurans and dioxin-like polychlorinated biphenyls from food and feed samples, *Journal of Chromatography A*, vol. 1138, 2007, pp.55–64.
- [96] B. Subedi, S. Usenko, Enhanced pressurized liquid extraction technique capable of analyzing polychlorodibenzo-p-dioxins, polychlorodibenzofurans, and polychlorobiphenyls in fish tissue, *Journal of Chromatography A*, vol. 1238, 2012, pp.30–37.
- [97] E.M. Robinson, S.J. Trumble, B. Subedi, R. Sanders, S. Usenko, Selective pressurized liquid extraction of pesticides, polychlorinated biphenyls and polybrominated diphenyl ethers in a whale earplug (earwax): A novel method for analyzing organic contaminants in lipid-rich matrices, *Journal of Chromatography A*, vol. 1319, 2013, pp.14–20.
- [98] M.G. Pintado-Herrera, E. González-Mazo, P.A. Lara-Martín, In-cell clean-up pressurized liquid extraction and gas chromatography-tandem mass spectrometry determination of hydrophobic persistent and emerging organic pollutants in coastal sediments, *Journal of Chromatography A*, vol. 1429, 2016, pp.107–118.
- [99] S. Lacorte, D. Raldúa, E. Martínez, A. Navarro, S. Diez, J.M. Bayona, D. Barceló, Pilot survey of a broad range of priority pollutants in sediment and fish from the Ebro river basin (NE Spain), *Environmental Pollution*, vol. 140, 2006, pp.471–482.
- [100] C.M.A. Iwegbue, L.C. Overah, G.O. Tesi, F.I. Bassey, B.S. Martincigh, Polycyclic Aromatic Hydrocarbon Profiles of Some Brands of Canned Fish in the Nigerian Market, *Human and Ecological Risk Assessment*, vol. 21, 2015, pp.157–168.
- [101] Q. Li, X. Zhang, C. Yan, Polycyclic aromatic hydrocarbon contamination of recent sediments and marine organisms from Xiamen Bay, China, *Archives of Environmental Contamination and Toxicology*, vol. 58, 2010, pp.711–721.
- [102] W. Tao, Z. Zhou, L. Shen, B. Zhao, Determination of dechlorane flame retardants in soil and fish at Guiyu, an electronic waste recycling site in south China, *Environmental Pollution*, vol. 206, 2015, pp.361–368.

- [103] T.M. Kolic, L. Shen, K. MacPherson, L. Fayez, T. Gobran, P.A. Helm, C.H. Marvin, G. Arsenault, E.J. Reiner, The analysis of halogenated flame retardants by GC-HRMS in environmental samples, *Journal of Chromatographic Science*, vol. 47, 2009, pp.83–91.
- [104] A. Note, A. Note, Extraction of PCBs from Environmental Samples Using Accelerated Solvent Extraction (ASE[®]), *Time*, vol. 3545, 2000, pp.1–4.
- [105] D.R. Luellen, M.J. LaGuardia, T.D. Tuckey, M.C. Fabrizio, G.W. Rice, R.C. Hale, Assessment of legacy and emerging contaminants in an introduced catfish and implications for the fishery, *Environmental Science and Pollution Research*, vol. 25, 2018, pp.28355–28366.
- [106] G. Ottonello, A. Ferrari, E. Magi, Determination of polychlorinated biphenyls in fish: Optimisation and validation of a method based on accelerated solvent extraction and gas chromatography-mass spectrometry, *Food Chemistry*, vol. 142, 2014, pp.327–333.
- [107] J.E. West, S.M. O'Neill, G.M. Ylitalo, Time Trends of Persistent Organic Pollutants in Benthic and Pelagic Indicator Fishes from Puget Sound, Washington, USA, *Archives of Environmental Contamination and Toxicology*, vol. 73, 2017, pp.207–229.
- [108] S. Panseri, L. Chiesa, G. Ghisleni, G. Marano, P. Boracchi, V. Ranghieri, R.M. Malandra, P. Roccabianca, M. Tecilla, Persistent organic pollutants in fish: biomonitoring and cocktail effect with implications for food safety, *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, vol. 36, 2019, pp.601–611.
- [109] Z. Gao, Y. Deng, W. Yuan, H. He, S. Yang, C. Sun, Determination of organophosphorus flame retardants in fish by pressurized liquid extraction using aqueous solutions and solid-phase microextraction coupled with gas chromatography-flame photometric detector, *Journal of Chromatography A*, vol. 1366, 2014, pp.31–37.
- [110] K. Kitamura, Y. Takazawa, S. Hashimoto, J. Choi, H. Ito, M. Morita, Effective extraction method for dioxin analysis from lipid-rich biological matrices using a combination of pressurized liquid extraction and dimethyl sulfoxide / acetonitrile / hexane partitioning, *Journal of Chromatography A*, vol. 512, 2004, pp.27–37.
- [111] K. Kim, G. Choo, H. Cho, B. Lee, K. Park, J. Oh, Science of the Total Environment The occurrence and distribution of polychlorinated naphthalenes (PCNs), focusing on tissue-specific bioaccumulation in crucian carp in South Korea, *Science of the Total Environment*, vol. 665, 2019, pp.484–491.
- [112] J.A. Layshock, G. Wilson, K.A. Anderson, Ketone and quinone-substituted polycyclic aromatic hydrocarbons in mussel tissue, sediment, urban dust, and diesel particulate matrices, *Environmental Toxicology and Chemistry*, vol. 29, 2010, pp.2450–2460.
- [113] B. Murphy, S. Lingam, B. Richter, R. Carlson, Simultaneous Extraction of PAHs and PCBs from Environmental Samples Using Accelerated Solvent Extraction, 2012, pp.1–5.
- [114] P. Wang, Q. Zhang, Y. Wang, T. Wang, X. Li, L. Ding, G. Jiang, 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, *Analytica Chimica Acta*, vol. 663, 2010, pp.43–48.
- [115] L. Webster, M. Russell, P. Walsham, L.A. Phillips, G. Packer, I. Hussy, J.A. Scurfield, E.J. Dalgarno, C.F. Moffat, An assessment of persistent organic pollutants (POPs) in wild and rope grown blue mussels (*Mytilus edulis*) from Scottish coastal waters, *Journal of*

Environmental Monitoring, vol. 11, 2009, pp.1169–1184.

- [116] M. Gassel, S. Harwani, J.S. Park, A. Jahn, Detection of nonylphenol and persistent organic pollutants in fish from the North Pacific Central Gyre, *Marine Pollution Bulletin*, vol. 73, 2013, pp.231–242.
- [117] L.L. Johnson, G.M. Ylitalo, M.S. Myers, B.F. Anulacion, J. Buzitis, T.K. Collier, Aluminum smelter-derived polycyclic aromatic hydrocarbons and flatfish health in the Kitimat marine ecosystem, British Columbia, Canada, *Science of the Total Environment*, vol. 512–513, 2015, pp.227–239.
- [118] A. Luna-Acosta, H. Budzinski, K. Le Menach, H. Thomas-Guyon, P. Bustamante, Persistent organic pollutants in a marine bivalve on the Marennes-Oléron Bay and the Gironde Estuary (French Atlantic Coast)-Part 1: Bioaccumulation, *Science of the Total Environment*, vol. 514, 2015, pp.500–510.
- [119] J. Parera, M. Ábalos, F.J. Santos, M.T. Galceran, E. Abad, Chemosphere paraffins and polybrominated diphenyl ethers in marine fish species from Ebro River Delta (Spain), *Chemosphere*, vol. 93, 2013, pp.499–505.
- [120] H. Wolschke, X. Meng, Z. Xie, R. Ebinghaus, M. Cai, 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, *MARINE POLLUTION BULLETIN*, 2015,.
- [121] M. Bujalance, I. Giraldez, A. Velasco, E. Morales, Determination of polychlorinated biphenyls in biota samples using simultaneous pressurized liquid extraction and purification q, vol. 946, 2002, pp.209–219.
- [122] A. Covaci, A. Gheorghe, O. Hulea, P. Schepens, Levels and distribution of organochlorine pesticides , polychlorinated biphenyls and polybrominated diphenyl ethers in sediments and biota from the Danube Delta , Romania, vol. 140, 2006, pp.136–149.
- [123] Y. Picó, D. Barceló, Emerging contaminants in biota, 2012, pp.2525–2526.
- [124] P. Zapata, R. Ballesteros-cano, P. Colomer, A. Bertolero, P. Viana, S. Lacorte, F. Javier, Science of the Total Environment Presence and impact of Stockholm Convention POPs in gull eggs from Spanish and Portuguese natural and national parks, *Science of the Total Environment*, vol. 633, 2018, pp.704–715.
- [125] M. Choi, I.S. Lee, R.H. Jung, Rapid determination of organochlorine pesticides in fish using selective pressurized liquid extraction and gas chromatography-mass spectrometry, *Food Chemistry*, vol. 205, 2016, pp.1–8.
- [126] Z.L. Shen, D. Yuan, H. Zhang, M. Hu, J.H. Zhu, X.Q. Zhang, Q. De Su, Matrix solid phase dispersion-accelerated solvent extraction for determination of OCP residues in fish muscles, *Journal of the Chinese Chemical Society*, vol. 58, 2011, pp.494–502.
- [127] A.G. Frenich, J.L. Mart, A.D.C. Sicilia, M.J. Gonz, Multiresidue analysis of organochlorine and organophosphorus pesticides in muscle of chicken , pork and lamb by gas chromatography – triple quadrupole mass spectrometry, vol. 558, 2006, pp.42–52.
- [128] R. Sühling, F. Busch, N. Fricke, D. Kötke, H. Wolschke, R. Ebinghaus, Distribution of brominated flame retardants and dechloranes between sediments and benthic fish - A comparison of a freshwater and marine habitat, *Science of the Total Environment*, vol. 542, 2016, pp.578–585.

- [129] D. Chen, Statewide Surveillance of Emerging Flame Retardant Contamination in Illinois Waters via Fish Monitoring, vol. 2013, 2013, pp.1–9.
- [130] P. Wardrop, J. Shimeta, D. Nugegoda, P.D. Morrison, A. Miranda, M. Tang, B.O. Clarke, Chemical Pollutants Sorbed to Ingested Microbeads from Personal Care Products Accumulate in Fish, *Environmental Science and Technology*, vol. 50, 2016, pp.4037–4044.
- [131] E.M. Chua, J. Shimeta, D. Nugegoda, P.D. Morrison, B.O. Clarke, Assimilation of polybrominated diphenyl ethers from microplastics by the marine amphipod, *allorchestes compressa*, *Environmental Science and Technology*, vol. 48, 2014, pp.8127–8134.
- [132] F. Neugebauer, A. Dreyer, N. Lohmann, J. Koschorreck, Determination of halogenated flame retardants by GC-API-MS/MS and GC-EI-MS: a multi-compound multi-matrix method, *Analytical and Bioanalytical Chemistry*, vol. 410, 2018, pp.1375–1387.
- [133] S.L. Klosterhaus, H.M. Stapleton, M.J. La Guardia, D.J. Greig, Brominated and chlorinated flame retardants in San Francisco Bay sediments and wildlife, *Environment International*, vol. 47, 2012, pp.56–65.
- [134] S. Losada, F.J. Santos, M.T. Galceran, Selective pressurized liquid extraction of polybrominated diphenyl ethers in fish, *Talanta*, vol. 80, 2009, pp.839–845.
- [135] X. Ma, H. Zhang, Z. Wang, Z. Yao, J. Chen, J. Chen, Bioaccumulation and trophic transfer of short chain chlorinated paraffins in a marine food web from liaodong bay, North China, *Environmental Science and Technology*, vol. 48, 2014, pp.5964–5971.
- [136] D. Xia, L. Gao, S. Zhu, M. Zheng, Separation and screening of short-chain chlorinated paraffins in environmental samples using comprehensive two-dimensional gas chromatography with micro electron capture detection, *Analytical and Bioanalytical Chemistry*, vol. 406, 2014, pp.7561–7570.
- [137] H. Li, J. Fu, W. Pan, P. Wang, Y. Li, Q. Zhang, Y. Wang, A. Zhang, Y. Liang, G. Jiang, Determination of short-chain chlorinated paraffins in multiple matrices of Arctic using gas chromatography-electron capture negative ion-low resolution mass spectrometry, *MethodsX*, vol. 5, 2018, pp.939–943.
- [138] B. Du, J. Ge, R. Yang, X. Han, H. Chen, J. Li, L. Zeng, Altitude-dependent accumulation of short chain chlorinated paraffins in fish from alpine lakes and Lhasa river on the Tibetan Plateau, *Environmental Pollution*, vol. 250, 2019, pp.594–600.
- [139] S. Bayarri, L.T. Baldassarri, N. Iacovella, F. Ferrara, A. Di Domenico, PCDDs, PCDFs, PCBs and DDE in edible marine species from the Adriatic Sea, *Chemosphere*, vol. 43, 2001, pp.601–610.
- [140] C. Munschy, N. Guiot, K. Héas-Moisán, C. Tixier, J. Tronczyński, Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) in marine mussels from French coasts: Levels, patterns and temporal trends from 1981 to 2005, *Chemosphere*, vol. 73, 2008, pp.945–953.
- [141] M. Nunes, P. Marchand, A. Vernisseau, B. Le Bizec, F. Ramos, M.A. Pardal, PCDD/Fs and dioxin-like PCBs in sediment and biota from the Mondego estuary (Portugal), *Chemosphere*, vol. 83, 2011, pp.1345–1352.
- [142] R. Lega, D. Megson, C. Hartley, P. Crozier, K. MacPherson, T. Kolic, P.A. Helm, A. Myers, S.P. Bhavsar, E.J. Reiner, Congener specific determination of polychlorinated

naphthalenes in sediment and biota by gas chromatography high resolution mass spectrometry, *Journal of Chromatography A*, vol. 1479, 2017, pp.169–176.

- [143] S.B. Gewurtz, N. Gandhi, K.G. Drouillard, T. Kolic, K. MacPherson, E.J. Reiner, S.P. Bhavsar, Levels, patterns, trends and significance of polychlorinated naphthalenes (PCNs) in Great Lakes fish, *Science of the Total Environment*, vol. 624, 2018, pp.499–508.
- [144] L. Cui, S. Wang, L. Gao, H. Huang, D. Xia, L. Qiao, W. Liu, Concentrations and trophic magnification of polychlorinated naphthalenes (PCNs) in marine fish from the Bohai coastal area, China, *Environmental Pollution*, vol. 234, 2018, pp.876–884.
- [145] J. Tan, X. Lu, L. Fu, G. Yang, J. Chen, Quantification of Cl-PAHs and their parent compounds in fish by improved ASE method and stable isotope dilution GC-MS, *Ecotoxicology and Environmental Safety*, vol. 186, 2019, pp.109775.
- [146] K. Xia, G. Hagood, C. Childers, J. Atkins, B. Rogers, L. Ware, K. Armbrust, J. Jewell, D. Diaz, N. Gatian, H. Folmer, Polycyclic aromatic hydrocarbons (PAHs) in Mississippi seafood from areas affected by the deepwater horizon oil spill, *Environmental Science and Technology*, vol. 46, 2012, pp.5310–5318.
- [147] M.I.H. Helaleh, A. Al-Rashdan, Automated pressurized liquid extraction (PLE) and automated power-prep™ clean-up for the analysis of polycyclic aromatic hydrocarbons, organo-chlorinated pesticides and polychlorinated biphenyls in marine samples, *Analytical Methods*, vol. 5, 2013, pp.1617–1622.
- [148] K.A. Blocksom, D.M. Walters, T.M. Jicha, J.M. Lazorchak, T.R. Angradi, D.W. Bolgrien, Persistent organic pollutants in fish tissue in the mid-continental great rivers of the United States, *Science of the Total Environment*, vol. 408, 2010, pp.1180–1189.
- [149] L.M. Chiesa, G.F. Labella, S. Panseri, R. Pavlovic, S. Bonacci, F. Arioli, Distribution of persistent organic pollutants (POPS) IN wild Bluefin tuna (*Thunnus thynnus*) from different FAO capture zones, *Chemosphere*, vol. 153, 2016, pp.162–169.
- [150] M.R. Arkoosh, S. Strickland, A. Van Gaest, G.M. Ylitalo, L. Johnson, G.K. Yanagida, T.K. Collier, J.P. Dietrich, Trends in organic pollutants and lipids in juvenile Snake River spring Chinook salmon with different outmigrating histories through the Lower Snake and Middle Columbia Rivers, *Science of the Total Environment*, vol. 409, 2011, pp.5086–5100.
- [151] B. Barhoumi, K. Le Menach, C. Clérandeau, W. Ben Ameer, H. Budzinski, M.R. Driss, J.Ô. Cachot, Assessment of pollution in the Bizerte lagoon (Tunisia) by the combined use of chemical and biochemical markers in mussels, *Mytilus galloprovincialis*, *Marine Pollution Bulletin*, vol. 84, 2014, pp.379–390.
- [152] A. Method, F.O.R. Simultaneous, D. Of, F.S. Pulkrabova, F. Chemistry, C.T. Prague, C. Republic, R pcb , pbde pah, *Chemical Technology*, vol. 71, 2009, pp.2230–2235.
- [153] P. Méndez-Fernandez, L. Webster, T. Chouvelon, P. Bustamante, M. Ferreira, A.F. González, A. López, C.F. Moffat, G.J. Pierce, F.L. Read, M. Russell, M.B. Santos, J.Ô. Spitz, J. V. Vingada, F. Caurant, An assessment of contaminant concentrations in toothed whale species of the NW Iberian Peninsula: Part I. Persistent organic pollutants, *Science of the Total Environment*, vol. 484, 2014, pp.196–205.
- [154] D.J. Greig, G.M. Ylitalo, E.A. Wheeler, D. Boyd, F.M.D. Gulland, G.K. Yanagida, J.T. Harvey, A.J. Hall, Geography and stage of development affect persistent organic

pollutants in stranded and wild-caught harbor seal pups from central California, *Science of the Total Environment*, vol. 409, 2011, pp.3537–3547.

- [155] F. Galbiati, L. Chiesa, G. Labella, R. Pavlovic, F. Arioli, S. Panseri, Determination of Persistent Organic Pollutants in Fish Tissues by Accelerated Solvent Extraction and GC-MS/MS, 2016,.
- [156] S. Fernando, A. Renaguli, M.S. Milligan, J.J. Pagano, P.K. Hopke, T.M. Holsen, B.S. Crimmins, Comprehensive Analysis of the Great Lakes Top Predator Fish for Novel Halogenated Organic Contaminants by GC×GC-HR-ToF Mass Spectrometry, *Environmental Science and Technology*, vol. 52, 2018, pp.2909–2917.
- [157] L. Drábová, J. Pulkrabová, K. Kalachová, J. Hradecký, M. Suchanová, M. Tomaniová, V. Kocourek, J. Hajšlová, Novel approaches to determination of PAHs and halogenated POPs in canned fish, *Czech Journal of Food Sciences*, vol. 29, 2011, pp.498–507.
- [158] P. Haglund, S. Sporning, K. Wiberg, E. Björklund, Shape-selective extraction of PCBs and dioxins from fish and fish oil using in-cell carbon fractionation pressurized liquid extraction, *Analytical Chemistry*, vol. 79, 2007, pp.2945–2951.
- [159] B. Subedi, L. Aguilar, E.S. Williams, B.W. Brooks, S. Usenko, Selective pressurized liquid extraction technique capable of analyzing dioxins, furans, and pcbs in clams and crab tissue, *Bulletin of Environmental Contamination and Toxicology*, vol. 92, 2014, pp.460–465.
- [160] N. Molbert, F. Alliot, R. Santos, M. Chevreuil, J.M. Mouchel, A. Goutte, Multiresidue Methods for the Determination of Organic Micropollutants and Their Metabolites in Fish Matrices, *Environmental Toxicology and Chemistry*, vol. 38, 2019, pp.1866–1878.
- [161] R. Draisci, C. Marchiafava, E. Ferretti, L. Palleschi, G. Catellani, A. Anastasio, Evaluation of musk contamination of freshwater fish in Italy by accelerated solvent extraction and gas chromatography with mass spectrometric detection, *Journal of Chromatography A*, vol. 814, 1998, pp.187–197.
- [162] C. Toledo-Neira, P. Enríquez, P. Richter, Integrated pressurized solvent extraction-cleanup for the rapid determination of polychlorinated biphenyls in meat samples, *Journal of the Brazilian Chemical Society*, vol. 24, 2013, pp.743–748.
- [163] METHOD 3630 c, n.d.,. <https://www.epa.gov/sites/production/files/2015-12/documents/3620c.pdf> (accessed September 19, 2020).
- [164] R.C. Brändli, T.D. Bucheli, T. Kupper, F.X. Stadelmann, J. Tarradellas, Optimised accelerated solvent extraction of PCBs and PAHs from compost, *International Journal of Environmental Analytical Chemistry*, vol. 86, 2006, pp.505–525.
- [165] Method 3610b, n.d.,. <https://xenco.com/pdf/tech/SW846/SW846-3000series/SW846-3610b.pdf> (accessed September 19, 2020).
- [166] Method 3620c, n.d.,. <https://www.epa.gov/sites/production/files/2015-12/documents/3620c.pdf> (accessed September 19, 2020).
- [167] F. KOC, E. KARAKUS, Organik Klorlu Pestisid Kalıntılarının Florisil Kolonda Ayrılmasından Sonra Gaz Kromatografi - Kütle Spektrometre ile Belirlenmesi, *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, vol. 17, 2009, pp.65–70.
- [168] B. Yuan, T. Wang, N. Zhu, K. Zhang, L. Zeng, J. Fu, Y. Wang, G. Jiang, Short chain chlorinated paraffins in mollusks from coastal waters in the Chinese Bohai Sea,

Environmental Science and Technology, vol. 46, 2012, pp.6489–6496.

- [169] L. Chen, Y. Huang, S. Han, Y. Feng, G. Jiang, C. Tang, Z. Ye, W. Zhan, M. Liu, S. Zhang, Sample pretreatment optimization for the analysis of short chain chlorinated paraffins in soil with gas chromatography-electron capture negative ion-mass spectrometry, *Journal of Chromatography A*, vol. 1274, 2013, pp.36–43.
- [170] N. Xiang, C. Jiang, T. Yang, P. Li, H. Wang, Y. Xie, S. Li, H. Zhou, X. Diao, Occurrence and distribution of Polycyclic aromatic hydrocarbons (PAHs) in seawater, sediments and corals from Hainan Island, China, *Ecotoxicology and Environmental Safety*, vol. 152, 2018, pp.8–15.
- [171] Dionex ASE 350, n.d., source: <https://www.thermofisher.com/order/catalog/product/083114> (accessed September 26, 2021).